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DNA FINGERPRINTING STUDIES OF SOME WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

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Abstract

Random amplified polymorphic DNA (RAPD) analysis was employed to estimate genetic diversity/genetic similarity among nine wheat (*Triticum aestivum* L.) genotypes. Out of 25 random 10-mer primers surveyed, 18 primers detected polymorphism among all the wheat genotypes, while 7 primers produced monomorphic pattern. A total of 175 bands were amplified, 60.57% of which were polymorphic. Genetic similarity matrix was generated on the basis of Nei and Li's coefficients whose values ranged from 61.29% to 88.03%. The coefficients were used to make clusters using unweighted pair group method of arithmetic means (UPGMA). Wheat genotypes were grouped in two main clusters (A and B). However, CMH-771917/PKV1600 and VEE/Trap # 1 were the most distant genotypes, which were 61.29 to 76.53% and 66.01 to 78.81% genetically similar with the other genotypes, respectively. The information generated here along with the use of most advanced DNA fingerprinting techniques like AFLP, SCAR, SSR and ESTs would be helpful for future genome mapping programs as well as for the application of intellectual breeder rights in the country. The study will also work as indicator for wheat breeders to evolve varieties with diverse genetic background to achieve sustainability in wheat production in the country.

Introduction

Wheat (*Triticum aestivum* L.) is the world's most important cereal crop and due to its different uses and nutritive value it is the a staple food for more than one third of the world's population. Being the main staple food of rapidly increasing population of Pakistan, wheat occupies central position in the agricultural policies of this country. It contributes 12.5% to the value added in agriculture and 2.9% to GDP. For 2002-2003 the wheat production was 19.50 million tones, which does not seem to be compatible to the international level (Anon., 2003). Wheat production can be enchanced through the development of improved cultivars with wider genetic base capable of producing better yield under various agroclimatic conditions and stresses. Diverse genetic base buffers against the spread of diseases in advance cereals (Zhu *et al*., 2000).

In the recent past, concerted efforts have resulted in the development of new high yielding cultivars by exploiting Borlaug's wheat improvement program. This transformation created a problem of lack of genetic diversity (Tillman, 1998). Moreover, in Pakistan wheat genotypes is facing a dual menace of biotic and abiotic stresses. 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). Hence breeding wheat genotypes with diverse genetic base is a factor to achieve a level of self-sufficiency and sustainability.

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Although to monitor and maintain germplasm biodiversity morphological markers (Porter & Smith, 1982) and biochemical markers (Miller *et al*., 1989) have been used, but these markers are affected by environment and limited to a small number of loci (Tanksley *et al*., 1989). However, concerns regarding the conventional marker systems were overcome with the advent of DNA markers. Many genetic studies in eukaryotic and prokaryotic genomes have been facilitated by deploying DNA marker technology (Erlich *et al*., 1991). The use of DNA markers for characterization and identification of genotypes is essential for the early detection of true inter and intra-specific hybrids, parentage of a cultivar and patent protection (Benedetti *et al*., 2000). DNA markers not only allow the easy and reliable identification of clones (Devarumath *et al*., 2002), breeding lines, hybrids (Bastia *et al*., 2001) and cultivars (Mohanty *et al*., 2001) but also facilitate the monitoring of introgression (Paterson *et al.,* 2003) and the assessment of genetic diversity (Iqbal *et al.,* 1997, Mukhtar *et al.,* 2002, Rahman *et al.*, 2002) and relatedness among germplasm (Milligan, 2003).

In recent years, one of PCR-based DNA marker techniques, random amplified polymorphic DNA (RAPD) markers (Williams *et al*., 1990; Welsh & Mc Clelland, 1990) has been used extensively for the identification of genotype in crop plants (Yamagishi, 1995) and gained importance due to its simplicity, efficiency and non requirement of sequence information (Karp *et al*., 1997). It involves the use of a single arbitrary primer in a PCR reaction and results in the amplification of several discrete DNA products. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite segments, that are complementary to the primer and sufficiently close together for the amlification to work (Williams *et al*., 1990; Welsh & Mc Clelland, 1990; Jones *et al*., 1997). RAPD assay has been extensively applied to assess genetic diversity in the genus *Triticum* (Cao *et al*., 1998; Mukhtar *et al*., 2002, Spielmeyer *et al*., 2003) showing the narrow genetic base. RAPD markers have also been used for cultivar identification (Rahman & Zafar, 2001; Rahman *et al*., 2002), evaluation and characterization of germplasm (Bai *et al*., 2003; Mohapatra *et al*., 2003), verification of hybridity in interspecific crosses (Benedetti *et al*., 2000), for fingerprinting of genomes (Welsh & Mc Clelland, 1990), tagging of genes (Martin *et al*., 1991) and marker-assisted selection (MAS) (Rahman, 2002; Sixin & Anderson, 2003)

The present research work was conducted to evaluate genetic diversity and similarity among nine lines of wheat (*Triticum aestivum* L.), especially to see the genetic distance of Marvi-2000 (a candidate wheat line) with other wheat lines/varieties as a control. This will also be helpful in identification of genetically diverse parents and varietal classification. Further more, this information along with use of most advanced DNA fingerprinting techniques like AFLP, SCAR, SSR and ESTs would be helpful for future genome mapping and protection of Intellectual Breeder's Rights (IBRs) (Rahman & Zafar, 2001).

Materials and Methods

Plant material: Seeds of 9 wheat genotypes viz., Marvi-2000, SD-1200/14 and SD-4085/3, CMH-771917/PKV1600, RL6010/6*SKA, VEE/Trap # 1, Soghat-90, Sarsabz and Sunco were received from Nuclear Institute of Agriculture (NIA), Tandojam. Plants were grown in pots in a greenhouse at NIBGE, Faisalabad.

Total genomic DNA isolation: Total genomic DNA was isolated from 10-12 young leaves bulked from 5-6 different plants of each wheat line by CTAB method of DNA extraction (Rahman *et al.*, 2002). After RNase treatment, the DNA concentration was determined both by DyNA Quant 200 and by comparison with standard DNA, electrophoresed on a 0.8% agarose gel and the DNA was diluted in 0.1 X TE buffer to a concentration of 2 ng/ul for PCR analysis.

PCR and primers: For polymerase chain reaction (PCR), concentration of the total genomic DNA, 10 X PCR buffer without $MgCl_2$, gelatin, $MgCl_2$, dNTPs (dATP, dTTP, dGTP and dCTP), 10-mer random primer and *Taq* DNA polymerase were optimized. Deca-mer random oligonucleotide primers were purchased from Operon Technologies Inc. Alameda, Calif. USA. *Taq* DNA polymerase together with 10 X PCR buffer, $MgCl_2$ and dNTPs were from MBI Fermentas, while gelatin from Sigma, St-Louis, was used. PCR amplifications were carried out in a 25 ul reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 0.1 mM each of dATP, dTTP, dGTP and dCTP, 30 ng of primer, 0.001% gelatin, 6 ng of genomic DNA and 1 unit of *Taq* DNA polymerase. A total of 25 random decamer primers were surveyed for PCR amplification. The PCR profile used was: a first denaturation step of 5 minutes at 94^0C followed by 40 cycles of 94^0C for 1 min., 36^0C for 1 min. and 72^0C for 2 min. After the completion of 40 cycles the reactions were kept at 72 ^{0}C for 10 min. and then held at 4^0C until the reaction tubes were removed.

Analysis of RAPD data: The PCR products were electrophoresed on 1.2% agarose gels using 0.5X Tris Borate EDTA (TBE) buffer and visualised by ethidium bromide staining under UV light and photographed using EagleEye, Gel Documentation System (GDS). Nine wheat genotypes were compared with each other using their amplification profiles and bands of DNA fragments were scored as present (1) and absent (0). Genetic similarity matrix was generated on the basis of Nei and Li's (1979) coefficients. A dendrogram based on these similarity cofficients was constructed by using unweighted pair group method of arithmetic means (UPGMA).

Results and Discussion

To estimate genetic diversity among autogamous crop species, genotypes or cultivars must be true breeding. In earlier findings, an individual plant analysis was conducted of three wheat genotypes representing different breeding institutes (Inqulab 91, PARC-3 and 8881) by surveying 20 plants of each genotype with random amplified polymorphic DNA (RAPD) analysis, produced monomorphic amplification profiles with ten primers (Mukhtar *et al*., 2002). Hence, in the present studies, purity testing was not undertaken.

Genomic DNAs of the 9 wheat (*Triticum aestivum* L.) genotypes were amplified using 25 random decamer primers. A total of 18 primers detected polymorphism (Fig. 1) while 7 primers produced monomorphic pattern. A total of 175 bands were produced, with an average of 7 bands per primer. Out of these amplified products 106 (60.57%) were polymorphic. The level of polymorphism was varied with different primers. A maximum of 12 bands were amplified with primers OPA-03 and OPI-01 while a minimum of 4 fragments were amplified with different primers like OPA-12, OPI-02, OPI-06, OPI-07, OPI-08 and OPI-12. The size range of amplified products was 170 bp to 2.8 kb. Maximum number of bands (123) was amplified by the genotype SD-4085/3, while minimum number of bands (105) was amplified by the genotype SD-1200/14.

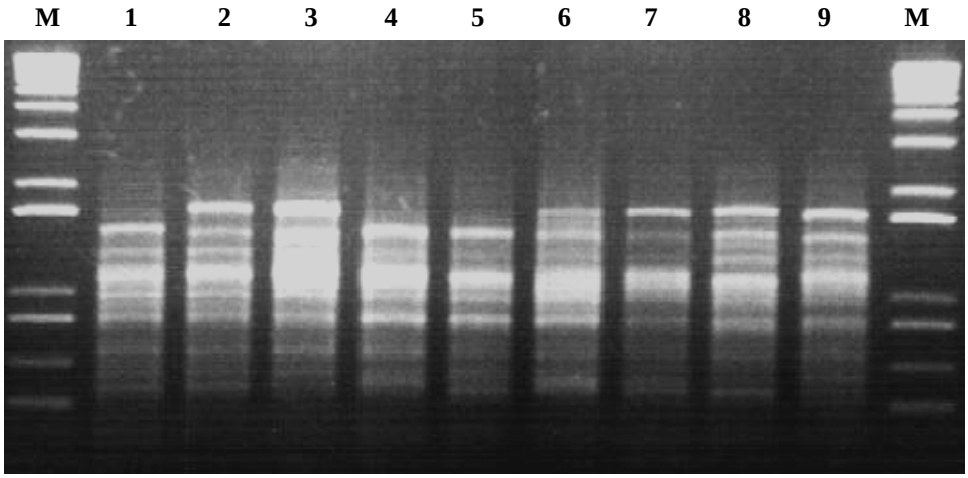

Fig. 1. Amplification profile of nine wheat genotypes with primer OPI-04 showing polymorphism. M= DNA marker, 1= Marvi-2000, 2= SD-1200/14, 3= SD-4085/3, 4= CMH-771917/PKV1600, 5= RL 6010/6*SKA, 6= VEE/Trap # 1, 7= Soghat-90, 8= Sunco and 9= Sarsabz.

The amplification of monomorphic loci is depicting sharing of common blood among the genotypes. In Pakistan, wheat cultivars and candidate lines have been developed by crossing indigenous genotypes with exotic parents or genotypes derived from exotic material mostly introduced from CIMMYT, Mexico. Selection of superior genotypes from segregating populations of such crosses have made a relatively small gene pool for all wheat cultivars in Pakistan (Mukhtar *et al*., 2002). Moreover, breeders usually share breeding material, so the tendency to the use of extensive similar parents in breeding programmes has led to a concern of lack of genetic diversity (Iqbal *et al*., 1997; Rahman *et al*., 2002). Similarly, breeders select more than one elite lines developed from the same cross, so the same breeding population would be used to select elite genotypes for future varietal improvement programs, which also results in close kinship. The need to broaden the genetic base of germplasm is an area of concern in modern agriculture. Conical crosses are suggested to increase the genetic diversity in the population (Fouilloux & Bannerot, 1988) that would be extremely helpful to create better segregants for improved wheat cultivars.

By using Nei & Li's (1979) coefficients similarity matrix (Table 1) were calculated to estimate genetic divergence and relatedness among wheat genotypes. Genetically most similar genotypes were Marvi-2000 and SD-4085/3 (88.03%), while genetically most dissimilar genotypes were CMH-771917/PKV1600 and Sarsabz (61.29%). Cluster analysis (Fig. 2) by the unweighted pair group method of arithmetic means (UPGMA) showed that the wheat genotypes can be clustered in two distinct groups A (similarity range 83.04 % to 88.03%) and B (similarity range 82.48% to 83.73%). However, the genotypes VEE/Trap # 1 (77.61%) and CMH-771917/PKV1600 (73.95%) were distantly related to these two groups. The information gathered here would be helpful in genome mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity.

Table 1. Similarity matrix for Nei and Li's coefficients of nine wheat genotypes

	A	B	C	D	E	F	G	H	I
A	1								
B	0.6635	1							
C	0.8803	0.6905	1						
D	0.6164	0.7188	0.6301	1					
E	0.6842	0.8236	0.6886	0.7653	1				
F	0.7762	0.7397	0.7806	0.6601	0.7399	1			
G	0.8193	0.7150	0.8535	0.6255	0.6760	0.7881	1		
H	0.6616	0.8373	0.6862	0.7085	0.8259	0.7409	0.7014	1	
I	0.8506	0.7257	0.8134	0.6129	0.7086	0.7688	0.8287	0.7171	1

A= Marvi-2000, B= SD-1200/14, C= SD-4085/3, D= CMH-771917/PKV1600, E= RL 6010/6*SKA, F= VEE/Trap # 1, G= Soghat-90, H= Sunco and I= Sarsabz.

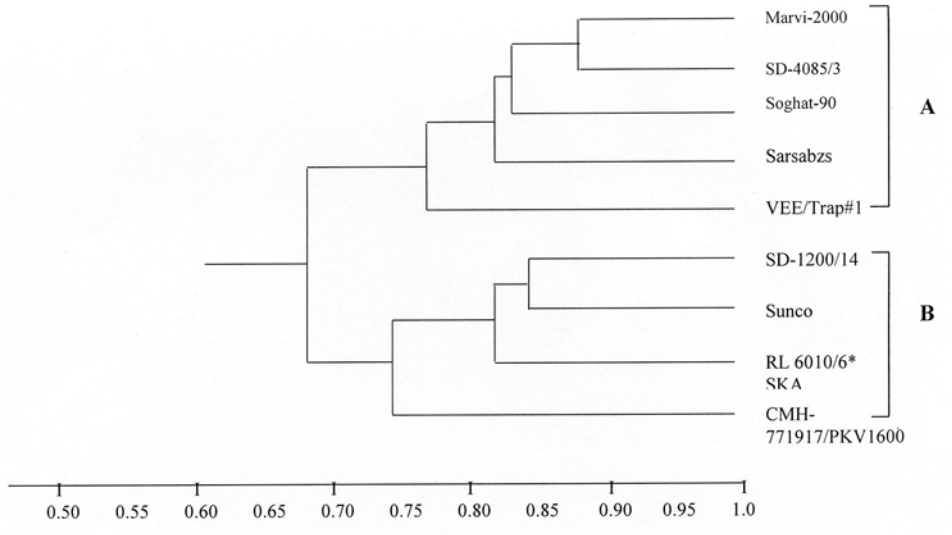

Fig. 2. Dendrogram of nine wheat genotypes developed from RAPD data using unweighted pair group method of arithmetic means (UPGMA).

The data obtained in this experiment confirmed the efficiency of the RAPD technique for determination and estimation of genetic distances and relatedness among different plant genotypes. The RAPD analysis has been found to a valuable DNA marker system to evaluate genetic diversity. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform. Because of the simple experimental procedures, the requirement of minimal amount of plant tissue and the possibility of automation (Terzi, 1997), RAPD analysis should be very useful in breeding for rapid and early identification of most diverse individuals in large seedling populations, allowing the detection of true to type genotypes for the improvement of our crop breeding programs. Keeping in view the useful information about the close genetic relationship, it is suggested that mission oriented breeding programs with the help of DNA fingerprinting technology will be helpful to produce distinct cultivars/ genotypes with diverse genetic background and improved productivity.

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