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**RESEARCH ARTICLE**

# Phenotypic stability and adaptability of sweet sorghum genotypes evaluated in different Brazilian regions

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## **ABSTRACT**

Sweet sorghum is a special purpose sorghum with a sugar-rich stalk, almost like sugarcane. The objective of this work was to evaluate the phenotypic stability and adaptability of sweet sorghum genotypes, in different Brazilian regions, for the production of bioethanol. Twenty-five sweet sorghum genotypes were evaluated in 10 environments distributed in the Southeast, Midwest, Northeast, and Southern regions of Brazil. The experimental design was a randomized complete block design with three repetitions. The following agroindustrial traits were evaluated: fresh biomass yield (FBY), total soluble solids content (TSS) and tons of Brix per hectare (TBH). The adaptability and stability analyzes were performed with the methods GGEbiplot and Annicchiarico methodologies. The Annicchiarico and GGEbiplot adaptability and stability study methods presented satisfactory and consistent results and can be used separately or together in sweet sorghum breeding programs, and B005 and B008 sweet sorghum genotypes presented superior performance, with similar classification in both methods studied.

**Keywords**: *Sorghum bicolor* (L.) Moench, bioenergy, renewable energy, biofuel, gas emissions, renewable crops.

#### **INTRODUCTION**

The global demand for petroleum fuel and its associated environmental concerns, such as greenhouse gas emissions, increases the use of renewable crops for the production of bioenergy (Appiah-Nkansah, Li, Rooney, & Wang, 2019). Among the renewable energy sources, biofuels, especially ethanol produced by fermenting carbohydrates-rich crops, have gained prominence in the world energy market (Leite et al., 2019).

Brazil is the second-biggest producer of ethanol in the world, second only to the USA (Renewable Fuels Association [RFA], 2019). Several factors have combined to stimulate the development of Brazil's ethanol industry: an increased capacity to produce sugarcane as an ethanol feedstock, and greater incentive to research and investment with new and complementary energy cultures. Currently, the use of ethanol as biofuel in Brazil has been the most successful program to replace fossil fuels worldwide. Several countries have started their own programs for the production and use of ethanol as fuel to reduce oil dependence and greenhouse gas emissions. However, the Brazil still needs to sustain production growth in the ethanol sector in order to meet increasing domestic demand and maintain its export share.

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a special purpose sorghum with a sugar-rich stalk, almost like sugarcane. Besides having rapid growth, high sugar accumulation, and biomass production potential, sweet sorghum has wider adaptability (Souza, Parrella, Souza, & Parrella, 2016; Silva et al., 2017; Appiah-Nkansah et al., 2019; Leite et al., 2019). Besides, it is can be grown with less irrigation and rainfall and purchased inputs compared to sugarcane (Ribeiro, Salvador, Oliveira, & Menezes, 2021). The sugar content in the juice extracted from sweet sorghum varies from 16 - 23% Brix. It has also a great potential for jaggery, syrup, and most importantly fuel alcohol production (Silva et al., 2017; Teixeira et al., 2017; Klasson, Qureshi, Powell, Heckemeyer, & Eggleston, 2018).

The commercial release of new sweet sorghum cultivars requires understanding the performance of potential genotypes in different environmental conditions. The differential response of genotypes in different environments is known as the genotype x environment interaction (GxE), and when recommending sorghum cultivars in the face of GxE, it is necessary to annually test a large number of genotypes in different environments before the final recommendation and multiplication is made (Almeida Filho et al., 2014). GxE can complicate the recommendation of cultivars for different environments, making adaptability and stability analyses necessary. The study of adaptability and stability allows the identification of genotypes with predictable behavior in specific or general environments, and the identification of genotypes sensitive to positive environmental variations (Cruz, Regazzi, & Carneiro, 2012).

There are several methods for analyzing the adaptability and stability of genotypes when grown in different environments. The choice of using certain analytical methods of experimental data depends mainly on the number of environments available, the accuracy required, and the type of information desired. The GGE biplot analysis is efficient because it enables predicting the mean genotype yield per specific environment, as well as helps identifying the most stable genotype for the region of interest (Yan, 2014; Santos, Amaral Júnior, Kurosawa, Gerhardt, & Fritsche Neto, 2017). According to Badu-Apraku, Akinwale, Fakorede and Oyekunle (2012), this analysis is more versatile and flexible than other models based on simple linear regression and on segmented linear regression, as well as

than non-parametric methods, because it allows better understanding the G×E interaction. Already, the Annicchiarico (1992) method has the confidence index (Ii) as its statistical index whose result is expressed as a percentage of the mean of the environments. The confidence index allows us to estimate the risk of using each genotype.

Therefore, the objective of this work was to evaluate the phenotypic stability and adaptability of sweet sorghum genotypes, in different Brazilian regions, for the production of bioethanol.

## **MATERIAL AND METHODS**

#### **Environments and genotypes**

The experiments were conducted in ten environments, distributed in different Brazilian regions: Southeast (Minas Gerais and São Paulo), Midwest (Distrito Federal, Mato Grosso do Sul and Mato Grosso), Northeast (Alagoas) and Sul (Rio Grande do Sul) in the 2015/2016 agricultural year (Table 1). Twenty-five sweet sorghum genotypes were evaluated, being 20 experimental hybrids, two experimental cultivars (CMSXS646 and CMSXS647), two commercial cultivars (BRS508 and BRS511), all belonging to the Embrapa Maize and Sorghum breeding program, and the commercial hybrid, CV198 (CanaVialis®). Sweet sorghum hybrids were developed using female sweet sorghum lines (A-lines) and restorative lines (Rlines) (Whitford et al., 2013).



**Table 1.** Geographic location of the environments and the mean cropping cycle of each sweet sorghum experiment evaluated in the 2015/2016 agricultural year.

<sup>1</sup>MG: Minas Gerais state; SP: São Paulo state; DF: Distrito Federal; MS: Mato Grosso do Sul state; MT: Mato Grosso state; AL: Alagoas state; RS: Rio Grande do Sul state.

#### **Experimental planning and conducting**

The experimental design was a randomized complete block design with three repetitions. The experimental unit consisted of two rows of five meters long, spaced 0.7 m between rows, except the Dourados environment, where the row

spacing used was 0.5 m and in Lavras 0.6 m. The plant population was 110,000 plants per hectare. The following agroindustrial traits were evaluated: fresh biomass yield (FBY; weight of all plants in the plot measured in kg using a digital hanging scale and expressed as ton.  $ha^{-1}$ ); total soluble solids content (TSS; determined in the extracted juice using hydraulic press from a sample of six stalks using a digital automatic refractometer in °Brix); and tons of Brix per hectare (TBH; determined in t. ha<sup>-1</sup> using the following expression: TBH = (FBY x TSS)/100).

#### **Statistical analysis**

Firstly, the analysis of variance was conducted for each environment. After verifying the homogeneity of residual variances using Bartlett´ test, the joint analysis of variance was performed, according to the following model:

$$
Y_{ijk} = \mu + g_i + a_j + ga_{ij} + b/a_{jk} + e_{ijk}
$$

where:  $Y_{ijk}$  is the observed phenotypic value of the i-th genotype, in the k-th block, within the j-th environment;  $\mu$  is the general mean;  $g_i$  is the effect of the i-th genotype;  $a_j$  is the effect of the j-th environment;  $ga_{ij}$  is the interaction of the i-th genotype within the j-th environment;  $b/a_{ik}$  is the effect of the k-th block, within the j-th environment;  $e_{ijk}$  is the experimental error.

The averages of the genotypes were grouped by the Scott-Knott test (Scott & Knott, 1974), also at 5% probability. Then, the adaptability and stability analyzes were performed with the methods GGEbiplot (Yan, 2001) and Annicchiarico (1992). The methodology proposed by Annicchiarico is based on the Wi confidence index, which aims to demonstrate the risk of adopting a cultivar with performance below the general mean. Thus, the ideal cultivar is the one that presents the lowest risk and can be recommended, that is, the cultivar with the highest confidence index. The model used is as follows:

$$
W_{i(g)} = \hat{\mu}_{i(g)} - Z_{(1-\infty)} \hat{S}_i,
$$

where:  $W_{i(g)}$  is the confidence index (%);  $\hat{\mu}_{i(g)}$  is the mean percentage of the genotype i;  $S_i$  is the standard deviation of the percentage values;  $Z_{(1-\infty)}$  is the percentile (1-α) of the cumulative normal distribution function, with  $α = 0.25$ .

For the GGE-Biplot analysis, only the main effect of genotype and G×E are important and must be considered together, and the main effect of the environment is not relevant for the selection of cultivars. The GGE biplot analysis was carried out according to the model expressed:

$$
y_{ij} = \mu + \beta_j + y_{1 \text{eilpjl}} + y_{2 \text{eilpjl}} + \varepsilon_{ij}
$$

where:  $y_{ij}$  is the mean grain yield of genotype i at environment j;  $\mu$  is the overall mean of observations;  $\beta_j$  is the main effect of the environment;  $y_1$  and  $y_2$ are the errors associated to first (PC1) and second principal component (PC2), respectively;  $\varepsilon_1$  and  $\varepsilon_1$  are the values of PC1 and PC2, respectively, for the genotype of order i; ρj1 and ρj2 are the values of PC1 and PC2, respectively, for the environment of order j; and  $\varepsilon_{ii}$  is the error associated to the model of the i<sup>th</sup> genotype and j<sup>th</sup> environment (Yan, Hunt, Sheng, & Szlavnics, 2000).

This analysis was performed using the GGEBiplotGUI, package of the R software (R Core Team 2015) and the software Genes (Cruz, 2013).

## **RESULTS AND DISCUSSION**

All plants that were exactly at the V2 developmental stage (V2+0D) showed epidermis with stomata and tector trichomes; angular collenchyma consisting of only one layer of cells; cortex of 6 to 9 layers, including aerenchyma and monocrystals were observed in the parenchyma cells of the primary phloem (Figure 1A). At this collection time (The significance was observed to effect of the genotypes at individual analysis. Then, the joint analysis of variance is shown in the Table 2. There was significance ( $p$ <0.01) for the sources of variation (genotype -  $G$ , environment - E, and the interaction GE) according to F test. A significant environmental effect (P<0.01) for all the traits was found, indicating differences among environments. These differences may be due to variations in macroenvironmental aspects, such as soil type, climate, altitude, geography, or differences related to the crop cycle in the studied environments (Table 1).

The effect of genotype was significant (P<0.01) for all the traits, demonstrating that the observed differences in performances had genetic causes and, thereby, offer the possibility of selection and genetic gains. There was significant GxE interaction (P<0.01), indicating differential response of genotypes to the environments evaluated, and there may even be genotypes that display specific adaptability (Table 2). The coefficient of variation was satisfactory, indicating precision experimental (Table 2) as demonstrated by Almeida Júnior et al. (2020) and Silva et al. (2020).



**Table 2.** Summary of joint analysis of variance for fresh biomass yield (FBY), content of total soluble solids (TSS) and tons of Brix per hectare (TBH) of 25 genotypes of sweet sorghum, grown in different environments, in 2015/2016 agricultural year.

\*\*: significant at 1% probability by the F test. DF: Degrees of freedom; CV: coefficient of variation, in %.

The mean of fresh biomass yield (FBY) evaluated in each environment ranged from 34.72 t ha<sup>-1</sup> in Pelotas to 94.69 t.ha<sup>-1</sup> in Dourados, showing higher yield when compared to other studies, such as Souza, Parrella, Tardin and Costa (2013), where the yield was 27.31 t.ha<sup>-1</sup> in Pelotas and 55.06 t.ha<sup>-1</sup> in Goiânia,

thus validating the bioenergetic potential of these cultivars. The mean for total soluble solids (TSS) ranged from 11.80° Brix in Pelotas and 19.03° Brix in Sete Lagoas. And for TBH, ranged from 4.09 t.ha<sup>-1</sup> in Pelotas to 14.35 t.ha<sup>-1</sup> in Dourados.

According to the Scott-Knott test, the genotypes were classified into four groups. Then, the following genotypes were highlighted for FBY: B002, B004, B007, B008, B009, B012, B018, B020, CMSXS 647, CV198, which presented above mean yield in Dourados, Lavras, Nova Porteirinha, Penedo and Sete Lagoas. For TSS, genotypes with above mean values were B004, B007, B008, B010, B011, B012, B014, B015, B016, B019, B020, CMSXS 646, BRS 508 and BRS 511 in the environments of Sete Lagos, Sinop, Jaguariúna and Nova Porteirinha. These genotypes presented good quality of the juice, being these values higher than the one recommended for processing according to Masson et al. And for TBH, the genotypes that presented above mean values were B004, B005, B006, B007, B008, B011, B012, B015, B020, CMSXS 646, BRS 508 and CV198, in Dourados, Nova Porteirinha, Lavras and Sete Lagoas.

According to the environment classification, by Annicchiarico (1992) methodology, the estimation of the environmental index (Ij) was calculated for the traits FBY, TSS, and TBH (Table 3). This estimation of the environmental index validated the mean results obtained in the experiments classified as favorable or unfavorable.



**Table 3.** Environmental indices (Ij) and classification of favorable or unfavorable environments for cultivation, according to the methodology of Annicchiarico (1992). Values obtained by evaluating the traits of fresh biomass yield (FBY), content of total soluble solids (TSS) and tons of Brix per hectare (TBH), for 25 sweet sorghum genotypes evaluated in 2015/2016 agricultural year.

> Given these results, it was observed that for the Pelotas, Passo Fundo and Planaltina the lowest *Ij* values were estimated and, consequently, unfavorable for all the traits. And the Nova Porteirinha presented the favorable classification for all the traits. Then, the confidence index  $(Wi)$  was calculated for favorable environments  $W_i(f)$  and for unfavorable environments  $W_i(d)$ . It is considered the

ideal genotype that provides the lowest risk of being adopted, in other words, the genotype that presents the greatest absolute value for the confidence index. The results are demonstrated in Tables 4, 5 and 6.

The genotypes CV198, B020, B004, B008, B005, B006, B009, B012, B019, B002, B017 and B014 had the lowest risk of having behavior below mean for FBY, considering 75% confidence (Table 4). For TSS, genotypes B008, BRS 508, B012, CMSXS647, B011, BRS511, B016, B015, B007, CMSXS646, B019 and B004. And for TBH, genotypes CV198, B006, B008, B005, CMSXS646, B010, B019, B009, B022, B017, B012, B016 and B004. Similar results were observed by Souza et al. (2013), that validate the use of the Annicchiarico method, using sweet sorghum genotypes.

The authors considering this methodology that is easy to implement and interpret, and it was possible through the reliability index to select genotypes that have adaptability and stability. The genotypes CV198 and B005 in favorable environments, and CV198 in unfavorable environments presented high Wi for FBY, with performances de 34.40% and 13.45% higher than mean. For TSS, the genotypes (B008 and BRS508) in favorable environments and (B008 and CMSXS647) in unfavorable environments. And for TBH, the genotypes (CV198 and B008) in unfavorable environments (B008 and B004). Thus, the CV198, B006, B008, B005 genotypes, in the general analysis, presented the lowest behavioral risks, the CV198 and B008 genotypes were the most responsive to environmental improvements, and the B008 genotype was highlighted because even in an unfavorable environment, it presented a behavior superior to the other genotypes.

The B008 genotype showed high Wi for TSS and TBH in the classification of the general analysis, in favorable and unfavorable environments, demonstrating that this genotype is widely adapted, in addition to having an mean content of SST 17.60° Brix, presenting quality agronomic potential in the juice. Thus, the B008 genotype is a promising sweet sorghum hybrid, developed through the research of the sweet sorghum breeding program of Embrapa Maize and Sorghum.

For the FBY the genotypes more adapted and stable, were CV198, B005, B006, B020 (Figure 1A). For TSS it was B012, B008, BRS 508, and CMSXS 646 (Figure 1B) and for TBH, B008, B012, B005, B020 (Figure 1C). The B008 and B012 genotypes were the more stable for both TSS and TBH, and B005 and B020 genotypes were the more stable for FBY and TBH. Some authors have found similar results when working with sweet sorghum. Alvels et al. (2020) aiming to select simultaneously, pre-commercial sweet sorghum hybrids with high adaptability and stability, via mixed models, in six different environments, evaluated 16 pre-commercial sweet sorghum hybrids, in the off-season of 2015/16 and 2016/17 in environments of the Brazilian Cerrado. This study demonstrated the possibility of obtaining high ethanol yield from sweet sorghum cultivated in the sugarcane off-season, using GGE biplot methodologies. Rono et al. (2016) evaluated eight sweet sorghum genotypes at five different locations in two growing seasons of 2014, and the GGE biplot showed high yielding genotypes EUSS10, ACFC003/12, SS14, and EUSS11 for cane yield; EUSS10, EUSS11, and SS14 for juice yield; and EUSS10, SS04, SS14, and ACFC003/12 for ethanol yield.

Based on the behavior of different genotypes in different environments, the formation of mega-environments is possible, where the mean of the genotype is compared to the mean of the mega-environment (Figure 2). For the FBY the environments were divided into two groups, starting from the blue lines that came from the origin of the biplot. For this trait, the environments were grouped as follows: Mega-environment 1: Penedo, Lavras, Pelotas, Sete Lagoas, and Sinop, where the best genotypes were those at the vertex of the group of environments, highlighting the CV198 genotype. And the Mega-environment 2: Jaguariúna and Planaltina, highlighting the B007 genotype (Figure 2A). For the TSS, only one megaenvironment was formed, consisting of Dourados, Lavras, Nova Porteirinha, Pelotas, Planaltina, Sete Lagoas and Sinop. For this mega-environment the genotypes B008 and B012 highlighting (Figure 2B). And for TBH two megaenvironments were grouped as follows: Mega-environment 1: Penedo, Lavras, Passo Fundo, Pelotas, Sete Lagoas, and Sinop, highlighting the CV198 genotype. And in the second mega-environment: Dourados, Jaguariúna and Planaltina, highlighting genotype B019 (Figure 2C).

**Table 4**. Parameters of adaptability and overall stability (Wi) in favorable environments (Wi(f)) and in unfavorable environments (Wi(u)) of 25 genotypes of sweet sorghum for fresh biomass yield (FBY), based on the methodology of Annicchiarico (1992), for 25 sweet sorghum genotypes evaluated in 2015/2016 agricultural year.



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**Table 5.** Parameters of adaptability and overall stability (Wi) in favorable environments (Wi(f)) and in unfavorable environments (Wi(u)) of 25 genotypes of sweet sorghum for total soluble solids (TSS), based on the methodology of Annicchiarico (1992), for 25 sweet sorghum genotypes evaluated in 2015/2016 agricultural year.



**Table 6.** Parameters of adaptability and overall stability (Wi) in favorable environments (Wi(f)) and in unfavorable environments (Wi(u)) of 25 genotypes of sweet sorghum for tons of Brix per hectare (TBH), based on the methodology of Annicchiarico (1992), for 25 sweet sorghum genotypes evaluated in 2015/2016 agricultural year.





**Figure 1.** Classification of 25 sweet sorghum genotypes according to the GGE biplot model in ten environments during the 2015/2016 agricultural year. A: fresh biomass yield (FBY); B: content of total soluble solids (TSS); and C: tons of Brix per hectare (TBH).



**Figure 2.** Groups of mega-environments according to the GGE biplot model in ten environments during the 2015/2016 agricultural year. A: fresh biomass yield (FBY); B: content of total soluble solids (TSS); and C: tons of Brix per hectare (TBH).

Figueiredo et al. (2015) used the GGEbiplot method and found that the CMSXS 647 genotype presented the best stability and adaptability for FBY and TBH. This genotype was also used in the present work but did not obtain similar behavior. This can be explained by the fact that the genotype was evaluated in only six similar environments, Dourados, Nova Porteirinha, Passo Fundo, Pelotas, Sete Lagoas, and Sinop.

Thus, the adaptability and stability study methods used in this work presented satisfactory and consistent results, aiming at the selection of more adapted and stable genotypes for bioenergy production. Also, B005 and B008 sweet sorghum genotypes, showed superior performance, with similar classification in both methods. However, new studies of the adaptability and stability in the different agricultural year and new environments are important to obtain more complete information on the performance of sweet sorghum genotypes for bioenergy production.

### **CONCLUSIONS**

The Annicchiarico and GGEbiplot adaptability and stability study methods presented satisfactory and consistent results and can be used separately or together in sweet sorghum breeding programs.

B005 and B008 sweet sorghum genotypes presented superior performance, with similar classification in both methods studied.

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