

Genetic diversity of *Cedrela fissilis* (Vell.):  
insights on conservation and productionDiversidade genética de *Cedrela fissilis* (Vell.):  
perspectivas para conservação e produçãoAndrea Chizzotti Cusatis<sup>1</sup>, Diego Tyszka Martinez<sup>2</sup>,  
Luciana Duque Silva<sup>3</sup> e Antonio Rioui Higa<sup>4</sup>**Resumo**

O objetivo deste trabalho foi estabelecer um critério de seleção para a conservação genética e crescimento de *Cedrela fissilis*, baseado em um teste de progênie com 48 famílias de *C. fissilis*, plantadas com *Pinus taeda* no Município de Rio Negrinho, Santa Catarina, Brasil. Os parâmetros genéticos foram estimados por meio da máxima verossimilhança restrita e as predições precoces de ganho genético foram estimadas utilizando a melhor predição linear não viciada e o Índice Multiefeito. As Análises de Deviance indicaram que as famílias tiveram origem em uma única população e que houveram diferenças significativas para sobrevivência, diâmetro de colo e altura entre progênies. Bifurcação, número de rebrotas, danos causados por *Hypsipyla grandella* e geada não foram significativos. Um Pomar de Sementes por Mudas foi proposto para a produção de sementes para conservação genética visando à restauração florestal de Reservas Legais brasileiras. Estima-se que tais sementes apresentem um ganho genético de 2% em sobrevivência e tamanho efetivo populacional de 78 por meio da seleção baseada nos valores aditivos de progênies. Para a produção de sementes melhoradas, via Pomar Clonal de Sementes, foi proposta a seleção de famílias em níveis independentes de eliminação, utilizado valores genéticos aditivos de sobrevivência e Índice Multiefeitos de diâmetro de colo. Neste caso, 15 genótipos foram selecionados, com um ganho predito de 4% para sobrevivência e 18% para diâmetro de colo. A variabilidade genética encontrada permite explorar o teste de progênies como um banco de germoplasma ativo, combinando conservação genética e uso sustentável de *C. fissilis*.

**Palavras-chave:** Cedro-rosa, Meliaceae, Restauração, Domesticação, Análise de Deviance, Modelos lineares Mistos.

**Abstract**

The objective of this study was to determine selection criteria for the genetic conservation of, and growth improvement in *Cedrela fissilis* using a progeny test composed of 48 *C. fissilis* families planted with *Pinus taeda* in Rio Negrinho Municipality, Santa Catarina State, Brazil. Genetic parameters were estimated using the restricted maximum likelihood method, and early predictions of genetic gains were estimated using best linear unbiased predictions and a multi-effects index. Deviance analyses indicated that the families originated from a single population, and there were significant differences in survival, collar diameter and height among progeny. Stem bifurcation, number of sprouts per stump, damage caused by *Hypsipyla grandella* and frost damage did not differ. A seedling seed orchard was proposed to produce seeds for genetic conservation aimed at forest restoration in the Brazilian "Legal Reserve". It is estimated that these seeds will have a genetic gain of 2% for survival and a population size of 78 through the selection based on the progeny's additive values. In order to produce genetically improved seeds through a clonal seed orchard, a family selection based on independent elimination levels was proposed, using additive genetic values for survival and a multi-effect index within plots for collar diameter. In this case, 15 genotypes were selected, with a predicted gain of 4% in survival and 18% in collar diameter. The genetic variability found enables the use of the progeny test as an active germplasm bank, combining genetic conservation and sustainability of *C. fissilis*.

**Keywords:** Cedro-rosa, Mahogany, Restoration, Domestication, Deviance analysis, Mixed linear models.

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## INTRODUCTION

*Cedrela fissilis* Vell., commonly known as “cedro-rosa” in Brazil, is a Latin American forest tree species susceptible to *Hypsipyla grandella*, a shoot borer that damages the stem. Other mahoganies, such as *Swietenia macrophylla*, show variations among families in their resistance to this insect and in growth height, indicating a genetic role in these characteristics (GRIJPMA, 1976, NEWTON et al. 1993; MAYHEW; NEWTON, 1998).

Despite of *C. fissilis*'s important place in the international wood market during the last century, because of its use in making fine furniture, and the already known genetic variations between provenances (INOUE, 1972), few breeding programs were developed to explore its potential to produce genetically selected materials for commercial plantations. This is mainly due to the low interest of landowners in planting this species because of the long growth period required prior to harvesting, which affects the plants economic competitiveness when compared with other options for wood production.

However, some changes in planting strategies should be expected after the approval of the New Forest Code by the Brazilian National Congress in 2012. This legislation established that the “Legal Reserve”, which represents at least 20% of Brazilian rural properties, has to be covered by local vegetation. If it does not, then it is required to be restored by 2034 with at least 50% of local species. It is estimated that 21 million ha of rural properties in Brazil do not meet this condition (SOARES-FILHO, 2013), which means that the demand for forest restoration projects will be increasing. Consequently, not less than 21 trillion different native seed tree species will need to be produced before the deadline. Rules for forest restoration are being formulated by the Federal Environment Ministry, which is in charge of controlling and inspecting the restoration. The planting composition should include a representative number of species from the local biome, which is highly diverse in Brazil, including hundreds of native forest species (SOARES-FILHO, 2013).

One feature of the “Legal Reserve” is the possibility of the management of the mixed forest without clear cutting by the landowner, for cash crops. This is a unique opportunity to use native species with high value woods, such as *C. fissilis*, which naturally grows at a low density (1 to 3 trees per ha). Planting *C. fissilis* mixed with one or more other species, increases diversity and should decrease the attacks by *H. grandella*. Additionally, this mix would approach original forest conditions.

A main limitation in this hopeful scenario to use native Brazilian forest species at a commercial level is the scarcity of selected genetic materials (HIGA; SILVA, 2006). In forest restoration, the problems to overcome are the low level of diversity in the species available (BRANCALION et al. 2010), as well as genetic contamination from non-local populations of the same species (DURIGAN et al. 2010).

In the present study, early selection was applied to establish two breeding strategies to produce seeds of *C. fissilis*: a) a seedling seed orchard; and b) a clonal seed orchard. We explored the genetic structure of natural populations of *C. fissilis* to contribute to its genetic conservation and sustainable use, and consequently, its domestication. This study also addressed the need for research on native forest genetic resources, as reported by the Commission on Genetic Resources for Food and Agriculture (FAO, 2014).

The main objective of this study was to evaluate the genetic variability of three native populations of *C. fissilis* and select genotypes, based on survival and initial growth height, in a progeny test that was planted with *Pinus taeda* in Rio Negrinho municipality, Santa Catarina State, Brazil.

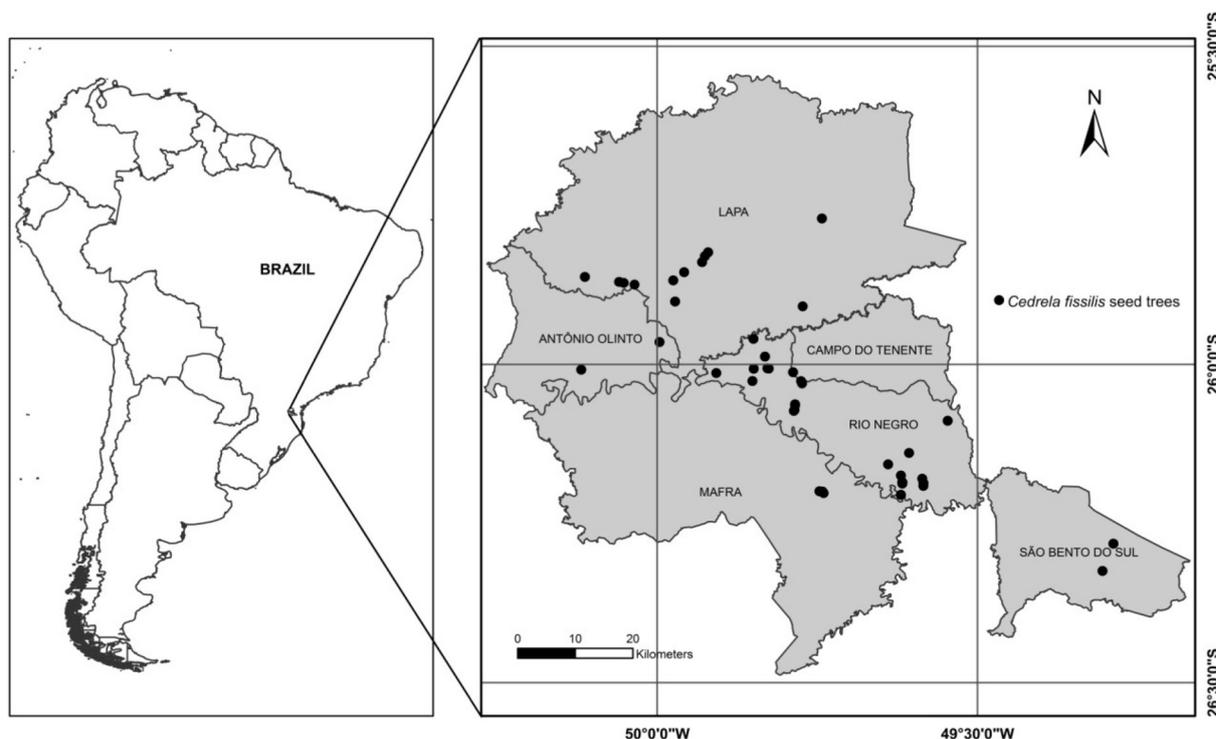
## MATERIAL AND METHODS

*C. fissilis* trees (48) were marked in 2008 in forest remnants in six municipalities on the borders of Paraná and Santa Catarina States, Brazil (Fig. 1) (BIERNASKI, 2010). In 2009, seedlings from these trees were planted in a progeny test design in the Rio Negrinho municipality.

The climate of the region is humid subtropical (Cfa); with a mean annual precipitation of 1,734 mm and annual mean, maximum, and minimum temperatures of 18 °C, 23 °C and 13 °C, respectively. Up to 10 frosts are registered per year (EPAGRI, 2011, not published). The region is part of the Atlantic Forest Region, with Araucaria Forest typology and landscapes characterized by mosaics of disturbed native vegetation in different stages of regeneration. Most of the land is used

for small- and medium-scale agriculture, as well as *Pinus* spp. and *Eucalyptus* spp. reforestation (BIERNASKI et al., 2012).

The progeny test of *C. fissilis* was planted with *P. taeda* at a density of 1,000 seedlings/ha of both species. Seedlings were distributed in randomized complete blocks design, with 48 families, 8 blocks and 10 plants per plot. The field test occupied 6 ha.



**Figure 1.** Location of the 48 seed trees of *Cedrela fissilis* distributed in six municipalities on the borders of Paraná and Santa Catarina States, Brazil.

**Figura 1.** Localização das 48 matrizes de *Cedrela fissilis* distribuídas em seis municípios na fronteira entre Paraná e Santa Catarina, Brasil

In 2012, at 43 months of age, the following variables were measured: survival, damage by *H. grandella* and frost; stem bifurcation; collar diameter; height and number of shoots per plant.

A likelihood ratio test was applied, based on a deviance analysis, to evaluate the hypothesis that the sampled trees belonged to a single or different populations. Trees were grouped based on the spatial distribution of 33 trees and their respective mean average altitudes of 916 m (range from 881 to 936 m), 868 m (811 to 920 m) or 880 (849 to 917 m) (BIERNASKI et al., 2012). This sorting could not be applied to 15 of the trees which were in different altitudes.

The deviance analyses were estimated using the restricted maximum likelihood (REML) calculated by the software SELEGEN (RESENDE, 2007). A deviance analysis tested the significance of the provenance effects using the mathematic model:

$$y = Xb + Za + Wc_1 + Qr + Uc_2 + e,$$

where  $y$  = data vector;  $b$  = vector of blocks effect (fixed);  $a$  = vector of additive genetic effects (random);  $c_1$  = vector of plot effects related to the progeny (random);  $r$  = vector of provenance effects;  $c_2$  = vector of random plot effects related to provenances;  $e$  = vector of errors or residuals (random); and  $X, Z, W, Q$  and  $U$  = incidence matrices for  $b, a, c_1, r, c_2$ , and  $e$ , respectively.

The deviance values for the progeny variables and genetic parameters were estimated using the statistic model (RESENDE, 2002):

$$y = Xb + Za + Wc + e,$$

Where:  $y$  = data vector;  $b$  = vector of block effects (fixed);  $a$  = vector of additive genetic effects (random);  $c$  = vector of the plot effects (random);  $e$  = vector of errors or residuals (random); and  $X, Z$  and  $W$  = incidence matrices for  $b, a$  and  $c$ , respectively.

In the analyses, *C. fissilis* was regarded as being a species with a mixed reproductive system and a self-fertilization rate of 20% (GANDARA, 1996; BIERNASKI, 2010).

The phenotypic and genetic correlations were estimated as described by Pires et al. (2011). The additive genetic correlation between the characters  $x$  and  $y$  are described by:

$$r_{a(x,y)} = \frac{COV_{a(x,y)}}{\sigma_{ax}\sigma_{ay}},$$

and the phenotypic correlation between the characters  $x$  and  $y$ :

$$r_{(x,y)} = \frac{COV_{a(x,y)} + COV_{e(x,y)}}{\sigma_x\sigma_y} = \frac{COV_{(x,y)}}{\sigma_x\sigma_y},$$

where  $COV_{a(x,y)}$  = additive genetic covariance between the characters  $x$  and  $y$ ;  $\sigma_{ax}$  = standard deviation for additive genetic character  $x$ ;  $\sigma_{ay}$  = standard deviation for additive genetic character  $y$ ;  $COV_{e(x,y)}$  = environmental covariance between the characters  $x$  and  $y$ ;  $COV_{(x,y)}$  = phenotypic covariance between the characters  $x$  and  $y$ ;  $\sigma_x$  = phenotypic standard deviation for the character  $x$ ; and  $\sigma_y$  = phenotypic standard deviation for the character  $y$ .

Considering the mixed reproductive system of the species with an open and large number of male progenitors, the effective population size was estimated by the formula (RESENDE, 2002):

$$N_e = \frac{2(2-S)f\bar{n}}{(1+S)^2 + \frac{\sigma_n^2}{n} + (1+S)^2\bar{n} + (3-2S-S^2)},$$

Where:  $S = 0.20$  self-fertilization rate for *C. fissilis* (GANDARA, 1996);  $f$  = number of seed trees (previous generation) selected;  $\sigma_n^2$  = variance of the number of individuals selected per progeny; and  $\bar{n}$  = average of the number of individuals selected per progeny.

For selection, the additive effects were estimated using the best linear unbiased prediction (BLUP) (HENDERSON, 1984) model:

$$y = X\beta + Za + Wp + e,$$

Where  $a$  is the vector of additive genetic effects (random).

Also used for selection, the multi-effect index developed by Resende and Higa (1994) was applied as described below:

$$I_1 = b_1(Y_{ijk} - \hat{Y}_{ij.}) + b_2(\hat{Y}_{i..} - \hat{Y}_{...}) + b_3(\hat{Y}_{ij.} - \hat{Y}_{i..} - \hat{Y}_{.j.} + \hat{Y}_{...})$$

Where:  $I_1$  = Multi-effect index;  $Y_{ijk}$  = individual value;  $\hat{Y}_{ij.}$  = plot average;  $\hat{Y}_{i..}$  = progeny average;  $\hat{Y}_{...}$  = general average;  $\hat{Y}_{.j.}$  = block average. Additionally,

$$b_1 = \frac{(1-\rho_a)h_a^2}{1-t_1},$$

$$b_2 = \frac{1 + (\sum n_i - 1)\rho_a h_a^2}{1 + (\sum n_i / \sum b_i - 1)t_1 + (\sum b_i - 1)(\sum n_i / \sum bi)t_2}$$

$$b_3 = \frac{(1-\rho_a)h_a^2}{1 + (n_{ij} - 1)t_1 - n_{ij}t_2}.$$

## RESULTS AND DISCUSSION

The results of the deviance analysis, estimated by the likelihood ratio test, of the seven characteristics evaluated indicated that the provenance effect was not significant (Table 1). Therefore, the 33 progenies sampled at different mean altitudes of 916 m, 868 m and 880 m, covering six municipalities, composed a single population or provenance.

**Table 1.** Results of the deviance analysis on the three provenances proposed for *Cedrela fissilis*, in a progeny test planted in Rio Negrinho, Santa Catarina, Brazil.

**Tabela 1.** Resultados das análises de deviance às três procedências propostas para *Cedrela fissilis*, em um teste de progênie, plantado em Rio Negrinho, Santa Catarina, Brasil.

Effect	Likelihood ratio test						
	Number of survivors	Collar diameter (cm)	Height (cm)	Individuals bifurcated (%)	Number of sprouts per individual	Damage by <i>H. grandella</i> (%)	Damage by frost (%)
Provenances	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>	-0.59 <sup>ns</sup>	0.16 <sup>ns</sup>
Progenies	5.67*	3.02 <sup>ns</sup>	3.29 <sup>ns</sup>	0.00 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.00 <sup>ns</sup>	0.01 <sup>ns</sup>
Average	23.94	3.17	57.28	97.25	2.55	2.22	3.72
Variation	6–33	2.38–4.05	37.53–91.06	89–100	1.64–3.68	0–11	0–18

<sup>ns</sup> not significant; \* $p < 0.05$

These results are different from those related by Inoue (1972) and Firkowski (1983), probably because they collected seeds of *C. fissilis* from a larger territorial extension of Brazil. Inoue (1972) tested five provenances of *Cedrela* spp. planted with and without *Eucalyptus* sp. at 6 and 12 months old, and found significant variations in growth height, frost damage and survival among the provenances. The different origins of the genetic materials, experimental designs, analyses, ages of evaluation, and especially the different site locations, are possible reasons for the higher growth and survival levels in the Inoue study in comparison with the present study.

While Firkowski (1983) sampled five provenances of *C. fissilis* from the same Brazilian states as the present study, the territorial range was broader. Although the seeds were collected in a smaller area than in Inoue's study, Firkowski also found significant variations in survival and initial growth, both diameter and height, among provenances of *C. fissilis* seedlings. Therefore, it is reasonable to assume that the trees sampled in this study, from a smaller territorial range and with lower environmental variations, represent only one population of *C. fissilis*.

Póvoa (2002), using an isozyme analysis, found higher levels of variation within three natural provenances of *C. fissilis* rather than among them. In fact, this pattern was found in more than 20 Brazilian tree species and could be a quite ordinary frequency distribution of variability in tropical forest species (ETTORI et al. 2006).

Progeny effects, when considering the 48 progenies as a single provenance, were significant ( $p < 0.05$ ) for the characteristics collar diameter and survival, and highly significant ( $p < 0.01$ ) for height. The others variables showed no significant variations (Table 2).

**Table 2.** Results of the deviance analysis on 48 progeny of *Cedrela fissilis*, in a progeny test, planted in Rio Negrinho, Santa Catarina, Brazil.

**Tabela 2.** Resultados das análises de deviance para 48 progênies de *Cedrela fissilis*, em um teste de progênies plantadas em Rio Negrinho, Santa Catarina, Brasil

Effect	Likelihood ratio test						
	Number of survivors	Average collar diameter (cm)	Average height (cm)	Individuals bifurcated (%)	Number of sprouts per individual	Damage by <i>H. grandella</i> (%)	Damage by frost (%)
Progenies	4.48*	6.02*	7.55**	0.00 <sup>ns</sup>	0.03 <sup>ns</sup>	0.01 <sup>ns</sup>	0.05 <sup>ns</sup>
Average	22.92	3.18	58.41	96.70	2.57	2.27	3.86
Variation	8–41	2.38–4.05	37.53–1.06	90–100	1.64–3.68	0–12	0–20

<sup>ns</sup> not significant; \* $p < 0.05$ ; \*\* $p < 0.01$

The results of the provenance, as well as the progeny, confirm the findings of a study using the same genetic material evaluated at the seedling stage (BIERNASKI et al. 2012). Those authors found significant variations in 13 characteristics and concluded that the variability was high enough for breeding programs and *ex situ* conservation. Thus, the results using genetic materials from our study stage reaffirmed their conclusions.

Among the genetic parameters (Table 3) the accuracy estimations ( $Ac_{prog}$ ) of the diameter (84%) and height (87%) variables were considered reliable because they were above 70% (RESENDE, 2002); however, the survival accuracy estimation was lower (60%). The values of the experimental coefficient of variations ( $CVe$ ) associated with experimental precision and measurement (BATISTA et al. 2012), were considered low for all of the estimated characteristics, including survival, although the  $CVe$  for height was greater than the others.

**Table 3.** Genetic parameters of the forestry characteristics of *Cedrela fissilis* in a progeny test established in Rio Negrinho, Santa Catarina, Brazil.**Tabela 3.** Parâmetros genéticos dos caracteres silviculturais de *Cedrela fissilis* em um teste de progênes, Santa Catarina, Brasil.

Variance components	Survival	Collar diameter	Height	Bifurcation	Sprouts per individual	Damage by <i>H. grandella</i>	Damage by frost
$\sigma_g^a$	0.0019	0.0594	46.0841	0.0000	0.0083	0.0000	0.0001
$\sigma_{plot}^b$	0.0132	0.0142	10.2222	0.0009	0.2839	0.0009	0.0085
$\sigma_e^c$	0.1442	1.8650	1100.7782	0.0199	2.1979	0.0199	0.0275
$\sigma_p^d$	0.1593	1.9387	1157.0845	0.0209	2.4900	0.0209	0.0361
$h_a^2$	0.0338 ±	0.0851 ±	0.1106 ±	0.0041 ±	0.0092 ±	0.0041 ±	0.0101 ±
$h_{ad}^2$	0.0140	0.0464	0.0529	0.0101	0.0155	0.0101	0.0160
$h_{mp}^2$	0.0239	0.0566	0.0744	0.0027	0.0067	0.0027	0.0085
$h_{mp}^2$	0.3599	0.7031	0.7540	0.0783	0.1163	0.0783	0.0854
$Ac_{prog}^h$	0.5999	0.8385	0.8683	0.2798	0.3410	0.2798	0.2923
$c_{plot}^2$	0.0826	0.0073	0.0088	0.0422	0.1140	0.0422	0.2364
$CV_d(\%)^j$	62.9235	61.5369	1897.6329	3.2095	81.4632	2.0631	2.8960
$CV_g(\%)^k$	0.8290	1.9599	79.4443	0.0000	0.3077	0.0000	0.0105
$CV_e(\%)^l$	0.0063	0.0062	0.1898	0.0002	0.0081	0.0002	0.0003

<sup>a</sup>genetic variance among families; <sup>b</sup>environmental variance among plots; <sup>c</sup>residual variance; <sup>d</sup>individual phenotypic variance; <sup>e</sup>narrow-sense heritability; <sup>f</sup>additive heritability within plot; <sup>g</sup>progeny average heritability; <sup>h</sup>accuracy of progeny selection; <sup>i</sup>determination coefficient of plot effects; <sup>j</sup>genetic coefficient of variation within progeny; <sup>k</sup>genetic coefficient of variation among progenies; <sup>l</sup>experimental coefficient of variation

The narrow-sense heritability ( $h_a^2$ ) and additive within plot heritability ( $h_{ad}^2$ ) were not high for any of the characteristics. The values for progeny average heritability ( $h_{mp}^2$ ) for collar diameter (70.31%) and height (75.40%) were greater than the other characteristics, indicating their potential. When comparing juvenile characteristics (BIERNASKI et al. 2012), the value closest to that in the present study was heritability for survival.

The values of the genetic parameters concerning *C. fissilis* seedling growth were higher, in general, than the estimated values in the field, indicating a high environmental effect for the materials. Such an inference could also explain the low levels of accuracy ( $Ac_{prog}$ ) for the bifurcation of stems, number of sprouts, and damage caused by *H. grandella* and frost. Although there are records of resistance to frost damage in *C. fissilis* (Inoue 1972), in the present study there was no evidence of genetic variance for this characteristic, neither for stem bifurcation or number of sprouts.

Most of the variability was found within progenies. The value of the genetic coefficient of variation within progenies ( $CV_d$ ) was higher than the  $CV_e$  and higher than as a juvenile characteristic (BIERNASKI et al. 2012). However, the variation in the field was much higher within and among progeny when comparing to the seedling variations, which could be due to the controlled conditions during seedling cultivation. A study using 13 forest native species over 9 years, also found higher levels of variability within progenies (ETTORI et al. 1996). These results indicate a higher gain in the selection within progenies than among them.

The phenotypic and genetic correlations were estimated for the 880 individual survivors to identify interactions among the forestry characteristics, aiming to choose the most important variables for genotype selection.

The phenotypic correlations among the characteristics of collar diameter, height, stem bifurcation and number of shoots was positive and moderate. The highest correlations between diameter and height ( $r = 0.67$ ;  $p < 0.01$ ) are in agreement with the values described in the literature for species in the same forest typology; but in adult trees (SANQUETTA et al. 2014; NEGRELLE; LEUCHTENBERGER 2001). The correlation may have been higher if there had been no damage to the shoots caused by frosts and *H. grandella*, which may have interfered with the expression of the height characteristic. Therefore, the correlation value may be an indication of the bad phytosanitary conditions of *C. fissilis* in the field.

The genetic correlations indicated the level of genetic association between the characteristics. Thus, it quantifies the influence that a particular characteristic exerts over another (PIRES et al. 2011). A moderate positive correlation ( $r = 0.75$ ) between diameter and height indicated a pleiotropic effect between them or a genetic linkage effect between loci, controlling the development of the characteristics (BATISTA et al. 2012). Therefore, the selection of one of the characteristics implies gains in the other.

However, environmental pressures influence the apical dominance and, consequently, the estimation of height. Moreover, diameter had the lowest characteristic value among the  $CV_e$ , at 0.0062%. Thus, we chose to use diameter as a characteristic for growth selection. Considering the low survival result, which is expected for a species under domestication, survival must also be included in selection.

The strong natural selection that the plants were under during the experiment, combined with BLUP selection, indicates the genotypes that are better adapted in forestry conditions under test.

The effective population size ( $N_e$ ) of the progeny test, when planned and planted was 121.08 (3,840 individuals and 48 progeny), considering a 20% rate of self-fertilization in the species. In November 2012, the survival was 23% (880 individuals and 48 progeny) and the  $N_e$  was 109.95. The decrease of 9.2% from the original  $N_e$  was not in the same proportion as the individual survival number (77%) because of the effective population size.

The remaining 880 individuals are from at least one of the 48 progenies originally established. Even though there were losses within progenies or among half-brothers, these had little effect on the alleles in the sampled population. Surprisingly, the genetic base of the sampled *C. fissilis* population was barely affected, despite the mortality rate of individuals of the progeny test.

From a conservation point of view, the  $N_e$  estimated was large enough to retain alleles with frequencies greater than 6% and, once it is above 50, to prevent inbreeding depression (VENCOVSKY, 1987; FRANKHAM, 1995).

The strategy proposed for the conservation of *C. fissilis* is the conversion of part of the progeny test into a seed orchard, which will produce improved seeds for survival. It is estimated that the first flowering of the progeny test will occur in 2017. After the selective genetic thinning, it could be possible to generate income from the commercialization of the improved seeds, which can be used for forest restoration.

Four blocks of the progeny test were chosen to be turned into a seed orchard; it had 32% survival, with 620 individuals and 48 families, representing a  $N_e$  of 105.36. A simulation of selection among and within the highest surviving progeny and the effect on gain and on effective population size are described in Table 4.

**Table 4.** Gain results with selection between and within progenies for survival in a progeny test of *Cedrela fissilis* in Rio Negrinho, Santa Catarina, Brazil.

**Tabela 4.** Resultado do ganho em sobrevivência à seleção entre e dentro de progênies em um teste de progênies de *Cedrela fissilis*, em Rio Negrinho, Santa Catarina, Brasil.

Number of progenies	48	46	44	42	40	38	36	34	32	30	28
Number of individuals selected	168	164	157	150	146	138	131	124	117	110	105
Effective population size	78	75	72	69	66	63	60	56	53	50	47
Gain	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
New average	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.35	0.35

We compared different genetic selections, gradually excluding the progeny with lower survival rates, based on the ranking of the additive genetic effects for progeny, until identifying the 28 progeny with best survival values. To avoid inbreeding, the selection within progenies resulted in one individual per plot per block and was based on the ranking of the individual additive genetic values.

In this simulation, the reduction in the number of progenies considerably impacts the effective population size. The gain in survival was practically constant and had no effect on the new average. The low values in gain for survival could be explained by the reduced values of heritability and accuracy for this characteristic.

Thus, in this context, the proposed conversion of the progeny test into a seed orchard should only include within-progeny selection, and not among progeny. It is expected that the seed orchard, composed of 48 progenies of *C. fissilis* with a  $N_e$  of 78, will produce improved seeds with a 34% gain in survival; 48% greater than the average survival of the progeny test. These values were estimated for an environment similar to that of the chosen blocks.

The recommendation for a seed orchard of native species aimed at conservation, should contain a minimum of 45 progenies in order to stabilize future populations with genetic variability and evolutionary potential. In addition, seeds must be from trees of the same genetic characteristics

as the restoration project, which increases the probability of success, mainly related to seedling survival and growth (SEBBENN, 2006). These criteria were considered in this proposal to explore the possibility of developing a seed orchard for forest restoration purposes.

We believe that the strategy used to generate the *C. fissilis* seed orchard, with selection aimed at producing improved seeds for forest restoration, as in the “Legal Reserve”, could be applied to other progeny tests of native forest species. The use of seeds for restoration purposes that came from seed trees with known survival rates and  $N_e$  values, provides a quality assurance of genetic diversity, and consequently, a reference for the permanence of the local population. Furthermore, planting seedlings from these seed orchards could also be a strategy for the *ex situ* conservation of native species and their populations, as well as providing a certification standard for forestry restoration and conservation projects.

The breeding strategy aimed at growth production, is to collect vegetative material; a technique quite simple for *C. fissilis* (XAVIER et al. 2003); by taking vegetative material from the best genotypes in the progeny test to create a clonal seed orchard. Assuming that the rescue of the genetic material could be completed in 2016; then from 2021 the production of improved *C. fissilis* seeds could start.

The early selection of the best genotypes was based on the independent elimination level (PIRES et al. 2011), combining BLUP and the multi-effect index, both applied in the narrow-sense. The multi-effect index was chosen because of the unbalanced data, once all the effects of the statistical model were randomized (RESENDE; HIGA 1994).

To predict gains, different strategies of selection, using the independent elimination level, were tested (Table 5). We compared the 15 best additive genetic values of progeny for survival, estimated by BLUP, with one, two or three of the best additive genetic values of individuals for diameter within those progenies as estimated by the multi-index or BLUP.

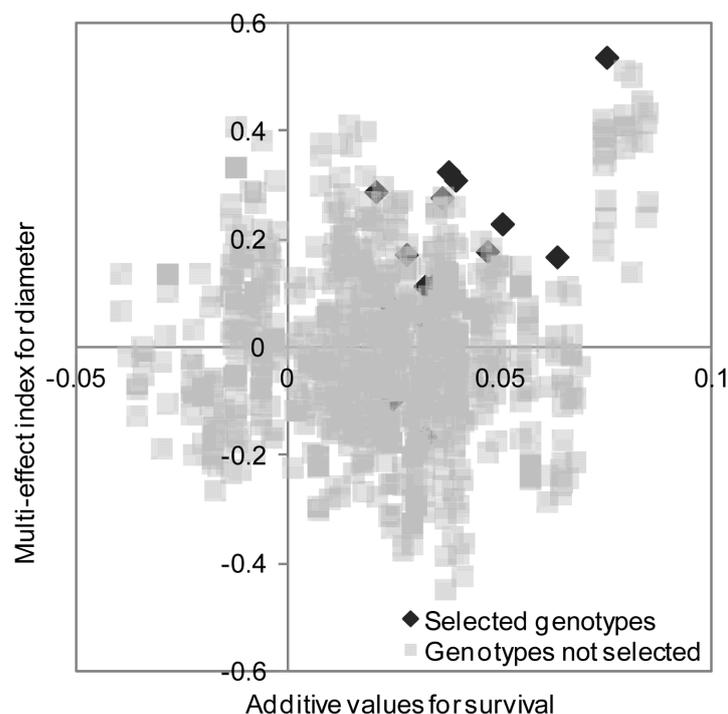
**Table 5.** Gain estimated using the independent elimination level of the 15 best progenies for survival and, one; two or three highest additive genetic values of individuals for diameter within those progeny as estimated by the multi-effect index and BLUP, in a progeny test of *Cedrela fissilis* in Rio Negrinho, Santa Catarina, Brazil.

**Tabela 5.** Estimativa de ganho em níveis independentes de eliminação das 15 melhores progênies para sobrevivência e; um, dois ou três maiores valores genéticos aditivos individuais para diâmetro entre progênies estimados pelo índice multiefeito e BLUP, em um teste de progênies de *Cedrela fissilis* em Rio Negrinho, Santa Catarina, Brasil.

Number of progenies selected	Number of individuals selected within progeny	$N_e$	Additive genetic values of progeny for survival by BLUP		Additive genetic values of individual for collar diameter by multi-effect index		Additive genetic values of individuals for collar diameter by BLUP	
			New average	Gain	New average	Gain	New average	Gain
15	45	24	0.27	0.04	3.31	0.13	3.13	0.10
15	30	20	0.27	0.04	3.33	0.15	3.14	0.11
15	15	14	0.27	0.04	3.36	0.18	3.17	0.14

The multi-effect index predicted a higher gain in comparison with the BLUP estimate, as expected. The gradual increase in the intensity of selection, when reducing three individuals per progeny to one, increased the gain in diameter, but consequently reduced the value of the effective population size. Therefore, the suitable proposal for the generation of the clonal seed orchard aimed at the production of seeds improved for survival and growth; was the selection of the 15 best progenies for survival and the best individuals within those progenies for diameter, as determined by the multi-effect index (Figure 2).

The predicted values for the seeds produced in the clonal seed orchard were 27% for survival rate and 3.63 cm for diameter, which were 4% and 18% higher, respectively, than the average of the previous generation (the progeny test). The intensity of the selection proposed (1:59), which aimed to increase the gains, did not take into consideration the effective population size, a parameter of low interest for production purposes. The  $N_e$  of the proposed clonal seed orchard was 14.



**Figure 2.** Graphic representation of *Cedrela fissilis* genotypes using the additive values of the multi-effects index for diameter and additive values for survival.

**Figura 2.** Representação gráfica dos genótipos de *Cedrela fissilis* utilizando os valores aditivos do Índice Multiefeito para diâmetro e valores aditivos para sobrevivência.

## CONCLUSIONS

The genetic material of the *C. fissilis* progeny test was composed of one single population distributed in six municipalities, ~5,700 km<sup>2</sup>, featuring a unique zone of seed collection.

Among the seven characteristics evaluated for the first cycle of the improvement program and domestication of *C. fissilis*, survival and collar diameter were significant among progenies, and therefore prioritized. The damage caused by *H. grandella* and frost, bifurcation of stems; and number of sprouts were not genetically controlled among progenies. The genetic parameters suggested that plants in the field were under intense pressure by natural selection, which is reasonable for a non-domesticated species.

Aiming at forest restoration, a seed orchard was proposed, with predicted gain for the next generation of 2% for survival and a *Ne* of 78. The production of seeds from the clonal seed orchard proposed predicts a 4% gain in survival and an 18% increase in collar diameter.

The progeny test had a large enough effective population size for *ex situ* conservation and the genetic breeding of *C. fissilis*. The selection proposals intended to explore the progeny test as an active germplasm bank combining the interests of the conservation and sustainable use of *C. fissilis*.

## ACKNOWLEDGMENTS

We thank the engineers Ulisses Ribas Jr., Diego Fernando Roters and Fernanda Silveira; the companies Battistella Florestal and Valor Florestal, and their research staff, who supported the field work; Fabrício Antonio Biernaski for planning and installing the progeny test; Laboratory of genetics and forest improvement (LAMEF) of Federal University of Paraná (UFPR) for practical and theoretical support; the Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI) for the climate data; the Forest Engineering Postgraduate Program of UFPR; the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Brazilian government and citizens for the PhD and Postdoctoral (CAPES - Proc. N° 99999.006171/2014-01) grants and the opportunity.

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Recebido em 19/01/2016

Aceito para publicação em 18/05/2016

