https://doi.org/10.71252/2177-34912025720027
ISSN: 2177-3491

Influence of 6-benzylaminopurine and culture medium on the regeneration of axillary shoots of *Psidium cattleyanum* Sabine cultivars Ya-cy and Irapuã

Alexandre Klas Bico[1], *Juliana Degenhardt*[2], *Luciana Lopes Fortes Ribas*[3*]

[1] Universidade Federal do Paraná, Programa de Pós-Graduação em Agronomia – Produção Vegetal, Curitiba, Paraná, Brasil. aleklas@gmail.com.

[2] Embrapa Florestas, Colombo, Paraná, Brazil. juliana.degenhardt@embrapa.br.

[3] Universidade Federal do Paraná, Departamento de Botânica, Curitiba, Paraná, Brazil. llfribas@ufpr.br.

*Corresponding author: llfribas@ufpr.br

Editors:
Danielle Fabíola Pereira da Silva
Ismael Diego Rocha

Submitted: March 10th, 2025.
Accepted: June 25th, 2025.

ABSTRACT

Psidium cattleyanum, commonly known as araçá, is a fruit tree valued for its nutritional benefits and is increasingly consumed fresh and processed. However, the genetic variability within its populations makes selecting and propagating individuals with desirable traits challenging. This study aimed to optimize the *in vitro* shoot regeneration from axillary buds of Irapuã and Ya-cy cultivars using a complete MS culture medium and half-strength MS basal salts (MS/2), supplemented with 6-benzylaminopurine (BAP). Apical shoots from *in vitro* germinated plantlets were placed on MS or MS/2 medium, supplemented with 2.2, 4.4, or 8.8 µM BAP, and a control without BAP. For the Irapuã cultivar, the highest shoot regeneration rate was achieved on MS/2 medium with 2.2 µM BAP during the third subculture, resulting in 100% of the explants producing shoots. In contrast, the Ya-cy cultivar showed the best results on MS/2 medium with 4.4 µM BAP, yielding up to 78.3% shoot formation in the first subculture. Both cultivars were rooted on MS/2 culture medium without plant growth regulators, with cv. Irapuã achieving up to 100% rooting and cv. Ya-cy 41.2%. A micropropagation protocol was successfully developed for the Irapuã and Ya-cy cultivars.

Keywords: araçá, Myrtaceae, native fruit, cytokinin, plant growth regulator, MS medium.

INTRODUCTION

Psidium cattleyanum Sabine, commonly known as araçá, is a fruit tree that grows in sunny, humid environments, reaching heights of 3 to 11 meters.[1,2] The two main varieties are the red araçá (*Psidium cattleianum* var. *purpureum* Mattos) and the yellow araçá *(Psidium cattleianum* var. *lucidum* Hort.).[2] Native to Brazil, this species thrives in the Caatinga, Cerrado, and Atlantic Forest, and is also found in northeastern Uruguay.[3] It is present in several Brazilian states, including Rio Grande do Sul, Santa Catarina, Paraná, and São Paulo.[3] *P. cattleyanum* has adapted well to tropical climates in Hawaii and the Caribbean and has also been cultivated in South and Central America.[4]

The fruit is a berry characterized by its red-vinaceous or yellow skin and white, light yellow, or red pulp when ripe.[2] It has a sweet, acidic, and slightly astringent flavor, which makes it valuable in the food industry.[1,5] Rich in vitamin C, minerals, fatty acids, polysaccharides, and phenolic compounds, the fruits provide essential nutrients and offer various biological benefits.[5] Studies have shown that *P. cattleyanum* has several beneficial properties, including antioxidant, antidiabetic, anticarcinogenic, antimicrobial, anti-inflammatory, and anti-aging effects.[5-7] Despite its potential, araçá remains under-exploited, with supply falling to meet market demand, creating opportunities for commercial cultivation for fresh consumption and agro-industry. This could support regenerative agriculture and crop diversification.[1,8] Pereira et al.[9] noted the fruit's significant bioactivity and potential for industrialization despite low production levels.

Currently, there are only two araçá cultivars developed through breeding program by Embrapa Temperate Climate: Ya-cy, which produces yellow fruit, and Irapuã, which produces red fruit.[10] Ya-cy is ideal for fresh consumption due to its sweetness and low acidity, while Irapuã is better for processing, offering larger, more acidic fruits with slight astringency.[10]

This species primarily propagates through seeds, produced annually.[2] Studies have reported divergences in seed dormancy and seeds showing either orthodox or recalcitrant behavior.[11,12] Although seed production and storage are not issues, significant genetic variability among individuals necessitates research into vegetative propagation of those with desirable traits.[8] No consensus exists on the effectiveness of using cuttings for this species. While Schwengber et al.[13] reported a rooting success of only 5.2%, Rodriguez et al.[14] found a much higher rate of 98% without plant growth regulators. Although cuttings can yield positive results, they require substantial plant material. Grafting techniques have low success rates, below 5%.[10] In contrast, micropropagation offers a promising alternative for mass propagation, enabling rapid growth in limited space throughout the year.[15]

There have been limited studies on the micropropagation of araçá. Freire et al.[16] noted challenges with microbial contamination in red araçá explants from the Irapuã cultivar. They suggested adding 500 mg L^{-1} of ampicillin to the Murashige and Skoog[17] culture medium and using 6.6 µM 6-benzilaminopurine (BAP) and 2.85 µM indole-3-acetic acid (IAA) for shoot induction. Additionally, Arruda et al.[18] found that red araçá cv. Irapuã seeds need a 1-minute immersion in 80 °C water to overcome dormancy before *in vitro* cultivation in liquid MS medium.

Micropropagation through axillary shoot induction reduces the risk of somaclonal variation and is generally simpler than techniques such as regeneration from adventitious shoots or somatic embryogenesis.[19] However, there is still a significant lack of research regarding the *in vitro* propagation of native forest species. Therefore, this study aimed to optimize the micropropagation protocol for the Irapuã and Ya-cy cultivars of *P. cattleyanum*, with a focus on enhancing the regeneration of axillary shoots.

MATERIALS AND METHODS

Plant material, disinfestation and in vitro establishment

Seeds were germinated *in vitro* at the Plant Micropropagation Laboratory of the Federal University of Paraná (UFPR) in Curitiba, PR. The micropropagation experiments were carried out at the Tissue Culture and Transformation Laboratory of Embrapa Forestry (Colombo, PR), using apical shoots from these seedlings as initial explants.

Seeds of yellow araçá (cv. Ya-cy) and red araçá (cv. Irapuã) were collected from Embrapa Temperate Climate in Pelotas, RS. They were first disinfested in 70% ethanol for 1 minute, then immersed in a 2% sodium hypochlorite solution with 0.1% Tween® 20 for 15 minutes. After treatment, the seeds were rinsed eight times with sterilized distilled water and placed in individual test tubes (2 cm in diameter and 10 cm long) with 10 mL of MS/2 culture medium, which is Murashige and Skoog[17] culture medium with salts halved. In a laminar flow chamber, the seedlings

were removed from the test tubes, and their roots were trimmed, leaving only the youngest pair of leaves on the explants (Figures 1A, 1B). The shoots were then cultured for one month in flasks (9.5 cm high and 6 cm in diameter) containing 30 mL of MS/2 medium with 30 g L^{-1} of sucrose and no plant growth regulators (PGRs). The pH was adjusted to 5.8 using 1 N NaOH or HCl before adding 7 g L^{-1} of agar (Reatec®).

Induction and development of explants

The explants were inoculated on MS or MS/2 media with 2.2 µM or 4.4 µM BAP and a control without cytokinin. For the Irapuã cultivar, they were also grown on MS/2 medium with BAP concentrations of 2.2 µM, 4.4 µM, and 8.8 µM. The culture media was supplemented with 30 g L^{-1} sucrose and 7 g L^{-1} agar (Reatec®). The pH of the media was adjusted to 5.8 with NaOH or HCl 1N. Each flask contained 30 mL of culture medium and was autoclaved for 20 minutes at 121 ºC. The cultures were kept in a growth room at 23 ± 2 ºC, a 16-hour photoperiod, and 40-45 µmol m^{-2} s^{-1} light intensity from Elgin® tubular LED lamps.

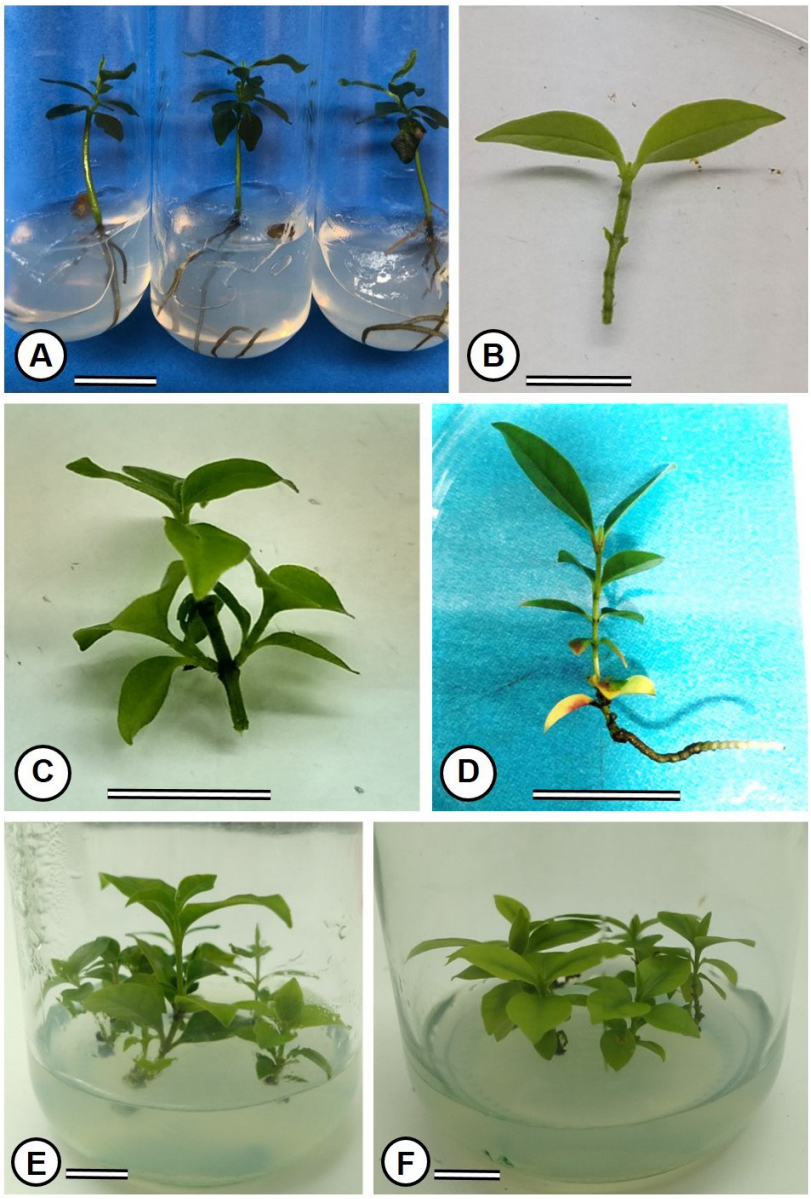

Figure 1. *Psidium cattleyanum* explants grown in Murashige and Skoog (17) (MS) or MS/2 (MS, with salts halved) culture media with different concentrations of 6-benzylaminopurine (BAP). A- Germinated seedlings. B- Initial explant. C- Explant with axillary shoots grown on MS culture medium with 4.4 µM BAP. D- Rooted explant grown on MS/2 medium. E- cv. Irapuã explants after three subcultures (24 weeks) in MS/2 culture media and 4.4 µM BAP. F- cv. Ya-cy explants after three subcultures (24 weeks) in MS/2 culture media and 4.4 µM BAP. Bars: 10 mm.

Effect of BAP concentrations and MS medium on in vitro multiplication of the cvs. Ya-cy and Irapuã

Apical shoots, 1.5 cm long, with at least three nodes and the youngest pair of leaves, were used as initial explants (Figure 1 B) and placed on MS or MS/2 culture media, either without BAP or with 2.2 µM or 4.4 µM of BAP. Each flask contained five explants, with eight replicates, totaling 40 per treatment for each cultivar.

The explants were subcultured to the fresh medium of the same composition for three subcultures every eight weeks. The percentage of shoots per explant (Figure 1C), the average number and length of shoots, and the rooting percentage were evaluated (Figure 1D).

Effect of BAP at higher concentration in the micropropagation of the cv. Irapuã

The shoots of cv. Irapuã were grown on MS/2 medium with 8.8 µM BAP, in addition to the previously tested concentrations of 2.2 and 4.4 BAP and the control without BAP. Five explants were inoculated per flask, with eight replicates. After three subcultures on media containing the same BAP concentration, the percentage of explants with shoots, the average number of shoots per explant, and the rooting percentage were evaluated.

Experimental design and statistical analysis

The experimental design was entirely randomized, with a factorial scheme of 2 (culture media: MS and MS/2) x 3 (BAP concentration: no BAP, 2.2 µM and 4.4 µM) for each cultivar. The variables of the percentage of explants with shoots and the percentage of rooting were analyzed using a generalized linear model (GLM), using the binomial family of distributions with a logito. For the GLMs, the deviance analysis used the likelihood ratio test, and the mean comparison tests were based on the ratio between the means. The average number of shoots was analyzed using the likelihood ratio test of the effects adjusted via GAMLSS modeling,[20] using the Poisson distribution altered to zeros in the case of the first experiment. The average number of shoots in the second experiment (higher concentration of BAP) was analyzed via GLM with a Quasi-Poisson distribution and a logarithmic link function. The average length of the explants was analyzed using ANOVA, and the data was transformed into square roots. The Shapiro-Wilk normality test with a p-value 0.05 was used to verify the normality of the residuals. Tukey's mean comparison test was used for all variables with a significance level of 0.05. The statistical analyses were conducted using the R language.[21]

RESULTS

Effect of BAP concentrations and MS medium on in vitro multiplication of the cv. Irapuã

The percentage of explants with shoots increased over the three subcultures, except for those grown on MS or MS/2 medium without BAP (Table S1). The best shoot regeneration responses occurred on MS or MS/2 medium containing 4.4 µM BAP (53.80 and 47.80%, respectively) (Table S1 and Figure 1E).

The percentage of explants producing shoots and the average number of shoots per explant was significantly affected by BAP concentration. There wasn't an interaction between BAP and culture medium. Explants cultured in a medium with BAP exhibited notably higher shoot percentages than those without BAP (Table 1). Explants cultivated in a culture medium containing 4.4 µM of BAP produced a significantly higher average number of shoots (2.09) compared to the control (1.50) (Table 1).

The rooting percentage of the explants showed an interaction effect between the culture medium and BAP concentration (Table 2). The highest rooting percentage (70.3%) was achieved on the MS/2 medium without PGR. It was significantly higher than the 32.7% rooting observed on the MS medium. The rooting percentage of shoots in the MS/2 medium without a PGR was significantly higher than in the medium with BAP (Table 2). There was no significant difference in rooting between explants with or without BAP in the MS medium (Table 2).

The analysis showed that optimal shoot multiplication of cultivar Irapuã occurred on the MS medium supplemented with 4.4 µM BAP during the third subculture. Additionally, rooting was successful in the MS/2 medium without PGRs.

Effect of higher concentration of BAP on the micropropagation of the cv. Irapuã

Even with a higher concentration of BAP, there was no significant difference in the percentage of explants with shoots (Table 3). However, the average number of shoots per explant was greater in the BAP treatments than in the control. The treatment without BAP achieved 100% rooting, significantly differing from the BAP treatments, especially the one with 8.8 µM BAP, which showed the lowest response (Table 3).

Table 1. Percentage of explants with shoots (%) and average number of shoots of *Psidium cattleyanum*, cultivar Irapuã, grown in MS or MS/2 media and different concentrations of 6-benzylaminopurine (BAP)

		explants with shoots (%)		
		BAP concentration (µM)		
Culture Medium	**0.0**	**2.2**	**4.4**	
MS/2	2.20 (1.54)	31.80 (4.05)	33.30 (4.15)	14.80 (3.20) ^{ns}
MS	6.10 (2.42)	21.10 (3.91)	28.00 (4.96)	15.90 (2.42) ^{ns}
	3.70 (1.47) b	26.10 (2.89) a	30.60 (3.28) a	
		average number of shoots		
MS/2	1.50 (0.66)	1.70 (0.15)	2.02 (0.16)	1.82 (0.26) ^{ns}
MS	1.50 (0.71)	1.87 (0.22)	2.39 (0.24)	2.06 (0.07) ^{ns}
	1.50 (0.32) b	1.77 (0.13) ab	2.09 (0.14) a	

Means with different lowercase letters differ according to Tukey's test ($p < 0.05$). ^{ns} not significant. Standard deviation in parenthesis. MS= Murashige and Skoog (17) MS/2 = MS, with salts halved. Data are means of the three subcultures.

Table 2. Rooting percentage (%) of *Psidium cattleyanum* explants of the Irapuã cultivar cultivated in MS or MS/2 media with different 6-benzylaminopurine (BAP) concentrations

		BAP concentration (µM)		
Culture Medium	**0.0**	**2.2**	**4.4**	
MS/2	70.30 (4.79) Aa	30.30 (4.00) Ab	31.80 (4.10) Ab	43.90 (2.89)
MS	32.70 (4.74) Ba	26.60 (4.23) Aa	15.90 (4.03) Aa	24.30 (2.64)
	51.70 (3.93)	28.40 (2.93)	22.90 (3.14)	

Means with different uppercase letters in the columns or lowercase letters in the rows differ by Tukey's test ($p < 0.05$). Standard deviation in parenthesis. MS= Murashige and Skoog (17) MS/2 = MS, with salts halved. Data are means of the three subcultures.

Table 3. Percentage of explants with shoots (%), average number of shoots per explant, and rooting percentage (%) of *Psidium cattleyanum* cultivar Irapuã, grown in MS/2 medium, with different concentrations of 6-benzylaminopurine (BAP)

		BAP concentration (µM)		
Variable	**0.0**	**2.2**	**4.4**	**8.8**
Shoots (%)	92.00 (18.00) ^{ns}	100.00 (0.00) ^{ns}	100.00 (0.00) ^{ns}	100.00 (0.00) ^{ns}
Number of shoots	1.12 (0.18) b	2.60 (0.95) a	2.72 (1.06) a	3.50 (1.45) a
Rooting (%)	100.00 (0.00) a	55.00 (33.00) b	57.80 (25.00) b	17.80 (25.00) c

Means with different lowercase letters in the rows differ by Tukey's test ($p < 0.05$). ^{ns} not significant. Standard deviation in parenthesis. MS= Murashige and Skoog (17) MS/2 = MS, with salts halved. Data are means of the three subcultures.

Table 4. Percentage of explants with shoots (%) of *Psidium cattleyanum*, cultivar Ya-cy, grown in MS or MS/2 media, with different 6-benzylaminopurine (BAP) concentrations

		BAP concentration (µM)		
Culture Medium	**0.0**	**2.2**	**4.4**	
MS/2	3.90 (1.92) Ac	18.50 (3.74) Bb	59.00 (4.17) Aa	19.20 (3.06)
MS	6.00 (2.37) Ab	36.90 (4.23) Aa	45.20 (4.43) Aa	23.90 (2.98)
	4.90 (1.53)	26.70 (3.01)	52.10 (3.10)	

Means with different uppercase letters in the columns or lowercase letters in the rows differ by Tukey's test ($p < 0.05$). Standard deviation in parenthesis. MS= Murashige and Skoog (17) MS/2 = MS, with salts reduced by half. Data are means of the three subcultures.

Effect of BAP concentrations and MS medium on in vitro multiplication of the cv. Ya-cy

For the Ya-cy cultivar, in contrast to Irapuã, the percentage of shoots decreased over the subcultures (Table S2), except for explants on MS/2 medium without BAP. The best results were observed in the first subculture using either MS or MS/2 medium with 4.4 µM BAP, achieving shoot rates of 78.6% and 70.3%, respectively (Table S3 and Figure 1F). However, the explants of the Ya-cy cultivar exhibited a loss of vigor (Table S2), while the Irapuã cultivar maintained its vigor throughout the subcultures (Table S1).

The interaction between the culture medium and BAP concentration influenced the percentage of explants with shoots. Explants grown on MS/2 culture medium with 4.4 µM BAP produced more shoots than those on 2.2 µM BAP, while the control (without BAP) had the lowest response (Table 4). In MS medium, explants with BAP had higher shoot percentages, though no significant differences were found between the BAP treatments. When comparing the culture media, explants grown on MS medium with 2.2 µM BAP exhibited a significantly higher percentage than those grown on MS/2 medium (Table 4).

The average number of shoots per explant for the cultivar Ya-cy, similar to Irapuã, was significantly influenced by the addition of BAP, though there was no interaction between BAP and the culture medium (Table 5). Explants grown in media containing 2.2 µM BAP produced a significantly higher average number of shoots per explant (2.07) compared to the treatment without BAP (1.50) (Table 5).

The average length of the Ya-cy explants was affected solely by the presence of BAP, with no influence from the culture medium or interactions between factors (Table 5). Interestingly, the average length of shoots grown in the medium without BAP was significantly greater than that of shoots grown in the medium containing the highest concentration of BAP (Table 5).

The rooting percentage of Ya-cy explants was influenced by both the culture medium and the concentration of BAP (Table 6). There wasn't an interaction between BAP and culture medium. Explants cultured on the MS/2 medium showed significantly higher rooting percentages than those grown on the MS medium. Furthermore, explants cultivated on media without BAP exhibited the highest rooting percentages (Table 6).

Table 5. Average number of shoots and average length (cm) of explants of *Psidium cattleyanum*, cultivar Ya-cy, grown in MS or MS/2 media with different 6-benzylaminopurine (BAP) concentrations

average number of shoots per explant				
	BAP concentration (µM)			
Culture Medium	**0.0**	**2.2**	**4.4**	
MS/2	1.25 (0.46)	1.70 (0.24)	2.02 (0.13)	1.93 (0.22) ^{ns}
MS	1.67 (0.25)	2.23 (0.18)	2.00 (0.15)	2.08 (0.05) ^{ns}
	1.50 (0.26) b	2.07 (0.16) a	2.01 (0.10) ab	
average length (cm) of explants				
MS/2	2.00 (0.06)	1.86 (0.06)	1.74 (0.05)	1.85 (0.03) ^{ns}
MS	1.92 (0.07)	1.82 (0.06)	1.76 (0.05)	1.83 (0.03) ^{ns}
	1.96 (0.04) a	1.84 (0.04) ab	1.75 (0.04) b	

Means with different lowercase letters are significantly different according to Tukey's test ($p < 0.05$). ^{ns} not significant. Standard deviation in parenthesis. MS -Murashige and Skoog (17), MS/2 -MS with salts reduced by half. Data are means from three subcultures.

Table 6. Rooting percentage (%) of *Psidium cattleyanum* explants of the Ya-cy cultivar, grown in MS or MS/2 media, with different 6-benzylaminopurine (BAP) concentrations

	BAP concentration (µM)			
Culture Medium	**0.0**	**2.2**	**4.4**	
MS/2	41.20 (4.87)	12.00 (3.13)	2.90 (1.42)	12.40 (2.25) A
MS	13.00 (3.36)	3.10 (1.51)	2.40 (1.36)	4.65 (1.22) B
	24.40 (3.30) a	6.20 (1.70) b	2.60 (0.90) b	

Means with different lowercase letters in the rows or different uppercase letters in the columns differ according to Tukey's test ($p < 0.05$). Standard deviation in parenthesis. MS= Murashige and Skoog (17) MS/2 = MS, with salts halved. Data are means of the three subcultures.

DISCUSSION

The micropropagation of Irapuã (red) and Ya-cy (yellow) araçá cultivars, initiated from *in vitro* germinated seedlings, was effective using the MS or M/2 medium combined with BAP. This cytokinin notably increased the percentage of explants with shoots. In the first experiment with the Irapuã cv., explants cultured in a medium containing 4.4 µM BAP achieved up to 53.80% shoot development by the third subculture. All explants formed shoots at the tested BAP concentrations in the second experiment with this cultivar. These results were expected in culture media with added BAP since the primary physiological effects of this class of PGR are the breaking of apical dominance and induced cell division.[22-24]

Shoot development responses seemed better at the beginning of cultivation for cv. Ya-cy. As the subcultures increased, the explants lost vigor, the average length was reduced, and they showed chlorotic leaves. On average of the three subcultures, the best results were observed in the combination between MS/2 medium and 4.4 µM of BAP, with 59.00% of explants with shoots. Considering the subcultures individually, the highest result was 78.6%, achieved in the first subculture with MS medium and 4.4 µM of BAP. This demonstrates that the requirements of the salts in the culture medium and BAP may differ among cultivars. *In vitro* cultivation of guava (*Psidium guajava* L.) cultivars, which belong to the same genus as the araçá, have shown different responses depending on the cultivar and the treatments with various PGRs.[25] We recommend testing alternative culture media and cytokinins, or, especially, reducing the subculture period from 60 to 30 days in future experiments to determine if Ya-cy performs better without exhibiting stress and chlorosis symptoms.

Adding BAP to the culture medium improved the average number of shoots per explant for both cultivars. For the Irapuã and Ya-cy, the explants grown on media containing 2.2 µM and 4.4 µM BAP showed similar responses. The addition of 8.8 µM, for Irapuã, yielded the highest average number of shoots, but this was not statistically different from the other treatments containing BAP. Considering both variables combined, the percentage of explants with shoots, and the average number of shoots, we recommend using MS/2 medium containing 2.2 µM BAP for the Irapuã and the same culture medium containing 4.4 µM BAP for the Ya-cy Freire et al.[16] found an average of 1.20 shoots for the Irapuã, recommending higher BAP concentrations, with 6.6 µM of BAP and 2.85 µM of AIA being optimal. In our study, using 8.8 µM of BAP produced the highest average of shoots per explant (3.5). In comparison, Sant'Ana et al.[26] observed that *Campomanesia rufa* explants cultivated on medium containing 4.5 µM of BAP yielded an average of 4.08 shoots after 90 days, which is higher than our results, but their evaluation lasted 30 days longer.

The joint analysis of the data of cv. Irapuã indicated that BAP should be added to the MS/2 medium at a concentration of 2.2 µM for optimal shoot production, with no significant difference observed between 2.2 and 8.8 µM. For cv. Ya-cy, it is recommended cultivating in MS/2 medium with 4.4 µM BAP for shoot regeneration.

The average length of Irapuã explants did not vary, regardless of the culture medium or the presence of BAP. However, for the Ya-cy cultivar, only the addition of cytokinin influenced this variable. The most favorable response was observed without BAP. In contrast, the length when using 4.4 µM BAP was shorter. This length reduction is likely due to BAP's role as a cytokinin, which promotes cell division and shoot regeneration, potentially at the expense of elongation.[22]

Both cultivars of araçá tested in this work do not require exogenous auxin for rooting. The best results were achieved using MS/2 medium without BAP, where Irapuã showed a rooting percentage of 70.3% in the first experiment and 100% in the second. In comparison, the cultivar Ya-cy had a lower rooting percentage of 41.20% in the same MS/2 medium without BAP. This indicates that the MS/2 medium is the most suitable for *in vitro* rooting of both cultivars. The absence of PGR in the rooting medium suggests that the explants contain sufficient endogenous auxin, which is beneficial as it reduces the cost of the plant production protocol. In contrast, Freire et al.[16] reported a different response for the same species, achieving a rooting percentage of 66.67% in MS medium with 8.8 µM BAP after 40 days of cultivation. In our study, we also tested 8.8 µM of BAP, which resulted in only 17.80% rooting, nearly a quarter of the rooting percentage reported by those authors.

Our experiments showed that BAP inhibited rhizogenesis. Generally, rooting is promoted by a higher auxin-to-cytokinin ratio, as noted by Mazzoni-Putman et al.[27] and Kurepa & Smalle.[28] In a second experiment with Irapuã, using MS/2 medium, we found that indole-3-butyric acid (IBA) was unnecessary, as the rooting percentage reached 100%. The Irapuã exhibited greater vigor and better rooting

than Ya-cy during subcultures with both MS and BAP concentrations. This suggests that Irapuã is more suitable for micropropagation and confirms its potential for large-scale plant production from axillary shoots.

CONCLUSIONS

To micropropagate axillary shoots of *P. cattleyanum*, it is recommended to use MS/2 medium supplemented with 2.2 µM BAP for the Irapuã cultivar and 4.4 µM BAP for the Ya-cy cultivar. Using MS/2 medium without any plant growth regulators is advisable for rooting both cultivars. A cost-effective micropropagation protocol has also been developed for araçá, which allows for the production of clonal seedlings.

DATA AVAILABILITY

The entire dataset supporting the results of this study was used in this article.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

To the Cargill Foundation for the master´s scholarship and the Federal University of Paraná and Embrapa Forests for providing the plant material and resources for the development of the experiments. There is no conflict of interest in conducting and publishing the work.

AUTHOR CONTRIBUTIONS

Conceptualization: Juliana Degenhardt, Luciana Lopes Fortes Ribas.

Data curation: Alexandre Klas Bico.

Formal analysis: Alexandre Klas Bico, Luciana Lopes Fortes Ribas.

Investigation: Alexandre Klas Bico.

Methodology: Alexandre Klas Bico, Juliana Degenhardt, Luciana Lopes Fortes Ribas.

Resources: Juliana Degenhardt, Luciana Lopes Fortes Ribas.

Supervision: Juliana Degenhardt, Luciana Lopes Fortes Ribas.

Validation: Luciana Lopes Fortes Ribas.

Visualization: Alexandre Klas Bico, Juliana Degenhardt, Luciana Lopes Fortes Ribas.

Writing – original draft: Alexandre Klas Bico, Luciana Lopes Fortes Ribas.

Writing – review & editing: Alexandre Klas Bico, Juliana Degenhardt, Luciana Lopes Fortes Ribas.

References

1. Lisbôa GV, Kinupp VF, de Barros IB. *Psidium cattleianum* Araçá. In: Coradin L, Siminski A, Reis A, editors. Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro – Região Sul. Brasília: Ministério do Meio Ambiente; 2011. p.205-8.
2. Lorenzi H, de Lacerda MT, Bacher LB. Frutas no Brasil nativas e exóticas (de consumo in natura). São Paulo: Instituto Plantarum de Estudos da Flora; 2015.
3. Tuler AC, Costa IR, Proença CE. *Psidium* in Flora e Funga do Brasil [Internet]. Rio de Janeiro: Jardim Botânico do Rio de Janeiro; 2024 [cited 2024 Nov 15]. Available from: https://floradobrasil.jbrj.gov.br/FB10858
4. Patel S. Exotic tropical plant *Psidium cattleianum:* a review on prospects and threats. Rev Environ Sci Biotechnol. 2012;11:243-8. https://doi.org/10.1007/s11157-012-9269-8
5. Pereira ES, Vinholesa J, Franzon RC, Dalmazob G, Vizzotto M, Norab L. *Psidium cattleianum* fruits: a review on its composition and bioactivity. Food Chem. 2018;258:95-103. https://doi.org/10.1016/j.foodchem.2018.03.024
6. Elsayed HE, El-Deeb EM, Taha H, Taha HS, Elgindi MR, Moharram FA. Essential oils of *Psidium cattleianum* Sabine leaves and flowers: anti-inflammatory and cytotoxic activities. Front Chem. 2023;11. https://doi.org/10.3389/fchem.2023.1120432
7. Seraglio SK, Schulz M, Silva B, Deolindo CT, Hoff RB, Gonzaga LV, et al. Chemical constituents and antioxidant potential of red guava (*Psidium cattleianum* Sabine) from southern Brazil in different edible ripening stages. Plant Foods Hum Nutr. 2024;79(1):166-72. https://doi.org/10.1007/s11130-024-01141-6
8. Santos PC, Gallo R, Silva GC, Nonato ER, Sousa MB, Silva CL. Genetic potential and breeding strategies in *Psidium cattleianum* Sabine: a bibliometric analysis. J Crop Improv. 2024;38(5):513-49. https://doi.org/10.1080/15427528.2024.2375274
9. Pereira ES, Raphaelli O, Radunz M, Camargo TM, Vizzotto M. Biological activity and chemical composition of native fruits: a review. Agrociência Uruguay. 2021;25:1-19. https://doi.org/10.31285/agro.25.815
10. Franzon RC, Campos LD, Proença CE, Sousa-Silva JC. Araçás do gênero *Psidium:* principais espécies, ocorrência, descrição e usos [Internet]. Planaltina: Embrapa Cerrados; 2009 [cited 2024 Nov 15]. Available from: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/697560/1/doc266.pdf
11. Camargo HV, Walter LS, Gabira MM, Kratz D. Pre-germinative treatments and seed storage of *Psidium cattleyanum* Sabine morphotypes. Colloq Agraria. 2020;16(4):102-9. https://doi.org/10.5747/ca.2020.v16.n4.a387
12. Tafarel AZ, Silvestre WP, Pansera MR, Rodrigues LS, Sartori VC. Seed dormancy and germination in *Psidium cattleyanum* Sabine (red and yellow araçá). Interdiscip J Appl Sci. 2021;5(9):20-7. https://doi.org/10.18226/25253824.v5.n9.03
13. Schwengber JE, Dutra L, Kersten E. Efeito do sombreamento da planta matriz e do PVP no enraizamento de estacas de ramos de araçazeiro (*Psidium cattleyanum* Sabine). Curr Agric Sci Technol. 2000;6:30-4.
14. Rodriguez EA, Pradella EM, Souza PV, Schafer G. Asexual propagation of araçá (*Psidium cattleianum* Sabine) by leaf and young branches cuttings. Rev Árvore. 2016;40:707-14. https://doi.org/10.1590/0100-67622016000400014
15. Abdalla N, El-Ramady H, Seliem MK, El-Mahrouk ME, Taha N, Bayoumi Y, et al. An academic and technical overview on plant micropropagation challenges. Horticulturae. 2022;8:677. https://doi.org/10.3390/horticulturae8080677
16. Freire CG, Gardin JP, Baratto CM, Vieira RL, Werner SS. Micropropagation's complete protocol of red araçá (*Psidium cattleianum*, Myrtaceae) from germinated seeds in vitro. J Agric Sci. 2018;10:234-5. https://doi.org/10.5539/jas.v10n2p234
17. Murashige T, Skoog F. A revised medium for rapid growth and bio

assays with tobacco tissue cultures. Physiol Plant. 1962;15:473-97. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

18. Arruda AL, Buss M, Silva PS, Nerbass FR, Kretzschmar AA, Rufato L. Estabelecimento in vitro de sementes de *Psidium cattleianum* Sabine. Acta Biol Catarinense. 2019;6:105-13.

19. Phillips GC, Garda M. Plant tissue culture media and practices: an overview. In Vitro Cell Dev Biol Plant. 2019;55:242-57. https://doi.org/10.1007/s11627-019-09983-5

20. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape. J R Stat Soc Ser C Appl Stat. 2005;54:507-54. https://doi.org/10.1111/j.1467-9876.2005.00510.x

21. R Core Team. R: a language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing; 2024 [cited 2024 Nov 15]. Available from: https://www.R-project.org/

22. Wybouw B, De Rybel B. Cytokinin – a developing story. Trends Plant Sci. 2019;24(2):177-85. https://doi.org/10.1016/j.tplants.2018.10.012

23. Sosnowski J, Truba M, Vasileva V. The impact of auxin and cytokinin on the growth and development of selected crops. Agriculture. 2023;13(3):724.

24. Svolacchia N, Sabatini S. Cytokinins. Curr Biol. 2023;33(1). https://doi.org/10.1016/j.cub.2022.11.022

25. Joshee N, Mutua M, Yadav AK, Zee F. In vitro shoot bud induction and plantlet regeneration in guava as influenced by genotype. Acta Hortic. 2004;632:279–85. https://doi.org/10.17660/actahortic.2004.632.36

26. Sant'Ana CRDO, Paiva R, Reis MVD, Silva DPCD, Silva LC. In vitro propagation of *Campomanesia rufa:* an endangered fruit species. Ciênc Agrotec. 2018;42:372-80. https://doi.org/10.1590/1413-70542018424011018

27. Mazzoni-Putman SM, Brumos J, Zhao C, Alonso JM, Stepanova AN. Auxin interactions with other hormones in plant development. Cold Spring Harb Perspect Biol. 2021;13:10. https://doi.org/10.1101/cshperspect.a039990

28. Kurepa J, Smalle JA. Auxin/cytokinin antagonistic control of the shoot/root growth ratio and its relevance for adaptation to drought and nutrient deficiency stresses. Int J Mol Sci. 2022;23:4. https://doi.org/10.3390/ijms23041933