

Genetic diversity of sugarcane hybrid cultivars by RAPD markers

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Abstract Genetic diversity among sugarcane hybrids (*Saccharum* spp) is pre-requisite for sugarcane improvement through breeding. Twelve decamer oligonucleotide random-amplified polymorphic DNA (RAPD) markers were utilized to investigate the genetic potential among 24 sugarcane cultivars. A total of 120 fragments were originated by 12 RAPD primers. An average number of fragments were obtained as 11.42 fragments per cultivar, which ranged from 4 to 21 fragments. The genetic similarity among 24 sugarcane cultivars ranged from 0.236 to 0.944 with the mean similarity value of 0.508. On the basis of phylogenetic analysis based on dendrogram, the cultivars were clustered into five groups. Two varieties Co 0118 and CoS 07250 were found as highly diverse sugarcane cultivars. Three most popular cultivars viz, Co 0238, Co 1158, and CoS 08272 were clustered a diverse among particular group. These clusters with their diverse genealogy indicated the influence of parental genome contribution to clustering. Diverse varieties developed for east region were grouped in the separate clusters which indicated the influence of adaptation of varieties to particular agro-climatic condition. Hence, these five diverse hybrid cultivars would be used in further breeding program to get the prominent sugarcane clones which may produced higher cane yield and sugar content.

Keywords Sugarcane hybrids · Varietal improvements · Genetic diversity · Dendrogram · Molecular markers · RAPD

Introduction

Modern sugarcane varieties are poly-aneuploid hybrids with unequal contribution from *S. officinarum* (80–90%) and *S. spontaneum* (10–20%) parental genomes and a small percentage of recombinant chromosomes (Jisen et al. 2013). Commercial sugarcane varieties have complex large poly-aneuploid genome that creates difficulty in breeding efforts (Cunff et al. 2008). Over the past two decades, studies utilizing various molecular techniques to unravel the complexity of this important crop species have provided a greater understanding of its complex genetic composition (Rossi et al. 2003).

Most of the sugarcane varieties are breed of *S. spontaneum* and *S. officinarum*. To minimize the negative effects of *S. spontaneum* and to retain the high sucrose producing ability of *S. officinarum* during crosses, a series of back-crosses were made between the inter-specific hybrids and the *S. officinarum* parents. This led to the “nobilization” of *Saccharum* spp. hybrids (Sreenivasan et al. 1987). This was a major breakthrough in sugarcane varietal improvement programs in the terms of improved sugar productivity, high disease resistance, and high ratooning ability. Although nobilization was highly successful, but due to limits of the gene pool exploited during the traditional breeding programs, very limited progress has been achieved in increasing sugar content (Pan et al. 2004; Singh et al. 2015).

The limited number of sugarcane parental clones involved in the crosses. Subsequently, the narrow genetic base of the modern varieties is reflected in the slow progress in sugarcane breeding program. The utilization of genetic variability of sugarcane commercial cultivars, related genera, *Saccharum* complex is more essential for further breeding study. Knowledge of genetic diversity

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among adapted cultivars or elite breeding materials has a considerable impact on the improvement of crop plants. It can be obtained from pedigree analysis, morphological traits or using molecular markers (Mohammadi and Prasanna 2003). Molecular markers offer the best estimate of genetic diversity, since they are independent of the perplexing effects of environmental factors. Molecular markers have been widely used for the characterization of germplasm in a variety of crops including sugarcane. Various types of markers such as DNA-based markers which include restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite or simple sequence repeat (SSR) etc. have been used in these studies for the analysis of phylogeny, inter-species relationships, and genetic diversity among the *Saccharum* species, related genera and their hybrids (Sharma et al. 2014; Ahmed and Gardezi 2017). In the light of this information, the phylogenetic and genetic variability analysis of sugarcane commercial cultivars has been proposed for the identification of potential specific groups and for optimising hybridisation and selection procedures for evaluating elite genotypes.

Materials and methods

Plant material

Commercial cultivars used in the experiment were grown at Sugarcane Research Institute. Twenty four Indian commercial hybrid cultivars viz, CoS 96260, Co 98014, CoS 767, CoS 802, CoJ 64, CoSe 92423, CoSe 03234, Co 0118, CoS 510, CoS 08272, CoS 95255, CoS 88230, CoSe 98231, Co 05011, CoS 8436, CoS 8432, CoS 07250, Co 1158, CoS 96268, Co 0238, UP 05125, CoSe 01235, CoS 96275, and Co 1007 were documented for experimentation, which are the commercial cultivars of tropical and subtropical parts of the Indian subcontinents (Table 1).

DNA extraction and genotyping

A total of 24 promising sugarcane cultivars including early and mid maturing were carried out for DNA extraction. Genomic DNA was extracted from young disease-free, immature leaves of all the 24 sugarcane varieties using modified CTAB method. Leaf samples (500 mg) were grounded to fine powder in liquid nitrogen and were transferred into 10 ml prewarmed CTAB buffer containing 2% (w/v) CTAB, 20 mM EDTA, 1.4 M NaCl, 100 mM Tris-HCl (pH 8.0), and 0.2% (v/v) β -mercaptoethanol and were incubated at 60 °C for 1 hour followed by incubation at room temperature for 15 min. Equal volume of

chloroform: isoamyl alcohol (24:1) was added and the mixture was centrifuged at 12,000 rpm for 10 min. After, centrifugation aqueous phase was pipette out in autoclaved tube. Equal volume of chilled iso-propanol was added into aqueous phase to precipitate the DNA. Precipitated threads of DNA were pipette out in microtube. White pellet was washed with 70% alcohol and air dried, and stored in 10 mM Tris. Unpurified DNA was treated with RNase for 1 h at 37 °C and was purified by phenol extraction (25 phenol:24 chloroform:1 isoamyl alcohol, v/v/v) followed by ethanol precipitation. Quantification for working DNA was determined @ 25 ng/ μ l by agarose gel electrophoresis using known concentration of λ DNA as standard.

RAPD amplification and agarose gel electrophoresis

Twelve decamer oligonucleotide random-amplified polymorphic DNA (RAPD) markers were utilized to investigate the genetic diversity among 24 sugarcane cultivars (Table 2). PCR was performed in the total volume of 25 μ l containing 1 μ l of genomic DNA as template, 2.5 μ l of 10 \times PCR buffer, 0.25 μ l each dNTPs (10 mM), 1 μ l of decamer primer (15 ng/ μ l), 1 U of Taq DNA polymerase (Genei), and rest PCR water. The optimized PCR conditions for RAPD analyses consisted of an initial denaturation at 94 °C for 5 min followed by 44 cycles of 60 s at 94 °C, 30 s at 37 °C, and 60 s at 72 °C and finally an extension of 7 min at 72 °C. PCR reactions were carried out on a Geneai Thermal Cycler. The amplified products were separated by horizontal electrophoresis on a 1.5% (w/v) Agarose gel using 1 \times TBE buffer (pH 8.0) and 0.5 μ g/ml ethidium bromide. Samples were electrophoresed at 100 V current for 4 h, and photographed under UV. The PCR analysis was conducted twice.

Data analysis

Clearly resolved bands were manually scored for their presence (1) or absence (0). Phylogenetic analysis among all the cultivars was computed using Jaccard's similarity coefficient using implemented module in the FreeTree software and clustered using the unweighted pair group method (UPGMA). The TreeView software was used for interactive visualization of the dendrogram.

Results and discussion

Fragments analysis

A total of 120 amplified fragments were well resolved generated by 12 decamer oligonucleotide RAPD primers. An average number of fragments were obtained as 11.42

Table 1 Sugarcane cultivars and their parentage used in diversity analysis

Sl. No.	Varieties	Parentage	Sl. No.	Varieties	Parentage
1	Co 0238	CoLk 8102 × Co 775	13	CoS 96275	CoS 8119 × Co 62198
2	CoS 07250	CoS 8436 × Co 775	14	CoS 96260	CoS 767 × Co 453
3	Co 0118	Co 8347 × Co 86011	15	CoSe 92423	BO 91 × Co 453
4	Co 1007	Co 683 × P 63/32	16	CoSe 03234	BO 91 GC
5	Co 1158	Co 421 × Co 419	17	CoS 95255	Co 1158 × Co 62198
6	CoS 767	Co 419 × Co 313	18	CoS 96268	Co 1158 × Co 62198
7	CoJ 64	Co 976 × Co 617	19	CoS 08272	CoSe 92423 GC
8	CoS 510	Co 453 × Co 557	20	Co 05011	CoS 8436 × Co 89003
9	CoS 802	Co 419 × Co 658	21	CoSe 98231	CoS 7927 × Co775
10	CoS 8432	MS 68/47 × Co 1148	22	CoSe 01235	CoS 8119 × Co 62198
11	CoS 88230	Co 1148 × Co 775	23	Co 98014	CoS 8436 × Co 8213
12	CoS 8436	MS 68/47 × Co 1148	24	UP 05125	GRL28/92 × CoSe 92423

fragments per cultivar, which were ranged from 4 (CoS 96268) to 21 (UP 05125) fragments. Primer K8 produced 21 bands from 24 diverse cultivars. The fragment sizes were ranged from 54 bp to 1172 bp using RAPD primers (Fig 1). The number and range of markers amplified in these sugarcane hybrids are higher or equal than reported earlier elsewhere (Piperidis 2003) and comparable with Indian sugarcane clones (Selvi et al. 2003; Hemaprabha et al. 2006). The high number of amplifications obtained in the species is possibly due to its polyploid origin and large genome size. It is probably due to large polyploid genome which may have more amplification sites. The fragments amplified were highly diverse revealing a high degree of polymorphism among the cultivars, which is not unusual for the genomes of polyploid species. The high level of polymorphism exhibited by the cultivars genome for RAPD fragments has been attributed to the high polyploidy, heterozygosity (Nair et al. 2002a, b). In comparison, an automated AFLP analysis of sugarcane germplasm detected 150 bands per primer combination (Besse and McIntyre 1998). In a previous study using RAPD and STMS markers, 15 primers amplified 221 fragments were screened in their study with sugarcane hybrids (Saranakumar et al. 2014).

Sugarcane cultivars show a range of variation in their parentage, cytotypes and adaptation. Since several agronomically important traits such as vigour, tolerance to biotic and abiotic stress are inherent in this species, it is considered to be the most important germplasm source in sugarcane breeding programs (Roach 1978). It has been widely accepted that the genetic variability among the modern sugarcane cultivars is largely due to their parentage diversification present in their genome (D'Hont et al. 1996).

Phylogenetic and cluster analysis

The RAPD pattern was clustered into five distinctive groups based on their diversification analysis. Robustness of clusters in the dendrogram was tested using Bootstraps analysis. The varieties Co 0118 and CoS 07250 were computed as diverse nature of genetic potential based on their parentages, comprising in an independent cluster under group first and second, respectively. Third cluster was divided into three sub-groups. The varieties Co 0238, Co 05011, CoS 8436, CoS 8432, CoS 88230, and CoSe 98231 were grouped together in first sub-cluster. CoS 8436 and CoS 8432 showed close relationship may be due to the same parentage (MS 68/47 × Co 1148). CoS 8436 and Co 05011 were closely associated due to parent. Likewise, CoS 88230 and CoSe 98231 also comprised in the same group, due to common male parent (Co 775). Five respective cultivars, namely, Co 1158, CoS 96268, CoS 95255, CoS 96275, and CoSe 01235, were comprised in the second sub-cluster. Similarly, CoS 96268 and CoS 95255 were more similar due to the same parent (Co 1158 × Co 62198). The third cluster contained CoS 08272, CoS 510, CoS 96260, CoSe 92423, and CoSe 03234 in the third sub-cluster. All sub-groups of third clusters were comprised of 16 varieties, and these varieties had widely used in previous breeding program. Fourth cluster comprised only three cultivars Co 98014, CoS 767, and CoS 802. The cultivars UP 05125, Co 1007, and CoJ 64 were grouped in the fifth cluster. The varieties Co 0238, Co 1158, and Co 08272 were found a diverse among particular sub-group. The varieties Co 0238 and Co 0118 having lower pollen fertility may be used as female parentage and CoS 8436 and CoSe 92423 having higher pollen fertility may be used as male parentage due to their diverse nature (Fig. 2).

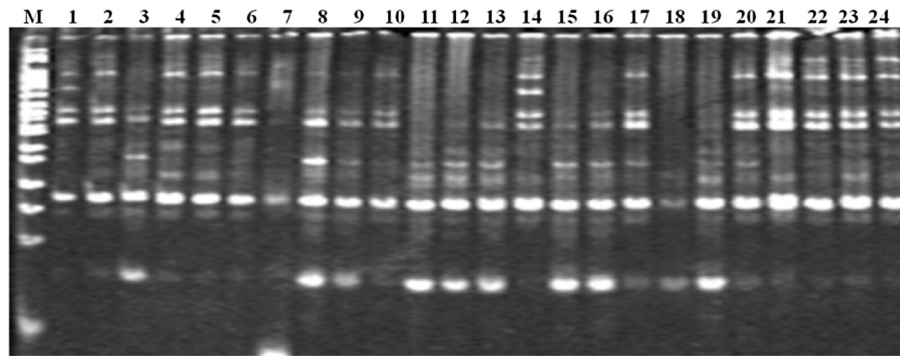


Fig. 1 DNA profiling of early sugarcane varieties with RAPD (A1) primer M- DNA ladder 100 bp, 1 CoS 96260, 2 Co 98014, 3 CoS767, 4 CoS 802, 5 CoJ 64, 6 CoSe 92423, 7 CoSe 03234, 8 Co 118, 9 CoS 510, 10 CoS 08272, 11 CoS 95255, 12 CoS 88230, 13 CoSe 98231, 14

Co 05011, 15 CoS 8436, 16 CoS 8432, 17 CoS 07250, 18 Co 1158, 19 CoS 96268, 20 Co 0238, 21 UP 05125, 22 CoSe 01235, 23 CoS 96275, 24 Co 1007

Table 2 Description of 12 decamer oligonucleotide RAPD markers

Sl. No.	Name of primers	Sequence (5'-3')
1	A1	AGT CAG CCA C
2	A6	GGT CCC TGA C
3	A10	GTG ATC GCA G
4	B 1	GTT TCG CTC C
5	B 8	GTC CAC ACG G
6	J 4	GAA TGC GAC C
7	J 14	ACC GAT GCT G
8	J17	ACC CCC TAT G
9	J18	GGC TAG GTG G
10	J 19	ACA GTG GCC T
11	J20	ACA CGT GGT C
12	K8	CTG TCA TGC C

The Jaccard's similarity indices based on RAPD profiles were subjected to UPGMA analysis. The dendrogram revealed the genetic similarity among the twenty four (24) sugarcane varieties, which were ranged from 0.236 to 0.94 with the mean similarity value of 0.508. This wide range of variation indicated high variability among the parental lines. The maximum similarity was calculated 0.94 between CoSe 01235 and CoSe 95255, CoS 96275 and CoSe 01235. The range of variation of inter genetic similarity was calculated from 0.28 to 0.94 with an average of 0.51. High genetic similarity among the sugarcane hybrid varieties grown in India (Hemaprabha et al. 2006; Nair et al. 2002a, b; Selvi et al. 2005a, b) and among the foreign varieties were reported by earlier researchers (Pan et al. 2004; Afghan et al. 2005; Alvi et al. 2008).

The values of genetic diversity parameters across the populations indicate that the genetic variation intra population (0.94) was much larger than that of inter population (0.51). This also indicates the wide genetic diversity among

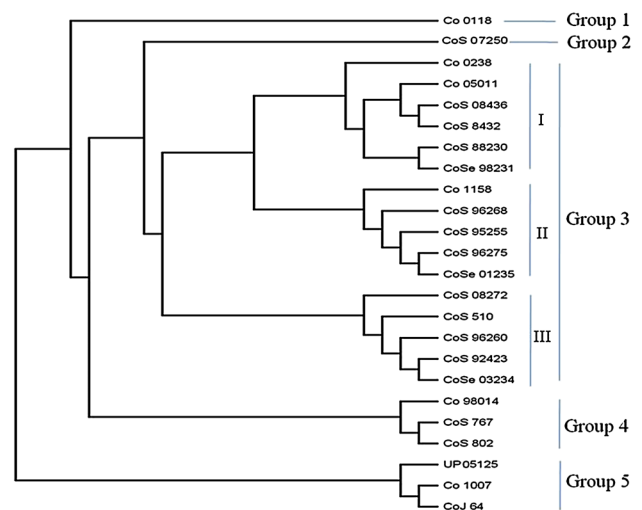


Fig. 2 Dendrogram derived from UPGMA cluster analysis using Jaccard's similarity indices based on RAPD profiles for twenty four sugarcane cultivars

the cultivars of different groups than those of same cluster. This suggested that genotypes occupying the same cluster have little diversity and selection of parents from within the cluster may not be considered promising for the development of elite sugarcane clones through hybridization program (Selvi et al. 2005a, b; Goel et al. 2005). A practical way that breeders could use genetic similarity among cultivars in breeding programs is to select the most genetically distant/similar genotypes among all possible cross combinations. In the present study, all 23 sugarcane cultivars in common parents group showed relatively lower diversity, compared with the diverse cultivars Co 0118 exhibited a new parents group. The other parentage, namely, CoS 07250, Co 0238, Co 1158, CoS 08272, Co 98014, and UP 05125, showed also diversity nature within own clusters. The diverse varieties Co 0118 and Co 0238 having lower pollen fertility may be used as female parentage and CoS

8436 and CoSe 92423 having higher pollen fertility used as male parentage due to their diverse nature. It indicates that the innovation of parents has showed a positive role in sugarcane breeding programs, since the group of new parents has higher genetic diversity, and thus, it will to some degree benefit the broadening of the genetic basis in sugarcane hybridization (Selvi et al. 2005a, b).

High level of genetic similarity revealed among the cultivars needed an urgent need to deploy genetically diverse genetic parents in sugarcane breeding program to broaden the genetic base of the cultivated varieties. Hence, introgression of diverse parents including new sugarcane hybrids must be increased to improve new clone which resistant to major pests and diseases. Consequently, it can be concluded that genetic similarity of the hybrids is primarily decided by their pedigree. In addition, selection of varieties for specific agro-climatic condition also influences genetic relatedness of the varieties.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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