


Recombination orchard of *Cordia trichotoma* (Vell.) Arráb. ex Steud.: Quantitative genetics, grafting and pollen management

Kethlin de Carvalho Santos Romão¹, Paulo Henrique Müller da Silva², Daniel Piotto¹, Samir Rolim¹, Lílian Alves Carvalho Reis³, Laura Vicente Guimarães³ and Andrei Caíque Pires Nunes^{1*} 

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Abstract: *Recombination orchards facilitate superior tree genotypes crossings and improved seed production. In this study we aimed to a) investigate genetic parameters in a progeny test of Cordia trichotoma (Vell.) Arráb. ex Steud.; b) establish a clonal recombination orchard using grafting of the selected trees by genetic values; and c) carry out pollen collection and processing. A provenance and progeny test was conducted and evaluated at nine years of age for growth traits. Quantitative genetic parameters were estimated, and superior genotypes were selected and preserved through grafting. Grafted plants were monitored, and pollen collection techniques were tested. The best 10 ranked individuals reached up to 33 m³ ha⁻¹ year⁻¹ MAI yield, with gains up to 55%. The grafting success rate reached up to 70% and pollen management was effective. Forest breeding programs for timber species like C. trichotoma were optimized by refining grafting and controlled pollination methods, making genetic recombination viable.*

Keywords: *Timber species, forest breeding, crossing trees, controlled pollination, louro-pardo*

INTRODUCTION

Genetic recombination among superior genotypes is fundamental for advancing breeding programs and generating improved seeds (Castro et al. 2021). Crossing trees is a hard task due to their height, which makes it difficult to reach the tree crown and collect improved seeds. Synchronizing the flowering of target individuals for recombination, as well as inducing early flowering to accelerate the breeding process are challenging steps (Sharma et al. 2024). Clonal recombination orchard is a nursery area for reproducing genetically superior trees at an accessible height in order to easily handle flower buds (control pollination), as well as for seed and pollen management (Garuzzo et al. 2021, Mendes et al. 2021, Santos et al. 2025). This strategy enables crossings among a large number of superior genotypes and efficiency in the genetically improved seed generation, which optimize tree breeding programs (Santos et al. 2023).

Knowledge regarding the establishment of recombination orchards, pollen management, and grafting is limited for most timber species and even more so for tropical species. Generating improved seeds quickly and developing pollen collection and management techniques is crucial for the advancement of forestry

***Corresponding author:**

E-mail: andrei.nunes@gfe.ufsb.edu.br

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¹ Universidade Federal do Sul da Bahia, Centro de Formação em Ciências Agroflorestais, Praça José Bastos, s/n, Centro, 45600-923, Itabuna, BA, Brazil

² Instituto de Pesquisas e Estudos Florestais, Avenida Comendador Pedro Morganti, 3500, Monte Alegre, 13415-000, Piracicaba, SP, Brazil

³ Symbiosis Investimentos e Participações, Rodovia BA 001, Estrada de Trancoso, Distrito de Trancoso, Fazenda Symbiosis, Coqueiro Alto, 45818-000, Porto Seguro, BA, Brazil

worldwide. Given the climate change scenario and the need to expand forest plantations for carbon sequestration and generation of timber products from planted areas, developing strategies to accelerate forest breeding programs is urgent for nature conservation and industry services (Laverdière et al. 2022, Chen et al. 2024, Xu et al. 2024). Especially for Brazilian native timber species, the lack of knowledge about their potential uses allied to the absence of genetic studies resulted in little interest of the private sector to establish commercial plantings (Santos et al. 2023, Pereira et al. 2024, Zaruma et al. 2024). However, companies have been buying large areas in Brazil in recent years to develop plantations with native timber species, aiming at timber production and carbon credit markets. Therefore, interest in species such as *Cordia trichotoma* (Vell.) Arráb. ex Steud (Boraginaceae) has been increasing and studies to subsidize its silvicultural development and planting need to be conducted (Rolim and Piotto 2024).

Tree species such as *C. trichotoma* are attractive species for timber and carbon projects due to its fast growth rate and industry favorable wood properties, such as medium wood density and hardness, low anisotropy, and a low tendency toward warping and twisting (Grzybowski et al. 2022). These characteristics make *C. trichotoma* a viable option for the sawn timber sector, furniture industry, civil and naval construction, as well as in landscaping (Grzybowski et al. 2022, Rolim and Piotto 2024). *C. trichotoma* has great geographical distribution around Brazil and Latin America, standing out in Atlantic Rainforest biome (Avinio et al. 2022). It has hermaphrodite flowers and a predominantly allogamous mating system (Souza 2008). The main floral visitors and consequently pollination vectors of *C. trichotoma* belong to the order Hymenoptera, with an emphasis on bees, as well as small insects from the orders Coleoptera, Diptera, and Lepidoptera (Albuquerque et al. 2020). This species additionally has large potential for sawn wood and good volume yield (Nunes et al. 2024). Considering all of its potential, it is imperative to develop studies for *C. trichotoma* crossings of superior genotypes inside recombination orchards and enable the generation of more productive individuals.

Clonal recombination orchard is an important strategy to optimize crossing among top ranked genotypes selected in provenance and progeny tests (Santos et al. 2025). Vegetative propagation techniques such as grafting can be applied to clone superior genotypes for improved seed production (Mendes et al. 2021). In the case of recombination orchards, the main goal is to clone the best trees from a progeny test for subsequent crossing. Therefore, the trees in a recombination orchard are short, about 1.5 m tall, and must be managed to produce a full canopy for the subsequent appearance of flower buds (Garuzzo et al. 2021). Flowering can be artificially induced, either to anticipate flowering or to increase the number of flowers and thus produce more seeds after controlled crosses (Castro et al. 2021, Mendes et al. 2021). Artificial controlled crosses allow specific crosses to be performed among selected and high genetic potential genitors, increasing genetic gains, and obtaining superior genotypes in a shorter time (Nunes et al. 2018, Castro et al. 2021). The step which precedes controlled crosses is pollen extraction and processing which requires special attention, as it determines the amount of viable pollen for pollination and successful recombination of superior genitors (Santos et al. 2021). Studies on pollen management and assembling of recombination orchards are scarce in the literature for timber species, especially for *C. trichotoma*.

In this study, we tested the viability of setting up a recombination orchard through grafting for *C. trichotoma* originating from genetic analysis of a provenance and progeny test. We also tested the eucalyptus methodology reported by Fonseca et al. (2010) for collecting and processing pollen of *C. trichotoma*. We hypothesize that it is possible to obtain a recombination orchard and isolated pollen from tested genotypes of *C. trichotoma*. In this context, we aimed to: a) investigate the genetic parameters of *C. trichotoma* in a provenance and progeny test; b) establish a clonal recombination orchard using grafting techniques; and c) carry out pollen collection and processing from *C. trichotoma* genotypes.

MATERIAL AND METHODS

Genetic material and experimental area

The *C. trichotoma* trial was established in May 2014 at a farm of Symbiosis Investimentos e Participações S.A. (lat 16° 34' 08.29" S, long 39° 10' 09.33" W, alt 56 m asl), in Porto Seguro, Southern Bahia, Brazil. Seeds originated from 23 half-siblings families, collected in 12 municipalities within rainforest remnants of Rio de Janeiro, Minas Gerais, and Espírito Santo states (detailed seed-trees information in supplementary material). Seed-trees were phenotypically evaluated for traits such as stem form and circumference at breast height (CBH), the latter measured with a standard tape.

The trial design followed a randomized complete block structure with single-tree plots, arranged at 4 × 4 m spacing, and distributed in 10 replications. Site preparation included deep tillage (60 cm) before planting. Each seedling received 200 g of single superphosphate at planting, with no further fertilization applied. Maintenance practices consisted of crowning during the first year and periodic weed control using glyphosate combined with clorimuron-ethyl; no pruning interventions were carried out until data collection.

According to the Köppen-Geiger classification, the site has a humid tropical climate (Af), with yellow argisol soils (Beck et al. 2023). Mean annual rainfall is about 1,500 mm, reaching up to 1,850 mm in wetter years. Temperatures peak between December and March and are milder from June to September, with an annual average close to 25 °C.

Measurements and statistical analysis

The trees from the provenance and progeny trial were assessed at nine years of age for circumference at breast height (CBH, measured at 1.30 m and converted to diameter at breast height - DBH in cm) and survival. Total tree height (H , m), stem volume (V , m³), and mean annual wood increment (MAI, m³ ha⁻¹ year⁻¹) were estimated using equations previously tested and reported for *C. trichotoma* by Rolim and Piotto (2018). Height was calculated according to Naslund's model, $H = (DBH/(\beta_0 \times DBH + \beta_1))^2$, with $\beta_0 = 0.1749$ and $\beta_1 = 1.608$, while volume followed the Schumacher-Hall (1933) model: $V = 1.11 \times 10^{-4} \times DBH^{2.0479} \times H^{0.6352}$. MAI was derived from volume, tree age, and plot spacing. In addition to these growth metrics, stem form and branch thickness were evaluated. Stem shape was scored on a scale from 1 to 4, with 1 indicating a straight stem, 2 a single bifurcation, 3 two bifurcations, and 4 multiple bifurcations. Branch thickness was categorized as thin (1), medium (2), or thick (3).

The genotypic selection was performed as per the statistical model described below and implemented in the Selegen REML/BLUP software (Resende 2016). The model used for the analyses of the provenance and progeny test was: $y = Xr + Za + Wp + e$, where y is the data vector, r is the vector of block effects and the overall mean (assumed to be fixed), a is the vector of additive genetic effects (assumed to be random), p is the vector of population or provenance effects (random), and e is the vector of errors or residuals (random). The incidence matrices and genetic parameter formulas for the above model are described in Santos et al. (2022), Santos et al. (2023) and Nunes et al. (2024). A likelihood ratio test (LRT) was used to determine the significance of each genetic parameter in the models.

The selection of families and progenies were based on genetic value of DBH and survival rate. Those with the highest genetic value for DBH were considered to select the best individual among the top 10 ranked families, with a score of 1 for stem shape and branch thickness, and free of phytosanitary issues. The genetic gain of the genotypes selected via DBH was calculated for MAI with the aim of reproducing yield on a scale comparable to other crops, such as eucalyptus. The gains were predicted by calculating the differences between the genetic values of superior genotypes and the general mean of the experiment. The 10 best individuals selected within the 10 best families were used to compose the grafts. Graphs were prepared in R software (R Core Team 2025) with the ggplot2 package (Wickham 2016).

Grafting and pollen management

The rootstocks were prepared from clonal seedlings of superior individuals of *C. trichotoma*, grown in 5-liter polyethylene pots, sterile growing medium primarily composed of Canadian peat moss, vermiculite and perlite, fertilized with 40 g of slow-release fertilizer with a 16-8-12 NPK formulation, in addition to 5% sulfur, 0.02% boron, 0.05% copper, 0.4% iron, 0.06% manganese, 0.015% molybdenum, and 0.02% zinc. These clonal seedlings were available at the partner company from a previous and independent project. The seedlings were managed until they reached around 1.5 cm in rootstock base diameter and 30 cm in height. The scion was obtained from the median portion of the canopy of the best trees, which were in healthy conditions. Scions with a thickness of approximately 1.2 cm were collected from the top 10 families, with one top ranked individual per family being grafted 10 times. The top wedge grafting technique was used (Mendes et al. 2021). Therefore, 100 grafts were made, corresponding to 10 rescued individuals, replicated 10 times. The grafted seedlings were kept in the covered nursery (approximately 50% shade) and watered once a day. The success grafting rate was assessed after 60 days of grafting, considering the scions with multiple leaf emergences.

Pollen was collected from *C. trichotoma* trees with flower buds in the provenance and progeny test. Pollen collection and processing was tested and adapted based on the methodology used in eucalyptus described by Fonseca et al. (2010).

The pollen was obtained through the direct method, in which the flower buds were directly extracted from the trees in the pre-anthesis period. The collected buds were placed on absorbent paper so that the material could be pre-dried. *C. trichotoma* pollen was processed by cutting the anthers and distributing the material into several Petri dishes to facilitate drying. The anthers were dried in desiccators containing silica gel at 25 °C temperature for 40 hours, which helped in the detachment of the pollen grains. The anthers with pollen were sieved through a metal sieve with an opening of 53 µm mm. The sieved pollen was collected on sheets of aluminum foil for later storage in cryogenic vials with a rubber seal on the lid, which were kept in a freezer at a temperature of approximately -16 °C. The material used for collecting and processing the pollen was sanitized with alcohol for each genotype handled to prevent unwanted contamination of the pollen. The amount of pollen obtained from each genotype was determined by measuring it on a precision scale.

RESULTS AND DISCUSSION

Quantitative genetics

The individual heritability among progenies (h_o^2) values were above 0.40 and statistically significant for diameter at breast height (DBH) and total tree height (H). The value of h_o^2 for mean annual increment in wood volume (MAI) was lower than those for DBH and H, and not statistically significant. The determination coefficient of provenance effects (c_{prov}^2) were lower than h_o^2 values, and not statistically significant. Accuracy of genotype selection (r_{gg}) ranged from 0.54 to 0.74 and coefficient of individual genetic variation (CV_{gi} %) ranged from 20.61 to 32.82. The values of coefficient of genetic variation among family (CV_{gp} %) ranged from 10.30 to 16.41, and coefficient of environmental variation (CVe %) values ranged from and 1.29 to 3.77, respectively. The DBH, H and MAI general means of the experiment (u) were 18.37 cm, 14.05 m and 21.24 m³ ha⁻¹ year⁻¹, respectively (Table 1).

Genetic variability in a provenance and progeny test is important to allow gains with selection. The values and statistical significance of h_o^2 for DBH and H indicates predominance of available genetic variability in relation to total phenotypic variation and possibility of obtaining gains with selection. Those values were relatively high, above 0.40, which has been considered a threshold for high values of individual heritability in tree breeding (Resende and Alves 2022). Especially for growth traits as DBH which normally presents low values of h_o^2 , the findings in our work are promising and indicate success with selection for this trait. Tree diameter is generally directly measured and allows a high degree of accuracy in the records (Santos et al. 2022, Romão et al. 2023). Considering these aspects, the DBH records of our work can lead a breeding program to a successful and reliable genetic gains scenario in the future and should be the target trait to be used due to this direct measurement and high level of genetic control.

In addition to h_o^2 , provenance effects analysis is important to understand the proportion of local adaptation effects of seed-trees to different environment conditions, and how this can affect selection (Moreno et al. 2022, Santos et al. 2022). The c_{prov}^2 of our work indicates low influence of provenance in the total variability of the test, especially due to lower values of this parameter compared to h_o^2 , and the non-statical significance. The populations of seed-trees accessed for seed collection possibly belonged to sites with similar climatic and soil conditions. Therefore, these provenances apparently did not demonstrate differences originating from the local-specific gene pool when evaluated at nine years of age through their progenies. The majority of genetic variance is found within population and not among populations or provenances (Barreto et al. 2023).

Table 1. Genetic parameters of the *Cordia trichotoma* (Vell.) Aráb. ex Steud provenance and progeny test, at nine years of age in Porto Seguro, Southern Bahia, for the trait's diameter at breast height (DBH in cm), total tree height (H in m), and mean annual increment in wood volume (MAI in m³ ha⁻¹ year⁻¹)

Genetic parameters	DBH	H	MAI
h_o^2	0.59*	0.80*	0.28
c_{prov}^2	0.09	0.08	0.10
r_{gg}	0.69	0.74	0.54
CV_{gi} (%)	23.08	20.61	32.82
CV_{gp} (%)	11.54	10.30	16.41
CVe (%)	1.72	1.29	3.77
u	18.37	14.05	21.24

h_o^2 : individual heritability among progenies; c_{prov}^2 : determination coefficient of provenance effects; r_{gg} : accuracy of genotype selection; CV_{gi} (%): coefficient of individual genetic variation; CV_{gp} (%): coefficient of genetic variation among family; CVe (%): coefficient of environmental variation; u = general mean of the experiment. *Statistically significant at 5% probability by the likelihood ratio test (LRT), considering a critical chi-square value of 3.84. The LRT values for each tested factor are: h_o^2 and DBH: 4.69; c_{prov}^2 and DBH: 1.03; h_o^2 and H: 8.22; c_{prov}^2 and H: 0.76; h_o^2 and MAI: 1.1; c_{prov}^2 and MAI: 1.43.

The r_{gg} values were higher for DBH than MAI, indicating that DBH is a reliable trait for genotype selection. This result corroborates with the low h_a^2 value found for MAI. The coefficient of variation values are in accordance with an accurate interpretation. The values of $CVe\%$ are below 10% for all traits, which would be recommended, while the genetic coefficients of variation are well above the residual. Despite this, it is important to analyze coefficients of variation linked to an accurate interpretation (Resende and Duarte 2007). DBH presented r_{gg} of moderate magnitude as per the classification by Resende and Duarte (2007) and Resende and Alves (2022), which indicates good experimental quality and reliability of the results. The r_{gg} value found for DBH can be considered intermediate and expected in early-stage populations, such as the one in the present study. Thus, these r_{gg} values indicate the possibility of following the tree breeding to estimate gains and selection of the best genotypes.

Genetic gains with selection compared to the general mean of the experiment of the top 10 individuals selected within the 10 best families ranged from 11% to 55%. MAI yield reached up to 33 $m^3 ha^{-1} year^{-1}$ for the top ranked genotype of the family 4, displayed in block 10. The other selected genotypes MAI yield ranged from 23.56 $m^3 ha^{-1} year^{-1}$ to 32.31 $m^3 ha^{-1} year^{-1}$ (Figure 1). Genetic gains demonstrate high potential of volumetric increments for *C. trichotoma*. Superior genotypes with an MAI yield up to 33 $m^3 ha^{-1} year^{-1}$ reveals the success in conducting breeding programs for this species. Furthermore, the need for investment is clear to generate new timber forest products on the market from different native species in Brazil, such as *C. trichotoma*. The selected superior individuals allow genetic gains of up to 55%, which substantially increases the genetic progress in breeding programs. Eucalyptus productivity in Brazil currently ranges from 20 to 41.1 $m^3 ha^{-1} year^{-1}$ and pine around 25.6 to 33.3 $m^3 ha^{-1} year^{-1}$ (IBÁ 2024). Thus, through *C. trichotoma* breeding and conducting controlled crosses of superior individuals, it is possible to achieve volumetric yield of eucalyptus and pine and offer different wood attributes to the forestry sector.

Grafting and pollen management

The mean successful rate of grafting was 18%, ranging from 0% to 70% among genotypes. The top-ranked individuals of the families 2, 12, 3 and 17 had no live grafts after two months of evaluation. Family 19 had the highest grafting success rate of 70% and families 15, 5, 16, 4 and 14 had this rate ranging from 10% to 30% (Figure 2). Controlled crosses are essential to allow important genetic combinations to be made, and hybridization orchards are needed to make them possible. The hybridization orchard is a nursery of grafted genotypes maintained at an accessible height to make crossings easy and seed collection possible (Garuzzo et al. 2021). The basal technique to initiate a hybridization orchard

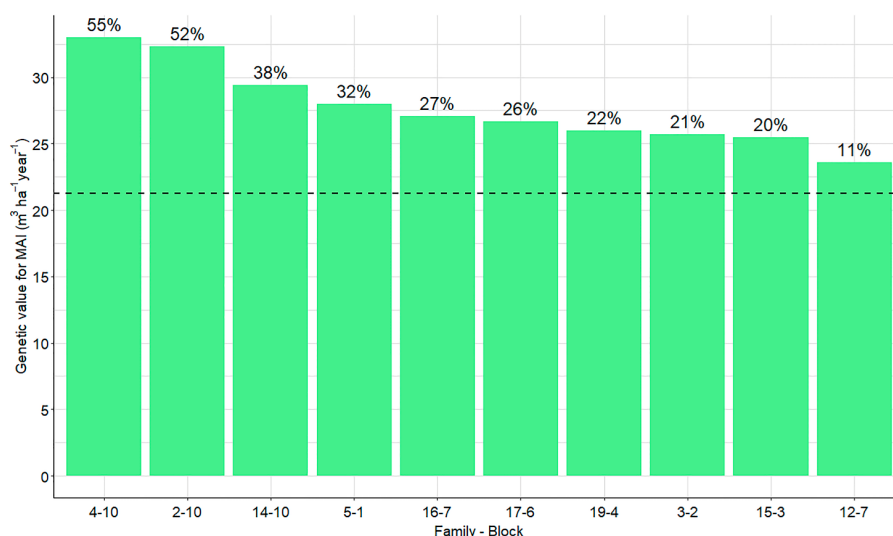


Figure 1. Genetic value and gains with selection (%) of the top-ranked *Cordia trichotoma* (Vell.) Arráb. ex Steud individuals from the best families for mean annual increment in wood volume (MAI, $m^3 ha^{-1} year^{-1}$). In the X axis there is the information of family (left side) and block (right side) codes and in the Y axis the MAI values. The dashed line shows the general MAI mean of 21.24 $m^3 ha^{-1} year^{-1}$ and the top-bars percentages represents the estimated gain with selection.

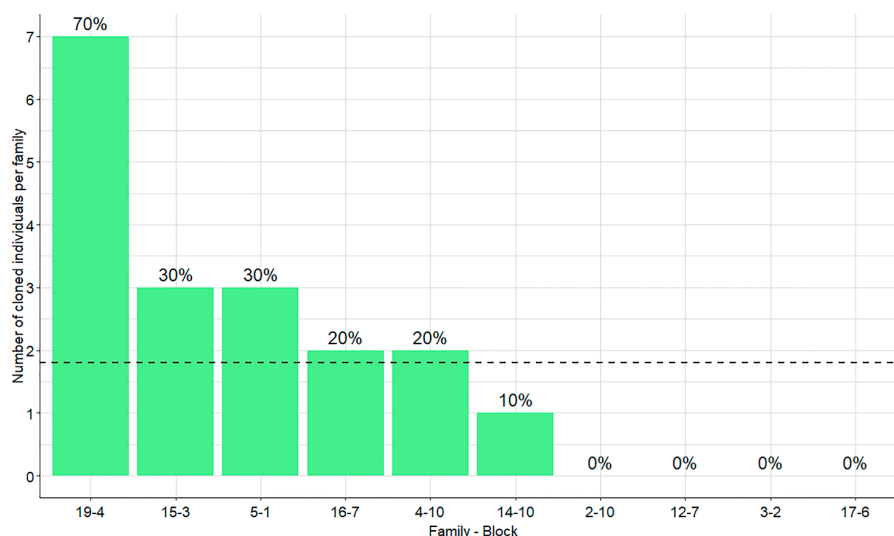


Figure 2. Number of *Cordia trichotoma* (Vell.) Arráb. ex Steud cloned individuals per family and success grafting rate (%). In the X axis there is the information of family (left side) and block (right side) codes and in the Y axis the number of grafted ramets per genotype. The dashed line shows the general mean for number of cloned individuals per family of 1.8 and the top-bars percentages represents the success grafting rate.

is grafting the top-ranked individuals to make controlled crossing possible after flowering induction (Warschefsky et al. 2016). The success of grafting is crucial for balancing crossings among genotypes and consequently large number of improved seeds (Santos et al. 2025). Our low grafting success rate demonstrates the difficulty of grafting woody species and especially the challenge that *C. trichotoma* presents. For grafting, all care was taken to control systematic variations, freeing the process from bias. Despite this, different grafting rates can be observed for different genotypes that ranged from 0% to 70%. Effects of different grafting rates for different genotypes were also reported by Garuzzo et al. (2021) for *Zeyheria tuberculosa* ranging from 20% to 80%. Thus, there are genotypes with a greater predisposition to grafting than others (Prodhomme et al. 2019).

The collection of *C. trichotoma* flower buds was performed in the pre-anthesis phase of the grafts placed in an outdoor orchard, which proved to be the best collection point (Figure 3 A). The processing to obtain the pollen occurred by removing the anthers with the aid of pruning shears, followed by placing the anthers in petri dishes for drying in a silica gel desiccator, without the need for pre-drying on paper towels (Figure 3 B, C, D). During the process, it was observed that the anthers needed to be dried for a longer time than the methodology used for eucalyptus and so the 48-h protocol was adopted. After drying, the anthers were placed on a 200 Mesh or 74 $\mu\text{m}/\text{mm}$ sieve, another necessary adaptation, since the 53 $\mu\text{m}/\text{mm}$ sieve used to process the eucalyptus pollen did not allow the passage of the pollen granules (Figure 3 E). After processing, the pollen obtained was stored in cryogenic tubes and kept in a freezer at an approximate temperature of $-16\text{ }^{\circ}\text{C}$ (Figure 3 F).

Studies on the collection, handling, and storage of tree pollen are limited in the literature, particularly for tropical timber species. Specifically, no manuscripts reporting such activities were found for *C. trichotoma*. However, there is little work in this area and new research needs to be developed. Importantly, two main modifications were made in our work regarding the adaptation of the eucalyptus pollen management protocol for *C. trichotoma*: a longer drying time for the pollen in the desiccator and an increase in the sieve mesh size. As a result, it was possible to store the male gametes of superior genotypes of *C. trichotoma* pollen for later use in controlled crosses.

This study enabled approaches and analyses regarding acceleration of forest breeding of timber species which could be applied to any species, including those at the beginning of the breeding steps. Our work enabled genetic analyses of provenance and progeny test, detection of available genetic variability, estimation of gains with selection and the creation of a hybridization orchard with subsequent collection, processing and storage of *C. trichotoma* pollen. Thus, all



Figure 3. Collection of flower buds and processing of *Cordia trichotoma* (Vell.) Arráb. ex Steud pollen. A) Flower buds of *C. trichotoma* in pre-anthesis; B) Flower buds; C) Anthers in petri dish; D) Drying of anthers in silica gel desiccator; E) Pollen of *C. trichotoma*; F) Pollen stored in cryogenic tube.

this generated information and applied technologies will be able to support advancing the selection and recombination cycles of *C. trichotoma*, in addition to being used as a basis for other forest species around the globe.

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