UNIVERSIDADE FEDERAL DO PARANÁ

VÂNIA BEATRIZ CIPRIANI

# OPTIMIZATION OF CLONING METHODOLOGIES FOR *Araucaria angustifolia*

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VÂNIA BEATRIZ CIPRIANI

# OPTIMIZATION OF CLONING METHODOLOGIES FOR *Araucaria angustifolia*

Tese apresentada ao Programa de Pós-Graduação em Engenharia Florestal, no Setor de Ciências Agrárias, da Universidade Federal do Paraná como requisito parcial para obtenção do título de Doutor em Engenharia Florestal.

Orientador: Dr. Ivar Wendling
Coorientadores: Dra. Kátia Christina Zuffellato-Ribas
Dra. Juliana Degenhardt Goldbach

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# TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ENGENHARIA FLORESTAL da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **VÂNIA BEATRIZ CIPRIANI** intitulada: **OPTIMIZATION OF CLONING METHODOLOGIES FOR *Araucaria angustifolia***, sob orientação do Prof. Dr. IVAR WENDLING, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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## RESUMO

O gênero *Araucaria* é composto por 20 espécies distribuídas em diversas regiões do planeta. No Brasil, *Araucaria angustifolia* (Bertol.) Kuntze possui grande relevância econômica, social, ambiental e cultural devido à sua madeira de alta qualidade e sua semente, o pinhão. A espécie atualmente está na lista de ameaçadas de extinção e, como consequência, a legislação vigente proíbe o manejo de indivíduos em áreas nativas. Desta forma, o estímulo à conservação mediante o uso tem se apresentado como a principal alternativa para evitar a extinção da espécie. Incentivar o seu plantio para fins madeireiros e não madeireiros requer a adoção de tecnologias como a propagação vegetativa, que tem sido pesquisada e aplicada em diversas espécies florestais, inclusive a araucária. Apesar dos avanços alcançados nos últimos anos, ainda restam lacunas para o aperfeiçoamento das técnicas. Neste sentido, o presente estudo teve como objetivo propor alternativas na aplicação das técnicas de miniestaquia e enxertia de *A. angustifolia*, contribuindo na elucidação de dúvidas e estímulo ao avanço dos estudos relacionados à espécie. O capítulo I, teve como objetivo determinar a influência de diferentes composições de substratos (casca de pinus, vermiculita, fibra de coco, turfa, casca de arroz carbonizada) e ambientes (casa de vegetação automatizada, casa de vegetação convencional e estufim) no enraizamento de miniestacas de *A. angustifolia*. Os resultados demonstraram não haver interação entre substrato e ambientes. A espécie não apresentou diferença de enraizamento em função dos tipos de substratos, podendo ser utilizados para a formulação de diferentes combinações entre casca de pinus, vermiculita, fibra de coco, turfa, casca de arroz carbonizada, dentre outros. Em contrapartida, o uso de casa de vegetação automatizada, com controle de umidade e irrigação por microaspersão favoreceu a sobrevivência (68%) e o enraizamento (18%) das miniestacas. O percentual de enraizamento registrado ainda não suporta o uso da técnica em escala comercial, sendo necessário buscar novas alternativas. O capítulo II teve como principal objetivo verificar a aplicabilidade da enxertia interespecífica, utilizando como modelo *A. angustifolia* e *A. bidwillii*, duas espécies do gênero produtoras de sementes comestíveis. Os resultados indicaram incompatibilidade tardia, com sintomas surgindo aos 7 anos após a realização da enxertia. A incompatibilidade é caracterizada pela diferença de crescimento do enxerto e do porta enxerto, sendo o diâmetro da *A. agustifolia* superior ao da *A. bidwillii*. Apesar de constatada a incompatibilidade, foi observada a produção de pinhas em *A. bidwillii*, a partir do sexto ano. O capítulo III, objetivou estudar os efeitos da procedência (Paraná, Santa Catarina, Minas Gerais e São Paulo), do sexo (feminino e masculino) e do período de maturação do pinhão (precoce, intermediário e tardio) de plantas matrizes de *A. angustifolia* na sobrevivência e crescimento dos enxertos. Constatou-se que matrizes femininas tem maior sobrevivência e crescimento dos enxertos, assim como, aquelas com período de maturação classificado como intermediário e tardio. Ademais, matrizes provenientes do Paraná e Santa Catarina são superiores às de Minas Gerais e São Paulo em relação a sobrevivência e crescimento dos enxertos de *A. angustifolia*.
**Palavras-chave:** Enxertia; Miniestaquia; Pomar de sementes; Procedências; Genótipos.

# ABSTRACT

The genus *Araucaria* comprises 20 species distributed in different regions of the planet. In Brazil, *Araucaria angustifolia* (Bertol.) Kuntze has great economic, social, environmental, and cultural relevance due to its high-quality wood and it is pine nut seed. The species is on the endangered list and, as a consequence, current legislation prohibits the management of individuals in native areas. In this way, encouraging conservation through use has been presented as the main alternative to avoid the extinction of the species. Encouraging its planting for timber and non-timber purposes requires the adoption of technologies such as vegetative propagation, which has been researched and applied to several forest species, including araucaria. Despite the advances made in recent years, there are still gaps in improving techniques. In this sense, the present study aimed to propose alternatives in the application of minicutting and grafting techniques for *A. angustifolia*, contributing to clarifying doubts and encouraging the advancement of studies related to the species. Chapter I aimed to determine the influence of different substrate compositions (pine bark, vermiculite, coconut fiber, peat, carbonized rice bark) and environments (automated greenhouse, conventional greenhouse, and mini-tunnels) on rooting of *A. angustifolia* minicuttings. We find that there was no interaction between the substrate and environments. The species did not show any difference in rooting depending on the type of substrate, and different combinations of pine bark, vermiculite, coconut fiber, peat, and carbonized pine bark, among others, could be used for composition. On the other hand, the use of an automated greenhouse with humidity control and microsprinkler irrigation favored the survival and rooting of the minicuttings. The percentage of rooting recorded does not yet support the use of the technique on a commercial scale, making it necessary to seek new alternatives. Chapter II main objective was to verify the applicability of inter specific grafting between species of the genus *Araucaria*, using as models *A. angustifolia* and *A. bidwillii*, two species of the genus that produce edible seeds. The results indicated late incompatibility, with symptoms appearing 7 years after grafting. The incompatibility is characterized by the difference in growth of the scion and rootstock, with the diameter of *A. angustifolia* being greater than that of *A. bidwillii*. Despite the incompatibility being confirmed, the production of pine cones was observed in *A. bidwillii*, from the seventh year onwards. Chapter III aimed to study the effects of origin (Paraná, Santa Catarina, Minas Gerais, and São Paulo), sex (female and male), and pine maturation period (early, intermediate, and late) on *A. angustifolia* grafts survival and growth. Female matrices have greater survival and growth of grafts, as well as those with a maturation period classified as intermediate and late. Furthermore, matrices from Paraná and Santa Catarina are superior to those from Minas Gerais and São Paulo in relation to survival and growth of *A. angustifolia* grafts.
**Keywords:** Grafting; Minicutting technique; Seeds orchard; Provenances; Genotypes.

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# 1 GENERAL INTRODUCTION

*Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae), commonly known as araucaria, Paraná pine, or Brazilian pine, is a Brazilian native conifer (Carvalho, 2002) associated with the Mixed Ombrophilous Forest (Seitz, 2009). The species occurs mainly in the Southern Region (Paraná, Santa Catarina and Rio Grande do Sul states) and some areas of the Southeast Region (São Paulo, Rio de Janeiro, Espírito Santo and Minas Gerais states) at altitudes above 800 m. Also, it can be found in northeastern Argentina and in eastern Paraguay (Ruschi, 1950; Wrege *et al*., 2017).

This species plays an important role in social and economic development of the southern of Brazil through the use of wood and consumption of its edible seeds (Zanette *et al*., 2017). During the last seven decades, araucaria has suffered from habitat loss and tree exploitation (Castro *et al*., 2020), due to human occupation activities and the high added value of wood (Souza; Aguiar, 2012). This exploration and commercialization without technical criteria resulted in a drastic reduction of individuals and genetic diversity (Kock; Correa, 2010). Consequently, the species was classified as endangered (Brasil, 2014), and since 2001, the harvest of native trees has been prohibited (Brasil, 1998).

Edible seeds of araucaria, known as Brazilian pine nuts or *pinhão*, are an important food source for humans and wildlife. The consumption of these seeds is part of regional culture. Research indicates that the *Kaingang* and *Xokleng* indigenous people have been using these seeds as food and have incorporated it into their traditional rituals since prehistoric times (Schimitz, 2009). Pine nuts are considered a functional food with a low glycemic index and high levels of starch, proteins, lipids, fiber, calcium, iron, magnesium, potassium, and phenolic compounds (Castrillon; Helm; Mathias, 2023).

The gathering of pine nuts is mainly a job undertaken by small farmers who live around remaining Araucaria Forests (Ávila *et al*., 2023). Commercialization can provide a monthly income of approximately 1 or 2 minimum wages for families in Brazil, making it one of the main sources of income for these households during the productive season (Tagliari *et al*., 2021). Plant Extraction and Forestry Production Report for 2022 highlights pine nuts among the most sold non-wood products in the country, with increases of 7.1% in the volume produced and 16.6% in the value collected compared with the previous year (IBGE, 2022). Although this trade stimulates the regional economy, the absence of orchards for pine nut production leads to the overexploitation of native areas and interferes with the natural regeneration of the species and, consequently, its genetic maintenance (Silveira *et al*., 2011; Zanette *et al*., 2017).

The prohibition of exploitation in native forests, initially implemented with the purpose of conservation, led to an economic devaluation of the species and decreased interest in this trade. As a result, creates a disincentive for property owners to support natural regeneration. To overcome this problem, conservation based on sustainable use of the species is considered a viable strategy. It is necessary to encourage property owners to see the economic potential of planting the species (Zanette *et al*., 2017).

The establishment of araucaria plantations for timber purposes is still legalized (Conama, 2001). Also, the implementation of orchards for araucaria seed production is an interesting alternative, considering the increasing trade (Danner; Zanette; Ribeiro, 2012). Significant research on araucaria silviculture has been conducted on wood and non-wood products. This research focused on the production of seedlings, genetic improvement, breeding, clonal forestry, among others (Wendling; Stuepp; Zuffellato-Ribas, 2016; Gaspar *et al*., 2017; Rickli-Horst *et al*., 2019; Maggioni *et al*., 2020).

Just like other conifers, the araucaria seedlings production is traditionally done using seeds for many reasons, like access to seeds, ease of application of the technique, and lower production costs compared to vegetative propagation (Wendling; Stuepp; Zanette, 2017). However, vegetative propagation is recommended for commercial plants production. An alternative to producing plants for timber purposes is the use of minicuttings technique (Wendling, 2015). A propagation technique that consists of rooting shoots from cuttings or seedlings produced by seeds (Xavier; Wendling; Silva, 2013). This technique has been used and studied for some conifers, like *Pinus pinaster* (Majada *et al*., 2011), *Pinus radiata* (Corrêa *et al*., 2015) and *Sequoia sempervirens* (Pereira *et al*., 2020). The advantages of minicutting technique include higher environmental, phytopathological, nutritional, and hydric control (Wendling; Xavier, 2003).

Minicutting technique is promising for taking advantage of the endogenous juvenile potential of the species, which is favorable for rooting, in addition to allowing the use of plant growth regulators to assist root initiation and development (Ferriani; Zuffellato-Ribas; Wendling. 2010). For araucaria, studies have already been conducted for some years and have demonstrated the species' recalcitrant behavior related to rooting and non-responsive behavior to auxin application (Pires; Wendling; Brondani, 2013; Pires *et al*., 2015; Maggioni *et al*., 2020), but adjustments such as mini-stump nutrition and propagation environment can be made (Pires, Wendling; Brondani, 2013).

On the other hand, the grafting technique has gained attention in the last few years from researchers, producers, property owners, and other professionals; this technique has enabled the implementation of orchards for pine nut production. The use of grafting allows a reduction in the time to first production from 12 or 15 to six to 10 years, a reduction in plant height, selection of plants by sex (female or male), nuts maturation season, color, size, and flavor of nuts (Wendling, 2011).

Protocols for araucaria grafting have been partially consolidated (Zanette; Oliveira; Biasi, 2011; Wendling; Stuepp; Zuffellato-Ribas, 2016; Constantino; Zanette, 2018; Rickli-Horst *et al.,* 2019). However, plant production takes an approximate one-and-a-half year depending on many factors, like rootstock quality, climatic conditions, grafter skill, and costs (Wendling, 2011). Furthermore, preliminary results indicate the influence of genotype on the success of grafting and development of grafted plants (Valente; Cipriani; Wendling, 2023). Additionally, orchards with grafted plants provide an opportunity to select and propagate trees with desirable genetic traits and contribute to improving efforts in native forest conservation.

In addition to *A. angustifolia*, two other species of the genus produce edible seeds, they are *Araucaria araucana* (Molina) K. Koch or Pehuén is found in southern Chile (VEBLEN, 1982)*;* and *Araucaria bidwillii* (Hook), known as Bunya Pine, is found in Southeast Queensland, Australia (Smith; Butler, 2009). Like Brazilian Pine, Bunya Pine species play an important cultural role. In Australia, however, bunya nuts are not commercialized to a large extent or processed, being available only in a few farmers markets around Queensland and only during the season (Nadolny *et al*., 2023).

Recent research found a similarity in the nutritional and chemical composition of the nuts of these three araucaria species, which allows interchange in knowledge, especially related to processing, and their sensory properties, indicating that these nuts are a unique option as ingredients in the food industry (Nadolny, 2023). Considering this knowledge, it is possible to infer the future of the consumer market for pine nuts and their derivatives. Using grafting techniques, a combination of two tree species is possible (Xavier; Wendling; Silva, 2013). This technique can enable expansion of the cultivated area, increasing the economic value of products and visibility for species inside and outside their natural range, and boosting the national and international nuts trade.

Based on the above information, the present thesis aimed to propose alternatives in the application of minicutting and grafting techniques for *A. angustifolia*, contributing to clarifying doubts and encouraging the advancement of studies related to the species, the following thesis was developed into tree chapters, with different approaches to minicutting and grafting techniques. The first chapter, "ROOTING OF ARAUCARIA MINI-CUTTINGS IN DIFFERENT ENVIRONMENTS AND SUBSTRATES," was published in the journal

“Floresta”, under volume 54, e-89570, in January 2024. This study aimed to determine the influence of different environments and substrates on *A. angustifolia* minicuttings rooting.

The second chapter, “INTERESPECIFIC GRAFTING ON ARAUCARIA GENUS” was submitted for publication in "Plant Biology". This chapter describes the late grafting incompatibility between these two species of the *Araucaria* genus from different continents that produce edible seeds.

The third chapter, “FIELD GRAFTING OF ARAUCARIA GENOTYPES FROM DIFFERENT PROVENANCES” was prepared for submission on "Journal of Forestry Research". This section presents an analysis of the influence of provenance, sex, and productivity characteristics on the survival and initial development of grafted plants.

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# CHAPTER I

## 2 ROOTING OF ARAUCARIA MINICUTTINGS IN DIFFERENT ENVIRONMENTS AND SUBSTRATES[1]

**Abstract**

Araucaria is a native conifer, with high economic importance, especially in the southern region of 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 $cm^3$ tubes, testing four different substrates, which are, 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). These minicuttings were maintained in three different environments for rooting: Automated Greenhouse (CVA) with 80% reduction in luminosity and mist irrigation, Simple Greenhouse (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.

### 2.1 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).

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

[1] Published in “Revista Floresta” v. 54, e-89570, 2024.

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 *Eucalyptus* 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*.

## 2.2 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; Wendling; Zanette, 2013), distributed three times a day, at a total flow rate of 5 L $m^{-2}$ (Table 2.1).

Table 2.1. Composition of the nutrient solution used for fertigation of *Araucaria angustifolia* mini-garden in a semi-hydroponic system.

| 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; Wendling and Zanette (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, Wendling and Zanette (2013). Subsequently, they were planted in 210 $cm^3$ plastic containers. Three commercial substrates were used (Table 2.2), 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 2.2. Characteristics of substrates used for rooting of *Araucaria angustifolia* minicuttings.

| 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 30 ºC, 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 30 ºC. 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 minicuttings, entirely green with or without roots) and the rooting percentage (minicuttings 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 minicuttings 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).

## 2.3 RESULTS

No interaction was found between environmental and substrate factors for the variables analyzed. Therefore, the simple effects of the treatments were assessed and presented next. Among the rooting environments, CVS promoted 80% survival of the minicuttings, which was higher than the other environments (Figure 2.1A). 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 minicuttings (Figure 2.1B).

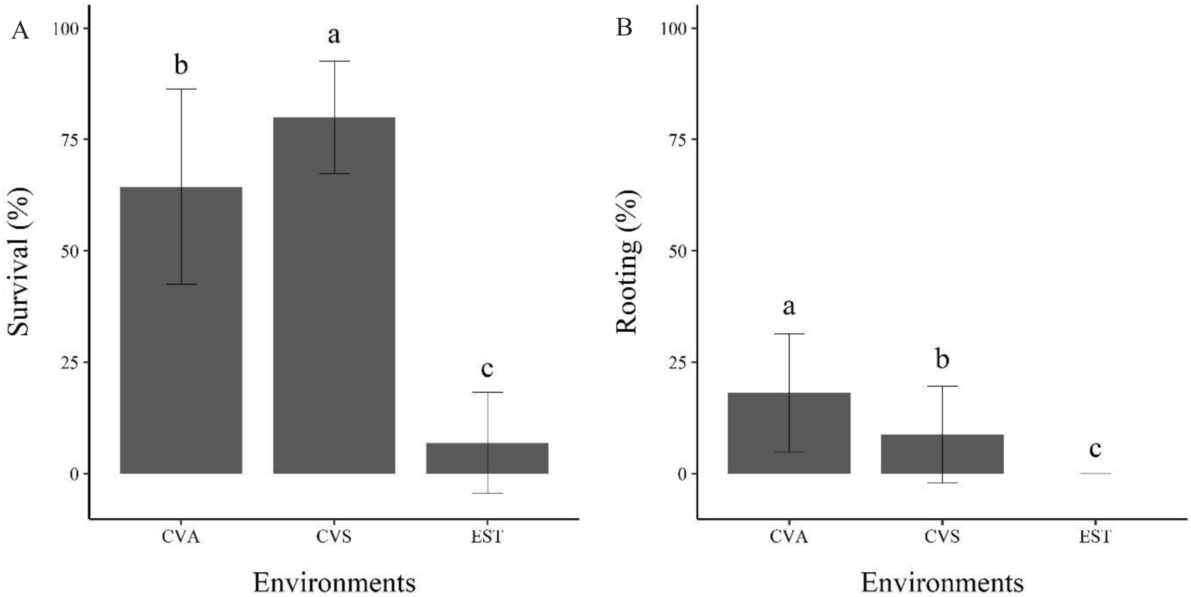


Figure 2.1. Survival (A) and rooting (B) of *Araucaria angustifolia* minicuttings submitted to different environments.
Averages followed by the same letter do not differ significantly at the 5% probability level using the Wilcoxon test. Source: The Author (2024).

With respect to the substrates, no significant difference was observed for both variables analyzed (Figure 2.2). The survival of the minicuttings 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 2.2B).

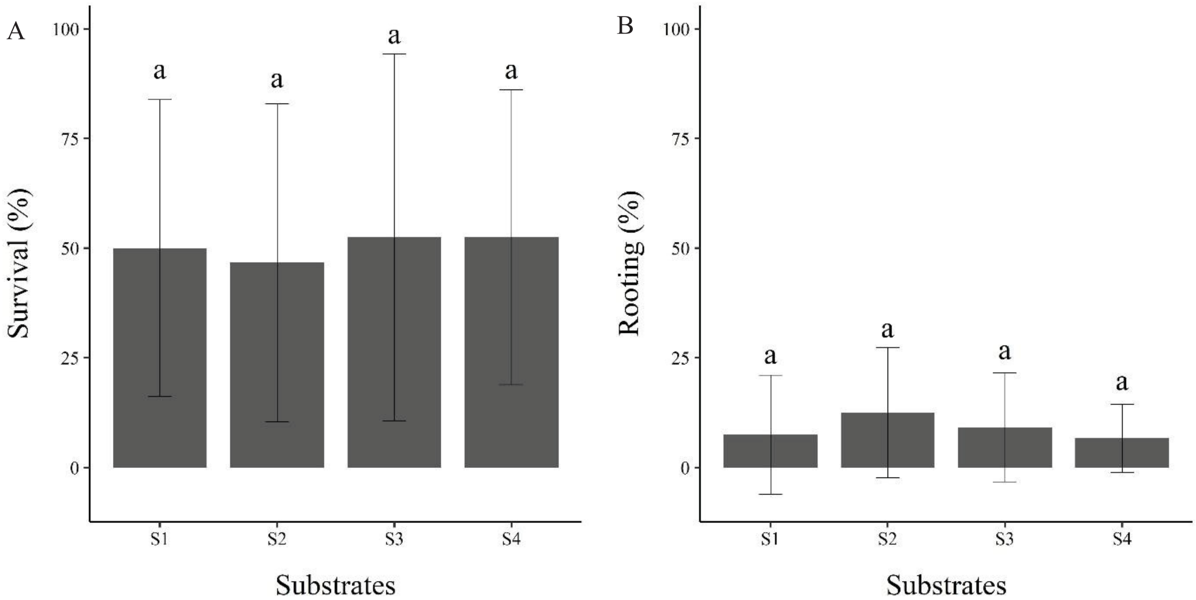


Figure 2.2. Survival (A) and rooting (B) of *Araucaria angustifolia* minicuttings submitted to four different substrates.
Averages followed by the same letter do not differ significantly at the 5% probability level using the Kruskal-Walli test. Source: The Author (2024).

Upon visually analyzing the minicuttings 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 minicuttings (Figure 2.3). This was observed mainly in the first 60 days following planting.

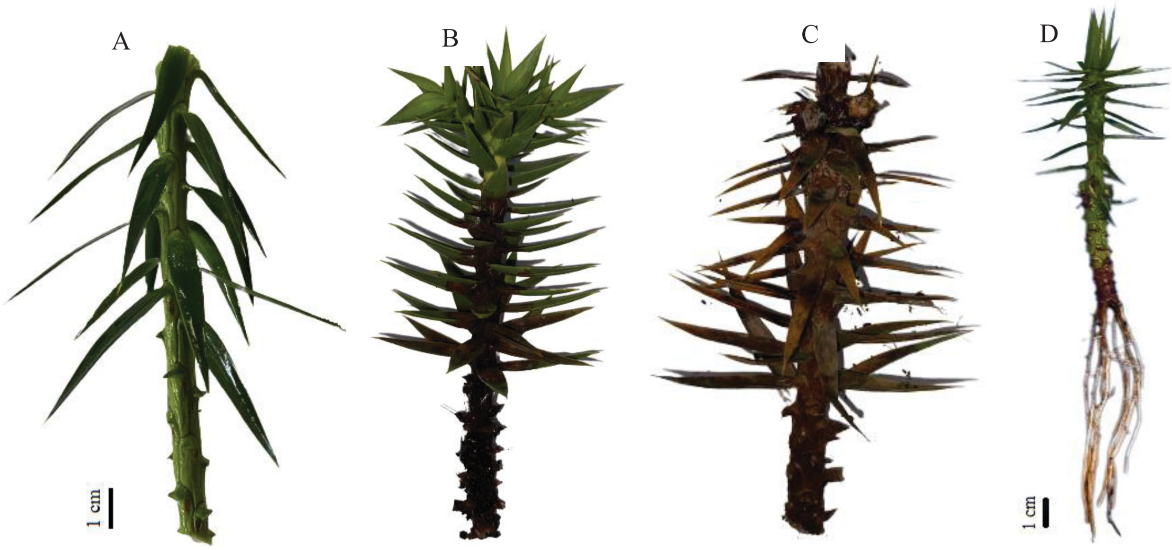


Figure 2.3. Visual aspect of rotting progression in *Araucaria angustifolia* minicuttings: (A) intact minicutting (alive); (B) minicutting with rotting starting at the base, (C) completely rotted minicutting and (D) rooted minicutting. Source: The Author (2024)

## 2.4 DISCUSSION

Although CVS provided the highest survival of the minicuttings, 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 minicuttings 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 minicuttings 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, Wendling and Silva (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 minicuttings, 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, Wendling and Zanette (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; Trueman; Xavier, 2014). Studies conducted by Wendling, Stuepp and Zuffellato-Ribas (2016a) have already demonstrated the difficulty of rooting cuttings and minicuttings 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, Stuepp and Zuffellato-Ribas, 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, Wendling and Zanette (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 minicuttings, 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.

## 2.5 CONCLUSIONS

- The Automated Greenhouse (CVA) promoted greater rooting of the minicuttings and is the most recommended environment for the production of clonal plants 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 minicutting technique, although the general rooting indices are still not considered viable.

## ACKNOWLEDGMENTS

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**CHAPTER II**

# 3 INTERESPECIFIC GRAFTING ON ARAUCARIA GENUS[2]

**Abstract**
*Araucaria angustifolia* and *Araucaria bidwillii* have social, cultural, and economic importance associated with pine nuts production, which has great potential for commercialization. The objective of this study was to describe the grafting compatibility between *A. angustifolia* and *A. bidwillii* as an alternative to promote the establishment of pine nut orchards. We also report the early production of pine cones by *A. bidwillii* grafted plants. Grafts of 10 plants were done using the patch-grafting technique. Three grafts of *A. bidwillii* as rootstock and *A. angustifolia* as scion, and seven grafts of *A. angustifolia* as rootstock and *A. bidwillii* as scion. After grafting, plants were established in the field at the experimental area of Embrapa Florestas, Colombo, Parana, Brazil and visually monitored. Seven years after grafting, differences in rootstock and scion diameters were observed in all grafted plants. This symptom is classified as anatomical incompatibility. *A. angustifolia* has a larger diameter in all plants. To date, no evidence of damage to growth and development caused by this incompatibility has been observed. In addiction after six years *A. bidwillii* grafted plants began pine cones production. The results of this study indicate the occurrence of late incompatibility between species; therefore, new studies are necessary to verify the future effects of these symptoms. Furthermore, the first record of the early production of *A. bidwilli* pine cones was observed in grafted plants, which could support the implementation of seeds orchards producing pine nuts of these species in Brazil.
**Keywords:** Araucariaceae; Brazilian pine, Bunya pine, Radial growth; Vegetative propagation.

## 3.1 INTRODUCTION

Araucariaceae is the most primitive family of coniferous trees still alive and occurs exclusively in the Southern Hemisphere (South America and Oceania). The genera Araucaria comprise 20 species (Aslam *et al*., 2013; Mill *et al*., 2017), however, only three of these species have been reported to produce edible seeds (Zanette *et al*., 2017). There are *Araucaria angustifolia* (Bert.) Kuntze, also known as Brazilian pine, occurs in Argentina, Paraguay, and mainly in Southern Brazil (Zanette *et al*., 2017); are *Araucaria araucana* (Molina) K. Koch or Pehuén is found in southern Chile (VEBLEN, 1982); *Araucaria bidwillii* (Hook), known as Bunya pine, is found in southern Queensland, Australia (Smith; Butler, 2009).

Phylogenetic research and fossil records indicate a common ancestry for these species, as well as others in the *Araucaria* genus (Escapa; Catalano, 2013). Both Brazilian Pine and Bunya Pine are long-lived trees with culturally and spiritually significant for indigenous groups (Schimitz, 2009; Cooke *et al*., 2024). Brazilian pine is a dioecious species that can reach 10–50

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m in height and 0.5–2.0 m in diameter (Lorenzi, 2002). Bunya pine is a monoecious species that can reach 30–45 m in height with a diameter of up to 1.5 m (Huth, 2009).

Pine nuts produced by both species have a similar nutritional composition, with starch as the main component in addition to proteins and lipids (Nadolny *et al*., 2023). The high starch content in these species suggests that they could be used as a flour or a novel source of starch (Cordenunsi *et al*., 2004; Nadolny *et al*., 2023). In the natural occurrence areas, the seed colleting is common, in the case of Brazilian pine, the trade of nuts has contributed to the regional economy (Ávila *et al*., 2023). Although this trade stimulates the economy, the absence of orchards for pine nut production leads to the overexploitation of native areas and interferes with the natural regeneration of the species and, consequently, its genetic maintenance (Silveira *et al*., 2011; Zanette *et al*., 2017).

Grafting is the most widely used vegetative propagation method to support the formation of seed orchards (Wendling, 2011). This method connects two pieces of living plant tissue, which unite and subsequently grow and develop into one plant. The rootstock is responsible for the support and absorption of water and nutrients, and the scion is a genetic material selected for a specific purpose (Xavier; Wendling; Silva, 2013). Grafted plants exhibit better adaptation to greater ecological ranges through the combination of different cultivars and can support early production (Hartmann *et al*., 2011).

This technique has already been used for *A. angustifolia* seed orchards with early production (Wendling 2011; 2015). Considering the similarity of these species, it is assumed that grafting could be applied to *A. bidwillii* with the same objective and contribute to increasing their economic value. The combination of these two *Araucaria* species could introduce a new quality of nuts to Brazil, stimulating increase of cultivation areas and the commercialization of nuts. The objective of this study is to describe the grafting compatibility between Brazilian Pine and Bunya Pine, as an alternative to promote the establishment of pine nut orchards. Also, report the pine conse production of *A. bidwillii* grafted plants.

## 3.2 MATERIALS AND METHODS

The experiment was carried out at the Forest Species Propagation Laboratory and the Experimental area of Embrapa Florestas, Colombo - PR, Brazil (25º 19,206 ‘S, 49º 9,514’ O, 928 m). The region’s climate is classified as Cfb – temperate, according to the Köppen-Geiger climate classification, with the coldest month temperature between -3 and 18 °C and humid, well-distributed rainfall throughout the year. Average annual rainfall is around 1,500 mm without the occurrence of a dry season (Alvarez *et al*., 2013).

To evaluate the applicability of the technique between species, three clones of *A. bidwillii* and two of *A. angustifolia* were combined. Ten plants were grafted: three grafts of *A. bidwillii* as rootstock and *A. angustifolia* as scion, and seven grafts of *A. angustifolia* as rootstock and *A. bidwillii* as scion. The rootstocks of both species were produced by seeds in plastic tubes with 3 L of capacity, filled with a commercial substrate of pine bark base, and 8 g $L^{-1}$ of Osmocote® 15-9-12 (NPK) slow-release fertilizer. At 1.5 years of rootstock age, the grafts were made using the patch graft technique, which has already been successfully used for *A. angustifolia* (Wendling, 2015; Rickli-Horst *et al*., 2021).

Seeds used for rootstock production of *A. angustifolia* were collected in Embrapa Florestas areas, in Colombo, Paraná, Brazil, and *A. bidwillii* were obtained from Colégio Marista, located in Curitiba, Paraná, Brazil. To obtain the scion, were used only adult orthotropic shoots of female trees established in clonal garden of Embrapa Florestas and Colégio Marista.

Planting in the field was established 12 months after grafting in an experimental area of Embrapa Florestas. Grafted plants were visually monitored, and it was possible to visualize the formation of cones and symptoms of incompatibility at the grafting point. To characterize this symptom, the diameters of the rootstock and scion (cm) and total height (m) were measured.

## 3.3 RESULTS AND DISCUSSION

Seven years after grafting, a difference in diameter above and below the grafting point was visually verified in all grafted plants. *A. angustifolia* had a larger diameter than *A. bidwillii*, regardless of whether it was used as a rootstock or scion (Figures 3.1A-B). These differences in the diameter growth between the scion and rootstock are considered incompatibility symptoms (Hartmann *et al*., 2011) and have often been observed in interspecific grafts of woody plants (Jayawickrama; Jett; Mckeand, 1991), as an example of grafting between *Pinus sibirica* and *Pinus sylvestris* (Scherba *et al*., 2020) or *Pinus patula* and *Pinus cembroides* (González-Jiménez *et al*., 2023).

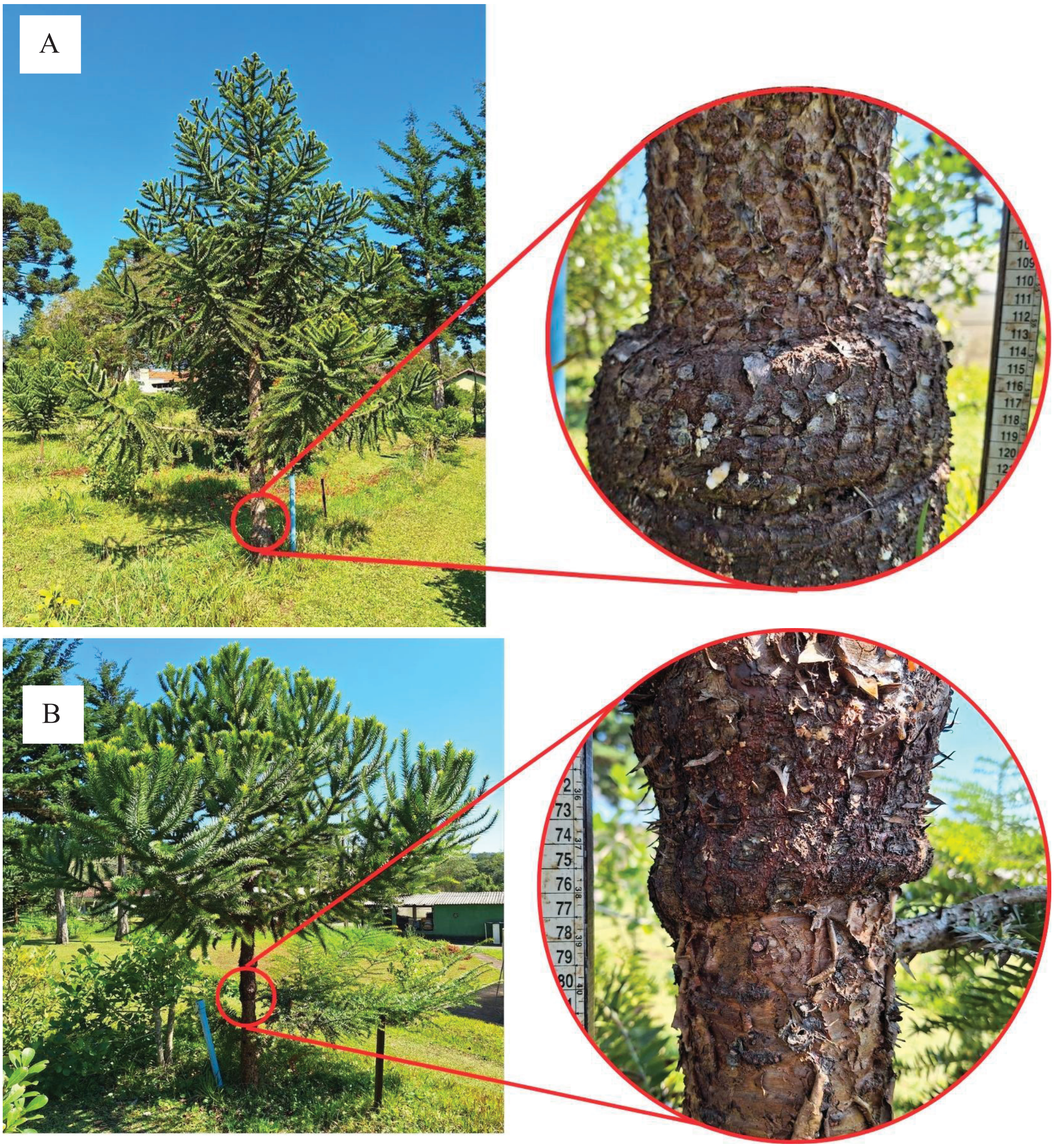


Figure 3.1. Visual aspect of plant grafted between (A) *A. angustifolia* (rootstock) and *A. bidwillii* (scion). (B) *A. angustifolia* (scion) and *A. bidwillii* (rootstock) Source: The Author (2024).

Graft compatibility depends on favorable physiological interactions between rootstock and scion, biochemical composts at the graft union, vascular tissue structure, contamination, and other factors (Hartmann *et al*., 2011). According to Goldschmidt (2014), taxonomic affinity is a prerequisite for grafting successfully, and incompatibility increases with genetic distance. Both species belong to *Araucaria* genus and, as previously mentioned, share common ancestors (Escapa; Catalano, 2013). However, the isolation of the species from different continents (*A. angustifolia* in South America and *A. bidwillii* in Australia) may have resulted in genetic and morphological distinctions between them (Kershaw; Wagstaff, 2001).

The most notable differences were observed when *A. angustifolia* was used as rootstock. *A. angustifolia* has a diameter 0.64 to 7.64 cm larger than *A. bidwillii*, which represents 10% to 38% higher (Table 3.1). Considering that all grafts were realized at the same time and the plants had the same age, this amplitude of difference on diameter between the grafted plants, can be explained due to the differences between the grafted genetic materials and the soil characteristics of the planting site, as the plants were established in different locations in the experimental area of the Embrapa Florestas.

Table 3.1. Total height (m), diameter of rootstock, scion and difference between rootstock and scion (cm), and percentual difference on diameter (PDD) of rootstock and scion on grafted plants.

| Plant | Total Height (m) | Diameter (cm) | | | PDD (%) |
|---|---|---|---|---|---|
| | | **Rootstock** | **Scion** | **Difference** | |
| | | *A. angustifolia* (rootstock) x *A. bidwillii* (scion) | | | |
| 1 | 4.6 | 19.74 | 12.10 | 7.64 | 38.71 |
| 2 | 5.5 | 18.78 | 12.41 | 6.37 | 33.90 |
| 3 | 1.8 | 4.46 | 3.02 | 1.43 | 32.14 |
| 4 | 2.1 | 4.77 | 3.50 | 1.27 | 26.67 |
| 5 | 3.1 | 11.14 | 8.28 | 2.86 | 25.71 |
| 6 | 2.1 | 6.37 | 4.93 | 1.43 | 22.50 |
| 7 | 2.2 | 6.05 | 5.41 | 0.64 | 10.53 |
| **Mean** | **3.1** | **10.19** | **7.09** | **3.09** | **27.17** |
| | | *A. bidwillii* (rootstock) x *A. angustifolia* (scion) | | | |
| 1 | 2.5 | 9.23 | 11.14 | - 1.91 | - 17.14 |
| 2 | 1.9 | 4.93 | 6.37 | - 1.43 | - 22.50 |
| 3 | 1.5 | 2.55 | 3.18 | - 0.64 | - 20.00 |
| **Mean** | **2.2** | **7.08** | **8.75** | **- 1.67** | **- 19.82** |

Source: The Author (2014).

Even in grafts where rootstock and scion share the same genetic background (same species), certain incompatibility may be present. It is common for such effects or symptoms to also be expressed between species. The time needed for the establishment of a functional graft union is considerably shorter in herbaceous plants than in trees, and species dependent (Goldschmidt, 2014). *Pinus radiata* for example, expressed the signs of incompatibility 15 or more years after grafting and, for *Prunus* at almost fifteen years after grafting the graft union may break off (Hartmann *et al*., 2011).

Incompatibility has already been reported in *Araucaria* intraspecific grafting. *Araucaria cunninghamii*, for example, is related to swelling at the union, chlorosis, and necrosis, occurring between 2 and 10 years after grafting (Haines; Dieters, 1990). For *Araucaria angustifolia* grafts using the same species as rootstock, no symptoms of incompatibility were observed. Further studies are necessary to understand the mechanisms involved in the symptoms observed in this study.

The symptoms associated with this condition may be associated with anatomical incompatibility (Hartmann et al., 2011), which is caused by different rates of cambium cellular division, leading to xylem vessel discontinuity (Rodrigues et al., 2004). To confirm this hypothesis, histological analysis of grafted plants was necessary. Despite that, plants with incompatible grafts can grow for several years without any external indication of incompatibility (Machado *et al*., 2017) or could even flower and produce seeds normally (Darikova *et al*., 2011).

To date, no effect of incompatibility on the formation of reproductive structures has been observed. Pine cones production in *A. bidwillii* grafted plants was observed beginning at seven years after grafting (Figure 3.2). All nuts present in the pine cones were empty due to a lack of pollen from the species during the pollination season. *A. bidwillii* is a monoecious species (male and female strobili in the same plant), with a female strobili in the upper part and a male strobili in the lower part of the crown (Huth, 2009). The scion was obtained from the top of the crown, so, up to now, all plants were female, and no pollen was produced.

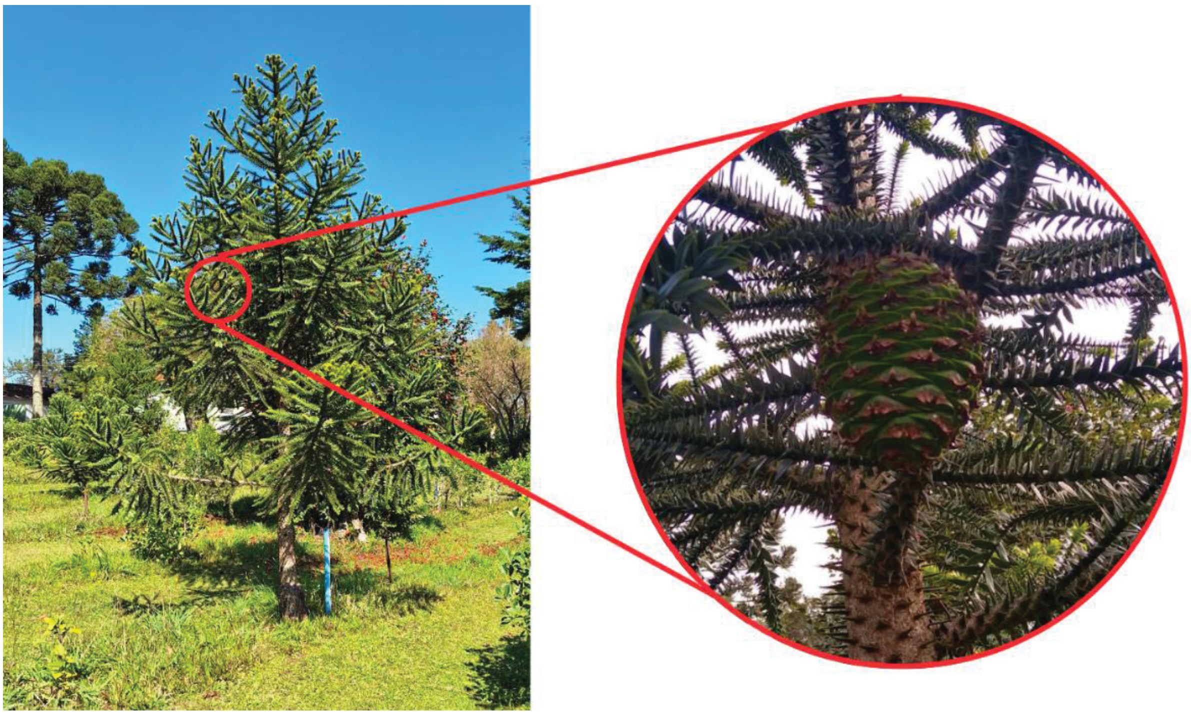

Figure 3.2. Pine cone produced at seven years by *A. bidwillii* grafted over *A. angustifolia* rootstock. Source: The Author (2024).

Early cone production has already been reported for *A. angustifolia* starting at six years after grafting (Wendling, 2011). According to Hartmann *et al.* (2011) this is possible because the use of mature propagules collected by tree crown. Plants produced by seeds in Australia start their reproductive period at about 14 years old (Huth, 2009). Therefore, this unprecedented result indicates the feasibility of applying the patch graft technique to induce early production for *A. bidwillii*. The fall of cones was observed between February and March of grafted plants established in experimental area of Embrapa Florestas, in Brazil. This observation is within the pine cone drop patterns that are naturally observed in Australia, where the dispersal occurs between December and March (Huth, 2009). On the other hand, seed dispersal of *A. angustifolia* occurs normally from April to June in the same region (Carvalho, 2002).

The reports presented in this study support the possibility of a combination between the two species. Despite the symptoms of incompatibility between rootstock and scion, no evidence of damage on growth and development of plants was observed to date. Based on these results, new studies are needed to verify the union of tissues from the moment of grafting and the progression or regression of differences in recorded diameters, among countless other possibilities.

Early cone production represents a significant step forward in boosting the Bunya seed production market. The next steps in this research are related to male pollen production, the formation of viable nuts, and the quantification of nut production. It is worth mentioning that both species share similar habitats (Frezza *et al*., 2020), which increases the possibility of establishing the species in Brazil and stimulus for the formation of orchards with a mix of seeds produced.

## 3.4 CONCLUSION

Grafts between *A. agustifolia* and *A. bidwillii* were functional, but showed symptoms of tardy incompatibility, as evidenced by the different radial growths between rootstock and scion. This symptom was observed in all grafted plants, with *A. angustifolia* having a larger diameter than *A. bidwillii*. In addition, early pine cone production from grafted *A. bidwillii* plants began at the age of six years, indicating the applicability of this technique to these species.

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# CHAPTER III

# 4 FIELD GRAFTING OF ARAUCARIA GENOTYPES FROM DIFFERENT PROVENANCES[3]


## Abstract

The grafting of mature vegetative material is used to establish pine nuts orchards of *Araucaria angustifolia*. Some of the gaps for advancing the establishment of productive areas include the selection of genetic materials with high productivity, different characteristics of pine nuts, as well as the increase in the period of seeds production. Based on these findings, the present study aimed to evaluate the influence of genetic materials from different provenances, seasons of nuts production, and mother tree sex on the survival and initial growth of *A. angustifolia* grafts. Were selected twenty-four genotypes, female and male, from four provenances (São Paulo, Minas Gerais, Paraná and Santa Catarina), classified according to their pine nut maturation time (precocious, intermediary and tardy). These genotypes were grafted directly in the field by patch graft technique. Graft survival assessments were performed at 60, 90, 120, 150, 340, and 540 days after grafting (DAG). The vigor of the shoots was evaluated using the diameter and height, number of whorls, and distance between whorls. The experiment was conducted in a randomized block design with 10 blocks composed of 2 plants of each genotype per block. Grafts survival varied according to genotype. Female genotypes showed higher survival and better initial shoot growth. Likewise, clones with intermediate and late pine nut maturation times were superior to those classified as early. Regarding origin, genetic materials originating from Paraná and Santa Catarina were superior in all variables analyzed. The better growth of genotypes from the southern region suggests better adaptation of these materials to local soil and climate conditions. A weak correlation was observed between rootstock vigor and graft survival and growth. Through these results, it was possible to verify the significant effects of genetic material characteristics on the graft survival and shoot growth. Highlighting female individuals from Paraná and Santa Catarina, with nuts maturation season defined as intermediate and tardy. This information can support the formation of commercial orchards in different regions of the country.

**Keywords:** Pine nut production; shoot growth; clonal forestry; field grafting.

## 4.1 INTRODUCTION

*Araucaria angustifolia* (Bertol.) Kuntze, Araucariaceae, is a native conifer from Brazil, eastern Paraguay, and northeastern Argentina, popularly known as araucaria or Brazilian pine. The species originally occurs in the Southern states (Paraná, Santa Catarina and Rio Grande do Sul) and in high altitude areas in the Southeast Brazilian states (São Paulo, Minas Gerais, Rio de Janeiro and Espírito Santo) (Ruschi, 1950; Carvalho, 2003; Wrege *et al.*, 2017).

Due to their excellent wood quality, araucaria trees underwent uncontrolled exploitation during the 19th century (Hess *et al.*, 2018). As a result, the araucaria is on the list

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of endangered species (Brasil, 2014), and Brazilian federal laws prohibit its exploitation (Conama, 2001), which discourages species plantation. An alternative to reducing this problem is to focus on the implementation of new araucaria areas with the main objective of producing edible seeds (Zanette *et al*., 2017).

The araucaria nut, known as *pinhão*, is a traditional food since prehistoric times (Danner; Zanette; Ribeiro, 2012). *Xokleng* and *Kaingang* indigenous peoples use the seeds as food and as part of their culture and spiritual rituals (Santos, 1973). The nuts exhibit significant nutritional values, containing approximately 48% starch, 4% proteins, and 2% lipids, in addition to calcium, iron, and phenolic compounds (Silva *et al*., 2022; Castrillon; Helm; Mathias, 2023), making them a functional food. Although the nuts are considered essentially a source of starch, its flour is comparable to other protein sources used in the human diet (Young; Pellet, 1994), and it is a gluten-free food (Basso *et al.,* 2015).

Pine nut extractive production in Brazil increased from 7,700 tons in 2017 to 10,600 tons in 2020 (Snif, 2024). Plant Extraction and Forestry Production Report for 2022 highlights pine nuts among the most sold non-wood products in the country, with increases of 7.1% in the volume produced and 16.6% in the value collected compared with the previous year (IBGE, 2022). The increase in prices of pine nuts (Ceasa/PR, 2023) leads to more intense exploitation of native araucaria, which could create problems for forest conservation. Danner, Zanette and Ribeiro (2012) and Wendling (2015) point out that an alternative to overcome this problem is the implementation of seed orchards with the aim of producing nuts.

Several studies have been conducted to generate knowledge for plant production of the species, highlighting the establishment of araucaria vegetative propagation protocols based on grafting of adult propagules (Zanette; Oliveira; Biasi, 2011; Wendling; Stuepp; Zuffellato-Ribas*,* 2016; Rickli-Horst *et al.,* 2019). Using grafting, it is possible to reduce the time required to initiate production and reduce tree size. Additionally, it allows the selection of genotypes with high yields, defined genders, and specific production seasons, among other benefits (Wendling, 2015).

The high genetic variability between provenances and matrices makes gains possible through selection between and within provenances, mainly for characters such as weight and diameter of the cone, weight and number of nuts per cone, and tree growth (Gerber *et al*., 2021). Considering the possible genetic gains with breeding programs of this species and the possibility of establishing nut production orchards, the present study aimed to evaluate the influence of genetic materials from different provenances, nuts production seasons, and mother tree sex on the survival and initial growth of *A. angustifolia* grafts.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Experimental area

The experiment was conducted in an area of 45,360 m² at the Salto Canoinhas Experimental Farm (Figure 4.1A), belonging to Epagri (Agricultural Research and Rural Extension Corporation of Santa Catarina), in the municipality of Papanduva, Santa Catarina, Brazil (26°22’16.7’ S, 50°16’34.8’ W, 792 m). According to Köppen classification, the climate in the region is Cfb, temperate, always humid with evenly distributed rainfall throughout the year, without dry season. The temperature during the hottest month is approximately 22 °C, and temperatures during the coldest month between -3 and 18 °C. Precipitation between 1,100 and 2,000 mm annually (Alvarez *et al*., 2013). With frequent occurrence of frosts in the region, mainly between May and September (Wrege *et al*., 2018).

The experimental area was previously cultivated with soybean for 12 years and corn for three years. Relief is characterized as undulated at gently undulated (Almeida *et al*., 2018). The original geological formation developed soils with high organic matter content. Table 4.1 contents the chemical soil analysis done before planting.

Table 4.1. Chemical analysis of soil in the experimental area (layer 0 to 20 cm).

| pH $H_2O$ | pH SMP | Ca | Mg | Al | H + Al | m* | V** |
|---|---|---|---|---|---|---|---|
| | | ------------------ cmol/dm³ ----------------- | | | | --------- % -------- | |
| 4.6 | 5.3 | 4.2 | 1.5 | 2.0 | 9.48 | 25.33 | 37.97 |

| P | K | CTC | M.O. | Argila | Ca/Mg |
|---|---|---|---|---|---|
| ------- mg/dm³ ------- | | cmol/dm³ | --------- % -------- | | |
| 4.4 | 43.8 | 15.28 | 3.8 | 30 | 2.87 |

* Aluminum saturation; ** Base saturation. Source: The Author (2014).


Figure 4. 1. Overview of experimental area (A); grafted plant of *A. angustifolia* with plastic tape (B); graft alive after plastic tape remove (C and D); graft dead after plastic tape remove (E and F), and grafted plant with identification of rootstock, scion and grafting point (G). Source: The Author (2024).

### 4.2.2 Rootstock production and area management

The rootstocks were produced in a nursery, using plastic tubes with 3,780 ml of capacity, filled with commercial substrate based on peat, vermiculite and roasted rice husk, and supplemented with 6 g $L^{-1}$ of slow-release Osmocote® (15-9-12) fertilizer (8 months). The seeds used for rootstock production were collected in native areas, close to the plantation region. Approximately 18 months after seeding, with 50-60 cm in height and 12 mm in stem diameter (Wendling, 2015), plants were selected for planting.

The rootstocks planting was carried out in October 2019, in pits of 40 x 40 x 40 cm. A quincunx system was used, with 9 x 9 m spacing between plants (Figure 4.2). Two replanting were carried out in 2019 and 2020. Two organic fertilizations with tanned cattle manure were realized in the winters of 2020 and 2021, with 15 and 30 $m^3$ $ha^{-1}$, i.e. a 1,5 and 3,1 kg per pit, respectively. Weed control was realized with periodically mechanized mowing. Systematic control of leaf-cutting ants was realized with granulated bait with Fipronil (0,01%) and weekly inspection.

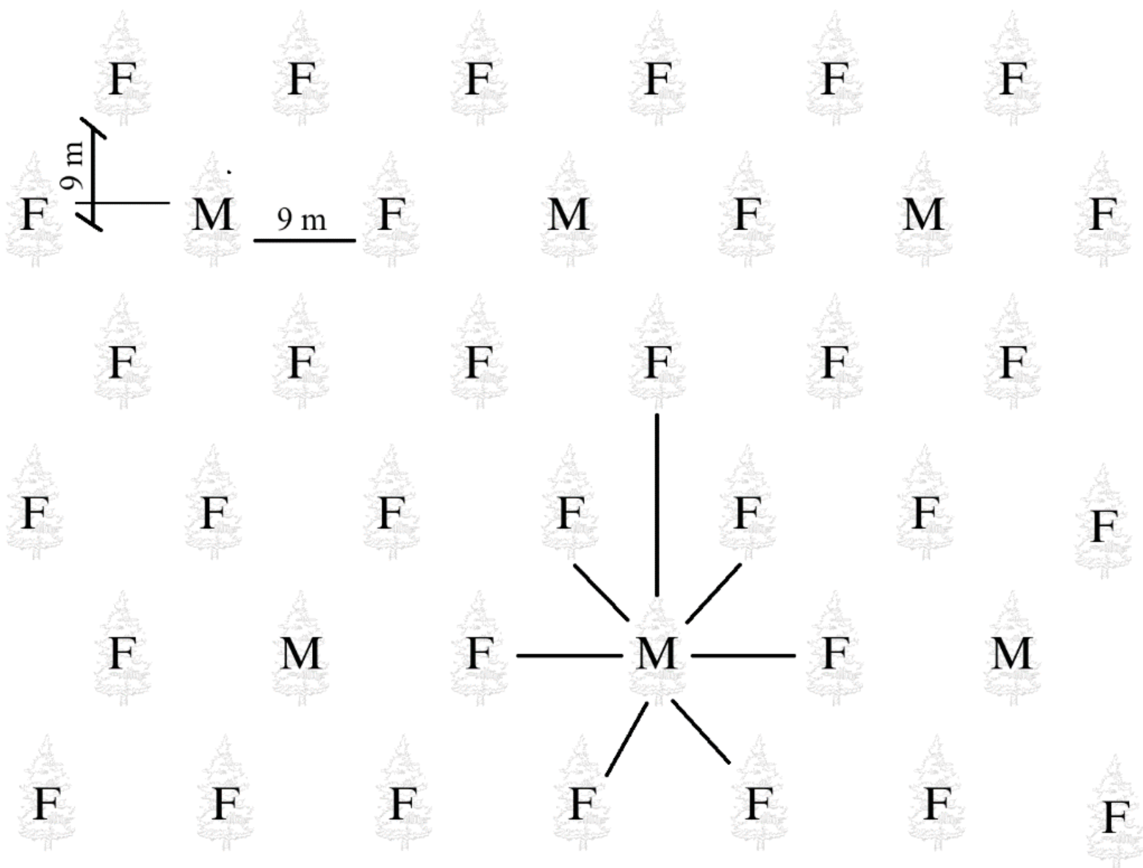


Figure 4.2. Design of plant distribution in planting blocks. F = female and M = male. Fonte: Adapted from Wendling; Stuepp and Zanette (2017).

### 4.2.3 Grafting

In September 2021, we grafted directly in the field the rootstocks previously planted, using 24 genotypes. These genotypes originate from four states: São Paulo (SP), Minas Gerais (MG), Paraná (PR), and Santa Catarina (SC), and were established at the experimental area of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Florestas) in Paraná. From where they were selected, rescued, and established in a clonal garden.

Table 4.2 lists the 24 genotypes collected from the clonal garden with identification code, mother tree gender, nuts maturation season, original provenance, selection provenance, and age of mother plant. Selection of genotypes was based on original provenance: MG, SP, PR and SC; gender: female and male plants; nut maturation season: precocious, intermediary and tardy. Maturation season classification was based on Coutinho (2007) and classified as: precocious with maturation before May; intermediary with maturation between May and July; and tardy with maturation after July.

Table 4.2. Identification and characterization of *Araucaria angustifolia* genetic materials inserted into the experimental area.

| **ID** | **Gender** | **Nuts maturation season** | **Provenance** | | **Mother's age** |
|---|---|---|---|---|---|
| | | | Original | Selection | |
| F4 | Female | Precocious | SP | PR | 35 |
| F12 | Female | Tardy | SC | PR | 35 |
| F14 | Female | Precocious | MG | PR | 35 |
| F17 | Female | Intermediary | PR | PR | 35 |
| F18 | Female | Intermediary | PR | PR | 35 |
| F24 | Female | Intermediary | SP | PR | 35 |
| F25 | Female | Precocious | MG | PR | 35 |
| F26 | Female | Tardy | PR | PR | 50 |
| F27 | Female | Precocious | PR | PR | 150 |
| F28 | Female | Precocious | SP | PR | 35 |
| F29 | Female | Precocious | MG | PR | 35 |
| F32 | Female | Tardy | PR | PR | 35 |
| F33 | Female | Precocious | PR | PR | 35 |
| F36 | Female | Tardy | PR | PR | 80 |
| F37 | Female | Intermediary | PR | PR | 35 |
| F42 | Female | Intermediary | SC | PR | 35 |

| | | | | | |
|---|---|---|---|---|---|
| F45 | Female | Tardy | SC | SC | 35 |
| F52 | Female | Intermediary | SC | SC | 9 |
| F53 | Female | Intermediary | SC | SC | 9 |
| M10 | Male | - | MG | PR | 35 |
| M13 | Male | - | PR | PR | 35 |
| M16 | Male | - | SP | PR | 35 |
| M18 | Male | - | PR | PR | 30 |
| M20 | Male | - | SC | PR | 35 |

ID – Identification; MG – Minas Gerais; SP – São Paulo; PR – Paraná; SC – Santa Catarina. Source: The Author (2024).

We used only orthotropic shoots to obtain the scions, and the grafting technique was patch graft (Wendling, 2011; Rickli-Horst *et al*., 2021). Patches with approximately 4 ± 1 cm were fixed into rootstock using plastic tape (Figure 4.1B); after 60 days, the plastic tape was removed, and 15 days later, the remaining rootstock aerial part was pruned just above the bud to stimulate the bud shoot. Rootstock shoots were removed every week until the graft sprouted.

At grafting time, we evaluated the morphological parameters of rootstocks: total height (Ht) and grafting point height (Hg) in centimeters; collar diameter (Dc) and grafting point diameter (Dg) in millimeters. Survival percentage of grafting was evaluated over time at 60, 120, 390, 480 and 540 days after grafting (DAG). Grafts with green color, no necrosis, and callus formation were considered alive. In contrast, black, detached, or rotten grafts were considered dead (Figure 4.1 C-F). Characterization of shoot vigor was determined by shoot height (Hs) and shoot diameter (Ds), the number of branch whorls (Nw) and distance between branch whorls (Dw) were measured at 540 DAG.

### 4.2.4 Experimental design and statistical analysis

The experiment was carried out in a randomized block design with ten blocks. Each block was composed of three rows with 48 plants total (two plants per clone) distributed at a spacing of 9 x 9 m in a quincunx arrangement (Figure 4.2).

Analyses were conducted using Generalized Linear Models, with a binomial distribution for survival results and Gamma distribution for other parameters. Multiple comparisons between treatments were performed using the Tukey test ($p<0.05$) for Gender, Nut maturation season and Provenance treatment and Skott-Knott test ($p<0.05$) for clones comparation. Spearman's correlation ($p<0.05$) was used to verify the relationship between survival, shoot growth and the morphological characteristics of the rootstocks.

## 4.3 RESULTS AND DISCUSSION

Graft survival was influenced by days after grafting (DAG), gender of the mother plant, seed maturation time, origin, and genotype. Genotype influenced all analyzed variables. In addition to survival, the gender of the mother plant influenced the diameter, height, and number of whorls in the shoots; the nuts maturation season exert influence on height of the shoots and the distance between whorls; and the provenance effects the height of the shoots and the number of whorls (Table 4.3).

Table 4.3. ANOVA of GLM (Binomial and Gamma function) for survival (%), Ds – shoot diameter (mm), Hs – shoot heigh (cm), Nw - number of whorls and Dw – distance between whorls (cm) of *Araucaria angustifolia* grafts by DAG – Days After Grafting, mother tree gender, nut maturation season, provenance and genotype.

| **Effect** | **Pr (>F)** | | | | |
|---|---|---|---|---|---|
| | **Survival (%)** | **Ds (mm)** | **Hs (cm)** | **Nw** | **Dw (cm)** |
| DAG | $2.52^{-07}$ | - | - | - | - |
| Gender | $1.28^{-11}$ | $3.42^{-03}$ | 0.02 | $9.78^{-03}$ | 0.96 |
| Nut maturation season | $< 2.20^{-16}$ | 0.24 | $6.00^{-03}$ | 0.42 | 0.04 |
| Provenance | $< 2.20^{-16}$ | 0.19 | 0.04 | $2.20^{-03}$ | 0.23 |
| Genotype | $< 2.20^{-16}$ | $4.80^{-04}$ | $2.50^{-03}$ | $< 1.00^{-16}$ | $3.80^{-03}$ |

Source: The Author (2024).

A higher percentage of graft survival occurred at 60 DAG (80.8%) when the plastic tape was removed (Figure 4.3). At 90, 120, and 150 DAG reduction of survival was recorded, with significant differences from the initial values. However, the largest decline in the survival percentage was observed after 150 DAG, significantly lower than previous evaluations, the survival at 540 DAG (last evaluation) was 16% lower than 60 DAG.

Survival rates in this study were similar to other results reported in the literature related to *A. angustifolia* field grafting (Wendling *et al*., 2017a). Also, survival decline by days after grafting has already been recorded for this species (Wendling *et al*., 2016; Rickli-Horst *et al*., 2019), as well as for other native species, like *Ilex paraguariensis* (Domingos; Wendling, 2006) and *Bertholletia excelsa* (Almeida *et al*., 2020). Survival declines during the first few months may be related to a lack of connection between the scion and rootstock tissues after plastic tape removal (Rickli-Horst *et al*., 2021). Plastic tape has two main functions: keep scion and rootstock tissues in contact for healing and maintaining tissue moisture (Xavier; Wendling; Silva, 2013). Consequently, after plastic removal at 60 DAG, the tissues were exposed to environmental humidity and temperature variations.

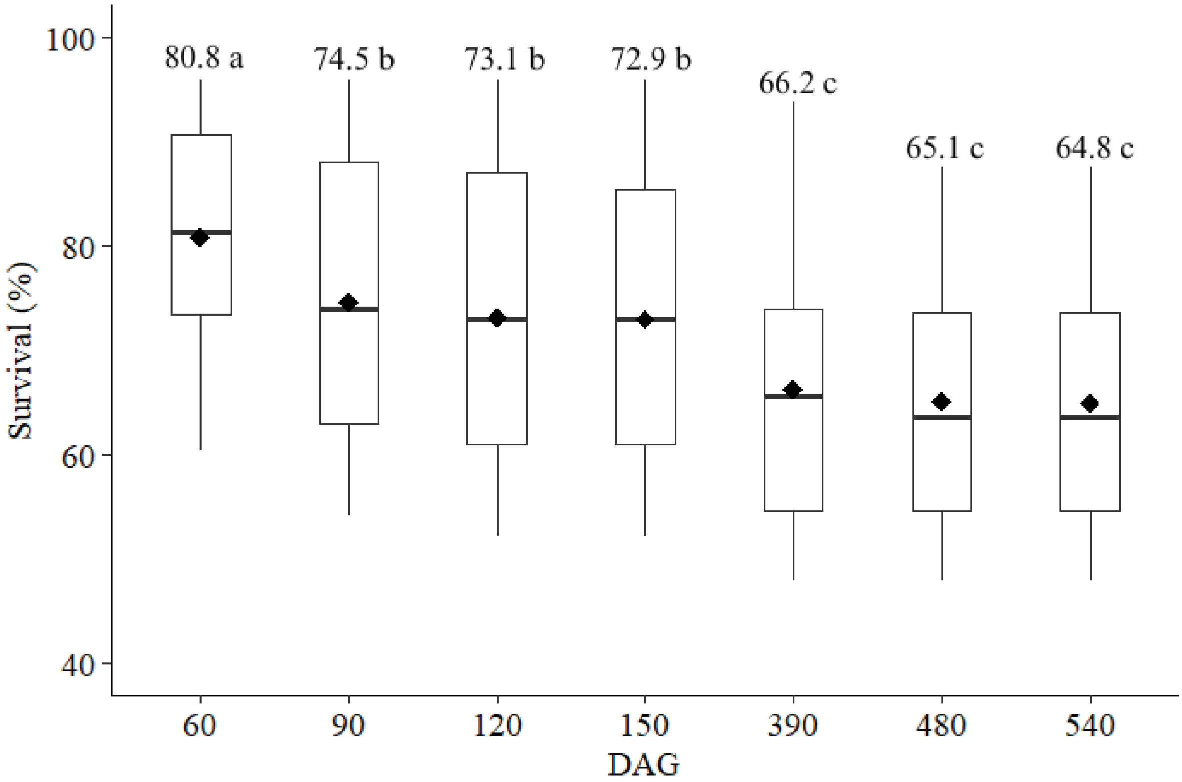


Figure 4.3. Survival percentage of *Araucaria angustifolia* grafts from 60 to 540 Days After Grafting (DAG). Source: The Author (2024).
♦ indicates the mean. Means followed by same letter do not differ among themselves by Tukey Test (p> 0.05).

Furthermore, field grafting is susceptible to external environmental conditions (Wendling *et al*., 2017b). The results showed lower survival after 150 DAG, which can be explained by adverse climatic conditions. The year 2022 was marked by an atypical cold wave that caused frosts between May and June in many Santa Catarina regions, including in the North Plateau (CNN, 2022; INMET, 2023), causing damage due to freezing tissue, death of scion, and in some cases, of rootstock.

Genotype gender influenced survival percentage, diameter, height, and whorls number of shoots (Figure 4.4). Female genotypes showed superior survival of grafts and initial growth of shoots compared to males. Differences between genders of genetic material have already been reported for *A. angustifolia* (Wendling *et al*., 2016; Gabira *et al*., 2022) and *Ilex* *paraguariensis* (Santin *et al*., 2015; Rakocevic *et al*., 2023). This behavior may be associated with ecological and morphophysiological differences among male and female dioecious species (Han *et al*., 2013).

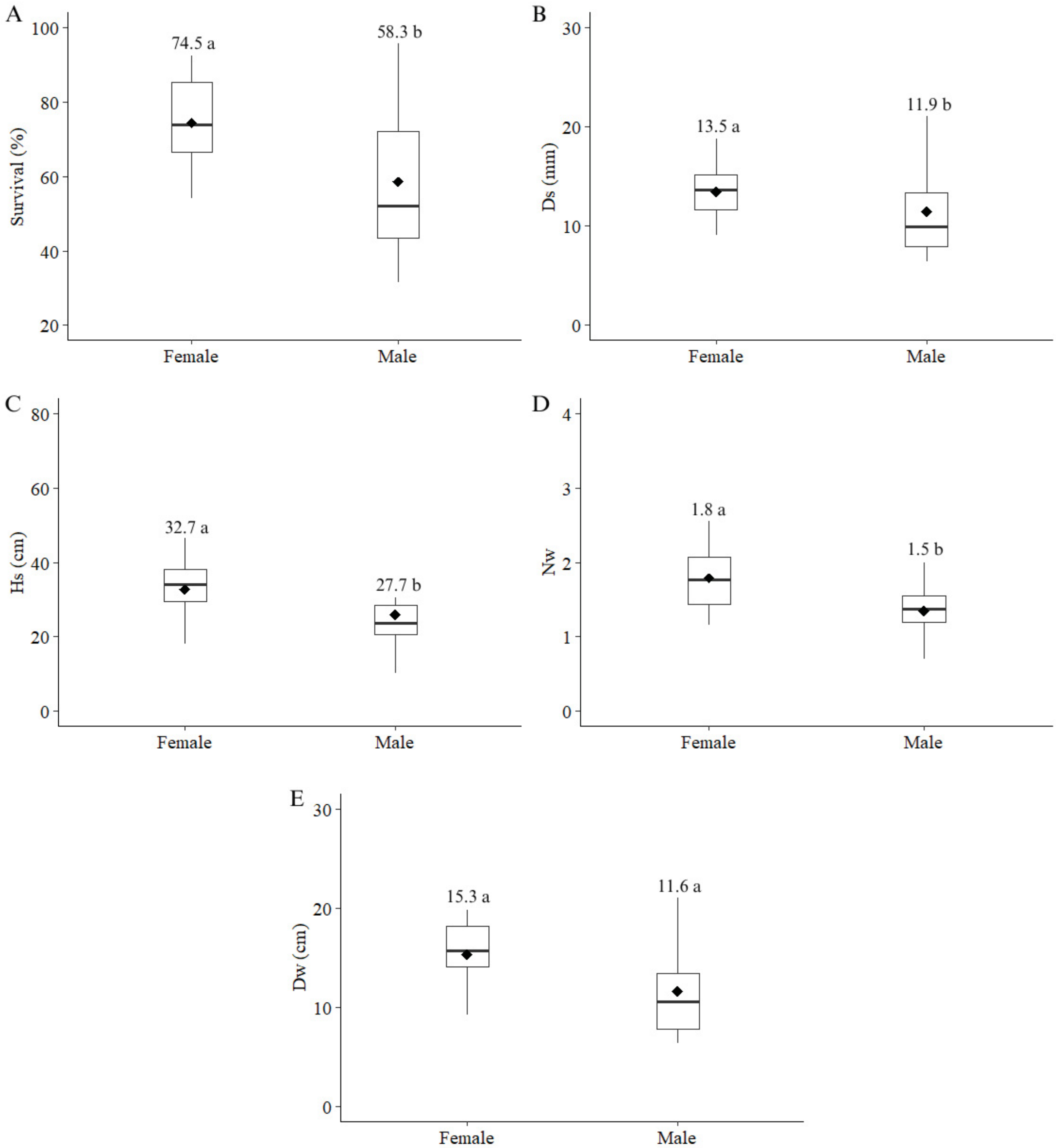


Figure 4.4. Influence of clone gender on *Araucaria angustifolia* grafts. (A) survival of graft, (B) Ds – shoot diameter, (C) Hs – shoot height, (D) Nw – number of branch whorls and (E) Dw – distance between branch whorls. Source: The Author (2024).
♦ indicates the mean. Means followed by same letter do not differ among themselves by Tukey Test ($p > 0.05$).

Mature female individuals tend to have higher biomass and nutrient allocation index, photosynthetic rate, and carbon absorption than males because of the energy required for seed and fruit production (Chailakhyan; Khrianin, 1987; Milla *et al*., 2006; Sánchez-Vilas; Turner; Pannell, 2011; Rakocevic *et al*., 2023). Furthermore, females can be distinguished by their higher water, carbohydrate, calcium, nitrogen, potassium, and phosphorus concentrations and the most sensibility of environmental differences (Dawson; Ward; Ehleringer, 2004).

Only the distance between whorls did not show significant differences for male and female plants. The mechanism underlying whorl formation in conifers suggests that whorls form at the end of each vegetative growth period. The distance between the whorls is directly related to the total height (Encinas; Silva; Pinto, 2005).

The nuts maturation season, classified as precocious, intermediary, and tardy, had a significant influence on graft survival, shoot height, and distance between whorls (Figure 4.5). For these variables, genotypes with an intermediary and tardy maturation season were significantly superior to precocious genotypes.

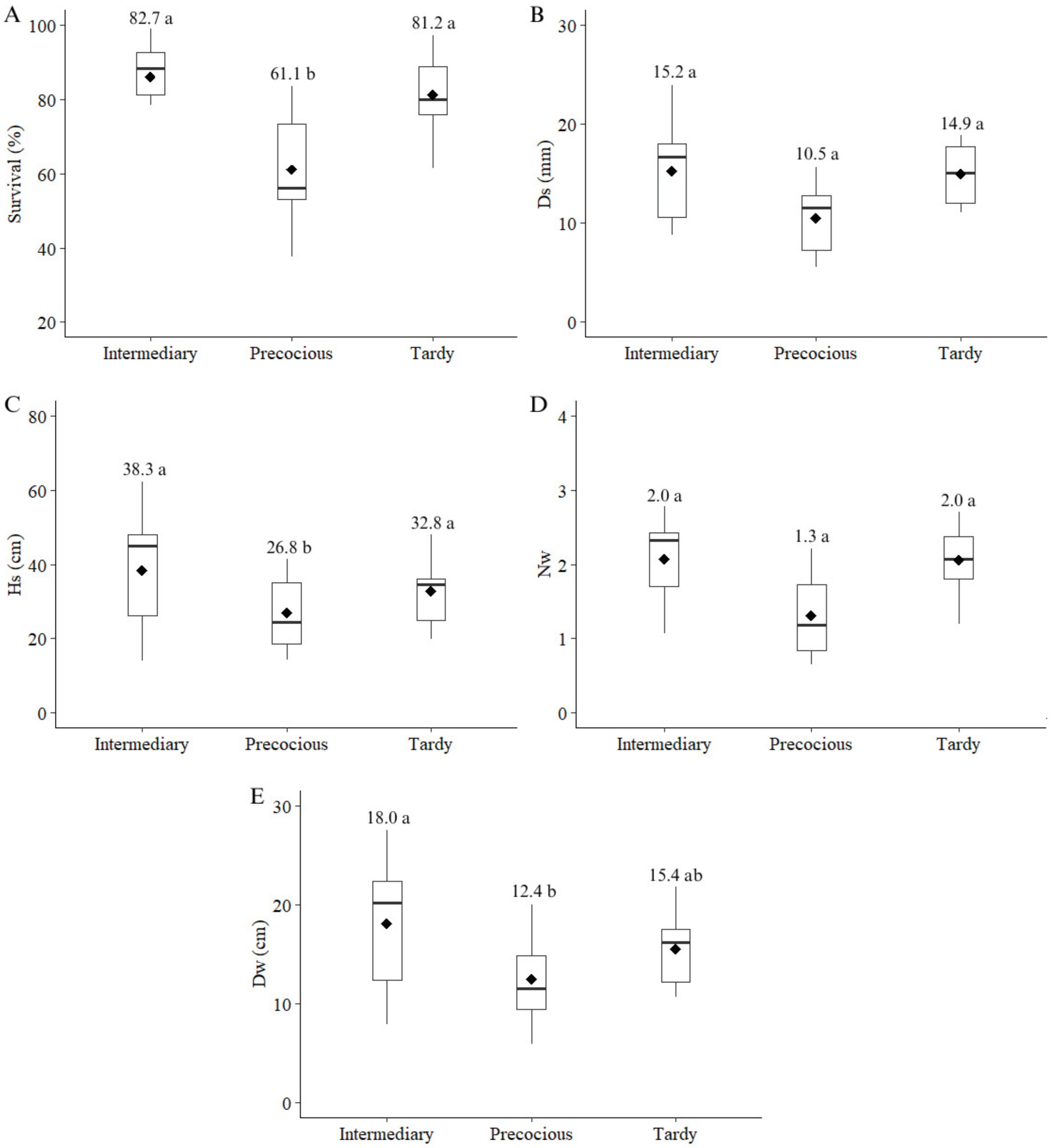


Figure 4.5. Influence of nut maturation season on *Araucaria angustifolia* grafts. (A) survival of graft, (B) Ds – shoot diameter, (C) Hs – shoot height, (D) Nw – number of branch whorls and (E) Dw – distance between branch whorls. Source: The Author (2024).
♦ indicates the mean. Means followed by same letter do not differ among themselves by Tukey Test ($p > 0.05$).

Similar to present work, Coutinho (2007) verified the difference in the initial growth of araucaria seedlings characterized by different nut maturation seasons. According to the author, the intermediary variety has better growth than the tardy variety. The aspects involved in these results have not yet been clarified, but one hypothesis is the existence of different ecotypes characterized by differences in plant physiology and morphology within the same species (Futuyama, 2003). Coutinho (2007) suggested that different varieties are caused by genetic polymorphism, not only by phenotypic plasticity. Also, the varieties maintain their behavior when cultivated in different sites from those of origin. Following this logic, it is expected that in the next years the pine nut maturation season of the genotypes established in the study area will be maintained.

Genotype origin also has a significant influence on graft survival, shoot high and number of whorls (Figure 4.6). Grafts from the southern region of the country (SC and PR) have higher survival (80.2 and 76.1%, respectively), followed by those from MG, and SP with lower values. In an equivalent way, genotypes provenance from PR and SC showed shoot heights (37.1 and 36.1 cm, respectively) and numbers of whorls (1.9 and 2.2, respectively) significantly superior to genotypes from SP (15.4 cm and 0.9 whorls, respectively).

We suppose that better performance for genotypes from the southern region can be explained by the graft adaptation of environmental conditions at the experimental site. Species with larger geographic distributions have great variability and can exhibit different growth responses according to the experimental environment (Moura 2011). The superior performance of genotypes under environmental conditions similar to those of local origin has already been reported for other species (Santos; Cabral; Costa, 2014; Menegatti; Mantovani; Navroski, 2016).

In araucaria populations, the greatest genetic variation occurs in space, suggesting that individuals from provenances differ due to evolutionary forces such as gene flow (Silva, 2016). This behavior can be explained by the ecotypic genetic structure associated with environmental variations (Shimizu, 2007) and the gradual change in phenotypes across the distribution of a species or population (Menegatti; Mantovani; Navroski, 2016).

Araucaria has prehistoric origins, and species belonging to this genus are distributed across different regions of the planet, adapting to various environmental conditions. In Brazil, *Araucaria angustifolia* has undergone a series of adaptations to different habitats (Fritzsons; Wrege; Mantovani, 2017). In SP and MG, the distribution of the species is not continuous, as in the southern regions, but rather fragmented. The species is mainly found in places with altitudes above 750 m, such as Serra da Mantiqueira and Serra do Mar (Fritzsons; Mantovani; Wrege, 2021), which contributes to the hypothesis of the development of races or ecotypes.

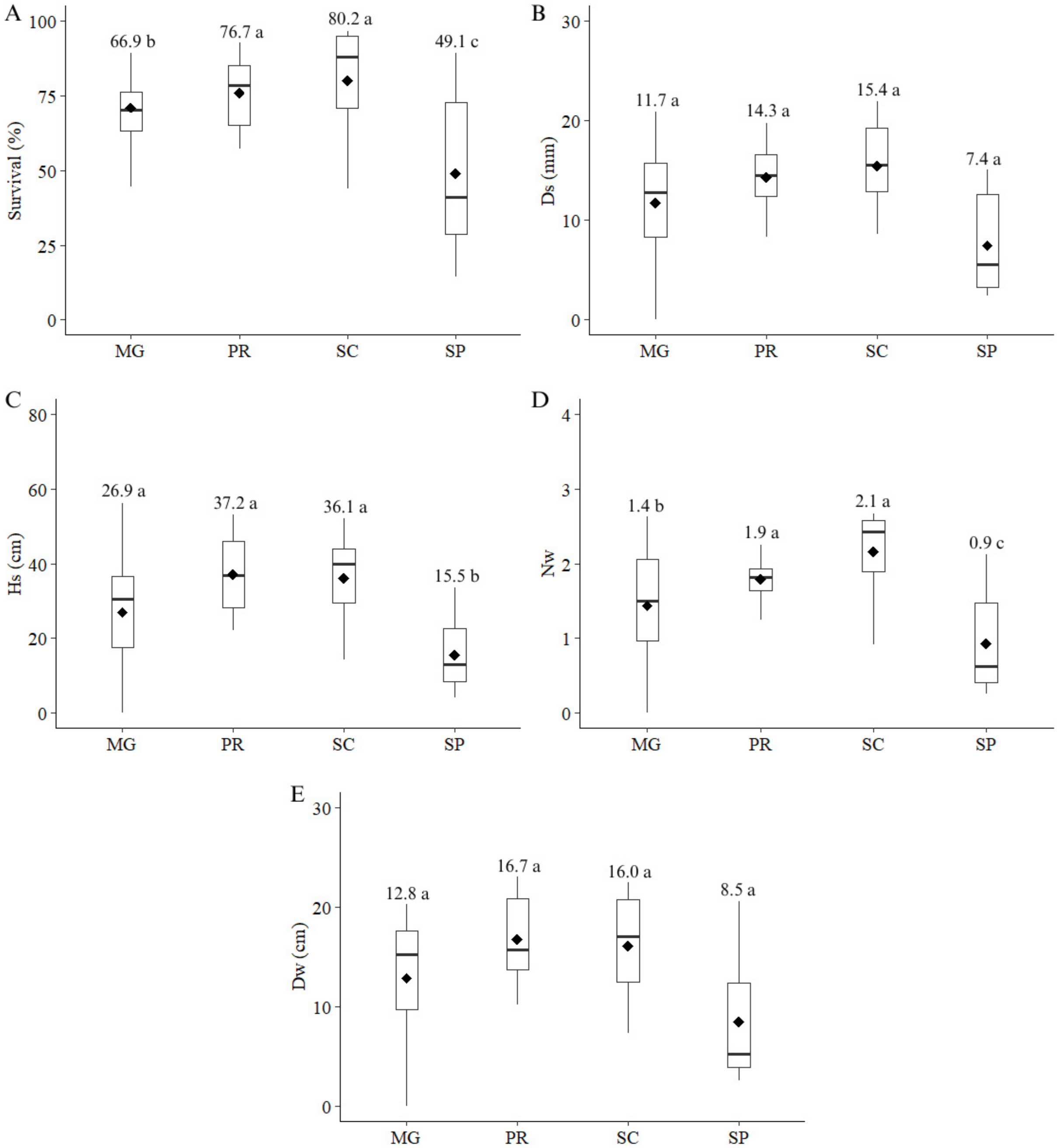


Figure 4.6. Influence of clone provenance on *Araucaria angustifolia* grafts. (A) survival of graft, (B) Ds – shoot diameter, (C) Hs – shoot height, (D) Nw – number of branch whorls and (E) Dw – distance between branch whorls. Source: The Author (2024).
MG= Minas Gerais, PR = Paraná, SC = Santa Catarina, SP = São Paulo. ♦ indicates the mean. Means followed by same letter do not differ among themselves by Tukey Test ($p > 0.05$).

Research applying molecular genetic estimators have distinguished the genetic basis of populations from the southeast and southern regions. Differences between the SP and PR populations amount to 11.3%; between SP and SC 21.9%, while the differences between Paraná and Santa Catarina are only 4.9% (Stefenon; Gailing; Finkelday, 2007). These ecotypes, or races that are adapted to specific environmental conditions, may be the result of genetic forces within a species that are influenced by environmental pressures such as altitude, latitude, soil, and climatic conditions. Also, the introduction of plants into environments distinct from their natural habitat can lead to unexpected development, growth, morphology, and phenology (Shimizu, 2007), which can be observed by the results presented here.

Research on *A. angustifolia* suggests that considerable genetic variation exists within populations or between progenies (Auler *et al*., 2002; Stefenon; Gailing; Finkelday, 2007; Souza *et al*., 2009). Although the results of this study are from clones and not seminal progenies, it is possible to verify this variation by individually analyzing the responses of each genotype (Table 4.4). Graft survival varies between 35 and 95% for M16 and F53, respectively. Nine genotypes had survival rates above 80%, seven of which were from PR and SC (F17, F18, F26, F36, F37, F52 and F53) and two from MG (F25 and M10).

Table 4.4. Influence of each genotype on *Araucaria angustifolia* grafts characteristics. Survival of graft, Ds – shoot diameter, Hs – shoot height, Nw – number of branch whorls and Dw – distance between branch whorls.

| **Genotype** | **Survival (%)*** | **Ds (mm)** | **Hs (cm)** | **Nw** | **Dw (cm)** |
|---|---|---|---|---|---|
| F4 | 72.1 e | 12.3 e | 28.4 e | 1.6 g | 12.9 e |
| F12 | 70.7 e | 12.4 e | 21.8 f | 1.6 g | 11.8 e |
| F14 | 40.0 h | 7.8 f | 23.2 f | 1.0 j | 7.4 g |
| F17 | 90.0 b | 16.9 b | 45.6 b | 2.4 d | 20.2 b |
| F18 | 82.9 c | 13.6 e | 27.0 e | 1.4 i | 16.6 c |
| F24 | 50.0 f | 8.2 e | 15.4 g | 0.9 k | 10.9 e |
| F25 | 80.7 d | 12.4 e | 26.9 e | 1.5 h | 14.5 d |
| F26 | 91.4 b | 17.5 b | 46.5 a | 2.8 b | 20.1 b |
| F27 | 63.6 f | 13.0 e | 31.9 d | 1.4 i | 15.9 c |
| F28 | 38.6 g | 5.3 f | 11.1 g | 0.6 l | 6.2 g |
| F29 | 60.0 f | 11.7 e | 30.2 d | 1.4 i | 13.0 d |
| F32 | 77.1 d | 15.0 d | 35.3 d | 2.0 e | 14.3 d |
| F33 | 72.9 e | 11.2 | 36.3 d | 1.5 h | 17.2 c |
| F36 | 92.9 b | 18.3 a | 38.4 c | 2.1 e | 21.2 b |
| F37 | 90.7 b | 16.3 b | 49.9 a | 2.7 b | 18.6 c |
| F42 | 76.4 d | 17.2 b | 48.4 a | 2.2 e | 18.7 b |
| F45 | 74.0 e | 11.8 e | 22.1 f | 1.7 f | 10.1 f |
| F52 | 94.3 b | 19.3 a | 46.6 a | 3.3 a | 17.0 c |
| F53 | 95.0 a | 15.3 c | 35.4 d | 1.5 h | 24.4 a |
| M10 | 87.1 c | 15.0 d | 27.7 e | 1.8 f | 16.5 c |
| M13 | 54.3 f | 13.3 e | 39.5 d | 1.5 h | 15.6 d |

| | | | | | |
|---|---|---|---|---|---|
| M16 | 35.7 h | 4.1 f | 7.1 h | 0.5 l | 3.8 h |
| M18 | 45.0 g | 8.3 e | 21.6 e | 1.0 j | 8.0 f |
| M20 | 70.7 d | 16.5 b | 42.6 d | 2.6 c | 14.1 d |
| **Total** | 71.1 | 13.0 | 31.6 | 1.7 | 14.5 |

Means followed by same letter do not differ among themselves by Scott-Knott Test (p> 0.05) and * by Tukey Test (p> 0.05). Source: The Author (2024).

The shoot height varied from 7 to 50 cm, and the shoot diameter ranged from 4 to 19 mm. The number of whorls per shoot varied from zero to 3, with a distance between whorls ranged from 3 to 24 cm. F17, F26, F32, F36, and F52 also exhibited better performance for all variables analyzed, all females from the PR and SC, with intermediary nut maturation season. These results reinforce the effect of provenance on graft survival and growth and demonstrate the superiority of female genotypes.

Through Spearman's correlation analysis, we observed that rootstock vigor variables (Dc, Ht, Dg and Hg) were not significantly correlated with graft survival up to 150 DAG (Figure 4.7A). After 150 DAG, there is a positive correlation between variables; however, according to Callegari-Jacques (2003) classification, the value indicates a weak correlation (≤ 0.20) between variables. It is suggested that plants with larger rootstock diameters and heights survive better when subjected to adverse conditions like frosts. Reis, Chalfun and Reis (2010) asserted that the efficiency of grafting depends, among other factors, on the capacity of rootstocks to adapt to different climes, as well as on the initial performance under different cultivation conditions.

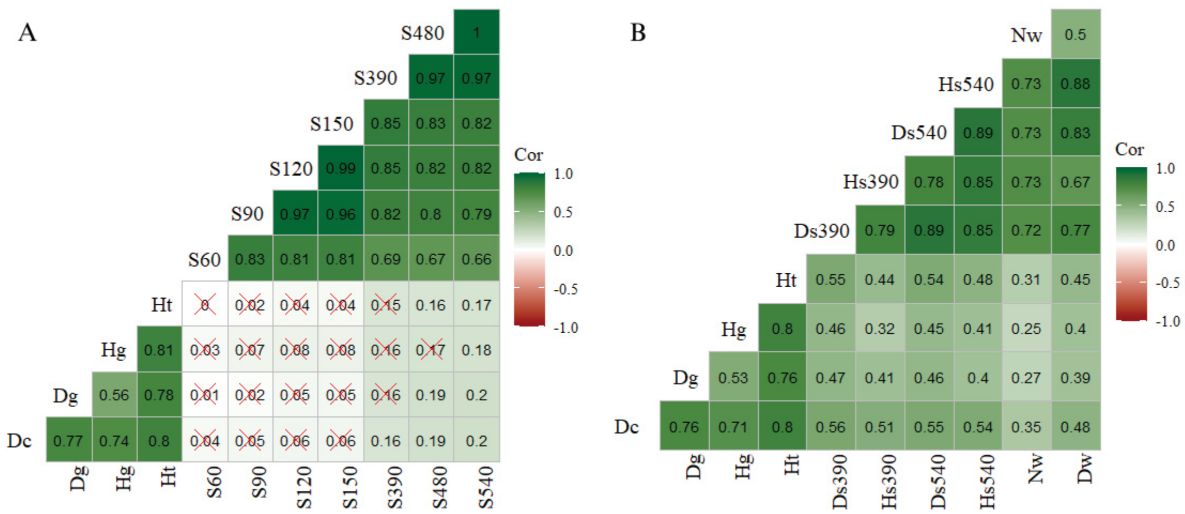


Figure 4.7. Spearman correlation (A) between Ht - total height, Hg - grafting point height, Dc - collar diameter, Dg - grafting point diameter and S - survival at 60, 120, 390, 480, 540 DAG. (B) between Ht - total height, Hg - grafting point height, Dc - collar diameter, Dg - grafting point diameter, Hs - shoot height, Ds - shoot diameter at 390 and 540 DAG, Nw - number of branch whorls and Dw - distance between branch whorls. Source: The Author (2024).
Values with × are not significant at 5% level (green: positive correlation; red: negative correlation).

Shoot growth (Ds, Hs, Nw and Dw) was positively correlated with rootstock vigor variables (Figure 4.6B). These correlations ranged from 0.25 between the height of the graft point and the number of whorls to 0.56 between the collar diameter of the rootstock and shoot diameter. So, all correlations between variables are considered weak or moderate (Callegari-Jacques, 2003).

Survival over days after grafting was positively correlated with each other, as well as the rootstock vigor variables (Dc and Ht) and the graft point variables (Dg and Hg). A strong correlation between the height and diameter of the rootstock has already been observed, and it is considered an essential indicator of vigor to determine the survival and growth of grafts (Spinelli *et al.,* 2024). The use of rootstocks with a larger diameter favors graft survival and shoot diameter growth (Gomes *et al*., 2010).

The results collected by this research represent a further step in the development of strategies to promote the cultivation, valorization, and even conservation of araucaria. Identifying and rescuing individuals, as was done in this research, is an arduous and necessary task. Through selection between and within populations, significant genetic gains can be achieved, especially for araucaria. Research has identified heigh genetic diversity and exclusive alleles in many populations of *A. angustifolia* in the mountainous region of Santa Catarina (Costa *et al.,* 2015). Establishing seed orchards from different plant origins can promote crossbreeding and increase diversity (Sebben *et al*., 2007).

To date, there are no records of other araucaria orchards with such diversity and the potential for future studies and discoveries. We hope that in the future, it will be possible to establish orchards with different nut qualities, such as size and taste. In the next few years, it will be possible to determine the productive characteristics of genotypes and estimate orchard productivity based on this proposition. Other information that can be identified includes interactions between genetic materials of different origins and potential crossbreeding among varieties.

## 4.4 CONCLUSION

Genetic material characteristics influence grafts establishment. Female genotypes from Paraná and Santa Catarina states, with intermediary and late nut maturation seasons have greater graft survival and shoot initial growth.

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# 5 FINAL CONSIDERATIONS

Over the last few decades, the struggle to contain the extinction process and make araucaria a species allied to rural properties has faced many challenges and has reached a paradigm shift due to the possibility of early pine nut production. Rural producers who previously removed Araucaria seedlings from their property currently maintain them due to the possibility of using these seedlings to graft productive genetic materials favoring early production.

The appreciation of the species provides new perspectives for research; in this sense, the results represent unprecedented advances in the clonal forestry of the species. The use of minicuttings technique has not yet been consolidated due to difficulties related to rooting. In the first chapter, we found that controlling air humidity and using microsprinklers in an automated greenhouse are favorable for the rooting of minicuttings, regardless of the type of substrate used. Although rooting percentages are not ideal for enabling the production of clonal plants on a large scale, this discovery can be a starting point for new studies aimed to increase the control of rooting conditions, as well as those for maintaining mini-stumps, given their importance in this process.

*Araucaria angustifolia* is not the only one of the genus capable of producing edible seeds. *A. bidwillii*, which is native from Australia, also produces pine nuts. The grafting combining these two species proved to be functional and with normal development until seven years of age when differences in diameter were observed between the scion and rootstock, indicating late incompatibility. There is no evidence to date that this symptom interferes with seed production or future graft growth. Additionally, the first occurrence of early pine cone production for *A. bidwillii* was reported in this study, making it possible to apply the technique to this species. Due to the lack of individuals with active pollen production, the cone seeds were empty; therefore, future observations are necessary to clarify effective seed formation. It is believed that the introduction of this species in Brazil will bring new quality pine nuts and new products derived from its seeds, in addition to the possibility of forming orchards with a mix of species.

Every year, the demand for grafted araucaria plants with high performance grows, consequently the need to understand how different individuals respond to grafting and cultivation in orchards also grows. The results suggest that genetic materials have better survival and growth under edaphoclimatic conditions similar to those of their provenance, reinforcing the need for producers or nurseries to select their matrices in adaptation to planting locations. Female with a pine maturation period classified as intermediary or tardy are the most recommended. From the experimental area formed, studies will be possible over the next few decades related to the growth of clones, the interaction of competition and crossing between individuals of different origins, pollen and pine nut productivity of the matrices, and other information. Furthermore, these results can be fundamental for planning new commercial orchards, not just with already registered clones.

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