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ARTICLE

Selecting for stable and productive families of *Eucalyptus urophylla* across a country-wide range of climates in Brazil

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Abstract: To identify stable and productive *Eucalyptus urophylla* S.T. Blake families across diverse climate zones in Brazil, we evaluated growth and survival of 322 open-pollinated families derived from 13 genetically improved seed sources in 10 trials across the country. Survival and growth data were analyzed using linear mixed models and REML/BLUP. Survival ranged from 51% to 92%, and the mean annual increment varied from 19 to 46 $m^3 \cdot ha^{-1} \cdot year^{-1}$. Although planted in suitable climatic zones, some trials had low survival and (or) productivity. Conversely, the highest productivity was recorded in a zone considered to be of low suitability. These results show the importance of assessing the climatic requirements of eucalypts beyond those determined from analyses of their natural distribution, especially when testing already improved seed sources. A number of productive and stable families were identified based on analysis of the interaction between genotype and environment, and from these, 144 individuals were selected and had their genetic diversity estimated using 19 microsatellite DNA markers. The genetic diversity of these selected trees was equivalent to that observed in previous studies of natural populations of *E. urophylla*, indicating that breeding programs of *E. urophylla* in Brazil still retain high levels of diversity for sustainable genetic gains.

Key words: genotype–environment interaction, productivity and stability, family selection, genetic diversity, abiotic and biotic stress.

Résumé : De façon à identifier des familles d'*Eucalyptus urophylla* S.T. Blake stables et productives pour diverses zones climatiques du Brésil, nous avons évalué la croissance et la survie de 322 familles à pollinisation libre provenant de 13 sources de semences génétiquement améliorées déployées dans 10 stations réparties dans tout le pays. Les données de survie et de croissance ont été analysées à l'aide de modèles linéaires mixtes et d'approches par le maximum de vraisemblance restreinte et par le meilleur prédicteur linéaire non biaisé. La survie variait de 51 à 92 % et l'accroissement annuel moyen de 19 à 46 $m^3 \cdot ha^{-1} \cdot an^{-1}$. Bien que les familles aient été plantées dans des zones climatiques favorables, la survie ou la productivité étaient faibles dans certaines stations. À l'inverse, la plus forte productivité a été observée dans une zone considérée comme étant peu favorable. Ces résultats montrent l'importance d'évaluer les exigences climatiques des eucalyptus au-delà de celles qui sont déterminées par l'analyse de leur répartition naturelle, surtout lorsqu'on teste des sources de semences déjà améliorées. Un certain nombre de familles productives et stables ont été identifiées sur la base de l'analyse de l'interaction entre le génotype et l'environnement. Parmi celles-ci, 144 individus ont été sélectionnés pour estimer leur diversité génétique à l'aide de 19 marqueurs d'ADN microsatellites. La diversité génétique de ces arbres sélectionnés était équivalente à celle observée lors d'études précédentes portant sur les populations naturelles d'*E. urophylla*, ce qui indique que les programmes de sélection d'*E. urophylla* au Brésil conservent toujours des niveaux élevés de diversité pour des gains génétiques durables. [Traduit par la Rédaction]

Mots-clés : interaction entre la génétique et l'environnement, productivité et stabilité, sélection de familles, diversité génétique, stress biotiques et abiotiques.

Introduction

Eucalyptus urophylla S.T. Blake (Timor mountain gum) is one of the most economically important forest trees in the world, planted both as a pure species and in hybrid combinations (Denison and Kietzka 1993; Potts and Dungey 2004; Harwood 2011). *Eucalyptus urophylla* is native to some of the Sunda Islands in Indonesia (Flores et al. 2016) and was introduced into Brazil in the 1970s and 1980s. In hybrid combinations, it provided a solution to

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Table 1. *Eucalyptus urophylla* seed sources used to establish the two experimental networks of field trials.

Seed source	Families	Origin	Level of breeding
Experimental network 1			
Turmalina and Timóteo, MG	45	Flores and Timor	SSO 2nd to 4th generation
Ipatinga, MG	50	Flores and Timor	SSO 2nd and 3rd generation
Avaré, SP	14	Flores and Timor	SSO 2nd and 3rd generation
Lençóis Paulista, SP	15	Remexio (East Timor)	SSO 2nd and 3rd generation
Altinópolis, SP	13	Flores and Timor	SSO 3rd generation
Itamarandiba, MG	15	Flores	SSO 2nd generation
Teixeira de Freitas, BA	15	Timor	SPA 1st generation
Experimental network 2			
Multi (Almeirim, PA)	16	Flores and Timor	SSO 2nd and 3rd generation
Mexico	29	Multi	SSO 1st generation
Três Marias, MG	29	Flores and Timor	SSO 2nd and 3rd generation
Eunapolis, BA	19	Flores and Timor	SSO 2nd and 3rd generation
Rio Claro, SP	18	Unknown	SPA 2nd generation
Anhembi, SP	44	Flores	SSO 2nd generation

Note: Brazilian states: MG, Minas Gerais; SP, São Paulo; BA, Bahia; PA, Para; SSO, seedling seed orchard; SPA, seed production area.

the *Cryphonectria cubensis* canker susceptibility of *Eucalyptus grandis*, the main species planted in the country (Ferreira 2015). Additionally, *E. urophylla* also contributed to improving coppicing ability, rust (*Austropuccinia psidii*) tolerance, rooting ability, drought tolerance, and wood density (Rocha et al. 2006). The hybrid *E. urophylla* × *E. grandis*, associating stress tolerance of *E. urophylla* to the fast growth of *E. grandis*, currently makes up the vast majority of commercial plantations in Brazil (Gonçalves et al. 2013).

Climate similarity analysis between the natural distribution of the species in its center of origin and candidate regions for trials and plantation worldwide has long been used for agro-ecological zoning of species of *Eucalyptus* (Booth and Pryor 1991; Jovanovic et al. 2000; Brawner et al. 2013; Garcia et al. 2014; Booth et al. 2017). Likewise, the Köppen climate types have been used to define agroclimatic zones (Maes et al. 2009; Gonçalves et al. 2013). Environmental conditions are directly linked to biotic and abiotic stresses, which cause significant impact on forest productivity (Brawner et al. 2011; Silva et al. 2013; Campoe et al. 2016; Binkley et al. 2017) and ultimately affect the selection of elite genotypes (Oliveira et al. 2018).

Besides the more general predictions of adaptability based on broad climate similarity, variation in environmental conditions at a more local scale results in different patterns in genotype–environment interaction (GEI). Scale-effect interactions happen when the relative performance of genotypes differ across environments, while rank-change interactions involve the change of relative rankings of genotypes in different environments (White et al. 2007). The estimation of genetic parameters and breeding values become more difficult when trials are located in different environments because of the GEI that needs to be accounted for (Zobel and Talbert 1984). When properly evaluated, the GEI allows the identification of genotypes (individuals or families) with good performance across a climatic gradient (ecological taxa zoning) and (or) select genotypes tailored to specific environments (van Buijtenen 1992; Hernández et al. 2009).

The high selection intensity applied to identify superior individual trees inevitably tends to decrease genetic diversity (White et al. 2007). On the other hand, managing the amount of genetic variability available in a breeding program is a key issue for sustainably advancing genetic gains, especially in light of the emergence of new pests and diseases and the increasing impact of climate fluctuations (Jurskis 2005; Wingfield et al. 2008; Brawner et al. 2013; Garcia et al. 2014). Genetic marker data have been increasingly used as a valuable tool to monitor the genetic diversity available and the actual relatedness of tree breeding populations, especially when population advancement is carried out by open pollination (Gaiotto et al. 1997; Grattapaglia et al. 2004).

In this study, we wanted to answer the following four questions. (*i*) Is it possible to identify stable and productive *Eucalyptus urophylla* families across a wide range of Brazilian environmental conditions? (*ii*) What is the magnitude of the GEI at the family level? (*iii*) Should environmental stratification be used to optimize family selection? (*iv*) What has been the impact of selective breeding in *E. urophylla* on the levels of genetic diversity when compared with natural populations in their native range?

Materials and methods

Genetic material, experimental networks, and design

The genetic material involved a total of 322 open-pollinated families from 13 seed sources. These seed sources correspond to genetically improved seeds developed by the breeding programs of forest-based companies that participate in the Genetic Improvement Cooperative Program (PCMF) of the Institute of Forestry Research and Studies (Instituto de Pesquisas e Estudos Florestais (IPEF)). These seeds were derived mostly from second- and third-generation seedling seed orchards, while some were derived from first-generation selections in seed production areas in different regions in Brazil (Table 1). The open-pollinated families were deployed in two experimental networks (EN), with EN1 established in 2009–2010 and EN2 established in 2012–2013. Each network included five trial sites in the land properties of the different forest-based companies across Brazil covering variably suitable climates for *Eucalyptus urophylla* (Fig. 1). Climate suitability was defined based on ecological zoning for *E. urophylla* in Brazil proposed by Flores et al. (2016), who used the Köppen climate classification and similarity of the Brazilian climates with the climates of the natural distribution of the species in its center of origin. Flores et al. (2016) used the Global Biodiversity Information Facility database (Yesson et al. 2007), from which 32 sites were found corresponding to the natural range distribution of *E. urophylla*. The climates sampled in the experimental networks were as follows: Af, tropical without dry season; Am, tropical monsoon; Aw, tropical with dry winter; Cwa, humid subtropical with dry winter and hot summer; Cfa, humid subtropical oceanic climate without dry season and with hot summer; and Cfb, humid subtropical oceanic climate without dry season and with temperate summer. The field trials were established using a randomized block design, with linear plots of six trees and four to six replications. The number of families in each trial ranged from 118 to

Fig. 1. Geographic location of trial sites for the two experimental networks EN1 and EN2 and their respective classification in terms of climate suitability (Flores et al. 2016) for *Eucalyptus urophylla*. [Colour version available online.]

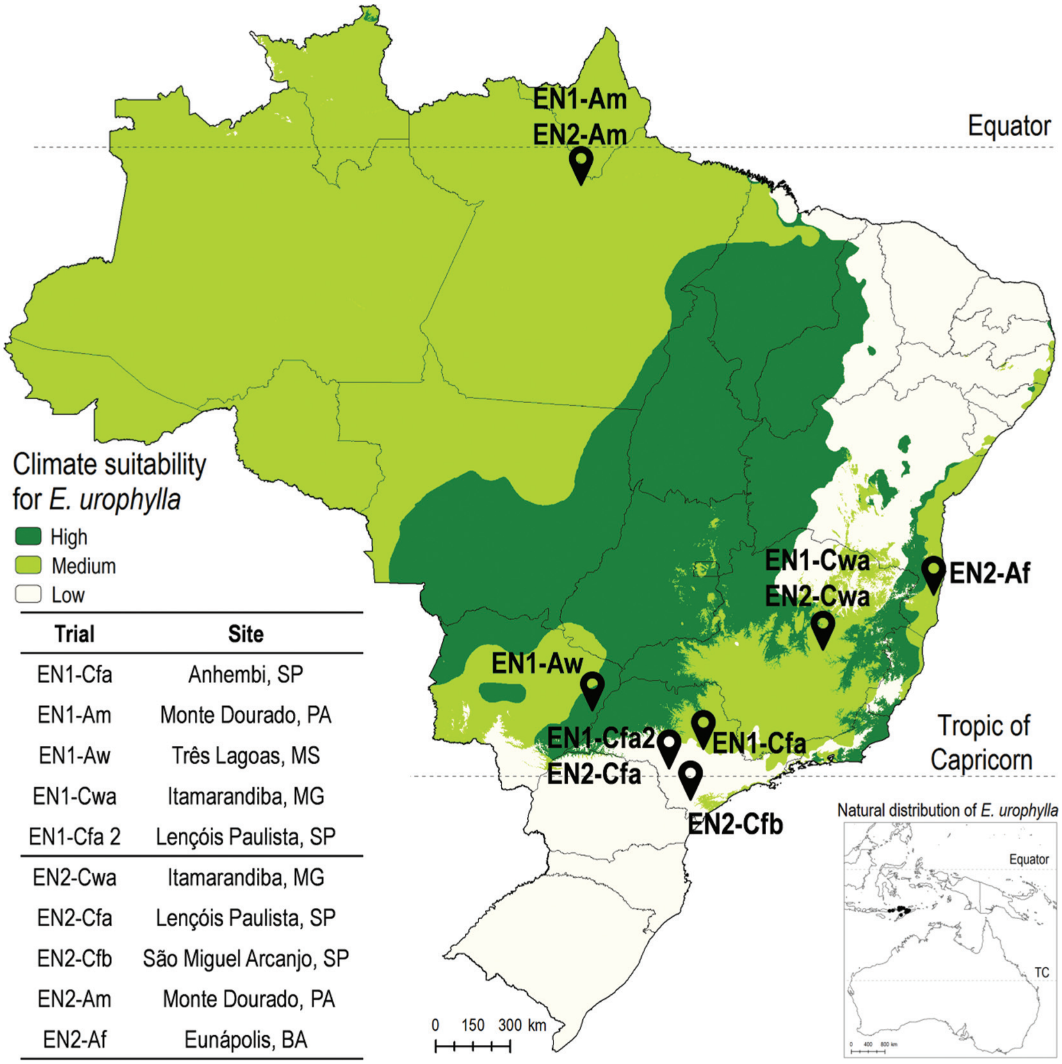

Table 2. Location of the trial sites and respective climate characteristics.

Trial	Municipality	Date planted (month/year)	Density	No. of families	Köppen	Temp (°C)	Rainfall (mm)	Elevation (m)	Latitude (S)	Longitude (W)
EN1-Cfa	Anhembi, SP	12/2009	1667	166	Cfa	22.3	1307	480	22°28′	48°04′
EN1-Am	Monte Dourado, PA	03/2010	1667	163	Am	26.1	2637	100	08°89′	52°60′
EN1-Aw	Três Lagoas, MS	03/2010	1116	166	Aw	24.2	1240	324	20°45′	51°40′
EN1-Cwa	Itamarandiba, MG	10/2009	1111	166	Cwa	22.6	1100	910	17°45′	42°46′
EN1-Cfa 2	Lençóis Paulista, SP	11/2009	1667	122	Cfa	19.7	1370	827	23°13′	48°34′
EN2-Cwa	Itamarandiba, MG	12/2012	1111	130	Cwa	22.6	1100	910	17°45′	42°46′
EN2-Cfa	Lençóis Paulista, SP	01/2013	1253	135	Cfa	19.7	1370	827	23°13′	48°34′
EN2-Cfb	São Miguel Arcanjo, SP	01/2013	1515	118	Cfb	20.4	1398	659	23°59′	48°03′
EN2-Am	Monte Dourado, PA	04/2013	1667	132	Am	26.1	2637	100	8°89′	52°60′
EN2-Af	Eunápolis, BA	08/2013	833	138	Af	23.8	1165	183	16°37′	39°58′

Note: Density, number of trees per hectare; Temp, mean annual temperature. Climate description: Af, tropical without dry season; Am, tropical monsoon; Aw, tropical with dry winter; Cwa, humid subtropical with dry winter and hot summer; Cfa, humid subtropical oceanic climate without dry season and with hot summer; Cfb, humid subtropical oceanic climate without dry season and with temperate summer. Köppen climate classification according to Alvares et al. (2013).

166 (Table 2). Site preparation involved soil ripping at a depth of >0.45 m with mineral fertilization using the site-specific optimized fertilization regime. Seedlings were irrigated with 3 to 4 L·tree^{-1} at planting. Leaf cutter ant control was applied using baits, and weeds were controlled by herbicide application until crown closure (12 months after planting).

Phenotypic measurements and data analysis

Survival, diameter at breast height (DBH), and height of all trees were evaluated three years after planting. These parameters were used to calculate stand survival (%) and mean annual increment (MAI) of volume growth. Biotic and abiotic stresses that could cause damage to the trials were also monitored. A deviance analysis was carried out using the maximum likelihood ratio test (LRT) to evaluate the significance of model effects. Based on the results of the deviance analysis and using DBH as the best proxy for growth, family adaptability and stability were estimated. Estimates of variance components and genetic parameters were obtained by the restricted maximum likelihood and best linear unbiased prediction (REML/BLUP). A linear mixed model analysis was used to analyze genotypes (families) across environments, allowing for the evaluation of data even with heterogeneity of variances (Malosetti et al. 2013).

DBH data were analyzed both separately for each trial site (individual analyses) and jointly, considering all sites, for each EN, using a linear mixed model analysis as described by Resende (2007). To assess adaptability and stability, the harmonic mean of relative performance of genetic values (HMRPGV) was used. Stability is the harmonic mean of genotypic values (HMGV), and adaptability is the relative performance (RPGV) of families in each trial (Resende 2016). The analysis enables one to work with unbalanced datasets, where the lack of a treatment in one of the experiments does not generate the exclusion of that treatment from the joint analysis. The following linear models were used in the analyses:

Individual analysis by trial site:

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{r} + \mathbf{Z}\boldsymbol{a} + \mathbf{W}\boldsymbol{pl} + \mathbf{T}\boldsymbol{s} + \boldsymbol{e}$$

Joint analyses (all sites):

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{r} + \mathbf{Z}\boldsymbol{a} + \mathbf{W}\boldsymbol{pl} + \mathbf{T}\boldsymbol{s} + \mathbf{Q}\boldsymbol{i} + \boldsymbol{e}$$

where the vectors are $\boldsymbol{y}$ (phenotypic observation) and $\boldsymbol{r}$ (replication, assumed as fixed) added to the mean; other vectors, $\boldsymbol{a}$ (additive genetic effect), $\boldsymbol{pl}$ (plot effect), $\boldsymbol{s}$ (seed source effect), $\boldsymbol{i}$ (genotype (family) × environment interaction), and $\boldsymbol{e}$ (errors or residuals), were assumed random. **X**, **Z**, **W**, **Q**, and **T** are the incidence matrices.

Narrow-sense heritabilities were estimated according to the following equations:

Individual analysis by site:

$$h_a^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_{pl}^2 + \hat{\sigma}_s^2 + \hat{\sigma}_e^2}$$

Joint analysis:

$$h_A^2 = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_A^2 + \hat{\sigma}_{Pl}^2 + \hat{\sigma}_S^2 + \hat{\sigma}_i^2 + \hat{\sigma}_E^2}$$

Estimates were obtained assuming the open-pollinated families as half-sibs or, alternatively, containing different levels of relatedness, considering a mixed mating system as described in Resende et al. (1995) and Tambarussi et al. (2018).

We estimated the additive genetic variance and narrow-sense heritability considering the coefficient $\frac{(1 + S)^2}{4}$ where self-fertilization (S) = $\frac{2F}{(1 + F)}$, instead of 0.25, the average coefficient of relatedness when considering half-sibs. The estimate of inbreeding, F, obtained from a genetic marker analysis using 19 microsatellite markers (see below), was used to calculate the coefficient used in the heritability estimates.

Genotypic correlations and coefficients of determination were estimated by the following expressions:

Genotypic correlation between family performance and sites:

$$r_{\text{g×site}} = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \hat{\sigma}_i^2}$$

Coefficient determination of the effects of family × site interaction:

$$c_i^2 = \frac{\hat{\sigma}_i^2}{\hat{\sigma}_A^2 + \hat{\sigma}_{Pl}^2 + \hat{\sigma}_S^2 + \hat{\sigma}_i^2 + \hat{\sigma}_E^2}$$

Coefficient determination of the effects of seed source:

$$c_s^2 = \frac{\hat{\sigma}_S^2}{\hat{\sigma}_A^2 + \hat{\sigma}_{Pl}^2 + \hat{\sigma}_S^2 + \hat{\sigma}_i^2 + \hat{\sigma}_E^2}$$

where $\hat{\sigma}_a^2$ and $\hat{\sigma}_A^2$ represent the additive genetic variance by site and in the joint analysis, respectively; $\hat{\sigma}_{pl}^2$ and $\hat{\sigma}_{Pl}^2$ represent the environmental variance between plots by site and in the joint analysis, respectively; $\hat{\sigma}_s^2$ and $\hat{\sigma}_S^2$ represent the genotypic variance between seed source by site and in the joint analysis, respectively; $\hat{\sigma}_e^2$ and $\hat{\sigma}_E^2$ represent the residual variance by site and in the joint analysis, respectively; $\hat{\sigma}_G^2$ represents the genotypic variance in the joint analysis; and $\hat{\sigma}_i^2$ represents the family × environment interaction variance.

Finally, family stability and adaptability were estimated as follows:

Stability:

$$\text{HMGV}_i = \frac{1}{\sum_{l=1}^{L} \frac{1}{\text{GV}_{il}}}$$

Adaptability:

$$\text{RPGV}_i = \frac{1}{l}\left(\frac{\sum_{l=1}^{L} \text{GV}_{il}}{M_l}\right)$$

Stability and adaptability, the harmonic mean of relative performance of genetic values:

$$\text{HMRPGV}_i = \frac{1}{\sum_{l=1}^{L} \frac{1}{\text{RPGV}_{il}}}$$

where l is the number of sites, GV is the genetic value, i is the genotype, and M_l is the mean of site l. The site pairwise correlation, i.e., the correlation of the estimated genotypic values, estimated by BLUP, was performed pairwise family by family between the two experiments.

DNA marker analyses

Following phenotypic data analyses, 79 individuals from EN1 and 65 from EN2 were selected, considering (*i*) family performance in the joint analysis (top families), (*ii*) the best 5% top trees of the local selection (BLUP), (*iii*) up to four individuals per family, and (*iv*) visual assessment of form, health, and growth in the field. Fresh leaves were harvested from these selected trees in the EN1-Cfa and EN2-Cwa trials. DNA extraction and microsatellite marker amplification and fluorescent detection analysis in an ABI 3100XL

Table 3. Survival, mean annual increment (MAI), narrow-sense individual heritability for DBH (h_a^2, considering half-sibs and mixed mating) and seed source coefficient (c_s^2) in *Eucalyptus urophylla* in the two experimental networks (EN) of trials.

	Survival (%)	MAI ($m^3 \cdot ha^{-1} \cdot year^{-1}$)	h_a^2 (half-sibs)	h_a^2 (mixed mating)	c_s^2
EN1					
EN1-Cfa	86	36	0.09±0.03	0.07±0.02	0.02
EN1-Am	54	20	0.21±0.06	0.16±0.04	0.04
EN1-Aw	91	28	0.25±0.05	0.19±0.04	0.03
EN1-Cwa	89	29	0.18±0.04	0.13±0.03	0.10
EN1-Cfa 2	88	21	0.29±0.05	0.21±0.03	0.02
EN2					
EN2-Cfa	92	43	0.32±0.06	0.19±0.03	0.06
EN2-Am	51	19	0.26±0.07	0.17±0.05	0.06
EN2-Af	64	22	0.29±0.06	0.15±0.03	0.05
EN2-Cwa	90	26	0.39±0.05	0.26±0.04	0.05
EN2-Cfb	89	46	0.15±0.05	0.10±0.04	0.03

Note: Cfa, Am, Aw, Cwa, Cfa 2, Af, and Cfb — Köppen climate classification according to Alvares et al. (2013).

automatic sequencer were carried out as described earlier (Faria et al. 2010, 2011). Nineteen microsatellite markers described by Grattapaglia et al. (2015) were used (EMBRA2; EMBRA3; EMBRA10; EMBRA11; EMBRA12; EMBRA21; EMBRA28; EMBRA32; EMBRA38; EMBRA45; EMBRA63; EMBRA128; EMBRA157; EMBRA204; EMBRA210; EMBRA681; EMBRA915; EMBRA1144; EMBRA1349). Average number of alleles per locus (*A*), number of private alleles (Pa), observed heterozygosity (Ho), expected heterozygosity (He), and Wright's fixation index (*F*) were estimated using GenALEx (Peakall and Smouse 2006). To evaluate the genetic diversity retained in this set of selected individuals from the improved seed sources, these diversity measures were compared with the same ones published for 12 microsatellites for geographically defined *E. urophylla* natural populations in its center of origin (Payn et al. 2008). To allow bona fide comparisons of genetic diversity for equivalent sets of markers, we took the averages of 100 random samples without replacement of these estimates for subsets of 12 markers out of the 19 genotyped.

Results

Overall tree survival and growth of the improved seed sources diverged from the expectations based on the suitability of the climatic similarity map. Survival ranged from 51% to 92%, with the best survival rates observed in the trials established in the southeastern Cfa and southwestern Aw climatic zones and the worst in the trials in the northernmost Am climatic conditions (Table 3). The highest MAI was recorded in trial EN2-Cfb, being twice as large as in some other trials. Family narrow-sense heritability in each trial ranged from 0.09 to 0.39 and 0.07 to 0.26 when considering open-pollinated families as half-sibs or mixed mating, respectively. The lowest heritabilities were found in trial EN1-Cfa and the highest in EN2-Cwa (Table 3).

Narrow-sense individual heritability was low (0.06–0.09 for the mixed mating model) in both ENs. The genotypic correlation in the joint analyses for GEI was similar for both ENs. The GEI variance at the family level corresponded to 30%–45% of the genotypic variance (Table 4).

The pairwise analysis between family genetic values showed high genetic correlations in the EN1. The trial EN1-Cfa had the highest average genetic correlation (0.87). A lower site pairwise genetic correlation was observed in the EN2. Trial EN2-Cfa showed good correlation with EN2-Cwa and EN2-Cfb but poor correlation with EN2-Am and EN2-Af, consistent with the widely different climatic zones (Table 5).

Selection of the top 15 families (<10% of the families for EN1, and 11.5% of the families for EN2) by the harmonic mean of relative performance of genetic values across all sites allowed selecting a larger number of productive and stable families in various locations in EN1 when compared with EN2 where a striking rank-change GEI was observed for seed source Multi developed in the almost equatorial climate of Almeirim, state of Pará. This seed source showed a poor performance in climates Cwa, Cfa, and Cfb but was the best seed source in the northernmost trials in the Am and Af climates (Table 6).

Total number of alleles (*k*) observed in the sets of selected individuals in EN1 and EN2 were 293 and 268, respectively (Table 7). The proportion of private alleles was higher in set EN1 (67 or 22% of the total number of alleles) than EN2 (39 or 15% of the total number of alleles). The observed (0.77 and 0.74) and expected (0.86 and 0.85) heterozygosities were essentially equivalent in the two selected sets, and the estimates of the fixation index (*F*) were 0.09 and 0.14, respectively. The average diversity measures of the selected sets of individuals based on downsized data sets of 100 random samples of 12 out of the 19 markers genotyped were essentially the same as those for the full dataset of 19 markers. The diversity of the EN1 and EN2 selected sets was slightly higher (7%–15%) than the diversity reported for a natural population by Payn et al. (2008) (Table 7).

Discussion

Previous studies with unimproved *Eucalyptus urophylla* seed sources reported survival rates ranging from 68% to 90.4% and productivity ranging from 10.9 to 20.5 $m^3 \cdot ha^{-1} \cdot year^{-1}$ three years after planting in trial networks across different countries (Hodge and Dvorak 2015). In our study, the *E. urophylla* improved seed sources showed a higher variation in survival and productivity when tested in variable climatic zones in Brazil. Survival ranged from 51% to 92% and MAI ranged from 19 to 46 $m^3 \cdot ha^{-1} \cdot year^{-1}$ three years after planting. This result is not surprising considering the directional selection history and consequent adaptation of the different improved seed sources across considerably different climatic regions (e.g., Cfa versus Am) in Brazil, which is expected to increase the overall genetic variance across seed sources when compared with what would be expected in more "buffered" unimproved seed sources from natural populations. As expected, the productivity of the *E. urophylla* seed sources was generally lower than the average productivity of planted eucalypts in Brazil — based mainly on highly selected *E. urophylla* × *E. grandis* hybrid clones — which currently stands at about 40 $m^3 \cdot ha^{-1} \cdot year^{-1}$ (Gonçalves et al. 2013). Still, the *E. urophylla* trials revealed important facets of the patterns of genetic variation, adaptability, and genotype × environment interaction of the different seed sources that should provide important pointers for their optimized use

Table 4. Estimates of genetic parameters for DBH in joint analyses of the five trials in each one of the two *Eucalyptus urophylla* experimental networks (EN).

Genetic parameters	EN1	EN2
Genotypic variance ($\hat{\sigma}_A^2$) (half-sibs)	0.88	1.55
Genotypic variance ($\hat{\sigma}_A^2$) (mixed mating)	0.65	0.95
Genotype × environment interaction variance ($\hat{\sigma}_i^2$)	0.25	0.47
Seed source variance ($\hat{\sigma}_{ss}^2$)	0.38	0.36
Residual variance ($\hat{\sigma}_E^2$)	8.52	8.30
Individual phenotypic variance ($\hat{\sigma}_{FI}^2$)	10.1	11.1
Narrow sense individual heritability (h_A^2) half-sibs	0.09±0.01	0.14±0.02
Narrow sense individual heritability (h_A^2) mixed mating	0.06±0.01	0.09±0.01
Coefficient of determination for GEI (c_i^2)	0.02	0.04
Coefficient of determination for seed source (c_s^2)	0.03	0.03
Genotypic correlation in all sites ($r_{g\times site}$)	0.47	0.45

Table 5. Pairwise site genetic correlation (considering the families tested on the two sites) for DBH in the two experimental networks.

EN1	Cfa	Am	Aw	Cwa	Cfa 2	Mean
Cfa	—	0.86	0.85	0.86	0.90	0.87
Am		—	0.86	0.83	0.87	0.85
Aw			—	0.82	0.84	0.84
Cwa				—	0.85	0.84
Cfa2					—	0.86
EN2	Cfa	Am	Af	Cwa	Cfb	Mean
Cfa	—					0.50
Am	0.28	—				0.50
Af	0.40	0.74	—			0.57
Cwa	0.71	0.56	0.57	—		0.62
Cfb	0.61	0.42	0.56	0.64	—	0.56

Note: Cfa, Am, Aw, Cwa, Cfa 2, Af, and Cfb — Köppen climate classification according to Alvares et al. (2013).

for the development of new *E. urophylla* hybrids by the several companies that took part in the trials.

A second relevant result of this study to the various *E. urophylla* breeding programs currently carried out in Brazil was that even though the trials were established in climatic zones considered adequate for *Eucalyptus urophylla* (Flores et al. 2016), some trials had a surprisingly low survival and (or) productivity. Although the few generations of selective breeding already contributed to shaping the overall growth and survival performance of the different seed sources in zones different from the ones in which they were developed, field observations indicated that such striking survival differences were mostly due to site-specific biotic and abiotic stresses. This result highlights the importance of including the evaluation of such stresses when considering productivity modeling in suitable climatic zones. One factor that strongly reduced productivity in our study was the low rainfall in the EN2-Cwa trial. It was only ≈600 mm·year^{-1} during the second and third year after planting, instead of the expected 1100 mm·year^{-1}. In this same region, when the water deficit reaches 250 mm year, Gonçalves et al. (2017) reported that for each 10 mm increase in water deficit, there was a decrease of 1 m^3·ha^{-1}·year^{-1} in productivity of clonal eucalypt plantations. The authors consider the productivity in regions classified as Aw and Cwa more susceptible to water stresses in Brazil due to a more variable rainfall distribution along the year. Clonal eucalypt trials across Brazil and Uruguay also revealed an important response to precipitation with an increase in wood production (Binkley et al. 2017). Our results contribute additional evidence that climatic variation among years can cause considerable shifts in expected productivity. Taking the historical average climatic conditions into account to estimate the productivity could therefore lead to large miscalculations in productivity prediction.

Besides water deficit, a second element responsible for reduced productivity in some of our trials was the occurrence of *Cylindrocladium* leaf disease, causing high mortality (~50%) in both trials in the Am climatic zone. Without considering the disease occurrence, several tropical regions with warm and continuously high air humidity would be considered appropriate for *Eucalyptus urophylla*; however, this increasingly prevalent disease results in high mortality under such conditions decreasing the productivity of susceptible individuals as reported for several eucalypt species (Silva et al. 2017). Productivity prediction models therefore have to take into account the risk of such biotic stresses that could be vastly different irrespective of having a match of climate classification with the one in the natural distribution of the species (Booth 2017). Silva et al. (2019), studying *Eucalyptus grandis* families under essentially the same climatic conditions, also described both stresses — water availability and *Cylindrocladium* disease. A higher mortality and decrease in productivity were, however, reported for *E. grandis* when compared with *E. urophylla*, consistent with the fact that *E. urophylla* is well known to improve the stress tolerance of *E. grandis* in commercial plantations when used in hybrid combinations (Rocha et al. 2006; Booth et al. 2017).

A third highlight of this study was the observation that the highest overall productivity was recorded in the Cfb climate, considered of low suitability for *E. urophylla* (Flores et al. 2016). The MAI in that site was 46 m^3·ha^{-1}·year^{-1}, 15% higher than the Brazilian average of clonal plantations. The Cfb climate does not exist near or in the islands where *E. urophylla* naturally occurs in latitudes ranging from 8°S to 10°S (Payn et al. 2008). As such, it would not be contemplated, in principle, as a suitable area for *E. urophylla* in a model based on climatic similarity. The Cfb climate is, however, known as a very productive climate for eucalypt stands in Brazil, clearly showing that very good adaptability can be found under climatic conditions that are different from those expected based on a simple model of climate correspondence. Similar results were observed for survival and initial growth of *Corymbia torelliana* across eight trials in South America (00°37′S to 32°10′S), showing that this tropical species can adapt well even to subtropical regions (Silva et al. 2017). Our results are in line and contribute to the overall concept laid out in a recent review by Booth (2017) on the importance of assessing the climatic requirements of tree species beyond those determined from analyses of their natural distributions. Systematically designed replicated trials such as the one described in this study not only allow a better understanding of the climatic requirements of tree species, provenances, and improved seed sources, but also provide important clues to optimizing efforts of introducing unanticipated germplasm in ongoing breeding programs.

The narrow-sense heritability for growth was generally lower than expected in the joint analyses of the two experimental networks, likely due to the high variation across trial environments. In the individual trial analyses, the lowest narrow-sense heritability was in EN1-Cfa, probably due to cattle damage two months after planting (>80% of the seedlings were damaged), which increased the stand heterogeneity. The highest heritability was seen

Table 6. Ranking position and genotypic value of seed sources in the joint and individual trial analyses.

EN1

Ranking in the joint analysis			Ranking position in the individual site analysis				
Position	Seed Source	Genotypic value	Cwa	Cfa	Cfa2	Aw	Am
1	Turmalina and Timóteo	0.71	1	1	3	1	4
2	Altinópolis	0.47	2	4	1	3	1
3	Avaré	0.34	3	2	2	2	3
4	Ipatinga	0.22	4	5	7	4	2
5	Teixeira de Freitas	−0.15	5	3	6	5	5
6	Lençóis Paulista	−0.67	6	6	4	6	6
7	Itamarandiba	−0.92	7	7	5	7	7
Mean		10.49					

EN2

Ranking in the joint analysis			Ranking position in the individual site analysis				
Position	Seed Source	Genotypic value	Cwa	Cfa	Cfb	Am	Af
1	Três Marias	1.02	1	1	1	2	2
2	Eunapolis	0.15	2	3	2	4	4
3	Anhembi	0.02	3	2	3	6	6
4	Multi (Almeirim)	−0.12	6	6	6	1	1
5	Mexico	−0.52	5	5	5	3	3
6	Rio Claro	−0.55	4	4	4	5	5
Mean		12.48					

Table 7. Genetic diversity in the two selected sets of *Eucalyptus urophylla* individuals and comparisons with published diversity measures for natural populations by Payn et al. (2008).

Selected set	*n*	*A*	SD	*k*	Pa	Ho (±SD)	He (±SD)	*F* (±SD)	*S* (±SD)
EN1 (19 markers)	79	16.3	5.2	293	67	0.774±0.03	0.856±0.01	0.094±0.03	0.172±0.05
EN2 (19 markers)	65	14.8	4.5	268	39	0.736±0.03	0.855±0.01	0.138±0.03	0.242±0.05
EN1 (12 markers)	79	16.2	4.1	195	39	0.778±0.02	0.855±0.01	0.094±0.02	0.171±0.05
EN2 (12 markers)	65	14.6	4.4	175	27	0.737±0.02	0.856±0.01	0.139±0.03	0.244±0.05
Payn et al. (2008)	357	10.1	1.3	192	4	0.668±0.03	0.739±0.02	0.074±0.04	0.138±0.06

Note: *n*, sample size; *A*, mean number of alleles; *k*, total number of alleles; Pa, number of private alleles; Ho, average observed heterozygosity; He, average expected heterozygosity; *F*, average Wright's fixation index; *S*, average self-fertilization coefficient; SD, standard deviation. "(12 markers)": to allow comparison of the genetic diversity in the selected samples with the diversity of natural population estimates of Payn et al. (2008), 100 random samples without replacement of subsets of 12 markers out of the 19 were taken and the average diversity measures were estimated.

in climate EN2-Cwa, where a prolonged water stress occurred, resulting in a greater genetic effect on the phenotypic variation. In both ENs, the narrow-sense heritabilities were intermediary in the individual analyses, which translates into good genetic control of growth and the possibility of genetic gain. When the narrow-sense heritabilities were estimated taking into account the estimates of inbreeding obtained from the microsatellite marker data, which in turn were converted into estimates of selfing rate, an average overestimation of 31% was seen when assuming half-sibs compared with the most truthful mixed mating. The overestimation varied from 22% to 48% in the individual trial analyses. Accounting for inbreeding effects and relatedness based on molecular marker data when estimating additive genetic parameters has been shown to be important in eucalypts to avoid significant upward bias of parameters and consequent erroneous ranking of breeding values (Bush et al. 2011, 2015). Similar results were also reported for two *Corymbia* species with a 21% to 32% overestimation (Tambarussi et al. 2018). The estimates of inbreeding obtained in our study are consistent with those in earlier reports showing the mixed-mating system of *Eucalyptus urophylla* (Gaiotto et al. 1997) and once again underlines the importance of properly estimating genetic parameters to avoid overestimating genetic gain.

Significant variation for survival and growth was reported among trials of *Eucalyptus urophylla* provenances established in different countries (Dvorak et al. 2008). Our study, however, was based on a considerably narrower genetic base because all seed sources traced back to only two islands in the natural range of the species, Flores and Timor, and to the past selective breeding history that the seed sources had gone through. Still, the variances due to GEI in both ENs corresponded to 38% and 49% of the genotypic variance when using the more accurate mixed mating estimates (Table 4). Shelbourne (1972) indicated as a "rule of thumb" that when the GEI term is less than 50% of the family (or provenance) genotypic component, then the effects of the interaction are likely to be unimportant for gains, selection, and testing. This rule of thumb is consistent with our observation that that GEI was only modest in EN1 but important in EN2 where it significantly impacted selection decisions. EN1 had a more stable behavior in the top families, displaying only scale-effect interaction among families and a higher genetic correlation among trials in the pairwise analysis. This higher stability of EN1 could be partially explained by the shared base populations used in the initial phase of the breeding programs that supplied the seed sources and the frequent exchange of genetic material between private and public institutions that took place during those times. A higher stability enables selection of a larger number of good families with plasticity in one site, as observed by, e.g., Miranda et al. (2015) in *Eucalyptus grandis*. On the other hand, EN2 displayed a lower stability of the best families and poor pairwise correlation between sites, as also observed in, e.g., *Eucalyptus saligna* in trials in southeastern Brazil (Oliveira et al. 2018). This result shows the importance of selecting specific genotypes for each environment due to significant family rank change. Not surprisingly, the variable performance of seed source Multi derived from second-generation selection in Almeirim, state of Pará (01°31′S, 52°34′W), highlights a clear case of rank-change GEI. This seed source corresponds to a land race developed for tropical regions (Am, Köppen classification) from material originally from Timor Island. As a land race, it therefore ranked as the best seed source in tropical regions and the poorest under subtropical conditions. Consistent with the behavior of this seed source, it was in the northernmost tropical climate Am that the greatest variation among seed sources was observed, advocating the need to apply an environmental stratification in Brazil in at least two separate major zones, tropical and subtropical, before carrying out genotype selection.

The microsatellite marker data analysis showed essentially the same levels of genetic diversity in the selected sets of individuals in the two experimental networks based on the observed and expected heterozygosity (Table 7). The estimates of inbreeding in both sets (0.09 and 0.14) were consistent with previous estimates for *Eucalyptus urophylla* (0.07 to 0.11) reported by Gaiotto et al. (1997), as well as for other species such as *Eucalyptus grandis* (0.03 to 0.14; Chaix et al. 2003; Jones et al. 2008; Bertoncini et al. 2017). The levels of genetic diversity estimated for the selected sets in both experimental networks are slightly higher than those reported in a survey of 19 natural populations throughout the geographic range of *E. urophylla* (Payn et al. 2008). The results were the same even after correcting for the number of microsatellite markers used by taking the averages of 100 random samples of 12 markers out of the 19 used. Although this result might seem at odds with the expectations, the slightly higher diversity could be explained by the fact that the selected individuals were derived from improved seed sources subject to the combined impact of selection and genetic drift. When individuals from such diverse seed sources that show genetic structure are jointly analyzed, the overall heterozygosity tends to increase. Another explanation, however, could be due to the microsatellite markers used in our study being largely different from the 12 markers used by Payn et al. (2008) (only two markers were in common, EMBRA2 and EMBRA27). The derived estimates are therefore subject to ascertainment bias in the selection of the markers such that the microsatellites used may display larger numbers of alleles, therefore providing different estimates of heterozygosity. Notwithstanding these caveats, as the diversity estimates are averages, it is reasonable to argue that at a minimum, the genetic diversity observed in the two sets of selected trees is equivalent to the one observed in samples of natural populations. This result actually reinforces the fact that directional selection in *Eucalyptus urophylla* breeding in Brazil is still at its early stages and that advancing generations by a usually large number of open-pollinated families is very efficient in maintaining the large amount of within-family variation for continued genetic gains.

Conclusions

In closing this report, we summarize our main conclusions in light of the questions posed. The overall magnitude of the GEI at the family level was significant, although more so in experimental network 2, likely to impact selection decisions. The trials allowed us to identify more stable and productive *Eucalyptus urophylla* families across a wide range of Brazilian environmental conditions. The rank change of seed sources also indicated that environmental stratification in at least tropical and subtropical climate conditions seems necessary when expanding the evaluations of seed sources to climates to the northernmost regions of the country where climates are considerably different. The genetic survey with molecular markers showed that the impact of individual selection on the level of genetic diversity in the seed sources evaluated is still largely inconsequential, indicating that a large amount of genetic variation is still available for future genetic gains, possibly as a result of the relatively weak selection intensity applied when advancing breeding generations by a large number of open-pollinated families. However, with the current trend of eucalypt breeding in Brazil now moving more to full-sib crosses from a small number of hybrid elite parents (Rezende et al. 2014), if similar restriction of the effective population size is applied to the pure species breeding programs, this scenario is likely to change. Additionally, our study showed the importance of assessing the climatic requirements of eucalypts seed sources beyond those determined from strict climate similarity analyses. Systematically designed field trials will confirm the expected genotype adaptation and provide useful clues to introducing unanticipated germplasm in ongoing breeding programs. Furthermore, even if trials are set up under historically suitable climatic conditions, only by sampling the climatic variation across years can one effectively derive a better prediction of stand productivity.

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References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., and Sparovek, G. 2013. Köppen's climate classification map for Brazil. Meteorol. Z. **22**: 711–728. doi:10.1127/0941-2948/2013/0507.

Bertoncini, G.H., Tambarussi, E.V., Sebbenn, A.M., de Moraes, C.B., Teixeira, L.G., Furtado, E.L., Zimback, L., and Mori, E.S. 2017. Rust resistance and mating system in *Eucalyptus grandis* Hill ex Maiden progenies. Sci. For. **45**: 405–413. doi:10.18671/scifor.v45n114.16.

Binkley, D., Campoe, O.C., Alvares, C., Carneiro, R.L., Cegatta, Í., and Stape, J.L. 2017. The interactions of climate, spacing and genetics on clonal *Eucalyptus* plantations across Brazil and Uruguay. For. Ecol. Manage. **405**: 271–283. doi:10.1016/j.foreco.2017.09.050.

Booth, T.H. 2017. Assessing species climatic requirements beyond the realized niche: some lessons mainly from tree species distribution modelling. Clim. Change, **145**: 259–271. doi:10.1007/s10584-017-2107-9.

Booth, T.H., and Pryor, L.D. 1991. Climatic requirements of some commercially important eucalypt species. For. Ecol. Manage. **43**: 47–60. doi:10.1016/0378-1127(91)90075-7.

Booth, T.H., Jovanovic, T., and Arnold, R.J. 2017. Planting domains under climate change for *Eucalyptus pellita* and *Eucalyptus urograndis* in parts of China and South East Asia. Aust. For. **80**: 1–9. doi:10.1080/00049158.2016.1275101.

Brawner, J.T., Lee, D.J., Hardner, C.M., and Dieters, M.J. 2011. Relationships between early growth and *Quambalaria* shoot blight tolerance in *Corymbia citriodora* progeny trials established in Queensland, Australia. Tree Genet. Genomes, **7**: 759–772. doi:10.1007/s11295-011-0372-8.

Brawner, J.T., Lee, D.J., Meder, R., Almeida, A.C., and Dieters, M.J. 2013. Classifying genotype by environment interactions for targeted germplasm deployment with a focus on *Eucalyptus*. Euphytica, **191**: 403–414. doi:10.1007/s10681-013-0892-4.

Bush, D., Kain, D., Matheson, C., and Kanowski, P. 2011. Marker-based adjustment of the additive relationship matrix for estimation of genetic parameters — an example using *Eucalyptus cladocalyx*. Tree Genet. Genomes, **7**: 23–35. doi:10.1007/s11295-010-0312-z.

Bush, D., Kain, D., Kanowski, P., and Matheson, C. 2015. Genetic parameter estimates informed by a marker-based pedigree: a case study with *Eucalyptus cladocalyx* in southern Australia. Tree Genet. Genomes, **11**: 798. doi:10.1007/s11295-014-0798-x.

Campoe, O.C., Munhoz, J.S.B., Alvares, C.A., Carneiro, R.L., de Mattos, E.M., Ferez, A.P.C., and Stape, J.L. 2016. Meteorological seasonality affecting individual tree growth in forest plantations in Brazil. For. Ecol. Manage. **380**: 149–160. doi:10.1016/j.foreco.2016.08.048.

Chaix, G., Gerber, S., Razafimaharo, V., Vigneron, P., Verhaegen, D., and Hamon, S. 2003. Gene flow estimation with microsatellites in a Malagasy seed orchard of *Eucalyptus grandis*. Theor. Appl. Genet. **107**: 705–712. doi:10.1007/s00122-003-1294-0. PMID:12750775.

Denison, N.P., and Kietzka, J.E. 1993. The use and importance of hybrid intensive forestry in South Africa. S. Afr. For. J. **165**: 55–60. doi:10.1080/00382167.1993.9629390.

Dvorak, W.S., Hodge, G.R., and Payn, K.G. 2008. The conservation and breeding of *Eucalyptus urophylla*: a case study to better protect important populations and improve productivity. South. For. **70**: 77–85. doi:10.2989/SOUTH.FOR.2008.70.2.3.531.

Faria, D.A., Mamani, E.M.C., Pappas, M.R., Pappas, G.J., and Grattapaglia, D. 2010. A selected set of EST-derived microsatellites, polymorphic and transferable across 6 species of *Eucalyptus*. J. Hered. **101**: 512–520. doi:10.1093/jhered/esq024. PMID:20231265.

Faria, D.A., Mamani, E.M.C., Pappas, G.J., and Grattapaglia, D. 2011. Genotyping systems for *Eucalyptus* based on tetra-, penta-, and hexanucleotide repeat EST microsatellites and their use for individual fingerprinting and assignment tests. Tree Genet. Genomes, **7**: 63–77. doi:10.1007/s11295-010-0315-9.

Ferreira, M. 2015. A aventura dos Eucaliptos. *In* Silvicultura do Eucalipto no Brasil. *Edited by* M.V. Schumacher and M. Vieira. UFSM, Santa Maria, Brazil. pp. 13–48.

Flores, T.B., Alvares, C.A., Souza, V.C., and Stape, J.L. 2016. *Eucalyptus* no Brasil: zoneamento climático e guia para identificação. IPEF, Piracicaba, Brazil.

Gaiotto, F.A., Bramucci, M., and Grattapaglia, D. 1997. Estimation of outcrossing rate in a breeding population of *Eucalyptus urophylla* with dominant RAPD and AFLP markers. Theor. Appl. Genet. **95**: 842–849. doi:10.1007/s001220050634.

Garcia, L.G., Ferraz, S.F.B., Alvares, C.A., Ferraz, K.M.P.M.B., and Higa, R.C.V. 2014. Modelagem da aptidão climática do *Eucalyptus grandis* frente aos cenários de mudanças climáticas no Brasil. Sci. For. **42**: 503–511.

Gonçalves, J.L.M., Alvares, C.A., Higa, A.R., Silva, L.D., Alfenas, A.C., Stahl, J., Ferraz, S.F.B., Lima, W.P., Brancalion, P.H.S., Hubner, A., Bouillet, J.D., Laclau, J., Nouvellon, Y., and Epron, D. 2013. Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. For. Ecol. Manage. **301**: 6–27. doi:10.1016/j.foreco.2012.12.030.

Gonçalves, J.L.M., Alvares, C.A., Rocha, J.H.T., Brandani, C.B., and Hakamada, R. 2017. Eucalypt plantation management in regions with water stress. South. For. **79**: 169–183. doi:10.2989/20702620.2016.1255415.

Grattapaglia, D., Ribeiro, V.J., and Rezende, G.D.S.P. 2004. Retrospective selection of elite parent trees using paternity testing with microsatellite markers: an alternative short term breeding tactic for *Eucalyptus*. Theor. Appl. Genet. **109**: 192–199. doi:10.1007/s00122-004-1617-9. PMID:15004676.

Grattapaglia, D., Mamani, E.M.C., Silva-Junior, O.B., and Faria, D.A. 2015. A novel genome-wide microsatellite resource for species of *Eucalyptus* with linkage-to-physical correspondence on the reference genome sequence. Mol. Ecol. Resour. **15**: 437–448. doi:10.1111/1755-0998.12317. PMID:25146326.

Harwood, C. 2011. New introductions — doing it right. *In* Developing a eucalypt resource. Learning from Australia and elsewhere. *Edited by* J. Walker. Wood Technology Research Centre, University of Canterbury, Christchurch, New Zealand. pp. 125–136.

Hernández, J.J.V., Upton, J.L., Valverde, G.R., and Harrand, L. 2009. Genetic parameters of growth traits and wood density in *Eucalyptus grandis* progenies planted in Argentina. Silvae Genet. **58**: 11–19. doi:10.1515/sg-2009-0002.

Hodge, G.R., and Dvorak, W.S. 2015. Provenance variation and within-provenance genetic parameters in *Eucalyptus urophylla* across 125 test sites in Brazil, Colombia, Mexico, South Africa and Venezuela. Tree Genet. Genomes, **11**: 57. doi:10.1007/s11295-015-0889-3.

Jones, M.E., Shepherd, M., Henry, R., and Delves, A. 2008. Pollen flow in *Eucalyptus grandis* determined by paternity analysis using microsatellite markers. Tree Genet. Genomes, **4**: 37–47. doi:10.1007/s11295-007-0086-0.

Jovanovic, T., Arnold, R., and Booth, T. 2000. Determining the climatic suitability of *Eucalyptus dunnii* for plantations in Australia, China and Central and South America. New For. **19**: 215–226. doi:10.1023/A:1006662718206.

Jurskis, V. 2005. Eucalypt decline in Australia, and a general concept of tree decline and dieback. For. Ecol. Manage. **215**: 1–20. doi:10.1016/j.foreco.2005.04.026.

Maes, W.H., Trabucco, A., Achten, W.M.J., and Muys, B. 2009. Climatic growing conditions of *Jatropha curcas* L. Biomass Bioenergy, **33**: 1481–1485. doi:10.1016/j.biombioe.2009.06.001.

Malosetti, M., Ribaut, J.-M., and van Eeuwijk, F.A. 2013. The statistical analysis of multi-environment data: modeling genotype-by-environment interaction and its genetic basis. Front. Physiol. **4**: 44. doi:10.3389/fphys.2013.00044. PMID:23487515.

Miranda, A.C., Moraes, M.L.T., Silva, P.H.M., and Sebben, A.M. 2015. Ganhos genéticos na seleção pelo método do índice multi-efeitos em progênies de polinização livre de *Eucalyptus grandis* Hill ex Maiden. Sci. For. **43**: 203–209.

Oliveira, T.W.G., de Paula, R.C., Moraes, M.L.T., Alvares, C.A., Miranda, A.C., and Silva, P.H.M. 2018. Stability and adaptability for wood volume in the selection of *Eucalyptus saligna* in three environments. Pesqui. Agropecu. Bras. **53**: 611–619. doi:10.1590/s0100-204x2018000500010.

Payn, K.G., Dvorak, W.S., Janse, B.J.H., and Myburg, A.A. 2008. Microsatellite diversity and genetic structure of the commercially important tropical tree species *Eucalyptus urophylla*, endemic to seven islands in eastern Indonesia. Tree Genet. Genomes, **4**: 519–530. doi:10.1007/s11295-007-0128-7.

Peakall, R., and Smouse, P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes, **6**: 288–295. doi:10.1111/j.1471-8286.2005.01155.x.

Potts, B.M., and Dungey, H.S. 2004. Interspecific hybridization of *Eucalyptus*: key issues for breeders and geneticists. New For. **27**:115–138. doi:10.1023/A:1025021324564.

Resende, M.D.V. 2007. Matemática e estatística na análise de experimentos e no melhoramento genético. Embrapa Florestas, Colombo. 362p.

Resende, M.D.V. 2016. Software Selegen-REML/BLUP: a useful tool for plant breeding. Crop Breed. Appl. Biotechnol. **16**: 330–339. doi:10.1590/1984-70332016v16n4a49.

Resende, M.D.V., Vencovsky, R., and Fernandes, J.S.C. 1995. Selection and genetic gains in populations of *Eucalyptus* with a mixed mating system. *In* Eucalypt plantations: improving fibre yield and quality, Proceedings of the CRCTHF-IUFRO Conference, Hobart, Tasmania, 19–24 February 1995. Cooperative Research Centre for Temperate Hardwood Forestry, Sandy Bay, Tasmania, Australia. pp. 191–193.

Rezende, G.D.S.P., Resende, M.D.V., and Assis, T.F. 2014. *Eucalyptus* breeding for clonal forestry. *In* Challenges and opportunities for the world's forests in the 21st century. *Edited by* T. Fenning. Springer, Dordrecht, Netherlands. pp. 393–424. doi:10.1007/978-94-007-7076-8_16.

Rocha, M.G.B., Pires, I.E., Xavier, A., Cruz, C.D., and Rocha, R.B. 2006. Avaliação genética de progênies de meio-irmãos de *Eucalyptus urophylla* utilizando os procedimentos REML/BLUP e E(QM). Cien. Flor. **16**: 369–379. doi:10.5902/198050981918.

Shelbourne, C.J.A. 1972. Genotype–environment interaction: its study and its implications in forest tree improvement. *In* Proceedings of the IUFRO Genetics–SABRAO Joint Symposia for the Advancement of Forest Tree Breeding, Tokyo, Japan. IUFRO. pp. 1–27.

Silva, P.H.M., Miranda, A.C., Moraes, M.L.T., Furtado, E.L., Stape, J.L., Alvares, C.A., Sentelhas, P.C., Mori, E.S., and Sebbenn, A.M. 2013. Selecting for rust (*Puccinia psidii*) resistance in *Eucalyptus grandis* in São Paulo State, Brazil. For. Ecol. Manage. **303**: 91–97. doi:10.1016/j.foreco.2013.04.002.

Silva, P.H.M., Lee, D.J., Miranda, A.C., Marino, C.L., Moraes, M.L.T., and de Paula, R.C. 2017. Sobrevivência e crescimento inicial de espécies de eucalipto em diferentes condições climáticas. Sci. For. **45**: 563–571. doi:10.18671/scifor.v45n115.13.

Silva, P.H.M., Marco, M., Alvares, C.A., Miranda, A.C., Lee, D.J., Moraes, M.L.T., and de Paula, R.C. 2019. Early selection of *Eucalyptus grandis* families across environmental conditions. Crop Breed. Appl. Biotechnol. In press.

Tambarussi, E.V., Pereira, F.B., Silva, P.H.M., Lee, D., and Bush, D. 2018. Are tree breeders properly predicting genetic gain? A case study involving *Corymbia* species. Euphytica, **214**: 150. doi:10.1007/s10681-018-2229-9.

van Buijtenen, J.P. 1992. Fundamental genetic principles. *In* Handbook of quantitative forest genetics. *Edited by* L. Fins, S.T. Friedman, and J.V. Brotschol. Springer, Dordrecht, Netherlands. pp. 29–68. doi:10.1007/978-94-015-7987-2_2.

White, T.L., Adams, W.T., and Neale, D.B. 2007. Forest genetics. CABI, Wallingford. doi:10.1079/9781845932855.0000.

Wingfield, M.J., Slippers, B., Hurley, B.P., Coutinho, T.A., Wingfield, B.D., and Roux, J. 2008. Eucalypt pests and diseases: growing threats to plantation productivity. South. For. **70**: 139–144. doi:10.2989/SOUTH.FOR.2008.70.2.9.537.

Yesson, C., Brewer, P.W., Sutton, T., Caithness, N., Pahwa, J.S., Burgess, M., Gray, W.A., White, R.J., Jones, A.C., Bisby, F.A., and Culham, A. 2007. How global is the global biodiversity information facility? PLoS One, **2**: e1124. doi:10.1371/journal.pone.0001124. PMID:17987112.

Zobel, B., and Talbert, J. 1984. Applied forest tree improvement. 4th ed. John Wiley & Sons, New York.