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ESTIMATION OF GENETIC VARIABILITY AMONG ELITE WHEAT GENOTYPES USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

SAJIDA BIBI[1], IMTIAZ A. KHAN[1], M. UMAR DAHOT[2], ABDULLAH KHATRI[1], M. H. NAQVI[1], M. AQUIL SIDDIQUI[1], SHAFQAT YASMEEN[1] AND NIGHAT SEEMA[1]

[1]*Plant Breeding and Genetics Division, Nuclear Institute of Agriculture, Tando Jam, Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan.*

Abstract

Twenty four wheat varieties/lines were assessed through RAPD for genetic diversity. Of forty primers, thirteen were able to amplify the genomic DNA and yielded 269 polymorphic bands. The percentage of the polymorphic loci was 86.22%. Nei's genetic diversity *(h)* ranged from 0.248 to 0.393, with an average of 0.330. Shanon's index (*I*) ranged from 0.382 to 0.567, with an average of 0.487. The proportion of genetic variation among the populations (*D*s) accounted for 28.58 % of the whole gentic diversity. The level of gene flow (Nm) was 1.25. Some specific RAPD bands were also identified, variety C-591, and QM-4531 contain a specific segment of 4.9kbp. Whereas SARC-1 and PKV-1600 amplified a specific DNA segment with primer A-09. Marvi-2000 contains two specific segments of 3.2kb and 200bp amplified with primer B-07. Genetically most similar genotypes were C-591 and Pasban-90 (76%) and most dissimilar genotypes were Rawal-87 and Khirman (36.1%). On the basis of results, 24 wheat varieties under study could be divided into 'two' groups and five clusters 'A' to 'E'.

Introduction

Every year a number of plant cultivars are released particularly for crops of world wide economic importance, such as hexaploid wheat (*Triticum aestivum* L.). Breeders usually select the new genotype mainly on the basis of morphological and biochemical markers. Morphological traits are the byproduct of gene and environmental interactions. The degree of gene expression is highly influenced by the conduciveness of the environment and genetic background in which gene is present. This difference is not actually by the genetic changes and breeder who is doing selection for such traits may do wrong selection on the basis of phenotypic traits (Kumar *et al*., 1998; Astarini *et al.*, 2004). Due to lack of the information, breeders have been using genetically similar parents extensively in a breeding programme leading to a narrow genetic base (Fouilloux & Bannerot, 1988; Xia *et al*., 2004; Rehman *et al*, 2002). DNA marker is a tool to provide the exact information of the genotype. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion (Manifesto *et al*., 2001). Thus, molecular markers generally detect more variability than other markers because they are not influenced by the environment.

The most common molecular markers are restriction fragment length polymorphism (RFLP) and marker based on polymerase chain reaction such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP). The genetic variability estimated through such markers distributed throughout the genome should (in principle) provide a mean to predict hybrid performance prior to making and evaluating the actual cross (Vienne *et al*., 1992). Molecular markers have been used to directly assess, the genetic diversity and/or genetic distance between parental genotypes of wheat (Sasanuma *et al*., 1996; Mayburg *et al*., 1997; Sun *et al*., 1998), Lens species (Ahmed & Mc Neil, 1996), *Alium cepa* (Le-Thierry *et al*., 1997) and other crops. RAPD is least complicated, most user-friendly, cost and time effective (Williams *et al*., 1990; Karp *et al*., 1997; Asif *et al*., 2005). Therefore, RAPD analysis was conducted to assess genetic diversity and genetic relationship among local collection of wheat genotypes for marker assisted breeding.

Materials and Methods

Plant material: Seeds of 24 wheat genotypes viz; C-591, Pasban-90, Barani-83, Punjab-81, LU-26-S, QM-4531, QM-4934, GA-2002, Bakhar-2002, 2K-CO-50, Chakwal-97, Rawal-87, Sarsabz, Kiran-95, Marvi-2000, Bhitai, Khirman, NIA amber, Abadgar-93, Inqilab-91, SARC-1, PKV-1600, Chakwal-86 and CM24/87 were grown in bowls at control condition at NIA Tando Jam, and seedlings were harvested after 15 days.

DNA extraction and amplification: DNA was extracted from fresh leaves of wheat genotypes using MATAB method (Bibi *et al*., 2010). The concentration of the extracted DNA was measured with spectrophotometer (BIOMATE 3) at absorbance 260nm and 280 nm. The quality of DNA was further checked on 0.8% agrose gel.

Thirteen primers from Gene Link, each ten bases in length, were used to amplify the DNA (Table 2). PCR reaction was carried out in 25µl reaction mixture and amplification reaction was performed in the Eppendorf Master Cycler according to the method described by Bibi *et al*., 2010.

Data analysis: Data was scored as presence of band as (1) and absence of bands as (0) on the basis of size in comparison with external standards using the UVitec Cambridge UK, gel documentation and analysis system with Band Map software for calculating the bands size from RAPD amplification profile. The following parameters were generated using the population genetic software package POPGENE: the observed number of alleles (Na), the effective number of alleles (Ne), Nei's gene diversity (h) and Shannon's index (Nei's, 1987).

The genetic structure was investigated as described by Nei's (1987) analysis. The proportion of the total genetic variation found among the population (G_{ST}) was calculated from $G_{ST}=D_S/H_T$. Gene flow was estimated from G_{ST} or G_{CS} according to the McDermott and McDonald (1993), $Nm=0.5(1-G_{ST})/G_{ST}$.

Genetic divergence between the populations was investigated using Nei's unbiased genetic distance (D) and genetic identities (I) (Nei's 1973). Genetic relationships among populations were quantified by the Nei's (1973) which is modified from NEIGHBOR procedure of PHYLIP Version 3.5 software. The matrix of the similarity between the pairs of individuals was subject to cluster analysis by the un-weight pair group method with the arithmetic averages (UPGMA).

Results

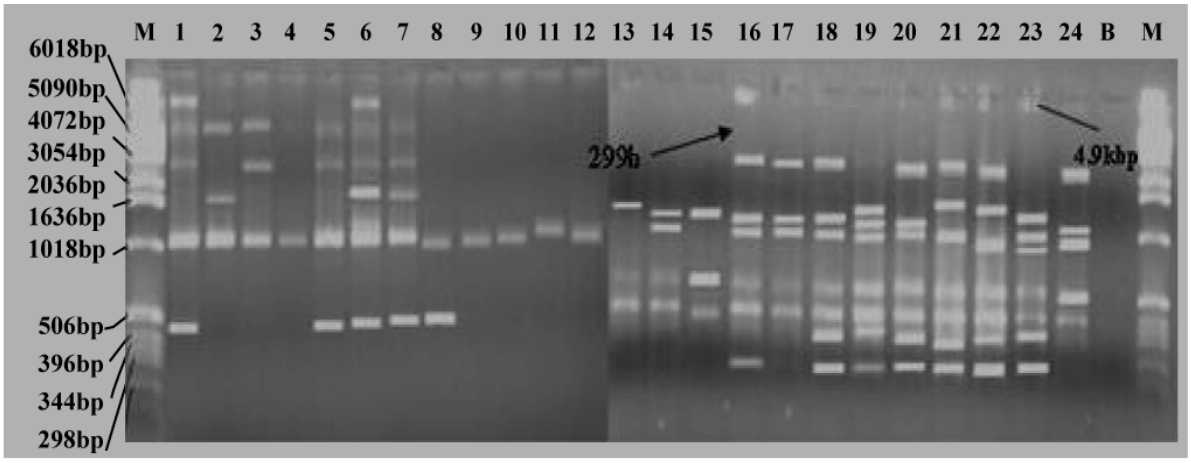
RAPD polymorphism: Genomic DNAs of wheat cultivars produced multiple fragments with ten base arbitrary primers. Of forty primers, thirteen were amplifying the genomic DNA. The total number of the amplified DNA products (bands) yielded across the set of 24 wheat cultivars was 269. The percentage of the polymorphic loci with an average was 86.22% (Table 1). Level of the individual genotype of the twenty four wheat varieties/lines produced polymorphism in which few monomorphic loci were observed. Primer A-09 amplified twelve segments which were polymorphic, with the range of 299bp-4.9kb. Only C591 and QM-4531 contain a specific segment of 4.9kb. SARC-1 contains a differentiate segment of 1.68kp. PKV-1600 contain a specific segment of 1.09kb All wheat genotypes contains segment of 1.45kb except Sarsabz, Marvi-2000, PKV-1600 (Fig. 1).

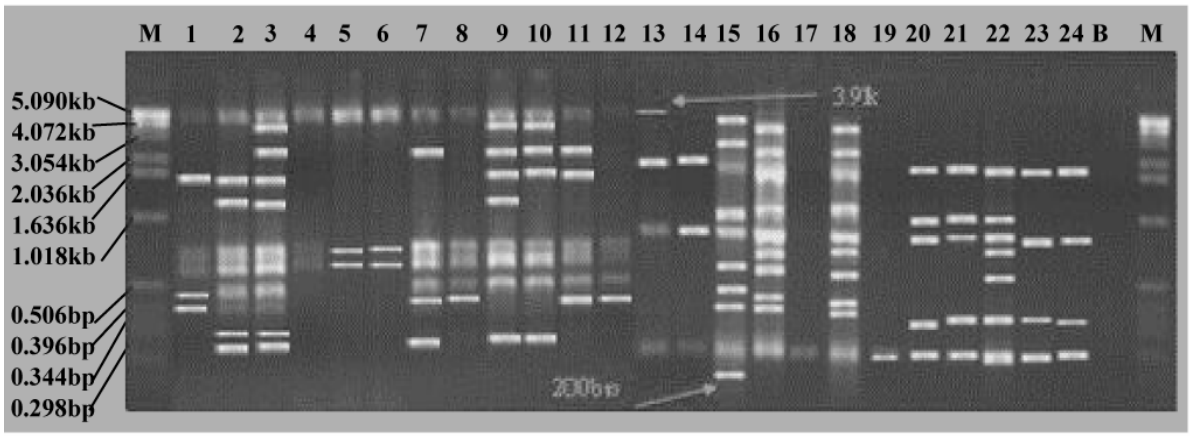
Primer-B-07 amplified seventeen bands ranged between 200bp-3.9kb and was polymorphic. Marvi-2000 contains two specific RAPD markers of 3.2kb and 200bp whereas a specific band (2.91kb) was also observed in Bhitai and NIA amber. Sarsabz, Kiran-95, Bhitai and NIA amber contain a specific segment of 2.1kb. Another specific segment of 1.39kb appeared in Pasban-90, Barani-83 and Bakhar-2002. Pasban-90 and Barani-83 containing a differentiated 396bp segment of DNA (Fig. 2).

Table 1. Genetic variation parameters of wheat genotypes based on RAPD

Population	No. Polymorphic loci	% of Polymorphic loci	Na	Ne	h	I
C-591	11	84.62	1.8462	1.4446	0.2812	0.4312
Pasban	13	100	2.0000	1.5249	0.3239	0.4976
Barani-83	11	84.62	1.8462	1.5580	0.3208	0.4738
Punjab-81	13	100	2.0000	1.6425	0.3754	0.5578
LU-26-S	11	84.62	1.8462	1.5679	0.3317	0.4891
QM-4531	10	76.92	1.7692	1.5066	0.2951	0.4366
QM-4934	10	76.92	1.7692	1.3969	0.2475	0.3815
GA-2002	12	92.31	1.9231	1.5278	0.3188	0.4821
Bakhar-2002	12	92.31	1.9231	1.6883	0.3864	0.5604
2K-CO-50	11	84.62	1.8462	1.5194	0.3047	0.4555
Chakwal-97	12	92.31	1.9231	1.6938	0.3897	0.5644
Rawal-87	09	69.23	1.6923	1.4655	0.2677	0.3945
Sarsabz	11	84.62	1.8462	1.5732	0.3275	0.4824
Kiran-95	12	92.31	1.9231	1.6799	0.3772	0.5473
Marvi-2000	12	92.31	1.9231	1.6480	0.3626	0.5308
Bhittai	10	76.92	1.7692	1.5346	0.3063	0.4493
Khirman	10	76.92	1.7692	1.4649	0.2718	0.4075
NIA amber	12	92.31	1.9231	1.6591	0.3707	0.5406
Abadgar-93	11	84.62	1.8462	1.4805	0.2892	0.4380
Inqilab-91	10	76.92	1.7692	1.5174	0.2948	0.4347
SARC-1	11	84.62	1.8462	1.6399	0.3586	0.5188
PKV-1600	12	92.31	1.9231	1.6474	0.3637	0.5322
Chakwal-86	11	84.62	1.8462	1.6189	0.3510	0.5104
CM-24/87	12	92.31	1.9231	1.7218	0.3932	0.5663
Mean	**269**	**86.22**			**0.3295**	**0.487**

Na, observed No of alleles/ locus; Ne, effective's number of alleles /locus; (h), Nei's genetic diversity; (I), Shanon's index





Figs. 1 & 2. Amplification profile of 24 wheat genotypes with primer A-09 & B-07 respectively by RAPD-PCR. M=1kb ladder, 1=C-591, 2=Pasban, 3=Barani-83, 4=Punjab-81, 5=LU-26-S, 6=QM-4531, 7=QM-4934, 8=GA-2002, 9=Bakhar-2002, 10=2K-CO-50, 11=Chakwal-97, 12=Rawal-87, 13=Sarsabz, 14=Kiran-95, 15=Marvi-2000, 16=Bhitai, 17=Khirman, 18=NIA amber, 19=Abadgar-93, 20=Inqilab-91, 21=SARC-1, 22=PKV-1600, 23=Chakwal-86, 24=CM 24/87, B=Blank

Genetic variation within population : Genetic variability among the population is presented in Table 1. In individual populations the per population percentage of polymorphic loci *(P)* ranged from 69.23% to 100%, with an average of 86.22%. The observed number of alleles *(Na*) varied from 1.692 to 2.000, while the effective number of allelles (*Ne*) varied from 1.397 to 1.722. Nei's genetic diversity *(h)* ranged from 0.248 to 0.393, with an averge of 0.330. Shanon's index (*I*) ranged from 0.382 to 0.566, with an average of 0.487.

Twenty four populations of *T. aestivum* showed diferent level of genetic diversity. The heighest level of genetic diversity was detected in population CM 24/87, while the lowest level was detected in population of QM 4934. The order of genetic diversity based on genetic parameters (*I*) was found to be CM 24/87 > Chakwal-97 > Bakhar-2002 > Punjab-81 > Kiran-95 > NIA amber > PKV-1600 > Marvi-2000 > SARC-1 > Chakwal-86 > Pasban-90 > LU-26-S > Sarsabz > GA-2002 > Barani-83> 2K-CO-50 > Bhittai > Abadgar-93 > QM-4531 > Inqilab-91 > C-591 > Khirman > Rawal-87 > QM-4934.

Population genetic structure and differentitiation : Wheat genotypes showed level of genetic differentiation among the populations (Table 2). The total gene diversity (H_T) and gene diversity among the populations (*Hs*) were 0.4614 and 0.3296 respectively. The proportion of genetic variation among the populations (*Ds*) accounted for 28.58% of the whole gentic diversity, indicating a low degree of genetic differentitation among the populations. The level of gene flow (Nm, the number of migrating individuals among the populations per generation) was estimated to be 1.25. The observed valued indicated that gene exchange between the populations was low.

Table 2. Population genetic structure of wheat genotypes.

Locus	Sample size	Ht	Hs	Gst	Nm*
Mean	196	0.4614	0.3296	0.2858	1.2494

Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst.

Population genetic relationships: The genetic relationshionship between the populations was determined by calculating genetic distances (*D*) and genetic identity (*I*) (Table 3). For populations pairs, the greatest genetic distance (D=0.639) was found between the populations Rawal-87 and Khirman followed by Rawal-87 and Bhittai (D=0.628) and the lowest genetic distance (D=0.024) was found between the popilations C-591 and Pasban-90 followed by LU-26-S and QM-4531 (D=0.026) and Inqilab-91 and SARC-1 (D=0.028).

Table 3. Similarity coefficient among the wheat cultivars calculated according to Nei & Li's coefficient.

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	****	0.9759	0.8893	0.8794	0.9259	0.9312	0.8752	0.7932	0.8335	0.7785	0.9140	0.8479	0.6906	0.8215	0.7686	0.6521	0.6999	0.6650	0.6880	0.7571	0.8005	0.6754	0.6707	0.6904
2	0.0244	****	0.8767	0.8997	0.9195	0.9254	0.8892	0.8452	0.8589	0.8323	0.9460	0.8421	0.7420	0.8466	0.7990	0.6956	0.7701	0.7223	0.7718	0.8209	0.8505	0.7491	0.7431	0.7637
3	0.1173	0.1316	****	0.7779	0.8418	0.8274	0.8036	0.8249	0.8700	0.7734	0.8302	0.8306	0.6765	0.8699	0.7731	0.6125	0.6468	0.6989	0.6762	0.6730	0.7403	0.7198	0.6962	0.7164
4	0.1285	0.1057	0.2511	****	0.8624	0.8011	0.8033	0.7670	0.8971	0.7987	0.9458	0.7772	0.8547	0.8211	0.8779	0.8106	0.7558	0.8416	0.7332	0.8699	0.8789	0.8112	0.7805	0.7965
5	0.0769	0.0839	0.1722	0.1480	****	0.9745	0.9338	0.7412	0.8785	0.7159	0.8902	0.7635	0.7354	0.8492	0.8137	0.7549	0.7735	0.7183	0.7651	0.8195	0.8347	0.7842	0.7494	0.7105
6	0.0713	0.0776	0.1895	0.2217	0.0258	****	0.9044	0.7628	0.8045	0.7226	0.8558	0.7765	0.6820	0.8131	0.7387	0.6889	0.7760	0.6705	0.7663	0.7991	0.8260	0.7341	0.7435	0.7303
7	0.1333	0.1174	0.2187	0.2190	0.0684	0.1004	****	0.6764	0.8869	0.6310	0.8891	0.6728	0.7622	0.8739	0.7675	0.8133	0.8159	0.7213	0.8251	0.8336	0.8356	0.8124	0.7737	0.6761
8	0.2317	0.1682	0.1925	0.2653	0.2995	0.2708	0.3910	****	0.7015	0.9141	0.7576	0.9155	0.7016	0.7408	0.8230	0.6181	0.6136	0.7712	0.7058	0.6909	0.7164	0.7644	0.7342	0.8682
9	0.1821	0.1522	0.1392	0.1086	0.1296	0.2176	0.1201	0.3545	****	0.7006	0.9242	0.6594	0.8473	0.9409	0.8193	0.8439	0.8163	0.8182	0.8081	0.8394	0.8873	0.8785	0.8268	0.7555
10	0.2504	0.1836	0.2570	0.2247	0.3341	0.3249	0.4604	0.0898	0.3558	****	0.8165	0.8613	0.6166	0.7149	0.8711	0.5559	0.5897	0.7505	0.6422	0.7016	0.7231	0.7260	0.6944	0.8096
11	0.0899	0.0555	0.1861	0.0557	0.1163	0.1557	0.1176	0.2776	0.0788	0.2028	****	0.7693	0.8142	0.8963	0.8390	0.8019	0.8287	0.8071	0.8250	0.8575	0.8843	0.8035	0.7965	0.7787
12	0.1650	0.1718	0.1856	0.2521	0.2698	0.2530	0.3963	0.0882	0.4165	0.1493	0.2623	****	0.5955	0.6821	0.8198	0.5335	0.5278	0.6582	0.5765	0.5943	0.6009	0.6016	0.5713	0.7398
13	0.3701	0.2984	0.3909	0.1571	0.3074	0.3827	0.2715	0.3544	0.1657	0.4835	0.2056	0.5184	****	0.8176	0.7403	0.9617	0.8511	0.9299	0.8792	0.8682	0.8857	0.8871	0.9117	0.8560
14	0.1967	0.1666	0.1394	0.1971	0.1634	0.2069	0.1348	0.3001	0.0609	0.3356	0.1095	0.3826	0.2013	****	0.7483	0.8110	0.8339	0.8080	0.8847	0.7786	0.8520	0.8708	0.8543	0.7793
15	0.2632	0.2244	0.2573	0.1302	0.2062	0.3028	0.2646	0.1948	0.1993	0.1380	0.1755	0.1987	0.3007	0.2900	****	0.7419	0.6391	0.8323	0.6493	0.8021	0.7652	0.8384	0.7364	0.7701
16	0.4275	0.3630	0.4902	0.2100	0.2812	0.3726	0.2067	0.4810	0.1697	0.5871	0.2207	0.6283	0.0391	0.2095	0.2986	****	0.8558	0.8962	0.8832	0.8497	0.8568	0.8904	0.8846	0.7950
17	0.3567	0.2612	0.4357	0.2800	0.2568	0.2537	0.2035	0.4884	0.2030	0.5282	0.1879	0.6390	0.1613	0.1816	0.4477	0.1557	****	0.7487	0.9155	0.8810	0.9005	0.8273	0.8886	0.7716
18	0.4080	0.3254	0.3583	0.1725	0.3309	0.3997	0.3267	0.2598	0.2006	0.2871	0.2143	0.4182	0.0727	0.2132	0.1835	0.1096	0.2895	****	0.8487	0.8624	0.8669	0.9226	0.9328	0.9278
19	0.3740	0.2590	0.3912	0.3103	0.2678	0.2661	0.1923	0.3484	0.2130	0.4428	0.1924	0.5507	0.1287	0.1225	0.4319	0.1242	0.0883	0.1641	****	0.8220	0.8551	0.8693	0.9225	0.8465
20	0.2783	0.1973	0.3960	0.1394	0.1991	0.2243	0.1820	0.3697	0.1751	0.3544	0.1537	0.5203	0.1414	0.2502	0.2205	0.1629	0.1267	0.1481	0.1960	****	0.9725	0.9058	0.9230	0.8452
21	0.2225	0.1620	0.3007	0.1291	0.1807	0.1912	0.1796	0.3335	0.1195	0.3241	0.1230	0.5093	0.1213	0.1601	0.2677	0.1546	0.1048	0.1428	0.1565	0.0279	****	0.9126	0.9442	0.8688
22	0.3924	0.2889	0.3288	0.2092	0.2430	0.3091	0.2078	0.2687	0.1296	0.3202	0.2188	0.5081	0.1198	0.1384	0.1762	0.1161	0.1896	0.0805	0.1401	0.0989	0.0914	****	0.9533	0.8836
23	0.3994	0.2969	0.3621	0.2478	0.2885	0.2963	0.2565	0.3090	0.1902	0.3647	0.2276	0.5599	0.0925	0.1574	0.3060	0.1227	0.1181	0.0695	0.0806	0.0801	0.0574	0.0478	****	0.9265
24	0.3705	0.2696	0.3335	0.2275	0.3418	0.3142	0.3914	0.1413	0.2804	0.2112	0.2501	0.3014	0.1555	0.2493	0.2612	0.2294	0.2594	0.0750	0.1667	0.1682	0.1407	0.1237	0.0764	****

1=C-591, 2=Pasban, 3=Barani-83, 4=Punjab-81, 5=LU-26-S, 6=QM-4531, 7=QM-4934, 8=GA-2002, 9=Bakhar-2002, 10=2K-CO-50, 11=Chakwal-97, 12=Rawal-87, 13=Sarsabz, 14=Kiran-95, 15=Marvi-2000, 16=Bhitai, 17=Khirman, 18=NIA amber, 19=Abadgar-93, 20=Inqilab-91, 21=SARC-1, 22=PKV-1600, 23=Chakwal-86, 24=CM24/87

Cluster analysis: On the basis of dendrogram, the varieties could be divided into two groups and five clusters A to E, C-591, Pasban-90, LU-26-S, QM-4531 and QM-4934 in cluster A, Punjab-81, Chakwal-97, Bakhar-2002 and Kiran-95 in cluster B, Sarsabz, Bhittai, NIA amber, CM 24/87, and Inqilab-91 in cluster C. Cluster D contained predominantly PKV-16000 and Chakwal-86. Cluster E comprises of Khirman, Abadgar-93, GA-2002, Rawal-87, 2K-CO-50 and Marvi-2000 which is showing genetically close related to each other. Cluster A, B C and D forming a group 'one'. Whereas, Cluster E forming a group two. Barani-83 showed 12-25% dissimilarity with cluster A and B, while SARC-1 showed 12-28% dissimilarity with cluster A B and C.

Discussion

Welsh & McClelland (1991) developed a PCR-based genetic assay (RAPD), this procedure detects nucleotide sequence polymorphisms in DNA by using a single random primer. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence variability between individuals (Franco *et al*., 2001; Semagn *et al*., 2006). The reproducibility of the RAPD technique can be influenced by variable factors, such as primer sequence, template quality and quantity, the type of thermocycler, and polymerase concentration (Semagn *et al*., 2006). However, the use of a standardized RAPD protocol can ensure a reproducible RAPD pattern. The random amplified polymorphic DNA (RAPD) technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences has been successfully used for the assessment of genetic diversity in diploid, tetraploid and hexaploid wheat (Sivolap *et al*., 1999). In hexaploid wheat, large genome size, a high proportion of repetitive DNAs, continuous inbreeding caused by self-pollination and a narrow genetic base represent the difficulties for use of molecular markers (Joshi & Nguyen, 1993). Knowledge of genetic diversity among adopted cultivars or elite breeding materials has a significant impact on the improvement of crop plants (Franco *et al*., 2001).

In this study, a total of 269 polymorphic bands were produced for thirteen primers combination in all 24 wheat genotypes. The percentage of the polymorphic loci with an average was 86.22%. Compared with previous studies, the level of genetic diversity was moderate. Mukhtar *et al*., (2002) was reported that total number of amplification products produced was 445, out of these amplified product 64.38% were polymorphic among 20 wheat cultivars. Khan *et al*., (2005) reported that 40.7% of the total score able bands were polymorphic and 26.1% of the polymorphic bands were observed most frequently among the 20 Pakistan wheat cultivars released from 1933-2002. Bibi *et al*., (2009) also reported that a total of 102 Scorable loci were amplified, out of which 89.2% were polymorphic among 12 wheat varieties/lines. Khan *et al*., (2010) observed that a total of 177 DNA fragments were amplified in the 75 accession of wheat with 20 RAPD primers. Of the total, 142 fragments were polymorphic among the wheat accession, which indicated 80.22% polymorphism.

Wright (1978) reported that an FST range of 0–0.05 indicates little differentiation, 0.05–0.15 moderate differentiation (Hartl, 1987), 0.15–0.25 large differentiation, and above 0.25 indicates a very large differentiation (Kiambi *et al*., 2005). In this study, basing on the AMOVA analysis, wheat population showed significant levels of genetic differentiation among the genotypes, with 28.6% of the total variation (Gst=0.286) due to differences among the populations. Loveless & Hemrick, (1984) reported that high genetic differentiation was attributed to restricted gene flow. When the distance is not a limiting factor, dispersal and gene flow is unlimited and population will form a single uniform genetic unit. According to Slatkin, (1977) and Hutchison & Templeton, (1999), in restricted gene flow population genetic differences will increase.

Kiran-95 and Inqilab-91 have the same parentage but Inqilab-91 developed through hybridization whereas Kiran-95 was out come of indirect mutagenesis (Table 4), showing the 25% dissimilarity between them. Kiran-95 and Inqilab-91 have different levels of genetic diversity. Effective number of alleles (Ne) 1.68 was observed in kiran-95 and 1.52 in Inqilab-91. Nei's genetic diversity (h) was 0.38 (kiran-95) and 0.3 (Inqilab-91), whereas, shanon's index was observed 0.55 in kiran-95 and 0.44 in Inqilab-91 (Table 1). The clear DNA fingerprinting results of kiran-95 and Inqilab-91 was showed in Figs. 1 and 2. This showed that both the methodologies have the potential to create new ideotypes which can be useful for enhancing the crop productivity. These DNA fingerprints will also provide documentary evidence of breeder rights Thus; it indicates the reliability of RAPD technique for the evaluation of duplicate accession (Congiu *et al*., 2000).

Twenty four wheat varieties/lines showing genetic relationship through banding pattern in Figs. 1 and 2. RAPD by definition uses randomly marked sections of the genome and associated genes are not necessarily critical in distinguish cultivar performance (Heneen & Jorgensen, 2001). Polymorphism revealed by RAPD could be a result of nucleotide changes at the primer annealing site, or due to addition or deletion between two priming sites, which results in different lengths of the amplification products (Williams *et al*., 1990). Moreover, the morphological features of the individual plants in the field were also uniform. The amplification of monomorphic loci is depicting sharing of common blood among the genotypes (Asif *et al*., 2005).

Barani-83 was showing maximum (88%) similarity with C-591 and minimum with Punjab-81 (75%), as well as another distinct genotype SARC-1 comprises of 88% similarity with Bakhar-2002 and 72% with Inqilab-91. The close genetic relationships are quite alarming and may impede further plant improvement. It has been very well documented that plant improvement is based on the information about the genetic relationships among accessions within and between species (Thormann *et al*., 1994).

Table 4. Parentage, origin and year of release of wheat varieties of Pakistan.

S. No.	Variety	Year of release	Parentage	Origin
1.	Sarsabz	1986	PI/FRND//MxP/3/PI/M20/79	NIA, Tando Jam
2.	Kiran-95	1996	WL 711/Crow 'S'	NIA, Tando Jam
3.	Marvi-2000	2003	CMH-77A917/PKV-1600//RL6010/6 SKA	NIA, Tando Jam
4.	Bhittai	2004	VEE/TRAP#1 Soghat-90	NIA, Tando Jam
5.	Khirman	2005	ULC/PVN//TAN/3/BUC	NIA, Tando Jam
6.	NIA amber	2010	VEE # 5 'S'/ SARA//Soghat-90	NIA, Tandojam
7.	Abadgar-93	1996	Yaktana54xNorin10-BervorxSon64	Wheat. Res. Stat. T. Jam
8.	Inqilab-91	1991	WL 711/CROW 'S'	AARI, Faislabad
9.	SARC-1		Advanced line	
10.	PKV-1600		ZA76 x Sonalika-64	
11.	Chakwal-86	1988	FORLANI/ACC//ANA	AARI, Faislabad
12.	CM 24/87		Advanced line	
13.	C-591	1934	T_9 x 8_B	AARI, Faislabad
14.	Pasban-90	1991	INIA66/A.DISTT//INIA66/3/GEN.81	AARI, Faislabad
15.	Barani-83	1984	BB/GLL/3/GTO/TC/BB/CNO	
16.	Punjab-81	1981	INIA/3/SON64/P4160 (E)//SON64	AARI, Faislabad
17.	LU-26-S	1977	Blue silver//Khushal69	UAF
18.	GA-2002	2002	FRET2/UKURU//FRET2	
19.	Bakhar-2002	2002	---	
20.	2K-CO-50		---	
21.	Chakwal-97	1997	---	
22.	Rawal-87	1987	MAYA/MON'S'//KVZ/TRM	BARI, Rawalpindi
23.	QM4934		Advanced line	NIA Tando Jam
24.	QM4531		Advanced line	NIA Tando Jam
25.	Sonalika (BLS)	1978	II-53-388/An/3/Yt54'NIOB//LR64	
26.	Soghat-90 (Pavon)	1991	VCM//CNO S'/7C/3KAL/BB	AARI, Faislabad

NIA= Nuclear Institute of Agriculture; AARI= Ayub Agriculture research Institute; BARI= Barani Agriculture Research Institute; UAF= University of Agriculture Faisalabad

Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weir, 1996). The genetic distance of 24 wheat genotypes ranging from 0.024 to 0.639 with an average of 0.332 suggested that the level of genetic diversity among the wheat genotypes is low. In several other studies, elite wheat germplasm showed genetic diversity (Joshi and Nguyen, 1993; Mukhtar *et al*., 2002; Asif *et al*., 2005; Khan *et al*., 2005; Bhutta *et al*., 2006; Bibi *et al*., 2009; Khan *et al*., 2010). Mukhtar *et al*., (2002) reported similarities as high as 76-93% among 20 elite wheat genotypes. Bhutta *et al*., (2006) observed that within population genetic variation ranged from 83% to 93% of the total. This low value of genetic distance is an indication that a large part of the genome of the genotypes under study is identical. This is primarily due to the lack of parental diversity, because all the genotypes may share somewhat similar parents in the pedigree.

Furthermore, a closer view of Fig. 3 revealed that 18 of 24 genotypes under examination were grouped together in the major group I comprising four clusters. However, 6 genotypes of group II were the most distinguishable ones, which were depicting sharing of the common blood among the genotypes. Moreover, breeders usually share the breeding material of other breeding station or use common exotic parents/lines, so the tendency to the use of extensive similar parents in breeding programme has led to a concern of lack of genetic diversity (Iqbal *et al*., 1997, Rahman *et al*., 2002). It is extremely important to study the genetic composition of the germplasm of existing modern-day cultivars in comparison with their ancestors and related species. This will not only provide information on their phylogenetic relationship but will also indicate a chance of findings new and useful genes, as the accessions with most distinct DNA profiles are likely to contain a greater number of novel alleles (Messmer *et al*., 1992).

NIA amber, QM-4934, QM-4531, Pasban-90, C-591, Punjab-81, Kiran-95 Barani-83, Bakhar-2002, Rawal-87 and Sarsabz developed from exotic material. Breeders usually developed the wheat cultivars/lines by crossing indigenous genotypes with exotic parents and also derived from the exotic material mostly introduced from the ICARDA/CIMMYT, Mexico. Such type of the selection has made a small gene pool for wheat cultivars (Mukhtar *et al*., 2002). Thus, conscious efforts need to be made to diversify the parental genetic base to ensure high genetic variability among the cultivated varieties. New sources from the interspecific/ intergeneric hybrid gene pool need to be used along with proven parents to generate the variability that will be both commercially viable and genetically diverse.

In the present study, it was found that RAPD marker is valuable system to evaluate the genetic variability. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform and endangering long term productivity gains (Messmer *et al*., 1992). Therefore, this information will be helpful for breeders to produce distinct genotypes that will maintain the steady genetic improvement.

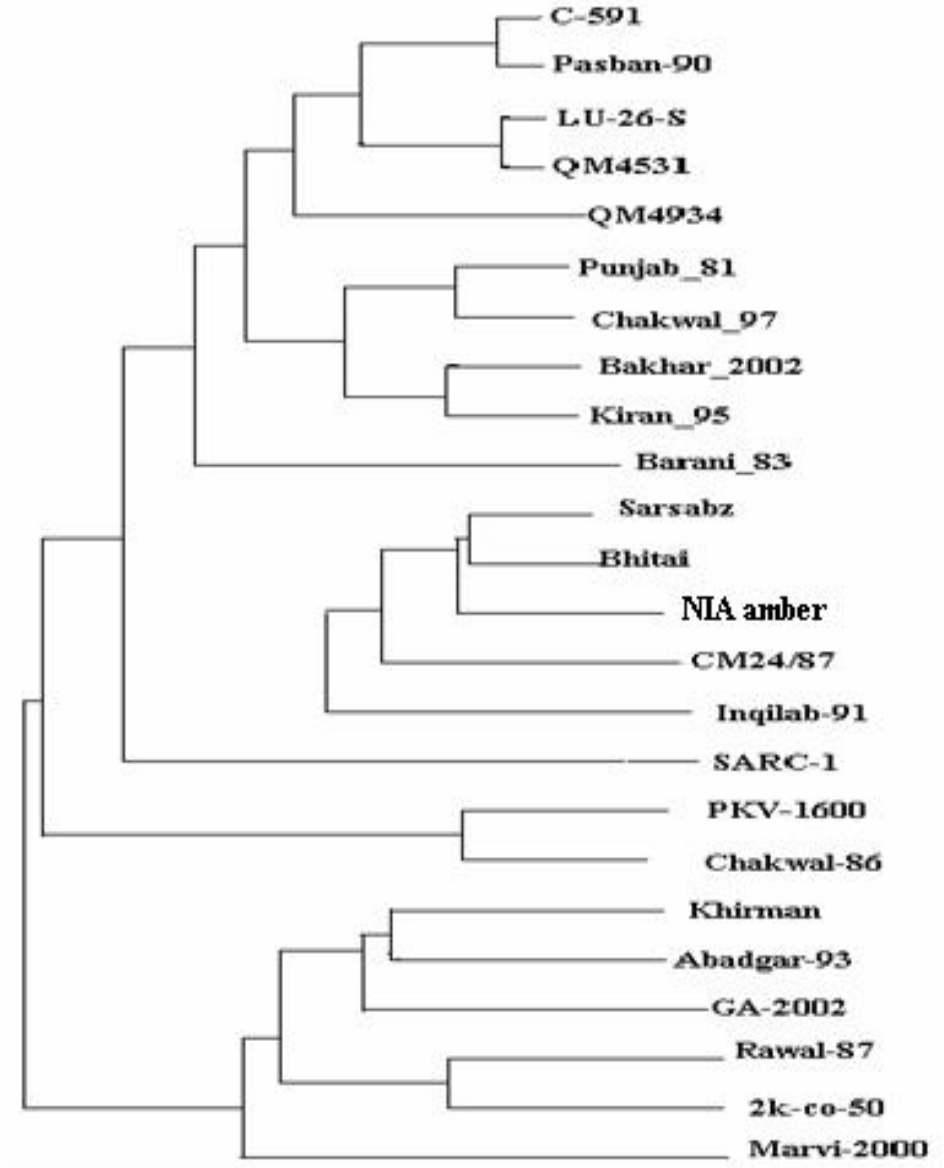


Fig. 3. Dendrogram of 24 wheat genotypes developed from RAPD data using un-weighted pair group method of arithmetic means (UPGMA).

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