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CHARACTERIZATION OF WHEAT GENOTYPES USING RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS

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Abstract

Genomic DNA from the genotypes *Khyber-87, Saleem 2000, Suleman-96, Pir Sabak-2004, Pir Sabak-2005, NRL-0320, NRL–0517, RAS-II, Tatara, RAS-I, Bathoor, Fakhare Sarhad, Bakhtawar-94, Inqilab-91, Haider-2002, Noshera-96,* and *Auqab-2000* was amplified using RAPD primers. Loci of 100-1400 bp sizes were amplified and on average 3.51 loci per genotype were amplified. Average genetic diversity using the twelve RAPD primers ranged from 30-90%. Phylogenetic relationship among the wheat genotypes was carried out using dendrogram analysis. The seventeen genotypes were clustered in five groups. It was found that the genotypes Pirsabak 2004 and *Ras 1* were most distantly related to *Khyber-87.* It is suggested that these two genotypes be crossed with Khyber 87 for creating maximum genetic variations.

Introduction

Wheat is the world most important cereal grain. Wheat is the staple food for 35% of the world's population and is grown on about 17% of the cultivated area in world (Kronstad, 1998). It provides more calories and protein to human diet than any other crop. There is also more wheat and wheat flour moving in the world trade than any other grain. In Pakistan it is grown on about 9062 thousand hectares with an annual production of 23421 thousand tones giving an average yield of 2585 kg per ha which is far more less than its potential (Anon., 2008-2009). Its production can be enhanced through the development of improved varieties capable of producing better yield under various agroclimatic conditions and stresses (Inamullah *et al*., 2006). It is a general agreement that germplasm diversity among elite breeding material is the fundamental element in plant breeding (Mukhtar *et al*., 2002). Wheat production can be increased through the development of new varieties having wider genetic base capable of better performance under various agroclimatic conditions (Inamullah *et al*., 2005).

It is therefore important to develop our own indigenous breeding and selection programs for wheat improvement. To develop indigenous breeding program, an important pre requisite is the information about existing genetic diversity in the germplasm. Recent development in DNA technology, especially the generation of diverse molecular markers revolutionized the utilization of variation present in DNA base pairs (sequence) for improvement of crops of commercial importance like Wheat, *Brassica*, Maize and Rice, (Paterson *et al*., 1991).

Genetic diversity assessment through Polymerase Chain Reaction (PCR) is easy and fast than other systems and hence is widely used in crop improvement (Roeder *et al.,* 1995). Among the PCR based techniques Randomly Amplified Polymorphic DNA (RAPDs) is more user's friendly as it does not require any sequence information of the host cell genome (Williams *et al.,* 1991). RAPD based PCR assays have been used earlier (Chikaiza *et al.,* 2006; Czaplicki *et al.,* 2000; Hamza *et al.,* 2004; Mukhtar *et al.,* 2002) for the estimation of genetic diversity in wheat germplasm. During present study, Seventeen genotypes of common wheat were collected from Agricultural Research Institute of Bafa, Mansehra for the estimation of genetic variability at molecular level. Randomly Amplified Polymorphic DNA Primers were used to identify differences at DNA level among the genotypes. Statistical analyses were carried out to estimate genetic diversity and phylogenetic relationship among the genotypes included in this study.

Material and Methods

Plant material: Seventeen varieties of wheat viz., *Khyber-87, Saleem 2000, Suleman 96, Pir Sabak 2004, Pir Sabak 2005, NRL 0320, NRL 0517, RAS II, Tatara, RAS-1, Bathoor, Fakhare-Sarhad, Bakhtawar-94, Inqilab-91, Haider-2002, Noshera-96* and *Auqab-2000* were used for RAPD analysis.

Genomic DNA isolation: Genomic DNA was isolated from young leaf tissues using the method of Weining and Langridge (1992).

RAPD procedure: Genomic DNA was used as a template for PCR amplification using 12 randomly selected primers (Table 1). The amplification was performed in a system "Nyxtechnik ATC201"cycler. The PCR was carried out in 25µl volume containing 50 ng of samples DNA, 1X PCR buffer, 3.4 mM $MgCl_2$, 10 mM each dNTP, 2 µM of each primer and 1µ Taq DNA polymerase.

The reaction was done under the following cycling conditions: 94°C for 4minutes, 40 amplification cycles and each with 94°C for 1minute (denaturation), 28°C for 1 minute (annealing), 72°C for 2minute (polymerization) and final extension at 72°C for 10minutes and a final 4°C hold.

Electrophoresis of the amplified PCR products were carried out using 1% (w/v) agarose gels and photographed by gel documentation system.

Statistical analysis: Every band was considered as a single locus. All the scorable Loci were considered for generation of bivariate 1-0 data matrix and genetic distances (GD) among the genotypes were estimated using Unwaited Pair Group of Arthematic Means (UPGMA) as described by Nei & Li (1979) and for estimation of genetic diversity dendrogram was constructed using the software Pop gene 32.

Table 1. Sequence of RAPD primers used.

S. No.	Primer name	Sequence (5'-3')	Molecular weight	GC contents
1.	GLA-13	CAGCACCCAC	2.942	70
2.	GLA-17	GACCGCTTGT	3.019	60
3.	GLA-20	GTTGCGATCC	3.035	60
4.	GLB-06	TGCTCTGCCC	2.955	70
5.	GLB-11	GTAGACCCGT	3.028	60
6.	GLB-12	CCGGTACGCA	2.988	60
7.	GLB-15	GGAGGGTGTT	2.722	60
8.	GLB-17	AGGGAACGAG	3.126	60
9.	GLB-20	GGACCCTTAC	2.988	60
10.	GLC-11	AAAGCTGCGG	3.013	60
11.	GLC-15	GACGGATCAG	3.077	70
12.	GLC-19	GAGCTACAGG	3.055	60

Results

RAPD analysis of seventeen wheat genotypes was carried out using twelve randomly selected primers (Table 1). Loci of 100-1400 bp were amplified using twelve RAPD primers and average 3.51 loci per genotype were amplified (Fig. 1). The average genetic distance estimated among seventeen genotypes ranged from 30%-90%. Maximum average genetic distance was estimated for Khyber-87 with Pir sabak 2004 and Ras I.

The results obtained from PCR amplification of twelve RAPD primers was used for estimation of phylogenetic relationship among the genotypes of wheat. Dendrogram was constructed using the software Popgene ver. 32 (Fig. 2). The seventeen genotypes of wheat were grouped into 5 clusters "A", "B", "C', "D" and "E" comprising 7, 3, 2, 3 and 2, genotypes respectively. It was found that Khyber- 87was most distantly related to Ras 1 and Pirsabak 2004. It is therefore recommended that these 2 genotypes (Pirsabak 2004 and Ras 1) should be crossed with Khyber-87 for generation of maximum genetic diversity and selection of transgressive segregants.

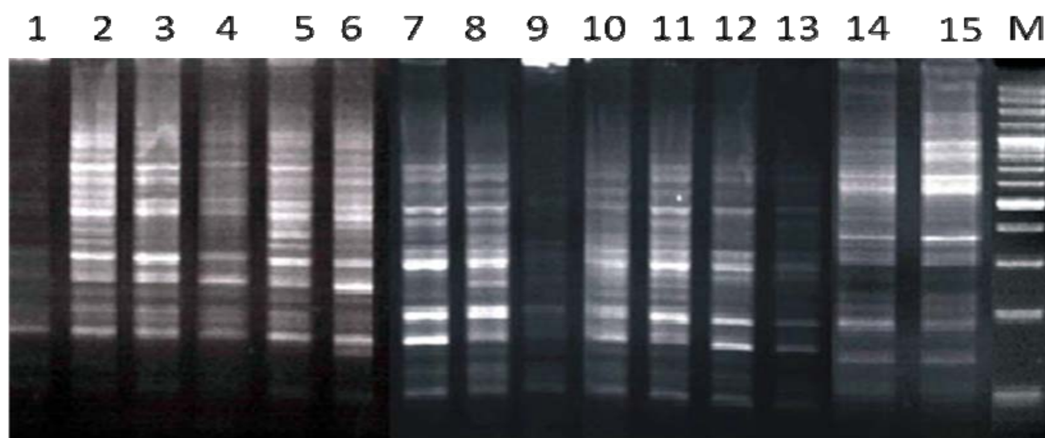

Fig. 1. Amplification profile of wheat genotypes using RAPD primer GLA-17, Khyber-87 (1), Saleem 2000 (2), Suleman-96 (3), Pir Sabak-2005 (4), NRL-0320 (5), NRL–0517 (6), RAS-II (7), Tatara (8), Bathoor (9), Fakhare Sarhad (10), Bakhtawar-94 (11), Inqilab-91 (12), Haider-2002 (13), Noshera-96 (14), Auqab-2000 (15) and Marker (M) Fermentas, Cat # SM0323.

Discussion

Estimation of genetic diversity is a prerequisite for improvement of any species through genetic means. Various procedures have been utilized in the past for the estimation of genetic diversity in various plants and animal species of commercial importance. With the recent introduction of DNA technology, Marker Assisted Selection (MAS) for the estimation of genetic diversity and selection of suitable genotypes has been utilized extensively. The PCR based Randomly Amplified Polymorphic DNA (RAPD) has an extra advantage that it does not require any sequence information.

Genomic DNA isolated from seventeen wheat genotypes was amplified using twelve RAPD primers i.e., GLB-06, GLB-15, GLB-11, GLC-15, GLA-20, GLA-13, GLB-20, GLC-11, GLC-19, GLB-17, GLA-17and GLB-12. Loci of 100-1400 bp size were amplified and on average 3.51 loci per genotype were amplified. Mukhtar *et al.,* (2002); Hamza *et al.,* (2004) and Fu *et al.,* (2006) have also reported similar results using PCR based assays while working on wheat.

Phylogenetic relationship among the wheat genotypes was also studied using dendrogram analysis. The seventeen genotypes were grouped into five clusters "A", "B" "C'' "D" and "E" comprising of 7, 3, 2, 3 and 2 genotypes, respectively. Groups A, B, C, D, and E, comprised (Khyber-87, NRL 0320, NRL 0517, Bathoor, Fakhare-Sarhad, Pir Sabak 2005 and Tatara), (Saleem 2000, Suleman 96 and Ras II), (Bakhtawar-94 and Noshera-96), (Inqilab-91, Haider-2002 and Auqab-2000), (Pir Sabak 2004 and Ras 1) respectively. It was noted that Khyber-87 was most distantly related *Ras 1* and Pirsabak 2004.

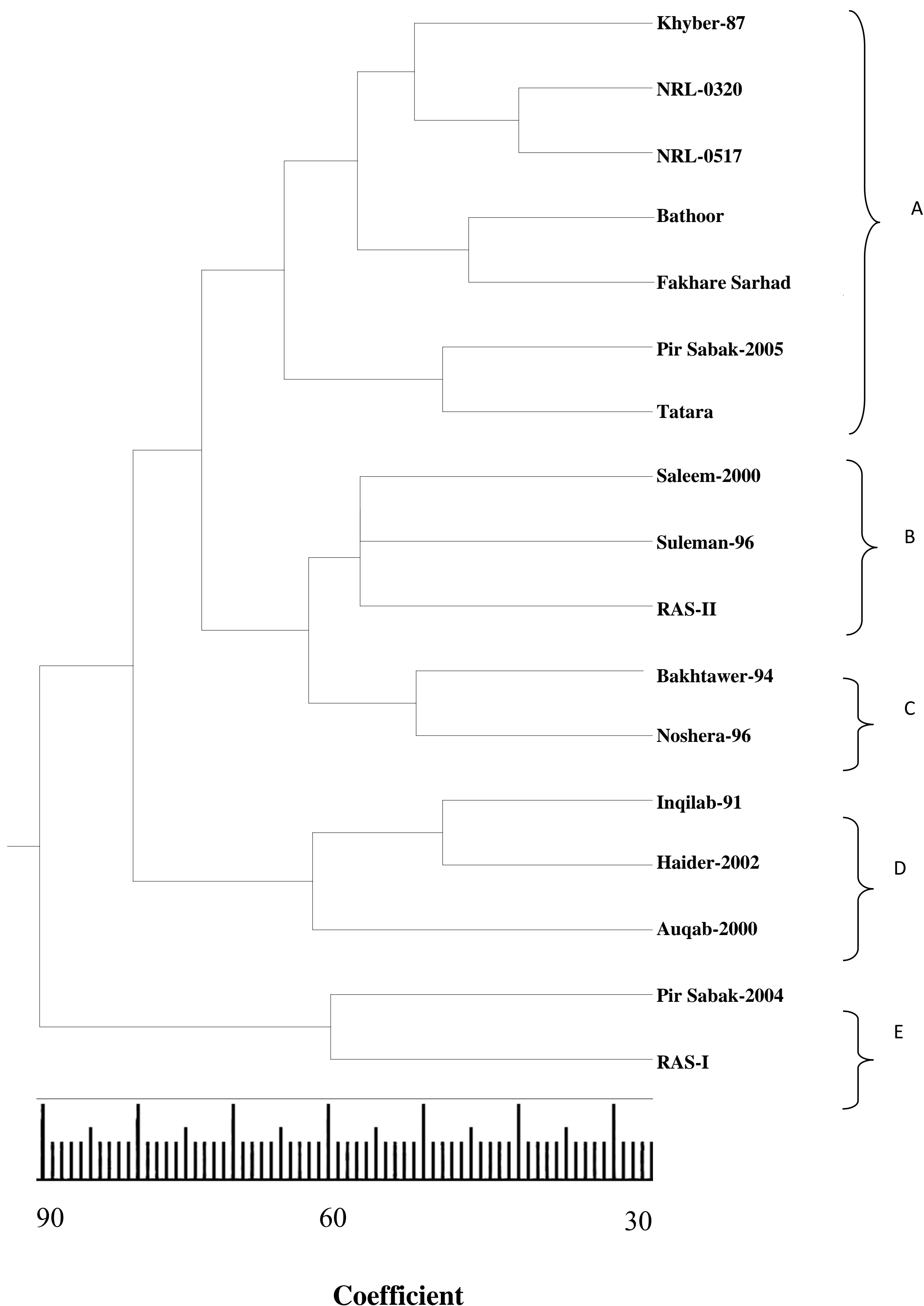

Fig. 2. Dendrogram for seventeen genotypes using the data from RAPD primers GLB-06, GLB-15, GLB-11, GLC-15, GLA-20 GLA-13, GLB-20, GLC-11, GLC-19, GLB-17, GLA-17 and GLB-12.

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