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Full Length Research Paper

Study on genetic diversity in Pakistani wheat varieties using simple sequence repeat (SSR) markers

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Common wheat (*Triticum aestivum* L.) is a grass species, cultivated world wide. Globally, it is the most important human food grain and ranks second in total production as a cereal crop behind maize. Genetic diversity evaluation of germplasm is the basis of improvement in wheat. In the present study genetic diversity of 10 varieties of wheat (*T. aestivum*) were analyzed using 14 simple sequence repeat (SSR) primer sets. To estimate the genetic diversity among the genotypes, bivariate data matrix was generated and genetic distances were calculated using unweighted pair group of arithmetic mean (UPGMA) procedure. A high degree of genetic polymorphism was observed among the wheat varieties with average genetic distances ranged from 16 to 67%. SSR primer gdm-3, gdm-19, gdm-61, gdm-62, gdm-64, gdm-86, gdm-88, gdm 93-2a, 93-4b, gdm-13 and gdm-115 amplified 3, 2.9, 4.1, 4.7, 3, 1.7, 2.7, 3.7, 5.8, 4.1 and 1.4 loci per variety, respectively. Most diverse varieties of wheat were identified (Punjab-81 and Indus-79) and recommendations were made to utilize these varieties in future breeding program.

Key words: *Triticum aestivum* L., SSR markers, genetic diversity.

INTRODUCTION

In Pakistan wheat is grown on 8358 thousand hectares with a total production of 21.6 million/tons giving an average yield of 2.39 tons ha^{-1}. Wheat occupies 70% of Rabi and 37% of total cropped area of Pakistan. In NWFP, wheat is grown on 40% of the total cropped area. (MINFAL, 2004 - 2005). The major reasons for low productivity and instability of wheat yield in Pakistan include (i) constant threat to cultivars by continually evolving new pathogen races and agro-ecological stresses like drought, cold, heat, soil salinization and water logging and (ii) complicated and intensified wheat based cropping systems demanding varieties with very specific traits.

Although yield of wheat continued to increased, as new varieties came under cultivation and with improved agricultural husbandry involving the use of chemical fertilizers, still Pakistan has to import millions of tones of wheat every year. To increase wheat production in the country, it is a pre-requisite that local wheat germplasm should be improved by using new technologies especially biotechnology which is fast, easy and reliable way to improve crop species. In the past, main source of new and improved wheat varieties in Pakistan has been the International Wheat and Maize Research Centre (CIMMYT, Mexico). This resulted in narrow genetic base of wheat that caused limited genetic diversity. Wheat breeding through hybridization also requires the selection of diverse genotypes, which can only be obtained and utilized if gene pool of local germplasm has sufficient amount of genetic variability.

To improve genetic diversity of local germplasm, it is important to know the extent of already existing genetic variability in the material. To estimate genetic diversity in wheat germplasm, various kinds of markers can be used. In the past morphological traits were used as markers for assessing genetic diversity but these markers are often influenced by the environment, and are unreliable. However, DNA based markers have enhanced the utilization of biotechnology in crop improvement (Miller et al., 1989; Tanksley et al., 1989). These DNA markers, when tightly linked to genes of interest, can be used to select indirect-

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Table 1. Pedigree of ten Pakistani wheat varieties used for genetic diversity analysis using simple sequence repeat (SSR) primer sets.

Variety	Year of release	Parentage	Pedigree
C-228	1934	HARD FEDSRATION X 9D	N.A
Mexi-pak	1965	PJG2/GB55	1185G-6
SA-75	1975	NAIGG/CB/51//S949/S/MEXIPAK	N.A
Pavon	1978	VCM//CNO/7C/O/KAL/DD	CM8399-D-4M-3Y-1M-1Y-1M-0Y-0PAK
Indus-79	1980	BB/15-13-5/SON64	OA-2A
Punjab-81	1983	1N1A/3/SON64/PU/60(E) //SON	PAK6841-2A-1A-0A
Tandojam-83	1985	TZPP/PL/7C	CM5287-J-1Y-2M-2Y-3M-OY
Punjnad-88	1988	K4500.2/DGY	CM40480-25Y-2M-1Y-3M-1Y-0B-OPAK
Pasban-90	1991	INIA66/3/GEN	N.A
Kaghan-93	1993	TTR/JUN	CM59123-3M-1Y-2M-1Y-2M-2Y-OM-OPAK

tly for the desirable allele and this represents the simplest form of marker assisted selection (MAS). Molecular markers are also used to study the level of genetic diversity among different cultivars, closely related species, gene bank accessions, etc (Mullis, 1990; Erlich et al., 1991). Molecular markers have also been very valuable for improving the understanding of genome structure and function and have allowed the isolation of genes of interest through cloning (Devos and Gale, 1992).

Polymerase chain reaction (PCR) based assays are efficiently being used to study genetic diversity of commercial crops like wheat, barley, maize, etc (Cao et al., 1998; Sun et al., 1998). At present, SSR is one of most promising molecular markers which are able to identify or differentiate genotypes within a species. SSRs are ubiquitously interspersed in eukaryotic genomes and can find applications as highly variable and multi allelic PCR based genetic markers (Brown et al., 1996). The application of SSR techniques to finger print plant species was first reported by Akkaya et al., (1992). The high level of polymorphism and easy handling has made SSRs extremely useful for different applications in crop improvement (Stachel et al., 2000). The present research work was carried out in order to study genetic variation at DNA level among various Pakistani wheat varieties using chromosome specific simple sequence repeat (SSR) markers and to find genetically most diverse genotypes of wheat which can further be used in hybridization programs to create genetically diverse germplasm of local wheat.

MATERIAL AND METHODS

Plant material

Ten wheat varieties released in Pakistan during 1934 - 1993 were used to study level of genetic polymorphism using chromosome specific SSR DNA markers. Parentage and pedigree of the varieties is presented in Table 1. The plants were grown in the green house of the Institute of Biotechnology and Genetic Engineering, NWFP Agricultural University Peshawar, Pakistan.

DNA isolation

Two to three young fresh leaves were collected in the green house and were placed in the eppendorf tubes and subsequently dropped in the liquid nitrogen to freeze the tissue. In the laboratory, leaf material was crushed with knitting needle to make a fine powder. Five hundred micro-liter extraction buffer (1% SDS, 100 mM NaCl, 100 mM Tris, 100 mM Na2 EDTA, pH = 8.5 by HCl) was added to each eppendorf tube containing the crushed leaf material and was mixed well with knitting needle. Equal volume (500 µl) of phenol: chloroform: isoamyl alcohol (25:24:1) was added and tubes shaken until a homogenous mixture were obtained. Samples were then centrifuged at 5000 rpm for 5 min. The aqueous phase was transferred to a fresh tube. One tenth volume (50 µl) sodium acetate (PH = 4.8) and equal volume (500 µl) isopropanol was added in the tube and was mixed gently to precipitate the DNA. Samples were centrifuged at 5000 rpm for 5 min to make the DNA pellet. After pouring the supernatant, the pellet was washed with 70% ethanol. Pellet was dried at room temperature for an h and re-suspended in 40 microlitre TE buffer (10 mM Tris, 1 mM Na_2 EDTA, PH = 8.0). To remove RNA, DNA was treated with 40 microgram RNAse-A at 37°C for 1 h. After RNase treatment, DNA samples were stored at 4°C. For use in polymerase chain reaction (PCR), 1:5 dilution of DNA was made in double distilled deionized and autoclaved water (Weining and Langridge, 1991).

Polymerase chain reaction (PCR)

All 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, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase (Devos and Gale, 1992). Amplification conditions was 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 55°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 gel and visualized by staining with ethidium bromide under ultra-violet (U.V) light and photographed using gel documentation system “Uvitec”. 14 simple sequence repeat primer sets (SSRs) were used for estimation of genetic diversity at DNA level in 10 Pakistani wheat varieties. Sequence information of the SSR primer sets are given in Table 2.

Table 2. Oligoname/locus, sequence, repeat and melting temperatures of 14 SSR primer sets used for the study of genetic diversity of Pakistani wheat varieties.

Locus	Sequence forward	Sequence reverse	Repeat	Tm
xgdm -3-5D	GTGTGATGTTTGAATACGCA	GTATCTCGGTGATGCAAGCAA	GT (15)	55
Xgdm-19-1D	ATTGACAGCAGATGGCAGTG	GCGTTCGAGTGACTTCCAAT	CA (24) TA (7)	55
xgdm -33-A	TACGTTCTGGTGGCGDCTC	GGCTCAATTCAACCGTTCTT	CA (21)	60
Xgdm-61-4D	CGCACTTTTTACTAGGGGTC	TTCTTTGCGTGTGTGCGT	GT (12)	60
Xgdm-62-3D	GATCTTGAAGCACTCTTGGT	GAAAGCCGTCCACTGCC	CT (13)	55
Xgdm-64-3B	GTCTCTTGCGTACACAGGCC	CCGCTAGTGTTTGTGTTTG	TT (14)	60
Xgdm-86-2B	GGCGCTCCATTCAATGG	GGTCACCCTCTCCCATCC	TG (13)	60
xgdm87-2D	CCCAAGCCCCAATCTCTCTCT	AATAATGTGGCAGACAGTCTTGG	GT (4) CT (7)	60
xgdm 88-4A	AAGGACAAATCCCTGCATGA	TCCCACCTTTTTGCTGTAGA	CT (17)	60
Xgdm-93-2A	GGAGGCATGGCTACATCCTTC	AAAAGCTGCTGGAGCATACA	GA (17)	55
Xgdm-93-4B	GGAGCATGGCTACATCCTTC	AAAAGCTGCTGGAGCATACA	GA (18)	55
xgdm-109-5A	AAAGCTGCTCATCGTGGTG	GGTCCGCCTGACAGACC	GC (8)	55
xgdm-113-6B	AAAATGCCCTTCCCAC	ACCCATCTGATATTTTGCGG	GA (9)	55
Xgdm-115-5D	AAGGTAGGACGAGGGCATG	TTTCCATGTCCTATGCCCC	CA (15)	60

Tm = Melting temperature (Annealing temperature = Tm-5°C).

Statistical analysis

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 generated. Genetic distances were calculated using "Unweighted Pair Group of Arithmetic Means" (UPGMA) procedure described by Nei and Li (1979).

GD = 1-dxy/dx+dy-dxy

Where GD = Genetic distance between two varieties, dxy = Total number of common loci (bands) in two varieties, dx = Total number of loci (bands) in variety 1 and dy = Total numbers of loci (bands) in variety 2. The genetic dissimilarity coefficient matrix of 10 varieties of wheat based on 14 SSR primer sets using UPGMA method was used to construct dendrogram using computer program "Popgene" (http:www.ualberta.ca/~fyesh.fyesh).

RESULTS AND DISCUSSION

Simple sequence repeats (also termed as microsatellites) are small (2 - 6 bp) DNA motifs, highly conserved and distributed among the genomes of all higher eukaryotes. SSR have been used extensively for designing primer sets which are not only highly polymorphic but also species specific (Pestova et al., 2000). Genetic diversity plays an important role in crop improvement and was demonstrated through SSR markers. In the present study, 14 wheat specific SSR primer sets were used to detect and estimate genetic polymorphism based on co-dominant marker system and to find out most diverse varieties for future breeding programs. The highest number of alleles per locus was detected using SSR primer 93-4b showing 52 alleles with an average of 5.2 alleles per variety. The lowest allele number per locus among the homologous chromosomes was observed using SSR primer 115 and SSR primer set 86 showing a total of 14 and 17 alleles with an average of 1.4 and 2.1 alleles per variety, respectively. Average genetic distances ranged from 16 to 67%. Highest genetic distance 67% was observed for one comparison (Punjnad-88 and Indus-79) (Table 2 and 3). Cluster analyses show the genetic dissimilarity coefficient matrix of 10 Pakistani varieties based on the data of 14 SSR primer sets using UPGMA method and was used to construct dendrogram using computer program "popgene32" (Figure 1). The varieties were grouped in 4 main clusters (A, B, C and D) .Groups A and B consist of two varieties, while groups C and D consist of three varieties each. Based on the dendrogram analyses, Punjab-81 and Indus-79 were more distantly related among the group of 10 Pakistani wheat varieties (Figure 1).

Genetic diversity is the basis for genetic improvement. Knowledge of germplasm diversity has significant impact on the improvement of crop plants. Due to modern breeding, it has been suggested that genetic diversity in wheat has been increasingly narrowed (Frankel, 1970; Sears, 1981). Narrow genetic diversity is problematic in breeding for adaptation to biotic and a biotic stresses. Therefore it is necessary to investigate the genetic diversity in wheat germplasm in order to broaden the genetic variation in future wheat breeding. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers such as RAPD (Joshi and Naguen, 1993), AFLP (Barret and Kidwell, 1998) and SSR (Roder et al., 1995).

During present study 14 Simple Sequence Repeat (SSR) primer sets were used to characterize 10 Pakistani wheat varieties to know about the diverse varieties for future breeding programs to enhance wheat production. Microstallites displayed a high level of polymorphism in the present study. A total of 458 alleles were amplified.

Table 3. Average estimates of average genetic distances among 10 Pakistani wheat varieties using 14 chromosome specific simple sequence repeat (SSR) primer sets.

Punjab-81	Punjab-81	Pavon	S.A-75	C-228	Punjnad-88	Mexi-pak	Tandojam-83	Kaghan-93	Pasban-90
Pavon	0.30								
S.A-75	0.42	0.32							
C-228	0.30	0.16	0.32						
Punjnad-88	0.35	0.30	0.36	0.37					
Mexi-pak	0.46	0.40	0.30	0.35	0.35				
Tandojam-83	0.44	0.35	0.30	0.42	0.38	0.60			
Kaghan-93	0.40	0.30	0.37	0.37	0.30	0.48	0.38		
Pasban-90	0.49	0.39	0.35	0.41	0.35	0.43	0.41	0.37	
Indus-79	0.49	0.49	0.47	0.59	0.67	0.50	0.53	0.51	0.42

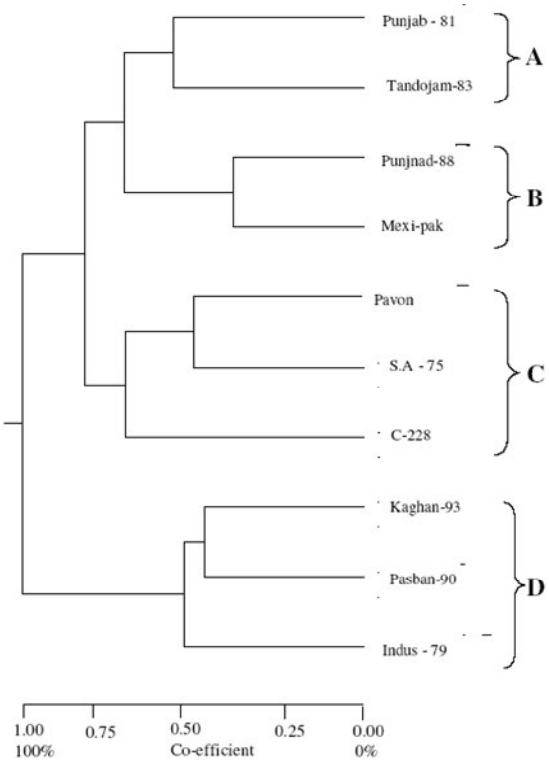

Figure 1. Dendrogram constructed for 10 wheat varieties using data obtained from 14 SSR primer sets.

The average number of alleles per-microstallite locus / variety was 3.4 alleles. It is recommended that diverse varieties Punjab-81and Indus-79 can be used in future breeding programs aimed at creating genetic variability in Pakistani wheat germplasm. The current data will enhance the breeding efficiency and will add the strength of marker assisted selection (MAS). In the light of information about the genetic diversity in 10 Pakistani wheat varieties, it is therefore suggested that the breeding programs with the help of DNA fingerprinting technology will be helpful to utilize the local varieties to produce cultivars / varieties by crossing them with different elite varieties.

REFERENCES

Akkaya MS, Bhagwat AA, Cregan PB (1992). Length polymorphism of simple sequence repeats DNA in Soybean. Genetic. 132: 1131-1139.

Barret BA, Kidwell KK (1998). AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci. 38: 1261-1271.

Brown SW, Szewe AK, Kresoviclw S (1996). Development and application of simple sequence repeat (SSR) loci for plant genome analysis. In; Janhar P.P (ed) methods of genome analysis in plants. CRC press, Bica Raton, florida. pp. 147-159.

Cao WG, Huel PSG, Chibbar RN (1998). Genetic diversity within spelta and Macha wheats based on RAPD analysis. Euphytica. 104: 181-189.

Devos KM, Gale MD (1992). The use of Randomly Amplified Polymorphic DNA markers in wheat. Theor. Appl. Genet. 84: 567-572.

Erlich A, Gelfand D, Sminsky JJ (1991). Recent advances in Polymerase Chain Reaction. Science. 252: 1643.

Joshi CP, Naguyen HT (1993). RAPD Analysis based intervarietal genetic relationships among wheat hexaploids. Plant Sci. 93: 95-103.

Miller PJ, Parfitt DE, Weinbaum SA (1989). Out crossing in Peach. Hort. Sci. 24: 359-360.

MINFALL, 2004-2005. Ministery of Food, Agriculture and Livestock Islamabad, Pakistan: 5-6.

Mullis KB (1990). Unusual origin of Polymerase Chain Reaction. Sci. Am. 262: 56-65.

Nei M, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76: 5269-5273.

Pestova F, Ganal MW, Roder MS (2000). Isolation and mapping of microstallite markers specific for the D-genome of bread wheat Genome 43: 689-697.

Roder MS, Plaschke J, Koing SU, Borner A, Sorrells ME, Tanksley SD, Ganal MM (1995). Abundance, variability and chromosomal location microstallites in wheat. Mol. Gen. Genet. 246: 327-333.

Sear ER (1981). Transfer of alien genetic material to wheat. In: evan LT, peacock WJ (eds) wheat science today and tomorrow Cambridge university press Cambridge 75-89.

Stachel M, Lelly T, Grausgruber H, Vollman J (2000). Application of microstallite in what for studying genetic differentiations caused by section for adaptation and use. Theor. Appl. Genet. 100: 242-248.

Sun QZ, Ni L, Gao J, Huang T (1998). Genetic relationships and diversity among Tibetan wheat, common wheat and European Spelt wheat revealed by RAPD markers. Euphytica. 99: 205-211.

Tanksley SD, Young ND, Paterson AH, Bonierable MW (1989). RFLP mapping in plant breeding: New tools for old science. Biotechnol. 7: 257.

Weining S, Langridge P (1991). Identification and mapping of polymorphism in cereals based on polymerase Chain reaction. Theor. Appl. Genet. 82: 209-216.