

Indole butyric acid application methods in 'Brite Blue' blueberry cuttings collected in different seasons

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ABSTRACT: The cultivation of temperate fruits such as blueberries is expanding in Brazil. However, lower technical information on plant cultivation and difficulties in propagation are among the limiting factors in the expansion of the blueberry cultivation. The objective of this study was to evaluate the influence of different methods of indole butyric acid (IBA) application on the rooting of 'Brite Blue' blueberry herbaceous cuttings, with and without lesions, collected in two different seasons. The experimental design was completely randomized, with five replicates, each consisting of 10 cuttings, in a 3x2x2 factorial arrangement. Two IBA application methods (talc and alcohol) and control (no application), with two collection seasons (autumn and summer), and cuttings with and without basal injury, consisted of two superficial cuts on the basal part, on opposite sides, were evaluated. The cuttings were put in carbonized rice husk substrate, inside perforated plastic boxes (44x30x7 cm), and maintained in a mist chamber with intermittent regime. After 200 days, it was verified that the 'Brite Blue' blueberry was influenced by the cutting collection season, with higher rooting percentage when basal lesion was performed in cuttings collected in the summer. The IBA application of 1000 mg L⁻¹ does not influence the rooting and root development of the cuttings.

Key words: IBA; propagation; rooting; talc; *Vaccinium* sp.

Métodos de aplicação de ácido indolbutírico e épocas de coleta de estacas de mirtilo 'Brite Blue'

RESUMO: O cultivo de plantas frutíferas de clima temperado como o mirtilheiro está em expansão no Brasil. Entretanto, o conhecimento técnico escasso sobre a cultura e a dificuldade de propagação estão entre os fatores limitantes à expansão da cultura. O objetivo desse trabalho foi avaliar a influência de diferentes formas de aplicação de ácido indolbutírico (AIB) no enraizamento de estacas herbáceas, com e sem lesão, coletadas em duas épocas do mirtilo 'Brite Blue'. O delineamento experimental foi inteiramente casualizado, com cinco repetições de 10 estacas por parcela, em arranjo fatorial 3x2x2, sendo avaliados duas formas de aplicação de AIB (talco e álcool) e controle (sem aplicação), duas épocas de coleta das estacas (outono e verão), e estacas sem e com lesão na base, que consistiram em dois cortes superficiais na parte basal, em lados opostos. As estacas foram colocadas em substrato de casca de arroz carbonizada, colocadas em caixas plásticas perfuradas (44x30x7 cm) e dispostas em câmara de nebulização com regime intermitente. Após 200 dias, verificou-se que o mirtilo 'Brite Blue' sofre influência da época de coleta, com maior enraizamento das estacas com lesão na base e coletadas no verão. A aplicação de 1000 mg L⁻¹ de AIB não influencia no enraizamento e desenvolvimento radicular das estacas.

Palavras-chave: AIB; propagação; enraizamento; talco; *Vaccinium* sp.

Introduction

The blueberries (*Vaccinium* sp.) are originated in some regions of Europe and North America. Its fruit has been widely consumed around the world and its production has been showing a significant increase worldwide (Wang et al., 2015). The production of blueberry is an activity that requires labor and intense work, but it offers a high economic return in a short period, being an alternative for small growers (Pasa et al., 2014; Affonso et al., 2015).

In subtropical and tropical regions such as Brazil, scarce technical knowledge about the crop and the difficulty of most cultivars propagation are among the limiting factors to the expansion of the blueberries cultivation (Shahab et al., 2018). The commercial production of nursery plants from this species is mainly carried out by means of cuttings, which allows obtaining a greater number of young trees per shoot in a shorter period of time (Vignolo et al., 2012). However, the higher mortality rate of cuttings is still a hindrance in the plants propagation.

Acceleration of the adventitious roots formation in the cuttings is possible by the application of plant growth regulators from the auxins group, that can cause the improvement of the tissues hormonal balance (Tofaneli et al., 2003), which in turn can decrease the loss of the nurseries by desiccation. Auxin can be applied at the basal portion of the cuttings by different methods, like liquid and talc (Yamamoto et al., 2010). The application of indole butyric acid – IBA, in the liquid form, allows a more uniform application (Hartmann et al., 2014). However, the powder application method is easy to apply, and promotes the long durability of the growth hormone (Fachinello et al., 2013). In 'Brite Blue' blueberry, it was observed that the application of the IBA alcoholic solution in different concentrations did not influence the rooting of the cuttings (Trevisan et al., 2008). However, different application methods can influence the release and absorption of the growth regulator.

Regarding the collection period of blueberries cuttings from the stock tree, it was observed that summer is the best time for 'Bluegem' and 'Powder Blue' blueberries (Marangon & Biasi, 2013). In this period, the vegetative growth is intense, shoots are more herbaceous, with sufficient carbohydrates, and tend to favor the formation of roots (Lima et al., 2011). However, for some blueberries such as 'Bluebelle', there is no effect of the collection season, allowing a good rooting of the cuttings throughout the year (Marangon & Biasi, 2013).

The objective of this work was to determine the influence from different forms of IBA application on herbaceous cuttings of 'Brite Blue' blueberry, with and without lesions, collected in two seasons, in order to optimize the production system of nursery trees.

Materials and Methods

The experiment was carried out from March to October 2015 and from January to August 2016, in the Fruit Sector

from the Agricultural Research Center, Londrina State University – PR, Brazil (23°23'S latitude, 51°11'W longitude, 566 m of elevation). Herbaceous cuttings of 10 to 12 cm were taken from the middle part of the shoots from stock plants of 'Brite Blue' blueberry (*Vaccinium* sp.). Stock plants were kept in the same institution in a greenhouse, from the collection of blueberry cultivars belonging to Embrapa Temperate Agriculture, Pelotas – RS, Brazil.

The experimental design was completely randomized, in a 3x2x2 factorial arrangement with five replicates, each one consisting of 10 cuttings. The following factors were evaluated: Seasons of cuttings collection (autumn and summer), application of IBA (talc and alcohol at the concentration of 1000 mg L⁻¹ and no IBA), and two types of cuttings (with and without lesion at the basal portion).

Before collecting the cuttings, the hydroalcoholic solution of IBA was prepared, weighing 0.1 g of 99.9% pure IBA (Sigma®) in a semi analytical balance and dissolving it in 50 mL of alcohol (P.A.). After the complete dissolution of the IBA, the volume was raised to 100 mL with distilled water, obtaining the IBA concentration of 1,000 mg L⁻¹. In order to prepare the IBA in talc, 0.1g of IBA was mixed in industrial inert talc (Quimidrol®) to 100g. For better homogenization, sufficient alcohol (P.A.) was added to form a paste, and then transferred to an oven at 40 °C where it remained until complete evaporation of the solvent.

Preparation of the cuttings consisted of a basal cut just below a node, with the removal of the leaves from the basal part, keeping a pair on the upper part, which were later cut in half. The injury at the base of the cutting was performed with two superficial cuts on the basal part, on opposite sides, removing a portion of the bark of about 2 mm wide and 2 cm in length, in order to expose the cambium tissues. During the preparation, the cuttings were placed in a bucket with water to avoid dehydration. After preparation of the cuttings, the liquid IBA treatment was applied by rapid immersion for 10 seconds.

The cuttings were placed in perforated plastic boxes (44x30x7 cm) containing the carbonized rice husk substrate for rooting and arranged in a mist chamber with intermittent controlled timer and solenoid valve. The valve was programmed to mist for 10 seconds every six minutes. The mist nozzle used (Model Mist Dan Sprinklers, Israel) presents a flow rate of 35 L hour⁻¹. The mist chamber is maintained in greenhouse with transparent polyethylene film cover and 30% shade.

For control of fungal diseases, cuttings were sprayed weekly with fungicide tebuconazole (1mL L⁻¹). Foliar fertilization was carried out every 15 days with Biofert Plus® fertilizer (8-9-9 + micronutrients) at 5 mL L⁻¹ concentration.

The experiments were evaluated identically in two periods of the year, i.e., from cuttings collected in autumn of 2015 (in mid-March) and summer of 2016 (in mid-January) (Figure 1).

After 200 days of the experiments install, the following variables were evaluated: survival of the cuttings (% of live cuttings); rooted cuttings (% of cuttings that emit at least one

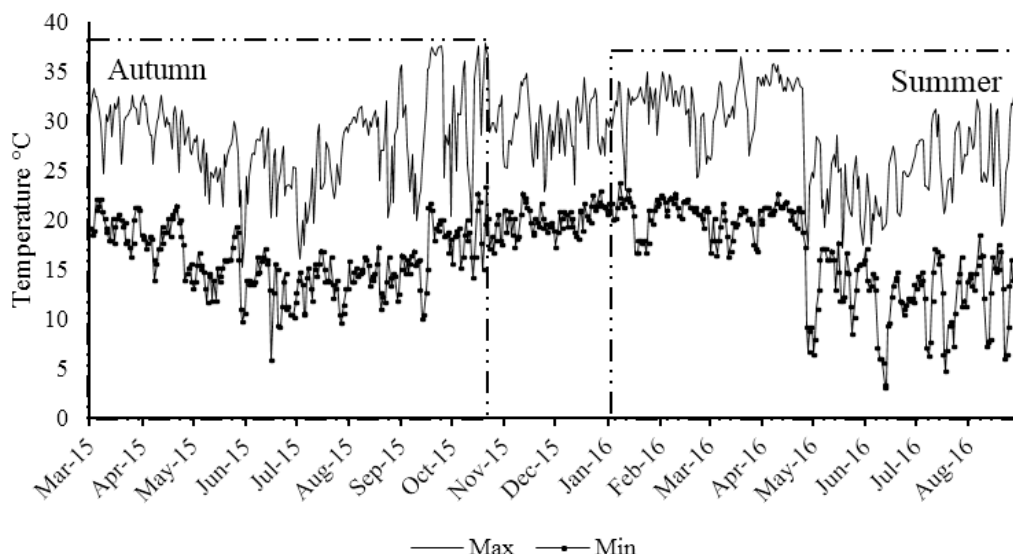


Figure 1. Daily maximum and minimum temperature (°C) during autumn and summer seasons.

root); leaf retention (% of cuttings that did not lost leaves), sprouted cuttings, number of roots per cutting; root length (cm), root dry weight (g) and cuttings with callus (% of live cuttings without roots). The root dry weight was obtained by means of drying in a oven with forced air circulation at 78 °C for 24 h.

The collected data were submitted to analysis of variance and the means were compared by the F and Tukey tests at 5% probability. The data transformation for sine arc $\sqrt{x / 100}$ was performed for the variables expressed as percentage and $\sqrt{x + 1}$ for the counting data.

Results and Discussion

The analysis of variance indicated that there was no significant interaction between season factors, injury and AIB for none of the evaluated characteristics (Table 1). On the other hand, significant interactions between the period (season) of collected cuttings and lesion factors were observed for the rooted cuttings. Significant effects of the lesion on rooted cuttings and survival were also observed, while the season factor presented significance for all evaluated variables.

Table 1. Analysis of variance of the characteristics: rooting percentage (RP), cutting survival (CS), leaf retention (LR), sprouted cuttings (SC), cuttings with callus (CC), dry root weigh per cutting (RW), number of roots (NR) root length (RL), all evaluated in 'Brite Blue' blueberry.

Source of variation	DF	Mean square ^{1/}							
		RP (%)	CS (%)	LR (%)	SC (%)	CC (%)	RW (g)	NR	RL (cm)
Season (S)	1	2.123 **	0.361 **	2.562 **	3.337 **	1.786 **	2.109 **	9.401 **	92.92 **
Incision (I)	1	0.194 *	0.162 *	0.096 ns	0.160 ns	0.131 ns	0.011 ns	0.001 ns	0.21 ns
IBA (A)	2	0.005 ns	0.024 ns	0.026 ns	0.037 ns	0.065 ns	0.110 ns	0.086 ns	29.35 ns
S x I	1	0.327 **	0.031 ns	0.008 ns	0.065 ns	0.157 ns	0.032 ns	0.042 ns	12.56 ns
S x A	2	0.052 ns	0.006 ns	0.038 ns	0.120 ns	0.045 ns	0.038 ns	0.038 ns	9.45 ns
I x A	2	0.103 ns	0.050 ns	0.044 ns	0.047 ns	0.073 ns	0.029 ns	0.223 ns	22.45 ns
S x I x A	2	0.049 ns	0.006 ns	0.056 ns	0.020 ns	0.034 ns	0.031 ns	0.014 ns	0.81 ns
Residue	32	0.050	0.041	0.031	0.044	0.038	0.038	0.212	11.11
CV (%)		33.93	28.76	23.79	26.75	23.86	29.45	23.02	21.27

ns, ** and * = not significant and significant at the 1 and 5% probability level by the F test, respectively.

A significant difference was observed in the rooted cuttings, for the interaction of the cuttings collection periods and the basal injury (Table 2). 'Brite Blue' blueberries cuttings with basal lesion collected in the summer showed 29% higher rooting than the cuttings without the lesion collected in the same season and 56% higher than the cuttings collected in the autumn. The incisions in the tissues exposed the cambium and the cortex region, increasing the absorption of water. Moreover, the removal of the physical barrier constituted by the cortical tissue by means of the mechanical injuries can stimulate the cellular division, producing root primordia, and this possibly occurs due to the increase in respiratory rate, accumulation of carbohydrates, auxins and ethylene synthesis

Table 2. Deployment of the interaction from seasons x incision for rooted cuttings characteristic evaluated in 'Brite blue' blueberry cultivar, Londrina, 2018.

Season	Rotting percentage	
	With IBA	Without IBA
Autumn	12.67 bB	17.33 bA
Summer	68.67 aA	39.33 aB

Means followed by the same lowercase letter in the column and capital letter in the lines do not differ among themselves by the F test at the 5% probability level.

Table 3. Rooting percentage (RP), cutting survival (CS), leaf retention (LR), sprouted cuttings (SC), cuttings with callus (CC), dry root weigh per cutting (RW), number of roots (NR) and root length (RL) of 'Brite Blue' blueberry in response to different collection seasons, incisions and application methods of IBA.

Seasons*	RP (%)	CS (%)	LR (%)	SC (%)	CC (%)	RW (g)	NR	RL (cm)
Autumn	15.00 b	43.00 b	7.33 b	10.00 b	23.33 a	0.031b	1.73 b	3.61 b
Summer	54.00 a	62.67 a	40.00 a	51.00 a	2.33 b	0.406 a	4.81 a	9.37 a
Incision*								
With	40.67 a	59.00 a	26.67 a	35.00 a	15.67 a	0.232 a	3.23 a	6.44 a
Without	28.33 b	46.67 b	20.67 a	26.00 a	10.00 a	0.204 a	3.31 a	6.53 a
AIB**								
Control	36.00 a	57.50 a	25.50 a	31.00 a	16.50 a	0.283 a	3.43 a	5.97 a
Talc	32.50 a	49.50 a	22.00 a	27.00 a	10.00 a	0.137 a	2.99 a	5.61 a
Quick dip	35.00 a	51.50 a	23.50 a	33.50 a	12.00 a	0.234 a	3.39 a	7.87 a

*Means followed by the same letter are not significantly different at 5% level of significance by the F test.

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

in the part with lesion (Fachinello et al., 2013; Hartmann et al., 2014).

Another factor that contributed to a higher rooting rate of cuttings with lesions was the period of collection. Perennial temperate plants have endogenous auxin levels that vary with the seasons, with lower concentrations in autumn and winter than those found in spring and summer (Wagner Júnior et al., 2004). Thus, the levels of endogenous auxins present in the cuttings during the summer collection period may possibly have contributed to root formation and development. A change in the chemical composition of cuttings is caused by the season of their collecting and it can influence the rooting. Since in summer season the quantity of sunlight hours is higher in subtropical areas, herbaceous cuttings under these conditions are favored by higher production of photosynthesized compounds (Hussain et al., 2017). The influences of the season of the year on root induction also can be caused by the nutrients reserve in the cambial tissues and the cambial activity, as well by the endogenous auxins presence in the cuttings or cuttings stage of development (Leite & Martins, 2007).

In summer, the cuttings showed higher survival and development of the nurseries, with a higher percentage of sprouted cuttings, leaf retention, root dry mass, root number, root length and lower incidence of cuttings with callus (Table 3). Cuttings collected during this period passed through a higher temperature in the first three months (Figure 1), which may have contributed to higher leaves survival and retention, stimulating root growth and sprouting.

In an experiment carried out with different cuttings lengths of the 'Woodard' blueberry, it was verified that cuttings with foliar retention showed 68% higher survival rate (Koyama et al., 2018). The presence of leaf on cuttings, being it a source of auxins and carbohydrates, is essential for the formation of new roots, reducing the time necessary for the formation of the nursery tree, and consequently reducing the cuttings death by dehydration (Xavier et al. 2009). In addition, in autumn, plants from temperate climate, such as the blueberries, decrease the activity in young tissues of secondary phloem, vascular flows and exchange, with greater accumulation of phenols and inhibitors, thus, slowing the formation of the cuttings root system (Martini et al., 2013; Hartmann et al., 2014).

The rooting and development of the cuttings were not influenced by the application of IBA at the tested concentration of 1000 mg L⁻¹. However, it was shown in previous studies with blueberry that the results may vary according to the concentration of IBA applied, the cultivar and the season of collecting cuttings (Peña et al., 2012; Marangon & Biasi, 2013).

Conclusion

'Brite Blue' blueberry is influenced by the season of its collection, with larger rooting of the cuttings that have lesions at the basal portion when collected in summer. The application of 1000 mg L⁻¹ IBA does not influence the rooting and root development of 'Brite Blue' blueberry cuttings.

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