

## ASEXUAL PROPAGATION OF ARAÇÁ (*Psidium cattleianum* Sabine) BY LEAF AND YOUNG BRANCHES CUTTINGS<sup>1</sup>

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**ABSTRACT** – *Psidium cattleianum* Sab., from Mirtaceae family, commonly known as araçá, is a native species from the South of Brazil, present in biomes such as the Atlantic Forest, a deciduous forest of Brazilian cerrado, strongly affected by the expansion of areas for agricultural and industrial production. Researches on plant breeding area, nutraceuticals and other areas have validated its potential and it detaches the need to promote it on a commercial scale. Mechanisms, that allow exploiting its genetic potential while maintaining its conservation areas, require adjustment in cloning techniques for fixing interest characteristics. In this investigation, seedlings of leaf cutting and young branch were assessed, with three treatments in leaf cutting ( $H_2PO_4$  0.2 g L<sup>-1</sup>, AIB 100 mg L<sup>-1</sup> and AIB and powder-based applied over cutting region). Moreover, leaf cuttings of five cm length and the two leaves were treated with AIB (0, 1,500, 3,000 and 6,000 mg L<sup>-1</sup>) in the absence and presence of BAP (500 mg L<sup>-1</sup>). Treatment with AIB (powder) in leaf cuttings showed higher survival of leave; however, there were callus formation, especially compared to other treatments of  $H_2PO_4$  and AIB that showed average rooting percentage of 18%. In plant cuttings, there were no significant difference for the variables evaluated between AIB doses; however, the use of cytokinin (BAP 500 mg L<sup>-1</sup>) was inhibitory. There was no difference at rooting percentage (98%) without treatment with regulator, maintaining similar with BAP doses.

**Keywords:** Growth regulator; Native fruit; Plant propagation.

## PROPAGAÇÃO ASSEXUADA DE ARAÇAZEIRO (*Psidium cattleianum* Sabine) POR ESTACAS DE FOLHAS E RAMOS JOVENS

**RESUMO** – *Psidium cattleianum* Sab., família Myrtaceae, popularmente conhecida no Brasil como araçazeiro é uma espécie nativa do sul do Brasil, presente em biomas como a Mata Atlântica, Floresta Estacional Decidua e o Cerrado brasileiro, fortemente afetados pela expansão das áreas de cultivo e agroindústria. Pesquisas na área fitotécnica, nutracêutica e outras têm validado o seu potencial e destacado a necessidade de promovê-la a escala comercial. Mecanismos que permitam explorar seu potencial genético, mantendo as áreas de preservação exigem o ajuste de técnicas de clonagem para a fixação de características de interesse. Nesta pesquisa, avaliou-se a produção de mudas por estacas de folhas e ramos jovens, com três tratamentos ( $H_2PO_4$  0,2 g L<sup>-1</sup>, AIB 100 mg L<sup>-1</sup> AIB em talco, aplicado por contato na região do corte). Além disso, estacas herbáceas de cinco centímetros de comprimento e duas folhas foram tratadas com AIB (0, 1500, 3000 e 6000 mg L<sup>-1</sup>) na ausência e na presença de BAP (500 mg L<sup>-1</sup>). O tratamento com AIB (talco) nas estacas de folha mostrou maior sobrevivência, porém houve principalmente a formação de calos quando comparado com outros tratamentos de AIB e  $H_2PO_4$  que mostraram porcentagem de enraizamento em média de 18%. Nas estacas de ramo



*jovem, não houve diferença significativa entre as variáveis avaliadas nas doses de AIB, por outro lado o uso de BAP (500 mg L<sup>-1</sup>) foi inibitório. Não houve diferença no percentual de enraizamento (98%) entre o tratamento sem regulador e as doses de BAP.*

*Palavras-chave: Frutífera nativa; Produção de mudas; Reguladores de crescimento.*

## 1. INTRODUCTION

The conservation of biodiversity allows the great mass of chemical compounds, genetic resources and other benefits of terrestrial species are available to provide solutions to existing problems and those that are yet to be discovered (WILLIAMS et al., 2001). *Psidium cattleianum* Sabine is considered one of the native fruit species of Southern Brazil of great potential interest for its fruit, and ornamental, pharmaceutical and nutraceutical use, besides the environmental interest for its high production of fruits and seeds, considered another interesting feature from environmental point of view to the use in the recovery of native fauna and flora in deforested areas of the Atlantic Forest.

As *P. cattleianum* is still a native species of little research, there are no programs to reduce the genotypic variability within a population necessary for its commercial advantage. According to Rocha et al. (2008), there are two morphotypes that show anatomical differences: yellow and red.

In order preserve the homogenous agricultural characteristics, asexual propagation is an alternative. As a source of explants, it mainly uses cuttings, depending on plant area that may be apical, intermediate or radicular, ranging on explants characteristics. Propagation by cuttings has been researched and used in woody species such, as *Pinus* spp. (ANDREJOW; HIGA, 2009; ALCANTARA et al., 2007), *Eucalyptus* spp. (NAVARRETE and VARGAS, 2005), *Psidium guajava* (ZIETEMANN; ROBERTO, 2007) and ornamental (KÄMPF, 2005).

For technique success, preparation of cuttings is an important element. For example, in plant cuttings, the presence of young leaves may promote the synthesis of auxin, a source of carbohydrates and in turn, facilitated by the formation of less specialized cells (HOPPE et al., 2004), that is an important characteristic for relocation and reorganization of air and root system required for asexually formation of seedlings.

However, morphogenesis depends on the interaction of physiological, chemical, histological factors, among others. In apical cutting, for example, for being the region of auxin synthesis, it facilitates the development of shoots; while in leaf cutting also reorient the translocation of photo-assimilated needed to induce the formation of shoot and root system.

In species of difficult response, the participation of propagation by cutting requires synthetic regulators, especially of the auxin group, which are more associated to the development of lateral roots (SMET et al., 2007). Meanwhile, the cytokinins group acts in various processes of growth and development of plants, including the promotion of cell division, delayed senescence and regulation of apical dominance, but it can also disturb the development of lateral roots by its increased concentration (DITENGOU et al., 2008) and trigger the programmed cell death in the zone of rhizogenesis response.

The application of regulators for rooting induction can be carried out on a commercial powder, concentrated alcoholic solutions or in low concentrations for leaf application. The immersion time of cuttings depends on the used concentration. Dilute solutions (0 to 500 mg L<sup>-1</sup>) require hours into contact with tissue, while in low concentration (> 500 mg L<sup>-1</sup>) require a few seconds (HARTTMAN et al., 2002).

In turn, the formation of plantlets by leaf cutting and bud represent also the cited above, histological restructuring and function changes. From an anatomical point of view, the cells of abaxial and adaxial surfaces differ in size and thickness, cell from apex and margin are generally thinner than the rest (CUTLER et al., 2011).

The physical aspects are also responsible for rooting induction. Suitable temperature affects the formation of roots, but its development is highly dependent on energy source (HARTMANN et al., 2002), this being the case of propagation by guava cuttings (*Psidium guajava*), wherein root formation depends on the maintenance of healthy leaves (ANDRADE, 2001).

Cuttings from young branches rooting faster; however, require adequate installations and adequate control of the factors that affect its formation, being crucial to maintain leaf retaining. The control of these factors associated with the use of strategies, such as exogenous application of synthetic auxin and branch etiolation can increase the rhizogenesis capacity in guava cuttings (MARINHO et al., 2008).

The aim of this study was to evaluate the potential of two sources of cutting rooting using rooting inducers, as well as the effect of the cut type in cutting base.

## 2. MATERIAL AND METHODS

The experiments were performed at the Biotechnology Laboratory of the Department of Horticulture and Forestry at Universidade Federal do Rio Grande do Sul, under greenhouse conditions without temperature control, with intermittent irrigation by misting, maintaining a relative humidity of 80% and an average temperature of 22.4 °C. Three experiments were performed, one of leaf cuttings and two using young branches. The stock plants were kept under greenhouse conditions with periodic fertilization on 5 L polypropylene bags and irrigated by dripping with three daily shifts of three minutes each.

For all three treatments, the experimental design was completely randomized, with four replications, and block was dependent on the location on greenhouse and experimental unit consists of ten explants arranged in honeycomb tray of plastic with cells from 20 mL, totaling 40 explants per treatment. It was used charred rice husk as substrate, previously sterilized by moist heat on 1.3 atm pressure and at 120 °C for 20 minutes.

When there was a significant difference in ANOVA, means were compared by Tukey test ( $P > 0.05$ ) using ASSISTAT (SILVA; AZEVEDO, 2002)

### 2.1 Experiment 1. Propagation by leaf cuttings

For propagation by leaf and node cutting, mature leaves were used of approximately 22 cm<sup>2</sup> of laminar area obtained from plants in young stage, with no presence of flower branches at the time of establishment. The cutting was taken maintaining a leave with bud and about one centimeter and the region involves bud. Leaves were placed on the substrate were in a semi-inclined position to increase the perception of light.

The three treatments by contact with the cutting area with the product for rooting induction were: immersion for 18 hours in AIB solution (0.1 g L<sup>-1</sup>); immersion for 18 hours in H<sub>2</sub>PO<sub>4</sub> solution (0.2 g L<sup>-1</sup>) and ascorbic acid (5 g L<sup>-1</sup>) for 10 seconds and commercial powder for rooting (AIB 10 g kg<sup>-1</sup>). The evaluated variables were: percentage of callus formation, rooting, survival and mass of roots or callus by cuttings.

### 2.2. Experiment 2. Propagation by cuttings of young branches: growth regulators action

For propagation by cuttings of young branches, mother-plants were pruned and arranged in semi-inclined position to increase the regrowth emission area. Cuttings of five cm length with two whole leaves were used as explants.

At the base of cuttings part of the phloem was removed and this region was submerged for ten seconds in solutions. Subsequently, cuttings were placed at an angle of 45 ° in relation to 20 mL plastic tray. It used the same substrate and conditions of the Experiment 1.

Four AIB concentrations were evaluated (zero, 1,500, 3,000 and 6,000 mg L<sup>-1</sup>) in hydro alcoholic solution at 40% in the absence and presence of BAP (500 mg L<sup>-1</sup>), in accordance with subdivided plots, with BAP as the main plot and subplots the doses of AIB. It was evaluated the following variables: rooting percentage, volume, number and dry weight (g) of roots per cutting:

### 2.3. Experiment 3. Propagation by cuttings of young branches: cut types

In parallel with the experiment 2, the effect of three cut types on the base of the cutting (bevel cutting, double and straight bevel) in the formation of roots were evaluated. The following variables were assessed: number, mass (g) and volume (mL) of roots per cutting: The experiment was conducted under the same conditions as the previous, without the application of growth regulators.

## 3. RESULTS

### 3.1. Experiment 1. Propagation by leaf cuttings and bud

The three treatments with solution of 100 mg L<sup>-1</sup> AIB and 0.2 g L<sup>-1</sup> of phosphoric acid (H<sub>2</sub>PO<sub>4</sub>) showed low percentage of rooting (18%), while the use of the regulator in powder (AIB 10 g kg<sup>-1</sup>) had a high percentage

of live cuttings with callus formation at the base and rooting percentage almost null (3%) (Table 1).

By the time of the evaluation of the experiment, cuttings of any treatments showed development of air structure (Figure 1b).

### 3.2. Experiment 2. Propagation by cuttings of young branches: growth regulators action

In the absence of BAP, AIB treatments showed more than 97% of rooting. The use of BAP (500 mg L<sup>-1</sup>) showed inhibitory effect for all variables with no statistical difference between treatments (Table 2). However, at the same concentration of BAP, in any of the assessed variables were significant differences between the doses of AIB (Table 2).

### 3.3. Experiment 3. Propagation by cuttings of young branches: cut types

Based on the cutting effect on the rooting, the cut types test on basis cutting without treatment with regulator showed that the number of roots per cut was significantly higher in straight cut compared to those that were beveled cut. The average volume of roots per cutting was 0.89 mL, with no significant difference between treatments (Table 3).

## 4. DISCUSSION

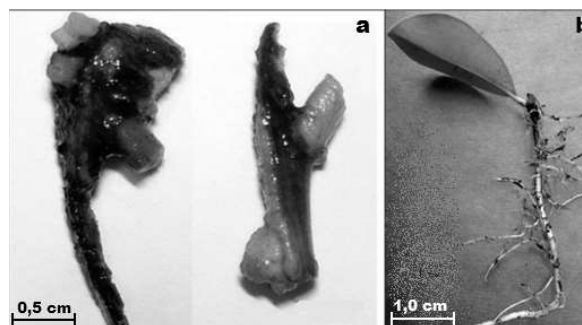
### 4.1. Experiment 1. Propagation by cuttings of leaves

Peixe et al. (2007) in the propagation by cuttings of *Olea europaea* L., observed that ontogeny of mass cell named callus, it would be closely linked by its subsequent differentiation from parenchyma cells, the subsequent formation of adventitious roots. In turn, Quadros (2009), in the propagation of plant cuttings of *Ilex paraguariensis* observed a positive correlation between the percentage of callus formation and survival;

**Table 1** – Effect of treatment on leaf cutting rooting after 120 days. Callus formation (C), rooting (R), death (M) root dry matter per cutting (R) and callus (C), UFRGS, Porto Alegre, 2016.

**Tabela 1** – Efeito do tratamento no enraizamento de estacas de folha após 120 dias de estabelecimento. Formação de calo (C), enraizamento (R), morte (M) e massa seca de raiz por estaca (R) e calo (C), UFRGS, Porto Alegre, 2016.

Treatment	Percentage			Dry matter (gr)	
	Callus	Root	Died	Callus	Root
1-IBA in solution (100 mg L <sup>-1</sup> )	45 <sup>b</sup>	18 <sup>a</sup>	35 <sup>b</sup>	0.25 <sup>ns</sup>	0.095 <sup>ns</sup>
2-H <sub>2</sub> PO <sub>4</sub> in solution (0.2 g L <sup>-1</sup> ) and Ascorbic acid 5 g L <sup>-1</sup>	18 <sup>c</sup>	18 <sup>a</sup>	65 <sup>a</sup>	0.23	0.95
3-IBA in industrial powder (10 g kg <sup>-1</sup> )	75 <sup>a</sup>	3 <sup>b</sup>	23 <sup>b</sup>	0.23	0.08
Mean (%)	46	13	41	0.24	0.09
C.V %	18.9	46	22.7	22.14	31.3



**Figure 1** – Cut detail of *P. cattleanum* leaf cuttings, thirty days after the establishment for root induction: a. callus formation. b. root formation, UFRGS, Porto Alegre, 2016.

**Figura 1** – Detalhe do corte em estacas de folha de araçazeiro trinta dias após estabelecimento em condições de sala de nebulização: a. formação de calo, b. formação de raiz, UFRGS, Porto Alegre, 2016.

Huetteman and Preece (1993), on the other hand, recognize that callus formation is a preliminary step in the regeneration of adventitious organs, but this may become inhibitory on woody species.

The propagation technique by leaf cutting has been successfully used in the propagation of other horticultural crops such as cassava and potato (CIAT, 1983) and with a moderate level of rooting on species such as avocado (*Persea americana*), but without the development of shoot, which was achieved only plant production using semi-woody cuttings (FERREIRA, 2008), similar to the results obtained in *Cedrela fissilis* (XAVIER et al., 2003).

The prioritization in the formation of root structures, reported with *P. americana* (FERREIRA, 2008) and *Cedrela fissilis* (XAVIER et al., 2003), is possibly caused by physiological structures prioritizing for the absorption of nutrients.

**Table 2** – Rooting percentage (%), Root volume per cutting (mL), number of roots per cutting, root dry mass (MSR) in the propagation of *P. cattleianum* by cutting using different doses of BAP and AIB. UFRGS, Porto Alegre, 2016.

**Tabela 2** – Percentual de enraizamento (%), volume de raiz por estaca (mL), número de raízes por estaca, massa seca de raiz (MSR) na propagação de *P. cattleianum* por estaca herbácea, utilizando diferentes doses de BAP e AIB. UFRGS, Porto Alegre, 2016.

BAP Concentration (mg L <sup>-1</sup> )	IBA Concentration (mg L <sup>-1</sup> )	Rooting (%)	Root volume by cutting (mL)	number of roots by cutting	root dry matter and mean
0	0	98 ns	2.23 ns	7.4 ns	21.59 ns
	1500	100	3.13	9.45	21.89
	3000	95	2.63	10.88	26.96
	6000	98	3.25	9.53	22.24
Mean		97.75 a	2.81a	9.315 a	23.17a
500	0	83	1.50	5.73	8.63
	1500	85	1.25	5.05	9.67
	3000	68	0.95	3.81	6.76
	6000	75	0.98	4.99	7.73
Mean		77.75 b	1.17 b	4.895 b	8.1975 b
Data transf.		arsen x	raiz (x+1)		
P value (BAP)		<0.001	<0.001	<0.001	<0.001P
value (IBA)		0.284	0.615	0.922	0.942 P
value (BAP x AIB)		0.908	0.179	0.314	0.557
CV %		27.2	35.1	16.96	35.5

**Table 3** – Effect of cut type on cuttings of young branches of *P. cattleianum*, a. number of roots, b. dry weight, c. volume of roots per cutting. UFRGS. Porto Alegre. 2016.

**Tabela 3** – Efeito do tipo de corte no enraizamento de estacas de ramos jovens de *P. cattleianum*, a. número de raízes, b. massa seca, c. volume de raízes por estaca enraizada. UFRGS, Porto Alegre, 2016.

Cut Type	Number of roots	Dry weight	Volume
Horizontal	7.41 a	30.16 a	0.75 ns
Simple bisel	5.13 b	20.39 b	1.02
Double bisel	5.67 ab	26.47 ab	0.92
Average	6.07	25.67	0.89
CV %	49.00	0.30	9.90
P value	0.012	0.004	0.34

Not differ at the level of 5% probability of error by Tuckey test. \*\* Ns = not significant.

#### 4.2. Experiment 2. Propagation by cuttings of young branches: growth regulators action

Hartmann et al. (2002) indicate that callus formation on cuttings is indicative of suitable environmental conditions for rooting. However, no formation in young seedlings but in adults is indicative of low juvenility. Under this premise, the incubation conditions of this experiment were favorable to morphogenesis.

Among treatments, the 6- benzylaminopurine (6-BAP) of cytokinins group associated to the stimulation of cytokinesis and subsequent development of axillary meristems, key in the propagation by cuttings evaluated in this experiment, has showed inhibitory. According to Skoog and Miller (1957), the hormonal

group of cytokinin, which BAP is part, is related with cytokinesis stimuli, generating responses in growth of axillary meristems (MOTYKA et al., 1996).

The response to auxin in tissues, of asymmetrical presence in stem and root is defined, among other factors, by its location. Based on the above, the presence of cells less specialized in juvenile tissue, site of auxin synthesis and lower degree of cell differentiation, promote the formation of adventitious roots (DAVIES, 2010).

A greater rhizogenesis response of tissues without the use of auxinic compounds of synthesis can be also correlated with low degree of lignification of cuttings, being this last can become limiting. In the propagation of *P. cattleianum*, young shoots of adult plants showed

greater ability of rooting compared to more lignified explants (FACHINELLO et al., 2005). Olesen (1978) suggest that the use of this kind of explants obtained from stock plants in juvenile stage can reduce the effect associated with topophysis.

Considering the high percentage of cuttings observed with callus formation, both, propagation by leaf cuttings and young branches, freehand cuts were performed in rooting region of cuttings in various stages of formation. The cuts suggest that the mass of callus is not a rooting pioneer (Figure 2d and Figure 2b), and this shown as a cellular mass that covers the cut region (Figure 2c) and that its formation can be avoided with early appearance of roots (KOMISSAROV, 1968), while the last ones differed from pro-exchange (2f) of the tissue where there was no cut (Figure 2a) and (Figure 2-e). Other authors reported that the formation of adventitious roots primordia begins by parenchyma cell division similar the divisions that originate lateral roots in pericycle primary roots (CALDERON et al., 2004), or this is favored when xylem and phloem tissues are exposed by cuts (FACHINELLO et al., 1995)

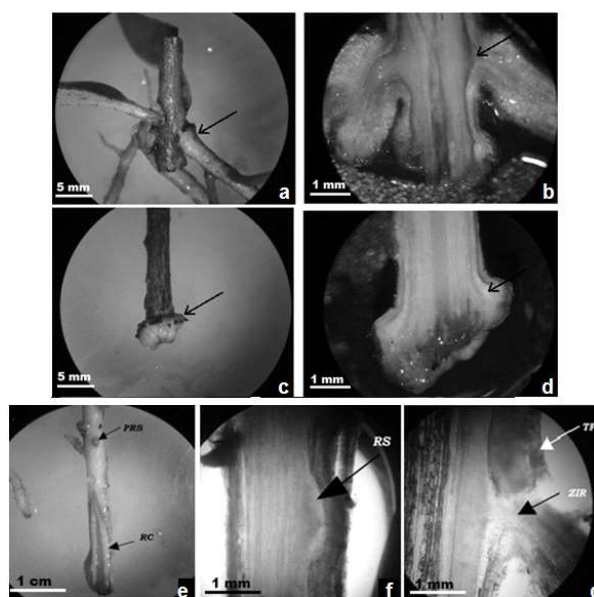
#### 4.3. Experiment 3. Propagation by cuttings of young branches: cut types

According to Baltierra et al. (2004), the formation of root adventitious occurs from cell divisions in the cortical parenchyma region, similar to those that originate secondary roots in pericycle. In *P. cattleianum*, roots formed in this section not affected by cut, whereas in the present, there was no formation of scar callus (Figure 2e), which shows that the practice of double cut does not increase the formation of roots of this species.

### 5. CONCLUSIONS

Under the experimental conditions, it is concluded that:

- *P. cattleianum* is easy rooting through the propagation by cuttings.
- There was root induction in plant cuttings without AIB use.
- The straight cut in the cuttings is most suitable for root formation.
- Propagation by leaf cutting shows low potential for root formation.



**Figure 2** – Freehand cut of rooting zone in cuttings of *P. cattleianum*. a. primary root. b. Detail of the formation of lateral roots. c. callus formation on cutting base. d. Freehand cut showing callus region, e: The formation of root primordia (PRS) and callus outside the cutting area (RC), f: longitudinal stem section showing the beginning of the formation of secondary root (RS), g: Development of secondary roots in vascular exchange region (ZIR) through the phloem (TF). UFRGS, Porto Alegre, 2016.

**Figura 2** – Corte a mão livre da zona de enraizamento em estacas herbáceas de *P. cattleianum*, a. raiz primaria, b. Detalhe da formação de raízes laterais, c. Formação de calo na base da estaca, d. Corte a mão livre da região de formação do calo na base de estaca, e: Formação dos primórdios radiculares (PRS) e calo fora da região do corte (RC), f: Corte longitudinal do caule mostrando o início de formação da raiz secundaria (RS), g: Desenvolvimento de raiz secundaria na região do câmbio (ZIR) (TF). UFRGS, Porto Alegre, 2016.

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