ASSESSMENT OF GENETIC VARIABILITY IN SORGHUM GENOTYPES FOR DROUGHT TOLERANCE BASED ON RAPD ANALYSIS

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ABSTRACT

In a study conducted in the Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan during the year 2008-09, 20 sorghum (Sorghum bicolor) genotypes were evaluated for their genetic variability in drought tolerance. These genotypes were analyzed using 10 RAPD primers. Total number of loci traced by these primers was 66, of which all were polymorphic showing 100 percent polymorphism. Size of scorable fragments ranged from 250-3000 bp. The genetic distance ranged from 51.5 to 95.5 percent for drought tolerance. The results obtained from RAPD showed 99.5 percent overall polymorphism among 20 genotypes for concerned trait. Genotypes were grouped in cluster by using cluster analysis and most diverse genotypes were identified. These results confirmed the availability of maximum amount of genetic variability for drought tolerance in the selected genotypes.

KEYWORDS: Sorghum bicolor; genotypes; DNA; Pakistan.

INTRODUCTION

Sorghum (Sorghum bicolor L. Moench) ranks fifth among cereals for economic importance with an annual production of 60 million tons in the world. It is an important food, feed, forage and provides raw material for producing of starch, fibre, 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 (5). It is an important summer season crop grown both for fodder and grain purposes. It is grown throughout Pakistan under irrigated and rainfed conditions (10). The new cultivars of sorghum are better for large scale cultivation under irrigated as well as rainfed conditions of the Punjab province in Pakistan (2).

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The drought tolerance mechanism is expressed in sorghum by the development of long root system. This crop is prone to drought when moisture stress occurs at early vegetative growth, panicle development, pre-flowering and post flowering stages (14). The sorghum genotypes differ in drought tolerance with respect to the growth and developmental stages. Sorghum genotypes with good tolerance during one developmental stage are found to be susceptible to drought during the other growth stages. This situation creates complication in improving the drought tolerance characteristics of sorghum.

The future success in sorghum improvement against drought depends upon the availability of genetic variability. Phenotypic variation does not reliably reflect genetic variation due to environmental interaction in determining the phenotype. Biochemical markers, on the other hand, reflect more truly the genetic variability being the direct products of genes (15).

In order to exploit this diversity at genotypic level, an efficient marker system is required. RAPD proved to be reliable, rapid and practical technique of revealing relationship among sorghum varieties. These markers have proved to be the most polymorphic markers in sorghum for drought tolerance and are highly useful for various applications within and several sorghum populations (12).

The present study was conducted to assess the genetic variability in different sorghum genotypes for drought tolerance by the use of RAPD markers.

**MATERIALS AND METHODS**

This study was conducted in the Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, during 2008-09. Seeds of 20 sorghum genotypes with known drought tolerance trait were obtained from National Agricultural Research Centre (NARC), Islamabad and Millets Research Station, Rawalpindi. Seeds were sown in earthen pots during normal growing season (July 2008). Fresh 8-10 leaf samples were collected two weeks after germination and immediately stored at -20°C for molecular studies.

**DNA extraction**

Fresh and newly emerged leaves of two weeks old sorghum seedlings were collected and used for DNA extraction. CTAB DNA extraction method as
described by Puchooa and Venkatasamy (13) was followed. Approximately one to two leaves were taken in an autoclaved mortar and were crushed to fine paste using 2 ml CTAB buffer. The homogenate was incubated at 65°C for 45 minutes in a block heater. Material was centrifuged at 13000 rpm for ten minutes. An equal volume of chloroform was added in supernatant. After vortexing the mixture, it was centrifuged for ten minutes at 13000 rpm. The supernatant was taken into another eppendorf 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 percent ethanol. The pellet was dried and mixed with 50 µl of RNase to digest RNA and obtain pure DNA.

**Primer sequence**

N series of RAPD primers were used in the study for genetic analysis. Ten polymorphic primers were used to amplify the genomic DNA of drought tolerant sorghum germplasm.

**Optimization of PCR conditions**

For polymerase chain reaction ten oligonucleotides (decamer) primers were used. PCR thermal cycler was used in the present study. The total reaction was performed in a volume of 20µl. The components for 20 µl reaction mixture were as under:

DNA template = 2 µl, 10X Buffer = 2µl, dNTPs = 3µl, dH2O = 8.8µl, Taq Polymerase = 0.2µl MgCl₂ = 2µl, Primers = 2µl

**DNA ladder**

The genetic ruler 1kb DNA Ladder (Catalogue # SMO313, Lot: 00018968, Concentration: 0.1g/ml) for RAPDs by Fermentas was used for sizing and approximate quantification of wide range double stranded DNA fragments on agarose gel. The ladder was premixed with 6x Loading dye solution for direct loading on gel.

**Agarose gel electrophoresis**

DNA quality was checked by running it on 0.5 percent agarose gel. The gel was stained in ethidium bromide solution. The gel was photographed by using Gel Documentation System (Kodak EDAS 290) and quality of DNA was assessed. The DNA was further quantified and diluted for further analysis.
DNA amplification

PCR reaction mixture (20 µl) contained 2 µl of genomic DNA, 2µl 10x reaction buffer, 3µl dNTPs, 2µl magnesium chloride, 2µl of primer and 2µl of taq polymerase and 6µl dH₂O. Amplification was performed in programmable thermal cycler, which was set for one cycle of 5 minutes at 94°C followed by denaturation step of 1 minutes at 94°C, an annealing step of 1 minute at 36°C and an extension step of 2 minutes at 72°C. Then from step 2 to 4 were repeated with a number of cycles 40, then after this 10 minutes for 72°C and then held at 4°C. The whole amount was loaded on 0.5 percent agarose gel in TAE buffer and stained with ethidium bromide. DNA fragments were visualized by illumination with UV light (Kodak EDAS 290). In all cases, DNA ladder (SM0383) from Fermentas, Canada was used as molecular marker.

Statistical analysis

The fingerprints were examined under ultra violet Transilluminator and photographed using Gel Documentation System (BIO RAD). The presence of a particular band was scored as 1 and absence as 0 for each of 20 varieties with all random primers. Bands with same mobility were treated as identical fragments. Ambiguous bands that could not be clearly distinguished were not scored. The position of PCR bands were compared with molecular weight standards. Data were analyzed using the Multivariate statistical package, version 3.13p (1985-2007). After processing the gel images, all pair wise similarity values were calculated with a similarity coefficient. Then the similarity matrix was converted into dendrogram using UPGMA (unweighted pair group method with the arithmetic average).

RESULTS AND DISCUSSION

Genetic diversity evaluation

The oligonucleotide sequence of these primers (Table 1) showed that 10 RAPD primers had given clear and polymorphic amplification patterns in terms of band number (Table 1, Fig. 1). Total numbers of loci traced by these primers were 66 and all these loci were polymorphic according to population genetic analysis, thus revealing 100 percent polymorphism among these genotypes (Table 1). The number of amplification products per primer pair ranged from 3 to 9 while size of amplified products ranged from 250-3000bp.
Fig. 1. Amplification profile of 20 sorghum genotypes shown by ten Primers OPN-02 (a), OPN-04(b), OPN-05(c), OPN-06(d) OPN-10 (e), OPN-11(f) OPN-12(g), OPN-15(h), OPN-16(i) and OPN-17(j). M = 1 Kb DNA ladder, 1 = RASIL, 2 = VI-1, 3 = SPV-462, 4 = CSV-13, 5 = DS-97-1, 6 = PARC-SS-1(Johar), 7 = RARI-S-3, 8 = RARI-S-4, 9 = YSS-9, 10 = RS-29, 11 = PARC-SS-2, 12 = YSS-98, 13 = YSS-10 (Red), 14 = YSS-10 (Cream), 15 = YSS-17, 16 = YSS-18, 17 = YSS-19, 18 = SV-10, 19 = JV-2002, 20 = CSV-15.
The variability in the number of alleles per locus may result from different locus specific mutation rates (4), whereas, this behaviour according to Nei (11), depends on both the number of alleles per locus and the respective allele frequency. Maximum number of bands (9) was amplified by OPN-16 and minimum number of bands (3) was amplified by OPN-06. The existence of some minor bands might affect the allele scoring process. However, some researchers reported that these minor bands could be useful during scoring for verification of the genotypes.

Table 1. Primers used for amplification and their polymorphism percentage.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’-3’)</th>
<th>Scored bands</th>
<th>Polymorphic bands</th>
<th>Polymorphism rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPN-02</td>
<td>AATCGGGCTG</td>
<td>07</td>
<td>07</td>
<td>100</td>
</tr>
<tr>
<td>OPN-04</td>
<td>AGGGGTCTTG</td>
<td>08</td>
<td>08</td>
<td>100</td>
</tr>
<tr>
<td>OPN-05</td>
<td>GTTCCCTGAC</td>
<td>08</td>
<td>08</td>
<td>100</td>
</tr>
<tr>
<td>OPN-06</td>
<td>GTGACGTAGG</td>
<td>03</td>
<td>03</td>
<td>100</td>
</tr>
<tr>
<td>OPN-10</td>
<td>TCGGCGATAG</td>
<td>07</td>
<td>07</td>
<td>100</td>
</tr>
<tr>
<td>OPN-11</td>
<td>CAGCACCCAC</td>
<td>07</td>
<td>07</td>
<td>100</td>
</tr>
<tr>
<td>OPN-12</td>
<td>GACCGCTTG</td>
<td>04</td>
<td>04</td>
<td>100</td>
</tr>
<tr>
<td>OPN-15</td>
<td>GTTGGATCC</td>
<td>06</td>
<td>06</td>
<td>100</td>
</tr>
<tr>
<td>OPN-16</td>
<td>TCGGCTTTT</td>
<td>09</td>
<td>09</td>
<td>100</td>
</tr>
<tr>
<td>OPN-17</td>
<td>GGGGCTTTT</td>
<td>07</td>
<td>07</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
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<td>66</td>
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<tr>
<td>Average</td>
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<tr>
<td>Range</td>
<td></td>
<td>3-9</td>
<td>3-9</td>
<td>100</td>
</tr>
</tbody>
</table>

A similarity matrix was generated by RAPD amplification data to estimate genetic diversity and relatedness among 20 selected sorghum genotypes for drought tolerance. Dendrogram drawn for the genetic distances is shown in Fig. 2. The results of genetic similarity analysis showed broad genetic base (ranging from 51.5 to 95.5%) using UPGMA method. Maximum similarity was observed between genotypes PARC-SS-1 and YSS-17 which is 95.5 percent while minimum similarity was observed in genotype DS-97-1 which is 51.5 percent. Minimum genetic distance (51.5%) was detected in DS-97-1. The genotypes which showed a value of maximum similarity ranging from 71.2 to 95.5 percent are RS-29 with VI-1 (71.2%), Rasili with CSV-15 (72.7%), SV-10 with YSS-98 (77.3%), YSS-18 with YSS-10 (C) (86.4%), PARC-SS-1 with YSS-17 (95.5%). Similar results were obtained by Dahlberg et al. (3). Highly reproducible amplification profiles were produced under constant conditions. In this 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 percent (3) and 75 to 85 percent (1). In present study 78.94 percent of amplification products from 10 selected primers were found to be polymorphic. These results agree to the findings of Ayana et al. (1) and Mehmood et al. (7) who also found high level of polymorphism (78.94%) in sorghum genotypes.

The cluster analysis clearly distinguished the 20 sorghum genotypes from each other. For making interpretation easy, dendrogram is divided into three main clusters A, B and C (Fig. 2). Group A consisted of only one genotype (SPV-462) and is the most genetically diverse genotype than other genotypes. Cluster B included 9 genotypes and divided into two sub clusters: B1 and B2. B1 contained two genotypes whereas B2 consisted of seven...
genotypes. In this cluster, the genotypes showing maximum genetic distance were more diverse. Cluster C included 10 genotypes and was divided into two sub clusters, C₁ and C₂. The genotypes with maximum genetic distance were genetically diverse for drought tolerance from all other genotypes. These results indicated that the selected sorghum genotypes possess maximum amount of genetic variability for drought tolerance, therefore these can be utilized for improvement of drought susceptible genotypes.

The development of drought tolerant sorghum varieties seems to be a very desirable solution to address the drought problem of sorghum growing countries where vast areas are affected by scarcity of water. The study revealed that amplifications of multiple loci from a single primer are possible with RAPDs and it is much simpler in operation than AFLPs and small amount of DNA is required. RAPDs are not generally reproducible and transferability of these markers to other genotypes is not possible. These problems confined RAPD to genetic diversity studies only and made it unsuitable for genome mapping (8, 9). Surveys of genetic polymorphism in sorghum have been made by Uptmoor et al. (16) and Dahlberg et al. (3) using RAPD, SSR, RFLP, etc. RAPD-PCR analysis in this study revealed high genetic variations among cultivators of sorghum for drought tolerance. This was indicated by the variation of pairwise similarity matrix (Table 3). In an earlier study (6), on the basis of genetic similarity matrices, accession pairs were classified as ‘unrelated’ or ‘related’ (loosely, moderately, and highly related). In present study after polymorphism analysis DS-97-1, SPV-462, YSS-10(R), YSS-19, JV-2002, PARC-SS-2 and CSV-13 were found genetically diverse. This shows that these genotypes are an important source for drought tolerance in crop improvement.

REFERENCES


