



Genetic divergence of sugarcane varieties collected in the region of Lavras, Minas Gerais, Brazil

L.E.V. Cesar, A.T. Bruzi, J.A.R. Nunes, L.A. de B. Andrade, M.F. Lopes, L.R. Sales and M.M. Mourão

Departamento de Agricultura, Universidade Federal de Lavras, Lavras, MG, Brasil

Corresponding author: L.E.V. Cesar

E-mail: levcesar@uol.com.br

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ABSTRACT. Genetic diversity among local accessions and varieties subsidize plant breeding programs, allowing the utilization of existing variability in plants that have already adapted to local climate conditions. An alternative to studying genetic variability is the study of diversity. The aim of this research was to study genetic diversity among sugarcane accessions and varieties used for the production of craft-distilled cachaça (distilled sugarcane alcohol) in the region of Lavras, Minas Gerais, Brazil. Using a one-way design, an experiment was conducted in the municipality of Perdões, Minas Gerais to evaluate 35 regional accessions derived from germplasm collection expeditions and four varieties. Using morphological descriptions of 46 multicategorical sugarcane characteristics, dissimilarity and Tocher cluster method analyses were performed. Based on the results, it was concluded that genetic diversity exists among the accessions evaluated for the target traits.

Key words: *Saccharum* spp; Characterization; Dissimilarity; Cluster analysis

INTRODUCTION

Studies of genetic diversity provide information about traits used to identify parents, and they facilitate the discovery of duplicates in germplasm banks (Rodrigues et al., 2002). Estimates of genetic diversity among local accessions and cultivars subsidize plant breeding programs, allowing the utilization of existing variability in plants that have already adapted to local climatic conditions. The morphological and agronomic characterizations of plants grown are of the utmost importance when determining the genetic divergence of available germplasm sets (Franco et al., 2001).

In this context, genetic diversity inferences can be made in both quantitative and predictive manners. Quantitative methods are diallel analyses that include crosses between the parents, followed by subsequent evaluation. The predictive methods have morphological differences and are based on nutritional, physiological, or molecular quality, which is quantified as the measurement of dissimilarity expressing the degree of genetic diversity between parents (Cruz and Carneiro, 2006). According to the same author, dissimilarity studies serve the various aims of breeders because they provide information on the degree of similarity or the difference between two or more genotypes, and they may be used as a tool for genetic divergence studies. However, the resulting number of dissimilarity estimates is relatively high when there is a large number of genotypes, and hence recognition of homogeneous groups by a simple visual examination is sometimes unfeasible. Therefore, the use of methods that cluster genotypes may be one of the best alternatives for data analysis and interpretation.

There are various clustering methods that differ by the type of result provided and by the different manners used to define the proximity between an individual and an already formed cluster (or between any two clusters). Among these, the optimization clustering method, or Tocher method (Cruz and Carneiro, 2006), constitutes a simultaneous clustering method, which separates genotypes into clusters in a single operation. This method uses a single clustering criterion, and it specifically presents a mean distance within clusters that is always less than the mean distance between clusters.

The Tocher method has been widely used in the study of genetic divergence among accessions. Souza et al. (2005) evaluated the genetic divergence among 31 watermelon genotypes using clustering techniques and canonical variables. In the study of Oliveira et al. (2004), the Tocher method was also used to evaluate genetic divergence among lettuce genotypes. Marchioro et al. (2003) used the Tocher clustering method for the evaluation of genetic dissimilarity among oat genotypes, and Silva et al. (2005) used the method to evaluate genetic diversity among sugarcane varieties. Moreover, Karasawa et al. (2005), when working with tomato crops, used the Tocher method to evaluate the genetic divergence among germplasm bank accessions, which facilitated the identification of potential parents for use in breeding programs.

Therefore, the aim of the present study was to examine genetic divergence among sugarcane accessions in the region of the municipality of Lavras, Minas Gerais, Brazil.

MATERIAL AND METHODS

The experiment was conducted in the commercial sugarcane production area of the Cachaça Artesanal João Mendes company (craft-distilled sugarcane alcohol company), located at the farm site Vó Zirica (highway BR-381, km 666) in the municipality of Perdões, Minas Gerais, Brazil, at 842 meters altitude, 21°05' S and 45°05' W. According to the Köppen classification, the

climate in Perdões is Cwb, which is characterized by a dry season from April to September and a rainy season from October to March. The region has a mean annual rainfall of 1493.2 mm and a mean temperature of 19.3°C (Dantas et al., 2007).

In this trial, 35 accessions were evaluated, and these were derived from a germplasm collection from rural properties with craft-distilled cachaça production in municipalities located in the region of Lavras, Minas Gerais (Coqueiral, Itutinga, Lavras, Nepomuceno, Perdões, and Ribeirão Vermelho) (Table 1). The germplasm collection was undertaken from November to December 2011 from four cultivars (RB867515, SP80-1842, SP79-1011, and SP81-3250) (Table 1).

Table 1. List of varieties and accessions examined in the experiment.

Collection	Local	Description	Collection	Local	Description
Accession 1	Lavras	L1	Accession 21	Lavras	L21
Accession 2	Lavras	L2	Accession 22	Nepomuceno	N22
Accession 3	Lavras	L3	Accession 23	Nepomuceno	N23
Accession 4	Perdões	P4	Accession 24	Rib. Vermelho	RV24
Accession 5	Perdões	P5	Accession 25	Rib. Vermelho	RV25
Accession 6	Perdões	P6	Accession 26	Rib. Vermelho	RV26
Accession 7	Lavras	L7	Accession 27	Lavras	L27
Accession 8	Lavras	L8	Accession 28	Nepomuceno	N28
Accession 9	Lavras	L9	Accession 29	Lavras	L29
Accession 10	Lavras	L10	Accession 30	Lavras	L30
Accession 11	Perdões	P11	Accession 31	Coqueiral	C31
Accession 12	Lavras	L12	Accession 32	Itutinga	I32
Accession 13	Perdões	P13	Accession 33	Lavras	L33
Accession 14	Perdões	P14	Accession 34	Perdões	P34
Accession 15	Lavras	L15	Accession 35	Perdões	P35
Accession 16	Perdões	P16	RB867515	RIDESA	V36
Accession 17	Perdões	P17	SP80-1842	Copersucar	V37
Accession 18	Perdões	P18	SP79-1011	Copersucar	V38
Accession 19	Lavras	L19	SP81-3250	Copersucar	V39
Accession 20	Lavras	L20	-	-	-

Experimental planting was conducted from December 5 to 12, 2011, and the trial was set up as a one-way type design with 39 genetic treatments. Plots consisted of three 3.0 m rows with 1.3 m spacing between rows. The experimental area chosen had a recent history of use as a pasture and for corn cultivation. Soil acidity had been amended, and the soil was well managed and conserved, with complete contour lines and the absence of erosion.

Prior to experimental planting, the soil was prepared through plowing and two diskings. Furrows were then opened at 30-40 cm depths and spaced at 1.3 m. For planting, a manual system was adopted where the cultivars and accessions were distributed within the plots according to a drawing designed prior to the experiment. The sugarcane billets were cut with three buds and distributed within the rows, and six billets were placed per linear meter for a total of 18 buds/m. The billets were manually covered using hoes, and a layer of approximately 10 cm soil was placed over the billets distributed in the planting rows.

Fertilization at planting was performed with 40 kg/ha N, 140 kg/ha P₂O₅, and 100 kg/ha K₂O. In February 2012, sidedressed fertilization was conducted with the application of 60 kg/ha N. For weed control, a chemical method was adopted using herbicides at the pre-emergence of the crops and weeds via 2 kg of a commercial product composed of 468 g/kg diuron + 132 g/kg hexazinone, which is a selective herbicide for sugarcane crops that is derived from a group of substituted triazinones and ureas.

Approximately twelve months after planting, the morphological characterization of each experimental plot was performed with the aid of the document “Descritores Botânicos da Cana-de-Açúcar” (“Botanical Descriptive Features of Sugarcane”) published in the Diário Oficial da União (Official Government Registry) from march five thousand nine hundred ninety-eight (n 43-Seção I, pages 95 to 98). This document contains the description of 46 morphological variables that differentiate the botanical structure of sugarcane accessions and varieties for the purpose of individualizing each genotype. This allows the use of this information in studies of genetic divergence where accessions and cultivars are clustered in different blocks, and it aids breeders in choosing parents with greater potential for use in sugarcane breeding programs.

The morpho-agronomic traits were used as multicategorical variables, thus estimating dissimilarity by means of a similarity index (S_{ij}).

$$S_{ij} = C / C + D \quad (\text{Equation 1})$$

where: C: agreement of the category; D: disagreement of the category.

With this index, it is possible to express the percentage of similarity coincidence considering the various traits analyzed. Thus an S_{ij} value equal to 0.50 reveals that the two genotypes (i and i') are similar in 50% of the multicategorical characteristics studied.

Dissimilarity (d_{ij}) is given by:

$$d_{ij} = C / C + D \quad (\text{Equation 2})$$

where: C: agreement of the category; D: disagreement of the category.

Cluster analyses were conducted using these estimates, and the Tocher optimization methods were adopted. In this analysis, it is necessary to obtain a matrix of dissimilarity where the most similar pair of accessions is identified. These individuals will form the initial cluster in which the possibility of inclusion of new individuals is evaluated; therefore, adopting the criterion that the mean intra-cluster distance must be less than the mean inter-cluster distance.

The inclusion or exclusion of the k accession in the cluster is made considering the following: - if $d_{(\text{group})k} / n \leq \Theta$, the k accession is included in the cluster; - if $d_{(\text{group})k} / n > \Theta$, the k accession is not included in the cluster, where n is the number of accessions that constitute the original cluster.

In this case, the distance between the individual k and the cluster formed by the accessions ij is given by:

$$D_{(j)k} = d_k + d_j \quad (\text{Equation 3})$$

Genes, a genetic statistical software, was used to conduct dissimilarity analyses and Tocher method clustering (Cruz, 2006).

RESULTS AND DISCUSSION

The information obtained via the dissimilarity matrix of sugarcane accessions, with the use of multicategorical variables utilized to generate Tocher method clustering data, resulted in the formation of eleven distinct clusters (Table 2). Cluster I, containing 18 treatments, was the largest set formed, including seventeen accessions and the cultivar SP80-1842 (SP71-1088 x H57- 5028), and it corresponded to 46.2% of the total treatments evaluated (Table 2).

Table 2. Representation of the clusters generated by the Tocher optimization method based on genetic dissimilarity among the 39 sugarcane accessions evaluated using 46 morphological characters.

Clusters	Accessions/Varieties										%
I	P11	I32	N23	L1	L12	L30	L21	L29	L20	L19	46.2
	RV24	N22	L33	RV25	C31	P34	L27	V37			
II	P4	N28	RV26								7.7
III	P16	P18	P13								7.7
IV	P6	L9	P35								7.7
V	L8	V39	L2	V36							10.3
VI	P5	L10									5.1
VII	L7	P14									5.1
VIII	V38										2.6
IX	L15										2.6
X	L3										2.6
XI	P17										2.6

Silva et al. (2005), using multivariate techniques to evaluate genetic divergence in 129 sugarcane cultivars, conducted an experiment in the municipality of Paranaíba (Paraná, Brazil), and they observed the formation of seven clusters using the Tocher method. Furthermore, the first of these clusters consisted of 108 cultivars that represented 83.72% of the studied samples. These results corroborate the results of the present study in that a greater number of treatments constituted the first group.

Cluster V, with four treatments, was the second largest with 10.3% of the total cultivars examined in this study (Table 2), including two accessions and the cultivars RB867515 (RB72454 x unknown) and SP81-3250 (CP70-1547 x SP71-1279). Clusters II, III, and IV were composed of three accessions that contained 7.7% of those studied. Clusters VI and VII had two accessions per cluster, representing 5.1% of the total analyzed for each cluster.

Cluster VIII, IX, X, and XI contained only a single accession. Of these, cluster VIII, composed of the cultivar SP79-1011 (NA5679 x Co775), stood out. The results show the genetic divergence between the accessions and sugarcane varieties used in the region of Lavras, Minas Gerais, Brazil, and they corroborate reports presented in the literature.

Elias et al. (2007) studied genetic variability in traditional black common bean germplasm, and concluded that the Tocher optimization method maintains homogeneity within clusters and heterogeneity between the clusters. Thus, a greater number of individuals in a determined cluster is indicative of greater genetic similarity, and individuals that fit within the last cluster exhibit greater divergence relative to those in the first cluster. Genetic divergence among the treatments may be observed in the minimum and maximum dissimilarities detected in the results. The pairing between treatments P11 and I32, which were allocated to cluster I, denoted a dissimilarity of 0.39 (39.0%) between them, and this was the minimum among all those studied. The other extreme lay in the

pairing of treatments P17 and L29, with a dissimilarity of 0.85, indicating divergence in 85.0% of the characteristics evaluated in these accessions.

Regarding the morphological characteristics obtained via the descriptors that led to the formation of the divergent groups, treatment P17 (cluster XI) stood out. P17 diverged from most of the treatments based on the characteristics studied, including the following: growth habit of the clump (classified in this case as “prostrate” on a scale of upright, lightly prostrate, and prostrate); dehusking (classified as “difficult” on a scale of natural, easy, medium, and difficult); and splitting (classified as “deep” on a scale of absent, shallow, medium, and deep). These characteristics indicated that this accession has traits that limit sugarcane production directed toward regional craft stills. Harvests are performed manually, without prior burning. Therefore, it is fundamental that the accessions exhibit natural or easy dehusking and upright growth habit. The occurrence of splitting reduces the quality and yield, and it is also a point of entry for insects and pathogens.

Relative to the other clusters, cluster X (accession L3) exhibited the characteristics of a “short” distance between nodes and the presence of hairiness (classified as “a lot” on a scale of absent, little, medium, or a lot). It may also be inferred that this accession has characteristics that do not match regional demands for cachaça. Furthermore, clusters VIII (SP79-1011), VII (accessions L7 and P14), and IV (accessions P6, L9, and P35) have dehusking classified as “difficult”, which is a non-desirable characteristic for craft still production because it hinders the cleaning of sugarcane at harvest and before milling.

It is fitting to mention that the existence of genetic variability among the studied accessions was evident, even though a large number of accessions were present in cluster I (Table 2).

It should also be emphasized that for the choice of varieties or accessions to be used in the production of craft-distilled cachaça, several important morphological traits (e.g., dehusking, the presence of hairiness, tillering, the presence of splitting, and growth habit) must be considered in addition to agronomic potential.

CONCLUSIONS

There was genetic divergence among the accessions and varieties studied. Accessions P17 and L29 were the most divergent, with a dissimilarity of 0.85.

Conflicts of interest

The authors declare no conflict of interest.

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