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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(1): 1446-1449
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www.thepharmajournal.com
Received: 08-11-2021
Accepted: 27-12-2021

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Genetic diversity analysis of sorghum (*Sorghum bicolor* L.) genotypes through RAPD

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Abstract
Fifteen sorghum (*Sorghum bicolor* L.) genotypes were evaluated for genetic diversity using RAPD markers. 17 primers were screened, out of which 14 primers generated total 100 bands with average level of polymorphism 93%. Based on the RAPD markers, dendrogram was constructed using the UPGMA method. The similarity coefficient ranged from 0.31 to 0.76 with an average of 0.53. The dendrogram clearly divided the 15 genotypes into six main clusters. The highest similarity coefficient (0.76) was recorded between ES-5 (G11) and EJ-4 (G-12) while the lowest similarity coefficient (0.31) was recorded between E-2 (G1) and CSV-23 (G9) and E-5 (G3) and CSV-2 (G9). Present investigation illustrates considerable potential of RAPD markers for estimating genetic diversity.

Keywords: Genetic diversity, RAPD markers, Sorghum

Introduction
Sorghum most popularly known in India as "Jowar" belongs to the family. Sorghum, most popularly known in India as "Jowar" is an often cross-pollinated crop, belongs to the family *Poaceae*, it is grown in about 90 countries over an area of about 44 million hectares in semiarid tropics. Sorghum (*Sorghum bicolor* L.) ranks fifth in worldwide economic importance among cereal crops with an annual production of 60 million tons. Besides being an important food, feed and forage crop, sorghum also provides raw material for the production of starch, fiber, dextrose syrup, biofuels, alcohol, and other products. More than half of the world's sorghum is grown in semi-arid tropics of India and Africa, where it is a staple food for millions of poor people (Mehmood *et al*., 2008) [9]. The advent of the RAPD assay (Williams *et al.* 1990) [16] provided an efficient method to detect DNA polymorphisms and generate a large number of molecular markers for genomic applications. RAPD markers are simple, rapid and have the advantage of no prior knowledge of genome sequence. RAPD technique can be used in laboratories with limited resources, but requires optimization for reproducible results for each species under investigation. Once the reaction conditions have been optimized the technique is reliable and informative.
RAPD markers for mapping and analysis of genetic diversity have been reported for a wide variety of plants including tobacco (*Nicotiana* species) (Lin *et al*., 2001) [6], cotton (*Gossypium*) (Khan *et al.,* 2000) [5], bambara groundnut (*Vigna subterranean*) (Massawe *et al*., 2003) [8], potato (*Solanum tuberosum* L.) (Sun *et al.,* 2003) [13], mustard (*B. carinata*) (Teklewold and Becker, 2005) [15] and safflower (*C. tinctorious* L.) (Mahasi *et al.,* 2010) [7].
The study comprising the 15 sorghum genotypes for exploiting the genetic diversity by RAPD markers as tools for assessing genetic variation.

Material and Methods
Plant material
Present investigation was conducted on 15 sorghum genotypes E-2, E-4, E-5, E-7, E-9, E-14, PJ-1430, CSV-15, CSV-17, CSV-23, CSV28, ES-5, EJ-4, EJ-11 and EJ-12. All the facilities related to present study were made available by Department of Molecular Biology and Biotechnology and Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur. The genotypes were obtained from Department of Plant Breeding and Genetics, MPUAT, RCA, Udaipur.

DNA Extraction

Total genomic DNA was isolated from young healthy leaves from 4 week old seedlings of all genotypes following CTAB extraction method of Doyle and Doyle (1990) [2]. DNA quantification and purity was checked by nano-spectrophotometric measurements.

Optimization of PCR conditions and RAPD analysis

PCR was performed according to the protocol of Williams *et al*. (1990) [16]. PCR reactions were carried out in gradient eppendorf thermocycler. Based on the results of initial experiments, PCR reaction mixture was used as described in Table 1. Quantity of DNA was diluted to final concentration of 50 ng/µl using TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0).

Table 1: PCR reaction mixture content

S. No	Components	Final concentration	Single tube (20µl)
1.	DNA template 50 ng/µl	50 ng	1.00µl
2.	Master mixture		
	1.dNTP mix	200 µM	1.6µl
	2. Taq DNA polymerase	1 U	0.33µl
	3. Reaction buffer (10X)	1 X	2.00µl
	4. Primer	0.5 µM	2.00µl
	5. dd H_2O		13.07µl

Agarose Gel Electrophoresis

Submerged gel electrophoresis unit was used for fractionating amplified PCR products on 1.2% agarose gel. The gel was prepared in 1X TAE buffer (Sambrook *et al*., 1989) containing (5.0µl/100ml) of ethidium bromide. The samples and loading dye were mixed in 8:1 ratio and loaded with micropipette. Electrophoresis was carried out at 50 V for 3 hr in 1X TAE buffer. The gel was viewed under UV transilluminator and photographed with the help of gel documentation system (Alpha DG DOC).

Scoring of the RAPD products

In order to score and preserve banding patterns, photographs of the gel were taken by a gel documentation system, under UV transilluminator. RAPD bands were designated on the basis of their molecular size ranging between 300-2500 bp. Molecular size of PCR products were estimated by referencing to the DNA ladder. The presence of each band was scored as '1' and its absence as '0'. Faintly visible bands were not scored, but a major band corresponding to faint bands was considered for scoring.

Statistical analysis for Similarity Coefficient

The scores (0 or 1) for each band obtained from photograph were entered in the form of a rectangular data matrix (qualitative data matrix). The pair-wise association coefficients were calculated from qualitative data matrix using Jaccard's similarity coefficient. The equation for calculating Jaccard's similarity coefficients 'F' between two samples A and B is:

$f = n_{xy} / (n_1 - n_z)$

n_{xy} = Number of bands common to sample A and sample B.

n_1 = Total number of bands present in all samples.

n_z = Number of bands not present in sample A or B but found in other samples.

Cluster analysis for the genetic distance was then carried out using UPGMA clustering method. The genetic distances obtained from cluster analysis through UPGMA were used to construct the dendrogram, depicting the relationships of the genotypes using computer program NTSYSpc version 2.02 (Rohlf, 1997) [10].

Results and Discussion

Total genomic DNA was isolated from young healthy leaves from 4 week old seedlings of all genotypes following CTAB extraction method of Doyle and Doyle (1990) [2]. DNA quantification and purity was checked by nano-spectrophotometric measurements. The concentration of DNA varied from minimum 179 ng/µl (CSV-23) to maximum 2740 ng/µl (E-14) (Table 2).

Table 2: DNA quantification in selected sorghum genotypes

S. No.	Sorghum genotypes	Code	Ratio of A260/A280	Conc. of DNA (ng/µl)
1.	E-2	G1	1.66	887
2.	E-4	G2	1.86	1390
3.	E-5	G3	1.88	890
4.	E-7	G4	1.84	858
5.	E-9	G5	1.94	554
6.	PJ-1430	G6	1.84	1738
7.	CSV-15	G7	1.77	679
8.	CSV-17	G8	1.92	377
9.	CSV-23	G9	1.97	179
10.	CSV-28	G10	1.95	321
11.	ES-5	G11	2.00	363
12.	EJ-4	G12	1.92	326
13.	EJ-11	G13	1.94	880
14.	EJ-12	G14	1.96	1934
15.	E-14	G15	1.94	2740

Assessment of amplified fragments obtained from RAPD

Each RAPD products was assumed to represent a single locus and data were scored as presence (1) and for its absence (0). Only those fragments which consistently amplified were considered for analysis. Electrophoresis pattern of RAPD profile on 1.2% agarose gel is illustrated in plate 1-7 with 14 specific primers. Total 17 primers were screened, out of which 14 primers generated total 100 bands. The total numbers of bands were observed for each primer separately and polymorphism percentage per primer was calculated subsequently. Three primers (RKAT-14, OPA-04, and OPK-4) gave no amplification. The total number of amplified bands varied between 3 (OPA-04) and 10 (OPB-10, OPC 8 and OPC 10) with an average of 5.88 bands per primer. The size of PCR amplified products ranged from 300-2500 bp (Table 3). Primer RKAT-17, OPB-10, OPC 1, OPC 3, OPC 5, OPC 6, OPC 7, OPC 8 and OPC 10 had amplified 8, 10, 9, 6, 7, 4, 7, 10 and 10 scorable bands showing 100% polymorphism.

A total 100 scorable bands out of which, 93 bands were found polymorphic. The average level of polymorphism was 93% (Table 4). The level of polymorphism achieved in the present investigation was in line with the findings of Ayana *et al.* (2000) [1], Jeyaprakash *et al.* (2006) [4] and Iqbal *et al*. (2010) [3]. Amplification data of present study indicated a high level of diversity in selected sorghum genotypes and also reflect the ability of RAPD primers to reveal high level of polymorphism.

Table 3: DNA polymorphism generated using 17 RAPD primers in 15 sorghum genotypes

S. No.	Primers	Total No. of bands (a)	Total No. of polymorphic bands (b)	Polymorphism % (b/a × 100)	Band size (bp)
1.	RKAT-14	0	0	0	NA
2.	RKAT-17	8	8	100	350-1600
3.	OPA-02	0	0	0	NA
4.	OPA-03	6	4	66	300-1100
5.	OPA-O4	3	2	66	500-1300
6.	OPB-10	10	10	100	400-1400
7.	OPC 1	9	9	100	550-2000
8.	OPC 2	8	6	75	300-1300
9.	OPC 3	6	6	100	450-1400
10.	OPC 4	7	6	85	300-1000
11.	OPC 5	7	7	100	350-1700
12.	OPC 6	4	4	100	500-950
13.	OPC 7	7	7	100	600-2000
14.	OPC 8	10	10	100	300-2500
15.	OPC 9	5	4	80	700-1900
16.	OPC 10	10	10	100	300-1500
17.	OPK-4	0	0	0	NA
	Total	100	93	93	
	Mean	5.88	5.47	93	

NA – Not amplified

Table 4: Details of the random primers used for amplification of genomic DNA of sorghum genotypes

Total number of primers	17
Number of primers which show amplification	14
Number of primers which did not show amplification	3
Number of primer which did not show polymorphism	0
Number of primers which show polymorphism	14
Total number of monomorphic bands	7
Total number of polymorphic bands	93
Total number of bands	100
Total number of polymorphic amplicon	652
Total number of amplicon produced	757

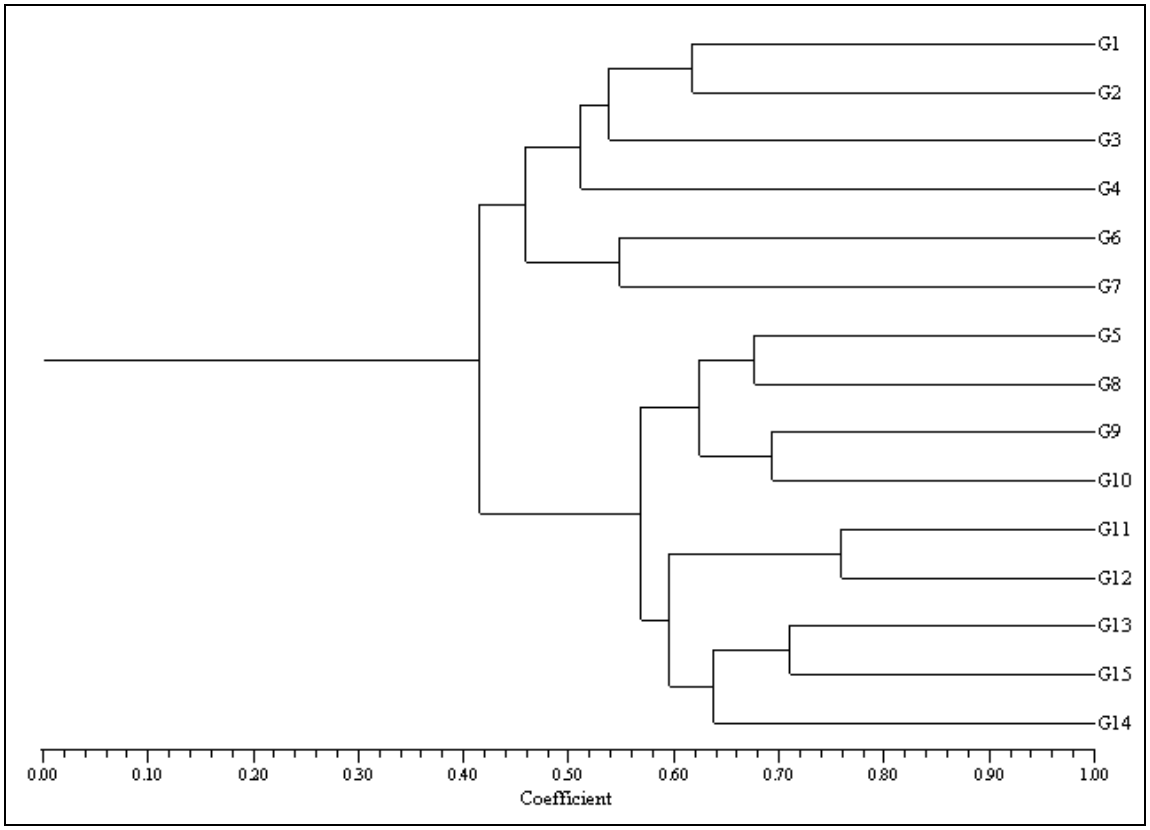

Fig 1: Dendrogram generated for fifteen sorghum genotypes using UPGMA cluster analysis based on Jaccard similarity coefficient

Table 5: Jaccard similarity coefficient for fifteen sorghum genotypes based on RAPD profiling

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
E-2	1.00														
E-4	0.62	1.00													
E-5	0.53	0.55	1.00												
E-7	0.47	0.57	0.50	1.00											
E-9	0.35	0.51	0.35	0.49	1.00										
PJ-1430	0.41	0.60	0.41	0.50	0.52	1.00									
CSV-15	0.34	0.54	0.43	0.44	0.58	0.55	1.00								
CSV-17	0.37	0.49	0.36	0.41	0.68	0.46	0.58	1.00							
CSV-23	0.31	0.49	0.31	0.44	0.63	0.45	0.56	0.68	1.00						
CSV-28	0.36	0.50	0.36	0.41	0.54	0.46	0.52	0.63	0.69	1.00					
ES-5	0.38	0.43	0.33	0.46	0.55	0.41	0.46	0.64	0.65	0.63	1.00				
EJ-4	0.38	0.40	0.32	0.39	0.48	0.35	0.42	0.56	0.57	0.57	0.76	1.00			
EJ-11	0.33	0.44	0.38	0.43	0.50	0.40	0.44	0.61	0.65	0.67	0.61	0.65	1.00		
EJ-12	0.40	0.41	0.40	0.42	0.39	0.39	0.38	0.51	0.51	0.61	0.57	0.56	0.61	1.00	
E-14	0.34	0.41	0.37	0.36	0.49	0.35	0.41	0.55	0.55	0.68	0.57	0.63	0.71	0.67	1.00

Cluster analysis signified that RAPD markers used in this study comprehensively distinguished the genotypes under investigation (Fig1). Cluster analysis revealed that highly susceptible genotypes (G12, G13 and G15), susceptible genotype (G14) except G4, were found genetically similar. A critical perusal indicated that out of 17 RAPD primers investigated, 9 RAPD primers (RKAT-17, OPB-10, OPC1, OPC3, OPC5, OPC6, OPC7, OPC8 and OPC10) were found to be polymorphic (Table 5, Fig: 2).

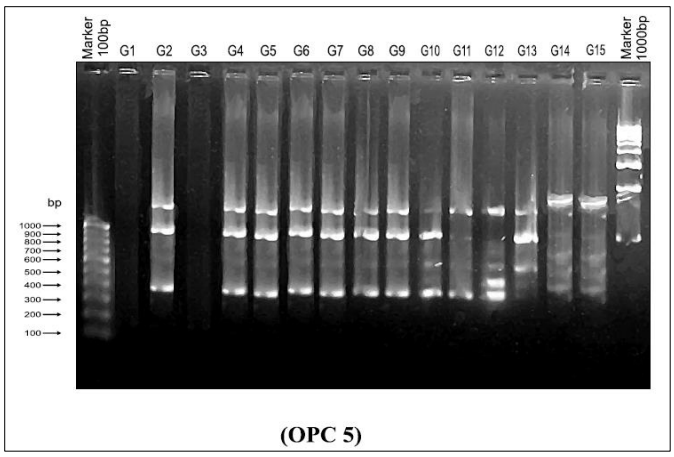

Fig 2: RAPD profile of Sorghum Genotypes (G1 –G15) generated with primer OPC-5

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