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Improvement of blackberry rooting using mini cuttings and different methods of IBA application

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Abstract

Among the fruit species for commercialization perspectives, blackberry (*Rubus* spp.) is one of the most promising in the group of small fruits. It can be propagated by softwood cuttings, woody roots, shoots and by tissue culture. Propagation through mini cutting is an efficient technique which enables the achievement of large quantity seedlings in a short time. Therefore, this research was designed with the aim to improve the rooting of blackberry 'Xavante' using mini cuttings and different methods of IBA applications. Mini cuttings were taken from stock plant established in green house. The experiment was carried out in two consecutive seasons, summer and autumn, using complete randomized design with five replications. The treatments included IBA application using quick dip method with concentrations 0, 1000, 2000 and 3000 $mg.L^{-1}$ and IBA application using talc powder with same concentrations. After 90 days, rooting, cutting survival, number of roots per cutting, length of major roots per cutting, fresh and dry weight of roots per cutting was recorded. The rooting of 'Xavante' blackberry using mini cuttings collected in summer showed to be an effective clonal propagation method, and IBA at the rate of 1000 $mg.L^{-1}$ increased the rooting percentage, and its application was more effective when combined to powder application method.

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Introduction

Small fruits such as blackberries (*Rubus* spp.) have caught the attention of researchers, fruit growers and consumers due to presence of basic nutrients, fibers, essential micronutrients, such as minerals and vitamins, and various phenolic compounds (Souza *et al.*, 2014). It is a perennial fruit tree which belongs to Rosaceae family, presenting shrubby structure and considered a good cultivation option for small scale growers because of low cost of development and maintenance of orchards, minimum level of insect and disease damage and higher nutritional value (Hussain *et al.*, 2014).

In 2005, the area planted with blackberry in Brazil was 250 ha, and in recent years it has increased by about 100%, reaching approximately 500 ha (Antunes *et al.*, 2014).

The 'Xavante' blackberry is one of the main growing cultivar in the country, and it is derived from seeds collected in Clarksville, Arkansas, USA, from a cross between the A1620 andA1507 varieties and is therefore the second generation of this cross. It has vigorous, upright stem without thorns that has good production (Botelho *et al.*, 2009).

This cultivar can be propagated by softwood cuttings, from woody roots, shoots and by tissue culture. The success of vegetative propagation through cuttings depends on several factors, such as the genetic potential of rooting, physiological conditions of the stock plant, season of year, hormonal balance, temperature, light and humidity (Soundy *et al.*, 2008).

In the traditional system of production of nursery plants by cuttings, it has been recommended the use of 10-15 cm long semi-hard or softwood cuttings, with two to three leaves in the upper portion which can be cut in half, so as to facilitate handling and avoid water loss (Antunes and Raseira, 2004).

However, the multiplication of some fruit trees by using mini cuttings has presented efficient results and has been relatively fast to obtain clones. This technique is based on the higher juvenility of mini cuttings obtained from shoots in successive collections, thus providing better rooting indices of plant material (Alfenas and Mafia, 2007; Tracey *et al.*, 2014).

The use of mini cuttings instead of cuttings has some advantages, such as the reduction in the size of the propagules, increase shoots productivity per area, better rooting, low cost, and reduced need of plant growth regulators. In fact, nowadays it has been successfully used in the propagation of selected clones of different species (Brondani *et al.*, 2012; Wendling *et al.*, 2010; Stuepp *et al.*, 2017).

The development control of adventitious roots is influenced by growth regulators, with an optimal concentration which may vary between species (Costa and Costa, 2003).The exogenous auxin used to promote rooting normally used is IBA (indole-3-butyric acid), and it is widely used on the stem cuttings for accelerating the formation of adventitious roots (Galavi*et al.*, 2013).

The root-promoting auxins are frequently applied to stem cuttings using a basal quick-dip in a concentrated liquid solution or as powder (talc) application. The use of liquid quick-dip solutions have the advantage of being highly uniform, consistent and easy to use, while talc-based products have the advantage of being less toxic, more sanitary than the liquid formulations and quicker and easier to apply (Cerveny and Gibson, 2005).

In this context, the objective of this work was to evaluate the multiplication of 'Xavante' blackberry using mini cuttings and different methods of IBA application, aiming to optimize the nursery production system.

Material and methods

Experimental area

The trial was carried out in two consecutive seasons, summer (from December 2016 to March 2017) and

autumn (from April 2017 to July 2017) at State University of Londrina (UEL), PR, Brazil (altitude 23°23 S, longitude 51°11 W and elevation of 566m). According to Köppen, the local climate is Cfa (subtropical humid).

Plant material

The genetic material of blackberry 'Xavante' was obtained from stock trees of Embrapa Temperate Agriculture, Pelotas, RS, Brazil. Healthy shoots were selected and softwood mini cuttings were prepared from middle portion of each shoot, measuring 5 cm long with a pair of leaves at the top.

Then, all mini cuttings were disposed in perforate plastic boxes (44x30x7cm) containing vermiculite as growth medium and place in a mist chamber controlled by an intermittent timer and a solenoid valve.

The valve was programmed to mist during 10 seconds every 10 minutes. The mist chamber was maintained in greenhouse covered with transparent polyethylene film and 30% shading.

Statistical design used

The Complete Randomized Design was used as a statistical design for the experiment, being the treatments arranged in 2x4 factorial systems with five replications, with 10 mini cuttings per plot.

The following factors were evaluated: a) IBA application (liquid basal quick-dip and talc powder); and b) IBA concentrations (0; 1000; 2000 and 3000 $mg.L^{-1}$).

Preparation of IBA solution

IBA from Sigma® was used for preparing the treatment solutions, which has 99% active ingredient. For quick dip method, the IBA was mixed in 50% alcohol solution, while for powder application method; it was first dissolved in 50% alcohol solution and then mixed with talc powder to turn it into a paste, which was then dried in order to provide a better adhesion to the mini cuttings.

Methods of IBA application

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

The powder application method involved dipping the basal portion [0.5 to 2 cm (0.2 to 0.8 in)] of a stem mini cutting 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 substrate (Blythe *et al.*, 2007).

Parameters evaluated

After 90 days, the following variables were evaluated: cutting rooting (% of cuttings which issued at least one major root), cutting survival (% of alive cuttings); number of roots per cutting, length of major roots per cutting (cm) and fresh and dry weight of roots per cutting (g). The root dry mass was obtained by drying it in an oven with forced air at 78 °C for 48h.

Statistical analysis

All data was subjected to analysis of variance using Sisvar® software. Mean values of treatments were compared by using Tukey´s test ($P \leq 0.05$) (Gomes, 2009).

Results

Rooting percentage

The rooting percentage (Table 1 and 2) was affected significantly by different levels of IBA druing summer season while in case of autmn it was not affected significantly by different IBA levels,regarding application methods, it was affected significantly during both seasons i.e., summer and autmn.

Minicuttings of blackberry treated with IBA 1000 $mg.L^{-1}$ were recorded with superior rooting percentage compared to other treatments in both seasons, while powder application method was superior among the application methods in both seasons.

Table 1. Rooting percentage, cutting survival percentage and number of roots of blackberry 'Xavante' mini cuttings in response to different levels and application methods of IBA in summer, 2016.

Treatments	Rooting (%)		Cutting survival (%)		Number of roots per cutting
	Talc	Liquid	Talc	Liquid	
IBA (I) (mg.L^{-1})					
0	14.0 Ab	22.0 Aa	26.0 Ab	34.0 Aa	2.1
1000	52.0 Aa	18.0 Ba	56.0 Aa	26.0 Ba	2.4
2000	26.0 Ab	14.0 Aa	36.0 Aab	24.0 Aa	2.0
3000	26.0 Ab	18.0 Aa	36.0 Aab	26.0 Aa	2.2
F	2.9*		1.4 ^{NS}		0.09 ^{NS}
Methods (M)					
Talc	29.5 a		38.5 a		2.9 a
Quick dip	18.0 b		27.5 b		1.5 b
F	6.6*		5.9*		5.5*
F (I x M)	3.7*		2.9*		1.8 ^{NS}
CV	39.2		22.4		29.2

Means followed by the same letter (upper case in lines and lower case in column) are not significantly different at 5% level of significance by Tukey´s test. ^{NS} = non-significant, *= significant at 5% level of significance.

Survival percentage

The survival percentage (Table 1 and 2) was not affected significantly by different IBA concentrations during both season, however, the method of application showed a significant effect over this characteristic during summer season, whereas powder application dominated the survival percentage for both seasons.

Number of roots

The number of roots of minicuttings was also not affected significantly by different IBA levels during both seasons (Table 1 and 2).

Table 2. Rooting percentage, cutting survival percentage and number of roots of blackberry 'Xavante' mini cuttings in response to different levels and application methods of IBA in autumn, 2017.

Treatments	Rooting (%)	Cutting survival (%)	Number of roots per cutting
IBA (I) (mg.L^{-1})			
0	21.0	40.0	4.3
1000	29.0	43.0	5.0
2000	21.0	44.0	4.0
3000	23.0	34.0	4.0
F	0.5 ^{NS}	1.1 ^{NS}	0.3 ^{NS}
Methods (M)			
Talc	29 a	44.0	5.4 a
Quick dip	18 b	37.0	2.9 b
F	4.2*	3.1 ^{NS}	7.9*
F (I x M)	1.9 ^{NS}	2.1 ^{NS}	1.4 ^{NS}
CV%	34.4	16.2	33.0

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

Among application methods, the powder application was recorded with higher means in summer and autumn seasons, while the liquid quick-dip application did not show any significant effect on this variable.

Root length

Regarding root length, there was no significant effect of different IBA levels and application methods during both seasons (Tables 3 and 4), and the powder application method also showed higher means in comparison to quick dip method during both seasons.

Dry and Fresh weight

Similarly, dry and fresh root weight for blackberry minicuttings was not affected significantly by different IBA concentrations (Table 3 and 4). However, the method of application showed a significant effect over these characteristics of the minicuttings, where powder application of IBA dominated the survival percentage in both seasons, and the liquid quick-dip application did not show any significant effect.

Discussion

The success of cuttings rooting depends upon the species and cultivar, condition of cutting wood, type of cuttings (hardwood, semi-hardwood cuttings, softwood and herbal cuttings), season and many other factors (Daneh-Loueipour *et al.*, 2006).

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).

Table 3. Root length, dry and fresh root weigh per cutting of blackberry blackberry 'Xavante' mini cuttings in response to different levels and application methods of IBA in summer, 2016.

Treatments	Root length (cm)	Dry root weight per cutting (g)	Fresh root weight per cutting (g)
IBA (I) ($mg.L^{-1}$)			
0	41.6	0.13	0.20
1000	44.0	0.30	0.73
2000	42.6	0.07	0.22
3000	44.4	0.11	0.63
F	0.06^{NS}	1.8^{NS}	2.7^{NS}
Methods (M)			
Talc	59.3	0.24 a	0.70 a
Quick dip	27.0	0.06 b	0.19 b
F	3.9^{NS}	6.4*	9.4*
F (I x M)	0.4^{NS}	0.8^{NS}	1.6^{NS}
CV%	72.3	9.7	16.5

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

Rooting is the most important characteristic in the ultimate survival of plants. During the summer of 2016, it was observed that rooting percentage was affected significantly by different IBA levels, and 1000 $mg.L^{-1}$ of IBA resulted in highest rooting percentage as compared with other treatments, including 2000 and 3000 $mg.L^{-1}$. This seems to be the optimal level of IBA concentration, resulting in efficiency of rooting percentage. In several studies, it this kind of behavior has been observed among different types of cuttings, such as the results found by Luciane *et al.* (2009) working with herbaceous cuttings of fig (*Ficuscarica* L.).

Table 4. Root length, dry and fresh root weigh per cutting of blackberry 'Xavante' mini cuttings in response to different levels and application methods of IBA in autumn, 2017.

Treatments	Root length (cm)	Dry root weight per cutting (g)	Fresh root weight per cutting (g)
IBA (I) ($mg.L^{-1}$)			
0	65.0	0.24	0.50
1000	62.0	0.29	0.73
2000	58.0	0.27	0.42
3000	63.0	0.27	0.83
F	0.02^{NS}	0.9^{NS}	0.03^{NS}
Methods (M)			
Talc	80.4	0.47 a	0.95 a
Quick dip	43.3	0.07 b	0.29 b
F	3.6^{NS}	10.4^{*}	13.3^{*}
F (I x M)	0.7^{NS}	1.7^{NS}	0.06^{NS}
CV%	65.2	12.4	18.7

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

In addition, the Paul and Aditi (2009) findings revealed that IBA at 1000 $mg.L^{-1}$ was more effective in enhancing the emergence of roots in air-layer of water apple (*Syzygium javanica* L.). Increasing the concentration of exogenous auxin applied on cuttings causes root stimulating effect up to a maximum value, from which any increase of auxins has an inhibitory effect (Fachinello *et al.*, 2005).This indicates that IBA can increase rooting of a plant cutting to a certain concentration, and if the concentration is increased above that level, rooting incidence is also negatively affected, eventually damaging the cutting basal portion. This may be due to the toxicity caused by higher IBA concentration that can lead to shoot death.

These observations suggest that high IBA concentration results in higher endogenous plant hormone (especially auxins), causing an imbalance to the normal hormonal concentrations. The use of auxins stimulates rooting of different propagules, but the concentration to be used for this purpose varies with the species, its level of maturity, the environment and the way the plant growth regulator is provided to the plants (Wendling *et al.* 2014a,b).

Whereas during autumn of 2017, rooting was not affected significantly by different IBA levels, it might be due to the fact that there is a complex relationship between the cutting and the season of the year.

The environmental conditions alter the chemical composition of cuttings and it can influence the rooting (Paula *et al.*, 2009).

The influence of the season of the year on root induction also can be caused due the reserve of nutrients in the cambial tissues and the cambial activity, as well due the endogenous auxins present in the cuttings or cuttings stage of development (Leite and Martins, 2007; Ohland *et al.*, 2009).

The survival percentage of blackberry minicuttings was not affected significantly by different IBA level. Similar results were observed by Bastos *et al.* (2006), where IBA increased the cutting rooting but had no significant effect on the cutting survival, and had no role in the cuttings survival of blackberry 'Xavante' (Yamamoto *et al.* 2013; Hussain *et al.*, 2014).

The number of roots per cutting and the root length were not significantly affected by different IBA levels

in the experiment. During evaluation of the woody cutting rooting of blackberry 'Xavante', it was reported that the application of IBA did not influence the average length of roots and number of roots (Maia and Botelho, 2008).

Mini cutting of blackberry 'Xavante' showed high rooting percentage in summer as compared to autumn and the powder application method showed positive response on rooting in comparison to liquid basal quick dip method.

Conclusion

The rooting of 'Xavante' blackberry using mini cuttings collected in summer is an effective clonal propagation method. IBA at the rate of 1000 mg.L^{-1}increases the rooting percentage and its application is more effective when combined to powder application method.

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