Abstract

Our aim was to verify the effect on gain and genetic diversity through the restriction of the number of trees per family in selection, in order to compose an elite population of *Eucalyptus urophylla* in two trials under distinct management levels. We studied 166 open-pollinated families of *E. urophylla* in Anhembi, Sao Paulo State, Brazil under commercial practices, and the same families in Selvíria, Mato Grosso do Sul State, Brazil under lower management level (mainly no mineral fertilization). Mortality, height and diameter at breast height (DBH) were measured. DBH was analyzed by the REML/BLUP to select the best 25 trees, with four levels of tree restriction per family (no restriction; 1; 2 and 3 per family). We evaluated heritability; genetic gain and effective size of number of total and private alleles; observed and expected heterozygosity; coancestry and fixation index. A large difference in survival (48 and 83 %) and productivity (MAI of 26 and 44 m$^3$ha$^{-1}$y$^{-1}$) was observed between trials due to the different levels of management applied. The highest restriction in number of individuals per family caused a small decrease in gain, corresponding to 7 % in the more productive trial and 3 % in the less productive one. Observed and expected heterozygosity, coancestry and fixation index were not significantly (lower than 5 %) affected by the restriction in both sites. The restriction of one tree per family allowed different alleles to be kept in the selected population and higher effective population size in order to insure variation for the next generations.

Keywords: Elite population, genetic diversity, heritability, inbreeding, tree breeding

Introduction

*Eucalyptus urophylla* S. T. Blake, or Timor mountain gum, is an important economic tropical tree species around the world as a pure species or in hybrid combination (Denison and Kietzka, 1993; Harwood, 2011). In Brazil, the species is one of the most important exotic planted trees, being introduced for improving stress tolerance in the 1970’s, to which the then usually planted *Eucalyptus grandis* was not adapted (Ferreira, 2015). Recently, commercial plantations in Brazil today yield a mean of 40 m$^3$ha$^{-1}$y$^{-1}$ (Gonçalves et al., 2013), based mainly on highly selected hybrid clones between the species *E. urophylla* x *E. grandis*.

The focus of tree improvement programs is to increase the productivity of commercial stands. In order to do that it is important to select the correct commercial genotypes, while considering environmental conditions and management practices (Gonçalves et al., 2013). However, high selection intensity to increase productivity may decrease genetic diversity and effective population size, as observed in individual selection of *E. grandis* (Oda et al., 1989) and *Eucalyptus benthamii*, which already has a narrow genetic base in its natural occurrence (Costa et al., 2016). It is important to keep a balance between gain and diversity in selection. Genetic diversity is needed in order to advance genetic gain and it is a buffer.
against biotic and abiotic stress which strongly decreases forest productivity (Jurskis, 2005; Wingfield et al., 2008; Brawner et al., 2013; Garcia et al., 2014; Silva et al., 2016; Silva et al., 2017). Genotype is one factor influencing tree growth. Other factors of growth decrease under stress are derived from silvicultural practices and environmental conditions (Brawner et al., 2011; Silva et al., 2013; Campoe et al., 2016).

Therefore, balancing selection intensity for high productivity with the size of the population being improved is a challenge to breeders in order to compose an elite population with a minimum of inbreeding. In such situations, a quantitative approach and genetic markers can be useful to estimate genetic variation, heritability, genetic gains, and effective number of parent trees (Kumar and Richardson, 2005; Steane et al., 2006; Payn et al., 2008; Resende, 2016). Thus, the aim of this study was to evaluate the effects of selection on the number of families and trees per family. We specifically wanted to determine how the restriction in number of trees selected per family affects genetic gain, effective size and genetic diversity.

Materials and Methods

**Sites and families’ trials**

Two trials with 166 open-pollinated families of *E. urophylla* from several Brazilian provenances were evaluated (Table 1). Both trials were established, using a randomized complete block design, with 166 treatments, four blocks and linear plots of six trees at 3 x 2 m spacing. Trial 1 was planted in Anhembi, São Paulo State (22° 28’ S, 48° 07’ W, altitude of 472 m), Climate: Aw; annual mean temperature of 21.8° C and annual mean precipitation of 1300 mm) under commercial Brazilian plantation practices as described by Gonçalves et al. (2013). It was done with minimum soil cultivation, mineral fertilization (during planting and after planting), leaf-cutting ant (baits application) and weed control (herbicides). Trial 2 was established in Selvíria, Mato Grosso do Sul State (20° 21’ S, 51° 24’ W, altitude of 375 m), Climate: Aw, annual mean temperature of 24.8° C, and annual mean precipitation 1300 mm) under lower level of silvicultural practices, such as no fertilization with weed and ant control only at planting. Survival, tree height (H) and DBH were measured in order to calculate stem volume using a form factor (f) of 0.5 at half the rotation age (close to three years) and at the harvest age (between five and six years) in both trials.

\[
Vol = \pi(DBH/2)^2 Hf
\]

**Analysis of variance and estimates of genetic parameters**

Our estimates were based on the selection of 25 trees with high DBH at four different levels of selection among and within families: i) no restriction in the number of trees per family; ii) selection of 1 tree per family; iii) selection of up to 2 trees per family; iv) selection of up to 3 trees per family. Variance components and genetic parameters were estimated for DBH by the method of maximum restricted likelihood and best unbiased linear prediction (REML/BLUP), using the Selegen-REML/BLUP software (Resende, 2016). In the analysis, the open-pollinated families were assumed to be half-sib families. We used the mixed model that considers the effect of replications (r) and measurement (age)-repetition (environment) (m) as fixed; and additive genetic variance (a), plot variance (p), environment (s), genotype x environment interaction (i) and error (e), as random (Resende, 2016):

Individual analyses:

\[
y = Xr + Za + Wp + e
\]

Joint analyses:

\[
y = Xm + Za + Wp + Ts + Qi + e
\]

Where capital letters represent the incidence matrixes for replications (r), families (a), and trees per family. We specifically wanted to determine how the restriction in number of trees selected per family affects genetic gain, effective size and genetic diversity.

**Table 1**

**Description of provenances, origins and breeding level**

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Origin</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flores and Timor, MG</td>
<td>SSO 2 to 4th generation</td>
</tr>
<tr>
<td>2</td>
<td>Itapura, MG</td>
<td>Flores and Timor</td>
</tr>
<tr>
<td>3</td>
<td>Arac, SP</td>
<td>Flores and Timor</td>
</tr>
<tr>
<td>4</td>
<td>Lençóis Paulista, SP</td>
<td>Remexco - Timor</td>
</tr>
<tr>
<td>5</td>
<td>Altinópolis, SP</td>
<td>Flores and Timor</td>
</tr>
<tr>
<td>6</td>
<td>Itamaranduba, MG</td>
<td>Flores</td>
</tr>
<tr>
<td>7</td>
<td>Teixeira de Freitas, BA</td>
<td>SPA 1st generation</td>
</tr>
</tbody>
</table>

\( \sigma^2_a \) is the number of families; SSO is Seedling Seed Orchard; SPA is Seed Production Area.

\( a = \) mean of family in a determined block (mean plot);

\( a = \) mean of family in a determined trial;

\( a = \) mean of family in a determined generation.

\( \sigma^2_p \) is the narrow sense mean family heritability:
Analyses of microsatellite loci

DNA extraction and genotyping were carried out at Genomax/Heréditas (Tecnology in DNA Analysis, Brasília Federal District, Brazil). DNA was extracted from 100-150 mg leaf material from 72 (40 in Anhembi + 32 in Selvíria) selected trees using the method of Doyle and Doyle (1987). Nineteen microsatellite loci were amplified based on Faria et al. (2010): EMBRA2, EMBRA3, EMBRA10, EMBRA11, EMBRA12, EMBRA21, EMBRA28, EMBRA32, EMBRA38, EMBRA45, EMBRA63, EMBRA128, EMBRA157, EMBRA204, EMBRA210, EMBRA681, EMBRA915, EMBRA1144 and EMBRA1349. One loci (EMBRA333) showed some individuals that did not amplify, so the locus was not considered in the analysis. The loci EMBRA2, EMBRA3, EMBRA10, EMBRA11, EMBRA12, EMBRA28, EMBRA38, EMBRA63, EMBRA128, MBRA157, EMBRA204, EMBRA210, and EMBRA681, present regular Mendelian segregation and are not genetic linked (Pupin et al., 2017).

Analyses of genetic diversity

Genetic diversity was characterized by the total number of alleles (k); mean number of alleles per loci (A); observed (H_o) and expected (H_e) heterozygosity and fixation index (F_i), using FSTAT software (Gouded, 1995). The statistical significance of F values was determined through the permutation of alleles among individuals, associated to a Bonferroni correction for multiple test (95 %, α= 0.05). Estimates of gene frequencies among individuals, associated to a Bonferroni correction for values was determined through the permutation of alleles using the SPAGEDI 1.3 software (Hardy and Vekemans, 2002). Negative values of F_s and θ were assumed as being zero, because θ was derived based on the inbreeding coefficient and the true co-ancestry coefficient between pairwise individuals (both indices range from zero to 1), and estimate of F_s and θ_s using a correlation coefficient (ranging from -1 to 1). The effective population size was calculated following Cockerham (1969) with base in the sample variance in gene frequencies by, Ne = 0.5/θ (Sebbenn, 2002; Gonzaga et al., 2016).

Results

Survival and mean annual increment (MAI) were higher in Anhembi than in Selvíria. The MAI was 2.4 (36/15) times higher for the rotation age and 1.7 (44/26) times higher at harvest age in Anhembi. The individual heritability was higher in Selvíria and increased from the middle of rotation to the harvest age. Sites genotypic correlation was 0.67 and the coefficient of determination of GxE was low (Table 2).

Table 2
Survival, mean annual increment volume (MAI) and narrow sense individual heritability (H_s^2) in Anhembi, Selvíria at half the rotation age and at harvest age. Narrow sense individual heritability (H_s^2), coefficient of determination for G x E (C_r^2) and genotypic correlation (r_g x site) in joint analysis in both sites and ages

<table>
<thead>
<tr>
<th></th>
<th>Anhembi</th>
<th>Selvíria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>Half rotation</td>
<td>Harvest age</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>83</td>
</tr>
<tr>
<td>MAI (44 m^3 ha^-1)</td>
<td>36</td>
<td>44</td>
</tr>
<tr>
<td>H_s^2 (KE)</td>
<td>0.13 (0.04)</td>
<td>0.16 (0.04)</td>
</tr>
</tbody>
</table>

Joint analysis (ages and trials)

- k^2 = 0.19 (0.02)
- r_g = 0.024
- F_s x site = 0.67

The number of selected families (n_s), genetic gain in percentage (g%), and the effective population size (N_e) with no restriction and 3, 2 and 1 selected individuals per family were higher in Selvíria than in Anhembi. In Selvíria, g% ranged between 78.5–91.8 % and N_e were up to 10.2 % higher than in Anhembi. The effect of restriction was stronger in Anhembi for number of families (n_f : 13–25) and for N_e (18.7–25) than for Selvíria (n_f : 18–25; N_e : 20.6–25). The coincidence of families in Anhembi selection with those in Selvíria ranged from 44–47 %. Coincidence in the selection of Selvíria with those in Anhembi improved from 33 % without restriction to 44 % with 1 tree selected per family restriction (Table 3).

In Anhembi the total number of alleles (k = 226 to 253) and mean number of alleles per loci (A = 11.9 to 13.3) increased with the increase of the restriction level (Table 4). The observed
Table 3
Results of the number of families ($n_f$), family coincidence ($fc$), genetic gain ($g$), effective size (N_e), co-ancestry ($\Theta$) for selection with and without restriction of individuals per family in Anhembi and Selvíria

<table>
<thead>
<tr>
<th></th>
<th>Anhembi</th>
<th>Selvíria</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n_f$</td>
<td>$%$</td>
<td>$%$</td>
<td>$%$</td>
</tr>
<tr>
<td>$\Theta$</td>
<td>$%$</td>
<td>$%$</td>
<td>$%$</td>
</tr>
<tr>
<td>N_e</td>
<td>$%$</td>
<td>$%$</td>
<td>$%$</td>
</tr>
<tr>
<td>g</td>
<td>$%$</td>
<td>$%$</td>
<td>$%$</td>
</tr>
<tr>
<td>1 tree selected</td>
<td>15.1%</td>
<td>18.7%</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

Note: other populations (selection restriction) had no private alleles.

Table 4
Results of genetic diversity in Eucalyptus urophylla under different restriction of the number of individuals per family in Anhembi and Selvíria.

<table>
<thead>
<tr>
<th></th>
<th>Anhembi</th>
<th>Selvíria</th>
<th>Anhembi</th>
<th>Selvíria</th>
<th>Anhembi</th>
<th>Selvíria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of alleles: $k$</td>
<td>226</td>
<td>235</td>
<td>225</td>
<td>235</td>
<td>226</td>
<td>235</td>
</tr>
<tr>
<td>Mean number of alleles: $A$</td>
<td>11.9</td>
<td>12.4</td>
<td>11.8</td>
<td>12.4</td>
<td>11.9</td>
<td>12.4</td>
</tr>
<tr>
<td>$95%$ CI: $R$</td>
<td>10.3–13.5</td>
<td>10.6–14.2</td>
<td>10.2–13.4</td>
<td>10.6–14.2</td>
<td>10.7–14.1</td>
<td>10.5–14.1</td>
</tr>
<tr>
<td>Number of private alleles: $P_a$</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Observed heterozygosity: $H_o$</td>
<td>0.77</td>
<td>0.73</td>
<td>0.78</td>
<td>0.73</td>
<td>0.79</td>
<td>0.73</td>
</tr>
<tr>
<td>$95%$ CI: $H_o$</td>
<td>0.72–0.82</td>
<td>0.65–0.81</td>
<td>0.73–0.84</td>
<td>0.65–0.81</td>
<td>0.73–0.85</td>
<td>0.64–0.81</td>
</tr>
<tr>
<td>Expected heterozygosity: $H_e$</td>
<td>0.84</td>
<td>0.85</td>
<td>0.85</td>
<td>0.85</td>
<td>0.86</td>
<td>0.85</td>
</tr>
<tr>
<td>$95%$ CI: $H_e$</td>
<td>0.81–0.88</td>
<td>0.82–0.89</td>
<td>0.82–0.89</td>
<td>0.82–0.89</td>
<td>0.83–0.90</td>
<td>0.82–0.89</td>
</tr>
<tr>
<td>Fixation index: $F$</td>
<td>0.09*</td>
<td>0.15*</td>
<td>0.08*</td>
<td>0.15*</td>
<td>0.08*</td>
<td>0.15*</td>
</tr>
<tr>
<td>$95%$ CI: $F$</td>
<td>0.03–0.14</td>
<td>0.06–0.24</td>
<td>0.02–0.14</td>
<td>0.06–0.24</td>
<td>0.02–0.15</td>
<td>0.06–0.24</td>
</tr>
</tbody>
</table>

Discussion
The difference in survival and productivity between the sites is mainly due to differences in silviculture practices, since both sites are suitable for E. urophylla (Flores et al., 2016). For the proper development of eucalypts, it is mandatory to use the correct pest and weed control and fertilizer (Gonçalves et al., 2013; Ferreira Filho et al., 2015). In Anhembi, the test was established using commercial Brazilian plantation practices, such as mineral fertilization during and after planting; leaf-cutting ant (use of bait) and weed control (herbicides). In Brazil, eucalypt plantations are very productive and one of the reasons is the adequate use of fertilizer (Silva et al., 2013a; Laclau et al., 2013; Melo et al., 2015). Mineral fertilization was not done in Selvíria.

The absence of soil fertilization probably resulted in high selection in Selvíria due to worse growth conditions, so individuals and families more adapted to this condition were favored, yielding higher additive genetic variance between

Table 5
Number of families ($n_f$) and trees ($n_t$) selected, and loci ($n_l$) with private alleles ($P_a$).

<table>
<thead>
<tr>
<th></th>
<th>$n_f$</th>
<th>$n_t$</th>
<th>$n_l$</th>
<th>$P_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anhembi: No restriction</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>1.3</td>
</tr>
<tr>
<td>Anhembi: 1 tree selected</td>
<td>8</td>
<td>8</td>
<td>28</td>
<td>3.5</td>
</tr>
<tr>
<td>Selvíria: 1 tree selected</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Note: other populations (selection restriction) had no private alleles.
individuals and increasing the values. As the same selection intensity was applied in both trials, the difference in genetic gain between trials was due to the lower heritability in Anhembi (0.16) than in Selvíria (0.22), resulting in largest genetic gains in Selvíria. In spite of growth discrepancy between individuals in Selvíria, some trees from several families ranked higher than the mean. Genetic gain was measured in percentage points above the mean of the population traits; the mean being low, trees with higher values for the traits resulted in high genetic gains.

Productive and stable families across sites and age in the joint analyses were found; there was only a change in the scale due to the good correlation between the trials, as already discussed about the same families of E. urophylla across six trials (Pupin et al., 2015).

Restriction in the number of individuals per family caused a decrease in genetic gain. It corresponded to 1.5 % (14.9-13.4 %) of total gain in Anhembi and 0.9 % (26.6-25.7 %) in Selvíria. A decrease in genetic gain was expected when applied at lower selection intensity (Borralho et al., 1992). In our study, the decrease was due to small difference among individual additive genetic values of individuals belonging to different families. On the other hand, the effective population size (N_e) in Anhembi was 25.2 % and in Selvíria was 16.8 % higher with the restriction of one individual selected per family than under no restriction. This is clearly a result of the numbers of families and individuals within family. The coincidence of families in the selection in Selvíria with no restriction had the smallest value of family coincidence, showing a specific behavior of the selected families. The estimates are based on the final selection of 25 plus trees; with no restriction, the number of families was lower, causing some individuals to be selected in the same family.

After the selection, there were some related offspring in the same family. As N_e is determined by the co-ancestry among individuals and inbreeding within individuals; and since without restriction some related individuals were selected, this resulted in a higher group of co-ancestry and lower N_e than when selecting one tree per family. Maintenance of a high N_e is important to avoid the loss of genetic diversity and allele fixation, which permits advancing genetic gain and is a buffer against stress (Lande and Barrowclough, 1987; Jurskis, 2005; Brawner et al., 2013; Bertoncini et al., 2017).

The selection of more than one tree per family may result in mating among related individuals and inbreeding in the improved seed produced. Inbreeding in eucalypt species causes negative effects due to inbreeding depression; such as decrease in survival, seed production, and growth under field conditions and in tree stem form (Hardner and Potts, 1995; Brawner, J.T., Lee, D.J., Meder, R., Almeida, A.C., Dieters, M.J. (2013). Classifying plant resistance to pests of Eucalyptus in managed forests and plantations. Euphytica 191:403–414. Available at http://dx.doi.org/10.1007/s10681-013-0892-4.

The higher restriction generated an increase in the number of private alleles in both trials that decrease the population genetic bottlenecks effect caused by changes in allelic frequencies through selection (Luikart et al., 1998). However there was one exception: the population no restriction in Anhembi (Anhembi-No restriction), in which private alleles appeared; these must have arrived with foreign pollen (open pollination).

Conclusion

The restriction of one tree per family, resulting in a slight decrease in the genetic gain, still allowed for different alleles to be kept in the selected population and higher effective population size; which insures variation for the next generations. This was more pronounced in the more productive trial.

Acknowledgement

Thanks are due to Amcel, Arborgen, Aperam, Copener, Duratex, Eldorado, Fibria, International Paper, Jari, Klinbin, Lwarcel, Montes del Plata, Palmosalia, Stora Enso, Vallowouec, Veracel and Votoratín Siderurgia (companies which are part of the Genetic Improvement Cooperative Program - IPEF), Suzano, University of São Paulo – USP/ESALQ, São Paulo State University – UNESP, COTEC-Instituto Florestal (Forest Institute in São Paulo State), and FAPESP (15/15651-2) for providing information, seeds, land and financial support.

References


Unauthentifiziert | Heruntergeladen 08.09.19 01:39 UTC