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RESEARCH ARTICLE

MULTICATEGORICAL VARIABLES FOR DETERMINING THE GENETIC DIVERGENCE AMONG BIOMASS AND SACCHARIN SORGHUM GENOTYPES

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ABSTRACT

This study aimed to evaluate the genetic diversity of biomass and saccharin sorghum genotypes, based on 19 agronomic traits by the means of multicategorical variables. The experiment was conducted in the experimental area of the University of the State of Mato Grosso/UNEMAT, in Cáceres. We evaluated 25 genotypes of saccharin sorghum and 36 genotypes of biomass sorghum, in a randomized complete-block design with three replications. The data were submitted to three clustering methods, Tocher optimization, UPGMA hierarchical and Projection distances in 3D plan and comparing their results. The study showed the most divergent genotypes and the behavior of the three methods, which have proved partially concordant in the grouping of genotypes. The combined use of the clustering methods enabled a more accurate assessment for inference, and evidence the existence of genetic variability among saccharin and biomass sorghum genotypes.

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INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the fifth most produced cereal in the world after corn, wheat, rice and barley, in which the United States leads as largest producer worldwide, followed by Mexico, Nigeria, and India (Conab, 2015). It is a well-adapted crop to some Brazilian regions, being tolerant to excess or deficit of soil moisture, resistant to high and low temperatures and greater adaptability to different soil and climatic conditions than most cereals crops (Rezende *et al.,* 2011). National Sorghum production is increasing annually, and the Midwest region holds 55% of national production, with a planted area of 366,600 ha. Among the Brazilian States that most produced sorghum in 2014/15 crop, are: Goiás, with 712,900 tons; Minas Gerais with 506,700 tons and Mato Grosso, occupying the third position in the rankings with a production of 335,600 tons, productivity approximately of 2406 kg ha^{-1}, harvested in a 139,500 hectare plantation area (Conab, 2015). In Brazil are grown different types of sorghum: grain, forage, broom, saccharin and recently biomass, among which, the saccharin and biomass sorghum have the particularity of being used in renewable energy generation. The sorghum has culms with broth similar to sugarcane, rich in fermentable sugars and can be used in the production of ethanol, using the same facilities used for sugarcane (Durães, 2011), since sorghum biomass is gaining prominence due to its versatility as a source of starch and lignocellulose, occupying a privileged position as a source for energy co-generation or second generation ethanol (Carrillo, 2014). Knowledge and exploitation of genetic variability are essential for raising the Brazilian agribusiness productivity, thus, in plant breeding programs, the knowledge of genetic diversity between a group of parents is critical for conservation of genetic resources and reduction of genetic vulnerability of the crop (Carvalho *et al.,* 2003). In this context, the use of multivariate techniques, given the simple inheritance and easy to measure, allows the breeder to identify different genotypes by studies of the most important traits in the discrimination of the diversity in a population (Hair Jr. *et al.,* 2009). The multicategorical descriptors have been widely used in germplasm characterization and also used to evaluate genotypes, aiming to characterize their genetic variability. Techniques of multivariate analysis, such as cluster analysis has been used to quantify the phenotypic divergence in several species (Sudré *et al.,* 2005; Sudré *et al.,* 2006; Bento *et al.,* 2007; Bertini, 2010; Kloster *et al.,* 2011; Benitez, 2011).

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The aim of this study was to evaluate the variability of saccharin and biomass sorghum genotypes, using 19 morphoagronomic traits, by the means of multicategorical variables, based on the minimum descriptors for sorghum.

MATERIALS AND METHODS

The experiment was conducted from December 2014 to June 2015, in the experimental area of the Laboratory of Genetic Resources & Biotechnology at the University of the State of Mato Grosso, located in the coordinates 16º11 "42' South and longitudes 57º40"51' West, at an average altitude of 118m. The soil of the region was classified as Oxisol dystrophic (Couto *et al.,* 2006). The annual mean temperature is 26.24ºC, annual rainfall of 1335 mm, with period of higher concentration from December to March (Neves *et al.,* 2011). We evaluated 25 genotypes of saccharin sorghum, as follows:1) CMSXS5010, 2) CMSXS639, 3) BRS 508, 4) CMSXS648, 5) V82392, 6) V82391, 7) CMSXS647, 8) BRS 509, 9) CMSXS5004, 10) Sugargraze, 11) CV 568, 12) CMSXS5003, 13) CMSXS5006, 14) CMSXS644, 15) CV 198, 16) CMSXS629, 17) V82393, 18) CMSXS646, 19) BRS 506, 20) CMSXS630, 21) CMSXS5008, 22) CMSXS643, 23) BRS 511, 24) CMSXS5007 and 25) CMSXS5009; and 36 biomass sorghum: 26) 201429B004, 27) 201429B019, 28) 201429B016, 29) 201429B029, 30) 201429B026, 31) 201429B027, 32) BRS655 (control forage sorghum) 33) 201429B018, 34) 201429B017, 35) 201429B009, 36) 201429B023, 37) 201429B020, 38) 201429B028, 39) 201429B010, 40) 201429B013, 41) 201429B025, 42) 201429B003, 43) 201429B033, 44) 201429B006, 45) 201429B012, 46) 201429B008, 47) 201429B022, 48) 201429B030, 49) 201429B015, 50) 201429B007, 51) 201429B005, 52) Volumax (control forage sorghum), 53) 201429B021, 54) 201429B002, 55) BRS 716, 56) 201429B024, 57)201429B014, 58) 201429B001, 59) 201429B011, 60) 201429B032 and 61) 201429B031.

The experimental design was a randomized block with three replications; the plots were composed by four lines of 5 m, spaced 0.70 m apart and seeding density of ten plants per linear meter. Fertilization planting consisted of 150 kg ha^{-1} of N-P_2O_5-K_2O. At 45 and 80 days after emergence was held on topdressing with 89 kg ha^{-1} of CH_4N_2O. From a random sample of 10 plants of each plot, we evaluated 19 descriptors belonging to the list of minimum descriptors for the sorghum crop (Mapa, 1997). Plants traits evaluated were: 01) seedling - pigmentation of the leaf blade by anthocyanin, 02) seedling - pigmentation of the coleoptile by anthocyanin, 03) seedling - pigmentation of the dorsal side of the 1st leaf by anthocyanin, 04) seedling - pigmentation of the leaf sheath by anthocyanin, 05) leaf - leaf insertion on the stem 06) leaf - green pigmentation of the leaf blade, 07) leaf - pigmentation of midrib (the 3rd leaf from the flag leaf), 08) leaf - sheathwax, 09) leaf - angle between the blade and the stem (measured in 3rd leaf from the flag leaf), 10) stem - tillering capacity, 11) panicle shape-physiological maturity, 12) panicle shape - density/physiological maturity, 13) panicle-main rachis length, 14) panicle - primary branch length, 15) shape and length of the peduncle (above the flag leaf), 16) leaf - blade length (from the flag leaf), 17) leaf - blade width of the 3rd leaf (from the flag leaf), 18) stem diameter and 19) plant height. The quantitative characteristics of stem diameter and plant height were recoded into Multicategorical data, using the software Genes (Cruz, 2015).

Data were evaluated following the methodology of multicategorical variables, where D is the category discord and C the category agreement. From this ratio the distance matrix based on qualitative data is generated (Cruz, 2006).

$$d_{ii} = \frac{D}{C+D}$$

With the dissimilarity measures, analyzes were performed: Grouping by the Tocher modified method, Middle Between Groups (UPGMA) and Projection Distance of the Plan 3D, using the software GENES (Cruz, 2015) and software R. (R Development Core Team 2012).

RESULTS AND DISCUSSION

The estimated dissimilarity based on 19 descriptors, for 61 sorghum genotypes, enable to identify that the greatest distance ($D^2_{ii'} = 0.84$) was between combinations of the forage sorghum genotype BRS655 with saccharin sorghum genotypes CMSXS503 and CV568,with 16 distinct traits, as follow: seedling - pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the dorsal side of the 1st leaf by anthocyanin; seedling-pigmentation of the leaf sheath by anthocyanin; leaf-leaf insertion on the stem; leaf - green pigmentation of the leaf blade; leaf-pigmentation of midrib (the 3rd leaf from the flag leaf); leaf-angle between the blade and the stem (measured in 3rd leaf from the flag leaf); stem-tillering capacity; panicle shape-physiological maturity; panicle shape - density/physiological maturity; panicle-main rachis length; panicle- primary branch length; shape and length of the peduncle (above the flag leaf); leaf - blade length (from the flag leaf) and leaf- blade width of the 3rd leaf (from the flag leaf). The most similar combinations ($D^2_{ii'} = 0.10$), occurred mostly between biomass sorghum genotypes: 201429B009 x 201429B004, 201429B018 x 201429B027, 201429B012 x 201429B029, 201429B010 x 201429B006 e 201429B001 x 201429B025;and only one between saccharin genotypes: BRS506 x BRS509. Among the 19 traits, genotypes 201429B009 x 201429B004 showed differences for the panicle - main rachis length and plant height; 201429B018 e 201429B027 showed differences for the seedling - pigmentation of the leaf blade by anthocyanin, seedling - pigmentation of the coleoptile by anthocyanin, leaf - angle between the blade and the stem (measured in 3rd leaf from the flag leaf), stem - tillering capacity and panicle - primary branch length; 201429B012 x 201429B029 showed differences for the traits: leaf - green pigmentation of the leaf bladeand leaf - blade length (from the flag leaf); 201429B010 x 201429B006 showed differences for panicle-main rachis length e panicle-primary branch length; and 201429B001 x 201429B025 showed differences for seedling - pigmentation of the leaf blade by anthocyanin and panicle- primary branch length and the combination of saccharin genotypes BRS506 X BRS509 showed differences for leaf - angle between the blade and the stem (measured in 3rd leaf from the flag leaf) and plant height.

The Tocher optimization method enabled the segregation of genotypes, forming eleven distinct groups (Table 1), and the genotypes that are part of the same group have greater similarity between them, based in the magnitude of their dissimilarity.

Table 1. Representation of custering generated by Tocher optimization method, based on the dissimilarity between the 61 genotypes of biomass and saccharin sorghum, Cáceres-MT in 2014/2015 crop

Group	Genotypes	% of Genotypes
I	8,19, 25, 2, 22, 23,17, 15,18,7, 24	18.03%
II	26, 34,38, 43, 35, 44, 49, 39,28,29,50,57,32,30,33,58,40	27.87%
III	4, 20, 3, 59	6.56%
IV	10,11	3.28%
V	37,56,27	4.92%
VI	41, 47, 51, 55, 48,42	9.84%
VII	5,6,21,1,16,13,36	11.48%
VIII	52,54,53	4.92%
IX	9,12,61	4.92%
X	31, 45	3.28%
XI	46, 60,14	4.92%
Total	61	100

Group I consisted of 18.03% of the genotypes, all of them saccharin type. The similar traits between them were: seedling - pigmentation of the leaf blade by anthocyanin; seedling - pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the dorsal side of the 1rd leaf by anthocyanin; seedling - pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem; leaf - green pigmentation of the leaf blade; leaf - pigmentation of midrib (the 3rd leaf from the flag leaf); leaf - sheath wax and leaf - blade width of the 3rd leaf (from the flag leaf). Group II was composed by a large number of genotypes, with a total of 27.87% of the genotypes, all belonging to biomass type. The similar traits between them were: seedling - pigmentation of the dorsal side of the 1rd leaf by anthocyanin; seedling - pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem; leaf - green pigmentation of the leaf blade; leaf - pigmentation of midrib (the 3rd leaf from the flag leaf) and leaf - sheath wax. Group III consists mostly of saccharin sorghum, with the exception of the biomass sorghum genotype 201429B011. The similar traits between them were: seedling - pigmentation of the leaf blade by anthocyanin; seedling-pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the dorsal side of the 1rd leaf by anthocyanin; seedling - pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem; leaf - sheath wax; panicle shape - physiological maturity; panicle shape-density/physiological maturity; panicle - primary branch length; shape and length of the peduncle (above the flag leaf); leaf - blade length (from the flag leaf) and leaf - blade width of the 3rd leaf (from the flag leaf).

Groups IV and X were less expressive, formed by only two genotypes each, suggesting that these are the most divergent genotypes analyzed via multicategorical variables. The group IV allocated only saccharin sorghum, presenting similarities for almost all traits, except for: leaf - angle between the blade and the stem (measured in 3rd leaf from the flag leaf); leaf - blade length (from the flag leaf) and stem diameter. The group X has allocated forage and biomass sorghum genotypes, presenting similarities for almost all traits, except for: seedling - pigmentation of the leaf blade by anthocyanin; stem - tillering capacity; panicle shape - density/physiological maturity; leaf - blade length (from the flag leaf); leaf - blade width of the 3rdleaf (from the flag leaf) and stem diameter. Groups formed by few individuals shows wide divergence, since the genotypes into smaller groups are more dissimilar at the total (Benitez *et al.,* 2011). Groups V, VIII, IX and XI were formed by only three individuals each, totaling 4.92% of the genotypes, wherein the group V and VIII allocated biomass sorghum genotypes, while group IX and XI grouped genotypes of both types of sorghum. Group V showed similarity in most of the traits evaluated, except for: seedling - pigmentation of the coleoptile by anthocyanin; leaf - angle between the blade and the stem (measured in 3rd leaf from the flag leaf); panicle shape -physiological maturity; panicle-main rachis length; leaf- blade width of the 3rd leaf (from the flag leaf); stem diameter and plant height. The Group VIII differed for allocating, in addition to the traits described above, the following traits: leaf - leaf insertion on the stem; leaf - green pigmentation of the leaf blade; leaf - pigmentation of midrib (the 3rd leaf from the flag leaf); leaf - sheath wax; stem - tillering capacity and shape and length of the peduncle (above the flag leaf). Group IX and XI allocated biomass and saccharin sorghum genotypes, wherein the group IX showed similarity to the following traits: seedling-pigmentation of the leaf blade by anthocyanin; seedling -pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem; leaf-sheath wax; panicle shape - density/physiological maturity; panicle- primary branch length; leaf - blade length (from the flag leaf); leaf - blade width of the 3rd leaf (from the flag leaf) and plant height. The group XI showed similarity to the following characteristics: seedling - pigmentation of the leaf blade by anthocyanin; seedling - pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the leaf sheath by anthocyanin; leaf-leaf insertion on the stem; leaf - sheath wax; stem-tillering capacity; stem diameter and plant height.

Group VI only grouped genotypes of biomass sorghum, representing 9.84% of total genotypes, being similar to eight traits; seedling - pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the dorsal side of the 1rd leaf by anthocyanin; seedling - pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem; leaf-green pigmentation of the leaf blade; leaf-pigmentation of midrib (the 3rd leaf from the flag leaf); leaf-sheath wax and panicle shape - density/physiological maturity. Group VII comprised seven genotypes, totaling 11.48%, six genotypes of saccharin sorghum, and one biomass sorghum genotype (201429B020), presenting similar traits to: seedling - pigmentation of the leaf blade by anthocyanin; seedling - pigmentation of the coleoptile by anthocyanin; seedling-pigmentation of the dorsal side of the 1rd leaf by anthocyanin; seedling-pigmentation of the leaf sheath by anthocyanin; leaf-leaf insertion on the stem; leaf - sheath wax; panicle-primary branch length and shape and length of the peduncle (above the flag leaf).

Several studies have employed multicategorical variables to characterize and evaluate the divergence based on qualitative variables. For example, Gonçalves *et al.* (2014), evaluating the genetic diversity of common bean accessions through qualitative traits of seeds, observed that the dissimilarity methods were effective in identifying the most divergent materials, and the grouping of similar accessions in the same group. Coimbra *et al.* (2010) evaluate the relationship of seven qualitative descriptors in 16 genotypes of maize. The authors concluded that the cluster analysis using dissimilarity matrices obtained from multicategorical data consists of a viable alternative to evaluate the difference between genotypes. Kloster *et al.* (2011) assessed the genetic diversity of 22 bean cultivars based on 28 morphological traits, which allowed the identification of cultivars to be evaluated by agronomic descriptors. The dendrogram containing 61 genotypes developed by the UPGMA method (Figure 1) allowed to observe the dissimilarity between biomass and saccharin

sorghum genotypes. Considering the optimal cut at 0.3 of genetic distance, we observed the formation of groups, which were allocated pairs of genotypes with greater similarity.

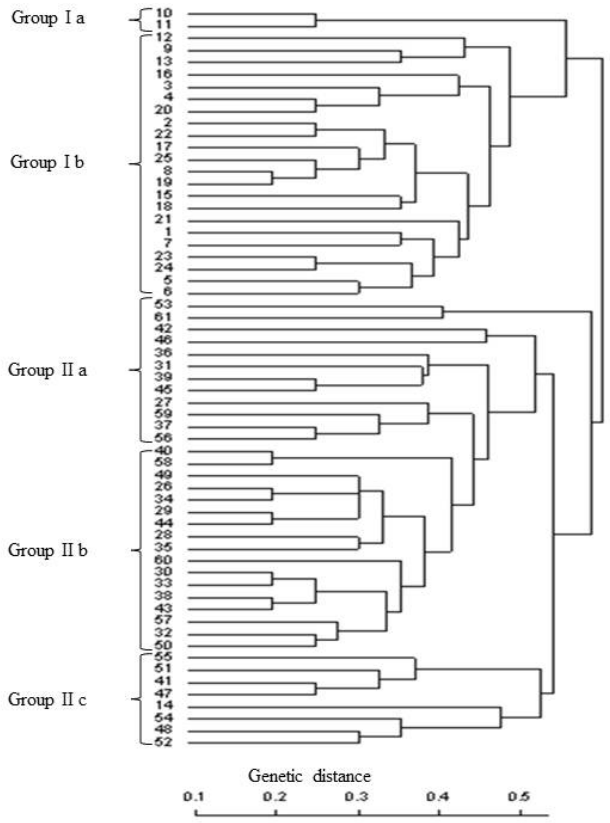

Figure 1. Representative Dendogram of the grouping of 61 genotypes of biomass and saccharin sorghum, using the UPGMA method, based on estimated dissimilarity from 19 morphological traits

The cophenetic correlation coefficient (CCC), applied to the T test grouping method, showed a value of medium and significant magnitude to the methods used, with r: 0.68% (P <0.01), demonstrating reliability in the relationship and dendrogram. The graphical representation of the dendrogram, according Bertan *et al.* (2006), should represent good accuracy with its original matrix. Group I was comprised exclusively of saccharin sorghum genotypes, subdivided into two subgroups: subgroup Ia, with Sugargraze and VC 568 genotypes, with the similar traits: seedling - pigmentation of the leaf blade by anthocyanin; seedling - pigmentation of the coleoptile by anthocyanin; seedling - pigmentation of the dorsal side of the 1st leaf by anthocyanin; seedling-pigmentation of the leaf sheath by anthocyanin; leaf-leaf insertion on the stem; leaf-green pigmentation of the leaf blade; leaf - pigmentation of midrib (the 3rd leaf from the flag leaf); leaf - sheath wax; panicle shape - density/physiological maturity; panicle - primary branch length; leaf - blade length (from the flag leaf) and leaf-blade width of the 3rd leaf (from the flag leaf); and subgroup Ib, composed by genotypes CMSXS5010, CMSXS639, BRS 508, CMSXS648, V82392, V82391, CMSXS647, BRS 509, CMSXS5004, Sugargraze, CV 568, CMSXS5003, CMSXS5006, CV 198, CMSXS629, V82393, CMSXS646, BRS 506, CMSXS630, CMSXS5008, CMSXS643, BRS 511, CMSXS5007 and CMSXS5009, showing similarity to the traits: leaf - leaf insertion on the stem; leaf-pigmentation of midrib (the 3rd leaf from the flag leaf) and leaf - sheath wax.

Group II was subdivided into three subgroups, which placed mostly sorghum genotypes biomass, except for one. The subgroup IIa was composed by genotypes 201429B021, 201429B031, 201429B003, 201429B008, 201429B023, 201429B027, 201429B010, 201429B012, 201429B019, 201429B011, 201429B020 and 201429B024. These genotypes showed similarity to the following traits: seedling-pigmentation of the leaf blade by anthocyanin; seedling-pigmentation of the leaf sheath by anthocyanin; leaf - leaf insertion on the stem and leaf - sheath wax. Subgroup IIb grouped the genotypes 201429B013, 201429B001, 201429B015, 201429B004, 201429B017, 201429B029, 201429B006, 201429B016, 201429B009, 201429B009, 201429B032, 201429B0026, 201429B018, 201429B028, 201429B033, 201429B014, BRS655 and 201429B007, showing similarity to the traits: leaf - leaf insertion on the stem and leaf - sheath wax; subgroup IIa was composed by seven biomass genotypes BRS716, 201429B005, 201429B025, 201429B022, 201429B002, 201429B030 and Volumax and one saccharin genotype CMSXS644, and were similar to the following characteristics: seedling - pigmentation of the leaf blade by anthocyanin; leaf - leaf insertion on the stem and leaf - sheath wax. Both methods, UPGMA and Tocher, showed similarity in the grouping of genotypes. However, the UPGMA method was more efficient in the separation of biomass and saccharin sorghum genotypes, the differences between the methods might be explained by the difference of each method when calculate the genetic variability (Geleta *et al.,* 2005). Corroborating the above information, Neitzke *et al.* (2010) found differences in the number of groups formed by Tocher and UPGMA method when study the genetic dissimilarity among pepper accessions with ornamental potential. Bertani *et al.* (2006) report that the UPGMA discriminate each accession, allowing a better view within each group. Büttow *et al.* (2010) also found some differences in the groups generated by the two methods, when compared *Capsicum annuum* accessions.

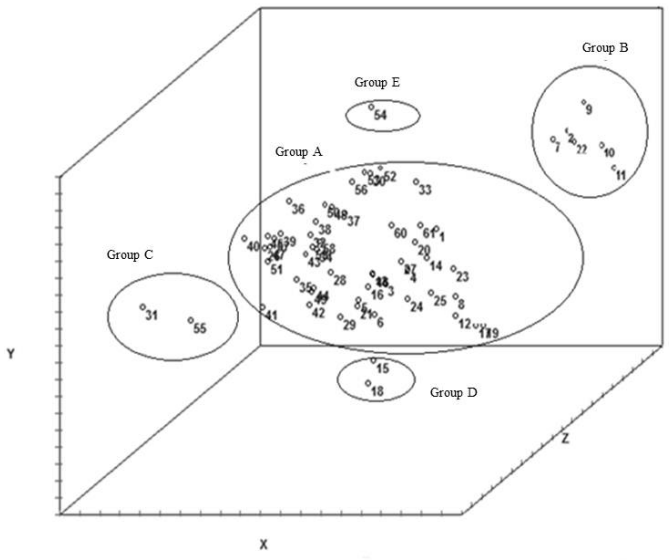

Figure 2. Projection distances in 3D plan of 61 genotypes of biomass and saccharin sorghum, based on 19 multicategorical variables

When analyzing the projection distances in the plane, shown in Figure 2, in the three-dimensional space, we observed the formation of five groups. Group A was the largest group, comprises 27.87% of saccharin sorghum genotypes

(CMSXS5010, BRS 508, CMSXS648, V82392, V82391, BRS 509, CMSXS5003, CMSXS5006, CMSXS644, CMSXS629, V82393, BRS 506, CMSXS630, CMSXS5008, BRS 511, CMSXS5007 and CMSXS5009) and 54.10% of biomass genotypes (201429B004, 201429B019, 2201429B016, 201429B029, 201429B026, BRS655, 201429B018, 201429B017, 201429B009, 201429B023, 201429B020, 201429B028, 201429B010, 201429B013, 201429B025, 201429B003, 201429B033, 201429B006, 201429B012, 201429B008, 201429B022, 201429B030, 201429B015, 201429B007, 201429B005, Volumax, 201429B021, 201429B024, 201429B014, 201429B001, 201429B011, 201429B032 and 201429B031). Similar traits for grouping the genotypes were: leaf - leaf insertion on the stem and leaf-sheath wax.

The B and D group were comprised only by saccharin genotypes, six and two genotypes, respectively, as follows: (CMSXS639, CMSXS647, CMSXS5004, Sugargraze, CV 568, CMSXS643) and (CV 198 e CMSXS6460). Group B presented as similar traits; seedling-pigmentation of the leaf blade by anthocyanin; leaf-leaf insertion on the stem; leaf - green pigmentation of the leaf blade; leaf-pigmentation of midrib (the 3rd leaf from the flag leaf); leaf-sheath wax; stem – tillering capacity; panicle shape-density/physiological maturity; leaf - blade width of the 3rd leaf (from the flag leaf). Group D showed similarity for all traits, except for: stem - tillering capacity panicle - primary branch length and plant height. The groups C and E were formed only by genotypes of sorghum biomass, with the group C comprising the genotypes 201429B027 and BRS 716 and the group E by the genotype 201429B002. The group C showed similarity to the following traits: leaf - leaf insertion on the stem; leaf- green pigmentation of the leaf blade; leaf - pigmentation of midrib (the 3rd leaf from the flag leaf); leaf-sheath wax; panicle shape-physiological maturity; panicle shape-density/physiological maturity; panicle- primary branch length; shape and length of the peduncle (above the flag leaf); leaf - blade length (from the flag leaf); leaf - blade width of the 3rd leaf (from the flag leaf) and plant height. Similar genotypes were sorted into similar groups, identifying and isolating the most divergent in different groups, indicating diversity among the 61 genotypes. According to the analysis made in the UPGMA and the Projection on the Plan, we observed agreement on the number of groups formed. However, when genotypes were compared with the UPGMA clustering, they were allocated differently.

Conclusion

The sorghum genotypes and biomass showed genetic divergence for all traits. Therefore, the combined use of these two clustering methods enabled a more accurate assessment for the inference of the evidence and the existence of genetic variability among genotypes. The selection of multivariate techniques assists breeders in determining the genetic diversity among genotypes, by studies of the most important traits in the existing diversity in a population.

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