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Genetic Diversity within Sweet Sorghum (*Sorghum bicolor* (L.) Monech) Accessions as Revealed by RAPD Markers

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ABSTRACT

Genetic diversity was evaluated among twenty seven sweet sorghum germpalsm genotypes using random amplified polymorphic DNA (RAPD) polymorphic markers. RAPD markers were efficient and detected 93.4 per cent polymorphism among the accessions. All the genotypes were grouped into three clusters of which all the females came under one single cluster with exception of ICSB 293. Similarly all the males were evenly distributed except one genotype, SSV 74 which formed a distinct cluster itself. Hence, RAPD markers proved to very useful in estimating the genetic diversity among sweet sorghum accessions.

Key words : Diversity, RAPD, sorghum, cluster, polymorphism.

One of the major facets of any crop improvement programme is understanding and analyzing the extent of genetic variation and its distribution in a crop species, so that we can sample genetic resources in a more systematic fashion for breeding and conservation purpose. Classifying sorghum germpalsm accessions based on few discrete morphological characters may not provide an accurate indication of the genetic divergence. To overcome this limitation, biochemical and molecular markers are now widely used as tools to assess and validate the taxonomic classification. Efficient utilization of molecular techniques for evaluating genetic diversity has been proved in many crops by Tanksley *et al.* (1989) and Paterson *et al.* (1991) and in sorghum by Nkongolo and Nsapato (2003) and Uptmoor *et al.* (2003). However, there are no reports available on analysis of genetic diversity in sweet sorghum using molecular markers. The use of molecular techniques in assessing genetic diversity is supported by the fact that the evolutionary forces such as natural selection and genetic drift produce divergent polygenic branching which can be recognized

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because the molecular sequences on which they are based share a common ancestor. Among different types of molecular markers, random amplified polymorphic DNA (RAPD) technique is simple and used to detect nucleotide sequence variation. It is quick and well adapted for non-radioactive DNA fingerprinting of genotypes (Agrama and Tunistra, 2003). With this in view, RAPD markers were used with the objective to analyze the genetic diversity in some of the sweet sorghum accessions obtained from International Crop Research Institute for Semi-Arid Tropics (ICRISAT) and to find the genetic distance patterns among the accessions.

MATERIALS AND METHODS

Plant material : The plant material for the present study comprised of 27 genotypes of sweet sorghum obtained from world collection depository of sorghum at ICRISAT. These accessions were maintained at the Botanical Garden of Department of Genetics and Plant Breeding, College of Agriculture, UAS, Dharwad.

DNA extraction : Total genomic DNA was extracted from 5-6 days old seedlings grown in the laboratory, by grinding one g of leaf sample in liquid nitrogen using chilled pestle and mortar following the procedure of Sajjanar (2002). CTAB extraction buffer [(N-lauryl sarcosine, cetyltrimethylammonium-bromide), 0. 14 M sorbitol, 0. 22 M Tris, pH 8.0, 0.22 M EDTA, 0.8 M NaCl], which was pre-warmed to 65°C and added to each tube and placed on a water bath (65°C) for 15 minutes and cooled to room temperature. Equal volume of chloroform: isoamylalcohol (24:1) was added and vortexed for few seconds and centrifuged at 13,000 rpm for 10-15 minutes. The supernatant was transferred to a new eppendorf tube. The DNA was precipated by adding equal volume of prechilled isopropanol and incubated overnight at -20° C. DNA was recovered by centrifugation and the pellet was washed with 70 per cent ethanol and dissolved in T_{10} E_1. (10mM Tris-HCl: 1mM EDTA, pH 8.0). DNA concentration was estimated with a spectrophotometer and by Gel analysis. RNase 5 µl solution (10 mg ml^{-1}) treatment was given to remove RNA from the samples. PCR amplifications were performed in a Master Thermal Cycler-5331-Eppendorf version 2.30, 31-09,Germnay, by loading 20 µl reaction [1x PCR assay buffer, 200 m dNTP mix (eppendorf), 20 ng primer (Operon Technologies Inc, Almeda, CA, USA), 2U Taq DMA polymerase(Bangalore Genei, India) and approximately 50 ng template DNA]. A total of 29 primers were used for screening in the present study. The thermal profile used as: 1 cycle of 95°C for 5 min, 40 cycles each of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and one cycle each of 72°C for 8 min for amplification. The PCR amplified products were resolved on 1.5 per cent agarose gel electrophoresis and visualized by ethidium bromide staining and the Gel was photographed in Gel Documentation System (UVI Tech, Cambridge, England).

RAPD product scoring and data analysis : Each accession was scored for presence (1) or absence (0) of each polymorphic band. RAPD bands within the accessions were scored as missing if they were

Table 1. Total number of bands and per cent polymorphism generated by RAPD markers in sweet sorghum accessions.

Primer	5'----3' Sequence	Total No. of bands	Polymorphic bands	Per cent polymorphism
RKAT-2	CAGGTCTAGG	10	9	90
RKAT-4	TTGCCTCGCC	12	12	100
RKAT-5	ACACCTGCCA	10	10	100
RKAT-6	CCGTCCCTGA	12	12	100
RKAT-8	TCCTCGTGGG	8	7	87.5
RKAT-9	CCGTTAGCGT	16	15	93.75
RKAT-11	CCAGATCTCC	6	6	100
RKAT-12	CTGCCTAGCC	7	6	85.71
RKAT-14	GTGCCGCACT	3	2	66.66
RKAT-17	AGCGACTGCT	10	8	80
OPK-4	CCGCCCAAAC	4	4	100
OPK-6	CACCTTTCCC	6	6	100
OPK-7	AGCGAGCAAG	8	8	100
OPK-10	GTGCAACGTG	3	3	100
OPK-9(A)	CCCTACCGACA	9	8	88.88
OPK-9©	CCCTACCGACC	3	3	100
A7(A)	GAAACGGGTGA	9	8	88.88
A7(G)	GAAACGGGTGG	9	8	88.88
OPB-10	CTGCTGGGAC	9	8	88.88
OPJ-6	TCGTTCCGCA	15	15	100
Total		169	158	93.49

poorly resolved on the gel or if the template DNA did not amplify appropriately. Genetic distance was calculated on the basis of Jaccard's coefficient method. A dendrogram was constructed using a TREE procedure by Numerical Taxonomy and Multivariate analysis System (NTSYS-pc) computer version 2. 0 (SAS Inc, 1994) based on Jaccard's similarity coefficient using Unweighted Pair Group Method using Arithmetic Average Method (UPGMA) from which a matrix of 27 x 27 was obtained.

RESULTS AND DISCUSSION

A total of 169 scorable bands were produced by 20 polymorphic primers. The number of bands produced per primer varied from three (RKAT-14, OPK-9(C) and OPK-10) to sixteen (RKAT-9). The average number of bands generated per primer was 8.45. Out of the 169 bands, 158 were found to be polymorphic (93.49 %) indicating large amount of genetic variation with average number of polymorphic bands of 7 to 9 per primer pair. Out of the total 20 polymorphic primers 10 primers produced 100 per cent polymorphism (Table 1). The remaining 10 exhibited polymorphism which varied from 80. 0 per cent (RKAT-17) to 93. 75 per cent (RKAT-9). Similarly, higher level of polymorphism in sorghum based on RAPD has been reported by Aldrich and Doebley (1992), Veirling *et al.* (1994) and Cui *et al.* (1995). The higher genetic diversity observed in the present investigation may be due to the fact that the sweet sorghum accessions in this study were varying from very low to very high for sugar content and they also represented the entire

Table 2. Genetic similarity matrix generated by RAPD markers in sweet sorghum accessions.

Accessions	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
ICSB264	1.00																										
ICSB293	0.28	1.00																									
ICSB297	0.91	0.30	1.00																								
ICSB77	0.87	0.30	0.84	1.00																							
ICSB213	0.80	0.31	0.85	0.85	1.00																						
ICSB657	0.83	0.32	0.86	0.88	0.90	1.00																					
ICSB404	0.82	0.28	0.86	0.80	0.80	0.85	1.00																				
ICSB654	0.78	0.32	0.84	0.76	0.83	0.82	0.88	1.00																			
ICSR93034	0.55	0.24	0.57	0.52	0.54	0.48	0.45	0.53	1.00																		
SSV74	0.26	0.32	0.28	0.30	0.31	0.30	0.31	0.33	0.22	1.00																	
SSV84	0.76	0.30	0.80	0.75	0.79	0.72	0.68	0.78	0.68	0.35	1.00																
ICSR56	0.83	0.27	0.87	0.83	0.85	0.80	0.80	0.82	0.62	0.31	0.85	1.00															
ICSR89012	0.77	0.32	0.80	0.75	0.76	0.73	0.71	0.75	0.69	0.33	0.86	0.88	1.00														
ICSR91005	0.66	0.27	0.69	0.64	0.66	0.62	0.72	0.72	0.58	0.34	0.75	0.77	0.84	1.00													
S35	0.76	0.31	0.78	0.72	0.73	0.68	0.69	0.71	0.68	0.28	0.81	0.83	0.87	0.79	1.00												
ICSV574	0.74	0.27	0.76	0.74	0.76	0.71	0.69	0.72	0.65	0.28	0.78	0.84	0.83	0.72	0.87	1.00											
ICSR93026-2	0.61	0.31	0.64	0.60	0.61	0.61	0.57	0.62	0.62	0.43	0.65	0.64	0.66	0.62	0.65	0.67	1.00										
ICSR93019-2	0.70	0.30	0.76	0.68	0.74	0.71	0.70	0.73	0.56	0.31	0.74	0.75	0.76	0.69	0.80	0.79	0.66	1.00									
ICSR92003	0.24	0.70	0.23	0.21	0.23	0.22	0.20	0.22	0.19	0.29	0.22	0.20	0.23	0.19	0.25	0.20	0.27	0.27	1.00								
ICSR196	0.25	0.66	0.24	0.23	0.24	0.23	0.22	0.24	0.24	0.29	0.22	0.22	0.25	0.21	0.27	0.22	0.29	0.25	0.93	1.00							
ICSR89058	0.69	0.28	0.72	0.69	0.70	0.67	0.67	0.70	0.69	0.31	0.77	0.80	0.84	0.76	0.80	0.83	0.70	0.75	0.24	0.26	1.00						
ICSV700	0.70	0.25	0.69	0.68	0.69	0.65	0.65	0.68	0.62	0.27	0.72	0.74	0.76	0.67	0.74	0.78	0.64	0.69	0.25	0.27	0.87	1.00					
ICSV93046	0.71	0.30	0.72	0.67	0.73	0.69	0.69	0.74	0.63	0.29	0.73	0.76	0.77	0.68	0.76	0.80	0.65	0.74	0.28	0.30	0.85	0.92	1.00				
GD65122	0.70	0.27	0.73	0.67	0.72	0.68	0.71	0.74	0.62	0.28	0.71	0.76	0.77	0.69	0.76	0.79	0.64	0.78	0.28	0.28	0.83	0.92	0.93	1.00			
CSV19SS	0.70	0.26	0.68	0.69	0.68	0.65	0.65	0.69	0.62	0.26	0.70	0.75	0.75	0.67	0.75	0.79	0.67	0.73	0.26	0.29	0.83	0.92	0.89	0.90	1.00		
M351	0.71	0.26	0.72	0.70	0.72	0.69	0.69	0.74	0.63	0.27	0.71	0.76	0.77	0.68	0.76	0.79	0.69	0.77	0.26	0.30	0.85	0.86	0.90	0.90	0.95	1.00	
Uttara	0.68	0.24	0.65	0.64	0.65	0.62	0.61	0.66	0.61	0.19	0.68	0.72	0.74	0.60	0.72	0.76	0.59	0.72	0.26	0.26	0.81	0.81	0.81	0.80	0.87	0.88	1.00

1-ICSB264, 2-ICSB293, 3-ICSB297, 4-ICSB77, 5-ICSB213, 6-ICSB657, 7-ICSB404, 8-ICSB654, 9-ICSR93034, 10-SSV74, 11-SSV84, 12-ICSR56, 13-ICSR89012, 14-ICSR91005, 15-S35, 16-ICSV574, 17-ICSR93026-2, 18-ICSR93019-2, 19-ICSR92003, 20-ICSR196, 21-ICSR89058, 22-ICSV700, 23-ICSV93046, 24-GD65122, 25-CSV19SS, 26-M351, 27-Uttara

germplasm source of sweet sorghum maintained at world sorghum collection of ICRISAT. Second fact may be that all the primers chosen had tremendous discriminating power producing distinct banding pattern of all the genotypes of sweet sorghum. Similar results were also noticed in case of grain sorghum by Thimmaraju (1999).

The genetic similarity matrix (Table 2) also revealed that the genotypes ICSR 92003 and ICSR 93034; ICSR 92003 and ICSR 91005 and Uttara and SSV 74 as distantly related which was indicated from the lowest genetic similarity coefficient (0.19), while CSV 19 SS and M 35-1 were closely related with a genetic similarity coefficient of 0.95. The dendrogram (Fig 1) constructed from the data partitioned all the genotypes into three different clusters. Further, it also revealed that all the female lines were very closely related as they were grouped in a single cluster with exception of ICSB 293. However, SSV 74 formed a distinct cluster by itself and was found to be genetically distant from all the genotypes.

High level of DNA polymorphism among sorghum genotypes has been observed in other studies [Aldrich and Doebley (1992), Veirling *et al.* (1994) and Cui *et al.* (1995)]. Several factors could contribute to the high level of genetic variation present in the cultivated sorghums (Dogett, 1988). In addition to that high rate of naturally occurring hybridization between landraces and their wild relatives can lead to highly polymorphic genotypes. Among the different races of sorghum, bicolor and guinea are widely distributed

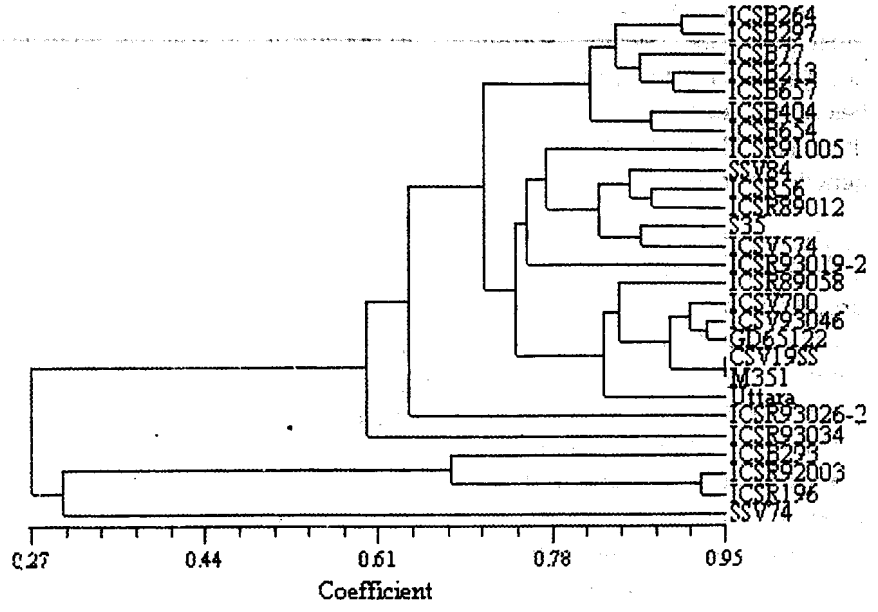

Fig. 1. Dendrogram constructed from RAPD markers on sweet sorghum accessions.

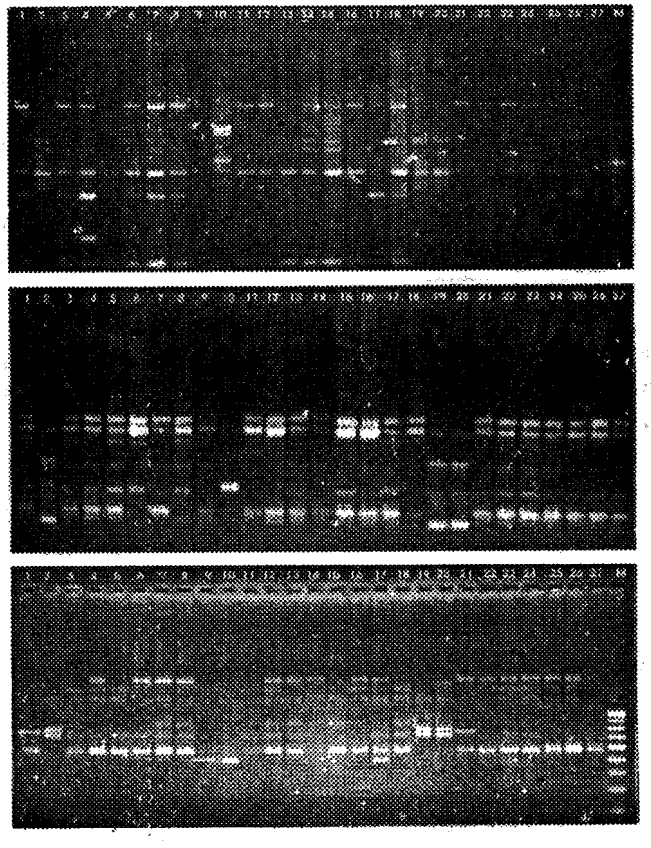

Photo 1. RAPD banding pattern of polymorphic primers in sweet sorghum (RKAT-09, RKAT-06, and OPJ-06)

and are likely to have greater genetic diversity resulting from differential selection pressure of broad array of ecological habitats as

reported by Menkir *et al.* (1997). As most of the sweet sorghum accessions in the present study belong to these two races therefore, they are likely to reveal higher genetic diversity.

Agrama and Tunistra (2003) also opined that eventhough sorghum is predominantly a self pollinated species it has unusual amount of genetic diversity. Menkir *et al.* (1997) also concluded that the multiple origin theory of domesticated sorghums and cross pollination between selected races and out crossing between domestic cultivar and highly variable wild species are also considered to be the factors contributing to the extensive genetic diversity observed in grain sorghum.

SSV 74 which formed a distinct cluster by itself may be due to the presence of unique alleles and such alleles are important because, they may be diagnostic for particular regions of the genome, specific to a particular type of sorghum.

Based on the results of the present study, it can be concluded that RAPD markers are extremely efficient and reliable tool for estimating genetic diversity and should be used on a continuing basis to document the available variability in sweet sorghum germplasm as a first step. Secondly, when such an high amount of genetic diversity has been realized in the sweet sorghum lines, which may be due to reshuffling of the alleles due to recombination, then there are better chances of getting transgressive segregants with higher mean performance for the trait when such lines are involved in the breeding programme.

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