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**IMPACT OF THE SHOOT MATURITY LEVEL ON ROOTING,
ACCLIMATISATION OF GREEN AND SEMI-HARDWOOD CUTTINGS
OF THE BLUEBERRY (*Vaccinium corymbosum* L.) CV. BLUECROP
STIMULATED WITH INDOL BUTYRIC ACID
AND NAPHTHALENE ACETIC ACID**

SUMMARY

In this research we have tested the rooting abilities of green and semi-hardwood cuttings with leaves in the ennobled blueberry (*Vaccinium corymbosum* L.) Bluecrop cultivar taken at the end of August, from shoots of the year when growth was stopped and they had not reached one year. These cuttings were stimulated with IBA (Indol butyric Acid) and NAA (Naphthalene Acetic Acid) in concentrations of 1500 ppm, 3000 ppm, 4500 ppm and in the variation control without treatment, in substrate turf, turf-perlite 2:1. The results in both types of cuttings show differences of statistical significance ($p = 0.05$) and ($p = 0.01$). Bearing in mind that the greatest rooting ability is in special time periods and based on results from several years, propagation with green cuttings did not produce a high percentage of rooting based on the fact that their rooting is difficult and has reached 37.5% whilst semi-hardwood shoots taken at the same time period have reached a higher percentage (42.5%) which is definitely related to the physiological condition of the shoot.

Keywords: green cuttings; semi-hardwood cuttings; acclimatization; IBA; NAA

INTRODUCTION

There are several ways for a successful vegetative propagation of blueberry that can potentially be used to spread blueberry *V. corymbosum* L. This is achieved using hardwood cuttings, semi-hardwood cuttings and soft-wood cuttings which are the most commonly used for spreading blueberry during which the rooting percentage reaches over 50% (Mainland, 1993; Miller *et al.*, 2006). And the blueberry propagation with green cuttings is applied on blueberry cultivars with various successes in the rooting process. Even if this type of blueberry propagation with green cuttings is applied less, it still has some advantages since in this way it can provide a larger amount of seedling material from a mother plant, which enables a rapid spread of new cultivars as well as cultivars which have difficulties propagating with hardwood cuttings (Stanić *et al.*, 2004). Vegetative propagation ensures the preservation of germplasm and

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enables the creation of homogenous descendents from the genetic aspect (clones), since it enables the transfer of all the genetic potential from the donor fruit to the new fruit (Zobel and Talbert, 1991; Barbat, 2006; Soudre, 2008). However, through vegetative or clonal propagation, the plant has the advantage of capturing all genetic superiority without including any segregation gene. Factors that have an impact on the successful rooting of cuttings were reported by (Garner and Chaudri, 1976), (Hartman *et al* 1990), as: the age of mother plant, the season when the cutting was taken, the type of cutting, presence of vegetative buds, the content of water and other ingredients. Therefore, the basic function of vegetative propagation is the formation of adventitious roots and in special cases of adventitious buds as well (Hartman *et al.*, 2002). One way to support the increase of areas with the blueberry culture *V. corymbosum* L. is to graft it over a plant which has a higher tolerance for pH and is easier adjusted to environmental conditions. One possible rootstock would be *V. arboreum*, which has the ability to grow in many fields which are unsuitable for commercial blueberries. Firstly, *V. arboreum* has a thick root system with long roots, and because of this, it is resistant towards draught and is able to tolerate draught more than *V. corymbosum*. *Vaccinium corymbosum* L. has specific requirements regarding soil pH, it can tolerate a pH at around 4,0-5,5 but *V. arboreum* can tolerate soil pH between 4 and 7 (Darnell and Hiss, 2006). In general through grafting *V. corymbosum* over *V. arboreum* the aim is to raise the crown (Ballington *et al.*, 1990), have a more powerful root system and that the blueberry is more tolerant towards soil reaction starting from pH 4-7 bearing in mind that the soil pH is a limiting factor for blueberry cultivation.

Lack of nitrogen or excessive amounts may have a negative effect on the metabolism of the plant, (Huang *et al.*, 2004). High nitrogen content decreases the rooting percentage, while nitrogen shortage prevents it. Carbohydrates are important for the root formation because they are the basic blocks of building structural elements and are used as energy sources in plants (Struve, 1981). The adventitious root formation from the stem has been known from ancient times and is used for the vegetative propagation of elite plants that have been or were selected by natural populations (De Klerk, 2002). Auxin was widely used in hardwood cuttings to accelerate the adventitious root formation (Galavi *et al.*, 2013). The initiation of adventitious roots and their growth is an intensive metabolic process which is promoted by auxin and other growth regulators that lead towards the increase of enzyme activities for the ARN and protein synthesis (Hartmann and Kester, 1983; De Klerk *et al.*, 1999; Legue *et al.*, 2014).

MATERIAL AND METHODS

The experiment was conducted during the vegetation period of 2016 in Llukë e Epërme village (Peja region). The experiment follows the method (G. Zec *et al.*, 2001). The propagation material used were one-year old green and semi-hardwood shoots with 6 - 8 mm thick leaves without fruit buds taken by parent plants of the ennobled blueberry, Bluecrop cultivar at the time when

shoots have stopped their vegetative growth. The shoots taken were cut at a length of 15 cm, i.e. several mm above the high bud and several mm under the lower bud. The cuttings taken before being placed in rooting bangos (boxes) filled in with substrate turf-only and turf-perlite 2:1 (substrate thickness 25 cm) are prepared, tied into tubes and their base part is dipped in 2.5 cm in a solution of IBA and NAA in various concentrations of 1500 ppm, 3000 ppm and 4500 ppm, in a duration of 5-7 seconds, whilst one row per box is not treated (controlled) at all.

At the bottom of boxes a layer of gravel was placed to ensure the drainage of excessive water. Such treated cuttings have stayed for 15 minutes (until they have well absorbed IBA and NAA), and after drying they were powdered at their base with captan powder mixed with talk (at a ratio 1:10 against rotting), than they were placed in boxes for rooting at a distance of 10 x 5 cm, at a depth of around $\frac{1}{2}$ of the length of the cutting, leaving at least two buds above the substrate where they have stayed for 4 - 5 weeks.

The experiment was placed in four boxes with four repetitions each, where one repetition = 40 cuttings, 40 x 4 = 160 cutting/box. The rooting of cuttings was carried out under a misting system, which has enabled the maintenance of a certain moisture and temperature. The boxes filled with substrate for the rooting of green and semi-hardwood cuttings were placed in a glasshouse where the relative air moisture is 85-90%.



a. green cuttings;

b. semi-hardwood cuttings

Figure 1. Rooted blueberry cuttings (*Vaccinium corymbosum* L.) Bluecrop cultivar

RESULTS AND DISCUSSION

Results show that semi-hardwood cuttings have reached a rooting percentage of 42.5 % compared to green cuttings where the rooting percentage reaches 37.5% taken at the same time in the same substrate (figure. 2).

This difference in the rooting percentage is definitely related to the shoot maturity level, reserve nutrients and hormone substances which help the rooting and in different stages of plant development are present in different amounts.

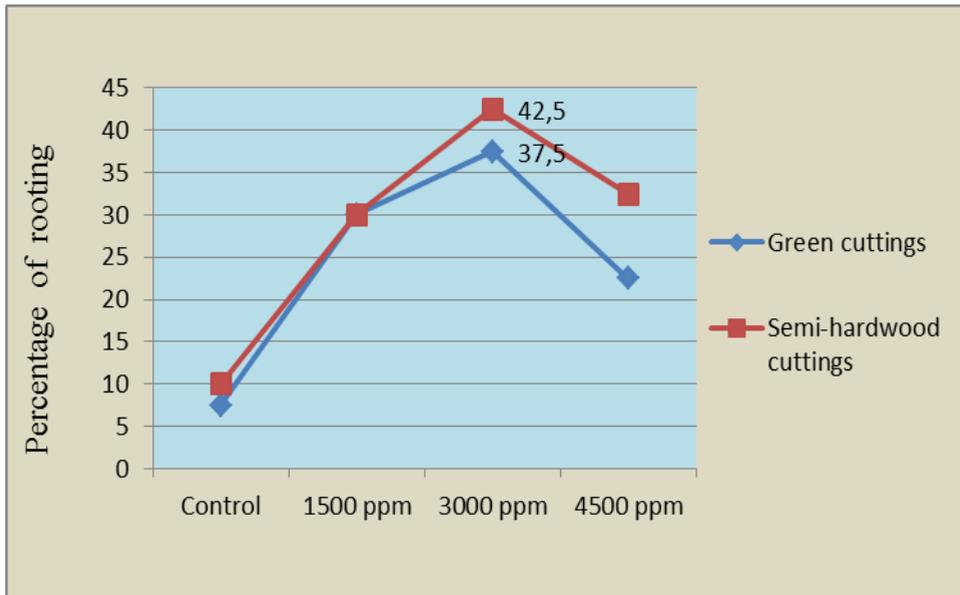


Figure 2. Averages of rooting percentage for green and semi-hardwood cuttings, Bluecrop cultivar, taken at the end of August, stimulated with IBA

Among green and semi-hardwood cuttings taken during the vegetation it was observed that the rooting ability increases from the base towards the top. This is because of auxin and other matter which is synthesized into new leaves and in the apical bud of the shoot. Moreover, the tissues of the top are less lignified, thus favouring the formation of adventitious roots. Our results are similar to the researches of other authors conducted previously, (Pokorny, F. A., and M. E. Austin. 1982), (Giroux *et al.*, 1999) (Gough, 1993; Lee *et al.*, 2004; Yong *et al.*, 2005). The differences in the rooting percentage among green and semi-hardwood cuttings were observed in all variants and in both types of substrate. The control variant also shows a small difference in the rooting percentage favouring a more mature shoot (more lignified).

These research results and the data of previous researchers provide a justification to recommend, respectively, favour the taking of cuttings from semi-hardwood and lignified shoots in order to increase the rooting percentage, because such shoots may live longer in a rooting substrate without their base part rotting and they are more resistant to dehydration. This dynamic of the rooting percentage (Figure 2) shows a dependency between the maturity level of the shoot and the rooting percentage of cuttings.

The analysis of variance (ANOVA) of the rooting percentage in green cuttings taken at the end of August (Table1) shows that differences were observed at the level of statistical significance ($p = 0.01$) in the middle of control (variant without stimulation) and variant with stimulation IBA, NAA 3000 ppm in both types of substrate. Whilst, between variants 1500 and 3000 ppm differences were observed at the level of statistical significance ($p=0.05$).

Table 1. The three way analysis of variance (ANOVA) on the rooting percentage of green cuttings with two leaves taken at the end of August.

Factor A - Substrate		Factor -B Growth regulators	Factor -C Concentration	Average (AB)	Average (A)			
Turf	Turf- Perlite							
0.50	0.75	Control	-	0.63	0.5**			
0.25	0.50	Control	-	0.38				
Average AC								
0.38	0.63							
2.0	3.0	IBA	1500 ppm	2.5	2.1*			
1.5	2.25	NAA	1500 ppm	1.8				
Average AC								
1.75	2.75							
2.5	3.75	IBA	3000 ppm	3.1	2.8**			
2.0	3.0	NAA	3000 ppm	2.5				
Average AC								
2.25	3.4							
1.5	2.25	IBA	4500 ppm	1.8	1.7**			
1.25	1.75	NAA	4500 ppm	1.5				
Average AC								
1.3	1.9							
Average C								
0.50	2.18							
Average BC				Average B				
0.5**	2.2**			1.35*				
2.8**	1.7**			2.25*				
Factors		A**	B*	C**	AB	AC	BC	ABC
LSD	1 %	0.20	0.36	0.71	0.59	1.15	1.15	2.24
	5 %	0.15	0.28	0.51	0.42	0.79	0.79	1.35

The three way ANOVA according to the test by Vukadinovic. **Significant 0.01% level. Numbers in bold show the most important attribute for variant.

The three way analysis of variance (ANOVA) on the rooting percentage of semi-hardwood cuttings (Table 2) shows that statistically verified changes were observed on both types of substrate at the level of statistical significance $p=0.01$ between the control variant and variants IBA, NAA 3000 ppm, as well as with variants 1500 and 4500 ppm.

Table 2. The three way analysis of variance (ANOVA) on the rooting percentage of semi-hardwood cuttings with leaves taken at the end of August

Factor A - Substrate		Factor -B Growth regulators	Factor -C Concentration	Average (AB)	Average (A)			
Turf	Turf- Perlite							
0.75	1.0	Control	-	0.88	0.75**			
0.50	0.75	Control	-	0.63				
Average AC								
0.63	0.88							
2.25	3.00	IBA	1500 ppm	2.62	2.25*			
1.75	2.00	NAA	1500 ppm	1.88				
Average AC								
2.00	2.50							
3.00	4.25	IBA	3000 ppm	3.62	3.25**			
2.50	3.25	NAA	3000 ppm	2.88				
Average AC								
2.80	3.80							
2.50	3.25	IBA	4500 ppm	2.88	2.56*			
2.00	2.50	NAA	4500 ppm	2.25				
Average AC								
2.25	2.88							
Average C								
0.75	2.25							
Average BC				Average B				
0.75**	2.25*			1.50**				
3.25**	2.56*			2.90**				
Factors		A**	B**	C**	AB	AC	BC	ABC
LSD	1 %	0.15	0.38	0.66	0.61	1.07	1.07	2.08
	5 %	0.11	0.28	0.48	0.44	0.73	0.73	1.25

The three way ANOVA according to the test by Vukadinović. **Significant 0.01% level. Numbers in bold show the most important attribute for variant

The stimulation of cuttings with auxin has played a central role in the development process of adventitious roots, and highly significant differences seem to exist between control variants and variants stimulated with IBA and NAA. Our results are also matching with the results of other authors (Smith and Thorpe 1975, De Klerk *et al.*, 1995, De Klerk *et al.*, 1999, Luckman and Menary 2002). The stimulation of cuttings with auxin apart from inducing the propagation of roots and their development, has also had an impact on reducing the time for rooting as well as on the uniformity of rooting of cuttings in general.

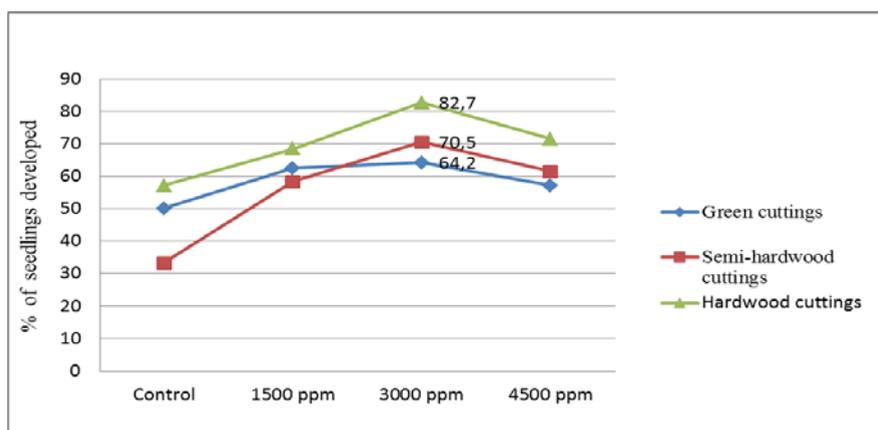


Figure 3. Average percentages of shoots developed at the end of acclimatization process, substrate turf-perlite 2:1 stimulated with IBA

Figure 3 shows that hardwood cuttings perform better during the acclimatisation process where the percentage of shoots developed at the end of the acclimatisation process reaches 82.7%. In these cuttings, the tissue is more mature and resistant towards the change of moisture and temperature which happens during the transplantation of shoots from rooting bangos in the vases. Before the removal of shoots, the amount of watering in the rooting bangos should gradually be reduced in order for the plant to be prepared for transplantation in vases, which should be placed in environments protected from the direct sunlight in order to avoid plant dehydration.

Whilst among semi-hardwood cuttings the percentage of shoots developed at the end of the acclimatisation process is 70.5% which is 12.2% lower than in hardwood cuttings. Yet, among green cuttings this percentage is even lower (only 64.2%) as a result of being more sensitive during the acclimatisation process and can very easily dehydrate, which is related to the level of shoot maturity.

CONCLUSIONS

- The green cuttings are very sensitive and cannot endure for long in the loam, and thus their base part gets rotten. However, this propagation method has an advantage since it can provide a greater amount of seedling material which

enables the rapid propagation of young cultivars and of those cultivars which have difficulties in rooting with hardwood cuttings.

- Results of the percentage of seedlings developed in hardwood and vegetative cuttings cv. 'Bluecrop' show that hardwood cuttings are more sustainable during the acclimatisation process and this is due to the physiological and anatomical status of the offshoot.

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