UDC 575. 630
https://doi.org/10.2298/GENSR2201275T
Original scientific article

POPULATION DIFFERENTIATION AND GENE FLOW OF *Glaucium flavum* (Papaveraceae)

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Ting S. and Y. Yibing (2022*). Population differentiation and gene flow of Glaucium flavum (Papaveraceae)*. - Genetika, Vol 54, No.1, 275-288.

Yellow hornpoppy (*Glaucium flavum* Crantz.) is a herbaceous plant with gray-green leaves in coastal sands, rocky areas, and heavily eroded soils up to 500 meters above sea level. *Glaucium flavum* is native to Northern Africa, temperate zones in Western Asia and Europe, and is indigenous to Iran. The plant has been widely recognized for its aporphine-type isoquinoline alkaloids, which are pharmacologically active. Thus, we conducted a combination of morphological and molecular data analysis on such species because of the plant species' relevance. One hundred seven randomly collected plants from 14 natural populations in 5 provinces were evaluated using ISSR markers and morphological traits. The evaluation of molecular variance (AMOVA) demonstrated significant genetic divergence between the examined populations. It indicated that 25% of overall genetic variability was related to intra-population variety, whereas 75% was due to inter-population genetic differentiation. ISSR primers discovered 156 bands, 139 (83 %) of which have been polymorphic, each primer containing an average of 13 bands. The Polymorphic Bands (PPB) Percentage (ISSR-6) varied from 50% to 100%. (ISSR-1, ISSR-4, and ISSR-5). The average polymorphic information content (PIC), Shannon's information indexes (I), and several effective alleles (Ne) were correspondingly 0.39, 0.26, and 1.2.

Keywords: Genetic diversity, Gene flow, Genetic differentiation, inter simple sequence repeat (ISSR)

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INTRODUCTION

Glaucium considers a genus in the subfamily Papaveraceae. According to KADEREIT (1993), Ernest's Chelidonoideae has roughly 23 species. FEDDE (1909) listed twenty species, ten varieties, and one subvariety; however, BOISSIER (1867) only listed 12. MORY (1979) categorized the genus's 22 species into two divisions depending on fruit dehiscence, morphological and anatomical properties of leaves, stems, seeds, as well as pollen grains: Four species of *G.* sect. *Acropetal* Mory has acropetal dehiscence, whereas eighteen species of *G.* sect. *Glaucium* possesses basipetal dehiscence. The genus is dispersed in a natural form of the Atlantic shores of Europe and the Canary Islands to Mongolian Altai (MORY, 1979), and ecologically, such a genus's species are discovered in both dry and open areas (KADEREIT, 1994). It was shown in Iran by eleven (CULLEN, 1966) to thirteen (MOBAYEN, 1985; GRAN and SHARIFNIA, 2008) species, five of which were indeed endemic: *Glaucium calycinum* Boiss., *Glaucium contortuplicatum* Boiss., *Glaucium mathiolifolium* Mobayen, and *Glaucium golestanicum* Gran & Sharifnia.

Glaucium flavum Cr. is considered a perennial plant that grows up to 90 cm tall. Several names for the plant include yellow horned poppy (KADEREIT, 1994; YIN *et al.*, 2021), yellow horn poppy, *G. luteum* L., and *Chelidonium Glaucium* L. (MORY, 1979). The common name "horned-poppy" refers to the plant's extremely high, bulging, and pointed capsules that sport horn-like protrusions. During June through August, the stems of *G. flavum* Cr. display yellow poppy-like flowers. From August through September, these blooms produce long-stemmed siliquiform capsules that contain seeds. According to CULLEN (1966), the yellow horned poppy could be found in the Mediterranean area, as well as throughout the Atlantic and the Black Sea coasts of Europe. Micromorphology of seeds and trichomes has been demonstrated in many taxonomic studies to be beneficial for taxonomic categorization and delineation at most levels of taxonomic and in different plant families (BARTHLOTT, 1981; KRAK and MRAZ, 2008; SALMAKI *et al*., 2009; SATIL *et al*., 2011; SALIMI MOGHADAM *et al*., 2015; TAVAKKOLI and ASSADI, 2016; ARABI *et al*., 2017; JIA *et al.*, 2020). GRAN and SHARIFNIA (2008) also examined the seed ornamentations of 14 *Glaucium* species in Iran. Light microscopy (LM) and scanning electron microscopy (SEM) were utilized to analyze the seeds and trichomes of fifteen species of the genus *Glaucium* that are scattered in Iran (TAVAKKOLI and ASSADI, 2019). Although the seeds are typically semicircular to reniform in shape, reniform and elongated reniform seeds have been discovered in *G. oxylobum* and *G. elegans*, correspondingly. Verrucate–rugulate (the most common kind), verrucate–granulate, verrucate–perforate, verrucate–lineolate, rugulate–granulate, rugulate, and ocellate are the sculpturing types of the testa surface. Their findings indicate that the micromorphological properties of seed and ovary trichomes give substantial knowledge for species and taxa inside species separation and a diagnostic key to the taxa. Their findings reveal that several of these features change across species, specifically in micromorphology and the development of clades in phylogenetic trees depending on matK and ITS3-6 DNA sequence data. The genus *Glaucium* of Turkey was separated into subsections Glabrousae and Pubescentae, relying on the results of DNA investigations backed by morphological evidence (stem trichomes). Molecular markers provide a powerful tool for studying genetic diversity. ISSR markers and morphologic traits have been used for the first time in Iran to investigate genetic variability in 107 *Glaucium flavum* accessions from 14 different populations.

MATERIALS AND METHODS

Plant materials

We employed 107 plant accessions (four to twelve samples from every population) from 14 distinct *Glaucium flavum* populations across East Azerbaijan, Tehran, Mazandaran, Guilan, and Esfahan Provinces of Iran July and August 2018 for the morphometric and ISSR analyses (Table 1). Table 1 and Fig. 1 provide further information regarding the accessions' geographical distribution 1. The plant individuals were identified morphologically using different literature (MOBAYEN, 1985; GRAN and SHARIFNIA, 2008).

Table 1. Voucher details and diversity within Iranian populations of Glaucium flavum in this study.

No	Subspecies	Locality	Latitude	Longitude	Altitude (m)
Pop1	*G. flavum* var. *serpieri* (Heldr.) Halácsy	Mazandaran, Amol to Sari	36 ˚ 52'37'	52 ˚ 23' 92'	122
Pop2	*G. flavum* var. *serpieri*	Tehran, Damavand	37°50′03″	49°24′28″	-6
Pop3	*G. flavum* var. *serpieri*	Mazandaran, Ramsar	36◦20′07″	50° 52′08″	13
Pop4	*G. flavum* var. *serpieri*	Esfahan, Najafabad	36 ˚ 52'373	54 ˚ 23' 92'	155
Pop5	*G. flavum* var. *serpieri*	Mazandaran, Behshahr	36° 57′12″	53° 57′32″	5
Pop6	*G. flavum* var. *serpieri*	Mazandaran, Chalous	36 ˚ 52'373	51 ˚ 23' 92'	180
Pop7	*G. flavum* var. *serpieri*	Tehran, Rudehen	36 ˚ 52'373	54 ˚ 23' 92'	98
Pop8	*G. flavum* var. *flavum* (Sm.) Fedde	Mazandaran, Sari	36°50′03″	53°24′28″	-3
Pop9	*G. flavum* var. *flavum*	Mazandaran, Babolsar	36°14′14″	52°18′07″	-14
Pop10	*G. flavum* var. *flavum*	Mazandaran, Amol	36◦36′93″	52°27′90″	44
Pop11	*G. flavum* var. *flavum*	Gilan, Bandar-e Anzali	37°07′02″	49°44′32″	-18
Pop12	*G. flavum* var. *flavum*	Azarbaijan, Arasbaran, Kolaleh	38◦57′22″	46°28′31″	1010
Pop13	*G. flavum* var. *flavum*	Azarbaijan, Arasbaran, Kolaleh	38◦07′24″	46° 59′06″	1108
Pop14	*G. flavum* var. *flavum*	Mazandaran, Ramsar	36◦57′22″	50°28′31″	310

ISSR analysis and DNA extraction

For each tested population, fresh leaves have been randomly selected from four to twelve samples. They were dried using silica gel. Genomic DNA was extracted using the CTAB-activated charcoal technique (ESFANDANI-BOZCHALOYI *et al*., 2019). Twenty-two primers from the (University of British Columbia) series have been evaluated for DNA amplification during

the ISSR study. Based on band repeatability, ten primers have been selected for the ISSR study of genetic variability (Table 2). Germany).

Table 2. Details about the banding pattern revealed by ISSR primers.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI
ISSR-1	DBDACACACACACACACA	26	26	100.00%	0.28	1.86
ISSR-2	GGATGGATGGATGGAT	15	13	91.00%	0.38	2.91
ISSR-3	GACAGACAGACAGACA	14	12	93.00%	0.46	5.34
ISSR-4	AGAGAGAGAGAGAGAGYT	10	10	100.00%	0.33	6.88
ISSR-5	ACACACACACACACACC	15	15	100.00%	0.25	3.23
ISSR-6	GAGAGAGAGAGAGAGARC	21	9	50.00%	0.35	4.66
ISSR-7	CTCTCTCTCTCTCTCTG	13	10	77.00%	0.44	5.21
ISSR-8	CACACACACACACACAG	13	11	92.00%	0.52	2.32
ISSR-9	GTGTGTGTGTGTGTGTYG	12	10	83.00%	0.25	3.56
ISSR-10	CACACACACACACACARG	25	22	91.00%	0.37	2.25
	Average	14	13	83.00%	0.39	3.9
	Total	156	139			

TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, PIC, polymorphism information content for each of ISSR primers

Data analysis

Morphological studies

The morphometric studies were conducted on four to twelve samples from every species. We examined 26 morphological features (**Appendix 1**). After normalizing the data (mean=0, variance=1), the Euclidean distance was calculated for clustering and ordination analysis (PODANI, 2000).

Molecular analysis

ISSR profiles were collected for every sample and evaluated as binary traits. Polymorphism information content (PIC) and marker index (MI) were utilized to assess the discriminating power of the employed primers to assess each primer's ability to discover polymorphic loci among genotypes (POWELL *et al*., 1996). The genetic differences across the populations have been demonstrated using the AMOVA (Analysis of molecular variance) test (with 1000 permutations) in GenAlex 6.4 (PEAKALL and SMOUSE, 2006) and Nei's GST analysis in GenoDive ver.2 (2013) (MEIRMANS and VAN TIENDEREN, 2004). Additionally, G(ST)est = standardized measure of genetic differentiation (HEDRICK, 2005; SI *et al*., 2021; BI *et al.,* 2021; CHENG *et al.,* 2021) and Dest = Jost measure of differentiation were used to examine population genetic differentiation (JOST, 2008). A heuristic technique relying on Bayesian clustering algorithms was employed to determine the population structure of *Glaucium flavum* accessions. On a similar data set, the clustering approach depending on the Bayesian model applied in the STRUCTURE program (PRITCHARD *et al*., 2000; FALUSH *et al*., 2007) was employed to discover

population substructures effectively. This clustering technique is based on an algorithm, which allocates genotypes to homogenous groups according to the number of clusters (K).

RESULTS

Morphometry

The morphological study indicated significant differences in flower features across the accessions. Fifty-seven of the 107 analyzed accessions were recognized as *G. flavum* var. *serpieri* and 50 as *G. flavum* var. *flavum*, depending on botanical features (Fig. 1). ANOVA indicated statistically significant variations ($P < 0.01$) among the populations investigated in quantitative morphological characteristics. The PCA analysis was performed to find the species with the most variable traits. The first three factors were shown to be responsible for more than 70% of the total variances. Corolla form, calyx shape, calyx length, bract length, and leaf shape exhibited the greatest association (>0.7) in the first PCA axis, accounting for 44 % changes. Leaf apex, corolla length, leaf length, leaf width were the traits influencing the PCA axes 2 and 3, respectively. Since the findings of various clustering and ordination techniques were comparable, the PCA plot of morphological features is provided here (Fig. 2). generally, plant samples of every population were grouped and formed into separate groups. The findings show that both quantitative and qualitative morphological characters separated *G. flavum* var. *serpieri* from *G. flavum* var. *flavum* into distinct groups. We found no intermediate types among the specimens we examined.

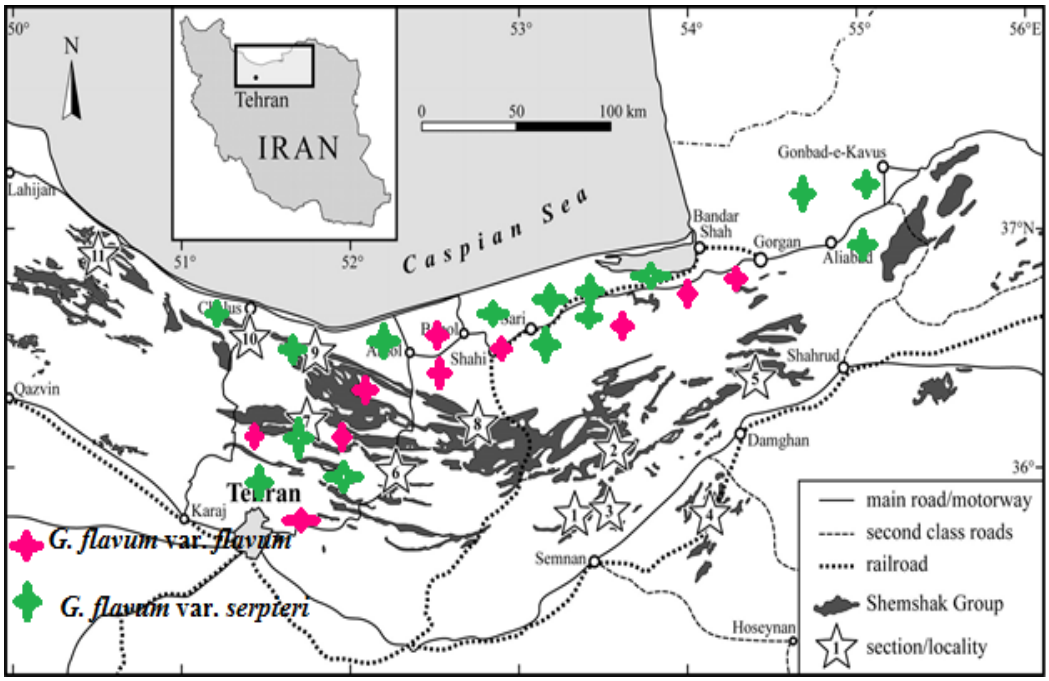

Fig. 1. Distribution map of studied populations of *Glaucium flavum* in Iran

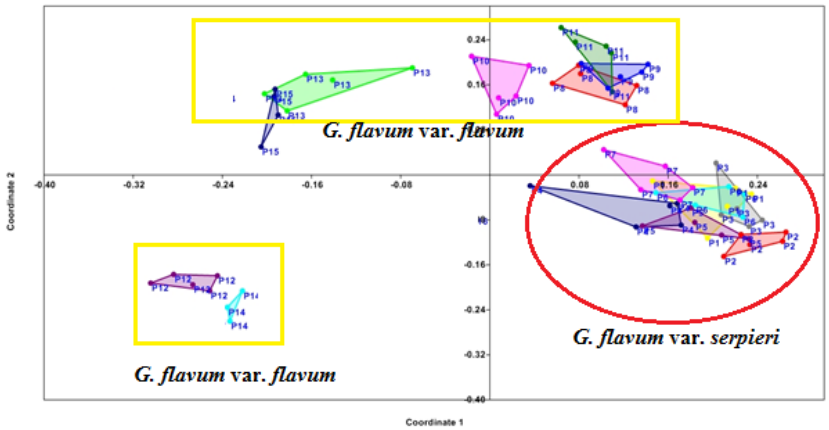

Fig. 2. PCA plot of *Glaucium flavum* populations based on morphological traits

The genetic diversity of populations

In the current research, ten of the 22 chosen ISSR primers amplified 156 identifiable bands, 139 (83 percent) of which were polymorphic, demonstrating the strong discriminative and resolving capacity of the ISSRs utilized in the examined germplasm.

Figure 3 depicts the gel electrophoresis pattern produced employing primers ISSR-9 and ISSR-3. The total number of bands per primer varied from ten (ISSR-4) to twenty-six (ISSR-1), with an average of fourteen. The overall number of bands generated by each primer ranged between ten (ISSR-4) and twenty-six (ISSR-1), with an average of fourteen. The amplified products have band diameters ranging from 100 to 3,000 bp.

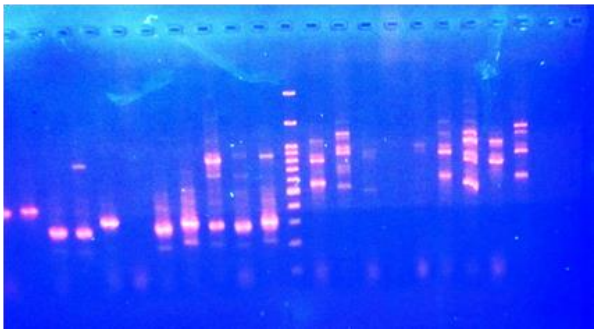

Fig.3. Electrophoresis gel of studied ecotypes from DNA fragments produced by ISSR-9 and ISSR-3 (Population numbers according to Table 1)

Various indicators, including the greatest percentage of polymorphic bands, Ne, I, and PIC, were computed to assess each primer's potential to discover polymorphism and assess the discriminating power of each primer in the examined germplasm. Primers ISSR-1, ISSR-4, and ISSR-5 generated the most significant proportion of polymorphic bands (100%), whereas primer ISSR-6 generated the most negligible percentage of polymorphic bands (0%). (50 percent). The average PIC value for all primers was 0.39. ISSR-8 had the greatest PIC value (0.52), whereas ISSR-5 and ISSR-9 had the lowest PIC values (0.25), respectively (Table 2).

Table 3. Genetic diversity parameters in the studied Glaucium flavum populations.

SP	N	Na	Ne	I	He	UHe	%P
Pop1	3.000	0.667	1.062	0.24	0.224	0.213	44.73%
Pop2	8.000	0.499	1.067	0.19	0.181	0.14	49.26%
Pop3	9.000	0.261	1.034	0.272	0.13	0.13	33.15%
Pop4	6.000	0.545	1.011	0.25	0.20	0.10	23.53%
Pop5	5.000	0.290	1.024	0.23	0.15	0.18	34.30%
Pop6	3.000	0.452	1.089	0.23	0.22	0.15	35.05%
Pop7	5.000	0.333	1.006	0.422	0.32	0.32	53.23%
Pop8	4.000	1.247	1.358	0.271	0.184	0.192	35.91%
Pop9	5.000	0.258	1.017	0.274	0.11	0.12	34.30%
Pop10	8.000	0.258	1.029	0.231	0.18	0.20	45.38%
Pop11	9.000	0.452	1.089	0.28	0.22	0.25	45.05%
Pop12	8.000	0.333	1.006	0.31	0.23	0.26	43.23%
Pop13	12.000	1.255	1.304	0.18	0.104	0.019	15.91%
Pop14	5.000	0.258	1.017	0.28	0.15	0.12	34.30%

Abbreviations: (N = number of samples, Na= number of different alleles; Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Table 3 displays the genetic variability factors observed in 14 regional populations of *Glaucium flavum*. The most significant value of polymorphism percentage (53.23%) was observed in Tehran, Rudehen (population No. 7, *G. flavum* var. *serpieri*), which illustrates a high value for the genetic variability (0.32) and Shannon's information index (0.42). The Azarbaijan, Arasbaran, Kolaleh population (No. 13, *G. flavum var. flavum*) possesses the minor percentage of polymorphism (15.91%) and the lowest values for Shannon's information index (0.18) as well as He (0.10).

The genetic distinction between populations

AMOVA demonstrated a significant difference between the examined populations (PhiPT = 0.55, P = 0.0010, Table 4.). It also indicated that 25% of overall genetic changes were related to population diversity, and 75% were due to genetic divergence across populations. The genetic resemblance (0.94) between the Mazandaran, Ramsar (pop. No. 3), and Mazandaran, Chalous populations (pop. No. 3) was found in a paired comparison of Nei's genetic identity

among the examined populations *Glaucium flavum* (Table 5.) (pop. No. 6), whereas Mazandaran, Ramsar (population No. 3) and Gilan, Bandar-e Anzali had the least genetic similarity score (0.67) (pop. No. 11).

Table 4. Analysis of molecular variance (AMOVA) of the studied Glaucium flavum populations

Source	df	SS	MS	Est. Var.	%	*ΦPT*
Among Pops	99	2201.364	92.789	61.154	75%	75%
Within Pops	332	224.443	8.905	9.905	25%	
Total	456	2355.807		70.060	100%	

df: degree of freedom; SS: sum of squared observations; MS: mean of squared observations; EV: estimated variance; *Φ*PT: proportion of the total genetic variance among individuals within an accession, ($P < 0.001$).

Table 5. Pairwise Population Matrix of Nei Unbiased Genetic Identity

pop1	pop2	pop3	pop4	pop5	pop6	pop7	pop8	pop9	pop10	pop11	pop12	pop13
1.000												
0.741	1.000											
0.802	0.828	1.000										
0.775	0.873	0.860	1.000									
0.818	0.896	0.874	0.862	1.000								
0.852	0.858	0.944	0.828	0.884	1.000							
0.712	0.846	0.800	0.796	0.881	0.794	1.000						
0.679	0.818	0.807	0.794	0.874	0.752	0.862	1.000					
0.727	0.821	0.829	0.826	0.705	0.742	0.745	0.775	1.000				
0.759	0.814	0.720	0.745	0.812	0.832	0.825	0.858	0.885	1.000			
0.743	0.838	0.679	0.738	0.787	0.768	0.773	0.798	0.754	0.842	1.000		
0.782	0.891	0.771	0.794	0.852	0.797	0.804	0.807	0.789	0.797	0.661	1.000	
0.636	0.813	0.759	0.731	0.810	0.858	0.846	0.804	0.766	0.819	0.867	0.737	1.000

The genetic affinity of populations

The UPGMA tree revealed two notable clusters (Fig. 4). Two sub-clusters were included within the first main cluster: The Gilan, Bandar-e Anzali population (population No. 11, *G. flavum* var. *flavum*) is different and is separated from the other populations for a long distance, constituting the first sub-cluster. The other populations formed the second sub-cluster from *G. flavum* var. *flavum* and *G. flavum* var. *serpieri*, which had a strong genetic affinity. The second main cluster had only *G. flavum* var. *flavum*, which diverged from the other populations studied and reconnected with the others across a long distance. The findings reveal that plant specimens from every analyzed subspecies were just not clustered, showing that ISSR molecular markers do not delineate subspecies. As a result, such an outcome does not support our

morphological findings. Furthermore, the Popgene software Nm research produced a mean Nm= 0.48, which is considered low gene flow between the investigated species. After 5000 permutations, the Mantel test indicated a significant relationship between genetic and geographical distance (r = 0.99, P = 0.001).

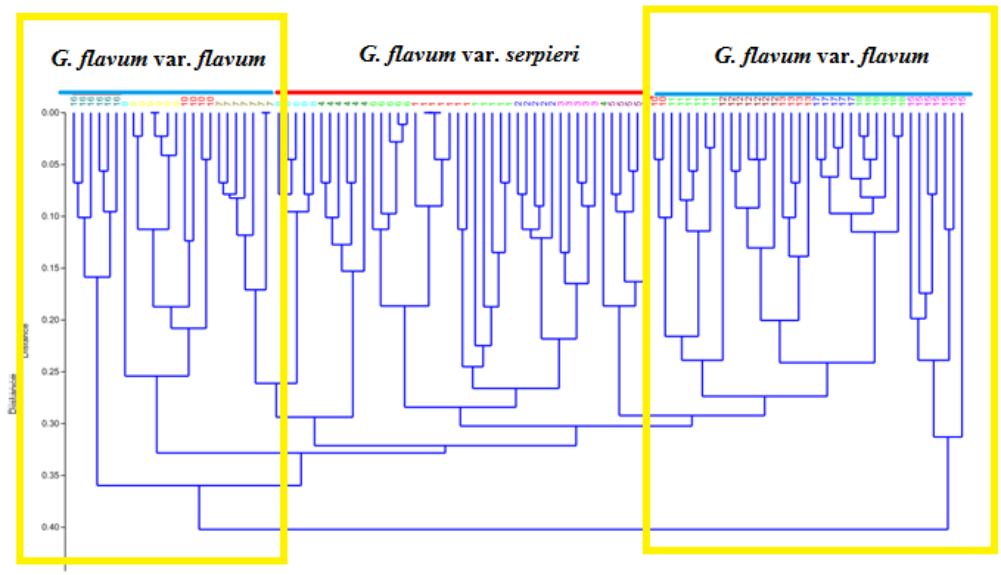

Fig. 4. UPGMA plot of populations in *Glaucium flavum* populations based on ISSR data (Population numbers according to Table 1)

Genetic structure of populations

K = 2 indicates the existence of two distinct genetic groups. Evanno test findings on STRUCTURE analysis garnered equivalent results, with a substantial peak at k = 2. (Figure 5.). Both studies demonstrated genetic stratification within *Glaucium flavum* populations. The genetic distinction in population 4-10 (red-colored) with other populations was disclosed by the STRUCTURE plot relying on k = 2 (Figure 5). However, it demonstrated genetic affinity between populations 11-14 (red) and populations 1-3 (green). Overall, the STRUCTURE analysis of the analyzed populations indicated that the plants in such populations are intermixed and did not completely delimit the investigated populations. There was a greater intermixture between *G. flavum* var. *serpieri* and *G. flavum* var. *flavum*. The mean Nm value of 0.48 was achieved for all ISSR loci, indicating modest gene flow across populations and corroboration of the genetic stratification shown by K-Means and STRUCTURE analysis. Nevertheless, the reticulogram was created using the least-square approach. (Figure not included) demonstrated that populations 3 and 8, populations 11, 9, and 10, and populations 1, 5, and 6 shared certain alleles. This result agrees with the grouping obtained with the UPGMA tree since the populations

remained clustered together. The shared alleles form a relatively small portion of the genomes in the populations, as indicated by the STRUCTURE plot depending on the admixture model. The data consistently reveal a significant degree of genetic stratification among *Glaucium flavum* populations.

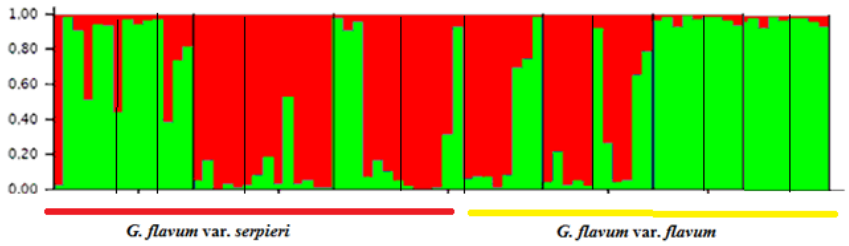

Fig. 5. STRUCTURE plot of *Glaucium flavum* populations based on k = 3 of ISSR data (Population numbers according to Table 1)

DISCUSSION

Antitussive, hypoglycemic, and hypotensive properties of *G. flavum* Cr. have previously been examined for their medicinal and pharmacological properties (PREININGER, 1986). The plant contains several medicinally useful secondary metabolites. It maintains numerous medicinal properties, making it suitable for inclusion on the International Union for Conservation of Nature's (IUCN) red list of vulnerable species (http://www.iucnredlist.org/). The current research demonstrated fascinating genetic diversity, stratification, and morphological difference in Iran's north and west. Genetic variety is vital for the survival of a species since it is utilized to effect the required adaptations to deal with environmental changes (LUBBERS *et al*., 1991). The degree of genetic variation within a species is significantly correlated with its mode of reproduction. The higher the degree of open pollination/crossbreeding, the greater the genetic variation is the examined taxon (SAEIDI *et al*., 2006). A primer's PIC and MI features help determine its efficiency in genetic variability analysis. According to SIVAPRAKASH *et al*. (2004), a marker technique's capacity for resolving genetic diversity could be directly connected to the degree of polymorphism. In general, a PIC value of 0 to 0.25 indicates extremely modest genetic variety across genotypes, a value of 0.25 to 0.50 implies mid-level genetic variability, and a value of ≥0.50 indicates significant genetic variability (TAMS *et al*., 2005). The PIC values of the ISSR primers varied from 0.25 to 0.52, with a mean value of 0.39, indicating that ISSR primers have a strong aptitude for detecting genetic variability among *Glaucium flavum* accessions.

All ten primer pairs from horned Poppy offered amplification, and *Glaucium flavum* displayed a high level of polymorphism. One hundred fifty-six alleles have been identified. The average number of alleles per locus was 13, and the total number of bands per primer varied from 9 to 26 polymorphic bands. It did not conform to the results of SAEIDI *et al*. (2006), who obtained these results: 7.3 mean and 4–12 range, and according to PESTSOVA *et al*. (2000) who obtained these results: 18.8 mean and 11–25 range, which was achieved by SSR marker. Most

research restricts the population to a few vegetative accessions (MEUSEL *et al*., 1965; UOTILA, 1996). The high FIS and low genetic variability in such a population suggest that there has been some genetic drift. The isolation of the population and absence of the gene flow led to fragmentation of the *Glaucium* populations.

Genetic diversity parameters and population size have shown positive correlations that confirmed various studies (LEIMU *et al*., 2006). The positive correlation between genetic variability and population size could be attributed to two factors (LEIMU *et al*., 2006). 1- A positive connection might indicate the occurrence of an extinction vortex, in which the decrease in population size reduces genetic variety, resulting in inbreeding depression. The second argument is that plant fitness allows populations to be distinguished by differences in habitat quality (VERGEER *et al*., 2003). According to BOOY *et al*. (2000), low levels of genetic variation may decrease plant fitness and limit a population's capacity to react to changing environmental circumstances via selection and adaptation. Twenty-five percent of genetic variation was gained within populations, while 75% of genetic variance was obtained between the examined groups. The breeding system of plant species is an important component in influencing the distribution of genetic diversity (DUMINIL, 2007). Couvet (BOOY *et al*., 2000) demonstrated that one migrant each generation is insufficient to ensure the long-term survival of relatively small populations. The number of migrants is determined by life history characteristics and population genetics (VERGEER *et al*., 2003). Genetic variances between the three groups were very similar but statistically important. There are two hypotheses for the absence of differences between isolated populations. The initial theory proposed genetic variation within and across populations demonstrates gene flow procedures that resulted in population fragmentation (DOSTÁLEK *et al*., 2010). The second hypothesis proposed that geographically close populations are more effectively linked via gene flow than populations separated by a wider distance. In conclusion, the findings of this research demonstrated the necessity to assess the genetic diversity of *Glaucium flavum*. ISSR-derived primers were more successful than other molecular markers.

ACKNOWLEDGEMENT

This is one of periodical research achievements of key project of Anhui Provincical quality engineering teaching:Exploration and Practice of “Liberal English+”Layered College English Teaching Reform. Item No.2019jyxm0481

Received, December 30th, 2020
Accepted November18th, 2021

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Appendix. Morphological characters in studied species.

No	Characters	No	Characters
1	Length of basal leaves (mm)	14	Peduncle length (mm)
2	Width of basal leaves (mm)	15	Stamen filament length (mm)
3	Length / Width of basal leaves (mm)	16	Style length (mm)
4	Number of segment basal leaves	17	Vegetation-forms
5	Length / Width of stem leaves (mm)	18	State of stem strength
6	Number of segment stem leaves (mm)	19	Petioles and Leaf hair:
7	Length of basal leaves petiole (mm)	20	Sepal hair:
8	Petal length (mm)	21	Peduncle and pedicel hair:
9	Petal width (mm)	22	Shape of segments cauline leaves :
10	Petal length / width (mm)	23	Petal shape
11	Fruit length (mm)	24	Leaf outline
12	Anthers color	25	Seed color
13	Stamen filament hair	26	Leaf tips

DIFERENCIJACIJA POPULACIJA I PROTOK GENA KOD *Glaucium flavum* (Papaveraceae)

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Izvod

Žuti mak (*Glaucium flavum* Crantz.) je zeljasta biljka sa sivo-zelenim listovima koja raste u priobalnom pesku, stenovitim predelima i jako erodiranim zemljištima do 500 metara nadmorske visine. *Glaucium flavum* je porijeklom iz severne Afrike, umerenih zona u zapadnoj Aziji i Evropi, a poreklom je iz Irana. Biljka je široko poznata po svojim izohinolinskim alkaloidima aporfinskog tipa, koji su farmakološki aktivni. Stoga smo, zbog relevantnosti biljne vrste, sproveli kombinaciju morfoloških i molekularnih analiza podataka.

Sto sedam nasumično prikupljenih biljaka iz 14 prirodnih populacija u 5 provincija je procenjeno korišćenjem ISSR markera i morfoloških osobina. Procena molekularne varijanse (AMOVA) pokazala je značajnu genetsku divergenciju između ispitivanih populacija. Pokazalo se da je 25% ukupne genetske varijabilnosti povezano sa varijabilnošću unutar populacije, dok je 75% bilo zbog međupopulacijske genetske diferencijacije. ISSR prajmeri su otkrili 156 traka, od kojih je 139 (83 %) bilo polimorfno, a svaki prajmer sadržao je u proseku 13 traka. Procenat polimorfnih opsega (PPB) (ISSR-6) varirao je od 50% do 100%. (ISSR-1, ISSR-4 i ISSR-5). Prosečan sadržaj polimorfne informacije (PIC), Šenonovi indeksi informacija (I) i nekoliko efektivnih alela (Ne) bili su 0,39, 0,26 i 1,2.

Primljeno 30.XII.2020.
Odobreno 18. XI. 2021.