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APPRAISAL OF GENETIC DIVERSITY OF DIFFERENT PEACH CULTIVARS AND GENOTYPES THROUGH RAPD MARKERS

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Abstract

The present study was amid to investigate the genetic diversity of twenty peach cultivars and genotypes by RAPD primers at the Institute of Biotechnology and Genetic Engineering, KPK Agricultural University Peshawar. The result indicated that fifteen primers (GLCO9, GLC20, GLA20, GLA13, GLB10, GLB20, GLB06, GLB19, GLA19, GLB19, GLD16, GLB15, GLA15, GLB12, GLB11) gave genetic distance among the peach cultivars and genotypes under study by PCR amplification. Average genetic diversity (estimated as genetic distance) ranged between 12 and 58%. The molecular size of most of the bands were from 150bp to 1000bp. Based on dandrogram analysis, Khyber 1 and Khyber 2 was grouped in cluster A, and Tex-A6-69 and BY-8-135 in cluster B, Candan and 6A were most closely related cultivars and genotypes among the 20 peach cultivars and genotypes while Lering, Flam crest, Tex x-9, early grand and Floradaking were distinctly grouped when compared with the rest of population.

Introduction

Peach (*Prunus persica* L. Batsch) is a common fruit tree specie grown mainly in the temperate region. It is considered the queen of temperate-zone fruits and, next to apple, is the world's most widely grown fruit tree (Bailey & Bailey, 1976). More than 5000 cultivars of peach are available throughout the world (Anon., 1985). United States Department of Agriculture alone recorded 800 cultivars in 1998 by breeding and introduction and more than 1000 cultivars were selected in China (Wang & Zhuang, 2001). Peach is also considered a traditional fruit crop of Northern Areas of Pakistan and is the second most important fruit crop after apple and ranks first in genus *Prunus*. Genetic diversity of germplasm plays an important role in the establishment of the peach fruit crop.

Molecular markers are used for the characterization and evaluation of genetic diversity among different plant species and population. It has been revealed that different markers revealed different classes of variation (Graham *et al.*, 2004; Lambert *et al.*, 2004; Martin *et al.*, 2004; Fu *et al.*, 2006; Sargent *et al.*, 2007; Lewer *et al.*, 2008; Brennan *et al.*, 2008; Mattia *et al.*, 2008; Cerbert *et al.*, 2008; Ahmed *et al.*, 2009). RAPD (Randomly amplified polymorphic DNA), a PCR based marker system can be readily used, requiring minute amount of genomic DNA and does not require expensive molecular biology techniques like blotting and radioactive detection. DNA finger prints can be generated with RAPD using short nucleotide sequences of arbitrary nature as primers (Fernandez *et al.*, 2002; Nazari & Pakniyat, 2008), does not need any prior knowledge of DNA sequence and also reveals a high level of polymorphism. RAPD PCR is currently used as a common tool to assess different genetic markers for the assessment of genetic variability between genotypes which can be useful in different breeding programmes.

Different molecular markers; randomly amplified polymorphic DNA (RAPD) (Yang *et al.*, 2001 a and b Yuan *et al.*, 2002; Dirlewanger *et al.*, 2002; Baranek *et al.*, 2006; Cheng, 2007; Bakht *et al.*, 2011 a and b; Farhad *et al.*, 2011), amplified fragment length polymorphism (AFLP) (Struss *et al.*, 2002, 2003; Tavaud *et al.*, 2003), Simple Sequence Repeat (SSR) (Wang *et al.*, 2002; Aranzana *et al.*, 2002, 2003; Schuelder *et al.*, 2003; Wunsch & Hormaza, 2004; Baranek *et al.*, 2006; Li *et al.*, 2008; Yong *et al.*, 2009), Random Amplified Microsatellite Polymorphism (RAMP) (Cheng *et al.*, 2001) have been fruitfully used in the identification and diversity of different plant species including peaches. Peach breeding is usually time consuming, especially for fruit-specific characters and therefore, molecular markers linked to these traits are of great importance for the identification and selection of plant genotypes with the aspiration characters long before the traits are expressed (Dirlewanger *et al.*, 1998). Keeping in view the role of RAPD in determination of genetic diversity, the present study was initiated to investigate genetic variation among different peach genotypes for future breeding.

Materials and Methods

Plant material: The present study investigated genetic diversity of twenty peach cultivars by RAPD primers at the Institute of Biotechnology and Genetics Engineering (IBGE), KPK Agricultural University Peshawar Pakistan (Table 1). Plant materials from these peach cultivars and genotypes were collected from Agricultural Research Institute Tarnab Peshawar KPK in liquid nitrogen and further processed at the Institute of Biotechnology and Genetics Engineering (IBGE), KPK Agricultural University Peshawar Pakistan.

Table 1. Different peach cultivars and genotypes used during the present study.

S. No.	Cultivar names	S. No.	Cultivar names
1.	Floradaking	11.	Lering
2.	Early grand	12.	6-A
3.	Khyber 1	13.	Prime Rose
4.	Khyber 2	14.	Tex-M-9
5.	Late florderson	15.	Havester
6.	Tex-A6- 69	16.	Tex-X-9
7.	By-8-135	17.	Tex-Y7-72
8.	By-8-1664	18.	Plain 4
9.	Candon	19.	Tex-Y2-7 (1569)
10.	Tex-Y5-67	20.	Flame crest

DNA extraction: DNA was extracted according to the methods of Weining & Langridge, (1999). Briefly, 100 mg leaf materials were crushed with a knitting needle to fine powder and mixed with extraction buffer (l% SDS, 100 mM NaCl, 100 mM Tris-HCI, 50 mM EDTA, pH 8.5). Equal volume of phenol: chloroform isoamylalcohol (ratio of 25:24:1) was added and centrifuged at 14000 rpm for 5 minutes. The aqueous phase was re-extracted with equal volume of chloroform (i.e. mixed, centrifuged at 14000 rpm for 5 minutes). One-tenth volume of 3 M sodium acetate (pH 4.8) and equal volume of isopropanol were added and centrifuged at 14000 rpm for 5 minutes. The pellet was washed with 100%, 80% and 70% ethanol respectively, dried at room temperature for 10 minutes and re-suspended in sterile distilled water. For complete dissolution of DNA, the samples were heated at 70^0C for 10 minutes. To remove RNA, DNA was treated with 40µg RNAse-A at 37^0C for 1 hour and samples were re-extracted first with phenol: chloroform: isloamyl alcohol, then with chloroform and finally precipitated with sodium acetate as described earlier. DNA samples were centrifuged, washed with ethanol (100%, 80% and 70%) dried and dissolved as before. DNA samples were stored at -80^0C until used. Before PCR, quality and quantity of DNA was determined by UV spectrophotometer at 260nm and 280nm.

Polymerase chain reaction: PCR reaction was carried out according to the protocols of Devos & Gale (1992) with certain modifications. For PCR, Randomly Amplified Polymorphic DNA primers obtained from Operon Technologies USA were used (Table 2). PCR reaction was carried out in 2µl reaction containing 100ng total genomic DNA template, 0.25µM of each primer, 200µM of each dATP, dGTP, dCTP, dTTP, 50mM KCI, l0mM Tris, 1.5mM Mg Cl_2 and 2.5 units of Taq DNA polymerase (Dweikat *et al.*, 1993). Amplification conditions for RAPD primers were an initial denaturation step of 4 minute at 94^0C followed by 40 cycles each consisting of a denaturation step of 1 minute at 94^0C, annealing step of 1 minute at 36^0C, and an extension step of 2 minutes at 72^0C. The last cycle was followed by 10 minutes extension at 72^0C. All amplification reactions were performed using A GeneAmp PCR System 2700 (Applied Biosystem) programmable thermocycler. The amplification products were electrophoresed on 1.5% agarose/TBE gel and visualized by staining with ethidium bromide under ultra-violet (U.V) light and photographed.

Table 2. Name, sequence and size of the 15 RAPD primers used in the present study.

S. No	Name	Sequence	Size (bp)
1.	GLC09	CTCACCGTCC	10
2.	GLC20	ACTTCGCCAC	10
3.	GLA20	GTTGCGATCC	10
4.	GLA13	CAGCACCAC	10
5.	GLB10	GCTGGGACC	10
6.	GLB20	GGACCCTTAC	10
7.	GLB06	TGCTCTGCCC	10
8.	GLB19	ACCCCCGAAG	10
9	GLB18	CCACAGCAGT	10
10.	GLA19	CAAACGTCGG	10
11.	GLD16	AGGGCGTAAG	10
12.	GLB15	GGAGGGTGTT	10
13.	GLA15	TTCCGAACCC	10
14.	GLB12	CCTTGACGCA	10
15.	GLB11	GTAGACCCGT	10

Statistical analysis: For statistical analysis every score able bands of RAPD was considered as a single locus. The loci were scored as present (1) or absent (0) to generate Bi-variate 1-0 data matrix. Genetic distances were calculated using the following Unweighted Pair Group of Arithmetic Means (UPGMA) procedure (Nei & Li, 1979) and cultivars and genotypes were clustered using computer program "Popgene32" version 1.31.

$GD_{xy} = l\text{-}d_{xy}/d_x+d_y\text{-}d_{xy}$,

Where GD_{xy} = Genetic distance between two genotypes,

d_{xy} = Total number of common loci (bands) in two genotypes,

d_x = Total number of loci (bands) in genotype,

d_x = Total numbers of loci (bands) in genotype 2.

Results and Discussion

For genetic diversity studies of 20 peach cultivars and genotypes, different Randomly Amplified Polymophic DNA (RAPD) primers were used (Table 2) and only fifteen primers gave reproducible amplified product. Genetic dissimilarity for each comparison was estimated using Bi-variate data. The different alleles amplified by these primers were 5.8, 6.6, 0.8, 8.1, 3.0, 1.75, 4.05, 5.5, 4.6, 5.5, 2.2, 3.1, 0.8, 11.8 and 3.8 alleles cultivar^{-1}. Among the fifteen studied primers, GLA13 amplified maximum alleles (8.1 cultivar^{-1}; Fig. 1) followed by GLC20 (6.6 alleles cultivar^{-1}; Fig. 2) while minimum (0.8 alleles cultivar^{-1}) by GLA20 (Fig. 3).The amplified fragments generated by RAPD primers ranged between 150 and 1000 base pair (bp). Over all genetic distance among 20 peach cultivars and genotypes ranged between 16% and 58% (Table 3). Similar results are also reported by Warburton *et al.*, (1996), Yang *et al.*, (2001 a and b) and Yuan *et al.*, (2002), Baranek *et al.*, (2006) and Li *et al.*, (2008).

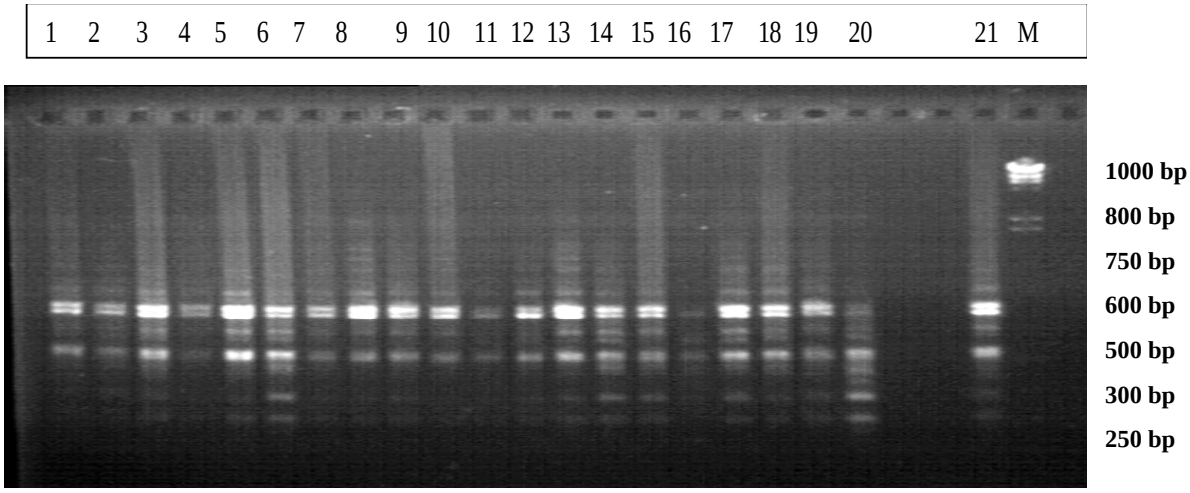

Fig. 1. Electrophoregram showing PCR based amplification product of twenty peach cultivars and genotypes by RAPD primer GLA13 (Line 1-20 = peach cultivars; M = molecular weight marker; for detail see Materials and Methods).

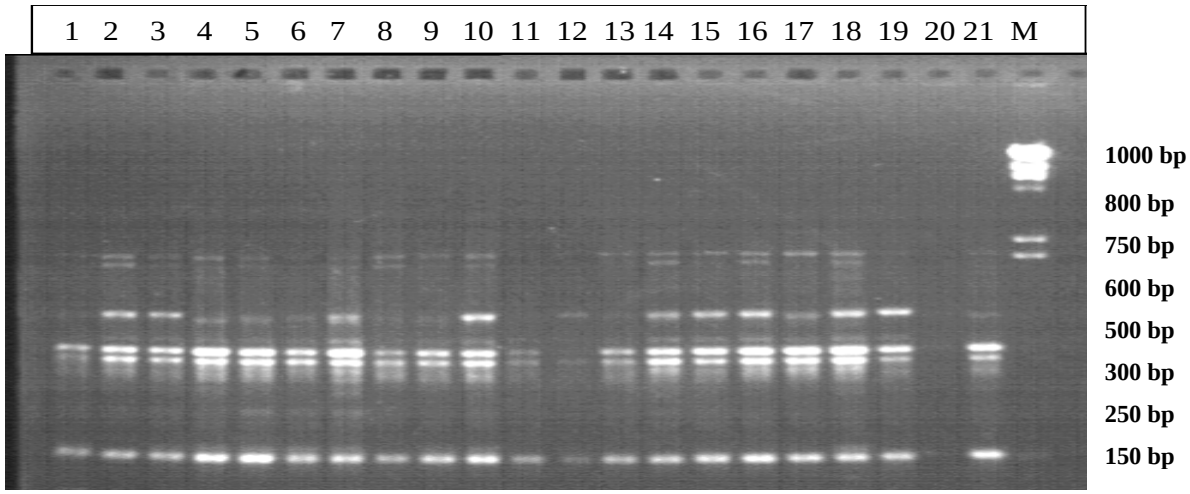

Fig. 2. Electrophoregram showing PCR based amplification product of twenty peach cultivars and genotypesby RAPD primer GLC20 (Line 1-20 = peach cultivars; M = molecular weight marker; for detail see Materials and Methods).

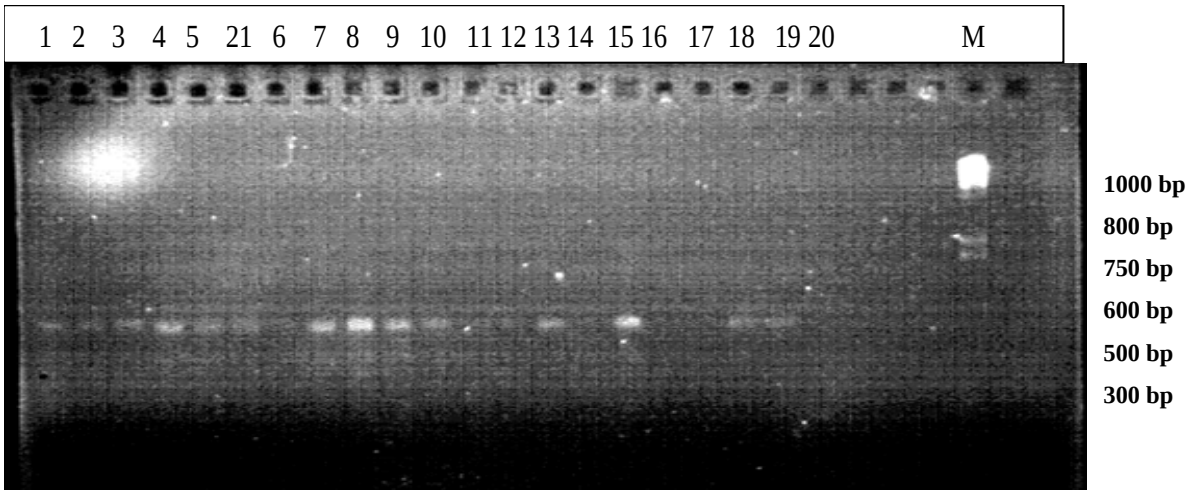

Fig. 3. Electrophoregram showing PCR based amplification product of twenty peach cultivars and genotypes by RAPD primer GLA20 (Line 1-20 = peach cultivars; M = molecular weight marker; for detail see Materials and Methods).

Table 3. Average estimates of genetic distance among 20 peach cultivars and genotypes using 15 RAPD primers.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	0.33																			
3	0.31	0.24																		
4	0.33	0.28	0.32																	
5	0.33	0.26	0.24	0.24																
6	0.45	0.26	0.32	0.39	0.28															
7	0.38	0.25	0.26	0.36	0.29	0.28														
8	0.40	0.26	0.34	0.30	0.32	0.42	0.29													
9	0.35	0.23	0.26	0.29	0.30	0.33	0.31	0.25												
10	0.37	0.27	0.25	0.29	0.25	0.30	0.26	0.25	0.31											
11	0.50	0.49	0.49	0.53	0.47	0.43	0.40	0.43	0.45	0.47										
12	0.40	0.27	0.33	0.35	0.27	0.37	0.34	0.23	0.12	0.29	0.45									
13	0.53	0.30	0.35	0.45	0.40	0.35	0.27	0.32	0.31	0.36	0.43	0.33								
14	0.44	0.28	0.30	0.34	0.21	0.35	0.19	0.19	0.20	0.30	0.42	0.24	0.19							
15	0.53	0.33	0.30	0.44	0.35	0.33	0.25	0.30	0.36	0.33	0.53	0.43	0.20	0.22						
16	0.42	0.20	0.27	0.40	0.36	0.40	0.35	0.36	0.25	0.31	0.54	0.25	0.25	0.26	0.20					
17	0.47	0.16	0.23	0.37	0.30	0.27	0.30	0.33	0.25	0.26	0.52	0.26	0.30	0.26	0.25	0.20				
18	0.35	0.34	0.36	0.36	0.26	0.32	0.30	0.31	0.32	030	0.40	0.34	0.34	0.23	0.34	0.35	0.26			
19	0.47	0.49	0.36	0.44	0.35	0.38	0.35	0.51	0.40	0.45	0.47	0.46	0.44	0.44	0.44	0.40	0.45	0.38		
20	0.32	0.39	0.37	0.40	0.33	0.36	0.37	0.30	0.26	0.32	0.58	0.30	0.36	0.27	0.32	0.26	0.30	0.30	0.37	

From 1 to 20 = Peach cultivars; for detail see Materials and Methods

The level of polymorphism observed in the present study with 15 primers was 31% which is close to the value reported by Dettori (2001) in peach through different RAPD primers. Similarly, Dirlewanger *et al.,* (1998), Warburton *et al.,* (1996) reported 17% and 39% genetic diversity respectively among different peach cultivars using various RAPD primers. Gerlach & Stosser (1998) reported 28% polymorphism in cherry. These differences in genetic diversity could be explained by the differences in primer sets used, criteria for selecting markers and the number and origin of cultivar analyzed. There is a correlation between the variability found in specie and it's mating system. It is low in self pollinating (self-compatible) species as peach while high in the self incompatible species such as almond. Similar results have also been reported in prunus using isozymes (Byrne & Littleton. 1989). The number of alleles in all loci ranged from 0.27 to 0.48 with mean of 0.32 among the 20 tested peach cultivars and genotypes. The order for different cultivars and genotypes of peach were as Lering, Floradaking, Tex-Y2-7(1569), Tex-Y5-67, Plain-4, Tex-A6-69, Tex-Y7-72, 6-A, Late florderson, Havester, Tex-X9, By-8-135, Prime rose, Tex-M9, Candan, By-8-1664, Khyber 1, Khyber 2 and Early grand (Cheng, 2007).

Cluster analysis: The genetic dissimilarity coefficient matrix of 20 peach cultivars and genotypes was based on the data of 15 RAPD primer using UPGMA method (Nei & Lie, 1979). The UPGMA grouped 20 cultivars and genotypes of peaches into two main clusters (Fig. 4). Cluster-A consisted of six cultivars (28.57% of the total population) and was further divided into three sub-clusters (I, II & III). Sub-cluster-I encircled Tex-Y5-67, sub-cluster-II Early grand and sub-cluster-III grouped four cultivars (Late florderson, Khyber 2, Khyber 1, and Flordaking). Among these 20 peach cultivars and genotypes, Khyber 2 and Khyber 1 showed highest level of genetic similarity. Cluster-2 contained By-8-1664 and Learing which were unique and distinctly related. Cluster-3 comprised of 13 cultivars (61.9% of the total population) and subdivided into three sub-clusters. Sub-cluster-I grouped Tex-Y2-7(1569) and Plain 4; sub-cluster-II clustered Flame crest, Tex-X-9, Tex-M9, 6-A, Tex-A6-69 and Candan while sub-cluster-III grouped Tex-Y7-72, Havester, Prime Rose and By-8-135. Out of the 13 cultivar, Tex-Y2-7(1569), Plain 4; A6 and Candan; By-8-135 and Tex-A6-69 showed highest level of genetic similarity. According to the dendrogram analysis, Lering, By-8-1664, Tex-Y5-67, Early grand and Floradaking were distinctly grouped and showed genetic dissimilarity when compared with the rest of the population used in the present study. These results agree with those reported by Zhen-Xiang *et al.,* (1996). The cluster analysis showed that there was a high similarity in A and B cluster among the different peach cultivars and genotypes under study. The possible explanation might be that frequent gene flow from one cultivar to another occurred because of the crossing for new cultivar breeding in history. Between genetically distinct cluster A and B, there were few chance of crossing and thus gene exchange was limited. It is possible to gain new cultivars or to create novel germplasm of peach through hybridization between genetically distinct species (Wang *et al.,* 2001).

Fig. 4. Dendrogram analysis of 20 peach cultivars and genotypes amplified by 15 different RAPD primers through PCR

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