International Journal of Bio-resource and Stress Management 2014, 5(3):381-385 DOI:10.5958/0976-4038.2014.00584.3

Full Research Article

RAPD Analysis in Sorghum [*Sorghum bicolor* (L.) Moench] Accessions

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Article History

Manuscript No. AR799
Received in
Received in revised form 13th August, 2014
Accepted in final form 16th August, 2014

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Keywords

Sorghum, Genetic diversity, RAPD, PIC, Cluster

Abstract

Genetic diversity is essential for the continued progress in breeding and to meet future environmental challenges. The present study was conducted to assess the genetic diversity among forty sorghum accessions using RAPD markers. A total of 107 polymorphic loci generated 65.35% polymorphism. The number of markers per primer ranged from 6-12. PIC value ranged from 0.34 to 2.54. Jaccard's similarity coefficient values ranged from 0.40 to 0.78. The cluster analysis resulted in five clusters revealing high homology between B-lines and R-lines. The clustering pattern of accessions also indicated that there was a wide genetic diversity between the grain sorghum groups and the forage sorghum groups. The variation was moderate among the sweet sorghum. The mutant lines showed more DNA polymorphism compared to the parents. Thus, RAPD analysis revealed the existence of a wide genetic diversity among the sorghum accessions providing scope for the development of well-defined heterotic groups for this crop.

1. Introduction

Sorghum (*Sorghum bicolor*) is the most drought tolerant of all the cereal crops and is the main staple food for the worlds poorest and most food insecure people. It is main source of food security in the semi-arid zone. Sorghum was domesticated in Africa, from where it was introduced to other regions of the world with diverse agro-climatic conditions. Therefore a wide diversity is found within and among the sorghum cultivars at both phenotypic and genotypic level. Besides being an important food, feed and forage crop, it provides raw material for the production of starch, fiber, dextrose syrup, biofuels, alcohol and other products. Genetic improvement of sorghum can help farmers in semi-arid regions where sorghum is a key food crop. Understanding the wealth of genetic diversity in sorghum will facilitate further improvement of this crop for its genetic architecture (Jayaramachandran et al., 2011). Knowledge of genetic diversity of a crop usually helps the breeder in choosing desirable parents for the breeding program. More diverse genotypes or accessions can be crossed to produce superior hybrids with resistance to abiotic and biotic stresses. Therefore, there is a need to evaluate the available accessions for the extent of genetic diversity.

Genetic diversity is normally assessed by common morphological traits. However, such traits are affected by effects of environment, development stage of the plant, also the type of plant material and require several replications to establish the genotypic contributions. Assessment of genetic diversity with molecular markers overcomes this problem. They are devoid of environmental effects and provide a true representation of the entire genome. Among the molecular markers, Random Amplified Polymorphic DNAs (RAPDs) have been extensively used in genetic research owing to their speed and simplicity. Several reports are available (Jeya Prakash et al., 2006; Sinha and Kumaravadivel, 2010; Orenthung and Changkija, 2013) on the genetic diversity studies among the sorghum accessions and had led to the development of effective strategies for genetic conservation and utilization. However, some doubts have also been raised regarding the reproducibility of RAPD band patterns. This problem can be solved by thoroughly optimizing PCR conditions and following the same protocol each time for more accurate analysis. The reaction should be performed twice, scoring only those bands that are reproducible in both the reactions (Singh et al., 2004). In the present study, an attempt was made to assess the genetic diversity among forty sorghum accessions using RAPD markers.

2. Materials and Methods

2.1. Experimental materials

The experimental material consisted of forty accessions of sorghum (*Sorghum bicolor* L.). Among these forty accessions, four accessions were sweet sorghum, seventeen were grain sorghum, two were forage sorghum, ten were mutant populations, three were maintainer lines and remaining four accessions were restorer lines (Table 1).

2.2. DNA extraction

The genomic DNA was extracted from fresh leaf tissue collected from 20 days old seedling of the sorghum accessions following Miniprep method described by Dellaporta et al. (1983). Quantity and quality of DNA was checked both by agarose gel eletrophoresis (0.8%) and Fluorometer. After quantification, the DNA was diluted to a concentration of 40 ng μl^{-1} for RAPD analysis.

2.3. RAPD analysis

PCR amplification was performed with 5 sets and 20 primers each thus a total of 100 primers (OPA01-20, OPB01-20, OPC01-20, OPD01-20 and OPE01-20) obtained from Operon technologies (Almeida, California, USA). Amplifications were carried out in 15 µl reaction volume containing 0.5U of Taq DNA polymerase (Bangalore Genei Ltd., India), 1X PCR buffer (Bangalore Genei Ltd., India), 0.25 µM primer (Operon Technologies Inc., USA), 0.25 mM dNTP (Fermentas Inc, USA), 1.5 mM $MgCl_2$ (Bangalore Genei Ltd., India) and 40 ng of template DNA. The thermal cycler (BIO-RAD iCycler, BIO-RAD laboratories, Inc.) was programmed as initial extended step of denaturation at 94^0C for 2 min followed by 40 cycles of denaturation at 94^0C for 1min, primer annealing at 37^0C for 1min and primer elongation at 72^0C for 2min, followed by

Table 1: List of sorghum accessions taken for the study

Sl. No	Accessions	Pedigree
	Sweet sorghum	
1	SSV84	Selection from Zera-zera sorghum IS2356
2	VMS98001	Selection from CSV15-1
3	VMS98002	Selection from NSS04-1
4	VMS98003	Selection from 208-1
	Grain sorghum	
5	APK1	Hybrid derivative of TNS30×CO26
6	BSR1	Multiple cross derivative (SC108-3×ICSV4) 16-3-1×(MR-801×R2751) 4-1-1
7	Paiyur2 (PYR2)	Pureline selection from IS15845
8	AKS96	Culture from Aruppukottai
9	AKS109	Culture from Aruppukottai
10	AKS112	Culture from Aruppukottai
11	TNS30	(CO18×CO22)×1022
12	TNS342	Culture from Coimbatore
13	TNS357	SPV1010×SPV881
14	TNS590	Culture from Coimbatore
15	K7	K3×M35-1
16	K8	IS12611×SV108
17	K11	K7×A6552
18	K12	Culture from Koilpatti
19	CO (S)28	CO25×SPV942
20	CO20	CO1×Bonganhilo
21	CO26	Derivative of MS8271×IS3691
	Forage sorghum	
22	CO27	CO11×S. halepense
23	CO(FS)29	TNS30×S. sudanense
	Mutants	
24	CO26-Tall plant mutant (TP)	γ ray (40 kR) induced mutant of CO26
25	CO26- High yield mutant (HY)	γ ray (40 kR) induced mutant of CO26
26	Co(S)28-Bold grain mutant (B)	γ ray (35 kR) induced mutant of CO(S)28
27	CO(S)28- High yield mutant (HY)	γ ray (35 kR) induced mutant of CO(S)28
28	CO(S)28-Tall plant mutant (TP)	γ ray (35 kR) induced mutant of CO(S)28
29	CO27-Tall plant mutant (TP)	γ ray (50kR) induced mutant of CO27
30	CO27-High biomass mutant (HB)	γ ray (50kR) induced mutant of CO27
31	CO(FS)29- Non shattering mutant (NS)	γ ray (60 kR) induced mutant of CO(FS)29
32	CO(FS)29- Tall plant mutant (TP)	γ ray (60 kR) induced mutant of CO(FS)29
33	CO(FS)29-25 High tiller mutant (TL)	γ ray (60 kR) induced mutant of CO(FS)29
	Maintainer lines	
34	CK60	Culture from Coimbatore
35	ICS111	Culture from Coimbatore
36	ICS2219	Culture from Coimbatore
	Restorer lines	
37	IS3541	Culture from Coimbatore
38	₹ 29	Culture from Coimbatore
39	₹ 673	Culture from Coimbatore
40	M-35-1	Culture from Coimbatore

an extended elongation step at 72^{0}C for 5min and finally held at 4^{0}C. The amplified PCR products were resolved in 1.5% agarose gel stained with ethidium bromide along with 1Kb DNA ladder as molecular marker. Gel was visualized under UV light and documented in gel documentation system (Alpha Imager 1200, Alpha Innotech Corp., USA).

2.4. Data analysis

RAPDPCR was repeated twice and the bands which consistently appeared in both the experiments and unambiguously polymorphic were scored visually for their presence (1) or absence (0) with each primer. Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc ver 2.02 (Rohlf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted Pair-Group method (UPGMA) (Sneath and Sokal, 1973).

The polymorphic information content (PIC) values of each primers were estimated using the formula $PIC=1-\Sigma pi^2$ where *pi* is the allele frequency for the ith allele (Nei, 1973).

3. Results and Discussion

3.1. RAPD analysis

Twenty random primers produced scorable and unambiguous bands out of the 50 RAPD primers tried in all the accessions were taken for scoring (Figure 1). The number of bands produced by each primer varied from 6 to 12 with an average of 8.1 bands and the size of the amplicons generated ranged from 200 to 3500 bp. A total of 162 loci were produced, out of which 107 loci were polymorphic. Polymorphism was observed to the extent of 66.04%. The primer OPE-04 proved to be highly polymorphic (88.89%).

3.2. Polymorphic information content

The Polymorphic information content (PIC) values for the 20 primers ranged from 0.340 in primer OPE-07 to 2.539 in primer OPE-04 (Table 2). The higher the PIC value, the more informative is the RAPD marker. Hence, primer OPE-04 was found to be highly informative. These results showed the ability of RAPD to discriminate among sorghum accession.

Table 2: Primers showing polymorphism across sorghum accessions

Sl. No	Primer code	Total number of loci	Number of polymorphic loci	PIC
1	OPA-01	8	5	1.72
2	OPA-02	8	6	1.382
3	OPA-03	12	10	1.844
4	OPA-04	7	5	1.812
5	OPA-07	8	3	0.768
6	OPA-08	9	6	2.051
7	OPA-09	7	6	2.051
8	OPA-11	8	6	2.433
9	OPA-13	7	4	1.766
10	OPA-15	6	4	0.853
11	OPA-18	10	8	1.964
12	OPA-20	6	4	1.465
13	OPC-03	7	4	0.588
14	OPC-10	7	4	1.527
15	OPE-03	8	5	1.421
16	OPE-04	9	8	2.539
17	OPE-07	7	3	0.34
18	OPE-09	8	4	0.56
19	OPE-15	8	5	1.713
20	OPE-18	12	7	1.717

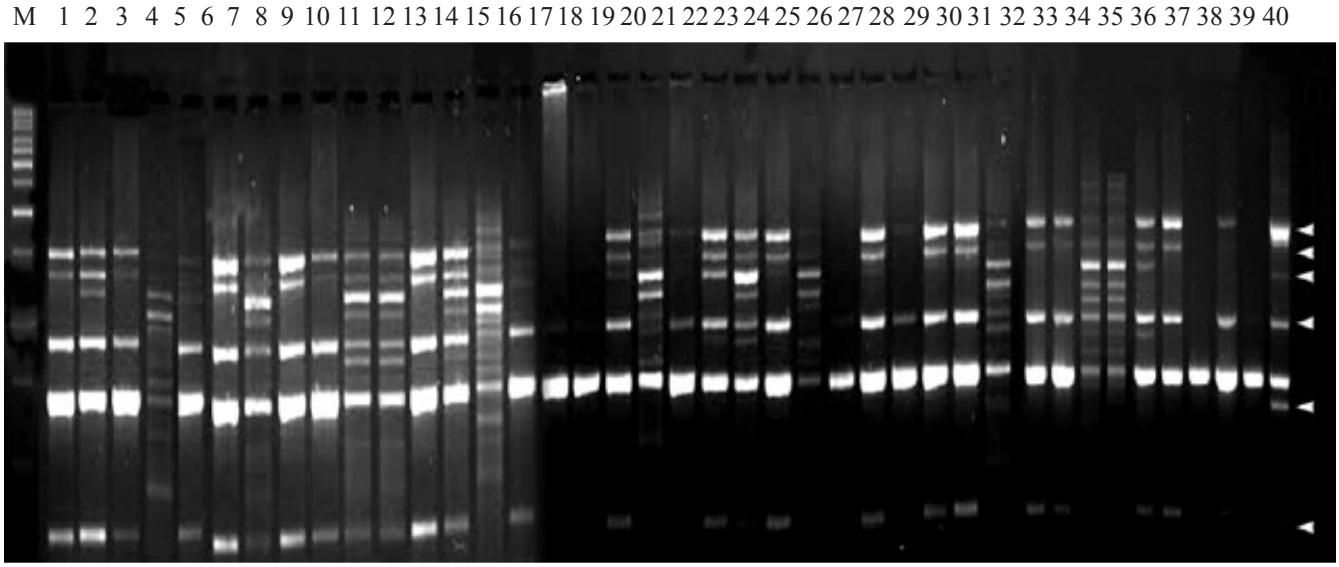

Figure 1: RAPD marer profile across sorghum accessions generated by the primer OPA-09. M=1kb ladder; △ = polymorphicband

3.3. Cluster analysis

The genetic relationships among the accessions are presented in the form of dendrogram (Figure 2). The Jaccard's similarity coefficient varied from 0.40 to 0.78. At nearly 53% similarity level, the dendrogram resulted in five clusters. Among the different clusters, the cluster size varied from 22 (cluster I) to 1 (Clusters IV and V). The cluster I and cluster III were highly heterogeneous. The cluster I consisted of sweet sorghum, grain sorghum, forage sorghum, CO26 mutants and CO(S)28 mutants. The cluster II consisted of grain sorghum, forage sorghum and CO(S) 28 mutants. The cluster III consisted of accessions of sweet sorghum, forage sorghum, maintainer lines and restorer lines. The cluster IV and cluster V consisted of grain sorghum. In cluster II, two accessions namely, CO27 and C27-Tall mutant were grouped together with similarity coefficient of 78%, which was the maximum among all the accessions. K7 and K8, which belong to grain sorghum group formed separate cluster, cluster IV and cluster V respectively.

This indicated that the accessions K7 and K8 were highly diverged from other accessions with similarity coefficient of

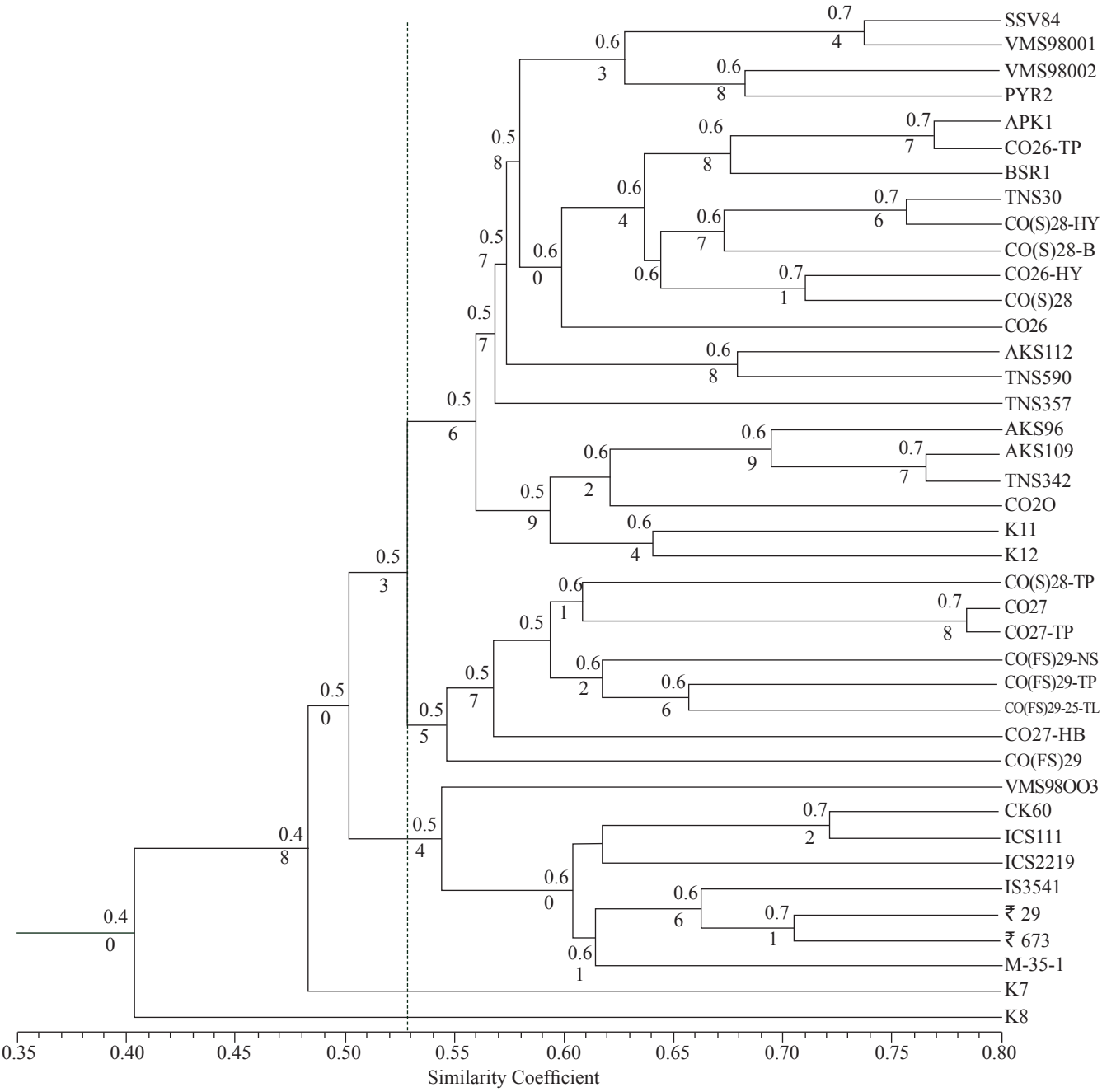

Figure 2: Dendrogram of sorghum accessions based on the RAPD data

40%. Although K7 is one of the parent of K11 but both these accession are quite divergent because selection for K11 could have taken place against K7 genome. Hence there may be differences in genome composition of both the lines that is identified by the RAPD markers due to recombination within amplified region.

Most of the grain sorghum genotypes were classified in cluster I except CO26, K7 and K8. Sweet sorghum viz., SSV84, VMS98001 and VMS98002 were grouped together in cluster I except VMS98003 (cluster III) and it was genetically dissimilar from them.

The maintainer lines (CK60, ICS111 and ICS2219) and restorer lines (IS3541, ₹ 29, ₹ 673 and M-35-1) were grouped together in cluster III indicating that they might all have a common pedigree. Fodder sorghum viz., CO27 and CO (FS) 29 falls into different clusters (cluster II and cluster III respectively) along with their mutants. CO26 and its mutants differ from each other, falls into different clusters but CO(S)28 and its mutants were genetically similar except CO(S)28 tall mutant.

4. Conclusion

The results of the above study confirm the reproducibility and reliability of RAPD analysis towards genetic diversity studies in sorghum. The clustering pattern indicated the existence of significant amount of variability among the grain sorghum. Thus, RAPD data were able to reveal the existence of a genetic diversity among the sorghum accessions used providing scope for the development of well-defined hetrotic g.1roups for this crop.

5. Future thrust

Validation of more number of molecular markers can provide better genome coverage and will facilitate easy discrimination of sorghum accessions at molecular level.

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