Analysis of genetic divergence in sweet corn genotypes through hierarchical optimization methods

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ABSTRACT. Sweet corn (*Zea mays* subsp. *saccharata*) is considered a special vegetable of high nutritional value. One of the barriers encountered by breeders has been a lack of adequate genetic variability of sweet corn, coupled with a need for appropriate methodologies to evaluate the existing genetic diversity. Our objective was to determine the best method to identify promising genotypes to improve sweet corn production. We used data from 181 open-pollinated sweet corn genotypes, cultivated during 2016 and 2017. Multivariate analyses were carried out to determine the genetic dissimilarity between the genotypes, obtaining the matrix of dissimilarity by Euclidean distance. Prior to calculating the distance between matrices, two data standardizations (Z_1 and Z_2) were performed for comparison. Genetic divergence was analyzed by four distinct hierarchical methods: Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA), Ward, Weighted Pair-Group Method Using Arithmetic Averages (WPGMA) and Single Linkage. Tocher's optimization method was also used. The Simple Linkage and UPGMA methods presented similar groupings, consistent with breeding program aims and with the highest values of co-phenetic correlation coefficient (CCC). The Ward's method was not efficient,

because it produced several clusters without isolating different genotypes. Furthermore, it was the method with the lowest CCC for both matrices. The standardized Z_2 matrix should be avoided, especially when a large number of genetic traits are measured, in order to prevent possible overlapping between traits, a variables with higher standard variations could contribute more to the clustering.

Key words: Genetic diversity; Genetic breeding; Data standardization; *Zea mays* subsp. *saccharata*

INTRODUCTION

Sweet corn (*Zea mays* subsp. *saccharata*) is considered a special vegetable of high nutritional value. In Brazil, about US$ 7.1 million were traded in sweet corn seeds, occupying approximately 50 thousand hectares in 2012 (ABCSEM, 2014). It is worth mentioning that the commercial cropping of sweet corn does not meet the current Brazilian demand (Moterle et al., 2012; Ribeiro et al., 2012; Vittorazzi et al., 2013). Sweet corn has distinct characteristics compared to common green corn (Pena et al., 2012), with reduced genetic variability available for direct use in breeding programs. Recurrent selection methods have been successfully applied (Amaral Junior et al., 2013; Freitas et al., 2013) to later obtain simple hybrids (Rangel et al., 2017). One of the barriers encountered by breeders has been the relatively low genetic variability of sweet corn, coupled with a lack of appropriate methodologies to evaluate the existing genetic diversity. Genetic variability determines the feasibility of a breeding program, being potentiated by cross-linking between contrasting genotypes (Silva et al., 2011; Ertiro et al., 2013; Silva and Dias, 2013; Fernandes et al., 2015; Silva et al., 2016).

Multivariate techniques have been used to estimate the genetic divergence between accessions, like biometric models estimated by the Euclidean Distance and hierarchical grouping methods (Cruz et al., 2014). However, there is no consensus regarding the best cluster analysis method to evaluate sweet corn germplasm. Also, for the characters to be estimated by the matrices data standardization is necessary; few studies have evaluated the impact of different data transformation methods.

When evaluating green corn genotypes, Silva et al. (2016) verified that the UPGMA method was more sensitive to detect differences between genotypes and consequently to form groups. In contrast, Rigon et al. (2015) evaluated only two distinct clustering techniques, suggesting that both (Tocher and Single Linkage) were useful in the study of genetic divergence in corn. Nowadays, the UPGMA method is mostly suggested to study genetic traits of a population (Silva and Dias, 2013); however, the efficiency of other clustering methods to evaluate sweet corn germplasm is unknown.

The objective was to determine the best method to identify promising genotypes to obtain segregant populations and improve sweet corn production, using four distinct hierarchical dendograms and Tocher's optimization method, based on Euclidian distance of 181 sweet corn genotypes, with two methods of data standardization.

MATERIAL AND METHODS

The experiment was conducted at the Vegetable Experiment Station of Federal University of Uberlândia (UFU), located in Monte Carmelo - Brazil (18º42'43.19" S; 47º29'55.8" W; 873 m above sea level), during the years of 2016 and 2017. The soil presented the following characteristics: pH (H_2O) = 5.9; P available = 30.1 mg.dm^{-3}; K^+ = 0.22 cmol$_c$.dm^{-3}; Ca^{+2} = 2.8 cmol$_c$.dm^{-3}; Mg^{+2} = 1.0 cmol$_c$.dm^{-3}; H + Al exchangeable = 3.40 cmol$_c$.dm^{-3}; organic matter = 4.2 dag.kg^{-1}; Index SMP = 3.40; Al^{+3} = 0.0 cmol$_c$.dm^{-3}; CEC pH 7.0 = 7.42 cmol$_c$.dm^{-3}; base saturation in CEC in pH 7.0 = 54 %; effective saturation by aluminum in CEC= 0%; Cu^{+2} = 2.3 mg.dm^{-3}; Zn^{+2} = 6.6 mg.dm^{-3} e Mn^{+2} = 6.6 mg.dm^{-3}.

The genetic material consisted of 181 open-pollinated sweet corn genotypes (S3) that belonged to the UFU's germplasm bank. The sowing was carried out on November 19, 2016. The experimental design was a complete randomized block design with two replications. The spacing between rows was of 0.9 m and the plots were consisted of two lines with four meters each. The experimental area was previously prepared by a conventional farming system. Sowing and groove fertilization were done manually. The planting and cover fertilization were performed as recommended for the crop and according to the soil analysis.

The following characteristics were evaluated between the stages of full female flowering (silking - R1 and blister - R2): plant height (cm) at 76 days after sowing (DAS); leaf number per plant at 78 DAS; cob insertion height (cm) at 80 DAS; number of cobs per plant at 88 DAS; days to silking; chlorophyll index (SPAD) on leaf at 81 DAS, using the chlorophyll determiner MINOLTA SPAD-502. Productivity variables were collected at maturity stage (R6): seed number per cob after threshing; production per cob (g); 1000 seeds weight (g); and plant production (g).

The data were subjected to analysis of variance by F test (P-value $\leq$ 0.05). Then, multivariate analyzes were carried out to determine the genetic dissimilarity between the genotypes, obtaining the matrix of dissimilarity by the Euclidean distance. Prior to calculating the distance between matrices, two data standardizations were performed for comparison. The standardization Z score (Z_1) was performed by:

$$Z_1 = (\frac{X - X_i}{s}) \qquad \text{(Eq. 1)}$$

where X was the original value, X_i was the mean and s was the standard deviation. Therefore, the transformed values present the mean equal to zero and the variance equal to one (Romesburg, 1984). The second standardization (Z_2) was calculated as:

$$Z_2 = \frac{X}{s} \qquad \text{(Eq. 2)}$$

The formula Z_2 produces transformed values with variance equal to one and a mean equal to $\overline{X}/s$. Genetic divergence was represented by four distinct hierarchical methods: Unweighted Pair-Group Method Using Arithmetic Averages (UPGMA), Ward, Weighted Pair-Group Method Using Arithmetic Averages (WPGMA) and Single Linkage. The Tocher's optimization method was also measured. The relative contribution of the quantitative traits was calculated according to Singh (1981). In order to validate the clusters, in other words, to verify the ability of the method to reproduce dissimilarity matrices, the cophenetic correlation coefficient (CCC) was estimated. The package *NbClust* (Charrad et

al., 2014) was used to determine the best number of clusters, based on 24 indexes that establish the criteria of compactness and separation between clusters. The data was analyzed using the R software version 3.4.1.

RESULTS

All the evaluated traits presented statistical difference by F test (P-value ≤ 0.05), expressing the genetic variability in the 181 sweet corn genotypes. The ANOVA results confirmed that the sweet corn genotypes, from the Germplasm Bank of UFU, can be explored for breeding. The coefficient of variation (CV) was lower than 3% for all the traits, indicating high experimental precision, except for seed weight with a CV of 32.1%.

The most important characteristic for genetic divergence, according to the Singh (1981) criterion, for Z_1 standardization was plant height, with 26.5% (Table 1). On the other hand, the characteristic that contributed least to genetic divergence among the genotypes was seed weight, with 0.3%. The evaluation of this characteristic can be eliminated in future trials, given its low contribution to genetic divergence and laborious measurement. For Z_2 standardization, days to silking was the most influential variable in the distances estimation, with 64.4% of contribution, in contrast to Z_1, which was one of the least important traits, with 0.4%. The large variation in the contribution of characters between the two matrices justifies that Z_1 standardization maintains all the values in the scale between zero and one, while the Z_2 standardization allows greater variation of the data by the division of the means by the standard deviation, which led the values of days to silking to be superior than the other means, with an overlap of variables by this method.

Table 1. Relative contribution of 10 agronomic traits to the genetic diversity of 181 sweet corn genotypes, according to Singh's criterion (Singh, 1981).

Variable	(%) Z_1	Z_2	Variable	(%) Z_1	Z_2
PH	26.5	1.5	DS	0.4	64.4
LN	7.3	16.6	SN	13.5	0.5
HC	13.6	5.6	PC	10.8	0.7
CN	9.0	3.0	SW	0.3	3.5
SPAD	12.1	3.2	PROD	6.5	1.0

PH: plant height; LN: leaf number per plant; HC: cob insertion height; CN: number of cobs per plant; DS: days to silking; SPAD: chlorophyll index; SN: seed number per cob after threshing; PC: production per cob; SW: 1000 seeds weight; PROD: plant production.

For the Euclidean matrix of the Z_1 standardization, fourteen indexes of the *Nbclust* function indicated the formation of two to four clusters. For Z_2 standardization, ten indexes indicated the formation of two clusters. The cutting of the dendrograms was done looking for the formation of the clusters recommended by the *Nbclust* indexes. Although the *Nbclust* indexes suggested the formation of up to four clusters, Tocher's optimization method presented the formation of six clusters, based on the Z_1 standardized matrix (Table 2). With CCC value closed to the dendrograms (Table 3), the method isolated genotype 2, genotypes 19 and 111, genotypes 108 and 172, and created three more large clusters (Table 2).

Table 2. Clustering of 181 sweet corn (*Zea mays* subsp. *saccharata*) genotypes by Tocher's optimization procedure from the Euclidean distances obtained based on 10 agronomic traits standardized by Z-scores (Z_1) and by the mean standard deviation (Z_2).

	Z_1	Z_2
Cluster 1	2	2
Cluster 2	19 111	Other genotypes
Cluster 3	108 172	
Cluster 4	12 124 140 23 57 34 10 73	
Cluster 5	76 107 27 115 98 37 156 135 36 81 55 46 146	
Cluster 6	Other genotypes	

Table 3. Coefficients of co-phenetic correlation (CCC) obtained between the matrix of average Euclidean distances and the matrix of co-phenetic distances for the different hierarchical methodologies by Z-scores (Z_1) and by the mean standard deviation (Z_2) standardization for analysis of sweet corn varities.

Methodology	CCC	
	Z_1	Z_2
Single Linkage	0.51	0.66
UPGMA	0.65	0.77
WPGMA	0.52	0.66
Ward	0.45	0.36
Tocher	0.55	0.47

Considering as a cut-off rule, the index between 60 and 70% of similarity, dendrograms by Simple linkage's and UPGMA's methods were efficient in determining four different clusters, also isolating genotype 2 (Figure 1). Genotype 111 was also isolated and genotypes 46 and 81 formed a new group that was not detected by the Tocher's method. The WPGMA's method was able to isolate genotypes 2 and 111 in different clusters and created three clusters with the rest of the genotypes. Ward's method was not able to isolate any genotypes, creating six clusters. Regarding CCC, the UPGMA's method was the most reliable of all the methods used, with 65%, and Ward's method less efficient, with 45% (Table 3).

On the other hand, based on Z_2 standardized matrix, Tocher's method allowed the formation of two clusters, only isolating the genotype 2 (Table 2). The number of clusters formed was the same as those recommended by the *NbClust* indexes; however the CCC value was low, with 47% of similarity, smaller than the same method for the Z_1 standardization (55%). Considering as a cut-off rule, the index between 70 and 80% of similarity, the dendrograms simple linkage and UPGMA also produced two clusters, isolating the genotype 2 (Figure 2). The WPGMA's method isolated genotype 2, but divided the remaining genotypes into two clusters (Figure 2). The Ward's method created five clusters and did not isolate any genotype (Figure 2). The standardized Z_2 matrix produced higher CCC values, with the exception of the Ward's method, which had a CCC of 36%, the lowest of all methods used (Table 3).

The standardized Z_2 matrix indicated that the genetic basis of the germplasm bank was narrow. The standardized Z_1 matrix allowed a larger group of genotypes to be isolated, resulting in the possibility of greater cross-breeding combinations in the hybrid exploration. The comparison between the Tocher's optimization method and the hierarchical

dendograms demonstrated the ability to identify a high number of clusters with the dendrograms. Therefore, this increased the chances to find genetic divergence, especially if the Z_2 standardized matrix is used.
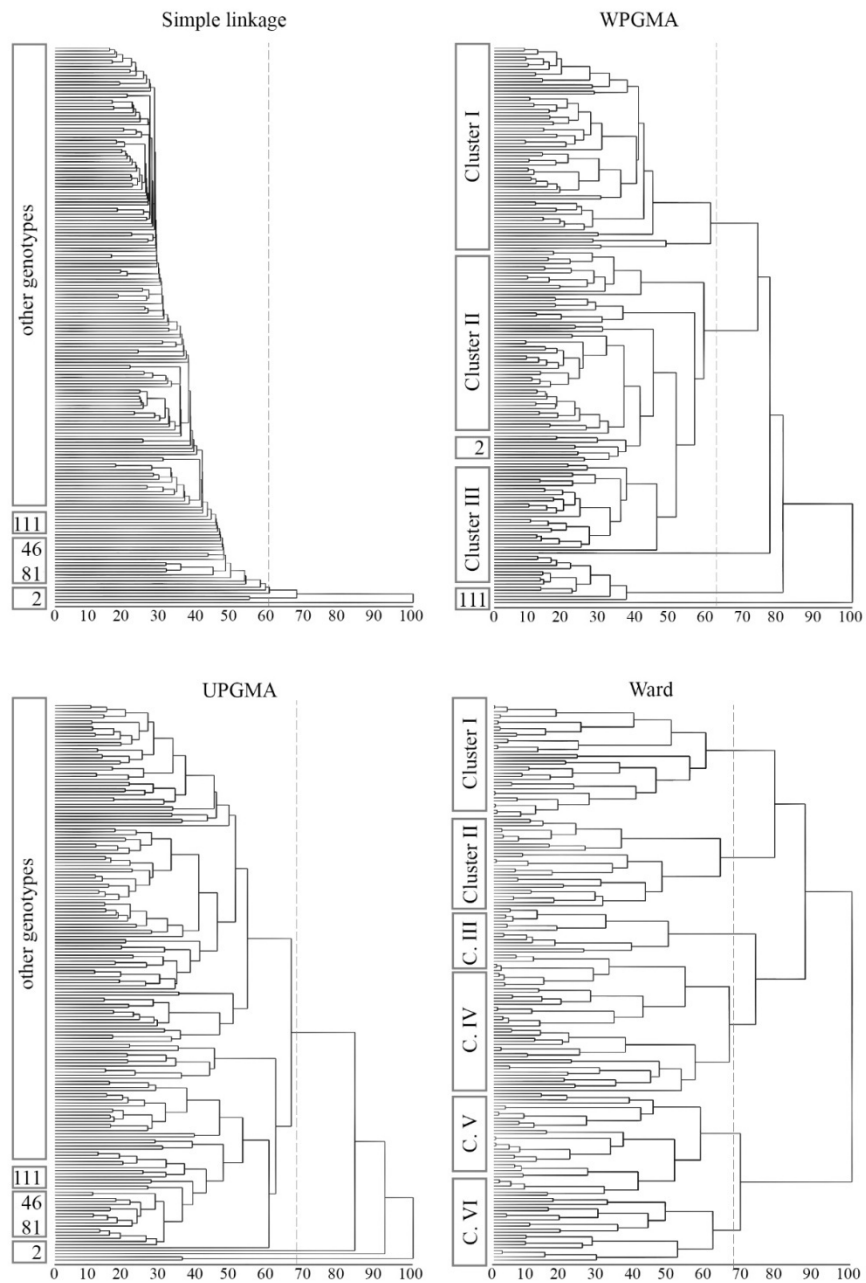
Figure 1. Clustering of 181 sweet corn genotypes using the Simple linkage, WPGMA, UPGMA and Ward method, from the Euclidean distances obtained based on 10 agronomic traits standardized by Z-scores (Z_1).
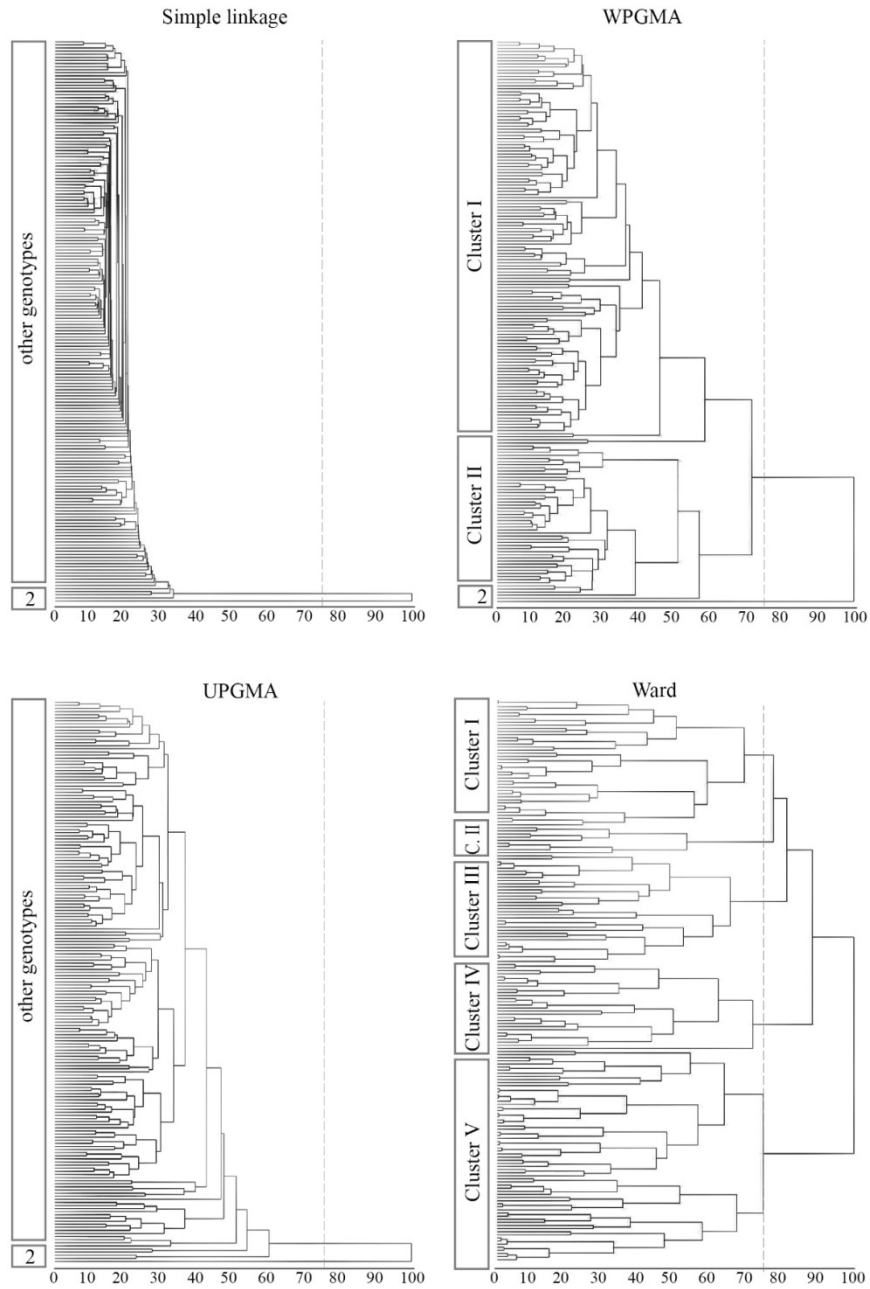
Figure 2. Clustering of 181 sweet corn genotypes using the Simple linkage, WPGMA, UPGMA and Ward methods, from the Euclidean distances obtained based on 10 agronomic traits standardized by dividing the values by the mean standard deviation (Z_2).

DISCUSSION

Sweet corn is widely used for human consumption around the world. Because of the importance of sweet corn, breeders have been devoted to the improvement of sweetness and other valuable traits, such as yield, disease and insect resistance, and tolerance to environmental stress. Genetic combinations of the endosperm have allowed higher sugar content in sweet corn varieties and with longer shelf life in the market (Lertrat and Pulam, 2007).

Although there are several publications about genetic diversity in corn germplasm, few describe the genetic variability in sweet corn (Gerdes and Tracy, 1995; Amorim et al., 2003; Bered et al., 2005; Kashiani et al., 2010). Among the characteristics analyzed, only seed weight presented a very high CV, greater than 28%, according to the criteria proposed by Scapim et al. (1995) for corn traits.

Researches on the heritability of sweet corn traits indicated that plant height and days to silking are the ones of higher heritability, with 79.9 and 66.9% respectively (Kashiani et al., 2010). The Z_1 standardized matrix considered plant height as one of the most important characters, but days to silking had little contribution in the Euclidean distance of the standardized matrices. For Z_2 standardized matrix, the inverse occurred for these traits. However, the overestimation for days to silking was estimated in 64.4%.

It is very common genetic dissimilarity studies apply only an agglomerative method, which leads to subjective results, given the difference between these methods (Fernandes et al., 2015). Therefore, it is advisable to use a variety of clustering methods in order to compare the results (Jackson et al., 1989; Rohlf, 1990). Our results confirm the need of more researches about the genetic diversity in sweet corn, in order to present data with a greater number of multivariate techniques.

Moreover, the use of microsatellite markers was efficient to analyze the genetic structure of *su1* sweet corn progenies with SSR markers, allowing estimating the genetic distance or similarity between the progenies (Lopes et al., 2014). Further studies combining the evaluation of morphological traits with microsatellite markers should be performed.

The dendrograms identifieded contrasting genotypes for future crosses. The first cluster in a dendrogram is formed by the genotypes that present the greatest similarities (Cruz et al., 2014). Clusters formed by a single individual suggest that the material is more divergent from the others. It was observed in all methods that genotype 2 was the most contrasting and for some methods, the genotypes 46, 81 and 111 were highlighted for future crosses. Parental selection should be based on the magnitude of the genetic divergence among the genotypes, when the objective is to perform crosses resulting from superior progenies in relation to the characteristics of interest (Abreu et al., 2004).

Rotili et al. (2012) recommend the use of the Single Linkage method with the Tocher's method for maize genotypes. Our results showed that inference through dendrograms is safer and less restricted and the UPGMA and Single Linkage methods are more useful and reliable. Cantelli et al. (2016) obtained similar results to our study in soybean, in which the UPGMA and Single Linkage methods were superior when compared to the other clustering methods. Moreover, the results of Vogt et al. (2010) converged in similarity to the UPGMA's and Tocher's methods.

Although it is a requirement that the database has to be standardized when the variables came from different measurements (Hair Junior et al., 2009), many researchers

forget about this procedure or execute it without knowing. There are no publications that reveal the impact of data transformation on cluster analysis, though there are several types of standardization. Our results showed that large differences were obtained using different forms of standardization. The standardization procedure Z_1 was superior to several other standardizations when used with the Ward's method (Milligan and Cooper, 1988). In fact, the only dendrogram with higher CCC using Z_1 standardization, was the Ward's method.

The Simple Linkage and UPGMA methods presented similar groupings, consistent with the aims of a breeding program and with the highest vales of CCC, being recommended for the study of genetic diversity in sweet corn. Ward's method was not efficient, because it created several clusters without isolating different genotypes. Furthermore, it was the method with the lowest CCC for both matrices. The standardized Z_2 matrix should be avoided, especially when a large number of genetic traits are measured, in order to prevent possible overlapping between the traits, as variables with higher standard variations could contribute more to the clustering.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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