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Clonal propagation of blueberries mini cutting sunder subtropical conditions

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Abstract

Blueberries are terrestrial shrubs and are economically important crop in many countries, however, increasing the production area of the fruit is limited by the availability of nursery quality. The multiplication of blueberries is mostly done through cuttings to preserve the genetic characteristics. This research aimed to find out the best level and application method of Indole Butyric Acid (IBA) to mini-cuttings of rabbit eye blueberry 'Woodard' (*Vaccinium ashei*). The experiment was carried out in two consecutive seasons, autumn from May 2015 to August 2015 and spring from October 2015 to January2016 using complete randomized design with 5 replications. The treatments included IBA application using quick dip method with concentrations 0; 1000; 2000 and 3000 mg.L^{-1} and IBA powder application method with the same concentrations. After 90 days, rooting parentage, cutting survival, number of roots per cutting, length of major roots per cutting, fresh and dry weight of roots per cutting was recorded. Major rooting parameters including rooting percentage, major roots per cutting, fresh and dry root weigh were recorded significantly higher in IBA treated mini-cutting at the level of 3000 mg. L^{-1}. While among method of application for treating cuttings with IBA, powder application was recorded with significantly higher values among all the studied parameters. IBA at the rate of 3000 mg. L^{-1} is superior in terms of rooting response, while powder application can be more effective instead of liquid quick-dip method for rooting of this blueberry cultivar.

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Introduction

Blueberry is an economically important crop in many countries. Interest in small fruits has recently increased because they are excellent sources of health-promoting vitamins, anti-oxidants and other valuable nutrients (Song and Sink, 2004). These fruits contain relatively elevated levels of antioxidants and anti-inflammatory compounds, which make them an important health promoting diet (Ehlenfeldt and Prior, 2001; Conner *et al.*, 2002; Zheng and Wang, 2003), and it is one of the few fruits that contain so wide range of anthocyanins. While many fruits contain only about 3 to 6 individual type of anthocyanins, various cultivated blueberries contain about 15 on the low end, rising to about 25 in wild blueberries (Bliss, 2002).

From 2003 to 2013, the worldwide area for blueberries production has increased. Canada and USA are among the top producers and exporters (FAO, 2015).However, increasing the production area of blueberries is in subtropical regions e.g. Brazil is limited by the availability of nursery quality (Fischer, 2007).

Blueberry can be propagated through seeds (sexual propagation) or by grafting or cuttings (asexual propagation). The most widely used form of blueberry spread is the cutting. High bush blueberries are generally multiplied by hardwood cuttings, taken during the period of winter (Antunes and Raseira, 2009), while the Rabbit eye blueberries, which are more suitable for subtropical condition, result best with softwood cuttings (Santos and Raseira, 2002). Moreover, stem cutting is considered the simplest and most economical method of vegetative propagation practiced in horticultural industry for mass production within a short time (Yoo and Kim, 1996).
In the traditional system of nursery production by cuttings, it has been recommended to use 10 to 15 cm long cuttings, keeping 2 to 3 upper leaves and removing rest (Antunes, Gonçalves and Trevisan, 2004). However, an alternative method is mini-cutting propagation used successfully on a commercial scale cultures (Ritzinger and Grazziotti, 2005). Mini-cutting practice is commonly used in clonal seedling nurseries, where vegetative propagules ranges from 4 to 8 cm long cuttings (Alfenas *et al.*, 2004) is a recent technique that has been used successfully in order to maximize the process of clonal propagation(Almeida *et al.*, 2007). Formation of adventitious root is the main step for successful vegetative propagation and development of the plants. The rooting process of cuttings is determined by the complex interaction between environmental factors and internal factors (Li *et al.*, 2009). So, the success of the propagation is dependent on variety of factors including seasonal timings, propagation technique, age and health of the stock plant, and environmental conditions. For internal factors, auxin, which is a plant growth regulator, plays a key role in root development (Overvoorde *et al.*, 2010). Some blueberry cultivars have the ability to develop roots on cuttings spontaneously, however, other require auxin application exogenously as root promoters (Strik *et al.*, 1993).

Although the natural form of auxin is indole-3-acetic acid (IAA), which can be found abundantly in nature, it has been reported that synthetic auxin indole-3-butyric acid (IBA) is the most commonly used and most effective to promote adventitious root formation in blueberries and other plants that are being multiplied from stem cuttings as well as tissue culture (Debnath, 2005; Litwinczuk *et al.*, 2005; Meiners *et al.*, 2007).

As root-promoting chemicals, auxins are frequently applied to stem cuttings using a basal quick-dip in a concentrated solution or as powder (talc) application. Liquid formulations offer the advantages of flexibility by allowing dilutions to various final concentrations and uniform application to the base of the cuttings, while powder formulations require no additional preparation prior to use (Blythe and Sibley, 2003).

Keeping in view the above facts, this research was designed with the main objective to find out the optimum level as well as the best method of applying

IBA to mini-cuttings of 'Woodard' blueberry, aiming to improve the nursery system production.

Material and methods

Experimental area and plant material

The trial was carried out in two consecutive seasons, autumn from May 2015 to August 2015 and spring from October 2015 to January 2016at State University of Londrina, PR, Brazil (altitude 23°23 S, longitude 51°11 W and elevation of 566m). The local climate is *Cfa* subtropical humid.

The cuttings were taken from the stock plants of blueberries 'Woodard' (*Vaccinium ashei*) obtained from Embrapa Temperate Agriculture. Softwood cuttings were used from healthy, non-diseased stock plants with a homogenous size of 5cm for each cutting. Then, cuttings were disposed in perforate plastic boxes (44x30x7cm) containing rice husk as growth medium.

The experiment was performed in a mist chamber controlled by an intermittent timer and a solenoid valve. The valve was programmed to mist during 10 seconds every 3 minutes. The mist chamber was maintained in greenhouse covered with transparent polyethylene film and 30% shading.

Design and treatments

The complete randomized design was used as a statistical design with 5 replications, and each plot consisted of 10 mini-cuttings. The treatments were arranged in 2x4 factorial systems, and the following factors were evaluated:

First, IBA applications methods: quick-dipliquid and powder application. Second, IBA concentrations: 0; 1000; 2000; and 3000 $mg.L^{-1}$

Growth regulator and treatment methodology

The quick-dip liquid application involved dipping the basal portion [0.5 to 2 cm (0.2 to 0.8 in)] of mini-cutting into a concentrated solution of IBA for 5-10seconds prior to inserting it into the rooting growth medium.

The powder application involved dipping the basal portion [0.5 to 2 cm (0.2 to 0.8 in)] of mini-cutting (often pre-moistened to enhance adhesion) into a blend of IBA and talc powder(Premium talc, Quimidrol® Commercial Ltd), followed by a light tap to remove excess powder prior to inserting it into the rooting growth medium (Blythe *et al.*, 2007).

IBA (Sigma®) was used for preparing the treatments, which has 99% active ingredient. For quick-dip liquid method, the IBA was dissolved in 50% alcohol solution, while for powder application; IBA was first dissolved in 50% alcohol solution and then mixed with talc powder to make it a paste. The paste was then dried in order to apply it to the cuttings.

Studied parameters

After 90 days, the following variables were evaluated: rooting percentage (% of mini-cuttings which issued at least one major root), mini-cutting survival (% of living mini-cuttings); number of roots per mini-cutting, length of major roots per mini-cutting (mm), fresh and dry weight of roots per mini-cutting (mg).

The root dry mass was obtained by drying it in an oven with forced air at 78 °C for 48hours.

Statistical analysis

The data was submitted to analysis of variance and means were compared by Tukey's test at 5% probability (Gomes, 2009). Sisvar statistical software was used for analyzing the collected data.

Results

Survival Percentage

The survival percentage for blueberry mini-cuttings was not affected significantly by different IBA concentrations during both seasons, i.e., autmn and spring (Tables 1 and 2).

However, the method of application showed a significant effect over the survival percentage of the mini-cuttings, where powder application of IBA dominated the survival percentage for both seasons.

Rooting percentage

The rooting percentage was significantly affected by different levels of IBA as well as different application methods during autmn and spring seasons (Tables 1 and 2). Mini-cuttings of blueberry treated with IBA 3000 mg.L^{-1}were recorded with superior rooting percentage compared to other treatments in both seasons, while powder application was superior among the application methods in both seasons for the mini-cuttings rotting.

Table 1. Cutting survival percentage, rooting percentage and number of roots of Rabbit eye blueberry 'Woodard' in response to different levels and application methods of IBA, Autumn 2015.

Treatments	Cutting survival (%)	Rooting (%)	Number of roots per cutting
IBA (I) (mg.L^{-1})			
0	87.0	19.0 b	0.46 b
1000	92.0	17.0 b	0.41 b
2000	88.0	27.0 ab	0.78 ab
3000	88.0	46.0 a	1.53 a
F	0.39 ^{NS}	5.98**	3.94**
Methods (M)			
Talc	95.5 a	50 a	1.49 a
Quick dip	82.0 b	4.5 b	0.10 b
F	14.52*	70.78*	28.52*
F (I × M)	2.37	1.88	1.94

Means followed by the same letter are not signifficantly different at 5% level of significance by Tukey´s test.

^{NS} = non-significant, *= significant at 5% level of significance.

Number of roots per minicutting

Similarly, the number of rootsper mini-cuttingwashigher when cuttings were treated with IBA 3000 mg.L^{-1},both in autmn and spring seasons (Tables 1 and 2),whereas powder application method overceeded the quick dip application method of IBA to mini-cuttings in relation to the number of roots per cutting.

Table 2. Cutting survival percentage, rooting percentage and number of roots of Rabbit eye blueberry 'Woodard' in response to different levels and application methods of IBA, Spring 2015.

Treatments	Cutting survival (%)	Rooting (%)	Number of roots per cutting
IBA (I) (mg.L^{-1})			
0	69.0	33.0 b	0.83 ab
1000	63.0	38.0 ab	0.89 b
2000	56.0	36.0 ab	0.77 b
3000	68.0	53.0 a	1.48 a
F	2.16 ^{NS}	3.81*	4.15*
Methods (M)			
Talc	69.0 a	47.0 b	1.25 a
Quick dip	59.0 a	33.0 a	0.74 b
F	6.11*	9.42*	10.19*
F (I × M)	0.37	0.42	1.85

Means followed by the same letter are not signifficantly different at 5% level of significance by Tukey´s test.

^{NS} = non-significant, *= significant at 5% level of significance.

Dry root weight

Regarding dry root weight,there was no significant effect of different IBA levels on mini-cuttings during autumn season (Table 3), however,during the summer season, there was a statistically significant effect recorded, where 3000 mg.L^{-1} IBA was superior but at par with control treatment (Table 4). On the other hand, the powder application method also showed higher dry root weight in comparison to quick dip method during both seasons.

Table 3. Root length, dry and fresh root weigh per cutting of Rabbit eye blueberry 'Woodard' in response to different levels and application methods of IBA, Autumn 2015.

Treatments	Root length (mm)	Dry root weight per cutting (mg)	Fresh root weight per cutting (mg)
IBA (I) (mg.L^{-1})			
0	7.40	4.6	13.8 b
1000	4.24	3.0	17.3 ab
2000	6.20	4.4	32.8 ab
3000	9.95	9.1	65.2 a
F	1.72 ^{NS}	4.66 ^{NS}	3.40*
Methods (M)			
Talc	1.279 a	9.6 a	59.0 a
Quick dip	1.100 b	0.9 b	5.5 b
F	41.22*	11.68*	17.72*
F (I × M)	0.686	2.24	1.49

Means followed by the same letter are not signifficantly different at 5% level of significance by Tukey test.
^{NS} = non-significant, *= significant at 5% level of significance.

Fresh root weight

The fresh root weight of blueberry mini-cuttingswas affected significantly by different levels of IBA, as well as by the different applying technique of IBA during autmn as well as spring(Tables 3 and 4).

IBA at the rate of 3000 mg.L^{-1} was higher but statistically equal to the control treatment where no IBA was applied to the mini-cuttings, while the powder application method affected the fresh root weight of mini-cuttings in comparison to quick dip method of application for both seasons.

Root length

The root length of the mini-cuttingswas also not affected significantly by different IBA levels during both season (Tables 3 and 4). Among application methods, the powder application was recorded with higher means in autumn and spring seasons, while the liquid quick-dip application did not show any significant effect on root length.

The daily means temperature (^{o}C) and relative humidity (%) during autumn and spring seasons are presented in Fig.1.The figure shows that there was not much difference regarding relative humidity during both seasons even though during autumn season it was slightly higher. However, the temperature difference between too seasons seems to be much higher and that might be the reason for the difference among the recorded data of two seasons.

Discussion

The obtained results indicate that different levels of IBA affected the key rooting aspects like number of roots, rooting percentage and dry weight of roots. The findings of Fischer *et al.* (2008) contradict with those findings, who studied rooting response of five rabbit eye blueberry cultivars and reported that 'Woodard' was not significantly affected by the different IBA concentrations.

This might be due to the fact that they used only hardwood cuttings for the experiment, while according to Santos and Raseira (2002) the Rabbit eye blueberry, which is more suitable for subtropical condition, results best with softwood cuttings. Secondly, the author applied IBA to the cuttings in a solution way, and from the results of this experiment it was clearly observed that liquid method of application, i.e., quick dip, did not showed any positive effect on rooting of this cultivar. It is not universal that IBA powder application is always superior to liquid quick-dip method. However, some plants root better with powder application depending on specie, time of the year and age of the cuttings and mother plant (Kroin, 2006).

Table 4. Root length, dry and fresh root weigh per cutting of Rabbit eye blueberry 'Woodard' in response to different levels and application methods of IBA, Spring 2015.

Treatments	Root length (cm)	Dry root weight per cutting (mg)	Fresh root weight per cutting (mg)
IBA (I) ($mg.L^{-1}$)			
0	1.03	51.2 a	161.5 ab
1000	1.04	38.1 ab	131.8 ab
2000	1.02	15.9 b	102.6 b
3000	1.20	52.6 a	187.2 a
F	9.66 ^{NS}	4.4*	3.25*
Methods (M)			
Talc	1.33 a	47.6	213.0 a
Quick dip	0.82 b	31.4	78.2 b
F	0.28*	4.2 ^{NS}	45.14*
F (I × M)	1.24	0.32	0.22

Means followed by the same letter are not signifficantly different at 5% level of significance by Tukey test.

^{NS} = non-significant, *= significant at 5% level of significance.

Blyth and Sibley (2007) compared different methods of auxin application by studding the work of different researchers over the last 70 years and reported either quick dip responded better or powder application responded better. Their study showed that optimum rooting varied by species, age and relative developmental stage of the shoot, time of year, auxin concentration, and method of application. Talc based products have the advantage of being less toxic, more sanitary than the liquid formulations and quicker and easier to apply. These factors may ultimately make powders more cost effective.

The mini-cutting technique follows the same concept as micro propagation, except this technique surpass the laboratory work (Titon, 2001).Originally developed for propagation of forest trees, this technique has been adopted for fruit trees, such as plum (*Prunus salicina*), fic (*Ficus carica*), barbados cherry (*Malpiglia glabra*), guava (*Psidium guajava*) and cattley guava (*Psidium cattleyanum*) (Xavier *et al.*, 2003; Wendling *et al.*, 2000; Tonietto *et al.*, 2001; Pio *et al.*, 2002a, b; Ritzinger and Grazziotti, 2005; Marinho *et al.*, 2009; Azevedo *et al.*, 2008).Thus, the reduced size of the blueberry cuttings enables more optimal use of the stock-plant and thus increases the number of propagules in each collection, thus, propagation through mini-cuttings holds more advantages over the conventional stem cuttings leading to technical, operational, economical and quality benefits (Assis, 1997; Ferreira *et al.*, 2004). The preparation and plantation along IBA application is much easier and faster to execute, thus requiring less labor. Similarly, nutrition, water management and disease control is simpler as compared to conventional stem cuttings management. Also, the juvenility of blueberry mini-cuttings gives it an edge over regular stem cuttings by more quick rooting initiation and root growth, giving rise to a better quality of nursery trees.

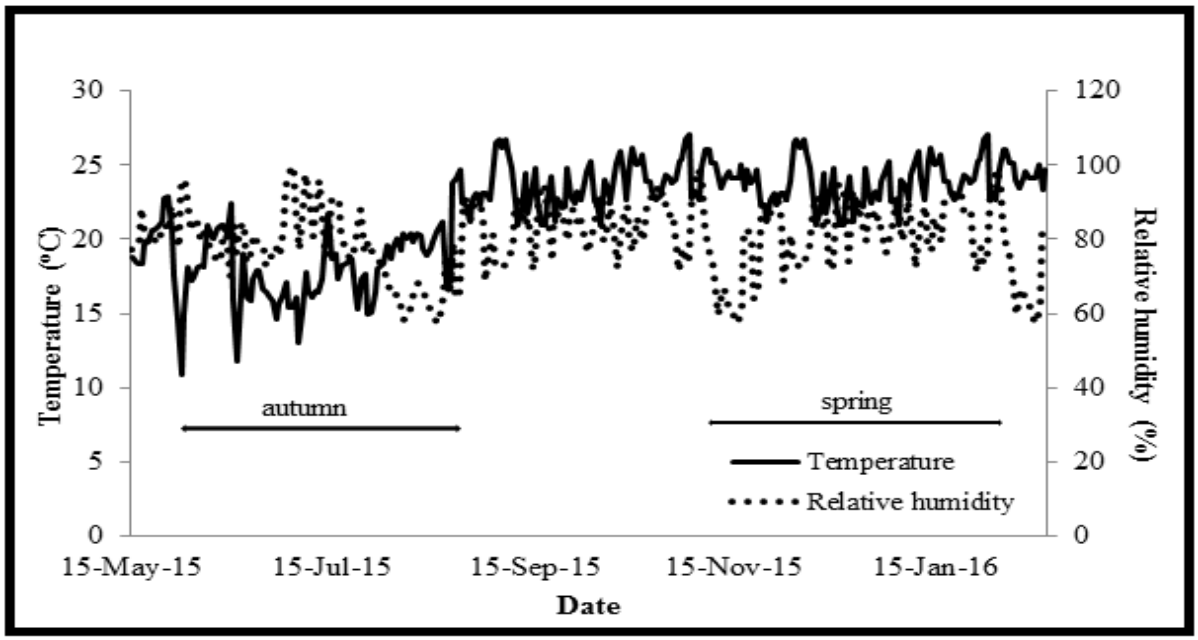

Fig. 1. Daily mean temperature (°C) and relative humidity (%) during autumn and spring seasons.

Conclusion

The multiplication of 'Woodard' blueberry using5 cm long mini-cuttings is an effective tool of clonal propagation method. For mass propagation of blueberries by using mini-cuttings, propagating material can be better utilized. IBA at the rate of 3000 mg. L^{-1} is superior in terms of rooting response, while powder application can be more effective instead of liquid quick-dip method for rooting of this blueberry cultivar.

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