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Adaptability and stability of saccharine sorghum cultivars

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The Brazilian bioenergy sector has been trying the use of saccharine sorghum in order to optimize ethanol production. However, there are few varieties fitted to this objective and little knowledge about their adaptability and stability. Then, the purpose of this work was to study the adaptability and stability of saccharine sorghum, taking into account the effects of the G x E interaction, to select superior genotypes and validate if the two selection methods for identification of genotypes with better phenotypic stability complement one another. Thus, the methodologies of Eberhart and Russell as well as Cruz et al. were used; and according to Eberhart and Russell, the BRS 511 genotype is preferred in ethanol production because it presents highly predictable and responsive behavior to changes in environments for all the traits evaluated in specific or broad conditions. The method also identified the genotypes CMSXS644, CMSXS647 and Sugargraze for green mass production; CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506 and BRS 509 for total soluble solid content. The methods used in this study were not complementary to selection genotypes.

Key words: Bioethanol, ethanol production, genotype and environment interaction, genetic breeding, *Sorghum bicolor* (L.) Moench.

INTRODUCTION

Brazil is a country with different renewable energy alternatives and with favorable weather to production,

one of the leaders in world ethanol production (Lamers et al., 2011; Li et al., 2018). The bioethanol is a fuel that is

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> produced in large quantities in the world, being motivated by the creation of flex-fuel vehicles and by the more accessible and lower price of this fuel in relation to gasoline. This biofuel can be obtained from sources saccharose rich agricultural crops, as it is traditionally done in a large scale from sugarcane in Brazil or starchrich crops as corn in the USA (AIE, 2010).

Sugarcane is considered the main crop for biofuel production; however, other species are also viable for this purpose such as the saccharine sorghum [Sorghum *bicolor* (L.) Moench]. This crop is a C₄ plant with a high photosynthesis efficiency being the most efficient energy producer in order to produce concentrated energy from renewable sources. Thus, the saccharine sorghum is a promising alternative to complement the supply of raw material for sugar-ethanol industry, since this crop has the potential to supply the demand for raw material for bioethanol production in the sugarcane harvesting season without cessation of service in the production (Dar et al., 2018). The main purpose of breeding programs involving sorghum hybrids is the identification of genotypes with high production potential, wide adaptability and good stability. According to Cruz and Carneiro (2006), the genotype adaptability indicates the ability to respond advantageously to environmental improvements, meanwhile genotype stability refers to the ability to present expected behaviors in relation to environmental variations. In Brazil, breeding programs aiming the production of sorghum varieties work intensively evaluating trails in several environments and years. The high cost of research activities requires a rational use of resources, since there is a decrease in the number of environments for testing and proper selection of the right method to do this analysis.

Furthermore, selection can be maximized by using methodologies permitting a better interpretation of genetic and environmental effects individually, which allows selection to be carried out considering only the genetic effects. In this case, the method proposed by Eberhart and Russel (1966) and Cruz et al. (1989) are recommended to be used in data analysis as an attempt to maximize selection. These methodologies mainly try to quantify genotype behavior in relation to genotype adaptability and stability in various environments, besides quantifying environmental differences when selecting the ideal environment and, therefore, providing high genetic gains. Although both methodologies are very informative separately, their association can provide a better understanding about genotype and environment interaction.

Eberhart and Russel (1966) method evaluate only a regression coefficient, which can be estimated by a unique analysis considering all the tested environments. In this method, the ideal genotype can be discarded because it has a double slope and, then, deviations that should be evaluated in different environments could be quite high in relation to the estimated straight line. Thus,

this method was improved by Cruz et al. (1989) with the use of bissegmented linear regression, which makes possible to adjust a straight-line segment to unfavorable and poor environments and also another straight-line segment to the best and favorable environments (Ramalho et al., 2012).

Thus, the purpose of this study was to analyze the adaptability and stability of saccharine sorghum, considering the effects of genotype and environment interactions for selecting superior genotypes for ethanol production, and compare the two methods of genotype identification with better phenotypic stability.

MATERIALS AND METHODS

Experimental design

Eight experiments (2013 -2014) were performed in order to obtain the crop values and uses as depicted in Table 1.

Sixteen sorghum saccharine genotypes obtained from a genetic breeding program of *Embrapa Milho e Sorgo* were evaluated in eight environments. Ten of these genotypes are fertility restoration lineages (BRS506, BRS508, BRS509, BRS511, CMSXS629, CMSXS630, CMSXS643, CMSXS644, CMSXS646 and CMSXS647) and the other six are hybrids (CV198, CV568, Sugargraze, V82391, V82392 and V82393).

The experiment was carried out in a randomized complete block design with 16 saccharine sorghums (treatments) and three replications. The plots (experimental units) were considered to be four rows of 5 m. Row spacing was 0.7 m and eight plants were left per linear meter. Two central rows were considered as useful plots. Weeding and pest control was performed as needed. Fertilization was performed using 400 kg ha⁻¹ of the 8N-28P-16K and 200 kg ha⁻¹ of urea in dressing. No micro nutrients were applied.

Evaluated traits

The evaluated traits were green mass production (GMP), content of total soluble solids (TSS) and tons of brix per hectare (TBH). The values for GMP were obtained by measuring the weight of all plants in each plot, with no panicles. The plants were harvested when the seed reached physiological maturity, and their weight was determined in kilograms (kg) for each plot in tons per hectare (t ha⁻¹). The values for TSS was determined from six plants per plot which were taken at random by using a juice sample from plants an automatic digital refractometer, with the reading in degrees Brix. Finally, the values for TBH was obtained by the multiplication of the values for GMP by the values for TSS (TBH = GMP x TSS).

Statistical analysis

All the statistical analysis described below was performed in the statistical genetic software GENES. The first analysis conducted before the analysis of variance (ANOVA) was performed to verify the following assumptions: If the errors follow a normal distribution, are independent and present a constant variance (homogenous variance) (RAMALHO et al., 2000), then, individual analysis of variance was performed considering each environment separately and all the evaluated traits. The statistical model adopted for the individual analysis of variance for each environment was the following:

Environment	Geographical coordinate												
Environment	Latitude	Longitude	Altitude (m)										
Santa Vitória-MG	18º 50' 19" S	50º 07' 17" O	498										
Sete Lagoas-MG	19º 27' 57" S	44º 14' 49" O	767										
Lavras-MG	21º 14' 43" S	44º 59' 59" W	919										
Nova Porteirinha-MG	15º 47' 00" S	43º 18' 00" O	533										
Piracicaba-SP	22º 43' 31" S	47º 38' 57" W	547										
Sinop-MT	11º 51' 51" S	55° 30' 09" W	345										
Planaltina–DF	17º 35' 03" S	47º 42' 30" W	1.100										
Dourados-MS	22º 13' 16" S	54º 48' 20" W	430										

Table 1. Municipalities where experiments with sorghum saccharine genotypes were conducted with their geographical coordinates, respectively.

$$Y_{ii} = \mu + g_i + b_i + e_{ii}$$

Where: Y_{ij} is the individual observation (plot) of the genotype *i* in the block *j*; μ represents the overall mean and is a constant associated to the observations; g_i is the effect of genotype *i* (*i* =1, 2,..., 16);

 b_j is the random effect of block j (j = 1, 2, ..., 3); and e_{ij} is the experimental error associated to Y_{ij} .

Before performing the combined analysis, the Cochran (1947) test was performed to verify the homogeneity of variances for all variables considered in this study. Since there are statistical differences between the mean squared errors, it used the method proposed by Cochran (1954) accordingly to recommendations of Pimentel-Gomes (2000), in which the residual mean squares of the combined analysis were obtained by the degrees of freedom adjusted of each variable. Then, it was possible to perform an analysis of variance combined with the decomposition of the sum of squares for environments. The following statistical model was fitted to the data when performing the analysis of variance combined between the environments:

$$Y_{ijk} = \mu + G_i + B/A_{jk} + A_j + GA_{ij} + e_{ijk}$$

Where: Y_{ijk} is the individual observation (plot) of the genotype *i* inside the environment *j* and in the block *k*; μ represents the overall mean and is a constant associated to the observations; G_i is the fixed effect of the genotype *i* (*i* =1, 2, ...,16); B/E_{jk} is the effect of the block *k* inside the environment *j*; E_J is the effect of the environment *j* (*j*=1, 2, ...,8); GE_{ij} is the effect of the interaction between the genotype *i* and the environment *j*; and e_{ijk} is the experimental error associated to Y_{ijk} .

Analysis of variance was first conducted for each environment. After verifying the assumptions of homogeneity of residual variances, analysis of variance for all sites and the Scott-Knott test (1974) at 5% probability were performed as illustrated in supplementary Tables S1, S2 and S3. Finally, adaptability and stability analysis was conducted, after determining significant genotype by environment interaction. The analysis of phenotypic adaptability and stability for the genotypes were performed using both the methodologies of Eberhart and Russell (1966) and Cruz et al. (1989). The method proposed by Eberhart and Russel (1966) is based on linear regression analysis and uses the mean of productivity as an adaptability parameter for each genotype (β_{0i}), the linear regression coefficient (β_{1i}) as a response pattern of the genotype in different environments, and finally the genotype stability is evaluated by the mean squared deviation of regression (σ^2_d) and/or by the coefficient of determination (R^2) . The following statistical model was fitted to the data when performing analysis

using the method of Eberhart and Russel (1966):

$$Y_{ij} = \beta_{0i} + \beta_{1i}I_j + \delta_{ij} + \varepsilon_{ij}$$

where: Y_{ij} is the mean of productivity for the genotype *i* in the environment *j*; β_{0i} is the mean of the genotype *i* in all environments; β_{1i} is the linear regression coefficient of the genotype *i*; I_j is the environmental index estimated by the difference between the environmental mean and the general mean; δ_{ij} is the regression deviation of the genotype *i* in the environment *j*; ε_{ij} is the mean of the experimental error associated to the observation Y_{ij} , presenting normal and independent distribution, with a mean of zero and constant variance. The adaptability parameter (β_{1i}) was estimated by using the following equation:

$$\hat{\beta}_{1i} = \frac{\sum Y_{ij}I_j}{\sum I_j^2}$$

Under the null hypothesis H_0 : β_i =1 and the alternative hypothesis H_1 : $\beta_i \neq 1$, the β_i estimates were evaluated by student's *t*-test using the following equation:

$$t = \frac{\hat{\beta}_{li} - 1}{\sqrt{\hat{V}(\hat{\beta}_{li})}} \hat{V}(\hat{\beta}_{li}) = \frac{\sigma^2}{r \sum_{j=1}^{2} \sigma^2}$$

The stability parameter (σ_{di}^{2}) was estimated by the following equation:

$$\sigma_{di}^2 = \frac{MSD_i - MSE}{r},$$

in which: MSD_i is the mean squared deviation of genotype i; MSE is the mean squared error; and *r* is the number of repetitions.

Then, under the null hypothesis of H_0 : $\sigma^2_{di}=0$ and the alternative hypothesis of H_1 : $\sigma^2_{di}\neq 0$, the estimates for σ^2_{di} were analyzed by *F*-test using the following equation:

$$\mathsf{F}_{\mathsf{calculated}} = \frac{MSD_i}{MSE}$$

Where: MSD_i is the mean squared deviation and MSE is the mean

squared error.

Thus, as proposed by Eberhart and Russel (1966), the environment effect can be split in two components: a linear and nonlinear component. The regression coefficient β_{1i} is associated to the linear component and indicates the genotype adaptability, which is the genotype ability to respond to environmental improvements. On the other hand, the regression deviations σ^2_{di} are associated to the nonlinear component and indicate the phenotypic stability.

The method of Eberhart and Russel (1966) reveals that a stable genotype occurs when $\sigma_{di}^2 = 0$ and a non-stable genotype is found when $\sigma_{di}^2 \neq 0$. Also, a genotype has wide adaptability if $\beta_{1i} = 1$; is adapted to favorable environments, in case that $\beta_{1i} > 1$, and adapted to unfavorable environments, if $\beta_{1i} < 1$. The coefficient of determination (R2) for each genotype was proposed by Pinthus (1973) as an additional measure for the Eberhart and Russel (1966) method and was also used in this study as a measure to define the phenotypic stability and to quantify the ratio of phenotypic variation of each genotype that is explained by linear regression.

According to Cruz et al. (2004), genotypes with higher means of productivity and presenting σ_{di}^2 statistically different from zero can occur and, then, it would be necessary the selection of some genotypes from the group with low stability. In these cases, an auxiliary measure of comparing genotypes is the coefficient of determination (R^2_i), which is estimated by the following equation:

$$R_i^2 = \frac{SS(Linear \text{Regression})}{SS(E/G_i)}.100,$$

Where; SS is the sum of squares.

For evaluating stability of the genotype, the method proposed by Cruz et al. (1989) was used in the analysis. This method is based on the bissegmented regression analysis and has the mean as an adaptability parameter (β_{0i}) and a linear response to both unfavorable ($_{1i}$) and favorable environments ($_{1i} + _{2i}$). Then, stability of the genotype is evaluated by the regression deviation of each genotype in relation to the environmental variations following this statistical model:

$$Y_{ij} = \beta_{0i} + \beta_{1i}I_j + \beta_{2i}T(I_j) + \delta_{ij} + e_{ij}$$

where: Y_{ij} is the mean of genotype *i* in the environment *j*; β_{0i} is the general mean of genotype *i*; β_{1i} is the linear regression coefficient associated to the variable l_j ; l_j is the environmental index; β_{2i} is the linear regression coefficient associated to the variable $T(l_j)$; if $I_j < 0$, $T(l_j)=0$; if $l_j > 0$, $T(l_j)=l_j - l_+$, in which *l*+ is the mean of the positive indexes l_j ; δ_{ij} is the linear regression deviation; and e_{ij} is the mean of the experimental error; β_{0i} indicates the maximum productivity found in all the experimental essay; $\beta_{1i} = 0.5$ and $\beta_{2i} = 1$ are values established by Cruz and Carneiro (2006). $\beta_{1i} = 0.5$ indicates a low response to unfavorable environments and $\beta_{1i} + \beta_{2i} = 1.5$ reveals the responsiveness to favorable conditions.

RESULTS AND DISCUSSION

The homogeneity of variances showed that the ratio between the bigger and lower mean squared error was higher to the approximate ratio of 7:1, therefore, the maximum F was statistically significant and then the null hypothesis was rejected for all the variables evaluated in this study. Then, the combined analysis was carried out and the results showed that the source of genotype variation was non-statistically significant only for GMP, indicating there are none significant statistical differences between the genotype means in the environments. On the other hand, for the variables TBH and TSS, it was possible to identify significant differences between genotype means in the environments. The G x E interaction was statistically significant (P<0.01) by F-test, indicating that the performance of sorghum cultivars evaluated in this study is statistically different in the environments. And furthermore, the coefficients of variation (C.V.) for the variables indicate good experimental precision; for GMP is 15.77%, for TSS is 12.37% and for TBH is 19.51% as illustrated in Table 2. In fact, Figueiredo et al. (2015) also studied about the adaptability and stability of saccharine sorghum and used the methods of GGEBiplot and Toler in data analysis. Their same variables GMP, TBH and TSS were also identified as statistically significant, indicating statistical differences between genotype means in the environments. For this reason, the significant differences between genotype means inside each environment reflects the need of doing a more detailed study, aiming to identify genotypes that present better adaptability and stability.

The variance analysis for the phenotypic adaptability and stability were evaluated by the linear regression method proposed by Eberhart and Russel (1966) and the results are shown in Table 3. Significant differences were observed for the effects of genotypes, environments and G x E interactions, which mean that genotypes presented different behaviors in non-similar environments, confirming the variability of evaluated genotypes and environments.

Significant effects of G x E interaction indicate that there are differences between regression coefficients of the genotypes being evaluated, and that a part of G x E interaction can be explained by a linear relationship between genotypes and environments. Furthermore, regression deviations inform about phenotypic stability and they were also identified to be statistically significant in this study. Thus, linear and nonlinear components of stability are involved in the performance of genotypes in the environments. Also, the significance of regression deviations informs that in general the evaluated genotypes showed an unstable and unpredictable behavior. However, by its magnitude, the linear environment was mainly responsible for the explanation of genotype behavior.

The mean estimates of GMP, TBH and TSS for genotypes ($\hat{\beta}_{0i}$), the regression coefficient (β_{1i}), the variance of regression coefficients (σ^2_d) and the coefficient of determination (\mathbb{R}^2) for each genotype were obtained by Eberhart and Russel (1966) method and the results are in Table 4. The genotypes BRS 506 and CMSXS643 presented $\mathcal{B}_1 > 1$ for GMP, which indicates a higher production than the general mean reflecting a specific adaptability to favorable conditions and adaptability to environments with high productivity.

Variable	Source of variation		Degree of freedom	Mean square	F
	Blocks/environments	-	16	172.23	-
	Environments (E)	-	7	6556.59	38.06**
	Genotype (G)	-	15	367.84	1.62 ^{n.s.}
GMP	GxE	-	81	227.08	2.94**
	Residue	-	179	77.15	-
	Mean	55,7	-	-	-
	Coefficient of variation (%)	15,77	-	-	-
	Blocks/environments	-	16	0.8	
	Environments (E)	-	7	52.33	64.64**
	Genotypes (G)	-	15	6.17	2.72**
ТВН	GxE	-	86	2.27	3.36**
	Residue	-	191	0.68	-
	Mean	4,21	-	-	-
	Coefficient of variation (%)	19,51	-	-	-
	Blocks/environments	-	16	3.47	-
	Environments (E)	-	7	143.12	41.22**
	Genotypes (G)	-	15	81.14	6.16**
TSS	GxE	-	77	13.16	3.88**
	Residue	-	168	3.39	-
	Mean	14,88	-	-	-
	Coefficient of variation (%)	12,37	-	-	-

Table 2. Summary of the combined analysis of variance of sixteen saccharine sorghum genotypes that were evaluated in eight environments and the following variables were considered in the analysis: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Values with * were statistically significant at 1%; values with ^{ns} are non-significant by F-test.

Similarly, Souza et al. (2013) also studied the adaptability and stability of the saccharine sorghum and, by using the method of Annichiarico, found out that the genotype BRS 506 showed a high stability and adaptability to favorable and unfavorable environments, meanwhile the CMSXS643 genotype did not present a high index for stability and adaptability.

Also, by using Eberhart and Russel (1966) method in this study, it is possible to recommend the BRS 511 genotype as a good cultivar for ethanol production because it shows a highly predictable and responsive behavior to variations in environments with specific or wide conditions to all the evaluated traits and shows good mean in variables of agronomist as presented in supplementary Table S1, S2 and S3. Similarly, Figueiredo et al. (2015) used the GGEBiplot method and verified that the following genotypes BRS 511 and CMSXS647 presented higher adaptability and stability for the variable TBH. This fact corroborates the results of this present work, in which the BRS 511 variety presents adaptability, stability and good means, making it possible to be recommended as a cultivar for ethanol production.

When analyzing the mean squared deviation (σ_d^2) , a parameter that classifies the stability of a genotype, it

was possible to identify that BRS 511, CMSXS647 and TBH genotypes presented a good foreseeability with σ_d^2 = 0. Genotypes with wide adaptability in the environments are the ones with $\beta_1=1$, and thus with unpredictable behaviors ($\sigma_{di}^2 \neq 0$ and $R^2 < 80\%$). In this situation, the CMSXS630, CMSXS643, BRS 506, CV 198, CV 568 and V82393 are superior genotypes for the variable GMP; CMSXS643, CV 568, Sugargraze and V82393 for the variable TBH; and finally, BRS 508, CV 198, Sugargraze, V82391 and V82393 for the variable TSS. According to Eberhart and Russel (1966), the superior genotypes in the analysis are the ones showing a higher mean than the overall mean, overall or wide adaptability ($\beta_1 = 1$), good foreseeability ($\sigma_d^2 = 0$) and with good response both in favorable and unfavorable environments. The results of this analysis are in Table 4 and it can be inferred that the CMSXS644, CMSXS647, BRS 511 and Sugargraze are superior genotypes for the variable GMP. The CMSXS629, CMSXS630, CMSXS646, CMSXS647, BRS 508, BRS 509, BRS 511 and CV198 genotypes are superior for the variable TBH. And finally, the CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506, BRS509 and BRS 511 genotypes are superior for the variable TSS.

	ANO	VA	
Variable	Source of variation	Degree of freedom	Mean square
	Environment (E)	7	6556.592**
	Genotypes (G)	15	367.840**
	E x G interaction	105	175.176**
CMD	Env/Gen. (E/G)	112	574.014**
GMP	Linear Env. (LE)	1	45896.146**
	G x EL interaction	15	128.869**
	Combined deviation (E/G)	96	171.463**
	Residue	179	1
	Environments (E)	7	52.332**
	Genotypes (G)	15	6.174**
	E x G interaction	105	1.861**
TDU	Env/Gen. (E/G)	112	5.016**
IBH	Linear Env. (LE)	1	366.324**
	G x LE interaction	15	1.265**
	Combined deviation (E/G)	96	1.838**
	Residue	191	1
	Environments (E)	7	143.116**
	Genotypes (G)	15	81.135**
	E x G interaction	105	9.653**
TOO	Env/Gen. (E/G)	112	17.995**
155	Linear Env. (LE)	1	1001.811**
	G x LE interaction	15	7.167**
	Combined deviation (E/G)	96	9.439**
	Residue	168	1

Table 3. Summary of analysis of variance combined with decomposition of the sum of squares of environments according to the method of Eberhart and Russell (1966).

Values with * were statistically significant at 1% by F-test. Sixteen saccharine sorghum genotypes were evaluated in eight environments and the following variables were considered in the analysis: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

The results of the adaptability and stability analysis for the variable GMP, TBH and TSS using the method proposed by Cruz et al (1989) are found in Table 5. The β_1 estimates genotype performances in unfavorable conditions. For both GMP and TBH, most of the genotypes did not differ from one (β_1 =1), with the exception of V82392 and BRS 506 that showed to be more demanding in this same condition (β_1 >1). And finally, for the variable TSS, all the genotypes did not differ significantly from one (β_1 =1). On the other hand, when considering unfavorable conditions, it was not possible to identify responsive genotypes to variations in the environment.

In relation to the linear response of favorable environments ($\beta_1+\beta_2$), the genotype CMSXS630 was the most responsive in this environment to the variable GMP and the genotype CMSXS643 to the variable TBH, which showed statistically significant results (linear response) higher than one ($\beta_1+\beta_2>1$). However, it was found that only the genotype CMSXS643 is adapted to favorable environments and responsive to environmental improvements, for having a higher mean than the overall mean. For the TSS variable, all the genotypes were nonstatistically significant from one ($\beta_1+\beta_2=1$).

Phenotypic stability or genotype predictability is evaluated by regression deviations in relation to the linear response to the environmental improvement. Then it was possible to identify the following genotypes CMSXS629, CMSXS630, CV198, CV568 and V82393 showing regression deviations different from zero ($\sigma^2_d \neq 0$) for the variable GMP; the genotypes CV568 and V82393 for the variable TBH, and finally the genotypes BRS508, CV 198, Sugargraze and V82391 for the variable TSS. Thus, these genotypes can be classified as unstable both on favorable and unfavorable environments.

By Cruz et al. (1989), the ideal genotype should present a high mean (high β_0), the phenotypic averages shown in supplementary Tables S1, S2 and S3 are less

						Variable						
Genotype		GN	/IP			TBI	4			TSS	6	
	Mean (ß₀)	ß 1	σ² _d	R² (%)	Mean (ß₀)	ß 1	σ^{2}_{d}	R² (%)	Mean (ß₀)	ß 1	σ^2_d	R² (%)
CMSXS629	51.93	0.83 ^{ns}	21.74 ^{ns}	70.12	4.42	0.74 ^{ns}	0.26 ^{ns}	54.22	15.91	0.79 ^{ns}	-0.92 ^{ns}	66.43
CMSXS630	53.42	1.16 ^{ns}	100.52**	62.93	4.32	0.68 ^{ns}	0.22 ^{ns}	51.33	16.1	0.64 ^{ns}	-0.46 ^{ns}	47.59
CMSXS643	56.04	1.23 ^{ns}	55.82**	74.98	4.53	0.89 ^{ns}	0.80**	47.33	15.9	0.50 ^{ns}	0.87 ^{ns}	23.1
CMSXS644	57.15	0.95 ^{ns}	8.54 ^{ns}	80.95	4	0.99 ^{ns}	-0.16 ^{ns}	87.70	14.22	0.60 ^{ns}	-1.69 ^{ns}	79.43
CMSXS646	52.58	0.71 ^{ns}	-14.77 ^{ns}	88.44	4.68	0.87 ^{ns}	0.00 ^{ns}	74.49	17.28	1.33 ^{ns}	-1.53 ^{ns}	92.61
CMSXS647	58.54	0.96 ^{ns}	-1.80 ^{ns}	86.22	4.57	0.92 ^{ns}	0.04 ^{ns}	74.24	14.76	1.32 ^{ns}	1.31 ^{ns}	64.39
BRS 506	58.16	1.13 ^{ns}	32.46*	77.87	4.57	1.55**	0.15 ^{ns}	86.43	14.93	1.07 ^{ns}	-0.36 ^{ns}	70.61
BRS 508	53.06	0.85 ^{ns}	-1.99 ^{ns}	83.36	4.33	0.77 ^{ns}	0.19 ^{ns}	59.18	17.45	0.55 ^{ns}	5.46**	12.39
BRS509	53.73	1.41*	8.25 ^{ns}	90.4	4.34	1.13 ^{ns}	0.14 ^{ns}	77.52	16.41	1.40 ^{ns}	-0.99 ^{ns}	86.86
BRS 511	57.1	0.81 ^{ns}	-3.52 ^{ns}	82.80	4.84	0.78 ^{ns}	-0.01 ^{ns}	70.54	16.9	1.00 ^{ns}	-0.05 ^{ns}	63.80
CV 198	59.56	0.93 ^{ns}	71.34**	58.92	4.34	1.10 ^{ns}	0.19 ^{ns}	74.81	15.24	0.63 ^{ns}	3.20*	20.91
CV 568	65.51	0.69 ^{ns}	99.84**	37.53	4.47	0.89 ^{ns}	1.05**	42.1	13.12	0.96 ^{ns}	0.01 ^{ns}	61.06
Sugargraze	59.14	0.90 ^{ns}	28.90 ^{ns}	70.27	4.17	1.31 ^{ns}	0.41*	74.59	13.36	1.20 ^{ns}	5.41**	40.19
V82391	51.61	1.08 ^{ns}	21.11 ^{ns}	80.15	3.32	1.24 ^{ns}	0.05 ^{ns}	83.72	12.15	1.17 ^{ns}	3.60*	46.07
V82392	52.86	1.34 ^{ns}	23.10 ^{ns}	85.53	3.32	1.18 ^{ns}	0.47*	69.02	12.07	1.42 ^{ns}	1.71 ^{ns}	65.34
V82393	50.83	1.04 ^{ns}	62.45**	66.22	3.17	0.96 ^{ns}	0.70**	53.23	12.25	1.42 ^{ns}	2.44*	61.03
Overall mean	55.7	-	-	74.79	4.21	-	-	67.53	14.88	-	-	56.36

Table 4. Estimates of the regression coefficients (β_0 and β_1), mean squared deviation of regression (σ_d^2) and coefficient of determination for the following variables: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Values for \mathbf{B}_1 , parameters with * were statistically significant at 1%, respectively; values with ^{ns} are non-significant by Student *t*-test. Values for σ_d^2 parameters with ** or *** were statistically significant at 1% or 5%, respectively; values with ^{ns} are non-significant by *F*-test. The genotypes of saccharine sorghum were analyzed following the method proposed by Eberhart and Russel (1966).

demanding to unfavorable environment (lower β_1), have the ability to respond to environmental improvements (the highest $\beta_1 + \beta_2$) and present a high stability in the evaluated environments ($\sigma_d^2=0$ or $\mathbb{R}^2 > 80\%$). According to the data on Table 5, there was no genotype satisfying this condition, and for this reason the results obtained by this method was not coincident to the results of the method proposed by Eberhart and Russel (1966), which do not allow a better description of genotypes in relation to genotype and environment interaction. In case of coinciding the

results between these two methodologies, it would be possible to identify genotypes with better adaptability and especially its discrimination in relation to the phenotypic stability in the different environments evaluated in this study. The difficulty in identifying the ideal cultivars by the method proposed by Cruz et al (1989) can be due to the positive correlation that exists between β_{1i} and β_{1i} + β_{2i} (Miranda et al., 1998).

Depending on the degree of association between the methodologies used in this study, they could be an auxiliary measure to choose the stability parameter which results in the best adjustment (Duarte and Zimmermann, 1995). However, there is a lack of association between the methodologies proposed by Eberhart and Russel (1966) and Cruz et al. (1989), which indicates that there exists a redundancy in the information provided by methods based on regression and, for this reason, they should not be used together. The association between the methodologies of Eberhart and Russel (1966) and Cruz at al. (1989) are mainly explained, by the fact that these methodologies use the same

							V	ariable							
Genotype			GMP					TBH					TSS		
	X (ß₀)	ß ₁	(ß ₁ +ß ₂)	σ^2_d	R² (%)	X(ß₀)	ß ₁	(ß1+B2)	σ^2_d	R² (%)	X(ß₀)	ß 1	(ß ₁ +ß ₂)	σ_d^2	R² (%)
CMSXS629	51.93	0.86 ^{ns}	0.74 ^{ns}	30.05 ^{ns}	70.39	4.42	0.66 ^{ns}	1.21 ^{ns}	0.43 ^{ns}	57.88	15.91	0.83 ^{ns}	0.36 ^{ns}	0.12 ^{ns}	68.23
CMSXS630	53.42	0.83 ^{ns}	2.28 **	53.68**	80.49	4.32	0.66 ^{ns}	0.77 ^{ns}	0.44 ^{ns}	51.51	16.1	0.59 ^{ns}	1.11 ^{ns}	0.66 ^{ns}	49.99
CMSXS643	56.04	1.08 ^{ns}	1.77 ^{ns}	55.21**	79.16	4.53	0.62 ^{ns}	2.50**	0.46 ^{ns}	73.44	15.9	0.54 ^{ns}	0.05 ^{ns}	2.26 ^{ns}	24.86
CMSXS644	57.15	0.89 ^{ns}	1.16 ^{ns}	12.30 ^{ns}	82.09	4	0.98 ^{ns}	1.00 ^{ns}	-0.02 ^{ns}	87.71	14.22	0.61 ^{ns}	0.55 ^{ns}	-0.74 ^{ns}	79.49
CMSXS646	52.58	0.74 ^{ns}	0.58 ^{ns}	-14.22 ^{ns}	89.33	4.68	0.79 ^{ns}	1.34 ^{ns}	0.11 ^{ns}	78.35	17.28	1.34 ^{ns}	1.27 ^{ns}	-0.54 ^{ns}	92.63
CMSXS647	58.54	1.00 ^{ns}	0.81 ^{ns}	1.07 ^{ns}	86.83	4.57	0.87 ^{ns}	1.16 ^{ns}	0.20 ^{ns}	75.13	14.76	1.19 ^{ns}	2.68 ^{ns}	2.15 ^{ns}	70.78
BRS 506	58.16	1.03 ^{ns}	1.45 ^{ns}	37.69 ^{ns}	79.71	4.57	1.54*	1.59 ^{ns}	0.35 ^{ns}	86.44	14.93	1.03 ^{ns}	1.50 ^{ns}	0.79 ^{ns}	71.67
BRS 508	53.06	0.83 ^{ns}	0.92 ^{ns}	1.79 ^{ns}	83.53	4.33	0.56 ^{ns}	2.03 ^{ns}	-0.01 ^{ns}	85.83	17.45	0.52 ^{ns}	0.89 ^{ns}	7.81**	12.81
BRS509	53.73	1.34 ^{ns}	1.63 ^{ns}	11.57 ^{ns}	91.07	4.34	1.04 ^{ns}	1.64 ^{ns}	0.27 ^{ns}	80.25	16.41	1.36 ^{ns}	1.81 ^{ns}	0.04 ^{ns}	87.54
BRS 511	57.1	0.82 ^{ns}	0.76 ^{ns}	0.12 ^{ns}	82.88	4.84	0.63 ^{ns}	1.67 ^{ns}	-0.04 ^{ns}	86.09	16.9	0.97 ^{ns}	1.31 ^{ns}	1.20 ^{ns}	64.36
CV 198	59.56	1.13 ^{ns}	0.27 ^{ns}	65.30 **	67.71	4.34	1.25 ^{ns}	0.25 ^{ns}	0.21 ^{ns}	82.5	15.24	0.57 ^{ns}	1.27 ^{ns}	4.98**	22.93
CV 568	65.51	0.91 ^{ns}	-0.06 **	92.78 **	50.64	4.47	1.16 ^{ns}	-0.72**	0.76 **	65.63	13.12	0.97 ^{ns}	0.82 ^{ns}	1.30 ^{ns}	61.18
Sugargraze	59.14	0.89 ^{ns}	0.90 ^{ns}	39.15 ^{ns}	70.27	4.17	1.47 ^{ns}	0.38 ^{ns}	0.44 ^{ns}	81.07	13.36	1.20 ^{ns}	1.14 ^{ns}	7.79**	40.2
V82391	51.61	0.98 ^{ns}	1.44 ^{ns}	22.79 ^{ns}	82.66	3.32	1.35 ^{ns}	0.55 ^{ns}	0.11 ^{ns}	88.08	12.15	1.25 ^{ns}	0.34 ^{ns}	5.34**	48.24
V82392	52.86	1.46**	0.91 ^{ns}	21.75 ^{ns}	88.13	3.32	1.32 ^{ns}	0.37 ^{ns}	0.56 ^{ns}	74.67	12.07	1.44 ^{ns}	1.22 ^{ns}	3.33 ^{ns}	65.47
V82393	50.83	1.21 ^{ns}	0.46 ^{ns}	60.37**	72.33	3.17	1.08 ^{ns}	0.25 ^{ns}	0.88 **	58.23	12.25	1.58 ^{ns}	-0.31 ^{ns}	3.07 ^{ns}	69.4
Overall mean	55.7	-	-	-	78.58	4.21	-	-	-	75.8	14.88	-	-	-	58.11

Table 5. Mean and parameter estimates of adaptability and stability for the following variables: green mass production (GMP), tons of brix per hectare (TBH) and the content of total soluble solids (TSS).

Under the null hypothesis H₀: $\beta_1 = 1$ and H₀: $(\beta_1 + \beta_2) = 1$ and the alternative hypothesis H₀: $\beta_1 \neq 1$ and H₀: $(\beta_1 + \beta_2) \neq 1$, the adaptability parameter was decomposed in favorable and unfavorable environments and then evaluated by the Student's *t*-test. Values with * and ** are statistically different at 1% and 5%, respectively, and ^{ns} are non-significant values. Under the null hypothesis H₀: $\hat{\sigma}_{\delta_i}^2 \neq 0$, the stability parameter was evaluated by *F*-test. Values with * and ** are statistically different at 1% and 5%, respectively, and ^{ns} are non-significant values. Sixteen genotypes of saccharine sorghum were analyzed in eight environments following the method proposed by Cruz et al. (1989).

stability parameters (σ^2_{di} and R^2).

Conclusion

The method of Eberhart and Russel (1996) was efficient in identifying the genotype BR511 as a cultivar for ethanol production. Considering each trait individually, the following superior genotypes were also identified: CMSXS644, CMSXS647 and Sugargraze for the trait GMP; CMSXS629 CMSXS630, CMSXS646, CMSXS647, BRS 508, BRS 509 and CV 198 for the trait TBH; and CMSXS629, CMSXS630, CMSXS643, CMSXS646, BRS 506 e BRS 509 for the trait TSS. The method of Cruz et al. (1989) was not appropriate to identify the superior genotypes to be recommended for ethanol production. The compared methodologies were noncomplementary, then the combined use of them is not recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of

interests.

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SUPPLEMENTARY TABLES

Table S1. Average of green mass production (GMP), *, in t ha⁻¹, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

Construct	Enviroment																								
Genotype	S. Vit	ória	a	Sete la	igo	as	Lav	ras		N. Poi	rteiı	rin	Piraci	icab	ba	Sin	ор		Plana	ltin	a	Dourados			Mean
CMSXS629	52.00	В	С	49.49	В	b	62.12	А	b	54.72	В	а	44.29	В	b	42.25	В	b	37.27	В	а	73.31	А	а	51.93
CMSXS630	47.25	В	С	50.99	В	b	92.19	А	а	57.78	В	а	37.66	С	b	53.31	В	а	39.31	С	а	48.89	В	b	53.42
CMSXS643	47.57	С	С	63.35	В	а	88.56	А	а	54.96	В	а	33.56	С	b	57.01	В	а	41.32	С	а	61.95	В	b	56.04
CMSXS644	52.75	В	С	59.97	В	а	76.67	А	b	46.67	В	а	52.52	В	а	57.18	В	а	39.64	В	а	71.81	А	а	57.15
CMSXS646	55.54	А	С	57.20	А	а	67.34	А	b	51.64	А	а	41.40	В	b	51.93	А	а	40.09	В	а	55.46	А	b	52.58
CMSXS647	54.37	В	С	67.89	А	а	75.73	А	b	54.25	В	а	48.33	В	а	56.69	В	а	40.16	В	а	70.91	А	а	58.54
BRS 506	51.40	С	С	67.15	В	а	85.72	А	а	48.57	С	а	53.16	С	а	43.26	С	b	45.04	С	а	71.00	В	а	58.16
BRS 508	47.38	В	С	60.50	А	а	70.83	А	b	49.70	В	а	41.48	В	b	53.13	В	а	38.98	В	а	62.46	А	b	53.06
BRS 509	52.88	В	С	59.21	В	а	86.09	А	а	56.80	В	а	31.84	С	b	44.36	С	b	35.26	С	а	63.36	В	b	53.73
BRS 511	60.22	В	С	61.64	В	а	75.38	А	b	56.02	В	а	41.27	С	b	56.62	В	а	45.71	С	а	59.98	В	b	57.1
CV 198	76.67	А	b	56.34	В	а	70.34	А	b	61.13	В	а	59.85	В	а	44.62	С	b	35.11	С	а	72.45	А	а	59.56
CV 568	89.78	А	а	61.54	С	а	74.84	В	b	65.14	С	а	64.48	С	а	59.91	С	а	43.70	D	а	64.68	С	b	65.51
Sugargraze	62.87	А	С	61.27	А	а	78.30	А	b	49.48	В	а	64.85	А	а	46.68	В	b	40.94	В	а	68.78	А	а	59.14
V82391	49.57	В	С	50.85	В	b	75.61	А	b	46.59	В	а	54.63	В	а	39.05	С	b	30.93	С	а	65.68	А	b	51.61
V82392	63.65	А	С	51.52	В	b	72.23	А	b	51.25	В	а	35.03	С	b	40.44	С	b	31.71	С	а	77.04	А	а	52.86
V82393	71.67	А	b	45.55	В	b	65.81	А	b	48.06	В	а	30.94	С	b	45.11	В	b	35.06	С	а	64.41	А	b	50.83
Means	58.47	-	-	57.78	-	-	76.11	-	-	53.3	-	-	45.96	-	-	49.47	-	-	38.76	-	-	65.76	-	-	55.7

*Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.

Table S2. Average of tons of brix per hectare (TBH)*, in t ha-1, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

	Enviroment																								
Genotype	S.Vi	tória	а	Sete I	agoa	IS	Lav	ras	;	N. Po	rteir	in	Pirac	cical	ba	Sir	пор		Plan	altiı	na	Dourados			Mean
CMSXS629	3.74	В	с	5.95	А	b	4.38	А	а	4.61	А	а	5.06	А	а	2.52	В	b	3.89	В	а	5.22	А	а	4.42
CMSXS630	4.40	В	с	5.36	А	b	6.22	А	а	3.49	В	а	4.00	В	b	3.70	В	а	3.96	В	а	3.43	В	а	4.32
CMSXS643	4.25	В	С	7.13	А	а	5.89	А	а	2.98	В	а	3.38	В	b	4.30	В	а	4.32	В	а	3.95	В	а	4.53
CMSXS644	3.84	В	С	5.63	А	b	4.70	А	а	2.67	В	а	4.79	А	а	2.40	В	b	3.57	В	а	4.39	А	а	4.00
CMSXS646	5.56	А	b	6.79	А	а	4.75	В	а	3.93	В	а	4.75	В	а	3.87	В	а	3.98	В	а	3.81	В	а	4.68
CMSXS647	4.80	В	С	6.63	А	а	4.74	В	а	4.04	В	а	5.60	А	а	3.68	В	а	3.37	В	а	3.71	В	а	4.57
BRS 506	4.44	В	С	7.27	А	а	5.58	А	а	2.31	С	а	5.95	А	а	2.18	С	b	4.35	В	а	4.51	В	а	4.57
BRS 508	3.37	В	С	6.53	А	а	4.89	В	а	3.28	В	а	4.34	В	а	3.68	В	а	4.03	В	а	4.51	В	а	4.33
BRS 509	5.25	А	b	6.49	А	а	5.85	А	а	3.55	В	а	3.40	В	b	2.81	В	а	3.48	В	а	3.91	В	а	4.34
BRS 511	5.20	В	b	6.96	А	а	5.15	В	а	4.27	В	а	4.49	В	а	3.95	В	а	4.67	В	а	4.02	В	а	4.84
CV 198	4.51	А	С	5.66	А	b	4.87	А	а	3.64	В	а	6.21	А	а	2.14	В	b	3.14	В	а	4.58	А	а	4.34
CV 568	7.23	А	а	5.16	В	b	4.56	С	а	3.95	С	а	5.30	В	а	2.76	С	а	3.51	С	а	3.29	С	а	4.47
Sugargraze	4.08	А	С	5.67	А	b	5.03	А	а	3.03	В	а	6.22	А	а	1.21	С	b	3.63	В	а	4.46	А	а	4.17
V82391	3.39	А	С	4.80	А	b	4.41	А	а	2.39	В	а	4.68	А	а	0.82	В	b	2.19	В	b	3.87	А	а	3.32
V82392	4.86	А	С	4.48	А	b	4.50	А	а	2.36	В	а	3.16	А	b	0.99	В	b	1.72	В	b	4.50	А	а	3.32
V82393	5.57	А	b	4.24	В	b	3.67	В	а	2.74	С	а	2.49	С	b	1.13	С	b	2.09	С	b	3.47	В	а	3.17
Means	4.65	-	-	5.92	-	-	4.95	-	-	3.33	-	-	4.61	-	-	2.63	-	-	3.49	-	-	4.10	-	-	4.21

* Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.

	Enviroment																								
Genotype	S.Vi	tória		Sete	lago	as	La	vras		N. Po	rteiri	n	Pirac	icab	а	Sir	пор		Plan	altina	a	Dour	ados		Mean
CMSXS629	15.07	Α	b	17.37	Α	а	17.07	Α	а	16.97	Α	а	18.22	Α	а	13.87	Α	а	14.45	Α	а	14.25	Α	а	15.91
CMSXS630	18.80	Α	а	15.23	Α	а	16.57	А	а	15.90	Α	а	17.05	А	а	17.03	Α	а	14.15	А	а	14.10	Α	а	16.10
CMSXS643	17.87	Α	а	16.63	Α	а	16.07	А	а	14.90	Α	а	16.42	Α	b	18.13	Α	а	14.44	А	а	12.78	Α	а	15.90
CMSXS644	14.63	Α	b	14.03	Α	b	14.97	А	а	15.60	Α	а	15.18	Α	b	14.27	Α	а	12.83	А	а	12.21	Α	а	14.22
CMSXS646	20.03	Α	а	17.67	Α	а	18.03	А	а	18.37	Α	а	19.94	Α	а	16.33	В	а	14.11	В	а	13.74	В	а	17.28
CMSXS647	17.60	Α	а	13.73	В	b	14.53	А	а	15.43	Α	а	18.66	Α	а	16.20	Α	а	11.33	В	b	10.58	В	а	14.76
BRS 506	17.23	Α	а	15.63	Α	а	16.07	А	а	14.10	В	а	18.13	Α	а	11.93	В	b	13.79	В	а	12.57	В	а	14.93
BRS 508	14.17	В	b	17.10	В	а	18.47	А	а	20.00	Α	а	19.36	Α	а	21.13	Α	а	15.02	В	а	14.33	В	а	17.45
BRS 509	19.87	Α	а	16.07	В	а	16.80	А	а	17.87	Α	а	19.36	Α	а	14.90	В	а	13.97	В	а	12.44	В	а	16.41
BRS 511	17.47	Α	а	17.80	Α	а	17.00	А	а	17.03	Α	а	20.25	Α	а	17.97	Α	а	14.26	В	а	13.41	В	а	16.90
CV 198	11.87	В	b	14.97	В	b	18.13	А	а	16.23	Α	а	17.68	Α	а	16.97	Α	а	13.39	В	а	12.70	В	а	15.24
CV 568	16.03	Α	b	12.00	В	b	14.40	А	а	15.53	Α	а	13.54	Α	b	11.00	В	b	12.27	В	а	10.18	В	а	13.12
Sugargraze	12.97	В	b	13.47	В	b	16.87	А	а	16.13	Α	а	15.87	Α	b	6.57	С	с	12.02	В	а	12.95	В	а	13.36
V82391	14.10	Α	b	13.77	Α	b	14.43	Α	а	12.90	Α	а	14.24	А	b	5.73	С	с	9.97	В	b	12.03	Α	а	12.15
V82392	15.20	Α	b	12.73	Α	b	14.90	А	а	12.07	Α	а	14.65	Α	b	7.63	В	с	7.62	В	b	11.72	Α	а	12.07
V82393	15.30	Α	b	13.67	Α	b	13.23	А	а	15.93	Α	а	13.25	Α	b	7.10	В	с	8.58	В	b	10.91	В	а	12.25
Means	16.14	-	-	15.12	-	-	16.10	-	-	15.94	-	-	16.99	-	-	13.55	-	-	12.64	-	-	12.56	-	-	14.88

Table S3. Average of total soluble solids (TSS) in °Brix, of 16 cultivars of sweet sorghum grown in five environments, in 2013/2014 season in Brazil.

*Means followed by the same lowercase and same capital letters do not differ at same column and row, respectively, by Scott Knott test at 5% probability.