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Genetic diversity studies on some pear genotypes using simple sequence repeats (SSRs) derived from apple and pear

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ABSTRACT

Genetic divergence and similarity among diverse 11 pear genotypes was estimated by 120 SSR markers. The primer pairs (120) resulted in amplification of 1915 putative alleles in 11 genotypes with an average of 174.09 alleles per genotype. The total number of alleles amplified with 120 SSR primers in each of the genotype ranged from 146 in Keifer to 192 in Sucker (*P. calleryana*). The number of alleles detected with 120 SSR primers ranged from 1 to 9 with an average of 3.62 alleles per primer pair. The highest number of alleles was amplified by CH02f06 (9), CH01d 01 (8), CH01e09 (7), CH01d09 (6), CH01f12 (6), CH02c06 (6), CH02d12 (6), CH04d10 (6), CH04e03 (6), CH04f10 (6), CH04g 09 (6), KU10 (6), BGT23b (6) and HGA8b (6). PIC value ranged from 0 to 0.84 with an average of 0.53 across 11 pear genotypes. On the basis of SSR data, dendrogram clustered the genotypes into two major groups (I and II) and six sub-groups. Genetic similarity values between genotypes ranged from 0.52 to 0.84. Smith and LeConte showed the highest similarity coefficient of 0.84, whereas lowest (0.52) value was observed between major groups I and II.

Key words: *Pyrus*, SSRs, genetic relationship, diversity.

INTRODUCTION

Genus *Pyrus* is believed to have originated during the tertiary periods (65-55 million years ago) in the mountainous area of western and south- western China and spread east and west from there. Vavilov (7) identified three centres of diversity for pears: China, Central Asia and Near Eastern/ Asia Minor. The genus *Pyrus* (2n = 34) contains 22 species having basic chromosome numbers of X = 8 and Y = 9 (Bell *et al.*, 4). Commercial pear production is mainly represented by *Pyrus communis* (European pear), *Pyrus pyrifolia* (Asian or Oriental pear) and their hybrids. Most of the cultivars belonging to *Pyrus communis* are suitable for cultivation in temperate climate, however, the *Pyrus pyrifolia* cultivars are well adapted to sub-tropical climate of north-western states of India. The Department of Horticulture (PAU, Ludhiana) is maintaining germplasm of a good number of varieties introduced from other countries or different parts of the country in order to broaden the genetic base. However, no information is available about the extent of genetic diversity in the existing germplasm, which is the basis for any genetic improvement programme.

Microsatellite or simple sequence repeat (SSR) markers have become valuable molecular tools for genetic finger printing due to their abundance, high degree of polymorphism, co-dominance and suitability for automation. Genomic microsatellite markers in pear are a recent development (Yamamoto *et al.*, 10) and have been used for mapping, genotype identification and determination of genetic relatedness. Apple microsatellite markers represent an additional source of markers for pear due to their reported cross transference (Bassil *et al.*, 3). The SSRs developed in apple show good syntany with pear and have been used for characterization of *Pyrus* germplasm (Yamamoto *et al.*, 9). Brini *et al.* (5) assessed genetic diversity in local Tunisian pears through SSR markers. With the help of molecular markers Bao *et al.* (2) showed that Chinese sand pear consisted of four groups with Chinese white pear, showing large diversity. Sixty three European cultivars have been characterized using microsatellites developed from apple (Wunch and Harmoza, 8). Hence, characterization at the genetic level with the help of SSR markers could be the first step towards efficient study of genetic diversity among various pear genotypes, which can be utilized for future breeding programmes.

MATERIALS AND METHODS

We analysed 11 pear genotypes which are commercially very important. DNA was isolated using CTAB (Cetyl trimethyle ammonium bromide) method with some minor modifications like treatment with copper acetate and other modifications so as to remove the polyphenols thereby preventing their interaction with DNA and yielding high quality DNA.

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A total of 120 SSR markers spanning all the linkage groups were surveyed on pear genotypes for genetic diversity analysis. A mixture 20 µl of various PCR reagents, based on the stock and final concentration of different components was prepared. The polymerase chain reaction (PCR) was programmed as follows: pre-denaturation for 4 min. at 94°C, then 35 cycles each consisting of a denaturation step for 1 min., an annealing step for 1 min. at appropriate annealing temperature and an extension step for 7 min. at 72°C. To 20 µl of the amplified product, 3.0 µl of 6X loading dye was added so as to make the final concentration of the loading buffer in the reaction samples to 1X. The PCR products were resolved on 2.5 per cent superfine resolution agarose gel. The gel was prepared in 0.5 X TBE buffer. Ethidium bromide was added at concentration of 0.5 µg/µl. 10 µl of sample was loaded onto each well and gel was run at 5V/cm, visualized under UV light and photographed using UVP gel documentation system (Model GDS 7600). 100 Kb ladder was used as a standard. The total number of alleles was recorded for each microsatellite marker in all the genotypes under study by giving the number to amplified alleles as 1, 2, 3 etc. Data matrices were prepared in which the presence of a band was coded as 1 (band present) and 0 (band absent) in a binary matrix. The lines that did not show any amplification were scored as null alleles since the amplification was repeated 2-3 times.

Numerical Taxonomic and Multivariate Analysis System NTSYS-pc) version 2.02e. Dendrogram was constructed using UPGMA (Unweighted Pair Group Method using Arithmetic Averages) by available in NTSYS. Dissimilarity coefficients were estimated for allelic data generated by 120 SSR primer pairs by using DARwin 5 software. Tree was constructed using neighbour joining on the basis of UPGMA.

RESULTS AND DISCUSSION

The summarized data of 120 SSR primers used for identification and evaluation of genetic diversity in 11 pear genotypes is presented in Table 1. The primer pairs (120) resulted in amplification of 1915 putative alleles in 11 genotypes with an average of 174.09 alleles per genotype. The total number of alleles amplified with 120 SSR primers in each of the genotype ranged from 146 in Keifer to 192 in Sucker (*P. calleryana*). The number of alleles detected for 120 SSR primers ranged from 1 to 9 with an average of 3.62 alleles per primer pair. The highest number of alleles was amplified by CH02f06 (9), CH01d 01 (8), CH01e09 (7), CH01d09 (6), CH01f12 (6), CH02c06 (6), CH02d12 (6), CH04d10 (6), CH04e03 (6), CH04f10 (6), CH04g 09 (6), KU10 (6), BGT23b (6) and HGA8b (6). The variation in the number of allele produced by SSR markers demonstrates heterozygosity in different alleles at a given locus, in which the heterozygosity could reflect greatly the state of genetic variability. All these amplified fragments produced different fingerprinting pattern that allowed all the varieties analysed to be distinguished. Wunsch and Hormaza (8) also studied 7 SSRs marker developed in apple to identify the 63 European pear cultivars, and a total number of 46 alleles were amplified with an average of 6.6 allele per locus. Kimura *et al.* (6) identified 60 Asian pear accessions from six *Pyrus* sp. by nine SSR markers with total of 133 alleles. In order to conduct diversity studies in five *Pyrus* species, Yamamoto *et al.* (11) used seven SSR primers and observed a total of 65 putative alleles with an average of 9 alleles per primer pair.

Table 1. Total number of alleles amplified in each of 11 pear genotypes using 120 SSR markers.

Genotype	No. of amplified fragments
Patharnakh	169
Kainth	166
Shiara	183
Smith	170
Sucker	192
LeConte	182
YaLi	157
Punjab Beauty	183
Shinseiki	186
Keifer	146
Baggugosha	183
	Av. (174.09)

The data pertaining to polymorphic information content (PIC) values and the number of alleles detected for each of the 120 SSR markers are presented in Table 2**.** PIC value ranged from 0 to 0.84 with an average value of 0.53 across 11 pear genotypes. Eighty five out of 120 SSR markers revealed PIC value of more than 0.5 and in remaining primers it was less than 0.5. Primer CH02f06 amplified 9 alleles and had a highest PIC value of 0.84. In many primers the amplified fragments were high but the PIC value was low. Primer CH02a03 amplified 5 alleles and had PIC value of 0.69, while CH02a04 amplified 4 alleles and had similar PIC value (0.69). Among 120 SSR primers used in this study, 85 primers detected a total of 434 amplicons, out of which 419 were polymorphic. The percentage of polymorphism varied from 50 to 100 per cent. The marker loci with an average number

Table 2. Polymorphic Information Content (PIC) value and number of alleles amplified by SSR markers.

S. No.	Primer	No. of alleles		Polymorphism (%)	PIC value	S. No.	Primer	No. of alleles		Polymorphism (%)	PIC value
		A	P					A	P		
1.	CH01a07	1	1	100.0	0.00	61	CH03b06	3	3	100	0.37
2.	CH01a09	5	4	80.0	0.71	62	CH03b10	1	1	100	0.00
3.	CH01b07	3	2	66.6	0.47	63	CH03c01	2	2	100	0.40
4.	CH01b09	4	4	100	0.66	64	CH03c02	3	3	100	0.73
5.	CH01b11	4	4	100	0.64	65	CH03d01	5	5	100	0.63
6.	CH01b12	3	2	66.6	0.66	66	CH03d02	1	1	100	0.00
7.	CH01c06	4	4	100	0.58	67	CH03d07	1	1	100	0.00
8.	CH01c08	4	4	100	0.64	68	CH03d08	3	3	100	0.61
9.	CH01c09	5	4	80	0.66	69	CH03d10	4	4	100	0.66
10.	CH01c11	2	2	100	0.29	70	CH03d11	1	1	100	0.00
11.	CH01d01	8	8	100	0.82	71	CH03d12	3	3	100	0.65
12.	CH01d03	4	4	100	0.70	72	CH03e03	2	2	100	0.39
13.	CH01d07	1	1	100	0.00	73	CH03g06	5	5	100	0.70
14.	CH01d08	2	2	100	0.29	74	CH03g07	5	5	100	0.63
15.	CH01d09	6	6	100	0.78	75	CH03g12	3	2	66.6	0.57
16.	CH01e01	1	1	100	0.00	76	CH03h03	4	4	100	0.69
17.	CH01e09	7	7	100	0.83	77	CH03h06	3	3	100	0.56
18.	CH01e12	4	4	100	0.64	78	CH04a06	5	5	100	0.71
19.	CH01f02	2	2	100	0.48	79	CH04a12	5	5	100	0.72
20.	CH01f03	5	5	100	0.72	80	CH04b10	1	1	100	0.00
21.	CH01f07	5	5	100	0.73	81	CH04c03	1	1	100	0.00
22.	CH01f09	1	1	100	0.00	82	CH04c06	4	4	100	0.66
23.	CH01f12	6	6	100	0.79	83	CH04c07	5	5	100	0.74
24.	CH01g05	1	1	100	0.00	84	CH04c07	5	5	100	0.72
25.	CH01g12	2	1	50	0.33	85	CH04d02	4	4	100	0.68
26.	CH01h01	3	3	100	0.61	86	CH04d07	1	1	100	0.00
27.	CH01h02	2	1	50	0.47	87	CH04d08	3	2	66.6	0.54
28.	CH01h10	4	4	100	0.69	88	CH04d10	6	6	100	0.75
29.	CH01h11	5	5	100	0.77	89	CH04e02	3	3	100	0.64
30.	CH02a03	5	5	100	0.69	90	CH04e03	6	6	100	0.77
31.	CH02a04	4	4	100	0.69	91	CH04e12	3	2	66.6	0.58
32.	CH02a08	3	3	100	0.61	92	CH04f03	4	3	75	0.63
33.	CH02a10	3	3	100	0.63	93	CH04f04	5	5	100	0.70
34.	CH02b03	3	3	100	0.57	94	CH04f06	5	5	100	0.47
35.	CH02b10	5	5	100	0.70	95	CH04f06	2	1	50	0.15
36.	CH02b11	4	4	100	0.64	96	CH04f07	1	1	100	0.00
37.	CH02b12	4	4	100	0.69	97	CH04f08	4	4	100	0.54
38.	CH02c02	2	2	100	0.27	98	CH04f10	6	5	83.3	0.68

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S. No.	Primer	No. of alleles A	No. of alleles P	Polymorphism (%)	PIC value	S. No.	Primer	No. of alleles A	No. of alleles P	Polymorphism (%)	PIC value
39.	CH02c02	2	2	100	0.49	99	CH04g04	4	4	100	0.68
40.	CH02c06	6	6	100	0.77	100	CH04g07	4	3	75	0.67
41.	CH02c11	4	3	75	0.70	101	CH04g09	6	6	100	0.77
42.	CH02d08	5	5	100	0.73	102	CH04g12	1	1	100	0.00
43.	CH02d10	4	4	100	0.70	103	CH04h02	3	3	100	0.62
44.	CH02d10	4	4	100	0.71	104	CH05a02	2	2	100	0.37
45.	CH02d11	5	5	100	0.67	105	CH05a04	3	3	100	0.55
46.	CH02d12	6	6	100	0.80	106	CH05a09	3	3	100	0.54
47.	CH02e02	3	3	100	0.51	107	KA4b	3	3	100	0.58
48.	CH02e12	2	2	100	0.49	108	KA5	4	4	100	0.66
49.	CH02f06	9	9	100	0.84	109	KA14	4	4	100	0.67
50.	CH02g01	3	3	100	0.60	110	KA16	5	5	100	0.57
51.	CH02g04	4	4	100	0.58	111	KB16	4	4	100	0.64
52.	CH02g09	3	3	100	0.48	112	KU10	6	6	100	0.72
53.	CH02h07	4	4	100	0.45	113	BGA35	1	1	100	0.00
54.	CH02h11	3	3	100	0.54	114	BGT23b	6	6	100	0.79
55.	CH03a02	3	3	100	0.56	115	HGA8b	6	6	100	0.75
56.	CH03a03	3	3	100	0.59	116	05g8	5	5	100	0.68
57.	CH03a04	1	1	100	0.00	117	28f4	3	3	100	0.60
58.	CH03a08	1	1	100	0.00	118	HGA8b	8	8	100	0.83
59.	CH03a09	3	3	100	0.51	119	05g8	2	1	50	0.48
60.	CH03b01	5	5	100	0.76	120	28f4	3	3	100	0.44
Avg.								3.62			0.53

A = Amplified; P = Polymorphic

of alleles running at equal frequencies will have the highest PIC values. The second reason for high PIC values could be due to differences in medium for resolving the amplified products. In the present study, a high average value of polymorphism information content (PIC) was found indicating that this could be a valid tool for discrimination of pear genotypes.

The similarity coefficient based on DNA amplification of 11 pear genotypes using SSR primers was estimated by dice similarity coefficient. The dendrogram generated based on UPGMA is depicted in Fig. 1. Genetic similarity values between genotypes ranged from 0.52 to 0.84 as depicted in dendrogram.

As is evident from the dendrogram that the 11 pear genotypes studied in present investigation constituted two major clusters (I and II). A considerable genetic variability was found between genotypes falling in cluster I and those in cluster II. The genotypes included in cluster I were well separated from genotypes in

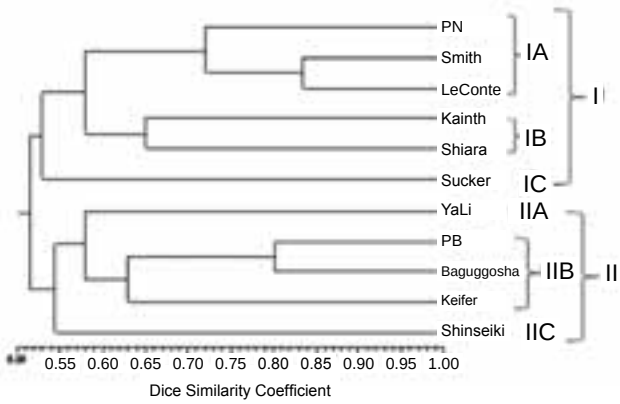

Fig. 1. Dendrogram showing similarity coefficient of 11 pear genotypes.

cluster II at a very low affinity level of about 0.52. The cluster I was further sub-divided into IA, IB and IC, while, cluster II was sub-divided into IIA, IIB and IIC. The sub-cluster IA comprised of 3 genotypes among which Smith and LeConte showed the highest affinity with similarity coefficient of 0.84, whereas both these genotypes shared 0.72 similarity coefficient with Patharnakh in the same sub-group. Phenotypically and morphologically, Smith, LeConte and Patharnakh were also associated for several traits like tree height, spread, fruit set etc. Rootstocks Kainth and Shiara (both are native to India) were included in sub-cluster IB sharing 0.64 similarity coefficient. Commercially, these genotypes were not so important with respect to fruit quality, but were found in wild forms as hardy plants and resistant against diseases and are used as rootstocks for commercial varieties of pears. However, another rootstock (*P. calleryana*) formed separate sub cluster IC and exhibited very low genetic relationship with all other genotypes. The main cluster I included five genotypes with YaLi and Shinseiki forming solitary clusters (IIA and IIC, respectively). YaLi exhibited low genetic similarity with genotypes falling in IIB. The similarity coefficient of this genotype was 0.57 with IIB. Such distant affinity of this genotype with other genotypes of pear indicates that it might be an independent clone. The genotypes Punjab Beauty, Baggugosha and Keifer fell in sub-cluster IIB with Punjab Beauty and Baggugosha sharing a maximum similarity coefficient of 0.84, while Keifer shared low genetic similarity and was separated at a similarity coefficient of 0.63 from Punjab Beauty and Baggugosha. Shinseiki (*P. pyrifolia*)-a Japanese cultivar formed a separate sub-group (IIC) and was the second most diverse genotype after Sucker among all the genotypes used in this study. It showed a similarity coefficient of 0.54 with YaLi (*P. pyrifolia*), which is native to China.

The results of present study indicated a considerable level of genetic variation in the most of the genotypes used. The clustering of genotypes in the dendrogram of 11 pear genotypes showed no relationship with their pedigree and origin. Furthermore, the relation among genotypes as revealed by molecular markers were not significantly correlated with those based on morphological observations for all the genotypes, suggesting that two systems give different estimates of genetic relations among genotypes. These differences may have arisen because diversity at molecular level is neutral, whereas, at morphological level environment plays an important role. *Pyrus pyrifolia* varieties were grouped with *Pyrus communis* × *Pyrus pyrifolia* hybrids. Some of clustering of genotypes were found in agreement with morphological data but not seen for all the genotypes under study. However, some cluster was found to be congruent with the geographical distribution, *e.g.* Kainth and Shiara both of Indian origin were clustered together. The similarity coefficient was only 0.53 indicating a wide diversity among genotypes which might be due to nature of genotypes under study, their pedigree and interspecific hybridization. Bao *et al.* (2), while studying genetic diversity in *Pyrus* cultivars in East Asia reported that Chinese sand pear consisted of four groups with Chinese White pear and Japanese pear, showing large diversity. Genetic diversity of *Pyrus* germplasm (63 accessions) were characterized using 12 microsatellite markers by Ahmed *et al*. (1) who observed similarity coefficients ranging from 0.00 to 1.00 in different pear genotypes and the data illustrated an unambiguous genetic diversity and relationship with the local accession KT53 (Btung), representing the highest genetic diversity to all genotypes, had a unique genetic base.

Neighbor joining tree was generated using UPGMA method and is depicted in Fig. 2. Tree obtained through DARwin 5.0 corresponded to the clustering pattern observed through NTSYS indicating that the analysis was reliable. The genotypes were divided into two main groups, *i.e.* I and II. The genotypes YaLi, Shinseiki, Keifer, Punjab Beauty and Baggugosha formed group I. It was further sub-divided into two

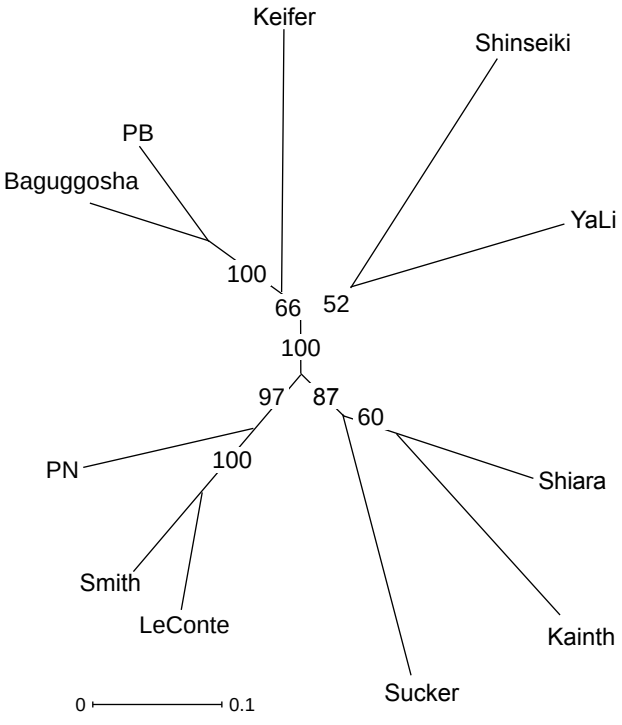

Fig. 2. Tree showing clustering of 11 pear genotypes using computer software DARwin 5.0.

sub-groups (IA and IB). The sub-group IA included YaLi and Shinseiki, while IB comprised of Keifer, Punjab Beauty and Baggugosha genotypes. Main group II included a total of six genotypes and was grouped into two sub-groups with rootstocks Shiara, Kainth and Sucker (*P. calleryana*) as sub-group IIA and cultivars Patharnakh, Smith and LeConte in IIB. The analysis done with either NTSYS or DARwin clearly distinguished genotypes into two major clusters having same genotypes falling in each cluster. This clearly reveals the precise genetic analysis of genotypes and any type of software can be applied for genetic diversity analysis. On the basis of present results, it is concluded that the SSR markers are useful in distinguishing pear genotypes.

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