Analysis of population structure and genetic diversity of Iranian Wild Salicornia (Salicornia iranica Akhani) population

Mohammad Aghaei1*, Abbas Hassani1, Hosein Nazemiyeh2, Babak Abdollahi Mandouulkani3 and Mohammad Saadatian4

Abstract

Background: Salicornia is a halophyte plant capable of being irrigated with seawater, which can be used as an alternative food. Given this, it is necessary to study the potentials of this plant’s morphological diversity in the natural environment. In this study, 33 wild populations of Salicornia were collected from different geographical areas around Urmia Lake during the flowering stage, and 55 morphological traits and 25 ISSR loci of the plant were analyzed. Based on morphological and molecular traits and the cluster analysis, Salicornia populations were divided into four and two groups, respectively.

Results: Overall, the high percentage of polymorphic loci (65.69%), the average number of effective alleles per locus (1.63), and the Shannon data index (0.540) indicate that ISSR markers was used to identify genetic diversity. Molecular data cluster analysis divided the studied populations into two main groups, which included 12.12% and 87.88% of the populations, respectively. Based on the effective analysis of the population's genetic structure and the precise classification of individuals into suitable sub-populations, the value of $K=2$ was calculated.

Conclusions: The research findings indicated that the populations of Salicornia have a considerable diversity in morphological traits. Furthermore, markers UBC823, B, A7, and K, as well as markers with the Shannon index, effective allele, and large heterozygosis values, are the most effective markers in comparison with other markers used in this study. The findings of this study will aid in parental selection studies for breeding programs of Salicornia in future.

Keywords: Genetic distance, Cluster analysis, ISSR, Morphological traits, Salicornia

Background

Genetic diversity in crops and orchards is an issue long considered by plant breeders searching for new sources of germplasm to perform gene transfer, phylogenetic testing, and marker selection, among other things [18].

Given the role of genetic diversity in advancing breeding programs and the importance of the local population, it is necessary to study the local population's genetic diversity [31]. A variety of natural genetic resources in an area can provide beneficial genes for plant breeding. These genes have been formed and stored mainly in native plants for centuries [25]. Many of these native species have been being introduced as new plants due to their medicinal and industrial properties [11]. It is necessary to study genetic diversity among different species using morphological features to find desirable traits for further production [26]. Morphological traits obtained from visible mutations in morphology include a wide range of genes that control morphological characteristics based on the phenotypes and serve as the first markers. They used time immemorial, that is, the location of a gene chromosome determined [24]. The
studied, the stored genetic material should be known. Samples can be evaluated in accordance with the purpose of germplasm usage, including pathological, agronomic, morphological, biochemical, molecular, and histological dimensions. With the evaluation of germplasm, information about the weaknesses and strengths of the genotypes and populations and their potentials can be obtained, and genetic basis of each trait can be determined by these evaluations. Investigating genetic diversity in plants is significant from various dimensions. Generally, when genetic diversity is determined, it is beneficial for researchers for managing collections, conservation, maintenance, and specification of plants, as well as usage of plant collections [27]. The use of molecular markers in scientific research has opened up new possibilities for identifying and manipulating particular genes. Molecular markers have become increasingly important in evaluating species diversity and evolutionary relationships [15]. For researchers, the genetic analysis of plants is a foundation for characterizing natural plant genetic resource, detecting genetic diversity or genetic homogeneity, and selecting plants with specific traits such as the synthesis of desired chemicals and stress tolerance mechanisms [8]. Salicornia consists of approximately 15 genus and 68 species [29]. However, it is challenging to classify this plant species due to self-pollination and diversity in local populations.

Besides the loss of leaves and morphological identification indices and the small amount of dry matter compared to wet tissue, the accurate identification of species is difficult [3]. Salicornia Akhani, an endemic species of Salicornia in Iran, grows in central Iran and is a diploid genus of Salicornia [1]. The habitats of this plant in Iran are Fars, Semnan, Gorgan, Bushehr, Hormozgan, Yazd, Khorasan, Khuzestan, Markazi, West and East Azerbaijan, Isfahan, Qom, and Tehran provinces [22]. According to studies, species collected from seven regions surrounding Urmia Lake have been identified as Salicornia iranica [22].

The Salicornia is important as a medicinal plant, and given the fact that there are not adequate and comprehensive studies in different fields of production. The current survey was conducted in order to (1) estimate the morphological and molecular variation among 33 wild Salicornia populations, (2) search for genetic structure of Salicornia populations and identify the most effective ISSR markers, and (3) identify the relationships between morphological characteristics and ISSR markers to partition the genetic variation within and among populations, and provide basic information for conservation and breeding programs. In this study, 33 populations of Salicornia grown around Urmia Lake were collected, and to evaluate the morphological and genetically diversity between different populations, 55 different morphological traits and 25 ISSR markers were studied; also, for future genetic modification and parent plant selection, the results can be made available to the breeders.

Methods
In this study, 33 wild populations of Salicornia in full bloom and plant seeds were collected from different geographical areas in the lake’s vicinity (Table 1, Fig. 1). At the time of data collection, features such as the geographic area’s location and characteristics (altitude and latitude) were recorded. Some populations were geographically less than a few hundred meters apart, which were considered separately, based on field observations.

Fifty-five morphological traits were evaluated. Fifteen specimens were sampled per population, and for each plant, all 55 traits were calculated (Table 2). The morphological traits were measured in the Plant Physiology Laboratory, Horticulture Department, Faculty of Agriculture, Urmia University, and Herbarium, Faculty of Pharmacy, Tabriz University. The properties were measured using a ruler, digital caliper, scrubber, and optical microscope [12, 14].

Studying genetic diversity
Molecular evaluation
CTAB approach [6] was used for extraction of individual genomic DNA. Spectrophotometry and 1% agarose gel electrophoresis were performed for evaluating the quantity and quality of the extracted DNA. Using 25 ISSR primers, genotypes were recorded in the subjects. Lodhi et al. [19] optimized PCR reactions and their temperature cycle. PCR was run in 15-μl reaction mixture, which consists of master mix 2× of 5 μl, primer 10pM of 1 μl, template DNA 50 ng/μl concentration of 2 μl, and sterile water of 7 μl. PCR amplification profile with 95 °C for 4 min of initial denaturation, followed by 30 cycles of 94 °C for 30 s, 41–58 °C for 1 min and 72 °C for 1 min, and followed by a final extension for 10 min at 72 °C. PCR amplicons were resolved on 0.8% agarose gel electrophoresis. Besides, using the GeneRuler’O Fermentas size indicator, the size of the band was determined.

The combination of markers was used for obtaining population structure according to the data by the use of STRUCTURE software 2.3.4 (30) with 50,000 MCMC repetitions and 50,000-in-Burn time in Admixture mode in varying values of K in a range of 1–20 (5 repetitions per k). This software was also used for estimating the membership share matrix (Q). With this matrix,
| Longitude | Latitude | Regions | Population | Code | Longitude | Latitude | Regions | Population | Code |
|-----------|----------|---------|------------|------|-----------|----------|---------|------------|------|
| 45° 5' 7.24" E | 38° 0' 2.55" N | West Azerbaijan | Qoshchi 1 | P1 | 45° 50' 23.82" E | 37° 49' 1.22" N | East Azerbaijan | Gogan khaslou II | P18 |
| 45° 5' 7.24" E | 38° 0' 2.55" N | West Azerbaijan | Qoshchi 2 | P2 | 45° 39' 2.32" E | 37° 52' 34.46" N | East Azerbaijan | Saray Road | P19 |
| 45° 47' 35.42" E | 37° 30' 27.75" N | East Azerbaijan | Port of Rahmanlu | P3 | 45° 21' 50.18" E | 37° 11' 34.29" N | West Azerbaijan | Sand Plant | P20 |
| 45° 26' 28.23" E | 37° 8' 3.149" N | West Azerbaijan | After medical sciences Univ. before Hasanlu dam | P4 | 45° 15' 59.99" E | 37° 31' 35.60" N | West Azerbaijan | Isa-Can I | P21 |
| 45° 41' 7.89" E | 37° 2' 9.74" N | West Azerbaijan | Dashkhan | P5 | 45° 15' 59.99" E | 37° 31' 35.60" N | West Azerbaijan | Isa-Can II | P22 |
| 45° 28' 21.76" E | 38° 10' 30.96" N | East Azerbaijan | Sharafkhaneh Port | P6 | 45° 26' 51.08" E | 37° 7' 48.66" N | West Azerbaijan | Shirin-Bulagh I | P23 |
| 46° 0' 31.73" E | 37° 24' 52.32" N | East Azerbaijan | Bonab plant | P7 | 45° 26' 51.08" E | 37° 7' 48.66" N | West Azerbaijan | Shirin-Bulagh II | P24 |
| 45° 25' 11.52" E | 37° 54' 15.67" N | East Azerbaijan | Islami Island | P8 | 45° 44' 55.30" E | 37° 52' 2.41" N | East Azerbaijan | Aji Chai River | P25 |
| 45° 15' 32.59" E | 37° 33' 15.63" N | West Azerbaijan | Chi-Chest | P9 | 45° 13' 57.39" E | 37° 43' 9.29" N | West Azerbaijan | Road Police | P26 |
| 45° 28' 49.08" E | 37° 6' 2.48" N | West Azerbaijan | Wetland in front of Hasanlu Dam I | P10 | 45° 45' 16.60" E | 37° 56' 17.47" N | East Azerbaijan | Hassanabad River | P27 |
| 45° 28' 49.08" E | 37° 6' 2.48" N | West Azerbaijan | Wetland in front of Hasanlu Dam II | P11 | 45° 49' 23.52" E | 37° 52' 29.76" N | East Azerbaijan | Radio station | P28 |
| 45° 35' 10.94" E | 37° 2' 39.24" N | West Azerbaijan | Solduz Wetland | P12 | 45° 37' 34.52" E | 37° 2' 13.09" N | West Azerbaijan | Gerda-ghit I | P29 |
| 45° 17' 17.52" E | 37° 21' 8.63" N | West Azerbaijan | Urmia Road Police | P13 | 45° 39' 10.45" E | 37° 1' 53.30" N | West Azerbaijan | Gerda-ghit II | P30 |
| 45° 16' 13.60" E | 37° 22' 34.67" N | West Azerbaijan | Before Urmia Road Police | P14 | 45° 19' 32.19" E | 37° 15' 3.44" N | West Azerbaijan | Dizaj-dol | P31 |
| 45° 34' 44.08" E | 37° 51' 55.05" N | East Azerbaijan | Saray | P15 | 45° 9' 4.67" E | 38° 18' 9.86" N | West Azerbaijan | Mighatlou | P32 |
| 45° 42' 7.39" E | 37° 56' 27.59" N | East Azerbaijan | Shekargah | P16 | 45° 18' 19.35" E | 37° 18' 20.48" N | West Azerbaijan | Cement factory | P33 |
| 45° 50' 23.82" E | 37° 49' 1.22" N | East Azerbaijan | Gogan khaslou I | P17 | | | | | |
it is shown that each member to what extent fits to the clusters. Using the same software, the average stabilization index (FST) was calculated for potential subgroups. The approach proposed by Evanno et al. [9] was used for determining the actual number of subpopulations. The basis of this approach is on $\Delta K$ statistic breaking a function’s slope when there is the maximum probability for a hypothetical number.

Statistical analysis of data

The ANOVA and variation within-group were expressed as coefficient of variation for quantitative descriptors calculated for each group and the whole collection. Principal components analysis (PCA) was performed using XLSTAT 2018.1 statistical software. The first and second principal component axes scores were plotted to aid visualization of origin group differences and detect morphological variation in the collection.

Analysis of data

Population structure was studied using bands from all marker matrices. Using different algorithms, such as UPGMA, single linkage, and complete linkage, cluster analysis was performed. These algorithms were employed as zero (absence) and one (presence) scoring. The clusters were drawn in the present work using Mega software. Also other data were analyzed using the following software: NTSYSpc version 2.0.1.5, SAS 9.2 (ANOVA analysis), SPSS (means), Mega (Molecular analysis), and PopGene (Molecular analysis).

Results

The variation and the mean traits were examined for different populations. Among the studied populations of *Salicornia*, the non-fertile parts on the longest secondary branch (V29) (84.75%), the fertile parts on the longest secondary branch (V28) (81.49%), and the flowering plants in the first lateral branch (V34) (66.13%) had the highest diversity (Table 3). According to the results, the highest and lowest number of primary lateral branches (V9) was observed in P27, 43, and P22, 13.4, respectively. Complete information about other variables is given in Table 3.

The first five of the 32 principal components (PCs) obtained have eigenvalues greater than 2. Together, they accounted for about 67.28% of the total variance of *Salicornia* traits (Fig. 2, Table 4). The first two PCs account for 42.32% of the total variability (25.76% and 16.56%, respectively) (Tables 4 and 5). PC1 represent ration of V7, V8, V11, V14, V16, V19, V25, V26, V31, V32, V37, V38, V39, V40, V41, V42, V44, V45, V46, V53, and V55. PC2 describe the ration of V1, V10, V13, V23, V24, V27, V30, and V43. Figure 2 and Table 4 show that traits lie around PC1 and PC2 center. The large variability of the traits allows observation such as V10, V31, V39, V41, and V45, where the amount of length of longest 1st primary branch, length of the terminal spike, height of central floret of 3rd fertile segment, height of side floret of 3rd fertile segment, and distance between florets on 2nd fertile segment.

According to the morphological traits results of cluster analyses by the Ward method, *Salicornia* populations were assigned to four groups (Fig. 3). The first group contained 8.18% of populations (P16, P18, P24,
Table 2  Morphological traits studied in *Salicornia* populations

| Code | Traits                                           | Measurement unit | Code | Traits                                           | Measurement unit     |
|------|--------------------------------------------------|------------------|------|--------------------------------------------------|----------------------|
| V1   | Height of plant from rooting point to apex       | (cm)             | V29  | Number of sterile segments on the longest secondary | (Number)             |
| V2   | Stem diameter                                    | (cm)             | V30  | Length of the longest tertiary branch            | (cm)                 |
| V3   | Height from rooting point to 1st branching point | (cm)             | V31  | Length of the terminal spike                     | (cm)                 |
| V4   | Number of internodes                             | (Number)         | V32  | Number of fertile segments on terminal spike     | (Number)             |
| V5   | Length of 1st internode                          | (cm)             | V33  | Number of sterile segments on terminal spike     | (Number)             |
| V6   | Length of 2nd internode                          | (cm)             | V34  | Number of spike in 1st (basal) primary branch    | (Number)             |
| V7   | Length of penultimate internode                  | (cm)             | V35  | Number of spike in penultimate branch            | (Number)             |
| V8   | Length of ultimate internode                     | (cm)             | V36  | Number of spike in ultimate branch               | (Number)             |
| V9   | Number of side primary branch                    | (Number)         | V37  | Height of 3rd fertile segment on terminal spike  | (mm)                 |
| V10  | Length of longest 1st (basal) primary branch     | (cm)             | V38  | Width of 3rd fertile segment on terminal spike   | (mm)                 |
| V11  | Average number of fertile segments on terminal spike in 1st primary branch | (Number) | V39  | Height of central floret of 3rd fertile segment | (mm)                 |
| V12  | Average number of sterile segments on terminal spike in 1st primary branch | (Number) | V40  | Width of central floret of 3rd fertile segment | (mm)                 |
| V13  | Length of longest 2nd primary branch             | (cm)             | V41  | Height of side floret of 3rd fertile segment     | (mm)                 |
| V14  | Average number of fertile segments on terminal spike in 2nd primary branch | (Number) | V42  | Width of side floret of 3rd fertile segment     | (mm)                 |
| V15  | Average number of sterile segments on terminal spike in 2nd primary branch | (Number) | V43  | Width across apex of 3rd fertile segment         | (mm)                 |
| V16  | Length of the longest penultimate branch         | (cm)             | V44  | Distance from tip of 3rd fertile segment to apex of middle floret | (mm)                 |
| V17  | Number of fertile segments in penultimate branch | (Number)         | V45  | Distance between florets on 2nd fertile segment | (mm)                 |
| V18  | Number of sterile segments in penultimate branch | (Number)         | V46  | Length of 1st sterile segment on terminal spike | (mm)                 |
| V19  | Length of ultimate branch                        | (cm)             | V47  | Length of last sterile segment on terminal spike | (mm)                 |
| V20  | Number of fertile segments in ultimate branch    | (Number)         | V48  | Height of central seed                           | (mm)                 |
| V21  | Number of sterile segments in ultimate branch    | (Number)         | V49  | Width of central seed                            | (mm)                 |
| V22  | Distance from apex to apex of ultimate branch    | (cm)             | V50  | Height of side seed                              | (mm)                 |
| V23  | Distance from apex to apex of 1st primary branch | (cm)             | V51  | Width of side seed                               | (mm)                 |
| V24  | Number of secondary branches in 1st primary branch | (Number)    | V52  | Weight 1000 seed                                 | (g)                  |
| V25  | Number of secondary branches in 2nd primary branch | (Number)    | V53  | Length of Stomata                                | (μm)                 |
| V26  | Maximum number of secondary on a primary branch  | (Number)         | V54  | Width of Stomata                                 | (μm)                 |
| V27  | Length of longest secondary branch               | (cm)             | V55  | Number of Stomata                                | (Number)             |
P31, P20, and P22). In this group, populations with a short height, long spike, greater weight of 1000 seed, low number of stomata, and the width across the apex on the third fertile segment were more abundant than other populations. The morphotype and inflorescences of this group were distinct from other groups. The second group covered 15.15% of the whole population (P3, P11, P23, P2, and P33), comprising populations that were within the average range of sizes for diverse traits. The third group hosted 15.15% of the population (P4, P6, P1, P8, and P10), and the fourth group included 51.51% of the population (P9, P30, P25, P27, P21, P26, P15, P12, P28, P7, P14, P17, P5, P19, P29, P13, and p32). These populations had a great height, more internodes, more lateral branches, more stomata, a great weight of 1000 seeds, and the width of the third fertile segment on the terminal spike. The accurate number of groups was identified using the detection function.

Genetic diversity of *Salicornia* populations

We evaluated genetic diversity in 33 *Salicornia* populations using 42 ISSR primers. Twenty-three primers out of 42 primers under study generated a polymorphic band at the suitable resolution, which were employed for the subsequent analysis phases (Table 6). In total, 204 alleles with an average 8.87 allele per marker were detected, 134 of them were polymorphic (65.69%). The ratio of markers to primer was 1 to 14, averagely 5.82 (Table 6, Fig. 4).

The number of effective (Ne) alleles ranged from 1.25 for UBC849 and 1.92 for in PB with an average 1.63 per locus. Maximum value of this statistic shows that alleles have identical frequency in this location, and this statistic’s minimum shows the rarity of other alleles and one allele’s high frequency in samples.

In investigating allelic diversity, the highest observed heterozygosity was found by B marker with 0.477; however, the lowest observed heterozygosity was noticed by UBC849 marker with 0.199. Besides, the highest

| Variable | CV  | SD  | Mean | Max  | Min  | Variable | CV  | SD  | Mean | Max  | Min  |
|----------|-----|-----|------|------|------|----------|-----|-----|------|------|------|
| V1       | 20.65 | 6.73 | 32.59 | 50.12 | 23.70 | V28      | 81.49 | 284.65 | 1473.2 | 349.32 |
| V2       | 21.47 | 0.16 | 0.76 | 1.23 | 0.41 | V29      | 84.75 | 90.78 | 107.12 | 444.40 |
| V3       | 76.91 | 0.98 | 1.28 | 6.38 | 0.56 | V30      | 37.36 | 3.16 | 8.45 | 15.60 |
| V4       | 23.16 | 4.73 | 20.42 | 29.40 | 12.00 | V31      | 43.03 | 2.16 | 5.02 | 9.08 |
| V5       | 18.87 | 0.22 | 1.15 | 1.55 | 0.59 | V32      | 39.66 | 6.75 | 17.01 | 30.60 |
| V6       | 18.17 | 0.25 | 1.09 | 1.48 | 0.72 | V33      | 26.65 | 0.61 | 2.29 | 4.60 |
| V7       | 20.73 | 0.23 | 1.09 | 1.48 | 0.72 | V34      | 66.13 | 99.76 | 150.84 | 366.80 |
| V8       | 21.99 | 0.21 | 0.94 | 1.36 | 0.62 | V35      | 40.55 | 2.94 | 7.25 | 12.80 |
| V9       | 24.74 | 7.02 | 28.36 | 43.00 | 13.40 | V36      | 44.93 | 2.44 | 5.42 | 14.60 |
| V10      | 24.87 | 6.57 | 26.41 | 41.00 | 15.10 | V37      | 16.05 | 0.56 | 3.47 | 4.86 |
| V11      | 37.90 | 5.49 | 14.48 | 28.70 | 4.60 | V38      | 21.13 | 0.60 | 2.84 | 4.30 |
| V12      | 997   | 0.21 | 2.13 | 2.90 | 2.00 | V39      | 18.23 | 0.46 | 2.53 | 3.33 |
| V13      | 25.98 | 6.27 | 24.13 | 37.46 | 13.28 | V40      | 17.20 | 0.36 | 2.11 | 9.20 |
| V14      | 37.27 | 5.42 | 14.54 | 28.76 | 4.60 | V41      | 22.00 | 0.33 | 1.50 | 2.20 |
| V15      | 11.90 | 0.26 | 2.17 | 2.84 | 2.00 | V42      | 25.80 | 0.38 | 1.46 | 2.57 |
| V16      | 31.41 | 2.25 | 7.16 | 11.92 | 3.34 | V43      | 27.52 | 0.13 | 0.46 | 0.93 |
| V17      | 54.55 | 36.7 | 67.32 | 154.0 | 20.20 | V44      | 32.26 | 0.18 | 0.55 | 1.01 |
| V18      | 48.81 | 7.46 | 15.28 | 36.40 | 6.40 | V45      | 18.59 | 0.62 | 3.35 | 5.03 |
| V19      | 29.49 | 1.77 | 5.99 | 9.72 | 2.60 | V46      | 28.39 | 0.62 | 2.18 | 3.53 |
| V20      | 47.00 | 23.6 | 50.38 | 112.0 | 17.00 | V47      | 29.81 | 0.33 | 1.11 | 1.89 |
| V21      | 44.66 | 4.79 | 10.73 | 27.60 | 4.60 | V48      | 10.88 | 0.20 | 1.87 | 2.27 |
| V22      | 47.83 | 6.44 | 13.46 | 43.00 | 5.64 | V49      | 13.09 | 0.13 | 0.96 | 1.27 |
| V23      | 31.19 | 14.4 | 46.34 | 77.50 | 9.76 | V50      | 14.37 | 0.23 | 1.58 | 2.11 |
| V24      | 34.44 | 6.11 | 17.73 | 29.60 | 6.20 | V51      | 19.25 | 0.16 | 0.84 | 1.47 |
| V25      | 33.77 | 6.24 | 18.48 | 33.40 | 7.60 | V52      | 31.21 | 0.12 | 0.38 | 0.73 |
| V26      | 32.15 | 7.24 | 22.50 | 39.00 | 9.20 | V53      | 17.78 | 2.75 | 15.47 | 19.40 |
| V27      | 31.57 | 5.59 | 17.71 | 27.94 | 2.74 | V54      | 22.40 | 1.93 | 8.61 | 16.40 |
| V28      | 31.57 | 5.59 | 17.71 | 27.94 | 2.74 | V55      | 21.69 | 1.52 | 6.99 | 11.00 |
expected heterozygosity was observed at approximately 0.484 by B marker, and the lowest expected heterozygosity was observed at approximately 0.203 in the UBC849 marker. Examining Shannon index (I) values showed that the highest value for this index was in marker B with a 0.670 and the lowest value was in UBC849 marker as 0.351 (Table 7).

The Jaccard similarity coefficient and UPGMA algorithm were used for dividing different populations into two separate groups. The first group contained 12.12% and the second group included 87.88% of the masses. Two subgroups were made in the first group, which the first one included P24, P22, P26, and P1. The second group contained the residual 29 populations (P13, P20, P18, P30, P32, P29, P19, P8, P15, P17, P5, P27, P12, P33, P28, P31, P16, P21, P23, P14, P4, P3, P2, P7, P25, P6, P10, P11, P9), which was classified into two subgroups. The first one is composed of just the P13 population. Also, this population was approximately different from other ones (Fig. 5).

Structure 2.3.1 software was used for analyzing genetic population structure and precise classifying individuals into proper subpopulations. As shown by a two-way diagram of optimal determination of K with ISSR indicator, the ISSR primer shows the best K as 2, i.e., two subpopulations (K = 2) in the cultivars under study. The group was specified (Tables 8 and 9).

The stabilization index (FST) is a common and appropriate measure for genetic differentiation among groups and populations. When the FST is higher, a better allele differentiation is obtained, with a higher allele stabilization rate. Potential subgroups in K = 2 show the difference among the populations under study in two potential groups. Besides, the individuals’ matrix of the share in these groups (Tables 4 and 5) indicated belonging populations with high coefficients to one group. Bar plot results demonstrated inclusion of 26 Salicornia populations in the first group (red) and 5 populations in the second group (green), with 2 populations had a complex structure (Fig. 6).

Discussion

The results showed that there was a significant difference between the studied populations in terms of traits in the question. Based on the mean of traits measured in the population, traits with a high percentage of variance had a wide range of trait quantities and offered a more extensive choice for traits. This difference is due to the impact of both genetic and environmental factors. Studies have shown that fluctuations in soil and water salinity lead to physiological and phenotypic changes in the plant. Also, high plant density in a population restricts the number of branches and glaciers formed in the plant [13]. Self-pollination in plants, especially in diploid species, due to the flower’s unique structure, leads to the formation of
various local populations in Salicornia [5]. The pheno-
typic variation coefficient between traits results in mor-
phologically different plants manifests distinct genetic
variations in different regions [28]. Together with the
weight of 1000 seeds, these traits undermine the plants'
ability to produce satisfying seeds. With an increase
in the number of internodes and lateral branches, the
weight of 1000 seeds drops. Most of the plant energy
comes spent on vegetative growth. Studies have focused
on Salicornia's two species in Iran (S. Biglovi and Salicor-
nia persica). In S. biglawi species, raising the salinity of
irrigation water to 45 dS/m reduces the height and dry
weight of the plant. In Persica species, increasing the irri-
gation water salinity had no effect on plant height but sig-
nificantly decreased the dry weight [28].

The cluster analysis results showed that (Fig. 3) the
clustering of populations is incompatible with geo-
graphical distribution. It may be due to sources of seed
diversity caused by migration to different areas. There-
fore, it may not be limited to different geographical
regions in selecting parents for breeding projects, but
it should be consistent with each population's specific
capacities. By studying Salicornia pusilla, researchers
have found that the plant seeds remain attached to the
inflorescence after ripening, and the spikes are trapped
by a separating layer of the plant isolated in the water

Table 4  Principal components for studied morphological traits in Salicornia populations

| Variable | F1   | F2   | F3   | F4   | F5   |
|----------|------|------|------|------|------|
| V1       | 0.005| 0.546*| 0.021| 0.314| 0.002|
| V2       | 0.001| 0.216| 0.05 | 0.253*| 0.075|
| V3       | 0.001| 0.224| 0.069| 0.006| 0.315*|
| V4       | 0.169| 0.213| 0.041| 0.400*| 0.013|
| V5       | 0.022| 0.007| 0.123| 0.042| 0.505*|
| V6       | 0.043| 0.02  | 0.006| 0.04 │ 0.592*|
| V7       | 0.297*| 0.235| 0.004| 0.048| 0  |
| V8       | 0.475*| 0.152| 0.061| 0.001| 0.009|
| V9       | 0.109| 0.21  | 0.065| 0.313*| 0.071|
| V10      | 0.002| 0.831*| 0.093| 0.01 | 0.004|
| V11      | 0.425*| 0.147| 0.142| 0.01 | 0.048|
| V12      | 0.03 | 0.103| 0.374*| 0.104| 0.14 |
| V13      | 0.009| 0.759*| 0.113| 0.002| 0.008|
| V14      | 0.388*| 0.153| 0.147| 0.03 | 0.067|
| V15      | 0.003| 0.015| 0.302*| 0.09 | 0.193|
| V16      | 0.529*| 0.154| 0.181| 0.003| 0.003|
| V17      | 0.225| 0.183| 0.402*| 0.016| 0.004|
| V18      | 0.048| 0.105| 0.587*| 0.134| 0.04 |
| V19      | 0.714*| 0.101| 0.088| 0.012| 0 |
| V20      | 0.185| 0.148| 0.366*| 0.001| 0 |
| V21      | 0.023| 0.112| 0.561*| 0.001| 0 |
| V22      | 0.176| 0.023| 0.169| 0.081| 0.005|
| V23      | 0.001| 0.646*| 0.164| 0.016| 0.009|
| V24      | 0.31 | 0.395*| 0.014| 0.002| 0.002|
| V25      | 0.304*| 0.277| 0.162| 0.003| 0 |
| V26      | 0.333*| 0.28 | 0.012| 0.049| 0.004|
| V27      | 0     | 0.686*| 0.046| 0.077| 0.005|
| V28      | 0     | 0.139| 0.1  | 0.344*| 0.175|
| V29      | 0.06  | 0.099| 0.107| 0.366*| 0.092|

*Significant at \( P < 0.05 \)

Table 5  Eigenvalue, proportion, and cumulative variation of analyzed components

|          | F1    | F3    | F3    | F4    | F5    |
|----------|-------|-------|-------|-------|-------|
| Eigenvalue| 14.17 | 9.11  | 6.43  | 4.53  | 2.77  |
| Variances| 25.76 | 16.56 | 11.7  | 8.23  | 5.03  |
| Cumulative variances| 25.76 | 42.32 | 54.02 | 62.24 | 67.28 |
Fig. 3  Hierarchical cluster analysis (HCA) of different *Salicornia* populations based on 55 main morphologic traits

| Table 6  | Markers name, annealing temperature (°C), total band, polymorphic band, and percent of polymorphic band |
|----------|-------------------------------------------------------------------------------------------------|
| **Markers' name** | **Primer sequences** | **Annealing temperature** | **Total bond** | **Polymorphic band** | **Polymorphic band percent** |
| 1  | A  | CACACACACACAGG | 44 °C | 10 | 8 | 80 |
| 2  | B  | CACACACACACAC | 41 °C | 11 | 6 | 54.55 |
| 3  | F  | GAGAGAGAGAGAGG | 44 °C | 8 | 3 | 37.50 |
| 4  | G  | GTGTTGTTGTTGTC | 44 °C | 10 | 4 | 40 |
| 5  | H  | AGAGAGAGAGAGAGGT | 50 °C | 4 | 2 | 50 |
| 6  | I  | AGAGAGAGAGAGAGGC | 52 °C | 5 | 4 | 80 |
| 7  | J  | ACAACACACACACCT | 50 °C | 10 | 9 | 90 |
| 8  | K  | ACAACACACACACCCG | 52 °C | 10 | 6 | 60 |
| 9  | A7 | AGAGAGAGAGAGAGAGT | 58 °C | 10 | 5 | 50 |
| 10 | A12 | AGAGAGAGAGAGGCC | 52 °C | 9 | 3 | 33.33 |
| 11 | A13 | GTGTTGTTGTTGCC | 55 °C | 14 | 13 | 92.86 |
| 12 | UBC818 | CACACACACACACAG | 56 °C | 5 | 2 | 40 |
| 13 | UBC825 | ACAACACACACACACT | 55 °C | 7 | 6 | 85.71 |
| 14 | UBC849 | GTGTTGTTGTTGTTGTCG | 55 °C | 3 | 1 | 33.33 |
| 15 | UBC811 | GAGAGAGAGAGAGAC | 54 °C | 11 | 8 | 72.73 |
| 16 | UBC844 | CTCCTCTCTCTCTCTCTC | 56 °C | 9 | 6 | 66.67 |
| 17 | UBC823 | TCTCTCTCTCTCTCTCTC | 48.5 °C | 4 | 3 | 75 |
| 18 | UBC834 | CTCTCTCTCTCTCTCTAC | 51.3 °C | 17 | 14 | 82.35 |
| 19 | UBC850 | GTGTTGTTGTTGTTGTTGTTGTC | 56.03 °C | 12 | 11 | 91.67 |
| 20 | UBC860 | TGTGTTGTTGTA | 40 °C | 6 | 3 | 50 |
| 21 | 201274 | CACACACACACARY | 42 °C | 14 | 11 | 78.57 |
| 22 | 201275 | CACACACACACARG | 43 °C | 6 | 3 | 50 |
| 23 | 201246 | AGAGAGAGAGAGAGYC | 47 °C | 9 | 3 | 33.33 |
| **Total** | | | 204 | 134 | 65.69 |
that may keep moving with the flow of water up to 3 months. They may even germinate but do not grow until the seeds are deposited in sediments [4]. This feature may explain the common seed origin in the studied populations. Using 22 growth parameters, the researchers evaluated 11 \textit{S. bigelovii} populations in the field and divided the cluster analysis of studied populations into four groups [20]. Contrary to our study, the results of research on the genetic diversity of six \textit{Salicornia ramossissima} populations in central Germany showed that it is consistent with geographical distribution [17]. A review of the genetic diversity of the two species of saline \textit{Salsola} manifested a significant difference in this plant and the environmental conditions of the plant, suggesting that disparity in salinity, nutrition, pH, and soil moisture changes the vegetative type of plants [30].

The results of analyzing the main components confirmed the clustering obtained from the cluster decomposition. The analysis of main components sheds light on the difference between individuals and allowing the identification of groups and the relationship between individuals and variables [21]. Based on the results of this analysis and multivariate analysis, four \textit{Salicornia} populations were divided into three separate groups: the first component (46.11%), the second component (41.35%), and the overall component (87.46%) of the entire diversity. The study of the morphological diversity patterns of 52 \textit{Salicornia} populations in 31 regions of Northern Europe using 28 morphological traits demonstrated diversity in the studied populations. The main

### Table 7

| Primer | Ne    | I     | He    | Ho    |
|--------|-------|-------|-------|-------|
| 201274 | 1.665 | 0.573 | 0.388 | 0.394 |
| p825-1 | 1.602 | 0.526 | 0.353 | 0.358 |
| A13    | 1.654 | 0.564 | 0.380 | 0.386 |
| l      | 1.725 | 0.595 | 0.409 | 0.415 |
| UBC811 | 1.724 | 0.599 | 0.411 | 0.418 |
| UBC823 | 1.819 | 0.640 | 0.448 | 0.456 |
| A7     | 1.805 | 0.624 | 0.435 | 0.442 |
| UBC850 | 1.673 | 0.574 | 0.390 | 0.396 |
| UBC849 | 1.249 | 0.351 | 0.199 | 0.203 |
| UBC860 | 1.654 | 0.574 | 0.388 | 0.394 |
| A      | 1.631 | 0.559 | 0.375 | 0.381 |
| B      | 1.917 | 0.670 | 0.477 | 0.484 |
| F      | 1.494 | 0.446 | 0.290 | 0.295 |
| G      | 1.406 | 0.391 | 0.247 | 0.251 |
| H      | 1.711 | 0.602 | 0.413 | 0.419 |
| J      | 1.483 | 0.463 | 0.299 | 0.303 |
| K      | 1.571 | 0.518 | 0.343 | 0.348 |
| UBC834 | 1.788 | 0.620 | 0.431 | 0.438 |
| UBC844 | 1.501 | 0.474 | 0.307 | 0.312 |
| UBC818 | 1.558 | 0.492 | 0.324 | 0.329 |
| 201275 | 1.422 | 0.396 | 0.257 | 0.261 |
| 201246 | 1.766 | 0.620 | 0.429 | 0.436 |
| Mean   | 1.628 | 0.540 | 0.378 | 0.384 |
Fig. 5 Dendrogram showing relationships among 33 population of *Salicornia*. Group I represents the four populations while group II represents other 29 populations based on ISSR marker (algorithm UPGM)
components’ analysis revealed the first five components accounted for 79.8% of the total diversity. In the first component, characteristic spikes included the length of fertile segment and length of the spike (explaining 40% of the diversity, and in the second component, they included the size of the plant and the branches (explaining 18.1% of the diversity) [1]. The findings are aligned with our results. Principal component analysis revealed that V7, V8, V11, V14, V16, V19, V25, V26, V31, V32, V37, V38, V39, V40, V41, V42, V44, V45, V46, V53, and V55 contributed mostly to diversity.

Though the association between regional diversity was not that evident, a close look at the scatter plot revealed some regional adaptation level was observed. Such regional variability could be due to geographic isolation and microclimatic differences between regions. Factors such as plant population isolation, adaptation to the environment due to declining lake water levels, and strong self-pollination within the plant population may contribute to Salicornia's population diversity. The degree of morphological differentials is significantly noticeable in different populations from four groups.

The research findings indicated that markers UBC823, B, A7, and K, and with the Shannon index, effective allele, and large heterozygosity values, are markers with the highest effectiveness compared to other markers utilized, and they are used better than other compounds in genetic distance.

As stated by Dirlewanger et al. [7], there is a relationship between the alleles number in each gene locus and the number of used markers and the samples’ number. According to the findings of research on the genetic diversity of six populations of Salicornia herbacea in South Korea, where 6 ISSR markers were used, 39 polymorphic bands were obtained out of 49 bands, with an average of the effective allele for each gene locus as 1.22. The mean genetic index was 0.249 and the mean Shannon index was 0.382. These researchers mentioned that for achieving high diversity in populations Salicornia, a wider research scope is required to be chosen [16]. Using ISSR markers to identify genotypic differences among the 23 genotypes of finger millet revealed a high degree of polymorphism supported by substantial differences in all marker parameters [33].

These populations were separately gathered because of varying morphological types compared to other populations. Also, this difference is shown in the results. The second subgroup included P26 and P1 populations, with different appearances compared to other populations. They had a taller plant than average, particularly the taller plant was observed in P26 population among all populations. Moreover, long glazes were observed in these two populations. Additionally, it shows all botanical properties of S. Iranica [1].

In earlier Iranian research works on Salicornia, 36 samples of Salicornia were collected by Heydarian [10] from different saline areas. He specified this plant's genetic diversity by the use of 17 RAPD markers, Jaccard similarity coefficient, and UPGMA approach. The subjects were categorized into 7 classes. Moreover, 18 Salicornia populations were evaluated by Mohammadi [23], which were collected from different regions in Iran. He used AFLP markers and categorized the individuals into 4 groups by the use of UPGMA method and Jaccard similarity coefficient. As shown by the research in this work, the researcher collected species from 7 regions near Lake Urmia and S. iranica are presented, all in a group. In this research, S. iranica species were separated from S. persica species using the AFLP marker, and they were placed in a subgroup. Additionally, the populations gathered from each area were put in a different subgroup. The genetic diversity in 11 Salicornia brachiata populations was evaluated in India using 15 ISSR and 15 RAPD primers [2]. The investigated populations showed high diversity. It was also observed in both markers of the populations under study. They were grouped into 3 groups.

**Table 8** Mean Stabilization Index (FST) of each cluster based on cluster analysis based on Bayesian model at $K = 2$

| Cluster | FST  |
|---------|------|
| I       | 0.0003 |
| II      | 0.2156 |

**Table 9** Population membership matrix in each cluster based on Structure 2.3.1 software calculations at $K = 2$

| Population | Group 1 | Group 2 | Population | Group 1 | Group 2 |
|------------|---------|---------|------------|---------|---------|
| P1         | 0.007   | 0.993   | P18        | 0.991   | 0.009   |
| P2         | 0.986   | 0.014   | P19        | 0.196   | 0.804   |
| P3         | 0.985   | 0.015   | P20        | 0.995   | 0.005   |
| P4         | 0.996   | 0.004   | P21        | 0.010   | 0.990   |
| P5         | 0.953   | 0.047   | P22        | 0.571   | 0.429   |
| P6         | 0.992   | 0.008   | P23        | 0.992   | 0.008   |
| P7         | 0.990   | 0.010   | P24        | 0.008   | 0.992   |
| P8         | 0.921   | 0.079   | P25        | 0.995   | 0.005   |
| P9         | 0.994   | 0.006   | P26        | 0.579   | 0.421   |
| P10        | 0.989   | 0.011   | P27        | 0.956   | 0.044   |
| P11        | 0.988   | 0.012   | P28        | 0.992   | 0.008   |
| P12        | 0.993   | 0.007   | P29        | 0.990   | 0.010   |
| P13        | 0.991   | 0.009   | P30        | 0.985   | 0.015   |
| P14        | 0.992   | 0.008   | P31        | 0.988   | 0.012   |
| P15        | 0.140   | 0.860   | P32        | 0.994   | 0.006   |
| P16        | 0.983   | 0.017   | P33        | 0.902   | 0.098   |
| P17        | 0.041   | 0.959   |            |         |         |
The resulting bar plot showed that when the membership percentage to a cluster for a genotype is higher than or equal to 0.7, the genotype is allocated to that cluster, while if the percentage is below it, it is considered as a mixed genotype (hybrid) [32]. Generally, when the average effective allele numbers per gene locus (1.63), the polymorphic gene loci percentage (65.69%), and the Shannon data index (0.540) are high, it is indicated that we can use ISSR markers for identifying genetic diversity.

Conclusions
This study showed that *Salicornia* populations growing around Urmia Lake had considerable diversity in morphological and ISSR characteristics. The incompatibility of population clustering with their geographical distribution may be due to different populations’ exact seed origins. The populations under the genetic study were divided into two major groups based on marker data, including 12.12% and 87.88%. The K value was obtained as two according to the practical analysis of the population’s genetic structure and the accurate individuals’ classification to suitable sub-populations. The populations under study were classified into two groups because *Salicornia* is a self-pollinated plant. Differences in morphological and genetic grouping may be due to the environment’s effect on morphological traits, while in genetic traits, the difference between the populations may be due to
the populations’ isolation due to the lowering of the lake water, and the plant was directed towards self-breeding. Combining morphological and ISSR data may be more effective for defining genetic variation and genetic diversity within the *Salicornia* population.

**Abbreviations**

ISSR: Inter Simple Sequence Repeat; CTAB: Cetyltrimethylammonium bromide; MCMC: Markov Chain Monte Carlo; UFGMA: Unweighted Pair Group Method with Arithmetic Mean; PCA: Principal component analysis.

**Acknowledgements**

The authors sincerely thank Urmia Lake Studies Research Institute for providing part of this research’s necessary funds (Grant No. 608/53)

**Funding**

The Urmia Lake Studies Research Institute partly supported this work under Grant No. (608/53)

**Availability of data and materials**

All data is provided in full in the results section of this paper. Expression all morphological and molecular data is openly available from Dryad at [https://doi.org/10.5061/dryad.83bk3j9](https://doi.org/10.5061/dryad.83bk3j9)

**Declarations**

**Ethics approval and consent to participate**

There is no need to provide ethics approval and consent to participate in this research.

**Consent for publication**

There is not any person's data in any form.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

1. Department of Horticulture, Faculty of Agriculture, Urmia University, Urmia, Iran.
2. Pharmaceutical Microtechnology Research Center, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.
3. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran.
4. General Science Department, Faculty of Education, Soran University, Soran, Erbil, Iraq.

Received: 16 December 2021 Accepted: 22 March 2022 Published online: 21 April 2022

**References**

1. Akhani H (2008) Taxonomic revision of the genus Salicornia L. (Chenopodiaceae) in Central and Southern Iran. Pakistan J Botany 40:1635–1655
2. Badlani Ak (2011) Molecular characterization of genetic diversity in Salicornia brachiata (Rosob) using RPFD and ISSR markers. Saudarashtra University, India, Saurashtra
3. Ball PW (1964) A taxonomic review of *Salicornia* in Europe. Feddes Repert 1:1–8
4. Dalby DH (1962) Chromosome number morphology and breeding behaviour in the British *Salicornia*. Watsonia 5:150–162
5. Davy AJ, Bishop GF, Costa CSB (2001) *Salicornia* L. (Salicornia pusilla J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P.W. Ball & Tutin, *S. nitens* P.W. Ball & Tutin, *S. fragilis* P.W. Ball & Tutin and *S. dolichostachya* Moss). J Ecol 89:681–1079
6. De Masl L, Silviero P, Esposito C, Castaldo D, Siano F, Laratta B (2006) Assessment of agronomic, chemical and genetic variability in common basil (*Ocimum basilicum* L.). Eur Food Res Technol 223:273–281
7. Dirlewanger E, Cosson P, Tavaud M, Arananza M, Poizat C, Zanetto A, Arus P, Laigret F (2002) Development of microsatellite markers in peach (*Punus persica* (L.) Batsch) and their use in genetic diversity analysis in peach and sweet cherry (*Punus avium* L.). Theoretical Appl Genet 105:127–138
8. El-Demerdeh E-SS, Elsherbeny EA, Salama YAH, Ahmed MZ (2019) Genetic diversity analysis of some Egyptian Origanum and Thymus species using AFLP markers. J Genet Eng Biotechnol 17:1
9. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620
10. Heydarian Z (2001) Collection and determination of genetic diversity of *Salicornia* plant based on Rapid molecular marker. Faculty of Science. Shiraz University, Shiraz p14
11. Heywood V (1999) Trends in agricultural biodiversity. Perspectives on new crops and new uses. ASHS Press, Alexandria, pp 2-14
12. Ingrouille M, Pearson J (1987) The pattern of morphological variation in the *Salicornia europaea* L. aggregate (Chenopodiaceae). Watsonia 16:269–281
13. Kadereit G, Ball P, Beer S, Mucina L, Sokoloff D, Teege P, Yaprak AE, Freitag H (2007) A taxonomic nightmare comes true: phyllonygeny and biogeography of glassworts (*Salicornia*, *Chenopodiaceae*). TAXON 56:1143–1170
14. Kaligaric M, Bohanec B, Simonovik M, Sajna N (2008) Genetic and morphologic variability of annual glassworts (*Salicornia L.*) from the Gulf of Trieste (Northern Adriatic). Aquatic Bot 89:275–282
15. Khodaeae L, Azizinezhad E, Emirinan AR, Khosroshahi M (2021) Assessment of genetic diversity among Iranian *Aegilops* triuncialis accessions using ISSR, SCOT, and CBDP markers. J Genet Eng Biotechnol 19:5
16. Kim S-K, Cho YS, Hur YB, Song JH, Jeong HD, Chung SG (2017) Genetic diversity of *Salicornia herbacea* according to Habitat Area by ISSR Markers. Kor J Environ Ecol 31:492–499
17. Krüger AM, Hellwig FH, Oberprieler C (2002) Genetic diversity in natural and anthropogenic inland populations of salt-tolerant plants: random amplified polymorphic DNA analyses of *Aster tropilum* L. (Compositae) and *Salicornia ramosissima* Woods (Chenopodiaceae). Mol Ecol 11:1647–1655
18. Liebhard R, Gianfranceschi L, Koller B, Ryder CD, Tarchini R, Van De Weg E, Gessler C (2002) Development and characterisation of 140 new microsatellites in apple (*Malus x domestica* Borkh.). Mol Breed 10:217–241
19. Lodhi MA, Ye G-N, Weeden NF, Riesch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13
20. Lyra DA, Ismail S, Kurt KURB, Jed Brown J (2016) Evaluating the growth and yield of *Salicornia bigelovii* populations under full strength seawater irrigation using multivariate analyses. Aust J Crop Sci 10:1429–1441
21. Martinez-Calvo J, Gissett AD, Alamr MC, Hernandorena R, Romero C, Llacer G, Badenes ML (2008) Study of a germplasm collection of *loquat* (*Eriobotrya japonica* Lindl) by multivariate analysis. Genet Resources Crop Ecol 5:695–703
22. Milic D, Lukovic J, Dan M, Zoric L, Obreh T, Veselic S, Anasiov G, Petanidou T (2011) Identification of salicornia population: anatomical characterization and RAPD fingerprinting. Arch Biol Sci 63:1087–1098
23. Mohammadi A (2012) Collection and determination of genetic diversity of *Salicornia* plant using markers AFLP. Faculty of Agriculture, vol. Master. Shiraz University, Shiraz p108
24. Naghavi MR, Ghareyazie, B. and Hossenei Salekdeh, GH (2007) Molecular method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13
25. Naghavi MR, Ghareyazie, B. and Hossenei Salekdeh, GH (2007) Molecular method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12:6–13
26. Noroozloo YA, Mirjalili MH, Nazeri V, Araghi ARM (2015) Evaluation of some ecological factors, morphological traits and essential oil productivity of *Stachys lavandulifolia* Vahl in four provinces of Iran. Iran J Med Aromatic Plants 30:985–997
27. Quilot B, Kervella J, Génard M, Lescourret F (2005) Analysing the genetic control of peach fruit quality through an ecophysiological model combined with a QTL approach. J Exp Bot 56:3083–3092
28. Rezapour M, Fattahi, M. (2018) Evaluation of diversity in some morphological characteristics and essential oil antioxidant activity among of wild-growing populations of Golpar Heracleum persicum Desf. ex Fischer, from Northwest of Iran. Eco-Phytochem J Med Plants 6:40-55
29. Shepherd KA, Macfarlane TD, Colmer TD (2005) Morphology, anatomy and histochemistry of salicornioidae (chenopodiaceae) fruits and seeds. Ann Bot 95:917–933
30. Shuyskaya E, Toderich K, Gismatullina L, Rajabov T, Khohlov S (2017) Genetic diversity of two annual Salsoś species (Chenopodiaceae) among habitat types in desert plant communities. Biologia (Poland) 72:267–276
31. Solouki M, Mehdikhani H, Zeinali H, Emamjomeh AA (2008) Study of genetic diversity in Chamomile (Matricaria chamomilla) based on morphological traits and molecular markers. Sci Hortic 117:281–287
32. Spataro G, Tiranti B, Arcaleni P, Bellucci E, Attene G, Papa R, Zeuli PS, Negri V (2011) Genetic diversity and structure of a worldwide collection of Phaseolus coccineus L. Theor Appl Genet 122:1281–1291
33. Venkatesan J, Ramu V, Sethuraman T, Sivagnanam C, Doss G (2021) Molecular marker for characterization of traditional and hybrid derivatives of Eleusine coracana (L.) using ISSR marker. J Genet Eng Biotechnol 19:178

**Publisher’s Note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.