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## PHYSIOLOGICAL AND MORPFOLOGICAL CHARACTERISTICS OF ONE-YEAR OLD SEEDLINGS OF COMMERCIAL MEDLAR CULTIVARS (Mespilus germanica L.) IN THE REGION OF NORTH MONTENEGRO

#### **SUMMARY**

The paper was written based on a study conducted in a nursery in the north of Montenegro during three successive years. The study had helped author of this paper to examine the possibility of producing one-year old seedlings of commercial medlar cultivars (*Mespilus germanica* L.) grafted on quince Ba 29 and wild pear generative rootstocks (*Pyrus communis* L.) during the first year after bud grafting. The aim of this study is to determine morphological characteristics, compatibility of commercial medlar seedling cultivars (Domestic medlar, Pomoravka - seedless medlar genotype, Royal medlar, Medlar without seeds) with the quince Ba 29 and wild pear generative rootstocks and water attaining capability of the leaves in one-year old seedlings of commercial medlar cultivars.

The results of this study also showed that the water attaining capability of the leaves in one-year old seedlings of commercial medlar cultivars (*Mespilus germanica L.*) as an indicator of their resistance to drought was genetic characteristics of the cultivars.

**Keywords:** Medlar (*Mespilus germanica L.*), morphological characteristics, one-year old seedlings, resistance to drought, water attaining capability.

#### **INTRODUCTION**

Medlar (*Mespilus germanica L.*) belongs to Rosaceae family, and it is called 'Döngel' or 'Beşbıyık' in Turkey, 'Ezgil' in Azerbaijan, 'Bushmala' in Georgia and 'German' or 'Germanic Medlar' in the most of European countries (Anonim.2009.). Its tree is generally 3-5 m tall, but it may reach nearly 8 meters. It is self-fertile and long-lived tree. It lives approximately 30-50 years. There are 100 year old trees in UK as well. The flowers are white-pink and hermaphrodite. Flower buds are formed in May-June, and each bud has one flower. Fruit shape may be variable. Fruits are uneatable during tree maturity stage because of tannin content. It becomes eatable when the skin color becomes chocolate brown. Medlar contains organic acids, sugars, pectin, vitamin C, and small amounts of vitamin A (Korbanova *et al.* 1998.).

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The commercial cultivars of medlar are reproduced by grafting. The best results are achieved by grafting on a sleeping bud. As good rootstocks for grafting, they showed: generative rootstock of medlar (*Mespilus germanica L*), sorb apple (*Sorbus domestica L*), a whitethorn (*Crataegus sp.*), waild pear (*Pyrus communis L*) and vegetative rootstock of pear and quince (Quince A, Quince C and Ba 29).

The rapid developments fruit tree nursery technology and rootstock research and introduction of new clonally propagated rootstocks opened in new area in fruit science (Ecisli *et al.* 2006). For this reason more recently modern orchards with different modern training systems to start establish with use of clonal quince (*Cydonia oblonga* L.) rootstocks such as Quince A, Quince C and Ba 29 in Europe. These clonal rootstocks with dwarfing characteristics well reported to increase precocity and fruit quality, especially in the high intensity modern orchard sand thus gained more importance (Lewko *et al.* 2007).

The selection of clonal quince (C. *oblonga*), such as Quince A (MA), Quince C (MC) and BA 29 in Europe, or of clonal *Pyrus communis* L., such as 'Old Home' × 'Farmingdale' (OHF) in the USA or in South Africa, as substitutes for valid pear seedling rootstock, have clearly improved the precocity, productivity and quality of some European cultivars (Ikinci *et al.* 2014).

From year to year, fruit production in Montenegro is more and more difficult without watering. The fertile buds production is extremely reduced in the drought conditions. Since these buds should bring the yield in the next year, dry year indicates low yields in the next one too.

In Dinaric mountains annual rainfall varies from 1.000 1/m<sup>2</sup> to 5.500 mm/m<sup>2</sup> in Crkvice (Montenegro) what is the European maximum. Though these regions abound in precipitation, the lack of water appears in the summer period because of porous soils and in some summers because of drought. Traditionally the people who live there collect rain from the roofs. The rainfall potential could be used in more economical way with building accumulations with concrete walls or by finding and protecting autochthonous varieties. In the most fertile valley in Bijelo Polje (Nedakusko-Rasovska ravnica), vegetable production depends on watering. Water capacity of the leaves of plum autochthonous varieties was tested as an indicator of their resistance to drought in Bjelo Polje, Montenegro (Šebek, 2016).

The development of drought resistant cultivars or lines of crops through selection and breeding is of considerable economic value for increasing crop production in areas with low participation or without any proper irrigation system (Subbarao *et al.* 2005). However, availability of genetic variation at intra – varietal level is of prime importance for selection and breeding for enhanced resistance to any stress (Serraj *et al.* 2005). In order to develop drought tolerant cultivars, it is imperative to develop efficient screening and suitable selection criteria. Various agronomic, physiological and biochemical selection criteria for drought tolerance are being used to select drought tolerant plants, such as seed yield, harvest index, shoot fresh and dry weight, leaf water potential, osmotic

adjustment, accumulation of compatible solutes, water use efficiency, stomatal conductance, chlorophyll fluorescence (Araus *et al.* 2002; Richards *et al.* 2002; Flexas *et al.* 2004; Ashraf and Foolad 2007; Tambussi *et al.* 2007).

Development of drought tolerance for a plant is the result of overall expression of many adaptive traits in a specific environment. The adaptive traits can be physiological and morphological, such as: selection of rootstocks resistant to drought, selection of autochthonous cultivars with greater water attaining capabilities, selection of cultivars tolerant to dry conditions, etc. Since many adaptive traits are effective only for certain aspects of drought tolerance and over a limited range of drought stress, there is no single trait that breeders can use to improve productivity of a given crop in a dry environment. In this context, Subbarao et al. (2005) suggested that those traits, whether physiological or morphological, that contribute to check water loss through transpiration, and enhance water use efficiency and yield are traits of interest. However, priority should be given to those traits that will maintain or increase yield stability in addition to overall yield, because traits for higher yield may in fact decrease yield stability (e.g. longer growth period). Thus, in order to improve crop productivity under drought stress conditions, selection of cultivars with short life span (drought escape), incorporation of traits responsible for well – developed root system, high stomatal resistance, high water use efficiency (drought avoidance) represent the traits responsible for increasing and stabilizing yield during drought stress period (drought tolerance).

Drought stress is highly variable in its timing, duration and severity, and this result in high environmental variation and  $G \times E$  variation (Witcombe *et al.* 2005).

Many studies interactions rootstock - scion show that the rootstock controls the overall growth, while scions affects the number and type of shoots (Ferree *et al.*, 2001a, b) as well as the number of buds that will become a flower (Hirst and Ferree, 1995).

Scion has a greater impact on the rootstock at a monthly growth rate of trees (Tworkoski *et al.*, 2007). Dwarfing apple rootstocks M9 combined with different scions consistently has the lowest, and seedling rootstocks of *Malus sylvestris* has the highest vegetative growth and tree trunk corpulence (Tworkoski *et al.*, 2007). Although the rootstock is used to control the size of the tree, the mechanism that is responsible and which is closely related to the action of the growth of the tree is still unclear (Atkinson *et al.*, 2001). The main difficulty in determining the influence of the rootstocks are connected with the fact that these cumulative effects of variations in the development of seedlings overlap from year to year (Barritt *et al.*, 1995; Ferree *et al.*, 1995).

Nursery material of high quality is the basic of intensive fruit growing (Baryla and Kaplan 2006). Namely, modern pear orchards are planted at at 2000 - 5000 trees per ha  $\overline{0}^1$  on under Hight Density Planting (HDP), if it is grafted on dwarf or semi-dwarf quince rootstocks, yielding at least 40-50 t per ha $\overline{0}^1$  (Wertheim, 2002). Intensive pear orchards are based on the concept of high

density planting, training systems of low tree height and high productivity on the basis of the unit area (ha or m<sup>2</sup>). However, quince is graft incompatible with some of the major pear cultivars such as Bartlett (Tukey, 1978; Hartmann et al., 1997). Vegetative rootstocks that are used for production of pear, quince and medlar seedlings are Anžerska quince MA and Provencal Ba 29 (Stančević et al., 1993). There are other Quince rootstocks in use such as 'Adams', 'Ba29' and the more recently introduced 'EMH' and 'Eline' and it must be assumed for the sake of caution that the incompatibilities mentioned here are probably expressed in some degree in all such Quince rootstock forms. This paper uses practical experience from the conducted study and also literature related to the compatibility of commercial cultivars of medlar with quince rootstocks clone Ba 29 and wild pear generative rootstocks (*Pyrus communis L*).

Quince is graft incompatible with some of the major pear cultivars such as Bartlett (Tukey, 1978; Hartmann et al., 1997). The study showed that the incompatibility of pear seedling sorts with the quince Ba 29 and wild pear seedlings expresses itself in several ways. Firstly, a poor 'bud' or 'graft' that was taken from the nursery would be evidently of not sufficient quality; secondly, even if growing is successful in the nursery, very often 'lifted' breakages (brittle unions) could occur between the rootstock and the scion; and thirdly, 'delayed' incompatibility may occur on a place where the union between the rootstock and the scion breaks suddenly in later years. The last process is unpredictable and can often happen when there is a heavy crop load assisted by strong autumnal winds.

### MATERIALS AND METHODS

The material of this study were commercial cultivars of medlar: 'Domestic medlar', 'Pomoravka - seedless medlar genotype', 'Rojal medlar', and 'Medlar without seed'. These commercial cultivars of medlar are grafted on vegetative rootstocks quince Ba-29. Comparative or control graft was performed on generative rootstock of wild pear (*Pyrus communis L*).

Seedless medlar genotype Pomoravka was found in Pomoravlje, in the close vicinity of Svilajnac (Serbia), 1994. It was grafted and transferred at three locations so as to be protected from deterioration. Apart from being used fresh, its fruit is very interesting for processing industry for making pastes, jelly, mash, liqueur etc., this genotype can be beneficial in the breeding aimed at development of seedless medlar cultivars of satisfactory fruit size. This genotype has a relatively small fruit (8.2 g) but high stone flesh ratio (96.5%) and it is highly qualitative especially when it is overripe. Due to its high using values this genotype should be more propagated especially in view of the fact that the presence of pathogen *Erwinia amylovora* has not been detected on any young tree of this medlar genotype (Nikolić,2005).

The experiment was conducted in the village Njegnjevo in the period from the year 2009 to the year 2012. The nursery was located at Njegnjevo near Bijelo Polje (43°05'N; 19 °05'E), North Montenegro. This is mainly an upland area, with an average altitude of about 320 m, characterized by temperate continental climate. The nursery soil was typically eutric land on alluvial and colluvial deposits, mildly acid (a pH of 5.41 in the topsoil), with a moderate organic matter (3.88%) and a very low Ntot content (0.18%), the values thereof gradually decreasing with the depth (data not show). The contents of available P2O5 and K2O in the 0-30 cm soil depth were 6,7mg•100g-1 and 14,07mg•100g-1, respectively. Fertilization treatments included applications of mineral nitrogen fertilizers at the rate of 80 kg N•ha-1 prior to growing season and following the cutting of the rootstock above the graft union, i.e. towards the end of March in three seasons.

During the year of 2009, the vegetative and generative rootstocks were cultivated. Seeds of wild pear were collected the year before from local trees, they were cleaned of flesh, dried and stratified in wet sand during the winter of 2008/2009. Wild pear seedlings were cultivated in 2009. The same procedure of producing generative and vegetative rootstocks was repeated two times more in order to have results from three different years. We already knew that generative rootstocks have diverse genetic characteristics but we included them in this project in order to compare them to the vegetative rootstocks. What we are hoping to accomplish in this project is to determine the compatibility between vegetative rootstocks (Ba-29) and commercial cultivars of medlar. In Montenegro, commercial cultivars of medlar were only grafted on generative rootstocks. This fact prevents raising of medlar orchards with intensive production. Budding of sleeping buds was conducted in the autumn (late August) during the years of 2009, 2010 and 2011. Due to poor production results there were other graftings that took place in the spring of years 2010, 2011 and 2012, in which the method of 'English linking' was used. Grafting height is 10 cm from the root collar of wild pear generative rootstock or vegetative rootstock for medlar (quince Ba-29). Scions for grafting were collected in the spring before the abrupt movement of buds and stored in the basement until the proper grafting conditions. Acceptance of grafting was monitored during three years of production. The study (2010 -2012) includes those morphometric characteristics of plants that are used as basic parameters for their classification according to outward, phenotypic characteristics. Determination of seedling growth indicators was done with a sample of of 80 rootstocks. One-year seedling height was measured with a meter. The diameter of seedlings on 3 cm from the seedling grafting point was measured with a micrometer of 0.01 mm precision. The dynamic of evolution of the one-year seedlings (seedling height and thickness) was followed during the growing season treatments: June, July, August and September. The results were analyzed using one-way analysis of variance (statistical program Systat 11) where the middle of treatment compared to the LSD test.

Examples of leaves for analysis were taken when it was dry weather: three times a year – at the end of June, the end of July and the end of August. The dynamics of leaf dehydration per measured interval was determined by method of Eremeev 1964 (cit. according to Šebek,2016). Eremeev's method

(Šebek,2016) is relevant for determination of water attaining capability of leaves. Loss of water at the time of transpiration will be monitored by measuring of the weight of cut leaves. (Slavik,1974 cit. according to Šebek,2016). Level of regained hydration will be monitored after 12h and 16h from cutting the leaves from one-years seedlings of commercial medlar cultivars. The loss of water due to transpiration followed by measuring the weight of leaves (Šebek,2016) The dynamics of leaf dehydration was measured in order to obtain initial resistance rate of commercial medlar cultivars towards drought conditions. The dynamics of leaf dehydration depends on the thickness of leaf cuticle and leaf average size. The results were analyzed using one-way analysis of variance (statistical program Systat 11) where the treatment was compared to the LSD test.

### **RESULTS AND DISCUSSION**

The average acceptance of autumn grafting process in the shape of the 'T' letter of comercial medlar cultivars with quince clone BA 29 seedlings (vegetative rootstocks) had been with the following percent of success: 73.30% ('Domestic medlar'); 90.30% (' Pomoravka - seedless medlar genotype '); 51.67% ('Royal medlar') and 44.00% ('Medlar without seeds'). The acceptance of autumn grafting process in the shape of the 'T' letter of comercial medlar cultivars with wild pear seedlings (generative rootstocks) had been with the following percent of success: 89.30% ('Domestic medlar'); 84.00% (' Pomoravka - seedless medlar genotype '); 96.70% ('Royal medlar')and 75.00% ( 'Medlar without seeds' ).Due to the results of the grafting process in the shape of the 'T' letter there was a need for repetition of the grafting process (next spring: English linking) for defining causes of low acceptance of seedlings regarding individual sorts and for increasing of production results. When the grafting process was repeated, satisfied results from the aspect of plantation production profitability had been achieved. Achieved percent after the repetition of the grafting process for comercial medlar cultivars with Ba 29 seedlings was: 99.30% ('Domestic medlar''); 99.00% (' Pomoravka - seedless medlar genotype '); 93.67% ('Royal medlar') and 85.67% (' 'Medlar without seeds' '). Achieved percent after the repetition of the grafting process for comercial medlar cultivars with wild pear seedlings (Pyrus communis L) was: 96.30% ('Domestic medlar'); 88.70% ('Pomoravka - seedless medlar genotype '); 99.3% ('Royal medlar'); and 99.00% ('Medlar without seeds'). Growth dynamic of one-year-old seedlings (height and corpulence of the seedlings) was monitored during vegetation in time treatments: June, July and August. Values of the monitored parameters (height and corpulence of the seedlings) showed differences in average values and seedling growth dynamic. By analyzing the data for medlar cultivar 'Domestic medlar" (rootstocks is quince Ba 29), average height of the seedling in June was 33 cm. In the month of the July average height was 58 cm. For August average height was 94 cm. The average corpulence of the seedlings, 10 cm from the grafting spot, of the same sort was 3.20 mm in June. In July, data for the average corpulence was 5.45 mm. In august, corpulence was 9.05 mm. Parallel data for

medlar sort ' Pomoravka - seedless medlar genotype ' (rootstocks is quince Ba 29), of studied parameters in three different time treatments were following: 36 cm; 61 cm; 98 cm; (height) and 3.30 mm; 6.25 mm; 9.55 mm (corpulence). Parallel data for pears sort 'Royal medlar' of studied parameters in three different time treatments were following: 41 cm; 65 cm; 113 cm; (height) and 3,80 mm; 7.05 mm; 10.30 mm (corpulence). Parallel data for pears sort 'Medlar without seeds' of studied parameters in three different time treatments were following: 43 cm; 73.5 cm; 120 cm; (height) and 3.55 mm; 7.80 mm; 11.05 mm (corpulence). Based on the data in Table 1, the highest average tree height (163.2 cm) had the variety 'Medlar without seeds' grafted on the rootstock guince Ba 29. Based on LSD values, we can note that the height of the seedling in interaction between ' 'Medlar without seeds'' and the rootstock quince Ba 29 was significantly higher compared to other seedling (interactions) height. The corpulence of the seedlings was the greatest in the variety ' Pomoravka - seedless medlar genotype medlar' grafted on the rootstock quince Ba 29 (21.80 mm), which is statistically significantly higher than all other. The results showed that the low amount of successfully grafted seedlings after the autumn grafting on the rootstock quince Ba 29 of cultivars 'Medlar without seeds' (41%) and 'Royal medlar' (51.67%) can be significantly improved in next spring grafting (85.67% 'Medlar without seeds' and 93.67% 'Royal medlar'). The same effect of improving was evidenced in grafting of the variety ' Domestic medlar', the percentage of successfully grafted seedlings was increased from 73.30% to 99.30%. The lowest effect of improving was evidenced in grafting of the varieties 'Medlar without seeds'.

Results of autumn grafting of comercial medlar cultivars 'Domestic medlar' (89.3%) and 'Royal medlar' (96.7%) on the generative rootstock (Pyrus communis L.) show that they have higher percentage of successfully grafted seedlings than varieties 'Pomoravka - seedless medlar genotype' (84%) and ''Medlar without seeds' (75%). Because of the re-grafting method in spring we had higher amount of successfully grafted seedlings on both generative and vegetative rootstocks.

Based on the data in Table 4 the highest average tree height (183 cm) had the variety 'Medlar without seeds' grafted on the generative rootstock (Pyrus communis L.). Based on LSD values we can note that the height of the seedling in interaction between 'Medlar without seeds' and the generative rootstock (Pyrus communis L.) was significantly higher compared to other seedling (interactions) height. Seedlings of all studied comercial medlar cultivars grafted on the generative rootstock (Pyrus communis L.) had significantly higher height than any seedlings grafted on the vegetative rootstock ('quince Ba -29').

Out of the studies autochthonous pears cultivars, the highest water attaining capability had the leaves of cultivar ' Pomoravka - seedless medlar genotype '(Table 5). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (one-year old seedlings) lost on average 35.50% of water. The lowest level of the stated capability was recorded with the leaves of cultivar 'Medlar without seeds' (40.44%). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the control (one-year old rootstocks Pyrus communis L) lost on average 35.08% of water.

the re gratting proces in spring (rootstocks is quince bu 2)									
Cultivar/ rootstocks	Percent of the grafting				Percent of the re-grafting proces in				
	process (%)				spring (%)				
	(autumn: ,,T"letter)				(spring: English linking)				
	2009	2010	2011	Х	2010	2011	2012	Х	
Domestic medlar/Ba 29	68	77	75	73.3dc	98	100	100	99.3a	
Pomoravka - seedless									
medlar genotype /Ba	90	87	94	90.3a	99	100	98	99a	
29									
Royal medlar/ Ba 29	50	53	52	51.67h	91	94	96	93.67b	
Medlar without seeds/	37	44	42	41ij	82	90	85	85.67c	
Ba 29									
LSD 0.05				4.6				3.4	
LSD 0.01				6.8				5.5	

Table 1. Achieved percent of the grafting process in autumn and the percent of<br/>the re-grafting proces in spring (rootstocks is quince Ba-29)

Table 2. Height and corpulence of one-year-old seedlings of medlar and achieved percent of the grafting process (rootstocks is quince Ba-29)

Cultivar/rootstocks	Height of the seedlings (cm) (average)				Corpulence of the seedlings (mm) (average)				
	June	July	August	Sept.	June	July	August	Sept.	
Domestic medlar/Ba 29	33	58	94	151.3cb	3.2	5.45	9.05	17.6c	
Pomoravka - seedless medlar genotype /Ba 29	36	61	98	149.8c	3.3	6.25	9.55	21.8a	
Royal medlar/ Ba 29	41	65	113	151.6cb	3.8	7.05	10.3	19b	
Medlar without seeds/ Ba 29	43	73.5	120	163.2ab	3.55	7.8	11.05	18.8bc	
LSD 0.05				9.3				1.6	
LSD 0.01				10.3				1.9	

Out of the studied autochthonous plum cultivars, the highest water attaining capability had the leaves of cultivar 'Crvena ranka' (Šebek,2016). Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (one-year old seedlings) lost on average 33,54% of water. The lowest level of the stated capability was recorded with the leaves of cultivar 'Obični piskavac' (41.74%). Out of the studied water attaining capability of leaves in autochthonous apple cultivars (Šebek, 2004), the highest water attaining capability had the leaves of cultivar 'Pašinka'. Over the monitored time interval (8 hours upon sample taking), leaves taken from the annual twigs of the studied cultivars (in situ) lost on average 38.09% of water.

The lowest level of stated capability was recorded with the leaves of cultivar 'Arapka' (40.64%). In terms of the selected wild apples (Šebek, 2004), the highest level of water attaining capability was registered in the leaves of type 2 (32,44%). Leaves taken from the annual twigs out of the studied selected types (in situ) lost on average level (36,61%) showed the leaves of type 6.

Cultivar/	Percer	nt of the g	grafting p	process	Percent of the re-grafting proces			
rootstocks		(% 	%) 		in spring (%)			
rootstoens	(	autumn:	"1 letter	)	(spring: English linking)			
	2009	2010	2011	Х	2010	2011	2012	Х
Domestic medlar/ P.communis	92	86	90	89.3b	98	91	100	96.3a
Pomoravka – seedless medlar genotype / P.communis	83	82	87	84c	90	86	90	88.7c
Royal medlar/ P.communis	92	98	100	96.7a	98	100	100	99.3a
Medlar without seeds/ P.communis	70	77	78	75e	99	98	100	99a
LSD 0.05				4.6				3.4
LSD 0.01				6.8				5.5

Table 3. Achieved percent of the grafting process in autumn and the percent of the re-grafting proces in spring (generative rootstocks is *Pyrus communis L*.)

## CONCLUSION

The research was conducted on 4 different medlar cultivars and that allowed us to obtain important morphological and physiological traits.

1. Seedlings of all studied comercial cultivars of medlar grafted on the generative rootstock (*Pyrus communis L.*) had significantly higher height than seedling grafted on the vegetative rootstock ('quince Ba-29').

2. We also have significant difference between the diameters of seedlings. Those differences are the consequences of lower verdure in vegetative rootstocks than in generative rootstocks.

3. The most important result of our research is the fact that we determined the compatibility between researched comercial cultivars of medlar and vegetative rootstocks ('quince Ba -29').

4. The method of re-grafting in spring is very useful because we had higher amount of successfully grafted seedlings on both generative and vegetative rootstocks after re-grafting.

5. Production of seedling material of comercial cultivars of medlar with vegetative rootstocks 'Quince Ba -29' will be enormous contribution for product of our ecological environment .

6. The results of this research show that the plant height, stem corpulence one-year old seedlings are characteristics of comercial cultivars of medlar, from which rapid growth and uniformity of scions depend.

7. The highest water attaining capability had the leaves of cultivar 'Pomoravka - seedless medlar genotype '. The lowest level of the stated capability was recorded with the leaves of cultivar 'Medlar without seeds'.

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