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Diversity analysis of sugarcane genotypes by microsatellite (SSR) markers

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Thirty (30) simple sequence repeat (SSR) primer pairs chosen randomly from the SSR primer collection were used to detect polymorphism in 17 sugarcane accessions. A total of 62 DNA fragments were generated by the 30 primers with an average of about 2.14 bands per primer. Bands that a primer yielded in the study ranged from 1 to 4. The genetic distances for SSR data using 17 sugarcane accessions, was constructed based on Nei (1978) and relationships between accessions were portrayed graphically in the form of a dendrogram. The value of genetic similarity ranging from 62.90 to 90.30% was observed among the 17 sugarcane accessions. The highest genetic similarity of 90.03% was seen among genotypes S-2003-US-118 and S-2003-US-312. From the present study, it may be concluded that SSRs markers are best tool for investigation of genetic diversity in sugarcane.

Key words: Simple sequence repeat (SSR), polymorphism, genetic diversity.

INTRODUCTION

Sugarcane (*Saccharum* spp. hybrids) is a genetically complex crop of major economic importance in tropical and sub-tropical countries (Khan et al., 2004). 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. The importance of sugarcane has increased in recent years because cane is an important industrial raw material for sugar and allied industries producing alcohol, acetic acid, butanol, paper, plywood, industrial enzymes and animal feed (Arencibia, 1998). Considering the current needs of cane industry it is imperative to breed high sugar producing varieties that also have other desired agro-nomic traits.

Saccharum is a complex genus characterized by high ploidy levels and composed of at least six distinct species - *Saccharum officinarum, Saccharum barberi, Saccharum sinensi, Saccharum spontaneum, Saccharum robustum* and *Saccharum edule* (Daniels and Roach, 1987). Sugar recovery can be increased from current average of 8.32 to 10-11% with the development of improved cane varieties. For development of improved varieties, genotypic studies of sugarcane are required. Described as an allopolyploid, modern cultivated sugar-cane have approximately 80-140 chromosomes with 8-18 copies of a basic set (x = 8 or x = 10 haploid chromo-some number) (Ming et al., 2001). Continuous selection for the same traits may narrow genetic diversity to the extent that it may be difficult to predict diversity based on pedigree history alone. With the advent of molecular markers, it is now possible to make direct comparison of genetic diversity at the DNA level without some of the over simplifying assumptions associated with calculating genetic diversity based on pedigree history (McIntyre et al., 2001). Rapid advances in the field of molecular bio-logy and its allied sciences made the use of molecular markers a routine practice providing plant breeders a precise tool in analyzing genetic diversity for plant improvement (Andersen and Lubberstedt, 2003).

The molecular markers are of many types e.g. RFLPs,

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Table 1. Description of seventeen genotypes used in genetic diversity study.

Genotype	Source of collection
CPF-247	AARI , Faisalabad
SPF-245	AARI , Faisalabad
S-2003-US-618	AARI , Faisalabad
S-2003-US-628	AARI , Faisalabad
S-2002-US-247	AARI , Faisalabad
HSF-240	AARI , Faisalabad
CPF-237	AARI , Faisalabad
CPF-234	AARI , Faisalabad
S-2003-US-718	AARI , Faisalabad
S-2003-US-778	AARI , Faisalabad
S-2003-US-165	AARI , Faisalabad
S-2003US-312	AARI , Faisalabad
HSF-242	AARI , Faisalabad
CP-77-400	AARI , Faisalabad
CP-72-2086	AARI , Faisalabad
SPF-246	AARI , Faisalabad
SPF-213	AARI , Faisalabad

TRAPs, RAPDs, SNPs, simple sequence repeats (SSRs) and AFLPs. In the present study, microsatellite or SSR marker was used to analyze genetic diversity of different sugarcane genotypes. Microsatellites or simple sequence repeats (SSRs), are stretches of DNA, consisting of tandemly repeated short units of 1-6 base pairs in length. They are ubiquitous in eukaryotic genomes and can be analyzed through PCR technology. The sequences flanking specific microsatellite loci in a genome are believed to be conserved within a particular species, across species within a genus and rarely even across related genera. Simple sequence repeats (SSR) markers reveal polymorphisms due to variation in the lengths of microsatellites at specific individual loci. Microsatellites are born from regions in which variants of simple repetitive DNA sequence motifs are already over represented (Tautz et al., 1989). It is now well established that the predominant mutation mechanism in microsatellite tracts is ‘slipped-strand mispairing’. This process has been well described by Eisen (1999). When slipped-strand mispairing occurs within a microsatellite array during DNA synthesis, it can result in the gain or loss of one, or more, repeat units depending on whether the newly synthesized DNA chain loops out or the template chain loops out, respectively. The relative propensity for either chain to loop out seems to depend in part on the sequences making up the array, and in part on whether the event occurs on the leading (continuous DNA synthesis) or lagging (discontinuous DNA synthesis) strand. SSR allelic differences are, therefore, the results of variable numbers of repeat units within the microsatellite structure; they are therefore, multiallelic and co-dominant in nature, thus proving to be very informative. Among the range of DNA-based molecular marker techniques, a promising polymerase chain reaction (PCR)-based technique used extensively for genetic mapping (McIntyre et al., 2001), as well as fingerprinting of sugarcane clones (Piperidis et al., 2000; Pan *et al*., 2002), is microsatellites or SSRs. SSR genetic markers are the best tool to demonstrate the genetic diversity in sugarcane (Smiullah et al., 2012).

The present study was undertaken to investigate the genetic diversity and establish the relationship between different sugarcane genotypes in Pakistan, using SSR markers. Obtaining accurate estimates of the genetic diversity among germplasm sources may increase the efficiency of plant breeding. Knowledge of genetic diversity and relationships among breeding genome, their polymorphic nature, codominance and materials has a significant impact on crop improvement.

MATERIALS AND METHODS

The genetic diversity studies were done as a collaborative research, in Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad and Agriculture Biotechnology Research Institute (ABRI), Ayub Agricultural Research Institute (AARI), Faisalabad during 2010-2012. The plant material used for the study of genetic diversity was comprised of seventeen sugarcane accessions (Table 1). These accessions were collected from the germplasm source in the Sugarcane Section of Ayub Agricultural Research Institute, Faisalabad. The genetic material includes commercial cultivars and elite lines.

PCR amplification

Fresh young leaves were collected from the field experiment for isolation of the DNA. Total genomic DNA of the plants was extracted by using modified (CTAB) method (Hoisington et al., 1994; Doyle and Doyle, 1990). DNA concentration was determined, using a Nano Drop spectrophotometer (ND1000). Primer selection was based on previous investigation on SSR analysis, carried out with sugarcane genotypes and somaclones in this laboratory. Primer pairs obtained from Gene link company (USA) were used in PCR reaction for each genotype. For SSR analysis, concentration of genomic DNA, l0 × PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs primers and taq DNA polymerase were optimized.

A reaction mixture of 20 µl was used to amplify genomic DNA in a thermal cycler (Eppendorf DNA Thermal Cycler 9600). To confirm that the observed bands were amplified genomic DNA and not the primer artifacts, genomic DNA was omitted from control reaction. A negative control was also run to confirm if the master/reaction mixture is correctly prepared or not. The PCR products were electrophoresed at 90 V, in 2% agarose gel for approximately 2 h, using 0.5 × tris-boric acids EDTA (TBE) buffer, along with a DNA molecular size marker.

The gel contained 0.5 µg/ml ethidium bromide to stain the DNA and photographed under UV light using gel documentation system. Reactions were duplicated to check the consistency of the amplified products. Only easily resolved bright DNA bands were scored as presence (1) and absence of bands (0). Coefficient of similarity among somaclones was calculated according to Nei and Li (1978). Similarity coefficient was utilized to generate a dendrogram by means of unweighted pair.

Data analysis

The data on bands generated by the 30 primers were selected for analysis of genetic diversity (Table 2). The bands were counted by starting from the top and ending with the bottom of the lanes. All segregating bands that were well resolved and unambiguous were scored for the presence (1) or absence (0) in the 17 genotypes. The data of the primers were used to estimate the dissimilarity on the basis of number of unshared amplified products and a dissimilarity matrix was generated using Nei's similarity indices (Nei, 1978). In addition, population relationships were inferred using the un-weighted pair group of arithmetic means (UPGMA) clustering method using the Popgen software (version 3.5).

RESULTS AND DISCUSSION

In recent years, the popularity of SSR-based markers has increased considerably. The main reasons which make microsatellites an especially attractive tool for a number of applications are: their high levels of allelic variation and their co-dominant character, which means that they deliver more information per unit assay than any other marker systems, thus reducing costs; microsatellites are assayed using PCR, so only small amounts of tissue are required.

Thirty (30) SSR primer pairs chosen randomly from the SSR primer collection were used to detect polymorphism in 17 sugarcane accessions. The PCR product was observed by running on agarose gel to study polymorphism, most of the primers were polymorphic except five primers which were monomorphic and produced only one fragment per primer (Figure 1). All the primers were found to give reproducible bands. A total of 62 DNA fragments were generated by the 30 primers with an average of about 2.14 bands per primer. Bands that a primer yielded in the study ranged from 1 to 4. Generally, the size and the number of bands produced were dependent upon the nucleotide sequence of the primer pair, size of the primer used and the source of the template DNA. In this study the primer used were of the size ranging from 300-420 bp. Reactions were duplicated form to check the consistency of the amplified products. Only easily resolved bright DNA bands were scored.

Cluster analysis

Pattern of polymorphism by SSRs

About 85.25% polymorphism was estimated as 55 out of 62 fragments were polymorphic with 30 primers used among the 17 sugarcane accessions. The rest of the 7 bands were monomorphic in the 17 accessions. In the present study, the 17 sugarcane accessions appeared to show variability with the 30 primers used. Although none of the primers individually was as informative as to differentiate all the accessions; highly polymorphic profiles were obtained with of the primers SMs35. (Sugarcane Microsatellite primer no.35) while five primer pairs such as SMs46, SMs47, SMs48 SMs49 and SMs50 were found to be monomorphic. Therefore, it may be concluded from the present results that SSRs can be used for identification of genetic diversity and the relationship between the members of the complex species. Jannoo et al. (2001) studied diversity in 96 sugarcane genotypes with just two primer pairs and reported a high level of heterozygosity. Cordeiro et al. (2001) applied 21 primer sets to five sugarcane genotypes, and among them, 17 pairs were polymorphic, but the level of polymorphism (PIC value) in the cultivars detected by these SSRs was low (0.23). The level of polymorphism indicates that distinction between any two varieties is possible with appropriate SSR primer pair. This supports the use of SSR markers, as an excellent tool, for diversity analysis and loci mapping.

Genetic distances/similarities between the accessions

The genetic distance for SSR data using 17 sugarcane accessions, was constructed based on Nei (1978) and relationships between accessions were portrayed graphically in the form of a dendrogram in Figure 2, the value of genetic similarity ranging from 62.90 to 90.30% was observed among the 17 sugarcane accessions. The lowest genetic distance of 62.90% was seen among genotypes S-2003-US-118 and S-2003-US-312. These two genotypes differed from each other only in 5 bands with 14 different primers. The most dissimilar of all the accessions was S-2003-US-118 and SPF-213 with genetic distance of 90.30%. Genomic SSRs have been shown to produce a greater number of alleles and higher PIC values than those from EST derived SSRs in sugarcane (Pinto et al., 2006).

In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed genetic diversity as well (Selvi et al., 2003; Cordeiro et al., 2003). Selvi et al. (2003) revealed a broad range (0.324-0.8335) of pair-wise similarity values when tested on 30 or 40 commercial sugarcane cultivars.

Clustering pattern

The cluster analysis based on similarity values has classified all the sugarcane accession in two of the four major groups (I, II, III and IV). The first major group consisted of two accessions CPF-247 and S-2003-US-165 forming the most distinct cluster I. Second major group was further grouped into IIA, IIB and IIC. Group IIA consisted of three accessions namely SPF-245, S-2003-US-618 and HSF-242. Group IIB consists of four genotypes viz. HSF-240, CP-72-2086, S-2003-US-778 and SPF-213. Group IIC contained two accessions CPF-

Table 2. Name of the primers used for detection of polymorphism in sugarcane genotypes.

Primer no.	Band size	Primer sequence (F/R)	Annealing temperature
SMs1	600-2000	GGTTTGTTACTCTACTCCCGT GGTTTGTTACTCTACTCCCGT	55
SMs2	550-900	CATCTGCTCCCTCTTCCT TGAGCAAAGAAAGAGAAGTAGTC	55
SMs3	400-550	CATCTGCTCCCTCTTCCT CTCTGGCGGCTTGGTCCTG	52
SMs5	400-800	CTCTGCGGCTTGGTCCTG CATCCTCCAAGCATCTGT	54
SMs6	500-600	GACTCCTGTCACCGTCTTC ATACTTCAACCGTCTCCTCC	55
SMs7	400-500	CTAAGCAAGAACACAGGAAAG AGCAACAGCAGAGAGCAG	54
SMs8	400-550	CTGACTAAGGAGGAAGTGGAG GACGACGATAGATGAAACA	55
SMs9	400-500	GAGCCGCAAGGAAGCGAC CATACAAGCAGCAAGGATAG	50
SMs10	500-700	CTCTCTTCTCGTCTCCTCATT GTCCTTCTTCTTCTCGTGGT	55
SMs11	400-500	ACACGCATCGCAAGAAGG AAGAACACTCAACAGAAGCAC	55
SMs12	400 - 600	AAATGTCTTCGCACTAACC AAGGAGATGCTGATGGAGA	55
SMs16	400 - 500	CCCAGAGGACAAGGAACT GTAATGGAAGGAAGCAACTGA	50
SMs17	400-450	GGCTCCTCCTACTCGTTC GAGCCTTTGGATGTGGTC	55
SMs18	400-600	CTACACATCTCCATTCCACAG TTTAGGGTTCGTTAGGGTAAG	55
SMs19	300-500	GGCTCCTCCTACTCGTTC GAGCCTTTGGATGTGGTC	53
SMs20	350-500	CTACACATCTCCATTCCACAG TTTAGGGTTCGTTAGGGTAAG	50
SMs21	400-600	GGCTCCTCCTACTCGTTC GAGCCTTTGGATGTGGTC	50
SMs22	300-400	CTACACATCTCCATTCCACAG TTTAGGGTTCGTTAGGGTAAG	55
SMs23	350-600	GGCTCCTCCTACTCGTTC GAGCCTTTGGATGTGGTC	50
SMs24	400-450	CTACACATCTCCATTCCACAG TTTAGGGTTCGTTAGGGTAAG	53
SMs31	550-650	TTCTCGCCCTCCCGCTAC TTCTCTCCTCCTCCTCTTTC	55
SMs35	400-850	TTCTCGCCCTCCCGCTAC TTCTCTCCTCCTCCTCTTTC	53
SMs42	400-500	GTTTCTCCACCTCCAACTC ACAGACACAGGCGGGCGA	55
SMs43	400-500	CCCAGTGCTTCCTCTCTC TAGCACTCCATTCAGCAAA	55
SMs45	400	CTTCCCTCCCTCTCCTCT AGCCTTCTACTAAACTATCTGCT	55
SMs46	400	GTGAGTGAGACCAGACCAG CCGTGCTGTAGTTGTTGTAG	50
SMs47	400	ATACGCTACTCTGAATCCCAC CAATCACTATGTAAGGCAACA	50
SMs48	400	ACTCCTCTTCCTCTTCCTCTT GTTGTTCCCGTTCCCGCC	53
SMs49	250 - 400	ACTCGGTCATCTCATCACTC GTTCTTCGGGTCATCTGG	55
SMs50	400-500	ACGGTGAGCGAGGACTAC CTTGGGTGGCATCAGGAA	55

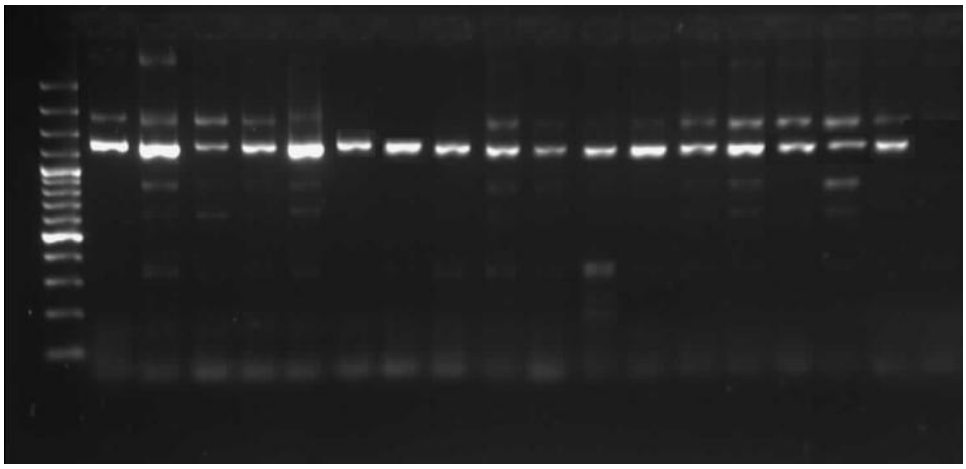

Figure 1. Result of electrophoresis of SSR product of 17 genotypes using sugarcane microsatellite primer no.18.

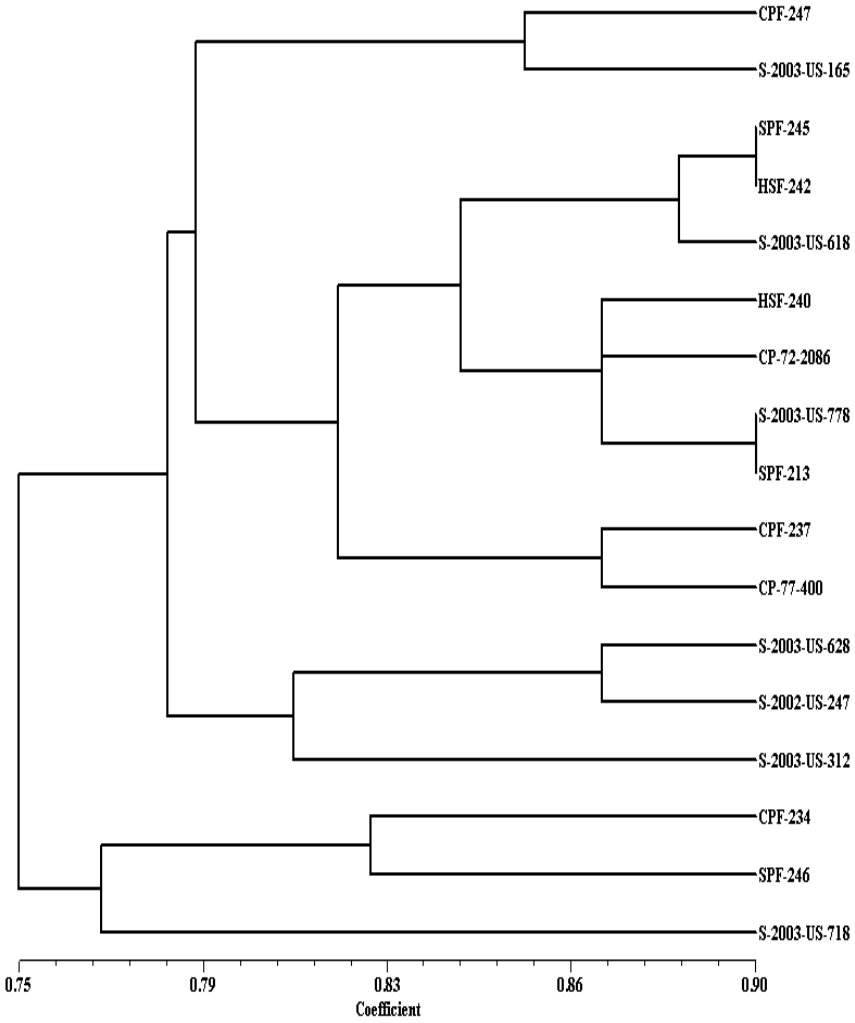

Figure 2. Dendrogram of 17 sugarcane accessions developed from SSRs data using unweighted pair group of arithmetic means (UPGMA) based on Nei's (1978) genetic distance.

237 and CP-77-400. Group III comprised of three genotypes viz. S-2003-US-628, S-2003-US-312 and S-2002-US-247. Group IV consisted of three accessions viz. CPF-234, SPF-246 and S-2003-US-718. Genotypes included in same cluster are more similar to each other but these are less similar to the genotypes in other clusters.

Conclusions

The analysis of variations in SSR fragments provides an effective tool for examining diversity to improve plant breeding strategies. Identifying useful SSRs is critical but in sugarcane this can be a lengthy and difficult process due to their abundance and the complexity of the sugarcane genome. Less information is available on the genetic diversity within and between *Saccharrum* 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. Future breeding efforts involving crosses between and within the groups identified in this study may provide useful strategies for combining beneficial genes and alleles in new sugarcane varieties while maintaining genetic diversity.

REFERENCES

Andersen JR, Lubberstedt T (2003). Functional markers in plants. Trends Plant Sci. 8:554-560.

Arencibia A (1998). Gene transfer in sugarcane. In: Biotechnology of Food Crops in Developing Countries. (Eds.): T. Hohn and K.M. Leisinger. Springer-Verlag, New York. pp. 79-104.

Cordeiro GM, Casu R, McIntyre CL, Manners JM (2001). Microsatellite markers from sugarcane (Saccharum spp.) ESTs cross transferable to Erianthus and Sorghum. Plant Sci. 160:1115-1123.

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

Daniels J, Roach BT (1987). Taxonomy and Evolution. In: Sugarcane Improvement through Breeding (Heinz DJ, ed.). Elsevier Press, Amsterdam. 7-84.

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus. 12:13-15.

Eisen JA (1999). Mechanistic basis for microsatellilte instability. In: Goldstein DB, Schlotterer, C (eds) Microsatellites: evolution and applications. Oxford University Press, Oxford. pp. 34-48.

Hoisington D, Khairallah M, González D (1994). Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. CIMMYT, Mexico. 165-173.

Jannoo N, Forget L, Dookun A (2001). Contribution of Microsatellites to Sugarcane Breeding Program in Mauritius. International Society of Sugar Cane Technologists, Proceedings of the XXIV Congress, Brisbane. pp. 637-639.

Khan IA, Khatri A, Nizamani GS, Siddiqui MA, Khanzada MH, Dahar NA, Seema N, Naqvi MH (2004). *In vitro* studies in sugarcane. Pak. J. Biotech. 1:6-10.

McIntyre L, Aitken K, Berding N, Casu R, Drenth J, Jackson P, Jordan D, Piperidis G, Reffay N, Smith G, Tao Y, Whan V (2001). Identification of DNA Markers Linked to Agronomic Traits in Sugarcane in Australia. In: International Society of Sugar Cane Technologists. Proceedings of the XXIV Congress, Brisbane, Australia, 17-21 September. Australian Society of Sugar Cane Technologists, Brisbane. 560-562.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individual. Genetics 89:583-590.

Pan YB, Cordeiro GM, Henry RJ, Schnell RJ (2002). Microsatellite Fingerprints of Louisiana Sugarcane Varieties and Breeding Lines. In: Plant, Animal and Microbe Genomes X Conference, 12-16 January, San Diego.

Pinto LR, Oliveira KM, Marconi T, Garcia AAF, Ulian EC, de Souza AP (2006). Characterization of novel sugarcane expressed sequence tag microsatellites and their comparison with genomic SSRs. Plant Breed. 125:378-378.

Piperidis G, Christopher MJ, Carroll BJ, Berding N, D'Hont A (2000). Molecular contribution to selection of intergeneric hybrids between sugarcane and the wild species *Erianthus arundinaceus*. Genome 43:1033-1037.

Selvi A, Nair NV, Balasundaram N, Mohapatra T (2003). Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. Genome 46:394-403.

Smiullah**,** Farooq AK, Abdullah, Aqeel A, Muhammad AJ, Zafar I, Rameez I, Javed IW (2012). In vitro Regeneration, detection of Somaclonal Variation and screening for SCMV in Sugarcane (*Saccharum* spp.) Somaclones. Afr. J. Biotech., 11(48):10841-10850.

Tautz D (1989). Hypervariablity of simple sequences as a general source for polymorphic DNA markers. Nucl. Acids Res. 17:6443-6471.