Indian J. Hort. 68(3), September 2011: 293-302

Analysis of genetic diversity in pear germplasm using morphological traits and DNA markers

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ABSTRACT

Variability among 47 pear genotypes was evaluated for characters like vegetative, reproductive and fruit traits. Among three pear groups, hard pear group was found earliest in flowering, semi-soft pear strains were observed with maximum fruit length, breadth and weight, whereas soft pear had the maximum number of fruits per spur. The fruit characters showed significant variation among three groups with maximum TSS in semi-soft pear strains and highest acid content in hard pear strains. Genetic divergence among these genotypes was also estimated using simple sequence repeats (SSR) marker. Thirteen SSR primer pairs amplified 733 bands in 47 genotypes with the average of 15.7 bands per genotype. On the basis of SSR data, the 47 genotypes clustered into four major groups with similarity coefficient of 65 per cent. The dendrogram based on five morphological markers showed 10 per cent similarity coefficient. The set of microsatellite markers used in the present study provided a positive assessment of the ability of SSR markers in producing unique DNA profiles and established discrete identity of pear genotypes, which, otherwise was not possible using only morphological traits.

Key words: Pear germplasm, SSR markers, genetic diversity.

INTRODUCTION

Genus *Pyrus* belongs to family Rosaceae and sub-family Pomoideae order Rosales. The genus is supposed to be native of mountainous regions of western China. Speciation has occurred mainly in eastern and central Asia, the Himalayas, Caucasus, Asia Minor and Eastern Europe (Bell and Hough, 2). Pear evolved as amphidiploids between two ancestral species having basic chromosome numbers of x = 17. The pear cultivars are grouped in three categories, *i.e.*, European, Asian and their hybrids. Asian pear (*P. pyrifolia*) is native to eastern Asia including China, Japan and Manchuria. European pear (*P. communis*) is native to Europe. Asian pears have the advantage of growing in sub-tropical region, whereas European pears grow mainly in temperate zones. Patharnakh belongs to *P. pyrifolia*, while Baggugosha is believed to be natural hybrid between these two groups. The University is maintaining germplasm of a good number of varieties collected from local selections, introduced from other countries or different parts of the country. However, no information is available about the extent of the genetic diversity in the available germplasm, which is the basis for any genetic improvement programme. Knowledge about the degree and distribution of genetic diversity and relationship among the breeding material has a significant impact on crop improvement. Early efforts to identify cultivars by means of phenotypic data proved useful for a limited number of cultivars under certain conditions (Shen, 15). However, the phenotypic variability seen amongst accessions of tree fruit grown in different areas with slightly different environments and production practices demonstrates number of problems with that approach (Gianfranceschi *et al.*, 5). Simple sequence repeats (SSRs) also designated as microsatellites have become the markers of choice in animal and plant species because of their abundance, high degree of polymorphism and suitability for automation. The SSRs developed in apple show good synteny with pear and have been used for characterization of *Pyrus* germplasm (Cipriani *et al.*, 4). Recently, a good number of SSR markers have been developed in pear (Kimura *et al.*, 10). In this study, we selected 13 SSRs developed from pear and used them for assessing genetic diversity of pear germplasm available at Punjab Agricultural University, Ludhiana. In addition to SSR markers, data on several phenotypic traits was also recorded and used for assessing overall genetic diversity in the germplasm.

MATERIALS AND METHODS

Forty seven pear accessions used in this study are presented in Table 1, including 10 strains of hard pear (*P. pyrifolia*), 25 strains of semi-soft pear (*P. communis* x *P. pyrifolia*), seven accessions of Asian soft pear (*P. pyrifolia*), four accessions of European pear (*P. communis*) and one unknown *Pyrus* species. All the 47 accessions are being maintained at Punjab Agricultural University, Ludhiana. The observations on

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morphological characters were recorded during 2007-2008. The 47 genotypes were evaluated for vegetative, flowering and fruiting behaviour. The vegetative characters like foliage colour at bud burst, leaf shape, leaf margin, leaf apex, leaf base and flowering initiation were recorded visually. The data on fruit characters and quality attributes were recorded in quantitative terms. Newly emerged leaves samples from 47 genotypes were harvested in month of February and stored at -80°C until DNA extraction. DNA was isolated using CTAB (Cetyl trimethyl ammonium bromide) method as modified by Saghai-Maroof *et al.* (13). Thirteen SSR primer pairs from pear (Yamamoto *et al.*, 18) were used for PCR amplification of DNA. PCR reaction mix consisted of 40-60 ng of genomic DNA, 1 unit of *Taq* polymerase (Life Technologies, USA) and 2 µl of forward and reverse primers. PCR was performed in an MJ Research PTC200 or Eppendorf Master Cycler using the following temperature profile : initial denaturation for 3 min. at 94°C followed with 1 min. at 94°C, 1 min. at an appropriate annealing temperature and 2 min. at 72°C, with a final elongation step of 10 min. at 72°C. The PCR products were fractionated in 2.5 per cent agarose in 0.5X TBE buffer. The gel was run at 10V/cm, visualized under UV light and photographed using UVP gel documentation system (Model GDS 7600).

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 and 4. The amplified bands were recorded as 1 (band present) and 0 (band absent) in a binary matrix and further analyzed the data with NTSYS-pc (Version 2.02i) software. A dendrogram of 47 genotypes was constructed by using UPGMA (Unweighted Pair Group Method using Arithmetic Averages) available in NTSYS based on Nei genetic identity (Nei and Li, 12). Analysis of variance was calculated for various traits for 47 genotypes, which were planted in CRBD with three replications each using computer programme CPCS1 (Cheema and Singh, 3).

RESULTS AND DISCUSSION

The data in Table 3 show the foliage colour of Greyed Red (179A) in all strains of hard pear, and cultivars like Orient, Florida Home, Tenn and Pack ham's Triumph of Asian soft pear, whereas, Yellow Green (147A) colour was observed in all strains of semi-soft pear, and Yellow Green (144B) in cultivars Nijisseiki, Shinseiki, Kosui, Hosui, T-Su-Li and Saharanpur (SH). However, the leaf shape of orbicular, serrulate leaf margin, acuminate leaf apex and reinform leaf base was found in hard pear strains. The semi-soft pear strains possessed ovate leaf shape, serrulate leaf margin, acute leaf apex and convex leaf base. Cultivars like Nijisseiki, Shinseiki, YaLi, Hosui, Kosui and T-Su-Li were observed with orbicular leaf shape, serrate leaf margin, acute leaf apex and convex leaf base. This variability was ascribed to the differences in genetic makeup of different pear genotypes. Similar variability in the foliage colour at bud burst was observed Yellow Green (144B) in Nijisseiki, Shinseiki, Kosui, and YaLi (Singh, 16). A crenate and serrate leaf margin was reported in Callery pear (Gilman and Watson, 6).

Earliest flowering commenced from 15th-20th February in strain I, strain II, strain V and strain VI of hard pear and 19th to 25th February in strains S20, S21, S22, S23, S24 and S25 of semi-soft pear (Table 3). Cultivars like Florida Home was observed to be earliest in flowering from 28th February, followed by Orient (1st March), Tenn (5th March), YaLi (7th March). Late flowering was observed in Kosui from 17th March followed by T-Su-Li (13th March) and Nijisseiki (10th March). Hard pear strains I and II showed earliest end of flowering (3rd to 14th March) and late in strains III and IV (6th to 14th March). In semi-soft group, it started on 8th March in strains S20, S21, S22, S23, S24 and S25 and extended up to 15th March. However, in cultivars like PAU-I and Saharanpur (SH) end of flowering started from (16th March) followed by Orient, Tenn, Florida Home, Packham's Triumph (20th March) and late was found in Kosui from 4th April. Like foliage traits, there was very little variability for flowering time and duration and may be attributed to the difference in chilling hours required for breaking the flower bud dormancy. Similar varietal variability in respect to end of flowering has also been reported by Griggs and Iwakiri (7).

In hard pear group, maximum number of fruits per spur was observed in strain IX (2.66) and minimum (1.33) in strains II and VI (Table 4). Number of fruits per spur ranged from 1.00 to 2.33 with maximum fruits per spur in strain S13 and lower in strains S17 and S23 of semi-soft pear group. Highest fruits per spur (4.00) was found in Kosui and lowest (1.00) in Florida Home, Orient, Tenn, Packham's Triumph and PAU-I. The average data of number of fruits per spur depicts that soft pear strains were observed with larger mean number of fruits per spur (1.92), followed by hard pear (1.86) and lower in semi-soft pear (1.62). Two fruits per spur were observed in Packham's Triumph, a cultivar (Wauchope, 17). However, the fruit length varied from 5.78-7.51 cm in different strains of hard pear with maximum value in strain X and lowest in strain XI. Among semi-soft pear strains, maximum (7.96 cm) fruit length was observed in strain S21 and minimum (5.93 cm) in strain S17. Table 4 reveals maximum (6.94 cm) fruit breadth in strain XII and minimum (5.32 cm) in strain XI of hard pear group, whereas in semi-soft pear group it varied from 5.69 to 6.94 cm with maximum fruit breadth in strain S24 and lowest in S8. Orient was recorded with

Table 1. Type and origin of the pear genotypes used in the study.

Genotype	Species/parentage	Origin
Strains of hard pear (Patharnakh)		
Strain I	*Pyrus pyrifolia*	India
Strain II	*P. pyrifolia*	India
Strain III	*P. pyrifolia*	India
Strain IV	P. pyrifolia	India
Strain V	*P. pyrifolia*	India
Strain VI	*P. pyrifolia*	India
Strain IX	*P. pyrifolia*	India
Strain X	*P. pyrifolia*	India
Strain XI	P. pyrifolia	India
Strain XII	*P. pyrifolia*	India
Asian soft pear and other varieties		
Nijisseiki	*P. pyrifolia*	Japan
Shinseiki	*P. pyrifolia*	Japan
YaLi	*P. pyrifolia*	China
Hosui	*P. pyrifolia*	Japan
Kosui	*P. pyrifolia*	Japan
T-Su-Li	*P. pyrifolia*	China
Saharanpur (SH)	*P. pyrifolia*	India
Orient	*P. communis*	North America
Florida Home (FH)	*P. communis*	North America
Tenn	*P. communis*	North America
Packham's Triumph (PT)	*P. communis*	Australia
PAU-I	*Pyrus* spp.	India
Strains of semi-soft pear		
S 1	*P. communis* x *P. pyrifolia*	India
S 2	*P. communis* x *P. pyrifolia*	India
S 3	*P. communis* x *P. pyrifolia*	India
S 4	*P. communis* x *P. pyrifolia*	India
S 5	*P. communis* x *P. pyrifolia*	India
S 6	*P. communis* x *P. pyrifolia*	India
S 7	*P. communis* x *P. pyrifolia*	India
S 8	*P. communis* x *P. pyrifolia*	India
S 9	*P .communis* x *P. pyrifolia*	India
S 10	*P. communis* x *P. pyrifolia*	India
S 11	*P. communis* x *P. pyrifolia*	India
S 12	*P. communis* x *P. pyrifolia*	India
S 13	*P. communis* x *P. pyrifolia*	India
S 14	*P. communis* x *P. pyrifolia*	India
S 15	*P. communis* x *P. pyrifolia*	India
S 16	*P. communis* x *P. pyrifolia*	India
S 17	*P. communis* x *P. pyrifolia*	India
S 18	*P. communis* x *P. pyrifolia*	India
S 19	*P. communis* x *P. pyrifolia*	India
S 20	*P. communis* x *P. pyrifolia*	India
S 21	*P. communis* x *P. pyrifolia*	India
S 22	*P. communis* x *P. pyrifolia*	India
S 23	*P. communis* x *P. pyrifolia*	India
S 24	*P. communis* x *P. pyrifolia*	India
S 25	*P. communis* x *P. pyrifolia*	India

Table 2. Details of the selected microsatellite markers.

Locus	Primer sequence (5'-------------- 3')	
	Forward	Reverse
NH001c	AATACTAATCCTTTTTGCTAA	TCCATTCAATCTGTCTCGGTC
NH002b	GGAGTCAGCGGCAAAAAAAG	CCCACTCCCTCCTCTTATTGT
NH004a	AGGATTTTTGGGACGAGTTTAGAG	CCACATCTCTCAACCTACCA
NH005b	TGAGAAGAATTAGCCATGATGA	TTACTACTTGCGTGCGTTCC
NH007b	TACCTTGATGGGAACTGAAC	AATAGTAGATTGCAATTACTC
NH008b	GGAAAAGAGAAGGAAGAAGAGAAGG	TGATAGGGGCATTTCGGTAA
NH009b	CCGAGCACTACCATTGA	CGTCTGTTTACCGCTTCT
NH011b	GGTTCACATAGAGAGAGAGAG	TTTGCCGTTGGACCGAGC
NH012a	CCGCCAGTACCCATCTCCA	ACCACTCAAACCCCCCCTC
NH013a	GGTTTGAAGAGGAATGAGGAG	CATTGACTTTAGGGCACATTTC
NH014a	CAAACCTAACCCTAAATACC	TGTTCATATATTCATCATC
NH015a	TTGTGCCCTTTTTCCTACC	CTTTGATGTTACCCCTTGCTG
NH017a	CAGAAAGGAGAGGGCTACAG	CCCTCACCCAATCAAAACTC

highest fruit breadth and lowest in Hosui. The average data of pear genotypes show that semi soft pear strains had significantly higher mean fruit weight 165.50 g over hard pear (163.74 g) and soft pear (110.52 g) group. The data showed a significant variation in fruit length, breadth and weight among different groups of pear and might be due to genetic difference of different varieties of pear and their interaction with the environment. In earlier studies, similar variation in fruit length was observed by Mukherjee and Rana (11). Among the soft pear varieties, Sandhu *et al.* (14) reported that the fruits of YaLi were heaviest followed by Hosui, Shinseiki, Nijisseiki and Kosui.

In hard pear group, total soluble solids were highest (11.00%) in strains IX, XI and XII and lowest (9.52%) in strain IV (Table 5). Among semi-soft group, maximum TSS was observed in strain S15, followed by strain S16 (15.08%) and S8 (14.37%) and ranged from 10.00 to 14.00 per cent in Asian soft pear group. A great variability with respect to total soluble solids might be due to differences in fruit development pattern in different pear genotypes. Similar results were presented by Gupta and Chohan (8) in different cultivars of pear with maximum TSS in LeConte, followed by Patharnakh and Smith. However, among hard pear strains, the highest acid content (0.50%) was observed in strain I and lowest (0.26%) in strains IV and V. On the other hand, in soft pear group, maximum acidity was observed in Packham's Triumph (0.40%) and lowest in Nijisseiki (0.19%). The average data of acidity showed a significant variation in acid content among different groups of pear varieties might be due to different rate of conversion of organic acids into soluble sugars by different genotypes. Hence, analysis of variance for 47 pear genotypes for various characters revealed statistically significant differences among the three pear group, *i.e.* hard pear group, Asian soft pear group and semi-soft pear group.

The 47 pear genotypes amplified a total of 733 fragments with an average of 15.7 fragments for each genotype. Number of bands amplified ranged from as low as 10 in S12 to as high as 21 in strain II (Table 5). Thus, some markers did not show any amplification in certain genotypes, while others amplified more than one band in certain genotypes (Fig. 1), which could be due to presence of multiple alleles or heterozygosity. Based on diversity analysis using UPGMA, the 47 genotypes could be grouped into four major clusters designated as I-IV (Fig. 2). The genetic similarity values among genotypes ranged from 0.65 to 1.0. Group I and II further forms two sub-clusters each, designated as IA, IB and IIA, IIB. The group IA comprised of nine

Table 3. Foliage and leaf characteristics and flowering duration of the pear germplasm.

Genotype	Foliage colour at bud burst	Leaf shape	Leaf margin	Leaf apex	Leaf base	Start of flowering	End of flowering
Strains of hard pear (Patharnakh)							
Strain I	GR (179A)[a]	Orbicular	Serrulate	Acuminate	Reinform	15-20 Feb.	3-14 March
Strain II	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	15-20 Feb.	3-14 March
Strain III	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	18-21 Feb	5-16 March
Strain IV	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	18-21 Feb	5-16 March
Strain V	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	15-20 Feb.	6-14 March
Strain VI	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	15-20 Feb.	6-14 March
Strain IX	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	16-22 Feb	6-14 March
Strain X	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	16 -22 Feb	6-14 March
Strain XI	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	16-22 Feb	6-14 March
Strain XII	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	16-22 Feb	6-14 March
Asian soft pear and other varieties							
Nijisseiki	YG (144B)	Orbicular	Serrate	Acute	Convex	10-15 March	28-30 March
Shinseiki	YG (144B)	Orbicular	Serrate	Acute	Convex	10-15 March	30-31 March
YaLi	YG (144B)	Orbicular	Serrate	Acute	Convex	7-10 March	26-27 March
Hosui	YG (144B)	Orbicular	Serrate	Acute	Convex	10-15 March	28-29 March
Kosui	YG (144B)	Orbicular	Serrate	Acute	Convex	17-19 March	4-6 April
T-Su-Li	YG (144B)	Orbicular	Serrate	Acute	Convex	13-15 March	26-27 March
Saharanpur (SH)	YG (144B)	Ovate	Serrulate	Acute	Convex	16-18 March	20-25 March
Orient	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	1-5 March	20-25 March
Florida Home (FH)	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	28-29 Feb	20-25 March
Tenn	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	1-5 March	20-25 March
Packha's Triumph (PT)	GR (179A)	Orbicular	Serrulate	Acuminate	Reinform	6-8 March	16-18 March
PAU-I	YG (144B)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
Strains of semi-soft pear							
S 1	YG (147A)	Ovate	Serrulate	Acute	Convex	8-10 March	19-22 March
S 2	YG (147A)	Ovate	Serrulate	Acute	Convex	8-10 March	19-22 March
S 3	YG (147A)	Ovate	Serrulate	Acute	Convex	8-10 March	19-22 March
S 4	YG (147A)	Ovate	Serrulate	Acute	Convex	6-8 March	15-17 March
S 5	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 6	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 7	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 8	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 9	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 10	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 11	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 12	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 13	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S14	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 15	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 16	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 17	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 18	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 19	YG (147A)	Ovate	Serrulate	Acute	Convex	7-9 March	16-20 March
S 20	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March
S 21	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March
S 22	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March
S 23	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March
S 24	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March
S 25	YG (147A)	Ovate	Serrulate	Acute	Convex	19-25 Feb	8-15 March

[a] Numbers in parentheses refer to the colour codes as per Wilson's Chart. GR = Greyed Red, YG = Yellow Green

Table 4. Fruit characters of pear genotypes.

Genotype	No. of fruits per spur	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)	TSS (%)	Acidity (%)
Strains of hard pear (Patharnakh)						
Strain I	2.00	6.14	5.91	125.10	10.03	0.50
Strain II	1.33	6.72	6.16	157.10	10.17	0.40
Strain III	2.00	6.90	6.22	158.96	10.03	0.27
Strain IV	1.33	6.46	6.11	153.00	9.52	0.26
Strain V	2.00	6.36	6.31	156.66	10.31	0.26
Strain VI	1.33	6.91	6.13	159.96	10.30	0.38
Strain IX	2.66	6.98	6.37	176.10	11.00	0.39
Strain X	2.00	7.51	6.40	173.90	10.13	0.38
Strain XI	1.66	5.78	5.32	179.00	11.00	0.28
Strain XII	2.33	7.29	6.94	197.58	11.00	0.36
Mean	1.86	6.70	6.19	163.74	10.35	0.35
Range	1.33-2.66	5.78-7.51	5.32-6.94	125.10-197.58	9.52-11.00	0.26-0.50
CD at 5%	0.80	0.12	0.13	0.47	0.09	0.01
Asian soft pear and other varieties						
Nijisseiki	3.00	5.92	5.75	84.50	11.00	0.19
Shinseiki	3.00	4.83	5.26	70.00	12.50	0.23
YaLi	2.00	5.31	5.22	117.20	11.00	0.30
Hosui	2.00	5.17	4.81	74.84	14.00	0.33
Kosui	4.00	4.76	5.93	95.15	12.00	0.23
T-Su-Li	2.00	7.56	5.73	141.36	10.97	0.26
Saharanpur (SH)	2.00	7.06	5.84	139.00	11.13	0.23
Orient	1.00	6.32	7.24	116.10	11.00	0.27
Florida Home (FH)	1.00	5.11	6.24	108.00	10.00	0.28
Tenn	1.00	5.42	5.31	100.00	12.03	0.39
Packham's Triumph (PT)	1.00	6.36	6.41	138.10	12.07	0.40
PAU-I	1.00	6.71	5.93	142.00	12.00	0.37
Mean	1.92	5.88	5.81	110.52	11.64	0.29
Range	1.00-4.00	4.76-7.56	4.81-7.24	70.00-142.00	10.00-14.00	0.19-0.40
CD at 5%	0.48	0.05	0.20	0.09	0.06	0.10
Strains of semi-soft pear						
S 1	1.33	6.81	6.30	145.36	12.50	0.17
S 2	1.66	7.52	5.98	153.89	12.80	0.20
S 3	1.66	6.71	5.92	176.92	13.07	0.20
S 4	1.33	7.63	6.31	170.10	14.27	0.26
S 5	1.66	7.11	6.00	152.16	14.00	0.28
S 6	2.00	7.13	6.0	153.00	13.03	0.27
S 7	2.00	6.99	6.61	137.00	12.51	0.21
S 8	1.66	6.91	5.69	163.06	14.37	0.21
S 9	1.33	7.53	6.41	165.86	13.95	0.25
S 10	1.66	7.39	6.92	169.96	14.00	0.24
S 11	2.00	7.40	6.20	159.03	13.98	0.26
S 12	1.33	6.03	6.20	147.20	13.67	0.25
S 13	2.33	6.80	6.14	139.99	10.00	0.25
S14	2.00	6.69	5.84	185.16	14.25	0.26
S 15	1.33	6.91	5.97	184.00	15.11	0.23
S 16	2.00	7.00	6.07	170.20	15.08	0.25
S 17	1.00	5.93	5.71	127.00	9.00	0.26
S 18	1.33	6.90	5.81	143.16	11.80	0.25
S 19	2.00	6.80	6.00	150.96	13.00	0.26
S 20	1.00	7.54	6.47	183.00	12.31	0.30
S 21	1.66	7.96	6.37	172.00	12.00	0.23
S 22	2.00	7.51	6.59	189.00	12.87	0.26
S 23	1.00	7.72	6.27	187.00	13.00	0.39
S 24	1.33	7.00	6.94	199.60	11.07	0.31
S 25	2.00	6.72	6.47	213.00	12.11	0.38
Mean	1.62	7.07	6.21	165.50	12.95	0.26
Range	1.00-2.33	5.93-7.96	5.69-6.94	127.00-213.00	9.00-15.11	0.17-0.39
CD at 5%	NS	0.09	0.05	0.23	0.14	0.02
Pooled range	1.00-4.00	4.76-7.96	4.81-7.24	70.00-213.00	9.00-15.11	0.17-0.50
Pooled CD at 5%	0.79	0.10	0.07	0.27	0.12	0.03

Table 5. Total number of bands amplified in each of 47 genotypes using SSR primers.

Genotype	No. of amplified fragments with 13 primers
Strain I	19
Strain II	21
Strain III	14
Strain IV	15
Strain V	14
Strain VI	15
Strain IX	15
Strain X	17
Strain XI	18
Strain XII	15
Nijisseiki	16
Shinseiki	12
YaLi	15
Hosui	12
Kosui	11
T-Su-Li	11
Saharanpur (SH)	16
Orient	15
Florida Home (FH)	17
Tenn	17
Packham's Triumph (PT)	10
PAU-I	16
S 1	18
S 2	15
S 3	13
S 4	17
S 5	17
S 6	15
S 7	19
S 8	18
S 9	15
S 10	21
S 11	17
S 12	10
S 13	12
S 14	16
S 15	18
S 16	11
S 17	18
S 18	20
S 19	16
S 20	17
S 21	17
S 22	13
S 23	17
S 24	17
S 25	15
Avg.	15.7

Contd...

genotypes of hard pear strains among which strain I and IX, and strains II and VI showed highest similarity coefficient of 1.0, whereas group IB contained six semi-soft pear strains and one hard pear strain. Strain XI of hard pear and S1 of semi-soft pear also showed 100% similarity based on 13 SSR markers. The subgroup IIA and IIB included seven and nine genotypes respectively and these comprised both semi-soft and soft pear types. Several genotypes like S2 and S17, S3 and S22 and S6 and S25 showed 100% similarity based on the variation in SSR markers. The subgroup IIA showed that Packham's Triumph (PT) and Orient sharing 0.92 similarity coefficients and clustered with semi-soft pear strains S17 and S2. However, Tenn and Florida Home (FH) clustered with Saharanpur (SH) which is of Indian origin. In sub-group IIA, semi-soft pear strains S3, S22, S6, S25, S8 and S12 clustered with Asian soft pear varieties, namely Kosui, Nijisseiki and YaLi. Group III comprised of nine genotypes, all being semi-soft, of these the germplasm lines S4 and S23 and S5 and S24 showed 100% similarity. The group IV was composed of two semi-soft pear strains (S21 and S13) and three Asian soft pear varieties Shinseiki, Hosui and T-Su-Li. Shinseiki and Hosui are native to Japan, T-Su-Li native to China and S21 and S13 evolved in India. Germplasm line S19 was different from all other genotypes. Thus based on variation in SSR markers, the hard pear strains showed least variability. The semi-soft genotypes PT, Orient, Tenn and FH belong to species *P. communis* and all clusters together. Genotype Saharanpur which belongs to *P. pyrifolia,* cluster with four *P. communis* genotypes. Thus, the taxonomic classification of genotype Saharanpur needs to be reconsidered. All the hard pear genotypes belong to *P. pyrifolia* group in one cluster whereas other six *P. pyrifolia* genotypes having their origin from China and Japan group in distinct clusters. The 25 semi-soft genotypes, which were developed through interspecific hybridization, show grouping with soft pear genotypes belonging to *P. communis* and *P. pyrifolia*. Several genotypes, which show 100% similarity between themselves, may either be duplicate types or need more markers to be distinguished from each other.

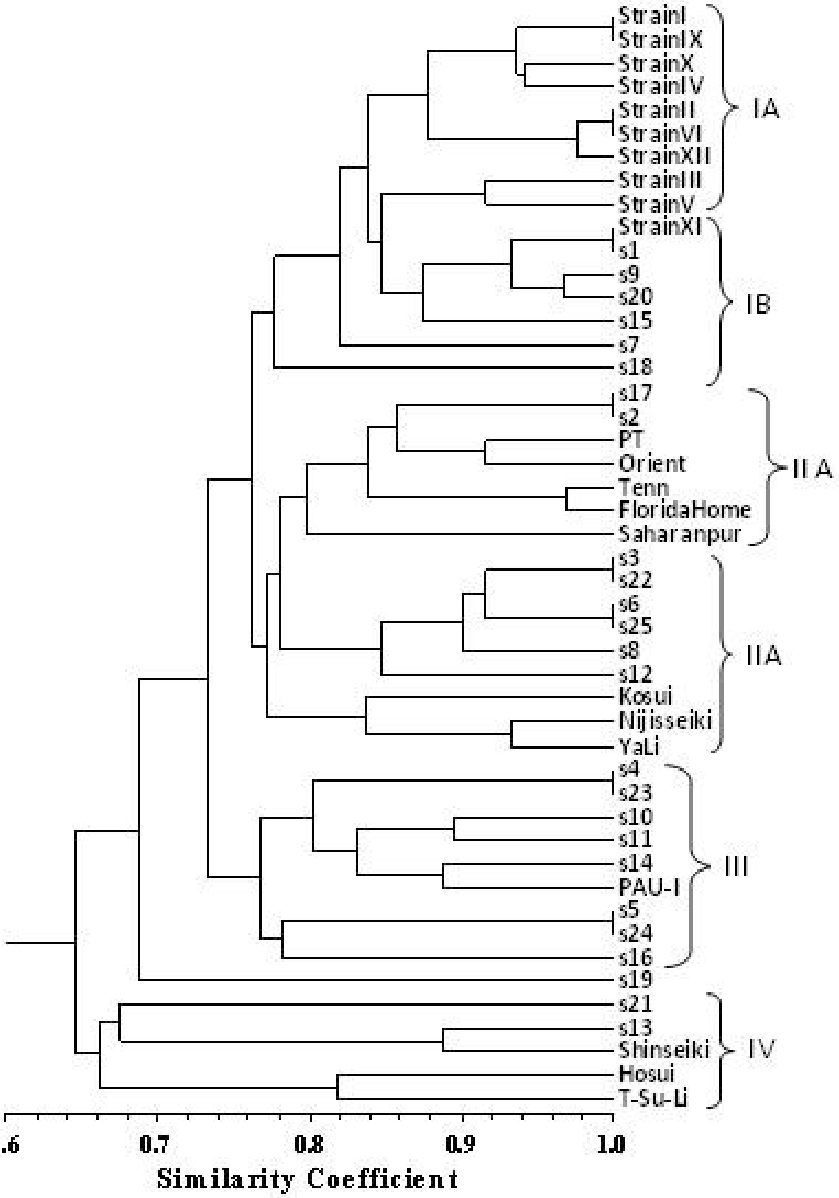

Fig. 2. Dendrogram for 47 pear genotypes based on 13 SSR molecular markers. The dendrogram was produced using the UPGMA method of Nei's genetic identity between the genotypes.

Based on a limited number of SSR markers used in the present study, the 47 genotypes belonging to different species, show sufficient overall variability. Variability among hard pear genotypes however is limited. Likewise, variability among *P. communis* genotypes seems limited, which could be due to very small sample, used in this study.

Based on morphological markers (foliage and leaf characters) which could be recorded as nominal data (Table 3), the 47 genotypes grouped into four distinct clusters designated as I to IV (Fig. 3). Ten hard pear strains and 25 semi-soft pear strains were clustered separately in group I and III respectively. Asian soft pear types from East (China and Japan) and West (North America) group in two distinct clusters. Hard pear strains shared 68% genetic similarity with soft pear types from West (North America), whereas, semi-soft pear strains and soft pear types from East Asia (China and Japan) showed 44% genetic similarity. The data on leaf characteristics classified all the genotypes

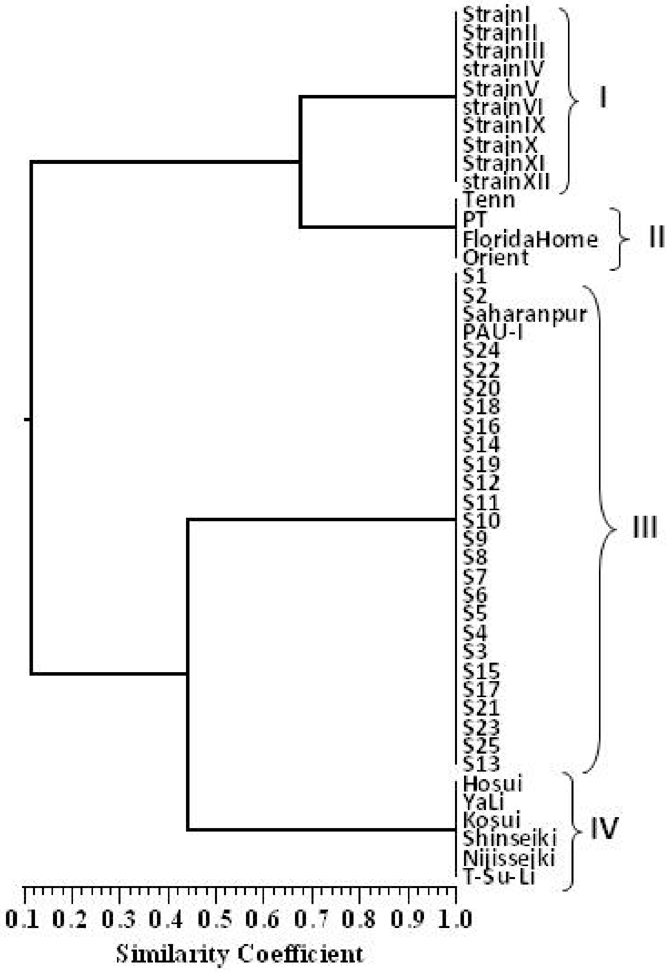

Fig. 3. Dendrogram of 47 genotypes evaluated for five morphological markers. The dendrogram resulted from UPGMA analysis based on Dice's similarity coefficient.

into four distinct groups, *viz.*, hard pear, *P. communis* (soft), *P. pyrifolia* (soft) and interspecific (semi-soft group). The genotype Saharanpur grouped among semi-soft interspecific group. This genotype, which has been classified under species *P. pyrifolia* needs to be changed. Similarly, the uncharacterised genotype, designated as PAU-1 may also have its origin from *P. communis* x *P. pyrifolia* inter-specific crosses.

The UPGMA based clustering showed that high level of diversity exits in soft pear and semi-soft groups but very limited diversity within hard pear. Thus crosses among the hard pear genotypes may not yield much variability whereas crosses between hard and semi-soft group may prove to be more valuable for developing new varieties. The collection analysed by Hokanson *et al.* (9) is a core subset of greater collection, and was designed to maximise the conserved variability, while the collection of varieties analysed in this work mainly includes *P. pyrifolia* from India and a smaller number of varieties from other countries. This may account for the lower level of variability detected in this work. Similarly, Yamamoto *et al.* (19) used seven SSR primers to conduct genetic diversity in five *Pyrus* species and observed a total of 65 putative alleles with an average of 9 alleles. A high level of genetic polymorphism with a mean of 28 putative alleles per locus in assessing genetic diversity and relationship of *Pyrus* cultivars native to East Asia was observed by Bao *et al.* (1). The SSR based diversity analysis among the 47 genotypes should guide the pear horticulturist/breeder for designing of the crossing programme. Thus combination of floral traits, fruit quality characteristics and marker based diversity may help breeders for attempting inter specific or intergroup crosses for further improvement of pear genotypes.

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Received: March 2011; Revised: May 2011;
Accepted : August 2011