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Estimation of genetic diversity among pear cultivars using PCR-based RAPD markers

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ABSTRACT

Diversity studies are desirable for the purpose of better management and conservation of the genetic resources and for planning the breeding strategies. In the present study genetic relatedness among twenty genotypes of pear growing in Himachal Pradesh was tested using RAPD molecular marker analysis using 26 RAPD primers. The average size of amplicons ranged from 100 to 2000 bp with minimum (OPP-12) of 100-900 bp and maximum (OPP-05) of 200-2000 bp. Among all the primers the highest polymorphism was found to be 100 per cent (OPA-03, OPA-20, OPC-01, OPC-08, OPE-15, OPE-18, OPH-14, OPL-12, OPP-05, OPP-12, OPU-01, OPU-11, OPU-20, OPY-16 and OPY-17). The level of polymorphism across the subjected genotypes was 96.15 per cent by RAPD markers. Therefore the high level of detected polymorphism would impress their applicability in development of superior progenies, QTL mapping, molecular breeding, investigation of population genetic diversity, comparative mapping and the selection of the parents in near future.

Keywords: DNA polymorphism; genetic relatedness; molecular markers; RAPD; pear

INTRODUCTION

The genus *Pyrus* belongs to the sub-family Pomoideae of family Rosaceae and its two main species *Pyrus communis* and *P pyrifolia* are commercially grown throughout the temperate zone of the world. The genus *Pyrus* has probably originated in mountainous region of western China. In India pear is grown in more than 9 states and area is 38160 ha with production of 334774 MT (Anon 2012). Assessment of genetic diversity to recognize groups with similar genotypes is very important to conserve, evaluate and utilize the genetic resources for studying the diversity of the germplasm as potential basis of genes that may be capable to improve the performance of cultivars and for determining the distinctness and uniqueness

of the phenotypic and genetic formation of genotypes with the purpose of protecting the intellectual property rights of the breeder. Diversity studies are also desirable for the purpose of better management and conservation of the genetic resources and for planning the breeding strategies (Badenes et al 2000). DNA-based molecular markers have become increasingly popular in the characterization and identification of genetic resources as they are not influenced by environmental factors and are more polymorphic. Random amplified polymorphic DNA (RAPD) the first PCR based marker has underscored the advantages to appraise the genetic diversity in different fruits like apple (Adebayo et al 2009) and pear (Lisek and Lozpara 2010). Keeping in view the above considerations the present study was framed to evaluate the genetic relatedness among different genotypes of pear growing in Himachal Pradesh using RAPD analysis.

MATERIAL and METHODS

Plant material and DNA extraction

Young green leaves of each pear genotype were procured from the NBPGR Regional Station, Phagli, HP for carrying out molecular marker studies (Table 1). Isolation of genomic DNA was done from the collected leaves of each subjected genotype by using CTAB method (Doyle and Doyle 1990) with some modifications. RNA contaminants in all the samples were digested with 100 µg/ml RNaseA for 30 minutes at 37°C. DNA concentration and purity was measured using UV/VIS spectrophotometer at 260 and 280 nm absorbance respectively.

DNA amplification and gel electrophoresis

DNA amplification was observed following polymerase chain reaction (PCR) to study the genetic diversity in various pear cultivars. PCR was carried out in a 15 µl reaction volume for each marker analysis containing Taq DNA polymerase (3U/ reaction), Taq DNA polymerase buffer (1X) with 1.5 mM $MgCl_2$, primers (10 pmol/ reaction), deoxynucleotide triphosphate (dNTPs) (25 mM) of Genei, Bangalore, India and template DNA (50 ng/reaction). A total of 40 RAPD primers were employed to characterize 20 genotypes of pear at their respective annealing temperatures using a Thermal Cycler (Applied Biosystems, USA) programmed to initial cycle of 4 min at 95°C followed by 35 cycles of 1 min at 94°C, annealing temperature depending upon T_m value of primer for 1 min, elongation step of 2 min at 72°C and a final extension step of 8 min at 72°C followed by a 4°C soak until recovery. Products were analysed by electrophoresis on agarose (GeNei, Bangalore, India) concentrations of 1.6 per cent for RAPD in 1X TAE buffer containing ethidium bromide (10 mg/ml) respectively and images were taken through gel documentation unit (Syngene, UK). The size of the amplified product was

determined by co-electrophoresis of 100 bp standard molecular weight markers (GeNei, Bangalore, India). Each reaction was carried out twice to establish the reproducibility of results. Only those primers which produced bands with all the samples were used to score for polymorphism.

Data analysis

NTSYS-PC ver 2.02i software was used to analyze the data after compiling the observations of bands on gel images of different primers and genotypes in molecular marker study. The data on band position on agarose gel was recorded by assigning '0' for the absence of band and '1' for presence of band. The similarity matrix generated using Jaccard coefficient was used for unweighted pair-group method based on arithmetic average (UPGMA) using software package NTSYS-PC ver 2.02i (Rohlf 1998) and the output data were graphically represented as dendrogram.

RESULTS and DISCUSSION

A varying level of genetic polymorphism was revealed in the banding pattern across all the subjected genotypes. For a total 26 RAPD informative primers out of 40 initially screened primers 286 bands were amplified of which 275 (96.15%) were polymorphic (Table 2). The average size of amplicons ranged from 100 to 2000 bp with minimum (OPP-12) of 100-900 bp and maximum (OPP-05) of 200-2000 bp. The number of bands varied from 7 with OPU-11 to 17 with primer OPP-05 respectively with fragment size ranging from 100-2000 bp (approx) for all the informative primers. Of the 40 random RAPD primers used only 26 were able to amplify the genomic DNA. 18 random primers showed 100 per cent polymorphism (like OPA-03, Fig 1) and eight were certainly varied. The rest of the fourteen primers failed to amplify the genomic DNA uniformly and were not included into further analysis. Similarly comparative analysis among 26 pear cultivars was made with 25 RAPD primers and 103 polymorphic DNA fragments were obtained as per Lisek and Rozpara (2010). Similarly the RAPD technique with the use of 25 primers was successful in the identification of 25 pear cultivars where depending on the primer used 8 to 17 total bands were obtained their size varying from 400 to 1500 bp (Monte-Corvo et al 2000). Nine primers generating from 5 to 11 total bands enabled identification of 16 genotypes belonging to different species of *Pyrus* including 4 cultivars *Pyrus communis* L (Sharifani and Jackson 2000). On an average total number of bands generated per primer was 11.00 (Table 2). Similarly Bayazit et al (2011) obtained 16 marker levels in quince of which 70 bands were amplified and 54.29 per cent were polymorphic. On an average total number of bands generated per primer was 22.86. The results obtained by Monte-Corvo et al

Table 1. List of pear genotypes used in molecular characterization studies

S/N	Genotype	Origin	S/N	Genotype	Origin
1.	Kashmir Pear	India (J/K)	11.	Korean Giant Pear	Korea
2.	Chugeryongshiki	Japan	12.	Baldwin Pear	USA
3.	Leconte	Japan	13.	Stirling	USA
4.	Tan-Yan Jhao	USSR	14.	Rakovslik	Hungry
5.	King Pear	-	15.	Wesatchee	USA
6.	Moodeung	Korea	16.	Babugosha	India
7.	Doynee Bussarch	-	17.	Nugget	Italy
8.	Hood	USA	18.	Harogen	USA
9.	Keiffer	Japan	19.	EC-566191	USA
10.	Nic-58127	India	20.	IC-20092	India

Table 2. Summary of the RAPD markers studied in pear genotypes

S/N	Primer	Sequence	Annealing temp (°C)	Amplified product range (bp)	# amplified bands	Polymorphism (%)
1.	OPA-01	CAGGCCCTTC	32	100-1100	11	90.90
2.	OPA-02	TGCCGAGCTG	37	150-950	11	81.81
3.	OPA-03	AGTCAGCCAC	30	150-1400	14	100
4.	OPA-04	AATCGGGCTG	32	200-1000	12	90.90
5.	OPA-05	AGGGGTCTTG	30	100-1400	10	88.89
6.	OPA-13	GAAACGGGTG	30	250-1100	11	90.90
7.	OPA-18	AGGTGACCGT	30	200-1000	10	80.00
8.	OPA-20	GTTGCGATCC	32	200-1000	15	100
9.	OPB-07	GGTGACGCAG	32	150-900	10	90.00
10.	OPB-08	GTCCACACGG	32	300-1100	10	80.00
11.	OPB-12	CCTTGACGCA	32	100-1200	12	91.67
12.	OPB-17	AGGGAACGAG	32	150-1300	13	92.30
13	OPC-01	TTCGAGCCAG	32	200-950	12	100
14.	OPC-08	TGGACCGGTG	32	150-1100	11	100
15.	OPD-12	CACCGTATCC	32	100-1100	10	90.00
16.	OPE-15	ACGCACAACC	30	150-1000	08	100
17.	OPE-18	GGACTGCAGA	32	200-1200	11	100
18.	OPH-14	ACCAGGTTGG	32	200-1100	10	100
19.	OPL-12	GGGCGGTACT	32	200-1000	09	100
20.	OPP-05	CCCCGGTAAC	32	200-2000	17	100
21.	OPP-12	AAGGGCGAGT	30	100-900	10	100
22.	OPU-01	ACGGACGTCA	32	150-1350	15	100
23.	OPU-11	AGACCCAGAG	32	200-1200	07	100
24.	OPU-20	ACAGCCCCCA	32	250-1100	13	100
25.	OPY-16	GGGCCAATGT	30	100-1200	12	100
26.	OPY-17	GACGTGGTGA	30	150-1300	10	100
Mean			-	-	11.00	96.15

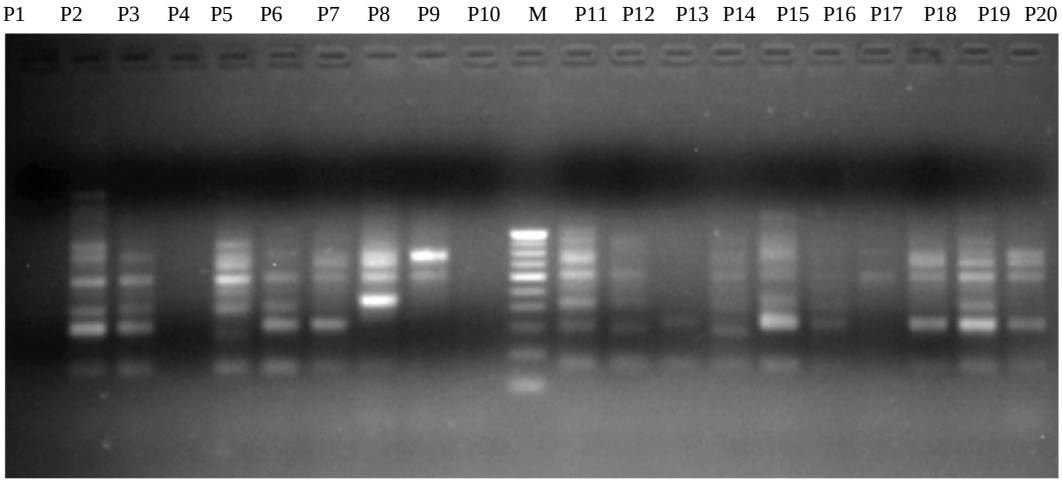

Fig 1. Gel showing DNA banding profiles using RAPD marker (OPA-03) where M= 100 bp ladder, (P1-P20) pear genotypes

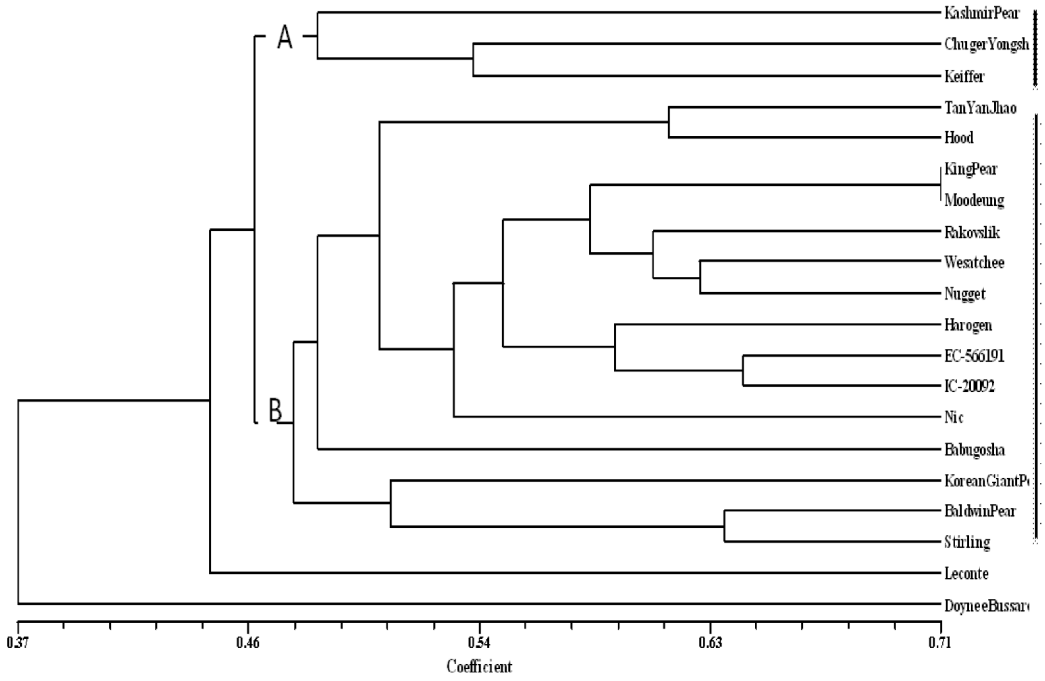

Fig 2. Dendrogram showing the relatedness among subjected pear genotypes

(2000, 2002) (73.80 to 84%) and Sharifani and Jackson (2000) (14 to 42.80%) in pear genotypes showed good level of polymorphism.

Based on DNA amplification using 26 informative primers genetic similarity among 20 genotypes of pear was estimated following Jaccard's coefficient by using NTSYS pc ver 2.02i. The similarity coefficient was as low as 0.37 to as high as 0.71 that indicated substantial diversity present in the germplasm (Fig 2). The dendrogram generated by RAPD analysis divided pear genotypes into two distinct clusters comprising of 3 (Cluster A) and 15 (Cluster B) genotypes truncated at similarity value of 0.37. Cluster A contained the pear genotypes named Kasmir Pear, Chugeryongshiki and Keiffer showed 48 per cent similarity with each other. Major Cluster B grouped 15 genotypes in which maximum similarity of 71 per cent was observed between King Pear and Moodeung. Genotypes Tan Yan Jhao and Hood were 62 per cent similar while Rakovslik, Wesatchee, Nugget, Harogen, EC-566191, IC-20092 and Nic showed 48 per cent similarity with Babugosha and in second sub-cluster Baldwin Pear and Stirling showed 52 per cent similarity with Korean Giant Pear. Further 18 genotypes grouped under two main clusters showed 45 per cent similarity with Leconte and 37 per cent similarity with Doynee Bussarch. Doynee Bussarch was found to be highly diversified and distinct genotype without showing any grouping with rest of the genotypes. Similarly clustering was also revealed by Gupta (2011) in pear genotypes after RAPD analysis.

Hence RAPD has the advantages of being technically simple and rapidly facilitated and has been used for plant genetics and phylogenetic studies. RAPD markers can be of great importance as a fast process for taxonomic studies. Although reproducibility of RAPD is open to debate RAPD markers are used in many plant and animal species for DNA fingerprinting and mapping studies. Results showed that RAPD primers should undergo precise selection if a higher degree of polymorphism is to be produced. Information on the genetic relationship within related crop varieties would be essential for a rationale use of genetic resources to estimate any possible loss of genetic diversity and to offer evidence of evolutionary forces shaping the genetic diversities etc. In the present study high level of polymorphism indicated the applicability in development of superior progenies, QTL mapping, molecular breeding, investigation of population genetic diversity, comparative mapping and the selection of the parents etc. Therefore these results would be helpful in framing more extensive studies on the determined taxa in near future.

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