Bulgarian Journal of Agricultural Science, 21 (No 3) 2015, 485-493
Agricultural Academy

GENETIC DIVERSITY IN *PAPAVER BRACTEATUM* AND *PAPAVER SOMNIFERUM* POPULATIONS REVEALED BY ISSR MARKERS

Z. MOHSENI[1], I. BERNOUSI[1,2], B. A. MANDOULAKANI[*1,2] and R. DARVISHZADEH[1,2]
[1] *Urmia University, Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia, Iran*
[2] *Urmia University, Department of Agricultural Biotechnology, Institute of Biotechnology, Urmia, Iran*

Abstract

MOHSENI, Z., I. BERNOUSI, B. A. MANDOULAKANI and R. DARVISHZADEH, 2015. Genetic diversity in *Papaver bracteatum* and *Papaver somniferum* populations revealed by ISSR markers. *Bulg. J. Agric. Sci.,* 21: 485–493

Inter-simple sequence repeat (ISSR) markers were used to assess genetic diversity and structure of two populations of *Papaver bracteatum* and one population of *Papaver somniferum*. Fifteen ISSR primers amplified 135 lociwhich 117 (86.6%) were polymorphic. The genetic diversity estimated by Shannon's information index (I) was 0.283 and 0.1 for *P. bracteatum* and *P. somniferum*, respectively. Eighty-three (61%) fragments were found to be useful as species-specific markers and 52 fragments were conserved between two species. Analysis of molecular variance (AMOVA) showed that the genetic variation mostly allocated to among species (64%). UPGMA clustering algorithm differentiated and classified studied genotypes and populations in three distinct groups. The result of the present study indicated a wide range of variation in the phenetic relationship among *P. bracteatum* and *P. somniferum*. Also, species-specific bands generated could be used to develop the species-specific sequence characterized amplified region (SCAR) markers.

Key words: AMOVA, shannon's information index, species-specific marker, UPGMA

Introduction

Genus *Papaver*, containing the highest level of botanical and phytochemical variability, has embraced many species with numerous subspecies and varieties, yields approximately 170 alkaloids from 13 important alkaloid groups (Madadi et al., 2009). Many of them have considerable pharmaceutical values. Morphine, codeine, thebaine, narcotine and papaverine are the most important alkaloids produced by the plants of this species and are exploited by the pharmaceutical industry as analgesics, antitussives and anti-spasmodic (Levy and Milo, 1998).

Papaver bracteatum Lindl (diploid, 2*n*=14) is perennial and an out-crossing species with gametophytic self-incompatibility (Goldblatt, 1974). It is one of the three species belonging to the section *Oxytona;* two other perennial species *P. orientale* and *P. pseudo-orientale* of this section are closely related to *P. bracteatum* (Milo et al., 1998). *P. bracteatum* is naturally distributed in high altitudes from 1500 to 2500 m. The species is found in three distinct areas: Alborz mountains in north of Tehran, in the Iranian Kurdistan and on the Northern slope of the Caucasus (Goldblatt, 1974). *P. somniferum* (Opium poppy), 2*n*=22, is affiliated to the section *Mecones* and is considered to be a predominantly self-pollinating species (Hammer and Fritsch, 1977). Although the diversity center of the opium poppy is identified as the near east and middle Asia, the plant can be successfully grown in such diverse areas as most parts of Europe, Asia, north and east Africa, south America and Australia (Singh, 1982).

The pharmaceutical utilization of medicinal and aromatic plants (MAPs) is strictly connected with the content of their active substances, which in turn depends on their genetic potential and the climatic conditions. Various populations of MAPs make stocks for pharmaceutical drugs with differential quality; and knowledge about its variability and quality is necessary for thoughtful use, conservation, and/or protection of genetic diversity, and also the existence of substantial variations in the available gene pool of a species is necessary for any successful breeding program (Dušek et al., 2010). The accurate estimation of genetic diversity can be invaluable in the selection of diverse parental combinations to gener-

*Corresponding author: b.abdollahi@urmia.ac.ir, zmohsenib88@yahoo.com, ibernosi@gmail.com, r.darvishzadeh@urmia.ac.ir

ate segregating progenies with maximum genetic variability. Heterosis has been reported in *P. somniferum* for agronomic and chemical characteristics (Sip et al., 1977). Heterotic effects can be used in a breeding program aimed at increasing the thebaine yield. *P. bracteatum* is suited for the production of hybrids because of the very large quantities of seeds that are produced in each capsule (Milo et al., 1998).

Genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant. In contrast, molecular markers, based on DNA sequence polymorphisms, are independent of environmental conditions and show higher levels of polymorphism (Domayti et al., 2011). In the recent decades, a number of PCR-based DNA markers, such as RAPD (random amplified polymorphic DNA, ISSR (inter-simple sequence repeat), AFLP (amplified fragments length polymorphism) and SSR (simple sequence repeat) have been widely used to investigate the genetic structure of a population (Esselman et al., 1999). Among various molecular marker tools, ISSRs have been widely used for population genetic studies of various plant species, including several medicinal plants (Li and Jin, 2008). ISSR primers of repeat motifs (microsatellites) that are abundant and dispersed throughout genomes anchored either at 5' or 3' end with one or a few specific nucleotides and amplify the sequences between the two microsatellite loci (Zietkiewicz et al., 1994). Because of higher annealing temperature and longer sequence of ISSR primers, they can yield reliable and reproducible bands than RAPDs (Qian et al., 2001), and the cost of the analyses is relatively lower than that of some other markers such as RFLP, SSR and AFLPs (Wang et al., 2008). There are a few reports on the use of molecular markers in *P. bracteatum* and *P. somniferum*. Acharya et al. (2009) evaluated genetic diversity of the 24 germplasm of *P. somniferum* using RAPD and ISSR markers and reported 62.52% polymorphism. AFLP markers was performed on 40 accessions of *P. somniferum* and two other control genera (*P. bracteatum* and *P. setigerum*) from a commercial breeding collection held in Tasmania and Australia. In this study the percentage of polymorphism is significantly low within these commercial breeding lines, reflecting narrow genetic diversity of the population (Saunders et al., 2001). Also, relationships among 53 accessions belonging to 3 important *Oxytona* species, i.e. *P. bracteatum* Lindl., *P. orientale* L. and *P. pseudo-orientale* have been studied using 19 different morphological characters, one chemical character and 15 RAPD primers. A total of 81 bands generated by 15 successful RAPD-PCR reactions and polymorphism rate were approximately 85% (Parmaks and Özcan, 2010).

In the present study, the genetic diversity within and among natural populations of *P. bracteatum* and *P. somniferum* sampled from Iran were assessed using ISSR markers to partition the genetic variation within and among populations, and provide basic information for conservation and breeding of this medicinal plant.

Materials and Methods

Plant materials and cultivation aspects

Two populations of *P. bracteatum* and one population of *P. somniferum* originated from Iran were used in the current study (Table 1). Twenty individuals were randomly selected from each population. Various treatments were applied to determine the suitable soil type, moisture and temperature for seed germination and growth. The germination rate was influenced by temperature. Temperature between 22-25°C increased the rate of germination. Also soil type affected the germination rate as germination rate and speed were much higher in light soil. A higher germination percentage was obtained in peat moss soil with minerals. Humidity of 60% and photoperiod of 16/8 h light/ dark were applied.

Chromosomal analysis

Cytogenetic analysis was performed to determine the ploidy levels of the *P. bracteatum* and separate the individuals of this species from *P. orientale* and *P. pseudo-oriantale* (*P. bracteatum*, *P. orientale* and *P. pseudo-oriantale* are outcrossing, and the seeds with different ploidy levels may be found on a single plant of *P. bracteatum*). Chromosome observation was carried out by aceto-orcein method (La Cour, 1941). Collected root tips were pretreated with a 0.002 M

Table 1
Location of three populations used in this study

Name	Location	Latitude (E)	Longitude (N)
Papver bracteatum (Pop1)	Salmas	44.75°	38.18°
Papver bracteatum (Pop2)	Alborz mountain	51.09°	35.956°
Papaver somniferum	Turkey	35.40°	39.10°

aqueous solution of 8-hydroxyquinoline for 5h and fixed in Carney's solution at 4°C for 24h. The root tips were washed and hydrolyzed in 1 N hydrochloric acid. Chromosomes were stained with aceto-orceinand counted in squash preparation. The 5 different karyotype asymmetry parameters including difference of relative length of chromosome (DRL) (Alishah and Omidi, 2008), total form percentage of karyotype (%TF) (Huziwara, 1962), relative length of short chromosome (%S) (Alishah and Omidi, 2008), intrachromosomal asymmetry index (A_1) (Romero Zarco, 1986) and interchromosomal asymmetry index ($^{A}{}_{2}$) (Romero Zarco, 1986) were measured.

DNA extraction and ISSR amplification

Total DNA was isolated from the leaves of 4-weeks-old seedlings according to the CTAB (Doyle and Doyle, 1990) method with slight modifications. DNA was quantified using spectrophotometer and electrophoresis on 0.8% (w/v) agarose gel. ISSR reactions were done in a volume of 20 µl containing 40 ng of genomic DNA, 1X PCR buffer (10mMTris-HCL, 50mM KCL, PH=8.3), 1.5 mM $MgCl_2$, 0.2 µM dNTP, 0.5U Taq polymerase and 10 pmol primer. Amplification of genomic DNA was done on 96-well Eppendorf Mastercycler Gradient (Type 5331, Eppendorf AG, Hamburg, Germany) and commenced with 4 min at 94^0C, followed by 35 cycles of 40 s at 94^0C, 40s annealing at 53^0C and 2 min extension at 72^0C, and a final extension cycle of 5 min at 72^0C.Aftercompletion of the PCR, 5µl of formamide dyes (98% formamide, 10Mm EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) were added to the amplified products and were resolved electrophoretically on 1.8% (w⁄v) agarose gels run at 65 V for 3 h in 0.5xTBE buffer visualized by staining with ethidium bromide (1.0 µg ml^{-1})and photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO, USA). The amplicon size was determined by comparison with the ladder (O'GeneRuler™ DNA ladder, Fermentas Co.)

Data analysis

The ISSR band profiles were scored for presence (1) or absence (0) for each DNA sample, excluding smeared and weak bands. Fragments of the same molecular weight were considered as the same locus. The binary data matrix was analyzed using GenAlEx Version 6.4 (Peakall and Smouse, 2010). Number of loci, percentage of polymorphic loci, number of alleles or loci with a frequency higher or equal to 5%, number of private loci or alleles, number of less common loci with frequency lower or equal to 25% and 50%, mean of expected heterozygosity (He), number of effective alleles (Ne) and Shannon's information index (I) were used to quantify genetic diversity of each population. Analysis of molecular variance (AMOVA) was conducted to calculate variance components among and within populations. Cluster analysis using Jaccard similarity coefficients and UPGMA algorithm were used to depict the genetic relationship among individuals. The cophenetic correlation coefficient between the matrix of genetic similarity and the matrix of cophenetic values were computed and the significance of cophenetic correlation was tested by using the Mantel matrix correspondence test. All these computations were implemented in NTSYSpc 2.02 (Rohlf, 2000). The confidence of clustering in the dendrogram was evaluated through bootstrap analysis using the program Winboot (Yap and Nelson, 1996) with 1000 permutations. Principal coordinate analysis (PCoA) was performed using NTSYSpc software to highlight the resolving power of the ordination. A genetic distance matric was established between populations using Nei genetic distance coefficient (Nei, 1978) in GenAlex 6.41 (Peakall and Smous, 2010).

Results

Cytogenetic analysis

Cytogenetic analysis was performed for two purposes: determination the ploidy levels of the species and separation of the collected seeds of *P. bracteatum* from *P. orientale* and *P. pseudo-oriantale*. Chromosome analysis revealed that two species are diploid but the basic chromosome number varied between two species. The basic chromosome number in *P. bracteatum* and *P. somniferum* wereX=7 (2*n*=2x=14) and X=11 (2*n*=2x=22), respectively (Figure 1).According to asymmetry indices and in comparison to *P. bracteatum*, *P. somniferum* represented the low value of TF% and %S and

Fig. 1. Somatic chromosomes

Somatic chromosomes of the representative species of *Papaver* showing varying chromosome number. A:*Papaver bracteatum* (2*n*=14); B. *Papaver somniferum* (2*n*=22)

the high value of DRL, A_1 and A_2 (Table 2). Hence, it might be concluded that *P. somniferum* is evolutionary more advanced than *P. bracteatum*.

ISSR polymorphism

A total number of forty ISSR primers were screened using a few DNA samples and fifteen ISSR primers produced clear and reproducible bands, were selected for amplifying sixty DNA samples. These fifteen primers generated a total of 135 bands ranging from 3 (UBC818) to 12 (UBC834 and UBC855) with an average of 9 bands per primer. Of the 135 bands produced, 117 (86.6%) were polymorphic. The number of polymorphic bands detected per primer ranged from 1 (UBC849) to 12 (UBC885), with an average of 7.8. The band sizes of amplified products were found between 200 bpto 3 kbp. The highest size difference (300 to 3000 bp) between the amplified products was obtained with the primers UBC855, UBC857 and UBC836, while lowest size difference (400 to 1000 bp) was obtained with primer UBC818. The He for 135 amplified loci were between 0.036(UBC849) and 0.412(UBC857) with an average of 0.311. Gelelectrophoresis pattern obtained using primersUBC836 and UBC857 are illustrated in Figures 2 and 3. The oligonucleotide sequences of primers and the resultant multiple band patterns are summarized in Table 3.

Table 2
Karyotype asymmetry parameters in studied *Papaver* species

Species	%S	DRL	A_2	A_1	TF%	2n
P.bracteatum	5.84	1.76	0.11	0.44	35.14	14
P.somniferum	3.4	3.4	0.22	0.51	32.19	22

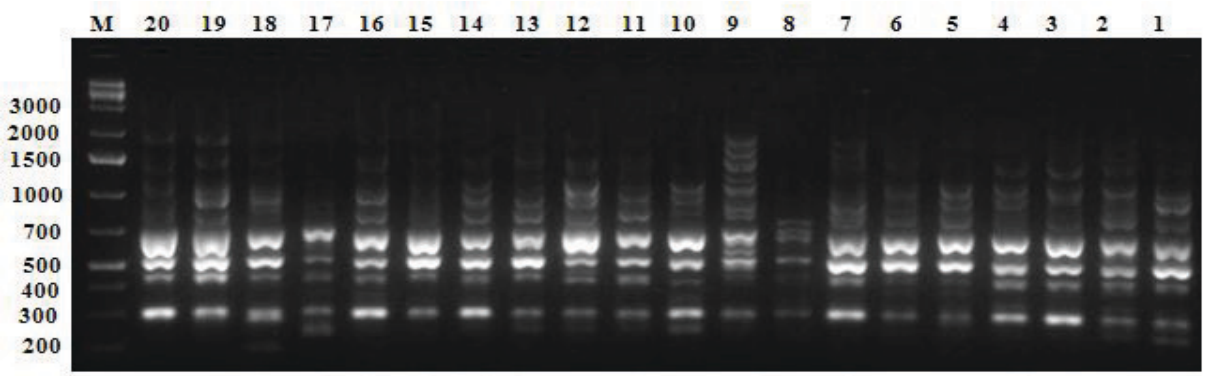

Fig. 2. PCR amplification products
PCR amplification products obtained with ISSR primer UBC836 in *P. bracteatum*, lanes 1-20: individuals of *P. bracteatum,* lane M: 1000bp DNA ladder

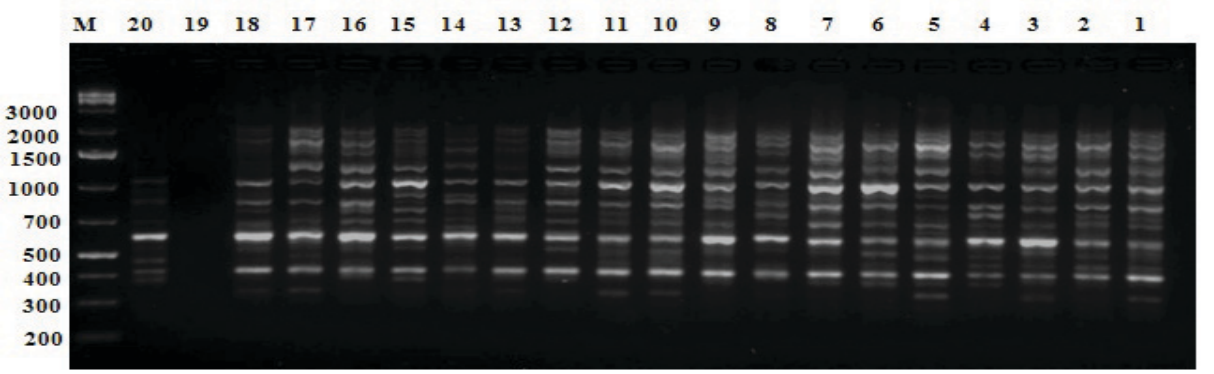

Fig. 3. PCR amplification products
PCR amplification products obtained with ISSR Primer UBC857 in *P. somniferum*, lanes 1-20: individuals of *P. somniferum*. Lane M: 1000bp DNA ladder

Table 3
Sequence and characteristics of the used ISSR primers in this study

Primer	Sequence	Tm (^{0}C)	TB[1]	PB[2]	PPB[3]	BS (bp)[4]	He[5]	Ne[6]	I[7]
UBC818	$(CA)_8G$	53	3	2	67	400-1000	0.281	1.489	0.408
UBC849	$(GT)_8CG$	53	4	1	25	500-1500	0.036	1.042	0.068
UBC 840	$(GA)_8YT$	53	8	8	100	500-2000	0.408	1.748	0.591
UBC 825	$(AC)_8T$	53	6	6	100	500-2000	0.373	1.619	0.557
UBC 848	$(CA)_8RG$	50	11	10	91	500-3000	0.278	1.435	0.434
UBC 885	HBH(AG)7	53	12	12	100	300-3000	0.384	1.652	0.570
UBC 857	$(AC)_8YG$	51	11	11	100	300-3000	0.412	1.723	0.6
UBC 816	$(CA)_8T$	53	11	8	73	400-2000	0.225	1.343	0.351
UBC 834	$(AG)_8YT$	53	12	10	83	300-2000	0.242	1.367	0.384
UBC 836	$(AG)_7YA$	53	8	4	50	300-3000	0.189	1.316	0.281
A12	$(GA)_6CC$	52	9	5	56	500-2000	0.171	1.264	0.267
A7	$(AG)_{10}T$	53	11	11	100	200-1500	0.403	1.718	0.589
A13	$(GT)_6CC$	52	11	11	100	500-2000	0.371	1.640	0.552
438	$(AC)_9G$	52	11	11	100	500-3000	0.346	1.571	0.523
A14	$(CA)_6/AC$	53	7	7	100	700-3000	0.368	1.661	0.540
Total	-	-	135	117	-	-	-	-	-
Mean	-	-	9	7.8	83	-	0.311	1.525	0.467

[1] total bands, [2]number of polymorphic bands, [3]percentage of polymorphic bands, [4]band size,
[5] mean of expected hetrosigosity, [6]number of effective alleles, [7]Shannon's information index.
R= purine (A/G), Y= pyrimidine (C/T), H = A, C, Tnot G, B = C, G, T not A

Eighty-three fragments were found to be useful as species-specific markers and fifty-twofragments were conserved between two species. A number of fifty-one positive specific markers were scored for the presence of unique bands for the *P. bracteatum*, while a number of thirty-two positive specific markers were scored for the presence of unique bands for the *P. somniferum* (Table 4). Primer UBC818 did not generate any specific marker for both species. The highest number of species-specific markers was generated by primer UBC848for *P. bracteatum* and primers UBC 816 and UBC834 for *P. somniferum*.

Genetic diversity parameters among all sixty individuals containing two populations of *P. bracteatum* and one population of *P. somniferum* are listed in Table 3. The analysis of data showed that the percentage of polymorphic band ranged from 25 to 100 with a mean value of 86.67. The overall Shannon's index (I) was 0.467. Also, genetic diversity variables were assessed among two species. A summary of the genetic data for each species is given in Table 5. The percentage of polymorphic loci varied between two species. *P. bractaetum* exhibited the high level of polymorphism (49.63%), whereas *P. somniferum* presented the low level of that (16.67%). Differences in the He and I were also significant among two species. The characteristics of amplified ISSR loci as well as genetic diversity parameters were also measured for two populations of *P. bracteatum* (Table 6). No consequential differences were found between two populations in view of the calculated parameters.

ANOVA analysis based on three populations and two species using ISSR data indicated that 64% of the genetic variation is attributed to differences among species. Genetic differences among populations (14%) were lower than within populations (21%). Pair wise comparisons of genetic differentiation (Φ_{ST}) showed that genetic differences among two species (Φ_{ST}=0.81, P<0.01) were more significant than genetic differences between two populations of *P. bracteatum* (Φ_{ST}=0.352, P<0.01).

Genetic relationships among populations

In order to estimate the genetic similarity among the individuals, the similarity matrix was computed using Jacquard similarity coefficient. The obtained matrix showed that coefficient of similarity values ranges from 0.247 to 0.973, averaging 0.595. The smallest similarity value (0.247) between two species suggested the high divergence between *P. bracteatum* and *P. somniferum* and the maximum similarity value (0.97) was scored between two individuals of *P. somniferum*. The UPGMA dendrogram using ISSR loci clustered the populations into the three groups (Figure 4). Population 1 from *P.*

Table 4
Species-specific markers in *P. bracteatum* and *P. somniferum* resulting from fifteen different ISSR primers

	Papaver bracteatum			*Papaver somniferum*		
Primer	AF[1]	CONF[2]	SM[3]	AF	CONF	SM
UBC818	3	3	0	3	3	0
UBC849	4	3	1	4	3	0
UBC 840	8	2	6	8	2	0
UBC 825	6	4	0	6	4	2
UBC 848	11	3	7	11	3	1
UBC 885	12	3	5	12	3	4
UBC 857	11	8	1	11	8	2
UBC 816	11	3	3	11	3	5
UBC 834	12	3	4	12	3	5
UBC 836	8	5	3	8	5	0
A12	9	5	3	9	5	1
A7	11	1	5	11	1	5
A13	11	3	5	11	3	3
438	11	2	5	11	2	4
CA,AC	7	4	3	7	4	0
Total	135	52	51	135	52	32

[1]Amplified Fragments, [2]Conserved Fragments, [3]Specific Marker

Table 5
Characteristics of amplified ISSR loci in *Papaver bracteatum* and *Papaver somniferum*

Species	*P. somniferum*	*P. bracteatum*
Number of loci	84	102
Percentage of polymorphic loci (%)	16.30	49.63
Number of loci with frequency of ≥5%	84	102
Number of less common loci (≤25%)	0	0
Number of less common loci (≤50%)	0	0
Number of private loci	32	51
Mean of heterozygosity (He)	0.069±0.014	0.192±0.018
Number of effective alleles (Ne)	1.127±0.024	1.336±0.033
Shannon's information index (I)	0.1±0.020	0.283±0.026

Table 6
Characteristics of amplified ISSR loci in two populations of *Papaver bracteatum*

Populations	*P. bracteatum* (Pop1)	*P. bracteatum* (Pop2)
Number of loci	98	93
Percentage of polymorphic loci (%)	51.96%	47.06
Number of loci with frequency of ≥5%	98	93
Number of less common loci (≤25%)	0	0
Number of less common loci (≤50%)	0	0
Mean of heterozygosity (He)	0.203±0.021	0.187±0.021
Number of Effective Alleles(Ne)	1.352±0.038	1.334±0.040
Shannon's Information Index (I)	0.299±0.030	0.272±0.030

bracteatum was in group I, group II contained population 2 of *P. bracteatum* and *P. somniferum* was in group III. Group I and group II were more closely related to each other than with of group III. The high bootstrap values in the forks of major clusters revealed high confidence level in the UPGMA clustering. The comparison of Jacquard similarity matrix with the matrix of cophenetic values of ISSR dendrogram using the Mantel test suggested that data in the Jacquard's similarity matrix was represented very well by the UPGMA dendrogram (r=0.99).

The result of PCoA analysis was comparable to the cluster analysis. Sixty genotypes formed three clusters in PCoA analysis (Figure 5). In PCoA, individuals with similar descriptions were gathered into the same cluster and were having high internal (within cluster) homogeneity and high external (between clusters) heterogeneity. The first two PCoA accounted for 48.57% and 9.58% of the total variance, respectively. Genetic distances among three populations were also estimated according to Nei's genetic distance. Similar to

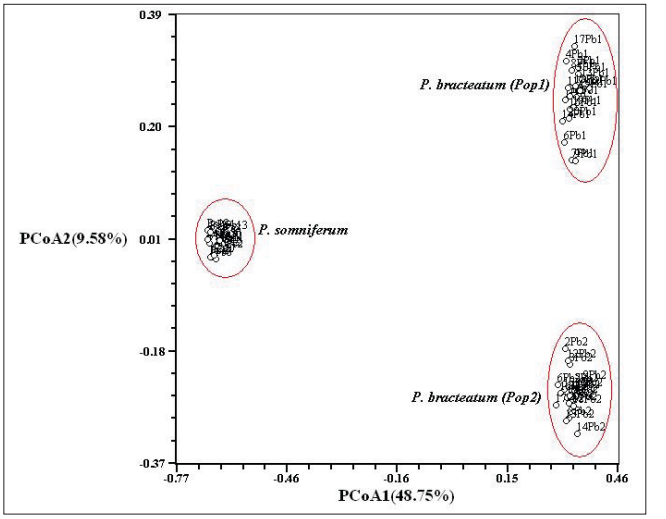

Fig. 5. Two-dimensional plot

Two-dimensional plot of the genetic relationship among 60 individual of *Papave rsomniferum* and *Papaver bracteatum*

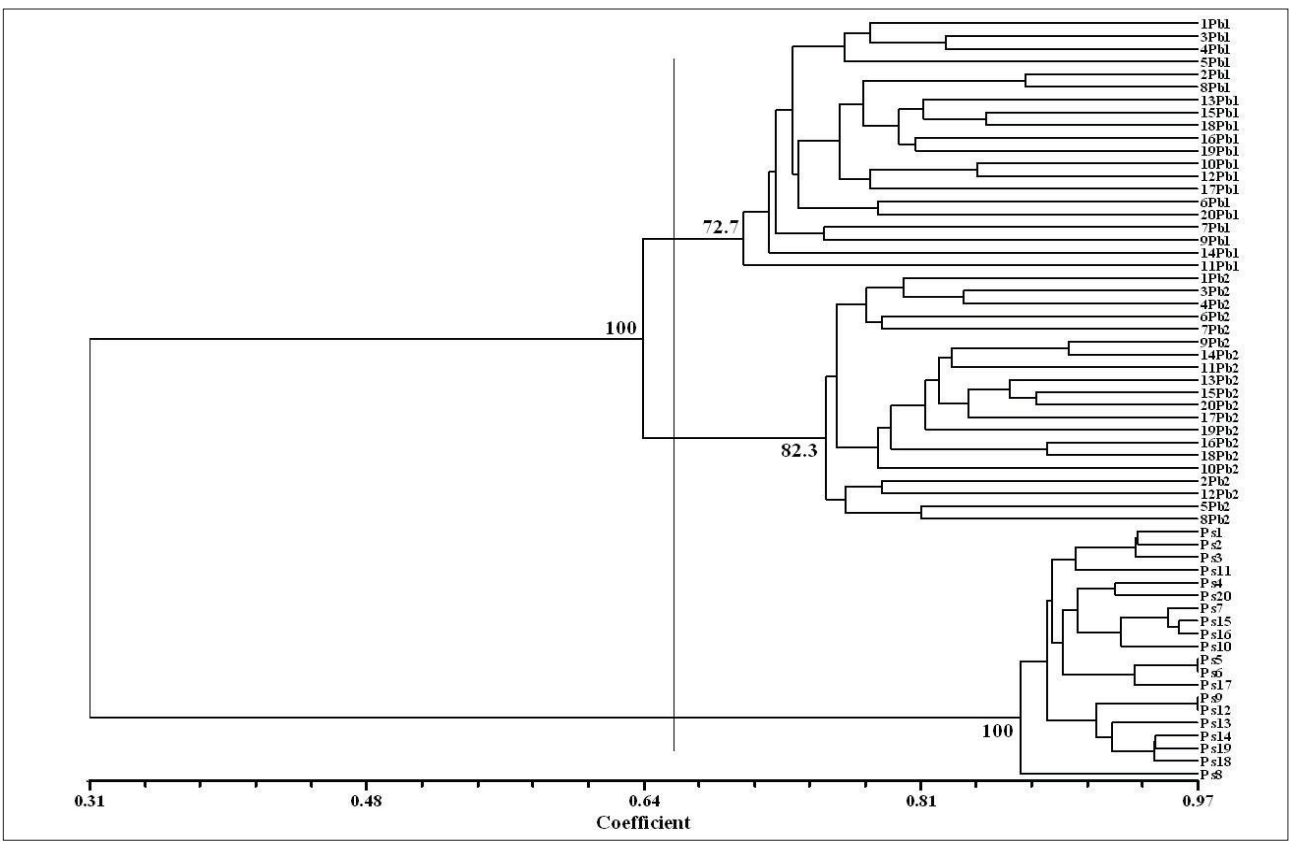

Fig. 4. UPGMA dendrogram

UPGMA dendrogram generated using 135 ISSR loci, the bootstrap values from 1000 replicates are given on the main forks. P.b1: Population 1 of *Papaver bracteatum*, P.b2: Population 2 of *P. bracteatum*, P.s: *P. somniferum*

what was found by UPGMA cluster and PCoA analysis, the most two closely related populations were two populations of *P. bracteatum* with the highest similarity index of 89%. On the other hand, low similarity index (50%) was obtained between two species.

Discussion

DNA based markers provide precise information on genetic diversity because of the independence of the confounding effects of environmental factors (Powell et al., 1995). Because ISSR markers have a better reproducibility and a much greater number of total polymorphic and discriminate fragments than RAPDs (Mattioni et al., 2000) and lower relative costs compared with RFLP (Yang et al., 1996), are of easier detection and at lower costs than AFLPs (Tian et al.,2008), and simpler to use than the SSR technique (Triest,2006) and may offer considerable variation among species (Wolf and Liston,1998), ISSR markers are increasingly applied since 2000 (Tian et al., 2008; Wang et al., 2008). Clearly, ISSR is one of the powerful approaches for the assessment of genetic variation among populations, especially for species in which no molecular genetic information was previously available. Here, we demonstrated that ISSR is highly efficient for the determination of genetic variations among selected populations of *P. somniferum* and *P .bracteatum*. In the current investigation, fifteen ISSR primers were used for fingerprinting and estimating genetic diversity of two populations of *P. bracteatum* and one population of *P. somniferum*. Using these primers, 135 discernible DNA fragments were generated with 117 polymorphic ones. High polymorphism (86.6%) revealed in the present study suggested that ISSR markers were polymorphic enough to detect the genetic diversity of selected populations at the DNA level. The overall I was relatively high, indicating that a relatively great genetic diversity lies in the *Papaver* populations. The high percentage of polymorphism is common for ISSR amplified products. Other workers obtained similar results in other medicinal plants: 61.53% in *Jatrophacurcas* (Mittal and Dubey, 2010), 67% for some medicinal plants in Sinai (Domyati et al., 2011), 97% in *Coptischinensis* (Shi et al., 2008) and 97.2% in *Ochreinauclea missions* (Chandrika and Ravishankar, 2009). Hamrick et al. (1991) stated that several factors affect genetic diversity level in a given species. Geographic range is strongly associated with the levels of genetic diversity. Generally endemic species have lower genetic diversity than widespread species. Other factors such as breeding systems, vegetative reproduction, and dispersal pattern and sample size also significantly influence the genetic diversity of a species. The percentage of polymorphic loci, I and Ne for *P. bracteatum* was superior to those of *P. somniferum.* The reason for their differences might be due to the breeding system of two species. *P. bracteatum* is an out-crossing species but *P. somniferum* is a self-pollinating species. Acharya and Sharma (2009) showed that *P. somniferum* genotypes have low genetic diversity. Also high polymorphism rate (85%) detected among 53 accessions belonging to 3 important *Oxytona* species, i.e. *P. bracteatum* Lindl., *P. orientale* L., and *P. pseudo-orientale.*

The higher proportion of genetic variation (64%) and significant Φ_{ST} (0.81) among two species showed divergence between two species. The relatively high Φ_{ST} between two populations of *P. bracteatum* is probably due to geographical distance. In addition to, genetic variation within populations was relatively higher than among populations, it may arise from out-crossing nature of *P. bracteatum*. The pollinators of this species are small insects (Goldblatt, 1974). The transfer of pollen by insects between the different individuals may increase the possibility of sexual recombination and subsequently increase within-population genetic diversity (Shi et al., 2008). Also the UPGMA dendrogram showed that plants from different geographical regions were distributed in different groups. Even in the case of *P. bracteatum,* two accessions from Iran were not grouped together. Therefore the correlation between geographic distance and genetic diversity was concluded.

Conclusion

In conclusion, all ISSR primers used could produce polymorphic markers to discriminate and identify the different genotypes. Two important findings have emerged from the present study. First, a wide range of variation is indicated in the phenetic relationship among *P. bracteatum* and *P. somniferum*. Second, species-specific bands were detected. The sequence information in these bands can be used to develop the species-specific SCAR markers. These bands confirm the presence of microsatellites, whose sequences are complimentary to that of the primers. These microsatellites can be used to develop STS (sequence tagged site) maps. Such maps which will be necessary to locate genes that impart alkaloids in two species.

References

Acharya, H. S. and V. Sharma, 2009. Molecular characterization of opium poppy (*Papaver somniferum*) germplasm. *American Journal of Infection Diseases*, **5:** 155–160.

Alishah, O. and M. Omidi, 2008. Laboratory methods of cytogenetics. *University of Tehran Press*, Tehran, Iran.

Chandrika,M. and R. Ravishankar, 2009. Genetic fidelity in micropropagated plantlets of *Ochreinaucleamissionis* an endemic, threatened and medicinal tree using ISSR markers. *African Journal of Biotechnology,* **4:** 2933–2938.

Domayti, F. M., A. A. Younis Rania, S. Edris, A. Mansour, G. Sabir and A. Bahieldin, 2011. Molecular markers associated with genetic diversity of some medicinal plants in Sinai. *Journal of Medicinal Plants Research,* **5:** 200–210.

Doyle, J. and J. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, **19:** 11–15.

Dušek, K., E. Dušková and K. Smékalová, 2010. Genetic Diversity of Selected Medicinal Plants in Protected Landscape Areas in the Czech Republic. *Czech Journal of Genetics and Plant Breeding*, **46:** (Special Issue) 34–36.

Esselman, E. J., J. Q. Li, D. J. Crawford, J. L. Windus and A. D. Wolfe, 1999. Clonal diversity in the rare *Calamagrostis porter* ssp. *Insperata* (Poaceae): Comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. *Journal of Molecular Ecology*, **8:** 443–451.

Goldblatt, P., 1974. Biosystematic studies in *Papaver section Oxytona*. *Annals of the Missouri Botanical Garden,* **61:** 264–296.

Hammer, K. and R. Fritsch, 1977. The question of ancestral species of cultivated poppy (*Papaver somniferum* L.). *Kulturpflanzen,* **25:** 113–124.

Hamrick, J. L., M. J. Godt, D. A. Murawski and M. D. Loveless, 1991. Correlations between species and allozyme diversity: implications for conservation biology. In: D. A. Falk and K. E. Holsinger (Editors), *Genetics and Conservation of Rare Plants, Oxford University Press*, New York, pp. 75–86.

Huziwara, Y., 1962. Karyotype analysis in some genera of Compositae. Further studies on the chromosomes of *Aster*. **American Journal of Botany**, **49:** 116–119.

La coure, L. F., 1941. *Stain Technology,* **16:** 169.

Levy, A. and J. Milo, 1998. Genetics and breeding of *Papaver somniferum*. In: J. Bernáth (Editor), *Poppy - The Genus Papaver, Harwood Academic Publisher,* Amsterdam, pp. 93–103.

Li, J. M. and Z. X. Jin, 2008. Genetic structure of endangered *Emmenopterys henryi* Oliv. based on ISSR polymorphism and implications for its conservation. *Genetica,* **133:** 227–234.

Madadi, R., R. Asghari-Zakaria and M. Fathi, 2009. Karyotype study in several populations of *Papaver dubium* from North West of Iran. *Biological Diversity and Conservation*, **3:** 18–22.

Mattioni, C., M. Casasoli and M. Gonzalez, 2002. Comparison of ISSR and RAPD markers to characterize three *Chilean Nothofagus* species. *Theoretical and Applied Genetics*, **104:** 1064 –70.

Milo, J., A. Levy and D. Palevitch, 1998. The cultivation and breeding of *P. bracteatum*. In: J. Bernáth (Editor), *Poppy - The Genus Papaver, Harwood Academic Publisher,* Amesterdam, pp. 279–289.

Mittal, N. and A. K. Dubey, 2010. A novel set of highly polymorphic chloroplast microsatellite and ISSR markers for the biofuel crop *Jatrophacurcas*. *Bios Sciences*, **4**: 119–131.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics,* **89:** 583–590.

Parmaksiz, I. and S. Özcan, 2011. Morphological, chemical, and molecular analyses of Turkish Papaver accessions (Sect.Oxytona).*Turkish Journal of Botany*, **35:**1–16.

Peakall, R. and P. E. Smouse, 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6:** 288–295.

Powell, W., C. Orozco-Castillo, K. J. Chalmer, J. Provan and R.Waugh,1995. Polymerase chain reaction-based assays for the characterization of plant genetic resources. *Electrophoresis,* **16:** 1726–1730.

Qian, W., S. Ge and D. Y. Hong, 2001. Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theoretical and Applied Genetics*, **102:** 440–449.

Rohlf, F. J., 2000. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. *Exeter Software*, New York.

Romero Zarco, C., 1986. A new method for estimating karyotype asymmetry. *Taxon,* **35:** 526–530.

Saunders, J. A., M. J. Pedroni, D. J. Penrose and A. J. Fist, 2001. AFLP analysis of opium poppy. *Crop Science*, **41:** 1596–1606.

Shi,W., C. F. Yang, J. M. Chen and Y. H. Guo, 2008. Genetic variation among wild and cultivated populations of the Chinese medicinal plant *Coptis chinensis* (Ranunculaceae). *Plant Biology,* **10:** 485–491.

Singh, H. G., 1982. Cultivation of opium poppy. In: C. K. Atal and B. M. Kapur (Editors), *Cultivation and utilization of medicinal plants, Regional Research Laboratory Council of Scientific and Industrial Research,* Jammu-Tawi, pp. 120–138.

Sip, V, V. Martinek and M. Skorpik, 1977. A study of the inheritance of economically important characters in poppy.*Genetika a Slechteni,* **13:** 207–218.

Tian, H. L., J. H. Xue, J. Wen,G. Mitchell and S. L. Zhou, 2008. Genetic diversity and relationships of Lotus (Nelumbo) cultivars based on allozyme and ISSR markers. *Scientia Horticulturae*, **116:** 421–429.

Triest, L., 2006. Molecular ecology and biogeography of mangrove trees towards conceptual insights on gene flow and barriers: a review. *Aquatic Botany,* **89:** 138–154.

Wang, C., H. Zhang, Z.Q. Qian and G. F. Zhao, 2008. Genetic differentiation in endangered *Gynostemma pentaphyllum* (Thunb.) Makino based on ISSR polymorphism and its implications for conservation. *Biochemical Systematics and Ecology*, **36:** 699–705.

Wolfe, A. D. and A. Liston, 1998. Contributions of PCR-based methods to plant systematic and evolutionary biology.In: D.E.Soltis et al. (Editors), *Plant Molecular Systematics.* Vol. II. *Kluwer*, Boston, p. 43–86.

Yang, W. P, A.C. De Oliveira, I. Godwin, K. Schertz and J. L. Bennetzen, 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: Variability in Chinese sorghums. *Crop Science*, **36:** 1669–1676.

Yap, I. V. and R. J. Nelson, 1996. Winboot: a program for performing bootstrap analysis of binary data to determine the confidence of UPGMA-based dendrograms. *International Rice Research Institute (IRRI),* Manilla, Phillipines.

Zietkiewicz, E, A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics,* **20:** 176–183.

Received June, 18, 2014; accepted for printing January, 20, 2015.