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Original scientific article

GENETIC DIVERSITY ANALYSIS OF THE MEDICINAL PLANT *Heracleum persicum* Desf. ex Fischer

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Persian hogweed (*Heracleum persicum* Desf. ex Fischer) is native to Iran but was introduced to Europe as an invasive plant. It has medicinal and edible values. Its fruits have been used to relieve flatulence, stomach aches as well as a flavoring as a digestive and an antiseptic. This medicinal plant forms many geographical populations in the country, particularly in mountainous regions; however, we have no data on the genetic structure and genetic diversity of this plant species. Such information can be used in gene pool identification and future genetic conservation and breeding programs. Therefore, the present study was performed with the following aims: 1- Provide data on genetic diversity of geographical populations, 2- Identify the potential gene pools for future genetic conservation and breeding programs. We used both Inter-simple sequence repeats (ISSR) and the Start Codon Targeted (SCoT) molecular markers as these are suitable molecular markers for genetic diversity investigations. There are highly polymorphic, reproducible, and relatively low cost markers. The present study revealed that both ISSR and SCoT markers produce data on the genetic variability and genetic affinity of the local populations. Both molecular markers revealed a good level of genetic variability within and among *Heracleum persicum* populations. Analysis of molecular variance (AMOVA) produced a significant differences between geographical populations for both markers. We could identify few ISSR as well as SCoT bands which can differentiate the studied populations. The moderate to a good level of genetic diversity we observed within each *H. persicum* may be due to cross pollination. In conclusion, we suggest using combination of ISSR and SCoT molecular markers to study population genetic variability

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in *H. persicum* geographical populations for future genetic conservation and germplasm collection of this medicinal plant.

Keyword: Heracleum persicum, ISSR, SCoT, genetic variability.

INTRODUCTION

Heracleum persicum (Persian hogweed) is native to the region of Iran and grows wild in humid mountainous regions. It seems that hogweed was introduced to Europe from Iran (ALM, 2013).

Heracleum persicum is one of the medicinal species known as Golpar or Persian Hogweed plant. This species is well known as a spices and medicinal plant. Golpar fruit has been used to relieve flatulence, stomach aches as well as a flavoring as a digestive and an antiseptic. It has also antioxidant capacity as its essential oils have protective effect on the liver (ROSHANAEI *et al*., 2017).

In spite of many geographical areas in which the Persian gulper is growing, we have no information on the genetic structure and diversity of these populations. For proper use of potential gene pools of medicinal plants, a through population genetic study concerned with genetic variability of geographical populations is a major task. Data produced on genetic variability and gene pool identification may be used in future medicinal plant conservation and breeding (KOOHDAR *et al*., 2015; SHEIDAI *et al*., 2018; AZIMISHAD *et al*., 2019). Therefore, the present investigation was performed with the following aims: 1- Provide data on genetic diversity of geographical populations, 2- Identify the potential gene pools for future genetic conservation and breeding programs. This study was based on molecular genetic analyses. Since both ISSR and SCoT molecular markers are highly polymorphic, reproducible and relatively low-cost markers, we use them for genetic diversity investigations (SHEIDAI *et al*., 2018; AZIMISHAD *et al*., 2019).

MATERIAL AND METHODS

Plant materials

Ten geographical populations were identified. In total, 70 plants were randomly collected from these populations and used for molecular and morphological studies (Table 1).

Table 1. Populations studied and their locality.

	Province	Locality
1	Mazandaran	Shourab
2	Mazandaran	Emamzadeh Hashem
3	Mazandaran	Chalus
4	West Azerbaijan	Azerbaijan
5	Mazandaran	Vali Abad
6	Mazandaran	Siah Bisheh
7	Mazandaran	Polour
8	Mazandaran	Gadook
9	Tehran	Lavasan
10	Gilan	Rudbar

DNA extraction and ISSR assay

Genomic DNA was extracted using CTAB activated charcoal protocol (SHEIDAI *et al*., 2018). The quality of extracted DNA was examined by running on 0.8% agarose gel.

Ten ISSR primers were used based on SHEIDAI (2018); MOHEBI ANABAT *et al*. (2020). The PCR reaction mixture consisted of 20 ng genomic DNA and 3 U of Taq DNA polymerase (Bioron, Germany); 50 mMKCl; 10 mMTris-HCl buffer at pH;8 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 lM of each primer in a total volume of 25 pi. DNA amplification was performed on a BIO RAD (T100 Thermal cycler). with the following program: 5 min at 94°C, 40 cycles of 30s at 94°C, 1 min at 55°C and 1 min at 72 C and a final cycle of 7 min at 72 C. The PCR amplified products were separated by electrophoresis on 2% agarose gels (Merck). The gels were stained with ethidium bromide and visualized under UV light or silver stained for added sensitivity. Fragment size was estimated by using a 100 base pair (bp) molecular size ladder (Fermentas, Germany).

Four primers (SCoT 1, SCoT 2, SCoT 36, and SCoT 41) based on Collard and Mackill (2009) for monocotyledons plants were selected (Collard and Mackill, 2009). These primer sequences are: SCoT 1: CAACAATGGCTACCACCA, SCoT 2: CAACAATGGCTACCACCC, SCoT 36: GCAACAATGGCTACCACC and SCoT 41: CAATGGCTACCACTGACA.

The PCR reaction mixture consisted of 20 ng genomic DNA and 3 U of Taq DNA polymerase (Bioron, Germany); 50 mMKCl; 10 mMTris-HCl buffer at pH;8 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 lM of each primer in a total volume of 25 pi. DNA amplification was performed on a BIO RAD (T100 Thermal cycler). with the following program: 5 min at 94°C, 40 cycles of 30s at 94°C, 1 min at 49–52 ºC and 1 min at 72 C and a final cycle of 7 min at 72 C. The PCR amplified products were separated by electrophoresis on 2% agarose gels (Merck). The gels were stained with ethidium bromide and visualized under UV light or silver stained for added sensitivity. Fragment size was estimated by using a 100 base pair (bp) molecular size ladder (Fermentas, Germany).

Data analyses

SCoT and ISSR bands were scored as binary characters (presence = 1, absence = 0). Genetic diversity parameters like Nei gene diversity(He), Shanon Information Index (I), and percentage of polymorphism (%P) estimated for genetic diversity as well as AMOVA was done with GenAlex 6.4 program (PEAKALL and SMOUSE, 2006).

Discriminating power of ISSR and SCoT markers investigated by Gst analysis as implemented in POPGENE32.

The grouping of the studied populations was done by unweighted pair group method (UPGMA) and principal coordinate analysis (PCoA) (Podani, 2000) with Paleontological statistics (PAST) version 3.01 program (HAMMER *et al*., 2012).

RESULTS

ISSR analyses

We obtained 67 ISSR reproducible bands. Details of ISSR bands for each population are given in Table 2. The highest number of ISSR bands (loci) were observed in population 1 (40

bands), followed by population 7 and 10 (37 bands). A few populations had private bands (1 or 2). These bands can differentiate geographical populations from each other.

Table 2. Details of ISSR bands in the studied populations of Heracleum persicum (Populations 1-10 are according to Table 1).

Population	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
No. Bands	40	24	33	25	35	31	37	25	32	37
No. Bands Freq. >= 5%	40	24	33	25	35	31	37	25	32	37
No. Private Bands	2	0	1	0	0	1	0	0	1	1
No. LComm Bands (<=25%)	6	1	1	0	2	3	2	1	3	5
No. LComm Bands (<=50%)	15	8	11	9	12	11	14	7	12	15

Genetic diversity parameters determined based on ISSR data are presented in Table 3. The genetic polymorphism percentage varied from 22 to 47% in the studied populations. The highest level of genetic polymorphism due to ISSR bands occurred in population 1 (47%), followed by populations 7 (44.78%).

Table 3. Genetic diversity parameters determined in populations of Heracleum persicum(Populations 1-10 are according to Table 1).

Pop	%P	Na	Ne	I	He	uHe
Pop1	47.76%	1.075	1.241	0.230	0.149	0.160
Pop2	22.39%	0.582	1.110	0.105	0.068	0.073
Pop3	35.82%	0.851	1.184	0.172	0.112	0.121
Pop4	19.40%	0.567	1.141	0.113	0.078	0.084
Pop5	38.81%	0.910	1.178	0.177	0.113	0.122
Pop6	31.34%	0.776	1.203	0.170	0.115	0.124
Pop7	44.78%	1.000	1.223	0.213	0.138	0.149
Pop8	14.93%	0.522	1.098	0.085	0.057	0.062
Pop9	35.82%	0.836	1.220	0.190	0.127	0.137
Pop10	41.79%	0.970	1.267	0.230	0.155	0.167

[a] N: No of plants studied; [b] Na: No. of alleles; [c] Ne: Effective No. of alleles; [d] He: Gene diversity; [e] UHe: Unbiased gene diversity; [f] %P: Polymorphism percentage.

Most of the studied populations had almost close range of mean Nei gene diversity (He 0.1), except populations 2, 4, and 8 (<0.1).

AMOVA performed based on ISSR data, produced significant genetic difference among the studied populations (Phipt = 0.50, P = 0.001). This result indicates that, the studied populations of *Heracleum persicum* are genetically differentiated and also contain a good level of within population genetic variability.

Grouping of the populations by UPGMA dendrogram is provided in Figure 1. In general, the studied populations have been placed in two major clusters. This indicates that we have two genetically differentiated gene pools in the studied populations of *Heracleum persicum.* The populations 1, 2, 3, 4, 7, and 9 are genetically closer to each other and comprise the first major cluster. Similarly, populations 5, 6,8, and 10, form the second major cluster.

Unweighted pair group method also supports AMOVA result, as almost the members of each population formed a single cluster, separated from the other studied populations, due to their genetic difference. Mainly, the members of populations 5 and 7 were intermixed with the other populations.

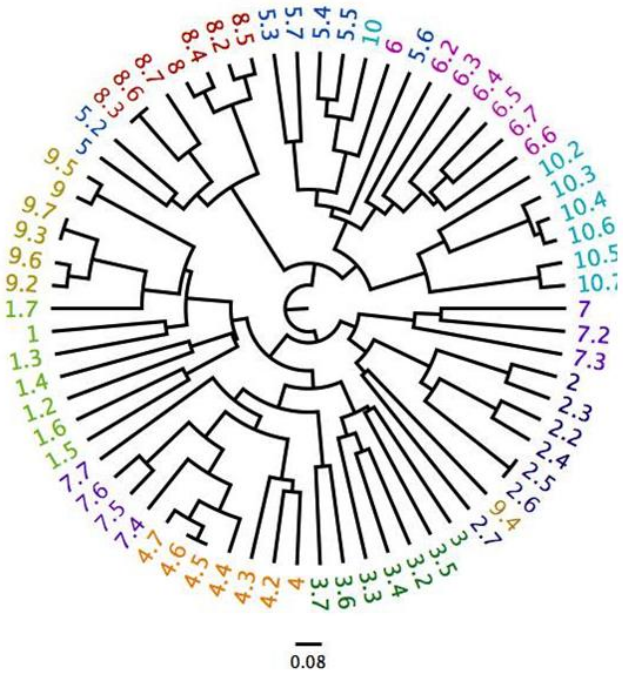

Fig.1. UPGMA dendrogram of the studied populations of *Heracleum persicum*, based on ISSR data.
(The numbers are in table1.

Nei's genetic identity versus the genetic distance of the studied populations is given in Table 4. The genetic similarity of these populations varied from 0.74 between populations 9 and 10, to 0.97 between populations 3 and 7(Table 4).

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) in Heracleum persicum based on ISSR bands (Populations 1-10 are according to Table 1).

pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.9093	0.9153	0.8743	0.8738	0.8440	0.9310	0.7818	0.9542	0.7566
2	0.0951	****	0.8856	0.8121	0.9304	0.8846	0.9077	0.8237	0.8923	0.8072
3	0.0885	0.1215	****	0.9109	0.8686	0.8231	0.9710	0.8271	0.8940	0.7518
4	0.1343	0.2082	0.0933	****	0.8082	0.7532	0.9384	0.8702	0.8398	0.6789
5	0.1349	0.0721	0.1409	0.2129	****	0.9415	0.8943	0.8744	0.8651	0.8859
6	0.1697	0.1227	0.1946	0.2835	0.0602	****	0.8322	0.8175	0.8239	0.9014
7	0.0715	0.0968	0.0294	0.0635	0.1117	0.1837	****	0.8654	0.9046	0.7557
8	0.2461	0.1940	0.1898	0.1390	0.1342	0.2014	0.1446	****	0.7572	0.7581
9	0.0469	0.1139	0.1121	0.1746	0.1449	0.1937	0.1003	0.2781	****	0.7478
10	0.2789	0.2142	0.2853	0.3873	0.1212	0.1038	0.2801	0.2770	0.2907	****

The ISSR loci obtained were screened for their discriminating power among the studied populations. ISSR loci with high Nm value (>1) have a higher degree of gene flow among populations, while loci with high Gst value (Table 5), can differentiate the studied populations.

Table 5. ISSR loci with high discriminating power (High GST value) in the studied populations of Heracleum persicum.

Locus	Sample Size	Ht	Hs	Gst	Nm*
Locus2	70	0.4772	0.0994	0.7918	0.1315
Locus4	70	0.4964	0.0851	0.8285	0.1035
Locus7	70	0.4999	0.1266	0.7468	0.1695
Locus16	70	0.2289	0.0506	0.7788	0.1420
Locus22	70	0.2179	0.0369	0.8306	0.1020
Locus27	70	0.4903	0.0536	0.8906	0.0614
Locus39	70	0.3715	0.0498	0.8661	0.0773
Locus41	70	0.1680	0.0137	0.9183	0.0445
Locus43	70	0.2179	0.0369	0.8306	0.1020
Locus54	70	0.1547	0.0262	0.8309	0.1018
Mean	70	0.2298	0.1113	0.5159	0.4692

SCoT marker analysis

The start codon targeted molecular marker study produced 72 reproducible bands. Details of SCoT bands for each population are provided in Table 6. The highest number of bands (41 bands), occurred in population 2. The highest number of private bands (5 bands) was observed in population 6, followed by population 10 with 3 private bands.

Table 6. Details of SCoT bands in the Heracleum persicum populations (Populations 1-10 are according to Table 1).

	Pop1	Pop2	Pop3	Pop4	Pop5	Pop6	Pop7	Pop8	Pop9	Pop10
No. Bands	30	41	34	25	34	34	26	22	27	26
No. Bands Freq. >= 5%	30	41	34	25	34	34	26	22	27	26
No. Private Bands	0	2	1	1	0	5	0	0	2	3
No. LComm Bands (<=25%)	1	6	6	1	2	2	1	0	1	2
No. LComm Bands (<=50%)	10	18	13	6	12	10	8	5	8	10

Genetic diversity parameters determine based on SCoT markers are given in Table 7. The studied populations had 22-43% genetic polymorphism, with the population 2 having the highest level of genetic variability. The populations with a high percentage of genetic polymorphism also had a higher value for Nei gene diversity (He = 0.1).

Table 7. Genetic diversity parameters in Heracleum persicum populations based on SCoT molecular markers (Populations 1-10 are according to Table 1).

Pop	%P	Na	Ne	I	He	uHe
Pop1	34.72%	0.764	1.187	0.169	0.111	0.119
Pop2	43.06%	1.000	1.230	0.213	0.139	0.150
Pop3	30.56%	0.778	1.138	0.135	0.086	0.093
Pop4	22.22%	0.569	1.153	0.125	0.085	0.092
Pop5	27.78%	0.750	1.170	0.148	0.099	0.106
Pop6	38.89%	0.861	1.186	0.177	0.114	0.123
Pop7	33.33%	0.694	1.196	0.170	0.113	0.122
Pop8	26.39%	0.569	1.169	0.144	0.097	0.105
Pop9	34.72%	0.722	1.224	0.187	0.127	0.137
Pop10	31.94%	0.681	1.168	0.151	0.099	0.106

Analysis of molecular variance performed based on SCoT data like ISSR data produced significant genetic differences among the studied populations (Phipt = 0.50, P = 0.001).

Based on PCoA plot, the studied populations have been placed in three major groups. The populations 7-10 are genetically similar to each other and are placed inter-mixed. They comprise the first major group.

The members of populations 1-3, are well differentiated from each other and altogether form the second major group. Similarly, populations 4-6 comprise the third group with the members of each population separated from the others. These results are in agreement with AMOVA and indicate genetic difference of the studied *Heracleum persicum* populations.

The Nei's genetic identity and genetic distance of the studied populations based on SCoT markers are provided in Table 8. The lowest value for genetic similarity in these

populations (0.78), occurred between populations 2 and 5, as well as populations 5 and 8. Similarly, the highest value for the same parameters (0.97), occurred between populations 7 and 9.

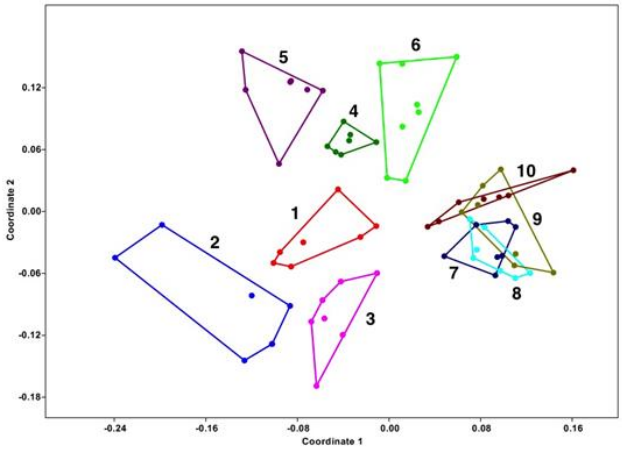

Fig. 2. PCoA plot of *Heracleum persicum* populations based on SCoT molecular data (The numbers are in table1).

Discriminating power of the SCoT markers determined by Gst analysis is provided in Table 9. The loci with high Gst score are given. These loci can differentiate the studied populations.

Table 8. Nei's genetic identity (above diagonal) and genetic distance (below diagonal), in Heracleum persicum populations based on SCoT molecular data (Populations 1-10 are according to Table 1).

pop ID	1	2	3	4	5	6	7	8	9	10
1	****	0.8984	0.9119	0.8879	0.8323	0.8889	0.9132	0.8761	0.8949	0.8971
2	0.1071	****	0.9161	0.8225	0.7845	0.8103	0.8342	0.8046	0.8321	0.8258
3	0.0922	0.0877	****	0.8195	0.7976	0.8100	0.8600	0.8277	0.8578	0.8240
4	0.1189	0.1954	0.1991	****	0.8994	0.9422	0.8679	0.8460	0.8591	0.8567
5	0.1835	0.2427	0.2261	0.1060	****	0.8603	0.8012	0.7832	0.8010	0.7848
6	0.1177	0.2103	0.2107	0.0596	0.1504	****	0.8973	0.8760	0.8998	0.8882
7	0.0908	0.1813	0.1508	0.1416	0.2216	0.1084	****	0.9811	0.9724	0.9448
8	0.1323	0.2174	0.1891	0.1672	0.2444	0.1324	0.0191	****	0.9696	0.9205
9	0.1110	0.1838	0.1534	0.1518	0.2219	0.1056	0.0280	0.0309	****	0.9552
10	0.1085	0.1915	0.1936	0.1547	0.2424	0.1186	0.0567	0.0828	0.0458	****

Table 9. Discriminating (High Gst score) SCoT loci in Heracleum persicum populations.

Locus	Sample Size	Ht	Hs	Gst	Nm*
Locus7	70	0.4982	0.0608	0.8780	0.0695
Locus23	70	0.4373	0.0399	0.9087	0.0502
Locus27	70	0.4360	0.1291	0.7038	0.2104
Locus30	70	0.3374	0.0275	0.9186	0.0443
Locus32	70	0.4805	0.1403	0.7080	0.2062
Locus37	70	0.4984	0.1429	0.7133	0.2010
Locus56	70	0.1918	0.0137	0.9284	0.0386
Locus59	70	0.4659	0.0897	0.8075	0.1192
Locus61	70	0.2146	0.0412	0.8080	0.1188
Mean	70	0.2137	0.1070	0.4991	0.5017

DISCUSSION

Identification of the gene pools for medicinal plants is of eminent importance for future conservation and breeding (SHEIDAI *et al*., 2018). Population genetic studies not only can identify the potential gene pools but can produce data on the genetic structure and degree of genetic variability in plant species (SHEIDAI *et al*., 2018). Such data are obtained from molecular investigation based on geographical populations.

The present study revealed that both ISSR and SCoT markers are produce data on the genetic variability and genetic affinity of the local populations.

Both molecular markers revealed a good level of genetic variability within and among *Heracleum persicum* populations.

RIJAL *et al*. (2015), while reconstructing the invasion history of *Heracleum persicum* into Europe, studied one of the Iranian populations (Mazandaran region) along with few European populations by using Simple sequence repeat (SSRs) molecular markers. They showed a higher level of genetic diversity in Iranian population compared with the European population. Moreover, PCoA ordination of microsatellites showed that the Iranian, Danish and Norwegian populations differ from each other. The population of Britain, Finland, Latvia and Sweden were among the former populations.

We could identify few ISSR as well as SCoT bands which can differentiate the studied populations. Usually, these private bands occur due to geographical populations, genetic differentiation (local mutations), which may be of adaptive nature (SHEIDAI *et al*., 2018).

Heracleum persicum is probably protandrous because it is a common feature in the Apiaceae family and has been reported for *H. Mantegazzianum* (PERGLOVÁ *et al*., 2006; PERGLOVÁ et al., 2007) a species that is often confused with *H. persicum*. It may be self-compatible, but cross-pollination seems just as likely if *H. persicum* is protandrous (PERGLOVÁ *et al*., 2006; PERGLOVÁ *et al*., 2007). Therefore, a moderate to good level of genetic diversity was observed in each *H. persicum* may be due to cross pollination.

Hybridization may occur between *H. persicum* and *H. mantegazzianum* since intermediate plants were observed which shared the characteristics of both *H. persicum* and *H. mantegazzianum* (ELVEN, 2005; FRÖBERG, 2010).

CONCLUSION

In conclusion we suggest using combination of ISSR and SCoT molecular markers to study population genetic variability in *H. persicum* geographical populations for future genetic conservation and germplasm collection of this medicinal plant.

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ANALIZA GENETIČKOG DIVERZITETA LEKOVITE BILJKE *Heracleum persicum* Desf. ex Fischer

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Izvod

Biljka *Heracleum persicum* Desf. Ek Fischer je poreklom iz Irana, ali je u Evropu uvedena kao invazivna biljka. Ima lekovite i jestive vrednosti. Njegovi plodovi su korišćeni za ublažavanje nadimanja, bolova u stomaku, kao i za aromatizaciju kao probavni i antiseptički sastojak. Ova lekovita biljka formira mnoge geografske populacije u zemlji, posebno u planinskim regionima; međutim, nemamo podataka o genetskoj strukturi i genetskoj raznolikosti ove biljne vrste. Takve informacije mogu se koristiti u identifikaciji genskog fonda i budućim programima za očuvanje i uzgoj genetike. Stoga je ovo istraživanje izvedeno sa sledećim ciljevima: 1- Obezbediti podatke o genetskoj raznolikosti geografskih populacija, 2- Identifikovati potencijalne genske *pool*-ove za buduće programe genetskog konyervacije i oplemenjvanja. Koristili smo ISSR i molekularne markere *Start Codon target* (SCoT), jer su oni pogodni markeri za ispitivanje genetičkog diverziteta. Oni su visoko polimorfni, ponovljivi i relativno jeftini markeri. Ova studija je otkrila da i ISSR i SCoT markeri daju podatke o genetskoj promenljivosti i genetskom afinitetu lokalnih populacija. Oba molekularna markera otkrila su dobar nivo genetske varijabilnosti unutar i među populacijama *Heracleum persicum*. Analiza molekularne varijanse (AMOVA) proizvela je značajne razlike između geografskih populacija za oba markera. Mogli bismo da identifikujemo nekoliko ISSR kao i SCoT opsega koji mogu razlikovati proučavane populacije. Umereni do dobar nivo genetske raznolikosti koji smo primetili u svakoj *H. persicum* može biti posledica unakrsnog oprašivanja. U zaključku predlažemo upotrebu kombinacije ISSR i SCoT molekularnih markera za proučavanje genetske varijabilnosti populacije u geografskim populacijama *H. persicum* za buduće genetičko konzerviranje i kolekcionisanje germplazme ove lekovite biljke.

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