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ARTICLE

Decoding the Genetic Diversity and Population Dynamics of *Phalaris minor* Through ISSR and SCoT Markers

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ABSTRACT *Phalaris minor* is a noxious weed that thrives in wet soils and swamps across various habitats in Iran. Inter Simple Sequence Repeat (ISSR) markers are effective tools for assessing both intra- and inter-species genetic variations. The relationship between geographical distribution and ecological adaptation in this genus has long been debated. This study aimed to investigate the genetic diversity of *P. minor* populations in Iran using ISSR and Start Codon Targeted (SCoT) markers to assess genetic variation within and between populations of this taxon. A total of 11 populations of *P. minor* were sampled. Genomic DNA was extracted from dried leaf samples using a modified Cetyl Trimethyl-Ammonium Bromide (CTAB) protocol. Seven ISSR and two SCoT primers, selected for their reliability and polymorphism, were employed. Genetic diversity indices, the correlation between genetic and geographical distances, and genetic differentiation within and among populations were analyzed. A high level of genetic diversity was observed in *P. minor* populations. The highest genetic diversity (61%) was found within populations, while genetic diversity between populations accounted for 39%. Genetic distance increased with geographical distance. The observed genetic diversity in *P. minor* may result from genetic drift, gene flow, and adaptation to environmental conditions.

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Introduction

Phalaris (Phalaridinae, Poeae, Poaceae) comprises 21 annual and perennial species distributed across both hemispheres (Voshell et al. 2011). These species are widely found in temperate and subtropical regions worldwide, including mountainous areas of Africa and South America (Voshell et al. 2011). The genus originated in the Mediterranean Basin and later spread to the New World through a secondary center of diversity in western North America (Voshell and Hilu 2014).

Species of *Phalaris* serve multiple purposes, including livestock and poultry fodder, soil erosion control, sand stabilization, wetland restoration, and biofuel production. Additionally, some species are cultivated as ornamental plants in green spaces (Anderson 1961; Baldini 1995; Kávová et al. 2018; Graper et al. 2021). *Phalaris* thrives in diverse habitats, ranging from natural ecosystems to disturbed areas, typically at low elevations in open fields, sandy soils, abandoned lands, and waste beds (Baldini 1995).

In Iran, the genus *Phalaris* includes four native and two introduced species: *Phalaris arundinacea* L., *Phalaris brachystachys* Link, *Phalaris canariensis* L., *Phalaris minor* Retz., *Phalaris paradoxa* L., and *Phalaris aquatica* L. (Bor 1970; POWO 2023). Among them, *P. minor* is a noxious weed, particularly in *Triticum* and *Hordeum* cultivations, and predominantly grows in wet soils and swamps within the Iranotournian region (Keshavarzi et al. 2007; Gherekhloo et al. 2011).

The taxonomy of *Phalaris* has long been considered complex (Baldini 1995). According to Baldini (1993), species within this genus exhibit two ploidy levels and can be classified into three diploid groups with $x = 6$ and $x = 7$, along with a tetraploid group. *P. minor* belongs to the tetraploid group. Nuclear Internal transcribed spacer (ITS) and *trnT-F* marker analyses by Voshell et al. (2011) revealed the presence of two distinct clades in the genus, highlighting correlations between geographic distribution and diploid-polyploid groups.

Molecular markers have been instrumental in assessing genetic diversity within *P.* species. Inter Simple Sequence Repeat (ISSR) markers have been effectively used to analyze intra- and inter-species similarities in *P. minor* in India (McRoberts et al. 2005). Similarly, SSR markers have been used to evaluate the genetic diversity of *P. arundinacea* cultivars (Jakubowski et al. 2011), revealing high genetic variation in European populations, suggesting natural or human-mediated germplasm distribution

Table 1. Geographical information and voucher numbers of the sampled populations of *P. minor*

Code	Population	Latitude and Longitude	Voucher no.	No. of individuals studied
Pop1	Lorestan, Khorramabad, 1319 m., Keshavarzi	N33°29´12", E48°20´25"	ALUH-83m7	10
Pop2	Tehran, Vanak Village, 1490 m., Keshavarzi	N35°46´00", E51°23´28"	ALUH-83m19	11
Pop3	Yazd, Meybod, 1102 m., Keshavarzi	N32°13´54", E54°00´05"	ALUH-83m14	10
Pop4	Khuzestan, Izeh, 847 m., Keshavarzi	N31°48´59", E49°52´07"	ALUH-83m20	10
Pop5	Fars, Kazerun, Baghe Nazar, 830 m., Keshavarzi	N29°37´14", E51°37´58"	ALUH-84m1	10
Pop6	Sistan and Baluchestan, Hirmand, Dehe Mirza Ali, 482 m., Ijbari and Keshavarzi	N31°03´30", E61°47´39"	ALUH-83m25	10
Pop7	Fars, Shiraz, 1665 m., Keshavarzi	N29°38´51", E52°30´32"	ALUH-84m10	10
Pop8	Fars, Sarvestan, Sasanian Palace, 1546 m., Keshavarzi	N29°11´44", E53°13´51"	ALUH-84m8	10
Pop9	Fars, Maharloo Lake, 1463 m., Keshavarzi	N29°31´47", E52°47´21"	ALUH-83m12	8
Pop10	Gilan, Rasht, Mother Blvd., 2 m., Oshib Nataj	N37°16´35", E49°34´05"	ALUH-84m11	7
Pop11	Gilan, Rasht to Tehran Road, Saravan, 80 m., Oshib Nataj	N37°04´02", E49°39´12"	ALUH-83m16	7

across Eurasia. Furthermore, ISSR markers applied to *Phalaris* species confirmed significant inter-populational genetic diversity (Nelson et al. 2014).

The genus *Phalaris* presents a valuable model for studying chromosomal evolution, speciation, hybridization, and gene flow due to its combination of endemic and non-native species, morphological variability, diverse chromosome numbers, and widespread distribution. However, the relationship between geographical distribution and ecological adaptation in *Phalaris* remains debated.

Effective weed management and mitigation of invasive impacts require a comprehensive understanding of genetic diversity, gene flow, and ecosystem sensitivity. Investigating the genetic structure of weed populations can provide sustainable, long-term strategies for their control. Despite the ecological and agricultural significance of *P. minor* in Iran, no studies have been conducted on its genetic diversity within the country.

This study aimed to assess the genetic diversity of P. minor populations in Iran using ISSR and Start Codon Targeted (SCoT) markers. Specifically, we seek to determine the number of genetic pools in Iran, quantify genetic differentiation within and among populations, and test whether genetic distance correlates with geographic distance.

Materials and methods

Plant material

This study investigated 11 populations (103 individuals) of *Phalaris minor* from various habitats across Iran (Fig. 1 and Table 1). Herbarium specimens from the Alzahra University Herbarium (ALUH) were utilized for this study. To ensure accurate species identification, all populations were examined using standard taxonomic references, including Flora Iranica (Bor 1970) and Flora of Turkey (Davis 1985). After verification, suitable specimens were

Table 2. Properties of primers used

Primer	Sequence	BR	PB	MB	PPB	PIC	MI
UBC 807	(AG)8T	100-1300	11	0	100	0.338583	0.001224
UBC 811	(GA)8C	100-800	8	0	100	0.384303	0.000502
UBC 841	(GA)8YT	100-1000	10	0	100	0.340256	0.001192
UBC (GA)9C	(GA)9C	100-1300	13	0	100	0.359243	0.000866
UBC (GA)9T	(GA)9T	200-1200	10	0	100	0.337315	0.001248
UBC (AGC)5GG	(AGC)5GG	50-1500	17	0	100	0.355805	0.000921
UBC (AGC)5GT	(AGC)5GT	100-1600	16	0	100	0.301960	0.002485
SCOT22	CCATGGCTACCACCGCAC	50-1500	17	0	100	0.365556	0.000769
SCOT24	CCATGGCTACCACCGCAG	50-1500	16	0	100	0.313584	0.001803

(BR: band range, PB: polymorphic bands, MB: monomorphic bands, PPB: percentage of polymorphic bands, PIC: polymorphism information content, MI: marker index)

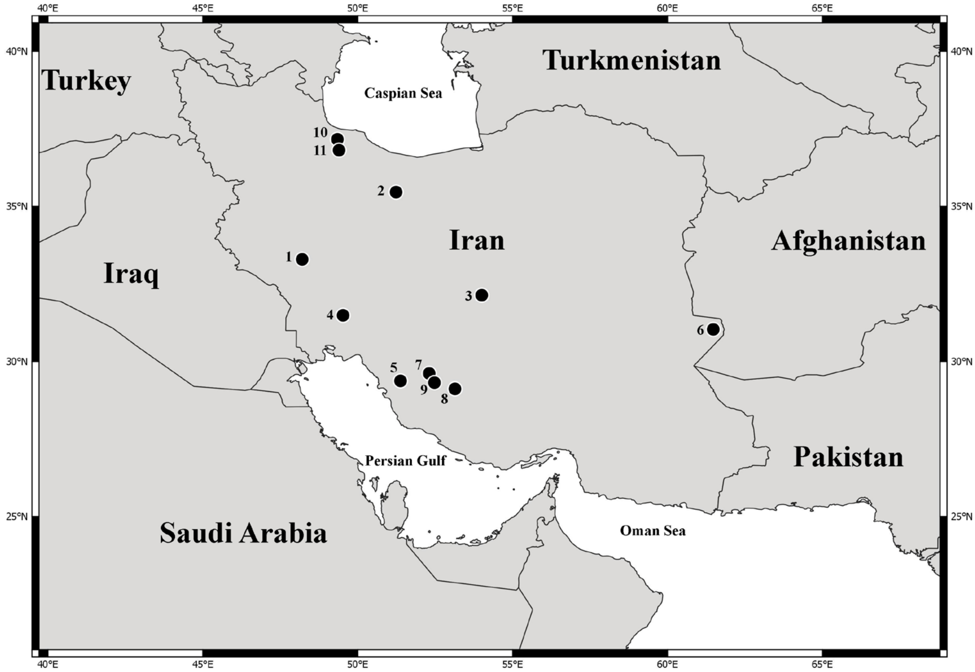


Figure 1. Geographical distribution of *P. minor* studied populations.

selected from each population, resulting in a total of 103 individuals being analyzed. Habitat characteristics, herbarium accession numbers, and the number of individuals per population are presented in Table 1.

DNA extraction

Genomic DNA was extracted from dried leaf samples using a modified CTAB (Cetyl Trimethyl-Ammonium Bromide) protocol (Murray and Thompson 1980). In this study, 16 ISSR and 9 SCoT (Start-Codon Targeted) primers were initially tested, and ultimately, 7 ISSR and 2 SCoT primers with reliable and polymorphic fragments were selected for analysis (Table 2). These primers were not specifically designed for *Phalaris* species but were commercially developed by the University of British Columbia. The quality of the extracted DNA was evaluated using 0.7% agarose gel electrophoresis.

ISSR and SCoT Assay

The amplification reaction was carried out in a total volume of 25 µl, consisting of 1 µl template DNA (20–50 ng), 12 µl dH_2O, 10 µl 2X Master Mix containing *Taq* polymerase, 1 µl DMSO, and 1 µl primer (100 pM/mL).

Polymerase Chain Reaction (PCR) amplification for ISSR markers was performed using a SensoQuest thermal cycler (SensoQuest GmbH, Göttingen, Germany) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. A final extension step was carried out at 72 °C for 7 min.

For SCoT markers, PCR was conducted under a similar thermal cycling program, with slight modifications: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 53–54 °C for 1 min, and extension at 72 °C for 1 min 30 sec. A final extension step was performed at 72 °C for 7 min. PCR products of both markers were stained with DNA Green Viewer and analyzed using 1% agarose gel electrophoresis alongside a 100 bp DNA ladder (Thermo).

Data analysis

ISSR and SCoT bands were scored as either present (1) or absent (0) based on their patterns observed on agarose gels. Genetic diversity indices were calculated using GenAlEx ver. 6.501 (Peakall and Smouse 2006). The polymorphism information content (PIC) and marker index (MI) for each primer were computed using the iMEC: Online Marker Efficiency Calculator (Amiryousefi et al. 2018).

The correlation between genetic distance and geographical distance was assessed for all populations using the Mantel test with 999 permutations in GenAlEx. Genetic differentiation within and among populations was determined through Analysis of Molecular Variance (AMOVA) with 1000 permutations.

Population structure analysis was performed using STRUCTURE ver. 2.3.4 (Pritchard et al. 2000) to assess genetic admixture. Simulations were conducted for K values (number of genetic clusters) ranging from 1 to 18, with five independent replicates per run. The Burn-in

Table 3. Performance parameters for ISSR and SCoT markers used in this study

Primers	H	PIC	E	H.av	MI	D	R
UBC 807	0.420071	0.338583	3.300971	0.000371	0.001224	0.910133	5.184466
UBC 811	0.29158	0.384303	1.417476	0.000354	0.000502	0.968783	0.873786
UBC 841	0.416069	0.340256	2.951456	0.000404	0.001192	0.913091	4.640777
UBC (GA)9C	0.367612	0.359243	3.15534	0.000275	0.000866	0.941225	4.640777
UBC (GA)9T	0.423077	0.337315	3.038835	0.000411	0.001248	0.90786	4.912621
UBC (AGC)5GG	0.376849	0.355805	4.281553	0.000215	0.000921	0.936676	7.961165
UBC (AGC)5GT	0.499705	0.30196	8.194175	0.000303	0.002485	0.737868	6.097087
SCOT 22	0.350019	0.365556	3.84466	0.0002	0.000769	0.948953	7.68932
SCOT 24	0.475875	0.313584	6.242718	0.000289	0.001803	0.847912	11.80583

H: Expected heterozygosity, PIC: Polymorphism information content, E: Effective multiplex ratio, H. av: Mean heterozygosity, MI: Marker index, D: Discriminating power and R: Resolving power.

period length was set to 10,000 iterations, and the Markov Chain Monte Carlo (MCMC) chain length was set to 100,000 iterations. The results from the five independent replicates for each K were integrated and summarized by averaging the Q-Matrices. The results were analyzed using Structure Harvester (Earl and von Holdt 2012), and the optimal K value was determined using the Evanno method (Evanno et al. 2005).

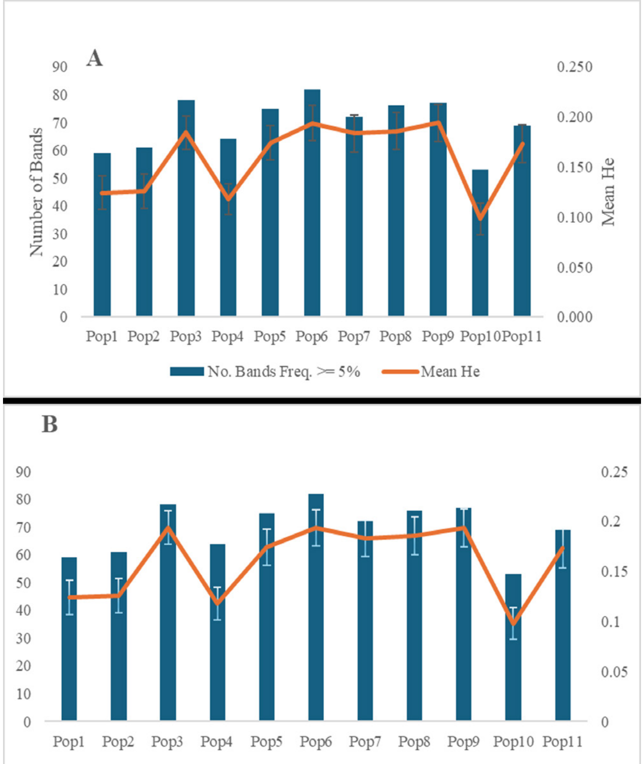


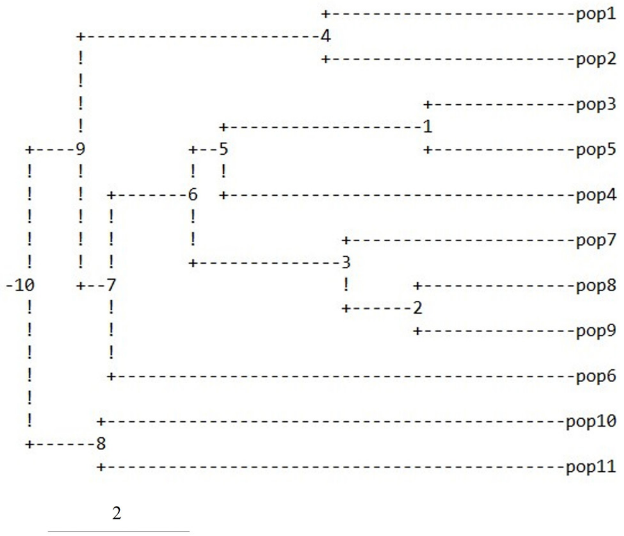
Figure 2. Genetic diversity parameters across eleven populations of P. minor. (A) Analysis of band distribution and heterozygosity; blue bars represent the number of bands with a frequency ≥5%, while the orange line indicates the mean expected heterozygosity (He). (B) Comparison of population-specific genetic indices. In both panels, error bars represent the standard error (SE) of the mean He.

A Neighbor-Joining (NJ) tree was constructed in PopGene ver. 1.32 (Yeh and Boyle 1997) based on Nei's genetic distance. Gene flow ($Nm = 0.5\ (1 - GST)/GST$) and Nei's genetic identity and distance (Nei 1978) were estimated using PHYLIP ver. 3.69 (Felsenstein 1989). Bootstrap analysis was conducted to assess the robustness of the phylogenetic tree, generating at least 100 trees to form a consensus tree (Huson and Bryant 2006).

The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) clustering method was used to evaluate genetic affinities among *P. minor* populations, implemented in POPGENE ver. 1.32 (Yeh et al. 1999). The UPGMA dendrogram was constructed using the Gower's genetic distance matrix, which was calculated based on the ISSR and SCoT marker data. To account for the potential limitations of ISSR and SCoT markers and to assess significant genetic differentiation among populations, Hickory ver. 1 was employed (Holsinger and Lewis 2003).

Reticulation analysis was performed using DARwin ver. 5 (2012) to examine gene exchange among populations. A phylogenetic tree was initially constructed using the NJ method, followed by an optimization algorithm that minimizes least-squares errors at each step (Makarenkov and Lapointe 2004).

Additionally, Principal Coordinate Analysis (PCoA) was conducted in GenAlEx. The PCoA analysis was performed based on the Gower's genetic distance matrix. To examine the correlation between ISSR markers and environmental variables (longitude, latitude, and elevation), a Latent Factor Mixed Model (LFMM) was applied using LFMM ver. 1.2 (Frichot et al. 2013).



```
                              +------------------------pop1
       +----------------------4
       !                      +------------------------pop2
       !
       !                                +--------------pop3
       !             +------------------1
  +----9          +--5                  +--------------pop5
  !    !          !  !
  !    !  +-------6  +---------------------------------pop4
  !    !  !       !
  !    !  !       !             +----------------------pop7
  !    !  !       +-------------3
-10    +--7                     !      +---------------pop8
  !       !                     +------2
  !       !                            +---------------pop9
  !       !
  !       +--------------------------------------------pop6
  !
  !      +--------------------------------------------pop10
  +------8
         +--------------------------------------------pop11

          2
```

Figure 3. UPGMA dendrogram of *P. minor* populations based on Nei's genetic distance (ISSR and SCoT markers). Distance scale (2 units) is shown.

Results

Genetic diversity

A total of 118 bands were generated from the nine selected primers, all of which were polymorphic. The fragment sizes ranged from 50 to 1600 bp. Among the ISSR primers, UBC $(AGC)_5GG$ produced the highest number of fragments (17 bands), while UBC 811 yielded the lowest (8 bands). The highest number of polymorphic bands was also observed for UBC $(AGC)_5GG$, whereas UBC 811 exhibited the lowest. None of the ISSR primers produced unique or species-specific bands. The Polymorphism Information Content (PIC) of the examined primers ranged from 0.3019 for UBC $(AGC)_5GT$ to 0.3843 for UBC 811, with an average PIC value of 0.3453 (Table 3).

The Mantel test revealed a significant correlation between geographical distance and genetic distance among the studied populations ($r = 0.2293$, $P = 0.0002$). This finding suggests isolation by distance, meaning that as the geographical distance between populations increases, the genetic similarity decreases, limiting gene flow among distant populations.

For ISSR primers, the highest genetic diversity index (*H*) was recorded for UBC $(AGC)_5GT$, whereas UBC 811 exhibited the lowest value. The highest evenness index (*E*) was observed for UBC $(AGC)_5GT$, while the lowest was for UBC 811. The UBC $(GA)_9T$ primer had the highest average heterozygosity (*H.av*), whereas UBC $(AGC)_5GG$ displayed the lowest value. The highest differentiation index (*D*) was associated with UBC 811, while the lowest was found in UBC $(AGC)_5GT$. The highest resolving power (*R*) was observed for UBC $(AGC)_5GG$, while UBC 811 exhibited the lowest (Table 3).

Among the SCoT primers, SCoT22 generated the highest number of bands (17), with the most polymorphic fragments. Similar to ISSR primers, none of the SCoT primers produced species-specific bands (Table 2). The PIC values of the SCoT primers ranged from 0.3135 for SCoT 24 to 0.3655 for SCoT 22, with an average PIC value of 0.3395. Additional performance parameters for the SCoT primers are summarized in Table 3.

Genetic diversity parameters for the studied populations are presented in Table 4. The highest percentage of polymorphism was observed in the Meybod population

Table 4. Genetic diversity parameters in the studied P. minor populations

Population	N	Na	Ne	I	He	uHe	P
		Mean	Mean	Mean	Mean	Mean	
Khorramabad	10	0.890	1.211	0.188	0.124	0.131	38.98%
Vanak Village	11	0.915	1.213	0.191	0.126	0.132	39.83%
Meybod	10	1.305	1.300	0.287	0.184	0.194	64.41%
Izeh	10	0.941	1.190	0.182	0.118	0.124	39.83%
Kazerun	10	1.229	1.284	0.270	0.174	0.183	59.32%
Dehe Mirza Ali	10	1.305	1.327	0.295	0.194	0.204	61.02%
Shiraz	10	1.153	1.311	0.276	0.183	0.193	54.24%
Sasanian Palace	10	1.203	1.321	0.279	0.186	0.195	55.93%
Maharloo Lake	8	1.212	1.330	0.291	0.194	0.207	55.93%
Rasht	7	0.746	1.167	0.148	0.098	0.106	29.66%
Saravan	7	1.051	1.301	0.257	0.173	0.186	46.61%

Abbreviations: N = number of individuals, Na = No. alleles, Ne = No. of effective alleles, I = Shannon's information index, He = expected heterozygosity, uHe = Unbiased expected heterozygosity, %P = Genetic polymorphism.

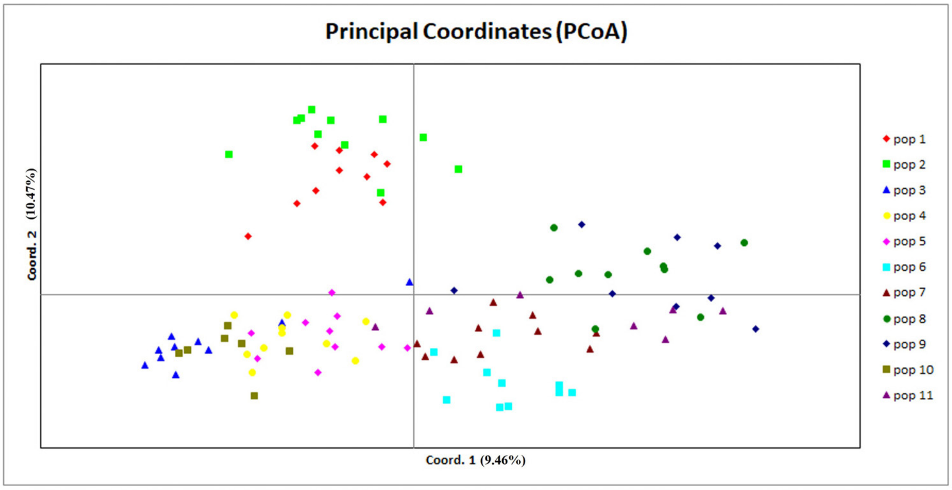


Figure 4. The PCoA diagram of the studied populations of *P. minor* based on molecular data.

(64.41%), while the lowest was recorded for the Rasht population (29.66%) (Table 4). The number of different alleles (*Na*) ranged from 0.746 to 1.305, with the highest values in the Meybod and Deh Mirza Ali populations (1.305) and the lowest in the Rasht population (0.746). The number of effective alleles (*Ne*) varied between 1.167 and 1.330, with the highest in the Maharloo Lake population (1.330) and the lowest in the Rasht population (1.167).

Shannon's information index (*I*) ranged from 0.295 in the Deh Mirza Ali population to 0.148 in the Rasht population. Expected heterozygosity (*He*) was highest in the Deh Mirza Ali and Maharloo Lake populations (0.194) and lowest in the Rasht population (0.098). Unbiased expected heterozygosity (*UHe*) followed the same pattern, with the highest value in the Maharloo Lake population (0.207) and the lowest in the Rasht population (0.106).

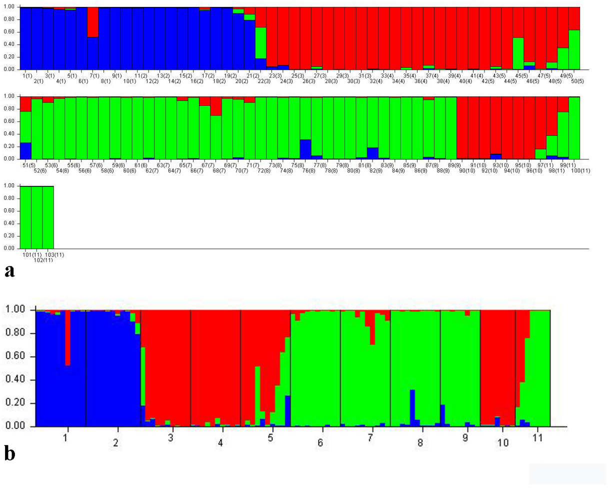


Figure 5. The population structure profile of *P. minor* accessions: based on the ISSR marker data set for K=3. Structure of a) individuals, b) populations studied STRUCTURE analysis for K=3 with burn-in = 10000 iterations and MCMC = 100000 iterations.

The highest number of bands was observed in the Deh Mirza Ali population, while the Rasht population exhibited the lowest. Specific bands were detected only in the Saravan, Deh Mirza Ali, and Meybod populations. Among them, the highest number of specific bands was recorded in the Deh Mirza Ali population, whereas the Saravan population had the lowest (Fig. 2).

The AMOVA analysis (Table 5) revealed that 39% of the total genetic variation was attributed to differences among populations, while 61% occurred within populations. According to Nei's genetic index, the highest genetic similarity was observed between the Kazerun (Pop5) and Meybod (Pop3) populations. In contrast, the lowest genetic similarity was recorded between the Rasht (Pop10) and Deh Mirza Ali (Pop6) populations.

Population genetic structure

The UPGMA tree based on Nei's genetic distance is presented in Fig. 3, illustrating the genetic similarity among populations. This tree consists of two main clusters: the Rasht and Saravan populations form the first cluster, while the remaining populations belong to the second cluster. Within the second cluster, the populations from the Sasanian Palace and Maharloo Lake are grouped, indicating a higher degree of genetic similarity.

The PCoA diagram derived from molecular data largely confirms the results of the UPGMA tree (Fig. 4). The Khorramabad and Vanak Village populations are

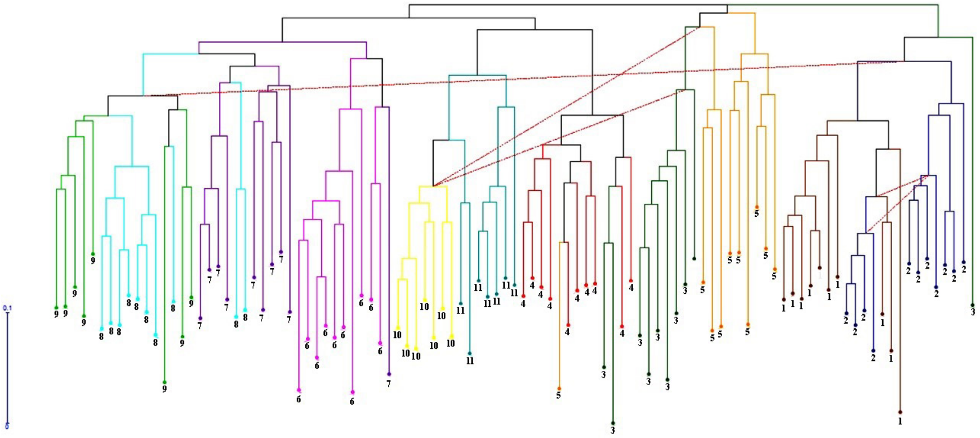


Figure 6. Neighbor-joining tree of the populations of *P. minor* in this study.

positioned close to each other. Similarly, the Sassanian Palace and Maharloo Lake populations form a distinct group, while the Shiraz population is more distant.

To determine the optimal genetic clustering, a preliminary test was conducted using the STRUCTURE program, followed by the Evanno test to identify the best K value with the aid of the STRUCTURE Harvester website. The results of the Evanno test indicate that K = 3 has the highest ΔK, suggesting that K = 3 represents the most suitable genetic grouping among the studied populations.

STRUCTURE analysis revealed three distinct genetic assemblages within P. minor populations (Fig. 5). The highest level of genetic admixture was observed in the Kazerun population, whereas the Khorramabad and Vanak Village populations exhibited lower genetic variation. Notably, genetic similarities were detected between the Dehe Mirza Ali and Shiraz populations, as well as between the Sasanian Palace and Maharloo Lake populations. To provide a comprehensive understanding of our data structure, we present the results for K = 3 (Fig. 5). The STRUCTURE results were consistent with the findings of the UPGMA tree.

The Neighbor-Joining tree was constructed to explore the relationships among populations and individuals under study. Additionally, reticulation analysis was performed to visualize gene flow between different individuals. The results indicate the presence of gene flow among individuals of the studied accessions (Fig. 6), which aligns with the findings of the STRUCTURE analysis (Fig. 5).

LFMM analysis identified 10 out of 118 ISSR loci as locally adaptive. While some of these loci exhibited low Nm values, others had high Nm values (>1.5) and were more frequently shared among the studied populations.

Table 5. Results of analysis of molecular variance (AMOVA)

Source	df	ss	MS	Est. Var.	Total
Among populations	10	804.279	80.428	7.362	39%
Within populations	92	1069.565	11.626	11.626	61%
Total	102	1873.845		18.988	100%

df: degree of freedom, ss: sum of squares, MS: mean square, Est. Var.: estimated variation

Gene flow

We employed various indirect approaches to assess the level of gene flow and ancestral shared alleles in *P. minor* populations. The Nm approach assumes an equal rate of gene flow among populations, which may not always hold. Our analysis yielded a mean Nm value of 0.786, which, being lower than 1, suggests a moderate degree of gene flow among populations. This finding is consistent with the results of the STRUCTURE analysis. Among the 118 ISSR loci examined, most displayed high Nm values (>1.00).

Discussion

Phalaris minor is a noxious weed that significantly affects various crops, particularly wheat fields. Given the limitations of cultural and chemical weed control methods, understanding its biology concerning environmental, edaphic, and management factors is crucial for enhancing weed management strategies (Om et al. 2004). Genetic diversity plays a key role in plant adaptation and resistance to adverse environmental conditions (Caliskan 2012). Therefore, effective weed management and mitigation of its invasive effects require comprehensive knowledge of genetic diversity, gene flow, and ecosystem sensitivity.

Genetic diversity parameters such as polymorphism information content (PIC), marker index (MI), and resolution are essential indicators for assessing marker efficiency. In this study, the ISSR primers exhibited PIC values ranging from 0.30196 to 0.384303, with UBC 811 showing the highest value, indicating its superior ability to differentiate genetic distances among samples. Conversely, UBC $(AGC)_5GT$ had the lowest PIC value.

The MI values for ISSR primers ranged from 0.000502 to 0.002485, with the highest MI observed in UBC $(AGC)_5GT$, demonstrating its high band-production potential, while UBC 811 had the lowest MI. The resolving power (R) of ISSR primers varied between 0.873786 and 7.961165, with UBC $(AGC)_5GG$ exhibiting the highest value and UBC 811 the lowest. These findings confirm that ISSR markers are valuable for assessing the genetic diversity of *P. minor* populations (McRoberts et al. 2005).

The calculated average expected heterozygosity (Hav) values observed across our populations (ranging from 0.000200 to 0.000411) are notably low compared to those typically reported in outcrossing plant species. This finding can be attributed to two main factors inherent to this study. Firstly, the use of dominant markers (ISSR and SCoT) inherently leads to a more conservative, lower estimate of heterozygosity because these markers cannot distinguish between heterozygous and dominant homozygous individuals (Holsinger and Lewis 2003). Secondly, the low diversity reflects the breeding system of *P. minor*. As a predominantly self-pollinating (selfing) species (Keshavarzi et al. 2007; Gherekhloo et al. 2011), *P. minor* is expected to exhibit high levels of homozygosity due to continuous inbreeding, resulting in suppressed Hav values. This result is consistent with genetic diversity trends observed in other self-pollinating weeds and grasses (McRoberts et al. 2005).

Among the two selected SCoT primers, both demonstrated suitability, although SCoT 24 performed better. Previous studies on other Poaceae species have also confirmed the efficacy of ISSR and SCoT markers for genetic diversity analysis (Khodaee et al. 2021; Tabaripour and Keshavarzi 2021).

Our findings revealed high genetic diversity within *P. minor* populations. According to AMOVA results, 61% of genetic variation was observed within populations, while 39% was attributed to differences between populations. These results align with the STRUCTURE analysis, further supporting the existence of substantial intra-population diversity. Similarly, a study by Matus and Hucl (1999) reported that *P. minor* exhibits greater genetic diversity compared to other species, predominantly at the intra-populational level. McRoberts et al. (2005) also noted comparable intra- and interspecies similarities in P. minor accessions from India using ISSR markers.

In a related study on *Phalaris arundinacea*, SSR markers indicated that intra-population genetic diversity exceeded inter-population diversity (Anderson et al. 2018). Similar results were observed in *Phalaris aquatica*, where SNP markers highlighted significant intra-population diversity (Gapare et al. 2021), supporting our findings in *P. minor*.

The estimated gene flow (Nm = 0.786) suggests moderate genetic connectivity among populations. Generally, when Nm > 1, populations maintain genetic continuity over time, whereas Nm < 1 indicates genetic divergence. Thus, our results suggest that gene flow occurs at a moderate level in some studied populations.

The Mantel test revealed a positive correlation between geographical and genetic distance, indicating limited gene flow between populations. According to the UPGMA tree, the Rasht and Saravan populations formed a distinct sub-cluster, signifying higher genetic similarity compared to other populations. This pattern supports the inverse relationship between geographical distance and genetic similarity, as closer populations experience greater genetic exchange. In contrast, Nei’s genetic index identified the Rasht and Dehe Mirza Ali populations as having the least genetic similarity, likely due to their considerable geographical separation. The observed genetic diversity within and between P. minor populations may be attributed to genetic drift, gene flow, and environmental adaptation.

Conclusion

This study revealed substantial genetic diversity in Iranian *P. minor* populations based on ISSR and SCoT markers. AMOVA indicated that most of the variation occurred within populations, while a considerable proportion was attributable to differences among populations. The significant Mantel test supported isolation by distance, suggesting that gene flow is more limited among geographically distant populations. Population structure analyses further indicated distinct genetic clusters and admixture in several populations. These findings provide a genetic baseline for future monitoring and may support long-term management strategies for this noxious weed.

Previous research has demonstrated substantial heterogeneity within *P. minor* populations, potentially contributing to variable herbicide efficacy across different locations. Some studies suggest that the local evolution of herbicide-resistant biotypes may explain this variation. Future research should focus on assessing the genetic composition of populations near agricultural fields with prolonged herbicide exposure. Based on genetic diversity parameters, both ISSR and SCoT markers proved to be effective tools for investigating the genetic diversity of *P. minor* populations.

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