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ROOTING OF ARAUCARIA MINI-CUTTINGS IN DIFFERENT ENVIRONMENTS AND SUBSTRATES

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Resumo

Enraizamento de miniestacas de araucária em diferentes ambientes e substratos. Araucária é uma conífera nativa, com elevada importância econômica, sobretudo na região Sul do Brasil. Considerando as dificuldades da produção de mudas clonais da espécie, objetivou-se determinar a influência de diferentes ambientes e substratos na rizogênese de miniestacas de *Araucaria angustifolia*. Brotações ortrotópicas foram coletadas em minijardim clonal, para confecção de miniestacas com 10 ± 1 cm, mantendo-se 1/3 das acículas, e imersas em solução hidroalcóolica de 3.000 mg L^{-1} de ácido indolbutírico por 10 segundos. Em seguida, foram plantadas em tubetes de 210 cm³, testando-se quatro substratos: casca de pinus, vermiculita e carvão vegetal (S1); casca de pinus e vermiculita (S2); casca de pinus, tufa e fibra de coco (S3) e casca de pinus, vermiculita, carvão vegetal e casca de arroz carbonizada (S4). As miniestacas foram mantidas em três diferentes ambientes para enraizamento: Casa de Vegetação Automatizada (CVA) com redução de 80% de luminosidade e irrigação por nebulização, Casa de Vegetação Simples (CVS) com redução de 84% de luminosidade e irrigação por microaspersão e Estufim (EST) com redução de 90% de luminosidade e irrigação por microaspersão. Após 120 dias foram determinadas a sobrevivência e o enraizamento das miniestacas. A CVA proporcionou melhor enraizamento das miniestacas, já EST resultou em alta mortalidade e nenhuma formação de raízes. Não houve influência dos substratos sobre as variáveis avaliadas. Portanto, recomenda-se a utilização de casas de vegetação com irrigação automatizada por nebulização para enraizamento das miniestacas de araucária, independente do substrato utilizado, embora os índices gerais de enraizamento ainda não sejam considerados viáveis.
Palavras-chave: *Araucaria angustifolia*, pinheiro brasileiro, casa de vegetação, rizogênese, propagação vegetativa.

Abstract

Araucaria is a native conifer, with high economic importance, especially in the southern region of the Brazil. Considering the difficulties of producing clonal plants of the species, the objective of the study was to determine the influence of different environments and substrates on root formation of *Araucaria angustifolia* minicuttings. Orthotropics shoots were collected in a mini clonal garden. Minicuttings were prepared with 10 ± 1 cm in length, keeping 1/3 of the needles, and immersed in a hydroalcoholic solution of 3,000 mg L^{-1} of indolbutyric acid for 10 seconds. Then they were planted in 210 cm³ tubes, testing four different substrates, which are, based on pine bark, vermiculite and charcoal (S1); based on pine bark and vermiculite (S2); based on pine bark, peat and coconut fiber (S3) and based on pine bark, vermiculite, charcoal and carbonized rice husk (S4). These minicuttings were maintained in three different environments for rooting: Automated Greenhouse (CVA) with 80% reduction in luminosity and mist irrigation, Simple Greenhouse House (CVS) with 84% reduction in luminosity and microsprinkler irrigation, and Mini-tunnel (EST) with 90% light reduction and micro sprinkler irrigation. After 120 days, minicuttings survival and rooting were determined. CVA provided better rooting of minicuttings, whereas EST resulted in high mortality and no root formation. There was no influence of the substrates on the evaluated variables. Thus, the use of greenhouses with automated irrigation by misting is recommended for rooting araucaria minicuttings, regardless of the substrate used, although overall rooting rates are not yet considered viable.
Keywords: *Araucaria angustifolia*, brazilian pine, greenhouse, rhizogenesis, vegetative propagation.

INTRODUCTION

Araucaria angustifolia (Bertol.) Kuntze is a conifer native to the southern region of Brazil, associated with the Mixed Ombrophilous Forest. Timber and non-timber products are obtained from this species, giving it significant economic and social importance. The physical and chemical properties, as well as the low density of the wood, make this species highly valued for construction purposes, as well as for long fiber cellulose production. Its seed, commonly known as "pinhão," is widely consumed in the region, used in various traditional recipes, and also serves as food for wildlife (ZANETTE *et al*., 2017).

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Over the decades, there has been a drastic reduction in the occurrence area and the number of individuals, leading to the species being listed as endangered (BRASIL, 1992), and as a consequence, the prohibition of cutting down native individuals (CONAMA, 2001). Encouraging the planting of selected genetic materials and the sustainable use of their products may be the key to reducing the existing pressure on remaining individuals. Cloning through minicutting is one of the most commonly used tools in the production of seedlings of forest species for productive purposes (STUEPP *et al*., 2018).

Through the minicutting of superior genotypes, it is possible to increase productivity, reduce the rotation period of the forest, control product quality, among other benefits (XAVIER; SILVA, 2010). Establishing protocols for this and other cloning techniques for Araucaria is a viable option to encourage the planting of commercial areas with Araucaria and further enhance the economic potential of the species (WENDLING; BRONDANI, 2015).

Despite minicutting being widely used for some species, such as those in the E*ucalyptus* genus (FERNANDES et al., 2018), there is still a need to establish protocols for native species like araucaria. Several factors can influence the success of the technique, including environmental conditions during rooting, such as temperature, humidity, light, and substrate (CUNHA et al., 2009). Brightness is directly related to photosynthetic activity, humidity influences all plant metabolic activities, and temperature plays an important role in metabolism and cell division.; thus, all these factors act together in rhizogenesis (TAIZ *et al*., 2017). The substrate is determinant in the rooting process, being responsible for propagule support, for providing water and nutrients in adequate quantities, and is free of contaminants and pathogens (KRATZ et al., 2013). Based on this, the objective of this study was to determine the influence of different environments and substrates on the rhizogenesis of minicuttings of *Araucaria angustifolia*.,

MATERIAL AND METHODS

The experiment was conducted between December 2021 and April 2022, at the Laboratory of Forest Species Propagation of Embrapa Florestas, located in Colombo – PR (25°20' S and 49°14' W). The clonal mini-garden used was established in 2014, consisting of a mix of clones obtained from basal shoot cuttings of selected mature trees in the field. The mini-garden was set up in a semi-hydroponic system, with minicuttings distributed in a spacing of 10 x 10 cm, in channels filled with sand. The system was located inside a greenhouse covered with plastic and with movable sides, without temperature control. The minicuttings were irrigated daily using a nutrient solution composed of macro and micronutrients (PIRES et al., 2013), distributed three times a day, at a total flow rate of5 L m^{-2} (Tabela 1).

Table 1. Composition of the nutrient solution used for fertigation of *Araucaria angustifolia* mini in a semi-hydroponic system.
Tabela 1. Composição da solução nutritiva utilizada para fertirrigação das minicepas de *Araucaria angustifolia* em sistema semi-hidropônico.

Fertilizer	Concentration (mg L^{-1})
Macronutrient	
Monoammonium phosfate (MAP)	80,0
Magnesium sulfate	356,0
Ammonium sulfate	112,0
Calcium chloride	134,0
Calcium nitrate	578,0
Potassium chloride	300,0
Micronutrient	
Boric acid	2,88
Manganese sulfate	3,70
Sodium molybdate	0,18
Zinc sulfate	0,74
Iron chelate	81,8

Source: Pires *et al.* (2013)

For the experiment, orthotropic shoots were used, which are characterized by their vertical growth and emergence at the apex of the minicutting. Such shoots were collected from the minicuting and stored in styrofoam boxes containing cold water to minimize the loss of cellular turgor pressure in the tissues. Immediately afterward, the minicuttings were prepared using the apical and intermediate portions of the shoots. The minicuttings were standardized to 10 ± 1 cm in length, the base was cut at an angle, and 1/3 of the needles were removed. After preparation, the base of the minicuttings was immersed in a hydroalcoholic solution (1:1) with 3,000 mg L-1 indolebutyric acid (IBA) for 10 seconds, following the recommendation of Pires *et al.* (2013). Subsequently, they were planted in 210 cm³ plastic containers. Three commercial substrates were used (Table 2), and a combination (S4) was composed of substrate S1 and carbonized rice husk in a 1:1 (v/v) ratio. All substrates were supplemented with 4 g L-1 of slow-release Osmocote® (15-9-12) fertilizer (8 months).

Table 2. Characteristics of substrates used for rooting of *Araucaria angustifolia* minicuttings.
Tabela 2. Características dos substratos utilizados para enraizamento de miniestacas de *Araucaria angustifolia*.

Substrate*	**Composition**	**Moisture (%)**	**WRC (%)**	**Density wet base (kg.m^{-3})**	**EC (mS.cm^{-1})**	**pH**
S1	Pine bark, vermiculite and charcoal	60	150	400	0,7 ± 0,3	5,8 ± 0,3
S2	Pine bark and vermiculite	58	60	375	0,8 ± 0,3	6 ± 0,3
S3	Pine bark, peat and coconut fiber	67	187	450	0,5 ± 0,3	6 ± 0,3

* Information provided by manufacturers; WRC: water retention capacity; EC: electrical conductivity

After the cutting process, the tubes containing the minicuttings were kept in three different environments for rooting: 1) Automated Greenhouse (CVA), covered with an external layer of heat-reflective mesh and an internal layer of plastic, resulting in an 80% reduction in light; a FOG-type mist irrigation system with a flow rate of 4 L per hour^{-1}, automatically activated every 8 minutes for 10 seconds whenever the relative humidity was below 90%, along with two exhaust fans to maintain the temperature below 30ºC, and without movable sides 2) Simple Greenhouse (CVS), covered with an external layer of plastic and an internal layer of shading cloth, resulting in an 84% reduction in light; a mini-sprinkler irrigation system with a flow rate of 144 L per hour^{-1}, automatically activated four times a day for 30 minutes each time, without fans, and with movable sides opened whenever the temperature exceeded 30ºC. 3) Mini-tunnels (EST) (PEREIRA et al., 2020), covered with an external layer of shading cloth and an internal layer of plastic, resulting in a 90% reduction in light; a micro-sprinkler irrigation system with a flow rate of 33 L per hour^{-1}, activated for 15 seconds every 30 minutes, without fans and with no movable sides. After 120 days, the survival rate (live mini-cuttings, entirely green with or without roots) and the rooting percentage (mini-cuttings with at least one root of 1 mm) were evaluated. The design used was completely randomized, in a 3 x 4 factorial scheme (environments x substrates) with four repetitions of 10 mini-cuttings each. The data were subjected to tests for normality distribution (Shapiro-Wilk test) and homogeneity of variances (Bartlett's test). As the data did not meet these assumptions, the non-parametric Kruskal-Wallis test was applied, and when necessary, the Wilcoxon test for pairwise mean comparisons ($p < 0.05$) was used. All analyses were carried out using the R software (R CORE TEAM, 2022).

RESULTS

Among the rooting environments, CVS promoted 80% survival of the mini-cuttings, which was higher than the other environments (Figure 1A). The EST environment resulted in less than 7% survival after 120 days. Despite promoting higher survival, CVS did not favor root system formation. The highest rooting was observed in CVA (18.13%), which was superior to the other environments. In addition to low survival, EST did not promote rooting of the mini-cuttings (Figure 1B).

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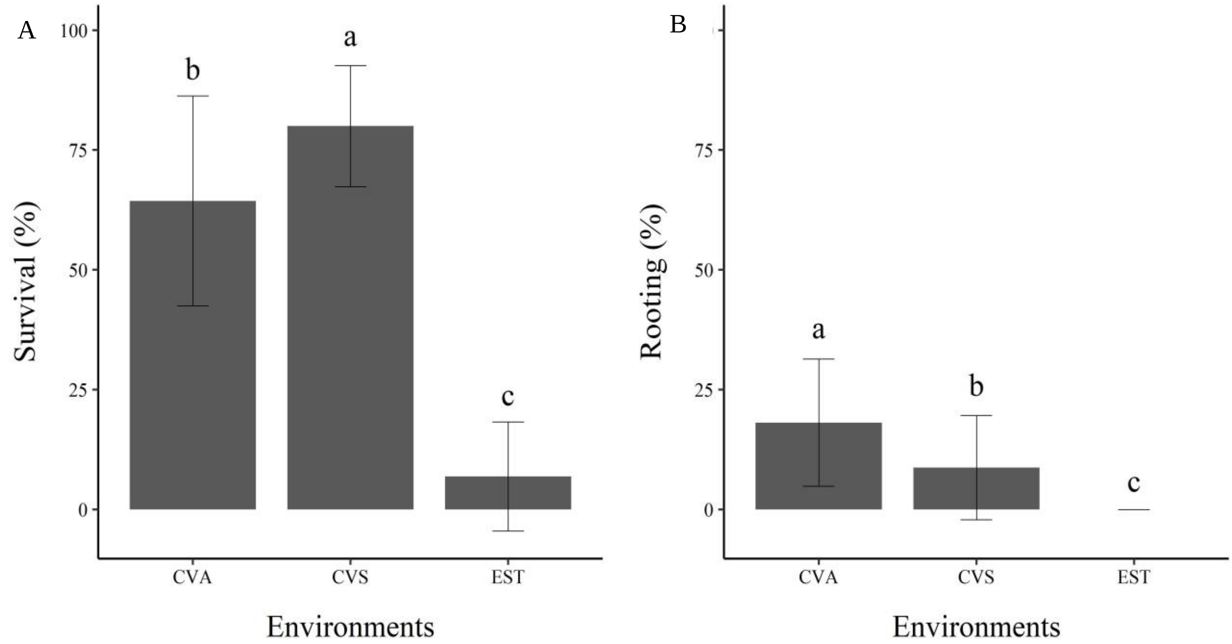

Figure 1. Survival (A) and rooting (B) of *Araucaria angustifolia* minicuttings submitted to different environments.

Figura 1. Sobrevivência (A) e enraizamento (B) das miniestacas de *Araucaria anustifolia* submetidas a diferentes ambientes.

Averages followed by the same letter do not differ significantly at the 5% probability level using the Wilcoxon test.

With respect to the substrates, no significant difference was observed for both variables analyzed (Figure 2). The survival of the mini-cuttings ranged between 46.7 and 52.5% with an overall average of 50.4%. Rooting varied between 6.7 and 12.5%, with an overall average of 8.9% (Figure 2B).

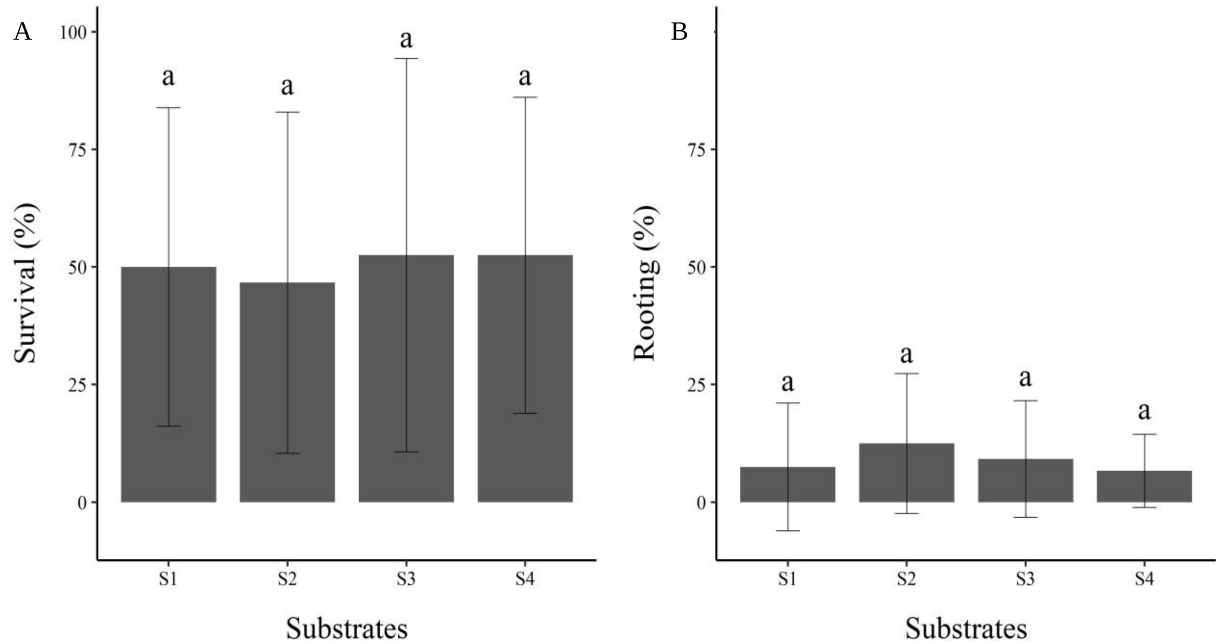

Figure 2. Survival (A) and rooting (B) of *Araucaria angustifolia* minicuttings submitted to four different substrates.

Figura 2. Sobrevivência (A) e enraizamento (B) das miniestacas de *Araucaria anustifolia* submetidas a quatro diferentes substratos.

Averages followed by the same letter do not differ significantly at the 5% probability level using the Kruskal-Walli test.

Upon visually analyzing the mini-cuttings throughout the experiment, it was found that the high mortality rate occurred due to rotting at the base, which progressed up to the apex of the mini-cuttings (Figure 3). This was observed mainly in the first 60 days following planting.

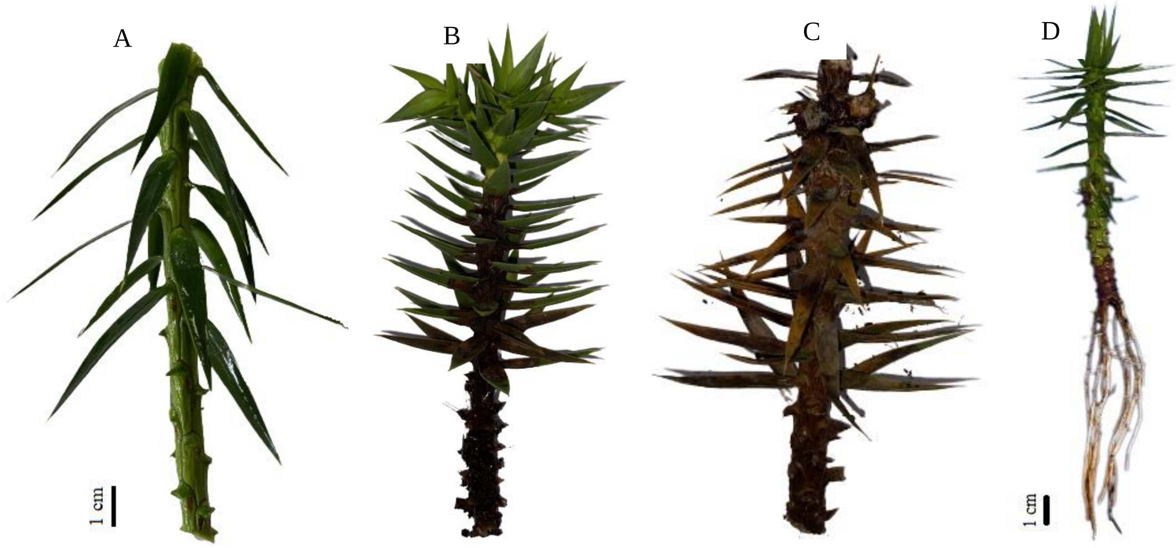

Figure 3. Visual aspect of rotting progression in *Araucaria angustifolia* minicuttings: (A) intact minicutting (alive); (B) minicutting with rotting starting at the base, (C) completely rotted minicutting and (D) rooted minicutting.

Figura 3. Aspecto visual da progressão do apodrecimento em miniestacas de *Araucaria angustifolia*: (A) miniestaca viva, (B) miniestaca com apodrecimento iniciando pela base, (C) miniestaca totalmente apodrecida e (D) miniestaca enraizada.

DISCUSSION

Although CVS provided the highest survival of the mini cuttings, this environment did not favor rhizogenesis, which was superior in CVA, indicating the need for a controlled environment for the development of roots in this species. The use of a system that is automatically activated based on the percentage of air humidity ensures a greater water supply, especially on days or at times with high temperatures (BRONDANI et al., 2007). Another advantage of CVA is the mist irrigation system, which, according to Milhem *et al.* (2014), ensures greater uniformity in water supply and maintenance of relative air humidity, thereby favoring the development of adventitious roots.

In relation to temperature, Araucaria is a subtropical species, adapted to milder temperatures throughout the year (PIRES et al., 2015), a factor that may influence the species' rhizogenesis. Therefore, it should be noted that in all rooting environments there was no temperature control, subjecting the mini-cuttings to variations during the rooting period, except in CVA, where temperature was reduced through exhaust fans whenever it reached 30 ºC. Through daily monitoring, it was found that the temperatures in CVA ranged between 21 and 30 ºC, for CVS between 18 and 28 ºC, and EST between 15 and 21 ºC during the 120-day period, a fact that may have influenced the rhizogenic potential of the propagules. In pine species, for example, Alcântara *et al.* (2007) found that winter is more favorable for collecting mini-cuttings of *Pinus taeda*, due to the lower temperatures. For *Pinus radiata*, it is recommended to root the minicuttings during the summer, from shoots collected at the end of winter (CORRÊA et al., 2015). Xavier *et al.* (2013) reported that air temperatures in the rooting environment should not be excessively high or low, as this could slow down the metabolism of the mini-cuttings, which may prolong the time needed for rooting or even be insufficient for the induction, development, and growth of roots. Climatic variations can affect not only the metabolism of mini cuttings but also hormonal and nutritional conditions, among other aspects that may be involved in rhizogenesis (HARTMANN *et al*., 2011).

The rooting percentages found in this study are below those already described in the literature by Pires *et al.* (2013) testing the application of growth regulators (32%), and by Pires *et al.* (2015) testing different nutrient solutions (83%) applied to the mini-stumps. However, the current study used adult genetic material, which naturally significantly increases the difficulty of rooting (WENDLING *et al*., 2014). Studies conducted by Wendling *et al.* (2016a) have already demonstrated the difficulty of rooting cuttings and mini-cuttings of A. angustifolia even with the application of plant regulators, a behavior described by Maggioni *et al.* (2020) as recalcitrance to vegetative propagation.

In addition to the environmental factors already mentioned, the physiological condition of the mini-stumps, associated with the juvenility of the propagative material, for example, can have a great influence on rooting (HARTMANN et al., 2011; WENDLING et al., 2016b). The minicuttings used in this study were produced from cuttings taken from adult trees that were selected in the field, suggesting that the material may still require rejuvenation to enhance root formation. In addition, the rooting of cuttings and mini-cuttings of Araucaria can be considered genotype-dependent, meaning it is influenced by the origin of the genetic material (MAGGIONI et al., 2020). The issue of rotting at the base of Araucaria minicuttings has also been reported in studies by Pires *et al.* (2013). This issue is common in less lignified propagules and in conifers, due to the presence of resin at the base of the cuttings. This resin creates problems with water absorption and aeration, leading to the development of necrotic tissue (HARTMANN et al., 2011). The moisture content of the substrate may have also facilitated the growth of fungi, which could harm root development and contribute to their rotting, however, this aspect was not evaluated in the present study, in this case, using more porous substrates could potentially reduce the likelihood of fungal growth.

The different compositions of the substrates tested in this work did not influence the rhizogenesis of the mini-cuttings, demonstrating that the environment was determinant in this process. There are various substrate options for seedling production of forest species, such as sand, peat, sawdust, carbonized rice husk, organic composts, coconut fibers, vermiculite, among others (DIAS et al., 2015). Combinations among these compounds, like the ones used in this study, are common. However, they don't always have an influence, given that the rooting process can be affected by various other factors that have already been mentioned. Other alternatives or combinations of substrates beyond those already tested could be considered in future studies, aiming to improve the physicochemical characteristics of the substrates and achieve better rooting results, or even better root development.

CONCLUSIONS

- The Automated Greenhouse (CVA) promoted greater rooting of the minicuttings is the most recommended environment for the production of clonal seedings of the species.
- The substrates based on pine bark; vermiculite and charcoal (S1); pine bark and vermiculite (S2); pine bark, peat and coconut fiber (S3) and pine bark, vermiculite, charcoal and carbonized rice husk (S4), can be used for the production of *A. angustifolia* through minicuttiong, although the general rooting indices are still not considered viable.

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