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THE USE OF GENETIC DISTANCE AND GROUPING METHODS TO PREDICT *Eucalyptus pellita* F. MUELL GENITORS FOR HYBRIDIZATION

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HIGHLIGHTS

UPGMA and Tocher's grouping methods showed contrasting results.

Tocher's method was more reliable according to the cophenetic correlation coefficient.

Limited genetic distance was found between *Eucalyptus pellita* provenances.

Heterotic groups can be validated through crossings between divergent parents.

Keywords:
Multivariate analysis
forest improvement
heterotic groups
hybrids

ABSTRACT

The objective of this study was to use quantitative traits to estimate the genetic distance among *E. pellita* provenances and progenies, to inform possible hybridization strategies in a species improvement program. A provenance and progeny test with 118 progenies from seven provenances was evaluated. The following quantitative traits were measured at seven years of age: diameter at breast height (DBH); height; and individual volume. The data were submitted to REML/BLUP analysis to obtain the predicted genetic value (BLUP). From this, the Mahalanobis (D^2) genetic distance was estimated for provenances and progenies, which were then grouped by Tocher's method, the unweighted pair group method using arithmetic averages (UPGMA), and principal component analysis (PCA). In total, 29 divergent groups were obtained among progenies based on Tocher's method, which showed greater reliability according to the cophenetic correlation coefficient than UPGMA. The opposite was found between provenances, where the results for UPGMA demonstrated greater clustering reliability. Based on principal component analysis (PCA), the M. Ray and Tully provenances were the most similar, while Connl. A and Orchard were the most divergent. Height was the most important trait in estimating genetic distance. The results obtained offer important insights for breeding programs; with this information, crosses can be designed between contrasting individuals among and within provenances to obtain *E. pellita* hybrids, validating the possible heterotic groups identified through the genetic distance and grouping methods.

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INTRODUCTION

In Brazil, *Eucalyptus pellita* F. Muell is an important species in hybridization, particularly in forest frontier regions, as there is a need to incorporate a range of characteristics, such as high levels of growth, basic density, and cellulose yield, into one single genotype (Resende and Assis, 2008). In addition, *E. pellita* has been exploited for its resistance to drought and diseases, such as eucalyptus rust (*Puccinia psidii*) and leaf spots caused by fungi of the genus *Cylindrocladium* (Resende and Assis, 2008; Assis et al., 2015). Therefore, in breeding programs, there is interest in incorporating these characteristics through the development of interspecific hybrids with high-productivity species, such as *Eucalyptus grandis* and *Eucalyptus urophylla* (Assis et al., 2015).

Due to the increasing attention given to using *E. pellita* parents to provide complementary alleles in hybridization, understanding the genetic divergence between populations and parents of these populations is crucial. For this, multivariate analysis methods to estimate genetic divergence between genotypes, such as Euclidean or Mahalanobis distance, are useful in defining potential parents in breeding programs (Cruz et al., 2014; Resende et al., 2014).

However, due to the large number of combinations between pairs of divergent parents, visual identification of groups is not feasible; thus, it is necessary to use grouping methods, such as Tocher's optimization method and the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA), to facilitate reliable analysis (Cruz et al., 2012). In recent studies, the generalized Mahalanobis distance showed satisfactory results when estimating genetic divergence in forest species such as *Pinus caribaea* var. *caribaea* (Silva et al., 2012), *Eucalyptus camaldulensis* (Costa et al., 2016), and *Corymbia citriodora* (Luz et al., 2018), along with Tocher's optimization method and UPGMA, which were applied to identify divergent groups.

A widespread concept in agricultural studies, particularly studies on corn, is the identification of a heterotic group, which is a set of related inbred lines that when crossed with lines from different groups tend to form potential hybrids (Ertiro et al., 2017). These heterotic groups are obtained from diallel crosses, where the potential genotypes are crossed with each other. As such, it is possible to estimate the heterosis of each crossing, along with the specific combining ability (SCA) which measures the complementarity of favorable alleles (Souza Junior, 2001).

Questions remain as to which genotypes should be used in hybridization, so that the crosses performed are effective and maximize the number of potential hybrids. Thus, it is essential to evaluate the feasibility of methodologies that group genetically divergent parents, which can help choose the parents to be used in diallelic designs.

Therefore, the objective of this study was to identify genetic distance and group *E. pellita* provenances and progenies based on quantitative traits, to support hybridization strategies in a breeding program for the species.

MATERIAL AND METHODS

Experimental data

The evaluated experiment is an open-pollinated provenance/progeny test of *E. pellita* belonging to the company Suzano S.A., located in the municipality of Mucuri, Bahia State, Brazil (latitude 18° 01' 23.14" S and longitude 39° 54' 45.18" W). The climate in the region according to the Köppen classification (Alvares et al., 2013) is Af, or rainforest without dry season, with long, hot summers, average annual rainfall of 1,583 mm, and average annual temperature of 24.3 °C. The soil classification of the site is yellow Argisol, and the elevation is 78 m.

The trial was installed using a randomized block design, with five replications in linear plots of nine plants, 119 treatments, 118 progenies from seven provenances (Table 1) and a commercial clone used as a control. The control is a *E. grandis* x *E. urophylla* hybrid from the 2nd generation of an improvement program located in the extreme south coast of Bahia, Brazil. The clone was obtained through open pollination of *E. grandis* (seeds Coffs Harbor, Australia and Flores Island, Indonesia) and *E. urophylla* (seeds from Anhembi, São Paulo, Brazil). The spacing used for planting was 3x2 m.

The provenances Helenvale, M. Ray, Tully, Connl. A, Black M. Road, and Creb Track are natural populations from the north coast of the state of Queensland, Australia. The Orchard provenance is from Papua New Guinea (Figure S1, Supplementary Material) and is a seed orchard of *E. pellita* seedlings, with an undefined degree of improvement. This orchard was established in the early 1990s from a collection of seeds belonging to the Commonwealth Scientific and Industrial Research Organization (CSIRO).

TABLE 1 *Eucalyptus pellita* provenances from Queensland, Australia and Papua New Guinea, present in the provenance/progeny test installed in Mucuri, Bahia, Brazil.

Provenance	Number of Progenies	Lat. (S)	Long. (L)	ELV. (m)	AAT (°C)	AAP (mm)	Improvement level
Helenvale	17	15° 48'	145° 13'	241	25.6	1814	Wild
M.Ray	21	15° 12'	144° 58'	282	25.6	1814	Wild
Tully	15	17° 54'	145° 41'	63	23.7	3311	Wild
Connl. A	4	18° 26'	146° 07'	40	23.9	2121	Wild
Black M. Road	19	16° 41'	145° 31'	424	25.0	1989	Wild
Creb Track	21	16° 03'	145° 18'	220	25.0	1989	Wild
Orchard	21	08° 20'	141° 26'	37	28.5	919	Seed Orchard

Lat. (S): south latitude in degrees; Long. (E): east longitude in degrees; ELV: elevation; AAT: average annual temperature; AAP: average annual precipitation.

At seven years of age, quantitative silvicultural traits were measured, including diameter at breast height (DBH, cm) and total height (m). The DBH, total height, and a form factor (f) of 0.42 were used to estimate the individual volume (m^3) with the equation 1, where, f is the form factor (0.42); π is the mathematical constant Pi; DBH is diameter at breast height; and H is total height.

$$Volume = \frac{\pi . DBH^2}{40,000} . H . f \quad [1]$$

Statistical analysis

To estimate the predicted genetic values (BLUP), a mixed linear model was adjusted from the statistical package *lme4* (Bates et al., 2015), which is suggested for analysis of mixed models, using the Restricted maximum likelihood/best linear unbiased prediction method (REML/BLUP). The "ranef" function was used to extract the random effects corresponding to adjustments to the model.

The mixed linear model was adjusted with the aid of the R statistical software (R Core Team, 2019): [2] in which Y_{ijkl} is the phenotypic value of the l^{th} individual of the k^{th} progeny of the j^{th} provenance in the i^{th} repetition; μ is the fixed term of the general average of the trait under analysis; b_i is the fixed effect of the i^{th} repetition; t_j is the random effect of the j^{th} provenance; $f_{j:k}$ is the random effect of the k^{th} progeny on the j^{th} provenance; $(tb)_{ij}$ is the effect of the interaction between the j^{th} provenance and the i^{th} repetition; $(fb)_{j:ki}$ is the effect of the interaction between the i^{th} repetition and the k^{th} progeny of the j^{th} provenance; e_{ijkl} is the effect of the relative experimental error of the i^{th} tree within the k^{th} progeny of the j^{th} provenance in the i^{th} repetition.

$$Y_{ijkl} = \mu + b_i + t_j + f_{j:k} + (tb)_{ij} + (fb)_{j:ki} + e_{ijkl} \quad [2]$$

Genetic distance and clusters

The genetic distance between provenances and progenies was estimated using the Generalized Mahalanobis Distance (Mahalanobis, 1936), in the *vegan* package with the "vegdist" function (Oksanen et al., 2018). As recommended by Resende et al. (2014), the predicted genetic values (BLUP) of DBH, height, and volume were used to calculate the Mahalanobis distance (D^2).

From the Mahalanobis distance (D^2), the progenies and provenances were grouped using the mutually exclusive Tocher's Optimization Method (Rao, 1952), with the "tocher" function in the *biotools* package (Silva et al., 2017). This method adopts as a criterion the inclusion of new progenies into the group, in which η = number of progenies that make up the original group; α = addition limit; $D^2_{(Group)i}$ = average intra-group distance, for the formation or inclusion of a new member.

if $\left(D^2_{(Group)i} / \eta\right) \leq \alpha \Rightarrow$ the progeny is included in the group;

if $\left(D^2_{(Group)i} / \eta\right) > \alpha \Rightarrow$ progeny "i" should not be included in the group;

The progenies and provenances were also grouped using UPGMA, as proposed by Nei (1978), using the *vegan* package (Oksanen et al., 2018), in which the grouping of individuals results in a dendrogram of the identified clusters. This method uses the arithmetic means (unweighted) of the dissimilarity measures (D^2), avoiding the use of extreme values between genotypes when forming the clusters.

In order to verify and guarantee reliability in the groups identified using the Tocher and UPGMA methods, the cophenetic correlation coefficient (CCC), as proposed by Sokal and Rohlf (1962), was estimated. The significance of CCC was assessed using the Mantel test (Mantel, 1967) with 1,000 bootstraps. The relative importance of each quantitative trait in the estimate of genetic distance was obtained through the methodology proposed by Singh (1981) and implemented in the *biotools* package.

All analyses were performed with the aid of the R statistical software environment (R Core Team, 2019).

Principal component analysis

In addition to the clustering methods used, the predicted genetic values (BLUP) of the progenies and provenances were subjected to principal component analysis (PCA) to assess the similarity of the progenies and provenances through graphic dispersion of the main components PC1 and PC2 in a biplot chart. The *ggbiplot* package (Vu, 2011) was used to obtain the components using the "princomp" function.

RESULTS

Genetic distance and clusters

Using the Mahalanobis distance matrix (Table S1, Supplementary Material), genetic distances ranged from $D^2 = 0.101$ to $D^2 = 5.702$ between progeny pairs. The smallest distance was found between progenies 116-O and 114-O, both from the Orchard provenance located in Papua New Guinea. The greatest genetic distance was found between progenies 99-CT and 54-C, from two different populations, Creb Track and Connl. A.

Based on Tocher's method, 29 divergent groups were obtained (Table 2) from the BLUPs of the 118 progenies. In general, no pattern in the formation of the groups in relation to the provenances was discernable, except group 1 which was formed only by progenies of Orchard, Papua New Guinea.

The grouping criterion between progenies was $\alpha = 1.08$, which was the value used for inclusion of the progenies in group 1 or the formation of new groups. Group 2 included the largest number of progenies, 22.9% of those evaluated, which came from five different populations: Black Mount Road, Creb Track, Helenvale, M. Ray, Tully.

The intergroup analysis revealed that the greatest genetic distance occurred between groups 7 and 26 ($D^2 = 5.702$). Group 7 is formed by progenies 110-O, 98-CT, 99-CT, belonging to Orchard and Creb Track, while group 26 was formed by a single progeny, 54-C, from Connl. A. The 48-T progeny belonging to group 25, has the highest productivity averages for DBH, height, and volume (Figure 1). Progenies 67-B, 76-B, and 28-M belonging to group 11 and 36-M belonging to group 24, also show growth above the test average.

Considering the results of UPGMA, some similarities were observed between the groupings obtained by Tocher's method (Figure 2), however they do not correspond completely.

TABLE 2 Grouping of *Eucalyptus pellita* progenies at seven years of age, using Tocher's optimization method based on the generalized Mahalanobis distance (D^2), and predicted genetic values (BLUP) for DBH, total height, and individual volume, in Mucuri, Bahia, Brazil.

Group	Progenies	Number of Progenies
1	114-O 116-O 113-O 47-T 112-O 44-T 41-T 117-O 102-O 101-O	10
2	26-M 68-B 24-M 74-B 62-B 65-B 21-M 13-H 43-T 94-CT 66-B 34-M 69-B 23-M 83-CT 19-M 33-M 73-B 91-CT 88-CT 35-M 61-B	22
3	105-O 107-O 109-O 119-O 104-O	5
4	10-H 9-H 25-M 86-CT 5-H 92-CT 15-H 97-CT 77-B 38-M 95-CT 37-M 27-M 82-CT 81-CT	15
5	100-O 80-CT 55-C 56-C 40-T 72-B 111-O	7
6	63-B 71-B 6-H 64-B 1-H 50-T 79-CT	7
7	110-O 98-CT 99-CT	3
8	14-H 84-CT 96-CT 31-M 4-H 3-H 11-H 2-H	8
9	49-T 8-H 89-CT 75-B	4
10	45-T 46-T 87-CT 120-O	4
11	67-B 76-B 28-M	3
12	12-H 16-H	2
13	60-B 7-H	2
14	29-M 70-B 39-T 30-M	4
15	103-O 115-O 52-T 53-T	4
16	42-T 57-C	2
17	18-M 59-B	2
18	106-O 118-O	2
19	20-M 51-T	2
20	108-O	1
21	17-H	1
22	22-M	1
23	32-M	1
24	36-M	1
25	48-T	1
26	54-C	1
27	85-CT	1
28	90-CT	1
29	93-CT	1

B= Black Mount Road; C= Connl. A; CT= Creb Track; H= Helenvale; M = M. Ray; O= Orchard; T= Tully

Some progenies were grouped in different clusters when comparing the two methods. Those progenies identified by Tocher's method as the group of progenies with the greatest similarity (group 1), were also grouped by UPGMA, but were included in two groups (the first and second groups clockwise).

Progenies 107-O, 108-O, 119-O, and 120-O belonging to the Orchard provenance were paired, with progenies 107-O and 119-O forming a group and 108-O and 120-O forming another. According

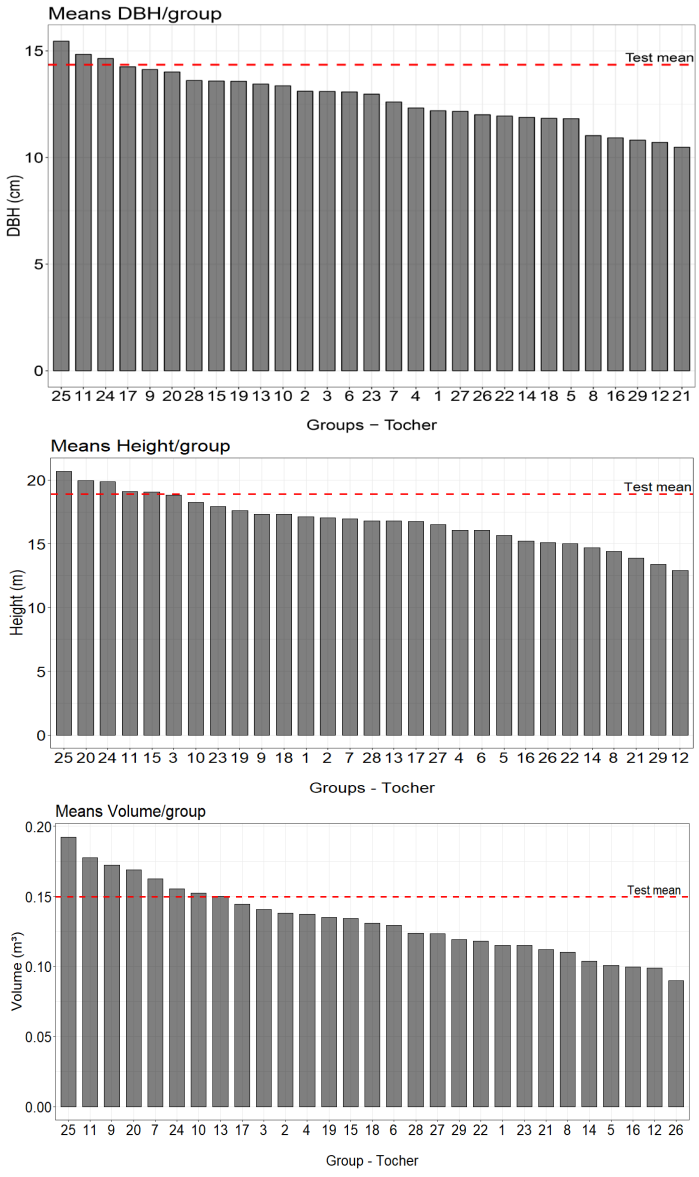


FIGURE 1 Mean DBH, height, and individual volume of the 29 groups formed by Tocher's method, for *Eucalyptus pellita* progenies assessed at seven years of age in Mucuri, Bahia, Brazil.

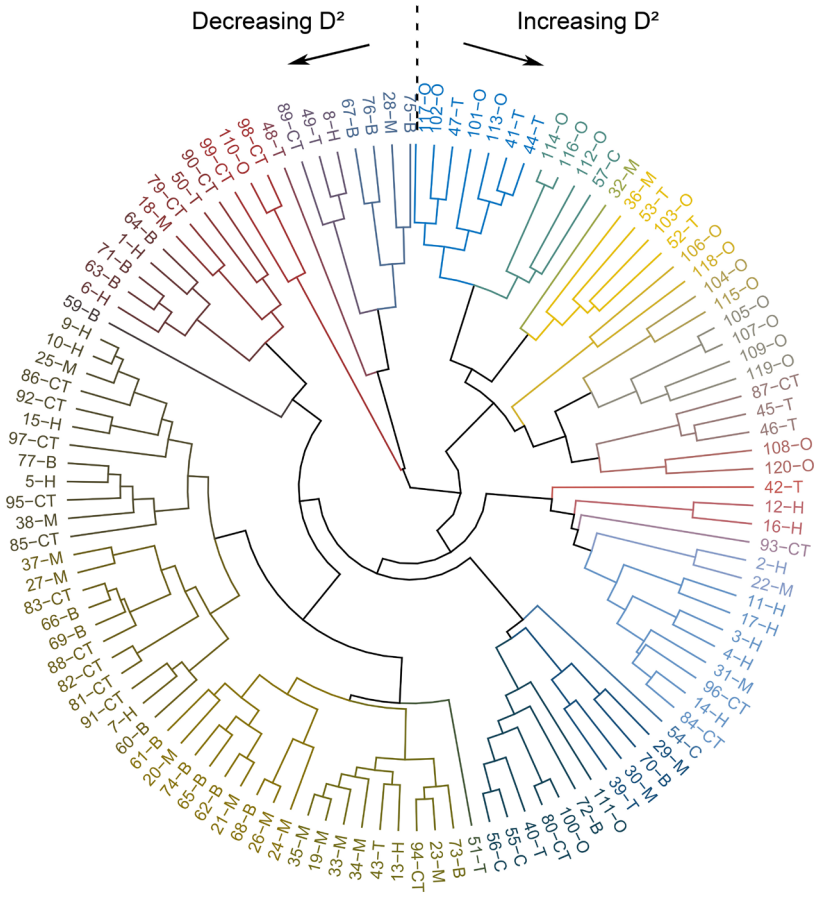
to the dendrogram (Figure 2), the progenies present in the branches beginning from the counterclockwise direction form four groups that are the most divergent of the 118 evaluated progenies.

The provenances were grouped by Tocher's method into three divergent groups, with group 1 made up of five of the seven provenances and Connl. A and Orchard making up groups 2 and 3, respectively (Table 3).

The grouping criterion was $\alpha = 2.41$, with provenances Connl. A and Orchard showing the greatest genetic distance ($D^2 = 3.37$). The least contrasting provenances were M. Ray and Tully, with

TABLE 3 Grouping of *Eucalyptus pellita* provenances at seven years of age, using Tocher's optimization method, based on the generalized Mahalanobis distance (D^2), and the traits DBH, total height, and individual volume, in Mucuri, Bahia, Brazil.

Groups	Provenances	Number of Provenances
1	M. RAY TULLY BLACK M. ROAD CREB TRACK HELENVALE	5
2	CONNL. A	1
3	ORCHARD	1



B= Black Mount Road; C= Connl. A; CT= Creb Track; H= Helenvale; M = M. Ray; O= Orchard; T= Tully

FIGURE 2 Dendrogram obtained by UPGMA based on the generalized Mahalanobis distance (D^2), forming 29 groups from the genetic values (BLUP) of 118 *Eucalyptus pellita* progenies at seven years of age, Mucuri, Bahia. Clockwise: increasing genetic distance; Counterclockwise: decreasing genetic distance.

an estimated minimum genetic distance of $D^2 = 0.853$. These were clustered in group 1, together with Black M. Road, Creb Track, and Halenvale.

Based on the UPGMA, the grouping of the provenances (Figure 3) was similar to that identified by Tocher's method, but they were grouped into four divergent groups by UPGMA, and three by Tocher. The Connl. A and Orchard provenances remained the most divergent; however, the Creb Track and Helenvale provenances were identified as a third group based on UPGMA, whereas using Tocher's method they were grouped together with the other five provenances in the group of greatest similarity.

Considering the two distinct grouping methods, UPGMA and Tocher's optimization method, the groups do not fully correspond. For the grouping of progenies, Tocher's presented a coefficient correlation coefficient (CCC) of 0.89, significant at 5% based on the Mantel test (Figure 4), while the grouping of the progenies by UPGMA showed a CCC of 0.63, significant at 5% based on the Mantel test.

For grouping the provenances using Tocher's method, the CCC was 0.69 and significant at 1% (Figure 4), while the grouping with UPGMA presented a CCC = 0.86, significant at 5%.

Considering the method described by Singh (1981), the variable that most contributed to the estimate of genetic divergence between the provenances and progenies at age seven was height, at 86.8% and 73.8%, respectively, followed by DBH at 12.6% and 25.7%, respectively (Figure 5). The individual volume contributed only 0.6% to the estimate of genetic divergence between the provenances and progenies.

Principal component analysis (PCA)

Principal component analysis for *E. pellita*

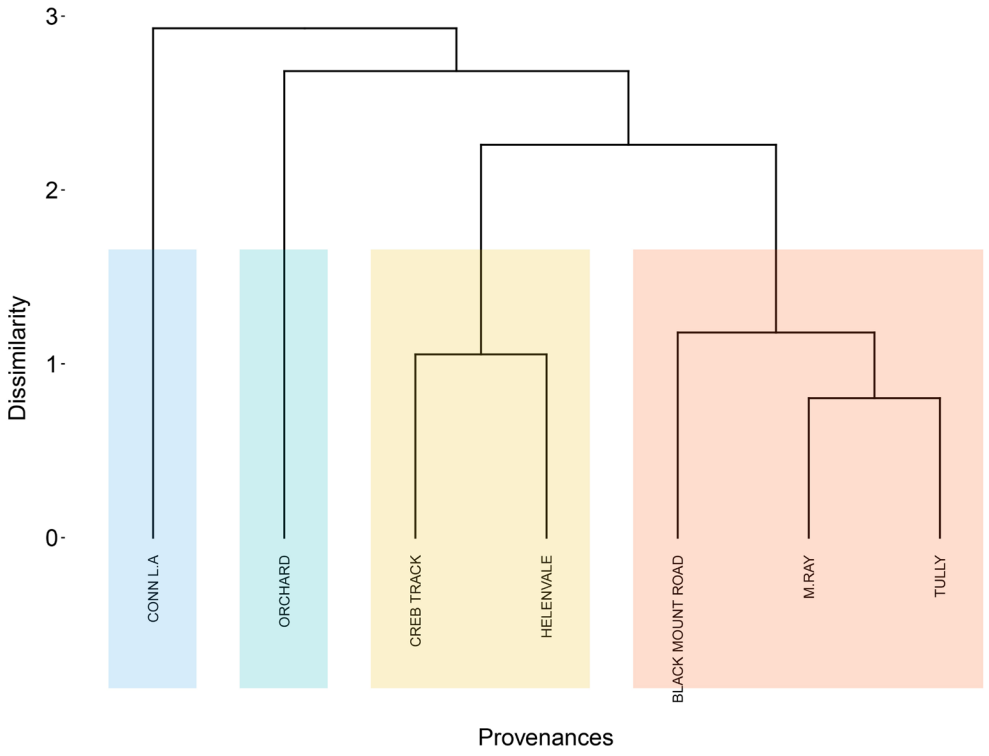


FIGURE 3 Dendrogram obtained by UPGMA based on the generalized Mahalanobis distance (D^2) for seven provenances of *Eucalyptus pellita* at age seven.

progenies showed that the first two principal components explained 98.3% of the variability between BLUPs for quantitative traits (Figure 6). Principal component 1 (PC1) and 2 (PC2) contributed 90.7% and 7.6%, respectively. Based on their BLUP, the quantitative traits contributed similarly to PC1, with results for volume of 0.59, DBH of 0.57, and height of 0.56. For PC2, DBH (0.63) showed greater discriminatory power, followed by volume (0.12), with height acting in reverse (-0.76).

Progenies 75, 28, 110, 7, and 120, all of which belong to different provenances, showed the best BLUP performance for DBH. For height, progenies 76, 108, 98, 109, and 119 were closer to the vector g_Height and belong to the provenances Orchard, Creb Track, and Black M. Road. For volume, progenies 67, 15, and 120 showed the best performance in terms of BLUP. We found no evident pattern of progeny distribution in relation to the provenances, where at least one progeny from each provenance stood out in relation to the traits evaluated.

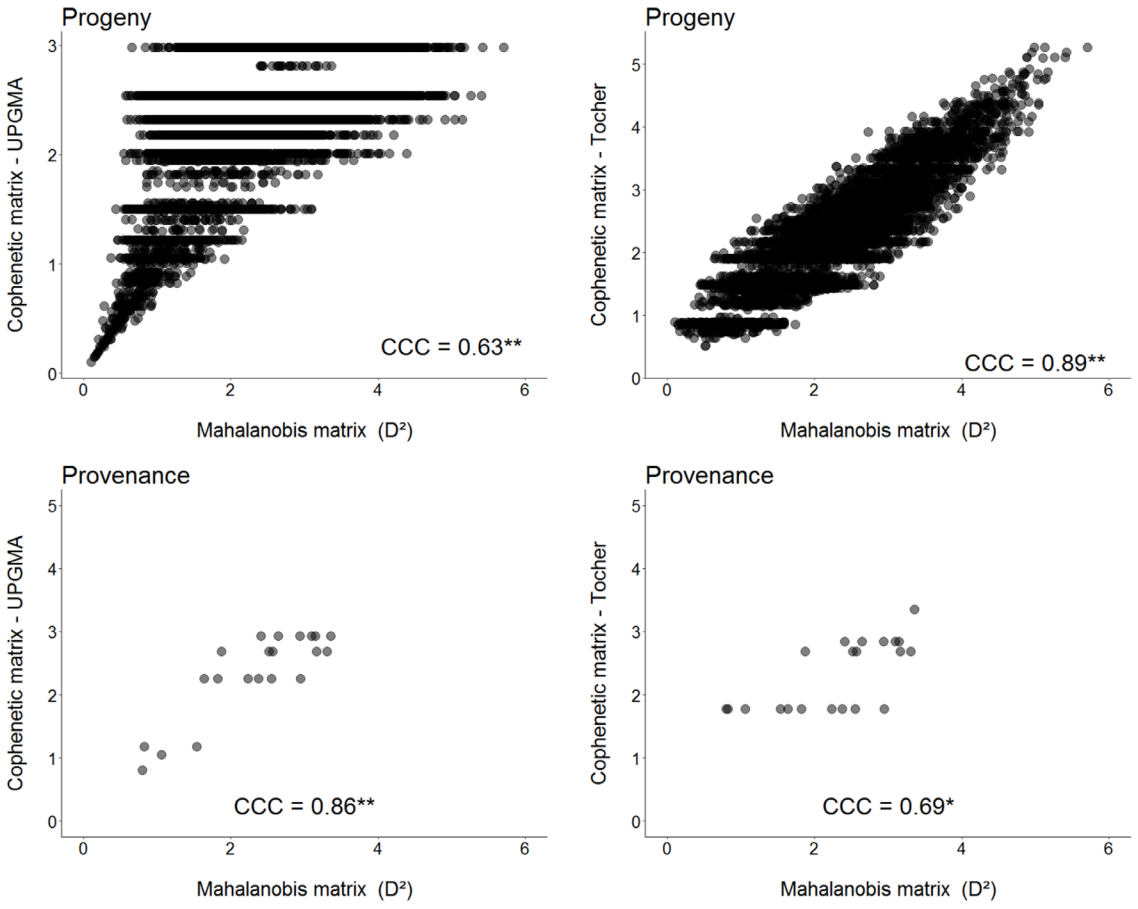
Through the dispersion of the scores of the principal components, it was also possible to assess the similarity of the provenances in terms of genetic values (BLUP) for DBH, height, and volume. The first two principal components explained 99.6% of the variability between BLUPs of quantitative traits (Figure 7). Principal component 1 (PC1) and principal component 2 (PC2) contributed 70.5% and 29.1%, respectively, with variance among *E. pellita* provenances. The trait with the greatest discriminatory power in PC1 was volume (0.68), followed by DBH (0.64) and height (0.35). For PC2, height (-0.92) again showed inverse discriminatory power in contrast to DBH (0.37) and volume (0.12).

A distinct group can be seen between Helenvale, Black M. Road, and Creb Track as these provenances performed better in terms of BLUP for DBH and volume. As observed in the grouping methods mentioned above, Connl. A and Orchard were distinct from other provenances. Connl. A showed performance for DBH and volume contrary to that presented by the other provenances and with a particularly poor performance for height. Meanwhile, the Orchard provenance showed the best performance for BLUP in terms of height.

DISCUSSION

Genetic distance and clusters

Although DBH, height, and volume are highly correlated and result in multicollinearity, these traits



Significant based on the Mantel test at 1% (*) and 5% (**) probability, with a 1000 bootstrap repetitions.

FIGURE 4 Cophenetic correlation coefficients (CCC) between the Mahalanobis distance matrix (D^2) of the *Eucalyptus pellita* provenances and progenies and the cophenetic matrices of Tocher's clustering method and UPGMA.

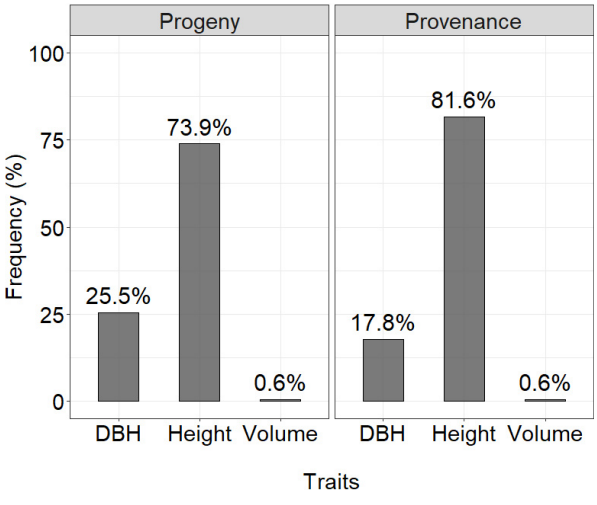
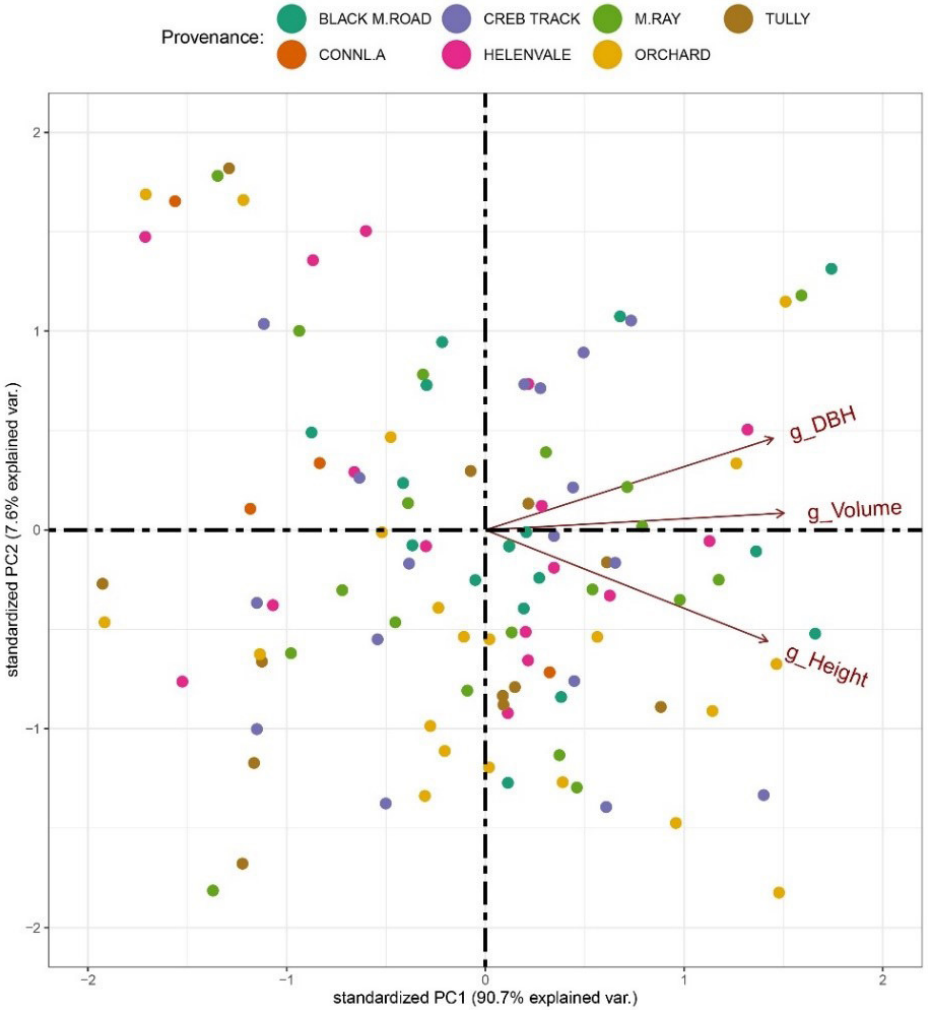


FIGURE 5 Relative importance of the traits to estimate genetic divergence in progenies and provenances of *Eucalyptus pellita* at seven years of age.

should not be disregarded in studies of divergence, as they are particularly important for forest improvement. To alleviate this problem, the Mahalanobis distance standardizes and compensates for the existing correlation between these traits, while also considering the magnitude of the variance and residual covariance. As such, it offers an alternative for studies with traits that present multicollinearity (Hair Júnior et al., 2009; Resende et al., 2014).

The Mahalanobis distance has been shown to be applicable in studies on a range of Eucalypts, such as *E. maculata*, *E. tereticornis*, *E. urophylla*, *E. grandis*, *E. cloeziana*, *E. camaldulensis*, *E. resinifera* and *E. pauciflora* (Trugilho et al., 1997; Gauli et al., 2015), and other forest species, such as *Dalbergia sissoo* (Kumar et al., 2016), *P. caribaea* var. *bahamensis* (Silva et al., 2012). The use of the Mahalanobis distance in conjunction with Tocher's method was also positively assessed by Costa et al. (2016), who classified both methods as effective



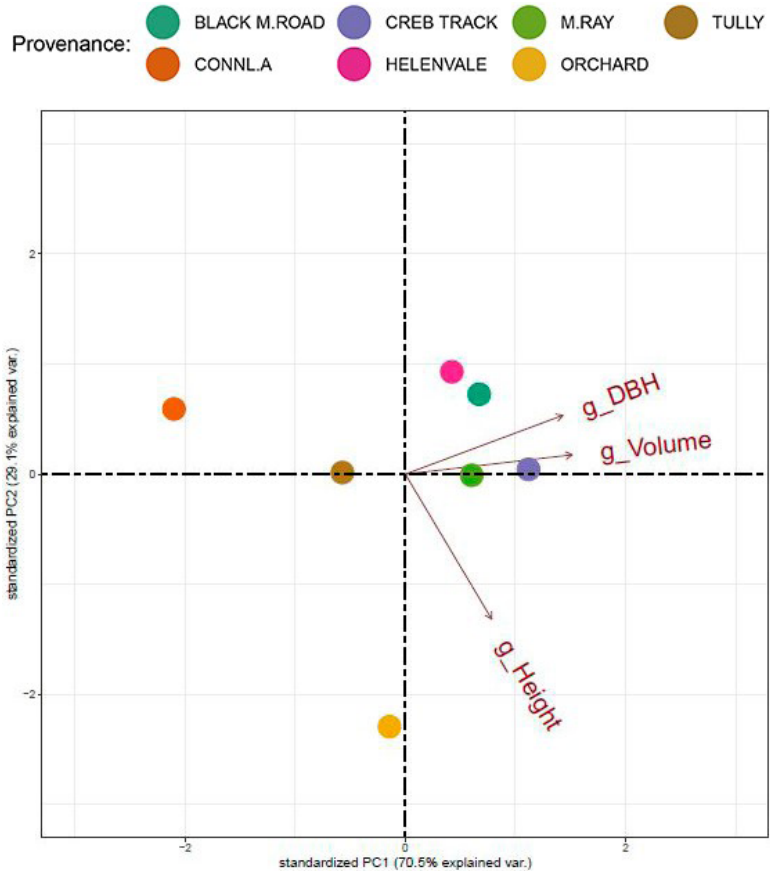
g_DBH: BLUP of DBH; g_ Height: BLUP of height; g_Volume: BLUP of volume.

FIGURE 6 Dispersion of *Eucalyptus pellita* progenies according to the scores of the two principal components, based on the predicted genetic values (BLUP) of the quantitative traits DBH, height, and volume, at seven years of age in Mucuri, Bahia, Brazil.

in evaluating quantitative traits such as DBH and height in *E. camaldulensis*.

The minimum distance between the progenies ($D^2 = 0.101$) is equivalent to the existing distance between the progenies belonging to group I, meaning that the progenies in this group are the most similar. This group was formed mostly by progenies from the Orchard provenance of Papua New Guinea (seven progenies) in addition to three progenies from the Tully provenance. The genetic similarity between progenies in group I may be the result of seed collection strategies used to develop the Orchard in Papua New Guinea, as some seeds from matrices in the Tully region may have been used. These provenances are geographically distant (Figure S1, Supplementary Material), thus preventing the occurrence of gene flow between these populations. Information about how the Orchard provenance was installed is important to understand this similarity between progenies and provenances. For example, it is unclear which populations were included in seed collection, or if there was previous use of genetic diversity methodologies in the formation of the Orchard. However, this information is unknown.

The concentration of 22.9% of the evaluated progenies in group 2, from five different provenances (Black Mount Road, Creb Track, Helenvale, M. Ray, Tully), may be related to the geographical proximity of these populations, which enabled gene flow between them (Figure S1, Supplementary Material). Orchard and Connl. A were the only provenances that did not contribute any progeny to group 2, possibly because they are isolated from the other populations (Table 2).



g_DBH: BLUP of DBH; g_Height: BLUP of height; g_Volume: BLUP of volume.

FIGURE 7 Dispersion of *Eucalyptus pellita* provenances according to the scores of the two principal components, based on the predicted genetic values (BLUP) of the quantitative traits DBH, height, and volume, at seven years of age in Mucuri, Bahia, Brazil.

From the intergroup analysis, we identified the existence of groups with productive progenies in terms of DBH, height, and volume, for example progeny 48-T belonging to group 25, progenies 67-B, 76-B, and 28-M belonging to group 11, and 36-M belonging to group 24 (Figure 1). However, these groups have low and/or moderate genetic distance. Therefore, crossing between individuals in these groups is not recommended as it is likely that there would be no heterotic effect for the evaluated traits (DBH, height, and volume). Groups 25, 11, 9, 20, 7, 24, and 1 are the most productive in terms of individual volume and are thus strategic groups that can be used in hybridization.

In terms of improvement, the highest levels of heterosis tend to occur when individuals from genetically divergent groups and populations are crossed (Lee, 1995; Souza Junior, 2001). Thus, individuals from the same group should not be crossed with each other, as kinship between related individuals may occur, which can result in inbreeding and compromise the continuity of the improvement program (Kageyama, 1981).

The number of dendrogram clusters (Figure 2) obtained by UPGMA was pre-defined based on the number of groups identified using Tocher's method. Some similarities were observed between the groupings obtained by both methods (Table 2), but the results are not identical, since some progenies were grouped in different clusters. Group 1, which Tocher's method identified as the group of progeny with the greatest similarity, was also similarly classified by UPGMA, where the first and second groups (from left to right) include the progenies in group 1.

We can see that groups were formed of progenies from different provenances, demonstrating that the groups did not follow a pattern based solely on geographical location. Other factors, such as genetic drift and natural selection, can contribute to the formation of groups with different progenies (Sebbenn et al., 2005).

According to Aslam et al. (2011), when groups include progenies from different provenances, one hypothesis is that the species has genetic uniformity, which may be the case in *E. pellita* and should be further investigated.

In this study, we did not evaluate progeny for tolerance to pests and diseases. Nevertheless, these are important characteristics of *E. pellita* that make the species attractive for forest improvement programs, and particularly for hybridization, which is the main focus of this study. However, we must consider including in crosses those individuals that may be disease/pest tolerant not only in groups with high- or intermediate-level growth, but also those in the least productive progenies. Therefore, it is important to identify *E. pellita* individuals with resistance to *Puccinia psidii* (rust), as previously reported in the literature (Santos et al., 2014), including in the Helenvale provenance (Roux et al., 2015). Resistance to other diseases, such as those caused by *Ceratocystis fimbriata* (ceratocystis wild) and *Cylindrocladium pteridis* (leaf spot and defoliation) (Guimarães et al., 2010), should also be considered in diallel designs along with individuals from fast-growing species such as *E. grandis*, to evaluate all possible crosses and identify the presence of heterosis of these crossings (Souza Junior, 2001).

The use of *E. pellita* to compose hybrids in combination with highly productive species has potential, as *E. pellita* has shown high levels of resistance to diseases caused by *Cylindrocladium* (Rodas et al., 2005). This is particularly important for regions in which the disease affects productivity, for example in Northern Brazil, which is also a forest frontier region (Assis et al., 2015). Therefore, it is important that the variability of *E. pellita* populations existing in Brazil is maintained, and forest improvement activities to adapt the species must not weaken existing tolerance (Silva et al., 2018).

The fact that the groupings of the provenances by UPGMA (Figure 3) and Tocher's method (Table 3) are similar demonstrates that both methods were able to identify the distances between each provenance pair. However, UPGMA was more robust as it was able to separate the provenances into four groups, instead of three as with Tocher. The robustness of UPGMA in the grouping of provenances is shown through the CCC = 0.86, which is higher than Tocher's method CCC = 0.69 (Figure 4). This coefficient describes the similarity between the Mahalanobis distance matrix and the cophenetic matrix of each evaluated method, that is, it indicates how representative the Mahalanobis distance is in the groups formed (Sokal and Rohlf, 1962).

For the progeny, the magnitude of the CCC (Figure 4) suggests that the groups formed by Tocher's method (CCC = 0.89) are more reliable than the groups formed by UPGMA (CCC = 0.63); according to Rohlf (1970), a CCC less than 0.7 indicates inadequate or limited reliability of the cluster.

Despite the fact that height had a greater relative importance in estimating the genetic distance between progenies and provenances (Figure 5) and volume was not very informative, this result must be considered with caution. At first glance, discarding the analysis of volume in estimating genetic distance would be indicated; however, the impact of discarding volume, or even DBH, must be weighed accordingly, given that it is one of the main traits used in selection. Santos et al. (2016) found contradictory results for *Pinus caribaea* var. *hondurensis* in terms of quantitative traits, where volume was the trait that contributed the highest percentage for genetic divergence (98%), followed by DBH (1.51%) and height (0.26%).

Considering the distribution of progenies in relation to BLUPs in the biplot graph (Figure 6), the fact that there is no evident pattern of progeny distribution in relation to provenances demonstrates a certain homogeneity across provenances, with greater divergence within provenances. The PCA analysis for provenances corroborates the results observed in the grouping methods, where Connl. A and Orchard were different from other provenances. The poor performance for the height of the Connl. A is likely one of the reasons for the distinct divergence from the other provenances, since height was the trait that most contributed to estimates of genetic divergence.

CONCLUSIONS

Groups of progenies identified by Tocher's optimization method based on the Mahalanobis distance are more consistent than those obtained through UPGMA. Therefore, we recommended that crosses are performed between divergent and productive parents in order to determine through the resulting progenies if the groups identified by Tocher's method can be used as heterotic groups in the hybridization of *E. pellita*. Australian provenances showed low genetic divergence, while the provenance from Papua New Guinea was the most genetically distant among all those studied. Height was the most important trait in estimating genetic distance.

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