Evaluation of Genetic Diversity in *Saccharum* **Species Clones and Commercial Varieties Employing Molecular (SSR) and Physiological Markers**

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Genetic diversity of the 38 sugarcane (*Saccharum* spp.) genotypes was evaluated using 50 microsatellite (SSR) markers and seven morphological markers. A complex PCR banding pattern was observed in all the accessions with SSRs markers. The allelic polymorphism information content (PIC) values ranged from 0.105 to 0.790 with an average of 0.37, indicating markers ability to detect high levels of polymorphism. The value of genetic similarity (GS) co-efficient ranged from 0.33 to 0.84, indicated a broad genetic diversity within sugarcane genotypes. Genetic similarity co-efficient indicated low level of genetic diversity among the *S. Officinarum* (0.84 similarity), relatively medium level of genetic diversity in *S. spontaneum* clones (0.78 similarity), and higher degree of genetic diversity in the *S. barberi* clones, and ISH genotypes (0.77 similarity). The SSRs derived from sugarcane were found to be more informative then the transferred SSRs from other related crops. Comparison between morphological and SSRs data revealed a low correlation among two data. These results suggested that the classification based on morphological characters and microsatellite markers will be useful for sugarcane breeders to plan crosses for agronomic traits. Genetically diverse parents could be identified for broadening the genetic base of sugarcane varieties and varietal development in sugarcane.

Key Words: Genetic diversity, *Saccharum* complex, Microsatellite (SSR) markers, Morphological diversity, *Saccharum* species, Sugarcane nobilization

Introduction

Sugarcane alone is responsible for the approximately 70% of row sugar, ~30% bio-ethanol production aswell-as molasses, and paper as byproducts at global level (FAO). As an alcohol crop for biofuel, output to input ratio of sugarcane is higher than the maize (Waclawovsky et al., 2010). It produces huge drybiomass with annual greater yields compared with other major lignocellulosic biofuel plants such as Miscanthus, switch grass, and maize (Heaton et al., 2008). Energy sugarcane has a potential to produce cellulosic biofuel since it has a high fiber and biomass, and all the fiber, cellulose, and lignin components can be easily converted to energy (bio-ethanol). Hence, it has been established as an important industrial as-well-as farmer's cash crop in many tropical and sub-tropical countries of the world (Singh et al., 2011). Though sugarcane have the potential of high biomass and sugar production, breeding programs yet not entirely utilized genetic resource of potential multiple stress resistance and the high yield capacity exists within sugarcane germplasm resources.

The detection of the genetic diversity within sugarcane germplasm is crucial, because diversity within a breeding genetic pool is required for making genetic gains in sugarcane (Dillon *et al.*, 2007; Singh *et al.*, 2012).

The genus Saccharum is a group of perennial grasses and belongs to the family Poaceae, tribe Andrpogoneae, which includes six species such as S. officinarum (noble canes), S. sinense (Chinese clones), S. barberi (North Indian canes), S. robustus, S. spontaneum and S. edule (Roach, 1972). Saccharum and other related genera i.e. Erianthus Michx., Miscanthus Anderson, Narenga Bor., and Sclerostachya make an interbreeding pool of genetic resources termed "Saccharum complex" (Daniels and Roach, 1987). The Modern sugarcane varieties derived from introgression between S. officinarum and S. spontaneum clones (Price, 1963). The F_1 progenies were backcrossed with the S. officinarum recurrent parent to expand genes for sucrose biosynthesis and accumulation, and this process is widely known as "nobilization" (Roach, 1972). Initially, few first hybrids were extensively intercrossed to generate sugarcane

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varieties by nobilization which leads to a narrow genetic base of modern sugarcane hybrid varieties than the other *Poaceae* crops (Creste *et al.*, 2010; Singh *et al.*, 2013a). Genetically, sugarcane is a highly polyploid and heterozygous crop with highly unstable genetic constitution.

The development of novel sugarcane varieties with high sugar content has proven to be difficult due to the genetic complexity and heterozygous nature (Singh *et al.*, 2011). The success for development of elite sugarcane varieties depends on the ability to select parents after efficient evaluation of the genetic diversity among the germplasm. It is the most important to make a new breeding program by using diversified parentage to produce hybrids with advanced agronomic traits and broader genetic base (Santos *et al.*, 2012).

Traditionally, morphological traits have been used to identify and characterize Saccharum species clones, and the extent of morphological diversity has been used for accurate identification of sugarcane species and commercial varieties. Although, there is a high levels of morphological variability within the genus Saccharum which the breeders have used in the past and it provides a large base for selection of agronomic characters. Currently, Molecular (DNA) markers are routinely used for accurate identification, conservation, and management of germplasm stocks of plant species (Karp et al., 1997). Moreover, genetic diversity within Saccharum germplasm has been analyzed by various molecular markers such as RFLP (Besse et al., 1997), RAPD (Selvi et al., 2008), SSRs markers (Singh et al., 2014a) and AFLP (Aitken et al., 2007). Microsatellite (SSRs) markers have preferentially been used due to their simplicity, abundance, variability, co-dominance inheritance, and high reproducibility (Singh et al., 2014b).

Sugarcane exhibits a wide range of phenotypic diversity within and between species in different geographical areas and climates. Sugarcane shows a great polymorphism in terms morphological characters such as sucrose content, cane height, girth (thickness), number of internodes, length of internodes, number of leaves, leaf length, leaf width, cane weight etc (Singh *et al.*, 2013b). These morphological characters have been used for various purposes including identification of parentage, taxonomical studies, assessment genetic diversity and correlation with characteristics of agronomic importance (CIAT, 1993). Morphological

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characterization is an important first step towards the assessment of sugarcane diversity (Prakash and He, 1996). Phenotypic characterization in combination with DNA markers based genetic diversity study would be more rewarding for the precise identification and description of closely related *Saccharum* species and commercial varieties.

The objective of the present study was to assess the genetic diversity among the various *Saccharum* species clones and commercial varieties (hybrids) using morphological traits and microsatellite markers.

Material and Methods

Plant Materials

Thirty eight *Saccharum* species clone and commercial varieties of sugarcane were used in assessment of genetic diversity based on morpho-physiological traits and SSRs markers. All the 38 sugarcane genotypes are listed below in Table 1 with their origin and pedigree.

The genotypes include five clones of *S. officinarum* (Badila, Otaheit, Bendjermassimhitam, IJ-76-564, Gunjera), five clones of *S. barberi* (Pathari, Agoule, Lalari, Hemja, Saretha), six clones of *S. spontaneum* (Baheri2, SES135B, WS18, SES515/7, N58, Ramsal), one clone of *S. robustum* (IJ-76-545), fourteen Indian commercial varieties, four Inter-specific hybrids and three non-Indian commercial hybrids (Table 1).

Evaluation of Morphological Traits

The measurements for seven morpho-physiological characters namely Stalk length (SL) in meter (m), Yield/ Clump (cane weight) in kg, No. of Internodes (INTN), Length of Internodes (INTL) in centimetres (cm), No. of Green leaves (NGL), Stalk diameter (Girth) in mm and sucrose content (HR brix %) were recorded on randomly chosen plants. These morpho-physiological characters are measured in most of the sugarcane breeding programmes for the selection of superior genotypes from mapping populations.

Extraction of Genomic DNA

Genomic DNA was extracted from disease free immature fresh leaves of all 38 sugarcane genotypes by the CTAB method (Hoisington *et al.*, 1992) with minor modification (Singh *et al.*, 2011). The extracted DNA was diluted to a final concentration of ~25ng/µl as determined by agarose-gel electrophoresis using known concentration of uncut λ DNA as standard.

S. No.	Name of Genotype/Variety	Place of Origin	Pedigree	
A. Saccharum officinarum	L. (2n=80)			
1	Badila	New Guinea	Natural	
2	Otaheit	Java, Indonesia	Natural	
3	Bendjermassimhitam	New Guinea	Natural	
4	IJ-76-564	Iryan, Java,	Natural	
5	Gunjera	Java, Indonesia	Natural	
B. Saccharum barberi Jesw	v (2n=81-124)			
6	Pathari	North-Eastern India	Natural	
7	Agoule	North-Eastern India	Natural	
3	Lalari	North-Eastern India	Natural	
)	Hemja	North-Eastern India	Natural	
0	Saretha	North-Eastern India	Natural	
C. Saccharum spontaneum	L. (2n=42-128)			
1	Ramsal	India	Natural	
12	WS18	WB, India	Natural	
13	SES515/7	UP, India	Natural	
14	N58	Bihar, India	Natural	
15	SES135B	UP, India	Natural	
6	Baheri2	UP, India	Natural	
D. Saccharum robustum (2	n = 60 - 200			
17.	IJ-76-545	New Guinea	Natural	
D. Indian commercial varie	eties (hybrids)			
18	CoS91269	Shahjahanpur, India	Bo91×Co1158	
19	CoS96268	Shahjahanpur, India	Co1158×Co62198	
20	CoS8436	Shahjahanpur, India	MS68/47×Co1148	
21	CoS510	Shahjahanpur, India	Co453×Co557	
22	CoS767	Shahjahanpur, India	Co419×Co313	
23	CoS94527	Shahjahanpur, India	Bo91×Co62198	
24	CoS95255	Shahjahanpur, India	Co1158×Co62198	
25	UP22	Shahjahanpur, India	Bo91×CoSe40/80	
26	UP0097	Shahjahanpur, India	Se1444/91×Se1854/91	
27	CoJ64	Jalandhar, India	Co976×CO617	
28	CoH70	Haryana, India	Unknown	
29	CoJ99192	SBI Coimbatore, India	Unknown	
30	CoLk92238	Lucknow, India	Unknown	
31	B34-104	Bihar, India	Unknown	
E. Inter-specific hybrids				
32	ISH135	Coimbatore, India	Inter specific hybrid	
33	ISH168	Coimbatore, India	Inter specific hybrid	
34	ISH148	Coimbatore, India	Inter specific hybrid	
35	ISH273	Coimbatore, India	Inter specific hybrid	
F. Non-Indian commercial	hybrids (NICH)			
36	PoJ2878	Java, Indonesia	NICH	
37	CP44-43	Canal Point, USA	NICH	
38	Q49	Queensland, Australia	NICH	

Table 1. Saccharum species clones, Indian commercial, Inter specific hybrids and Non-Indian commercial varieties with their respective plac	e
of origin and pedigree	

Polymerase Chain Reaction and SSR Analysis

Microsatellite (SSRs) primers were designed from the flanking regions of the simple repeats motifs of ESTs sequences using batchprimer3 online tool. Primers were synthesized by commercial services provider Bangalore GeNeiTM, India. Total 4500 EST sequences were retrieved from EST database of National Center for Biotechnology Information (http://www.ncbi.nlm. nih.gov). All the collected ESTs were the functional parts of the sugarcane metabolic pathways which many regulatory functions in biosynthetic process.

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S.No.	Genotype Name	HRBrix% (Jan)	Yield/clump (kg)	INTN	INTL (cm)	NGL	SD (mm)	SL (m)
1.	Badila	19.8	3.25	20	10.06	11	23	2.01
2.	Otaheit	22.3	4.35	22	10.64	12	24	2.34
3.	Bendjermasimhitam	21.6	3.5	19	9.74	14	26	1.85
4.	IJ76-564	21.6	6.25	25	13.82	10	27	3.45
5.	Gunjera	17.03	5.1	25	13.2	10	27	3.30
5.	Pathari	12.8	3.4	26	10.22	13	23	2.65
7.	Agoule	18.3	3.2	25	11.4	11	22	2.85
3.	Lalari	19.5	2.15	24	10.62	13	17	2.54
).	Hemja	20.1	2.5	22	11.24	10	18	2.47
0.	Saretha	14.6	2.5	23	11.74	11	15	2.70
1.	IJ76-545	10.87	6.5	22	14.78	12	30	3.25
2.	Ramsal	4.6	2.0	31	15.68	11	15	4.86
3.	WS18	6.4	2.5	37	11.92	11	18	4.41
4.	SES515/7	10.1	5.0	37	15.34	11	23	5.67
5.	N58	5.8	1.5	24	18.44	10	14	4.42
6.	SES135B	13.0	2.73	26	15.34	11	18	3.98
7.	Baheri-2	3.6	2.4	25	14.98	12	21	3.74
8.	CoS91269	14.77	3.2	24	14.46	12	23	3.47
9.	CoS96268	22.8	5.4	29	15.53	11	22	4.50
0.	CoS8436	20.6	4.1	22	16.1	9	24	3.54
1.	CoS510	21.6	2.6	28	11.74	13	15	3.28
2.	CoS767	20.8	3.8	25	13.32	9	21	3.33
3.	CoS94527	18.2	4.6	24	15.92	11	23	3.82
4.	CoS95255	22.4	4.5	27	15.92	11	24	4.29
5.	UP22	17.2	2.9	29	13.2	11	18	3.82
6.	UP0097	20.01	3.1	26	16.08	6	18	4.36
7.	CoJ64	22.4	2.8	24	15.44	13	24	3.70
	CoH70	13.83	5.02	24	17.84	10	26	4.28
9.	CoJ99192	19.17	2.3	21	16.76	12	18	3.51
0.	CoLk92238	19.5	3.6	19	15.7	11	21	2.98
1.	B34-104	18.26	5.3	26	10.98	10	27	2.85
32.	ISH135	17.5	5.5	25	14.94	13	27	3.73
3.	ISH168	9.85	2.4	24	13.32	13	20	3.19
4.	ISH148	17.4	2.4	22	13.62	13	27	3.00
5.	ISH273	13.83	4.5	23	15.14	9	25	3.48
6.	PoJ2878	20.1	3.6	25	12.06	11	26	3.01
7.	CP44-43	17.2	4.1	27	11.46	13	21	3.09
38.	Q49	18.51	2.4	27	13.4	12	16	3.61
	Average	16.52	3.603	25.10	13.73	11.210	21.763	3.45
	Range	3.6-22.8	1.5-6.5	19-37	9.74-18.44	6-14	14-30	1.85-5.67

Table 2. The average and size range of the morpho-physiological characters recorded for phenotypic diversity analysis

Identification of unique molecular markers associated with the sugar traits is major objective for maker assisted selection (MAS) in sugarcane energy crop (Singh *et al.*, 2012). Accordingly, the newly developed EST-SSRs primers were screened for robust polymorphism by bulk segregation assay (BSA) using a bulk DNA of contrast (high and low sugar) segregating lines of sugarcane mapping population. Total 50 simple sequences repeat (SSRs) or microsatellite polymorphic markers were used to estimate the genetic diversity among 38 sugarcane genotypes. The information regarding the PCR amplification and polymorphism are given in Table 3. SSRs motifs regions were amplified by polymorphic SSR primer pairs in 10 μ l reaction volume (Singh *et al.*, 2010; 2011).

Diversity Analysis by SSR Markers

To measure the in formative potential of the microsatellite markers, the polymorphism information content (PIC) for each primer pair was calculated according to the formula of Milbourne *et al.* (1997) as follows.

$$\left[PIC = 1 - \sum_{J=1}^{N} p_{JJ}^{2}\right]$$

Where P_{IJ} is the frequency of the *j*th allele for marker *i* and summation extends over *n* alleles. Frequency of the ith allele in the set of 38 genotypes/varieties investigated. DNA bands were scored for the presence (1) or absence (0) in all 38 genotypes and binary data was used to calculate the Jaccard's similarity coefficient using module of free tree. Genetic distance between each pair were estimated as D=1-JS. Clustering was based on a similarity matrix using Unweighted Pair Group Method with Arithmetic average (UPGMA) algorithm; of freeware program Free Tree (Hampl *et al.*, 2001). Most universal resampling technique bootstrapping was used to estimate the level of inferred relationships. Tree View, drawing software was used for interactive visualization of the dendrogram (Page, 1996).

Diversity Analysis with Morphological Traits

Cluster analysis was carried out on standardized morphological data based on the Euclidian distance coefficient and Unweighted pair group method with arithmetic means (UPGMA) algorithm using NTSYSpc version 2.11 (Sokal and Michener, 1958). The dendrogram was generated employing SAHN program of NYTSYS (Rohlf, 2000).

Results

Development of SSR Markers from EST Sequences

Total 189 (4.2%) simple sequence repeat motifs were identified from the non-redundant EST sequences. Among the identified SSRs, tri-nucleotide repeats were found to be most abundant (47.1%) class followed by

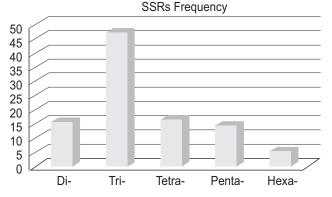


Fig. 1. Frequency distribution of different SSRs & types identified in *Saccharum* species ESTs

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tetra-nucleotide repeats (16.6%), di-nucleotide repeats (15.8%), penta-nucleotide repeats (14.4%) and hexanucleotide repeats (5.5%) (Fig. 1). Eighty seven primer pairs were designed from the flanking regions of the microsatellite repeats (SSRs) and fifty primer pairs were used in the present genetic diversity analysis. These developed EST-SSR markers were able to reveal genetic variability existing within the expressed region of the sugarcane genome which is the more informative then the genetic diversity detected by other genomic SSRs markers (Singh *et al.*, 2013c).

The genetic variability prevailing in the functional coding regions of the plants could not be analyzed by many of the molecular markers and most of the molecular markers detects genetic variability in non-coding region of the plant genome. However, these EST based microsatellite markers are less polymorphic then the other genic (derived from genomic sequences) molecular markers, though these exclusively explains the variability in exists in evolutionarily conserved regions of the plants genome (Cordeiro *et al.*, 2001; Parida *et al.*, 2009; Singh *et al.*, 2013c).

Microsatellite Polymorphism and Cluster Analysis

Distinctive PCR banding patterns were found in most of the EST-SSRs markers in all the 38 sugarcane genotypes indicating the variability in expressed regions of the genome. The PCR amplified DNA profiles revealed the potential of microsatellite markers distinguish between inter as-well-as intra-species clones of sugarcane (Fig. 2). Present results corroborate to earlier reports (Singh *et al.*, 2013a). Moreover, some previous reports on SSR markers based genetic diversity analysis were in accordance to the present research findings (Selvi *et al.*, 2003; Brown *et al.*, 2007).

A total of 412 DNA bands were amplified and their size ranged from 50 to 1250 bp with average of 8.29 bands per primer (Table 3). The polymorphism information content (PIC) of markers varied from 0.137 to 0.790 with an average of 0.373 and indicated a good discriminatory power of the functional EST-SSRs.

Morphological Traits' Analysis

The *Saccharum* species clones and cultivated varieties selected for morpho-physiological characterization exhibited high morphological variation in aerial part of the sugarcane. An analysis of variance illustrated that all the characters evaluated were significantly different (P<0.01)

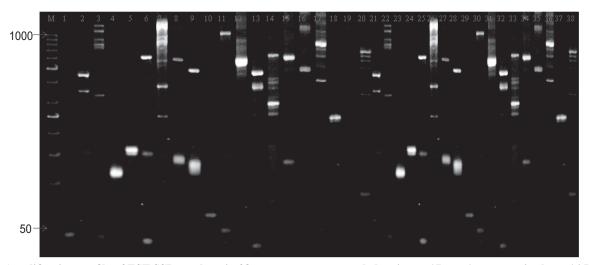


Fig. 2. Amplification profile of EST-SSR markers in 38 sugarcane genotypes belonging to 17 *Saccharum* specie clone, 14 Indian commercial varieties (hybrids), 4 Interspecific hybrids and 3 Non-Indian commercial hybrids. Lane 1-Badila, 2-Otahite, 3-Benjdermassimhitam, 4-Gunjera, 5-IJ76-564, 6-Baheri2, 7-SES135B, 8-WS-18, 9-SES515/7, 10-N58, 11-Ramsal, 12-Pathari, 13-Agoule, 14-Lalari, 15-Hemja, 16-Saretha, 17-CoS91269, 18-CoS96268, 19-CoS94527, 20-UP0097, 21-CoS767, 22-PoJ2878, 23-CP44-43, 24-Q49, 25-B34-104, 26-CoJ64, 27-CoH70, 28-CoJ99192, 29-CoLk92238, 30-IJ76-545, 31-CoS95255, 32-CoS510, 33-UP22, 34-CoS8436, 35-ISH135, 36-ISH273, 37-ISH148, 38-ISH168, L: 50bp DNA ladder (MBI Fermentas, Lithuania). Uncommon DNA banding pattern is showing distinctive nature of genotype by SSR markers.

between the genotypes. The dendrogram generated using phenotypic characters separated genotypes into two major clusters I and II with Euclidian distance ranging from 0 to 140 (Fig. 4). Cluster I contains 37 genotypes and divided into two groups. Group (A) included Baheri2, WS18, Ramsal, and ISH168. Group (B) further divided into two sub groups (a) and (b), Sub-group (a) included CoS91269, Patheri, IJ76-545 and CoJ99192, UP22, CoS91269, CoH70, ISH273, Otaheit, Badila, Bendjermasimhitam, CoS94527, SES135B, Saretha, CP44-43, and Q49. Sub-group (b) includes PoJ2878, ISH135, B34-104, UP0097, Agoule, CoS767, Hemja, CoLk92238, IJ76-564, CoS95255, CoS96268, ISH148, CoS510, and CoJ64. N58 (S. spontaneum) formed distinct cluster, which diverged from all the 38 sugarcane genotypes. It was the most diversified wild genotype along with Baheri2, WS18, Ramsal (S. spontaneum) and ISH168 genotypes. The genotypes did not form specific groups according to morphological characters in the dendrogram and genotypes related to a common species did not show close similarities in the morphological study. All the recorded physiological parameters with their size range and average are listed in Table 2.

Correlation between Morphological and SSR Data

The mental test showed quite low correlation between

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morphological and molecular marker based dendrograms (r=-0.03). Both morphological and genetic analysis allowed separation of the sugarcane genotypes into two different clusters. Despite the low correlation between morphological and SSR similarity matrices, there were similar grouping of genotypes in the respective dendrogram.

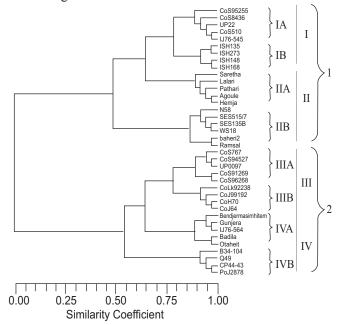


Fig. 3. Dendrogram showing genetic relationship among the 38 sugarcane genotypes based on SSR polymorphic markers. Scale indicates Jaccard's similarity coefficient values

Table 3. Details about the microsatellite (SSRs) marker's name, primer sequences, total no. of DNA bands, size range of bands and polymorphic
information content (PIC)

2 SNR2 ACCACTTCACACAGGAGTC TATTCATCGGTTCCC 07 155-1181 0.482 3 SNR3 GGGCAAGATGTATAGACA TTTATCGCCGGATAAGTGAT 04 126-1040 0.598 4 SNR5 AGACCCCCACATACACAC CTGTGTGTGTTTTGT 09 58-629 0.514 6 SNR5 CAGAATACCOCCTATCAGAC CTGTGTGTGTTTGGTTG 01 92-840 0.185 7 SNR5 TGGGCAAGAAGAAGAC GTGCATATTGACACACAAGAGAC 05 59-160 0.210 0 SNR50 GGGCAAGCAAGAAGAGGC TATTGCCACGTAACAGAAGGAC 12 59-918 0.416 10 SNR51 GGGCAAGAAGAGAGACACACAC AGCTTCTCATCAAGAGGACA 12 59-918 0.416 11 SNR11 ACAATGGAGAACAAC ACTCTTCGTTTGTTT 10 56-923 0.421 12 SNR11 ACAATGAGGGAAAACAAC ATTGCGGAGGAGGAAACAAC 10 51-980 0.412 13 SNR51 GTTCTAGTCTCATCATCCACC TAGAGAGCATGTGAGACA 10 51-980 0.414 14	S.No	Marker name	Forward primer (3'- 5')	Reverse primer (3'- 5')	Total Band	Band size (bp)	PIC Value
2 SNS2 ACCACTTCACAACAGCAGT TATTGATGGGTTCGTTTCC 0 155-1181 0.482 3 SNS3 GGGCAGGATGTATAGCCA TTTTGTCGCGAATAGTGATAGTGA 04 125-1040 0.598 4 SNS4 TATAAACCACACCACGGGAA TTTGTTCATAGCACCACATA 06 235-532 0.521 6 SNS6 CAGAATACCGCCACATACACACA CTGTGTGTTGGTTGGT 09 28-629 0.514 6 SNS5 CAGGATACCTAGAAGAAG GTCCTGTGCTTTGGTTGGT 16 23-532 0.432 7 SNS7 TTAGGGCCAAGCAACAAGAAG GTCGGATGATGTTGGT AGCCCTGCCAAGAAGAGA 5 59-160 0.210 10 SMS10 GGAGATGTTGGATGGGG AGAGTAGCATAAGGGAA 05 59-160 0.210 11 SMS10 GGAGATGTTAGAGGGAA AGAAGAGCCATAAGGGAA AGAAGAGCCAGAGAAACAAC 10 51-867 0.521 12 SMS13 CCTTGAGAGTGTTAGCTGACACA 10 51-867 0.521 13 SMS14 AAGAAGAGCCGTGAAAACAAC 10 51-867 0.521 <t< td=""><td>1</td><td>SMS1</td><td>GGTGTGTTTGAGGTTTAGGT</td><td>TGTAATGGCAAGCTCACATA</td><td>12</td><td>50-900</td><td>0.622</td></t<>	1	SMS1	GGTGTGTTTGAGGTTTAGGT	TGTAATGGCAAGCTCACATA	12	50-900	0.622
3 SMS3 GGGCAAGAATGATAGACCA TTTATCGCCGAATAAGTGAT 0 125-192 0.521 5 SMS5 AAGACCGCACCAGTACAAATC CTGTGTGGTGCTTTGGTTG 0 58-529 0.514 6 SMS6 CAGAATACCGCCTACGACGA CTGTGTGTGTTTGGTTGG 0 67-0440 0.298 7 SMS7 TAGAGGCAACGAAGAAGAG CTGGATTAATGAGCGGCT 1 92-840 0.185 8 SMS8 TGGCGAAGGAACGTGTCT ACCCTCTCACCAGTAGCACA 0 51-299 0.432 9 SMS9 GTGCGAGAGGAACTGTGT ACCCTCTCACCAGTATCAAGGAGGCAG 12 59-180 0.210 10 SMS10 GGACATGGAATTTGCAC TATTCTCCACGATTAATGAGGCAG 12 57-175 0.414 11 SMS11 ACCAATGGAGTTTAACTCCAC TATTCTCCACCATTCTGTCT 10 5-6-233 0.212 12 SMS13 CTTGTAGTCTCACATTCTCCAC TATCATCTGACGGCTGTCAC 10 51-890 0.412 13 SMS14 AAGAAGGCGTGAAAAAAAAGATTGTTACTCCTCC 0 6-0423 0.216 14	2	SMS2	ACCACTTCACAACAGGAGTC	TATTGTATGGGTTCGTTTCC	07	155-1181	0.482
4 SMS4 TTATAAACACACACCAGGGAA TTGTTCATAGCACCGACAT 06 235-322 0.521 6 SMS6 CAGAATACCGCCACAGTACAAATC CTGTGTGGTTTGGTT 09 58-629 0.514 6 SMS6 CAGAATACCGCCTATCAGAC CTGGTTAGTATGGATGGTT 10 92-840 0.185 8 SMS8 TGGGCAGGAAGGAAGGAA GCCCTGCTAACAGGAG 05 59-160 0.210 10 SMS10 GGAGAGGAACGTGTTGACAC AGCCTCTCCTAACAGGAG 05 59-160 0.210 11 SMS11 ACAGTGTTGAGAGGGAA AGCCTCCCTCAACAGGAG 05 59-160 0.210 12 SMS13 ACTCTCACATCCACC ATCTTCCACATCGACC ATCTTGGCTTGGTTG 12 59-018 0.412 13 SMS13 CCTTGATGTCAGGCATACACC ATGAGGAGGGGGATGAAC 0 51-880 0.422 14 SMS14 AAGAAGACCGCGTAGAACACA ATGAGGAGGAGGTGAAC 0 51-880 0.412 15 SMS16 GTTTAGACAGCGTAGAGAGT TACAAGAGACATGGGTGTC 0 52-830 0.613	3	SMS3			04	126-1040	
6 SNS6 CAGAATACCGCCTATCAGAC GTTCTTGTGCTTTGG 06 70-640 0.288 8 SNS8 TGGGCAGGAAGAAGAG GTGGTATATTGAGCTGGTC 11 92-840 0.185 8 SNS9 GTGCGAGGAACGAGGAACTGTGT AGCCTACCAACAAGGA 05 59-160 0.210 10 SNS10 GGACAGTGTTTGAGAGGAAA AGCCTACCAACAAGGACGA 12 57-175 0.414 11 SNS12 ACATCGACTCACACCAATCACCACA AGCCTTGCTTGGTTT 10 56-92.3 0.421 12 SNS12 ACTTGAGGTGTGAATTGG CCGATTCGCCGGGGGAGAAC 10 51-980 0.412 13 SNS14 AAGGAAGGCGTGAGGTT ATCGTTGTGTGTGGTGC 0 60-1250 0.213 15 SNS16 GTTTCATACGACGTGTGTGATA TAGAGGACATGAGGGGTGCGAAT 0 57-842 0.613 18 SNS18 GTGCGATAGCAAGAAGAAC TACAATTTACAACCACAAGGG 09 58-433 0.152 19 SNS19 CTGCATACAGCAGTTCCTC GCACAAGCAAGAGG 08 60-145 0.511 2	4				06		
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11 SMS11 ACATGGAGTGTATTIGGC TATTIGCCACGTGATACTCA 2 \$7.175 0.414 13 SMS13 CCTTGATGTCACATCACCA ATCGTCCTGCTTGGTTGGG 0 \$6-93 0.421 13 SMS13 CCTTGATGTTCAGATAGTTGG CCGATTCAGCCCTGGTC 05 \$7-687 0.521 14 SMS14 GATGCTAGACACGCTAGAAACACA ATGGGGGGGTCC 09 60-1250 0.213 15 SMS15 GTTCTTAGCCACGGTAGTA ATGGAGGACAGGGGGGCACT 10 \$7-820 0.613 16 SMS18 GTTGTCGAGAGTGATACAGAAGATG TACATATTACACACACACACACAGGG 09 \$5-843 0.152 19 SMS19 CTGCAGTACGTCGCGGAATC GTACAATATTACACACACACACACAGGG 08 60-145 0.511 20 SMS20 TCCATCAAGCGGTACATCCCCACTAC CTCACCAGGAGAGAGAAGAACAAGAC CCCCAAGCAGAGACAGAGG 08 60-495 0.539 21 SMS21 ACCCATCAAGCGGAGAAGAACAAGAC CACCAGAAGAGACAGAGAGAGAGAGAGA 05 209-943 0.487 21 SMS23 AGACAGGGAGAAGAACAAGAC CACCAGAAGACAGAGA 05 209-943 0.487 22 SMS25	9	SMS9	GTGCGAGAGGAACTGTGT	AGCCCTGCCTAACAAGGA	05	59-160	0.210
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27 SMS27 TACTATGGAGGCGGAGG TAGAAGAGCACAGAGCAAAC 10 65-925 0.649 28 SMS28 TCAAACCAGGATCTAAGCTCAC GGTAGTGCCATTGAGGTTGC 08 55-879 0.200 29 SMS29 GCGAGAGAGATGAGGGAGAA AGGTGCCGTTCATGAGGTGC 11 51-798 0.236 30 SMS30 AATATACTTCTCGATTAATCACCG CTACTACTACTACCAGGTGC 03 62-431 0.526 31 SMS31 ACTAACTCTCTTCAACTTCCTCG AGCGGTGTCACCAGGAGC 16 51-246 0.171 32 SMS32 CACGCAGAGCGGCACACAGAACC AGGAACTCACACTACTGGTGC 19 54-1141 0.228 33 SMS33 AATCGGCGCTGACCATGGACTC AGGAACTCACACATACTCTGTTT 6 51-452 0.428 34 SMS34 AGAAGGTACTACTCCTAAGGACAA AACGATTCATATGCCTATATGGACTACAGGTGATGTG 18 51-556 0.476 35 SMS35 TAGGAGAAATGCACACGA AGACTGACACCTTTGAGGTGAGA 19 56-465 0.176 36 SMS38 TTTCTTGGTATATGCACACAGA AGGACACACTTTGAGATGA 19 56-465 0.176 37 SMS37 TAGGAGAAATAGCAACAGA	25						
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30SMS30AATATACTTCTCGATTAATCACCGCTACTACTACTACCAAGTACGGCG0362-4310.52631SMS31ACTAACTCTCTTCAACTTCCTCGAGCTGTTCCTCTTAGCTAGTC0451-2460.17132SMS32CACCGCAGCCTGACACAGAACCAGGAACTCAGCATACTCGTGAC1954-11410.22833SMS33AATCGGCGCTGACCATGGACCAGAACACAACTTTCACCTTGT0651-4520.42834SMS34AGAAGGTGATCCTCAAGGACAAGAACTGATCCCTCTTTCATATATTC0459-2880.24335SMS35TAGCAATCTACTCCCTACGTCTGGTTGACGCTGGACAG0954-1980.16936SMS36AAAGACTCCAAGCACCTGTGTGCAAGTTTATAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCACAGGAGACTGACACTTTGAGATGA1956-4650.17638SMS38TTTCTTTGGTATACTGACTAGCGGGACAACTAATGTACAGGATCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACCTAATGTACAGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69544SMS44GCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT							
31SMS31ACTAACTCTCTTCAACTTCCTCTGAGCTGTTCCTCTTAGCTAGTTC0451-2460.17132SMS32CACCGCAGCCTGACACAGAACCAGGAACTCAGCATACTCGTGAC1954-11410.22833SMS33AATCGGCGCTGACACAGAACCAGAACACAACTTTCACCATGTT0651-4520.42834SMS34AGAAGGTGATCCTCAAGGACAGAACTGATCCTCTTTCATATATTC0459-2880.24335SMS35TAGCAATCTACTCCCTACGTCTACGTTGACGTTGATCAGCCCGTTG0851-5560.47636SMS36AAAGACTCCAAGGCACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACTATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAGGAACTTAACACTAATGTAACTGATTCT0651-5000.13740SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGGTGAGCCTGAAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCCTCCCTCCTTCTCCTCTTGTT0760-6030.26344SMS44GCTCTCCTCTCTCTCTCTCTCCGCAACTCAGGCGTGAGAT0954-9900.29445SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGGCGTGCAACG0759-1990.42846SMS46CAGGACTACAGGGCGTAAAGAGTAAACCAAGGCTGCATAAC0954-9900.29447SMS47ACGCGTAGGGCGTACAAGGTAAACCAAGGCTCAAGGCGTGAACA0954-9900.29448SMS48							
32SMS32CACCGCAGCCTGACAGAACCAGGAACTCAGCATACTCGTGAC1954-11410.22833SMS33AATCGGCGCTGACCATGGACCAGAACACAACTTTCACCTTGTT0651-4520.42834SMS34AGAAGGTGATCCTCAAGGACAAGAACTGATCCTCTTTCATATATTC0459-2880.24335SMS35TAGCAATCTACTCCCTACGTCTACGTTGACGTTGATCAGCCGTTG0851-5560.47636SMS36AAAGACTCCAAGCTCCTGTGTGCAAGTTTATAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTGAACCGATGAGAAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS43AGGTCATCTCTCTCTCTCTCTCTCTCCTTCTCTCTCTTGTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCTCTCCCGCCACTTTATCAGCGTGGAA0866-8300.69545SMS45TTTGTGTCCTCTCTCTCTCTCTCTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGATACAGGGAACAAAGGTTAAACCTCAGGCGTGAGAT0954-9900.29447SMS47ACGCGTAGGGCGTACAAAGGTTAAACCTCAGGCGTGATAC0859-8840.20048SMS48ACGAGTTCAGGGCGTAAGAGGTTAAACCTCAAGGCGTAACATAC0859-8840.20049 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>							
33SMS33AATCGGCGCTGACCATGGACTCAGAACACAACTTTCACCTTGTT0651-4520.42834SMS34AGAAGGTGATCCTCAAGGACAAGAACTGATCCCTCTTTCATATATTC0459-2880.24335SMS35TAGCAATCTACTCCCTACGTCTACGTTGACGTTGATCAGCCCGTTG0851-5560.47636SMS36AAAGACTCCAAGCTCTGGTGGCAAGTTTATAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGAA1956-4650.17638SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGATCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAAGGGCAA0551-950.18442SMS43AGGTCATCTCTCTCTCTCTCTCTGTCTCCTTCTCCTCTTTGT0760-6030.26344SMS44GCTCTCCTCCTCTCTCTCTCTCTCGCCACTTTATCAGCGTGAAC0866-8300.69545SMS45TTTGTGTCCTCTCTGTTCATTGCAAGGATCAGGGTCACATCA0954-9900.29446SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACAAAGGTAAACCTCAGCGTGAAC0859-8840.20048SMS48ACGAGTTCAGGCGTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>							
34SMS34AGAAGGTGATCCTCAAGGACAAGAACTGATCCCTCTTTCATATATTC0459-2880.24335SMS35TAGCAATCTACTCCCTACGTCTACGTTGACGTTGATCAGCCCGTTG0851-5560.47636SMS36AAAGACTCCAAGCTCCTGTGTGCAAGTTTATAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS38TTTCTTGGTATATCTGACTTGACGGGACAACTAATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0866-8300.69542SMS42ATGATGACGAGAACGATGGCAAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTCCCTCCTTCTCCTCTTGTT0760-6030.26344SMS44GCTCTCCTCCTCTCTCTCTCTCCGCAACTAAGGGTCACAGTGTTCATC1258-10390.48645SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGGGTCACATGTCTC1258-10390.42846SMS44ACGCGTAGGCCGTACAAAGGTAAACCTCAGGCCTCATAGTCTAGTCT1258-10390.42847SMS47ACGGGTAGGCGGTACAAAGGTAAACCTCAGGCCTCACATGT0954-9900.29447SMS48ACGAGTTCAGGGCCGTACAAAGGTAAACCTCAGGCCTAAGTGTAGAC0954-9900.294 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>							
35SMS35TAGCAATCTACTCCCTACGTCTACGTTGACGTTGATCAGCCCGTTG0851-5560.47636SMS36AAAGACTCCAAGCTCCTGTGTGCAAGTTTATTAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGAATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGGAGAGACCCAAC0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTCTCTCCTCCTTCTCCTCAGTT09130-11480.40745SMS45TTTGTGTGTCCTCTCTCTCTCTCTGCAAGGATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACACTCAGGCCGTACTTAC0859-8840.20048SMS48ACGAGTTCAGGGCGCTGATAGAGATCACACAGGCTATAGTCCTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCTCCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCTGCCCCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>							
36SMS36AAAGACTCCAAGCTCCTGTGTGCAAGTTTATTAGGGTCTGCAAG0954-1980.16937SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTCTCTCCTTCTCCTCTTGTT0760-6030.26344SMS44GCTCTCCTCCTCTCTCTCTCTGCCAACTAATGCAGGTTCATC1258-10390.48645SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9000.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGGCGTGAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCCTCCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTTGCATTGCATTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412					* .		
37SMS37TAGAGGAAATAGCAGAACAGGAGACTGACACCTTTGAGATGA1956-4650.17638SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTAACTGATTCT0651-5000.13740SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTCTGTCTCCTTCTCCTCTTGTT0760-6030.26344SMS44GCTCTCCTCCTCTCTGTCATTGCAAGGATCAGTGTCATCT1258-10390.48645SMS45TTTGTGTCCTCTCTGTTCATTGCAAGGCTCACTTCA0954-9000.29447SMS47ACGCGTAAGGCGCTGATAGAGGTTAAACCTCAGCGTGAAC0859-8840.20049SMS49GCCGAAGCTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCGCATTAACATATTCATAGCCCAATT1158-11100.411Total412-							
38SMS38TTTCTTTGGTTATACTGACTTGACGGGACAACTAATGTAACTGATTCT0651-5000.13739SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTCTGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCTCTCTCGCCACTTTATCATCATCAGGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCTCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
39SMS39GTTAAGCTACTATGGACAACAGGACTTAACACTATGTCAGGTCTCAA0551-780.57340SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCTCTCCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412-							
40SMS40CGTTATGGAAAGCACGACCTTGATGCCGTTGAAGAA1257-10290.79041SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCTCTCCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
41SMS41AAGATTCCAAACGCTGAAAGAGATAGACTCAAAGGGCAA0551-950.18442SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCTGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCCTCCTCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCTCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412-							
42SMS42ATGATGACGAGAACGATGGCAAGGGTGAGCGTGGAA0866-8300.69543SMS43AGGTCATCTCTCTCTCTCGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCCTCCTCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
43SMS43AGGTCATCTCTCTCTCTCGTCTCCTTCTCCTCTTGT0760-6030.26344SMS44GCTCTCCTCCTCCTCCTCCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412-							
44SMS44GCTCTCCTCCTCCTCCCGCCACTTTATCATCCTCAGTT09130-11480.40745SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
45SMS45TTTGTGTCCTCTCTGTTCATTGCAAGCATCAGTGTTCATC1258-10390.48646SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCTCCGTCATCAATGACAGAGAGTGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
46SMS46CAGGACTACAGGGAACAATAAGAAATACCAGGCTCACTTCA0954-9900.29447SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCTCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
47SMS47ACGCGTAGGCCGTACCAAAGGTTAAACCTCAGCCGTGAGT0759-1990.42848SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCTCCTCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
48SMS48ACGAGTTCAGGGCGCTGATAGAGATCACGACGTCATAGTCCGTAAC0859-8840.20049SMS49GCCGAAGCCTCTCCTCCTCCGTCATCAATGACAGAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412							
49SMS49GCCGAAGCCTCTCCTCCTCCGTCATCAATGACAGAGATGTAGAC0959-4280.41350SMS50GCGTCTCTGCTCTGCACTCTGCATTAACATATTCATAGCCCAATTT1158-11100.411Total412	47						
50 SMS50 GCGTCTCTGCTCTGCACTCTGC ATTAACATATTCATAGCCCAATTT 11 58-1110 0.411 Total 412 - -	48						
Total 412	49						
	50	SMS50	GCGTCTCTGCTCTGCACTCTGC	ATTAACATATTCATAGCCCAATTT		58-1110	0.411
Mean 8.29 - 0.373	Total				412	-	-
	Mean				8.29	-	0.373

UPGMA dendrogram grouped all the 38 sugarcane genotypes into two major clusters 1 and 2. The cluster 1 divided into two sub-clusters I and II (Fig. 3). Sub-cluster I was further divided into two sub-sub-clusters IA and IB; sub-sub-cluster IA included CoS95255, CoS8436, UP-22, CoS510, IJ76-564 (Indian commercial hybrids, UPCSR), and sub-sub-cluster IB included ISH135, ISH273, ISH148 and ISH168 (Interspecific hybrids). Sub-cluster II also divided into two sub-sub-clusters IIA and IIB; IIA included Saretha, Lalari, Patheri, Agoule and Hemja (*S. barberi*), and IIB included N58, SES515/7, SES135B, WS18 Baheri2 and Ramsal (*S. spontaneum*). Cluster II also divided into two sub-clusters IIIA and IIB; IIIA included CoS767, CoS94527, UP0097, CoS91269 and CoS96268 (Indian commercial hybrids) and IIB included CoLk92238, CoJ99192, CoH70, and CoJ64 (Indian commercial hybrids, from different parts of India). Sub-cluster IV also divided into two sub-sub-clusters IVA and IVB; IVA included Bendjermassimhitam (BMH), Gunjera, IJ76-564, Badila and Otaheite (*S. officinarum*) and IVB included B34-104, Q49, CP44-43 and PoJ2878 (Foreign commercial hybrids; NICH). Clustering pattern in the present dendrogram is with the accordance to the origin or pedigree, and the genotypes and commercial varieties that shared a common name showed genetic similarities.

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Discussion

EST based molecular markers termed EST-SSR were developed from publically available EST database at NCBI website. The publically available EST resources offer an opportunity to develop informative molecular markers for field crops without expenditure. These EST based SSR markers are relatively more informative since they reveals genetic diversity within the SSR streches dispersed in expressed regions of the plant genome (Oliveira et al., 2009; Parida et al., 2009). The high level of polyploidy and heterozygous nature of sugarcane is responsible for intense PCR amplification pattern (Singh et al., 2011). The observed genetic diversity among the 38 sugarcane genotypes was comparatively lower than the earlier study of the five Saccharum species and related Erianthus (Cordeiro et al., 2003). Polymorphic fragment amplified in sugarcane genotypes revealed size variation and the length polymorphism observed between diverse accessions could be due to an accumulation of mutation events during DNA replication and recombination (Parida *et al.*, 2006). The genesis of the simple sequence repeats loci is result of the errors in DNA metabolism due to the slippage of DNA polymerase at time of replication (Litt and Lutty 1989; Singh *et al.*, 2014a). Thus, SSR markers have been proved more frequently occurring types of markers in sugarcane as well as other crop plants. A better representation of the genetic diversity in sugarcane varieties was obtained with SSR markers based analysis (Brown *et al.*, 2007; Singh *et al.*, 2012). Similarly, previous study has also shown that the SSR loci give good discrimination between closely related genotypes (Powell *et al.*, 1996).

Physiological parameters and SSR markers have been previously employed to the study of genetic diversity in sugarcane varieties and different views regarding genetic base of sugarcane have been predicted. A comparative analysis of the genetic variation in sugarcane is essential for genetic conservation strategies and selection of parents for breeding of desirable economic trait (Singh *et al.*, 2013a). Moreover successful conservation of any given

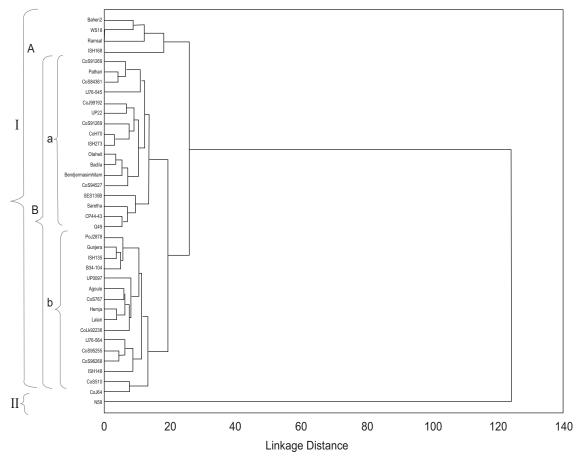


Fig. 4. A dendrogram of 38 *Saccharum* species clones, Indian commercial varieties, Interspecific hybrids and Non Indian commercial hybrids (NICH) based on seven morphological markers

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gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al.*, 1999).

It could be suggested that diverse germplasm sources (other than S. spontanem and S. officinarum) should be used as parental lines to develop sugarcane varieties. The mental test for association among the matrices derived from SSR and morphological data indicated a poor matrix correlation. It showed that both the methods discriminated very differently among the genotypes. Lesser correlation between morphological and molecular markers has been reported earlier in plants and suggested that it might be due to the independent nature of morphological and molecular variations (Bushehri et al., 2005). The poor correlation also could be due to the fact that higher level of genetic variation detected by molecular markers are non adaptive and the selection pressure is influenced by the environment (Vieira et al., 2007). Genotypes with the most distinct DNA profiles are likely to carry unique and potentially agronomically useful genes. This makes genomic diversity estimates a potentially valuable predicting source for selecting diverse parent genotypes for favourable heterotic combination that aims to broaden the genetic bases.

Genetic diversity based on morphological traits was higher on an average than SSR markers analysis, which reflects the influence of the environment on the performance of the genotypes. Due to this fact DNA markers and morphological traits could not necessarily gained closely corresponding results (Mart *et al.*, 2005). Moreover, mainly two reasons are advocated to explain the limited correlation between DNA markers and morphological studies. First DNA markers cover a larger proportion of the genome than the morphological markers; second DNA markers are less subjected to artificial selection compared to morphological markers (Mart *et al.*, 2005).

The development new EST-SSRs will have significant implication for the genetic study and utilization of genetic resources of the sugarcane and related genera. These functional molecular tools will also provide more direct estimate of functional genetic diversity in *Saccharum* species (Oliveira *et al.*, 2009).

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