# Stability of annual biomass energy production of elephant grass (Pennisetum purpureum Schum.) genotypes in the Northern region of the Rio de Janeiro State, Brazil 

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#### Abstract

The objectives of this study were to estimate the stability of dry biomass production of elephant grass genotypes under an annual harvest regime, in soil-climatic conditions of the Northern region of the Rio de Janeiro State, Brazil, and to compare methodologies for stability analyses of Yates and Cochran (YC), Plaisted and Peterson (PP), Annicchiarico (ANN), Lin and Binns (LB), Huenh (HU), and Kang and Phan (KP). A randomized block design with 83 treatments and two replicates was adopted. Four annual harvests were performed


(2012-2015) and dry matter yield (DMY, t.ha ${ }^{-1}$. year ${ }^{-1}$ ) was evaluated. Individual and combined analyses of variance for DMY revealed significant effects for genotypes, harvests, and for the genotype x harvest interaction at the probability levels of 1 and $5 \%$. Genotypes indicated by the YC and PP methods are associated with higher stability and lower DMY. The weighting of KP with YC and PP was highly effective in associating stability with DMY. The LB and ANN methods showed strong agreement with each other and produced similar classifications as to phenotypic stability, and so we recommend using one or the other. Genotypes Elefante Cachoeiro do Itapemirim, Cuba-116, Taiwan A-46, P241 Piracicaba, Taiwan A-144, Cameroon Piracicaba, 10 AD IRI, Guaçu/I,Z,2, Mineirão IPEACO, Taiwan A-121, IJ7125 cv EMPASC308, 903-77, Mole de Volta Grande, and Porto Rico 534-B showed high stability and DMY, standing out as promising genotypes for the soil-climatic conditions of the Northern region of the Rio de Janeiro State. The methodologies based on ANOVA and nonparametric analyses were complementary and increased reliability in the recommendation of genotypes.

Key words: Genotype x environment interaction; Bioenergy; Elephant grass; Pennisetum purpureum Schum.

## INTRODUCTION

In the current historical context, humanity is notably facing three major inter-related problems: the threats to food safety, to energy safety, and climatic changes (Sachs, 2007). In the light of the information available today, energy production from renewable sources represents the most viable strategy to reverse the global dependence on fossil fuels (Santos et al., 2012).

Brazil, in turn, has great potential in the renewable-energy sector, mainly for the production of bioenergy and biofuels from plant biomass. In fact, the plant biomass is an only short-term raw material with the ability to be carbon-neutral and sustainable in the long term (InterAcademy Council - IAC, 2007).

The most largely used raw materials for bioenergy production in Brazil and worldwide are sugarcane, eucalyptus, soy, and corn (Carbonari et al., 2012). The plant biomass derived from elephant grass; however, has aroused the interest of big consumers and entrepreneurs of the energy sector, mainly due to its high yield, short cycle, and many quality attributes related to biomass that allows its use for these purposes (Morais et al., 2008; Patelini et al., 2013).

Despite many favorable attributes of this crop, research focusing on genetic breeding aimed at its use as an energy source is recent and not very expressive when compared with many of the commodities (Daher et al., 2014). Considering this need, the Elephant Grass Breeding Program of Universidade Estadual do Norte Fluminense Darcy Ribeiro has intensified efforts in the development of studies aimed at obtaining highly productive elephant grass genotypes with appropriate biomass quality for use as energy source, to meet mainly the demand of the ceramic industries from the Northern region of Rio de Janeiro State, Brazil (de Lima et al., 2011; Oliveira et al., 2014; Rossi et al., 2014; Rocha et al., 2015; Menezes et al., 2014, $2016 a, b)$. To this end, a better understanding of the genotype versus environment interaction is
indispensable for the breeding of this crop (Pereira and Lédo, 2008; Cruz et al., 2012).
To detail the interaction effect, a study can be carried out on the stability and adaptability of genotypes, based on experiments replicated in more than one environment with different genotypes, with successive harvests, and periodical assessments over time. This procedure analyzes the variation occurring between environments, for each genotype, allowing the identification of those with predictable behavior and that respond to environmental variations, in either specific or general condition (Cruz et al., 2012).

Among the methods proposed for the study and quantification of the genotype x environment interaction, those of stability and adaptability based on analysis of variance (ANOVA) (Yates and Cochran, 1938; Plaisted and Peterson, 1959; Wricke, 1965), simple linear regression (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966), multiple linear regression (Cruz, et al., 1989), and non-parametric methods (Annicchiarico, 1992; Lin and Binns, 1988; Huehn, 1990; Kang and Phan, 1991) stand out. The criterion for the choice of the method should be that of simple execution and easy interpretation.

In this scenario, the present study was conducted to i) estimate the biomass production stability of 83 genotypes of elephant grass under the regime of annual harvests in the soilclimatic conditions of the Northern region of the Rio de Janeiro State, Brazil and ii) to compare methodologies of analysis of parametric and non-parametric stability.

## MATERIAL AND METHODS

The experiment was developed at Estação Experimental do Centro Estadual de Pesquisas em Agroenergia e Aproveitamento de Resíduos (PESAGRO-RIO), located in Campos dos Goytacazes ( $21^{\circ} 19^{\prime} 23$ " S latitude and $41^{\circ} 19^{\prime} 40^{\prime \prime} \mathrm{W}$ longitude; 25 m average altitude), Northern region of the Rio de Janeiro State, Brazil.

The climate of the Northern region of the Rio de Janeiro State is a hot and wet tropical Aw type, with dry winters and rainy summers (Köppen, 1948) and annual precipitation around 1152 mm .

The soil of the experimental area is characterized as a dystrophic Argisol (Santos et al., 2013), which showed the following characteristics in the top $0-20 \mathrm{~cm}$ layer: pH (water) 5.5; $\mathrm{P}\left(\mathrm{mg} / \mathrm{dm}^{3}\right)-18 ; \mathrm{K}\left(\mathrm{mg} / \mathrm{dm}^{3}\right)-83 ; \mathrm{Ca}\left(\mathrm{cmolc} / \mathrm{dm}^{3}\right)-4.6 ; \mathrm{Mg}\left(\mathrm{cmolc} / \mathrm{dm}^{3}\right)-3.0 ; \mathrm{Al}(\mathrm{cmolc} /$ $\mathrm{dm}^{3}$ ) $-0.1 ; \mathrm{H}+\mathrm{Al}\left(\mathrm{cmolc} / \mathrm{dm}^{3}\right)-4.5$; and C (\%) - 1.6 .

A randomized block design was adopted, with two replicates and 83 elephant grass genotypes. These genotypes were originated from Banco Ativo de Germoplasma of Embrapa Gado de Leite, located in Coronel Pacheco, Minas Gerais, Brazil. Each plot consisted of one genotype planted into $0.10-\mathrm{m}$ deep and $5.5-\mathrm{m}$ long furrows spaced 2.0 m apart. The available area comprised $2 \mathrm{~m}^{2}$ in the center of the plot.

The following elephant grass genotypes were used: Elefante Colômbia (1), Mercker (2), Três Rios (3), Napier Volta Grande (4), Mercker Santa Rita (5), Pusa Napier N ${ }^{\circ} 2$ (6), Gigante de Pinda (7), Napier N ${ }^{\circ} 2$ (8), Mercker S, E, A (9), Taiwan A-148 (10), Porto Rico 534-B (11), Taiwan A-25 (12), Albano (13), Hib.Gig. Colômbia (14), Pusa Gigante Napier (15), Elefante Híb. 534-A (16), Costa Rica (17), Cubano Pinda (18), Mercker Pinda (19), Mercker Pin. México (20), Mercker 86 México (21), Taiwan A-144 (22), Napier S.E.A. (23) Taiwan A-143 (24), Pusa Napier N ${ }^{\circ} 1$ (25), Elefante de Pinda (26), Mineiro (27), Mole Volta Grande (28), Porto Rico (29), Napier (30), Mercker Comum (31), Teresopólis (32), Taiwan A-46 (33), Duro de Volta Grande (34), Mercker Comum Pinda (35), Turrialba (36), Taiwan A-146 (37), Cameroon - Piracicaba (38), Taiwan A-121 (39), Vrukwona (40), P241 Piracicaba
(41), IAC-Campinas (42), Elefante Cachoeiro Itapemirim (43), Capim Cana D’África (44), Gramafante (45), Roxo (46), Guaçu/I,Z,2 (47), Cuba-116 (48), Cuba-169 (49), King Grass (50), Roxo Botucatu (51), Mineirão IPEACO (52), Vruckwona Africano (53), Cameroon (54), CPAC 55 Guaçu (56), Napierzinho (57), IJ7125 cv MPASC308 (58), IJ7126 cv MPASC310 (59), IJ7127 cv EMPASC 309 (60), IJ7136 cv EMPASC307 (61), IJ 7139 (62), IJ7141 cv EMPASC 306 (63), Goiano (64), CAC-262 (65), Ibitinema (66), 903-77 (67), 13 AD (68), 10 AD IRI (69), Pasto Panamá (70), BAG - 92 (71), 09 AD IRI (72), 11 AD IRI (73), 05 AD IRI (74), 06 AD IRI (75), 01 AD IRI (76), 04 AD IRI (77), 13 AD IRI (78), 03 AD IRI (79), 02 AD IRI (80), 08 AD IRI (81), União (82), and PesagroBord (83).

Planting took place on $02 / 23 / 2011$ and $02 / 24 / 2011$, using two whole stems per furrow that were arranged with the base of a plant touching the apex of another plant, and then these were cut to pieces containing two or three buds. Fifty days after planting, a plot-leveling cut was made, and plots with flaws were replanted. Fertilization was defined based on the analyses of the soil of the experimental area and recommended fertilization practices for the State of Rio de Janeiro proposed by Freire et al. (2013). Evaluations were carried out in an annual harvest regime, in November 2012, November 2013, December 2014, and December 2015, totaling four harvests, in which the variable dry matter yield (DMY), in t.ha ${ }^{-1}$. year ${ }^{-1}$, was analyzed.

For the statistical analysis of the data, we first performed ANOVA and the F-test for each annual harvest. After the individual ANOVA, we analyzed the homogeneity of the residual variances of the experiments (residual mean squares, RMS), as proposed by PimentelGomes (2012), who suggested that combined ANOVA should only be performed if the ratio between the residual variances of the experiments is lower than 7. The split-plot statistical model was adopted for the combined ANOVA, following Steel et al. (1997).

Subsequently, the phenotypic stability was analyzed using the methods of Yates and Cochran (YP) (1938), Plaisted and Peter (PP) (1959), Kang and Phan (KP) (1991), Annicchiarico (ANN) (1992), Lin and Binns (LB) (1988), and Huenh (HU) (1990). We used the Spearman rank correlation to evaluate the degree of association between the different stability methodologies (Steel et al., 1997). Therefore, all statistics were classified in descending order (Cargnelutti Filho et al., 2007; Pena et al., 2012; Marinho et al., 2013; Almeida Filho et al., 2014; Rocha et al., 2015). All statistical analyses were processed using the Genes software (Cruz, 2016).

## RESULTS AND DISCUSSION

The results of combined ANOVA revealed significant differences between DMY means for all genotypes (Table 1). There were significant differences, at the $1 \%$ probability level, between genotypes and between harvests, as well as significant effects for the genotype x annual harvest interactions ( $\mathrm{G} \times \mathrm{E}$ ) at the $5 \%$ probability level by the F-test.

These results indicate that there is variability for selection between genotypes, as well as a variation between the studied annual harvests. The significance of the GxE interaction demonstrates the occurrence of a differential response of genotypes to environments, which justifies the study of phenotypic stability aiming at the identification of the most stable and productive genotypes.

The RMS obtained in the individual ANOVA referring to DMY (Table 1) resulted in a ratio of 6.57 between the highest and the lowest RMS, which indicates relative homogeneity of variances, allowing the use of all evaluated harvests in the combined ANOVA.

Table 1. Summary of combined analysis of variance for dry matter yield (DMY) in $t \cdot h a^{-1} \cdot$ year ${ }^{-1}$ evaluated in four annual harvests and 83 elephant grass genotypes (Campos dos Goytacazes - RJ).

| Source of variation | d.f. | Mean squares |
| :--- | :---: | :---: |
| Block | 1 | 582.99 |
| Genotype | 82 | $507.9^{* *}$ |
| Error A | 82 | 234.74 |
| Harvests | 3 | $14073.02^{* *}$ |
| Error B | 3 | 137.24 |
| G x | 246 | $128.74^{*}$ |
| Error C | 246 | 96.87 |
| Mean |  | 30.96 |
| CV $\%$ ) |  | 31.79 |
| RMS/<RMS |  | 6.57 |

** and *Significant at 1 and $5 \%$ probability levels, respectively, by the F-test. RMS $=$ residual mean squares.
The coefficient of variation was $31.79 \%$, which is considered acceptable for the DMY variable, since it is a quantitative characteristic whose genetic control involves many genes and thus undergoes a strong influence of the environment (Hallauer et al., 2010). Note that this value is consistent with values normally reported in other studies with culture also under field conditions (Oliveira et al., 2014; Rossi et al., 2014; Rocha et al., 2015).

## Analysis of stability estimates

Phenotypic stability estimates of the genotypes by each methodology are presented in Table 2. For a comparison between the different stability methodologies, the statistical results are discussed based on the selection of $15 \%$ of the population, which corresponds to the first twelve genotypes.

## Traditional (YC) method

The evaluation of genotype performance stability based on the traditional method (Table 2) showed that the most stable genotypes for DMY, for showing the lowest variation in the environment, were, in descending order of stability, $8,81,55,1,24,83,51,31,46,13$, 5 , and 70 , with mean square values of environments within genotypes ( $\mathrm{MSE} / \mathrm{G}_{\mathrm{i}}$ ) of 8.627 , $20.027,32.861,33.698,37.467,52.231,60.292,63.108,66.252,66.802,70.480$, and 76.256 , respectively. However, the classification of these genotypes regarding the average DMY in the four harvests was not satisfactory, and they were ranked 80 th, 82 nd , 39 th, 57 th, 78 th, 77 th, 68th, 69th, 66th, 56th, 52 nd , and 81st, respectively.

There was a trend for the most productive genotypes to be the most unstable, since a correlation coefficient of -0.73 was observed between classifications for the average DMY and the $\mathrm{MSE} / \mathrm{G}_{\mathrm{i}}$ (Table 3). These results are in line with the main disadvantage of the method reported in the literature, that genotypes with lower variances between environments are, in general, the least productive (Cargnelutti Filho et al., 2007; Cruz et al., 2012).

## P method

The most stable genotypes for DMY, by this methodology, were $63,64,82,14,26,80$, $72,75,66,53,62$, and 68 , for presenting the lowest $\% \theta$ i values $(-1.13,-1.09,-1.04,-1.01,-1.00$, $-0.99,-0.76,-0.69,-0.64,-0.63,-0.57$, and -0.57 , respectively), and were thus the genotypes
Table 2. Mean values for dry matter yield (DMY) and estimates given by the Yates and Cochran (YC), Plaisted and Peterson (PP), and Kang and Phan
(KP) methods applied to the Yates and Cochran (KP+YC), Plaisted and Peterson (KP+PP), Annichiarico (ANN), Lin and Binns (LB), and Huenh (KP+HU)
methods with their respective positions (P) for the 83 elephant grass genotypes (G) (Campos dos Goytacazes, RJ, 2016).


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Table 2. Continued.

$\approx$


 HUS $_{1}$ : Statistical S1 Huenh, HUS $_{2}$ : Statistical S2 Huenh, HUS 3 : Statistical S3 Huenh.

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that least contributed to the interaction (Table 2). Of these, only genotypes 66, 53, and 62 had higher average DMY than the overall mean. Thus, the stability of genotypes based on PP is independent of the average yield and the response to changes in environmental conditions.

Table 3. Estimates of Spearman ( $\rho$ ) correlations between rankings for average dry matter yield (DMY) and different stability methods obtained in 83 elephant grass genotypes (Campos dos Goytacazes, RJ, 2016).

| Method | Traditional | Plaisted and Peterson | Annichiarico | Lin and Binns | $\begin{gathered} \hline \text { Huenh } \\ \mathrm{S}_{1} \\ \hline \end{gathered}$ | $\begin{gathered} \hline \text { Huenh } \\ \mathrm{S}_{2} \\ \hline \end{gathered}$ | $\begin{gathered} \hline \text { Huenh } \\ \mathrm{S}_{3} \\ \hline \end{gathered}$ | Kang and Phan + Traditional | Kang and Phan + Plaisted and Peterson |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| DMY | -0.730** | -0.323** | 0.990** | 0.986** | -0.297** | -0.284** | $-0.041^{\text {ns }}$ | 0.320** | 0.557** |
| Tradition |  | 0.379** | -0.660** | -0.778** | $0.168^{\text {ns }}$ | $0.160^{\text {ns }}$ | -0.065 ns | 0.397** | -0.261* |
| Plaisted | d Peterson |  | -0.266* | -0.253* | 0.428** | 0.444** | -0.295** | $0.086^{\text {ns }}$ | 0.565** |
| Annichia |  |  |  | 0.972** | -0.289** | -0.275* | $-0.067^{\text {ns }}$ | 0.403** | 0.603** |
| Lin and B |  |  |  |  | -0.256* | -0.243* | $-0.054^{\text {ns }}$ | 0.231* | 0.601** |
| Huenh S |  |  |  |  |  | 0.996** | 0.606** | -0.157 ${ }^{\text {ns }}$ | $0.128^{\text {ns }}$ |
| Huenh S |  |  |  |  |  |  | 0.595** | $-0.152^{\text {ns }}$ | $0.156^{\text {ns }}$ |
| Huenh S |  |  |  |  |  |  |  | $-0.131^{\text {ns }}$ | -0.260* |
| Kang and Phan + Traditional |  |  |  |  |  |  |  |  | 0.372** |

$\mathrm{ns}=$ non-significant at 1 and $5 \%$ probability by the $t$-test; *significant at $5 \%$ probability; ${ }^{* *}$ significant at $1 \%$ probability.

Analyzing the association of rankings by the PP and YC methods with the average DMY, a negative correlation is observed. Overall, the genotypes considered most stable according to these methodologies were not the most productive, corroborating the results observed by Rocha et al. (2015), who worked with 73 of these genotypes in a semiannual harvest regime.

## KP method

By the YC method, the genotypes indicated as the most stable showed low yields; however, when they were re-classified by the KP method, the best positions were occupied by genotypes with better yields. In this case, estimates highlighted, in descending order of stability, genotypes $55,69,52,38,62,18,19,48,3,1,54$, and 5 (Table 2).

Reclassification via KP+PP indicated genotypes 22, 62, 38, 66, 32, 53, 37, 3, 47, 69, 52 , and 80 as those with highest stability (Table 2). Before the weighting, the twelve most stable genotypes occupied very low positions for DMY, with the PP correlation with average DMY being $\rho=-0.323$. However, after reclassification, all recommended genotypes displayed a DMY above the overall experimental mean, except for genotype 80 .

As shown in Table 3, the correlation between the indications of both $\mathrm{KP}+\mathrm{YC}$ and $\mathrm{KP}+\mathrm{PP}$ with the average DMY turned positive, but with low magnitudes. Therefore, KP's weighting is highly effective in associating stability and productivity. These results corroborate Marinho et al. (2013), Pena et al. (2012), and Rocha et al. (2015).

## ANN method

By the ANN methodology, considering the analysis of all harvests, genotypes 38, $39,43,67,47,41,22,69,58,33,52$, and 48 will be $65.53,58.23,48.54,48.54,38.55,36.19$, $33.78,29.74,22.31,21.25,21.13$, and $20.99 \%$ higher than the average of the environments, respectively. In addition to these, another 16 also had confidence indices above $100 \%$ (Table 2). There was a high agreement between the ANN parameter and the average DMY ( $\rho=$
0.990 ); thus, the genotypes indicated as having greatest stability are the most productive, corroborating Cargnelutti Filho et al. (2007) and Rocha et al. (2015).

## LB method

The Pi stability index estimates ranged from 44.60 , for genotype 43 , to 978.63 , for genotype 70 . The twelve genotypes with greatest stability were $43,38,67,39,41,47,33,22$, $58,69,11$, and 28 , which had the lowest Pi values: $44.60,45.66,59.92,62.70,82.36,114.62$, $140.30,141.48,182.66,208.31,211.39$, and 219.23 , respectively (Table 2 ).

The percentage values of the genetic contribution of the studied genotypes ranged from 43.22 to $98.83 \%$. Of the twelve aforementioned genotypes, $43,41,33$, and 22 are noteworthy, as they had the 1st, 5 th, 7 th, and 8 th lowest Pi values, the 4th, 5 th, 7 th, and 8 th highest yields, and $92.77,88.61,87.79$, and $92.56 \%$ of genetic contribution, respectively.

Genotypes 38,39 , and 67 , in turn, were the most productive, with DMY of 52.039, 50.934, and 49.857 t /ha, and were ranked as the 2nd, 4th, and 3rd most stable, respectively. However, they expressed the lowest genetic contribution percentages for the interaction (43.22, 43.51 , and $59.78 \%$, respectively), suggesting an intense influence of the effect of variation among the studied harvest years and that a large portion of the high performance may not be attributed to the genetic effect of the genotypes.

When the results of the LB method were compared regarding average DMY, there was a trend for the most stable genotypes to be the most productive, given the high positive and significant correlation at $1 \%$, equivalent to $\rho=0.986$ (Table 3). Rocha et al. (2015) also obtained widely adapted and stable genotypes, associated with high yields. Also, the ranking of genotypes by LB and ANN was practically equal (Table 2), indicating a strong association between these methodologies based on Spearman's correlation coefficient: $\rho=0.972$ (Table 3).

## HU method

According to HU's stability measures $\mathrm{S}_{1}, \mathrm{~S}_{2}$, and $\mathrm{S}_{3}$ described in Table 2, the genotype with maximum stability has $S_{1}, S_{2}$, and $S_{3}$ equal to zero. However, estimates obtained in this study varied in the ranges of 3.833 to $44.833,8.9167$ to 1279.6 , and 0.2156 to 4.4622 , for $\mathrm{S}_{1}$, $S_{2}$, and $\mathrm{S}_{3}$, respectively.

The most stable genotypes for DMY were $40,43,8,42,48$, and 23 , for showing the lowest $\mathrm{S}_{1}, \mathrm{~S}_{2}$, and $\mathrm{S}_{3}$ values. However, more productive genotypes are desired, and, among the previously mentioned genotypes, 43 and 48 stand out due to their more stable performance and good DMY, ranking 4th and 11th, respectively, in average DMY.

Genotypes $64,82,63,75,14$, and 76 also had good $S_{1}$ and $S_{2}$ stability estimates. By contrast, they occupied the 64th, 76th, 79th, 54th, 70th, and 73rd positions for average DMY, and thus became uninteresting options, since desired genotypes should encompass high yield and stability.

## CONCLUSIONS

By ANOVA-based methods, the genotypes classified as most stable have a lower DMY. With the non-parametric methods, however, there was a greater association between stability and productivity of genotypes, especially by the LB and ANN methodologies.

The LB and ANN methods showed strong agreement with each other and produced similar classifications regarding phenotypic stability; therefore, either methodology is recommended.

The most productive and stable genotypes were Elefante Cachoeiro do Itapemirim (43), Cuba-116 (48), Taiwan A-46 (33), P241 Piracicaba (41), Taiwan A-144 (22), Cameroon - Piracicaba (38), 10 AD IRI (69), Guaçu/I,Z,2 (47), Mineirão IPEACO (52), Taiwan A-121 (39), IJ7125 cv EMPASC308 (58), 903-77 (67), Mole de Volta Grande (28), and Porto Rico 534-B (11).

## Conflicts of interest

The authors declare no conflict of interest.

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