The Effects of Indol Butyric Acid and Naphthalene Acetic Acid of Adventitious Root formation to Green Cuttings in Blueberry cv. (*Vaccinium corymbosum* L.)

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Abstract: ***The purpose of this research was to determine the influence of the time when green cuttings were taken ½ and the influence of the position of the cutting in the branch in inducing adventive roots. The experiment has shown that the best results in rooting were achieved by ½ lignified cuttings with two leaves prepared in the middle of the branch and the ½ lignified cuttings with leaves taken from the branch-base (cuttings were taken from the year's runners which have stopped growing and are less than one year old) since these parts are richer with nutrients and rhizogenic matter (auxins, phenolic materials and carbohydrates). Green cuttings were taken twice, on July 15th, 2015 in which rooting has reached 27.5 % whilst in the cuttings taken one month later, on August15th, 2015 during the same vegetation; the rooting % was higher, reaching 35%. To help induce the adventive roots, growth bio-regulators IBA and NAA were used in concentrations (-1500, 3000 and 4500 ppm-) and the control (without treatment). The low rooting % in green cuttings is referred to the physiologic condition of the cutting, the hormonal balance and the nutrition condition of the cutting related to the seasonal period and the fruit development phase. The best promoter in inducing adventive roots was IBA, whilst the most favourable substrate was torf-perlite at a 2:1 ratio.***

Keywords: *Vaccinium corymbosum* L., green cuttings, growth regulator, rooting, substrate; turf-perlite.

1. Introduction

The highbush blueberry requires acidic soils at a pH range of 4.0 -5.5, with a high amount of organic matter, Fe and N in the form of NH_4^+ [5] Also, the soil must be well drained and aerated and with relatively sustainable amounts of moisture. For this reason, the blueberry cultivation requires well-aerated soils. When temperatures are high, respiration of plants increases causing the consumption of sugars that reduce the amount of available transported sugars towards the roots that participate in the metabolism of ammoniac nitrogen NH_4^+ [3]. Vegetative propagation may be described as a method of plant propagation through the use of separated vegetative parts of plants, when placed under favourable conditions for regeneration, they will develop into a new plant, similar in all characteristics with the parent plant[11], cutting may be done in vegetative parts of plants, such as the stems, leaves or roots. Vegetative propagation is the one method of keeping the desired characteristics of a superior cultivar, especially when it is heterozygote and polyploidy. The use of green cuttings for blueberry propagation is one of the methods of vegetative propagation [1] where the rooting % is under 50%. As a result of quite plant unique requirements, the blueberry production has the potential to increase if adverse condition can be overcomed. *V. corymbosum* has a shallow rooting system, roots which are sensitive towards draught [13]; therefore, root rehydration during the night may be very important for preserving the roots in the surface soil layers where the root density is higher, where the soil surface in many regions may be dry for a longer period of time[4]. The success of rooting depends on the type (specie), the cultivar and the conditions of the cuttings (woody, semi-lignified or green), the season and many other factors [11,6]. The rooting ability is a feature which changes during different stages of plant development). Cuttings taken from horizontal branches have better rooting abilitie [15]. while IBA is proven to be more effective in rooting of cuttings comparing to NAA [9,14]. Blueberries like other Ericaceae are typically propagated by stem cuttings [12,17], although these are known to be very hard rooted when multiplied by this method. Their rates of rooting are low and time for emission of adventitious roots goes beyond 3 to 4 weeks [10], therefore, auxin applications tend to increase the percentage of rooting in cuttings [7,8,2].

2. Materials and Methods

The propagation material used was comprised of semi-lignified green branches without fruit buds taken during the vegetation period on July 15th and Aug.15th , 2015. The branches around 5 mm thick were cut in length of 15 cm leaving 2 leaves in the upper part. The cuttings for rooting were taken from the middle of the branch ½ lignified since the middle part of the branch is richer in nutrients and rhizogenic factors which help the process of inducing adventives roots. Such cuttings were prepared, tied in tubes and their base part was dipped into 2.5 cm of growth bio-regulator solutions IBA and NAA with various concentrations of (1500, 3000 and 4500 ppm), for 5-7 sec., whilst one row in each box was left untreated (control). These cuttings after treatment have stayed for 15 min. (until they fully absorbed IBA and NAA), and after drying were powdered in their base with powder captain mixed (at a ratio 1:10 – against rotting), then they were placed in boxes for rooting at a distance 10 x 5 cm, and depth around ½ of the cutting length leaving at least 2 buds over the substrate where they have stayed for 5 weeks. The boxes were filled with torf-only rooting substrate and torf-perlite at a ratio 2:1 (substrate thickness 20 to 25 cm). At the bottom of the boxes a layer of gravel was placed to ensure the drainage of excessive water. The experiment was placed in 4 boxes with 4 repetitions each; 1 repetition = 40 cuttings 4 x 40 = 160 cutting/box. The experiment was conducted twice with the same number of cuttings, whilst the cuttings were treated with the same concentrations of IBA and NAA. The boxes filled with substrate for the rooting of green cuttings were placed in a glass greenhouse where the relative air moisture was between 75 and 80 %. This relative air moisture necessary to conduct the rooting of green cuttings was ensured through the hydrofogger (misting system) which was placed inside greenhouses under controlled conditions of temperature, moisture and lighting. The opening and closure of sprayers was regulated through the electronic leaf or photo-cell and depending on evapotranspiration.

3. Results and discussion

The rooting the of green cuttings in the turf-perlite substrate taken on August 15th is higher and reaches up to 35 % compared to the cuttings taken on July 15th where rooting reaches up to 27.5 % in the case of IBA 3000 ppm in the same turf-perlite substrate (since the presence of perlite helps in aerating the substrate and in inducing the roots bearing in mind that all processes of root formation are aerobe processes). This difference (7.5%), in the rooting of the same cuttings is related to the interaction between the time period – hormonal equilibrium and the effect of IBA and NAA. Indol Butyric Acid (IBA) in all concentrations prove to be more efficient in inducing adventive roots among green cuttings in comparison to Naphthalene Acetic Acid (NAA). The statistical analysis ANOVA shows that there are high significant differences in the % of treatment rooting (treated wood cuttings) compared to the control (untreated cuttings)as well as at the time of taking the cuttings at the reliability level ($P = 0.05$).The growth regulators concentration effects of for IBA at 3000 ppm (had up to 35% rooting) (Fig.3) were important when compared to the control (had up to 10% rooting) (Fig.3).At the treatments with IBA and NAA in the concentrations of 1500 and 4500 ppm, there were no significant differences except in few cases. Regarding the turf substrate and turf-perlite 2:1, there are significant differences among cuttings taken at the same time (in favor of substrate turf-perlite 2:1). Regarding the concentration, the one of 3000 ppm shown to be best in rooting of cuttings bearing in mind that the concentrations of auxins over 3000 ppm may prevent the blooming of cutting buds and contribute to the decomposition of the cutting base part.

Table 1: Data averages for green cuttings by repetition taken July 15th , 2015

Factor-A	Factor-B	Factor-C	Repetition				Average
			I	II	III	IV	
Turf	IBA	Control	0.00	0.00	0.00	0.00	0.00
		1500 ppm	1.00	0.00	2.00	1.00	1.00
		3000 ppm	2.00	2.00	1.00	2.00	1.75
		4500 ppm	1.00	0.00	1.00	1.00	0.75
	NAA	Control	0.00	0.00	0.00	0.00	0.00
		1500 ppm	2.00	1.00	0.00	1.00	1.00
		3000 ppm	1.00	2.00	2.00	0.00	1.25
		4500 ppm	1.00	1.00	0.00	1.00	0.75
Turf-Perlite	IBA	Control	1.00	0.00	1.00	0.00	0.50
		1500ppm	1.00	2.00	3.00	1.00	1.75
		3000 ppm	2.00	3.00	2.00	4.00	2.75
		4500 ppm	2.00	1.00	0.00	2.00	1.25
	NAA	Control	1.00	0.00	0.00	0.00	0.25
		1500 ppm	2.00	1.00	1.00	2.00	1.50
		3000 ppm	2.00	2.00	1.00	3.00	2.00
		4500 ppm	1.00	2.00	0.00	1.00	1.00

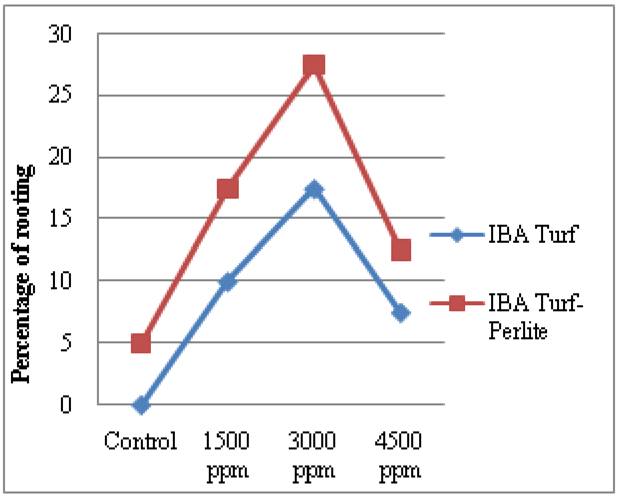

Figure 1: Rooting percentage of green cuttings in turf, turf-perlite substrate (cuttings taken in July 15th , 2015)

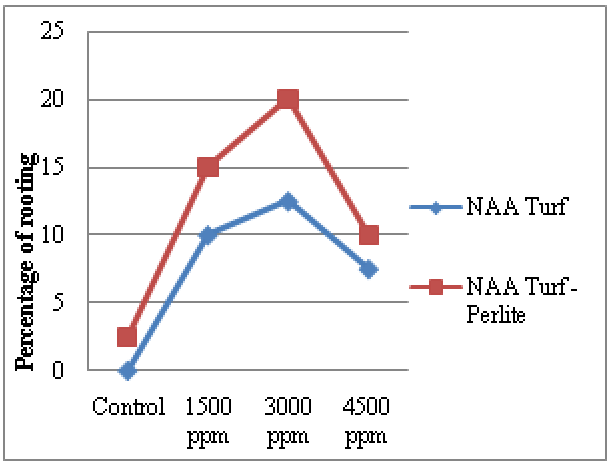

Figure 2: Rooting percentage of green cuttings in turf, turf-perlite substrat (cuttings taken in July 15th , 2015)

Table 2: The influence of the substrate and the concentration of IBA and NAA in inducing of the adventive roots (ANOVA three way for green cuttings taken July 15th , 2015)

Factor A Substrate		Factor B Growth regulators	Factor C Concentration	Average (AB)	Average (A)
Torf	Turf-Perlit				
0.00	0.50	IBA	Control	0.25	0.18**
0.00	0.25	NAA	Control	0.12	
Average AC					
0.00	0.38				
1.00	1.75	IBA	1500 ppm	1.37	1.25*
0.75	1.50	NAA	1500 ppm	1.12	
Average AC					
0.87	1.62				
1.75	2.75*	IBA	3000 ppm	1.75	1.93**
1.25	2.00	NAA	3000 ppm	2.75	
Average AC					
1.50	2.37				
0.75	1.25	IBA	4500 ppm	1.00	1.00*
1.00	1.00	NAA	4500 ppm	1.00	
Average AC					
1.00	1.62				
Average C					
1.06	1.12				
Average BC				Average B	
0.18	1.00**			0.59**	
1.93*	1.25			1.59**	

Factors		A	B	C*	AB	AC	BC**	ABC
LSD	1 %	0.62	0.43	0.49	1.12	1.28	0.78	2.48
	5 %	0.45	0.32	0.37	0.77	0.88	0.57	1.49

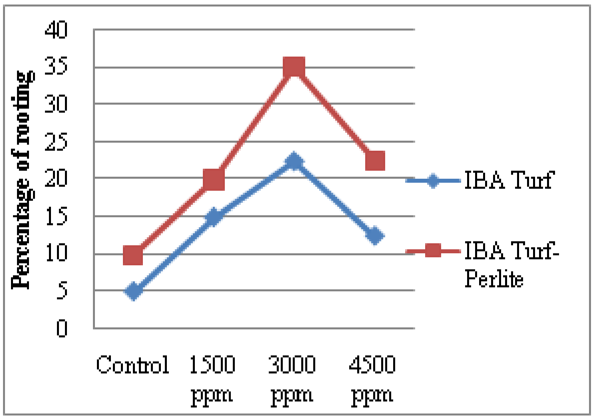

Figure 3: Rooting percentage of green cuttings turf- perlite substrate (Cuttings taken in August 15th, 2015)

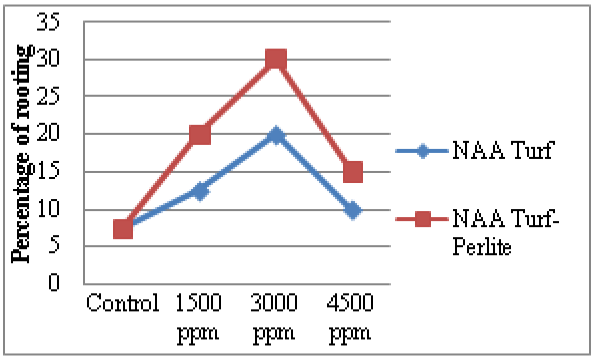

Figure 4: Rooting percentage of green cuttings in turf, in turf, turf perlite substrate (Cuttings taken in August 15th, 2015)

Table 3: Data averages for green cuttings by repetition taken August 15th , 2015

Factor-A	Factor -B	Factor -C	Repetition I	II	III	IV	Average
Turf	IBA	control	1.00	-	-	1.00	0.50
		1500 ppm	1.00	2.00	2.00	1.00	1.50
		3000 ppm	2.00	3.00	1.00	3.00	2.25
		4500 ppm	1.00	2.00	1.00	1.00	1.25
	NAA	control	1.00	-	1.00	1.00	0.75
		1500 ppm	1.00	2.00	1.00	1.00	1.25
		3000 ppm	1.00	2.00	3.00	2.00	2.00
		4500 ppm	1.00	1.00	-	2.00	1.00
Turf-Perlite	IBA	control	1.00	-	2.00	1.00	1.00
		1500ppm	2.00	3.00	1.00	2.00	2.00
		3000 ppm	3.00	4.00	4.00	3.00	3.50
		4500 ppm	2.00	2.00	3.00	2.00	2.25
	NAA	control	1.00	-	1.00	1.00	0.75
		1500 ppm	1.00	2.00	3.00	1.00	1.75
		3000 ppm	3.00	4.00	2.00	3.00	3.00
		4500 ppm	2.00	1.00	1.00	2.00	1.50

Table 4: The influence of the substrate and the concentration of IBA and NAA in inducing of the adventive roots (ANOVA three way for green cuttings taken August 15th)

Factor A Substarte		Factor B Growth regulators	Factor C Concentration	Average (AB)	Average (A)
Turf	Turf-Perlite				
0.50	1.00	IBA	control	0.75	0.75**
0.75	0.75	NAA	control	0.75	
Average AC					
0.63	0.88				
1.50	2.00	IBA	1500 ppm	1.75	1.63*
1.25	1.75	NAA	1500 ppm	1.50	
Average AC					
1.38	1.87				
2.25	3.50	IBA	3000 ppm	2.87	2.69**
2.00	3.00	NAA	3000 ppm	2.50	
Average AC					
2.12	3.25				
1.25	2.25	IBA	4500 ppm	1.88	1.57*
1.00	1.50	NAA	4500 ppm	1.25	
Average AC					
1.37	1.87				
Average C					
1.28*	1.90				
Average BC				Average B	
0.75**	1.63			1.19	
2.69**	1.57			2.13	

Factors		A**	B	C**	AB	AC	BC	ABC
LSD	1 %	0.84	0.42	0.47	1.09	1.22	0.75	2.37
	5 %	0.61	0.32	0.35	0.75	0.84	0.54	1.43

The data from Figures 1, 2, 3 and 4 show that the green cuttings rooting ability strongly depends on the time period when they are taken. This is related to the physiologic condition and nutritional condition of the cuttings related to the seasonal period and plant development stage. Rooting capacity for stem cuttings will be determined by the interaction of hereditary factors in stem cells and the following factors: auxins level, the amount of carbohydrate reservoir in the cuttings, stage of plant growth,stem location and the type of the cutting tissue [16].

In general, the rooting % of green cuttings in the turf-only substrate taken on August 15th reaches up to 22.5 % (Fig.3) compared to the cuttings placed on July 15th (the same substrate - turf), where rooting reaches to 17.5 %(Fig.1) in the case of IBA 3000 ppm. The turf-perlite (2:1) substrate is more favourable for the rooting of green cuttings. Indol Butyric Acid (IBA 300 ppm had up to 35% rooting, cuttings taken in August 15th) seems to have better results in rooting regardless of concentration comparing to Naphthalene Acetic Acid (NAA 3000 ppm had up to 30% rooting, cuttings taken in August 15th), whilst the best results were obtained with concentration of 3000 ppm.

4. Conclusions

The rooting ability among green cuttings increases from the base towards the top of the runner; thus, in green cuttings the highest result was achieved by the middle part of the runner (branch), taking into account that this part is richer in nutrients and rhizogenic matter which helps the induction of adventives roots. The time of taking the cuttings is of crucial importance for successful rooting (including the set date) since in different time periods the presence of various hormonal substances also changes as well as the nutritional substances which favour rooting.

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