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Studies on Genetic Diversity in Pakistani Wheat Varieties using Randomly Amplified Polymorphic DNA

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Abstract: To improve yield and quality of wheat, presence of genetic diversity is an important prerequisite. To estimate the extent of genetic variability, DNA markers including Randomly Amplified Polymorphic DNA (RAPD) have been widely used in various crops of agronomic importance. During present study, RAPDs were used to estimate genetic relationship among 10 Pakistani wheat varieties and to identify genetically diverse genotypes which can be used in future breeding programs. RAPD primers used during present study produced an average of 1.9 alleles per primer. Size of scorable fragments ranged from 250-1000 bp. A high level of genetic dissimilarity (GD = up to 63%) was estimated among the wheat varieties. Ten wheat varieties were grouped in 3 clusters using dendrogram analysis. Clusters A, B and C comprised land races, derivatives of Mexi-Pak and elite lines having alien gene, respectively.

Key words: Wheat, DNA marker, RAPD, genetic diversity, cluster analysis

INTRODUCTION

Bread wheat (*Triticum aestivum* L. em Thell. genomically AABBDD, 2n = 6x = 42) is the most important cereal crop of the world. Pakistan is the 7th largest wheat producer, but the national average of wheat in Pakistan (a little more than 2 t ha^{-1} Anonymous, 2003)) is less than half as compared to major wheat producing countries such as USA, Australia and Canada (more than 4 t ha^{-1} FAO, 2003). Diseases especially rusts are an important factor responsible for low national average of wheat crop in Pakistan. Crop yield losses of 30-55% from leaf (caused by *P. recondita*) and stripe (caused by *P. striiformis*) rusts have been reported in susceptible wheat cultivars in Pakistan during 1978 and it was estimated to have caused economic losses of $US86 million (Hussain *et al*., 1980). The use of genetic resistance has been the method of choice for wheat breeders all over the world for economical and environmentally friendly way to control the disease (McIntosh *et al*., 1995). For improvement of wheat crop and incorporation of agronomically desirable genes in local cultivars, information about existing genetic diversity in the local germplasm is of prime importance. For characterization of germplasm accessions and estimation of genetic variability of breeding material, morphological, cytological and biochemical markers have been utilized in the past but because of limitations, these methods are not considered suitable for handling large breeding populations (Khan, 1999). The recent developments in molecular biology to generate large number of molecular markers over almost the whole genome of wheat and related species has provided the opportunity for utilizing Marker Assisted Selection (Paterson *et al*., 1991). Different molecular marker techniques are currently being used in crop breeding programmes aimed at identifying genetically diverse genotypes (Rafalski *et al*., 1996). Among commonly used DNA markers, Randomly Amplified Polymorphic DNA (RAPD) provides virtually unlimited number of markers to compare individual genotypes and considering easy handling and cheaper cost per assay, it is possible to carry out large scale screening of breeding populations and genetic resources (Demeke *et al*., 1996).

A number of wheat varieties have been released in Pakistan during past 50 years. These varieties can broadly be divided into 3 main groups viz., (1) varieties released from 1947-1970 (mainly comprised of anticipated land races) (2) varieties released between 1971-1990 (mainly comprised derivatives of Mexi-Pak) and (3) varieties released between 1991-2004 (mainly comprised of elite material infused by alien resources e.g., 1BL-1RS translocation).

The main objective of the current study was to evaluate genetic diversity at DNA level among 10 Pakistani wheat varieties selected from 3 different groups using Randomly Amplified Polymorphic DNA (RAPDs).

MATERIALS AND METHODS

The plant material used in present study comprised 10 wheat varieties (viz., C-270, C-291, C-518, Potohar, PARI-73, Pak-70, Sandal, Salman-98, Marvi-2000, Saleem

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2000) released in Pakistan during different periods. Plants were grown in the glass house of Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University Peshawar during 2005-2006.

Leaf samples were used to isolate total genomic DNA using protocols described by Weining and Langridge (1991). To remove RNA, DNA was treated with 40 μg RNAse-A at 37°C for 1 h. After RNAse treatment, DNA samples were stored at 4°C. To use in Polymerase Chain Reaction (PCR) a 1: 4 dilution of DNA was made in doubled distilled, deionized and autoclaved water.

The Polymerase Chain Reactions were carried out using standard protocole (Devos and Gale, 1992). Six Randomly Amplified Polymorphic DNA primers (GL-A06, -B11,-B19,-C10,-D07 and-D17) were obtained from Gene Link, Inc. NY 10532, USA. PCR reactions were carried out in 25 μL reaction containing 50-100 ng total genomic DNA template, 0.25 μM of each primer, 200 μM of each dATP, dCTP, dGTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. Amplification conditions were an initial denaturation step of 4 min at 94°C followed by 40 cycles each consisting of a denaturation step of 1 min at 94°C, annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. The last cycle was followed by 10 min extension at 72°C. All amplification reactions were performed using GeneAmp PCR System 2700 (Applied Biosystem) programmable thermocycler. The amplification products were electrophoresed on 1.5% agarose/TBE gel and visualized by staining with ethidium bromide under ultra-violet (U.V) light. Sequence information and molecular weight of the RAPD primers are presented in Table 1.

For statistical analysis every scorable band was considered as a single locus/allele. The loci were scored as present (1) or absent (0). Bi-variate 1-0 data matrix was used to estimate genetic distances (GD) using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure (Nei and Li, 1979).

$Gd_{xy} = 1-d_{xy}/d_x+d_y-d_{xy}$, where Gd_{xy} = Genetic distance between two genotypes, d_{xy} = Total number of common loci in two genotypes, d_x = Total number of loci in genotype 1 and d_y = Total numbers of loci in genotype 2. Dendrogram was constructed using computer program PopGene32 version 1.31 (http://www.ualberta.ca./~fyeh/fyeh).

Table 1: Sequence, size, molecular weight and %GC content of 6 RAPD primers used to study genetic diversity among 10 wheat varieties

Name	Sequence	Size	Mol. Wt.	GC (%)
GL-A06	CGTCCCTGAC	10	3003	70
GL-B11	GTAGACCCGT	10	3028	60
GL-B19	ACCCCCGAAG	10	2981	70
GL-C10	TGTCTGGGTG	10	3090	60
GL-D07	TTGGCACGGG	10	3084	70
GL-D17	TTTCCCACGG	10	2979	60

RESULTS AND DISCUSSION

The 10 wheat lines used in present study showed various banding patterns using six RAPD primers. An example of PCR amplification obtained using RAPD primer GL-D18 is presented in Fig. 1. A total of 10, 14, 31, 20, 26 and 14 alleles were amplified in 10 genotypes using RAPD primers GL-A06, -B11,-B19,-C10,-D07 and-D17, giving averages of 1.0, 1.4, 3.1, 2.0, 2.6 and 1.4 alleles per genotype, respectively. Similar results were reported by Mukhtar *et al.* (2002) who reported 2.1 alleles per genotype amplified by the RAPD primer they used. The size of amplified fragments (estimated by using molecular size marker, 1 Kbp DNA ladder, Gene Link, USA) ranged from approximately 250 to 1000 bp. Average Genetic distances (GD) estimated among the 10 wheat varieties ranged from 0 to 63% (Table 2). Maximum genetic distance estimate (GD = 63%) was observed between varieties Sandal and Salman-98, closely followed by comparison between varieties PARI-73 and Salman-98 (GD = 60%). Comparison between varieties C-270 and C-291 showed complete homozygosity for the loci detected using 6 RAPD primers (GD = 0%).

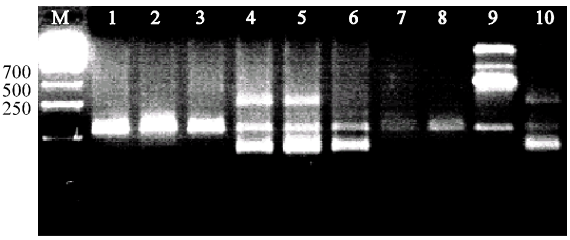

Fig. 1: PCR amplification profile of 10 wheat lines using RAPD primer GL-D18. M = Molecular size marker (molecular weights in bp are given on left). From left to tight, lane 1 = C-270, 2 = C-291, 3 = C-518, 4 = Potohar, 5 = PARI-73, 6 = Pak-70, 7 = Sandal, 8 = Salman-98, 9 = Marvi-2000, 10 = Saleem 2000

Table 2: Average genetic distance estimates of 10 wheat varieties using 6 RAPD primers

	1	2	3	4	5	6	7	8	9
1									
2	0.00								
3	0.17	0.17							
4	0.51	0.51	0.54						
5	0.40	0.40	0.49	0.13					
6	0.35	0.35	0.43	0.31	0.19				
7	0.18	0.18	0.32	0.40	0.28	0.23			
8	0.54	0.54	0.57	0.51	0.60	0.50	0.63		
9	0.42	0.42	0.46	0.49	0.57	0.45	0.50	0.13	
10	0.33	0.33	0.47	0.50	0.42	0.45	0.36	0.39	0.36

1 = C-270, 2 = C-291, 3 = C-518, 4 = Potohar, 5 = PARI-73, 6 = Pak-70, 7 = Sandal, 8 = Salman-98, 9 = Marvi-2000, 10 = Saleem 2000

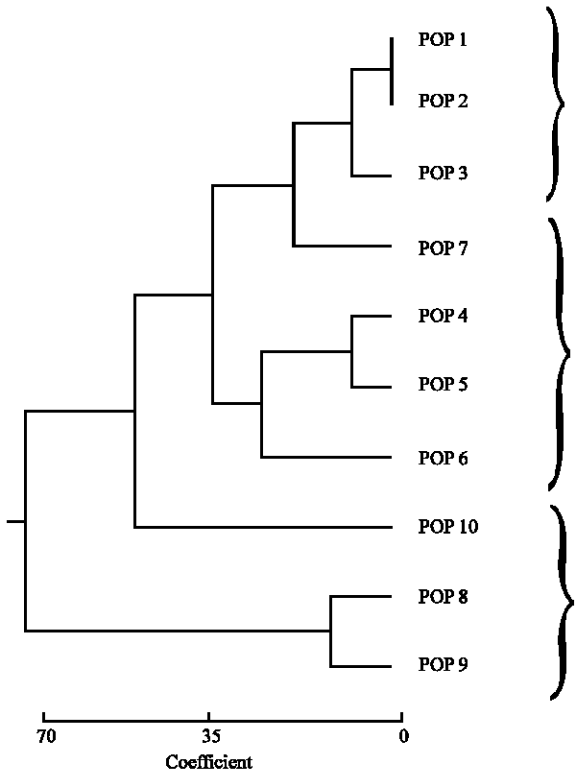

Fig. 2: Dendrogram of 10 wheat varieties constructed by using data generated from 6 RAPD primers (GL-A06, -B11, -B19, -C10, -D07 and -D17) using Unweighted Pair Group of Arithmetic Means (UPGMA) and computer program POPGENE. POP1 = C-270, 2 = C-291, 3 = C-518, 4 = Potohar, 5 = PARI-73, 6 = Pak-70, 7 = Sandal, 8 = Salman-98, 9 = Marvi-2000, 10 = Saleem 2000

The genetic dissimilarity coefficient matrix of 10 wheat varieties based on the data of 6 RAPD primers using UPGMA method (Nei and Lie, 1979) was used to construct a dendrogram using computer program Popgene (Fig. 2). The genotypes were grouped in 3 main clusters A, B and C. These groups comprised 3, 4 and 3 genotypes, respectively. Groups A, B and C consisted of genotypes belong to set 1 (landraces), set 2 (Mexi-Pak derivatives) and set 3 (elite material infused by alien resources), respectively. Based on dendrogram analyses, wheat varieties C-270 and Marvi-2000 were found most distantly related and it is recommended that these varieties should be used in future breeding programs aimed at creating genetic variability in local wheat germplasm.

Present study strengthened earlier reports that Randomly Amplified Polymorphic DNA can be used for estimation of genetic diversity in crop improvement programs (Sun *et al*., 1998; Czaplieki *et al*., 2000) for cultivars identification (Hu and Quirose, 1991; Malik *et al*., 1996) and for fingerprinting of genomes (Welsh and McClelland, 1990) and tagging of genes (Kelly *et al*. 1993).

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