Pak. J. Bot., 45(S1): 327-332, January 2013.

GENETIC DIVERGENCE AMONG PAKISTANI BREAD WHEAT VARIETIES AND ADVANCED LINES FOR RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

RAHEELA REHMAN[1*], KAUSAR NAWAZ SHAH[2], M. SHAHID MASOOD[1], M. ARSHAD[1] AND ABDUL GHAFOOR[1]

[1]*National Agricultural Research Centre (NARC), Park Road, Islamabad, PAKISTAN*
[2]*Department of Plant Breeding and Genetic PMAS-Arid Agricultural University, Rawalpindi, PAKISTAN*
[*]*Corresponding author's e-mail: raheelarehman87@gmail.com*

Abstract

A large number of wheat varieties have been developed over the years but very few have been investigated for genetic divergence at molecular level. The present paper explains the genetic diversity for Randomly Amplified Polymorphic DNA (RAPD) markers among wheat varieties as well as advanced lines. Twenty RAPD decamer primers were used to determine the extent of genetic differences among 48 genotypes. Among 20 primers ten were monomorphic and others generated 71 DNA fragments with an average of about 7.1 bands per primer. The primers, *viz*., OPE-01, OPB-13 and OPB-09 have 17%, 14% and 5.6% share to the total polymorphism among total variation. Forty seven genotypes were amplified with the primer OPA-09 and 11 with OPA-16. Six genotypes NR-346, NR-373, NR-389, NR-383, WSP-148 and WSP-196 were the most diverse from rest of the genotypes for RAPD analysis. The information about genetic similarity and differences will be helpful to avoid any possibility of elite germplasm becoming genetically uniform.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops and due to its multifaceted use and nutritive value it is staple food for more than one third of the world's population including Pakistan (Asif *et al*., 2005). Wheat is the 'King of cereals' due to its acreage, productivity and the conspicuous position in international food grain trade (Shashikala, 2006). Approximately 681 million tons wheat is produced annually on 225 million hectare of the total cultivated land in the world and supports nearly 35% of the world's population (http://www.earth-policy.org/data_center/C24). World demand for wheat is expected to be much higher in near future because of rapid increase in human population but the resources available for wheat production are likely to be significantly lower. The challenge of food security is critical in the developing countries than it is in the developed world while wheat is the main crop to ensure food stability especially in Pakistan. Wheat contributes 2.8% to Gross Domestic Product (GDP) and 13.1% to the value added in agriculture (Annon, 2009). The estimated population of Pakistan in 2030 is 24.63 millions that demand wheat production at the rate of 2% year^{-1} increase (Annon, 2011). In the recent past, significant development of new high yielding cultivars has been attained that is still going on to meet the emerging requirements of wheat. The mechanized modern wheat production has damaged genetic diversity that is required to evaluate and conserve prior to erosion (Sofalian *et al*., 2008). It is widely accepted that information about germplasm diversity and genetic relatedness among elite breeding material is a fundamental element in plant breeding (Mukhtar *et al*., 2002). The advance bread wheat varieties have been reported with narrow genetic base, hence breeding wheat genotypes with diverse genetic base is a vital factor to achieve not only the level of self-sufficiency and sustainability but also to enter in the export market (Reif *et al*., 2005).

In the recent past, RAPD markers have been used extensively for the identification of genotype in crop plants (Malviya & Yadav, 2010; Pervaiz *et al*., 2010) and gained importance due to its simplicity, efficiency and non requirement of sequence information (Skaria *et al*., 2011, Jan *et al*., 2011). Although RAPD is first generation molecular technique but this is the best to initiate genomic work on any crop because this involves the use of a single arbitrary primer in a Polymerase Chain Reaction (PCR) without any background genomic knowledge rather it is a bench mark for molecular genetics (Abdellatif & AbouZeid, 2011). Pakistan lags behind in molecular markers and few reports are available on RAPD markers in wheat (Mukhtar *et al*., 2002 and Asif *et al*., 2005). The present study is focused on estimation of genetic distance between locally developed wheat varieties and advanced lines based on RAPD markers. Although it is a preliminary work but the data generated from the study can be used for genotype identification in combination of various fragments and the information gathered here would be helpful in future for genomic mapping studies leading to development of wheat cultivars with broader genetic background to obtain improved crop productivity.

Materials and Methods

This study was conducted at Germplasm Evaluation Laboratory of Institute of Agro-biotechnology and Genetic Resource (IABGR), National Agricultural Research Centre (NARC). Eleven approved varieties and 9 advanced lines were obtained from IABGR and Wheat Salinity Project at National Institute for Genomic and advanced Biotechnology (NIGAB) while remaining 28 were obtained from wheat program at NARC which were included in the National Uniform Wheat Yield Trials (NUWYT) during the year 2011. All the 48 genotypes were planted in plastic pots under control conditions. The total DNA was extracted from young leaves by a method proposed by Rogers & Bendich (1988) and concentration of DNA was measured at 260nm in a spectrophotometer (S-30 Spectrophotometer BOECO Germany). The quality of DNA was assessed by gel electrophoresis on 0.8 %

agarose gel (1 x TBE buffer and stained with ethidium bromide) and it was checked for contamination by RNA (which usually runs ahead) while DNA was evaluated by comparing it with a standard undigested DNA sample (50ng/µl and 100ng/µl). All the samples were diluted up to 25ng/µl.

The primer series, *viz*., OPA, OPB, OPC, OPD, OPE, OPJ and OPK of decamer were obtained from Operon technologies Inc. Alamedos, USA. Genomic DNA amplification was performed in total volumes of 20µl containing 2µl 10X PCR buffer, 2.4 µl (3mM) $MgCl_2$, 0.4 µl dNTPs (dATP, dCTP, dGTP, dTTP), 1 µl primer, 1 µl of DNA template, 0.2 µl of Taq Polymerase and 13 µl ddH2O. The RAPD amplification was carried in a 96 well thermo cycler programmed for initial denaturation step of 5 min. at 94°C followed by 40 cycles of 1 min at 94°C, 1 min. at 36°C and 2 min. at 72°C. The PCR tubes were kept at 72°C for 10 min. and then held at 4°C until the tubes were removed. After the completion of the PCR, the products were stored at 4°C until the gel electrophoresis was done. The detailed protocol is available at IABGR (Masood *et al*., 2003). The amplified products were separated and visualized by 1.5% agarose gel stained with ethidium bromide (Sambrook *et al*., 1989) and the gels were digitized by gel documentation system. The data were recorded as binary file and the fragment size was determined by 1kb DNA ladder. The data was analyzed for genetic diversity with the help STATISTICA 7.0 version and genetic distances were calculated as described by Nei & Li, (1979). Clustering was performed using Unweighted Pair Group Method of Arithmetic Means (UPGMA) and dendrogram was constructed using graphic function in the program.

Results

Among 20 decamer primers only half showed their reaction in PCR (Table 1). All the primers generated 71 fragments with an average of 7.1 bands per primer, although all the primers varied in number of bands (Table 2). Only polymorphic bands were included in this analysis as the monomorphic fragments do not resolve any taxonomic issue. The Fig. 1 shows the polymorphic bands among wheat genotypes amplified by the primer "OPE-01" [-*CCCAAGGTCC*-]. A considerable variation was observed and this primer generated the maximum polymorphic bands that was 16.9% of the total diversity at molecular level. The number of bands in this study ranged from 4 (OPB-09) to 12 (OPE-01) and the size of the amplified products ranged from 500bp to 10kb while the polymorphism among various molecular weight is given in ranges (Table 2). Every single band was considered as a single locus for all the genetic analyses, hence the intensity of the fragment was not considered. The loci were scored as binary data, i.e., 1 (presence of band) and 0 (absent of band) and were used to estimate genetic distances. The maximum 12 polymorphic bands were produced by the primer OPE-01 followed by OPB-13 10 bands and OPB-09 that generated only 4 fragments. The primer OPE-01, OPB-13 and OPB-09 contributed 17%, 14% and 5.6% respectively to the total polymorphism among 48 genotypes of wheat. Maximum 47 genotypes were amplified with the primer OPA-09 and minimum 11 with the primer OPA-16.

Table 1. Primer used for preliminary DNA amplification in wheat.

Sr.No.	Primer	Sequence		Sr. No.	Primer	Sequence	
1.	OPA-02	TGCCGAGCTG	+ (present)	11.	OPB 13	**TTCCCCCGCT**	**+ (present)**
2.	OPA-07	GAAACGGGTG	+ (present)	12.	OPJ 13	CCACACTACC	- (absent)
3.	OPA 11	CAATCGCCGT	- (absent)	13.	OPC-13	AAGCCTCGTC	+ (present)
4.	OPA 12	TCGGCGATAG	- (absent)	14.	OPD 02	GGACCCAACC	- (absent)
5.	OPA-16	AGCCAGCGAA	+ (present)	15.	OPB 05	TGCGCCCTTC	- (absent)
6.	OPA-18	AGGTGACCGT	+ (present)	16.	0PB 06	TGCTCTGCCC	- (absent)
7.	OPA-19	CAAACGTCGG	+ (present)	17.	OPB 09	TGGGGGACTC	+ (present)
8.	OPB-09	TGGGGGACTC	- (absent)	18.	OPF 09	CCAAGCTTCC	- (absent)
9.	OPB 11	GTAGACCCGT	- (absent)	19.	OPE-01	CCCAAGGTCC	+ (present)
10.	OPB 14	TCCGCTCTGG	- (absent)	20.	OPK-06	CACCTTTCCC	+ (present)

Represents amplification of primer = +, No amplification = -

Table 2. RAPD fragments observed by using ten primes in 48 wheat genotypes.

S. No.	Primer name	Sequence	No. of polymorphic bands	Polymorphic bands for molecular weight (bp)			Percentage
				>750	500-750	<500	
1	OPE-01	CCCAAGGTCC	12	5	4	3	16.90
2	OPB-09	TGGGGGACTC	4	1	2	1	5.60
3	OPK-06	CACCTTTCCC	6	2	3	1	8.45
4	OPA-02	TGCCGAGCTG	8	1	5	2	11.27
5	OPA-07	GAAACGGGTG	6	1	4	1	8.45
6	OPC-13	TTCCCCCGCT	6	2	3	1	8.45
7	OPB-13	AAGCCTCGTC	10	4	4	2	14.08
8	OPA-16	AGCCAGCGAA	7	3	3	1	9.86
9	OPA-18	AGGTGACCGT	6	3	2	1	8.45
10	OPA-19	CAAACGTCGG	6	2	3	1	8.45
Total:			71	24	33	14	100

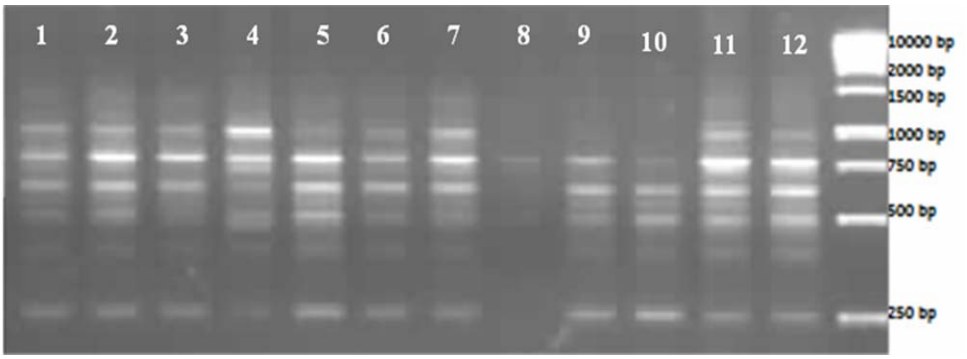

Fig. 1. RAPD fragments with primer OPE-01.

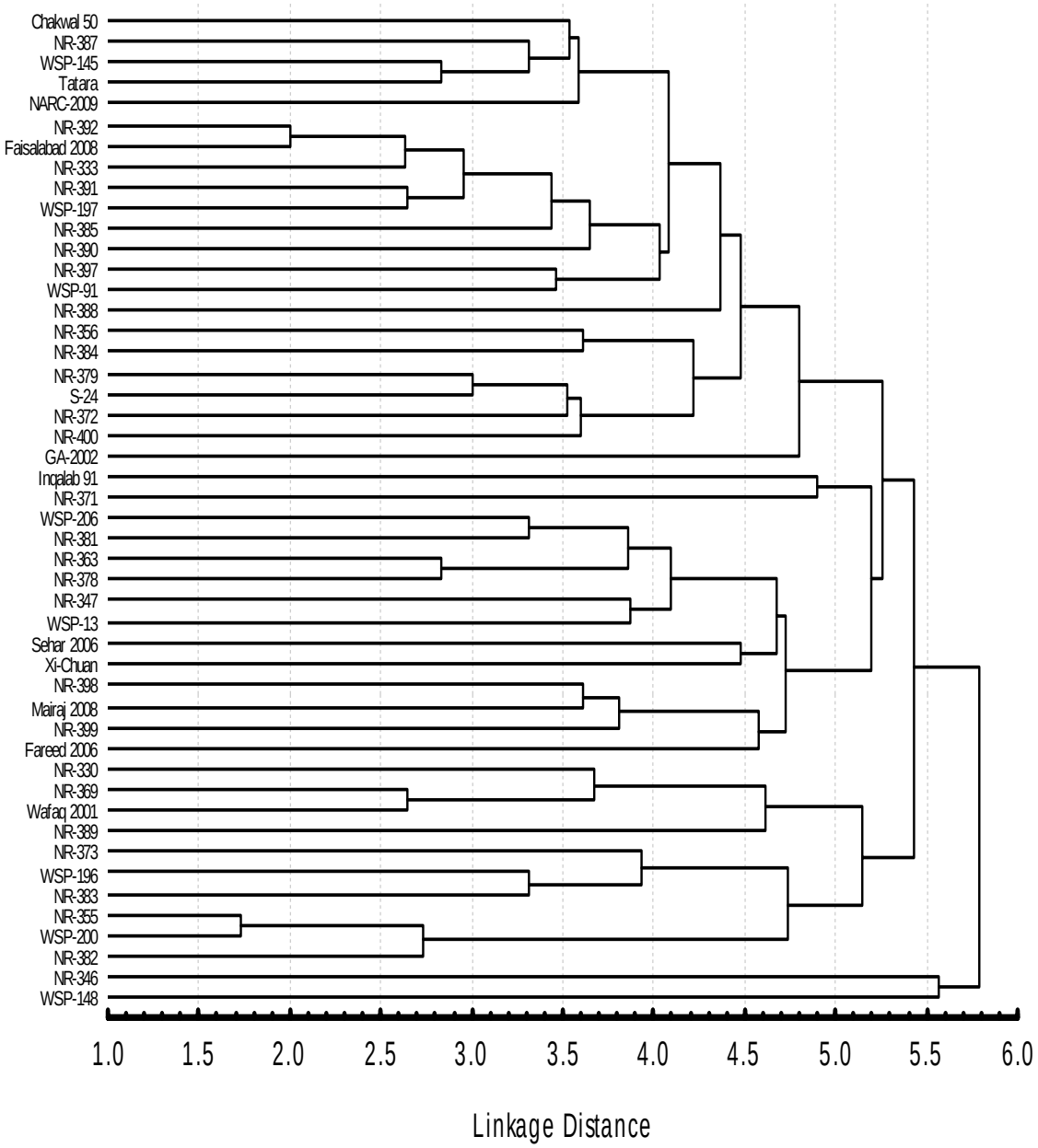

Fig. 2. Dendrogram based on 71 RAPD fragments IN 48 wheat lines.

The level of polymorphism was different with different primers among various genotypes. Out of 48 wheat genotypes, WSP-148 and Wafaq-2008 produced 49 and 12 fragments respectively with all of 10 primers. The dendrogram based on genetic distances was constructed to investigate genetic diversity among 48 genotypes and relatively higher magnitudes of genetic distance were observed (Fig. 2). The average genetic distances ranged from 2.00 (Faisalabad-08 and NR-392) to 6.86 (NR-382 & NR-346, NR-389 & NR-371). Six genotypes NR-346, NR-373, NR-389, NR-383, WSP-148 and WSP-196 were observed diverse as compared with other genotypes. Cluster diagram of 48 wheat genotypes is presented in Fig. 2 revealed that 5 main clusters that were further subdivided into sub clusters. The cluster 1 comprised of 2 genotypes (WSP-148, NR-346), the cluster 2 consisted 10 genotypes and was sub divided into 2 sub-clusters, i.e., 2a and 2b. The sub cluster 2a consisted of 6 genotypes (NR-382, WSP-200, NR-355, NR-383, WSP-196 and NR-373) and 4 genotypes grouped in the sub cluster 2b. The cluster 3 has twelve genotypes and this cluster was sub divided into 3 sub-clusters 3a, 3b and 3c. The sub cluster 3a comprised of 4 genotypes (Fareed-2006, NR-399, Mairaj-2008 and NR-398), sub cluster 3b consisted of 2 genotypes (XI-Chuan & Sehar-2006) and the sub cluster 3c consisted of 6 genotypes (WSP-13, NR-347, NR-378, NR-363, NR-381 and WSP-206). The cluster 4 consisted 2 genotypes (NR-371 and Inqalab-91), the cluster 5 was subdivided into 3 sub-clusters, i.e., sub cluster 5a consisted one genotype (GA-2002), the sub cluster 5b comprised of 6 genotypes (NR-400, NR-372, S-24, NR-379, NR-384 and NR-356) and sub cluster 5c consisted 15 genotypes (NR-388, WSP-91, NR-397, NR-390, NR-385, WSP-197, NR-391, NR-333, Faisalabad-2008, NR-392, NARC-2009, Tatara, WSP-145, NR-387 and Chakwal-50).

Discussion

Efficient and effective crop improvement program depends on the extent of genetic diversity either existing or created (Naz *et al*., 2006, Akbar *et al*., 2011). Wheat breeding has achieved hallmark progress that is able to feed ever increasing population of Pakistan that has been further assisted by modern agricultural biotechnology in the recent years (Khan *et al*., 2005). The ultimate trends concerning the loss of genetic diversity have been reported due to modern breeding practice by Fufa *et al*., 2005, Iqbal *et al*., 2007 and Khan *et al*., 2010, Turi *et al*., 2012, hence these markers have been recently employed for screening and development of a-biotic stresses including salinity (Shahzad *et al*., 2012). Prior to have project on crop improvement, it is imperative to understand the levels and distribution of genetic diversity in existing crop gene pools, as a basis for developing strategies of resource management and exploitation prior to its elimination (Abdellatif & Khidr, 2010; Abdellatif & AbouZeid, 2011). Field evaluation has been the priority for conventional plant breeding that has changed recently with the intervention of molecular markers that can support a more detailed characterization of genetic resources in limited span (Zarkti *et al*., 2010). In the present study, it was observed that half of the RAPD primers were polymorphic that can be used for other wheat germplasm for investigation of genetic diversity. In addition to direct measure of genetic diversity, the molecular markers also provide opportunity to go beyond indirect diversity based on agronomic traits or geographic origin (Mir *et al*., 2012). The continuous breeding efforts has been focused only for higher yields under optimum growth conditions and all the advanced lines included in the present study were selected by the breeders and in addition information on this material will have significance for genotyping and ensuring intellectual property rights (Ahmad *et al*., 2010). The data reported in this paper is suggested to relate with agronomic performance of these genotypes that could be used for indirect selection for high yielding wheat genotypes (Liu *et al*., 2012). Genetic diversity studies reported in this manuscript confirmed the findings from previous researchers (Malik *et al*., 2000, Asif *et al*., 2005, Bhutta *et al*., 2005, Tahir, 2008, and Bibi *et al*., 2009) but with the inclusion of new material that has not been analyzed before. Forty eight wheat genotypes studied with RAPD markers showed a close similarity among them that could be due to limited number of primers used in the present study or the material that were ultimate selections converging main focus to yield potential, these types of results have already been reported by Mukhtar *et al*., (2002) and Khan *et al*., (2005) in Pakistani wheat using different material in their studies. Sun *et al*., (2003) observed up to 25.6% genetic distance among 40 wheat lines based on RAPDs, whereas in another study, RAPDs detected from 16 to 67% genetic distance among 14 winter wheat lines from Croatia (Maric *et al*., 2004). Ahmad *et al*., (2010) also reported low level of genetic diversity among 32 advanced breeding lines of Pakistani wheat using 15 polymorphic RAPD markers. The level of genetic diversity is expected higher when this type of investigation is accomplished in early segregating populations or germplasm derived for distinct characteristics.

In the present study, RAPD analysis was found to be a valuable diagnostic tool to evaluate genetic diversity and in addition it could served the purpose of genotyping if the number of primers are increased, and preferably the DNA markers be made specific either converting the reported fragments to SCAR (Gupta & Varshney, 2000) or using new types of markers, including SSR, AFLP, RFLPs etc (Khan *et al*., 2010). Six genotypes NR-346, NR-373, NR-389, NR-383, WSP-148 and WSP-196 were observed diverse as compared with other genotypes based on RAPD and these have been obtained from Wheat Research Program (4 genotypes) and Wheat Salinity Project (2 genotypes), NIGAB, NARC. The information on genetic similarity based on DNA markers will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains (Nawaz *et al*., 2009). In the view of the close kinship of wheat genotypes reported, it is proposed that objective oriented breeding programs should be strengthened coupled with DNA markers that would be helpful to produce distinct

genotypes for steady genetic improvement (Ojaghi & Akhundova, 2010, Zarkti *et al*., 2010). The use of RAPDs, remained under criticism because the sensitivity of this technique to working conditions and equipment used can influence the results (Weder, 2002), anyhow this technique is the basis to initiate molecular research on any crop (Ghafoor & McPhee, 2012) and particularly for conversion of new marker system where basic genomic knowledge is a prerequisite. Another problem is the possibility of duplications of genetic similarity estimates because fragments with the same size could have different origins and this is specific particularly in hexaploid wheat because of the complexity of its genome (Maric *et al.,* 2004).

References

Abdellatif, K.F. and Y.A. Khidr. 2010. Genetic diversity of new maize hybrids based on SSR markers as compared with other molecular and biochemical markers. *Journal of Crop Science and Biotechnology*, 13(3): 139-145.

Abdellatifa, K.F. and H. M. AbouZeib. 2011. Assessment of genetic diversity of Mediterranean bread wheat using randomly amplified polymorphic DNA (RAPD) markers. *Journal of Genetic Engineering and Biotechnology*, 9: 157-163.

Ahmad, F.A., M. Iqbal, M.S. Masood, M.A. Rabbani and M. Munir. 2010. Assessment of genetic diversity among Pakistani wheat (*Triticum aestivum* L.) advanced breeding lines using RAPD and SDS-PAGE. *Electron. J. Biotechnol*., (online): 13.

Akbar, F., M.A. Rabbani, M.S. Masood and Z.K. Shinwari. 2011. Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from pakistan using RAPD markers. *Pak. J. Bot*., 43(4): 2153-2160.

Anonymous. 2009. *Agriculture Statistics of Pakistan.* Finance Division, Economic Advisor's Wing. Islamabad, Pakistan.

Anonymous. 2011. *Economic survey of Pakistan*. Finance Division, Economic Advisor's Wing. Islamabad, Govt. of Pakistan.

Asif, M., M. Rahman and Y. Zafar. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pak. J. Bot*., 37(2): 271-277.

Bhutta, W.M., A. Shahzad, J. Akhtar and M. Ibrahim. 2005. Assessment of genetic divergence among wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Biologia Bratislava*, 60(6): 671-674.

Bibi, S., M.U. Dahot, I.A. Khan, A. Khatri and M.H. Naqvi. 2009. Study of genetic diversity in wheat (*Triticum aestivum* L.) using random amplified polymorphic DNA (RAPD) markers. *Pak. J. Bot*., 41(3): 1023-1027.

Fufa, H., P.S. Baenziger. B.S. Beecher, Dweiket, R.A. Graybosch and K.M. Eskridge. 2005. Comparison of' phenotypic and molecular marker based classifications of hard red winter wheat cultivars. *Euphytica*, 145: 133-146.

Ghafoor, A. and K. McPhee. 2012. Marker assisted selection (MAS) for developing powdery mildew resistant pea cultivars. *Euphytica*, 186(3): 593-607.

Gupta, P.K. and R.K. Varshney. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, 113: 163-185.

Iqbal, A., A.S. Khan, I.A. Khan, F.S. Awan, A. Ahmad and A.A. Khan. 2007. Study of genetic divergence among wheat genotypes through random amplified polymorphic DNA. *Genet. Mol. Res*., 6(3): 476-481.

Jan, H.U., M.A. Rabbani and Z.K. Shinwari. 2011. Assessment of genetic diversity of indigenous turmeric (*Curcuma longa* L.) germplasm from Pakistan using RAPD markers. *J. Med. Plants Res*., 5(5): 823-830.

Khan, A.A., A. Iqbal, F.S. Awan and I.A. Khan. 2010. Genetic diversity in wheat germplasm collections from Balochistan province of Pakistan. *Pak. J. Bot*., 42(1): 89-96.

Khan, I. A., F.S. Awan, A. Ahmed, Y.B. Fu and A. Iqbal. 2005. Genetic diversity of Pakistani wheat germplasm as revealed by RAPD markers. *Genet. Resour. & Crop Evol*., 52: 239-224.

Liu, Y., Z. He, R. Appels and X. Xia. 2012. Functional markers in wheat: current status and future prospects *Theoretical and Applied Genetics*, 125: 1-10.

Malik, T.A., A. Price and D. Wright. 2000. Bulked segregant analysis and RAPD markers for drought resistance in wheat. *Pak. J. Agri. Res.*, 16(2): 82-88.

Malviya, N. and D. Yadav. 2010. RAPD Analysis among Pigeon pea [*Cajanus cajan* (L.) Mill sp.] cultivars for their genetic diversity. http://astonjournals.com/gebj, GEBJ-1, 1-9.

Maric, S., S. Boleric, J. Martinicic, I. Petic and V. Kozumplic. 2004. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficient of parentage. *Plant Breeding*, 123: 366-369.

Masood, S., A. Ghafoor and A. Javaid. 2003. Laboratory manual-Characterization and evaluation of plant genetic resources, Plant Genetic Resources Institute, NARC, Islamabad, Pakistan, pp. 37.

Mir, R.R., N. Sreenivasulu, R.K. Varshney, M. Zaman-Allah and R. Trethowan. 2012. Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theoretical and Applied Genetics*, 125: 625-645.

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

Nawaz, M., S.A. Hussain, I. Ullah, M. Younus, M.Z. Iqbal and S.M. Rana. 2009. Estimation of genetic diversity in wheat using DNA markers. *American-Eurasian J. Sustainable Agric*., 3(3): 507-511.

Naz, A., Z.A. Swati and I.A. Khan. 2006. Studies on genetic diversity in Pakistani wheat varieties using randomly amplified polymorphic DNA. *Pak. J. Biolo. Sci*., 9(15): 2858-2861.

Nei, M. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings *of the National Academy of Sciences of the United States of America*, 76: 5269-5273.

Ojaghi, J. and E. Akhundova. 2010. Genetic diversity in doubled haploids wheat based on morphological traits, gliadin protein patterns and RAPD markers. African J. Agric. Res., 5(13): 1701-1712.

Pervaiz, Z.K., M. A. Rabbani, Z. K. Shinwari, M. S. Masood and S. A. Malik 2010 Assessment of Genetic Variability in Rice (*Oryza sativa* L.) Germplasm from Pakistan Using RAPD Markers. *Pak. J. Bot*., 42(5): 3369-3376.

Reif, J.C., P. Zhang, S. Dreisigacker, M.L. Warburton, M. van Ginkel, D. Hoisington M. Bohn and A.E. Melchinger. 2005. Wheat genetic diversity trends during domestication and breeding. *Theor. Appl. Genet.*, 110:859-864.

Rogers, S.O. and A.J. Bendich. 1988. *Extraction of DNA from plant tissues.* In: Gelvin SB, Schilperoort RA (eds) Plant Molecular Biology Manual, pp A6:l-10. Boston, MA: Kluwer Academic Publishers.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular cloning*. 2nd Edition. Cold Spring Harbor Laboratory, New York.

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed and G.M. Ali. 2012. Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. *Genetics and Molecular Research*, 11(1): 679-692.

Shashikala, S.K. 2006. *Analysis of genetic diversity in wheat.* M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, India.

Skaria R., S. Sen and P.M.A. Muneer. 2011. Analysis of genetic variability in rice varieties (*Oryza sativa* L.) of kerala using RAPD markers. *Genet. Eng. & Biotech. J*., 24: 1-9.

Sofalian, O., N. Chaparzadeh, A. Javanmard and M.S. Hejazi. 2008. Study the genetic diversity of wheat landraces from Northwest of Iran based on ISSR molecular markers. *Int. J. Agri. Biol*., 10: 465-468.

Sun, G., M. Bond, H. Nass, B. Martin and Z. Dong. 2003. RAPD polymorphisms in spring wheat cultivars and lines with different levels of *Fusarium resistance*. *Theor. Appl. Genet*., 106: 1059-1066.

Tahir, N.A.R. 2008. Assessment of genetic diversity among wheat varieties in sulaimanyah using Random Amplified Polymorphic DNA (RAPD) Analysis. *Jordan J. Biolo. Sci*., 1: 159-164.

Turi, N.A., Farhatullah, M.A. Rabbani and Z.K. Shinwari. 2012. Genetic diversity in the locally collected brassica species of Pakistan based on microsatellite markers". *Pak. J. Bot*., 44(3): 1029-1035.

Weder, J.K.P. 2002. Influence of experimental conditions on the reproducibility of RAPD-PCR identification of legumes and cereals. *Leheosm-Wise u-Technol*., 35: 233-238.

Zarkti, H., H. Ouabbou, A. Hilali and S.M. Udupa. 2010. Detection of genetic diversity in Moroccan durum wheat accessions using agro-morphological traits and microsatellite markers. *African J. Agri. Res*., 14(5): 1837-1844.

(Received for publication 1 September 2012)