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Genetic divergence and parent selection of sugarcane clones

Valéria Rosa Lopes[1], João Carlos Bespalhok Filho[1*], Ricardo Augusto de Oliveira[1], Edson Perez Guerra[2], José Luís Camargo Zambon[1], and Edelclaiton Daros[1]

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ABSTRACT - *The objective of this study was to estimate the genetic divergence of 140 sugarcane clones of the series RB97, in phase T3 of the Sugarcane Genetic Improvement Program of the Universidade Federal do Paraná, at three locations by multivariate analysis, using the linear mixed model and grouping analysis by the Tocher procedure, based on Mahalanobis' generalized distance. The evaluated traits were number of stalks per plot, mass of ten stalks, Brix and Brix per plot in kg. The number of groups varied according to the evaluated environment. Based on the results, combinations of one of the most divergent clones RB975008, RB975112, RB975019 RB975153 and RB975067 with any one of the most productive clones RB975269, RB977533, RB975102, RB975317 and RB975038 are recommended.*

Key words: Mixed models, plant breeding, *Saccharum* spp., genetic distance

INTRODUCTION

The cross is one of the most essential steps in plant breeding since it is the source of all genetic variability available for the selection of new plants (Barbosa 2001, Bonato et al. 2006). The correct planning of the crosses raises the probability of developing superior cultivars because it maximizes the use of favorable genes, besides reducing the costs of breeding programs (Cruz et al. 2004a).

Sugarcane is a species with assexual reproduction. The objective of hybridization in sugarcane is therefore to create genetic variability for the selection of superior and productive plants that are propagated to become the future cultivars. In this case, the genetic potential is fixed from the clone selection onwards and is not altered through the generations (Calija et al. 2001).

Depending on the objectives of a breeding program, different methods can be used to select the parents (Cruz et al. 2004b). In general, breeders try to

1 Departamento de Fitotecnia e Fitossanitarismo, Universidade Federal do Paraná, R. dos Funcionários, 1540, 80035-050, Curitiba, PR, Brazil. *E-mail: bespa@ufpr.br
2 Centro de Ciências Agrárias e Ambientais, Pontifícia Universidade Católica do Paraná, BR 376, Km 14, C.P. 129, 83010-500, São José dos Pinhais, PR, Brazil

use crosses between divergent and high-yielding plants (Carpentieri-Pípolo et al. 2003, Cruz et al. 2004b). Knowledge on genetic divergence is therefore fundamental to identify and organize the available genetic resources aiming at the production of promising cultivars (Palomino et al. 2005).

The genetic divergence can be determined by multivariate analysis, a procedure that is widely used in different crops for parent selection (Cruz et al. 2004b).

The objective of this study was to evaluate the genetic divergence of sugarcane clones through the multivariate analysis procedure and grouping by Tocher´s optimization method for parent selection.

MATERIAL AND METHODS

For the genetic divergence analyses, data of experiments of clone competition of the RB97 series were used, in the third phase of selection (phase T3), of the Sugarcane Genetic Improvement Program of the Universidade Federal de Paraná (PMGCA/UFPR); see Matsuoka et al. (2005) for further information on different stages of sugarcane improvement. The experiments were performed at three sites in the state of Paraná: Paranavaí (23°05' S, 52°27' W, 503 m), Colorado (22°50' S, 51°54' W, 400 m) and Iguatemi (23°21' S, 52°05' W, 580 m). Besides two commercial standard cultivars (RB72454 and RB835486), 138 sugarcane clones were evaluated.

At each location, an experiment was installed in an augmented block or Federer block design (Federer, 1956) with two replications (Field "A" and "B"). Each block contained 18 promising clones of the RB97 series plus two commercial standard cultivars: RB72454 and RB835486. The experimental plot consisted of two 5-m-long rows spaced 1.4 m apart in Paranavaí and 1.1 m apart in Colorado and Iguatemi.

The trials were planted in May 2003. Six stalk segments meter^{-1} with three buds each were used for planting. Fertilization consisted of 500 kg ha^{-1} of the formula 04:25:25 (N:P:K) with 20 kg ha^{-1} N, 100 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_20; and 600 kg ha^{-1} of the formula 20:00:20 (N:P:K) applied to the first ratoon, with 120 kg ha^{-1} N and 120 kg ha^{-1} K_20.

The first harvest (cane) was cut in May 2004 (data not shown) and the second (ratoon) in June 2005.

The evaluated traits were: number of stalks per meter (NSP), mass of 10 stalks (M10), Brix and Brix weight per plot in kilograms (BWP).

The variance components were estimated and the genetic values predicted using the REML/BLUP (Restricted Maximum Likelihood/ Best Linear Unbiased Prediction) procedure. For Deviance analysis (ANODEV) the Model 74 of Selegen - REML/BLUP software was used (Resende 2006).

The multivariate analysis followed the sequence: estimation of dissimilarity values by Mahalanobis´ generalized distance (D^2) and plant clustering in similarity groups by Tocher´s optimization procedure (Cruz et al. 2004a), using Model 104 of the SELEGEN - REML/BLUP software (Resende 2006). In this Model, Mahalanobis´ generalized distance was used to identify the most similar plant pairs. These constituted the initial group, into which new plants were included, following the rule that the mean intra group distance should be smaller than the inter group distance. Using this approach, the plants were grouped until the last one was included or formed a new group (Cruz 2001).

The Pearson correlation between the three environments (localities) for the clone dissimilarity matrix (matrix 140 x 140) was estimated using Microsoft Excel 2003.

RESULTS AND DISCUSSION

The Deviance analysis (Table 1) revealed that there are significant differences between the genotypes (clones of the RB97 series) for almost all evaluated traits in all environments, at 1% significance, except for BWP in Paranavaí (10% significance) and Colorado (non-significant). The Deviance analysis is similar to the Variance analysis (ANOVA), but allows the analysis of unbalanced data with higher precision of the variance components and accuracy in the estimation of genetic values than by ANOVA (Resende 2006).

These results indicate a favorable condition for breeding of these traits, since superior genotypes can be selected efficiently due to the existence of genetic variability (Ferreira et al. 2005). In this case, it is therefore possible to achieve considerable gains with these plants in plant breeding programs using adequate methods. It further indicates that, due to the existence of genetic variability among the T3 stage clones, it is possible to evaluate the genetic divergence by

Table 1. Results of Deviance analysis (ANODEV) for the genotypic effect of 138 clones of RB97 series plus the standard cultivars RB835486 and RB72454 for four traits evaluated in three environments in the state of Paraná

Location	Trait	Deviance	$\chi^{2\,(1)}$	p-value
Colorado	M10	754.47	21.56 **	<0.01
	NSP	1948.47	11.49 **	<0.01
	Brix	439.99	10.23 **	<0.01
	BWP	1272.3	0.72 ns	0.3961
Paranavaí	M10	766.55	18.91 **	<0.01
	NSP	2165.2	19.58 **	<0.01
	Brix	425.9	59.93 **	<0.01
	BWP	1488.33	3.55 *	0.0595
Iguatemi	M10	768.18	21.99 **	<0.01
	NSP	2296.45	61.06 **	<0.01
	Brix	389.43	72.99 **	<0.01
	BWP	1501.95	16.16 **	<0.01

(1) χ^2 - Chi-square tabled value: 2.71 and 6.63 at significance levels of 10% (*) and 1% (**) probability, respectively, and (ns) non-significant.

multivariate analysis.

By Tocher grouping, the number of similarity groups varied according to the tested environment. Four similarity groups were formed in Colorado (Table 2), eight in Paranavai (Table 3) and six in Iguatemi (Table 4). In general, the clones within each group varied in different environments (localities), in other words, there was not a standard response for group formation in the different environments. The Pearson correlation between the three environments for the clone dissimilarity matrix varied between -0.02277 and -0.06317 (statistically insignificant). These results demonstrate the poor repeatability of the distance between the clones in different environments due to genotype-environment interaction.

In a study with sugarcane Silva et al. (2005) also found variation in the number of similarity groups and genotypes within each group, according to the environment where the genetic divergence was evaluated. The interpretation of the divergence analysis among genotypes is difficult due to the non-repeatability of the divergence values. The number and composition of the groups can vary according to the evaluated traits, genotype environment interaction and

Table 2. Similarity groups formed by Tocher's optimization procedure, based on four evaluated traits of 138 sugarcane clones and two standard cultivars, by Mahalanobis' generalized distance (D^2), in Colorado, PR

Group	RB Clones
1	977534, 976320, 976324, 975015, 976331, 975038, 976319, 975086, 976328, 976300, 976311, 975177, 975019, 975088, 975057, 976315, 976336, 976338, 976310, 975005, 975007, 975092, 975087, 975299, 975151, 975183, 975205, 975153, 975185, 977619, 975021, 975338, 975350, 975249, 975207, 975157, 975188, 975211, 975175, 975204, 975253, 975172, 975279, 975174, 975228, 975179, 975294, 975163, 975345, 975173, 975241, 975286, 975219, 975323, 975285, 975115, 975244, 975100, 975243, 975164, 975081, 975102, 975221, 975169, 975238, 975080, 975111, 975068, 975166, 975046, 975053, 975282, 975112, 975051, 976339, 975224, 975114, 975165, 975269, 975235, 975290, 975013, 975202, 975029, 975184, 975329, 975170, 975293, 975298, 975353, 975101, 975206, 975317, 975073, 975289, 975256, 975061, 975339, 975200, 975103, 975270, 975337, 975212, 975022, 975070, 975033, 975024, 975032, 975089, 975094, 975026, 977533, 975000, 977543, 975004, 975083, 975031, 975010, 975006, 975045, 975002, 975027, 977529, 975049, 975008, 976341, 975047, 975069, 975048, 975108, 975055, 975067, 975078, 975090, 976317
2	976306, 975079
3	835486, 72454
4	975217

Table 3. Similarity groups formed by Tocher's optimization procedure, based on four evaluated traits of 138 sugarcane clones and two standard cultivars, by Mahalanobis' generalized distance (D^2), in Paranavaí , PR

Group	RB Clones
1	72454, 975211, 975205, 975179, 975298, 975177, 975244, 975290, 975169, 975183, 975185, 975202, 975293, 975329, 975249, 975157, 975188, 975207, 975338, 975350, 975172, 975153, 975204, 975235, 975228, 975166, 975170, 975151, 975174, 975224, 976339, 976341, 975061, 975101, 975269, 975049, 975007, 977543, 975083, 975008, 975078, 975006, 975002, 975032, 975000, 975027, 975238, 975045, 975339, 975015, 977533, 977529, 975092, 975021, 975026, 975081, 975219, 975173, 975285, 975243, 975212, 975053, 975048, 975031, 975067, 975057, 975005, 975069, 975115, 975323, 975108, 975111, 975112, 975184, 975241, 975055, 975068, 975079, 975033, 975022, 975029, 975013, 975004, 975010, 975221, 975282, 975270, 975317, 975353, 975337, 975299, 975289, 976336, 976310, 975047, 975051, 975046, 975103, 975100, 975114, 975024, 975073, 975217, 975286, 975345, 975256, 975253, 975294, 975164, 976324, 976331, 976338, 976315, 976317, 976319, 976300, 976328, 976306, 975038, 975175, 975206, 975087, 975090, 977534, 976320, 975089, 975086, 975094, 975019, 976311, 975200, 975080, 975279
2	975163, 975088
3	977619
4	975070
5	835486
6	975102
7	975165

Table 4. Similarity groups formed by Tocher's optimization procedure, based on four evaluated traits of 138 sugarcane clones and two standard cultivars, by Mahalanobis' generalized distance (D^2), in Iguatemi, PR

Group	RB Clones
1	977534, 835486, 975293, 975207, 975323, 975027, 975024, 977543, 975055, 975029, 975206, 975238, 975015, 975217, 975200, 975163, 975179, 975157, 975282, 975270, 975205, 975279, 975172, 975286, 975183, 975289, 977529, 975338, 975249, 976315, 975115, 975244 975329, 975083, 975337, 976324, 975211, 975170, 975185, 975173, 975350, 975053, 975353, 975184, 976338, 976328, 976300, 975317, 975204, 976336, 975219, 976320, 975051, 976311, 975038, 975000, 975078, 975111, 975169, 975094, 975290, 975151, 975032, 976317, 975067, 975221, 975235, 975080, 975007, 975002, 975228, 975010, 975243, 975033, 975013, 975005, 975061, 975108, 975070, 975212, 975045, 976331, 976319, 975102, 975004, 975073, 976306, 975202, 976310, 975049, 975224, 975177, 975047, 975256, 975079, 975006, 975086, 975298, 976341, 975021, 975165, 975175, 977619, 975294, 977533, 975164, 975112, 975269, 976339, 975100, 975241, 975088, 975092, 975022, 975048, 975339, 975103, 975046, 975174, 975057, 975068, 975253, 975081, 975345, 975188, 975101, 975069, 975166, 975026
2	975089, 975008 975087
3	975299, 72454 975114
4	975153, 975031
5	975285, 975019
6	975090

also the growing season (Silva et al. 2001).

When the genotype-environment interaction is complex, the response of the clones to the diverse environments differs. The use of Selegen-REML/BLUP minimizes this effect, since the clone comparison is based on the genotypic values. One way to improve the discrimination of divergent groups is the use of data from various environments and more than one growing season, because the inheritance of the majority of the evaluated traits is quantitative, in other words, the environmental influence is strong. The presence and importance of genotype-environment interaction in sugarcane breeding has been mentioned by several authors (Jackson et al. 1995, Jackson and Mcrae 1998, Calija et al. 2001).

Another possibility to improve discrimination of divergent groups is to use more traits with lower response to environmental conditions (high heritability), making results more precise, provided that

these traits are relevant for plant breeding.

On average 92 to 96% of the plants remained in group 1, while the other groups comprised only 1 or 2% of the clones, in all environments. This result is common in this kind of analysis, where the first groups contain most plants and the last groups a lower number. The high percentage of plants in only one group indicates the low divergence found. These results are similar to the ones reported by Silva et al. (2005) who found a low number of groups studying genetic divergence of sugarcane clones of RB91 series in two environments.

The selection in sugarcane improvement programs is directed to traits of agronomic interest and, in advanced stages, a great number of genotypes has been discarded. So, clones of the T3 stage are phenotypically much more similar genotypes, due to previous selection in early stages that alter the genotypic mean in the desirable direction, which can partly explain the low number of groups formed.

Based on this information, the possibility to identify divergent plants would be higher through the study of divergence in initial stages of the breeding program prior to selection, although without replication data.

In relation to the distance between clones, the most similar clones in Colorado were RB975279 and RB975204 (originated from different crosses), with a distance of 0.0482. At this site, the standard cultivar RB72454 and the clone RB976306 were the most divergent, with a distance of 86.25, according to the Mahalanobis´ generalized distance (matrix 140 x 140, not shown). In Paranavaí, the closest clones were RB975174 and RB975100 (0.0654); and RB975114 in relation to clone RB976311 (0.0654), while the most divergent were RB976310 and RB975165 (79.8778); and RB975202 with RB975088 (79.8778). In Iguatemi, the most similar clones were RB975317 and RB975207 (0.0461), RB977529 and RB975006 (0.0553) and the most divergent RB975153 and RB975114 (79.7935) and RB975153 in relation to clone RB975299 (67.9110).

For comparison, a genetic divergence analysis using all environments together was performed (Table 5). By the joint analysis, nine similarity groups were formed, and the plants (clones) in each group differed from the results obtained in each environment. The highest percentage of plants in a same group was 76% (group 1), while the others groups contained from 1 to 7% each. The group formation indicated a higher genetic divergence by the joint analysis, which can be associated to the genetic complexity of sugarcane. Sugarcane is a highly heterozygous plant, and due to the genetic complexity can have alleles that are only expressed in certain environmental conditions (Ferreira et al. 2005). Considering a greater amount of data (environments) in the joint analysis, a better discrimination of the genetic divergence in the tested

Table 5. Similarity groups formed by Tocher´s optimization procedure, based on four evaluated traits of 138 sugarcane clones and two standard cultivars, by Mahalanobis´ generalized distance (D^2), in three environments (Colorado, Paranavaí and Iguatemi, PR) - joint analysis

Group	Clones RB
1	835486, 72454, 977534, 976320, 976324, 975015, 976331, 976319, 975086, 976328, 976300, 976311, 975177, 976306, 975057, 976315, 976336, 976338, 976310, 975007, 975092, 975151, 975183, 975205, 975185, 975021, 975338, 975350, 975207, 975157, 975188, 975175, 975204, 975253, 975172, 975279, 975174, 975228, 975179, 975294, 975163, 975173, 975241, 975286, 975323, 975285, 975115, 975100, 975243, 975164, 975081, 975221, 975169, 975238, 975080, 975111, 975068, 975166, 975046, 975053, 975282, 975051, 975224, 975269, 975235, 975290, 975013, 975202, 975029, 975184, 975329, 975170, 975293, 975298, 975101, 975206, 975073, 975289, 975256, 975061, 975339, 975200, 975103, 975270, 975337, 975212, 975022, 975033, 975024, 975089, 975094, 975026, 977543, 975004, 975010, 975006, 975045, 975002, 975027, 977529, 975049, 976341, 975047, 975069, 975048, 975055, 976317
2	975299, 977619, 975211, 975345, 975219, 975079, 975353, 975000, 975108, 975078
3	975249, 975165, 975217, 975031, 975090
4	975088, 975005, 975087, 975070, 975032
5	975038, 975244, 975102, 976339, 975317, 977533, 975083
6	975114, 975067
7	975019, 975153
8	975112
9	975008

clones was therefore observed.

Although the results evidenced the existence of genetic variability in the sugarcane clones tested, this variability should be further increased by divergent crosses to raise the probability of finding superior clones. Crosses of divergent genotypes raise the heterotic effect (Silva et al. 2005) and avoid future problems with inbreeding depression (Ferreira et al. 2005), which improves the chances to select superior clones in the segregating populations derived from these divergent crosses.

The results obtained by Tocher´s grouping in the joint data analysis and in the performance of the clones of the tested series, suggest that crosses between the most divergent with the highest-yielding clones could be indicated in order to improve variability. The most promising combinations are: RB975008, RB975112, RB975019 RB975153 and RB975067 (the most divergent clones) with any of the clones RB975269, RB977533, RB975102, RB975317 and RB975038 (the most productive clones).

The results found here for sugarcane clones of the RB97 series indicate that more important traits should be aggregated to broaden the genetic divergence underlying the parent selection for genetic improvement programs. Moreover, the use of more precise techniques such as molecular markers is recommended, whose results are independent of altering environmental and temporary conditions (Borém and Caixeta 2006). The efficiency of molecular markers has been pointed out in numerous reports using RFLP (Jannoo et al. 2004 and Jordan et al. 2004); PCR (Alix et al. 1998), QTL (Ming et al. 2001); and AFLP markers (Hoarau et al. 2001).

Lastly, the use of more techniques associated to multivariate analysis is recommended, as for example an evaluation of combining ability of the parents indicated here, to corroborate their superiority.

CONCLUSION

Based on the results obtained by the analysis of genetic divergence, it can be concluded that there is genetic divergence among the evaluated clones, however the low number of groups formed indicates the restricted genetic base of this series. The variation in the group composition according to the environment were the divergence was evaluated was wide, demonstrating the great environmental influence in this kind of evaluation, regardless of the techniques used to minimize this effect. When a higher number of data (environments) was considered in the joint analysis, the genetic divergence among the studied clones was discriminated more clearly. It is recommended to use other techniques associated to multivariate analysis for more accurate results in the parent selection.

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Divergência genética e seleção de parentais em clones de cana-de-açúcar

RESUMO - *Este trabalho teve como objetivo estimar a divergência genética entre 140 clones de cana-de-açúcar da série RB97, na fase T3 do Programa de Melhoramento Genético de Cana-de-Açúcar da UFPR, em três diferentes localidades por meio de análise multivariada, usando a metodologia de modelos lineares mistos e análise de agrupamento pelo método de Tocher, com base na distância generalizada de Mahalanobis. Os caracteres avaliados foram número de colmos por parcela, massa de dez colmos, Brix e quilograma de Brix por parcela. O número de grupos formados variou de acordo com o ambiente estudado. Com base nos resultados recomendam-se os cruzamentos entre os clones mais divergentes RB975008, RB975112, RB975019 RB975153 e RB975067 com qualquer um dos clones RB975269, RB977533, RB975102, RB975317 e RB975038, por serem os mais produtivos.*

Palavras chave: modelos lineares mistos, melhoramento de plantas, distância genética, Saccharum spp.

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