Indian J. Plant Genet. Resour. 31(1): 17-26 (2018)
DOI 10.5958/0976-1926.2018.00003.7

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

Ram Baran Singh[1,3]*, Balwant Singh[2] and RK Singh[3]
[1]*Department of Biotechnology, Punjab Technical University (PTU), Jalandhar-144601, Punjab, India*
[2]*Swami Satyanand College of Management and Technology, Amritsar-143001, Punjab, India*
[3]*Sugarcane Research Institute, U.P. Council of Sugarcane Research, Shahjahanpur-242001, Uttar Pradesh, India*

(Received: 21 August 2015; Revised: 11 July 2016; Accepted: 22 October 2016)

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 as-well-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 dry-biomass 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

**Author for Correspondence: Email- rvs_doc@rediffmail.com*
Present Address: Quality Lab, ICAR-Indian Institute of Wheat and Barley Research (IIWBR), Karnal-132001, India

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 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.

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

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 (2n=81-124)			
6	Pathari	North-Eastern India	Natural
7	Agoule	North-Eastern India	Natural
8	Lalari	North-Eastern India	Natural
9	Hemja	North-Eastern India	Natural
10	Saretha	North-Eastern India	Natural
C. *Saccharum spontaneum* L. (2n=42-128)			
11	Ramsal	India	Natural
12	WS18	WB, India	Natural
13	SES515/7	UP, India	Natural
14	N58	Bihar, India	Natural
15	SES135B	UP, India	Natural
16	Baheri2	UP, India	Natural
D. *Saccharum robustum* (2n= 60-~200)			
17.	IJ-76-545	New Guinea	Natural
D. Indian commercial varieties (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

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 GeNei^{TM}, 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.

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

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
6.	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
8.	Lalari	19.5	2.15	24	10.62	13	17	2.54
9.	Hemja	20.1	2.5	22	11.24	10	18	2.47
10.	Saretha	14.6	2.5	23	11.74	11	15	2.70
11.	IJ76-545	10.87	6.5	22	14.78	12	30	3.25
12.	Ramsal	4.6	2.0	31	15.68	11	15	4.86
13.	WS18	6.4	2.5	37	11.92	11	18	4.41
14.	SES515/7	10.1	5.0	37	15.34	11	23	5.67
15.	N58	5.8	1.5	24	18.44	10	14	4.42
16.	SES135B	13.0	2.73	26	15.34	11	18	3.98
17.	Baheri-2	3.6	2.4	25	14.98	12	21	3.74
18.	CoS91269	14.77	3.2	24	14.46	12	23	3.47
19.	CoS96268	22.8	5.4	29	15.53	11	22	4.50
20.	CoS8436	20.6	4.1	22	16.1	9	24	3.54
21.	CoS510	21.6	2.6	28	11.74	13	15	3.28
22.	CoS767	20.8	3.8	25	13.32	9	21	3.33
23.	CoS94527	18.2	4.6	24	15.92	11	23	3.82
24.	CoS95255	22.4	4.5	27	15.92	11	24	4.29
25.	UP22	17.2	2.9	29	13.2	11	18	3.82
26.	UP0097	20.01	3.1	26	16.08	6	18	4.36
27.	CoJ64	22.4	2.8	24	15.44	13	24	3.70
28.	CoH70	13.83	5.02	24	17.84	10	26	4.28
29.	CoJ99192	19.17	2.3	21	16.76	12	18	3.51
30.	CoLk92238	19.5	3.6	19	15.7	11	21	2.98
31.	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
33.	ISH168	9.85	2.4	24	13.32	13	20	3.19
34.	ISH148	17.4	2.4	22	13.62	13	27	3.00
35.	ISH273	13.83	4.5	23	15.14	9	25	3.48
36.	PoJ2878	20.1	3.6	25	12.06	11	26	3.01
37.	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

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 µl 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.

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

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 NTSYS-pc 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 tetra-nucleotide repeats (16.6%), di-nucleotide repeats (15.8%), penta-nucleotide repeats (14.4%) and hexa-nucleotide 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).

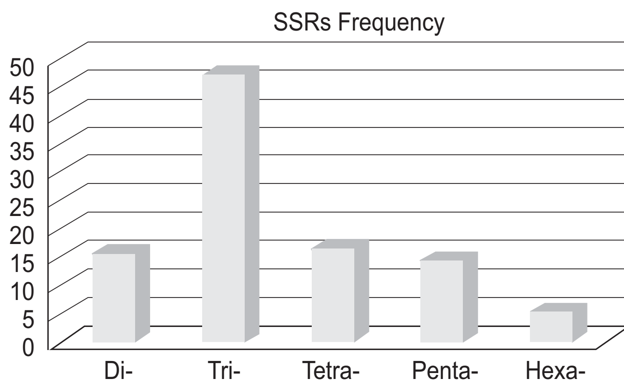

Fig. 1. Frequency distribution of different SSRs & types identified in *Saccharum* species ESTs

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)

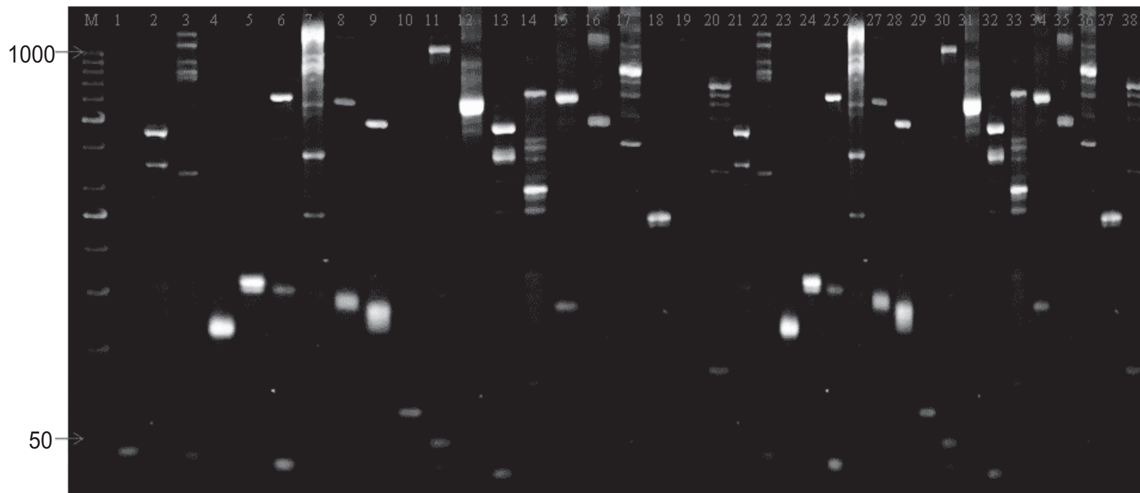

Fig. 2. Amplification profile of EST-SSR markers in 38 sugarcane genotypes belonging to 17 *Saccharum* specie clone, 14 Indian commercial varieties (hybrids), 4 Interspecific hybrids and 3 Non-Indian commercial hybrids. Lane 1-Badila, 2-Otahite, 3-Benjdermassimhitam, 4-Gunjera, 5-IJ76-564, 6-Baheri2, 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 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.

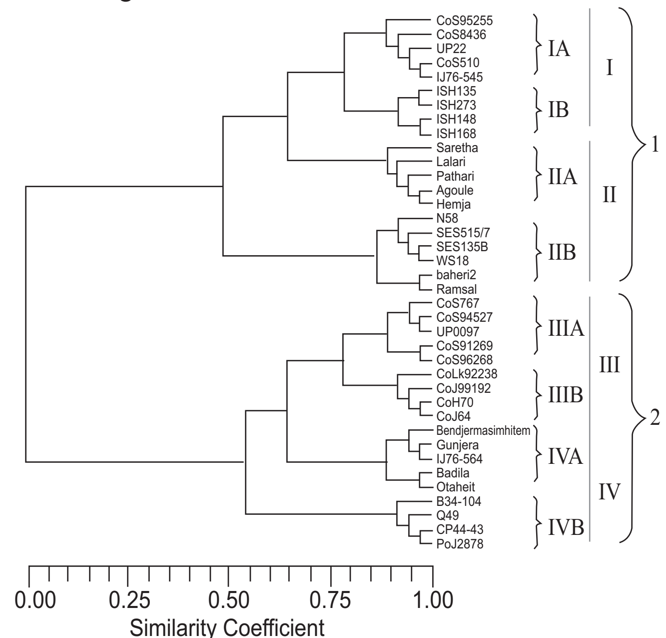

Fig. 3. Dendrogram showing genetic relationship among the 38 sugarcane genotypes based on SSR polymorphic markers. Scale indicates Jaccard's similarity coefficient values

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)

S.No	Marker name	Forward primer (3´- 5´)	Reverse primer (3´- 5´)	Total Band	Band size (bp)	PIC Value
1	SMS1	GGTGTGTTTGAGGTTTAGGT	TGTAATGGCAAGCTCACATA	12	50-900	0.622
2	SMS2	ACCACTTCACAACAGGAGTC	TATTGTATGGGTTCGTTTCC	07	155-1181	0.482
3	SMS3	GGGCAAGAATGTATAGACCA	TTTATCGCCGAATAAGTGAT	04	126-1040	0.598
4	SMS4	TATAAACACACACACGGGAA	TTTGTTCATAGCACCGACTA	06	235-532	0.521
5	SMS5	AAGACCGCACAGTACAAATC	CTGTGTGGTGCTTTGCTT	09	58-629	0.514
6	SMS6	CAGAATACCGCCTATCAGAC	GTTCTGTGCTTTGGTTGG	06	70-640	0.298
7	SMS7	TAGAGGCAACGAAGAAAGAG	CTGGATTAATTGAGCTGGTC	11	92-840	0.185
8	SMS8	TGGGCTAGCTATCCTTACAC	AGCTTCTTACCAGTATGCCA	07	51-529	0.432
9	SMS9	GTGCGAGAGGAACTGTGT	AGCCCTGCCTAACAAGGA	05	59-160	0.210
10	SMS10	GGAGATGTTTGAGAGGGAA	AGAGTAGCATAAAGGAGGCAG	12	59-918	0.416
11	SMS11	ACAATGGAGTTGTATTTGGC	TATTTGCCACGTTGTACTTG	12	57-175	0.414
12	SMS12	ATCTTCACATCCATCATCCAC	ATCTCTCCTTGCTTTGGTTT	10	56-923	0.421
13	SMS13	CCTTGATGTTCAGATAGTTGG	CCGATTCAGCCCTTCGTC	05	57-687	0.521
14	SMS14	AAGAAGAGCCGTAGAAACAAC	ATTGAGCGAGGGATGAAC	10	51-980	0.442
15	SMS15	GTTCTTAGTCCAGCCGTAGTT	ATCGTTGTTGTCGGTGTC	09	60-1250	0.213
16	SMS16	GTTTAAGACAAGATGGTGTAGATG	TACATATTTACATTGTTACTCCGC	04	56-632	0.276
17	SMS17	GCGTCTTCATCATCTGCAAC	TAGAGAGACATGGGGTGCAT	10	57-820	0.613
18	SMS18	GTTGTCGAGATGATACAGAAGTAA	GTACAATATTACACACACAAAGGG	09	55-843	0.152
19	SMS19	CTGCAGTACGGTCCGGAATC	GTACCACCATGGCTCTAGCTTC	07	150-555	0.287
20	SMS20	TCCATCAAGCCGTTCCTC	GCCAAGCAGATAAAGAAGTG	08	60-145	0.511
21	SMS21	ACTCCTCCCGCCTCCACTAC	CTCACCGAAGCAATCAAG	08	66-945	0.539
22	SMS22	AGAGAGGGAGAAGAACAAGAC	ACCAGAAGGACAGAGATGG	09	53-707	0.173
23	SMS23	ATGACAGCAGCACAATGA	CACCCAGTTGAATAAGTGA	05	209-943	0.487
24	SMS24	GAAGCGAATGTGAACTGG	GAGAGCAGCGAGGACAGG	09	146-750	0.219
25	SMS25	CACTTCCCAGAGACCCAG	GACCTTAGCAATCAAGACAGA	06	186-666	0.223
26	SMS26	CTCCCAAAGCAAACCCTT	GTTCTTGACCTTCTTCCTGTC	03	371-954	0.105
27	SMS27	TACTATGGAGGCGGGAGG	TAGAAGAGCACAGAGCAAAC	10	65-925	0.649
28	SMS28	TCAAACCAGGATCTAAGCTCAC	GGTAGTGCCATTGAGGTTGC	08	55-879	0.200
29	SMS29	GCGAGAGAGATAGAGGGAGAGA	AGGTGCCGTTCATGAGGTAGT	11	51-798	0.236
30	SMS30	AATATACTTCTCGATTAATCACCG	CTACTACTACTACCAAGTACGGCG	03	62-431	0.526
31	SMS31	ACTAACTCTCTTCAACTTCCTCTG	AGCTGTTCCTCTTTAGCTAGTTC	04	51-246	0.171
32	SMS32	CACCGCAGCCTGACACAGAACC	AGGAACTCAGCATACTCGTGAC	19	54-1141	0.228
33	SMS33	AATCGGCGCTGACCATGGACTC	AGAACACAACTTTCACCTTGTT	06	51-452	0.428
34	SMS34	AGAAGGTGATCCTCAAGGACAAG	AACTGATCCCTCTTTCATATATTC	04	59-288	0.243
35	SMS35	TAGCAATCTACTCCCTACGTCTAC	GTTGACGTTGATCAGCCCGTTG	08	51-556	0.476
36	SMS36	AAAGACTCCAAGCTCCTGTGTG	CAAGTTTATTAGGGTCTGCAAG	09	54-198	0.169
37	SMS37	TAGAGGAAATAGCAGAACAGG	AGACTGACACCTTTGAGATGA	19	56-465	0.176
38	SMS38	TTTCTTTGGTTATACTGACTTGAC	GGGACAACTAATGTAACTGATTCT	06	51-500	0.137
39	SMS39	GTTAAGCTACTATGGACAACAGG	ACTTAACACTATGTCAGGTCTCAA	05	51-78	0.573
40	SMS40	CGTTATGGAAAGCACGAC	CTTGATGCCGTTGAAGAA	12	57-1029	0.790
41	SMS41	AAGATTCCAAACGCTGAA	AGAGATAGACTCAAAGGGCAA	05	51-95	0.184
42	SMS42	ATGATGACGAGAACGATG	GCAAGGGTGAGCGTGGAA	08	66-830	0.695
43	SMS43	AGGTCATCTCTCTCTTCTCGT	CTCCTTCTCCTCCTTCTTGT	07	60-603	0.263
44	SMS44	GCTCTCCTCCTCCTCTCC	GCCACTTTATCATCCTCAGTT	09	130-1148	0.407
45	SMS45	TTTGTGTCCTCTCTGTTCATT	GCAAGCATCAGTGTTCATC	12	58-1039	0.486
46	SMS46	CAGGACTACAGGGAACAATAA	GAAATACCAGGCTCACTTCA	09	54-990	0.294
47	SMS47	ACGCGTAGGCCGTACCAAAG	GTTAAACCTCAGCCGTGAGT	07	59-199	0.428
48	SMS48	ACGAGTTCAGGGCGCTGATAGAG	ATCACGACGTCATAGTCCGTAAC	08	59-884	0.200
49	SMS49	GCCGAAGCCTCTCCTCTCCTCC	GTCATCAATGACAGAGATGTAGAC	09	59-428	0.413
50	SMS50	GCGTCTCTGCTCTGCACTCTGC	ATTAACATATTCATAGCCCAATTT	11	58-1110	0.411
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 III and IV. Sub-cluster III further divided into two sub-sub-clusters IIIA and IIIB; 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.

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

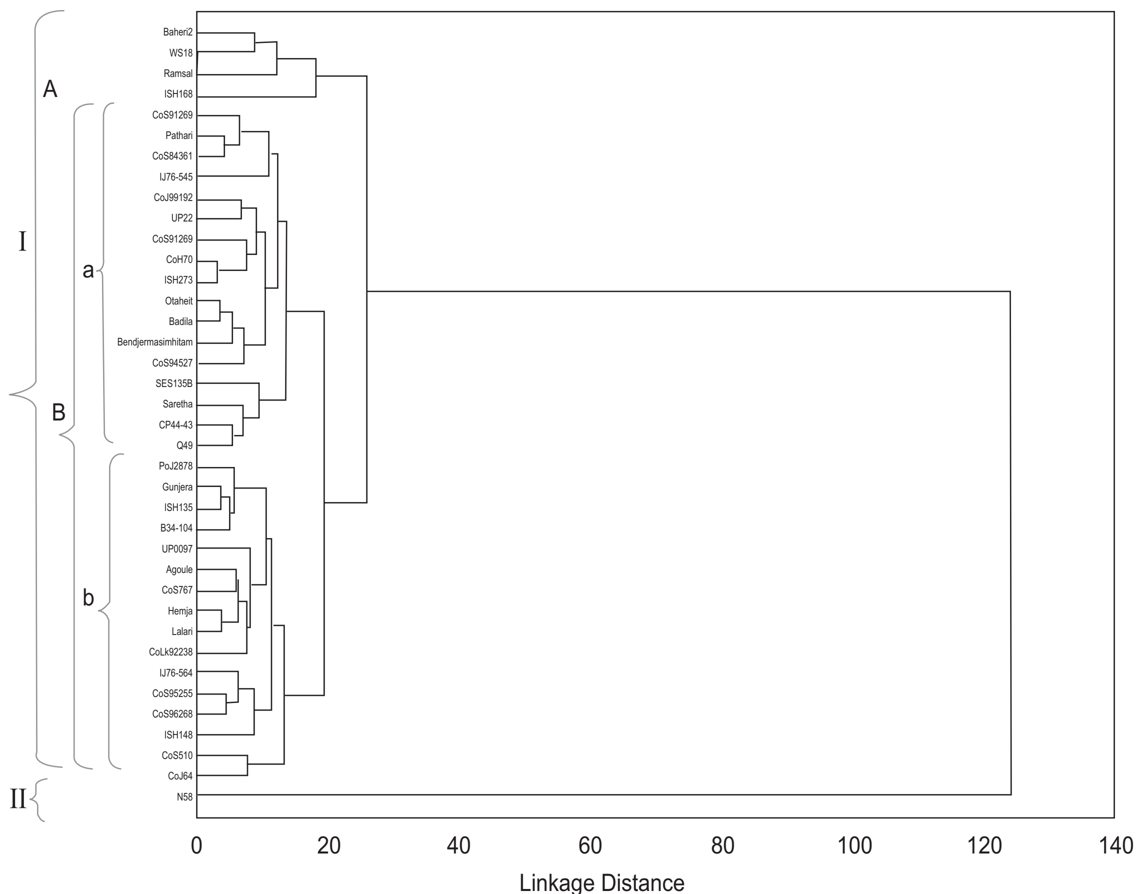

Fig. 4. A dendrogram of 38 *Saccharum* species clones, Indian commercial varieties, Interspecific hybrids and Non Indian commercial hybrids (NICH) based on seven morphological markers

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).

Acknowledgement

Authors are thankful to the Director Sugarcane Research Institute (UPCSR), Shahjahanpur-242001, Uttar Pradesh (India) for providing necessary research facilities.

References

Aitken K, J Li, L Wang, C Qing, YH Fan and P Jackson (2007) Characterization of intergeneric cultivars of *Erianthus rockii* and *Saccharum* using molecular markers. *Genet. Resour. Crop Evol.* **54**: 1395-1405.

Besse P, CL McIntyre and N Berding (1997) Characterisation of *Erianthus* sect. Ripidium and *Saccharum* germplasm (*Andropogoneae-Saccharinae*) using RFLP markers. *Euphytica* **93**: 283-292.

Brown S, RJ Schnell, EJ Power, Stephanie, DL Douglas and N Kuhn (2007) Analysis of clonal germplasm from five *Saccharum* species*: S. barberi, S. robustum, S. officinarum, S. sinense* and *S. spontaneum*. A study of inter- and intra species relationships using microsatellite markers. *Genet. Resour. Crop Evol.* **54**: 627-648.

Bushehri A, S Torabi and M Ghannadha (2005) Comparison of genetic and morphological distances with heterosis with RAPD markers in hybrids of Barley. *Int. J. Agric. Biol*. **7**: 592-595.

CIAT (1993) Biotechnology Research unit, Annual report, Cali, Columbia International Potato Centre (CIP), Asian Vegetable Research and Development Centre (AVDRCE), International board for plant genetic resources (IBPGR). *In:* Huaman, Z (ed) Descriptors for Sweet Potato. 43-130.

Creste S, KAG Accoroni, LR Pinto, R Vencosvskv, MA Gimenes, MA Xavier *et al* (2010) Genetic variability among sugarcane genotypes based on polymorphism in sucrose metabolism and drought tolerance genes. *Euphytica* **172**: 435-446.

Cordeiro GM, R Casu, CL McIntyre, JM Manners and RJ Henry (2001) Microsatellite markers from sugarcane (*Saccharum s*p.) ESTs cross-transferable to Erianthus and sorghum. *Plant Sci.* **160**: 1115-1123.

Cordeiro GM, YB Pan and RJ Henry (2003) Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Sci*. **165**: 8-181-189.

Daniels J and BT Roach (1987). Taxonomy and evolution. In: Heinz D.J. (ed.), Sugarcane Improvement through Breeding. Elsevier, *Amsterdam*.

Dillon SL, FM Shapter, RJ Henry, G Cordeiro, L Izquierdo and LS Lee (2007) Domestication to crop improvement: genetic resources for sorghum and *Saccharum* (Andropogoneae). *Ann. Bot.* (London). **100:** 975-989.

Hampl V, A Pavlicek and J Fleger (2001) Construction and Bootstrp analysis of DNA fingerprinting based phylogenetic trees with a freeware program FreeTreee: Application to trichomonad parasaites. *Int. J Syst. Evol. Microbio*. **51**: 731-735.

Heaton EA, FG Dohleman and SP Long (2008) Meeting US biofuel goals with less land: the potential of Miscanthus. *Glob. Chang. Biol.* **14**: 2000-2014.

Hoisington D (1992) Laboratory protocol. CIMMYT applied molecular genetics laboratory, CIMMYT, Mexico DF, CIMMYT.

Karp A, K Ovich, KV Bhat, WE Ayad and T Hodgkin (1997). Molecular Tools in Plant Genetic Resources Conservation:

A Guide to the Technologies. IPGRI Technical Bulletin no. 2, Rome: IPGRI.

Litt M and JA Lutty (1989) A hyper variable microsatellite revealed by in vitro amplification of a dinucluotide replete within the cardiac muscle actin gene. *Am. J. Hum. Genet*. **44**: 397-401.

Mailbourne D, RC Meyer, JE Bradshaw, E Baird, N Bonar, J Provan, W Powell and R Waugh (1997) Comparison of PCR-based marker systems for the analysis of genetic relationship in cultivated potato. *Mol. Breed*. **31:** 27-136.

Mart N, LP Cavagnaro and R Masuilli (2005) Evolution of diversity among Argentine parapvine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. *Elect. J. Biotechnol*. **63:** 7-45.

Oliveira KM, LR Pinto, TG Marconi, M Mollinari, EC Ulian, SM Chabregas, MC Falco, W Burnquist, AAF Garcia and AP Souza (2009) Characterization of new polymorphic functional markers for sugarcane. *Genome* **52**: 191-209.

Page RDM and TreeView (1996) An application to display phylogenetic trees on personal computers. *Comp. Aappl. Biosci.* **12**: 357-358.

Parida SK, KA Rajkumar, V Dalal, NK Singh and T Mohapatra (2006) Unigene derived microsatellite markers for the cereal genomes. *Theor. Appl. Genet*. **112**: 808-817.

Parida SK, SK Kalia, S Kaul, V Dalal, G Hemaprabha, A Selvi, A Pandit, A Singh, K Gaikwad, TR Sharma, PS Srivastava, NK Singh and T Mohapatra (2009) Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. *Theor. Appl. Genet*. **118**: 327-338.

Powell W, C Morgante, M Andre, V Hanafey, MJ Tingey and SV Rafalski (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol. Breed*. **5:** 222-235.

Prakash CS and G He (1996) DNA marker based genetic relatedness in United States sweet potato cultivars. *J. American Hortic. Sci.* **121**: 1059-1062.

Price S (1963) Cytogenetics of modern sugarcanes. *Econo. Bot.* **17:** 97-105.

Roach BT (1972) Nobilization of sugarcane. *Proceed. Internl. Soci. Sugarcane Technol*. **14**: 206-216.

Rohlf FJ (2000) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics Exerter Publishing Ltd New York.

Selvi A, N Mukunthan, RM Shanthi, P Govindaraj, B Singaravelu and TK Prabu (2008) Assessment of genetic relationships and marker identification in sugarcane cultivars with different levels of top borer resistance. *Sugar Tech.* **10**: 53-59.

Santos JM, LSC Duarte Filho, ML Soriano, PP Silva *et al.* (2012). Genetic diversity of the main progenitors of sugarcane from the RIDESA germplasm bank using SSR markers. *Ind. Crops Prod*. **40**: 145-150.

Selvi A, NV Nair, N Balasundram and T Mohapatra (2003) Evalution of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. *Genome*. **46**: 349-403.

Singh RK, RB Singh, SP Singh and ML Sharma (2011) Identification of sugarcane microsatellites associated to sugar content in sugarcane and transferability to other cereal genomes. *Euphytica* **182**: 335-354.

Singh RK, RB Singh, SP Singh and ML Sharma (2012) Genes tagging and molecular diversity of red rot susceptible/tolerant sugarcane cultivars using c-DNA and unigene derived markers. *World J. Microbio. Biotechnol.* **28**: 1669-1679.

Singh RK, RB Singh, SP Singh, N Mishra, J Rastogi, ML Sharma and A Kumar (2013a) Genetic diversity among the *Saccharum spontaneum* clones and commercial hybrids through SSR markers. *Sugar Tech.* **15**: 109-115.

Singh RK, SP Singh, DK Tiwari, S Srivastava, SB Singh, ML Sharma, R Singh, T Mohapatra and NK Singh (2013b) Genetic mapping and QTL analysis for sugar yield-related traits in sugarcane. *Euphytica* **191**: 333-353.

Singh RK, SN Jena, S Khan, S Yadav, N Banarjee, S Raghuvanshi, V Bhardwaj, SK Dattamajumder, R Kapur, S Solomon, M Swapna, S Srivastava and AK Tyagi (2013c) Development, cross-species/genera transferability of novel EST-SSR markers and their utility in revealing population structure and genetic diversity in sugarcane. *Gene* **524**: 309-329.

Singh RB, S Srivastava, AK Verma, B Singh and RK Singh (2014a) Importance and progresses of microsatellite markers in Sugarcane (*Saccharum* spp. hybrids). *Indian J. Sugarc. Technol*. **29**: 1-12.

Singh RB, S Srivastava, J Rastogi, GN Gupta, NN Tiwari, B Singh and RK Singh (2014b) Molecular markers: Exploited in crop improvement practices. *Res. Environ. Life Sci.* **7:** 223-232.

Sokal RR and CD Michener (1958) A statistical method for evaluating systematic relationship, *University of Kansas*, *Scientific Bulletin* **38**: 1409-1438.

Vieira E, F Carvalho, I Bertan, M Kopp, P Zimmer, G Benin, DA Sliva, I Haetiwg, G Malone and A Oliviera (2007) Association between genetic distance in wheat (*Triticum astivum* L.) as estimated by AFLP and morphological markers. *Gen. Mol. Bio.* **30**: 392-399.

Waclawovsky AJ, PM Sato, CG Lembke, PH Moore and GM Souza (2010) Sugarcane for bioenergy production: an assessment of yield and regulation of sucrose content. *Plant Biotechnol. J.* **8:** 263-276.

Zhang, DP, D Carbajula, L Ojeda, G Rossel, S Milla, Herrera and M Ghilson (1999) Microsatellite analysis of genetic diversity in sweet potato varieties from *Latin America*. Program report 1999-2000 CIP International potato centre Lima Peru.