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Statistical strategies design based on competition classes of *Eucalyptus* clones

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ABSTRACT

A detailed knowledge of different types of trial designs is essential to establish adequate *Eucalyptus* breeding strategies. This study compared clone's development in single-tree-plot (STP) and square-plot (SP) experiments to analyze differences in estimated genetic parameters, ranking, predicted genotypic value and competition ability. Experiments were carried out in the CMPC Celulose Riograndense Company, in the state of Rio Grande do Sul, Brazil. A total of 239 clones were used, 214 of which were common to the STP and SP trials. Results showed that STP is a very promising trial design for accurately ranking the genotypes. The STP and SP tests showed high coincidence in ranking selection, but the best clone yields were overestimated in the STP trial and the yields of the worst clones were underestimated in the STP trial. Therefore, an STP design should be used in initial and intermediary clonal tests, and an SP design should be used in the final stages of a breeding program, in order to estimate the clonal yield at a semi-operational scale. The estimated yield decreasing of clones planted in SP compared to STP is 26%. This value should be used in future STP experiments in the same area and with the same germplasm evaluated in this work. In addition, this research identified classes of aggressive clones, sensitive clones, and clones that were homeostatic to the competitiveness effect. Based on this classification, the optimized multiclonal plantation were suggested aiming to maximize yield by using aggressive and homeostatic clones.

1. Introduction

Developing more productive clonal forests is one of the major challenges of a breeding program. A successful generation of superior genetic materials depends on realistic approaches about plant yield (Osorio et al., 2003; Resende et al., 2005; Crossa et al., 2017). In the final stages of a forest-breeding program, clonal tests have defined which genotypes can be commercially exploited (Scarpinati et al., 2009; Mendes et al., 2013). To conduct a meaningful clonal experiment, it is essential to have detailed knowledge about the best trial design, a correct estimation of genetic parameters, an establishment of adequate improvement strategies, and to be able to predict gains and select the best genetic materials (Osorio et al., 2003; Binkley et al., 2017). A plant breeding dilemma is to select on a mixed population and expect high yielding in pure stands (Griffing, 1966).

Experimental designs must allow genetic selection to occur in an optimized and accurate way (Cappa and Cantet, 2006; Mendes et al., 2013). A commonly used method is establishing field tests with a single-tree-plot (STP) as the experimental unit (Jansson et al., 1998). Several authors have used STP trial designs for research on *Eucalyptus* (Petroli et al., 2012; Bartholomé et al., 2013; Suontama et al., 2015; Li et al., 2016; Resende et al., 2016; Santos et al., 2016). The STP design allows for small experimental areas and the testing of a large number of genotypes with many replicates, leading to increased selective accuracy, high selection intensity, and elevated genetic gains (Jansson et al., 1998; Resende, 2002; Zhang et al., 2015). Despite these advantages, the use of STP trial designs can produce biased estimates of genotype yielding, masking the real performance of individuals (Stanger et al., 2011; Pavan et al., 2011). Thus, the conditions of superior materials selection may differ from those of commercial stands, affecting the success of the breeding program as a result of inter-genotypic competition (Pavan et al., 2011).

To ensure a realistic value of yield in experimental evaluations, square-plot (SP) tests can be used (Stringer et al., 2011). The SP design relies on large-block plots and allows for symmetric competition of related individuals or clones (Pavan et al., 2011; Zhang et al., 2015).

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The use of multiple-tree-plot (MTP) designs mimics the operational deployment that occurs in large tracts of genetically identical individuals in commercial stands (Jansson et al., 1998; Zhang et al., 2015). Unfortunately, SP trials often include a limited number of genotypes with little or no replication, and require large experimental areas (Stanger et al., 2011; Stringer et al., 2011; Zhang et al., 2015). With few genotypes being tested, the chance of identifying material that is more productive than the control decreases substantially. In addition, the small number of replications results in low selective accuracy, which biases the genetic merit assessment of the individuals (Cappa and Cantet, 2006; Stanger et al., 2011).

Few *Eucalyptus* breeding studies (Scarpinati et al., 2009; Stanger et al., 2011) have used a small number of clones to compare STP with MTP designs. Aside from these studies, information about the performance of a large number of *Eucalyptus* clones in STP and SP (5 × 5 m) designs is extremely scarce. The complexity of establishing distinct trials containing hundreds of clones has prevented attainment of a robust conclusion about genetic correlation, ranking, predicted genetic gain, and predicted genotypic value differences between STP and SP designs (Cappa and Cantet, 2006). To identify the optimal breeding program strategy, it is therefore imperative to investigate the differences between these two types of trial designs (Silva et al., 2012).

Considering the need for an enhanced knowledge about *Eucalyptus* clone performance and ordering between STP and SP trial designs, the goals of this study were to: (i) use 214 *Eucalyptus* clones to estimate genetic parameters within STP and SP experiments; (ii) determine coincidence index and genetic correlation between these two trial designs; (iii) predict the genotypic value and genetic gain with selection using the two designs; (iv) quantify the yield difference between *Eucalyptus* clone performance in STP and that in SP trial designs; and (v) investigate the competition ability of clones.

2. Material and methods

2.1. Experimental design

Experiments were conducted in the CMPC Celulose Riograndense Company, in the municipality of Arroio dos Ratos (Lat 30°03′36,26″ S, Long 51°44′38,12″ W, average temperature 19.5 °C, annual rainfall 1320 mm, and 79 m), located in the state of Rio Grande do Sul, Brazil (Cfa climate, according to the Köppen classification). The experimental area was considered by the company as homogeneous in terms of fertility gradient and altitude.

In 2010, 235 *Eucalyptus* clones were planted in an STP randomized block design, using 20 replications. Seedlings were planted within a 4.0 × 2.25 m grid. The clonal tests evaluation was performed when the trees were 4 (2014) and 5 years old (2015). These clonal tests included three experiments at the same site for all trials.

An SP design using 218 *Eucalyptus* clones with 25 trees per plot, with one replication was established in 2010 at the same site as that of the STP trial. Seedlings were planted within a 4.0 × 2.25 m grid and the SP experiment was evaluated when the trees were 5 years old (2015). Of the 218 clones used in the SP trial, 214 were common to the STP experiment. Thus, there are 214 clones common among the two experiments, which will be the focus of this work.

2.2. Trait measurements

Tree-growth data were collected at 4 and 5 years of age in the STP trial and at 5 years of age in the SP trial. Attributes measured included tree diameter at breast height (DBH, cm) and total height (TH, m). DBH was measured with the aid of a diameter tape, and TH was measured with a relascope.

Tree volume (VOL, m^3) was calculated according to Schumacher and Hall (1933) as described below:

$$VOL = \frac{\pi \times DBH^2 \times TH \times f}{40{,}000} \quad (1)$$

where VOL = volume of trees in m^3; DBH = diameter at breast height in cm; TH = total height in m; f = taper factor adopted by the company (0.45); and π = the ratio between the circumference and diameter of a circle (3.14159).

The mean annual increment (MAI, $m^3\,ha^{-1}\,y^{-1}$) was calculated using the VOL of individual trees with a spacing of 4.0 × 2.25 m (9 m^2), extrapolated to 1 ha and divided by age.

For the yield at 4 years of age, MAI was calculated as:

$$MAI = \frac{VOL \times 10{,}000}{36} \quad (2)$$

where MAI = mean annual increment in $m^3\,ha^{-1}\,y^{-1}$; VOL: = volume of individual trees in m^3, at 4 years of age.

For the yield at 5 years of age, MAI was calculated as:

$$MAI = \frac{Vol \times 10{,}000}{45} \quad (3)$$

where MAI = mean annual increment in $m^3\,ha^{-1}\,y^{-1}$; VOL: = volume of individual trees in m^3, at 5 years of age.

2.3. Statistical analysis

For the STP trial, the statistical model used was y = Xr + Zg + Wb + e, where: y, r, g, b, and e are vectors of data, general mean (fixed), genotypic effects (random), block effects nested in experiment (random), and random errors, respectively. In addition, X, Z, and W are the incidence matrices for r, g, and b. In order to analyze the SP experiment, the statistical model used was y = Xr + Zg + e, where: y, r, g, and e are vectors of data, general mean (fixed), genotypic effects (random), and random errors, respectively. In addition, X and Z are the incidence matrices for r and g. This analysis of both experiment was conducted using the Selegen-REML/BLUP software (Resende, 2016). Pictures were made using the package ggplot2 in the software R (R Core Team, 2017).

Using the genotypic values of all clones in the STP and SP trials, coincidence ranking and genotypic correlations between these two designs were made for DBH, TH, and MAI. In addition, using the predicted direct effect (τ) of a clone as its genotypic value in the STP trial, the indirect effect (φ) was calculated as the difference between the genotypic value in the SP trial and τ. Correlation between direct and indirect effects (r_τφ) was evaluated to determine the effect of inter-genotypic and intra-genotypic competition.

The indirect effect was transformed in percentage to obtain the yield decrease of a clone when it's planted in a STP compared to its development in SP trial, according to the formula below:

$$YD(\%)_i = \frac{\varphi}{\tau} \times -100 \quad (4)$$

Where: $YD(\%)_i$ = yield decrease in percentage of a clone when planted in a SP compared to its development in STP; τ = predicted direct effect of a clone as its genotypic value in the STP trial; φ = indirect effect of a clone in its neighbors given by the difference between the genotypic value in the SP trial and τ.

The yield decreasing of the most ten aggressive clones can be given according to the formula below:

$$YD(\%)_{10} = \frac{\sum_i^{10} YD(\%)_i}{10} \quad (5)$$

Where: $YD(\%)_{10}$ = estimated yield decreasing of the most ten aggressive clones; $YD(\%)_i$ = yield decreasing in percentage of a clone when planted in a SP compared to its development in STP.

So, with the factor named $YD(\%)_{10}$, it is possible to calculate the estimated yield of a clone in monoclonal plantation if this clone is evaluated in a STP trial design, discounting the competitiveness effect,