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STUDY OF GENETIC DIVERSITY IN WHEAT (*TRITICUM AESTIVUM* L.) USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

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Abstract

Twelve wheat genotypes developed through hybridization programme were screened for genetic diversity through RAPD marker. A total of 102 loci were amplified with 14 primers out of which 91 (89.2%) were polymorphic and only 11(10.8%) were monomorphic. Fragments size ranged from 142bp-5.3kb and fragments produced by various primers ranged from 1-11 with an average of 7.1 fragments per primer. The highest number of loci (13) was amplified with primer A-10, while the lowest number (1) with primer B-10. Results revealed that variety SARC-1, PKV-1600 and Chakwal-86 contain a specific segment of 478 bp while SARC-1 contains another specific segment of 957 bp amplified with primer A-07. Genetically most similar genotypes were SARC-1 and Chakwal-86 (70%) while most dissimilar genotypes were Sarsabz and PKV-1600 (33%). On the basis of results achieved, the varieties could be divided into 3 groups, Kiran-95, Marvi-2000 and Sarsabz in one group, Bhitai, ESW-9525, Inqilab-91, Khirman and Abadgar-93 clustered in second group and SARC-1, Chakwal-86, PKV-1600 and CM 24/87 in the third group.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops in the world, used mainly for human consumption and support nearly 35% of the world population. Nearly 95% of wheat grown today is hexaploid, used for the preparation of bread and other baked products (Debasis & Khurana, 2001). Wheat being a staple food of Pakistan, identification of high yielding lines is a main thrust of the wheat breeders in the country (Asif *et al*., 2005). Identification based on morphological characters is time consuming and requires extensive field trials and evaluation (Astarini *et al*., 2004), while morphological differences may be epigenetic or genetic based characters (Tahir, 2001; Mukhtar *et al*., 2002; Migdadi *et al*., 2004). During last three decades genetic diversity was studied in plants through isoenzymes (Hemrick & Godt, 1990). The development of molecular (DNA) marker provides new dimension, accuracy and perfection in the screening of germplasm (Tar'an *et al*., 2005). The development in molecular genetics in wheat has been relatively slow, especially when compared to other crops such as maize, rice or tomatoes, this is mainly because of wheat's ploidy level, the size and complexity of its genome (Gupta *et al*., 1999), the very high percentage of repetitive sequence and low level of polymorphism (Hoisington *et al*., 2002). Evaluation of germplasm diversity can help to identify landraces with the greatest novelty and thus are most suitable for rescue or incorporation into crop improvement program (Peterson *et al*., 1991; Devos & Gale, 1992; Asif *et al.*, 2005). Efficient and quick screening of such genotypes speedup the process of varietal evaluation, thus molecular marker plays pivotal role in this regard.

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Molecular markers are considered constant landmarks in the genome. In this study the random amplified polymorphic DNA (RAPD) technique has been used (Williams *et al*., 1990; Welsh & McClelland, 1990) for the identification of improved genotypes (Manifesto *et al*., 2001) to screen the genetic similarity/dissimilarity between some wheat germplasm. The most distinct genotypes will be used in the breeding program to increase the genetic diversity in wheat and will be used in marker assisted breeding as well as genome mapping.

Materials and Methods

Plant material and DNA extraction: A RAPD study was conducted to estimate the genetic diversity among commercially grown lines (Sarsabz, Kiran-95, Marvi-2000, Bhitai, Khirman, ESW-9525, Abadgar-93, Inqilab-91, SARC-1, PKV-1600, Chakwal-86 and CM 24/87). Genomic DNA was isolated through DNA isolation kit (Gentra system, Minnesota, USA) and DNA was quantified on spectrophotometer (Bio-mate 3), at absorbance of 260/280nm. The quality was further checked on 0.8% agarose gel.

PCR with random primers: Fourteen primers from gene link (New York, USA) were used to amplify the DNA (Table 1). PCR reaction was carried out in 25µl reaction mixture containing 2.6ng/µl of template (Genomic DNA), 2.5mM $MgCl_2$, 0.33mM of dNTPs (Eppendorf, Hamburg, Germany), 0.1U of Taq polymerase (Eppendorf, Hamburg, Germany) and 1µM of primer in 1x reaction buffer (Eppendorf, Hamburg, Germany). The amplification reaction was performed in the Eppendorf Master cycler with an initial denaturation for 5 minute at 94^{o}C, then 33 cycles: 1 minute denaturation at 94^{o}C; 1 minute annealing at 52^{o}C; 2 minute extension at 72^{o}C. Final extension was carried out at 72^{o}C for 10 minutes. Amplified products were electrophoresed on 1.5% agarose gels containing 0.5 x TBE (Tris Borate EDTA) and 0.5µg/ml Ethidium bromide to stain the DNA. The PCR product was electrophoresing at 72 volts for 2 hours. Photograph was taken under UV light using gel documentation system (Vilber Lourmat).

Data analysis: Data was scored as presence of band as (1) and absence of band as (0) from RAPD of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei & Li's (1979). Similarity coefficient was utilized to generate a dendrogram by means of un-weighted pair group method of arithmetic means (UPGMA).

Results and Discussions

Genomic DNA of commercially grown lines produced multiple fragments with 10 base arbitrary primers. Of 40 primers, 14 were amplifying the genomic DNA (Table 2). A total of 102 scorable loci were amplified, out of which 91 (89.2%) were polymorphic and only 11(10.8%) were monomorphic. Fragments ranged in size from 142bp-5.3kb and fragments produced by various primers ranged from 1-11 with an average of 7.1 fragments per primer. The highest number of loci (13) was obtained with primer A-10, while the lowest number (1) was obtained with primer B-10 (Table 1). Level of the individual genotype of the 12 wheat varieties produced polymorphism in which few monomorphic loci were observed (Fig. 1). The amplification of monomorphic loci is depicting sharing of common blood among the genotypes (Loucou *et al*., 1998; Asif *et al*., 2005).

Table 1. Sequence of the primers.

Primer	Sequence	Range of amplified loci	Polymorphic loci	Monomorphic loci	Total no. of loci
A-02	TGCCGAGCTG	1.1kb-5.3kb	07	01	08
A-07	GAAACGGGTG	478bp-957bp	02	Nil	02
A-09	GGGTAACGCC	350bp-3.98kb	11	01	12
A-10	GTGATCGCAG	204bp-2.9kb	13	Nil	13
A-13	CAGCACCCAC	258bp-1.26kb	07	Nil	07
A-15	TTCCGAACCC	326bp-2.9kb	07	01	08
A-18	AGGTGACCGT	316bp-1.6kb	05	03	08
A-20	GTTGCGATCC	214bp-2.16kb	07	01	08
B-06	TGCTCTGCCC	298bp-1.4kb	08	Nil	08
B-10	CTGCTGGGAC	2.4kb & 2.5kb	Nil	01	01
B-17	AGGGAACGAG	298bp-2.14kb	04	03	07
C-02	GTGAGGCGTC	142bp-1.3kb	09	Nil	09
C-05	GATGACCGCC	211bp-1.5kb	06	Nil	06
C-08	TGGACCGGTG	220bp-1.2kb	06	Nil	06
			91 (89.2%)	11 (10.8%)	102

Table 2. Similarity coefficient among the wheat cultivars calculated according to Nei & Li's coefficient.

	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
L2	1											
L3	0.550	1										
L4	0.528	0.582	1									
L5	0.437	0.430	0.54	1								
L6	0.436	0.461	0.484	0.541	1							
L7	0.441	0.392	0.467	0.600	0.494	1						
L8	0.399	0.415	0.438	0.491	0.481	0.498	1					
L9	0.465	0.398	0.419	0.567	0.565	0.577	0.511	1				
L10	0.407	0.411	0.429	0.556	0.519	0.467	0.479	0.633	1			
L11	0.329	0.351	0.357	0.446	0.389	0.453	0.439	0.542	0.639	1		
L12	0.403	0.404	0.364	0.429	0.422	0.449	0.507	0.574	0.701	0.642	1	
L13	0.407	0.417	0.396	0.399	0.406	0.405	0.44	0.556	0.63	0.531	0.659	1

L2=Sarsabz, L3=Kiran-95, L4=Marvi-2000, L5=Bhittai, L6=Khirman, L7=ESW-9525, L8=Abadgar-93, L9=Inqilab-91, L10=SARC-1, L11=PKV-1600, L12=Chakwal-86, L13=CM24/87.

In the present experiment some specific RAPD bands were identified; thus reflecting the RAPDs application for the identification of wheat, which may correlate with morphological trait. It was observed that variety SARC-1, Chakwal-86 and PKV-1600 contain a specific band of 478bp while SARC-1 contains another specific band of 957bp amplified with primer A-07. Souza *et al.*, (1994) and Manifesto *et al*., (2001) found some specific RAPD marker while examining genetic diversity in spring wheat cultivars grown in the Yaqui Valley of Mexico and the Punjab of Pakistan.

Nei's and Lei's coefficient similarity matrix were calculated to estimate the genetic divergence and relatedness among wheat genotypes. Genetically most similar genotypes were SARC-1 and Chakwal-86 (70%) while most dissimilar genotypes were Sarsabz and PKV-1600 (33%). (Table 2) Moreover, breeders usually share breeding material and the tendency to use genetically similar parents in breeding programmes have led to a concern of lack of genetic diversity (Iqbal *et al*., 1997; Rehman *et al*., 2002). The need to broaden the genetic base of germplasm is an area of concern in modern agriculture. Pyramiding crosses are suggested to increase the genetic diversity in the population (Fouilloux & Bannerot, 1988) and will be helpful in developing improved wheat cultivars.

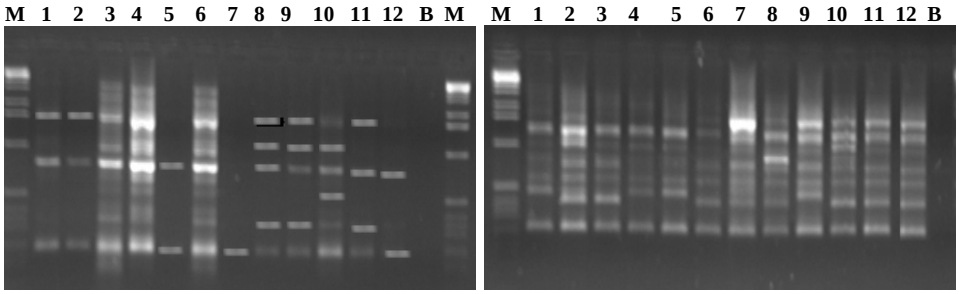

Fig. 1. Amplification profile of twelve wheat genotypes with primer A-09 and primer B-05 respectively by RAPD-PCR. M=1kb ladder, 1=Sarsabz, 2=Kiran-95, 3=Marvi-2000, 4=Bhittai, 5=Khirman, 6=ESW-9525, 7=Abadgar-93, 8=Inqilab-91, 9=SARC-1, 10=PKV-1600, 11=Chakwal-86, 12=CM24/87, B=Blank

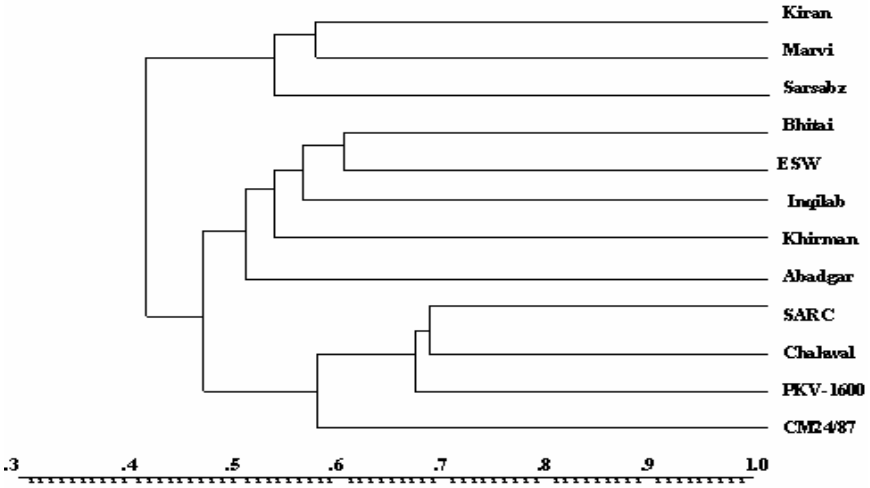

Fig. 2. Dendrogram of twelve wheat genotypes developed from RAPD data using unweight pair group method of arithmetic means (UPGMA).

The dendrogram (Fig. 2) constructed on the basis of the similarity matrix showed that the varieties of wheat studied could be divided into three groups. Kiran-95, Marvi-2000, Sarsabz were clustered in first group in the dendrogram showing more genetic similarity among each other. Bhitai, ESW-9525, Inqilab-91, Khirman and Abadgar-93 clustered in second group, were genetically close to each other. Third group was formed among SARC-1, Chakwal-86, PKV-1600 and CM 24/87.

The RAPD analysis has been found to be a valuable DNA marker system to evaluate genetic diversity. The information about genetic similarity will be helpful to avoid any possibility of elite germplasm becoming genetically uniform. Efficiency and speed of plant breeding programs can be accelerated by (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.

References

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

Astarini, A.I., A.J Plummer, A.R Lancaster and G. Yan. 2004. Fingerprinting of cauliflower cultivars using RAPD markers. *Aust. J. Agri. Res*., 55: 112-124.

Debasis, P. and P. Khurana. 2001. Wheat biotechnology: A minireview. *E.J.B ISSN*: 0717-3458.

Devos, K.M. and M.D. Gale. 1992. The use of random amplified DNA markers in wheat. *Theor. Appl. Genet*., 84: 567-572.

Fouillloux, G. and H. Bannerot. 1988. Selection methods in the common bean (*Phaseolous vulgaris* L.). In: *Genetic Resources of Phaseolous Bean.* (Ed.): P. Gepts. Kluwer, Dordrecht, pp. 503-542.

Gupta, P.K., R.K. Varshney, P.C. Sharma and B. Ramesh. 1999. Molecular markers and their application in wheat breeding. *Pl. Breed*., 118: 369-390.

Hemrick, J.L. and M.J.W. Godt. 1990. Alloenzyme diversity in plant species, In: *Plant Population Genetics, Breeding and Genetic Resources.* (Eds.): A.H.D. Brown, M.T. Clegg, A.L. Kahler, B.S. Weir. Sinauer Associates Inc., Sunderland MA, pp. 43-63.

Hoisington, D., N. Bohorova, N.S. Fennell, M. Khairallah, A. Pellegrineschi and J.M. Ribout. 2002. *Plant production and protection series*. Available from http://www.fao.org (FAO document respository).

Iqbal, M.J., N. Aziz, N.A. Saeed, Y. Zafar and K.A. Malik. 1997. Genetic diversity of some elite cotton varieties by RAPD analysis. *Theor. Appl. Genet*., 94: 139-144.

Loucou, V., K. Haurogne, N. Ellis and C. Rameau. 1998. Genetic mapping in pea. 1. RAPD based genetic linkage map of (*Pisum sativum* L.). *Theor. Appl. Genet*., 97(5-6): 905-915

Manifesto, M.M., A.R. Schlatter, H.E. Hopp, E.Y. Suárez and J. Dubcovsky. 2001. Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.,* 41: 682-690.

Migdadi, H.M., A.T. Majid and S. Masoud. 2004. Genetic diversity in some Aegilops species in Jordan revealed using RAPD. *PGR Newsletter, FAO-IPGRI,* 139: 47-52.

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.

Nei, N. and W. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*., 76: 5269-5273.

Peterson, A.H., S.D. Tanksley and M.E. Sorrells. 1991. DNA markers in crop improvement. *Adv. Agron*., 46: 39-90.

Rehman, M., D. Hussain and Y. Zafar. 2002. Estimation of genetic divergence among elite cotton cultivars genotypes by DNA fingerprinting technology. *Crop Sci*., 42: 2137-2144.

Souza, E., P.N. Fox, D. Byerlee and B. Skovmand. 1994. Spring wheat diversity in two developing countries. *Crop Sci*., 34: 774-783.

Tahir, M.S. 2001. *Reaction of different wheat* (*Triticum aestivum* L.) *genotypes in response to salt stress and genetic mapping of QTL for salt tolerance using AFLP markers*. Ph.D. Thesis Univ, Keil, Germany.

Tar'an, B., C. Zhang, T. Warkentin, A. Tullu and Vandenberg. 2005. A genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on molecular markers, and morphological and physiological characters. *Genome*, 48: 257-272.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids. Res*., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids. Res*., 18(22): 6531-6535.

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