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Relationship between isozyme diversity of parental clones and progeny performance in sugarcane (*Saccharum* spp.)

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Abstract

Isozyme marker based estimation of genetic diversity among 49 parental clones used in sugarcane varietal development was carried out using peroxidase, esterase and phosphorylase systems. Based on similarity index values, all 1176 possible parental combinations were categorized into high, medium and low diversity groups. Maximum number of combinations showed medium diversity (SI = 0.60 to 0.80) reflecting the existence of moderate genetic diversity in sugarcane parental clones. Selection percent per cross, based on H.R. Brix, cane diameter and single cane weight, calculated in 14 crosses belonging to all the three groups in the Ist clonal trial, was correlated with similarity index. A significant negative correlation (–0.6221) was observed between them. Selection percent was lower in the combinations with high SI values (above 0.80) compared to the other two groups indicating the importance of diversity information in indirectly identifying less productive combinations. Moderate levels of diversity were sufficient to generate a higher proportion of selectable types, as selection % did not improve with increasing diversity. Thus isozyme analysis of parental clones provided useful information in identifying and thereby avoiding genetically more similar combinations, which would otherwise lead to close breeding. Use of more efficient marker systems is needed to identify heterotic cross combinations and to predict progeny performance in sugarcane.

Key words: Sugarcane, isozyme diversity, parental clones, cross combinations, selection per cent, similarity index

Introduction

Any method that could predict progeny performance with some degree of accuracy prior to field evaluation is of interest to sugarcane (*Saccharum* spp.) breeders in view of the long span of 12-15 years needed for varietal development. In this regard, molecular markers offer a potential method for estimating genetic divergence in the breeding material. Use of molecular markers for estimating genetic distance among breeding stocks for predicting heterosis was investigated in several crops [1, 2]. Isozymes are the oldest among the molecular/ biochemical markers. Their use in sugarcane was proposed to rationalize the exploitation of genetic diversity in the breeding of modern varieties [3]. This study is an extension of an earlier study [4] wherein peroxidase diversity among the commercial hybrids was suggested to be useful in identifying diverse cross combinations for deriving heterotic hybrids. In this study, the relationship between the isozyme diversity of parental combinations and the performance of their progenies in terms of selectable types was estimated in order to assess the utility of isozyme based diversity estimation in sugarcane varietal improvement.

Materials and methods

Forty nine parental clones commonly used in hybridization program by the breeders of the country to develop economically important sugarcane cultivars were analyzed for isozyme diversity based on three isozyme systems *viz.*, peroxidase, esterase and phosphorylase. Leaf extracts from young leaf tissues were taken under cold conditions in 0.1 N sodium phosphate buffer and subjected to polyacrylamide gel electrophoresis on 7.5 per cent non-denaturing gel [5]. For phosphorylase analysis, the gel was supplemented with 2 per cent soluble starch. The gels were stained for three isozyme systems using standard protocols [6-8] and isozymes were visualized as bands. Relative mobility of each isozyme, calculated as the fraction of the distance of the band from origin to the total distance run, was used to estimate similarity index (SI) values [9]. Dendrograms depicting the genetic diversity were drawn using UPGMA programme [10].

Parental combinations were classified into three categories based on SI as diverse combinations (SI < 0.60), combinations with medium diversity (SI = 0.60 to 0.80) and closely related combinations (SI > 0.80). Hybridization was effected in the three categories and progenies raised and transplanted to ground nursery. All normal seedlings were sett-planted in 1 clonal trial with a plot size of 2m × 0.9 m. Progenies were evaluated for three economic traits *viz.*, H. R. Brix, single cane weight and cane diameter at 360 days after planting. Those clones combining single cane

weight ≥ 0.90 Kg, cane diameter ≥ 2.4 cm, Hand Refractometer (H.R) Brix ≥ 18.0 percent and with no adverse morphological traits were selected and carried forward to 2 nd clonal trial. Selection per cent (SP) of each cross having more than 60 progenies was calculated from number of selected clones over the total number of clones tested in the Ist clonal trial and was compared with similarity index values to understand the relationship between isozyme diversity and cross performance. Correlation between SI and SP was estimated using standard statistical procedures [11]. Dissimilarity index (DS) percent was calculated as (1-SI) × 100 for plotting the graph showing the relationship between selection percent and isozyme diversity of individual crosses.

Results and discussion

All the clones showed variable isozyme profile with eleven polymorphic bands for peroxidase, 14 for esterase and five for phosphorylase, accounting to 30 different isozyme loci. Widespread nature of peroxidase and esterase [12,13], coupled with multimeric profile indicated the usefulness of these two isozymes in quantifying genetic diversity in sugarcane. UPGMA dendrogram constructed based on presence and absence of individual bands showed that the clones fell into seven distinct groups (Fig. 1). In general, clones of common descent clustered together as in the case of ISH clones (ISH 1 to ISH 100) which are interspecific hybrids involving *S. robustum*, a wild species of *Saccharum*. Exceptions were also observed as in the case of ISH 11, probably due the segregation of progenies of sugarcane characterized by high degree of heterozygosity and polyploidy.

SI values for 1176 possible combinations of the 49 parental clones surveyed for isozymes were calculated and number of combinations with high, medium and low diversity is summarized in Table 1. The SI values ranged from 0.45 between Co 7201 and CoLk 8102 to 0.97 between ISH 45 and ISH 100. It was also observed that out of 1176 combinations, only 57 were diverse with SI values below 0.60 and 278 combinations were less diverse (or more similar) with SI above 0.80. In the latter category, SI values in 127 combinations were between 0.80 and 0.85, while 44 were least diverse with SI above 0.90 among which 23 combinations were between those ISH clones having a similar origin. This observation also supported the efficiency of isozyme analysis in reflecting and estimating genetic similarity in the crop. Such combinations within ISH series are not generally made for regular breeding programme considering their similar genetic make up. The remaining vast majority of combinations (numbering 898) had SI values between 0.60 and 0.80, showing that moderate genetic variability existed in sugarcane

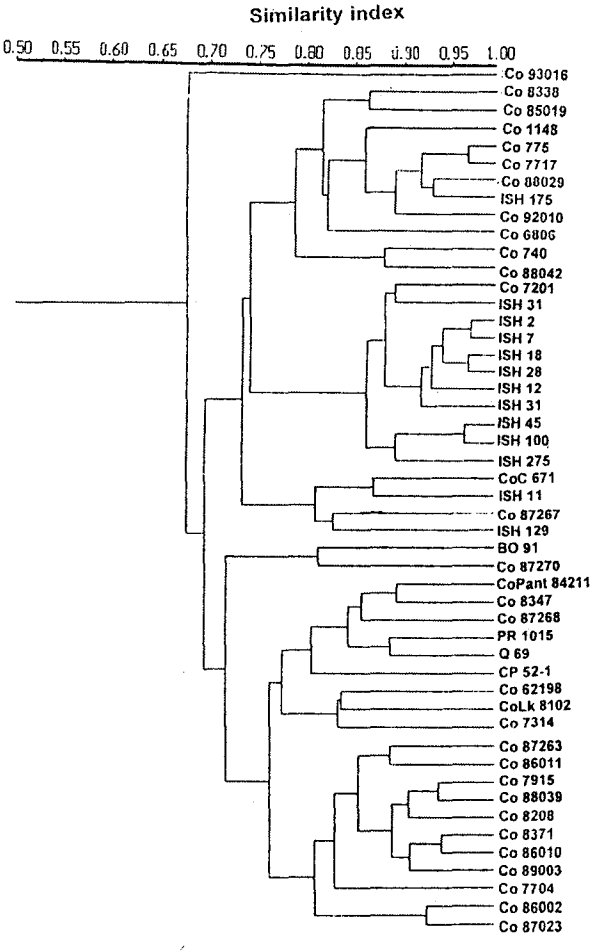

Fig. 1. Dendrogram indicating genetic similarity among 49 hybrid parental clones in sugarcane based on three isozyme systems

Table 1. Number of combinations with high, medium and low diversity based on isozyme marker polymorphism among 49 parental clones in sugarcane

Number of parental combinations with similarity index values				
Below 0.60	Between 0.60 and 0.80	Above 0.80		
		Below 0.85	Between 0.85 and 0.90	Above 0.90
57	898	127	50	44
			Total = 221	

Number of possible combinations = 1176

parental clones and provided possible reason for the continued success of intervarietal hybridization adopted in crop improvement activities.

Based on flowering intensity and synchrony in flowering, 38 crosses were made within the three groups representing high, medium and low diversity. Fourteen cross combinations that yielded sufficient population size (more than 60 progenies per cross) were considered in the present study, considering the reports that a

minimum sample size of 40-200 seedlings/cross is needed to evaluate an experimental cross in sugarcane at seedling stage [14-16]. Cross evaluation was based on the proportion of selectable clones and not based on mid parental values as the parental clones included subtropical types which showed a relatively poor performance at Coimbatore. Criteria of selection were based on evaluation of individual clone for H.R. Brix as an index of juice quality and single cane weight and cane diameter being the components of yield. Among these, H.R. Brix and single cane weight having high heritability are more effective in selection [17]. Out of 941 clones tested 582 clones that surpassed the threshold fixed for each character (as indicated in Materials and methods) was carried forward to 2 nd clonal trial for further evaluation.

The cross combinations Co 775 × Co 1148, CoLk 8102 × Co 62198 and Co 86002 × Co 8347 showed SI above 0.80, while six combinations *viz.*, Co 7201 × CoLk 8102, CoC 671 × Co 87270, Co 8347 × BO 91, Co 7201 × Co 7314, Co 7201 × BO 91 and CoLk 8102 × Co 775 recorded lower similarity values i.e., below 0.60 and the remaining five crosses showed intermediate values.

Details of crosses effected, number of clones evaluated and selected per cross, selection percent, SI values and DS per cent are given in Table 2. Correlation between SP and SI was –0.6221 (Fig. 2.), indicating significant negative relationship between them and hence the usefulness of selecting parents based on isozyme diversity for deriving a higher proportion of selectable types in sugarcane. Relationship between SP and DS% for individual crosses is given in Fig. 3. The crosses that gave higher selection percent were CoC 671 × Co 87270 (SI = 0.545; SP = 92.1), CoC 671 × Co 62198 (SI = 0.670; SP = 88.3) and CoLk 8102 × Co 775 (SI = 0.552; SP = 84.0) where the SI values were low or moderate. It was interesting to see that

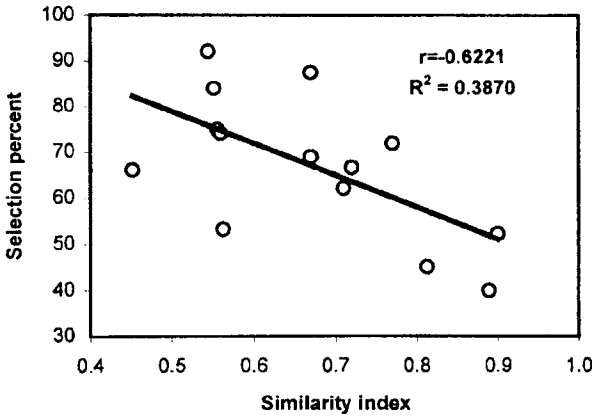

Fig. 2. Relationship between selection percent and similarity index

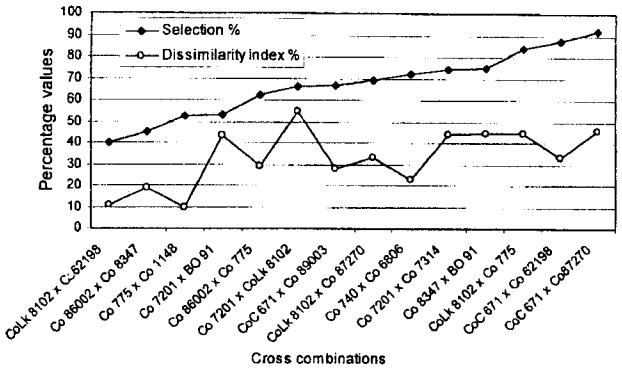

Fig. 3. Relationship between selection percent and dissimilarity index based on isozyme diversity in sugarcane

Table 2. Number of progenies screened and selected per cross, selection per cent and similarity index of cross combinations studied in the Ist Clonal trial

Sl. No.	Cross combination	No. of progenies		Selection percent	Similarity index	Dissimilarity index %
		Tested	Selected			
1.	CoLk 8102 × Co 775	75	63	84.0	0.552	44.8
2.	CoC 671 × Co87270	63	58	92.1	0.545	5.5
3.	CoLk 8102 × Co62198	60	24	40.0	0.889	11.1
4.	Co 86002 × Co 8347	62	28	45.2	0.813	18.7
5.	CoLk 8102 × Co 87270	61	42	69.0	0.670	33.0
6.	Co 7201 × CoLk 8102	74	49	66.2	0.452	54.8
7.	Co 740 × Co 6806	68	49	72.0	0.770	23.0
8.	Co 7201 × BO 91	69	37	53.3	0.563	43.7
9.	Co 8347 × BO 91	60	45	75.0	0.556	44.4
10.	Co 775 × Co 1148	65	34	52.3	0.900	10.0
11.	Co 7201 × Co 7314	66	49	74.2	0.560	44.0
12.	Co 86002 × Co 775	95	59	62.1	0.710	29.0
13.	CoC 671 × Co 89003	63	42	66.7	0.720	28.0
14.	CoC 671 × Co 62198	60	53	88.3	0.670	33.0
	Total	941	582			

selection per cent was higher in all the crosses with SI values below 0.80, while the crosses with higher SI such as Co 775 × Co 1148, CoLk 8102 × Co 62198 and Co 86002 × Co 8347, recorded low selection per cent (52.3, 40.0 and 45.2. per cent respectively). The result thus showed the need for selecting those crosses with SI below 0.80 (genetically less similar) for obtaining higher rate of selection per cross.

In spite of significant negative correlation between the two traits, selection did not increase with increasing diversity, showing that moderate levels of diversity were sufficient to produce a higher proportion of selectable types in sugarcane. It was also not possible to select heterotic combinations based on isozyme analysis. Isozyme diversity information was, therefore, helpful in identifying less productive combinations (those with SI above 0.80). Avoiding such combinations would lead to higher rate of success in sugarcane breeding in terms of higher percentage of selection from the populations handled by the breeders.

As intercrossing of elite hybrids is adopted in the progressive synthesis of varieties, judicious use of genetic variability existing among the parental material lies in identifying the most productive combinations. Isozyme analysis of parental clones could partially aid such an effort by which genetically similar combinations yielding lesser number of selections could be avoided. In the light of reports of DNA based molecular markers in crop breeding for identifying heterotic combinations and in establishing correlation between molecular diversity and cross performance [1, 18-20], it is important to use more efficient marker systems like microsatellites and AFLP in sugarcane. Such an exercise might offer a reliable and effective means for assessing genetic diversity among parental clones and providing alternative means of predicting hybrid performance thereby paving way to a precision breeding strategy in this genetically complex crop.

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