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Characterization of *Prunus armeniaca* L. Germplasm using RAPDs

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ABSTRACT

The present study characterizes apricot germplasm through the use of RAPD markers. Twelve apricot cultivars ('Chuli', 'Suffaida', 'Shakarpara', 'Kaisha', 'Nari', 'Royal', 'Charmagaz', 'Shipley Early', 'New Castle', 'Tilton', 'Turkey' and 'Moorpork') were selected. RAPD analysis was carried out using 18 random decamer primers, 16 of which showed polymorphism. A total of 79 bands were amplified, out of which 56 were polymorphic and 10 were specific RAPD markers. Total polymorphism was 70.89%. The similarity value ranged from 0.59 to 0.86. A low similarity value was obtained between 'Shakarpara' and 'Chuli' cultivars and high between 'Moorpork' and 'Suffaida' cultivars. On the basis of a similarity matrix, a dendrogram was constructed by the UPGMA method. RAPD analysis is useful in a certification scheme for releasing certified plant material. It could also be useful for assessing genetic variation among different apricot accessions.

Keywords: apricot, genetic diversity, molecular characterization, molecular markers

INTRODUCTION

Apricot (*Prunus armeniaca*) is one of the most important stone fruits grown in temperate regions of the world. It is generally cultivated between 900-1800 m above sea level. Apricot occupies the third place in the world among the stone fruits in economic importance after peach and plum (Hurtado *et al.* 2002). In India apricots are commercially cultivated in Himachal Pradesh, Uttranchal and Jammu and Kashmir. A substantial quality of wild apricot commonly known as '*chulli*' is also grown in various parts of Himachal Pradesh, Uttranchal and Jammu and Kashmir.

Apricots are attractive, delicious and highly nutritious. They are a rich source of Vitamin A and contain more carbohydrate, protein, phosphorus and niacin than the majority of other similar fruits (Teskey and Shoemaker 1972). In the apricot kernel the oil content was found to be as high as 54.25% and it is used for edible purpose with out any toxic and side effect (Dwivedi and Ram 2008).

In Himachal Pradesh the mid hills are especially suitable for early maturing cultivars of apricot like 'Newcastle', 'Early Shipley', 'Nugget' whereas 'Charmagaz', 'Kaisha', 'Nari', 'Suffaida' and 'Shakarpara' are suitable for cold hills. 'Newcastle', 'Shakarpara' and 'Early Shipley' are the main cultivars grown in Shimla and Solan district of Himachal Pradesh. In Kinnaur district 'Suffaida', 'Kaisha','Nari' and 'Shakarpara' are the main apricot cultivars grown normally (Sharma *et al*. 2005).

The development of new genotypes requires fast and reliable techniques to study the genetic diversity in germplasm and to identify and protect newly released genotypes (Sánchez-Pérez *et al*. 2005). Molecular markers have been used for characterization of different apricot germplasm (Henry 1999). Molecular techniques like random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) are useful option for cultivar identification (Lin *et al*. 1996; Aradhya *et al*. 2004; Khan *et al.* 2006). RAPD analysis is a powerful tool for detection of sequence polymorphism (Welsh and McClelland 1990). The polymorphism of DNA markers (RFLPs and RAPDs) has been used for studying the genetic diversity of apricot cultivars (Gogorcena *et al*. 1994; De Vicente *et al*. 1998; Marinello *et al*. 2002; Wu and Chen 2003) and also for finding out the relationships between Japanese, Chinese and European apricots (Takeda *et al*. 1998). RAPDs marker was also used by Hurtado *et al*. (1999) and Ercisli *et al*. (2009) for studying genetic diversity and genetic similarity in different apricot cultivars. In the present studies we identified the characteristics molecular markers for apricot cultivars and to use stable molecular markers for assessing variations in apricot cultivars.

MATERIALS AND METHODS

Plant material

Leaves for the study of genetic diversity and DNA fingerprinting of 12 apricot cultivars were procured from Kinnaur, Shimla and Solan districts of Himachal Pradesh, India (**Table 1**).

DNA extraction

The leaves were frozen in liquid nitrogen and stored at -80°C. Genomic DNA was isolated using the method of Doyle and Doyle (1990) with some modifications. Genomic DNA was extracted from powdered (grounded with liquid nitrogen). Approximately 250-300 mg tissue samples from each plant species were snap frozen in liquid nitrogen in 2 ml Eppendorf tubes. 750 µl DNA extraction buffer [100 mM Tris-HCl (pH 8.0); 50 mM EDTA (pH 8.0); 500 mM NaCl; 2% CTAB; 2% 2-mercaptoethanol] was added and mixed well. The mixture was incubated at 65°C in a water bath for 1 h with intermittent shaking at 5-min intervals. The mixture was centrifuged at 15,000 rpm for 20 min at 4°C, the supernatant was transferred into a new 2.0 ml tube and mixed with equal volume of chloroform: isoamylalchol (24: 1), and centrifuged. The supernatant was collected and mixed with 2/3 volume of freezer-cold isopropanol, left at -20°C for 1 h. The DNA was pelleted by centrifugation (12,000 rpm, 10 min) and the isopropanol was poured off and washed with 70% ethanol; the DNA was allowed to air-dry before being dissolved in 100 µl of TE buffer.

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Table 1 List of apricot germplasm and their availability.

Shakarpara	Private Orchard, Dist. Shimla (H.P.)
Kaisha	Horticulture Research Station, dept. of Horticulture, Dutt Nagar, Dist. Shimla (H.P.)
Nari	Horticulture Research Station, dept. of Horticulture, Dutt Nagar, Dist. Shimla (H.P.)
Royal	Private Orchard, Dist. Shimla (H.P.)
Charmagaz	Regional Horticulture Research Station, Sharbo, Dist.Kinnaur (H.P.)
Suffaida	Regional Horticulture Research Station, Sharbo, Dist. Kinnaur (H.P.)
Shiplay Early	Regional Horticulture Research Station, Sharbo, Dist. Kinnaur (H.P.)
New Castle	Department of Pomology, UHF, Nauni, Solan (H.P.)
Moorpork	Regional Horticulture Research Station, Kandaghat, Dist. Solan (H.P.)
Tilton	Regional Horticulture Research Station, Kandaghat, Dist. Solan (H.P.)
Turkey	Regional Horticulture Research Station, Kandaghat, Dist. Solan (H.P.)
Chuli (wild apricot)	Rampur Bushahr Dist. Shimla (H.P.)

Table 2 Total number of amplified fragments and number of polymorphic fragments generated by PCR using 16 random decamer primers.

Primers	Size (bp)	Sequence (5′-3′)	Number of Amplified bands	Number of Polymorphic bands	Number of Monomorphic bands	Number of Unique bands
OPA-03	515-1203	AGTCAGCCAC	6	3	2	1
OPA-04	1210	AATCGGGCTG	1	1	0	0
OPA-06	371-2195	GGTCCCTGAC	7	7	0	0
OPA-20	585-1973	GTTGCGATCC	6	3	2	1
OPB-09	1136	TGGGGGACTC	1	1	0	0
OPC-05	274-1386	GATGACCGCC	9	5	3	1
OPC-08	228-2019	TGGACCGGTG	8	4	0	4
OPC-15	362-1521	GACGGATCAG	6	4	0	2
OPD-12	535-1188	CACCGTATCC	4	3	1	0
OPD-16	665-1310	AGGGCGTAAG	3	2	0	1
OPE-03	548-1275	CCAGATGCAC	6	5	1	0
OPE-07	575-771	AGATGCAGCC	3	3	0	0
OPE-13	401-1598	CCCGATTCGG	4	2	2	0
OPE-14	350-1310	TGCGGCTGAG	5	5	0	0
OPE-16	566-1377	GGTGACTGTG	5	4	1	0
OPE-18	329-1572	GGCCTATCGG	5	4	1	0
Total			79	56	13	10

Optical density was recorded at 260 and 280 nm and $A_{260}/A_{280} > 1.8 \pm 0.1$ was considered useful (Lodhi *et al*. 1994).

PCR amplification and product electrophoresis

Amplification of genomic DNA was carried out using 18 random decamer oligonucleotide primers (M/S Operon Technologies, Inc., Alameda USA) in a Perkin Elmer Gene Amplification PLX system 2400.

The reaction mixture of 25 µl contained 15 µl sterilized water, 0.25 µl *Taq* DNA polymerase (1 U), 2.5 µl *Taq* DNA polymerase buffer (10X), 1.25 µl dNTPs mixture (2 mmol each), 2 µl random primer (10 ρmole/reaction) and 4 µl genomic DNA (100 ng/reaction). PCR was carried out in the thermal cycler with a total of 45 cycles. Each cycle consisted of 1 min denaturation step at 94°C, 1 min annealing at 35.5°C and 2 min extension at 72°C. All the PCR samples were given 5 min pre-amplification at 95°C and 10 min post-amplification at 72°C. Electrophoresis of the amplified DNA was carried out in 1.4% agarose containing 1X TAE buffer and 0.5 µg/ml aqueous solution of ethidium bromide (10 mg/ml) under submerged conditions using 1X TAE as tray buffer. To each PCR-amplified sample 3 µl of 6X loading dye (bromophenol blue) was added. 10 kbp DNA ladder (GENEI Banglore) was used as standard and the gel was run at 80V until the loading dye reached the gel front. The amplified DNA was viewed under a UV trans-illuminator and the image was taken through flour S^{TM} Multi-imager and saved in a Bio Rad Computer.

Data analysis

Each amplified product was scored for all the genotypes of apricot with 18 primers for the presence and absence of bands. Co-migrating bands were considered to represent the same locus and thus treated as the same band while scoring. The presence of an amplified product was designated as '1' and its absence was marked as '0'. The intensity of the products was not taken into account while scoring. A pair-wise similarity matrix was constructed by using Dice's similarity index. This was further subjected to UPGMA clustering analysis and a dendogram was constructed. NTSYS- PC v. 2.02 (Rohlf 1998) (Numerical Taxonomy system, Exeter software) was used to perform cluster analysis of the complete RAPD data.

RESULTS AND DISCUSSION

Molecular characterization of 12 *P. armeniaca* cultivars were investigated using 18 RAPD primers. Out of 18 primers, 16 showed polymorphism yielding 79 scorable bands with an average of 4.39 bands/primer (**Table 2**). Unique bands were observed with different primers for 5 cultivars. Unique banding patterns were obtained with primers OPA-03 (1203 bp), OPC-08 (2019, 5866 and 363 bp) and OPD-16 (948 bp) in 'Shakarpara'. In 'Charmagaz' two unique bands (228 and 362 bp) were obtained with primers OPC-08 and OPC-15, respectively. Primer OPA-20 (665 bp) produced a unique band in 'Newcastle' (**Fig. 1**). In 'Suffaida' one unique band (552 bp) was obtained with primer OPC-05. Primer OPC-15 produced a unique band (762 bp) in 'Royal'.

In the present study DNA amplification of 18 random decamer RAPD primers was used, 16 of which showed polymorphism. A total of 79 bands were obtained, 56 of which were polymorphic, 13 monomorphic and 10 were unique bands which could be used for identifying particular cultivars. 70.89% of bands were polymorphic. Hurtado *et al*. (1999) used 40 random decamer primers to study variation in 18 apricot cultivars collected from North America, France and Spain. Only 22 primers produced 45 reproducible and polymorphic bands. Hormaza (2001) suggested that both RAPDs and microsattalite markers could be used for releasing certified material. Jun *et al*. (2003) obtained four unique fragments in 95 peach cultivars, which were found to be linked to the flesh adhesion gene. Dogra (2006) obtained unique RAPD markers and could distinguish all the 8 grape cultivars on the basis of reliable bands on choosing the right primer. Ercisli *et al*. (2009) used 40 random deca-

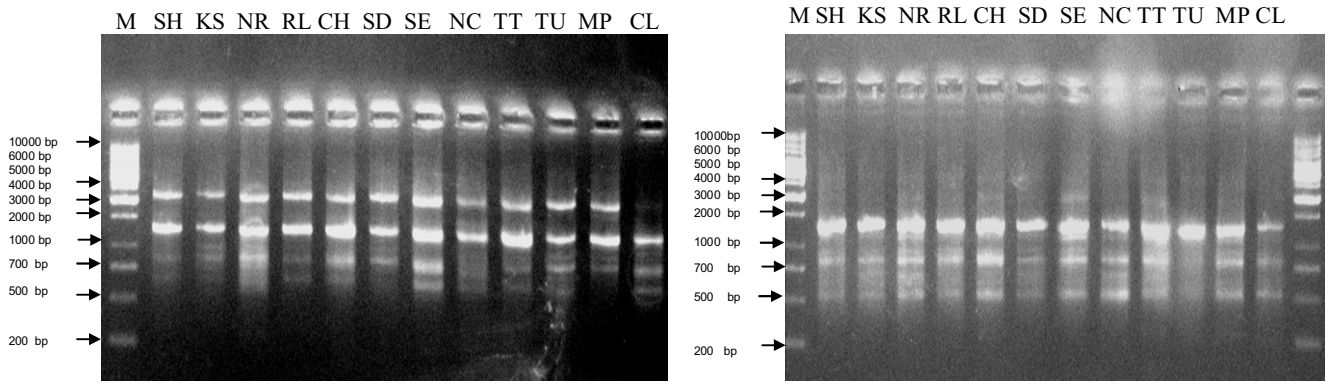

Fig. 1 RAPD pattern of *Prunus armeniaca* cultivars using primer OPA-20 (left) and OPE-03 (right). M – Gene ruler 10 kbp molecular marker; SH – Shakarpara; KS – Kaisha; NR – Nari; RL – Royal; CH – Charmagaz; SD – Suffaida; SE – Shipley Early; NC – New Castle; TT – Tilton; TU – Turkey; MP – Moorpork; Cl – Chuli.

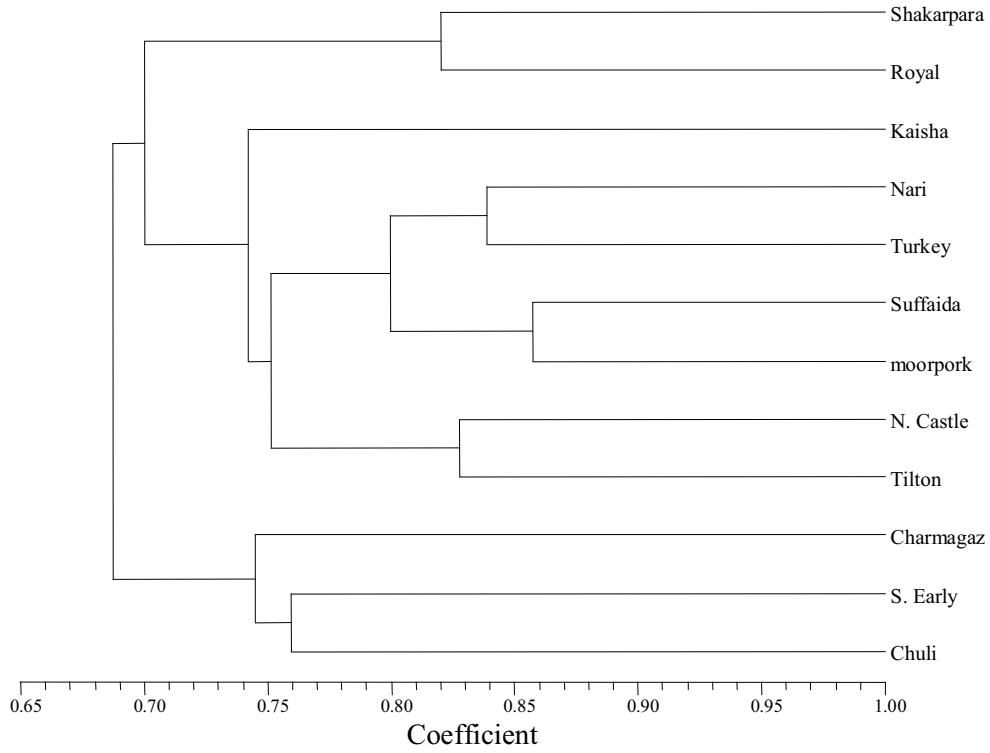

Fig. 2 RAPD-derived dendrogram.

mer primers to study variation in 23 apricot cultivars, only 12 random primers produced polymorphic bands. They found 97.5% polymorphic bands. In our study, two main clusters, A and B, were obtained in the dendrogram. Cluster A was further divided into subclusters C, D, E, F and G. The Chinese cultivar 'Shakarpara' along with American cultivar 'Royal' formed subcluster C. The second subcluster D included American cultivar 'Kaisha'. The cultivars 'Nari' and 'Turkey' introduced from Turkey formed the subcluster E. The fourth subcluster F included Chinese cultivar 'Suffaida' and American cultivar 'Moorpork'. The cultivars 'Newcastle' and 'Tilton' introduced from America formed the fifth subcluster G. The main cluster B was further divided into two subcluster H and I. The subcluster H included the cultivar 'Charmagaz' introduced from China. The 'Chuli' (wild apricot) grouped along with Spanish cultivar 'Shipley early' in subcluster I (**Fig. 2**). This shows that the American cultivars grouped along with Chinese cultivars are genetically similar to each other, where as some other American cultivars showed similar profile. Ercisli *et al*. (2009) also found that similarity rate of apricot cultivars varied among groups of regions, however dissimilarity rate was very low within the groups. Our findings also support the fact that apricot cultivars are severely restricted in their ecological adaptation (Mehlenbacher *et al*. 1991).

The cultivars collected from Shimla and Solan district were grouped into different clusters and showed similar profile, whereas the cultivars collected from district Kinnaur grouped along with the cultivars collected from Solan district. Our findings also support the fact that suitability of apricot cultivars to the different geographical locations (mid hills and cold hills in Himachal Pradesh) for their cultivation (Sharma *et al*. 2005).

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