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Research Article

Identification of genetically diverse parental combinations in *saccharum* complex utilizing unigene microsatellite markers

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Abstract
Unigene sequence tagged microsatellite markers were used to analyze the genetic diversity among 30 sugarcane clones with different genetic background to identify diverse parents for utilizing in crossing program. Jaccards similarity co-efficient value based on the molecular marker indicated that eight new ISH and IGH clones *viz.*, IA 1504, SSCD 941, N_1 0107, 98 N_1 1305, 98 N_1 1303, GU 07-291, SSCD 1755 and SSCD 682 had low genetic similarity with commonly used parents. Genetic similarity coefficient and clustering of genotypes were worked out utilizing UPGMA method and the highest genetic similarity was observed between CoC 671 and Co 88025 (0.94) wherein Co 88025 was the somaclone of CoC 671. This demonstrated the efficiency of these molecular markers to differentiate even very closely related individuals. The lowest similarity was observed between ISH 100 and 9870101 (0.42) followed by 87 A 380 and ISH 100 (0.42). Clustering based on genetic similarity coefficient values grouped the genotypes into 8 distinct clusters. A total of 14 diverse parental combinations were identified and their utilization in crossing program is discussed. In addition, four clones *viz*., 87 A 380, IA 1504, ISH 100, N_1 0107 were identified by the presence of specific markers which will be helpful in identification of true hybrids when these clones are involved in hybridization programme.

Keywords
Saccharum, unigene, microsatellite markers, molecular diversity

Introduction

Sugarcane is the chief source of sugar which accounts for nearly 80% of total sugar produced in the world. In 2016-17, the total global sugar production was 171.47 million metric tonnes. India ranked 2nd in total sugar production next to Brazil with 22.2 million tonnes in 2016-17 accounting for 13 % of total world production (USDA, Sugar: world market and trade). Sugar production in India was highly fluctuating in the last decade between the lowest production of 12.7 million tonnes in 2004-05 and the highest production of 28.3 million tonnes in 2014-15. The average sugar production in the last five years was 24.64 million tonnes against the average consumption of 24.48 million tonnes which resulted in the carryover of excess sugar every year. Excess sugar production, lower sugar price and high cost of sugar and sugarcane production has dwindled the profitability of the sugar mills. Improving sucrose accumulation in commercial varieties and increasing the sugar production per unit area are the possible solutions to address these problems and also to meet the growing sugar consumption. Sugarcane is the classical example where wild species contributed directly to the revolution in improving sugarcane and sugar yield. Among the six species of *Saccharum* (*S. officinarum, S. spontaneum, S. robustum, S. barberi, S. edule and S. sinense*) only *S. officinarum* was the major contributor of sucrose genes in the development of commercial varieties**.**

Critical analysis of the pedigree and ancestry of the modern cultivars revealed that they are essentially derived from around 15-20 nobilized clones initially developed in India and Java utilizing only two *S. spontaneum* accessions (Roach., 1989). Repeated use of such genetically related nobilized clones for creating variability and selection of cultivars resulted in the narrow genetic base of the present-day cultivars globally. The immediate concern is the breakdown of these varieties to new pests and diseases, drought, salinity, extreme atmospheric temperature etc. due to global warming. It is imperative that urgent action is required to broaden the genetic base of the working sugarcane germplasm for use as parents to develop commercial varieties. Broadening of parental gene pool of sugarcane can be achieved by introducing new accessions of basic species in breeding program. In order to exploit the new sugar genes from *Saccharum* complex, ICAR-

Sugarcane Breeding Institute (ICAR-SBI), Coimbatore, India had initiated base broadening program and developed an array of genetic stocks involving new *S. officinarum* and other related species. Analysis of genetic diversity among these clones in improved gene pool poses several limitations due to polyaneuploidy (Sreenivasan *et al*., 1987), larger genome size (D'Hont and Glaszmann., 2001), presence of part of the genomes from different species and long duration.

Genetic base broadening program requires identification of diverse parents through genetic diversity studies utilizing different marker systems. Among these markers, molecular markers are more stable than morphological and biochemical markers. Microsatellite markers are very important among the available DNA based markers, as they are codominant and more evenly distributed throughout the genome. Sugarcane with large genome size requires large number of polymorphic markers to employ them in genetic diversity studies, DNA finger printing, identification of hybrids, selection of diverse parents and marker aided selection. Microsatellites markers which generally exhibit high polymorphism information content had been successfully used in sugarcane crop for fingerprinting studies (Pan *et al.*, 2000, Pan *et al.*, 2007), cross transferability among *Saccharum* and related genera (Singh *et al.*, 2011a, Singh *et al.*, 2011b), genetic diversity using chloroplast SSR markers (Raj *et al.*, 2016), DArT arrays (Heller-Uszynska *et al.*, 2011) and association mapping for red rot (Singh *et al.*, 2016). Although microsatellite markers were more reliable and used for different purposes, a major disadvantage of the EST-derived microsatellites is the sequence redundancy that yields multiple sets of markers at the same locus. The problem of redundancy in EST databases can be managed by assembling the random EST sequences into unique gene sequences called unigenes (Parida *et al.*, 2006). In barley, around 41,600 EST sequences were efficiently assembled into 1,240 unigenes. The Unigene-based microsatellite markers would therefore, have the advantages of unique identity and positions in the transcribed regions of the genome. With the availability of large unigene databases, it is now possible to systematically search for microsatellites in the unigenes. Unigene derived microsatellite markers (UGSM) can be used for accurately assaying functional diversity in the natural populations and the available germplasm collections as well as for comparative mapping and evolutionary studies as anchor markers (Parida *et al.*, 2006). Thus, unigene microsatellite markers are one of the most efficient markers but limited information is available in sugarcane (Singh *et al.*, 2014).

ICAR-SBI is pioneer in developing pre-breeding genetic stocks utilizing different species and genera in *Saccharum* complex. Before employing them in breeding program for developing commercial varieties, genetic diversity among these clones must be estimated and the best diverse combinations of parents must be identified to generate larger variability. The study was aimed to identify newly developed pre-breeding clones which were genetically diverse.

Materials and Methods

Diverse sugarcane genetic materials including commonly used parents (CoC 671, Co 88025, Co 89010, Co 0218, Co 94008, Co 0230, Co 8371, BO 91, ISH 100), new ISH, IGH pre-breeding clones (986158, 985921, 971236, 973908, 9860088, ISH 12, N_1 0107, 984860, 98 N_1 1305, 98 N_1 1303, 98 N_1 1401, 98 N_1 1405, GU 07-291, SSCD 941, SSCD 1755, SSCD 682, SSCD 479, IA 1504) which were developed from new accessions of *Saccharum* species, near commercial hybrids (9870101, 87 A 380) and a *Saccharum officinarum* clone (NG 77-18) were used (Table1) for molecular marker analysis. Closely related clones viz., CoC 671 and Co 88025 (somaclone of CoC 671) were included to test the efficiency of the unigene markers in discriminating these two genetically related clones.

DNA was isolated from the fresh leaves using the method described by Doyle and Doyle (1990). Purification of DNA was done using Phenol: Chloroform method. Quantification of DNA was done using a Nanodrop USA (v 2.1) and stored at -20°C for further use. Working stock was adjusted to 10ng/µl with double distilled water.

Twenty-one UGSM primers developed by Singh *et al.*, (2011b) were used in the study (Table 2). Microsatellite marker analysis was done using the genomic DNA of the 30 clones as templates. Every 10µl PCR mixture contained 20 ng template DNA, 0.5U Taq polymerase (Merck, India), 1µL 10X reaction buffer, 200 µmole dNTP's (Merck, India), 7.5 µmole of forward and reverse primers. 35 cycles of PCR were carried out in Eppendorf Master Cycler gradient programmed as initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 1 minute and appropriate annealing temperature for 1 minute (temperature dependent on primer), extension at 72°C for 1 minute and final extension at 72°C for 7

minutes. The PCR products were size separated by 8% polyacrylamide gel electrophoresis, visualized by silver staining and documented using Alphaimager gel documentation unit.

All clear, unambiguous markers were scored as 1 and 0 for the presence or absence of markers and 100bp DNA ladder was used for determining the molecular weight of the markers using AlphaeaseFC software (v4.0). Similarity analysis was performed through Jaccard co-efficient method and clustering of genotypes was carried out by UPGMA method using NTSYSpc v 2.0 software. PIC was calculated using the formula $1-\sum P_{ij}^2$ (Anderson *et al.,* 1993).

Results and Discussion

Unigene derived STMS markers were used to analyze the genetic diversity among 30 sugarcane clones sharing gene pool involving *S. officinarum, S. robustum, S. spontaneum* and *E. arundinaceus*. Use of such microsatellite markers as an effective tool in germplasm analysis, MAS and parent selection (Cordeiro *et al.,* 2000) and differentiating closely related sugarcane clones (Hemaprabha *et al.,* 2006) were well demonstrated. The unigene microsatellite markers were derived from various predicted functional genes, which corresponded mostly to sugar metabolic enzymes, structural proteins, hypothetical proteins, transcription and translation factors, unknown proteins and signal transduction pathway genes (Singh *et al.,* 2011b). Among the 30 unigene primers used, UGSM 573 produced a maximum of 16 markers and primer UGSM 515 produced a minimum of 2 markers. The average number of markers produced by all the primer pairs was 9.80 while Singh (2011b) reported an average of 14.37 alleles in a highly diverse study material consisting of nineteen accessions including six sugarcane cultivars, one inter- specific hybrid, five related species, four related genera, and three diverse genera by using 27 UGSM primers.

Polymorphism Information Content (PIC) is an effective parameter in evaluating the efficiency of molecular markers. High variation for PIC was observed among the 21 primers based on the markers generated by them (Table 2). Maximum PIC of 0.97 were recorded by the primers UGSM 573 and UGSM 542 respectively and the lowest PIC of 0.79 was observed with the primers UGSM 515. UGSM 407 had the high PIC value of 0.96 while Singh *et al.,* (2014) also reported high value of 0.86 with this primer. Primers UGSM 432, UGSM 452, UGSM 576 and UGSM 581 recorded 100% polymorphism while the primers UGSM 574, UGSM 515 and UGSM 443 showed 50% polymorphism. The high level of polymorphism accounted to the complex genetic structure of *Saccharum* complex and high efficiency of the markers employed. Effectiveness of unigene microsatellites in dissecting the complex genetic structure of sugarcane was described by Singh *et al.,* (2014). Correlation coefficient between the number of markers generated and number of polymorphic markers was high (0.90) and statistically significant. All the primers which generated ≤4 markers recorded the lowest polymorphism percentage (50 %) compared to the primers producing large number of markers (10≤) showing 100% polymorphic markers. Hence for diversity studies the primers generating more number of markers will be very useful for wide coverage of sugarcane genome.

Efficiency of UGSM markers were evaluated utilizing two genetically related clones viz., CoC 671 and Co 88025 (somaclone of CoC 671). The highest genetic similarity was observed between CoC 671 and Co 88025 (0.94). Out of 21 primers used, 5 primers (UGSM 436, UGSM 482, UGSM 519, UGSM 574, UGSM 581) generated polymorphic markers between them which accounted to 23.81% of the primers. The efficiency of the unigene microsatellite markers were demonstrated with the identification of 6 polymorphic markers between them generated by these five primers. This indicated the efficiency of UGSM in discriminating closely related clones and justified using them in genetic diversity analysis.

Estimates of genetic similarity coefficient revealed high genetic variation among the clones. The average genetic similarity among clones was 0.62. The lowest similarity co-efficient was observed between ISH 100 X 9870101 (0.42) and 87 A 380 X ISH 100 (0.42). While 9870101 and 87 A 380 were near commercial clones developed by several generation of backcrossing with noble canes/commercial canes, ISH 100 was an earlier generation interspecific hybrid involving *S. officinarum, S. spontaneum* and *S. robustum*. Another interspecific hybrid ISH 12 had relatively high genetic similarity of 0.59 and 0.70 with 9870101, 87 A 380 compared to ISH 100. This may be due the fact that ISH 12 had only *S. officinarum* and *S. robustum*, the latter is considered as the progenitor of *S. officinarum* whereas ISH 100 had additional genome of *S. spontaneum*, a wild species which contributed much variability to the commercial hybrids in addition to *S. officinarum* and *S. robustum*. Mean genetic similarity between commonly used 9 parents and 21 genetic stocks was 0.58, which

showed diversity present among the clones. Less than 0.5 genetic similarity was observed between 23 pairs of clones.

Genetic relatedness of *S. robustum* with *S. officinarum* was analysed by estimating genetic similarity values between NG 77-18 (*S. offcinarum*) with four groups of genotypes with different species constituents. In addition to *S. officinarum* group 1 had *S. robustum*, group 2 had S. *spontaneum* & *S. robustum*. Group 3 had *S. spontaneum* and group 4 had *S. spontaneum* and *Erianthus arundinaceous*. The genetic similarity value between NG 77-18 and group 1 with *S. robustum* was the highest (0.845) indicating that *S officinarum* and *S. robustum* are very closely related. With the additional genome of *S. spontaneum* in the group 2 the genetic similarity value was 0.72 indicating the diversity provided by *S. spontaneum*. Low genetic similarity value of 0.63 with group 3 and 0.62 with group 4 revealed that *S. spontaneum* and *E. arundinaceous* are diverse from *S. officinarum*. *Saccharum robustum* Brandes & Jesw. ex Grassl the wild species found in Papua New Guinea had been considered as the immediate progenitor species of cultivated sugarcane (*S. officinarum* L.) (Artschwager and Brades., 1958, Brandes 1929). Irvine (1999) also reported that *S. officinarum* and *S. robustum* were closely related in morphology, cytology and physiology, differing primarily in fiber and sugar content. Because of the genetic relatedness between *S. officinarum* and *S. robustum* the later was used less in genetic base broadening program, compared to *S. spontaneum*.

Clustering of 30 clones based on the similarity coefficient was done through UPGMA method and 8 major clusters were constructed (Fig.1). Cluster VIII had single genotype ISH 100 which was a trispecies hybrid. The Cluster VI also had a unique clone Co 94008, a progeny of two commercial hybrids Co 7201 and Co 775. The members of the cluster IV *viz*., N_1 0107, 98 N_1 1305, 98 N_1 1303, 98 N_1 1401, 98 N_1 1405 were the immediate progenies of the cross N_2 1902 X IK 76-81. Alienation of clones with *E. arundinaceus* (IK 76-81) from the other clones with other *Saccharum* species background indicated wide genomic difference between these two genera. Strong molecular differentiation between *Erianthus* and *Saccharum* was previously demonstrated with SSR markers (Govindaraj *et al*., 2012), DNA spacers (Al-janabi *et al.,* 1994), RFLP (Burnquist *et al.,* 1995), AFLP (Besse *et al.,* 1998), 5S RNA intergenic spacers (Pan *et al.,* 2000) and TRAP markers (Alwala *et al.,* 2006). The phylogram analysis also distinguished *Erianthus* from the rest of *Saccharum* complex based on the cytoplasmic markers (Raj *et al.,* 2016). It was interpreted that among the species and genera in *Saccharum* complex, *Erianthus* was considered to be primitive (Daniels and Roach 1987) and might have evolved in different historical pathway (Sobral *et al.,* 1994). Of late *E.arundinaceus* has been used in introgression breeding program, to generate new and larger variability for high biomass, high tillers, fast growth, resistant to biotic and abiotic stresses. In general, cluster analysis clearly revealed that pedigree of the clones and their genome composition had significantly contributed to the genetic relatedness among them.

Molecular diversity analysis brought out several potential cross combinations involving one commonly used parent and other parent from newly developed ISH and IGH hybrids viz., IA 1504, SSCD 941, N_1 0107, 98 N_1 1305, GU 07-291, 98 N_1 1303, SSCD 1755 and SSCD 682. These identified clones had wild species (*S. spontaneum, S. robustum, E. arundinaceus*) as one of their immediate parents which are known for their ability to withstand biotic and abiotic stress. IA 1504 was an immediate progeny of the cross between Co cane and *S. spontaneum.* This was found to be a good male parent for making diverse crosses with parents viz., CoC 671, Co 8371 and ISH 100 (Table 3). Similarly, GU 07-291, a relatively high sucrose clone can be used for making crosses with ISH 100 and Co 94008 to generate potential progeny. Another hybrid (Co cane X *S. spontaneum*) clone SSCD 941, was found to be genetically diverse from ISH 100, hence this specific cross combination can be attempted. The identified cross combinations can be utilized in breeding programs to impart stress tolerance in progenies. Among the ISH clones, ISH 100 was also found to have low genetic similarity with parents CoC 671, Co 88025, Co 94008 and BO 91 which can also be utilized in crossing programs, especially for imparting drought tolerance as ISH 100 possess high tolerance to water stress.

In the identified diverse cross combinations, CoC 671 was widely used as parent possessing early ripening, smut resistance, high sucrose (20.88%) and juice purity (94.56%) (Table 5). Co 0218 had moderate resistance to red rot, resistance to smut, drought and salinity tolerance, good ratooning ability and better quality jaggery production. It also had 20.82% of sucrose with high single cane weight of 1.23 kg. Co 8371 was a mid-late maturing clone which was resistant to smut, moderate resistance to red rot and tolerant to water logging and drought. In

general, all female parents have relatively more height (> 200cm). IA 1504 and SSCD 941 had *S. spontaneum* in their immediate pedigree and this wild species, had contributed significantly in incorporating biotic and abiotic tolerance, high tillering and wider adaptability to the modern sugarcane varieties. Critical analysis of mean performance for agronomical trials indicated that one of the parents in selected combinations had high sucrose content, cane diameter and single cane weight, while the other parent possessed cane height, number of millable canes etc., the main yield contributing traits thus complementing for improving cane yield and juice quality. In the two identified cross combinations viz., CoC 671 X IA 1504 and Co 0218 X SSCD 941, the female parents namely CoC 671 and Co 0218 had high sucrose accumulation and cane girth while IA 1504, SSCD 941 had high NMC and cane height for combining cane yield and juice quality in the progeny. Likewise, Co 94008 had high sucrose accumulation, cane girth and single cane weight which can be crossed with IA 1504 and ISH 100 possessing high NMC and cane height. Co 8371 had low NMC and medium sucrose accumulation which can be crossed to IA 1504 for improving tillering ability.

Sugarcane microsatellite had been a valuable tool in clone identification (Govindaraj *et al*., 2013) and analysis of genetic makeup of sugarcane genome (Corderio *et al.,* 2000, Perera *et al.,* 2012, Aitken *et al.,* 2014). Critical observation on the markers generated by 21 UGSM identified 9 specific markers unique to 6 clones (Table 4). Four clones were identified by the presence of specific markers viz., 87 A 380 (UGSM 361_{532}, UGSM 407_{529}), IA 1504 (UGSM 358_{450}, UGSM 436_{161}), ISH 100 (UGSM 436_{220}) and N_1 0107 (UGSM 432_{683}). Unique markers which were absent in specific clones were also identified. Three clones were identified by the absence of markers viz., N_1 0107 (UGSM 576_{234}), Co 89010 (UGSM 482_{191}) and Co 94008 (UGSM 407_{319}). Although limited number of markers used, unigene microsatellite markers could alienate specific clones with the presence or absence of marker. Cordeiro *et al*., (2003) demonstrated the ability of unigene derived markers to produce reproducible results and to assess the sugarcane germplasm. These unique markers cab be used in identification of true hybrids when these clones are involved in crossing programme.

The stability and reproducibility of microsatellite markers that are derived from Unigene markers proved to be a viable tool in sugarcane germplasm evaluation studies (Cordeiro *et al.,* 2000). In the present study, unigene SSR primers demonstrated the superior power of discriminating even the closely related clones *viz*., CoC 671 and its somaclone Co 88025. Varietal identification and finger printing of poly-aneuploidy sugarcane varieties can be precisely done with this group of markers. High polymorphism also indicated that these markers can be effectively used for genetic diversity studies (Raj *et al.,* 2016) in the genetic enhancement program. Fourteen new diverse parental combinations identified in the present study can be utilized in sugarcane breeding programs for developing new improved varieties with high yield, juice quality, and wider adaptability.

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Table 1. Pedigree and genome constitution of the genetically diverse parents used

S.No.	Clone	Remarks	Parentage	Genome constitution
1	CoC 671	Commercial hybrid	Q 63 x Co 775	SS, SO
2	Co 88025	Commercial hybrid	Somaclone of CoC 671	SS, SO
3	986158	Near commercial clone	BGC 25021 x Co 87045	SS, SO
4	Co 89010	Commercial hybrid	Co 6304 x Co 775	SS, SO
5	985921	Near commercial clone	BGC 25021 x Co 8365	SS, SO
6	971236	Near commercial clone	931236 x Co 775	SO, SS
7	973908	Near commercial clone	Co 86010 x CN2237272	SO, SS
8	9860088	Near commercial clone	BGC 25021 x Co 88037	SO, SS
9	Co 0218	Commercial hybrid	Co 8353 x Co 86011	SO, SS
10	87 A 380	Commercial hybrid	CoC 671 x CoA 7602	SO, SS
11	Co 94008	Commercial hybrid	Co 7201 x Co 775	SO, SS
12	Co 0230	Commercial hybrid	CoLk 8102 x Co 775	SO, SS
13	9870101	Near commercial clone	Co 8353 x Co 86011	SO, SS
14	ISH 12	Interspecific hybrid with *S. officinarum* and *S. robustum* genomes	(57 NG 110 x *S. robustum*) (57NG 77 x 57NG 80)	SO, SR
15	Co 8371	Commercial hybrid	Co 740 x CoC 671	SO, SS
16	N_1 0107	First generation hybrid with *E. arundinaceus* genome	N_2 1902 x IK 76-81	SO, SS, EA
17	BO 91	Commercial hybrid	BO 55 x BO 43	SO, SS
18	NG 77-18	*S. officinarum*	*S. officinarum*	SO
19	984860	Near commercial clone	CN1C850 x Co 8353	SO, SS
20	ISH 100	Third back cross progeny of *S. robustum* genome	Co 7202[CoC 671(57NG 110 x *S. robustum*)]	SO, SS, SR
21	98 N_1 1305	First generation hybrid with *E. arundinaceus* genome	N_2 1902 x IK 76-81	SO, SS, EA
22	GU 07-291	Near commercial clone	Co 99006 x GUK 00-909	SO, SS
23	98 N_1 1303	First generation hybrid with *E. arundinaceus* genome	N_2 1902 x IK 76-81	SO, SS, EA
24	98 N_1 1401	First generation hybrid with *E. arundinaceus* genome	N_2 1902 x IK 76-81	SO, SS, EA
25	SSCD 941	Co cane x *S. spontaneum* hybrid	Co 8371 x SES 574	SO, SS
26	SSCD 1755	Co cane x *S. spontaneum* hybrid	Co 86249 x SES 590	SO, SS
27	98 N_1 1405	First generation hybrid with *E. arundinaceus* genome	N_2 1902 x IK 76-81	SO, SS, EA
28	SSCD 682	*S. spontaneum & S. officinarum* hybrid	Co 1148 x SES 404	SO, SS
29	IA 1504	Indo-American hybrid between co cane and *S. spontaneum*	Co Cane x *S. spontaneum*	SO, SS
30	SSCD 479	*S. spontaneum & S. officinarum* hybrid	BO 130 x IND 82-228	SO, SS

SS – *Saccharum spontaneum* SO – *Saccharum officinarum* EA – *Erianthus arundinaceous* SR – *Saccharum robustum*

Table 2. Sequence information and polymorphism information content of the primers

S.no	Primer	Sequence 5' – 3' Fwd & Rev	No. of bands	No. of polymorphic bands	Poly morphism percentage	PIC
1	UGSM 597	ACTCTAATGACTCTTCTTCCCA GTGAATGCTGCTTACTTTGTC	5	4	80.00	0.89
2	UGSM 573	CAGAGCATCACCAGCACC CTTGAGCAGCGTCTTGTT	7	4	57.14	0.95
3	UGSM 361	GTTCTTAGTCCAGCCGTAGTT ATCGTTGTTGTCGGTGTC	12	10	83.33	0.95
4	UGSM 387	TAAATATGGTGGAGCAAAGTATTA ACTAGAGCTCTTAAATTCCACAGT	7	6	85.71	0.90
5	UGSM 394	ACTCCTCCCGCCTCCACTAC CTCACCGAAGCAATCAAG	14	12	85.71	0.95
6	UGSM 351	AAGAAGAGCCGTAGAAACAAC ATTGAGCGAGGGATGAAC	14	13	92.85	0.95
7	UGSM 354	ACTGACACACACGCACAC TGGAAGTGAATGAAGCGA	14	13	92.85	0.95
8	UGSM 358	ACCCTTCCCATTCCCATC CTCCAGGTTCGCCACCAC	10	8	80.00	0.95
9	UGSM 407	AGGAGGACTACGAGGAGAAG AACAAGAACAATCACAAGGAA	15	11	73.33	0.96
10	UGSM 432	CTCCGAGAACGTCTGCGTGT TGTTCTCAAACCTGGTGTAAC	10	10	100.00	0.93
11	UGSM 436	CACTTCCCAGAGACCCAG GACCTTAGCAATCAAGACAGA	12	7	58.33	0.96
12	UGSM 443	AGTGAGTGAAGAAGAGCCAG AAGAACAACCGAAGGAGATT	4	2	50.00	0.91
13	UGSM 452	CACCGCAGCCTGACACAGAACC AGGAACTCAGCATACTCGTGAC	11	11	100.00	0.94
14	UGSM 482	GTGAATCTGCAGGCTGCTGGAAG GCACTAGTCACTACTACACACGC	7	6	85.71	0.91
15	UGSM 513	GAACCACGGAACCCACTC CTACGACCACCAGTCACAC	8	7	87.50	0.92
16	UGSM 515	ATCTTTCTCGTCCGCCTC CCTTGGTTCGGTCTATGTT	2	1	50.00	0.79
17	UGSM 542	ACCTCCACCTCCACCTCAGTTC CGTTCAGCTTCAGGGTGTCGAT	13	8	61.53	0.97
18	UGSM 573	CAGAGCATCACCAGCACC CTTGAGCAGCGTCTTGTT	16	10	62.50	0.97
19	UGSM 574	GCTTCCTCGCTCCTCCTC TACTTCTACCTCGTCTGCTTC	4	2	50.00	0.89
20	UGSM 576	TATTCAGTCATTCGTTTCGTT GTTCCATACAAGCAGTAGCC	11	11	100.00	0.94
21	UGSM 581	CACACTGACACCTACCAATGA GCCAAATACAACGAACGA	10	10	100.00	0.93

Table 3. Diverse cross combinations identified based on similarity coefficient between the pair of clones

S. No	Commonly used Parents	Genetically Diverse clone
1	CoC 671	IA 1504
2	Co 88025	-
3	Co 89010	-
4	Co 0218	SSCD 941
5	Co 94008	N_1 0107, ISH 100, 98 N_1 1305, GU 07-291, 98 N_1 1303, IA 1504
6	Co 0230	-
7	Co 8371	IA 1504
8	BO 91	-
9	ISH 100	GU 07-291, SSCD 941, SSCD 1755, SSCD 682, IA 1504

Table 4. Clone specific markers identified with UGSM based DNA fingerprints

S. No	Clone	Presence of Marker	Absence of markers
1	87 A 380	UGSM 361_{532}, UGSM 407_{529}	-
2	IA 1504	UGSM 358_{450}, UGSM 436_{161}	-
3	N_1 0107	UGSM 432_{683},	UGSM 576_{234}
4	ISH 100	UGSM 436_{220},	-
5	Co 89010	-	UGSM 482_{191}
6	Co 94008	-	UGSM 407_{319}

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Table 5. Agronomic performance of diverse cross combinations identified

		Performance of Female Parent						Performance of Male Parent					
S. No	**Diverse Cross**	**Cane height (cm)**	**Single cane weight (kg)**	**Girth (cm)**	**NMC**	**Sucrose % juice**	**purity%**	**Cane height (cm)**	**Single cane weight (kg)**	**Girth (cm)**	**NMC**	**Sucrose % juice**	**purity%**
1	CoC 671 xIA 1504	200	1.10	3.05	26	20.88	94.56	200	0.25	1.52	31	11.94	78.14
2	Co 0218 x SSCD 941	230	1.23	3.04	51	20.82	92.41	290	0.41	2.09	36	15.02	82.85
3	ISH 100 x Co 94008	285	1.81	3.10	43	16.72	87.80	170	0.53	2.81	38	17.55	87.79
4	Co 94008 x IA 1504	170	0.53	2.81	38	17.55	87.79	210	0.25	1.52	31	11.94	78.14
5	Co 8371 x IA 1504	250	1.36	3.34	30	16.84	86.93	210	0.25	1.52	31	11.94	78.14
6	ISH 100 x GU 07-291	285	1.81	3.10	43	16.72	85.34	235	0.31	1.68	85	11.38	80.25
7	ISH 100 x IA 1504	285	1.81	3.10	43	16.72	87.79	210	0.25	1.52	31	11.94	78.14

NMC- Number of millable canes per 20 feet row

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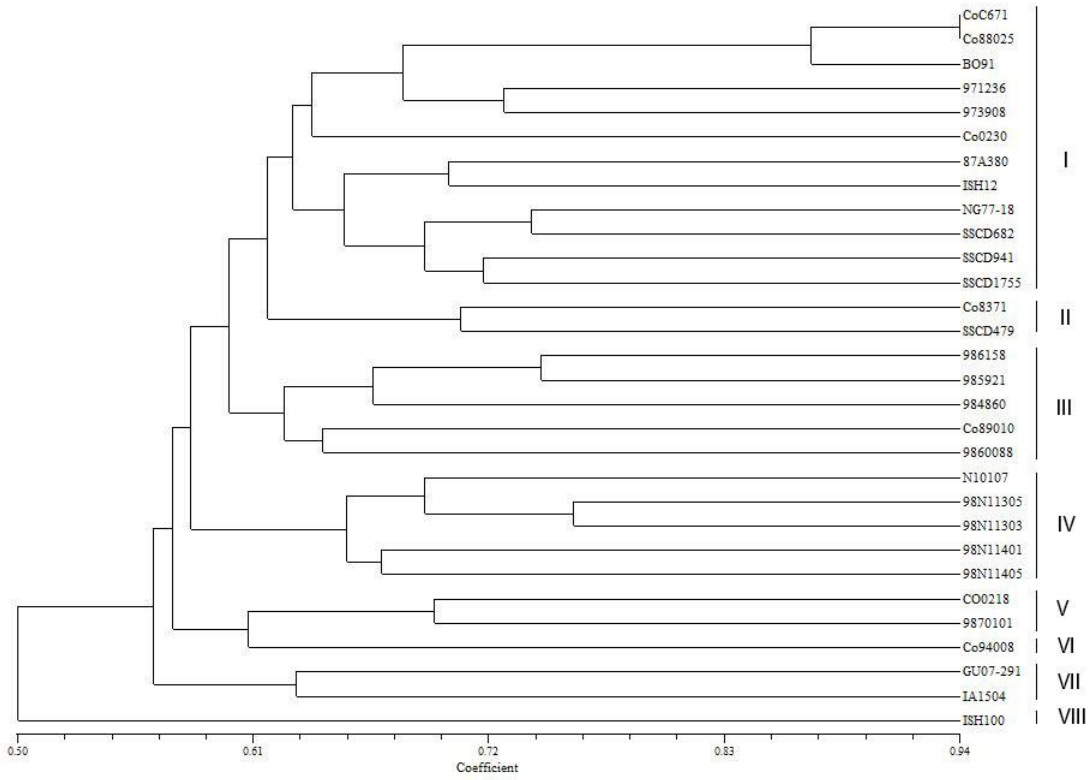

Fig. 1. Grouping of commonly used parents and new genetic stocks based on marker data generated by UGSM primers