academicJournals

Vol. 13(11), pp. 526-533, 15 March, 2018
DOI: 10.5897/AJAR2017.12913
Article Number: 4C7CCF056288
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic divergence in *Agave* accessions through ISSR markers and phenotypic traits

Silmara Chaves de Souza[1], José Jaime Vasconcelos Cavalcanti[2], Jean Pierre Cordeiro Ramos[3], Isaías Alves[2], Roseane Cavalcanti dos Santos[2] and Liziane Maria de Lima[2*]

[1]Postgraduate in Agrarian Sciences, Universidade Estadual da Paraíba, Rua Baraúnas, 351, Bairro Universitário, Campina Grande-PB, CEP 58429-500, Brazil.
[2]Embrapa Algodão, R. Osvaldo Cruz, 1143, Centenário, Campina Grande-PB, 58428-095, Brazil.
[3]Postgraduate in Agronomy, Universidade Federal da Paraíba, Campus II, Rodovia PB 079- km 12, Areia-PB, CEP 58397-000, Brazil.

Received 30 November, 2017; Accepted 23 January, 2018

The *Agave* genus is composed of about 200 species, but the cultivation of sisal for fiber production in Brazil is restricted to two species: *Agave sisalana* and *Agave fourcroydes,* both have several accessions with wide variability. The collection of *Agave* of Embrapa has 37 accessions maintained *in situ* and periodically evaluated agronomical traits. Most of these accessions have phenotypic similarities, although they differ in fiber quality, which are widely used for commercial purposes. The identification of promising accesses contributes to the advance in improvement works, focusing on commercial indication. In order to estimate the genetic divergence of this collection, a cluster analyses was performed based on Inter-Simple Sequence Repeat (ISSR) markers and phenotypic traits. Genomic DNA from these accessions were used in polymerase chain reaction (PCR) with thirty ISSR oligonucleotides. For phenotypic characterization, twelve descriptors were adopted based on morphological and agronomic data. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Tocher multivariate methods were adopted. Thirteen groups were formed by the Tocher Method and six by UPGMA; however UPGMA method was more representative in the group formation. The comparison of the band patterns among accessions derived from the shoots showed that genetic variability is generated during asexual reproduction in these plants. The four lines generated from Tatui were the most divergent accessions. These plants are tallest, with higher mass values of fresh and dry mucilage, fresh and dry fiber mass, fiber length and presence of spines at the edges. The accessions from Instituto Agronômico de Campinas (IAC) showed the lowest genetic distances, indicating a possible narrow genetic base and high kinship degree. The crossings between H-RN, H-Kenya, H-400 fls, and H-11648 with Tatuí 1, 2, 3, and 4 can be a valorous strategy to broadening genetic diversity among commercial and native sisal germplasm.

Key words: Sisal, *Agave sisalana,* molecular markers, morphoagronomic, diversity, cropping breeding.

INTRODUCTION

Agave is a xerophytic and monocot plant that grows natively in semi-arid, subtropical, and tropical regions from the southern United States to northern South America and throughout the Caribbean (Infante et al.,

2006). Based on Angiosperm Phylogeny Group (APG) III, Bremer et al. (2009) cited the new classification for the genus *Agave*, which became part of the Asparragaceae family. The genus *Agave,* which originated from arid and semi-arid regions of Mexico, contains about 200 species, with restricted geographic distribution (Gentry, 1982; Garcıa-Mendoza and Galvan, 1995). *Agave sisalana* Perr. and *Agave fourcroydes* (henequen) are broadly cultivated due to their fiber qualities (Judd et al., 2007; Martin et al., 2009). The reproductive mechanisms of most agaves involve reproduction by seeds and through rhizomes, which appear in early stages of plant development, forming new individuals (Infante et al., 2006). The plant has hermaphrodite flowers and dehiscent fruits. The flowering takes place only once, during the whole cycle. The fiber is the hardest among the fibrous species and has wide use in the artisanal segment, civil construction and automobile industry (Soto et al., 2013). In addition, the mucilage derived from defibration process can be used in ruminant feeding (Silva et al., 1999; Brandão et al., 2011; Macedo et al., 2015).

Brazil is the world's largest producer and exporter of thread and manufacturer of sisal (*A. sisalana*) (CONAB, 2016). It is an important crop in semi-arid region, where total rainfall is often scarce and irregular. Despite the widespread use of sisal, information about the genetic basis of genotypes grown in the Northeast region is limited. It is assumed that the cultivars must have the same genetic basis, although *A. sisalana* is not a genetically pure germplasm (Lock, 1962). According to Moreira and Vieira (1999), the lack of knowledge on heritability of the fiber-related traits has limited the progress of sisal improvement, although they report that the resistance and fiber percentage have high heritability, and therefore can provide selection gains in breeding program. The Brazilian Agricultural Research Corporation (Embrapa) has an *Agave* collection, containing *A. sisalana* and *A*. *fourcroydes* accessions, maintained *in vivo* in a semi-arid environment, in the Cariri region of Paraíba, Brazil. Phenotypic descriptors, based on morphological and agronomic traits are periodically recorded. Knowledge of the genetic diversity of the collection is limited, although such information is essential to estimate the potential of the genotypes for later use in breeding program. The analysis of genetic divergence in germplasm banks provides several useful information on genetic resources, based on a data set. The multivariate methods are important tools to assist the selection procedures in improvement programs and provide broad contribution to classification and identification of germplasm. Among the most used multivariate techniques, the unweighted paired group method using arithmetic averages (UPGMA) is widely adopted by breeders because it is consistent with regard to the allocation of clusters. When different types and number of traits were used, it also revealed higher cophenetic correlation coefficient (Sneath and Sokal, 1973). Other widely used method is the Tocher's optimization method that uses the criterion of optimization, minimizing the average distance intra-cluster and maximizing the average distance inter-cluster (Rao, 1952). However, the Tocher's method does not involve a construction of a phenogram to perform the clustering. The clustering is normally used together with UPGMA, revealing correspondence to the allocation of elements in the groups (Arriel et al., 2006).

Although studies involving genetic diversity in sisal collections are limited, some findings are reported in the literature. Navarro-Quezada et al. (2003) analyzed the genetic differentiation of *A. deserti* complex, comprising *A. deserti*, *A. cerulata* and *A. subsimplex*, using Nei's genetic distance from random amplified polymorphic DNA (RAPD) markers. According to authors, Nei's genetic distances between the three species were low compared to the values obtained from other Agavaceae, and there was no clear correlation with taxonomic divisions. In an UPGMA analysis, *A. subsimplex* and *A. cerulata* formed exclusive monospecific clusters, whereas the *A. deserti* populations appeared in more than one cluster together with other species. The results were consistent with a pattern of genetic isolation by distance. With the Tocher method, Moreira and Vieira (1999) used agronomic traits collected in seven accessions to estimate the genetic divergence, based on agronomic data. Despite the reduced number of accessions, the authors were able to discriminate them in five groups, with 11648 and IAC 069 being the most divergent and therefore those were recommended for later use in breeding program. The perspective of acquiring heterotic expression with these genotypes is high, considering the genetic base of the genotype 11648 which is an interspecific hybrid between *A. angustifolia* × *A. amaniensis* (Doughty, 1938).

The *Agave* collection of Embrapa has been periodically evaluated to agronomical traits and some biochemical studies have been carried out as to mucilage proprieties of *A. sisalana* accessions (Oliveira et al., 2016). Based on evaluations carried out by Moreira and Vieira (1999), most accessions have phenotypic similarities, although they differ in some fiber traits. The identification of promising accesses is relevant to improvement of sisal, because it contributes to further indication of commercial genotypes.

The aim of this work is to determinate the genetic

*Corresponding author: E-mail: liziane.lima@embrapa.br. Tel: +55 (83) 3182 4442. Fax: +55 (83) 3182 4367.

divergence among the *Agave* accessions from Embrapa performed by a cluster analysis using UPGMA and Tocher methods, based on ISSR markers and phenotypic traits.

MATERIALS AND METHODS

Genetic resources and ISRR-PCR assays

In this study, 37 accessions of the sisal collection were used, maintained in Monteiro, PB, Brazil (07°53'22 "S, 37°07'40" W, 599 m). A summary of the phenotypic data of the accessions is found in Table 1.

Fresh leaves of each accession were used for DNA extraction based on CTAB method (Ferreira and Grattapaglia, 1998). The PCR assays were performed in a Mastercycler Gradient (Amplitherm Thermal Cyclers). The reaction mixture (25 µl) contained 10× PCR buffer (Fermentas), 25 mM $MgCl_2$, 100 mM dNTP, 1.2 µMol.L^{-1} of each ISSR primer (USB), 60 ng of genomic DNA, and 1 U of Taq DNA polymerase (Ludwig Biotec). Ten ISSR oligonucleotides were used to PCR assays. The samples were submitted to follow the program: initial cycle of denaturation at 95°C/5 min, followed by 40 cycles of denaturation at 95°C/1 min, annealing at 50°C/1 min, and extension at 72°C/2 min. A final extension cycle was added to reaction at 72°C/7 min. The amplified products were analyzed by electrophoresis on 2.5% agarose gel. All reactions were performed in triplicate.

Analysis of genetic divergence and clustering techniques

For the multivariate analysis, molecular and phenotypic data were used. In the molecular data, each band generated by ISSR-PCR was coded with 1 and 0 for presence and absence, respectively, resulting in a binary matrix, which was used to quantify the genetic similarity (Sij) among the accession pairs based on the coefficient of Jaccard, following the expression:

$$S_{ij} = \frac{a}{a} + b + c$$

Where: *a* means the sum of coincidences of type 1-1 for each pair of accession, *b*, the sum of the discordances of type 1-0 for each pair of accession, and *c*, the sum of discordances of type 0-1 for each pair of accession. The arithmetical complement 1- S_{ij} was also used to transform the similarity matrix into a dissimilarity matrix, which is used in cluster analysis. The estimated dissimilarity matrix for the phenotypic data was obtained based on the Gower distance (Gower, 1971), allowing the treatment of phenotypic data simultaneously. The sum of the two matrices was performed to obtain a representative matrix of the molecular and phenotypic data, which was used to perform the cluster analysis. The dissimilarity values of each matrix were standardized through the expression:

$$d_{pj} = \frac{d_{ij}}{\sigma d}$$

Where, d_{pj} is the standardized dissimilarity between an individual *i* and individual *j*; d_{ij} is the dissimilarity between *i* and *j*, and σ_d is the standard deviation of dissimilarity.

The correlation between the matrices was also made with the purpose of estimating the level of relation between them, obtaining the significance from the t and Mantel tests, indicating or not, the agreement of the two methods in expressing the existing genetic difference between pairs of accessions (Cruz, 2008).

The clustering analyses were carried out through Tocher's optimization and UPGMA methods. The cophenetic correlation coefficient was estimated in order to eliminate the non-hierarchical effects. Cluster analysis was performed using the software GENES, version 2016.6.0 (Cruz, 2013). The following phenotypical traits were used for analysis: number of leaves, plant height, leaf length, fresh leaf mass, dry mucilage mass, fresh fiber mass, dry fiber mass, fiber length, presence of spines on the leaf edge, tillering and high folding endurance.

RESULTS AND DISCUSSION

Phenotypic traits (Table 1) and ISSR markers were used to estimate the genetic divergence among thirty-seven sisal accessions, maintained *in situ*, in the semi-arid zone Cariri of Paraíba, Brazil (Figure 1A-F). Adult plants were used for agronomical characterization, with subsequent molecular analysis, using leaf tissues.

In order to optimize molecular analysis, thirty oligonucleotides were previously tested for polymorphic selection, and therefore contributory to the evaluation processes. Of the total, ten oligos were selected, with high polymorphism rate, revealed by amplicons obtained from agarose gels, with an average of 13/oligo (Table 2). Although we used a relatively small population, maintained asexually, it was possible to identify differences among accessions through profiles obtained via ISSR-PCR. Several bands were common among the accessions; however, some were unique and therefore contributory to the analysis of genetic divergence of the collection. Figure 1G shows a pattern of amplicons obtained with UBC 812, with 11 bands distributed between 0.1 and 1 Kb.

Most molecular markers are robust to identify variability in sexually or asexually propagated populations. In literature, ISSR, amplified fragment length polymorphism (AFLP) and microsatellites markers are the most reported (Barraza-Morales et al., 2006; Abraham-Juárez et al., 2009; Santos et al., 2015). In *Agave*, the production is supported by intensive clonal propagation and the suppression of the sexual reproduction, leading to reduction in genetic variability plantations (Gil-Vega et al., 2001; Infante et al., 2006; Abraham-Juárez et al., 2009).

Abraham-Juárez et al. (2009) analyzed the genetic variability by AFLP markers, using three reproductive forms of *A. tequilana*, and found 75.08 and 86.06% of polymorphic loci, respectively, from offsets and bulbils generated from the same matrix plant. As to authors, although a significant level of polymorphism was observed between rhizome offsets, the levels were even higher between bulbils, reaching levels comparable to those found between plantlets produced from seeds (90.1%). Infante et al. (2006) explained the origin of the asexual variability in *Agave* species based on molecular markers. According to authors, in plant shoots, the whole cells are derived from the apical meristem, including the germline cells. In asexual reproduction, meristems are the source of the mitotically derived offprints. The only source of genetic differences in these materials comes

Table 1. Phenotypic traits of the sisal collection, based on an average of 3 years.

Accession	Name	NL	PH	LL	FLM	FMM	DMM	FFM	DFM	FL	PS	NT	HFE
SS-01	H-11648	69.3	104.8	77.1	283.6	220.4	26.8	22.5	10.6	76.5	2	3	2
SS-02	H-400 fls	75. 6	151.1	89.1	304.6	199.3	25.2	31.5	10.6	84.5	2	3	2
SS-03	Cabinho	34.6	170.0	111.2	604.1	317.3	38.5	79.7	28.2	109.8	1	2	1
SS-04	IAC 034	36.0	133.3	86.5	306.3	178.5	19.9	66.4	19.0	91.3	2	3	2
SS-05	H-RN	65.0	124.2	84.7	306.6	168.5	23.1	31.7	18.8	82.1	2	3	2
SS-06	H-Quênia	62.8	147.0	104.9	428.5	162.1	20.1	59.1	15.2	77.5	2	3	2
SS-07	*A. fourcroydes*	33.3	162.5	97.9	686.9	392.3	47.5	80.1	27.3	109.5	1	2	2
SS-08	Tatuí	17.3	117.1	67.2	895.5	477.2	51.3	63.8	18.5	99.5	1	2	1
SS-09	Espinho	27.8	152.0	89.9	303.1	132.9	24.3	35.8	14.0	90.7	1	3	1
SS-10	Valente	29.3	112.0	70.6	318.6	230.7	25.9	27.3	11.4	88.5	1	2	2
SS-11	H-Teixeira	57.2	126.6	91.9	276.0	196.0	18.6	31.2	11.4	74.6	1	3	2
SS-12	H-Imaculada	66.3	145.6	106.0	392.2	160.7	21.6	31.2	15.0	89.8	2	3	2
SS-13	Ornamental	25.0	98.5	61.7	135.6	83.7	12.2	19.5	6.8	61.1	1	1	2
SS-14	Tatuí 3	27.0	183.5	105.2	855.6	479.8	47.6	107.6	29.3	131.2	1	3	1
SS-15	Tatuí 4	33.2	187.8	110.5	1588.2	904.1	92.4	146.5	45.5	132.8	1	3	1
SS-16	Mutante 1	38.0	149.2	93.6	521.9	269.6	30.9	45.5	20.5	96.1	1	3	1
SS-17	Hoxa México	35.0	135.3	85.1	364.0	200.6	25.3	41.2	17.0	88.3	2	3	2
SS-18	Tatuí 1	32.0	179.0	105.5	1419.6	853.5	82.1	142.1	39.1	122.7	1	3	1
SS-19	Tatuí 2	19.5	153.0	86.2	670.1	322.2	38.3	102.5	28.5	112.1	1	3	1
SS-20	Tanzânia	49.5	133.5	91.5	342.3	147.2	18.3	29.6	13.6	82.6	2	3	2
SS-21	Mutante PB	26.8	124.8	75.8	357.8	209.4	21.6	38.7	17.1	86.0	1	3	1
SS-22	IAC 0101	27.6	106.6	67.1	204.6	140.7	15.8	45.8	10.8	73.1	2	2	2
SS-23	IAC 84193	19.6	80.4	50.0	115.2	82.8	8.4	34.9	6.9	58.5	2	1	2
SS-24	Mutante BA	31.1	133.5	82.3	409.5	179.7	17.3	41.1	18.5	87.1	2	2	2
SS-25	IAC 84003	26.0	121.5	73.7	274.5	243.3	27.6	46.3	17.3	94.5	2	3	2
SS-26	IAC 84051	34.8	94.6	64.7	191.1	152.6	20.9	30.0	11.7	65.8	2	2	2
SS-27	IAC 00200	29.0	93.2	61.1	267.0	142.2	18.5	42.8	16.1	74.1	2	2	2
SS-28	IAC 0067	44.0	129.0	86.5	299.5	230.4	33.9	35.7	15.9	90.5	2	2	2
SS-29	IAC 84001/4	38.0	104.0	71.0	338.9	190.7	19.8	29.8	11.6	83.0	2	3	2
SS-30	IAC 84005	50.6	134.4	92.5	265.1	229.7	44.8	42.3	16.7	79.6	2	3	2
SS-31	IAC 0056	36.0	98.8	67.4	374.3	121.1	16.5	30.9	9.8	73.5	2	3	2
SS-32	84001/2	38.0	104.0	71.0	218.4	115.4	16.6	32.6	13.4	70.1	2	3	2
SS-33	IAC 84-019	44.3	107.5	75.9	241.3	153.2	23.9	69.1	27.1	88.0	2	1	2
SS-34	IAC 840096	33.3	89.0	61.1	126.6	65.0	8.5	23.2	7.9	60.6	2	2	2
SS-35	IAC 0097	28.4	105.8	67.1	140.8	59.4	9.3	34.7	12.8	73.4	2	1	2
SS-36	IAC 0069	30.0	101.0	65.5	110.3	57.0	9.2	13.9	5.4	64.2	2	3	2
SS-37	H- Itaporanga	51.0	126.7	88.8	293.2	260.7	22.0	22.1	9.9	76.5	2	3	2

H- hybrid; number of leaves (NL); height of the plant from the base to the crown (PH); length of fully expanded mature leaf (LL); fresh leaf mass (FLM); fresh mucilage mass (FMM); dry mucilage mass (DMM); fresh fiber mass (FFM); dry fiber mass (DFM); fiber length (FL); presence of spines at the edges (PS): 1 = has spines, 2 = has no spines; number of tiller (NT): 1 = without tiller, 2 = between 4 and 5 tiller, 3 = ≤ 5 tiller; high folding endurance (HFE): 1 = no resistance, 2 = resistant.

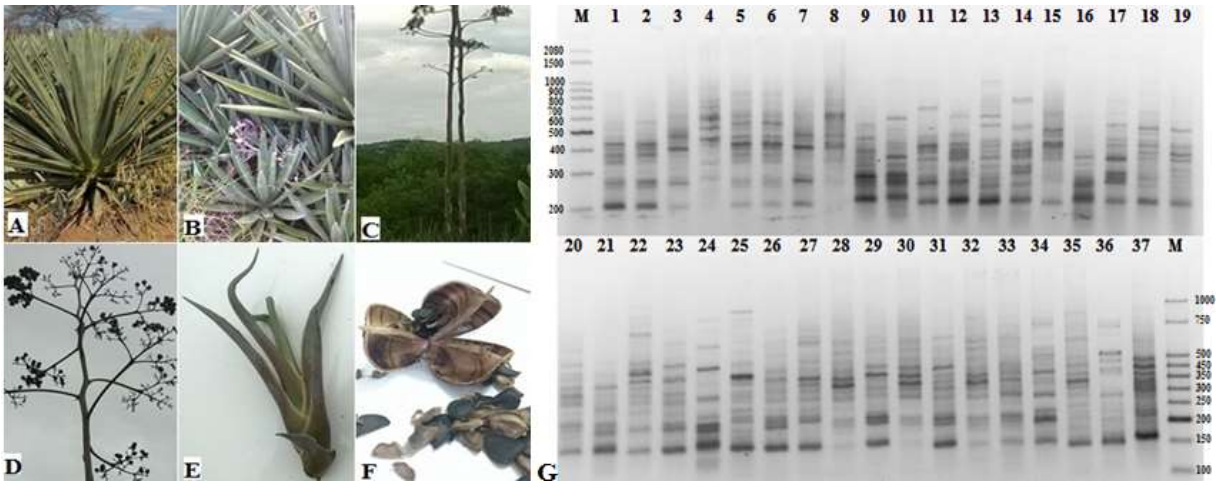

Figure 1. Details of a sisal accession, maintained in the Cariri environment (Monteiro-PB, Brazil). A- Adult plant, B- Tiller originated from the matrix plant, C- Floral scape, D- Panicle with bunch flowers, E- Bulbils located at floral scape, F- Mature capsule and round-triangular seeds, G- Amplicons obtained by ISSR-PCR, using UBC 812 oligonucleotide. M- Molecular marker (Ludwig Biotec). List of accessions in Table 1.

Table 2. Sequence of ISSR oligonucleotides used in the genetic analysis of sisal accessions and total number of bands (TNB) obtained by ISSR-PCR.

Oligonucleotides	Sequence $(5' \rightarrow 3')$	TNB
UBC 808	AGA GAG AGA GAG AGA GC	19
UBC 809	AGA GAG AGA GAG AGA GG	15
UBC 812	GAG AGA GAG AGA GAG AA	11
UBC 823	TCT CTC TCT CTC TCT CC	11
UBC 824	TCT CTC TCT CTC TCT CG	8
UBC 825	ACA CAC ACA CAC ACA CT	13
UBC 827	ACA CAC ACA CAC ACA CG	11
UBC 830	TGT GTG TGT GTG TGT GG	12
UBC 853	TCT CTC TCT CTC TCT CRT	12
UBC 881	GGG TGG GGT GGG GTG	11

from somatic mutations.

Although the *Agave* has both forms of propagation, the sexual process that is responsible for segregation and high variability is not frequent and only occurs under special conditions, such as when the tassel is decapitated before the emission of floriferous branches leading the plants to produce viable seeds and fruits. However, it is a time-consuming process and when it occurs, the progenies often show marginal leaf spines, an undesirable trait for clones destined for commercial exploitation (Abraham-Juárez et al., 2009).

The clustering analyses by Tocher and UPGMA are found in Table 3 and Figure 2, respectively. Through Tocher method, 13 groups were formed, of which 6 had only one accession. The group 1 contained the majority of accessions (40.5%), with medium height and high number of leaves. Among them, stands out the hybrids RN, Kenya, 400 fls, 11648 and *A. sisalana*, all established in Brazilian semi-arid region. According to Silva et al. (2008), the hybrid 11648 and *A. sisalana* are widely grown in Northeast region, due to acceptable traits to fiber market. The group 2 contained accessions with high height and long fibers, represented by Cabinho and *A. fourcroydes*. The group 3 clustered the accessions from the Instituto Agronômico de Campinas (IAC 840096, IAC 0069, IAC 0097, IAC 0101) and an ornamental accession (ornamental Sisalana). These materials have medium and short fibers and have high folding endurance. The group 4 contained two IAC accessions (IAC 84003, IAC 84001/4), all showing low number of leaves and no spines at the edges. The group 5 grouped three lines from Tatuí, all of them are tall plants, with greater leaf weight and extra-long fibers. These attributes are interesting for further use in breeding work. The groups 6 and 7 clustered two accessions each, with tall plants, medium fibers, spines at the edges and tillering, but those of group 6 (Valente, Teixeira) had high folding endurance, which does not occur in group 7 (Espinho, Mutant Paraíba).

In clustering analysis via UPGMA, the number of

Table 3. Grouping of sisal accessions by Tocher optimization method, based on phenotypic and molecular data.

Groups	Accessions
1	H-RN, H-Quênia, H-400 folhas, H-11648, IAC 84005, IAC 0067, 84001/2, Sisalana Tanzânia, IAC 0056, H-Itaporanga, IAC 00200, H-Imaculada, Hoxa México, IAC 84051, IAC 84-019
2	Cabinho, *A. fourcroydes*
3	IAC 840096, IAC 0069, IAC 0097, Sisalana ornamental, IAC 0101
4	IAC 84003, IAC 84001/4
5	Tatuí 4, Tatuí 1, Tatuí 3
6	Valente, Teixeira
7	Espinho, Mutante Paraíba
8	Mutante 1
9	IAC 034
10	Mutante Bahia
11	Tatuí 2
12	IAC 84193
13	Tatuí

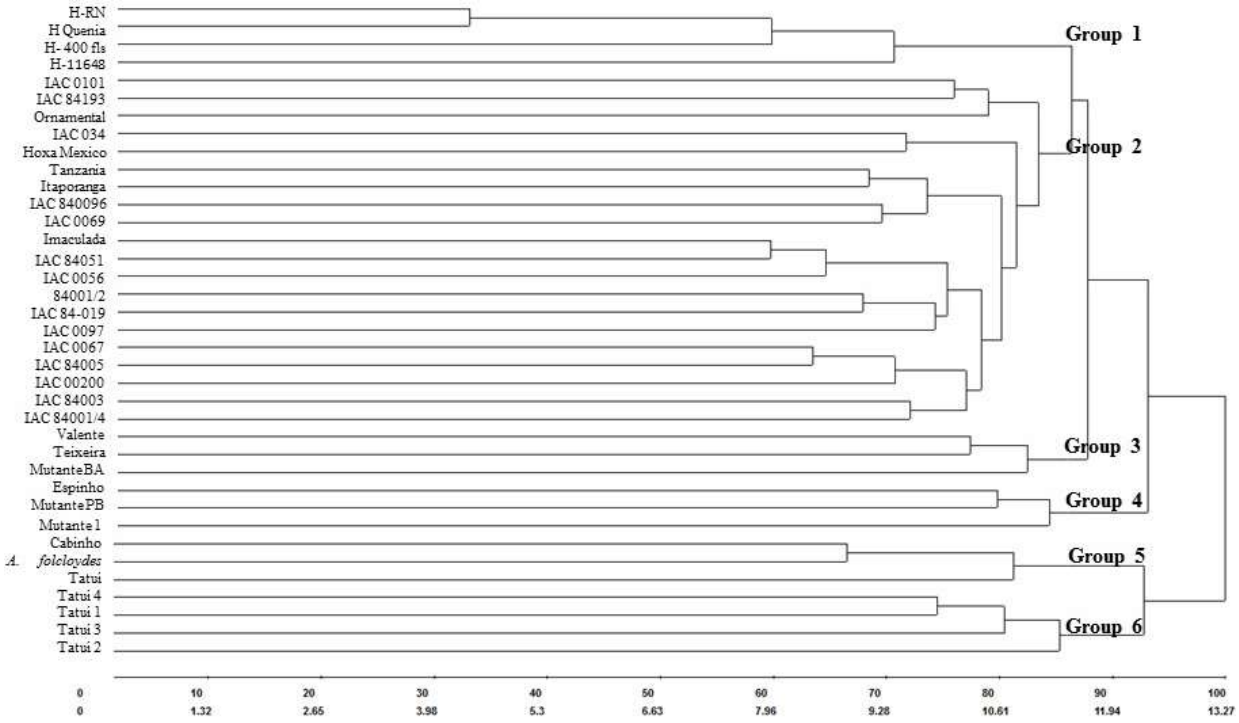

Figure 2. Dendrogram obtained by UPGMA method, generated from a similarity matrix with 37 agave genotypes. Cophenetic correlation coefficient: 0.77. A similarity index up to 85% was adopted ($p \leq 0.01$, F test).

grouping was more condensed, distributed in six clusters. The degree of association between dissimilarity matrices obtained from phenotypic and molecular data was significant, based on t and Mantel test ($p \leq 1\%$), indicating that both sets of data were adequate to represent the genetic divergence in the collection of sisal. The distribution of accessions through UPGMA was more contributive to discriminate divergent groups than Tocher's and provides more possibilities for choice of parents in breeding work aiming at production and fiber quality. UPGMA method tends to generate higher values of the cophenetic correlation coefficient, allowing to infer that the groups of accessions indicated in the graphic have an adequate fit between the dissimilarity matrix and dendogram obtained (Cruz and Carneiro, 2003).

As shown in Figure 2, the hybrids H-400 fls, H-Kenya, H-RN, and H-11648 were clustered in a group separated from the others. All of them are bred genotypes, with

special traits to the natural fiber market. Overall, the fiber of these hybrids shows strength and yield near to 750 g/force and 2.7 t.ha^{-1}, respectively (Amorim Neto and Beltrão, 1999). The most IAC accessions were clustered in group 2 that contained 20 accessions, and most showed short fiber. The groups 3 and 4 contained three accessions each, constituted by mutants collated in Paraíba and Bahia states, and also local types. The group 5 contained *A. fourcroydes*, Cabinho and Tatuí, all characterized by presence of spines at the leaf edges. The last, group 6, clustered the four lines derived from Tatuí, characterized by high weigh of leaf (fresh mass) and dry mucilage, high weigh of fiber (dry and fresh), large fiber length and presence of spines at the leaf edges. They are similar lines, spreading in different places in Sao Paulo State, located at Sudeste region, Brazil.

Based on results obtained from clustering analysis, we can consider that the variability found in the Embrapa-*Agave* collection offers possibilities to implement a breeding program in order to meet the demands of the natural fiber market. The richness of this information lies in the fact that, although *Agave* is originated from Mexico, Brazil has several ecotypes that have as their main skill the broad adaptation to the semi-arid and tropical environments. There is no information on the existence of variability in fiber quality in different environments. However, in the literature, the phenotypical differences in plant canopy are marked when grown in less arid environments (Silva et al., 2008). Thus, depending on the purpose of the breeding program, it is possible to identify in these results divergent genotypes that may contribute to the generation of promising hybrids.

CONCLUSION

Agronomical and molecular variability was found in *Agave* collection, maintained by Embrapa, in Monteiro, PB, Brazil. This represent a valorous strategy to broadening genetic diversity among commercial and native sisal germplasm.

CONFLICT OF INTERESTS

The authors declare no conflict of interest between the partners with the release the results.

REFERENCES

Abraham-Juárez MJ, Ramírez-Malagón R, Gil-Vega K, Simpson J (2009). Análisis AFLP de la variabilidad genética en tres formas de reproducción de *Agave tequilana*. Rev. Fitotec. Mex. 32(3):171-175.

Amorim Neto M, Beltrão NEM (1999). Clima e solo. In: Silva ORRF, Beltrão NEM. O agronegócio do sisal no Brasil. Brasília, 4:41-52.

Arriel NHC, Di Mauro AO, Di Mauro SMZ, Bakke AO, Unêda-Trevisoli SH, Costa MM, Capeloto A, Corrado AR (2006). Técnicas multivariadas na determinação da diversidade genética em gergelim, usando marcadores RAPD. Pesq. Agropec. Bras. 41(5):801-809.

Barraza-Morales A, Sánchez-Teyer FL, Robert M, Gardea MEA (2006). Variabilidad genética em *Agave angustifólia* Haw. de la sierra sonorense, México, determinada con marcadores AFLP. Rev. Fitotec. Mex. 29(1):1-8.

Brandão LGN, Pereira LGR, Azevedo JAG, Santos RD, Aragão ASL, Voltolini TV, Neves ALA, Araújo GGL, Brandão WN (2011). Valor nutricional de componentes da planta e dos coprodutos da *Agave sisalana* para alimentação de ruminantes. Arq. Bras. Med. Vet. Zootec. 63(6):1493-1501.

Bremer B, Bremer K, Chase MW, Fay MF, Reveal JL, Soltis DE, Soltis PS, Stevens PF (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161:105-121.

CONAB – Companhia Nacional de Abastecimento (2016). Conjuntura especial. Sisal: Exportações em queda. Available in: < http://www.conab.gov.br/OlalaCMS/uploads/arquivos/16_07_28_17_46_03_sisal_conjuntura_jul_2016.pdf>. Acess in: 18 Fev 2017.

Cruz CD (2008). Programa GENES: Biometria: Versão Windows: aplicativo computacional em genética e estatística. Viçosa: UFV.

Cruz CD (2013). GENES – a software package for analysis in experimental statistics and quantitative genetics. Acta Sci. 35(3):271-276.

Cruz CD, Carneiro PCS (2003). Modelos biométricos aplicados ao melhoramento genético. Viçosa: UFV, 585p.

Doughty LR (1938). Experimental breeding as fiber agaves in East Africa. II. The progress of breeding at amani. E. Afr. Agric. J. 3:268-278.

Ferreira ME, Grattapaglia D (1998). Introdução ao uso de marcadores moleculares em análise genética. 3.ed. Brasília: EMBRAPA-CENARGEN, 220p.

Garcia-Mendoza A, Galvan R (1995). Riqueza de las familias Agavaceae y Nolinaceae en Mexico. Bol. Soc. Bot. México 56:7-24.

Gentry HS (1982). Agaves of Continental North America. The University of Arizona Press, Tucson, Arizona, 670p.

Gil-Vega K, Chavira GM, Martínez De La Vega O, Simpson J, Vandemark G (2001). Analysis of genetic diversity in *Agave tequilana* var. Azul using RAPD markers. Euphytica, 3ª. Ed. 119:335-341.

Gower JC (1971). A general coefficient of similarity and some of its properties. Biometrics 27(4):857-874.

Infante D, Molina D, Demedey JR, Gámez E (2006). Asexual genetic variability in Agavaceae determined with Inverse Sequence-Tagged Repeats and Amplification Fragment Length Polymorphism analysis. Plant Mol. Biol. Report. 24:205-217.

Judd WS, Campbell CS, Kellog EA, Stevens PF, Donoghue MJ (2007). Plant systematics: a phylogenet-ic approach. 3. ed. Massachusetts: Sinauer Associates Inc., 565p.

Lock GW (1962). Sisal. London: Longman, 355p.

Macedo ADB, Sousa APM, Oliveira JAM, Mattos MA, Santana RAC, Campos ARN (2015). Bioconversão da palma forrageira e do sisal como alternativa para alimentação animal. Blucher Chemistry Proceedings 3(1):630-639.

Martin AR, Martins MA, Mattoso LHC, Silva ORRF (2009). Caracterização química e estrutural de fibra de sisal da variedade *Agave sisalana*. Polímeros 19(1):40-46.

Moreira JAN, Vieira RM (1999). Melhoramento genético do sisal. In: Silva ORRF, Beltrão NEM. O agronegócio do sisal no Brasil. Brasília, 205p.

Navarro-Quezada A, González-Chauvet R, Molina–Freaner F, Eguiarte LE (2003). Genetic differentation in the *Agave deserti* (Agavaceae) complex in the Sonoran Desert. Heredity 90(3):220-227.

Oliveira LHG, Sousa PAPS, Hilário FF, Nascimento GJ, Morais JPS, Medeiros EP, Sousa MF, Nunes FC (2016). *Agave sisalana* extract induces cell death in *Aedes aegypti* hemocytes increasing nitric oxide production. Asian Pac. J. Trop. Biomed. 6:396-399.

Rao RC (1952). Advanced statistical methods in biometric research. New York: J. Wiley, 390p.

Santos SLB, Passos AR, Queiroz SROD, Nascimento MN, Carneiro FS (2015). Genetic variability in populations of *Agave sisalana* PERRINE detected by inter simple sequence repeats. Biosc. J. 31(6):1624-1633.

Silva ORRF, Bandeiras DA, Beltrão NEM (1999). Aproveitamento dos

resíduos do desfibramento. In: SILVA ORRF, BELTRÃO NEM. O agronegócio do sisal no Brasil. Brasília, cap. 8: 125-143.
Silva ORRF, Coutinho WM, Cartaxo WV, Sofiatti V, Filho JLS, Carvalho OS, Costa LB (2008). Cultivo do Sisal no Nordeste Brasileiro. Circular Técnica 123. Embrapa Algodão. Campina Grande, Paraíba.
Sneath PH, Sokal RR (1973). Numerical taxonomy: The principles and practice of numerical classification. San Francisco: Freeman WH P 573.
Soto II, Ramalho MA, Izquierdo OS (2013). Post-cracking behavior of blocks, prisms, and small concrete walls reinforced with plant fiber. Rev. IBRACON Estrut. Mater 6(4):598-612.