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ESTABILIDADE FENOTÍPICA DE CARACTERES MORFOLÓGICOS EM CLONES DE *Eucalyptus dunnii* MAIDEN

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RESUMO – (ESTABILIDADE FENOTÍPICA DE CARACTERES MORFOLÓGICOS EM CLONES DE *Eucalyptus dunnii* MAIDEN) *Eucalyptus dunii* é amplamente cultivada em regiões temperadas do mundo para a produção de madeira para celulose e existem programas de reprodução em muitos países onde uma combinação de mudas e clones são usados para testes genéticos e estabelecimento de plantações. No presente estudo foram comparados clones de *E. dunnii*, em uma área de plantio florestal localizada no municipio de Ponta Grossa, PR. A população estudada era formada por indivíduos de 36 meses de idade, ao longo do plantio de 4,0 há. Com o objetivo de determinar a estabilidade fenotípica dessa população foram considerados nesse trabalho caracteres morfológicos submetidos a tratamentos estatísticos. A análise estatística descritiva somente para altura das árvores, a variância ficou próximo de 0 (zero), nas outras características observou-se que a variância extrapolava o critério para verificação de estabilidade entre indivíduos. O teste de Kruskal-Wallis, para as mesmas características, entre parcelas, evidenciou diferenças entre os locais que pode se justificar pela composição química do solo. Dessa pode se verificar a existência de uma instabilidade fenotípica da população estudada.

Palavras-chave: Genótipo e meio ambiente. *Eucalyptus dunii*. Morfologia.

ABSTRACT – (PHENOTYPIC STABILITY OF MORPHOLOGICAL TRAITS OF *Eucalyptus dunnii* MAIDEN CLONES) *Eucalyptus dunii* is widely cultivated in temperate regions of the world for the production of pulpwood with breeding programs in many countries where a combination of seedlings and clones are used for genetic testing and establishment of plantations. In the present study, clones of *E. dunnii* located in forest plantations in the municipality of Ponta Grossa, PR were compared. The study population consisted of organism 36 months old along the 4.0 acre plantation. The population used underwent morphological statistical treatments to determine its stability. The height of the trees had a statistical variance close to 0 (zero), and thus was used as the criterion for stability check between organisms. The Kruskal - Wallis test for the same characteristics between plots showed differences between sites justified by the chemical composition of the soil. This can verify the existence of a phenotypic instability of the studied population .

Keywords: Genotype and environment. *Eucalyptus dunii*. Morphology.

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1. INTRODUCTION

Normally in commercial plantations, most traits of economic interest that are considered important for growing trees are quantitative in nature, with variations resulting from a combination of environmental factors, genetic effects or interaction (Namkoong et al., 1988). Until recently, accurate information about such characteristics and environmental influences were little studied (Bundock et al., 2008).

Many Eucalyptus species are planted in temperate regions of the world for the production of pulpwood, and there are breeding programs in many countries. Many crops are established using seedlings derived from selected matrixes, open pollinated orchid seeds, and a more recent method, systems of artificial pollination. Although there may be additional costs involved in the cloning process, this can potentially increase the genetic gain due to an improvement in selection (Costa e Silva et al., 2013).

The selection of clones with superior characteristics, ie with better growth, volume and content of cellulose, which are stable and adapted to a specific environment, is one of the main goals of eucalyptus breeding programs in Brazil (ROSY et al. , 2012).

Clone eucalyptus plantations have been successful in many countries (Cossalter; PYE - SMITH, 2003; FONSECA et al. 2010; KIEN et al., 2010). The *E.dunii* forestry is used for cloning since it is easily propagated using vegetative methods and micro and macropropagation.

The characteristics related to crop production are under genetic control of the organisms itself, the environment in which it is grown and the interaction between these two factors. The different phenotypic responses to changes in environmental conditions result in different behaviors of genotypes, characterizing the interactions. If the expression of a given genotype depends on the genes and the environment in which it is evaluated, the GE interaction should be another factor to consider in the analysis. Thus , improving the selection process also depends on this interaction, unless the unexpected result in performance of a tested organism (YAMAMOTO, 2006) occurs.

Squilace (1969) demonstrates some factors that would be directly related to the genotype x phenotype interaction. Factors that can influence interactions are differences within small areas, sites or a

region; between repetitions in time, artificially created organisms, cultivation and spacing. Shelbourne and Campbell (1976) reports that other influential factors include the environment, soil, climate, photoperiod, nutrition, competition, disease, pests and cultural effects .

Burdon (1977) points out that it is well known that changes in the behavior of environments effect different genotypes in different ways and that genotypes that are superior in one environment may not be necessarily superior in any other location. Mora (1986 apud Patino - Valera, 1986) comments that the phenotype to change the character height, circumference at breast height and volume are influenced by small variations in the environment.

Terms related to adaptability and stability of phenotypes have been defined in various ways. Resende (2001 apud Cruz 1989) and Vencovsky & Barriga (1992) use the term "adaptability " to designate the capability of genetic materials to benefit from environmental stimulus, and to characterize the stability of genotypes to show highly predictable behavior due to stimulation.

The use of phenotypic plasticity is relatively new, however, a very important aspect studied is the ability to generate a genotype of a wide variety of phenotypes depending on the environment. In the literature, several types of phenotypic plasticity are recognized, acting at different levels from one generation to the next. Several methods to characterize and quantify the plasticity have also been described and implemented. However, it is necessary to further investigate this issue regarding forest species, mainly in Mediterranean environments, to understand the impact that global climate change may have on current populations (Chambel et al . , 2005) .

Studies with phenotypic variations (Kageyama and Vencovsky, 1983; Sebbenn et al, 2008) have always shown a wide variation in the morphology of plants of different genotypes (open pollinated). More recently, it has been found that these phenotypic changes also occur in genotypically identical populations (clones).

The interactions between the genetic material and micro conditions - climate and soil , can generate variation in production, even in small areas, which is not desirable. The causes of this behavior are not fully understood suggesting that it is possibly a consequence of the genetic structure of the matrix and its interactions with the environment.

Grace (1987 apud EMBRAPA, 1990) comments that in the vegetative propagation of forest species, the most

usual method in plantation cloning is by rooting cuttings. This process is recommended for the species *Eucalyptus dunnii* Maiden, by restricting reproduction through seeds.

According IPEF (2012), the E.dunnii have characteristics similar to E. grandis and thus studies show they can be used for pulp and paper . Today, there is a restriction of seeds in the Brazilian market with a difficulty of importing amounts that satisfy the market demand. Having production of seedlings or seeds, the *E. dunniis* will become one of the species with the greatest potential in the bioclimatic regions of Brazil.

According Alfenas et al. (2004), micropropagation is one of the techniques that can be used in the rejuvenation of mature trees. The serial propagation through successive subcultures is necessary to rejuvenate adult tissues. For In vitro cultures, insertion is complex in micropropagation because tissue contamination by bacteria and yeasts endogenous are difficult to eliminate. Even with its limitations, the micropropagation of species and hybrids of Eucalyptus is part of the seedling production process for some forest companies (Dutra et al , 2009).

Braga (2008 cited by Ferreira et al., 2006) comments that phenotypic stability of cloned plants depend on their interaction with the environment, influenced by soil fertility, water regime, and photoperiod. Having prior knowledge of the conditions listed above is critical for selecting potential genetic materials.

Stability alone should not be used in a selection process, but in combination with the average performance to determine the suitability of each of the origins being selected (JAYASEKERA, 1983).

This study aimed to verify phenotypic stability of a population of clones in the region of *E. dunnii* Maiden planted in Ponta Grossa, Paraná to 36 months of age, where we specifically collected morphological data from field studies.

2. MATERIAL AND METHODS

The choice of method for the characterization of genotypes when there is adaptibility, is dependent on experimental data available, the precision required and the type of information desired by the breeder (Cruz et al . , 2004).

This experiment was conducted in a stand of clone *E. dunnii* Maiden in Catanduva Out / Ponte Preta Ponta Grossa , PR . Located at 25 º 01 ' 34.98 " S 49 º 59'11 .02 " W, with altitude 943 meter , with a subtropical climate, annual average temperature of 21.5 º C with accordance to

the Köppen climate classification - Geiser , Cfb : humid temperate climate with mild summer and annual rainfall of aproximadamente 1500 mm.

The area belongs to the company Mueller Forest. The planting took place in December 2009, with an approximate area of 4.0 acres, with spacing of 3.0 m x 2.0 m. Planting corresponds to 100 % of the available area of the property. The population is about 36 months old.

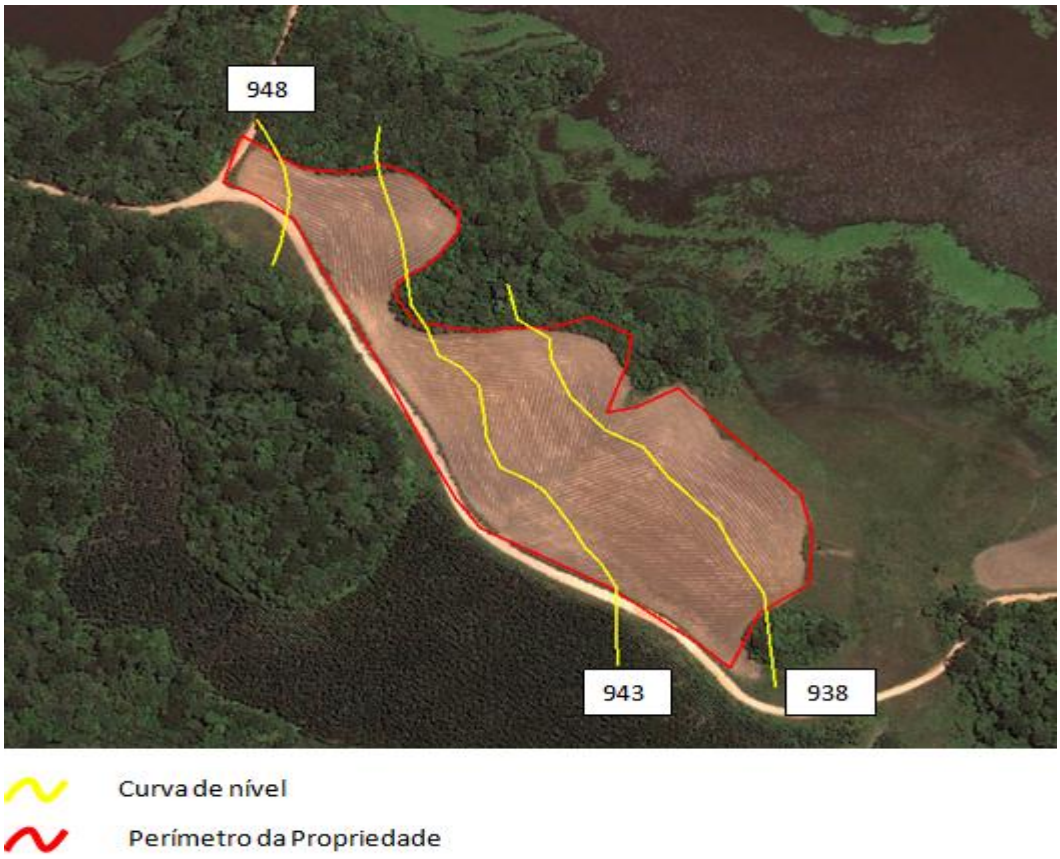

Figure 1. Altimetry Plan Eucalyptusdunnii planting site, the company deployed Forest Mueller, Ponta Grossa, PR.
Figura 1. Plano altimétrico do local plantio de *Eucalyptusdunnii*,implantado pela empresa Florestal Mueller, município de Ponta Grossa, PR.

In this study, 3 plots of 20m x 20m were installed, totaling 180 plants in the experiment. The choice of local allocations of plots occurred in random order. Measurements in plants took place in the month of September of this year. The plants on the perimeter were disregarded, since all portions of the blocks were installed in the interior of the plot.

Plant trait values such as height of plants were collected with a hypsometer that measures heights of trees. The

circumference at breast height (CBH) was done with tape. Crown diameter (CD) was determined using the methodology described below to obtain canopy diameter; the measurement of two perpendicular diameters were taken, the first being taken on the side of greatest crown width. These measurements were obtained with the use of tape, and their limits are defined by visual observation of each end. The methodology for measuring the diameter of the canopy is presented below (Figure 2).

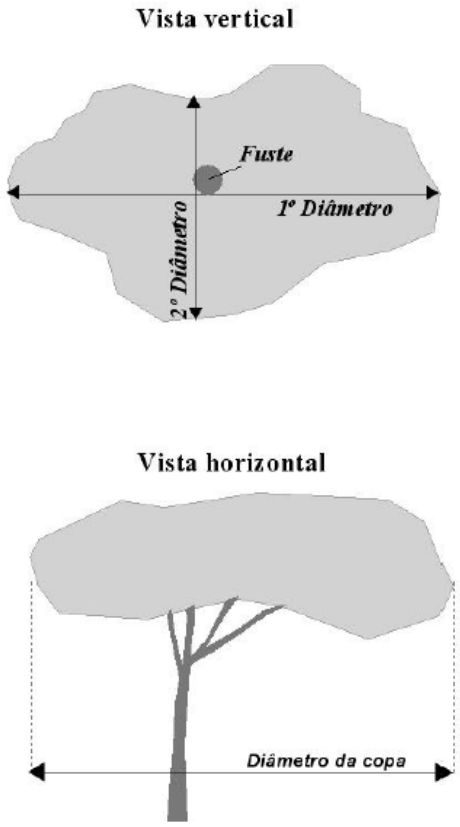

Figure 2. Methodology for measuring crown diameter.
Figura 2. Metodologia de medição de diâmetro de copa.

Source: THE WEBER KSMANEJO bracatinga (*Scabrella mimosa* Benth) BASED ON THE GROWTH OF INDIVIDUAL TREES diameter. UFPR, Curitiba, 2007, 114 f. Dissertation (Postgraduate Diploma in Forestry).

Living branches present until the height of the CAP (GV) and dead branches present until the height of the CAP (GM) were also observed. Figure 5 shows the general appearance of individuals E. dunnii utilized for evaluation.

Samples were collected from different locations of the planting, adding in all 10 samples, these samples were homogenized and taken for chemical analysisby the founding company ABC.

The data collected from all characteristics considered were subjected to descriptive statistics, in order to determine the mean, variance, skewness and kurtosis, in addition to the minimum and maximum values and standard deviation between individuals in the plot. To this end, the equations were used:

a) Mean value

$$\bar{x} = \sum_{i=1}^{n} \frac{X_i}{n}$$

$\bar{x}$= mean of the characteristic

x_i= observed value of the characteristic of individual i

n= number of observations

b) Variance

$$S_x^2 = \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}\right)^2}{n-1}$$

S_x^2= variance of the characteristic

$\bar{x}$= mean of the characteristic

x_i= value oberserved of the characteristic of individual i

n = number of observations

c) correlation coefficient

$$CV = \frac{S_x}{\bar{x}} \times 100$$

CV = coefficient of variation of the trait;

s_x = Standard deviation of the characteristic;

$\bar{x}$ = Average value of the characteristic.

d) Measure of asymmetry

$$\hat{s} = \frac{\sum_{i=1}^{n}\left(\frac{x_{i-\bar{x}}}{S_x}\right)^3}{n}$$

$\hat{S}$ =Measure of asymmetry of the characteristic;

x_i =Observed value of the individual i in the feature;

$\bar{x}$= Characteristic value of the average;

s_x = Standard deviation of the characteristic;

n= Number of observations.

e) Kurtosis value

$$\hat{K} = \frac{\sum_{i=1}^{n}\left(\frac{x_{i-\bar{x}}}{S_x}\right)^4}{n}$$

$\hat{k}$= Measure of kurtosis feature;

x_i = Observed value of the individual i in the feature;

$\bar{x}$ = characteristic value of the average;

s_x = Standard deviation of the characteristic;

n = Number of observations.

Analysis of variance between plots (local) and the contrasts between means using the Kruskal-Wallis test was also performed, according to the expression:

$$H_{cal} = \frac{12}{n(n+1)}\sum_{i=1}^{t}\frac{R_i^2}{n_i} - 3(n+1)$$

t = number of cases;

n_i = Number of observations in the case i;

n = Total number of observations;

R_i = Sum of the ranks for each case.

As phenotypic stability criteria were considered: S_x^2=0; $\hat{S}$=0; $\hat{k}$=<3; H_{cal}^{ns}.

3. RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics of the plot between individuals one (1) for characteristics plant height (H), circumference at breast height (CBH), canopy diameter (DC), live branches (GV) and dead branches (GM), clone *E.dunnii*, 36 months old, taken individually in plants.

Table 1. Results of descriptive statistics of the plot between individuals 1
Tabela 1. Resultados da estatística descritiva da entre indivíduos da parcela 1

Session 1					
Results	**CAP**	**Diameter of Canopy**	**Height**	**GV to CAP**	**GM to CAP**
Medium	0,4253	5,1315	11,0909	0,0909	15,924
Variance	0,0055	10,3792	31,8314	0,5455	18,010
Kurtosis	2,5857	6,1440	2,0357	66,0000	-0,607
Correlation Coefficient	17,3872	62,7822	50,8699	812,4038	26,650
Measure of Asymmetry	-1,2743	-1,8124	-0,8803	8,1240	-0,095
Max. Value	0,5500	6,5000	14,0000	6,0000	24,000
Min. Value	0,1400	1,3000	6,0000	0,0000	7,000
Standard Deviation	0,0739	0,8435	1,4752	0,7385	4,244

The following table shows the descriptive statistics of the plot between individuals two (2) for the same characteristics presented in part 1. Similarly at 36 months of age, taken individually in plants.

Table 2. Results of descriptive statistics between individuals of plot 2
Tabela 2. Resultados da estatística descritiva da entre indivíduos da parcela 2

Session 2					
Results	**CAP**	**Diameter of Canopy**	**Height**	**GV to CAP**	**GM to CAP**
Medium	0,4157	4,6662	11,7778	0,3519	17,648
Variance	0,0099	1,2682	4,0912	0,4965	39,100
Kurtosis	-0,6351	0,5058	-0,8338	3,7425	-0,808
Correlation Coefficient	23,9835	24,1342	17,1736	200,2638	35,432
Measure of Asymmetry	-0,2039	-0,7988	-0,2974	2,0650	0,301
Max. Value	0,6100	6,5500	15,0000	3,0000	32,000
Min. Value	0,1900	1,8500	8,0000	0,0000	8,000
Standard Deviation	0,0997	1,1261	2,0227	0,7046	6,253

Shown in Table 3, are the results of descriptive statistics of individuals between plot three (3) for the same characteristics presented in part 1 and 2, with the same standard 36 months old, taken individually in plants.

Table 3. Results of descriptive statistics between individuals of plot 3
Tabela 3. Resultados da estatística descritiva da entre indivíduos da parcela 3

Session 3					
Results	**Results**	**Results**	**Results**	**Results**	**Results**
Medium	0,4058	5,1696	12,0333	0,2667	20,467
Variance	0,0057	0,7064	2,0243	1,7582	40,219
Kurtosis	2,4081	5,7735	3,0081	51,3850	0,035
Correlation Coefficient	18,5578	16,2579	11,8236	497,2381	30,986
Measure of Asymmetry	-1,1826	-1,9754	-1,2979	6,9785	0,236
Max. Value	0,5400	6,2000	14,5000	10,0000	39,000
Min. Value	0,1300	1,7500	7,0000	0,0000	9,000
Standard Deviation	0,0753	0,8405	1,4228	1,3260	6,342

From the values obtained in the three plots, averages between all 3 are presented below in Table 4.

Data was analyzed using the Kruskal-Wallis test for analysis of variance and multiple comparisons of means, whose results are presented in table five (5).

The averages followed by the same letter do not differ at 5% probability level and 1% probability, using the Kruskal-Wallis test.

Table 4. Results of descriptive statistics of average between the three (3) plots
Tabela 4. Resultados da estatística descritiva da média entre as 3(três)

Session 4					
Results	**Results**	**Results**	**Results**	**Results**	**Results**
Medium	0,4156	4,9891	11,6340	0,2365	17,1019
Variance	0,0070	4,1179	12,6490	0,9334	31,1820
Kurtosis	1,4529	4,1411	1,4033	40,3758	-0,7146
Correlation Coefficient	19,9762	34,3914	26,6224	503,3019	32,0401
Measure of Asymmetry	-0,8869	-1,5289	-0,8252	5,7225	0,1607
Max. Value	0,5667	6	15	6	29
Min. Value	0,1533	2	7	0	8
Standard Deviation	0,0830	0,9367	1,6402	0,9230	5,5110

Tabela 5. Results of the Kruskal-Wallis test for analysis of variance and multiple comparisons of means.
Tabela 5. Resultados do Teste de Kruskal-Wallis, para análise de variância e comparações múltiplas de médias.

Trat	H	Trat	CAP	Trat	DC	Trat	GV	Trat	GM
1	11.09091 c	1	0.42530 a	1	5.13227 b	1	0.00000 c	1	15.92424 a
2	11.77778 b	2	0.41574 a	2	4.66685 c	2	0.35185 a	2	17.64815 a
3	12.03333 a	3	0.40583 a	3	12.03333 a	3	0.26667 b	3	17.73333 a
H = 14.7120		H =2.6373		H =122.2459		H = 18.1046		H = 2.6401	

How phenotypic stability criteria were considered: S_x^2=0; $\hat{S}$=0; $\hat{k}$=<3; H_{cal}^{ns}.

Table 3 shows the characteristics analyzed. Note that the height (H has variance close to 0 (zero), showing stability in this variable. Circumference at breast height (CBH), canopy diameter (DC), live branches gifts to the CAP and dead branches to the present CAP, presented variances that may be indicated as instability among individuals.

The asymmetry variable observed negative values for H, CAP and DC, similar to studies by (Braga, 2008), that exceed the criterion for stability in *E. urograndis.*

In kurtosis only (CD and FG) of the five characteristics evaluated showed values above 3 (three), which may indicate stability in this variable.

The results in Table 5 show that the analyzed characteristics differ between local plantations, except for the characteristic dead branches (GM) that did not show this behavior, which does not support the work of (Braga, 2008) for *E . urograndis*, that showed stability for this test (H and CAP), which directly interferes with the production of wood.

4. CONCLUSION

Under the conditions of this study, analysis of clone *E. dunni* demonstrates variances between the charactertistics studied related to the plants in the sites sampled, demonstrating the existence of phenotypic

instability, observed mainly in statistical analysis performed by the Kruskal-Wallis test not supporting stability for height, circumference at breast height, crown diameter and live twigs.

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