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PHYLOGENETIC ANALYSES OF SUGARCANE CULTIVARS USING SIMPLE SEQUENCE REPEAT MARKERS

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Abstract

Sugarcane (Saccharum sp.) is one of the world's most commercial and extensively grown crops. The breeding of sugarcane is keystone of all advanced sugarcane industries and several research institutes. The success of sugarcane breeding program lies in the appropriate selection of genetically rich and diverse genotypes. Research rationale of present study was to analyze genetic diversity among 24 promising flowering and non-flowering sugarcane cultivars. Molecular marker based screening was done with PCR analysis using 10 SSR primers. SCM-32 primer showed highest polymorphic bands. The genetic similarity and UPGMA clustering were analyzed for all 24 sugarcane cultivars. The similarity index values for S19, S21, S22 and S23 suggested them as closest ones and S17 as the most distant one. UPGMA clustering based dendrogram showed that the correlation between Jaccard coefficient and similarity index is high and significant. All 5 clusters showed a mixture of flowering and non-flowering cultivars, indicating that molecular marker can play a potential role in sugarcane breeding programs than morphology based analysis.

Key words*: cluster analysis, genetic similarity coefficients, simple sequence repeats, sugarcane.*

INTRODUCTION

Sugarcane (*Saccharum* sp.) is the most prominent source of sugar and bio-fuel across the globe. Thus, it is one of the most commercially important crops. More than 75% of world sugar production is achieved from sugarcane which is grown across 100 countries. The sugarcane breeding is the mainstay of all advancements in sugarcane based industries and technologies in most of the research institutes. Hence, the application of modern molecular techniques will strengthen varities to achieve high levels of yield (Silva & Bressiani, 2005).

The success of sugarcane breeding program lies in the appropriate selection of genetically rich and diverse genotypes. However, understanding the genetic diversity among the cultivars may provide platform for improved traits.

Studies have shown that genetic markers greatly aid in early identification of genotypes as they are rapid, reliable and reproducible. Thus, allowing early identification of genotypes when compared with conventional techniques, viz., morphological and biochemical markers (Sindhu et al., 2011).

Generally, molecular markers are found to be useful in genetic diversity, systematic and phylogenetic analysis. Nevertheless they have also proved to be advantageous in construction of genetic maps and genetic linkage studies in combination with other markers (Anderson, 2007; Bosland et al., 2012).

Microsatellites (Simple Sequence Repeats) are ubiquitous on eukaryotic genomes. Apparently, their sequence patterns are capable to induce hyper-variability in multiple repeats at particular locus, during DNA replication and recombination. This kind of variation among microsatellite markers has proved to be advantageous for genetic marking.

Further, these markers exhibit high level of polymorphisms and extensively exploited for evaluating genetic diversity, in construction of genetic map and cultivar identification. Microsatellite markers are likely to have many applications in sugarcane genetics and breeding including germplasm analysis, cultivar

identification, parent evaluation and marker assisted breeding (Cordeiroa et al., 2000).

Several types of molecular markers have been successfully employed for better understanding genetic relatedness among the commercial crops such as Wheat (Mohapatra et al., 2003) and Phaseolae (Vir et al., 2009). Among sugarcane crops different molecular markers have been used. Recently, genetic markers such as RAPD, ISSR and ITS, have been used to study genetic diversity among *Erianthus* and *S. spontaneum* species (Zhang et al., 2004). Singh et al. (2010) analyzed the genetic diversity among eighty four genotypes of *Saccharum barberi*, *S. spontaneum* and *S. officinarum* origin which included Indian and non-Indian commercial cultivars. Thirty two microsatellite markers consisting of sugarcane cDNA derived microsatellite markers (SCM), genomic microsatellites and unigene sugarcane microsatellite markers (UGSM) were used. More recently, Sindhu et al. (2011) reported the use of sequence tagged microsatellite sites (STMS) in genetic diversity analysis and concluded that 12 unique markers may aid in varietal identification. In the present study, SSR markers were employed to assess genetic diversity of 24 commercial cultivars. Similarly, Singh et al. (2017) investigated genetic relatedness among 24 sugarcane cultivars using 12 RAPD markers. The objective of the present study was to analyze genetic diversity among 24 promising flowering and non-flowering sugarcane cultivars.

MATERIALS AND METHODS

Plant Material

In the present study, 24 sugarcane commercial cultivars were used for the analysis of genetic diversity which is widely grown across the Northern Karnataka, India (Table 1). All cultivars were maintained at S. Nijalingappa Sugarcane Research Institute, Belgavi, Karnataka. Young leaf samples were collected and kept at -20°C until further analysis.

Genomic DNA Extraction

Genomic DNA was extracted following the protocol of Doyle and Doyle (1987). The DNA quality was confirmed by agarose gel electrophoresis (0.8%) and quantified with the aid of Nano-Drop spectrophotometer (ND-1000, version 3.1.1, USA). The DNA samples were diluted to 20 ng μl^{-1} for polymerase chain reaction (PCR) amplification.

Table 1. List of Sugarcane Cultivars – Flowering and Non Flowering

Sample Code	Name of the Cultivars
Flowering	
S1	Co 2012-109
S2	Co06027
S3	Co 11024
S4	Co 10023
S5	Co 10024
S6	Co 2001-15
S7	CoC 671
S8	Co SNK 0632
S9	Com 0265
S10	Co SNK 09268
S11	Co 13006
S12	Co 10027
Non-flowering	
S13	Co 2012-23
S14	Co 2012-24
S15	Co 11023
S16	Co SNK 7658
S17	Co SNK 07337
S18	Co SNK 07680
S19	Co SNK 09227
S20	Co SNK 09293
S21	Co SNK 09232
S22	CO SNK 0811324
S23	Co SNK 83495
S24	Co 86032

Simple Sequence Repeat Marker Analysis

Ten microsatellite markers were selected based on the previous studies of Singh et al. (2010, 2017). PCR reactions were carried out in a 25µl reaction volume comprising genomic DNA (20 ng), forward primer (0.5 µl), reverse primer (0.5 µl), dNTP (1.5 µl), *Taq* buffer (1×), *Taq* DNA polymerase (1.5U) and finally making the volume to 25 µl using nanopure water. The PCR amplification was performed using Mastercycler gradient (Eppendorf) with the following conditions; the initial denaturation at 95°C for 5 min, following 40 cycles of denaturation at 94°C for 1 min; annealing condition was set depending on the standardized annealing temperature (Table 2) of each SSR primer with common time duration of 1 min extension at 72°C for 2 min and ended with final extension step for 10 min. The PCR reactions were repeated thrice for each primer for better reproducibility. Only highly reproducible and polymorphic primers were chosen for the data analysis.

Scoring of DNA Bands

Fragments that were clearly readable were considered for data analysis. Each amplified product was considered to be a unit character and the populations were scored for their presence (1) or absence (0) of a band on the gel (Botstein et al., 1980; Anderson et al., 1993) and the cluster analysis was performed. Dendrogram was plotted with the aid of DendroUPGMA online server and similarity matrix was calculated using Jaccard's coefficient.

RESULTS AND DISCUSSIONS

The present investigation was undertaken to evaluate genetic relatedness within 24 cultivars of *Saccharum* sp. These 24 accessions were maintained by S. Nijalingappa sugar institute, Belgaum, Karnataka, India. Among 24 cultivars, 12 were flowering (S1-S12) and 12 were non flowering (S13-S24) (Table 1).

Sugarcane is a heterozygous and considered to be genetically complex aneu-polyploid species. Studies revealed that sugarcane readily undergoes inbreeding depression upon selfing (Stevenson, 1965). Hence, it is highly essential to understand the genetic diversity of *Saccharum* sp. in order to work on genetic improvement of the germplasm for commercial purpose. Thus, in this study, we have made an effort to decipher the genetic diversity of this species using microsatellite markers especially SSR. A total of 10 SSR primers were used which are specific for *Saccharum* sp. Among ten primer sets, eight primers showed reproducible bands (Figure 1). The detailed information about the primers used in this study is tabulated in Table 2.

We noted that primer SCM-32 amplified highest polymorphic bands as compared with other selected primers. The above mentioned primers amplified a total of 56 alleles out of 15 loci with a range of 1 to 6 alleles and an average of 5.3 alleles per locus; proving the efficiency of these SSR markers as a potential tool for detecting genetic variations in cultivars of studied *Saccharum* sp.

Based on generated SSR profiles, cluster analysis was performed using Jaccard similarity index and unweighted pair group method with arithmetic mean (UPGMA) to plot a dendrogram representing genetic diversity among 24 accessions (Figure 2). The genetic similarity indices among cultivars ranged from 0.083 to 1 (Table 3). The cultivar S17 showed least genetic similarity; the cultivar S18 with similarity index 0.083 and S19 was 100% similar to S21, S22 and S23 with the similarity index of 1. Further, cluster analysis categorized the cultivars into five clusters (Figure 2).

Table 2. List of SSR Primers with their Annealing Temperatures used in the present study

Oligo (Name)	Primer (Sequence)	AT
SCM4-F	CATTGTTCTGTGCCTGCT(18)	52
SCM4-R	CCGTTTCCCTTCCTTCCC(18)	
SCM21-F	CCCTCCCATAACACACAC(18)	55
SCM21-R	TTGACAGCCCAAAGAGTT(18)	
SCM27-F	TTCTCTGACTTCCAATCCAA(20)	56
SCM27-R	ATCAAGCACGCCCGCCTC(18)	
SCM32-F	GATGAAGCCGACACCGAC(18)	55
SCM32-R	AGTTGCCTGTTCCCATTT(18)	
SOMS58-F	CCGCTTTCAACCTCTACAC(19)	52
SOMS58-R	GGCTTGGTGATTCTTCTCT(19)	
SOMS118-F	GAGGAAGCCAAGAAGGTG(18)	57
SOMS118-R	TAGAGCGAGGAGCGAAGG(18)	
SOMS135-F	TCTTCAACTTCCTCTGCCT(19)	55
SOMS135-R	GTTCCTGACTGTTCCCTTG(19)	
SOMS148-F	GATGACTCCTTGTGGTGG(18)	52
SOMS148-R	CTTGACGACCCTGCTGCT(18)	
UGSM60-F	CGACTCCACACTCCACTC(18)	55
UGSM60-R	CCGAACACCACCTTCTTG(18)	
UGSM542-F	ACCTCCACCTCCACCTCAGTTC(22)	55
UGSM542-R	CGTTCAGCTTCAGGGTGTCGAT(22)	

AT: Annealing Temperature (ºC)

Cluster 1 consists of one cultivar, S9. Cluster 2 consists of two groups; first group consists of S7, S12, S18, S20 cultivars. Second group consists of S4, S6, S8, S11 and S15. S17 with lowest genetic similarity is situated in the cluster 3. Cluster 4 consists of three groups. Cultivars S13, S16, S24 are situated in the first group, cultivars S19, S21, S22, S23 with 100% similarity are situated in the second group and cultivars S2, S3 are situated in the third group. Cluster 5 consists of S1, S5, S10 and S14. All clusters showed the mixture of flowering and non-flowering cultivars which suggests the application of large number of SSR markers for precise differentiation of cultivars. However, the present SSR markers used in the study is revealing the wide genetic diversity in studied cultivars suggesting that SSR markers are important tool to assess the genetic diversity and relatedness in *Saccharum* sp. commercial cultivars (Singh et al., 2010).

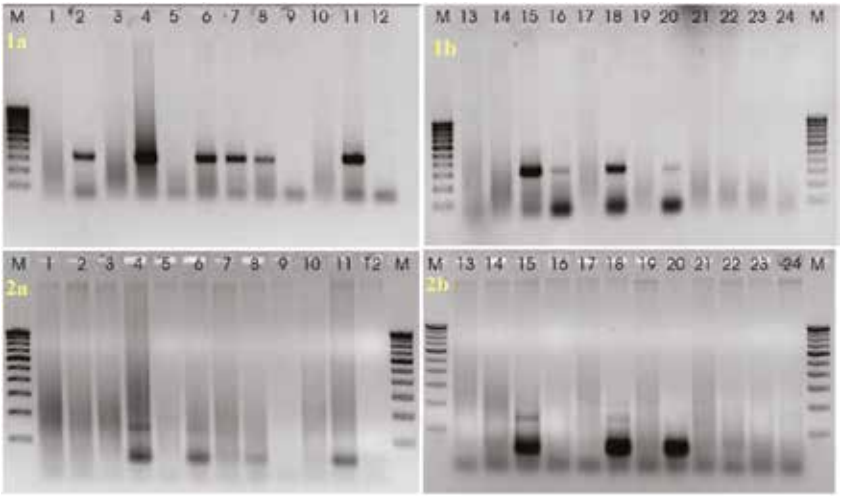

Figure 1. SSR Marker Analyses of Sugarcane Cultivars (1a: SOMS118-F, 1b: SOMS118-R, 2a: UGSM542-F, 2b: UGSM542-R)

Table 3. Similarity Index Computed with Jaccard Coefficient

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
S1	1	0.625	0.625	0.263	0.667	0.250	0.231	0.385	0.125	0.700	0.211	0.143
	0.429	0.545	0.429	0.250	0.222	0.231	0.571	0.154	0.571	0.571	0.571	0.429
S2		1	0.714	0.211	0.556	0.267	0.364	0.417	0.143	0.455	0.222	0.250
	0.286	0.333	0.462	0.500	0.250	0.250	0.667	0.273	0.667	0.667	0.667	0.286
S3			1	0.211	0.556	0.267	0.364	0.417	0.143	0.600	0.222	0.250
	0.286	0.333	0.462	0.286	0.250	0.250	0.667	0.273	0.667	0.667	0.667	0.286
S4				1	0.316	0.765	0.444	0.556	0.118	0.421	0.941	0.529
	0.176	0.421	0.579	0.111	0.167	0.529	0.105	0.471	0.105	0.105	0.105	0.111
S5					1	0.400	0.417	0.462	0.250	0.636	0.263	0.308
	0.222	0.500	0.500	0.222	0.200	0.417	0.500	0.333	0.500	0.500	0.500	0.222
S6						1	0.571	0.600	0.154	0.438	0.706	0.692
	0.143	0.438	0.625	0.143	0.214	0.692	0.133	0.615	0.133	0.133	0.133	0.067
S7							1	0.667	0.222	0.357	0.471	0.800
	0.091	0.357	0.467	0.333	0.182	0.636	0.182	0.700	0.182	0.182	0.182	0.200
S8								1	0.182	0.615	0.588	0.538
	0.273	0.400	0.600	0.273	0.071	0.538	0.250	0.583	0.250	0.250	0.250	0.273
S9									1	0.200	0.125	0.222
	0.250	0.200	0.154	0.250	0.200	0.222	0.200	0.250	0.200	0.200	0.200	0.250
S10										1	0.368	0.267
	0.300	0.538	0.643	0.182	0.167	0.357	0.400	0.286	0.400	0.400	0.400	0.300
S11											1	0.562
	0.188	0.368	0.526	0.118	0.176	0.471	0.111	0.500	0.111	0.111	0.111	0.110
S12												1
	0.091	0.267	0.467	0.200	0.182	0.800	0.083	0.889	0.083	0.083	0.083	0.091
S13	1	0.300	0.143	0.200	0.167	0.091	0.400	0.100	0.400	0.400	0.400	0.500
S14		1	0.438	0.182	0.400	0.267	0.400	0.200	0.400	0.400	0.400	0.300
S15			1	0.231	0.133	0.571	0.308	0.500	0.308	0.308	0.308	0.143
S16				1	0.167	0.200	0.400	0.222	0.400	0.400	0.400	0.500
S17					1	0.083	0.333	0.091	0.333	0.333	0.333	0.167
S18						1	0.083	0.889	0.083	0.083	0.083	0.091
S19							1	0.091	1.000	1.000	1.000	0.400
S20								1	0.091	0.091	0.091	0.100
S21									1	1.000	1.000	0.400
S22										1	1.000	0.400
S23											1	0.400
S24												1

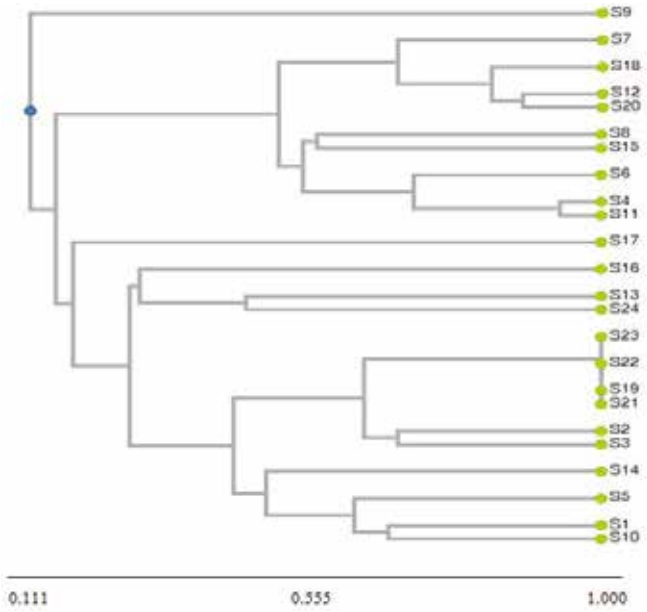

Figure 2. Phylogenetic Tree Analyses Based on Jaccard Coefficient

CONCLUSIONS

In sugarcane, cultivars morphological tools have limited implication in progeny identification as all sugarcane cross often consist of hybrids, selfs, and off-types. Hence molecular markers can play vital role in sugarcane breeding program. Among the various molecular markers, SSR markers may efficiently be used to evaluate the genetic polymorphism to establish the breeding and conservation strategies for cultivated plants like *Saccharum* sp.

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