



## Air layering in *Caryocar brasiliense* – effect of stem diameter

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**ABSTRACT:** Seed propagation of *pequi* (*Caryocar brasiliense*) has many disadvantages, including low germination rates and high genetic variability of the resulting plants. Therefore, vegetative propagation techniques, such as the air layering process, are potentially important for this species. This study evaluated the effects of stem diameter and parental plant on the air layering efficacy of *pequi*. The air layering process was carried out in mid-December 2019, in the following stem diameters: less than 15 mm; 15-19 mm; 20-24 mm; 25-29 mm and above 29 mm. The treatments comprised four replicates of 10 air layers. After 3 months, the percentages of survival, callus formation and rooting were evaluated, in addition to the rooting vigor. The percentages of survival and callus formation observed in the air layering, regardless the parental plant, were higher than 85%, showing the high potential of this technique in the propagation of *pequi*. The callus differentiation rate in roots varied between the parental plants. Considering the evaluated parameters, stem diameters ranging from 20 to 24 mm are the most suitable ones for the air layering process in *pequi*. These results indicated the high potential of the air layering technique for the commercial propagation of *pequi*.

**Key-words:** callus formation, plant propagation, rooting, stem diameter.

## Alporquia em *pequi* – efeito do diâmetro do caule

**RESUMO:** A propagação seminal de *pequi* acarreta grande variabilidade na taxa de germinação e no genótipo das mudas produzidas, mostrando a necessidade do desenvolvimento de técnicas para a propagação vegetativa dessa espécie, como a alporquia. Os objetivos deste estudo foram avaliar a eficácia da alporquia, bem como o efeito do diâmetro do caule e da planta-mãe na sobrevivência, no calejamento e no enraizamento de alporques de *pequi*. O processo de alporquia foi realizado em meados de dezembro de 2019, nos seguintes diâmetros de caule: inferior a 15 mm; 15-19 mm; 20-24 mm; 25-29 mm e superior a 29 mm. Os tratamentos foram testados em quatro repetições de 10 alporques. Após três meses, avaliaram-se as porcentagens de sobrevivência, de calejamento e de enraizamento, além do vigor de enraizamento. As porcentagens de sobrevivência e de calejamento nos alporques, independentemente da planta matriz, foram superiores a 85%, mostrando o grande potencial dessa técnica na reprodução de *pequi*. A taxa de diferenciação de calos em raízes variou entre as matrizes. Considerando os parâmetros avaliados, diâmetros de caule de 20 a 24 mm são os mais adequados para a alporquia em *pequi*. Esses resultados indicam o potencial da técnica de alporquia para a produção comercial de mudas de *pequi*.

**Palavras-chave:** calejamento, *Caryocar brasiliense*, enraizamento, diâmetro de ramo, propagação de plantas.

The commercial potential of *pequi* is increasing nowadays, due to the remarkable organoleptic characteristics of the fruits, which are suitable for a large number of uses, such as oil production, culinary purposes, pharmaceutical industry, nuts and liquor production and as an animal fodder (GERASEEV et al., 2011; AFONSO et al., 2015; BEZERRA et al., 2015).

This species, as well as other native tree species, is normally propagated through seeds, especially due to the lower costs involved and the lack

of information about other propagation methods (DIAS et al., 2012). However, this propagation technique implies some difficulties including dormancy, which results in low and often variable germination rates (DOMBROSKI et al., 2010; NASSORY & CUNHA, 2012). The fruit pulp (mesocarp) contains substances that prevent germination (NASSORY & CUNHA, 2012), the endocarp offers mechanical resistance to seed germination (ROCHA, 2009; PEREIRA et al. 2004) and the seeds also show embryonic dormancy, which can be partially alleviated by exogenous

application of growth regulators such as gibberellic acid (ROCHA, 2009, DOMBROSKI et al., 2010, SILVA & LEONEL, 2017, SOUSA et al., 2017). However, even in the presence of this substance, the germination rates often do not reach 50% (NASSORY & CUNHA, 2012, SILVA & LEONEL, 2017). Large amplitudes in the germination rate of pequiizeiro seeds have also been related to genotype effects, which is probably due to dormancy level effects, among other factors (PEREIRA et al., 2004). Genotype effects on seed germination rates were also reported in other tree species (PEREIRA et al., 2004, COSTA et al., 2005, COSTA et al., 2009).

Besides dormancy restrictions, pequiizeiro plants produced from seeds also have great genetic variability due to its high allogamy rate, which causes enormous phenotypic variability in the orchards. Therefore, vegetative propagation studies have been carried out in order to overcome the limitations of sexual propagation (VALENTINI et al., 2011; DUTRA et al., 2012; PEREIRA et al., 2017). The main advantages associated to vegetative propagation include the maintenance of the genetic identity of the stock plants (clonal propagation), the uniformity of plants development, the fast initial growth and also the possibility of using this technique to recover the genetic diversity of some native species (DIAS et al., 2012; STUEPP et al., 2018). The grafting process has been widely used as a vegetative propagation method for fruit plants (PEREIRA et al., 2002), including pequiizeiro. However, even in this method the rootstocks are obtained from seeds, with the limitations described above. Thus, other cloning methods have been studied in pequiizeiro, especially via cutting, but still with low success rates (SANTOS et al., 2006; LEITE et al., 2007; PEREIRA, 2017).

The air layering technique, which induces the formation of adventitious roots in the stems still connected to the stock plants, is used as a successful propagation method in some fruit species such as jabuticabeira (*Plinia cauliflora*) and lichia (*Litchi chinensis*). It is simple, does not require a sophisticated structure (misting house, greenhouse etc.) and generates more rustic plants with environmental pre-adaptation. This technique induces the formation of adventitious roots in the stems still connected to the stock plants and; although, more laborious, it is often more efficient than the cutting method in some species, such as peach trees (CASTRO & SILVEIRA, 2003). In addition, this technique is often successful in propagating species that are difficult to root by cuttings (HARTMANN et al., 2014), which is the

case of pequiizeiro. Rhizogenesis is favored in this situation by the constant supply of water and minerals from the xylem, which is intact; and the accumulation of photo assimilates and hormones from leaves and buds in the air layering region (HARTMANN et al., 2014).

Studies with the air layering technique have shown that many aspects can interfere with the formation of callus and rhizogenesis in the stems. These aspects include the exogenous application of indole-3-butyric acid (LEITE et al., 2007), the season of the year in which the air layering process is carried out (CASSOL et al., 2015), the characteristics of the coating involving the layering (CASSOL et al., 2015) and the diameter of the stem used for this purpose (SASSO et al., 2010). According to SASSO et al. (2010) the rate of rooting in branches of jabuticabeira (*Plinia cauliflora*) with 2.5 cm of diameter was 87.5%, while in branches with 1.5 cm it was 50%. The number and length of roots was also higher in branches with a larger diameter. TCHOUNDJEU et al. (2010) observed rooting rates of around 47% in air layering carried out on branches with 2-3 cm, while the highest mortality occurred at 4-5 cm diameter branches in *Irvingia gabonenses*, a native species from the African continent.

Therefore, many aspects can affect the efficacy of the air layering process. Considering the low efficiency of propagation techniques, coupled with the scarcity of studies in air layering in pequiizeiro, the objective of the present study is to evaluate the effects of the stem diameter and of the parental plant in the survival, in the callus formation and in the rooting of pequiizeiro air layering.

Due to the low number of suitable branches for layering in a single plant, aiming to reduce the damage to the stock plants, four pequiizeiro trees (*Caryocar brasiliense* Cambess), located at Fazenda Água Limpa-UnB (15°56'49.3" S, 47°55'47.3" W), Distrito Federal, were used in the experiment (Figure 1B). The stock plants were naturally grown, with estimated age of 30 to 40 years, homogeneous in terms of phenotypic characteristics, well developed, with 5-6 m height and each of them were considered a replicate in this study.

The experiment was carried out in a randomized block design, with 5 treatments (branch diameters) and four replications (stock plants). The following stem diameters were tested: smaller than 15 mm; 15-19 mm; 20-24 mm; 25-29 mm and above 29 mm. Each experimental unit (plot) comprised 10 air-layers in lignified branches, due to the high mortality rate of the new branches (green) submitted to the

layering process (unpublished data). The air layering process was undertaken in the basal (lower) part of the stock plants and as closer as possible to the branch end (where the leaves are located in this species). The air-layers were assembled on four successive days in mid-December 2019, at the flowering plant growth stage (BBCH-scale). Once the air layering process was accomplished, the flowers and eventual fruits were removed from the branch end.

The air layering process comprised the following steps: the branches were girdled with the aid of pliers adapted for air layering (Figure 1A), in order to completely remove the bark, resulting in a 3.0 cm width ring; the ringleted region was wrapped out with a transparent plastic bag (10 x 20 cm), cut longitudinally in one side, containing the commercial Bioplant Plus® substrate (produced by Bioplant, Brazil) and tied up with transparent adhesive tape. Each air-layer was labeled according to the treatment. The substrate, which was previously moistened with water, avoiding excess, contains *sphagnum* peat, coconut fiber, pine bark and vermiculite enriched with macro and micronutrients.

Three months after assembly, the air-layers were cut off from the stock plants using pruning shears, 10 cm above and below the girdled region. The survival, callousness (callus formation) and

rooting percentages were evaluated. Rooting vigor (intensity) was also evaluated in each calloused air-layer, using the following scale (defined according to preliminary tests results), with grades ranging from 0 to 5, in which: 0 – callus formation without rooting; 1 - from 1% to 20% of the calluses emitted roots; 2 - from 21% to 40% of the calluses emitted roots; 3 – from 41% to 60% of the calluses emitted roots; 4 - from 61% to 80% of the calluses emitted roots; 5 - from 81% to 100% of the calluses emitted roots (Figure 2). The means of each treatment for survival, callus formation and rooting of air-layers were subjected to analysis of variance and, in case of significance, to the Scott-Knott clustering test ( $p$ -value  $\leq 0.05$ ). The average rooting vigor for each stem diameter was analyzed using polynomial regression. Statistical analyses were carried out using the Genes software (CRUZ, 2013).

The stem diameter affected the survival and the callus formation percentages of the pequizeiro air-layers (Table 1). Practically all branches with diameter above 20 mm survived and produced callus when submitted to the air layering process. Conversely, the survival of the branches was reduced by half when the air layering process was carried out in stems smaller than 15 mm of diameter. The callus formation capacity of the surviving branches

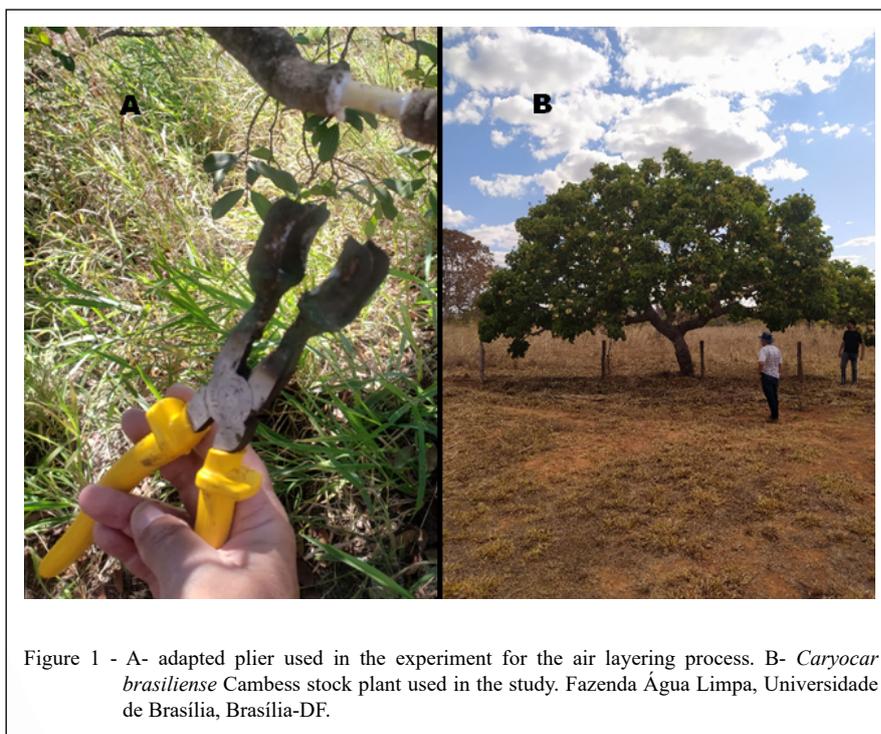
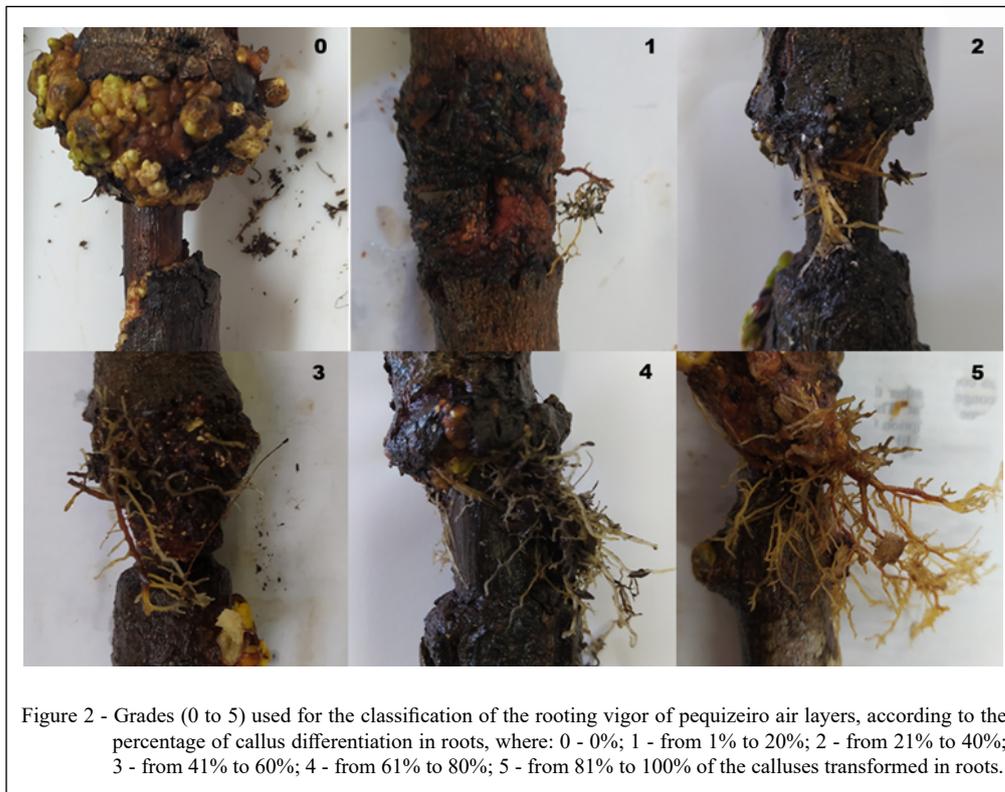


Figure 1 - A- adapted plier used in the experiment for the air layering process. B- *Caryocar brasiliense* Cambess stock plant used in the study. Fazenda Água Limpa, Universidade de Brasília, Brasília-DF.



was nearly complete, once the survival and callus formation percentages were practically the same for the different stem diameters (Table 1).

According to HARTMANN et al. (2014) and SINGH & ANSARI (2014), callus formation precedes the formation of adventitious roots in some species, while in others these processes are independent. There is still no classification of pequi in the literature regarding this aspect, but

the observations of the present study indicated that the callus formation precedes the rooting process in this species. Even though the presence of calluses is not a safe indicator for root formation, it is of fundamental importance in air layering, as it indicates that the plant responded to the treatment (CASSOL et al., 2015).

Significant effects of the stem diameter on callus formation and rooting have been observed in

Table 1 - Effect of stem diameter on survival, callus formation and rooting of *Caryocar brasiliense* air-layers. Fazenda Água Limpa, Universidade de Brasília, Brasília-DF.

Stem diameter (mm)	Survival (%)	Callus (%)	Rooting (%)
<15	52b	50b	12a
15-19	87a	87a	45a
20-24	97a	97a	42a
25-29	100a	100a	40a
>29	100a	100a	40a
Average	87	87	36
CV (%)	11.7	13.5	50.5

Means followed by the same letter in each column are not different by the Scott-Knott Test ( $p$ -value  $\leq 0.05$ ).

other species, such as jabuticabeira (SASSO et al., 2010). In that study, the rooting rate ranged between 50% and 87% in different stem diameters, which was attributed by the authors to the higher amount of carbohydrates in branches of larger diameter. Conversely, ABDON et al. (2015) did not observe significant differences in the rooting process of air-layers in branches with diameters between 1-3 cm in *Prosopis africana*, but they did observe that the rooting rate of this species is influenced by the climate of the region, mainly by humidity.

Apparently, the rhizogenesis process occurred from the calluses, in 40-45% of the air layers, in diameters above 15 mm (Table 1). The stock plants did not affect the rates of survival and callus formation, but it affected the rooting rate, which ranged from 6 to 72% according to the stock plant (Table 2). These results showed that the rooting response to the air layering process depend on the stock plant, despite their visual uniformity. Genotype effects have been observed in the rooting rates of other species, such as plum (MOZUNDER et al., 2017) and olive tree (AFZAL et al., 2017). In olive trees, this effect was observed regarding the rooting term and number of roots per layering in addition to the rooting rate (AFZAL et al., 2017). SAUCO et al. (2018) reported higher rooting rates of lychee air-layers in young plants, compared to the mature ones. In the cutting process, genotype effect has also been observed in the rooting capacity of pequiizeiro tested in a greenhouse (GUIMARÃES et al., 2019). Further studies are necessary to elucidate the factors related to the stock plant that can affect the rooting vigor of both air-layers and cuttings in pequiizeiro.

In addition to the rooting rate, the stem diameter also affected the rooting vigor. Stems with diameter between 20 and 24 mm (Figure 3) showed the highest vigor of the root development (average score of 1.4 on the scale). Similar results were

obtained by SASSO et al. (2010) in the jabuticaba tree layering, whom observed the largest number and length of roots in layering with diameter between 20 and 24 mm.

The pequiizeiro air-layers rooting rates observed in the present study (up to 72%) are relatively high compared to the rooting rates observed in other studies. LEITE et al. (2007) and CASSOL et al. (2015) observed an average of 9% and 20% of rooting in pequiizeiro and jabuticaba trees, respectively, with IBA. DUTRA et al. (2012) also reported rooting rates below 30% in umbuzeiro air layering.

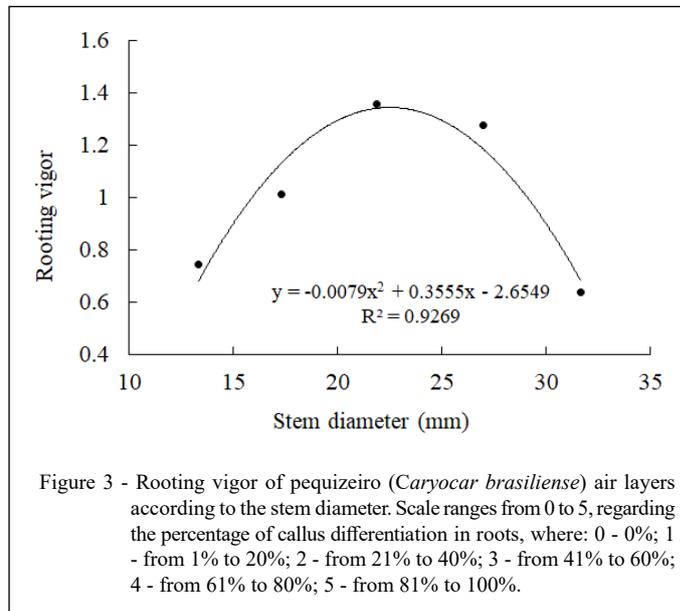
These results confirmed the effect of the stem diameter on the efficacy of the air layering process in pequiizeiro and also demonstrated the potential of this technique for pequiizeiro propagation, once the survival and the callus formation reached 100% in some treatments. They also demonstrate the viability of the air layering process for the pequiizeiro cloning, considering the high survival rates, callus formation and rooting observed. The importance of selecting stems with an adequate diameter for the air layering process was demonstrated, once this parameter affects the branches survival. In order to maximize the success rate of this technique in pequiizeiro, further studies are recommended relating to the factors of the parental plants (genotypes) that can affect the rate and vigor of rooting.

It was concluded that pequiizeiro cloning via air layering is feasible, generating high rates of survival and callus formation, and, in some stock plants, rooting above 70%; the rate of differentiation of calluses in roots varies between parental plants; the stem diameter affects the efficacy of the air layering. Considering survival, callus formation, rooting rate and vigor, stem diameters ranging from 20 to 24 mm are ideal for the air layering process.

Table 2 - Stock plant effect on survival, callus formation and rooting of *Caryocar brasiliense* air-layers.

Stock plant	Survival (%)	Callus (%)	Rooting (%)
1	94a	94a	14b
2	86a	86a	72a
3	82a	80a	52a
4	88a	88a	6b
Average	88	87	36
CV (%)	11.7	13.5	50.5

Means followed by the same letter in each column do not differ by the Scott-Knott Test ( $p$ -value  $\leq 0.05$ ).



## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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