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ASSESSMENT OF GENETIC DIVERGENCE AMONG SUGARCANE GENOTYPES (*Saccharum officinarum L.*) BASED ON MOLECULAR MARKERS

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ABSTRACT
The success of any crop improvement program depends upon the available genetic diversity as well as the kind and magnitude of variation. In the present studies a set of fifty-two visually chosen agronomically superior genotypes were analyzed for genetic diversity at molecular level. From a total of forty-one simple sequence repeats markers, thirty - two polymorphic markers revealed eighty-four alleles/bands indicating 2.64 alleles per locus. Polymorphism information content (PIC) value ranged from 0.17 to 0.74 with a mean value of 0.54 indicating a moderate level of genetic diversity in the test material. On the basis of DARwin's Dice dissimilarity coefficient fifty-two genotypes were grouped into seven distinct clusters. The estimated values of genetic dissimilarity ranged from 0.224 to 0.535. The cluster I was the largest with 15 genotypes while cluster VI and VII had only one genotype each. The parentage of genotypes did not have any effect on clustering pattern and the genotypes of bi-parental crosses were found to be distributed more widely. Genotypes CoJ 85 and S877/07 were the most divergent. The material under our study has indicated a good level of variability and genetic diversity. The potential of SSR markers in diversity analysis is also demonstrated.

INTRODUCTION

Sugarcane is a highly complex crop in terms of high ploidy level and heterozygous gene composition. The crop accounts for two- thirds of the world sugar production besides the allied uses of different sugarcane parts/bye products for the production of chemicals, paper, cattle feed, electricity etc. The crop has recently gained more attention because the ethanol derived from sugarcane represents an important renewable biofuel source (Menossi *et al.,* 2008).

Inspite of the diversity of sugarcane genetic resources and a long history of sugarcane genetic improvement, the progress in the varietal development during the past decade has been slow. This has mainly been attributed to narrow genetic base of sugarcane (kawar *et al.,* 2008). Patade and Suprasanna (2008) reviewed the past eighty years of cane breeding, and reported that the modern commercial cultivars were derived from a germplasm of twenty nobles and fewer than ten *S. spontanuem* derivatives. Molecular diversity studies also revealed the limited genetic base of present commercial varieties (Nair *et al.,* 2002). The availability and use of diverse germplasm is must in breeding program of a crop to enhance its productivity and sustainability. The careful choice of rich and genetically diverse parents can be based on geographical origin, agronomic traits, and pedigree data or molecular markers data (Melchinger, 1999). Keeping in view the importance of knowledge about the type and magnitude of variability in sugarcane improvement program, the present study was planned with the objective of assessment of genetic diversity in a set of genotypes using SSR markers.

Morphological character's based genetic diversity estimates suffer from the drawback that these are limited in number and influenced by the environment (Van Beuningen and Busch, 1997). On the other hand, molecular markers are powerful tools to estimate the complex genetic variation of sugarcane without environmental impact (Zala *et al.*, 2014; Bahurupe *et al.*, 2013; Ninghot *et al.*, 2015). Among the various types of molecular markers, microsatellite markers have gained considerable importance in plant genetics and breeding owing to their many desirable attributes and have been employed in linkage mapping, varietal identification, parental selection, marker assisted breeding and diversity studies etc. (Markad *et al.*, 2014; Ahmed and Gardezil, 2017). The paper deals with the results of studies conducted to assess the magnitude of genetic diversity in a set of selectively chosen sugarcane genotypes on molecular basis.

MATERIALS AND METHODS

A set of fifty-two genotypes comprising of five released varieties and crosses belonging to twenty-three cross combinations (three biparental crosses and twenty general crosses) were chosen (Table 1). The genotypes were selected visually on the basis of their performance in field conditions, from a set of

seventy-two genotypes being assessed for diversity analysis by Mahalonobis D^2 statistics detailed by Rao (1952) in a parallel study.

The test genotypes were sown in randomized complete block design with four rows of each genotype (six-meter length) in three replications. The standard cultural practices were carried out as per recommendations of Package of Practices for raising the crop. A total of 41 Simple sequence repeats (SSR) markers (Govindraj *et al*., 2005) were chosen for the assessment of genetic diversity in sugarcane.

Total genomic DNA was extracted from leaf tissues using CTAB (Cetyltrimethylammonium bro-mide) method (Murray and Thomson, 1980). Polymerase Chain Reactions were conducted following a procedure described by reagents manufacturer (Thermo Scientific™) with little modifications. Reaction volume was 20 μl containing reagents 5X Taq Buffer 4.0 μl, 25 mM $MgCl_2$, dNTP's mix 1 mM, 5 μM forward and reverse primers each, Taq DNA polymerase 5 U/μL, 40ng/μl DNA from each genotype and MilliQ H_2O 8.2 μl. The PCR amplification reactions were conducted in a Mini Opticon Real-Time PCR System BIO RAD™ under the programme of 105°C pre-heating, 94°C for 5 min initial denaturation, 35 cycles of: 94°C denaturation for 30 secs, annealing ranging 48-68°C depending on primer length for 45 secs, and extension 72°C for 2 min. Final extension at 72°C for 7 min and hold at 4°C. The PCR products were analyzed by electrophoresis on a 2.5% (w/v) agarose gel using 10 μl sample mixed with 2 μl 6X loading dye (Thermo Scientific™). Gel images were captured under gel documentation system (UV tech™).

Bands visualized after agarose gel electrophoresis were scored for their presence as 1 and absence as 0. Binary matrices consisting of 0's and l's were analyzed to obtain Dice dissimilarity coefficients among the genotypes using DARwin pc software (Version 5.0.158). Dice coefficient was clustered using the hierarchical clustering program selecting the unweighted pair group method with arithmetic average (UPGMA) algorithm in DARwin pc.

RESULTS AND DISCUSSION

Knowledge on genetic divergence is fundamental requirement to identify and organize the available genetic resources aiming at the production of promising cultivars (Palomino *et al*., 2005). Molecular markers have been recognized as one of the versatile tools for exploring the genetic phenomena. In the present studies out of 41 SSR markers used for diversity analysis, nine markers viz. NKS 1, NKS 3, NKS 7, NKS 8, NKS 9, NKS 11, NKS 17, NKS 30, and NKS 31 produced a single monomorphic band and were not included in the diversity analysis. The rest of 32 markers scored a total number of 84 alleles/bands and the number of alleles/bands per locus varied from 0 to 4 with an average of 2.65 alleles per locus (Table 2).

The markers NKS 5, NKS 6, NKS 34, NKS 38 were highly polymorphic in the material under study and revealed maximum of four bands (Plate 1), the markers NKS 26, NKS 45 revealed a maximum of three bands while the SSR markers NKS 12, NKS 14, NKS 15, NKS 21, NKS 22, NKS 23, NKS 25, NKS 40, NKS 48 and NKS 49 could reveal only two bands.

Comparatively lesser number of bands were observed from each marker in current studies than the number of bands documented by other researchers. Govindraj *et al*., (2005) used the same set of SSR markers for the genotyping of 48 germplasm lines and reported that the number of alleles (bands)

Table 1: Genotypes with their parentage used for molecular marker analysis

S. No.	Genotype	Parentage	S. No.	Genotype	Parentage
1	CoJ83	Released varieties	27	S716/07	CoS92263 G.C
2	CoJ85	Released varieties	28	S721/07	CoH110 G.C
3	CoJ88	Released varieties	29	S211/07	CoS87216 G.C
4	CoJ89	Released varieties	30	S875/07	Co 1148 G.C
5	CoH119	Released varieties	31	S608/07	CoS96869 G.C
6	S84/07	CoJ82315 X ISH 176	32	S1331/07	CoH76 G.C
7	S113/07	CoJ82315 X ISH 176	33	S832/07	Co 1148 G.C
8	S562/07	CoS96869 G.C	34	S1217/07	ISH 175 X CoS 510
9	S44/07	CoJ82315 X ISH 176	35	S643/07	CoS92263 G.C
10	S335/07	CoJ77 G.C	36	S1017/07	Co 93009 G.C
11	S233/07	CoS87216 G.C	37	S782/07	ISH 69 G.C
12	S206/07	CoS87216 G.C	38	S818/07	Co 1148 G.C
13	S677/07	CoS92263 G.C	39	S1333/07	CoH76 G.C
14	S722/07	CoH110 G.C	40	S1020/07	Co 93009 G.C
15	S196/07	CoH92 G.C	41	S823/07	Co 1148 G.C
16	S474/07	CoS8436 G.C	42	S570/07	CoS96869 G.C
17	S459/07	CoS8436 G.C	43	S850/07	Co 1148 G.C
18	S473/07	CoS8436 G.C	44	S854/07	Co 1148 G.C
19	S201/07	CoS87216 G.C	45	S737/07	CoJ83 G.C
20	S371/07	CoJ77 G.C	46	S844/07	Co 1148 G.C
21	S739/07	CoJ83 G.C	47	S116/07	CoJ82315 X ISH 176
22	S673/07	CoS92263 G.C	48	S931/07	Bo 91 G.C
23	S548/07	CoS96869 G.C	49	S893/07	Co 1148 G.C
24	S212/07	CoS87216 G.C	50	S861/07	Co 1148 G.C
25	S456/07	CoS8436 G.C	51	S1254/07	Cose 92423 G.C
26	S564/07	CoS96869 G.C	52	S1101/07	CoJ64(TC) X Co 86011

Table 2: List of polymorphic markers showing no. of bands and PIC value

S. No.	Name	No. of Band	No. of polymorphic bands	Per cent polymorphism	PIC
1	NKS 2	3	1	33.3	0.61
2	NKS 5	4	3	75	0.68
3	NKS 6	4	4	100	0.70
4	NKS 12	2	1	50	0.45
5	NKS 14	2	1	50	0.47
6	NKS 15	2	1	50	0.29
7	NKS 16	3	3	100	0.66
8	NKS 20	3	2	66.6	0.54
9	NKS 21	2	2	100	0.49
10	NKS 22	2	2	100	0.50
11	NKS 23	2	1	50	0.46
12	NKS 24	2	2	100	0.48
13	NKS 25	2	2	100	0.50
14	NKS 26	3	3	100	0.61
15	NKS 27	2	2	100	0.50
16	NKS 28	3	3	100	0.56
17	NKS 29	2	2	100	0.49
18	NKS 32	3	2	66.6	0.64
19	NKS 33	2	2	100	0.49
20	NKS 34	4	4	100	0.74
21	NKS 38	4	3	75	0.73
22	NKS 40	3	3	100	0.62
23	NKS 42	2	2	100	0.50
24	NKS 43	2	1	50	0.46
25	NKS 45	3	3	100	0.65
26	NKS 46	2	2	100	0.17
27	NKS 48	3	2	66.6	0.57
28	NKS 49	2	2	100	0.49
29	NKS 50	2	2	100	0.49
30	NKS 51	2	2	100	0.50
31	NKS 52	3	3	100	0.67
32	NKS 53	2	2	100	0.48

varied from 3 to 11.

Similar results were obtained in a study conducted by (Deng *et al.*, 2015) using 10 SSRs markers with as a set of 125 sugarcane varieties. They reported high ratio of polymorphism with variation ranging from 7-14. The differences observed in the number of alleles/bands could be due to the nature of germplasm used for the assessment of genetic diversity. The research material used in the present studies represented the progenies of commercially released varieties or parents used in crossing programs for a long time while genetic stocks belonging to different species of sugarcane i.e. *Saccahrum officinarum, S. spontaneum, S. robustum, S. sinense, S. barberi* and different sugarcane clones from Hawaii, Fiji, Puerto Rico, Mauritius, Indonesia, Taiwan, USA (Canal Point, Barbados, Australia and India) were used by Govindraj *et al.*, (2005). Similarly, in the diversity analysis of 54 Indian genetic stocks comprising of hybrids, inbreds, induced mutants and soma-clones with 18 STMS markers (Hemaprabha *et al.*, 2005), which included 11 common markers, a total of 221 bands were observed and the differences in the number of bands again could be attributed to the inherent diversity in the genetic stocks used for diversity analysis.

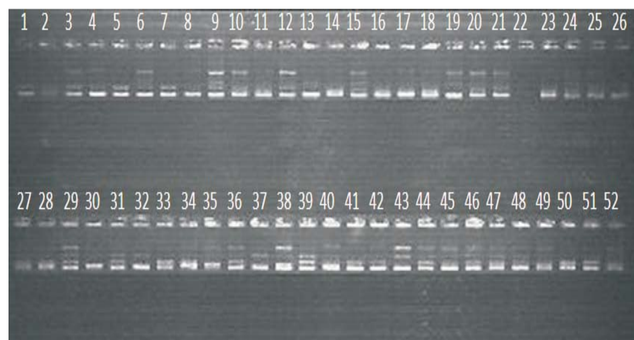
The polymorphism information content value, a measure of the relative ability of the marker to detect the genetic variability (Table 2) was found to range from 0.17 to 0.74 with a mean value of 0.54. This indicates that some markers revealed a fairly high level of genetic diversity in the current set of sugarcane genotypes while others exhibited a low level (Oliveira *et al.*, 2017). Overall, a moderate level of genetic diversity has been revealed by the current set of markers in the material under investigation.

The PIC values observed in current studies were nearly similar to those (0.34 to 0.78) obtained by Filho *et al.*, (2010) in genetic similarity assessment on 30 commercial cultivars from the breeding program of Republic of Brazil with 18 SSR markers.

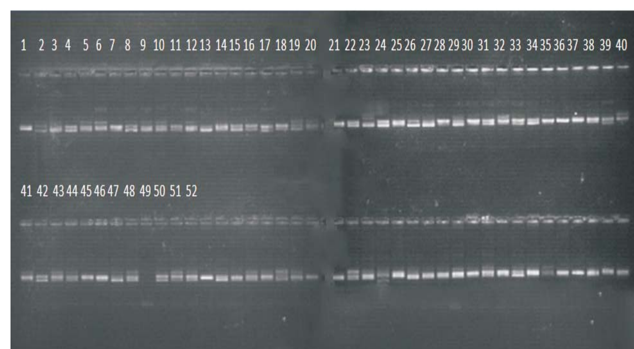
The high Polymorphism Information Content (PIC) represents a significant tool abetting in QTL mapping and identification there by facilitating marker assisted breeding by representing association with functional regions of genome (Marconi *et al.*, 2011).

On the basis of clustering pattern all the genotypes were clearly divided into seven distinct clusters. Cluster I was the biggest with 15 genotypes followed by cluster V with eleven genotypes. The clusters VI and VII were smallest having one genotype each viz. S854/07 and CoJ85, respectively. The pictorial representation of clustering pattern in the form of dendrogram is represented in Fig.1.

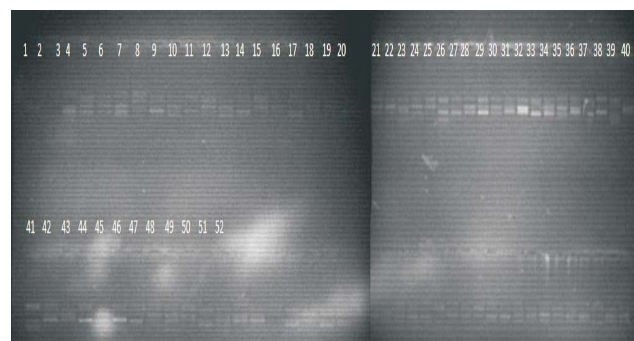
The pair wise genetic distances were found to range from 0.224 (between S854/07 and S844/07) to 0.535 (between



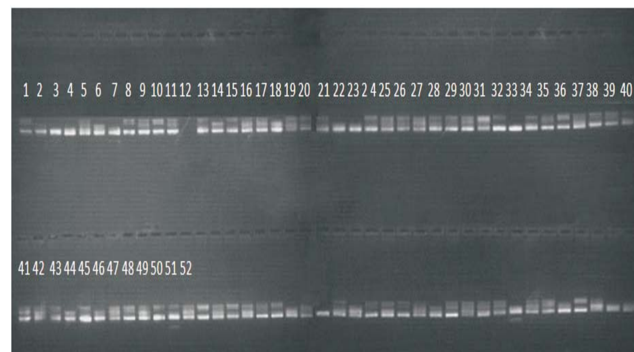
NKS 5



NKS 6



NKS 34



NKS 38
Plate 1

S677/07 and CoJ 85). High degree of dissimilarity was also found between S844/07 and CoJ85 (0.500). The CoJ 85 variety has been derived from the cross Q 63 x CoJ 70 and thus has Queensland blood in its genetic makeup due to which it may have exhibited the greatest level of diversity at molecular level.

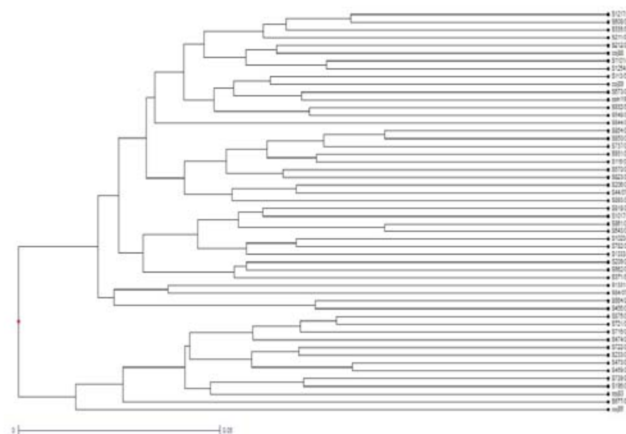


Figure 1: Dendrogram depicting the genetic diversity based on molecular markers

The genotypes S854/07 and S844/07 were found to be least divergent (0.224) followed by S459/07 and S473/07 (0.225). These genotypes shared the common parentage Co 1148 G.C.

The clustering pattern revealed that parentage of genotypes did not have any contribution towards clustering pattern. For example, the genotypes S823/07, S875/07 and S818/07 belongs to the same parent Co 1148 G.C but are grouped in the different clusters viz. cluster II, IV and III respectively. The genotypes from biparental crosses were found to be distributed more widely. Similar types of results were reported by Nair *et al.,* (2002). Further, the grouping of progenies of a same cross into different clusters could explain a high level of heterozygosity at the parental level. Sugarcane is a highly heterozygous polyploid crop with varying levels of aneuploidy (Grivet and Arruda, 2002).

So, a moderate to high level of genetic diversity was observed in the material under study at DNA level. The genotypes with high levels of genetic diversity can be chosen in a crossing program which may yield transgressive segregants. Moreover, strong pre- breeding strategies aiming at diversifying the germplasm base of sugarcane needs to be initiated for developing improved sugarcane varieties catering the future needs of growers, processing industry and consumers.

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