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Evaluation of microsatellite markers for genetic diversity analysis among sugarcane species and commercial hybrids

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Abstract

Increasing sugar productivity is the main concern of sugarcane (*Saccharum officinarum*) breeding programs. The complexity and size of the sugarcane genome is a major limitation in genetic improvement. Characterization of sugarcane wild germplasm provides essential information on genetic diversity that breeders utilize for crop improvement. The objective of this study was to evaluate the utility of 7 sugarcane cDNA derived microsatellite markers (SCM), 9 genomic microsatellites and 16 unigene sugarcane microsatellite markers (UGSM) for accessing genetic diversity and inter-relationships in sugarcane germplasm collections. Eighty four genotypes from the *Saccharum barberi, S. spontaneum, S. officinarum,* Indian and non Indian commercial cultivars were used in the study. The UGSM and SCM yielded a higher mean number of alleles per locus and superior polymorphism information content (PIC) values than the sugarcane genomic markers (SGM). The number of amplified fragments ranged from 4 (UGSM312) to 14 (UGSM667) indicating that high polyploidy and hetrozygosity exist in sugarcane. The *Saccharum spontaneum* clones retained significantly higher number of DNA fragments (20%) than *S. barberi* clones (14%). Based on cluster analysis, the 84 *Saccharum species* clones and commercial cultivars were grouped into 10 distinct classes. Clusters showing grouping of individual genetic relationship among the sugarcane species clones, Indian and non Indian sugarcane commercial cultivars based on geographical origin, available pedigree information, adaptation zone and morphological characters. The cultivar B29-228, *S. spontaneum* clones N-56, N-75 and Inter-specific hybrids (ISH-112) were found most diverse pairs but unfortunately these were not extensively utilized in the development of other sugarcane cultivars. These sources which have desirable agronomic characteristic should be used as progenitors for the creation of cultivars with a wider genetic base.

Keywords: SSR, RFLP, RAPD, TRAP, UPGMA, Jaccard's Similarity coefficient; Boot Strap Values

Abbreviations: EDTA-ethylenediaminetetraacetic acid; UGSM- Sugarcane Unigene derived microsatellite markers; SCM-cDNA derived; microsatellite marker; SGM-sugarcane genomic microsatellite markers; ISH-interspecific Hybrids; NICH-non-Indian commercial hybrids

Introduction

Sugarcane (*Saccharum* spp. hybrids) is a genetically complex crop of major economic importance in tropical and sub tropical countries. It is mainly used for sugar production but recently gained increased attention because of its employment generation potential and recent emphasis on production of bio-fuels. Ethanol is an alternate source of energy and its production from sugary or starchy material also makes sugarcane as a future bio-fuel plant. Considering the current needs of cane industry it is imperative to breed high sugar producing varieties that also have other desired agronomic traits. Modern sugarcane genome is a complex blend of aneuploidy and polyploidy. It is derived from the interspecific hybridization involving different *Saccharum* species particularly *S. officinarum* and *S. Spontaneum*. The importance of the wild species *S. spontaneum* L. (x=8, 2n=40-128) was realized after its successful hybridization with the domesticated sugar-producing species. It is characterized by low sugar content, thin stalks, high fiber, high ratooning ability and high resistance to biotic and abiotic stresses. Among the five species of the genus *Saccharum, S. spontaneum* has the widest distribution, extending from Afghanistan in the west to the Malaya peninsula, Taiwan and the South pacific island in the east (Alexander, 1973). *S. officinarum* (x=10, 2n=8x=80) on the other hand is best represented by the two commercial

hybrid varieties Co205 and Co285 which replaced the indigenous cultivated varieties of northern India (Sreenivasan et al., 1987). It represents cultivated sugarcane and is characterized by high sugar content, thick stalks, low fiber and low disease resistance. Most of the varieties in the world are breed of these two species of genus *Saccharum*. To minimize the negative effects of *S. spontaneum* and to retain the high sucrose producing ability of *S. officinarum* during crosses, a series of back-crosses were made between the interspecific hybrids and the *S. officinarum* parents. This led to the "nobilization" of *Saccharum spp*. hybrids (Sreenivasan et al., 1987).

This was a major breakthrough in sugarcane varietal improvement programs in the terms of improved sugar productivity, high disease resistance and high ratooning ability. Although nobilization was highly successful due to limits of the gene pool exploited during traditional breeding programs, very limited progress has been achieved in increasing sugar content (Sreenivasan et al., 1987; Lima et al., 2002; Pan et al., 2004). The success of sugarcane breeding program therefore lies in the proper choice of rich and genetically diverse parents. The search of genetically diverse parents can be based on geographical origin, agronomic traits, and pedigree data or molecular markers data (Melchinger, 1999).

Morphological characters based genetic diversity estimates suffer from the drawback that theses are limited in number and influenced by the environment (Van Beuningen and Busch 1997). Therefore, techniques which can measure the genetic relationship without any influence of environmental factors and phenotype properties are the need of future breeding program.

Molecular marker analysis offers an efficient measure of genetic relationships on the basis of genetic characteristics. Among these molecular marker techniques, DNA based markers which include restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite or simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) are of significance in crop improvement (Ali MA. et al., 2008). These markers are extensively being used to increase the understanding of genetic and taxonomic complexity of various agricultural crops. The desirable attributes of these markers encouraged its development (Cordeiro et al., 2000) and utilization to achieve agronomical desirable traits in sugarcane (Roshi et al., 2003; Aitken et al., 2005). *Saccharum sp.*, which has a complex polyploid genome, requires more number of such type of informative markers for various applications in genetics and molecular breeding. Microsatellites or Simple Sequence Repeats (SSRs) are tandem repeats, of one to six nucleotides long DNA motifs, present in eukaryotic genome. Microsatellites has gained considerable importance in molecular breeding due to their desirable genetic attributes like hypervariability, wide genomic distribution, co-dominant inheritance, reproducibility, multi-allelic nature and chromosome specific location. These markers are suitable for paternity determination, mapping of useful genes, marker- assisted selection and for establishing evolutionary relationship (Parker et al., 2002; Aitken et al., 2005). SSR markers have been extensively used in genetic diversity studies in many plants, including jute cultivars (*Corchorus* sp. Akter et al., 2008), Sweet sorghum (*Sorghum bicolor L*, Ali ML. et al., 2007), triticale (*X-Triticosecale Wittmack*; Kuleung et al. 2006, wheat (*Triticum aestivum L.*; Fufa et al. 2005; Mahmood et al. 2004), and pearl millet (*Pennisetum glaucum L.*; Budak et al. 2003). This work includes evaluation of SSR markers, their polymorphic potential, and assessment of genetic diversity among 84 sugarcane genotypes. Sugarcane genotypes used from the assessment of genetic diversity includes sugarcane species clones, commercial cultivars of Indian and non-Indian commercial hybrids. Two hundred eighty four SSRs primer pairs were evaluated on a group of fourteen randomly chosen genotypes. On the basis of their capacity to generate polymorphic bands only thirty two pair of highly polymorphic SSRs was used to estimate the diversity among all the 84 the accessions (Table 2). Out of these 32 microsatellite markers used, 7 were developed from sugarcane cDNA microsatellite markers (SCM), 9 sugarcane genomic microsatellite markers (SGM), and 16 unigene derived microsatellite markers (UGSM). Genetic relationships would be useful in utilization and management of the genotypes during breeding programs. The information obtained from diversity analysis is useful in making the crosses and selection of divergent parents to maximize heterosis.

Materials and method

Plant material

Genotypes used in the experiment were grown at our Institute's farm. These eighty four genotypes include twenty one non Indian commercial hybrid viz., B29-228, B34-104, B-49-228, POJ2818, POJ2878, POJ2883, CP33-130, CP33-320, CP349-377, CP36-105,CP44-120, CP5011, Q49, Q68, PR-1048, H6538, H5174, TUC521, HM223, HP89 and H35-263, three Saccharum sinense clone viz., UbaWhite, Malani and Kheli, three Saccharum barberi clone, viz., Dhaulu, Khatuya and Maneria-IPM-1552, twenty one *saccharum spontenium* clone, viz., SES594, SES51517, Calcutta, Ramsal, Gajraula, BG-10, BG-15, Pusa-2, Pusa-9, Lal-Kuan-1, Baheri-2, N-91, N-129, N-56, N-144, N-290, N-75, N-58, N-87, N-176 and WS-18 and five inter specific hybrids viz. ISH112, ISH35, ISH43, ISH163 and ISH111 and rest thirty were the commercial cultivars of tropical and subtropical parts of the Indian subcontinent, viz., CoS510, CoS687, CoS767, CoS8432, CoS8436, CoS91269, CoSe92423, CoSe95422, CoS95255, CoS96269, CoS96275, CoSe96436, CoS97261, CoS97264, CoSe98231, CoSe01235, Co213, Co331, Co621, Co337, Co1111, Co1148, Co1158, Co89003,

Table 1. The parentage, place of origin and the nature of genotypes used in the study

Genotype(Origin)	Parent	Genotype (Origin)	Parent	Genotype (Origin)	Parent
***Saccharum Barberi* Jesw 2n= 81-124**		***Saccharum officinarum* L 2n= 80**		**National Commercial Hybrids**	
ManeriaIPM-1552 (India)	Wild	Dr Lal (India)	Wild	CoS97261 (UPCSR, India)	$70A_2GC$
Khatuya(India)	Wild	**Non-Indian Commercial Hybrids**		CoS8436 (UPCSR, India)	MS6847xCo1148
Dhaulu(India)	Wild	CP33-130 (USA)	NICH	CoS97264 (UPCSR, India)	$70A_2GC$
***Saccharum spontaneum L* 2n=42-128**		CP349-377(USA)	NICH	CoS510 (UPCSR, India)	Co453xCo557
Pusa-2 Bihar (India)	Wild	CP33-320(USA)	NICH	CoS95255 (UPCSR, India)	Co1158xCo62198
Pusa-9 Bihar (India)	Wild	CP36-105(USA)	NICH	CoS8432 (UPCSR, India)	MS6847xCo1148
Baheri-2 (UP, India)	Wild	CP44-120(USA)	NICH	CoS96269 (UPCSR, India)	BO108xCo1148
SES594 (UP, India)	Wild	CP5011(USA)	NICH	CoS96275 (UPCSR, India)	CoS8119xCo62198
SES51517 (UP, India)	Wild	Q49 (Australia)	NICH	CoS91269 (UPCSR, India)	BO91xCo1158
BG-10 (UP, India)	Wild	Q68(Australia)	NICH	CoS767 (UPCSR, India)	Co419xCo313
BG15 (UP, India)	Wild	H6538 (Hawai)	NICH	CoS687 (UPCSR, India)	Co976xCo312
N-91 (Bihar, India)	Wild	H5174 (Hawai)	NICH	CoSe95422 (UPCSR, India)	BO91xCo453
N-129 (Bihar, India)	Wild	PoJ2818 (Indonesia)	NICH	CoSe01235 (UPCSR, India)	CoS8119xCo62198
N-56 (Bihar, India)	Wild	PoJ2878 Indonesia	NICH	CoSe92423 (UPCSR, India)	BO91xCo453
N-75 (Bihar, India)	Wild	PoJ2883 Indonesia	NICH	CoSe96436 (UPCSR, India)	BO91xCo62198
N-58 (Bihar, India)	Wild	B49-228 (Barbados)	NICH	CoSe98231 (UPCSR, India)	CoS7927xCo775
N-87 (Bihar, India)	Wild	B29-228 (Barbados)	NICH	UP39 (UPCSR, India)	Wild
N-176 (Bihar, India)	Wild	B34-104 (Barbados)	NICH	Co89003(Coimbatore,India)	Co7314xCo775
N-290(Bihar, India)	Wild	Tuc521 (Tucuman)	NICH	Co331 (Coimbatore, India)	Co213xCo214
N144(Bihar, India)	Wild	PR10-48 (China)	NICH	Co1158 (Coimbatore, India)	Co421xCo419
WS18 (WB, India)	Wild	Hm-223 (Hawai)	NICH	Co331 (Coimbatore, India)	Co213xCo214
Calcutta(WB, India)	Wild	HP89 (Hawai)	NICH	Co213 (Coimbatore, India)	POJ213xCo291
Gajraula (India)	Wild	H35-263 (Hawai)	NICH	Co1111 (Coimbatore, India)	Co421xCo312
Lal-kuan (India)	Wild	**Inter Specific Hybrids**		Co1148 (Coimbatore, India)	P4383xCo301
Ramsal (India)	Wild	ISH-112(Coimbatore, India)	ISH	CoH56 (Haryana, India)	Wild
***Saccharum sinense* Roxb; 2n=111-120**		ISH-35 (Coimbatore, India)	ISH	CoLk8102(Lucknow, India)	CO1158GC
Malani (P.R.China)	Wild	ISH-43 (Coimbatore, India)	ISH	BO52 (Bihar, India)	Wild
Kheli (P.R.China)	Wild	ISH-163(Coimbatore, India)	ISH	BO28 (Bihar, India)	Wild
Uba white (P.R.China)	Wild	ISH-111(Coimbatore, India)	ISH	BO91 (Bihar, India)	BO55xBO43

NICH; Non-Indian Commercial Hybrids
ISH; Inter Specific Hybrids

CoH56, CoLk8102, UP39, BO52, BO28 and BO91 (Table1).

DNA extraction

For Genomic DNA extraction, disease free, whorl, young, immature leaves were collected from the institute form. Sample was freeze-dried and then stored at -86^0 C. Genomic DNA was extracted from young leaf tissues of each genotype using modified CTAB method (Hoisington et al., 1994). Approximately 500 mg leaves were separately ground to fine powder in liquid nitrogen using pre-chilled mortar pestle. Fine powder was transferred in to 25ml of tube containing 10ml pre-warmed CTAB buffer. CTAB buffer was prepared using 2% (w/v) CTAB, 20 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), and 0.2% (v/v) β-mercaptoethanol. |Sample was incubated in water bath up to one hour at 60^0C. Sample was taken out from water bath and putted in room temperature for 15 minutes. Then equal volume of choloroform:Iso-amyl alcohol (24:1) was added and followed by centrifugation at 12,000 rpm for 10 minute. Sample was taken out from ultracentrifuge and aqueous phage was pipette out in a 25 ml autoclaved tube. Equal volume of chilled Iso-propanol was added in aqueous

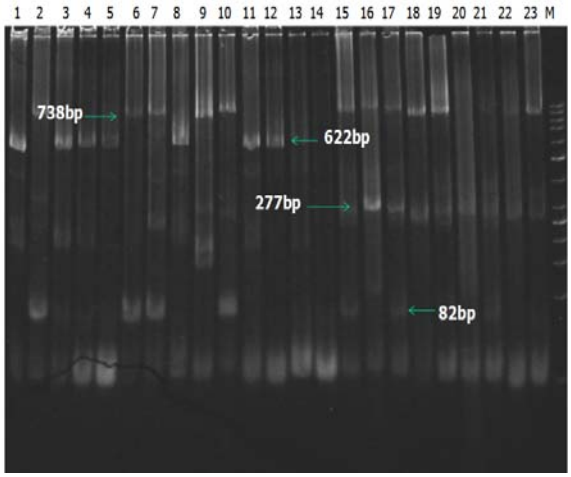

Figure 1. Molecular Diversity of S*accharum spp.* clones along with non-Indian commercial hybrid showing polymorphism with UGSM-302, Lane 1-N-290, 2-Pusa 9, 3-Baheri, 4- Lalkuan, 5-N-144, 6-BG 15, 7-Pusa 2, 8-N 56, 9-N 129, 10-Gajraula, 11-N 91, 12-Ramsal, 13-BG 10, 14-Colcutta, 15-SES 515/7, 16-H-5174, 17-Q 68, 18-SES 594, 19- Uba White, 20-H 35-263, 21-CP 44-120, 22-Khatuiya, 23- CP 36-105.

Table2. Details of SSR markers with their annealing temperature (Ta), sequences (forward and reverse), product size, range of product size and total number of amplified bands and total number of polymorphich bands given by the primer (in small bracket) (Parida et al., 2008).

	Primer Name	Repeat Motif	Forward Primer (5-3) Reverse Primer (3-5)	Ta (^{0}C)	Range of Product Size(bp)	No of Bands[a]
1	SCM4	(CGGAT)4	CATTGTTCTGTGCCTGCT CCGTTTCCCTTCCTTCCC	52	705-134	10(10)
2	SCM15	(CAG)5	GGAGATGTTTGAGAGGGAA AGAGTAGCATAAAGGAGGCAG	55	145-624	6(5)
3	SCM16	(TCCG)4	GTGCGAGAGGAACTGTGT AGCCCTGCCTAACAAGGA	50	123-792	11(11)
4	SCM18	(ATAC)3	CATCAGTATCATTTCATCTTGG CAGTCACAGTCGGGTAGA	55	199-694	10(10)
5	SCM21	(GGCC)3	CCCTCCCATAACACACAC TTGACAGCCCAAAGAGTT	50	514-774	8(7)
6	SCM27	(GCAA)4	TTCTCTGACTTCCAATCCAA ATCAAGCACGCCCGCCTC	55	279-714	6(6)
7	SCM32	(TCG)4	GATGAAGCCGACACCGAC AGTTGCCTGTTCCCATTT	50	156-770	11(11)
8	SOMS58	(ACC)7	CCGCTTTCAACCTCTACAC GGCTTGGTGATTCTTCTCT	52	99-1237	8(7)
9	SOMS118	(AT)9cacca...(A)12	GAGGAAGCCAAGAAGGTG TAGAGCGAGGAGCGAAGG	55	82-1018	11(10)
10	SOMS120	(GAG)6ctctga..(GTGA)6(GA)11	GCATCTATCGGTCTTCTGG ATCCAATCCTTCATCTTCTTC	52	84-1155	11(11)
11	SOMS124	(AT)6gatat...(A)87	TAGAGGAAATAGCAGAACAGG AGACTGACACCTTTGAGATGA	52	93-1057	8(8)
12	SOMS135	(TA)6aatat...(T)10	TCTTCAACTTCCTCTGCCT GTTCCTGACTGTTCCCTTG	52	210-929	6(6)
13	SOMS143	(AC)6cttc..(A)17	TGACTTGGAATAACACAAAGAA ATGGGATGGATAATAAGCAGT	55	137-555	8(7)
14	SOMS148	(GAA)5gggca...(GAG)5	GATGACTCCTTGTGGTGG CTTGACGACCCTGCTGCT	52	193-1008	8(7)
15	SOMS154	(AAG)5gaga..(CAG)5	CTCGTTTCATAGCAGACCTT GCAACTGGAGGAACTGATG	52	54-1101	5(5)
16	SOMS156	(CGG)5ctgg..(TTC)5	ATCGTCTCTGGTTGTTGGT ATCCTCCATTTCCACCTC	52	62-593	7(6)
17	UGSM60	(CGA)7	CGACTCCACACTCCACTC CCGAACACCACCTTCTTG	55	92-759	7(6)
18	UGSM193	(GA)8	AGATATAACACACACACACAAA GGCCATCGAGGAGGAGTTCAAG	55	53-787	13(13)
19	UGSM296	(TC)7	ATTATCTACATTCAGACACGTCAC ATCTTTGTTAGCAATCCATTAAG	55	357-1054	5(4)
20	UGSM301	(TC)7	GAAGAAGAAGAAGAAGAAGAAGAA ACTCGTCCTACAACCACGACTAC		79-725	8(7)
21	UGSM302	(AT)7	GAAGAAGAAGAAGAAGAAGAAGAA ACTCGTCCTACAACCACGACTAC	50	82-738	8(7)
22	UGSM312	(GA)7	AACGTATCTTTATTTCCATTCTTC CTTTCAGTTCAACTTTGGATAAAT	58	200-583	4(3)
23	UGSM504	(GCT)7	TAGAGGAAATAGCAGAACAGG AGACTGACACCTTTGAGATGA	56	168-224	5(5)
24	UGSM542	(GA)9	ACCTCCACCTCCACCTCAGTTC CGTTCAGCTTCAGGGTGTCGAT	55	53-1123	12(11)
25	UGSM565	(GCA)6	CATAGCAAGCACCACCTC TCTTCTTCTCGTCCACCC	53	348-539	8(8)
26	UGSM574	(ACG)6	GCTTCCTCGCTCCTCCTC TACTTCTACCTCGTCTGCTTC	53	65-1026	13(12)
27	UGSM575	(AGC)6	CTGTTTCCTTCCTTCTCGT CAATCATAGCCCAGACACC	53	66-901	12(11)
28	UGSM585	(CGT)6	GAAGAGGAGGAGAGGAGAAG TGGGATGGTTGTTGACTG	53	62-648	12(11)
29	UGSM665	(CAC)6	GTTACCATCCCATCCCAC TGTCCCTCGTTCACAGAC	53	147-770	10(10)
30	UGSM667	(CTC)6	CTATCCTCTTGTTGGGTCCT TCCGCACCTCCGTTCACC	56	54-1063	14(13)
31	UGSM671	(AGG)5	TCCCTACTTCTATGAATATCCTTC TTGACAAATTGCTTGATGTAGT	55	96-571	6(6)
32	UGSM681	(AC)8	ACACATCGCTTTCCCACA GCATACCTGTCGTCGTCT	55	94-592	10(9)

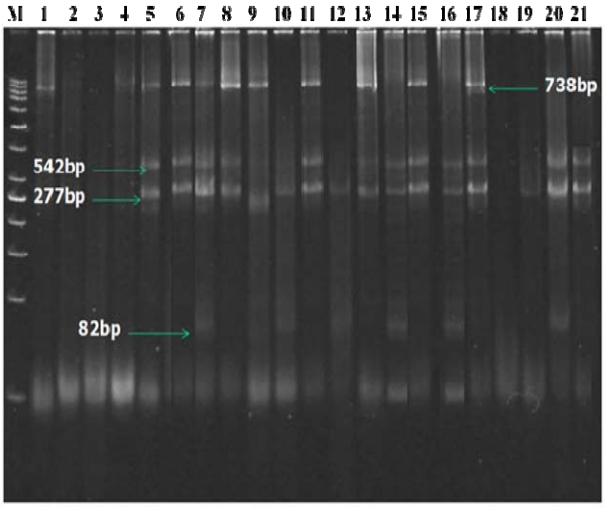

Figure 2. Molecular diversity of elite commercial clone of Indian and non-Indian commercial hybrid showing polymorphism with UGSM 302, Lane 1-CoS97261, 2-CoS8436, 3-CoS97264, 4-Co621, 5-Co89003, 6-CoS95255, 7-Co337, 8-CoS8432, 9-CoS96275, 10-CoS91269, 11-CoS767, 12-Co1111, 13-Co1148, 14-CoS687, 15-CoH56, 16-B 49-228, 17-CoSe01235, 18-CoSe92423, 19-CoSe96436, 20-CoSe98231, 21-B34-104.

phage to precipitate the DNA. Precipitated threads of DNA were pipette out in a 2ml appendoff using wide bore tip followed by centrifugation at 12,000 rpm for 10 min. Milky white pellet was washed with 70% alcohol; air dried, and resuspended in 10 mM Tris at pH 8.0. To degrade RNA content, RNAse treatment was given for 1hour at 30^0C. The quality and quantity of the genomic DNA was checked on 0.8% agarose gel and diluted appropriately for working concentration of 25ng/µl concentration.

PCR amplification and PAGE

Thirty two SSR primers pairs were used to determine the diversity. SSR primers were developed at National Research Centre on Plant Biotechnology (NRCPB), Indian Agricultural Research Institute (IARI), New Delhi, India under our collaborative 'National Network Project on Sugarcane Genome' funded by Department of Biotechnology, Govt. of India (Parida et al., 2008). These sequences of oligonucleotide were synthesized from Bangalore Ge Nei^{TM} ferm by Sugarcane Research Institute, Shahjahanpur, India (Table 2). PCR reaction was carried out in a total of 10 µl volume containing 25 ng template DNA, 1.0 µl of each forward and reverse primer, 100 mM of dNTPs, 0.5 U of taq DNA polymerase, 1.0 µl of 10X PCR buffer and 2.5 mM of $MgCl_2$. Amplifications were performed in a Peltier thermal cycler (MJ Research) in following conditions: initial denaturation at 94°C for 5 minute followed by 25 cycles amplifications. Each amplification cycle was initially at 94°C for 1 minute fallowed by annealing temperature (Ta) for 1 minute and then 72°C for 2 minute; final extension at 72°C for 7 minute was allowed. The amplified products were stored at 4°C. The amplified products were separated by 7.5% denaturing polyacrylamide gel electrophoresis in 0.5X TBE buffer. Visualized of the bands was done by 0.5µg/ml ethidium bromide staining. Gel photographs were taken under UV light in GelDoc system (Alpha Innotech).

Genetic diversity estimation

Bands were scored for the presence (1) or absence (0) in all 84 genotype and this binary data was used to calculate the Jaccard's Similarity coefficient (JS) module of the software FreeTree (Hampl V et al., 2001). Genetic distances between each pair of lines were estimated as D = 1-JS. Clustering was based on a similarity matrix using a heuristic and well resolved algorithm unweighted pair group method with arithmetic average (UPGMA), with freeware program FreeTree (Hampl V et al., 2001). Most universal re-sampling technique bootstrapping was used to estimate confidence levels of inferred relationships. TreeView, drawing software was used for interactive visualization of the dendrogram (Page RDM, 1996).

Results

Use of sugarcane microsatellite markers as a potential cost effective method for molecular diversity analysis of sugarcane is described. Molecular diversity was analyzed in 84 genotypes of various S*accharum spp.* and hybrids. After repeating the result three times on PAGE only clear and unambiguous bands were taken for scoring (Fig. 1, 2). An example of the obtained SSR profile is shown in Fig.1&2. A total of 281 loci were generated, out of which 94.0% were polymorphic with a mean of 8.78 alleles per SSR locus (Table 2). The highest level of polymorphism was detected in UGSM followed by SCM and SGM. Complex banding patterns were encountered in sugarcane with the number of amplified fragments ranging from 4 (UGSM312) to 14 (UGSM667) (Table 2). Maximum number of bands (14) produced by SSR primer UGSM667 and least (4) by UGSM312. Fragments size ranged from 62 (SGM156 and UGSM585) to 1237 bp (SGM58) in length.

Analysis of four *Saccharum* species and the fifty six cultivars including interspecific hybrids presented high level of genetic distances. Estimated genetic similarities (Jaccard's similarity) of elite sugarcane germplasm result a dendrogram (Fig. 3) and redial diagram (Fig. 4). Analysis of the SSR data using Jaccard's similarity coefficient measures of genetic identity and genetic distance showed that genetic similarity (GS) value ranged from 0.056 (B29-228 and N-75) to 0.778 (Co1158 and Co213) with a mean of 0.34 among sugarcane population, where as a low degree of similarity was also found between ISH112 & N56 (0.059), ISH112 & BG-15

(0.060), B29-228 & Gajraola (0.063). In the dendrogram, B29-228 and N-75 are in different clusters, diverse from each other and easily distinguishable among the genotypes tested (Fig 3, 4).

The cluster analysis grouped the 84 *Saccharum species* clones and commercial cultivars of sugarcane from tropical/subtropical part of India, PR China, Australia, Hawai, Indonesia, Barbados, Argentina and USA into 10 main groups. Different clusters of the tree indicated a clear pattern of division among the sugarcane genotypes. Internodes reveal a general complex structure between cultivars when examined comparatively in terms of pedigree information, geographical origin, adaptation zone and morphological characters.

Seven genotypes were clustered in group I, namely, Malani, CoH56, CoSe01235, CoSe92423, B49-220, Co1148 and CoS 687. CoSe01235, CoSe92423, Co1148, B49-220, CoH56 and CoS 687. Most of them share a common genetic background while Malani (*Saccharum sinense*), is unrelated from rest of the entries with regard to the apparent pedigree relationship. Group II included accessions CoS767, CoS8432, CoSe96436, CoSe98231, CoS8436, CoS97264, CoS95255 and CoS96275. Out of these eight cultivars, CoS767, CoS8432, CoSe98231, CoS8436, CoS95255 and CoS96275 have common parent. They all was high sugar, high yielding varieties which was very popular in subtropical part of India. In group III, out of nine, four genotypes, ISH-111, ISH-35, ISH-163 and ISH-43, developed at Sugarcane Breeding Institute, Coimbatore (TN), India. They clustered together as they shared the common parent in their pedigree. They are the product of second nobilization because they also have genes for red rot resistance and high sugar content. Interspecific crosses have been predominantly responsible for nobilization in sugarcane. Inclusion of Dr Lal (*S. officinarum*) in this group could not be explained by parentage information. Rest of the genotypes in this cluster has been developed from common *S. officinarum* ancestors. In group IV, three cultivars, BO28, BO52 and BO91, have common parent and origin. They were developed at Sugarcane Research Institute, Pusa, Bihar, India. In the group V, 4 genotypes were clustered together even they were from different geographical origin. But the reason behind the close similarity in clustering is the common parents. In group VI, seven genotypes were included; out of which Kheli and Khatauya are the basic clones of *S. sinense* and *S. barberi* respectively. These are closely related to CoLk8102 because Kheli was one of the parents of CoLk8102 (Table 1, Fig. 3, 4). Similarly grouping of two foreign commercial hybrids CP36-105 and CP44-120 together could be explained by their parental relationship. The group VII clusters included twenty six genotypes of which twenty one were wild S. *spontaneum* clones collected from different states of the Indian subcontinent. Four non-Indian commercial cultivars viz; HP35-263, CCP50-11, Q-68 and H-5174 were from the different biographical origin but the have *S. spontaneum* dominated blood in their genome. Presence of UbaWhite (*S. sinense*) could not be explained based on their genomic identity. In group VIII only two foreign commercial hybrids HP89 and PR10-48 were clustered together. Both of them have common ancestor but geographically they are different. In group IX, sixteen national and foreign commercial hybrids were clustered together due to involvement of common parents during interrogation (Table1, Fig 3, 4). Although some of the commercial cultivars in this cluster are from different geographical origin but due to the use of same *S. spontaneum* clones in their development, they share a close similarity. Last group X have only two wild clones, namely, Dhaulu and MineriaIMP-1552, which are showing similarity (0.32) in the dendrogram, because of their origin from same species (*S. barberi*). These results indicate the presence of large amount of genetic diversity between the majorities of genotypes analyzed. The most closely related commercial cultivars of tropical and subtropical Indian subcontinent (parent-descendant) yield similarities among them. Most of them occupy there place close to each other. This explains that recent cultivar has gained a saturation level in the characters and we to identify desired traits for future breeding programs. Cultivars are located in particular region of the tree and they do not form outstanding branches. The genotypes derived from the breeding program in Sugarcane Research Institute, Shahjahanpur (UP), India, exhibit a wide distribution, concentrated in the lower right portion of the tree in Fig. 4. Elite cultivars dispersed in the rest of the tree (Fig. 4), suggest the high ploidy level and the tradition of germplasm exchange among breeders in breeding programs.

Discussion

This is the first report on the use of genomic and cDNA derived microsatellite markers for finger printing and evaluation of genetic relationships in germplasm and commercial cultivars used at Sugarcane Research Institute, Shahjahanpur (UP), India. The present study determines the feasibility of the use of sugarcane derived genomic and cDNA microsatellite markers in establishing genetic relationship among the sugarcane species and commercial cultivars. Our study revealed a large degree of SSR polymorphisms within the material under study; only 6% of the markers being monomorphic. High levels of polymorphism and hetrozygocity were detected with an average number of 8.78 polymorphic fragments per primer pair. This relationship will be beneficial to explore their potentiality in varietal improvement programs. The level of polymorphism indicates that distinction between any two varieties is possible with appropriate SSR primer pair. This supports to the use of SSR markers, as an excellent tool, for diversity analysis and loci mapping. Polymorphism had been also reported by Nair et al. (2002) using RAPD markers on twenty eight tropical and subtropical Indian sugarcane cultivars. Up to the present time there are no reports on sugarcane genetic diversity using very high number of polymorphic

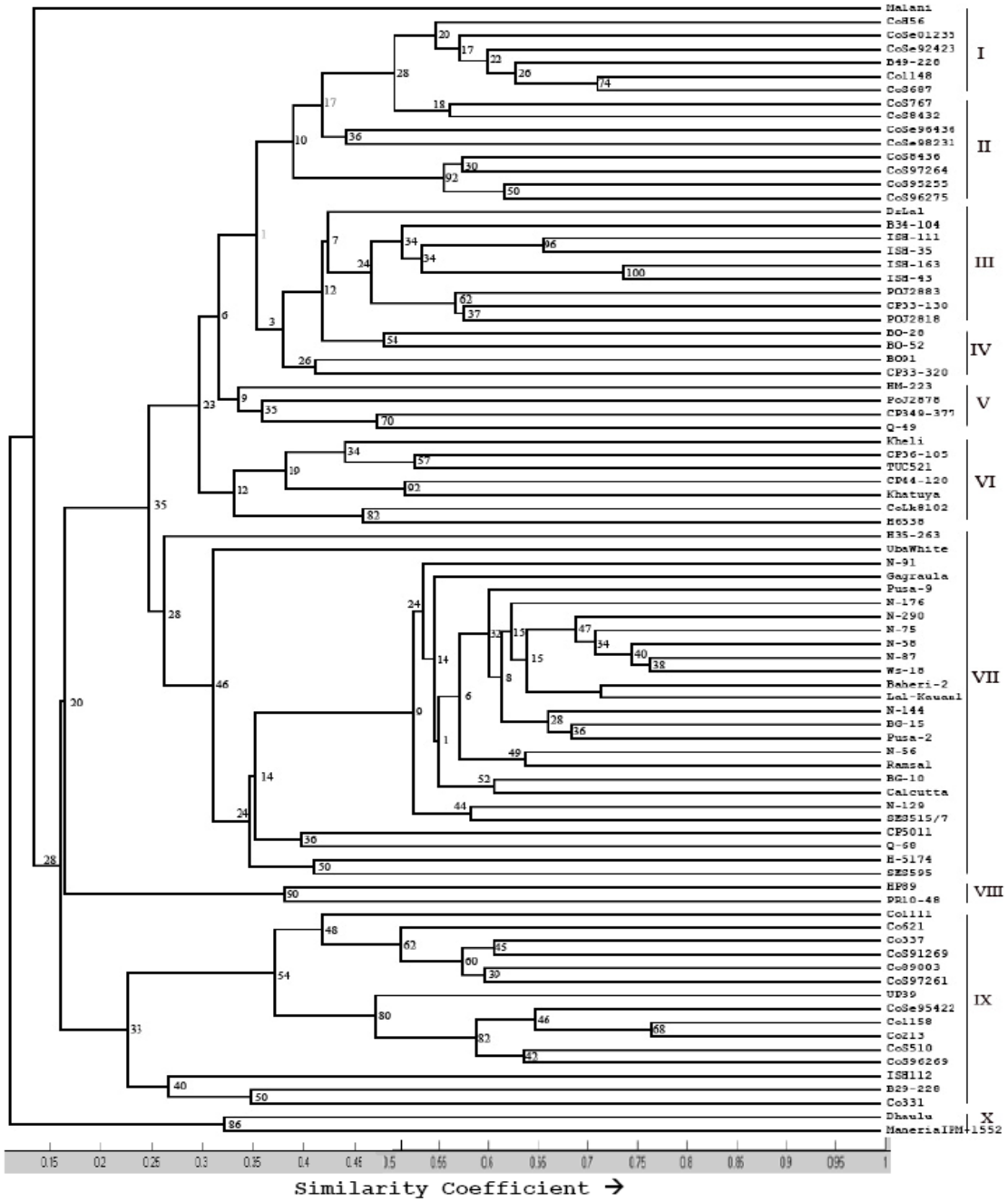

Fig 3. UPGMA tree showing genetic relationships among the sugarcane species clones, commercial cultivars of Indian and non-Indian commercial hybrids based on Jaccard's similarity coefficient. Numbers shown in the internodes of the tree represent percentage confidence level for particular branch, obtained in the bootstrap analysis.

bands such as those obtained in this research. With the objective of evaluating the genetic diversity among sugarcane cultivars, Lima et al. (2002) used 21 AFLP primers combination, evaluating a total 1121 polymorphic loci but they were not found high level of genetic diversity between the cultivars analyzed. Afghan et al. (2005) used 38 RAPD primers, evaluating a total of 258 discrete markers in 10 sugarcane genotypes. Ali et al. (2008) used Forty-one SSR markers that generated 132 alleles to estimate the genetic diversity among 72 sweet sorghum germplasm. The necessity of a high number of the marker for the complete mapping of sugarcane genome is justified by the complex genome, polyploidy structure and relatively narrow genetic base of this species. This large amount of molecular variation will allow a thorough analysis of the organization of quanti-

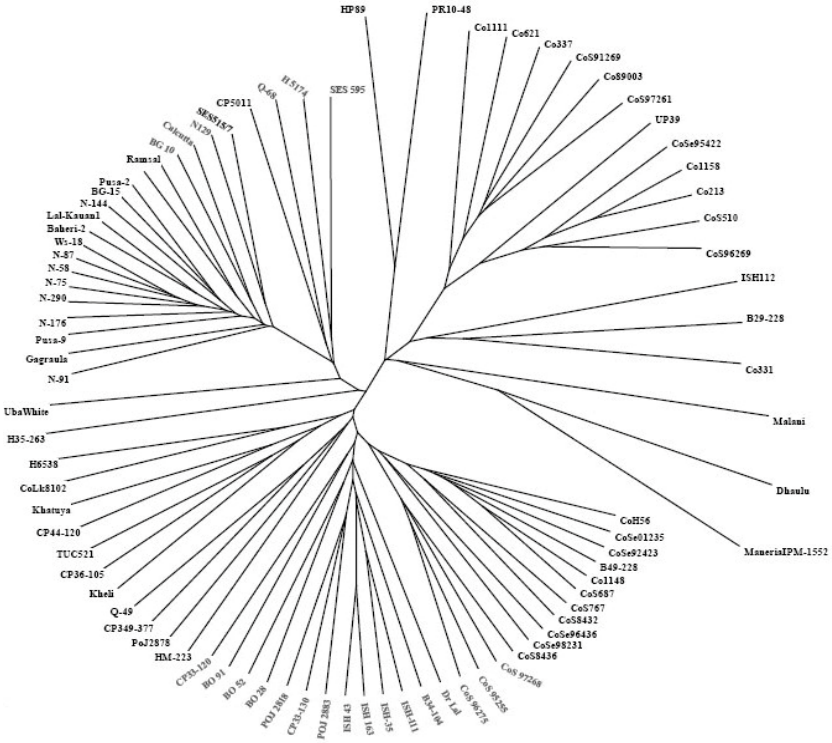

Fig 4. Unrooted UPGMA tree, calculated from SSR data (281 Loci), assembling 84 genotypes which were sugarcane species clones, commercial cultivars of Indian and non-Indian commercial hybrids.

tative traits in *Saccharum Species* and the commercial cultivars studied. The level of polymorphism detected by UGSM and SCM markers was comparatively higher than the microsatellite markers derived from the genomic sequences (SGM). It could be due the use of highly conserved coding sequences as against less conserved genomic sequences in their development. More Number of the polymorphic bands was observed in twenty one accessions of *Saccharum spontaneum* which shows that it is the most variable and diverse species among the *Saccharum* species. This also support that the main structuring part of the variability among the cultivars is due to alleles inherited from *S. spontaneum*. This finding is in agreement with Mary et al., (2006). Our results illustrate the large genetic contribution of this species and provide indications that it may be related to the specialization of the cultivars. This study supports that interspecific hybrids and commercial cultivars shares a great part of their genome with *S. officinarum*. This also explains that why these cultivars shared more unique bands with *S. Officinarum.* (Sreenivasan et al., 1987; d'Hont et al., 1996; Suman et al., 2008)

The tropical and subtropical identities of the cultivars also did not contribute to the clustering pattern because individual clusters include cultivars from both places. This supports that genetically similar cultivars are present in both the regions. Among the cultivars, Co312, B29-228, POJ2878 were found to be distinct and divergent from rest of the cultivars. The study results species which can be used new sources of germplasm in breeding programs. Commercial cultivars are showing more similarity with *S. officinarum* clones where as less similarity with *S. spontaneum*. It explains that principal component of diversity between cultivars may be due to the genetic influence of *S. spontaneum*. A very weak global topology observed in the dendrogram is in agreement with the profuse exchanges of parental materials between sugarcane breeding stations. Traces of linkage disequilibrium can be attributed to the distribution of *S. spontaneum* chromosomes among sugarcane cultivars (Mary et al., 2006). Genetic similarity coefficient ranging from 60.5 % to 88.5% has earlier been reported by Pan et al., 2004 in a collection of *S. spontaneum* clones using RAPD markers. *S. spontaneum* clones proved their wide distribution because of the presence of morphological as well as cytological (2n = 40-128) variation in their genome. They are divergent among all the genotypes used in the study (Table 1, Fig 2, 3), supporting the earlier reports based on RFLP (Burnquist et al., 1995). The low Jaccard's similarity of B29-228 with N-75 can be explained by the fact that farmer is an elite foreign commercial hybrid and later belongs to wild *Saccharum spontaneum* collection. This shows that these genotypes were geographically diverse and evolved independently either by interrogation (ISH112 and BG29-228) or by their wild nature (wild

collections of *S. spontaneum*). They had not been utilized in breeding programs, due to lack of the knowledge but have unique features for the improvement of high fiber, high ratooning ability and high resistance to biotic and abiotic stresses in sugarcane commercial cultivar. This is in agreement with the cultivar ploidy and its high heterozygocity in the genotypes because of number of alleles incorporated in the initial interspecific crossings (Lima et al., 2002). Ten major clusters illustrated in the dendrogram were connected at a similarity level of 0.34 with GS values ranging from 0.056 to 0.778. Low degree of similarity between genotypes from different species and geographical origin like ISH112 & N56, ISH112 & BG-15, B29-228 & Gajraola supports the use cultivars for future breeding programs. The S. spontaneum clones clustered in a clearly separate group, which is supported by previous findings of RAPD markers (Nair et al., 1999), TRAP markers (Alwala et al., 2006) and SRAP markers (Suman et al., 2008). Dr Lal (*S. officinarum* germplasm), shown great similarity with modern cultivated sugarcane cultivars and interspecific hybrids. It supports the origin of modern sugarcane cultivars, by crossing the *S. officinarum* clones with *S. spontaneum*, followed by a few backcrosses to *S. officinarum*. Present clustering analysis based on the segregation at SSR loci can resolve the genetic background issues of the cultivars with unknown pedigree. These relationships between Saccharum spp., hybrids of unknown genetic origin and cultivars with known parentage will help sugarcane breeders to select appropriate parents in their breeding programs to maximize yield as well as to maintain genetic diversity. Thus, it can be concluded that estimates of genetic diversity based on molecular markers may provide more accurate information to plant breeders than the pedigree method. It could help breeders in making reliable crossings on a short term basis or to strategically plan the breeding program on a long term basis. Genetic analysis has been hindered in sugarcane due to lack of sufficiently informative markers. Less information is available about the genetic diversity within and between Saccharum cultivars which has been based mainly on morphological characteristic. Thus, it can be concluded that estimates of genetic similarity based on molecular markers may provide more accurate information to plant breeder. This data will support the exploitation of sugarcane germplasm on molecular basis. SSR markers used in the study may also be used by researcher for genetic mapping and gene tagging in sugarcane. Locus mapping ability of these SSR markers will provide more information than those available through diversity. These markers may be used for construction of genetic map in sugarcane.

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