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Determination of genetic variation among chickpea genotypes and their F_1 crosses with RAPD markers

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ABSTRACT. The intensity of genetic diversity amongst chickpea genotypes and their crosses is unknown. The current study investigated the genetic diversity of chickpea genotypes and their F_1 crosses by using random amplified polymorphic DNA (RAPD) markers. We assessed the variation among 6 chickpea genotypes and 15 F_1 crosses with the RAPD markers. The 6 parents and their 21 hybrids were carefully studied based on the presence or absence of bands. The level of polymorphism varied with different primers. Of 28 primers

used, 21 amplified the genomic DNA in all the varieties, 15 generated polymorphic bands among all the varieties, and 6 produced similar banding patterns.

Key words: RAPD markers; DNA fingerprinting; Genetic diversity; Chickpea; Hybrids

INTRODUCTION

Chickpea is an edible, protein-rich legume and is one of the earliest cultivated grain legumes. Almost 7500-year-old remains have been found in the Middle East (Duke, 1981; Tanno and Wilcox, 2006; Redden and Berger, 2007). In accordance with FAO data, 8.7 million tons of chickpea are produced per annum from 10.6 million hectares, with an average seed production of 819 kg per hectare (FAO, 2006). It is grown in large areas around the world, but with comparatively low yield (Cani and Toker, 2009; Toker, 2009; Upadhyaya et al., 2001). Containing 17-24% proteins, 41-50.8% carbohydrates, and a high percentage of other mineral nutrients and saturated linoleic and oleic acid, chickpea is one of the most important crops for human consumption (Hulse, 1991; Huisman and van der Poel, 1994; Kerem et al., 2007). With a low production cost, wide climate adaptation, and the ability to be used in crop rotation and atmospheric nitrogen fixation, chickpea is one of the most important legume plants in a sustainable agriculture system (Smithson et al., 1985; Singh and Ocampo, 1997; Cani and Toker, 2009).

The classification of diverse genotypes of crop species is vital when various successions of crop germplasm are to be exemplified. With the advent of polymerase chain reaction (PCR) technology, it has become possible to study the genetic differences in plants and animals. DNA fingerprinting, gene mapping, and polymorphic studies have benefited tremendously from PCR. One variation of PCR is random amplified polymorphic DNA (RAPD), which generates DNA fingerprinting with a single synthetic oligonucleotide primer (Williams et al., 1990). RAPDs are inherited in simple Mendelian fashion and are usually dominant markers. Gene mapping using RAPD markers has several advantages over restriction fragment length polymorphism. The RAPD procedure is less expensive, faster, requires less DNA (0.5-50 ng), and does not involve radioisotopes. Simple DNA fingerprinting of multifaceted genomes can be created using single randomly selected primers and PCR, and strains can be identified by evaluating polymorphisms in fingerprints, such as in *Staphylococcus*, *S. pyogenes*, and *Oryza sativa* (Welsh and McCielland, 1990; Williams et al., 1990).

PCR-based RAPD markers were used to assess diversity in 23 chickpea genotypes. Forty of 100 random primers exhibited polymorphism. Most of the primers revealed a single band and only 14 were polymorphic (Rakesh et al., 2002). Another study by Rao et al. (2007) predicted heritable associations among 19 chickpea cultivars and 5 accessions of their wild parents with RAPD markers. On average, 6 bands per primer were observed in RAPD analysis with 51.7 and 50.5% polymorphic bands among the wild accessions and chickpea cultivars, respectively. The markers generated by RAPD can provide useful information for the management of genetic resources. The RAPD technique has been used extensively for checking the genetic diversity in diverse crops, which

yielded a varied array of bands exhibiting polymorphism, e.g., cotton, chickpea, gram, black gram, etc. (Souframanien and Gopalakrishna, 2004; Talebi et al., 2009; Khan et al., 2010). These studies demonstrated the efficient and reliable use of RAPDs for analyzing genetic variation in colored and white-linted genotypes of cotton.

Keeping in view the present status of chickpea in Pakistan, we planned this present study to determine the genetic diversity and genetic relationship among 6 varieties of chickpea and their F_1 crosses.

MATERIAL AND METHODS

Plant sample

The seeds of 6 parental genotypes, i.e., CM-98, AUG-786, Bittal-98, Balksar-2000, Wanhar-2000, Punjab-2000, and their F_1 single crosses were grown in earthen pots and supplied with the optimum amount of water and nutrition through the Hoagland solution. Later, leaf tissues were collected for DNA extraction.

DNA extraction

The total genomic DNA was extorted by the CTAB method (Doyle and Doyle, 1990). Young leaves (4-5) were washed, dried, and ground into powder with liquid nitrogen. The resultant paste was transferred to a 15-mL Falcon tube to which 15 mL hot CTAB (65°C) was added. The tube was inverted gently several times to mix the suspension and subsequently incubated at 65°C for 30 min. Soon after, 15 mL chloroform-isoamyl alcohol (24:1) was added and gently inversion mixed to form an emulsion. The mixture was centrifuged for 10 min at 9000 rpm. The supernatant was dispensed to a new Falcon tube. The above steps were repeated twice and 0.6 volumes 60% isopropanol (pre-chilled) were added and mixed gently to precipitate the DNA. The tubes were centrifuged at 9000 rpm for 5 min and the supernatant was discarded. The pellet was washed with 70% ethanol twice or thrice and air-dried at room temperature, following which 0.5 mL 0.1X TE buffer was added. RNAase (7 µL) was added, mixed gently, and incubated for 1 h at 37°C. An equal volume of chloroform-isoamyl alcohol (24:1) was added and mixed gently; 3 M NaCl was added and the mixture was gently mixed. The DNA was precipitated with absolute ethanol (cold, 7 mL). The sample was spun at 13,000 rpm for 10 min and the resultant supernatant was discarded. The pellet was washed with 70% ethanol, air-dried, and resuspended in 0.1X TE buffer or d_3H_2O, and the concentration of DNA was measured at 260 nm.

Estimation of DNA quantity

For the estimation of total genomic DNA from 21 samples, absorbance was measured at 260 nm with a spectrophotometer. A solution with an OD_{260} contained 50 µg DNA/mm, following which the DNA quantity was calculated with the following formula:

DNA concentration (µg/mL) = absorbance at 260 nm x dilution factor x 50.

RAPD analyses

DNA concentration in the working solution of approximately 12 ng/µL in d_3H_2O was confirmed by spectrophotometer. For RAPD analysis (Williams et al., 1990), the concentration of genomic DNA, 10X PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs, Taq DNA polymerase (Fermentas), and 10-mer random primers (Table 1) (Gene Link Company, USA) were optimized. The DNA amplification reactions were performed in a thermal cycler. The PCR profile consisted of 1 cycle of 94°C for 5 min, 40 cycles of 94°C for 1 min, 36°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min.

Table 1. RAPD polymorphic primers used in this study.

No.	Primer	Sequence (5'→3')
1	GLD-11	TAGTAAGCTTTAGTAGGCGTTCGCCTAC
2	GLA-01	GCCTCTCGAGCTTAGGCTTCGTGAGAGA
3	GLD-01	AAGAGCCACCATGGCATTATAACACATAC
4	GLB-14	GCTCAGATCAAGTCGTGAACGCTGGGCCG
5	GLC-20	CAGGAGTCCCTAACACATGSTGCAAGTCGC
6	GLA-09	TGGCTCAGAGGCCTTGAACGCGCGGCAG
7	GLA-02	GTTTAACGAACGCTAGCTAAGCATGGGC

Analysis of RAPD data

Amplification products were analyzed by electrophoresis on 1.2% (w/v) agarose gel and were identified by ethidium bromide staining. All visible and definite scorable fragments amplified were counted. The profiles of all 21 chickpea genotypes were compared with each other and DNA bands were scored as present (1) or absent (0). The data were used to calculate the resemblance (Nei and Li, 1979). Resemblance coefficients were utilized to generate a dendrogram by means of unweighted pair group method of arithmetic means (UPGMA).

RESULTS AND DISCUSSION

The generated RAPDs were used to establish the genetic diversity between 6 parents and their 21 Pakistani chickpea hybrids. Numerous polymorphisms were observed among the chickpea cultivars. Amplification of genomic DNA was performed by using 28 primers, in which 21 exhibited amplification in all the varieties, 15 primers generated polymorphic bands among all the varieties, and 6 produced similar banding patterns (Figures 1-7). The 6 parents and their 21 hybrids were carefully studied based on the bands. The level of polymorphism varied with different primers. We used 28 primers for the PCR. Of these 28 primers, 21 amplified the genomic DNA in all the varieties, 15 primers amplified polymorphic DNA bands among all the varieties, and 6 produced similar banding patterns. Approximately 25% of the total reactions could not amplify genomic DNA. This was due to contamination in the reaction mixture, which may have caused primer degeneration and resulted in complete failure of amplification.

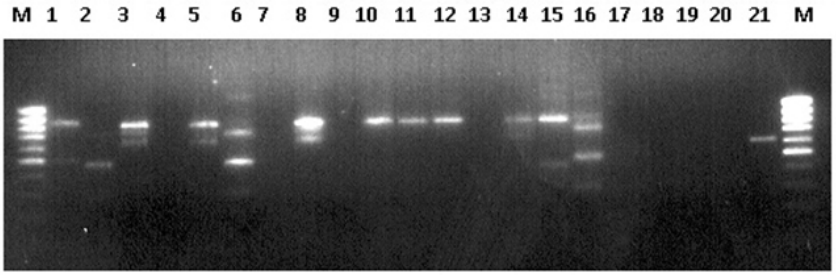

Figure 1. Amplification profile of 21 chickpea genotypes with primer GLD-11. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

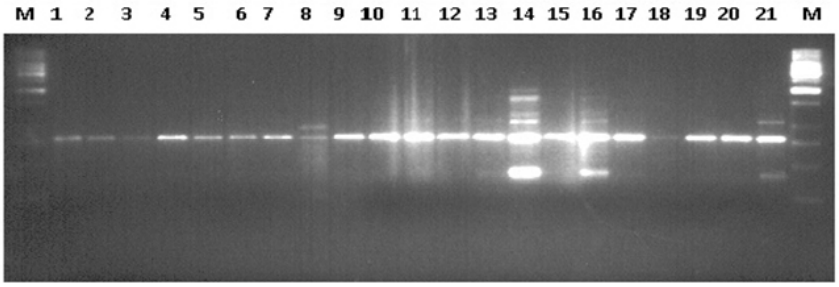

Figure 2. Amplification profile of 21 chickpea genotypes with primer GLA-01. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

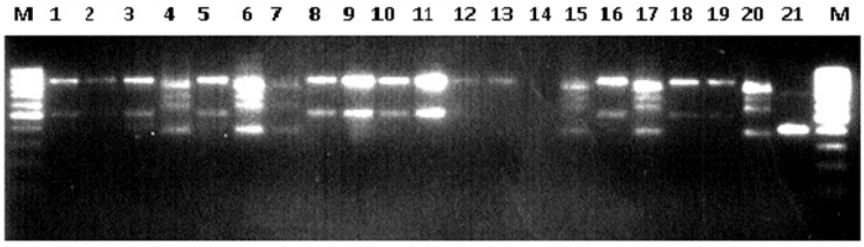

Figure 3. Amplification profile of 21 chickpea genotypes with primer GLD-01. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

Figure 4. Amplification profile of 21 chickpea genotypes with primer GLB-14. *Lane M* = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

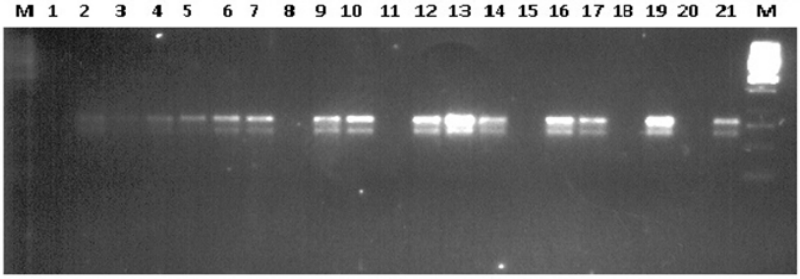

Figure 5. Amplification profile of 21 chickpea genotypes with primer GLC-20. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

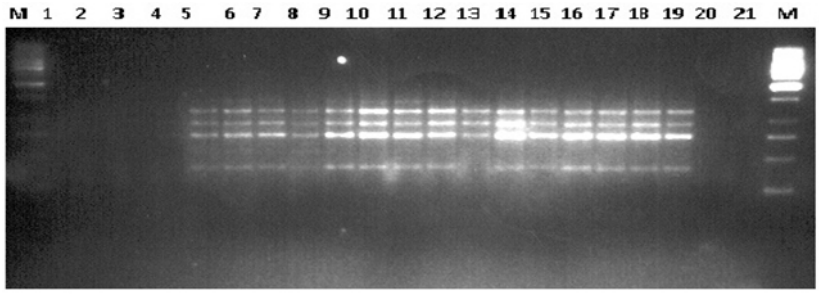

Figure 6. Amplification profile of 21 chickpea genotypes with primer GLA-09. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786 ; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

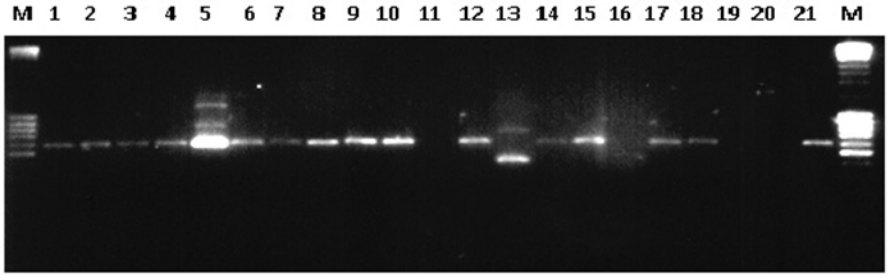

Figure 7. Amplification profile of 21 chickpea genotypes with primer GLA-02. *Lane* M = 1-kb ladder; *lane* 1 = Balksar-2000 x Bittal-98; *lane* 2 = Wanhar-2000 x Punjab-2000; *lane* 3 = Wanhar-2000 x Bittal-98; *lane* 4 = Bittal-98 x CM-98; *lane* 5 = Punjab-2000 x Balksar-2000; *lane* 6 = Aug-786 x Punjab-2000; *lane* 7 = Bittal-98 x Aug-786; *lane* 8 = Wanhar-2000 x Balksar-2000; *lane* 9 = Bittal-98 x Punjab-2000; *lane* 10 = CM-98 x Wanhar-2000; *lane* 11 = Aug-786 x CM-98; *lane* 12 = CM-98 x Punjab-2000; *lane* 13 = Balksar-2000 x Aug-786; *lane* 14 = Aug-786 x Wanhar-2000; *lane* 15 = Balksar-2000 x CM-98; *lane* 16 = Punjab-2000; *lane* 17 = Wanhar-2000; *lane* 18 = Balksar-2000; *lane* 19 = CM-98; *lane* 20 = Aug-786; *lane* 21 = Bittal-98.

With primer GLA-09, most of the amplified bands among the chickpea cultivars were monomorphic (Figure 6). A maximum number of polymorphism and monomorphism among the chickpea cultivars were observed by using primer GLD-01 (Figure 3). We amplified 70 bands from 21 random primers in the different chickpea genotypes. Of these arbitrary primers, almost all exhibited polymorphism among the chickpea cultivars. Monomorphic bands are constant bands that cannot be used to study diversity, while polymorphic bands reveal differences and can be used to study and create a logical association among genotypes (Hadrys et al., 1992). Broad DNA polymorphism has been reported by means of RAPD in quite a few other crops (Hilu and Stalker, 1995; Iruela et al., 2002; Hou et al., 2005). The quantity of RAPD portions produced per primer varied from 2 to 10 fragments, which was the highest number of fragments produced. The results confirm the findings of Kernodle et al. (1993), who stated that the deviation in the number of bands amplified by primers is influenced by inconsistent aspects such as primer configuration, template amount, and few annealing sites in the genome. Mahmood et al. (2009) reported parallel results in *Gossypium* spp. The sensitivity of the working environment and equipment used can influence RAPD amplification (Devos and Gale, 1992). The use of PCR markers is an influential means that discloses broad DNA polymorphism, and it has turned out to be helpful in genetic analysis (Davierwala et al., 2000; Porreca et al., 2001; Neeraja et al., 2002; Saker et al., 2005). Since RAPD does not involve prior sequencing and uses a randomly chosen short primer, as compared to a routine PCR to amplify genomic DNA, excess polymorphic DNA markers may be easily generated.

After scoring the bands, a similarity matrix was developed after multivariate analysis by means of the Nei and Li (1979) coefficients presented in Table 2. The similarity coefficients ranged from 0.0889 to 0.631. Maximum similarity (63.1%) was detected among Wanhar-2000 x Bittal-98; Bittal-98 x CM-98 and Punjab-2000 x Balksar-2000, and the lowest similarity (8.89%) was observed between Bittal-98 x Punjab-2000 and CM-98 x Wanhar-2000. The similarity coefficient values in the present study are almost similar to the observation of Rasul et al. (2007), who reported similarity coefficient values ranging from 0.36 to 0.86 among teasel gourd cultivars, with an average similarity value of 0.72, and this value indicated slight genetic variability in the improved varieties. The results are also similar to those of Talebi et al. (2008a,b), who reported that the average polymorphic information content was 0.43, ranging from 0.68 to 0.12 among elite lines of chickpea.

Table 2. Similarity matrix for Nei and Li's original measures of genetic identity and genetic distance of 21 chickpea genotypes.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Balk-2000 x Bitt-98	Wanh-2000 x Pb-2000	Wanh-2000 x Bitt-98	Bitt-98 x CM-98	Pb-2000 x Balk-2000	A-786 x Pb-2000	Bitt-98 x A-786	Wanh-2000 x Balk-2000	Bitt-98 x Pb-2000	CM-98 x Wanh-2000	A-786 x CM-98	CM-98 x Pb-2000	Balk-2000 x A-786	A-786 x Wanh-2000	Balk-2000 x CM-98	Pb-2000	Wanh-2000	Balk-2000	CM-98	A-786	Bittal-98
1	****																				
2	0.3536	****																			
3	0.5179	0.2666	****																		
4	0.4490	0.3238	0.2392	****																	
5	0.4829	0.4162	0.3844	0.2666	****																
6	0.3844	0.3844	0.2392	0.3536	0.3238	****															
7	0.5179	0.5921	0.2948	0.2948	0.2666	0.2392	****														
8	0.4829	0.4829	0.3844	0.4490	0.6313	0.3844	0.5921	****													
9	0.3536	0.3536	0.3844	0.3844	0.2948	0.2126	0.3238	0.2948	****												
10	0.4162	0.2948	0.3238	0.2666	0.2392	0.2666	0.3238	0.3536	0.0889	****											
11	0.4490	0.3238	0.2948	0.2948	0.3844	0.2392	0.3536	0.2666	0.1125	0.1613	****										
12	0.3536	0.4162	0.3238	0.4490	0.3536	0.1613	0.3238	0.3536	0.1366	0.2392	0.1125	****									
13	0.4490	0.5179	0.4829	0.6313	0.3844	0.2948	0.4829	0.3238	0.2126	0.3238	0.2392	0.1125	****								
14	0.4162	0.2948	0.4490	0.3844	0.2948	0.2126	0.3238	0.4829	0.1866	0.2392	0.2126	0.2392	0.3238	****							
15	0.3844	0.4490	0.5543	0.4162	0.4490	0.3536	0.4829	0.3844	0.2666	0.3844	0.3536	0.3238	0.4162	0.3238	****						
16	0.4829	0.5543	0.5921	0.3844	0.4162	0.4490	0.3844	0.2948	0.2392	0.2392	0.2666	0.2948	0.2666	0.2948	0.3238	****					
17	0.4162	0.3536	0.2666	0.3238	0.2392	0.2126	0.2666	0.4162	0.1366	0.0889	0.2666	0.2948	0.3844	0.2948	0.4490	0.2948	****				
18	0.3238	0.3238	0.2392	0.2392	0.2126	0.1366	0.2392	0.3844	0.1613	0.1125	0.1866	0.1613	0.2948	0.2666	0.3536	0.2666	0.1125	****			
19	0.3536	0.4162	0.3238	0.1613	0.1866	0.2126	0.2666	0.4829	0.2392	0.1866	0.2666	0.2948	0.3844	0.2948	0.3844	0.2948	0.1866	0.1125	****		
20	0.2666	0.2126	0.4162	0.3536	0.3844	0.4162	0.4829	0.5921	0.3238	0.3844	0.4162	0.4490	0.5543	0.3238	0.4162	0.4490	0.3238	0.3536	0.2666	****	
21	0.3238	0.3844	0.6313	0.4829	0.3844	0.4829	0.4829	0.5921	0.3238	0.3238	0.3536	0.3844	0.4162	0.3238	0.5543	0.2666	0.3238	0.2948	0.2666	0.2392	****

Pb-2000 = Punjab-2000; Wanh-2000 = Wanhar-2000; Balk-2000 = Balksar-2000; CM-98 = CM-98; A-786 = Aug-786; Bitt-98 = Bittal-98.

The similarity indexes were used to build a dendrogram by means of UPGMA analysis in order to determine the cluster of the varieties (Figure 8). As shown in the dendrogram, the cluster of 6 chickpea varieties and their hybrids revealed associations among the experimental materials. Chickpea genotypes were classified into 6 main clusters, namely a, b, c, d, e, and f. Cluster a was genetically more diverse than the others were. Cluster c was further subdivided into 4 subclusters. Two parents, Wanhar-2000 and Balksar-2000, belonged to one cluster. The parents Punjab-2000 and Bittal-98 also belonged to the same cluster. The positions of Wanhar-2000 x Balksar-2000 and Balksar-2000 x CM-98 at the end of the dendrogram revealed maximum variation from the rest of the cultivars. This shows that a large amount of genetic variation exists among chickpea genotypes, and this can be utilized in breeding programs to develop high-yield cultivars. The RAPD-based dendrogram of chickpea genotypes displayed the genetic relationships between these accessions, which are in accordance with previous studies on chickpea (Ahmad et al., 1992; Tayyar and Waines, 1996; Iruela et al., 2002). It was evident from the results that the RAPD marker-based dendrogram was in accordance with the dendrogram based on morphological traits, as reported for other crops (Loarce et al., 1996; Fernandez et al., 2002).

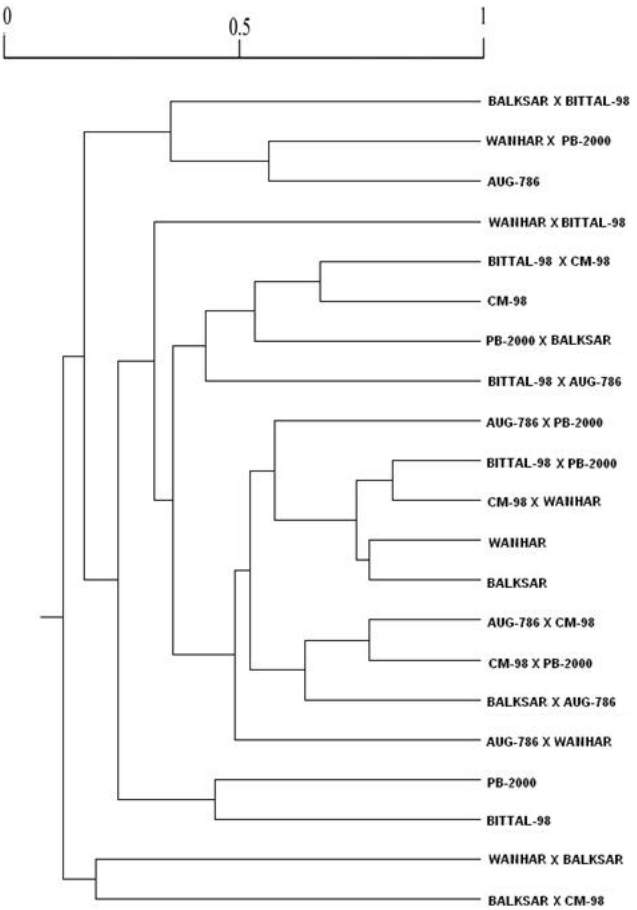

Figure 8. UPGMA clusters analysis-based dendrogram depicting genetic relationships among 7 chickpea cultivars.

The results of our findings are indicative of the genetic association of chickpea genotypes. To increase diversity further, breeders should use parents with diverse origins and it will be helpful for further breeding programs.

CONCLUSIONS

A broad conclusion from the current investigation is that RAPD is capable of disclosing the practical stage of DNA polymorphism amongst chickpea varieties. These findings endorse the ideas of incorporating RAPD markers in chickpea propagation. The limited appraisal of chickpea lines in this study led to the identification of genetic variation, which exists in the accessions. In addition, high genetic deviation, which prevails among chickpea genotypes, can be used proficiently for genome mapping to explore favorable traits. This is suggested based on the findings of the present study in that the chickpea cultivars Wanhar-2000 x Balksar-2000 and Balksar-2000 x CM-98 will produce greater hybrid vigor when they are used in breeding programs because they are genetically distinct from other chickpea cultivars and have minimum genetic similarity.

REFERENCES

Ahmad F and Slinkard AE (1992). Genetic relationships in the genus *Cicer* L. as revealed by polyacrylamide gel electrophoresis of seed storage proteins. *Theor. Appl. Genet.* 84: 688-692.

Cani H and Toker C (2009). Evaluation of annual wild *Cicer* species for drought and heat resistance under field conditions. *Genet. Resour. Crop Evol.* 56: 1-6.

Davierwala AP, Chowdari KV, Kumar S, Reddy AP, et al. (2000). Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica* 108: 269-284.

Devos KM and Gale MD (1972). The use of random amplified polymorphic DNA markers in wheat. *Theor. Appl. Genet.* 84: 194-200.

Doyle JJ and Doyle JI (1990). Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Duke JA (1981). Handbook of Legumes of World Economic Importance. Plenum Press, New York.

FAO (2006). Food and Agriculture Organization of the United Nations. Available at [faostat.fao.org].

Fernandez E, Figueiras M and Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet.* 104: 845-851.

Hadrys H, Balick M and Schierwater B (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* 1: 55-63.

Hilu KW and Stalker HT (1995). Genetic relationships between peanut and wild species of *Arachis* sect. *Arachis* (Fabaceae): evidence from RAPDs. *Plant Syst. Evol.* 198: 167-178.

Hou YC, Yan ZH, Wei YM and Zheng YL (2005). Genetic diversity in barley from west china based on RAPD and ISSR analysis. *Barley Genet. Newl.* 35: 9-22.

Huisman J and Van der Poel AFB (1994). Aspects of the Nutritional Quality and use of Cool Season Food Legumes in Animal Feed. In: Expanding the Production and Use of Cool Season Food Legumes (Muehlbauer FJ and Kaiser WJ, eds.). Kluwer Academic Publishers, Dordrecht, 53-76.

Hulse JH (1991). Nature, Composition and Utilization of Grain Legumes. In: Uses of Tropical Legumes: Proceedings of the Consultants' Meeting, March 27-30, 1989. ICRISAT Center. ICRISAT, Patancheru, 11-27.

Iruela M, Rubio J, Cubero JI, Gil J, et al. (2002). Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. *Theor. Appl. Genet.* 104: 643-651.

Kerem Z, Lev-Yadun S, Gopher A, Weinberg P, et al. (2007). Chickpea domestication in the neolithic levant through the nutritional perspective. *J. Archaeol. Sci.* 34: 1289-1293.

Kernodle SP, Cannon RE and Scandalios JG (1993). Concentration of primer and template qualitatively affects product in RAPD-PCR. *Biotechniques* 1: 362-364.

Khan S, Mirza KJ, Anwar F and Abdin MZ (2010). Development of RAPD markers for authentication of *Piper nigrum*.

Environ. Int. J. Sci. Technol. 5: 53-62.
Loarce Y, Gallego R and Ferre E (1996). A comparative analysis of the genetic relationship between rye cultivars using RFLP and RAPD markers. *Euphytica* 88: 107-115.
Mahmood Z, Raheel F, Dasti AA, Saima S, et al. (2009). Genetic diversity analysis of the species of *Gossypium* by using RAPD. *Afr. J. Biotechnol.* 8: 3691-3697.
Neeraja CN, Sarla N and Siddiq EA (2002). RAPD analysis of genetic diversity in Indian landraces of rice (*Oryza sativa* L). *J. Plant Biochem. Biotechnol.* 11: 93-97.
Nei N and Li W (1979). Mathematical model for studying genetic variation in terms of restriction endo nuclease. *Proc. Natl. Acad. Sci. U. S. A.* 76: 5269-5273.
Porreca P, Sabina MR, Martelli G, Sunseri F, et al. (2001). Genetic variability among Italian rice (*Oryza sativa* L.) cultivars investigated by RAPDs analysis. *J. Genet. Breed.* 55: 349-355.
Rakesh S, Prasad DC, Vibha S and Gurinder JR (2002). Analysis of genetic diversity in *Cicer arietnium* L. using RAPD markers. *J. Pl. Biochem. Biotech.* 11: 2.
Rao LS, Rani PU, Deshmukh PS, Kumar PA, et al. (2007). RAPD and ISSR fingerprinting in cultivated chickpea (*Cicer arietinum* L.) and its wild progenitor *Cicer reticulatum* Ladizinsky. *Genet. Resour. Crop. Evol.* 54: 1235-1244.
Rasul MG, Hiramatsu M and Okubo H (2007). Genetic relatedness diversity and cultivar identification by randomly amplified polymorphic DNA RAPD markers in teasle gourd *Momordica dioica* Roxb. *Sci. Hort.* 111: 271-279.
Redden B and Berger J (2007). History and Origion of Chickpea. In: Chickpea Breeding and Management (Yadav SS, Redden B, Chen W and Sharma B, eds.). CAB International, Wallingford, 1-13.
Saker MM, Youssef SS, Abdullah NA, Bashandy HS, et al. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *Afr. J. Biotechol.* 4: 882-890.
Singh KB and Ocampo B (1997). Exploitation of wild *Cicer* species for yield improvement in chickpea. *Theor. Appl. Genet.* 95: 418-423.
Smithson JB, Thompson JA and Summerfield RJ (1985). Chickpea (*Cicer arietinum* L.). In: Grain Legume Crops (Summerfield RJ and Roberts EH, eds.). Collins, London, 312-390.
Souframanien J and Gopalakrishna T (2004). A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. *Theor. Appl. Genet.* 109: 1687-1693.
Talebi R, Fayaz F, Mardi M, Pirsyedi SM, et al. (2008a). Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers. *Int. J. Agric. Biol.* 8: 1560-8530.
Talebi R, Naji AM and Fayaz F (2008b). Geographical patterns of genetic diversity in cultivated chickpea (*Cicer arietinum* L.) characterized by amplified fragment length polymorphism. *Plant Soil. Environ.* 54: 447-452.
Talebi R, Jelodar NAB, Mardi M, Fayaz F, et al. (2009). Phylogenetic diversity and relationship among annual *Cicer* species using RAPD markers. *Gen. Appl. Pl. Physiology.* 35: 3-12.
Tanno K and Willcox G (2006). The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L. early finds from Tell el-Kerkh, north-west Syria, late 10th mellinnium B.P. *Veg. Hist. Archaeobot.* 15: 197-204.
Tayyar RI and Waines JG (1996). Genetic relationships among annual species of *Cicer* (Fabaceae) using isozyme variation. *Theor. Appl. Genet.* 92: 245-254.
Toker C (2009). A note on the evolution of kabuli chickpea as shown by induced mutations in *Cicer arietinum* Ladizinsky. *Genet. Resour. Crop Evol.* 56: 7-12.
Upadhyaya HD, Bramel PJ and Singh S (2001). Development of a chickpea core subset using geographic distribution and quantitative traits. *Crop Sci.* 41: 206-210
Welsh J and McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218.
Williams JG, Kubelik AR, Livak KJ, Rafalski JA, et al. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18: 6531-6535.