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Genetic diversity within sucrose rich parental pool of sugarcane and its application in sugarcane breeding through hybridization and selection

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Abstract

Genetic diversity in 82 high sucrose genotypes of sugarcane was estimated using 30 sugarcane specific STMS primers. The overall SI values using three diversity measures *viz*., Dice, Jaccard's and simple matching methods were 0.69, 0.54 and 0.74 respectively, indicating the existence of moderate diversity among the clones and the scope of improving sucrose content through breeding. The dendrogram analysis among the 82 sugarcane types showed that two pairs of clones (Co 200002 and Co 86005 and, Co 91017 and 89005) were identical. Though the clustering of clones reflected the pedigree relationship between the cultivars, deviations from this were observed and could be attributed to the high heterozygosity and polyploidy of the genus *Saccharum* that lead to gross differences in phenotype and genotype. The more diverse clones were Co 87009, Co 86002, Co 90006, Co 86014, Co 775, Co 87011 and Co 85037. Out of 3321 possible combinations, 443 combinations were genetically more similar (SI = 0.83) which might not provide incremental gains through hybridization, while 813 combinations (SI= 0.69) were genetically more diverse and 2206 combinations showed moderate diversity (SI = 0.70-0.82). Such diverse clones and combinations have immediate application in breeding for improving efficiency and precision in sugarcane breeding. Based on diversity estimates, hybridization involving four genetically diverse, three genetically similar and three with intermediate similarities were affected and progeny performance correlated with genetic diversity. A strong correlation (–0.7265) between genetic diversity and cross performance and cross selection rate from crosses with high and medium diversity indicated the importance of diversity estimates in the choice of parents and to estimate genetically more similar crosses.

Key words: Genetic diversity, STMS markers, hybridization, sugarcane

Introduction

A basic understanding of the genetic diversity existing in the germplasm available for breeding is fundamental to the success of a breeding program. Though sucrose content is a major contributing factor for sugar yield, improvement in sucrose levels has been slow compared to higher gains achieved in cane yield. One of the major concerns has been the limited genetic diversity available within the sugarcane breeding pool [1, 2]. Assessment of genetic diversity available in the sugar rich germplasm is important for improvement by selection. Molecular markers are important in accelerating breeding process through quantifying the genetic diversity available for crop improvement programme. A detailed molecular characterization of sugar rich breeding pool is therefore essential to improve breeding efficiency and to increase precision in quality breeding in sugarcane. Microsatellites have been ideal in sugarcane for estimating genetic diversity [3-6] to optimize and facilitate the breeding process, through selection of the best parents and cross combinations. The present investigation involves a detailed study on sucrose rich genotypes used in sugarcane improvement programmes in India with an aim to estimate the genetic diversity available within breeding pool and to identify more diverse sources and combinations for genetic improvement of the trait and to compare the selection percent of crosses based on genetic diversity.

Materials and methods

A total of 82 commercial hybrids of sugarcane with high sucrose content (> 18 % sucrose at 360 days after planting) were taken for the study (Table 1).

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Table 1. List of 82 sugar rich sugarcane clones from the germplasm used for genetic diversity study using STMS markers

S. No	Hybrids	S. No	Hybrids
1	Co 20000-01	2	Co 20000-02
3	Co 20000-03	4	Co 99014
5	Co 99012	6	Co 98004
7	Co 98005	8	Co 98014
9	Co 97001	10	Co 97005
11	Co 97007	12	Co 97012
13	Co 96002	14	Co 96011
15	Co 96023	16	Co 95002
17	Co 95003	18	Co 95008
19	Co 95021	20	Co 94002
21	Co 94003	22	Co 94009
23	Co 94011	24	Co 94015
25	Co 93010	26	Co 93019
27	Co 93020	28	Co 93023
29	Co 92002	30	Co 92005
31	Co 92007	32	Co 92008
33	Co 92010	34	Co 92023
35	Co 91002	36	Co 91017
37	Co 90001	38	Co 90006
39	Co 90013	40	Co 89002
41	Co 89005	42	Co 89006
43	Co 89015	44	Co 89022
45	Co 89025	46	Co 89035
47	Co 89038	48	Co 88001
49	Co 88006	50	Co 88007
51	Co 88008	52	Co 88023
53	Co 88027	54	Co 87005
55	Co 87007	56	Co 87009
57	Co 87011	58	Co 87024
59	Co 87257	60	Co 86001
61	Co 86005	62	Co 86007
63	Co 86013	64	Co 86014
65	Co 86032	66	Co 85004
67	Co 85037	68	Co 85048
69	Co 85286	70	Co 85287
71	CoC 671	72	Co 97014
73	Co 94012	74	Co 86011
75	Co 85002	76	Co 97015
77	Co 7201	78	Co 86002
79	Co 98010	80	Co 775
81	NS 83472	82	ISH 147

PCR Amplification and Electrophoresis

DNA from the 82 clones were isolated using CTAB method [7] and quantified in DNA RNA Quantifier (Nanodrop 100). Thirty sugarcane specific STMS primers with high polymorphism information content were used to screen these clones (Table 2). PCR reactions were performed in MJ Thermal cycler PTC 100 with a total reaction volume of 10 µl containing 25 ng of template DNA, 1pMol of Forward and Reverse Primers, 0.2 mM of dNTPs, 1.75 mM $MgCl_2$ and 0.5 U Taq polymerase. The first cycle was carried out for 4 minutes at 94^{o}C, followed by denaturation, annealing and extension in 30 cycles of two minutes at 92^{o} C, 30 seconds at the appropriate annealing temperature (ranging from 51^{o} C to 59^{o} C depending on the primer) and 50 seconds at 72^{o} C, followed by a final extension of 5 minutes at 72^{o} C.

PCR products were resolved on a 7.5% non-denaturing polyacrylamide gel using 1X TBE Buffer and stained in Ethidium Bromide. The gels were visualized in UV in a 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. The data was analysed with NTSYS-pc software [8] using Dice's, Jaccard's and Simple Matching (SM) coefficients [9, 10]. Bootstrap analysis for validating the clustering was performed with 1000 replications with the software WINBOOT [11].

Hybridization, progeny evaluation

Parental combinations were classified into three categories based on SI as diverse combinations, combinations with medium diversity and closely related combinations. From these, twelve parents were utilized in making ten crosses based on flowering synchrony The hybrid seed (fluff) was used to raise seedling nursery. All the healthy seedlings were advanced to the first clonal trial without practicing selection for any characters. The first clonal trial was carried out during 2006-2007. All normal seedlings were sett-planted in Ist clonal trial in a plot size of 2m x 0.9 m. The progeny were screened for number of millable canes (NMC), cane diameter, cane height and Brix at 360 days of crop age. Based on the threshold values fixed for the four characters, the selections were forwarded to the second clonal trial. Selection percent (SP) for each cross was worked out based on the number of clones advanced to the next stage over total number of progeny expressed in percentage. Correlation between percent selection and similarity index was calculated using standard procedures [12].

Results and discussion

Among the 30 STMS primers used for genetic diversity

Table 2. List of STMS primers used for genetic diversity analysis of 82 sugar rich clones of sugarcane from germplasm

S.No.	Primer	Source sequence	EST/ genomic	SSR motif	Forward primer (5'- 3')	Reverse primer (5' - 3')	Polymorphism %
1	NKSCSSR 1	AA577668	EST	(gaa)6	tggcatgtgtcatagccaat	ccccaactgggacttttaca	43
2	NKSCSSR 2	AA961302	EST	(ga)13	gctgtcccgttccaagttac	gcgaccggattatgatgatt	0
3	NKSCSSR 3	AF062734	EST	(tgc)5	cgtgttcctcttcaacaacg	tgcttcgctatatatgggttca	71
4	NKSCSSR 5	SHY293476	Genomic	(gt)28	atagctcccacaccaaatgc	ttggcaaaattgacccaaat	50
5	NKSCSSR 6	SHY293477	Genomic	(tg)32	tccaaattgcctgttgttttc	cttacacatgcacaggcaca	88
6	NKSCSSR 7	SHY293478	Genomic	(cgg)9	ttacagcctggagctcgttt	cgaagcctctcctctcctc	80
7	NKSCSSR 8	SHY293479	Genomic	(cgg)6	gtgacagcggcttgttcag	ttaaacacgcagccattcag	60
8	NKSCSSR 9	SHY293481	Genomic	(cgc)6	ctttcagtggccatctccat	gaatgcgcagggataggata	77
9	NKSCSSR 12	SHY293484	Genomic	(ag)23	cagccacgtgatgctttct	ccgatccatcagtttcaggt	60
10	NKSCSSR 14	SHY293487	Genomic	(ga)22	ttccaccagtgacattcagc	ccaacagcagcttcttcctt	80
11	NKSCSSR 15	SHY293488	Genomic	(ag)19	aacccattgaccagatccag	tagtggccctaggcgtaaaa	50
12	NKSCSSR 16	SHY293489	Genomic	(ag)23	gacagaatatgccatggataacaa	cgttctctggtcctattgagc	60
13	NKSCSSR 21	SHY293495	Genomic	(ga)20	taagccattgggaagaggtg	ctgatgcctgggaatctttc	67
14	NKSCSSR 23	SHY293498	Genomic	(ga)18	taaacccccgaaaaagaacc	tccggaggtagatccatttg	25
15	NKSCSSR 24	SHY293499	Genomic	(ga)34	tatatggcgaggacagatgc	gggttcagaattagagcaatcg	58
16	NKSCSSR 25	SHY293500	Genomic	(ag)27	tccatgcatgcgtgtagttt	agtgcacaacgttcttgctg	70
17	NKSCSSR 27	SHY293502	Genomic	(ga)20	tggatttgggtaaggatgga	taatgcctctgggctcaaat	71
18	NKSCSSR 28	SHY293503	Genomic	(ag)27	gtgctgggattctgagcttc	gcaagttcttggcctttgtt	75
19	NKSCSSR 30	SHY293561	Genomic	(cgg)7	ctccttctccttcgcatcct	cacctttctggagcacgtta	40
20	NKSCSSR 31	SHY293562	Genomic	(cgg)8	aaccaccactcatcgtcctc	caccgagttcccattgttct	50
21	NKSCSSR 32	SHY293563	Genomic	(tc)36	ccaactcactcaccccagtt	atgagagtgcagatgcatgg	40
22	NKSCSSR 33	SHY293564	Genomic	(tgt)6	acaggagcgcttggagatta	gagcagaagggctagaagca	67
23	NKSCSSR 34	SHY293573	Genomic	(gt)18 (ga)31	cgtcttgtggattggattgg	tggattgctcaggtgtttca	36
24	NKSCSSR 38	SHY401316	Genomic	(ag)15	tgaactcggcaacagttttt	cccaccaagtcgttctgaat	44
25	NKSCSSR 42	SHY401320	Genomic	(tg)35	accgattgttcagtgggaag	aacctagcaatttacaagagaattaga	67
26	NKSCSSR 45	SHY401325	Genomic	(tg)35	gtcggtcgtgagaaggaaag	cacgtataaaggccctgtgg	97
27	NKSCSSR 46	SHY401326	Genomic	(tg)24	acaataaccccgcagacatc	taatgcgtcatttggagcag	80
28	NKSCSSR 52	SHY401333	Genomic	(gt)24	ggcctatggaacgaagttca	Cagccttttcttcgcaaaac	6
29	NKSCSSR 53	SHY401334	Genomic	(gt)28	agctcacgtgtgtgtgtgtg	Gtgcagtgtcaggggaccta	83
30	NKSCSSR 56	SHY401337	Genomic	(tg)19	ctatacggcaaacgcaacct	tatacgtcgcatgcaccatc	58 61

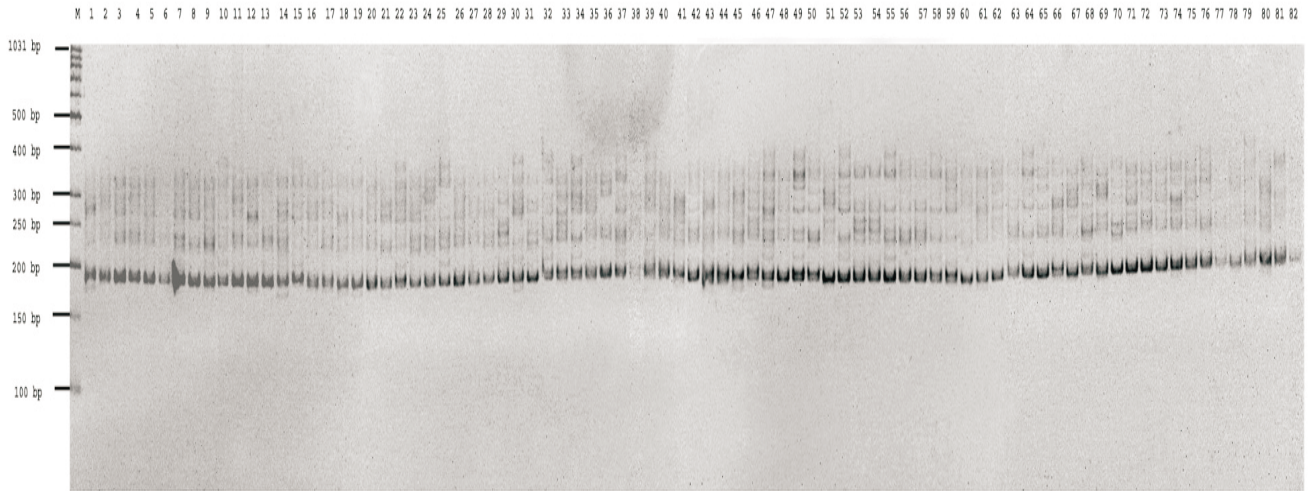
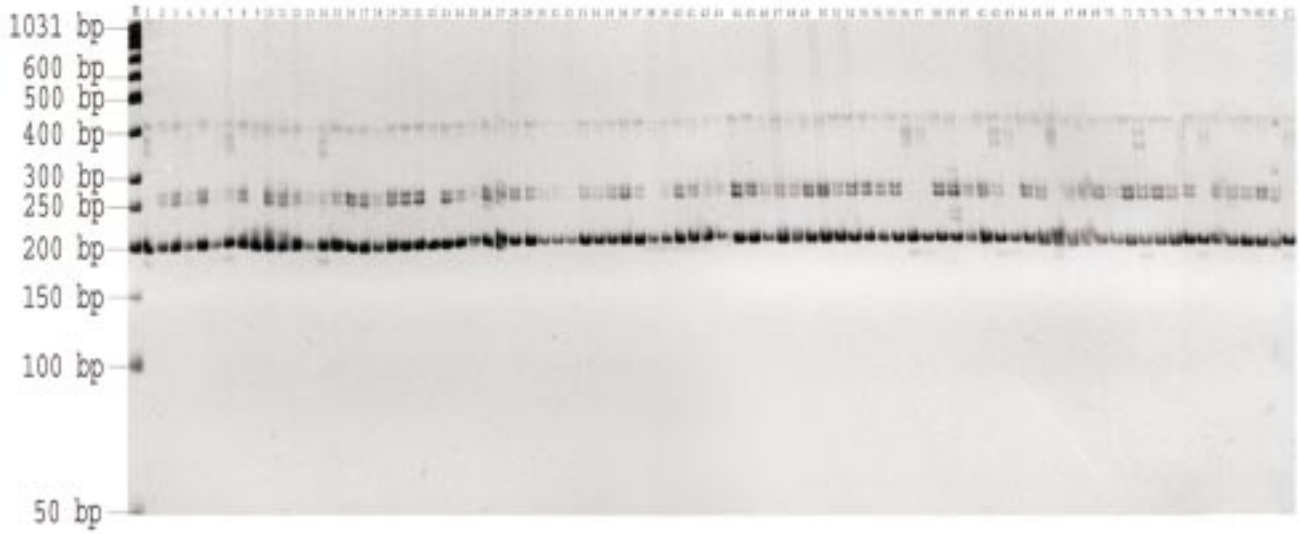


Fig. 1. Molecular analysis of 82 sugar rich sugarcane varieties from the germplasm using STMS primer NKSCSSR 7



M - 50 bp ladder

1	- Co 2000 01	13	- Co 96002	25	- Co 93010	37	- Co 90006	49	- Co 88007	61	- Co 86005	73	- Co 94012
2	- Co 2000 02	14	- Co 96011	26	- Co 93019	38	- Co 90013	50	- Co 88008	62	- Co 86007	74	- Co 86011
3	- Co 2000 03	15	- Co 96023	27	- Co 93020	39	- Co 89002	51	- Co 88023	63	- Co 86013	75	- Co 97015
4	- Co 99014	16	- Co 95002	28	- Co 93023	40	- Co 89005	52	- Co 88027	64	- Co 86014	76	- Co 7201
5	- Co 99012	17	- Co 95003	29	- Co 92005	41	- Co 89006	53	- Co 87005	65	- Co 86032	77	- Co 86002
6	- Co 98004	18	- Co 95008	30	- Co 92007	42	- Co 89015	54	- Co 87007	66	- Co 85004	78	- Co 98010
7	- Co 98005	19	- Co 95021	31	- Co 92008	43	- Co 89022	55	- Co 87009	67	- Co 85037	79	- Co 775
8	- Co 98014	20	- Co 94002	32	- Co 92010	44	- Co 89025	56	- Co 87011	68	- Co 85048	80	- BS 83472
9	- Co 97001	21	- Co 94003	33	- Co 92033	45	- Co 89035	57	- Co 87016	69	- Co 85286	81	- ISH 147
10	- Co 97005	22	- Co 94009	34	- Co 91002	46	- Co 89038	58	- Co 87024	70	- Co 85287	82	- Co 94008
11	- Co 97007	23	- Co 94011	35	- Co 91017	47	- Co 88001	59	- Co 87257	71	- CoC 671		
12	- Co 97012	24	- Co 94015	36	- Co 90001	48	- Co 88006	60	- Co 86001	72	- Co 97014		

Fig. 2. Molecular analysis of 82 sugar rich sugarcane varieties from the germplasm using STMS primer NKSCSSR 8

study, three (NKSCSSR 1, 2, 3) were EST generated primers and the remaining 27 primers were generated from genomic library sequences. Table 2 gives the repeat sequences contained in each primer and the number and range of fragments generated. Of the 30 STMS primers, six contained GA repeats, six AG repeats, five TG repeats, three GT repeats and one TC repeat among the dinucleotide repeats and four CGG repeats, one CGC repeat, one GAA repeat, one TGC repeat and one TGT repeats among the trinucleotides and one GT/GA repeat. The predominance of dinucleotide repeats specially $(AC)_n$ and $(GA)_n$ repeats has been reported [13]. The maximum number (31) of fragments was amplified by the simple dinucleotide repeat $(TG)_{35}$, while $(GA)_{13}$ repeats amplified the minimum number of four bands. The primer NKSCSSR 2 gave four bands which were all monomorphic, while primer NKSCSSR 45 gave more number of polymorphic bands (30). The number of amplified products was more than the earlier reported two to twelve fragments in agarose gels [14] but was in agreement to the reports of [6] and [3] and was attributed to the complex polyploid and heterozygous nature of sugarcane.

The size of the fragments amplified ranged from 114 bp to 1,265 bp, while the majority of the fragments were in the size range of 150bp - 300bp. The lowest fragment size of 114 bp was amplified by the primer NKSCSSR 23. Clearly resolved and repeatable bands which were produced and were polymorphic among the 82 sugar-rich clones were 203 out of 332 bands, accounting to 61 % of the overall number of bands. On an average the polymorphism revealed by EST derived sugarcane primers were 44.4 %, while the genomic library derived sugarcane primers were 61.7 % (Table 2). Similar results were also reported by [15] as higher

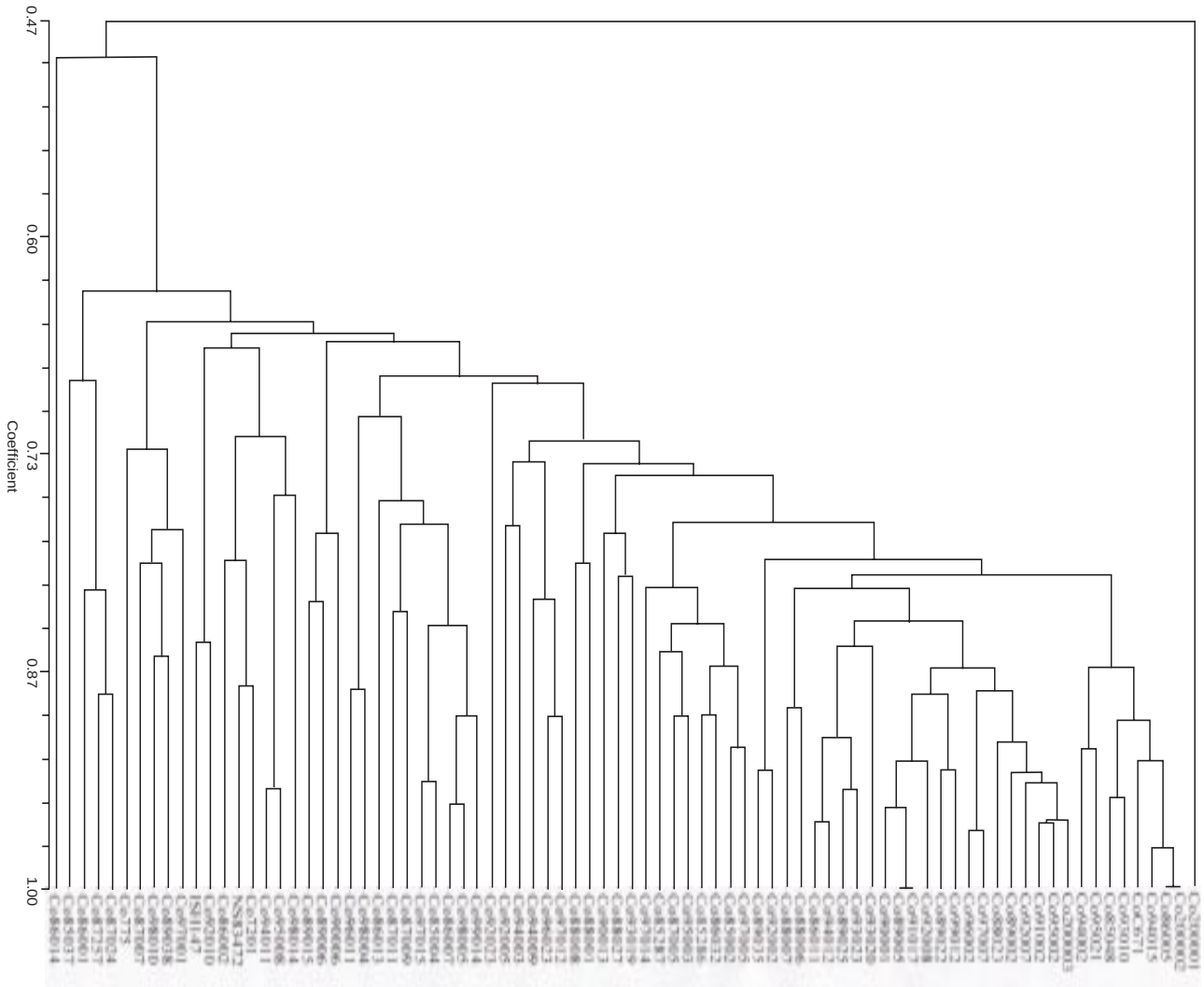


Fig. 3. Dendrogram of 82 sugar rich genotypes using Dice coefficient method

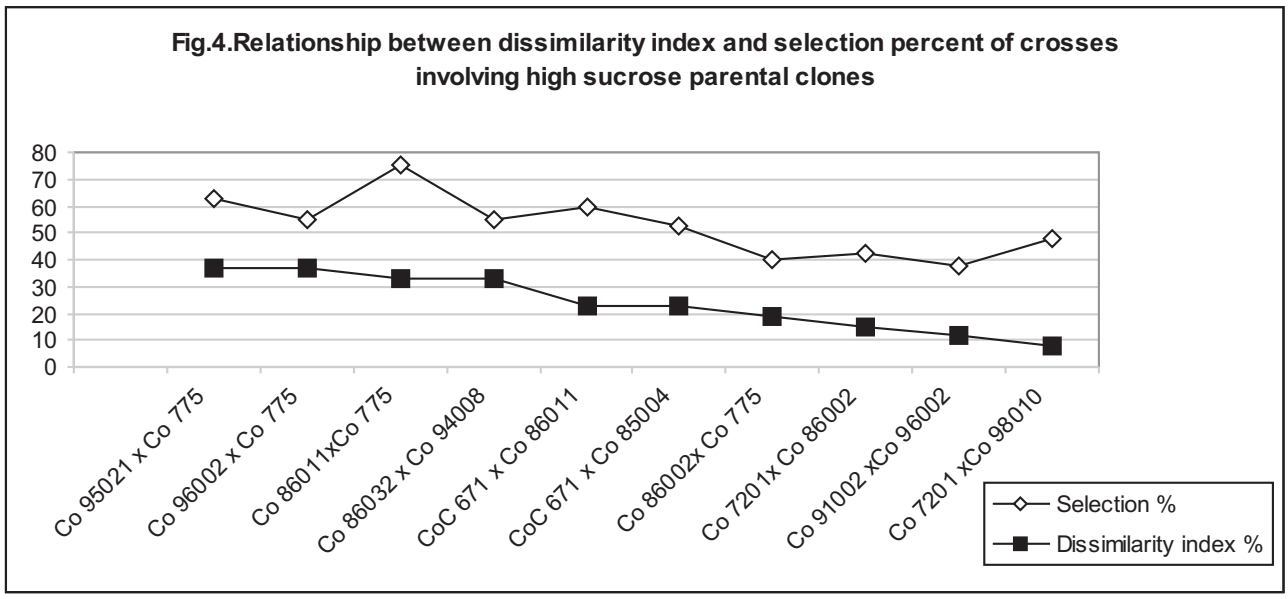


Fig. 4. Relationship between dissimilarity index and selection percent of crosses involving high sucrose parental clones

level of polymorphism was detected in sorghum from sorghum genomic SSR markers than from sorghum EST-SSR markers and in rice and durum wheat [16]; [17]. EST sequences being associated with the coding regions, are conserved and hence low amount of polymorphism is expected when compared with the genomic sequences.

Polymorphism generated by the primers ranged from 6% in NKSCSSR 52 to 97% in NKSCSSR 45. Though the PIC values of Genbank derived markers were reported to be low compared to those from the genomic libraries [6], the results of the present study involving Genbank derived sequences showed that the 30 STMS markers analysed were informative and produced distinctly different banding patterns in the investigated material through PAGE due to its ability to accurately resolve minor differences in length (Fig. 1 and 2). The primers NKSCSSR 1, 2, 23, 30, 32, 34, 38 and 52 were particularly useful in genetic diversity studies on account of their higher PIC values and more number of polymorphic markers.

Diversity estimates among the high sucrose genotypes from germplasm

The genetic diversity available within the primary high sucrose gene pool is a cause of concern, as the vast majority of commercial cultivars in the world owe their origin to a few early Coimbatore and Java interspecific hybrids [1] and for sucrose the variability available is not appreciative. Genetic diversity estimated in the investigated material based on three methods *viz*., Dice, Jaccard's and Simple Matching method were used to assess the diversity within the high sucrose parental material and to identify the more diverse types for quality improvement.

Using dice coefficient method

The overall mean genetic similarity was 0.69. Among the 82 clones, the highest mean similarity value was 0.81 in Co 200003, while Co 200001 had the lowest overall genetic similarity of 0.47. Higher genetic similarity of 0.90 was observed between Co 94015 and Co 200002 and Co 94015 and Co 86005. The phylogenetic tree drawn using Dice coefficient method gave two clusters (A and B). The cluster A is divided further into 13 sub clusters, while cluster B had a single clone *viz*., Co 200001. The sub cluster 1 and 12 are subdivided further into two groups having two and four sub-clusters respectively. The dendrograms which depicted the genetic similarity among the 82 sugar rich clones were shown in Fig. 3. The dendrograms showed that Co 91017 and Co 89005 were identical within the cluster 12c. The cluster 13 showed Co 200002 and Co 86005 as identical clones.

Using Jaccard's coefficient method

The overall mean SI among the 82 sugar rich types was found to be 0.54. The highest mean SI was found in Co 200003 (0.69), while the lowest mean SI was 0.31

Table 3. Number of crosses, with mean and standard deviation of the four traits, selection percent and dissimilarity index values of cross combinations studied in first clonal trial

		Mean				Standard deviation				Selection %	Dissimilarity index %
		NMC	C.dia	Brix	C.ht	NMC	C.dia	Brix	C.ht		
1	Co 95021 x Co 775	15.28	2.49	20.37	164.88	7.18	0.21	1.81	33.16	62.5	37
2	Co 96002 x Co 775	16.95	2.47	19.67	180.38	7.95	0.26	1.92	30.31	55.0	37
3	Co 86011xCo 775	15.03	2.46	20.56	173.88	6.74	0.23	1.50	30.46	75.0	33
4	Co 86032 x Co 94008	18.50	2.39	19.59	175.13	9.50	0.28	2.24	30.58	55.0	33
5	CoC 671 x Co 86011	12.83	2.43	19.29	170.63	8.20	0.26	3.20	38.27	60.0	23
6	CoC 671 x Co 85004	13.33	2.43	19.77	169.75	7.28	0.26	2.50	32.38	52.5	23
7	Co 86002x Co 775	11.73	2.44	18.86	177.35	8.87	0.28	3.15	32.42	40.0	19
8	Co 7201x Co 86002	13.25	2.42	18.70	155.13	9.40	0.30	3.28	34.13	42.5	15
9	Co 91002 xCo 96002	9.40	2.67	19.78	130.75	7.78	0.21	1.66	38.00	37.5	12
10	Co 7201 xCo 98010	12.58	2.45	19.80	161.38	7.05	0.24	1.80	38.93	47.5	8
	Mean	**13.89**	**2.46**	**19.64**	**165.92**	**7.99**	**0.25**	**2.31**	**33.86**		
	Mean –SD	5.89	2.21	17.33	132.06						

found in Co 200001. Genetic similarity of 0.95 was found between the pairs Co 94015 and Co 86005 and Co 94015 and Co 200002. The lowest genetic similarity of 0.10 was found between Co 86013 and Co 86014. The dendrogram is divided into cluster A and B. The cluster A was subdivided into 15 sub clusters, while cluster B had only one clone Co 200001. The cluster 14 is subdivided further into four sub clusters 14a to 14d. The clones Co 200002 and Co 86005 were identical and were grouped under the cluster 15. In cluster 14c, the clones Co 91017 and Co 89005 were seen as identical.

Using simple matching method

The overall mean SI using Simple Matching coefficient was 0.74. The highest mean SI was 0.87 found in Co 7201, while the lowest overall similarity index of 0.57 was observed in Co 96011. The lowest genetic similarity of 0.40 was found between Co 86001 and Co 86013 and Co 87009 and Co 86002. The clones Co 91017 and Co 89005 were identical and were in the cluster 15a, so too the clones Co 200002 and Co 86005 and were included in cluster 15b.

STMS marker diversity based on Jaccard's matrix gave less weight to matching bands than the Dice index. This was used in an attempt to minimize errors resulting from scoring different bands as identical. Though the SI values (0.69, 0.54 and 0.74 in Dice, Jaccard's and SM method respectively) varied generally these analysis did not reveal any difference in genetic relationships between the three measures, showing that fortuitous identical scoring of different STMS markers was either minimal or undetectable in the present study. This was supported by 72% bootstrap values. All the three coefficient analysis showed similar results with regard to the pairs of more similar genotypes. Though the highest and the lowest SI values were different, all the three types of analysis showed similar trend in similarity among the clones. The pairs with the highest similarity and dissimilarity were also the same. The existence of moderate genetic diversity in the investigated material was revealed by the overall mean SI values. Earlier studies with AFLP system detected an average polymorphism rate of 48 per cent in Brazilian cultivars [18] and 62.8 per cent in Indian cultivars [19] that detected higher level of polymorphism in Indian sugarcane cultivars. In this study the information generated with 30 primers was used to give a high confidence level in clonal identification and genetic diversity study.

The observation of less amount of genetic diversity among the mutants and somaclones (0.83 and 0.76, 0.73 and 0.62, 0.86 and 0.79 in Dice, Jaccard's coefficient and SM method SI values respectively) compared to that derived through the conventional breeding programmes (mean genetic similarity of 0.73, 0.59 and 0.78 in Dice, Jaccard's coefficient and SM

method respectively) clearly showed that the extent of genetic differences brought about by somaclonal variation and induced mutation in sugarcane is very minor compared with conventional breeding.

Based on the SI values generated by SM method, 443 combinations out of 3321 possible combinations, were genetically more similar (SI = 0.83), 813 combinations were genetically more diverse (SI = 0.69) and 2206 were intermediate (SI = 0.70-0.82).Large number of diverse combinations in the available breeding pool explained the continued success of intervarietal hybridization in sugarcane aimed at evolving commercial hybrids in India. Genetic similarity measured by STMS markers in this study was lower than the earlier studies on a similar set of commercial hybrids using RAPD markers [20] and isozymes [21], but was in line with previous studies with STMS markers [6].

The present study, in general, reflected the existing pedigree relationships between the cultivars. The clones of common descent clustered together as in the case of the popular commercial varieties *viz.*, CoC 671 and Co 200002, Co 86032 and Co 97005. Similarly Co 94011 (Co 7201 x Co 8214) and Co 7201 in cluster three, four and twelve in Dice, Jaccard's and SM method respectively; Co 94015 (CoC 671 x Co 86250) and Co 93010 (CoC 671 x Co 62198) in cluster thirteen, fifteen and fifteen in Dice, Jaccard's and SM method respectively also clustered together. However, this kind of relationship was not always visible. For instance, Co 89038 and Co 87009 evolved from the cross CoC 671 x [57 NG 110 x *S. robustum*] and Co 7704 x CoC 671 showed more diversity (SI = 0.44). Poor correspondence between pedigree and molecular markers in sugarcane, in light of previous reports [3], [21] could be explained as the existence of high level of heterozygosity at the parental level, whereby the progeny differ substantially in phenotype and genotype. This finding has applied value in that use of hybrid derivatives from the same parental combination as specific combiners need not always lead to close breeding due to the existence of sufficient genetic divergence between them.

Hybridization and progeny analysis of crosses based on molecular diversity

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. Molecular marker technology is presently assisting the genetic improvement of sugarcane by identifying novel genetic variation, improving the heritability and expediting the production of elite genetic material, as well as providing improved understanding of the sugarcane genome [22]. Based on molecular diversity, flowering intensity and synchrony in flowering, ten crosses were made involving high sucrose parents. The crosses included four genetically diverse crosses, three genetically similar and three crosses with intermediate similarity values. Evaluation of 40 randomly selected progeny in the first clonal trial was followed, as this is the minimum population size to assess the cross performance in sugarcane [23, 24].Clonally propagated progeny was used in evaluation on account of low correlation observed between seedling and settling stages of progenies for economic characters [25]. Criteria of selection were based on evaluation of individual clones for H.R. Brix as an index of juice quality and NMC, cane height and cane diameter being the components of cane yield. Among these, H.R.Brix having high heritability is more effective in selection [25]. In the first clonal trial, those clones clones combining cane diameter = 2.4 cm, Hand Refractometer (H.R) Brix = 18.0 percent, number of millable canes (NMC) = 12, and cane height = 180 cm and with no adverse morphological traits were selected and carried forward to second clonal trial for further evaluation. The cross combinations viz. Co 7201 x Co 98010 (SI= 0.92), Co 91002 x Co 96002 (SI=0.88) and Co 7201 x Co 86002 (SI= 0.85) were genetically more similar, while Co 95021 x Co 775 (SI =0.63), Co 96002 x Co 775 (SI= 0.63), Co 86011x Co 775 (SI = 0.67) and Co 86032 x Co 94008 (SI= 0.67) were the most diverse combinations. The rest of the crosses viz. CoC 671 x Co 86011 (SI= 0.77), CoC 671 x Co 85004 (SI= 0.77) and Co 86002 x Co 775 (SI= 0.81) recorded intermediate values (Table 3). Details regarding the crosses made, number of clones evaluated, selection percent and dissimilarity index values are given in Table 3. The SP *values,* based on the number of progeny selected over the number of clones evaluated showed that the cross Co 86011 x Co 775 was the best with 75 % selection, while Co 91002 x Co 96002 (SP = 37.5 %) was the poorest.

Relationship between SP and dissimilarity index expressed in percentage (DS %) for individual crosses is given in Fig. 4. The crosses that gave higher selection percent were Co 86011 x Co 775 (SI = 0.67, SP = 75 %; Co 95021 x Co 775 (SI = 0.63, SP = 62.5%) and CoC 671 x Co 86011 (SI = 0.77, SP = 60%), while lower selection percent was observed in the crosses Co 91002 x Co 96002 (SI = 0.88, SP = 37.5%) and Co 86002 x Co

775 (SI = 0.81, SP = 40.0%). Selection percent was higher in all the crosses with SI values below 0.81, while the crosses with higher SI such as Co 91002 x Co 96002, Co 86002 x Co 775, Co 7201 x Co 86002 and Co 7201 x Co 98010 recorded low selection percent (37.5, 40.0, 42.5 and 47.5 percent respectively). The result thus showed the need for selecting those crosses with SI below 0.81 (genetically less similar) for obtaining higher rate of selection per cross. The result has applied value as *apriori* selection of cross combinations based on molecular diversity of parents including those from common descent would lead to deriving more number of elite types, thereby making varietal evolution process more efficient in the genetically complex crop of sugarcane.

Correlation between SP and SI was – 0.7265 (Fig. 4), indicating significant negative relationship between them and hence the usefulness of selecting parents based on STMS diversity for deriving a higher proportion of selectable types. However inspite of negative correlation between the two traits, selection did not increase with increase in diversity showing that moderate levels of diversity were sufficient to produce a higher proportion of selectable types in sugarcane. Thus, genetically more similar combination based on SI value could be identified for avoiding hybridization between them as a mean of preventing close breeding. The relationship between parental divergence and hybrid performance in grass family has been investigated in maize [26], oat [27], wheat [28] and rice [29], and both high and low correlations have been reported. However the extent that marker distance related to hybrid performance was found to depend on the genetic background of the prospective parents [30]. Significant positive correlation between Amplified Fragment Length Polymorphism (AFLP) and coefficient of parentage has been demonstrated in sugarcane and the study suggested the use of AFLP data to more accurately quantify the degree of relationship among sugarcane cultivars [18]. Diversity measures based on isozyme markers and STMS markers [31] and [6] have shown the need to identify genetically close combinations in order to avoid close breeding. It was concluded that estimates of genetic similarity based on molecular markers may provide more accurate information to plant breeding than the pedigree method, allowing breeders to perform hybridization more efficiently on a short term basis or strategically design the breeding program on a long term basis. The present study, in the light of these findings, highlights the use of molecular marker based diversity estimates for identifying genetically similar parental clones so as to prevent close breeding especially for quality improvement programmes. The results of the study have also shown that pedigree records alone may not be sufficient in selecting parents for heterosis breeding in sugarcane.

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