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# Progeny Selection and Genetic Diversity in a *Pinus taeda* Clonal Seed Orchard

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**Abstract:** The present article describes the development of an improved *Pinus taeda* clonal seed orchard adapted to the edaphoclimatic conditions of Uruguay. Initially, 2068 hectares distributed in nine companies were prospected, and 124 plus trees were identified based on growth, straightness, and health traits. These trees were clonally propagated via grafting to establish a clonal seed orchard. For the genetic evaluation of the orchard, two progeny tests were carried out in the Rivera and Paysandú municipalities. Quantitative genetic analyses allowed us to identify a simple genotype–environment interaction and an expected genetic gain for volumes of 17%, 13%, and 8% for selection intensities of 12%, 25%, and 50%, respectively. Moreover, the genetic diversity of the 124 clones of the orchard was assessed using 10 microsatellite markers. The fingerprinting profiles allowed us to identify a total of 224 alleles. The polymorphism information content of the different markers was in the range of 0.594 to 0.895. The combined probability of identity and probability of identity among siblings had a discrimination power of $8.26 \times 10^{-14}$ and $5.91 \times 10^{-5}$, respectively. Analysis of the genetic structure demonstrated that the seed orchard population was not structured by the supplier.

**Keywords:** loblolly pine; genetic selection; heritability; genetic gain; molecular markers



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## 1. Introduction

*Pinus taeda* L., commonly known as loblolly pine, is a conifer species native to North America. It has acquired significant importance worldwide because of its rapid growth and wide adaptability, which render it a valuable option for wood production and greenhouse gas mitigation [1–3]. Genetically improved loblolly pine materials are well adapted to local conditions, thus ensuring good volumetric growth and high survival rates, and thereby enhancing the success of commercial plantations [3,4]. *Pinus taeda* wood is used in housing construction, furniture, packaging, and various industrial applications, which has led to a steady increase in its global demand [5].

In the southeast region of the United States, there are 10 million hectares of natural pine forests and 14 million hectares of commercial plantations [4,6]. In Latin America, the area forested with *Pinus* and *Eucalyptus* species has expanded significantly because of the promotion policies of the producing countries [7]. The species in the genus *Pinus* in Brazil, Chile, Argentina, and Uruguay collectively occupy 4.7 million hectares [8–11]. The region has vast expanses of land suitable for the cultivation of this species, and its adaptability to different climatic and edaphic conditions renders it a profitable option for large-scale timber production [7].

In Uruguay, *P. taeda* has acquired a significant role in the forestry sector. The country has extensive plantations of this species, particularly in the northern region, where environmental conditions are favorable for its development. The production of wood from *P. taeda* has consolidated itself in recent decades, rendering Uruguay one of the main exporters of forest products in Latin America. Furthermore, proper management of *P. taeda* plantations contributes to the diversification of the country's productive matrix and the generation of employment in rural areas [7].

The origins of *P. taeda* planted in Uruguay trace back to various locations in the United States and South Africa, and such plantations were established in Uruguay by more than a dozen companies during the 1990s. To optimize the production and conservation of *P. taeda*, it is essential to establish seed orchards of high genetic quality, which can be achieved by selecting trees that excel in growth, resistance to pests and diseases, and wood quality. Seed orchards allow obtaining seeds with a uniform and improved quality, thus ensuring the production of superior plants and the transmission of desirable characteristics to future generations [12,13].

In recent years, Latin America has experienced significant advancements in forest genetic improvement techniques. The emergence of high-density SNP marker-based chips has enabled the implementation of genomic selection, allowing the prediction of an individual's genetic value based on their complete genetic information, thereby accelerating the selection process by avoiding reliance solely on observable phenotypes. In particular, genomic selection is useful for long-cycle forest species, such as *Pinus* and *Eucalyptus*, as it reduces the time and cost associated with identifying superior individuals [14–16]. High-density chips have enabled genome-wide association studies (GWAS), a technique that identifies genetic markers associated with complex traits such as disease resistance or drought tolerance [17]. This is crucial for forest species where adaptation to changing conditions is a priority. Additionally, microsatellites (SSRs) are repetitive DNA sequences widely used in genetic diversity studies, clonal identity, and variety protection due to their high variability and reproducibility [18,19]. These markers have been essential for monitoring genetic diversity in breeding programs, ensuring that improved populations do not lose their genetic variability, which is a key aspect for adapting to future environmental conditions [20].

The genetic characterization of *P. taeda* using microsatellite markers has proven to be a powerful tool for evaluating genetic diversity, population structure, inbreeding, gene flow between populations, and relatedness between individuals [21–24]. This technique allows the identification of genetically valuable trees, an understanding of the population structure, and the design of management strategies that ensure the continuous genetic improvement and adaptability of the species [25–28].

Genetic gain and genetic diversity are two key concepts in the field of forest genetic improvement. Genetic gain enables the selection and promotion of desirable traits in tree populations, thereby enhancing productivity and wood quality. Conversely, genetic diversity is essential for resistance to pests, diseases, and environmental changes, thus ensuring the adaptability and survival of forests in the face of natural threats [12,13].

To sustain ongoing genetic gain, breeding program selection must factor in both production traits and genetic diversity [29,30]. Therefore, individuals with close genetic ties should not be part of the same recombination strategies. By limiting the number of progenies selected from the same family, crosses between related individuals can be minimized, thereby reducing the risk of inbreeding [31–33]. Maintaining a high level of genetic diversity and restricting the number of progenies from the same family in breeding crosses helps conserve rare alleles within the base population [31]. These rare alleles are crucial for preserving long-term genetic diversity and for achieving genetic improvements in each selection cycle [20].

The aim of this study was to select superior clones in a clonal test to establish a *P. taeda* seed orchard for the production of improved seeds adapted to Uruguayan conditions. To this end, 124 trees were selected for growth vigor, stem form, and health across nine

commercial *P. taeda* plantations in Uruguay. These trees were cloned to establish a clonal test, and open-pollinated seeds were collected to implement a progeny test, which was repeated at two sites. The estimated genetic gains for three different intensities of selection among progenies in the two progeny tests were used to select superior clones in the clonal test and establish the seed orchard. In addition, 10 microsatellite loci were used to characterize the genetic diversity of the 124 orchard clones and whether the clones were structured according to the company of origin.

## 2. Materials and Methods

### 2.1. Prospecting of Plus Trees

The morphological selection of plus trees was carried out in 2003 through a survey of nine forestry companies covering an area of 2068 ha (Table 1). Initially, trees that stood out based on their growth, trunk straightness, branch diameter, angle of insertion, and health were classified as candidate trees. For the growth trait, the diameter at breast height (DBH, cm) was measured. Straightness was estimated on a scale of 1 to 5, with trees with a score of 5 being perfectly straight and trees with a score of 3 having slight twists; trees with a score < 3 were not included in the study. The diameter of the branches was evaluated using a scale of 1 to 3, with 3 being the score for trees with very thin branches and 1 for those with thick branches; trees with a score < 1.5 were not included in the study. The angle of branch insertion was classified using a scale of 1 to 3, with 3 being perpendicular insertion; trees with a score < 1.5 were not included in the study. The health evaluation was subjective, with visibly healthy trees being selected and those affected by pests or diseases being discarded.

**Table 1.** Plus tree code, provider, and locations.

| N° | Provider | Location | Prospected Area (ha) | Quantity of Clones | Clone Code |
|---|---|---|---|---|---|
| 1 | Caja Bancaria | Paysandú, Uruguay | 15 | 1 | 906ACB |
| 2 | Caja Notarial | Rio Negro, Uruguay | 225 | 21 | 983ACN, 968ACN, 953ACN, 985ACN, 980ACN, 966ACN, 987ACN, 963ACN, 984ACN, 949ACN, 950ACN, 956ACN, 958ACN, 967ACN, 960ACN, 947ACN, 957ACN, 964ACN, 982ACN, 917ACN 948ACN |
| 3 | Arazatí | San José, Uruguay | 30 | 4 | 993AR, 994AR, 995AR, 998AR |
| 4 | I.M.T | Tacuarembó, Uruguay | 11 | 3 | 961IMT, 459IMT, 961BIMT |
| 5 | I.N.C | Tacuarembó, Uruguay | 58 | 12 | 963LZ, 962LZ, 950LZ, 964LZ, 966LZ, 972LZ, 974LZ, 945LZ, 971LZ, 973LZ, 975LZ, 976LZ |
| 6 | La Rosada | Tacuarembó, Uruguay | 227 | 21 | 925LR, 922LR, 941LR, 985LR, 987LR, 944LR, 985LR, 920LR, 905LR, 946LR, 990LR, 923LR, 937LR, 932LR, 930LR, 906LR, 934LR, 936LR, 986LR, 989LR, 919LR |
| 7 | FYMNSA | Rivera, Uruguay | 1392 | 52 | 430FY, 469FY, 426FY, 454FY, 422FY, 437FY, 456FY, 466FY, 421FY, 412FY, 460FY, 413FY, 468FY, 462FY, 428FY, 411FY, 455FY, 440FY, 463FY, 451FY, 450FY, 442FY, 438FY, 439FY, 444FY, 423FY, 433FY, 435FY, 434FY, 415FY, 994AFY, 464FY, 995AFY, 429FY, 407FY, 458FY, 419FY, 445FY, 432FY, 418FY, 467FY, 469bFY, 470FY, 408FY, 449FY, 443FY, 993AFY, 436FY, 406FY, 998AFY, 992AFY, 996AFY |

**Table 1.** *Cont.*

| N° | Provider | Location | Prospected Area (ha) | Quantity of Clones | Clone Code |
|---|---|---|---|---|---|
| 8 | M. Zinger | Rivera, Uruguay | 70 | 9 | 927AZ, 941AZ, 971AZ, 942AZ, 932AZ, 934AZ, 936AZ, 944AZ, 970AZ |
| 9 | Consular S.A. | Tacuarembó, Uruguay | 40 | 1 | 928A |
| - | Total | - | 2068 | 124 | - |

In a second evaluation phase, the trees that had been classified as candidates were compared regarding the DBH trait with the 30 closest surrounding trees. Those that proved to have a significantly higher DBH than that of the neighboring trees were considered plus trees. The percentage of superiority was calculated according to the following equation:

$$\text{Superiority } \% = 100 \left( \frac{\text{DBHplustree} - \text{DBHmean}}{\text{DBHmean}} \right) \quad (1)$$

### 2.2. Vegetative Propagation of Selected Plus Trees

A total of 8600 seedlings were produced from seeds in a nursery. The origin of these seeds was IFCTA158 and IFCTA197 (South Carolina, GA, USA, Atlantic Coastal Plane Area second-generation seed orchard). The technique used for grafting was "apical grafting", according to the protocol described by McKeand and Jett [34].

### 2.3. Installation of the Seed Orchard

An open-pollinated clonal seed orchard was established using the materials produced by grafting during 2004. Land preparation included weed and ant control. The land is located at the National Research Institute of Agriculture Research Tacuarembó experimental station and care was taken to ensure that there were no stands of *P. taeda* within 300 m of the orchard location. The total number of plants was 2220 and the total number of clones was 124. The average number of plants per clone was 15. The seed orchard was established in a randomized block design, with 124 treatments (clones), 33 blocks, and one plant per plot.

### 2.4. Progeny Trials and Measurements

Two open-pollination progeny tests were installed in 2004, one in the municipality of Rivera at the FYMSA company (Rivera, Uruguay) and the other in the municipality of Paysandú at the LUMIN company (Tacuarembó, Uruguay). The plantings followed the spacing of 4 m × 2.5 m, covering an area of 10 $m^2$ per plant. The trials were implemented in a randomized complete block design with 124 treatments (progenies), one plant per plot, and 20 (Rivera) and 25 (Paysandú) blocks. A total of 124 families harvested from the original plus trees were planted per test. The DBH (cm) and height (H, m) traits were measured in Rivera and Paysandú at the ages of nine (10 September 2012) and eight (14 January 2013) years, respectively. To estimate the real individual volume (VOL, $m^3$) without bark, the methodology described by Clutter et al. [35] was used, according to the instructions described by Rachid et al. [36].

### 2.5. Estimates of Genetic Parameters

For the analysis of the trials established in Rivera and Paysandú, 124 progenies originating from mothers that had been phenotypically selected as plus trees were considered [37]. The joint analysis of the two progeny tests was carried out using model 22, whereas the individual analysis was carried out using model 19 in SELEGEN software version 1.0 [38], which is indicated for half-sibling progenies in complete random block delineations in several locations with one observation per plot.

The statistical model used was as follows: y = Xr + Za + Wi + e, where "y" is the data vector, "r" is the vector of the repetition effects (assumed to be fixed) added to the overall mean, "a" is the vector of the individual additive genetic effects (assumed to be random), "i" is the vector of the effects of the genotype × environment interaction (random), and "e" is the vector of the residuals (random). The capital letters (X, Z, and W) represent the incidence matrices for the mentioned effects. From these models, the following variance components were estimated: additive genetic variance ($\sigma_a^2$), variance of the genotype × environment interaction ($\sigma_{ge}^2$), residual variance (environmental + nonadditive) ($\sigma_e^2$), and individual phenotypic variance $\sigma_p^2 = \sigma_a^2 + \sigma_{ge}^2 + \sigma_e^2$. The parameters estimated from the variance components were as follows:

Individual heritability in the strict sense ($h_a^2$) :

$$h_a^2 = \frac{\sigma_a^2}{\sigma_p^2} \tag{2}$$

Mean heritability among progenies ($h_m^2$) :

$$h_m^2 = \frac{0.25\sigma_a^2}{0.25\sigma_a^2 + \frac{\sigma_{ge}^2}{i} + \frac{\left(0.75\sigma_a^2 + \sigma_e^2\right)}{nr}} \tag{3}$$

Coefficient of individual additive genetic variation ($CV_{gi}$%):

$$CV_{\text{gi}}\% = 100\frac{\sqrt{\sigma_a^2}}{m}, \tag{4}$$

where $m$ is the mean trait on analysis.

Precision of progeny selection ($r_{aa}$), assuming complete survival, was as follows:

$$r_{aa} = \sqrt{h_m^2} \tag{5}$$

Model 102 in SELEGEN software was used to estimate the genetic correlations ($r_{x,y}$) among the DBH, H, and VOL variables, as follows:

$$r_{x,y} = \frac{COVa(x,y)}{\sqrt{\sigma_{a(x)}^2 \sigma_{a(y)}^2}} \tag{6}$$

$r_{x,y}$ = Value of the genetic correlation between variables $x$ and $y$
$COV$ = Covariance operator
$a(x)$ = Estimated genotypic value of the variable ($x$)
$a(y)$ = Estimated genotypic value of the variable ($y$)
$\sigma_{a(x)}^2$ = Additive genetic variance of the variable ($x$)
$\sigma_{a(y)}^2$ = Additive genetic variance of the variable ($y$)

The G × E interaction coefficient $\left(C_{ge}^2\right)$ was calculated as follows:

$$C_{ge}^2 = \frac{\sigma_{ge}^2}{\sigma_a^2} \tag{7}$$

The genotypic correlation coefficient between sites ($r_{gloc}$) was calculated as follows:

$$r_{gloc} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{ge}^2} \tag{8}$$

The genetic gain ($G$) was estimated according to the following formula:

$$G = h_a^2 Ds, \quad (9)$$

with ($h_a^2$) being the individual heritability in the strict sense and ($Ds$) being the selection differential. The expected percentage genetic gain ($G\%$) was calculated as follows:

$$G\% = (G100)/m, \quad (10)$$

where $m$ is the general mean of the experiment.

### 2.6. DNA Extraction from the Trees in the Seed Orchard and Genotyping

Needles were collected from each of the 124 tree genotypes from the seed orchard. Approximately 100 mg of fresh tissue was ground with of CTAB2x in a TyssueLyser II (Qiagen, Hilden, Germany) at maximum frequency for 5 min. The remainder of the extraction protocol was carried out as described by Bruegmann et al. [39]. For microsatellite analysis, 10 SSR markers labeled with 6-FAM and HEX (Macrogen, Seoul, Republic of Korea) were used. To increase specificity and reduce stutter, a pigtail sequence was added (5′–GTTTCTT–3′) at the 5′ end of the reverse primers without labeling, as proposed by Grattapaglia et al. [21]. The 10 SSRs were distributed in three multiplex PCRs, as indicated in Grattapaglia et al. [21]. PCRs were performed in a final volume of 10 µL using the Multiplex PCR Kit (Qiagen) according to the manufacturer's protocol. The cycler was programmed using the following conditions: 95 °C for 15 min; followed by 30 cycles of 94 °C for 30 s, 60 °C for 1.5 min, and 72 °C for 1 min; and a final extension 60 °C for 30 min. The molecular weights of the 124 genotyped clones were determined using the Genescan service (Macrogen) and Peak Scanner 1.0 (Applied Biosystems, Waltham, MA, USA).

### 2.7. Analysis of Genetic Diversity

The following genetic parameters for each marker were estimated and included in the analysis: number of alleles per locus ($k$); allele size range; observed heterozygosity ($H_o$); polymorphism information content ($PIC$) [40]; probability of identity ($PI$) [41], which corresponds to the probability of two random individuals displaying the same genotype; major allele frequency ($MAF$); probability of finding two full-sib individuals from a population that have the same genotype by chance ($PI_{sibs}$) [42]; paternity exclusion probability ($PE$) [43], which corresponds to the power with which a locus excludes an erroneously assigned individual tree from being the parent of an offspring; and the frequency of null alleles ($F_{Null}$). CERVUS 3.7 [44] was used to estimate $k$, $H_o$, $PIC$, and $F_{Null}$, whereas IDENTITY 1.0 [45] was used to calculate $PI$, $PI_{sibs}$, $PE$, and $MAF$.

### 2.8. Dendrogram and Genetic Structure Analysis

The genetic relationships among clones were calculated using a matrix of dissimilarity with a simple matching coefficient in DARWIN 6 [46]. The dissimilarity matrix was estimated using a setting of 1000 bootstraps, allelic data, and missing data. A dendrogram was also constructed in DARWIN 6 using the clustering unweighted pair group method with arithmetic mean. The graphic representation was prepared using FIGTREE [47].

A Bayesian assignment analysis, performed in STRUCTURE 2.2.3 [48], was used to identify distinct genetic clusters in germplasm from trees in the clonal seed orchard. A Bayesian analysis of population structure was performed using the admixture and allele-frequency-correlated allele models with a burn-in of 100,000 and 100,000 Markov chain Monte Carlo repetitions. The range of the number of clusters (K) was set from 1 to 10, with 10 replicates for each K. The most probable number of clusters (K) was selected by computing the second-order rate of change of the likelihood function with respect to K [49] using STRUCTURE HARVESTER [50].

## 3. Results

### 3.1. Prospection of Plus Trees and Installation of the Clonal Seed Orchard

In the 2068 hectares that were surveyed, 287 candidate trees were selected. After the second selection stage, 199 additional trees were identified. Approximately one plus tree was selected for every 10 hectares surveyed. For 60% of the plus trees, information on the country of origin of the seed, and, in some cases, the specific place of origin was available. The minimum age of the selected trees was nine years, and the maximum age was 35 years. The average DBH across the nine locations surveyed was 37.6 cm, and the average percentage of superiority of the plus trees was 29.8%. In terms of stem form, the classification ranged from 4 to 4.8, with an overall average value of 4.2. The score of the diameter of the branches ranged from 1.7 to 2.3, with an average value of 2. The angle of the branches was scored between 1.8 and 2.5, averaging 2.2. Among the total 199 plus trees assessed, 75 were lost during the process of grafting, clonal propagation, and orchard installation. Consequently, the seed orchard included 124 selected genotypes.

### 3.2. Quantitative Genetic Analysis

From the analysis of variance, we observed that the genotypic correlation coefficient of performance between the environments ($r_{gloc}$) was >0.9 for all three traits evaluated (Table 2). This is a high value that translates into a simple genotype–environment interaction, indicating that there was no significant effect of the environment on the tests and, consequently, no significant changes in the genotype rankings between the two sites. This means that the two sites can be statistically analyzed together, as established in Model 22 in SELEGEN.

**Table 2.** Estimation of genetic parameters for DBH, H, and VOL considering Rivera, Paysandú, and the two locations together.

| Parameter | Rivera | | | Paysandú | | | Joint | | |
|---|---|---|---|---|---|---|---|---|---|
| | DBH | H | VOL | DBH | H | VOL | DBH | H | VOL |
| $h_a^2$ | 0.374 | 0.367 | 0.485 | 0.514 | 0.751 | 0.698 | 0.428 | 0.469 | 0.511 |
| SE($h_a^2$) | 0.068 | 0.068 | 0.080 | 0.088 | 0.852 | 0.103 | 0.054 | 0.057 | 0.059 |
| $C_{ge}^2$ | - | - | - | - | - | - | 0.002 | 0.008 | 0.006 |
| $r_{gloc}$ | - | - | - | - | - | - | 0.980 | 0.935 | 0.955 |
| $h_m^2$ | 0.673 | 0.669 | 0.734 | 0.786 | 0.852 | 0.841 | 0.874 | 0.871 | 0.703 |
| *CVgi*% | 8.7 | 5.0 | 20.1 | 9.0 | 7.5 | 22.9 | - | - | - |
| Mean | 22.82 | 13.34 | 0.211 | 20.53 | 10.55 | 0.128 | 21.54 | 11.79 | 0.165 |

$h_a^2$, individual heritability in the strict sense; SE, standard error; $C_{ge}^2$, G × E interaction coefficient; $r_{gloc}$, genotypic correlation coefficient between sites; $h_m^2$, mean heritability among progenies; *CVgi*%, coefficient of individual additive genetic variation.

The coefficient of determination of the effects of the G × E interaction $\left(C_{ge}^2\right)$ yielded values close to 0 for the three traits analyzed in these tests (Table 2). A value close to 0 indicates that the G × E interaction has little or no effect on the phenotypic variation. The mean heritability among progenies ($h_m^2$) represents the proportion of the total phenotypic variance that is attributable to the average genetic differences between the progenies. In the case of Rivera, values > 0.820 were obtained for the three traits under study, whereas for Paysandú, they were close to 0.9. For the joint analysis of the two sites, a value of 0.703 was obtained for VOL and a value of 0.874 was obtained for H. In all cases, the values were closer to 1, which indicates that the phenotypic variation was mainly caused by genetic differences between the progenies.

The estimates of narrow-sense heritability ($h_a^2$) ranged from moderate (DBH = 0.428; H = 0.469) to high (VOL = 0.511) for the combined test. For the individual trials, the values varied from 0.514 (DBH) to 0.751 (H) in Paysandú, and from 0.367 (H) to 0.485 (VOL) for Rivera (Table 2). For the Rivera and Paysandú sites, the coefficient of individual additive

genetic variation (*CVgi*%) was lower for DBH (maximum of 9%) and H (maximum of 7.5%) than for VOL (minimum of 20%).

The genetic correlations between the three traits were positive and high, ranging from 0.81 between DBH and H to 0.96 between DBH and VOL (Table 3). Therefore, selecting for one of the traits implied indirectly selecting for the remaining two. To estimate the genetic gain (G%), we chose the individual VOL trait at three different selection intensities to observe the different scenarios. Selecting 50% of the plus trees (67 clones) gave a genetic gain G% = 8%. Increasing the intensity to 25% (33 clones), we obtained a G% value of 13%, whereas the selection of an intensity of 12% (16 clones) yielded G% = 17% (Table 4). This information is relevant when planning thinning.

**Table 3.** Values of the genetic correlations between growth DBH, H, and VOL.

| Trait | H | VOL |
|---|---|---|
| DBH | 0.81 | 0.96 |
| H | | 0.92 |

**Table 4.** Selection intensity and expected genetic gain.

| Scenario | Selection Intensity (%) | Expected Genetic Gain (%) |
|---|---|---|
| 1 | 12 | 17 |
| 2 | 25 | 13 |
| 3 | 50 | 8 |

### *3.3. Genetic Diversity*

The 10 microsatellite loci analyzed here were highly polymorphic. A total of 224 alleles were observed, ranging among loci from seven to 32 alleles (Table 5). Moreover, there were 195 rare alleles with a frequency of <1%, representing 87% of the total alleles. Conversely, the MAF was in the range of 0.197 (ript0031) to 0.665 (pttx3011), with an average of 0.331. The observed heterozygosity ($H_o$) ranged among loci from 0.444 (pttx3011) to 0.887 (ript0031), with an average of 0.641. The PIC value ranged among loci from 0.534 (pttx3011) to 0.895 (ript0031).

**Table 5.** Genetic diversity parameter of the 10 SSR markers for the 124 clones of the seed orchard.

| Marker | k | PI | $PI_{sibs}$ | PE | $H_o$ | PIC | $F_{Null}$ | MAF | Linkage Group |
|---|---|---|---|---|---|---|---|---|---|
| pttx3011 | 20 | 0.218 | 0.699 | 0.375 | 0.444 | 0.534 | 0.109 | 0.665 | 7 |
| pttx3117 | 11 | 0.142 | 0.457 | 0.456 | 0.645 | 0.647 | 0.027 | 0.399 | 11 |
| sifg3145 | 14 | 0.1 | 0.419 | 0.535 | 0.726 | 0.708 | 0.008 | 0.415 | 3 |
| pttx4093 | 23 | 0.059 | 0.375 | 0.637 | 0.597 | 0.781 | 0.141 | 0.367 | 8 |
| pttx2080 | 21 | 0.04 | 0.342 | 0.699 | 0.573 | 0.828 | 0.187 | 0.274 | 12 |
| ript0255 | 27 | 0.034 | 0.336 | 0.72 | 0.46 | 0.84 | 0.298 | 0.298 | 10 |
| pttx4137 | 32 | 0.035 | 0.335 | 0.718 | 0.661 | 0.84 | 0.127 | 0.266 | 6 |
| pttx2037 | 24 | 0.033 | 0.329 | 0.729 | 0.734 | 0.849 | 0.082 | 0.226 | 2 |
| pttx3081 | 27 | 0.018 | 0.306 | 0.8 | 0.685 | 0.891 | 0.137 | 0.209 | 9 |
| ript0031 | 25 | 0.016 | 0.304 | 0.805 | 0.887 | 0.895 | 0.005 | 0.197 | n.d. |
| Mean | 22.4 | 0.091 | 0.406 | 0.593 | 0.641 | 0.781 | 0.112 | 0.331 | - |
| Overall | 224 | $8.26^{-14}$ | $5.91^{-5}$ | - | - | - | - | - | - |

k, number of alleles; PI, probability of identity; $PI_{sibs}$, probability of sibs; PE, probability of exclusion; $H_o$, observed heterozygosity; PIC, polymorphism information content; $F_{Null}$, frequency of null alleles; MAF, major allele frequency.

Based on the genotyping data, an identity analysis of the 124 clones was performed, confirming that each genotype had a unique fingerprinting profile. The PI ranged among loci from 0.016 to 0.218. Because the loci were found in different linkage groups (Table 5)

and segregated independently, in this case, the probability of randomly identifying two individuals sharing the same fingerprinting profile was very low ($8.26 \times 10^{-15}$). The combined probability of two clones that were randomly sampled in the population being siblings ($PI_{sibs}$) was low ($5.91 \times 10^{-5}$, ranging among loci from 0.281 to 0.304).

### *3.4. Dendrogram and Genetic Structure*

The genetic distance between the 124 clones is indicated in the dendrogram presented in Figure 1. The clones were not grouped according to the company of origin, indicating that the *P. taeda* germplasm appeared to be mixed, without a clear correlation with the supplier. A genetic structure analysis of the 124 clones was performed, and a maximum value of $\Delta K = 25.3$ was obtained for K = 2 (Figure 2A). This indicates that the germplasm represented by the 124 clones could be divided into two classes according to the value of K. However, the individuals did not appear to be clearly subdivided into two classes, as depicted in Figure 2B. Furthermore, there was no correlation between the genetic structure and the germplasm-supplying companies, which was in accordance with the dendrogram data.

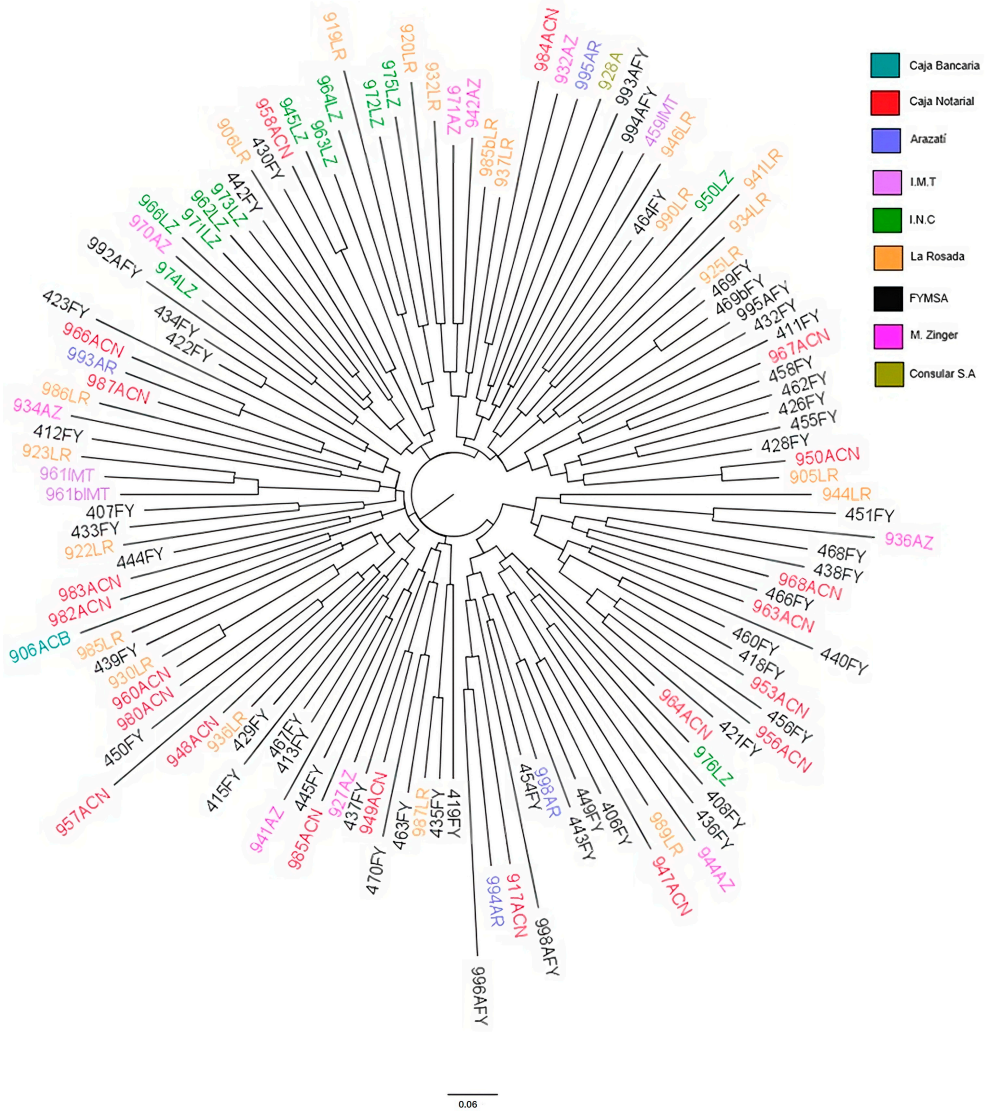


**Figure 1.** Dendrogram of the 124 clones that made up the seed orchard.

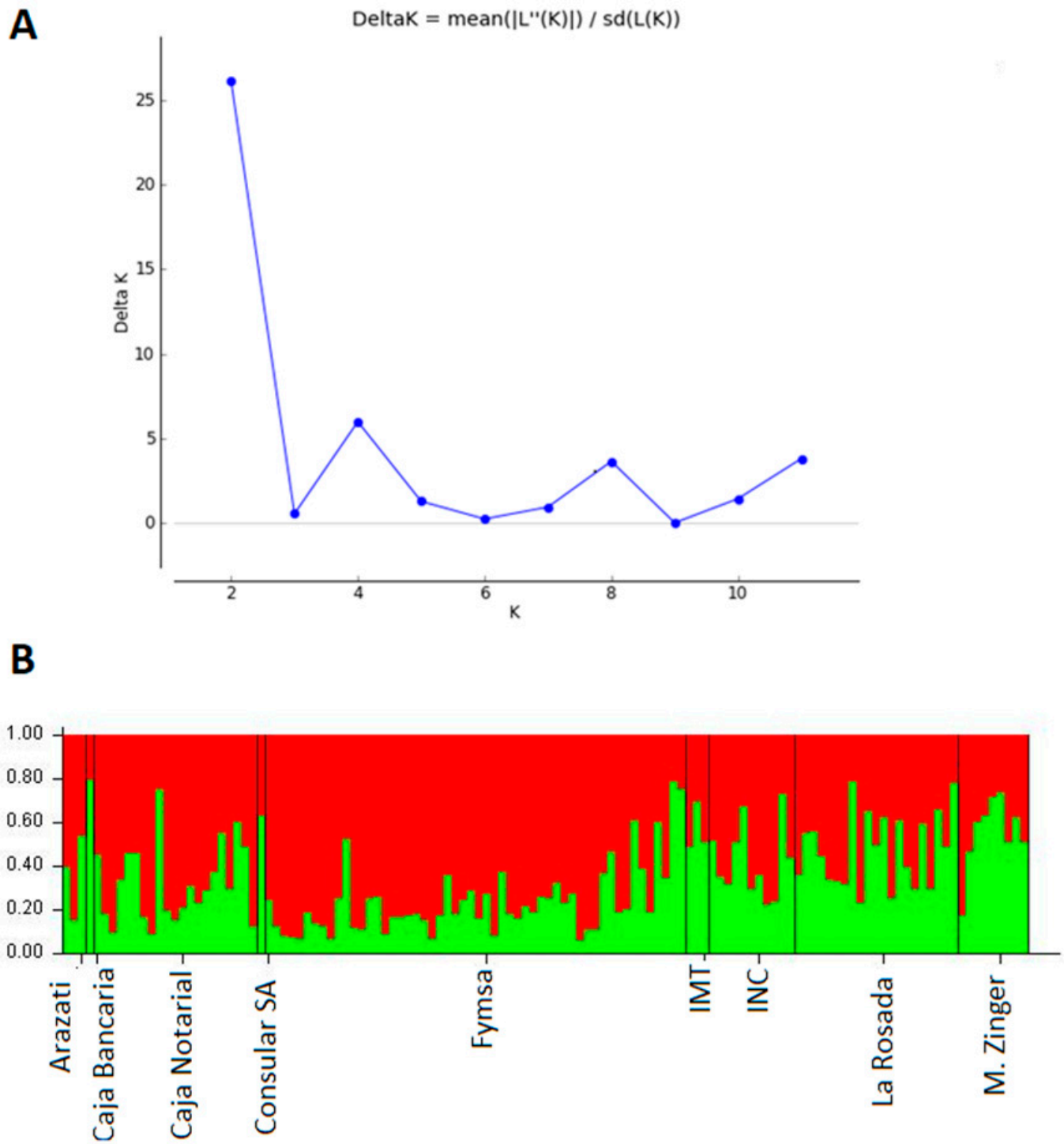


**Figure 2.** Genetic structure analysis. (**A**) Delta K estimation. (**B**) Barplot and cluster determination.

## 4. Discussion

### *4.1. Vegetative Propagation of Plus Trees*

The apical graft technique described in McKeand [34] was successfully applied for the propagation of *P. taeda* germplasm in Uruguay. The optimal time to graft *P. taeda* was between the months of June and July, when the buds are dormant. A significant variability in grafting success was observed among the different cloned genotypes. Spikes with good vigor that were collected from the upper third of the crown with a length of approximately 8 cm (ranging from 6 to 10 cm) and a diameter of 8 mm (ranging from 6 to

10 mm) were found to be ideal for grafting *P. taeda*. Regarding the rootstock, those with a height between 0.8 and 1 m and with a base diameter between 1 and 1.5 cm yielded the best results. The highest success rates were achieved when grafting occurred within 24 h after harvest, although the time between harvest and grafting could be extended up to 5 days. Conducting the grafts in a greenhouse with potted plants resulted in good grafting efficiency [37].

### 4.2. Quantitative Genetics

According to Resende [51], $h_a^2$ values > 0.5 are considered high, those between 0.15 and 0.5 moderate, and those <0.15 low. For the three traits analyzed in this report, the $h_a^2$ values were high for VOL and moderate for both DBH and H in the two-test joint analysis. When examining the trials individually, high $h_a^2$ values were observed for all three traits in Paysandú, whereas moderate values were found in Rivera (Table 2). These values were higher than those reported by Ishibashi et al. [52], who stated that the genetic selection of *P. taeda* in multiple environments ranged from 0.04 to 0.16, and they were also higher than those reported by Marchetti et al. [4], which ranged from 0.01 to 0.19. In the present study, the high genetic control, as indicated by the $h_a^2$ values, suggests the significant genetic influence of DBH, H, and VOL, indicating the potential for achieving genetic gains through the selection of the best clones.

The mean heritability among progenies, $h_m^2$, was high for all three traits in both environments and in the joint analysis of the sites. For instance, Walker et al. [53] observed values for $h_m^2$ ranging from 0.76 to 0.85 in 269 progenies, which are very similar to those obtained in the present test. This heritability indicates that a substantial proportion of the variability in the trait has a genetic origin, suggesting that selection for that trait will be effective [51,54].

For the VOL trait, *CVgi*% values > 20% were obtained, which is considered high, and indicates a significant relative genetic variability of the trait in the population. This result was above the range of 8.01%–12.2% reported by Silva et al. [55] and below the range of 20%–29.9% obtained by Santos et al. [56]. This high value of *CVgi*% implies that there is a wide range of genetic variability that can be exploited through genetic selection. All these parameters indicate that there is a good potential for genetic selection in the *P. taeda* breeding population.

The information provided by the progeny tests enables the determination of the best mothers in terms of growth. In turn, this allows us to define the selection intensity for future thinning operations. For initial thinning, a selection intensity of 50% would be applied according to these results, which would generate an expected genetic gain of 8% (Table 4). The increase in the thinning of selection will allow us to incorporate traits such as the modulus of elasticity. Based on our results, we can achieve a genetic gain of up to 17% in the seed orchard.

This work began in 2004 and implements a classical breeding strategy combined with an estimation of genetic diversity using molecular markers. Current breeding efforts aim to shorten selection times by employing genomic selection strategies or detecting specific genetic variants through GWAS [13–17].

### 4.3. Genetic Diversity Analysis

The markers used in this study were highly polymorphic, as reflected by a mean PIC value of 0.781 (Table 5). In this study, we classified PIC values as low for PIC $\leq$ 0.25, moderate for 0.25 < PIC $\leq$ 0.5, and high for PIC > 0.5. As shown in Table 5, all markers exhibited high PIC values. This indicates that they are highly informative and useful for genetic diversity studies. The PIC express the degree of genetic diversity among plants, and its evaluation is beneficial for establishing plant genetic pools and accelerating the improvement process [40,57].

The high percentage of rare alleles (87%) identified in our study population indicates a substantial level of genetic diversity, which is favorable for maintaining long-term genetic

variability through successive thinnings. This also suggests a reduced risk of inbreeding, as rare alleles tend to be less evenly distributed among individuals. Furthermore, the abundance of rare alleles holds significant implications for the adaptive potential of the population, enhancing its capacity to withstand potential threats such as pests or other environmental stresses, thereby improving the orchard's overall resilience.

Here, in this study, as $H_o$ ranges from zero to 1, values $\leq 0.25$ were considered as low, >0.25 to 0.5 as moderate, >0.5 to 0.75 as high, and >0.75 as very high. The mean $H_o$ value obtained in the present work was 0.641 (high), which suggests a relatively high genetic diversity, with most individuals being heterozygous. The $H_o$ estimate further indicates that the genetic base is broad enough to subject the orchard to successive thinnings, increasing genetic gain at higher selection intensities. This result is greater than the 0.438 reported by Yang et al. [26] in a second-generation *Pinus tabuliformis* seed orchard. In *P. taeda*, Grattapaglia et al. [21] reported an $H_o$ value of 0.708 among a total of 300 genotyped individuals. In another *P. taeda* breeding program, five subpopulations were analyzed using markers from the PtTX series, obtaining observed heterozygosity $H_o$ values ranging from 0.31 to 0.38, which are interpreted as moderate [24].

Genetic diversity is one of the most important parameters to consider in genetic improvement programs. It is defined as the total amount of genetic variation present within or between individuals and genetic units due to their evolutionary pathways, and it forms the basis of their response to biotic and abiotic factors. Therefore, genetic diversity is considered a prerequisite for adaptability and evolution [12,58].

To develop a reasonable and effective genetic improvement strategy, it is important to analyze the genetic diversity of the seed orchard using SSR molecular markers. SSR markers have the advantages of codominance, stable amplification, and good repeatability; therefore, they are a method that is commonly used for the analysis of species in the genus *Pinus* [26,28,58–60]. Moreover, SSRs have a strong specificity and yield clear bands, and afford precise data, which renders them ideal for the construction of fingerprint profiles for many genotypes [56,61]. In this work, we screened 10 *P. taeda* SSR loci in 124 selected clones of *P. taeda* and obtained an average number of alleles per locus (k) of 22.4, which was higher than the value of 6.8 obtained by Yan et al. [58] in their analysis of 161 *Pinus koraiensis* clones from different populations in China. Conversely, other authors used the same methodology to obtain an average number of alleles per locus (k) of 13.2 among 300 individuals from a clonal seed orchard [14].

Monitoring and managing genetic diversity are important concerns for breeders because its reduction is associated with serious consequences. Genetic drift leads to unpredictable random changes in allele frequencies, whereas co-ancestry and inbreeding accumulate over generations, causing a decrease in the physical fitness of affected individuals, which is a phenomenon that is known as inbreeding depression [12].

### 4.4. Genetic Structure

In the study by Eckert et al. [22], 23 markers from the PtTX series, which are also used in this work, were combined with 3059 SNP markers in native populations of loblolly pine. The study highlighted that the identification of genetic clusters may be challenging or inappropriate for species like loblolly pine, which are continuously distributed across large geographical expanses. On the other hand, the genetic structure of a *P. taeda* population represented by 120 open-pollinated families was inferred using 15 microsatellites from the PtTX series, yielding a K value of 5, indicating five genetic clusters [24]. In our genetic structure analysis, no clusters were observed. However, the material analyzed in this study was sourced from nine different companies, all of which tend to purchase seeds from the same suppliers in the United States and South Africa, which may explain the absence of well-defined clusters.

## 5. Conclusions

The present study aimed to establish a clonal seed orchard with selected individuals of *P. taeda* that were adapted to the soil and climate conditions of Uruguay, to produce seeds with high genetic quality.

Genetic analyses of progeny tests demonstrated a significant genetic gain in future generations.

The estimated genetic diversity of the clonal seed orchard was high and could be used in plantings and as a base population for the genetic improvement of *P. taeda*.

The findings of this study ensure the availability of improved seeds for Uruguay and create the opportunity for germplasm exchange with national companies and international institutions. This exchange helps to further expand the genetic base of *P. taeda* in Uruguay, thereby strengthening and enhancing the species' breeding program.

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