

Divergência genética em genótipos de sorgo biomassa via caracteres agrônômicos e físico-químicos

Genetic divergence in biomass sorghum genotypes through agronomic and physical-chemical characters

Divergencia genética en genotipos de biomasa sorgo a través de caracteres agronómicos y físico-químicos

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Resumo

A presente pesquisa teve como objetivo avaliar a divergência genética em 34 genótipos de sorgo biomassa via caracteres agronômicos e físico-químicos. O delineamento utilizado foi de blocos casualizados com três repetições. As características agronômicas e físico-químicas avaliadas foram: dias para florescimento, número de colmos, altura da planta, número de folhas, produção de massa verde, produção de massa seca, determinação de cinzas totais, determinação do teor de voláteis, lignina insolúvel e determinação teor de carbono fixo. Os dados foram submetidos à análise de variância e em seguida, para estimar divergência foi utilizada como medida de dissimilaridade a distância generalizada de Mahalanobis. Com base nesta matriz, foram empregados os métodos de agrupamentos de otimização de Tocher e método Hierárquico de Agrupamento Médio Entre Grupos (UPGMA), e análises de variáveis canônicas, e a projeção com base nas duas primeiras variáveis canônicas dispostas no espaço bidimensional. Utilizou-se, também, o critério de Singh para quantificar a contribuição relativa dessas características na divergência genética. Os genótipos avaliados apresentaram diferenças significativas para as todas as características avaliadas. A combinação entre os pares de genótipos 201429B001 e 201429B028 (394.98) foi a mais divergente e a combinação entre os genótipos 201429B015 e 201429B031 (6.31) a mais similar. O agrupamento gerado pelo método de Otimização de Tocher, hierárquico UPGMA e dispersão gráfica demonstraram semelhança no agrupamento dos genótipos. As duas primeiras variáveis canônicas foram suficientes para explicar cerca de 81,78 % da variação total observada. Os resultados demonstraram ampla diversidade genética entre os 34 genótipos de sorgo biomassa.

Palavras-chave: *Sorghum bicolor* (L.) Moench; Análise multivariada; Melhoramento genético.

Abstract

The present research aimed to evaluate the genetic divergence in 34 sorghum biomass genotypes via agronomic and physicochemical characters. The design used was randomized blocks with three replications. The agronomic and physical-chemical characteristics evaluated were: days for flowering, number of stems, plant height, number of leaves, green mass production, dry mass production, determination of total ash, determination of volatile content, insoluble lignin and determination of fixed carbon content. The data were submitted to analysis of variance and then, to estimate divergence, the generalized *Mahalanobis* distance was used as a measure of dissimilarity. Based on this matrix, the methods of Tocher's optimization clusters and the Hierarchical method of Average Grouping Between Groups (UPGMA) were used, and analysis of canonical variables, and the projection based on the first two canonical variables arranged in two-dimensional space. Singh criterion was also used to quantify the relative contribution of these characteristics to genetic divergence. The evaluated genotypes showed significant differences for all the evaluated characteristics. The combination between the 201429B001 and 201429B028 (394.98) genotype pairs was the most divergent and the combination between the 201429B015 and 201429B031 (6.31) genotypes was the most similar. The grouping generated by the Tocher Optimization method, hierarchical UPGMA and graphical dispersion showed similarity in the grouping of genotypes. The first two canonical variables were sufficient to explain about 81.78% of the total variation observed. The results showed a wide genetic diversity among the 34 genotypes of sorghum biomass.

Keywords: *Sorghum bicolor* (L.) Moench; Multivariate analysis; Genetic breeding.

Resumen

Esta investigación tuvo como objetivo evaluar la divergencia genética en 34 genotipos de biomasa de sorgo mediante caracteres agronómicos y fisicoquímicos. El diseño utilizado fue de bloques al azar con tres repeticiones. Las características agronómicas y físico-químicas evaluadas fueron: días de floración, número de tallos, altura de la planta, número de hojas, producción en masa verde, producción en masa seca, determinación de cenizas totales, determinación de contenido volátil, lignina insoluble y determinación del contenido de carbono fijo. Los datos se sometieron a análisis de varianza y luego, para estimar la divergencia, se utilizó la distancia de Mahalanobis generalizada como medida de disimilitud. A partir de esta matriz, se utilizaron los métodos de optimización de clusters de Tocher y el método Jerárquico de Agrupación Promedio entre Grupos (UPGMA) y el análisis de variables

canónicas, y la proyección a partir de las dos primeras variables canónicas ordenadas en un espacio bidimensional. También se utilizó el criterio de Singh para cuantificar la contribución relativa de estas características a la divergencia genética. Los genotipos evaluados mostraron diferencias significativas para todas las características evaluadas. La combinación entre los pares de genotipos 201429B001 y 201429B028 (394.98) fue la más divergente y la combinación entre los genotipos 201429B015 y 201429B031 (6.31) fue la más similar. El agrupamiento generado por el método de Optimización de Tocher, UPGMA jerárquico y dispersión gráfica mostró similitud en el agrupamiento de genotipos. Las dos primeras variables canónicas fueron suficientes para explicar alrededor del 81,78 % de la variación total observada. Los resultados mostraron una amplia diversidad genética entre los 34 genotipos de biomasa de sorgo.

Palabras clave: *Sorghum bicolor* (L.) Moench; Análisis multivariable; Mejora genética.

1. Introduction

The cultivation of biomass sorghum [*Sorghum bicolor* (L.) Moench] has proved to be a widespread crop in the world, with a high content of fermentable fibers and sugars, which can be explored on a large scale and with great adaptability to various edaphoclimatic conditions (Carrillo et al., 2014). In addition, the short production cycle of an average of six months for high biomass yield, as well as less water requirements, in relation to other energy crops, favor sorghum in terms of energy efficiency (Nagaiah et al., 2012).

Due to this importance, several researches are carried out with this culture, among them, the study of the divergence of sorghum biomass aiming at the production of bioenergy, it is important to identify, characterize and quantify genetic diversity, since the success of any genetic improvement program, is based on the presence of variability for the characteristic to be improved (Oykunle, 2015).

Genetic divergence can be estimated by assessing dissimilarity through the use of biometric techniques, using a set of phenotypic and genotypic information accessed through the observation of morphological, physiological or molecular differences. After quantification, genetic diversity can be better evidenced by applying multivariate statistical techniques (Cruz et al., 2014).

Johnson and Wickern (1988) consider that the prediction of genetic divergence can be performed using the technique of canonical variables, generalized Mahalanobis distance, Tocher grouping methods and dispersion in cartesian axes. Cruz et al. (2014), report that the

advantage of multivariate methods is linked to the fact that they allow combining the multiple information contained in the experimental unit, enabling the characterization of genotypes based on a complex of variables. In this context, this research aimed to estimate genetic divergence between sorghum biomass genotypes based on agronomic and physical-chemical characteristics, using multivariate analysis techniques.

2. Material and Methods

The experiment was conducted at the experimental station of the Laboratory of Genetic Resources & Biotechnology, belonging to the State University of Mato Grosso (UNEMAT), located in the county of Cáceres, state of Mato Grosso, Brazil (16°11'42"S and 57°40'51"), in the agricultural year 2014/15. The experimental area has a red yellow dystrophic Latosol soil, altitude of approximately 118 m. According to Dallacort et al. (2014), the characteristic climate of the region, according to the Köppen classification, is of the tropical, hot, humid and dry winter (Awa) type, with period of rain regime varying from October to April, and drought from May to September.

A total of 34 sorghum biomass genotypes were evaluated, all of them hybrids sensitive to the photoperiod: 201429B001, 201429B002, 201429B003, 201429B004, 201429B005, 201429B006, 201429B007, 201429B008, 201429B009, 201429B010, 201429B011, 201429B012, 201429B013, 201429B014, 201429B015, 201429B016, 201429B017, 201429B018, 201429B019, 201429B020, 201429B021, 201429B022, 201429B023, 201429B024, 201429B025, 201429B026, 201429B027, 201429B028, 201429B029, 201429B030, 201429B031, 201429B032, 201429B033 and BRS716, from the Embrapa Corn and Sorghum Improvement Program.

The experimental design adopted was randomized blocks with three replications, in which the plots were composed of four lines of 5 m, spaced 0.70 m, with a density of ten plants m⁻¹. Only the two central rows were considered as a useful area where the data collection and evaluation was carried out.

The harvest was performed manually, evaluating ten plants per plot, at an average of 180 days after sowing. The quantitative agronomic characteristics evaluated were:

Days to Flowering (FLOWER): number of days from sowing to the beginning of pollen release in 50% of the plants in the plot.

Number of Stems (NS): average number of stems obtained from ten plants of the useful area of the plot;

Plant Height (PH): average height in meters of ten plants from each plot, measured at the base of the plant to the apex of the panicle;

Number of Leaves (NL): average number of leaves obtained from ten plants in the useful area of the plot;

Green Mass Production (GMP): determined in Kg/plot, by weighing ten whole plants (without panicle) of the plot's useful area. GMP data will be converted to $t\ ha^{-1}$.

Dry Mass Production (DMP): determined by the difference in weight between samples of the newly collected material (GMP) and after being subjected to drying in a forced aeration oven at $65^{\circ}\ C$ for 72 hours. DMP data has been converted to $t\ ha^{-1}$.

Physical-chemical analyzes were carried out at the Biomass laboratory at Embrapa Agrossilvipastoril – Sinop, state of Mato Grosso, Brazil, in which it evaluated:

Determination of Total Ashes (TA): Estimated as the ratio between the weight of the calcified sample in a muffle furnace at $525^{\circ}\ C$ for 4 hours and the weight of the final dry matter (ABNT NBR 13999, 2003).

Determination of Volatile Content (VC): Estimated by the difference between the weight of the initial sample + crucible and the weight of the final sample + crucible placed on the muffle door previously heated to ($950^{\circ}\ C$), for 3 minutes, and inside for 7 minutes with the door closed. At the end, this difference was divided by the weight in grams of the biomass sample according to the ABNT NBR 8112 (1983).

Insoluble Lignin (IL): Determined according to the Klason method modified by Rocha et al. (1997).

Determination of Fixed Carbon Content (FCC): Estimated by the difference between the ash content and the volatile content according to the ABNT NBR 8112 (1983).

The data were subjected to analysis of variance and then, to estimate genetic divergence between the genotypes, the generalized Mahalanobis distance was used as a measure of dissimilarity. Based on this matrix, the methods of Tocher's optimization clusters and the Hierarchical method of Average Grouping Between Groups (UPGMA) were used, and analysis of canonical variables, and the projection based on the first two canonical variables arranged in two-dimensional space. Also, Singh (1981) criterion to quantify the relative contribution of these characteristics in genetic divergence. To perform all statistical analyzes, the Genes software computational resources were used (Cruz, 2013).

3. Results and Discussion

The effects of treatments showed significant differences at the level of 1% probability by the F test for the variables: FLOWER, NS, PH, NL, GMP, TA, VC, IL and FCC and only the DMP characteristic showed a significant difference at the 5% probability level by the F test (Table 1). These results are indicative of the presence of genetic diversity among sorghum biomass genotypes, analyzed in this research. This fact is favorable to the improvement of these characteristics in order to identify genetic materials with agroenergetic potential.

Table 1 - Summary analysis of variance of agronomic and physical-chemical characters related to the evaluation of 34 sorghum biomass genotypes evaluated in the county of Cáceres – Mato Grosso, agricultural year 2014/15.

FV	Medium Squares ^{1/}										
	GL	^{2/} FLOWER	NS	PH	NL	GMP	DMP	TA	VC	IL	FCC
Block	2	0.0093	4.8450	0.6514	49.3425	1001.9532	22.0453	0.0123	0.0025	0.0226	0.0072
Genótype	33	0.2460**	3.0981**	0.1789**	3.3816**	242.2658*	99.8055**	0.1687**	0.0360**	0.1072**	0.2177**
Error	66	00041	0.7342	0.0443	0.7237	134.5534	40.3622	0.0046	0.0074	0.0171	0.0501
Average		11.18	19.37	5.05	19.30	72.01	32.83	2.16	9.03	4.40	3.69
CV(%)		0.58	4.42	4.16	4.41	16.11	19.35	3.16	0.95	2.97	6.08

¹FLOWER = number of days to flower; NS = average number of stems; PH = average plant height; NL: average number of leaves; GMP = green mass production; DMP = dry mass production; TA: total ash content; VC: volatile content; IL = insoluble lignin; FCC = fixed carbon content e CV: coefficient of variation. ²Flowering data transformed into square root. ** and * significant 1% and 5% probability, respectively, by the F test. Source: Authors.

The values of the variation coefficient ranged from low to medium, according to the classification proposed by Gomes (2009), ranging from 0.58 % for the FLOWER variable to 19.35% for the DMP variable, thus confirming good experimental precision. These results demonstrated that there was little influence of the uncontrollable experimental variations and corroborate the results found in other sorghum research (Perazzo et al., 2014; Silva et al., 2016).

From the generalized *Mahalanobis* distance, using 10 characteristics in 34 sorghum biomass genotypes, it was possible to verify that the combination between 201429B001 and 201429B028 was the most dissimilar, presenting the longest distance (394.98), and the genotypes 201429B015 and 201429B031 were the most similar, presenting the shortest distance (6.21).

The existence of divergence between the evaluated genotypes, provides the opportunity for heterotic gain and manifestation of superior genotypes in segregating generations

(Falconer, 1981; Paixão et al., 2008). However, Amaral Júnior (1996) ponders that in addition to the dissimilarity, it is necessary to observe the performance of each individual genotype in a breeding program.

The use of the Tocher Optimization method, based on the dissimilarity expressed by the Mahalanobis distances, made it possible to distribute the 34 genotypes in eight different groups (Table 2). Group I comprised eight genotypes, corresponding to 23.52% of the total analyzed, presenting a lower genetic dissimilarity of 6.21 between the genotypes 201429B015 and 201429B031 and greater dissimilarity between 201429B001 and BRS716 (45.88).

Table 2 - Representation of the cluster generated by the Tocher Optimization method, based on the dissimilarity of the 34 sorghum biomass genotypes evaluated in the county of Cáceres – Mato Grosso, in the agricultural year 2014/15.

CLUSTER	GENÓTIPOS	%
I	201429B015, 201429B031, 201429B005, BRS716, 201429B032, 201429B008, 201429B001 and 201429B013	23.52
II	201429B014, 201429B018, 201429B017, 201429B023, 201429B010, 201429B029, 201429B009, 201429B007, 201429B011, 201429B004, 201429B003 and 201429B027	35.29
III	201429B020, 201429B033 and 201429B026	8.82
IV	201429B025, 201429B024, 201429B021 and 201429B028	11.79
V	201429B012 and 201429B030	5.88
VI	201429B002 and 201429B022	5.88
VII	201429B016 and 201429B019	5.88
VIII	201429B006	2.94
TOTAL	34	100

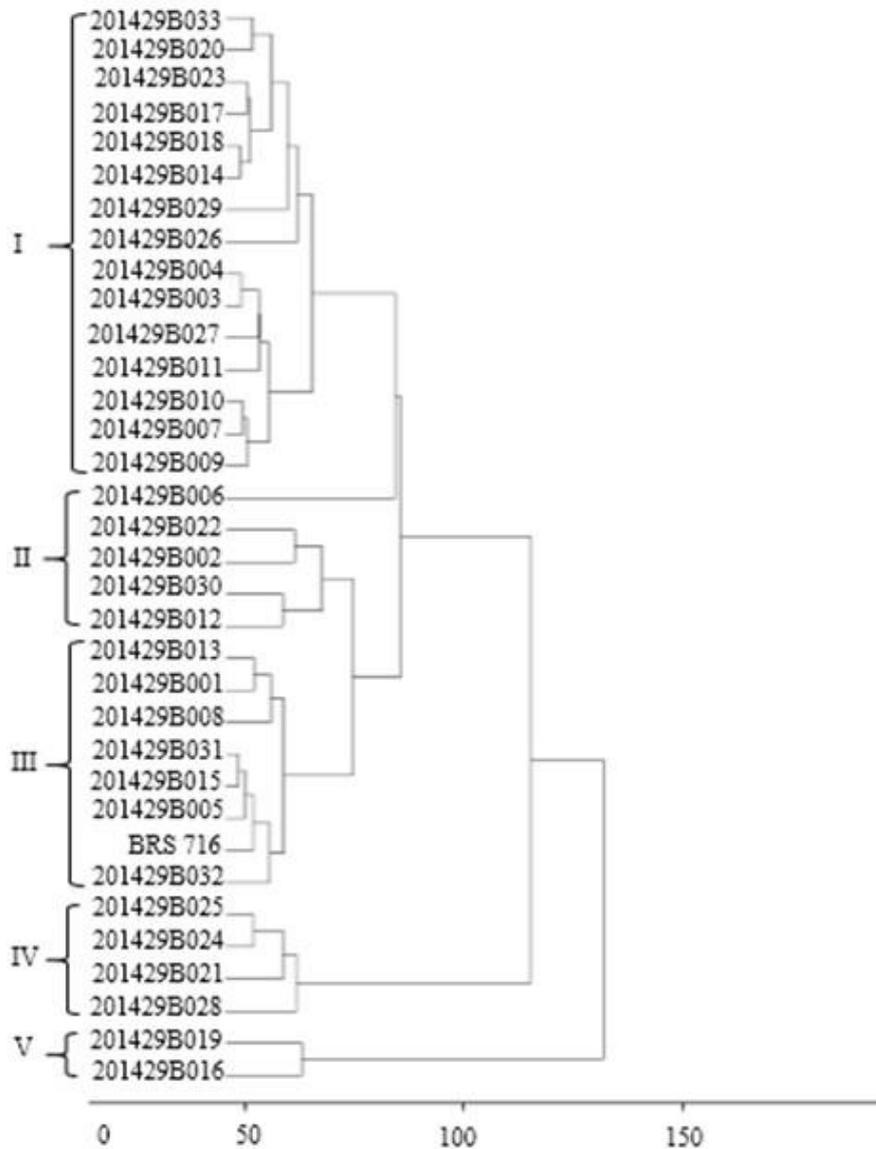
Source: Authors.

Group II was the most numerous constitution, gathering 35.29% of the sorghum biomass genotypes, with the greatest dissimilarity in this group represented between the genotypes 201429B017 and 201429B027 (50.67), and the lowest dissimilarity between 201429B004 and 201429B003 (7.68). In group III the highest dissimilarity was observed between the genotypes 201429B020 and 201429B026 (25.49) and the lowest between 201429B020 and 201429B023 (17.03), in which this group formed by three genotypes, representing 8.82%. Group IV allocated four genotypes (11.79%) showing similarity between the combinations 201429B024 and 201429B025 (13.25) and dissimilarity between 201429B021 and 201429B028 (39.41) (Table 2).

Group V, VI and VII were formed by two genotypes each (5.88%), being the genotypes 201429B012 and 201429B030 (26.95), 201429B002 and 201429B022 (32.62) and 201429B016 and 201429B019 (35.91) respectively, showing a broad genetic basis, characterizing thus a good degree of genetic divergence (Table 2). Group VIII was represented only by the genotype 201429B006 demonstrating to be the most distant in relation to the other genotypes, presenting satisfactory behavior in relation to the production of green and dry mass, volatile content, lignin content and fixed carbon content.

Regarding the similarity dendrogram obtained by the UPGMA method, it enabled the formation of five groups (Figure 1). Group I is made up of 44.11% of the genotypes because they present mostly lower averages for number of leaves.

Figure 1 - Representative dendrogram of the grouping of 34 biomass sorghum genotypes, using the UPGMA method, based on the dissimilarity estimated from ten characteristics, evaluated in the county of Cáceres - Mato Grosso, 2014/15 agricultural year.



Source: Authors.

Group II allocated 14.70% of the genotypes and their main characteristic is high productivity of green mass. Group III is formed by 23.52% of the genotypes because they have longer days for flowering, that is, longer cycle. For biomass sorghum, late cycles increase the vegetative period, leading to an increase in the production of green and dry mass. Group IV, formed by 11.76% of the genotypes for having lower dry mass productivity and shorter days for flowering, and group V, forming only 5.88% of the total evaluated genotypes, presented low ash content (Figure 1).

The UPGMA method grouped similarly to the Tocher method the biomass sorghum genotypes, with Tocher groups II and III now incorporating UPGMA group I and Tocher group V, VI and VII met in group II of UPGMA.

Table 3 contains the eigenvalue estimate (λ_i) corresponding to the Canonical Variables (VC_i), the percentage and accumulated variances and the weighting coefficients (eigenvectors) associated with the original variables. The results showing in the table in which the first two canonical variables explained about 81.78% of the variation, being 53.01% for the first and 28.76% for the second, which were used to estimate the genetic divergence of the 34 genotypes in a two-dimensional space facilitating the geometric interpretation. Cruz et al. (2014), points out that the feasibility of using the canonical variable technique is restricted to the concentration of variability between the first two variables as above 80%.

Table 3 - Eigenvalue estimates (λ_i) corresponding to the percentages of variation, explained by canonical variables (VC_i), and weighting coefficient (eigenvectors) of ten characteristics, evaluated in 34 sorghum biomass genotypes, evaluated in the county of Cáceres - Mato Grosso, agricultural year 2014/15.

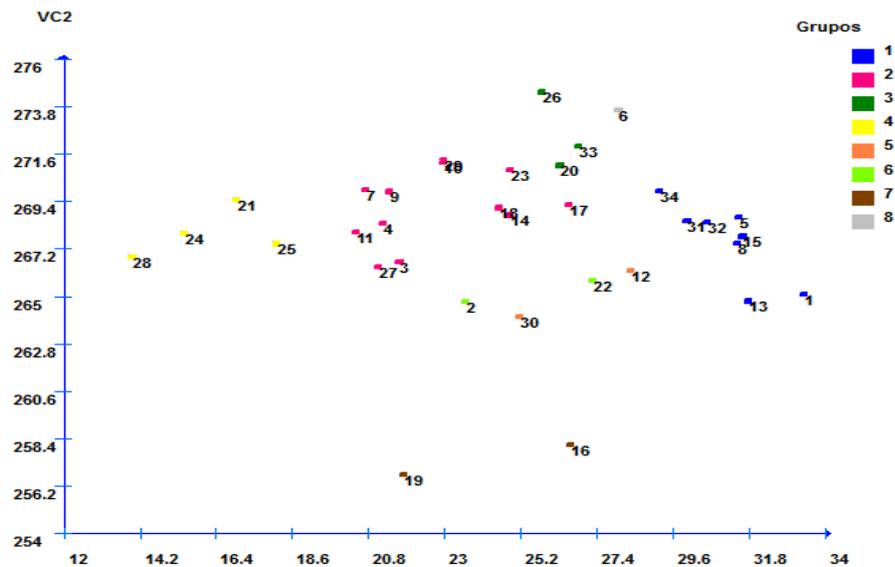
VC _i	Eigenvalues		Autovetores associados ^{1/}									
	λ_i	Acum. (%)	FLOWER	NS	PH	NL	GMP	DMP	TA	VC	IL	FCC
VC ₁	53.01	53.01	15.76	0.08	1.47	0.16	-0.03	0.001	-5.74	-13.45	-1.11	-5.98
VC ₂	28.76	81.78	3.59	-0.03	-0.03	-0.06	-0.02	0.02	18.59	17.73	1.36	6.64
VC ₃	4.93	86.72	0.77	0.12	1.63	-0.55	-0.02	0.009	4.26	32.13	3.73	9.07
VC ₄	4.05	90.77	1.08	0.57	-0.81	0.15	-0.01	0.16	-0.47	1.43	6.63	2.56
VC ₅	2.98	93.75	-3.27	0.33	2.43	0.71	-0.06	0.71	6.09	25.77	-1.10	9.90
VC ₆	2.03	95.79	0.98	-0.68	-1.03	0.27	-0.02	0.10	13.00	58.09	1.32	22.60
CV ₇	1.81	97.61	0.27	-0.55	3.93	-0.51	0.002	0.66	2.10	9.41	0.28	5.82
CV ₈	1.10	98.71	-0.12	0.75	-2.58	-0.53	0.07	-0.05	11.89	54.94	-0.97	21.67
CV ₉	0.89	99.61	-0.95	-0.16	0.19	-0.23	0.01	0.11	-6.35	-28.95	0.11	-12.55
CV ₁₀	0.38	100.0	0.29	0.21	0.11	-0.55	-0.12	0.21	-3.64	-14.43	-0.54	-5.63

^{1/}FLOWER = number of days to flower; NS = average number of stems; PH = average plant height; NL: average number of leaves; GMP = green mass production; DMP = dry mass production; TA: total ash content; VC: volatile content; IL = insoluble lignin; FCC = fixed carbon content. Source: Authors.

Based on the graphic dispersion related to the first and second canonical variable, in two-dimensional space, it was possible to visualize the formation of eight distinct groups, where it is observed that the genotype 201429B006 was the most distant in relation to the others (Figure 2). Comparing Tocher's method to graphic projection, it was possible to observe agreement between these two methods, and their constituent elements were concordant in the amount of group formation and of its elements within each group, therefore

reliable in identifying parents with high divergence, in the sense to guide promising intersections.

Figure 2. 2D graphic projection of the 34 genotypes of sorghum biomass, based on the measure of generalized dissimilarity of Mahalanobis, in which the genotypes grouping are based on the information provided by the Tocher optimization method estimated from ten characteristics evaluated in the county of Cáceres - Mato Grosso, agricultural year 2014/15.



Source: Authors.

In plant breeding programs, it is interesting to identify the character with the greatest contribution to the dissimilarity process. The magnitude corresponding to the contribution of the characters to the genetic dissimilarity process of the 34 genotypes of sorghum biomass, according to the method of Singh (1981), are shown in Table 4.

Thus, the characters FLOWER and TA showed the greatest relative effect in the dissimilarity process, explaining 44.80% and 33.42% of the total variation, respectively. It was found that the other characters IL, PH, FCC, NL, NS, VC, GMP and DMP are of less importance, as they presented less than 10% of the variation.

Table 4. Percentage relative contribution of ten characters evaluated for the divergence between 34 genotypes of sorghum biomass evaluated in Cáceres - Mato Grosso, agricultural year 2014/15.

Rated characters ^{1/}	S _j	Contribution (%)
FLOWER	25103.16	44.80
TA	18727.24	33.24
IL	2689.80	4.80
PH	2183.19	3.89
FCC	2041.53	3.64
NL	1819.00	3.24
NS	1316.35	2.34
VC	933.71	1.66
DMP	635.88	1.13
GMP	579.49	1.03

¹FLOWER = number of days to flower; NS = average number of stems; PH = average plant height; NL: average number of leaves; GMP = green mass production; DMP = dry mass production; TA: total ash content; VC: volatile content; IL = insoluble lignin; FCC = fixed carbon content. Source: Authors.

According to Cruz et al (2014), the characters dispensable in divergence studies involve those that are relatively non-variant between the studied genotypes and present instability with the change in environmental conditions, or are redundant, as they are correlated with other characteristics. Leite et al. (2017), report that the relative contribution of each character to estimate genetic divergence is of great importance in helping to discard those who contribute little to the discrimination of genotypes.

4. Final Considerations

There is genetic diversity among the sorghum biomass genotypes evaluated in the present research for the agronomic and physicochemical characteristics analyzed. The use of the Mahalanobis generalized distance techniques, Tocher's method, and the analysis of canonical variables, allow a better understanding of the genetic distances between the 34 genotypes of sorghum biomass.

This research adds important information for the pre-breeding of biomass sorghum developed by the State University of Mato Grosso, and from the genetic divergence analyzes we can verify the segregating and superior cultivars that could be used in the future for crossbreeding aiming to improve cultivars sorghum for the state of Mato Grosso.

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