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Rooting of Azalea Cuttings *(Rhododendron x simsii Planch.)* Under Indolebutyric Acid and Boron Concentrations

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Abstract— **Azalea plants produce flowers of different colors, and are one of the most cultivated ornamental plants. Their propagation is performed mainly by cuttings, but it still results in low commercial return. The objective of this study was to evaluate the indolebutyric acid and boron influence on the rooting of cuttings of two azalea varieties. One produces white flowers, while the other produces pink ones. Softwood cuttings were treated with five concentrations of IBA (0, 100, 200, 300 and 400 mg L^{-1}), with or without boron (25 mg L^{-1}), along 14 hours without light. The experimental design was randomized in plots, in a factorial arrangement 2x5x2 (two azalea varieties, five IBA concentrations and the presence or absence of boron), with a total of 500 cuttings. Rooting, mean root number, mean root length and mean root dry mass were analyzed. Rooting of both varieties were not influenced by boron, but all treatments showed high percentage for this variable, as the lowest value was 91% at the absence of IBA and boron. The pink variety was superior to the white one what concerns the mean root number, the mean root length and the root dry mass, but the best treatments for it were the ones with low concentrations of IBA with boron. The white variety rooted better without boron. Both varieties, however, showed high rooting power, regardless the treatment used.**

Index Terms - **Stem cuttings. Plant growth regulators. Ornamental plants.**

I. Introduction

Intensely cultivated in pots, borders and massifs, the azalea (*Rhododendron x simsii* Planch., Ericaceae) comprises a large group of woody shrubs from China where it has been hybridized and improved. As one of the main ornamental crops in Europe, its flowers are of varying colorations that appear in autumn-winter (LORENZI, SOUZA, 2008). The distinction between varieties with flowers of different colors is dependent on genetic mapping (DE KEISER et al., 2009), and is difficult to identify visually.

Plants of the genus Rhododendron L. can be multiplied by means of seeds, but without success. Among the general causes, there is the precarious germination of some species and the production of seeds in a small quantity, besides the loss of desirable characteristics of the mother plant (SAI-CHIT; CORLETT-RICHARD, 2000), however, in Brazil there is no commercialization of azalea seeds, because the commercial propagation is vegetative, by means of cuttings.

The propagation by cutting is recommended to maintain the characteristics of the matrix plant, however, several factors can contribute to the development and differentiation of roots, among them growth regulators, genetic characteristics, juvenile propagating material (GRIMALDI et al., 2008) , as well as the concentration of nutrients at different times of the year (BAÑADOS et al., 2012).

Root induction is one of the most common and exploited physiological effects of auxins, and occurs through the stimulation of cellular dedifferentiation (MAUAD et al., 2004). Among the auxins used for this purpose, indolebutyric acid is the most effective and least toxic for vegetable tissues.

The boron element, as a plant micronutrient, is essential for plants to complete their life cycle and, in the process of rooting cuttings, is still little known. Its use has presented contradictory effects, which seem to depend on the species tested.

The objective of this work was to evaluate the influence of indolebutyric acid and boron micronutrient on the rooting of herbaceous cuttings of two varieties of azalea.

II. MATERIAL AND METHODS

Herbaceous cuttings were collected from two *Rhododendron x simsii* parent plants, one with white flowers and the other with pink flowers. These plants were cultivated in the Faculty of Agrarian and Veterinary Sciences - UNESP, Jaboticabal. The experiment was conducted at the Seed Analysis Laboratory of the Plant Production Department (FCAV / UNESP) at coordinates 21 ° 17 'S and 48 ° 17' W, at an altitude of 590 m. According to the classification of Köppen, the climate is of the type Cwa (subtropical climate with dry winter and humid summer).

The experiment was installed in a greenhouse with intermittent nebulization for 10 minutes every hour, located

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The experimental design was a randomized block design with 2x5x2 triple factorial treatments (two varieties x five concentrations of IBA x presence or absence of B), with 20 treatments, five replications and five cuttings per replication, totaling 500 cuttings.

The cuttings were collected in the summer in the morning, and prepared with about 5 cm in length, with three to four leaves at the apex and the base cut into a bevel. On the same day, in the afternoon, the cuttings were immersed in five concentrations (0, 100, 200, 300 and 400 mg L-1) of indolebutyric acid (IBA), with or without boron (B) (0 and 25 mg L-1). The cuttings had 2 to 3 cm of their bases immersed and remained in the solutions for a period of 14 hours, in the absence of light. After this time, their bases were washed in running water.

The cuttings were then arranged for rooting in styrofoam trays of 128 cells, and the substrate used was vermiculite of medium granulometry.

At the end of 75 days, the following evaluations were performed: rooting, number of roots per pile, average length of roots per pile and root mass per pile.

The averages of the resulting data were compared by Tukey test at 1% and 5% significance, and also analyzed by polynomial regression.

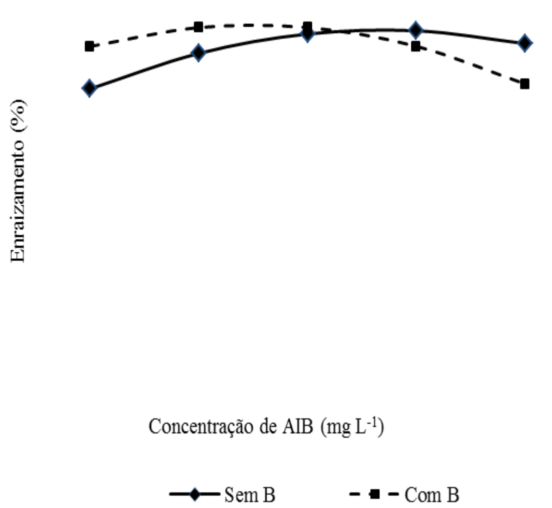
III. RESULTS AND DISCUSSION

The two varieties of azalea tested showed high overall percentages of rooting, regardless of the AIB concentration or the B application. There was a significant difference only in the interaction between the three factors (variety x AIB x B) (Table 1). In the unfolding of the linear, quadratic and cubic regressions, there was a quadratic regression adjustment only for the white variety (Figure 1).

Table 1. Rooting percentage and average number of roots of azalea cuttings (Rhododendron x simsii), under different concentrations of indolebutyric acid and boron (Jaboticabal / SP).

Causes of variation	D.F.[1]	Rooting (%)[2]	Roots number[3]
Variety (V)	1	254,052^{NS}	2,696*
IBA (A)	4	165,839^{NS}	1,489*
Boro (B)	1	0,000^{NS}	2,442*
V x A	4	95,269^{NS}	0,644^{NS}
V x B	1	28,228^{NS}	1,080^{NS}
A x B	4	88,212^{NS}	1,778*
V x A x B	4	257,580*	2,379**
Blocks	4	77,627^{NS}	0,803^{NS}
Residue	76	85,055	0,593
CV (%)		10,755	17,359

[1]Degrees freedom; [2]Values transformed in arc sin $\sqrt{x/100}$; [3]Values transformed in $\sqrt{x+0,5}$; ^{NS}not significant (p>0,05); *significant (p<0,05); **significant (p<0,01).



Sem B = 72,69480 + 0,1229580x - 0,0002277 x^2; R^2=0,91
Com B = 84,68700 + 0,07969499x - 0,00026565x^2; R^2=1,00

Figure 1. In the presence and absence of boron (B), for the white variety of azalea (*Rhododendron x simsii*) (Jaboticabal / SP)

When B was used, the rooting percentage for the white variety was higher at the AIB concentrations of 0, 100 and 200 mg L^{-1}. The isolated use of B at the concentration of 25 mg L^{-1} resulted in 99% rooting. In the absence of B and AIB, rooting was 91%. Initially, a positive effect was observed on the combination of AIB and B.

However, the AIB, when used in higher concentrations without the presence of B, showed higher rooting percentage, unlike that found by Salvador et al. (2005), who did not observe the effect of IBA on the rooting of another species of azalea, but same genus (*Rhododendron indicum* (L.) Sweet.).

LONE et al. (2010) also obtained high rooting values (from 96 to 99%) for herbaceous azalea cuttings, regardless of the application of IBA and the substrate used, among charcoal rice husk, vermiculite and coconut fiber, although the bark of charred rice and coconut fiber have provided greater root length than vermiculite. Other authors also obtained the same results, such as Mauad et al. (2004), which achieved satisfactory rooting results (above 79%) of azalea herbaceous cuttings under different concentrations of ANA (25, 50 and 75 mg Kg^{-1}) in the sand and charcoal rice husk substrates. The rooting at the 0 mg L^{-1} concentration, although it was lower than the rooting provided by the ANA, was also high (66% in sand and 86% in charred rice husk). Also, Carvalho et al. (2002), had rooting percentages above 70% for semi-hardy azalea stakes, also under different concentrations of naphthalene-acetic acid (ANA). However, the application of higher concentrations (2500 and 5000 mg L^{-1}) did not present significant results regarding the 0 mg L^{-1} concentration.

The substrate can also influence the rooting success, especially in relation to the percentage of rooting and also to the quality of the roots formed (LONE et al., 2010). In the case of yellow jasmine (*Jasminum mesnyi* Hance) for example, two concentrations of ANA and two types of substrates were evaluated in the rooting of their cuttings. The addition of the plant regulator did not influence the results, but the vermiculite showed to be the most suitable substrate for the rooting of cuttings of this species (ALTHAUS et al., 2007). Only this substrate was evaluated in this work.

The rooting of cuttings of the pink variety was not influenced by the application of B. However, for both varieties, the stakes treated with the micronutrient presented greater rooting when the IBA was used in the concentrations of 100 and 200 mg L^{-1}, while the stakes that did not have the application of B rooted better under the 300 mg L^{-1} concentration of AIB.

The use of B also did not affect the rooting of woody cuttings of *Ginkgo biloba* L. (VALMORBIDA; LESSA, 2008) or of semi-hardwood cuttings of *Laurus nobilis* L. (HERRERA et al., 2004), but root formation was intensified, in *G. biloba*, with the application of AIB to 2000 mg L^{-1}. In the case of *L. nobilis*, the cuttings presented 54% rooting when there was no application of B and 50 mg L^{-1} of IBA.

As for seedlings of a sunflower cultivar (*Helianthus annuus* L. 'Giganteus'), the root development was completely dependent on the availability of B, because when no micronutrient was applied, no roots were formed (JOSTEN, KUTSCHERA, 1999). Since auxins were not used, the authors consider that endogenous levels were sufficient for root formation. They also affirm that the cellular organization and the consequent differentiation of these organs into roots was only detected in the presence of B.

Good results were also reached for rooting of rootstocks of 'Riparia do Traviú' grapevine (*Vitis riparia* Michx x V. rupestris Scheele 'Cordifolia 106-8') under different concentrations of AIB and ANA and 150 mg L- 1 of B, used separately or together with the aforementioned regulators. However, the data obtained did not differ significantly from the cuttings that were treated with water only (LEONEL; RODRIGUES, 1993), which may indicate a species of easy natural rooting. (*Litchi chinensis* Sonn.), With the difference that the isolated application of B was not efficient for this species (LEONEL et al., 1995), nor was it for the rooting of stakes of a coffee cultivar (*Coffea arabica* L. cv. Mundo Novo). The treatments that were significantly different for cuttings of this species were all constituted of auxins and B, and the addition of B to the plant regulators benefited the rooting (ONO et al., 1992a).

For some species still, the influence of B, added to the IBA or the ANA, varies according to the collection season of the cuttings. For example, the cultivar Abbott kiwi (*Actnidia chinensis* Planch.), B only positively influenced the rooting during the summer, the same season of collection of the stakes of this study, autumn and winter, presenting the best results when applied with the ANA (ONO et al., 1995). However, the 'collection time' factor may be unrelated to the use of B, ie the experiment could present similar results even if B had not been tested. For the rooting of 'Ripária do Traviú' vine cuttings, B, AIB and ANA were also tested at different sampling times, but the results, which indicate only the best picking period, did not show any interaction with the application of B (LEONEL; RODRIGUES, 1993).

Regarding the mean number of roots, there was a significant difference between the varieties, and the rose presented the best results (Table 1). There was a quadratic regression adjustment for this variety, both in the presence and absence of B, and for the white variety only in the absence of B (Figure 2).

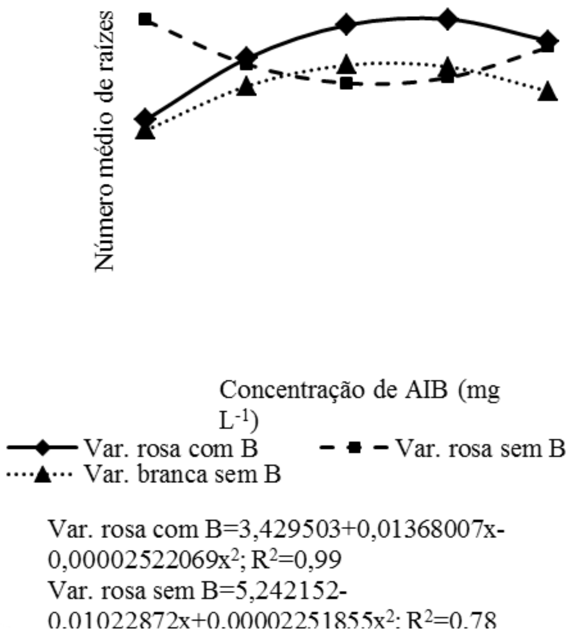


Figure 2 - In the presence and absence of boron (B), for the pink and white varieties of azalea (*Rhododendron x simsii*), the regression curves between the mean number of roots (values transformed with and different concentrations of indolebutyric acid (IBA) (Jaboticabal / SP).

The cuttings of the pink variety that presented the best results for root number were those treated with B and low concentrations of IBA. However, those that received high concentrations of AIB without the application of B also presented good results, as occurred for the percentage of rooting. The lowest concentrations of AIB, in the absence of B, did not present significant results, indicating the inefficiency of low concentrations of this plant regulator for the proposed objective.

For the white variety, the highest number of roots was obtained when IBA was used at concentrations 100, 200 and 300 mg L^{-1}, without the application of B.

Eucalyptus benthamii minicuttings increased the percentage of rooting with a presence of 2000 mg L^{-1} IBA and B concentrations in nutrient solutions (BRONDANI et al., 2014), agreeing with the results found by Schwambach et al. (2005).

This has also been proven for mung bean (*Phaseolus aureus* Roxb.), In the rooting of cuttings removed from seedlings. Few roots developed in the absence of auxins and in the presence of B at 10 mg L^{-1}. The use of B at concentrations of 10 to 100 mg L^{-1} caused leaf chlorosis and inhibitory effect on root growth. The B, although essential in the development and root growth, presents antagonistic action to the effects of the auxins when these are present in low concentrations (JARVIS et al., 1983). However, B was efficient in stimulating root growth. In addition, it was found that rootstocks were also inhibited in the presence of B and auxins (JOSTEN and KUTSCHERA, 1999).

The effects of the use of B, either alone or with auxins, have been very dependent on the species, since this micronutrient was efficient in the formation of roots for other species, for example the vine 'Ripária do Traviú', when used with high doses of AIB (1000 and 5000 mg L^{-1}). These same doses of AIB did not show significant results when they were used without B (LEONEL; RODRIGUES, 1993).

In addition to the cell division, root development and meristematic activity in general, B could be used after root induction promoted by auxins (JARVIS et al., 1983; JOSTEN; KUTSCHERA, 1999). An adequate level of B at the stake would increase the availability of carbohydrates in the meristem root growth region (NICOLOSO et al., 1999). This was also confirmed by the length of roots of rootstock cuttings of 'Ripária do Traviú' vine and of lychee. The treatments that had the use of B, either alone or in conjunction with AIB or ANA, presented the best results (LEONEL; RODRIGUES, 1993; LEONEL et al., 1995).

In relation to the average root length, for the stakes of the two azalea varieties, there was also a significant difference between them, between AIB and B concentrations, and for the interaction between varieties and B (Table 2). Regardless of the variety and application of B, AIB concentrations 200 and 300 mg L^{-1} were those which resulted in higher lengths.

Tabela 2. Length and dry mass of roots of azalea cuttings (Rhododendron x simsii), under different concentrations of indolebutyric acid and boron (Jaboticabal / SP).

Causes of variation	D.F.[1]	Root length (cm)[2]	Mass of roots (mg)[2]
Variety (V)	1	0,920**	354,268**
IBA (A)	4	0,241**	18,181^{NS}
Boro (B)	1	1,206**	54,494^{NS}
V x A	4	0,025^{NS}	5,839^{NS}
V x B	1	0,021*	32,013^{NS}
A x B	4	0,010^{NS}	9,530^{NS}
V x A x B	4	0,035^{NS}	15,890^{NS}
Blocks	4	0,153*	21,550^{NS}
Residue	76	0,052	14,609
CV (%)		20,522	81,153

[1]Degrees freedom; [2]Values not transformed; ^{NS}not significant (p>0,05); *significant (p<0,05); **significant (p<0,01).

When B was used without AIB, the cuttings had shorter roots. The concentrations of 100, 200 and 300 mg L^{-1} of AIB were the ones that presented the best results, regardless of the use of B. OB also did not affect the root length of *G. biloba* (VALMORBIDA; LESSA, 2008) Similarly, the use of 400 mg L^{-1} of IBA without the application of B promoted the root length of *Arundina bambusifolia* Lindl. (MENGARDA et al., 2013).

The varieties also differed from each other for the variable mass of roots (Table 2), and the rose variety once again surpassed the white one significantly.

According to Ono et al. (1992b), the addition of B to the cuttings is able to increase the availability and translocation of sugars to the roots, resulting in greater accumulation of fresh and dry mass, besides favoring the development of roots, since it regulates the endogenous levels of auxin (HERRERA et al., 2004). The increase of this micronutrient positively influenced the growth and development of the pitaya (*Hylocereus undatus* (Haw.) Britton & Rose) root system (SANTOS et al., 2015).

Thus, the well-formed root system favors the absorption of nutrients and water, aiding the success of vegetative propagation (CARDOSO et al., 2011).

IV. Conclusion

The two varieties of azalea, white and pink, presented great rooting capacity, regardless of the treatment used.

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