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EVALUATION OF GENETIC DIVERSITY IN DIFFERENT PAKISTANI WHEAT LAND RACES

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Abstract

Wheat is one of the main sources of nutrition worldwide. Genetic improvement of the seed makes wheat a source of high quality flour for human consumption and for other industrial uses. With the help of molecular markers, the available germplasm of wheat can be assessed for future breeding programs. Therefore, the aim of the present work was to analyze the genetic diversity among 15 Pakistani wheat land races based on Random Amplified Polymorphism DNA (RAPD) markers. A total of 284 DNA fragments were amplified, ranging in size from 200bp to 1100bp by using six primers. The number of DNA fragments for each primer varied from 2 (OPC-6) to 9 (OPC-8) with an average of 6 fragments per primer. Out of 284 amplified products, 120 were monomorphic and 137 were polymorphic showing an average of 7.8% polymorphism per primer. One specific marker was detected both for OPC-1 and OPC-8, two for OPC-5, while no RAPD specific marker was detected for the remaining primers. The genetic similarity index values ranged from 0.36 to 0.93, with an average of 0.64. Maximum genetic similarity (91%) was observed between Sur bej (3) and Khushkawa (11). On the contrary, minimum genetic similarity (32%) was observed in Khushkaba-1 (5) and Khushkawa (11). The dendrogram resulting from the NTSYS cluster analysis showed that the studied genotypes are divided into two main clusters from the same node. The first cluster contained 13 land races, while the second cluster contained only 2 land races. The dendrogram clustered the genotypes into 5 groups and showed efficiency in identifying genetic variability. These results indicated the usefulness of RAPD technique in estimating the genetic diversity among wheat genetic resources.

Introduction

Wheat (*Triticum aestivum* L.) production in Pakistan has a central position in agricultural policies because it is a staple food and supplies 72% energy and protein in the average daily diet in Pakistan (Khalil, 2006). Globally it is cultivated on approximately 17% of the world's cultivable land (Jones, 2005) accounting for over one quarter of total cereal production. Annual wheat production in Pakistan was recorded 23.2 million tons in 2006–07 which now stands at about 24 million tons in 2008–09 (Anon., 2009). Being a staple food for Pakistani population, development of high yielding and disease resistant wheat cultivars is a main thrust of breeders in the country (Asif *et al*., 2005).

Utilization of diverse genetic resources is paramount in the genetic improvement of wheat, as it results in increase in its potential for grain yield. Characterization of genetic diversity and genetic relatedness is a fundamental element in crop improvement strategies (Zhu *et al*., 2000). The genetic diversity of wheat has been studied in morphological and agronomic traits or pedigree information (Bernad *et al*., 1998). A number of methods are currently used for analysis of genetic diversity in germplasm accessions, breeding lines and segregating populations. These method were based on pedigree, morphological, agronomic performance, biochemical and molecular (DNA-based) data (Mohammadi &

Prasanna, 2003). Morphological traits can be used to evaluate genetic diversity but are often influenced by environmental factors, while the expression of molecular markers being the direct product of genes is not influenced. Further by using molecular markers, a large number of germplasm lines can be characterized in a short period of time and reflect more accurate genetic variability (Perry & Mclntosh, 1991; Masood *et al*., 2000).

Like other crops, the first step in the wheat improvement is the complete assessment of the local materials including collection, evaluation and molecular characterization of available germplasm. Knowledge about germplasm diversity and genetic relationships among wheat breeding materials could be valuable for making crop improvement strategies (Mohammadi & Prasanna, 2003). The diversity patterns allow plant breeders to better understand the evolutionary relationships among accessions and to incorporate useful genotypes in the breeding programs (Thompson *et al*., 1998). According to Zhou *et al*., (1995) the wheat germplasm by means of DNA fingerprinting techniques provides a tool for precise germplasm identification and a quantitative estimate of genetic diversity. The level of molecular variation in durum wheat, as observed in common wheat is low (Figliuolo *et al*., 2007). Similarly, Iqbal *et al*., (2007) estimated the degree of genetic divergence in 7 wheat genotypes (6 exotic genotypes and one local variety) through 15 random primers with an average of about 7.4 bands per primer.

RAPD markers were used as a powerful tool for the generation of potential fingerprinting diagnostic markers for cultivars (Matos *et al*., 2001; Fernandez *et al*., 2002). The use of molecular markers (RAPD) are routine methods for quickly and efficiently estimating relationships between lines and populations of many plant species. It is assumed that these markers are randomly spaced throughout the genome (Mark *et al*., 1999). In this study the random amplified polymorphic DNA (RAPD) technique has been used to explore genetic diversity among wheat land races and to identify RAPD primers useful to study genetic diversity.

Material and Methods

Seeds of 15 wheat land races were arranged from National Agriculture Research Centre (NARC) Islamabad, Pakistan. The local names, accession numbers and other information of these land races are mentioned in Table 1.

Table 1. Status, accession numbers and local names of fifteen wheat land races.

Accession	Local name	Origin	Province	District	Altitude	Longitude	Latitude
Pak-16342	Kanak	Pakistan	Sindh	Dadu	0040	67 49	26 47
Pak-16344	Kholum	Pakistan	Balochistan	Quetta	1680	68 58	30 12
Pak-16355	Sur bej	Pakistan	Balochistan	Quetta	1870	67 09	30 05
Pak-16359	Sur Ghanum	Pakistan	Balochistan	Quetta	1870	67 09	30 05
Pak-16357	Khushkaba-1	Pakistan	Balochistan	Quetta	1400	66 54	30 18
Pak-16360	Sufaid Ghanum	Pakistan	Balochistan	Quetta	1400	66 54	30 18
Pak-16361	Bali Ghanum	Pakistan	Balochistan	Quetta	1400	66 54	30 18
Pak-16362	Ghanum	Pakistan	Balochistan	Quetta	1340	66 56	30 23
Pak-16372	Khushkaba-2	Pakistan	Balochistan	Kharan	1400	66 25	29 40
Pak-16376	Sur Ghalla	Pakistan	Balochistan	Kalat	1560	66 35	29 21
Pak-16377	Khushkawa	Pakistan	Balochistan	Kalat	1680	66 39	29 25
Pak-16432	Ghom	Pakistan	Northern Areas	Gilgit	1350	74 25	35 50
Pak-16434	Desi Ghanum	Pakistan	Northern Areas	Gilgit	1300	74 35	35 40
Pak-16877	Gandam	Pakistan	Punjab	Chakwal	0490	72 20	33 15
Pak-16494	Ghandam	Pakistan	NWFP	Swat	1610	72 73	34 36

Isolation of genomic DNA and RAPD-PCR: The seeds of each variety were germinated in growth chamber for 2 weeks at 32ºC. Total genomic DNA was extracted from fresh leaves by CTAB method described by Richards (1997), with few modifications. The quality of the genomic DNA was checked by running it on 1% agarose gel, while concentration was estimated by spectrophotometric analysis.

PCR reactions were conducted by using 10 arbitrary decamer primers of OPC series (OPC1-10). Out of 10, six primers have given score-able banding profiles. The sequence of these 6 primers was OPC1: TTCGAGCCAG, OPC2: GTGAGGCGTC, OPC4: CCGCATCTAC, OPC5: GATGACCGCC, OPC6: GAACGGACTC and OPC8: TGGACCGGTG. Amplification reaction mixture was prepared for each sample, containing 1µl (50 ng) of template, 10.5 µl of water nuclease-free, 12.5 µl of PCR master mix (MBI Fermentas), and 1µl (25pm) of primer. After different optimization reactions, denaturation at 94ºC for 5 minutes followed by 40 cycles of denaturation at 94ºC for 1 minute, annealing at 36ºC for 30 seconds and extension at 72ºC for 2 minutes proved to be more suitable for amplification reactions. Final cycle was same except extension for 7 minutes at 72ºC. After that, PCR contents were held at 4ºC till use. 1.5% agarose gel prepared in 0.5x TAE buffer was subjected to electrophoresis at 70 volts for approximately 45 minutes. The gel was stained for 30 minutes with Ethidium bromide and visualized using Wealtec Dolphin Doc plus gel documentation system. The molecular weight of amplified fragments was estimated with the help of 100bp plus (MBI, Fermentas) DNA ladder.

Statistical analysis: Polymorphic bands from RAPDs were individually identified by their specific migration rates in the electrophoretic analyses. Once bands were properly and distinctively identified, binary (0/1) matrices were constructed to compare the patterns. A dendrogram was constructed on the basis of the similarity index by Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc) version 2.02 (Rohlf, 1997).

Results and Discussion

Screening of RAPD primers: The selected primers should present a high number of bands and/ or to produce discriminative and reproducible patterns among the samples under study. Ten primers of OPC series (OPC1-OPC10) were tested during the screening of the RAPD primers. The primers OPC1, OPC2, OPC4, OPC5, OPC6 and OPC8 gave clear and reproducible banding pattern.

RAPD profile: Ten RAPD primers were applied to 15 land races of wheat for DNA amplification. The results showed different primers generated variable numbers of fragments with different lengths of DNA amplified products (Table 2). The six random decamer primers generated a total 284 amplification product of which 37 (48%) were found to be polymorphic with an average of 7.8% polymorphism per primer. Maric *et al*., (2004) reported that RAPD markers can show a high level of polymorphism among the cultivars and the breeding lines. The size of amplified products varied between 190bp (generated with OPC2) to 1100bp (generated with OPC1, OPC5 and OPC8). Maximum cumulative numbers of 77 bands were amplified with OPC2 and generated highest number of polymorphic fragments (59) in all land races. RAPD markers showed high level of polymorphism among the wheat varieties of Pakistan. Bhutta *et al*., (2005) used RAPD technique to estimate the genetic divergence in 7 wheat genotypes from a diverse location of Pakistan.

Table 2. Number of bands amplified and polymorphism as revealed by RAPD.

S. No.	Primers	Total bands	Monomorphic bands	Polymorphic bands	Rare bands	Specific bands	Polymorphism %
1	OPC-1	40	15	20	4	1	50%
2	OPC-2	77	15	59	3	0	77%
3	OPC-4	57	45	12	0	0	21%
4	OPC-5	50	30	16	2	2	32%
5	OPC-6	14	0	0	0	0	0%
6	OPC-8	46	15	30	0	1	65%
All primers		**284**	**120**	**137**	**9**	**3**	**48.20%**

Table 3. RAPD based genetic similarities within groups.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.71	1.00													
3	0.84	0.75	1.00												
4	0.56	0.53	0.65	1.00											
5	0.84	0.68	0.75	0.65	1.00										
6	0.65	0.68	0.68	0.78	0.62	1.00									
7	0.78	0.68	0.93	0.59	0.68	0.62	1.00								
8	0.71	0.75	0.75	0.78	0.75	0.75	0.68	1.00							
9	0.71	0.50	0.75	0.59	0.75	0.56	0.75	0.62	1.00						
10	0.68	0.78	0.78	0.75	0.71	0.78	0.71	0.84	0.59	1.00					
11	0.78	0.68	0.93	0.65	0.36	0.68	0.87	0.68	0.68	0.71	1.00				
12	0.65	0.68	0.81	0.59	0.68	0.62	0.87	0.68	0.68	0.65	0.75	1.00			
13	0.71	0.75	0.81	0.53	0.68	0.52	0.75	0.75	0.62	0.78	0.75	0.68	1.00		
14	0.78	0.81	0.87	0.53	0.68	0.62	0.87	0.75	0.62	0.78	0.81	0.81	0.87	1.00	
15	0.71	0.68	0.87	0.59	0.62	0.75	0.81	0.75	0.62	0.71	0.87	0.75	0.75	0.81	1.00

1: Kanrak, 2: Kholum, 3: Surbej, 4: Sur Ghanum, 5: Khushkaba-1, 6: Sufaid Ghanum, 7: Bali Ghanum, 8: Ghanum, 9: Khuskaba-2, 10: Sur Ghalla, 11: Khushkawa, 12: Ghom, 13: Desi Ghanum, 14: Gandam, 15: Ghandam.

The primer OPC1 generated a specific fragment of 350bp, observed only in Sur Ghanum. Similarly OPC1 showed a common band of 500bp in Khushkaba-2 and Desi Ghanum which is absent in rest of land races. Amplification with OPC8 in Kanrak showed one unique band with molecular weight of 690bp. These results are in agreement with those of Guadagnuolo *et al*., (2001) who reported that RAPD can produce a large set of markers, which can be used for the evaluation of genetic variation both between and within species. In another study Cao *et al*., (2005) observed that the RAPD markers are useful in pedigree assessment of common wheat and for the identification of wheat varieties.

Dendrogram analysis: Similarity indices were developed on the basis of the obtained amplified fragments of the 15 wheat genotypes using 6 RAPD primers (Table 3). The genetic similarity values ranged from 0.36 to 0.93 with a mean of 0.64. These results agreed with those of Mandoulakaui *et al*., (2003) who reported that similarity coefficient ranged from 0.40 to 0.91 with an average of 0.64 and the dengrogram indicated two main clusters. The highest value (91%) was found between Sur bej (3) and Khushkawa (11), while the lowest genetic similarity (32%) was observed between Khushkaba-1 (5) and Khushkawa (11). On the contrary based on the dendrogram analyses, Kanrak (1) and Sufaid Ghanum (6) most distantly related to one another. It was observed that these land races may have originate more or less at equal interval of time but proceeded in opposite directions and have maintained their genomic integrity. The dendrogram revealed that 15 genotypes can be divided into two distinct clusters CI and CII (Fig. 2).

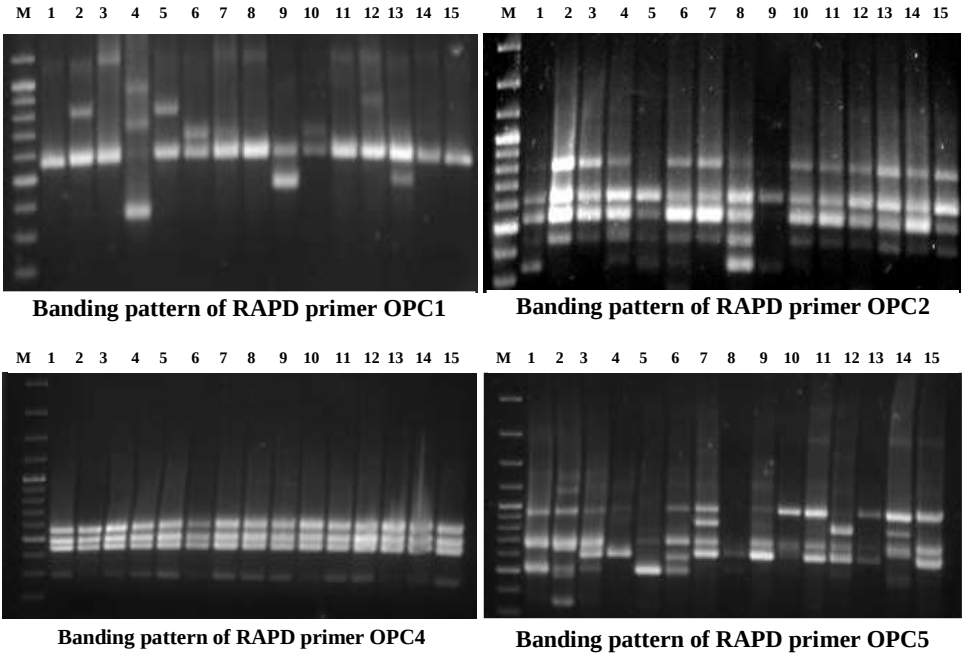

Fig. 1. RAPD amplification profiles of fifteen wheat land races with different primers. M: DNA ladder marker, 1: Kanrak, 2: Kholum, 3: Surbej, 4: Sur Ghanum, 5: Khushkaba-1, 6: Sufaid Ghanum , 7: Bali Ghanum, 8: Ghanum, 9: Khuskaba-2, 10: Sur Ghalla, 11: Khushkawa, 12: Ghom, 13: Desi Ghanum, 14: Gandam, 15: Ghandam.

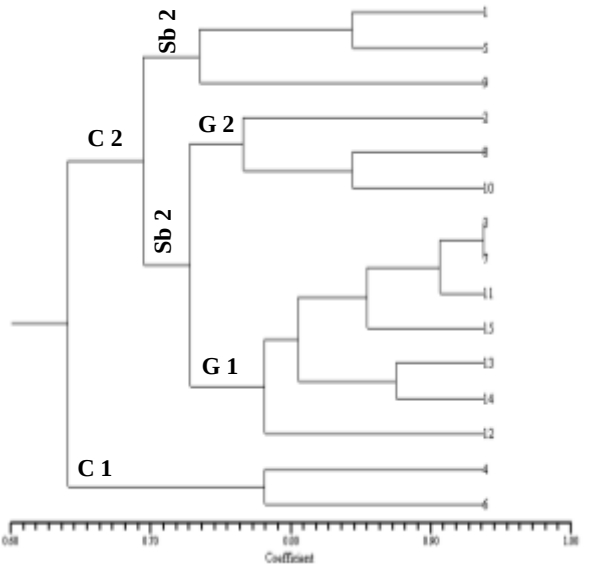

Fig. 2. Dendrogram representing the genetic relationships among fifteen wheat land races using NTYSYS clustral analysis generated from six RAPD primers. C: Cluster, Sb: Sub cluster, G: Group.
1:Kanrak, 2: Kholum, 3: Surbej, 4: Sur Ghanum, 5: Khushkaba-1, 6: Sufaid Ghanum, 7: Bal Ghanum, 8: Ghanum, 9: Khuskaba-2, 10: Sur Ghalla, 11: Khushkawa, 12: Ghom, 13: Desi Ghanum, 14: Gandam, 15: Ghandam.

Cluster-I (CI) comprised of two land races Sur Ghanum and Sufaid Ghanum which had 79% similarity level and appeared into two sub clusters, whereas all other land races appeared in another cluster. Cluster-II further showed divergence at 70% similarity level and formed two sub clusters. Sub cluster-I (sbI) had two groups. Group-I had six land races and showed similarity level up to 79%. Sur bej and Bali Ghanum showed highest level of similarity (95%) while Group-II consisted of three land races (Ghanum, Kholum and Sur Ghalla). In Group-II Kholum showed divergence at 78% similarity level and appeared separately. Sub cluster-II (sbII) consisted of three land races (Kanrak, Khushkaba and Khuskaba). Kanrak and Khuskaba showed close genetic relatedness.

The results showed that RAPD markers are useful for exploring germplasm diversity which is raw material for developing new varieties. In this study, cluster analysis grouped the accessions into two main clusters (63% similarity), with four accessions remaining ungrouped. The information on genetic similarity will be useful to establish a germplasm information bank of wheat landraces. Based on the observed patterns of variation, it is concluded that variation is present in these 15 wheat land races which suggested their suitability to be used in wheat breeding programs.

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