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GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIP AMONG DIFFERENT PEACH GENOTYPES THROUGH RAPD MARKERS

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Abstract

Peach is a traditional crop of northern areas of Pakistan. It is the second most important fruit crop after apple and ranks first in the genus *Prunus*. For the well establishment of the peach crop, presence of genetic diversity in the germplasm is very important. During the present study, genetic diversity in peach genotypes was analyzed using different RAPD molecular markers through PCR. Variation matrix for each primer followed by an average was calculated using bi-variate data set. Analysis of the banding profile of these primers showed different level of genetic polymorphism among the various peach genotypes under study. The level of genetic polymorphism (estimated as genetic distance using RAPD primers) ranged between 20% and 80%. Cluster analysis revealed that the various peach genotypes were grouped into 5 classes. Group I and II and V had only one genotype each. Group III was comprised of 3 genotypes. Group IV had 6 genotypes. Genetically diverse peach genotypes were identified which can be used in future breeding programs to develop cultivars with broad genetic base.

Introduction

Peach (*Prunus persica* (L.) Batsch) is an extensively investigated species and serves as a model genome for Rosaceae family (Abbott *et al.,* 2002). The main growing habitat of the genus *Prunus* L. is the temperate region of the Northern hemisphere with some extensions into the Southern hemisphere in both old and new worlds (Krussman *et al.,* 1984). It includes many species which are economically important sources of fruits, nuts, oil, timber and ornamentals. It is also called the queen of temperate zone fruits and is the world's most widely grown fruit tree next to apples (Baird *et al.,* 1994). In the process of peach domestication, there were more than 5000 cultivars in the World (Anon., 1985) and because of breeding development and introduction more than 100 cultivars were selected in China (Wang & Zhuang, 2001).

Molecular markers are valuable tools in the characterization and evaluation of genetic diversity among different species and population. It has been reported 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; Lewers *et al.,* 2008; Brennan *et al.,* 2008; Mattia *et al.,* 2008; Ananga *et al.,* 2008; Ahmed *et al.,* 2009; Mahmood *et al.,* 2009; Bakht *et al.,* 2011a, b). RAPD (Randomly amplified polymorphic DNA), a PCR based marker has many advantages i.e. readily being used, requiring minute amount of genomic DNA, does not need blotting and radioactive detection etc. DNA finger prints can be easily generated with Random Amplified Polymorphic DNA (RAPD). RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide sequences of arbitrary nature as primers (Fernandez *et al.,* 2002; Nazari & Pakniyat, 2008). RAPD does not need any prior knowledge of DNA sequence, however, still reveals a high level of polymorphism. RAPD PCR is currently used as a tool for the assessment of genetic variability among genotypes in different breeding programmes.

Different molecular markers; randomly amplified polymorphic DNA (RAPD) (Yang *et al.,* 2001; Yuan *et al.,* 2002; Dirlewanger *et al.,* 2002; Bakht *et al.,* 2012), amplified fragment length polymorphism (AFLP) (Struss *et al.,* 2002, 2003; Tavaud *et al.,* 2003), Simple Sequence Repeat (SSR) (Aranzana *et al.,* 2002, 2003; Wang *et al.,* 2002; Schuelder *et al.,* 2003; Wunsch & Hormaza, 2004; Li *et al.,* 2006), Random Amplified Microsatellite Polymorphism (RAMP) (Cheng *et al.,* 2001, 2007) have been fruitfully used in the identification and determination of genetic diversity of peaches. Peach breeding is time consuming, especially for fruit-specific characters as the tree require at least 3 or 4 years before they bear fruit. Therefore, molecular markers associated to these traits are of economic importance for the identification and selection of plant genotypes with the desirable characters long before the traits are expressed (Dirlewanger *et al.,* 1998). Keeping in view the importance of RAPD markers in different breeding programmes, the present study was initiated to determine the genetic diversity and phylogenetic relationship among different peach genotypes for future breeding.

Materials and Methods

The present study was initiated at the Institute of Biotechnology and Genetic Engineering (IBGE) The University of Agricultural Peshawar, KPK Pakistan to determine molecular variations among 12 peaches genotypes using different RAPD primers.

Plant material and DNA extraction: Leaf materials were collected from each genotype grown at Agricultural Research Institute Tarnab Peshawar KPK Pakistan, freezed in liquid nitrogen and kept at -70°C until used for DNA extraction. DNA was extracted by Cetylmethylammoniumbromide (CTAB) method. About 200 mg frozen young leaves were crushed via pistol and mortar fine powder, transferred to eppendrof tubes and mixed with DNA extraction buffer (2% CTAB). Samples were mixed thoroughly by inversion and incubated for 1

hour at 65°C. Five hundred micro liters (500 µl) of chloroform and isoamylalcohol (24:1) was added, mixed and centrifuged at 12000 rpm for 10 min. The upper aqueous phase was mixed with 600 µl of 1% CTAB, incubated at room temperature for 3 hours and centrifuged at 8000 rpm for 10 min at 4°C. Five hundred micro liters of 1M NaCl was added to pellet to dissolve DNA. Then, 500 µl of 100% ethanol was added and kept at -30°C for overnight. Samples were centrifuged at 8000 rpm for 15 min at 4°C and the pellet was washed with 70% ethanol. Samples were again centrifuged at 7000 rpm for 10 min at 4°C and dried at room temperature. To remove RNA, the samples were treated with 40 µg RNAse enzyme A at room temperature for one hour. DNA was re-extracted as described earlier. DNA pellets were dried, dissolved in 50 µl TE buffer and stored at -80 ^{0}C until used. Before PCR, concentration of DNA and protein was determined by spectrophotometer using 260 nm and 280 nm.

Table 1. Peach genotypes used during the present study.

S. No.	Genotype
1.	A6-69
2.	Sun brite
3.	Sun light
4.	Carmon
5.	Spring crest
6.	Early king
7.	Summer gold
8.	Arim king
9.	Tex-y 4-55
10.	Muria emilea
11.	Sun free
12.	Flavor top

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

S. No.	Name	Sequence	Size (bp)
1.	GLC 20	ACTTCGCCAC	10
2.	GLA 20	GTTGCGATCC	10
3.	GLB 20	CGACCCTTAC	10
4.	GLA 15	TTCCGAACCC	10
5.	GLB 11	GTAGACCGT	10

Polymerase Chain Reaction: Polymerase chain reaction **(**PCR) was carried as described by Devos & Gale (1992) with certain modifications using Randomly Amplified Polymorphic DNA primers (Operon Technologies USA). For PCR, 25 µl reaction containing 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM each of dATP, dGTP, dCTP, dTTP, 50 mM KCI, l0 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase was used (Dweikat *et al.,* 1994). Amplification conditions for RAPD primers were an initial denaturation temperature of 94°C for 4 minutes followed by 40 cycles each consisting of a denaturation temperature of 94°C for 1 minute, annealing temperature 36°C for 1 minute, and an extension temperature of 72°C for two minutes. The last cycle was followed by 10 minutes extension temperature of 72°C. 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. Forty decamer Randomly Amplified Polymorphic DNA (RAPD) primers were used to study the level of genetic polymorphism among the 12 peach genotypes (Table 1). Only 5 primers amplified reproducible DNA bands while thirty five failed to produce any band in any of peach genotype. Sequence and size information of the primers used in the present study are given in Table 2.

Data analysis: Data on all unambiguous polymorphic RAPD fragments were identified and scored as presence (1) or absence (0). Similarity matrix, generated according to the coefficient of Dice (Dice, 1945) was used for the un-weighted pair-group method with arithmetic averaging (UPGMA) (Sokal & Michener, 1958). A dendogram indicating the estimated similarity among the peach genotypes was constructed with computer programme "Popgene 32" version 1.31.

Results and Discussion

For the present study of genetic diversity among different peach genotypes, forty randomly amplified polymorphic DNA (RAPD) primers were used and only 5 primers produced amplified product. Genetic difference for each assessment was predicted using bi-variate data. The five RAPD primers used during the present study were GLC20, GLA20, GLG20, GLA15 and GLB11 (Table 2). These primers amplified 0.92, 1.5, 2.08, 1.08 and 0.6 alleles per genotype respectively (Fig. 1). Among the 5 tested RAPD primers, GLB11 amplified maximum alleles (2.08 per genotype) followed by GLA20 (1.5 alleles per genotype) while minimum (0.6 alleles per genotype) by GLB20 (Fig. 1). The amplified fragments generated using RAPD primers ranged from 150 bp to 1000 bp. The overall genetic distance among the 12 peach genotypes ranged between 20% and 80% (Table 3). Our results strengthened the earlier reports that RAPDs can be used for the estimation of genetic diversity in crop enhancement programs (Warburton *et al.,* 1996; Yang *et al.,* 2001; Yuan *et al.,* 2002; Li *et al.,* 2006; Bakht *et al.,* 2011 a and b; 2012). The average number of alleles in all loci ranged from 0.2 to 0.8 with a mean of 3.4 among the 12 peach genotypes. The order for the diverse genotypes of peach were shown as A6-69, Sun Brite, Early King, Summer Gold, Carmon, Arim King, Tex-y4-55, Spring Crest, Sun Free, Flavor Top, Sun Light and Muria Emilea. Similar results were also reported by Cheng (2007).

Cluster analysis: Figure 2 present genetic similarity coefficient matrix of 12 peach genotypes based on the

data of 5 RAPD primers. At 0.57 dice similarity coefficient five groups were formed. Group I, II and V had only one genotype each (Muria Emilea, Sun-Light and A6-96 respectively). Group III was composed of three genotypes i.e. flavor-Top, Sun Free and Spring-Crest. Group IV comprised of 6 genotypes i.e., Tex-y4-55, Arim King, Carmon, Summer Gold, Early King and Sun Brite, showing the highest level of genetic similarity among them these genotypes. According to the dendrogram analysis, Muria Emilea, Sunlight and A6-69 were distinctly grouped genotypes when compared with the rest of the genotypes used in the present study (Table 1). Similar results were also reported by Zhen-Xiang *et al.,* (1996). The cluster analysis showed that there was high similarity among peach genotypes in their relevant cluster. The possible explanation might be that frequent gene flow from one genotype to another occurred. Because of the crossing for new cultivar breeding in history between genetically distinct clusters, there were few chance of crossing thus gene exchange was limited. It is possible to breed new cultivars or to create novel germplasms of peach through hybridization between genetically different species (Wang *et al.,* 2002; Dirlewanger *et al.,* 2002).

Table 3. Average estimates of genetic distance among 12 peach genotypes using RAPD primers.

	1	2	3	4	5	6	7	8	9	10	11	12
1.	--											
2.	0.36	--										
3.	0.26	0.2	--									
4.	0.3	0.26	0.06	--								
5.	0.4	0.43	0.13	0.2	--							
6.	0.4	0.53	0.33	0.3	0.3	--						
7.	0.4	0.53	0.33	0.3	0.3	0.8	--					
8.	0.36	0.4	0.4	0.26	0.23	0.53	0.53	--				
9.	0.5	0.6	0.23	0.4	0.4	0.5	0.5	0.43	--			
10.	0.5	0.16	0.26	0.4	0.3	0.4	0.7	0.26	0.4	--		
11.	0.6	0.43	0.33	0.3	0.5	0.3	0.6	0.53	0.8	0.5	--	
12.	0.32	0.5	0.25	0.3	0.43	0.37	0.38	0.45	0.43	0.32	0.38	--

Column 1 to 12 = Peach genotypes; for detail see Materials and Methods

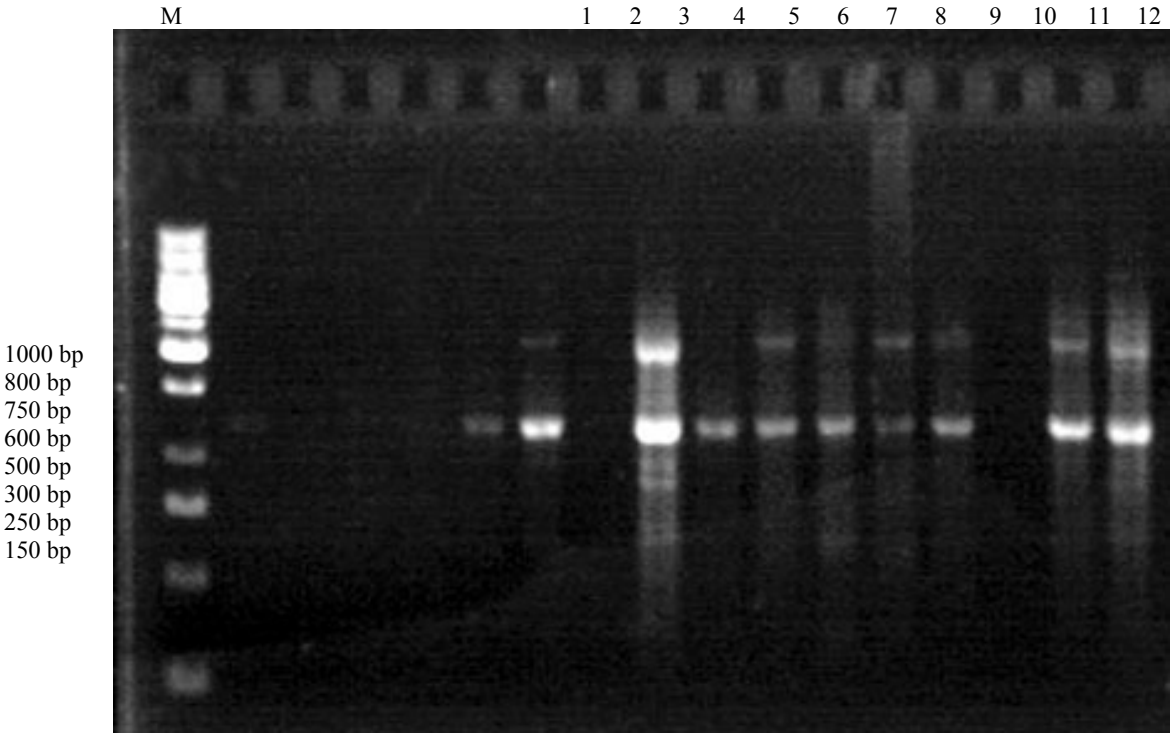

Fig. 1. Electrophoreogrm showing PCR based amplification product of twelve peach genotypes by RAPD primer GLCA20 [Line 1-12= Peach genotypes; M=molecular weight markers; for detail see Materials and Methods]

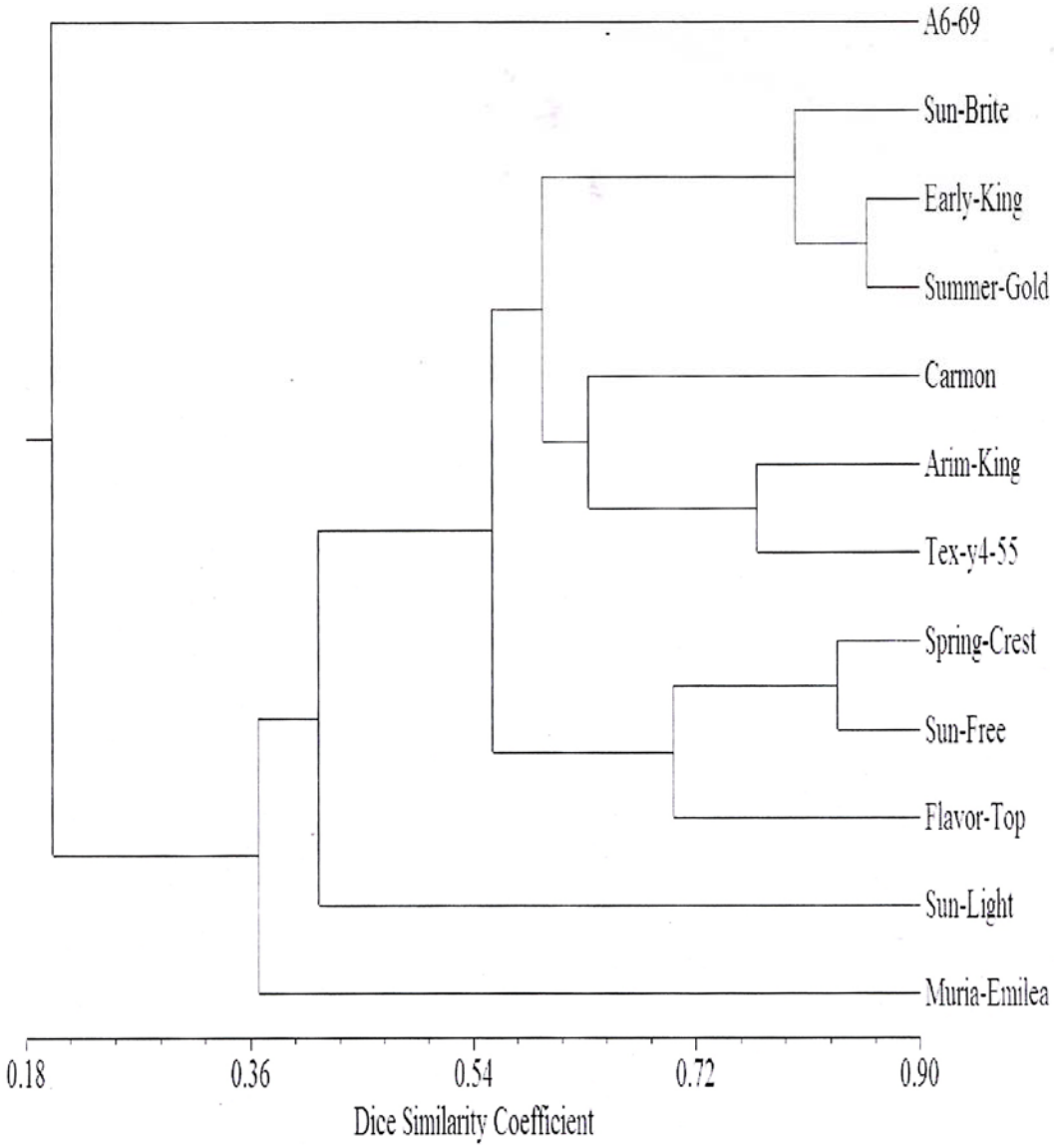

Fig. 2. Cluster analysis of 12 peach genotypes using RAPD primers (for detail see Materials and Methods)

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