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Genetic Diversity of pear (*Pyrus spp)* Germplasm Assessed by Simple Sequence Repeat (SSR) and Morphological Traits

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Abstract
This study was conducted to identify and recognize relations and diversity among accessions of the Iranian pears (*Pyrus spp*). A total of 34 pear accessions, derived presumably from at least six species, were subjected to simple sequence repeat (SSR) analysis. The Japanese and the Chinese pear samples were; "Housui" and "Yali" cultivars, example of *Pyrus pyrifolia and P. bretschneideri*, respectively. Some European pears and Iranian germplasm were analyzed. Seven SSR markers (KU10, BGA35, BGT23b, NH011b, NH013a, NH004a and NH015a) were used for the analysis. One hundred six visible amplified fragments (putative alleles) acquired for 34 pear samples NH011b and NH015a loci exhibited high heterozygosities of 0.82 and 0.79, respectively. BGA35 produced eight putative alleles, while NH013a created 22 putative alleles. The average value of allele per locus was 15. A phenogram was constructed based on the similarity-matrix data using the unweighted pair-group and arithmetic average methods. There was no association between the SSR and morphological phenograms. This work revealed relatedness of Iranian pear samples to the four species of *P. communis, P. syriaca, P. salicifolia,* and *P. glabra*.

Keywords: Genetic diversity, Persian pear, *Pyrus*, simple sequence repeats

Introduction

Pear (*Pyrus* spp.) is one of the major fruit crops in broad temperate regions throughout the world. The wild species of this genus are widespread in Europe, North Africa and Asia (Kajiura et al., 1983). Identification of Pyrus species is crucial because each species is a separate gene pool expressing unique characteristics of flowering, fruiting and resistance to biotic and abiotic stresses, which can be applied in breeding programs. Consequently, identification of new genes or physiological traits in the species could be a benefit to biologists, horticulturists, breeders and growers. Rubtsov (1944) proposed 35 species for the genus *Pyrus*. Kitamura (1979) listed 50 species for this genus. Browicz (1993) listed pears in two categories, one containing 38 species, the other including 47 taxa of hybrids and feral accessions. Different morphological characteristics, including leaf

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characteristics and seed traits (e.g. weight, length and width) of pears have been used for species identification. Previous studies (Challice and Westwood, 1973; Aldasoro et al., 1996) have identified 22 species using both morphological and chemical characteristics. Precise determination of the true number of *Pyrus* species is difficult, due to synonym names, low morphological variability intra- and inter-specific hybridization, introgression, mutations and polyploidy inductions, all of which contribute to the creation of pear sub-species (Bell et al., 1996). Vavilov and Bell et al. (1996) have convincingly demonstrated that China can be considered the main centre of origin for the *Pyrus* species. However, some species appear to be native to Iran (Rubtsove, 1944, Vavilov, 1951, Khatamsaz, 1992). In this respect the Caucasus Mountains and south of Caspian Sea was mentioned as one of the three centers of diversity for the genus *Pyrus* (Vavilov, 1951). Authentication botanical studies on genus *Pyrus* were led to the identification of 12 pear species in Iran (Khatamsaz, 1992). Those were adapted to different climatic and geographical conditions. Iranian pear germplasm not only are distributed south of the Caspian Sea Pyrus also it extends over to northwest and mid west of Iran. Beside this, Vavilov (1951) explained Pyrus sources in western Asia; specifically, in northern India, Pakistan, Afghanistan, Tajikistan and Uzbekistan. Further resources of genus *Pyrus* in the region are located in Caucasus Mountains (Asanidze et al., 2011). In this respect, Volk et al. (2012) identified different *P. communis* in the Caucasian region similar to those available in the germplasm found in Oregon in the USA.

In recent years, application of molecular markers for pear identification has been increased. SSR markers are a good source of polymorphism for eukaryotes because of co-dominant inheritance and large number of alleles per locus and frequency in genome. Ghosh et al. (2006) used SSR primers to identify pears from Europe and Asia. Brini et al., (2008) used seven SSRs primers to identify pears from Tunisia. Accordingly, they obtained 12 different fingerprinting patterns. The patterns could distinguish 25 Tunisian cultivars. Erfani et al. (2012) produced 174 alleles using 28 SSR primers to identify a set of 47 pear samples. Results showed one Iranian cultivar had similarity with *P. bretschneideri*, a Chinese species. Gasi et al., (2013) discriminated 64 pear germplasm of Bosnia and Herzegovina with an average of 14.5 alleles per locus using 11 SSR pair primers. Fan et al., (2013) developed 67 useful primer pairs and discriminated among 8 pear species. Further application of the primers gave discrimination among 8 pear species.

The objectives of this study were; to evaluate relationship and diversity among Iranian pears, using SSRs and morphological traits. In addition, efficacy of each locus to find diversity was the other mean of this investigation.

Materials and Methods

Plant materials and DNA extraction

Thirty-four pear accessions were used in this study, including 15 Iranian cultivars and 15 accessions of several wild species from Iran and four cultivars grown in Japan (Table 1). The 14 samples of Iranian cultivars were as; Iranian Bartlett, Shah Miveh Esfahan, Shah Miveh Mashhad, Sebri Mashhad, Sebri Esfahan, Tabrizi, Dome Kaj, Belderjani, and the ancient cultivars Laleh and Jose Ghand. These were collected from different regions in Iran and divided into two categories; known wild accession species and unknown wild species. Code numbers were used to simplify identification of all Iranian accessions.

Four cultivars were used as reference cultivars: the Chinese pear Yali as the ecotype of *P. pyrifolia* (Teng et al., 2002), the Japanese pear (*P. pyrifolia*) Housui, and the European pears (*P. communis*) Bartlett and La France (Table 1). Genomic

DNA was extracted from 200 mg of fresh leaf tissue, using the QIAGEN genomic tip method. The quality of the extracted DNA was measured using a ratio of A260/A280, through reading via spectrophotometer. This ratio for standard-quality DNA samples ranged between 1.7 and 2; if the ratio was out of this limit, the DNA was re-extracted.

SSR analysis

Seven SSR primers were used for SSR-PCR amplification (Table 2) as follow: NH004a, BGA35, BGT23b, KU10, NH011b, NH013a and NH015b (Yamamoto et al., 2002a, 2002b). The motif for each primer was as follow; KU10, (CT)10, BGA35, (AG)8, BGT23b; (TC)18.5, NH011b; (AG)9 AA (AG)7, NH012a; (AG)23, NH013a; (AG)13, NH004a; (GA)19, NH015a; (AG)19. The PCR reaction was performed in a 20 µL solution consisting of 10 mM Tris-HCl (pH 8.3); 0.01 % gelatin; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM for each of dNTPs; 10 pmoles of each primer, with forward primers having a fluorescent label (FAM or TET or HEX) and the reverse primer being unlabeled; 10 ng genomic DNA; and 0.5 unit of Taq polymerase (Life Technology, USA). For the SSR loci, KA16, KU10, BGA35, and BGT23b, amplifications were conducted under the following conditions: initial denaturation condition at 94 °C for 2 minutes followed by 10 cycles of denaturation at 94 °C for 1 minute, reduced by 0.5 °C per cycle, at 72 °C for 2 minutes for extension, at 25 cycles at 94 °C for 1 minute, at 55 °C for 1 minute, at 72 °C for 2 minutes, then at the final stage for 10 minutes. For the other five SSR loci (NH004a, NH011b, NH013a, NH014a, and NH015a), the amplification program was 35 cycles at 94 °C for 1 minute, at 50-55 °C for 1 minute, and at 72 °C for 10 minutes, for denaturation, annealing and primer extension, respectively. PCR products were separated and detected using a PRISM 377 DNA sequencer (PE Applied Biosystems, USA). The size of amplified bands was calculated based on an internal standard DNA (GeneScan-350TAMRA, PE Applied Biosystems, USA) using GeneScan software (PE Applied Biosystems, USA).

Evaluation of morphological traits

Seven morphological characteristics of leaf and fruit were evaluated: leaf margin, leaf base, leaf shape, leaf tip, leaf petiole length, fruit shape, and fruit size. Leaf petiole length < 2 cm was considered small, 2-4 cm medium and > 4cm long. Fruit size was categorized based on height and diameter and divided into small, medium and large (Table 3). Fruit shape was categorized as ovate, round or pyriform (Table 3). Characteristics were coded as digits (0, 1, 2 to 7 states for some characteristics) and states in the observed specimens recorded in a characteristic-taxon matrix.

Statistical analysis

The observed and expected heterozygosities of pears for each SSR locus were calculated using CERVUS version 2.0 software (Marshall et al., 1998). The observed heterozygosity (HO) was calculated as the number of heterozygous genotypes at a given locus divided by the total number of genotypes scored at that locus. The expected heterozygosity (HE) was calculated based on the frequency of each allele according to the formula: $HE=1-\Sigma pi2$, where pi is the frequency of the ith alleles of each SSR locus (Roder et al., 1995). A phenogram of 34 accessions was constructed using UPGMA (unweighted pair-group method using arithmetic averages) based on Nei's genetic identity (Nei, 1972). NTSYS-pc version 2.01 software was used to construct the phenogram (Rohlf, 1998). Clustering analyses of morphological characteristics were performed using the UPGMA method based on a Euclidean distance matrix.

Table 1. List of pear species, cultivars and the collected regions of pear germplasm that were used in this study.

Code	Germplasm name	Species	Collected region	Altitude (m)
101	101	unknown	south central, Iran	1,650
102	102	unknown	south central, Iran	1,650
103	103	unknown	south central, Iran	1,650
120	Tabrizi Cv2	*P. communis*	north east, Iran	980
130	Sebri Mashhad	unknown	north east, Iran	980
160	Sebri Esfahan	unknown	central, Iran	1,570
170	Ghara Sorkhi	unknown	central, Iran	1,570
180	Jose Ghand	unknown	central, Iran	1,570
195	Laleh Cv	unknown	central, Iran	1,570
451	451	*P. hyracana*	north, Iran	1,480
453	453	*P. syriaca*	west, Iran	1,380
457	457	*P. boissieriana*	north, Iran	1,480
459	459	*P. salicifolia*	north west, Iran	1,350
461	461	*P. salicifolia*	north west, Iran	1,350
463	463	*P. glabra*	central west, Iran	1,320
465	465	*P. syriaca*	west, Iran	1,360
469	469	*P. syriaca*	west, Iran	1,320
471	471	*P. syriaca*	west, Iran	1,380
473	473	*P. syriaca*	west, Iran	1,250
477	477	*P. syriaca*	west, Iran	1,270
479	479	*P. syriaca*	west, Iran	1,380
547	Natanzi Cv	*P. communis*	central, Iran	980
551	Iranian Bartlett	*P. communis*	north east, Iran	980
623	Dome Kaj	*P. communis*	north east, Iran	980
625	Shah Miveh Mashhad (625)	*P. communis*	north east, Iran	980
627	Shah Miveh Mashhad (627)	*P. communis*	north east, Iran	980
629	Sebri Mashahd (629)	unknown	north east, Iran	980
631	Shah Miveh Esfahan Cv	*P. communis*	central, Iran	1,570
633	Tabrizi Cv1	*P. communis*	north east, Iran	980
635	Belderjani	*P. communis*	north east, Iran	980
Ba	Bartlett	*P. communis*	NIFTS, Japan	877
Ho	Housui	*P. pyrifolia*	NIFTS, Japan	877
La	La France	*P. communis*	NIFTS, Japan	877
Ya	Yali	*P. bretschneideri*	NIFTS, Japan	877

Results

SSR amplification of pears

Seven SSR primers; NH004a, BGA35, BGT23b, KU10, NH011b, NH013a, and NH015a – were used in this study. One hundred six visible amplified fragments (putative alleles) for 34 pear samples were obtained using seven primer pairs. The seven SSR markers generated a range of putative alleles from 8 (BGA35) to 22 (NH013a), with an average value of 15.1 (Table 2). The SSRs NH013a and BGT23b (Table 2 and Fig. 1) generated 22 and 19 as the highest putative alleles, respectively. High heterozygosity values were obtained for NH011b (HO=0.82, HE= 0.92), NH015a (HO= 0.79, HE = 0.87), and NH004a (HO = 0.71, HE =0.89) (Table 2). The size of amplified fragments diverged from 87 bp (NH004a) to 249 bp (KU10). The average number of allele per locus was 15.1 (Table 2). The highest value of He were detected 0.94 for NH013a, while the lowest value was observed for BGT23b.

Cluster analysis

A phonogram was constructed based on Nei's genetic similarity using seven SSR loci for 34 Iranian pears. The genetic similarity was ranged from 97% to 76%. Relationship among the 34 individuals of Pyrus spp and cultivars was being established using genetic similarity values.

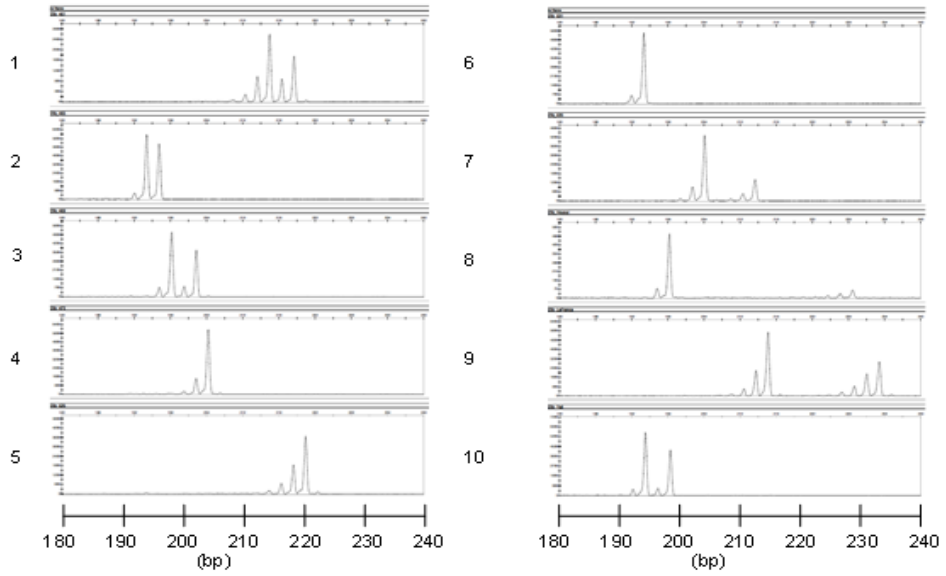

Fig.1. Amplified fragment patterns of BGT23b SSR from 10 pears. Lane 1 to 10 display amplified fragments for 10 pear species and cultivars as the following; 1; *P. salcifolia,* 2; *P. syriaca,* 3; *P. syriaca,* 4*; P. syriaca,* 5; "Shah Miveh Mashhad" 6; "Shah Miveh Esfahan", 7; "Belderjani' 8-"Hosoui', 9; "La France' 10; "Yali'

This further constructed by UPGMA cluster analysis via Nei's genetic distance coefficient. The obtained phenogram separated the pear accessions into two major clusters. "Belderjani" (635) (Fig. 2), was the only accession distant from all other cultivars at 76% similarity. The phenogram was composed of two main clusters; one of those consisted of four sub-clusters. The first cluster was comprised of seven samples. These included two East Asian cultivars "Housui" and "Yali", which joined together in the first clade of cluster I separated at 81% similarity from other genotypes. Five wild samples *P. hyracana* (451) and *P. boissieriana* (457); *P. syriaca* (469); and two unknown ancient species (101, 102) were placed in the second clade of cluster I (Figure2). Though, *P. syriaca* (469) was separated at 78% similarity from other samples of this clade (Figure 2). The second main cluster on figure (1) consisted of four sub-clusters: I (seven accessions), II (four accessions), III (nine accessions) and IV (six accessions) (Fig. 2). Sub-cluster I contained "Bartlett" maintained in Japan and "Bartlett" (551) from Iran, presented identical SSR genotypes. "Dome Kaj" (623) showed a close relationship to the "Bartlett" samples (Fig. 2). Results showed that all accessions in sub-cluster I belong to *P. communis* or owned close similarity, (e.g. 479). It was considered that Iranian cultivars "Tabrizi" Cv1 (633), "Tabrizi" Cv2 (120), and "Dome Kaj" (623) were all *P. communis*. Sub-cluster II consisted of two species: *P. salicifolia,* (samples 459 and 461) and two *P. syriaca* (samples 471 and 477). The samples of *P. salicifolia* had round fruits with willow leaves (Table 2). Sub-cluster III contained an unknown germplasm (103), "Natanzi" (547), "Shah Miveh Esfahan" Cv (631). In this sub-cluster "Sebri Esfahan" (160), and "Ghara Sorkhi" (170) recognized genetically identical. Further, *P. glabra* (463), collected from Esfahan province, *P. syriaca* (465) from Kurdistan province, "Sebri Mashhad" (130), "Sebri Esfahan" (160), and 'Sebri Mashahd (629) from northern Iran, were positioned in this sub-cluster. "Shah Miveh Esfahan" and "Natanzi" cultivars are two Iranian important commercial cultivars.

Genotypes and cultivars in this sub-cluster had different genetic distance to each other. In this respect, "Shah Miveh Esfahan" illustrated 3% genetic distance with *P. glabra*. In sub-cluster IV "Laleh" (195), and two samples of *P. syriaca* (473) indicated similarity of 95%, Two samples of "Shah Miveh Mashhad" (accession 625 and 627), showed high genetic similarity. However "Shah Miveh" from Esfahan (631) and Mashhad (625, 627) were placed in two different sub-clusters. Furthermore, both "Sebri" (130 and 160) were positioned together in sub-cluster III, suggesting that these two accessions had common origin with 97% similarity (Fig. 2). At least four species –*P. communis*, *P. syriaca*, *P. salicifolia*, and *P. glabra* were placed in these sub-clusters. It is possible inter-specific crosses among these species already occurred and interspeceficly hybrid extended over via seed propagation. In this research, high diversity was discovered within *P. syriaca* samples.

Evaluation based on leaf and fruit characteristics

Among the Pyrus species, *P. boissieriana* with local name of Telka, grows in both low and high altitudes (Table 1). The other species (Table 1) are spread over higher altitudes. *P. boissieriana*, is a pea pear which is considered to be the most primitive *Pyrus* species in the region of south Caspian Sea. The fruit of the recent contained three locules or three ovaries each consisted of two ovules. Few of the species had round fruits; these were included *P. boissieriana* and *P. hyracana*. The genetic distance ranged from 0 to 0.48, with an average of 0.24, and cluster analysis based on morphological data assigned the genotypes into two main groups (Figure 3). Two samples of *P. salicifolia* (459, 461) were similar on the morphological dendrogram, however, those revealed 15% genetic distance.

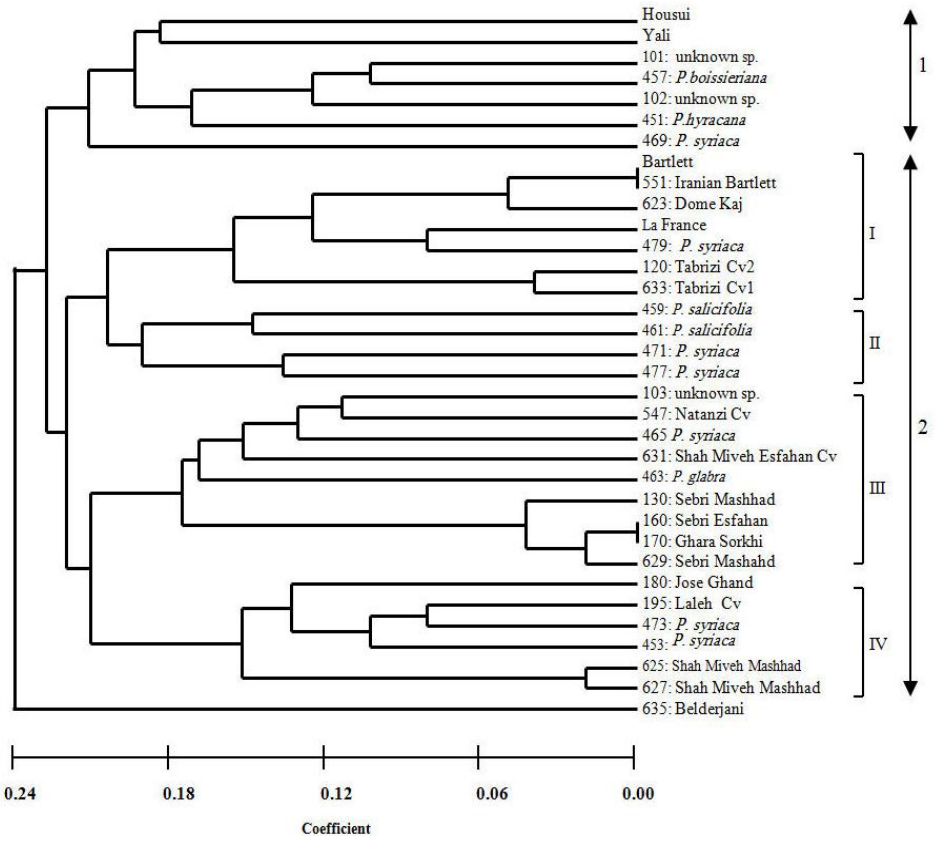

Fig. 2. A dendrogram for 34 pear cultivars and species, using Nei's genetic distance, based on SSR analysis

Table 2. Characteristics of SSR markers that were used for evaluation of Persian pears

SSR Locus	Primer sequences	Number of putative alleles(bp)	Size range	H_O Z)	H_E Z)
NH004a	F: AGGATGGGACGAGTTTAGAG R: CCACATCTCTCAACCTACCA	15	88-118	0.71	0.89
BGA35	F:AGAGGGAGAAAGGCGATT R: GCTTCATCACCGTCTGCT	8	127-138	0.50	0.76
BGT23b	F:AGAGTCGGTTGGGAAATGATTG R:CACATTCAAAGATTAAGAT	19	168-240	0.65	0.94
KU10	F:AGTATGTGACCACCCCGATGTT R: AGAGTCGGTTGGGAAA	12	220-269	0.32	0.80
NH011b	F: GGTTCACATAGAGAGAGAGAG R: TTTGCCGTTGGACCGAGC	17	155-196	0.82	0.92
NH013a	F: GGTTTGAAGAGGAATGAGGAG R: CATTGACTTTAGGGCACATTTC	22	160-227	0.68	0.94
NH015a	F: TTGTGCCCTTTTTCCTACC R: CTTTGATGTTACCCCTTGCTG	13	98-135	0.79	0.87
Average		15.1		0.64	0.87

Z) *HO* and *HE* denote the observed and expected heterozygosities, respectively. F means forward primer and R: means reverse primer.

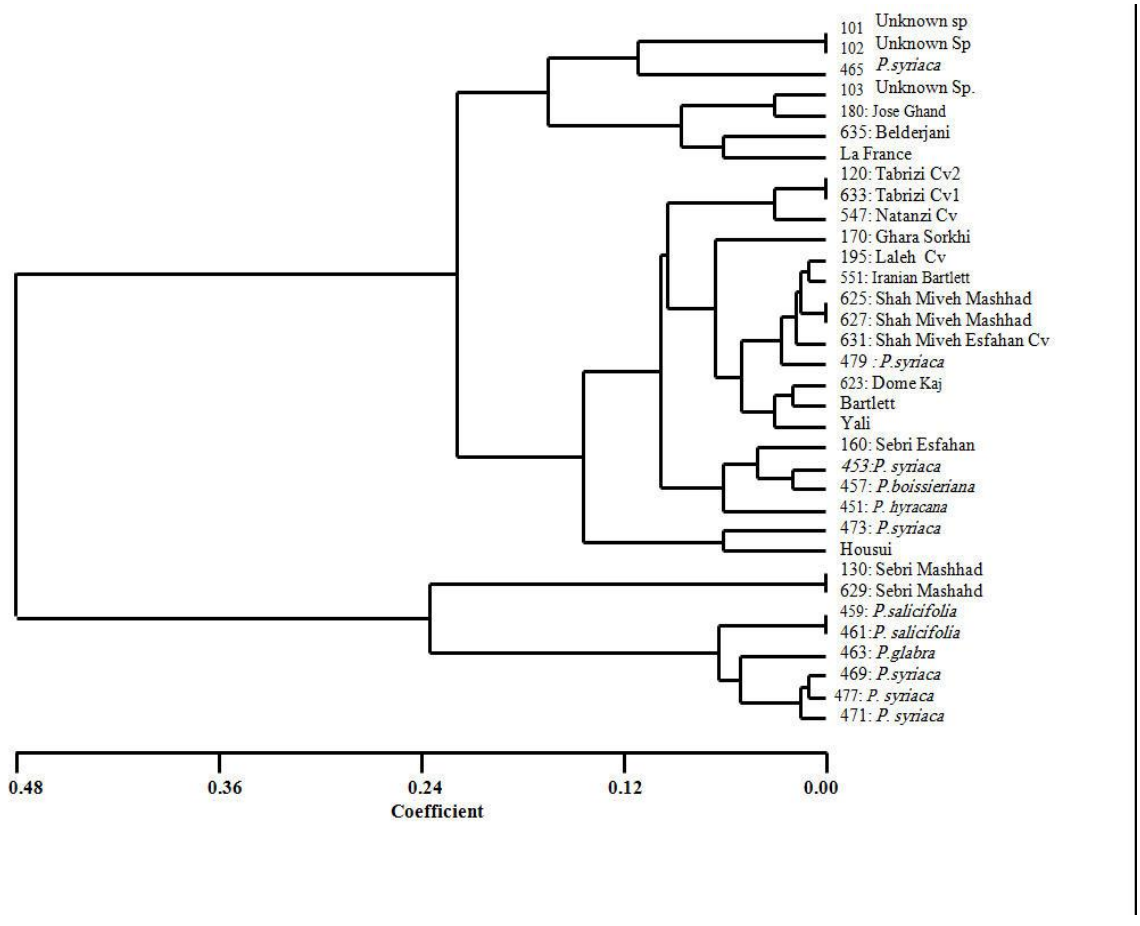

Fig. 3. A dendrogram for 34 pear cultivars and species, using Nei's genetic distance, based on morphological data

Table 3. Morphological traits of pears species and cultivars that were used in this study.

Germplasm name	Leaf margin	Fruit shape	Leaf base	Leaf shape	Fruit size	Leaf petiole	Leaf tip
101	entire	round	cuneate	lanceolate	small	small	acute
102	entire	round	cuneate	lanceolate	small	small	acute
103	serrate	pyriform	cuneate	ovate	medium	small	obtuse
Tabrizi Cv2	serrate	oblong	round	orbicular	large	medium-large	marinate
Sebri Mashhad	serrate	ovate	cuneate	elliptic	large	large	cuspidate
Sebri Esfahan	deep serrate	round	round	orbicular	small	medium	cupsidate
Ghara Sorkhi	serrate	ovate	cuneate	oblong	medium	medium	cuspidate
Jose Ghand	serrate	ovate	round	orbicular	medium	small	cuspidate
Laleh Cv	serrate	oblong	round	ovate	medium	medium	sub-acute
451	entire	round	round	orbicular	small	large	round
453	serrate	oblong	round	ovate	small	large	sub-acute
457	serrate	round	round	orbicular	small	large	sub-acute
459	fimbriate	round	cuneate	elliptic	small	small	acute
461	fimbriate	round	cuneate	elliptic	small	small	acute
463	serrate	pyriform	cuneate	lanceolate	small	medium	acute
465	serrate	round	round	orbicular	medium	medium	obtuse
469	serrate	round	cuneate	lanceolate	small	medium	acute
471	deep serrate	round	cuneate	ensiform	small	medium	acute
473	serrate	round	cuneate	lanceolate	small	medium	acute
477	deep serrate	round	cuneate	lanceolate	small	medium	acute
479	serrate	oblong	cuneate	ovate	small	large	sub-acute
Natanzi Cv	serrate	oblong	round	ovate	medium	large	cupsidate
Iranian Bartlett	serrate	pyriform	round	ovate	medium	medium	sub-acute
Dome Kaj	serrate	pyriform	round	ovate	large	medium	sub-acute
Shah Miveh Mashhad (625)	serrate	ovate	cuneate	elliptic	large	large	sub-acute
Shah Miveh Mashhad (627)	fimbriate	pyriform	round	ovate	medium	medium	sub-acute
Sebri Mashahd (629)	serrate	ovate	cuneate	elliptic	large	large	cupsidate
Shah Miveh Esfahan Cv	fimbriate	pyriform	round	ovate	medium	medium	cupsidate
Tabrizi Cv1	serrate	oblong	obovoid	round	large	medium-large	mucronate
Belderjani	entire	pyriform	cuneate	round	large	medium	obtuse
Bartlett	serrate	pyriform	round	orbicular	large	medium	cupsidate
Housui	deep serrate	round	round	ovate	large	medium	acute
La France	entire	oblongb	round	orbicular	large	medium	obtuse
Yali	deep serrate	ovate	round	orbicular	large	medium	Cuspidate

Discussion

Bassil and Postman (2010) obtained the amplified alleles using the PYC series of primers. They noticed most of the primers produced lower number of alleles for *P. pyrifolia* in comparison with *P. communis* and *P. ussriensis*. The recent finding was in accordance with Yamamoto et al. (2002a). Cao et al. (2011) acquired 19 alleles for locus of BGT23b while we obtained 12 for the same locus. However, Bao et al. (2007) obtained lower number of alleles for *P. communis* in comparison with Asian pears and for the same loci. Erfani et al. (2012) attained 7, 5 and 7 alleles for the loci NH013a, NH011b and NH004a, respectively. Furthermore, within our study 22, 17 and 15 alleles were detected for the same loci, correspondingly. Locus BGT23b produced 17 putative alleles with the size range from 188 bp to 236 bp in five *Pyrus* species (Yamamoto, 2002c). Furthermore, this locus produced 9 alleles in the work of Martinelli et al., (2008) and 10 alleles in the work of Erfani et al., (2012). Nevertheless, application of automated electrophoresis in our work indicated 19 alleles. The values of observed heterozygosity (Ho) in the work of Yakovin et al. (2011) varied from 0.19 (KU10) to 0.79 (NH004a), while expected heterozygosity assorted from 0.7 (KU10) to 0.88 (BGT23b) and further Erfani et al. (2012) reported Hob 0.85 for NH004a.

This is while in our current study, it was varied from 0.32 to 082. In addition, during the current study, the highest observed heterozyosity of 0.82 was gained for the locus NH011b. Brini et al. (2008) reported the average number of 5.1 alleles per locus and Zhang et al. (2011) informed average number of 5.4 alleles per locus for 29 accessions of apple. Erfani et al., (2012) demonstrated in some of their samples such as *P. mazandaranica*, "Chojuro," "Sebri," unknown 1, and "Domkaj Zard," three alleles were observed. In accordance with what Erfani et al., (2012) discovered, we found common bands between the two Asian pears and few Iranian pears. In addition, the recent authors revealed band ranges from 81 bp to 290 bp among 28 SSR loci. The band sizes in our work were ranged from 88 bp for NH004a to 269 bp for the locus for KU10.

In this study, Dome-Kaj, Tabrizi, and Bartlett cultivars and other accessions as *P. communis* pears were separated from the first sub-cluster, which contained two Asian pears. The species *P. glabra* and *P. syriaca* were probably the genetic sources for some Iranian cultivars, but further research using more and variable markers will be necessary to characterize the genetic backgrounds of these germplasms. Khtamsaz (1992) used morphological traits to classify *P. syriaca* into three variety types: var. oxyprion, var. syriaca, and var. microphylla. In the current study, *P. syriaca* was distributed into two major and minor clusters and four sub-clusters, indicating a wide genetic diversity among the population of this species in Iran. This was in accordance with Mozafari et al. (1996). Previous results of morphological analysis based on IBPGR descriptors (Thibault et al., 1982, Tahzibi-Hagh et al., 2011, Erfani et al., 2012) confirmed that Tabrizi and Dome-Kaj (623) cultivars were examples of *P. Communis*. One species in sub-cluster III, *P. glabra* (463), had probably been the germplasm source for both "Natanzi" Cv (547) and "Shah Miveh Esfahan" Cv (631). Possibly the two above cultivars might originate from *P. glabra*. The results of morphological traits analysis were in accordance with Aldasoro et al. (1996) and Asanidze et al. (2011). Even though Asian pears grouped in a separate clade in this research, however we could not separate those in a separate cluster. It will be necessary to evaluate more samples of Asian pears and more number of primers with high PI index value to reveal more relation of Iranian pears with Asian pears. Finally, we considered SSR markers as a reliable tool to identify native pear species and cultivars and relation among them.

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