DOI: 10.5965/223811711632017247

Induction of mutation and measurement of genetic variability in sugarcane genotypes

Indução de mutação e mensuração da variabilidade genética em genótipos de cana-de-açúcar

Maicon Nardino*, Ivan Ricardo Carvalho, Mauricio Ferrari, Alan Junior de Pelegrin, Vinicius Jardel Szareski, Diego Nicolau Follmann, Braulio Otomar Caron, Denise Schmidt e Velci Queiróz de Souza

Recebido em 01/03/2016 / Aceito em 02/03/2017

ABSTRACT

The widespread usage of commercial varieties of sugar that are adapted to the agricultural system and come from parents with a small genetic distance can lead to a narrowing of the genetic basis of sugarcane. This study aimed to induce mutations and evaluate the genetic distance among the sugarcane mutants produced by different multivariate techniques. Mutations were induced and then the genetic distance among these sugarcane mutants was evaluated with the use of multivariate techniques. The study was conducted in the experimental area of the UFSM, Frederico Westphalen, RS. The genotype used for mutation induction was IAC 873396. The induction process proceeded with the placement of buds in a solution of the mutagenic agent MMS (Methyl Methane Sulfonate). A total of 22 mutations and six commercial checks were evaluated for 12 traits of agronomic interest. The analyses were: the evaluation of individual averages, Tocher's grouping, average Euclidean distance, an analysis of principal components, and the relative contribution of characteristics. The mutation induction process generated significant patterns of genetic variability among sugarcane mutants verified by three multivariate analyses. The UPGMA clustering methods, Tocher and principal component analysis revealed similar results related to the dissimilarity of sugarcane genotypes. Genotypes 20, 24, and 10 were greater than the general average of genotypes and have a high genetic dissimilarity for traits. The yield of stems, bagasse mass, and the yield sugarcane broth are sufficient to characterize a set of sugarcane genotypes.

KEYWORDS: agronomic performance, genetic variability, *Saccharum officinarum L.*.

RESUMO

O uso generalizado de variedades comerciais adaptadas ao sistema agrícola, oriundas de genitores muito próximos, com pequena distância genética pode levar ao estreitamento da base genética em cana-de-açúcar. O trabalho teve por objetivo induzir mutação e avaliar a distância genética entre os mutantes produzidos de cana-de-açúcar, por diferentes técnicas multivariadas, almejando a identificação de mutantes com caracteres contrastantes, com interesse para o melhoramento de cana-de-açúcar no Sul do Brasil. O trabalho foi desenvolvido na área experimental da Universidade Federal de Santa Maria, Campus de Frederico Westphalen, RS. O genótipo utilizado para indução de mutação foi o IAC 873396. O processo de indução transcorreu com a alocação de gemas em solução do agente mutagênico MMS. Foram avaliados 22 mutantes e seis testemunhas comerciais para 12 caracteres de interesse agronômico. As análises realizadas foram: avaliação das médias dos indivíduos, agrupamento de Tocher, distância euclidiana média, análise de componentes principais e a contribuição relativa dos caracteres. O processo de indução de mutação gerou padrões significativos de variabilidade genética entre os mutantes de cana-de-açúcar verificada pelas três análises multivariadas. Os métodos de agrupamento UPGMA, Tocher e a análise de componentes principais revelam resultados próximos quanto à dissimilaridade dos genótipos de cana-de-açúcar. Os genótipos 20, 24 e 10 são superiores a média geral dos genótipos e apresentam elevada dissimilaridade genética. O rendimento do colmo, massa do bagaço e rendimento de caldo são caracteres suficientes para realizar a discriminação do conjunto de genótipos de cana-de-açúcar.

Universidade Federal de Pelotas, Pelotas, RS, Brasil.
*Autor para correspondência <nardinomn@gmail.com>

PALAVRAS-CHAVE: desempenho agronômico, variabilidade genética, *Saccharum officinarum L.*.

INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is a major crop in Brazil and plays a very important role as a source of raw material for sugar and ethanol production. The production of sugar cane for harvest 15/16 is estimated at 658.7 million tons (CONAB 2015). Genetic improvement is a science that has ensured, and continues to ensure, the sustainability of this type of crop through the generation of sugarcane varieties tolerant or resistant to pests and diseases and thereby increasing production with the emergence and availability of new plant varieties adapted to the conditions of a given region (GOES et al. 2009).

The production and extension of genetic variability is a crucial aspect for genetic improvement in the search for agronomically desirable parents for different locations and cropping systems. A widely known mechanism to increase genetic variability is through induced mutations which present an alternative for plant breeders by producing genetic diversity without the need to perform artificial crosses (NARDINO et al. 2016). This variability is extremely important to genetic improvement. Mutation is any heritable change that is specific to a gene, or a consequence of a change in chromosome number or structure. Mutation induction has been a positive contribution to our classic breeding methods (ALLARD 1971).

The generation of variability by crosses or induced mutations in sugarcane aims to identify agronomically superior individuals that will be disseminated. The genetic potential is determined after obtaining a clone that does not change over generations (BORÉM & MIRANDA 2005). The widespread use of commercial varieties from very close and a short genetic distanced ancestors adapted to the agricultural systems can lead to the narrowing of the genetic base and an increased risk of genetic vulnerability of biotic and abiotic stress.

Predominantly, plant breeders have successfully relied on a common genetic base derived from an ancestor with a restricted genetic formation, obtained in the early twentieth century from interspecific crossing and backcrossing (MATSUOKA et al. 2005). Knowing the genetic dissimilarity between commercial varieties is essential to plant breeders for identifying and organizing genetic resources (PALOMINO et al. 2005). FERREIRA et al. (2005) showed in their sugarcane studies significant values of inbreeding depression for brix characteristic, stem tones, and diameter and average weight of stems in plants produced (obtained) by selfing. Thus, the cross between individuals next to each other may result in low genetic gain or even some loss by inbreeding.

Whether the expansion of variability occurs by artificial crossing or by induction of mutations, superior and contrasting parents are of interest for genetic improvement. Knowing the problems of sugarcane cultivation in the south of Brazil where the crop does not complete its cycle due to the occurrence of frosts during winter, it becomes difficult to increase genetic variability by crossing. The use of mutagens can increase genetic variability and therefore be beneficial to plant improvement and genetic development. In this context, the study aimed to induce mutations and evaluate the genetic distance among sugarcane mutants by using different multivariate techniques. Thus, identify mutants with contrasting characteristics in order to improve sugarcane breeding in southern Brazil.

MATERIAL AND METHODS

The study was conducted in the experimental area of the Federal University of Santa Maria, Frederico Westphalen Campus, RS, in the Plant Breeding Production Laboratory. The geographic coordinates were: latitude: 27º39’56“, longitude: 53º42’94”, with an altitude of 490 meters above sea level. The soil of the region is classified as Oxisol Alumino Ferric and the climate, according to Köppen is Cfa, subtropical (EMBRAPA 2006).

The genotype used for mutation induction was the cultivar IAC 873396. This specific type was chosen because it has been outstanding between 19 sugarcane genotypes regarding some characteristics such as the brix broth, broth yield, and stem mass per hectare during the harvests of 2008, 2009, and 2010 in southern Brazil. Twenty-plant pieces were placed on the mutagenic chemical were approximately. The induction process was accomplished by the allocation of individual buds immersed in solution of the chemical mutagen Methyl Methane Sulfonate (MMS) at a concentration of 5 ppm for 12 hours. Later, these buds were washed in order to removing excess alkylating agents and later placed in indole acetic acid

for 60 minutes in order to induce root development. Subjected to this procedure, the buds that were subjected mutation induction were transferred to a greenhouse in sandy soil along with six checks, where they remained until reaching a height of 0.3 meters.

The mutagenic plants were transferred to a field when they reached a height of over 0.3 meters, and aligned with a spacing of 1.0 meter between plants and 1.2 meters between lines. The plot was seven meters in length. The experimental design was in blocks with intercalate checks. Each genotype was evaluated in the same plot during the three years, 22 mutants were evaluated. Each mutagenic sampling and check was evaluated by counting the number of stems and two representative stems were analyzed for characteristics of agronomic interest. The evaluations were conducted in the years 2011, 2012 and 2013.

The evaluated traits were:

Stem length (SL): measured from the soil basis to the apex of the plant using a graduated scale in centimeters (cm);

Number of buds (NB): The total number of buds on each stem was counted;

Base diameter, middle and apex (BD, MD and AD): measured with a millimetric caliper at the bottom, the middle and the apex of the plant;

The mass of two stems using an analytical scale, the unit is kg ha^{-1};

Bagasse mass (BM): quantification of residues after extracting the broth using an analytical scale, the unit is kg ha^{-1};

Broth yield (SBY): the amount of broth removed from two plants with a cane grinder containing three cylinders, and measured with a graduated cylinder. Then, the amount of broth in liters per hectare (L ha^{-1}) was estimated.

Initially, the average of sugarcane mutants was computed and then the average standard deviation was calculated from the general average of the experiment. The data was submitted for analysis of the principal components such as the relative importance of characteristics for each trait and the total dissimilarity that was observed (SINGH 1981). In the study of genetic dissimilarity a Tocher optimization method was used and for clustering analysis the used method used was the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Analyses were performed using the computer program Genes (CRUZ 2013).

RESULTS AND DISCUSSION

There is evident amplitude between the sugarcane mutants and the checks to the averages of the evaluated traits (Table 1). Such results are promising for evaluating a possible genotype with desired agronomic performance for the conditions found in southern Brazil. Thus, there may be new alternatives for producers and future industries, generating economic alternatives (FABRIS et al. 2013).

The production of sugarcane in the state of Rio Grande do Sul meets less than 1% of its energy demand. Thus, the government encourages the expansion of crop area and the installation of sugarcane power plants, which allows for the production of alcohol (BELLÉ et al. 2014). In this context, the analyses of genotypes that optimize characteristics related to the broth yield are justified.

Superior to at least one standard deviation are the mutants 20, 14, 15, 26, 22, 24, and 12 due to the length of the stem which is 170.8 cm. Genotypes with the highest number of buds were 17 (16); 14 (15.5); 20 (15.3); check 2 (14.8); 18 (14.8); 23 (14.5) and 10 (14.3). Regarding the diameter of the stem mutants, 13, 16, and 10 have the largest diameter of the base, middle and apex of the stem.

Regarding the stem brix degree, there were differences between genotypes related to the brix content in the base, middle and apex of the stem. Mutant 24 had the highest brix content in the base (19.1), in the middle of the stem (18.5) and also a high brix degree was seen in the stem apex (15.6); the values for soluble solids were considered satisfactory (MARQUES et al. 2001, DEON et al. 2010).

For the stem mass, 16, 20, 10, 13, and 22 were observed and compared to all checks as well as the broth yield to mutants 16, 20, 10, and 25, which are all superior to at least one standard deviation from the overall average of the genotypes. The identification of superior mutant genotypes for traits related to agribusiness in areas yet not well known is extremely important to maximize crop exploration (MAULE et al. 2001). The association of sugarcane genotypes that have a high production of stem mass with high broth yield and low production of residue have high production efficiency. These characteristics are highlighted in mutant 10 which had highest SM, high JY and average bagasse production of the entire group of genotypes.

Table 1. Average results for six commercial checks and 22 sugarcane mutants referring to agronomically important traits.

Genotypes	SL*	NB	BD	MD	AD	BB	MB	AB	SM	SBB	BM	SBY
Check_1	142,8	9,5	29,9	29,5	29,9	19,0	17,9	14,3	1035,1	15,5	1007,7	922,5
Check _2	168,0	14,8	27,7	27,5	29,0	18,6	18,5	15,5	1086,9	15,0	1098,4	917,5
Check _3	164,3	12,3	29,3	28,7	30,5	16,5	17,5	15,8	1144,5	15,8	1277,7	1075,0
Check _4	130,8	11,8	25,0	26,3	26,6	17,6	17,4	13,4	737,9	15,0	756,1	687,5
Check _5	161,0	10,0	27,9	26,6	25,7	18,6	16,8	13,8	873,7	15,0	857,6	730,0
Check _6	181,0	13,8	27,5	27,0	28,6	18,7	17,9	14,0	1149,5	14,9	1052,5	1100,0
7	142,5	10,8	29,0	26,8	28,7	18,9	17,3	14,3	1024,9	15,0	949,4	1020,0
8	166,8	10,5	30,5	28,6	29,5	17,9	16,2	10,8	1121,5	14,1	1019,2	1120,0
9	124,0	9,5	24,4	23,6	22,4	17,1	15,8	11,7	605,5	12,5	573,9	550,0
10	168,8	14,3	30,4	31,2	30,8	18,4	16,8	10,3	1391,5	14,3	1184,5	1365,0
11	180 ,3	11,3	26,7	25,0	25,7	18,4	17,3	11,9	988,8	14,8	1133,5	913,0
12	188,0	13,0	28,6	26,9	29,5	17,9	16,8	12,4	1224,6	15,5	1223,0	1065,0
13	175,3	13,0	33,4	31,6	32,5	18,4	17,9	11,6	1364,4	14,9	1357,0	1215,0
14	197,5	15,5	27,3	25,6	27,3	17,8	18,4	13,4	1125,8	16,0	1138,9	947,5
15	192,3	12,0	29,5	28,1	27,7	18,7	17,7	13,0	1271,1	14,8	1205,1	1160,0
16	171,3	12,8	33,4	31,1	32,2	17,4	16,5	11,8	1579,8	14,9	1286,5	1620,0
17	160,3	16,0	29,1	29,0	30,1	14,5	18,4	12,4	1217,4	15,2	1208,6	1145,0
18	158,3	14,8	26,9	26,5	26,3	18,9	17,7	11,4	1043,2	14,1	1069,6	955,0
19	170,8	12,0	28,3	25,8	27,1	17,0	16,1	12,2	1186,6	14,1	1018,5	1080,0
20	204,8	15,3	30,2	28,8	30,4	18,2	18,1	14,4	1533,8	16,5	1374,7	1465,0
21	179,8	12,0	29,2	28,2	30,2	17,8	16,8	13,5	1215,0	15,5	1202,3	1170,0
22	190,8	12,3	30,0	27,9	29,8	18,0	17,7	12,5	1312,8	15,0	1159,3	1220,0
23	151,8	14,5	27,1	26,2	27,4	18,3	14,4	14,1	1042,0	14,1	994,1	972,5
24	188,8	13,0	25,0	25,0	27,6	19,1	18,5	15,6	1149,6	15,2	1166,3	1060,0
25	185,8	13,3	29,3	27,1	28,8	18,5	18,3	14,5	1281,4	15,9	1157,2	1265,0
26	192,3	13,3	27,5	26,9	26,9	18,6	17,0	13,9	1107,5	15,0	1092,7	1010,0
27	185,0	11,0	27,0	23,9	24,5	18,1	17,1	14,7	1192,5	13,6	1021,4	1060,0
28	159,5	13,8	29,4	29,8	30,4	18,4	17,9	13,9	1188,1	15,5	1085,2	1109,0
Average	170,8	12,7	28,54	27,5	28,4	18,0	17,3	13,2	1149,8	14,9	1095,4	1068,6
DP	15,8	1,45	1,63	1,63	1,86	0,64	0,72	1,22	143,6	0,57	124,4	153,5

*SL: stem length (cm); NB: number of buds in the stem; BD: diameter of the base of the stem (mm); MD: diameter measured in the middle of the stem (mm); AD: diameter at the stem apex (mm); BB: Brix content of the base of the stem (% w / v); MB: Brix content measured in the middle of stem (% w / v); AB: brix content in the stem apex (% w / v); SM: Total stem mass (g); SBB: brix content of the sugarcane broth (% w / v); BM: total residue mass (g); SBY: sugarcane broth yield (L).

Regarding the broth brix degree, the genotypes 20, 14, 25, check 3, check 1, 10, 21 and 28 were higher than the general average.

The purpose of analyzing the averages of genotypes is to identify genotypes with higher averages for traits of interest and associate them to individuals with greater genetic distance because according to CARPENTIERI-PÍPOLO et al. (2000), identification based only on genetic distance, regardless of performance relative to the average, cannot be an effective strategy for improved breeding.

The dissimilarity measures estimated by using the Euclidean distance between the 22 mutants and six commercial checks are evidenced in Figure 1. Among the analyzed mutants, smaller dissimilarity values were observed between mutant 22 and 27 (0.1023) and the highest observed distance occurred between Check 1 and mutant 19 (0.5109). The average distance between the genotypes was 0.2399 ± 0.0928. These results show that mutation induction with a chemical mutagen generated genetic variability; such results are promising for breeding. It can be asserted that this variability enables the identification of genotypes with greater tolerance to cold, with high productivity and high-quality broth. Those genotypes may be suitable for the southern region.

Grouping genotypes by the Tocher optimization method provided the formation of five distinct groups. Group I consists of the largest number of genotypes represented by 22 genotypes of sugarcane which is 71% of the total 28 genotypes studied. Group II consists of three genotypes, and groups III, IV, and V of one genotype (Table 2). The Tocher method formed an initial group composed of the two more similar individuals identified in the dissimilarity matrix. In addition, we estimated the possibility new individuals by adopting a criterion where the average intra-group distance must be less than the average inter-group distance (CRUZ et al. 2011, PUIATTI et al. 2014). Thus, it is demonstrated that the formation of the first group will contain the highest number of genotypes and other groups will be formed by individuals with higher inter-group averages. The discrimination of genotypes in groups is related to their different average values, which can be seen in Table 1, especially for genotypes 20, 24, and 10 that turn out to be superior to others by at least one agronomic characteristic.

Inter-group dissimilarity obtained by the Tocher optimization method allowed us to distinguish between formed groups which turn out to be more genetically distant (Table 3). The shortest intra-group distance was observed in group I (0.25) and the greatest distance was in the group II (0.26). For the other groups, intra-group distances were not estimated because they had only one genotype.

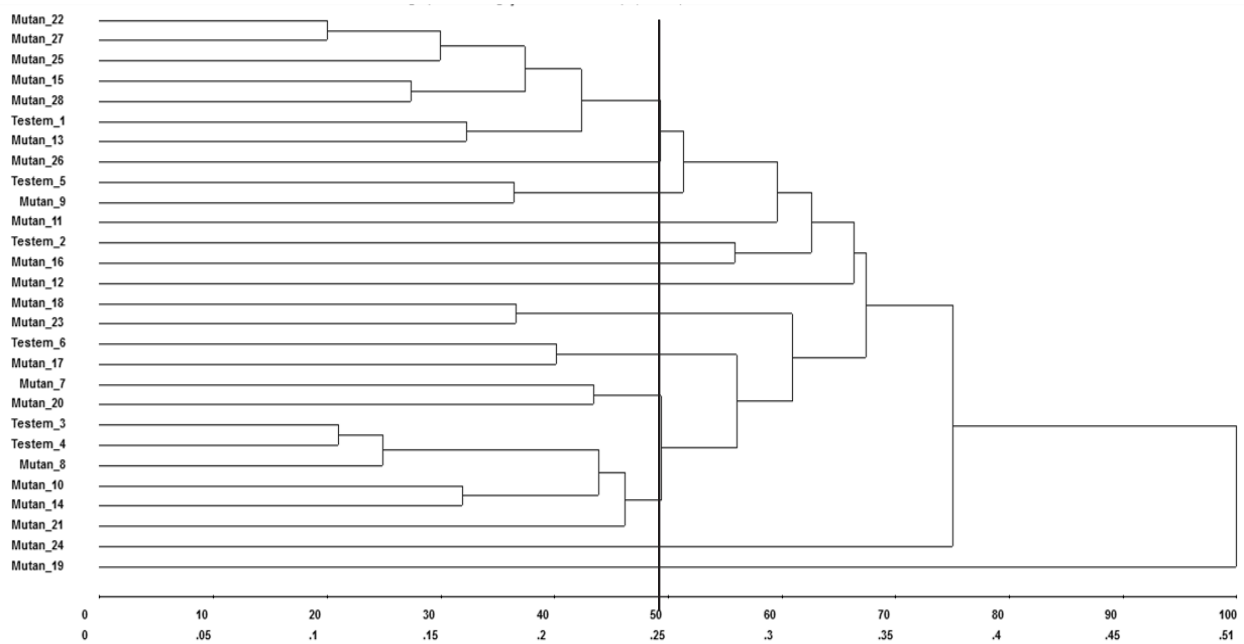

Figure 1. Representative dendrogram of genetic dissimilarity between 22 sugarcane mutants and six commercial checks, using the Euclidean distance Average Standardized obtained by the method of average connections (UPGMA).

Table 2. Results for the grouping of 22 mutants and six commercial checks by the Tocher optimization method based on the standardized mean Euclidean distance.

Groups	Sugarcane Mutants
I	15, 22, 12, 21, 26, 6, 25, 28, 2, 14, 3, 11, 19, 7, 18, 8, 5, 1, 27, 13, 4, 23
II	10, 16, 20
III	17
IV	24
V	9

Table 3. Results of average inter-groups distance estimated by the Tocher optimization method. It involves 22 mutants and six commercial checks of sugarcane, considering 12 important agronomic characteristics.

Groups	I	II	III	IV	V
I	0,25	0,34	0,36	0,39	0,50
II		0,26	0,34	0,48	0,69
III			-	0,50	0,63
IV				-	0,64
V					-

The greatest average distances between groups were observed between groups II and IV (0.69), followed by Group III and IV (0.63) and the groups IV and V (0.64). On the other hand, the shortest distances between groups were observed between groups I and II (0.34), II and III (0.34), and I and III (0.36). Thus, it can be inferred consistently that mutation inductions with the chemical agent MMS generated genetic variability and therefore the genotypes can be explored the most promising genotypes for breeding can be selected and based on these methods. In the hierarchical method, genotypes are grouped through a process that is repeated for several levels by establishing a tree or dendrogram diagram (CRUZ & CARNEIRO 2006). Hence, the UPGMA method associated with Tocher optimization method provides greater efficiency in distinguishing genotypes by their genetic distances.

An analysis of principal components (Figure 2) was conducted and based on the first three components, since the accumulated variance in the first two principal components explained only 52% of the total variation. The first three principal components explained 62% of the total variation: the first explained 35%, the second 16%, the third 11%, and the fourth 8%, components layout only allows the projection of the first three principal components.

Relying on the principle that the relative importance of the principal components decreases from the first to the last, it can be said that the last components are responsible for explaining a minimum fraction of the total variance (CRUZ & REGAZZI 1997). A dispersion of the scores related to the position of each genotype was performed in three-dimensional Cartesian coordinates in order to enable the best viewing angle for distinguishing the sugarcane genotypes (Figure 1). It can be seen that the genotypes 9, 17, 24, 10, 16, 13 and 20 presented higher dispersion of scores in the first three principal components. They can be considered the most dissimilar ones.

It can be observed that both methods partially agreed on the clustering structure, particularly for discrimination of the dissimilarity where the methods based on Euclidean distance indicated that the genotypes 9 and 20 have the highest dissimilarity and were consistent with the dispersion of scores of the major components. Along these lines, the Tocher grouping method revealed the formation of similar groups by other techniques. The first three principal components explained only 62% of the total variation; below the stated 80% (CRUZ & REGAZZI 1997), the dispersion of genotypes showed the same consistency that was demonstrated in the methods of Euclidean

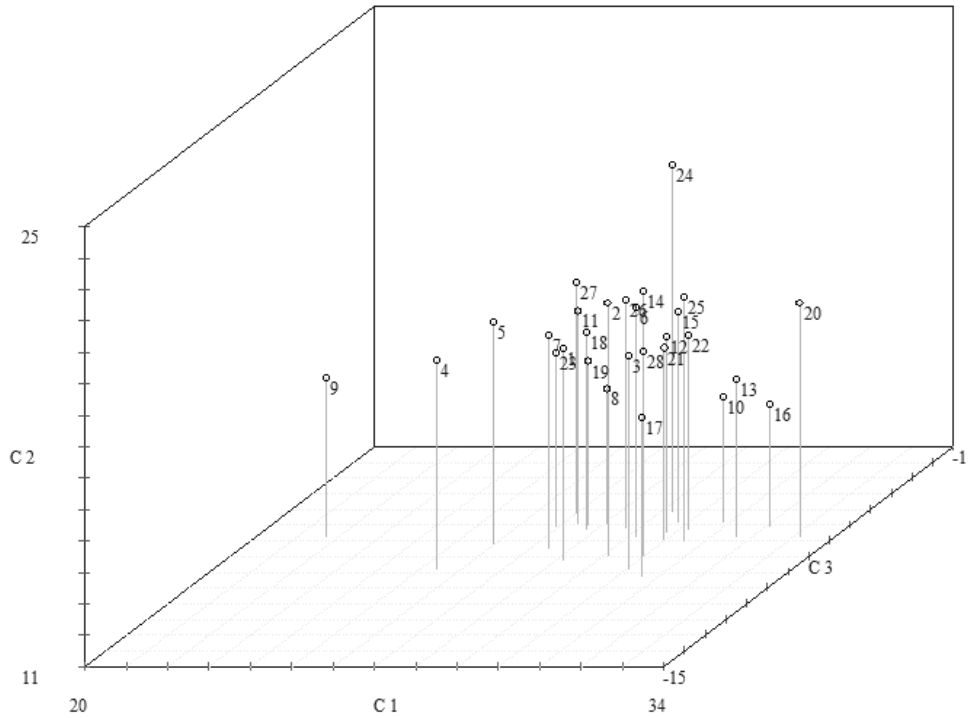

Figure 2. Results for graphic dispersion analysis of the scores in the first three principal components C1, C2, and C3 of the 22 mutant clones and six sugarcane commercial checks (soca).

Table 4. Results of the relative contribution of the 22 mutants and six commercial checks by the SINGH method (1981).

Characteristics	S.j	Valor (%)
Stem length	303213,18	0,302
Number of buds per stem	2423,25	0,002
Base diameter	9377782,40	9,34
Middle stem diameter	3198,81	0,003
Apex of the stem diameter	4024,18	0,004
Brix content in the stem base	663,60	0,001
Brix contente in the middle of the stem	677,41	0,001
Brix contente in the apex of the stem	1607,38	0,002
Stem yield	31958654,38	31,83
Brix content in the juice	496,30	0,001
Bagasse mass	22280795,35	22,19
Juice content	36451594,75	36,31

distance and Tocher. This similarity originated from different clustering methods in the discrimination of genotypes for genetic distance, and collaborates with studies by other authors from different cultures (NEITZKE et al. 2009, BÜTTOW et al. 2010, SIMON et al. 2012).

The estimate relative contribution of the 12 characteristics among 22 mutant and six commercial checks are shown in Table 4. Concerning the characteristics that contributed most to genotypes discrimination by SINGH method (1981), the broth yield (36.31%), stem yield (31.83%), and mass of bagasse (22.19%) stand out and are sufficient to account for approximately 87% of the genetic dissimilarity among the evaluated genotypes of sugarcane. According to DOTTO et al. (2010), it is

essential to know the influence of the main features for genetic discrimination of individuals because they will give direction for the improvement studies.

By contrast the characteristic of brix content of the base, middle and apex (0.001, 0.001 and 0.002%), broth brix content (0.001%), the number of buds per stem (0.002%), diameter of the center and stem apex (0.003; 0.004%) are the characteristics that have contributed least to explaining the genetic dissimilarity observed among genotypes of sugarcane. In this regard, the variables that contribute less to distinguish genotypes are also important because they enable the disposal of variables with low contribution in the discrimination of genotypes, and allow a reduction of hand labor, time, and costs spent on experiments (CRUZ & REGAZZI 1997).

The results obtained in this study reveal prospects for future research to further explore genetic variability through mutation induction processing, and thus aid in the exploration of mutants and lead to the production of superior clones associated with high average broth yield and high brix content, even in regions with lower temperature.

CONCLUSION

The mutation induction process with the MMS agent generated significant patterns of genetic variability among sugarcane mutants verified by the three multivariate analyses. UPGMA and the Tocher clustering methods and the main component analysis revealed similar results in relation to the dissimilarity of sugarcane genotypes.

Genotypes 20, 24 and 10 were greater than the overall average of the evaluated characteristics and had a high genetic dissimilarity.

REFERENCES

ALLARD RW. 1971. Princípios do melhoramento genético das plantas. São Paulo: Edgard Blücher. 381p.

BELLÉ C et al. 2014. Fitonematoides associados à cultura da cana-de-açúcar no Rio Grande do Sul, Brasil. Nematropica 44: 207-217.

BÜTTOW MV et al. 2010. Diversidade genética entre acessos de pimentas e pimentões da Embrapa Clima Temperado. Ciência Rural 40: 1264-1269.

BORÉM A & MIRANDA GV. 2005. Melhoramento de Plantas. 4.ed. Viçosa: UFV. 525p.

CARPENTIERI-PÍPOLO V et al. 2000. Seleção de genótipos parentais de acerola com base na divergência genética multivariada. Pesquisa Agropecuária Brasileira 35: 1613-1619.

CONAB - Companhia Nacional de Abastecimento. 2015. Acompanhamento da safra brasileira – Cana de açúcar. Safra 2015-2016. Terceiro levantamento. 70p.

CRUZ CD & REGAZZI AJ. 1997. Modelos biométricos aplicados ao melhoramento genético. 2.ed. Viçosa: UFV. 390p.

CRUZ CD & CARNEIRO PCS. 2003. Modelos biométricos aplicados ao melhoramento genético. 2ª ed. Viçosa: UFV. 585p.

CRUZ CD et al. 2011. Biometria aplicada ao estudo da diversidade genética. Visconde do Rio Branco: Suprema. 620p.

CRUZ CD. 2013. GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum 35: 271-276.

DEON MD et al. 2010. Produtividade e qualidade da cana-de-açúcar irrigada com efluente de estação de tratamento de esgoto. Pesquisa Agropecuária Brasileira 45: 1149-1156.

DOTTO MA et al. 2010. Divergência genética entre cultivares comerciais de milho em baixas altitudes no Tocantins, safra 2007/2008. Revista Ciência Agronômica 41: 630-637.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. 2006. Sistema Brasileiro de classificação de solos. 2.ed. Rio de Janeiro: Embrapa Solos. 306p.

FABRIS LB et al. 2013. Produtividade e desempenho de cana soca cultivada em diferentes espaçamentos e doses de adubação nitrogenada em cobertura. Revista Agrarian 6: 252-258.

FERREIRA FM et al. 2005. Effects of inbreeding on the selection of sugar cane clones. Crop Breeding and Applied Biotechnology 5: 174-182.

GOES T. 2009. Novas fronteiras tecnológicas da cana-de-açúcar no Brasil. Revista de Política Agrícola 1: 50-59.

MARQUES MO et al. 2001. Tecnologia do açúcar: produção e industrialização da cana de açúcar. Jaboticabal: FUNEP. 166p.

MATSUOKA S et al. 2005. Melhoramento da cana-de-açúcar. In: BORÉM A (Ed.). Melhoramento de espécies cultivadas. 2.ed. Viçosa: UFV. p. 205-251.

MAULE RF et al. 2001. Produtividade agrícola de cultivares de cana-de-açúcar em diferentes solos e épocas de colheita. Scientia Agricola 58: 295-301.

NARDINO M et al. 2016. Associações entre caracteres de genótipos mutantes de cana-de-açúcar. Scientia Plena 12: 1-7.

NEITZKE RS et al. 2009. Caracterização morfológica e dissimilaridade genética entre variedades crioulas de melão. Horticultura Brasileira 27: 534-538.

PALOMINO EC et al. 2005. Genetic diversity of common bean genotypes of Carioca commercial group using RADP markers. Crop Breeding and Applied Biotechnology 5: 80-85.

PUIATTI GA et al. 2014. Comparação dos métodos de

agrupamento de Tocher e UPGMA no estudo de divergência genética em acessos de alho. Revista da Estatística da Universidade Federal de Ouro Preto 3: 275-279.
SIMON GA et al. 2012. Divergência genética em milho de primeira e segunda safra. Semina: Ciências Agrárias 33: 449-458.
SINGH D. 1981. The relative importance of characters affecting genetic divergence. The Indian Journal of Genetics e Plant Breeding 41: 237-245.