Pak. J. Bot., 40(5): 2015-2021, 2008.

MOLECULAR CHARACTERIZATION OF REGIONAL *SORGHUM BICOLOR* VARIETIES FROM PAKISTAN

SAJID MEHMOOD[1], AMINA BASHIR[1], ASIF AHMAD[1], ZAHID AKRAM[2], NYLA JABEEN[3] AND MUHAMMAD GULFRAZ[1*]

[1]*Department of Biochemistry, PMAS Arid Agriculture University, Rawalpindi-46300, Pakistan*
[2]*Department of PBG, PMAS Arid Agriculture University, Rawalpindi-46300, Pakistan*
[3]*Department of Bio Chemistry, Quaid-e-Azam University, Islamabad, Pakistan*

Abstract

Systematic characterizations and evaluation of plant genetic resources are prerequisites for the efficient use of material through conventional methods. Pakistan is an agro-based country and has a variety of important crops which includes sorghum. Traditionally, the data on agronomic, morphological and physiological plant traits are used to estimate the genetic diversity. But now biochemical and molecular markers have received much attention in recent years for genetic diversity studies. A study was conducted to assess the genetic diversity and phylogenetic relationship among different sorghum varieties by Genome DNA fingerprinting as revealed by Random Amplified Polymorphic DNA (RAPD) analysis. A total of 10 sorghum varieties were evaluated using RAPD. DNA was extracted from leaves, quantified and subjected to PCR analysis by using different primers. PCR products will be evaluated using 1.5% agarose gel electrophoresis. Data analysis was done using Gel compare II software program. Results show the appreciable amount of genetic diversity among the sorghum varieties. Out of the 95 amplification products scored, 75 bands (78.94%) were found to be polymorphic. The pairwise similarity values shows that variety RARI-S3 and RARI-S-4 (both from Chakwal) are showing closest relationship with highest similarity value i.e., 95.6% while variety YSS-9 and 84G01 (Bonus) show distant relationship with similarity value 67.8%. In this experiment RAPD proved to be a reliable, rapid and practical technique of revealing relationship among sorghum varieties.

Introduction

Plant genetic resources play an important role in generating new crop varieties with the high yield potential and resistance to biotic and abiotic stresses. The germplasm of a particular crop collected from the local sources provides greater genetic variability and can furnish useful traits to broaden the genetic base of the crop species. Morphological, biochemical and molecular procedures are currently being employed in evaluating plant genetic resources. Until recently, most of the characterization and evaluation has been based on the recording of either qualitative or quantitative morphological characters. Biochemical markers have received more attention in recent years as the data reflect more truly the genetic variability because they are the direct products of genes (Perry McIntosh, 1991). Besides biochemical markers, DNA based markers provide powerful and reliable tools for discerning variation within crop germplasm and to study evolutionary relationships (Gepts, 1993). PCR based techniques have been used successfully in DNA fingerprinting of plant genomes and in genetic diversity studies. These techniques include RAPD (Randomly amplified polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeat) and AFLP (Amplified fragment Length Polymorphism). In RAPD technique short oligonucleotides

*Corresponding author E-mail: gulfrazsatti@uaar.edu.pk, Tel.: +92-51-9062268

of arbitrary sequences are used singly to support the amplification of the plant genome and amplification products are separated by gel electrophoresis. RAPD technique has also been used in plants for the construction of genetic maps (Reitar *et al*., 1992), genotype identification, taxonomical studies in species such as *Arabidopsis*, banana and slash pine (Gaunge *et al*., 1996).

Pakistan is an agro-based country and has a variety of important crops. There are a number of crops whose genetic diversity is required to be found out due to their importance in different fields. One such crop includes *Sorghum bicolor* (L.). Monech which is ranked the 5th most important cereal crop in the world. Sorghum is a drought resistant low input cereal grain grown throughout the world. In most of the countries it is used primarily as animal feed, but in Africa and India it is used as human feed, where it is a staple food for millions of people (Agrama & Tunistra, 2003). It is one of the important crops that can be utilized for the production of bioethanol and electricity. It can produce 7000 liters of ethanol per hectare. Therefore keeping in view importance of sorghum as a valuable crop and RAPD as a power technique, the study was conducted with followings aims and objectives.

1. To assess the genetic diversity of 10 different *Sorghum bicolor* varieties, and
2. To find out the phylogenetic relationships among these varieties.

Materials and Methods

Sorghum seeds used in this study were collected from Millet station Rawalpindi, Pakistan under National Uniform Sorghum Yield Trial (NUSYT) program. A total of 10 varieties were collected and analyzed for molecular analysis. Seeds of these selected varieties were grown in Green house and 4-5 weeks old seedlings were taken and stored at -80°C.

DNA extraction: DNA extraction was done following the modified method of Puchooa (2004) using CTAB. Approximately two or three leaves were taken in an autoclaved mortar and crushed to fine paste using 2 ml CTAB buffer. The homogenate was incubated at 65°C for 45 minutes in block heater (Stuart Scientific). Material was centrifuged at 13000 rpm for 10 minutes. An equal volume of chloroform was added in supernatant, mixture vortexed and centrifuged for 10 minutes at 13000 rpm. The supernatant was transferred to another eppendorf tube and mixed with an equal volume of isopropanol and 0.1 volume of 4 M ammonium acetate. DNA was recovered as a pellet by centrifugation and washed with 70% ethanol. The pellet was dried and stored in 50 µl of TE buffer and 2 µl of RNase to digest RNA and to obtain pure DNA.

Agarose gel electrophoresis: The quality of DNA was checked by running it on 1.5% agarose gel and stained in Ethidium bromide solution. The stained gel was photographed and quality of the DNA was assessed using gel documentation system (Kodak EDAS 290). The DNA was further quantified by spectrophotometer at 260 and 280 nm and dilutions were made for further RAPD analysis.

DNA ampification: For polymerase chain reaction 10 oligonucleotide (decamer) primers were used. The PCR reaction mixture (15 µl) contained 20 ng of genomic DNA, 1.5 µl 10x reaction buffer, 1.5 µl 10x dNTPs, 0.5 µl Magnesium chloride (25 mM), 1µl of primer, 1 µl of taq polymerase and 8.5 µl nano pure water. Amplification was performed in programmable thermal cycler which was set for one cycle of 3.5 min., at 92°C, 1min., at 35°C and 2 min., at 72°C. Then 44 cycles of 1 min at 92°C, 1 min at 35°C and 2 min at

72°C and hold at 15°C. The PCR product was loaded on 1.5% agarose gel in TAE buffer and stained with ethidium bromide. DNA fragments were then visualized by illumination with UV light (Kodak EDAS 290). In all cases DNA Ladder (SM0383) from Fermentas was used as molecular marker.

Data analysis: Photographs from Ethidium bromide stained agarose gel were used to score RAPD data for analysis. The presence of a particular band was scored as 1 and absence as 0. Bands with same mobility were treated as identical fragments. The positions of PCR bands were compared with molecular weight standards. Data analysis was performed using the software package Gelcompar II version 4.602 Applied Maths Inc USA. After processing the Gel images, all pair wise similarity values were calculated with a similarity coefficient. The similarity matrix was converted into Dendrogram using UPGMA (i.e., unweighted pair group method with the arithmetic average) clustering algorithm.

Results and Discussion

A total of 10 oligonucleotides primers (10-mer) generated strong amplification and resulted in informative and polymorphic products (Table 1 and Fig. 1). Out of the 95 amplification products scored, 75 bands (78.94%) were found to be polymorphic. The average number of scoreable bands per primer was 9.5 (ranging from 3-15 brands per primer and the average number of polymorphic bands was 7.5 (ranging from 2-15 bands per primer) respectively.

Abundant polymorphism was detected with all primers used in this experiment. The percentage of polymorphic bands was 100% with OPC4, OPC6, OPC7, OPC9 and OPC10. OPC8 exhibited the lowest level of variability and the percentage of polymorphic band was only 33%.

The similarity matrix (Table 2) based on pairwise similarity values (which are calculated with similarity coefficient) reveals the polymorphism among 10 sorghum varieties. Similarity values varied from 67.8-95.6. Seventy percent of similarity matrix values were higher than 82.4 and remaining values are lower than 82.4. A UPGMA dendogram based on the similarity matrix displayed more detail genetic relationships among the sorghum varieties (Fig. 2). Ten Sorghum varieties were clustered into one group at the similarity level of 76.94%.

Genetic variation among individuals within a species and their interrelationships have conventionally been assessed using morphological and agronomical traits or by a biochemical test (Ford & Ball, 1991). Molecular techniques provide an alternative approach for evaluating genetic diversity in crop plants since they are not subject to environmental effects and are independent of the developmental stage of the plant; these methods have been used to identify cultivators in a wide range of plants. The development of molecular techniques has resulted in alternative DNA-based procedure of detecting polymorphism. The RAPD technique reveals an extensive amount of variation leading to clear cultivator identification (Ayana *et al*., 2000).

Surveys of genetic polymorphism in sorghum have been made by Gebisa & Ejeta (1997), Uptmoor *et al*., (2003), Agrama (2000), Ayana *et al*., (2000) and Dahlberg *et al*., (2002) using RAPD, SSR and RFLP etc. In these studies RAPD analysis proved to be powerful tool with a number of advantages i.e., easy generation of data, no requirement of previous knowledge of the genome.

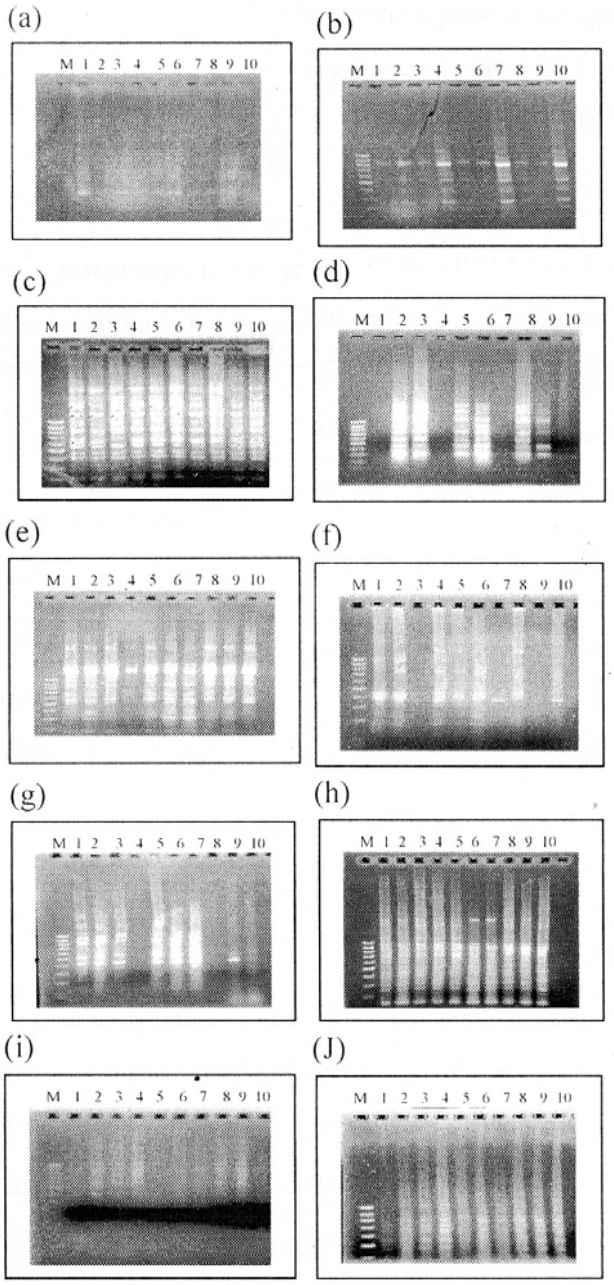

Fig. 1. RAPD pattern of sorghum varieties with different OPC primers: (a) OPC 1; (b) OPC 2; (c) OPC 3; (d) OPC 4: (e) OPC 5; (f) OPC 6; (g) OPC 7; (h) OPC 8; (i) OPC 9 and (j) OPC 10. The DNA used as a template from 1, YSS 9; 2, RARI S-3; 3, RARI S-4, 4, DS-97-1; 5, 84-Y-00; 6, 84-Y-01; 7, 85-G-83; 8, Mr. Buster; 9, 86-G-87 and 10, PARC SS-1.

Table 1. List of RAPD primers along with Percentage polymorphism detected.

Primer	Sequence (5'-3')	Scored bands	Polymorphic bands	Polymorphism rate (%)
OPC 1	TTCGAGCCAG	03	02	66.6
OPC 2	GTGAGGCGTC	09	08	88.8
OPC 3	GGGGGTCTTT	12	05	41.6
OPC 4	CCGCATCTAC	09	09	100.0
OPC 5	GATGACCGCC	15	12	80.0
OPC 6	GAACGGACTC	07	07	100.0
OPC 7	GTCCCGACGA	15	15	100.0
OPC 8	TGGACCGGTG	12	04	33.0
OPC 9	CTCACCCTCC	05	05	100.0
OPC 10	TGTCTGGGTG	08	08	100.0
Total		95	75	78.94
Average		9.5	7.5	78.94
Range		3-15	2-15	33-100

Table 2. Similarity matrix of pair wise distance constructed from RAPD banding

	DS-97-1	84-Y-00	RARI-S-3	RARI-S-4	Mr. Buster	86-G-87	84-Y-01	85-G-83	PARC-SS-1	YSS-9
DS-97-1	-									
84-Y-00	93.0	-								
RARI-S-3	90.0	91.8	-							
RARI-S-4	94.4	93.7	96.8	-						
Mr. Buster	92.0	92.6	88.8	91.4	-					
86-G-87	88.2	89.2	85.7	85.9	94.3	-				
84-Y-01	86.5	92.0	89.1	87.8	86.0	86.2	-			
85-G-83	82.4	88.5	87.6	85.2	88.7	92.0	94.2	-		
PARC-SS-1	96.8	77.4	73.8	76.5	87.0	88.4	74.5	85.2	-	
YSS-9	79.6	75.9	80.0	79.7	77.8	77.3	67.8	75.4	78.8	-

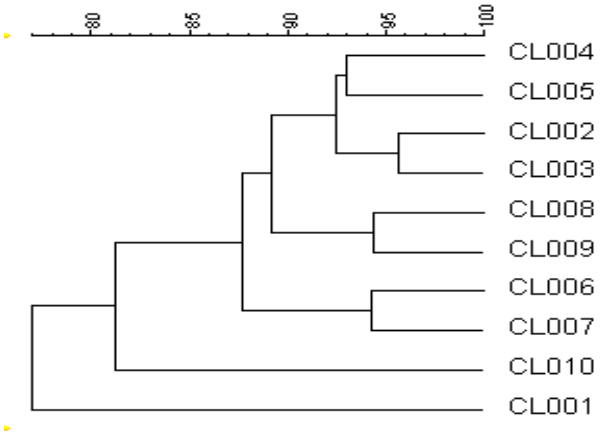

Fig. 2. UPGMA Dendogram with all tested primers showing the pair wise similarities values among the ten sorghum varieties: CL001, YSS 9; CL002, RARI S-3; CL003, RARI S-4, CL004, DS-97-1; CL005, 84-Y-00; CL006, 84-Y-01; CL007, 85-G-83; CL008, Mr. Buster; CL009, 86-G-87 and CL010, PARC SS-1.

In RAPD study on sorghum, reproducible results were obtained by maintaining constant conditions Dahlberg *et al*., (2002). In the present study, highly reproducible amplification profiles were produced under constant conditions. In our image analysis, high resolution was used, only reproducible characters were recorded and more genetic variation could be seen in RAPD-PCR bands. Previous studies reported that level of polymorphism in sorghum detected by RAPD were 70% (Dahlberg *et al.,* 2002) and 75%-85% (Ayana *et al.,* 2000). In our results 78.94% of amplification products from 10 selected primers were found to be polymorphic. These results are agree with the conclusion of Ayana *et al*., (2000) that RAPD analysis is an effective method of detecting high level of polymorphism in sorghum.

The genetic variability of plants results from interaction of mutation, selection, random migration. Mutation pressure and selection pressure are major factors changing the level of genetic equilibrium. Geographical, ecological and reproductive isolation have all had marked effects on level of genetic diversity within species (Max *et al.,* 1978).

RAPD-PCR analysis in this study revealed high genetic variations among cultivators of sorghum from different region this was indicated by the variation of pairwise similarity matrix. The pairwise similarity values shows that variety RARI-S3 and RARI-S-4 are showing closest relationship with highest similarity value i.e., 96.8% while variety YSS-9 and 84-Y-01 show distant relationship with similarity value 67.8%. Variety DS-97-1 and 84-Y-00 are showing close relationship with similarity value 93%. Similarly variety Mr. Buster and 86-G-87 were showing 94.3% similarity and variety 84-Y-01 was showing closer relationship with variety 85-G-83. Variety PARC SS-1 and YSS-9 are showing distant relation with all the varieties.

In conclusion, our results demonstrate that RAPD analysis can be applied to assess the genetic diversity and phylogenetic relationship of plant species.

Acknowledgment

The authors thank to Higher Education Commission (HEC), Pakistan, for providing the funds under the project No. 286 to Dr. Muhammad Gulfraz. We are also thankful to Applied Maths Inc USA for providing software package Gelcompar II version 4.602.

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(Received for publication 5 February 2008)