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RESEARCH PAPER

Analysis of genetic diversity among high sucrose genotypes of sugarcane (*Saccharum* spp.) derived from CoC 671 using morphological and sugarcane specific microsatellite markers

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Abstract **:** Twenty eight genotypes derived from a common donor parent for juice quality *viz*., CoC 671 through crossing as male parent, as female parent, selfing, through somaclonal variation and induced mutagenesis were evaluated based on morphological markers and sugarcane specific STMS markers to estimate genetic using Dice, Jaccard's and SM methods. The correlation between the mean similarity index values of molecular diversity and morphological diversity was +0.7506, showing strong relationship between the two characters. STMS markers were found to be suited to detect more differences among the clones of common descent as supported by the lower similarity index values. Mean genetic similarity among the somaclones (SI value = 0.72), mutants (SI value = 0.81), clones derived from CoC 671 as a female (SI value = 0.73) parent and male parent (SI value = 0.70) clearly showed that the clones with CoC 671 as the male parent were more diverse and indicated the role of male parent in creating a more segregating population than when used as female parent. Tissue culture based selection through somaclonal variation and induced mutations were less efficient in creating variability than the conventional methods. More diverse clones and combinations were identified to be used in hybridization for sucrose improvement in sugarcane.

Key Words **:** Genetic diversity, CoC 671 derivatives, Morphological markers, STMS markers, Breeding methods, Sugarcane

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INTRODUCTION

A major objective of sugarcane improvement programmes worldwide is to produce more sugar per unit area. In spite of the diversity of sugarcane genetic resources and a long history of genetic improvement, the progress in varietal development during the past decade has been slow, especially in improving sucrose levels of commercial varieties. Genes concerned with high juice quality are almost entirely inherited from *Saccharum officinarum* ancestors. Successful efforts to improve sucrose content through the adoption of different selection strategies and choice of appropriate parents have been reported (Legendre, 1995). Building genetic stocks for sucrose content has been in progress in all the world sugarcane breeding stations and donor parents identified have been extensively utilized in the progressive synthesis of several high sucrose derivatives. This repeated intercrossing necessitates an assessment of genetic base of such high sucrose parental clones to identify the more diverse clones in breeding for better gains. Molecular markers have been used in genetic improvement programmes to study genetic diversity and to select parents for planning crossing between parents from divergent backgrounds. Microsatellite repeats have the potential to be used in studies on genetic diversity (Selvi *et al*., 2003; Cordeiro *et al*., 2003; Hemaprabha *et al*., 2005 and Hemaprabha *et al*., 2006). In this study the derivatives of a common donor parent for juice quality *viz*., CoC 671 bred

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through crossing as male parent, as female parent, selfing, through somaclonal variation and induced mutagenesis were evaluated based on morphological and molecular markers in order to identify the more diverse types to be used as parents and to compare the efficiency of different breeding methods to generate more variability for imparting selection.

MATERIALS AND METHODS

A total of 28 commercial hybrids of sugarcane having CoC 671 as one of the parents (either female or male) along with the original parent CoC 671 were taken for the study (Table A).

Cane morphological characters based on 28 morphological descriptors used in describing a sugarcane

Table A : List of 28 varieties taken for the study

Sr. No.	Breedig approach	genotypes
1.	Somaclones of CoC 671	Co 99012, Co 94003, Co 89005, Co 89025, Co 88006 and Co 94012
2.	Induced mutants	Co 200002, Co 200003 and Co 91017
3.	Derived from CoC 671 as male parent	Co 87007, Co 87009, Co 87257, Co 86032, Co 92002 and Co 85002
4.	Derived from CoC 671 as female parent	Co 97005, Co 96002, Co 91002, Co 94015, Co 90001, Co 89002, Co 93010, Co 93020, Co 89038, Co 92005 and Co 88023
5.	Selfs of CoC 671	Co 92008 and Co 92023

Table B : Sugarcane specific STMS primers and the bands amplied

Sr. No.	Primer	SSR motif	Fragment size (bp)	Total no. of bands	No. of polymorphic bands	Polymorphism %
1.	NKSCSSR 1	(gaa) 6	215-392	7	3	43
2.	NKSCSSR 2	(ga) 13	241-484	4	0	0
3.	NKSCSSR 3	(tgc) 5	181-340	7	5	71
4.	NKSCSSR 5	(gt) 28	183-330	5	2	50
5.	NKSCSSR 6	(tg) 32	165-1250	17	15	88
6.	NKSCSSR 7	(cgg) 9	182-326	10	8	80
7.	NKSCSSR 8	(cgg) 6	161-323	10	6	60
8.	NKSCSSR 9	(cgc) 6	138-1265	13	10	77
9.	NKSCSSR 12	(ag) 23	153-290	10	6	60
10.	NKSCSSR 14	(ga) 22	138-204	5	4	80
11.	NKSCSSR 15	(ag) 19	158-400	12	6	50
12.	NKSCSSR 16	(ag) 23	198-450	10	6	60
13.	NKSCSSR 21	(ga) 20	143-287	6	4	67
14.	NKSCSSR 23	(ga) 18	114-368	16	4	25
15.	NKSCSSR 24	(ga) 34	158-467	12	7	58
16.	NKSCSSR 25	(ag) 27	182-444	10	7	70
17.	NKSCSSR 27	(ga) 20	190-393	14	12	71
18.	NKSCSSR 28	(ag) 27	123-568	12	9	75
19.	NKSCSSR 30	(cgg) 7	176-399	10	4	40
20.	NKSCSSR 31	(cgg) 8	214-491	6	3	50
21.	NKSCSSR 32	(tc) 36	178-371	10	4	40
22.	NKSCSSR 33	(tgt) 6	134-297	6	4	67
23.	NKSCSSR 34	(gt)18(ga)31	145-312	14	5	36
24.	NKSCSSR 38	(ag) 15	237-685	16	7	44
25.	NKSCSSR 42	(tg) 35	129-243	6	4	67
26.	NKSCSSR 45	(tg) 35	148-712	31	30	97
27.	NKSCSSR 46	(tg) 24	178-303	5	4	80
28.	NKSCSSR 52	(gt) 24	243-681	18	1	6
29.	NKSCSSR 53	(gt) 28	193-629	18	15	83
30.	NKSCSSR 54	(tg) 19	181-547	12	7	58
Total number of fragments				332	202	61

variety were recorded at the crop age of 300 days. The characters considered were stool habit, stem colour (exposed), stem colour (unexposed), ivory marks, corky patches, internode shape, internode alignment, internode diameter, splits, wax, node swelling, root zone colour (exposed), root zone colour (unexposed), number of root eyes, bud size, bud shape, bud wings, bud groove, growth ring colour, lamina colour, leaf carriage, leaf sheath colour, leaf sheath waxiness, leaf sheath spines, leaf sheath clasping, dewlap colour, ligular process and shape of ligule. Scoring was done based on the expression of characters in CoC 671, presence marked as 1 and absence as 0.

PCR amplification and electrophoresis:

DNA from the 28 clones along with the parent CoC 671 was isolated using CTAB method (Murray and Thompson, 1980) and are quantified by Nanodrop 100. Thirty sugarcane specific STMS primers with high polymorphism information content were used to screen these clones (Table B). PCR reactions were performed in MJ Thermal cycler PT 100 with a total reaction volume of 10 µl containing 25 ng of template DNA, 1pMol of Forward and Reverse Primers, 2mM of dNTPs, 1.75 mM $MgCl_2$ and 0.5 U Taq polymerase. Cycling conditions were: One cycle of 5 minutes at 94°C, 30 seconds at appropriate annealing temperature (ranging from 51° C to 59° C depending on the primer) and 50 seconds at 72° C, with a final extension of 5 minutes at 72° C. PCR products were resolved on a 7.5 per cent non-denaturing polyacrylamide gel using 1X TBE buffer and stained in ethidium bromide. The gels were visualized in UV using the gel documentation system, Alpha Innotech. Bands were scored as '1' for presence and '0' for absence and the binary data were used for statistical analysis. Data on molecular analysis were analysed with NTSYS-pc software (Rohlf, 1993) using Dice's, Jaccard's and simple matching (SM) coefficients (Nie and Li, 1979; Sneath and Sokal, 1973). The formulae for estimating the diversity are given below.

The Dice coefficient $S_D = [2N11/(2N11 + N10 + N01)$

Jaccard's coefficient $S_J = N11/(N11 + N10 + N01)$

Simple Matching Coefficient $S_{SM} = [(N11/ (N11 + N00)/ (N11 + N10 + N01 + N00)]$

where N11 is the number of bands present in both individuals, N00 is the number of bands absent in both individuals, N10 is the number of bands present only in the 1st individual, N01 is the number of bands present only in the 2nd individual and N represents the total number of bands.

RESULTS AND DISCUSSION

The results of the present study alongwith relevant discussion have been presented as under:

Similarity matrix analysis using molecular and morphological data:

STMS marker diversity quantified with three different genetic similarity coefficients, which showed minor differences in the estimation of similarity based on the number of shared bands. Jaccard's matrix gave less weight to matching bands than the Dice index, while in simple matching coefficient, the number of bands absent in both the individuals was also considered. Three types of analyses were used in an attempt to minimize errors resulting from scoring different bands as identical so as to get a better estimate of diversity.

Morphological diversity:

For each morphological character, the clones were scored for the presence/absence in comparison with CoC 671 as given in Table 1. Similarity matrix was generated using Dice, Jaccard's coefficient and simple matching methods. All the three matrices revealed high morphological similarity in the investigated material. Based on Dice's coefficient (Table 2) the maximum similarity value (0.98) was between Co 88006 and Co 88023, while minimum similarity (0.59) was observed between Co 87009 and Co 99012. Using Jaccard's coefficient, two pairs of clones Co 91017 and Co 89038 and Co 88006 and Co 88023 had the maximum similarity value (0.95) and a minimum similarity value (0.42) was between Co 87007 and Co 99012. Using simple matching method the two pairs of clones *viz*., Co 88006 and Co 88023 and Co 91017 and Co 89038 had the maximum morphological similarity (0.96), Co 87007 and Co 94012 showed the minimum similarity value (0.48).

Dendrogram was generated by simple matching method, Dice coefficient and Jaccard's coefficient methods depicting the morphological similarity of the clones dendrogram based on Dice coefficient method as shown in Fig. 1 and had six clusters. Jaccard's coefficient and simple matching methods also showed six clusters in the dendrogram. Both the Dice and Jaccard's cluster analysis showed that the grouping of clones in different clusters was almost similar. All the three analysis showed that the clone Co 94012 had the maximum diversity.

Though the clones showed continuous variation for the morphological characters, the overall SI values analyzed among these were 0.79, 0.66 and 0.73 in Dice, Jaccard's coefficient and SM method, respectively. The somaclones Co 88006 (Dice SI value = 0.90) and mutant clone Co 200002 (Dice SI value = 0.84) showed less morphological variation. The progeny derived from CoC 671 as male parent was the most diverse. (Co 87009 with Dice SI value = 0.74)

Molecular diversity:

Molecular profiles were generated with 30 STMS primers. The molecular profiles generated with the primers NKSCSSR 33 are given in Fig. 2.

The mean SI values of the above clones, estimated by Dice coefficient, Jaccard's coefficient and simple matching methods based on the data generated with 30 sugarcane specific STMS markers, was 0.69, 0.54 and 0.74 in Dice,

Table 1 : Details of morphological characters taken for scoring the 28 clones having CoC 671 as a common parent

Sr. No.	Morphological characters	As in CoC 671	Different from CoC 671
1.	Stool habit – semi-erect	1	0
2.	Stem colour (exposed)–orangish pink	1	0
3.	Stem colour (unexposed) – yellowish pink	0	1
4.	Ivory marks – Absent	1	0
5.	Corky patches – Light	1	0
6.	Internode shape – Cylindrical	1	0
7.	Internode alignment – Zig zag	0	1
8.	Internode diameter – 3	0	1
9.	Splits – Absent	0	1
10.	Wax – Absent	1	0
11.	Node swelling – Absent	1	0
12.	Root zone colour (exposed) – Greenish yellow	1	0
13.	Root zone colour (unexposed) – yellowish green	1	0
14.	Number of root eyes – medium	1	0
15.	Bud size – Medium	1	0
16.	Bud shape – Oval	0	1
17.	Bud wings – Present	1	0
18.	Bud groove – Absent	1	0
19.	Growth ring colour – Pink	1	0
20.	Lamina colour – Green	1	0
21.	Leaf carriage – Open	1	0
22.	Leaf sheath colour – Green with purple blotches	0	1
23.	Leaf sheath waxiness – Absent	1	0
24.	Leaf sheath spines – Heavy	1	0
25.	Leaf sheath clasping – Loose	1	0
26.	Dewlap colour – Dark brown	1	0
27.	Ligular process – 'L'	1	0
28.	Shape of ligule - Deltoid	1	0

Table 2 : The clones grouped based on Dice's similarity %

Sr. No.	Similarity %	Varieties
1.	High (> 0.82)	Co 200002, Co 200003, Co 99012, Co 91002, Co 91017, Co 89005
2.	Medium (0.77-0.83)	Co 97005, Co 96002, Co 94015, Co 93020, Co 92002, Co 92005, Co 92008, Co 92003, Co 89025, Co 88023, Co 86032, Co 85002, Co 94012
3.	Low (< 0.76)	Co 94003, Co 93010, Co 92002, Co 89002, Co 89038, Co 88006, Co 87007, Co 87009, Co 87257

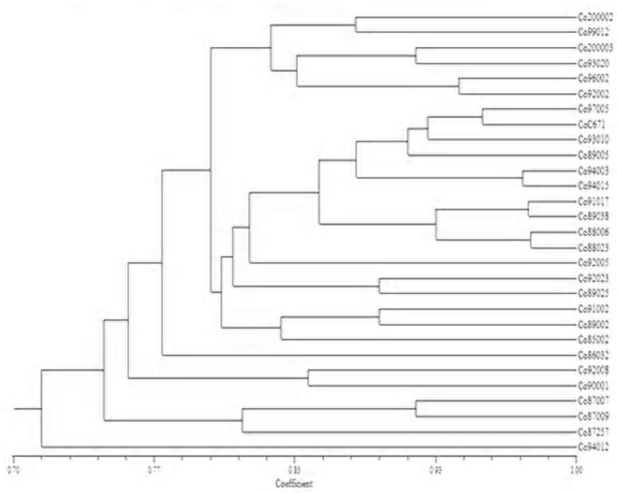

Fig. 1: Dendrogram based on morphological characters of 28 clones having CoC 671 as a common parent Dice coefficient method

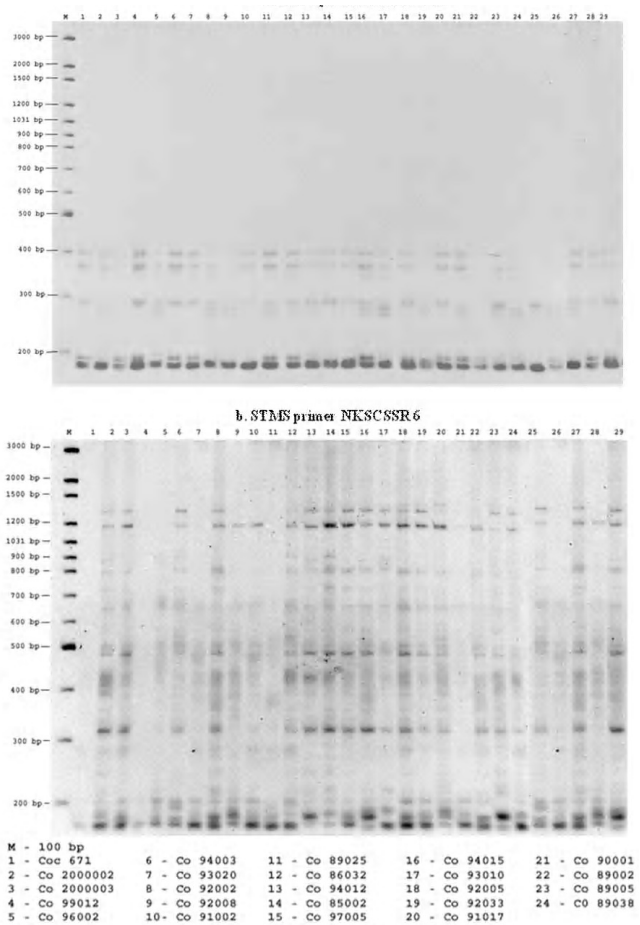

Fig. 2 : Dendrogram based on STMS based genetic similarly of 28 clones having CoC 671 as a common parent Dice coefficient method

Table 3 : Genetic similarity among CoC 671 derived STMS markers through Dice's coefficient

Jaccard's coefficient and SM method, respectively showing moderate genetic diversity (Table 3).

Dendrogram analysis:

The dendrogram constructed using Dice coefficient method was divided into three main clusters (Fig. 3).

In the first cluster, the clones Co 91017 and Co 89005 were seen to be identical. The clones Co 92002 and Co 94003 were separately branched and did not come under any cluster.

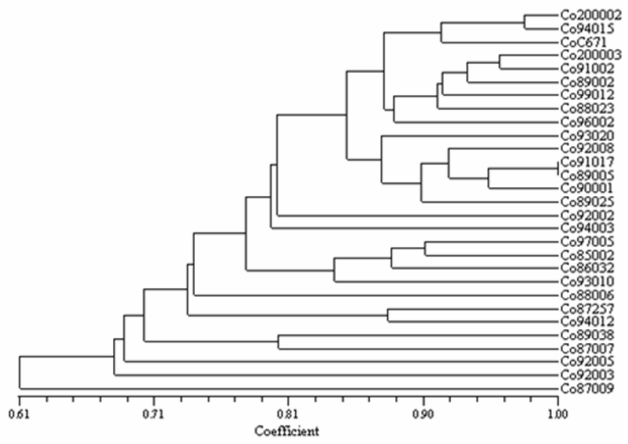

Fig. 3 : Dendrogram based on STMS based genetic similarly of 28 clones having CoC 671 as a common parent Dice coefficient method

The clone Co 87009 fell separately in the dendrograms generated by Jaccard's coefficient and simple matching coefficient. The clones Co 87009 and Co 87007, generated from the cross Co 7704 x CoC 671, shared a similarity per cent of 0.52 based on simple matching coefficient, exhibiting divergence. The highest SI value of 1.00 (Dice coefficient) was observed between Co 91017 and Co 89005. The close morphological resemblance supported by high molecular similarity showed that both were genetically very similar. Mean genetic similarity of the clones based on Dice's coefficient is given in Table 2. Accordingly Co 87007, Co 87009, Co 87257, Co 88006, Co 89002, Co 89038, Co 92002, Co 93010 and Co 94003 were the most diverse (Table 2). Among the possible 378 combinations, 72 were more similar (Table 3) that might not give incremental gains when crossed, while the rest would be useful in quality improvement programmes. The diversity among these clones of common descent could be realized through the high amount of genetic recombination in the heterozygous genetic background of sugarcane (Heinz, 1987). High selection pressure exerted while screening for high sucrose is another explanation (Sobral *et al.,* 1994).

Comparison of genetic diversity of progeny derived from five crop improvement methods:

The mean genetic similarity of the five different crop improvement approaches *viz.*, selfing, derived through somaclonal variation, induced mutation, hybridization using CoC 671 as male parent and female parent using the three genetic similarity estimates is given in Table 4. The mutants of CoC 671 showed the highest level of similarity with an overall mean SI value of 0.81, followed by those derived with CoC 671 as the female parent (SI=0.73) and the somaclones (SI=0.72), while those derived from Co 671 as the male parent exhibited similarity of 0.70 and the two selfs had a SI of 0.68.

The clones which had CoC 671 as male parent gave high

Table 4 : Mean genetic similarity of different groups of progeny derived from CoC 671 parent

Sr. No.	CoC 671 derivatives	Dices coefficient	Jaccard's Coefficient	Simple matching coefficient	Mean
1.	Self	0.72	0.57	0.76	0.68
2.	Somaclones	0.76	0.62	0.79	0.72
3.	Mutants	0.83	0.73	0.86	0.81
4.	As female parent	0.75	0.61	0.83	0.73
5.	As male parent	0.73	0.59	0.79	0.70

Table 5 : Genetic similarity measured using Dice, Jaccard and simple matching coefficients and similar and diverse clones identified

Characters	Similarity coefficient	Mean similarity	Clones with highest similarity with CoC 671	Clones with lowest similarity with CoC 671
Morphological characters	Dice	0.79	0.95 (Co 97005)	0.74 (Co 92008, Co 87009
	Jaccard's	0.66	0.90 (Co 89038)	0.58 (Co 87009)
	Simple matching	0.65	0.93 (Co 97005)	0.63 (Co 87009)
Molecular analysis	Dice	0.69	0.93 (Co 200002)	0.63 (Co 87257)
	Jaccard's	0.54	0.87 (Co 200002)	0.42 (Co 87257)
	Simple matching	0.74	0.94 (Co 200002)	0.60 (Co 87257)

diversity when compared to those having CoC 671 as female parent, indicating the role of male parent in creating a more segregating population than the other methods. Hasu *et al.* (1996) has reported the role of female parent in the inheritance of H.R. brix and sucrose content. This information is useful in that using high quality clones like CoC 671 as female parent can lead to more frequency of high quality clones, while such parents when used as males can lead to more variability among the progeny. Among the somaclones, the clone Co 94012 exhibited more high diversity based on morphological and molecular markers, which has resulted in lower genetic similarity among somaclones in relation to induced mutations. The results clearly showed that tissue culture based sugarcane improvement methods *viz.*, somaclonal variation and induced mutation did not lead to gross genetic and morphological changes in the clones compared to conventional breeding approaches of crossing or selfing. The two selfs taken for the study were more diverse. Though this type of dissimilarity is not normally expected, inheritance of segregation leading to genetic differences could occur in this complex polyploidy crop. Morphologically, also these two clones *viz.*, Co 92008 and Co 92023 differed from CoC 671 to explain their diversity from the parent. Thus the selfs considered in this study may not be a representative sample.

Comparison between molecular and morphological similarity:

The mean SI values obtained through molecular and morphological diversity analysis using three methods are given in Table 5 and Fig. 4. Correlation between molecular diversity and morphological diversity was estimated to be + 0.7506, showing strong positive relationship between the two characters. However, molecular similarity values were lower than those based on morphological markers, indicating the high discriminatory power of STMS markers to detect differences among progeny of common descent

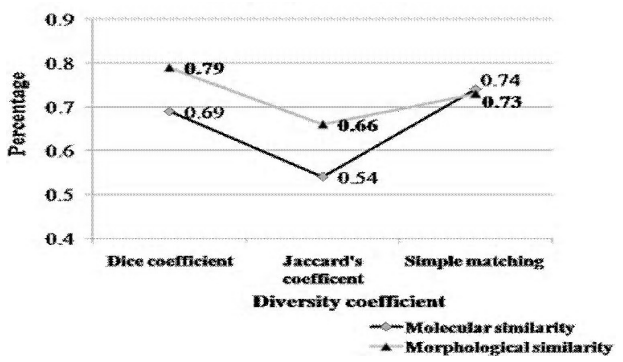

Fig. 4 : Similarity index values based on molecular and morphological diversity

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