GENETIC VARIATION FOR FROST TOLERANCE IN AN URUGUAYAN BASE POPULATION OF *Eucalyptus grandis*

Balmelli, G.D.[1] and **Rockwood, D.L.**[2]
[1]Programa Forestal, INIA-Uruguay, Ruta 5 km 386, Tacuarembo, Uruguay - Phone (0632) 2407 - E-mail gubal@iniaen.org.uy
[2]University of Florida, Gainesville, FL, USA, 32611-0410

ABSTRACT

Genetic variation for frost tolerance was studied in a 2.5-year-old 1st-generation base population of *Eucalyptus grandis*. Seventy open-pollinated families, representing 14 provenances, were screened in three electrical conductivity tests in winter 1996. Provenance and family effects on relative conductivity (RC) measurements were not significant. Trees within families was highly significant and had a large contribution (28%) to the total variation. However, 53% of the total variation was found within trees and repeatabity of observations for RC was low (0.35). Individual tree and family heritability estimates for RC were 0.11 $\pm$ 0.13 and 0.20 $\pm$ 0.22, respectively. Improvement of frost tolerance in this population should rely mainly on intensive selection of individual trees rather than family or provenance selection. However, the gains predicted for different strategies of selection were very low.

INTRODUCTION

The frost susceptibility of *Eucalyptus grandis* (**EG**), probably the most widely planted eucalypt in temperate and subtropical latitudes, is the main factor limiting its expansion to cold areas (Golfari, 1983). In Uruguay, young EG are often seriously damaged by the erratic occurence of severe or out-of-season frosts. The improvement of **EG** frost tolerance will increase the area suitable for its planting and reduce the risks of losses due to frosts.

Significant provenance variation has been reported in **EG** for frost tolerance (Mullin and Barnes, 1977; Franklin and Meskimen, 1983; Marco, 1987; Marco *et al.* 1991), but studies on the magnitude of additive genetic variation are very limited. Low individual tree heritability for frost tolerance ($h^2 = 0.10$) was reported by Van Wyk (1976) but Meskimen *et al.* (1987) found a broad sense heritability of 0.53 for cold damage in 1.6-year-old clones.

A first-generation base population of **EG**, composed of 5,400 trees from 180 individual seedlots from Australia and from locally selected trees, was installed in 1993 in Tacuarembo, Uruguay, by the National Institute of Agricultural Research (INIA) (Bennadji *et al.*, 1997). No severe freeze occured during the 1994 and 1995 winters, and therefore there was no experimental information regarding genetic variation of frost tolerance in the Uruguayan base population. This study reports genetic parameters of frost damage based on electric conductivity measurements carried out during the winter of 1996.

MATERIALS AND METHODS

Plant material

A subset of 630 fully hardened trees, representing 70 open-pollinated families from 14 provenances (five families per provenance) in a 2.5-year-old first-generation base population of *E. grandis* was screened in July and August 1996. As three tests, through a four-week period, was necessary to conduct the screening, ten trees were sampled as checks to indicate any change in relative hardening over time. A total of nine trees per family were sampled by taking two recently fully expanded leaves from the middle part of the crown of each tree. Leaves were immediately placed in sealed plastic bags, packaged carefully in insulated coolers, and shipped to the University of Florida at Gainesville for analysis. Total elapsed time between a field sample collection and laboratory freeze was 72 hours or less.

Frosting Method

The screening for frost tolerance was based on the electrical conductivity method developed by Eldridge *et al.* (1983) and Raymond *et al.* (1986). The equipment consisted of a cold bath containing aqueous ethylene glycol solution in which racks of 12 x 75 mm glass test tubes were immersed. Test tubes were initially filled with 3.5 ml of distilled deionized water and placed in a freezer ovemight to allow ice formation. Discs 6 mm in diameter were cut from leaves with a paper punch and single discs were placed in test tubes. Racks of tubes were placed and held in the cold bath at -7°C for one hour. Then, racks of tubes were removed and placed at room temperature for 24 hours. Electrical conductivity of the diffusate from the leaf discs was then measured with a conductivity meter. Racks of tubes were then placed in an autoclave for 20 minutes. After 24 hours at room temperature, electric conductivity was measured again. The two measures of conductivity, conductivity for the test temperature (Ct), and the absolute conductivity (Ck) after killing the discs were converted to relative conductivity (RC) values to assess the degree of damage suffered by the leaf tissue as defined by Raymond *et al.* (1986).

$$RC = (Ck - Ct/Ck)^{1/2}$$

Four replications (two discs from each of the two leaves from each tree) were run in each freeze test to provide and indication of the degree of variation within tree.

Statistical Analysis

A pooled analysis of variance for RC was conducted utilizing a linear model containing terms for run (frosting time), provenance, run by provenance interaction, family within provenance, run by family within provenance interaction, tree within family, leaf within tree and disc within leaf. All factors were assumed random. Variance component were estimated using PROC VARCOMP Method REML (SAS Institute, 1989).

Repeatability estimates for multiple RC observations were obtained using the relationship given by Falconer and Mackay (1989) as

$$\rho = \sigma_t^2/(\sigma_t^2 + \sigma_l^2 + \sigma_d^2)$$

where σ_t^2, σ_l^2 and σ_d^2 are the variance components due to tree, leaf within tree and disc within leaf, respectively. Individual tree and family heritability for RC were estimated as follows:

$$h^2 = 4\,\sigma_f^2/(\sigma_f^2 + \sigma_{rf}^2 + \sigma_t^2) \quad \text{and}$$

$$h_f^2 = \sigma_f^2/(\sigma_f^2 + \sigma_{rf}^2/r + \sigma_t^2/rt)$$

where σ_f^2, σ_{rf}^2 and σ_t^2 are the variance component due to family, run by family interaction and trees within family, respectively. The coefficients r and t are the number of runs and number of trees within family per run.

Phenotypic and genetic correlations between RC and height, DBH and volume at age 30 months were computed. Covariance components were estimated using PROC GLM, MANOVA (SAS Institute, 1989), and used to calculate genetic correlations as

$$rA = COV(xy) / (Var(x)\,Var(y))^{1/2}$$

where Var (x), Var (y) and Cov (xy) are the additive variance of RC and the growth trait and the additive covariance of the two traits, respectively.

To determine the most effective method of an eventual convertion of the INIA base population into a seed orchard with frost tolerance as breeding objective, seven different selection strategies were considered: mass selection of 54 best trees or 180 best trees; combined selection of 10 best families (18 trees per family), 20 best families (9 trees per family), 60 best families (3 trees per family), 90 best families (2 trees per family); and within family selection, i.e., the best tree from each of the 180 families.

RESULTS AND DISCUSSION

Genetic Variation for RC

Despite having used the same temperature in the three runs, a highly significant effect of run and a significant run by family interaction on RC were observed (Table 1). The overall mean RCs for the three runs were 0.72, 0.79

and 0.75. The differences among runs could be due to random sampling or to hardening and/or dehardening conditions between runs, as suggested by the checks, whose RCs for the three runs averaged 0.71, 0.78 and 0.73, respectively.

Provenance and family within provenance effects on RC were not significant and contributed only with 0.4 and 1.4%, respectively, to the total variation. The effect of trees within families was highly significant and had a large contribution (28.4%) to the total variation for RC. However, most of the variation for RC was found within trees (53%). Leaf effect accounted for 26% of the within tree variation and the residual effect (including the within leaf variation) represented 74% of the within-tree variation. A within-tree sample size of two leaves and two discs per leaf was found the best compromise between the desired precision and the practical limitations of sampling and shipment (Balmelli, 1997), and agrees with results obtained by Raymond **et al.** (1992). The same within-tree sampling scheme was used also by Rockwood (unpublished data) in a study comparing **EG**, *E. amplifolia* and *E. regnans*, and effectively differentiated among species groups at -2.3 and -5.5°C for unhardened and hardened seedlings. In this case, however, the low repeatability found for RC, $\hat{r}$ = 0.35, suggests that a larger within-tree sample size would have been required in order to have a more reliable indicator of plenotypic values.

The small variation among families, the interaction between families and runs, and the large variation between and within trees for RC suggests that this trait is under weak genetic control. The individual and family heritabilities for RC were 0.11 $\pm$ 0.13 and 0.20 $\pm$ 0.22, respectively. Heritability for RC, estimated for other eucalypts, suggest that this trait is under moderate or high genetic control. Volker *et al.* (1994) found individual heritabilities ranging from 0.23 to 0.44 in control-pollinated families of *E. nitens* and from 0.29 to 0.50 in *E. globulus*. Even higher heritabilities, ranging from 0.46 to 0.66, were reported by Tibbits *et al.* (1991) in *E. nitens*.

Discrepancies between the low heritability found in this study and the moderate to high values reported elsewhere could be attributed at least to four factors. First, although storage of leaves for 24 hours has been reported to have no effect (Raymond *et al.* 1986), the 72 hours required in our case could have affected the leakage of electrolytes. Second, as discussed previously, the low $\hat{r}$ obtained for RC suggests that a larger sample size could have reduced the within-tree variance and thus increased the heritability estimate. However, $\hat{r}$ sets the upper limit for heritability (Falconer and Mackay, 1989), and our $\hat{r}$ of 0.35 is still low compared with previous reports. Third, larger additive variation could possibly have been found with a larger number of families tested. Fourth, most studies of heritability for RC have used seedlings, which may have different anatomical or physiological mechanisms of hardiness than 30-month-old trees. For

TABLE 1. Mean squares, variance components and their contribution (%) to the total variation for RC.

Source	df	MSRC	$\hat{\sigma}^2$	%
Run R	2	1.265**	0.0015006	14.4
Provenance (P)	13	0.035	0.0000423	0.4
R*P	26	0.015	0.0000000	0
Family/P	56	0.027	0.0001425	1.4
R*F/P	112	0.023*	0.0002576	2.5
Tree/F/P/R	420	0.018**	0.0029656	28.4
Leaf/T/F/P/R	630	0.007**	0.0014202	13.6
Error (w/leaf)	1260	0.004	0.0041079	39.3
Total	2519			

* and ** significant at the 5 and 1% level, respectively

example, survival of 6-month-old potted **EG** plantlets, cuttings, or seedlings screened as whole plants at -5° C only somewhat paralleled their field resilience (Rockwood *et al.* 1989).

Relationship Between RC and Growth Rate

Both phenotypic and genetic correlations between RC and growth traits at age 30 months were positive (Table 2). Phenotypic correlation coefficients were low but statistically highly significant. Although very high genetic correlations were found, their precision was obviously low, particularly the correlations between RC and DBH and between RC and volume, whose values lie outside the theoretical range. Positive genetic correlations (0.49 and 0.45) between field frost hardiness and height in **EG** were reported by Van Wyk (1976) and Franklin and Meskimen (1983), respectively. The latter authors attributed that relationship to the fast early growth of **EG**, which allow the trees to escape inversion freezes. However, this hypothesis does not apply in this case and therefore other anatomical or physiological mechanisms must be involved in the frost tolerance measured through the electrical conductivity method.

Predicted gains

The patterns of genetic variation indicate that selection pressure for increased frost tolerance at the provenance and family level would be practically ineffective. Although considerable variation was found within-family, the low individual and family heritability estimates found for RC suggest that expected gains through any strategy must rely on high selection intensity.

The same pattern of genetic variation was assumed for the entire population in order to predict genetic gains for RC under different selection strategies of converting the INIA base population to a seed orchard. The gains predicted for RC over the population mean for all strategies were very low, and even with a fairly intensive selection pressure of 1%, the predicted gains are below 3% (Table 3).

Under different circumstances, within family selection coupled with continuous introduction of new materials and mass selection coupled with vegetative propagation and testing of candidates have shown to be effective strategies to improve **EG** frost tolerance in Florida (Franklin and Meskimen, 1983; Meskimen *et al.*, 1987). A two-stage evalution process consisting of preliminary laboratory screening and

TABLE 2. Additive genetic and phenotypic correlations for RC and growth traits at age 30 months.

	r_A	r_p	P>r
Height	0.68	0.132	0.001
DBH	1.15	0.127	0.001
Volume	1.15	0.141	0.001

TABLE 3. Predicted genetic gains for RC over population mean for different selection strategies.

Selection strategy	Genetic gain (%)
Mass selection	2.9
54 best trees	2.4
180 best trees	
Combined selection	
10 best families (18 trees per family)	2.0
20 best families (9 trees per family)	2.2
60 best families (3 trees per family)	2.2
90 best families (2 trees per family)	2.1
Within family selection	
180 families (1 tree per family)	1.7

a later field test for vigor can be used to test new introductions or selections. A large number of plants assessed, resulting in high selection intensities, combined with an adequate exploitation of the fast generation turnover allowed by **EG**, can help to increase the gains per unit of time in the INIA population.

CONCLUSIONS

The results suggest need for a critical evaluation of all stages of the methods utilized in this study in order to improve the precision of the technique. After that, more accurate estimation of genetic parameters can be obtained by further tests with larger number of provenances and families. The results must also be verified throught field evaluation of natural freezes if the method is to be used as a tool to improve frost tolerance in the INIA population of **EG**.

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