


Minimum number of measurements for an accurate evaluation of growth traits in eucalyptus species¹

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¹ This work is part of the research project of the last author

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Editors:

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Submitted: December 10th, 2021.

Accepted: September 5th, 2022.

ABSTRACT

The objective of this research was to identify the most effective method to estimate the repeatability coefficients in species of eucalyptus and to predict the minimum number of measurements necessary for growth traits. The experimental design was randomized blocks, with five species, with four repetitions. Data were collected from five measurements during the period from 2014 to 2016, evaluated according to the diameter, chest height and total height. The repeatability coefficient (r) was estimated using different strategies: analysis of variance (ANOVA), principal component analysis based on the correlation matrix (PCCOR), principal components based on the phenotypic variance and covariance matrix (PCCOV), and structural analysis based on the correlation matrix (SACOR). The PCCOR and PCCOV provide accurate estimates of the repeatability coefficient and the number of measurements required. At least five measurements are necessary to predict the real value, with a minimum accuracy of 80%.

Keywords: *Eucalyptus spp.*; diameter at breast height; total height; repeatability.

INTRODUCTION

In Brazil, eucalyptus field occupies about 6.97 million hectares, which corresponds to more than 77% of the total area of planted forests in Brazil⁽¹⁾ The wide availability of species means that the crop is adapted to Brazilian edapho-climatic conditions and is present in 23 states of the national territory,⁽²⁾ of which the regions of Minas Gerais, Mato Grosso do Sul, and São Paulo stand out, respectively.

⁽¹⁾ Due to the large increase in areas and advances in forest silviculture, Brazil has consolidated itself as a leading and important country in this sector.⁽³⁾ The increase in commercial eucalyptus plantations has been noticeable in Mato Grosso do Sul in the last decade, which is mainly due to the establishment of large companies in the paper and cellulose sector in the state.

During the selection process of eucalyptus trees, whether for cloning or hybridization, it is important to identify the genetic superiority of individuals. For this, many repetitions and measurements of the same individual are performed.⁽⁴⁾ Dimensioning the number of measurements to be carried out using the repeatability coefficient is necessary for forest species such as eucalyptus, as it generally provides the approximation of the maximum value of the heritability of a given trait.⁽⁵⁾ Such information makes it possible to direct forest plant breeding programs, as it allows foreseeing which is the smallest possible selection cycle and optimizing the financial and human resources for the process.⁽⁶⁾

The dimensioning of the number of measurements in *Pinus*⁽⁷⁾ and *Bertholletia excelsa*⁽⁸⁾ has allowed advances in plant breeding of forest species. Overall, these studies have demonstrated that multivariate methods are more comprehensive in relation to the univariate method, but they recommend that more research be carried out before generalizing these results. The choice of the species to be cropped is directly linked to the economic interest linked to the activity performed, so they are closely related to the structure, development and behavior of plants in the field.⁽⁹⁾

In Brazil, the species *Eucalyptus grandis*, *E. urophylla*, *Corymbia citriodora* and *E. camaldulensis* are among the most used. It is common for forest improvement programs to carry out inter-specific hybridizations between two or more species to obtain genotypes more adapted to the growing regions.⁽²⁾ In this sense, evaluating the number of measurements to be performed to select the most promising genotypes of different species is essential. Thus, the repeatability coefficient can help in choosing the top specimen

that will be ideal for planting. Therefore, the objective of this research was to identify the most effective method to estimate the repeatability coefficients in species of *Eucalyptus spp* and to predict the minimum number of measurements required for growth traits.

MATERIAL AND METHODS

Experimental design

The experiment was conducted between 2014 and 2016 crop seasons, at experimental area of the Federal University of Mato Grosso do Sul, Chapadão do Sul campus. The average altitude is 810 m, with latitude: 18 ° 46 '44' 'South and longitude: 52 ° 36 '59' 'West. The soil is classified as medium-textured red latosol. According to the Köppen classification system, the climate is tropical humid (Aw) with a rainy season from October to April and a dry season between May and September. The average precipitation varies from 750 to 1,800 mm year⁻¹ and the average annual temperature varies from 20 to 25°C.

Fertilization management was determined by chemical soil analysis: pH (CaCl₂): 4.9; organic matter: 31.5 g dm⁻³; phosphorus: 13.6 mg dm⁻³; hydrogen + aluminum (H + Al): 5.4; potassium: 0.29 cmol dm⁻³; calcium: 2.8 cmolc dm⁻³; magnesium: 0.5 cmolc dm⁻³; cation exchange capacity (CTC): 9.0 cmolc dm⁻³; base saturation: 39.9%. The proportions of clay, sand and silt were 46, 46 and 8%, respectively. Phytosanitary management such as crowning, weeding, ant control and herbicide application were carried out when necessary.

The experimental design was a randomized block design, with 20 plants within each experimental plot, with four replications. The treatments consisted of five species of *Eucalyptus*: *E. camaldulensis*, *E. urophylla*, *E. saligna*, *E. grandis* and *E. urograndis*.

Variables evaluated

The mean diameter at breast height (DBH) and total height (Ht) were obtained by measuring five trees from each experimental unit. A measuring tape was used to measure the DBH, which made it possible to obtain the circumference at chest height, which was later converted to DBH (cm). Ht (m) was obtained with the aid of a Haglof hypsometer. Five measurements were made during August 2014, January and August 2015, January and August 2016. Figure 1 illustrates the weather conditions of the experimental area during the evaluation period.

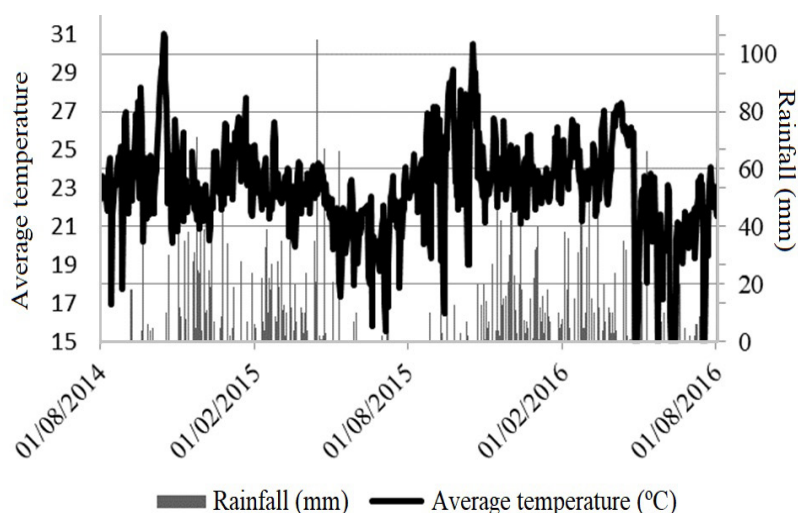


Figure 1: Weather conditions during the period from August 2014 to August 2016.

Statistical analysis

Initially, the data were submitted to ANOVA and the adopted statistical model considered two factors of variation:

$$Y_{ij} = \mu + S_i + M_j + \varepsilon_{ij} \quad (1)$$

where: Y_{ij} is the observation regarding i-th species of eucalyptus, in the j-th measurement; μ is the overall average; S_i is the random effect of the i-th species under the influence of the permanent environment; M_j is the fixed effect of the temporary environment on the j-th measurement; and ε_{ij} is the experimental error established by the temporary effects of the environment on the j-th measurement of the i-th eucalyptus species. The averages were compared by the Scott and Knott test at 5% probability of error.

To estimate the repeatability coefficient (r), four procedures were used: analysis of variance (ANOVA), principal components based on the correlation matrix (PCCOR), principal components based on the phenotypic variance and covariance matrix (PCCOV), and structural analysis based on the correlation matrix (mean r - SACOR) according to the procedures shown in Cruz et al.⁽⁵⁾

- For each trait, the minimum number of measurements necessary to predict the real value of the individuals was determined (η), based on a coefficient of determination (R^2) pre-established (80 and 85%), according to Cruz et al.⁽⁵⁾ Statistical analyzes of the data were performed with the aid of the Genes software.⁽¹⁰⁾

RESULTS AND DISCUSSION

The F test (Table 1) demonstrated genetic variability ($p < 0,01$) among eucalyptus species for all traits evaluated, indicating the possibility of selecting superior genotypes. Alves et al.⁽¹¹⁾, in an experiment with *Eucalyptus* spp. under different water availability, found that the traits of DBH and Ht responded positively, promoting proportional gains. It is worth mentioning that the water regime influences the material used since irrigation is traditionally used at the beginning of planting during the seedling establishment phase.

There was an increase in the traits evaluated over the five measurements (Figure 2). It is worth mentioning that *E. camaldulensis* (E1) had a better performance, presenting better averages for DBH and Ht. However, from the third measurement made in August 2015, the species *E. urograndis* (E2) and *E. grandis* (E5) showed improved performance, presenting the best averages of the variables at the end of the period. Data exposed in Figure 2, demonstrate that one of the species that stood out best in both DBH and Ht was E2. Such results are in agreement with those found by Silva et al.⁽²⁾, who evaluated the total height of the eucalyptus species, indicating the superior growth in Ht of *E. grandis*.

The choice of species to be used is essential to obtain high productivity in forest plantations. In this research, *E. grandis* stood out in relation to the others due to its growth in the region. According to Resende & Silva⁽¹²⁾, this species is one of the main species used in commercial forests due to its wide adaptation, tolerance to cancer, being raw material for pulp and paper and more recently as lumber for sawmills.

Table 1: Summary of analysis of variance for growth traits evaluated in five species of eucalyptus, between the period 2014 to 2016

Variation sources	GL	Diameter at breast height	Total height
Measurements	4	55,30*	128,71*
Species	4	0,86*	1,06*
Error	16	0,66	0,58
Coefficient of variation (%)		10,59	9,27

* Significant effect by F test at 1% probability of error; GL: degrees of freedom.

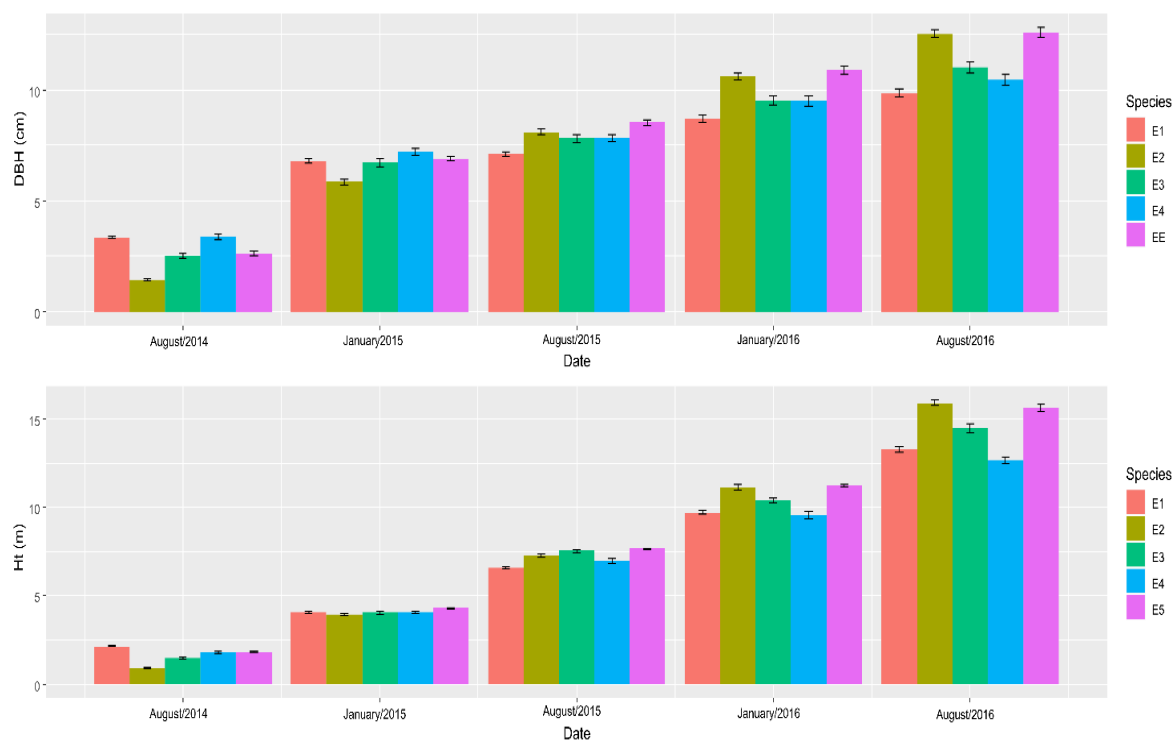


Figure 2: Mean values of diameter at breast height (DBH) and total height (Ht) for five species of eucalyptus evaluated from 2014 to 2016. E1: *E. camaldulensis*; E2: *E. urograndis*; E3: *E. saligna*; E4: *E. urophylla*; E5: *E. grandis*. The bars indicate the mean standard error.

Table 2 shows that the repeatability coefficients (r) estimated by the ANOVA method were lower than the other methods, regardless of the trait in question. The estimates of r using the principal components (PCCOR and PCCOV) were superior to the other methods, causing a smaller number of measurements necessary to identify species of superior *Eucalyptus* spp.

When the measurements factor is significant, the values can be affected by physiological, regular, irregular or systematic changes.⁽⁶⁾ This effect can vary in different ways and intensities between species of *Eucalyptus* spp. The ANOVA method, usually used to estimate r , does not allow isolating the measurement factor, which, when it occurs, is included in the experimental error, which increases its value and causes the underestimation of repeatability and overestima-

tion of the number of necessary measurements.⁽⁵⁾

The method based on structural analysis provided estimates of r and R^2 (Table 2) in magnitudes similar to ANOVA for both traits. This method estimates the repeatability coefficients by the arithmetic mean of the phenotypic correlations between the species, considering each pair of measurements.⁽¹³⁾ However, due to the non-linear behavior of the growth traits, r may be underestimated.

In this case, the principal component method, which takes into account the linear behavior of the trait, is the most recommended to estimate the r with greater accuracy. This is because in this methodology, the eigenvector, whose elements have the same sign and close magnitudes, is the one that expresses the tendency of species to maintain their relative positions in the measurement periods.⁽⁵⁾

Table 2: Estimates of the repeatability coefficient (r) and determination (R^2) for the traits diameter at breast height (DBH) and total height (Ht) evaluated in five species of eucalyptus, from 2014 to 2016

Trait	Parameter	ANOVA	CPCOR	CPCOV	AECOR
DBH	r	0,19	0,91	0,56	0,22
	R^2 (%)	54,21	98,04	86,28	58,72
Ht	r	0,06	0,83	0,71	0,06
	R^2 (%)	26,26	96,18	92,30	23,21

ANOVA: analysis of variance method; CPCOR: principal component analysis method based on the correlation matrix; CPCOV: principal component analysis method based on the phenotypic variance and covariance matrix; AECOR: structural analysis method based on the correlation matrix (mean r).

Similar results were obtained with other perennial species such as *Bertholletia excelsa*⁽¹⁴⁾, *Jatropha curcas*⁽⁶⁾, *Psidium cattleianum* and *Eugenia uniflora*.⁽¹⁵⁾ However, further work with perennial species is recommended for using methods based on the principal components (PCCOR and PCCOV) to estimate r , R^2 and the number of measurements to select genotypes with greater prediction of the actual value.

The values of r and R^2 by the PCCOR and PCCOV methods for both evaluated traits were higher than the limits ($r \geq 0.40$; $R^2 \geq 0.75$).^(16,17) The r values demonstrated good species' ability to repeat the expression of a trait throughout the measurements (Figure 2), while the R^2 estimates expressed the genetic superiority of the selected species.

In all the analysis methods, it was possible to notice that the DBH allowed a reduction in the number of measurements when compared to Ht (Table 3). Therefore, the selection for DBH in species of *Eucalyptus spp* can be carried out earlier in forest plant breeding programs, reducing time and labor costs. On the other hand, a minimum determination coefficient of 80% requires more measurements for Ht, which indicates a higher effect of the environment on this trait, that is, the environmental variance is greater in

relation to the genetic variance existing between species.

However, establishing a minimum number of measurements necessary for the evaluation of species allows greater effectiveness and use of time in forest research, since the evaluation time can be reduced but still maintain the accuracy of the results. Ht is considered one of the major targets in forest plant breeding programs and can be estimated directly or indirectly to provide the forest's productive potential.⁽¹⁸⁾ The traditional measurement of heights in forest species is considered an activity that demands time compared to measurements of diameter and circumference.⁽¹⁹⁾

Other errors can occur in the density of the stand, making it impossible to visualize a point, making it difficult for the operator to find the top of the tree, the inclination of the trees (especially in the second rotation eucalyptus species), and the presence of strong winds that can promote errors in measurements. This information must be observed and can influence the increase of the minimum coefficient of determination of this trait. Therefore, from the results reported here, eucalyptus breeding programs will be able to optimize their planning, since four and five measurements are needed to select superior genotypes for DBH and Ht, respectively.

Table 3: Estimates of the number of measurements associated with different determination coefficients (R^2) for the traits diameter at chest height (DBH) and total height (Ht) evaluated in five species of Eucalyptus spp, from 2014 to 2016

Trait	R^2	ANOVA	CPCOR	CPCOV	AECOR
DBH	80	17	4	5	17
	85	24	5	6	24
Ht	80	20	5	6	19
	85	27	6	7	25

ANOVA: analysis of variance method; CPCOR: principal component analysis method based on the correlation matrix; CPCOV: principal component analysis method based on the phenotypic variance and covariance matrix; AECOR: method of structural analysis based on the correlation matrix (mean r).

CONCLUSIONS

The PCCOR and PCCOV methods provide accurate estimates of the repeatability coefficient and number of measurements required. At least four and five measurements are required to predict the true value with a minimum accuracy of 80% for the DAP and Ht, respectively.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

The authors would like to thank the Universidade Federal de Mato Grosso do Sul (UFMS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) – Grant number 303767/2020-0, and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) TO 88/2021, 07/2022 and 172/2024, and SIAFEM 30478 and 31333 and SIAFIC 000921. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) – Financial Code 001.

The authors declare there is no conflict of interest in carrying the research and publishing the manuscript.

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