University of Montenegro
Biotechnical faculty
Univerzitet Crne Gore
Biotehnički fakultet

Agriculture and Forestry
Poljoprivreda i šumarstvo
1

Agriculture and Forestry, Vol. 63. Issue 1: 1-366, Podgorica, 2017

ISSN 0554-5579; E-ISSN 1800-9492; DOI: 10.17707/AgricultForest
COBIS.CG-ID: 3758082 www.agricultforest.ac.me

Agriculture and Forestry - *Poljoprivreda i šumarstvo*
PUBLISHER - *IZDAVAČ*
University of Montenegro – Univerzitet Crne Gore
Biotechnical faculty, Podgorica - Biotehnički fakultet, Podgorica
Bul. M. Lalića 1, 81000 Podgorica, Crna Gora (Montenegro), P.Box 97,
Tel.: +382 20 268434; +382 20 268437; Fax: +382 20 268432
Web: www.agricultforest.ac.me; E-mail: agricultforest@ac.me

Indexed in (by alphabethical order): AGRICOLA, AGRIS, CAB Abstracts, CAB Direct, CABI full text, COBISS, CrossRef, DOI, DOAJ, Directory of Research Journal Indexing (DRJI), Directory Indexing of International Research Journals, EBSCO (currently included in EDS EBSCO Discovery Service), Elektronische Zeitschriftenbibliothek EZB (Electronic Journals Library), Genamics JournalSeek, Global impact factor (GIF), Google Scholar, HathiTrustt, Index Copernicus, International Scientific Indexing (ISI), Israel Union List of Electronic Journals (ULE), J-Gate, JournalRate (Academic journal search tool), Journals Impact factor (JIF) by Global Society for Scientific Research (GSSR), KoBSON, MALMAD, National Agricultural Library – USA, ProQuest, Research bible, RoMEO, Regional Information Center for Science and Technology (RICeST) – Iran, Rubriq database, The Open University Library, The Rudjer Bošković Institute Library, Ulrichsweb, Universal Impact Factor, WorldCat, XML, Zeitschriftendatenbank (ZDB), Zoological Record of the Thomson Reuters, ...

The journal "Agriculture and Forestry" is funded by the Biotechnical faculty, Co-funded by the Architecture Faculty of the University of Montenegro, the Ministry of Science & the Ministry of Agriculture and Rural Development of Montenegro

CIP – Каталогизација у публикацији
Централна народна библиотека Црне Горе, Цетиње
ISSN 0554-5579
COBIS.CG-ID 3758082

CONTENT

DOI: 10.17707/AgricultForest.63.1.01

Carlo De MICHELE,
Massimo NATALIZIO, Guido D'URSO[1]

APPLYING EARTH OBSERVATION TO DETECT NON-AUTHORISED IRRIGATION: THE CASE STUDY OF CONSORZIO SANNIO ALIFANO (ITALY)

SUMMARY

In addition to reducing global water availability unauthorized irrigation and over-consumption can have social consequences in terms of conflicting water use. In its Water Framework Directive, the European Union (EU) has outlined an agenda for future water policy, emphasizing that, to ensure a sustainable use of water resources, these practices should be strongly opposed. In order to address this problem efficiently, water managers need to map irrigated area, plan the rational use of water resources under limited availability, and prevent unauthorized irrigation. We are currently developing an innovative system to do this based on a series of multi-spectral satellite acquisitions from two sensors having different spatial and temporal resolutions (DEIMOS, Rapid Eye). In this system, the irrigated area is identified based on temporal pattern recognition, exploiting the differing developmental rates between irrigated and not irrigated crops. This method was applied in the district of Consorzio Sannio Alifano, located in Southern Italy, where irrigation is required for most crops including corn, alfalfa, fruit trees and vegetables. An accuracy assessment of the methodology has been performed and has demonstrated positive results of this approach. Future system upgrades will exploit information derived from short-wave infrared data obtained using of the newly developed Sentinel-2 sensor. The approach described herein is the technological basis of a recently-funded EU H2020 project, named ***D****etection and* ***I****ntegrated* ***A****ssessment of* ***N****on-authorised water* ***A****bstractions using Earth Observation* or DIANA.

Keywords: *Detection of non-authorised irrigation, EO & illegal irrigations, agricultural advisory services, satellite monitoring of irrigated areas, management irrigation systems.*

INTRODUCTION

European agriculture is by far the largest user of water in this region. For example, in Spain, Greece and Portugal, respectively, 64%, 88% and 80% of

[1]Carlo De Michele, Ariespace s.r.l., Spin-off Company of the University of Naples Federico II, Centro Direz. Is.A/3, Naples, ITALY, Massimo Natalizio (corresponding author: massimonatalizio@gmail.com) Irrigation Consorzio Sannio Alifano, Viale della Libertà 61, Piedimonte Matese (CE), ITALY, Guido D'Urso Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, Portici (NA), ITALY
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

total water resources are destined to agriculture, with significant environmental and economic impacts (OCSE 2006). The dominance of agricultural water usage isa critical issue especially considering that, in Europe, about 75% of 16 million hectares of equipped agricultural fields is centered in Mediterranean countries (France, Greece, Italy, Portugal and Spain), a region periodically exposed to periods of crisis in water availability. In these countries, proper irrigation management and efficient utilization of water resources is a strategic goal. After an initial period of huge financial investment, based on the concept that modernization of irrigation required new irrigation infrastructure, increasing emphasis has been placed on the development of systems and technologies for better management of the modernized infrastructure.

One of the critical issues in this second phase of irrigation improvement is linked to the availability of updated data concerning the actual extension of irrigated areas and the evaluation of crops' water needs. At present, in effect, the methods applied in EU Member States to estimate irrigation water volumes are mostly cognitive, administrative or indirect. They include questionnaires, coefficients of water use, water rights, time of pumping stations and other empirical methods (Nagy, Lenz, Windhofer, Fuerst, Fribourg-Blanc,2007). Although the water directive (2000/60 CE) states that each water district must know the quality and the quantity of water allocated for various uses, to date, a comprehensive framework concerning both the localization and the amount of irrigation use is still in the future (Wriedt *et al.*,2009). In particular, the extension and the distribution of irrigated areas still remain unknown, despite the importance that these information would have on both food safety and the water/energy cycle (Vörösmarty, 2002).

In this context, a replicable and updated methodology allowing the identification of irrigated areas would be a valuable tool to monitor the use of water resources for irrigation purposes. Several authors point out that satellite-based technology, particularly those related to Earth observation, may be an effective tool for mapping irrigated areas all over the world and at different spatial scales (global, regional, local) (Ozdogan *et al.*, 2010) (D'Urso *et al.*, 2010).

The aim of this study is the definition of an innovative methodology based on Earth observation data for mapping irrigated areas on a local scale in semi-arid environment. The following example shows how the method was applied to map the irrigated area of a part of the general district served by the Consorzio Sannio Alifano, in Southern Italy, illustrating aspects of the study which can be generalized in favor of Authorities and management bodies committed to ensuring a sustainable and legal use of water resources in agriculture.

MATERIALS AND METHODS

The basic assumption of the proposed methodology is that, under conditions of hydrologic deficit typical of semi-arid environments, high crop growth trends are compatible only with external irrigation supplies. Based on this

assumption, the detection of the irrigated areas can be conducted independently of the actual cultivated crops. Practically, this means that a detailed knowledge of the spatial distribution of the different crops is not required. Rather, it is enough to take into account the timing of some indexes able to represent the vegetative vigor, such as NDVI.

The proposed approach is based on the use of two time series of satellite multispectral images (Double Series Irrigation Mapping - DSIM) acquired from the beginning to the end of the irrigation season. The first series, consists of data with low spatial resolution and high temporal frequency, in order to follow the phenological development of crops. The second, consists of 1 or 2 images with high spatial resolution, acquired in coincidence of the peak crop growth, in order to obtain a better segmentation of individual fields.

From an operational point of view, the procedure consists of the following stages:

a) Choice of data and preliminary processing

In this phase the types of sensors most suitable for the study are chosen based on the requirements in terms of spatial resolution, temporal and spectral.

b) Production of multi-time series of vegetation index NDVI maps

At this stage, a spectral index map NDVI is produced for each of the acquisitions of the low spatial resolution series. Subsequently, a temporal stack (layer stack) is created (a secondary activity conducted is the manual masking of areas not of interest, typically urban, mountain and wetlands such as rivers, lakes and water basins in these maps).

c) ISODATA unsupervised classification applied to time series of NDVI index maps.

d) Automatic extraction both of temporal NDVI index and vegetation peak pattern.

e) Labeling of vegetative areas

Identification of classes of vegetation categories that, given the water deficit conditions, show NDVI pattern compatible only with irrigation;

f) Supervised classification of the high-resolution image

This is done using such training pixels the irrigated areas identified via the multi-time classification of NDVI index made on the long series of low-resolution images. This phase is aimed at improving the spatial resolution of the data.

g) Mapping of irrigated areas along with possible integration with GIS data.

This process is illustrated schematically in Figure 1.

In summary, the time series maps of NDVI index collects all the information needed, pixel-by -pixel, parcel-to-parcel, to establish whether or not a given area is irrigated. However, the final solution to the problem of identification requires a single map for the whole irrigation season. Ideally, this would include a binary information (irrigated/non-irrigated) in which regions are divided into classes (irrigated with high, medium or low probability).

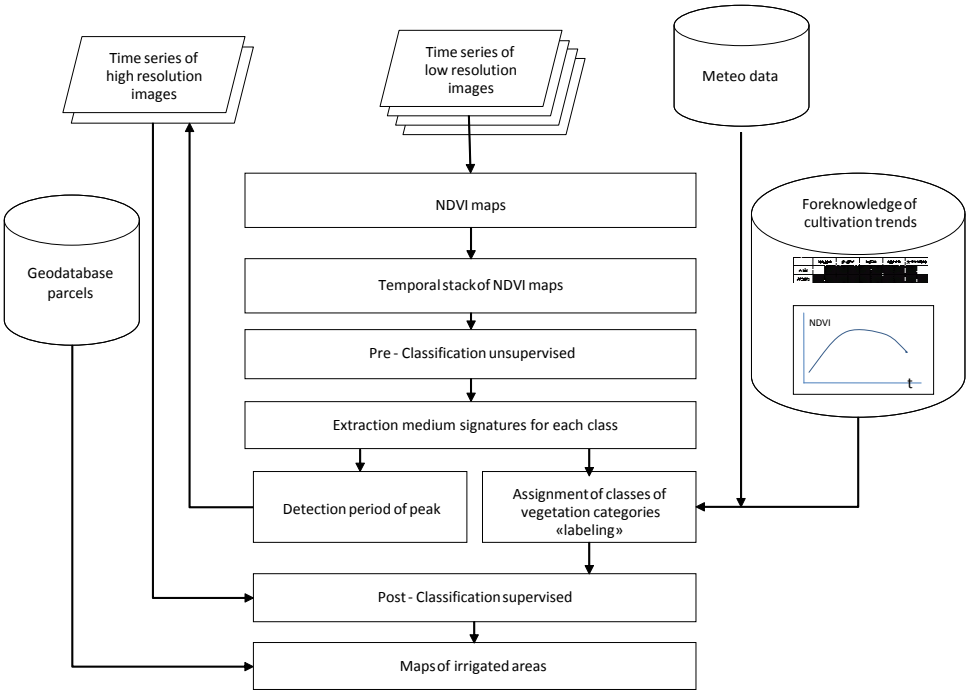


Figure 1. - Work flow of the detection process of irrigated areas

Given the complexity of the 3-dimensional problem (two defined by the plane of the investigated surface and the third, time, i.e., growth trends) that develops, an automatic classification of the temporal series of NDVI maps was performed. This is equivalent to eliminating the time dimension from the derived solution: the index NDVI trend is analyzed, pixel by pixel, and the result of this analysis assigns the pixel to a class (irrigated/non-irrigated).This operation is carried out automatically by a statistic algorithm that divides the pixels into clusters with similar characteristics, i.e. temporal trends NDVI index which can be gathered together. Finally, with the help of metrics and model diagrams, the different classes irrigated and non-irrigated can be recognized by following the procedure illustrated in Figure 2.

The application of this method leads to the classification of irrigated areas into three types:

1. **Class A irrigated areas**: characterized by a strong trend of crop growth, that is irrigated with high probability (mainly crops such as corn);
2. **Class B irrigated areas**: characterized by a time trend of NDVI index with "saw tooth shape", definable as irrigated with medium probability (mainly crops like alfalfa); in case of satellite acquisitions very spaced in time their recognition is very difficult;
3. **Class C irrigated areas**: characterized by a fairly steady and high-value NDVI index, definable as irrigated with low probability; they are mainly permanent crops i.e. some tree crops (including vineyards; in such cases)

where it is difficult to recognize the practice of irrigation because of the stress condition in which they are normally subjected during the growth or for limited vegetation dynamics.

4. **Non-irrigated areas**.

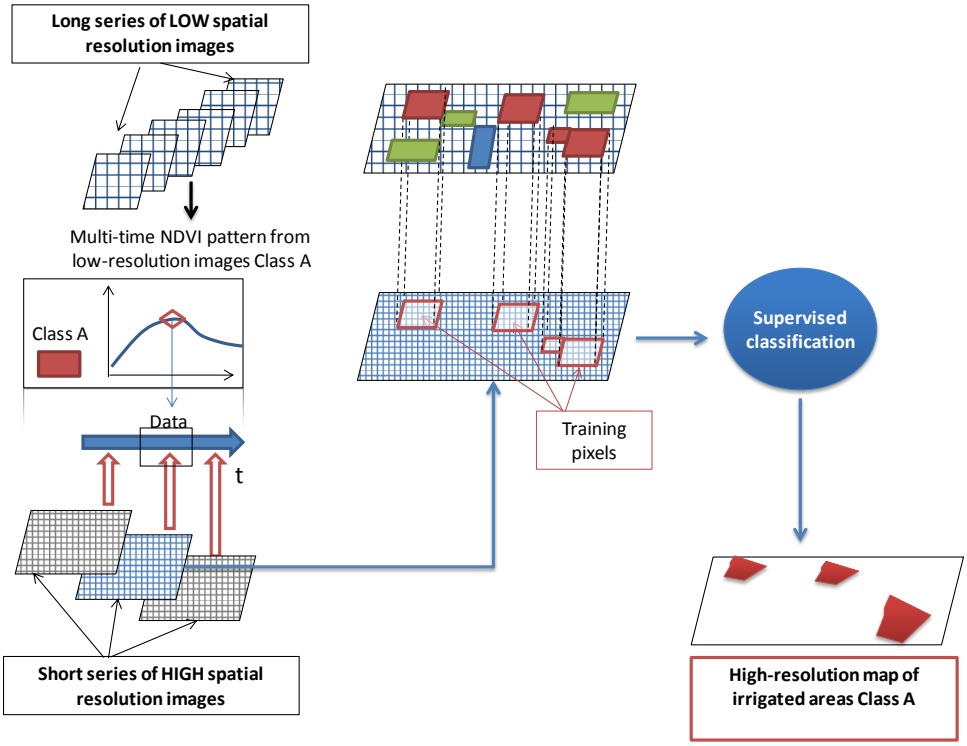


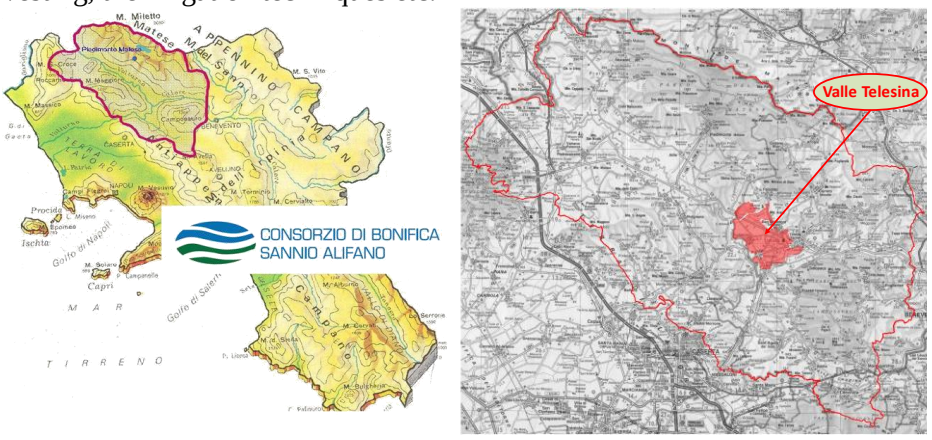
Figure 2. Procedure for the classification of high-resolution images (short series) by choosing the training pixels on the basis of multi-time classification NDVI index made on the long series of low-resolution images**.**

RESULTS AND DISCUSSION

The methodology described above was applied to map the irrigated area of a part of the overall district served by Cosorzio Sannio Alifano, located in Southern Italy, encompassing a surface of about 5,000 hectares and named Valle Telesina. This region is shown in Figure 3.The study area is characterized by agriculture irrigation in the period from May to September, with main crops grown corn, alfalfa, fruit trees and vegetables. The average size of each plot is about 2 hectares.

An important source of knowledge for this study has been the irrigation information system used by the Cosorzio Sannio Alifano. In 2013, the Consorzio set up a geographic information system (GIS) to streamline irrigation management. The system, designed by the academic spin off company Ariespacesrl., which is consulted and updated via web, allows the Consorzio to

generate single irrigation plot mapping referred to land parcels, irrigation districts, distribution networks, etc. and also integrates information supplied by the farmers about the type of cultivated crops, the time of planting and harvesting, the irrigation techniques etc.



a) b)

Figure 3. a) The overall district of Cosorzio Sannio Alifano (area of about 195,000 ha) outlined in red within the larger Campania region; b) The study area (in red) named "Valle Telesina" (about 5,000 ha).

A portion of this map is shown in Figure 4. For the purposes of this study, the access to the geographical database, allowed the improvement of the segmentation and resolution of the maps produced, by using the information vector cadastral layer to compare the data declared by farmers with the real land use and detect non-authorized irrigation.

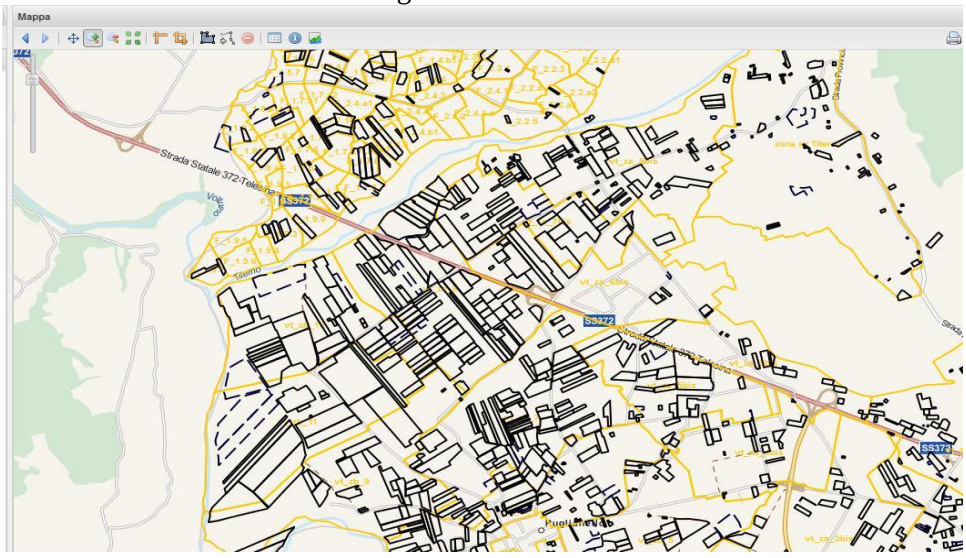


Figure 4. - View of a portion of the Consorzio Web-GIS map

The study was based on 8 DEIMOS images (long multi-time series at a lower spatial resolution, 22m) and 4 Rapid Eye images (short multi-time series with higher spatial resolution, 6.5 m), acquired in the period May 1 - September 30.The results showed a high accuracy of the maps could be achieved (see Figure 5). In particular, the reliability of the classification was assessed by comparison with the ground truth obtained from irrigated land database and, sometimes, through field inspection of plots evenly distributed in the study area.

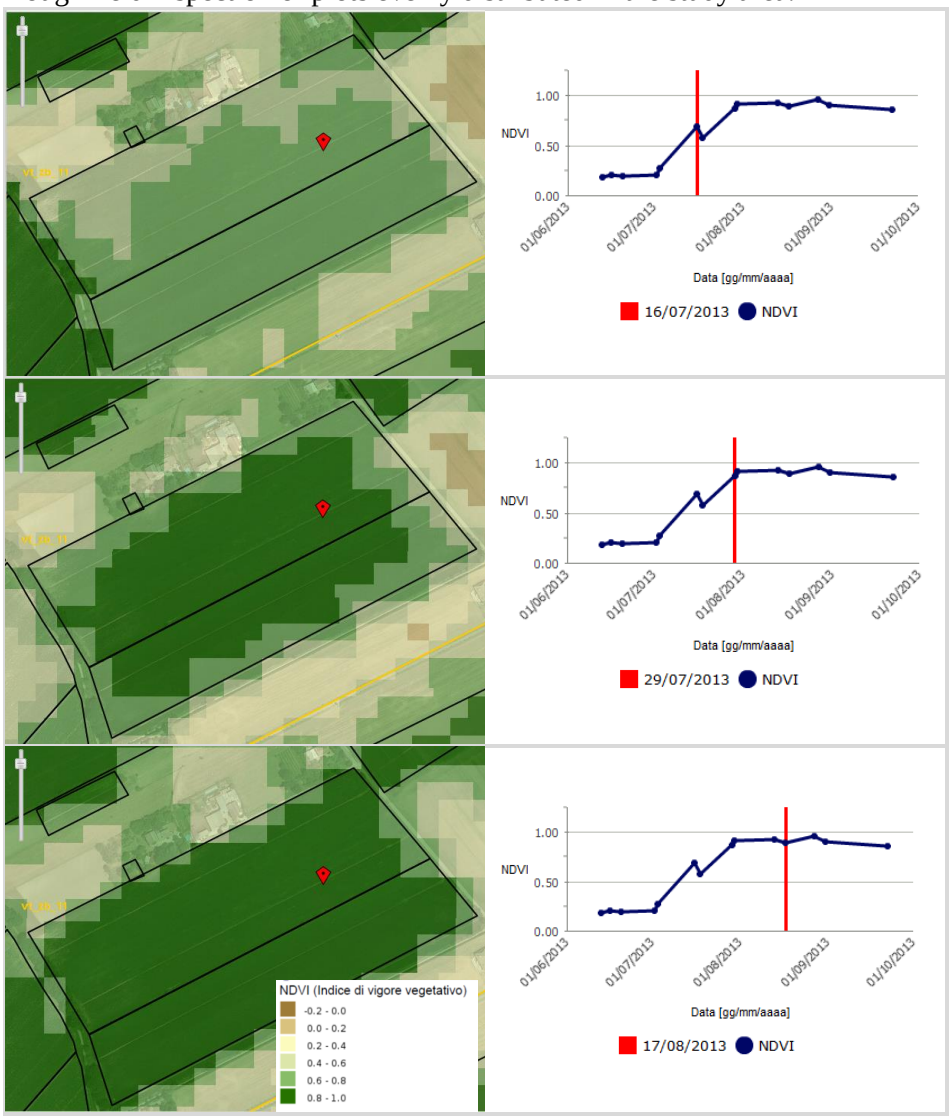


Figure 5. Time trends of NDVI index of an irrigated parcel (corn, Class A): notice the growth trend of NDVI index during the period June-September 2013 (dates: July 16; July 29; August 17).

Table 1. - Error matrix and values of accuracy measures of the procedure

		Data reference / ground truth				**Total per class in the map**	**UA**	
		Class A	Class B	Class C	No irrigation			
Classification data	Class A	25	1	0	0	27	93%	
	Class B	1	2	0	0	8	63%	
	Class C	0	3	1	1	6	50%	
	No irrigation	0	0	0	8	9	89%	
Total per truth class		26	6	6	9	50		
PA		96%	56%	50%	89%		82%	GA

The accuracy of the method was assessed by the following error matrix, in which the quality indexes have been calculated in terms of global (overall) accuracy (GA), user accuracy (UA) and producer accuracy (PA), as shown in Table 1.GA was defined by the ratio of the total of the agreements to the total test samples, and represents the probability that a point taken at random is correctly classified in the thematic map. It can be seen from Table 1that the estimated GA was 82%.

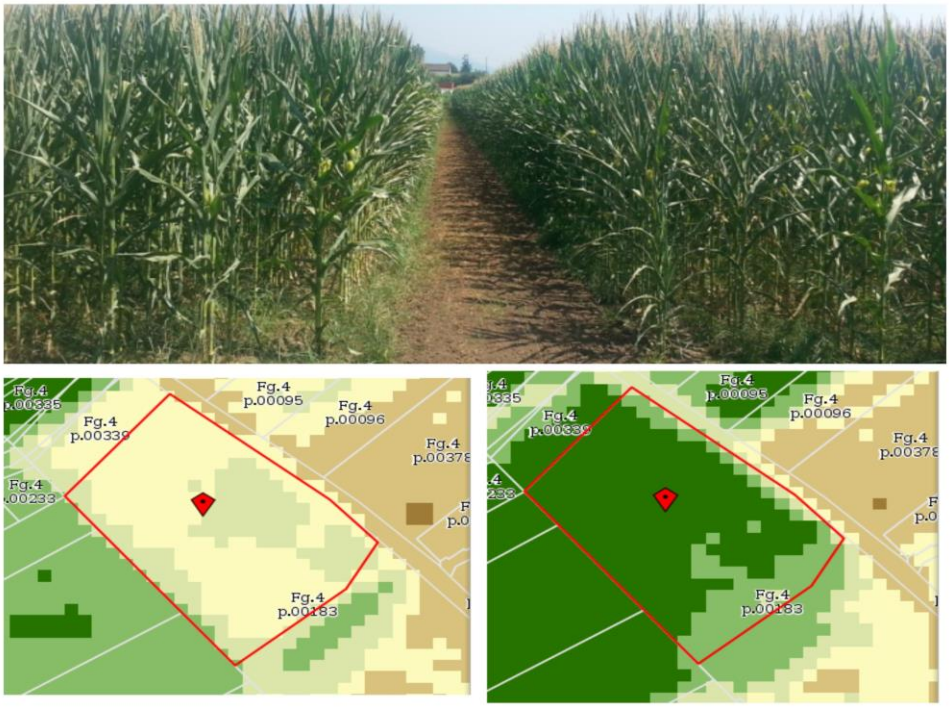


Figure 6. Inspection of a corn field for the detection of the ground truth, originated from the observation of the time trends of NDVI index. a) picture of the inspected field; b) NDVI map at beginning of the season; c) NDVI map at the period of field inspection.

As a result of the analysis, computer experts of Ariespacesrl Company identified and promptly indicated to the staff of Consorzio the lists and the maps of land parcels to be checked. The information was provided in terms of cadastral coordinates - Municipality, Sheet and Parcel - and identity of land owners. These parcels (266 in total) correspond to approximately 114 hectares (compared to a total surface of 920 hectares registered as "irrigated") to be checked because they were identified as potentially irrigated but lacking of irrigation demand. The field inspection carried out by the staff of Consorzio have verified the accuracy of the predictions (see Figure 6), given that in 89% of the cases the presence of irrigated crops in the reported lands was observed.

CONCLUSION

This study has demonstrated that the mapping of irrigated areas obtained by EO data is a reliable support tool for irrigation management capable of identifying unauthorized irrigation in agriculture rapidly and remotely. The system used has the following strengths:

- qualitative/quantitative information data is provided on the real performance and the status of crops growth;
- programming many acquisitions during the entire irrigation season is easy;
- large areas can be covered with a single image;
- processing of the data is rapid (images are usually supplied already pre-processed);
- support can be readily provided to water managers, i.e. it's possible to carry out targeted controls on the ground based on satellite information, thus maximizing the utilization of dedicated personnel;
- procedures can be simply automated;
- information provided at an affordable cost.

This same methodology can also be applied to give farmers and water managers an additional service, i.e. the "irrigation advice" (timely information about crop water requirements), without appreciable increased costs, amplifying the advantages of EO systems.

Future developments of the proposed methodology are related both to the possibility of improving the analysis of the temporal vegetation indexes and the improvement of the segmentation using classifiers, i.e. "object oriented" instead "pixel based". In particular, the study on vegetation indexes can benefit greatly from the opportunity to use the new generation of Sentinel-2 constellation sensors that allows to frequently updating the mapping of land cover. Moreover, using short-wavelength infrared data (SWIR), can improve the evaluation of biophysical parameters of vegetation, taking into account the water content of the vegetation.

REFERENCES

D'Urso G., Richter K., Calera A., et al. (2010). Earth Observation products for operational irrigation management in the context of the PLEIADeS project. Agricultural Water

Management, Vol. 98, No. 2, pp. 271-282. doi:10.1016/j.agwat.2010.08.020. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0378377410002878 [Accessed March 11, 2014].

Nagy M., Lenz K., Windhofer G., Fuerst J., Fribourg-Blanc B. (2007). Data Collection Manual for the OECD/Eurostat Joint Questionnaire on Inland Waters Tables 1-7, Version 2.1.

Ozdogan M., Yang Y., Allez G., Cervantes C. (2010). Remote Sensing of Irrigated Agriculture: Opportunities and Challenges. Remote Sens. Vol. 2, Issue. 9, pp. 2274-2304. doi:10.3390/rs2092274. Available at: http://www.mdpi.com/2072-4292/2/9/2274/ [Accessed February 11, 2014].

Vörösmarty C. (2002). Global water assessment and potential contributions from Earth Systems Science. Aquatic Sciences, Vol. 64, Issue. 4, pp. 328-351 doi:10.1007/PL00012590. Available at: http://link.springer.com/article/10.1007/PL00012590 [Accessed April 5, 2014].

Wriedt G., Van der Velde M., Aloe A., F. Bouraoui (2009). Estimating Irrigation Requirements in Europe. Journal of Hydrology, Vol. 373, Issue. 3-4, pp. 527-544. doi:10.1016/j.jhydrol.2009.05.018. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022169409003175 [Accessed February 25, 2014].

DOI: 10.17707/AgricultForest.63.1.02

Milena MOTEVA, Georgi KOSTADINOV, Velibor SPALEVIC, Veska GEORGIEVA, Vjekoslav TANASKOVIK, Natalia KOLEVA[1]

SWEET CORN - CONVENTIONAL TILLAGE VS. NO-TILLAGE IN HUMID CONDITIONS

SUMMARY

The goal of the paper is to compare the impact of conventional tillage and no-tillage technology on the growth, the yield and yield components of sweet corn, cultivated on chromic luvisols. A field experiment with Super Sweet 71,12 R hybrid was carried out in 2014 in the region of Sofia, Bulgaria. The impact of both systems on the total fresh ear yield, marketable fresh ear yield, total ear number, marketable ear number, single marketable fresh ear mass, marketable ear row number, one row kernel number of a marketable ear, marketable fresh ear kernel mass, plant height, leaf number per plant, ear legth, and tassel length was established. Analysis of variance was applied to all data obtained. The experiment was carried out on chromic luvisols, in a temperate-continental climate and in a very humid year. The results showed that the conventional tillage in such nature conditions have had better performance than the no-tillage technology. The yield of marketable fresh ears under conventional tillage was twice higher than that under no-tillage, i.e. 8.5 Mg/ha vs. 4.2 Mg/ha; kernel mass of a single fresh ear was with 22.6% higher, i.e. 163.8 g vs. 133.6 g, the 1000-kernel mass was with 14.4% higher, i.e. 337.2 g vs. 293.0 g. Analogously, the plants were longer and had thicker stems with greater leaf number, resulting in 12.5% greater fresh-ear length – 20.7 cm. The total fresh biomass under conventional tillage reaches 633.0 g/plant vs. 414.6 g/plant under no-tillage and the dry matter - 145.6 g vs. 103.7 g/plant. The protein content was 13.8% vs. 12.7%. The production under conventional tillage was more profitable. The price of a marketable corn ear was much lower - 0.0358 EUR/pc vs. 0.0512 EUR/pc. No-till requires precise preliminary estimation of the nature conditions and weather prognoses and cannot be recommended to very humid areas and

[1]Milena Moteva University of Architecture, Civil Engineering and Geodesy, Faculty of Geodesy, Department of Land Management and Agrarian Development, BULGARIA, Georgi Kostadinov (corresponding author: gdkostadinov@gmail.com), Institute of Soil Science, Agro-Technology and Plant Protection – Agricultural Academy, BULGARIA, Velibor Spalević University of Montenegro, Faculty of Philosophy, Niksic Department of Geography, MONTENEGRO, Veska Georgieva National Institute of Meteorology and Hydrology – Bulgarian Academy of Sciences, Department of Agrometeorology, BULGARIA, Vjekoslav Tanaskovik University St. Cyril and Methodius, Faculty of Agricultural Sciences and Food, Skopje, MACEDONIA, Natalia Koleva, Forestry University, Faculty of Agronomy, Department Agriculture and Herbology, Sofia, BULGARIA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

conditions. In very humid conditions it should be applied on weed-free areas after several-year control through herbicides.

Keywords: *No-till, Conventional tillage, Sweet corn, Yield, Yield components, Bulgaria*

INTRODUCTION

One of the strict requirements of market economy is high-quality and competitiveness of the agricultural products. The resource-saving and environmentally friendly agricultural technologies play a major role in meeting these issues. Recently, a new outlook to soil preservation by applying conservation tillage has gained popularity. The traditional systems of soil cultivation are regarded as causing soil degradation and being inconsistent with the environmentally friendly use of land. Any kind of conservation tillage (zero, strip, mulch, chisel, ridge and plow tillage, the latter with special plows that retain at least 30% plant residuals or plant mulch on the surface) provides safe land use from environmental, social and economic points of view. According to Derpsh (2009), if the farmers want to have the opportunity not only to survive, but also to achieve an environmentally sound and economically viable farming, they have to replace the traditional tillage with new tillage methods and farming. The conservation tillage technologies take control on water and wind erosion, preserve and improve soil fertility, improve the humus balance, reduce the loss of nutrients and moisture, improve crop productivity and labor efficiency, reduce the specific energy consumption and the production costs. These cultivation practices are considered as the most promising (Vrazhnov, 2013). Currently, 25% of the whole cultivated area in the world is under no-till. No-till is now practiced in all latitudes and altitudes, in extremely dry conditions and extremely rainy areas, in all kinds of farm sizes, in soils, in all crops as well. In the South American countries, Canada and the United States, 90% of the area sown with cereals is cultivated by conservation technologies, including up to 50-60% no-till (Calistu, Jităreanu, 2014). Despite its advantages, this technology is not yet widely used in Europe. The share of the conventional soil preparation technology is 70-75%, subsurface - 20-25%, while direct sowing in the untreated soil - less than 5%, (Gapon, 2014, Bulavin, 2016). The area of grain crops under no-tillage technology in Russia does not exceed 2% (Vrazhnov, 2013). According to Vrazhnov, (2013) a number of shortcomings hinder the widespread use of soil conservation technologies - disruption of the biological processes in the top soil layer, lack of N, unharmonious and weakened shoots, an increase of the weeds application of a large amount of herbicides additional application of N fertilizer, lack of necessary machines and tools, high cost of special stubble seeders. The direct seeding technology does not always ensure the growth of productivity and profitability (Bulavin, 2016)

Sweet corn (*Zea mays saharata* Korn.) has gained popularity in Bulgaria over the past 10 years. Nowadays the consumers' demand for fresh sweet corn is strong. The crop is attractive for its excellent taste and nutritional value due to

the high content of sugars. Though the crop is profitable and easily marketed, Bulgarian market of sweet corn is still underdeveloped. Production of sweet corn needs a production technology that provides maximum return and meets the environmental principles of the sustainable production (Sevov, 2014).

The goal of the paper is to compare the impact of conventional tillage and no-tillage technology on the growth and the yield and yield components of sweet corn, grown on chromic luvisols.

MATERIALS AND METHODS

A field experiment with Super Sweet 71,12 R hybrid was carried out in 2014 in the region of Sofia, Bulgaria. The site is near Sofia at 550 m a.s.l. The climate is temperate-continental and the region is one of the coldest and most humid in Bulgaria (Moteva et al., 2015). The experiment was put in a randomized complete block design in three replications. No-till vs. conventional tillage was tested. Conventional tillage consisted of deep plowing and repeating cultivation before sowing, while no-tillage consisted of direct sowing in a field with plant canola residues. Dicamba in a dose of 0.15-0.30 l/ha and working fluid 250-400 l/ha was applied against weeds during the 3-4 leaf stage (Tosheva, 2006). The N-fertilizer was divided into two portions - N_{60} before sowing and N_{60} as feeding up during the vegetation stage, totally 120 kg/ha. The following production elements were investigated – total fresh ear yield, marketable fresh ear yield, total ear number, marketable ear number, single marketable fresh ear mass, marketable ear row number, one row kernel number of a marketable ear, and marketable fresh ear kernel mass. Plant characteristics such as plant height, leaf number per plant, ear legth, and tassel length were read. Analysis of variance was applied to all data obtained.

According to air temperature, the conditions were average to cool. According to the rainfalls, the period was very humid. The May-August rainfall total was 444.7 mm and has 10% probability of exceedance (Table 1). The daily rainfalls were evenly distributed through all the season. No need in irrigation occurred. These very humid conditions hindered the weed control. Corn was sown on 10th May. The duration of the growing season was 103 days.

RESULTS AND DISCUSSION

The results from the analysis of variance showed that the yields obtained under no-till technology were statistically lower than the yields obtained under conventional tillage (Table 1).

The yield of the marketable fresh ears under conventional tillage was 8.530 Mg/ha, while under no-tillage - 4.229 Mg/ha, which was 102% less. The mass of the total fresh ears was 17% less respectively. The green biomass (vegetative mass plus ears) under conventional tillage was 35.3% less that the one under no-tillage. The yields in the experiment correspond to the reported in literature yields. (Tosheva, 2006; Jett, 2006; O'Neill, 2008).

Table 1. Yields

Tillage variant	Total green mass + ears			Fresh ears total			Marketable fresh ears		
	Yield	Difference		Yield	Difference		Yield	Difference	
	Mg/ha	Mg/ha	%	Mg/ha	Mg/ha	%	Mg/ha	Mg/ha	%
Conventional	25.365			11.565			8.530		
No-till	18.741^{00}	6.624	35.3	9.873^{00}	-1.692	-17.1	4.229^{0}	-4.307	-102.0
$GD_{5\%}$ Mg/ha	±2.082			±0.579			±2.847		
$GD_{1\%}$ Mg/ha	±4.802			±1.334			±6.566		
$GD_{0.1\%}$ Mg/ha	±15.289			±4.248			±20.903		

^{0}significant at P=5%, ^{00}significant at P=1%

Both tillage technologies affected the yield components similarly (Table 2). There were obtained 215.5 g vs. 177.9 g average weight per marketable ear, 163.8 g vs. 133.6 g average kernel mass of a marketable ear and 337.2 g vs. 293.9 g for 1000-kernel mass, respectively. Those differences were significant at P=5% and P=1%. The average number of kernels of an ear was not affected by tillage technologies.

Table 2. Yield components of the marketable ears

			Tillage variant		$GD_{5\%}$	$GD_{1\%}$	$GD_{0.1\%}$
			Conventional	No-till	g, pcs	g, pcs	g, pcs
Single ear	Mass	g	215.5	177.9^{0}	±12.3	±28.4	±90.3
	Difference	g		-37.7			
		%		-21.2			
Kemel mass of a single ear	Mass	g	163.8	133.6^{0}	±14.6	±33.7	±107.2
	Difference	g		-30.2			
		%		-22.6			
Kemel number of a single ear	Pieces	pcs	485.7	454.2	±66.7	±153.8	±489.8
	Difference	pcs		-31.5			
		%		-6.9			
1000-kemel mass	Mass	g	337.2	293.9^{0}	±40.4	±93.2	±296.6
	Difference	g		-43.2			
		%		-1.47			

^{0}significant at P=5%, ^{00}significant at P=1%

Twice as many marketable ears per 100 plants (80 pcs) were obtained under conventional tillage, compared with those under the no-tillage technology (Table 3).

The tiller number was much higher - 14 pcs vs. 1 pcs, respectively. The number of non-marketable ears was the same under both tillage technologies. The total number of ears under conventional tillage (142 pcs) was greater vs. those obtained under no-tillage. Plant height and the height of betting of the first ear were significantly influenced by the tillage technology.

Table 3. Number of tillers and ears

	Tillage variant	Per 100 plants	Per hectare
Marketable ears	Conventional tillage	80	36000
Non marketable ears		60	26850
Number of tillers		14	4050
Total number of ears		142	62850
Marketable ears	No-till	48	36000
Non marketable ears		66	29550
Number of tillers		1	450
Total number of ears		113	51000

The plants affected by no-till are significantly shorter – less by 21.5 cm, and the height of betting of the first ear - less by 6.4 cm (Table 4). The number of leaves and length of the tassel showed no differences. The ear under no-till was significantly shorter – 18.4 cm vs. 20.7 cm.

Table 4. Analysis of variance for the biometric characteristics

			Tillage variant		$GD_{5\%}$	$GD_{1\%}$	$GD_{0.1\%}$
			Conventional tillage	No-till	cm, pcs	cm, pcs	cm, pcs
Plant height	Height	cm	137.3	158.0^{0}	±9.4	±21.6	±68.9
	Difference	cm		-21.5			
		%		-18.6			
Height of betting of the first ear	Height	cm	26.3	19.9^{0}	±4.7	±10.9	±34.6
	Difference	cm		-6.4			
		%		-32.2			
Leaves number	Number	pcs	7.6	7.2^{0}	±2.19	±5.05	±16.09
	Difference	pcs		-0.3			
		%		-4.6			
Tassel length	Length	cm	31.2	30.2^{0}	±5.43	±12.53	±39.87
	Difference	cm		-1.0			
		%		-3.3			
Fresh ear length	Length	cm	20.7	18.4^{00}	±1.0	±2.2	±7.0
	Difference	cm		-2.3			
		%		-12.5			

^{0}significant at P=5%, ^{00}significant at P=1%

The absolute growth of plant height, leave number, tassel length and fresh ear length was greater under the conventional soil tillage. The maximum plant height was read at harvesting - 137.3 cm vs. 115.8 cm (Fig. 1a). The maximum number of leaves under both tillage technologies was reached for about 75 DAS and was 7.6 vs. 7.2 (Table 4 and Fig 1b).

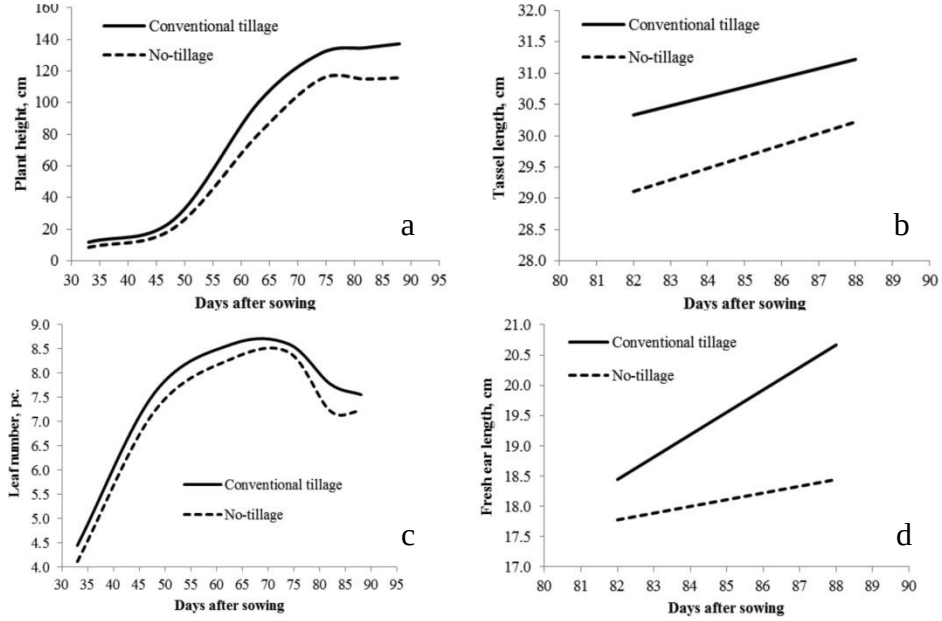


Figure 1. Absolute growth of biometric characteristics: a) plant height; b) leaf number; c) tassel length; d) fresh ear length

The rate of growth was most intensive in the period from 48th to 75th DAS (stem elongation stage) and the formation of leaf mass - from 35th to 50th DAS. The latter completed with the appearance of 8th leaf under conventional tillage vs. 7th leaf under no-tillage (Fig 1ab). Leaf number was 8.5 on the 75th DAS under both tillage technologies. The tassels and the ears increased their length to the end of the vegetation period (Fig. 1cd).

The accumulation of fresh biomass was most intensive during the period 60th-75th after sowing in the course of stem elongation stage (Fig. 2).

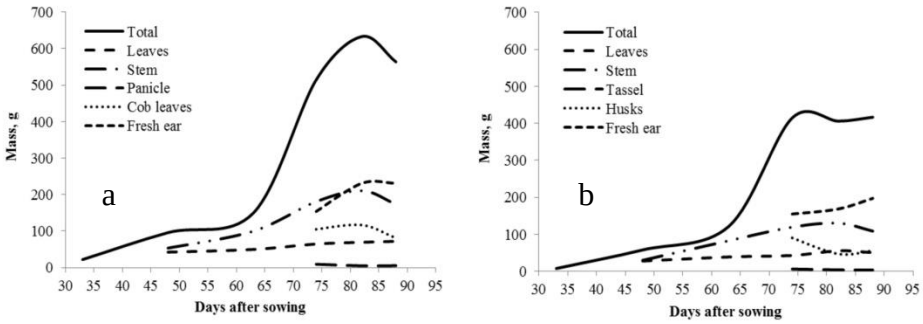


Figure 2. Fresh biomass accumulation under:
a) conventional tillage; b) no-tillage

The greates part of the biomass was accumulated in the stems and in the ears. Least mass was accumulated in the husks and tassel. The total fresh biomass was greatest at the stage of tasseling under conventional tillage - 633.0 g/plant. Dry matter reached 145.6 g vs. 103.7 g/plant, respectively (Fig. 3).

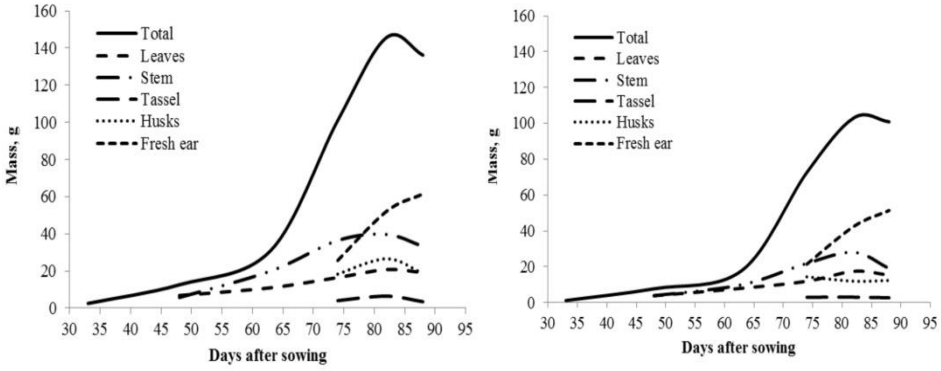


Figure 3. Dry biomass accumulation under:
a)conventional tillage; b) no-tillage

Regardless of the absolute values of biomass distribution, the relative values of fresh and dry biomass at tasseling under both tillage technologies were similar. The ear constituted 53-55% of the whole plant fresh biomass, the stem - 32-33% and the leaves – 11-14%. The ratio between the biomass of the husks and the fresh ear was more favorable at no-tillage. The conventional tillage contributed for a greater mass of the husk leaves - 18.4% vs. 11.6% and for smaller kernels+cob mass – 36.5% vs. 41.6%, respectively. The tendencies in the dry mass were like those of the fresh biomass. The proportion of the relative dry weight of the kernels to the cob under the impact of both tillage methods was the same - about 74% kernel mass and 26% cob mass (Table 5).

Table 5. Proportions in the fresh and dry biomass per organs, %

Plant organs	Fresh biomass		Dry biomass	
	Conventional	No-till	Conventional	No-till
Leaves	11.0	13.7	14.3	16.9
Stem	33.3	32.1	27.2	27.2
Tassel	0.8	1.0	4.5	2.9
Ear, incl.:	54.9	53.2	54.0	52.9
Kernels+cob	*36.5*	*41.6*	*35.8*	*41.3*
Husks	*18.4*	*11.6*	*18.3*	*11.6*
Total	100.0	100.0	100.0	100.0

Table 6. Distribution of the dry biomass in the fresh ear

Component	Conventional		No-till	
	g	%	g	%
Fresh ear, incl.:	61.5	100	51.4	100
Kernels	*45.3*	*73.6*	*37.9*	*73.8*
Cob	*16.2*	*26.4*	*13.5*	*26.2*

Table 7. Proteins and fat at 71.1% average humidity of the kernel (weighing percent)

Tillage technology	Proteins	Fat
Conventional	13.80	5.02
No-till	12.70	6.00

The protein content in the grain under conventional tillage was higher than of that of no tillage - 13.8% vs. 12.70% (of the abs. dry matter at 71.1% moisture of the kernels) (Table 6) and the fat content was less - 5.02% vs. 6.00% (Table 7). The production of sweet corn under conventional tillage was more profitable. The rate of profitability under conventional tillage was around 150% higher and the cost price per piece was 0.00154 EUR lower. The price under conventional tillage amounted to 0.0358 EUR/pc. vs. 0.0512 EUR/pc. under no-tillage (Table 8).

Table 8. Economic indices

	Yield	Production (x 0.30 EUR/pc.)	Expences	Income	Cost price	Rate of profitability
	pcs/ha	EUR/ha	EUR/ha	EUR/ha	EUR/pc	%
Conventional	36000	552.645	1,217.87	4,308.58	0.0358	353.8
No-till	21450	329.284	1,120.64	2,172.20	0.0512	193.8

CONCLUSION

An experiment that was carried out with sweet corn on chromic luvisols in a temperate-continental climate and in a very humid year, showed better results for the conventional tillage technology than for the no-tillage one. The yield of the marketable fresh ears was twice higher – 8.5 Mg/ha vs. 4.2 Mg/ha, the kernel mass of a single fresh ear was with 22.6% higher – 163.8 g vs. 133.6 g, 1000-kernel mass was with 14.4% higher – 337.2 g vs. 293.0 g. Analogously, the plants were longer and had thicker stems with greater leave number, resulting in 12.5% greater length of a single fresh-ear – 20.7 cm vs. 18.4 cm. The total fresh biomass under conventional tillage reached 633.0 g/plant in the stage of tasseling, while under no-tillage reached 414.6 g/plant. Dry matter reached 145.6 g vs. 103.7 g/plant, respectively. The protein content was 13.8% vs. 12.7%.

The production was more profitable under conventional tillage and according to that, the price of a marketable corn ear was much lower - 0.0358 EUR/pc. vs. 0.0512 EUR/pc. In high humidity conditions, despite of spraying,

no-tillage didn't guarantee clear fields and the weeds have probably affected the production results. No-till in very humid conditions should be applied on weed-free areas after several-year control on them with herbicides.

REFERENCES

Bulavin, L.A., A.P. Gvozdov, S.S. Nebishinetz. (2010). Minimizing tillage: Reality and Perspectives. Belarus Agriculture http://old.agriculture.by/archives/1615 Accessed on 18.03.2016

Calistru, A.E., G. Jitareanu (2014). No-tillage around the world. Journal "Life Sciencies, a Challenge for the Future", Seria Agronomie, 57, 2, Ed. "Ion Ionescu de la Brad", Iasi, Romania, (11-16)

Derpsh, R. (2009). The main steps for the implementation of no-tillage. http://no-till.ru/view_articles.php?id=11 Accessed on 1/03/2016

Gapon, N. (2014). Classical tillage technology, http://agroinfo.com/selxoztexnika-2/ Accessed on 01/03/2016

Growing Guide: Sweet Corn Growers Handbook. (2005). Queensland Government, www.deedi.qld.gov.au Accessed on 18/03/2016

Jett, L. W. (2006). Growing Sweet Corn in Missouri. http://extension.missouri.edu/p/G6390 Accessed on 18/03/2016

Moteva, M., V. Kazadjiev, V. Georgieva (2015) Climatological and Meteorological Information for Future Sustainable Agriculture in Bulgaria. In: Environment, Ecology and Sustainability at the Beginning of 21st Century, Chief ed. Prof. Recep Efe, St. Kliment Ohridski University Press, Sofia, Bulgaria, (91-111)

O'Neill, C. J., E. Humphreys, J. Louis, A. Katupitiya. (2008). Maize productivity in southern New South Wales under furrow and pressurised irrigation. Australian Journal of Experimental Agriculture, Vol. 48 (3), (285–295)

Sevov, A., B. Yankov, P. Zorovski, E. Valcheva, R. Popova, N. Palagacheva, D. Stoychev. (2014). A Technology for Sweet Corn Production. Acad. Ed. Agrarian University, Plovdiv, Bulgaria

Tosheva, T. (2006). Technologies for Growing and Processing of Sweet and Popcorn. PublishSaiSet, Sofia, Bulgaria

Vrazhnov, A.V. (2013). Efficiency of minimizing of tillage. Journal "Zauralie fields", Vol. 9(109), October 2013, http://svetich.info/publikacii/agronauka/yeffektivnost-minimalizacii-obrabotki-po.html, Accessed on 01/03/2016

DOI: 10.17707/AgricultForest.63.1.03

Teresa BORRALHO, Anabela DURÃO, Adelaide ALMEIDA, Ana PARDAL, Fátima CARVALHO, Humberto CHAVES, Maria IMAGINÁRIO, Isabel SIMÕES, Ivone CASTANHEIRA, Mariana RAPOSO[1]

IRRIGATION WATER QUALITY IN ALENTEJO (PORTUGAL) STUDY OF THE IRRIGATION PERIMETER OF THE RESERVOIR OF ROXO

SUMMARY

Alentejo is a region of Portugal located in the south of the country and the main economic activity is agriculture. In Alentejo the precipitation is very irregular. The problem of droughts often occurs.

The Alqueva reservoir is the biggest reservoir in Europe and provides water for more than 120,000 hectares for agriculture. Before the use of the reservoir, the main agricultural products were cereals. Now farmers grow vegetables and they make an intensive culture of olive trees and almond trees.

The Roxo reservoir is one of those will receive water from Alqueva in 2016. The increase of water in quantitative and qualitative terms can provide very significant increases in the growth of the soil production that we must monetize in the best way.

The aim of this study was evaluate the quality of water resulting from the monitoring carried out during 2014/2015 in the hydraulic system and in the drainage network of the irrigation perimeter of Roxo's reservoir to be made a more sustainable management of irrigated area. The main conclusions were as follows: i) the water of the irrigation channel and drainage network show a mild to moderate degree of salinity risk; ii) doesn't show characteristics to modify the soil infiltration conditions; iii) indicates degree of restriction slight to moderate due to the amounts of Na^+ and Cl^-; iv) the drainage network presents toxicity due the chlorides and boron; v) the ammonia nitrogen and nitrate values were low. The application of nitrogenous fertilizers seems to influence the results.

Keywords: *Irrigation water quality, Sustainable management, Droughts, Alentejo, Portugal.*

INTRODUCTION

In 2016 the construction of the Alqueva Multipurpose Project (AMP) will provide an increase in quantitative terms of the water available in Roxo reservoir (Alentejo - Portugal).

[1]Teresa Borralho (corresponding author: mtcarvalhos@ipbeja.pt), Adelaide Almeida, Ana Pardal, Fátima Carvalho, Humberto Chaves, Maria Imaginário, Isabel Simões, Ivone Castanheira, Mariana Raposo Technology and Applied Sciences Department, Higher School of Agriculture, Polytechnic Institute of Beja, Beja, PORTUGAL Anabela Durão Engineering Department, Higher School of Technology and Management, Polytechnic Institute of Beja, Beja, PORTUGAL

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

The irrigation water and soil are determinant resources for the growth of the irrigated areas production. The available water can provide very significant increases in the growth of the soil production that we must monetize in the best way. However, we must keep in mind the decline and the collapse of many irrigation perimeters along the history resulting in the loss of the cultures production and in an increasing regional desertification so difficult to stop and to invert (Oliveira, 2012; Annetta and Colonna, 2004). In the origin of this situation are several ways of soil degradation and specially those related to the irrigation water quality deterioration (Oliveira, 2012).

In a hydro-agricultural project the problem diagnosis must be realized including the soil and water usage, farming, production means and others. Relatively to the soil and water usage the problems are related to the low efficiency of the irrigation at the parcel level, irrigation methods unadapted to the cultures and soil characteristics (Russo, 2013). The bad quality of the irrigation water can provoke serious problems in the soil quality of the irrigation perimeter (Sequeira *et al*., 1995 in Gonçalves *et al,* 2007), becoming particularly important when is used in slow kinetic soils which is the case of the thin texture soils existing in Alentejo (Gonçalves *et al*, 2007).

The aim of this study was: i) to evaluate the quality of water resulting from the monitoring carried out during 2014/2015 in Roxo hydro-agricultural holding (irrigation channel and drainage network); ii) to constitute a database for further comparison with the water quality resulting from the mixture after the connection to the Alqueva Multipurpose Project (AMP) in order to provide the implantation and maintenance of irrigation cultures of high lucrativeness and that may contribute indirectly to the inversion of the desertification in Alentejo.

MATERIALS AND METHODS

The Roxo Irrigation Perimeter, (Figure 1), including the Roxo's Reservoir and Dam, is in Baixo Alentejo (Portugal) and serves presently 7,730 ha. With the connection to the Alqueva System will reach the 15,000 ha in 2016 (Parreira and Marques, 2012).

The monitoring campaign of the water quality of the Roxo perimeter was carried out monthly, between July 2014 and September 2015. Samples were taken in 11 important spots of the irrigation channel and of the drainage network (Figure 1 and Table 1). The irrigation channel includes 7 sampling points. The drainage network includes the 4 remaining sampling points. The parameters analyzed were namely, pH, Electrical Conductivity (EC), Bicarbonates, Dissolved Oxygen (DO), Demand Chemical Oxygen (DQO), Chloride (Cl^-), Sodium (Na^+), Calcium (Ca^{2+}), Boron (B^{3+}), Nitrates (NO_3^-), Ammonia nitrogen (NH_4^+), Kjeldahl nitrogen, Total Phosphorus (P_{total}), Phosphates (PO_4^{3+}), Potassium (K^+), Magnesium (Mg^{2+}) and Sulphates (SO_4^{2-}). Experimental determination was made according to the standard methods for the water analysis (APHA, 1998).

Figure 1. Representation of the Roxo Irrigation Perimeter.

The calculation of the relation of sodium adsorption ($RAS_{adjust.}$) was made to obtain a correct amount of the ion calcium that remains in the water after the link with the bicarbonates (Ayers and Westcot, 1994). The results were treated in the computer program *Statistica 8* grouped by parameters, place and period (wet and dry). The results were analyzed according to the Guide of FAO (Ayers and Westcot, 1994), compared with the maximum values recommended in the Portuguese legislation, VMR (Decree-Law nº 236/98 of the 1st August) (Water quality for irrigation) and specialized literature.

Table1. Sampling points: Localization, designation and GPS coordinates

Point	Localization	Designation	East	North	Quotation
1	Irrigation Channel	B_Roxo	4517,319	-192957	110,7138
2	Irrigation Channel	ROuteiro	12556,92	-190068	140,6319
3	Irrigation Channel	E_Amendoal	-5993,59	-189011	99,76268
4	Drainage Network	S_RRoxo	-15893	-189330	48,97927
5	Irrigation Channel	E_Milho			
6	Irrigation Channel	E_Olival	-13672,5	-189232	56,85863
7	Drainage Network	B_Xacafre	-6346,04	-190688	79,28525
8	Irrigation Channel	C_Geral	-4922,26	-188301	101,599
9	Irrigation Channel	RLouriçais	10210,16	-200178	151,791
10	Drainage Network	E_RRoxo	-718,215	-191362	78,7748
11	Drainage Network	E_Agua Forte	-2371,76	-191491	76,83632

RESULTS AND DISCUSSION

The sampling points were grouped according to their localization but the results were analyzed and discussed separately. The figure 2 shows the results related to the irrigation channel.

The sampling points, Outeiro creek (ROuteiro) and Louriçais creek (RLouriçais) flow to the Roxo's reservoir and they have different characteristics.

The first one is located upstream the reservoir and receives discharges from a Wastewater Treatment Plant. The second one is also located upstream the reservoir and receives the run-off from nearby livestock farming. These characteristics justify the differences found in the measured values. The junction of those creeks in B_Roxo allows the obtainment of a water quality more or less regular along the irrigation channel. The pH values from that point presents alkaline characteristics, with values in the range of VMR and without significant variations in the two periods of reference. As for DO, the values obtained from B_Roxo don´t present significant variations ($p<0.005$), being however high in the dry period in the E_Amendoal tree and E_Milho points which can be justified for the huge water quantity that is going to the cultivation parcels and consequently is suffering a bigger oxygenation. The values of DQO, from the B_Roxo, are more or less regular but relatively high, approaching the recommended by VMR for a superficial water of bad quality. In Louriçais creek and Outeiro creek we can see high values probably due to the sampling spots characteristics previously described.

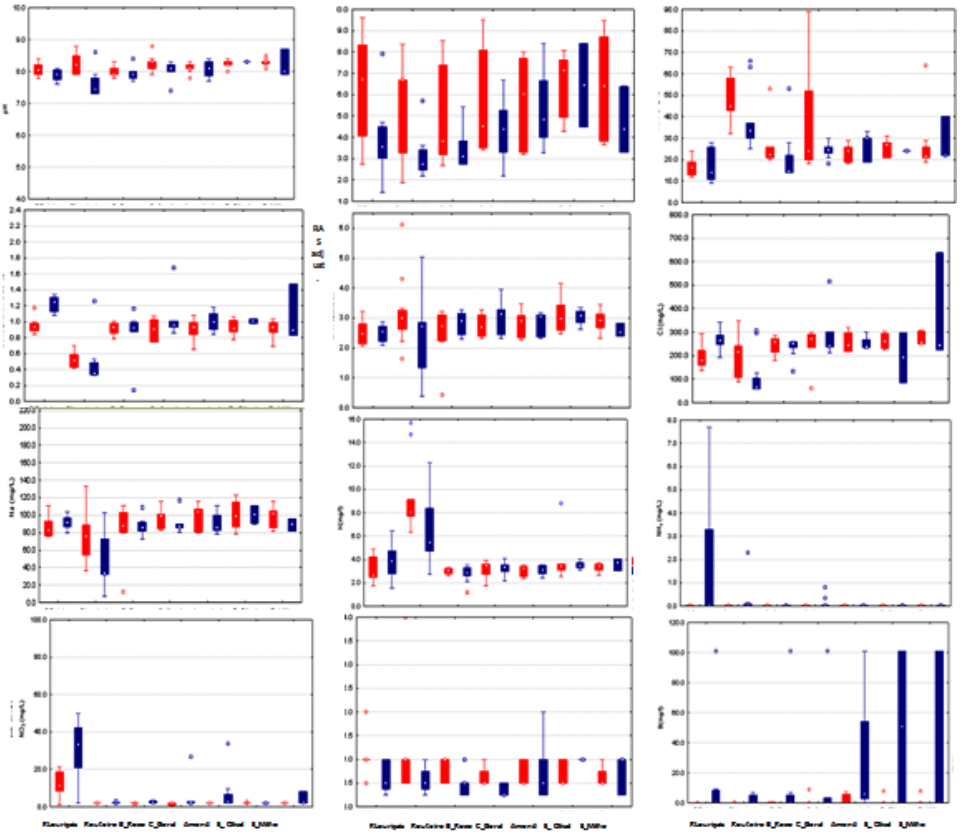


Figure 2. Box-and-Wisker graph diagram of the results obtained from the sampling points of the irrigation channel, ordered from upstream to downstream and categorized by wet period (blue) and dry (red), for the parameters: pH, DO, DQO, EC, RAS adjust, NO_3^-, NH_4^+, Cl^-, Na^+, K^+, P_{total} and B^{3+}.

Concerning to the salinity risk, (evaluated in terms of EC), and considering 0.7mS/cm limits (below it there is no restriction of use) and 3.0mS/cm (above it the level of restriction is severe) (Ayers and Westcot, 1994), we can verify that in the first two sampling points, the conductivities are not altered with the reference periods. After the junction of Outeiro creek (ROuteiro) and Louriçais creek with the Roxo´s reservoir, the resulting water body shows that it has in all points of the irrigation channel, a mild to moderate degree of salinity risk because the values obtained are between 0.7mS/cm and 3.0mS/cm (Ayers and Westcot 1994). The values are very close to VMR=1mS/cm.

After the junction of Outeiro creek and Louriçais creek with the Roxo´s reservoir, the resulting water body shows that it has in all points of the irrigation channel, a mild to moderate degree of salinity risk because the values obtained are between 0.7mS/cm and 3.0mS/cm (Ayers and Westcot 1994). The values are very close to VMR=1mS/cm.

To evaluate the degradation problems of soils permeability by the action of water quality, we analysed the *RAS adjust* with the EC. As a matter of fact, for a determinate RAS the infiltration rate increases when the water salinity increases (RECOQUAR, 2006).

Consequently, the water of the irrigation channel doesn´t have characteristics to modify soil infiltration conditions (Richards, 1954). To do the analysis of the toxicity of some specific ions that can affect the cultures susceptibility, the results are presented relating to the parameters Na^+ and Cl^- that cause the highest number of problems and for being the most common in the irrigation waters (Ayers and Westcot, 1994). Consequently, both ions don´t change significantly for reference period after the point 1, but the very high parametric values indicate a mild to moderate restriction degree in water usage (Ayers and Westcot, 1994). The chloride ion exceeds always the VMR of 70mg/L. The ammonia nitrogen and nitrate values along the irrigation channel were always low and the concentrations of NO_3^- always lower than VMR=25mg/L. The K^+ values and the phosphorus present slightly higher values to those considered typical for irrigation waters (Almeida, 2010). The phosphates were also monitored but their value was always lower than the detection limit of the analytical method. The Boron presents higher values and a high variability in the wet period in the sampling point E_Olival tree. The results for the drainage network are presented in figure 3.

The water pH presents alkaline characteristics except the entrance of *E_RAgua Forte* whose characteristics are acid (pH<7). This situation is due to the effect of the water coming from the Aljustrel´s mine in the wet period. However, as the Roxo's creek water is alkaline, it's junction with the previous *E-RAgua Forte* lowers the pH according to VMR (6.5<pH<8.4). Although the pH is just an indicator, any value out of the VMR may cause a nutritional unbalance or have a toxic effect (RECOQUAR, 2006). Regarding to the DO, the values obtained don´t show significant changes ($p<0.005$), for period, for each sampling point except in B_Xacafre, with higher values in the dry period probably due to

the water withdrawal for the irrigation causing a bigger oxygenation in the water body.

The DQO is similar to the irrigation channel in all sampling points with slightly higher values in the dry period.

In relation to the salinity risk, analyzed in terms of electrical conductivity, there is in all the sampling points a mild to moderate degree of salinity risk because the values obtained are between 0.7mS/cm and 3.0mS/cm (Ayers and Westcot 1994). Most values are higher than VMR=1mS/cm. Regarding the sodium and salinity risks in the soil, the water quality of the drainage network kept the same risk degree as the water quality of the irrigation channel, that is, high salinity risk and low sodium risk (Richards, 1954).

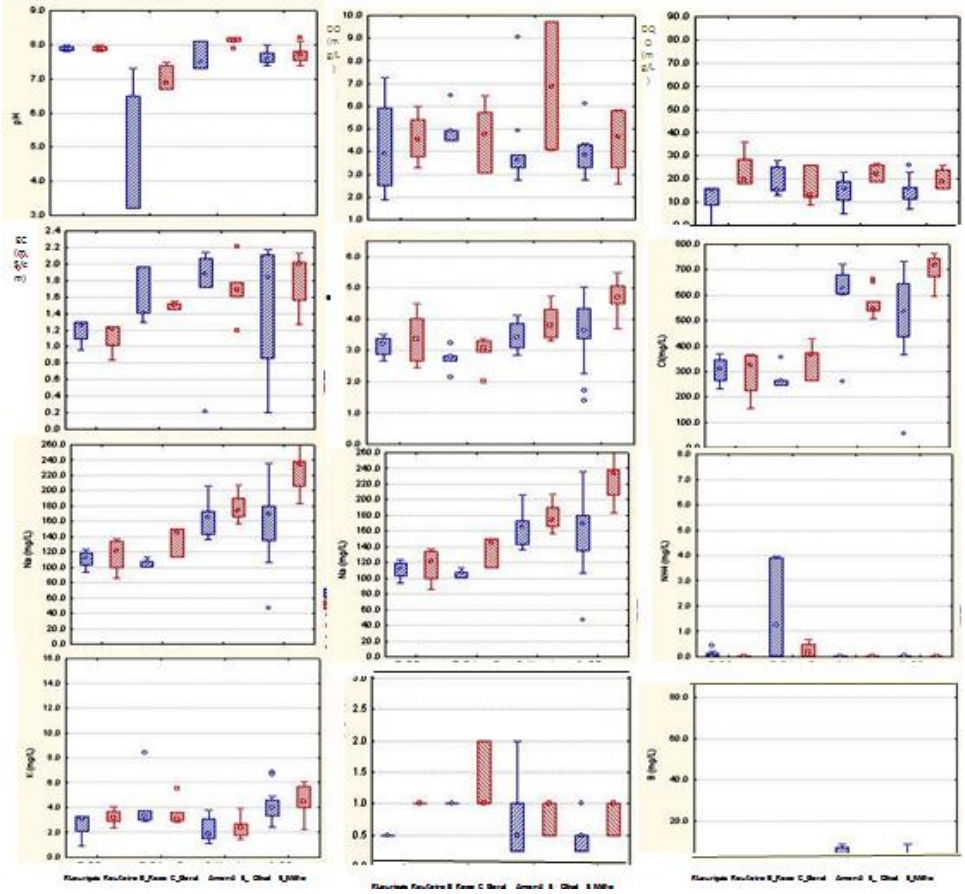


Figure 3. Box-and-Wisker graph diagram of the results obtained from the sampling points of the drainage network, ordered from upstream to downstream and categorized by wet period (blue) and dry (red), for the parameters: pH, DO, DQO, EC, RAS $_{adjust}$, NO_3^-, NH_4^+, Cl^-, Na^+, K^+, P $_{total}$ and B^{3+}.

CONCLUSIONS

The water of the irrigation channel of Roxo´s reservoir, after the junction of the side branches; Louriçais creek and Outeiro creek; has in all analyzed points a mild to moderate degree of salinity risk which is kept in the drainage network.

The water of the irrigation channel and of the drainage network don´t present characteristics to modify the conditions of infiltration in the soil. Regarding the sodium and salinity risks in the soil, the water quality of the drainage network kept the same risk degree as the water quality of the irrigation channel, that is, high salinity risk and low sodium risk.

The highly parametric values of Na^+ and Cl^- ions in the irrigation channel indicate a mild to moderate restriction degree in the water usage which is kept in the drainage network with higher values to downstream of the irrigation perimeter. The ammonia nitrogen and nitrate values along the irrigation channel were always low, but in the drainage network, the application of nitrogenous fertilizers, in the parcels of the Almond tree, seems to influence the point B_Xacrafre in the wet period.

REFERENCES

Almeida, O.A. (2010). Qualidade da água de irrigação. 1ª ed. Saulus Santos da Silva, Lisboa.(Irrigation Water Quality. 1st ed.Saulus santos da Silva,Lisbon). http://ainfo.cnptia.embrapa.br/digital/bitcreek/item/26783/1/livro-qualidade-agua.pdf. Acessed on 28/01/2016.

Annetta, M., Colonna, N. (2004). Land Care in Desertification Affected Areas. http://geografia.fcsh.unl.pt/lucinda/booklets/B3_Booklet_Final_PT.pdf. Acessed on 30/01/2016.

APHA (1998). Standard Methods for the Examination of Water and Wastewater. American Public Health Association (APHA), Washington DC, USA.

Ayers, R. and Westcot, D. (1994). Water quality for agriculture. FAO Irrigation and Drainage. Paper 29, Rev. 1. Rome, Italy.

Gonçalves, M. C.; Martins, J. C.; Neves, M. J.; Pires F. P.; Ramos, T. B.; Vilar, M. T.; Bica, J. (2007). Balanço de sais em Monólitos de solo após 4 ciclos de rega com águas de diferentes qualidade. II Congresso Nacional de Rega e Drenagem. (Salts balance in soil monoliths after 4 irrigation cycles with water of different quality. II National Congress on Irrigation and Drainage, Fundão) 26-28 October.

Oliveira I. (2012). Técnicas de Regadio (Technical of Irrigation). Editing author-2nd Edition.Lisbon.

Parreira, A.M., Marques, C.M.P. (2012). Os Desafios de Alqueva. O caso do Aproveitamento Hidroagrícola do Roxo. XIX Congresso Nacional da Ordem dos Engenheiros. Lisboa, (The Alqueva challenges. The case of Hydroagricultural of Roxo. XIX National Congress of the Order of Engineers. Lisbon,) 19-20 October. http://www.ordemengenheiros.pt/fotos/dossier_artigo/19102012_roxo_95574038550b793d5e020e.pdf. Acessed on 29/01/2016.

RECOQUAR (2006)- Rede de Controlo da Qualidade da Água de Rega. Relatório Anual – 2005. Centro Operativo e de Tecnologia de Regadio.(Quality Control Network of Irrigation Water. Annual Report – 2005. Operations Center and Irrigation Technology).

http://www.cotr.pt/cotr/documentos/RELAT%C3%93RIO%20%20RECOQUAR_1.pdf, Acessed on 25/01/2016.

Richards, L. A. (1954). Diagnosis and Improvement of Sa1ine and A1ka1i Soi1s. US Dep. Agric. Handb. 60.Washington, USA.

Russo, A.M.T. (2013). Hidráulica Agrícola e Desenvolvimento Rural. Estudos e Projectos: Relatório Detalhado Integrador e Crítico da Actividade Profissional. Dissertação de Mestrado em Engenharia Agronómica. Instituto Superior de Agronomia, Universidade de Lisboa. (Hydraulic Agricultural and Rural Development. Studies and Projects: Detailed Report Integrator and Critical Professional Activity. Dissertation in Agronomic Engineering. Institute of Agronomy, University of Lisbon).

Simões J., Barroso S., Roque M.A., Capitão F., Ferreira I., Gamboa M., Matos M., Fernandes J. (2002). Projecto do Plano de Ordenamento da Albufeira do Roxo. Agência Portuguesa do Ambiente (Planning Plan Project of Roxo Reservoir-Portuguese Environment Agency).

DOI: 10.17707/AgricultForest.63.1.04

Vida CHALAVI, Sodabeh ZAREH[1]

GENETIC ENGINEERING OF ROSE SCENTED GERANIUM WITH BACTERIAL GENES FOR PHYTOREMEDISTION OF POLYCHLORINATED BIPHENYLD

Polychlorinated biphenyls (PCBs) are major water and soil contaminants worldwide. These harmful compounds to human health are highly stable and resistance to degradation. Biphenyl dioxygenase (BPDO) is a bacterial enzyme from *Burkholderia xenovorans* which has catabolic degradation activity of PCBs. In present study we report simultaneous genetic transformation of three coding components of BPDO enzyme,*bphA*, *bphE*, and *bphG* genes, into rose scented geranium (*Pelargonum graveolens L.*), a fast growing plant with high phytoremediation potential. Toward this end, the *bphA*, *bphE, and bphG* genes were cloned into pGreen0029 vector. Then, pGreen0029 vector carrying all three genes along with pSoup plasmid were introduced into *Agrobacterium tumefaciens* strain LBA4404 which then were used for transformation of rose scented geranium. Fully green putative transformant plantlets produced in selection medium were successfully rooted in presence of 50 mgl^{-1} kanamycin. The transgenic nature of fully green and rooted rose scented geranium plantlets were confirmed by Polymerase Chain Reaction (PCR) analysis. Specific primers of *bphA* gene (one of the three tandem cloned genes in the vector of this experiment) was used for PCR analysis and produced a 1380 bp fragment which is the exact size of this gene. Transgenic plants were successfully transferred into soil and continued their growth.

Keywords: *chlorinated biphenyls, rose scented geranium, Agrobacterium LBA4404, pGreen vector.*

INTRODUCTION

Polychlorinated biphenyls (PCBs), widely used in different industries from 1920s to 1980s, are organic chlorinated biphenyl *rings* with stability and resistance to degradation (Van Aken *et al*., 2010). These compounds are highly toxic and harmful for human health; therefore their production was banned in 1980s. Nevertheless, worldwide contamination of water and soil with PCBs is one of the important environmental problems at present time (Passatore *et al*., 2010).

A practical method for soil clean up is phytoremediation and the use of plant-microbe interaction (Furukawa and Fujihara, 2008). Plants can be used to

[1]Vida Chalavi (corresponding author: v.chalavi@sanru.ac.ir), Sodabeh Zareh Department of Horticulture, Sari Agricultural Sciences and Natural Resources University, Sari, IRAN
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

initiate pollutants degradation to be followed by bacteria catabolic processes (Mohammady *et al*., 2007). A bacterial enzyme that involves in catabolic pathway of major soil contaminants, such as polychlorinated biphenyls (PCBs) is biphenyl dioxygenase (BPDO) from *Burkholderia xenovorans*. Three components of BPDO are a heterodimer oxygenase, (a subunit, Mr = 51,000 and b subunit, Mr = 22,000), a ferredoxin (Mr = 12,000) and a ferredoxin reductase (Mr = 43,000). The encoding genes for these enzyme components are *bphA* (a subunit), *bphE* (b subunit), *bphF* (ferredoxin) and *bphG* (ferredoxin reductase) (Mohammady *et al*., 2007).

A way to reduce PCBs contamination is transformation and expression of biphenyl dioxygenase (BPDO) genes having catabolic degradation activity of polychlorinated biphenyls into plants. Plant genetic transformation is being used as a fast track in crop improvement and it is a valuable research tool in plant biology. Transgenic technology has been used for a better yield, resistance to herbicides, improved tolerances to insect infestation as well as better nutritive content, biofuel production, phytoremediation and soil clean up. Among different phytoremediation technologies, transgenic approaches have been described as efficient and promising strategies (Mohammady *et al*., 2007).

Perhaps the integration and expression of entire genes of a specific biochemical pathway is the ultimate goal of many genetic engineering approaches. However, the majority of experiments reported to date involve the introduction and expression of single genes and the manipulation of multiple genes is still a challenging task. A variety of different methods have been used to transfer multiple genes into plants. These strategies have been sexual crossing plants carrying different transgenes, successive retransformation and cotransformation with several plasmids or single plasmids harboring multiple gene assembly.

Rose scented geranium (*Pelargonum graveolens L.*) grows fast and has phytoremediation potential (Mahdieh *et al*., 2013) which could be enhanced by plant genetic transformation. The aim of present study was developing a method for simultaneous transformation of bphA, bphE, and bphG genes, coding for the components of BPDO enzyme, into rose scented geranium plants.

MATERIALS AND METHODS

Plant material and sensitivity to kanamycin

For developing an efficient regeneration system, shoots of rose scented geranium cv. Attar Rose were surface sterilized and cultured on different combinations of cytokinin (BA) and auxin (NAA).Experimental treatments were 3 concentrations of BA in combination with 3 NAA concentrations with 4 replications in a completely randomized design. Furthermore, sensitivity to kanamycin was tested by exposing in vitro rose scented geranium shoots to 0, 10, 25, 50, 100 $\mu g\ ml^{-1}$ of kanamycin.

Plasmid constructs

Binary pGreen0029 (Hellens *et al*., 2000) vector was chosen for *Agrobacterium* mediated plant transformation in this study. The T-DNA of pGreen0029 vector contains a kanamycin (Kan) selection marker gene between the nopaline synthase (*nos*) promoter and *nos* terminator internal to the T-DNA LB for plant transformation.

For co-transformation with linked transfers in a single plasmid, the following constructs were made. In the first construct, the gel purified EcoRV fragment of 35S-bphA was ligated to blunted KpnI site of the pGreen0029-bphE to produce pGreen0029-bphE+bphA. In the second constract, the gel purified EcoRV fragment of 35S-bphG was cloned into blunted NotI site of the pGreen0029-bphE+bphA (pG-AE) to produce a triple transgenes vector, pGreen0029-bphE+bphA+bphG (pG-AEG). All above recombinant constracts were carefully characterised by restriction mapping.

Plant Transformation

For producing transgenic rose scented geranium plants,recombinant pGreen0029-bphE+bphA+bphG (pG-AEG)vector along with pSoup plasmid were introduced into *Agrobacterium tumefaciens* strain LBA4404 (Hoekma et al., 1983) in a mix electroporation. *In vitro* shoots of rose scented geranium were transferred by leaf disk transformation method (Horsch *et al*., 1985). Trandgenic shoots were recovered and rooted on MS (Murashige and Skoog, 1962) medium containing 50 mgl^{-1} kanamycin for selection and 500 μg ml^{-1}carbenicillin. Positively identified transgenic plantlets were propagated and maintained under 16-hr photoperiod (50-70 μmol $m^{-2}s^{-1}$ light intensity) at a temperature of $24^{o}C \pm 1$.

DNA isolation and PCR analysis

Modified CTAB method was used for DNA extraction from rose scented geranium plants. Primers derived from the 5' (5' – ATG AGT TCA GCA ATG AAA -3') and 3' (5' – GGG CTC GGA CAT CAT GCG -3') ends of bphA gene were used for PCR analysis. Amplified DNA fragments were subjected to electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

Analysis of data

Efficient regeneration system was analyzed using a one-way ANOVA. A level of statistical significance at $p < 0.05$ was used in all analyses. The data were analyzed using Procedure-General Linear Model of SAS (version 9.1) software.

RESULTS AND DISCUSSION

Adventitious shoot regeneration and sensitivity to kanamycin

In a series of experiments, different plant growth regulator combinations were tested for shoot regeneration of rose scented geranium cv. Attar Rose. A high rate of shoot regeneration (100%) obtained in the treatment of 2.5 mgl^{-1} BA in combination with 0.5 mgl^{-1} NAA and each leaf explants regenerated a few shoots (Figure 1).

Shoot regeneration from leaf explants of rose scented geranium was completely inhibited at 50 mgl^{-1} kanamycin. On medium containing 100 μg ml^{-1}

kanamycin all shoots became necrotic within 2-3 weeks. On medium containing 100 µg ml^{-1} kanamycin all leaf explants became necrotic within 2-3 weeks. Therefore, in transformation experiments 50 mgl^{-1} of kanamycin was used in the selection medium.

Production of transgenic plants

Transformation of 3 *bphA, bphE, and bphG* genes cloned into pGreen vector were done successfully for rose scented geranium by using Agrobacterium LBA4404. The transformation experiments, resulted in transgenic shoots which regenerated on the medium containing 50 mgl^{-1} kanamycin. Fully green putative transformant plantlets produced in selection medium were successfully rooted in presence of 50 mgl^{-1} kanamycin. Rooted transgenic plants were transferred to soil. With regard to phenotypic appearance, there were no discernible differences between transgenic and non-transgenic plants.

DNA extraction and PCR analysis

High quality of extracted DNA was confirmed by spectrophotometery and agarose gel electrophoresis. The transgenic nature of fully green and rooted rose scented geranium plantlets were confirmed by Polymerase Chain Reaction (PCR) analysis. Specific primers of *bphA* gene (one of 3 tendom cloned genes in the vector of this experiment) was used for PCR analysis and produced a 1380 bp fragment which is the exact size of this gene (Figure 2).No amplification was observed for non-transformed rose scented geranium plants (Figure 2). Transgenic plants were successfully transferred into soil and continued their growth.

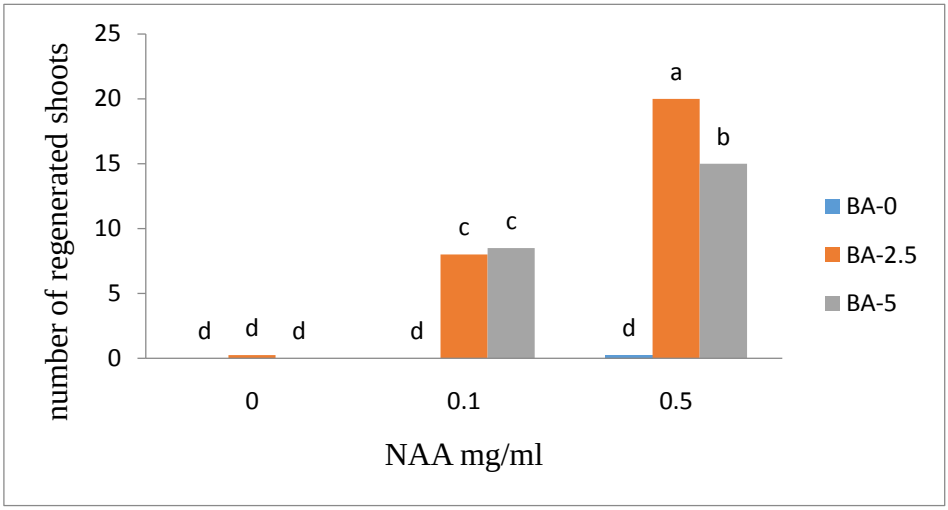


Figure 1**.** The Effect of different combinations of BA and NAA on adventitious shoot regeneration from leaf explants of rose scented geranium cv. Attar Rose.

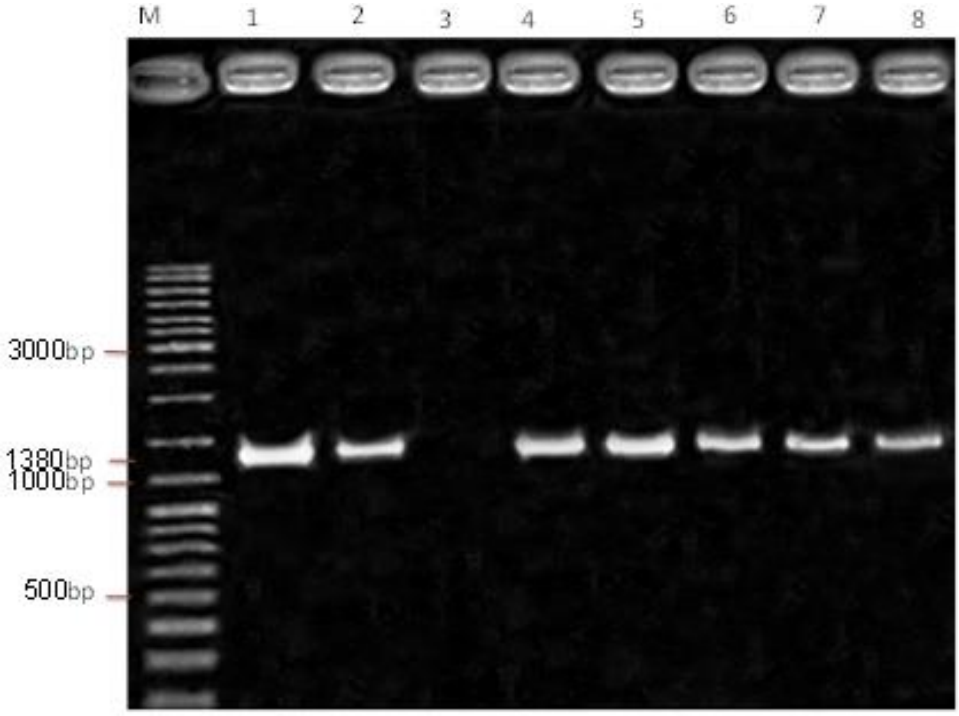


Figure 2**.** Polymerase chain reaction (PCR) amplification of *bphA* gene. Lane M, the DNA size standards. Lane 1, DNA from **pGreenAEG** vector, containing bphE, bphA and bphG genes.lane 3, DNA from non-transformed rose scented geranium plant. Lane 2 and 4-8 DNA from different transgenic rose scented geranium cv. Attar Rose plants.

CONCLUSIONS

This is a report of successful genetic transformation of rose scented geranium using leaf disk regeneration system. In this study we developed an efficient regeneration and transformation procedure for the rose scented geranium. Adventitious shoot regeneration of this cultivar was made possible by using the optimum combinations of BA and NAA. Kanamycin resistance phenotype combined with PCR analysis confirmed the stable integration of insert genes into rose scented geranium genome. The transgenic lines produced in this study show that plant genetic transformation can be used for improving the phytoremediation potential of rose scented geranium.

REFERENCES

Furukawa, K., Fujihara, H. (2008): Microbial degradation of polychlorinated biphenyls: Biochemical and molecular features. J. Biosci. Bioeng 105:433–449.

Hellens, R.P., Edwards, E.A., Leyland, N.R., Bean, S., Mullineaux, P.M. (2000): pGreen: a versatile and flexible binary Ti vector for Agrobacterium mediated plant transformation. Plant MolBiol 42: 819–832.

Horsch, R.B., Fry, J.E., Hoffmann, N.L.Eichholtz, D., Rogers, S.G., Fraley, R.T. (1985): A simple and general method for transferring genes into plants. Science 227: 1229-1231.

Mahdieh, M., Yazdani, M., Mahdieh, SH. (2013): The high potential of Pelargonium roseum plant for phytoremediation of heavy metals. Environ Monit Assess. 7877–788.

Mohammady, M., Chalavi, V., Sura, M., Laliberté, J.F., Sylvestre, M.(2007): Expression of the bacterial Biphenyl Dioxygenase gene in tobacco plants (Biotechnology & Bioengineering. Biotechnol.Bioeng. 97: 496-505.

Murashige, T., Skoog, F. (1962): A revise medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Passatore, L., Rossetti, Juwarkar, A. Massacci, A. (2014): Phytoremediation and bioremediation of polychlorinated biphenyls (PCBs): state of knowledge and research perspectives. Journal of hazardous materials 278: 189-202.

Van Aken, B. A., Correa, P, Schnoor, L. (2010): Phytoremediation of Polychlorinated Biphenyls: New Trends and Promises. Environmental science and Technology, 44(8): 2767-2776.

DOI: 10.17707/AgricultForest.63.1.05

Metodija TRAJCHEV, Dimitar NAKOV,
Milena PETROVSKA, Gordana JANKOSKA[1]

MASTITIS PATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY IN EARLY LACTATING DAIRY COWS

SUMMARY

A two years cross-sectional longitudinal survey was carried out for determination of udder pathogens prevalence and their antimicrobial profile in one commercial dairy farm. Dairy cows were selected in relation to date of calving and period of early lactation in order to minimize environmental impact. A udder quarters milk samples were obtained from totally 211 black-white dairy cows. The quarter milk-samples were screening using the California Mastitis Test (CMT) for detection of abnormal milk secretion (AMS) and from quarters with AMS were obtained milk-samples for microbiological examination in order to detect intramammary infections (IMI). The milk-samples were obtained in two periods: period from beginning of lactation until 21st day in lactation and period from 22nd to 42nd day in lactation. The prevalence of IMI in period from beginning of lactation until 21st day in lactation and period from 22nd to 42nd day in lactation was 4.03% and 4.38%, respectively. Based on CMT, there was found totally 162 udder quarters with AMS. From these quarters, the overall proportion of microbiological negative quarters was 56.17% and mastitis pathogens were isolated from 43.83% of udder quarters, from which the dominant mastitis pathogens were: *Streptococcus agalactiae (*19.14%), *Enterococcus spp.* (8.02%), *Candida non-albicans* (6.79%), *Staphylococcus aureus* (6.17%), *Escherichia coli* (1.85%), *Aspergilus niger* (1.23%) and *Pseudomonas aeruginosa* (0.62%). The results indicated that contagious pathogens were the most common patogens of IMI in early lactation. There was significant difference in the prevalence of intramammary infection (χ^2=35.136, df=1, p<0.001) when comparing the front and rear udder quarters. The isolated bacteria were examined for determination of susceptibility to 27 most used antimicrobial agents for mastitis treatment. The most resistant strain was *Staphylococcus aureus.*

Keywords: *dairy cow, intramammary infection, lactation*

INTRODUCTION

Maintaining adequate herd health remains a major issue for the majority of dairy herds, and is an area where constant attention to detail is required.

[1]Metodija Trajchev (corresponding author: metot@fznh.ukim.edu.mk), Dimitar Nakov University „St. Cyril and Methodius“,Faculty of Agricultural Sciences and Food, Institute of Animal Biotechnology, Department of Animal Health and Animal Welfare, Skopje, Republic of MACEDONIA Milena Petrovska, Gordana Jankoska University „St. Cyril and Methodius“, Medical Faculty, Institute of Microbiology with Parasitology Skopje, Republic of MACEDONIA
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Parturition and the onset of lactation represent the most hazardous periods of drastic physiological changes and intense metabolic demands in dairy cows which leads to increased incidence of metabolic disorders and infectious diseases (Sordillo and Aitken, 2009). Equally as important but however, mastitis in dairy cows are the most important diseases of the mammary gland which incidence increases as a consequence of parturition when host defense mechanisms are compromised. Mastitis has high economic implications due to losses in milk production and the risk posed by consumption of infected milk to public health. Bacterial infection and growth in the udder is the main cause of bovine mastitis. More than 150 Gram (+) and Gram (-) bacteria are identified as mastitis pathogens. However, the most prevalent mastitis pathogens are coagulase (+) and coagulase (-) bacteria from genus *Staphylococcus,* than bacteria from genus *Streptococcus* and Gram (-) Coliforms, especially *Escherichia coli* (Contreras and Rodriguez, 2011). However, approximately 10–40% of clinical mastitis cases yield ‘‘no significant growth’’ in routine clinical culture assays, and one study has also indicated that the number of such cases may be on the rise, although the reason for this is not currently known (Persson et al., 2011).

Traditionally, all udder pathogens have been classified as either ‘contagious’ or ‘environmental’, depending on their primary behavior and their route of transmission (White et al., 2006). The major contagious udder pathogens on dairy farms are: *Staphylococcus aureus, Streptococcus agalactiae* и *Mycoplasma bovis* (Trajcev et al., 2009). There are a wide range of environmental opportunistic pathogens that caused mastitis: *Escherichia coli, Klebsiella spp., Enterobacter spp., Serratia spp., Pseudomonas spp., Proteus spp., Corynebacterium pyogenes, Streptococcus uberis* and *Streptococcus dysgalactiae*, else more coagulase (-) bacteria from genus *Staphylococcus* (Taponem and Pyorala, 2009). However, Piessens et al. (2012) revealed that this dichotomous in transmission of udder pathogens is loosing and from epidemiological aspect there is no strict border between contagious and environmental mastitis.

According the literature data from around the world, the prevalence of udder pathogens that cause subclinical and clinical mastitis can vary widely (Ericsson et al., 2009; Persson et al., 2011). In the past, the most common udder pathogens in dairy herds were *Streptococcus agalactiae* and *Staphylococcus aureus.* However, the adoption of modern milking practices and implementation of the mastitis control programs has resulted in a considerable decline in the prevalence of these organisms in many modern dairy farms (Makovec and Ruegg, 2003). Today, the common environmental organisms include CNS, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Klebsiella* spp., and *Escherichia coli* (Taponen and Pyorala, 2009).

The most common udder pathogens from subclinical cases of mastitis in dairy farms in Republic of Macedonia were *Streptococcus agalactiae, Staphylococcus aureus, Enterococcus spp* and environmental *Streptococcus spp* (Trajcev et al., 2010).

The literature data differ significantly in reporting the prevalence of etiological agent for mastitis depending from the region where the surveys were done. For example, the prevalence of *Staphylococcus aureus* in Finland and Germany vary from 3,0 to 3,5% up to 38,5% in Zimbabwe and Australia (Kloppert et al., 1999; Milne et al., 2002). Kalmus et al. (2011) reported that *Streptococcus agalactiae* is the second predominant etiological agents of mastitis in dairy farms but cure rate with antibiotic therapy during lactation is very high.

The program for prevention and eradication of mastitis in dairy herds include regular implementation of screening methods for early detection of mastitis, like *California Mastitis Test* (CMT), measurements of milk conductivity and the presence of chlorides and sodium in milk as results of udder inflammation (Sharma *и* cop., 2011). The early mastitis detection in dairy herd has many potential benefits, out of which it will allows implementation of proactive management strategies that will avoid negative effects of disease and will leads to better cure rates (Deluyker et al., 2005). Several studies have reported an increased trend over time in resistance among mastitis pathogens due to uncontrolled use of antimicrobials for mastitis treatment (Trajcev et al., 2009). There is a concern that antimicrobial therapy of mastitis in dairy cows can lead to residues occurrence in milk which are potential hazards to public health (Andersson et al., 2011).

The objective of this study was to identify the udder pathogens in one dairy herd in period of early lactation and to determine their antimicrobial susceptibility. in order to establish a more effective therapy for udder infections.

MATERIALS AND METHODS

A two year longitudinal survey in period from 1st of April, 2012 until 31st of March, 2014, was carried out for determination of udder pathogens prevalence and their antimicrobial profile in dairy cows during the period of early lactation. The study was carried out in a conventional dairy farm localized near city of Strumica in south-eastern part of Macedonia. Totally, 211 primiparous and multiparous black-white dairy cows in beginning of lactation were selected. The dairy cows were allocated into groups related to the season of calving in order to minimize the influence of environment on mastitis pathogens prevalence. Cows were kept in the same farm providing with similar house conditions and feeding regime during 2 years of experiment to exclude the influence of additional environmental factors, such as feeding. Cows consumed ordinary diet which differed in accordance with the stage of pregnancy and lactation but was the same during consecutive events and included corn and grass silage, hay, commercial concentrate (maize, wheat, barley) as well as vitamin and mineral premix. The udder health status was followed from calving to 42nd day in lactation. This period of early lactation was subdivided into 2 periods: period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. Daily, during the trial period, each cow on milking line was observed clinically for presence of clinical signs of mastitis (rubber, tumour, colour, dolour

and *function laesa*) and determination of abnormalities in milk (presence of watery milk, flakes, clots, blood, pus, discoloured milk, etc). Additionally, there was used Califonia Mastitis Test (CMT) as predicted tool for detection of udder quarters with abnormal milk secretion (Schalm и Noorlander, 1957). The screening of udder health status was done on quarter level.

Samples for bacteriological culture from each quarter positive to CMT, were collected aseptically in sterile 10 mL tubes, without additives, according to the National Mastitis Council (Hogan et al., 1999) and kept at 4 ºC during transport. Samples were analyzed within 12 hours of collection. Bacterial species were identified according standard microbiological procedure using accredited methodology based on the National Mastitis Council standards (NMC, 2001). From each sample, 10 µl of milk was cultured on Columbia blood agar (Oxoid – UK) for enrichment and isolation of specific udder pathogens. Parallel, 10 µl of milk was cultured on STRB agar (BioMérieux - France) for isolation of β-hemolytic *Streptococcus spp.* group B (Smith et al., 1985). Incubation was done for 48 h. at 37 ^{0}C and 24 h more on room temperature for grout of eventually present of fungi. Sometimes, for better fungi grout, the incubation was lasting up to 5 days. A minimum of five colonies of the same type of bacterium was recorded as bacteriological positive, and growth of more than two types of bacterial colonies was categorized as mixed growth. No bacterial growth was recorded when fewer than five colony-forming units were detected during 48 h of incubation. Based on results from screening using clinical examination, CMT and bacteriological culturing, all cows in observed population were allocated into three groups: healthy cows without udder health problems, cows with persistent abnormal milk secretion and cows with persistent intramammary infection. The difference in prevalence of IMI between front and rare udder quarters and their significance was estimated by Chi-square test.

Once they had been isolated and identified, pure cultures of udder pathogens were tested for antibacterial susceptibility with the disc diffusion assay on Mueller-Hinton agar using commercial antimicrobial discs (Oxoid - UK). Testing was performed according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The list of antibiotics in susceptibility testing may vary in order to find accurate treatment after getting the laboratory test results. The criteria for the interpretation of zone of inhibition diameter were: sensitive, intermediate sensitive and resistant.

RESULTS AND DISCUSSION

The prevalence of udder quarter health disorders in observed population of dairy cows in early lactation during the two years survey is shown in Table 1.

The prevalence of udder quarters with abnormal milk secretion was 5.33% and 5.45%, respectively for period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. The prevalence of IMI showed the same ratio and therefore, the prevalence in the first period in lactation from calving to21st day in lactation was 4.03% and the prevalence in the period from 22nd to

42nd day in lactation was 4.38%. The total prevalence of udder health disorders on quarter level was 9.36% and 9.83%, respectively for period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation.

Table 1. The prevalence of udder health disorders in dairy cows on quarter level in two periods of early lactation

Period	Total	CMT(-)		CMT(+)		AMS		IMI	
	n	n	%	n	%	n	%	n	%
Day_21	844	765	90,64	79	9,36	45	5,33	34	4,03
Day_42	844	761	90,17	83	9,83	46	5,45	37	4,38

In Table 2 is shown the ratio between udder quarters with IMI versus udder quarters that have positive CMT reaction depending from quarters udder position.

Table 2. The ratio between IMI and AMS on udder quarter level regarding their udder position

Total quarters				FL			FR			RL			RR		
Period	CMT	IMI	IMI (%)	CMT	IMI	IMI (%)	CMT	IMI	IMI (%)	CMT	IMI	IMI (%)	CMT	IMI	IMI (%)
Day 21	79	34	43,04	16	6	37,50	16	10	62,50	20	6	30,00	27	12	44,44
Day 42	83	37	44,58	17	8	47,06	19	10	52,63	28	10	35,71	19	9	47,37

Generally, the rare udder quarters are more prevalent to have IMI in relation with front quarters. There was statistically significant difference in prevalence of IMI in rare udder quarters than front udder quarters (χ^2=35.136, df=1, p<0.001). Table 3 represents the distribution of udder pathogens in milk samples from udder quarters with abnormal milk secretion that showed positive reaction to CMT.

Table 3. Udder pathogens isolated in milk sample from quarters with positive CMT

	n	%		n	%
CMT (+) quarters	162	100,00			
Microbiologically negative	91	56,17			
Microbiologically positive	71	43,83	***Streptococcus agalactiae***	31	19,14
			Enterococcus Spp.	13	8,02
			Candida non-albicans	11	6,79
			Staphylococcus aureus	10	6,17
			Escherichia coli	3	1,85
			Aspergillus niger	2	1,23
			Pseudomonas aeruginosa	1	0,62

During the two year observation of dairy cow population, there was found 162 udder quarters that have abnormal milk secretion and showed positive reaction on CMT out of totally 844 tested quarters. Of them, 56.17% were microbiologically negative and 43.83% microbiologically positive: *Streptococcus agalactiae* was isolated in milk samples from 19.14% quarters, *Enterococcus spp.* in 8,02%, *Candida non-albicans* in 6,79%, *Staphylococcus aureus* in 6,17%, *Escherichia coli* in 1,85%, *Aspergilus niger* in 1,23% and *Pseudomonas aeruginosa* was isolated in milk samples from 0,62% of quarters.

Antimicrobial sensitivity of isolated udder pathogens is shown in Table 4.

Table 4. Antimicrobial susceptibility of udder pathogens

		Str. agalactiae	**St. aureus**	**E. coli**	**Enterococcus spp.**	**P. aeruginosa**
β-laktam	Penicillin (10 IU)	S	R		S	
	Ampicillin (10 μg)	S	R	I	S	
	Amoxicillin (10 μg)	S	I	R	S	
	Amoxyclav (20/10 μg)	S	S	S	S	R
	Imipenem (10μg)			S		S
	Meropenem (10 μg)			S		S
Glycopeptid	Vancomicin (30 μg)		S		S	
Ureidopenicillin + β-laktam	Piperacilin-tazobactam (100/10 μg)			S		S
Aminoglycosid	Gentamicin (10 μg)			S	I	S
	Amikacin (30μg)		S	S		S
Cephalosporin I	Cefadroxil -ALICEF (30 μg)	S	S	S		
	Cefalexin	S		I		
Cephalosporin III	Cefpodoxime (TRICEF) (10 μg)	S	S			
	Cefuroxim (30μg)		R	S		R
	Ceftriaxon (30μg)	S	S	S		S
	Cefotaxim (30 μg)	S	S	S		S
	Ceftazidim (30μg)			S		S
	Cefixim (Pancef) (5 μg)		R	S		R
Cefalosporini IV	Cefepim (30 μg)			S		S
Linkosamid	Clindamycin (2 μg)	S	S			
Macrolid	Erythromycin (15 μg)	S	S		I	
	Azithromycin (15 μg)	S	S			
	Clarithromycin (5 μg)	S				
Trimethoprim + Sulfamethoxasol	Co-trimoxazol (1,25/23,75 μg)	R	S	I		R
Fluoroquinolon	Ciprofloxacin (5 μg)	S	S	S	S	S
	Moxifloxacin (5μg)	S				
Polymyxin	Colistin (10 μg)			S		S

The isolates of *Streptococcus agalactiae* were mostly susceptible to antimicrobials tested, with exception to *Co-trimoxazol* from the group of *Trimethoprim+Sulfamethoxasol*.

The isolates of *Staphylococcus aureus* were resistant to most of the antimicrobials. The isolates were resistant to *Penicillin, Ampicillin, Cefuroxim, Cefixim,* partly susceptible to *Amoxicillin* and showed susceptibility to the other antimicrobials tested.

The isolates of *Escherichia coli* were resistant to *Amoxicillin*, intermediate susceptible to *Ampicillin, Cefalexin* and *Co-trimoxazol*. The isolates were susceptible to the other antimicrobials tested.

The isolates of *Enterococcus spp.* were intermediate susceptible to *Gentamicin* and *Erythromycin*, while the isolates were susceptible to other antimicrobials tested.

Pseudomonas aeruginosa was resistant to *Amoxyclav, Cefuroxim, Cefixim* and *Co-trimoxazol*, while the isolate was susceptible to other antimicrobials tested.

Accurate mastitis detection and effective mastitis control strategies has a influent economic impact in dairy farms followed by sustainable milk production (Wallace et al., 2002).

In general, there is no ideal screening test for prompt and quick diagnosis of IMI. Culturing examination is the "gold standard" for detection of infected udder quarters, but very offen this methods are very expensive, time consuming for routine screening followed by lack for on-farm assessment (Sargeant et al., 2001). However, as IMI are usually followed by an influx of leucocytes and other macrophages into the milk, an increase in its SCC (Somatic Cell Count) has been used widely as indicating mastitis.

Comparing to culturing methods and determination of SCC, the field screening methods for diagnosis of mastitis are easy and routine methods that give prior information for antibiotic treatment of infected udder quarters and early drying off. Among the others, the California Mastitis Test (CMT) is widely used for on-farm detection of mastitis in dairy herds (Sharma et al., 2011). Calderon and Rodrigues (2008) reported some insufficiency of the CMT regarding their sensitivity and specificity in determination of IMI. The main weakness of the CMT is its low specificity for determination of udder quarters infected with a major or minor mastitis pathogens. In general, the CMT is a rapid and inexpensive test to indirectly determine the somatic cell concentration in milk and is a practical, easy method for demonstrating IMI by testing milk samples on-farm.

The results obtained by Saidi (2013) showed a good correlation between the results of CMT and isolation for the identification of intra-mammary infections in cows. Other authors reported negative bacteriological findings in 17.7 to 26.5% from cases of clinical mastitis and 28.7 to 38.6% from cases of subclinical mastitis (Roesch et al., 2007).

A higher incidence of *Staphylococcus spp.* and *Streptococcus spp*. was revealed from cases of subclinical mastitis in dairy cows (Roesch et al., 2007; Kalmus et al., 2011). Saini et al. (2013) reported higher prevalence of

environmental mastitis pathogens and *Escherichia coli* in dairy herds, predominantly in early lactation.

The other study (Kivaria and Noordhuizen, 2007) performed on dairy farms in Tanzania, reveled 29% microbiologically negative milk samples. From microbiologically positive samples, there were isolated *Staphylococcus aureus* (25,7%), *Streptococcus agalactiae* (15.4%), *Klebsiella pneumonia* (14.3%), *Escherichia coli* (14.1%), *Pseudomonas aeruginosa* (7.5%), *Streptococcus dysgalactiae* (5.2%) and *Streptococcus uberis* (4.2%). The most prevalent fungus in infected mammary gland was *Candida spp.* (30%).

Similar results as obtained in this research were reported in the research of Rodrigues (2006), when the most prevalent mastitis pathogens were *Streptococcus agalactiae* and *Staphylococcus aureus*, isolated in 35% to 45% of infected udder quarters.

The recent research from Sweden reported that the most prevalent pathogens from infected mammary gland were: *Staphylococcus aureus* (19%), coagulase negative *Staphylococcus spp.* (16%), *Streptococcus dysgalactiae* (9%), *Streptococcus uberis* (8%), *Escherichia coli* (2.9%) and *Streptococcus spp.* (1,9%). The rate of microbiologically negative samples was 22% from udder quarters that have positive reaction on CMT (Persson и сор., 2011).

The prevalence of udder pathogen microorganisms in cases of intramammary infections in dairy herds in Sicily for period 2000 to 2006, was: microbiologically negative 47.4%, than coagulase negative *Staphylococcus spp.* (22.6%), *Staphylococcus aureus* (20.6%), *Streptococcus spp.* (11.1%), *Streptococcus agalactiae* (2.3%), environmental coliforms (2.9%) and other pathogens (5.8%) (Ferguson et al., 2007).

The study from Croatia, country with similar climatic conditions like Macedonia and similar management of dairy farms, reported that predominant pathogen microorganisms in cases of intramammary infection in period prior drying were: *Streptococcus spp.* Lancefield group B (7.79%), *Staphylococcus aureus* (6,56%), coagulase negative *Staphylococcus spp.* (2.87%), *Pseudomonas spp*. (2.46%), *Streptococcus agalactiae* (2.05%), and 1.23% from intramammary infections were caused by other pathogen microorganisms (Maćešić et al., 2012).

According our research, *Streptococcus agalactiae* isolated from milk samples showed good sensitivity to all tested antimicrobials, with exception to *Co-trimoxazol*. The most resistant were isolates of *Staphylococcus aureus.* Other isolated microorganisms were susceptible to antimicrobials tested.

Similar results were reported by Persson et al. (2011) when the most of the isolated udder pathogens were susceptible to wide range of antimicrobials, with exception to *Penicillin G*. Opposite, the other authors reported the high level of resistance of udder pathogens to antimicrobials (Kalmus et al., 2011).

In the research performed on dairy farms in Iran (Ebrahimi et al., 2007), the prevalence of udder pathogens in dairy farms was as similar as in our research, while the most of isolated microorganisms showed resistance to *Penicillin, Streptomycin, Oxytetracyclin* and *Colistin*. The most of the researchers indicate on increased antimicrobial resistance between udder pathogens while *Staphylococcus aureus* was the most resistant microorganism (Bengtsson et al., 2009).

CONCLUSIONS

The total prevalence of udder health disorders on quarter level was 9.36% and 9.83%, respectively for period from calving until 21st day in lactation and period from 22nd to 42nd day in lactation. The high prevalence of contagious udder pathogens in observed population of dairy cows indicate on bad hygienic condition during milking of cows and possibility for infection of health udder quarters through the equipment and hands of the workers. The isolates of udder pathogens were mostly susceptible to antimicrobials tested, with exception to the isolates of *Staphylococcus aureus* that were resistant to most of the antimicrobials.

REFERENCES

Andersson, I., Andersson, H., Christiansson, A., Lindmark-Månsson, H., Oskarsson, M., Persson, Y. and Widell, A. (2011). System analys Celltal. Stockholm: Svensk Mjölk Forskning. Nr. 7091.

Bengtsson, B., Ericsson, H.U., Ekman, T., Artursson, K., Nilsson, M., Persson, K. (2009). Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. Veterinary Microbiology 136, pg. 142-149.

Calderon, A., and Rodriguez,. V. (2008). Prevalencia de mastitis bovina y su etiologia infecciosa en sistemas especializados en produccion de leche en el altiplano cundiboyacense (Colombia). Rev. Colomb. Cienc. Pecu. 21: pg. 582-589.

Contreras, G.A. and Sordillo, L.M. (2011). Lipid mobilization and inflammatory responses during the transition period of dairy cows. Comparative Immunology, Microbiology and Infectious Diseases, 34, pg. 281-289.

Deluyker, H.A., Van Oye, S.N., Boucher, J.F. (2005). Factors affecting cure and somatic cell count after pirlimycin treatment of subclinical mastitis in lactating cows. J. Dairy Sci. 88, pg. 604-614.

Ebrahimi, A., Pirali Kheirabadi, K.H., Nikookhah, F. (2007). Antimicrobial susceptibility of environmental bovine mastitis pathogens in west central Iran. Pakistan Journal of Biological Sciences 10 (17): pg. 3014-3016.

Ericsson, U.H., Lindberg, A., Persson, W.K., Ekman, T., Artursson, K., Nilsson-Ost, M., Bengtsson, B. (2009). Microbial aetiology of acute clinical mastitis and agent-specific risk factors. Vet. Microbiol. 137: pg. 90-97.

Ferguson, J.D., Azzaro, G., Gambina, M. and Licitra, G. (2007). Prevalence of Mastitis Pathogens in Ragusa, Sicily, from 2000 to 2006. J. Dairy Sci. 90: pg. 5798-5813.

Hogan, J.S., Gonzalez, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W. and Smith, K.L. (1999). Testing procedures. Page 207 in Laboratory Handbook on Bovine Mastitis. 1st ed. Nat. Mastitis Counc., Inc., Madison, WI.

Kalmus, P., Auasme, B., Kurssin, A., Orro, T., Kask, K. (2011). Udder pathogens and their resistance to antimicrobial agents in dairy cows in Estonia. Acta Vet. Scand., 53, 4: pg. 1-7.

Kivaria, F.M. and Noordhuizen, J.P.T.M. (2007). A retrospective study of the aetiology and temporal distribution of bovine clinical mastitis in smallholder dairy herds in the Dar es Salaam region of Tanzania. Vet. J 173, pg. 617-622.

Kloppert, B., Wolter, W., Risse, K. and Zschöck, M. (1999). Erregerspektrum bei boviner subklinischer Mastitis in hessischen Milcherzeugerbetrieben. In: Tagungsbericht des 23. Kongresses der Deutschen Veterinärmedizinischen Gesellschaft e.v. in Bad Nauheim. pg. 350-355.

Maćešić, N., Karadjole1, T., Bačić, G., Benić, M., Karadjole, M., Vince, S., Lipar, M. and Cergolj, M. (2012). Aetiology and prevalence of bovine intramammary infection at drying off. Vet Arh. 82 (2), pg. 125-131.

Milne, M.H., Barrett, D.C., Fitzpatrick, J.L., Biggs, A.M. (2002). Prevalence and aetiology of clinical mastitis on dairy farms in Devon. Vet. Rec. 151, pg. 241–243.

National Committee for Clinical Laboratory Standards (NCCLS). (2002). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 2nd ed., approved standard M31-A2. NCCLS, Wayne, PA.

National Mastitis Council (NMC). (2001). National Mastitis Council Recommended Mastitis Control Program. Http://www.nmconline.org/docs/NMC10steps.pdf. 2001.

Persson, Y., Nyman, A.J. and Grönlund-Andersson, U. (2011). Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden Acta Veterinaria Scandinavica, 53:36.

Piessens, V., De Vliegher, S., Verbist, B., Braem, G., Van Nuffel, A., De Vuyst, L., Heyndrickx, M., Van Coillie, E. (2012). Intra-species diversity and epidemiology varies among coagulase-negative Staphylococcus species causing bovine intramammary infections. Vet Microbiol 155: pg. 62-71.

Rodriguez, G. (2006). Comportamiento de la mastitis bovina y su impacto economico en algunos hatos de la Sabana de Bogota, Colombia. Rev. Med. Vet.12: pg. 35-55.

Roesch, M., Doherr, M.G., Schären, W., Schällibaum, M., Blum, J.V. (2007). Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. J Dairy Res, 74: pg. 86-92.

Saidi, R., Khelef, D., Kaidi, R. (2013). Bovine mastitis: Prevalence of bacterial pathogens and evaluation of early screening test Afr. J. Microbiol. Res. Vol. 7(9), pg. 777-782.

Saini, V., McClure, J.T., Scholl, D.T. DeVries, T.J. and Barkema, H.W. (2013). Herd-level relationship between antimicrobial use and presence or absence of antimicrobial resistance in gram-negative bovine mastitis pathogens on Canadian dairy farms. Dairy Sci. 96 : pg. 1-12.

Sargeant, J.M., Leslie, K.E., Shirley, J.E., Pulkrabek, B.J. and Lim, G.H. (2001). Sensitivity and specificity of somatic cell count and California mastitis test for identifiying intramammary infection in early lactation. J. Dairy Sci. 84, pg. 2018-2024.

Schalm, O., Noorlander, D., 1957. Experiments and observations leading to the development of California mastitis test. Journal of American Veterinary Medical Association 130, pg. 199-204.

Sharma, N., Singh, N.K., Bhadwal, M.S. (2011). Relationship of somatic cell count and mastitis: an overview. Asian-Australas J Anim Sci 24: pg. 429-438.

Sordillo, L.M. and Aitken. S.L. (2009). Impact of oxidative stress on the health and immune function of dairy cattle. Vet. Immunol. Immunopathol. 128: pg. 104-109.

Taponen, S., Salmikivi, L., Simojoki, H., Koskinen, M.T. and Pyorala, S. (2009). Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. J. Dairy Sci. 92: pg. 2610-2617.

Trajcev, M., Popovski, Z.T., Nakov, D., Dimitrievska, B.P., Porcu, K., Petrovska, M., Jankoska, G. 2009. Characterisation of resistance of *Streptococcus Spp.* and *Staphylococcus Spp.* isolated from cow's milk. 4-th international congress of microbiologists of Macedonia - Book of abstracts, Macedonian medical review, year 63 (supl. 77), pg. 1-87.

Trajcev, M., Petrovska, M., Nakov, D., Jankoska, G. 2010. Therapy of subclinical mastitis in dairy cows. Yearbook of the Faculty of agricultural science and food, vol. 55, Skopje, pg. 117-128.

Wallace, J.A., Leslie, K.E., Dingwell, R.T., Schukken, Y.H., Baillargeon, P. (2002). An evaluation of a diagnostic and treatment protocol for intramammary infections in early postpartum dairy cows. Proc Annu Meet National Mastitis Council: pg. 159-160.

White, L.J., Lam, T.J., Schukken, Y.H., Green, L.E., Medley, G.F., Chappell, M.J. (2006). The transmission and control of mastitis in dairy cows: a theoretical approach. Prev. Vet. Med. 74, pg. 67-83.

DOI: 10.17707/AgricultForest.63.1.06

Nina LIOGCHII,
Adam BEGU, Vladimir BREGA[1]

STATUS AND ROLE OF NATURAL ECOSYSTEMS IN NORTH REGION OF THE REPUBLIC OF MOLDOVA

SUMMARY

Regional development in Moldova is a necessity assumed by Law no. 438-XVI from 28.12.2006. The regional development model provides the division of the country territory into 6 regions and is based on the country's sustainable economic development. In this regard, the natural areas protected by the state have a special role. Due to their special protection status they ensure the conservation of natural resources in the region.

The work is focused on some aspects of the ecological status of state protected natural areas in the Northern Region. The results were obtained on the basis of a comprehensive study conducted during field expeditions and laboratory research over several years. There are highlighted valuable items specific for protection category, the ecological state of biotic and abiotic components and the human impact on protected natural areas in the region.

As a result of the research it was found that the specific elements for the categories of investigated areas are in satisfactory condition and the transboundary sources are the main sources with negative impact on these studied areas. The investigated natural ecosystems, especially those located in the forestry sector, provide favourable conditions for a rich diversity of plant and animal species, among them rare species protected at the national and international level. Interconnection between biotic and abiotic components of protected areas and surrounding habitats, especially agrocoenoses ensure ecological balance in the region.

Keywords: *state protected natural areas, valuable items, human impact*

INTRODUCTION

Sustainable regional development is a priority determined by the alarming state of the environment which, over the last time, threatens the survival of humanity. In this context, the key issue of sustainable development is the reconciliation between two human aspirations: the need to continue economic and social development, and environmental protection and improvement as the only way for the welfare of both the present generation and those to come. This

[1]Nina Liogchii (corresponding author: ninaliogchii@mail.ru), Adam Begu, Vladimir Brega Institute of Ecology and Geography of Academy of Science of Moldova, Chisinau, Republic of MOLDOVA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

concept is placed at the base of Regional Development in Moldova, which is a necessity assumed by Law no. 438-XVI from 28.12.2006. The Model of Regional Development provides the division of the territory into 6 regions and is based on the sustainable development of the country's economy.

At the the basis of the transition to a sustainable development model is the research, that can be achieved in such pilot areas as protected areas. The natural areas have a special status of protection. They ensure the conservation of natural resources and contribute to maintaining the ecological balance in the region.

Those referred above come to argue on the research timeliness and need regarding the ecological status assessment of State Protected Natural Areas (SPNA) in the North Region of Economic Development (NRED) of Moldova. The study is motivated also by the very small share of protected areas (4.65% of the country) and their uneven spread in the country.

MATERIALS AND METHODS

The study includes field researches to assess the status of specific components of the categories of protected areas, the natural ecosystems in the main phonological development phases of efemeroide vegetation, annual and perennial and of animal life (Doniţă I., Doniţă N., 1975; Begu A. *et al.*, 2005; Munteanu A. *et al.*, 2004), to record rare species and collect samples of plants and animals to determine the systematic affiliation (Negru A., 2007;ГейдеманТ. С., 1975).

The abundance and coverage degree of the substrate by rare species were determined according to Braun-Blanquet (Braun-Blanquet J., 1964). The category of threat to rare species was determined by the classifier IUCN and the protection status at the national, regional and international levels according to the List of Rare Plants from Spontaneous Flora of RM (R),2012, Red Book of Moldova (MRB), 2015, Romania (RRB), 2005, Ukraine (URB), 2009, European Red List (ERL), 2011, the Annexes of Conventions from Bern (CBern), 1979, Bonn (CBonn), 1979, Washington (CWash.), 1979 and Rio de Janeiro, 1992.

Air quality assessment was carried out on the basis of emissions from local and transboundary pollution sources (Evidentele statistice, 2014). The emissions of pollutants SO_2, NO_x from stationary sources were established on the basis of the statistical data and yearbooks of the State Ecological Inspectorate and the Department of Statistics and Sociology, while the emissions of the mobile sources were calculated using the methodology EMEP Corinair (Transboundary Acidifying Air Pollution in Europe…, 1998). The Roentgen fluorescent spectrometry method was used to determine the heavy metal concentration (Методикавыполнения.., 2002).

RESULTS AND DISCUSSION

The object of research is the SPNA in the Northern Region of the Republic of Moldova, which are divided into three physico-geographic regions: The Region of Plateaus and Forest Steppe Plains of North Moldova, The Region

of Steppe Plains and Hills of North Moldova, and The Region of Codrii Plateau. The physical and geographical conditions, including type of substrate, favour various economic activities based on natural resource exploration that ultimately determine the ecological state of the environment.

Human impact

Human impact comes from local and transboundary pollution sources. Analyzing the results on air pollution in the region, we find that about 25 and, respectively, 40% of the overall pollution in the country rests with NO_x and SO_2 pollution. The data in tab. 1 state that SO_2 and NO_x air pollution in the region comes from transboundary sources and the local impact is predominantly from mobile sources. During the 1990s the major stationary sources were presented in the towns of Balti and Floresti. However, actually, the largest share in pollution is owned by the mobile sources. The local impact of air pollution with NO_x is higher than the transboundary one, while that with SO_2 is, practically, at the same level for each settlement. For the current period, the precipitations with SO_2 in rural areas of Moldova are in the range from 0.780 to 0.825 mg/m^3 and with NO_x are from 0.619 to 0.885 mg/m^3, which is approximately 30 times less than the critical limit (CL) value for forest ecosystems (tab. 1).

Table1.The content of NO_x and SO_2 in the emissions from local and transboundary sources

Settlements	Stationary Sources, t				Mobile Sources, t		Local Sources, mg/m^2		Transboundary Sources, mg/m^2	
	1990		2013		2013		2013		2013	
	SO_2	NO_x	SO_2	NO_x	SO_2	NO_x	SO_2	NO_x	SO_2	NO_x
Edinet	3215	132	61	41.3	21	869	331	854	424	291
Falesti	3460	69	60	37.3	20	98	280	234	431	288
Floresti	7460	300	27	5.8	71	288	365	573	386	316
Balti	10406	722	35	54	285	901	6954	48142	424	281
The range for other North Districts	950-3000	88-360	8-108	8-48	16-72	539	234-573	294-402	386-501	277-316
The range for RM in precipitations, mg/m^2									385-518	267-326
The range for RM in atmospheric air, for SO_2 0.780-0.825 μg/m^3, for NO_x – 0.619 – 0.885 μg/m^3										
CL forest vegetation/lichens for SO_2 μg/m^3 20/10, for NO_x 30/30μg/m^3										

Calculation of the impact from the local sources, according to Ecolog Programme and EMEP (Monitoring and Evaluation European Programme) data, indicates a pollution of 0,005mg/m^3 (or 0.1CMA, RM standard) with SO_2 and

NO_x in the rural settlements with an insignificant influence to forests ecosystems.

The content of heavy metals in soil

Among noxious pollutants, depending of toxicity and degree of impairment of the ecosystem components, a particular role is played by the heavy metals that are released into the atmosphere with exhaust gases, emissions from industrial enterprises, chemicals used in agriculture etc. In this context it was determined the content of heavy metals in soil samples (0-20 cm) from objects of study.

On the basis of the graduation scale of the level of heavy metals concentration in soils in Moldova (Кирилюк В., 2006) it was found that the content of heavy metals in the top layer of soil from the protected areas located in forests was within five of six levels, being *very low*, *low*, *medium*, *increased* and *high*, excluding the *very high level*. Only in two protected areas - LRs Cosauti and Holosnita– it was recorded that the cobalt content has exceeded the alert threshold (Kloke A., 1980). However, the registered concentrations of heavy metals do not reach values exceeding the threshold for intervention, the more of the pollution one, which do not show danger of toxicity to plants and soil organisms, but does not exclude the possibility of their transmission through the food chain to the higher levels and their accumulation in the animal bodies.

State of representative elements

The research objects are the protected areas placed in the NRED of the Republic of Moldova. Due to the function of SPNA is to preserve and protect the representative and rare components along with environmental factors within their boundaries, we will refer to some aspects of the state of valuable biotic and abiotic elements specific for protection categories.

From the point of view of diversity of protection categories, the fund of SPNA in the NRED are presented by various categories of protected areas such as scientific reserves, nature reserves, landscape reserves, resource reserves, multifunctional management areas, natural monuments, landscape architectural monuments and wetlands of international importance. Their surface constitutes 3.2% of the surface of NRED or about 20% of the surface of the country's protected areas.

The famous geological and paleontological elements of the natural heritage of the northern sector of the country are protected in the geological and paleontological natural monuments (GPNM) which, alternating with agricultural ecosystems, create unrepeatable and quite attractive landscapes (Begu A. et al., 2012). Our research has focused on highlighting the value of these landforms as habitats for certain rare rocky and epiphytic plant species and rare animal species like reptiles, birds of prey and rodent mammals. Thus, true reservoirs of flora and fauna, important due to the value of registered species, are recorded: GPNMs Defile Duruito area, Defile Varatic, Stinca Mare (Great Rock), Gorge Butesti, and Tectonic Fault Line near the Village of Naslavcea.

In all these reserves are present the rocky ferns from the genus *Asplenium*. On the rocks of GPNM Defile Duruitoarea and Gorge Butesti was recorded abundantly the endangered species *Sempervivum ruthenicum*, in GPNM Varatic and Defile Duruito area - the vulnerable species *Schivereckia Podolite* which has international status of protection being protected by the European Red List and Berne Convention. In the GPNMs Gorge Butesti and Stinca Mare is met solitarily the species of lichens *Peltigera polydactila* endangered in the Republic of Moldova and in GPNMT ectonic Fault Line near the Village of Naslavceais protected the endangered species *Pulsatilla grandis*.

On top of limestone cliffs from the most GPNMs are nesting species of raptors such as: *Falco tinnunculus*, *Buteo lagopus* and *Accipiter gentilis* while on forested areas we meet *Dendro coposmedius*, *Turdus philomelos*, *Parus major*, *Carduelis spinus*, *Corvus corax* and others. Among the fauna species are reported the vulnerable species *Oryctes nasicornis* and *Papilio machaon* as well as abundant species common for the Republic of Moldova, but protected by the Bern Convention, such as: *Lacerta viridis*, *L. muralis*, *Helix pomatia*.

Exploitation of deposits of limestone and gypsum and burning tyres at lime illegal obtaining in GPNMs Defile Fetesti and Trinca, and Mine Criva lead to intense environmental pollution and, respectively, to a considerable decrease of species diversity, including there are ones.

The most favorable conditions for the preservation of biotic and abiotic components of the environment are provided by protected areas located in the forest fund. Thus, the botanical natural monuments (BNMs) comprise precious sectors with forest vegetation and trees. They aim to conserve the unique or typical habitats of endemic and relicts plant species, their communities, as well as some rare or endangered species of plants and secular trees.

The evaluation of the four BNM from NRED allows us to state their importance in conserving the forestry sectors with *Quercus robur* in Caracuseni, *Quercus pubescens* in Calinestii Mici, *Quercus pertaea* in Rudi-Gavan and the artificial spruce stand of *Piceaabies* with a high productivity in Lipnic. In the mentioned sectors are protected copies of secular trees and rare grassy plant species with national, regional and international protection status (Fig. 1). Among the valuable species we mention the endangered species of lichens *Cetrelia cetraroides* and the bird species *Dryocopus martius* and *Picus viridis* in the BNMs noticed in BNM Caracuseni and Lipnic, respectively.

The BNM Rudi Gavan is the richest in rare species. There were recorded 14 species of plants included in the Moldova's Red Book among which the most common are *Galanthus nivalis*, *Hepatica nobilis*, *Pulsatilla grandis*, *Scopolia carniolica*, *Poa versicolor*, *Polystic humaculeatum*, and pteridofite species on rocks: *Dryopterisfilix-mas*, *Athyrium filix-femina*, *Phyllitis scolopendrium*. Here were reported rare animal species among which *Lutra lutra*, *Mustela erminea*, *Felis silvestris*, *Hyla arborea*, *Rana temporaria* are found in Moldova's Red Book.

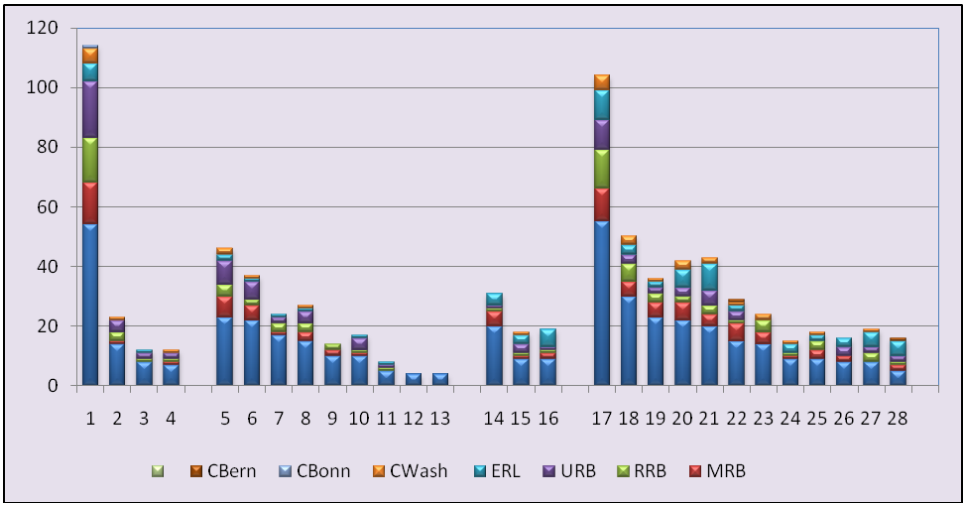


Figure 1. The numbers and the protection status of rare species of plants

The category of protected areas called Natural Reserves (NR) is the natural space, scientifically valuable for preserving or restoring one or more components for maintaining the ecological balance of nature. In the research area are located 12 NR, of which 9 belong to natural forest reserves (FNR) and 3 to natural reserves of medicinal plants (NRMP).

The specific objective of FNR is the protection of fundamental natural stands and rare species of plants and animals. In the most FNR from NRED, regarding tree species, natural stands of oak with durmast and cherryare protected. In the FNR Rososeni, the oak and birch are the basic species; in the FNR Mestecanis the species of birch (*Betula pendula*) is protected. This area shows the South-Western edge of birch growth in the Eastern Europe. On the basis of state assessment of the FNR Mestecanis were registered only a few copies of birch trees which according to their health state can be attributed to the category of healthy trees. Many copies have broken tops, affected branches and stem(about 20%) with frequent hollows. A perspective in preserving the value of this reservation is the conservation of about 80 copies of birch saplings located on the bank of the steep ravine along the border with Ukraine.

The presence of valuable species of plants and animals in the framework of a protected habitat is also beneficial to establish mutual sustainable relations and create conditions for ecological balance. From this point of view, the greatest number of rare species is recorded in FNRs Rososeni and Saptebani (23 and 22, respectively).In both of them are protected the following species listed in MRB: *Fritillaria montana, Dryopteris filix-mas* and *Climacium dendroides.* The critically endangered species*Gladiolus imbricatus* were signalled in the FNR Rososeni and the species *Pulsatilla grandis, Coronella austriaca, Oryctes nasicorni, Morimus funereus* were signalled in the FNR Saptebani.

Another category of state protected areas are organized to preserve and reproduce the rare species of medicinal plants. The specific floristic elements of

NRMP in the NRED are represented by the species of medicinal plants that form a compact carpet with a luxurious development on some plots. For example, in NRMP Radoaia were attested some plots of *Convallaria majalis*, with coverage of 50-70% and *Fritillaria montana*, with coverage of 25-30%. Here were also reported other rare plant species such as *Lilium martagon* and *Veratrum nigrum* and animals such as *Meles meles*, *Capreolus capreolus* and *Lucanu scervus* with different protection status.

In the herb layer of the NRMP Rososeni were recorded the following rare species: *Dryopteris filix-mas, Scopolia carniolica, Doronicum hungaricum* and *Allium ursinum*, and in that of NRMP Cernoleuca the dominant species of medicinal plants are *Arctium lappa, Leonurus cardiac* and*Urtica dioica* reaching 1.0-1.5 m height. Here are also recorded some rare species of plants like *Asparagus officinalis and Rhamnus tinctoria* and animals like *Martes martes, Felis silvestris, Hyla arborea* and *Coronella austriaca* which enrich the value of protected area (Fig. 2).

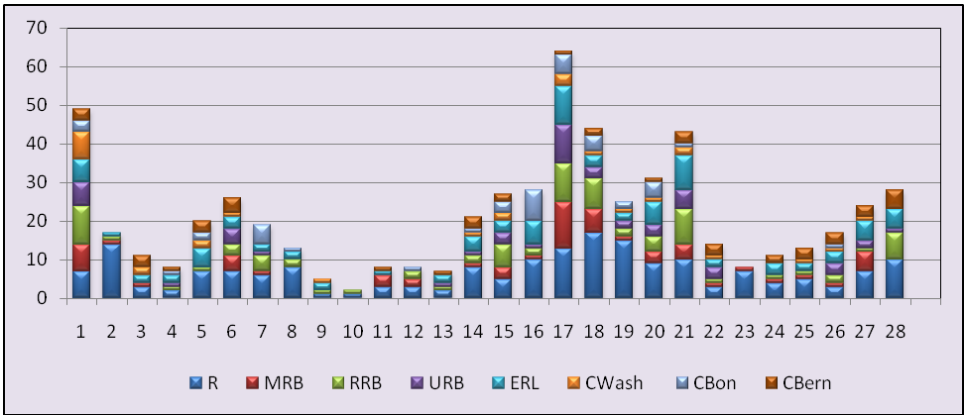


Figure 2. Number and protection status of rare species of animals

Legend to Figures 1 and 2: 1 - BNM Rudi-Gavan, 2 - BNM Lipnic, 3 – BNM Caracuseni, 4 - BNM CalinestiiMici, 5 – FNR Rososeni, 6 – FNR Saptebani, 7 – FNR Baxani, 8 – FNR Climauti, 9 - FNR Ocnita, 10 - FNR Stinca, 11 – FNR Pociumbeni, 12 – FNR Mestecanis, 13 - FNR Lucaceni, 14 – NRMP Rososeni, 15 – NRMP Cernoleuca, 16 – NRMP Radoaia, 17 - LR Rudi-Arionesti, 18 – LR Cosauti, 19 - LR La 33 de Vaduri, 20 - LR Holosnita, 21 – LR Tetcani, 22 – LR Fetesti, 23 – LR Calarasovca, 24 - LR Zabriceni, 25 - LR Izvoare-Risipeni, 26 - LR La Castel, 27 – LR Suta de Movile, 28 - LR Lopatnic.

Approximately 21% of the natural areas in the region belong to landscape reserves (LR) that according to the Law of SPNAF, 1998 presents a natural homogeneous system of forest, steppe and meadow, swamp and marsh, with scientific value, ecological, recreational, aesthetics, instructive and educational value, intended to maintain its natural qualities and carry out regulated economic activities.

The landscape elements of this type of protected areas are complemented by their rich diversity of flora and fauna. The analysis of quantitative presence of rare species in the 12 LRinthe research area allows us to state that the richest and most diverse is the flora and fauna in the LRs Rudi Arionesti, Cosauti, La 33 de Vaduri, Holosnita and Fetesti. They also feature a varied relief, which creates favourable conditions for different types of vegetation. In the mentioned areas were recorded the greatest number of rare species of plants with protection status at the national, regional and European level, proved by their presence on the List of Rare Species and in MRB, RRB,URB, ERL and Annexes of Environmental Conventions.

However, the quantitative criterion does not always reflect the value of the protected object for the conservation of one or another species. For example, it is incontestable the value of LRs Lopatnic and Fetesti as favorable habitats for the species *Sempervivum ruthenicum* and *Schivereckia podolica,* LRsIzvoare-Risipeni and Holosnita for *Scopolia carniolica,* LRsCalaraseuca and La 33 de Vaduri for the most extensive areas of the species *Galanthus nivalis* and *Hepatica nobilis* and LRsRudi-Arionesti for *Polystichum aculeatum, Melittis sarmatica* and *Alnus glutinosa*. The most of those reserves are favorable habitats for many species of animals such as *Felis silvestris*, *Martes martes*, *Capreolus capreolus*, *Meles meles, Upupa epops, Buteo buteo*, *Coronella austriaca*, *Lacerta viridis, Rana dalmatina* and *Lucanus cervus*.

The function of regulated management of natural resources goes to the multifunctional management areas (MMA), which care also of nature conservation. During the investigation period there were evaluated 10 representative sectors with meadow vegetation. The investigated areas are characterized by a biodiversity specific to meadow ecosystems with higro- and mesophyte species, aquatic and marsh systems with hygro- and hydrophilic species and steppe ecosystems with xerophyte species. Among them, the most rich in vegetation are MMA Meadow with Stoloniferous Grass, the River Raut, where were frequently encountered 35 species of herbaceous plants and MMAReedy Marshy Meadow with about 25 species. Both are dominated by grasses and cyperaceae.

An important role is played by the windbreaks nearby the city of Balti, which were created according to the planting method of nests arranged in square. *Quercus robur* is the main species and *Fraxinus lanceolata, Robinia pseudacacia, Cerasus avium, Acer tataricum, Acer pseudoplatanus, Acer tataricum, Gleditsi atriacanthos, Armeniaca vulgaris* and others are the accompanying ones. Among shrubs prevail the species *Swidas anguinea, Rhamnus catharctica, Prunus spinosa, Rosa canina, Humulus lupulus, Cerasus mahaleb*, *Crataegus monogyna*, *Ligustrum vulgare*, *Loniceraxylosteum* and *Sambucus nigra.* In their canopy are sheltering the birds species *Carduelis carduelis*) – ERL, CBern; *Carduelis cannabina* – ERL, CBern; *Cocco thraustes cocco-thraustes* – ERL; CBern; and *Passer domesticus* – ERL, CBern. Windbreaks help to maintain the ecological balance, protect from drought and

improve microclimate in field crops, i.e. it is of great importance their proper management.

CONCLUSIONS

Along with the specific elements of protection categories, the protected areas, particularly those located in forests, provide favorable conditions for a rich diversity of plant and animal species, among them rare species protected nationally and internationally. In investigated areas 36 rare plant species and 26 rare animal species were registered.

The analysis of the system of protected areas, by establishing the compliance criteria for integration, extension and space distribution, emphasizes the fact that protected areas are spatially isolated and require the establishment of connection corridors with the national and international surrounding ecosystems, which also would contribute to the conservation of biological diversity in the region.

The interconnection between biotic and abiotic components of protected areas and surrounding habitats, agrocoenoses in particular, ensure the ecological balance in the region.

REFERENCES

Begu A., David A., Liogchii N. ş. a. (2012). Starea mediului şi patrimoniul natural al bazinului Dunării în limitele Republicii Moldova). In English: *State of the Environment and Natural Heritage of the Danube River Basin* (*in the limits of Moldova*). Noosfera. Chişinău, 300 p.

Begu A., Manic Ş., Şalaru V., Simonov Gh. (2005). Lumea vegetală a Moldovei. Ciuperci, plante fără flori. In English: *Moldova's Vegetable World. Fungi, Flowerless Plants*), v. I.Ştiinţa.Chişinău.204 p.

Bilz M., Kell Sh. P.,Maxted N., Lansdown R.V. (2011). European Red List of Vascular Plants. Luxembourg: Publications Office of the European Union.144 p.

Botnariuc N., Tatole V. (2005). Cartea Roşie a vertebratelor din Romania.(in English: *Red Book of Romania's Vertebrates*). Muzeul Naţional de Istorie Naturală "Gr. Antipa". Bucureşti. 260 p.

Braun-BlanquetJ. (1964). Pflanzensoziologie.(in English: *Plant associations*). 3Aufl. Wien, N. Y. 865 p.

Cartea Roşie a Republicii Moldova (2015).(in English: *Red Book of Moldova*). Ed. a 3-a. Ch., Î.E.P. Ştiinţa. 492p.

Checklist of CITES species and Annotated CITES appendices and Reservations. Washington. 1979. 417 p.

Convention on Biological Diversity. Rio de Janeiro. 1992.

Convention on Migratory Species. Bonn. 1979.

Convention on the Conservation of European Wildlife and Natural Habitats. Bern. 1979.

Doniţă I., Doniţă N. (1975). Metode practice pentru studiul ecologic şi geografic al vegetaţiei. (in English: *Practical Methods for Ecological and Geographical Study of Vegetation*). Centrul de multiplicare a Universităţii din Bucureşti. Bucureşti. 47p.

Evidenţele statistice (2014). (in English: *Statistical Evidence*). Rapoartele Inspectoratului Ecologic de Stat pentru perioada 1990-2013. Chişinau.

Kloke A. (1980). Orientierungsdaten fur tolerierbare gesamtgehalte einiger elemente in kulturboden, mitt.(in English: *Orientation Data for Tolerable Overall Contents of Some Elements in Culture Soil*). VDULFA, H1-3. p. 9-11.

Lege Nr. 438 din 28.12.2006 privind dezvolt area regională în Republica Moldova.(in English. *Law* no. 438 from 28.12.2006 *on Regional Development in the Republic of Moldova*).

Legea privind fondul ariilo rnaturale protejate de stat (2002).(in English: *Law on the State Natural Protected Areas Fund*). Chişinău.

Lumea animală. Cartea Roşie a Ucrainei (2009). (in English: *World of Animals. Red Book of Ukraine*). Maister-print.Kiev. 608 p.

Lumea vegetală. Cartea Roşie a Ucrainei (2009).(in English:*World of Plants. Red Book of Ukraine*).Global consulting Kiev. 912 p.

Munteanu A., Lozan M. (2004). Mamifere. Lumea animală a Moldovei.(in English: *Mammals Animal World of Moldova*).Ştiinţa. Chisinau. 132 p.

Negru A. (2002). Plantele rare din flora spontană a Republicii Moldova.(in English:*List of RarePlants from Spontaneous Flora of Moldova*).CEUSM. Chişinău. 198p.

Negru A. (2007). Determinator de plante din flora Republicii Moldova (*Identification Manual of Plants from Moldova's Flora*). Univers.Chisinau.391 p.

Transboundary Acidifying Air Pollution in Europe, EMEP, Co-operative programme for monitoring and evaluation of the long range transmission of Air pollutants in Europe. MSC-W Status Report 1998. Part 2: Numerical Agendum.

ГейдеманТ. С. (1975). ОпределительвысшихрастенийМолдавскойССР. (in English: *Identification Manual of Higher Plants of Moldova*). Штиинца.Кишинев .636р.

Кирилюк В. П. (2006). Микроэлементы вкомпонентах биосферы Молдовы. (in English: *Microelements in Moldova's Biosphere Components*) Pontoc, Chişinău. 156 p.

Методикавыполнениязмерениймассовойдолиметалловиоксидовметалловвпорошковых пробах почв методом рентгено флуоресчентного анализа(2002).(in English: *Measurement Methods of Mass Fraction of Metals and Metal Oxides in Soil Powder Samples by Renthgeno-fluoreschent Analysis*).НПО С. Петербург.

DOI: 10.17707/AgricultForest.63.1.07

Luiza DAWIDOWICZ, Marek SIWULSKI [1]

COMPARSION OF GROWTH OF MYCELIUM OF *PLEUROTUS CYSTIDIOSUS* (MILLER) ON VARIOUS AGAR MEDIA

SUMMARY

Pleurotus cystidiosus occurs in natural areas on all continents except Antarctica and South America. Fruiting bodies of *P. cystidiosus* are valued as a source of nutrients and biologically active substances. *P. cystidiosus* has antitumor, anti-inflammatory and antioxidant, antihyperglycemic activity. This species is cultivated mainly in North America and Asia. The aim of the study was selection the best agar medium for the fastest mycelium growth and the best mycelium hyphal quality. In the experiment, two cultivars of *P. cystidiosus* from the collection of the Department of Vegetable Crops Poznan University of Life Sciences were used: B1 and B122. Growth of mycelium on five agar media: wheat, potato, synthetic Hansen`s, maltose and sawdust was compared. Measure of this parameter was a diameter of agar medium overgrown by hyphae after 7, 10, 14 and 21 days of incubation. Experiment was conducted in Biological Laboratory of the Department of Vegetable Crops Poznan University of Life Sciences. It has been shown that growth of mycelium of tested strains on different agar media was different. Research of mutual dependence between morphological and qualitative characteristics of agar medium type allows production optimization. Development of low-cost, simple method for production of *P. cystidiosus* can contribute to introduction of this species into intensive cultivation in Poland.

Keywords: *Abalone oyster mushroom, summer oyster mushroom, cultivation of edible mushrooms, medicinal mushrooms, mycelium growth*

INTRODUCTION

Pleurotus cystidiosus belongs to the kingdom of *Fungi*, phylum *Basidiomycota*, class *Agaricomycetes*, order *Agaricales*, family *Pleurotaceae*, genus *Pleurotus*. This species was described first time in North America by O.K. Miller in 1969. It is widespread on angiosperm trees wood (Moncalvo 1995, Zervakis et al. 2004). Name Abalone oyster mushroom, Miller`s oyster mushroom and summer oyster mushroom can be found in the literature, as well as Chinese name *bao yu gu* and japanese *Ohiratake*. Caps of *Pleurotus cystidiosus* are convex to hollow with dark greyish to brown centre and yellowish

[1]Luiza Dawidowicz (corresponding author: loocy7@op.pl), Marek Siwulski Department of Vegetable Crops, Faculty of Horticulture and Landscape Architecture, Poznan University of Life Sciences, POLAND

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

brown smooth margins; stems are long and white (Hanelt 2001, Croan 2004, Lechner et al. 2004, Abdullah et al. 2012, Usami et al. 2014). *Pleurotus cystidiosus* produces darkly pigmented arthroconidia forming a black pigment on mycelium or basidiomata (Petersen et al. 1997, Zervakis 1998).

In natural conditions *Pleurotus cystidiosus* occurrs on all continents except Antarctica and South America (Vilgalys et al. 1996, Zervakis and Balis 1996, Lechner et al. 2004). In natural environment it is encountered on dead tree stumps, especially deciduous trees. *Pleurotus cystidiosus* occurs often in form of groups consisting of several larger and smaller specimens that arise from a common base or imbricately are arranged one above the other. It can be grown on substrates prepared on base of straw and various types of agricultural, horticultural and forestry wastes (Cohen et al. 2002, Croan 2004, Lau et al. 2013, Usami et al. 2014). It is cultivated mainly in South-West Asia, especially in China (Hanelt 2001, Usami et al. 2014).

The genus of oyster mushroom (*Pleurotus*) is represented by many species (Vilgalys et al. 1996, Bao et al. 2004). The best-known oyster mushroom is *Pleurotus ostreatus*, which is widely cultivated in Poland. Poland is the largest producer and exporter of *Pleurotus ostreatus* in European Union. Oyster mushroom, beside common button mushrooms (*Agaricus* L.), is among the most frequently purchased and consumed mushrooms in Poland (Siwulski and Sobieralski 2004). In addition to culinary qualities of fruiting bodies of *Pleurotus cystidiosus* it has high nutritional value (proteins, fibre, vitamins, and minerals) and contain biologically active substances with proven health-promoting properties. The biological activity of these mushrooms has been confirmed in several laboratory tests and clinical trials, which showed among others their antibacterial, antiviral, antifungal, antitumor, immunomodulatory, antiallergic, anti-inflammatory, antiatherosclerotic, hepatoprotective, lowering blood sugar and blood cholesterol effect. This mushroom is low in calories due to the limited content of lipids. In China and many other countries it is considered a healthy and functional food (Manzi and Pizzoferrato 2000, Wasser 2002, Croan 2004, Rajewska and Bałasińska 2004, Bernaś et al. 2006, Thekkuttuparambil and Kainoor 2007, Kalbarczyk and Radzki 2009, Karaman et al. 2010, Abdullah et al. 2012, Patel et al. 2012, Lau et al. 2013, Siwulski et al. 2013, Siwulski et al. 2014, Usami et al. 2014).

During last several years there has been a very dynamic development of production of edible and medicinal mushrooms. This development was mainly caused by high availability of low-cost, waste materials from agricultural, textile and wood processing industry, which may pose a potential base for their cultivation including *Pleurotus cystidiosus* (Vilgalys et al. 1996, Usami et al. 2014). Poland, in comparison to European Union countries, is still an ecologically clean area. That is why our country has a great potential to use uncontaminated with heavy metals and pesticides substrates for growing mushrooms. *P. cystidiosus* is tolerant for high temperatures during growth and yielding, therefore can be grown in adverse to *P. ostreatus*, species requiring

lower temperatures. This feature makes *P. cystidiosus* an alternative for oyster mushroom producers and at the same time may diversify the market offer of edible mushrooms. (Siwulski and Sobieralski 2004, Zervakis et al. 2004, Selvakumar et. al. 2008, Usami et al. 2014). This feature is important because it allows cultivation in chambers without air conditioning during summer time, which is impossible for other oyster species of greatest economic importance. Introduction to commercial cultivation species of *P. cystidiosus* will be a perfect solution for small producers of oyster mushroom, who until now were forced to stop production in the summer time. Literature says that *P. cystidiosus* can be grown on a many different kinds of waste, such as: chopped cocoa pods, cotton wastes, chopped and corn stover, waste from palm oil production , tobacco straw, tea leaves, rice straw, bagasse, sawdust of different kinds of trees (Vilgalys et al. 1996, Croan 2004, Lau et al. 2013, Usami et al. 2014). Use of these waste materials in oyster mushroom production will significantly increase profitability of the crop and reduce its costs. There will also be a new solution for those waste disposal, which causes enormous problems in agricultural production.

Development of low-cost and simple method for production of *P. cystidiosus* can contribute to introduction of this species into commercial cultivation in Poland. Determination of morphological and qualitative characteristics of substrate type and growing conditions will allow optimizing production, in order to obtain the best yield of fruiting bodies with the best characteristics, for consumption and as raw material for obtaining biologically active substances.

Pleurotus cystidiosus is a species requiring evaluation. Currently in Poland this mushroom is unknown for large scale cultivation. Research of this project will aim to clarify mechanisms of growth of *P. cystidiosus* and the yield depending on various factors, especially the type of agar medium. The aim of experiment was selection of agar medium on which mycelium growth was the fastest and mycelium hyphae had the best quality.

MATERIALS AND METHODS

The subject of research in all experiments was two strains of *Pleurotus cystidiosus* from the Collection of Edible and Medicinal Mushrooms, Department of Vegetable Crops, Poznan University of Life Sciences – B1 and B122.

Laboratory experiments were conducted in Biological Laboratory of the Department of Vegetable Crops, Faculty of Horticulture and Landscape Architecture, Poznan University of Life Sciences. All laboratory experiments were established in fully randomized design, in 3 replications in 2 series.

Influence of type of agar medium for mycelium growth – five different agar media, was investigated: wheat – extract from 200 g of wheat grains, 22 g of agar, 3 g of glucose per 1 litre of medium; potato – extract from 200 g of potatoes, 22 g of agar, 3 g of glucose per 1 litre of medium; Hansen`s – 0.3 g of KH_2PO_4, 0.2 g of $MgSO_4$, 22 g of agar, 5 g of glucose, 1 g of peptone per 1 litter of medium; maltose – 30 g of maltose, 22 g of agar, 5g of peptone per 1 litter of

medium; sawdust – extract from 200 g of sawdust of alder and sawdust of beech (1:1), 22 g of agar, 3 g of glucose per 1 litter of medium. The measure of this parameter was a diameter of agar medium overgrown by hyphae after 7, 10, 14 and 21 days of incubation.

The results of research were statistically analyzed. When comparing the experimental results, the analysis of variance for factorial experiments was applied (ANOVA, level of significance α=0.05).

RESULTS AND DISCUSSION

Comparison of growth of mycelium of two strains of *Pleurotus cystidiosus* after 7, 10, 14 and 21 days of incubation is presented on Figure 1. It has been shown that growth of mycelium of strain B1 was significant fastest than strain B122. This is apparent especially after 14 and 21 days of incubation.

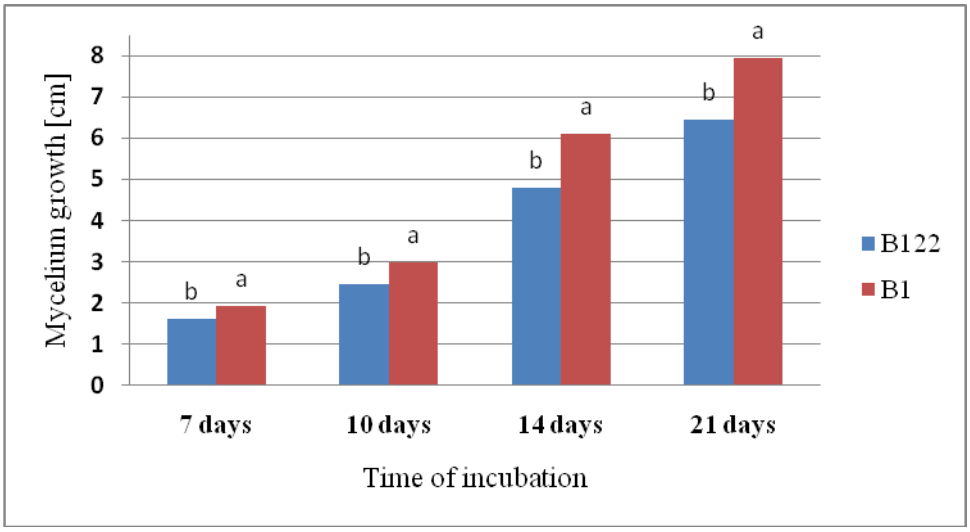


Figure 1. Growth of mycelium of two strains of *P. cystidiosus*

Comparison of mycelium growth of *Pleurotus cystidiosus* on various agar media after 7, 10, 14 and 21 days of incubation is presented on Figure 2. It has been shown that growth of mycelium on different agar media differed significantly. The best growth of mycelium after 7 days of incubation occurred on wheat agar medium. After 10 and 14 of incubation the best growth of mycelium occurred on wheat and sawdust agar media. After 21 days of incubation the best growth of mycelium occurred on wheat, sawdust and maltose agar media. It has been shown that growth of mycelium on wheat and sawdust agar media was significantly fastest than growth of mycelium on potato, Hansen`s and maltose agar media. This may be due to the fact this mushroom in natural environment grows on dead wood, so sawdust is its natural habitat for growth. In potato agar medium may consist from too much starch and therefore ratio of nitrogen to carbon can be unfavourable for good mycelium growth. The

slowest growth of mycelium after 7, 10, 14 and 21 days of incubation occurred on Hansen`s agar medium. This may be due to the fact, that Hansen`s agar medium is a synthetic agar medium and it is low in nutrients.

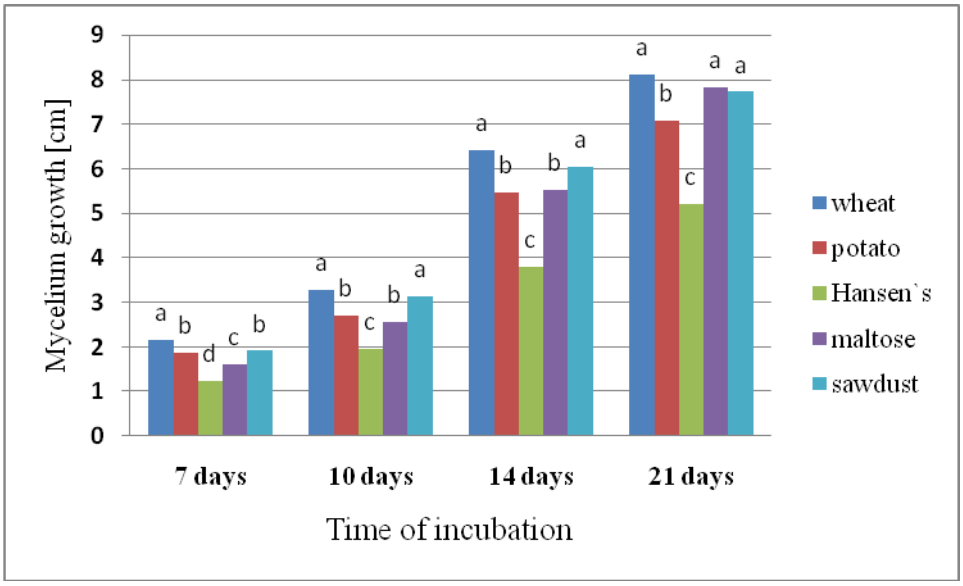


Figure 2. The growth of mycelium of *P. cystidiosus* on various agar media

Comparison of mycelium growth of two strains of *Pleurotus cystidiosus* on five various agar media are presented in Table 1. It has been shown that growth of mycelium of two strains – B122 and B1 – on different agar media was various.

Table 1. Growth of mycelium of two strains of *P. cystidiosus* on various agar media [cm]

Strain	Agar medium				
	Wheat	**Potato**	**Hansen`s**	**Maltose**	**Sawdust**
After 7 days of incubation					
B122	1.93 a	1.63 b	0.91 c	1.61 b	1.91 a
B1	2.38 b	2.11 c	1.56 d	1.61 d	1.91 c
After 10 days of incubation					
B122	3.03 b	2.15 d	1.40 de	2.55 c	3.20 a
B1	3.53 c	3.28 d	2.51 e	2.58 e	3.06 de
After 14 days of incubation					
B122	6.05 a	4.45 c	2.45 d	5.05 b	5.98 a
B1	6.78 c	6.46 c	5.16 d	6.00 c	6.08 c
After 21 days of incubation					
B122	7.73 c	6.21 b	3.23 cd	7.16 c	7.91 a
B1	8.50 c	7.95 cd	7.18 d	8.50 c	7.53 d

It has been shown, that myceliumgrowth of strain B122 after 7 days of incubation was the fastest on wheat and sawdust agar media and the growth of mycelium of strain B1 was the fastest on wheat agar medium. After 10 days of incubation the growth of mycelium of strain B122 was the fastest on sawdust agar medium and strain B1 on wheat agar medium. After 14 days of incubation the growth of mycelium of strain B122 was the fastest on wheat and sawdust agar media and the growth of mycelium of strain B1 comparatively fast on all agar media except for the synthetic Hansen agar medium. After 21 days of incubation the growth of mycelium of strain B122 was the fastest on sawdust agar medium and the growth of mycelium of strain B1 on wheat and sawdust agar media.

For the reason the literature for this topic is limited, mycelial growth of *Pleurotus cystidiosus* was compared to the growth of mycelium of other species from the genus *Pleurotus*. According to the Sobieralski et al. (2012) mycelial growth of *Pleurotus eryngii* (DC.:Fr.) Quèl was also the fastest on wheat agar medium. Comparing mycelial growth of various species of oyster mushrooms on potato agar medium and Hansen`s agar medium has been shown, that *Pleurotus eryngii* and *Pleurotus precoce* (Fr.) Quel. grow faster on Hansen`s agar medium. Whereas mycelial growth of *Pleurotus citrinopileatus* (Singer) and *Pleurotus djamor* (Rumph. ex Fr.) Boedijn was fastest on potato agar medium (Gapiński et al. 2007). According to Shen et al. (2005) mycelial growth of *Pleurotus nebrodensis* (Inzengae) Quél. was fastest on potato-corn agar medium.

The mycelium growth depends on many factors, among others genus, species, variety and agar medium this was also confirmed in research made by Ziombra (1998).

CONCLUSION

Strain B1 of *Pleurotus cystidiosus* due to faster growth of the mycelium shows a greater ability to be utilised in large scale cultivation, where it is important to obtain of mother spawn quickly.

Wheat and sawdust agar media due to faster mycelium growth of *Pleurotus cystidiosus* shows a greater ability to be used in commercial cultivation.

Strain B1 of *Pleurotus cystidiosus* showed the fastest growth on wheat agar medium and next on sawdust agar medium.

Strain B122 of *Pleurotus cystidiosus* showed fastest growth on sawdust agar medium and next on t wheat agar medium.

REFERENCES

Abdullah N., Ismail S., Aminudin N., Shuib A., Lau B. (2012). Evaluation of Selected Culinary-Medicinal Mushrooms for Antioxidant and ACE Inhibitory Activities. Evidence-Based Complementary and Alternative Medicine, Vol. 2012, Article ID 464238, 1-12.

Bao D., Kinugasa S., Kitamoto Y. (2004). The biological species of oyster mushrooms from Asia based on mating compatibility tests. Journal of Wood Science, 50(2), 162-168.

Bernaś E., Jaworska G., Lisiewska Z. (2006). Edible mushrooms as a source of valuable nutritive constituents. Acta Sci. Pol., Technol. Aliment. 5(1), 5-20.

Cohen R., Persky L., Handar Y. (2002). Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Appl Microbiol Biotechnol. 58, 582-94.

Croan S. (2004). Conversion of conifer wastes into edible and medicinal mushrooms. Forest Products Journal, 54(2), 68-76.

Gapiński M., Woźniak W., Ziombra M., Murawska J. (2007). Oyster mycelium on the liquid medium. Acta Mycol. 42(1), 125-128.

Hanelt P. (2001). Mansfeld's Encyklopedia of Agricultural and Horticular Corps. Springer-Verlag Berlin Heidelberg, 29-30.

Kalbarczyk J., Radzki W. (2009). Uprawiane grzyby wyższe jako cenny składnik diety oraz źródło substancji aktywnych biologicznie (Cultivated mushrooms as a valuable diet constituent and a source of biologically active substances). Herba Polonica, vol. 55, no. 4, 224-232.

Karaman M., Jovin E., Malbasa R., Matavuly M., Popovic M. (2010). Medicinal and edible lignicolous fungi as natural sources of antioxidative and antibacterial agents. Phytother. Res. 24 (10), 1473-1481.

Lau C., Abdullah N., Shuib A. (2013). Novel angiotensin I-converting enzyme inhibitory peptides derived from an edible mushroom, *Pleurotus cystidiosus* OK Miller identified by LC-MS/MS. BMC Complementary and Alternative Medicine, 13(1), 1-10.

Lechner B., Wright J., Albertó E. (2004). The genus *Pleurotus* in Argentina. Mycologia, 96(4), 845-858.

Manzi P., Pizzoferrato L. (2000).β-glucans in edible mushrooms. Food Chem.,68(3), 315-318.

Moncalvo J. (1995). *Pleurotus cystidiosus* var. *formosensis* var. nov.: an unusual *Pleurotus* collection of subgenus Coremiopleurotus from Taiwan. Mycal. Res. 99(12), 1479-1482.

Patel Y., Naraian R., Singh V. (2012). Medicinal properties of *Pleurotus* species (oyster mushroom): a review. World Journal of Fungal and Plant Biology 3(1), 01-12.

Petersen R., Nicholl D., Hughes K. (1997). Mating systems of some putative polypore-agaric relatives. Plant Syst Evol 201, 135-158.

Rajewska J., Bałasińska B. (2004). Związki biologicznie aktywne zawarte w grzybach jadalnych i ich korzystny wpływ na zdrowie (Biologically active compounds of edible mushrooms and their benefi cial impact on health). Postępy Higieny i Medycyny Doświadczalnej, tom 58, 352-357.

Selvakumar P., Rajasekar S., Periasamy K., Raaman, N. (2008). Isolation and characterization of melanin pigment from *Pleurotus cystidiosus* (telomorph of *Antromycopsis* macrocarpa). World Journal of Microbiology and Biotechnology, 24(10), 2125-2131.

Shen J., Guo H., Cheng Y., Wei X., Guo G., Liu G., Jia S. (2005). The exploitation and cultivation of *P. nebrodensis* in China. In: Q. Tan (eds.). Proceedings 5th International Conference on Mushroom Biology and Products, Acta Edulis Fungi, 354-359.

Siwulski M., Sobieralski K., Jędryczka M. (2013). *Ganodrma lucidum* – znaczenie, substancje biologicznie czynne i właściwości lecznicze (*Ganoderma lucidum* – importance biologically active substances and medicinal properties). In: A. Kwieciński, M. Siwulski (eds.). Reishi *Ganodrma lucidum* – właściwości lecznicze (Reishi *Ganoderma lucidum* – medicinal properties). Wyd. Uniwersytetu Przyrodniczego w Poznaniu, Centrum Innowacji Transferu Wiedzy i Technologii NeoMED 3B w Bielsku Białej, 11-35.

Siwulski M., Sobieralski K., Sas-Golak I. (2014). Wartość odżywcza i prozdrowotna grzybów (Nutritive and health-promoting value of mushrooms). Żywność. Nauka. Technologia. Jakość, 1(92), 16-28.

Siwulski M., Sobieralski K. (2004). Uprawa grzybów jadalnych i leczniczych w warunkach naturalnych (Cultivation of edible and medicinal mushrooms in natural conditions). Wyd. Kurpisz, Poznań, s. 158.

Sobieralski K., Siwulski M., Lisiecka J., Sas-Golak I., Jasińska A., Nowak-Sowińska M. (2012). *Pleurotus eryngii* – mało znany, wartościowy gatunek boczniaka (*Pleurotus eryngii* – little known, valuable species of oyster mushroom). Kosmos, 61(4), 647-656.

Thekkuttuparambil A., Kainoor K. (2007). Indian medicinal mushrooms as a source of antioxidant and antitumoragents. J. of Clinical Biochemistry and Nutrition 40 (3), 157-162.

Vilgalys R., Moncalvo J., Liou S., Volovsek M. (1996). Recent advances in molecular systematics of the genus *Pleurotus*. Mushroom Biology and Mushroom Products, 91-101.

Usami A., Motooka R., Nakahashi H., Okuno Y., Miyazawa M. (2014). Characteristic odorants from bailingu oyster mushroom (*Pleurotus eryngii* var. *tuoliensis*) and summer oyster mushroom (*Pleurotus cystidiosus*). Journal of Oleo Science, 63(7), 731-739.

Wasser S. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology 60, 258-274.

Zervakis G. (1998). Mating competence and biological species within the subgenus *Coremiopleurotus*. Mycologia 90, 1063-1074.

Zervakis G., Balis C. (1996). A pluralistic approach in the study of *Pleurotus* species with emphasis on compatibility and physiology of the European morphotaxa. Mycological Research, 100(6), 717-731.

Zervakis G., Moncalvo J., Vilgalys R. (2004). Molecular phylogeny, biogeography and speciation of the mushroom species *Pleurotus cystidiosus* and allied taxa. Microbiology, 150(3), 715-726.

Ziombra M. (1998). Wpływ niektórych czynników na wzrost grzybni i plonowanie boczniaka (The effect of some factors on the growth of mycelium and yielding of oyster mushroom). Rozprawy Naukowe AR Poznań, 278, 75.

DOI: 10.17707/AgricultForest.63.1.08

Marta POGRZEBA, Szymon RUSINOWSKI, Jacek KRZYŻAK[1]

MACROELEMENTS AND HEAVY METALS CONTENT IN *PANICUM VIRGATUM* CULTIVATED ON CONTAMINATED SOIL UNDER DIFFERENT FERTILIZATION

SUMMARY

Heavy metal contamination of soils is a major problem worldwide. As a result, arable land polluted with heavy metals is unsuitable for food production. Utility of energy crops which allow the commercial exploitation of these soils by establishing biofuel feedstock production systems can offer a solution. Additionally, plant cultivation offers opportunities for site remediation.

Field experiments have been performed on heavy metal contaminated arable soil located in southern Poland, in the vicinity of a former smelting factory. Although heavy metal concentration exceeded standards, the area has been used for agricultural purposes. Experiments involved testing Switchgrass (*Panicum virgatum*) cultivated with standard NPK fertilizers and commercially available microbial inoculum. Biomass water, macronutrients (N, P, K, Mg, Ca) and heavy metal (Cd, Pb, Zn) content in aboveground plant organs were assessed at the end of two growing seasons.

Switchgrass biomass water content was higher after the second year for nearly 40%. Additionally, after the first as well as the second year fertilizers increased it. Magnesium content, essential in chlorophyll biosynthesis, was higher in the first year and additionally more evident in fertilized variants after every year. Heavy metals accumulation in aboveground organs was lower after the second year compare with the first year. Similar trend was observed for Ca and N plant accumulation. However P and K accumulations were higher after the second year of experiment.

In conclusion, due to acclimatization, switchgrass reduce heavy metal uptake, what could result in increase of two biogenic elements (P, K) essential in plant growth.

Keywords: *Switchgrass, Heavy metals, Macronutrients, Inoculum, Biomass composition.*

INTRODUCTION

Heavy industry (*i.e.*, smelters, coal mine) is main emitter of heavy metals (HMs) to the environment. The most dangerous directly for human health is spreading HMs to vicinity agricultural areas where it can be introduce to food

[1]Marta Pogrzeba (corresponding author: mag@ietu.katowice.pl), Szymon Rusinowski, Jacek Kryżak Institute for Ecology of Industrial Areas, Katowice, POLAND

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

chain by accumulation in consumed plant organs (Järup, 2003). Such contaminated arable lands should be excluded from agricultural production. The alternative for such production can be establishing biofuel feedstock production systems on those areas. Except biomass production for energy purposes there is additional benefit in those land management what protect soil from erosion (Meers*et al.*, 2010). Mineral macro- and micronutrients are essential in plant development and proper growth of plants is undeniable depends on its content in soil. Mineral macronutrients are need for plants in higher amount in comparison to micronutrients. Mineral macronutrients can be divided in to two groups: primary mineral macronutrients (N, P, K) and secondary mineral macronutrients (Ca, Mg, Fe, S) (Tripathi *et al.*, 2014). There are reports indicates negative impact of HM on macronutrients accumulation in above ground organs in agricultural plant as well as in plant cultivated for medical purposes (Bello *et al.*, 2004, Siedlecka, 2014). There is a dearth of papers concerning on macronutrients status in energy crop cultivated HMs contaminated soils. *Panicum virgatum* (Switchgrass) is C4 photosynthesis perennial grass belongs to second generation energy crops, however it can be also cultivated for feed purposes. It is originated from prairie of Northern America where it is dominant species. Switchgrass due to those features is plant very resistant to drought conditions (Parrish and Fike, 2005).

The aim of the study was to describe relationships in HMs and mineral macronutrients accumulation in fertilized Switchgrass during two growing season (2014 and 2015) after plantation establishment. Plants were treated with standard NPK fertilizer and commercial available microbial inoculum (EmFarma, Probiotics Magdalena Górska, Poland).

MATERIALS AND METHODS

Site description

The trial was established in Bytom (Upper Silesia Region, 50°20'43.0"N 18°57'19.6"E, Poland) on arable land contaminated with HMs. Soil was contaminated over the last century due to dust fall with HMs deposition (Zn, Cd and Pb) emitted by already non-existent Zn/Pb smelter. The climate conditions at the site are moderate, with monthly average temperature and precipitation presented in Figure 1 (based on Institute of Meteorology and Water Management data).

Experiment design

Switchgrass plots were established at the beginning of May 2014 from the seedlings obtained from seeds, pre-cultivated in growing room. On each of the three plots 49 plants were planted with density of 3plants per 1 m^2. Due to high soil homogeneity (Tab. 1) and apprehension of uncontrolled fertilizer application pseudo-replication were performed. On each plot four section were distinguish (Fig. 2): edge plants excluded from further analysis(i) and three section (pseudo-replication) from which samples were taken(ii). Each plot was treated in a different way and 4 meter buffer zone between each experimental plot was set:

•**P - C** - control (no treatment);
•**P - NPK** - NPK standard fertilization, applied directly to the soil once before planting (nitrogen 70 kg ha^{-1}, phosphorus 30 kg ha^{-1} as P_2O_5 and potassium 45 kg ha^{-1} as K_2O);
•**P - INC** - commercial microbial inoculum (EmFarma, ProBioticsPolska Magdalena Górska, Poland) applied on seedlings roots before plantation and on the leaves as aerosol in the middle of every month during the growing seasons (from May to September 2014 and 2015).
Initial soil samples were collected before planting, one from each plot sections. The plant samples for HMs and mineral macronutrients analysis were collected in the middle of October each year: one sample were collected from the middle plot section and two samples were collected from upper and down plot section.

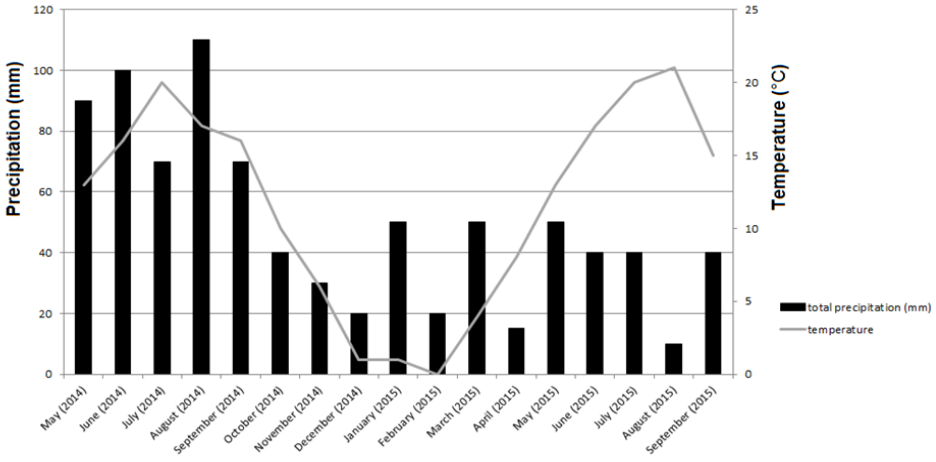


Figure 1. Meteorological data for growing seasons 2014-2015

Soil and plant samples analysis

Soil pH was measured in H_2O (1:2.5 m/v) with a combination glass/calomel electrode (OSH 10-10, METRON, Poland) and a pH-meter (CPC-551, Elmetron, Poland) at 20°C.
The conductivity was determined by an ESP 2ZM electrode (EUROSENSOR, Poland) according to the Polish norm PN-ISO 11265:1997.

Soil texture was evaluated by the hydrometric method according to the Polish norm PN-R-04032:1998.

The total content of metals in the soil and plant tissues was obtained using hot plate digestion (HNO_3 and $HClO_3$, ratio 4:1) and flame atomic absorption spectrometry (SpektrAA 300, Varian INC., USA).
Total nitrogen content (N) in plant (PB.06 edition 1 from 2011.09.01) and soil (ISO 13878:1998), available phosphorus (P) in plant (PB.03 edition 1 from 2011.09.01) and soil (PN-R-04023:1996) as well as potassium (K) content in

plant (PB.01 edition 1 from 2011.09.01) and soil (PN-R-04022:1996) samples was assessed in Institute for Ecology of Industrial Areas laboratory.

Soil organic matter (OM) was measured by loss on ignition as follows: air dry soil was sifted to pass a 2 mm sieve, dried at 105°C for 24 h and then (5 g) treated with 550°C for 4 h. Soil organic carbon (C-org) was assessed according to PN-ISO 14235:2003.

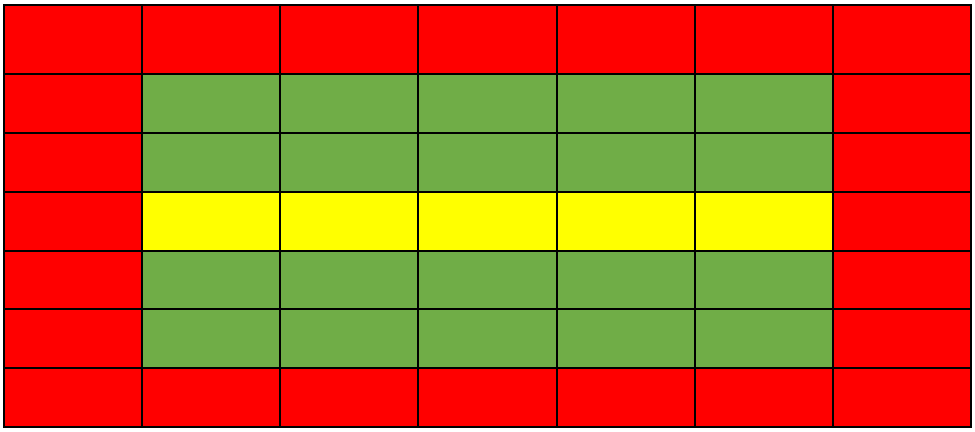


Figure 2. Section on plot distinguished for sample collection. Red - section excluded from sampling, green – section where two samples were collected, yellow – section where one sample was collected

Statistical analysis

Data were analyzed using a one-way ANOVA, followed by a *post hoc* comparison using the Fisher LSD test ($P < 0.05$). Statistical analyses were performed using Statistica 10 (Statsoft, USA). Spider charts were constructed using Excel MS Office (Microsoft, USA) and standardization of data for charts construction were performed using Statistica 10 Software (Statsoft, USA).

RESULTS AND DISCUSSION

Results of soil analyses indicate that among experimental plots there were no significant differences between measured parameter, except Zn (Tab. 1). Soil Zn content was slightly higher on P – INC plot when compare to control. There were no significant differences between P – NPK plot and P – INC plot. Soil texture on the experimental field was classified as silty loam. According to obtained results and regulation of Polish law (D.2002. r.165 poz.1369) soil HMs content exceed the limits defined by government regulation. In this case experimental area should be classified as marginal, moreover whole agriculture production should be abandoned (Gopalakrishnan *et al.*, 2011). Spider charts (Fig. 3) were used as a tool for assess values pattern changes of measured parameters in above ground organs of Switchgrass among different treatments, as well as annual changes for two growing seasons (2014 and 2015). Spider charts can be divided in to three representative sections: water with secondary

macronutrient content (Ca, Mg) (1), primary macronutrients content (N, P, K) (2) and HMs content (Zn, Cd, Pb) (3). Overall, the highest HMs accumulation in above ground organs were found for control plants and it was conditioned by Cd accumulation in the first growing season. The lowest values of Mg and Ca for control plants could be associated with that highest HMs uptake. Hermans *et al.* (2011) reported that in *Arabidopsis thaliana* old leaves the content of Cd was lower forplants treated with Cd and Mg, when compare to plant treated only with Cd. It can be assumed with agreement to presented results that higher Mg content can reduce Cd accumulation in elder plants. It is well known that magnesium plays significant role in chlorophyll biosynthesis due to this is essential for photosynthesis process (Cakmak, 2014). Other measured parameters were equal among each treatment in the first growing season. In the second growing season it was found, that there is similar trend among treatments for Mg and Ca accumulation, however it was found that those parameter values are lower when compare to the second growing season.

Table 1. Soil physico-chemical characteristic, heavy metal content and macronutrients content in initial soil samples

	Soil physico-chemical characteristic				
	pH (H_2O)	pH (KCl)	EC ($\mu S\ cm^{-3}$)	OM (%)	C-org (%)
P - C	6.49 ± 0.03a	5.97 ± 0.05a	89.92 ± 1.41a	5.39 ± 0.02a	2.18 ± 0.03a
P - NPK	6.57 ± 0.04a	6.06 ± 0.07a	82.28 ± 2.80a	5.52 ± 0.06a	2.11 ± 0.03a
P - INC	6.57 ± 0.03a	6.12 ± 0.02a	89.33 ± 2.89a	5.44 ± 0.06a	2.10 ± 0.01a
	Soil heavy metals content				
	Pb ($mg\ kg^{-1}$)		Cd ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	
P - C	514.77 ± 14.26a		17.94 ± 0.05a	1659.50 ± 7.22b	
P - NPK	487.30 ± 3.18a		18.06 ± 0.05a	1700.00 ± 6.35ab	
P - INC	496.50 ± 4.33a		18.02 ± 0.11a	1750.33 ± 34.04a	
	Soil primary mineral macronutrients content				
	N_{total} (%)		P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	
P - C	0.15 ± 0.00a		834.30 ± 6.64a	950.30 ± 17.32a	
P - NPK	0.15 ± 0.00a		833.20 ± 8.66a	970.60 ± 1.15a	
P - INC	0.15 ± 0.00a		835.87 ± 15.90a	1014.27 ± 24.62a	
	Soil secondary mineral macronutrients content				
	Fe ($mg\ kg^{-1}$)		Mg ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	
P - C	9854.50 ± 44.74a		1557.00 ± 30.60a	3015.00 ± 49.07a	
P - NPK	10135.00 ± 13.28a		1592.00 ± 4.04a	2998.50 ± 3.17a	
P - INC	10219.00 ± 164.93a		1669.33 ± 51.49a	3185.17 ± 98.48a	

P – *Panicum virgatum*, C – control plot; NPK – NPK fertilized plot, INC – microbial inoculated plot. Values are means ± SE (n=3). A lower case letters (a, b, c, d) denotes significant differences among soils samples taken from different plots at $P \leq 0.05$ according to Fisher LSD test.

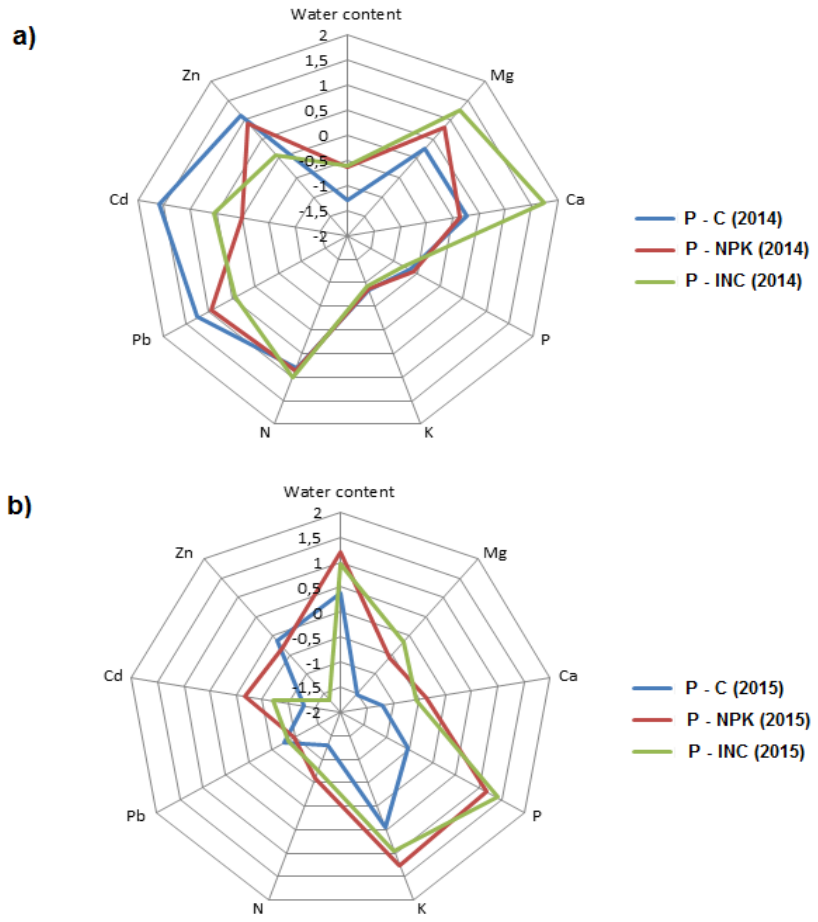


Figure 3. Spider charts constructed on macronutrients, heavy metals and water content show patterns indicated changes of those parameters; among treatment (**C** – control, **NPK** – NPK fertilizer treated plant, **INC** – microbial inoculum treated plant) and growing season (a-**2014** and b-**2015**) for *Panicum virgatum*(**P**). **Mg, Ca, P, K, N, Pb, Cd, Zn** – elements accumulation in above ground plants organs. For better data visualization all presented values were standardized. Each measurement was performed in 5 replicate (n = 5)

It also has been found that there is overall decrease in accumulation of HMs and N for plants from each plot, except Cd accumulation in NPK treated plants when compare to the first growing season. Increased K concentration in plant samples was found for each variant and P was found to be increased on each plot treated with fertilizer, when compare to the first growing season. Gonçalves *et al.* (2009) reported, that higher Cd concentration in hydroponic solution can decrease K uptake to above ground organs. This paper is with agreement with obtained results, however the tendency is only contrary to NPK treated plants. Water content in plant stems was 40% higher for plant in the

growing season 2015 when compare to the growing season 2014 and treatments slightly increased it. This phenomena can be associated with ability to water capture, which is correlated to root distribution. It is known that perennial grasses root system become fully developed after 4th growing season (Ferchaud *et al.*, 2015). In this case water content could be increased due to annual increase of roots density. Spider charts with description corresponds to overall annual and treatment changes in biomass HMs, water and mineral macronutrient content. Its allows to track changes in tendencies while detailed statistical analysis for measured parameters is presented in Table 2.

Table 2. Matrix of statistical significant differences among analyzed parameters presented on spider charts (Fig. 2)

	P-C (2014)	P-NPK (2014)	P-INC (2014)	P-C (2015)	P-NPK (2015)	P-INC (2015)
Water content	b	b	b	a	a	a
Mg	bc	ab	a	e	d	cd
Ca	ab	ab	a	b	ab	ab
P	b	b	b	b	a	a
K	b	b	b	a	a	a
N	a	a	a	b	b	b
Pb	a	a	b	c	c	c
Cd	a	bc	ab	c	bc	bc
Zn	a	a	a	ab	ab	b

P – *Panicum virgatum*, **C** – control; **NPK** – NPK fertilized plants, **INC** – microbial inoculated plants.

A lower case letters (a, b, c, d, e, f – where "a" corresponds to the highest value and "f" to the lowest) denotes significant differences among plants elements and water content taken from different plots at $P \leq 0.05$ according to Fisher LSD test. Each measurement was performed in 5 replicate (n = 5)

CONCLUSION

It could be concluded that HMs in soil affect mineral macronutrients status in *Panicum virgatum*, especially Mg and K. Additionally there are differences mostly in each measured parameters when compare growing seasons. It can be associated with acclimatization of plant to contaminated site. There is a dearth of papers concerning mineral nutrients as well as HMs status in the first two years after establishment, because most of them are focused on fully establishment trials, where perennial energy crops achieves its maturity after 3rd -4th year after establishment.

ACKNOWLEDGEMENT

Research financed by the PHYTO2ENERGY project – 7FP EU, Grant Agreement No. 610797 and IETU statutory funds by Polish Ministry of Science and Higher Education.

The authors wish to extend their appreciation to dr Włodzimierz Majtkowski from Plant Breeding and Acclimatization Institute, National Research Institute (Poland), for providing seeds of *Panicum virgatum*.

REFERENCES

Bello, M. O., Ibrahim, A. O., Ogunwande, I. A., Olawore, N. O. 2004. Heavy trace metals and macronutrients status in herbal plants of Nigeria. Food Chemistry 85, 67-71.

Cakmak, I. 2014. Major functions of calcium and magnesium in crop plants. In World Fertilizer Congress , 16, 30-32.

D.2002. nr.165 poz.1369 – Regulation of the Polish Ministry of Environment on Soil and Ground Standards (in Polish).

Ferchaud, F., Vitte, G., Bornet, F., Strullu, L., Mary, B. 2015. Soil water uptake and root distribution of different perennial and annual bioenergy crops. Plant and Soil, 388, 307-322.

Gonçalves, J. F., Antes, F. G., Maldaner, J., Pereira, L. B., Tabaldi, L. A., Rauber, R., Rossato, L. V., Bisognin, D. A., Dressler, V. L., de Moraes Flores, E. M., Nicoloso, F. T., 2009. Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions.Plant Physiologyand Biochemistry. 47, 814-821.

Gopalakrishnan, G., Cristina Negri, M., and& Snyder, S. W. 2011. A novel framework to classify marginal land for sustainable biomass feedstock production.Journal of environmental quality, 40, 1593-1600.

Hermans, C., Chen, J., Coppens, F., Inzé, D., Verbruggen, N. 2011. Low magnesium status in plants enhances tolerance to cadmium exposure. New phytologist, 192, 428-436.

ISO 13878:1998 - Soil quality - Determination of total nitrogen content by dry combustion ("elemental analysis")

Järup, L. 2003. Hazards of heavy metal contamination.British medical bulletin, 68, 167-182.

Meers, E., Van Slycken, S., Adriaensen, K., Ruttens, A., Vangronsveld, J., Du Laing, G., Witters, N., Thewys, T., Tack, F. M. G. 2010. The use of bio-energy crops (*Zea mays*) for 'phytoattenuation'of heavy metals on moderately contaminated soils: a field experiment. Chemosphere, *78*, 35-41.

Parrish, D. J.and Fike, J. H. 2005.The biology and agronomy of switchgrass for biofuels. BPTS, 24, 423-459.

PB.01 edition 1 from 2011.09.01 – Potassium content, Flame photometry method (in Polish)

PB.03 edition 1 from 2011.09.01 – Phosphorus content, Spectrophotometric method (in Polish)

PB.06 edition 1 from 2011.09.01 - Total nitrogen content (Kjeldahl), Titration method (in Polish)

PN ISO 14235:2003 Soil Quality - Determination Of Organic Carbon By Sulfochromic Oxidation

PN-ISO 11265:1997 -Soil quality - Determination Of Electrical Conductivity

PN-R-04022:1996 - Assessment of assimilable potassium content in mineral soils (in Polish)

PN-R-04023:1996 - Assessment of available phosphorus content in mineral soils (in Polish)

PN-R-04032:1998 – Soil and mineral pieces – Soil sampling and texture assessment (in Polish)

Siedlecka, A., 2014. Some aspects of interactions between heavy metals and plant mineral nutrients.ActaSocietatisBotanicorumPoloniae 64, 265-272.

Tripathi, D. K., Singh, V. P., Chauhan, D. K., Prasad, S. M., Dubey, N. K., 2014. Role of macronutrients in plant growth and acclimation: recent advances and future prospective, in: Ahmad, P., Wani, M. R., Azooz, M.M., Tran, L.-S. P. (Eds.), Improvement of Crops in the Era of Climatic Changes. Springer, New York, pp. 197-216.

DOI: 10.17707/AgricultForest.63.1.09

Anatoly SMYKOV, Yuriy IVASHCHENKO,
Iuliia IVASHCHENKO, Olga FEDOROVA[1]

PHOTOSYNTHETIC ACTIVITY OF PEACH LEAF IN CONNECTION WITH DROUGHT TOLERANCE

SUMMARY

The aim of our research was to study the leaf chlorophyll fluorescence parameters of peach and selection of cultivars with increased resistance to drought. Studies were conducted in the laboratory on intact leaf plates of 8 cultivars of foreign selection (Veteran, Zempush, Gavazuri, Tszyu-Yus-Tszyuy, Hidistavsky Belyiy, Baby Gold-7, Pintu, Favorita Morettini) in two periods - August and September. The control cultivar was Kryimsky Shedevr of Nikitska Botanical Garden selection.

Photosynthetic activity was characterized by chlorophyll fluorescence parameters (Kautsky effect). It was established that, as a result of 24-hour water deficit parameter, Fm operates more stable in cultivars Hidistavsky Belyiy, Tszyu-Yus-Tszyuy and Baby Gold-7. More prone to dehydration were peach cultivars Zempush and Pintu. Suppression index have reached 21-22%. High stability parameter Fm combined with a high water-holding capacity was found in grade Tszyu-Yus-Tszyuy. The leaves of this cultivar have lost least amount of moisture by dehydration (45%). On an indicator of $(F_m-F_0)/F_m$ the highest value was recorded in cultivar Pintu. Cultivars Pintu, Gavazuri and Tszyu-Yus-Tszyuy are the best according to the indicators $(F_m -F_{st})/F_m$ and (F_m/F_{st}). Their photoactivity is 10-12% higher in comparison with other cultivars and control cultivar Kryimsky Shedevr. They are characterized by a high water-holding capacity and a significant degree of recovery turgidity of tissues (82-100%). Cultivars Hidistavsky Belyiy, Veteran are promising for further study. Some cultivars have a high photoactivity at various stages of photosynthesis (Zempush, Baby Gold-7), indicating a wide range of applications in order to diagnose the state of the photosynthetic apparatus in fluorimetry method of dehydration of the leaves.

Keywords: *Peach, cultivars, drought resistant, parameters fluorescence*

INTRODUCTION

Peaches (*Persica vulgaris* Mill.) - one of the most popular fruit crops in the south of Russia. This is due to its early appearance of fruit, high yield

[1]Anatoly Smykov (corresponding author: fruit_culture@mail.ru), Yuriy Ivashchenko, Iuliia Ivashchenko, Olga Fedorova Nikita Botanical Gardens – Federal Reseach Centre of RAS, Yalta, RUSSIAN FEDERATION

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

potential, outstanding flavor, dietary and medicinal properties of fruits, their suitability for different types of processing. Crimea refers to insufficient moisture zone with a large deficit of irrigation water in the summer, so the creation of new drought-resistant varieties - a very important issue for the development of peach culture.

The parameters of the water regime, which determine the degree of drought resistance, are closely related to leaf apparatus performing photosynthesis function. Violation of the functional state of the photosynthetic apparatus, which occurs under the influence of the arid conditions of the environment, expressed in leaf tissue damage and reduced yields of peach trees (Smykov*et al*., 2001; Kershanskaya, 2007; Ivashchenko, 2008).

Photosynthesis and fluorescence are interrelated processes and reflect the efficiency of the distribution of the absorbed light energy (Bilger*et al*., 1990). Disturbances in the process of photosynthesis, cause characteristic changes in the fluorescence intensity: the less energy used in photochemical processes, the higher the level of fluorescence (Brionet al., 2000; Stirbet, 2011.).

The study of photosynthetic processes based on the phenomenon of fluorescence allows to estimate the resistance of plants to drought at the subcellular level, operation of the of chlorophyll-protein complexes under the action of high temperatures, insufficient moisture and wind drying up in the summer.

It is also important in practical and methodical purposes to establish the influence of a complex of conditions that lead to a deficiency of moisture in the tissues of the leaf plates, on photoactivity of a leaf.

The aim of our study was to investigate the influence of extreme conditions of summer period to the change of chlorophyll fluorescence parameters of peach leaves and selection of cultivars with improved drought tolerance.

MATERIALS AND METHODS

Studies conducted in laboratory conditions on intact leaf plates of 8 peach cultivars (Veteran, Zempush, Gavazuri, Tszyu-Yus-Tszyuy, Hidistavsky Belyiy, Baby Gold-7, Pintu, Favorita Morettini) different ecological and geographical origin. The plants grew on peach collection sites of Nikita Botanical Garden, Yalta, Crimea. Samples of leaves were collected in the first week of August and September 2015. During the research observed hydrothermal stress in plants peach. The average daily air temperature was 27.7°C (August) and 28.5°C (September), which is 4.3-5.1 °C above normal, the maximum reached – 35.6 °C (Table 1).

Air humidity decreased to 20% and 28%. During the first week were fell 7.9 mm precipitation in August and 24.8 mm – in September. The reserves of productive moisture in a meter layer of soil under the peach amounted to 26 and 25 mm. Maximum soil surface temperature was raised to 58 °C and 56.2 °C.

Table 1.Agrometeorological parameters of the experiment in August-September, Yalta, 2015.

Agrometeorological parameters	August	September
	the first week	the first week
The average air temperature	27.7°C	28.5°C
The maximum air temperature	35.6°C	34.8°C
The amount of rainfall	7.9 mm	24.8 mm
Minimum humidity	20.0%	28.0%
Maximum temperature of the soil	58.0°C	56.2°C
The stock of productive moisture in a meter layer of soil	26.0mm	25.0mm

Indicators of the water content in the leaves, the moisture loss in the course of wilting and ability to restore turgor determined according to generally accepted guidelines (Lishchuk *et al*., 1991, 1999). Changes in fluorescence intensity were carried out on a portable fluorometer "Floratest". Leaves were taken in triplicate of each cultivar and before measuring fluorescence parameters adapted to the dark for 8 minutes. In the spectral range of operation of photosynthetically active forms of chlorophyll (690 nm) was recorded multicomponent photo induction fluorescence curve (Kautsky effect), which proved to be very informative in studying drought resistance of peach plants (Buschmann, 1986; Krause, Weis, 1991). We used the following parameters: F_0 - background or a minimum fluorescence level excited by very low intensity of measuring light to keep PS II reaction centers open; F_m - maximum fluorescence level meets both the beginning of producing photosynthetic processes, CO_2 fixation and activation of enzymes of the Calvin cycle; F_{st} - stationary level of fluorescence, indicating on establishing a stable and most intense level of photosynthesis; F_v - variable fluorescence, corresponding to the difference between the maximum and minimum levels (F_m-F_o), which determines the ability of the chlorophyll apparatus to the photosynthesis; F_v/F_o - the parameter has been related tothe maximum and effective photochemical quantum yield of PSII; (F_m-F_{st})/F_m – coefficient of fluorescence induction; F_m/F_{st} - fluorescence decrease rate (Brion *et al.*, 2000; Korneev., 2002). Changing the photoactivity of the studied cultivars were presented in comparison with the control cultivar Kryimsky Shedevr of Nikitska Botanical Garden selection.

RESULTS AND DISCUSSION

The photosynthetic activity of the leaves is made up of a number of different quality stages of photosynthesis. Usually the steps forming of the primary processes of photosynthesis, including capture or absorption of light energy of pigment-protein complexes (parameter F_m), the transfer of energy to the primary electron acceptors, which characterizes the efficiency index (F_m-F_0)/F_m).

Table 2. Change of leaf fluorescence indicators of introduced cultivars of peach in different conditions of water regime, August 2015.

Peach cultivars, the conditions of the water regime	**Changes in fluorescence indicators rel. un**						**The total water content, %**	**The loss of water in the leaves, %**	**Leaves restore. turgor, %**
	F_0	F_m	F_{st}	F_v	$\frac{F_m - F_{st}}{F_m}$	$\frac{F_m}{F_{st}}$			
Kryimsky Shedevr (control)									
After saturation	485	2085	507	1600	1.01	4.11			
After 24h moisture loss	592	1493	1184	901	0.21	1.26	46.7	52.6	
After the restoration of turgor	352	544	384	192	0.29	1.42			63.5
Veteran									
After saturation	421	1723	475	1302	0.72	3.63			
After 24h moisture loss	485	1195	1045	710	0.13	1.14	49.0	54.2	
After the restoration of turgor	475	1398	712	689	0.49	1.96			55.0
Zempush									
After saturation	475	2229	528	1754	0.76	4.22			
After 24h moisture loss	736	1765	1029	1029	0.41	1.72	49.0	48.9	
After the restoration of turgor	528	2224	512	1696	0.77	4.34			60.0
Tszyu-Yus-Tszyuy									
After saturation	459	2213	576	1754	0.74	3.84			
After 24h moisture loss	667	1963	832	1296	0.58	2.36	50.0	44.8	
After the restoration of turgor	640	1973	667	1333	0.66	2.96			97.5
Hidistavsky Belyiy									
After saturation	592	2277	603	1685	0.74	3.78			
After 24h moisture loss	693	2186	1472	1493	0.33	1.49	48.6	49.9	
After the restoration of turgor	673	1973	709	1300	0.64	2.78			85.0
Pintu									
After saturation	456	2336	552	1880	0.76	4.23			
After 24h moisture loss	768	2976	795	2208	0.73	3.74	54.2	49.6	
After the restoration of turgor	564	2399	603	1834	0.75	3.97			100.0
Baby Gold-7									
After saturation	459	1920	475	1461	0.75	4.04			
After 24h moisture loss	565	1664	1221	1099	0.27	0.27	43.8	50.8	
After the restoration of turgor	389	1227	796	741	0.35	1.54			48.0
Favorita Morettini									
After saturation	395	1637	405	1242	0.75	4.04			
After 24h moisture loss	581	1211	1034	630	0.15	1.17	42.7	58.8	
After the restoration of turgor	467	1104	537	637	0.51	2.05			43.0

Further processes determining the photoactivity associated with the functioning of the dark, photochemical reactions of photosynthesis (index (F_m - F_{st}) /F_m), and conjugation processes of absorption and transmission of light energy to process of disposal (F_m/F_{st}).For leveling conditions of wilting the leaf blade applied approach proposed by Lishchuk *et al*. consisting in complete saturation of the material up to 100% humidity. In the process of the research revealed that the leaves of studied varieties in 24 hours of wilting lost by 42.7 to 54.2% of moisture. Under these parameters, moisture loss, restoring turgor of leaves ranged from 43.0% (Favorita Morettini) to 100.0% (Pintu).

Table 2. presents data on changes in indicators photoactivity introduced cultivars of peach (fluorometric indicators and their ratio) under different drainage regimes. From 8 introduced cultivars of peach belonging to different ecotypes, were identified in a row of promising indicators of photoactivity.

Comparative analysis of the value of the pool complexes of light trapping (F_m), shows that the cultivar characteristics are appear significantly. Light trapping structure of the most stable functioning the varieties Hidistavsky Belyiy, Tszyu-Yus-Tszyuy and Baby Gold-7. As a result of 24 hours of dehydration they occurred the decline of index within the 4-13%. The greatest decrease F_m, within the limits of 21-22% was observed in cultivars Zempush and Pintu.

A strong dehydration and unstable functioning of light trapping complexes appeared in the cultivars Kryimsky Shedevr, Favorita Morettini and Veteran. In the cultivar Kryimsky Shedevr observed decline F_m by 28% Favorita Morettini by 26% at the cultivar Veteran by 30%. More importantly these cultivars is reduced variable fluorescence (F_v). In conditions of dewatering its index decreased by 44-49%.In the context of itsof dehydration performance decreased by 44-49%. Within 24 hours of dehydration leaves of cultivars Kryimsky Shedevr, Favorite Morettini and Veteran also lost more than half of the original moisture content of the leaf. Less significant was the recovery in turgor characterized cultivars (43-64%). It should be noted that the cultivars of leaves Favorita Morettini after dehydration regained turgor just 43%. This is 20% less than that of the control cultivar Kryimsky Shedevr but photoactivity indicators reduce to 2 times better than the control cultivar. According to the cultivar characteristics, high stability of operation of light-harvesting complexes (F_m), distinguished cultivar Tszyu-Yus-Tszyuy. The leaves of this cultivar have lost an average of 1.3 times less moisture than cultivar weakly resistant to dehydration. The same trend was observed in relation to the indicator variable fluorescence (F_v) and others available to us for research parameters – (F_m- F_{st})/F_m and F_m/F_{st}.

These indicators reflect the effectiveness of the passage of the primary processes of photosynthesis and the level of transformation of light energy into photochemical reactions of the chloroplast membrane systems. It can be assumed that a disturbance of the primary processes of photosynthesis and whole photoactivity as a result of dehydration leaf plate reaches the small size of cultivars Pintu (reduction of 10%), Hidistavsky Belyiy (29% reduction) and Tszyu-Yus-Tszyuy (reduction of 29%).

A more significant change in dehydration indicators of photoactivity was obtained from cultivars Kryimsky Shedevr, Veteran, Favorita Morettini (reduction of 64-67%). How to appear differences between cultivars can be seen in Figure 1, which shows a disturbance of the kinetics of photo induction fluorescence curves (Kautsky effect) native peach leaves as a result of changes in the water regime.

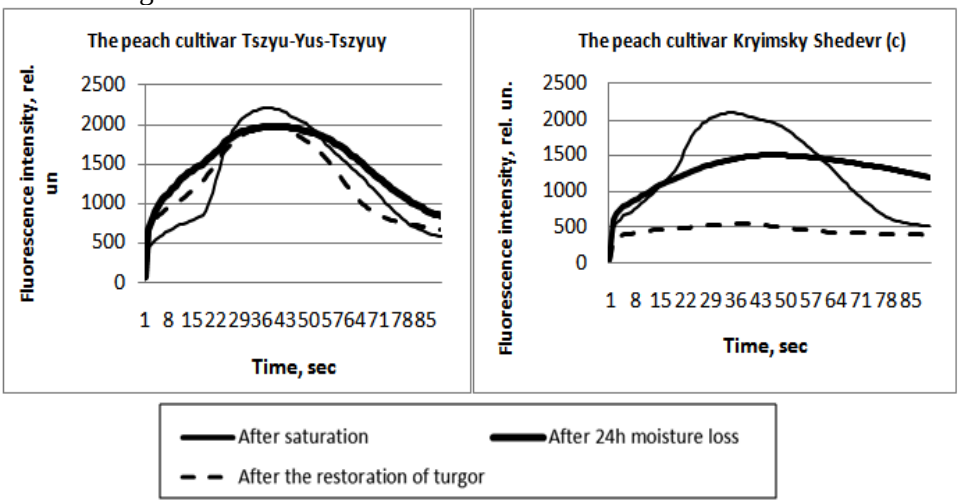


Figure 1.Kinetics photo induction curve of the Kautsky effect in the cultivars of peach Kryimsky Shedevr (control) and Tszyu-Yus-Tszyuyas a result of different conditions of water regime of leaf plates, August, 2015.

Kinetics photoinduction curve reflects all the stages of the primary processes of photosynthesis (Lichtenthaler, 1992) and is a reliable and proven tool to assess plant photoactivity. Photo induction curve at cultivar Tszyu-Yus-Tszyuynon monotonically at all stages of the water regime, but changing its kinetics insignificant. The sharp decrease in the fluorescence intensity and the disturbance of the kinetics of the curve in a state of dehydration and restore turgor leaf plate is fixed at cultivar Kryimsky Shedevr.

These results allow a more objective divided peach cultivars in their sensitivity to waters carcity. Dehydration of the leaf blade results to repression of photoactivity in all stages of the primary photosynthesis. Research foliage photoactivity conducted with the involvement of promising cultivars (Gavazuri) in the first ten days of September, also showed the susceptibility of the light-harvesting complex as a result of dehydration (Table 3).

Loss of moisture in the leaves of the most cultivars was in 8 hours - 23 to 35%, in 24 hours - 40 to 51%. The leaves, when saturated with water, restored turgor from 42 to 100%. Cultivars were identified with high water-holding capacity: Gavazuri and Pintu (95 and 100%, respectively), which is significantly higher than the control cultivar Kryimsky Shedevr. In terms of the water regime of the leaves (the degree of dehydration when wilting, restoring turgor) Gavazuri cultivar were the most hardy, Pintu and Veteran.

Table 3.The change in the fluorescence indices of the leaves of introduced peach varieties in different conditions of water regime, September 2015

Peach cultivars, the conditions of the water regime	Changes in fluorescence indicators rel. un						The total water content,%	The loss of water in the leaves,%	Leaves restore. turgor,%
	F_0	F_m	F_{st}	$\frac{F_v}{F_0}$	$\frac{F_m - F_{st}}{F_m}$	$\frac{F_v}{F_m}$			
Kryimsky Shedevr (control)									
To saturation	379	1813	565	3.8	0,69	0.8			
After saturation	389	1632	464	2.9	0,72	0.7	100.0		
After 8h moisture loss	475	1984	512	3.2	0,74	0.8	75.9	33.0	
After 24h moisture loss	501	1243	901	1.5	0,27	0.6	55.2	49.3	
After the restoration of turgor	293	475	347	0.6	0,27	0.4			42.0
Gavazuri									
To saturation	325	1509	443	3.6	0,71	0.8			
After saturation	395	1728	475	3.4	0,73	0.8	100.0		
After 8h moisture loss	421	1552	555	2.7	0,64	0.7	85.0	22.5	
After 24h moisture loss	352	1168	427	2.3	0,63	0.7	62.5	39.6	
After the restoration of turgor	421	1552	555	2.7	0,64	0.7			94.5
Zempush									
To saturation	672	2912	848	3.3	0,71	0.8			
After saturation	656	2896	747	3.4	0,74	0.8	100.0		
After 8h moisture loss	656	2891	747	3.4	0,74	0.8	72.1	34.8	
After 24h moisture loss	885	1701	1370	0.9	0,19	0.5	47.5	50.7	
After the restoration of turgor	475	1057	587	1.2	0,44	0.6			60.0
Pintu									
To saturation	501	2651	677	4.3	0,74	0.8			
After saturation	565	2656	699	3.7	0,74	0.8	100.0		
After 8h moisture loss	634	2624	683	3.1	0,74	0.8	73.6	29.9	
After 24h moisture loss	603	1861	901	2.1	0,52	0.7	52.8	43.4	
After the restoration of turgor	507	1760	571	2.5	0,68	0.7			100.0
Veteran									
To saturation	397	1877	539	3.8	0,71	0.8			
After saturation	421	1872	560	3.5	0,70	0.8	100.0		
After 8h moisture loss	432	1696	475	2.9	0,72	0.7	77.1	32.9	
After 24h moisture loss	475	1520	731	2.2	0,50	0.7	56.3	46.3	
After the restoration of turgor	379	1163	485	2.1	0,58	0.7			82.5
Hidistavsky Belyiy									
To saturation	565	2805	715	4.0	0,75	0.8			
After saturation	667	3045	768	3.6	0,75	0.8	100.0		
After 8h moisture loss	688	2805	688	3.1	0,76	0.8	76.5	31.8	
After 24h moisture loss	725	2181	1221	2.0	0,44	0.7	47.1	50.8	
After the restoration of turgor	581	1664	704	1.7	0,57	0.7			90.0

Their leafy plate with wilting lost at least half of the total moisture content (39-46%) and reduce the turgor in the range 83-100%. Hardiness of photosynthetic structures in these cultivars was also high. In the cultivar Gavazuri parameter F_v/F_0 as a result of dehydration decreased by 32%. Behind him allocate cultivars Pintu, Veteran and Hidistavsky Belyiy. Change index F_v/F_0 have been recorded within the 37-44%.

It was noted that maintaining a high level of photoactivity of these cultivars was due to conservation a stable pool of light-harvesting complex (F_m and F_v/F_m) and reliable functioning of photochemical, "dark" processes the transformation of light energy (indicators (F_m-F_{st}) / F_m). On average, the suppression of these indicators in dehydration was 1.5-1.8 times less than in the group of resistant cultivars (Gavazuri, Pinto, Veteran and Hidistavsky Belyiy), compared with cultivars react more significantly to water deficit (Kryimsky Shedevr, Zempush).

It was noted that maintaining a high level of photoactivity of these cultivars was due to conservation a stable pool of light-harvesting complex (F_m and F_v/F_m) and reliable functioning of photochemical, "dark" processes the transformation of light energy (indicators $(F_m-F_{st})/F_m$). On average, the suppression of these indicators in dehydration was 1.5-1.8 times less than in the group of resistant cultivars (Gavazuri, Pintu, Veteran and Hidistavsky Belyiy), compared with cultivars react more significantly to water deficit (Kryimsky Shedevr, Zempush).

Thus, the most favorable period for carrying out diagnostic measures for drought resistance (August, September), selected the cultivars of peach Tszyu-Yus-Tszyuy, Gavazuri, Veteran, Hidistavsky Belyiy, combining high water-holding capacity with a stable maintenance of the level of functioning of the light-harvesting complexes. Changing the process of transformation of energy from the light-harvesting structures in pigment-protein complexes associated with the transmission and utilization of energy, quite objectively reflected in the dynamics of F_v/F_0 and $(F_m-F_{st})/F_m$.

On an indicator of F_v/F_0 the greatest value and, consequently, increased the proportion of photosynthetic active complexes recorded in cultivars Pintu, Gavazuri somewhat reduced, by about 5-8% in cultivars Tszyu-Yus-Tszyuy and Veteran. Unstable cultivars have proven Zempush and BabyGold-7.

In the first measurement period (august) cultivar Hidistavsky Belyiy differed by good photosynthetic activity, increased water-holding capacity and reliable regenerative capacity. In the second measurement period (september) of this cultivar in the process of dehydration, decreased the amount of photosynthetically active forms and worsened water-holding capacity. At the same time reducing power remained at a high level.

Indicators Fv/F_0 and $(F_m-F_{st})/F_m$ consistently characterize the state of the collection chain transfer and saving of the light energy depending on the degree of dehydration of leaf tissue. Still, allocated cultivars Pintu, Gavazuri and Tszyu-Yus-Tszyuy. Their contribution to the preservation of photoactivity is 10-12%

higher compared with other cultivars. Along with this they also have a high water-holding capacity and a significant degree of recovery turgidity of tissues (82-100%).

The peachcultivars Hidistavsky Belyiy and Veteran are promising for further study of drought resistance. Some cultivars also have a high photoactivity at various stages of photosynthesis (Zempush, Baby Gold-7), indicating that the problems that exist in the interpretation of the mechanisms that determine the state of the photosynthetic apparatus in leaves dehydration.

CONCLUSIONS

The functional state of the photosynthetic apparatus of peach leaf depends on the cultivar characteristics and temperature. Three peach cultivars (Pintu, Gavazuri and Tszyu-Yus-Tszyuy) with a stable rate of photoactivity, high water-holding capacity and significant degree of recovery turgidity of tissues have been selected. These cultivars are interesting for breeding.

In general, it should be noted ample opportunity of fluorimetric method of analysis used for the isolation of promising cultivars of peach and their further use in breeding for economically valuable traits.

ACKNOWLEDGMENT

This study was funded by a research grant № 14-50-00079 of the Russian Science Foundation.

REFERENCES

Bilger W., Schreiber U. (1990).Chlorophyll luminescence as an indicator of stress-induced damage to the photosynthetic apparatus. Effects of heat-stress in isolated chloroplast.Photosynth.Res., 1990, 25 №3, pp. 161-171.

Brion O., Korneyev D., Snegur O., Kitayev O. (2000). Instrumental research of photosynthetic apparatus by means of chlorophyll fluorescence induction (Procedural Guidelines).Kiev, 2000.11 p.

Buschmann C. (1986) Fluoreszenz- und Wärmeabstrahlung bei Pflanzen - Anwendung in der Photosyntheseforschung.Naturwissenschaften 73: 691-699. [Fluorescence and heat dissipation in plants - application to the study of photosynthesis].

Ivashchenko Y.V. (2008). Asssesment of peach cultivars and forms by activity level oftheir photosynthetic apparatus: methodological aspects. Collection of scientific papers of the NBG-NSC, 2008.Vol. 129.pp. 57-70.

Kershanskaya O. (2007). Photosynthetic fundamentals of production process of wheat. Almaty, 2007.244 p.

Korneyev D. (2002). Information Capabilities of Chlorophyll Induction Technique. Kiev. Alterpres, 2002.188 p.

KrauseG., Weis E. (1991). Chlorophyll fluorescence and photosynthesis: the basics, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 313–349.

Lichtenthaler H. (1992). The Kautsky effect: 60 years of chlorophyle fluorescence induction kinetics. Photosynthetica, 1992, Vol. 25, № 1-2, pp. 45-55.

LishchukA., Pilkevich R. (1999). The Field Technique for Assessment of Resistance to Drought and High Temperatures Intensification of Breeding of Fruit Crops: Collection of scientific papers. Yalta, 1999.Vol. 118. pp. 113-116.

Physiological and biophysical techniques used in the breeding of fruit crops (Guidelines). Under edition of A.I. Lishchuk.Moscow, 1991. – 67 p.

SmykovA., LukyanovaN., Ivashchenko Y. (2001). Assessment of feachures of photosynthetic apparatus in diagnostics of yielding capacity of fruit crops. Basic directions and techniques used in the breeding of pomaceous fruit grops: collection of scientific papers. Orel, 2001. pp. 88-89.

Stirbet A. (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient, J. Photochem. Photobiol. B: Biol., 2011, pp. 1-22.

DOI: 10.17707/AgricultForest.63.1.10

Svetlana SHEVCHENKO,
Valentina GORINA, Irina MITROFANOVA [1]

THE INFLUENCE OF VIRAL STATUS ON POLLEN CHARACTERISTICS OF SOME APRICOT CULTIVARS

SUMMARY

Apricot is one of the leading temperate zone fruit-bearing crops and obtaining of its new cultivars with the highest economically-valuable features is an actual problem. The most effective method of new cultivars breeding is the hybridization. Considering the modern ecological situation, it is extremely important to create virus-free forms with high adaptive potential. During the monitoring of the viral diseases spread within apricot collection plots of Nikita Botanical Gardens – National Scientific Center (NBG-NSC)the analysis of mature pollen of 11 apricot cultivars was investigated. It was found out that studied cultivars are characterized by resistance to pathogens and affection by viruses. Two cultivars of NBG-NSC breeding (Krymsky Amur and Krymsky Medunec) and two introduced cultivars (Harcot and Markuleshti) demonstrated the difference to specified parameters. For example, the cultivar Krymsky Medunec is resistant to viral pathogens; its mature pollen has a large percentage of morphologically normal pollen grains characterized by high germination (up to 90% and 32%, respectively). At the same time, cultivars Markuleshti and Harcot are strongly affected by *Plum pox virus*; their ripe pollen is morphologically normal, but low vital capacity level.. On 15% sucrose solution pollen grains of cultivar Harcot did not germinate, and cultivar Markuleshti had only 1% of germinating pollen grains. It should be noticed that complex application of biotechnological methods (chemotherapy and meristem culture), further using and propagation of virus-free explants *in vitro* let us get healthy plant material. Thus, the results suggest the potential for creating new perspective cultivars of apricot with preset characteristics for further breeding.

Keywords: *apricot, cultivar, generative sphere, pollen grains, in vitro*

INTRODUCTION

One of the most popular stone fruit crops is apricot *Prunus armeniaca* L., (syn. *Armeniaca vulgaris* Lam). Modern scientists distinguish from three to six possible places of apricot origin, the most likely of which is considered the area of the Tien Shan Mountains in China (Dalby, 2003; Folta, 2009). This culture has fruits with high taste quality, containing pectin substances, vitamins, sugars,

[1]Svetlana Shevchenko (corresponding author: shevchenko_nbs@mail.ru), Valentina Gorina, Irina Mitrofanova Nikita Botanical Gardens – National Scientific Center, Yalta, 298648, RUSSIA
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

organic acids, mineral salts, etc. The apricot fruits are used as a food for thousands years and they are cultivated in warm temperate regions of Europe, Central Asia, the Caucasus, Ukraine and southern regions of Russia. During the cultivation of apricot many varieties were derived, but there is still a need for new genotypes and the creation of new varieties with a complex of economically-valuable traits that would be adapted to growing conditions, as well as free from viral pathogens (Fideghelli and Della Strada, 2008; Gorina, 2014). It's known, that due to influence on plants biotic and abiotic stress, including viruses, there is a change of physiological-biochemical parameters, which cause violation of biochemical equilibrium and affect the anatomic-morphologic characteristics. Such morphological changes like rugosity, spotting and dwarfing are used for visual evaluation of the degree of the plant penetration of the plant and its viability as a whole (Grin et al., 1990; Mitrofanova et al., 2000).State of male gametophite is one of the most affordable and effective signs, that gives us information about the level of stressful influences of various factors on the plant and its viability (Ruguzov et al., 1979; Bessonova et al., 1997; Mitrofanova et al, 2000 a).The aim of our research is to monitor the spread of viral diseases in apricot collection plantations of the Nikita Botanical Gardens, as well as an assessment of morphophisiological state of the pollen grains of several cultivars of apricot with visual signs of defeat viruses and asymptomatic plants to determine the effects of a viral infection on their generative sphere.

MATERIALS AND METHODS

The objects of the research were 11 apricot cultivars: Krymsky Amur, Harcot, Krymsky Medunec, Dionis, Mamaia, Ananasnyi Zhiurupinsky, Krasnyi Vimpel, Magistr, Holovousy, Markuleshti, Harlayne. Pollen analysis was carried out on temporal preparations stained with aceto-orcein and the pollen germination were determinate by putting pollen in 15% sucrose solution (Pausheva, 1990). For invigoration the plant material complex of biotechnological methods were applied: chemotherapy and meristem culture. Material was put on modified nutrition media of Murashige & Skoog (1962), and Gamborg & Eveleigh (1968). For the chemotherapy during *in vitro* cultivation in the first phase in culturemedia 6 mg/l ribavirin was added. Seedlings with an extensive root system and shoots were planted in vessels with the ground to adapt to native conditions.

RESULTS AND DISCUSSION

As a monitoring result of viral diseases spread in apricot collection of Nikita Botanical Gardens (NBG-NSC) were determined visually clean individuals and specimens infected by virus pathogens were marked out for comparative analysis of the mature pollen. It was established that viruses on different cultivars causes different effects on morphophisiological characteristics of pollen grains (table 1, 2). Despite the different temperature parameters in winter 2015 and 2016 (the average temperatures in December 2014 and January

2015 were +2.7°C and +1.2°Crespectively; in December 2015 +2.5°C and -0.3°C in January 2016), the reaction of almost all the study case varieties on contamination by viruses is identical. For example, in Krymsky Medunec, which is rather resistant to viral pathogens, the mature pollen has a large percentage of morphologically normal pollen grains characterized by high vitality. This cultivar, bred by the NBG scientists, is a medium size, fast-growing tree with a rounded crown, regular good yields (160-180 kg/ha), resistance to stress low and high temperature of air (Figure 1).

Figure 1. Shoot fragment with fruits of KrymskyMedunec cultivar

The cultivar Krymsky Amur was also quite resistant to viral pathogens, bred by NBG scientist. The tree is vigorous with spherical crown, quite high productivity (90-120 centners/hectare) and large fruits (until 90 g) (Figure 2).

Mature pollen of the visually virus-affected individuals is characterized by high number of morphologically normal pollen grains.Pretty steady in aspect of viral pathogens was the cultivar Ananasnyi Zhiurupinskyas well with high content of morphophisiological normal pollen grains (Tables 1 and 2).

Cultivars Markuleshti and Harcot were strongly affected by Sharka virus. Mature pollen of Markuleshti has lots of morphologically normal pollen, but with low vitality. Majority part of pollen grains in cultivar Harcot is defective (table 2), and on 15% sucrose solution they do not germinate. Only 1% of germinating pollen grains was identified for cultivar Markuleshti. Cultivar Magistr has more than 10% of germinated pollen grains in the 15% solution in 6 hours after sowing.

Figure 2. Fragment of shoot with fruits of Krymsky Amur cultivar

Complex use of biotechnological methods (chemotherapy and meristem culture *in vitro*) made it possible to introduce primary explants of cultivars Krymsky Amur on Gamborg & Eveleigh medium and induce their development. Among study cases only cultivar Krymsky Amur possessed high morphogenetic potential (65-70%). Ribavirin in culture medium was inhibited the growth and development of microshoots, and the number of developed meristems of cultivars KrymskyMedunec and Harcotdid not exceed 15%. Further microcuttings of virus-free explants *in vitro* and their cultivation induced formation of adventive microshoots.

On the stage of root formation adding of NAA and IBA in concentrations of 1-1,5 mg/l to Murashige & Skoog medium stimulated rhizogenesis in 57% microshoots of cultivar Krymsky Amur. Therefore, Sharka virus-affected plants of this cultivar were obtained *in vitro*. A similar effect was observed with ribavirin cultivation of undeveloped hybrid embryos of apricot (Shevchenko and Mitrofanova, 2015).

During of selection the parent forms and hybridization we allowed for not only pollen quality but also structure of the flower, because flowers with short pistil embryonic sacs are underdeveloped, the egg apparatus was not differentiated, what doesn't favor the effective fertilization. Study cultivars had different number of flowers with defective pistils. For example, more than 70% flowers of Markuleshti cultivar have undeveloped pistil, while for cultivar Krymsky Amurthis number until 30% (Figure 3).

Table 1. Characteristics of mature pollen of some apricot cultivars in April 2015

Cultivar	Total number of pollen grains		No of morphologically normal pollen grains		No of defective pollen grains		Percentage of defective pollen grains (%)	
	1*	2*	1	2	1	2	1	2
Ananasnyi Zhiurupinsky	1090	-	1000	-	90	-	8,025	-
Krasnyi Vimpel	1000		840		160		16,0	
Holovousy	-	1420	-	1000	-	420	-	29,6
Mamaia	-	1200	-	200	-	1000	-	83,3
Harlayne	-	1200	-	630	-	570	-	47,5
Harcot	-	1060	-	60	-	1000	-	94,3
Markuleshti	1120	-	1000	-	120	-	10,7	-
Krymsky Amur	1100	-	1000	-	100	-	9,1	-
Krymsky Medunec	-	1320	-	1000	-	320	-	24,2
Dionis	-	2400	-	1000	-	1400	-	58,3
Magistr	-	1250	-	1000	-	250	-	20,0

1*- visually clean apricot plants
2*- plants affected by viral pathogens

Table 2.Mature pollen characteristics of some apricot cultivars in March-April 2016

Cultivar	Total number of pollen grains		No of morphologically normal pollen grains		No of defective pollen grains		Percentage of defective pollen grains (%)	
	1	2	1	2	1	2	1	2
Ananasnyi Zhiurupinsky	1390	2940	1220	2500	170	440	12,2	15,0
Krasnyi Vimpel	1340	-	1250	-	90	-	6,7	-
Holovousy	1330	3790	970	2500	390	1290	29,3	34,0
Mamaia	1280	2200	1160	210	120	1990	9,37	90,45
Harlayne	1120	2040	930	960	190	1080	16,9	52,9
Harcot	1200	1610	1050	80	150	1530	12,5	99,5
Markuleshti	1270	2830	1180	2310	90	520	71	18,4
Krymsky Amur	1080	1820	980	1620	100	200	9,26	11,0
Krymsky Medunec	1230	4900	1100	4400	130	500	10,6	10,2
Dionis	990	4090	520	1890	470	2200	47,5	53,8
Magistr	1520	1840	1480	1520	40	320	2,6	17,4

1*- visually clean apricot plants
2*- plants affected by viral pathogens

Figure 3. Fragments of Markuleshti cultivars flower (A) and Krymsky Amurflower (B)

CONCLUSIONS

Based on the research results we can conclude that the morphological and physiological characteristics of pollen depend not only on the genotype, but also on the stability of cultivars affected by viral pathogens. Cultivars Krymsky Amur, Krymsky Medunec, Magistr and Ananasnyi Zhiurupinsky we recommended to use as parent cultivars for creation of new genotypes, which are resistant to environmental stress factors. Application of complex biotechnological methods (chemotherapy and meristem culture *in vitro*) allows getting and multiplying invigorative valuable plant material. In general, to increase efficiency of breeding process requires complex approach that involves biological and phenological researches, the study of generative sphere, improvement of plant health, adaptation and reproduction of obtained valuable genotypes.

ACKNOWLEDGMENT

This study was funded by a research grant № 14-50-00079 of the Russian Science Foundation.

REFERENCES

Bessonova V.P., Fendûr L.M., Peresypkina T.N. (1997). Environmental pollution media on male fertility decorative flower crops. Bot. Zhurn. Vol. 82, No. 5. 38-44.

Gorina V.M. (2014). Scientific basis for the selection of apricot and plum for the Crimea and southern Ukraine.PhD Thesis, Michurinsk, 479p.

Grin N., Staut U., Teylor D. (1990). Biology: in 3 volumes. Vol. 1. - M.: Mir. - 368 p.
Dalby A. (2003). Food in the ancient world from A to Z. P. 20.
Gamborg O.L., Eveleigh D.E. (1968). Culture method and detection of glucanases in suspension cultures of wheat and barley. Can. J. Biochem., 46, 5, 417-421.
Fideghelli C., Della Strada G. The breeding activity on apricot in the world from 1980 through today.ActaHorticulturae 862, 93-98.
Folta, K.M.(2009). Cenetics and Genomics of Rosaceae. Springer-Verlag. New-York.
Ruguzov I.A., Shevchenko, S.V., Molchanov E.F. (1979). Methodical recommendations for citoembryological analysis of plants to assess air pollution (in connection with the problem of environment) / (1979). Comp. Yalta. - 23 p.
Mitrofanova O., Lesnikova N., Mitrofanova I., Vogegov S. (2000a). Application of biotechnology method for obtaining the valuable cultivars of stone fruits. Horticulture and Vegetable Growing 19. 3. Part 1. 367-374.
Mitrofanova O.V., Slavgorodska-Kurpieva, L. MitrofanovaI.V., Lukicheva L.A. (2000) Diagnostics of viral diseases and biotechnological techniques for obtaining virus-free planting material stone fruit crops. -Yalta: Krympress. - 45 p.
Murashige T., Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15, 3,473-497.
Pausheva Z.P. (1990). Practicum by plant cytology. V. : Kolos. – 283 p.
Shevchenko S., Mitrofanova I. (2015) Investigation of apricot reproductive structures, creation and propagation of new forms.Agriculture & Forestry, Vol. 61, Issue 4:161-167.

DOI: 10.17707/AgricultForest.63.1.11

Fatma ACHEUK, Wassima LAKHDARI, Khemais ABDELLAOUI, Messaouda BELAID, Rabea ALLOUANE, Fatma HALOUANE[1]

PHYTOCHEMICAL STUDY AND BIOINSECTICIDAL EFFECT OF THE CRUDE ETHONOLIC EXTRACT OF THE ALGERIAN PLANT *ARTEMISIA JUDAICA L.* (ASTERACEAE) AGAINST THE BLACK BEAN APHID, *APHIS FABAE* SCOP.

SUMMARY

Plants are the nature's biochemical factories. They bio-synthesize a diverse array of different natural products, such as alkaloids, terpenes and terpenoids, phenolic compounds, flavonoids and coumarins through their structural mechanisms to reduce insect attacks, both constitutive and inducible, while insects have evolved strategies to overcome these plant defenses.

There is a widespread effort to find new pesticides, and currently it is focused on natural compounds such as flavonoids, coumarins, terpenoids, and phenolics from diverse botanical families from arid and semi-arid lands. Algeria by the diversity of its habitats has a very diverse flora. Some of these plants have very interesting insecticidal properties. The aim of this study is to evaluate the insecticidal effect of the plant *Artemisia judaica* L. (Asteraceae). The crude ethanol extract of the plant *A. judaica* was tested on the black bean aphid *Aphis fabae* Scop. Four doses (12.5, 6.25, 3.12 and 1.56 mg mL^{-1}) were tested on contact wingless adults. The results have showed that the tested extract has been very powerful to aphids. At the highest dose 12.5 $mgmL^{-1}$, the 100% of mortality were recorded 2 hours after treatment, and for the lowest dose (1.56 $mgmL^{-1}$) it was after 96 hours. The LD50 calculated 2 hours after treatment from the regression lines Probit = *f* (doses) shows that it is 2.75 $mgmL^{-1}$. This powerful insecticidal activity of the tested crude extract could be due to the richness of the plant on phenolics compounds known for their bio-insecticide action.

Keywords: *Artemisia judaica, Crude extract, Aphis fabae, Insecticidal activity, Phytochemical study*

INTRODUCTION

In Algeria the bean, *Vicia faba* L., is the crop that is part of farming systems since a long time in different agro-ecological areas of the country. It's the most important food legume with 58000 hectares or 44.3% of the total area

[1]Fatma Acheuk, (corresponding author: fatma.acheuk@yahoo.fr), Messaouda Belaid, Rabea Allouane, Fatma Halouane, Valcore Laboratory, Department of Biology, Faculty of Sciences, University of Boumerdes, Boumerdes, 35000 ALGERIA, Wassima Lakhdari, National Institute of Agronomic Research, Station of Sidi Mehdi, Touggourt, ALGERIA, Khemais Abdellaoui, Department of Biological Sciences and Plants Protection, High Institute of agriculture, Chott-Mariem, Sousse University, TUNISIA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

reserved for this crop category (Bouznad *et al.*, 2001). In addition to abiotic stresses (cold, frost, heat and salinity), beans crop are exposed to the harmful effects of weeds, fungal and viral diseases, nematodes and insects (Maatougui, 1996). Among subordinate insects, aphids have a special place. The black bean aphid, *Aphis fabae*, can severely damage bean plants by checking growth, decreasing yield and impairing the quality of the seed (Banks and Macaulay,1967). Honey dew excretion and growth of sooty molds create also an indirect damage through impending some physiological processes in plant (Hurej and Van Der Werf, 1993). Beside the mechanical damage caused, they also serve as the largest group of vectors of plant viruses (Blackman and Eastop, 2006).

However the yields and quality of agricultural products depend largely on the use of synthetic chemical insecticides needed to control the populations of these pests. These compounds are often found as residues in food and pose significant risks to human health and the environment. In the interests of environmental respect and in the context of sustainable development, it should greatly to reduce the amount of synthetic pesticides and develop alternative control strategies. These new approaches should be based on the combined use of biomolecules provided with bioinsecticidal properties. Many molecules that exhibit toxic and defensive action against plant pests have been identified and more than 2000 plant species with insecticidal properties have been identified (Benayad, 2008).

The genus *Artemisia* is one of the largest and most widely distributed genera of the family Astraceae (Compositae). It is a heterogenous genus, consisting over 500 diverse species distributed mainly in the temperate zones of Europe, Africa, Asia and North America (Kundan and Anupam, 2011).

Artemisia juadaica L. is a perennial fragrant shrub, with pubescent leaves, which grows widely in the deserts (Abd-Elhady, 2012) and is a very common anthelmintic drug in most North African and Middle-Eastern countries where it is known by the Arabic name of "*Shih*" (Van Wyk and Wink, 2004). The plant has been used also to treat gastro-intestinal disorders, poor eyesight, cardiovascular disease, skin disorders, and weak immune systems as well as to decrease the risk of atherosclerosis, cancer, and arthritis (Liu *et al.*, 2004; Abd-Elhady, 2012).

The aim of this study was to investigate the insecticidal effect of methanolic extract of the aerial parts of *A. judaica* against the black bean aphid, *A. fabae* Scop. under laboratory conditions.

MATERIALS AND METHODS

Plant collection and preparation of crude ethanolic extract Aerial parts of *A. judaica*were collected during spring seasons 2012 in the Tamnraset region (South of Algeria). The plant was taxonomical identified and confirmed by PrAbdelkrim from the National High College of Agriculture, Algiers, Algeria. The crude ethanolic extract of the above-ground portion of the plant was prepared from leaves, flowers and stems dried in the shade and ground into a fine powder using electrical blender. The extraction was carried out by macerating the

powder for 3 days in ethanol, followed by filtration and evaporation at 40°C. The percentage of yield was calculated and the dried extract was kept at 4 °C until further use.

Phytochemical screening

The ethanolic extract was tested for plant secondary metabolites, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, iridois and coumarins. Phytochemical screening of the extract was carried out according to the standard method (Dohou *et al.*, 2003). Visible color change or precipitate formation was taken into consideration for presence (+) or absence (-) of particular active constituents.

Test organisms and bioassays

Stock of adults wingless aphids used in this study was collected randomly from infested bean filed in Ain Taya, Algiers area. The bioassays were conducted on Petri dishes under laboratory conditions at temperature of 22 ± 2 °C, 40-80% relative humidity and 16L:8D light regime. Forty aphids were transferred to Petri dishes on fresh leaves of *V.fabae* serving as a support for the aphids. Wet cotton discs were placed under the bean leaves to keep them fresh during the test period. Four doses of crude ethanolic extract were prepared (12.5, 6.25, 3.12 and 1.56 $mgmL^{-1}$). An appropriate quantity of *A. judaica* extract was dissolved in ethanol to obtain each test solution. The insecticide solution was applied by topic application (contact) to adult aphids using micropipette. Controls were treated with only absolute ethanol. Mortalities percentages were determined for each treatment after 2, 4, 24, 48, 72 and 96h. The LD50, the concentration that produces 50% mortality, was determined by log probit analysis.

Enzymatic assays

The AChE activity was carried out following the method of Ellman *et al.* (1961) using acetylthiocholine as a substrate. Aphids were sampled from control and treated groups (at low dose 1.56 $mgmL^{-1}$). Pools of twenty adults aphid were homogenized in the solution containing 38.03 mg of ethylene glycol tetraacetic (EGTA), 1mL Triton X-100, 5.845 g NaCl and 80 mL Tris buffer (10Mm, pH 7). The homogenate was centrifuged (5000 g for 5 min at 4°C), and the resulting supernatant was used for enzymatic assay. The AChE activity was measured in aliquots (100µL) of resulting supernatants added to 100 µLof 5-5' dithiobis-(2-nitrobenzoic acid) (DNTB) in Tris buffer (0.01 M, pH 8)and 1 mL Tris (0.1 M, pH 8).After 5 min, 100µLof acetylthiocholine was added. Measurements were conducted at a wavelength of 412 nm with a run time of 20 minutes. GST activities were determined with the soluble fraction as enzyme source. GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Habig *et al.* (1974). Treated (at low dose 1.56 $mgmL^{-1}$) and control aphids were homogenized in sodium phosphate buffer (0.1 M, pH 6) and centrifuged (14000 g, 30 min). Two hundred microliter of the resulting supernatant was added to 1.2 mL of reaction mixture containing 1Mm of CDNB and 5 Mm of reduced glutathione (GST) in the homogenization buffer. Changes in absorbance were recorded at 340 nm. Total protein content was determined according to

method of Bradford (1976) using bovine serum albuminasa standard. Enzyme activities were expressed as µmolmin^{-1}mg^{-1}proteins.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured. Data were submitted to a monofactorial ANOVA using XLSTAT 7.5.2. Means were compared using Tukey's HSD test ($P< 0.05$).

RESULTS AND DISCUSSION

Phytochemical screening

The crude ethanolic extract of *A. judaica* was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using classical methods of analysis. The results of phytochemical study (Table 1) revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins and comarins. Antocyans and iridoiswere absents.

Table 1.Qualitative phytochemical screening of crude ethanolic extract of *A. judaica*

Alkaloids	Antocyans	Comarins	Tannins	Saponins	Iridois	Flavonoids
+	-	+	+++	+	-	+++

Insecticidal activity

The results of the toxicity assay against of the black bean aphid, *A.fabae*, are given in the figure 1 and the table 2. The test compound showed high insecticidal activity for all tested concentrations.

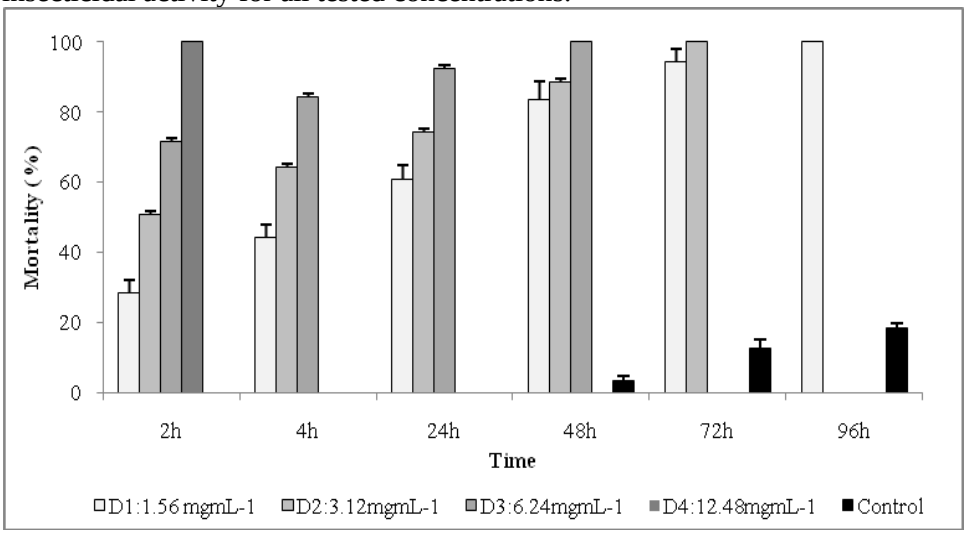


Figure 1. Effect of crude ethanolic extract of *A. judaica* on mortality of the the black bean aphid, *A.fabae* (Maen ± SD). N=60 aphids /replicate, ($p <0.05$).

At the highest dose 12.5 $mgmL^{-1}$, the 100% of mortality were recorded 2 hours after treatment, and for the lowest dose (1.56 $mgmL^{-1}$) it was after 96 hours. Mortality rates ranged from 50.83 to 71.66% at 2 h after treatment for the average concentration 3.12 and 6.24 $mgmL^{-1}$ respectively. Total mortality (100%) was achieved 48h and 72h after treatment respectively for the concentrations 6.24 and 3.12 $mgmL^{-1}$.

Table 2. LD50 values of *A. judaica* crude methanolic extract against the black bean aphid, *A.fabae*

Time (h)	LD50 values ($mgmL^{-1}$)
2	2.79
4	1.91
24	1.24
48	1.04
72	0.82

The results of probit analysis showed that *A. fabae* was susceptible to the crude ethanolic extract of *A.judaica*. The LD50 was obtained 2h after treatment.

Enzymathic effects

The effect of the crude ethanolic extract of *A. judaica* on enzymatic activities (GST and AchE) is presented in figures 2 and 3. The results showed an inhibition of AchE activity in treated aphids at high concentration of the crude extract of *A. judaica*. However, an activation of GST activity was observed on treated aphids.

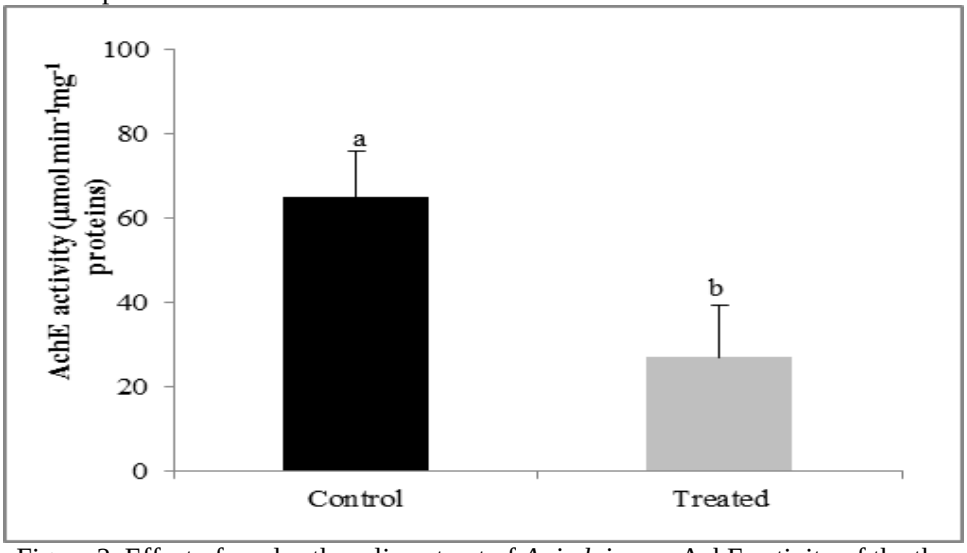


Figure 2. Effect of crude ethanolic extract of *A. judaica* on AchE activity of the the black bean aphid, *A. fabae* (Maen ± SD), N=60 aphids. Different letters denote significant differences (Tukey's test, $p < 0.05$).

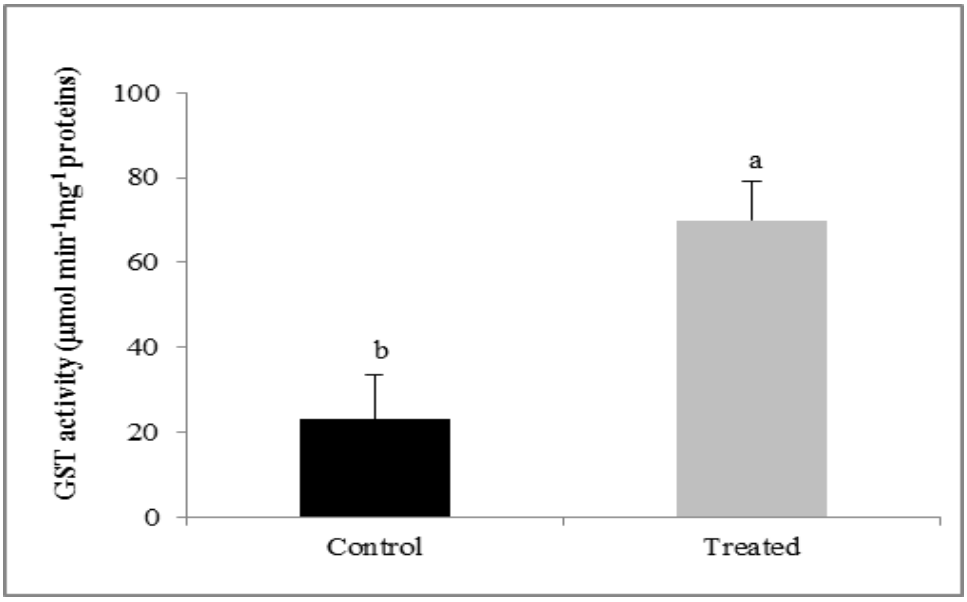


Figure 3. Effect of crude ethanolic extract of *A. judaica* on GST activity of the the black bean aphid, *A.fabae* (Maen± SD), N=60 aphids. Different letters denote significant differences (Tukey's test, $p < 0.05$).

Certain natural products can be suitable alternatives to synthetic pesticides owing to their generally reduced negative impacts on humans, beneficial insects and the environment. Higher plants constitute a diverse source of highly bioactive agents that include some that have contributed significantly to the successful use of natural products and analogues for crop protection (Akhtar and Isman, 2013). In this study, phytochemical screening and the insecticidal activity of the crude ethanolic extract of the Algerian plant *A. judaica* were studied. The results of phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins and comarins. These results are in agreement with knows compositions of many *Artemisia* species (Masotti *et al*., 2012). Kundan and Anupam (2011) reported that the *Artemisia* species comprise mainly terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes.

Many researchers have reported on the effectiveness of plant extracts against insects (Acheuk *et al.*, 2012; Abdellaoui *et al.*, 2013, 2016; Pavela,2004 and Nathan *et al.*, 2006). In the present work, the crude ethanolic extract of *A. judaica* showed potent insecticidal effects against the black aphid, *A.fabae*. Total mortality (100%) was achieved 2h after treatment with the higher concentration. This toxic effect of the extract might be due to the various bioactives compounds that exist in the aerial part of the plant. Indeed, the crude extract of plant is a mixture of potentially bioactive substances which may act synergically (Acheuk *et al.*, 2012) or independently (Kabir, 2013). Previous studies demonstrated the insecticidal effects of *Artemisia* plant extracts against other insects pest. Abd-

Elhady (2012) reported that volatile oils from the aerial parts of *A. judaica*, were found to have an insecticidal effect against *Callosobruchus maculatus* (Fab.). A concentrated extraction of 50% was shown to have the highest mortality. As our results, Masotti *et al.* (2012) reported that ethanolic extracts of *A. molinieri* and *A. campestris* Varglutinosa showed larvicidal activity against mosquito *Culex pipiens*. However, extracts of *A. molinieri* revealed a higher larvicidal activity than those of *A. campestris*. The biocide differences found for the tested extracts can be explained by their different chemical compositions, with aromatic polyacetylene dominant fraction for *A.molinieri* ethanolic extracts. In the same way, Dane *et al.* (2016) reported that the methanolic extract from *A. absinthium* showed potent toxicity for *Sitophilus oryzae* and causing 100% in 24h at a dose of 60 $mgcm^{-2}$.

Many natural plant compounds used in the control of insect pest are known to exhibit effects in the enzymatic profiles (Smirle *et al.*, 1996; Zhang *et al.*, 2013). The finding of our study showed that, the crude ethanolic extract of *A. judaica* inhibited AchE activity on treated aphids. It's known that AchE regulate nerve impulse transmission across cholinergic synaps. Monoterpenoids tested by López and Pascual-Villalobos (2010), showed inhibitor effect on acethylcholinesterase enzyme. From the all compounds tested by these authors, fenchone, S-carvone and linalool produced the highest inhibition. Similarly, the study conducted by Abdel-Aziz *et al.* (2015), demonstrated that, rosacide treatment showed the highest AchEpercent of inhibition (72.06%) on *Aphis craccivora*. This inhibition is due to the presence of high amount of the monoterpenoid 1, 8-cineolein rosacide, which is known for its insecticidal, feeding, deterrent and repellent properties. It inhibits acetylcholinesterase by occupying the hydrophobic site of enzyme's active centre. In agreement, Nathan *et al.*(2008) found that Azadirachtin significantly inhibits the activity of AchE in *Nilaparvata lugens*.

In insects, allelochemical defense system include P450, gluthathione S-transferase and esterases which are typically concentrated in midgut allowing rapid elimination of ingested toxic substances (Rattan, 2010). Elevated detoxification enzymes activity in insects tissues are often associated with enhanced detoxification of allelochemicals (Valles *et al.*, 1999). GST play a pivotal role in detoxification and antioxidant defense of insects against natural and synthetic exogenous xenobiotics including insecticides, allelochemicals and endogenously activated compounds (Papadopoulos *et al.*, 2004). GSTs catalyse the addition of a tripeptide glutathione to a wide variety of electrophilic substrates including host plant secondary compounds (Yu and Hsu, 1993). In our study, the GST activity in *A. fabae* was significantly stimulated by the low dose of the crude ethanolic extract of *A. judaica*. Similarly, different plant secondary compounds; catechol (phenolics), gramine (alkaloid) and L-ornithine-HCl (non protein amino acid), tested in the artificial diet activated the GST activity in the English grain aphid *Sitobion avenae* and a significant correlation was also observed between the concentration of each compound in diet and GST activity

in aphids (Zhang *et al*., 2013). Our findings are also in agreement with those of Abdel-Aziz *et al.* (2015) who reported that the green insecticide Cura (curcuma oil mixed with mineral and vegetable oils) increased the GST activity with (10.16%) than control in *Aphis craccivora*.

CONCLUSIONS

The results of our study demonstrate the potent aphicidal activity of the crude ethanolic extract of the Algerian Asteraceae *A. judaica*. However, further studies for guided isolation of the insecticidal compounds and their effects on the non target organisms are strongly needed.

REFERENCES

Abdel-Aziz N.F., Abdou W.L., Abdel-Hakim E.A., El-Hawarya F.M., El-Bakry A.M., Sammour E.A. (2015). The effect of some green insecticides from essential oils on *Aphis craccivora* and their side effects. Journal of Entomological Research, 39 (4), 275-286.

Abd-Elhady H.K. (2012). Insecticidal activity and chemical composition of essential oil from *Artemisia judaica* l. against *Callosobruchus maculatus* (f.) (Coleoptera: Bruchidae). Journal of Plant Protection Research, 52 (3), 347-352.

Abdellaoui K., Miladi M., Ben Marzouk I., Bahloul N., Acheuk F., Chaira N., Ben Halima-Kamel M. (2016). Antifeedant and antigonadotropic effects of *Ruta chalepensis* methanolic extract against *Locusta migratoria*. Tunisian Journal of Plant Protection, 11, 91-104.

Abdellaoui K., Ben Halima-Kamel M., Acheuk F., Soltani N., Aribi N., Ben Hamouda M.H. (2013). Biochemical and histological effects of gibberellic acid on *Locusta migratoria migratoria* fifth instar larvae. Pesticide Biochemstry and Physiology, 107, 32-37.

Acheuk F., Cusson M., Doumandji-Mitiche B. (2012). Effects of a methanolic extract of the plant *Haplophyllum tuberculatum* and of teflubenzuron on female reproduction in the migratory locust, *Locusta migratoria* (Orthoptera: Oedipodinae), Journal of Insect Physiology, 58, 335-341.

Akhtar Y., Isman M.B. (2013). In IshaayaI., Palli S. R.,Horowitz, A. R. Plant Natural Products for Pest Management: The Magic of Mixtures. Ed Spring, 231-247.

Banks C.J., Macaulay E.D.M. (1967). Effects of *Aphis fabae* Scop.and of its attendant ants and insect predators on yields of field beans (*Vicia faba* L.). Annals of Applied Biology, 60, 445-453.

Benayad N. (2008). Les huiles essentielles extraites des plantes médicinales marocaines: moyen efficace de lutte contre les ravageurs des denrées alimentaires stockées (Essential oils extracted from Moroccan medicinal plants: Effectiveness against fight against pests of stored foods). Projet de recherche. UNISCO. Université Mohamed V. Faculté des Sciences de Rabat, 1-63.

Blackman R.L., Eastop V.F. (2006). Aphids on the world's her because plants and shrubs: Vol. 2. Chichester: John Wiley & Sons.

Bouznad Z., Louanchi M., Allala L., Mérabti N. (2001). Les maladies de la fèveen Algérie cas de maladie à tache chocolat causée par *Bortrytis* spp. (Diseases of bean in Algeria: case of chocolat spot disease caused by *Botrytis* spp.) Quatrièmes journées scientifiques et techniques phytosanitaires, INPV, 12-13 Novembre 2001, 2 p.

Bradford M.M. (1976).A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72, 248-254.

Dane Y., Mouhouche F., Canela-Garayoa R., Delpino-Rius A. (2016). Phytochemical analysis of methanolic extracts of *Artemisia absinthium* L. 1753 (Asteraceae), *Juniperus phoenicea* L., and *Tetraclinis articulata* (Vahl) Mast, 1892 (Cupressaceae) and evaluation of their biological activity for stored grain protection. Arabian Journal for Science and Engineering, 41(6), 2147-2158.

Dohou W., Yamni K., Tahrouch S., Idrissi Hassani L.M., Bado A. Guira N. (2003). Screening phytochimiques d'une endemique ibére-macrowave, *Thymelaea lythroides* (Phytochemical screening of the endemic Ibero-macrowave, *Thymelaea lythroides*). Bulletin de la Société de Pharmacie de Bordeaux**,** 142**,** 61-78.

Ellman G.L., Courtney K.D., Andres V., Featherstone A. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7, 88-95.

Habig W.H., Pabst M.J., Jakoby W.B. (1974). Glutathione S-transferases, the first enzymatic step in mercapturic acid. Journal of Biological Chemistry, 249, 7130-7139.

Hurej M., Van Der Werf W. (1993). The influence of the black bean aphid, *Aphis fabae* Scop and its honeydew on the photosynthesis of sugar beet. Annals of Applied Biology, 122,189-200.

Kabir K.E., Choudhary M.I., Ahmed S., Tariq R.M. (2013). Growth-distruping, larvicidal and neurobehavioral toxicity effects of seed extract of *Seseli diffusum* against (L.) (Diptera: Culicidae). Ecotoxicology and environmental Safety, 90, 52-60.

Kundan S.B. Anupam S. (2011). The Genus *Artemisia*: A Comprehensive Review. Pharmaceutical Biology, 49(1), 101-109.

Liu C.Z, Murch S.J, El-Demerdash M., Saxena P.K. (2004).*Artemisia judaica* L., micropropagation and antioxidant activity. Journal of Biotechnology, 110, 63-71.

López M.D., Pascual-Villalobos M.J. (2010). Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products, 31, 284-288.

Maatougui M.E. (1996). Situation de la culture des fèvesen Algérie et perspectives de relance (Bean crop situation in Algeria and recovery prospects), In rehabilitation of fababean. Ed. Actes, Rabat, 17-30.

Masotti V., De Jong L., Moreau X., Rabier J., Laffont Schwob I., Thiery A. (2012). Larvicidal activity of extracts from *Artemisia* species against *Culex pipiens*L. mosquito: Comparing endemic versus ubiquist species for effectiveness. Comptes Rendus Biologies, 335, 19-25.

Nathan S.S., Chung P.G.Murugan K. (2006). Effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocismedinalis* (Guenée) (the rice leaffolder) (Insecta: Lepidoptera: Pyralidae). Ecotoxicology and Enviromrental Safety, 64,382-389.

Nathan S.S, Choi Y.M, Seo YH, Paik H.C, Kalaivani K., Kim D.J. (2008). Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål). Ecotoxicology and Environmental Safety, 70, 244-250.

Papadopoulos, I.A., Polemitou, I., Laifi, P., Yiangou, A. and Tananaki, C. (2004).Glutathione S-transferase in the developmental stages of the insect *Apis mellifera macedonica*. Comparative Biochemistry and Physiology, Part C, 139, 87-92.

Pavela, R., (2004). The effect of ethanol extract from plants of family Lamiaceae on colorado potato beetle adults (*Leptinotarsa decemlineata* Say). National Academy Science Letters, 27, 195-203.

Rattan R.S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection, 29, 913-920.

Smirle M.J., Lowery D.T., Zurowski C.L. (1996). Influence of Neem Oil on Detoxication Enzyme Activity in the Oblique banded Leafroller, *Choristoneura rosaceana*. Pesticide Biochemistry and Physiology 56, 220-230.

Valles, S.M., Koehler, P.G. Brenner, R.J. (1999). Comparative insecticide susceptibility and detoxification enzyme activities among pestiferous blattodea. Comparative Biochemistry and Physiology pat C, 124, 227-232.

Van Wyk B.E. Wink M. (2004). Medicinal Plants of the World; Briza Publications: Pretoria, South Africa, 54-56.

Yu S.T., Hsu E.L. (1993). Induction of detoxification enzymes in phytophagous insects: role of insecticide synergists, larval age and species. Archive of Insect Biochemistry and Physiology, 24, 21-32.

Zhang M., Fang T., Pu G., Sun X., Zhou X. Cai Q. (2013). Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avanae* (F.) (Hemiptera: Aphididae). Pesticide Biochemistry and Physiology, 107, 44-49.

DOI: 10.17707/AgricultForest.63.1.12

Reşat DEGIRMENCI,
Huseyin VAROGLU, Lutfi TAHTACIOGLU [1]

DETERMINATION OF SOME BARLEY (*Hordeum vulgare L.*) VARIETIES SUITABLE FOR CYPRUS ECOLOGICAL CONDITIONS

SUMMARY

This research was carried out on 6 barley varieties (two-rowed; Otis, Nure, Pewter Fouga, Prestige and six rowed; Athenais) in a Randomized Complete Block Design with three replications in 2008-2009 growing season in Cyprus; Turkmenkoy, Kumkoy, Tepebas and Haspolat locations. It was determined that the effects of genotype, environment and genotype x environment interaction were significant at 1% level of probability for grain yield. Grain yield varied from 61.0 – 410.5 kg/da. Kumkoy location had the highest grain yield (303.7 kg/da) while Turkmenkoy location had the lowest grain yield (118.3 kg/da). While, cultivars Nure and Pewter had the highest yield (250.0 and 261.4 kg/da), cultivars Fouga and Prestige had the lowest grain yield (156.3 and 158.0 kg/da) for all environments. While the highest grain yield is found in Kumkoy location with Nure (410 kg/da) and Pewter (400 kg/da), Turkmenkoy location has the lowest grain yield with Fouga (61 kg/da) variety.

Keywords: *barley, location, grain yield, cultivars*

INTRODUCTION

Cyprus is the third largest island in the North East of the Mediterranean Sea with 9251 km^2 area. However; the area of Northern Cyprus is 3298 km^2. By looking at the geographical location of the island; there are Besparmak Mountains which forms the Turkish side of the island and range from east to the west. Trodos Mountains on the other hand form the Greek side of the island and range on the middle part. There is arid Mesarya lowland between these two mountains and most of this part is this lowland. The western part of this region is called West Mesarya Lowland which is arable for irrigated farming as a result of groundwater springs in that region whereas on the East Mesarya dry farming is held mostly.

On the island where typical Mediterranean climate reigns over, the average precipitation is between 350-400 mm/year as well as altering per years. Precipitation is generally in dense in December-May (Statistics and Planning Division, 2011).

[1]Reşat Degirmenci (corresponding author: rdeirmenci@yahoo.com), Huseyin Varoglu The Ministry of Food, Agriculture and Energy, Agricultural Research Institute, CYPRUS, Lufti Tahtacioglu Crop Husbandry Technical Assistance Team, CYPRUS

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

134.3 million tons of barley is produced on 48.6 million hectare area in the world. The yield per decare is 276.3 kg In Turkey, the barley cultivation area is 3.4 million hectares and production amount is 7.6 million tons. Yield per decare is 232.0 kg and it is the second after wheat in grains (FAO, 2011). In North Cyprus, there is 124,000 ha cultivation area existing and Grain is cultivated in 84,000 ha area which corresponds to this land's %65-70. The largest share is barley (58,325 ha) with %93 ratio in grain lands then respectively wheat (4046 ha) with %6.5 ratio, oat (530 ha) with %1 ratio (Statistics and Planning Division, 2010).

The demand for barley has been increasing day by day which has a large usage area in feed and food industry. Although the livestock industry has improved in the country, the dry farming has spotlighted the barley due to insufficient pasture areas, limited water and precipitation and inability to cultivate roughage.

The barley cultivation area of North Cyprus is 80.7 thousand hectare and yield per decare is 208 kg which means below the average of Turkey and the world. This amount is not sufficient for the country and 51.000 tons of barley is imported average per year (Statistics and Planning Branch, 2011).

As being unable to increase the current cultivation sites; increasing the production amount from unit site is important and the variable to be used will contribute most importantly. In this study barley varieties which origin from Italy, France and Greece is tested in 4 different locations of Cyprus in terms of yield capability.

MATERIALS AND METHODS

This study is held in the producer lands of 4 different locations (West Mesarya-Kumkoy, North Coast- Tepebasi, Middle Mesarya- Haspolat, EastMesarya- Turkmenkoy) which represent different conditions of North Cyprus in 2008-2009 within the conditions without irrigation. 6 barley cultivars are used in the experiment and related information is given in Table 1.

Table 1.Information related to Barley varieties used in the Experiment

Cultivar Name	Country of Origin	Row Number
Athenais	Greece	6
Pewter	Greece	2
Otis	Italy	2
Nure	Italy	2
Prestige	France	2
Fouga	France	2

Areal precipitation per month, average relative humidity and average temperature per month related to the production years are given on Table 2. The soil types are different where the experiments were held and its characteristic features are given on Table 3.

Table 2. Climate Data related to 2008-2009 years

Months										
		Nov	**Dec**	**Jan**	**Feb**	**March**	**April**	**May**	**June**	**Period aver/ total**
Avarege temperature (C^0)	Turkmenkoy	17.2	11.9	16.6	17.0	18.8	24.6	28.7	34.6	21.2
	Tepebaşı	18.2	13.2	15.4	15.5	16.8	21.4	25.1	31.1	19.6
	Kumkoy	18.4	13.6	16.8	16.5	17.9	22.6	26.0	31.6	20.4
	Haspolat	17.4	12.0	11.3	11.6	12.2	17.1	21.8	28.1	16.4
Avarege relative humidity(%)	Turkmenkoy	64.0	67.7	72.0	76.3	70.7	62.0	51.9	44.2	63.6
	Tepebaşı	68.3	68.3	70.8	72.6	65.3	65.4	58.6	52.7	65.3
	Kumkoy	59.8	59.8	66.7	69.7	65.5	62.7	57.6	52.8	61.8
	Haspolat	63.4	67.7	71.6	78.3	70.1	61.7	50.9	41.6	63.2
Areal Precipitation (mm)	Turkmenkoy	20.6	61.9	49.5	40.4	51.4	27.4	18.3	0.7	270.2
	Tepebaşı	16.6	84.2	78.6	95.3	63.0	11.7	11.6	0	361.0
	Kumkoy	2.8	67.7	79.8	93.7	57.7	15.5	10.5	0	327.7
	Haspolat	9.2	49.3	41.3	28.9	40.1	22.5	9.4	0	200.7

Table 3. Soil Characteristics of Lands where the experiments were established

Location	**pH**	**Lime (%)**	**Total Salt (%)**	**Organic substance (%)**	**Texture**
Tepebası	7.4	22.0	0.06	2.30	Sandy-Loam
Turkmenkoy	7.8	36.6	0.05	1.22	Sandy-Loam
Kumkoy	7.3	14.1	0.05	1.30	Loamy-Sand
Haspolat	7.5	22.0	0.28	1.68	Argillaceous-Loam

The experiments were established in 3 replications according to Randomized Complete Block Design. The planting was done in parcels with 1.2 m width and 5m height to have 500 seeds per m^2. Each parcel included 6 plant lines with 20cm gaps. The parcels were dressed according to 8 kg/da pure N and 5 kg/da P_2O_5/da. The half of the Nitrogen and total Phosphorus was applied during the plantation and the remaining half of the Nitrogen will be applied at the beginning of bolting. In full-fledging period; from the sides of parcels 1 line each and from the parcel heads 50 cm each were cut and the remaining parts were harvested and blended by parcel combine harvester. The data obtained from the experiment were analyzed by JMP statistical package program and the differences in averages were compared by LSD test.

RESULT AND DISCUSSION

Turkmenkoy

The plantation of the experiment was done on 19.12.2008 with a grain drill. The experiment was established on a land where the organic substance and productivity is low and the soil is calcareous and its structure was destroyed

excessively. This land was chosen deliberately and rolling was done after plantation.

After about 15 days of planting, there was a good rain there was a perfect output in every types. Until the earing period, the plant progress began to regress due to decreasing rains and these decreasing rains in grain forming period have affected the productivity of all cultivars negatively. The experiment was harvested with parcel combine harvester on 11.05.2009.

In terms of rainfall during growing season, the second disadvantageous region is Turkmenkoy. Also, the obtained yield illustrates this openly. Great differences in terms of yield between variables are observed. On this location, the highest yield is obtained from Pewter with 162 kg yield decare, and then Athenais and Nure the followers of it. Again among these types; the earliest type is Athenais and latest is Fouga. Despite all the applications such as foreign plant control and tillage were in the optimum level, Turkmenkoy with 118 kg/da grain yield has been the location with the lowest average yield. It is obvious that; this is caused by bad soil conditions, rain amount and distribution on the plant growing period completely.

Tepebasi

The planting of experiment was done on 10.12.2009 with a grain drill and harvested on 01.06.2009. As can be seen the graph below, the obtained grain yield for all cultivars are above the country average and the average yield of this location is 233 kg/da.

Despite the antecedent precipitation and differences of soil structure, the performances of cultivars are similar to Turkmenkoy. While the highest yield in Tepebasi is obtained from Nurecultivar with 310 kg/da, Pewter (283 kg/da) has been the follower of it whereas the Athenais has been on the 4th line.

In case the environment becomes more convenient in terms of soil and climate; some cultivars which have high yield potential in good environment have become prominent. However; the types which have good performance in weak environment have dragged.

Kumkoy

This study was held in Kumkoy; a land 2 km far from the sea, very salty and previously hesperidium planting was done. However; the salinity was observed as not having a significant effect on barley.

The experiment was planted on 18.12.2009 with a grain driller and harvested on 13.05.2009.

Regarding the precipitation amount and soil conditions; the highest yields in all cultivars were obtained from this location. The average yield of the location is 304 kg/da. The highest yield is obtained from Nure cultivar with 410 kg/da and however the Pewter which had shown highest yields other locations has been on the second line with its 400 kg/da yield. Athenais on the other hand, has been on the fifth line with its 240 kg/da yield.

This experiment held in Kumkoy is a proof for obtaining very high yields in even one year from barley on the condition that using most suitable types for ecology.

Haspolat

On this field where it is close to water treatment unit of the municipality in Haspolat, planting was done with a grain driller on the 09.01.2009 and harvested on 11.05.2009.

Haspolat region is also the less rained land during plant growing period so; compared to other locations relatively, even though it has better soil conditions the grain yields here has been in very low level with 146 kg/da. The highest yield on this location is obtained from Nure cultivar with 185 kg/da and Pewter and Otis respectively. Athenais however has been on the fifth line.

General Evaluation of the Locations

The performances of types used in the experiment in different locations are given on the table below.

Table 4. The yields of cultivars in the experiments in different locations

	Turkmenkoy	Tepebasi	Kumkoy	Haspolat	Average
	Yield kg/da	Yield kg/da	Yield kg/da	Yield kg/da	Yield kg/da
Otis	113 ı-k	205 d	308 b	170 f	**199.0**
Nure	140 gh	310 b	410 a	185 ef	**261.4**
Athenais	142 gh	200 de	240 c	120 h-j	**175.4**
Prestige	92 k	157 fg	245 c	138 g-ı	**158.0**
Pewter	162 fg	283 b	400 a	155 fg	**250.0**
Fouga	61 l	240 c	219 cd	105 jk	**156.2**
Average	**118.3**	**232.5**	**303.7**	**145.5**	**200.0**
CV (%): 8.2 Lsd (0.05); Cultivar:13.5, Location: 11.0, Cultivar x Location: 26.9					

When the experiments are evaluated statistically; the effects of genotype, environment and genotype x environment interaction were significant at 1% level of probability for grain yield. While the highest grain yield is in Kumkoy location with Nure (410 kg/da) and Pewter (400 kg/da) types, the lowest grain yield is in Turkmenkoy location with Fouga (61kg/da) type.

When the locations are considered; the highest average yield is from Kumkoy with 304 kg/da and the lowest average yield is from Turkmenkoy with 118 kg/da. This difference is mainly caused by precipitation and distribution as well as subsidiary factors such as soil structure, etc.

When evaluating the types used in the experiments; the highest yield is obtained from Nure type with 261 kg/da according to average of grain yield in all locations and then respectively, Pewter 250 kg. and Athenais with 176 kg/da as

on the 4th line. The obtained results of Degirmenci et al., 2014 have shown similar results of the Degirmenci and Varoglu 2015 and Serinay et al.,2013 and Akerzurumlu 2014. However; these obtained results are found to be lower than Kirtok etal., 2001 results.

Although the results are for one year, while evaluating all cultivars and locations in this study can be said to be hint for barley agriculture.

CONCLUSIONS

Since the main limit in dry farming is water, the water efficiency of the cultivars used is expected to be high. Even the Athenais is used broadly and has more performance in weak and less rained environments; using this cultivar in the country in general is not a right choice. Giving advice according to the environment will provide better yields in normal rained years and better environments from the high potential cultivars and will make barley farming more economic.

Eliminating the effects of drought completely might be impossible with today's technology. However; spreading a science based farming in the system and minimizing the effects of drought should be the main target. With this regards; adding different variables to the production for different ecological zones of the country is possible and also integrating agronomic techniques which increase the water use efficiency makes barley farming more economic possible.

REFERENCES

Akerzurumlu E. (2004). A Study On Determining The Suitable Nitrogen Rate and Sowing Density For Barley In Cyprus conditions. M.Sc. (Agri.) Thesis, University of Cukurova, Adana.

Degirmenci et al.(2014). A Research On Yield And Yield Components Of Some Barley Cultivars. Scientific Conference. Challenges in Modern Agricultural Production, December 11, 2014, Skopje.

Değirmenci, R., Varoğlu H.(2015). A Research On Yield And Yield Components Of Some Barley Cultivars In The Mesoria Plain. 2nd Inner Anatolia region of agriculture and food congress, 28-30 April 2015, Nevsehir.

FAO (2011). Statistical database. http://www.fao.org.Accesed on 07/08/2011

Statistics and Planning Division (2010). Agricultural Structure and Production 2010.The Ministry of Agriculture and Forestry. Nicosia.

Statistics and Planning Division (2011).Agricultural Structure and Production2011.The Ministry of Agriculture and Forestry. Nicosia.

Kirtok et al. (2001).Investigation To Determine High Yielded Barley Cultivars In Irrigation Conditions Of Turkish Republic Of Northern Cyprus. The 4th Field Crops Congress, 17-21 September 2001, Tekirdag.s:147-150.

Serinay et al. (2013).Response of Three Barley Cultivars (Hordeum vulgare L.) to Various Nitrogen Rates In The Mesoria Plain. International Agriculture Congress, 26-29 September 2013, *Antalya*.

DOI: 10.17707/AgricultForest.63.1.13

***Valentine BRAILKO, Olga MITROFANOVA, Nina LESNIKOVA-SEDOSHENKO, Svetlana CHELOMBIT, Irina MITROFANOVA*[1]**

ANATOMY FEATURES OF *LAVANDULA ANGUSTIFOLIA* MILL. AND *LAVANDULA HYBRIDA* REV. PLANTS *IN VITRO*

SUMMARY

Lavender and lavandin are widely used in food, pharmaceutical, cosmetic industries and ornamental gardening. Gene pool collection of *Lavandula angustifolia* and lavandin is presented in Nikita Botanical Gardens plots and the same collection is being created *in vitro*.

Initial explants of *L. angustifolia*, its cultivars 'Belyanka', 'Record' and *L. hybrida* – 'Rabat' and 'Snezhnyi Bars' were introduced *in vitro*. Plant regeneration occurred on MS medium supplemented with 0.3-0.5 mg l^{-1} kinetin, 0.025 mg l^{-1} NAA, 0.25 mg l^{-1} GA_3. Samples were analyzed after 2 and 8 months of culture.

Structural analysis of regenerated plants vegetative organs enabled assessment of their morphogenetic capacity. Under *in vitro* culture 2-5 microshoots/explants were produced with the height of 2.3 - 8.2 cm. Leaves were opposite, sessile linear or linear-lanceolate, with entire edges curved outward, densely pubescent, and 10-30 leaves/microshoot. Leaf lamina length was 0.9-1.5 cm. Leaves were bifacial, amphystomatic, lamina thickness – 132-215 μm, palisade rate was 0.38-0.41 after 2 months culture and 0.56-0.58 – after 8 months. Epidermal thickness was 8-19 μm, cells were relatively small with thin, cellulose walls covered with cuticle. Stomata are of anomocyte type, small, raised above the epiderm cells, 48-150 pcs/mm^2 on adaxial surface, 115-432 pcs/mm^2 – on abaxial one. Essential oils are produced in simple and glandular, unicellular and multicellular hairs of epidermal origin, located above the leaf chlorophyll tissue. Features of xeromorphic structure appeared at a long-term *in vitro* cultivation.

Keywords: *lavender, lavandin, regenerant, leaf anatomy, xeromorphic structure.*

INTRODUCTION

In Eastern Europe, over 60 species of medicinal plants are cultivated. *Lavandula angustifolia* is among the three leading medicinal and aromatic crops. Lavender breeding in Nikita Botanical Gardens engaged since 1926. Cultivars

[1]Valentine Brailko (corresponding author: brailko@yandex.ua), Olga Mitrofanova, Nina Lesnikova-Sedoshenko, Svetlana Chelombit, Irina Mitrofanova Nikita Botanical Gardens - National Scientific Centre, Nikita, Yalta, 298648, RUSSIA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

with high oil yield, great part of esters, excellent fragrance evaluation, different terms of blossom, winter-hard and suitable for mechanized cultivation have been created (Mitrofanov et al., 2005).

As it is known, lavender could be successfully propagated both by seeds and vegetatively – by cuttings, layering and shrub dividing. Despite the fact that seed propagation method is the simplest and economically costly, it is not widely used as it is resulted in the offspring splitting (Rabotyagov et al., 2014). In this regard, the more efficient way is vegetative propagation. However, the problem of viral pathogens damages in collection plots and industrial plantings hinders vegetative propagation, and as a result, reduces possibility of promising lavender cultivars large-scale cultivation. Nowadays, producing of high-quality virus-free planting material identical to the parent plants is provided with biotechnological methods of plant cleaning up and micropropagation (Milošević et al., 2012;. Bhojwani & Dantu, 2013; Mitrofanova et al., 2014.).

Present study included cultivars of interspecific lavender hybrids (*L. angustifolia* Mill × *L. latifolia* Medie.) – Lavandin (*L. hybrida* Rev.) that are characterized by biological and economically valuable features exceeded the original species.

Some researchers (Sudria et al., 1999; Toma et al., 2004, Nikolakaki and Christodoulakis, 2006) indicated the possibility of essential oil obtaining from vegetative mass of Labiatae species and cultivars under *in vitro* conditions. Herewith, great attention was turned to the processes of histogenesis and organogenesis in cultured cultivars.

In this regard, the aim of our research was to study regeneration features in lavender and Lavandula plants, to evaluate morphological and anatomical parameters of tissues in various cultivars under *in vitro* conditions, to reveal those features variability according to the genotype, and thus, to determine morphogenetic capacity of regenerants.

MATERIALS AND METHODS

For study as a plant material microshoots of valuable lavender (Belyanka, Record) and Lavandin (Rabat, Snezhnyi Bars) cultivars from the collection of the Nikita Botanical Gardens, cultured *in vitro* were used.

From field grown intact plants the apical meristems of axillary buds were introduced in aseptic culture. Virus-free microshoots were cultured on modified MS medium with 0.3 mg l^{-1} Kinetin, 0.025 mg l^{-1} NAA and 0.25 mg l^{-1} GA_3. Explants in the culture vessels were kept in a growth chamber at 25 ± 1°C under 16-h photoperiod supplied by cool-white fluorescent lamps giving 37.5 μmol m^{-2} s^{-1}. Investigation material was collected after 2 and 8 months of culture.

For the analysis we took the third pair of leaves at microshoot apex from 10 microshoots in each treatment. Anatomic slides were prepared by common methods (Pausheva, 1990). Histological and anatomical studies of leaves in regenerants was carried out under a light microscope AxioScope A.1 (Zeiss,

Germany) using software Axio Vision Rel applications. 4.8.2. Essential oil presence in leaf tissues was set by Sudan III staining.

RESULTS AND DISCUSSION

During the study, morphological differences between the studied cultivars were already noticed at the initial stages of culture (Fig. 1). They appeared both in leaf color of microshoots: light-green narrow-linear or lanceolate in the cultivars Belyanka and Record and dark green in the cultivars Rabat and Snezhnyi Bars, and some quantitative parameters: number of microshoots produced per explant, microshoot height, size and number of leaves (Table 1).

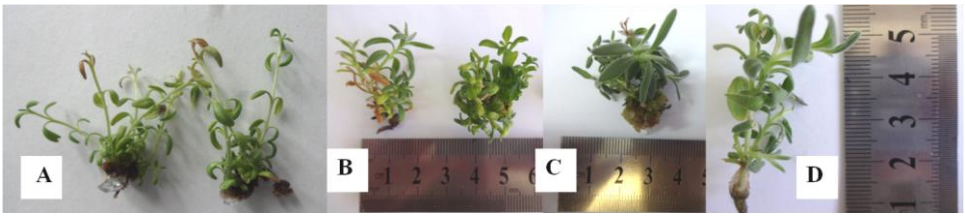


Figure 1. Regenerants appearance in lavender cultivars: A – Belyanka, B – Record and Lavandin cultivars: C – Rabat, D – Snezhnyi Bars after 2 months culture.

Table 1. Quantitative characteristics of regenerant morphological structures in lavender and lavandin (M ± SE)

Cultivar, time of culture			Microshoot number per explants, pcs	Microshoot height (cm)	Number of leaves (pcs)	Leaf size, cm
lavender	Belyanka	1*	3.4 ± 1.3	5.8 ± 1.5	22.8 ± 9.2	1.2 × 0.3
		2	3.2 ± 1.5	3.5 ± 0.8	14.8 ± 8.6	1.2 × 0.2
	Record	1	3.2 ± 1.6	2.5 ± 0.4	12.5 ± 5.0	1.0 × 0.2
		2	2.8 ± 1.2	4.1 ± 1.2	19.4 ± 6.5	1.1 × 0.2
lavandin	Rabat	1	1.7 ± 0.5	2.3 ± 1.0	8.0 ± 4.6	1.4 × 0.4
		2	3.1 ± 1.6	3.8 ± 0.5	14.0 ± 5.0	1.5 × 0.3
	Snezhnyi Bars	1	1.2 ± 0.8	4.9 ± 1.1	13.3 ± 8.4	1.6 × 0.4
		2	2.0 ± 1.0	4.5 ± 0.8	18.4 ± 4.5	1.4 × 0.3

1* - the first passage, 2 months culture; 2 - the fourth passage, 8 months culture.

Greater number of microshoots (5 pcs) with maximum small leaves per shoot formed Belyanka cultivar. In Lavandin cultivars 1-2 microshoots with a denser leaf arrangement in the cultivar Rabat and extended internodes in the cultivar Snezhnyi Bars formed. Leaves in Lavandin cultivars were larger and less acuminate at the top than in lavender cultivars. Leaf blade length exceeded its width 4-5 times.

Those morphological features are characteristics of leaves under growing in the field (Rabotyagov, 2014).

Under prolonged culture (4th passage) in all studied cultivars 2-4 microshoots were noticed in the average, branching occurred at the second or third pair of leaves on microcuttings. Cultivar Rabat had the largest leaves in all investigated culture stages.

Leaf blades were bifacial (Fig. 2), differentiated mesophyll was observed from early terms of microshoot culture *in vitro*. Leaf thickness increased under a long term culture (Table 2). Adaxial epidermis consisted of small isodiametric (cultivars Snezhnyi Bars and Record) or slightly elongated (cultivars Rabat and Belyanka) thin-walled cellulose cells with flexuous outlines, covered with thin cuticle layer (Table 3). Leaf abaxial epidermis in all studied cultivars consisted of smaller cells.

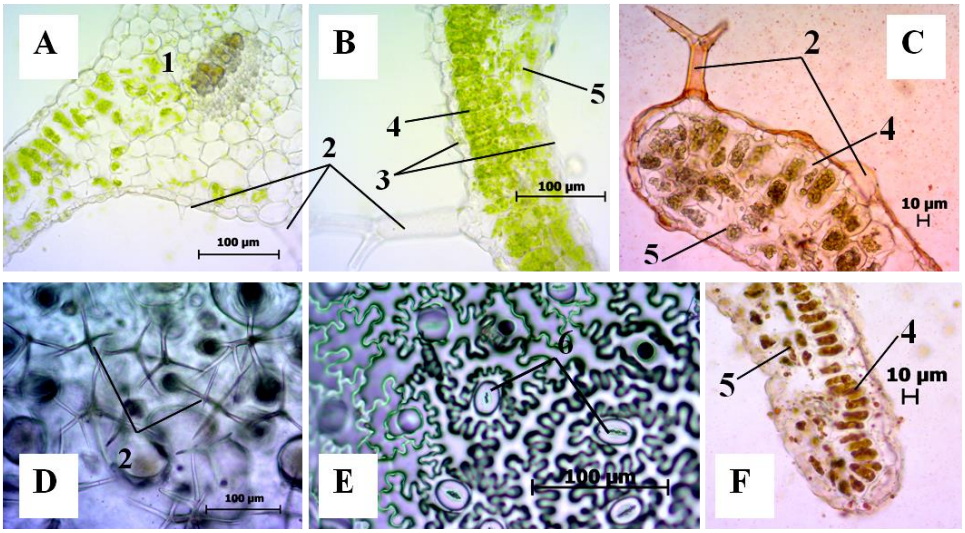


Figure 2. Leaf blade structure in lavandin cultivar Rabat *in vitro*: A, B – cross-section, C, F –cross sections stained with Sudan III; D, E – molds of abaxial epidermis; 1 – vascular bundle, 2 – trichomes, 3 – epidermis, 4 – palisade mesophyll, 5 – spongy mesophyll, 6 – stomatal apparatus.

Table 2. Anatomical characteristics of leaf blade transverse sections in lavender and lavandin regenerants (M ±SE, µm)

Indexes of anatomical structure		**Lavender**				**Lavandin**			
		Belyanka		Record		Rabat		Snezhnyi Bars	
		1*	2	1	2	1	2	1	2
Leaf blade thickness		130± 12	169± 16	145± 15	174± 43	151± 24	190± 26	159± 13	168± 21
Epidermis thickness	**Adaxial**	9±3	14±5	9±2	15±3	10±3	19±7	10±4	14±2
	Abaxial	5±2	9±3	6±2	8±2	8±2	11±5	6±2	9±4
Mesophyll thickness	**Palisade**	45±13	80±12	49±7	85±10	55±15	90±8	58±9	82±7
	Spongy	71±16	61±15	81±15	62±16	78±12	68±12	86±12	59±5
Palisade index		0.39	0.56	0.38	0.57	0.36	0.56	0.40	0.58

1* - the first passage, 2 months culture; 2 - the fourth passage, 8 months culture.

Table 3. Anatomical characteristics of leaf blade epidermis structure in lavender and Lavandula microshoots (after 8 months culture, M ± SE, μm)

Indexes of anatomical structure/Cultivars		**Lavender**		**Lavandula**	
		Belyanka	Record	Rabat	Snezhnyi Bars
Epidermis cells size	1*	60×33	32×23	52×35	29×27
	2	46×28	59×33	39×30	28×22
Stomata pore size (length×width)	1	27×13	17×7	25×11	25×14
	2	29×14	31×15	26×10	17×10
Stomata number per 1mm^2 surface	1	102±16	150±19	127±24	48±16
	2	180±21	115±13	432±52	283±27
Trichome number per 1mm^2 surface	1	21±8	48±6	38±6	26±9
	2	52±6	75±13	72±6	60±10

1* - on adaxial surface, 2 – on abaxial surface of the leaf blade

Vascular bundles in leaves are collateral. They are located in the middle layers of mesophyll (Fig. 2). Bundles of lateral veins consisted of three or four xylem and phloem vessels. Leaves were amfistomatic, under prolonged culture their significant predominance on abaxial surface was noticed. Stomata are anomocytic (ranunculaceous), and differed in sizes, raised above the base of the epidermis cells.

It is worth saying that density of stomata distribution had significant differences in the studied cultivars. Thus, in lavender cultivars it was 102-180 stomata/mm^2, stomata arranged substantially uniformly. In the cultivar Record numerous small stomata located on adaxial side, and the largest (31×15 μm) one in a lower number were on the abaxial side. Maximum number of stomata and their largest size were found in the cultivar Rabat. Guard cells are oval.

Our study demonstrated that pubescence of leaves' *in vitro* regenerated plants consist of single or double-celled simple, slightly branched trichomes and 2-4-, rarely 6-cell capitate glandular hairs. Their number is also greater on abaxial side (52-75 trichomes/mm^2). Due to leaves cross-sections analysis it was established that the part of glandular hairs was 23-38% of the total trichomes number. That corresponds to the data presented A. Nikolakaki and N.S. Christodoulakis (2006) who are reported that in *Lavandula vera* L. cultivars number of cells with secreting activity was not more than 30%.

M. R. Zuzarte et al. (2010) during morphological analysis demonstrated that trichomes on *Lavandula pedunculata* leaves *in vitro* are similar to the ones in intact plants. Histological investigation by Avato P. (2005) and Kamatou G.P.P. (2007) over *Salvia officinalis, S. albicaulis* and *S. dolomitica* plants on different stages of aseptic culture demonstrated the presence of shield-shaped glandular scales that produced high quality essential oils. Glandular trichomes were also described on leaf surface in *Mentha piperita* regenerants (Santoro et al., 2013). Glandular hairs are formed from epidermal outgrowths that divide asymmetrically to produce basal cells (Turner et al., 2000).

At the same time we are observed that the greatest accumulation of essential oil in dispersed state occurred in capitate glandular hairs. Also, essential oil droplets were formed in small amounts in the vacuoles of mesophyll cells. They were especially strongly expressed in the cells of palisade parenchyma. In lavandin cultivar Rabat drops of essential oil were more numerous and larger than in the other studied cultivars. For that cultivar their formation was also noted in the cells of parenchymal sheath of vascular bundles.

Mesophyll was multi-layered, consisting of 1-2 palisade chlorenchym rows with elongated, tightly arranged cells and 2-3 rows of spongy tissue with loose cell arrangement, contact with one another with protrusions. In all studied leaves from the microshoots of first passage palisade tissue consisted of a single layer, 45-58 μm thick. Palisade index was maximum in the cultivars Snezhnyi Bars (0.40) and Belyanka (0.39). Cells had oval-round shape. On the leaf sections from the 4th passage 2 layers of palisade cells were often observed. Maximum development palisade chlorenchym had in Rabat cultivar (90±8 μm). Palisade index increased to 0.56-0.58.

As V.D. Rabotyagov et al. (2014) pointed out; in interspecific hybrids intermediate type of anatomical structure inheritance is expressed. This phenomenon can be explained by the increase of homeostasis, which enables them to better adapt to low and high air humidity and substrate.

CONCLUSIONS

Thus, we not found any significant difference between lavender and lavandin cultivars in development of vegetative organs, formation leaves and internodes under long-time *in vitro* culture. Differences in the studied cultivars identified in leaf blades shape, color and size.

Anatomical analysis of microshoot leaves demonstrated that together with mentioned morphological features of vegetative organs, there were differences in such parameters as leaf blade thickness, stomata number on abaxial surface and essential oil localization.

Chlorenchym in the studied cultivars differentiated into palisade and spongy tissue. Sophistication of palisade leaf tissue in the cultivars Rabat and Record, cutinized cover tissues, dense pubescence confirm the presence of xeromorphic features and ability to water stress adaptation under *ex vitro* conditions. At the same time, large cells and loose arrangement of mesophyll in the cultivars Belyanka and Snezhnyi Bars demonstrated ability to develop normally both in xeromorphic environment and in wet habitats. Essential oil was accumulated in glandular and simple hairs, as well as in vacuoles of mesophyll cells and cells of mechanical leaf tissues in dispersed state.

Knowledge of leaf anatomy in the studied cultivars under *in vitro* conditions could be used in lavender and lavandin breeding works.

ACKNOWLEDGMENT

This study was funded by a research grant № 14-50-00079 of the Russian Science Foundation.

REFERENCES

Avato, P., Fortunato, I. M., Ruta, C., & D'Elia, R. (2005). Glandular hairs and essential oils in micropropagated plants of *Salvia officinalis* L. Plant Science,169(1), 29-36.

Bhojwani S.S., Dantu P.K. (2013). Production of Virus-free Plants. In: Plant Tissue Culture: An Introductory Text, 227-243. doi: 10.10007 / 978-81-322-1026-9_16.

Kamatou, G. P. P., Viljoen, A. M., Figueiredo, A. C., Tilney, P. M., Van Zyl, R. L., Barroso, J. G., & Van Vuuren, S. F. (2007). Trichomes, essential oil composition and biological activities of *Salvia albicaulis* Benth. and *S. dolomitica* Codd, two species from the Cape region of South Africa. South African Journal of Botany, 73(1), 102-108.

Milošević S., Cingel A., Jevermovic S., Stancovic I., Bulagic A., Krstić B., & Subotic A. (2012). Virus elimination from ornamental plants using in vitro culture techniques. Pestic. Phytomed, 27 (3), 203-211. doi: 10.2298 / PIF1203203M.

Mitrofanov V.I., Rabotyagov V.D., Samoylov Yu.K., Azarova Z.F., & Aksenov Yu.V. (2005). Lavender: elite nurseries, Yalta, NBG-NSC, 60 p.

Mitrofanova O.V., Mitrofanova I.V., Lesnikova-Sedoshenko N.P., & Ivanova N.N. (2014). Using of biotechnological methods for plants improvement and propagation of virus-free planting material of perspective ornamental plants. Works of the State Nikit. Botan. Gard., 138, 5-56.

Nikolakaki A., Christodoulakis N.S. (2006). Histological investigation of the leaf and leaf-originating calli of *Lavandula vera* L. Israel Journal of Plant Sciences, 54 (4), 281-290, doi: 10.1560 / IJPS_54_4_281.

Pausheva Z.P. (1990) Practicum in plant cytology. M., Kolos, 283 p.

Rabotyagov V.D., Svidenko L.V., & Boyko M.F. (2014). Anatomical and morphological features of interspecific F1 hybrids in comparison with the original diploid species and tetraploid. Chornomors'k. bot.z., 10 (1), 127-137. doi: 10.14255 / 2308-9628 / 14.101 / 14.

Santoro, M.V., Nievas, F., Zygadlo, J., Giordano, W., & Banchio, E. (2013). Effects of growth regulators on biomass and the production of secondary metabolites in peppermint (*Mentha piperita*) micropropagated *in vitro*. American Journal of Plant Sciences, 4, 49-55. doi: 10.4236/ajps.2013.45A008.

Sudria C., Pinol M.T., Palazon J., Cusido R.M., Vila R., Morales C., Bonfill M., & Canigueral S. (1999). Influence of plant growth regulators on the growth and essential oil content of cultured *Lavandula dentate* plantlets. Plant Cell Tissue Organ. Cult., 58 (3), 177-184. http://dx.doi.org/10.1023/A:1006377003962

Toma I., Toma C., Ghiorghita G. (2004). Histo-anatomy and *in vitro* morphogenesis in *Hyssopus officinalis* L. (Lamiaceae). Acta Botanica Croatica, 63 (1), 59-68.

Turner, G.W., Gershenzon, J., & Croteau, R.B. (2000). Development of peltate glandular trichomes of peppermint. Plant Physiology, 124(2), 665-680.

Zuzarte, M.R., Dinis, A.M., Cavaleiro, C., Salgueiro, L.R., & Canhoto, J.M. (2010). Trichomes, essential oils and *in vitro* propagation of *Lavandula pedunculata* (Lamiaceae). Industrial Crops and Products, 32(3), 580-587.

DOI: 10.17707/AgricultForest.63.1.14

Mehmet Salih SAYAR[1]

ADDITIVE MAIN EFFECTS AND MULTIPLICATIVE INTERACTIONS (AMMI) ANALYSIS FOR FRESH FORAGE YIELD IN COMMON VETCH (*VICIA SATIVA* L.) GENOTYPES

SUMMARY

The study was held to evaluate genotype × environment interactions and stability status of twenty common vetch (*Vicia sativa* L.) genotypes in terms of fresh forage yield trait by using additive main effects and multiplicative interactions analysis (AMMI). Field trials of the study were carried out during 2008-09, 2009-10 and 2010-11 growing seasons under the rainfed conditions of two different locations of the Southeastern Anatolia region of Turkey. The field trials were established according to randomized blocks design with three replications. Additive main effects and multiplicative interactions analysis (AMMI) showed that the effect of environments on genotype × environment interactions were found quite high levels for fresh forage yield trait. The first three principal component axes (IPCA 1 IPCA 2 and IPCA 3) were found highly significant (P<0.01), and they accounted for 93.44% of the total genotype by environmental interaction. Furthermore, the effect of environments on forage yields of common vetch genotypes was found to be highest (42.23%), it was followed by genotype×environment interaction (GEI) (36.13%) and genotypic effects (21.64%). AMMI analysis revealed that with their high yield means, and lower IPCA-1 scores, close to zero, respectively, D-135 (G6), IFVS-2541 (G4) and IFVS-715 (G3) were considered to be possessing high stable fresh forage yields. Therefore, these genotypes should be preferred for forage yield production in Southeastern Anatolia region conditions.

Keywords: *AMMI analysis, fresh forage yield, common vetch, genotype × environment interactions, stability*

INTRODUCTION

Common vetch (*Vicia sativa* L.) is one of the most highly cultivated annual legume forage species in Turkey and worldwide. It is typically grown for its forage and seeds and is also used as green manure (Cakmakci *et al.*, 2003). As a legume species, common vetch contributes to increased nitrogen content in the soil and prevents disease in subsequent crops in crop rotation systems (Ayed *et al.*, 2001; Caballero *et al.*, 2001). Common vetch forage is nutritious due to its

[1]Mehmet Salih Sayar (corresponding author: msalihsayar@hotmail.com) Department of Crops and Animal Production, Bismil Vocational Training High School, Dicle University, 21500, Bismil, Diyarbakır, TURKEY

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

high protein content and it is eagerly consumed by livestock due to its taste, without an accompanying risk of swelling (Acikgoz, 2001). A shortage of quality roughage is a serious problem for animal husbandry in Turkey, particularly in the Southeastern Anatolia Region (Sayar *et al.,* 2010). Plant breeders attempt to alleviate this deficiency by improving and introducing higher yield forage cultivars (Sayar *et al*. 2013).

Yield is a complex trait that depends on many other traits and is greatly affected by environmental factors. However; plant breeders and farmers demand stable varieties, has high mean yield but showing little fluctuation in performance when grown over various environments. Therefore; genotype–environment interactions (GEI), response of genotypes to different environments, are extremely important in plant breeding programs when introducing new crop cultivars (Sayar *et al*., 2013; Kendal and Dogan, 2015).

The additive main effects and multiplicative interaction (AMMI) analysis is one of the most useful and the most commonly used method in determining and evaluation of the GEI. AMMI model is a hybrid model combines the analysis of variance (ANOVA) and the principal component analysis (PCA) in a single model (Gauch and Zobel, 1996; Mirosavljević *et al.,* 2014; Islam *et al.,* 2014). Among the reasons for the preference of AMMI analysis by researchers are that firstly, this method is quite effective revealing GEI, secondly, it can show impact degree of genotypes, environments and GEI on performance of genotypes separately, (Ebdon and Gauch, 2002; Tarakanovas and Ruzgas, 2006; Asfaw *et al*., 2009), finally, the results of AMMI analysis shown in common graphs are called biplot (Gabriel, 1971) and these AMMI biplot graphs enable us to find out explicitly which genotypes suitable for which environment and stability status of genotypes. Accordingly, the objectives of the study were to (i) explicate GEI obtained by AMMI analysis of yield performances of twenty common vetch genotypes over five environments, (ii) visually evaluate variation of yield performances across environments based on the AMMI biplot and (iii) determine genotypes with high fresh forage yield stability.

MATERIALS AND METHODS

Plant genetic materials: The study materials consisted of 20 common vetch (*Vicia sativa* L.) genotypes, seven cultivars, and thirteen promising lines. The used cultivars were Alinoglu-2001, Dicle, Gorkem, Kralkizi, Kubilay-82, Ozveren and Uludag. On the other hand, among the used five promising lines, IFVS-715, IFVS 2427, IFVS 2541, IFVS 3091 and IFVS 3889 were supplied from International Center for Agricultural Research in Dry Areas, Aleppo, Syria (ICARDA). In addition, the three promising lines, D-71, D-72 and D-135 belonged to GAP International Agricultural Research and Training Centre (GAP IARTC). Only Line-22 was supplied from Cukurova University Agriculture Faculty Field Crops, Adana, Turkey. The remaining four promising lines, GAP-2604, GAP 2490, GAP 61721, GAP 59998, were obtained from the Eastern Mediterranean Agricultural Research Institute, Adana, Turkey.

Table 1. Climatic and soil information of the environments where the genotypes tested

Environments	Growing seasons	Locations	Altitude (m)	Soil properties	Sowing date	The average temperature (°C)	Total rain fall (mm)
E1	2008-2009	Diyarbakır	603	pH=7.86 clay-loam	14.11.2008	12.40	455.0
E2	2009-2010	Diyarbakır	607	pH=7.86 clay-loam	20.11.2009	14.30	517.9
E3	2010-2011	Diyarbakır	605	pH=7.86 clay-loam	11.11.2010	13.60	553.0
E4	2008-2009	Hazro	815	pH=7.65 clay-loam	06.11.2008	11.90	927.4
E5	2009-2010	Hazro	808	pH=7.64 clay-loam	17.11.2009	13.80	1055.6

Soil and climatic properties: Soil, climatic and other data of environments where the experiments were conducted are given in Table 1. The environments soils (0–30 cm) had a clay loam texture, with a slightly alkaline pH. The soils were rich in calcium and potassium content, but they were poor in organic matter (0.5–3%) and useful phosphorus. The Southeastern Anatolia region is one of Turkey's seven census-defined geographical regions, and the region is characterized by a continental climate. In this region, summers are dry and hot, whereas winters are cool and rainy.

Field experiments

The field trials in Diyarbakır location were carried out in the experimental fields of the GAP International Agricultural Research and Training Centre (GAP IARTC) in Diyarbakir, Turkey, and in Sarıcanak village, Hazro district were carried out in the farmer's fields. All of the experiments were conducted under rainfed conditions according to a randomized complete block design with three replications. Each plot consisted of 6 rows with 5 m in length, and rows were spaced 20 cm apart. Diammonium phosphate fertilizer (DAP 18-46-00) (150 kg ha^{-1}) was applied in the experimental plots with the sowings. Weeds appearing in the experimental area were controlled by hand. The seeding rate was 200 seeds m^{-2} (Acikgoz, 2001). The sowings were made by using an experimental drill. At harvest a half-meter at the beginning and end of each plot was neglected to account for edge effects. For determining fresh forage yield genotypes; each plot was harvested separately in full flowering time. Then, the fresh forage yield was weighed immediately without weight losing. And, the determined fresh forage yield value was converted to hectare for each plot.

Statistical analysis

ANOVA and Genotype-Environment Interaction (GEI) were estimated by the AMMI model (Zobel *et al.*, 1988). All data were subjected to analysis using GenStat 14.1. software package (VSN International, 2011).

RESULTS AND DISCUSSION

The AMMI analysis of variance of fresh forage yield (t ha^{-1}) of the 20 common vetch genotypes tested in five environments showed that the environments (E), genotypes (G), genotype × environment interactions (GEI) were found statistically highly significant ($p< 0.01$). In addition, 42.23% of the total sum of squares (SS) was attributable to environmental effects, and 21.64% to genotypic effects (G), and 36.13% to GEI effects (Table 2). Similarly, many researchers reported that environmental effects were greater than genotypic effects on yield characters of genotypes (Kaya *et al*.,2002; Ilker *et al*., 2011; Sayar *et al*., 2013; Kiliç, 2014; Georgieva and Kosev, 2016; Kendal, 2016). A large sum of squares for environments indicated that the environments were significantly different each other, and the differences among environmental means causing most of the variation in fresh forage yield. The magnitude of the GEI sum of squares was 1.6 times larger than that for genotypes, indicating that there were a great variation in rankings of fresh forage yields of the common vetch genotypes with changing the environmental changes. AMMI analysis of the forage yield of genotypes were revealed that the mean squares of the first three IPCAs (Interaction Principal Component Axes) were significant at $P< 0.01$ level. And IPCA 1, IPCA 2 and IPCA 3 cumulatively contributed to 91.46% of the total GEI (Table 2). Hence, the model was adequate enough to explain the total genotype x environment interaction component.

In the AMMI-1 biplot, x axis represents the genotypes and environment main effect. If an environment has higher yield than grand mean represents favourable environmental condition. And it locates in right side of the x-coordinate. Accordingly, in the study; E1 and E4 environments had higher forage yield than grand forage mean, for they represented favourable environmental conditions. In contrast, forage yield of E2, E3 and E5 environments were to be lower than grand mean, and these three environments represented unfavourable environmental conditions. In the same way, if its yield is lower than grand mean, it locates in left side of the x-coordinate, and it represents unfavourable environmental conditions. Similarly, if a genotype locates on the right side of x axis, it represents adaptability to favourable environmental conditions. If a genotype locates on the left side of x axis, it represents adaptability to unfavourable environmental conditions. In the study, exclusively G4, G6, G13, G17, G18 had much higher fresh forage yield than grand mean, and they showed adaptability to favourable environmental conditions. However, G7, G8, G16, G19 had less forage yield than grand mean, for they showed adaptability to unfavourable environmental conditions. On the other hand, G1, G2, G3, G5, G9, G15 gave almost as much as grand mean forage yield, and they generally located in middle of x- axis, close to grand mean line, in the AMMI-1 biplots. Therefore they showed average adaptability to all environmental conditions (Table 3 and Figure 1). In the AMMI-1 biplot, y axis represents the effects of interaction (Figure 1).

Table 2. AMMI variance analysis for fresh forage yield (t ha^{-1}) of 20 common vetch genotypes tested in 5 environments

Resource of variance	Degree of freedom (DF)	Sum of squares (SS)	Mean squares (MS)	F Value	G+E+GE SS Explained (%)	GE SS Explained (%)
Genotypes	19	2073	109.1	10.08**	21.64	
Environments	4	4046	1011.6	34.62**	42.23	
Block	10	292	29.2	2.7**		
GEI	76	3462	45.6	4.21**	36.13	
IPCA1	22	1911	86.9	8.03**		51.18
IPCA2	20	871	43.5	4.02**		25.62
IPCA3	18	448	24.9	2.3**		14.66
IPCA4	16	232	14.5	1.34^{ns}		8.54
Residuals	0	0	0.0	0.00		
Error	190	2057	10.8			
Total	299	11930	39.9			

Table 3. Fresh forage yields (t ha^{-1}) means of 20 common vetch genotypes across five environments and IPCAg[1] scores of AMMI analysis

Genotypes		Grand means (t ha^{-1})	IPCAg[1]
G1	IFVS 3091	20.36	-1,481
G2	IFVS 2427	21.25	1,668
G3	IFVS 715	21.24	-0,125
G4	IFVS 2541	22.56	-0,227
G5	IFVS 3889	20.94	-0,414
G6	D-135	26.13	0,082
G7	Kubilay-82	16.60	0,377
G8	Dicle	19.38	0,119
G9	Alınoglu 2001	21.13	-1,037
G10	Kralkızı	19.46	0,486
G11	Gorkem	19.41	-0,742
G12	D-71	15.85	1,403
G13	Line-22	23.71	-0,667
G14	D-72	20.58	0,115
G15	Uludag	20.20	-0,624
G16	Ozveren	19.31	0,415
G17	GAP 2604	25.89	0,648
G18	GAP 2490	23.62	-0,983
G19	GAP- 61721	18.31	0,258
G20	GAP 59998	24.50	0,732

Genotypes with IPCA-1 scores close to zero have small interactions and hence show wider adaptation to the tested environments. Namely, genotypes having small IPCA1 values are more stable. (Carbonell *et al.*, 2004; Mirosavljević *et al.*, 2014). In the study, IPCA-1 scores of G3, G4, G6, G8, G14 were to be the closest to zero line, they located close to zero line on the y axis in the AMMI-1 biplot graph. Therefore, these genotypes were considered to conserve fresh forage yield trait in differing environments, with high stability. Despite their lower IPCA-1 scores, close to zero, G8 and G14 can not be recommended due to their lower forage yields, under the grand mean. However, with their high yield means, and lower IPCA-1 scores, close to zero, respectively, G6, G4 and G3 were considered to be possessing high stable forage yields. Therefore, these genotypes should be preferred for forage yield production in Southeastern Anatolia region conditions. A large genotypic IPCA-1 score (either positive or negative) have high interaction and reflects more specific adaptation to the environments with IPCA-1 values of the same sign (Bose *et al.*, 2014). In the study, the highest IPCA-1 scores were recorded in the G1 (-1.481) and G2 (1.668) by far (Table 3 and Figure 1). Hence, these two genotypes were found to be the most unstable genotypes for forage yield trait according to AMMI-1 biplot.

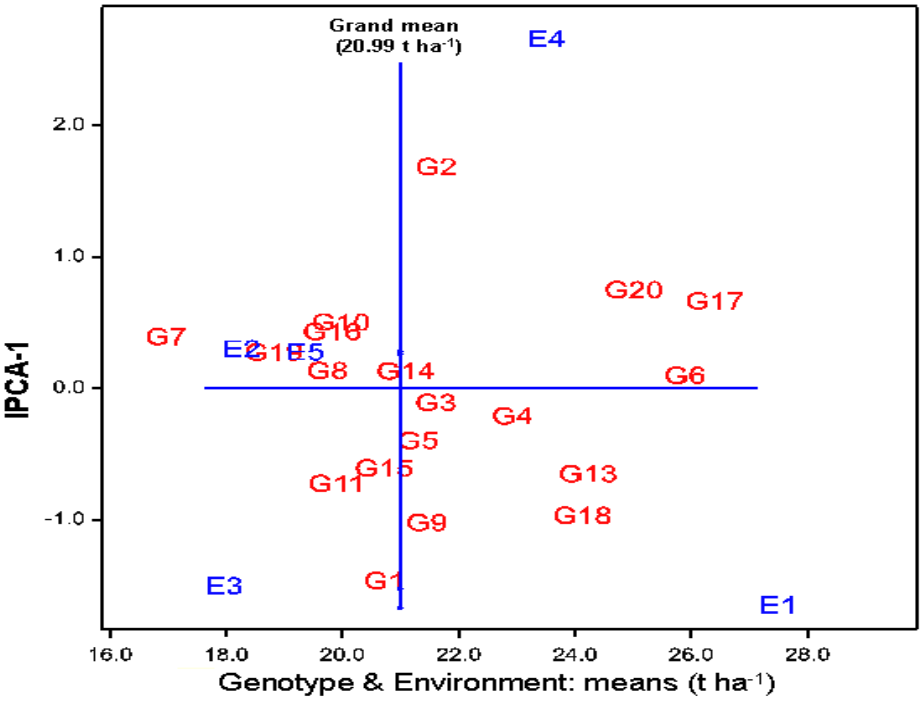


Figure 1. AMMI-1 biplot display fresh forage yields and IPCA-1 scores of 20 common vetch genotypes across five environments

AMMI-2 biplot analysis display both similarity among environments and

relations between genotypes and environments. Accordingly; E1 and E5 were the most resemble environments for forage yield, because the narrowest angle was found between two the vectors of the environments. Conversely, the most differences showing environments from the other environments were respectively E2 and E4, due to the fact that angels of two vectors were the largest ones among the environments (Figure 2). Also, the data shown in the Table 4 confirm this statement.

Table 4. Fresh forage yield means (t ha^{-1}) and IPCA scores of the tested five environments, recommended the first four common vetch genotypes for each environment

Number	Environment	Means	IPCAe[1]	1	2	3	4
1	E1	27.17	0.446	G4	G9	G18	G1
2	E2	17.93	-4.365	G17	G18	G20	G6
3	E3	17.63	1.700	G3	G6	G13	G9
4	E4	23.18	1.674	G2	G3	G4	G20
5	E5	19,03	0.546	G14	G17	G13	G6

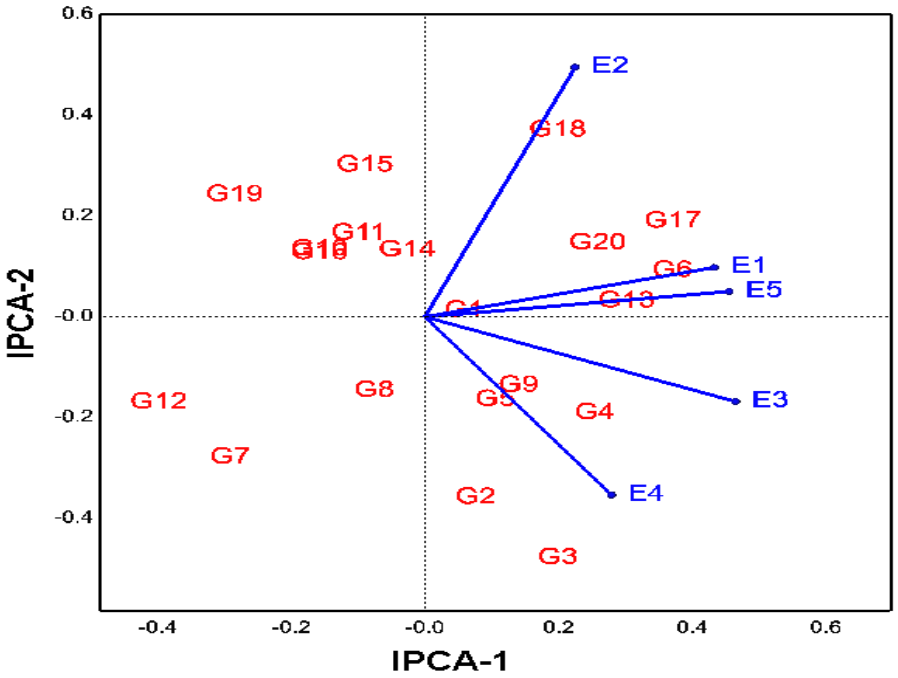


Figure 2. AMMI-2 biplot display response of 20 common vetch genotypes across five environments for fresh forage yield trait.

When genotype×environment interactions were evaluated from Figure 2 and Table 4, recommended common vetch genotypes for environments showed differences among the environments. Accordingly, respectively, G4, G9, G18, and G1 were recommended for E1 conditions, while G17, G18, G20 and G6 were

recommended for environmental conditions of E2. Additionally, although G3, G6, G13, G6 genotypes were advised for E3 conditions; G2, G3, G4 and G20 were found to be suitable for forage production in E4 conditions. Also, G14, G17, G13 and G6 respectively were suggested for E5 conditions.

CONCLUSIONS

Substantial variation was observed in the forage yield mean performance of all the tested genotypes (G) over environments (E) and on the genotype × environment interactions (GEI). The results indicated that the most effective factor on forage yield performance of common vetch genotypes was the environmental effect (42.23%). It was followed by genotype × environment interactions effect (36.13%) and genotype (21.64%) effect. AMMI analysis revealed that among the environments E1 and E4 were found as favorable environments, and D-135 (G6), IFVS-2541 (G4) and IFVS-715 (G3) respectively had the best stability in terms of fresh forage yield trait in Southeastern Anatolia region conditions.

REFERENCES

Acikgoz E. (2001). Forage Crops (3rd Press). Uludag University, Faculty of Agriculture, Lecture Book, Publication Number, 182:94-95, Bursa.

Asfaw A, Alemayehu F, Gurum F, Atnaf M, (2009). AMMI and SREG GGE biplot analysis for matching varieties onto soybean production environments in Ethiopia. Scientific Research and Essay, vol. 4 (11) pp. 1322-1330.

Ayed M.H., Gonzalez J., Caballero R., Alvir M.R. (2001). Effect of maturity on nutritive value of field-cured hays from common and hairy vetch. Animal Research, vol. 50, pp. 31-41.

Bose L.K., Jambhulkar N.N., Singh O.N (2014). Additive main effects and multiplicative interaction (AMMI)analysis of grain yield stability in early duration rice. The Journal of Animal & Plant Sciences, vol. 24(6), pp.1885-1897.

Caballero R., Alzueta C., Ortiz L.T., Rodriguez M.L., Barro C., Rebole A. (2001). Carbonhydrate and protein fractions of fresh and dried common vetch at three maturity stages. Agronomy Journal, vol. 93, pp.1006-1013.

Cakmakci S., Aydinoglu B., Karaca M. (2003). Determining relationships among yield and yield components using correlation and path coefficient analyses in summer sown common vetch (*Vicia sativa* L.) genotypes. Pak. J. Bot., vol. 35, pp. 387-400.

Carbonell S.A., Filho J.A., Dias L.A., Garcia, A.A., Morais L.K. (2004). Common bean cultivars and lines interactions with environments. Scientific Agriculture, vol. 61(2), pp. 169-177.

Ebdon J.S., Gauch H.G. (2002). additive main effect and multiplicative interaction analysis of national turfgrass performance trials. II cultivar recommendations. Crop Science, vol. 42, pp. 497–506.

Ilker E., Geren H., Unsal R., Sevim I., Tonk F., Tosun M. (2011). AMMI-Biplot Analyses of yield performances of bread Wheat cultivars grown at different locations. Turkish Journal of Field Crops, vol. 16(1), pp. 64-68.

Gabriel K.R. (1971). The biplot graphic display of matrices with application to principal component analysis. Biometrika, vol. 58, pp. 453-467.

Gauch H.G, Zobel R.W. (1996). AMMI analysis of yield trials. In Genotype-by-environment Interaction (Kang, M.S. and H.G. Gauch, eds.), CRC Press, Boca Raton, FL: 85-122.

Georgieva N.A., Kosev V.I. (2016). Comparative productivity and adaptive ability of forage pea (*Pisum sativum* L.) and Vetch (*Vicia sativa* L.) Cultivars. Journal of Agricultural Science, vol. 8, Issue 6, pp.109-119.

Islam MR, Anisuzzaman M, Khatun H, Sharma N, Islam Z, Akter A, and Biswas PS, (2014) .AMMI Analysis of yield performance and stability of rice genotypes across different Haor areas. Eco. Friendly Agril. J. 7 2:20-24.

Kaya Y, Palta Ç, Taner S, (2002). Additive main effects and multiplicative interactions analysis of yield performances in bread wheat genotypes across environments. Turkish Journal of Agricultural Forestry, 26, 275-279.

Kendal E and Dogan Y. (2015). Stability of a candidate and cultivars (*Hordeum vulgare* L.) by GGE biplot analysis of multi-environment yield trial in spring barley. Agriculture and Forestry, vol. 61, Issue 4, pp. 307-318.

Kendal, E. (2016). GGE Biplot Analysis of multi-environment yield trials in barley (*Hordeum vulgare* L.) cultivars. Journal of Crop Breeding and Genetics. vol. 2, Issue 1, pp. 90-99.

Kilic H, (2014). Additive main effect and multiplicative interactions (AMMI) Analysis of grain yield in barley genotypes across environments. Tarım Bilimleri Dergisi-Journal of Agricultural Science, vol. 20, pp. 337-344.

Mirosavlievi M.N., Pržulj N, Čanak P. (2014). Analysis of new experimental barley genotype performance for grain yield using AMMI Biplot. Selekcija Semenarstvo, vol: broj 1.pp: 27-36.

Sayar M.S., Anlarsal, A.E. and Basbag, M. (2010). Current situation, problems and solutions for cultivation of forage crops in the Southeastern Anatolian Region. J. Agric. Fac. HR. U., vol. 14, Issue, 2, pp. 59-67.

Sayar M.S., Anlarsal A.E. and Basbag M. (2013). Genotype-environment interactions and stability analysis for dry-matter yield and seed yield in Hungarian vetch (*Vicia pannonica* CRANTZ.). Turk. J. Field Crops, vol. 18, Issue 2, pp. 238-246.

Tarakanovas P., Ruzgas V. (2006). Additive main effect and multiplicative interaction analysis of grain yield of wheat varieties in Lithuania. Agronomy Research, vol. 4(1), pp. 91-98.

VSN International, (2011). GenStat for Windows 14th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk

Zobel R.W., Wright M.J., Gauch H.G., (1988). Statistical analysis of yield trial. Agronomy Journal, vol. 80, pp. 388-393.

DOI: 10.17707/AgricultForest.63.1.15

Milan BIBERDŽIĆ, Dragana LALEVIĆ,
Saša BARAĆ, Slaviša STOJKOVIĆ[1]

PRODUCTIVE TRAITS OF TRITICALE DEPENDING ON SOWING RATE AND METEOROLOGICAL CONDITIONS IN TESTED YEARS

SUMMARY

The results of the examination of sowing density and meteorological conditions on productive traits of several genotypes of winter triticale are presented in this paper. The examination was conducted in the vicinity of Bijelo Polje, in the northern part of Montenegro in the period 2009-2012. The experiment, set in randomized block system with three replications included five varieties of winter triticale (Odysseus, Kg-20, Triumph, Rtanj and Tango) and two sowing rates (density) – 600 and 800 germinated seeds per m^{-2}. Common agronomical practices were used in the experiment, with the application of NPK fertilizer combinations 60:80:80. The following parameters were assessed: the number of grains per spike, 1000 grain weight, hectoliter weight and grain yields of triticale.

The results of the examination showed significant differences in reproductive traits of winter triticale, depending on the variety, sowing density and weather conditions in the years of research. Variety Tango had the highest average grain yield (5610.0 kg ha^{-1}) and 1000 grain weight (48.4 g), while Kg-20 had the lowest grain yield (4465.6 kg ha^{-1}). Variety Odysseus had the highest number of grains per spike (38.5), while the highest value of hectoliter weight was determined in variety Triumph. Significantly higher yields were achieved by applying higher sowing density in comparison to the standard application of lower sowing density. The results of the examination showed that the demonstrated differences of observed parameters of the varieties which were included in this examination are the results of the specificity of varieties, of agro technical practices and climatic conditions in the years of study.

Keywords: *triticale, productive traits, sowing rate, meteorological conditions.*

INTRODUCTION

Triticale (x *Triticosecale* Wittm.), as a new highly successful species of small grains, was created by crossing wheat and rye. It was designed with the idea to obtain a cereal, which combines good quality grain yield from wheat parent with tolerance to abiotic and biotic stress (Villegas et al., 2010). In recent years it has become increasingly important, both for our producers and in the

[1]Milan Biberdžić, Dragana Lalević (corresponding author: dragana.lalevic@gmail.com), Saša Barać, Slaviša Stojković University in Pristina, Faculty of Agriculture, Lešak, SERBIA
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

world. Ugarte et al. (2007), Estrada-Campuzano et al. (2008) reported that triticale is an interesting crop for unfavorable conditions where productivity of common crops is more or less limited. Also, triticale has high resistance to diseases and pests, and extremely good endurance to drought, acid soils and poor quality soils. New varieties of triticale are equal or superior to other cultures for grain yield, forage and biomass production for human food, animal feed and industrial applications (Nefir and Tabără, 2011).

Optimum sowing rate and advanced agricultural techniques, with a special turn to fertilizing, have a vital role in increasing yield and quality of plants. Seed rates above the optimum level impose nutrients, light, moisture stresses and hence adversely affect crop yield while seed rate below optimum level usually has low yield (Mut et al., 2005).

Joshi et al. (2002) reported that the desirable cultivars for high grain yield and quality traits need to express genetic potential in different environment of growing. High temperatures after anthesis have negative influence in grain filling. Díaz Zorita et al. (2001) reported that water deficiency during the period of anthesis is critical moment to normal cereal production, causing significant yield losses.

The aim of this study was to establish some productive traits of triticale in dependence of sowing rate, agroclimatic conditions of locality and varieties.

MATERIALS AND METHODS

This field experiment was carried out in the vicinity of Bijelo Polje (Montenegro), on 43º 01' 45" north latitude and 19º 44' 44" east longitude during the 2009-2012. The trial was performed in a randomized block system with three replications on alluvial type of soil and the size of experimental plot was 6 m^2. The experiment included five varieties of winter triticale (Odysseus, Kg-20, Triumph, Rtanj and Tango) and two sowing rates (density) – 600 and 800 germinated seeds per m^{-2}. The fields were prepared with standard production practices for triticale varieties, with the application of NPK fertilizer combinations 60:80:80. The total amount of phosphorus and potassium were used in equal amounts (80 kg ha^{-1}) before the sowing period, while 1/3 of nitrogen was used before the sowing period, and the rest of the planed amount was applied after hibernation at the tillering stage in early spring. Sowing was carried out by manual method in optimal sowing date (October).The harvest was conducted, also, manually at the stage of full maturity, where the number of grains per spike, 1000 grain weight were measured, as well as the hectoliter weight, and the grain yield was corrected for 14% moisture. The average results of number of grain per spike, yield of dry grain, hectoliter weight, 1000 grain weight are presented in this paper, for the analyzed period of three years.

The obtained results were statistically processed using method of variance analysis, whereby the significance of average treatments was tested with LSD test, with significance threshold of 1 and 5 %.

RESULTS AND DISCUSSION

The soil on which the experiment was carried out belongs to the alluvial type of soil. It is weakly calcareous, with total content of carbonate being 2.4-2.44%. The soil is quite humic: 3.35-3.96% by low content of available phosphorus 5.12-4.24 mg/100 g soil) and potassium content (7.5-3.8 mg/100g soil).

Meteorological conditions, especially the precipitation and the temperature, largely determine the success of the production of winter triticale and other small grains.

The data in Table 2. indicate that the years in which this research was conducted varied in climate conditions. In the growing season 2009/10 there were 881 mm of rainfall which is 116 mm or 329 mm more than in the second and third year of the research.

Table 1. Meteorological data for Bijelo Polje

Year	Months										Sum
	X	XI	XII	I	II	III	IV	V	VI	VII	
Monthly rainfall (mm)											
2009-10	135	94	94	101	80	70	78	80	63	86	**881**
2010-11	65	131	147	36	76	31	46	121	33	79	**765**
2011-12	36	7	55	79	183	57	47	46	34	8	**552**
Average monthly temperatures (°C)											
2009-10	9.77	5.95	4.06	1.31	2.4	6.39	10.93	15	18.11	20.95	**9.5**
2010-11	10.12	8.54	2.05	-0.65	0.94	6.03	10.54	14.5	18.9	21.23	**9.2**
2011-12	9.3	3.25	2.17	-1.72	-3.52	5.96	10.8	15.02	20.67	24.63	**8.7**

Average temperatures were similar during the first (9.5°C) and the second (9.2°C) research years. In 2011/12, average temperature was lower than in the previos both years. From the data in the Table 2 it can be noted that conditions for germination and autumn plant development were considerably favorable during the first two years compared to the third year of research.

Also, the amount of rainfall in the period from April to June in the first year of research was higher compared to the other two years of research. Considering that the amount of rainfall and temperature in these months are extremely important for the development of small grains, the first year of research can be characterized as the most optimal in terms of weather conditions for growing triticale in this area.

Productive characteristics directly influence the forming of yield. Especially important is the advantage of agro-climatic factors. Table 3. provides an overview of some productive characteristics of triticale depending on variety and meteorological conditions in tested years.

The number of grains per spike is one of the most important components of yield and with the 1000 kernels weight dictates grain yield per spike (Milovanović et al., 2014).

Table 2. Number of grains per spike in dependence of variety, sowing rate and agroclimatic conditions

Variety		Number of grains per spike								
		Year								
		2009/10		Average	2010/11		Average	2011/12		Average
		G_1	G_2		G_1	G_2		G_1	G_2	
Odisey		47.0	45.0	46.0	33.3	31.7	32.5	38.0	36.3	37.1
Kg-20		33.7	31.7	32.7	34.6	31.7	33.1	29.1	28.2	28.6
Triumph		28.8	27.5	28.1	27.6	26.4	27.0	28.1	25.9	27.0
Rtanj		33.0	32.0	32.5	34.1	33.6	33.8	33.2	31.8	32.5
Tango		34.3	33.7	34.0	33.9	31.1	32.5	30.8	30.2	30.5
Average		**35.4**	**33.9**	**34.6**	**32.7**	**30.9**	**31.8**	**31.8**	**30.5**	**31.1**
		A	**B**	**AB**	**A**	**B**	**AB**	**A**	**B**	**AB**
LSD	0.05	0.903	0.571	1.277	1.148	0.726	1.623	1.097	0.694	1.551
	0.01	1.281	0.810	1.811	1.638	1.029	2.303	1.555	0.984	2.199

*****G_1 - sowing rate (600 germinated seeds per m^2)
G_2 - sowing rate (800 germinated seeds per m^2)

The highest average number of grains per spike (46.0) was recorded in variety Odysseus in the first year of research. With the application of the lower sowing rate a significantly higher number of grains per spike was achieved compared with the application of the sowing rate of 800 germinated seeds per m^2 in all tested varieties in all three years of research. Our results are in agreement with the results of Bokan and Malesevic (2004), which indicate a decrease in the number of grains per spike with the increase of sowing rate.

The1000 grain weight is one of the properties that directly affect the grain yield. Our results showed statistically significant differences in the 1000 grain weight between the first and the other two years of study. Our results are in agreement with the results of Jelic et al. (2002) who point out that the 1000 grain weight is a varietal characteristics, but also with Djekic et al. (2010) who found that the 1000 grain weight varies considerably depending on the weather conditions in each year of study. Also the data show that there is a statistically significant difference in the values of the 1000 grain weight depending on the sowing rate. Miric et al. (2007) reported that the 1000 grain weight decreases with the increasing sowing rate, or planting density, but it is very specific to each variety, which is confirmed by our results.

Hectoliter weight is also an important indicator of the technological value of seed. The obtained results are in agreement with the literature data, which point out that beside the variety and nutrition, meteorological conditions show significant influence on hectoliter weight. The highest hectoliter weight, in the three-year average, had the variety Triumph (72.99 kg), which is in agreement with the results of Milovanovic et al. (2004), who point out that the mentioned variety, beside the high and stable yield is characterized by good quality parameters.

Table 3. Mass of 1000 grain in dependence of variety, sowing rate and agro-climatic conditions

Variety		Mass of 1000 grain (g)								
		Year								
		2009/10		Average	2010/11		Average	2011/12		Average
		G_1	G_2		G_1	G_2		G_1	G_2	
Odisey		45.1	44.0	44.6	40.5	40.2	40.3	42.0	40.3	41.2
Kg-20		36.2	35.2	35.7	36.3	35.7	36.0	33.3	31.7	32.5
Triumph		48.5	47.8	48.1	42.0	40.7	41.3	42.2	41.8	42.0
Rtanj		46.3	45.8	46.1	47.5	46.2	46.9	45.7	44.6	45.2
Tango		49.2	48.5	48.8	49.7	48.0	48.8	48.0	46.8	47.4
Average		**45.1**	**44.3**	**44.7**	**43.2**	**42.2**	**42.7**	**42.2**	**41.0**	**41.6**
		A	**B**	**AB**	**A**	**B**	**AB**	**A**	**B**	**AB**
LSD	0.05	0.689	0.436	0.975	0.899	0.569	1.271	0.607	0.384	0.859
	0.01	0.978	0.619	1.383	1.264	0.796	1.802	0.862	0.545	1.218

*****G_1 - sowing rate (600 germinated seeds per m^2)
G_2 - sowing rate (800 germinated seeds per m^2)

Table 4. Hectoliter weight in dependence of variety, sowing rate and agro-climatic conditions

Variety		Hectoliter weight (kg)								
		Year								
		2009/10		Average	2010/11		Average	2011/12		Average
		G_1	G_2		G_1	G_2		G_1	G_2	
Odisey		73.9	76.2	75.1	67.0	67.6	67.3	67.1	70.8	68.9
Kg-20		61.8	67.4	64.6	61.2	63.2	62.2	59.6	69.5	64.5
Triumph		73.4	75.7	74.5	69.5	70.4	69.9	66.4	69.5	67.9
Rtanj		67.9	69.1	68.4	65.6	67.8	66.7	63.1	64.9	64.0
Tango		65.9	72.7	69.3	66.4	69.6	68.0	64.5	65.3	64.9
Average		**68.6**	**72.2**	**70.4**	**65.9**	**67.7**	**66.8**	**64.1**	**68.0**	**66.1**
		A	**B**	**AB**	**A**	**B**	**AB**	**A**	**B**	**AB**
LSD	0.05	0.697	0.441	0.985	0.579	0.366	0.819	0.800	0.506	1.312
	0.01	0.988	0.625	1.394	0.821	0.519	1.161	1.123	0.725	1.605

*****G_1 - sowing rate (600 germinated seeds per m^2)
G_2 - sowing rate (800 germinated seeds per m^2)

Also, the results showed that in the third year, which was the least favorable according to the climate characteristics, the lowest average value of the hectoliter weight was recorded. The influence of meteorological factors on the value of the hectoliter weight was confirmed earlier by other authors in their researches (Lalevic et al., 2012).

The grain yield of the tested genotypes of triticale showed considerable variation depending on the amount of seed used for planting and the weather conditions in the years of research.

In all three years of research, the application of the higher sowing rate caused a significantly higher yield in all tested varieties compared to the application of the lower sowing rate. The highest average yield, in all three years of research, of 6135.5 kg ha^{-1} was obtained with variety Tango at the highest sowing rate (800 germinated seeds per m^2). The lowest yield, in the three-year average, was obtained with the variety Kg-20 with the application of both sowing rates.

During the research, the yield variation was noticed, which was the result of different climatic factors (precipitation and temperature). The highest grain yield was achieved in the first year, which in terms of quantity and distribution of rainfall was the best.

The most unfavorable weather conditions for the cultivation of triticale were in the third year of research, which was reflected on the yield. Significant yield variation depending on the conditions and sowing rates were pointed out previously by other authors (Pecio, 2010; Biberdzic et al., 2013).

Table 6.Grain yield in dependence of variety, sowing rate and agro-climatic conditions

Variety		**Number of grains per spike**								
		Year								
		2009/10		**Average**	**2010/11**		**Average**	**2011/12**		**Average**
		G1	**G2**		**G1**	**G2**		**G1**	**G2**	
Odisey		47.0	45.0	46.0	33.3	31.7	32.5	38.0	36.3	37.1
Kg – 20		33.7	31.7	32.7	34.6	31.7	33.1	29.1	28.2	28.6
Triumph		28.8	27.5	28.1	27.6	26.4	27.0	28.1	25.9	27.0
Rtanj		33.0	32.0	32.5	34.1	33.6	33.8	33.2	31.8	32.5
Tango		34.3	33.7	34.0	33.9	31.1	32.5	30.8	30.2	30.5
Average		35.4	33.9	34.6	32.7	30.9	31.8	31.8	30.5	31.1
		A	**B**	**AB**	**A**	**B**	**AB**	**A**	**B**	**AB**
LSD	0.05	0.903	0.571	1.277	1.148	0.726	1.623	1.097	0.694	1.551
	0.01	1.281	0.810	1.811	1.638	1.029	2.303	1.555	0.984	2.199

*****G_1 - sowing rate (600 germinated seeds per m^2)
G_2 - sowing rate (800 germinated seeds per m^2)

CONCLUSIONS

Based on the studying the influence of sowing rate and meteorological conditions on productive traits of triticale, it can be concluded that:

All tested varieties, in all three years of research, achieved significantly higher yield with the application of the higher sowing rate compared to the application of the lower sowing rate;

The average yield of tested varieties of triticale was highest in the first and lowest in the third year of the study;

On average, the highest number of grains per spike and the 1000 grain weight of the tested winter triticale varieties, was achieved with the application of the lower sowing rate in the first year of research;

Variety Tango achieved the highest yield in the first year of research with the application of the higher sowing rate, while the lowest yield was recorded at variety Kg-20 in the third year of research with the application of the lower sowing rate;

The highest hectoliter weight was achieved at variety Triumph (75.7kg) in the first year of research with the application of the higher sowing rate;

The differences of the observed parameters at the tested varieties are the result of varietal specifics, the applied agro technical practices and the climate conditions in the years of research.

ACKNOWLEDGMENT

The study published in this paper is a part of the project ”The development of new technologies of small grains cultivation on acid soils using contemporary biotechnology” financed by the Ministry of Education and Science of the Republic of Serbia, grant No TR-31054.

REFERENCES

Biberdžić, M., Jelić, M., Knežević, B., Barać, S., Maksimović, G., Lalević, D. (2013): The effect of climatic conditions and variety on some morphological and productivity characteristics of triticale. Research Journal of Agricultural Science, 45 (3), 24-29.

Bokan, N., Malešević, M. (2004): The planting effect on wheat yield structure. Acta Agriculturae Serbica, Vol. Ix, 18, p. 65-79.

Díaz Zorita, M., Fernández-Canigia, M.V., Grosso, G.A. (2001): Applications of foliar fertilizers on yield and quality in sunflower (*Helianthus annuus* L.). Pacistan J. Biol. Sci. 7: 384-388.

Djekić, V., Milovanović, M., Staletić, M., Perišić, V. (2010): Ispitivanje komponenti prinosa kragujevačkih sorti ozimog tritikalea (Investigation of yield components Kragujevac varieties winter triticale). Zbornik naučnih radova instituta PKB Agroekonomik 16, 1-2, 35-41.

Estrada-Campuzano, G., Miralles, D.J., Slafer, G.A. (2008): Genotypic variability and response to water stress of pre- and post-anthesis phases in triticale. European Journal of Agronomy, 28 (3): 171-177.

Jelić, M., Stojanović, J., Stojanović, S., Živanović, S. (2002): Optimalna tehnologija proizvodnje kragujevačkih sorti strnih žita (The optimal production technology of Kragujevac varieties of small grains). Agroinovacija br. 3, 163-171.

Joshi, S. K., Sharma, S. N., Singhania, Sain, R.S. (2002): Genetic analysis of quantitative and quality traits under varying environmental conditions in bread wheat. Wheat Information Service, 95: 5-10.

Lalević, D., Biberdžić, M., Jelić, M., Barać, S. (2012): Some characteristics of triticale cultivated in rural areas, Agriculture & Forestry, Vol. 58 Issue 2, p27-34. 8p.

Milovanović, M., Perišić, V., Staletić, M. (2004): New cultivar of winter triticale *Triumph*. Book of Abstracts of the III Congress of Serbian, Subotica, p. 168.

Milovanovic, M., Perishic, V., Staletic, M., Djekic, V., Nikolic, O., Prodanovic, S., Lukovic, K. (2014): Diallel analysis of grain number per spike in Triticale, Bulgarian Journal of Agricultural Science, Agricultural Academy 20 (No 5) 2014, 1109-1115.

Mirić, M., Selaković, D., Jovin, P., Hojka, Z., Filipović, M. (2007): Masa 1000 semena u teoriji i praksi, Selekcija i semenarstvo, Plantbreeding and seedproduction, Vol. XIII, No. 3-4 (2007), str. 49-58, Novi Sad.

Mut, Z., Sezer, I., Gulumser, A. (2005): Effect of different sowing rates and nitrogen levels on grain yield, yield components and some quality traits of triticale, Asian Journal of Plant Sciences, 4 (5), 533-539.

Nefir, P., Tabără, V. (2011): Effect on products from variety fertilization and triticale (*Triticosecale* Wittmack) in the experimental field from Răcăşdia Caras-Severin country. Res. J. Agric. Sci. 43(4): 133-137.

Pecio, A. (2010): Productivity of triticale affected by nitrogen fertilization and weather conditions. Fertilizer and fertilization, 40: 101-116.

Villegas, D., Casadesus, J., Atienza, S., Martos, V., Maalouf, F., Karam, F., Aranjuelo, I., Nogues, S. (2010): Tritodeum, wheat and triticale yield components under multi-local mediterranean drought conditions. Field. Crops Research, 100: 240-248.

Ugarte, C., Calderini, D. F., Slafer, G. A. (2007): Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. Field Crops Research, 116: 68-74.

DOI: 10.17707/AgricultForest.63.1.16

Nina MORAVČÍKOVÁ,
Radovan KASARDA, Ondrej KADLEČÍK[1]

THE DEGREE OF GENETIC ADMIXTURE WITHIN SPECIES FROM GENUS *CERVUS*

SUMMARY

The aim of present study is to estimate the level of admixture within species originating from genus *Cervus* (family *Cervidae*) that can reflect not only their phylogeny origin, but mainly the impact of introgression and hybridization in Red deer population inhabit the forest area of Slovakia. It was happened over the last few centuries. Recently, the introduction and hybridization by non-native species is considered as the major risk of diversity loss within autochthonous Red deer populations in area of Central Europe. The genomic data were obtained from in total 61 animals (species *A. axis*, *D. dama*, *C. elaphus*, *C. Nippon*, *C. canadensis*) based on applying the cross-species SNP genotyping (Illumina BovineSNP50 BeadChip). The quality control of genotyping data has been performed to eliminate any SNPs with genotyping errors (loci with >10% missing genotypes), less informative markers (MAF<0.01) and markers deviating from Hardy-Weinberg equilibrium limit of 0.0001. From totally 43.21% of successfully genotyped markers up to 843 were informative for subsequent estimation. As expected the presence of admixed individuals was found within Red deer and Wapiti populations, but the signal of cross-species admixture was low. The membership probabilities of individuals were higher than 90% within each of analysed cervid group. Our study showed that the Slovak Red deer population can provide valuable gene pool within Central European deer populations in respect to the management of genetic resources conservation strategies.

Keywords: *deer, diversity, cross-species genotyping, hybridization.*

INTRODUCTION

Worldwide, human-mediated translocations increase the rates of introgression from non-native into native taxa. In addition, range shifts, especially in temperate areas, also involve increased population admixture. Potential negative effects include introgression of non-native gene copies, loss of local adaptations and breaking up of co-adapted gene complexes (Haanes *et al.*, 2013). Introgression that induces transfer of genes from one species to another by

[1]Nina Moravčíková (corresponding author: nina.moravcikova@uniag.sk), Radovan Kasarda, Ondrej Kadlečík Department of Animal Genetics and Breeding biology, Slovak University of Agriculture in Nitra, Nitra, SLOVAKIA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

repeated backcrossing and hybridization that results in admixture of parental genes from 2 different species are a major threat to biodiversity as they can cause the loss of fitness through outbreeding depression and in some cases, even extinction (Malcolm, 2015). In addition, ongoing hybridization can cause the native population to be replaced by one genetically and phenotypically resembling the non-native form in some traits, while individuals containing non-native genetic material might be morphologically indistinguishable from those containing an intact native genome (Biedrzyck a*et al.*, 2012).

To determine the outcome of introgression and admixture from non-native into native taxa, long-term monitoring is suggested. Non-indigenous introduction have been common in game management, especially in red deer (*Cervus elaphus*) that is one of the most widespread and important game species in Europe (Haanes *et al.*, 2013). The populations of Red deer have been subjected to a variety of anthropogenic interventions (historic or relatively recent) that have potentially affected the genetic structure of local populations (Krojerová-Prokešová *et al.*, 2015). Currently, the conservation status of native Red deer populations are threatened with extinction in some parts of Central Europe. The major cause is the hybridisation with an Asian species of the Japanese Sika deer (*Cervus nippon*) and North America Wapiti (*Cervus canadensis*) (Macháček *et al.*, 2014). The Sika deer have been introduced to many countries and the hybridisation with native Red deer population has been demonstrated in British Isles (Malcolm, 2015), Czech Republic (Bartoš *et al.*, 1982; Macháček *et al.*, 2014), Poland (Biedrzycka *et al.*, 2012) or Germany (Herzog *et al.*, 2016). However, in addition to local hybridization with sika three major conservation issues exist: threatened genetic lineages, and blurring of natural genetic structuring through translocations and reintroductions; selective hunting; and reduced effective population sizes due to habitat fragmentation (Zachos and Hartl, 2011). For long-term conservation and development purposes, it therefore appears compulsory to manage Red deer wildlife to maintain both species survival and within species genetic diversity.

The aim of present study was to assess the level of admixture within species originating from five species from tribe *Cervini* and especially genus *Cervus* (family *Cervidae*) that can reflect not only their phylogeny origin, but mainly the impact of introgression and hybridization in Red deer population inhabit the forest area of Slovakia.

MATERIALS AND METHODS

To assess the degree of genetic admixture within five species from tribe *Cervini* the dataset consisted of genotyping data from in total of 61 individuals: 29 Red deer (*Cervus elaphus* – Cela), 8 Sika deer (*Cervus nippon* – Cnip), 8 Wapiti (*Cervus canadensis* – Ccan), 8 Fallow deer (*Dama dama* – Ddam), and 8 Axis deer (*Axis axis* – Aaxi) has been used. The final dataset was created by merging of new data from 29 free living and farmed red deer inhabit the forest area in southwestern part of Slovakia that were genotyped by the Illumina

BovineSNP50 v2 BeadChip and previously published data of 32 cervids obtained by using the BovineSNP50 v1 BeadChip (Illumina, Inc. San Diego, USA) as described Decker *et al.* (2009). Overall 36,451SNPs common to both analysed datasets were retained in reduced panel of loci. The subsequent quality control of genotyping data has been conducted to exclude any SNPs with call rate lower than 90%, minor allele frequency lower than 0.01 and HWE limit of $1x10^{-4}$ using PLINK (Purcell *et al.*, 2007).

After applying quality control, 843loci across autosomes were used to analyse the state of genetic diversity within each of analysed species based on indices derived from allelic frequency; observed (H_o) and expected (H_e) heterozygosities and Wright's F_{IS} index using Genalex v6.1 software (Peakall and Smouse, 2012). The degree of genetic differentiation among cervid species was assessed based on Nei's genetic distances using R package *StAMPP* (Pembleton *et al.*, 2013)and visualised using R package *ape* (Paradis *et al.*, 2004).

To examine population genetic structure the discriminant analysis of principal components (DAPC) adopted in R package *Adegenet* (Jombart and Ahmed, 2011) was applied on genotyping data. The optimum numbers of clusters was determined by running a K-means algorithm that use Bayesian Information Criterion (BIC). Overall 10^9 iterations of the model were used to search for convergence and obtainment of the likelihood associated with each value of K between 1 and 30. The DAPC analysis was also used to assign individuals and to obtain the membership probability which present the overall genetic background of an individual.

The population structure without prior information of individual's origin was also evaluated based on Bayesian clustering approach proposed by Pritchard *et al.* (2000) implemented in Structure v2.3 software. The analysis was carried out based on a model with admixture and correlated allele frequencies using burn-in period of 100,000 followed by 1,000,000 MCMC replications. Runs for each selected value of K (1 – 10) were repeated ten times. The optimal value of clusters was selected based on evaluation of the log probability of delta K (ΔK) according to Evanno *et al.* (2005).

RESULTS AND DISCUSSION

Of the total 36,451 loci retained in reduced panel, 15,754 autosomal SNPs have been successfully genotyped in at least 90% of individuals with call rate at level of 61.04%. This marked decrease of genotyping call rate in comparison to species from family *Bovidae* was caused mainly by the fact that the applied genotyping array has been primarily designed to *Bos Taurus*. In relation to this Miller *et al.* (2012) found that the cross-species application of such array can result in 1.5% decrease of call rate per each million year divergence between species.

Despite that the 43.21% of loci from the bovine array could be genotyped, most of them were monomorphic and only 2.31% of SNPs showed polymorphism. The number of identified informative loci was comparable to

results presented by Wu *et al.* (2013) that similarly used cross-species genotyping to evolutionary related water buffalo. Relative higher proportion of polymorphic SNPs at level of 5% found Hayes and Latch (2012) and Kharzinova *et al.*(2015) within the species *Odocoileus virginianus* and *Rangifer tarandus*, respectively. However, the results of cross-species genotyping have to be described with cation mainly due to the differences between cervid and bovid genome that resulted in favour of conserved genomic regions that still retain polymorphisms ancestral to the divergence between *Cervidae* and *Bovidae* families(Haynes and Latch, 2012).

The level of observed heterozygosity ranged from 0.012±0.001 (Axis deer) to 0.13±0.07 (Red deer). The species from genus *Cervus* (Red deer, Wapiti and Sika) showed higher level of expected heterozygosity in comparison to genera *Axis* (Axis deer) and *Dama* (Fallow deer). The prevalence of homozygotes in particular groups and on the level of whole population confirmed also the value of Wright's F_{IS} (0.294±0.008) and F_{IT} indexes (0.821±0.012). The results showed lower degree of genetic variability within Slovak red deer population than presented Maršálková *et al.* (2014) using microsatellite markers. In addition, the value of F_{IS} index can signalize the potential risk of inbreeding increase accompanied by the decrease of genetic diversity within population.

The value of F_{ST} index (0.913±0.071) indicated that most of the genetic variation was conserved within separate species that clearly showed also subsequently estimated genetic relationships based on Nei's distances. The average genetic distances ranged from 0.05 (Red deer and Wapiti) to 0.697 (Axis and Fallow deer). The observed distances suggested the distribution of individuals into the three maingenetic clusters. The groups were formed by individuals from genera *Cervus, Axis* and *Dama* (Figure 1A).

The DAPC approach similarly confirmed a strong distinction among species in relation to their phylogenetic origin. The division of individuals in to the clusters produced by BIC analysis showed that the inferred clusters corresponded to the initial groups with optimal value of K=5. The first and second discriminant function clearly demonstrated the closest genetic affinity between the species from genus *Cervus* and division of individuals in to the three clusters(Figure 1B and 1C). Similarly, the Bayesian assignment analysis and the method proposed by Evanno *et al.* (2005) favoured a model with three clusters. Based on this it can be stated that the panel of 843 selected polymorphic loci applied to analysis of population structure significantly reflected the evolution and phylogeny of analysed species and is prospective for future management of Red deer population in order to maintain genetic diversity.

The membership probability outputs suggested that the allele frequencies varied continuously across the regions composed from genera *Cervus*, *Dama* and *Axis* (Figure 1E). The evidence of admixture has been found only based on DAPC analysis in genus *Cervus*. The presence of admixed individuals was identified between the species Red deer and Wapiti (Figure 1D).

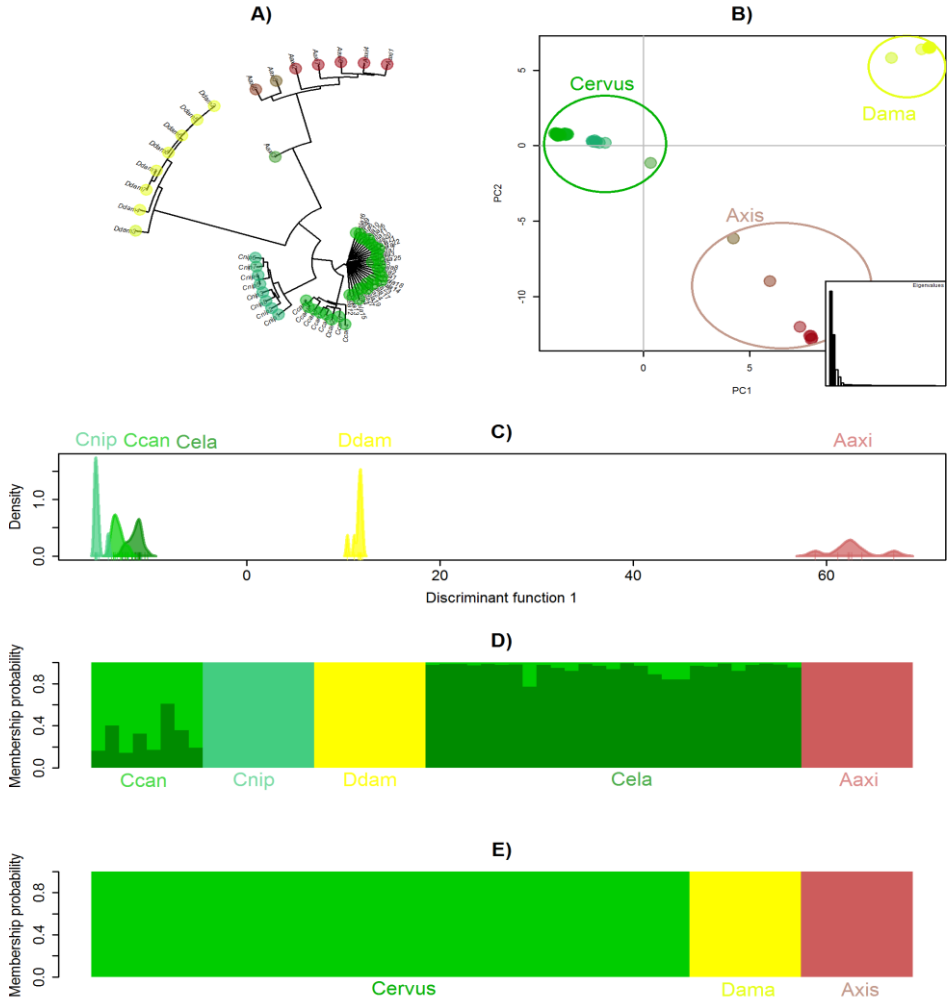


Figure 1. The phylogenetic tree representing genetic relationship based on Nei's distances (A), the structure of population based on PCA analysis (B), genetic clusters determined using first discriminant function (C), and membership probability resulting from DAPC analysis (D) and Bayesian clustering approach implemented in STRUCTURE (E)

This happened probably as a consequence of the introduction of red deer with various origin (*Cervus Elaphus Hippelaphus*, *C. Elaphus Sibiricus*, and *C. Elaphus Canadensis*) into the Slovak population during the 19th century (Apollonio *et al.*, 2010). Despite that the population of Red deer inhabits the Slovakia is often described as the subspecies *Cervus Elaphus Hippelaphus*, but in practice is rather a mongrel mixture of genotypes (Bališ *et al.*, 1980). Any level of admixture between the Slovak Red deer and Sika, which could be caused by

its introduction in to the neighbouring countries (for example Czech Republic), was not confirmed. In practical use this can be beneficial not only for the improvement of deer farming in Slovakia, but also for other local populations mainly in respect to the conservation of its genetic resources, sustainable management and prevention of genetic diversity loss.

CONCLUSIONS

Our study showed that the gene pool of Slovak Red deer population is not affected by introduction of Sika as non-native species into the local population inhabits neighbouring countries. The membership probability outputs suggested that the allele frequencies varied continuously across the three main regions composed from genera *Cervus*, *Dama* and *Axis*, while the presence of admixed individuals has been identified only within genus *Cervus* and species Red deer and Wapiti as a consequence of deerfarming history in Slovakia. Based on the fact that many areas of Central Europe are threatened by risk of loss of autochthonous populations due to the hybridisation with Sika, the Slovak Red deer population can be regarded as valuable gene poolmainly in respect to the conservation strategies of its genetic resources.

ACKNOWLEDGMENT

This work has been supported by the Slovak Research and Development Agency (Contracts No. APVV-14-0054 and SK-AT-2015-0016). Part of the work was done during the stay of the authors at BOKU Vienna, supported by the Austrian Agency for International Cooperation in Education and Research (OeAD-GmbH, project SK 07/2016). We would like to thank for cooperation the Xcell Slovakia Breeding Services s.r.o. and namely Ing. J. Pokorádi, Phd., who provided the biological samples of Red deer.

REFERENCES

Apollonio, M., Andersen, R., Putman, R. (2010). European Ungulates and Their Management in the 21st Century, Cambridge University Press, Cambridge, United Kingdom.

Bališ, M. (1980). Jelenia zver, Príroda, Bratislava, Slovakia.

Bartoš, L., Žirovnický, J. (1982). Hybridization between red deer and sika deer. II. Phenotype analysis, Zoologischer Anzeiger, 207, 271 - 287.

Biedrzycka, A., Solarz, W., Okarma, H. (2012). Hybridization between native and introduced species of deer in Eastern Europe, Journal of Mammalogy, 93, 1331 - 1341.

Decker, J. E., Pires, J. C., Conant, G. C., McKay, S. D., Heaton, M. P., Chen, K., Cooper, A., Vilkki, J., Seabury, C. M., Caetano, A. R., Johnson, G. S., Brenneman, R. A., Hanotte, O., Eggert, L. S., Wiener, P., Kim, J. J., Kim, K. S., Sonstegard, T. S., Van Tassell, C. P., Neibergs, H. L., McEwan, J. C., Brauning, R., Coutinho, L. L., Babar, M. E., Wilson, G. A., McClure, M. C., Rolf, M. M., Kim, J., Schnabel, R. D., Taylor, J. F. (2009). Resolving the evolution of extant and extinct ruminants with high-throughput phylogenomics, Proc Natl AcadSci U S A, 106, 18644 - 18649.

Evanno, G., Regnaut, S., Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study, Molecular Ecology, 14, 2611 - 2620.

Haanes, H., Rosvold, J., Røed, K. H. (2013). Non-indigenous introgression into the Norwegian red deer population, Conservation Genetics, 14, 237 - 242.

Haynes, G. D., Latch, E. K. (2012). Identification of novel single nucleotide polymorphisms (SNPs) in deer (Odocoileus spp.) using the BovineSNP50 BeadChip, PLoS One, 7:e36536.

Herzog, S., Gehle, T. (2016). Hybridization in the Genus Cervus: Evidence for Hybridization between Red and Sika Deer in Germany, Academic Journal of Applied Sciences Research, 1, 7 - 9.

Jombart, T., Ahmed, I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data, Bioinformatics, 1, 3070 - 3071.

Kharzinova, V. R., Sermyagin, A. A., Gladyr, E. A., Okhlopkov, I. M., Brem, G., Zinovieva, N. A. (2015). A Study of Applicability of SNP Chips Developed for Bovine and Ovine Species to Whole-Genome Analysis of Reindeer Rangifer tarandus, Journal of Heredity, 106, 758 - 761.

Krojerová-Prokešová, J., Barančeková, M., Koubek, P. (2015). Admixture of Eastern and Western European Red Deer Lineages as a Result of Postglacial Recolonization of the Czech Republic (Central Europe),Journal of Heredity, 106(4), 375 - 385.

Macháček, Z., Dvořák, S., Ježek, M., Zahradník, D. (2014). Impact of interspecific relations between native red deer (Cervuselaphus) and introduced sika deer (Cervusnippon) on their rutting season in the Doupovskéhory Mts, Journal of forest science, 60(7), 272 - 280.

Malcolm, S. (2015). An Ethical Assessment on the Management of Introduced Sika Deer (*Cervus nippon*) in Scotland; With Particular Reference to Hybridisation with Native Red Deer (*C. elaphus*), Journal of Student Research, 4(2), 13 - 27.

Maršálková, L., Židek, R., Pokoradi, J., Golian, J., Belej, Ľ. (2014). Genetic diversity and relatedness among seven red deer (Cervuselaphus) populations, Potravinárstvo, 8, 15 - 19.

Miller, J. M., Kijas, J. W., Heaton, M. P., McEwan, J. C., Coltman, D. W. (2012). Consistent divergence times and allele sharing measured from cross-species application of SNP chips developed for three domestic species, Molecular Ecology Resources, 12, 1145 - 1150.

Paradis, E., Claude, J., Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language, *Bioinformatics*, 20, 289 - 290.

Peakall, R., Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update, Bioinformatics, 28, 2537 - 2539.

Pembleton, L. W., Cogan, N. O. I., Forster, J. W. (2013). StAMPP: an R package for calculation of genetic differentiation and structure of mixed-ploidy level populations, Molecular Ecology Resources, 13, 946 - 952.

Pritchard, J. K., Stephens, M., Donnelly, P. (2000). Inference of population structure using multilocus genotype data, Genetics portions from molecular data, Molecular Biology and Evolution, 15, 1298 - 1311.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., Maller, J., Skalp, P., De Bakker, P. L., Daly, M. J., Sham, P. C. (2007). PLINK: a tool set for whole genome association and population-based linkage analysis, Am J Hum Genet, 81, 559 - 575.

Wu, J. J., Song, L. J., Wu, F. J., Liang, X. W., Yang, B. Z., Wathes, D. C., Pollott, G. E., Cheng, Z., Shide, S., Liu, Q. Y., Yang, L. G., Zhang, S. J. (2013). Investigation of transferability of BovineSNP50 BeadChip from cattle to water buffalo for genome-wide association study, Molecular Biology Reports, 40, 743 - 750.

Zachos, F. E., Hartl, G. B. (2011). Phylogeography, population genetics and conservation of the European red deer Cervuselaphus. Mammal Review, 41, 138 - 150.

DOI: 10.17707/AgricultForest.63.1.17

Tossaporn INCHAROEN, Tinnagon TARTRAKOON, Anat ONGKAN, Wandee TARTRAKOON[1]

UNSATURATED TO SATURATED FATTY ACIDS RATIO ADJUSTMENTIN DIETS ON DIGESTIBILITY AND PERFORMANCE OF GROWING PIGS

SUMMARY

Fat and oils are the most concentrated energy source for pigs. Various fat sources differ considerably in their physical-chemical characteristics and fatty acid profile. Therefore, the current study aimed to evaluate the effect of diets containing 3% fat with different ratios of unsaturated to saturated fatty acids (U:S) on the nutrient digestibility, energy utilization and growth performance of growing pigs. In experiment 1, a total of 6 crossbred, castrated male pigs (Duroc × Large White × Landrace) were used to determine the nutrient digestibility and energy utilization according to a repeated 3 × 3 Latin square design. Each of the two pigs was fed with one of the three diets containing 3% fat with a U:S ratio of 3, 4 or 5, respectively. No differences ($P>0.05$) in the nutrient digestibility were observed among groups, but the highest digestible and metabolizable energy ($P<0.05$) appeared in pigs receiving the dietary U:S ratio of 4. In experiment 2, a total of 30 pigs (with an equal number of entire males and females) were allotted into 3 groups in a randomized complete block design. Pigs were fed a diet without fat (T1), or the diets containing 3% fat with U:S ratio of 4 (T2) or 5 (T3), respectively.

Although there were no significant difference in any growth criteria, average daily gain and feed conversion ratio was slightly improved ($P>0.05$) in the T2 group. In conclusion, the present results suggest that diets containing 3% fat with a U:S ratio of 4 is optimal for improving energy utilization in growing pigs and results in a slight enhancement of performance.

Keywords: *Digestibility, Energy utilization, Fatty acids profile, Growing pigs, Growth performance*

INTRODUCTION

Fats and oils are the most concentrated energy source for livestock animals, and their inclusion in the diet is a common practice in modern animal production to increase energy density. Park et al. (2012) noted that dietary fat plays a considerable role in the development of growing-finishing pigs because

[1]Tossaporn Incharoen (corresponding author: tossaporni@nu.ac.th), Anat Ongkan, Wandee Tartrakoon Faculty of Agriculture Natural Resource and Environment, Naresuan University, Phitsanulok 65000, THAILAND, Tinnagon Tartrakoon Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna, Phitsanulok Campus, THAILAND
Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

of its high energy content. Moreover, it is recommended for use in growing-finishing pigs being reared under environmental heat stress due to a lower heat increment than other feedstuff (Spencer et al., 2005). Jung et al. (2003) found that the replacement of animal fat with vegetable oil rich in unsaturated fatty acids, such as soybean oil or corn oil, resulted in an improved growth performance in weaning pigs. Results from the poultry model reported that an optimal combination of vegetable oil and animal fat sources in broiler diets enhanced growth performance and carcass traits (Poorghasemi et al., 2013).

On the other hand, some reports described that vegetable oil (expressed as unsaturated fatty acids) or animal fat (representing extremes of saturated fatty acids) did not affect the fat digestibility of pigs (Jørgensen and Fernández, 2000; Kil et al., 2011). Recently, Tartrakoon et al. (2016) published that a different dietary U:S ratio greatly affect the performance and meat quality of finishing pigs. Gatlin et al. (2005) also reported that the apparent digestibility of dietary fat in finishing swine was improved with increasing values of the U:S ratio. This seems to suggest that unsaturated lipids may be more easily digested than saturated lipids (Scheeder et al., 2003). To our knowledge, there is limited data contributing to an understanding of the most optimal dietary U:S ratio for use in swine nutrition and production.

Therefore, the present trial was conducted to evaluate the effect of diets containing 3% fat with different U:S ratios on the nutrient digestibility, energy utilization and growth performance of growing pigs. These different U:S ratios in diets were adjusted by a combination of poultry fat and soybean oil.

MATERIALS AND METHODS

In experiment 1, a total of 6 crossbred, castrated male pigs (Duroc × Large White × Landrace) was used to determine nutrient digestibility and energy utilization according to a repeated 3 × 3 Latin square design. Each of two pigs was fed with one of the three diets containing 3% fat with U:S ratio of 3 (T1), 4 (T2) or 5 (T3), respectively (Table1).

Additionally, all diets contained 0.5% titanium dioxide as an indigestible marker to calculate the apparent total tract digestibility of nutrients and energy value. Feed was limitedly provided for 2.5 times the metabolizable energy (ME) requirements for maintenance (Kraler et al., 2014). Feces were collected daily, weighed and stored immediately in a -20°C freezer to prevent microbial activity until further processing. All samples of experimental diets and feces were prepared for analyses by drying in a hot air oven at 60 ºC for 72 h.

Feed and fecal samples were finely ground through a 1-mm mesh screen using an electric blender. Titanium dioxide was analyzed in the diet and feces according to Myers et al. (2004) with a minor modification. Dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and ash in the feces and diet samples were analyzed according to the standard methods of AOAC (2000). The gross energy of the diets and feces was determined through bomb-calorimeter.

Table 1. Ingredients and chemical composition of the experimental diets

	Experiment 1			Experiment 2		
Item	T1	T2	T3	T1	T2	T3
Ingredients (g/kg as fed basis)						
Corn	480	480	480	626	480	480
Rice bran	180	180	180	50	180	180
Soybean meal	285	285	285	299	285	285
Poultry fat	23	9	0	0	9	0
Soybean oil	7	21	30	0	21	30
Di-calcium phosphate	14	14	14	14	14	14
$CaCO_3$	5	5	5	5	5	5
$NaCl_2$	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin and mineral premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Analytical composition (g/kg DM basis)						
Gross energy (kcal/kg) 3823		4004	3786	3760	3848	3851
Crude protein	185.6	185.8	185.9	180.5	185.8	185.9
Ether extract	33.8	34.1	33.7	5.3	34.1	33.7
Calculated composition (g/kg DM basis)						
Metabolizableenergy(kcal/kg)	3307	3310	3312	3234	3310	3312
Lysine	10.3	10.3	10.3	10.4	10.3	10.3
Methionine+cystine	6.4	6.4	6.4	6.4	6.4	6.4
Tryptophan	4.9	4.9	4.9	5.6	4.9	4.9
Threonine	7.2	7.2	7.2	7.2	7.2	7.2
Total unsaturated fatty acids (U)	35.7	37.9	39.4	14.7	37.9	39.4
Total saturated fatty acids (S)	11.6	9.4	7.9	3.6	9.4	7.9
U:S ratios	3.08	4.03	4.99	4.08	4.03	4.99

[1]Vitamin and mineral premix provided per kilogram of diet: 450 mg Fe; 400 mg Cu; 250 mg Zn; 150 mg Mn; 0.5 mg I; 0.25 mg Se; 8,000 IU vitamin A; 2,000 vitamin D_3; 37.5 mg vitamin E; 0.925 mg vitamin K-3; 8.43 mg vitamin B_2; 0.04 mg vitamin $B_{12;}$ 34.5 mg nicotinic acid; 26 mg pantothenic acid.

The apparent total tract digestibility of DM, CP, CF, EE, ash, and DE contents were calculated according to Chen et al. (2013). ME was calculated using the equation according to NRC (1998) as shown in following: ME = DE × [1.012-(0.0019 × %CP)]. In experiment 2, a total of 30 pigs (with an equal number of entire males and females) were allotted into 3 groups in a randomized complete block design. Pigs were fed a diet without fat (T1), or diets containing 3% fat with a U:S ratio of 4 (T2) or 5 (T3), respectively. All pigs used in the current experiment were selected based on BW (initial 20 kg) during the pre-experimental period. They were housed in individual pens (1.0 m × 0.75 m) and provided feed and water ad libitum; the room had negative pressure ventilation with an evaporative cooling system. Animal management and care was performed by trained researchers under Naresuan University's Animal Care and Use Committee (NUACUC). Statistically, all data were subjected to statistical

analysis by one-way analysis of variance (ANOVA) followed by Duncan's least-significance multiple range test. Differences between means were considered to be significant at $P<0.05$.

RESULTS AND DISCUSSION

Data on the apparent total tract digestibility of the nutrient and energy values of growing pigs fed diets containing 3% fat with different U:S ratios is presented in Table 2.

Table 2. Apparent total tract digestibility of nutrient and energy value of growing pig fed diets containing 3% fat with different U:S ratios

Item	Apparent total tract digestibility (%)					Energy value	
	DM	CP	CF	EE	Ash	DE[2]	ME[3]
Treatment[1]							
T1	89.50	92.72	70.46	90.21	79.4	3,365[b]	3,352[b]
T2	89.62	92.69	68.73	90.30	79.54	3,604[a]	3,587[a]
T3	89.64	92.39	69.03	88.19	79.20	3,332[b]	3,318[c]
SEM[4]	0.37	0.25	1.11	0.74	0.41	36.07	32.33
P-value	0.99	0.86	0.81	0 .45	0.89	0.01	0.01

Growing pigs were fed with the diets containing 3% fat with a U:S ratio of 3 (T1), 4 (T2) or 5 (T3), respectively. [2]Digestible Energy (kcal/kg); [3]Metabolizable Energy(kcal/kg); [4]Standard error of the means;[a-c] Means within a column having dissimilar superscripts are significantly different ($P<0.05$).

No differences ($P>0.05$) in the apparent total tract digestibility of DM, CP,CF, EE and ash were observed among groups, but the highest DE and ME ($P<0.05$) appeared in pigs receiving the dietary fat at a U:S ratio of 4. Increasing the level of tallow from 3 to 6% decreased the apparent total tract digestibility of GE in pigs (Kellner et al., 2014). This finding seems to suggest that the lower digestibility might be due to the higher saturated fatty acids in the tallow, suggesting that an optimal U:S ratio in dietary fat could be an important consideration. Similarly, Powles et al. (1993) reported that DE content improved exponentially as a function of the U:S ratio. Gatlin et al. (2005) also reported that the apparent digestibility of dietary fat in finishing swine improved with increasing values of the U:S ratio. This seems to suggest that unsaturated lipid may be easier to digest than saturated lipids (Scheeder et al., 2003). Conversely, previous papers described that vegetable oil (expressed as unsaturated fatty acids) or animal fat (representing extremes of saturated fatty acids) did not affect the fat digestibility in pigs (Jørgensen and Fernández, 2000; Kil et al., 2011). The results on the growth performance of growing pigs fed a diet without fat or a diet containing 3% fat with different U:S ratios are shown in Table 3.

All experimental animals remained in good health throughout the study, with no mortalities. No correlations ($P>0.05$) between the diets and the sexes in regard to growth performance of the growing pigs was found.

Table 3.Growth performance of growing pigs fed a diet without fat or a diet containing 3% fat with different U:S ratios

Item	Experimental group[1]				Sex		
	T1	T2	T3	SEM[2]	Barrows	Gilts	SEM[2]
Period of trial, d	50.50	47.20	49.30	0.67	48.21	49.57	0.67
Average daily feed intake, kg/d	1.49	1.48	1.46	0.02	1.51	1.44	0.02
Average daily gain, kg/d	0.60	0.63	0.62	0.01	0.63	0.61	0.01
Feed conversion ratio	2.49	2.35	2.38	0.04	2.43	2.37	0.04
Feed cost/kg of weight gain, Baht	32.70	31.77	32.96	0.48	32.82	32.10	0.48

[1]Growing pigs were fed a diet without a fat source (T1), or the diets containing 3% fat with a U:S ratio of 4 (T2) or 5 (T3), respectively.
[2]Standard error of the means.

Although there were no significant differences in any growth criteria, average daily gain and feed conversion ratio slightly improved ($P>0.05$) in the T2 group. However, Jung et al. (2003) noted that the replacement of animal fat with vegetable oil, such as soybean oil or corn oil (with an increased value of the U:S ratio), resulted in an improved growth performance in weaning pigs. Tartrakoon et al. (2016) also reported that a dietary U:S ratio of 5.0 has the potential to improve the body weight gain and feed conversion ratio of finishing pigs, resulting in a higher quality of pork. According to the current results, improved performance with the highest DE and ME contents ($P<0.05$) occurred in pigs receiving the diet containing 3% fat with a U:S ratio of 4.

CONCLUSIONS

The present results suggest that diets containing 3% fat with a U:S ratio of 4 is optimal for improving the DE and ME contents in growing pigs, resulting in a slight enhancement of growth performance such as average daily gain and feed conversion ratio.

ACKNOWLEDGMENT

This study was financially supported by a research personal account (RPA) under the Faculty of Agriculture, Natural Resources and Environment, Naresuan University.

REFERENCES

AOAC. (2000). Official Methods of Analysis, 17th Edition. Association of Official Analytical Chemists, Gaithersburg.

Chen, L., Zhang, H.F., Gao, L.X., Zhao, F., Lu, Q.P., Sa, R.N. (2013). Effect of graded levels of fiber from alfalfa meal on intestinal nutrient and energy flow, and hindgut fermentation in growing pigs. Journal of Animal Sciences,91:4757–4764.

Gatlin, L.A., See, M.T. and Odle, J. (2005).Effects of chemical hydrogenation of supplemental fat on relative apparent lipid digestibility in finishing swine. Journal of Animal Sciences, 83: 1890–1898

Jørgensen, H. and Fernández, J.A. (2000). Chemical composition and energy value of different fat sources for growing pigs. Acta agriculture Scandinavica. Section A, Animal science, 50:129–136.

Jung, H.J., Kim, Y.Y. and Han, In K. (2003). Effects of fat sources on growth performance, nutrient digestibility, serum traits and intestinal morphology in weaning pigs. Asian- Australasian Journal of Animal Sciences, 16 (7): 1035-1040.

Kellner, T.A., Prusa,K.J. and Patience, J.F. (2014). Impact of dietary fat source and concentration and daily fatty acid intake on the composition of carcass fat and iodine value sampled in three regions of the pork carcass. Journal of Animal Sciences, 92:5485–5495.

Kil, D.Y., Ji,F., Stewart, L.L., Hinson, R.B., Beaulieu, A.D., Allee, G.L., Patience, J.F., Pettigrew, J.E. and Stein, H.H. (2011). Netenergy of soybean oil and choice white grease in diets fed togrowing and finishing pigs. Journal of Animal Science, 89:448-459.

Kraler, M., Schedle, K., Domig, K.J., Heine, D., Michlmayr, H. and Kneifel, W. (2014). Effects of fermented and extruded wheat bran on total tract apparent digestibility of nutrients, minerals and energy in growing pigs. Animal Feed Science and Technology, 197: 121–129.

Myers, W.D., Ludden, P.A., Nayigihugu, V. and Hess, B.W. (2004). Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. Journal of Animal Sciences,82: 179–183.

NRC. (1998). Nutrient requirements of swine. 10th edn. National Academy Press, Washington, DC.

Park, J.C., Kim, S.C., Lee, S.D., Jang,H.C., Kim, N.K., Lee, S.H. Jung, H.J., Kim, I.C., Seong, H.H. andChoi, B.H.(2012). Effects of dietary fat types on growth performance,pork quality, and gene expression in growing-finishing pigs. Asian - Australasian Journal of Animal Sciences,25(12): 1759-1767.

Poorghasemi, M., Seidavi, A., Qotbi, A.A.A., Laudadio, V. and Tufarelli, V. (2013). Influence of dietary fat source on growth performance responses and carcass traits of broiler chicks.Australasian Journal of Animal Sciences, 26 (5): 705-710.

Powles, J., Wiseman, J., Cole, D.J.A.and Hardy, B. (1993). Effect of chemical structure of fats upon their apparent digestible energy value when given to growing/finishing pigs. Animal Science, 57:137–146.

Scheeder, M.R.L., Gumy, D., Messikommer, R., Wenk, C. and Lambelet, R. (2003). Effect of PUFA at sn-2 position in dietarytriacylglycerols on the fatty acid composition of adipose tissues in non-ruminant farm animals. European journal of lipid science and technology, 105:74–82.

Spencer, J.D., Gaines, A.M., Berg, E.P. and Allee, G.L. (2005).Diet modifications to improve finishing pig growth performance and pork quality attributes during periods of heat stress. Journal of Animal Science, 83: 243-254.

Tartrakoon, W., Tartrakoon, T. and Kitsupee, N. (2016). Effects of the ratio of unsaturated fatty acid to saturated fatty acid on the growth performance, carcass and meat quality of finishing pigs. Animal Nutrition, 2: 79-85.

DOI: 10.17707/AgricultForest.63.1.18

***Nurper GUZ, Asli DAGERI*[1]**

MOLECULAR EVIDENCE ON SYMBIOTIC RELATIONSHIPS BETWEEN *BRACOVIRUS* AND *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

SUMMARY

Bracoviruses (BVs) from the Polydnaviridae family are symbionts of parasitic braconid wasps. BVs are used by parasitoid wasps to manipulate their lepidopteran host physiology. The virus is transmitted into the haemocoel of the host during oviposition, together with the parasitoid egg and other maternal protein secretions. Viral products encode proteins that lower host immunity; allowing the development of parasitoid wasp larvae in the host they ensure wasp survival in the lepidopteran larvae. The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), is an important pest that causes extensive damages in many vegetable, fodder, and fiber crops. Although resistance has been developed to different types of insecticides, chemical-based control methods are still used as a management strategy for *S. littoralis*.

In the present study, two transcripts that are members of the C-type lectin family encoding *BV* proteins were annotated from a cDNA library generated from the hemolymph of the fifth instar larvae of *S. littoralis*. Characterizations of the nucleotide and deduced amino acid sequences of these genes approved the bracoviral deriving genes, and also showed sequence similarity to lectin proteins of a parasitoid wasp, *Cotesia* sp. (*Hymenoptera*: Braconidae). A phylogenetic analysis showed a relationship between the C-type lectins from Lepidoptera and Hymenoptera suggesting that there is *bracovirus-mediated* gene flux *between* two orders. We suggest that the *symbiotic* relationship between *bracovirus* and S. littoralis might have an important role in the benefit of wasps, resulting in a suppressed host immune system and also for the evolution process of the interacted genomes.

Keywords: *Bracovirus, Spodopteralittoralis, Lectins.*

INTRODUCTION

Insects have unique adaptation strategies in order to survive. They have developed several defense mechanisms, including the immune system. The immune system of insects involves physical barriers, cellular responses and humoral responses that allow them to interact with microorganisms recognize and remove pathogens, and repair wound and tissue damage (Gillespie *et al.*,

[1]Nurper Guz (corresponding author: nurperguz@ankara.edu.tr), Asli Dageri Department of Plant Protection, Insect Molecular Biology Laboratory, Faculty of Agriculture, Ankara University, TURKEY

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

1997). Sclerotized cuticle, the integument, the peritrophic matrix of the midgut epithelium and the chitin lining of the tracheal system serve as initial barriers against wounds and invading microorganisms (Lavine and Strand, 2002; Wigglesworth, 1972). The hallmark of humoral defense reaction is the production of antimicrobial peptides (AMPs), reactive intermediates of oxygen and nitrogen species (ROS, RNS), and the complex enzymatic cascades leading to melanisation and hemolymph coagulation (Meister *et al.*, 2000 and Lowenberger, 2001; Bogdan *et al.*, 2000 and Vass and Nappi, 2001; Castro *et al.*, 2009; Lavine and Strand, 2002). In contrast, cellular defense refers to hemocyte-mediated immune responses like phagocytosis, nodulation and encapsulation (Strand and Pech, 1995; Schmidt *et al.*, 2001).

A key step before the initiation of an immune response is the detection and recognition of microorganisms. Insects produce some elicitors in order to recognize characteristic molecular patterns of microbial walls known as pathogen-associated molecular patterns (PAMPs) (Gillespie *et al.*, 1997; Jiravanichpaisal *et al.*, 2006). The recognition of these PAMPs is achieved predominantly by peptidoglycan recognition proteins (PRPs) (Elftherianos *et al.*, 2007). The best characterized PRPs in insects are the C-type lectins (CTLs). CTLs have a key role in the recognition of different kinds of pathogens during the early phase of microbial infection (Watanabe *et al.*, 2006). Invertebrate CTLs are involved in immune responses including PPO (*prophenoloxidase*) activation (Yu and Kanost, 2000), hemocyte nodule formation (Koizumi *et al.*, 1999), opsonization and microbial clearance (Jomori and Natori, 1992; Yu and Kanost, 2003). Lepidopteran C-type lectins can also recognize the insects' hemocytes and enhance hemocyte-related responses (Chai *et al.*, 2008).

On the other hand CTLs associated with the bracoviruses have been identified from Hymenopteran parasitoid viral genomes during parasitization which are involved in host immune and developmental suppression (Glatz *et al.*, 2003; Teramato and Tanaka, 2003). Polydnaviruses, comprise two genera Ichnoviruses (IVs) and Bracoviruses (BVs), are a group of insect viruses that have a symbiotic relationship with parasitic wasps.

The primary function of most polydnavirus genes expressed in lepidopteran larvae appears to be suppression of the immunity of its*host.* The endoparasitoid, *Cotesia* (Hymenoptera: Braconidae) maintain its development by attacking larval stages of Lepidopteran insects. During parasitoid oviposition the bracoviral particles are transmitted into the hemocoel of the host, and finally polydnaviruses express their own genes to manipulate the host physiology (Stoltz, 1993; Webb and Strand, 2005).

Here, we report two bracoviral proteins encoded by different transcripts of CTL family found in the transcriptome of the lepidopteran *S. littoralis*. Phylogenetic reconstruction and structural bioinformatics of each gene revealed two major clades that represent the two different CTLs. We further discussed our findings in the context of bracoviruses-host-parasite associations.

MATERIALS AND METHODS

Insect cultures were maintained on an artificial diet at 25 ± 1°C with 60% relative humidity and 16:8 h light-dark photoperiod (Sorour *et al.* 2011). Total RNA from last instar larvae was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was treated with DNase-free (Ambion, Austin, TX, USA) using 1.5 units/µg of total RNA. Quantification and integrity was assessed by using ethidium bromide-stained 1% agarose gel and Nanodrop ND-1000 spectrophotometry (Thermo Scientific, Waltham, MA, USA), with a cut-off value of 1.8 for the A260: 280 ratio.

RNA was fragmented with a zinc chloride solution. Fragmented RNA was quantified using the Agilent 2100 Bioanalyzer system using PicoGreen dsDNA Assay Kit (Invitrogen). cDNA was synthesized using the cDNA Synthesis System Kit with random hexamer primers (Roche Applied Science, Indianapolis, IN, USA). The cDNA fragments were subjected to ligation to the sequencing adaptors provided with the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Science), and small fragments were removed with AMPure XP (Beckman Coulter, Fullerton, CA, USA). Sequencing was performed on a GS FLX platform with Titanium chemistry (Roche/454) using a Small region of a Pico Titer Plate (PTP) per library, following the manufacturer's instructions.

Annotations of assembled sequences were carried out by BLASTx against NCBI (National Center for Biotechnology Information) non-redundant protein sequence databases using the software Blast2GO (Conesa *et al.*, 2005). The partial cDNA and deduced amino acid sequences were compared using the BLASTx tool and EXPASY. Sequences were aligned using the MUSCLE software. Phylogenetic trees were inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were performed with MEGA6 (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

We have constructed cDNA library from the hemolymph of the fifth instar larvae of *S. littoralis* and identified two different contigs encoding bracovirus-like lectin proteins. Contig 00225 corresponded to a transcript identified as *S. littoralis bracovirus-like lectin 1* (*Spli-BLL1*) while Contig 00253 corresponded to *S. littoralis bracovirus-like lectin 2* (*Spli-BLL2*). The *Spli-BLL1* was 1053 bp in length comprising of putative open reading frame (ORF) encoding 131 aa, while Spli-*BLL2* was 653 bp in length containing of putative ORF encoding 147 aa. BLAST analysis revealed that the *Spli-BLL1* showed highest similarity to *Spodoptera exigua BLL2* with 69% identity and *Spli-BLL2* shows highest similarity to *Spodoptera frugiperda CTL* with 87% identity.

Figure 1. Phylogenetic analysis of Spli-BLLs. The name of the used species and accession numbers of the GenBank of the aminoacid sequences are as follows:*Spodopterafrugiperda* proteins obtained from Spodobase (http://bioweb.ensam.inra.fr/spodobase) *S.frugiperda* lectin 3 (Sf1H08856-3-1) and *S. frugiperda* lectin 5 (Sf2H07501-5-1); *Spodopteraexigua*bracovirus lectin like 1-6 KP406769-74; *S. exigua* lectin like AKP99429.1; C-type-lectins from bracoviruses of *Cotesia* species CCQ71085.1; AEE09562.1; AEE09593.1; AGO14401.1; AAS10157.1; AAO74641.1; *B. mori* C-type lectin 19; NP_001165396.1; *B. mori* C-type lectin 21 NP_001037056.1; *Helicoverpaarmigera* lectin ABF83203.1; *H. armigera* C-type lectin 8 AFI47453.1; *H. armigera* C-type lectin 6 AFI47451.1; *H. armigera* C-type lectin 2 ACI32834.1; *Antheraeapernyi* C-type lectin AGN70857.1; *Mamestraconfigurata* C-type lectin AEA76325.1; *Anopheles stephensi* C-type lectin ACP43727.1; *Aedesaegypti* C-type lectin ABF18196.1; *Drosophila melanogaster* C-type lectin NP_001260046.1; *Musca domestica* C type lectin XP_005189940.1

The molecular phylogeny of *S. littoralis* BLLs were examined using amino acid sequences from different *insects and bracovirus genes* (Figure 1). Phylogenetic analysis result showed that Spli-BLL1 and Spli-BLL2 were clustered closely with the BLLs from other *Spodoptera* species and also Spli-BLL2 was clustered together with the Polydnavirus Lectins from *S. frugiperda.* Furthermore, both Spli-BLLs were situated on the same branch with the Bracovirus C-Type Lectin from Hymenoptera. It might be considered that Spli-BLLs have a mutual evolutionary background with BLLs, Polydnavirus Lectins from Lepidoptera and CTLs from Hymenopteran species. Previous bioinformatic analysis listing a series of bracovirus insertions suggested that there is a bracovirus-mediated gene flux between Hymenopteran and Lepidopteran orders (Gasmi *et al.*, 2015). Furthermore it was previously shown that bracoviral DNA integration into hosts and integration back into the wasp genome in a same manner involving a conserved viral site named Host Integration Motif (Beck et al., 2011; Herniou *et al.*, 2013). Altogether, we can suggest that there might be an integration of the BLLs to the ancestral *S. littoralis*, due to the transmission of the bracoviral DNA into the germlines of the Lepidopters.

Humoral or cell-associated lectins are the key component of innate immune responses of animals with the ability to recognize the exposed glycans on the cell surface of potential pathogens (Sun *et al.* 2008). Lectins from hemolymph of cockroaches have been reported to specifically bind bacterial LPS (Jomori and Natori, 1991) and to stimulate hemolymph phenol oxidase activation (Chen *et al.*, 1995). Immulectin synthesis is expressed in response to bacterial challenge in the hemolymph of *Manducasexta* and appears to interact with bacterial LPS to activate the prophenol oxidase system in plasma (Yu *et al.*, 1999). Similarly, bracovirus C-lectin sequences were highly expressed in the *S. exigua* hemocytes (Gasmi *et al.* 2015). We have also detected *Spli-BLL1* and *Spli-BLL2* genes in the hemolymph of larvae using transcriptomic analysis suggesting that these genes might be involved in the immune response of *S. littoralis*.

Several bracovirus sequences expressed by Lepidoptera have been reported that could result in adaptive advantages for the host (Gasmi *et al.*, 2015). Therefore further functional analysis will highlight the association of host parasitoid and bracoviral products in terms of host immune responses and development. Also full length sequences of the Spodopteran *BLL* genes will give more information about the mechanism involved in the integration of the viral gene into Lepidopteran *S. littoralis*.

CONCLUSIONS

Bracoviruses are symbiotic viruses of parasitic wasps which are used to manipulate host physiology. On the other hand CTLs are one of the important molecules involved in pathogen recognition. Here we report novel *Spli-BLL1* and *Spli-BLL2* genes from *S. littoralis* transcriptome analysis. Expression of both genes in the hemocytes of *S. littoralis* suggests that they could be implicated in

the immune response of the cotton leaf worm. We also hypothesize a possible integration of the bracoviral DNA into the germlines of the *S. littoralis* may lead to have a common phylogenetic history among different orders. However further investigation needs to be conducted about the acquisition and integration mechanisms of the bracoviral genes and their role in insect immune system.

REFERENCES

Beck M.H., Zhang S., Bitra K., Burke G.R., Strand M.R. (2011). The encapsidated genome of*Microplitis demolitor bracovirus integrates into the host Pseudoplusia includes*.J Virol. 85: 11685-11696.

Bogdan C., Rollinghoff M., Diefenbach A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Curr. Opin. Immunol. 12, 64-76.

Castro D.P., Figueiredo M.B., Genta F.A. *et al*. (2009). Physalin B inhibits *Rhodnius prolixus* hemocyte phagocytosis and microaggregation by the activation of endogenous PAF-acetyl hydrolase activities Journal of Insect Physiology, 55 (6) 532-537.

Chai L.Q., Tian Y.Y., Yang D.T., Wang J.X., Zhao X.F. (2008) Molecular cloning and characterization of a C-type lectin from the cotton bollworm,*Helicoverpa armigera*.Dev Comp Immunol. 32: 71-83.

Chen C., Durrant H.J., Newton R.P., Ratcliffe N.A. (1995). A study of novel lectins and their involvement in the activation of the prophenoloxidase system in *Blaberus discoidalis*. Biochemistry Journal. 310: 23.

Conesa A., Götz S., García-Gómez J.M., Terol J., Talón M., Robles M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21 (18), 3674-3676.

Eleftherianos I., Gokcen F., Felfoldi G., Millichap P.J., Trenczek T.E., ffrench-Constant R.H.,

Reynolds S.E. (2007). The immunoglobulin family protein hemolin mediates cellular immune responses to bacteria in the insect *Manduca sexta*. Cellular Microbiology 9 (5), 1137-1147.

Felsenstein J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Gasmi L., Boulain H., Gauthier J., Hua-Van A., Musset K., Jakubowska A.K., *et al*. (2015). Recurrent Domestication by Lepidoptera of Genes from Their Parasites Mediated by Bracoviruses. PLoS Genet 11(9): e1005470.

Gillespie J.P., Kanost M.R.,Trenczek T. (1997). Biological mediators of insect immunity. Annual Reviews of Entomology 42, 611-643.

Glatz R., Schmidt O., Asgari S., (2003). Characterization of a novel protein with homology to C-type lectins expressed by *Cotesia rubecula* bracovirus in larvae of the lepidopteran host, *Pieris rapae*. Journal of Biological Chemistry 278, 19743-19750.

Herniou E.A., et al. (2013) When parasitic wasps hijacked viruses: genomic and functional evolution of polydnaviruses.Philos Trans R Soc Lond B Biol Sci. 368: 20130051.

Jiravanichpaisal P., Lee B.L., Söderhäll K. (2006). Cell mediated immunity in arthropods: hematopoiesis, coagulation, melanization and opsonization. Immunobiology 211: 213-236.

Jomori T., Natori S. (1991). Molecular cloning of cDNA for lipopolysaccharide binding protein from the hemolymph of the American cockroach, *Periplenata americana*. J. Biol. Chem. 266: 13318-13323.

Jomori, T., Natori, S.(1992). Function of the lipopolysaccharide-binding protein of *Periplaneta americana* as an opsonin. FEBS Lett. 296, 283-286.

Koizumi N., Imamura M., Kadotani T., Yaoi K., Iwahana H., Sato R. (1999). The lipopolysaccharide-binding protein participating in hemocyte nodule formation in the

silkworm *Bombyx mori* is a novel member of the C-type lectin superfamily with two different tandem carbohydraterecognition domains. FEBS Lett. 443(2): 139-143.

Lavine M.D., Strand M.R. (2002). Insect hemocytes and their role in immunity. Insect Biochem Mol. Biol., 32, pp 1295-1309.

Lowenberger C. (2001). Innate immune response of *Aedes aegypti*. Insect Biochem. Mol. Biol. 31, 219-229.

Meister M., Hetru C., Hoffmann J.A. (2000). The antimicrobial host defense of *Drosophila*. In: Du Pasquier, L., Litman, G.W. (Eds.), Origin and Evolution of the Vertebrate Immune System. Current Topics in Microbiology, vol. 248. Springer-Verlag, Berlin, pp. 17-36.

Saitou N.,Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. MolBiolEvol 4: 406-425.

Schmidt O., Theopold U., Strand M.R. (2001).Innate immunity and evasion by insect parasitoids.BioEssays 23: 344-351.

Sorour M.A., Khamiss O., El-Wahab A.S.E., El-Sheikh M.A.K., Abul-Ela S. (2011). An economically modified semi-synthetic diet for mass rearing the Egyptian cotton leaf worm *Spodoptera littoralis*. Academic Journal of Entomology 4(3): 118-123.

Stoltz D.B. (1993). The PDV life cycle. pp. 167-187, in Parasites and pathogens of insects, Vol. 1, Parasites, Eds. S.N. Thompson, B.A. Federici and Beckage N.E. Academic Press, San Diego, CA.

Strand M.R., Pech L.L. (1995). Immunological basis for compatibility in parasitoid-host relationships. Annu. Rev. Entomol. 40, 31-56.

Sun Y.D., Fu L.D., Jia Y.P., Du X.J., Wang Q., Wang Y.H., Zhao X.F., Yu X.Q., Wang J.X. (2008). A hepatopancreas-specific C-type lectin from the Chinese shrimp *Fenneropenaeus chinensis* exhibits antimicrobial activity. Mol Immunol 45: 348-361.

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution, 30 (12), 2725-2729.

Teramato T., Tanaka T. (2003). Similar polydnavirus genes of two parasitoids, *Cotesia kariyai* and *Cotesia ruficrus*, of the host *Pseudaletia separata*. Journal of Insect Physiology 49: 463-471.

Watanabe A., Miyazawa S., Kitami M., Tabunoki H., Ueda K., Sato R. (2006). Characterization of a novel C-type lectin, *Bombyx mori* multibinding protein, from the *B. mori* hemolymph: mechanism of widerange microorganism recognition and role in immunity. J. Immunol. 177 (7): 4594-4604.

Webb B.A., Strand M.R. (2005).The biology and genomics of polydna viruses.pp. 323-360, in Comprehensive molecular insect science, Eds. L.I. Gilbert, K. Iatrou and S.S. Gill. Elsevier, New York.

Wigglesworth V.B. (1972). The principles of insect physiology. Digestion and nutrition, Chapman and Hall, London (1972), pp. 476–552.

Vass E., Nappi A.J. (2001). Fruit fly immunity. BioEssays 51, 529-535.

Yu X.Q., Gan H., Kanost M.R. (1999). Immulectin, an inducible C-type lectin from an insect, *Manduca sexta*, stimulates activation of plasma prophenol oxidase. Insect Biochemistry and Molecular Biology, 29 (7), 585-597.

Yu X.Q., Kanost M.R. (2000). Immulectin-2, a lipopolysaccharide-specific lectin from an insect, *Manduca sexta*, is induced in response to gram-negative bacteria.J Biol Chem. 275: 37373-37381.

X.Q., Kanost M.R. (2003). *Manduca sexta* lipopolysaccharide-specific immulectin-2 protects larvae from bacterial infection. Dev. Comp. Immunol. 27 (3): 189-196.

DOI: 10.17707/AgricultForest.63.1.19

Jasmina Balijagić, Jelena Bošković,
Jovan CRNOBARAC, Veselinka ZEČEVIĆ,
Dragoja RADANOVIĆ, Gordana DOZET, Miodrag JOVANČEVIĆ [1]

MORPHO - PHYSIOLOGICAL CHARACTERISTICS OF GENTIAN (*Gentiana lutea* L.) GENOTYPE SEEDLINGS FROM NATURAL POPULATIONS IN MONTENEGRO

SUMMARY

The aim of this paper is to examine the possibility of gentian generative propagation, because the production of high-quality planting material is very important not only for the beginning of revitalization of this plant species in their natural habitat but also for its large-scale production in Montenegro. Cultivation of gentian, as well as other medicinal plants, represents a safe way for their protection and conservation in the wild. For the purpose of choosing the best population, seed samples from 9 natural habitats were collected in the mountains of the northern part of Montenegro. The field trial in which dynamics of gentian seedlings growth was monitored in the first and the second year of vegetation was set up by sowing of seeds collected in natural conditions at the locality *Korita*.

The average fresh root weight of the sowed annual seeds was 0.17 g and the maximum weight had seedlings originating from the locality called *Jovanova koliba.* The average dry matter content in the roots was 29.18%. The average root length of biennial seedlings of the tested population was 14.6 cm and maximum length had seedlings originating from *Prelija*. The average root fresh weight of the analyzed population of biennial seedlings was 0.96 g and maximum weight had seedlings from *Jovanova koliba* (1.57 g).

Keywords: *Gentiana lutea, natural populations, seedling, root*

INTRODUCTION

Gentian lutea L. (yellow, *big* gentian or *Gentianella austriaca*) is a perennial, heliophilous, herbaceous rugged mountain plant of wide ecological valence. Its habitats are spread across the *Alps*, the *Apennines*, the *Carpathian mountains*, the *Iberian* and the *Balkan Peninsula* (Hesse *et al*., 2007; Vender *et al*., 2010) 300-2800 m above sea level, on different soil types, which as a rule

[1]Jasmina Balijagić (correspondging author: jas.be@t-com.me), Miodrag Jovančević University of Montenegro, Biotechnical Faculty, Podgorica, MONTENEGRO, Jelena Bpšković Business Academy University in Novi Sad, Faculty of Economics and Engineering Management, Novi Sad, SERBIA, Jovan CRNOBARAC University of Novi Sad, Faculty of Agriculture, Novi Sad, SERBIA, Veselina Zečević, Gordana Dozet John Naisbitt University Belgrade, Faculty of Biofarming in Bačka Topola, SERBIA, Dragoja Radanović Institute for Medicinal Plant Research "Dr. Josif Pančić" Belgrade, SERBIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

contain more than 6% of humus (Stepanović and Radanović, 2011). Gentian is more or less found on almost all Montenegrin mountains: *Sinjajevina, Bjelasica, Prokletije (Plav-Gusinje), Hajla, Komovi, Durmitor, Ljubišnja, Morača Mountains, Orjen, Lovcen, Rumija, Volujak, Vojnik, Golija, in the plateau of Korita* as well as in *Štedim* **(**Balijagić *et al*., 2012a; 2012b).

The demand for gentian use in herbal medicine and the beverage industry has been growing steadily (Sezik *et al*., 2005; Kušar and Baričević, 2006; Balijagić *et al*., 2012a; 2012b). Only in Europe, 400-500 tons of dry root (Radanović *et al*., 2014) is consumed annually. Thus, large amounts of yellow gentian root cannot be collected from natural populations over a longer period of time without the risk of its destruction. In almost all countries, yellow gentian is under a regime of strict control against exploitation, and in most countries of South Eastern Europe its use from natural populations is prohibited (Radanović *et al*., 2014).

In the last century, irrational exploitation of gentian caused its eradication or great reduction in most of our mountains. Sustainable use of natural resources can only be achieved with the introduction of plants, as well as cultivation, which is an important way to protect endangered plant species (Menges, 2000). Therefore, in recent decades of the last and at the beginning of this century, abroad, but also in Serbia and Slovenia great efforts had been made to develop a technology for gentian cultivation, especially in rural mountainous areas (Kušar and Baričević, 2006; Radanović *et al*., 2007b; 2007c; Radanović, 2008; Franz, 2012; Radanović *et al*., 2013; 2014; Balijagić, 2013; González-López *et al*., 2014; Sand, 2015).

The aim of this study was to examine the possibility of reproduction of gentian seeds and choose the most suitable populations of plant species in agricultural conditions of Montenegro. The production of high-quality planting material is very important for the start of the revitalization of plant species in their natural habitats as well as for their large-scale production in Montenegro. The cultivation of gentian as well as other medicinal plant species represents a safe way for protection and conservation of wild plants whose survival is endangered due to overexploitation. Besides that, the cultivation of endangered species has an important socio-economic dimension, as it can enable the rural development of the impoverished population in mountain regions.

MATERIAL AND METHODS

In 2009, the gentian seeds were collected from nine natural habitats in the mountains of northern part of Montenegro that are located at altitudes from1.400 to 2.076 m (Table1). The seeds were collected in accordance with the Law on Environmental Protection. Parts of collected inflorescence were dried in the shade, in the drafty place to air dry state, followed by manual threshing of seeds.

The same year, the field trial was set up under natural conditions at the locality of *Korita* (Sušica), which is at an altitude of 1.300 m and has good soil conditions for gentian cultivation (Table 2).

Table 1. Localities where in 2009 the seeds of yellow gentian were collected, the mountains in northern part of Montenegro

Population		**Number of plants (average)**	**The average fresh root (g) mass**	**The average dry root (g) mass**
Strmenica		24.00	0.178	0.051
Kobilja glava		24.50	0.138	0.038
Durmitor		22.25	0.277	0.075
Gutavica		31.25	0.095	0.033
Konjska rijeka		15.50	0.123	0.037
Prošćen Mountains		16.50	0.111	0.029
Prelija		13.50	0.142	0.043
Jovanova koliba		17.50	0.301	0.088
Ljubišnja		16.00	0.164	0.048
Average		20.11	0.170	0.049
LSD	1%	-	0.131	0.039
	5%	-	0.097	0.029
F – probability (p)			0.001	0.003

During September 2009, the sowing in the field trial was conducted in already prepared beds, according to the method of non-hormonal production of gentian seedlings in open winter beds (Radanović *et al*., 2007b).

According to the agrochemical analyses of the soil (Table 2), the farmyard manure was added to the soil before planting, at a dose of 100 m^3 ha^{-1} and mineral fertilizer NPK (8:20:30) at a dose of 150 kg ha^{-1}. The basic agrochemical analyses of the soil are determined by standard methods prescribed for soil analysis. Weed control in experimental plots was performed manually by hoeing and weeding, and during the summer beds were semi-shaded by a protective net. The field trial was irrigated as needed.

Table 2. Agrochemical soil analyses (0-30 cm) from the localities where the trial was set up

Number of samples	**pH**		**$CaCO_3$** (%)	**Humus** (%)	**P_2O_5**	**K_2O**
	u H_2O	u KCl			mg/100 g of soil	
1	6.43	5.85	2.8	4.81	2.2	11.5
2	6.17	5.60	2.6	4.48	2.6	10.4

For each of the studied population, four furrows (4 replications) were formed in beds, and each was sown by 250 seeds. At the end of the first year of vegetation, from each furrow, in four replications, at row length of 15 cm, the seedlings were removed. Firstly, the number of seedlings was determined, and this was followed by measurements of roots' neck width, roots' length, roots'

mass per seedling, as well as dry matter content in the root of seedling. The same measurements were also performed at the end of the second vegetation period.

The weight of plant was measured by precise laboratory beam scale while the length and thickness of seedlings' parts were measured by a vernier caliper.

Statistical data processing included a factorial analysis of variance and the averages of treatments were compared by LSD test (Witte and Witte, 2013) and a standard deviation (SD) was calculated as a measure of variation.

RESULTS AND DISCUSSION

Morpho-physiological characteristics of the annual seedlings

The average number of plants in analyzed samples of all populations was 20.11. The minimum number of plants (13.50) belonged to the population originating from locality *Prelija* locality while the maximum (31.25) from the locality called *Gutavica* (Table 3).

Table 3. The number of the studied plants and morho-physiological characteristics of the annual seedlings

Population		Number of plants (average)	The average fresh root (g) mass	The average dry root (g) mass
Strmenica		24.00	0.178	0.051
Kobilja glava		24.50	0.138	0.038
Durmitor		22.25	0.277	0.075
Gutavica		31.25	0.095	0.033
Konjska rijeka		15.50	0.123	0.037
Prošćen Mountains		16.50	0.111	0.029
Prelija		13.50	0.142	0.043
Jovanova koliba		17.50	0.301	0.088
Ljubišnja		16.00	0.164	0.048
Average		20.11	0.170	0.049
LSD	1%	-	0.131	0.039
	5%	-	0.097	0.029
F – probability (p)			0.001	0.003

The average fresh root weight of the annual seedlings was 0.170 g. The minimum average fresh root weight (0.095 g) was recorded on plants from locality *Gutavica*, while the maximum (0.301g) on gentian seedlings from locality *Jovanova koliba*. F-test of variance analysis (0.001) shows that the localities had significant effect on the fresh root weight of annual seedlings (Table 3).

The average weight of air dried roots in all seedlings was 0.049 g. The minimum weight (0.029 g) of air dried roots had the plants obtained by sowing

seeds of natural populations from the locality called *Prošćen mountains* and the maximum (0.088 g) from the locality called *Jovanova koliba*. F-test of variance analysis (0.003) shows that the localities had a significant effect on the mass of air dried roots (Table 3). The values of annual seedling mass were significantly lower in comparison to those obtained in the study of RADANOVIĆ *et al*. (2013), in which at the end of the first growing season in Serbia the average seedling fresh mass of gentian was 1.79 g. These differences are presumably caused by high quality seeds obtained by cultivated gentian, as well as by better conditions in seed beds seedlings' production in Serbia.

Morpho- physiological characteristics of biennial seedlings

The average number of plants of biennial seedlings in the studied samples of yellow gentian was 28.3. The maximum number of plants (42.0) was obtained from the seeds originating from the natural population of *Durmitor* locality and the minimum (15.3) from seeds of *Prošćen mountains*. F-test of variance analysis shows that localities had no significant effect on the number of plants (Table 4). Previous experiences in cultivation of yellow gentian obtained in the southern and South-eastern Europe demonstrated that the climatic conditions and the slow rooting and development of seedlings in the first two years of cultivation are the main limiting factors which cause great losses of plants and thus the low root yield (RADANOVIĆ *et al., 2014*).

Table 4: The number of the studied morpho - physiological characteristics of biennial seedlings

Population	Number of plants (average)		The average root neck widht (mm)	The average root length (cm)	The average fresh root mass (g)	The average dry root mass (g)
Strmenica	16.8		3.0	12.2	0.57	0.17
Kobilja glava	33.3		3.3	15.8	0.67	0.20
Durmitor	42.0		4.2	14.0	1.01	0.29
Gutavica	29.3		4.4	15.5	1.29	0.35
Konjska rijeka	28.8		3.5	13.3	0.72	0.19
Prošćen mountains	15.3		3.0	13.5	0.48	0.28
Prelija	30.0		4.0	16.2	1.10	0.13
Jovanova koliba	17.5		4.8	15.4	1.57	0.24
Ljubišnja	41.8		4.1	15.7	1,23	0.41
Average	28.3		3.8	14.6	0.96	0.25
LSD	1%	30.27	1.0	4.0	0.71	0.15
	5%	22.33	0.8	2.9	0.52	0.11
F - probability	0.14		<.001	0.103	0.003	<.001

The average width – roots' neck diameter of the examined populations was 3.8 mm. The minimum average diameter of 3.0 mm had seedlings originating from locality *Strmenica* and the maximum of 4.8 mm seedlings produced from the seeds of *Jovanova koliba* locality. F-test of variance analysis (<.001) shows that localities had significant effect on the diameter of the root neck of biennial gentian seedlings (Table 4)

The average value of the standard deviation (SD) for the width of the root neck for nine populations of the studied seedlings was 1.26 mm (Table 5). The minimum Sd value of 1.04 was obtained from seedlings originating from seeds from locality *Durmitor* and the maximum (1.81) from seedlings obtained from seeds from locality *Jovanova koliba*.

For nine populations, the average value of the coefficient of variation (CV) width of roots' neck was 33. 44%. The lowest coefficient of variation (24.81) was between seedlings from *Durmitor* locality and the highest (43.96) from locality *Strmenica*. Individually, the smallest root neck width of a biennial gentian (0.90 mm) was recorded in seedlings from locality *Prelija* and the highest (9.90 mm) in seedlings obtained from seeds of *Gutavica* locality. The obtained values of the width of root neck of biennial seedlings in this trial are lower in comparison to those obtained in the study of RADANOVIĆ *et al*. (2013) who at the end of the second year of growing obtained plants with average diameter of roots' neck of 13.03 mm. The plants were grown out of cultivated gentian seeds from Serbia.

Table 5*:* Indicators of biennial seedlings variability of root neck width

Population	SD (mm)	CV (%)	min	max
For all 9 population	1.26	33.44	0.90	9.90

The average root length of the studied populations was 14.6 cm. The minimum average root length (12.2 cm) had seedlings originating from *Strmenica* locality and the maximum (16.2 cm) had seedlings originating from *Prelija* locality. F-test analysis of variance (0,103) shows that the localities had no significant effect on the root length (Table 4).

The average value of SD for root length for nine populations was 4.98 (Table 6). The minimum value (3.35) was recorded in seedlings from locality *Jovanova koliba*, and the maximum (14.53) in seedlings from locality *Kobilja glava.* The average value of the coefficient of variation between root lengths (CV) in the examined population was 33.67.

The highest coefficient of variation (91.91) in root length had the seedlings of the population from locality *Kobilja glava*, and the smallest (20.75), the seedlings of the population from locality *Jovanova koliba.* The minimum root length (4.50 cm) measured separately, had seedlings obtained from the seeds of locality *Prelija*, and the maximum (30.80 cm) seedlings of the population from locality *Durmitor*.

Table 6: Indicators of biennial seedlings' variability of root length

Population	SD (cm)	CV (%)	min	max
Strmenica	4.29	34,98	4.70	21.30
Kobilja glava	14.53	91,91	7.1	23.30
Durmitor	4.04	28.94	6.30	30.80
Gutavica	3.75	24.18	7.50	24,80
Konjska rijeka	3.51	26.28	7.60	26.90
Prošćen mountains	3.90	24.89	8.30	24.50
Prelija	3.55	26.25	4.50	20.50
Jovanova koliba	3.35	20.75	9.50	24.70
Ljubišnja	3.93	24.85	5.40	24.00
For all 9 population	4.98	33.67	4.50	24.8

The average value of the fresh root mass of biennial seedlings was 0.96 g. The minimum average mass (0.48 g) had seedlings obtained from seeds from locality *Prošćen mountains*, and the maximum (1.57 g) had seedlings of the population from locality *Jovanova koliba*. F-test analysis of variance (<.001) shows that localities had significant effect on fresh root mass (Table 4).

The average value of standard deviation of the examined biennial seedlings' fresh root mass was 0.85 (Table 7). The minimum value of SD (0.44) was recorded in seedlings of the population from locality *Prelija* and the maximum (1.42) had seedlings of the population from *Ljubišnja* locality. Coefficient of variation value (CV) for the studied population fresh root weight was 92.21%. A minimum variation of fresh root weight (59.33%) was within the population of locality *Durmitor* and a maximum (124.58%) had seedlings from locality *Konjska Rijeka*. The minimum mass obtained by the separate measurement of seedlings fresh root weight (0.02 g) had seedlings from locality *Prelija* and the maximum (8.42 g) had seedlings of the population from locality *Gutavica.*

Table 7. Indicators of biennial seedlings' variability of fresh roots mass

Population	SD (g)	CV (%)	min	max
Strmenica	0.62	112.8	0.04	2.49
Kobilja glava	0.69	102.01	0.08	3.59
Durmitor	0.60	59.33	0.17	3.34
Gutavica	1.30	101.14	0.16	8.49
Konjska rijeka	0.90	124.58	0.15	4.85
Prošćen mountains	0.81	66.08	0.28	4.07
Prelija	0.44	91.86	0.02	1.69
Jovanova koliba	0.88	80.25	0.10	4.20
Ljubišnja	1.42	91.87	0.12	6.79
For all 9 population	0.85	92.21	0.02	8.49

The average of air dried root mass of the studied populations was 0.25 g. The minimum average mass had seedlings obtained from seeds collected in *Prelija* locality (0.13 g) while the maximum mass had seedlings from mountain *Ljubišnja* (0.41). F-test analysis of variance (<.001) shows that localities had a significant effect on air dried root mass of seedlings (Table 4).

The average root length of biennial seedlings was 14.6 cm. F-test analysis of variance indicates that the localities did not affect seedlings root length. Our results are in accordance with the statements of Radanović *et al*. (2007a): the length of annual seedlings in September were about 10 cm because there was a great probability that root of annual seedlings would grow for 1.25 cm more by October, the time when the measurements were performed.

In the third year of gentian cultivation in the pots, Galambosi and Galambosi (2010) achieved the one plant root yield of 78 g with 3 plants per pot, up to 44.3 g with 10 plants per pot.Our studies also confirmed that higher seedling number and density per unit of area reduce weight of seedlings root.

CONCLUSIONS

The origin of seeds i.e. the population had a significant effect on annual and biennial seedlings fresh and dry root mass, as well as on the width of roots' neck, while the origin of seeds had no significant effect on the relation between fresh and dry root mass, as well as on the length of both kinds of seedlings.

The population of seeds from the locality called *Jovanova koliba* should be used for the production of gentian seedlings. That seed had the highest average fresh root yield in both, annual and biennial seedlings with the lower coefficient of variance for this trait in comparison with the average value for all populations. Along with the gained experience in cultivation, this population would provide the best results in the production of gentian seedlings.

ACKNOWLEDGEMENTS

We would like to express the deepest gratitude to the Ministry of Education and Science of Montenegro for the financial support of three- year scientific research project "The examination of the possibility for gentian seedling production as well as the environmental and biodiversity protection" (No. 06/3-2302/7).

REFERENCES

Balijagić, J., T. Janković, G. Zdunić, J. Bošković, K. Šavikin, D. Godevac, T. Stanojković, M. Jovančeić, N. Menković (2012a): Chemical profile, radical, seevenging and cytotoxic activity of yelow Gentian leaves (*Gentitaneae luteae folium*) grown in Northern Regions of Montenegro. Natural Product Communications, *7* (11): 1487-1490.

Balijagić, J., D. Radanović, S. Antic Mladenović, J. Crnobarac, J. Bošković, V. Zečević, G. Dozet, M. Jovančević (2012b): Mineral content of autochthonous gentian (*Gentiana lutea* L.) root from natural habitats in northern parts of Montenegro. In Dajić-Stevanović Z. and D. Radanović (Eds). Proceedings of the seventh conference on medicinal and aromatic plants of Southeast European countries, Subotica, May 27th-31st, 2012, pp. 255-264.

Balijagić, J. (2013): Populations of yellow gentian (*Gentiana lutea* L.) in the northern part of Montenegro and possibilities of growing. (in Serbian). PhD. thesis, Megatrend University Belgrade, Faculty of Biofarming Backa Topola, Serbia, pp. 1-108.

Franz, CH. (2012): Enzian, Gelber (*Gentiana lutea* L.). In: Handbuch des Arznei-und Gewürzpflanzenanbaus, Band 4, Arznei-und Gewürzpflanzen A-K, Verein fur Arznei-und Gewürzpflanzen Saluplanta e.V. Bernburg, pp. 375-385.

Galambosi, B., Z.S. Galambosi (2010): Seedling quality and seed yield of *Gentiana lutea* L. Acta Hortic. *860*, 255-258. http://dx.doi.org/10.17660/ActaHortic.2010.860.38

González-López, Ó., P.A. Casquero (2014): Effects of GA_3 pregerminative treatment on *Gentiana lutea* L. *var. aurantiaca*. Germination and seedlings morphology. The Scientific World Journal, *vol. 2014*: 1-6. http://dx.doi.org/10.1155/2014/751279

Hesse, E., M. Rees, H. Müller-Schärer (2007): Seed bank persistence of clonal weeds in contrasting habitats: implications for control. Plant Ecol., 190: 233-243.

Kušar, A., D. Baričević (2006): Cultivation trials of yellow gentian (*Gentiana lutea* L. subsp. *Symphyandra* Murb.) in west part of Slovenia. Acta Agriculturae Slovenica, *87*: 213-22.

Menges, C.E. (2000): Population viability analyses in plants: challenges and opportunities. Trends Ecol Evol. *15*: 51-56.

Radanović, D., T. Marković, T. Janković (2007a): Morphological and chemical parameters of importance for cultivation of *Gentiana lutea* L. in mountain region of Serbia, 1st International scientific conference on medicinal, aromatic and spice plants, Nitra, Slovakia, December 5-6, 2007, Book of scientific papers, 28-32.

Radanović, D., T. Marković, B. Spasojević (2007b): Without the hormone production process of seedlings of yellow gentian (*Gentiana lutea* L.) in open seed beds. (in Serbian). The technical solution, Institute for Medicinal Plant Research "Dr. Josif Pančić" Belgrade, 1-16.

Radanović, D., S. Antić Mladenović, M. Jakovljević, M. Kresović (2007c): Content of heavy metals in *Gentiana lutea* L. roots and galenic forms. J. Serb. Chem, Soc. *72* (2): 133-138.

Radanović, D. (2008): New advances in the cultivation of yellow gentian (*Gentiana lutea*) and arnica (*Arnica montana*) in Serbia. (in Serbian). Plenary lecture, XXVIII Conference on medicinal and aromatic plants, Section for medicinal plants, Vršac, 8-11. 10. 2008, Proceedings, 12-18.

Radanović, D., T. Marković, S. Antić Mladenović (2013): Production of yellow gentian (*Gentiana lutea* L.) nursery plants suitable for transplanting and cultivation under dry farming conditions in mountain region of Serbia. Ratar.Povrt. *50* (3): 13-21.

Radanović, D., T. Marković, N. Aielo, P. Fusani (2014): Cultivation trials on *Gentiana lutea* L. in Southern and South-eastern Europe**.** Journal of Applied Research on Medicinal and Aromatic Plants, *1*(4):113-122.

Sand, S.C. (2015): Peculiarities for agricultural techonolgy of gentiana lutea. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, *15*, (1): 449-454.

Stepanović, B., D. Radanović (2011): The technology of cultivation of medicinal and aromatic plants in Serbia. (in Serbian). Institute for Medicinal Plant Research "Dr. Josif Pančić" Belgrade, pp. 1-243.

Vender, C., N. Aiello, S. Piovesana (2010): Survey of yellow gentiana populations of the Central Alps and record of their main morphological and qualitative characteristics. Acta Hortic. *860*, 101-104. http://dx.doi.org/10.17660/ActaHortic.2010.860.13.

Witte, S.R., J.S. Witte (2013): Statistics, 10th Edition. Publisher: Wiley, ISBN-13: 978-1118450536. P. 576.

DOI: 10.17707/AgricultForest.63.1.20

Alexander IVANOV,
Anastasia ERMOLAEVA, Julia YUDICHEVA[1]

PATTERNS OF SETTLEMENT OF MYCORRHIZA-FORMING-FUNGI IN THE BROAD-LEAVED FORESTS OF VOLGA FOREST-STEPPE

SUMMARY

Species composition of mycorrhiza-forming fungi of the class *Agaricomycetes* of broad-leaved forests of the Volga forest-steppe in the area of the Penza region was studied. Those forests have the richest species composition of the fungi of the studied group among forest community of the region in which they are presented by 134 species. The most common species, identified in all studied associations, are *Amanita crocea, A. pantherina, A. phalloides, A.rubescens, A.vaginata, B. reticulatus, Clytopilus prunulus, Hebeloma sinapizans, Lactarius quietus, L. piperatus, L. vellereus, , Neoboletus erythropus Russula foetens, R. delica, R. lutea, R. pseudointegra, Xerocomellus chrysenteron.* Due to the diversity of soil and hydrological conditions, the settlement of the related symbiotrophic species seems rather complex. Impoverished species composition of the symbiotrophic species is typical for *Quercus robur+Tilia cordata* associations on acid soils with pH from 4.3 to 4.5 and much richer for the broad-leaved forests on soils with pH of 6-7. The forest-steppes in flood plains of the water-collecting area of the Volga river are easternmost point of settlement of South-European nemoral species. Therefore, they are important from the point of view of the protection of rare species of agaricomycetes**.**

Keywords: calciphiles**,** mycorhizal symbiosis, rare species, *broad-leaved forests*, symbiotrophic species.

INTRODUCTION

Among of forest communities of Russian Plain the broad-leaved forests are the least studied regarding mycorrhiza-forming fungi. Brief information of them is represented in the publications of Ganzha, Bedenko, Svetashevoy, Rebriyeva and others (1960, 1979, 2015). They have been studied concerning the Volga forest-steppe by Ivanov (1987). However, to date, previously published materials have been significantly clarified and supplemented. The study of the mycorrhiza-forming fungi is critical from the understanding of

[1]Alexander Ivanov (corresponding author: rcgekim@mail.ru), Anastasia Ermolaeva, Julia Yudicheva Penza State Agricultural Academy, Penza, 30 Botanicheskaya str. 440014 RUSSIA
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

biological features of *Qercus robur* point of view which is the main forest forming species in these conditions.

MATERIALS AND METHODS

Study results conducted from 1978 to 2016 in broad-leaved forests of the Volga forest-steppe in the area of the Penza region were used as a basis for this paper. This area is located about 600 km south-west of Moscow (fig.1).
The climate of the considered area is moderately continental with recurrent droughts. The average temperature in July is +19°C - + 19.5° C, in January - -12,5°C - -13° C. The amount of precipitation in the different regions of the area is from 300 to 650 mm per year (Zhakov, 1970).

Figure 1. Geographical position of the Penza region

The relief throughout majority of the studied area is flat with intense erosion partitioning (?). Grey forest soils and salt washed black soils prevail in a soil cover. Underlying parent materials are represented by sands, flasks, noncalcareous and calcareous clay (Milkov, 1953).

Vegetation has a typical forest-steppe appearance. Forests are presented by separate areas of different sizes surrounded by agricultural holdings. Native types of forest vegetation are pine and broad-leaved forests. *Betula pendula* Roth(Author names for all species after first appearance in the text and *Populus tremula* L.forests are also widespread. Mycological studies were conducted for Broad-leaved forests mainly composed of *Qercus robur* L., *Tilia cordata* Mill.

and *Corylus avellana* L. (. *Carex pilosa* Scop. and *Aegopodium podagraria* L. prevail in a grass cover. Studied forests were cut down during the Second World War. They are of the same age (65-70 years) and have a vegetative origin.

We have studied species composition of mycorrhiza-forming fungi in the studied area from 1978 to 2016 by us. Herborization and identification of collected samples of the fungi were conducted by commonly used methods, using a light microscopy to study the morphology of the microstructures (Bondarcev, Zinger, 1950; Moser, 1978; Funga Nordica, 2008). Monographic reports were used to identify certain taxa Bakker, 2005; Muñoz,2005 et al.).

Censuring of fruit body and species variety of fungi was conducted by route and stationary methods. We have paved four routes taking into account the diversity of forest growth conditions (fig 2). The routes were visited in summer and autumn periods weekly because climatic conditions in these periods are favorable for growing fruits bodies. We have carried out the route studies from 1984 to 2016.

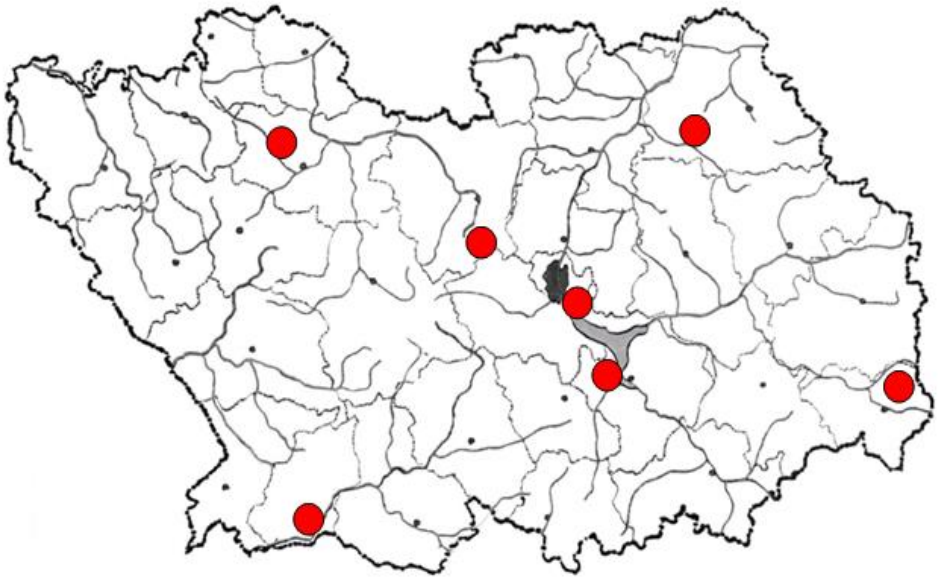


Figure 2. Venues route and stationary studies IT is not cear what is on the map. More precise Explanation should be given

The route studies were supplemented with stationary studies. We had laid four testing transects of 10x100 m to register species composition of mycorrhiza-forming fungi and yield of their fruit bodies from 2007 to 2009. Fruit bodies were collected with an interval of five days, dried and weighed. Drying of the fungi was carried out in the New Zealand dehydrator Ezidri snackmaker FD500 manufactured by Hydroflou indastris. The need for the drying was determined by the fact that water content in fruit bodies of fungi is not the sane in different

weather. Therefore, drained weight is a more objective index of biological productivity as compared with the weight of fresh fungi.

Characteristics of plant associations, soils, species composition and productivity of fungal fruit-bodies are given in table 1. Determination of pH of a salt extract of soil was carried out by means of pH meter pH-410 manufactured by NPKF "Akvilon" (Russia). The relevance of the names of fungi and their accuracy has been verified according to the database Index fungorum (2016).

Table 1. Species diversity and yields of Agaricomycetes mycothallus in broad-leaved forests on different types of soil

Association name	**Soil characteristic**			**Mycobiota characteristic**	
	Soil variety	Underlying rock	pH of salt extract	Number of species	Yield of fruiting body, g
Quercus robur+tilia cordata- Aegopodium podagraria	heavy loam gray forest soil	Noncalcareous clay	4.3-4.5	12	763
Quercus robur+tilia cordata-Carex pilosa	light sandy loam gray soil	Flask	4.2-4.5	18	3065
Quercus robur+tilia cordata- Carex pilosa	light loam gray forest soils	Marl	6.5-7	25	4950
Quercus robur+tilia cordata- Carex pilosa+ heterogerbosa	sandy loam gray forest soil	Sand	6-6.5	27	4944

RESULTS AND DISCUSSION

Among the forest communities of the examined region, the broad-leaved forests have the richest species composition of mycorrhiza-forming fungi. They are presented by 134 species, most of them are connected with symbiotic relationships with the main forest-forming species *Qercus robur*. 85 species are connected with *Pinus silvestris* L., 60 with *Betula pendula*, 39 with *Populus tremula*, 46 with *Alnus glutinosa* (L.) Gaertn. (Ivanov, 1987; 1992; 1994). However, the variety of species diversity of symbiotrophic species is not combined with a large-statured abundance. According to the attendance of testing areas, the average yield of fruit bodies of mycorrhiza-forming fungi in board-leaved forests is lower than in other types of forests. There is only 36805 g/ha in terms of the equivalent amount of dry substance, but in pine forests, the value is 55520 g/ha and 104700 g/ha in birch forest (Ivanov, 1992).

In the present research work the volume of a trophic group of symbiotrophic species is understood in accordance with the (Tedersoo et al., 2010). Most of its fungi in broad-leaved forests of the studied area are associated with *Qercus robur* which is the only highly mycotrophic species. Very few of

them form mycorrhizas with *Tilia cordata* (*Cortinarius rigens* Fr., *Neoboletus erythropus* (Pers.) C. Hahn, *Russula aurea* Pers, *R.delica* Fr. and *Corylus avellana* (*Lactarius pyrogalus* (Bull.) Fr., *L.volemus* (Fr.) Fr.) as evidenced by observations in pure stands of the last-mentioned.

The most common species, identified in all routes and most of testing area, are *Amanita crocea* (Quél.) Singer, *A. pantherina* (DC.) Krombh., *A. phalloides* (Vaill. ex Fr.) Link, *A. rubescens* Pers, *A.vaginata*(Bull.) Lam., *Boletus reticulatus* Schaeff., *Clitopilus prunulus* P. Kumm, *Hebeloma sinapizans* (Paulet) Gillet, *Lactarius quietus* (Fr.) Fr., *L. piperatus* (L.) Pers., *L. vellereus* (Fr.) Fr, *Neoboletus erythropus, Russula foetens* Pers., *R. delica, R.risigallina* (Batsch) Sacc., *R. pseudointegra* Arnould & Goris, *Xerocomellus chrysenteron* (Bull.) Šutara. These species have a broad ecological valence. They are observed in all sites, but with different degree of abundance.

Due to the variety of soil and hydrological conditions, the settlement of symbiotrophic species related to broad-leaved forests is rather complex. Species composition of symbiotrophic species in the most favorable conditions for growth of trees, on moister and humus rich grey forest soils, usually occupied by association *Quercus robur+Tilia cordata-Aegopodium podagraria,* is significantly impoverished, their abundance is also minor. It corresponds with the theory of Bierkman, which suggests that the mycorrhiza-forming fungi become weaker with good support of trees with mineral elements and moisture. (Bowen, 1973; Brundrett, 2004; 2009, Bruns, 1995, 2002; Buscot, 2000; Smith, Read 1997).

The testing transect No. 1 was laid in woodland consisting of *Quercus robur* and *Tilia cordata* of vegetative origin at the age of 65-70 to study species composition of symbiotrophic species and their yield. Underbrush consists of *Corylus avellana* and *Euonimes verrucosa* L., projective cover is 70%. *Aegopodium podagraria* prevails in the grass cover. The projective cover is about 95%. The soil is heavy loamy gray forest soil, underlain by carbonate-free clay, and pH is 4.5. 12 species were identified in the testing area. Ten more species were revealed in the course of the route studies of the present association. These species, identified in studied association, include all above mentioned species and *Cortinarius hinnuleus* Fr., *C.infractus* (Pers.) Fr., *C. rigens* (Pers.) Fr., *C. trivialis* (J.E. Lange) A.H. Sm., *Lactarius pyrogalus, L. vellereus, Leucocortinarius bulbiger* (Alb. & Schwein.) Singer. They are the fungi with broad ecological valence. They are not typical for locality under consideration. The yield of mycorrhiza-forming fungi in these localities was only U7630 g / ha. It is a minimal index for concerned forest communities.

Association of *Quercus robur+tilia cordata-Carex pilosa* occupies drier localities, in comparison with lipo -*Quercus robur+tilia cordata- Aegopodium podagraria,* related to different variety of grey forest soils. Their most common variant in the study area is a sandy loam light-gray soil formed on silicate rock detritus flask with pH from 4.2 to 4.5. The testing transect No. 2 was laid in association of *Quercus robur+tilia cordata- Carex pilosa+ heterogerbosa* of

vegetative origin at the age of 65-70 to study species composition of symbiotrophic species and their yield. Projective cover of the underbrush is 55% and consists of *Corylus avellana* and *Euonimes verrucosa. Carex pilosa* Scop prevails in the gross cover. Its projective cover is 80-90%. 19 species of mycorrhiza-forming fungi were revealed in the testing transect No.2. 25 more species of symbiotrophic species were revealed in the course of the route studies of the present association.

These species, identified in studied association, include all above mentioned species and *Amanita crocea* (Quél.) Singer, *A.vaginata, Boletus edulis* Bull., *Cortinarius anomalus, C.hinnuleus, C. infractus, C. subviolascens* Rob. Henry ex Nezdojm., *Lactarius fuliginosus*(Fr.) Fr., *L. pyrogalus, L. volemus, Russula aurea, R. chloroides* (Krombh.) Bres., *R. farinipes* Romell, *Suillellus luridus*(Schaeff.) Murrill, *Xerocomellus pruinatus* (Fr. & Hök) Šutara. These are widespread species with sufficiently broad ecological valence. They are not typical for locality under consideration. *Cortinarius argenteolilacinus* M.M. Moser, Secr. ex J.E. Lange *C. caerulescens*(Schaeff.)Fr., *C. citrinus* J.E. Lange ex P.D.Orton should be noted as rare species identified only in these conditions. The yield of mycorrhiza-forming fungi in these localities was 30650 g /ha, i.e. it is near to the average index for broad-leaved forests.

The maximum abundance and variety of symbiotrophic species for broad-leaved forests is typical for association of *Quercus robur+Tilia cordata-Carex pilosa* on light loam gray forest soils with pH of 6.5-7. They are formed on the slopes of river valleys on the yields of marl`s carbonate rock. Strengthening the process of mycorrhiza-forming in these conditions is because the phosphorus there is tied by calcium ions and passes in forms which are unavailable for plants (Remezov, Pogrebnyak, 1965). Mycorrhiza-forming fungi are capable to accumulate this element in its mycelium and transfer it in the process of symbiotic relationship to trees, contributing to their growth in these conditions (Smit, Rid, 1997).

The testing transect No. 3 was laid to study symbiotrophic species in considered coil conditions. The tree stratum consists mainly of *Qercus robur* of coppice regeneration at the age of 60-45 with a slight impurity of *Tilia cordata.* Underbrush has been formed by *Corylus avellana* with the projective cover of 40-50%; the grass cover is with the projective cover *Carex pilosa* of 60-70%. The soil is forest dark grey; pH is 7, underlain by marl. The transect is located on the slope of the valley of the river Sura with steepness of 15-20 degrees. 25 species of symbiotrophic species were revealed in the course of the stationary studies. They included all species with broad ecological valence, identified in transects No.1 and No.2.

58 more species were revealed in the course of the route studies. Among them, first of all, it is necessary to specify calciphilous highly specialized species, which are found mainly on calcareous soils in the conditions of the region under study. They include *Amanita strobiliformis,* (Paulet ex Vittad.) Bertill., *Butyriboletus fechtneri*(Velen.) Arora & J.L. Frank, *Caloboletus radicans* (Pers.)

Vizzini, *Cortinarius sodagnitus* Rob. Henry, *C. sulfurinus* Rob. Henry, *Hemileccinum depilatum* (Redeuilh) Šutara, *Hemileccinum impolitum* (Fr.) Šutara, *Hygrophorus arbustivus* Fr., *H. dichrous* Kühner&Romagn., *H. eburneus* (Bull.) Fr., *H. penarius* Fr., *H. russula* (Schaeff.) Kauffman, *Lactarius acerrimus* Britzelm., *Leccinellum crocipodium* (Letell.) Della Maggiora&Trassin., *Suillellus luridus* (Schaeff.) Murrill. In associations *Quercus robur+Tilia cordata-Carex pilosa* adapted to grey forest soils, underlain by cretaceous marls, the yield of symbiotrophic species achieves maximum of the considered forest type and equals to 49500g/ha.

Abundant species composition is characteristic of the testing transect No.4. In these conditions, there is maximum diversity of the studied fungi. It is confirmed by monitoring on the regular routes. 67 species, except for the specified in the Table, of symbiotrophic species were revealed in alluvial broad-leaved forests, associated with grey faintly acid and dark grey forest soils. There are a lot of rare fungi, widespread only in considered ecotopes.

Broad-leaved forests in the area are common not only in the watersheds but also in the floodplains. The maximum abundance and diversity of mycorrhiza-forming fungi in these conditions is characteristic of broad-leaved forests, adapted in well-drained slightly acidic and neutral alluvial sandy soil. They are spread along the high banks of cutoff lakes. High levels of pH of 6-6.5 are not associated with underlying rocks, and with high levels groundwater saturated by carbonates, the pH of which is usually 7-7.5.

The strengthening mycorrhizal formation in these ecotopes can also be associated with a strong ferritization of sands underlying soil. They are saturated with ferric carbonate due to the hardness of close-lying groundwater. The content of the last-mentioned one has a negative effect on the growth of autotrophic plants (Remezov, Pogrebnjak, 1965):). Iron salts have a negative impact on herbaceous plants, and therefore the grass cover in these forests is strongly partly stocked. It is also favorable for mycorrhiza-forming fungi (Burova, 1976).

The transect No. 4 was laid to study mycorrhiza-forming fungi in these conditions. Forest consists of *Qercus robur* and *Tilia cordata* of coppice regeneration at the age of 65-80. Underbrush has been formed by brier and *Euonimes verrucosa*. Its projective cover does not exceed 30%. *Carex pilosa* prevails in the grass cover. Its projective cover in different parts of transect is 40-60%. The soil is sandy loam gray forest sail; pH is 5.5, underlain by ferruginous alluvial sands. The transect is located on the elongated ridge in the Sura River valley between the former riverbed and sedge fen. Groundwater occurrence level is 1.7 m. These ecotopes are characterized by high abundance and diversity of mycorrhizal fungi (Table 1). 87 species of mycorrhizal fungi were revealed in these ecotopes. 27 species are presented on the testing transect.

As well as on the gray forest loamy soils underlain by marl, calciphilous species are found there. However, only a few of them are common for these ecotopes. They are *Butyriboletus fechtneri, Caloboletus radicans* (Pers.) Vizzini, *Cortinarius sodagnitus, Hemileccinum depilatum, Lactarius acerrimus,*

Leccinellum crocipodium, Suillellus luridus. Some species which are typical for the broad-leaved forests on gray forest soils is omitted under these conditions. Some of them are rare - *Amanita strobiliformis, H. penarius, H. russula* and widespread - *L. volemus, Russula aurea*. 16 species are specific for the floodplain broad-leaved forests. Most of them are rare, for example *Suillellus rhodoxanthus* (Krombh.) Blanco-Dios and *Rubinoboletus rubinus* (W.G. Sm.) Pilát & Dermek listed in the Red-book of Russia (2008), as well as *Suillellus rhodopurpureus* (Krombh.) Blanco-Dias, *Cortinarius caesiocortinatus* Jul. Schäff., *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang, which are listed in the red-book of the Penza region. The species *C. balteatoalbus* Rob. Henry, *C. rubellus* Cooke, *C. callochrous* (Pers.) Gray, *Entoloma lividoalbum* (Kühner & Romagn.) Kubička, *Tricholoma lascivum* (Fr.) Gillet, *T. orirubens* Quél., *T. ustaloides* Romagn., *Rheubarbariboletus armeniacus* (Quél.) Vizzini, Simonini & Gelardi are also rare, but they don't have a conservation status.

Described ecotopes have a large value from the protection of basidial macromycetes point of view. In the terms of collecting area of the Volga river, they are easternmost point of distribution most of the South-European nemoral species. Similar ecotopes of the last one are registered in Volgo-Ahtubinskoi flood-plain in the Volgograd region (Rebriev et al. 2012).

Pasture and the consequent trampling down of the underbrush and sold formation influence on settlement of symbiotrophic species. Species diversity of mycorrhiza-forming fungi in similar clear-boled forests of a park type is reduced. Large-fruited species, whose basidiomes easily pass through a dense sod that increase its abundance mainly develop in these conditions. They are presented by *B. reticulatus, Hebeloma sinapizans, Neoboletus erythropus, Rubinoboletus rubinus, Russula pseudointegra, Xerocomellus pruinatus* on acid light gray forest sandy soils as well as some species that grow under the forest canopy on soils with a pH of 6 or higher, for example *Butyriboletus fechtneri, Caloboletus radicans, Hemileccinum impolitum, Suillellus luridus, S. rhodopurpureus, Rubroboletus satanas* will join them in flood-lands.

CONCLUSIONS

Broad-leaved forests among the forest community of the considered area have the richest species diversity of mycorrhiza formation fungi, which are presented by 164 species.

Due to diversity of soil and hydrological conditions, the settlement of symbiotrophic species associated with broad-leaved forests seems rather complex. Impoverished species diversity of symbiotrophic species is typical of *Quercus robur+Tilia cordata* on acid soils with pH from 4.3 to 4.5 and more abundant for broad-leaved forests soils with pH 6-7.

The broad-leaved forests in flood-plain of water-collecting area of the Volga river are easternmost point of distribution most of the South-European nemoral species (ad some examples - species names).

REFERENCES

Bakker, H.C. (2005): Diversity in Leccinum. A molecular phylogenetic approach.160 p.

Bedenko, E.P. (1979): Macromycetes of Belgograd region // *Novosti sistematiki nizshih rasteniy*. 16: 33-42 (in Russian)

Brundrett, M.C. (2004): Diversity and classification of mycorhizal associations. *Biol. Rev.* 79: 473-495

Brundrett, M.C. (2009): Mycorrhizal associations and other means of nutrition of vascular plants: understanding global diversity of host plants by resolving conflicting informations and developing reliable means of diagnosis. *Plant Soil.* 320:37-77

Bruns, T. D. (1995). Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. Plant Soil. 170:63-73.

Bruns, T.D., Bidartondo, M.I., Taylor, L.D. (2002): Host Specificity in Ectomycorrhizal Communities: What Do the Exceptions Tell Us INTEG. AND COMP. BIOL. 42:352–359

Bowen, G.D. (1973): Mineral nutrition of ectomicorrhizae und Ectomycorrhiza, Their Ecology and Physiology., Ed. Marx G.C., Kozlowsky T.T. AP.151-205.

Buscot, F, Munch JC, Charcosset JY, Gardes M, Nehls U, Hampp R. (2000): Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. FEMS Microbiology Reviews. 24:601-614.

Burova, L.G., (1976): Influence of moss and grass cover on the development and distribution of macromycetes in broad-leaved spruce forests of the Moscow region.II //*Mycologia i Phytophathologia*. 10(2): 81-85 (in Russian).

Ganzha, R.V. (1960): Pileate fungi of oak forestes of river-valley Vorksla // *Botanicheckiy zhurnal.* 45(5): 758-764 (in Russian).

Index Fungorum (2016) lndexfungorum.org/names/names.asp Accessed 18 november 2016.- web source , on the end of the list www. Indexfungorum…

Ivanov, A.I.(1987): Macromycetes of oak in Penza region. II. Symbiotrophic species //*Mycologia i Phytophathologia*. 21(5): 419-424 (in Russian).

Ivanov, A.I. (1992): Biota of macromycetes of right-bank forest-steppe of the Volga region, Diss. Moscow. 289 (in Russian).

Ivanov, A.I., Vlasov, A.S., Vlasova, T.G., Sashenkova, S.A. (2012): Woody plants in the Penza region. Penza: RIO PGSHA. 252 (in Russian).

Ivanov, A.I. (1994): Macromycetes of pine forests of forest-steppe of the right bank of the Volga // *Mycologia i Phytophathologia*. 28(2):7-15. (in Russian).

Knudsen, H., Vesterholt, J. (2008): Funga Nordica Agaricoid, Boletoid and Cyphelloid genera. Copengagen: Nordvamp. 966.

Milkov, F.N. (1953): Middle Volga. Moscow: AN USSR publ. 153 (in Russian).

Moser, M. (1978): Die Rohrlinge und Blatterpilze. Kleine kriptogamenflora, lib/2.Studgart, Nev York: Gustav Ficher Verlag. 553.

Muñoz J.A. (2005): Boletus s.l. Edizioni Candusso. 951 .

Rebriev, Yu. A., Rusanov, T.S., Bulgacov, T. Yu., Svetasheva, I.V., Zmitrovich, I.V., Popov, E.S .(2012): Mycobiota of the arid the south-west of Russia. Rostov na Donu: Isdatelstvo Yznogo federalnogo universiteta. 88 (in Russian).

Red-book of the Penza region. Fungi, lichens, mosses, vascular plants, publ.2 (2013): Penza: OAO Penzeckaja pravda. 300 (in Russian).

Red-book of the Russian Federation (plants and fungi) (2008): Moscow:TNK KMK publ. 855 (in Russian).

Remezov, N.P., Pogrebnjak, P.S. (1965): Forestry, soil science. M.: Lesnaja promichlennost. 324 (in Russian).

Smith, S., Read D.J. (1997). Mycorrhizal symbiosys. London UK: Academic press. 605

Tedersoo, L., May, T.W., Smits, M.E. , 2010. – Ectomycorrhizal lifestile in fungi: global diversity, distribution and evolution of phylogenetic lineages. *Mycorrhiza.* 20: 217-263

Zhakov, S.I. (1970) Climate // Nature of the Penza oblast. Saratov: Privolzyskoye kniznoe isdatelstvo. 47 – 82 (in Russian).

DOI: 10.17707/AgricultForest.63.1.21

Vera ĐEKIĆ, Vera POPOVIĆ,
Snežana BRANKOVIĆ, Dragan TERZIĆ, Nenad ĐURIĆ[1]

GRAIN YIELD AND YIELD COMPONENTS OF WINTER BARLEY

SUMMARY

The interaction of genotype x environment on yield and 1000 grain weight and test weight at four KG cultivars of barley (Rekord, Jagodinac, Maksa and Grand) was researched in this paper. The experiment was established on the experimental field of the Small Grains Center in Kragujevac (Serbia) during two growing seasons. The highest grains yield per area unit had, on an average, Maksa cultivar (4.935 t/ha). Average grains yield observed in the two-year period was the highest at Maksa cultivar, while the lowest yield was obtained by Jagodinac cultivar (3.882 t/ha). During the both years of investigation the highest average value of test weight achieved the Rekord cultivar (72.14 kg/hl and 73.22 kg/hl). The largest two-year average value of test weight was found in the cultivar Rekord (72.68 kg/hl), and lowest in cultivar Grand (65.69 kg/hl). During the both years of investigation the highest average value of 1000 grain weight achieved the Maksa cultivar (49.62 g and 51.86 g). The highest average 1000 grain weight was determined for cultivar Maksa (50.74 g). During 2010/2011, statistically significantly higher grain yield per area unit as well as 1000 grain weight was achieved, compared with 2009/2010. Investigations on genotype and environment interaction present the basis for further refinement and barley zoning.

Keywords: *cultivar, grain yield, yield components, winter barley*

INTRODUCTION

Agronomic cultivar value depends not only on its genetic potential for yield, but also on its ability to achieve genetic potential under different conditions of production (Mladenović *et al.,* 2009, Đekić *et al.*, 2012.a). Barley cultivars that were in production until the end of the eighties were characterized by the lower yields, good technological quality and higher stem sensitive on lodging. Due to lower resistance on lodging, that cultivars were grown at modest soils and therefore they had lower yields (Đekić *et al*., 2012.b). New varieties are characterized by good technological quality, better resistance on lodging and diseases, shorter stem and more efficient assimilates usage (Pržulj *et al.,* 2004,

[1]Vera Đekić (corresponding author: verarajicic@yahoo.com), Small Grains Research Centre, Save Kovacevica 31, Kragujevac, SERBIA; Vera Popović, Institute of Field and Vegetable Crops Maxim Gorky St. 30, Novi Sad, SERBIA; Snežana Branković, University of Kragujevac, Faculty of Science, Institute of Biology and Ecology, 34000 Kragujevac, SERBIA; Dragan TERZIĆ, Institute for forage crops, Globoder bb, Kruševac, SERBIA; Nenad Đurić, Univerzity John Naisbitt, Belgrade, Faculty of Biofarming, Backa Topola, SERBIA
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

2014). Yield is largely dependent on the genetic potential, which could be defined as yield of variety which was grown in conditions on which it had been adapted, with adequately amounts of water and nutrients and efficient control of pests, diseases, weeds and other stresses (Đekić *et al.*, 2011). Grain yield is a value, which is not only genetically determined but also dependent to a high degree on the growing conditions (Popović *et al.*, 2011, Sabaghnia at al., 2013, Chamurliyski *et al.*, 2015). Yields considerably vary primarily as a result of agro-ecological conditions during the growing season (Đekić *et al.*, 2014). Experimental research needs to be carried out over multiple environment trials in order to identify and analyse the major factors that are responsible for genotype adaptation and final selection (Popovic *at al.*, 2011, Mirosavljevic *et al* 2014; Kendal, E and Dogan, Y. (2015), Dogan *et al* 2016). Malting barley is expected to have 1000 grain weight of 40 and 46 g and test weight of 68 to 75 kg/hl (Paunović *et al.*, 2006).

Barley is in the usage as an important component of beer and malt production, important and quality component in animal feeding, partially in human nutrition as well as a component in processing industry (Đekić *et al.*, 2012.a). As a top-quality forage crop plant, barley is used as fodder unit in meals balancing in animal feed (Đekić *et al.*, 2010).

The aim of this study was determination of the cultivars and ecological environmental factors influence on differences in stability and adaptability of cultivars regard the grain yield, 1000 grains weight and test weight of tested winter barley cultivars, as well as specificity cultivars exploring regard growing seasons conditions.

MATERIAL AND METHODS

Experimental design

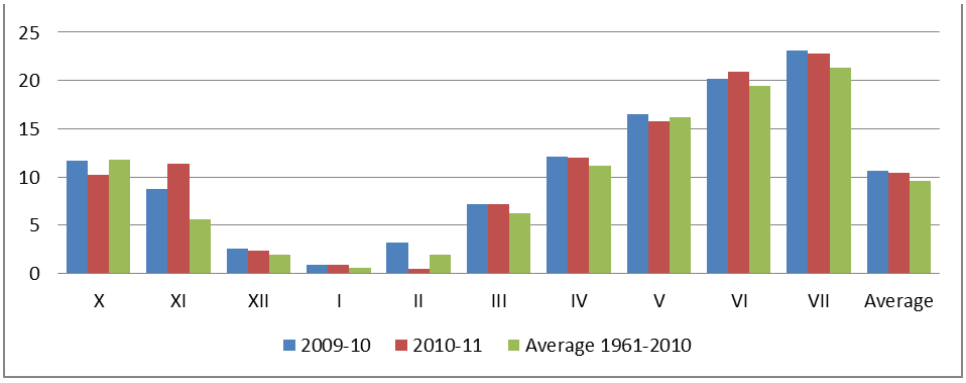
During the 2009/2010 and 2010/2011 growing seasons, four cultivars of winter barley (Rekord, Jagodinac, Maksa and Grand), cultivated at the Center for Small Grains in Kragujevac (Serbia) were investigated.

Experiments have been conducted in randomized block systems, with a plot size of 10 m^2 (2 m x 5 m) in two replicates. The usual techniques for barley production were applied, and it was done in the optimum sowing time in late October. 400 kg/ha of fertilizer NPK 15:15:15 was added in the fall on the investigated plots, while during the spring fertilization, 300 kg/ha (KAN) was supplemented. The following properties were analyzed: grain yield (t/ha), test weight (kg/hl) and 1000 grain weight (g).

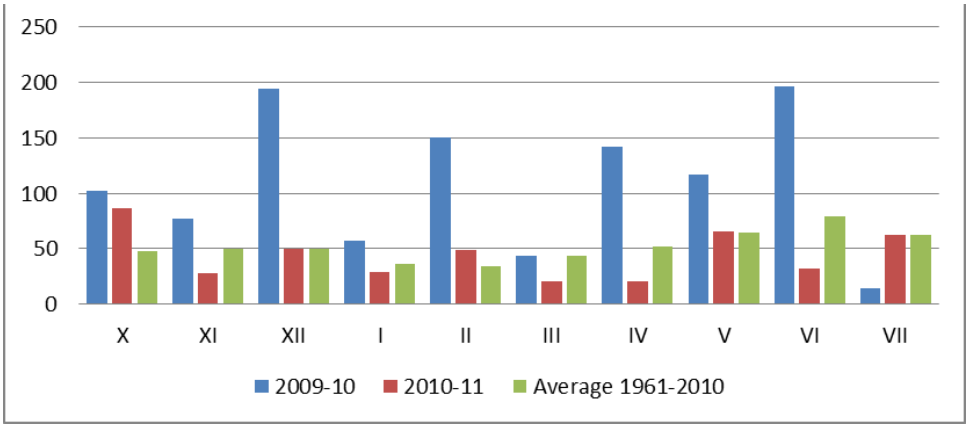
Meteorological conditions

Kragujevac area is characterized by a moderate continental climate, which general feature is uneven distribution of rainfall by months.

Data in Graph 1 and 2 for the investigated period (2009-2011) clearly indicate that the years in which the researches were conducted differed from the typical multi-year average for Kragujevac region, regarding the meteorological conditions.



Graph. 1. Mean monthly air temperature, °C, Kragujevac, Serbia



Graph. 2. Total monthly precipitation, mm, Kragujevac, Serbia

The average air temperature in 2009/2010 was higher by 1.01°C and 2010/2011 was higher by 0.79°C. The sum of rainfall precipitation in 2009/10 was higher by 576.2 mm, where the sum of rainfall in 2010/2011 was 75.1 mm lower than the average of many years and with a very uneven distribution of precipitation per months. Spring months April and May in 2009/2010 were surplus of precipitation, what affected unfavorable on the crops. During the April in 2009/2010 it was 142.2 mm of rainfall, what was 90.7 mm more compared with the perennial average. During the month of June in 2009/2010 it was 196.7 mm of rainfall, what was 117.4 mm more compared with the perennial average. Regard the high importance of sufficient rainfall amounts during the spring months, particularly May for small grains production, the distribution and amount of rainfall over the growing season 2010/2011 were considerably more favorable, what resulted with increment of yields during that year, graph. 1 and 2. Apart from the rainfall deficiency during the spring months and the non-uniform distribution of rainfall across months, an increasing in average air temperatures was also observed.

Based on the fact that sufficient amounts of rainfall in these months are very important for the successful production of cereal crops it can be concluded that the 2009/2010 year in which the researches were conducted were not favorable for the barley growing.

Soil and weather conditions

Before the commencement of the experiment soil samples were taken from the sample surface and the chemical analysis of soil was performed. On the basis of obtained results it was revealed that the soil belongs to the smonitza type, with relatively high clay content, and unfavorable physical properties. The humus content in the surface layer of soil was low (2.38-2.64%), and a substitution and total hydrolytic acidity were quite high (pH H_2O=5.99, KCl=4.56). The soil was medium provided with total nitrogen (0,11-0,13% N) and easily accessible potassium (10-14 mg/100 g soil K_2O), while the available phosphorus content was low (under 10 mg/100 g of soil P_2O_5).

Statistical Analysis

On the basis of achieved research results the usual variational statistical indicators were calculated: average values, error of the mean (arithmetic) and standard deviation. Statistical analysis was made in the module Analyst Program SAS/STAT (SAS Institute, 2000).

RESULTS AND DISCUSSION

Average values of yield (t/ha), test weight (kg/hl) and 1000 grain weight (g) at investigated Kragujevac's winter barley cultivars grown at the Center for Small Grains in Kragujevac during two growing seasons, 2009/2010 and 2010/2011, are presented in the Tab. 1.

Table 1. Average values of investigated barley cultivars characteristics

Traits	Cultivar	2009/2010			2010/2011			Average		
		x	S	Sx	x	S	Sx	x	S	Sx
Grain yield (t/ha)	Rekord	3.200	0.366	0.163	5.666	0.419	0.187	4.433	1.351	0.427
	Jagodinac	2.642	0.222	0.099	5.122	0.264	0.118	3.882	1.327	0.420
	Maksa	3.930	0.190	0.085	5.940	0.401	0.179	4.935	1.100	0.348
	Grand	3.444	0.138	0.062	5.554	0.263	0.117	4.499	1.130	0.357
Test weight (kg/hl)	Rekord	72.14	0.948	0.424	73.22	0.634	0.283	72.68	0.950	0.300
	Jagodinac	70.27	2.112	0.944	71.05	1.149	0.514	70.66	1.655	0.523
	Maksa	70.71	1.183	0.529	71.72	0.421	0.188	71.21	0.992	0.314
	Grand	64.17	2.528	1.131	67.20	0.469	0.210	65.68	2.343	0.741
1000 grain weight (g)	Rekord	48.18	1.310	0.586	50.66	2.019	0.903	49.42	2.070	0.654
	Jagodinac	41.52	0.958	0.428	49.70	0.678	0.303	45.61	4.382	1.386
	Maksa	49.62	0.444	0.198	51.86	1.816	0.812	50.74	1.717	0.543
	Grand	40.36	0.654	0.293	40.40	0.894	0.400	40.38	0.739	0.234

During the first year of investigations, cultivar Maksa achieved the highest grains yield (3.930 t/ha), while the lowest yield was at Jagodinac cultivar

(2.642 t/ha). During the second year of investigations (2010/2011), the yield of Maksa cultivar was the highest with 5.940 t/ha, while the slightly lower yield was realized by Rekord and Grand cultivars (5.666 t/ha and 5.554 t/ha). Average grains yield observed in the two-year period was the highest at Maksa variety (4.935 t/ha), while the lowest yield was obtained by Jagodinac cultivar (3.882 t/ha). Considerable variation in yield depending on years of research have established Jelić et al. (2007), Đekić et al. (2011), Malešević et al. (2010) and Popović et al. (2011).

Achieved statistically significantly higher yields in 2010/2011 were, primarily, the result of heavy rainfalls and their good distribution as well as favorable air temperatures during the vegetation period (Graph 1 and 2). Đekić et al. (2014) in his research states that the air temperatures and the rainfall amount and distribution during the barley growing season have the greatest impact on high yields and grain quality.

The barley cultivar Rekord has achieved the highest test weight in both years of investigation compared to other tested barley cultivars (72.14 kg/hl and 73.22 kg/hl). The average two-year value of test weight at Rekord cultivar was 72.68 kg/hl, Maksa 71.21 kg/hl, Jagodinac 70.66 kg/hl, while the lowest average was at Grand cultivar (65.68 kg/hl), Table 1.

Table 2. Analysis of variance of the tested parameters (ANOVA)

Effect of year on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 1, 38	p-level
Grain yield (t ha^{-1})	51.3702	0.23402	219.517***	0.000000
Test weight (kg hl^{-1})	21.7563	9.11184	2.388	0.130582
1000-grain weight (g)	104.6523	20.67320	5.062*	0.030324
Effect of cultivar on the traits analyzed				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 36	p-level
Grain yield (t ha^{-1})	1.8663	1.518442	1.229	0.313291
Test weight (kg hl^{-1})	92.3302	2.528208	36.520***	0.000000
1000-grain weight (g)	215.8162	6.744028	32.001***	0.000000
Effect of the year x cultivar interaction				
Traits	Mean sqr Effect	Mean sqr Error	F(df1,2) 3, 32	p-level
Grain yield (t ha^{-1})	0.14639	0.089204	1.641	0.199369
Test weight (kg hl^{-1})	2.72775	1.908625	1.429	0.252430
1000-grain weight (g)	30.18425	1.486875	20.300***	0.000000

**Statisticaly significant difference (P<0.05) **Statisticaly high significant difference (P<0.01)*

Grain of investigated barley cultivars was characterized by good physical characteristics; especially regard the test weight and 1000 grain weight. Realized

average values of these characteristics in the study were slightly higher than the values obtained by Jelić *et al.* (2007) and Đekić *et al.*, (2011).

Table 2 shows the impact of year, cultivar and interaction of year x cultivar on yield, test weight and 1000-grain weight. The ANOVA indicated very highly significant effects of the year and grain yield (F_{exp}= 219.517***) and significant effects of the 1000 grain weight (F_{exp}=5.062*). Our results are consistent with the results Madić *et al.* (2009), where the authors state that the growing conditions in the observed years had a significant impact on yield. Very highly significant differences in test weight and 1000-grain weight at investigated barley were found in relation with the cultivar. The interaction of 1000-grain weight and year x cultivar very highly significant differences at investigated barley cultivars.

CONCLUSIONS

Based on the gain results during two-year investigation on four Kragujevac's winter barley cultivars, it can be concluded that the highest yield achieved the cultivar Maksa (4.935 t/ha). Rekord and Jagodinac cultivars have achieved satisfactory results, while the poorest results were achieved by the Grand cultivar. During 2010/2011, statistically significantly higher grain yield per area unit as well as 1000 grain weight was achieved, compared with 2009/2010. Very highly significant differences in 1000 grain weight at investigated barley cultivars were found relative to the cultivar and very significant differences at grain yield relative to environmental factors. Very highly significant influence of the interaction of 1000-grain weight and year x cultivar differences at investigated barley cultivars.

ACKNOWLEDGEMENTS

Investigations necessary for this papers are part of the projects: TR 31054 "Development of new cereals cultivation technologies on acid soils by usage of modern biotechnology", and TR 31022; financed by the Ministry of Education, Science and Technology Development of Republic of Serbia.

REFERENCES

Chamurliyski P., Atanasova D., Penchev E. (2015): Productivity of foreign common winter wheat cultivars (*Triticum aestivum* L.) under the conditions of dobrudzha region. Agriculture & Forestry, Podgorica. Vol. 61, Issue 1: 77-83.

Dogan Y., Kendal E., Oral E. (2016): Identifying of relationship between traits and grain yield in spring barley by GGE biplot analysis. Agriculture & Forestry, Vol. 62, 4. p: in press.

Đekić V., Staletić M., Glamočlija Đ., Branković S. (2010): Variability of yield and grain yield components of KG winter barley cultivars. Zbornik radova XV Savetovanja o biotehnologiji sa međunarodnim učešćem, 26-27. Mart 2010, Čačak, Vol. 16 (17), 223-226.

Đekić V., Milovanović M., Glamočlija Đ., Staletić M. (2011): Yield and components yield grain in Kragujevac of winter barley varieties. Proceedings. 46 Croatian and 6 International Symposium on Agriculture, 14-18. Februar 2011, Opatija, Croatia, 601-604.

Đekić V., Staletić M., Milivojević J., Jelić M., Popović V. (2012.a): Parameters of spring barley grain yield. Zbornik radova XVII Savetovanja o biotehnologiji sa međunarodnim učešćem, 6-7. April 2012, Čačak, Vol. 17 (19), 53-57.

Đekić V., Milovanović M., Staletić M., Popović V., Jelić M. (2012.b): Effect of genotype and environment on winter barley quality (*Hordeum vulgare* L)**.** Proceedings, XVI International Eco-Conference® 2012 "Safe food", 26-29. September 2012, Novi Sad, 145-153.

Đekić V., Milovanović M., Popović V., Milivojević J., Staletić M., Jelić M., Perišić V. (2014): Effects of fertilization on yield and grain quality in winter triticale. Romanian Agricultural Research, No.31, p. 255-266, DII 2067-5720 RAR 2012-255.

Jelić M., Đalović I., Savić N., Knežević D. (2007): The effect of amounts of seed and dose of nitrogen fertilizers to grain yield and quality of forage winter barley and oat. ječma. *A* Periodical of Scientific Research on Field and Vegetable Crops, Vol. 44, 481-486.

Kendal, E and Dogan, Y. (2015). Stability of a Candidate and Cultivars (*Hordeum vulgare* L) by GGE Biplot analysis of Multi-environment Yield Trials in Spring Barley. Agriculture & Forestry, Vol. 61, Issue 4: 307-318, 2015, Podgorica, DOI: 10.17707/AgricultForest**.**61.4.37.

Madić M., Paunović A., Knežević D., Zečević V. (2009): Grain yield and yield components of two-row winter barley cultivars and lines. Acta Agriculturae Serbica, Vol. 14, No. 27, 17-22.

Malešević M., Glamočlija Đ., Pržulj N., Popović V., Stanković S., Živanović T., Tapanarova A. (2010): Production characteristics of different malting barley genotypes in intensive nitrogen fertilization. International Scientific Journal Genetics, „Genetika" Belgrade, Vol. 42, No. 2, 323-330.

Mladenović G., Kovačević N., Malešević M., Štatkić S. (2009): NS small grain seed production and marketing in the period 1998–2007. A Periodical of Scientific Research on Field and Vegetable Crops, Vol. 46, No. 2, 379-384.

Mirosavljevic, M., Przulj, N., Bocanski, N., Stanisavlievic, D., Mitrovic, B. (2014). The application of AMMI model for barley cultivars evaluation in multi-year trials. Genetika, Vol: 46, No. 2, 445-454.

Paunovic A., Madic M., Knezevic D., Djurovic D. (2006): The interdepedence of productive and technological traits in two-rowed spring barley. Acta Agriculturae Serbica, Vol. XI, No. 22, 37-43.

Popović V., Glamočlija Đ., Malešević M., Ikanović J., Dražić G., Spasić M., Stanković S. (2011): Genotype specificity in nitrogen nutrition of malting barley. Genetika. Belgrade, Vol. 43, No. 1, 197-204.

Pržulj N. (2001): Cultivar and year effect on grain filling of winter barley. Plant Breeding and Seed Science. Vol. 45, No. 2, 45-58.

Pržulj N., Momčilović V., Petrović N. (2004): Physiological basis of barley yield under near optimal and stress conditions. Plant Breeding and Seed Production, Vol. X, 1-4, 15-26.

Przulj, N., Momcilovic, V., Simic, J. and Mirosavljevic, M. (2014).Effect of growing season and variety on quality of spring two-rowed barley. Genetika, 46: 59-73.

Sabaghnia N., Mohammadi, M., Karimizadeh, R., 2013. Interpreting genotype x environment interaction of bread wheat genotypes using different nonparametric stability statistics. Agriculture & Forestry, Podgorica, MNE, Vol. 59, Issue 2: 21-35.

DOI: 10.17707/AgricultForest.63.1.22

Ammer MOHAMADI –NASAB
Alireza MOTALEBI-AZAR, Sam MOKTARZADEH[1]

THE EFFECT OF NAPHTHALENEACETIC ACID AND TWO CYTOKININS ON CALLUS AND SHOOT INDUCTION FROM HYPOCOTYLS THIN CELL LAYER EXPLANTS IN TOMATO (*LYCOPERSICON ESCULENTUM* MILL)

SUMMARY

This research was carried out for evaluating the morphogenesis responses of hypocotyls thin cell layers (TCL) of *Lycopersicon esculentum* Mill with various concentrations of NAA- Naphthaleneacetic Acid (NAA) and two types of cytokinins. The TCLs (0.1-0.5 mm) of hypocotyls were cultured to MS medium, containing various concentrations of NAA (0, 0.3, 0.5, 1 and 1.5mg/l) with 2 mg/l of BAP and kin. The obtained callus was transferred to the regeneration medium. The results showed that callus induction occurred in the all cultures containing auxin and cytokinin. But, callus induction was not observed on the medium containing only BAP and kin. So, the presence of auxin is essential for callus induction. The percentage of root and shoots, callus weight and diameter with BAP significantly differ in various concentrations of NAA. BAP and NAA concentrations of 2 mg/l and 1.5 mg/l of had maximum effects on callus weight and diameter, respectively. However, the maximum shoots were observed with the concentration of 1 mg/l of NAA. Different concentrations of NAA with kin significantly affected callus weight and diameter root and shoots. The maximum callus weight and diameter as well as percentage of shoots happened in NAA concentrations of 2 mg/l and 1 mg/l kin, respectively. The maximum number of root was observed with the concentrations of 2 mg/l kin and 0.3 mg/l NAA. The comparison between media containing BAP and kin showed that responses were better on the medium containing kin. Also, the amounts of roots, shoots and callus fresh weight were higher than those of BAP.

Keywords: *hypocotyls, kin, Lycopersicon esculentum, shoots, thin cell layer culture*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most important species of Solanaceae and is cultivated in most parts of the world (Devi et al. 2008). Nowadays, in vitro techniques are important instruments for genetic

[1]Ammer Mohamadi –Nasab Faculty of Natural Science, University of Tabriz, Tabriz, IRAN Alireza Motalebi-Azar, Sam Moktarzadeh Department of horticultural Science, Faculty of Agriculture, Tabriz University, IRAN

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

achievements and it is required to increase the usage of their reform styles by optimizing effective factors in plant regeneration (Taji et al. 2002). In the tomato plant, regeneration has been reported from various explants such as shoot tips, cotyledon, stem, petiole, leaf, anther and inflorescence (Raziuddin et al. 2004; Costa et al. 2000). Tomato regeneration was observed with callus of leaf and cotyledon or directly through the thin layer cell (TCL) of inflorescences (Compton and Veilluxe, 1991). In vitro plant regeneration depends on many factors such as genotype, explant, chemical medium composition, growth regulators, intensity and quality of light and photoperiod and temperature (Reed, 1999). performed studies using four medium (N6, LS, B5 and MS) containing various hormone combinations showed that N6 medium resulted in the maximum number of shoots length, which probably can be attributed to the increase of the sulphate ammonium medium concentration, contributing to plant regeneration (Sheeja et al. 2004).

In addition to this type of explant (Chaudhry et al. 2004), medium composition and genotype (Ishag et al. 2009) and hormonal treatments (Philip et al. 1996) can affect plant regeneration in tomato. For the first time, thin cell layer (TCL) technology was originally used by Tran et al. (1974) for controlling the development of flowers, roots, shoots and somatic embryos in tobacco pedicel longitudinal TCLs. TCL culture techniques have mostly focused on size and origin of explant and, as units of proper development, they have been used for regeneration and morphogenesis (Teixeire et al. 2007). The advantage of TCL culture is that thin cells layers are similar in physiological terms and can begin all morphogenesis patterns; however, only one pattern can be observed in each treatment (Kiem and Tran, 1981). In addition, direct organogenesis in most plants like *Begonia tuberous* (Nhut et al. 2006), *Lilium longiflorum* (Nhut et al. 2001), *Citrus* (Carimi et al. 1999), *Brassica napus* (Ghnaya et al. 2008) and *Digitaria sanguinalis* (Le et al. 1997) has been reported using TCL. Since morphogenesis from hypocotyls TCL has not been reported in *Lycopersicon esculentum* Mill, in this research, morphogenesis (callus, shoot, root or somatic embryogenesis induction) containing various concentrations of NAA and two types of cytokinins (BAP and kin) were studied in the hypocotyl TCL of *Lycopersicon esculentum* Mill seeding.

MATERIALS AND METHODS

Study placement and preparation of TCL explants

This experiment was conducted at the tissue culture laboratory of the Department of Horticultural Sciences at the University of Tabriz, Iran. The seeds of tomato cultivar "Super Chief" were the surface sterilized using alcohol 70% (for 2 min) followed by 2.5% Sodium hypochlorite for 15 min. Then the seeds were rinsed with distilled water for five times. Aseptic seeds were cultured on hormone free MS medium (Murashige and Skoog, 1962). Hypocotyls were obtained from the 6 to 7 days old seedlings and were cut to 0.1 to 0.5 mm explants (TCL) using a scalpel under a laminar flow in an aseptic condition.

Plantation of explants and treatments

TCL explants were transferred into the MS medium containing various concentrations of NAA (0, 0.3, 0.5, 1 and 1.5 mg/l) and two types of cytokinins, BAP and kin (each with 2 mg/l) (both addition before autoclaving) for callus induction under darkness at 24 ± 2°C for 4 weeks. The percentage of callus induction, callus diameter, callus color and number of the roots were recorded. After 4 weeks, the samples were transferred to the regeneration medium (MS free hormone) (Gubi et al. 2004).

Statistical Analysis

The experiment was conducted a factorial arrangement based on completely randomized design with three replications and 15 TCLs for each treatment combination. Data analysis was accomplished using the SPSS statistical software (Version 16.0) and the means were compared by Duncan New Multiple Range test at 5% probability level.

RESULTS AND DISCUSSION

After 4 to 5 days, callus induction was observed from TCL explants and with all hormonal treatments, except in the medium containing only cytokinin. Figure 1 A and B shows TCL explants and creamy calli produced from them, respectively. Callus tissue was hard and dry, but not juicy. However, depending on the hormonal combination treatments the root and shoot signs were observed from the callus of different media. The high number of root was observed on the medium containing kin. In addition, the roots had significantly longitudinal growth (Figure 1, C). In contrast, with the media containing BAP, the root growth was stopped in the early stages (Figure 1, D).

The rooting response in this case is seemingly associated with the external NAA employed as well as with the internal auxin. Shoot induction was mainly occurred indirectly and from calli. The shoots contained several small trachoma, as one of the feature of Solanaceae (Figure 1, E). Afterwards, the roots were originated from the end of the produced shoots into the rooting medium; then the rooted shoots were transferred to the hormone free MS medium for the complete plant development (Figure 1, F).

Apart from the hormonal amount and combination, callus induction was occurred on all the media containing both hormones; auxin and cytokinin (100% callus induction from explants). So, the analysis and mean comparisons were not performed for the percentage of callus induction. However, Chaudhry et al. (2004) reported 57 to 82/5% callus induction on the MS medium containing various hormonal combinations (4 mg/l Kin, 2 mg/l BAP, 2 mg/l NAA and 2 mg/l IAA) using leaf and hypocotyl explants. On the other hand, callus induction was not observed on the medium containing only BAP or kin. This means that any type of auxin is an essential factor for callus induction.

In a similar research, it was determined that callus proliferation from the leaf and hypocotyl explants was greatly depended on the hormone composition of the medium (Sheeja et al. 2004).

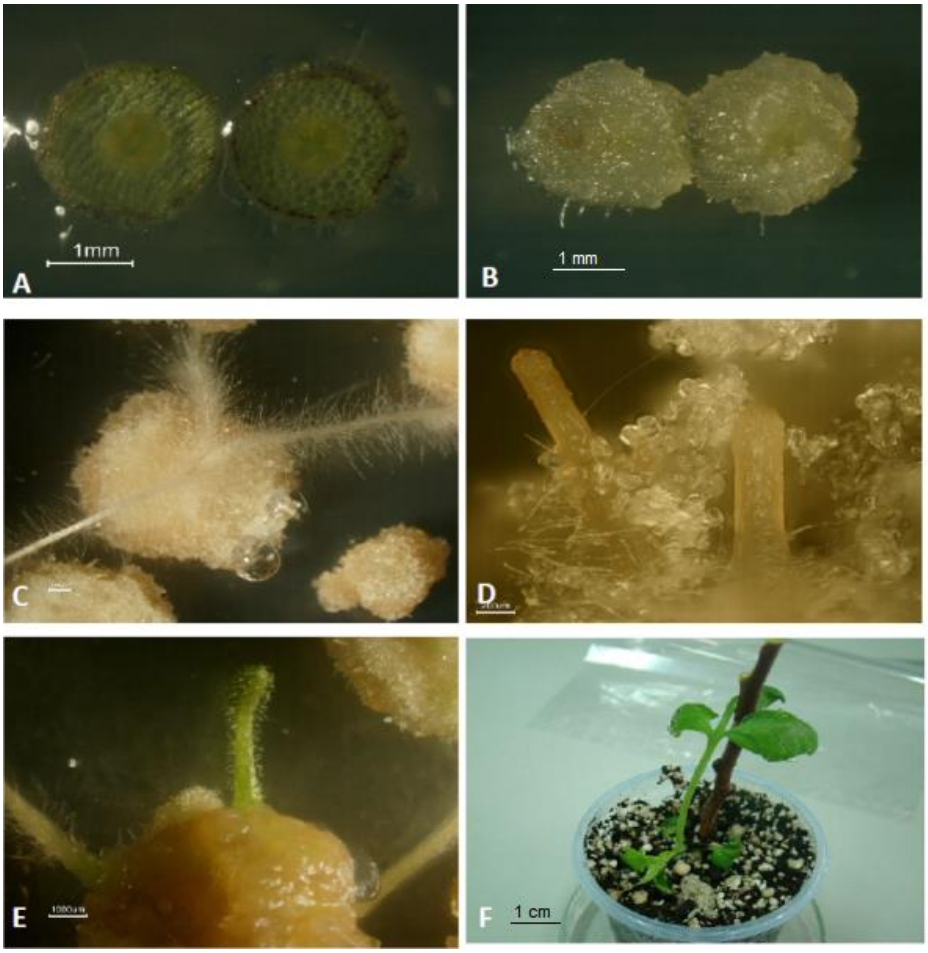


Figure 1. Thin Cell Layer (TCL) explants (A); Creamy callus produced from TCL (B); Root formation on the medium containing kin (C); Stopped root growth on the medium containing BAP (D). Shoot induction from callus with small trachoma (E). Plant regeneration on the hormone free MS medium (F).

A research conducted by Osman et al. (2010) verified that the callus induction potential from the hypocotyl explants of tomato was the highest on the MS medium containing one of the hormones of 2,4-D, TDZ and BAP with 0.5 mg/l NAA or a combination of 0.1 mg/l NAA and 0.5 mg BAP. However, the highest callus percentage was obtained with 2 to 3 mg/l NAA using cotyledon explants. In addition, regeneration through the callus from cotyledon was better than hypocotyl. Considering, TCLs are the explants of choice for the callus induction and its subsequent growth. By the way, the establishment and the capacity for the feasible and fast absorption of nutrients and growth regulators have been defined as the principle advantages for this explant type. One more advantage of TCL technique is the reliable determination of effective

concentration of the external hormones with morphogenesis studies (Tran, 1981). In line, defined levels of external plant growth regulators along with internal phytohormons affect the morphogenesis potential of explant. Overlays, TCLs are undoubtedly the best explant types for the comprehensive studies of morphogenesis patterns. The results showed that kin was more effective than BAP regarding the studied traits. Callus diameter was influenced by various concentrations of NAA ($p<0.01$) on the medium containing either kin or BAP (Table 1). The effect was more pronounced with NAA concentration higher than 0.3 mg/l. The maximum callus diameter of callus was observed in the media containing 1.5 mg/l NAA. The results showed that the increase in callus diameter was independent from the cytokinin type (Fig. 2A). Various concentrations of NAA significantly affected the callus fresh weight on the medium containing both BAP and kin ($p<0.01$) (Table 1). However, variation in of callus weight was dependent on the NAA concentration and cytokinin type. In the same way, on the media containing BAP, any increase in NAA level led to elevated calli fresh weight.

Table 1. Analysis of variance for callus diameter and weight, root and shoot induction percentage in response to NAA concentrations and two types of cytokines

Mean Squares					
Source of variations	**df**	**Callus diameter**	**Callus Weight**	**Root (%)**	**Shoot (%)**
NAA	4	2.66*	564.3**	0.251**	0.215*
Cytokinins	1	0.95	256.1**	0.122*	0.987**
NAA x Cytokinins	5	5.47**	756.8**	1.256**	1.002**
Error	20	0.58	50.2	0.045	0.061

The highest value for calli fresh weight was achieved using 1.5 mg/l NAA and with the presence of BAP. No significant difference was observed among the NAA concentrations ranging from 0.3 to 1 mg/l. However, there was a significant difference between these concentrations and 1.5 mg/l NAA (Figure 2B). With media containing kin, callus fresh weight was increased using NAA ranging from 0.3 to 1 mg/l, but it significantly decreased by 1.5 mg/l NAA (Figure 2B). As shown in Figure 2B, kin was more effective on callus fresh weight compared with BAP. Root initiation was more evident on the media containing kin and NAA when being compared with BAP and NAA. In addition, root growth was more intensified on media containing kin.

Beyond root initiation, the subsequent root growth was stopped with the media containing BAP. Root induction from callus was influenced by the different concentrations of NAA and cytokinin type ($p<0.01$) (Table 1). With media containing BAP, rooting percentage was declined with any increase in the concentrations of NAA.

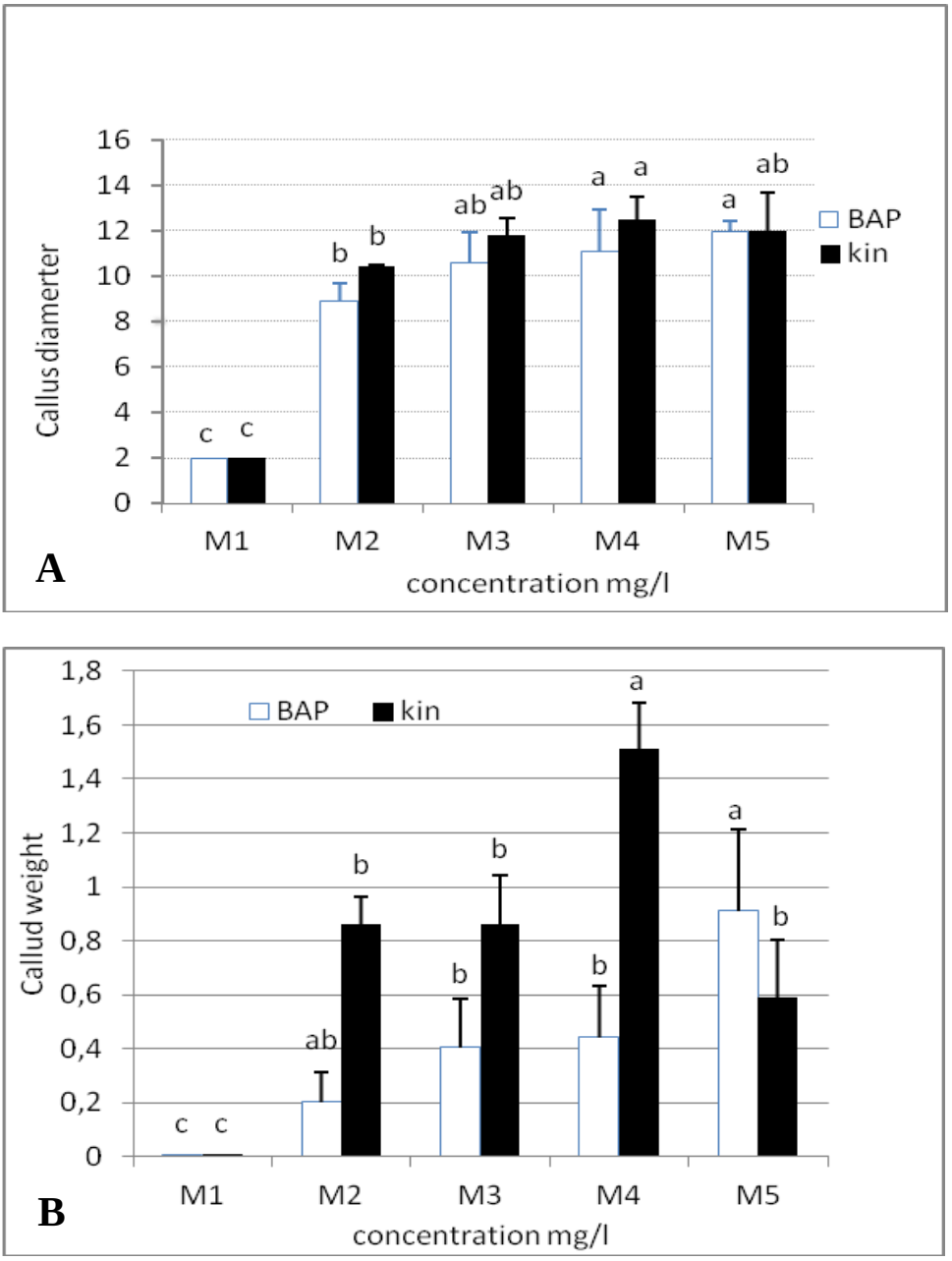


Figure 2. The average of callus diameter (A) and weight (B) in different concentration of NAA (0. 0.3, 0.5, 1, 1.5mg/l show that by M1-M5) in presence of 2 mg/l BAP or kin. Different letters show significant difference based on Duncan's multiple range test, p = 0.05.

The maximum number of roots was observed in the media enriched with 0.3 and 0.5 mg/l NAA and the least data were occurred in the media containing 1

and 1.5 mg /L NAA (Fig. 3). Also, in the media containing kin, the rooting percentage was decreased with any raise in the amount of NAA. The maximum of rooting percentage of roots was observed in 0.3 mg/l NAA with significant difference from higher concentrations. The lowest data for this trait was observed in the concentration of 1.5 mg/l (Fig. 3). So, as routinely expected, the higher auxin levels were failed root induction. Fig. 3 shows that with the NAA presence, kin was more efficient than BAP considering rooting percentage.

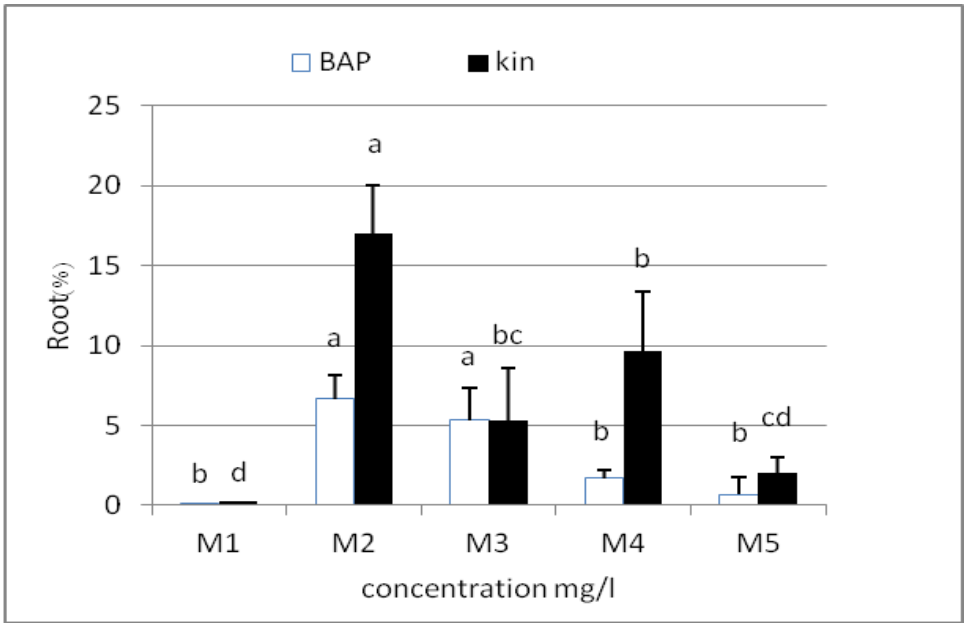


Figure 3. The average of root in different concentration of NAA (0. 0.3, 0.5, 1, 1.5mg/l show that by M1-M5) in presence of 2 mg/l BAP or kin. Different letters show significant difference based on Duncan's multiple range test, p = 0.05.

There is strong evidence that kin progressively improved the rooting potential in alfalfa (Arcioni et al. 1990). Moreover, the rooting on the regeneration medium significantly was dependent on the various concentrations of NAA. Also, two cytokinin types held the same response. With both media containing BAP or kin, the increase in shoot induction was correlated with the increase in NAA concentration till 1 mg/l. Any further increase in NAA levels led to the pronounced decrease in shoot induction potential. This indicates that for shoot induction and proliferation the participation of both hormones (auxin and cytokinin) is necessary, but their certain concentration needs special attention. Besides, as can be seen in Fig. 4, the medium containing kin had more percentage of shoots compared with media enriched with BAP in various concentrations of NAA. Also, shoots number was more on the medium containing kin rather than BAP. Several previous studies have notified that the

type and amount of cytokinins are main factors affected shoot induction and subsequent growth (Abdellatef and Khalafallah, 2007). In tomato, Ishag et al. (2009) reported the shoot regeneration on the MS medium containing kin was more than the medium incorporated with BAP and 2ip. Also, with our study, it was determined that kin was more efficient than BAP in case for the shoots related traits and the shoots regeneration was also complete with the media containing kin. Furthermore, this study revealed that the combination of NAA with BAP and kin was not enough potentiate for the proliferation of tomato plant compared to the medium that only contains cytokinin. Similarly, Gulati and Jaiwal (1992) reported that NAA adding to the medium containing cytokinin did not improve the shoot multiplication.

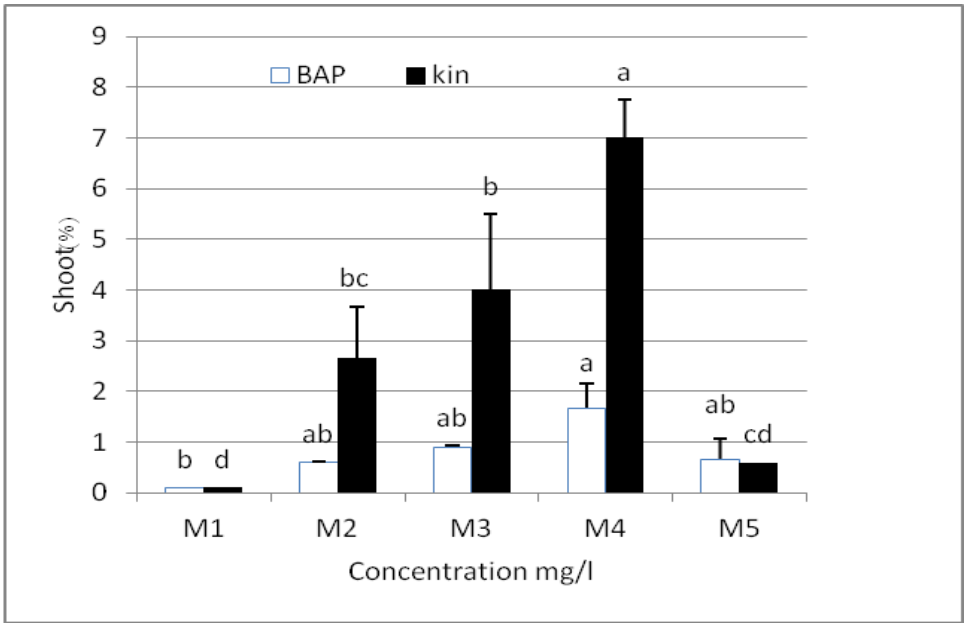


Figure 4. The average of shoot in different concentration of NAA (0. 0.3, 0.5, 1, 1.5mg/l show that by M1-M5) in presence of 2 mg/l BAP or kin. Different letters show significant difference based on Duncan's multiple range test, p = 0.05.

CONCLUSIONS

Results of these experiments show the influence and importance of growth regulators on the callus diameter and weight as well as root and shoot induction from TCL explants of tomato. Although plants have endogenous growth hormones, they are sometimes required to be supplemented under *in-vitro* conditions to obtain optimal results. This is corroborated by the reports of Jozef et al. 2004, that the addition of growth regulators in media enhanced the number of shoots regenerated from tomato cotyledons and hypocotyls. The *in-vitro* morphogenic responses of *in*-vitro cultured plant tissues are therefore affected by

the different components of the culture media, especially by concentration of growth hormones. These responses are also dependent on cultivar and explants types. The addition of plant growth hormones to the shoot regeneration medium could therefore enhance shoot regeneration in this cultivar and explants.

REFERENCES

Abdellatef, E., and Khalafallah, M.M. 2007. Adventitious shoot formation and plant regeneration in medium staple cotton (*Gossypium hirsitum* L.) cultivar (Barac B-67). International Journal of Agriculture and Biolbiology; 9, 913- 916.

Arcioni, S.F., Pezzoti, M., and Lupotto, E. 1990. Alfalfa, Lucern (*Medicago* spp). In: Biotecnology in Agriculture and forestry: Legumes and oilseed crops (Ed. Y.P. Bajaj). Spring Verlag, Berlin Germany. 1990. 197-241.

Beck, M.J., and Caponetti, J.D. 1983. The effect of kinetin and naphthaleneacetic acid on in vitro shoot multiplication and rooting in the fishtail fern. American Journal of Botany. 70, 1–7.

Carimi, F., Pasquale, F., and Crescimanno, D. 1999. Somatic embryogenesis and plant regeneration from pistil thin cell layers of citrus. Plant Cell Report. 18, 935-940.

Chaudhry, Z., Habib, D., Rashid, H., and Qureshi, S.A. 2004. Regeneration from various explant of in vitro seedling of Tomato (*Lycopersicon esculentum* L. c.v. Roma). Pakistan Journal of Biological Sciences. 7, 269-272.

Compton, M.E., and Veilluxe, R.E. 1991. Shoot, root and flower morphogenesis on tomato inflorescence explants. Plant Cell, Tissue and Organ Culture. 24, 223-231.

Costa, M.G.C., Nogueira, F., Figueira, M.L., Otoni, W.C., Brommonschenke, S.H., and Cecon, P.R. 2000. Influence of the antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum* Mill.) cultivars. Plant Cell Reports. 19, 327-332.

Devi, M., Dhaliwal, M.S., Kaur, A., and Gosal, S.S. 2000. Effect of growth regulators on in vitro morphogenetic response of tomato. Indian Journal of Biotechnology. 7, 526- 530.

Ghnaya, B.A., Charles, G., and Branchard, M. 2008. Rapid shoot regeneration from thin cell layer explant excised from petioles in four cultivars of *Brassica napus* L. Plant Cell, Tissue and Organ Culture. 92, 25-30.

Gubi, J., Lajchova, Z., Farago, J., and Jurekova, Z. 2004. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.). Biologia, Bratislava. 59: 405-408.

Gulati, A., and Jaiwal, P.K. 1992. In vitro induction of multiple shoots and plant regeneration from shoots tip of mung bean (*Vigna radiate* (L.)). Plant Cell Tissue and Organ Culture. 29, 199-205.

Jozef, G., Zuzana, L., & Zuzana, J. 2004. Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicum esculentum* Mill.). Biologia Bratislava, 59(3), 405-408.

Ishag, S., Osman, M.G., and Khalafalla, M.M. 2009. Effects of growth regulators, explant and genotype on shoot regeneration in tomato (*Lycopersicon esculentum* c.v). Omdurman. Int. J. Sustain. Crop Prod. 4, 7-13.

Kiem, M., and Tran, T.V. 1981. Control of morphogenesis in in vitro Cultures. Ann. Rev. Plant Physiology. 32, 291-311.

Le, B.V., Thao, N.D., Gendy, C., Vidal, J., and Van, T.K. 1997. Somatic embryogenesis on Thin Cell Layers of a C4 species, *Digitaria sanguinalis* (L.) Scop. Plant Cell, Tissue and Organ Culture. 49:, 201–208.

Murashige, T, and Skoog F, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiological Plant. 15: 473-497.

Nhut, D.T., Bui, L., Tran, T.V.M, and Aswath, C.R. 2001. Thin cell layer cultures system in Lilium: Regeneration and transformation perspectives. In Vitro Cell. Dev. Biol. Plant. 37, 516-523.

Nhut, D.T, Phan, M.X., Hai, N.T., Son, P.D.T., Huyen, P.X., and Hang, N.T.T. 2006. Thin cell layer technology and bioreactor culture in rapid propagation of Begonia tuberous. Proceedings of International Workshop on Biotechnology in Agriculture. 127- 130.

Osman MG, Elsadig A, Elhadi B, Khalafalla MM. Callus formation and organogenesis of tomato (*Lycopersicon esculentum* Mill, C.V. Omdurman) induced by thidiazuron. African Journal of Biotechnology. 2010; 9: 4407-4413.

Philip ON, Sankaran K, Praveen KS. Regeneration of Tomato (*Lycopersicon esculentum* L.): Somatic embryogenesis and shoot organogenesis from hypocotyl explants induced with 6-Banzyl adenine. Int. J. Plant Sci. 1996; 157: 554-560.

Philip ON, Newman SK, Praveen K, Saxena A. Regeneration of Tomato (*Lycopersicon esculentum* Mill.): Somatic Embryogenesis and Shoot Organogenesis from hypocotyl explants induced with 6-Benzyladenine. Int. J. Plant Sci. 1996; 157: 554-560.

Raziuddin S, Salim HJ, Chaudhary T, Mohammad A. Hormonal effect on callus induction in Tomato. Sarhad J. Agric. 2004; 20: 223-225.

Reed BM. Design a micropropagation system: Workshop presentation from the 1998 SIVB Congr. On in vitro Biology. In Vitro Cell Dev. Biol. Plant. 1998; 35: 275-284.

Ruzic DJ, Vujovic VT. The effects of cytokinin types and their concentration on in vitro multiplication of sweet cherry cv. Lapins (*Prunus avium* L.). Hort. Sci. (Prague). 2008; 35: 12–21.

Sheeja TE, Asit B, Monda F, Rathore RKS. Efficient Plantlet Regeneration in Tomato (*Lycopersicon esculentum* Mill.). Plant Cell Tissue Organ Culture. 2004; 14: 45-53.

Taji A, Kumar PP, Lakshmanan P. In Vitro Plant Breeding, Food Products Press, New York, 2002.

Teixeire DSAJ, Tran TVK, Biondi S, Nhut DT, Altamura MM. Thin cell layer: Developmental building block in ornamental biotechnology. Floriculture and Ornamental Biotechnology. 2007; 1: 1-13.

Tran TVK, Dien N, Chlyah, A. Regulation of organogenesis in small explant of superficial tissue of *Nicotiana tabacum* L. Planta. 1974; 119: 149-159.

Tran TVK. Control of morphogenesis by inherent and exogenously applied factors in thin cell layers. Int. Rev. Cytol. Suppl. 1981; 11: 75-194

DOI: 10.17707/AgricultForest.63.1.23

Sanja PAJIĆ, Ljiljana KEČA[1]

AUCTION IMPLEMENTATION OF OAK TECHNICAL ROUNDWOOS IN SREM FOREST AREA

SUMMARY

Financial investments in Srem forest area (SFA), despite small support of state, is achieved by providing funds from regular production in forests, whose primary purpose is the production of timber. The profit of a decade of work is implemented at the time of the sale of wood assortments. The maximum financial suficit in existing social - market conditions can be achieved by public sales of the technical roundwood (TRW). The aim is to study the SFA from the aspect of production and sale of TRW broadleaves. The study focused at determine the maximum gain and seller profit of TRW in existing market conditions and the legislative framework. The subjects of research are: production and sale of wood assortments in period of 1965-2015, which recorded a mild rising trend (production +0,4 % sale +0,5 %) in the analyzed period in the SFA, statistical analysis of produced structures TRW of common oak recorded a negative growth rate, that is especially high among the finest veneer (F) assortments (for F is -6,9 %), selection of the appropriate sales mode in the current conditions of increased demand and a trend of reduced supply of high quality and quality assortment of common oak.

Keywords: *Auction, timber trade, Srem forest area, roundwood, analysis.*

INTRODUCTION

Forestry is a relatively young branch of the economy and occurs as a resultant of two components: natural factors and socio - political conditions (Schmithüsen et al. 2006). Serbia is currently in the transition process, which involves a wave of radical socio-economic changes, for that purpose, the bid may serve as a way for selling, which would help wood market liberalization (Pajic, 2016). Sale by price-list, which ia today affected by state through various legal modalities (Rankovic and Keča, 2007, 2011), partially could be replaced by this method. Collected and processed statistics contribute to a more detailed consideration in the adjustment of sales method TRW, as the final forests product, for these purposes, in certain market conditions. The survey was created with the aim to introduce possible ways of trading with forest products in today's conditions. Achieving greater financial effects (Li and Perrigne, 2003; Roesch-McNally et al. 2016), and consequently a greater investment in sustainable forest

[1]Sanja Pajić, (corresponding author: pajicsanja@yahoo.com), PE „Vojvodina šume“ Petrovaradin, FE Sremska Mitrovica, SERBIA, Ljiljana Keča, (corresponding author: ljiljana.keca@sfb.bg.ac.rs), University of Belgrade, Faculty of Forestry, SERBIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

management (SFA) can be succeed by changing the ways of sales. At the auctions of the high worth TRW might be achieved higher price per units, instead of sales by the price-list, and as well at the market all customers would be equal (Oreščanin and Redžić 199; Hagenberg, 1999; Ranković and Keča, 2011).

MATERIALS AND METHODS

In the research are used qualitative and quantitative methods of socio - economic sciences (Bernard and Bernard, 2012; Creswell, 2013). For this type of research, in order to determine a representative sample, the data are defined on production and sale of TRW oak in the SFA, produced assortment structure of TRW oak in the SFA. Data were collected in PE "Vojvodina šume" Petrovaradin, FE Sremska Mitrovica za 93,4 % surface of this area, for period between 1965 and 2015. For private forests (6,6 % areas of SFA) there are no reliable sources of information.

The subject of research is the SFA, which covers an area of 45,673 ha, with a wood volume of 11,640,096 m^3 (Forest management plan SFA, 2014). State-owned forests make up 93,4%, and were given for care and management to the PE "Vojvodina" Petrovaradin, Sremska Mitrovica FE, private forests cover 6.6% of the area. This ownership forest structure has focused research on state forests because they can be regarded as representative in this area. From the aspects ratio of tree species in the SFA, as the dominant species of forest management stands out Quercus robur which is spread over 22 844 ha, with a volume of 6 231 043 m^3 and a share of 53,5% in volume compared to other tree species. Mixed stands cover 65,89% of the area, while pure stands make up to 34,11%. Planned fellng quantity of common oak for the period between 2005 and 2014 amounted to 559 945 m^3 in gross volume, 68% of it make it volume yield, a 32% preliminary yield (Forest management plan SFA, 2014). Common oak is a natural resource of special value, includes preserved forests that generate significant income through their sustainable use (Medarević, 2006). The demand for them in the last decades is beyond of production (Internal documentation of commercial services PE "Vojvodinašume" Petrovaradin FE Sremska Mitrovica, 2016).

Menagment and finance PE in Serbia is defined by the Law on Forests, Law on Nature Protection and Law on Public Enterprise. The last law regulates the relationship with the founder. It adopts programs of management, business reports, price list of products and services, advertising and other acts which the PE must confirm. If we analyze the structure of business revenue it's obvious that 83,77% of income (Independent Auditor's Report, 2016) PE "Vojvodina šume", FE Sremska Mitrovica in the period 2006 - 2015 comes from sale of goods and services, and only 2,84% of the subsidies, donations, grants and premiums (Independent Auditor's Report, 2016). Wood sale is made on the basis of the applicable price-list by contract. The way to achieve higher incomes in the existing legal framework is open competition, a bid TRW (Li and Perrigne, 2003). The study analyzes the production and sale of TRW common oak in state

forests of SFA for the period 1965 - 2015, as well as the assortment structure of TRW in the last 20 years (between 1996 and 2015).

RESULTS AND DISCUSSION

By observing the period of production and sales of the last 50 years (1965 - 2015) it can be concluded that the average production of TRW common oak ranges from 18 000 to 22 000 m^3 annually in state forests SFA and sales followed production. There are no problems for the placement of these products (Internal documentation of commercial services PE "Vojvodina šume" Petrovaradin FE Sremska Mitrovica, 2016). Statistical analysis showed that production and sales have the linear trend and recorded a slight increase, the annual rate of exponential growth of production is 0,4% and 0,5% of sales. Production and sales of this assortment are stable without significant fluctuations (Table 1).

Table 1. Statistical analysis of the production and sale of TRW of oak for the period 1965 - 2015

Production of TRW of oak						
Parameters		*T*	*R*	*F*	Y= 85,767x - 150052	
a	-150052	-1,26372	0,201153	2,066279		
b	85,7667	1,437456			Annual exponential growht rate	0,40%
Sale of TRW of oak						
Parameters		*T*	*R*	*F*	Y= 94,198x - 166909	
a	-166909	-1,37897	0,216027	2,398647		
b	94,19819	1,548757			Annual exponential growht rate	0,50%

This paper analyzes the produced assortment structure TRW of common oak in the SFA in state forests for the period between 1996 and 2015, and the following results are:

The analysis of the production of veneer logs of common oak we get a second degree polynomial, with a strong correlation relationship (0,917) and the correlation coefficient is statistically significant, as indicated by an error of about 0,05% (*Significance F*), the parameters are significant (for the level of faults α=0,05%) as the corresponding *P-value* indicates an error of about 0,05%. The growth rate is negative and amount to -6,9% (Figure 1).

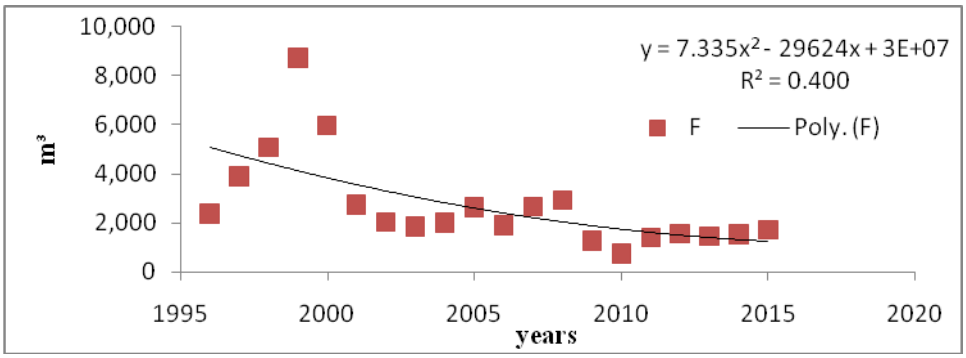


Figure 1. Production of veneer logs of common oak, SFA

Production of class I sawmill logs of common oak is represented by a polynomial of the second degree, with a strong bond correlation (0,927) and the correlation coefficient is statistically significant, as indicated by an error of about 0,000005% (*Significance F*), the parameters are significant (for the level of faults α=0,05%) as the corresponding *P-value* indicates an error of about 0,00007%. The growth rate is also negative and amount to -4,0% (Figure 2).

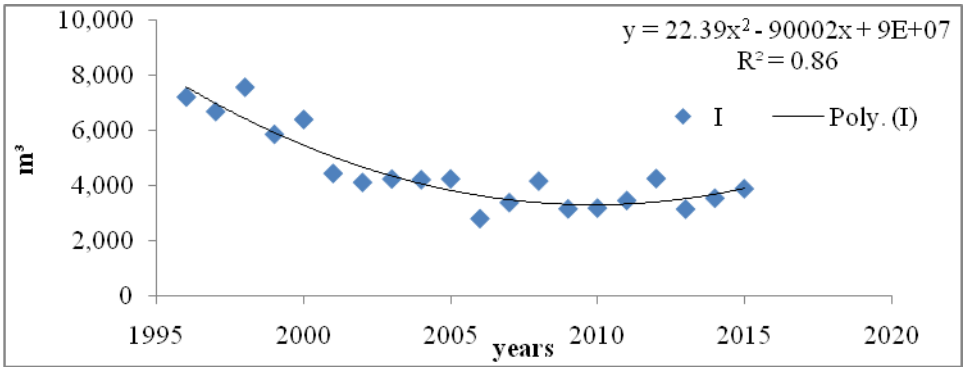


Figure 2. Production of class I sawmill logs of common oak, SFA

In the production of sawmill common oak class II the polynomial of the second degree is obtained, with a strong bond correlation (0,932) and the correlation coefficient is statistically significant, as indicated by an error of about 0,00003% (*Significance F*), the parameters are significant (for the level of faults of α=0,05%) because the corresponding *P-value* indicates an error of about 0,02%, the growth rate is negative and amount to -4,4% (Figure 3).

Production of sawmill logs of common oak III class is a polynomial of the second degree with a strong correlation relationship (0,834) and the correlation coefficient is statistically significant, as indicated by an error of about 0,004% (*Significance F*), the parameters are significant (for the level of error of α=0,05%) as the corresponding *P-value* indicates an error of about 0,2%. The growth rate is also negative and amount to -3,6% (Figure 4).

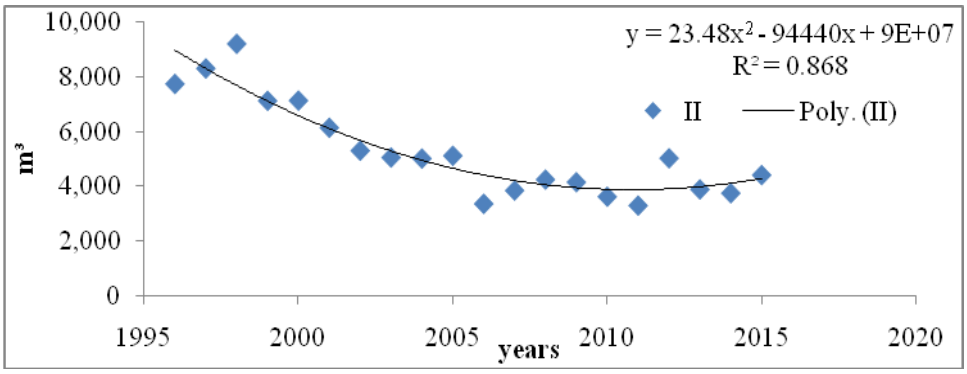


Figure 3. Production of II class sawmill logs of common oak, SFA

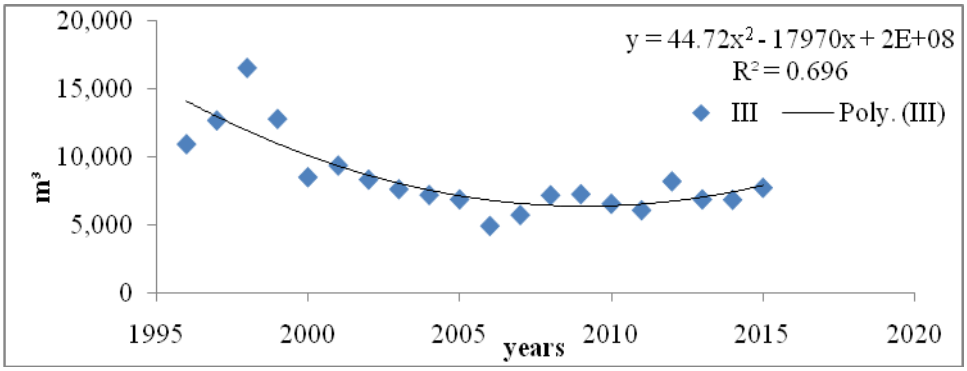


Figure 4. Production of III class sawmill logs of common oak, SFA

If we look at the production of TRW common oak as a whole, we get a polynomial of the second degree, with a strong correlation (0,839) and the correlation coefficient is statistically significant, as indicated by an error of about 0,0000003% (*Significance F*), the parameters are significant (for error level of α=0,05%) as the corresponding *P-value* indicates an error of approximately 1%, and the rate of -3,6% (Figure 5).

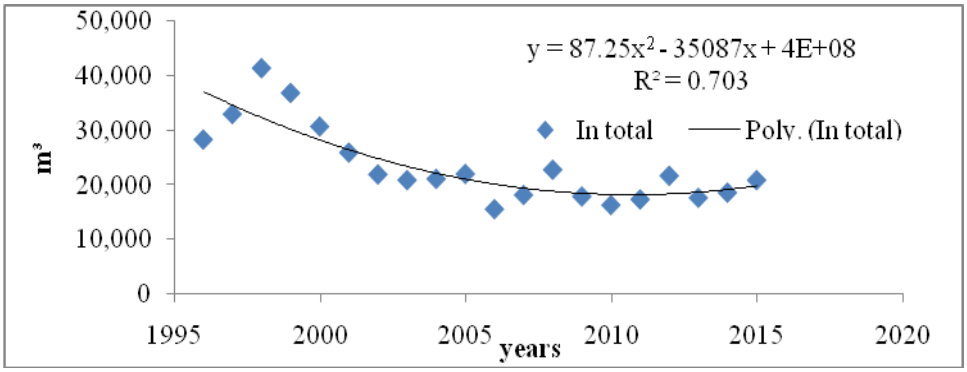


Figure 5. Production TRW of common oak, SFA

Over 90% of income is realized through the sale of TRW in the domestic market, but only a small part abroad and mostly for products for which there is sufficient demand in the same (soft pulpwood Hardwood) (Keča et al., 2009).

All wood processors that supply from the SFA have the capacity significantly larger than the current production volume, which means that, if necessary, they can processed more raw material (Pajić, 2016). Forest yield opportunities are less than the total processing capacity in the forest area, which their needs for raw materials complement from other forests. Even a significant increase in yield from the forests of this area would not lead to problems in the placement on the market (Internal documentation PE „Sremska Mitrovica“, 2016). From the above, it follows that all interested buyers, the fair competition in the auction would allow them reach the desired goods, which is not enough in the market, and is conditioned by the fact that the offer is limited in quantity and can not be increased (respecting the principles of sustainable development) (Schmithüsen et al., 2006; Medarević, 2006, Ranković and Keča, 2011).

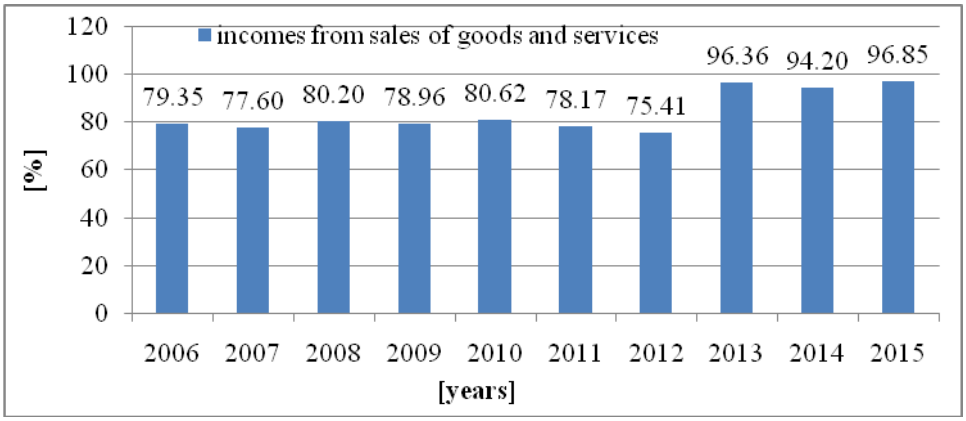


Figure 6. The share of incomes from sales and services in the total all revenues

The method of comparing income (Schmithüsen et al., 2006), which belongs to the static methods of investment calculations, can help in the assessment of PE able to identify the bid as a way of selling. This method takes into account, under the assumption of equal cost, the difference in expected incomes. From more investment opportunities, the variant that offers the highest profit is selected. Procedure for the comparison method is similar to the method of comparing costs. Expected profit can be calculated per unit of product [m^3], is useful for simple investments (Schmithüsen et al., 2006). If we take into account that the cost of production TRW are same regardless of the method of sale, that the initial price is formed based on the current value of assortments (the value of the lot is calculated according to the current price list, a value per unit obtained when the value is divided by quantity) we conclude that no significant increase of the initial price the company would realize a profit, because the cost of sales do not increase significantly. In situations of high demand and low supply of goods

on public sales growth trend is seen price growth trend and then sellers, as opposed to customers, prefer public sale (Hagenberg, 1999; Schwartz, 2012).

Sales of goods can be regarded as one of the functions of goods trade, but also as the second phase of the reproduction process, which follows the stages of production (Oreščanin and Redžić, 1994), which should be maximizing the seller's income. Situation on the market and the quality of the wood determines the type and volume of sales. Each of the methods has its advantages and disadvantages in certain market conditions (Oreščanin and Redžić, 1994; Hagenberg, 1999). In this case, the high quality and quality TRW common oak for which there is an increased demand, production is limited with feeling volume and cannot be increased. Based on the statistical analysis of assortment structure, that shows negative growth race, -3.6% average for all assortments, we can conclude that there will still be an increased demand due to reduced supply. These conditions correspond to the selection of the bid, as a sales method that would achieve the maximum financial effect (Oreščanin and Redžić 1994; Hagenberg, 1999; Rankovic and Keča 2011; Trifunovic, 2012) in the existing legislative framework of a market in Serbia. On the markets of the seller is in the forefront of the type and quantity of offers and products traded. For forest holdings in the markets of the seller, due to the fact that there is a lack of resources, these conditions put them in an advantageous position and enable them to earn more income from the sale of TRW through the auction, reduced for the cost of conducting auctions (Schmithüsen et al., 2006).

CONCLUSIONS

From exposed statistical analysis can be concluded that the production and sale of TRW from SFA stable without significant fluctuations. There is a negative growth rate of production for all assortments of common oak (F, I, II i III klasa). Practically every year, if it continues this trend offers will be lower, and on the other hand we have the growing demand. In such cases when supply is less than demand, the bid should be practiced as a way of sales, in order to achieve greater financial effects. This conclusion is justified by the fact that the sales of products and services is base of income of the enterprise, and achieving the maximum financial effects of the sale, in the existing legal framework, is possible in public sales.

The results can be successfully used as a basis for further research on the relationship between supply and demand in the sphere of auction. The results obtained in this way can be added to new influencing factors and construct a new, more complex models, and can be carried out and additional research that would more suit the nature of the observed values.

REFERENCES

(1965 – 2016): *Internal documentation of commercial services and services of forest management planning PE Vojvodinašume Petrovaradin, FE Sremska Mitrovica*, PE Vojvodinašume Petrovaradin, Serbia

(2006 - 2016): *Izvestaj nezavisnog revizora*, JP Vojvodinašume Petrovaradin, http://www.vojvodinasume.rs/publikacije-kategorije/izvestaji (pristupljeno novembar 2016)

(2014): *Opšta osnova Sremskog šumskog područja 2005-2014*, JP Vojvodinašume Petrovaradin, ŠG Sremska Mitrovica, Srbija

Bernard H. R. (2012): *Social research methods: Qualitative and quantitative approaches*, Sage.

Creswell J. W. (2013): *Research design: Qualitative, quantitative, and mixed methods approaches*, Sage publications.

Hagenberg K. (1999): *Guidelines for the strengthening and establishment of log auction systems*, Roma, FAO.

Keča Lj., Ranković N., Pajić S. (2009): *Analysis of commercial profitability of poplar plantations aged 29 years applying some methods of investment calculation*, Works of the Faculty of Forestry University of Sarajevo, Vol.1, Sarajevo (53-62).

Li T., Perrigne, I. (2003). *Timber sale auctions with random reserve prices, Review of Economics and Statistics*, 85(1), 189-200.

Medarević M. (2006): *Planiranje gazdovanja šumama*, Univerzitet u Beogradu – Šumarski fakultet, Beograd (1-401).

Oreščanin D., Redžić A. (1994): *Trgovina drvetom*, Univerzitet u Beogradu - Šumarski fakultet, Beograd (7-10)

Pajić S. (2016): *Istraživanje mogućnosti prodaje tehničkog oblog drveta licitacijom na području Ravnog Srema*, Univerzitet u Beogradu - Šumarski fakultet, Beograd (Magistarski rad)

Ranković N., Keča Lj. (2011): *Trgovina i marketing šumskih proizvoda*, Univerzitet u Beogradu - Šumarski fakultet, Beograd.

Ranković N., Keča Lj. (2007): *Šumarska politika Srbije*. Univerzitet u Beogradu, Šumarski fakultet.

Roesch-McNally G. E., Rabotyagov S., Tyndall J. C., Ettl G., Tóth S. F. (2016): *Auctioning the Forest: A Qualitative Approach to Exploring Stakeholder Responses to Bidding on Forest Ecosystem Services, Small-scale Forestry*, 1-13.

Schmithüsen F., Kaiser B, Schmidhauser A., Mellnghoff W.A. (2006): *Preduzetništvo u šumarstvu i drvnoj industriji, Osnove menadžmenta i poslovanja*, Centar za izdavačku delatnost Ekonomskog fakulteta, Beograd.

Schwartz R. A. (Ed.). (2012): *The electronic call auction: Market mechanism and trading: Building a better stock market* (Vol. 7). Springer Science & Business Media.

Trifunović D. (2012): *Aukcije*, Centar za izdavačku delatnost Ekonomskog fakulteta u Beogradu (4-8, 225).

DOI: 10.17707/AgricultForest.63.1.24

Ilija RISTESKI,
Karolina KOČOSKA, Valentina PELIVANOSKA[1]

RESULTS OF INVESTIGATIONS OF GENOTYPE AND ITS INFLUENCE ON THE YIELD, QUALITY AND ECONOMIC EFFECT OF VIRGINIA TOBACCO IN R. MACEDONIA

SUMMARY

Investigations were carried out in 2014 and 2015 on the experimental field of the Scientific Tobacco Institute - Prilep. They included four introduced fertile varieties and three domestic promising hybrid lines in CMS form created in the Institute, with the standard Australian variety Ca-757 used as a check. The trial was set up in randomized blocks with 4 replications. The highest average yield per stalk (161,7 g) and per hectare (3 593 kg) was achieved in line V-79/09 CMS F_1. The lowest yield per stalk (125,3g) and hectare (2 784kg) was recorded in the standard Australian variety Ca-757.

The highest quality of tobacco raw, expressed through the average price, was obtained in the line V-79/09 CMS F_1 (1,51 USD/kg) and lowest in the variety Ca-757 (1,28 USD/kg). Also, the gross income was the highest in line V-79/09 CMS F_1 (5 434 USD/ha) and the lowest in the variety Ca-757 (3 583 USD/kg).

With some variety lines, for some characteristics, there were statistically significant differences with 5% and 1% significant levels compared to a Ca-757.

Keywords*: tobacco, Virginia, yield, income.*

INTRODUCTION

The raw material of Virginia tobacco is inevitable component in the manufacturing of blended cigarettes. According to Beljo (1996) and Uzunoski (1985), Virginia belongs to the group of large-leaf, high tobaccos (200 cm). Its growth and development requires precisely determined agro-ecological conditions and cultural practices, and specific way of curing (flue-cured). This tobacco was grown in certain regions of the Republic of Macedonia until 2002, after which the production has stopped and now Macedonian cigarette factories is fully dependent on imports of this type. To overcome this situation it is necessary to restart the production according to world standards and to create raw material similar or equal to the imported one. In production chain of Virginia tobacco, variety is a very important factor which has a large impact on yield and quality of

[1]Ilija Risteski, (corresponding author: ilija.r@t.mk), Karolina Kočoska, Department of genetics, selection and seed control, Scientific Tobacco Institute – Prilep, Kičevska bb, Prilep, MACEDONIA, Valentina Pelivanoska, Department of agrotechnics, Scientific Tobacco Institute – Prilep, Kičevska bb, Prilep, MACEDONIA

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

the raw material. In recent years, many hybrid varieties (lines) in CMS form have been created in Tobacco Institute - Prilep. The results of comparative trials show that this newly created varieties and (lines) might be also interesting for tobacco growers.

MATERIAL AND METHODS

Six varieties, four of them in fertile form, were used as material for work: Ca-757 was used as a check, Vissana (Australia), V-3816 (Bulgaria), V-36/13 (Macedonia) and the male-sterile hybrid lines V-99/11 CMS F_1, and V-79/09 CMS F_1 created in Tobacco Institute - Prilep. The trial was set up on colluvial soil in randomized block system with four replications at 90×50cm planting density, on previously prepared site (one autumn and two spring ploughings, fertilization with 300 kg/ha NPK 8:22:20 and application of herbicide). Before the second hoeing, manual feeding of stalks was made with 3 g/stalk 26% KAN. The plants were also treated with chemicals for their protection from pests and diseases. Harvested tobacco was stringed, yellowed and then dried. Qualitative assessment of cured tobacco was made according to the rules for assessment of quality of dry Virginia tobacco (Rules on criteria for qualitative and quantitative assessment of raw tobacco leaves "Official Gazette" of R. Macedonia, No. 16/2007, amended and supplemented No. 144/2010 and No. 20/2011). Corrected yield per stalk and hectare was calculated by the method of Rimker and the gross income (USD/ha) by multiplying yield per hectare and the average price of 1 kg raw tobacco. Conversion in USD was calculated by 2015 average middle exchange rate of National Bank of Republic of Macedonia (50,65 ден for 1 USD).The results were statistically processed using the analysis of variance and LSD test.

RESULTS AND DISCUSSION

Tobacco yield mainly depends on leaves, their number and size. This trait is genetically controlled in each variety (genotype), but it is also highly affected by the environmental conditions during the growing season. Dražič (1986) explains that the yield is directly influenced by the genotype and environment. Carriers of the yield and quality of raw material in Virginia tobacco are the middle belt leaves, their size and color obtained after curing. According to Beljo (1996), tobacco yield and quality also depend on cultural practices applied during the growing season (fertilization, irrigation, harvesting time, yellowing, curing etc.). Results of our investigation of the above traits are presented in Tables 1, 2, 3 and 4.

Yield per stalk, g/stalk

Data on the obtained yield per stalk from the investigated varieties and lines are presented in Table 1.

According to data, the highest average yield per stalk was recorded in line V-79/09 CMS F_1 (161,7 g) and the lowest in the check variety Ca-757 (125,3 g). In other varieties in the trial, the average yield per stalk ranged from 135,8 g in

variety V-3816, to 141,3g in line V-99/11 CMS F_1. It can be also noted that higher yields were achieved in the more humid conditions of 2014. In both years of investigation, only the variety V-79/09 CMS F_1, achieved statistical significance at 1% level compared to the check. The line V-99/11 CMS F_1 achieved statistical significance at 1% level compared to the check only in 2015. The variety Vissana in 2014 showed statistical significance at 5% level.

Table 1. Corrected yield per stalk (g/stalk)

Variety	Year	g/stalk	Average 2014/2015 g/stalk	Difference		
				Absolute	Relative	Rank
Ca-757 Ø	2014	125,25	125,3	/	100,00	6
	2015	125,34				
Vissana	2014	148,80^{+}	140,3	+15,0	111,97	3
	2015	131,87				
V-3816	2014	135,28	135,8	+10,5	108,38	5
	2015	136,31				
V-36/13	2014	143,62	137,4	+12,1	109,66	4
	2015	131,15				
V-99/11 CMS F_1	2014	141,12	141,3	+16,0	112,76	2
	2015	141,50^{++}				
V-79/09 CMS F_1	2014	162,41^{++}	161,7	+36,4	129,05	1
	2015	160,97^{++}				

LSD 2014 $5\%^{+}$ = 19,15 g/stalk $1\%^{++}$ = 26,57 g/stalk 2015 $5\%^{+}$ = 5,91 g/stalk $1\%^{++}$ = 8,19 g/stalk

Dražič *et al.* (2012) made investigations with 13 domestic and introduced varieties and lines in 2011 at various locations in Serbia and found that the yield per stalk ranged from 105 g/stalk to 257 g/stalk in Nova Pazova and from 101 g/stalk to 298 g/stalk in Starčevo. Risteski (1999) reported that the stalks of MV-1,grown at nutritional area of 0,25 m^2achieved an average yield of 79,7 g/stalk and those grown at 1 m^2 achieved 198,2 g.

Yield per hectare, kg/ha

Data on the obtained yield per hectare are presented in Table 2.

Data reveals a very close relationship between yield per stalk and per hectare. The highest average yield was obtained in line V-79/09 CMS F_1 (3 593 kg/ha) and the lowest in check variety Ca-757 (2 784 kg/ha). In other varieties investigated, the yield ranged from 3 017 kg/ha in variety V-3816 to 3 140 kg/ha in line V-99/11 CMS F_1. In 2014 and 2015, statistical a significant difference at 1% wasachieved only the line V-79/09 CMS F_1. Difference like this in 2015 was achieved by line V-99/11 CMS F_1, and variety V-3816. In 2014 and 2015, variety Vissana achieved statistically significant differences at 5% level.

Table 2. Corrected yield per hectare (kg/ha)

Variety	Year	kg/ha	Average 2014/2015 kg/ha	Difference		
				Absolute	Relative	Rank
Ca-757 Ø	2014	2.783	2.784	/	100,00	6
	2015	2.785				
Vissana	2014	3.306^{+}	3.118	+334	111,99	3
	2015	2.931^{+}				
V-3816	2014	3.006	3.017	+233	108,37	5
	2015	3.029^{++}				
V-36/13	2014	3.191	3.052	+268	109,63	4
	2015	2.914				
V-99/11 CMS F_1	2014	3.136	3.140	+356	112,78	2
	2015	3.144^{++}				
V-79/09 CMS F_1	2014	3.609^{++}	3.593	+809	129,05	1
	2015	3.577^{++}				

LSD 2014 $5\%^{+}$ = 425,48 kg/ha; $1\%^{++}$ = 589,28 kg/ha; 2015 $5\%^{+}$ = 131,23 kg/ha; $1\%^{++}$ = 181,75 kg/ha

Jovovič (1957) stated that according to the results of tests performed and the experimental field of Bar (Montenegro) in the fifties of the last century, the yield per hectare of type Virginia was 1 707 kg/ha. Devčič *et al.* (1982) reported that by application of good cultural practices, Croatian hybrid varieties H-30, H-31 and H-32 can reach over 2 000 kg/ha. Hawks (1978) presented the yields of Virginia tobacco in the United States in different periods of time. According to the data, the average yield in the period 1934-1938 was only 959 kg/ha and in 1964-1967 it increased to 2 224 kg/ha. Risteski *et al.* (2012) reported that higher yields were achieved in Virginia tobacco lines created in the Scientific Tobacco Institute - Prilep, reaching up to 3 549 kg/ha in line V-53 CMS F_1.

Average price, USD/kg

The quality of tobacco raw expressed in monetary value gives the average price per kg. It is closely related to the variety, proper and timely applied cultural practices, yellowing, curing, etc. Data on average price in the varieties investigated are presented in Table 3. According to the above data, the average price ranged from 1,28USD/kg in check variety Ca-757 *to* 1,51 USD/kg in line V-79/09 CMS F_1.

In the other varieties and lines, the average price ranged from 1,33 USD/kg in variety V-36/13 to 1,46 USD/kg in line V-99/11 CMS F_1. Statistically significant differences at 1% level in both years of investigation were observed only in line V-79/09 CMS F_1. Statistical significance differences at 1% level in 2014 and 2015, was achieved only by the line V-79/09 CMS F_1.

Table 3. Average price (USD/kg)

Variety	Year	USD/kg	Average 2014/2015 USD/kg	Difference		
				Absolute	Relative	Rank
Ca-757 Ø	2014	1,25	1,28	/	100,00	6
	2015	1,32				
Vissana	2014	1,31	1,35	+0,07	105,45	3
	2015	1,39				
V-3816	2014	1,32	1,34	+0,06	104,60	4
	2015	1,36				
V-36/13	2014	1,31	1,33	+0,05	103,90	5
	2015	1,35				
V-99/11 CMS F_1	2014	1,48^{++}	1,46	+0,18	114,06	2
	2015	1,44^{+}				
V-79/09 CMS F_1	2014	1,51^{++}	1,51	+0,23	117,96	1
	2015	1,51^{++}				

LSD 2014 5%$^{+}$ = 0,11 USD/kg 2015 5%$^{+}$ = 0,11 USD/kg
1%$^{++}$ = 0,15 USD/kg 1%$^{++}$ = 0,15 USD/kg

The line V-99/11 CMS F1 achieved statistical significant difference at 1% in 2014, and in 2015 achieved statistical significant difference at 5%. Kočoska *et al*. (2004) in investigations with six varieties and lines in the region of Prilep during 2002 and 2003 reported that the highest average price was achieved in line V-53 (1,30 USD/kg) and the lowest in line V-69 (1,13 USD/kg).

Table 4. Gross income (USD/ha)

Variety	Year	USD/ha	Average 2014/2015 USD/ha	Difference		
				Absolute	Relative	Rank
Ca-757 Ø	2014	3 488	3 583	/	100,00	6
	2015	3 678				
Vissana	2014	4 334^{+}	4 200	+617	117,22	3
	2015	4 067				
V-3816	2014	3 976	4 048	+465	112,98	5
	2015	4 121^{+}				
V-36/13	2014	4 175^{+}	4 053	+470	113,12	4
	2015	3 932				
V-99/11 CMS F_1	2014	4 641^{++}	4 591	+1 008	128,13	2
	2015	4 541^{++}				
V-79/09 CMS F_1	2014	5 446^{++}	5 434	+1 851	151,66	1
	2015	5 422^{++}				

LSD 2014 5%$^{+}$ = 656 USD/ha 2015 5%$^{+}$ = 420 USD/ha
1%$^{++}$ = 909 USD/ha 1%$^{++}$ = 582 USD/ha

Gross income, USD/ha

The data presented in Table 4 determine the average price per 1 kg of raw tobacco and yield per hectare achieved by the investigated varieties and lines. Compared to the check variety Ca-757 (3 583 USD/ha), the highest gross income was recorded in line V-79/09 CMS F_1 (5 434 USD/ha). In other varieties and lines, gross income ranged from 4 048 USD/ha in V-3816 to 4 591 USD/ha in line V-99/11 CMS F_1.

In the both years of investigation (2014 and 2015) statistical significant difference at 1% level, were achieved by the lines V-79/09 CMS F_1 and V-99/11 CMS F_1. In 2014 variety Vissana and the line V-3613 achieved statistical significant difference at 5% level, and in 2015 the variety V-3816 also achieved statistical significant difference at 5% level.

CONCLUSIONS

The highest average yield per hectare and stalk was recorded in line V-79/09 CMS F_1 (161,7 g/stalk and 3 593 kg/ha) and the lowest in variety Ca-757 (125,3 g/stalk and 2 784 kg/ha). The highest average price of raw tobacco was achieved in line V-79/09 CMS F_1 (1,51 USD/kg) and the lowest in variety Ca-757 (1,28 USD/kg).The highest gross income was obtained in line V-79/09 CMS F_1 (5 434 USD/ha), and the lowest in variety Ca-757 (3 583 USD/ha). The above data lead to a conclusion that genotype has high influence on the investigated traits and therefore more attention should be paid to the selection of varieties for mass production.

REFERENCES

Beljo J., 1996. Postupak za identifikaciju kultivara duhana. Izvješče o znanstvenom i stručnom radu u 1994 god. Duhanski institut – Zagreb.

Devčič K., Triplat J. 1982. Neka svojstva novih flue-cured sorti uzgajanih u Duhanskom institute – Zagreb. Тутун/Tobacco, XXXII, N^o 11-12, 5-10

Dražič S. 1986. Prilog proučavanju nasledjivanja kvantitativnih stvojstava duvana. Univezitet u Beogradu – Poljoprivredni fakultet – Doktorska disertacija.

Jovovič V. 1957. Neki američki tipovi duvana i mogučnost njihovog gajenja na crnogorskom primorju. Agriculure and forestry: 3,4: 59-68

Hawks S.N., 1978. Osnovi proizvodnje virginiskog duhana. Zagreb (translation)

Кочоска К., Ристески И., Димитриески М., Мицеска Г. 2004. Компаративни испитувања на некои новосоздадени вирџиниски сорти тутун. 60 год. Јубилеј на научна конференција с международно участие. Стр. 162-167. Пловдив

Ристески И. 1999. Корелација помеѓу храниливата површина и некои производно-технолошки својства на тутунот од сортата вирџинија МВ-1. Магистерски труд. Земјоделски факултет. Скопје

Risteski I., Kocoska K. 2012. Results of broadleaf tobacco breeding in Scientific tobacco institute – Prilep. International symposium on agriculture and food. 12-14 Dec. 2012. Skopje. Republic of Macedonia. p. 643-648.

Slobodan Dražič[1]. Ilija Risteski[2]. Kiril Filiposki[2]. Karolina Kočoska[2]. 2012. Results of studies on recently developed virginia tobacco genotypes under different growing conditions in Serbia. Тутун/Tobacco. Vol. 62, N^o 1-6, p.03-08

Филипоски К. 2011. Статистички методи во земјоделските истражувања – одбрани поглавја. Научен институт за тутун – Прилеп.

DOI: 10.17707/AgricultForest.63.1.25

Frederico MARANGON, Lucas Emanuel SERVIDONI,
Carlos Wilmer COSTA, Velibor SPALEVIC, Ronaldo Luiz MINCATO[1]

LAND USE AND GROUNDWATER RESOURCES POTENTIAL VULNERABILITY TO CONTAMINATION

SUMMARY

Municipality master plan approach to the expansion processes of human activities is presently essential once poor and badly planned use of natural resources cause direct or indirect impacts on the quality and quantity of groundwater resources. Concerning the problem of contamination of this resource, the analysis of land use and coverage integrated to physical variables can contribute to improve perception of the problem. In this paper, a Chart of Groundwater Resources Potential Vulnerability to Contamination at the Municipality of Alfenas (849.97 km²), southern Minas Gerais State, Brazil, was developed. Multicriteria analysis was applied supported by decision matrices and overlapping imagery, which classified potential vulnerability to contamination in the levels: extreme, high, moderate, low and negligible. The matrices are characterized by overlapping and comparing environmental features and human actions in Geographic Information System. Results showed that the areas classified as of moderate or extreme potential vulnerability correspond to, respectively, 51.16% and 23.38% of the study area and must be a major issue for mitigation actions of their potential vulnerabilities.

Keywords: *Spatial analysis, environmental planning, soil and water conservation, geotechnologies...*

INTRODUCTION

The increase of domestic, agribusiness and industrial water consumption has raised pressure on surface and underground hydric resources. Considering the economic value of water because it is essential for many species and for biogeochemical processes, proposals of sustainable water use is essential. On the other hand, inappropriate and non-planned use affects negatively quality and quantity of this resource. Besides irregular distribution, harmful changes in chemical, physical and biological characteristics of groundwaters associated to human activities in rural and urban areas are also verified.

[1] Frederico Marangon, Lucas Emanuel Servidoni Post-graduation Program in Environmental Science, Universidade Federal de Alfenas – UNIFAL-MG, Alfenas, Minas Gerais, BRAZIL; Carlos Wilmer Costa, Post-graduation Program in Environmental Sciences, Universidade Federal de São Carlos – UFSCAR, São Carlos, São Paulo, BRAZIL; Velibor Spalevic, University of Montenegro, Faculty of Philosophy, Department of Geography, MONTENEGRO; Ronaldo Luiz Mincato (corresponding author: ronaldo.mincato@unifal-mg.edu.br), Instituto de Ciências da Natureza - Universidade Federal de Alfenas – UNIFAL-MG, Alfenas, Minas Gerais, BRAZIL.
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Rural areas present both diffuse impacts, due to intensive farming and cattle raising activities, and punctual or local, due to inappropriate disposal of residues and absence of basic sanitation. In urban areas, impacts are punctual and associated to illegal disposal of industrial effluents and poor sanitation services, which are absent or present precarious collection concerning treatment of drain water and discharge of residues. In Brazil, however, the concern with groundwaters contamination is limited because, in general, groundwaters's users ignore the severe effects of their pollution. This is because groundwaters contamination is not visible and exploitation is broadly shared, making the identification of the problem difficult. In general, when contamination is noticed, it has reached large extensions (Ribeiro *et al*., 2007). Besides, many compounds originating from polluting activities interact and are inserted as different chemicals in the hydrological cycle by natural and human processes, which may change hydrogeochemical properties of aquifers and of phreatic table water (Costa *et al*., 2015).

In this scenario, studies similar to those by Costa *et al*. (2015), which intend to explain contamination risk of groundwater resources by residues associated with human activities, are essential.

Several assessment studies of groundwaters contamination were carried out in Brazil. It is worth pointing out those of Löbler *et al*. (2014), which studied the municipality of Nova Palma using GOD (Groundwater occurrence, Overall lithology of the unsaturated zone, Depth to the water table) method to assess vulnerability; and, Löbler *et al*. (2013), which mapped groundwaters potential spots of contamination and natural vulnerability in the municipality of Restinga Seca, both in Rio Grande do Sul State. In the State of São Paulo, Cereda Junior and Röhm (2014) used multicriteria methodologies to assess environmental fragility of Rio Monjolinho basin in São Carlos Municipality. In Rondonópolis Municipality, Mato Grosso State, Cutrim and Campos (2010) estimated Furnas Aquifer vulnerability to contamination.

Therefore, the representation of vulnerability of underground hydric resources demands graphic illustration, in adequate spatial scale, physical environmental characteristics and human activities in progress there. Thus, the groundwater resources potential vulnerability to contamination by different land uses in the Municipality of Alfenas, in southern Minas Gerais State, was assessed by means of multicriteria analysis in geographic information systems (GIS).

MATERIAL AND METHODS

Alfenas, in southern Minas Gerais State, (Figure 1), is equidistant from the main metropolitan regions in Brazil: São Paulo, Rio de Janeiro and Belo Horizonte. Estimated population is of 78.712 inhabitants and it total area is of 849.97 km^2 (IBGE, 2015). Climate, as per Köppen classification, is CwB (mesothermal, tropical) (Sparovek *et al*., 2007).

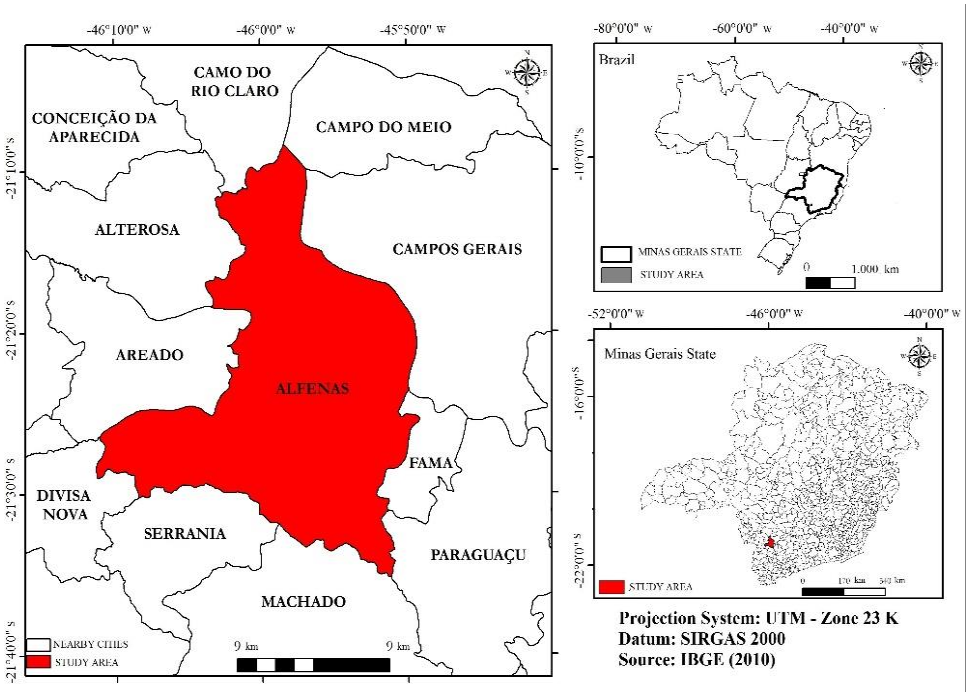


Figure 1: Site of the Study area

Alfenas is at the highlands of South of Minas Gerais or Alto Rio Grande (Ab'Saber, 1975). Altitudes vary from 760 to 960 m, in undulating relief. The region is in the transition of 'Cerrado' (Brazilian Savannah) and 'Atlantic Forest' (Alfenas, 2006) biomes, with only small remaining fragments of natural vegetation. Farming and cattle raising activities are responsible for 15% of the municipality Gross Domestic Product - GDP (IBGE, 2012).

The geological structure is formed of charnockitic and granolithic polymetamorphic gneisses from the Precambrian period (Hasui, 2010), with complex hydrogeological domains distributed in five distinct geological units: Andrelândia and Carrancas Groups and the Campos Gerais, the Varginha-Guaxupé and the Lavras complexes (CPRM and UFRJ, 2010).

Andrelândia Group is a unit of diversified metasedimentary rocks, with predominance of quartzites in the basement followed by garnet schists, leptinites and marbles. In Alfenas Municipality, there are two types of lithofacies from the Andrelândia group (Peternel *et al*., 2005). The first corresponds to the association of basal lithofacies positioned discordantly, by thrust faults, onto the basement orthogneisses formed by biotite gneisses, of fine grain and thin bands. The second is concordant in relation to the biotite gneisses, formed by quartzite successions and subordinate intercalation of muscovite-quartz schists.

The Carrancas Group (FUPAI, 2013) is an alochthonous succession of metapsamitic rocks that transform into mica-graphite schists to the top, admitted

as of Neoproterozoic passive margin of the meridional border of the Sanfranciscana Plate (Ribeiro *et al*., 1995).

The Basal Granulitic unit, the Intermediate Magmatitic Orthogneissic unit and the Migmatite High-grade paragneissic unit emerge in the Municipality of Alfenas from the Varginha-Guaxupé Complex. The first is formed by enderbite, granulite and mafic granulite of basement composition. The second is compound of anatexite, granitic gneiss, stromatic migmatites and tonalitic gneiss; and the third of paragneisses, mica schists and biotite gneiss (FUPAI, 2013).

The Archean terrains of the Lavras and the Campos Gerais complexes belong to the Southeastern Brazilian Gneissic-Migmatitic Archean Domain, represented by bodies of great spatial expression, intensely deformed by tectonic events. They are formed by old lithologies, as tonalitic, granitic and migmatitic orthogneisses and amphibolites (FUPAI, 2013).

Soil classes were defined as per the Soil Map of Minas Gerais State in the scale 1:650.000 (UFV *et al*., 2010) as: Dystrophic Red Latosol with clayey texture; Dystrophic Red-Yellow Latosol with clayey texture; Humic Dystrophic Yellow Latosol with clayey texture; typical Dystrophic Yellow Latosol with clayey texture; Dystrophic Red Argisol with medium/clayey textures; typical Eutrophic Red-Yellow Argisol with medium/clayey textures, and Dystrophic Melanic Gleysol with prominent clayey texture, plus Hydromorphic Quartzarenic Neosol, and Sapric Haplic Organosol, all field phase, flat relief.

Geographic-georeferenced information were created, developed and analysed in ArcGIS software ® 10.3.1 (ESRI, 2014). Database include topographic charts of Alfenas (SF-23-I-I-3), Areado (SF-23-V-D-I-4), Campestre (SF-23-D-IV-2), Campos Gerais (SF-23-V-D-I-4), Conceição da Aparecida (SF-23-V-D-I-2), and Machado (SF-23-I-III-1), all of them in the scale 1:50.000 (IBGE, 1971). Geologic charts include Alpinópolis (SF-23-V-B-IV), Machado (SF-23-V-D-V), Alfenas (SF-23-V-D-II), Guapé (SF-23-V-B-V), Campo Belo (SF-23-V-B-VI), Nepomuceno (SF-23-V-D-III), and Varginha (SF-23-V-D-VI), all of them in the scale 1:100.000 (CPRM and UFRJ, 2010).

Metadata from the Geodiversity Map of Minas Gerais State, scale 1:750,000 (CPRM, 2006), the Brazilian Hydrogeological Domains and Subdomains Map, scale 1:1,000,000 (CPRM, 2007), Soils Map of the State of Minas Gerais, scale 1:650,000 (UFV *et al*., 2010), imagery from Shuttle Radar Topography Mission - SRTM (Miranda, 2005), were also considered. These data and the topographic charts (IBGE, 1971) supplied altitude information in the detail compatible with the aims of the research. Slope classes were adjusted in accordance with EMBRAPA (2013). These materials were used in order to validate geological features found in the geological charts (CPMR and UFRJ, 2010). However, despite the Soils Map of the State of Minas Gerais (UFV *et al*., 2010), the Brazilian Hydrogeological Domains and Subdomains Map (CPRM, 2007), and the Geodiversity Map of Minas Gerais State (CPRM, 2006) being generalist, they are compatible with the aims of this work, since they allowed considering physical variables of the area.

Land use and management mapping dated of 2015 was carried out from Landsat-8 Operational Imager OLI imagery in band 2 (green), band 4 (near infrared) and band 5 (mid infrared). Imagery was obtained from the United States Geological Survey (USGS, 2015), the United States of America. Imagery was from the dry period, which allows detailing among the following uses: perennial crops (coffee), temporary crops (sugar cane, corn, beans and soybeans), grazing areas and bare soils.

The research was developed based on the multicriteria analysis (Costa *et al.*, 2015), whose procedure is based on decision matrices, overlapping of images and multidisciplinary assessment of selected criteria. Selected variables were the Hydrogeological Domains Map, the Pedologic Map, the Slope Map, the Geological Faults Map and the Chart of Land Use and Management of Alfenas Municipality.

In order to assess groundwater resources potential vulnerability to contamination, multicriteria analysis is characterized by overlapping physical variables and human actions in GIS. This research model is effective in management and planning of the geographic space, in exploiting natural resources and in the conservation of soil and water (Costa *et al*., 2015). However, multicriteria analysis demands definition and standardization of environmental factors relevant to the subject (Valle Junior, 2015).

Multicriteria analysis has been used in studies concerning environmental planning, and should be preferred in cases in which only one variable does not represent the reality of the phenomenon (Santos, 2010). Multicriteria analysis is based on the mapping of variables by information plans and on the definition of pertinence index of each plan and of each one of their legend components for the construction of results, with the adoption of consideration factors in order to relatively assess and compare different criteria (Moura, 2014).

Thus, it is possible to carry out the analysis of the environmental variables and of the actual scenarios observed in the study area from a multi-disciplinary approach. The methodological process (Figure 2) is supported by the multicriteria analysis by overlapping imagery and decision matrices of selected variables. The procedure was carried out by setting up the database by mapping in GIS, using geoprocessing techniques.

The approach of analysis of the potential contamination was defined simultaneously with the elaboration of decision matrices. Such matrices are descriptive tables, which inform the characteristics to be overlapped in the process for each variable. Decision matrices are descriptive tables that inform the potential contamination of each attribute of the variable in relation to groundwater contamination. In the first stage, the Synthesis Map of Hydrogeological Domain Classes *versus* the Synthesis Map of Soil Classes were overlapped, and the first overlapping result was obtained. Then, the result of the first overlapping was geoprocessed with the third information plan, the Slope Map.

The result of the second overlapping was geoprocessed with the Map of Productivity Classes and Geological Faults, the result of which was processed with the last variable, the Map of Land Use and Management. In short, in the GIS environment, each layer is converted into raster format and reclassified and the result of this reclassification is a new product that is used in the new overlapping.

This process is repeated to the end, by performing the last overlapping and decision matrix, which results in the Chart of Groundwater Resources Potential Vulnerability to Contamination in Alfenas - MG, as final product.

RESULTS AND DISCUSSION

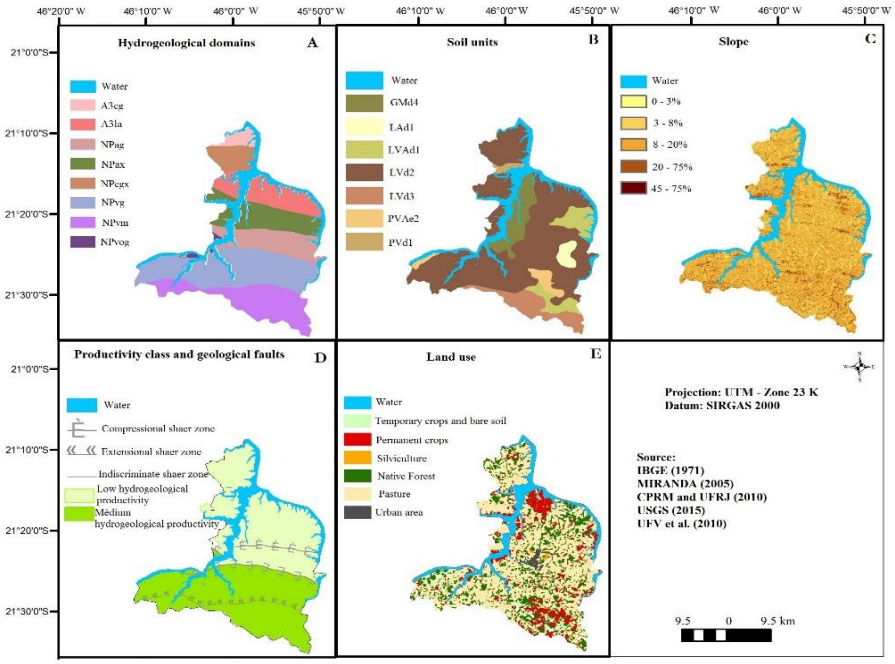
In this paper, four overlappings of the groundwater resources potential vulnerability to contamination were analysed considering the levels: Extreme, High, Moderate, Low and Negligible (Foster *et al*., 2013).

Groundwater resources potential vulnerability to contamination maybe defined by the correlation of physical variables (Figures 2A, 2B, 2C and 2D) with classes of use and soil coverage (Figure 2E).Correlation is carried out as follows:

1) Urban area class is located on the Varginha-Guaxupé Hydrogeological complex with basal granulite Unit to the South, and the Andrelândia Group with kyanite-garnet granulites to the North. Urban area, along its history, expanded in the North-Northwest direction, moving forward onto the Hydrogeological Domain of Andrelândia Group. With approximately 1,462 ha, the urban area represents, in 2015, 1.73% of the municipality. It is located on dystrophic Red Latosols of clayey texture and restrict dystrophic Melanic Gleisol, in flat to gently undulating relief (0.00% to 3.00% and 3.00% to 8.00%).

In the analysis of the overlapped imagery of the Synthesis Map of Hydrogeological Domain Classes and the Synthesis Map of Soil Classes, it was observed that in the urban area class, the groundwater resources potential vulnerability to contamination was low to the North, and moderate to the South, a consequence of the lithologic resistance to percolation of groundwaters, and of the predominance of clayey texture soils. In overlappings number three and number four, it is shown the urban area location in the class of low hydrogeological productivity and on a geological fault, where groundwater resources potential vulnerability to contamination reached the levels high and moderate. This classification represents 4% of the high potential vulnerability illustrated in the mapping.

2)The perennial crops class, mainly the coffee crop, is homogeneously distributed in 13.36% of the municipality, covering all the hydrogeological domains, especially the Campos Gerais Complex to the North and the Varginha-Guaxupé Complex to the South. It occurs in all classes of soil in the gently undulating and undulating slopes, respectively, 3.00% to 8.00% and 8.00% to 20.00%. From the first overlapping, it was observed that the class is in the moderate level (1.67%) and low level (0.57%) of potential vulnerability because of the lithologic characteristics of igneous and metamorphic rocks and of Clayey Soils Pedology.



Legend: A3cg: Campos Gerais Complex; A3la: Lavras Complex; NPag and NPax: Andrelândia Group; NPcqx: Carrancas Group; NPvg, Complex NPvog and NPvm: Varginha-Guaxupé; LVd2: dystrophic Red Latosol; LVd3: dystrophic Red Latosol + dystrophic Red Yellow Latosol; LAd1: humic dystrophic Yellow Latosol + dystrophic Yellow Latosol; PVd1: dystrophic Red Argisol; PVAe2: eutrophic Red Yellow Argisol; GMd4: Melanic Gleisol + Neosol + Organosol.

Figure 2: Information plans of variables related to physical characteristics and land use.

In the second overlapping, an increase to groundwater resources potential vulnerability to contamination was diagnosed because plantations occur in gently undulating and undulating slopes, which allows a higher infiltration of water and of possible contaminants.

In overlappings three and four, agricultural practices in relation to perennial crops consume relatively fewer quantities of natural resources. However, as they are produced by conventional techniques without conservationist management, they introduce chemical elements and compounds, which may trigger important ecological unbalance, with groundwater resources potential vulnerability to contamination in the extreme and high levels. In fact, the research showed that perennial crops represent 58.93% of extreme vulnerability and 64.23% of high vulnerability at the end (Figure 3 and Table 1).

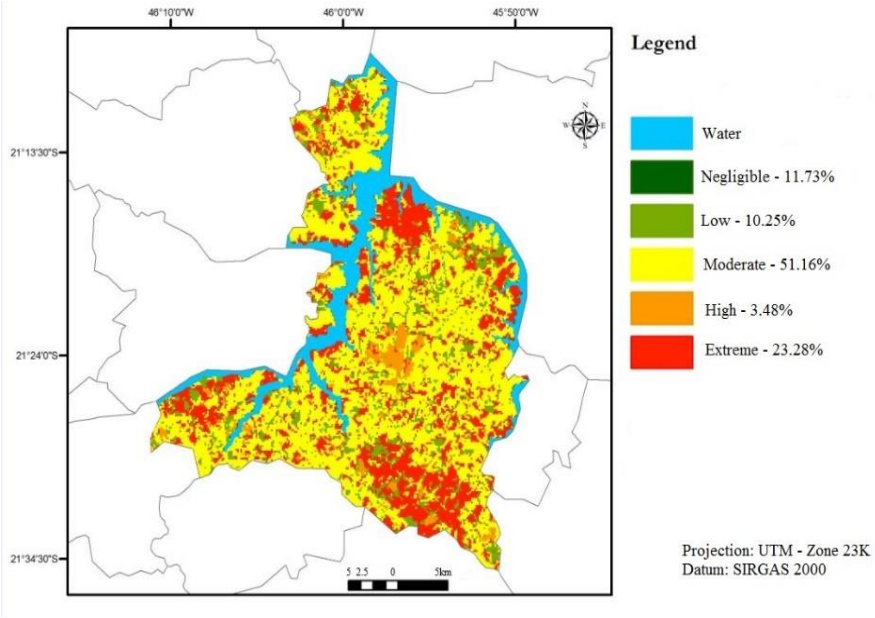


Figure 3. Chart of Groundwater Resources Potential Vulnerability to Contamination in Alfenas - MG Municipality, showing percentage area of each vulnerability level.

The classes bare soil and prepared soil for temporary crops represent 6.21% of the area and are found in every hydrogeological domains and in every class of soil in flat relief and in gently undulating and undulating slopes. As far the potential groundwater resources vulnerability to contamination is concerned, it was observed that these classes are predominant in clayey texture soils. Soils with such characteristics result in moderate to low potential vulnerability in the first overlapping.

In the second overlapping, potential contamination reached moderate and high levels due to crops being produced in the same way as perennial crops, with conventional agricultural techniques using toxic products, without conservationist management. In the fourth overlapping, the areas with temporary crops and with bare soil next to geological faults, with low hydrogeological productivity, were in the moderate and high potential vulnerability levels.

The areas of soil prepared for temporary crops and bare soil respond to 25.25% of high potential vulnerability and 9.70% of extreme vulnerability. However, satellite imagery classification did not differentiate spectral behaviour between soil prepared for temporary crops and bare soil.

Therefore, soil prepared for temporary crops was taken for bare soil. It is in this phase that chemical elements and compounds are introduced and affect, directly or indirectly, the ecosystem balance of the areas.

Table 1. Land use versus groundwater resources potential vulnerability to contamination: area and percentage

Levels of vulnerability / Variables	E	%	A	%	M	%	B	%	N	%
VN	-	-	-	-	-	-	8.56	96.65	-	-
AU	0.21	0.89	0.33	4.00	0.37	0.21	0.42	1.29	-	-
LPS	2.27	9.70	1.00	25.25	3.78	4.43	0.09	0.37	-	-
CT	7.02	30.02	0.80	4.13	2.72	3.76	-	-	-	-
CP	13.78	58.93	1.30	64.24	1.24	1.63	0.22	0.57	-	-
CA	-	-	-	-	-	-	-	-	4.00	32.68
RHE	-	-	-	-	-	-	-	-	7.73	67.32
AF	0.13	0.76	0.05	0.38	1.02	0.94	-	-	-	-
AP	-	-	-	-	42.03	89.03	0.96	1.12	-	-
AT	194.47	-	29.60	-	87.14	-	87.14	-	103.87	-
T	-	100	-	100	-	100	-	100	-	100

VN: Native Vegetation; AU: Urban Area; LPS: Prepared Soil/Bare Soil; CT: Temporary Crop; CP: Perennial Crop; CA: Waterbody; RHE: Furnas Hydroelectric Reservoir; AF: Agroforest; AP: Grazing area; AT: Total Area in km^2; T: Total Area in %; Levels: E: Extreme Vulnerability; A: High Vulnerability; M: Moderate Vulnerability; B: Low Vulnerability; N: Negligible Vulnerability.

3) Consolidated temporary crops class represent 12.03% of the area and is homogeneously distributed in all variables of the studied physical structure.

In the first overlapping, it was observed that this class shows groundwater resources potential vulnerability to moderate contamination (3.76% of the total). In the second overlapping, it was observed a predominance of these crops in flat and gently undulating slopes, which favor infiltration of possible contaminants into the soil, subsoil, and phreatic waters.

Furthermore, it was found that these crops are located on geological faults with medium hydrogeological productivity. Because of these characteristics, the class of consolidated temporary crops, which used relevant quantities of toxic products, able to impact the environmental systems, reached the extreme (30.02%) and high (4.13%) potential vulnerability.

4) The grazing area class is distributed in 46.32% of the municipality in all hydrogeological domains, in every class of soils, above all in the dystrophic Red Latosols and the dystrophic Red Latosol with dystrophic Red-Yellow Latosols, in all classes of slopes, with low and medium hydrogeological productivity and on geological faults.

Soil with clayey textures, when overlapped with the Lavras, Campos Gerais and Varginha-Guaxupé complexes and with the lithological features of the Carrancas and Andrelândia Groups resulted in the negligible and low levels of potential vulnerability. Further research revealed that the grazing area is associated to removal of native vegetation, which leads to changes in the soils characteristics. Thus, groundwater resources potential vulnerability to contamination of this class was in the moderate level (89.03%).

5) The agroforest class of the study area (0.77% of the total) was mapped and inserted in two hydrogeological domains: The Andrelândia Group – kyanite-garnet garnulites Lower Unit, with more resistant and impermeable rocks, and on the Varginha-Guaxupé Complex – migmatitic paragneissic Upper Unit, with good permeability and secondary porosity. As far as soil classes are concerned, agroforest occurs on dystrophic Red Latosol and dystrophic Red-Yellow Latosol in gently undulating and undulating slopes far from geological faults and with medium hydrogeological productivity.

In the first overlapping, it was verified that this culture presents negligible to low groundwater resources potential vulnerability to contamination due to the predominance of more resistant and impermeable rocks and of clayey soils. In the second overlapping the moderate potential vulnerability level was reached because it is in gently undulating and undulating slopes, which favors infiltration and percolation of possible contaminants.

Now, in the third and fourth overlappings, potential vulnerability to contamination was kept in the moderate level (0.94%), the presence of this culture being irrelevant in the high (0.38%) and extreme (0.76%) levels.

Native vegetation present in the area (7.36%) is found in small fragments. This class occurs in all hydrogeological domains, soil classes, and slope degrees, and low and medium classes of hydrogeological productivity, dissociated from geological faults. In this class, the overlappings showed that 96.65% of groundwater resources potential vulnerability to contamination is low.

6) Despite waterbodies being strictly related to geological faults, which may influence the drainage and the infiltration of contaminants resulting from human activities, the Furnas Hydroelectric Reservoir and other waterbodies (12.22% of the total) were not considered because they presented negligible groundwater resources potential vulnerability to contamination of, respectively, 67.32% and 32.68%.

CONCLUSIONS

The multicriteria analysis based on GIS as integration tool of selected variables showed to be efficient for environmental planning and management.

Alfenas Municipality presents groundwater resources potential vulnerability to contamination in the extreme level (23.38%), which results from a combination of: 1) perennial and temporary crops, 2) soils with medium clayey texture, and 3) good water storage and lithological structure transmission capacity. These factors, in combination with gently undulating and undulating slopes, with medium hydrogeological productivity and proximity to geological faults contributed with the perennial and temporary crops classes to increase potential vulnerability.

Areas in the moderate level (51.16% of the total) of groundwater resources potential vulnerability to contamination are associated to basement crystalline rocks, which, in general, present features that prevent water infiltration and percolation. Low (10.25%) and negligible (11.73%) potential vulnerability areas

are associated with undulating slopes, far from geological faults, with low hydrogeological productivity, and land use and coverage which present low potential of groundwater resources to contamination, as, for instance, grazing and agroforest areas.

Assessment of groundwater resources potential vulnerability to contamination, in Alfenas - MG, by the multicriteria analysis of physical variables and human activities was effective in generating primary and secondary knowledge about the subject. Therefore, the mapping of potential vulnerability allows planning actions in order to adjust, mitigate and manage environmental passives in areas of extreme and high potential vulnerability.

ACKNOWLEDGEMENTS

The authors are thankful to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarships.

REFERENCES

Ab'Saber, A. N. (1975): Formas de relevo: texto básico. 80p. São Paulo: Edart.

Alfenas, Prefeitura Municipal de Alfenas. 2006. Plano Diretor de Alfenas. 189p. Alfenas: Prefeitura Municipal.

Cereda Junior, A., Röhm, S. A. (2014): Analysis of Environmental Fragility Using Multi-Criteria Analysis (MCE) for Integrated Landscape Assessment. Journal of Urban and Environmental Engineering 8(1): 28-37.

Companhia de Pesquisa de Recursos Mineirais - CPRM, Universidade Federal do Rio de Janeiro - UFRJ (2010): Cartas Geológicas do Programa de Geologia do Brasil. Folhas Geológicas de Alpinópolis (SF-23-V-B-IV), Machado (SF-23-V-D-V), Alfenas (SF-23-V-D-II), Guapé (SF-23-V-B-V), Campo Belo (SF-23-V-B-VI), Nepomuceno (SF-23-V-D-III) e Varginha (SF-23-V-D-VI). Escala: 1:100.000. Brasília: CPRM.

Companhia de Pesquisa de Recursos Mineirais – CPRM. (2007): Mapa de domínios e subdomínios hidrogeológicos do Brasil. Escala: 1: 2.500.000. Projeto SIG de Disponibilidade Hídrica do Brasil (SDHB). Brasília: CPRM.

Costa, W. C., Cançado, C., Moraes, M. P., Marangon, F., Guerrero, J. V. R., Lorandi, R., Lollo, R., Moschini, L. E. (2015): Potencial de Contaminação de Aquíferos da Bacia do Ribeirão do Meio – Município de Leme – SP. Revista Brasileira de Recursos Hídricos 20(1): 218-225.

Cutrim, A. O., Campos, J. E. G. (2010): Avaliação da vulnerabilidade e perigo à contaminação do aquífero Furnas na cidade de Rondonópolis (MT) com aplicação dos métodos GOD e POSH. Rio Claro: Geociências, 29(3): 401-411.

Ducci, D., Sellerino, M. (2013): Vulnerability mapping of groundwater contamination based on 3D lithostratigraphical models of porous aquifers. Science of Total Environmental 447: 315-322.

Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA (2013): Sistema Brasileiro de Classificação de Solos. 3.ed. 412p. Rio de Janeiro: EMBRAPA.

Environmental Systems Research Institute – ESRI (2014): ArcGIS for the desktop 10.3.1.

Fundação de Pesquisa e Assessoramento à Indústria – FUPAI (2013): Plano Diretor de Recursos Hídricos da Bacia Hidrográfica do Entorno do Lago de Furnas: Relatório 1 – Diagnóstico da Bacia Hidrográfica. 305p. Itajubá: FUPAI.

Foster, S., Hirata, R., Andreo B. (2013): The aquifer pollution vulnerability concept: aid or impediment in promoting groundwater protection? Hydrogeology Journal 21: 1389-1392.

Hasui, Y. (2010): A grande colisão pré-cambriana do Sudeste Brasileiro e a estruturação regional. Rio Claro: Geociências 29(2): 141-169.

Instituto Brasileiro de Geografia e Estatística (IBGE). 1971. Cartas Topográficas: Folhas Topográficas de Alfenas (SF-23-I-I-3), Areado (SF-23-V-D-I-4), Campestre (SF-23-V-D-IV-2), Campos Gerais (SF-23-V-D-I-4), Conceição da Aparecida (SF-23-V-D-I-2) e Machado (SF-23-I-III-1). Escala 1:50.000.

Instituto Brasileiro de Geografia e Estatística - IBGE (2015): Estimativas da População Residente nos Municípios Brasileiros com Data de Referência em 1° de julho de 2015.

Instituto Brasileiro de Geografia e Estatística - IBGE (2012): Produção Agrícola Municipal, v.38, 97p. Rio de Janeiro: IBGE.

Löbler, C. A., Terra, L. G., Silva, J. L. S. 2014. Dados da CPRM/SIAGAS e imagens SRTM como base cartográfica na elaboração de mapeamento em recursos hídricos subterrâneos em escala municipal: o caso de Nova Palma, RS. Pernambuco: Revista Brasileira de Geografia Física 7(3): 513-523.

Löbler, C. A., Silva, J. L. S., Martelli, G. V., Ertel, T. (2013): Pontos Potenciais de Contaminação e Vulnerabilidade Natural das Águas Subterrâneas do Município de Restinga Seca – RS. Pernambuco: Revista Brasileira de Geografia Física 6(3): 500-509.

Miranda, E. E. (2005): Brasil em Relevo. Campinas: Embrapa Monitoramento por Satélite. Available: <http://www.relevobr.cnpm.embrapa.br> Acess in: December 20 of 2015.

Moura, A. C. M. (2014): Geoprocessamento na Gestão e Planejamento Urbano. 3.ed. 312p. Rio de Janeiro: Interciência.

Peternel, R., Trouw, R. A. J., Schmitt, R. S. (2005): Interferência entre duas faixas móveis Neoproterozoicas: O caso das Faixas Brasília e Ribeira no Sudeste do Brasil. São Paulo: Revista Brasileira de Geociências 3(35): 297-310.

Ribeiro, M. L., Lourencetti, C., Pereira, S. Y., Marchi, M. R. R. (2007): Contaminação de águas subterrâneas por pesticidas: avaliação preliminar. São Paulo**:** Química Nova 30(3): 688-694.

Ribeiro, A., Andreis, R. R., Trouw, R. A. J., Paciullo, F. V. P., Valença, J. G. (1995): Evolução das bacias proterozóicas e o termo-tectonismo brasiliano na margem sul do Cráton do São Francisco. São Paulo: Revista Brasileira de Geociências 24(4): 235-248.

Santos, A. A. (2010): Geoprocessamento aplicado à identificação de áreas de fragilidade ambiental no parque estadual da Serra do Rola Moça. (Especialização em Geoprocessamento) 39p. Belo Horizonte: UFMG.

Santos, A. P., Rocha, S. F., Abreu, M. V. S., Calijuri, M. L., Santos, P. M. (2012): O uso da análise multicritério no mapeamento da fragilidade social da área urbanizada do Município de Viçosa – MG. Rio de Janeiro:Revista Brasileira de Cartografia 64(5): 635-643.

Sparovek, G., Lier, Q. J. V., Dourado Neto, D. (2007): Computer assisted Köppen climate classification: case study for Brazil. Internacional Journal of Climatology 27(2): 257-266.

United States Geological Survey (USGS). Landsat_8. Scene: LC82190752015314LGN00. Available: <http://earthexplorer.usgs.gov/> Acess in: January 23 of 2014.

Universidade Federal de Viçosa (UFV); Fundação Centro Tecnológico de Minas Gerais (CETEC); Universidade Federal de Lavras (UFLA) e Fundação Estadual de Meio Ambiente (FEAM). (2010): Mapa de Solos do Estado de Minas Gerais. 49p. Belo Horizonte. FEAM, Available: <http://www.feam.br/noticias/1/949-mapas-de-solo-do-estado-deminas-gerais>. Acess: June 03 of 2014.

Valle Junior, R. F., Varandas, S. G. P., Fernandes, L. F. S., Pacheco, F. A. L. (2015): Multi Criteria Analysis for the monitoring of aquifer vulnerability: A scientific tool in environmental policy. Environmental Science Polici 48: 250-264.

DOI: 10.17707/AgricultForest.63.1.26

Miomir JOVANOVIĆ, Miljan JOKSIMOVIĆ, Ljiljana KAŠĆELAN and Aleksandra DESPOTOVIĆ [1]

CONSUMER ATTITUDES TO ORGANIC FOODS: EVIDENCE FROM MONTENEGRIN MARKET

SUMMARY

This paper examines the general attitude of consumers towards organic agriculture products. A polling questionnaire with questions on consumer attitudes on organic food based on Likert scale was used for the purpose of analysing the attitudinal structure of respondents. The results obtained were analysed and statistically processed using the descriptive statistics methods as well as SPSS program. The respondents' attitude is that organic food implies no risk to human health. At the same time, consumers do not have a defined attitude on whether organic food production risks are higher than its benefits. The respondents are of the opinion that domestic organic food price is high and that it is more expensive than conventionally produced food. Furthermore, in opinion of 48.6% of respondents, imported organic food is cheaper than organic food from domestic producers. More than 50% of respondents are of the opinion that domestic producers do not have a wide range of organic products and 73.6% of respondents state that domestic producers do not produce sufficient quantities of organic food that would meet the demand of consumers. Although respondents agree that the supply of organic products is not sufficient, 81.4% are not ready to pay - in their view - high price of organic products. On average, consumers agree with the attitude that further promotion effort is needed as well as larger areas under organic farming (90% of consumers). With a high interest in consumption of products grown in line with organic farming principles, the number of respondents who fully agree that they would like to engage in organic food production is significant (41.4%).

Keywords: *Preferences, Attitudes, Consumer, Organic foods, Likert-scale*

INTRODUCTION

Organic food production combines traditional and innovative food production methods with modem marketing principles. Organic production of foods is not the only, but it is the most far-reaching of currently applied measures to increase the sustainability of food production (Thøgersen, J., 2009). Demand

[1]Miomir Jovanović, Miljan Joksimović, Aleksandra DESPOTOVIĆ (corresponding author: alexd@t-com.me), Biotechnical Faculty, Department for Agro-Economic Research, University of Montenegro, MIhaila Lalića 1, 81000 Podgorica, MONTENEGRO, Ljiljana Kašćelan Faculty of Economics, Podgorica, MONTENEGRO

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

for organic food has increased considerably over the past decade or so, particularly in West European Countries. Denmark stands out as the country with the highest increase in demand of these products per capita worldwide (Wier, M., and Calvery, C., 2002). However, share of organic products consumption in overall food consumption still remains at a relatively low level (Wier, M., Mette, 2003).

Numerous researches have been conducted worldwide as regards stances and motives of consumers towards organic products. Aspects from which attitudes of consumers towards organic products can be viewed are numerous. Apart from economic factors, the health aspect is also quite important. According to Padel, S., & Foster, C., (2005) most consumers associate organic at first with vegeTables and fruit and a healthy diet with organic products. Organic food buyers considered themselves more responsible for their own health and were more likely to undertake preventive health action than the general population. Organic food consumption is part of a way of life (Schifferstein, H., and Peter AM Oude Ophuis, 1998). Food consumption in most developed countries has attained a saturation point in quantity terms, and consumer food choices are broader than in the past. In this saturated market environment, distribution channels, marketing activities, diversification strategies, and food quality are increasingly important. In addition, consumers have become more concerned about the nutrition, health, and quality of food they eat (Gil et al., 2000). Greek consumers seem to be informed about environmental and health issues. They seek information about the nutritional value of food and demand more products free from chemical residues (Tsakiridou, E., Boutsouki, C., Zotos, Y., & Mattas, K., 2008).

The organic and broader food industries must better understand the variety of motivations, perceptions, and attitudes consumers hold regarding organic foods and their consumption if their own long-term interests, as well as those of other of food marketing, are to be best served (Hughner, R., et al. 2007). Croatian consumers consider organically-grown products as very healthy, of good quality and tasty. However, these products are perceived as rather expensive and of questionable appearance (Radman, M., 2005). Consumers' attitudes towards health and environmental benefits provided by organic foods are the most important factors explaining, both, the intention to purchase and the final decision. Finally, income and organic knowledge positively influences the final decision to buy organic food products (Gracia, A., & de Magistris, T., 2013). Surveys on consumer attitudes toward organic products show different results among countries. In the United Kingdom, it has been established that a small part of the population has only the slight notion of what organic agriculture is (4% have never heard of organic production) and support the assertion that citizens in the United Kingdom connect organic products more with human health and nutrition and less with environmental factors (Beharrell, B., & MacFie, J. H., 1991). Food familiarity aspect was negatively associated with organic food purchases. Females and young people buy organic foods on a regular basis, as do

the more politically liberal and moderately religious (Onyango, B. M., Hallman, W. K., & Bellows, A. C., 2007).

Montenegrin organic food market is still emerging. Although the volume of organic production in Montenegro is still at a quite modest level, it is realistic to expect the upward trend, judging by the number of producers entered into the Register of Organic Agricultural Production Entities. The increase of area in plant production is noTable (around 420 hectares), with field and vegeTable crops almost equally represented. The area from which organic medicinal herbs are collected is above 143,400 hectares, while only three producers are engaged in organic livestock farming. For the time being, domestic produces are able to "find" the buyers and market the food produced in Montenegro. Most of organic food is delivered to hotels and restaurants. It is also marketed in health food stores and partly through grocery markets. In general, organic farming provides significant opportunities for further economic and rural development of Montenegro (Jovanović., M, Despotović, A., 2012). However, taking into account the overall consumption of agricultural-food products in Montenegro, import of agricultural-food products had a more dynamic growth than export (Jovanović, M and Despotović, A., 2014). The situation in Montenegro is generally characterised by continuous lack of co-operation between food producers and tourism industry in particular, which could be improved at the level of the regulator (state) and producers (Jovanović, M., 2004). Income is the dominant variable in consumer behavior and income growth necessarily brings changes of consumption structure and consumption level (Jovanovic, M., 2016). The objective of this research was to determine consumer attitudes for organic foods in Montenegro.

MATERIAL AND METHODS

Questionnaire surveys were conducted in 2015. The survey was implemented in the municipalities: Podgorica, Nikšić, Kolašin, Mojkovac, Pljevlja, Berane and Bar. A polling questionnaire was used with questions on consumer attitudes on organic farming and organic food. It was designed as a two-part questionnaire: the first part consists of a set of questions concerning the health aspect of organic food production and consumption, while the second part includes questions concerning the economic aspect of consumers to organic farming products.

Likert scale was used for analysing the attitudinal structure of respondents (Vagias, Wade M., 2006), in order to determine the extent of agreement or disagreement of respondents with an assertion on a continuum from absolutely positive to absolutely negative attitude towards the topic of research interest. On the assessment scale, the respondent presented stances concerning, primarily, agreement. In our case, the answers offered are divided into five attitudes: 1. Strongly agree, 2. Agree, 3, Neither agree nor disagree, 4. Disagree and 5. Strongly disagree. The survey was conducted in December 2015. It was carried out by trained survey takers. The survey included students respondents above 18

years of age. On a sample N=140 respondents, metric properties of the scale were assessed. The scale reliability is high. Results obtained were analysed and statistically processed using the descriptive statistics methods and *SPSS* program.

RESULTS AND DISCUSSION

The obtained results of the survey concerning the consumer attitude on health safety of organic food consumption show as statistically significant a group of respondents of 42.9% who strongly agree with the assertion that organic food implies no **risk to human health**. Furthermore, 42.1% of respondents agree with the assertion that organic food implies no risk to human health (Table 1- Q1). Analysis of the median value measured shows that 85% of the population polled has a positive attitude as regards health safety of organic food consumption which makes them a statistically significant category (Table 2 - Q1).

As regards the previously defined assertion on health aspect of organic food consumption, attitudes are strongly divided with regard to consumers' attitude concerning the **organic food production risks.** Review of the values of the results obtained shows as two statistically non-significant categories consumers who strongly agree, only 6.4% and consumers that strongly disagree 11.4%. Namely, 82.1% of respondents have almost equally divided opinion and among them the statistically significant group within the disagree category is that **organic food production risks are higher than benefits** of 32.1% (Table 1- Q 2). Values of statistical analysis of arithmetic mean and median in this particular case show that consumers actually do not have a formed opinion on whether the organic food production risks are higher than benefits. However, the most frequently given answers were that consumers disagree with the assertion, which substantiates the modus value (Table 2 - Q 2).

Whether organic food consumption is just a **fashion trend**, is a question that gave quite divided opinions. Of the total population surveyed, statistically significant groups are consumers that disagree that organic food consumption is a fashion trend – 32.9%, while 27.1% agree with the assertion (Table 1- Q3). However, taking into account the descriptive statistics values, the median value practically shows that, on average, consumers do not have specific attitude towards this assertion. Furthermore, the modus value shows that consumers most frequently responded that they disagree with the stance that organic food consumption is a fashion trend (Table 2- Q 3).

Of the total number of respondents, 65.7% of consumers stated their view that they strongly agree or agree with the stance that **organic food is genetically modified**, while 34.3% of respondents are undecided or disagree with the assertion. Thus, consumers who agree with the assertion that organic food is genetically modified, form quite a significant group (Table 1- Q4). Values of the statistically processed results obtained show that on average and at the highest frequency consumers gave the response that they agree with the offered assertion that organically produced food is genetically modified (Table 2 - Q4).

Table 1. Questions and answers according to Likert-scale

	QUESTION (Q)	Strongly agree	Agree	Neither agree or disagree	Disagree	Strongly disagree	Frequency
1.	Consumer attitudes on whether organic food implies risk to human health	**60**	**59**	**8**	**10**	**3**	**140**
		42.9	42.1	5.7	7.1	2.1	100%
2.	Consumer attitude on whether organic food production risks are higher than benefits	**9**	**39**	**31**	**45**	**16**	**140**
		6.4	27.9	22.1	32.1	11.4	100%
3.	Consuming organic food as a fashion trend	**27**	**38**	**23**	**46**	**6**	**140**
		19.3	27.1	16.4	32.9	4.3	100%
4.	Is organic food genetically modified	**35**	**57**	**33**	**13**	**2**	**140**
		25.0	40.7	23.6	9.3	1.4	100%
5.	Organic food is not treated with chemical products	**40**	**59**	**22**	**13**	**6**	**140**
		28.6	42.1	15.7	9.3	4.3	100%
6.	Mineral fertilizers are not used in organic food production	**35**	**41**	**34**	**26**	**4**	**140**
		25.0	29.3	24.3	18.6	2.9	100%
7.	Organic food has more nutritive elements than conventionally produced food	**51**	**51**	**28**	**10**	**-**	**140**
		36.4	36.4	20.0	7.1	-	100%
8.	Should the share of organic food in sales facilities be higher	**80**	**44**	**9**	**5**	**2**	**140**
		57.1	31.4	6.4	3.6	1.4	100%
9.	Is the price of organic food in Montenegro high	**65**	**34**	**18**	**16**	**7**	**140**
		46.4	24.3	12.9	11.4	5.0	100%
10.	Is the quality of imported organic food higher than that of organic food produced by domestic producers	**4**	**10**	**31**	**41**	**54**	**140**
		2.9	7.1	22.1	29.3	38.6	100%
11.	Is organically produced food more expensive than conventionally produced food	**66**	**42**	**10**	**13**	**9**	**140**
		47.1	30.0	7.1	9.3	6.4	100%
12.	Organic food from imports is cheaper than the organic food of domestic consumers	**18**	**50**	**39**	**27**	**6**	**140**
		12.9	35.7	27.9	19.3	4.3	100%

13.	Domestic producers do not have a wide range of organic products	**28**	**74**	**18**	**12**	**8**	**140**
		20.0	52.9	12.9	8.6	5.7	100%
14.	Domestic producers do not produce sufficient quantities of food to meet the needs of consumers in Montenegro	**42**	**61**	**20**	**14**	**3**	**140**
		30.0	43.6	14.3	10.0	2.1	100%
15.	Consumers are not ready to pay the high price of organic products in sales facilities	**50**	**64**	**11**	**9**	**6**	**140**
		35.7	45.7	7.9	6.4	4.3	100%
16.	Organic products need to be promoted more	**67**	**59**	**12**	**1**	**1**	**140**
		47.9	42.1	8.6	0.7	0.7	100%
17.	Larger agricultural area should be under organic farming	**81**	**45**	**8**	**4**	**2**	**140**
		57.9	32.1	5.7	2.9	1.4	100%
18.	Does Montenegro have good conditions for organic food production?	**86**	**37**	**12**	**4**	**1**	**140**
		61.4	26.4	8.6	2.9	0.7	100%
19.	Would you like to engage in organic food production	**58**	**40**	**23**	**12**	**7**	**140**
		41.4	28.6	16.4	8.6	5.0	100%

Source: own calculation

The tabular overview below shows that the whole of 70.7% of consumers strongly agree or agree with the assertion that **organic food is treated with organic products** (Table 1- Q 5). Overall, the most significant and most frequent consumer group consists of consumers with the attitude of agreeing that organically produced food is not treated with chemical products, which is shown by the descriptive statistics values measured (Table 2- Q5).

Attitudes as regards **use of mineral fertilizers** in organic food production are positive among more than 50% of consumers; 29.3% of respondents agree with the assertion, while 25% strongly agree. However, almost half of the respondents does not share this view on the assertion, as the whole of 45.7% are undecided or disagree with the assertion (Table 1- Q6). Results obtained support the group of consumers agreeing with the assertion, which was also the most frequently selected response in this case. The whole of 72.9% of respondents strongly agree or agree with the assertion that organic food has **more nutritive elements** than conventionally produced food. The remaining 27.1% of respondents are undecided. In this case, there were no disagreements with the

assertion (Table 1- Q7). Since respondents' attitudes have been evenly distributed along the responses offered – agree or strongly agree - the median value has not been confirmed. On average, respondents agree with the assertion that organic food has more nutritive elements than conventionally produced food (Table 2 - Q 7). Consumers' attitudes as regards **availability** of organic products in sales facilities are in a statistically significant percentage positive and confirmatory with 88.6%. The most frequent responses in this case were strongly agree with the assertion given (Table 1- Q8).

Results of this survey and respondents attitudes show that **organic food price** in Montenegro is high. Thus, 46.4% of respondents strongly agree with the assertion on high price of organic food while 24.3% stated that they agree with the assertion (Table 1- Q9). In the majority of repetitions, respondents stated they agreed with the assertion, while on average, statistically significant group was formed of respondents that strongly agree with the assertion.

Since in overview of results obtained above attitudes were focused on agreement with the assertion, the assertion analysing the view of respondents on the **quality of organically produced** food by domestic producers and organically produced products from imports are opposite. The whole of 38.6% of respondents strongly disagrees with the assertion, while 51.4% of respondents either disagrees or are undecided (Table 1- Q10). On average, respondents replied that they disagreed with the assertion; however, the most frequent responses were given in the group of overall disagreement with the assertion.

In respondents view, more than 50% have the perception that **organic food is more expensive than conventionally produced food**; 47.1% strongly agrees with the assertion, while 30% agrees (Table 1- Q11).

Respondents whose attitude is also statistically significant most frequently responded that they strongly agree with the given assertion.

Organic **food from imports** is cheaper than organic food of domestic producers in opinion of 48.6% of respondents, while 23.6% disagree with this attitude, with a significant number of undecided (Table 1- Q12).

Respondents who are undecided as regards this assertion also gave the highest number of such responses, while respondents who agree with the assertion form a statistically significant group.

More than 50% of respondents agree that domestic producers do not have a wide **range of organic products**. This is confirmed also by the attitude of consumers who strongly agree with the assertion - 20% (Table 1- Q13).

Along with the higher frequency of responses that agree with the assertion, on average, respondents shared the same opinion. Producers in Montenegro do not produce **organic food quantities** that would meet the demand of consumers is the attitude of 73.6% of respondents; 14.3% are undecided and 10% disagree with the assertion (Table 1- Q14).

However, on average consumers agree that organic food production in Montenegrin market is not sufficient to meet the domestic needs. Although consumers agree that sales facilities in Montenegro do not have sufficient

quantity of organic products in their supply, 81.4% of them **are not ready to pay - in their view - high price of organically produced products** (Table 1- Q15). Values of both median and modus confirm that they agree with the assertion given and in most cases the responses were such (Table 2- Q15). Consumers opinion that organic products need to be **promoted more** is prevailing, which is confirmed by attitudes of 90% of respondents (Table 1- Q16). On average, consumers agree with the stance that they need to be promoted more, while in majority of cases they strongly agree with the assertion. Consumers agree that prices of organically produced products are high and that the products need to be promoted more, they also believe that **larger agricultural area** should be under organic farming – 90% of respondents (Table 1- Q17). In this particular case, the stance is accepted both on average and in the highest number of repetitions. This analysis is confirmed also by the stance Montenegro has good **conditions for organic food production**. The percentage of respondents who strongly agree is 61.4% and those who agree 26.4% (Table 1- Q18). Statistical analysis elements show strong agreement with the assertion. Considering the interest in consumption of organically produced products, a very significant number of respondents strongly agree that they **would like to engage in organic food production** – 41.4% of them. However, the share of undecided is not small – 16.4% (Table 1- Q19).

Tables 2 and 2a. Descriptive statistics

	Q1	**Q2**	**Q3**	**Q4**	**Q5**	**Q6**	**Q7**	**Q8**	**Q9**	**Q10**
Mean	1.41	3.08	2.56	1.96	1.90	2.20	1.61	1.04	1.88	3,91
Std. Error of Mean	.117	.108	.129	.112	.121	.126	.114	.110	.116	,098
Std. Deviation	1.383	1.281	1.528	1.322	1.436	1.490	1.344	1.305	1.375	1,162
Variance	1.912	1.641	2.334	1.747	2.062	2.219	1.807	1.704	1.892	1,351
	Q11	**Q12**	**Q13**	**Q14**	**Q15**	**Q16**	**Q17**	**Q18**	**Q19**	
Mean	1,51	2,54	2,07	1,81	1,62	1,16	1,00	,94	1,66	
Std. Error of Mean	,138	,109	,113	,117	,120	,100	,108	,107	,134	
Std. Deviation	1,638	1,294	1,339	1,383	1,422	1,185	1,275	1,265	1,586	
Variance	2,683	1,675	1,793	1,912	2,021	1,404	1,626	1,600	2,515	

Respondents agree with the assertion in most cases, and also, on average, they provided similar answers. Analysis of the results obtained implies also the examination of correlation between certain questions. In difference to examination of correlation between a larger number of questions, for example, questions No. 1 and 7, or 18 and 19, significant correlation was not established,

while between questions 8 (*The share of organic food in sales facilities should be higher*) and 12 (*Organic food from imports is cheaper than organic food of domestic producers*), the correlation was confirmed and was .298** (Table 3-5).

Table 3: Correlations between 1 and 7

		Question _1	**Question _7**
Question _1	Pearson Correlation	1	-,072
	Sig. (2-tailed)		,397
	N	140	140
Question _7	Pearson Correlation	-,072	1
	Sig. (2-tailed)	,397	
	N	140	140

Table 4: Correlations between 8 and 12

		Question _8	**Question _12**
Question _12	Pearson Correlation	,298**	1
	Sig. (2-tailed)	,000	
	N	140	140
Question _8	Pearson Correlation	1	,298**
	Sig. (2-tailed)		,000
	N	140	140
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 5: Correlations between 18 and 19

		Question _18	**Question _19**
Question _18	Pearson Correlation	1	-,036
	Sig. (2-tailed)		,670
	N	140	140
Question _19	Pearson Correlation	-,036	1
	Sig. (2-tailed)	,670	
	N	140	140

CONCLUSIONS

Analysis – examination of consumer attitudes towards products from organic agriculture has not been done before using Likert scale and *SPSS* program. The objective of this paper was to determine the attitude of consumers towards these products both from the health and the economic aspects. The results obtained show a positive attitude towards health safety of organic food consumption by 85% of the population included in the survey, which makes them a statistically significant category (Padel, S., & Foster, C., 2005). Values of statistical analysis of arithmetic mean and median in this particular case show that consumers actually do not have a defined attitude on whether organic food production risks are higher than benefits. Taking into account the values of descriptive statistics, the median value practically shows that on average, consumers do not have a defined attitude on this assertion. Also, modus value shows that consumers most frequently responded that they disagreed with the

assertion that organic food consumption is a fashion trend (Schifferstein, H., and Peter AM Oude Ophuis, 1998; Gil et al., 2000; Tsakiridou, E. et al., 2008).

Values of results obtained from statistical processing show that on average and most frequently consumers responded that they agreed with the assertion that organically produced food is not treated with chemical means, which is shown by descriptive statistics values measured. On average, respondents agree with the assertion that, compared to the conventional food production principle, organic food contains more nutritive elements. The attitude of respondents regarding the share of organically produced products in sales facilities is positive and confirmatory in a statistically significant percentage (88.6%). The most frequently given response in this case was strongly agree with the assertion.

Results of this survey and attitude of respondents show that the price of organic food in Montenegro is high. Thus, 46.4% of respondents strongly agree with the assertion on high prices of organic food, while 24.3% stated that they agree (Radman, M., 2005). Since in overview of results obtained above attitudes were focused on agreement with the assertion, the assertion analysing the view of respondents on the quality of organically produced food by domestic producers and organically produced products from imports are opposite. Namely, 38.6% of respondents strongly disagree with the assertion while 51.4% of respondents either disagree or are undecided. The view of more than 50% of respondents is the perception that organic food is more expensive than conventionally produced food. The total of 47.1% strongly agrees with such a stance while 30% agrees.

Organic food from imports is cheaper than organic food produced by domestic producers in opinion of 48.6%, while 23.6% disagrees with this assertion and a significant share of respondents is undecided.

More than 50% of respondents agree that producers do not have a wide range of organic products. Producers in Montenegro do not produce organic food in quantities that would meet the demand of consumers is an attitude shared by 73.6% of respondents; 14.3% are undecided while 10% disagrees with the assertion.

Although consumers agree that quantities of organic products in supply in sales facilities in Montenegro are not sufficient, 81.4% of them are not ready to pay what in their view is a high price of organically produced products (Gracia, A., & de Magistris, T., 2013).

On average, consumers agree with the assertion that the organic products need to be promoted more, while they most frequently strongly agree with the assertion (Beharrell, B., & MacFie, J. H., 1991). Since consumers agree that the prices of organically produced products are high and should be promoted more, they also consider that larger agricultural area should be under organic crops – this view is shared by 90% of consumers. This analysis is confirmed by the attitude that Montenegro has good conditions for organic food production. With this attitude 61.4% of respondents fully agree, 26.4% agree. Considering the interest in consumption of products produced in line with the organic production principles, a significant number of respondents strongly agree that they would

like to engage in organic food production – 41.4%. However, the share of undecided on this issue is not small (16.4%).

Determining consumer attitudes under the circumstances of an increasing overall supply of these products is aimed at ensuring better positioning of domestic consumers based on better understanding of organic products consumers. In major and traditional organic-making countries consumption of organic products is fairly predicTable as it is mainly driven by designations of origin. In emerging markets, the commercial strategies are, for the most part, based on certain specificities and are quite interesting for surveys.

REFERENCES

Beharrell, B., & MacFie, J. H. (1991). Consumer attitudes to organic foods. *British Food Journal*, *93*(2), 25-30.

Gil, J. M., Gracia, A., & Sanchez, M. (2000). Market segmentation and willingness to pay for organic products in Spain. *The International Food and Agribusiness Management Review*, *3*(2), 207-226.

Gracia, A., & de Magistris, T. (2013). Organic food product purchase behaviour: a pilot study for urban consumers in the South of Italy. *Spanish Journal of Agricultural Research*, *5*(4), 439-451.

Hughner, Renée Shaw, et al. (2007), "Who are organic food consumers? A compilation and review of why people purchase organic food." Journal of consumer behaviour 6.2-3: 94-110.

Jovanovic, M., (2016): Empirical analysis of income changes impact on food consumption expenditure. Agriculture and Forestry, 62 (3): 49-56. DOI:10.17707/AgricultForest.62.3.04

Jovanović., M, Despotović, A., 2012: The analysis of socioeconomic conditions for organic production in Montenegro, Economics of Agriculture 2/2012, UDC: 631.147:339.13(497.16), EP 2012 (59) 2 (207-216).

Jovanović, M., Despotović Aleksandra, 2014: Analysis of position of agro-food trade in Montenegro's foreign trade. Agriculture & Forestry, vol. 60. ISSUE 1: 235-243, Podgorica, UDC (UDK) 631.11(497.16) ,

Jovanović, M., 2004: Food Market from the tourism consumption aspect. Agriculture & Forestry, vol. 50. Issue 1-2: 127-137, Podgorica, UDK: 339.133

Onyango, B. M., Hallman, W. K., & Bellows, A. C. (2007). Purchasing organic food in US food systems: A study of attitudes and practice. *British Food Journal*, *109*(5), 399-411.

Padel, S., & Foster, C. (2005). Exploring the gap between attitudes and behaviour: Understanding why consumers buy or do not buy organic food. *British food journal*, *107*(8), 606-625.

Radman, M. (2005). Consumer consumption and perception of organic products in Croatia. *British food journal*, *107*(4), 263-273.

Schifferstein, Hendrik NJ, and Peter AM Oude Ophuis "Health-related determinants of organic food consumption in the Netherlands." Food quality and Preference 9.3 (1998): 119-133.

Tsakiridou, E., Boutsouki, C., Zotos, Y., & Mattas, K. (2008). Attitudes and behaviour towards organic products: an exploratory study. *International Journal of Retail & Distribution Management*, *36*(2), 158-175

Thøgersen, John, 2009. Consumer decision-making with regard to organic food products. Traditional food production and rural sustainable development: A European challenge: 173-192.

Vagias, Wade M. (2006) Likert-type scale response anchors, Clemson International Institute for Tourism & Research Development, Department of Parks, Recreation and Tourism Management. Clemson University

Wier, M. et al. 2003. Consumer preferences for organic foods." Organic agriculture: Sustainability, markets and policies: 257-271.

Wier, M. and C. Calverley, 2002. Market Perspectives for Organic Foods in Europe, British Food Journal, Vol.104, pp.45-62.

DOI: 10.17707/AgricultForest.63.1.27

Laleh PARVIZ, Mahsa PAYMAI [1]

COMPARISON OF THE EFFICIENCY OF CLASSICAL AND FUZZY REGRESSION MODELS FOR CROP YIELD FORECASTING WITH CLIMATOLOGICAL ASPECT

SUMMARY

This paper presents the application of fuzzy concepts in the field of crop yield forecasting. In this regard, classical and fuzzy, using symmetric and non-symmetric triangular fuzzy number coefficients; regression models for wheat and oil seeds yield forecasting were used in Zanjan, West and East Azarbaijan Provinces (1984/2013). The predominance of various climatological parameters was determined using maximum correlation coefficient between climatological parameters and crop yield. The sensitivity analysis of climatological parameters indicated the diversity of climatological parameters in different Provinces. RRMSE criteria decreased 37.76% in symmetric fuzzy regression compare to classical and 15.6% in non-symmetric fuzzy compare to symmetric fuzzy regression. Based on error criteria, fuzzy regression has better performance in relation to the classical regression. There were not major differences between the performance of symmetric and non-symmetric fuzzy regression.

Keywords: *Climatological parameters, Regression, Fuzzy, Performance.*

INTRODUCTION

The knowledge concerning crop yield is one of the most important challenges in recent years since accurate crop yield forecasting is essential for the planning and policy making of related agricultural organizations. Climate variability is one of the most significant factors influencing annual crop production, even in high-yield and high-technology agricultural areas. Therefore, more and more attention has been paid to the risks associated with climate change, which will increase uncertainty with respect to food production (Kang et al., 2009).Crop production depends on climate change therefore, representation the dependency using efficient method is necessary. Some models have been widely applied the dependency of crop production to the climate factors. The first models used for large-scale yield simulation were statistical. Average yields from large areas for many years were regressed on time to reveal a general trend in crop yields (Basso et al., 2013).Furthermore, the most investigated statistical crop-yield-weather models are multivariate regression models. An agro-meteorological crop yield forecasting using a multiple regression was introduced by Gommes (2001) to develop an approach used by the FAO and a number of

[1]Laleh PARVIZ (corresponding author: laleh_parviz@yahoo.com), Mahsa Paymai, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, IRAN

developing countries for crop forecasting that would provide a good compromise between input requirements and ease of validation (Gommes, 2001). However, considering the inherent and irreparable disadvantages of the multiple regression models, such as variable interdependence or multi-collinearity, stringent linearity and normality assumptions, a more scientific methodology to incorporate weather data into crop yield models, is still under exploration, and remains of great importance to the government, and private sector insurers, and reinsurers(de Leona and Jalaob, 2013).Some regression forms of model which are used for crop yield forecasting are mentioned in the following part.

De Leona & Jalaob applied multiple linear regression for corn yield predicting in Quezon Province (de Leona and Jalaob, 2013). They presented new research possibilities for the application of modern classification methodologies to the problem of yield prediction. Four climatic variables such as temperature, solar radiation, rainfall and humidity as well as data about weather disturbance are gathered. 14 agronomic variables related to corn production are gathered. Full attribute set has better performance in corn yield prediction. Their result indicated that corn yield is greatly affected by planting practices; particularly by the application of right amount of fertilization(de Leona and Jalaob, 2013).An artificial neural network (ANN) approach was used to model the wheat production. From an extensive data collection involving 40 farms in Canterbury, New Zealand, the average wheat production was estimated at 9.9 t/ha. The final developed ANN model was capable of predicting wheat production under different conditions and farming systems using direct and indirect technical factors. The final ANN model could predict wheat production based on farm conditions, machinery condition and farm inputs in Canterbury with an error margin of ±9% (±0.89 t/ha)(Safa et al., 2015). Kumar used adaptive neuro fuzzy inference system (ANFIS) technique based on time series of 27 years to forecast rice yield in India (Kumar, 2011). The visual observation based on the graphical comparison between observed and predicted values and the qualitative performance assessment of the model indicates that ANFIS can be used effectively for crop yield forecasting (Kumar, 2011).Kumar and Kumar provided a number of modified techniques for time series based forecasting for the yield of any crop year (Kumar and Kumar, 2012). The study can contribute to the inventory management of wheat yield and management of storage space. They used the data of previous years and proposing a new method by using the fuzzy time series forecasting technique. The research results were remarkably near to the actual annual production. The time series work almost perfectly if there is no such a sudden rise or fall in production (Kumar and Kumar, 2012).

The present study was carried out to develop regression models in order to forecast wheat and oil seeds yield in some Provinces which are located in the north-west of Iran. In this regard, classical and fuzzy regression models were compared for crop yield forecasting. The inapplicability of fuzzy regression for crop yield forecasting in the previous researches is the reason of using fuzzy regression model in this study. Calibration and validation periods were divided in

two parts: (1984/2003) and (2004/2013). Effective meteorological parameters were selected based on the maximum correlation coefficient of climatological parameters and crop yield. Optimization method was used to determine the coefficient of symmetric and non-symmetric membership function.

MATERIALS AND METHODS

Several regression-based methods have been used for crop yield forecasting so that classical and fuzzy regression models were used in this research.

Regression analysis is one of the most widely used methods in yield forecasting and various regression models and techniques have been developed. This technique predicts the response variable, i.e. yield, in terms of explanatory variables such as weather, soil properties, input, and technology (de Leona and Jalaob, 2013).

Classical Regression: Regression analysis is the art and science of fitting straight lines to data patterns. In a linear regression model, the intended variable is predicted from other variables using a linear equation which can be explained such as Equation 1.

$$Y = A_0 + A_1 x_1 + \ldots + A_n x_n \quad (1)$$

Where Y is a dependent variable,x_1, $x_{2,\ldots,}$ x_n are independent variables,A_0,A_1,...,A_n are coefficients of equation.

Fuzzy Regression: In conventional regression analysis, the deviations between observed and estimated values are assumed to be due to random errors. However, most cases, these are due to the indefiniteness of the structure of a system or imprecise observations. Thus, uncertainty in this type of regression model becomes fuzziness and not randomness. Fuzzy linear regression is a fuzzy type of classical regression analysis in which some elements of the model are represented by the fuzzy number.

Fuzzy linear regression was originally introduced by researchers (Asai et al., 1982). They formulated a linear regression model with fuzzy response data, crisp predictor data and fuzzy parameters as a mathematical programming problem. Diamond proposed the approach of fuzzy least squares to determine fuzzy parameters by defining a metric between two fuzzy numbers(Diamond, 1988). However most of the articles on fuzzy regression analysis use the linear programming to estimate the parameters. In this regard, each additional observation results in several additional constraint sand the linear programming problem become unwieldy very quickly, especially if the fuzzy triangular numbers involved are not symmetric(Arabpour and Tata, 2008; Ghosh; Kumar). Modeling fuzzy linear systems has been addressed in fuzzy linear regression analysis. The following model shows the dependence of the output variable on the input variables.

$$\tilde{Y} = \tilde{A}_0 + \tilde{A}_1 x_1 + \ldots + \tilde{A}_n x_n \quad (2)$$

$$(3)\ \tilde{A} = (\tilde{A}_0, \tilde{A}_1, ..., \tilde{A}_n)$$

Where $\tilde{Y}$ is the fuzzy output, $x = [x_1, x_2, ..., x_{n1}]^T$ is the real- valued input vector, $\tilde{A}$ is a set of fuzzy numbers.

Then the regression analysis problem is defined as: given a set of crisp data points (x_1,y_1), (x_2,y_2), …,(x_n,y_n) we find a set of fuzzy parameters $\tilde{A}_0, \tilde{A}_1, ..., \tilde{A}_n$ for which Equation 2 express the best fit to the given data points, according to some criteria of goodness of fit.

If $\tilde{A}_i s$ have triangular membership functions, then each number coefficient $\tilde{A}_i$ can be uniquely defined by Equation 4.

$$\tilde{A}_i = \{a_i^L, a_i^C, a_i^U\} \qquad (4)$$

Where a_i^L is the lower limit, a_i^U is the upper limit and a_i^C is the point having the property of $\mu_{\tilde{A}_i}(a_i^C) = 1$. The property of the symmetry of the fuzzy coefficient $\tilde{A}_i$ enables us to establish the following two relations as Equation 5 and 6.

$$a_i^C = \frac{a_i^L + a_i^U}{2} \qquad (5)$$

$$a_i^S = a_i^C - a_i^L = a_i^U - a_i^C \qquad (6)$$

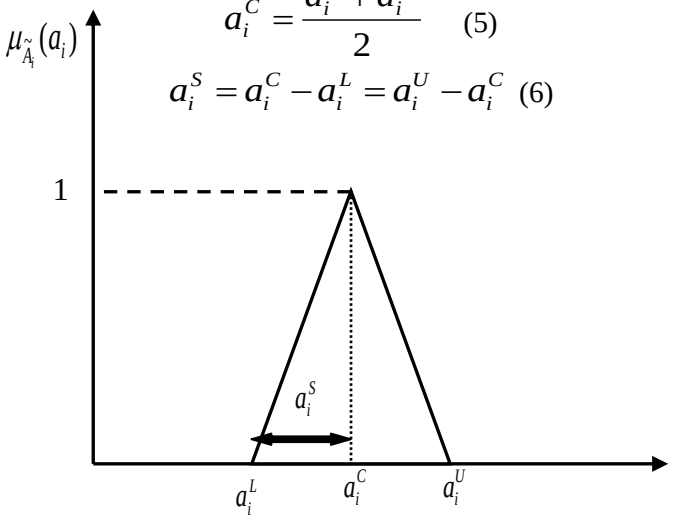


Figure 1. Membership function for the symmetric triangular fuzzy coefficient $\tilde{A}_i$.

Where a_i^C is the center, a_i^S is the spread of $\tilde{A}_i$. Then each symmetric fuzzy number coefficient $\tilde{A}_i$ can be uniquely described by only two parameters (Figure 1), either a_i^C and a_i^S or a_i^L and a_i^U, as $\tilde{A}_i = \{a_i^C, a_i^S\}$ or $\tilde{A}_i = \{a_i^L, a_i^U\}$.

From the above discussion, the fuzzy coefficient set $\tilde{A} = (\tilde{A}_0, \tilde{A}_1, ..., \tilde{A}_n)$ can be represented in a vector form in terms of a_i^C and a_i^S as $\tilde{A} = \{a^C, a^S\}$ where
$a^C = [a_1^C, a_2^C, ..., a_n^C]^T$ and $a^S = [a_1^S, a_2^S, ..., a_n^S]^T$.

The objective of the fuzzy regression method with non-fuzzy data is to determine the parameter $\tilde{A}_i$ so that the fuzzy output set, $\{y_j\}$ is associated with a membership value greater than *h*.

$$\mu_{\tilde{Y}}(y_j) \geq h, \quad j = 1, ..., m \tag{7}$$

Where the value of *h* is chosen for the purpose of generating the best-fitting model.

In this regard, the goal is to find the fuzzy coefficients that minimize the above-mentioned spread of fuzzy output for all the data sets. The cost function, *Z*, to be minimized can be written as equation 8.

$$\begin{aligned} &Objective\ function \quad a_0^S + \sum_{i=1}^{n} a_i^S \sum_{j=1}^{m} |x_{ij}| \\ &Subject\ to \\ &a_0^C + \sum_{i=1}^{n} a_i^C x_{ij} - (1-h)\left[a_0^S + \sum_{i=1}^{n} a_i^S x_{ij} \right] \leq y_j \\ &a_0^C + \sum_{i=1}^{n} a_i^C x_{ij} + (1-h)\left[a_0^S + \sum_{i=1}^{n} a_i^S x_{ij} \right] \geq y_j \end{aligned} \tag{8}$$

Where *y* is a dependent parameter, *x* is an independent parameter, a_i^C is the center and a_i^S the spread of $\tilde{A}_i$, *h* is the confidence level parameter.

Asai and co-workers formulated a linear programming problem (LPP) to determine the fuzzy number coefficients $\tilde{A}_i$ of the fuzzy linear model(Asai et al., 1982). Therefore, the minimization of the objective function in the LPP is equivalent to the minimization of the total fuzziness of the linear model f(x, $\tilde{A}$).

If the triangular, is not symmetric, minimally three parameters are need. For example, $\tilde{A}_i$ can be described by the triplets $\{a_i^L, a_i^P, a_i^U\}$ or by

$\{s_i^L, a_i^P, s_i^R\}$where a_i^p is the point inw hich $\mu_{\tilde{A}_i}(a_i^P)=1$,peak point, s_i^L is the left-side spread from the peak point a_i^p and s_i^R represents the right-side spread as shown in Figure 2.

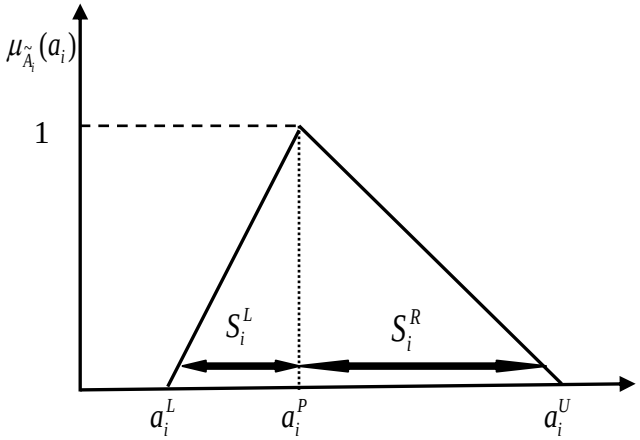


Figure 2. Non-symmetric triangular fuzzy coefficients.

Another representation is also possible, if the spreads are normalized. Since $s_i^L = a_i^P - a_i^L$ and $s_i^R = a_i^U - a_i^P$, spread can be used as the base to normalize the other one. If s_i^L is chosen as the base, then s_i^R can be expressed as $s_i^R = k_i\, s_i^L$ where k_i are the skew factors and are positive real numbers. The cost function, Z in non-symmetric case can be expressed by following equations.

$$\begin{aligned} &\textit{Objective function} \quad (1+k_0)S_0^L + \sum_{i=1}^{n}\left[(1+k_i)S_i^L \sum_{j=1}^{m}\left|x_{ji}\right|\right] \\ &\textit{Subject to} \\ &(1-h)S_0^L + (1-h)\sum_{i=1} S_i^L\left|x_i\right| + \sum_i a_i^P x_i + a_0^P \le y_j \\ &(1-h)k_0 S_0^L + (1-h)\sum_{i=1} k_i S_i^L\left|x_i\right| - \sum_i a_i^P x_i - a_0^P \le -y_i \end{aligned} \tag{9}$$

Where y is a dependent parameter, x is an independent parameter, h is the confidence level parameter, a_i^P is the point in which $\mu_{\tilde{A}_i}(a_i^P)=1$,$k$ is the skew factor, s_i^L is the left-side spread from the peak point a_i^P (Yen et al., 1999).

The sensitivity analysis must be conducted on two parameters of symmetric and non-symmetric membership function of fuzzy regression: confidence level parameter and skew factor.

The main objective of the research is the comparison of classical and fuzzy regression performance in the field of crop yield forecasting. In this regard some criteria were used which their mathematical forms are brought in Equation 10 and 11. The minimum values of criteria are related to the best performance of model.

$$RRMSE = \frac{\sqrt{\frac{1}{n}\sum_{i=1}^{n}(O_i - S_i)^2}}{\overline{O}} \quad (10)$$

$$MRE = \frac{\sum_{i=1}^{N}\left|\frac{O_i - S_i}{O_i}\right|}{n} \quad (11)$$

Where O_i are observation data, S_i are simulation data, *RRMSE* is relative root mean square error and *MRE* is mean relative error.

Case Study: According to the objective of study which is crop yield forecasting based on climatological parameters, forecasting of wheat and oil seeds yield in Zanjan, East and West Azarbaijan Provinces was investigated. The analysis of crop fluctuations due to the impact of climate change is one of the major issues in the mentioned Provinces. The climate of Provinces based on De Marton classification in 1984/2013 periods is semi-arid (De Marton climate index of East Azarbaijan =10.65, West Azarbaijan=14.1, Zanjan=11.83). Figure 3 shows the location of Provinces in Iran.

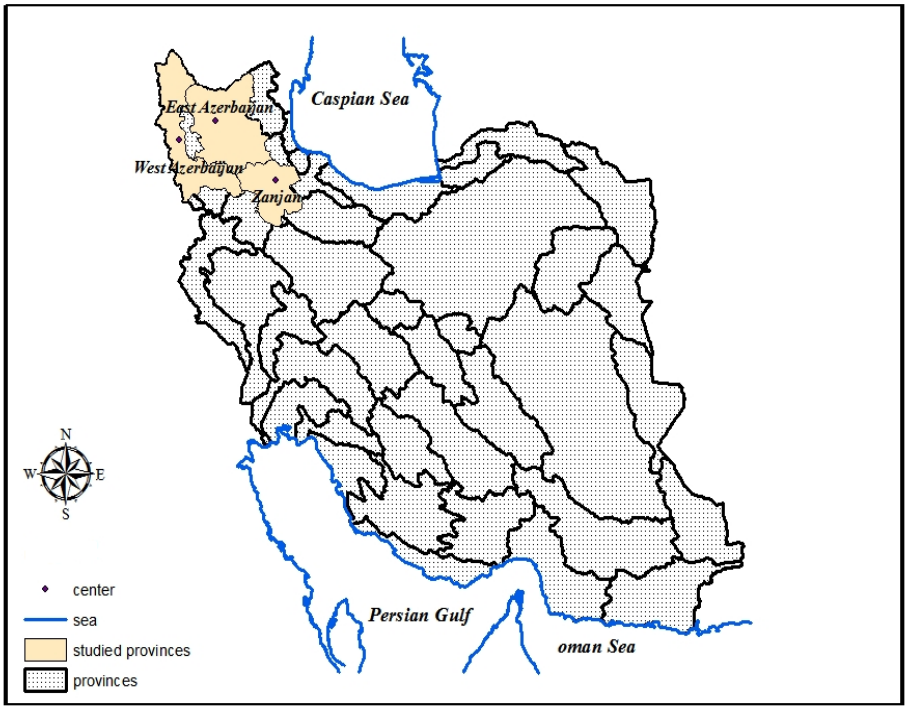


Figure 3. Location of studied Provinces in Iran

RESULTS AND DISCUSSION

Determination the effective climatological parameters: Climatological parameters have a significant impact on crop yield variations. In this research, the investigated climatological parameters to evaluate the wheat and oil seeds yield forecasting are: air temperature, maximum and minimum temperature, wind speed, air pressure, vapor pressure, relative humidity, maximum and minimum relative humidity, precipitation, sunshine hours, number of cloudy days and dew point temperature. The results of the correlation between climatological parameters and crop yield are presented in Table 1.

Table 1. The results of correlation coefficients between climatological parameters and crop yield

Climatological Parameters	Wheat			Oil seeds		
	East Azarbaijan	West Azarbaijan	Zanjan	East Azarbaijan	West Azarbaijan	Zanjan
Temperature	0.49	0.48	0.84	0.52	0.3	0.19
Max Temperature	0.48	0.31	0.84	0.5	0.29	0.2
Min Temperature	0.51	0.5	0.82	0.55	0.32	0.18
Sunshine Hours	0.54	0.29	0.74	0.44	0.19	0.14
Wind Speed	0.55	0.41	0.81	0.59	0.31	0.14
Pressure	0.43	0.45	0.85	0.37	0.16	0.19
Vapor Pressure	0.42	0.33	0.83	0.36	0.05	0.16
Relative Humidity	0.37	0.33	0.84	0.25	0.002	0.17
Max Relative Humidity	0.38	0.28	0.84	0.28	-0.07	0.18
Min Relative Humidity	0.3	-0.015	0.83	0.13	-0.3	0.17
Precipitation	0.11	-0.09	0.68	0.06	-0.2	0.09
Number of Cloudy Days	0	-0.11	0.74	-0.011	-0.3	0.21
Dew Point Temperature	0.34	-0.16	0.42	0.34	-0.19	-0.06

Based on the maximum correlation coefficient between crop yield and climatological parameters, four parameters were selected. In East Azarbaijan Province, the maximum correlation coefficient of wheat yield is related to wind speed, sunshine hours, minimum temperature and air temperature, in addition the maximum correlation coefficient of oil seeds yield is related to wind speed, minimum temperature, air temperature and maximum temperature. In Zanjan

Province, the maximum correlation coefficient of wheat yield is related to the air pressure, maximum temperature, maximum relative humidity and air temperature. Furthermore the maximum correlation coefficient of oil seeds yield is related to the number of cloudy days, maximum temperature, air pressure and air temperature. In West Azarbaijan Province, the maximum correlation coefficient of wheat yield is related to minimum temperature, air temperature, air pressure and wind speed. Moreover, the maximum correlation coefficient of oil seeds is related to minimum temperature, wind speed, air temperature and maximum temperature. Based on the results, in each crop and province, it can be mentioned that air temperature has a more important role in increasing the correlation coefficient. To investigate the impact of indices and climatological parameters on the wheat yield of Hamadan Providence in the research of Sabziparvar and co-workers, the multivariate correlation of 90% scenarios is significant with the range of 0.67-0.97(Sabziparvar et al., 2012).The relative humidity, minimum and maximum temperature have the maximum impact on wheat yield in the study of researchers in India(Parekh and Suryanarayana, 2012).

Cropyield forecasting using regression models: Modeling crop time series is the next step after effective climatological parameters selection. Modeling of this research is based on classical and fuzzy regression. Classical regression was conducted regarding four climatological parameters for each province and crop. Symmetric and non-symmetric membership functions were used for fuzzy regression modeling which confidence level parameter must be determined in this regard. Confidence level parameter determination is based on the model performance investigation using different values of confidence level parameter and at least the parameter selection is related to the model performance with minimum error or maximum efficiency.

The method used to convert the output variable from fuzzy state to the deterministic one is the center of area method. The results of optimization which is indicative of fuzzy regression coefficients were investigated with different values of confidence level parameter. The variation of fuzzy regression performance based on the confidence level parameter is low but the coefficient variation of the first climatological parameter for wheat yield performance in East Azarbaijan and for forth climatological parameter of the oil seeds in West Azarbaijan are illustrated in Figure4.

The fuzziness of each variable has a close relation with the spread parameter of membership function. According to Figure 4, the sensitivity analysis indicated that changing the value of confidence level parameter will not change the center of each A_i but will influence the values of the spread. In fact, the variation of the spread of fuzzy number coefficient is influenced by confidence level parameter. The spread increasing of fuzzy number coefficient and no changes of center with increasing the confidence level parameter are the results of the research conducted by researchers (Yen et al., 1999).

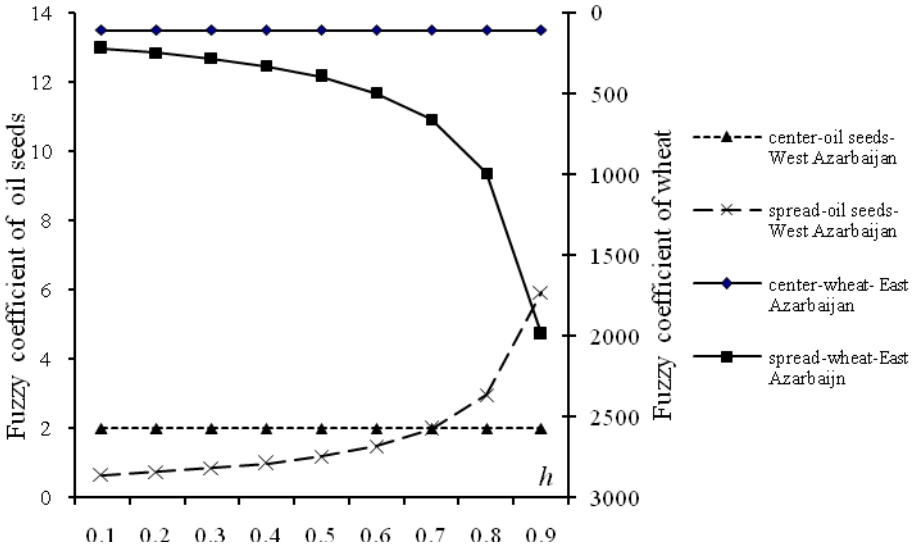


Figure 4. Variation of fuzzy coefficients with confidence level parameter.

In the case of non-symmetric membership function modeling, the sensitivity analysis is based on two steps: in the first step sensitivity analysis is related to the skew factors and in the second step, sensitivity analysis is related to the confidence level parameter based on the selected skew factors of the first step. The results of oil seeds skew factors in East Azarbaijan with confidence level parameter equal to 0.5 are presented in Table 2.

Table 2. Sensitivity analysis of skew factor oil seeds; East Azarbaijan

k_0	k_1	k_2	k_3	k_4	RRMSE
1.1	1.25	1.4	1.7	1.8	0.31
1.4	1.6	1.9	1.8	1.9	0.28
1.9	2.3	2.6	1.9	2	0.26
1	1	1	1	1	0.33
1.25	1	1	1	1	0.3
1.4	1	1	1	1	0.28
1	1.4	1	1	1	0.33

According to the sensitivity analysis in Table2, k_0=1.9,k_1=2.3,k_2=2.6,k_3=k_4=1.9 have the minimum error and they can be selected as the major skew factors. In the research of Yen et al. (1999) in the case of non-symmetric membership functions as the skew factor increases, the value of the spread S_0^L decreases and the center, a_0^P , increases. These results can be found in this research which is illustrated in Figure 5.

Yen et al.(1999) indicated that the variation of skew factors have influence on only a_0^P but not on the other coefficients. The results were investigated in the case of wheat yield and Zanjan Province in Table 3.

The peak point of constant parameter changes with skew factor variations but skew factor variations cannot change the peak point of other coefficients.

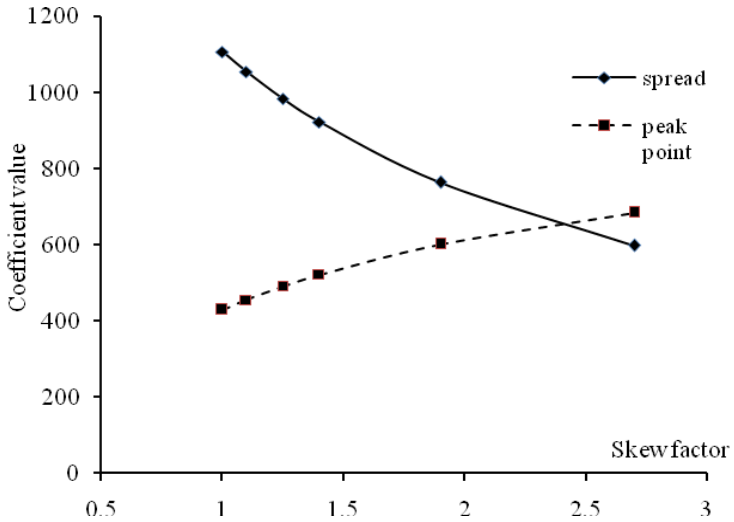


Figure 5. Variation of coefficient value with skew factor.

Table 3. Variation of peak point of fuzzy coefficients

k_0	k_1	k_2	k_3	k_4	a_0^P	a_1^P	a_3^P
1.1	1.25	1.4	1.7	1.8	455.99	2.66	14.22
1.4	1.6	1.9	1.8	1.9	521.91	2.66	14.22
1.9	2.3	2.6	1.9	2	601.47	2.66	14.22
2.7	2.9	3.2	3.5	3.8	684.03	2.66	14.22
1	1	1	1	1	429.63	2.66	14.22
1.25	1	1	1	1	491.15	2.66	14.22

Comparison regression models: After fuzzy coefficient determination, wheat and oil seeds yield were determined using two types of modeling; namely classical and fuzzy regression which the results of wheat modeling of East Azarbaijan are illustrated in Figure 6.

It is clear that the differences between observation and classical regression yield are high. Using symmetric and non-symmetric fuzzy regression, the differences between observation and modeling data are reduced. Some criteria were used for modeling methods comparison and the results are presented in Table 4.

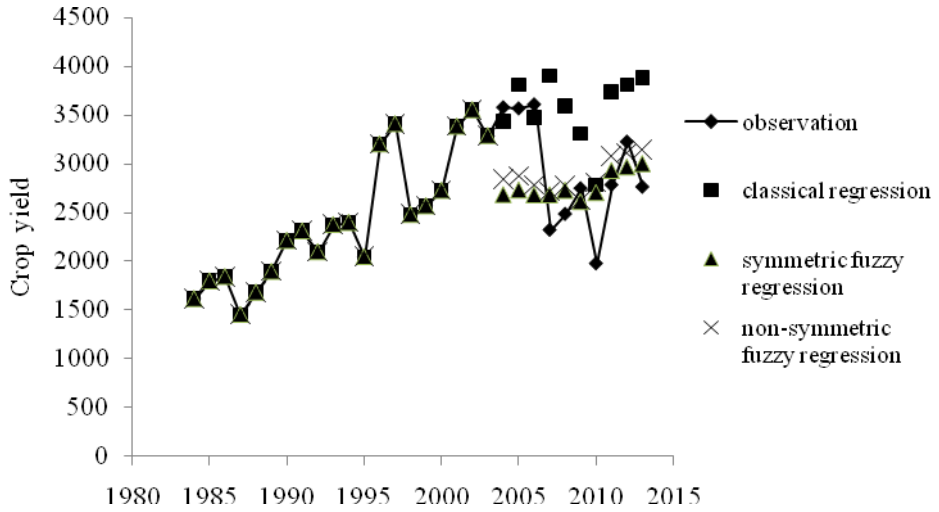


Figure 6. Wheat crop yield of observation, classical, symmetric and non-symmetric fuzzy regression

Table 4. Results of comparison regression models

Crop	Provinces	RMSE			MRE		
		Classical	Symmetric fuzzy	Non-symmetric fuzzy	Classical	Symmetric fuzzy	Non-symmetric fuzzy
Wheat	East Azarbaijan	0.29	0.19	0.18	0.28	0.16	0.16
	West Azarbaijan	0.26	0.24	0.2	0.24	0.22	0.18
	Zanjan	1.13	0.16	0.16	1.15	0.14	0.14
Oil Seeds	East Azarbaijan	0.32	0.33	0.26	0.23	0.21	0.2
	West Azarbaijan	0.26	0.24	0.16	0.24	0.21	0.15
	Zanjan	0.52	0.57	0.5	0.56	1	0.84

RMSE criteria decreased 37.76% in symmetric fuzzy regression in comparison to the classical ones and 15.6% in non-symmetric fuzzy in comparison to the symmetric fuzzy regression; *MRE* criteria decreased 28.14% in symmetric fuzzy regression in relation to the classical ones and 13.91% in non-symmetric fuzzy in relation to the symmetric fuzzy regression. Error criteria decreasing from classical to fuzzy regression model is obvious, therefore the results of forecasting are improved using the efficient regression models. In this regard, some researches such as Safa et al. 2015 and Kumar 2011 used another regression based models like ANN with improvement forecasted results.

For wheat case, *MRE* criteria decreased 68.866% in symmetric fuzzy regression in relation to the classical ones and 7.69% in non-symmetric fuzzy in relation to the symmetric fuzzy regression. For oil seeds case, *MRE* criteria decreased 37.86% in symmetric fuzzy regression in relation to the classical and 16.19% in non-symmetric fuzzy in relation to the symmetric fuzzy regression. The average *RRMSE* of wheat in all regression models is 0.48, 0.22 and 0.23 for Zanjan, East and West Azarbaijan Provinces, respectively. The average *RRMSE* of oil seeds in all regression models is 0.3, 0.36 and 0.38 for Zanjan, East and West Azarbaijan Provinces, respectively.

Except oil seeds of Zanjan, classical regression model has the highest error. In the comparison between symmetric and non-symmetric regression, in most cases, non-symmetric fuzzy regression has the minimum error. In the oil seeds of Zanjan, two missing data exist in the validation period and the lack of similar trend of criteria with the other Provinces and crops can be the result of missing data.

CONCLUSIONS

Crop yield estimation using efficient method is one of the major issues in the agricultural policy. In this study, regarding the issue, regression modeling was performed using fuzzy concepts. Increasing of confidence level parameter increased the spread parameter of fuzzy regression that has not any impact on the center parameter. The left-side spread decreases with the increase of skew factor and the peak point is increased. Based on the mentioned criteria, fuzzy regression improved crop yield forecasting. The difference between symmetric and non-symmetric performance is low but the non-symmetric is acceptable. The decreasing value of error for wheat is less than that for oil seeds. The average error of all regression models in East and West Azarbaijan is similar, which is less than that in Zanjan. The suggestion of this study is the comparison of fuzzy and artificial neural network models or another regression based models which had the improvement results.

REFERENCES

Arabpour, A. & Tata, M.(2008): Estimating the parameters of a fuzzy linear regression model. Iran. J. Fuzzy Sys. 5(2): 1-19.

Asai, H., Tanaka, S. & Uegima,K.(1982): Linear regression analysis with fuzzy model. IEEE Trans. Syst. Man Cybern, 12: 903-907.

Basso, B., Cammarano, D. & Carfagna,E.(2013): Review of crop yield forecasting methods and early warning systems, Proceedings of the First Meeting of the Scientific Advisory Committee of the Global Strategy to Improve Agricultural and Rural Statistics,FAO Headquarters, Rome, Italy, pp. 18-19.

de Leona, M. & Jalaob, E. (2013): A prediction model framework for crop yield prediction. Asia Pacific Industrial Engineering and Management System.

Diamond, P. (1988): Fuzzy least squares. Inf. Sci. 46(3): 141-157.

Ghosh, H. Fuzzy linear regression. cabgrid.res.in.

Gommes, R. (2001): An Introduction to the art of agrometeorological crop yield forecasting using multiple regression. Fao.org.

Kang, Y., Khan, S.& Ma,X.(2009): Climate change impacts on crop yield, crop water productivity and food security–A review. Prog. Nat. Sci. 19(12): 1665-1674.

Kumar, A. Fuzzy linear regression.iasri.res.in.

Kumar, P. (2011): Crop yield forecasting by adaptive neuro fuzzy inference system. Math. Theo. Model. 1(3): 1-7.

Kumar, S. & Kumar,N.(2012): Fuzzy Time Series based Method for Wheat production Forecasting. Int. J. Comp. App.44(12): 5-10.

Parekh, F. & Suryanarayana,T. (2012): Impact of Climatological Parameters on Yield of Wheat Using Neural Network Fitting. Int. J. Mode. Engin. Res.2(5): 3534-3537.

Sabziparvar, A. A., Torkaman, M. &Maryanajiz,Z. (2012): Investigating the effect of agroclimatic indices and variables on optimum wheat performance (Case study: Hamedan Province). J. Water Soil, 26(6): 15554-1567.

Safa, M., Samarasinghe, S.& Nejat,M.(2015): Prediction of Wheat Production Using Artificial Neural Networks and Investigating Indirect Factors Affecting It: Case Study in Canterbury Province, New Zealand. J. Agr. Sci. Technol.17(4): 791-803.

Yen, K., Ghoshray, S. & Roig,G.(1999): A linear regression model using triangular fuzzy number coefficients. Fuzzy sets and Syst.106(2): 167-177.

DOI: 10.17707/AgricultForest.63.1.28

Dušica RADONJIĆ, Božidarka MARKOVIĆ, Dragana DROBNJAK and Milan MARKOVIĆ [1]

LINEAR SCORING OF BROWN SWISS CATTLE BREED IN MONTENEGRO

SUMMARY

Brown cattle has become one of the globally most important breed for milk production. Many of its characteristics, such as longevity, production capacity, adaptability and fertility, have contributed to becoming a cosmopolitan breed. Also in Montenegro, Brown cattle breed takes very important place, especially in the northern part of the country where the largest number of animals of this breed is reared.

Linear scoring of brown cows was done in three areas: Pljevlja, Berane and Bijelo Polje. Results were analyzed separately for the first three lactations, while all subsequent lactations were treated together.

The linear scoring showed that certain type traits do not deviate much from the optimum (feet and legs), some characteristics deviate moderately (udder and some feet and legs traits) while there are those that have the largest deviation from the desired score (frame and rump).

The final linear score for all lactating cows places them in the category of very good. In terms of type traits Montenegrin Brown breed is not much inferior to European countries. Descriptive statistics for all traits was done. Statitical analysis showed significant differences between lactations for body measures (body depth, body lenght) but only for rear udder height an teat placement of all type traits ($P<0.05$).

Keywords: *Brown Swiss, linear scoring, type traits.*

INTRODUCTION

Cattle selection only on milk yield may adversely affect some properties, especially those which are of importance to the length of using cows. Therefore, it is very important that the selection criteria, in addition to milk production traits, include information on the type which will help in the selection of profitable long-lived cows.

To prolong productive life of cows for milk production special attention should be paid to the characteristics of the type and constitution. The disadvantages of type traits have leads to lower production, poor health and

Dušica Radonjić (corresponding author: dradonjić5@gmail.com), Božidarka Marković, Dragana Drobnjak and Milan Marković, University of Montenegro, Biotechnical faculty, Podgorica, MONTENEGRO

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online

premature exclusion from the exploitation of cows (Casanova, 1993). Selection of the exterior has multiple purposes in the breeding programs. Mindful of the exterior of offspring greatly reduce the possibility of spreading undesirable properties (exterior faults, inherited diseases), which bulls can have as hidden (recessive), which due to the application of artificial insemination can rapidly spread in the population (Pantelić et al., 2005).

Phenotypic differences between the type traits and production characteristics are positive but relatively low, so the assessment of the exterior can not perform the selection in terms of production. Yonikovski and Todorov (2009) reported that improving most of the type traits increasing milk production, but on the other side traits of body frame have low heritability. Milk yield in the standard lactation, and milk yield in one milking are positively correlated with the distance between the fore and rear teats before milking (Tilki et al, 2005).
Wiggans et al (2006) showed that traits of legs and feet are in very poor correlation with production traits, but they are positively correlated with the ability of survival and functional life of the flock.

Many authors indicate that the udder is the most important part of the body of the dairy cow and its morphological and physiological characteristics affect health of cows (Gulyas and Ivancsics, 2002; Umzay et al., 2003; Weiss et al., 2004; Tancin et al., 2007). Berglund et al. (2007), Forsbäck et al. (2011), Haghkhah et al. (2011), De Jong (1997) also argue that development of the cow's udder quarters is very important for better milk production. Slyzius et al. (2014) approved correlation between mastitis appearance and udder characteristics. Length of production life of dairy cows is positively correlated with several production and linear type traits, and data from these correlated traits are available earlier in life and are more highly heritable than is production life (Weigel et al., 1998).

De Haas et al. (2007) indicate genetic correlations with production traits (milk, fat and protein yield) and type traits differed between the dairy breeds. Stronger correlations were found in Brown Swiss and Red & White breed. Thurl width correlated positively with milk yield traits in Holstein and Red & White, but negatively in Brown Swiss. Results shows that conformation traits can be used as predictors for various purposes in dairy cattle breeding, but require adaptation for each breed.

Linear scoring system does not qualify traits as good or bad. The traits are described from 1 to 9, which represent the biological extremes (Berry et al., 2004). All traits are classified into four groups which in proportion to the importance participating in the final score: body frame, rump, legs and feet (functionality) and udder. The largest share in the overall score take udder traits, as much as 40%, the frame of body part as well as the feet and legs of 25% while the lowest part occupied the rump traits, only 10%. Linear scoring method is gradually introduced into the breeding programs of all breeders associations of dairy cattle and combined cattle.

Table 1: Linear traits

GROUP	Traits	Share in total csore		Optimum (desired score)
		Tarits	Groupe	
FRAME	Stature	5	25%	143 – 148
	Chest width	7		8
	Body depth	7		8
	Topline	6		7
RUMP	Rump lenght	2	10 %	8
	Rump position	4		5
	Thurl with	2		8
	Rump angle	2		7
FEET AND LEGS	Rear legs (side view)	8	25 %	5
	Bone structure	4		9
	Foot angle	6		6
	Foot height	7		8
UDDER	Fore udder lenght	3	40 %	8
	Fore udder attachment	4		9
	Rear udder witdh	3		9
	Rear udder height	4		9
	Udder cleft	4		7
	Udder depth	8		7
	Udder balance	4		5
	Teat lenght	2		5
	Teat thickness	1		5
	Teat placement	2		5
	Fore teat placement	3		6
	Rear teat placement	2		5

Swiss Brown cattle Breeders Association (1897-1997); Plesnicar (2010)

MATERIAL AND METHODS

Linear scoring was done according to the recommendations of the Swiss Brown cattle Breeders Association (1897-1997) and Plesnicar (2010). The scoring was done on farms in the municipalities of Pljevlja (5 farms), Berane (4 farms) and Bijelo Polje (4 farms) in the year 2011-2012. The survey included farms that have a tradition of breeding Brown breed and where grown animals that meet the standard of the breed.

Animals for which exist data for milk production were scored first, after that younger animals (the first, second and third lactation) and older animals at the end. We scored 16 cows in first lactation, 19 cows in second lactation, 21 cows in third lactation and 24 older cows, 80 cows were scored in total.

For taking body measurements Lydtin stick and measuring tape were used.

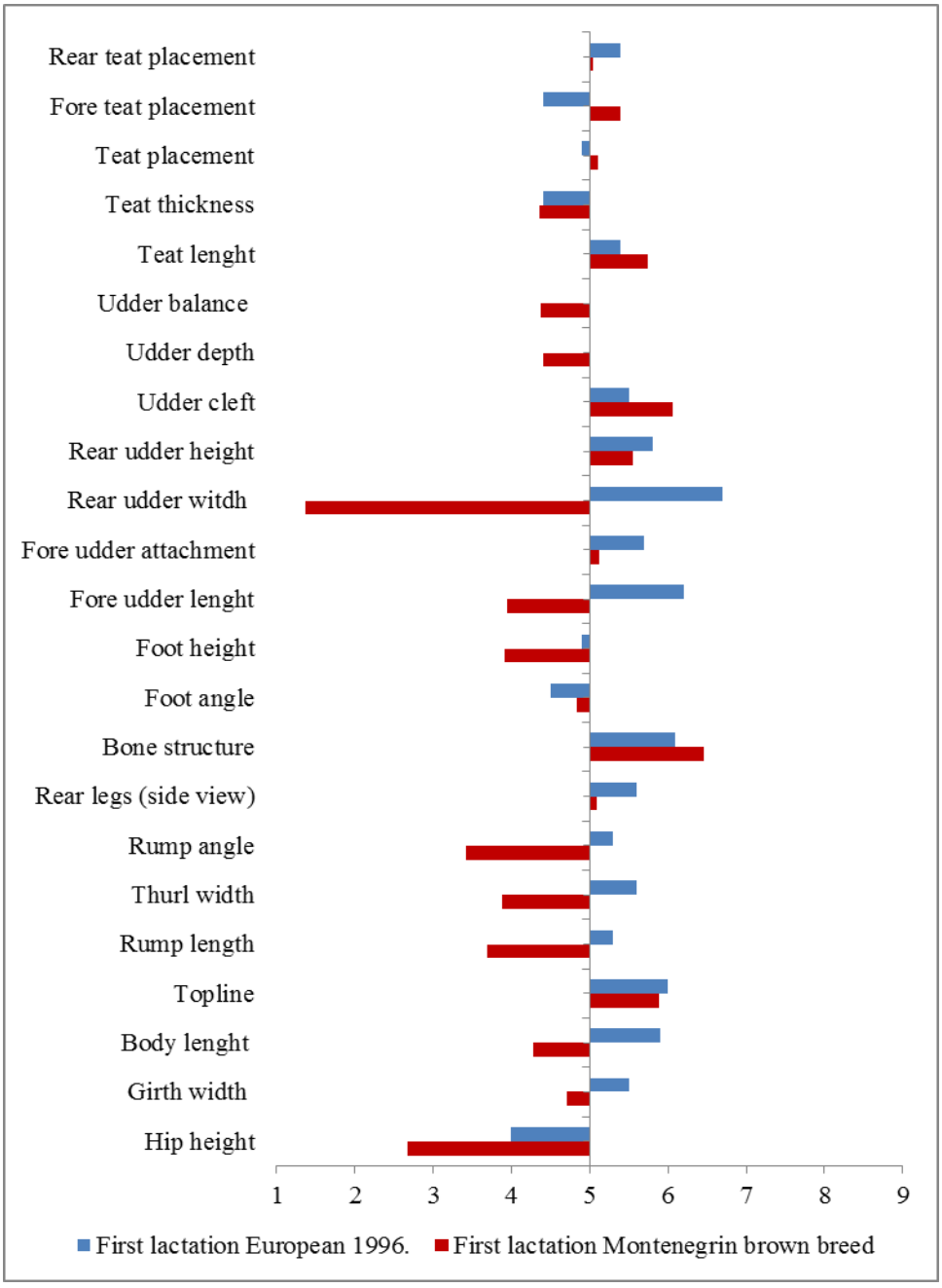
With Lydtin stick were measured:
-Stature
-Body depth
With tape were measured:
-Heart girth
-Body length
-Ramp length
-Ramp width
Traits scored from 1 to 9:
-Topline
-Ramp angle
-Rear legs (back view)
-Rear legs (side view)
-Foot angle
-Foot height
-Fore udder attachment
-Fore udder length
-Rear udder height
-Udder cleft
-Udder depth
-Rear udder width
-Fore teat placement
-Teat length
-Teat thickness
-Rear teat placement
-Teat placement
-Udder balance
-Muscularity
-Dairy character.

Those results were analysed by lactation. Comparison with optimized scores was made and the average score for each lactation separately and the average score for this breed was calculated. The data were processed using the computer programme STATISTICA 12. Descriptive statistics was done and LSD test for testing statistical significance between lactations ($p \leq 0.05$).

RESULTS AND DISCUSSION

The average heart girth of cows in first lactation was 184.34 cm and the average body length 158 cm. These physical measures were less for cows in first lactation compared with other lactations.

Comparative score for first lactation brown cows indicated by the Brown Swiss Cattle Federation (1897-1997), and first lacation cows in Montenegro is given in Graph 1. According to these data, it is evident that the Montenegrin cows in firs lactation is exterior lagging behind the European Brown breed.



Graph1: Comparison scores of first lactation Montenegrin Brown breed and European first lactation 1996.

The greatest deviation has the ramp width, rump length, body depth. Udder traits has a slight difference, but at least deviating traits of legs and topline.

All traits have a lot of variation (table 2). Muscularity of brawn cows in first lactation was evaluated with 5.5, and dairy character also. These scores are below desirable scores, for dairy character desirable score is 9, and 7 for muscularity. Some traits are very close to the required score (foot angle, teat length, teat placement), while some traits has somewhat larger deviation (udder balance, fore teats placement, feet position).

Other traits more deviate from desirable score, some of them moderately (topline, suspensory ligament, udder depth, teat thickness, rear teat placement). The maximum deviation from the optimum demonstrated the traits: chest width, body depth, rump length, thurl width, rump angle, feet height, bone structure, fore udder lenght, fore udder attachment, some of them are almost opposite extremes (rear udder width).The final score is obtained by multiplying the scores for each trait, with the participation of the group of traits in the overall assessment. The final score of first lactation puts them in a group of very good cows on a scale up to 50.

The average chest girth of the cows in the second lactation was 193 cm and the average body length 162 cm. Muscularity of cows in second lactation was scored with 8, a dairy character with 7. Muscularity is near perfect score. Preferred scor for dairy character is 9 so this trait deviates slightly more.

Very close to the desired assessment were: teat length, teat placement, placement of the fore and rear teats, while some features had larger deviation (udder balance, feet position).

The maximum deviation from the optimum demonstrated: body depth, rump length, thurl width, rump angle, feet height, fore udder length, rump height and the fore udder atachment, and some of them are almost opposite extremes (rear udder width).

Cows in second lactation has a slightly heighter overall score, but they are still scored as very good.

The average heart girth of cows in the third lactation was 189.45 cm and the average body length 161.38 cm. Muscularity of cows in the third lactation was evaluated with 5.14 and dairy character with 5.09. Very close to the desired assessment were: hock angle, teat length, fore teat placement, while some traits have larger deviation (udder balance, rear legs, udder cleft, teat thickness, teats placement, topline).

The maximum deviation from the optimum demonstrated: body depth, rump lenght, thurl width, rump angle, fore udder length and the fore udder attachment. Almost opposite extreme was the rear udder width. The cows in the third lactation had similar final score as cows in the first and second lactation.

The average heart girth of older cows was 193.9 cm, and the average body length 161.25 cm. Muscularity was assessed with 5.79, a dairy character with 6.54. Bearing in mind the desirable score for these features, the deviation in these cows is quite large.

Close to the desired score were: rear legs, teat placement, teat thickness, while some traits had larger deviation (udder balance, fore teat placement, teat

length, topline). Maximum deviation from the optimum demonstrated: rump length, thurl width, rump angle, fore udder attachment. In older cows, also maximum deviation occurs in the rear udder width. The heighest overall score had older animals, but it deviates slightly from the previous lactation. Table 2 shows the average scores for type traits for all lactations.

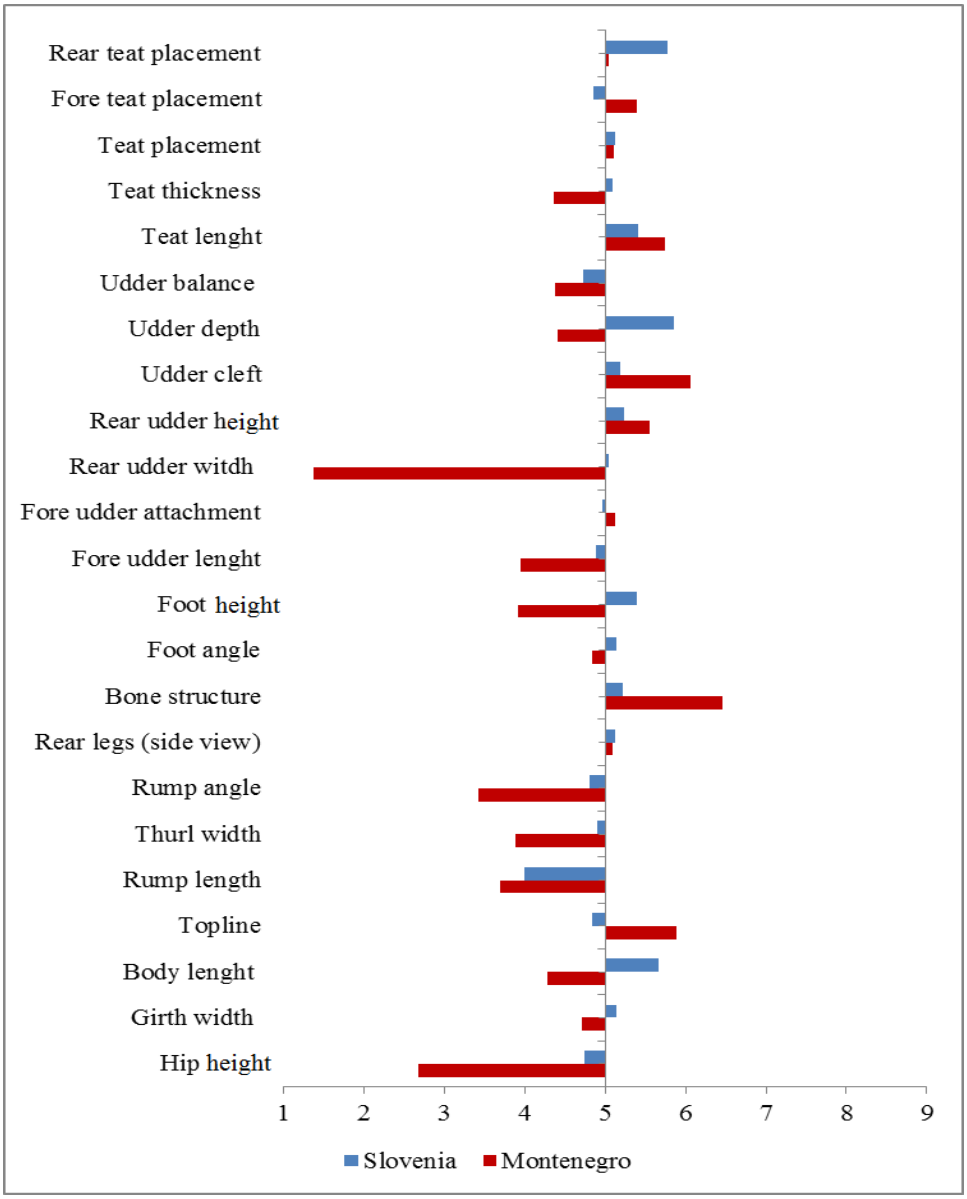
Table 2: Descriptive statistics of linear scores for Montenegrin Brown breed

Traits	**$\bar{X}$**	**SE**	**SD**	**CV**	**Min**	**Max**
Hip heightt, cm	2,66	0,18	0,30	11,57	2,25	3,10
Girth width, cm	4,71	0,46	0,78	16,70	3,85	6,00
Body lenght, cm	4,28	0,37	0,63	14,92	3,18	4,75
Topline	5,89	0,13	0,21	3,70	5,57	6,10
Rump lenght, cm	3,69	0,20	0,33	9,18	3,18	4,14
Thurl width	3,88	0,30	0,50	13,11	3,18	4,54
Rump angle	3,41	0,04	0,06	1,92	3,31	3,50
Rear legs (side view)	5,09	0,10	0,17	3,36	4,81	5,25
Bone structure	6,45	0,15	0,26	4,03	6,15	6,87
Foot angle	4,83	0,14	0,24	5,01	4,55	5,18
Foot height	3,91	0,12	0,20	5,16	3,68	4,23
Fore udder lenght	3,94	0,19	0,32	8,28	3,66	4,50
Fore udder attachment	5,11	0,16	0,28	5,48	4,80	5,54
Rear udder witdh	1,36	0,02	0,03	2,47	1,31	1,40
Rear udder height	5,55	0,10	0,16	3,06	5,31	5,79
Udder cleft	6,05	0,11	0,18	3,12	5,81	6,30
Udder depth	4,40	0,58	1,01	22,94	3,00	5,75
Udder balance	4,37	0,16	0,27	6,33	4,00	4,77
Teat lenght	5,74	0,29	0,50	8,74	5,00	6,41
Teat thickness	4,36	0,53	0,91	21,02	3,25	5,62
Teat placement	5,10	0,12	0,20	4,03	4,8	5,31
Fore teat placement	5,39	0,16	0,26	4,98	5,15	5,81
Rear teat placement	5,04	0,10	0,13	3,30	4,93	5,33

The average heart girth is 189.99 cm, which is approximately equal for the Slovenian Brown breed, which, according to Spehar et al (2012), is 190.2 cm. The average body length is 160.66 cm.

Comparing those average scores with optimal scores for the Brown breed, it can be seen that the deviation from the optimum is different from single traits and groups. Similar scores fore udder traits found Mazza et al. (2013) in Valdostana cattle. Graph 2 shows comparative linear scoring of Brown breed in Montenegro and Slovenia. Most of the traits are quite different compared to the Slovenian Brown breed. Traits that deviate the most from the optimum differ from Slovenian scores. Rear udder deviates, then the feet height, fore udder length and rump angle. Teats placement, fore and back, heart gitrh and rear legs

are rated similar as Slovenian Brown breed. For all traits statistical analysis were done.



Graph 2: Comparative linear scoring of Brown breed in Montenegro and Slovenia

LSD test shows significant differences between different lactation groups for some body measures (body depth, rear udder height and body length) but only for rear udder height and teat placement of all type traits ($P<0.05$). For other linear traits statistically significant differences between lactations was not found.

Table 3: LSD test for all traits

Traits	Sum of Squares	Mean Square	F	Sig.
Stature	8.055	2.685	.849	.472
Heart girth	28.207	9.402	3.050	.034
Body depth	18.609	6.203	7.806	.000*
Topline	3.997	1.332	1.422	.243
Body length	24.693	8.231	6.416	.001*
Ramp length	8.299	2.766	2.854	.043
Ramp width	.306	.102	.067	.977
Topline	2.079	.693	.591	.623
Ramp angle	4.643	1.548	1.755	.163
Rear legs (back view)	4.193	1.398	.731	.537
Rear legs (side view)	3.152	1.051	.463	.709
Foot angle	6.908	2.303	2.561	.061
Foot height	9.487	3.162	1.222	.308
Fore udder attachment	2.021	.674	.584	.627
Fore udder length	.078	.026	.056	.982
Rear udder height	80.422	26.807	12.364	.000*
Udder cleft	2.623	.874	.466	.707
Udder depth	3.165	1.055	.702	.554
Rear udder width	6.305	2.102	1.348	.265
Fore teat placement	19.343	6.448	2.055	.113
Teat length	66.564	22.188	4.015	.011
Teat thickness	5.183	1.728	1.025	.387
Rear teat placement	2.039	.680	.390	.760
Teat placement	975.288	325.096	4.230	.008*
Udder balance	183.821	61.274	1.000	.398
Muscularity	6.394	2.131	1.705	.173
Dairy character	20.211	6.737	2.068	.111

*Significant differencies

CONCLUSIONS

Linear scoring of first lactation cows was closest to the required scores. Leg traits a little more deviate from the desired scores. Moderate deviation showed a part of the udder traits and topline. Frame and rump trait had a maximum deviation from the optimum, as well as some leg traits. The final linear

score for the first lactation is very good. Cows in second lactation have similar linear scores as first lactation cows. Udder traits had at least deviate from optimum, with the exception of the rear udder width. Legs traits a little more deviate from the desired scores. Frame and rump traits had maximum deviation from the optimum, as well as some leg traits. The final linear score is very good.

Linear scoreing cows in the third lactation was found at least deviation some udder and legs traits. Most legs traits was a little further from the desired scores. Rump traits hase a moderate deviation from the optimum. Maximum deviation has frame traits, as well as part of the udder traits. Cows in the third lactation also had a very good final score.

Linear score for the older cows is not much different than the younger ones. Closest to the desired traits were udder traits. Legs traits a little more deviate from the desired score. Moderate deviation had some traits of the udder and topline. Frame and rump traits had maximum deviation from the optimum, as well as some legs traits. Older cows have a very good final score, as well as all previous lactations.

Comparing the average linear score for individual traits with optimum, it can be seen that traits had various deviations. Based on these results it can be concluded that part of the legs traits and some udder traits were closest to the desired score. Moderate deviate some udder traits, topline and leg traits. Furthest from the optimal score were frame and rump traits.

Statitical analysis showed significant differences between lactations for body measures (body depth, body lenght) but only rear udder height and teat placement of all type traits ($P<0.05$).

REFERENCES

American Brown Swiss Federation: Description of linear type traits

Bach, A. and Busto, I. (2005): Effects on milk yield of milking interval regularity and teat cup attachment failures with robotic milking systems. Journal of Dairy Research, 72: 101–106.

Berglund, I., Pettersson, G., Östensson K. and Svennersten Sjaunja K. (2007): Quarter Milking for Improved Detection of Increased SCC. Reprod. Dom. Anim., 42: 427–432

Berry, D.P., Buckley. F., Dillon, P. Evans, R.D. and Veerkamp, R.F. (2004): Genetic relationships among linear type traits, milk yield, body weight, fertility and soatic cell count in primiparous dairy cows. Irish Journal of Agricultural and Food Research 43: 161–176.

Casanova, L. (1993): Genetic evaluation of linear type traits for Swiss Braunvieh. Bull. No. 8. INTERBULL. Uppsala, Sweden.

De Haas, Y., Janss, L.L.G., Kadarmideen H.N.(2007): Genetic and phenotypic parameters for conformation and yield traits in three Swiss dairy cattle breeds. Animal breedeng and genetics, Volume 124, 12–19.De Jong, G. (1997): Scoring legs & feet in the Dutch conformation scoring system. Interbull Bull 15, 130-135

Forsbäck, L., H. Lindmark-Mansson, K. Svennersten-Sjaunja, L. Bach Larsen and Andrén, A. (2011): Effect of storage and separation of milk at udder quarter level

on milk composition, proteolysis, and coagulation properties in relation to somatic cell count. J. Dairy Sci., 94: 5341–5349.

Gulyas, L. and Ivancsics, J. (2002): Relationship between the somatic cell count and certain udder-morphologic traits. Acta Vet. Hung., 50: 373–383.

Haghkhah, M., Ahmadi, M. R., Gheisari, H. R. and Kadivar, A. (2011): Preliminary bacterial study on subclinical mastitis and teat condition in dairy herds around Shiraz. Turk. J. Vet. Anim. Sci., 35: 387–394.

Mazza, S., Sartori. C., Berry, D., Mantovani, R. (2013): Factors affecting linear type traits of Valdostana cattle. Agriculturae Conspectus Scientifi cus . Vol. 78, No. 3 (207-211).

Plesnicar Pavla (2010): Ocenjevanje goved za rjavo pasmo goveda, Kmetijsko gozdarska zbornica Slovenije, KGZS- Zavod Nova Gorica.

Slyzius, E., Juozaitiene, V., Juozaitis, A., Zilaitis, V., Slyziene, B. and Cereskiene, E. (2014): udder quarters morphological and milking traits risk factors influencing productivity and subclinical mastitis in dairy cows. Bulgarian Journal of Agricultural Science, 20 (No 6), 1502-1507.

STATSOFT, STATISTICA 12 (2012): Copyright © StatSoft. http://www.statsoft.com/

Tancin, V., Ipema, B., Hogewerf, P. and Macuhova, J. (2006): Sources of variation in milk flow characteristics at udder and quarter levels. J. Dairy Sci., 89: 978–988.

Tilki, M., Inal, S., Colak, M., Garip, M. (2005): Relationships Between Milk Yield and Udder Measurements in Brown Swiss Cows. Turk J Vet Anim Sci 29, 75-81.

Yonikovski, M., Todorov, M. (2009): Evaluation of phenotypic and genetic parameters related to classification by type and productivity of cows of the dairy population in Bulgaria. Biotechnology in Animal Husbandry 25 (5-6), p 605-612.

Uzmay, C., Kaya, Y., Akbas Y. and Kaya, A. (2003): Effects of udder and teat morphology, parity and lactation stage on subclinical mastitis in Holstein cows. Turk. J. Vet. Anim. Sci., 27: 935–941.

Wiggans, R. (2006): Parameters and Evaluation of Rear Legs (Rear View) for Brown Swiss and Guernseys GAnimal Science Unit, Gembloux Agricultural University, B-5030 Gembloux, Belgium

Weiss, D., Weinfurtner, M. and Bruckmaier, R. M. (2004): Teat anatomy and its relationship with quarter and udder milk flow characteristics in dairy cows. J. Dairy Sci., 87: 3280–3289.

Weigel, K. A., Lawlor, JR. T. J. Vanraden, P. M. and Wiggans, G. R. (1998): Use of Linear Type and Production Data to Supplement Early Predicted Transmitting Abilities for Productive Life. J Dairy Sci 81:2040–2044.

DOI: 10.17707/AgricultForest.63.1.29

Mahmoud Reza KARIMISHAHRI, R.C. SHARMA[1]

INHERITANCE OF RESISTANCE TO MAYDIS LEAF BLIGHT IN MAIZE

SUMMARY

Maize suffers from the threat of various diseases resulting in considerable yield losses. One of them is maydis leaf blight (MLB) an economically important disease incited by *Bipolaris maydis* (Nisikado and Miyake) Shoemaker. Analysis of variance for combining ability indicated that both GCA and SCA variances were highly significant for disease reaction studied. High GCA variance for disease reaction suggested operation of additive gene action**.** Out of the 6 parents 3 exhibited –ve and 3 exhibited +ve GCA effects for resistance to *B. maydis*. Resistance in inbred V-17 had the highest negative GCA effects hence it is the best general combiner for resistance. The estimates of SCA effects indicated several crosses showing -ve SCA effects. Out of the 15 hybrids only 5 hybrids showed significantly –ve SCA effects. Highest estimates of SCA effects were observed in the cross V-335 x V-13 followed by V-327 x V-17 and V-128 x V-17. Parent V-13 (with significant negative GCA effect) when crossed with parent V-335 (with significant negative GCA effects) gave hybrid V-335 x V-13 which had significant negative SCA effects. Parent V-17 (with significant negative GCA effect) when crossed with parent V-128 (with significant positive GCA effects) resulted in a hybrid which had significant negative SCA effects. Amongst resistant x resistant cross only V-335 x V-13 indicated heterosis both over mid parents and better parental values. The crosses involving V-13, V-17 and V-335 with susceptible lines indicated maximum heterosis for resistance. While the mean effects were highly significant for the eight resistant crosses the a and d effects were significant for only 4 crosses of the a and d effects. Though each of the three digenic epistatic effects dd was playing a greater role followed by ad effects in the resistance crosses. In V-335 x V-13 cross, dd effect was significant and negative. The relatively high magnitude and positive effects of dd in most of the crosses indicated that these interactions was enhancing susceptibility, while considering the CM-128 x V-335, CM-128 x V-13, CM – 128 x V-128, CM-128 x V-17 and V-327 x V- 335 crosses were observed that ad and dd effects were highly significant and positive value.

Keywords: *India, GCA, SCA, Maize, Bipolaris maydis*

INTRODUCTION

Like other extensively cultivated crops, maize suffers from several diseases as well. In India 61 diseases have been reported on maize (Payak and

[1]Mahmoud Reza Karimishahri (corresponding author: mrkarimi@iripp.ir), R.C. Sharma, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, INDIA

Sharma, 1985). Among them Maydis Leaf Blight (MLB) prevalent in many parts of India, is a major threat to maize cultivation when grown in warm and humid climates. Maize has great genetic diversity for resistance to pathogens, which makes the use of resistant cultivars the most economic and efficient form of disease control (Balmer & Pereira, 1987; Silva, 2001).

A logical requisite to launch a breeding programme for resistance to the disease is to investigate the mode of inheritance and to described the genetic variance present. Knowledge concerning the inheritance of characters showed increases the effectiveness of selection for this trait. Pate (1954) and Van Eijnatten (1961) have indicated that reaction to the disease shows a continuous variation in maize population and behaves as a quantitative trait. Although individual gene effects usually are not measurable in quantitative characters, statistical procedures have been developed. Since the development and use of resistant genotypes are the best means of obtaining practical control of foliar diseases, genetic information relating to host resistance would provide more relevant basis for making breeding decisions. The first recorded work on the inheritance of disease reactions to *H. maydis* is that of Ullstrup (1941) who reported that susceptibility is inherited as a monogenic recessive trait. Pate and Harvey (1954) observed a wide range of reactions which suggested polygenic inheritance.

Falugyi and Olorode (1984) demonstrated at resistance in two varieties is homogenic recessive. The genes for resistance in the two varieties are allelic. Thompson and Bergquist (1984) reported resistance in seedling stage was recessive but the mature plant resistance being independent of seedling reaction. The resistance could be explained by additive effects, is concerned with relatively few loci, and may be incorporated into susceptible inbreds by backcrossing. The application of concepts of heterosis, general and specific combining abilities (GCA and SCA, respectively) has been utilized for grain-producing crop breeding; GCA is relatively more important than SCA for nonselected endogamic lines, while the opposite is true for previously-selected lines (Sprague & Tatum, 1942; Hallauer & Miranda Filho, 1988; Nass et al., 2000). These concepts are useful both for the characterization of lines in crosses and for establishing heterotic standards between maize populations (Hallauer & Miranda Filho, 1988; Beck et al., 1990; Crossa et al., 1990; Han et al., 1991; Vasal et al., 1992), and in maize disease genetic resistance studies (Nelson & Scott, 1973; Lim & White, 1978; Callaway et al., 1990). Lingam *et al.* (1989) reported that analysis of data on disease severity revealed highly significant GCA and SCA variances, with the GCA component higher, indicating the predominance of additive gene action. Dey *et al*. (1989) revealed that the general combining ability (GCA) component is more important than the specific combining ability (SCA) component in the inheritance of resistance. The resistant lines generally had lower GCA effects and the crosses between moderately resistant and susceptible lines had negative SCA values.

MATERIAL AND METHODS

The present study was undertaken to find out the pathogenic reaction under field conditions.

Field experiments

The study was conducted on six inbred lines known for their reaction to *Bipolaris maydis* (race O). Detailed pedigree and disease reaction of these lines are provided in (Table 1). These lines and their crosses including (reciprocals mixed) were grown in a Randomized Block Design with four replications under recommended agronomic practices. All these material namely, 6 parental inbreds, 15 F_1 crosses, 15 sets of F_2 progenies, 15 BC_1 and 15 BC_2 progenies were planted in rows of 5 m length in replicated trials in disease nursery conducted during Kharif seasons at IARI field. Hundred plants for each of parents, F_1, F_2 and BC_1, BC_2 progenies per replication were sampled for disease scoring (a rating scale 1-5, Payak and Sharma, 1983).

Table 1: Source of seed material -*VPKAS (ICAR), Almora*

Parents	Pedigree
P_1	**CM 128**
P_2	**V 327** (P33C-ICH462-1/KC12/Suwan)-2/D1478)Ä-F- ##- Äb- Ä-1- Äb)
P_3	**V 335** (TZI-25-F-##- Äb- Ä-4-1- Äb- Äb)
P_4	**V 13** (P41-C13-MH-526-1-2-f-f-F-#- Ä-13-#-5- Äb)
P_5	**V 128** (VL 16 Ä-237-1-1-f- Ä-5-#- Äb)
P_6	**V 17** (Pob 45-C2-HC-151-7-1-1-2-1-2- ÄB-####- Ä-1-f-26-#-3- Äb Äb)

Mass multiplication of inoculum

Mass multiplication of inoculum was done as per procedure describing by Sharma (1983). Sorghum grains were soaked in fresh tap water for 24hrs, after through washing. Excess water from the soaked grains was drained off through several layers of cheesecloth. The grains were dispensed in flasks to one third of its value, following by autoclaving at 120^{o}C for 40 min. The sorghum grains were inoculated with freshly growing culture blocks of the pathogen, maintained at plant pathology laboratory DMR, IARI. Cultures were shaken every 2 days and incubated for 16-20 days at 24ºC and shaken every 2 days. Cultures were then mixed and air-dried at 20ºC inoculations were made by powder of sorghum grains into individual leaf whorls.

Disease scoring

The disease scoring was done by rating scale provided by Payak and Sharma (1983). The scale consists of five broad categories designated by numerals 1 to 5. The rating scale is as follows:

1.0- Very slight-to-slight infection, one or two to few scattered lesion on lower leaves.

2.0- Light infection, moderate number of lesion on lower leaves only.

3.0- Moderate infection, abundant lesions on lower leaves, few on middle leaves, extending to upper leaves.

4.0- Heavy infection, lesion abundant on lower and middle, extending to upper leaves.

5.0- Very heavy infection, lesions abundant on almost all leaves, plants prematurely dry or killed by the disease.

Transformation of data

The intensity of the disease shows a continuous variation and, therefore, it was measured on individual plant basis. A slightly modified version of the scale was suggested by Fisher and Yates (1963). Since the data were recorded on the rating scale of 1-5, it was subjected to transformation before analysis. Fisher and Yates (1963) transformation have been widely used in this kind of data. Nevertheless test of normality was also conducted to confirm the revision of the rating data to normality. The transformed values (for 9 classes) are as under:

Table 2: Actual and transformed value for 9 classes

Actual value	Transformed value
1.0	-1.49
1.5	-0.93
2.0	-0.57
2.5	-0.27
3.0	0.00
3.5	+0.27
4.0	+0.57
4.5	+0.93
5.0	+1.49

Analysis of variance

The analysis of variance (ANOVA) was carried out according to Randomized Complete Block Design (RCBD). The analyses were performed according to the procedure outlined by Hayman (1954a) and Griffing (1956a) using the mean values of each replication.

Combining ability analysis

The combining ability analysis was carried out according to Griffing's (1956b) Model-I (fixed effect model) and Method–II (parents and F_1s but no reciprocals). In this method experimental material is regarded as population, about which the inferences are to be drawn and combining ability effects of the parents could be compared when parents themselves are used as testers to identify good combiners. The combining ability effects (GCA or SCA) so obtained were tested for their significance, calculating 't' value as under and comparing it against the table 't' value (at 5% and 1% level of significance) at error degrees of freedom. The critical difference (CD) was calculated as a product of the standard error and 't' value.

Heterosis

The average of F_1 over replication were used for the estimation of heterosis expressed in percentage over mid parent values (MP), better parent values (BP) and standard check values. The computation of values was done as per Hayes et al. (1955)

Test of significance

Significance of heterosis was tested by 't' test. The calculated 't' was compared with table value of 't' at error degree of freedom from ANOVA comprising parents and F_1's at P=0. 05 and P= 0. 01. 't' value was estimated as follows (Fisher and Yates, 1963):

Estimate of gene action

All the six components of generation means, m, a, d, aa, ad and dd representing mean, additive, dominance, additive x additive, additive x dominance and dominance x dominance gene effects respectively were calculated from the population means of P_1, P_2, F_1 based on the method given by Hayman (1958).

RESULTS AND DISCUSSION

Disease reaction

The disease was recorded for parental, F_1, F_2, BC_1, BC_2 following 1-5 disease scoring method, where 1 indicates no disease and 5 indicates maximum disease. The observation were pooled and analyzed. For calculating the disease intensity individual plants were scored (Table 3).

Table 3: Mean performance of the parental, F1, F2 and backcross generation for *Bipolaris maydis* (original and transformed scale)

Disease rating (original scale)					**Disease rating (original scale)**					
	P1	P2	F1	F2	P1	P2	F1	F2	BC1	BC2
CM-128x V327	2.92	3.48	3.08	3.01	-0.04	0.27	0.04	0.01	-0.01	0.15
CM-128xV-335	2.92	2.25	2.11	2.30	-0.04	-0.42	-0.52	-0.40	-0.25	-0.21
CM-128 x V-13	2.92	2.74	2.32	2.58	-0.04	-0.15	-0.39	-0.23	-0.22	-0.37
CM-128xV-128	2.92	3.28	2.39	2.84	-0.04	0.16	-0.34	-0.09	-0.10	-0.17
CM-128xV17	2.92	2.10	1.87	2.53	-0.04	-0.53	-0.69	-0.27	-0.29	-0.60
V-327xV-335	3.48	2.25	2.54	2.89	0.27	-0.42	-0.25	-0.06	0.03	-0.05
V-327xV-13	3.48	2.74	2.53	2.73	0.27	-0.15	-0.26	-0.15	-0.08	-0.36
V-327xV-128	3.48	3.28	3.11	3.55	0.27	0.16	0.06	0.32	0.17	-0.19
V-327xV17	3.48	2.10	1.83	2.38	0.27	-0.53	-0.63	-0.36	-0.15	-0.70
V-33xV-13	2.25	2.74	1.28	2.23	-0.42	-0.15	-0.60	-0.45	-0.63	-0.40
V-335xV-128	2.25	3.28	2.04	2.42	-0.42	0.16	-0.57	-0.33	-0.29	-0.21
V-335xV17	2.25	2.10	1.41	1.83	-0.42	-0.53	-1.06	-0.71	-0.60	-0.66
V-1xV-128	2.74	3.28	2.05	2.32	-0.15	0.16	-0.56	-0.39	-0.37	-0.31
V-13xV17	2.74	2.10	1.51	1.92	-0.15	-0.53	-0.97	-0.65	-0.64	-068
V-128xV17	3.28	2.10	1.56	2.06	-0.15	-0.53	-0.92	-0.56	0.43	-0.65

Analysis of variance for the design of the experiment

The statistical analysis was carried out for disease reaction. Analysis of variance is given in (Table 4) for one character. The 'F' test indicated that variance due to parents and their progenies were significant for disease reaction at 1% probability. The analysis of variance revealed that the variance within parents and their progenies were highly significant for disease reaction studied.

Table 4: Analysis of variance for randomized block design

	Mean original scale		**Mean transformed scale**
SOURCE	D F	MSS	MSS
REPLICATION	3	1.10**	0.09**
TREATMENT	65	1.13**	0.37**
PARENTS	5	1.19**	0.40**
F_1	14	1.26**	0.44**
F_2	14	0.81**	0.29**
BC_1	14	1.06**	0.24**
BC_2	14	1.00**	0.34**
BETWEEN GROUPS	4	0.10	0.02
ERROR	195		
CD at 1%		0.562	0.249

**Significant at 1%

Combining ability effects

The disease data both on original and transformed scale were subjected to combining ability analysis following Griffing's(1956a). Method II, modal I was utilized in diallel cross analysis for combining ability analysis of the maydis leaf blight in maize.

Analysis of variance for combining ability

Analysis of variance for combining ability indicated the both GCA and SCA variances were highly significant for disease reaction studies. General combining ability variances was approximately five times higher in magnitude than the specific combining ability variance (Table 5). These data thus indicated that, although, variance due to GCA and SCA were important for disease resistant, major role in the expression of disease reaction in the present set of material is played by GCA. High GCA variance was found for disease reaction suggested the operation of additive gene action for this character**.**

Table 5: Analysis of variance for combining ability for reaction to *Bipolaris maydis*

SOURCE	DF	MSS
GCA	5	0. 997**
SCA	15	0. 195**
ERROR	0.195	0.024

**Significant at 1%

General Combining Ability effects

The estimates of GCA effects of the six parents in respect of disease reaction are given in (Table 6). Out of the 6 parents included in the present study 3 exhibited –ve and 3 exhibited +ve GCA effects for resistance to *B. maydis*. Resistance in inbred V-17 has the highest negative GCA effects of all the parents hence it is the best general combiner for resistance resulting in low infection. V-335 and V-13 also showed significant negative GCA effects. Inbreds CM-128, V-327 and V-128 showed significant positive GCA effects and are poor general combiners for resistance. V-128 and V-327 have significant positive GCA effects, and thus transmitted susceptibility to their progenies. Similar results were obtained on both original and transformed scale.

Table 6: Estimates of general combining ability effects of the parental lines for *Bipolaris maydis*

Inbred lines	Orginal scale	Transformed scale
CM-128	0.184**	0.101*
V-327	0.488**	0.287**
V-335	-0,281**	-0.132**
V-13	-0.121*	-0.035ns
V-128	0.198**	0.098*
V-17	-0.468**	-0.318
CD	At 5%	at 1%
SEgca	0.098	0.128

Specific Combining Ability effects

The estimate of SCA effects are presented in (Table 7). It was observed that several crosses showed -ve SCA effects. Out of the 15 hybrids only 5 hybrids showed significantly –ve SCA effects. Highest estimates of SCA effects were observed in the cross V-335 x V-13 (resistant x resistant) followed by V-327 x V-17 and V-128 x V-17 (susceptible x resistant). The V-17 as a good general combiner for resistance has transmitted in resistance with susceptible combination, while the cross V-335 x V-13 (resistant x resistant) produced the resistant hybrid as expected. Parent V-17 (with significant negative GCA effect) when crossed with parent V-327 (with significant positive effect) gave hybrid V-327 X V-17 which had significant negative SCA effects. Parent V-13 (with significant negative GCA effect) when crossed with parent V-335 (with significant negative GCA effects) gave hybrid V-335 x V-13 which had significant negative SCA effects. Parent V-17 (with significant GCA effect) when crossed with parent V-128 (with significant positive GCA effects) resulted in a hybrid which had significant negative SCA effects. Results based on transformed scale were similar to original scale. All three above crosses showed the negative SCA effect.

Table7: Estimate of specific combining ability effects of the 15 crosses for *Bipolaris maydis* at field condition

	Sca effects		**Mean Rating**		
Crosses	Original scale	Transformed scale	Original scale	Transformed scale	Reaction Type
CM-128xV-327	0.102ns	0.053ns	3.08	0.04	Susceptible
CM-12xV-335	-0.095ns	-0.087ns	2.11	-0.52	Intermediate
CM-128xV-13	-0.049ns	-0.057ns	2.32	-0.39	Intermediate
CM-128xV-128	-0.300*	-0.142*	2.39	-0.34	Intermediate
CM-128xV17	-0.151ns	0.072*	1.87	-0.69	Resistance
V-327xV-335	0.025ns	0.010ns	2.54	-0.25	Susceptible
V-327 xV-13	-0.140ns	0.113ns	2.53	-0.26	Susceptible
V-327xV-128	0.118ns	0.078ns	3.11	0.06	Susceptible
V-327xV17	-0.492**	0.202**	1.83	-0.63	Resistance
V-335xV-13	-0.622**	-0.037**	1.28	-0.60	Resistance
V-335xV-128	-0.181ns	-0.137*	2.04	-0.57	Intermediate
V-335xV17	-0.146ns	-0.210**	1.41	-1.06	Resistance
V-13xV-128	-0.329*	-0.221**	2.05	-0.56	Intermediate
V-13xV17	-0.206ns	-0.217**	1.51	-0.97	Resistance
V-128xV17	-0.475**	-0.300**	1.56	-0.92	Resistance

** Significant at the level of 0.01

* Significant at the level of 0.05

Heterosis

Heterosis was calculated as the percent increase or decrease of F_1 performance above the mean performance of parental lines as well as over better parent for resistance for each cross. The magnitude of heterosis expressed as percentage increase or decrease over middle parent and better parent for character studied is presented in (Table 8). Heterotic response in the F_1 generation was observed for disease incidence on both original and transformed scales. On the original scale amongst resistant x resistant cross only V-335 x V-13 indicated heterosis both over mid parents and better parental values. The crosses involving V-13, V-17 and V-335 with susceptible lines indicated maximum heterosis for resistance. The negative sign of mid parents indicated that crosses were in the direction of resistance.

Gene action (Results based on original data)

Generally in the resistance crosses the effects were relatively larger than either additive or dominance effects. The mean effects were also larger than the absolute magnitude of epistatic effects (Table 9). While the m effects were highly significant for the eight resistant crosses the additive and dominance effects were significant for only 4 crosses of the a and d effects. The d effects were more important. The negative dominance gene effects for most of the crosses suggested that dominance was in the direction of resistance. In case of V-327 x V-13 followed by V-13 x V-128, the d effects was significant and had negative value.

Table 8: Mean values of parents and F1s and heterotic response for *Bipolaris maydis* (original scale)

		Mean Rating					Heterosis	
Crosses		P1	P2	MID. PAR	BET.PAR	F1	MID.PAR	OVER.BET.PAR(%)
CM-128xV-327	F1	2.92	3.48	3.20	2.92	3.08	-3.72	5.45
CM-128xV-335	F1	2.92	2.25	2.59	2.25	2.11	-18.29	-6.21
CM-128xV-13	F1	2.92	2.74	2.83	2.74	2.32	-18.02	-15.27
CM-128xV-128	F1	2.92	3.28	3.10	2.92	2.39	-23.06	-18.26
CM-128xV17	F1	2.92	2.10	2.51	2.10	1.87	-25.56	-11.15
V-327xV-335	F1	3.48	2.25	2.86	2.25	2.54	-11.43	12.59
V-327xV-13	F1	3.48	2.74	3.11	2.74	2.53	-18.48	-7.47
V-327xV-128	F1	3.48	3.28	3.38	3.28	3.11	-8.01	-5.33
V-327xV17	F1	3.48	2.10	2.79	2.10	1.83	-34.29	-12.90
V-335xV-13	F1	2.25	2.74	2.49	2.25	1.28	-48.63	-43.13
V-335xV-128	F1	2.25	3.28	2.77	2.25	2.04	-26.29	-9.44
V-335xV17	F1	2.25	2.10	2.18	2.10	1.41	-35.25	-32.97
V-13xV-128	F1	2.74	3.28	3.01	2.74	2.05	-31.80-	-24.98
V-13xV17	F1	2.74	2.10	2.42	2.10	1.51	-37.80	-28.25
V-128xV17	F1	3.28	2.10	2.69	2.10	1.56	-42.09	-25.87

Table 9: Estimate of gen actions effects for the 15 crosses for *Bipolaris maydis* (original scale)

Crosses		m	a	d	axa	axd	dxd
CM-128xV-327	F1	3.010ns	-0.340*	0.176ns	0.294ns	-0.124ns	-0.076ns
CM-128xV-335	F1	2.301**	0.687ns	-0.838*	-0.365*	0.707*	0.928*
CM-128xV-13	F1	2.584**	0.291*	-0.8988	-0.389*	0.399*	0.733*
CM-128xV-128	F1	2.842*	0137*	-1.077*	-.0.361ns	0.638*	0.330ns
CM-128xV17	F1	2.532**	0.506**	-1.860*	01.218*	0.197*	1.073*
V-327xV-335	F1	2.886*	0.154*	0.041ns	0.368*	-0.915*	-1.477*
V-327xV-13	F1	2.727**	0.493**	-1.057*	-0.483*	0.247*	1.333*
V-327xV-128	F1	3.555**	-0.026ns	-1.230*	-0.960*	-0.243*	0.677*
V-327xV17	F1	2.378**	0.880**	-1.270**	-0.314*	0.389*	0.364ns
V-335xV-13	F1	2.228**	-0.357**	-1.645*	-0.431*	-0.231*	-0.497*
V-335xV-128	F1	2.419**	-0.876*	-1.689ns	-0.962*	-0.721*	1.868*
V-335xV17	F1	1.833**	0.073*	-0338ns	0.430ns	-0.002ns	-1.011ns
V-13xV-128	F1	2.318**	-0.809*	-2.169**	-1.212*	-1.072*	3.277*
V-13xV17	F1	1.924**	0.050ns	-1.030*	-0.119*	-0.532*	0.407*
V-128xV17	F1	2.062**	0.420*	-0.907ns	0.227*	-0.338*	-0.191ns

m=F2a=additive axd=additive x dominance dxd= dominance x dominance
d= dominance axa= additive x additive
** Significant at 1% level
* Significant at 5% level

Each of the three digenic epistatic effects, in general, appeared to be important in the resistance crosses. Of these three effects, dd was playing a greater role followed by ad effects. The negative epistatic effects were in the direction of resistance. In V-335 x V-13 cross, dd effect were significant and negative. The negative value of dd effects indicated that were controlling the lesser manifestation of disease incidence. The relatively high magnitude and positive effects of dd in most of the crosses indicated that this interactions was enhancing susceptibility, while considering the CM-128 x V-335, CM-128 x V-13, CM –128 x V-128, CM-128 x V-17 and V-327 x V- 335 crosses were observed that ad and dd effects were highly significant and positive value. The positive sign associated with most of the estimates indicated that these epistatic effects were in the direction of susceptibility.

Results based on the transformed data

The interpretation of sign is reversed when the effects were obtained on the transformed scale. In V-335 x V-13 cross gave similar results to those obtained on the original scale. V-327 x V-17 and V-13 x V-17 crosses showed significant dominance gene effects and also negative dominance gene effects. Therefore, these crosses were in the direction of resistance. These results were also similar to those obtained on the original scale. In the crosses of CM-128 x V-335, CM-128 x V-13, CM-128 x V-128 and CM-128 x V-17. In CM-128 x V-335 the estimates of parameters m were highly significant while both a and d effects were significant in all crosses except for a effect. The ad and dd effects had positive value indicating dominance toward susceptible parent. Except for ad and dd effect in CM-128 x V-335 cross but these effects had positive value on the transformed scale. In case of the V-327 x V-17 the m effects were highly significant and both a and d parameters also were significant. The negative sign of d effects also indicated that the dominance effects were enhancing resistance. The estimates of parameter aa were more prominent. The negative sign of aa effects indicated that additive x additive effects were enhancing resistance. The results were same as obtained from original scale.

Maydis leaf blight caused by *Bipolaris maydis* is one of the destructive diseases of maize. The disease is prevalent in all warm and humid areas whatever maize is grown. Knowledge of the inheritance of disease is also must importance for the development of resistant genotype cultivars. Also for the adoption of an appropriate breeding procedure it is essential to understand the type of gene action involved. The present study was undertaken with an objective of understanding genetics of resistant of the disease.

The investigations were carried out both on the original (Ullstrup *et al.*, 1941) and transformed scale (Fisher and Yates, 1963). The results obtained on these two scales indicated only minor differences. Hence, in the discussion the results obtained on the transformed scale only will be taken into consideration. Analysis of variance revealed that the treatment effects were highly significant

for disease incidence. Highly significant between generation means were revealed for the characters under study. Highly significant differences were also observed within generation means. This indicated that the choice of parents was appropriate and that the parents were quite distinct in relation to the character studied (Table 4).

The concepts of combining ability were enunciated by Sprague and Tatum (1942). General combining ability was defined as the average performance of a line in several hybrid combinations and specific general ability as the deviation from the expectation based on average performance.

Griffing (1965) suggested that combining ability analysis could also be carried out by using further segregation generation. Combining ability computed on a diallel cross of six inbred lines of maize in the generations revealed that variances due to general and specific combining ability for *B. maydis* were highly significant. However, the variances for general combining ability were of a much higher magnitude. The GCA variance was higher in magnitude than SCA variance indicating the presence of additive effects for this trait but both GCA and SCA variance were significant the presence of dominance and over dominance was not ruled out.

Since general combining ability is a result of additive gene effects and SCA is dependent up on non-additive gene effects (Sprague and Tatum, 1942). It was concluded that additive gene action was playing the predominant role in this material. However, predominantly additive effects have been reported to contribute to the expression of reaction to this disease (Lim, 1975). Bogyo (1958) and Jha (1970) working with the inheritance of *H. turcicum* came to similar conclusions. Combining ability analysis is also useful in identifying parents with high general combining ability effects.

V-17 had the highest GCA estimate and was significantly different from other parents (Table 6). It also had distinctly lower disease reaction than other inbred lines and thus was the best line for the resistance to maydis leaf blight. It is suggested that this parent can be widely used in hybridization programme to achieve resistance. This parent when crosses also gave higher percentage of heterosis over better parent. V-335 and V-13 had the high and negative GCA estimate. These two parents also had distinctly lower disease reaction

The cross V-335 x V-13 will be of special significance as it can be exploited as commercial hybrid as well as for conventional breeding programme. V-128 x V-17 and V-327 x V-17 showed significant SCA effects. The three resistant lines, V-17, V-335 and V-13 had highest values for GCA effects, thereby indicating that these lines possessed the maximum combining for resistance in next generation. CM-128, CM-327 and V-128 had significant positive GCA effects. Thus they transmitted susceptibility to their progenies. Among them line CM-128 proved to be the poorest general combiner for imparting resistance. It was followed by V-327 and V-128.It is interesting to note that combinations between resistant lines, which were also good general combiners. It was reflected by relatively high and negative value (original scale)

for specific combining ability effects for single crosses between these lines. Most of the specific combining effects contributed by these combinations were not significant. High specific combining ability effects can be related with heterosis as in its expression both the dominance and the epistatic effects preponderate. If the crosses showing high specific combining ability involved both the parents, which are also good general combiners, they could be exploited for practical breeding. The present investigation revealed that highest estimated of SCA effects were observed in the crosses of V-335 with V-13 (resistant x resistant), which are good combiners, and they could be exploited for practical breeding.

Studies on gene action of quantitative traits should make use of different approaches to biometrical analysis, such as combing analysis (Griffing, 1956b) and estimates of gene effects (Hayman, 1958a). The combining ability is useful in identification of parents with high general combing ability effects and in detecting cross combinations showing high SCA effects. The estimation of gene effects made it possible to make a though study of the different gene effects in individual crosses, particularly in resistant x susceptible combinations. Estimation of genetics component from parental and F_1 data revealed that in the material under investigations both additive and non-additive gene action were important in the control of resistance. In the three resistant x resistant crosses the estimates of parameter m were larger in magnitude relative to parameter a and d or the absolute magnitude of epistatic effects. These results confirmed the findings from specific combining ability studies between resistant x resistant lines, where such combining did not impart resistant to the F_1 hybrid, superior to performance and was reflected in the relatively high and negative values for specific combining effects (Table 7).

The estimates of the six parameters for the various gene effects considered show that additive gene effects made the major contribution to variation in resistance to *B. maydis*. Epistatic effects were also important contributors to variation for resistance to *B. maydis* in the most of the crosses. The magnitude and significance of the estimates for aa, ad, and dd, over 15 crosses indicated that epistatic gene effects were present and important in the basic genetic mechanism of disease resistance in the population studied with regards to individual epistatic gene effects, additive x dominance and dominance x dominance effects appeared to contribute more to resistance to *B. maydis* in these crosses than do the additive x additive gene effects.

Dominance gene effects were exhibited by 5 of the 6 crosses. Its sign indicated the importance of dominance gene effects. The negative sign associated with these effects indicated that dominance was in the direction of resistance. The present investigation revealed that both additive and non-additive gene effects were involved in resistance to *B. maydis*. Reciprocal recurrent selection as suggested by Comstock *et al.,* (1949) would appear to be the best breeding approach in concentrating genes for resistance to *B. maydis* since epistasis, additive and dominance gene effects appeared to be important in the inheritance of resistance. There is hardly any literature available where studies either on

combining ability, estimation of genetic components or gene effects have been reported. Sharma *et al*. (1993) reported that dominance as well as epistasis both contributing to resistance along with additive gene effects, creates considerable hindrance in efficient utilization of additive gene in the enhancement of resistance level by any cyclic breeding procedures. Khehra *et al*. (1984) demonstrated the SCA component was found to be of greater importance then GCA component in the inheritance of maydis leaf spot. Inbred line 'H 3191' was best general combiner for disease resistance and also had high SCA variance. The nature of gene controlling resistance to *H. maydis* (race O) in some commonly used maize genetic stock. Some resistant genetic stocks, which have good combining ability and can be used in breeding programmes have also been identified (Saini *et al.,* 1979).

The exploitation of heterosis to raise the yield levels has been tried by several workers. The level of heterosis as well as selection advance in segregation generation to depend upon the genetic among the parents. The heterosis component is largely dependent on parental diversity as suggested by several workers in both self and cross pollinated crops. Therefore, the choice of diverse parents with good GCA is a pre requisite for carrying out an efficient hybridization programme. In practical heterosis, it is necessary to select combination with high degree of specific combining ability as well as parents with high general combining ability. In the present study it was shown that heterotic response in the F_1 generation was observed for disease incidence on both original and transformed scales. On the original scale amongst resistant x resistant cross only V-335 x V-13 indicated heterosis over mid parents and better parental values. The crosses involving V-13, V-17 and V-335 with susceptible lines indicates maximum heterosis for resistance. The negative sign of mid parents indicated that crosses were in the direction of resistance. Lim, (1975) reported that variation attributed to average heterosis, by line, and specific heterosis, between lines, were highly significant and resistance was partially dominant. Based on disease rating resistant inbreds contributed less heterotic effects for resistance to single crosses than did susceptible inbreds.

CONCLUSIONS

The analysis of variance revealed that the variance within parents and their progenies was highly significant for disease reaction studies indicating that the choice of the parents was appropriate for the present study. Analysis of variance for combining ability indicated that both GCA and SCA variances were highly significant for disease reaction studied. High GCA variance for disease reaction suggested operation of additive gene action. Out of the 6 parents 3 exhibited –ve and 3 exhibited +ve GCA effects for resistance to *B. maydis*. Resistance in inbred V-17 had the highest negative GCA effects hence it is the best general combiner for resistance.The estimates of SCA effects indicated several crosses showing -ve SCA effects. Out of the 15 hybrids only 5 hybrids showed significantly –ve SCA effects. Highest estimates of SCA effects were

observed in the cross V-335 x V-13 followed by V-327 x V-17 and V-128 x V-17. Parent V-13 (with significant negative GCA effect) when crossed with parent V-335 (with significant negative GCA effects) gave hybrid V-335 x V-13 which had significant negative SCA effects. Parent V-17 (with significant negative GCA effect) when crossed with parent V-128 (with significant positive GCA effects) resulted in a hybrid which had significant negative SCA effects. Amongst resistant x resistant cross only V-335 x V-13 indicated heterosis both over mid parents and better parental values. The crosses involving V-13, V-17 and V-335 with susceptible lines indicated maximum heterosis for resistance.

REFERENCES

Balmer E, Pereira O A P.1987. Doenças do milho. In: Paterniani E, Viegas G P. (Ed.) *Melhoramento e produção de milho*. 2.ed. Campinas: Fundação Cargill, v.2, cap.14, p.595-634.

Beck D L, Vasal S K, Crossa J.1990.Heterosis and combining ability of CIMMYT'S tropical early and intermediate maturity maize (*Zea mays* L.) germplasm. *Maydica*, v.35, p.279-285.

Bogyo T P, de Mueleneare H J H.1958.Preliminary investigation on the effect of the gene Y on the carotene content of maize seeding leaves.Proc.I Cong. *South Africa Genet.Soc*.77-79

Callaway M B, Smith M E, Coffman W R.1990. Diallel analysis of resistance to anthracnose stalk rot in maize inbreds. *Crop Science***,** v.30, p.335-337.

Carson M L, Stuber C W, Senior M L.1996. Identification of quantitative trait loci (QTLs) for resistance to two foliar diseases in a mapping population of recombinant inbred (RI) lines of maize. *Phytopathology*, v.86, p.59.

Carson M L.2001. Inheritance of resistance to *Phaeosphaeria* leaf spot of maize. Plant Disease**,** v.85, p.798-800.

Comstock R E, Robinson H F, Harvey P H.1949.A breeding procedure designed to make maximum use of both general and specific combining ability. *Agron. J*. 41: 360-367.

Crossa J, Vasal S K, Beck D L.1990.Combining ability estimates of CIMMYT's tropical late yellow maize germplasm. *Maydica*, v.35, p.273-278.

Dey S K, Mahajan V, Pal S S, Dhillon B S, Malhi N S, Khehra A S. 1989.Resistance to *Drechslera maydis* in maize inbred lines and their combining ability. *Indian Phytopath.* 42: 261-264.

Faluyi J O, Olorode O. 1984.Inheritance of resistance to *Helminthosporium maydis* blight in maize (*Zea mays* L.). *Theorical Apply Genet.* 67: 341-344.

Fisher R A, Yates F. 1963.Statistical Table for biological Agriculture and Medical Research. Oliver and Boynd Ltd.,Edinberg.

Griffing B.1956a.A generalized treatment of use of diallel crosses in quantitative inheritance. *Heredity*, 10: 31-50.

Griffing B.1956b. Concepts of general and specific combining ability in relation to diallel systems. *Aust. J.Biol.Sci*. 9**:** 463-493.

Griffing B.1958. Application of sampling variable in the identification of methods which yield unbiased estimates of genotypic variation components. *Aust. J. Biol*. 2: 219-245.

Hallauer A R, Miranda Filho J B.1988. Quantitative genetics in maize breeding. Ames: Iowa State University Press. 375p.

Han G C, Vasal S K, Beck D L, Elias E.1991.Combining ability of inbred lines derived from CIMMYT maize (*Zea mays* L) germplasm. *Maydica*, v.36, p.57-64.

Hayes H K, Immer F R, Smith D C.1955.Methods of Plant breeding. McGraw-hill book company, Inc.New York.

Hayman B I.1954*a*.A mathematical theory of gene action and interaction. Ph.D. Thesis, University of Birmingham Library.

Hayman B I.1958.The separation of epistatic from additive and dominance variation in generation of means. *Heredity*, **12**: 371-390.

Jha T D, Dhawan N L.1970.Genetic analysis of resistance to *Helminthosporium turcicum* Pass. in inbred lines of maize. pp.388-396 in S.P.Raychaundhuri *et al*.(Ed.)Plant disease problems. *Indian Phytopath. Soc*.,I.A.R.I., New Delhi.

Khehra A S, Rao, A V B S, Dey S K, Dehillon B S, Malhi N S.1984. Inheritance of resistance to maydis leaf blight in maize. *Indian J. agric Sci*. 54: 881-883.

Lim S M, Hooker A L.1976.Estimates of combining ability for resistance to *Helminthosporium maydis* race 0 in a maize population. *Maydica*, 21: 121-128.

Lim S M.1975.Heterotic effects of resistance in maize to *Helminthosporium maydis* race O. *Phytopathol.* 65: 1117-1120.

Lingam S S, Balasubramanian K A, Reddy P R R.1989.Combining ability analysis for resistance to *Helminthosporium turcicum* leaf blight in maize (*Zea mays* L.). *Annals of Agricultural Research*. 10: 405-409.

Nass L L, Lima M, Vencovsky R, Gallo P B.2000.Combining ability of maize inbred lines evaluated in three environments in Brazil. *Scientia Agricola*, v.57, p.129-134.

Pate J B, Harvey P H.1954. Studies on the inheritance of resistance in corn to *Helminthosporium maydis* leaf spot. *Agron. J*. 46: 442.

Payak M M, Sharma R C.1983. Disease rating scale in maze in India.Techniques of scoring for resistance to important disease of maize.All India co-ordinate maize improvement project. IARI. New Delhi, 1-4 pp.

Payak M M, Sharma R C.1985.Maize disease and approaches to their management in India.*Tropical Pest Management*. 31: 302-310.

Saini R G, Gurmukh Singh J, Grewal R k, Goal R K, Gupta A K.1979.Inheritance of resistance to *Helminthosporium maydis*(Race O) in corn.*Genet.Agr*.33:201-208

Sharma R C, Payak M M, Mukerjee B K.1993.Durable resistance in maize-A case study from India .In Durability of disease resistance.(eds .Jacobs,T. and Parlevliet,J.E).227-229 pp.Kluwer Academic Publishers,Dordrecht,Netherlands

Sharma R C.1983. Maydis leaf blight of maize. Techniques of screening for resistance to important disease of maize improvement project, IARI , New Delhi,5-15-pp

Silva H P.2001. Análise genética da resistência a *Puccinia polysora* Underw em milho (*Zea mays* L). *Piracicaba*: USP/ESALQ, 87p. (Tese - Doutorado)

Sprague G F, Tatum L A.1942.General v/s specific combining ability in single crosses of corn. *J.Amer.Soc.Agron*. 34: 923-932.

Thompson D L, Bergquist R R.1984. Inheritance of mature plant resistance to *Helminthosporium maydis* race O in maize. *Crop Science*, 24: 807-811.

Ullstrup A J.1941. Inheritance of susceptibility to infection by *Helminthosporium maydis* race I in maize. *J. Agric. Res.* 63: 331-334.

Van Eijnatten C L M.1961.Susceptibility of leaf blight caused by *Cochliobolus heterostrophus* in Nigerian varieties of maize. *Nature* 191: 515-516.

Vasal S K, Srinivasan G, Han G C, Gonzales C F.1992.Heterotic patterns of eighty-eight white subtropical CIMMYT maize lines.*Maydica*, v.37, p.319-327.

DOI: 10.17707/AgricultForest.63.1.30

Sabina DELIĆ, Ljiljana KEČA, Aida IBRAHIMSPAHIĆ, Azra ČABARAVDIĆ and Damir BEHLULOVIĆ[1]

VALUE CHAIN ANALYSIS OF NON-WOOD FOREST PRODUCTS IN FUNCTION OF SUSTAINABLE DEVELOPMENT OF FOREST RESOURCES AND RURAL DEVELOPMENT IN BOSNIA AND HERZEGOVINA

SUMMARY

Forest resources in Bosnia and Herzegovina present rich sites of various non-wood forest products. In the concept of sustainable use of forest resources and assurance the economic, environmental and social effects, non-wood forest products have great importance, especially in the strategic commitments of rural development. However, information on the potentials of non-wood forest products in Bosnia and Herzegovina is very scant, whereas institutional and procedural framework relevant to this sector is underdeveloped. In this paper value chain analysis of non-wood forest products in Bosnia and Herzegovina have been carried out in order to identify the participants in the value chain, their mutual relations, and the analysis of organizational and institutional issues that affect the economic aspects of certain stages of the value chain. Survey method was used for primary data collection in the Federation of Bosnia and Herzegovina, where the relevant information from participants in the chain of non-wood forest products has been obtained. The study included a sample of 156 collectors, who had continuity in the collection and delivery, and 18 companies engaged in purchasing, processing and distribution, which have agreed to participate in the research. For data processing and interpretation of the results classical methods of analysis, synthesis, induction, deduction and comparison, and statistical methods of trend analysis were used. Technique of SWOT analysis was used in order to identify the positive and negative factors, as the basis for defining the strategic direction of non-wood forest products sector development.

The obtained results indicate on the presence of numerous problems in the value chain. The share of individual groups of non-wood forest products in the analyzed period is: 50% of berries, 40% of medicinal and aromatic plants and 10% of mushrooms. The average annual growth rate of purchased and processed amounts of medicinal and aromatic plants was 17%, 28% of forest berries, and

[1]Sabina Delić (corresponding author: sabinadelic@yahoo.com), Aida Ibrahimspahić, Azra Čabaravdić, Faculty of Forestry University of Sarajevo, Zagrebačka street 20, 71000 Sarajevo, BOSNIA AND HERZEGOVINA; Ljiljana Keča, University of Belgrade, Faculty of Forestry, Kneza Višeslava street 1, 11030 Belgrade, SERBIA; Damir Behlulović, Federal Office of Agropedology, Dolina street 6, 71000 Sarajevo, BOSNIA AND HERZEGOVINA

34% of mushrooms. Approximately 93% of these products are distributed in the international market, whereas achieved prices are twice as high as compared to the domestic market. Sales trend has been increasing both in the domestic and foreign markets. The average annual growth rate of sales in the domestic market for mushrooms is 120%, for forest berries 85%, and for medicinal and aromatic plants 64%. The average annual growth rate of sales in foreign markets is 15% for medicinal and aromatic plants, 26% for berries and 33% for mushrooms. The most important foreign markets are Germany, Italy and Austria, and also the United States and Canada for essential oils. The research results show that it is necessary to take certain measures on improvement the institutional and procedural framework relevant for the sector of non-wood forest products, for purpose of its affirmation and contribution to overall national economy. Observed in a broader socio-economic context, it can significantly affect the definition of the forest policy objectives, multifunctional use and determining the strategic direction of sustainable forestry development.

Keywords: *non-wood forest products, value chain, sustainable development of forest resources, rural development, Bosnia and Herzegovina*

INTRODUCTION

In recent decades, worldwide interest and demand for healthy food and organic products is increasing. Different non-wood forest products (hereinafter NWFPs) belong to this group of products since they meet the criteria of organic production. Many important international organizations (FAO, World Bank, CIDA, IDRC, CIFOR, IUCN) have NFWPs in its research portfolio and promote their importance. There are different definitions of NWFPs proposed by many authors (Arnold, 1995; Chamberlain *et al.* 1998), but in general practice universally accepted definition is proposed by FAO, by which the NWFPs all products are goods of biological origin other than wood derived from forest, other wooded land and trees outside forests (FAO, 2008). It includes: medical, edible and aromatic herbs, mushrooms, berries, honey, resin, moss, peat and similar (Pettenella *et al.*, 2006; Adepoju, Salau, 2007).

In the concept of forest resources sustainability and sustainable development of the forestry sector, NWFPs have great importance. Economic, environmental and social effects of the NWFPs use are undisputed, but they are, even in the most developed countries, still insufficiently known. The reason for this is, above all, the lack of data on the quantities and quality of collected and distributed products (Turtiainen, Nuutinen, 2012). For successful commercialization of NWFPs it is necessary to be aware of the available potential, as well as the factors and constraints that affect the usage (Keča, 2015). The commercialization of the NWFPs have been widely promoted as a concept in the rural development approach, poverty alleviation and conservation of natural resources (Schreckenberg, K. *et al.,* 2006). NWFPs as raw materials are linked to final consumers through a chain of value creation, whose optimization can

contribute to more efficient production of these products, global markets access and improvement of competitiveness (Keča, Lj. *et al.*, 2013).

Bosnia and Herzegovina (hereinafter B&H), together with other countries of Southeast Europe (Albania, Montenegro, Croatia, Macedonia and Serbia), constitute a key area for NWFPs collection, which plays important role in the local, regional and international trade (FAO, 2010). Based on various reports on NWFP potentials (Tanović, 2011; LAMP, 2004; FARMA, 2010), it is estimated that B&H has 200-300 plant species in nature, which are treated as medicinal, edible and aromatic (Vojniković *et al*., 2013). More precise data on NWFPs potentials in B&H does not exist, whereas the institutional and procedural framework, relevant to this sector, is underdeveloped. In the most cases actors of the NWFP sector in B&H show professional and organizational weaknesses, which impede the achievement and maintenance of competitive stability (Behlulović, 2015). The participants of the NWFP value chain (collectors, buyers and processors) in business philosophy are distant from each other, without regulated long-term relationships and a sufficient knowledge of market opportunities.

In recent years, a significant increase in exports of the NWFPs from B&H has been recorded, mainly raw materials, while the share of processed substances have been negligible. Estimates on NWFP values export in the Federation of B&H (hereinafter F B&H) are ranging from 14 to 15 million € per year, with an annual growth rate of 7-10% (Foreign Trade Chamber of B&H). It is also estimated that annualy from natural population in the F B&H 1 800 t of dried plant material and mashrooms have been collected, of which 95% are exported, while the plantation amounts of medicinal and aromatic plant species are negligible (Behlulović, 2015. according to Tanović, 2014). Global demand trend for medicinal and aromatic herbs and mushrooms is increasing, which gives the opportunity for access to the new markets. The NWFP value presents a significant component to the forest ecosystems value, which can affect the changes in the forest management practice. These are positive signals to the forest users (forest management companies) for rational and sustainable use of all available resources (Delić & Pozderac, 2011a). In that sense, forest owners (private and public) must be able to recognize the economic effects of their use or custom businesses for the purpose of providing these products and services. This creates presumptions and possibilities for development of small and medium entrepreneurship, which can stimulate economic development of rural areas, especially areas with agrravated living conditions. Sustainable use of NWFPs is the most direct and the most profitable method of linking the use and conservation of forests (Peters *et al.*,1989).

The results of this research have theoretical and practical significance and can contribute to the sustainable development of the NWFP sector, and thus forestry as bussines (Delić *et al*., 2011b).

MATERIAL AND METHODS

The research was conducted within the entity of the F B&H, which takes 51% of the total area of B&H. Forests and forest lands of F B&H, as sites of NWFPs, occupy an area of 1 518 600 hectares (Ministry of spatial planinng of F B&H, 2012). In certain phases of research, methods of analysis, synthesis, induction, deduction and comparation were applied. For the analysis of NWFP value creation chain, overall value chain was breakdown to the relevant sections or stages, which start from collection of the basic raw materials, purchase, procession, transportation, to placement for the end-users.

Using the technique of interviewing, primary data were collected for the period from 2010 to 2014. For that purposes two types of the questionnaire were created, one for collectors and other for processors (legal entities), whose primary business is related to NWFP. The questionnaire was modified according to Keča *et al*., (2013).

In the F B&H there is no register on collectors, therefore their total number is not known (Behlulović, 2015). This study includes a sample of 156 collectors, whereas the criteria for selection were: continuity in the collection and delivery of NWFPs, and territorial distribution. Questionnaire for collectors included questions related to socio-demographic profile, the basic aspects of NWFPs collecting (realized revenue, structure and volume of the collection, quality, conditions and problems in the collection, ownership of equipment, organization of collecting and collectors' education, membership in associations of collectors), and market aspects and factors that affect the purchase price.

Questionnaire for processors was consisted of several groups of questions which, in addition to socio-demographic profile processors, provided the following information: owning and utilization of capacites for NWFPs finishing and processing, structure and participation in the processing of certain types of NWFPs, purchase and transport, business environment and cooperation with other companies, market etc. In the F B&H 20 small and medium enterprises in the private property has been registered which belongs to the Group for herbs and berries within the Foreign Trade chamber of B&H (GEA, 2012). The survey included 18 companies that have given consent to participate in the research.

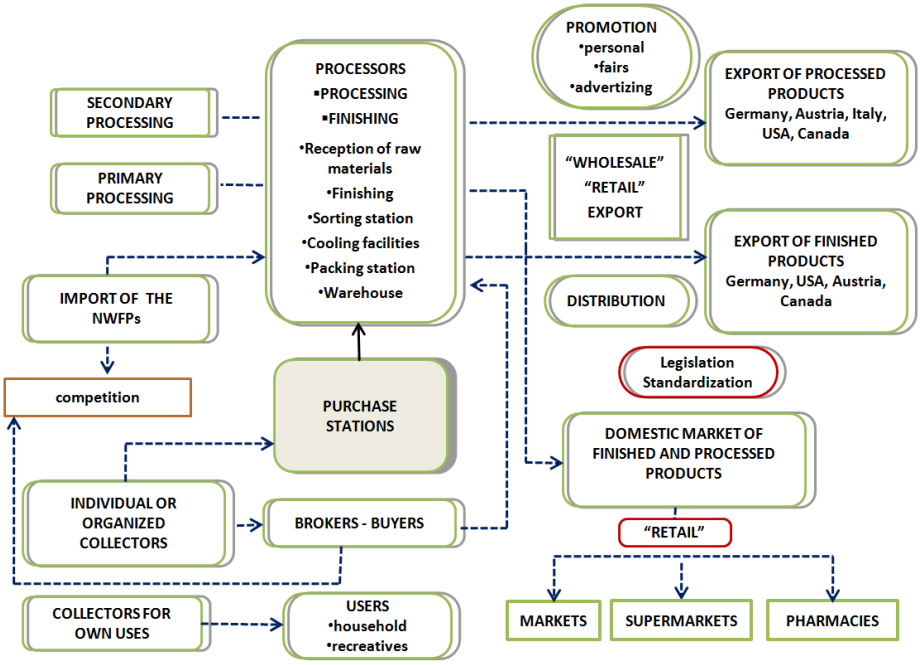
Statistical method of trend analysis was used in the addressing collected data, whereby the tendencies for development of purchase, finishing and processing of the considered NWFP types in the analyzed period were presented by a linear trend. In addition to the size of parameters functions trend, the size of the determination coefficient was determined as well, and also standard and relative errors of trend as a measure of estimation accuracy and quality assessment (Somun-Kapetanović, 2006). Based on the chain index the average annual growth rate of the observed sizes for the stages of collection, purchase, processing and sales was determined.

Technique of SWOT analysis was used for identication of positive and negative factors that present basis for defining the strategic direction of NWFP sector development and the setting of priorities in the strategic planning.

RESULTS AND DISCUSSION

The characteristics of the NWFP value chain in the F B&H

The participants in the value chain and key actors in the implementation of these activities are collectors, buyers and processors, who have an important impact on the NWFP value chain and have the opportunity to contribute in increasing the efficiency and effectiveness of production in this sector, and as well increase competitiveness in the global market. Three groups of NWFP were analyzed: medicinal and aromatic plants, forest berries and mushrooms for all relevant segments. The chain of activities from procurement to usage of the NWFPs is quite complex and influenced by many factors (Sheme 1).



Sheme 1. NWFP value chain in the F B&H

Collectors of NWFP in F B&H form the largest group of participants of chain that from natural populations (forest resources) collect NWFPs in its original form. Their contribution to the creation of additional and the total value is quite modest, because the collected products are usually sold as a raw material (pure substance) or just some primary form of processing is done (e.g., drying). They are a heterogeneous group, which aggravates their organization and realization of common goals. Of the total number of interviewed collectors, over 60% belong to the age category of 25-45 years, whereas 20% is younger than age 25. Most collectors belong to rural and unemployed population, out of which over 60% are female with elementary or secondary education. For the 44% of collectors NWFPs are the main source of income, for 38% additional, and rest

engaged collect NWFP for their own consumption. About 70% of the collectors believe that collected NWFPs are high quality, whereas only 4% assessed quality as bad. Problems that collectors highlight are mainly related to low prices, unfairness of purchasers and time-consumption. The largest number of respondents (34%) ween that the costs of the raw materials are low, and that in the NWFP value chain there is no equitable distribution among the participants, which in facts always negatively affect the collectors. About 26% of respondents pointed out the problem of time-consuming for collecting, 20% think that buyers are not fair, while 16% think that there are no enough raw materials, because the collection is not carried out in a sustainable way, which results in reducing the spots of some species (e.g. blueberries and cranberries).

Majority of interviewed collectors (82%) does not hold equipment for collecting and picking of the NWFPs, and those who possess, very rarely used it. Most collectors (85%) do not have knowledge about the basic principles and methods for identifying species, rules and modes of sustainable collection and other procedures related to this issue. Only 15% of the collectors comply with the rules of good collection practice. The collection of NWFPs is mainly unorganized, and only 17% of collectors carried out these activities with organized manner under the supervision of buyers (processors). About 63% of the collectors have not attended any training concerning the collection of NTFPs, while other hold certificate for successfully completed training on identifying and collecting medicinal and aromatic plants and mushrooms. The largest number of interviewed (80%) do not have membership in any association of collectors, 5% are members of the Association of citizens for medicinal and aromatic plants, mushrooms and their production of F B&H, and the rest belong to some of the municipal or cantonal non-governmental organizations.

Purchase and processing of NWFPs in the F B&H for the most cases takes place within the small businesses that are privately owned and still under development. Analysis of the processors socio-demographic profile indicates that the average age is 44 years, with the dominant representation of males (78%). About 45% of managers in surveyed companies have a master degree, 22% bachelor degree, and 33% secondary school education.

The available facilities for reception of the raw materials, storage, processing and finishing, as well as equipment, are not in accordance with the needs of processors, and do not meet basic standards. Only three of processing capacities fulfill all necessary requirements and standards for certification. Three processors have distilleries for distillation of the medicinal and aromatic plants and evergreen needle trees. Only two processors have cooling facilities and laboratory for their own basic analysis. About 90% of raw materials have been purchased directly from collectors in narrower and wider areas of the F B&H, and the rest through the purchase stations and brokers.

Over 70% of surveyed companies tweak purchased raw materials, while others also process it. At the level of F B&H there are no installed capacities for processing of mushrooms, although in some companies there are concrete ideas

and initiatives. Purchased amounts of mushrooms processors subject to the aspects of necessary tweaks, which refers to the drying, freezing, brining and the like. Although yield and quality of NWFPs largely dependent on natural and weather conditions, only 18% of respondents believe that this is the main influencing factor on prices. About 30% said that wholesales affect to the price fluctuations, buyers (28%) and processors (24%).

It is important to note that about 95% of processors did not signed a contract on the use of resources, thus they should be paying compensation for the use of resources.

The amount and structure of purchased NWFPs in the analyzed period was different per years. The share of forest berries is the highest (about 50%) on annual average, medicinal and aromatic plants about 40% and mushrooms about 10% (Table 1). The average annual growth rate of purchased and processed amount of medicinal and aromatic plants was 17%, forest berries 28% and mushrooms 34%.

Table 1. Structure and amount of the purchased raw materials for the period 2010-2014

NWFP	Amount (t/year)					The average annual growth rate (%)
	2010	2011	2012	2013	2014	
Medicinal and aromatic plants	591	686	870	978	1101	17
Forest berries	577	890	1229	1272	1449	28
Mushrooms	103	141	211	268	329	34

17 surveyed companies operate with purchasing and processing of medicinal and aromatic plants, whereas over 70% of total production is covered by the three large companies. The market most interesting medicinal and aromatic plant species are: wild garlic (*Allium ursinu L.*), hawthorn (*Crataegus monogyna L.*), mistletoe (*Viscum album L.*), Iceland moss (*Cetraria islandica L.*), sage (*Salvia officinalis L.*), juniper (*Juniperus communis L.*), silver linden (*Tilia tomentosa L.*), curry plant (*Helichrysum italicum L.*).

About 80% of the surveyed companies operate with finishing and processing of forest berries, whereas over 75% of production is concentrated in three large companies. Ten companies operate with buying and processing of the mushroom, whereas over 80% of production takes place in four companies. The market most interesting species from the group of forest berries are: blueberry (*Vaccinium myrtillus L.*), cowberry (*Vaccinium vitis idaea L.*), woodland strawberry (*Fragaria vesca L.*), dog-rose (*Rosa canina L.*), blackthorn (*Prunus spinosa L.*), and from the group of mushrooms are: porcini (*Boletus edulis L.*), chanterelles (*Cantharellus cibarius L.*), morel (*Morchella conica L.*), Caesar's mushroom (*Amanita caesarea L.*), fairy ring mushroom (*Marasmius oreades L.*).

In the analyzed period, trend of purchasing, finishing and processing of all types of the NWFPs has been growing (Figure 1). Identified linear trends for all three categories of NWFPs are characterized by a high coefficient of determination (93% or 99%), whereas the relative error of the estimate is 2.1 to 7.4%. The quality of these trends, given the determined size of estimation accuracy indicators and quality assessment is quite good or very good (Somun-Kapetanović, 2006).

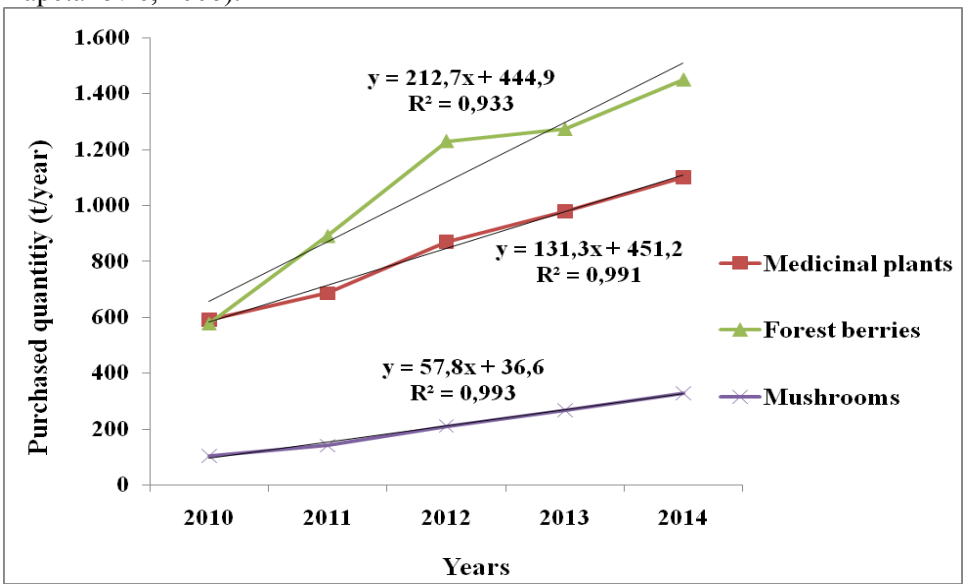


Figure 1. The trend of purchasing, finishing and processing of NWFPs for the period 2010-2014

Placement directions of NWFP

Approximately 93% of NWFPs are distributed to the international market, and majority of surveyed representatives of processors (78%) are not interested in the domestic market, due to low prices, low demand and uncertainty of business.

Market of the NWFPs in the F B&H is strongly influenced by the gray market (unregistered collection and transport), which has a negative impact on relations between collectors and purchasers/processors, with the presence of distrust and unfair competition. Their mutual relations usually are not regulated by contract, thus collectors are free in choosing purchasers, and this has an impact on the purchase prices and stability in the supply of raw materials. The largest quantity of products that have been traded in the domestic market are medicinal and aromatic plant species, then forest berries and least mushrooms (Table 2). The domestic market of medicinal and aromatic plants in B&H is still under development, and as end products, being sold in the domestic market, are mainly mono-component teas, tea mixtures, cosmetic, tinctures, drops, capsules etc.

Table 2. Sale of NWFPs in the domestic market for the period 2010-2014

NWFP	Amount (t/year)					The average annual growth rate (%)
	2010	2011	2012	2013	2014	
Medicinal and aromatic plants	16	25	58	76	104	64
Forest berries	12	15	49	65	101	85
Mushrooms	3	3	15	21	26	120

And despite the small quantity and low share of the domestic market in sales of the NWFPs, an extremely high growth rate of sales for all products in the analyzed period have been achieved. The highest average annual growth rate of sales in the domestic market is for mushrooms (120%), for forest berries 85% and for medicinal and aromatic plants 64%.

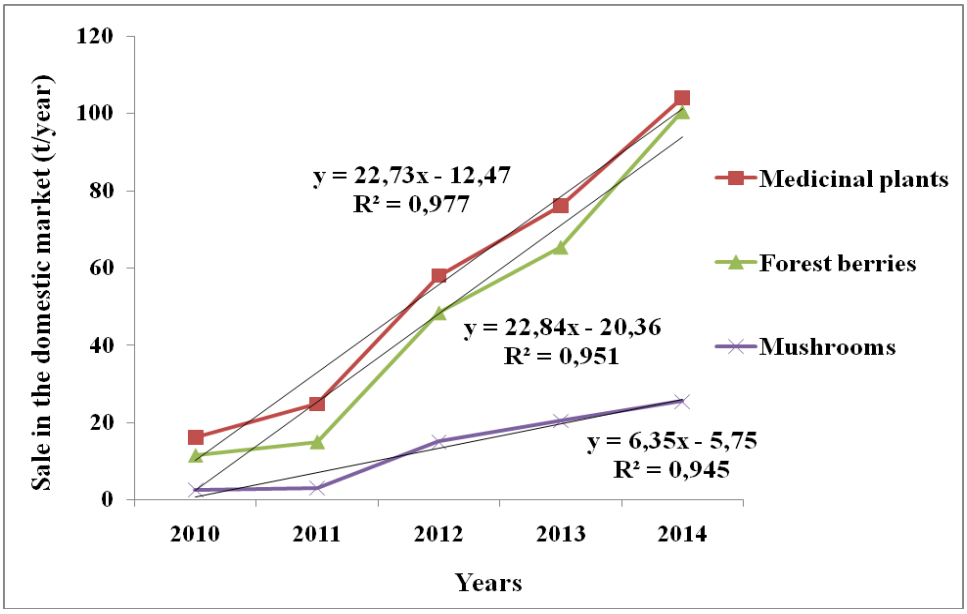


Figure 2. Sales trend of NWFPs in the domestic market for the period 2010-2014

The established linear growth trends are characterized by a high coefficient of determination (94-97%), whereas the relative error of the estimate is 8.8 to 16.2%, which indicates the quite good and very good quality evaluations of these trends (Figure 2).

The export trend of NWFPs has been also growing with the lower growth rate compared to the domestic market. Pure finished substance was mainly exported, whereas lower share refers to distillates of medicinal and aromatic

plants. In total exported quantities of the NWFPs for the period 2010-2014 participation of forest berries is over 50%, medicinal and aromatic plants about 40% and mushrooms about 10% (Table 3).

Table 3. Sale of NWFPs in the international market for the period 2010-2014

NWFP	Amount (t/year)					The average annual growth rate (%)
	2010	2011	2012	2013	2014	
Medicinal and aromatic plants	568	652	814	905	1000	15
Forest berries	561	872	1184	1210	1354	26
Mushrooms	100	136	197	248	305	33

The sale trend in the international market has been growing with an average annual growth rate of 15% for medicinal and aromatic plants, 26% for forest berries and 33% for mushrooms. Linear exports trends for all categories of the NWFPs are characterized by the high coefficient of determination (91-99%) (Figure 3).

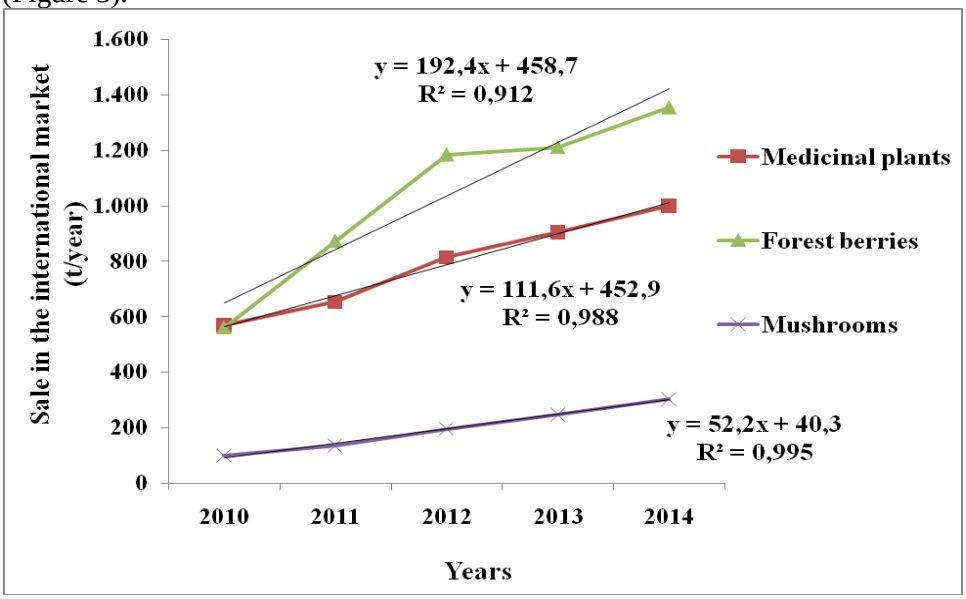


Figure 3. Sale trend of the NWFPs in the international market for the period 2010-2014

The relative error of the estimate ranges from 2.1 to 8.2%, which indicate a very good evaluation of trends quality and estimation accuracy of tendency. The largest amount of all types of the NWFPs are exported to Germany, then Italy and Austria, while the essential oils are placed to USA and Canada market. The trend of exports increase of these products for the analyzed period in all foreign markets has been growing (Figure 4).

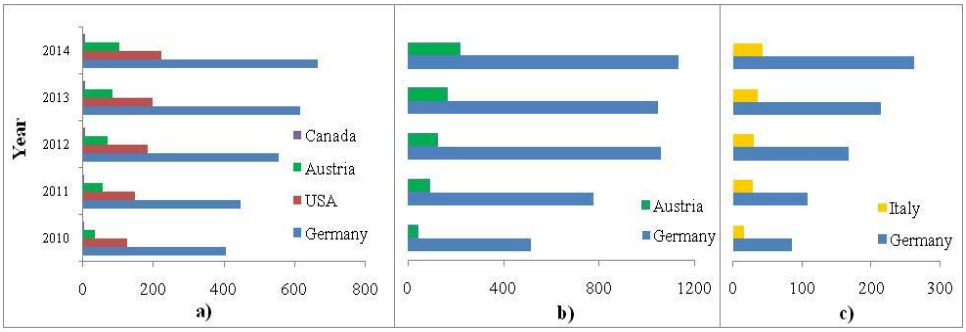


Figure 4. Export of NWFPs (t) by placement directions for the period 2010-2014: a)medicinal and aromatic plants; b) forest berries; c) mushrooms

Prices and promotion of NWFPs

Prices of all product categories in the NWFP value chain were different for the analyzed period, whereas the lowest share in the value chain has collectors. The majority of surveyed collectors believe that the purchase prices are low, while purchasers think that prices are fair.

Only 22% of purchasers believe that collectors should have a larger share in the NWFP chain. Market prices of all analyzed species are 2-3 times larger (even 10 times for some species of mushroom) in relation to the purchase price, which indicates on a high trade margins in the value chain, as well as the inadequate distribution of values within the value chain. Average realized prices of NWFPs in the domestic market were about twice lower compared to the prices in the international market.

The basic promotion method for the majority processors takes place through direct contact, or at the fairs, and it applies via advertising through a web presentations and advertising pamphlets. Approximately 67% of processors are certified by HACCP system, and/or certified by Organic Food Standard system.

Analysis of internal and external factors (SWOT analysis)

Based on the analysis and obtained information regarding the NWFPs, matrix for segments of purchase, processing and distribution of NWFPs have been formulated in which was reviewed the internal and external factors that directly affect the NWFP chain (Table 4). The results of internal strengths and weaknesses analysis, and external opportunities and threats, can serve as good basis for defining strategic development of this sector.

Based on the evaluation of certain internal and external influence factors on the NWFP chain strategy for development of NWFP sector in the F B&H can be defined, which should be built based on the maximum use of internal strength of the NWFP sector, with as much as possible use of the possibilities from the environment. The maximal internal strengths are reflected in the great wealth and diversity of the NWFPs, high-quality of raw materials, and competitiveness on the international market. The opportunities which should be used are in increased demand for the NWFPs, investing in processing capacities and increasing the

level of processing, better cooperation and improvement of relations of participants in NWFP chain, increase product sales in the domestic market and entering new markets, and the use of potential sources of funding for sustainable use of natural resources.

Table 4. Internal and external factors in the sector of NWFPs - SWOT Analysis

STRENGHTS	**WEAKNESSES**
The great wealth and diversity of the NWFPs	Lack of associations of collectors and skilled labor
The quality of raw materials at a high level	Insufficient utilization of processing capacities
Acceptance of standardization	Outdated equipment
Competitive prices and quality of products	Insufficient financial inputs for incentives to the rural areas
Good export potential	The low level of processing
Profitable business	Lack of information on market opportunities
The possibility of plantation production of certain NWFP	Inadequate system of collection points and transport of the NWFPs
The availability of low cost labor	Lack of marketing activities
OPPORTUNITIES	**THREATS**
Increased demand for the NWFPs	The existence of a "gray market" and unfair competition
Better cooperation and relations between participants in the NWFP chain	The lack of state support
Adequate training of the workforce	Climate changes
The increase of the product sale in the domestic market	Problems in export - Customs Procedures
Conquering of a new markets	Violation of biodiversity
Investment in processing capacities and increment of the level of processing	Strong competition in the international market
The use of pre-accession funds	Lack of legislation
The introduction of new product lines and new products	

CONCLUSIONS

B&H has a great potential, wealth, diversity and distribution of the NWFPs, but this sector is underdeveloped and disorganized. The whole system is very sensitive to weather conditions, market factors, characteristics of the participants in the chain, the business environment, as well as horizontal and vertical integration within the value chain of all stakeholders.

Collectors and processors recognize the economic importance of the NWFPs as well as the environmental and social aspects, evaluating them as very important, especially in the rural areas. Many problems and conflicts in relationships

between collectors and buyers are evident, and some of them are: unsatisfactory quality of the product due to poor procedures and rules in collection, insufficient training and professionalism of collectors, poor organization of purchase and transport of collected NWFPs, and dissatisfaction with the distribution of the value in the NWFP value chain. The lowest value gain collectors (two to three times less than the processors), while the share of trade margins in the market prices is very high.

The level of knowledge in the procedures for all participants in the value chain is very low. Impact of processors on collectors for the selection of appropriate methods for sustainable collecting and good "collection practices" is minimal, which is a major threat to break in an integrated value chain. Therefore, it is necessary to provide education to all participants in the value chain in order to facilitate their effective networking and the establishment of partnerships.

Processing of the NWFPs is mainly primary and refers to the phases of drying and freezing, whereas only a small part relates to the production of the essential oils. Capacity building for processing with higher degree of finalization would influence the creation of higher value for all participants in the value chain, whereas encouraging entrepreneurship would have positive effects on rural development and poverty reduction.

There is an evident growing sale trend of all the NWFP types. The basis for achieving competitiveness in international markets is improvement of the technical and technological aspects in proceedings with the NWFPs, standardization and certification, development of the new products, and implementation of marketing activities, in order to provide transparent information on market opportunities for all participants in the value chain.

The legal regulation of this field in accordance with international conventions and regulations on sustainable use and protection of endangered species, and the establishment of monitoring, would have resulted in the conservation of biodiversity and the sustainable use of the NWFPs.

Finally, it should be noted importance of improvement the institutional and procedural framework relevant for the NWFP sector in B&H with the aim for its affirmation and contribution to the overall national economy. The NWFPs represent a significant part of the forest ecosystems values, and thus can be a driver of development and motivation for change in the concept of the use of forest resources. Seen in a broader socio-economic context, it can significantly affect the definition of the forest policy objectives, multifunctional use and determine the strategic direction of the sustainable forestry development.

REFERENCES

Adepoju, A. A., Salau, A.S. (2007): Economic valuation of non-timber forest products (NTFPs). (Available at: http://mpra.ub.uni-muenchen.de/2689/), access, 20.09.2016.

Arnold, J.E.N. (1995): Socio-economic benefits and issues in non-wood forest products use, Non-wood forest products 3, FAO, Rome: 89–123.

Behlulović, D. (2015): Value chain and economic potential of non-wood forets products in the Federation of Bosnia and Herzegovina, Master thesis, Faculty of Forestry University of Sarajevo

Chamberlain, J.L.; Bush R.J.; Hammett A.L. (1998): Non-timber forest products: The other forest products. Forest Product J.48 (10):2–12.

Delić, S., Pozderac, M. (2011a): Value of forests and forest lands, Final report of the Study, Federal Ministry of Agriculture, Water management and Forestry (http://fmpvs.gov.ba/)

Delić, S., Pružan, E., Pozderac, M., Bajrić, A. (2011b): Planning, economics and marketing of forestry business, Final report of the Study, Federal Ministry of Agriculture, Water management and Forestry (http://fmpvs.gov.ba/)

FAO (2008): An Information Bulletin on Non-Wood Forest Products. Non-Wood News, FAO, Vol 17, Rome, pp. 12-21

FAO (2010): Report on Expert Consultation "Institutional Needs for Sustainable Non-Wood Forest Product Sector in South-East Europe". Food and Agriculture Organization of the United Nations and TRAFFIC International, Budapest, 2010, p.18 .

FARMA (2010): Medicinal and aromatic plants in Bosnia and Herzegovina, Project on improvement of agricultural markets, USAID, SIDA (Report)

GEA (2012): Biodiversity conservation through the opening of "green" jobs in the agriculture and forestry sectors, Study, 2012, (http://www.gea.ba/wp-content/)

Keča, Lj., Keča, N., Marčeta, M. (2015): Non-wood forest products, Socio-economics and ecologic aspects, University of Belgrade Faculty of Forestry, Belgrade (ISBN 978-86-7299-232-8)

Keča, Lj.; Keča, N.; Rekola, M. (2013): Value chains of Serbian non-wood forest products - International Forestry Review Vol.15 (3), (315-335), (ISNN 1465-5489)

LAMP (2004): Medicinal and aromatic plants – Market profile and report on competitiveness, Project – Linking farmers with market, USAID, ARD Inc. (Report)

Marshall, E., Newton, A.C., Schreckenberg, K., (2003), Commercialising non-timber forest products: Factors influencing success. International Forestry Review 5

Peters, C.M., Gentry, A.H., Mendelsohn, R.O. (1989): Valuation of an Amazonian. Forest Nature 339:655-656

Pettenella, D., Ciccarese, L., Dragoi, S., Hegedus, A., Hingston, A., Klöhn, S., Matilainen, A., Posavec, S., Thorfinnsson, T. (2006): NWFP&S marketing: Lessons learned from case studies in Europe.In: NISKANEN, A. Issues affecting enterprise development in the forest sector in Europe. University of Joensuu, Faculty of Forestry. Research Notes 169: 367–403.

Foreign trade chamber of Bosnia and Herzegovina (2012): Available at: http://www.komorabih.ba/

Schreckenberg, K., Marshall, E., Newton, A., te Velde, D. W., Rushton, J., Edouard, F. (2006): Commercialisation of Non-Timber Forest Products: What Determines Success?, Forestry Briefing Number 10, March 2006, ODI /Overseas Development Insistute, London

Somun-Kapetanović, R. (2006): Statistics in Economics and Management, University of Sarajevo. Faculty of Economics

Tanović, N. (2011): Identification of medicinal plants of the FB&H (Final report), Federal Ministry of Agriculture, Water management and Forestry, Sarajevo

Turtiainen M., Nuutinen T. (2012): Evaluation of information on wild berry and mushroom markets in European countries. Small-scale Forestry 11(1): 131–145. http://dx.doi.org/10.1007/s11842-011-9173-z.

Vojniković, S., Balić, B., Višnjić, C. (2013): Sustainable use of medicinal, edible and aromatic forest plants, Faculty of Forestry Sarajevo, (ISBN 978-9958-616-23-5)

Federal Ministry of Physical Planning (2012): Spatial plan of FB&H 2008-2028. (Available at: http://www.vladatk.kim.ba/)

DOI: 10.17707/AgricultForest.63.1.31

Mohammad Reza Safari MOTLAGH, Saber MOHAMMADIAN [1]

IDENTIFICATION OF NON-PATHOGENIC FUNGI OF RICE AND THE EVALUATION OF THEIR EFFECT ON BIOLOGICAL CONTROL OF *BIPOLARIS ORYZAE*, THE CAUSAL AGENT OF RICE BROWN SPOT DISEASE *IN VITRO*

SUMMARY

Rice brown spot disease is caused by *Bipolaris oryzae* is an important disease of rice in Iran and other parts of the world. In this research, 137 infected samples of rice were collected from paddy fields of Guilan province in Iran and 68 fungal isolates were isolated. PDA and WA media were used for isolation and identification of fungi. Morphological characteristics such as colony, conidia and conidiophores morphology were used for identification of these fungi. It was found that the isolated fungi belonged to *Bipolaris oryzae*, *Alternaria tenuissima*, *Preussia* sp., *Fusarium verticillioides*, *Alternaria infectoria*, *Alternaria citri*, *Trichoderma harzianum* and *Trichoderma virens*. Twenty isolates that didn't cause disease on rice or pathogenicity of them in rice was very low were selected for biocontrol studies and to do so, various methods were used. It was shown that seven isolates, *T. harzianum*, *T. virens*, *A. tenuissima*, *Preussia* sp., *F. verticillioides*, *A. infectoria* and *A. citri*, had the highest suppression percentage of mycelial growth of *B. oryzae*, respectively, in dual culture and culture filtrate methods. In volatile metabolites method, *T. harzianum*, *T. virens*, *A. tenuissima*, *A. infectoria*, *A. citri*, *Preussia* sp. and *F. verticillioides*, had the highest inhibition percentage of mycelial growth of *B. oryzae*, respectively. In hyperparasitism test, no coiling of *Trichoderma* spp. was observed around the hyphae of *B. oryzae*, however, *Alternaria* spp. penetrated into the mycelium of *B. oryzae* once they reached them and then, they tore the fungal mycelium and deformed it. Based on obtained results, *T. harzianum* was the most effective isolate in inhibiting the mycelial growth of *B. oryzae*. Analysis of variance and means comparison by least significant difference showed significant differences among the fungi used *in vitro*.

Keywords: *antagonist, rice, biological control, Bipolaris spp., non-pathogenic fungi*

INTRODUCTION

Rice brown spot disease is one of the most important diseases of rice whose symptoms may be displayed from nursery to farm (Safari Motlagh, 2000).

[1]Mohammad Reza Safari Motlagh, (corresponding author: ssafarimotlagh@yahoo.com), Department of Plant Pathology, Faculty of Agriculture, Rasht Branch, Islamic Azad University, Rasht, Guilan province, IRAN; Saber Mohammadian, Master of Microbiology, Department of Microbiology, Rasht Branch, Islamic Azad University, Rasht, IRAN

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

The fungus creates small round brown spots on host seedlings which may completely encircle coleoptile deforming the primary and secondary leaves (Khodaparast and Sahragard, 2004). In extreme cases, the seedlings in nursery are burned and the spikes are hollowed and blackened in farm (Safari Motlagh, 2000). The disease is caused by *Bipolaris* spp. (Safari Motlagh, 2000). This genus includes numerous species which mostly attack wheat, rice, barley and other grains. Brown spot, caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, is one of the most important seed borne diseases of rice and is an economically important foliar disease (Ou, 1985). In 1942, an outbreak of the disease caused yield losses of 90% which resulted in famine in Bengal (Nazari et al., 2015) and was one of the major reasons for the death of 2 million people (Nazari et al., 2015). It causes seedling blight and damages the foliage and panicles of rice, particularly when rice is grown in nutritionally deficient or unfavorable soils (Nazari et al., 2015). Biological control of plant pathogens is a relatively slow process, it has long-running, cheap and environmentally-friendly effects and can be a good alternative for chemicals (Kazemzadeh Chekosari, 2003). Microorganisms which are suitable for the biological control of plant pathogens have been identified among fungi and bacteria (McSpadden Gardener and Fravel, 2002).

Elad et al. (1980) examined the impact of a *Trichoderma harzianum* isolate on *Rhizoctonia solani* and *Sclerotium rolfsii* and found that *T. harzianum* grew faster than *S. rolfsii* in culture medium and attacked its mycelium.

The biological control of *R. solani* in cotton has been reported by *Trichoderma* and *Gliocladium* in field and by *Gliocladium virens* in greenhouse (Howell, 1982; Lewis and Papavizas, 1991).

In an *in vitro* and greenhouse study on the effect of isolates of *Trichoderma* on *Fusarium oxysporum* f. sp. *lycopersici* pathogen, Niknejad and Sharifi Tehrani (1993) found that gaseous compounds and extra-cellular exudations of *Trichoderma* had significant impact on the inhibition of mycelial growth of the pathogen.

The role of chitinase produced by *Stenotrophomonas maltophilia* in biological control of *Bipolaris sorokiniana* in *Festuca arundinacea* was studied and it was cleared that this enzyme can be effective in biological control of *B. sorokiniana* (Zhang and Yuen, 2000).

Salehpour et al. (2005) studied biological control of *B. sorokiniana,* the causal agent of wheat root rot by isolates of *Trichoderma* and results showed that *T. viride* was the most effective species in reducing the severity of infection in plant.

Abdel-Fattah et al. (2007) investigated the antagonistic mechanisms of *T. harzianum* against *B. oryzae*. The *in vitro* antagonistic effect of *T. harzianum* was brought about by its growth on *B. oryzae* and also, *T. harzianum* antifungal metabolites suppressed the linear growth of *B. oryzae*.

In an *in vitro* study on antifungal activities of *Pseudomonas fluorescens* strains against *Alternaria cajani*, *Curvularia lunata*, *Fusarium* spp. and *Bipolaris*

spp., it was revealed that all strains had good antagonistic effects against *A. cajani* and *C. lunata* (Srivastava and Shalini, 2008).

The antifungal activity of 86 isolates of *Bacillus* sp. was studied against *Bipolaris sorokiniana* as the causal agent of wheat brown spot and the isolate E64 was found to be the most effective (Carissimi et al., 2009).

The antagonistic potential of 135 local isolates of *Trichoderma* was, also, examined against *Phytophthora palmivora*, the causal agent of cocoa black pod, and the isolate T17 belonging to *T. virens* was found to be the most effective biocontrol agent hindering the mycelial growth of pathogen by over 97% (Mpika et al., 2009)

In a study, the biocontrol effect of some actinomycete isolates obtained from different habitats of Manipur, India was examined on major rice pathogens including *Curvularia oryzae¸ Pyricularia oryzae*, *Bipolaris oryzae* and *Fusarium oxysporum*. LSCH-10C isolated from Loktak Lake was found to be a promising biocontrol agent (Ningthoujam et al., 2009).

In greenhouse and *in vitro* studies on the effect of 200 *Trichoderma* strains isolated from soil, plant debris and phyllosphere in paddy fields of Mazandaran province on *Rhizoctonia solani*, Naeimi et al. (2011) revealed that some strains belonging to *T. harzianum, T. virens* and *T. atroviride* controlled the disease agent effectively.

In a study on biological control of *Bipolaris oryzae* in India, it was found that *Aspergillus niger, A. fumigatus, A. flavus, A. sulphureus, A. terreus, Penicillium chrysogenum, P. janthinellum, T. viride* and *T. harzianum* significantly reduced growth of *B. oryzae* (Manimegalai et al., 2011).

Khalili et al. (2012) investigated the influence of local isolates of *Trichoderma* isolated from rice fields of the Guilan and Mazandaran provinces on controlling rice brown spot caused by *B. oryzae* in which 145 *Trichoderma* isolates belonging to *T. atroviride, T. harzianum* and *T. virens* were screened in greenhouse and *in vitro* to find the best species for effective biocontrol of *B. oryzae*. Two strains belonging to *T. harzianum* significantly controlled the disease and two strains belonging to *T. atroviride* improved seedling growth (Khalili et al., 2012).

In a study, biological control of *B. oryzae* by *Pseudomonas synxantha* and *Bacillus* sp. was studied and it was cleared that disease severity decreased in rice infected seeds treated with bacteria (Moura et al., 2014).

Soltani Nejad et al. (2014) investigated biological control of *B. oryzae* by isolates of *Streptomyces* sp. and it was found that G isolate had more antagonistic activity.

Rice brown spot disease which hollow the seeds and impose heavy losses on paddy fields are observed from the nurseries to the fields in Guilan province. Since the methods to control the disease including chemical and agronomical methods have shortages, a new approach must be looked for like the use of antagonistic fungi to counteract the disease. The general objective of the present

study was to find fungus (fungi) in natural rice plant flora for *in vitro* suppression of *Bipolaris oryzae*, without causing disease on rice.

MATERIAL AND METHODS

Collection and culture of fungal isolates

Leaves with symptoms of the diseased rice were collected in Guilan province of Iran, then cut to appropriate sizes and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in petri dishes. Then, petri dishes were incubated at 28^0C in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and then, monoconidial isolates of the recovered fungi were maintained on half-strength potato dextrose agar (PDA) slants in test tubes as stock cultures (Safari Motlagh, 2010).

Study and identification of fungi

Morphological studies were carried out on water agar (WA) medium. Cuts of colonies were placed onto PDA medium for 2-3 days. Then, section of colonies was transferred to WA medium for 7-30 days in incubator at 27°C and 12h photoperiod. Afterwards, morphological observations were taken based on colony, conidium and conidiophore morphology and other morphological charactersitics (Ellis, 1971; Sivanesan, 1987; Gams and Bissett, 1998; Arenal et al., 2004; Arenal et al., 2007; Cain, 1961; Leslie and Summerell, 2006; Simmons, 1986).

Pathogenicity test

The pathogenicity test of the isolated fungi was done in desiccator under completely controlled conditions for which some farm soil was poured into Erlenmeyer flask and was sterilized in autoclave (twice, each time for 30 minutes) and then, some of this soil was put in sterile petri dishes. Afterwards, an amount of seeds of rice cv. Hashemi was disinfected in sodium hypochlorite solution 30% for one hour and then, 10 seeds were laid in soil in petri dishes. This was done in two desiccators, one as treatment and one as control. Two petri dishes were put in each desiccator. The petri dishes were poured with distilled water so that they were waterlogged during the experiment. Inoculation was done 16-18 days later when the seedlings in petri dishes were at two-leaf stage for which distilled water was first sprayed on all seedlings in control and treatment desiccator by hand sprayers (under sterile hood) and then, the spore suspension required for the inoculation was prepared (Safari Motlagh et al., 2005). In all experiments, a suspension containing 4×10^4 spores per ml distilled water was used which were counted by hemocytometer. In addition, Tween® 20 with the ratio of 1% was used for improving surface absorption. It should be mentioned that desiccators were kept in incubator at 26°C, >90% moisture, and 12/12 day/night light periods (Safari Motlagh et al., 2005).

Biological control studies

Inhibition of *B. oryzae* growth by culture filtrate

The isolates of the studied fungi were cultured in 250-ml Erlenmeyer flasks containing potato dextrose broth (PDB) culture medium and they were shook at 26°C at 70 rpm for 10 days. Then, they were extracted by biological filters and vacuum pump. Next, the extract was added to PDA culture medium. In control, the extract added to PDA culture medium lacked antagonistic fungus. A mycelial disc from 3-day culture of *B. oryzae*was placed at the center of treatment and control petri dishes and then these petri dishes were transferred into incubator at 26°C. After 10 days, radial growth of *B. oryzae* was calculated in control and treatment. Radial growth reduction was calculated by:

$$\text{Percentage of inhibition of radial mycelial growth} = \frac{C-T}{C} \times 100 ,$$

where *C* is the radial growth of *B. oryzae* in control petri dishes and *T* is its radial growth in the presence of other fungi (Dennis and Webster 1971a; Sivakumar et al., 2000).

Slide culture technique (hyperparasitism test)

A laboratorial slide was placed inside a 12-cm petri dish on two L-shaped glass bars and was sterilized. Then, some of molten 2% water agar culture medium was poured on the slide as so a thin layer of agar was formed. Small mycelial discs of the desired antagonistic fungus and *B. oryzae* were placed on slide with 2-cm spacing. A few milliliters of sterilized distilled water were added to each petri dish to avoid their drying. Petri dishes were kept at 26°C. As soon as the mycelia of the fungi were reached to each other, the slides were studied under optical microscope (Sivakumar et al., 2000).

The effect of volatile metabolites on inhibition of *B. oryzae* growth

A mycelial disc with the diameter of 5 mm from the 3-day culture margin of *B. oryzae* was placed in the center of a petri dish containing PDA medium. Forty-eight hours later, a disc with the diameter of 5 mm from the 3-day culture of the studied fungi was placed in the center of another petri dish containing PDA. Then, the caps of these petri dishes were removed under sterile hood and the dish containing *B. oryzae* was placed upside-down on the petri dish containing the studied fungi. In control, the studied fungi were replaced by a disc from PDA medium. Inhibition percentage was calculated 10 days later (Dennis and Webster, 1971b; Sivakumar et al., 2000).

Dual culture method

A mycelial disc with the diameter of 5 mm taken from margins of 5-7-day culture of *B. oryzae* was placed under sterile hood in an 8-cm petri dish containing PDA with 2 cm spacing from the wall of petri dish. Then, the petri dish was placed in incubator at 26°C for 48 hours so that the fungus started its growth. Then, a mycelial disc with the diameter of 5 mm taken from the margins of 5-7-day fungus was placed at a distance of 3 cm from the pathogenic fungus. The petri dishes were placed at 26°C and the measurements were recorded 7-10 days later (Sivakumar et al., 2000). In disease controls, a mycelial disc from the

margins of 5-7-day culture of *B. oryzae* was placed in the center of an 8-cm petri dish under sterile conditions. The control petri dishes were also placed in incubator at 26°C. At the end of incubation, the radial growth of *B. oryzae* was measured in control and treatment. The reduction of radial growth comparison to control was calculated (Sivakumar et al., 2000).

Data analysis

The study was based on a randomized complete design with seven treatments and three replications. Data analysis was done using SAS software. In order to compare average values, least significant difference (LSD) method was used.

RESULTS AND DISCUSSION

The pathogenic nature of all *Bipolaris* spp. isolates was proved on rice and characteristics of these isolates as follows: grey to dark grey colonies grew and spread rapidly. Aerial mycelium was fluffy, cottony, grey olivaceous with brownish tinge. Conidiophores were single or in small groups, straight to flexuous, sometimes geniculate, pale to mid brown or olivaceous brown, pale towards the apex, septate 430–580×4–7 µm (average 500×5 µm). Conidia were usually curved, navicular, fusoid or obclavate, occasionally almost cylindrical, pale to mid golden brown, smooth, 5–12 distoseptate, 46.5–125×10–26 µm and hilum was minute dark or light, often protruding, slightly papillate. The first septum was sub-median, the second delimited the basal cell and the third formed toward the apex of the conidium. Conidia germinated from polar cells and germ tube from the basal cell usually emerged immediately adjacent to the hilum and grows in the direction of the long axis (Figure 1). The general characteristics of this group are similar to *Bipolaris* Shoemaker, but special characteristics, such as shape and color of colony, morphology of conidium and conidiophore are similar to *Bipolaris oryzae* (Ito & Kurib) Drechsler ex Dastur (Ellis, 1971; Sivanesan, 1987).

After preliminary identification of fungal isolates at genus level, 20 isolates that were not pathogenic on rice were selected for *in vitro* biological control studies and were further identified at species level. Accordingly, the following fungal groups were identified:

Characteristics of Group I: Colonies blackish brown with fast growth. Conidiophores simple or branched individually or in simple or branched groups, straight or curved groups, almost cylindrical, septate, light yellow or light brown, smooth, up to 115 µm in length and 4.6 µm in thickness. Conidia solitary or in short chains, straight or curved, obclavate or with the body of the conidium ellipsoidal tapering gradually to the beak which is up to half the length of the conidium, usually shorter, sometimes tapered to a point but more frequently swollen at the apex where there may be several scars, light yellow to golden brown, usually smooth, sometimes minutely verruculose, generally with 4-7 transverse and some longitudinal or oblique septa, slightly or not constricted at the septa, overall length: 22-95 µm (54), 8-19 µm thickness in the widest part, 2-

4 μm at tip and 4-5 μm in the broadest part (Figure 2). Characteristics of this group of isolates were consistent with *Alternaria tenuissima* (Kunze) Wiltshire (Ellis, 1971).

Characteristics of Group II: Colonies effuse, olive green to black. Conidiophores simple or branched, straight or flexuous, septate, light brown to moderate or olive brown, up to 300 μm in length, 3-5 μm in thickness, with terminal scar and sometimes with one or two lateral scars. Conidia solitary or simple or in 2-7 branched chains, straight to slightly curved with various shapes but generally obclavate or oval, mostly rostrate, light brown or moderate brown or sometimes dark or olive brown, smooth to verruculose with up to 8 traverse and numerous longitudinal or oblique septa, constricted at the septa, 8-60 (42) μm long, 6-24 (17) μm thickness in widest part (Figure 3). The characteristics of this group of isolates conformed to those of *Alternaria citri* (Ellis & Pierce) (Ellis, 1971).

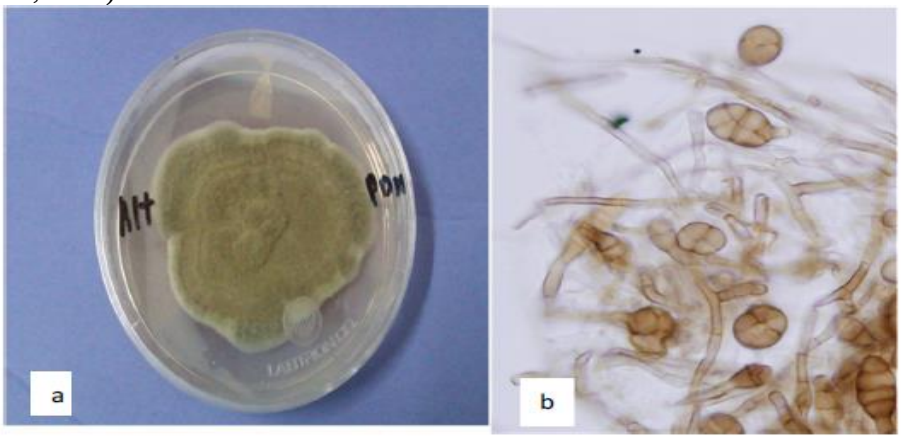


Figure 3. *Alternaria citri*: a) Colony on PDA, b) Conidia and conidiophores (× 1200)

Characteristics of Group III: Colonies 5 cm diam, with 3-4 pairs of well-defined concentric circles of growth and sporulation. Colonies exhibited a series of strongly delimited, dark, concentric circles of dense sporulation concentrated near the substrate surface in light-exposed zones. These rings alternate with less dense zones of long, ascending and variously twisted aerial hyphae plus a few longer, trailing hyphae or hyphal ropes that wander over the aerial surface. The aerial texture appears openly wooly and slightly arachnoid. Conidiophores that sporulate in the surface mass commonly were unbranched but had 1-3 geniculate extensions and conidiogenous loci incorporated in a total length of 50-100 μm. Each conidiogenous site produced a single conidium or more commonly a short chain or a branching chain of conidia, yielding a terminal cluster that was loose or crowded in density. The conidium population of a solitary, relatively small and open clump can be counted (at 50X) as 50-100. Clumps crowded within a surface ring were estimated to contain at least two or three times that number, with many comprising several thousand conidia. The architecture of an individual large head

of sporulation derives from the primary plurigeniculate conidiophore; primary conidia at each geniculate site of the initial conidiophore, with each conidium generating its own apical, plurigeniculate secondary conidiophore of variable length; and enormous numbers of relatively small conidia in closely branching chains. The long hyphal elements that make up the open aerial layer of the colony may be simple or in funiculose ropes of 2-3 parallel hyphae, commonly 1 mm in length and 3-4 µm wide. They were variously twisted and curved, branching and intersecting, but each unit was distinct and can be traced visually through the layer. They produced scattered lateral conidiophores of variable length, each with a few conidia. Each of these long aerial axes also produced several branches near its tip. The largest conidia usually were primary and basal to a branching sporulation mass; each reached a size range of 35-40 × 7-9 µm, with up to 7 transverse septa and no longisepta, or with a single longiseptum in 1-4 of the transverse segments. The spore body of these conidia was narrow-ellipsoid or long-ovoid with a pyramidal apical cell. Usually the apical cell generated a secondary conidiophore of variable length, commonly 30-110 × 3-4 µm in size. Each secondary conidiophore had several geniculate extensions, commonly 4-6, with conidiogenous sites that generated branching chains of secondary conidia. Conidia of intermediate size were ellipsoid or narrow-ovoid, reach a size range of ca 15-30 × 5-7 µm, and had 3-5 transverse septa and no longisepta. These conidia were very abundant and were critical components in the elaboration of the branching system, in that each had a short apical secondary conidiophore with 1-3 geniculate extensions that generated terminal chains of spores. Conidia that constitute the terminal chains were produced in enormous numbers. They were ca 7-15 × 4-7 µm in size and had 0-3 transverse septa and usually no longisepta. The apical secondary conidiophore on each spore in a terminal chain was either a short single cell, a slight bulge differentiated in the apex of a conidium, or simply an apical perforation. Most conidia were almost smooth-walled and a medium, clear greenish brown. Largest conidia that were most advanced in septation may became slightly roughened and a darker brown at maturity (Figure 4). The characteristics of this group of isolates conformed to those of *Alternaria infectoria* E. G. Simmons (Simmons, 1986).

Characteristics of Group IV: Colonies on PDA medium attaining 80 mm diameter in 14 d at 23ºC.Texture cottony, adpressed and partially submerged, light brown to pink. Ascomata scattered to aggregated, developed superficially or partially immersed in culture media when young. Pseudothecia globose to spherical, smooth, almost glabrous, usually not ostiolate, light brown to dark brown. Ascomata ornamentation consisting on septate and flexuose hyphae, 5-10 × 2-2.5 µm. Asci 80-110 × 10-13 µm, eight spored, cylindrical to clavate, broadly rounded above and gradually to abruptly tapering into a robust stipe of 10 × 5 µm. Pseudoparaphyses 10-15 µm, filiform, septate and longer than the asci, mixed with them and bifurcate. Ascospores 32-47 × 6-10 µm, two-celled, cells easily separable at the central septum, cylindrical, hyaline to olivaceous. When young and finally becoming olivaceous brown to dark brown when mature;

transversely septate, constrictions at septa broad and shallow, middle cells of equal length and broader than terminal cells, provided with rounded apices; germ slit diagonal, oblique or parallel and straight to sinuous; gelatinous sheath hyaline and narrow, less than 4 μm wide (Figure 5). The characteristics of this group corresponded with *Preussia* sp. Fuckel (Cain, 1961; Arenal et al., 2004; Arenal et al., 2007).

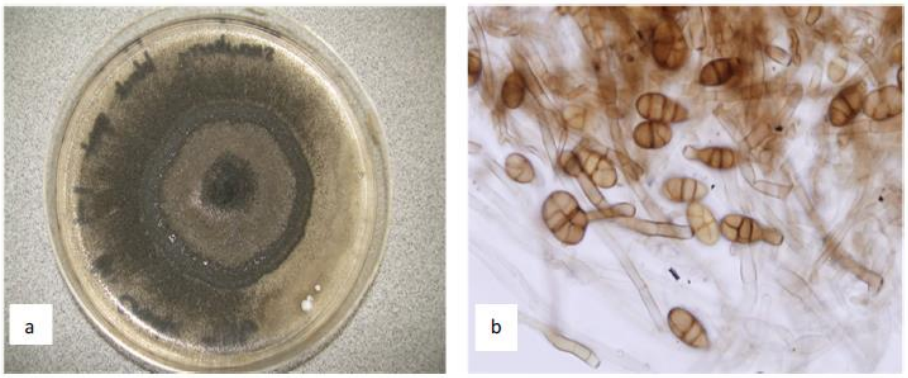


Figure 4. *Alternaria infectoria*: a) Colony on PDA, b) Conidia and conidiophores (× 1200)

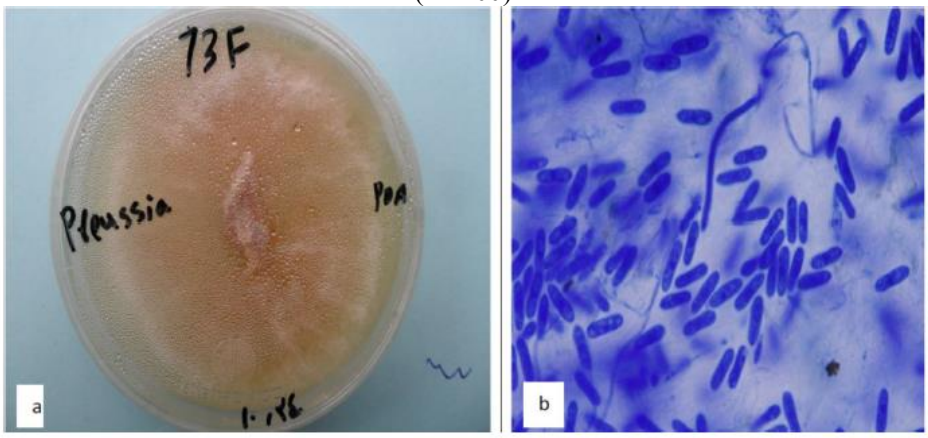


Figure 5. *Preussia* sp.: a) Colony on PDA, b) Ascospores (× 1200)

Characteristics of Group V: Initially cultures had white mycelia but occasionally developed violet pigments with age. Blue-black sclerotia developed in some isolates. Macroconidia relatively long and slender, slightly falcate or straight and thin walled. Apical cell curved and often tapered to a point, basal cell notched or foot shaped and 3-5 septa. Microconidia oval to club shaped with a flattened base and usually without septa. Conidiogenous cells monophialidic which were occasionally produced in pairs. Chlamydospores were not produced (Figure 6). The characteristics of this group of isolates conformed to those of *Fusarium verticillioides* (Saccardo) Nirenberg (Leslie and Summerell, 2006).

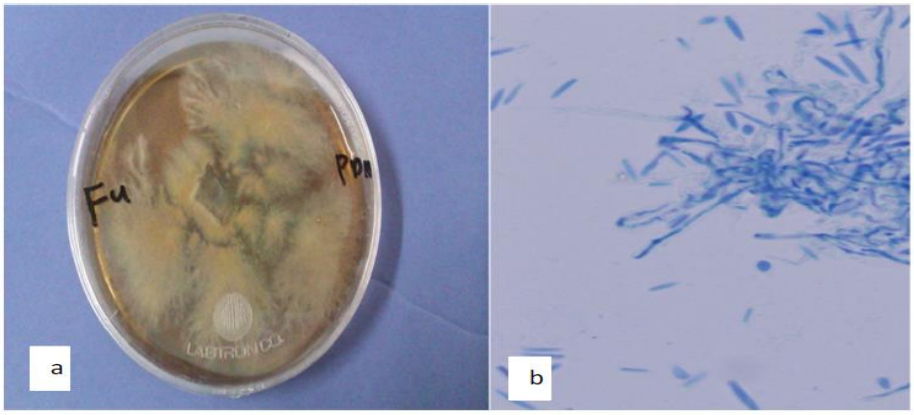


Figure 6. *Fusarium verticillioides*: a) Colony on PDA, b) Conidia (× 1200)

Characteristics of Group VI: Colonies growing rapidly (most isolates 7–9 cm). Conidiation predominantly effuse, appearing granular or powdery due to dense conidiation; rapidly turning yellowish-green to dark green, or producing tufts or pustules fringed by sterile white mycelium. Reverse colourless to dull yellowish, buff or drab. Odour indistinct or faintly earthy. Conidiophores as in the section, tending to be regularly verticillate forming a pyramidal structure. Phialides ampulliform to lageniform, usually 3–4-verticillate, occasionally paired, mostly 3.5–7.5 × 2.5–3.8 µm, terminal phialides up to 10 µm long. Conidia subglobose to obovoid, mostly (2.5–) 2.7–3.5 × 2.1–2.6 (–3.0) µm, smooth-walled, subhyaline to pale green (Figure 7). The characteristics of this group corresponded with *Trichoderma harzianum* Rifai (Gams and Bissett, 1998).

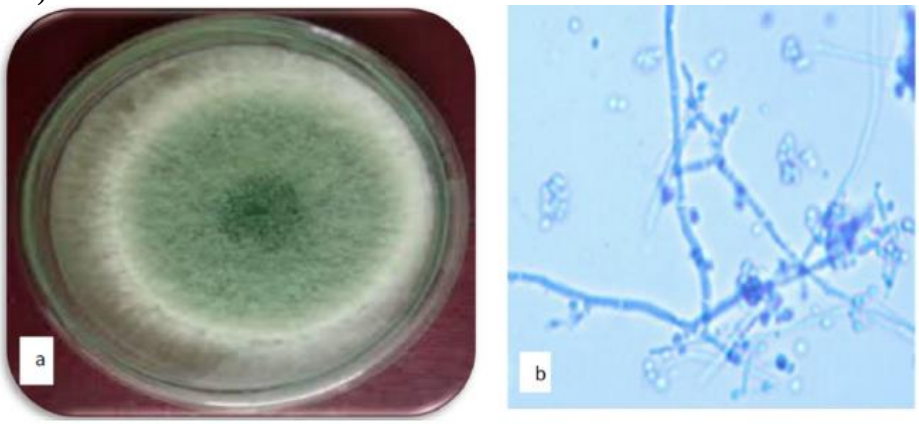


Figure 7. *Trichoderma harzianum*: a) Colony on PDA, b) Conidia and conidiophores (× 1200)

Characteristics of Group VII: Colonies growing rapidly (6–7 cm). Conidiation mostly predominantly effuse, covering the entire surface of the plate, or forming spreading, flat pustules concentrated near the margin of the plate or

arranged concentrically; quickly turning dark bluishgreen. Reverse colourless, or slowly developing dull yellowish to amber shades. Odour indistinct. Conidiophores in areas of effuse conidiation arising as lateral branches from undifferentiated aerial mycelium, at the base frequently sterile and unbranched for about half the length, toward the apex branching irregularly with each branch terminated by a cluster of 3–6 closely appressed phialides; macronematous conidiophores branching irregularly, the upper part fertile to the apex and the apex frequently bearing a terminal whorl of appressed branches and phialides; primary branches usually arising singly or in opposite pairs immediately beneath septa, the entire branching system irregular and uncrowded. Phialides from complex conidiophores lageniform to ampulliform, mostly 4.5–10 (–13) × 2.8–5.5 µm, mostly arising in closely appressed verticils of 2–5 on terminal branches, occasionally solitary or in pairs laterally on the conidiophore and branches; phialides from effuse areas of conidiation lageniform to subulate, up to 20 μm long × 2.5–3 µm. Conidia broadly ellipsoidal to obovoid, mostly 3.5–6.0 × 2.8–4.1 µm, smooth-walled, dark green, conidia from adjacent phialides often coalescing into large gloeoid masses (Figure 8). The characteristics of this group corresponded with *Trichoderma virens* (J. Miller, Giddens & Foster) von Arx, Beih (Gams and Bissett, 1998).

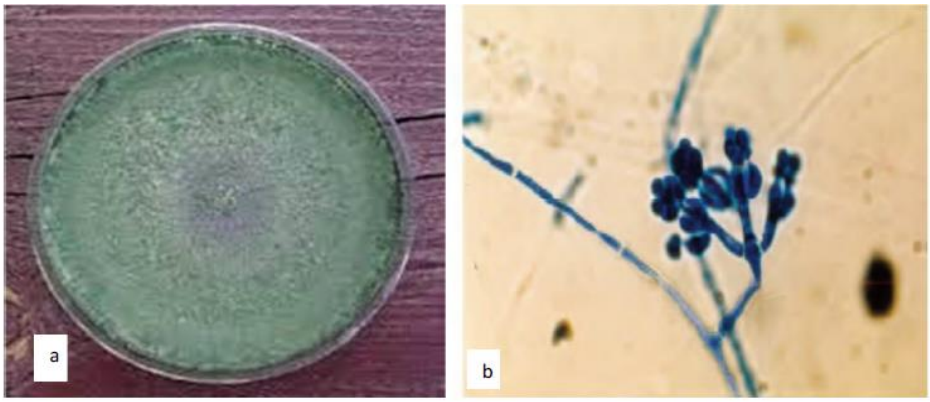


Figure 8. *Trichoderma virens*: a) Colony on PDA, b) Conidia and conidiophores (× 1200)

In evaluation of inhibition of B. oryzae growth by culture filtrate, it was found that T. harzianum had the highest inhibitory effect of 66.79% on the growth of B. oryzae colony. The isolates of T. virens, A. tenuissima, Preussia sp., F. verticillioides, A. infectoria and A. citri had the next highest efficiencies in reducing the growth of colony of B. oryzae (Table 1). Analysis of variance of the growth inhibition showed significant differences among the studied fungi at the 1% probability level.

Table 1. Comparison of means of growth inhibition by Least Significant Difference (LSD) in culture filtrate method

Fungal isolates	Growth inhibition (%)
F. verticillioides	43.37 bc
A. tenuissima	51.34 b
T. harzianum	66.79 a
T. virens	65.72 a
A. citri	41.39 c
A. infectoria	43.13 bc
Preussia sp.	47.51 bc
LSD 5%	9.562

Treatments having at least one similar letter do not show a significant difference at P=0.05.

In evaluation of hyperparasitism test, the hyphae of *T. harzianum* and *T. virens* did not coil around the mycelium of the fungal agent of rice brown spot disease. The hyphae isolates of *Preussia* sp. and *F. verticillioides* penetrated into the mycelium of *B. oryzae*, but were not able to deform them. The hyphae of *A. tenuissima*, *A. infectoria* and *A. citri* penetrated into the mycelium of *B. oryzae* once they reached them and then, they tore the fungal mycelium and deformed them.

In evaluation of inhibitory effect of volatile metabolites on *B. oryzae* growth, *T. harzianum* had the highest inhibitory effect of 83.13% on mycelial growth of *B. oryzae*. The next highest inhibitory effect on reducing mycelial growth of brown spot fungus was exerted by the isolates of *T. virens*, *A. tenuissima*, *A. infectoria*, *A. citri*, *Preussia* sp. and *F. verticillioides*, respectively (Table 2).

Table 3. Comparison of means of growth inhibition by Least Significant Difference (LSD) in dual culture method

Fungal isolates	Growth inhibition (%)
F. verticillioides	34.63 bc
A. tenuissima	41.99 b
T. harzianum	52.87 a
T. virens	51.63 a
A. citri	32.63 c
A. infectoria	34.57 bc
Preussia sp.	38.23 bc
LSD 5%	8.058

Treatments having at least one similar letter do not show a significant difference at P=0.05.

According to the analysis of variance of inhibition percentage in this method, the treatments showed significant differences at the 1% probability level. It was revealed that fungi had significant differences in inhibition percentage.

In evaluation of dual culture method, the growth of *B. oryzae* in the absence of *F. verticillioides* averaged 60.1 mm (in control), whilst the growth of these isolates in the presence of *F. verticillioides* averaged 39.29 mm implying

that *F. verticillioides* suppressed the mycelial growth of *B. oryzae* isolates. The suppression percentage of *B. oryzae* growth by *F. verticillioides* was 34.63% (Figure 9).

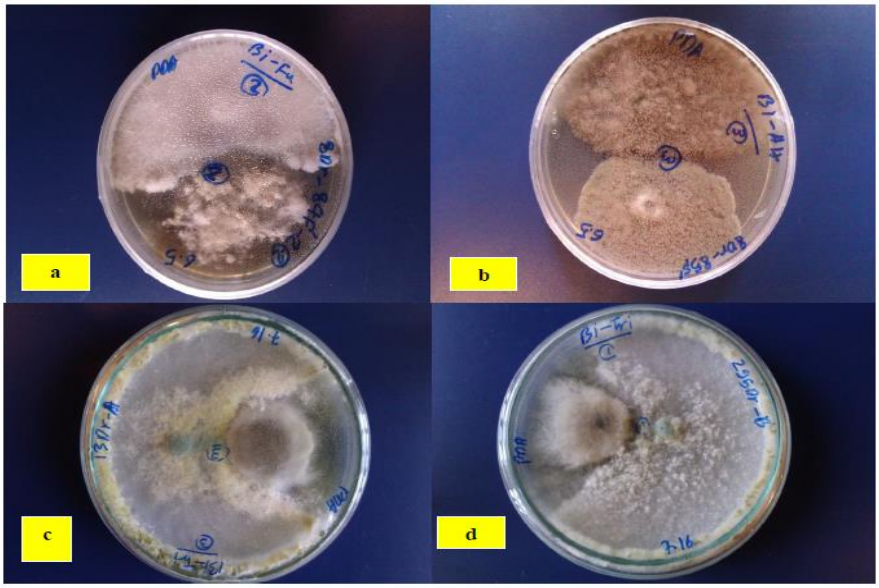


Figure 9. Dual culture method: a) *B. oryzae* × *F. verticillioides*, b) *B. oryzae* × *A. tenuissima*, c) *B. oryzae* × *T. harzianum*, d) *B. oryzae* × *T. virens*

Mean growth of *B. oryzae* isolates was 60.77 mm in the absence of *A. tenuissima* (in controls), whilst mean growth of these isolates was 35.26 mm in the presence of *A. tenuissima*. It shows that *A. tenuissima* stunted the mycelial growth of *B. oryzae* isolates by 41.99% (Figure 9).

The growth of *B. oryzae* isolates averaged 60.82 mm in the absence of *T. harzianum* antagonist (in controls) whilst it was 28.66 mm in the presence of *T. harzianum* implying the role of *T. harzianum* in inhibiting the mycelial growth of *Bipolaris* spp. isolates by 52.87% (Figure 9).

Mean growth of *B. oryzae* isolates was 60.8 mm (in controls) in the absence of *T. virens* and 29.41 mm in its presence. It concludes that *T. virens* inhibited the mycelial growth of *B. oryzae* isolates by 51.63% (Figure 9).

B. oryzae isolates grew up to 65.31 mm in the absence of *A. citri* (in controls), while they reached 44 mm in the presence of *A. citri* implying that *A. citri* hindered the mycelial growth of *B. oryzae* isolates by 32.63% (Figure 10).

The growth of *B. oryzae* isolates averaged 65.3 mm (in controls) in the absence of *A. infectoria*, whereas it was 42.72 mm in the presence of this fungus. It reveals that *A. infectoria* inhibited the mycelial growth of *B. oryzae* isolates by 34.57% (Figure 10). Mean growth of *B. oryzae* isolates was 75 mm (in controls) in the absence of *Preussia* sp. and 46.33 mm in its presence. It concludes that *Preussia* sp. inhibited the mycelial growth of *B. oryzae* isolates by 38.23% (Figure 10).

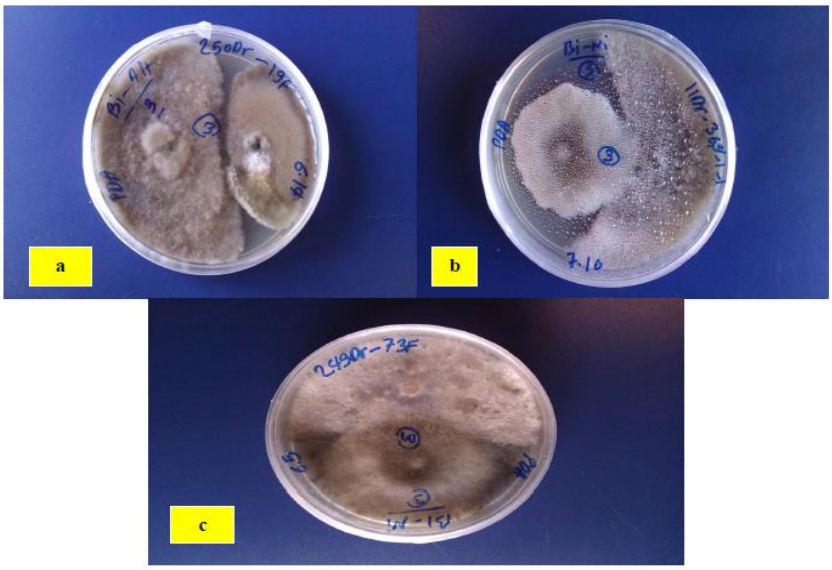


Figure 10. Dual culture method: a) *B. oryzae* × *A. citri*, b) *B. oryzae* × *A. infectoria*, c) *B. oryzae* × *Preussia* sp.

Inhibition percentage was calculated according to the results of dual culture, and out of 20 isolates used in dual culture, seven isolates suppress the growth of *B. oryzae* isolates more than others: *F. verticillioides*, *A. tenuissima*, *T. harzianum*, *T.virens*, *A. citri*, *A. infectoria* and *Preussia* sp. Following *T. harzianum* which had the highest inhibition percentage for *B. oryzae*, the isolates of *T. virens*, *A. tenuissima*, *Preussia* sp., *F. verticillioides*, *A. infectoria* and *A. citri* had the highest suppression percentage, respectively. Analysis of variance of inhibition percentage in dual culture showed significant differences among fungi at the 1% probability level (Table 4). Means comparison for inhibition percentage by least significant differences (LSD) method led to these results: the highest suppression percentage was related to treatments 3 and 4, i.e. control with *T. harzianum* and *T. virens*, which exhibited significant differences with treatments 1, 2, 5, 6 and 7 and the lowest suppression percentage was related to treatment 5, i.e. control with *A. citri* which had no significant differences with treatments 1, 6 and 7 (*F. verticillioides*, *A. infectoria* and *Preussia* sp.) (Table 3). Therefore, controlling with *T. harzianum* and *T. virens* showed the best efficiency among all studied fungi.

In the present study, *Bipolaris* spp., *Alternaria* spp., *Nigrospora* spp., *Fusarium* spp., *Trichoderma* spp. and *Preussia* sp. and some saprophytic fungi were isolated from rice from paddy fields of Guilan province. After elementary identification at genus level, 68 isolates were used for pathogenic studies and their pathogenicity of all isolates of *Bipolaris* spp. (34 isolates) was proved on rice. *Alternaria citri* which is pathogenic on citrus and also, *Preussia* sp. were among the fungi that were isolated from rice and were reported for the first time on rice from Iran.

Table 4. Variance analysis of inhibition mycelial growth

SOV	DF	MS
Treatment	6	207.191**
Error	14	20.518
C.V.	-	11.064

** Significance of the probability level of 1%

SOV: sources of variations; DF: degree of freedom; MS: squares mean

Among fungal isolates used *in vitro*, seven isolates including *Fusarium verticillioides*, *Alternaria tenuissima*, *Trichoderma harzianum*, *Trichoderma virens*, *Alternaria citri*, *Alternaria infectoria* and *Preussia* sp. suppressed mycelial growth of *Bipolaris oryzae* more efficiently than other isolates. In dual culture and culture filtrate methods, the isolates of *T. harzianum*, *T. virens*, *A. tenuissima*, *Preussia* sp., *F. verticillioides*, *A. infectoria* and *A. citri* inhibited the mycelial growth of *B. oryzae* more than other isolates, respectively but in volatile metabolites method, the most effective isolates in inhibition were respectively: *T. harzianum*, *T. virens*, *A. tenuissima*, *A. infectoria*, *A. citri*, *Preussia* sp. and *F. verticillioides*.

The isolates applied in these methods were all more efficient in volatile metabolites method in biological control of rice brown spot disease.

T. harzianum suppressed the growth of *B. oryzae* isolates by 52.87-83.13% *in vitro* and found to be the most effective isolate which was in agreement with Khalili et al. (2012) who stated that *Trichoderma* spp. isolates considerably inhibited the *in vitro* mycelial growth of *B. oryzae* and also was in agreement with Abdel-Fattah et al. (2007) who stated that *T. harzianum* antifungal metabolites suppressed the linear growth of *B. oryzae*.

T. virens isolates suppressed the *in vitro* mycelial growth of *B. oryzae* by 51.63-76.41%. It was consistent with Ru and Di (2012) and Khalili et al. (2012) who found that *T. virens* had a good influence on biological control of potato dry rot and rice brown spot diseases, respectively.

In another study, the biological control effect of *Alternaria infectoria* against *Ceroplastes rusci* as a plant pest was examined and it was evaluated as to be effective on the biological control of this pest (Shabana and Ragab, 1997).

Naeimi et al. (2011) studied antagonistic effect of *Trichoderma* strains on *R. solani*. *In vitro Trichoderma* strains effectively reduced the growth of *R. solani*. Some strains inhibited the production of *R. solani* sclerotia *in vitro* and in field conditions and suppressed the germination and growth of developed sclerotia. Seven *Trichoderma* strains that were shown in greenhouse assessments to have the highest effect were antagonist in dual culture too. In contrast, no relationship was found between their *in vitro* biological control activities and their usefulness in the control of rice sheath blight in greenhouse. For instance, *T. harzianum* AS12-2 did not hinder the *in vitro* formation of sclerotia, but was the most effective isolate in the control of rice sheath blight in greenhouse. The spray

of these antagonists' spores on rice plants infected by *R. solani* provided an effective transferring system for the control of this disease.

Akrami et al. (2011) examined the effect of *Trichoderma* spp. isolates on *Fusarium* sp. The results of dual culture revealed that medium was soon colonized with *Trichoderma* isolates and that all evaluated *Trichoderma* isolates were effective in the control of *Fusarium* isolates. As well, the assessment of the production of volatile and non-volatile substances showed promising performance in the suppression of pathogenic mycelial growth. They, also, stated that *T. vierns* was very effective in the control of *Fusarium* sp. at 35°C in damp soil and three isolates, *T. harzianum*, *T. asperellum*, and *T. virens*, were effective against lentil *Fusarium* rot.

In a study, it was indicated that rice inoculation with spore suspension of *Trichoderma* isolates reduced the germination of *B. oryzae* spores on plant significantly (Tsahouridou and Thanassoulopoulosh, 2002).

Khalili et al. (2012) studied antagonistic activity of *T. harzianum* against *Bipolaris oryzae*. *In vitro* tests showed that local isolates of *Trichoderma* sp. significantly suppressed the mycelial growth of *B. oryzae*. According to the results, there was clearly a competition between *T. harzianum* and *B. oryzae*. *Trichoderma* isolates grow faster and outperform the pathogenic fungus in the competition for space and nutrients resulting in the inhibition of the growth of the target organism. Microscopic observations showed no mycoparasitism between *Trichoderma* and *B. oryzae* isolates that was in agreement with present study.

In a study on the antagonistic effect of seven isolates including *F. verticillioides*, *A. tenuissima*, *T. harzianum*, *T. virens*, *A. citri*, *A. infectoria* and *Preussia* sp. on the mycelial growth of *B. victoriae* in laboratorial and greenhouse conditions, it was revealed that *T. harzianum* was the most effective antagonist in suppressing the mycelial growth of *B. oryzae* under laboratorial conditions and *Preussia* sp. and *T. harzianum* were the most effective isolates on reducing the intensity of brown spot disease under greenhouse conditions (Mohammadian, 2013) which was consistent with our findings in the present study *in vitro*.

CONCLUSIONS

Biological control is one of the best control methods against some plant pathogens. This strategy of control is ecologically clean and compatible with different models of agriculture organic biological and pathogen management.

This study indicated that *T. harzianum*, *T. virens* and *A. tenuissima* were the most effective fungal isolates in biological control of rice brown spot disease agent. So, they can be introduced as antagonistic fungi.

The study revealed that there are some fungi in natural rice flora that have potential antagonism for biological control of the causal fungus of rice brown spot disease. The identification and examination of these fungi *in vitro*, greenhouse and paddy fields levels can be promising about the efficiency of biological control in the management of rice brown spot disease.

ACKNOWLEDGMENT

This experiment was supported by the Islamic Azad University, Rasht Branch, Iran.

REFERENCES

Abdel-Fattah, G. M., Shabana, Y. M., Ismail, A. E. & Rashad, Y. M. (2007). *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. Mycopathologia, 164 (2), 81-89.

Akrami, M., Golzary, H. & Ahmadzadeh, M. (2011). Evaluation of different combinations of *Trichoderma* species for controlling *Fusarium* rot of lentil. African Journal of Biotechnology, 10(14): 2653-2658.

Arenal, F., Platas, G. & Peláez, F. (2004). Variability of spore length in some species of the genus *Preussia* (*Sporormiella*). Mycotaxon, 89: 137-151.

Arenal, F., Platas, G. & Pelaez, F. (2007). A new endophytic species of *Preussia* (*Sporomiaceae*) inferred from morphological observations and molecular phylogenetic analysis. Fungal Diversity, 25:1-17.

Cain, R.F. (1961). Studies of coprophilous ascomycetes. VII. *Preussia*. Canadian Journal of Botany, 39: 1633-1666.

Carissimi, M., Giraudo, M. S., Germani, J. C. & Van Der Sand, S. T. (2009). Antifungal activity of *Bacillus* sp. E164 against *Bipolaris sorokiniana*. Biociencias, 17(1):48-58.

Dennis, C. & Webster, J. (1971a). Antagonistic properties of species-groups of *Trichoderma*. I. Production of nonvolatile antibiotics. Transactions of the British Mycological Society, 57(1): 25-39.

Dennis, C. & Webster, J. (1971b). Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. Transactions of the British Mycological Society, 57(1): 41-48.

Elad, Y., Chet, I. & Katan, J. (1980). *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology, 70:119-121.

Ellis, M. B. (1971). Dematiaceous Hyphomycetes. CMI, Kew, England.

Gams, W. & Bissett, J. (1998). Morphology and identification of *Trichoderma*. In: Kubicek, C. P., Harman, G. E., (eds). *Trichoderma* and *Gliocladium*. Vol. 1. Basic Biology, Taxonomy and Genetics, Taylor and Francis Ltd., London: 3-34.

Howell, C. R. (1982). Effect of *Gliocladium virens* on *Pythium ultimum*, *Rhizoctonia solani* and damping-off of cotton seedlings. Phytopathology, 72:496-498.

Kazemzadeh Chekosari, M. (2003). A feasibility study on biological control of rice sheat blight disease (*Rhizoctonia solani*) by some bacterial biocontrol agents. Tehran University, Abureyhan Higher Education Institution (M.Sc. thesis). (in Persian with English abstract).

Khalili, E., Sadravi, M., Naeimi, Sh. & Khosravi, V. (2012). Biological control of rice brown spot with native isolates of three *Trichoderma* species. Brazilian Journal of Microbiology, 43 (1): 297-305.

Khodaparast, S. A. & Sahragard, A. (2004). Rice diseases. The University of Guilan Press. (in Persian) .

Leslie, J. F. & Summerell, B. A. (2006). *The Fusarium*, laboratory manual. Blackwell Publishing.

Lewis, J. A. & Papavizas, G. C. (1991). Biocontrol of cotton damping-off caused by *Rhizoctonia solani* in the field with formulations of *Trichoderma* spp. and *Gliocladium virens*. Crop Protection, 10:396-402.

Manimegalai, V., Ambikapathy, V. & Panneerselvam, A. (2011). Biological control of paddy brown spot caused by *Bipolaris oryzae*. European Journal of Experimental Biology, 1 (4):24-28.

McSpadden, G. B. B. & Fravel, D. R. (2002). Biological control of plant pathogens, Research commercialization and application in the USA. Plant Health Progress, 10.1094/PHP-2002-0510-01-RV.

Mohammadian, S. (2013). Biological control of *Bipolaris spp*., the causal agent of brown spot disease of rice by some antagonistic fungi in Guilan province. Islamic Azad University, Science and Research Branch, Guilan, Rasht (M.Sc. thesis). (in Persian with English abstract).

Mpika, J., Kébé, I. B., Issali, A. E., N'Guessan, F. K., Druzhinina, S., Komon-Zélazowska, M., Kubicek, C. P. & Aké, S. (2009). Antagonist potential of *Trichoderma indigenous* isolates for

biological control of *Phytophthora palmivora*, the causative agent of black pod disease on cocoa (*Theobroma cacao* L.) in Côte d'Ivoire. African Journal of Biotechnology, 8(20):5280-5293.

Moura, A. T., Ludwig, J., Santos, A. G., Schafer, J. T., Soares, V. N. & Correa, B. O. (2014). Biocontrol and seed transmission of *Bipolaris oryzae* and *Gerlachia oryzae* to rice seedlings. Journal of Seed Science, 36(9): 407-412.

Naeimi, Sh., Okhovvat, S. M., Javan-Nikkhah, M., Vágvölgyi, C., Khosravi, V. & Kredicks, L. (2011). Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. Phytopathologia Mediterranea, 49 (3):287-300.

Nazari, S., Javan Nikkhah, M., Khalil-Berdi, F., Khosravi, V. & Alizadeh, A. (2015). *Bipolaris* species associated with rice plant: pathogenicity and genetic diversity of *Bipolaris oryzae* using rep-PCR in Mazandaran province of Iran. Journal of Crop Protection, 4 (4): 497-508.

Niknejad, M. & Sharifi Tehrani, A. (1993). A study on antagonistic effect of *Trichoderma* on the causal agent of tomato *fusarium* wilting disease, *Fusarium oxysporum* f. sp. *lycopersici in vitro* and in greenhouse. 11th Iranian Congress of Plant Protection, 27 Augest - 1 September, Rasht, pp. 165. (in Persian with English abstract).

Ningthoujam, D. S., Sanasam, S., Tamreihao, K. & Nimaichand, S. (2009). Antagonistic activities of local actinomycetes isolates against rice fungal pathogen. African Journal of Microbiology Research, 3(11):737-742.

Ou, S. H. (1985). Rice diseases. Commonwealth Mycological Institute, 2nd ed.

Ru, Z., Di, W. (2012). *Trichoderma* spp. from rhizosphere soil and their antagonism against *Fusarium sabbucinum*. African Journal of Biotechnology, 11(18):4180-4186.

Safari Motlagh, M. R. (2010). Isolation and characterization of some important fungi from *Echinochloa* spp., the potential agents to control rice weeds. Australian Journal of Crop Science, 4(6):457-460.

Safari Motlagh, M. R. (2000). Etiology of rice brown spot disease in Guilan province. Tehran University (M.Sc. thesis). (in Persian with English abstract).

Safari Motlagh, M. R., Padasht Dehkaee, F. & Hedjaroud, G.A. (2005). Rice brown spot disease and evaluation of the response of some rice cultivars to it. Journal of Science and Technology of Agriculture and Natural Resources, 9(2): 171-182. (in Persian with English abstract).

Salehpour, M., Etebarian, H. R., Roustaei, A., Khodakaramian, G. & Aminian, H. (2005). *Biological control of common root rot of wheat* (*Bipolaris sorokiniana*) *by Trichoderma isolates*. Plant Pathology Journal, 4(1): 85-90.

Shabana, Y. M. & Ragab, M. E. (1997). *Alternaria infectoria*, a promising biological control agent for the Fig Wax Scale, *Ceroplastes rusci* (Homoptera: Coccidae), in Egypt. Biocontrol Science and Technology, 7:553-564.

Simmons, E. G. (1986). *Alternaria* themes and variations (22-26). Mycotaxon, 25(1):287-308.

Sivakumar, D., Wilson Wijeratnam, R. S., Wijesundera, R. L. C., Marikar, F. M. T. & Abeyesekere, M. (2000). Antagonistic effect of *Trichoderma harzianum* on postharvest pathogens of Rambutan *(Nephelium lappaceum)*. Phytoparasitica, 28 (3):240-247.

Sivanesan, A. (1987). Gramainicolous species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their telemorphs. CAB International Mycological Institute.

Soltani Nejad, M., Shahidi Bonjar, G. H. & Padasht Dehkaei, F. (2014). Control of *Bipolaris oryzae* the causal agent of rice brown spot disease by soil *Streptomyces* sp. isolate G. International Journal of Advanced Biological and Biomedical Research, 4(2): 310-317.

Srivastava, R. & Shalini, R. S. (2008). Antifungal activity of *Pseudomonas fluorescens* against different plant pathogenic fungi. Journal of Environmental, Agricultural and Food Chemistry, 7(4):2789-2796.

Tsahouridou, P.C. & Thanassoulopoulosh, C. C. (2002). Proliferation of *Trichoderma koningii* in the tomato rhizosphere and the suppression of damping off by *Sclerotium rolfsii*. Soil Biology & Biochemistry, 34:767-776.

Zhang, Z. & Yuen, G. Y. (2000). The role of chitinase production by *Stenotrophomonas maltophilia* strain C3 in biological control of *Bipolaris sorokiniana*. Phytopathology, 90:384-389.

DOI: 10.17707/AgricultForest.63.1.32

Anto MIJIĆ, Ivica LIOVIĆ, Aleksandra SUDARIĆ, Drena GADŽO, Zoran JOVOVIĆ, Mirjana JANKULOVSKA, Antonela MARKULJ KULUNDŽIĆ, Tomislav DUVNJAK[1]

THE EFFECT OF ENVIRONMENT ON THE PHENOTYPIC EXPRESSION OF GRAIN YIELD, OIL CONTENT AND OIL YIELD IN SUNFLOWER HYBRIDS

SUMMARY

Continuous sunflower breeding work at the Agricultural Institute Osijek results in the creation of new hybrid combinations which are then tested in a network of micro and macro trials. This paper presents the results of the investigation on seven sunflower hybrids: one standard and six new hybrid combinations (OS-H-2 to OS-H-7) from the Agricultural Institute Osijek, in a period of four years (2012-2015) at a location in Osijek. Trials were set as RCBD and the most important sunflower traits were analyzed: grain yield, oil content and oil yield. The results indicate that the values of analyzed traits were significantly impacted by year (and respectively by weather conditions), hybrid and the year x hybrid interaction. The highest yield was observed in 2013, when grain yield achieved 6.243 t/ha and oil yield 2.889 t/ha. The highest oil content was recorded in the year 2012 (51.35%). The highest grain yield was achieved by the hybrids OS-H-4 (6.257 t/ha) and OS-H-2 (5.911 t/ha), and oil content by hybrids OS-H-3 (51.83%) and OS-H-4 (51.62%). The hybrid OS-H-4 had the highest oil yield (2.944 t/ha), and it can therefore be considered as a promising hybrid for application on the recognition process in the country and abroad.

Keywords: sunflower, hybrid, grain yield, oil content, oil yield.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an extremely important field crop. Primarily, it is grown for oil used in human consumption. Sunflower oil is a valuable source of tocopherols and phytosterols and has a positive impact on human health (Patel and Thompson, 2006, Gotar et al., 2008, Jocković et al., 2014).

It is important as raw material for the processing industry, used as animal feed and in beekeeping. It is grown on about 24 million hectares around the world, with total annual production of about 33 million tons of grain. In the EU,

[1]Anto Mijić, Ivica Liović, Aleksandra Sudarić, Antonela Markulj Kulundžić, Tomislav Duvljan Agricultural Institute Osijek, Južno predgrađe 17, 31103, Osijek, CROATIA, Drena Gadžo University of Sarajevo, Faculty of Agriculture and Food Sciences, Zmaja od Bosne 8, 71000, Sarajevo, BOSNIA AND HERZEGOVINA, Zoran Jovović University of Montenegro, Biotehnical faculty, Mihaila Lalića 1, 81000, Podgorica, MONTENEGRO, Mirjana Jankulovska St. Cyril and Methodius University in Skopje, Faculty for Agricultural Sciences and Food, blvd. Aleksandar Makedonski BB 1000 Skopje, MACEDONIA

sunflower production is organized on about 4 million hectares with an average grain yield of 1.37 t/ha. Grain yield in Croatia is at top of the EU (about 2.66 t/ha), and areas of sunflower are approximately 34000 ha (average 2005-2015) (FAOSTAT Database, 2016). The large fluctuations, both in terms of grain yield and in terms of areas of sunflower, should be emphasized (Liović et al., 2006, Mijić et al., 2011).

Higher grain yield, oil content, and oil yield per unit area in a variety of environments are the primary goals of every sunflower producer (Kaya and Kolsarici, 2011, Škorić, 2012). Therefore, these traits are the focus of the majority of sunflower breeders in the world. Grain yield, oil content and oil yield are quantitative traits determined by genetic potential with significant variation influenced by environmental factors. The phenotypic expression of these properties is conditioned by genetic factors, factors from the external environment, as well as their interaction (Krizmanić et al., 2012). The environmental conditions are often a limiting factor in sunflower production (Miklič et al., 2007, Škorić, 2012). The occurrence of drier vegetation season, especially in summer, with less rainfall, higher temperatures, extremely high daily temperature maximums, or fluctuations and weather parameters in the short term (daily, weekly) significantly affects all the living world and consequently field crops. This impact is mostly negative, which is reflected through reduced value of the most important agronomic traits, both in quantitative (grain and oil) and qualitative (oil quality) terms. In addition to the weather conditions, the soil is also an important factor for successful production, as well as the level of applied technology. More intense, more comprehensive technology significantly reduces the negative impact of weather on yield (Pepo and Molnarova, 2010).

The aim of this study was to compare grain yield, oil content and oil yield of seven sunflower hybrids during the period of four years in eastern Croatia, with emphasis on the rainfall impact and mean air temperature and to select the best hybrid combinations for further breeding work and the process of recognition.

MATERIAL AND METHODS

The basic material in the study included seven sunflower hybrids, out of which six are new hybrid combinations (OS-H-2 to OS-H-7) created at the Agricultural Institute Osijek. An introduced hybrid, which for many years has occupied a large area of sunflower in Croatia, was used as a standard. Hybrids were sown in duration of four years (2012 – 2015), according to a randomized block design with three replications at the experimental fields of the Agricultural Institute Osijek. The soil is eutric cambisol with good pedodynamic properties, medium supplied with phosphorus and potassium, pH in KCl 6.3. Other elements of the experiment were: the length of the basic plot of 5 m, width of the basic plot of 2.8 m, inter row spacing of 0.7 m, space in row 0.23 m, the distance between the blocks 1.5 m, the recommended stand density of 62112 plants per hectare.

During all years of research the standard agricultural practices were applied. The most important sunflower agronomic traits were analyzed. Grain yield per plot was recalculated per hectare according to the standard (9% moisture and 2% impurities). The oil content was determined by the nuclear magnetic resonance method (MQA 7005 NMR Analyser), and the oil yield was calculated based on grain yield and oil content.

The values obtained for individual traits were systematized by hybrids and years and statistically analyzed by the analysis of variance (ANOVA) with the LSD test at level P<0.05. For each hybrid combination minimum and maximum values were calculated and compared to the average of experiments (%). Analysis of stability was made by means of regression coefficient (b_i) that represents the ratio k of the genotype according to various environments and deviation from regression (s^2d_i) concerning the unpredictable variability of a part of the genotype was obtained from the sum of squared differences between the expected and the obtained yield (Becker and Leon, 1988).

Table 1: Monthly rainfall (mm) and mean monthly temperatures (°C)

Year	I-III	IV	V	VI	VII	VIII	IX	Sum
Precipitation (mm)								
2012	82.6	47.3	93.5	67.9	47.8	4.0	32.3	375.4
2013	213.1	44.5	132.1	61.2	44.8	86.8	106.3	688.8
2014	109.5	67.2	142.6	63.9	68.8	69.9	74.9	596.8
2015	167.9	10.6	108.6	44.9	7.6	50.8	29.1	419.5
Average	143.3	42.4	119.2	59.5	42.3	52.9	60.7	520.1
Average 1961-2014	124.9	54.2	64.8	85.3	63.4	62.1	56.1	510.8
Temperature (ºC)								Average
2012		12.5	16.9	22.5	24.8	24.1	18.9	19.9
2013		13.0	17.5	20.2	23.6	23.7	16.4	19.1
2014		13.5	16.4	20.8	22.2	21.2	17.4	18.6
2015		12.4	17.9	21.3	24.9	24.1	19.0	19.9
Average		12.9	17.2	21.2	23.9	23.3	17.9	19.4
Average 1961-2014		11.7	16.8	20	21.6	21	16.6	18.0

*source: Meteorological and Hydrological Service of the Republic of Croatia

Table 2: Analysis of variance for grain yield, oil content and oil yield

Source of variation	Grain yield	Oil content	Oil yield
	MS (mean squares)	MS (mean squares)	MS (mean squares)
Hybrid	1.845**	26.674**	0.438**
Year	4.468**	89.684**	1.488**
Hybrid x Year	1.020*	4.163**	0.246**

*F test significant on level P<0.05

** F test significant on level P<0.01

The analysis of variance indicated that the hybrid, year and their interaction had a significant impact on the grain yield, oil content and oil yield. It also revealed that the variability of studied traits in some hybrids is derived from the genetic diversity of the material selected for the research, diversity of environments (years) in which the traits were realized, and their interaction as well (Table 2).

Grain yield

Table 3 shows the average values for grain yield of the hybrids over the years. The highest yield was achieved in 2013 (6.243 t/ha) and the lowest in 2012 (5.193 t/ha). The year with the highest grain yield (2013) was characterized by good reserve of winter moisture (I-III month - 213.1 mm), and the total rainfall and its distribution by months and decades during this growing season was exceptionally good. This is in agreement with the research of Gadžo et al., (2011) who pointed out that sunflower crop responds very well to the abundance of accumulated moisture in the soil during winter. Also, the mean monthly temperatures were on the average level and the number of hot days was acceptable (Table 1). The reason for the low grain yield in 2012 is the fact that this was the warmest year during the research period and with higher temperatures compared to the multi-year average. During June, July and August, average monthly temperatures were higher compared to 2013 and 2014, and compared to 2015 this year had many more warm days. It should also be noted that during winter (January to March) the precipitation was only 82.6 mm, and during the growing season (April-September) 292.8 mm, which is considerably less compared to the other years of research and to the multi-year average. In August, at the stage of intensive grain filling, there was only 4 mm of rainfall. Similar results were presented by Bošnjak (1999) and Josipović and Mađar (2004).

Table 3: Grain yield (t/ha) of analyzed sunflower hybrids and range (%)

Hybrid	2012	2013	2014	2015	Average	Range (%)
OS-H-4	5.963	7.000	5.920	6.146	6.257	95-112
OS-H-2	5.660	6.075	5.798	6.111	5.911	96-103
STANDARD	4.253	6.795	6.184	6.399	5.908	72-115
OS-H-7	5.408	6.289	5.260	6.623	5.895	89-112
OS-H-6	4.593	6.326	5.613	6.125	5.664	81-112
OS-H-5	5.819	5.855	4.800	4.967	5.360	90-109
OS-H-3	4.654	5.362	4.913	5.183	5.028	93-107
Average	5.193	6.243	5.498	5.936	5.718	91-109
Min.	4.253	5.362	4.800	4.967	5.028	
Max.	5.963	7.000	6.184	6.623	6.257	

$LSD_{0.05}$ hybrid (A) 0.567
$LSD_{0.05}$ year (B) 0.429
$LSD_{0.05}$ AxB 1.134

The experimental hybrid OS-H-4 had an average grain yield of 6.257 t/ha. Also, this hybrid had the highest individual value, and in the most yielding 2013 achieved grain yield of 7.000 t/ha. Another hybrid which also achieved very good results, better than the standard, was OS-H-2 with grain yield of 5.911 t/ha.

The significance of the hybrid x year interaction indicates that some hybrids are better in wet and others in dry years. The standard hybrid had a grain yield of 4.253 t/ha in dry year 2012, which was the lowest yield in the experiment. In 2014, with above average rainfall during the growing season, especially in July and August, it was the best hybrid in the trial. The grain yield of this hybrid varied from 72% to 115% in relation to the average grain yield (Table 3).

Oil content

The highest oil content was achieved in the dry 2012 year (51.35%) and the lowest in 2014 (47.04%) (Table 4).

Table 4: Oil content (%) of analyzed sunflower hybrids and range of variation

Hybrid	2012	2013	2014	2015	Average	Range (%)
OS-H-3	53.69	53.31	48.73	51.59	51.83	94-104
OS-H-4	53.24	53.42	47.98	51.82	51.62	93-103
STANDARD	51.96	51.64	47.83	50.16	50.40	95-103
OS-H-7	51.05	51.89	46.56	50.44	49.99	93-104
OS-H-5	49.90	48.96	47.61	48.73	48.80	98-102
OS-H-6	49.43	47.18	47.14	48.62	48.09	98-103
OS-H-2	50.19	49.45	43.46	48.89	48.00	91-105
Average	51.35	50.83	47.04	50.04	49.82	94-103
Min.	49.43	47.18	43.46	48.62	48.00	
Max.	53.69	53.42	48.73	51.82	51.83	

$LSD_{0.05}$ hybrid (A) 0.77
$LSD_{0.05}$ year (B) 0.59
$LSD_{0.05}$ AxB 1.57

High values for oil content in 2012 can be explained by the fact that in this year the lowest grain yields and the lowest weight of 1000 grains were registered. A smaller volume of grain means a lower proportion of shell and endosperm in relation to the nucleus, and thus higher oil content. Vranceanu (1977) reported similar results, which explained that increasing the number of plants per unit area resulted in the development of seeds with smaller size, with lower shell relative to the nucleus. Flowering took place in late June and early July, in the period when rainfall was slightly lower, which allowed numerous flights of insects, and thus higher fertilization and higher oil content. Balalić et al. (2008) and Miklič et al. (2014) also point out weather conditions as an important factor in the phenotypic expression of this trait. Sunflower is a typically open pollinated,

entomophile plant and for higher fertilization insects are extremely important, as confirmed by researches of Waghchoure and Rana (1988) and Puškadija et al. (2009).

During the four-year research, statistically significant higher values of oil content in relation to the standard were found in OS-H-3 (51.83%) and OS-H-4 hybrid (51.62%). The experimental hybrid OS-H-7 had similar values as the standard. These results point to the progress in breeding for this very important sunflower characteristic, and good preconditions for further breeding work in creating high oil sunflower hybrids.

The significance of the hybrid x year interaction, apart from grain yield, was also established for oil content. The highest variation in relation to the average had OS-H-2 hybrid and the lowest the hybrids OS-H-5 and OS-H-6. The hybrid OS-H-5, which in the rainy year 2014 was the fourth, had significantly worse results in the remaining three years of study. Similar results were observed in the hybrid OS-H-6, which except in 2014 had the lowest value for oil content in all other years.

Oil yield

The research results in Table 5 indicate that the highest oil yield (2.889 t/ha) was achieved in 2013, then in 2015 (2.704 t/ha) and in 2012 (2.426 t/ha). The lowest oil yield was determined in 2014 (2.352 t/ha). As with grain yield, favorable weather conditions in 2013 had an extremely important role in the expression of this trait. A satisfactory reserve of winter moisture, good distribution of rainfall, a mean monthly temperature and not too high temperature maximums allowed the highest oil yields in this year. This is in agreement with the research of Krizmanić et al. (2013) and Kaya (2016) that highlight the daily mean temperature and moisture level as a significant factor in the determination of this trait.

It should be pointed out that 2012 was a very dry year (in August only 4 mm of rainfall), characterized by an extreme deficit of rainfall, warm days and shorter vegetation period and grain filling phase. Although the environmental conditions were not so favorable for sunflower growth and development, they did not significantly affect grain and oil yield. It can be said that sunflower, unlike other spring field crops (corn, soybean) significantly reduces the negative effects of stress caused by drought and achieves satisfactory results. The reason lies in the fact that sunflower has a strong root system, but also because of the specific anatomical structure of the stem and leaf. Similar results were reported by Škorić (2009). The significant influence of year on oil yield and also oil content was previously emphasized by Balalić et al. (2007). The experimental hybrid OS-H-4 had the highest oil yield during the research (2.944 t/ha). Also, this hybrid had the highest individual value of 3.403 t/ha in 2013. The lowest oil yield during the study was detected in the experimental hybrid OS-H-3 (2.372 t/ha). Individually, the hybrid which was used as standard achieved the lowest oil yield (2.011 t/ha) in 2012, but in 2013 was among the best yielding hybrids.

Table 5: The values of oil yield (t/ha) and range (%).

Hybrid	2012	2013	2014	2015	Average	Range (%)
OS-H-4	2.889	3.403	2.585	2.898	2.944	88-116
STANDARD	2.011	3.193	2.692	2.921	2.704	74-118
OS-H-7	2.512	2.970	2.229	3.040	2.688	83-113
OS-H-2	2.585	2.734	2.293	2.719	2.583	89-106
OS-H-6	2.066	2.716	2.408	2.710	2.475	83-110
OS-H-5	2.642	2.609	2.080	2.203	2.383	87-111
OS-H-3	2.274	2.601	2.179	2.433	2.372	92-110
Average	2.426	2.889	2.352	2.704	2.593	91-111
Min.	2.011	2.601	2.080	2.203	2.372	
Max.	2.889	3.403	2.692	3.040	2.944	

$LSD_{0.05}$ hybrid (A) 0.261
$LSD_{0.05}$ year (B) 0.198
$LSD_{0.05}$ AxB 0.523

It can be said that the main goal of every breeder is to increase the genetic potential of the most important agronomic traits, particularly oil yield in case of sunflower. This is a complex trait determined primarily by grain yield components (number of plants per unit area, number of seeds per plant, 1000-grain weight, test weight, the proportion of shell, plant height, head diameter, stem diameter) and by length of the growing season, resistance to dominant pathogens, resistance to lodging as well as by oil content in grain. The objective quantification of the expression of this trait should not be guided by the values achieved in certain environments, but by the total value of each cultivar, which allows estimation of stability and adaptability. Thus, in addition to the level of yield in particular environments, the response of the individual genotype (hybrid) to environmental conditions, i.e., genotype by environment interaction is also analyzed, which is the basis for a quantitative assessment of phenotypic stability (Piepho, 1999; Sudarić and Vratarić, 2001).

Based on the statistical parameters like regression coefficient (b_i), deviation from regression (s^2d_i) and the level of oil yield (Table 6), hybrids in the research can be classified into 4 groups:

- The first group ($b_i \approx 1$, and the low value of s^2d_i) consists of hybrids OS-H-4 ($x = 2.944$, $b_i = 1.21$, $s^2d_i = 0.04$) and OS-H-6 ($x = 2.475$, $b_i = 0.97$, $s^2d_i = 0.05$). They are stable, widely adaptable hybrids, which would on improved production conditions (higher investment) respond by significant increase of oil yield regardless of environment.
- To the second group belong hybrids that have $b_i < 1$, and $s^2d_i > 0.00$. These are hybrids that have good performance in low yielding environments, and in high yielding environments achieve small changes in oil yields. We consider them adaptable to low yielding environments. In our research, it is the OS-H-5 hybrid ($x = 2.383$, $b_i = 0.42$, $s^2d_i = 0.11$).

- The third group includes hybrids which are characterized by values $b_i > 1$ and $s^2d_i > 0.00$. The standard hybrid (x = 2.704, $b_i = 1.54$, $s^2d_i = 0.16$) and the hybrid OS-H-7 (x = 2.688, $b_i = 1.41$, $s^2d_i = 0.09$) belonged to this group. They are adapted to high yielding environments, which means that in low yielding environments they generally have lower oil yields.
- For hybrids OS-H-2 and OS-H-3 it is hard to determine whether they belong to stable or unstable genotypes. For these genotypes the s^2d_i parameter value indicates stability, but $b_i < 1$ indicates unstable genotypes. Therefore, for these genotypes additional evaluation is needed.

Table 6: The average oil yield (2012- 2015), regression coefficient (b_i) and deviation from regression (s^2d_i).

Hybrid	Oil yield (t/ha)	b_i	s^2d_i
STANDARD	2.704	1.54	0.16
OS-H-2	2.583	0.70	0.02
OS-H-3	2.372	0.74	0.01
OS-H-4	2.944	1.21	0.04
OS-H-5	2.383	0.42	0.11
OS-H-6	2.475	0.97	0.05
OS-H-7	2.688	1.41	0.09
Average	2.593		

CONCLUSIONS

Based on the analyzed data for grain yield, oil content and oil yield in sunflower hybrids the following conclusions can be made:

1. For all studied traits statistically significant differences for years of research, hybrids and hybrid x year interactions were determined,
2. Satisfactory reserve of winter moisture, good distribution of rainfall and mean monthly temperature, and not too high temperature maximums in 2013 resulted in the highest grain yield (6.243 t/ha) and oil yield (2.889 t/ha),
3. The highest oil content (51.35%) was recorded in 2012, which was accompanied by a distinct drought, slightly lower grain yield, smaller mass of 1000 seeds which resulted in a higher share of nucleus,
4. OS-H-4 and OS-H- 6 are stable, widely adaptable hybrids. These hybrids, in improved production conditions (higher investment), will respond with significant increase in oil yield regardless on the environment,
5. OS-H-5 is a hybrid adapted to low yielding, and OS-H-7 to high yielding environments,
6. The hybrid OS-H-4, with respect to the achieved results of grain yield (6.257 t/ha), oil content (51.62%) and oil yield (2.944 t/ha) can be considered a perspective hybrid for recognition and commercial production.

REFERENCES

Balalić, I., Crnobarac, J., Dušanić, N. (2007). Planting date effects on oil yield in sunflower (Helianthus annuus L.). Helia. 30, 47: 153-158.

Balalić, I., Miklič, V., Jocić, S., Hladni, N., Marinković, R., Gvozdenović, S. (2008): Rezultati mikroogleda NS hibrida suncokreta i preporuka sortimenta za 2008. godinu. Zbornik radova, Institut za ratarstvo i povrtarstvo, Novi Sad, 45(II): 111-123.

Becker, H.C., Leon, J. (1988). Stability Analysis in Plant Breeding. Plant Breeding. 101: 1- 23.

Bošnjak, Đ. (1999). Navodnjavanje poljoprivrednih useva. Univerzitet u Novom Sadu, Poljoprivredni fakultet Novi Sad.

FAOSTAT Database. 2016. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor

Gadžo, D., Đikić, M., Mijić, A. (2011). Industrijsko bilje. Poljoprivredno- prehrambeni fakultet Sarajevo. 12-31.

Gotar, A.A., Berger, M., Labalette, F., Centis, S., Dayde, J., Calmon, A. (2008). Estimation of breeding potential for tocopherols and phytosterols in sunflower. In: Proc. of the 17th International Sunflower Conference, Cordoba, Spain. 555-559.

Jocković, M., Prodanović, S., Jocić, S., Marinković, R., Marjanović Jeromela, A., Jocković, B., Čanak, P. (2014). Genne Effects and Combining Abilities for Oil Content in Sunflower. Journal on Field and Vegetable Crops Research. 51 (2): 106-109.

Josipović, M., Mađar, S. (2004). Proizvodnja suncokreta u uvjetima natapanja u redovnoj i postrnoj sjetvi. In Suncokret *Helianthus annuus* L. Vratarić (ed.). Poljoprivredni institut Osijek. Hrvatska. 245-258.

Kaya, M.D., Kolsarici, O. (2011). Seed yield and oil content of some sunflower (*Helianthus annuus* L.) hybrids irrigated at different growth stages. African J. Biotehnol. 10(22): 4591-4595.

Kaya Y. (2016). Sunflower. In: S.K. Gupta (Ed), Breeding Oil Seed Crops for Sustainable Production. Opportunities and Constraints Academic Press, Elsevier Inc, USA, 55-88.

Krizmanić, M., Liović, I., Mijić, A., Krizmanić, G., Šimić, B., Duvnjak, T., Bilandžić, M., Marinković, R., Gadžo, D., Markulj, A. (2012). Utjecaj okolina na kvantitativna svojstva novih OS-hibrida suncokreta. Sjemenarstvo. 29, 3-4: 121-135.

Krizmanić, M., Mijić, A., Liović, I., Sudarić, A., Sudar, R., Duvnjak, T., Krizmanić, G., Bilandžić, M. (2013). Utjecaj okoline na sadržaj ulja i sastav masnih kiselina kod novih OS-hibridnih kombinacija suncokreta. Poljoprivreda. 19, 1: 41-47.

Liović, I., Kovačević, V., Krizmanić, M., Mijić, A., Šimić, B. (2006). Precipitation influence on edible oil production from sunflower crop in Croatia. Cereal Research Communications. 34, 1: 573-576.

Miklič, V., Škorić, D., Balalić, I., Jocić, S., Jovanović, D., Hladni, N., Marinković, R., Joksimović, J., Gvozdenović, S. (2007). Rezultati ispitivanja NS hibrida suncokreta u ogledima i preporuka za setvu u 2007. godini. Zbornik radova Instituta za ratarstvo i povrtarstvo. 43, 1: 115-128.

Miklič, V., Balalić, I., Jocić, S., Marinković, R., Cvejić, S., Miladinović, D., Jocković, M., Hladni, N. (2014). Rezultati ispitivanja NS hibrida suncokreta u

mikroogledima i preporuka za setvu u 2014. godini. Zbornik referata sa 48. savetovanja agronoma Srbije, Zlatibor. 4-24.

Mijić, A., Sudarić, A., Krizmanić, M., Duvnjak, T., Bilandžić, M., Zdunić, Z., Ismić E. (2011). Grain and Oil Yield of Single-Cross and Three-Way Cross OS Sunflower Hybrids. Poljoprivreda. 1: 3-8.

Patel, M.D., Thompson, P.D. (2006): Phytosterols and vascular disease. Atherosclerosis. 186, 1: 12-9.

Pepó, P., Molnárová, J. (2010). Sustainable, environmental friendly field crops production in changing climate conditions (monograph), (Ed.: J. Molnárová, P. Pepó). Slovak University of Agriculture of Nitra. 191.

Piepho, H.P. (1999). Stability analysis using the SAS System. Agronomy Journal. 91: 154- 160.

Puškadija, Z., Mijić, A., Zdunić, Z., Gadžo, D., Štefanić, E., Đikić, M., Opačak, A., Florijančić, T. (2009). Utjecaj oprašivanja medonosnom pčelom (*Apis melifera carnica* L.) na agronomska svojstva suncokreta. Radovi Poljoprivrednog fakulteta Univerziteta u Sarajevu. 59, 2: 181-187.

Sudarić, A., Vratarić, M. (2001). Stabilnost i adaptabilnost nekoliko OS- kultivara soje u urodu zrna. Poljoprivreda. 7, 1: 18- 24.

Škorić, D. (2009). Sunflower breeding for resistance to abiotic stresses. Helia 32, 50: 1-16.

Škorić, D. (2012). Sunflower breeding. In Sunflower Genetics and Breeding. D. Škorić, Z. Sakač (ed.), International Monography, 165-354.

Vranceanu, A.V. (1977). Sunflower. Edicions Mudi Presa.

Waghchoure, E.S., Rana, M.A. (1988). Effect of honeybee pollination on seed setting, yield and oil content of sunflower (*Helianthus annuus* L.). In Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia. Int. Sunflower Assoc., Paris, France. 439- 440.

DOI: 10.17707/AgricultForest.63.1.33

Mykola NAZARENKO[1]

INFLUENCE OF NITROSOALKYLUREAS ON WINTER WHEAT PLANTS AT FIRST GENERATION AFTER MUTAGEN ACTION

The strategy of investigation combined the identification of genotypes carrying specific low-sensitive to mutagen factor using cytological and morphometrical analyzes screening of mutagen treated wheat populations with the approach of comparing different varieties by breeding methods to reveal its connections and differences, specific sensitive to mutagens effects on cell and plant level. The main purposes of investigations in this area were identification more suitable varieties for planting on mutagen-polluted area or as an object for mutation breeding.

Here we report cytogenetic, plant growth and development characteristics of mutation induction variability of the new wheat varieties and some relationships between means of plants grows and developments, morphometrical parameters, cytogenetic characteristics and different concentrations and types of mutagens at first generation after mutagen treatment.

Keywords: *chemical mutagenesis, winter wheat, nitroso alkylureas, chromosomal aberration*

INTRODUCTION

There were next reasons for conducting so types of investigations. Firstly, more than 40 % of total Ukrainian territory is constantly under action of chemical pollution. For arable lands this measure is about 80 %, for Dnipropetrovsk region (as place of investigation) is about 90 % (about a half of this territory at high or very high level). We apparently need in deployment of suit varieties for farming on these lands, especially for winter wheat because this zone is main for grain production in Ukraine. Secondary, we exploited chemical mutagenesis as a one of the main methods for breeding process of winter wheat (about 40 % of wheat varieties, which official released in Ukraine are mutation varieties and 56 % of these varieties have been obtained with chemical mutagens, for example new type of Ukrainian national varieties obtained by combined of field hybridization and nitrosoalkylureas action) (Nazarenko, 2015; Kharytonov et al, 2016). The improvement of crop productivity under stress conditions requires genotypes with good agronomic traits, drought tolerance and yield stability (Mohammadi and Abdulahi, 2016 (citate Blum, 1989); Popović,

[1]Mykola NAZARENKO (corresponding author: nik_nazarenko@ukr.net), Department of Plant Breeding and Seeds Management, Faculty of Agronomy, Dnipropetrovsk State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

2015; Popović et al, 2012; Djekic et al, 2013; Jankovic et al, 2015).

Nitrosoalkylureas are related to special group of mutagens "suoermutagens" (as classified by Rapoport). Special ability of this group is induction mutations on level of comparable mutagen without high damages, which influence on survival ability of plant material (Jovtcheva et al, 2002; Özel et al, 2015). Supermutagens induct 50-60 times more mutations than relevant by their consequences for surviving and plant development doses of gamma rays or fast neutrons (Albokari M., 2014).

Other feature (general for all chemical mutagens) is induction of target mutations (or site-specific mutations). It is depends on chemical nature of specific mutagen. That's why chemical mutagenesis is one of the important methods for modern genetics investigations (as for example for reverse genetics, for different types of tilling's methods). We can predict (in certain limits) types of future mutations and their rates (according to preferable DNA sequences for mutagen action) (Juchimiuk-Kwasniewska, 2002; Natarajan, 2002).

Mutagenic effects of chemicals have been assessed by both analysis of chromosomal aberrations (Rakhmatullina and Sanamyan, 2007) and investigation plant development and grows at first generation under field conditions. Chromosomal abnormalities in irradiated mitotic cells range from breaks, through exchanges, laggards and anaphase bridges, dicentric and centric ring formations, terminal fragments with telomeric signal at only one end and interstitial fragments that appear as double minutes without any telomeric signals (Rakhmatullina and Sanamyan, 2007). For crops like wheat, individual tillers (side branches) originate from different cells of the embryo of the treated seeds. If an aberration occurs in one of these cells, it will be carried in the tiller developed from that cell (Hossain and Alam, 2001; Huaili et al, 2005; Shu et al, 2011).

Mutated plants typically show reduced fertility, mainly caused by chromosomal rearrangements and genomic mutations during meiosis. We developed plant surviving, pollen fertility and yield structure for identification of mutagen depression (or stimulation) effects (Karthika, Subba, 2006; Nazarenko, 2015; Nazarenko and Kharytonov, 2016).

The main purposes of investigations in this area were determination of the chemical mutagen-polluted area suitability for agriculture, identification more suitable varieties for planting on proper chemical-polluted area or as a object for exploited in mutation breeding or genetics.

MATERIAL AND METHODS

Presoaking seeds of (in brackets method of obtaining varieties or used mutagens) Favoritka, Lasunya, Hurtovina (irradiation of initial material by gamma rays), line 418, Kolos Mironovschiny (field hybridization), Sonechko (chemical mutagenesis, nitrosodimethilurea (NDMU) 0.005%) and Kalinova (chemical mutagenesis, DAB 0.1%), Voloshkova (termomutagenesis – low plus temperature at plant development stage of vernalizaion has been used as mutagen

factor) of winter wheat (*Triticum aestivum* L.) were subjected to chemical mutagens: nitrosomethilurea (NMU) 0.0125 and 0.025 %, nitrosoethilurea (NEU) – 0.01 та 0.025 %. Exposure to chemical mutagens lasts 18 hours. These concentrations and exposure are optimal for the breeding process that has been repeatedly established earlier (Shu et al, 2011). Non-treated varieties were used as a check.

Treated seeds were grown in rows with inter and intra-row spacing of 50 and 30 cm, respectively, to raise the M_1 population. The untreated seeds of mother varieties (parental line/variety) were also planted after every ten rows as control for comparison with the M_1 population. M_1 plant rows were grown in three replications with check-rows of untreated varieties in every ten-row interval. Data on seed germination and surviving plants were recorded considering whole plots of M_1 population. Data on yield structure components (plant height, general number of culms, number of productive culms, spike length, spikelets per spike, number of grain per spike, grain weight per spike and plant, 1000 grains weight) were taken from 50 randomly selected plants of each treatment representing more or less all types of morphological plants (Sanamyan et all, 2010).

The seeds used in this study were of the M_0 generation. After mutagen treatment dry seeds were germinated in Petri dishes under 24 – 72 hours (depends on presoaking and mutagen action), temperature $+25^0$C. After wards central primary roots were cut and fixed in solution of alcohol and acetic acid (in proportion 3:1) for 24 hours. Fixation material was stored in 70% alcohol solution under temperature 2 ^{0}C (20 – 25 roots per variant). Cytological analysis was carried out by the standard method at temporary press-time preparations of root tips (1 – 1.5 mm) stained with acetocarmine (has been prepared by Remsderh). Tissue maceration (if it needs for analysis) was carried out at 45% solution of acetic acid (during 5 minutes on bane-marie under 60^0C). Anaphase of cell division was observed by light microscope JNAVAL. No less than 1000 cells in proper phases of mitosis were observed in each variant (Lifang et al, 2001; Rank et al, 2002,; Natarajan, 2005; Nikolova et al, 2015).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by ANOVA. Used the standard tools of the program Statistica 8.0 for factor analysis (ANOVA module).

RESULTS AND DISCUSSION

Analysis of grows and development of plants

In M_1 population, observations were recorded seed germination and plant surviving, pollen fertility, plant height, spikes/plant, spike length, kernels/spike, 1000-grain weight, yield/plant (table 1 – 3). Standard error (±SE) values of the treated populations are at tables too.

The results on germination of seeds, survival rate of plants derived from treated and untreated seeds are tabulated (Table 1). Germination and survival abilities of seeds reduce compared to untreated seeds of the initial variety in all cases.

Germination and survival abilities of seeds reduce compared to untreated seeds of the initial variety in all cases except one (Sonechko, NEU). Plant survival ability ranges from 68 (Sonechko, Kalinova) to 63% (Lasunya) at 0.025% NMU, while it ranged from 98 to 92% under untreated control. As for the impact of chemical mutagens on the germination and survival abilities, it is the usual effect in plants for most crops previously observed by many researchers in wheat as well (Bolzarn, Bianchi, 2006). However, we can see that chemical mutagens are more specific at the comparable effects than the gamma-rays. Some varieties have been shown this parameter without significant difference from check (Voloskova, Hurtovina, Sonechko, NEU 0.01 %).

Table 1. Main parameters of grown of winter wheat plants at M_1 generation

Trial	Germination, %	Survival after winter, %	Germination, %	Survival after winter, %
Variety	Kolos Mironivschini		Kalinova	
Check	98±0.57	91±0.93	94±0.94	88±0.98
NMU, 0.0125%	80±1.05*	78±1.01*	74±0.61*	73±0.56*
NMU, 0.025%	66±1.01*	65±0.87*	69±0.49*	68±0.48*
NEU, 0.01 %	81±0.94*	81±0.94*	82±0.92*	82±0.92*
NEU, 0.025 %	74±0.70*	73±0.67*	76±0.80*	76±0.78*
Variety	Voloshkova		Sonechko	
Check	92±0.57	87±0.93	94±0.94	89±0.98
NMU, 0.0125%	78±0.90*	78±0.81*	79±1.02*	78±0.82*
NMU, 0.025%	69±0.70*	67±0.69*	69±1.04*	67±0.94*
NEU, 0.01 %	90±0.81	88±0.74	87±1.10*	87±1.0
NEU, 0.025 %	82±0.78*	80±0.68*	79±1.40*	78±1.25*
Variety	Favoritka		Hurtovina	
Check	98±0.57	91±0.93	92±0.94	84±0.98
NMU, 0.0125%	79±0.93*	78±0.87*	78±1.01*	77±0.93*
NMU, 0.025%	70±1.30*	69±1.0*	68±0.76*	68±0.74*
NEU, 0.01 %	88±0.82*	87±0.74*	86±0.90*	85±0.36
NEU, 0.025 %	82±1.04*	80±0.98*	81±1.02*	79±0.99*
Variety	Lasunya		Line 418	
Check	98±0.57	94±0.93	93±0.94	92±0.98
NMU, 0.0125%	76±1.20*	75±1.0*	82±1.40*	80±1.2*
NMU, 0.025%	64±1.50*	63±1.1*	73±0.81*	68±0.34*
NEU, 0.01 %	88±1.10*	85±0.88*	88±1.12*	85±1.02*
NEU, 0.025 %	81±1.30*	79±1.1*	78±1.50*	78±1.04*

* - difference is statistically significance from check at $P_{0.05}$

In general, the correlation between the dose value and survival abilities of plants is at the level of -0.8.

Correlation between the concentration of mutagens and pollen fertility was -0.85. We cannot see any difference in plant response on mutagen action, only

heightening of pollen sterility depended on concentration on mutagen. NMU was more active in induce decreasing of male sterility.

Table 2. Pollen fertility after mutagen action, %

Trial	Kolos Mironivschini	Kalinova	Voloshkova	Sonechko	Favoritka	Hurtovina	Lasunya	Line 418
Check	95.0	93.1	89.7	96.7	95.7	98.6	96.8	93.0
NMU, 0.0125%	89.1*	84.3*	87.6	79.8*	90.1*	88.5*	89.2*	89.6*
NMU, 0.025%	85.2*	72.3*	84.3*	64.2*	85.4*	84.4*	86.6*	85.4*
NEU, 0.01 %	90.2*	88.0*	88.9*	84.6*	93.0*	95.4*	93.2*	90.1*
NEU, 0.025 %	88.4	84.2*	87.0*	80.1*	90.6*	88.7*	90.8*	88.3*

* - difference is statistically significance from check at $P_{0.05}$

All parameters of the crop yield structure have been studied. Components such as plant height, 1000 grain weight, grain weight per plant, number of grains per spike, grain weight per spike, general number of culms, number of productive culms, spike lengths have been developed. Only four (plant height, grain weight per spike, grain weight per plant and 1000 grain weight) showed statistically difference level of mutagen depression under any dose action. And for this type of mutagens grain weight per plant is more reliability parameter in assessment of mutagen depression than grain weight per plant (in case of gamma-rays this index wasn't sufficient for correct evolution (Nazarenko, 2016).

Regarding the plant height, correlation between the concentration and the indicator constituted -0.82, (high invert correlation). This parameter decreases if the concentration increases. Gradual decrease in height is a tendency, but we ran on with substantial differences between the varieties. Sonechko and Kalinova has been shown stimulation effect after NEU 0.01 action and plant height was on level of check after NEU 0.025.

The indicator of grain weight per spike was so informative; weight was falling statistically valid with every increase in dose. Sonechko responded to mutagenic effect in the same manner (non-depression at NMU 0,0125%). The correlation coefficient was -0.8.

Due to the grain weight per plant, correlation between the concentration and the parameter is -0.84, (high invert correlation). This parameter decreases if the concentration increases. We cannot see depression in case of Kalinova and in one concentration (same as for previous parameter) for Sonechko.

The thousand grain weight is the most informative indicator (similar as gamma-rays). Depression value at each concentration is clear and statistically

valid. The correlation coefficient was -0.89. Variety Kalinova was non-depressed under NMU 0.0125%.

Sonechko and Kalinova are less sensitive to both mutagens in compare with other genotypes for all variants.

Table 3. Correlation between gamma-rays dose and some components of yield structure of M_1 varieties

Parameter	Plant height	No. of culms	Spike lengths	No. of spikelets	No. of grains per spike	Grain weight per spike	Grain weight per plant	1000 grain weight
Dose	-0.82	-0.22	0.18	0.23	-0.60	-0.80	-0.84	-0.89

Chromosomal aberrations analysis

The results of our investigation are represented at table 4 (total number of observing mitosis, number of division cells with chromosomal aberrations, general frequency of aberrations). Standard error (± SE) values of the treated populations are at tables too. As we can see from table 4 frequencies were changed from 4.44 (Sonechko, NEU 0.01 %) to 22.69 (Voloshkova, NMU 0.025 %) percents from total number of mitosis. All the variables are statistically significantly different from each other and from the check.

Variety Sonechko is the most resistance genotype for mutagen action with highest level of difference and this variety was obtained with nitrosoalkylureas action (mutagen was other, but it's belong to this type of mutagens). NMU initiated more rates of chromosome aberrations, than NEU.

After spectra of chromosomal aberrations had been investigated next types was identified: chromosomal bridges and double-bridges, fragments of chromosomes and double-fragments, micronucleus, lagging chromosomes. Cases with two or more types of aberrations in one cell and fragments-bridges ratio were calculated separately (Table 4).

After this date had been analyzed we identified some correlations between mutagen concentrations and parameters of spectra. Quantity of any type of chromosomal aberrations was increased with concentration increased (correlation coefficients 0.7 – 0.8, it was less than for gamma-rays, but enough for correct asset (Nazarenko, 2015). In our past investigations gamma-rays induced more bridges than fragments (fragments-bridges ratio lower than 1) (Nazarenko, Kharitonov, 2016). After chemical mutagens more fragments and double-fagments have been observed (fragments-bridges ratio more than 1). These parameters can be used for mutagen nature identification, but only for limit kinds of chemical substances. As we can see, we will be able to use this parameter for identify nature of unknown mutagen for nitrosoalkylureas agents. We know from other investigation that it was not correct (in some variants) for other type of supermutagens. Both mutagens (NMU and NEU) induced similar types of aberrations in similar rate. Amount of any types of chromosome's changes were increased with concentrations growth, but in case of complicated aberrations,

lagging chromosomes and micronucleus it was not significant. More rates of complicated aberrations and micronucleus are characterized for nitrosoalkylureas than for gamma-rays (Zhang et al, 2015).

Table 4. Frequency of chromosomal aberrations in M_1 generation of winter wheat varieties

Variable	Mitosis, number	Chromosomal aberrations		Mitosis, number	Chromosomal aberrations	
		n.	%		n.	%
	Favoritka			Line 418		
Check	984	19	1.93±0.31	962	11	1.14±0.11
NMU, 0.0125%	1048	139	13.26 ± 1.29*	906	106	11.70 ± 1.12*
NMU, 0.025%	934	179	19.17 ± 1,48*	983	188	19.12 ± 1.57*
NEU, 0.01 %	1020	91	8.92 ± 0.89*	1021	93	9.11 ± 0.84*
NEU, 0.025 %	940	141	15.00 ± 1.39*	900	156	17.33 ± 1.22*
	Lasunya			Hurtovina		
Check	1056	15	1.42±0.19	1034	12	1.16±0.11
NMU, 0.0125%	1019	121	11.89 ± 1.28*	1005	143	14.22 ± 1.37*
NMU, 0.025%	844	161	19.09 ± 1.43*	1022	223	21.83 ± 1.59*
NEU, 0.01 %	1003	97	9.67 ± 0.92*	1018	103	10.12 ± 0.99*
NEU, 0.025 %	1015	159	15.67 ± 1.33*	1024	184	17.97 ± 1.42*
	Sonechko			Voloshkova		
Check	1026	8	0.78±0.04	1003	31	3.09±0.34
NMU, 0.0125%	1027	56	5.45 ± 0.34*	1002	142	14.17 ± 1.17*
NMU, 0.025%	981	108	11.01 ± 0.99*	912	207	22.69 ± 1.64*
NEU, 0.01 %	1013	45	4.44 ± 0.44*	1005	116	11.54 ± 1.02*
NEU, 0.025 %	972	97	9.98 ± 0.98*	976	169	17.32 ± 1.44*
	Kalinova			Kolos Mironivschini		
Check	1047	9	0.86±0.11	909	10	1.10±0.13
NMU, 0.0125%	1009	106	10.51 ± 1.07*	1016	129	12.70 ± 1.22*
NMU, 0.025%	851	133	15.63 ± 1.27*	917	190	20.72 ± 1.61*
NEU, 0.01 %	984	78	7.93 ± 0.64*	1014	89	8.78 ± 0.82*
NEU, 0.025 %	846	135	15.96 ± 1.33*	951	155	16.30 ± 1.34*

* – difference statistically significant on $P_{0,01}$

In some preliminary works researches mentioned about exploited of double-fragment rate as measure for chemical mutagens efficiency on cell level (Natarajan, 2005; Shu et al, 2011), but no one said about dominance of fragments regarding to nitrosoalkylureas and about applying fragments-bridges ratio for mutagen nature identification. Our method enables to solve this problem and we sure in effectiveness one under any conditions for any plant object.

Thereby, we propose this parameter (prevalence of fragments under bridges as fragments-bridges ratio) for mutagen nature identification. Complicated (or combined) aberrations are more typical for chemical mutagens than for physical. Genotype, obtained with nitrosoalkylureas is less sensitive for repeated action of the same mutagen. Previous rule for gamma-rays was confirmed for one type of chemical supermutagens too.

CONCLUSIONS

The most informative parameters to determine the degree of mutagenic depression in the first generation for plant growth and development were germination and survival rates, pollen sterility. However, distinctly of gamma-rays, we have four inconsiderate morphometrical parameters for mutagen depression identification. There are plant height, grain weight per spike, grain weight per plant and 1000 grain weight. We developed stimulation effect in case of NEU 0.01 % for some varieties. The least level of mutagen depression by morphometrical indicators we observed in case of Sonechko and Kalinova. Therefore, chemical mutation varieties are less sensible for to same chemical mutagens

Chemical mutations varieties were less sensitive to same chemical mutagens. As we can see varieties Sonecko and Kalinova are more preferable for growth under this action. We can predict less number of mutations when these varieties would be used for mutation breeding purposes.

The higher rates of chromosomal aberrations are typical for varieties obtained by used field hybridization without any mutagen treatment or when initial material for breeding has been changed by low temperature action (variety Voloshkova).

Comparing between bridges and fragments is a reliable mean for identification of mutagen nature (chemical or gamma-rays). In first case more fragments have been induction, in second – bridges.

In general, the frequency of any type of chromosomal aberrations is linearly increased with increase concentrations of the mutagen.

As object for mutation breeding we propose varieties obtained by used field hybridization without any mutagen treatment or mutation varieties which obtained with other mutagen than using in breeding program. We offer for planting in mutagen nitrosoalkylureas pollution areas chemical mutation varieties like as Sonechko and Kalinova.

REFERENCES

Albokari M., 2014. Induction of mutants in durum wheat using gamma irradiation. In: Pakistan Journal of Botany, 46, 317–324.

Bolzarn, A. D., Bianchi, M. S., 2006. Telomeres, interstitial telomeric repeat sequences, and chromosomal aberrations. In: Mutation Research, 612, 189 – 214.

Jovtcheva, G., Stergiosa, M., Schubert, I., 2002. A comparison of N-methyl-N-nitrosourea-induced chromatid aberrations and micronuclei in barley meristems using FISH techniques . In: Mutation Research, 517, 47–51.

Juchimiuk-Kwasniewska, J., Brodziak, L., Maluszynska, J., 2011. FISH in analysis of gamma ray-induced micronuclei formation in barley. In: Journal of Applied Genetics, 52, 23 – 29.

Hossain M. F., Alam M. S., 2001. Effect of gamma irradiation on the callus, developed from indica rise. In: Pakistan Journal of Biological Sciences, 6, 670–671.

Huaili Q., Lanming X., Fei H., 2005. Biological effect of the seeds of Arabidopsis thaliana irradiated by MeV protons. In: Radiation Effects & Defects in Solids, 160, 131–136.

Karthika I R., Subba B., 2006. Effect of Gama Rays and EMS on Two varieties of Soybean. In: Asian Journal of Biological Sciences, 5, 721–724.

Kharytonov, M.M., Pashova, V. T., Mitsik, O.O., Nazarenko, M.M., Bagorka, M.O., 2016 Estimation of winter wheat varieties suitability for difference growth of landscape conditions. In: Agricultural and mechanical engineering: Materials of International Symposium ISB-INMA TECH (Bucharest, 27-29 October, 2016), Buchares, INMA, 401 – 406.

Lifang Y., Zengliang W., 2001. Radiobiological effects of a low-energy ion beam on wheat. In: Radiat Environ Biophys, 40, 53–57.

Natarajan, A.T., 2002. Chromosome aberrations: past, present and future. In: Mutation Research, 504, 3–16.

Natarajan, A.T., 2005. Chromosome aberrations: Plants to human and feulgen to FISH. In: Current Science 89, 335-340.

Nazarenko, M., 2015. Osobennosti vozdeystviya gamma-luchey na hromosomnyiy apparat kletki na primere pshenitsyi myagkoy ozimoy [The frequency and spectrum of chromosomal aberrations in root meristem cells of wheat under the action of mutagens]. Tambow State Unuversity Reports. Ser.: Natural and Technical sciences, 2, 449-452, (in Russian).

Nazarenko, M., 2015. Negativnyie posledstviya mutagennogo vozdeystviya [Peculiarities of negative consequences of mutagen action]. In: Ecological Genetics, 4, 25-26. (in Russian)

Nazarenko, M., 2016. Parameters of winter wheat growing and development after mutagen action. In: Bulletin of Transilvania University of Brasov - series II – Forestry, Wood Industry, Agricultural, Food Engineering, 9 (58), 2, 109–116.

Nazarenko, M., Kharytonov, M., 2016. Characterization of wheat mutagen depression after gamma-rays irradiated. In: Agriculture and Forestry, 62, 4, 267–276.

Nikolova, I., Georgieva, M., Kruppa, K., Molnor-Long, M., Liu, L., Manova, V., Stoilov, L., 2015. Cytogenetic effects in barley root apical meristem after exposure of dry seeds to lithium ion beams. In: Genetics and Plant Physiology, 5, 3 – 9.

Özel, H.B., Kirdar, E., Bilir, N. 2015. The effects of magnetic field on germination of the seeds of oriental beech (Fagus orientalis Lipsky.) and growth of seedlings. Agriculture and Forestry, 61 (3): 195-206.

Popović, V. (2015). The concept, classification and importance of biological resources in agriculture. (Ed) Milovanovic J., Đorđević S.: Conservation and enhancement of biological esources in the service of ecoremediation. Monograph. Belgrade. ISBN 978-86-86859-41-9; 29-51**;** 1-407.

Popović, V., Malešević, M., Glamočlija, Dj., Vučković, S., Tatić, M., Mladenović, G., Hristov, N. (2012): Effect of agro-ecological factors on wheat seed production. Scientific Journal of Univerzity of Szeged, Faculty Agriculture. Hodmezovasarhely, Vol. 6 (2),150-157.

Đekić, V., Staletić, M., Jelić, M., Popović, V., Branković, S. 2013. The stability properties of wheat production on acid soil. 4th International Symposium "Agrosym 2013", Jahorina, p. 84-89.

Janković, S., Ikanović, J., Popović, V., Rakić, S., Pavlović, S., Ugrenović, V., Simić, D, Dončić, D. (2015): Morphological and productive traits of spelt wheat – *Triticum spelta* L. "Agriculture and Forestry", Podgorica, Vol. 61, 2, 173-182.

Mohammadi, R., Abdulahi, A. (2016): Selection for traits associated with drought tolerance in durum wheat. Agriculture and Forestry, 62 (3): 139-150.

Rakhmatullina, E.M., Sanamyan, M.F., 2007. Estimation of efficiency of seed irradiation by thermal neutrons for inducing chromosomal aberration in M_2 of cotton Gossypium hirsutum L. In: Russian Journal of Genetics 43(5), 518-524.

Rank, J., Lopez, L.C., Nielsen M.H., 2002. Genotoxicity of maleic hydrazide, acridine and DEHP in Allium cepa root cells performed by two different laboratories. In: Hereditas 136, 13–18.

Sanamyan, M. F., Petlyakoval, J. E., Sharipoval, E. A., Abdurakhmonov, I. Y., 2010. Morphological characteristics and identification of new monosomic stocks for cotton (Gossypium hirsutum L.) In: Advances in Bioscience and Biotechnology 1, 372-383.

Shu, Q.Y., Forster B.P., Nakagava H., 2011 Plant Mutation breeding and Biotechnology, Vienna, CABI publishing.

Zhang, J., Jiang, Y., Guo, Y., Li, G., Yang, Z., Xu, D., 2015. Identification of Novel Chromosomal Aberrations Induced by 60Co-γ-Irradiation in Wheat-Dasypyrum villosum Lines. In: International Journal of Molecular Sciences, 16, 29787–29796.

DOI: 10.17707/AgricultForest.63.1.34

Lovro SINKOVIČ, Nataša MIRECKI, Dragan ŽNIDARČIČ[1]

EFFECT OF POLYPROPYLENE COVER AND PLANT DENSITY ON YIELD AND ASCORBIC ACID CONTENT OF BELL PEPPER FRUITS

SUMMARY

This study aims to evaluate the effect of polypropylene cover and plant density on the marketable yield and ascorbic acid content of bell pepper fruits. Seedlings of cultivar 'Bianca F1' were planted in four plant densities of 21.8, 13.2, 10.9 and 6.6 plants m^{-2}. The two-factorial experiment in four repetitions was used, where the 1st factor was covering (covered and uncovered) and 2nd factor was plant density in four steps (above mentioned). The polypropylene cover over the plants was laid on plastic hoops, while control plants remain uncovered. Plants were grown on soil covered with black polyethylene mulch film. Mean daily air temperature under the polypropylene cover was 2.3 – 5.8 °C higher than the air temperature in the uncovered crop. Polypropylene cover was removed after eight weeks, when the daily air temperature reached 30 °C. Yield component analysis indicated that fruits from covered plants had greater fresh weight in comparison to fruits from uncovered plants for all plant densities. The marketable yield under polypropylene cover was significantly higher (over 70 %) compared to uncovered control. Increasing plant density enhanced the marketable yield, but the interactions of cover and plant density were not significant. The cover had strong influence on the yield at the second harvest time. Ascorbic acid contents were found significantly higher for bell pepper fruits grown under cover at all plant densities.

Keywords*: ascorbic acid content, Capsicum annuum L., polypropylene cover, plant density, yield component analysis*

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is a warm-season crop with a high heat-unit requirement (Díaz-Pérez and Carlos, 2010). Bell pepper is an important vegetable used in our daily consumption and represent good sources of vitamins C and E, pro-vitamin A, and carotenoids (Materska and Perucka, 2005). These compounds are antioxidants and can reduce harmful oxidation reactions in human body (Sun et al., 2007). Open field production of bell pepper in central

[1]Lovro Sinković Crop Science Department, Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, SLOVENIA, Nataša Mirecki Biotechnical Faculty, Mihaila Lalića 1, MN-81000 Podgorica, MONTENEGRO, Dragan Žnidarčić (corresponding author: dragan.znidarcic@bf.uni-lj.si) Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, SLOVENIA

Slovenian area is limited, since the unfavourable climatic conditions from April to September. Therefore, planting of developed seedlings is used in commercial vegetable production in order to promote the early fruit harvesting and to improve the yields. Several studies have been conducted to increase the yield and quality of fruits or vegetables (Kacjan Maršić et al., 2009; Usenik et al., 2009; Veberič et al., 2010). To follow these trends in horticultural practice, growers have applied row covers, as a low-cost technique to protect both cool-season and warm-season vegetable crops (Gimenez et al., 2002). The most often used plant cover is the floating row cover made of spun bonded polypropylene (Demšar et al., 2009). The use of polypropylene covers and high plant densities have great potential and economic value for bell peppers production in order to increase the yields (Kasirajan and Ngouajio, 2012).

The number and weight of marketable pepper fruits generally decreases per plant and increases per hectare as plant density increases (Cavero et al., 2001). Enhancement of growth and yield has been attributed to higher daily air temperatures under the covered tunnels, compared with ambient readings (Gimenez et al., 2002). High micro-environmental temperatures under row tunnels early in the season may result in increased vegetative growth and subsequent increased fruit yield (Waterer, 2003). However, if the cover remains over the crop too long the fruits could be impaired and yield reduced. The optimum number of accumulated heat units cannot be precisely calculated, but it is suggested that the optimum is between 650 and 700, using a base temperature of 10 °C (Gerber et al., 1988). The optimum temperature for the vegetative growth of bell peppers ranges between 20 and 25 °C. When temperature falls below 15 °C or exceeds 32 °C, growth is usually retarded and yield decreases (Saha et al., 2010). A reduction in the early yield of bell peppers is thought to be caused by excessively high temperatures under the covers, which result in flower abortion. Pollination and fruit set can be inhibited by maintained continuous daily temperature above 32 °C (Gerber et al., 1989). Thus the removal time of the polypropylene covers is a critical phase. A wide range of different covers have been tested. However, polypropylene covers remain most popular for open field vegetable production in Slovenia. These materials are permeable for water and air, but the transmission of solar energy and the retention of heat energy are both very small (Jolliffe and Gaye, 1995).

The objective of this research was to determine the effects of polypropylene cover material and plant density on the marketable yield of bell pepper cultivar ‘Bianca F1’ in the central Slovenia as well as ascorbic acid content in fresh fruits. Cultivar ‘Bianca F1’ is one of the leading cultivars in this area, since the shape and size of the fruits are very acceptable for the fresh vegetable market.

MATERIAL AND METHODS

The experiment was conducted during the 2012 growing season, at the experimental fields of Biotechnical Faculty, University of Ljubljana, Slovenia

(46 °04' N, 14 °31' W, 300 m a.s.l.). Seeds of the bell pepper cultivar 'Bianca F1' were sown in seedling trays filled with fertilized peat. The seedlings were grown under greenhouse conditions, irrigated every day and fertilized once a week with a commercial nutrition solution, "Peters" (0.75 g N, 0.55 g P_2O_5 and 1.45 g K_2O per litre).

The open field experiment was carried out in randomized blocks in four repetitions, where each plot contained 30 plants. The soil type at open field was a heavy clay loam fertilized in early spring with 4 kg of cattle manure per m^2. A total of 50 g m^{-2} of compound fertilizer (N-P-K, 15-15-15) was broadcast-applied and incorporated one week prior the transplanting of developed seedlings with six true leaves. Black polyethylene mulch was laid on soil before planting. The bell pepper seedlings were transplanted by hand at the beginning of June 2012. Various planting distances between plants were used: 30×15 cm (21.8 plant m^{-2}), 30×30 cm (10.9 plant/m^2), 50×30 cm (6.6 plant/m^2), and two plants in parallel 50×30 cm (13.2 plant m^{-2}). At the same day after transplanting, plastic hoops were placed over the seedlings and polypropylene cover (17 g m^{-2}) was applied. A thermograph was used to record the temperature under the covers. The air temperature was measured 25 cm above the plot surface. In order to supply data for the comparison, temperature recordings were also measured at the same height in the open field with no cover. Cover was removed after eight weeks at the end of July, when the daily temperatures reached 30 °C. Irrigation and disease control were applied regularly according to the standard agronomic practice. The marketable fruits were harvested manually in three technological maturities on August 1 (first harvest), on August 25 (second harvest) and on September 25 (final harvest). Fruit size, number of fruits per plant and weight of fruits per plant were measured.

The content of ascorbic acid was determined in the pericarp of six randomly selected fruits at the end of second harvest. Bell pepper fruit pericarps (10 g) were homogenized with 15 ml of metaphosphoric acid (2% in water) using laboratory homogenizer (Ultraturax T25) and stored at -80 °C until further analysis. Determination was performed on HPLC system (Agilent 1260) with a diode array detector. The separation was carried out on a 100×2 mm i.d., 3µm Scherzo SM-C18 column (Imtakt, Japan) using a flow rate of 0.3 ml min^{-1}. The mobile phase consisted of (A) water and (B) acetonitrile both containing 0.3% (v/v) formic acid; the following elution gradient was used for solvent B: 0-3 min, 0-10%; 3-4 min, 10-100%; 4-6 min, 100%. Concentrations were calculated using external standard and expressed as mg ascorbic acid per 100 g fresh weight (FW). The results obtained during experiment were analysed through a general linear model (GLM) procedure and least-squares mean tests, with a 0.05 level of significance.

RESULTS AND DISCUSSION

With the advance of the growing season, the values of daily mean temperatures under the cover and in open field were increasing and consequently

the differences between covered and no covered treatments were noticed. The mean and maximum decade temperatures during the growing season are presented in Figure 1. From the beginning of June until the end of the second decade of July the mean decade temperature was below 20 °C, while the mean temperature under the cover was 3.6 °C higher. During the period from 20th to 30th July the mean daily temperatures outside the cover were 22.2 °C and the temperatures under cover were 28.0 °C. In general, the air temperatures under the cover were 2.3 °C – 5.8 °C higher compared to outside temperatures. Maximum daily outside temperature reached highest value at the end of July (29.5 °C). This was a critical phase, while temperature under the cover reached 35.4 °C. When temperatures during the growth increased greatly covers were removed due to the risk of flower abortion.

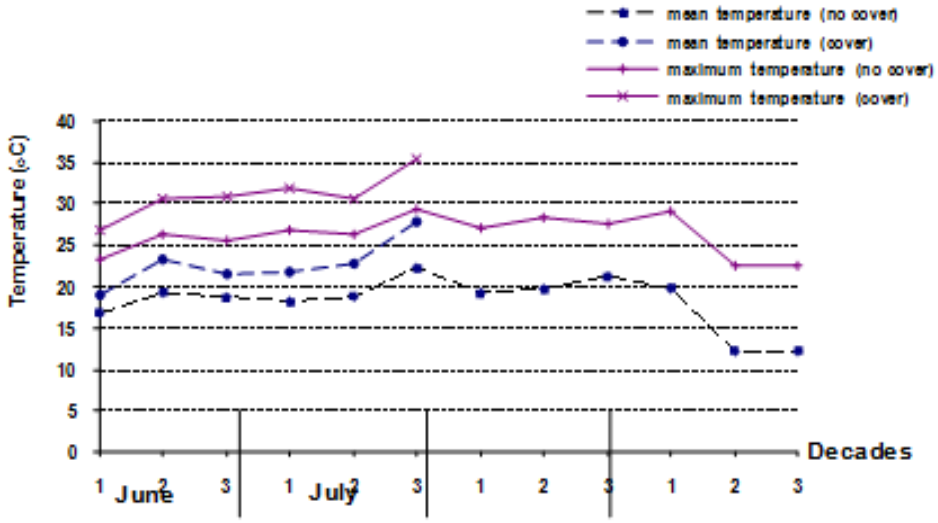


Figure 1. Mean and maximum decade temperatures during the growing season under polypropylene cover and in open field

The fruits of bell pepper grown under covers had significantly greater weight compared to the fruits of uncovered control plants for all plant densities; on average fruit weighted 121.2 g and 86.8 g, respectively (Table 1). The effects of plant density on fruit weight were not statistically significant. Not significant cover-by-plant-density interaction was indicated for the fruits weight. The marketable bell pepper fruit numbers increased significantly per plant as plant density decreased. Reducing the distance between plants (21.8 plant m^{-2}) resulted in decreases in the number of fruits per plant for covered and uncovered plants. The number of fruits per plant was higher in covered compared to uncovered plants. The increase in total weight of fruits per plant was attributed to a larger number of fruits per plant. This response has been observed by Gaye et al. (1992).

Table 1. Effect of covering and plant density on the yield parameters and ascorbic acid content of bell pepper fruits at second harvest

Covering	Plant density (plant m^{-2})	Fruit weight (g)	Number of fruits per plant	Weight of fruits (g $plant^{-1}$)	Ascorbic acid (mg $100g^{-1}$ FW)
No cover	21.8	88.8 ^{b}	3.5 ^{e}	310.8 ^{e}	140.2 ^{b}
	13.2	89.2 ^{b}	4.8 ^{cd}	428.2 ^{d}	139.6 ^{b}
	10.9	88.6 ^{b}	4.9 ^{cd}	434.1 ^{d}	141.5 ^{b}
	6.6	80.5 ^{b}	6.1 ^{b}	491.1 ^{cd}	138.9 ^{b}
Means		86.8	4.8	416.1	140.1
Polypropylene cover	21.8	122.2 ^{a}	4.3 ^{d}	525.5 ^{c}	165.9 ^{a}
	13.2	122.4 ^{a}	5.4 ^{c}	661.0 ^{b}	159.6 ^{a}
	10.9	123.8 ^{a}	5.6 ^{c}	693.3 ^{b}	166.1 ^{a}
	6.6	116.4 ^{a}	7.2 ^{a}	838.1 ^{a}	158.3 ^{a}
Means		121.2	5.6	679.5	162.5

Mean values in a column followed by the same superscripts do not differ significantly at $P = 0.05$.

In general, covered bell pepper plants contributed significantly to higher total marketable yields. Total marketable yield had also increased in response to increasing plant density (Figure 2).

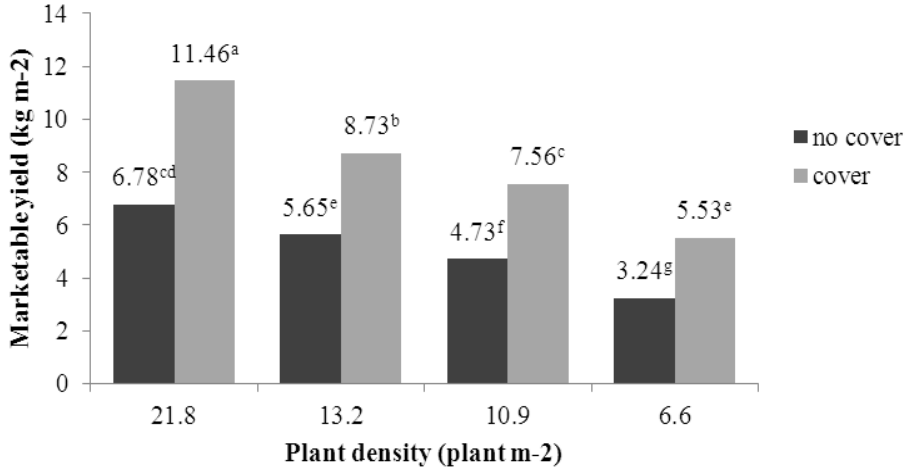


Figure 2. Effect of plant density and the covering on the marketable yield of bell pepper fruits ($P < 0.05$).

Due to the compensatory effects of higher number of plants per unit area, the total yield per unit area differed significantly. On average, the marketable yield of the first harvest was higher for the covered plants in comparison to the uncovered (Figure 3).

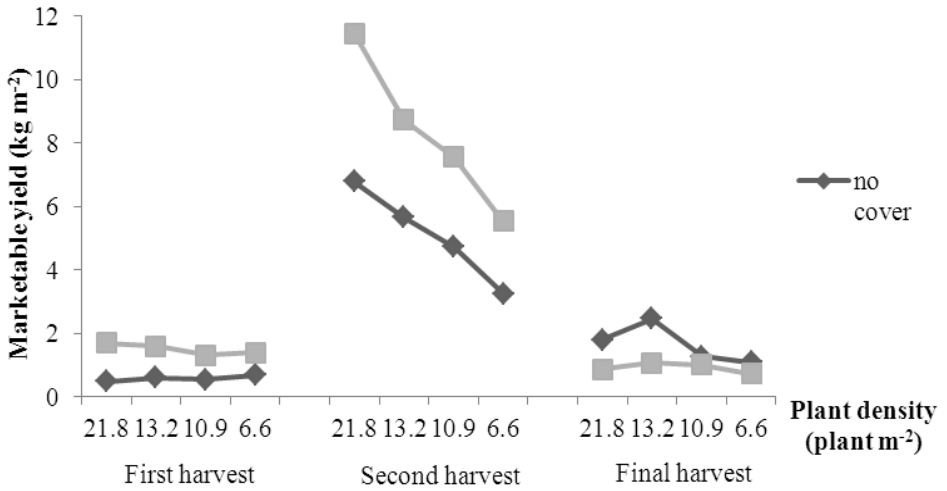


Figure 3. Effect of covering and plant density on the marketable bell pepper yield at different harvest time

These differences, however, were not significant. The increasing plant density did not influence the yield in the first harvest. There was a strong effect of covering plants on the yield in the second harvest. All of the covered plants had a higher yield compared to the uncovered plants (on average: 8.3 kg m^{-2} and 5.1 kg m^{-2}, respectively or 61 % greater yield). The higher temperature under the polypropylene cover might have been the main factor contributing to the yield increase in this harvest period. There was a significant effect of plant density on yields in the second harvest for the uncovered plants. Plant density of 21.8 plants m^{-2} resulted in significantly higher yields for covered and uncovered plants. At the end of the growing season, the marketable yield under the polypropylene cover was 68.3 % greater than for the uncovered. Ascorbic acid content varied between 138.9 to 166.1 mg/100 g fresh weight among different plant densities (Table 1). The results of the analysis of the ascorbic acid content in the pericarp of the bell pepper fruits showed significantly higher values for covered plants in comparison to uncovered, while plant density had no significant effect. Bae et al. (2014) had reported similar ascorbic acid values for greenhouse-grown bell peppers, wherein the significantly higher contents were found in mature fruits.

CONCLUSIONS

In Slovenia, the earliness and the marketable yield of bell peppers are the decisive factors for determining prices and consequently profit. A comparison of the growth pattern of the bell pepper plants under different growing conditions in the open field showed that the polypropylene cover and the plant density both have a significant effect on the total marketable yield of the bell pepper fruits. On the basis of this data, it can conclude following for the central Slovenian area:

use of polypropylene cover increases the total yield of bell pepper; the production under polypropylene cover influences the higher weight of fruits; total yield per unit area increases in response to increasing plant density; and bell pepper fruits grown under polypropylene cover contain more ascorbic acid.

REFERENCES

Bae H., Jayaprakasha G.K., Crosby K., Yoo K.S., Leskovar D.I., Jifon J., Patil B.S. (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. Journal of Food Composition and Analysis 33: 195-202.

Cavero J., Ortega R.G., Gutierrez M. (2001). Plant density affects yield, yield components, and color of direct-seeded paprika pepper. HortScience 36: 76-79.

Demšar A., Znidarčič D., Gregor-Svetec D. (2009). The comparison of properties of polypropylene fibres intended for the production of agrotextiles. Acta Agriculturae Slovenica 93: 211-217.

Díaz-Pérez J.C., Carlos J. (2010). Bell pepper (*Capsicum annum* L.) grown on plastic film mulches: effects on crop microenvironment, physiological attributes, and fruit yield. HortScience 45: 1196-1204.

Gaye M. M., Jolliffe P. A., Maurer A. R. (1992). Row cover and population density effects on yield of bell peppers in south coastal British Columbia. Canadian Journal of Plant Science 72: 901-909.

Gerber J.M., Mohd-Khir I., Splittstoesser W.E. (1988). Row tunnel effects on growth, yield and fruit quality of bell pepper. Scientia horticulturae 36: 191-197.

Gerber J.M., Splittstoesser W.E., Choi G. (1989). A heat unit system for predicting optimum row tunnel removal time for bell peppers. Scientia horticulturae 40: 99-104.

Gimenez C., Otto R., Castilla N. (2002). Productivity of leaf and root vegetable crops under direct cover. Scientia Horticulturae 94: 1-11.

Jolliffe P.A., Gaye M.M. (1995). Dynamics of growth and yield component responses of bell peppers (*Capsicum annuum* L.) to row covers and population density. Scientia Horticulturae 62: 153-164.

Kacjan Maršić N., Šircelj H., Kastelec D. (2009). Lipophilic antioxidants and some carpometric characteristics of fruits of ten processing tomato varieties, grown in different climatic conditions. Journal of agricultural and food chemistry 58: 390-397.

Kasirajan S., Ngouajio M. (2012). Polyethylene and biodegradable mulches for agricultural applications: a review. Agronomy for Sustainable Development 32: 501-529.

Materska M., Perucka I. (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). Journal of Agricultural and Food Chemistry 53: 1750-1756.

Saha S., Hossain M., Rahman M., Kuo C., Abdullah S. (2010). Effect of high temperature stress on the performance of twelve sweet pepper genotypes. Bangladesh Journal of Agricultural Research 35: 525-534.

Sun T., Xu Z., Wu C.T., Janes M., Prinyawiwatkul W., No H.K. (2007). Antioxidant Activities of Different Colored Sweet Bell Peppers (*Capsicum annuum* L.). Journal of Food Science 72: S98-S102.

Usenik V., Štampar F., Veberič R. (2009). Anthocyanins and fruit colour in plums (*Prunus domestica* L.) during ripening. Food chemistry 114: 529-534.

Veberič R., Jurhar J., Mikulič-Petkovšek M., Štampar F., Schmitzer V. (2010). Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). Food Chemistry 119: 477-483.

Waterer D. (2003). Yields and economics of high tunnels for production of warm-season vegetable crops. HortTechnology 13: 339-343.

DOI: 10.17707/AgricultForest.63.1.35

Gordan S. KARAMAN [1]

NEW SUBTERRANEAN SPECIES OF THE FAMILY NIPHARGIDAE FROM GREECE, *NIPHARGUS DENARIUS*, SP. N. (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 295)

ABSTRACT

One new species of the family Niphargidae (Crustacea: Amphipoda: Gammaridea), *Niphargus denarius*, sp. n. is described and figured from the subterranean water of Efimia, Cephalonia, Greece, and its relation to some other members of the genus *Niphargus* Schiödte, 1949 from Greece and some adjacent regions is discussed. This species belongs to the *Supraniphargus* group of taxa.

Keywords: taxonomy, Amphipoda, *Niphargus denarius*, Greece, new taxa, subterranean waters

INTRODUCTION

Based on its specific geographical, geological and climatic position in Europe, the subterranean fauna of Amphipoda in Greece is very rich and highly endemic, although only partially investigated. Within the family Niphargidae, three genera are known from Greece: *Niphargus* Schiödte, 1849, *Exniphargus* GK 2016b and *Niphargobatoides* G. Karaman 2016b; these genera are established based on morphological, ecological and zoogeographical data.

The subterranean genus *Niphargus* Schiödte, 1849 (Amphipoda: Gammaridea: Niphargidae) from Greece has been investigated by various scientists (S. Karaman, G. Karaman, A. Ntakis, C. Anastasiadou, V. Zakšek, C. Fišer, etc.), and nearly 15 species of this genus are known from this region; the most of them are endemic. Recently we have described a new taxa from Greece, *Niphargus spasenijae* G. Karaman 2015 from Tasos Island as well as *N. impexus* G. Karaman, 2016a and *N. lakusici* G. Karaman 2017 from Crete Island.

During our recent studies of Amphipoda from Balkan peninsula, we discovered one new species from Cephalonia, described here based on material given us for study by Dr Giuseppe Pesce from Aquilla, Italy. This species, with elevated number of spines on dactylus of pereopods, belongs to the *Supraniphargus* group of taxa.

Stanko Karaman (1960) established subgenus *Supraniphargus* [typus subgeneris: *Niphargus illidzensis* Schäferna, 1922] for the species with elevated number of spines of dactylus of pereopods. This division was very reasonable because in that period relatively small number of *Niphargus* species was known.

[1] Gordan S. Karaman (corresponding author: karaman@t-com.me)
Montenegrin Academy of Sciences and Arts, Podgorica, Montenegro
Note: The authors declare that they have no conflicts of interest. Authorship Form signed online.

During last 50 years numerous new taxa of genus *Niphargus* have been discovered with various characters, showing that many taxa with this character probably have different origin. This fact doesn`t exclude the possibility that *Niphargus illidzensis* Schäferna 1922 [locus typicus: Ilidža near Sarajevo, Bosnia and Herzegovina] with limited numerous similar taxa represent probably one entity based also on genetically and other characters, although for final decision is necessary to provide further genetic, molecular and taxonomical investigations. For the moment, we use the term *Supraniphargus* for an artificial group of taxa with elevated number of spines on dactylus of pereopods..

MATERIAL AND METHODS

The studied material was preserved in the 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerin and water (40:60) for study and drawing. The body-length of examined specimens was measured from tip of head to end of telson using camera lucida. All illustrations were inked manually. After the end of the study, the dissected body-parts were submerged in Liquid of Faure on slides and covered by thin cover glass. Some morphological terminology and seta formulae follows Karaman`s terminology (Karaman, G. 1969; 1970; 2012).

The advantage of use of Liquid of Faure is the possibility to remove dissected body-parts from Liquid of Faure by water, and study it again in the various positions under the microscope. The new species is established based on provided morphological, zoogeographical and ecological investigations and data.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS DENARIUS, sp. n. Figs 1-8.

MATERIAL EXAMINED:

GREECE:G-2 (G-61) Cephalonia, loc. Efimia (Crini), 8.5.1977, 15 exp. (leg. G. Pesce).

DIAGNOSIS

Body with relatively short and strong extremities, metasomal articles poorly setose along dorsoposterior margin; urosomal segments 1-2 with scarce number of setae and/or spines. Telson gaping, with dorsal, lateral and facial short spines. Coxae short, coxa 4 without lobe. Epimeral plate 3 almost subrounded, with convex posterior margin. Pleopods 1-3 with 2 retinacula, peduncle of pleopod 3 with 3-4 lateral plumose setae. Maxilla 1 outer plate with 7 spines (6 spines with one lateral tooth); maxilliped well developed. Gnathopods 1 and 2 of poorly unequal size, with propodus not larger than corresponding coxa, slightly trapezoid, with one S-, one R- and 3 L-spines, and 4-5 facial M-setae. Pereopods

3-7 are strong, with additional number of strong spines along inner margin (sometimes dactylus of some pereopod 5 or pereopod 6 is with one spine only); basipodit of pereopods 5-7 without distinct lobe. Uropod 1 peduncle without ventrodistal tubercle in males; inner ramus much longer than outer ramus in males. Uropod 3 elongated in males, with long distal article of outer ramus; in female uropod 3 is shorter, with second article of outer ramus much shorter than first one. Sexual dimorphic characters present (epimeral plates, uropod 1, uropod 3).

DESCRIPTION:

MALE 15.0 mm (holotype). Body moderately slender, metasomal segments 1-3 along dorsoposterior margin with 5-6 short setae (fig. 3E). Urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with one spine and one seta, or with 2 spine-like setae and one seta; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior side with one spine near basis of uropod 1 peduncle (fig. 5H).

Epimeral plates 1-2 almost subrounded, with marked ventroposterior spine-like seta, along posterior convex margin appear a row of short setae. Epimeral plate 3 almost subrounded, with convex ventral and posterior margin, ventroposterior corner with spine-like setae and a row of numerous short setae along posterior convex margin (fig. 3E). Epimeral plate 2 with 2-3 subventral spines; epimeral plate 3 with 4-5 subventral spines (fig. 3E).

Head with short rostrum and subrounded lateral cephalic lobes, ventroanterior excavation well developed (fig. 1B), eyes absent.

Antenna 1 slightly exceeding half of body-length (ratio: 60:150). Peduncular articles 1-3 progressively shorter (ratio: 59:43:21), scarcely setose (fig. 1A); main flagellum slender, consisting of 23-24 articles (most of them with one short aesthetasc). Accessory flagellum 2-articulate, short (fig. 1A).

Antenna 2 moderately setose; peduncular article 3 with distoventral bunch of setae; peduncular article 4 poorly longer than article 5 (ratio: 54:51), both articles along ventral margin with 4 bunches of setae (the longest setae reaching or slightly exceeding the diameter of articles themselves). Flagellum slender, longer than last peduncular article (ratio: 70:51), consisting of 9 articles bearing setae as long as or longer than articles themselves (fig. 1C). Antennal gland cone short (fig. 1C).

MOUTHPARTS: Labrum much broader than long, with slightly convex distal margin (fig. 5A). Labium much broader than long, with entire subrounded outer lobes and small but well developed inner lobes (fig. 5B).

Mandible well developed, molar triturative. Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, with several teeth and 6 rakers. Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth and 6 rakers. Palpus 3-articulate: article 1 naked; article 2 with 10-11 strong setae (fig. 1D); article 3 subfalciform, longer than article 2 (ratio: 70:57), with nearly 27 D-setae and 5-6 E-setae; on outer face appear one group of 7 A-setae, on inner face appear 8 B-setae sitting in 3 groups (fig. 1E).

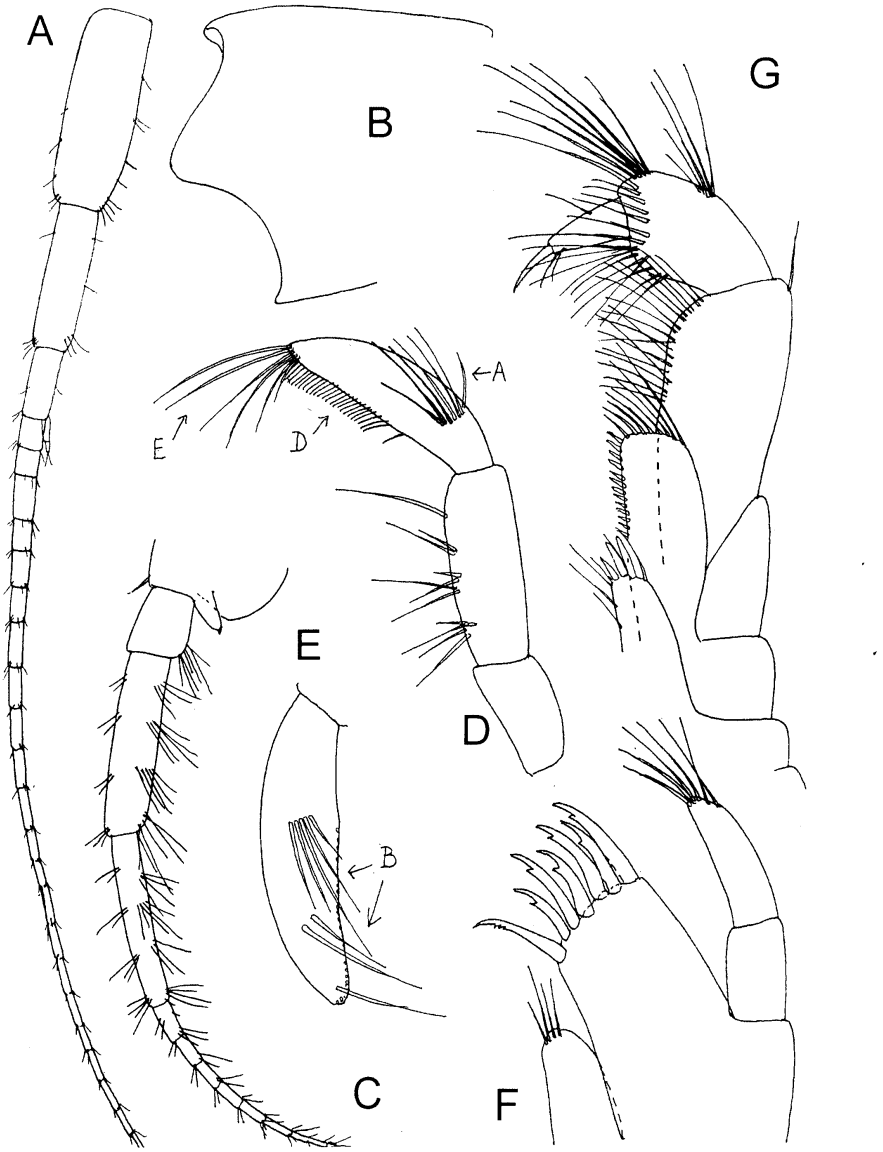


Fig. 1. *Niphargus denarius*, sp. n., Efimia, Cephalonia, male 15.0 mm (holotype): A= antenna 1; B= head; C= antenna 2; D= mandibular palpus, outer face [A= facial A-setae; D= lateral D-setae; E= distal E-setae]; E= distal palpus article of mandible, inner face [B= facial B-setae]; F= maxilla 1; G= maxilliped

Maxilla 1: inner plate with 3-4 simple setae (fig. 1F), outer plate with 7 spines (6 spines with one lateral tooth, one spine with 3 small lateral teeth); palpus 2-articulate, not reaching tip of outer plate spines and provided with 6-8 distal setae.

Maxilla 2: both plates with numerous distal setae (fig. 5C).

Maxilliped: inner plate short, not reaching outer tip of palpus article 1 and bearing 3-4 pointed distal spines accompanied by several setae (fig. 1G). Outer plate not exceeding half of palpus article 2, with numerous spines along distolateral (mesial) margin. Palpus 4-articulate, article 2 along mesial margin with numerous simple setae; article 3 along outer margin with one medial and one distal bunch of setae; article 4 at inner margin with 2 setae near basis of the nail, along outer margin appear one medial seta (fig. 1G).

Coxae are relatively short, not covering to each other. Coxa 1 is broader than long (ratio: 63:48), with broadly subrounded ventroanterior corner; along margins of coxa 1 appear a row of nearly 15 short setae (fig. 2A). Coxa 2 slightly broader than long (ratio: 64:57), along ventral convex margin appear a row of nearly 20 short setae (fig. 2D).

Coxa 3 slightly broader than long (ratio: 73:67), along ventral convex margin with a row of nearly 18 short setae (fig. 3A). Coxa 4 broader than long (ratio: 72:60), along ventral slightly convex margin are attached nearly 17 short setae, ventroposterior lobe absent (fig. 3C).

Coxa 5 broader than long (ratio: 72:52), bilobed, anterior lobe nearly as long as coxa 4, posterior lobe with several short marginal setae (fig. 4A). Segment above coxa at ventroposterior corner is provided with 2-3 marginal setae (fig. 4A).

Coxa 6 smaller than coxa 5 but bilobed also, broader than long (ratio: 60:42), posterior lobe with several short marginal setae (fig. 4C). Segment above coxa at ventroposterior corner is provided with 5-6 short marginal setae (fig. 4C).

Coxa 7 entire, broader than long (ratio: 54:27), with convex ventral margin and 2-3 setae along posterior margin (fig. 4E). Segment above coxa at ventroposterior corner is provided with 5-6 short marginal setae (fig. 4E).

Gnathopods 1 and 2 are relatively small, with propodus nearly as large as corresponding coxa (fig. 2A, D). Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae (fig. 2A); article 3 at posterior margin with one medial group of setae; article 4 with marked posterior bump; article 5 only slightly shorter than propodus (ratio: 42:52), at distoanterior tip with 1-2 groups of setae. Propodus trapezoid, slightly longer than broad (ratio: 97:83), along posterior margin with 6 transverse rows of setae (fig. 2B); palm poorly convex, inclined almost half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and 5 long facial M-setae (fig. 2C), on inner face by one subcorner R-spine (fig. 2C). Dactylus reaching remarkably posterior margin of propodus, along outer margin appear a row of 7 single or paired setae, along inner margin appear a row of several short setae (fig. 2B).

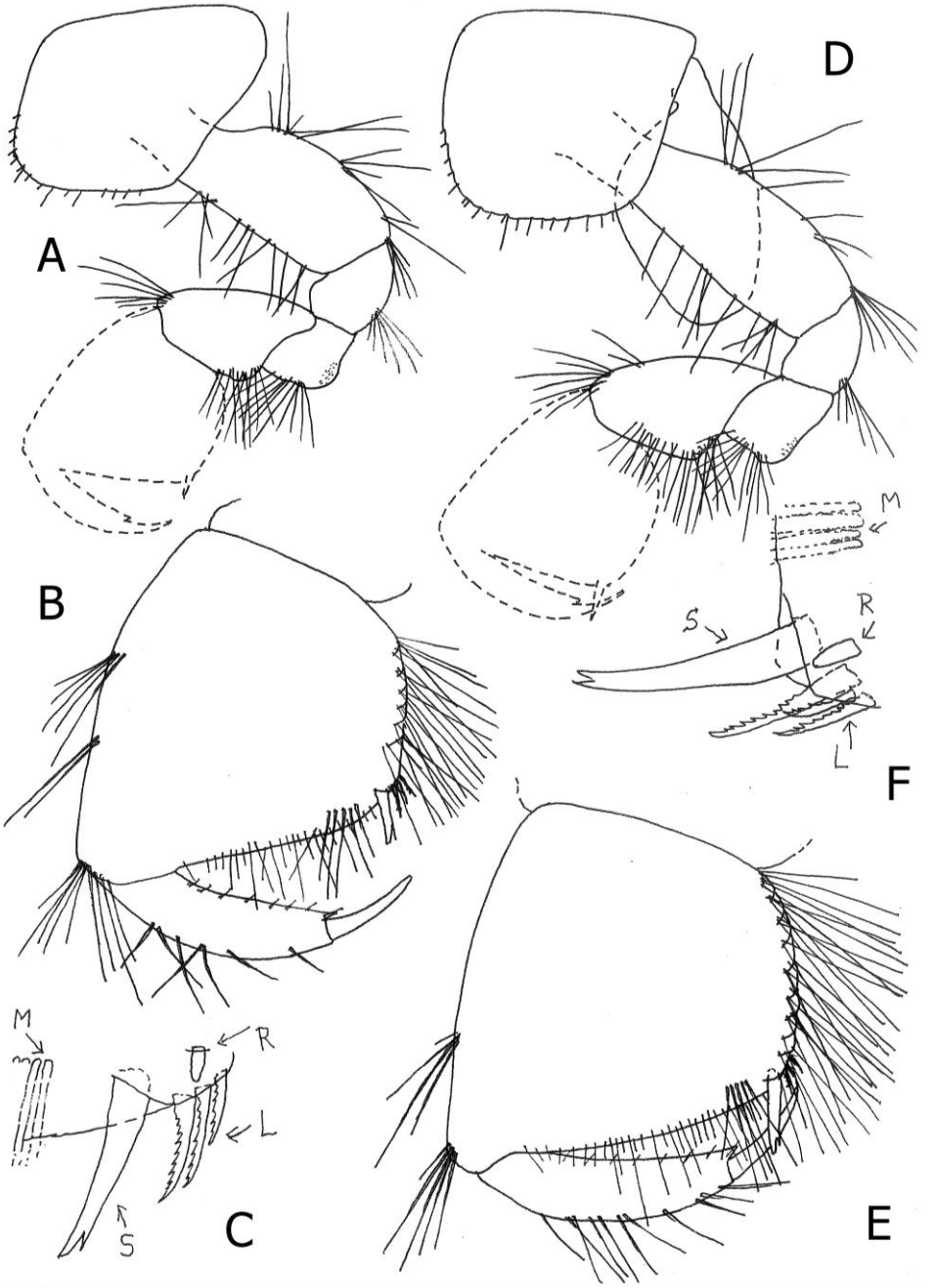


Fig. 2. *Niphargus denarius*, sp. n., Efimia, Cephalonia, male 15.0 mm (holotype): A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, inner face [S= corner S-spine; L= lateral serrate spines; M= facial M-setae; R= subcorner R-spine]; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus, inner face [S= corner S-spine; L= lateral serrate spines; M= facial M-setae; R= subcorner R-spine].

Gnathopod 2 is poorly larger than gnathopod 1; article 2 along anterior and posterior margin with row of long simple setae (fig. 2D); article 3 at posterior margin with one bunch of setae; article 4 along posterior margin with marked bump (fig. 2D); article 5 slightly shorter than propodus (ratio: 50:55), along anterior margin with 2 groups of setae. Propodus trapezoid, slightly longer than broad (ratio: 111:90), along posterior margin with 9 transverse rows of setae (fig. 2E); palm slightly convex, inclined slightly less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 slender L-spines and 5 facial long M-setae (fig. 2F), on inner face by one subcorner R-spine (fig. 2F). Dactylus remarkably reaching posterior margin of propodus, along outer margin provided with a row of 11 single or paired setae, along inner margin with a row of short setae.

Pereopods 3 and 4 rather similar to each other, moderately stout. Pereopod 3: article 2 along anterior margin with several long proximal setae and a row of mediodistal short setae, along posterior margin with several bunches of long setae. Articles 4-6 of unequal length (ratio: 65:45:50); article 4 along posterior margin with 4 bunches of setae (the longest setae reaching or hardly exceeding diameter of article itself); article 5 along posterior margin with 3 bunches of setae (the longest setae much exceeding diameter of article itself); article 6 along posterior margin with 5 single or paired short spines accompanied by 0-1 short seta (fig. 3A). Dactylus much shorter than article 6 (ratio: 20:50), short and strong, along inner margin with 3 strong spines, along outer margin with one medial plumose seta (fig. 3B); nail strong, shorter than pedestal (ratio: 29:35).

Pereopod 4: article 2 along anterior margin with 5-6 long proximal setae and a row of nearly 10 short mediodistal setae (fig. 3C); articles 4-6 of unequal length (ratio: 63:44:50): article 4 along posterior margin with 5 bunches of setae (the longest setae are nearly as long as diameter of article itself); article 5 along posterior margin with 3 bunches of spines and short setae; article 6 along posterior margin with row of 5 single or paired short spines accompanied by single short setae. Dactylus strong, much shorter than article 6 (ratio: 16:50), along inner margin with 3 strong spines, along outer margin with one medial plumose seta (fig. 3D); nail is nearly as long as pedestal.

Pereopods 5-7 relatively stout. Pereopod 5 is slightly shorter than pereopods 6 and 7, with article 2 longer than broad (ratio: 70:47), along anterior margin with 7 groups of short spines and/or setae; along posterior margin with nearly 20 short setae, ventroposterior lobe not fully developed (fig. 4A); articles 4-6 of unequal length (ratio: 45:48:50), along anterior margin mainly with bunches of short setae, along posterior margin with bunches of short spines sometimes accompanied by single short seta. Article 2 is longer than article 6 (ratio: 70:50). Dactylus is much shorter than article 6 (ratio: 17:50), along inner margin with 2 strong spines, along outer margin with one median seta (fig. 4B); nail slightly shorter than pedestal (ratio: 26:31).

Pereopod 6: article 2 remarkably longer than broad (ratio: 76:49), along anterior margin with 7 groups of short spine-like setae or setae, along posterior poorly medially concave margin with nearly 22 short setae, ventroposterior lobe

not fully developed (fig. 4C); articles 4-6 of unequal length (ratio: 55:60:71), along both margins with groups of short spines and setae. Article 6 is only slightly shorter than article 2 (ratio: 71:76). Dactylus is short and strong, much shorter than article 6 (ratio: 18:71), along inner margin with 3 strong spines, along outer margin with one median seta (fig. 4D); nail is shorter than pedestal (ratio: 33:50).

Pereopod 7: article 2 much longer than broad (ratio: 86:51), along anterior margin with 7 single or paired spine-like setae, along posterior poorly convex margin with nearly 23 short setae, ventroposterior lobe not fully developed (fig. 4E). Articles 4-6 of unequal length (ratio: 48:61:75), along margins with groups of short spines and short setae not reaching diameter of articles themselves. Article 2 is slightly longer than article 6 (ratio: 86:75). Dactylus is much shorter than article 6 (ratio: 23:75), strong, along inner margin with 4 strong spines, along outer margin with one medial seta (fig. 4F); nail is shorter than pedestal (ratio: 34:55).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 with 4-5 setae at distoanterior part (fig. 5D); peduncle of pleopod 2 with 2 distal setae at anterior margin and one medial short seta at posterior margin (fig. 5E). Peduncle of pleopod 3 along posterior margin with 3 strong setae, along outer margin with 4 plumose and one simple seta (fig. 5F).

Uropod 1 is long, peduncle with dorsoexternal row of spines and with dorsointernal row of setae (fig. 5H), ventrodistal tubercle absent. Inner ramus nearly as long as peduncle, with several lateral and distal short spines, as well as with 5 bunches of simple setae along ventral margin. Outer ramus reaching nearly half of inner ramus-length, with several lateral and distal short spines (spines never reaching diameter of article itself) (fig. 5H).

Uropod 2: peduncle with single lateral and distal spines; inner ramus bearing several lateral and distal short spines (fig. 5G) and 3 simple short setae at outer margin; outer ramus hardly shorter than inner one, with several lateral and distal short spines.

Uropod 3 long: peduncle much longer than broad (ratio: 49:25), with single distal spines; inner ramus slightly shorter than peduncle (ratio: 36:49), with 3 lateral and one distal spine accompanied by distal bunch of short setae (fig. 5 I); outer ramus 2-articulated, narrow; first article along both margins and tip with bunches of single or paired very short spines and simple setae; second article exceeding half of first article-length (ratio: 97:135), along both margins and tip with bunches of short simple setae.

Telson slightly broader than long (ratio: 92:80), gaping, incised nearly 2/3 of telson-length; each lobe with 3 short distal spines, along outer margin with one spine, along inner (mesial) margin with 2 single spines or one spine and 2 setae; a pair of facial spines and 0-1 seta appear on each lobe (fig. 3F).

Coxal gills 1-4 ovoid, moderately large, not exceeding ventral tip of corresponding article 2 (figs. 2D; 3A, C); coxal gills 5 and 6 much smaller, ovoid (fig. 4A, C).

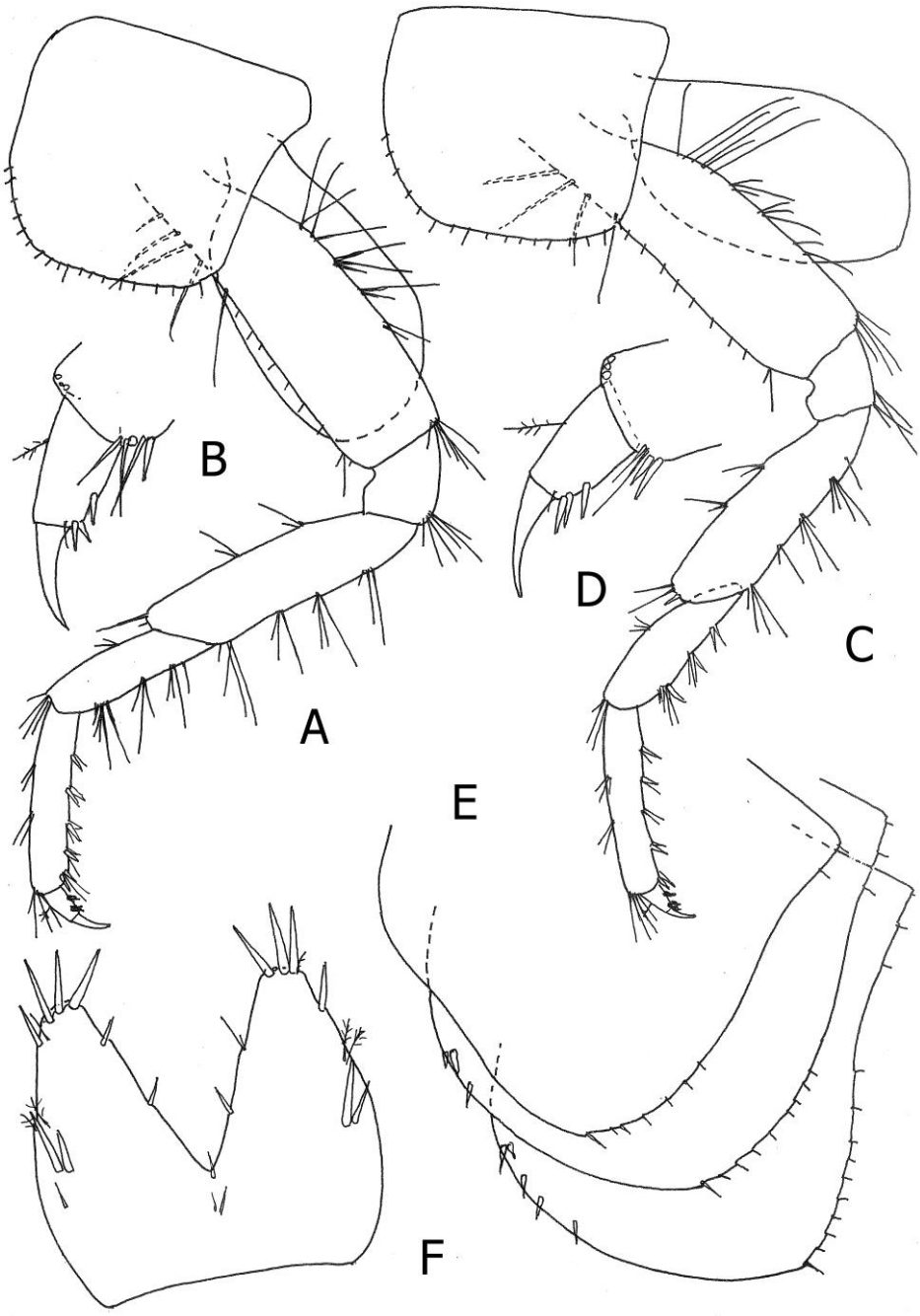


Fig. 3. *Niphargus denarius*, sp. n., Efimia, Cephalonia, male 15.0 mm (holotype): A= pereopod 3; B= dactylus of pereopod 3; C= pereopod 4; D= dactylus of pereopod 4; E= epimeral plates 1-3; F= telson.

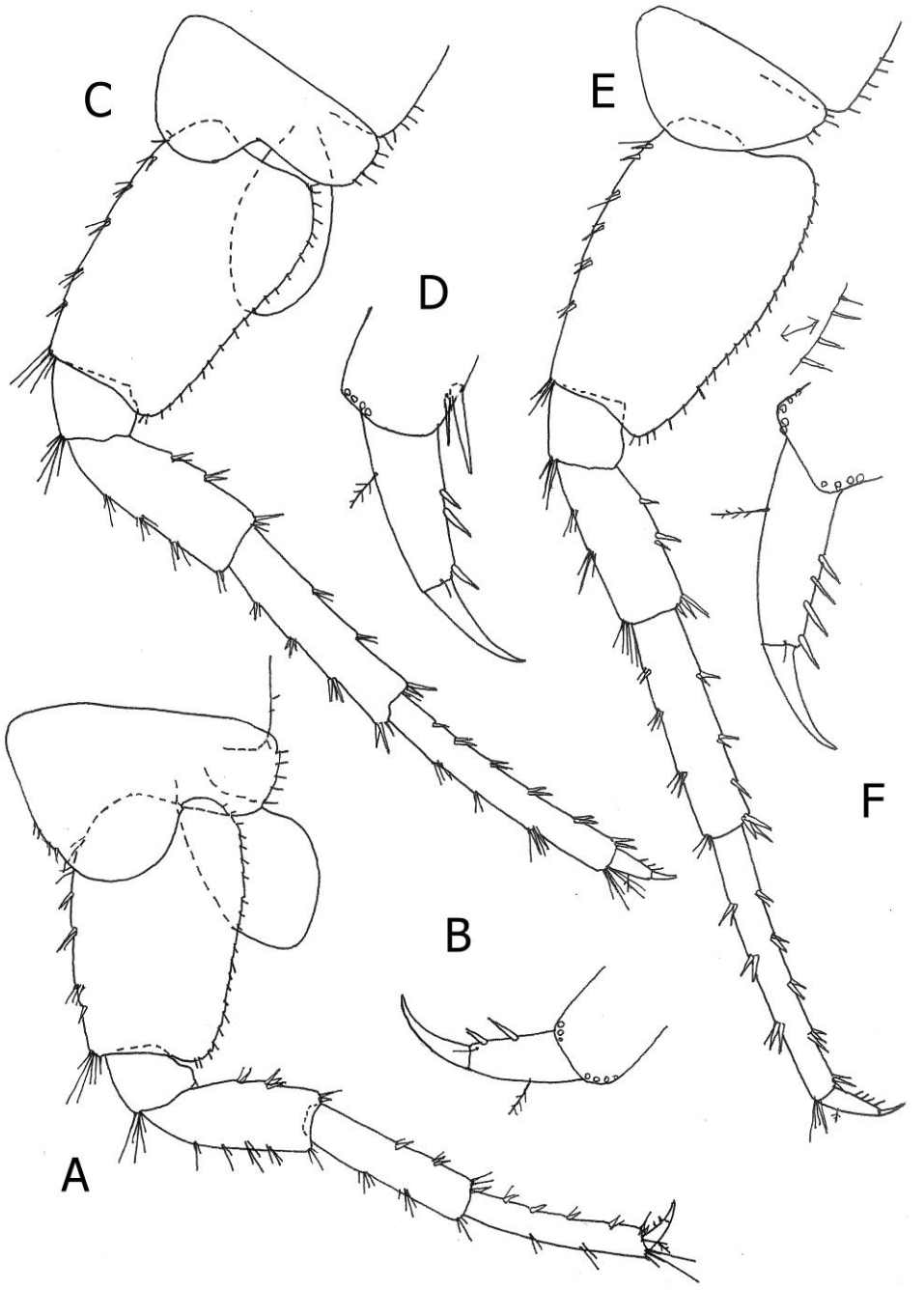


Fig. 4. *Niphargus denarius*, sp. n., Efimia, Cephalonia, male 15.0 mm (holotype): A= pereopod 5; B= dactylus of pereopod 5; C= pereopod 6; D= dactylus of pereopod 6; E= pereopod 7; F= dactylus of pereopod 7.

FEMALE 9.5 mm with setose oostegites (paratype). Body moderately slender, metasomal segments 1-3 at dorsoposterior margin with 5 short setae (fig. 5J); urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with one spine and one seta, or 2 spines and one seta; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig. 8A).

Epimeral plate 1 with marked ventroposterior corner, posterior convex margin bearing a row of short setae, ventral margin slightly concave in the middle (fig. 5J). Epimeral plate 2 almost subrounded, with well-marked ventroposterior corner spine-like seta and convex posterior margin bearing a row of short setae, ventral margin is convex. Epimeral plate 3 is poorly angular, with marked ventroposterior corner and corner spine-like seta, ventral margin is strongly convex, posterior margin slightly convex, with a row of short setae (some setae are spine-like setae) (fig. 5J). Epimeral plates 2 and 3 are with 2 subventral spines each.

Head like that in male. Antenna 1 almost reaching half of body-length (ratio: 45:95); main flagellum consisting of 20 articles. Accessory flagellum 2-articulated, slightly exceeding half of last peduncular article.

Antenna 2 peduncular articles 4 and 5 with 4-5 bunches of ventral setae; flagellum consisting of 9 articles. Antennal gland cone is short.

Mouthparts mainly like these in male. Mandibular palpus article 1 naked, article 2 with 7-9 setae; palpus article 3 with nearly 23-24D-, 5-6E-, 6A- and 4-5 B setae.

Inner plate of maxilla 1 with 3 setae, outer plate with 7 spines (6 spines with one lateral tooth, one spine with 2-3 small lateral teeth), palpus provided with 6-setae.

Maxilliped: inner plate with 3 spines, palpus article 3 at outer margin with 2 bunches of setae; article 4 at inner margin with 2 setae near basis of the nail.

Coxae 1-4 are rather longer than these in male. Coxa 1 is rhomboid, slightly longer than broad (ratio: 50:45), with broadly subrounded ventroanterior corner, and bearing nearly 21 short marginal setae (fig. 6A). Coxa 2 is longer than broad (ratio: 60:47), along ventral convex margin with nearly 17 short unequal setae (fig. 6C). Coxa 3 only poorly longer than broad (ratio: 60:57), along ventral margin with 15 short setae (fig. 6E). Coxa 4 is slightly longer than broad (ratio: 62:54), along ventral margin with nearly 18 short unequal setae, ventroposterior lobe is not developed (fig. 6F).

Coxa 5 is much broader than long (ratio: 72:50), anterior lobe almost as long as coxa 4, broadly subrounded, posterior lobe with 5-6 short posterior marginal setae (fig. 7A). Segment above coxa at ventroposterior corner is provided with 2-3 marginal setae (fig. 7A).

Coxa 6 is smaller than coxa 5, bilobed, broader than long (ratio: 60:42), posterior lobe along posterior margin with nearly 5 short setae (fig. 7C). Segment above coxa at ventroposterior corner is provided with 5-6 short marginal setae (fig. 7C).

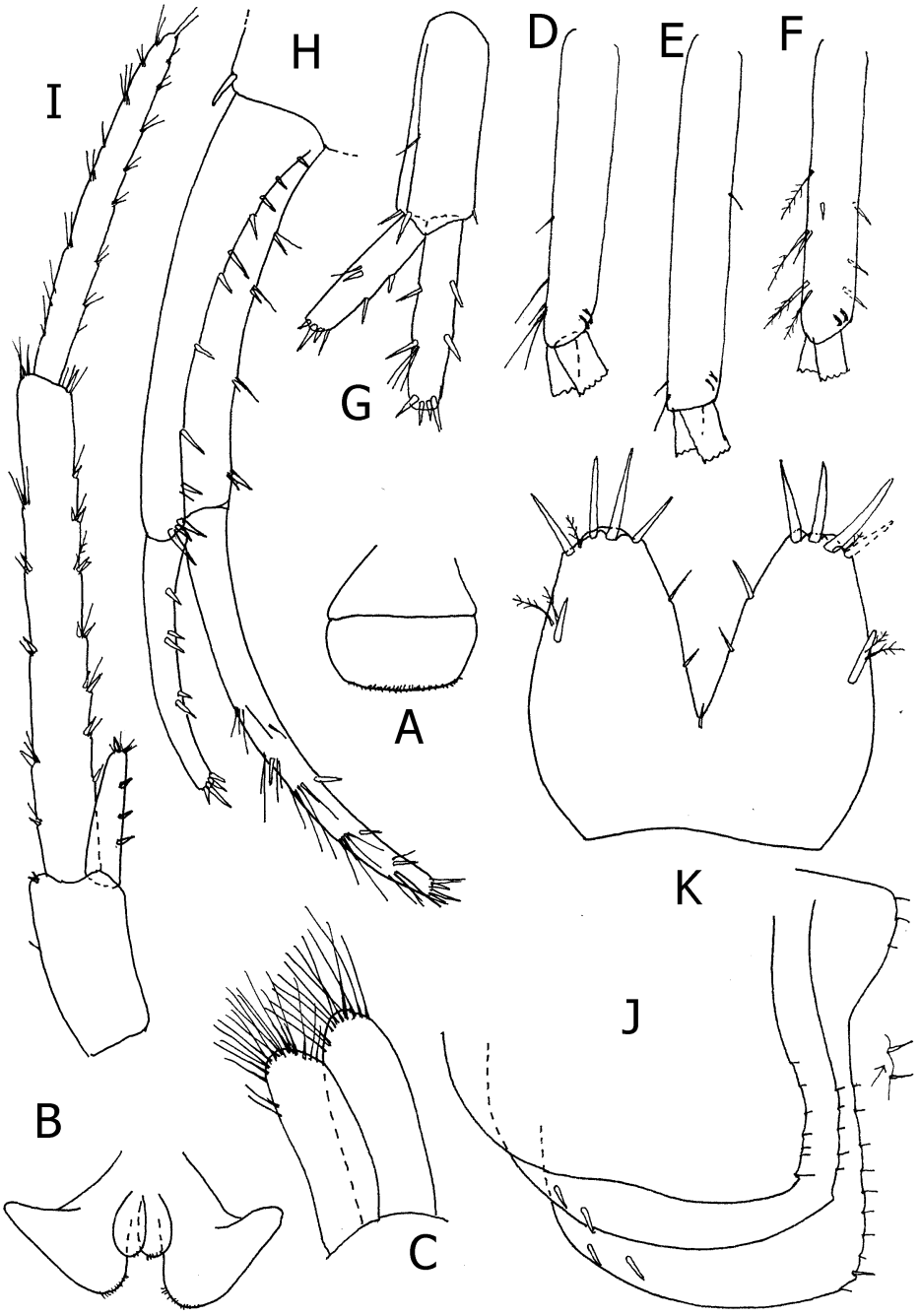


Fig. 5. *Niphargus denarius*, sp. n., Efimia, Cephalonia, male 15.0 mm (holotype): A= labrum; B= labium; C= maxilla 2; D= peduncle of pleopod 1; E= peduncle of pleopod 2; F= peduncle of pleopod 3; G= uropod 2; F= uropod 1; I= uropod 3. Female 9.5 mm (paratype): J= epimeral plates 1-3; K= telson.

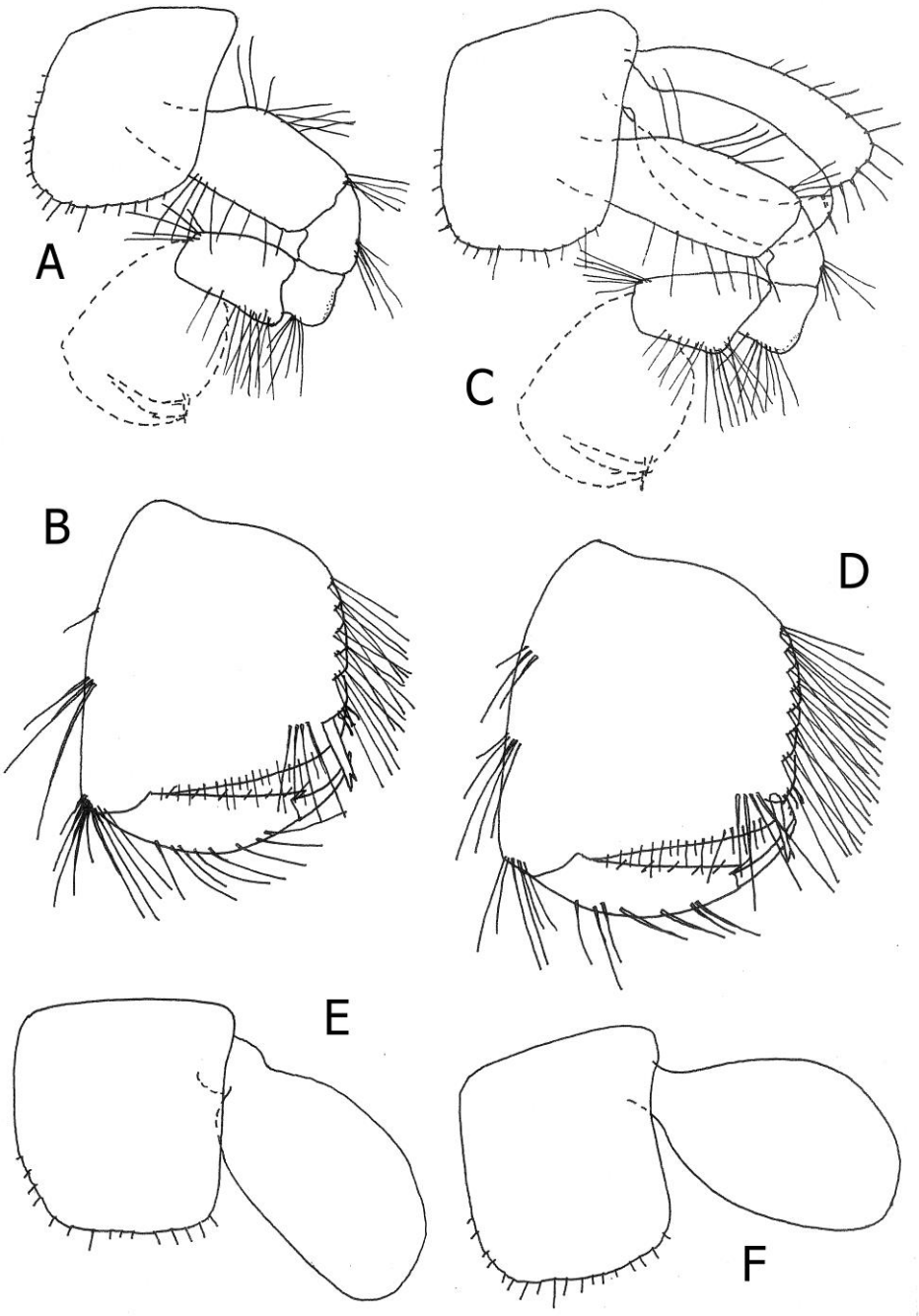


Fig. 6. *Niphargus denarius*, sp. n., Efimia, Cephalonia, female 9.5 mm (paratype): A-B= gnathopod 1, outer face; C-D= gnathopod 2, outer face; E= coxa 3; F= coxa 4.

Coxa 7 is much broader than long (ratio: 55:27), entire, with convex ventral margin and 2-3 setae at posterior margin (fig. 7D). Segment above coxa at ventroposterior margin is provided with 3-4 short setae (fig. 7D).

Gnathopods 1 and 2 are relatively small, poorly unequal in size and with propodus poorly smaller than corresponding coxa (fig. 6A, C). Gnathopod 1: article 2 stout, along anterior and posterior margin with numerous long simple setae (fig. 6A). Article 3 at posterior margin with one bunch of setae; article 4 with visible posterior bump; article 5 shorter than propodus (ratio: 28:42), along anterior margin with one distal bunch of setae. Propodus almost quadrate, longer than broad (ratio: 82:69), along posterior margin with 5 transverse rows of setae (fig. 6B). Palm inclined nearly 1/3 of propodus length, poorly convex, defined on outer face by one S-spine accompanied laterally by 3 slender L-spines and 4 long facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with row of 8 single or paired medial setae, along inner margin with several short setae (fig. 6B).

Gnathopod 2 is only poorly larger than gnathopod 1; article 2 along both margins with long setae; article 3 at posterior margin with one bunch of median setae (fig. 6C); article 4 with marked posterior bump; article 5 along anterior margin with one distal bunch of setae. Propodus almost quadrate, slightly longer than broad (ratio: 85:78), along posterior margin with 7 transverse rows of setae (fig. 6D), palm slightly convex, inclined nearly 1/3 of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 L-spines and 5 long facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with 7 medial setae, along inner margin with several short setae.

Pereopods 3 and 4 like these in male, with dactylus bearing 2 strong spines along inner margin and one median plumose seta at outer margin.

Pereopods 5-7 rather stout and short. Pereopod 5: article 2 longer than broad (ratio: 74:50), along anterior margin with 5 groups of spine-like setae, along posterior convex margin with 12 short setae, ventroposterior lobe not fully developed (fig. 7A). Articles 4-6 of unequal length (ratio: 46:48:55), articles along both margins with short spines and setae. Article 2 is longer than article 6 (74:55). Dactylus is much shorter than article 6 (ratio: 18:55), strong, along inner margin with one spine, along outer margin with one medial plumose seta (fig. 7B); nail is shorter than pedestal (ratio: 31:38).

Pereopod 6: article 2 distinctly longer than broad (ratio: 85:55), along anterior margin with 6 groups of spine-like setae, along posterior poorly convex margin with nearly 15 short setae, ventroposterior lobe not fully developed (fig. 7C). Articles 4-6 of unequal length (ratio: 54:63:77), along both margins with bunches of short spines and short setae. Article 2 is slightly longer than article 6 (ratio: 85:77). Dactylus is much shorter than article 6 (ratio: 23:77), strong, along inner margin with 1-2 spines, along outer margin with one medial seta; nail is shorter than pedestal (ratio: 25:40).

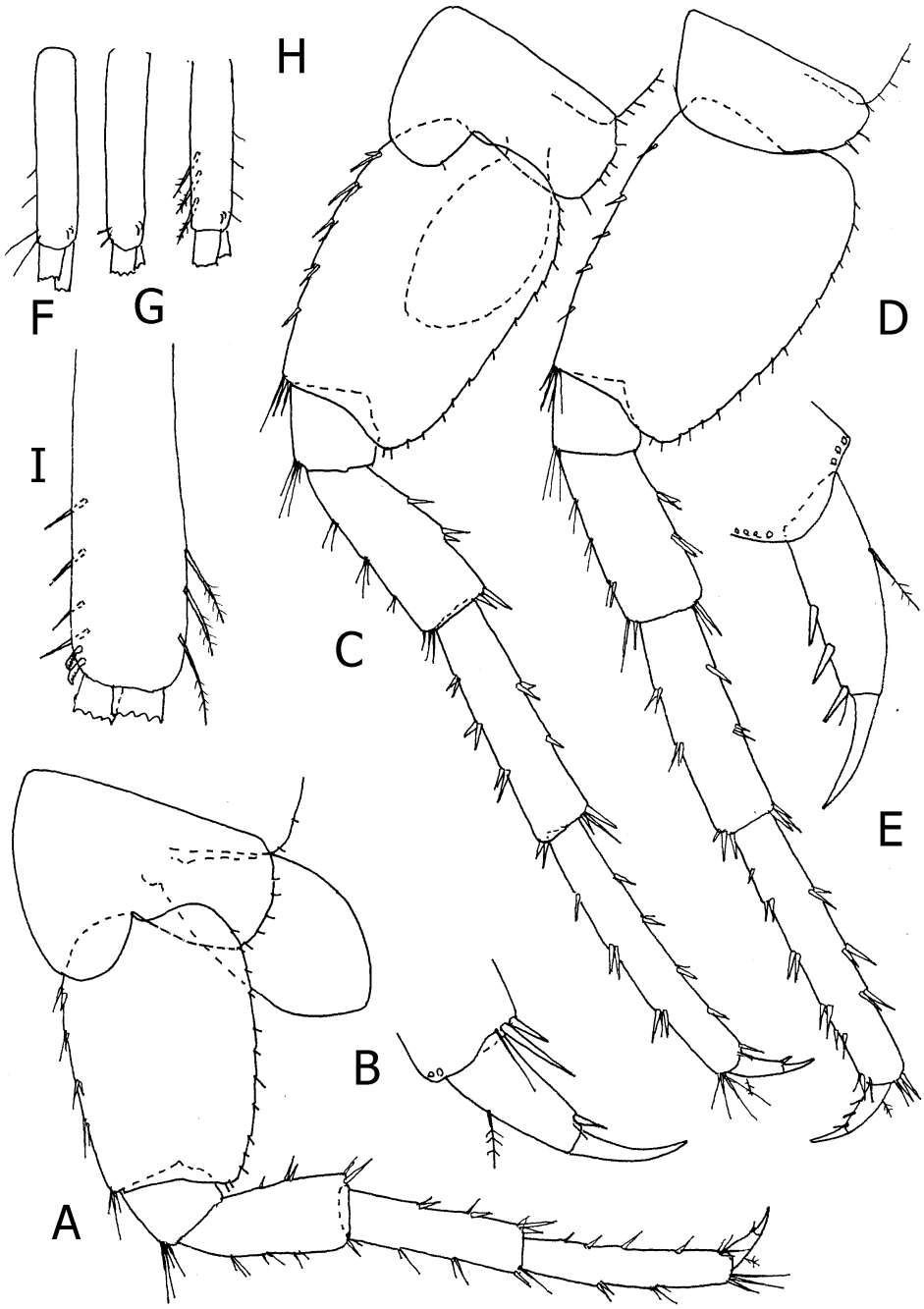


Fig. 7. *Niphargus denarius*, sp. n., Efimia, Cephalonia, female 9.5 mm (paratype): A= pereopod 5; B= dactylus of pereopod 5; C= pereopod 6; D= pereopod 7; E= dactylus of pereopod 7; F= pleopod 1 peduncle; G= pleopod 2 peduncle; H-I= pleopod 3 peduncle.

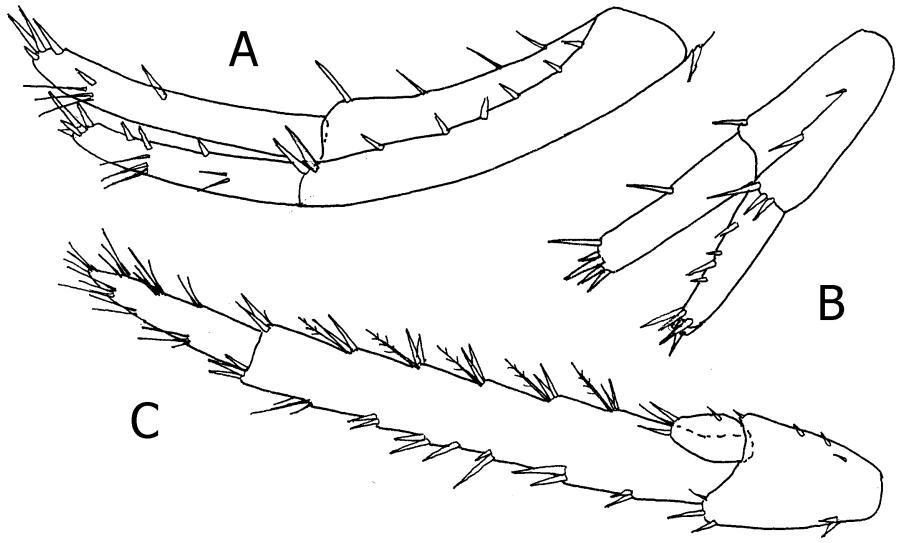


Fig. 8. *Niphargus denarius*, sp. n., Efimia, Cephalonia, female 9.5 mm (paratype): A= uropod 1; B= uropod 2; C= uropod 3.

Pereopod 7: article 2 is longer than broad (ratio:88:59), along anterior margin with 6 spine-like setae and one bunch of distal setae, along posterior slightly convex margin with 17 short setae, ventroposterior lobe not fully developed (fig. 7D). Articles 4-6 of unequal length (ratio: 50:60:78), articles along both margins with bunches of short spines and setae. Article 2 is slightly longer than article 6 (ratio: 88:78). Dactylus is shorter than article 6 (ratio: 27:78), along inner margin with 3 strong spines, along outer margin with one medial seta (fig. 7E); nail is much shorter than pedestal (ratio: 32:61).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 with 4 anterior marginal setae (fig. 7F); peduncle of pleopod 2 with 2 distoanterior setae (fig. 7G); peduncle of pleopod 3 at posterior margin with 4 spine-like setae, along outer margin with 3 plumose setae (fig. 7H, I).

Uropod 1: peduncle with dorsoexternal row of spines and with dorsointernal row of setae (except distal spine) (fig. 8A). Inner ramus is shorter than peduncle, bearing only 1-2 lateral spines and 3 simple setae, as well as 5 distal short spines. Outer ramus reaching nearly ¾ of inner ramus-length, bearing several lateral short sp ines and 3 bunches of short simple setae; ramus ended with 4-5 short spines.

Uropod 2: peduncle with single lateral and distal spines (fig. 8B); inner ramus provided with one lateral and 5 distal spines; outer ramus is almost as long as inner ramus, with 4 lateral and 5 distal short spines.

Uropod 3 is relatively short: peduncle only slightly longer than broad (ratio: 45:30), bearing single lateral and distal spines; inner ramus short, scale-like, with one lateral and 2 distal spines accompanied by one smooth seta (fig.

8C). Outer ramus is 2-articulated; first article broad, along outer margin with 8 groups of strong short spines, along inner margin with 6 bunches of spines accompanied by single short plumose setae; second article narrower and shorter than first one (ratio: 48:128), bearing 3 bunches of simple setae along both margins and tip.

Telson gaping, slightly broader than long (ratio: 90:80), incised nearly 3/5 of telson-length (fig. 5K); each lobe is provided with 4 distal short spines, as well as with 2 setae or spines along mesial margin; one spine appears on the face of each lobe; a pair of short plumose setae is implanted near the middle of each lobe.

Coxal gills ovoid, moderately long (figs. 6C, F; 7A, C), never exceeding ventral tip of corresponding basipodit.

Oostegites large, with long marginal setae (fig. 6C).

VARIABILITY.

Epimeral plates in females are, in general, slightly more angular than these in males. Scarce number of dorsoposterior short setae on metasomal segments 1-3 (4-6 setae) is present in males and females.

Epimeral plate 2 in males is with 2-3 subventral spines, epimeral plate 3 with 3-5 subventral spines. Maxilla 1 inner plate is with 3-4 setae; maxilliped inner plate with 3-4 distal pointed spines. Basipodit of pereopods 5-7 along posterior margin is with short setae in males and females; often some of these setae are stronger, spine-like setae (fig. 4E), ventroposterior lobe never fully developed.

Dactylus of pereopods 3-7 in males and females is provided with 2-3 spines, occasionally dactylus of pereopods 5 or pereopod 6 is with only one spine at inner margin.

Plumose setae on peduncle of pleopod 3 in males and females are always present. Uropod 2 in males and females is with almost subequal rami or with inner ramus scarcely longer than outer one.

HOLOTYPE: male 15.0 mm; paratype: female 9.5 mm with setose oostegites. Holotype and paratypes are deposited in the Karaman`s Collection in Podgorica, Montenegro.

DISTRIBUTION: Known from type-locality only.

REMARKS AND AFFINITIES.

The new species, *Niphargus denarius*, sp. n. is characterized by presence of additional number of spines on pereopods 3-7, the character observed in various taxa from Europe and Near East, but not mentioned among known taxa from Greece.

N. denarius is rather similar to the species *Niphargus rotundus* G. Karaman, 2016c known from well in Montelupo Albeze, 450 m a.s.l. (Cuneo, Italy) (epimeral plates, short strong pereopods with strong dactyls bearing

additional spines, gaping telson, elongated uropods 1 and 3 in males, etc.), but *N. denarius* differs from it by lower number of M-setae on propodus of gnathopods, by presence of plumose setae on peduncle of pleopod 3 etc.

Another species with the presence of additional spines on dactylus of pereopods 3-7 is *N. stankoi* G. Karaman 1974, known from spring near village Bukovo on road Resen-Ohrid, Macedonia. This species differs from *N. denarius* by different shape of propodus of gnathopods, different epimeral plates, by subequal rami of uropod 1 in males, strongly spinose telson etc.

In western Dinaric region (Croatia, Bosnia and Herzegovina, Montenegro) are present several taxa of the *Niphargus illidzensis*-complex with elevated number of spines on dactylus, but they differ from our species by various other taxonomical characters, including more acute epimeral plates, pleopods, etc. (S. Karaman, 1950).

In Italy are known several other species with additional number of spines on dactylus of pereopods 3-7 [*N. sestoputeanus* G. Karaman, 2016c (loc. typ.: Sesta Godano, Passo del Rastrello, 1000 m a.s.l., N. of La Spezia, Italy); *N. elegans* Garbini 1894 (loc. typ.: S. Pancrazio near Verona, Italy), etc.] but *N. denarius* differs from all of them by combination of different taxonomical characters.

In Bulgaria is present endemic species *Niphargus bulgaricus* Andreev 2001 [loc. typ.: Lake Bolata (Shabla, N. of cap Caliakra, Varna reg.] with elevated number of spines on dactylus of pereopods, but this species is characterized by acute epimeral plates.

In the subterranean waters of France are present various *Niphargus* taxa with elevated number of spines on dactylus of pereopods (*N. ladmiraulti* Chevreux 1901, *N. ciliatus* Chevreux 1906, *N. balazuci* Schellenberg 1951, etc.), but these species differ from our species by various characters.

DERIVATIO NOMINIS. The name “denarius” should allude to the shape of old Roman money “denarius”, according to the subrounded epimeral plates of this species.

CONCLUSION

Fauna of Amphipoda in Greece is still only partially known, and nearly 15 taxa of genus *Niphargus* are known from this region. *Niphargus denarius*, sp. n. described from Cephalonia is rather similar to the species *Niphargus rotundus* G. Karaman 2016 from Italy, and *Niphargus puteanus*-complex in general, but differs from later by several morphological characters. Elevated number of spines on dactylus of pereopods 3-7 is present in numerous taxa of genus *Niphargus* over Europe, including Balkan peninsula, but all of them differs from *N. denarius* by combination of taxonomical characters. Although the presence or absence of additional spines on dactylus of pereopods 5-7 is good morphological and taxonomical characters, we don`t know its real value for higher taxonomical categories.

ACKNOWLEDGEMENTS

I am thankful to Prof. Dr. Giuseppe Pesce from the University of Aquilla for the collected material used in this study.

REFERENCES

Andreev, S. 2001. Niphargus bulgaricus sp. n. et deux especes nouvelles pour la faune de Bulgarie-Niphargopsis trispinosus Dancau et Gammarus roeseli (sic!) Gervais (Amphipoda: Niphargidae, Gammaridae).- Historia Naturalis Bulgarica 13: 79-87.

Chevreux, E. 1901. Amphipodes des eaux souterraines de France et d`Algerie. I-VI. Bulletin de la Société zoologique de France: 168-222.

Chevreux, E. 1906. Niphargus ciliatus n. sp., in: Peyerimhoff, P.: Recherches sur la faune cavernicole des Basses-Alpes.- Annales de la Societe Entomologique de France, 75: 203-222.

Garbini, A. 1894. Gammarus ciechi in acque superficialis basse. - Academia d`Agricoltura Arti e Commercio di Verona, 70 (3): 107-112.

Karaman, G. 1969. XXVII. Beitrag zur Kenntnis der Amphipoden. Arten der Genera Echinogammarus Stebb. und Chaetogammarus Mart. an der jugoslawischer Adriaküste. Glasnik Republičkog zavoda zazaštitu prirode i Prirodnjačke zbirke u Titogradu. 2: 59–84.

Karaman, G. 1970. XXV. Beitrag zur Kenntnis der Amphipoden. Kritische Bemerkungen über Echinogammarus acarinatus (S. Kar., 1931) und Echinogammarus stocki n. sp. - Poljoprivreda i šumarstvo, Titograd, 16 (1–2): 45–66.

Karaman, G. 1974. XLVI. Contribution to the Knowledge of the Amphipoda. Two subterranean Niphargus species from Yugoslavia, N. stankoi, n. sp. and N. ilidzensis Schäferna (Fam. Gammaridae). - Glasnik Republičkog. zavoda za zaštitu prirode- Prirodnjačkog muzeja Titograd, 6: 35-57 (1973).

Karaman, G. 2012. Further investigations of the subterranean genus Niphargus Schiödte, 1849 (fam. Niphargidae) in Serbia. (Contribution to the Knowledge of the Amphipoda 264). - Agriculture and Forestry, Podgorica, 58 (2): 45–64.

Karaman, G. 2015. New data of genus Niphargus Schiödte, 1849 (Fam. Niphargidae) from Greece (Contribution to the knowledge of the Amphipoda 284).- Agriculture & Forestry, 61 (4): 43-60, Podgorica.

Karaman, G. 2016a. On two new or interesting species of the family Niphargidae from Greece and Croatia. (Contribution to the Knowledge of the Amphipoda 286).- Ecologica Montenegrina 5: 1-17.

Karaman, G. 2016b. Two new genera of the family Niphargidae from Greece (Contribution to the Knowledge of the Amphipoda 287). - Agriculture & Forestry, 62 (1): 7-27.

Karaman, G. 2016c. On two new Niphargus species (Fam. Niphargidae) from Italy (Contribution to the Knowledge of the Amphipoda 288).- Ecologica Montenegrina 5: 70-89.

Karaman, G. 2017. A new member of the genus Niphargus Schiödte, 1849 (Amphipoda Gammaridea, fam. Niphargidae) from Crete Island, Greece (Contribution to the Knowledge of the Amphipoda 293).- Ecologica Montenegrina, 10: 1-10, 4 figs.

Karaman, S. 1950. Niphargus ilidzensis Schaeferna i njegovi srodnici u Jugoslaviji. (= Supraniphargus ilidzensis Schäferna und seine Nächstverwandten in

Jugoslavien).- Srpska Akademija Nauka, Posebna Izdanja knj. 158, Odelenje Prirodno-matematičkih nauka, Beograd, 2: 51-85, figs. 1-40.

Karaman, S. 1960. Weitere Beiträge zur Kenntnis der Jugoslavischen Niphargiden.- Glasnik Prirodnjačkog muzeja Beograd, Ser. B, 15: 75-90, figs. 1-19.

Schäferna, K. 1922. Amphipoda balcanica, with notes about other freshwater Amphipoda. - Vestnik Královské. české Spolecnosti nauk, Trida matematičko-prirodovedecká, Praha, 1921-1922, 2: 1-111, 31 figs., 2 pls.

Schellenberg, A. 1951. Un Niphargus nouveau du Sud-est de la France. - Bulletin du Muséum National d`Histoire Naturelle, Paris, 2-e ser., 23 (2): 187-189.

DOI: 10.17707/AgricultForest.63.1.36

Biljana LAZOVIĆ,
Mirjana ADAKALIĆ, Draga JOVANOVIĆ [1]

THE FLOWERING AND POLLINATION STUDY ON OLIVE VARIETY ARBEQUINA GROWN IN MONTENEGRO CONDITIONS

SUMMARY

Arbequina, Spanish olive variety was introduced in Montenegro in the last decade. With small tree size, tolerant to low temperatures and self pollinated, Arbequina was well accepted by olive growers. New plantations were established in several locations on the Coast and in the area of Podgorica. Since there was no previous study of this variety in our ecological condition we set an aim to investigate its flowering and pollination ability. During two years (2014-2015) at two locations (Ulcinj and Podgorica) characteristics of flowering and yield potential were studied. The date and duration of flowering as well as the degree of flowering were assessed. Isolation of the branches with inflorescences for self- pollination was performed before flowering. At the same time branches were labelled for open pollination. Before bagging the number of inflorescences was counted. The structure of inflorescences was analyzed, counted the number of flowers and the number of perfect flowers. Flowering was lasting 12-13 days on both locations. Fruit set in open pollination was 1.45% in average and in self pollination plot 0.1%, respectively.

Keywords: *self pollination, open pollination, monovarietal orchards, inflorescence, self sterile, fruit set*

INTRODUCTION

Montenegro is a country with a small agricultural surface. In the relief structure dominates mountainous and hilly regions. Along the Montenegrin coast where are the traditional olive groves prevail slope areas at the foot of the Orjen, Lovcen and Rumija mountains. Of the total agricultural land in Montenegro, only 15% are flat surfaces (Markovic *et al*., 2010). Available areas for new plantations are mainly on the coast in the vicinity of cities Ulcinj, Budva and Tivat, in terms of areas suitable for olive cultivation and in the hinterland around Podgorica. The area of Podgorica is exposed to appearance of excessively low temperatures and therefore risky for olive production. For this reason, and also the reasons of limited surface, it was necessary to choose a variety that is tolerant to low temperatures and with lower requirements in terms of living space.

[1]Biljana Lazović, Mirjana Adakalić (corresponding author: adakalic@yahoo.com), Draga JOVANOVIĆ, University of Montenegro, Biotechnical faculty, Podgorica, Centre for Subtropical Cultures, Bar, MONTENEGRO

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Popularity of growing olive trees in the intensive system of high density planting is resulted with increased interest of olive growers in planting variety Arbequina, to better utilize the existing relatively small areas. The variety Arbequina is represented worldwide in intensive plantations. Recommended as a variety of low vigour, early entry to the productive period, self fertile with high and regular yield, with high oil content in the fruit, which is of good quality. The first orchard of high density with this variety was planted in 2006 in the area of Podgorica, and since 2010 at several locations in the area of Podgorica and on the coastal area, mainly in monovarietal plantings. Spreading of Arbequina variety has continued in the area of Ulcinj in 2011 and 2012.

The aim of this study was to investigate the capacity or yield potential of Arbequina olive variety that is new in Montenegro and predominantly grown in monovarietal plantings.

MATERIALS AND METHODS

The trial was set up in olive orchards in two areas, the hinterland around Podgorica, where Arbequina variety was grown with other olive varieties, and on the coast in the vicinity of Ulcinj in more or less isolated monovarietal orchards. Studies were carried out in the period 2014-2015 year. At each of the two areas ten trees were marked. The following parameters were observed: the degree of flowering, determined on the basis of the presence of floral elements in relation to the crown (1-5); the duration of flowering, number of days from the beginning (5% open flowers) and the end of flowering (95% fallen flower petals); inflorescence length was measured on a sample of 40 inflorescences taken from the middle part of the annual twigs from all over the crown at the man height, according to the descriptor for the olive (Barranco *et al*., 2000); number of flowers were counted in the inflorescence and the presence of imperfect flowers.

For the pollination trial, at each site five trees were selected for self-pollination (SP) and for free/open pollination (OP). For SP branches with inflorescences were isolated when the flowers were at the stage of the balloon; open flowers were manually removed. Flowering branches were isolated with paper bags. In all branches marked the number of inflorescences for self-pollination and open pollination were counted. Removal of the bags and counting of fruit set in self-pollination was carried out 45 days after the end of flowering. Based on the number of fruit set it was assessed the percentage of fruit set in relation to the number of inflorescences and the number of flowers.

The obtained results were statistically processed by analysis of variance (ANOVA), and the difference testing was performed by LSD test 0.05.

RESULTS AND DISCUSSION

In this study, over two years period (2014-2015), blossom characteristics, the ability to self-pollination and potential fruit set in open pollination in a variety Arbequina were studied.

Flowering and inflorescence characteristics

Flowering of Arbequina variety (Table 1) took place in the second half of May, almost simultaneously on both research sites, and lasted in average for 13 days, one day longer in the area of Ulcinj. The degree of flowering, however, differed significantly between the two areas (for 42%) and was higher in the area of Ulcinj, or in average 3.8.

Period of flowering in Arbequina in Podgorica coincides with the blooming of other foreign varieties, eg. Leccino variety flowered from 20-29.05. in average (unpublished data), with 3 days delay when compared to the coastal area of Bar (Lazovic *et al*., 2004), and also lasts two days longer. Also, the indigenous variety Zutica, which predominates on the coast specifically in the area of Ulcinj, and the most potential pollinator for variety Arbequina, flowered in period from 19-31. May or in average of 12 days (Miranović, 1976) and possibly 1-5 days earlier (Lazovic, 2000; Lazovic and Adakalic, 2012).

Generally speaking, Arbequina variety shows uniform characteristics of flowering period on both studied areas. The same can be said for the structure of inflorescence (Table 1), the capacity to produce fruit or the potential for satisfactory fruit set in productive plantations, respectively. Inflorescence length was significantly higher in the area of Ulcinj (3.04 cm). Number of flowers in inflorescence was in average 17.80, and slightly, but not significantly higher (about 4%) in the area of Ulcinj. Number of flowers in Arbequina variety is much higher than in the domestic variety Zutica (Lazovic, 2000), as well as in Spanish variety Cornicabra (Rojo *et al*., 2015) and some other Spanish and Italian varieties (Ferrara *et al*., 2007; Aguilera and Ruiz, 2012), including Arbequina (13.4 flower/inflorescence) in conditions of Puglia, Italy (Ferrara *et al*., 2007). This implies a greater capacity for fruit set in Ulcinj. However, this advantage is reduced in Arbequina in the area of Ulcinj due to higher number of imperfect flowers which was about 18% higher than in the area of Podgorica.

Table 1: Flowering and inflorescence characteristics of Arbequina variety in Montenegro (2014-2015)

Locality	Flowering			Degree of flower.	Flower. lasting (days)	Inflorescence characteristics		
	Begin.	Full	End			Length (cm)	No. of flowers	Imperfect flowers (%)
Podgorica	19.05.a[(q)]	23.05.a	31.05.a	2.8b	13a	2.58b	17.42a	16.97a
Ulcinj	19.05.a	24.05.a	30.05.a	4.8a	12a	3.04a	18.17a	20.44a
p-value[(r)]	0.4112ns	0.5021ns	0.0501ns	0.0000**	0.3784ns	0.0228**	0.5204ns	0.1667ns
$LSD_{0.05}$	1.4420	1.1784	1.2381	0.3508	1.1205	0.1404	0.8611	3.7911

[(q)] Values of traits marked with different letter in same column are statistically significant on the level $p<0.05$ (LSD test)

[(r)] *p*-values are highly significant (**), significant (*) or not significant (ns)

Inflorescence length, number of flowers and number of imperfect flowers in Arbequina variety is slightly above the value characteristic for autochthonous variety Zutica (Lazovic, 2000; Lazovic and Adakalic, 2012), and is well above

the value for the variety Leccino in Podgorica (unpublished data), or data for the variety Arbequina and Leccino in the area of Puglia, Italy (Ferrara *et al*., 2007). This indicates that the productive potential of Arbequina varieties is satisfactory for good yields, to which contributes the relatively low level of imperfect flowers. However, the amount of fruit per tree is not dependent on the percent of male flowers, as only 1-3 flowers per inflorescence set fruit (Lavee, 2007). Although, the olive pollen production is influenced by weather conditions, regulated at first by temperature, water availability and solar radiation (Rojo *et al*., 2015).

The fruit set

Fruit set in self and open pollination (Table 2 and 3) was observed at the studied sites in the two years of research. For about one third more flowers were marked for open pollination in the area of Ulcinj, and just above the number for self-pollination in the area of Podgorica. All data obtained were statistically highly significant.

Open pollination

Around 20% more inflorescences were marked for OP (Table 2) in the area of Ulcinj, or about 33% more flowers. However, the number of fruit set was significantly higher in Podgorica (1585) in relation to the area of Ulcinj (1214). Percentage of fruit set in relation to the isolated inflorescence was higher in Podgorica area (32.45%) as well as in relation to the number of isolated flowers (1.86%). According to the findings of Moutier (2002), fruit set below 33% compared to the number of inflorescences in hybridization represents the presence of the pollen of poor pollinators. On the other hand, the number of fruit set of 1-3%, compared to the number of flowers can be regarded as sufficient for a quality yield (Lavee, 2007), what we have recorded in the area of Podgorica.

Table 2: Fruit set of Arbequina variety in open pollination in Montenegro (2014-2015)

Locality	No. of isolated inflorescences	No. of isolated flowers	No. of fruit set	% of fruit set in relation to number of inflorescence	% of fruit set in relation to the number of flowers
Podgorica	4884 b[(q)]	85079.3 b	1585 a	32.45 a	1.86 a
Ulcinj	6081 a	110491.8 a	1214 b	19.96 b	1.10 b
p-value[(r)]	0.0065**	0.0047**	0.0007**	0.0039**	0.0033**
$LSD_{0.05}$	471.42	8317.9	44.142	3.6637	0.2041

[(q)] Values of traits marked with different letter in same column are statistically significant on the level $p<0.05$ (LSD test)

[(r)] *p*-values are highly significant (**), significant (*) or not significant (ns)

Higher number of fruit set in the OP in area of Podgorica could be due to better pollination in the plantation with mix of varieties (11 varieties), suggesting

presence of suitable pollinator. However, the topic of possible pollinators for varieties Arbequina in Montenegro needs further consideration and research.

Self pollination

From a total of 14,695 inflorescences, or approximately 261,916 flowers isolated on both studied areas for self pollination of Arbequina variety (Table 3), a total of 273 fruit was registered, almost equally to both studied areas (144 and 124), which is an average of 0.11% in relation to the number of flowers.

About 1,100 inflorescences or about 25,352 flowers were more isolated in the area of Ulcinj. However, the number of fruit set was 18% higher in the area of Podgorica. The percentage of fruit set in relation to the number of inflorescences and of isolated flowers was 2.12% and 0.12% respectively.

Table 3: Fruit set of Arbequina variety in self-pollination trial in Montenegro (2014-2015)

Locality	No. of isolated inflorescences	No. of isolated flowers	No. of fruit set	% of fruit set in relation to no. of inflorescence	% of fruit set in relation to the no. of flowers
Podgorica	6790 b[(q)]	118281.8 b	144 a	2.12 a	0.12 a
Ulcinj	7905 a	143633.9 a	124 b	1.57 b	0.09 b
p-value[(r)]	0.0070**	0.0044**	0.0136**	0.0114**	0.0299**
$LSD_{0.05}$	456.04	8011.8	12.170	0.2989	0.0304

[(q)] Values of traits marked with different letter in same column are statistically significant on the level $p<0.05$ (LSD test)

[(r)] *p*-values are highly significant (**), significant (*) or not significant (ns)

The reason for the appearance of the fruit set in the SP may be the presence of airborne pollen or pollen already present on the branches and inflorescences before bagging (Diaz de la Guardia *et al*., 2003; Rojo *et al*., 2015).

However, the results indicate almost complete incompatibility of varieties Arbequina in monovarietal plantings. Similar results were obtained in earlier research (Moutier, 2002; Diaz *et al*., 2006; Marchese *et al*., 2016).

Influence of the study year

In 2014, the colder weather (data not shown) with the rain during flowering (Table 1), especially evident in the area of Podgorica resulted with significantly lower fruit set per inflorescence compared to the area of Ulcinj (67.9%) (Table 4). In addition, in the area of Ulcinj where monovarietal plantations of Arbequina are situated, the presence of pollen from traditional varieties in the vicinity is assumed to have contributed to a better fruit set. In 2015, lack of flowering in traditional varieties in Ulcinj area, resulted with lower fruit set in comparison to the area of Podgorica.

The fruit set in 2015 in area of Podgorica was above 33% of isolated inflorescences that indicates possible presence of partially good pollinators (Moutier, 2002) among varieties in the mixed plantation.

Table 4: Percentage of the fruit set under open and self pollination of Arbequina in Montenegro during two years

Locality (A)	Year (B)	Open		Self	
		Fruits per inflor. (%)	Fruits per flower (%)	Fruits per inflor. (%)	Fruits per flower (%)
Podgorica	2014	17.25 c[(q)]	0.99 b	0.17 bc	0.01 c
	2015	37.7 b	2.16 a	2.88 a	0.15 a
Ulcinj	2014	67.9 a	3.77 a	2.84 ab	0.15 ab
	2015	12.36 c	0.72 b	0.10 c	0.05 bc
p-value[(r)] (A x B)		0.0002**	0.0110**	0.0120**	0.0106**
$LSD_{0.05}$		11.865	2.4489	2.6233	0.1476

[(q)] Values of traits marked with different letter in same column are statistically significant on the level $p<0.05$ (LSD test)
[(r)] *p*-values are highly significant (**), significant (*) or not significant (ns) for AxB

Further studies on pollination in Arbequina variety in Montenegro is necessary moreover because the varieties mentioned in the previous studies as potential pollinators for Arbequina (Moutier, 2002; Diaz *et al*., 2006; Marchese *et al*., 2016) are not grown in Montenegro.

CONCLUSION

Olive variety Arbequina shows uniform characteristics of flowering period on both studied areas. However, our study showed that Arbequina variety is not self fertile, inducing a high concern for the productivity of single variety orchards. The research should be continued towards study on the acceptable pollinators for this variety in Montenegro.

ACKNOWLEDGEMENTS

The results presented were obtained through the scientific project financed by the Ministry of Science of Montenegro and by the Academy of Science and Arts of Montenegro (CANU).

REFERENCES

Aguilera, F. & Ruiz Valenzuela, L. (2012): Microclimatic-induced fluctuations in the flower and pollen production rate of olive trees (Olea europaea L.), Grana, 51:3, 228-239, DOI: 10.1080/00173134.2012.659203.

Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafini, F. & Trujillo, I. (2000): World Catalogue Of Olive Varieties. Consejo Oleícola Internacional, Madrid, pp. 360.

Diaz de la Guardia, C., Alba, F., Trigo, M.M., Galan, C., Ruiz, L. & Sabariego, S. (2003): Aerobiological analysis of *Olea europaea* L. pollen in different localities of southern Spain. Forecasting models. Grana 42:234-243.

Diaz, A., Martin, A., Rallo, P., Barranco, D. & De la Rosa, R. (2006): Self-incompability of 'Arbequina' and 'Picual' olive assessed by SSR markers. J.Amer. Soc. Hort. Sci. 131:250-255.

Ferrara, G., Camposeo, S., Palasciano, M. & Godini, A. (2007): Production of total and stainable pollen grains in Olea europaea L, Grana, 46:2, 85-90, DOI: 10.1080/00173130701314292.

Lavee, S. (2007): Bienial bearing in olive (*Olea europaea* L.). Annaes Ser. Hist. Nat. 17:101-112.

Lazović, B. (2000): Rodnost ispitivanih sorti masline (*Olea europaea* L.). Voćarstvo. Vol.34. br. 131-132 (2000/3-4), 167-175. Čačak.

Lazović, B., Adakalić, M. & Perović, T. (2004): Bio-pomological properties of introduced olive varieties in the South of Montenegro. Agriculture and foretry, Vol. 50(1-2), 55-61.

Lazović, B. & Adakalić, M. (2012): Važnije karakteristike za razlikovanje autohtonih sorti masline (*Olea europaea* L.) iz crne Gore. In Novi raziskovalni pristup v oljkarstvu, Zbornik znanstvenih priprevkov z mednarodnega posveta (p. 23-32). Univerzitetna založba Annales, Koper, Slovenia ISBN 978-961-6862-16-5,

Marchese, A., Marra, P.F., Costa, F., Quartararo, A., Fretto, S. & Caruso, T. (2016): An investigation of self- and inter-compatibility of the olive cultivars 'Arbequina' and 'Koroneiki' in the Mediterranean climeate of Sicily. Australian journal of Crop Science AJCS 10(1):88-93.

Marković, M., Lazović, B. & Marković, B. (2010): Životna sredina i održivi razvoj – Obezbjeđivanje hrane, u Crna Gora u XXI stoljeću – u eri kompetitivnosti, Crnogorska akademija nauka i umjetnosti, 73/2 , str. 283-313, ISBN 978-86-7215-244-9.

Miranović, K. (1976): Izučavanje elajografskih karakteristika masline cv. žutice i njenog reagovanja na primijenjenu elajotehniku. Arhiv za poljoprivredne nauke, god.XXIX, Sv. 105, str. 49-88. Beograd.

Moutier, N. (2002): Self-fertility and inter-compatibilities of sixteen olive varieties. Acta Hort. 586: 209-212.

Rojo, J., Salido, P. & Perez-Badia, R. (2015): Flower and pollen prosuction in the 'Cornicabra' olive (*Olea europaea* L.) cultivar and the influence of environmental factors. Trees 29:1235-1245 DOI 10.1007/s00468-015-1203-6.

INSTRUCTIONS TO AUTHORS

The "Agriculture and Forestry" journal publishes original scientific papers, review papers, short communications on agriculture, veterinary medicine, forestry, biology and other natural sciences. It is the endeavour of the journal to give place to papers of high scientific quality and international interest, authored by all the scientists from the South Eastern European Region, as well as other international scientist in order to stimulate contacts and exchange of knowledge fostering scientific productivity.

Manuscripts, submitted via electronic journal web system should be prepared in Microsoft Word ***(Times New Roman font, 11 pt)*** and submitted in format 17 x 24 cm ***(File / Page setup / Paper / Width = 17 cm; Height = 24 cm)***, with single line spacing ***(Format / Paragraph / Line spacing = Single)***, 2 cm margins all around ***(File / Page setup / Margins / Top = 2 cm; Bottom = 2 cm; Left = 2 cm; Right = 2 cm)***, that is approximately 44 lines per page in this format. All technical details are available in section AUTHORS / Check-list for Authors.

Manuscripts are published in English. Papers that have been published elsewhere, in whole or extracts (excerpts) of their important findings, will not be accepted. A manuscript should not exceed 10 pages. Exceptions can be made if content and quality of the paper justify it (at the discretion of the Editor).

Full research papers should include the following sections:

- Author/s name/s, Title with **DOI number**

The author's name should be placed above the title and with the author's appellation and affiliation in a footnote at the bottom of the front page. Author(s) affiliation should indicate name and address of institution, including the e-mail address of the corresponding author. Title should provide a concise but also an informative synthesis of the study (recommended not more than 100 characters including spaces). Short title (not more than 70 characters) should also be provided in order to be included in the header of the Manuscript. Ensure that the title contains the most important words that relate to the topic.

- Abstract

The summary, in English language, should provide basic data on the problem that was treated and the results obtained. It should be brief, preferably one paragraph only, up to 250 words, but sufficient to inform the reader of the character of the work, its results and its conclusions. Include the keywords and phrases you repeated in your abstract.

- Key words

Keywords should provide 4-6 words or compound words, suitable for an information retrieval system. Choose the appropriate keywords and phrases for your article. Think of a phrase of 2-4 words that a researcher might search on to find your article. Repeat your keywords and phrases 3-4 times throughout the abstract in a natural, contextual way.

Main text of the manuscript includes the following sections:

- INTRODUCTION

The introduction should answer the questions what was studied, why was it an important question, what was known about it before and how the study will advance our knowledge.

- MATERIAL AND METHODS

Material and methods explain how the study was carried: the organism(s) studied; description of the study site, including the significant physical and biological features, and the precise location (latitude and longitude, map, etc); the

experimental or sampling design; the protocol for collecting data; how the data were analyzed. In this section also should be provided a clear description of instruments and equipment, machines, devices, chemicals, diagnostic kits, plants/animals studied, technology of growing/housing, sampling sites, software used etc.

- RESULTS followed by **DISCUSSION**

Results and Discussion may be combined into a single section (if appropriate) or it can be a separate section.

The results objectively present key results, without interpretation, in an orderly and logical sequence using both text and illustrative materials (tables and figures).

The discussion interpret results in light of what was already known about the subject of the investigation, and explain new understanding of the problem after taking results into consideration.

The International System of Units (SI) should be used.

- CONCLUSIONS

The conclusion should present a clear and concise review of experiments and results obtained, with possible reference to the enclosures.

- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

- REFERENCES (LITERATURE)

References should cover all papers cited in the text. The in-text citation format should be as follows: for one author (Karaman, 2011), for two authors (Erjavec and Volk, 2011) and for more than two authors (Rednak *et al*., 2007). Use semicolon (Rednak *et al*., 2012; Erjavec and Volk, 2011) to separate multiple citations. Multiple citations should be ordered chronologically. The literature section gives an alphabetical listing (by first author's last name) of the references. More details you can find in the Annex to the INSTRUCTIONS TO AUTHORS / Bibliographic style on the web page of the Journal: www.agricultforest.ac.me.

Short communication should include the following sections: Title, Abstract, Key words, Main text, Acknowledgments, References, Tables and Figures with captions.

SUPPLY OF ARTWORK, PHOTOS: Diagrams and graphs should be provided as finished black and white line artwork or colour images. Electronic graphics included in your manuscript should be either inserted in the word document or as .gif or .jpg formats. Please check with the editor if you wish to submit any other type of graphic for conversion suitability. Photos should be supplied un-screened in original form or in electronic form. All illustration (diagrams, graphs, tables, photos) must be fully captioned. When there are a number of illustrations, the author should endeavour to reduce the amount of text to accommodate the illustrations in the limited space available for any article.

THE REVIEW PROCESS: Submitted manuscripts are reviewed anonymously by 2 referees (duble blind review): one from the journal Editorial board, one must be an international reviewer, working out of the University of Montenegro. All tracking of manuscripts and reviewers is done by the Editor. All attempts will be made to ensure submissions will be reviewed within three months after the submission. Manuscripts will be returned to the coresponding authors when each review is completed.

Examples available on **www.agricultforest.ac.me**, section **On-line Journals.**