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The interactive effect of synthetic auxins and genotypes on the rooting of guarana cuttings

O efeito interativo de auxinas sintéticas e genótipos no enraizamento de estacas de guaranazeiro

Maísa Silva dos Santos Lemos[1] ; Anderson Adriano Martins Melo[2] ; Firmino José do Nascimento Filho[3] ; Eva Maria Alves Cavalcanti Atroch[4] ; André Luiz Atroch[5*]

[1]Federal University of Amazonas, Postgraduate Program in Tropical Agronomy – UFAM/PPGATR; [2,3,5] Embrapa Western Amazon; [4] Institute of Biological Sciences, Laboratory of Plant Tissue Culture, Federal University of Amazonas – UFAM. *corresponding author: andre.atroch@embrapa.br

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Resumo

O uso de reguladores de crescimento para aumentar o enraizamento de clones altamente produtivos de guaraná é uma alternativa para melhorar a propagação comercial desta espécie. Este trabalho foi realizado com o objetivo de avaliar o enraizamento de genótipos de guaranazeiro imersos nas soluções enraizadoras AIB, 2,4-D e ANA. As estacas de guaraná foram retiradas de ramos herbáceos e imersas por 10 s em 2 g L^{-1} de cada solução, imediatamente plantadas no substrato e mantidas por 90 dias em condição de viveiro. O experimento foi conduzido no Campo Experimental da Embrapa Amazônia Ocidental em Manaus, no delineamento de blocos ao acaso em arranjo fatorial com 16 genótipos x três reguladores de crescimento + 01 testemunha adicional, com duas repetições e 10 plantas por parcela. Após 90 dias, foi avaliada a porcentagem de estacas enraizadas, estacas com calos e estacas mortas, número de raízes, comprimento da maior raiz (cm), volume de raízes (mL), massa fresca de raízes (g) e massa seca de raízes (g). Os tratamentos com AIB e ANA melhoraram a qualidade do sistema radicular das estacas. A maior mortalidade foi observada nas estacas com 2,4-D, com 69% de estacas mortas comparado a 48% de ANA e 55% de AIB. Todas as outras caraterísticas foram influenciadas pelas soluções enraizadoras, exceto a porcentagem de estacas com calos. Os genótipos apresentaram grande variabilidade quanto ao enraizamento, destacando-se os genótipos CMU 1004, CMU 1005, CMU 1006 e CMU 1014 com maiores porcentagens de estacas enraizadas, variando de 53 a 63% enquanto os genótipos CMU 1016 e CMU 1022 apresentaram as menores taxas de enraizamento (5%). Houve interação significativa entre genótipos x soluções enraizadoras para volume, peso fresco e peso seco de raízes. O contraste fatorial *versus* controle foi não significativo, assim, o uso de soluções enraizadoras não influencia no enraizamento de estacas de guaranazeiro.

Palavras-chave: Propagação vegetativa; AIB; ANA; 2,4-D, *Paullinia cupana* Kunth.

Abstract

Using growth regulators to increase rooting of high-production guarana clones is an alternative to improve commercial propagation of this species. This work aimed to evaluate the rooting of guarana genotypes immersed in IBA, 2,4-D and NAA rooting solutions. Guarana cuttings were removed from herbaceous branches and immersed for 10 s in 2 g L^{-1} of each solution, immediately planted in the substrate and kept for 90 days under nursery condition The experiment was carried out in the Experimental Farm of Embrapa Western Amazon, in a randomized complete block design, with a factorial arrangement of 16 genotypes x three growth regulators + 1 additional control, with two replicates and 10 plants per plot. Percentage of rooted cuttings, cuttings with callus and dead cuttings, root number, root length (cm), root volume (mL), root fresh weight (g) and root dry weight (g) were assessed after 90 days. IBA and NAA treatment improved the quality of the root system of cuttings from all guarana genotypes. The highest mortality was observed in the treatment with 2,4-D, with 69% of dead cuttings compared to 48% of NAA and 55% of IBA. All other characteristics were influenced by the different rooting solutions, except the percentage of cuttings with callus. The genotypes showed great variability regarding rooting, especially the CMU 1004, CMU 1005, CMU 1006 and CMU 1014 with the highest percentages of rooted cuttings, ranging from 53 to 63%, while CMU 1016 and CMU 1022 showed the lowest rooting rates (5%). There was a significant interaction between genotypes x rooting solutions for volume, fresh weight and dry weight of roots. However, the factorial *versus* control contrast was not significant; thus, the use of rooting solutions does not influence the rooting of guarana cuttings.

Keywords: Vegetative propagation, IBA, NAA, 2,4-D, *Paullinia cupana* Kunth.

1. Introduction

Guarana (*Paullinia cupana* Kunth) can be propagated both sexually (seeds) and asexually (cuttings). However, sexual propagation is not commercially viable due to disadvantages such as high variability, susceptibility to diseases, rapid loss of viability (recalcitrant seeds), in addition to high heterozygosity, which increases gene segregation and consequent loss of desirable genetic traits (Arruda et al., 2007; Atroch et al., 2007).

Plant propagation from cuttings is a method that consists of removing plant segments such as roots, stems, leaves, buds, parts with meristems and even petioles which, under suitable conditions, can emit roots and form a new plant identical to its parent (Lu et al., 2008; Hartmann et al., 2011). Thus, the cutting technique has many advantages, for instance, maximizing the number of seedlings per time, allowing a single matrix plant to generate several identical individuals (clones) at low cost and easy execution, besides producing crop uniformity and enabling a more efficient selection when compared to sexual reproduction (Neves et al., 2006).

In recent years, there has been a great evolution in research aimed at maximizing the rooting of cuttings from guarana clones. A classification of guarana genotypes was proposed by Atroch & Nascimento Filho (2005), regarding the rooting potential of their cuttings: a) easy rooting (above 80%); b) intermediate rooting (around 50%); and c) low rooting (13% to 30%). Research has shown that the increase in IBA doses inhibits the rooting of guarana cuttings (Atroch et al., 2007).

For research purposes, auxins, cytokinins and gibberellins are among the most well-known plant hormones of interest in the vegetative propagation of plants, (Fronza & Hamann, 2015). Auxin and ethylene interact in a complex signaling network on the formation and development of adventitious roots where lower concentrations of auxin stimulates root formation during earlier

induction stages, whereas high concentration of auxin negatively impact root emergence and growth in a dependence of ethylene with inhibition of cell elongation genes (Bai et al. 2020). Thus, depending on concentration, exogenous application of auxin can favor the emission of roots in cuttings, mainly for species of difficult rooting (Hartmann et al., 2011). The main auxins used in plant propagation are the natural auxin IAA (indoleacetic acid) and the synthetic auxins IBA (indolebutyric acid), NAA (naphthaleacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) (Fronza & Hamann, 2015).

Indolebutyric acid (IBA) is the preferred synthetic auxin for the hormonal treatment of cuttings, and the most effective for rooting of a large number of plants, as it has the ability to promote the formation of root primordia and is chemically stable for a longer period (Fronza & Hamann, 2015).

Naphthalene acetic acid (NAA) is part of the group of synthetic auxins that have considerable agricultural importance, being used in several techniques for rooting plants, including *in vitro* cultivation protocols (Mercier, 2012). Weaver (1982) points out that although NAA can induce root formation in some species, sometimes even better than IBA, it can also cause undesirable effects as it is usually toxic to plant tissues. The use of NAA promoted rooting of water apple (*Syzygium javanica* L.) cuttings (Paul & Chaudhuri, 2009) and azalea (*Rhododendron simsii* Planch.) (Mauad et al., 2004), however in rapid treatment with Atemoya cv. Gefner had the lowest rooting averages, differing from IBA and 2,4-D.

2,4-dichlorophenoxyacetic acid (2,4-D) is one of the growth regulators that has activity like auxins in promoting lateral rooting and inhibition of root stretching (Simon & Petrášek, 2011). According to Oliveira Junior et al. (2011), 2,4-D has high rooting activity, because it can stimulate plant development in much smaller doses than IBA and NAA. However, the product is commercially registered as an herbicide (MAPA, 2019), and is toxic to the cuttings in high concentrations, inducing the formation of thick and stunted roots.

As rooting inducer, 2,4-D has been successfully used in St. John's wort (*Hypericum perforatum*) (Lu et al., 2008) and in cuttings of Atemoya (*Annona cherimola* Mill. X *Annona squamosa* L.) stems in rapid immersion (Ferreira & Ferrari, 2010), however, in grapevine (*Vitis* spp.) 2,4-D did not promote the formation of adventitious roots (Tofanelli et al., 2014).

Research has been devoted to increase rooting of guarana cuttings with IBA (Atroch et al., 2007), however, with current results, it is evident that the use of rooting solutions in guarana cuttings still need to be explored, mainly in relation to the effect of different substances in each one of the most promising clones, aiming at improving rooting and allow the commercial propagation of different clones. Studies on rooting of guarana cuttings treated with NAA and 2,4-D are nonexistent, especially in clones with high production, which subsides the development of technologies for the commercial propagation of the species. The objective of this work was to evaluate the effect of IBA, NAA and 2,4-D solutions on the rooting of cuttings from 16 guarana genotypes.

2. Material and methods

Guarana cuttings of 15 to 20 cm in length were collected in June 2019 from herbaceous branches released in the year, in parent plants grown in the experimental field of Jayoro Farm, located on BR 174, Km 120, in the municipality of Presidente Figueiredo - AM (1º 34’ 47” S and 60º 9’ 60” W). The preparation of cuttings, treatments, growing conditions, and evaluations were carried out at Embrapa Western Amazon, at the km 29 of the AM - 010 road, in the municipality of Manaus - AM(2º 53’ 25” S and 59º 58’ 06” W). The average altitude is 50 m and the annual average temperature is 25.6 °C, with an average annual rainfall of 2200 mm. The climate is of the type Af- humid tropical or equatorial climate, according to the Köppen-Geiger classification.

Cuttings were transported to seedling production nursery of Embrapa. The preparation of the substrate and cuttings was carried out following the same procedures described by Pinto et al. (2020). The treatment occurred by immersion for 10 s (quick immersion) in each of the three growth

regulators indolebutyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), and naphthalene acetic acid (NAA), all at the dose of 2 g L^{-1}. The nursery conditions consisted of a shade screen with light interception capacity reduced of 70% and irrigation through intermittent mist. The nursery beds contained a 10% slope, with a stone layer of 10 cm thick, to facilitate drainage and prevent the soaking of the bags.

A randomized block design was used with 48 treatments in a 16 x 3 factorial + 1 additional treatment (mean of 16 clones without rooting solutions), corresponding to cuttings from 16 guarana clones (BRSMaués, CMU1002, CMU1004, CMU1005, CMU1006, CMU1008 , CMU1009, CMU1010, CMU1012, CMU1013, CMU1014, CMU1015, CMU1016, CMU1017, CMU1018 and CMU1022), distributed in three blocks with 10 cuttings per clone. After 90 days in the nursery, the cuttings were removed from the substrate by washing the roots in running water using a 4 mm sieve to prevent loss of roots. Then, the cuttings were placed separately in plastic trays, identified, and transported to the Genetic Resources Laboratory of Embrapa Western Amazon, where the intact root system was collected to note the following characteristics: Rooted cuttings (RC %): only cuttings that emitted at least one adventitious root were considered to be rooted; Cuttings with Callus (CC %): live cuttings, with undifferentiated cell mass formation at the base and without roots, were considered cuttings with callus; Dead Cuttings (DC %) - dead cuttings were those with necrotic tissue; Root number (RN) - was obtained by counting the number of formed adventitious roots; root length RL (cm) - was measured using a millimeter ruler (± 0.05 mm) from the emergence zone to the end of the longest root; Root volume (RV) (mL) - was measured by the water displacement caused by the introduction of the roots in a graduated cylinder; Root Fresh Weight (RFW) (g) - was obtained by weighing the roots in a precision digital scale Shimadzu® (± 0.005 g), after collecting the root system; Root Dry Weight (RDW) (g) - was obtained by weighing the roots, after drying in an oven with forced air circulation at a temperature of 65 ºC until reaching constant mass, for 72 h.

The results obtained were subjected to analysis of variance and F test, for the sources of variation genotypes, rooting solutions, interaction between these factors and for contrast factorial versus control. For the significant sources of variation, the Tukey multiple comparison test was carried out at 5% probability. Data was transformed to $\sqrt{x}$ + 0.5 for the variables derived from the percentage of rooted cuttings, cuttings with callus and dead cuttings. The software GENES (Cruz, 2016) was used in the statistical analyzes.

3. Results and Discussion

Tables 1 and 2 show the results of the analysis of variance of the studied factors in guarana cuttings, that is, the combination of three different rooting solutions and 16 genotypes plus an additional control (without rooting solutions).

The rooting solutions factor was not significant for percentage of cuttings with callus (CC), and it showed significant differences for the percentage of rooted cuttings (RC) and percentage of dead cuttings (DC) at 1% probability by the F Test. The genotype factor), differed significantly at 5% and 1% probability for the three characteristics evaluated (CC, RC and DC) (Table 1). The interaction between rooting solutions and genotypes was not significant for the three variables under analysis. The contrast between the factors and the additional control was also not significant by the F Test for the variables rooted cuttings (RC), cuttings with callus (CC) and dead cuttings (DC) (Table 1). This indicates that the means of rooting solutions x genotypes factorial were not different from the means of not applying rooting solutions (additional treatment), that is, the non-application of rooting solutions causes the same effect of the application for the presented variables.

Table 1. Anova summary for rooted cuttings (RC), cuttings with callus (CC) and dead cuttings (DC) of guarana as a function of different genotypes, with and without rooting solutions.

Sources of Variation	d.f.	Mean Squares		
		RC	CC	DC
Blocks	1	1.938^{ns}	3.266^{ns}	0.001^{ns}
Rooting solutions (S)	2	41.101**	3.492^{ns}	18.789**
Genotype (G)	15	23.001**	8.997**	15.411**
Interaction (S x G)	30	2.976^{ns}	1.552^{ns}	2.162^{ns}
Factorial vs Additional	1	0.004^{ns}	0.000^{ns}	0.117^{ns}
Residual	48	2.765	2.715	1.320
Factorial Average		5.42	2.06	7,32
Additional Average		5.46	2.07	7.08
C.V. (%)		36.92	97.22	27.32

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

The genotype factor was significant at 1% probability by the F Test for the characteristics root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry weight (RDW). On the other hand, except for RN and RL, all variables were significantly influenced by the different rooting solutions (Table 2). There was significant difference in the interaction among the factors only for root volume (RV), root fresh weight (RFW) and root dry weight (RDW). For the other variables evaluated, there was no effect of interaction between the factors (Table 2). None of the observed variables had significant effect in the contrast factorial versus additional control, that is, the means of the factorial rooting solutions x genotypes were not different from the means of not applying rooting solutions (Table 2).

Table 2. Anova summary for root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry weight (RDW) of guarana cuttings as a function of different genotypes, with and without rooting solutions.

Sources of Variation	d.f.	Mean Squares				
		RN	RL	RV	RFW	RDW
Blocks	1	0.642^{ns}	193.679*	3.972*	8.470**	0.209**
Rooting Solutions (S)	2	6.173^{ns}	128.003^{ns}	3.305*	4.076**	0.093*
Genotypes (G)	15	11.503**	207.156**	4.664**	4.612**	0.124**
Interaction (S x G)	30	5.535^{ns}	36.250^{ns}	1.748**	1.651**	0.043**
Factorial vs Additional	1	0.872^{ns}	3.054^{ns}	0.154^{ns}	0.136^{ns}	0.003^{ns}
Residual	48	3.647	41.581	0.744	0.643	0.019
Factorial Average		3.60	14.06	1.97	1.88	0.29
Additional Average		2.94	12.82	1.69	1.62	0.32
C.V. (%)		62.31	18.85	88.37	92.22	536.99

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

Table 3 shows that the solutions IBA and NAA did not differ for any of the characteristics studied by the Tukey test at 5% probability. The average of rooted cuttings (RC) was 46.6% for the NAA treatment, which did not differ from IBA, and was approximately 25% higher than 2,4-D, with 21.9%. For the percentage of cuttings with callus (CC) there was no difference among the evaluated regulators. As for the percentages of dead cuttings (DC), those treated with 2,4-D had the highest mortality, with 68.7% (Table 3). The mortality was influenced by the 2,4-D, which at the concentration used (2 g L^{-1}), symptoms of toxicity were observed in the cuttings, with the presence of necrotic tissues, while regulators IBA and NAA promoted the formation of adventitious roots.

Table 3. Percentage of rooted cuttings (RC), cuttings with callus (CC) and dead cuttings (DC) of guarana in response to three rooting solutions.

Rooting solutions	Means*		
	RC (%)	CC (%)	DC (%)
IBA	38.4 a	6.3 a	55.3 b
NAA	46.6 a	5.6 a	47.8 b
2,4–D	21.9 b	9.4 a	68.7 a

*Means followed by the same letters, in column, do not differ by the Tukey test at 5% probability.

The results agree with those obtained for other species. Silva et al. (2012), working with *Melaleuca alternifolia*, found that the use of IBA and NAA in the concentration of 2 g L^{-1} for 10 s, did not differ statistically, obtaining an average of 49.3% of rooted cuttings, which did not compromise the survival of the cuttings for the species.

In a study with grapevine rootstocks, the mean rooting of the concentrations (0, 1, 2 and 3 g L^{-1}) of 2,4-D was 51.1%, lower than the mean of IBA (84.2%), while cuttings treated with 2,4-D showed the highest mortality, with 47.2%, in relation to the 15.6% of IBA (Tofanelli et al. 2014).

In contrast, rapid immersion of atemoya cv. 'Gefner' cuttings in IBA, NAA and 2,4-D promoted an average rooting of 91% in cuttings treated with IBA and 2,4-D, regardless of concentration, differing from NAA. On the other hand, 2,4-D in the concentrations of 2 and 4 g L^{-1} promoted lower percentages of calli, differently from IBA and NAA, with the highest averages (Ferreira and Ferrari, 2010).

The treatment of *Cordia trichotoma* by rapid immersion (10 s) in concentrated solutions of IBA and NAA resulted in low rooting rate of cuttings, whereas IBA in the concentration of 8 g L^{-1} promoted the best rooting (28.9%), however, IBA and NAA at such high dose resulted in increased mortality of the cuttings (32.3%) (Faganello et al., 2015).

Pimenta et al. (2007) treated cuttings from six species of *Lippia* with IBA, NAA and 2,4-D, and found that 2,4-D besides not promoting the emission of adventitious roots caused death of 100% of the treated cuttings. These results can be attributed to the effect of 2,4-D as an herbicide. Unlike natural auxins, which are rapidly inactivated in pant tissues, 2,4-D can last longer and upregulate genes of ABA and ethylene biosynthesis, resulting in herbicide-related symptoms such as growth abnormalities, increased ROS production leading to senescence and tissue death (Song, 2014).

The treatment of cuttings with IBA at the concentration of 2 g L^{-1} is the standard vegetative propagation method used for the production of guarana seedlings (Pinto et al., 2020). However, research regarding rooting of cuttings treated with NAA and 2,4-D is non-existent for this species.

Arruda et al. (2007), using IBA at 2 g L^{-1}, found that the highest average rooting of guarana was 88.1%, regardless of the substrate used. On the other hand, Pinto et al. (2020), obtained the highest percentage of adventitious roots (75%) using the concentration of 4 g kg^{-1} of IBA, but the authors also agreed that the application of the highest dose of IBA on the basis of the guarana cuttings influenced their mortality.

Table 4 shows that different rooting solutions did not influence root number (RN) and root length (RL). For root volume (RV), root fresh weight (RFW) and root dry weight (RDW), the best results were found in cuttings treated with IBA, with significant difference only in relation to the 2,4-D.

Table 4. Root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry weight (RDW) of guarana cuttings in response to three rooting solutions.

Rooting solutions	Means*				
	RN	RL (cm)	RV (mL)	RFW (g)	RDW (g)
IBA	4.03 a	15.30 a	2.22 a	2.20 a	0.36 a
NAA	3.62 a	15.13 a	2.0 ab	1.96 ab	0.35 ab
2,4-D	3.16 a	11.76 a	1.60 b	1.50 b	0.26 b

*Means followed by the same letters, in column, do not differ by the Tukey test at 5% probability.

Silva et al. (2019b) obtained significant difference when comparing concentrations from 0.25% (w/v) to 1% (w/v) of IBA and NAA applied as talcum powder on the quality of the root system in three species of the genus *Annona*, IBA treated cuttings had longer roots than NAA immersed ones. In *Cordia trichotoma* cuttings, rapid immersion (10 s) in IBA resulted in longer roots (3.7 cm) in comparison to NAA (1.2 cm), and the IBA at the concentration of 8 g L^{-1} promoted highest root number, with an average of 4 roots per cutting (Faganello et al., 2015). This difference between IBA and NAA was not observed in this work, probably because of the distinct application method and

concentration, which vary with many factors, including species. In jabuticaba (*Plinia cauliflora*) cuttings, only the two highest concentrations of a range from 0, 1, 3, 5 and 7 g L^{-1} of IBA and NAA were the best for improve root number and length.

In a study with 2,4-D as an alternative to IBA in vine rootstocks, Tofanelli et al. (2014), showed undesirable effects of 2,4-D in the concentrations 1, 2 and 3 g L^{-1}, for root number and length, highlighting its herbicidal effect when applied in supra-optimal doses. The cellular auxin level is tightly regulated via biosynthesis, transport, degradation and conjugation (Taiz et al., 2017), and the commercial use of 2,4-D as a stable form of auxin with the purpose of promoting growth and cell elongation has to be well controlled in terms of dose. Even at low doses, 2,4-D presents a distinct metabolic turnover via amino acid conjugation when compared to endogenous auxins, and their conjugated metabolites can also inhibit growth (Eyer et al., 2016). Silva et al. (2012), working with *Melaleuca alternifolia*, demonstrated that the IBA in concentrations 1, 2 and 4 g L^{-1} promoted greater root volume (0.36 mL) in relation to cuttings treated with NAA (0.16 mL). Moreover, the use of IBA in the concentration of 4 g L^{-1} provided greater length (57.71 cm) and root dry mass (42.98 mg), in relation to the same concentration of NAA. When testing different concentrations of IBA, Pinto et al. (2020) observed that the increase in IBA doses directly influenced the quality of the root system of guarana cuttings, providing longer roots, larger root volume and better accumulation of root dry matter.. Exogenous application of auxins can promote endogenous IAA synthesis in cuttings and induce adventitious rooting, in addition to differentiation of calli to root primordia (Quan et al., 2022). IBA effectiveness in promoting rooting is associate with its stability to degradation and its capacity to be converted into IAA to induce adventitious roots (Frick & Strader, 2018). The same is not true for NAA, since it has been shown that exogenous NAA can reduce endogenous IAA concentration in red-stem cuttings of *Camellia sinensis* with corresponding downregulation of NAA-induced IAA and *GH3* genes (Wang et al., 2022). As for the concentrations applied, Sourati et al. (2022) observed better rooting parameters in mulberry (*Morus alba*) cuttings treated with NAA at 200 mg L^{-1} and IBA at 200-400 mg L^{-1}, while Yan et al. (2014) reported stimulus on rooting percentage, root number and RDW in whip grass (*Hemarthria compressa*) cuttings immersed in increasing NAA concentrations up to 200 mg L^{-1} with clear inhibition at higher concentrations. These findings reinforce the idea that IBA usually works at a broader range of concentrations as compared to NAA and 2,4-D, which might be due to its chemical similarity to IAA, with possible faster and more efficient interconversion in active endogenous auxin, as well as degradation and transport routes.

The highest results of rooted cuttings (RC) were observed in the genotypes CMU 1004, CMU 1005, CMU 1006 and CMU 1014, with percentages above 53%, being classified as intermediate rooting genotypes (around 50%), differing statistically only from CMU 1012, CMU 1013, CMU 1016 and CMU 1022, which had low rooting (from 10% to 30%), with the highest averages of dead cuttings confirming the rooting difficulties of these genotypes (Table 5).

Among the evaluated genotypes, 40% of rooting was registered for BRS Maués, which differed from the results obtained by Albertino et al. (2012), who obtained an average of 81.3% of rooted cuttings and from those found by Arruda et al. (2007) (71.9%) for the same cultivar rooted in commercial substrate (Plantmax®), and 79.6% when rooted in chicken manure + soil substrate, a growth condition closer to that used in this work. These discrepancies suggest that the rooting percentage of this genotype is greatly influenced by edaphoclimatic and nursery management conditions. In general, the rooting of guarana cuttings was significantly influenced by the genotypes, with variations from 5% to 63.3% in rooting.

Table 5. Percentage of rooted cuttings (RC), cuttings with callus (CC) and dead cuttings (DC) of 16 guarana genotypes.

Genotypes	Means*		
	RC (%)	CC (%)	DC (%)
BRS Maués	40.0 abc	6.7 ab	53.3 bcdef
CMU 1002	46.7 abc	6.7 ab	46.7 cdef
CMU 1004	63.3 a	5.0 ab	31.7 f
CMU 1005	53.3 a	16.7 ab	30.0 ef
CMU 1006	58.3 a	6.7 ab	35.0 def

Genotypes	Means*		
	RC (%)	CC (%)	DC (%)
CMU 1008	50.0 ab	13.3 ab	36.7 def
CMU 1009	20.0 abcde	0.0 b	80.0 abc
CMU 1010	28.3 abcd	15.0 ab	56.7 abcde
CMU 1012	15.0 cde	1.7 ab	83.3 ab
CMU 1013	16.7 bcde	1.7 ab	81.7 abc
CMU 1014	53.3 a	0.0 b	46.7 cdef
CMU 1015	28.3 abcd	5.0 ab	66.7 abcd
CMU 1016	5.0 de	0.0 b	95.0 a
CMU 1017	43.3 abc	21.7 a	35.0 def
CMU 1018	43.3 abc	13.3 ab	43.3 def
CMU 1022	5.0 e	0.0 b	95.0 a

*Means followed by the same letters, in column, do not differ by the Tukey test at 5% probability.

Such results are corroborated by the studies of Pinto et al. (2020), who observed variations from 31.5% to 57% among three tested genotypes. Results obtained by Albertino et al. (2012), show variations between six cultivars, with averages ranging from 49.3% to 70.3%. For eleven evaluated genotypes, Atroch et al. (2007), registered variations from 16.6% to 85.2%, concluding that there is genetic variability for the rooting percentage. Other works showed even larger variation in rooting, such as the one by Arruda et al. (2007), ranging from 15.0 % to 88.1%, for twelve guarana cultivars. The influence of genotype on the formation of adventitious roots is also observed for other species such as tea plant (*Camellia sinensis*) , with variations from 31.9% to 41.6% (Lima et al., 2016), and peach (*Prunus persica*), ranging from 13.3% to 66.7% for the percentage of rooted cuttings (Da Rosa et al., 2017).

Some evaluated genotypes demonstrated difficulties in forming adventitious roots, such as CMU 1012, CMU 1013, CMU 1016 and CMU 1022, even using synthetic auxins to stimulate the development of the root system (Table 5). These difficulties are one of the main factors limiting the use of cuttings to produce guarana seedlings. The factors associated with the rhizogenesis of cuttings of a plant species are of endogenous origins, such as plant age, content of sugars, minerals and other molecules and exogenous, such as light, temperature, additional growth regulators, which act as signals that redirect cell differentiation, cell division, initiation of root primordia, emergence and elongation (Bellini et al. 2014). Ranjan et al. (2022) studying molecular bases for genotypic differences in rooting of two tree clones (hybrid poplar and hybrid aspen) observed that easiness of rooting was related to increased expression of genes encoding reactive oxygen species scavenging proteins, especially from the peroxidase and glutathione-S-transferase superfamilies, while difficulty of rooting was related to up-regulation of jasmonate producing genes in the cambium cells.

For cuttings with callus (CC), the CMU 1017 showed the highest percentage (21.7%) among the evaluated genotypes. However, comparing with the other genotypes, there was difference among CMU 1009, CMU 1014, CMU 1016 and CMU 1022, which had no callus development (Table 5).

In the present study, there was a variation among all tested genotypes from 0 to 21.7% of cuttings with callus, a range that was lower than those reported by Pinto et al. (2020) (from 6.5% to 58.5%) in different guarana cultivars.

Upon removal from the parent plant, the stem tissue at the base of the cuttings suffers a process of histological healing. In this region, a mass of disorganized and poorly differentiated parenchymal cells is often formed, called callus (Scariot et al., 2017). In some plant species, the formation of callus can be a precursor of rooting, however, it is an independent process, which will not essentially lead to the formation of adventitious roots (Mendonça et al., 2018). Moreover, the presence of callus in cuttings is associated with species that are difficult to root, being considered an indirect organogenesis process, since the formation of adventitious roots occurs after the formation of callus (Xavier et al. 2013).

Genotypes with higher percentages of cuttings with callus could still form root system if they remained in the substrates for a longer period (Scariot et al., 2017). According to Hartmann et al.

(2011), another factor influencing the rooting of cuttings is the internal structure of the stem, considering that the formation of adventitious roots in cuttings of plants with secondary growth usually originate from the young tissue of the secondary phloem, but they can also originate from the cells of the interfascicular cambium, between vascular bundles (Naija et al., 2008), from the cambium or from callus produced at the base of the cuttings.

The genotypes CMU 1016 and 1022 had the highest percentages of dead cuttings (DC), both with 95%, being the least suitable for propagation by cuttings, as they obtained the worst results in terms of survival (Table 5). Evaluating the percentage of dead cuttings for different guarana genotypes, Albertino et al. (2012), observed that the average mortality varied from 6.5 to 48.8%, and the cultivar BRS Maués presented 26.7% mortality, differing from what was found in this study, which was 53.3% of dead cuttings. Results obtained by Pinto et al. (2020) corroborate with the influence of the genotype factor on mortality of cuttings, with variations from 10.5 to 45% of dead cuttings among guarana cultivars.

For other species, Lima et al. (2016) observed that the average mortality of the cuttings was directly influenced by the genotypes of *Camellia sinensis* L. (from 24.6% to 35.7% of dead cuttings), while in grapevine rootstocks the percentage of mortality varied from 37.5% to 60.8% in the treatment with 2,4-D, and from 11.7% to 19.2% when treated with IBA (Tofanelli et al. 2014).

Table 6 shows that the root number (RN) per cutting differed statistically between the clones CMU 1014 and CMU 1016 at the highest and lowest range, respectively. The CMU 1022, had shorter roots in number, and significantly lower in comparison to CMU 1006, 1009 and 1014, while other genotypes did not differ in relation to the studied trait.

In adventitious rooting, the number of roots is an important data, since the vigor of the seedlings is directly related to the number of roots. According to Rima et al. (2011), the greater amount of physiologically active roots determines larger root surface area, reflecting an increase in the volume of soil to be explored, which may increase the activity of water and nutrient absorption, as well as having positive influences on production, and greater ability of plants to adapt to the environment under adverse conditions (Borcioni et al. 2016) especially when transplanting the seedling to the field.

Pinto et al. (2020) reported that there is an influence of cultivar on the root number in guarana, with a variation from 8.7 to 21.95. Results obtained by Albertino et al. (2012), also show variations in guarana genotypes, ranging from 4.73 to 15.96, with fertilization, a planting condition closer to that used in this work, and the cultivar BRS Maués presented an average amount of roots of 9.19, different from what was found in this work, an average of 4.12 roots per cutting (Table 6).

As for root length (RL), the clone CMU 1006 showed the best average (21.52 cm), differing statistically only from the clones CMU 1016, CMU 1018 and CMU 1022, with 3.83 cm; 7.52 cm and 1.17 cm, respectively (Table 6). Longer roots developed by cuttings allows deeper exploitation of the soil, to improve the absorption of water and nutrients, allowing for the development of new leaves in cuttings. Another factor influencing the root length in cuttings is the genetic potential of each cultivar (Da Rosa et al., 2017; Dawa et al., 2018). According to Ferraz et al. (2018), genetic potential, besides the endogenous hormonal balance and tissue consistency of the cuttings are essential for root growth and development.

Previous research with guarana cuttings also shows that the genotype factor influenced the root length. For the cultivar BRS Maués, the average length of the longest root was 11.68 cm, smaller than the average length found in this work (17.02 cm of roots per cutting) (Albertino et al., 2012). Pinto et al. (2020) also reported an influence of cultivar on the length of guarana roots, ranging from 12.31 to 18.86 cm.

Table 6. Root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry weight (RDW) of cuttings from 16 guarana genotypes.

Genotype	Means*				
	RN	RL (cm)	RV (mL)	RFW (g)	RDW (g)
BRS Maués	4.12 abc	17.02 abc	2.17 ab	2.58 abc	0.37 ab
CMU 1002	3.17 abc	12.67 abcd	1.72 bcd	1.65 abcde	0.26 bcd
CMU 1004	4.52 ab	16.83 abc	2.17 ab	2.01 abcd	0.35 abc
CMU 1005	2.33 abc	16.37 abc	2.12 abc	1.96 abcd	0.34 abc
CMU 1006	4.88 ab	21.52 a	3.53 a	3.23 a	0.57 a
CMU 1008	3.18 abc	13.15 abcd	1.35 bcd	1.10 cde	0.23 bcd
CMU 1009	4.93 ab	18.90 ab	1.92 abc	1.78 abcd	0.39 ab
CMU 1010	3.18 abc	15.45 abc	2.72 ab	2.70 abc	0.47 ab
CMU 1012	4.12 abc	13.87 abcd	2.27 ab	2.15 abc	0.38 ab
CMU 1013	4.05 abc	19.88 ab	2.73 ab	2.33 abc	0.41 ab
CMU 1014	5.68 a	19.90 ab	2.82 ab	3.11 ab	0.43 ab
CMU 1015	4.03 abc	17.35 ab	2.35 ab	2.16 abc	0.43 ab
CMU 1016	1.17 bc	3.83 cd	0.33 cd	0.38 de	0.07 cd
CMU 1017	3.40 abc	9.60 abcd	1.57 bcd	1.53 bcde	0.22 bcd
CMU 1018	4.48 ab	7.52 bcd	1.63 bcd	1.43 cde	0.26 bcd
CMU 1022	0.38 c	1.17 d	0.07 d	0.04 e	0.01 d

*Means followed by the same letters, in column, do not differ by the Tukey test at 5% probability.

The clone CMU 1006 had the highest mean root volume (RV) (3.53 mL) when compared to the CMU 1002, CMU 1008, CMU 1016, CMU 1017, CMU 1018 and CMU 1022 genotypes (Table 6).

Aiming to improve the quality of the root system of guarana cuttings, Pinto et al. (2020) tested the combination of three different clones with doses of IBA and found significant effect of the evaluated genotypes on the root volume, which varied from 1.10 to 2.35 mL. Albertino et al. (2012), in another study with guarana cuttings, observed that the root volume varies according to the genotype, from 2.45 to 3.31 mL. These results corroborate the results of this research, with an average of 0.07 to 3.53 mL of roots per cutting. In this sense, the need to apply exogenous regulators varies with the genetic characteristics of the plant material, and the younger the tissues to be propagated, the higher the endogenous levels of auxin and lower those of gibberellin, a condition that favors the rooting of stem cuttings (Botelho et al., 2005).

Root weight was influenced by the genotypes, with variation between the characteristics evaluated. The genotypes CMU 1016, and CMU 1022 provided the worst results for root fresh weight (RFW) and dry weight (RDW) when compared to the clones BRS Maués and CMU 1006 and CMU1009-CMU1015 (Table 6). CMU 1006 was also superior in terms of root mass, differing significantly from these two worst genotypes, besides CMU 1008, 1016, 1017 and 1018.. According to Albertino et al. (2012), the guarana genotypes evaluated influenced the root dry matter, varying from 0.39 g to 0.57 g, and the cultivar BRS Maués, presented an average of 0.44 g of root accumulation, a different result to that found in this work, with 0.37 g of roots per cutting.

In Table 7, Anova results show a significant effect at 5% and 1% probability among the genotypes within all rooting solutions by the F Test, for the variables root volume (RV), root fresh weight (RFW) and root dry weight (RDW). The factor rooting solutions was significant at 5% and 1% probability by the F Test, whereas the factorial versus additional control was not significant among the variables observed (Table 7). This means that the factorial rooting solutions x genotypes was not different from the non-application of regulators (additional treatment).

Table 7. Anova summary for root volume (RV), root fresh weight (RFW) and root dry weight (RDW) of guarana cuttings as a function of different genotypes within rooting solutions.

Sources of Variation	d.f.	Mean Squares		
		RV	RFW	RDW
Blocks	1	3.972*	8.470**	0.209**
Rooting Solutions	2	3.305*	4.076**	0.093*
Genotypes within 2,4-D	15	3.191**	2.938**	0.077**
Genotypes within IBA	15	2.626**	2.752**	0.068**
Genotypes within NAA	15	2.343**	2.224**	0.065**
Factorial vs Additional	1	0.154^{ns}	0.136^{ns}	0.003^{ns}
Residual	48	0.744	0.643	0.019
Factorial Average		1.97	1.88	0.33
Additional Average		1.69	1.62	0.29
C.V. (%)		88.37	92.22	536.99

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

In the results for root volume (RV) (Table 8) significant effect was observed by the F Test between the rooting solutions within the clones CMU 1004, CMU 1006 and CMU 1017 at 5% probability and the CMU 1012 and CMU 1013 genotypes at 5% and 1% probability.

When analyzing the effect of rooting solutions within each of the clones, solutions affected significantly root fresh weight (RFW) in the clones CMU 1002, CMU 1004 and CMU 1006 at 5% probability and CMU 1012 and CMU 1013 at 5% and 1% probability. However, for root dry weight (RDW), significance was observed only between rooting solutions within the CMU 1012 and CMU 1013 genotypes at 1% probability (Table 8).

The factor genotype was significant at 5% and 1% probability by the F Test, while, for the factorial versus additional control, there was no significance for the observed characteristics (Table 8), i.e., the means of the factorial rooting solutions x genotypes was not different from those averages of non-application of the solutions (additional treatment).

Table 8. Anova summary for root volume (RV), root fresh weight (RFW) and root dry weight (RDW) of guarana cuttings as a function of different rooting solutions within genotypes.

Sources of Variation	d.f.	Mean Squares		
		RV	RFW	RDW
Blocks	1	3.972*	8.470**	0.209**
Genotypes	15	4.664**	4.612**	0.124**
Solutions within BRS Maués	2	0.632^{ns}	0.939^{ns}	0.023^{ns}
Solutions within CMU 1002	2	2.082^{ns}	2.135*	0.039^{ns}
Solutions within CMU 1004	2	2.412*	2.733*	0.055^{ns}
Solutions within CMU 1005	2	0.062^{ns}	0.020^{ns}	0.007^{ns}
Solutions within CMU 1006	2	3.022*	2.523*	0.034^{ns}
Solutions within CMU 1008	2	0.015^{ns}	0.046^{ns}	0.002^{ns}
Solutions within CMU 1009	2	1.752^{ns}	1.442^{ns}	0.053^{ns}
Solutions within CMU 1010	2	0.687^{ns}	0.363^{ns}	0.033^{ns}
Solutions within CMU 1012	2	7.712**	6.940**	0.213**
Solutions within CMU 1013	2	5.952**	6.472**	0.149**
Solutions within CMU 1014	2	0.302^{ns}	1.071^{ns}	0.018^{ns}
Solutions within CMU 1015	2	0.485^{ns}	0.283^{ns}	0.029^{ns}
Solutions within CMU 1016	2	0.572^{ns}	0.829^{ns}	0.027^{ns}
Solutions within CMU 1017	2	2.502*	1.917^{ns}	0.030^{ns}
Solutions within CMU 1018	2	1.307^{ns}	1.118^{ns}	0.027^{ns}
Solutions within CMU 1022	2	0.027^{ns}	0.007^{ns}	0.000^{ns}
Factorial vs Additional	1	0.154^{ns}	0.136^{ns}	0.003^{ns}
Residual	48	0.744	0.643	0.019
Factorial Average		1.97	1.88	0.33
Additional Average		1.69	1.62	0.28
C.V. (%)		88.37	92.22	536.99

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

As for the root volume (mL) (Table 9), the cuttings treated with IBA and NAA presented the largest volumes for the evaluated genotypes, when compared with cuttings treated with 2,4-D, except for the CMU 1013, which was superior with the use of 2,4-D.

In general, when analyzing the volume of the root system of the 16 genotypes using the three growth regulators, the CMU 1016 and CMU 1022 clones obtained the worst results, with 0.33 and 0.07 mL, respectively while the CMU 1006 had the largest root volume (3.53 mL) (Table 9).

Pinto et al. (2020) also observed a significant interaction between clones x IBA in guarana cuttings, and regardless of the cultivar tested, the maximum root volume was reached with the highest dose of IBA, with an increase of around 38% of the IBA.

In contrast, studies with IBA and NAA treatment in *Melaleuca alternifolia* showed that root volume of IBA treated cuttings was higher than NAA, independent of concentration, and provided cuttings with better conditions for maintaining viability of the seedlings (Silva et al., 2012).

Table 9. Root volume (RV) of guarana in response to 16 different genotypes and three rooting solutions.

Genotypes	RV (mL)*			
	IBA	NAA	2,4-D	Genotype Average
BRS Maués	2.30 abcA	1.55 abcA	2.65 abcA	2.17 ab
CMU 1002	2.60 abcA	1.95 abcA	0.60 bcA	1.72 bcd
CMU 1004	2.85 abcA	2.75 abcA	0.90 bcA	2.17 ab
CMU 1005	1.95 abcA	2.10 abcA	2.30 abcA	2.12 abc
CMU 1006	4.50 aA	3.95 aAB	2.15 abcB	3.53 a
CMU 1008	1.40 abcA	1.25 abcA	1.40 abcA	1.35 bcd
CMU 1009	2.60 abcA	2.30 abcA	0.85 bcA	1.92 abc
CMU 1010	2.95 abcA	2.05 abcA	3.15 abA	2.72 ab
CMU 1012	3.35 abA	3.45 aA	0 cB	2.27 ab
CMU 1013	1.00 bcB	2.75 abcAB	4.45 aA	2.73 ab
CMU 1014	2.70 abcA	3.25 abA	2.50 abcA	2.82 ab
CMU 1015	2.75 abcA	2.50 abcA	1.80 abcA	2.35 ab
CMU 1016	0.95 bcA	0.05 cA	0.00 cA	0.33 cd
CMU 1017	2.85 abcA	1.05 abcA	0.80 bcA	1.57 bcd
CMU 1018	0.70 bcA	2.10 abcA	2.10 abcA	1.63 bcd
CMU 1022	0.00 cA	0.20 bcA	0.00 cA	0.07 d
Hormone Average	2.22 A	2.08 AB	1.60 B	1.97

*Means followed by the same letters, lowercase in column and uppercase in line do not differ by the Tukey test at 5% probability.

Taking the average root fresh weight (Table 10), it can be noted that the application of IBA and NAA in the concentration of 2 g L^{-1}, produced the best results of RFW (g) for all the genotypes, except for the CMU 1013, which presented the highest mass (4.22 g) with the use of 2,4-D. Among the evaluated genotypes, CMU 1022 showed a lower average root fresh weight, (0.04 g), indicating low potential for differentiation of adventitious roots.

Table 10. Root fresh weight (RFW) of guarana in response to sixteen different genotypes and three rooting solutions.

Genotypes	RFW (g)*			
	IBA	NAA	2,4-D	Genotype Average
BRS Maués	3.07 abA	1.80 abA	2.88 abA	2.58 abc
CMU 1002	2.50 abcA	1.95 abAB	0.50 bB	1.65 abcde
CMU 1004	2.88 abcA	2.47 abAB	0.68 bB	2.01 abcd
CMU 1005	1.98 abcA	2.08 abA	1.88 abA	1.96 abcd
CMU 1006	4.30 aA	3.33 aAB	2.06 abB	3.23 a
CMU 1008	1.27 bcA	0.98 abA	1.07 bA	1.10 cde
CMU 1009	2.55 abcA	1.92 abA	0.87 bA	1.78 abcd
CMU 1010	3.02 abA	2.22 abA	2.86 abA	2.70 abc
CMU 1012	3.13 abA	3.32 aA	0 bB	2.15 abc
CMU 1013	0.64 bcB	2.12 abB	4.22 aA	2.33 abc

Genotypes	RFW (g)*			
	IBA	NAA	2,4-D	Genotype Average
CMU 1014	3.25 abA	3.77 aA	2.32 abA	3.11 ab
CMU 1015	2.26 abcA	2.49 abA	1.75 abA	2.16 abc
CMU 1016	1.12 bcA	0.01 bA	0 bA	0.38 de
CMU 1017	2.66 abcA	1.08 abA	0.86 bA	1.53 bcde
CMU 1018	0.59 bcA	1.69 abA	2.01 abA	1.43 cde
CMU 1022	0.00 cA	0.11 bA	0 bA	0.04 e
Hormone Average	2.20 A	1.95 AB	1.50 B	1.88

*Means followed by the same letters, lowercase in column and uppercase in line do not differ by the Tukey test at 5% probability.

For root dry weight (RDW) most of the evaluated genotypes showed the same behavior, when analyzed the combined effect of the regulators IBA, NAA and 2,4-D, not differing from each other by the Tukey test at 5%, except for the CMU 1013 with lower RDW for IBA, obtaining values of 0.14 g and CMU 1012 for 2,4-D, which did not produce roots (Table 11).

The CMU 1013, CMU 1018 and CMU 1022 genotypes had the lowest average RDW when combined with IBA, obtaining values of 0.14 g, 0.13 g and 0, respectively. In relation to NAA, the CMU 1016 and CMU 1022 were lower with 0.01 g and 0.02 g. Within the 2,4-D regulator, the worst combinations were with the CMU 1002, CMU 1004, CMU 1012, CMU 1016, CMU 1017 and CMU 1022 genotypes.

In guarana cultivars, Pinto et al. (2020) found that the increase in IBA concentration provided the largest accumulation of RDW, and the dose of 2 g kg^{-1} of IBA, promoted a dry root weight of approximately 0.35 g/stem cutting, results similar to those obtained in this study, where the dose of 2 g L^{-1} of IBA showed 0.36 g of RDW on average.

Table 11. Root dry weight (RDW) of guarana in response to 16 different genotypes and three rooting solutions.

Genotypes	RDW (g)*			
	IBA	NAA	2,4-D	Genotype Average
BRS Maués	0.45 abcA	0.25 abcA	0.42 abA	0.37 ab
CMU 1002	0.35 abcA	0.33 abcA	0.10 bA	0.26 bcd
CMU 1004	0.47 abcA	0.43 abcA	0.16 bA	0.35 abc
CMU 1005	0.28 abcA	0.36 abcA	0.39 abA	0.34 abc
CMU 1006	0.71 aA	0.56 aA	0.45 abA	0.57 a
CMU 1008	0.27 abcA	0.23 abcA	0.21 abA	0.23 bcd
CMU 1009	0.45 abcA	0.52 abA	0.21 abA	0.39 ab
CMU 1010	0.59 abA	0.34 abcA	0.48 abA	0.47 ab
CMU 1012	0.53 abA	0.60 aA	0.00 bB	0.38 ab
CMU 1013	0.14 bcB	0.39 abcAB	0.69 aA	0.41 ab
CMU 1014	0.47 abcA	0.50 abcA	0.32 abA	0.43 ab
CMU 1015	0.43 abcA	0.55 aA	0.31 abA	0.43 ab
CMU 1016	0.21 abcA	0.01 cA	0.00 bA	0.07 cd
CMU 1017	0.36 abcA	0.18 abcA	0.13 bA	0.22 bcd
CMU 1018	0.13 bcA	0.30 abcA	0.36 abA	0.26 bcd
CMU 1022	0.00 cA	0.02 bcA	0.00 bA	0.01 d
Hormone Average	0.36 A	0.35 AB	0.26 B	0.32

*Means followed by the same letters, lowercase in column and uppercase in line do not differ by the Tukey test at 5% probability.

In the evaluation among the genotypes in the absence of growth regulators, there was a significant effect at 5% and 1% probability by the F Test for the variables rooted cuttings (RC) and dead cuttings (DC), while cuttings with callus (CC) were not significantly influenced by genotypes (Table 12).

Table 12. Anova summary for rooted cuttings (RC), cuttings with callus (CC) and dead cuttings (DC) of guarana as a function of different genotypes and without rooting solutions.

Sources of Variation	d.f.	Mean Squares		
		RC	CC	DC
Blocks	1	2.832^{ns}	2.634^{ns}	0.014^{ns}
Genotypes	15	16.188**	4.400^{ns}	11.304**
Residual	15	1.700	1.997	0.815
Average		5.46	2.07	7.08
C.V. (%)		23.87	68.18	12.75

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

There was no significant difference among genotypes in the absence of rooting solutions for the variables root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry weight (RDW) (Table 13).

Table 13. Anova summary for root number (RN), root length (RL), root volume (RV), root fresh weight (RFW) and root dry root weight (RDW) of guarana cuttings as a function of different genotypes and without rooting solutions.

Sources of Variation	d.f.	Mean Squares				
		RN	RL	RV	RFW	RDW
Blocks	1	3.638^{ns}	256.964*	5.561*	4.844^{ns}	0.109^{ns}
Genotypes	15	4.441^{ns}	63.903^{ns}	1.389^{ns}	1.382^{ns}	0.032^{ns}
Residual	15	2.934	51.361	0.791	1.086	0.029
Average		2.94	12.81	1.69	1.62	0.29
C.V. (%)		58.29	55.91	52.76	64.32	59.98

*Significant at 5% probability by the F Test; **Significant at 1% probability by the F Test; ns – non-significant.

The highest averages of rooted cuttings (RC) were presented by the genotypes CMU 1004, CMU 1005, CMU 1006 and CMU 1014, with 95%; 75%; 70% and 70%, respectively, differing statistically from the CMU 1012, CMU 1013, CMU 1015, CMU 1016 and CMU 1022 clones (Table 14).

Table 14. Percentage of rooted cuttings (RC), dead cuttings (DC) and cuttings with callus (CC) of guarana in response to 16 different genotypes without the use of rooting solutions.

Genotypes	Means without rooting solutions*		
	RC (%)	CC (%)	DC (%)
BRS Maués	40.0 ab	0.0 a	60.0 abcd
CMU 1002	45.0 ab	5.0 a	50.0 abcd
CMU 1004	95.0 a	0.0 a	5.0 f
CMU 1005	75.0 a	15.0 a	10.0 ef
CMU 1006	70.0 a	5.0 a	25.0 def
CMU 1008	30.0 abc	5.0 a	65.0 abcd
CMU 1009	50.0 ab	5.0 a	45.0 abcde
CMU 1010	20.0 abc	25.0 a	55.0 abcd
CMU 1012	10.0 bc	0.0 a	90.0 abc
CMU 1013	5.0 bc	5.0 a	90.0 abc
CMU 1014	70.0 a	0.0 a	30.0 def
CMU 1015	5.0 bc	5.0 a	90.0 abc
CMU 1016	5.0 bc	0.0 a	95.0 ab
CMU 1017	35.0 abc	25.0 a	40.0 bcde
CMU 1018	50.0 ab	15.0 a	35.0 cde
CMU 1022	0.0 c	0.0 a	100.0 a

*Means followed by the same letters, in column, do not differ by the Tukey test at 5% probability.

It is observed that in guarana, certain genotypes showed high rooting results, even when not submitted to treatments with growth regulators, reaching averages of up to 95% of rooting. Results obtained by Atroch et al. (2007) show a higher rooting in cuttings without application of IBA,

concluding that there is genetic variability among genotypes for the rooting potential. As stated earlier, differences in rates of auxin metabolism are inherent to each compound. The rates of absorption, transport, oxidation and conjugation can also differ among genotypes.

The fact that the controls have rooted is related to the presence of endogenous hormones in the cuttings. Endogenous auxins are very important in the process of root formation, as auxin produced in new leaves and buds naturally moves basipetally, accumulating at the bottom of the cut, along with sugars and other nutrients (Lone et al., 2010).

Root formation depends on an optimal level of auxin (Carvalho et al. 2002), which in certain concentrations promotes cell division by regulating the transcription of induced auxin genes, in addition to the interaction with environmental and endogenous factors (Geiss et al. 2018). Anatomical and physiological dynamics of adventitious root formation in cuttings is linked to wounding stimulus, which induces the expression of auxin transporter genes and accumulation of auxin in founder cell initiation sites in pericycle and later in meristematic cells of adventitious root primordia in tomato (Guan et al., 2019).

The tissue of cuttings that have herbaceous consistency has intense meristematic activity and a low degree of lignification (Santos, 2016), generally with high levels of endogenous auxin (Oliveira et al., 2012), thus presenting adequate physiological conditions for the rapid emission of adventitious roots, however, with a greater propensity to dehydration and death (Fachinello et al. 2005) requiring greater care with irrigation systems and maintaining humidity in the environment (Lima et al., 2006).

Guarana cuttings are sensitive to the excess of water, at any stage of rooting, causing mortality in the case of waterlogging (Arruda, Pereira & Moreira, 2007). However, in this work, the condition of the spray irrigation system was constant in the nursery, providing high humidity in the environment, at least in the first four months after planting the cuttings in substrate, so as not to negatively affect seedling establishment or causing accentuated dehydration.

In the case of the substrate mixture of soil + sand used in the present work, in a 4: 1 ratio, a more clayey substrate was obtained, which was easily soaked, and remained so for hours, making it difficult to drain irrigation water, and may have influenced the mortality of the cuttings induced by hypoxia of the tissues that would give rise to the root system.

4. Conclusion

The application of IBA and NAA provided an improvement in the quality of the root system of cuttings from guarana genotypes whereas using 2,4-D caused the highest mortality. The genotypes showed great variability in rooting, especially the CMU 1004, CMU 1005, CMU 1006 and CMU 1014 with the highest percentages of rooted cuttings, while the CMU 1016 and CMU 1022 genotypes showed the lowest rooting rates and lowest root quality measured as root number, length, and volume. Although an interactive effect of genotype x rooting solution was observed, it is not possible to affirm that the different rooting solutions tested have an influence on rooting of guarana cuttings, since the contrast of the factorial with untreated genotypes was not significant.. Future testing of different concentrations of the same growth regulators should be addressed, which might include other modes of application such growth regulators embedded in gel or in powder dilutions in order to maintain the substance for longer time in contact with the cutting ends.

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Author contribution:
Maísa Silva – Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – First Writing, Roles/Writing – original draft.
Anderson Melo – Methodology, Supervision, Validation, Visualization, Roles/Writing – original draft.
Firmino José – Conceptualization, Supervision, Validation, Roles/Writing – original draft.
Eva Atroch – Methodology, Supervision, Validation, Writing – review & editing.
André Atroch – Conceptualization, Data curation, Formal Analysis, Founding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Roles/Writing – original draft, Writing – review & editing.

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