Genetic diversity and morphological variability in Geranium purpureum Vill. (Geraniaceae) of Iran

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Original scientific paper

GENETIC DIVERSITY AND MORPHOLOGICAL VARIABILITY IN *Geranium purpureum* VILL. (GERANIACEAE) OF IRAN

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Genetic variability and populations' structure were studied in 15 geographical populations of *Geranium purpureum* Vill. (Geraniaceae). Genetic diversity parameters were determined in these populations. AMOVA and Gst analyses revealed the presence of genetic variability within populations and significant molecular difference among the studied populations. Mantel test showed positive significant correlation between genetic distance and geographical distance of the studied populations. STRUCTURE and K-Means clustering revealed populations' genetic stratification. UPGMA dendrogram of populations based on morphological characters was in agreement with NJ tree of molecular data. These results indicated that geographical populations of *G. purpureum* are well differentiated both in genetic content as well as morphological characteristics. Consensus tree based on morphological and genetic data separated some of these populations from the others suggesting the existence of ecotypes within this species.

Keywords: Gene flow, Network, ISSR, *Geranium purpureum*

INTRODUCTION

The genus *Geranium* L. contains about 400 species that are distributed in temperate and tropical regions of the world (AEDO *et al*., 1998). These species are annual, biennial or perennial plants. The annual species are commonly small-flowered, selfing weedy colonizers of disturbed habitats, while the outcrossing perennial species have showy flowers (FIZ *et al*., 2008). More than

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260 *Geranium* species are cultivated. Many species can hybridize and produce fertile hybrids. For example, *Geranium* 'Brookside', a superb, long-flowering garden plant is a hybrid between *G. clarkei* and *G. pratense*.

G. x *cantabrigiense*, is a hybrid between *G. macrorrhizum* and *G. dalmaticum*. It makes an excellent groundcover and produce pink to white flowers (YEO, 2001).

Some of the *Geranium* species are of medicinal value and used as antidiabetic, hemostatic, antihemorrhoidal, and antidiarrheal (BAYTOP, 1999; BATE-SMITH, 1973). These species were used as a remedy for tonsillitis, cough, whooping cough, urticaria, dysentery, pain, fevers, and gastrointestinal ailments (CALZADA, 2005).

The genus *Geranium* is divided into three subgenera: subgen. *Erodioidea* (Picard) Yeo, subgen. *Robertium* (Picard) Rouy, and subgen. *Geranium* (YEO, 1984).

G. purpureum (Subgenus *Robertium* Picard; section *Ruberta* Dumortier.) is a leafy annual or biennial plant, grows in height up to 30–65 cm (occasionally more). It is branched at base, and bright or dark green, sometimes slightly reddish-tinged in color. The plant is fragile, with a strong disagreeable smell. Different geographical populations of this species show morphological variations, particularly in growth habit (erect or prostrate) and the amount of hairs in different parts (YEO, 1973).

For *G. purpureum* and *G. robertianum* Van Loon (1984b) reported, respectively, 2*n* = 32 and 2*n* = 64 and as Baker found 2*n* = 64 of *G. robertianum*,Yeo (1985) states that reports of chromosome numbers of 32 for *G. robertianum* are in need of substantiation (the closely related and similar *G. purpureum* has a chromosome number of 32). In Iran, *G. purpureum* occurs in different habitats as rocky or stony places, on cliffs, dry hills and hedgebanks or in stabilized areas at the rear of shingle beaches of northern and north-western parts (up to 1500 m.) (PARSA, 1951; WIGGINTON, 1999).

Taxonomic controversy exits about the rank of *G. purpureum* and *G. robertianum*. Some authors consider them as two different species, or two different subspecies, while, some authors consider *G. purpureum* as a subspecies or variety of *G. robertianum* (RADLEY, 1994; YEO, 2003).
Population genetic studies produce information about the species genetic variability, gene flow, inbreeding versus outbreeding, effective population size and population bottleneck. These data are helpful in choosing effective management in conservative strategies (ELLIS and BURKE, 2007), and also through light on the presence of infra-specific taxonomic forms (SHEIDAI *et al*., 2014).

G. purpureum exhibits great extent of morphological variability, and form many geographical populations in Iran. These geographical populations have variable eco-geographical features, some of which are in close vicinity, while some others are distributed in distant regions. We have no information on genetic variability, gene flow and genetic structure of *G. purpureum* populations. Moreover, due to extensive morphological variability of this species in the country, there is possibility of having infra-specific taxonomic forms in this species. Therefore, we carried out population genetic analysis and morphometric study of 15 geographical populations for the first time in the country.

For genetic study, we used ISSR (Inter-simple sequence repeats). These molecular markers are highly reproducible, cheap, easy to work and are known to be efficient in population genetic diversity studies and species or infra-specific taxonomic delineation (SHEIDAI *et al*., 2012, 2013, 2014).

MATERIALS AND METHODS

Plant materials

In present study 82 plant samples were collected from 15 geographical populations. Different references were used for the correct identification of species (*G. purpureum*), (DAVIS, 1967; SCHÖNBECK-TEMESY, 1970; ZOHARY, 1972; AEDO *et al.*, 1998; JANIGHORBAN, 2009). Details of sampling sites are mentioned (Table 1, Fig.1). Voucher specimens are deposited in Herbarium of Shahid Beheshti University (HSBU).

Table 1 Populations studied, their locality and ecological features.

Population	Locality	Latitude	Longitude	Altitude (m)	Voucher no.
1	Gilan, Before Rasht, Road to Sangar	37° 06' 57"	49° 44' 06"	41	HSBU 201600
2	Gilan, Bandar Anzali, Pine artificial woodland	37 °27'34"	49° 35' 50"	-25	HSBU 201601
3	Guilan, Bandar Anzali, The Beach	37° 28' 59"	49° 28' 28"	-29	HSBU 201602
4	Gilan, Siahkal, Upstream of Gole rodbar river	37° 09' 08"	49° 55' 02	27	HSBU 201603
5	Gilan, Siahkal,Downstream of Gole rodbar river	37° 10' 05"	49° 56' 38"	15	HSBU 201604
6	Gilan , Lahijan Sheytan kouh hill side	37° 12' 04"	50° 03' 12"	9	HSBU 201605
7	Gilan , Lahijan , Highlands of Sheytan Kouh	37° 11' 52"	50° 03' 17"	159	HSBU 201606
8	Gilan, Bandar Anzali, Road side	37° 27' 48"	49° 33' 20"	-23	HSBU 201607
9	Mazandaran, Tonekabon, Neamat abad	36° 49' 02"	50° 52' 20"	-16	HSBU 201608
10	Mazandaran, Tonekabon, Shirodi Ring Road	36° 51' 10"	50° 48' 15"	-23	HSBU 201609
11	Mazandaran, Noshahr, Sisangan Beach Park	36° 35' 04"	51° 48' 24"	-29	HSBU 201610
12	Mazandaran, Royan, 30km Noshahr	36° 34' 13"	51° 55' 43"	-22	HSBU 201611
13	Mazandaran, Noshahr, Kheyrud kenar Forest	36° 38' 05"	51° 29' 05"	-16	HSBU 201612
14	Mazandaran, Chaloos	36° 30' 28"	51° 19' 59"	1591	HSBU 201613
15	Mazandaran, 20km Noshahr	36° 34' 14"	51° 53' 08"	-21	HSBU 201614

Fig. 1. Distribution map of the studied populations.

DNA extraction and ISSR assay

Fresh leaves were used randomly from 5-10 plants in each of the studied populations. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (SHEIDAI *et al*., 2013). The quality of extracted DNA was examined by running on 0.8% agarose gel. 10 ISSR primers; (AGC) 5GT, (CA) 7GT, (AGC) 5GG, UBC 810, (CA) 7AT, (GA) 9C, UBC 807, UBC 811, (GA) 9T and (GT) 7CA commercialized by UBC (the University of British Columbia) were used. PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications· reactions were performed in Techne thermocycler (Germany) with the following program: 5Min initial denaturation step 94°C, followed by 40 cycles of 1min at 94°C; 1 min at 52-57°C and 2 min at 72°C. The reaction was completed by final extension step of 7-10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

Morphological studies

In total 80 morphological (42qualitative, 38 quantitative) characters were studied. Five plant specimens were randomly studied for morphological analyses (Table 2).

Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) and Ward (Minimum spherical characters) as well as ordination methods of MDS (Multidimensional scaling) and PCoA (Principal coordinate analysis) were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters

among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Table 2. Evaluated morphological characters

No	Characters	No.	Characters
1	Plant height (mm)	20	Mericarp length (mm)
2	Length of stem leaves petiole (mm)	21	Mericarp width (mm)
3	Length of stem leaves (mm)	22	Mericarp length/ Mericarp width (mm)
4	Width of stem leaves (mm)	23	Seed length (mm)
5	Length of stem leaves / Width of stem leaves(mm)	24	Seed width (mm)
6	Width of stem leaves/ Length of stem leaves (mm)	25	Seed length/ Seed width(mm)
7	Number of segment stem leaves (mm)	26	Stipules length (mm)
8	Length of basal leaves petiole (mm)	27	Stipules width (mm)
9	Length of basal leaves (mm)	28	Stipules length/ Stipules width (mm)
10	Width of basal leaves (mm)	29	Bract length (mm)
11	Length of basal leaves / Width of basal leaves (mm)	30	Bract width (mm)
12	Width of basal leaves / Length of basal leaves (mm)	31	Bract length / Bract width (mm)
13	Number of segment basal leaves	32	Pedicel length (mm)
14	Calyx length (mm)	33	Peduncle length (mm)
15	Calyx width (mm)	34	Rostrum length (mm)
16	Calyx length/ Calyx width (mm)	35	Style length (mm)
17	Petal length (mm)	36	Stamen filament length (mm)
18	Petal width (mm)	37	Fruit length (mm)
19	Petal length / Petal width (mm)	38	Number of flowers per inflorescence
39	Type root	60	Bract shape
40	Vegetation-forms	61	Stipules shape
41	State of stem strength	62	Bract and Stipules hair density
42	State of stem branches	63	Bract and Stipules hair
43	Leave shape	64	Shape of segments cauline leaves
44	Phyllotaxy	65	Shape of calyx
45	Leaf tips	66	Calyx apex
46	Shape of segments basal leaves	67	Petal shape
47	Stamen filament color	68	State of petale ligule
48	Stigma hair	69	Shape of petal lobes
49	Mericarp shape	70	State of petale ligule hair
50	Mericarp surface	71	Stamen filament hair
51	Mericarp hair	72	Mericarp hair density
52	Mericarp Rostrum hair	73	Mericarp color
53	Sepale hair	74	Seed color
54	Sepale hair density	75	Seed shape
55	Peduncle and pedicel hair	76	Seed surface ornamentation
56	Anthers colour	77	Peduncle and pedicel hair density
57	Stem hair	78	Petioles hair
58	Stem hair density	79	Petioles hair density
59	Leaf hair	80	Leaf hair density

Molecular analyses

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism were determined (WEISING *et al*, 2005; FREELAND *et al*., 2011).

Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al*., 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic distance of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMER *et al*., 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) software.

AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006), and Nei's Gst analysis as implemented in GenoDive ver.2 (2013) (MEIRMANS and VAN TIENDEREN, 2004) were used to show genetic difference of the populations. Moreover, populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation (HEDRICK, 2005), and D_est = Jost measure of differentiation (JOST, 2008).

The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (PRITCHARD *et al*., 2000), and maximum likelihood-based method of K-Means clustering of GenoDive ver. 2. (2013). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al*., 2007). The Evanno test was performed on STRUCTURE result to determine proper number of *K* by using delta *K* value (EVANNO *et al*., 2005). In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC), provide the best fit for k (MEIRMANS, 2012).

We used latent factor mixed models (LFMM) (FRICHOT *et al*., 2013) that tests correlations between environmental and genetic variation while estimating the effects of population structure. The analysis was done by LFMM program Version: 1.2 (2013).

Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed in Genodive ver. in GenoDive ver. 2. (2013). The presence of shared alleles was determined by drawing the reticulogram network based on the least square method by DARwin ver 5. (2012).

RESULTS

Populations' genetic diversity

Genetic diversity parameters determined in 15 geographical populations of *G. purpureum* are presented in Table 3. The highest value of percentage polymorphism (56.47%) was observed in Gilan, Bandar Anzali, (population No.2) which shows high value for gene diversity (0.192). and Shanon' information index (0.289). Population Gilan, Road side Bandar Anzali (No.8) has the lowest value for percentage of polymorphism (4.71%) and the lowest value for Shanon, information index (0.027), and He (0.019).

Population genetic differentiation

AMOVA (PhiPT = 0.49, P = 0.010), and Gst analysis (0.599, p = 0.001) revealed significant difference among the studied populations. It also revealed that, 40% of total genetic variability was due to within population diversity and 59% was due to among population genetic

differentiation. Pairwise AMOVA produced significant difference among the studied populations. Moreover, we got high values for Hedrick standardized fixation index after 999 permutation (G'st = 0.615, P = 0.001) and Jost, differentiation index (D-est = 0.231, P = 0.001). These results indicate that the geographical populations of *G. purpureum* are genetically differentiated from each other.

Table 3. Genetic diversity parameters in the studied populations. (N = number of samples, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Pop	Na	Ne	I	He	UHe	%P
Pop1	1.094	1.309	0.267	0.179	0.189	49.41
Pop2	1.176	1.327	0.289	0.192	0.201	56.47
Pop3	0.647	1.182	0.152	0.103	0.111	27.06
Pop4	0.506	1.104	0.090	0.061	0.067	16.47
Pop5	0.694	1.131	0.126	0.081	0.087	27.06
Pop6	0.482	1.090	0.077	0.052	0.059	14.12
Pop7	0.459	1.115	0.089	0.062	0.068	15.29
Pop8	0.329	1.036	0.027	0.019	0.021	4.71
Pop9	0.388	1.081	0.068	0.046	0.056	11.76
Pop10	0.318	1.058	0.050	0.034	0.045	8.24
Pop11	0.835	1.206	0.179	0.119	0.132	34.12
Pop12	0.541	1.118	0.104	0.070	0.084	18.82
Pop13	0.718	1.162	0.147	0.097	0.106	29.41
Pop14	0.918	1.225	0.197	0.132	0.159	35.29
Pop15	0.576	1.144	0.122	0.083	0.095	21.18

Populations' genetic affinity

NJ tree and Neighbor-Net network produced similar results therefore only Neighbor-Net network is presented and discussed (Fig. 2). We have almost complete separation of the studied population in the network, supporting AMOVA result. The populations 1 and 2 are distinct and stand separate from the other populations with great distance. The populations 3 and 4, as well as populations 5 and 6 show closer genetic affinity and are placed close to each other.

Genetic divergence and separation of populations 1-4, as well as 7 and 8 from the other populations is evident in PCoA plot of ISSR data after 900 permutations (Fig.3). The other populations showed close genetic affinity. Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these populations (r = 0.28, P = 0.002). Therefore, the populations that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in *G. purpureum*.

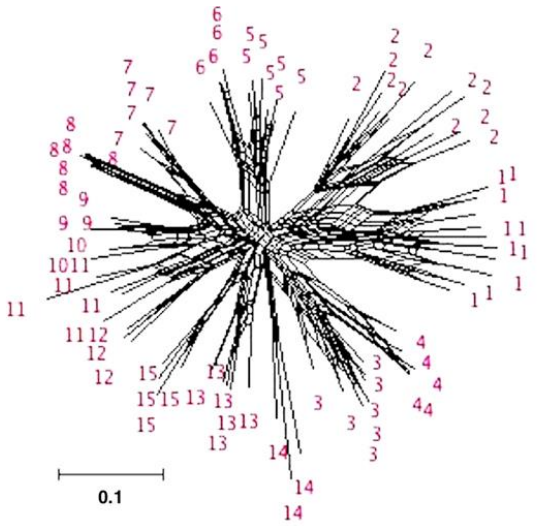

Fig.2. Neighbor-Net network of populations in *G. purpureum* based on ISSR data.

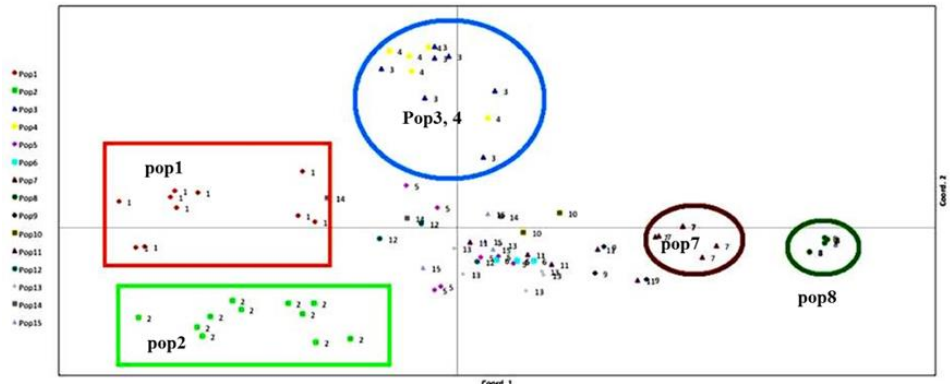

Fig. 3. PCoA plot of populations in *G. purpureum* based on ISSR data.

Populations genetic structure

K-Means clustering (Table 4) showed k = 8 according to pseudo-F and k = 14 according to BIC. K = 14 is in agreement with NJ grouping and AMOVA. K = 8 reveal the presence of 8 genetic group. Similar result was obtained by Evanno test performed on STRUCTURE analysis which produced a major peak at k = 8 (Fig.4). Both these analyses revealed that *G. purpureum* populations show genetic stratification.

STRUCTURE plot based on k = 8, revealed genetic difference of populations 1 and 2 (differently colored), as well as 7 and 8. But it showed genetic affinity between populations 3 and 4 (similarly colored), populations 5 and 6, as well as populations 9-12 and populations 13-15. The mean Nm = 0.22 was obtained for all ISSR loci, which indicates low amount of gene flow among

the populations and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. Population assignment test also agreed with Nm result and could not identify significant gene flow among these populations. However, reticulogram obtained based on the least square method (Fig. 5), revealed some amount of shared alleles among populations 3 and 4, and between 2 and 15 and 13, also between 7, 8, and 9. This result is in agreement with grouping we obtained with PCoA plot, as these populations were placed close to each other. As evidenced by STRUCTURE plot based on admixture model, these shared alleles comprise very limited part of the genomes in these populations and all these results are in agreement in showing high degree of genetic stratification within *G. purpureum* populations.

Table 4. K-Means clustering result. (Best clustering according to Calinski and Harabasz' pseudo-F: k = 8. Best clustering according to Bayesian Information Criterion: k = 14).*

K	SSD(AC)	SSD(WC)	SSD(WC)	r-squared	pseudo-F	BIC
1	1119.354	0	0	0	0	580.088
2	1119.354	114.9	1004	0.103	9.148	575.617
3	1119.354	210.5	908.9	0.188	9.147	571.822
4	1119.354	292.3	827.1	0.261	9.189	568.493
5	1119.354	367.5	751.9	0.328	9.409	565.084
6	1119.354	438.8	680.5	0.392	9.801	561.316
7	1119.354	498.3	621	0.445	10.03	558.22
8*	1119.354	545.8	573.5	0.488	10.061	556.102
9	1119.354	581	538.4	0.519	9.846	555.325
10	1119.354	612.7	506.7	0.547	9.674	554.75
11	1119.354	643.4	475.9	0.575	9.598	554.029
12	1119.354	671.7	447.7	0.6	9.548	553.413
13	1119.354	697.1	422.3	0.623	9.493	553.028
14&	1119.354	719.8	399.5	0.643	9.425	552.895
15	1119.354	740.3	379	0.661	9.347	552.989

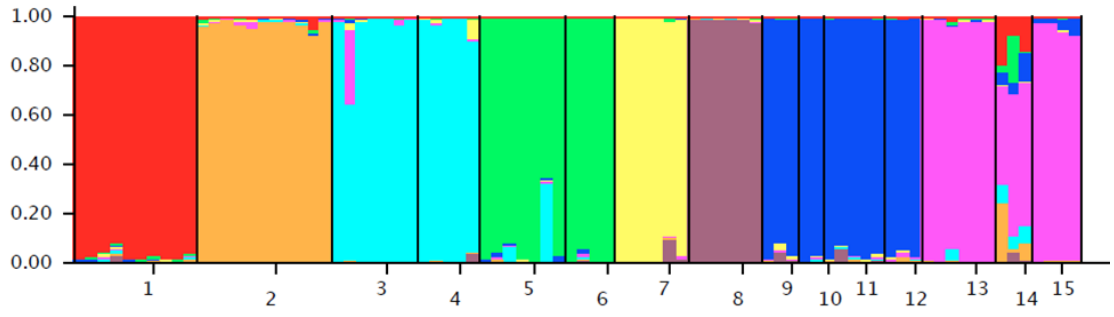

Fig. 4. STRUCTURE plot of *G. purpureum* populations based on k = 8 of ISSR data.

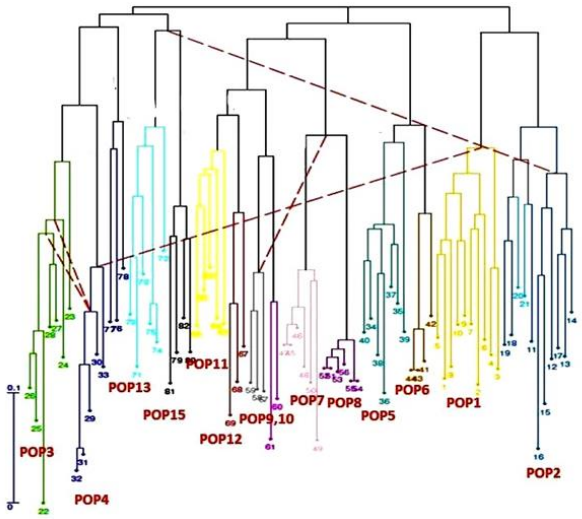

Fig. 5. Reticulogram of *G. purpureum* populations based on least square method analysis of ISSR data. (Population numbers are according to Table 1, pop1 (1-10), pop2 (11-20), pop3 (21-28), pop4 (29-33), pop5 (34-40), pop6 (41-44), pop7 (45-51), pop8 (52-56), pop9 (57-59), pop10 (60-61), pop11 (62-66), pop12 (67-69), pop 13(70-75), pop14 (76-78), pop15(79-82).

In total 82 ISSR bands (loci) were obtained, out of which 13 bands were private. Populations 1-5, 8, 11 and 13 contained 1-4 private bands. LFMM analysis revealed that 14 out of 85 ISSR loci had >1.1 -log 10 value > 1 (P <0.01) and may be considered as adaptive loci. Some of these loci had low Nm value <1, for example ISSR loci 34-36, 48, 67, 70, 73, 76, 78, 79, 83, and 84 and are less exchanged among populations. However, some loci like ISSR loci 59 and 61 had nm >1. Therefore, both ISSR loci that were partly shared by these populations and loci with lower admixture value/ private alleles were used by *G. purpureum* plants to adapt to their environment.

Morphometric analyses

In present study 82 plant samples were collected from 15 geographical populations. ANOVA test revealed significant difference in quantitative morphological characters among the studied populations ($P < 0.05$). Clustering and PCA plot of *G. purpureum* populations based on morphological characters produced similar results therefore only PCA plot is presented and discussed (Fig. 6). The result showed morphological difference/ divergence among most of the studied populations. This morphological difference was due to quantitative characters only. For example, character (Basal leaf of petiole length), separated population No. 1, character (Bract length) separated population No. 3, while character pedicel length, separated populations 6 and 7 from the other populations.

A consensus tree was obtained for both ISSR and morphological trees (Fig. 7), to reveal the populations that are diverged based on both morphological and molecular features. Interesting enough, it showed divergence of almost all populations at molecular level as well as morphological characteristics. Detailed comparison of the characteristics in these populations

revealed that, for example, population No. 1, has the longest peduncle length (46 mm), the highest bract length (1.32 mm), and the largest ratio of length/width of sepal (4.2 mm), among the studied populations. Similarly, population No. 5 had, the longest stem-leaf length (30 mm) and the broadest basal-leaf width (60 mm). Population No. 8 had, the narrowest stipule width (0.5 mm), and the highest ratio of stipule length/width.

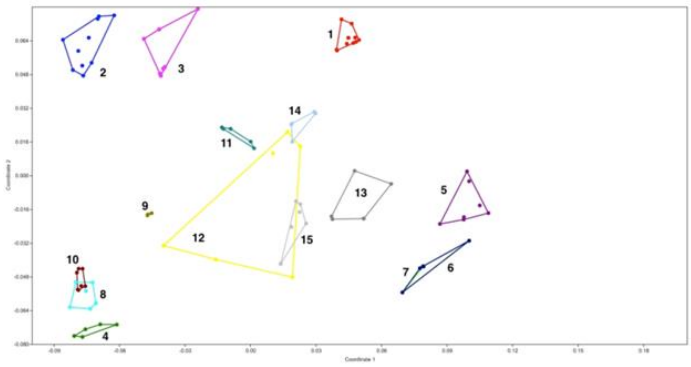

Fig. 6. PCA plot of *G. purpureum* populations based on morphological characters.

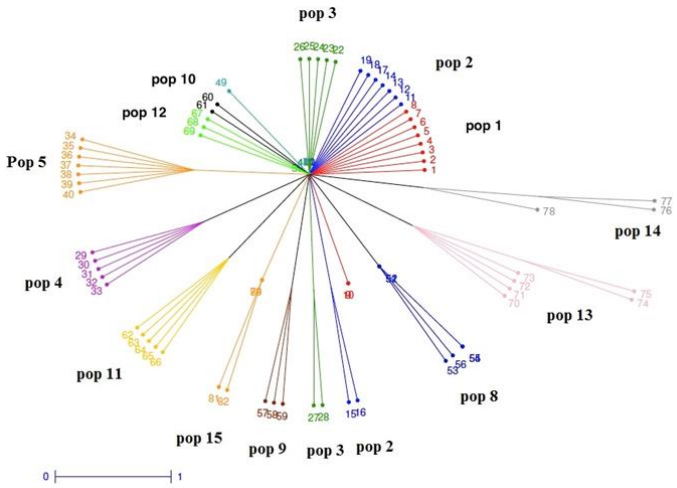

Fig. 7. Consensus tree of morphological and molecular data in *G. purpureum* populations.

DISCUSSION

Population genetic study provides valuable information about genetic structure of plants, the stratification versus gene flow among the species populations, genetic divergence of the populations, etc. (SHEIDAI *et al*., 2014). These information have different applications, and from pure understanding of biology of the species to conservation of endangered species, choosing of proper parents for hybridization and breeding and phylogeography and mechanism of invasion (FREELAND *et al*., 2011). *Geranium purpureum* is of wide spread in our country and it has several medicinal applications (PROESTOS *et al*., 2006), however we had no information on its genetic structure and detailed taxonomic information. The present study revealed interesting data about its genetic variability, genetic stratification and morphological divergence in north part of Iran.

The studied populations had low to moderate level of genetic diversity. The Genetic diversity is of fundamental importance in the continuity of a species as it is used to bring about the necessary adaptation to the cope with changes in the environment (ÇALIŞKAN, 2012; SHEIDAI *et al*., 2013, 2014). Degree of genetic variability within a species is highly correlated with its reproductive mode, the higher degree of open pollination/ cross breeding brings about higher level of genetic variability in the studied taxon (FREELAND *et al*., 2011). *Geranium purpureum* is mainly self-pollinating species (BAKER, 1955), therefore, low level of genetic variability within populations in this species might be related to the closer nature of breeding in this taxon.

Another well-known feature of self-pollinating species is high among-population genetic and morphological divergence. This happens due to limited amount of gene flow or its complete absence among geographical population in a single species (FREELAND *et al*., 2011). The present study also revealed significant morphological and genetic difference among *Geranium purpureum* populations, quite in agreement with the mentioned assumption. This is particularly supported by STRUCTURE plot that identified 8 separate genetic groups within this populations and by consensus tree of both morphological and genetic data. Different mechanisms like isolation, drift, founder effects and local selection may act to bring about among population differentiation and therefore, populations differ in phenotypic traits and allelic composition (JOLIVET and BERNASCONI, 2007). We should state that, the studied populations differed in quantitative morphological characters and we do not know how much of the morphological difference among the studied populations is genetically controlled; they may be under influence of environmental conditions. Therefore we do not attempt to suggest new taxonomic forms bellow the species level for this taxon and consider them as different ecotypes only.

The present population divergence may be under influence of isolation-by distance across the distribution range of the studied *G. purpureum* populations. The dispersal of these populations might be constrained by distance and gene flow is most likely to occur between neighboring populations. As a result, more closely situated populations tend to be more genetically similar to one another (SLATKIN, 1993; HUTCHISON and TEMPLETON, 1999; MEDRANO and HERRERA, 2008).

The populations' divergence may be accompanied by local adaptation. When we use multilocus molecular markers (such as SSR, AFLP, RAPD, ISSR, etc.) for population genetic studies we understand that these are neutral molecular markers (they are not directly acting as adaptive genes), but they may be linked to a gene or a genetic region with adaptive value (FREELAND *et al*., 2011). The LFMM analysis in present study revealed that some of the genetic loci were significantly correlated with the studied environmental features and possibly are adaptive and may be used by local populations to adapt to their environment. Therefore, combination of

genetic divergence, limited gene flow and local adaptation have played role in diversification of *G. purpureum* population in the country.

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GENETIČKI DIVERZITET I MORFOLOŠKA VARIJABILNOST KOD *Geranium purpureum* VILL. (GERANIACEAE) U IRANU

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Izvod

Genetička varijabilnost i struktura populacije su proučavane u 15 geografskih populacija *Geranium purpureum* Vill. (Geraniaceae) i u njima su utvrđeni parametri genetičkog diverziteta. AMOVA i Gst analize su utvrdile genetišku varijabilnost unutar populacija i značajne razlike između njih na molekularnom nivou. Mantel test pokazao je pozitivnu i značajnu korelaciju između genetičke distance i geografske distance proučavanih populacija. STRUCTURE i K-Means klasterom prikazana je genetička stratifikacija populacija. UPGMA dendrogram populacija zasnovan na morfološkim svojstvima bio je u skladu sa NJ zasnovanom na molekularnim podacima. Rezultati ukazuju da su geografske populacije *G. purpureum* dobro diferencirane na osnovu i genetičkih i morfoloških karakteristika. "Konsenzus" drvo zasnovano na morfološkim i genetičkim podacima razdvojilo je neke populacije od drugih ukazujući na postojanje ekotipova unutar ove vrste.

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