

Indole Butyric and Boric Acids in the Rooting of Atemoya “Thompson” Stem Cuttings

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Abstract: The current study aimed to evaluate the effect of indole butyric acid (IBA) and boric acid to induce rooting on atemoya “Thompson” cuttings. A randomized block design with 10 treatments and four blocks was laid out. The treatments consisted in the use of different levels of IBA (i.e., 0, 500, 1,000, 2,000 and 4,000 mg/L) with or without boric acid at 150 mg/L. The cuttings were placed to root in expanded polystyrene (EPS) trays with 72 cells and kept in a greenhouse under misting. At 120 d after planting, leaf dry and fresh masses (g), root dry and fresh masses (g), percentage of rooting, live cuttings (LC), live cuttings with callus (LCC), number of roots per cuttings and number of emitted leaves (NEL) were evaluated. Data were submitted to analysis of variance and comparison of means by Tukey test at 5% probability. Results indicated no statistically significant differences in both application methods (separate or associated with boric acid) for all evaluated variables, except for LC, in which boric acid provided higher survival rates. It could be concluded that the propagation method using either separate IBA application or associated with boric acid is unsuitable to induce rooting on atemoya “Thompson” cuttings.

Key words: Plant growth regulators, boron, propagation.

1. Introduction

Species of the Annonaceae family have sparked interest in the national and international market of fresh fruits, such as varieties of soursop (*Annona muricata* L.), cherimoya (*Annona cherimola* L.), sugar-apple (*Annona squamosa* L.) and recently, the hybrid atemoya [1]. The atemoya tree, result of the cross-breeding between sugar-apple and cherimoya, stands out for the rapid growth of its cultivated area and establishment in the Brazilian market [2].

Increased production can be achieved by improving propagation techniques for species of this botanical family. Clonal propagation emerges as an alternative to avoid possible influences of genetic variability and guarantee increased productivity [3]. Furthermore, clonal propagation reduces pathogen incidence in the seedling phase and guarantees production uniformity.

The process of root formation on cuttings is affected by many factors, such as physiological conditions of the mother plant, collection period and use of plant regulators [4, 5].

Regarding the use of plant regulators in promoting rooting of stem cuttings, it is necessary to know their appropriate concentration, as well as consider that plants already have endogenous levels of hormones [6]. Among regulators, auxins are remarkable in the induction of rooting, being indole butyric acid (IBA) one of the most used and most efficient [7, 8]. IBA is used in rooting of fruit tree cuttings such as peach [9], blueberry [10], soursop [11], sugar-apple [12], raspberry and Brazilian grape-trees [13].

However, some difficult-to-root cuttings do not emit roots because they do not have rooting cofactors in sufficient amounts. Thus, for a satisfactory rooting, both quantitative and qualitative cofactor presences are necessary in cuttings, which enable the process of root formation when combined with auxins [14]. In

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this case, the boron element constitutes an important cofactor in the induction and formation of adventitious roots [15].

The cutting method is not adequate for some species of Annonaceae, making the increment in rooting necessary. Therefore, the search for new propagation strategies for species of this family becomes crucial in the formation of new individuals. At present, the use of plant regulators may be the most viable technology in the formation of propagative material. Thus, the present work aimed to study the separate and combined effect of indole butyric and boric acid on the induction of rooting of atemoya (cv. Thompson) stem cuttings.

2. Materials and Methods

2.1 Place of Study

The experiment was conducted from September 2015 to January 2016 at the Department of Forestry Science, UNESP, Botucatu, SP, Brazil (22°51'22" S, 18°26'0" W), with 810 m of altitude and climate of the Cwa type, according to Köppen classification, and annual average precipitation of 1,524 mm [16].

Temperature and precipitation daily values in the

conduction period of the experiment were obtained through the meteorological station located in Botucatu-SP (22°50'48" S, 48°26'06" W, 817.74 m). The maximum and minimum average temperatures were 27.4 °C and 18.18 °C, with total precipitation of 1,234.7 mm (Fig. 1).

2.2 Plant Materials and Treatments

Cuttings were collected from eight-year-old trees in a commercial orchard located in the municipality of Pardinho-SP, in September 2015 (spring). Semilenous branches (10-15 cm) from the medial portion were collected. Two leaves cut in half were left remaining in the apical region of each cutting.

Basal portions of the cuttings (approximately 2.5 cm) were dipped for 1 s in 1% sodium hypochlorite solution, then immersed in distilled water, with subsequent application of the treatments for 10 s. Cuttings were rooted in expanded polystyrene (EPS) trays containing 72 cells filled with Carolina® substrate. One third of the cuttings were buried in the substrate and kept in intermittent misting chamber. Dan Sprinklers® foggers were set to 10 s of mist, every 10 min, from 9:00 am to 5:00 pm, during the entire experiment period.

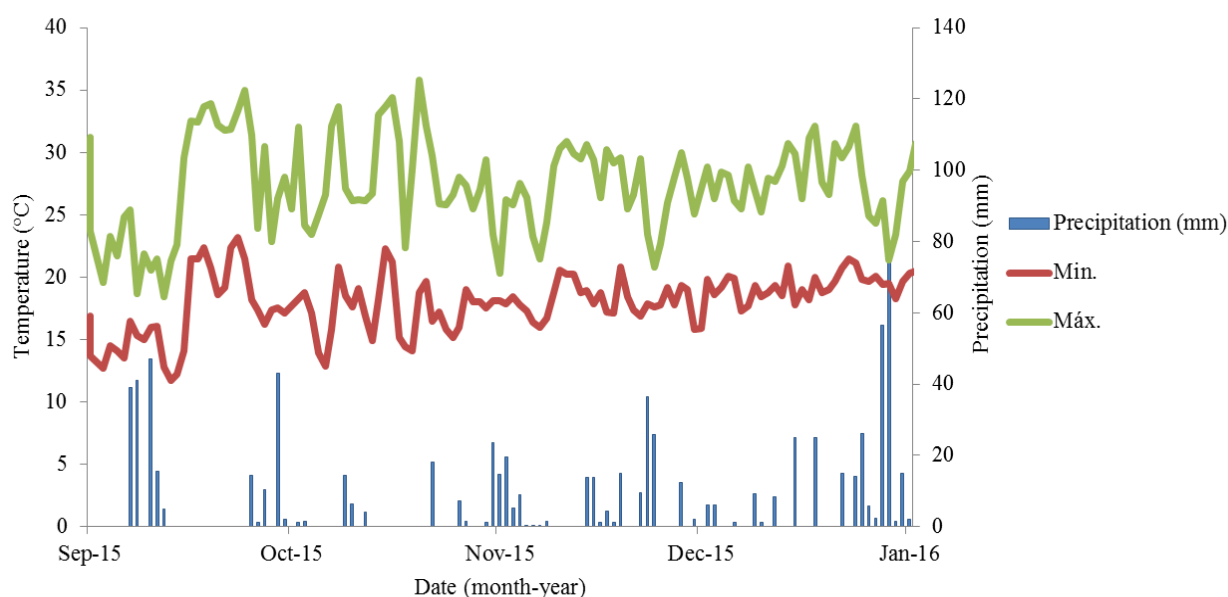


Fig. 1 Temperature and precipitation daily values during the conduction period of the experiment.

2.3 Experimental Design

A randomised-block design was used and a factorial scheme, consisting of 5×2 blocks (five IBA concentrations with and without the addition of boric acid), totaling 10 treatments with four replicates of 12 cuttings each, summing up to 480 cuttings. Treatments consisted in the application of different concentrations of IBA (0, 500, 1,000, 2,000 and 4,000 mg/L), associated or not to boric acid (150 mg/L).

2.4 Variables Analyzed

After 120 d of planting the cuttings, the following variables were analyzed: percentage of rooting, live cuttings (LC), live cuttings with callus (LCC); average root number per rooted cutting; fresh and dry shoot mass; fresh and dry root mass (emitted leaves and buds); number of emitted leaves (NEL) per cutting.

2.5 Statistical Analysis

Data were submitted to analysis of variance and means were compared by the Tukey test at 5% probability. For percentage data, arcsine square root transformation of $x/100$ was used [17].

3. Results and Discussion

Tables 1-3 are the results obtained for the rooting of atemoya stem cuttings for all variables analyzed. The

results of the analysis of variance show that there was no significant interaction between IBA concentrations and the presence or absence of boric acid (Table 1).

Regarding shoot fresh and dry mass, significant differences between the masses of cuttings were not found (Table 2). This result differs from that observed by Paulus et al. [18], who verified in rooted rosemary cuttings, in the absence of IBA, lower fresh and dry masses of shoots and roots. Studies by Paulus et al. [19] with citron (*Aloysia triphylla* (L'Hér.) Britton.) show that the concentration of 1,500 mg/L increased shoot fresh and dry masses.

As a result of the low rooting index, it was not possible to verify how IBA and boric acid influence root fresh and dry masses. Also, there were no differences in root emission per cutting. Hence, cuttings collected from the medial portion of productive branches do not favor their rooting. Ferreira et al. [20] observed in atemoya tree (cv. Gefner) greater number of buds in medial and basal cuttings. Lima et al. [21] found that “espinheira-santa” (*Maytenus muelleri* Schwacke., Celastraceae) grown during spring of 2005 presented low rooting rates compared to other seasons of the year. In this respect, it can be suggested that spring had a negative effect on the rooting process in atemoya cuttings, given that in this period, anona branches are in phase of reserve accumulation for the

Table 1 Mean square values and significance levels for shoot fresh mass (SFM), shoot dry mass (SDM), rooting (R), LC, LCC and NEL, in atemoya (cv. Thompson) stem cuttings with different concentrations of IBA and in the presence or absence of boric acid (B).

Sources of variation	Degrees of freedom (df)	Mean square					
		SFM (g)	SDM (g)	R (%)	LC (%)	LCC (%)	NEL (unit)
IBA	4	10.28326 ^{ns}	0.18205 ^{ns}	0.18205 ^{ns}	0.01789 ^{ns}	0.07356 ^{ns}	1.91920 ^{ns}
B	1	6.79141 ^{ns}	0.91537 ^{ns}	0.91537 ^{ns}	0.24414 ^{**}	0.06823 ^{ns}	2.21652 ^{ns}
IBA × B	4	11.44982 ^{ns}	0.74231 ^{ns}	0.74231 ^{ns}	0.01138 ^{ns}	0.02691 ^{ns}	1.75292 ^{ns}
Residual	27	7.41069	0.46983	0.46983	0.02955	0.03653	1.01829
Means		3.785	0.733	0.733	63.540	21.827	1.340
Minimum significant difference (MSD)				1.077			

^{**} Significant *F* value at 1%; ns: not significant.

formation of reproductive organs, which reduces tissue energy levels. In contrast, Heintzei et al. [22] and Hussein [23] observed in *Thunbergia mysorensis* and *Thunbergia grandiflora* higher rooting percentage in cuttings collected in the spring.

In Table 2, it was observed that there were no differences in the rooting percentage of stem cuttings treated with IBA and boric acid applied separately, as well as IBA and boric acid applied combined. These results corroborate with studies conducted by Casas et al. [24] with leafy cuttings of sour sop (*Annona muricata* L., Annonaceae), treated with various plant regulators, in which they did not detect rooting. Additionally Ferreira et al. [25], in experiments with “leiteiro” (*Sapium glandulatum* (Vell.) Pax., Euphorbiaceae), using concentrated solution of IBA (2,000 mg/L and 4,000 mg/L) and adding the same concentration of boric acid (150 mg/L), reported that boric acid associated with IBA did not increase rooting percentage. Salibe et al. [26], with “VR 043-43” grapevine rootstock, demonstrated that the use of boric acid (150 µg/L) was not efficient in the rooting process. Ferreira et al. [20] reported that the formation of atemoya “Gefner” seedlings by cutting can be performed through apical cuttings without the need for treatment with plant regulators.

Leonel and Rodrigues [27], in a study with grapevine rootstocks, found that the use of plant regulators provided favorable effects on rooting induction, with IBA at 2,000 ppm providing a higher percentage of rooted cuttings (88.87%) and greater root average length (84.33 mm). They also stated that cuttings treated with 1,000 mg/mL and 5,000 mg/mL of IBA + 150 mg/mL of boric acid increased rooting percentage, root length and root number, when compared with the isolated IBA treatment.

In relation to the number of LCC, it was verified that there were no significant differences among cuttings in the different IBA treatments (Table 2). However, as to the number of LC, IBA associated with boric acid showed higher survival rates (Table 3). Upon propagating of blackberry tree, with application of 0, 1,000, 2,000 and 3,000 mg/L of IBA, Villa et al. [28] did not observe significant differences in the percentage of cuttings with callus of the cultivars Brazos and Guarani.

Valmorbida and Lessa [29], in a study with *Ginkgo biloba* (*Ginkgo biloba* L., Ginkgoaceae), concluded that boron affects cutting survival. Nevertheless, Ferreira et al. [30] reported that the percentage of LC in *Sapium glandulatum* (Vell.) Pax was not influenced by IBA (4,000, 6,000 and 8,000 mg/L) and boric acid (150 mg/L) treatments.

Table 2 Analysis of variance and comparison of means for SFM, SDM, R, LC, LCC and NEL, in atemoya (cv. Thompson) stem cuttings with different concentrations of IBA in the presence or absence of boric acid (B).

Treatments (mg/L)	SFM (g)	SDM (g)	R (%)	LC (%)	LCC (%)	NEL (unit)
0 IBA	2.211 ^a	0.498 ^a	2.083 ^a	54.160 ^a	10.415 ^a	0.979 ^a
500 IBA	3.594 ^a	0.897 ^a	0.000 ^a	58.335 ^a	25.000 ^a	1.188 ^a
1,000 IBA	7.447 ^a	1.352 ^a	0.000 ^a	64.583 ^a	28.688 ^a	3.085 ^a
2,000 IBA	2.332 ^a	0.399 ^a	2.083 ^a	54.165 ^a	14.585 ^a	0.855 ^a
4,000 IBA	5.400 ^a	1.275 ^a	2.083 ^a	49.998 ^a	22.918 ^a	1.770 ^a
500 IBA + 150 B	4.207 ^a	0.792 ^a	0.000 ^a	72.915 ^a	16.668 ^a	1.168 ^a
1,000 IBA + 150 B	3.574 ^a	0.32 ^a	0.000 ^a	72.918 ^a	35.415 ^a	1.333 ^a
2,000 IBA + 150 B	3.745 ^a	0.667 ^a	4.165 ^a	77.083 ^a	22.918 ^a	1.210 ^a
4,000 IBA + 150 B	2.482 ^a	0.411 ^a	2.083 ^a	68.750 ^a	27.083 ^a	0.603 ^a
0 IBA + 150 B	2.855 ^a	0.717 ^a	0.000 ^a	62.498 ^a	14.583 ^a	1.210 ^a
Coefficient of variation (CV) (%)	71.93	93.54	240.03	17.37	42.17	75.31
MSD	6.628	1.669	0.256	0.418	0.465	2.457

Means followed by the same letter in the column do not differ at $p < 0.05$ (Tukey test).

Table 3 Comparison between averages of LC of atemoya cv. Thompson shoot branches with different concentrations of IBA in the presence or absence of boric acid (B).

Treatments	LC (%)
0 mg/L of B	56.248 ^b
150 mg/L of B	70.833 ^a
Mean	63.540
CV (%)	17.37

Means followed by the same letter in the column do not differ at $p < 0.05$ (Tukey test).

Table 2 shows that leaf number was not influenced by the addition of IBA and boric acid separately or combined. In this case, regardless of the treatments, the photoassimilates present in the cuttings were a source for leaf formation and maintenance, affecting rooting of the cuttings.

Another important fact to be mentioned is that in nearly all the cuttings, leaves were maintained, from the planting to the end of the experiment. This result is partially similar to that observed by Ferreira and Ferrari [31], where the application of IBA induced the highest survival percentage values and rooting in the cuttings whose remaining leaves were maintained. In the present study, it was not possible to correlate leaf presence with rooting of cuttings, since significant differences were not observed in rooting percentage and NEL (Tables 2 and 3). These results resemble those of Yamamoto et al. [32], in which it was observed that foliar retention on cuttings of guava (*Psidium guajava* L., Myrtaceae) cv. "Século XXI" treated with 1,000 mg/L and 2,000 mg/L of IBA did not affect rooting. However, the authors found out that applying this plant regulator in hydroalcoholic solution resulted in a lower survival percentage of the cuttings (62.5%).

Rios et al. [33] obtained higher rooting percentage of umbuzeiro (*Spondias tuberosa* Arruda., Anacardiaceae) at the concentration of 6,000 mg/L of IBA. Therefore, for higher rooting of atemoya cuttings, superior concentrations of IBA may be required. In addition, the execution time of the present work might have been short for root formation. It is also relevant to point out that, cutting is not satisfactory in anonas species, even with the use of synthetic auxins such as

IBA.

4. Conclusions

Considering the results obtained and under the conditions of this experiment, it can be concluded that the use of IBA alone or associated with boric acid was inadequate for rooting of atemoya (cv. Thompson) stem cuttings. For future studies the use of indolyl butyric acid could be evaluated in the rooting of cuttings collected at different times of the year. In addition, it may be that the adoption of different concentrations and times of contact with this plant regulator, as well as the use of new sources of plant regulators, such as naphthalene acetic acid (NAA), are more promising in the rooting of atemoya cuttings.

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