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UFSM

Articles

How to enhance grafting in *Eucalyptus grandis* Hill ex Maiden for the cloning of superior genotypes?

Como potencializar a enxertia em *Eucalyptus grandis* Hill ex Maiden para a clonagem de genótipos superiores?

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ABSTRACT

Grafting is a vegetative propagation technique used for forest genetic improvement. It involves the multiplication of selected matrices to produce improved seeds. In this study, we evaluated three grafting techniques for *Eucalyptus grandis*. The experiment was conducted using a completely randomized design, analyzing the techniques of cleft grafting with grafting pliers, bark grafting, and cleft grafting with a grafting knife. The technique with grafting pliers showed 50% establishment, superior to bark grafting (33.3%) and grafting using a knife (33.3%). The shoot length was significantly greater when using pliers (9.9 cm) and bark grafting (4.9 cm) than when using a grafting knife (2.6 cm). We conclude that the cleft grafting technique with pliers is the most suitable for this species due to operational practicality.

Keywords: Vegetative propagation; Grafting pliers; Cleft grafting; Genetic improvement; Silviculture

RESUMO

Dentre as técnicas de propagação vegetativa utilizadas no melhoramento genético florestal está a enxertia, a qual é utilizada na multiplicação de matrizes selecionadas, visando à produção de sementes melhoradas. O estudo teve como objetivo avaliar três técnicas de enxertia em *Eucalyptus grandis*. O experimento foi conduzido em delineamento inteiramente casualizado, analisando as técnicas de garfagem em fenda cheia com alicate de enxertia, sob casca e garfagem em fenda cheia com canivete de enxertia. A técnica com alicate de enxertia apresentou pegamento de 50%, sendo superior a técnica de enxertia sob casca (33,3%) e fenda cheia com canivete (33,3%). O comprimento de brotos foi significativamente superior com o uso do alicate (9,9 cm) e sob casca (4,9 cm) em relação ao canivete (2,6 cm). Pela praticidade operacional, verifica-se que a técnica de enxertia em fenda cheia com alicate é a mais indicada para a espécie.

Palavras-chave: Propagação vegetativa; Alicate de enxertia; Fenda cheia; Melhoramento genético; Silvicultura

1 INTRODUCTION

Cultivating commercial forests is essential for supplying raw materials for wood and non-wood industries to meet society's diverse demands (Lima *et al*., 2022). The estimated area of planted forests in Brazil is approximately 9.94 million hectares, with a greater concentration in the southeastern and southern regions. Approximately 76% of the total planted areas are destined for forest plantations with the most diverse species and hybrids of the *Eucalyptus* genus (IBÁ, 2023).

Owing to the edaphoclimatic conditions that enabled the expansion of the *Eucalyptus* genus throughout the national territory, Brazil became prominent in international trade as one of the main producers of cellulose, attracting many investments in research and innovation from private companies and universities (Gonçalves *et al*., 2017). The most planted species in Brazil is *Eucalyptus grandis* W. Hill ex Maiden, as well as its interspecific hybrids (Konzen *et al*., 2017), due to its excellent silvicultural responses, such as good shape and fast growth, in addition to its desirable timber properties for multiple uses (Soares; Carvalho; Vale, 2003).

Grafting stands out among vegetative propagation techniques used in forming clonal orchards aimed at producing improved seeds, mainly species of the genera

Pinus and *Eucalyptus*, with the aim of recombining superior genotypes (Alfenas *et al*., 2009; Pérez-Luna *et al*., 2019).

According to Fonseca, Resende and Alfenas (2010), hybridization programs were introduced in companies in the forestry sector, with the creation of in and outdoor orchards from the 1990s onwards. Artificial hybridization techniques have become fundamental tools for breeding programs, enabling the selection of parents with desired characteristics and obtaining hybrids via specific controlled crossings that are evaluated and selected in progeny and clonal tests until superior clones are obtained for future commercial plantations (Castro *et al*., 2016).

Matrices are propagated via grafting to form controlled pollination orchards, aiming for early flowering because of their greater ontogenetic age and reduced crown size, which facilitates manual pollination and seed collection (Golle *et al*., 2009).

Grafting involves the union of two genotypes, the rootstock and scion, to form a new plant (Pereira *et al*., 2014). The grafting technique is affected by several factors ranging from graft skill to anatomical, physiological, biochemical, and environmental aspects (Ribeiro *et al*., 2005). Furthermore, there are variations in the grafting method, of which cleft grafting, air layering and side-veneer grafting are the most commonly used, as they present varying responses depending on the species and type of material used in the process (Fachinello; Hoffmann; Nachtigal, 2005).

Therefore, the aim of this study was to evaluate different grafting techniques for *E*. *grandis* to improve vegetative propagation methods for forest improvement programs.

2 MATERIALS AND METHODS

The experiment was conducted from May to October 2022 at the Silviculture and Forest Nursery Laboratory (29°43’13”S; 53°43’16”W) of the Department of Forestry Sciences of the Federal University of Santa Maria (UFSM), Santa Maria, Rio Grande do Sul (Brazil). According to the Köppen classification, the region’s climate is type Cfa, i.e., subtropical with hot summers (Alvares et al., 2013). Meteorological data were obtained from a station of the National Institute of Meteorology in Santa Maria, Rio Grande do Sul (INMET, 2022). The average temperature and relative humidity (RH) during the study period were 15.7 °C and 81.78%, respectively.

The *E. grandis* seedlings used as rootstocks were produced via seed in polypropylene tubes with a volume of 50 cm^3 filled with Maxfertil® commercial substrate and were subsequently transferred to 3.5 L pots containing subsoil. The rootstock containers were exchanged and placed in 5 L pots filled with commercial substrate plus 4 g L^{-1} of controlled-release fertilizer. The seedlings were provided with 200 mL of a nutrient solution composed of macro and micronutrients every 30 days. The rootstocks used were 24 months old, with an average height of 120 cm and stem diameter of 11.43 mm.

The branches used to obtain the grafts were collected in May 2022 from a single adult mother tree of *E. grandis* in a plantation on the UFSM headquarters campus before flowering. The criteria for selecting the upper tree were dominant height, straight trunk, and absence of pest and disease symptoms. The branches were sectioned to obtain grafts measuring 12–15 cm in length and 7.5–12 mm in diameter, with at least three axillary buds. They were kept in a plastic container and covered with moistened paper towels to maintain graft turgidity.

The experiment was conducted in a greenhouse (up to 30 days after the start of the experiment) and a shaded house (from 30 days until the end of the experiment, i.e., 150 days after grafting). A completely randomized design was used to evaluate three grafting techniques: cleft grafting with grafting pliers (CGP), bark grafting (BG), and cleft grafting with a grafting knife (CGK). Each treatment consisted of six replicates (one plant per replicate for a total of six grafts per treatment). The rootstocks were not pruned during the seedling growth phase; cuts were made using the respective techniques only at the time of grafting. The cutting height on rootstocks varied depending on the position of the “lung branches”.

Four branches called "lung branches" were left on the rootstocks to maintain the photosynthetic function of the plant, and were removed after 60 days of applying the technique, thus allowing the development of shoots on the grafts. Subsequently, a parafilm-type tape was used in the union between the rootstock and scion to assist in the healing process, moisture retention, and protection against pathogens.

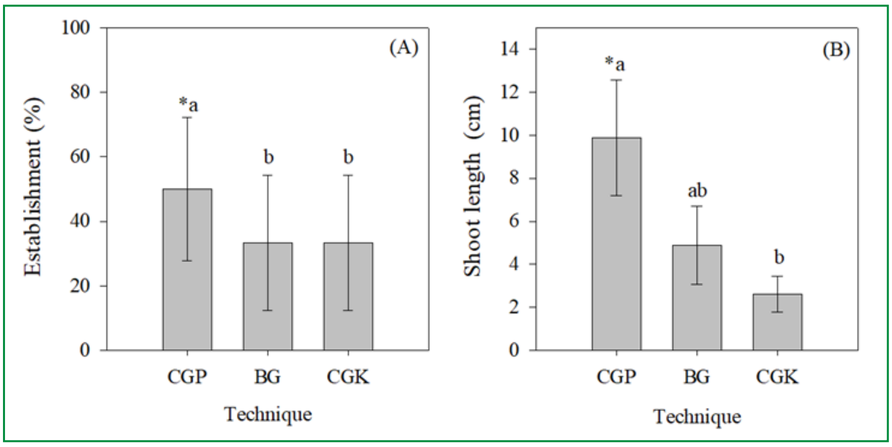
The following variables were analyzed: percentage of establishment, number of shoots formed in the graft, shoot length (cm), and shoot diameter (mm). A ruler and digital caliper were used to measure the length and diameter of the shoots. The analyses were performed 150 days after the start of the experiment.

The data were tested for normality using the Shapiro-Wilk test and homoscedasticity using the Bartlett test. Subsequently, the means were compared using Tukey's test at a 5% probability level. The data were transformed by $\sqrt{x+1}$ (Ferreira, 1991), aiming to meet statistical assumptions. To evaluate the percentage of establishment, a chi-square test was used at a 5% significance level. Statistical analyses were performed using the statistical software R Development Core Team version 4.1.3.

3 RESULTS AND DISCUSSIONS

The percentage of *E. grandis* grafts establishment was significant (p = 0.0199), with the technique using grafting pliers showing a establishment of 50%, which was superior to the bark grafting (33.3%) and cleft grafting with knife (33.3%) (Figure 1A). There was also a significant effect (p = 0.04931) on shoot length, with grafting using pliers and bark grafting showing higher averages of 9.9 cm and 4.9 cm, respectively. The cleft grafting with knife technique resulted in shorter shoots (2.6 cm) (Figure 1B). Shoot diameter and number did not differ significantly (p = 0.13281 and p = 0.70189, respectively), with an overall average of 0.89 mm and 1.64 shoots formed per plant.

Figure 1 – Percentage of graft establishment (A) and length of shoots (B) of *Eucalyptus grandis* in relation to the grafting technique



Source: Authors (2024)

In where: * Significant at the 5% level using chi-square (A) and Tukey (B) tests. CGP: cleft grafting with grafting pliers; BG: bark grafting and CGK: cleft grafting with a grafting knife. Vertical bars indicate the standard error.

The results of the percentage of establishment in the present study demonstrated the potential gain in the use of cleft grafting with pliers (16.3 percentage points) when compared to bark grafting and cleft grafting with a knife. In a study by Santos *et al*. (2012) on different species of the *Eucalyptus genus*, the cleft grafting technique showed an average establishment of 46 %. Using the lateral cleft grafting technique with *E*. *urophylla* and *E*. *urophylla* x *E*. *grandis*, Moraes *et al*. (2013) obtained 55% and 62% establishment, respectively, at 180 days.

Several factors influence the establishment of grafts, with the correct management of plants during and after grafting being of utmost importance (Franzon *et al*., 2008). This includes the environmental temperature, which must be between 20 and 30 °C, and an environment free of contaminating agents (Xavier; Wendling; Silva, 2013).

In this study, the growth environment of the grafts (greenhouse and shade house) and season (autumn and winter) were suitable for grafting (Figure 2). The most suitable seasons for grafting are spring and summer, as the temperatures are higher and greater cambium activity between the graft and rootstock is guaranteed (Hartmann *et al*., 2014). The grafting period used in the present study was adequate for the specific species. Furthermore, the use of parafilm ensured adequate union between propagules, avoiding the accumulation of moisture and preventing the development of diseases that could harm graft adherence.

Figure 2 – *Eucalyptus grandis* shoots 60 days after the start of the experiment using the cleft grafting with pliers (A) and healing of the grafts after one year using the pliers grafting techniques (B), bark grafting and (C) cleft grafting with knife (D)

Source: Authors (2024)

Other factors limiting the success of grafting include the technique used for each species, physiological and nutritional conditions of the rootstock and scion, asepsis during cutting, graft skill, and management after graft production (Fachinello; Hoffmann; Nachtigal, 2005). Skill is required to make the graft in species of the *Eucalyptus* genus so that the cuts made are not damaged, aiming at tissue healing (Assis, 2012). Cuts must be made uniformly to leave a good contact area for tissue connections.

The cleft grafting with pliers technique enables for more precise and shear-free cuts, comparing with cleft grafting with knife and bark grafting, and the cutting blades can be adapted according to the diameter of the propagules in addition to regulating the depth of the slit produced. The cleft cut performed using the pliers enabled the same dimensions to be obtained between the rootstock and scion, contributing to the establishment of the materials.

Another factor that may have contributed to the establishment of grafts was the production of rootstocks, with seeds obtained from several *E. grandis* trees coming from the same area where the branches were collected. Incompatibility between genetic materials greatly influences the grafting of *Eucalyptus* species (Xavier; Wendling; Silva, 2013). Indicators of graft incompatibility (premature death, failure to stick, yellowing, and leaf fall) may occur at the beginning or two years after grafting. No symptoms of incompatibility were observed among the materials used during the evaluation period.

Among the problems generated by incompatibility are nutritional deficiency and hormonal imbalance, mainly auxin and ethylene (Aloni *et al*., 2008). Problems can be solved with self-grafting, in which propagules (seeds and branches) from the same parent tree are used, in addition to maintaining the “lung branches” on the rootstock until the first shoots appear (Hartmann *et al*., 2014).

The main grafting techniques used by forestry companies for the *Eucalyptus* and *Corymbia* genera are bark grafting and cleft grafting with a grafting knife, with the use of pliers not being common. Thus, this study introduced a new grafting alternative using this equipment that guarantees greater grip and length of the shoots and can help forestry companies in the propagation process. The tool used facilitated the preparation of the graft and rootstock, ensuring greater adhesion between the parts and providing greater success in establishment, without requiring skills, when compared to the other techniques covered by this study.

Despite the initial cost of purchasing the pliers, time was optimized to carry out the grafting process, in addition to reducing the loss of plant material, considering the costs involved with seeds, inputs, labor for rootstock production, and the collection of propagules in the field.

4 CONCLUSIONS

Owing to operational practicality, the cleft grafting technique using grafting pliers appears to be the most suitable for *Eucalyptus grandis*, providing satisfactory establishment and greater growth of the shoots formed on the grafts.

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