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**INFLUENCE OF IBA AND NAA (0.8%) + (IBA 0.5%)
PHYTOREGULATORS TO THE RISOGENESIS
OF THE MATURE LEMON TREE-SHOOTS
(*Citrus limon* (L.) Burm. and *Citrus meyeerii* Y. Tan.)**

SUMMARY

This paper presents results of investigations on the influence of exogenous phytohormonal substances of indol-3-butyric acid (IBA) and alfa-naphtyl acetic acid in combination with IBA (NAA+IBA) on the percentage of rooting of the mature lemon tree-shoots of the two observed varieties, Meyer and Mjesečar.

The average value of tree-shoots rooting upon application of IBA phytohormone varied from 25.00% with the Mjesečar variety (IBA-1000 ppm) to 85.25% with the Meyer variety (IBA-3000 ppm). The average value of the lemon tree-shoots rooting with the two observed varieties under IBA concentrations amounted to 54.83%.

The average value of the tree-shoots rooting of the investigated lemon varieties that were treated with the phytohormone NAA (0.8%) + (0.5%) IBA amounted to 51.88%. The best average value of the rooted tree-shoots of 78.75% was recorded with the Meyer variety, whilst the least rooting of 25.00% was with the Mjesečar variety.

The variance analysis and the LSD test established differences between investigated varieties, whereby the rooting success depends on exogenous treatment of tree-shoots with different types and concentrations of phytohormones.

Keywords: lemon, variety, tree-shoot, phytohormone, rooting.

INTRODUCTION

Lemon seedlings can be produced by the generative method from seeds, as well as by the vegetative method though grafting (transplantation) of seedlings (*Poncirus trifoliata* L. I *Citrus bigaradia* L.) with noble cultivars, margotting and rooting of mature and green tree-shoots (Popović et al. 1998).

Plants obtained from the tree seeds are called seedlings and are very heterogeneous in terms of morphological characteristics, verdure and fruit quality. Offspring obtained by this reproduction manner carry in themselves,

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more or less, the pronounced characteristics of their ancestors, even of the wild forms. Fruit trees obtained from seeds go into the fruiting very late (Lučić *et al.* 1996).

The generative reproduction of plants goes through all onto stadiums of development, whilst the vegetatively reproduced plants continue with developmental path of ontogenetic development of the main plant (Hadžiabulić, 2010). That is why the fruit production varieties are not reproduced from seeds, but vegetatively (Lučić *et al.* 1996).

Vegetative reproduction ensures genetic identity of offspring, the procedure is easier, ensures mass production of planting material, fruit trees go through earlier fruiting, have more regular yield and provide high fruit quality.

Success of vegetative reproduction depends on biological factors of fruit trees, such as: tendency of the specie and variety towards vegetative reproduction, age of organs, volume of nutritive matters in tissues and health condition.

External factors, relevant for vegetative reproduction are: type of substrate, humidity, heat, light, air and reproduction technique (Popović and Čizmović, 2010).

The method mostly used in practice is transplantation (grafting) of lemon, by T-budding on the sprouting and dormant buds in the open and protected area (greenhouse and glasshouse) with application of appropriate climate conditions, primarily (18 – 20°C) for the successful callusing and coalescence on the joint between the rootstock and budstick (Popović *et al.* 1998).

However, the lemon reproduction by rooting of tree-shoots is recently being increasingly used. Certain lemon varieties (Meyer and Mjesečar) are also well rooted with exogenous application of phyto-synthetic hormonal substances that result in many useful reactions and influence to development of callus in the base zones of the tree-shoot rooting and to higher percentage of rooting and more branched root system. (Bakarić, 1974).

Phytohormones and bio-regulators are biological compounds that regulate growth and development and influence to the direction and intensity of metabolic processes in plants. If added they artificially cause risogenesis process. (Kastori, 1998).

Particularly successful results in nursery production are reached by the use of synthetic phytohormonal matters of indol-butric acid (IBA) and alfa-naphtyl – acetic acid (NAA) in different concentrations.

It is not good to thin tree-shoots too deep, since air can hardly reach deeply thinned parts, which results in poorer rooting. The optimum air temperature for lemon tree-shoots rooting is from 17 to 25 °C. Root usually emerges on tree-shoots during the second week since commencement of rooting, which depends on conditions that existed at that moment. (Bakarić, 1974).

Rooted tree-shoots are transplanted into containers with compost that must be of loose structure and which ensures undisturbed air access, holds the moisture and releases the water surplus, provides adequate and balanced supply

of plant nutrients in all phases of development, contains no harmful organisms and matters (Popović and Čizmović, 2010).

Many authors, and we are mentioning some of them, investigated issues and methods related to rooting of woody tree-shoots: Pravdin (1938), Ali and Westwood (1968), Howarad and Nahlawi (1969), Bakarić (1974, Markovski, Arsov; Spirovska (2000). They stated that tree-shoot rooting depends, to a large extent, on the volume of collected reserve substances, thus they recommend the period February-March for tree-shoots rooting.

Markovski, Arsov; Spirovska (2000) established that the influence of IBA on the percentage of lemon tree-shoots rooting is large. Tree-shoots treated with 2000 ppm IBA achieved rooting of 97.01%, whilst application of lower IBA concentration (0.3%) recorded rooting of 85.71%.

In addition to the above stated ways of lemon reproduction, there are also other ways, but they are not so frequent in practice, considering that they are difficult for implementation or too expensive, or lemons produced that way are quickly damaged due to diseases (Bakarić, 1974).

Application of phytohormones in the risogenesis process of lemon tree-shoots is less investigated in the world and domestically than of other fruit species. The following authors studied the problems of rooting with certain fruit species: Kapetanović et al. (1975), Popović (1984, 1995, 1997, 2000), Veličković et al. (1985), Tabain (1975), etc.

The objective of this paper is to determine types of phytohormones that influence, act on rooting process of the mature lemon tree-shoots, for the purpose of obtaining high quality seedlings on own roots and to provide recommendations for production and placement on the market.

MATERIAL AND METHODS

Investigation on the influence of exogenous phyto-synthetic hormones Indol-3-Butyric acid (IBA) and Alfa – Naphtyl acetic acid in combination with IBA (NAA+IBA) on the rhyogenesis of the mature lemon tree-shoots was conducted in the glasshouse (protected area) of the nursery of the Centre for Subtropical Cultures in Bar during 2014.

The research included two lemon varieties, Meyer and Mjesečar, and one-year-old mature tree-shoots were used for rooting, which were taken from the production-intended lemon trees from the Bar area in the beginning of April.

According to Tutberidz (1971), Meyer is considered to be a hybrid of lemon and unshiu. It produces medium rich trunk and is shaped like bush. Fertility is high and regular. The fruit is of medium size (around 80 grams), oval, with smooth skin and specific aroma. The fruit matures early, even from the 1st of July. Fruits maturity is simultaneous. It is often used for growing in plant pots, and there are several bigger plantations of this lemon at the Montenegrin coast. Of all varieties, this variety is the most abundant.

Mjesečar is a well known variety at the Montenegrin coast. It has a very rich trunk with loose (gangly) crown. Fruit is prone to uneven shapes, which is at the same time the shortcoming of this variety. It is of medium size, of around 60

grams, and of oblong shape. Fruit color is bright lemon-yellow, the apex of the fruit is nipple-like, and the bottom part is oval. Skin is smooth and quite thick with 11 to 12 slices that contain from 12 to 22 seeds. Juice is slightly sweet, aromatic, rich, very sour and of pleasant taste. It is mostly grown around houses since it blossoms and produces yield several times (two or three times a year) and since it gives good yield.

The experiment used: liquid solution of Indol-3-butyric acid (IBA) in three different concentrations of solution (1000, 2000 and 3000 ppm) and powdered growth simulator alfa-naphtyl acid in combination with IBA (NAA+IBA) as the manufactured finished product of INCIT K. The active ingredient of the phytohormone INCIT K consists of 0.8% of alfa-naphtyl acetic acid (NAA) in combination with 0.5% of IBA. It is intended for rooting of conifer plants, which are more difficult for rooting.

The indol-3-butyric acid (IBA) is a stable substance and is used for many species, representing the most common phytohormone used in nursery production. Alfa-naphtyl acetic acid (NAA) is relatively stable and mostly used after IBA.

The length of tree-shoots varied from 10 to 15 cm, depending on the length of interdonia and number of buds. For the assimilation purposes, 2 leaves were left on tree-shoots, which were cut to ½, whilst the others were removed.

Diagonal cuts were made on the base of tree-shoots on the opposite side of the main bud, in order to increase the active contact surfaces of phytohormones IBA and NAA+IBA, which was followed by epidermis smoothening for better callusing.

Before treated with risogene substance, the basal part of the tree-shoot was treated with fungicide Benlate 0.1% for the prevention purposes against development of fungal diseases.

Lower (base) parts of tree-shoots were dipped into the liquid solution of IBA (1000, 2000 and 3000 ppm) and held in the solution for 30 seconds, and then dried for 30 minutes on the room temperature, which was followed by thinning of tree-shoots in agroperlite. Tree-shoots treated with hormone NAA 0.8% + 0.5% IBA were also treated in their lower (base) parts by the powder hormone NAA 0.8% + 0.5% IBA. Before treatment of tree-shoots with the powdered NAA 0.8% + 0.5% IBA, the tree-shoots were made wet so that the powder could better stick to the base parts. A sponge cushion, previously wetted with water, was used for wetting. When the wetting of tree-shoots was completed, tree-shoots were dipped into a vessel containing NAA 0.8% + 0.5% IBA. After the take out of tree-shoots from NAA 0.8% + 0.5% IBA, they were gently shaken off against the vessel walls, with the aim to remove powder surplus, and then the tree-shoots were thinned in the rooting substrate (agroperlite).

Tree-shoots of the controlled option were not treated with any of the above stated phytohormones.

The tree-shoots were thinned at the distance of 5 x 5 cm, and the thinning depth was 5 to 10 cm. During the rooting process, nebulization system operated constantly, creating adequate air and substrate moisture in the glasshouse. The substrate temperature during rooting was 15 – 20°C and the air humidity in the glasshouse was 75 – 80%.

During the second decade of July, the rooted lemon tree-shoots were transplanted into 3-litre-PVC containers, containing the peat and manure substrate.

The obtained research results are processed by variance analysis and assessment of significance was done by the LSD test.

RESULTS AND DISCUSSION

Results of investigations on the influence of the Indol-butric acid (IBA) and Alfa-naphtyl acetic acid (NAA+IBA) and the control without phytohormones on the rooting of mature lemon tree-shoots are presented in the Table 1 and 2 and the Figure 1.

Table 1 - The average rooting value of the investigated lemon varieties with application of solutions of indol-3-butric acid (IBA) and alfa-naphtyl acetic acid in combination with IBA (NAA 0.8% + 0.5% IBA) in %, for the entire experiment

Variety	Control	NAA 0.8%+ 0.5% IBA	IBA/ppm				\bar{X}
			1000	2000	3000	\bar{X}	
Meyer	60,00	78,75	76,25	82,50	85,25	81,33	76,55
Mjesečar	16,25	25,00	25,00	28,75	31,25	28,33	25,25
\bar{X}	38,13	51,88	50,63	55,63	58,25	54,83	

It can be established from the Table 1 that there is variation of rooting percentage with mature lemon tree-shoots, depending on the investigated variety, application of phytohormones and different concentrations of IBA and NAA+IBA.

With tree-shoots not treated with phytohormones (control), the highest value of tree-shoot rooting was reached with Meyer variety (60.00%), whilst the lowest average value was registered with the Mjesečar variety (16.25%). The average rooting value with the controlled tree-shoots of investigated varieties was 38.13%. The average value of rooted tree-shoots of the investigated lemon varieties treated with phytohormone NAA 0.8% + 0.5% IBA amounted to 51.88%. It was recorded that the best average value of rooted tree-shoots of the two investigated varieties, treated with phytohormone NAA 0.8% + 0.5% IBA was with the Meyer variety and amounted to 78.75 %, whilst the lowest rooting of 25.00% was with the Mjesečar variety.

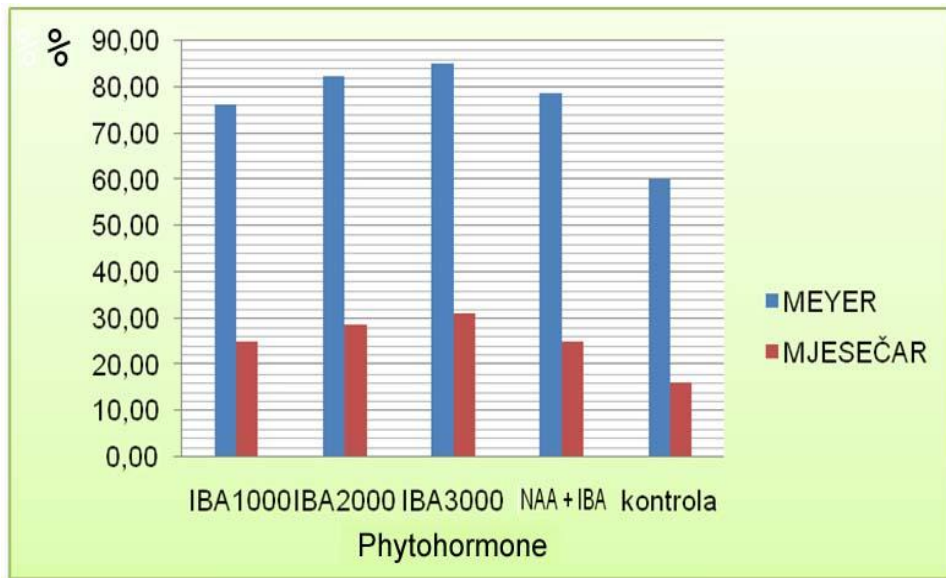


Fig.1 The average rooting value of the investigated lemon varieties under application of phytohormone IBA, NAA+IBA and the control (without phytohormone treatment)

The average rooting variety of tree-shoots by application of phytohormone IBA varied from 25.00% with Mjesečar (IBA-1000ppm) to 85.25% with Meyer variety (IBA-3000 ppm).

The average value of rooted lemon tree-shoots for two investigated varieties under all IBA concentrations amounted to 54.83%. Moreover, taking into consideration all applied treatments of IBA solutions, the average rooting value was the best with Meyer (81.33%), and the lowest with Mjesečar (28,33%).

The results obtained from investigations are similar to ones reached by Popović et al. 1998, when they established a high percentage of lemon tree-shoots rooting, which varied from 20.00% with Mjesečar to 86.66% with Meyer. The highest tree-shoot rooting was recorded with Meyer variety (86.66%) treated with 3000 ppm solution of indol-butric acid (IBA), and the lowest with Mjesečar variety (20,00%) treated with 0.1% alfa-naphtyl acetic acid.

The influence of IBA and NAA on the rooting of mature lemon tree-shoots showed different effects, which is a consequence of genotype specificities towards rooting, state Popović et al. 1998.

The variance analysis of the average rooting of lemon tree-shoots with investigated varieties is shown in the Table 2. According to all investigated parameters of the entire experiment (Table 2) for the applied phytohormones during the risogenesis of lemon tree-shoots, it can be established that there was a statically significant difference in rooting success, in relation to varieties (0.0000**) and to treatments (0.0000**).

Table 2: The variance analysis of the average value of rooted tree-shoots for the entire experiment

Source of Variance	The average value of rooted tree-shoots VARIETY (A), PHYTOHORMONE (B)				
	DF	SS	MS	F	p-level
Variety (A)	1	26316.9	26316.9	1012.84	0.0000 **
Treatment (B)	4	1924.60	481.150	18.52	0.0000 **
A*B	4	152.600	38.1500	1.47	0.2365 ns
Residual	30	779.500	25.9833		
Total	39	29173.6			

Regarding the interaction (AxB) of the two analyzed factors (phytohormone and variety) there was no statistically significant difference.

CONCLUSION

Rooting of the mature lemon tree-shoots was performed in the glasshouse (protected area) of the nursery in the Center for Subtropical Cultures in Bar during 2014.

It was established that there are diverse influences of applied phytohormonal substances of IBA (1000, 2000 and 3000 *ppm*) and NAA (0.8%) + IBA (0.5%) to the rooting success of the mature lemon tree-shoots, as well as to the root length and vegetative adherence of the rooted lemon tree-shoots.

The best rooting of the mature lemon tree-shoots, treated with the stated phytohormones, was recorded with application of IBA in the solution concentration of 3000 *ppm*, which, for the two investigated varieties in this experiment, amounted to 58.25%, and the least rooting was recorded with application of IBA in the solution concentration of 1000 *ppm*, which, for the two investigated varieties of this experiment, amounted to 50.63%.

The highest average rooting value with the controlled tree-shoots (not treated by the stated phytohormones) for the two investigated varieties amounted to 38.13%.

Regarding the rooted tree-shoots of the investigated lemon varieties treated with the phytohormone NAA 0.8% + 0.5% IBA, the highest average value was 51.88%.

Out of two investigated lemon varieties, and with application of all treatments, the Meyer variety had the best rooting (76.55%), and the Mjesečar variety had the least (25.25%), which is most likely a consequence of the variety genotype.

The best rooting was recorded with the tree-shoots of the Meyer variety (85.25%) treated with the IBA solution in the concentration of 3000 *ppm*, and the least with the tree-shoots of the Mjesečar variety (25.00%) treated with IBA solution in the concentration of 1000 *ppm*.

The variance analysis and the LSD test established differences between the investigated varieties, where the rooting success depends on exogenous treatment of tree-shoots with different types and concentrations of phytohormones.

The variance analysis established that application of IBA phytohormone under the three applied concentrations indicate a statistically very significant increase in rooting. The variance analysis also showed that application of NAA 0.8% phytohormone in combination with 0.5% IBA in the rizogenesis process provides statistically very significant increase in rooting percentage.

Application of different concentrations of indol-3-butric acid IBA (1000, 2000 and 3000 *ppm*) and alfa-naphtyl acetic acid NAA (0,8%) in combination with (0.5%) IBA proved the justified effect in rooting of the lemon tree-shoots in comparison to the control, and the rooting success depends on type and concentration of phytohormones used for tree-shoots treatments, as well as on the variety itself.

Use of higher IBA concentrations (3000 *ppm*) proved to be the best and as such can be recommended for the technology of the lemon seedling production.

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