Genetic diversity among sugarcane clones (*Saccharum* spp.)

Clandio Medeiros Silva*, Maria Celeste Gonçalves-Vidigal, Pedro Soares Vidigal Filho, Carlos Alberto Scapim, Edelclaiton Daros and Lucas Silvério

*Departamento de Agronomia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020- 900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: clandiomeddeiros@uol.com.br*

ABSTRACT. The objective of this study was to evaluate of the genetic similarity among 129 sugarcane clones. For that, it was evaluated the number of stalks per plot, the average mass of the stalks, the mass of 10 stalks, the medium brix and the brix production in kilograms for each plot. The data were analyzed through several Canonic Variables and Tocher method using the Mahalanobis (D^2ii') distance. The results obtained by Tocher method and graphic dispersion shown that the most dissimilar clone was TUC77519. On the other hand, the most similar clones were RB 975056 and RB 915006. The number of stalks per plot (NSP) and the brix production per kilograms per plot (BKP) were characteristics that most contributed most for the genetic variability.

Key words: *Saccharum* spp., genetic diversity, multivariate analysis.

RESUMO: Divergência genética entre clones de cana-de-açúcar (Saccharum spp.). O objetivo do presente trabalho foi avaliar o grau de similaridade genética entre 129 clones de cana-de-açúcar. Para tanto, avaliou-se o número de colmos por parcela, a massa média de colmo, massa de 10 colmos, brix médio e produção de brix em quilograma por parcela. Os dados foram analisados por meio das variáveis canônicas e análise de agrupamento pelo método de Tocher, para o qual foi adotada a distância generalizada de Mahalanobis (D^2ii'). Os resultados pelo método de Tocher e pela dispersão gráfica mostraram que o clone mais dissimilar foi o TUC77519. Por outro lado, os clones mais semelhantes foram RB 915056 e RB915006. As características número de colmos por parcela (NCP) e produção de brix em quilograma por parcela (KBP) foram as que mais contribuíram para a variabilidade genética.

Palavras-chave: *Saccharum* spp., divergência genética, análise multivariada.

Introducion

The sugarcane stands out in the Brazilian economy due to the fact that it constitutes a "removable" natural source (SEAB, 1999). From its processing it is obtained sugar, alcohol, cachaça and other products. There is also the utilization of its industrial waste for the production of fertilizes and the remained product used as a source of energy in the paper production (Lucchesi, 1995). The Paraná State is the second producer of sugarcane in the country, and it has been cultivated and processed for 22 years in this region. It is economically important, since the sugarcane crop occupies approximately 320 thousand cultivated hectares and generates about 74 thousand direct jobs, 65 thousand in the field and 9 thousand in the industry (Daros *et al.*, 1999).

Brazil leads the production and exportation of sugar and yield cultivated sugarcane. It is also the first to used sugarcane as a source of a liquid energy, the alcohol, being the only to utilize it as an alternative combustive for vehicles (Matsuoka *et al.*, 1999).

The productive chain of the sugarcane generates financial founds for the country, contributes to the reduction of the environment pollution and also constitutes an important economic source for small communities in Brazil, located far from the urban region, fact that sets out the reduction of the migration from the country side to the urban side (Matsuoka *et al.*, 1999).

Nowadays, the genetic variability present in the sugarcane cultivars, cultivated by the farmers, has hybrid origin, generally, from the sixth to the tenth generation. The *Saccharum officinarum* have been contributing for genetic variability in sugarcane more than *S. spontaneum, S. sinense* e *S. barberi* (Matsuoka *et al.*, 1999).

It is important to remember that the most disseminated cultivars in the 90s, in Paraná State, were RB 72454 and SP 70-1143, which occupied more than 50% of the cultivated area there. This fact shows the necessity to obtain new genetic combinations to support the production system, avoiding the genetic uniformity.

In the genetic breeding program of sugarcane the main goal is to obtain new cultivars with more productivity and best industrial characteristics (Bicudo, 1987).

In the last 20 years, several breeding programs developed studies with the goal to expand the genetic sugarcane basis, although none of them well succeed (Berding and Roach, 1987). Nowadays the plant breeding has been based on a common genetic base obtained by the pioneer ones from the beginning of the century, through inter crosses and retro crosses of *S. officinarum* (Matsuoka *et al.*, 1999). This fact is evident when the main genealogic trees of the most cultivated cultivars in the world (Levi, 1990), and (Matsuoka, 1989) in Brazil, were analyzed.

The cultivars of sugarcane obtained from the breeding programs in Brazil occupy today more than 90% of the commercial cultivated area, definitely contributing for the high levels of productivity in the its sector (Pires, 1993). Although, it is known that the new productivity increasing have been becoming difficult to be obtained, especially when the breeding programs are based on an exploration of the genetic variability from few ancestors (Pires, 1993).

The modern genetic breeding requires crosses between productive and genetic divergent parents, in order to have better heterotic effect and variability in the segregant generations (Cruz, 2001). Nowadays, researchers, to obtain the genetic divergence estimate of the performance 'per se' of the parents, have used the methods of the multivariate technique and molecular markers.

The indeed of a deep study in the genetic constitution and the genetic divergence between sugarcane cultivars cultivated in Brazil, in order to provide more information to facilitate the breeding programs and to overcome the productivity levels presented today, motivated this work. This work has the main objective to quantify the genetic divergence between sugarcane clones from the series RB91 using multivariate methods for the genetic divergence analysis.

Material and methods

Experiments were carried out in the counties Paranavaí (Estação Experimental de Paranavaí) and Campo Mourão (Estação Experimental da Cooperativa Agrícola Mourãoense de Campo Mourão), which are located in the Northwest of Paraná State. The soil predominant unit in the experimental area in Paranavaí is Dark Red Latosol soil, whereas in the experimental area in Campo Mourão is the Eutrophic Red Latosol soil. The experimental design was a randomized complete blocks with two replications. Each experimental unit was composed by two lanes with 5 meters of length and 1.30 meters of width.

The characteristics evaluated were: number of stalks per plot (NSP); mass of 10 stalks (M10); mean stalk mass (MSM); medium brix (BX); brix production in kilograms per plot (BKP).

The program developed by Cruz (2001) was used in the multivariable analysis. The divergence among 129 sugarcane clones was estimate through the use of Mahalanobis Generalized Distance (D^2ii') as a measure of genetic dissimilarity, combined with Tocher Method and Principal Components.

Results and discussion

The variance analysis of each characteristic had the objective to verify the existence of variability between the varieties studied. The variance data analysis referred to the average mass of 10 stalks (M10), number of stalks per plot (NSP), medium brix (BX), mean stalk mass (MSM) and brix production in kilograms per plot (BKP), indicated a significant difference of 1% of probability from the test F (Tables 1 and 2). This indicated that among the clones evaluated, considering the five characteristics, at least one of them is significantly different from the others, which corresponds 1% of probability. Therefore, it is prominence the existence of the genetic variability between the studied clones, which demonstrated a favorable situation to practice the breeding program.

The Mahalanobis (D^2ii') distance method was used to predict the genetic distance. In Paranavaí, the most dissimilar clones were Q136 and TUC77519, presenting the maximum value of D^2ii'= 109.03. From that, we concluded that the clones Q136 and TUC77519 were the most divergent. Beyond that, the clones considered the most divergent do not belong to the series RB 91, since the clones Q136 and TUC77519 are originated from Australia and Argentina, respectively.

Table 1. Analysis of variance of five agronomic traits assessed, based on plot's average, Campo Mourão, Paraná state.

SV	DF	Mean square[1/]				
		M10	NSP	BX	MSM	KBP
Block	1	334.34	24731.30	12.51	3.34	3.89
Genotypes	128	1487.09**	166150.94**	2.88**	0.12**	118.34**
Error	128	303.50	51027.70	1.44	0.02	30.75
Mean		12.10	103.32	19.11	1.23	24.21
CV (%)		12.51	19.33	6.28	12.51	22.90

[1/] M10 = mass of 10 stalks; NSP = number of stalks per plot; BX = medium brix; MSM = average stalk mass; BKP = brix production per plot in kilograms; ** = significant at 1% level.

Table 2. Analysis of variance of five agronomic traits assessed, based on plot's average, Paranavaí, Paraná state.

SV	DF	Mean square[1/]				
		M10	NSP	B$\bar{x}$	MSM	KBP
Block	1	63.31	144.38	39.30	0.63	121.31
Genotypes	128	13.40**	79196.96**	255.79**	17.16**	12374.79**
Error	128	5.34	18216.12	63.12	6.83	5609.43
Mean		17.52	92.61	21.37	1.75	34.41
CV (%)		13.18	13.15	3.29	13.18	19.24

[1/] M10 = mass of 10 stalks; NSP = number of stalks per plot; BX = medium brix; MSM = average stalk mass; BKP = brix production per plot in kilograms; ** = significant at 1% level.

On the other hand, it was verified that the most similar clones were RB915056 and RB9155006, due to the fact that those ones belong to the series RB91 and they were selected in the same place.

In case of the data obtained in Campo Mourão, it was verified that the most dissimilar clones were RB915055 and TUC77519, presenting a maximum value of D^2ii' = 70.46. Again, it is observed the presence of exotic materials among the most divergent ones. In this case it is recommended the recombination of the divergent material, since it will be expected a bigger heterotic effect among the genetic contrasting populations (Falconer, 1981). However, it is necessary to observe each genotype, relating it to its performance before the utilization of breeding programs (Amaral Júnior, 1996).

The Tocher method was used to cluster the clone groups through the dissimilar Mahalanobis D^2 distance. The largest cluster was 1 with 83.72%. The others clusters, 2,3,4,5,6,7 had the following percentage of 6.97%, 2.32%, 2.32%, 3.10%, 0.77% and 0.77%, according to Table 3, respectively.

On the other hand, the results obtained from the experiment carried out in Campo Mourão, demonstrated larger genetic diversity than those from Paranavaí, clustering the clones in 19 groups through Tocher method according to Table 4. The different clusters, composed in the two localities, are unalike due to the fact that the evaluated characteristics were quantitative traits, which are influenced by the environment.

Table 3. Cluster Analysis (Tocher Method), based on the Mahalanobis Distance (D^2ii'), using five characteristics from 129 clones of sugar cane. Paranavaí, state of Paraná, 2001.

Group	Clones
1	11, 113, 28, 34, 17, 16, 9, 58, 13, 22, 61, 110, 31, 52, 106, 75, 126, 122, 100, 93, 84, 53, 72, 6, 99, 89, 77, 32, 127, 25, 24, 105, 108, 114, 67, 117, 30, 55, 123, 88, 121, 26, 41, 111, 129, 97, 20, 102, 128, 3, 12, 19, 91, 2, 98, 40, 5, 21, 90, 63, 46, 115, 71, 8, 86, 107, 10, 38, 29, 37, 78, 39, 42, 118, 87, 74, 49, 23, 109, 69, 4, 27, 104, 81, 34, 94, 114, 1, 45, 82, 44, 96, 59, 101, 76, 33, 85, 47, 118, 125, 73, 92, 66, 43, 68, 80, 79, 51
2	103, 116, 56, 120, 62, 60, 65, 36, 7
3	50, 64, 48
4	83, 95, 112
5	54, 57, 70, 15
6	119
7	124

Table 4. Cluster Analysis (Tocher Method), based on the Mahalanobis Distance (D^2ii'), using five characteristics from 129 clones of sugarcane. Campo Mourão, Paraná state, 2001.

Group	Clones
1	37, 91, 47, 72, 44, 105, 11, 8, 49, 65, 93, 85, 19, 16, 92, 108, 63, 122, 118, 1120, 89, 114, 20, 71, 87, 117, 109, 24, 30, 67, 78, 129, 76, 102, 106, 4, 5, 90, 69, 75, 39, 107, 112, 35, 15, 55, 77, 74, 31, 12, 58, 98, 59, 18, 64, 1, 82
2	13, 40, 70, 127, 2, 79, 100, 94, 101, 116, 110, 41, 22
3	25, 97, 99, 23, 121, 61, 73, 45, 33
4	83, 86, 119, 48, 88, 53, 32, 80, 36
5	52, 60, 96, 27, 126
6	66, 81, 7, 125, 3
7	50, 57, 14, 84
8	28, 111, 21, 54, 115, 29, 17, 34, 46, 95, 113
9	6, 104
10	10, 38, 43
11	26, 124, 56
12	103
13	123
14	68
15	42
16	51
17	62
18	128
19	9

Table 5. Auto values (λi) corresponding to the percentage variation, explained by the Canonic Variables (VC_i), and the coefficients (auto vectors) of five characteristics evaluated in 129 sugarcane clones, Paranavaí, Paraná state.

VCi	Accumulated auto values (%)	Coefficients Associated A[1]

		M10	NSP	BX	MSM	KBP
VC_1	91.4969	-0.028	0.9592	-0.0072	-0.0028	0.2812
VC_2	99.5118	0.4443	-0.2394	0.0200	0.0444	0.8619
VC_3	99.9676	-0.6032	-0.0917	0.7420	-0.0603	0.2714
VC_4	99.9999	0.6543	0.1168	0.6701	0.0653	-0.3232
VC_5	100.000	-0.0994	0.000	0.0001	0.9950	0.000

[1] M10 = mass of 10 stalks; NSP = number of stalks per plot; BX = medium brix; MSM = average mass of 1 stalk and KBP = brix production per plot in kilograms.

Table 6. Auto values (λi) corresponding to the percentage variation, explained by the Canonic Variables (VC_i), and the coefficients (auto vectors) of five characteristics evaluated in 129 sugarcane clones, Campo Mourão, Paraná state.

VCi	Accumulated auto values (%)	Coefficients Associated A[1]				
		M10	NSP	BX	MSM	KBP
VC_1	95.9404	0.0153	0.9714	-0.0013	0.0015	0.2370
VC_2	99.7182	0.4338	-0.2193	0.0582	0.0434	0.8709
VC_3	99.9602	-0.4816	-0.0343	0.8564	-0.0482	0.1764
VC_4	100.000	0.7548	0.0845	0.5130	0.0755	-0.3927
VC_5	100.000	-0.0995	0.000	0.000	0.000	0.000

[1] M10 = mass of 10 stalks; NSP = number of stalks per plot; BX = medium brix; MSM = average mass of 1 stalk and KBP = brix production per plot in kilograms

Tables 5 and 6 show the estimates of the auto values (λi), corresponding to the Canonic Variables (Vc_i) and its coefficients associated with the original variables, respectively in Campo Mourão and Paranavaí. However, in Table 5 the first two Canonic Variables explain about 99.51% (91.49% for the first canonic variable and 8.01% for the second one) of the total variation from the data of the experiment carried out in Paranavaí. Although, Table 6 present that the first two Canonic Variables explained 99.71% (95.94% for the first canonic variable and 3.77% for the second one) of the total variation in the experiment carried out in Campo Mourão.

The p-dimensional graphic dispersion of the cultivars for the bi-dimensional would contain a minimum distortion scale, in order to explain the total variation through the first two Canonic Variables. Therefore, the two variables must present a total variation of 80% (Cruz and Carneiro, 2003).

According to Figures 1 and 2, the Canonic Variables were enough to explain about 99.51% and 99.71% of the total variation in the two experiments in Paranavaí and Campo Mourão, respectively. Despite that, the genetic divergence transposition of the p-dimensional space (in this case p = 5) for the bi-dimensional, with a minimum scale distortion caused by the clones distance is verified. Further, it is also observed that the genotype 124 (TUC 77519) was the most distant among the others. This result is also similar to the one obtained by the Tocher method.

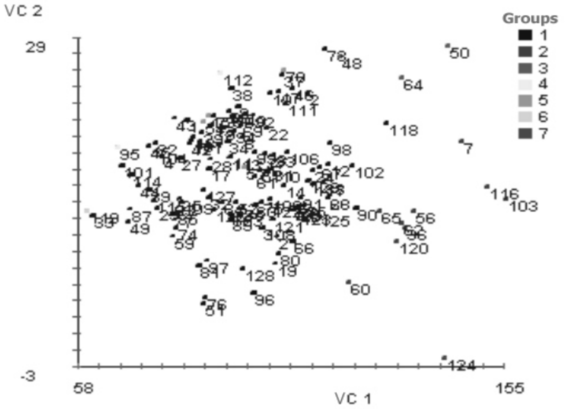

Figure 1. Graphic dispersion of 129 sugarcane clones in two dimensional space, based on the main components, Paranavaí, state of Paraná.

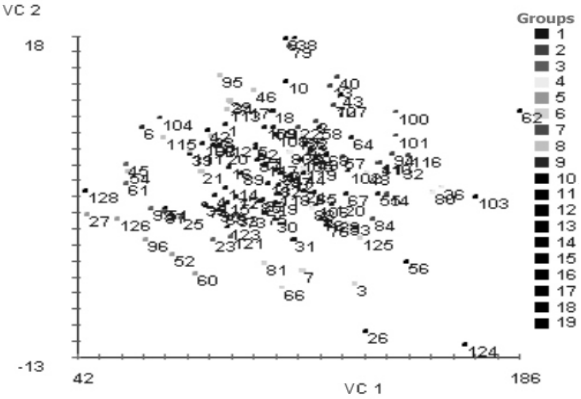

Figure 2. Graphic dispersion of 129 sugarcane clones in two dimensional space, based on the main components, Campo Mourão, state of Paraná.

Comparing the Tocher method and the graphic dispersion, it was possible distinguish four groups similar to the ones obtained by the first method. There were: Group 2, containing the clones 103 (RB915073), 116 (RB778573), 56 (N18), 120 (N19), 62 (RB877603), 60 (RB731136), 65 (LCP8110), 36

(RB915079), 7 (RB915118); Group 3, with the clones 50 (RB915086), 64 (RB865360), 48 (RB915095); Group 4, the 119 (Q136) and Group 7, the 124 (TUC77519). The others groups 1, 4 and 5 due to the large number of clones were not possible to identify with graphic dispersion.

The genetic divergence revealed by the clones when analyzed by the data characteristics in Campo Mourão, the results were similar in graphic dispersion and Tocher methods clustering the clones in 19 groups. Among them the following groups were pointed out: Group 5, which includes the clones 52 (RB915061), 60 (RB731136, 96 (RB915071), 27 (RB915036), 126 (RB8470); Group 9, 6 (RB915042), 104 (RB915040); Group 11, 26 (R915123), 124 (RB915120), 56 (N18); Group 12, 103 (RB915073); Group 17 62 (RB877603) and Group 18, 128 (RB742254). The others groups 1, 2, 3, 4, 6, 7, 8, 9, 13, 14, 15, 16 and 19 due to the elevated number of evaluated clones, were not possible to identify with the graphic dispersion analysis. Hence, the genetic divergence study for those clones is variable when graphic dispersion between the first two Canonic Variables is utilized. The Tocher method showed partial similarity when compared to the graphic dispersion. The Canonic Variables from the two experiments carried out in Paranavaí and Campo Mourão explained 99.51% and 99.71% of the total variation, respectively. The concordance of the two experiments demonstrates the importance of graphic dispersion method in the identification of divergent and also promising clones for future breeding programs to obtain superior hybrids.

Conclusion

1. There is a genetic similarity among the RB91 series clones, indicating a genetic restrict base.
2. The genetic divergence through the Tocher methods of and Canonic Variables characteristics revealed a concordance in the results, which included a large number of clones on the same group.
3. The Canonic Variables explained 99.51% and 99.71% of the total variation, respectively, in the experiments carried out in Paranavaí and Campo Mourão. The number of stalks per plot (NSP) and the brix production in kilograms per plot (BKP) characteristics contributed the most for the genetic variability.
4. The results obtained by Tocher method and graphic dispersion shown that the most dissimilar clone was TUC 77519. On the other hand, the most similar clones were RB 975056 and RB 915006. The clone TUC 77519 should be indicated for interpopulational breeding programs for brix production in kilograms per plot.

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