Indole-3-butyric Acid Improves Root System Quality in Guarana Cuttings

Karla Gabrielle Dutra Pinto, Sônia Maria Figueiredo Albertino, Bruna Nogueira Leite, Daniel Oscar Pereira Soares, Francisco Martins de Castro, Laís Alves da Gama, and Débora Clivati
Department of Animal and Plant Production, Universidade Federal do Amazonas, Av. Dr. Rodrigo Octávio Jordão, 1200, Coroado I, Manaus, Amazonas, Brazil 69067-005

André Luiz Atroch

Empresa Brasileira de Pesquisa Agropecuária, Centro de Pesquisa Agroflorestal da Amazônia Ocidental, Cpa. Rodovia AM-010, KM 30, Manaus, Amazonas, Brazil 69011-970

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Abstract. The economic potential of guarana relies on the energetic and medicinal properties of its seeds, which can be used to produce soft drinks, sticks, powder, and syrup. Brazil is the only guarana producer on a commercial scale, and the guarana crop system is the main agricultural activity in Maués, Amazonas. Although several types of technology have been developed to reduce costs and increase guarana productivity, the most important optimization of seedling production by cutting still needs to improve the rooting percentage and reduce mortality rates. However, the use of rooting inducers for guarana is still unestablished. Therefore, we evaluated the rooting potential of herbaceous cuttings from three guarana cultivars under different indole-3-butyric acid (IBA) concentrations. We recorded qualitative data from the roots of the cuttings. The IBA doses did not increase the percentage of rooted cuttings; however, they increased the root system quality of the guarana cuttings. We present this rooting method for the guarana plant as the most appropriate and least costly for small producers.

Paullinia cupana var. *sorbilis* (Mart.) Ducke, commonly known as guarana, is a wild vine growing in the Brazilian Amazon rainforest that has been used for centuries by the indigenous Sateré-Mawé because of its stimulant and medicinal properties (Clement et al., 2010; Schimpl et al., 2013). The fact that guarana seeds may have up to five-times more caffeine than coffee seeds has stimulated the worldwide demand by the pharmaceutical and food industries, subsequently leading to the need for expanded guarana crops (Schimpl et al., 2013). Therefore, agricultural research efforts have rapidly increased the knowledge of

guarana cultivation during the last decade (Albertino et al., 2012; Angelo et al., 2014; Gama et al., 2019; Pinto et al., 2018; Soares et al., 2019). However, several remaining gaps in relation to the cultivation of guarana must be addressed, mainly those regarding small farmers who represent part of the total Brazilian production. Vegetative propagation by stem cutting is the main technique used for guarana seedling formation; it provides high productivity, yield stability, and resistance to anthracnose, which is the most common disease in guarana plants. Nevertheless, multiple factors may affect the complex mechanisms of rooting, such as different hormone balances between guarana cultivars (Azevedo et al., 2015; Pacurar et al., 2014). Therefore, the endogenous concentration of rooting promoters or inhibitors may dispense or require the application of exogenous auxin for the rooting of stem cuttings (Ramos et al., 2003). The rooting method used for guarana seedling production suggests 70% shading, intermittent overhead mist, and the application of 2000 ppm indole-3-butyric acid (IBA); however, this concentration is being studied because the optimal value has not yet been found. The addition of exogenous auxin may be used for the rooting of guarana cutting when mineral nutrients are supplied to stock plants because it enhances the percentage of rooting and decreases cutting mortality (Albertino

et al., 2012). Moreover, microdoses of IBA require precision technology, which is unaffordable for Amazonian smallholder guarana farmers who produce without much technology using cultivation techniques that include traditional practices and popular knowledge and in a small area. Therefore, we assessed the quantitative and qualitative variables of root development for stem cuttings of guarana plants treated with different concentrations of IBA to evaluate whether exogenous auxin is useful for this purpose. We showed that no dose of IBA increased the rooting percentage; however, some doses increased the root system quality of the guarana plant cuttings. This is suitable for guarana seedling production by Amazonian smallholder farmers, nurseries, or even large guarana-producing companies.

Materials and Methods

Propagation experiments. We collected 600 herbaceous stems from three guarana cultivars: BRS Amazonas, BRS-CG372, and BRS-CG611; there were 200 cuttings of each cultivar from healthy plants from the experimental field of Embrapa Amazônia Ocidental, Manaus, Amazonas. Cuttings had variable lengths from 15 to 20 cm because of the high morphological variability of the different cultivars. We discarded too young, too old, and woody stem cuttings. Stems were placed in a cooler, transported to the greenhouse, and processed on the same day of their collection. Each stem cutting had one pair of leaflets cut in half to avoid dehydration by transpiration. Basal ends of cuttings were dipped in dry powder with 0, 1000, 2000, 3000, and 4000 ppm of IBA. Cuttings were subsequently planted in 23-cm × 18-cm × 0.15-mm plastic bags with 24 holes with diameters of 5 mm to drain excess water. Plastic bags were filled with substrate compound with wild forest soil and sand in a proportion of 4:1. We added 3 kg of regular superphosphate for each 1 m³ of substrate. After filling the plastic bags with the substrate, we placed a thin layer (1–2 cm) of sand. Plastic bags were placed in a bed over a 10-cm-thick layer of pebbles with a 10% slope to facilitate drainage. The experiment was conducted as a 3 × 5 fully random factorial design with 15 treatment combinations and 4 repetitions with 10 cuttings, with a total of 40 cuttings per experimental plot. Three cultivars of guarana tree (BRS-Amazons, BRS-CG372, and BRS-CG611) and five doses of IBA were used. Cuttings were placed in a constant misting system that was controlled by evaporation weigh; it was triggered according to the transpiration of plants.

Environment conditions. The experiment was conducted on a plant bed with 70% shading and natural temperatures and photoperiods (not controlled). Temperatures and humidity were obtained using a digital thermo-hygrometer (Thermo-Hygrometer Digital, Incoterm 7666.02.0.00; Hospinet

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S.M.F. is the corresponding author. E-mail: sonia.albertino@gmail.com.

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Table 1. Averages of the percentage of root formation and cuttings with callus and dead cuttings and averages of the length, number, volume, and weight of dry matter of the roots of cuttings of three guarana cultivars regardless of the application of indole-3-butyric acid.

Cultivar	Rooted (%)	With callus (%)	Dead (%)	Averages			
				Length (cm)	Number	Volume (mL)	Dry matter weight (g)
BRS-CG372	49.00 a ²	6.00 b	45.00 a	16.12 a	21.95 a	2.35	0.54 a
BRS-CG611	57.00 a	9.50 b	33.50 a	16.86 a	14.60 b	1.30 b	0.28 b
BRS Amazonas	31.50 b	58.00 a	10.50 b	12.31 b	8.70 c	1.10 b	0.19 b

²Means followed by the same letters in each column do not differ at $P = 0.5$ according to the Tukey test.

Fig. 1. Regression graphs showing the (A) root length, (B) root volume, (C) root dry matter weight, and (D) percentage of dead cuttings (%) of guarana cuttings submitted to five doses of indole-3-butyric acid (IBA).

Produtos Médicos e Hospitalares, Curitiba-PR, Brazil) located near the height of the cuttings.

Data collection and analysis. Cuttings were evaluated 90 d after planting, when propagation success and root system quality were assessed. First, cuttings were uprooted and rinsed gently to remove substrate. Each cutting was rated based on the success of propagation according to Atroch (2007) and classified as follows: class 1, easy rooting (>80% of cuttings rooted); class 2, intermediate rooting ($\approx 50\%$); class 3, difficult rooting (30% to 40%); and class 4, bad rooting (<30%). These classes were assigned to all cuttings that survived and formed at least one adventitious root at least 1 cm in length. Dead stem cuttings and cuttings with callus were also counted. Then, the entire root system was removed at the base of insertion on the stem cutting, dried to a constant weight in a hot drying room at 70 °C, and weighed to

determine root dry weights using a digital precision scale. Roots were counted and measured with a ruler; then, the average length was calculated per the experimental unit. Root volume was determined by water displacement introducing roots in graduated beakers. The dry weight of the roots was obtained with a precision digital scale. Data analysis was conducted using the statistical software GENES version 2015 5.0 (Cruz, 2001) and Assistat 7.7 (Silva and Azevedo, 2016). Percentage data were analyzed by transforming $\sqrt{x + 0.5}$ to approximate the data to a normal distribution. We used the Lillifors test to assess data normality and homogeneity. Then, we analyzed the data variance; the averages were compared using the Tukey test with 5% probability. Descriptive statistics (mean and sd) were calculated for each measured parameter. The significant variables for IBA doses and the

significant interactions for cultivar and IBA dose were used in the regression analysis adopting the linear model. To select the equation, the significance of the F test, the value of the coefficient of determination, and the best fit equation for the original data combined with the biological explanation of the characteristic were considered. The results of each factor were presented and discussed individually and with their significant interactions.

Results

Rooting classification. The cultivars BRS-CG372 and BRS-CG611 had the highest percentages of root formation but also the highest cutting mortality; therefore, they were classified as class 2 (intermediate rooting). The BRS Amazonas cultivar, however, had the lowest cuttings mortality and

Fig. 2. Regression graphs of the percentage of rooted cuttings from three guarana cultivars with different doses of indole-3-butyric acid (IBA).

the highest percentage of callus formation (Table 1); therefore, it was classified as a late cultivar.

Root system quality. The root system quality of the cultivar BRS-CG372 had the highest evaluated variables, except the root length, which was similar to that of BRS-CG611 (Table 1). When subjected to the five concentrations of IBA, the variables of root length, root volume, and root dry weight increased with increasing doses; however, the number of dead cuttings also increased (Fig. 1).

Cultivar vs. IBA. Responses were different between cultivars for each phytohormone dose. The cultivar BRS-CG611 had the highest rooting in all applied doses, including the control treatment, without IBA (Fig. 2). The root volume varied between cultivars with each dose of IBA. Without IBA, the cultivar BRS Amazonas had the lowest volume. At 1000 ppm, there was no significant difference in root volumes among cultivars; at 2000 ppm, the root volume of BRS Amazonas was also lower than that of the others cultivars; at 3000 ppm, only the volume of cultivar BRS-CG372 was higher; and at 4000 ppm, the volume of BRS-CG611 was lower than that of all the cultivars (Fig. 3). The aver-

age temperature during the experiment was 33 °C and the relative humidity averaged 70% (Fig. 4).

Discussion

Rooting classification. The root formation of guarana stem cuttings is highly unstable, varying between 5.6% and 90.6% among cultivars (Albertino et al., 2012; Arruda et al., 2007). The formation of roots starts with the swelling of the basal end, forming a callous tissue where the cells undergo progressive differentiation and constitute the root system of the cutting (Benda et al., 1960); this is the process that occurs in guarana cuttings. The possibility of adventitious rooting increases as the acceleration of this process increases (Dias et al., 2012). The percentage of rooting of BRS-CG611 corresponds to the intermediate class (Table 1). Similar results were found by Atroch et al. (2007), with 67.89% of rooted cuttings. However, the results are higher than those described by Arruda et al. (2007), who found 41.40% of rooted cuttings, probably because their experiment was conducted in small tubes (250 cm³) with less space for root growth and development.

Root system quality. A high-quality root system provides greater plant support and greater field survival. Therefore, the number and length of roots, in addition to the percentage of rooted cuttings, are the most relevant variables for seedling production (Lopes et al., 2015). A larger number of physiologically active roots and a larger root surface area are reflected in the exploration of a larger volume of soil, positively influencing production due to the greater capacity of plants to adapt to the environment under adverse conditions (Borcioni et al., 2016). Although root length is used to determine root density and growth (Benedetti et al., 2017), cuttings with larger roots are more likely to lose or damage seedling transposition. Depending on the concentration and the time of exposure to auxin, tissue growth and differentiation are inhibited or stimulated with optimal levels for these physiological responses (Alarcón et al., 2013). Therefore, the addition of IBA increased the root length (Fig. 1A).

The root volume increased proportionally with the increase in IBA doses (Fig. 1B). The maximum volume point was reached with the highest dose of IBA, with an increase of ≈38%, regardless of the cultivar. The increased root volume can enhance plant nutrition and support (Moreira et al., 2013). The accumulation of dry matter in the roots also increased with the addition of IBA; however, the nutritional status of the stock plant of the cutting also directly influences this variable (Galindo et al., 2018). Cell division promoted by the hormonal balance increases the fresh and dry mass of roots, indicating better absorption of nutrients (Fig. 1C) (Neumann et al., 2018). The higher dry matter weight of the cultivar BRS-CG372 (Table 1) may be related to the greater number and length of roots. However, redirection of assimilates to other activities, such as the large production of callus, may have decreased the roots dry biomass of the BRS Amazonas cultivar (Steffen et al., 2011). Our positive results indicate the efficiency of IBA to improve the root system quality of guarana plant cuttings and, consequently, help the establishment of the seedling in the field and plant development (Silva et al., 2012).

Although IBA application contributed to increase the root system quality, it reduced the rooting percentage, thereby causing the mortality of cuttings (Fig. 1D). The highest percentage of rooting and the lowest mortality rate of guarana plant cuttings are obtained without the application of IBA, regardless of the cultivar (Rodrigues and Lucchesi, 1987).

Cultivar × IBA. The auxin concentration can be abundant, scarce, or absent in plant tissues according to the physiological condition and genetic variability of the cuttings among and within cultivars (Atroch et al., 2007; Pizzatto et al., 2011), resulting in different responses of cultivars at different doses of IBA (Fig. 2). This may be the case for the BRS-CG372 cultivar, which rooted without IBA. Exogenous auxin may be toxic

Fig. 3. Regression graphs of the root volume (RV; mL) of cuttings from three guarana cultivars with different doses of indole-3-butyric acid (IBA).

Fig. 4. Averages of the minimum and maximum temperatures and minimum and maximum relative humidity during the rooting of guarana cuttings.

to this cultivar because we observed that higher IBA doses resulted in lower rooting percentages. This mechanism is well-described because inducers can stimulate the roots up to a maximum value, and eventual increases from that point can produce toxic effects (Inocente et al., 2018). In contrast, the BRS Amazonas cultivar had a higher percentage of rooted cuttings using the highest dose of IBA.

When a natural imbalance of auxin levels is found, the exact dose of exogenous regu-

lators must be investigated to optimize rooting without burdening the seedling production process. These regulators affect the root system quality, such as the root volume (Fig. 3), thus influencing seedling development and survival to transplanting (Lima et al., 2018). In some cases, the application of these regulators (such as IBA) does not influence the development of the root or aerial system of the seedlings (Lafetá et al., 2016). This null stimulus to the growth of the propagating material can occur when the

level of endogenous auxin in the stretching region is close to the optimum point; alternatively, inhibition can be attributed to ethylene biosynthesis induced by auxin (Alarcón et al., 2013). Therefore, the use of synthetic auxin, such as IBA, can induce adventitious root formation, thus increasing the rooting percentage because cuttings at different points of physiological maturation can present different responses in relation to rooting (Winhelmann et al., 2018). Other factors must be considered for good rooting, such as physiological conditions, the growth habits of the plant, and environmental factors (water availability, substrates, light, temperature, and relative humidity) (Denaxa et al., 2012). In this context, the high temperatures (average of 33 °C) observed during this experiment may have favored the loss of water from the stem cuttings, thus impairing their rooting and survival (Fig. 4). However, cell division is favored by the temperature increase, which helps the formation of roots and the production of shoots, and the high temperatures also increase transpiration and water loss by the leaves (Paiva et al., 2009). However, low temperatures decrease the metabolism of cuttings, leading to less production of sprouts and longer times for rooting, or do not provide appropriate conditions for induction, development, and root growth (Corrêa and Fett-Neto, 2004). The ideal average temperature range for rooting cuttings of most species is 21.1 to 26 °C, whereas the ideal air humidity is 80% to 100%, which allows the maintenance of the turgor of the tissue and survival (Benda et al., 1960). The avoidance of critical moisture levels, especially for cuttings with leaves, prevents the risk of dehydration and death before root formation (Zang et al., 2013). Nevertheless, the intermittent overhead mist system allowed the maintenance of 70% average humidity, thus protecting the leaf surfaces from dehydration. The intermediate to low rooting percentages (BRS-CG372 49%, BRS-CG611 57%, and BRS Amazonas 31.50%) might have been influenced by this environmental factor and genetic factors.

We demonstrated that IBA does not affect the rooting of guarana stem cuttings. Even though cultivars show different rooting capacities, all guarana cultivars rooted without exogenous IBA. Therefore, clonal production by stem cuttings can use rooting inducers for this purpose, allowing Amazonian smallholder farmers to produce guarana plant seedlings at reduced costs. Furthermore, the production of other species by cuttings also does not require IBA for rooting, such as some vine cultivars (Faria et al., 2007; Lone et al., 2010b; Machado et al., 2005), azalea (Lone et al., 2010a), different eucalyptus clones (Almeida et al., 2007), and mini-cuttings of purple ipê (Oliveira et al., 2015).

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