Advances in Agriculture & Botanics
International Journal of the Bioflux Society

OPEN ACCESS
Research Article

Assessment of genetic distance among wheat genotypes through RAPD markers

[1]Shahzadi Mahpara, [2]Jehanzeb Farooq, [1]Zulfiqar Ali, [3,4]I. Valentin Petrescu-Mag, [1]Fida Hussain

[1] Department of Plant Breeding and Genetics, University of Agriculture Faisalabad; [2] Cotton Research Institute, Ayub Agricultural Research Institute, Faisalabad; [3] Postdoctoral School in Agriculture and Veterinary Medicine (POSDRU), University of Agricultural Sciences and Veterinary Medicine Iasi, Romania; [4] SC Bioflux SRL, Cluj-Napoca, Romania.

Abstract. PCR technology has made it possible to find out genetic diversity in crop plants. The present research work was carried out to determine the percentage of genetic distance among seven wheat genotypes by random amplified polymorphic DNA markers based on PCR technique. A total of 588 DNA fragments was obtained through 20 decamer primers with an average of 4.2 bands per primer. Out of 588, 189 (32%) DNA fragments showed polymorphism between seven wheat genotypes, while rest of DNA fragments was monomorphic. The genetic similarity of these genotypes was high ranging from 34.29 % to 71.43 %. The genotypes Iqbal-2000 and Uqab-2000 had the greatest similarity (71.43 %), while the lowest genetic similarity was found between the genotypes MH-97 and Uqab-2000. These results indicated that RAPD technique is useful to estimate the extent of genetic diversity in wheat as well as other crop species, these results can help to select such parental genotypes having wide differences compared to other existing genotypes for contributing high grain yield, wider adaptability and other related traits in wheat plant.

Key Words: wheat genotypes, genetic diversity, RAPD markers.

Corresponding Author: Jehanzeb Farooq, jehanzeb1763@hotmail.com

Introduction

Wheat is an important cereal crop used as a major human consumable commodity in most areas of the world. In Pakistan wheat is used for making chapatti, which is more commonly used to eat than for making bread, especially in the areas where it is grown. Its diverse uses, nutritive importance and storage qualities have made it a staple food for more than one-third of the world's population. Wheat has been cultivated in Southwestern Asia, its geographic centre of origin, for more than 10,000 years (Sleper & Poehlman 2006). Wheat has large genomic size (approximately 17000 Mb) but very little information about its genome sequence is available.

Genetic diversity among crop plants is desirable for improving plant population. Determination of genetic diversity for various crop plants is one of the important tools in plant breeding because if more diversity occurs in crop species, chances for better plant selection will increase. Morphologic, physiologic and cytogenetic plant traits used at present as a selection criteria, are not stable and affected greatly by environmental conditions; but the selection based on molecular markers is more stable than above mentioned traits. Biochemical markers like isozymes, seed storage proteins and molecular markers i.e., restriction fragment length polymorphism (RFLPs), microsatellites, single nucleotide polymorphisms (SNPs) and random amplified polymorphic DNA (RAPD) are mostly used to determine the genetic relationship among various crop plants. These techniques are widely used for genotypic identification, genetic purity in seed testing (McDonald 1995) and for estimation of the genetic diversity among wheat genotypes. Among these techniques, RAPD is a known technique based on polymerase chain reaction (PCR) (Williams *et al* 1990) which is convenient, economical and sensitive compared to other techniques.

RAPDs use primers that are 9 or 10 nucleotides long and amplification products are separated on agarose gel electrophoresis and these DNA fragments become visible after staining with ethidium bromide. More consistent results were obtained by RAPD technique in presence of optimum reaction conditions. RAPDs are used to determine the polymorphism of genomic DNA (Welsh & McClelland 1990) and are more extensively used for genetic study of wheat (Mukhtar *et al* 2002; Iqbal *et al* 2007). RAPDs have been proved as useful genetic markers for self-pollinated species with comparatively low level of intraspecific polymorphism like bread wheat (Devos & Gale 1992; Joshi & Nguyen 1993) and barley (Chalmers *et al* 1993; Tinker *et al* 1993). Considering the above mentioned properties, RAPD markers were used to evaluate the variability and interrelationship of seven wheat genotypes. These results provide opportunity

to select such promising wheat parental genotypes having wide genetic distance compared to other existing genotypes.

Material and Method

Plant material

The experimental material used in the present study consisted of six wheat genotypes viz., Shahkar-95, Parwaz-94, Iqbal-2000, Uqab-2000, MH-97 and Punjab-96 and one line 4072. The plant material was grown in small plastic pots in a growth chamber (22°C).

DNA extraction

The total genomic DNA was isolated from young leaves of the seedlings using CTAB method (Doyle & Doyle 1990). Pellets of DNA obtained after treatment with RNase, were washed out with 70% ethanol and rehydrated in 150 µL 0.1 X TE (Tris EDTA). The concentration of extracted DNA was quantified at 260 nm in a spectrophotometer (CECIL, CE 2021). The quality of isolated DNA was observed by running the DNA samples on 0.8% agarose gel. Figure 1 shows DNA extraction of seven wheat genotypes/lines.

RAPD analysis

Genomic DNA was used as template for PCR amplification (Williams *et al* 1990). A set of 20 random decamer primers (Table 1) were used for amplification of DNA. For PCR reaction, concentration of DNA (10 ng/ 25µL) of seven wheat genotypes, 10 X PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, gelatin, dNTPs (dATP, dCTP, dGTP and dTTP), decamer random primers and Taq DNA polymerase were optimized. Taq polymerase buffer, $MgCl_2$, dNTPs and gelatin were purchased from Fermentas (Italy). DNA amplification was performed in a thermal cycler programmed for 1st cycle of 5 minutes at 94°C (hot start), followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 36°C (annealing) and 2 min at 72°C (extension) and final extension for 10 min.

RAPD data analysis

After PCR amplification, its products were subjected to electrophoresis on 1.2% agarose gel in 0.5 X TBE buffer (Tris borate EDTA) stained with Ethidium Bromide and photographed by gel-documentation system. The bands produced were counted. All bright/visible fragments were scored as present or absent. The data of the primers were used to estimate similarity (Table 1) on the basis of number of amplified products (Nei & Li 1979). Genetic similarity or difference between seven wheat genotypes was determined by dendrogram (Figure 5) constructed by unweighted pair group of arithmetic means (Sneath & Sokal 1973).

Results and Discussion

In this study, DNA analysis was applied to identify the diversity among wheat varieties. RAPD analysis was successfully used to confirm interspecific hybridization (Mei *et al* 2004). During amplification of RAPD markers, reproducibility is of major concern (Jones *et al* 1998). Bright DNA fragments were counted to resolve the problem of reproducibility. DNA of seven wheat varieties was amplified with 20 different random decamer primers (Table 1).

A total of 588 DNA fragments were generated by the 20 random primers with an average of 4.2 bands/primer.

Table 1. List of 20 decamer random primers

1	GL DecamerA-02	TGCCGAGCTG
2	GL DecamerA-04	AATCGGGCTG
3	GL DecamerA-07	GAAACGGGTG
4	GL DecamerA-09	GGGTAACGCC
5	GL DecamerA-15	TTCCGAACCC
6	GL DecamerA-18	AGGTGACCGT
7	GL DecamerA-19	CAAACGTCGG
8	GL DecamerB-04	GGACTGGAGT
9	GL DecamerB-07	GGTGACGCAG
10	GL DecamerB-15	GGAGGGTGTT
11	GL DecamerB-20	GGACCCTTAC
12	GL DecamerC-02	GTGAGGCGTC
13	GL DecamerC-03	GGGGGTCTTT
14	GL DecamerC-10	TGTCTGGGTG
15	GL DecamerC-11	AAAGCTGCGG
16	GL DecamerC-14	TGCGTGCTTG
17	GL DecamerC-17	TTCCCCCCAG
18	GL DecamerC-19	GTTGCCAGCC
19	GL DecamerD-09	CTCTGGAGAC
20	GL DecamerD-12	CACCGTATCC

The size and the number of bands produced were strictly dependent upon the nucleotide sequence of the primer used for template DNA. Reactions were duplicated for many times to check the consistency of the amplified products. Only bright DNA bands were considered and scored. All the varieties showed diversity towards each other on their amplification profiled based on these 588 DNA bands amplified by 20 primers. 189 out of 588 DNA fragments (32%) showed polymorphism in seven wheat varieties while the rest of the bands were monomorphic in all the varieties. All the seven varieties could be identified with a primer as shown in Figure 2 and 3. Therefore, RAPD markers can be used for identification of wheat varieties. Out of seven wheat varieties studied, the variety Parwaz-94 produced maximum number of DNA amplified fragments (96) while minimum number (65) was produced by the variety MH-97. The mean number of amplified RAPD loci due to each decamer primer was comparatively lower than the earlier studies conducted on the wheat and other crop species (Tao *et al* 1993; Ayana *et al* 2000; Mukhtar *et al* 2002; Tabbasam *et al* 2006; Iqbal *et al* 2007). Various factors affect the reproducibility of RAPD technique like sequence of primer, template quality, thermocycler type and concentration of DNA polymerase enzyme (Hernandez *et al* 1999).

However, the utilization of a standard RAPD protocol can ensure a reproducible RAPD pattern. The concentrations of MgCl2, Taq DNA polymerase and template DNA concentration were kept optimum for PCR reactions. DNA concentration of 10 ng/ 25 µL was found to produce the most consistent and reproducible banding patterns. 3mM $MgCl_2$ produced specific amplification during RAPD analysis. Similarly, one unit concentration of Taq DNA polymerase enzyme was optimum for amplification of genomic DNA. Similarly other reaction conditions were kept constant so consistent and reproducible results of genomic DNA was found. RAPD reaction of seven wheat lines with GL Decamer D-09 and GL Decamer D-12 is shown in Figure 4.

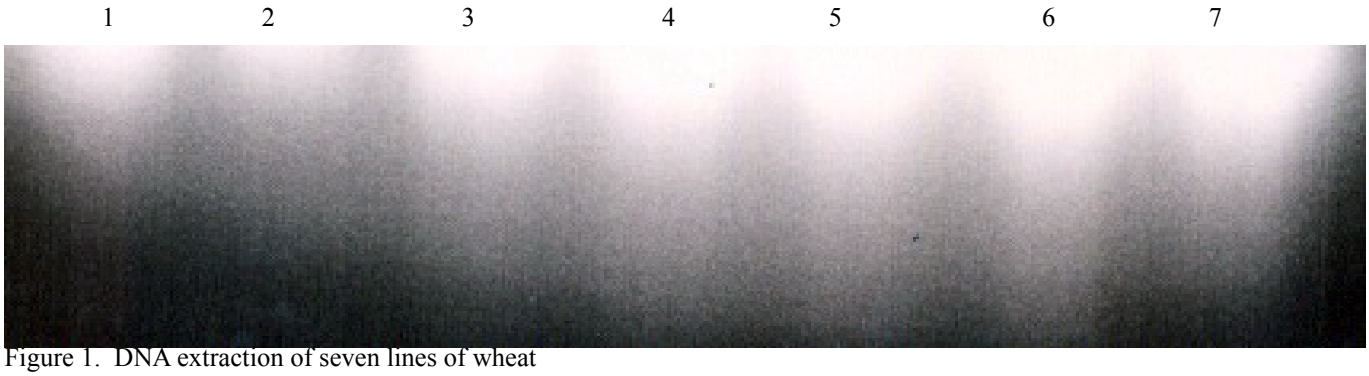

Figure 1. DNA extraction of seven lines of wheat

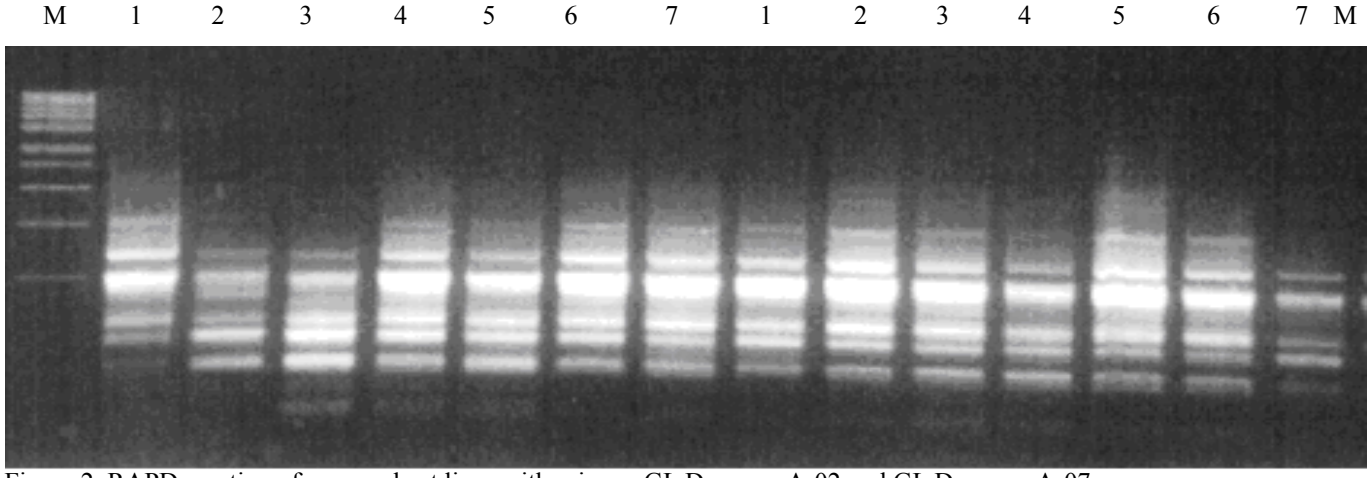

Figure 2. RAPD reaction of seven wheat lines with primers GL Decamer A-02 and GL Decamer A-07

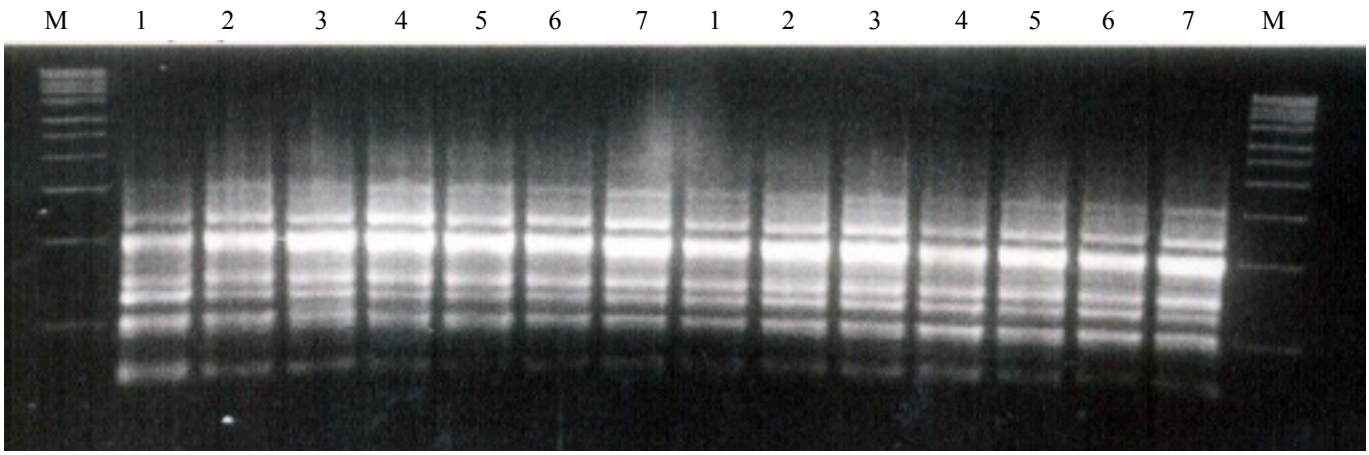

Figure 3. RAPD reaction of seven wheat lines with GL Decamer C-03 and GL Decamer C-10 respectively

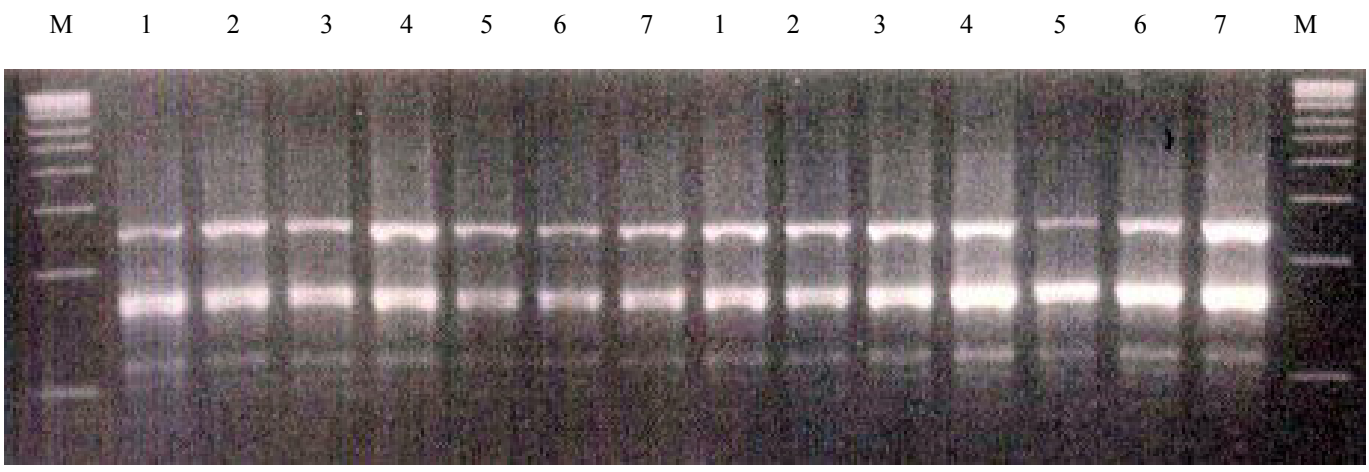

Figure 4. RAPD reaction of seven wheat lines with GL Decamer D-09 and GL Decamer D-12 respectively

Table 2. Similarity matrix of seven wheat genotypes obtained from RAPD markers

Genotypes	Shahkar-95	Parwaz-94	Iqbal-2000	Uqab-2000	MH-97	4072	Punjab-96
Shahkar-95	****	-	-	-	-	-	-
Parwaz-94	0.6000	****	-	-	-	-	-
Iqbal-2000	0.6000	0.4857	****	-	-	-	-
Uqab-2000	0.5429	0.4857	0.7143	****	-	-	-
MH-97	0.6286	0.6286	0.5143	0.3429	****	-	-
4072	0.6571	0.4857	0.5429	0.6000	0.4000	****	-
Punjab-96	0.6286	0.5714	0.4571	0.5143	0.6571	0.5143	****

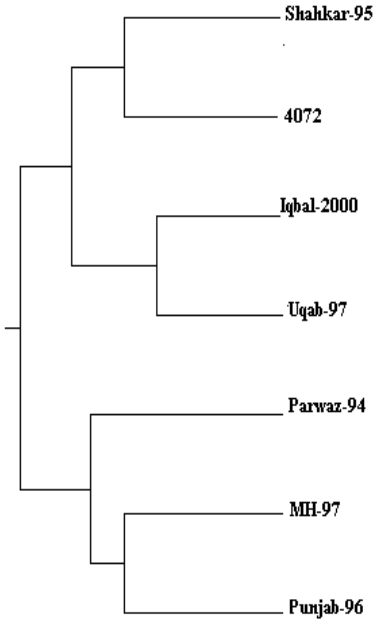

Figure 5. Dendrogram of seven wheat genotypes developed from RAPD data

Similarity matrix analysis of seven wheat lines according to Nei & Li's (1979) coefficient of similarity was done (Table 2). Twenty RAPD primers were used to check the genetic diversity. According to this table, the genetic similarities of these lines were high (34.29 % to 71.43 %). Variety Iqbal-2000 and Uqab-2000 had the greatest (71.43 %) similarity (Figure 5). These two varieties differed from each other from seventeen bands with 20 different polymorphic primers. The genetic similarity between genotypes 4072 and Shahkar-95 was the second highest (65.71%), the varieties Punjab-96 and MH-97 also fell in the same range (65.71). While lowest genetic similarity (34.29 %) was found between the lines MH-97 and Uqab-2000. Tabbasam *et al* (2006) found 78.8 % genetic similarity between two sorghum hybrids while Iqbal *et al* (2007) found narrow genetic base with a range of 86.2 to 93 % genetic similarity among seven wheat genotypes.

Present study suggested that DNA based markers like RAPDs play an important role in exploring genetic diversity among seven wheat genotypes to what extent they are different from each other and could be further utilized for gene mapping. These findings would help in selection of parents for further breeding program to produce high yielding hybrids with various desirable traits.

References

Ayana, A., Bryngelsson, T., Bekele, E., 2000. Genetic variation of Ethiopian and Eritrean sorghum (*Sorghum bicolor* (L.) Moench) germ plasm assessed by random amplified polymorphic DNA (RAPD). Genetic Resources and Crop Evolution 47:471-482.

Chalmers, K. J., Barua, U. M., Hackett, C. A., Thomas, W. T. B., Waugh, R., Powell, W., 1993. Identification of RAPD markers linked to genetic factors controlling the milling energy requirement of barley. Theoretical and Applied Genetics 87:314-320.

Devos, K. M., Gale, M. D., 1992. The use of random amplified polymorphic DNA markers in wheat. Theoretical and Applied Genetics 84:567-572.

Doyle, J. J., Doyle, J. L., 1990. Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Hernandez, P., Martin, A., Dorado, G., 1999. Development of SCARs by direct sequencing of RAPD products: A practical tool for the introgression and marker assisted selection of wheat. Molecular Breeding 5:245-253.

Iqbal, A., Khan A. S., Khan, F. S., Ahmad, A., Khan, A. A., 2007. Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA. Genetics and Molecular Research 6(3):476-481.

Jones, C. J., Edwards, K. J., Castiglione, S., Winfield, M. O., Sala, F., Vander-Weil, C., Vosman, B. L., Matthes, M., Daly, A., Brettschneider, R., Bettini, P., Buiatti, M., Maestri, E., Marmiroli, N., Aert, R. L., Volckaert, G., Rueda, J., Vazquez, A., Karp, A., 1998. Reproducibility testing of molecular tools for screening biodiversity. Chapman & Hall, London, UK, pp.176-179.

Joshi, C. P., Nguyen, H. T., 1993. RAPD (Random amplified polymorphic DNA) analysis based on intervarietal genetic relationships among hexaploid wheats. Plant Science 93:95-103.

McDonald, M. B., 1995. Genetic purity: From protein electrophoresis to RAPDs. In: Proceedings of the fiftieth annual corn & sorghum industry research conference. pp. 256-271.

Mei, M., Syed, N. H., Gao, W., Thaxton, P. M., Smith, C. W., Stelly, D. M., Chen, Z. J., 2004. Genetic mapping and QTL analysis of fiber-related traits in cotton (*Gossypium*). Theoretical and Applied Genetics 108:280-291.

Mukhtar, M. S., Rahman, M., Zafar, Y., 2002. Assessment of diversity among wheat (*T. aestivum* L.) cultivars from a range of localities across Pakistan using random amplified polymorphic DNA analysis. Euphytica 128(3):417-425.

Nei, N., Li, W., 1979. Mathematical model for studying genetics variation in terms of restriction endonucleases. Acta Horticulturae 262:305-310.

Sleper, D. A., Poehlman, J. M., 2006. Breeding Field Crops. 5th ed. Iowa State University Press. USA.

Sneath, P., Sokal, R., 1973. Numerical Taxonomy. Freeman, San Francisco.

Tabbasam, N., Rahman, M., Zafar, Y., 2006. DNA-based genotyping of sorghum hybrids. Pakistan Journal of Botany 38(5):1599-1604.

Tao, Y., Manners, J. M., Ludlow, M., Henzel, R. J., 1993. DNA polymorphisms in grain sorghum (*Sorghum bicolor* (L.) Moench). Theoretical and Applied Genetics 86:676-688.

Tinker, N. A., Fortin, M. G., Mather, D. E., 1993. Random amplified polymorphic DNA and pedigree relationship in spring barley. Theoretical and Applied Genetics 85:976-984.

Welsh, J., McClelland, M., 1990. Fingerprinting genomes using PCR with arbitrary primerss. Nucleic Acids Research 18:7213-7218.

Williams, J. G. K., Kubelik, A. R. K., Livak, J. L., Rafalski, J. A., Tingey, S. V., 1990. DNA polymorphisms amplified by random primers are useful as genetic markers. Nucleic Acids Research 18:6531-6535.

Authors

•Shahzadi Mahpara, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan.

•Jehanzeb Farooq, Cotton Research Institute, Ayub Agricultural Research Institute, Faisalabad 38040, Pakistan, jehanzeb1763@hotmail.com

•Zulfiqar Ali, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan.

•Ioan Valentin Petrescu-Mag, SC Bioflux SRL, 54 Ceahlau Str., Cluj-Napoca 400488, Romania, zoobiomag2004@yahoo.com

•Ioan Valentin Petrescu-Mag, Postdoctoral School in Agriculture and Veterinary Medicine (POSDRU/89/1.5/S/62371), University of Agricultural Sciences and Veterinary Medicine, Iasi, 3 Mihail Sadoveanu Street, Iasi, Romania, European Union.

•Fida Hussain, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38040, Pakistan.

Citation	Mahpara, S., Farooq, J., Ali, Z., Petrescu-Mag, I. V., Hussain, F., 2012. Assessment of genetic distance among wheat genotypes through RAPD markers. Advances in Agriculture & Botanics 4(1):31-35.
Editor	Miklos Botha
Received	15 February 2012
Accepted	13 May 2012
Published Online	30 June 2012
Funding	None Reported
Conflicts / Competing Interests	None Reported