

#### **ARTICLE**

# Selection of *Eucalyptus grandis* families across contrasting environmental conditions

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**Abstract:** The objective was to select productive and stable E. grandis families across contrasting sites in Brazil and Uruguay. Survival and growth of 130 open pollinated families were evaluated three years after planting. Survival ranged from 28 to 89% and mean annual increment from 20.5 to 48.4 m³ha² y¹. Low productivity in one site was attributed to unusually low rainfall and in the other, because of Cylindrocladium leaf disease. Heritability among families and the correlation family by environment were intermediate. Genetic pairwise correlations ranged from 0.03 to 0.81 across sites. The lowest genetic correlation between sites was observed where Cylindrocladium leaf disease occurs. Selection of top families across sites allowed selecting the best ones in each site. However, the effect of genotype—environment interactions was observed where climatic conditions are not adequate to the E. grandis, favoring the occurrence of Cylindrocladium disease.

**Keywords**: GEI, biotic and abiotic stress, productivity, adaptability, ecological zoning.

## **INTRODUCTION**

Eucalyptus grandis W. Hill ex Maiden (Flooded or Rose gum) is one of the most important species in tropical and subtropical environments. As a pure species or as parent of hybrid varieties, *E. grandis* can reach high productivity in suitable sites and produce timber with amenable properties to a wide range of uses (Denison and Kietzka 1993, Silva et al. 2013). *E. grandis* is among the group of "big nine" related species of subgenus *Symphyomyrtus* identified by Harwood (2011) that, along with its interspecific hybrids, account for around 90% of the world's eucalypt plantation resources across 90 countries (Silva et al. 2016a).

*E. grandis* has two contiguous occurrences with several disjunctions in its distribution in Australia. In the South, it occurs on coastal and sub-coastal ranges from near Bundaberg, Queensland-QLD (lat 25° 19′ S, long 151° 55′ E, and alt 130 m asl) to near Newcastle, New South Wales-NSW (lat 32° 55′ S, long 151° 46′ E, and alt 10 m asl). In the North, it occurs in QLD from the Windsor Tableland west of Daintree (lat 16° 15′ S, long 145° 24′ E, and alt 1100 m asl) to near Paluma (lat 18° 25′ S, long 146° 15′E, and alt 850 m asl) and extends down to altitudes of only 400 m around Copperlode Dam (around lat 16° 59′ S, long 145° 40′ E) on flat and lower slopes (Brooker and Kleinig 2004, Boland et al. 2006).

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<sup>6</sup> Universidade Estadual Paulista, 14.884-900, Jaboticabal, SP, Brazil E. grandis is an important species of commercial forest plantations and thus has been subject to genetic improvement programs for many decades in several countries, e.g. South Africa (van Wyk 1976), Australia (Burgess et al. 1996), Brazil (Ferreira 2015), Sri Lanka (Bandara and Arnold 2017) with improvements in generations by recurrent selection and open pollinated breeding. However, the species is susceptible to several diseases in some environmental conditions, namely Cryphonectria cubensis (eucalypt canker); Cylindrocladium leaf blight; and Astropuccinia psidii (eucalypt rust) (Conradie et al. 1992, Rodas et al. 2005, Silva et al. 2013, Alvares et al. 2016). Hybridization, particularly with E. urophylla and E. camaldulensis, has allowed the development of successful commercial plantation varieties in environments where E. grandis, as a pure species, is not well adapted (Brawner et al. 2011, Campoe et al. 2016).

Eucalypt breeding programs have focused on genotype selection through within and between species variability to ensure the best germplasm for plantation establishment (Gapare et al. 2003, Miranda et al. 2013, Bandara and Arnold 2017). Genetic variability is essential, not only for advancing genetic gain, but also as a buffer for various threats, such as pests, diseases and climate change (Jurskis 2005, Wingfield et al. 2008, Brawner et al. 2013).

In Brazil, Edmundo Navarro de Andrade introduced *E. grandis* at least a hundred years ago in Rio Claro Experimental Station; however, it was only in 1970 when provenance and recurrent selection of open pollinated seed orchard was intensified by various institutions. Provenances from around Coffs Harbour exhibited the greatest growth potential in the state of São Paulo, where subtropical conditions prevail (Ferreira 2015). However, Eldridge et al. (1993) reported that *E. grandis* from the Atherton region consistently display better disease tolerance than that of NSW provenances in tropical and subtropical environments.

The estimation of genetic parameters and breeding values are complex when calculated from trials on different sites, due to genotype by environment interactions (GEI). GEI evaluation allows breeders to identify genotypes performing well across the environment and select specific genotypes for each environment (Van Buijtenen 1992). GEI can occur as no crossover or crossover interaction (Acquaah 2007). If no crossover interactions are detected, then a single site may be used to select families for deployment across all sites. To handle heterogeneity of variances in environment correlations, evaluation needs to be properly carried out by a mixed model (Malosetti et al. 2013).

This study tests the selection of stable and productive *E. grandis* families, three years after planting, across divergent environmental conditions in South America.

#### **MATERIAL AND METHODS**

The study included up to 130 open-pollinated *E. grandis* families from five seed orchards (Table 1). The germplasm belongs to the Genetic Improvement Cooperative Program (PCMF) of Institute of Forestry Research and Studies (Instituto de Pesquisas e Estudos Florestais – IPEF). The trials were planted between May and December 2012 across four sites in Brazil and Uruguay (Figure 1).

The experimental layout was a randomized complete block design of four to six replicates with contiguous five-tree line plots planted across each of the four trials. The number of open pollinated families ranged from 114 to 130 per trial. As the trials were planted on land that belongs to forest companies (Figure 1), the routine tree stocking (spacing) and plantation management system of the company was used. Before planting, all sites were deep ripped to at least 45cm and lime were applied (total area). All sites were fertilized using the standard fertilization regime and the seedlings were

Table 1. Climatic conditions of seed sources, origin and number of breeding generations

Seed Source		Clima	itic Classificat	ion	Outsta	Company	Number of families	
266	ed Source	Rainfall (mm)	AAT (°C)	Koppen	Origin	Generation	Number of families	
1	Rio Claro-SP, BR	1294	20.3	Cwa	Unknown	SPA 1st	18	
2	Redenção da Serra and Itapetin- inga-SP, BR	1277 and 1175	18.3 and 18.9	Cfb and Cfa	Coffs Harbour	SSO 3 <sup>rd</sup>	12	
3	Tres Bocas-Rio Negro, UR	991	17.6	Cfa	Various	SSO 2 <sup>nd</sup> and 3 <sup>rd</sup>	16	
4	Concordia, AR	1278	18.6	Cfa	Various	SSO 2 <sup>nd</sup> and 3 <sup>rd</sup>	64	
5	Anhembi-SP, BR	1237	20.8	Aw/Cwa	Coffs Harbour	SSO 3 <sup>rd</sup>	22	

SPA = seed production area, SSO = seedling seed orchard, AAT = annual average temperature

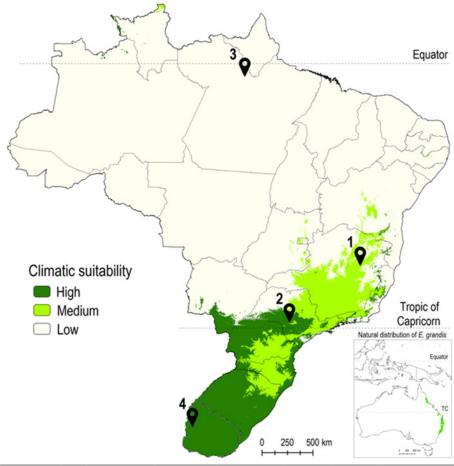
irrigated with 3 to 4 L tree<sup>-1</sup> at planting. Leaf cutter ant control (using baits) was applied and weeds were controlled by herbicide application until crown closure was reached.

Traits evaluated across all sites included survival, diameter at breast height (DBH), and height (H) of trees at 3.14 to 3.59 years after planting. DBH and H were used to calculate the mean annual increment (MAI), using a stem factor (f) of 0.45 and the total trial area (TA) for each experiment.

$$Vol_{ind} = \frac{\pi \cdot (DBH)^2 \cdot H \cdot f}{4}$$

$$MAI = \frac{\Sigma Vol_{ind}}{TA. age}$$

DBH data were analyzed separately for each site (individual analyses) and the joint analysis using a linear mixed model analysis, as described by Resende (2016).



Site – Location-State	Planting date	N	Evaluation Age	Stand density	Soil	Koppen	AAT (°C)	Rainfall (mm)	Alt (m)	Lat (S)	Long (W)
1- Itamarandiba-MG	Dec-12	117	3.14	1111	Red Ferralsols	Cwa	21.8	1100	910	17° 45′	42° 55′
2- Paulistânia - SP	May-12	130	3.17	1253	Red Ferralsols	Aw/Cwa*	21.9	1292	546	22° 29′	48° 15′
3- Monte Dourado -PA	Dec-12	114	3.15	1667	Yellow Ferralsols	Am	26.1	2637	100	08°89′	52° 60'
4- Paysandu- UR**	Nov-12	125	3.59	1667	Ferralsols	Cfa	18.4	1157	24	32° 10′	58° 05′

Figure 1. Map of the trials of the experimental network under distinct species suitability by climatic similarity with origin (adapted from Flores et al. 2016). N: number of open pollinated families; R: replication; AAT: annual average temperature; \* climate transition area; \*\*Uruguay; Am: Tropical monsoon; Aw: Tropical with dry winter; Cfa: Humid Subtropical with hot summer; and Cwa: Humid subtropical with dry winters and hot summers.

Individual analyses: y = Xr + Za + Wp + Ts + e,

Where the vectors are: y – phenotypic observation; r - replicate (assumed as fixed) added to the general average; other vectors assumed as random with a – additive genetic effect; p – plot effect; s – seed source effects; and e - error or residuals.

Inclusion of seed source to the models did not improve genetic parameter estimates and was therefore excluded from the joint analysis.

Joint analysis: y = Xr + Zg + Wp + Ti + e,

Where the vectors are: y = phenotypic observation; r = replicate of all locations (assumed as fixed) added to the general average; other vectors assumed as random with <math>g = genotypic effects; p = plot effects; i = family x environment interaction; and e = error or residuals.

As effects are associated to all assessment traits, unstructured variance—covariance matrices were estimated for each effect: X, Z, W and T.

A deviance analysis was performed using the likelihood ratio test of family survival and DBH to check the significance of random effects. Variance component and genetic parameters were estimated using the REML/BLUP method.

The Harmonic Mean of Relative Performance of Genetic Values - HMRPGV (Spinelli et al. 2015, Resende 2016) was used to estimate stability and adaptability of DBH data. Stability corresponds to Harmonic Mean, which penalizes treatments with greater variance and adaptability refers to Relative Performance of family effects within each site, considering the mean of each trial. An advantage of the HMRPGV method is that it enables unbalanced datasets to be analyzed, where the lack of treatments (families) in one of the experiments does not require excluding that treatment from the joint analysis (Resende 2007).

The average coefficient of relatedness was considered to estimate genetic parameters in a mixed mating system, rather than half-sibs, as described in Tambarussi et al. (2018). An average coefficient of 0.16 self-fertilization was considered, according to Miranda et al. (2013).

#### **RESULTS AND DISCUSSION**

In this experimental network, we observed different productivity across the sites and Paulistânia-SP (MAI =  $48.4 \,\mathrm{m}^3\,\mathrm{ha}^{-1}$  y<sup>-1</sup>) as two-fold more productive than Itamarandiba-MG and Monte Dourado-PA. There was a three-fold range in survival (28% at Monte Dourado-PA to 89% in Paulistânia-SP-Table 2). Paulistânia-SP has Aw/Cwa climatic conditions (Koppen classification), considered optimal for *E. grandis* production (Garcia et al. 2014, Flores et al. 2016). The least productive

**Table 2.** Survival, height (H), diameter at breast height (DBH) and narrow sense DBH heritability (DBH h²<sub>a</sub>) for each trial and genetic parameters in the joint analysis for five *E. grandis* family trials for DBH

Ct.	Survival	Н	DBH	MAI	<b>DBH</b> h <sup>2</sup> <sub>a</sub>	
Site	(%)	(m)	(cm)	(m³ ha-1 y-1)		
Individual analyses						
1- Itamarandiba	85	14.7	10.4	20.5	0.39+-0.05	
2- Paulistânia	89	18.0	13.1	48.4	0.19+-0.04	
3- Monte Dourado	28	15.1	11.0	20.6	0.25 +-0.09	
4- Paysandu	65	16.8	9.1	41.1	0.26+-0.05	
Joint analysis for DBH						
Deviance (Likelihood Ratio Test)					29675**	
Narrow sense heritability					0.07 +/- 0.01	
Coefficient of determination for G x E					0.08	
Average heritability among families					0.70	
Family correlation for G x E					0.46	
Coefficient of individual family variation					5.94	

For survival: no significate effect (deviance) was detected at 1% confidence level.

sites were Itamarandiba-MG and Monte Dourado-PA for distinct reasons. The climate in Itamarandiba is Cwa (Koppen classification) and should have better conditions than Monte Dourado-PA (Am Koppen classification) for *E. grandis* growth (Figure 1). However, the rainfall in Itamarandiba during the trial period was nearly half of the historical average (2014 and 2015 rainfall < 600 mm), resulting in moderate and prolonged water stress, known to reduce eucalypt productivity (Silva et al. 2016b, Gonçalves et al. 2017). On the other hand, in Monte Dourado, the disease *Cylindrocladium* leaf spot was observed. This disease resulted in high mortality of trees (72%), which decreased productivity of the stand. This corroborates results of Rodas et al. (2005) that when *E. grandis* is grown as a pure species, it is not well adapted to the Am climatic condition in Monte Dourado, where high temperatures and humidity favor *Cylindrocladium* leaf disease. In this site, the disease attacks the trees since the first summer (wet and hot) and probably is the main mortality cause of susceptible material in the climatic conditions of the site, as discussed by Silva et al. (2019).

Even with high mortality, it was still possible to find some good trees in Monte Dourado-PA. More resources available (i.e. nutrients, water, and light) favored the remaining trees because of lower inter tree competition, which leveraged their growth (Wilson and Tilman 1993, Boyden et al. 2008). Therefore, some individuals from families 248 and 290, stood out from other trees and families in the site. Both families were well ranked in Harmonic Mean of Relative Performance of Genetic Values in the joint analysis (Family 248 is the 12<sup>th</sup> and 290 is the 11<sup>th</sup>) having the largest trees of all sites. Based on their good overall performance and growth of some individuals under conditions favorable to *Cylindrocladium* leaf disease, both families may be the best option for hybridization (Denison and Kietzka 1993, Potts and Dungey 2004) with other species (e.g. *E. pellita*) for the production of elite hybrid trees for sites that are favorable to the disease. The hybridization program should result in the development of new genetic combinations that could improve biotic tolerance and improve stand productivity after adequate selection (Assis et al. 2005).

The selection of top 12 families (<10 % of families) by the Harmonic Mean of Relative Performance of Genetic Values (HMRPVG) in the joint analysis across all sites resulted in a cohort of families that included at least four of the top ranked families in each site. The smallest number of top families was in Paysandu-UR, where three of the best families were not evaluated and the others were closely ranked. The largest number was observed in Itamarandiba-MG, where seven were in the 12 top families (Table 3) and the two other families were ranked 13<sup>th</sup> and 14<sup>th</sup>. Some of the best families identified in HMRPVG were not planted across all sites due to seedling availability. In addition, few top families showed

**Table 3.** DBH ranking of stability and adaptability of top 12 *E. gra*ndis families based on Harmonic Mean of Relative Performance of Genetic Values across all sites and the actual family rank in each site; DBH Pairwise site coefficient of determination for GEI ( $c_i^2$  – above diagonal) and pairwise family genotype correlation ( $r_a$  – below diagonal) for *Eucalyptus grandis* 

Family	Stability and adaptability	Adaptability	Stability	ItamarandibaMG	Paulistânia SP	M Dourado PA	Paysandu UR
188	1	1	1	1	3	NI	1
207	2	2	13	3	8	6	NI
244	3	3	3	5	6	2	15
241	4	4	9	8	1	25	4
189	5	5	2	2	5	24	2
206	6	6	10	11	15	4	13
226	7	7	11	14	43	3	19
233	8	8	15	31	59	1	14
287	9	10	14	10	9	15	16
300	10	9	35	48	12	8	NI
290	11	11	47	43	13	16	NI
248	12	12	21	13	31	31	6

Site	Itamarandiba	Paulistânia	Monte Dourado	Paysandu	Average
Itamarandiba		0.029	0.219	0.035	0.094
Paulistânia	0.81		0.133	0.033	0.065
Monte Dourado	0.04	0.03		0.084	0.145
Paysandu	0.77	0.72	0.24		0.050
Average	0.54	0.52	0.10	0.58	

 $\mbox{NI}$  –  $\mbox{Not}$  included due to problems in seedling production.

poor rankings in specific sites. For example, family 233 is within the top 8<sup>th</sup> families in the joint analysis; however, it displayed a different behavior from the other top performing families selected in joint analysis. Family 233 displayed a complex GEI with good adaptability (ranked 8<sup>th</sup>); however, with not so good stability (ranked 15<sup>th</sup>). This was attributed to the poor performance of this family in Itamarandiba-MG (ranked 31<sup>st</sup>) and Paulistânia-SP (ranked 59<sup>th</sup>), to the excellent performance in Monte Dourado-PA (ranked 1<sup>st</sup>) and intermediary performance in Paysandu-UR (ranked 14<sup>th</sup>). These different behaviors may be attributed to the tolerance of the family to *Cylindrocladium* leaf spot.

In the individual and joint deviance analyses, no significant effects and no family correlation between sites was observed for survival across the four sites. On the other hand, DBH heritability between families and family by environment correlation were 0.70 and 0.46, respectively, indicating strong genetic control with families and moderate control for productivity across site stability.

The absence of significant differences between seed sources, although selected under different environmental conditions in Argentina, Brazil and Uruguay, could in part could be explained due to greater variation within than between seed sources. Other authors (Marcó and White 2002, Harrand et al. 2009) have reported this variation within the seed source. However, the seedlots were obtained from mother trees selected from breeding populations grown under subtropical conditions, where provenances from the Coffs Harbor region generally exhibit better performance (Ferreira 2015). Although they are from different breeding programs, all seed sources may be genetically similar (all Coffs Harbour derived). If other provenances (e.g. Atherton Tableland sources) of *E. grandis* were tested, more interprovenance variation would be expected (Eldridge et al. 1993, Bandara and Arnold 2017). Another issue is the outcrossing level between seed sources. Burgess et al. (1996) conducted a study on *E. grandis* in Coffs Harbour and observed that outcrossing levels strongly influenced growth traits and differences between provenances were not significant.

DBH heritability within each site range from 0.19 to 0.39 is considered intermediate for this growth character. This range is similar to improved *E. urophylla* families evaluated across ten sites in Brazil (Silva et al. 2019), with a range from 0.09 to 0.29, considering mixed mating. The highest DBH heritability was obtained in Itamarandiba-MG, indicating lower environmental effects on phenotypic variation. This may be attributed to moderate and prolonged water stress that occurred in this site during the trial.

The narrow sense heritability in the joint analysis was low, due to the large environmental variation across the sites. The intermediary genetic correlations across sites and the results of the sites pairwise correlation indicated that simple GEI were occurring between three trials; however, one site (Monte Dourado-PA) showed a low coefficient of determination for GEI (Table 3). The lowest pairwise genetic correlation between sites was found in Monte Dourado-PA, an area of Am climatic conditions. Monte Dourado-PA showed a coefficient of determination lower than 0.05 in pairwise with Itamarandiba-MG and Paulistânia-SP. However, despite complex GEI, the selection of some families for all sites was possible. Similar to the findings for *Eucalyptus urophylla*, which was planted across five sites in Brazil, Pupin et al. (2015) found for *E. urophylla* that it was possible to select families in a site with intermediate climatic condition, which showed the highest genetic correlation with the other trials.

This suggests even that a broadly based breeding program could be used to select elite families for deployment across all of the environments tested. Genetic by environmental interaction generates a strong effect on specific sites and stratification of environments is necessary. In addition, the lack of genetic variation between the five sources for growth and survival traits may imply that infusions of *E. grandis* material from other populations (e.g. Atherton) could be beneficial to future development of this species.

## **CONCLUSION**

Few families were the most productive and stable across the sites with contrasting environments in Uruguay and Brazil. These families were selected due to lower GEI, where the families selected were the most adaptable and stable.

Selection of elite families in intermediate climatic growing conditions allows selection of a suitable germplasm across the diverse range of climatic conditions evaluated. Nevertheless, attention should also be given to families that perform well in specific climatic or site conditions to ensure the use of opportunities to select species for pest and disease or adaptation to facilitate genetic gain in the improvement of pure species and development of new hybrid clones.

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