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Assessment of genetic diversity of indigenous turmeric (*Curcuma longa* L.) germplasm from Pakistan using RAPD markers

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Molecular genetic fingerprints of indigenous turmeric (*Curcuma longa* L.) genotypes were developed using Randomly Amplified Polymorphic DNA (RAPD) marker to elucidate the genetic diversity among the genotypes. DNA was isolated using CTAB method. The amplification was accomplished by using 10 primers and the specific PCR working program. Ten decamer-primers generated 95 RAPD fragments, of which 92 fragments were polymorphic with 96.84% of polymorphism. Some of the RAPD markers were useful for genotypes discrimination and identification. Most of the RAPD markers studied showed different level of genetic polymorphism. Amplified fragment sizes ranged from 200 to 3640 bp. Pair-wise Nei and Li's similarity coefficient value ranged from 0.00 to 0.71 for 20 genotypes of turmeric. A dendrogram was constructed based on the unweighted pair group method using arithmetic averages. Cluster analysis of data using UPGMA algorithm placed the 20 genotypes of turmeric into four groups that are somewhat congruent with classification based on morphological characters proposed by earlier works. This analysis grouped all genotypes from Bannu with two Haripur genotypes and clearly differentiated Kasur and Bannu genotypes into separate groups. This method of analysis can be helpful in selecting diverse parents and give broadness to the germplasm base of turmeric breeding programs in the future.

Key words: *Curcuma longa*, RAPD markers, genetic diversity, polymorphism, UPGMA, Pakistan.

INTRODUCTION

Turmeric (*Curcuma longa)* is a plant of the family Zingiberaceae commonly known as the ginger family and comprises about 70 species (Smart and Simmonds, 1992). India is the largest producer, consumer and exporter of turmeric. Turmeric is found throughout South and Southeast Asia with a few species extending to China, Australia and the South Pacific. The highest diversity is concentrated in India and Thailand, with at least 40 species in each area, followed by Burma, Bangladesh, Indonesia and Vietnam. Daod and Aslam (1996) have explained that turmeric has been specialized in Kasur, Sahiwal and Okara areas in Punjab and Bannu, Pubbi and Haripur areas in the Province of Khyber Pakhtunkhwa Pakistan.

Scientists are increasingly recognizing the importance of various spice-crops, especially turmeric. Vavilova (1990) reported that the powdered root of turmeric has been used for making a deep yellow dye for fabrics. It is also used as a coloring material for medicines at times. Turmeric plays an important role in the food industry, as a substitution of synthetic coloring, besides being used for its medicinal and pharmacological qualities (Scartezzini and Speroni, 2000). Turmeric has attracted much attention due to its significant medicinal potential (Cousins et al., 2007). A compound Curcuminoid is present in turmeric and acts as an inhibitor of the Human Immunodeficiency Virus Type1 (HIV-1) integrase (Mazumder et al., 1995). Turmeric oil is composed of several monoterpene and sesquiterpene compounds such as zingiberene, arturmerone and turmerone (Apisariyakul et al., 1995). The

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main biological activities of the oil are carminative, antiflatulence, antifungal as well as an antiplatelet agent (Lee, 2006).

Turmeric has been used in traditional medicines as a household remedy for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis (Ammon et al., 1992). From Pakistan, such information has been compiled and reported by Shinwari (2010) and Gilani et al. (2010). In old Hindu medicine, it was extensively used for the treatment of sprains and swelling caused by injury (Ammon and Wahl, 1991). In addition to helping cure some common diseases, Curcuma species also show some medicinal properties for the treatment of snake bites (Ratanabanangkoon et al., 1993) and as an antitumor (Baatout et al., 2004). The World Health Organization has recommended the use of this spice (Vavilova, 1990). Turmeric also demonstrated anti-fungal properties (Afaq et al., 2002). Turmeric has been reported to possess anti-inflammatory, hepatoprotective, antitumor, antiviral (Ammon and Wahl, 1991) and anticancer activities (Polasa et al., 1991).

Molecular markers RAPD, RFLP, SSR, etc were used to study inter- and intra-specific diversity in monocots and dicots. While to higher ranks, various genes (*matK, rbcL, rpl16 intron, rps16 intron, and 3′ trnK intron*). DNA sequencing was used to clear taxonomic confusion (Shinwari et al., 1994; Shinwari, 1998). Pinheiro et al. (2003) analyzed the genetic divergence in 20 accessions of *C. longa* based on molecular RAPD markers, and showed a small divergence between accessions. Recently in an attempt, Symkumar (2007) developed genetic fingerprints of 15 *Curcuma* species using RAPD and ISSR markers. Shamina et al. (1998) studied isozyme polymorphism in a germplasm collection of *C. longa* and used acid phosphatase, superoxide dismutase, esterase, polyphenol oxidase, peroxidase and catalase which showed good polymorphism in the 15 accessions studied. Analysis of the genetic of Zingiber, *Curcuma* and *Alpinia* using rice microsatellites as RAPD markers also demonstrated high polymorphism and confirmed the usefulness of these in genetic diversity studies of the Zingiberaceae (Jatoi et al., 2006). Apavatjrut et al. (1999) studied an isozyme polymorphism to identify some early flowering *Curcuma* species that include only seven species. An RAPD based assay revealed that the genetic diversity of *Curcuma zedoaria* was maintained to a greater extent in hilly populations than in plain and plateau populations (Islam et al., 2005). Significant variation among 17 elite cultivars of *C. longa* was determined using cytological and RAPD markers (Nayak et al., 2006) and investigated the single nucleotide sequence of *trnk* gene to identify *Curcuma longa*, *Curcuma phaeocaulis, Curcuma zedoaria* and *Curcuma aromatica*). Cao and Komatsu (2003) established simple and rapid molecular identification method for six medicinal *Curcuma* species from China using the *trnk* nucleotide sequences.

In order to conserve the genetic resources and get consistent variability, genetic studies involving molecular markers are used to detect relationship and genetic variability among germplasm. For landraces identification and utilization with novel character, polymorphism based on DNA is the best approach among molecular genetic fingerprinting. Among molecular marker techniques, one most progressively more used marker is random amplified polymorphic DNA (RAPD) due to its simplicity and low-cost nature (Williams et al., 1990). The present work involves the fingerprinting of turmeric crop genome without any prior information of genetic sequence and has been used for estimating genetic polymorphism at the species level. DNA markers are a consistent means to measure genetic drift in crop germplasm and studying the heterogeneity among the genotypes (Fukuoka et al., 2006). Genotypes and RAPD fingerprinting have been used for detecting genetic diversity in landraces and identification of accessions present in duplicate (Saker et al., 2005).

The genetic composition of *C. longa* species needs to be assessed for efficient maintenance and conservation. There has been no previous report on the use of this method to elucidate the genetic diversity of *C. longa* in various eco-geographical zones of Pakistan. The objective of this study was to evaluate the presence and pattern of genetic variability and relatedness among intraspecific genotypes of turmeric collected from selected areas of Pakistan by RAPD markers.

MATERIALS AND METHODS

Plant materials

Fresh plants of twenty genotypes of *C. longa* comprising of three populations were used in the present study for comparison and they were collected from Kasur, Haripur and Bannu, covering almost the three ecological zones of turmeric-growing in Pakistan. The germplasm of the plant is also preserved in the Genebank, PGRP, NARC, Islamabad. Twelve samples of *C. longa* were collected from Bannu, five samples from Haripur, while three samples were collected from Kasur population (Table 1). The sampling was done from September 2009 to February 2010. The research work comprised of two phases conducted under field and laboratory conditions.

RAPD analysis

Polymerase Chain Reaction (PCR) was carried out on 20 µl volume (19 µl master mix + 1µl template DNA) in a thermocycler ABI (Applied Biosystem Inc, USA) for amplification. A modified RAPD method based on Williams et al. (1990) was used with a thermocycler model Veriti 96 wells. Amplification reaction mix of 20 µl containing 1×PCR buffer (10 mM Tris-HCl; pH 8.3; 50 mM KCl), 1.5 mM $MgCl_2$, 200 µM each of deoxynucleotide triphosphate (dNTP), 0.2µM of 10-mer primer, 1 unit *Taq* DNA polymerase and 20 ng of template genomic DNA was used. Taq DNA polymerase and reaction buffer were supplied from Applied Biosystem, USA.

Table 1. Sampling of indigenous turmeric (*C. longa* L.) genotypes from selected areas of Pakistan.

No. of genotype	Genotype code	Collection area
12	B1-B12	District Bannu
5	H1-H5	District Haripur
3	K1-K3	District Kasur

RAPD-PCR temperature profile

Amplification was programmed to 1 cycle of 5 min at 94°C for initial strand separation. This was followed by 45 cycles of denaturation for 40 s at 94°C, 40 s at 36°C for primer annealing and 40 s at 72°C for primer extension. Finally, 1 cycle of 10 min at 72ºC was used for the final extension and kept hold temperature at 4°C forever. Amplified products were resolved on a 1.5% agarose gel in 1×TBE (10mM Tris-Borate, 1mM EDTA) buffer containing 0.5 µg ml^{-1} of ethidium bromide along with 1 kb DNA ladder (Fermantas). After electrophoresis, the gels were documented using UV illuminator system. After PCR reaction, DNA was analyzed through gel electrophoresis.

Data analysis

Amplified agarose gel pictures were compared with each other, by considering the presence of bands represented by "1" and absence represented by "0". The molecular size of the amplification product was calculated with a 1 kb molecular size weight marker (Fermantas). All the amplified profiles were analyzed together to form a binary data matrix for the estimation of genetic distance among the 20 genotypes. Estimates of genetic similarity (F) were calculated between all pairs of the genotypes by the Dice algorithm which is identical to that of Nei and Li (1979). The resulting similarity coefficients were used to evaluate the relationship among all the genotypes with a cluster analysis using an Un-Weighted Pair Group Method with Arithmetic Averages (UPGMA) and then plotted in the form of a dendrogram. All these were conducted using a computer software; Numerical Taxonomy and Multivariate Analysis System (NTSYS/ RAP Distance) PC Version 2.01 (Roulf, 2002).

RESULTS

Extraction of genomic DNA

The method of DNA extraction from fresh leaves samples of turmeric included two major steps, (i) treatment by DNA extraction buffer, and (ii) grinding of fresh leaves in the buffer, after incubation. Using this method of DNA extraction, we got DNA yield of 1.0~2.0 µg per single sample. The quality and quantity of DNA was obtained with normal methods. The purity of DNA determined from the A260/280 ratio ranged from 1.63 to 2.58 and averaged 2.32 for all the samples.

DNA amplification and genotypes identification

The amplification profiles generated by all the primers across a number of genotypes are described in Figure 1. In majority of the cases, all the genotypes exhibited different banding patterns. The RAPD exhibited several bands that were shared among the Bannu and Haripur genotypes, whereas a few bands were shared among Haripur and Kasur genotypes. Genotypes H4, H5, K1, K2, and K3 shared a limited number of bands with all the other genotypes, showing their most distant relationship to all other genotypes.

Among the 10 decamer oligonucleotides primers used for the amplification of the turmeric genomic DNA, 3 primers performed poorly and produced either faint bands or did not amplify the DNA in some of the genotypes. Primers varied greatly in their ability to resolve variability among the genotypes. Some primers generated several bands, while others generated only a few. A summary of the all amplified products from this study is given in Table 2. Data obtained shows comparable number and size of amplified products. As expected from related turmeric genotypes, only 3 amplified fragments were monomorphic. A total of 95 reproducible and scorable amplification products were generated by 10 primers across 20 genotypes out of which 92 (96.84%) fragments were polymorphic. The number of amplification products generated by each primer varied from 6 (OPA-07 and OPA-08) to 14 (OPC-07). The size of the amplified fragments ranged from 200 (OPA-10) to 3640 bp by (OPD-08). The study showed that most of the genotypes of turmeric were genetically different from each others.

Similarity matrix

A similarity matrix based on the proportion of shared RAPD fragments was used to establish the level of genetic relatedness among Bannu, Haripur, and Kasur genotypes studied during the present investigation (Table 3). As expected, genetic distances between the Bannu and Haripur genotypes were comparatively lower than the Kasur genotypes, which show a high genetic distance from Bannu genotypes as compared to the Haripur genotypes. Pair-wise estimate of similarity for 20 genotypes belonging to three different populations ranged from 0.00 to 0.71. Two pairs of genotypes B2 and B3 and B4 and B5 were the closest genotypes with the same highest similarity index of 0.71. It was followed by another two pairs of genotypes B6 and B7 and B9 and B10 with a

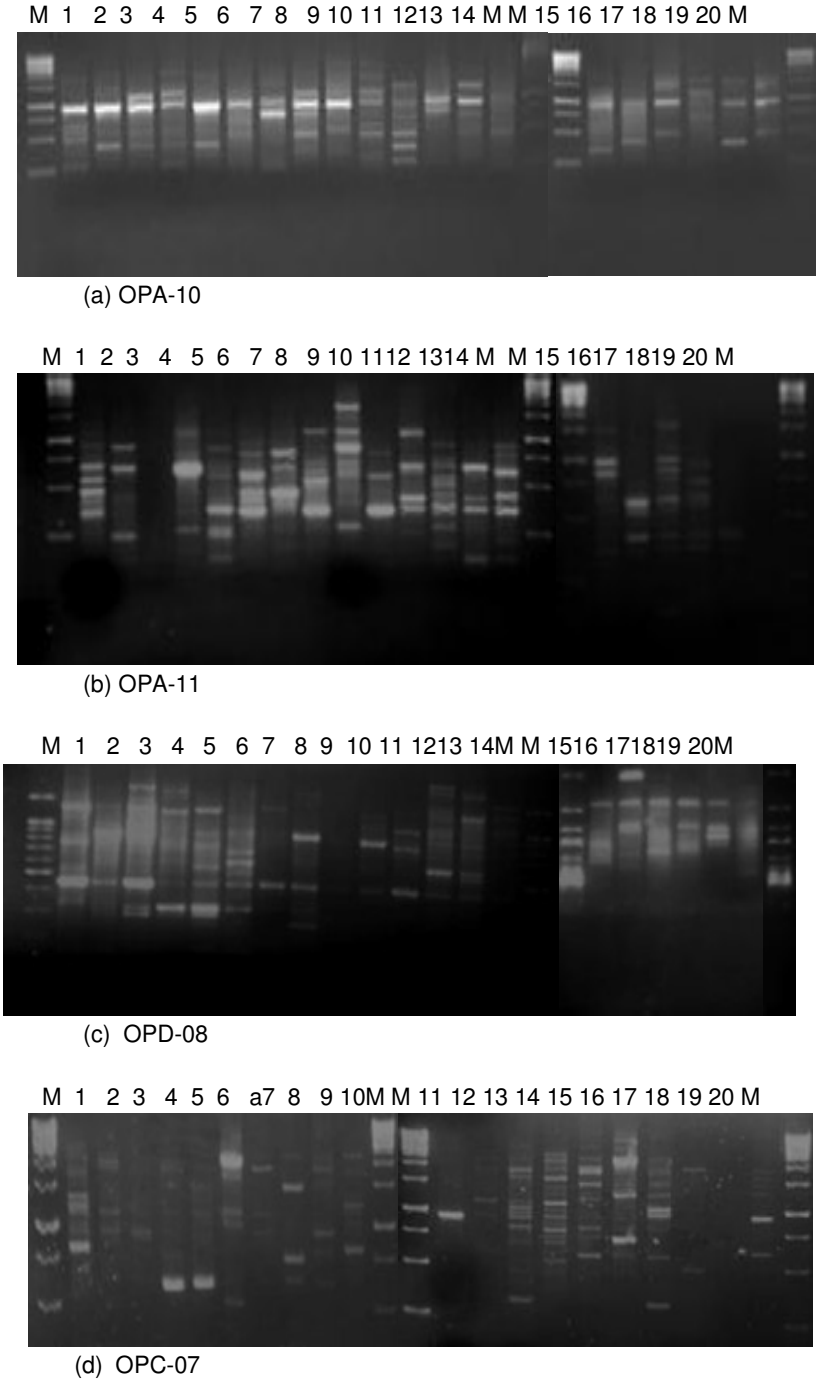

Figure 1. RAPD banding pattern of 20 genotypes of turmeric comprising of three populations (Bannu, Haripur, and Kasur) generated by random primers (a) OPA-10, (b) OPA-11, (c) OPD-08 and (d) OPC-07. Numbers denote 1-B1, 2-B2, 3-B3, 4-B4, 5-B5, 6-B6, 7-B7, 8-B8, 9-B9, 10-B10, 11-B11, 12-B12, 13-H1, 14-H2, 15-H3, 16-H4, 17-H5, 18-K1, 19-K2, and 20-K3 genotypes represented, respectively.

similarity index of 0.63 which are the closest genotypes belonging to Bannu.

Minimum co-efficient of similarity (0.00) was recorded between Genotypes K2 and B4, belonging to Kasur and

Table 2. Description of the primers and RAPDs products in turmeric genotypes of Pakistan.

RAPD primer	Sequence of primer (5' - 3')	Amplified bands (a)	No. of polymorphic bands (b)	% of polymorphism	Size range of amplicons (bp)
OPA-07	5′GAAACGGGTG3′	6	5	83.33	350-900
OPA-08	5′GTGACGTAGG3′	6	6	100	350-1250
OPA-10	5′GTGATCGCAG3′	9	9	100	200-1800
OPA-11	5′AAAGCTGCGG3′	10	10	100	400-1680
OPA-12	5′TGTCATCCCC3′	7	7	100	350-1450
OPA-18	5′AGGTGACCGT3′	11	10	90.9	400-1800
OPA-19	5′CAAACGTCGG3′	9	9	100	300-2500
OPB-07	5′GGTGACGCAG3′	11	10	90.9	430-1660
OPC-07	5′GTCCCGACGA3′	14	14	100	300-3400
OPD-08	5′GTGTGCCCCA3′	12	12	100	500-3640
Total	**10 primers**	**95**	**92**	**96.84**	**200-3640**
	Average	**9.5**	**9.2**		

Table 3. Dice coefficients of similarity based on RAPDs showing the relationship between turmeric genotypes.

Genotype	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	H1	H2	H3	H4	H5	K1	K2	K3
B1	1.00																			
B2	0.51	1.00																		
B3	0.48	0.71	1.00																	
B4	0.50	0.44	0.47	1.00																
B5	0.58	0.52	0.51	0.71	1.00															
B6	0.51	0.58	0.45	0.48	0.62	1.00														
B7	0.39	0.41	0.34	0.57	0.52	0.63	1.00													
B8	0.39	0.36	0.24	0.26	0.35	0.33	0.29	1.00												
B9	0.49	0.37	0.28	0.55	0.39	0.38	0.43	0.28	1.00											
B10	0.44	0.40	0.35	0.46	0.36	0.41	0.35	0.31	0.63	1.00										
B11	0.36	0.32	0.30	0.38	0.35	0.33	0.35	0.45	0.50	0.57	1.00									
B12	0.29	0.34	0.42	0.28	0.34	0.36	0.16	0.32	0.19	0.25	0.33	1.00								
H1	0.50	0.55	0.47	0.43	0.53	0.48	0.30	0.36	0.46	0.49	0.48	0.43	1.00							
H2	0.41	0.42	0.24	0.41	0.38	0.39	0.25	0.33	0.44	0.51	0.40	0.27	0.58	1.00						
H3	0.16	0.45	0.38	0.24	0.22	0.32	0.06	0.27	0.15	0.13	0.17	0.30	0.28	0.27	1.00					
H4	0.27	0.18	0.19	0.21	0.12	0.33	0.20	0.14	0.25	0.19	0.19	0.21	0.18	0.29	0.16	1.00				
H5	0.33	0.29	0.30	0.28	0.29	0.37	0.23	0.30	0.44	0.35	0.41	0.37	0.45	0.27	0.42	0.34	1.00			
K1	0.16	0.26	0.22	0.24	0.18	0.19	0.11	0.28	0.27	0.17	0.26	0.31	0.29	0.23	0.32	0.22	0.47	1.00		
K2	0.15	0.24	0.40	0.23	0.15	0.16	0.14	0.20	0.09	0.15	0.24	0.31	0.23	0.00	0.40	0.27	0.29	0.33	1.00	
K3	0.15	0.18	0.27	0.06	0.10	0.16	0.14	0.27	0.18	0.15	0.24	0.31	0.33	0.05	0.24	0.13	0.39	0.25	0.56	1.00

Bannu, respectively. When Kasur genotypes were compared with Haripur and Bannu genotypes, Kasur genotypes were found to be more similar to each other than those of Haripur and Bannu genotypes. The study shows that the genotypes of Kasur population had the greatest dissimilarity with all the other tested genotypes. Although comparison with Kasur genotypes indicated that these were closely related with no genotype of this population being exactly like any other genotype in the study. Among the 20 genotypes analyzed, the similarity coefficient of Haripur genotype H1, with all the other genotypes of the Bannu population ranged from 30 to 49, while genotype H3 of Haripur showed a similarity coefficient of 0.06 to 0.45 with all the genotypes belonging to the Bannu population. Similarly, the similarity co-efficient of Genotypes K1, K2, K3 belonging to Kasur with all the other genotypes belonging to Bannu and Haripur ranged from 0.11 to 0.47, 0.00 to 0.40, and 0.06 to 0.56 respectively, while Genotypes K1 and K2, and K2 and K3, showed 0.33 and 0.56 similarity index with one another.

UPGMA cluster analysis based on similarity level

Genetic diversity acquired from RAPD data was used to make a cluster diagram. Based on analysis performed on Nei and Li's distance matrix using UPGMA, 20 genotypes were grouped into several clusters and a genotype "16" belonging to Haripur as an independent genotype. As expected from the similarity estimates, cluster analysis placed most of the genotypes close to each other belonging to different localities and showing a high level of genetic relatedness.

UPGMA cluster can be divided into Clusters I (CI) and II (CII) at 0.22 similarity levels. Cluster I had nineteen genotypes (B1 to B12, H1, H2, H3, H5, K1, K2 and K3) collected from Bannu, Haripur and Kasur, respectively, while Cluster II had a single genotype (H4) collected from Haripur, and remained unresolved showing 22% similarity with the other genotypes of the same species.

Cluster I showed a high level of genetic variation among the genotypes and was further sub-divided into two sub-clusters. The Sub-Cluster I (SCI) showed a 24% similarity level with the Sub Cluster II (SCII) genotypes. Sub-Cluster I had thirteen genotypes (B1 to B11, H1 and H2) belonging to different areas of Bannu and Haripur, while Sub-Cluster II contained six genotypes (B12, H3, H5, K1, K2, and K3) belonging to all three populations.

Sub-Cluster I (SCI) is further sub-divided into two clusters; SCI 'A' and SCI 'B' showing 0.34 similarity level. Sub-Cluster SC1 'A' is further divided into two clusters 'A1' and 'A2' showing 0.40 similarity level. Cluster 'A1' comprised of seven genotypes (B1, B2, B3, B4, B5, B6 and B7); all of them belonged to the same area as Bannu showing 0.46 similarity level with all the genotypes. Similarity levels in the cluster is thus: Genotype (B1) showing 48% similarity, Genotypes (B4, B5) showing 72% similarity, Genotypes (B6, B7) showing 0.60 similarity level, and Genotypes (B2, B3) showing 72%. Cluster 'A2' contained five genotypes (B9, B10, B11, H1 and H2) belonging to different areas of Bannu and Haripur and showed 0.64, 0.54 and 0.58 similarity level with one another, respectively. Sub-cluster SCI 'B' had only one Genotype (B8) belonging to Bannu and remained unresolved and showed 34% similarity with the other genotypes of the same species.

The Sub-Cluster II (SCII) is further sub-divided into two clusters, SCII 'C' and SCII 'D' at 0.32 similarity level. Subcluster II 'C' contained four Genotypes (B12, H3, H5 and K1) belonging to different areas of Bannu, Haripur and Kasur with 34% similarity level of genotype 'B12', 38% similarity level of genotype 'H3', and 48% similarity level of genotypes 'H5 and 'K1', while Sub-Cluster II 'D' contained two Genotypes (K2 and K3) with 56% similarity level with one another belonging to the same area i.e., Kasur (Figure 2).

DISCUSSION

The main emphasis of the present study was to assess the genetic diversity at intra specific level using RAPD markers and differentiation of genotypes collected from three different populations in Pakistan at molecular level. As mentioned by Padua et al. (1999), the overall appearance of many species of *Curcuma* is very similar as they differ in small morphological details, DNA fingerprinting can be used to support the morphological data.

The Random Amplified Polymorphic DNA method is based on the Polymerase Chain Reaction (PCR) using short (usually ten nucleotides) primers of arbitrary sequences. Polymorphism of amplified fragments is caused by: (a) base substitution or deletion in the priming sites, (b) insertion that renders priming sites too distant to support amplification, or (c) insertion or deletion that changes the size of the amplified fragment (Williams et al., 1990). In the present study, a dendrogram was constructed based on the PCR (RAPD) marker which showed that 96.84% of the bands were polymorphic between 20 genotypes of turmeric (*C. longa* L.) collected from three different populations of Pakistan. The work of Islam et al. (2007) supported our results that reported a high level of genetic diversity within *C. zedoaria* populations. Hamrick and Loveless (1989) also reported a common trend of maintaining high genetic diversity within populations in tropical plants. Paisooksantivatana et al. (2001) investigated the genetic diversity of *C. alismatifolia* Gagnep in Thailand using allozyme polymorphism and found high levels of genetic diversity within a population. Furthermore, the above result was relatively very high when compared with reports relating to other RAPD findings such as *Alternaria* species (Wilkie et al., 1993), celery (Yang and Quiros, 1994) and wheat (Asif et al.,

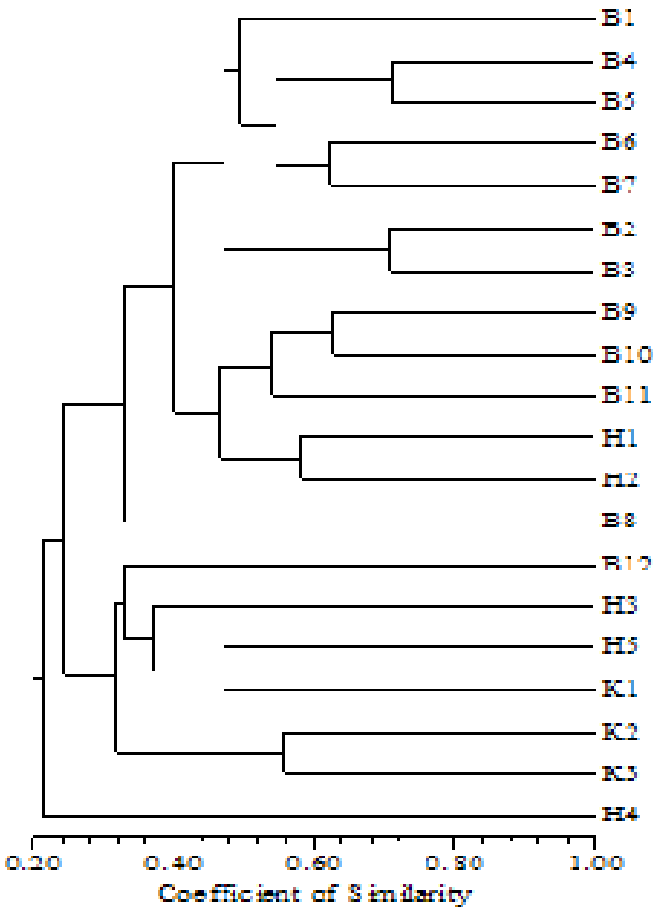

Figure 2. UPGMA cluster analysis showing the relationship (diversity) among turmeric genotypes using 95 RAPD fragments produced by 10 random primers.

2005). The main cause for the high level of polymorphism could be intra- specific variation as reported by Nayak et al. (2006) who demonstrated that high number of polymorphic loci revealed profound intra-specific variation among the turmeric cultivars. Our results are also supported by Huang et al. (2003) who reported significant (high) genetic variations by RAPD markers in other species at cultivar level. Thaikert and Paisooksantivatana (2009) suggested and also supported our results that within-species, high genetic variations do exist in *C. longa* L. even though this plant species is clonally propagated. The reason could be that the overall genetic diversity of all populations of *C. longa L.* was high possibly due to a wide range of ecological conditions within the distribution area of its populations in Pakistan. Our results are in uniformity with the findings of Thaikert and Paisooksantivatana (2009) who reported that the overall genetic diversity of all populations of *C. alismatifolia* was high possibly due to a wide range of ecological conditions within the distribution area of its populations in Thailand.

The present study of indigenous turmeric genetic diversity generated a total of 95 reproducible and scorable amplification products by 10 primers across 20 genotypes, of which 92 fragments (96.84%) were polymorphic. The total number of amplification products generated by each primer varied from 6 (OPA-07 and OPA-08) to 14 (OPC-07). Our results are in conformity with those reported by Thaikert and Paisooksantivatana (2009) and Angle et al. (2008).

The cluster analysis with all the genotypes tested showed a genetic relationship between different genotypes. According to the dendrogram, Genotypes B1, B2, B3, B4, B5, B6, B7, B9, B10 and B11 grouped together, were more genetically similar with each other as compared to other genotypes, which is reasonable because all belong to the population from Bannu and perhaps, have same ancestors as well as same ecological conditions. Genotypes H1, and H2 were also associated with this cluster representing closely relatedness between Bannu and Haripur genotypes. Similarly, Genotypes (K1, K2, and K3) nbelonging to the population from Kasur clustered together thus showing similar genetic relatedness and phenotypic traits.

Our study showed that pair-wise estimates of similarity for 20 genotypes belonging to three different populations ranged from 0.00 to 0.71. Two pairs of Genotypes; B2, B3 and B4, B5; were the closest genotypes with the same highest similarity index of 0.71. It was followed by another two pairs of Genotypes; B6, B7 and B9, B10; with the same similarity index of 0.63 were the closest genotypes belonging to Bannu indicating the highest genetic variability from other populations of indigenous turmeric from Pakistan. Similarly, Yu and Nguyen (1994) found out that rice genotypes IRAT13 and IAC25 were highly associated with each other by a similarity coefficient of 0.79.

In all the 20 genotypes analyzed, the similarity coefficient of Genotype H1, with all the other genotypes of the Bannu population ranged from 30 to 49, while Genotype H3 of Haripur showed a similarity coefficient of 0.06 to 0.45 with all the genotypes belonging to Bannu population. Similarly, the similarity coefficient of Genotypes K1, K2 and K3 belonging to Kasur with all the other genotypes belonging to Bannu and Haripur ranged from 0.11 to 0.47, 0.00 to 0.40, and 0.06 to 0.56, respectively. While Genotypes K1, K2, and K2, K3, showed 0.33 and 0.56 similarity indexes with one another. Knowledge about genetic relationships will be useful to avoid the chance of using genetically similar genotypes/landraces and will also be supportive in future breeding programmes to select genetically diverse parents of turmeric genotypes.

Our investigation affirmed the ability of RAPD markers to differentiate between different populations genotypes / landraces of turmeric and also revealed a primary but key step in using RAPD markers as a means for the assessment of genetic diversity of indigenous turmeric genotypes

in Pakistan.

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