ARTICLE

Genetic variability in progenies of *Eucalyptus dunnii* Maiden for resistance to *Puccinia psidii*

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Abstract – *This study investigated the genetic variability in progenies of* Eucalyptus dunnii *Maiden for resistance against rust (*Puccinia psidii*). Field experiments were installed in two regions with different soil-climatic conditions. Open-pollinated progenies were established in a randomized complete block design. Sixty and 48 progenies were evaluated under field conditions at two sites, respectively, with six replications and eight trees per plot. In another experiment in a controlled environment, 53 progenies were evaluated in randomized blocks with six replications and nine plants per plot. The following traits were evaluated: plant height, severity of pest attack and the most susceptible stage to the leaf disease. The genetic variability for rust resistance in the* E. dunnii *population under study was high, with a genetic coefficient of variation of 36.07%; 7% of the evaluated progenies were rust-resistant. It indicates a high potential for selection and breeding of the species.*

Key words: Eucalyptus*, genetic parameters, progeny testing, forest breeding.*

INTRODUCTION

Brazil is one of the world's largest producers of *Eucalyptus* wood. The share of the forest sector in the national economy, which has a participation of approximately 4.5% of GDP, clearly shows the importance of this genus for the country is grown on an area of 6.5 million hectares and generates about 4.73 million jobs (ABRAF 2012).

With the expansion of eucalyptus cultivation to warmer and more humid regions and with the impact of climate changes, conditions have become favorable for the occurrence of diseases (Telechea et al. 2003, Glen et al. 2007). Consequently, the planting of susceptible species and the uninterrupted use of the same cultivation areas increase the chances of pest attacks.

One of the major diseases of *Eucalyptus* today is rust. The economic problems resulting from rust are related to planting in the field (Ferreira 1983), where fungicide treatments are practically unfeasible. The vegetative growth of *Eucalyptus grandis* trees infected with this pathogenic fungus can be reduced by 28% to 35%, compared to unaf-

fected plants, resulting in direct losses in productivity and economic gains.

Nowadays, several forms of rust control are being applied, for example: fungicide treatment, tree harvest for regrowth in disease-unfavorable seasons and cultivation of resistant plants. For a number of reasons, the use of resistant varieties is the most indicated measure: it is cheap, practical and has less environmental impacts for requiring less fungicide application (Carvalho et al. 1998.).

Resistant plants can be selected in the field in progeny and clonal tests (Zobel and Talbert 1984, Alfenas et al. 2004, Teixeira et al. 2009), in areas where the disease is severe, endemic or even by infection through artificial inoculation in a controlled environment (Xavier et al. 2001).

A series of studies on rust have been conducted for the genus *Eucalyptus* (Dianese et al. 1984, Freeman et al. 2008, Zauza et al. 2010, Miranda et al. 2013, Silva et al. 2013) in view of the great commercial importance for the country. However, there are few reports in the literature about the disease in the species *Eucalyptus dunnii* Maiden, although

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the occurrence of the disease in the field has been observed. Therefore, this study evaluated the genetic variability in *E. dunnii* progenies for rust resistance caused by *Puccinia psidii*.

MATERIAL AND METHODS

Two trials of *E. dunnii* open-pollinated progenies were set up in the field (Itapetininga and Itatinga, SP, see Table 1), in July 2009. The trials were arranged in a randomized block design with 60 and 48 progenies, both with eight plants per plot with six replications, in a 3 x 2 m spacing The trial into the controlled environment (inoculation chamber) was carried out in the Department of Crop Science in the Sector of Plant Protection and Plant Breeding of São Paulo State University (UNESP), in Botucatu, SP, Brazil, by the randomized blocks with 53 progenies, with nine plants per plot and six replications, totalizing 2,544 plants. The total used progenies in the controlled environment trial, 48 were set up at Itapetininga, and all of 53 were at Itatinga.

Inoculation was performed with an uredospore solution of *P. psidii* from plants of a spontaneously infected rose apple *(Syzygium jambos)* tree, in a controlled environment. The spores were scraped from the leaves with a stylus and suspended in distilled water plus Tween 20% to reach a concentration of 9 x 104 spores mL^{-1}. The suspension was sprayed on all plants with an air compressor (Chiaperini® 2,3pcm, Model E48C) to ensure an even distribution of the spore suspension. The test temperature was maintained at 22 °C, the average relative humidity was 80% and the photoperiod 12 hours, for 15 days, until evaluations.

The following characteristics were evaluated in progeny field trials: tree height (H) and severity of fungal attack, to correlate the attack intensity with the development of the progenies. Evaluations were performed every three months for severity, with four assessments, and every six months for height, with two evaluations. Data on the severity of rust attack were collected in the field, based on grade criteria from 0 to 4, where: 0 - resistant plant; 1 - sporadic sporulation; 2 - generalized sporulation, but no apparent damage to the plant; 3 - generalized sporulation on leaves and branches, causing major damage and 4 - advanced disease, with loss of apical dominance. To determine resistance levels in *E.*

dunnii progenies we used a grade scale adapted by Aparecido et al. (2003), and the data were previously transformed to $\sqrt{x + 0.5}$.

The genetic parameters for each individual test were estimated using the program SELEGEN (Resende 2007) model 93, by the statistical model: $y = Xr + Za + Wp + e$, where *y* is the data vector, *r* the vector of effects of replications (assumed as fixed) added to the overall mean, *a* is the vector of individual additive genetic effects (assumed as random), *p* is the vector of plot effects (random) and *e* is the error or residue vector (random). Capital letters represent the incidence matrices for these effects. *X, Z* and *W* are known incidence matrices formed by the values zero and one, which associate the unknown *r*, *a* and *p* to the data vector *y*, respectively. By the mixed model methodology *r* can be estimated by the generalized least square procedure and predict *a* and *p* by the BLUP procedure. The REML procedure (method of maximum likelihood) was performed based on Expectation-Maximization (EM) algorithms, where the resolutions of the matrices provide estimates of adjusted effects of the calculated vectors. The following genetic parameters were estimated:

a) Additive genetic variance ($\hat{\sigma}_a^2$)

$\hat{\sigma}_a^2 = [\hat{a}' A^{-1} \hat{a} + \hat{\sigma}_e^2 tr (A^{-1} C^{22})] / q$

b) Environmental variance between plots ($\hat{\sigma}_c^2$)

$\hat{\sigma}_c^2 = [\hat{c}' \hat{c} + \hat{\sigma}_e^2 tr C^{33}] / S_1$

c) Residual variance (environmental + non-additive) ($\hat{\sigma}_e^2$)

$\hat{\sigma}_e^2 = [y' y - \hat{r}' X' y - \hat{a}' Z' y - \hat{c}' W' y] / [N - r(x)]$

where C^{22} *and* C^{33} are the inverse of C.

C: matrix of the coefficients of the mixed model equations

tr: trace operator matrix.

r(x): rank of matrix X.

N, q, s: number of data, plants and plots, respectively.

d) Individual phenotypic variance ($\hat{\sigma}_f^2$)

$\hat{\sigma}_f^2 = \hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2$

Table 1. Characterization of the two trial sites

Location	Number of assessed progenies	Latitude S	Longitude W	Altitude (m)	TMA (°C)	Tm (°C)	TM (°C)	Rainfall (mm)	Soil
Itatinga (SP)	48	23° 06'	48° 36'	845	20.0	16.3	21.9	1.350	LVA
Itapetininga (SP)	60	23° 35'	48° 03'	656	20.4	16.8	23.8	1.182	LVAd

TMA: Mean annual temperature; Tm: Mean temperature in the coldest month; TM: Mean temperature in the warmest month; LVd: Dystrophic Red Latosols; LVAd Dystrophic Yellow Red Latosols.

e) Individual narrow-sense heritability, i.e., the additive effects: $\hat{h}_a^2 = \dfrac{\hat{\sigma}_a^2}{\hat{\sigma}_f^2}$

f) Heritability of progeny means:

$\hat{h}_m^2 = \dfrac{(1/4)\hat{\sigma}_a^2}{(1/4)\hat{\sigma}_a^2 + \dfrac{\hat{\sigma}_c^2}{r} + \dfrac{(0.75\hat{\sigma}_a^2 + \hat{\sigma}_e^2)}{nr}}$

g) Additive heritability within plot: $\hat{h}_{ad}^2 = \dfrac{0.75\hat{\sigma}_a^2}{0.75\hat{\sigma}_a^2 + \hat{\sigma}_e^2}$

h) Coefficient of individual additive genetic variation:

$CV_{gi}(\%) = \dfrac{\sqrt{\hat{\sigma}_a^2}}{\hat{m}}.100$

i) Coefficient of genotypic variation among progenies:

$CV_{gp}(\%) = \dfrac{\sqrt{0.25\hat{\sigma}_a^2}}{\hat{m}}.100$

j) Coefficient of experimental variation:

$CV_e(\%) = \dfrac{\sqrt{[(0.75\hat{\sigma}_a^2 + \hat{\sigma}_e^2)/n] + \hat{\sigma}_c^2}}{\hat{m}}.100$

k) Coefficient of relative variation: $CV_r(\%) = \dfrac{CV_{gp}}{CV_e}.100$

l) The combined analysis (mathematical model 4) and genetic and phenotypic correlations (mathematical models 105 and 102) were estimated using SELEGEN software.

RESULTS AND DISCUSSION

The coefficient of experimental variation for rust resistance was 19.2%, 15.7% and 18.9%, in the three trials, respectively (Table 2). These values indicate good experimental accuracy for field trials and controlled environments in species of the genus *Eucalyptus* (Garcia 1989).

The values of the estimates for individual narrow-sense heritability were moderate ($\hat{h}_a^2 = 0.48$ and 0.37) and high

for mean progeny heritability ($\hat{h}_m^2 = 0.84$ and 0.64) into the inoculation chamber and in Itapetininga trial, indicating low influence of the environment on the rust resistence (Resende 2007). The values were similar to those reported by Mori et al. (2004) for rust resistance in *E. grandis* progenies under field conditions.

In the region of Itatinga, heritability was generally low, indicating the influence of environmental factors on trait expression. In the case of rust resistance, this can be influenced by the low availability of inoculum in the environment.

In general, estimates of the coefficient of individual genetic variation (CV_{gi}) were higher than the variation among progenies (CV_{gp}) for rust resistance. Lowest values of CV_{gi} (2.1%) and CV_{gp} (1.0%) were observed for rust severity in the Itatinga field trial, and highest values of CV_{gi} (36.1%) and CV_{gp} (18.0%) were found in the progeny trial in the inoculation chamber. The high CV_{gi} values show possibilities for selection in breeding programs. Miranda et al. (2013) found very similar CV_{gi} values (11.7% - 36.7%) for *Eucapyptus grandis* by nine different locations.

The relative coefficients of variation (CV_r) were low, medium and high, in the experiments in Itatinga, Itapetininga, and the controlled environment, respectively (Table 2). According to Vencovsky and Barriga (1992), the higher the CV_r value, the greater is the genetic control of traits and the lower is the influence by environmental factors, favoring selection.

The experimental coefficient of variation (CV_{exp}) for H (Table 3), evaluated after six and 12 months, was high in both field trials. The highest CV_{exp} values were found in Itapetining (19% after 6 months and 21.7% after 12 months). In Itatinga, CV_{exp} was adequate (15.2% after 6 months and 14.3% after 12 months), which is close to values found in

Table 2. Estimates of genetic parameters for rust susceptibility, in *Eucalyptus dunnii* progenies, in three environments [(Inoculation chamber (**IC**); Itapetininga, SP, Brazil (**R1**) and Itatinga, SP, Brazil (**R2**)]

Genetic parameters	Environments		
	IC	**R1**	**R2**
h_a^2	0.485 ± 0.83	0.370 ± 0.074	0.005 ± 0.009
h_{mp}^2	0.841	0.645	0.017
h_{ad}^2	0.430	0.404	0.006
CV_{gi} (%)	36.0	17.2	2.1
CV_{gp} (%)	18.0	8.6	1.0
CV_{exp} (%)	19.2	15.7	18.9
CV_r	0.94	0.55	0.054
Mean	1.07	0.87	0.86

Individual heritability (h_a^2), heritability of progeny mean (h_{mp}^2), additive heritability within progenies (h_{ad}^2), coefficients of genetic variation at individual level (CV_{gi}) and of progenies (CV_{gp}), coefficient of experimental variation (CV_{exp}), coefficient of relative variation (CV_r) and rust susceptibility.

Table 3. Estimates of genetic parameters, for plant height in *Eucalyptus dunnii*, progenies in two soil-climatic regions (Itapetininga and Itatinga, SP, Brazil)

Genetic parameters	Regions			
	Itapetininga		Itatinga	
	6 months	12 months	6 months	12 months
h_a^2	0.108±0.040	0.075±0.033	0.608±0.098	0.583±0.096
h_{mp}^2	0.317	0.235	0.822	0.819
h_{ad}^2	0.113	0.081	0.614	0.579
CV_{gi} (%)	10.6	9.8	26.7	24.8
CV_{gp} (%)	5.3	4.9	13.3	12.4
CV_{exp} (%)	19.0	21.7	15.2	14.3
CV_r	0.28	0.23	0.88	0.87
Overall mean	2.03	2.81	2.39	3.62

Individual heritability (h_a^2), heritability of progeny mean (h_{mp}^2), additive heritability within progenies (h_{ad}^2), coefficients of genetic variation at the individual level (CV_{gi}) and of progenies (CV_{gp}), coefficient of experimental variation (CV_{exp}), coefficient of relative variation (CV_r) and mean of plant height (H).

Table 4. Estimates of genotypic (r_g) and phenotypic correlations (r_f) for rust susceptibility, in three environments, for open-pollinated progenies of *Eucalyptus dunnii*

Traits	correlations	Rust chamber	Rust Itapetininga	Rust Itatinga	Plant height 6 months Itatinga	Plant height 12 months Itapetininga	Plant height 6 months Itatinga
Plant height 12 months Itatinga	r_g	-0.02	0.09	-0.07	0.28	0.26	0.95
	r_f	0.01	0.03	-0.04	0.08	0.07	0.90
Rust chamber	r_g		0.50	-0.03	0.22	0.07	-0.03
	r_f		0.04	0.00	0.01	-0.02	0.01
Rust Itapetininga	r_g			-0.12	0.30	0.17	0.10
	r_f			0.03	0.04	0.03	-0.02
Rust Itatinga	r_g				0.11	0.12	-0.08
	r_f				0.04	0.03	-0.04
Plant height 6 months Itatinga	r_g					0.91	0.28
	r_f					0.90	0.09
Plant height 12 months Itapetininga	r_g						0.24
	r_f						0.07

the literature (Santos et al. 2004, Souza et al. 2011).

The heritability at the individual plant level, in the narrow sense ($\hat{h}_a^2$) was low for H in Itapetininga (0.10 ± 0.04 after 6 months and 0.07 ± 0.03 after 12 months) and high in Itatinga trial (0.60 ± 0.09 after six months and 0.58 ± 0.09 after 12 months). This may indicate that about 90% of the variation in Itapetininga and 40% in Itatinga may be induced by the environment. The $\hat{h}_a^2$ values found in Itatinga, at the two ages for H, were higher than values found in the literature (Rocha et al. 2007, Rosado et al. 2009), in their studies with *E. urophylla*. For mean progeny heritability ($\hat{h}_{mp}^2$), the highest values found for Itatinga show that the environment had little influence on the phenotypic expression of H.

The coefficients of individual genetic variation (CV_{gi} %) were higher than those of genetic variation of progenies (CV_{gp} %) in both experiments and at both ages. In the tests, CV_{gi} % varied from 9.8% for Itapetininga/SP, after 12 months, to 26.7% for Itatinga/SP after 6 months. The same trend was found for CV_{gp} %. The results show the genetic variability for the trait ALT, under the experimental conditions of the study site.

The coefficients of relative variation (CV_r) were high in Itatinga, with an approximate value of 0.87, at both ages. In Itapetining however, the values were close to 0.27, similar to the values found for open-pollinated progenies of *E. urophylla* (CV_r =0.21) after 17 years for plant height, in

Table 5. Genetic parameters estimated in the joint analysis for rust resistance in three environments (Inoculation chamber (**CI**); Itapetininga (**R1**) and Itatinga (**R2**), in open-pollinated progenies of *Eucalyptus dunnii*)

Genetic parameters	Rust			
	3 environments	CI/R1	CI/R2	R1/R2
h_a^2	0.082±0.021	0.214±0.039	0.008±0.001	0.004±0.005
h_{mp}^2	0.359	0.547	0.033	0.019
h_{ad}^2	0.077	0.195	0.008	0.005
Overall mean	0.94	0.97	0.97	0.87

Individual heritability (h_a^2), heritability of progeny mean (h_{mp}^2), additive heritability within progenies (h_{ad}^2).

Selvíria, MS (Souza et al. 2011). According to Vencovsky and Barriga (1992), these values are considered low. The low CV_r values indicate that the genetic control of the trait is low and highly influenced by the environment. According to Vencovsky (1978), the CV_r estimates close to 1 are recommendable.

The genetic and phenotypic correlations are presented in Table 4. The correlations were highest for the character height (H), between different ages in the same environment. The high values show that the performance of the genotypes does not vary with increasing age. This behavior is important in the case of early selection.

For rust, the highest correlation was found between Itapetininga and inoculation chamber, where a genetic correlation (r_g) of 0.50 was observed. However, the same was not true for the phenotypic correlation (r_f), where a value of 0.04 was calculated. When progeny performance, tree height, and rust susceptibility were correlated, the values were very low, indicating that the poor performance of the plant material had no influence on rust attack. This may have occurred because of the low rust-susceptibility of the species, or to the low availability of inoculum in the area of the field trials. In general, the correlations found in this study were low, hampering indirect selection for the studied traits.

The estimates of genetic parameters in the combined analysis for rust in all three environments in pairwise analysis (controlled environment/Itapetininga, controlled environment/Itatinga and Itapetininga/Itatinga) and for the trait plant height in Itapetininga/Itatinga are shown in Table 5.

The combined analysis showed mean and low values for heritability. The values were highest in the analysis between tests of inoculation chamber and Itapetininga. The lowest values on the other hand were observed in the combined analysis of the two field tests for rust resistance.

The genotypes x environments (GE) interactions were high in the rust analysis in three environments, between the test controlled environment/Itapetininga and at both ages for plant height. This shows the presence of a complex GE interaction. In this case, the decision on selection is more difficult, since the selection of genotypes adapted to specific environments is required. Miranda et al. (2013) observed strong genetic control associated with rust resistance between provenances of *E. grandis*. For resistance in interspecific *Eucalyptus* hybrids to *P. psidii*, Alves et al. (2012) observed that quantitative additive and epistatic trait loci explain between 29.8 and 44.8% of the phenotypic variation, respectively. This is evidence of a more complex inheritance pattern of the trait. The genotypic correlation between the progeny performance in various environments ($\hat{r}_{gloc} = 0.21$) was considered low. Still, this interaction could be exploited in a positive way in breeding programs since it allows directing a particular genotype towards a specific region, maximizing the phenotypic trait expression in this environment. However, if the genotype is directed towards another region, its phenotypic value might be reduced.

In summary, this study shows the great potential of the studied populations of *E. dunnii* for selection and breeding, with a view to rust resistance, since 70% of the progenies obtained grade zero (resistant plants) and the genetic variability for rust resistance in the studied population was good ($CV_{gi} = 36.07\%$). The genetic correlation between the test in the controlled environment and field test in Itapetininga was also good ($r_g = 0.50$); this shows that the controlled environment is a good alternative for early selection of plant material for rust resistance to obtain progress in the *E. dunnii* breeding program for rust. It is noteworthy that the genetic correlation between the controlled environment and Itatinga (CI/R2) is zero, which prevents a selection under controlled conditions for response to selection in Itatinga. Even the field data showed no genotypic correlation between Itatinga and Itapetininga.

Variabilidade genética em progênies de *Eucalyptus dunnii* Maiden para resistência à *Puccinia psidii*

Resumo – *Avaliou-se a variabilidade genética em progênies de* Eucalyptus dunnii *Maiden para resistência à ferrugem (*Puccinia psidii*). Experimentos de campo foram instalados em duas regiões edafoclimáticas. Progênies de polinização aberta foram estabelecidas em um delineamento em blocos casualizados. Em condições de campo foram avaliadas 60 e 48 progênies em dois locais, com seis repetições e oito plantas por parcela. Instalou-se também um experimento em ambiente controlado, com 53 progênies, em blocos casualizados, com seis repetições e nove plantas por parcela. Foram avaliadas altura da planta e a severidade de ataque que determinam a fase mais suscetível à doença foliar. A população de* E. dunnii *estudada apresentou alta variabilidade genética para resistência à ferrugem, com coeficiente de variação genética de 36,07% e 70% das progênies avaliadas foram imunes a ferrugem. Isto indica alto potencial para seleção e melhoramento da espécie.*

Palavras-chave: Eucalyptus*, parâmetros genético, teste de progênies, melhoramento genético florestal.*

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