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Full Length Research Paper

Molecular characterization of Pakistani wheat cultivars using random markers

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The genetic diversity among fifteen varieties of wheat was studied by random amplified polymorphic DNA (RAPD) analysis. By applying 20 RAPD primers, 182 fragments were amplified, out of which 118 were polymorphic (64.84%). The number of fragments amplified per primer ranged from 10 to 24 with an average of 17 fragments per primer. Primer K-17 produced the maximum number of fragments (24) and all the fragments were polymorphic. Range of polymorphism percentage was from as low as 0% (I to 15) to as high as 100% (K-11). The number of fragments produced per wheat genotype varied from 36 to 56 with an average of 47.2 fragments per genotype. The variety Shahkar-95 produced maximum number of fragments (56). Cluster analysis classified fifteen varieties of wheat into two main groups; three varieties were placed in group I and the rest of the varieties were placed in group II. Second group (group II) was further divided into three subgroups; IIA, IIB and IIC. The pair wise similarity values ranged from 54.88 to 82.93% and showed that genotypes Kohinoor-83 and Pak-81 were the closest with highest similarity value (82.93%), while genotypes Kohinoor-83 and Kohistan-97 were most distinct with minimum similarity value (54.88%).

Key words: Cultivar, polymorphism, random amplification of polymorphic deoxyribonucleic acid (RAPD), cluster analysis, genotype.

INTRODUCTION

Wheat is a staple food for a major part of humanity supporting about 35% of the world population. Its great popularity as a human food is due to its mild, acceptable flavor, and the unique ability of its principle proteins to form gluten when mixed with water (Debasis and Khurana, 2001). Wheat production in Pakistan has a crucial role in agricultural policies because it is a staple food and supplies 72% of energy and protein in the average daily diet in Pakistan (Khalil, 2006). Production target for wheat was set 25 million tons in the 2009 to 2010 crops season, but the yield of wheat crop from 9.1316 million hectares was 23.3108 million tons which was 7.76% less than the target for this year (Anonymous, 2010).

Development of high yielding and disease resistant wheat cultivars is a main thrust of breeders in Pakistan (Asif et al., 2005). The first step in the improvement of wheat is the complete estimation of the local materials, which includes collection, evaluation and genetic characterization of available germplasm. Morphological traits used to estimate genetic divergence are time consuming and requires extensive field trials, while morphological variations may be genetic based as well as epigenetic; influenced by ecological factors. In contrast, the expression of molecular markers is the direct product of genes that are not influenced by environment (Migdadi

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Table 1. RAPD primers, their sequences and properties generated in 15 wheat varieties.

Primer	Sequence
GL B-06	TGCTCTGCCC
GL B-07	GGTGACGCAG
GL B-11	GTAGACCCGT
GL B-12	CCTTGACGCA
GL B-13	TTCCCCCGCT
GL B-14	TCCGCTCTGG
GL B-15	GGAGGGTGTT
GL B-16	TTTGCCCGGA
GL B-17	AGGGAACGAG
GL B-18	CCACAGCAGT
GL B-19	ACCCCCGAAG
GL B-20	GGACCCTTAC
GL D-07	TTGGCACGGG
GL D-20	ACCCGGTCAC
GL I-06	AAGGCGGCAG
GL I-07	CAGCGACAAG

et al., 2004).

Polymerase chain reaction (PCR) technology has promoted the development of a number of molecular assay systems which detect polymorphisms at molecular level. RAPD markers are used as a powerful tool for the generation of potential fingerprinting diagnostic markers for cultivars (Matos et al., 2001). The use of RAPD markers for efficient and quick estimation of relationships among lines and populations of various plant species is a routine method (Mark et al., 1999). RAPD analysis is easy to perform and it uses arbitrary primer sequences. It requires small amount of genomic DNA and does not require radiolabeling. RAPD primers have been used for tagging disease resistance genes and for other characters (Milla et al., 2005). The objective of proposed study was to investigate and compare the genetic relationships among wheat varieties, using molecular data obtained from RAPD profiles and by using bioinformatics applications.

MATERIALS AND METHODS

Seeds of fifteen wheat varieties; Chakwal-50, Mirij-2008, Kohistan-97, Faisalabad-2008, Punjab-96, Pasban-90, Lasani-2008, Inqlab-91, Barani-83, Iqbal-2000, Shahkar-95, PBW-222, Wattan-92, Pak-81 and Kohinoor-83 were obtained from Ayub Agricultural Research Institute (AARI) Faisalabad, where different lines are exchanged all over the Pakistan. Plants were grown in pots in green house under standard agricultural practices.

Total genomic DNA was extracted from 8 to 10 bulked leaves, obtained from 4 to 5 randomly selected plants of the same genotype, by CTAB method (Doyle and Doyle, 1990). The DNA quality and quantity was determined by NanoDrop-1000 3.3.1 spectrophotometer and comparison was done with standard DNA electrophoresis on 0.8% agarose gel. A total of 20 RAPD primers were employed for finger printing of wheat genotypes. PCR amplifications were performed in 25 µL reactions containing DNA Template (15 ng/µl, 2.5 µl), 10x Buffer + $(NH_4)_2SO_4$ (2.5 µl), $MgCl_2$ (50 mM, 3 µl), gelatin (0.025%, 2.5 µl), dNTPs (2.5 mM each, 4 µl), Primer (15ng/µL, 2.0 µl) and Taq DNA polymerase (0.2U/µl, 0.2 µl). The PCR was carried out on a DNA thermocycler (Eppendorf) programmed as (95°C/5 min) (1), (95°C/1 min, 36°C/1 min, and 72°C/2 min) (40), (72°C/10 min) (1). The PCR products were separated on 1.2% agarose gel in TBE buffer with added ethidium bromide (10 ng/100 ml) and agarose gels were photographed with UV light.

All amplifications were repeated and only reproducible bands were scored for analysis. The wheat accessions screened for RAPD primers were scored for presence (1) and absence (0) of bands from top to the bottom of each lane. The RAPD data on the number of bands for the 75 wheat accessions thus collected was subjected to Popgene software (Version 1.44) using unweighted paired group of arithmetic means (UPGMA) and similarity matrix (Nei and Li, 1979).

RESULTS AND DISCUSSION

Fifteen wheat cultivars were analyzed by means of 20 RAPD primers. Sixteen primers produced different, easily detectable fragments of variable intensities (Table 1). A total of 182 fragments were amplified for fifteen varieties of wheat, out of which 118 were polymorphic, showing overall 64.84% polymorphism. The number of fragments amplified per primer ranged from 10 to 24 with an average of 17 fragments per primer. Primer K-17 produced the maximum number of fragments (24), and only ten fragments were produced by the primer I to 15. Range of polymorphism percentage was from as low as 0% (I to 15) to as high as 100% (K-11). The number of fragments produced per wheat genotype varied from 36 to 56 with an average of 47.2 fragments per genotype. Variety Shahkar-95 produced maximum number of fragments (56), while the minimum numbers of fragments were produced by the variety Chakwal-50 (36).

The primer B-13 amplified a specific fragment, only observed in variety Lasani-2008 and also amplified another fragment that was observed only in variety Kohinoor-83. Similarly, the primer B-6 generated a specific band which amplified only in variety PBW-222. Primer B-14 showed a common band in Kohistan-97 and Faisalabad-2008 and also another common band in Barani-83 and Iqbal-2000 which are absent in rest of the varieties. Similarly a specific band is amplified in varieties Mirij-2008 and PBW-222 and another band in varieties Faisalabad-2008 and Pasban-90 by the primer K-17. The primers B-07 and B-17 (Figures 1 and 2) produced also a specific band in Lasani-2008 and Inqlab-9, and in varieties Pak-81and Kohinoor-83 respectively and the primer B-18 showed two bands in varieties Lasani-2008 and Kohinoor-83 which are absent in rest of the varieties.

Nei and Li's (1979) coefficient similarity matrix were calculated to estimate the genetic divergence and relatedness among wheat genotypes. Among the fifteen varieties of wheat, percentage of similarity is ranged from

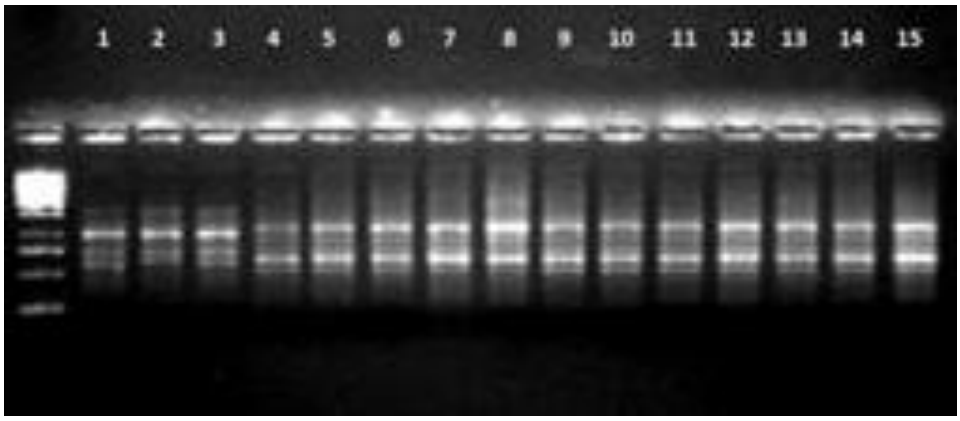

Figure 1. Banding pattern of RAPD primer GL B-07. Lane 1: Chakwal-50, lane 2: Mirij-2008, lane 3: Kohistan-97, lane 4: Faisalabad-2008, lane 5: Punjab-96, lane 6: Pasban-90, lane 7: Lasani-2008, lane 8: Inqlab-91, lane 9: Barani-83, lane 10: Iqbal-2000, lane 11: Wattan-92, lane 12: Shahkar-95, lane 13: PBW-222, lane 14: Pak-81, lane 15: Kohinoor-83.

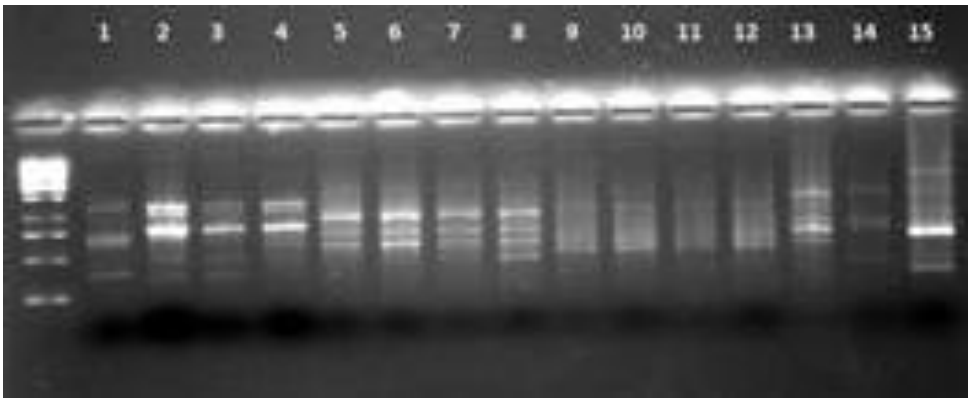

Figure 2. Banding pattern of RAPD primer GL B-17. Lane 1: Chakwal-50, lane 2: Mirij-2008, lane 3: Kohistan-97, lane 4: Faisalabad-2008, lane 5: Punjab-96, lane 6: Pasban-90, lane 7: Lasani-2008, lane 8: Inqlab-91, lane 9: Barani-83, lane 10: Iqbal-2000, lane 11: Wattan-92, lane 12: Shahkar-95, lane 13: PBW-222, lane 14: Pak-81, lane 15: Kohinoor-83.

54.88 to 82.93%. The maximum similarity was observed between variety Pak-81 and Kohinoor-83 (82.93%) while the most diverse varieties were Kohistan-97 and Kohinoor-83 (54.88%).

Genetic similarities obtained from RAPD data were used to generate a cluster diagram called dendrogram (Figure 3). Cluster analysis classified fifteen varieties of wheat into two main groups. Wattan-92, Pak-81 and Kohinoor-83 are placed in group I and rest of the twelve varieties Chakwal-50, Mirij-2008, Kohistan-97, Faisalabad-2008, Punjab-96, Pasban-90, Lasani-2008, Inqlab-91, Barani-83, Iqbal-2000, Shahkar-95 and PBW-222 are placed in group II. Second group (group II) is further divided into three subgroups; IIA, IIB and IIC. In group II, subgroup IIA contains five varieties Pasban-90, Inqlab-91, Barani-83, Shahkar-95 and PBW-222. The subgroup IIB has three varieties Punjab-96, Iqbal-2000 and Lasani-2008. Varieties Chakwal-50, Mirij-2008, Kohistan-97 and Faisalabad-2008 are placed in subgroup group IIC. Results obtained in the present research work showed that the polymorphism percentage observed in this experiment (82.92%) is comparable to the reports of several RAPD studies by various workers such as Farrakh et al. (2011), Siddiqui et al. (2010) and Mantzavinou et al. (2005), they found 81.48, 81 and 83.3% polymorphism, respectively. Cluster analysis by UPGMA clustered fifteen varieties of wheat into two main groups; group one contained 3 varieties and all the other 13 varieties are placed in second group. Sayed et al. (2001) also obtained nearly the same results in their study; they assessed the genetic diversity among 16 land races of wheat. The studied genotypes were also clustered into two main groups.

In the present study, percentage of genetic similarity among fifteen varieties of wheat is ranged from 54.88 to 82.93%. Grewal et al. (2007) also observed the

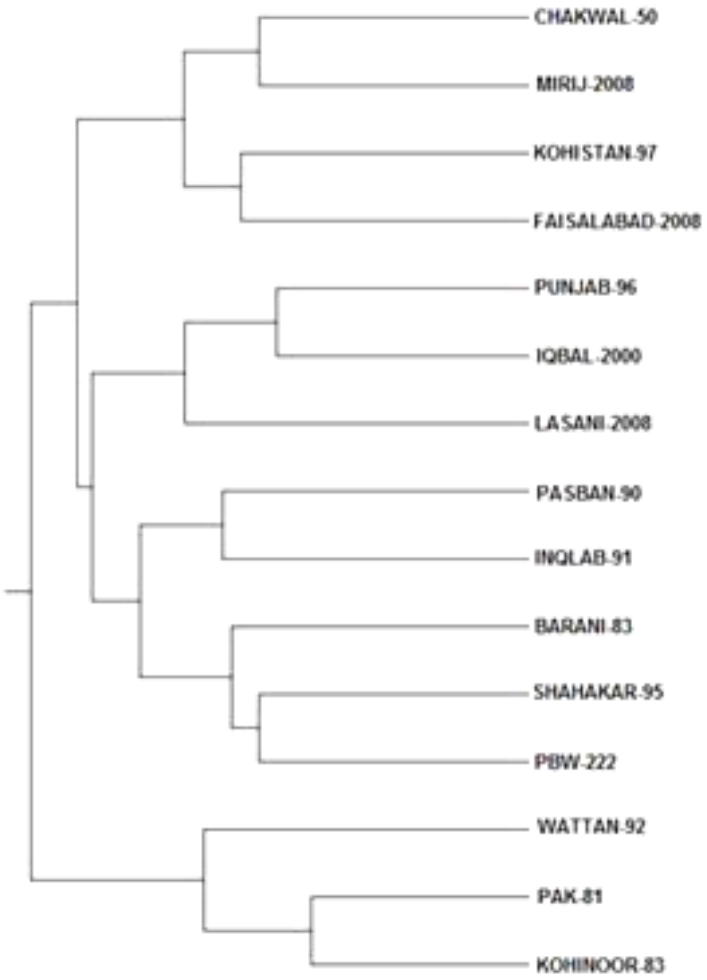

Figure 3. Dendrogram illustrating genetic relationships among 20 wheat genotypes, generated by UPGMA cluster tree analysis.

similarity from 52 to 82% among 20 Indian wheat accessions. The use of RAPD analysis in the present study revealed an extensive amount of divergence leading to cultivar identification. The information about genetic similarity will be useful, to avoid any possibility of elite germplasm becoming genetically uniform. Efficiency and speed of plant breeding programs can be accelerated by marker assisted selection (MAS) and permit persistent progress in the advancement of selected material. The information gathered here would be helpful in genomic mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity.

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