

Investigating the genetic diversity of plane (*Platanus orientalis* and *P. occidentalis*) in different regions of Iran using SRAP markers

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Abstract

Genetic diversity among 76 *Platanus orientalis* and three *P. occidentalis* genotypes from six distinct geographical regions of Iran was investigated using sequence related amplified polymorphism (SRAP) markers. Thirteen SRAP primer combinations (PCs) amplified 237 fragments, of which 61 were polymorphic. Average PIC value over all PCs was 0.153. UPGMA dendrogram, based on Jaccard's similarity coefficient ($r = 0.995$), grouped the genotypes into two main clusters according to species and regardless of the regions. AMOVA analysis also showed significant differences among the species ($P < 0.001$). Sum of the first three principal components in PCA analysis could represent most of (87.2%) the total variation in the original dimensions and confirm the results of cluster analysis. No significant genetic differences between old and young *P. orientalis* trees and also, among geographical regions could be detected due to the propagation method of plane trees through cutting. It can be concluded that the SRAP marker is an effective tools for the assessment of genetic diversity among *Platanus* species and low genetic variation within *P. orientalis* may be related to the propagation method.

Keywords: *Platanus*, plane, Genetic diversity, SRAP marker, Iran.

Abbreviations: SRAP_Sequence related amplified polymorphism; PIC_Polymorphism information content; PCA_Principal component analysis; AMOVA_Analysis of molecular variance; UPGMA_Unweighted pair group method with arithmetical averages.

Introduction

Platanus orientalis L. and *P. occidentalis* L. are two important species of the family Platanaceae. *P. orientalis* is a woody perennial tree (Mitrokotsa et al., 1993) with important riparian species (Schnitzler et al., 2005) that occur naturally from southeastern Europe to southwest Asia (especially Iran and Turkey) (Besnard et al., 2002). It is a large deciduous tree, native to temperate regions (Huxley, 1992). The buds of the tree are used in folk medicine as antiseptic and antimicrobial remedies of the urinary system (Mitrokotsa et al., 1993). Plane has advantages such as rapid growth rate, easy propagation, lush foliage and strong air purification ability. In slightly polluted regions, leaves of plane trees can absorb and block a portion of the harmful gas and chemical smokes such as ozone, benzene and phenol sulfide. For avenue and street plantation, it is very useful because it is tolerant of air pollution (Sajid et al., 2012). The wood structures of plane are meticulous and beautiful with moderate hardness, so it has been widely cultivated as an industrial material in the world (Cai et al., 2008). *P. orientalis* can be found naturally in almost all forestlands, inside the streams, and river bottoms in Iran. Some of them are monumental, long-lived trees that can grow taller up to 25-35 meters with a trunk diameter of more than 5 m and live for 2000 years. It is widely used in landscape design as urban open green spaces (parks and arboretum), water fronts, industrial areas, shade bearers, and street trees. From earliest days, *P. orientalis* has been an important tree in Persian gardens built around water and shade. There, it is known as

the *Chenar*. *P. occidentalis* L., American sycamore, is a world famous garden tree with some advantages such as rapid growth rate, easy propagation, lush foliage, strong air purification ability and soil adaptability. It has also been widely cultivated as an industrial material in America due to its wood structure (Cai et al., 2008). Plane is generally propagated through hardwood cuttings when practiced in the dormant season (Eldeen and Elgimabi, 2009). It is, therefore, expected that the plane trees would not be much variable, but considerable variation in the morphology could be detected. Plane cultivars distinctions are mainly based on morphological characteristics such as trunk diameter, bark and leaf color, leaf and fruit shape, and petiole length. As morphological characteristics are affected by agro-ecosystems, it is likely that the same genotypes may have different phenotypes in different regions. In recent years, molecular markers have been applied as useful complements to morphological characters in plant's system because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification in the early stages of development. The sequence-related amplified polymorphism (SRAP) technique is a relatively simple and highly reproducible DNA marker that targets coding sequences in the genome using a unique design of primer pairs, thereby resulting in the identification of a number of co-dominant markers (Li and Quiros, 2001). SRAP was shown to be more informative than other PCR-based techniques in detecting genetic diversity due to being more consistent and repeatable,

Table 1. Number of the total and polymorphic bands and PIC values for each SRAP primer combination.

Primer combination	NT ^a	NP ^b	PP ^c (%)	PIC ^d
Me1-Em17	31	8	25.80	0.166
Me1-Em2	9	1	11.11	0.197
Me2-Em6	11	3	27.27	0.197
Me2-Em17	7	2	28.57	0.134
Me3-Em6	17	5	29.41	0.147
Me3-Em17	21	7	33.33	0.162
Me4-Em4	29	10	34.48	0.153
Me5-Em1	11	4	36.36	0.150
Me5-Em4	32	6	18.75	0.187
Me5-Em5	8	1	12.50	0.197
Me5-Em17	26	9	36.61	0.134
Me6-Em2	18	3	16.66	0.071
Me6-Em5	17	2	11.76	0.104
Mean	18.23	4.69	24.66	0.153
Total	237	61	-	-

^aNT-Number of total fragments. ^bNP-Number of polymorphic fragments. ^cPP-Percentage of polymorphic fragments. ^dPIC-Polymorphic information content.

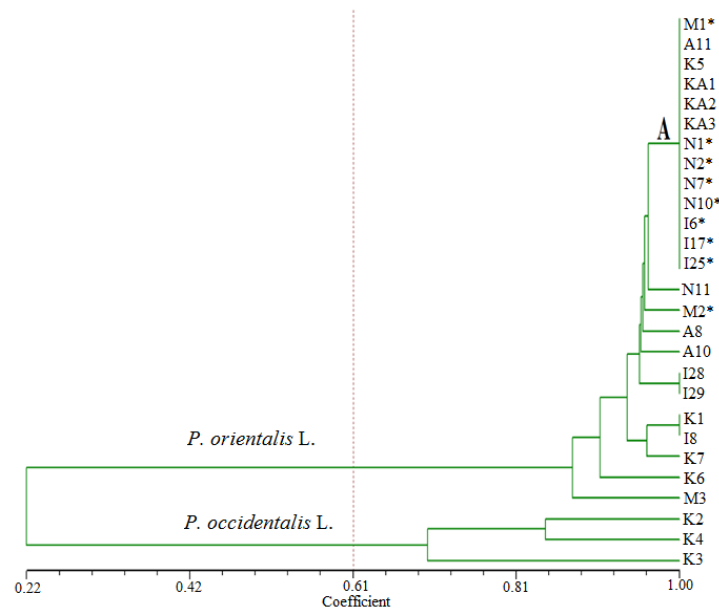


Fig 1. UPGMA dendrogram of 27 plane genotypes based on SRAP marker data. The codes refer to the abbreviations of the genotypes as shown in Table 3. *these genotypes are more than 700 years old.

and less labour-intensive and time-consuming (Ferriol et al., 2003; Budak et al., 2004). SRAP markers have been successfully used to study genetic diversity and relationships in several plants (Ferriol et al., 2003; Budak et al., 2004; Riaz et al., 2004; Esposito et al., 2007; Fu et al., 2008; Feng et al., 2009; Uzun et al., 2009; Abedian et al., 2012). *P. orientalis* is an important and widely used tree in landscape design in Iran; therefore, the objective of this study was to assess genetic diversity among different genotypes of *P. orientalis* in the central area of Iran and their relationships with *P. occidentalis* using SRAP molecular markers.

Results

SRAP amplification

The thirty SRAP primer combinations (PCs) were examined and 13 of them with stable and reproducible amplification patterns in two repetitive experiments were selected. A total of 237 fragments ranging from 80 to 1200 bp were scored among 79 genotypes, of which 61 (25.7%) were

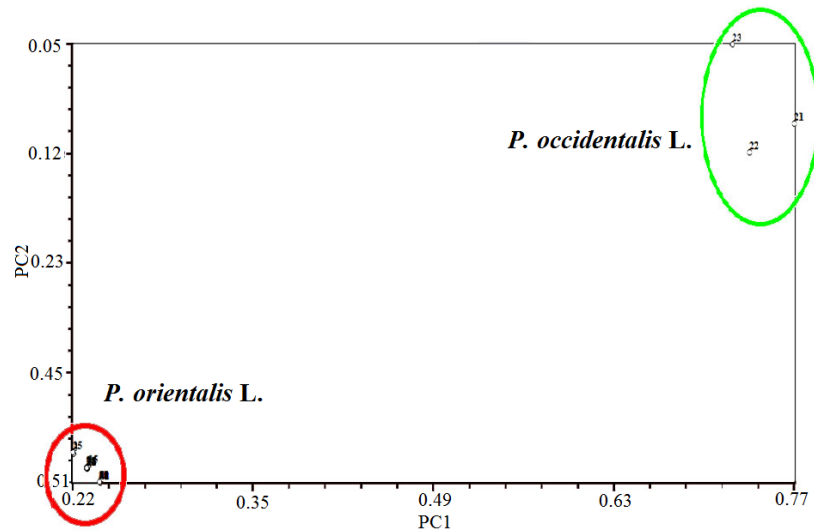
polymorphic, with an average of 4.69 polymorphic bands per PC (Table 1). The different SRAP PCs amplified the number of bands from seven (Me2- Em17) to 32 (Me5- Em4). The number of polymorphic fragments detected by each PC ranged from 1 to 10. The most polymorphism was shown by Me4- Em4 combination, which produced 10 polymorphic bands. The PIC values for PCs ranged from 0.071 to 0.197 with an average of 0.153 (Table 1).

Cluster and PCA analysis

An identical banding pattern amplified by all PCs was observed among 65 out of 79 genotypes. Due to high genetic similarity among these genotypes, NTSYS-pc software could not construct the dendrogram. Therefore, 52 genotypes with completely similar bands patterns were removed and the data obtained from SRAP analysis of 27 genotypes was subjected to cluster analysis. All removed genotypes belonged to the A section of dendrogram (Fig 1). Among different clustering and similarity coefficient methods, the highest cophenetic correlation coefficient value ($r=0.995$) was observed for

Table 2. Plane genetic diversity indexes within species based on SRAP data

Species	Population size	Polymorphic locus percent	Number of polymorphic locus	Shannon index	Nei's Genetic diversity	Number of effective alleles	Number of observed alleles
<i>P. occidentalis</i>	3	18.033%	11	0.0383	0.019	1.021	1.180
<i>P. orientalis</i>	24	29.51%	18	0.1878	0.13	1.236	1.295

**Fig 2.** Principal component analysis (PCA) based on SRAP marker data in *P. orientalis* and *P. occidentalis*.

UPGMA clustering method based on Jaccard's similarity coefficient, thereby suggesting that the cluster analysis could represent the similarity matrix. The UPGMA dendrogram grouped the 27 genotypes into two main clusters (Fig 1). All 24 *P. orientalis* and three *P. occidentalis* genotypes were grouped together as cluster I and II, respectively. This tree failed to discriminate the genotypes from different geographical regions and old trees. Genetic relationships among the genotypes were also investigated by principal component analysis (PCA). The first three PCs explained 45.09%, 38.06% and 4.05% of the total variation, respectively, confirming the results of UPGMA clustering (Fig 2). Analysis of molecular variance (AMOVA) was performed to estimate the percentage of intra and inter-species genetic variation which revealed a significant variation among the studied species ($P < 0.001$). Estimates of the genetic diversity among and within the species are summarized in Table 2. Nei's genetic diversity and Shannon's information index were used as two useful intra-species gene diversity indices. The values of these parameters were more in *P. occidentalis* than *P. orientalis*.

Discussion

To our knowledge, the present study can be regarded as the first report of genetic diversity among and within plane populations using SRAP markers. Our results confirmed that the SRAP markers could be powerful, simple, informative, reproducible and suitable tools for the detection of genetic diversity among and within species (Li and Quiros, 2001; Li et al., 2009; Chen et al., 2010). Huang et al. (2009) determined the genetic stability of *Platanus acerifolia* using ISSR markers. They found 16 out of 38 ISSR primers with clear and reproducible bands, resulting in a total of 103 distinct bands with an average of 6.44 scorable bands per primer. Among them, 17 bands were polymorphic across all 20 plants tested (16.5% polymorphism). The results of this

study showed 25.7% polymorphism, confirming the suitability of SRAP in comparison to ISSR markers. In addition to the polymorphism rate (P), the polymorphic information content (PIC) was also used to measure the genetic diversity. High, medium or low loci polymorphism was in accordance with $PIC > 0.5$, $0.5 > PIC > 0.25$ and $PIC < 0.25$, respectively (Vaiman et al., 1994; Xie et al., 2010). In this study, PCs had a $PIC < 0.25$, with an average of 0.153, thereby indicating that the SRAP markers could develop low loci polymorphism. UPGMA dendrogram constructed on the basis of SRAP data was separated in the two species, *P. orientalis* and *P. occidentalis*. AMOVA analysis also showed significant differences among the species ($P < 0.001$), confirming the results of cluster analysis. Based on the SRAP data analysis, *P. occidentalis* was more diverse than *P. orientalis*, which probably correlated with the propagation method. It is known that *Platanus* species can be propagated by means of generative (seed) and vegetative (stem cutting) methods and this varies from species to species. However, propagation through seeds is a commonly used method. Due to the negative influence of external natural factors, the rate of germination in seeds differs among the species (Hartman et al., 2011). Clonal propagation with vegetative methods produces a similar plant when compared to seed propagation. Therefore, based on the results of this study, probably the commonly used propagation methods in *P. orientalis* and *P. occidentalis* are stem cutting and seed, respectively. The results revealed no significant genetic differences between old and young *P. orientalis* trees and also, among different geographical regions. Probably, the propagation method of plane trees through cutting could be the reason for this genetic similarity. Assessment of genetic diversity and phenetic relationships in London plane tree samples from Schenley Park, Pittsburgh, PA, USA, and from several nurseries, using AFLP markers, separated the nursery trees from the Schenley Park samples, correlating with the propagation methods (Morton and Gruszka, 2008). The

Table 3. The list of genotypes evaluated in this study with the species and geographical distribution.

No	Code	Species	Geographical region	Latitude	Longitude	Height from sea level (m)
1	M1*	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
2	M2*	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
3	M3	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
4	M4	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
5	M5	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
6	M6	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
7	M7	<i>P. orientalis</i> L.	Mahalat-Markazi-Iran	33°54' N	50°27'E	1752
8	A1	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
9	A2	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
10	A3	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
11	A4	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
12	A5	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
13	A6	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
14	A7	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
15	A8	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
16	A9	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
17	A10	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
18	A11	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
19	A12	<i>P. orientalis</i> L.	Arak-Markazi-Iran	34°49'N	49°41'E	1755
20	K1	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
21	K2	<i>P. occidentalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
22	K3	<i>P. occidentalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
23	K4	<i>P. occidentalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
24	K5	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
25	K6	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
26	K7	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
27	K8	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
28	K9	<i>P. orientalis</i> L.	Krarj-Alborz-Iran	35°49'N	50°58'E	1328
29	KA1	<i>P. orientalis</i> L.	Kashan-Isfahan-Iran	33°59'N	51°26'E	940
30	KA2	<i>P. orientalis</i> L.	Kashan-Isfahan-Iran	33°59'N	51°26'E	940
31	KA3	<i>P. orientalis</i> L.	Kashan-Isfahan-Iran	33°59'N	51°26'E	940
32	N1*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
33	N2*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
34	N3*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
35	N4*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
36	N5*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
37	N6*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
38	N7*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
39	N8	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
40	N9	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
41	N10*	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
42	N11	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
43	N12	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
44	N13	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
45	N14	<i>P. orientalis</i> L.	Natanz-Isfahan-Iran	33°30'N	51°55'E	1629
46	I1	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
47	I2	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
48	I3	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
49	I4	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
50	I5*	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
51	I6	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
52	I7	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
53	I8	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
54	I9	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
55	I10	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
56	I11	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
57	I12	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
58	I13	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
59	I14	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
60	I15	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
61	I16*	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
62	I17	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
63	I18	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
64	I19	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
65	I20	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575

66	I21	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
67	I22	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
68	I23	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
69	I24*	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
70	I25	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
71	I26	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
72	I27	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
73	I28	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
74	I29	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
75	I30	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
76	I31	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
77	I32	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
78	I33	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575
79	I34	<i>P. orientalis</i> L.	Isfahan-Isfahan-Iran	32°39'N	51°40'E	1575

*these genotypes are more than 700 years old.

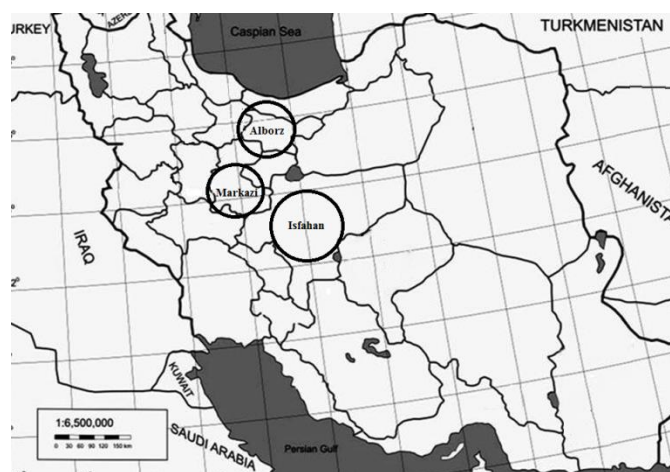


Fig 3. Sampling locations of *Platanus* sp. populations taken from different geographical regions of Iran and used in this study (Alborz, Isfahan, and Markazi provinces).

Table 4. SRAP primers sequence used in this study.

Forward (5'-3')	Reverse (5'-3')
Me 1: 5'TGAGTCCAAACCGGATA3'	Em 1: 5'GACTGCGTACGAATTAAT3'
Me 2: 5'TGAGTCCAAACCGGAGC3'	Em 2: 5'GACTGCGTACGAATTTGC3'
Me 3: 5'TGAGTCCAAACCGGAAT3'	Em 4: 5'GACTGCGTACGAATTGAC3'
Me 4: 5'TGAGTCCAAACCGGACC3'	Em 5: 5'GACTGCGTACGAATTAAC3'
Me 5: 5'TGAGTCCAAACCGGATG3'	Em 6: 5'GACTGCGTACGAATTGCA3'
Me 6: 5'TGAGTCCAAACCGGTAA3'	Em 17: 5'GACTGCGTACGAATTCCA3'

Schenley Park plants had been grown from seed or seedlings, while the nursery plants were clonally propagated.

Materials and Methods

Plant materials

A total of 79 genotypes were collected from three provinces of Iran (Isfahan, Alborz and Markazi). They included 76 *P. orientalis* and 3 *P. occidentalis* genotypes. (Table 3, Fig 3). Among them, 13 genotypes were monumental with an age more than 700 years (Table 3).

DNA extraction

For genomic DNA extraction, young leaves from each genotype were collected. Total genomic DNA was extracted using the modified CetylTrimethyl Ammonium Bromide (CTAB) method of Murray and Thompson (1980). The

concentration and quality of DNA were determined using agarose gel electrophoresis against known concentrations of unrestricted lambda DNA and verified by spectrophotometric measurements. All DNA samples were diluted to 20 ng/μl and stored at -20 °C until use.

SRAP amplification

The SRAP analysis was performed as described by Li and Quiros (2001). The PCR reactions were performed in a total volume of 15 μl containing 1×PCR buffer, 0.2 mM dNTPs, 0.4 μM of each forward and reverse primers (Bioneer, Daejeon, Korea; Table 4), 1.5 mM MgCl₂, 1 unit of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania) and approximately 20 ng of total genomic DNA. All reactions were carried out in a Eppendorf Thermal Cycler (Mastercycler Gradient) with the following PCR program: 3 min of initial denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C and 1

min of elongation at 72°C, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min; this was followed by a final extension for 10 min at 72 °C. The PCR products were mixed with 5 µl of formamide loading buffer (95% formamide; 20 mM EDTA, pH 8.0; 0.03 % of Xylene cyanol and 0.03 % of Bromophenol blue) and separated on 8% non-denatured polyacrylamide gels in 1x TBE buffer along with 100 bp DNA ladder (Fermentas, Vilnius, Lithuania) as size marker. Amplified fragments were visualized by silver staining (Bassam et al., 1991).

Data analysis

All intense, reliable and clearly distinguished SRAP products were scored as presence (1) and absence (0). This was followed by the construction of a matrix of SRAP data. The polymorphism information content (PIC) values for all selected markers amplified by a particular primer pair were calculated for the SRAP markers to characterize the capacity of each primer to detect polymorphic loci among and within the populations. The PIC value was calculated using the formula $PIC = 1 - \sum P_i^2$, where P_i is the frequency of the i th allele (Smith et al., 1997). The data obtained from the SRAP profiles with different individual primers, as well as in collection, were subjected to the construction of a similarity matrix using Jaccard's coefficients of similarity (Jaccard, 1908). Based on the unweighted pair group method with arithmetical averages (UPGMA), a dendrogram was analyzed using NTSYSpc version 2.02e software (Rohlf, 1998). The cophenetic correlation coefficient was estimated to verify the adjustment between similarity matrices and the respective dendrogram derived matrix. Genetic relationships among genotypes were also analyzed by the principal component analysis (PCA) of a similarity matrix according to the extracted Eigen vectors in NTSYSpc version 2.02e. Analysis of molecular variance (Excoffier et al., 1992; Excoffier and Smouse, 1994) was performed to estimate variance components for SARP data and partition the variation into within and among species using Arlequin 3.0 software (Excoffier et al., 2005). Popgene 1.32 software (Yeh et al., 1999) was used to compute the indices of population level genetic diversity such as shannon's information index (I) among the species.

Conclusion

The results of this study indicated a high level of homogeneity among the *P. orientalis* genotypes, which corresponded to the clonal propagation in the Iranian planes. It can be concluded that the SRAP marker is an effective tools for the assessment of genetic diversity among *Platanus* species and low genetic variation within *P. orientalis* which may be related to the propagation method.

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References

Abedian M, Talebi M, Golmohammadi HR, Sayed-Tabatabaei BE (2012) Genetic diversity and population structure of mahaleb cherry (*Prunus mahaleb* L.) and sweet cherry (*Prunus avium* L.) using SRAP markers. *Biochem Syst Ecol.* 40: 112-117.

Bassam BJ, Caetano-anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal Biochem.* 196: 80-83.

Besnard G, Tagmaount A, Baradat P, Vigouroux A, Bervillé A (2002) Molecular approach of genetic affinities between wild and ornamental *Platanus*. *Euphytica.* 126: 401-412.

Budak H, Shearman RC, Parmaksiz I, Dweikat I (2004) Comparative analysis of seeded and vegetative biotype buffalograsses based o phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theor Appl Genet.* 109: 280-288.

Cai X, Yang LW, Shi JS (2008) Study on characteristics differences between *Planatusoccidentalis* clones by tissue culture. *J Jiangsu Agric Sci.* 4: 89-93.

Chen M, Feng F, Sui X, Li M, Zhao D, Han S (2010) Construction of a framework map for *Pinus koraiensis* Sieb.et Zucc. using SRAP, SSR and ISSR markers. *Trees.* 24: 685-693.

Eldeen M, Elgimabi NE (2009) Improvement of propagation by hardwood cuttings with or without using plastic tunnel in (*Quisqualis indica*). *Adv Biol Res.* 3: 16-18.

Esposito MA, Martin EA, Cravero VP, Cointy E (2007) Characterization of pea accessions by SRAP's markers. *Sci Hortic.* 113: 329-335.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinfo.* 1: 47-50.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplo types: application to human mitochondrial DNA restriction data. *Genetics.* 131: 479-491.

Excoffier L, Smouse PE (1994) Using allele frequencies and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. *Genetics.* 136: 343-359.

Feng F, Chen M, Zhang D, Sui X, Han S (2009) Application of SRAP in the genetic diversity of *Pinuskoraiensis* of different provenances. *Afr J Biotech.* 8: 1000-1008.

Ferriol M, Pico B, Nuez F (2003) Genetic diversity of a germplasm collection of *Cucurbitapepo* using SRAP and AFLP markers. *Theor Appl Genet.* 107: 271-282.

Fu X, Ning G, Gao L, Bao M (2008) Genetic diversity of *Dianthus* accessions as assessed using two molecular marker systems (SRAPs and ISSRs) and morphological traits. *Sci Hortic.* 117: 263-270.

Hartman HT, Kester DE, Davies FT, Geneve RL (2011) Hartmann and Kester's Plant Propagation: Principles and Practices, 8th edn. Prentice Hall, NJ, USA.

Huang WJ, Ning GG, Liu GF, Bao MZ (2009) Determination of genetic stability of long-term micropropagated plantlets of *Platanus acerifolia* using ISSR markers. *Biol Plantarum.* 53: 159-163.

Huxley A (1992) RHS Dictionary of Gardening. MacMillan Press ISBN N0-333-47494-5.

Jaccard P (1908) Nouvelles recherches sur la distribution florale. *Bulletin de la Societe. Vaudoise des Sciences Naturelles.* 44: 223-270.

Li G, Quiros F (2001) Sequence related amplified polymorphisim (SRAP), A new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theor Appl Genet.* 103: 455-461.

Li Y, Fan X, Shi T, Zhang Q, Zhang Z (2009) SRAP marker reveals genetic diversity in tartary buckwheat in China. *Front Agric China.* 3: 383-387.

- Mitrokotsa D, Mitaku S, Demetzos C, Harvala C, Mentis A, Perez S, Kokkinopoulos D (1993) Bioactive compounds from the buds of *Platanus orientalis* and isolation of a new kaempferol glycoside. *Planta Med.* 59: 517-20.
- Morton CM, Gruszka P (2008) AFLP assessment of genetic variability in old vs. new London plane trees (*Platanus × acerifolia*). *J Hort Sci Biotechnol.* 83(4): 532-537.
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucl Acid Res.* 8: 4321-4325.
- Riaz A, Potter D, Stephen M (2004) Genotyping of peach and nectarine cultivars with SSR and SRAP molecular markers. *J Am Soc Hortic Sci.* 129: 204-211.
- Rohlf M (1998) NTSYSPC. Numerical Taxonomy and Multivariate Analysis System, Version 2.02, Department of Ecology and Evolution, State University of New York, NY, USA.
- Sajid M, Pervaiz M, Rab A, Jan I, Haq I, Wahid F, Shah ST, Ali I (2012) Response of plane tree (*Platanus orientalis*) to cuttings and planting dates. *J Animal Plant Sci.* 22(2): 420-424.
- Schnitzler A, Hale BW, Alsum E (2005) Biodiversity of floodplain forests in Europe and eastern North America: a comparative study of the Rhine and Mississippi Valleys. *Biodivers Conserv.* 14: 97-117.
- Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Zeigle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): Comparison with data from RFLPs and pedigree. *Theor Appl Genet.* 95: 163-173.
- Uzun A, Yesiloglu T, Aka-Kacar Y, Tuzcu O, Gulsen O (2009) Genetic diversity and relationships within *Citrus* and related genera based on sequence related amplified polymorphism markers (SRAPs). *Sci Hortic.* 121: 306-312
- Vaiman D, Mercier D, Moazai G (1994) A set of 99 cattle microsatellite, characterization, synteny mapping and polymorphism. *Mamm Genome.* 5: 288-297.
- Xie W, Zhang X, Cai H, Liu W, Peng Y (2010) Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.). *Biochem Syst Ecol.* 38: 740-749.
- Yeh FC, Yang RC, Boyle T, Ye ZH, Mao JX (1999) POPGENE, the User Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.