Assessment of genetic diversity in *Salvadora persica* L. based on inter simple sequence repeat (ISSR) genetic marker

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**Abstract**

Studies on the genetic variation in marginal populations and differentiation between them are essential for assessment of best gene conservation strategies and sampling schemes. In this study, ISSR markers were used to establish the level of genetic relationships and polymorphism 50 genotypes of *Salvadora persica* collected from 6 different regions of Hormozgan province. The ISSR analysis with 9 anchored primers also generated 105 scorable loci, of which 85 were polymorphic (80.95%). Parameters of genetic diversity and its partitioning were calculated. The genetic analysis demonstrated that *S. persica* maintain relatively high genetic diversity (PIC was 0.63, Na was 1.27 and Ho and He were 0.15 and 0.17 respectively). The coefficient of genetic differentiation among populations based on FST equaled 0.20. Genetic identities between population’s pairs were high (mean I = 0.88). These values are high as compared with other widespread congener species. Cluster analysis based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) revealed 3 main clusters for the ISSR data. The levels of genetic diversity maintained within populations of *S. persica* indicate that an appropriate sampling design for ex situ safeguarding should capture the majority of genetic diversity found within these taxa to help ensure the long term viability of this species. Furthermore, it could be inferred that ISSR markers are suitable tools for the evaluation of genetic diversity and relationships within the Salvadora persica.

**1. Introduction**

Conservation of medicinal plants is an important component of biodiversity conservation [1]. For successful conservation and breeding of a medicinal species, it is important to evaluate its genetic diversity [2]. The conservation and use of plant genetic resources are essential to the continued maintenance, improvement of agricultural and forestry production in addition to thus, sustainable development and poverty alleviation. Technological advances in molecular biology have contributed substantially to our understanding of plant genetic diversity. *Salvadora persica* L. (Family: Salvadoraceae) is a subtropical tree or evergreen shrub and perennial halophyte [3]. Its Arabic name is Shajar-el-Miswak, Al-arak or Khardal. The English name is Tooth brush tree or Mustard tree [4]. It has many medicinal potentialities and is widely used in folk medicine especially in Eastern Africa and in Middle East. Despite its multipurpose utility *S. persica* has not received due attention of cytologists and geneticists to estimate the range and quantum of existing natural variation which is essential for framing meaningful genetic improvement program, aimed at sustainable utilization. Associated with decreasing population sizes are increased extinction risk from stochastic factors (e.g. food, drought), environmental factors (e.g. decreased pollinator service) and genetic factors (e.g. increased inbreeding and decreased genetic diversity) [5]. Knowledge of the level and distribution of genetic variation both within and among populations facilitates the conservation of gene resources and helps in developing strategies for conservation and tree improvement programs [6]. For genetic variation studies, the choice of appropriate genetic markers assumes a great significance. Although morphological characters have been used traditionally to characterize levels and patterns of diversity, these traits alone represent only a small portion of plant genome and also influenced by the environmental factors [7].

Molecular marker technique is an efficient tool for genetic variation evaluation in plants [8–10]. Up to now, various types of molecular markers have been developed, widely applied, and can be classified into several groups, such as hybridization-based molecular markers (RFLP), polymerase chain reaction (PCR)-based...
markers (random amplified polymorphic DNA (RAPD) amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and inter simple sequence repeats (ISSR)), and DNA sequence based -markers (single-nucleotide polymorphism (SNP)) [11,12]. Their application is to bring out the genome-wide variability though each of which combines some desirable properties and is based on different principles [12].

The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP analysis and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism [13–15]. Also SNPs seem very exciting markers but they require extensive investment in equipment and manpower. Hence, SNPs are highly unlikely to be taken up by the national agricultural institutes and Universities in developing countries [9].

The application of inter-simple sequence repeats (ISSRs) is a quick, cost-effective and high reproducibility technique based on PCR amplification of inter-microsatellite sequences to target multiple loci in the genome. They also require small amounts of DNA and no prior genomic information is required for their use. ISSRs are found to be useful in analyses of genetic variation below the species level, mainly in studying population structure and differentiation [12,16].

Extraction and purification of high quality DNA is essential for PCR based DNA amplification. Plants of arid regions contain large amount of secondary metabolites which may interfere in the extraction and purification of PCR amenable DNA amplification. In current study PCR amenable genomic DNA has been extracted and used for the genetic diversity analysis by ISSR.

In this paper, we report the level and pattern of genetic variability in six populations of S. persica in their natural range in south of Iran. The objective is to provide valuable information for future conservation and breeding programs on S. persica.

2. Material and methods

2.1. Plant material

Leaves of S. persica were collected from 6 different regions of Hormozgan province of Iran in September 2016 and transferred to –80 °C freezer in Biotechnology Research Center of Hormozgan University (Table 1 and Fig. 1).

2.2. DNA isolation and quantification

S. persica was notoriously recalcitrant to many common DNA extraction methods due to high glucosinolates level that hindered the isolation of its DNA with a sufficient quantity and high purity. Total genomic DNA was isolated by modified Doyle and Doyle [17] method and Cetyl Trimethyl Ammonium Bromide (CTAB) method with minor modification in quantity of leaf tissue taken, concentration of NaCl, incubation duration and temperature. 0.07–0.2 g of fresh leaves were crushed using liquid nitrogen. The powder obtained was transferred to 2 mL micro centrifuge tube with extraction buffer [1.4 M NaCl, 100 mM Tris HCl (pH-8), 20 mM EDTA, 2% CTAB, 1% PVP]. This mixture was incubated at 70 °C for 1 hour. The tubes were centrifuged at 8000 rpm, for 5 min at 4 °C and the supernatant was transferred to a clean 2 mL micro centrifuge tube. An equal volume of chilled chloroform: isoamyl alcohol (24:1) was added, mixed by gentle inversion and centrifuged at 10,000 rpm for 10 min at 4 °C. The half of supernatant taken up and 5 mM salt (NaCl) was added. Then micro tubes were placed on ice for 30 minutes. 0.1 mL of supernatant taken up and sodium acetate was added.

DNA was precipitated by adding equal amount of pre chilled iso-propanol, incubated it at – 20 °C for 1 h followed by centrifugation at 12,000 rpm, for 15 min at 4 °C. Supernatant was discarded and pellet was washed by using 70% ethanol. The pellet was air dried and re-suspended in 100 μL of Tris–EDTA (TE) buffer. Each sample was diluted to 50 ng/μL with TE buffer (10 mM Tris–HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0) and stored at 4 °C for further use. The quantity and quality of DNA was analyzed by spectrophotometrically method at 260 nm and 280 nm. Purity of DNA was also checked by using 1% agarose gel, visualized under a UV transilluminator and photographed using the SYNGENE (Fig. 2).

2.3. ISSR amplification

Amplification reaction according to the Zeitkiewicz et al. [16] with slight variations in the final volume was 25 μL. Volume con-

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Table 1: Location of S. persica genotypes collected from Hormozgan province of Iran.

| Population | Population size | Location | Rainfall | Latitude (E) | Longitude (N) | Above sea level (m) |
|------------|-----------------|----------|----------|--------------|---------------|---------------------|
| 1          | 10              | Gachin   | 78.69    | 27° 54’      | 55° 54’       | 16                  |
| 2          | 10              | Geno     | 132.35   | 27° 21’      | 56° 9’        | 579                 |
| 3          | 10              | Minab    | 132.35   | 27° 8’       | 57° 4’        | 46                  |
| 4          | 9               | Sirik    | 264      | 26° 32’      | 57° 07’       | 25                  |
| 5          | 3               | Jask1    | 92.66    | 25° 43’      | 5° 58’        | 15                  |
| 6          | 8               | Jask2    | 92.66    | 25° 33’      | 58° 54’       | 11                  |

Fig. 1. Geographical distribution of selected S. persica genotypes in Hormozgan province as has been described in Table 1.

Fig. 2. Quality of the DNA in 1% (w/v) agarose gels in 0.5% TBE buffer.
Details of banding pattern revealed through ISSR primers. 

**Table 2**

Inter simple sequence repeat (ISSR) primers used in 50 *S. persica* genotypes.

| Primer   | Sequence                | %GC | Annealing temperature (°C) |
|----------|-------------------------|-----|---------------------------|
| UBC817   | CACACACACACACACAA       | 47.1| 52.0                      |
| UBC823   | TGGTGTGTGTGTGTGC        | 52.9| 54.2                      |
| UBC824   | TCTCTCTCTCTCTCTCG       | 52.9| 54.6                      |
| UBC825   | ACACACACACACACACT       | 52.9| 52.2                      |
| UBC834   | AGAGAGAGAGAGAGACT       | 44.4| 52.0                      |
| UBC836   | AGAGAGAGAGAGAGAGCA      | 44.4| 53.0                      |
| UBC841   | GAGAGAGAGAGAGAGGCC      | 50  | 56.2                      |
| UBC844   | CTTCTCTCTCTCTCTAC       | 50  | 52.0                      |
| UBC809   | AGAGAGAGAGAGAGAGG       | 47.4| 52.9                      |
| UBC810   | GAGAGAGAGAGAGAGAT       | 47.1| 50.4                      |
| UBC816   | CACACACACACACACAT       | 47.1| 50.4                      |
| UBC806   | ACACACACACACACACCA      | 47.1| 52.5                      |
| UBC801   | GAGAGAGAGAGAGAGAC       | 52.9| 52.4                      |
| UBC808   | AGAGAGAGAGAGAGAGGC      | 52.9| 52.4                      |
| ISSR3    | CTTCTCTCTCTCTCTGCC      | 50.2| 53.9                      |
| ISSR1    | CACACACACACACACT        | 47.1| 51                        |
| ISSR2    | GAGAGAGAGAGAGAGAGG      | 52.9| 49                        |
| ISSR5    | AGTGTAGTGTAGTGC         | 52.9| 45                        |
| ISSR6    | GATGTAGATAGATAGATAGA    | 44.1| 47                        |
| ISSR7    | TCTCTCTCTCTCTCTCT       | 50  | 45                        |

Electrophoresis

PCR products were electrophoresed on 1.5% (w/v) agarose gels, in 1X TBE Buffer at 100 V for 1.5 h. Molecular weight marker (100 bp DNA ladder) was also loaded on either side of the gel. Gels with amplification fragments were visualized and photographed under UV light and the intensity of bands was observed. Out of 20 primers used 9 primers produced recognizable bands (Table 2). The photographs of gels were used to score data for ISSR markers.

Data analysis

Each DNA fragment amplified by a given primer was considered as a unit character and the ISSR fragments were scored as present (1) or absent (0). Molecular data by software GeneAleX, POPGENE and DARWIN were analyzed. For each primer, No. of Effective Alleles, expected heterozygosity (He), observed heterozygosity (Ho), fixation index, percentage of polymorphic loci (95% criterion) (p); observed number of alleles per locus (Na), effective number of alleles per locus (Ne) and F-statistics (Fst and Fis) was estimated. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The polymorphism information content (PIC) was calculated by the formula:

\[ PIC = 2p_i (1 - p_i) \]

where, \( p_i \) is the frequency of occurrence of polymorphic bands in different primers. Genetic diversity measures were tested using Nei’s [19] gene diversity statistics. Shannon’s information index (I) was also used to examine partitioning of genetic diversity within and among populations. Analysis of molecular variance (AMOVA) was also used to obtain the variation component among individuals within populations and among populations.

For each population, genetic diversity parameters were assessed in terms of mean number of alleles per locus (na), expected heterozygosity (He) and unbiased expected heterozygosity (uhE). The genetic relationships among all populations were assessed by estimating Nei’s genetic distances for all population pairs.

### Table 3

Details of banding pattern revealed through ISSR primers.

| Primer   | No. of bands | No. of polymorphic bands | Percentage | No. of monomorphic bands | PIC  |
|----------|--------------|--------------------------|------------|--------------------------|------|
| UBC811   | 17           | 14                       | 82.35      | 3                        | 0.683|
| UBC825   | 12           | 10                       | 83.33      | 2                        | 0.703|
| UBC834   | 13           | 11                       | 84.61      | 2                        | 0.644|
| UBC836   | 13           | 12                       | 92.30      | 1                        | 0.847|
| UBC844   | 8            | 8                        | 100.00     | 0                        | 0.725|
| UBC809   | 7            | 3                        | 42.85      | 4                        | 0.140|
| UBC808   | 10           | 7                        | 70.00      | 3                        | 0.564|
| UBC856   | 12           | 10                       | 83.33      | 2                        | 0.752|
| UBC810   | 13           | 10                       | 76.92      | 3                        | 0.613|
| Mean     | 11.6         | 9.4                      | 80.95      | 2.22                     | 0.636|

### 3. Result

#### 3.1. ISSR amplification

Nine of 20 RAPD primers used could produce reproducible bands, ranging in size from 280 to 3000 bp. In total, 85 polymorphic bands/loci and 20 monomorphic bands were obtained, with an average of 37.46 bands for each primer (Table 3). The mean value of polymorphism for ISSR loci was 80.95%, with the highest value for the primer UBC844 (100%) and the lowest value for the UBC809 primer (42.85%). The PIC value ranged from 0.14 (UBC809) to 0.84 (UBC836) with a mean of 0.63. The represented agarose gel electrophoresis pictures of PCR fragments were shown (Fig. 3).

The highest number of effective alleles was obtained for the UBC844 primer (1.45) while the lowest value was obtained with the UBC856 primer (1.30). The mean value of the Shannon index as a measure of genetic diversity was 0.35 for all primers, with the highest value for the UBC844 primer (0.44) and the lowest value for the UBC809 primer (0.27). The highest value of Nei’s genetic diversity (H) for ISSR loci was found for the UBC844 (0.28) primer and the lowest value was observed with the UBC856 primer (0.19) (Table 4).

The expected heterozygosities (He) and observed heterozygosities (Ho) were relatively high and varied from 0.122 to 0.21 and from 0.13 to 0.26 with an average of 0.16 and 0.18 respectively (Table 4). The overall FIS values were nearly equal to zero, indicating that the populations were in Hardy-Weinberg equilibrium. Amount of genetic differentiation at 85 polymorphic loci in six nat-
ural populations of *S. persica* was calculated in terms of Wright’s Fixation Index (FST). The heterozygosity at the polymorphic loci was partitioned within population as well as between population components. The value of total heterozygosity (HT) ranged from 0.18 at UBC809 to 0.27 at UBC834. The value of FST at 85 polymorphic loci ranges from 0.11 (UBC809) to 0.35 (UBC811) with average value equaling 0.20 (Table 4).

### 3.2. Genetic diversity as revealed by percent polymorphism, Shannon and gene diversity values

From all the population studied, Gachin 56%, Geno 51.43%, Minab 55.24%, Jask1 49.5%, Jask2 26.67 were polymorphic. Gachin showed more percent polymorphism; while the least polymorphism was detected in population from Jask2 region (Table 5).

![Amplified PCR products generated using UBC825 primer for 50 genotypes of *S. persica.*](Image)

**Table 5**

| Population | % Polymorphic |
|------------|---------------|
| Gachin     | 56.19         |
| Geno       | 51.43         |
| Minab      | 55.24         |
| Jask1      | 49.5          |
| Jask2      | 26.67         |
| Sirik      | 48.57         |
| Error      | 4.43          |
| Mean       | 47.94         |

![Table 4](Image)

### Table 4

| Primer | Band frequency | Effective no. of alleles (Ne) | Nei’s genetic diversity (H) | Shannon’s information index | Expected heterozygosity He | Observed heterozygosity Ho | Total heterozygosity | FST | FIS |
|--------|----------------|-------------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|----------------------|-----|-----|
| UBC811 | 0.4517         | 1.3691                        | 0.2399                      | 0.3748                      | 0.1290                     | 0.1370                     | 0.2505               | 0.3517 | -0.0620 |
| UBC825 | 0.4250         | 1.3121                        | 0.2045                      | 0.3267                      | 0.1475                     | 0.1570                     | 0.2092               | 0.1875 | -0.0644 |
| UBC834 | 0.5100         | 1.4451                        | 0.2731                      | 0.3942                      | 0.1803                     | 0.1932                     | 0.2725               | 0.2372 | -0.0715 |
| UBC836 | 0.3046         | 1.3176                        | 0.2203                      | 0.3621                      | 0.1851                     | 0.2643                     | 0.2259               | 0.1550 | -0.4278 |
| UBC844 | 0.3900         | 1.4597                        | 0.2866                      | 0.4420                      | 0.2177                     | 0.2401                     | 0.2925               | 0.2654 | -0.1028 |
| UBC809 | 0.9228         | 1.3656                        | 0.1973                      | 0.2798                      | 0.1312                     | 0.1383                     | 0.1816               | 0.1189 | -0.0541 |
| UBC808 | 0.568          | 1.3768                        | 0.2215                      | 0.3381                      | 0.1624                     | 0.1783                     | 0.2366               | 0.1783 | -0.0979 |
| UBC856 | 0.3766         | 1.3068                        | 0.1973                      | 0.3157                      | 0.1506                     | 0.1620                     | 0.2089               | 0.1752 | -0.0756 |
| UBC810 | 0.5184         | 1.3676                        | 0.2272                      | 0.3335                      | 0.1534                     | 0.1697                     | 0.2251               | 0.2158 | -0.1062 |
| Mean   | 0.4752         | 1.3689                        | 0.2297                      | 0.3518                      | 0.1615                     | 0.1820                     | 0.2336               | 0.2094 | -0.1180 |

No unique bands were observed for either the accessions or the populations (Table 6). Among the *S. persica* genotypes evaluated using ISSR marker, samples from Gachin exhibited the highest gene diversity (*H* = 0.28). The average gene diversity for the total population (*HT*) was 0.167 (Table 6). The highest and lowest numbers of effective alleles, Shannon index and Nei’s genetic diversity were also obtained for Gachin and Jask2 populations. Gachin population showed the highest percentage of polymorphism, the highest numbers of effective alleles, Shannon index, and Nei’s genetic diversity (Table 6). The lowest values of percentage polymorphism, number of effective alleles, Shannon index, and genetic diversity were obtained for the Jask2 population (Table 6).

Nei’s genetic distance determined between pairs of populations for ISSR data (Table 7) revealed that Sirik and Jask2 populations had the greatest genetic distances, while Geno and Minab populations had the shortest genetic distances.
3.3. Analysis of molecular variance

The partitioning of variations within and among populations was analyzed by AMOVA, which revealed occurrence of 26% of total genetic variations among the different regions. It implies that remaining 74% was due to variations within a region (Table 8).

3.4. Principle component analysis (PCOA)

All the data obtained using the nine ISSR primers were used in PCOA analysis using Jaccard’s coefficient of similarity. The first three PC1, PC2 and PC3 explained with total variation of 64.81 (24.67, 20.67 and 19.48, respectively) (Table 9). Therefore, the grouping of individuals using two coordinates is indicated in Fig. 4. PCoA analysis also categorized genotypes into three different groups without following their genetic information (Fig. 4).

3.5. Clustering analysis

UPGMA tree construction methods were used to construct dendrogram for six populations. The dendrogram derived from UPGMA analysis of the whole ISSR data based on regions of collection of S. persica revealed three major groups (Fig. 5). The first cluster contained Geno and Gachin; while the second cluster contains Sirik and Jask origins. The final major cluster contained the M group (Fig. 5). Most of the individual genotypes collected from the same region tend to spread all over the tree without forming their own grouping. The wider distribution of S. persica genotypes all over the tree showed the low divergence among population from different localities.

4. Discussion

Genetic analysis using genetic markers in plant have shown the occurrence of very high genetic diversity, especially with in populations, whereas low differentiation has been observed among populations [20]. Genetic variation and genetic relationships were efficiently determined using the ISSR markers. Polymorphism in six populations of S. persica collected from different locations of Hormozgan was observed for estimating the genetic variability present in this species. The level of ISSR diversity was quite high among the six populations of S. persica studied (Na = 1.27; Ne = 1.47; Ho = 0.15; He = 0.17). In comparison to that found in previous reports S. persica had a mean expected heterozygosity (He = 0.15)

Table 6
Summary of genetic variations as revealed through ISSR markers among 6 populations of S. persica.

| Population | Na  | Ne  | Ne/Na | I   | H   | He  | uHe | Fis |
|------------|-----|-----|-------|-----|-----|-----|-----|-----|
| Gachin     | 1.3141 | 1.5619 | 0.8413 | 0.1895 | 0.2874 | 0.190 | 0.199 | -0.0473 |
| Geno       | 1.2831 | 1.5143 | 0.8461 | 0.1693 | 0.2572 | 0.169 | 0.178 | -0.0532 |
| Minab      | 1.3115 | 1.5524 | 0.8471 | 0.1817 | 0.2742 | 0.182 | 0.191 | -0.0494 |
| Sirik      | 1.2674 | 1.4857 | 0.8530 | 0.1580 | 0.2396 | 0.158 | 0.167 | -0.0569 |
| Jask1      | 1.2666 | 1.4952 | 0.8471 | 0.1599 | 0.2437 | 0.160 | 0.171 | -0.0687 |
| Jask2      | 1.1791 | 1.2667 | 0.9308 | 0.1032 | 0.1524 | 0.103 | 0.124 | -0.2038 |
| Mean       | 1.2703 | 1.4793 | 0.8606 | 0.1602 | 0.2424 | 0.1566 | 0.1716 | -0.0798 |

Table 7
Nei’s genetic distance between pairs of S. persica populations.

| Population | Gachin | Geno | Minab | Sirik | Jask1 | Jask2 |
|------------|--------|------|-------|-------|-------|-------|
| Gachin     | 0      | 0.071 | 0.109 | 0.078 | 0.087 | 0.195 |
| Geno       | 0.071  | 0    | 0.060 | 0.086 | 0.085 | 0.203 |
| Minab      | 0.109  | 0.060 | 0     | 0.085 | 0.100 | 0.169 |
| Sirik      | 0.078  | 0.086 | 0.085 | 0.100 | 0.062 | 0.204 |
| Jask1      | 0.087  | 0.085 | 0.085 | 0.100 | 0    | 0.099 |
| Jask2      | 0.195  | 0.203 | 0.169 | 0.204 | 0.099 | 0     |

Table 8
Analysis of molecular variance using ISSR markers.

| V.S | df | MS    | SS   | Est. Var. | Value (%) |
|-----|----|-------|------|-----------|-----------|
| Among Pops. | 5  | 43.302 | 216.511 | 3.908 | 26%       |
| Within Pops. | 44 | 11.322 | 498.189 | 11.322 | 74%       |
| Total        | 49 | 714.700 | 15.230 | 100%     |           |

Table 9
Analysis of principle component (PCO) using ISSR markers.

| Factor | 1 | 2 | 3 |
|--------|---|---|---|
| %      | 24.67 | 20.67 | 19.48 |
| % cumulative | 24.67 | 45.33 | 64.81 |
higher than that of other widespread tree species such as: *Narcissus longispatus*, *He* = 0.139 [21]; *Ulmus laevis*, *He* = 0.088 [22].

Genetic identities are influenced both by polymorphic loci and the number of monomorphic loci; Out of 106 loci, 85 loci exhibited polymorphism. *S. persica* showed high genetic diversity as indicated by percent polymorphic loci (*p* = 80%). Brito et al. [23] studied the genetic diversity between *Varronia curassavica* accessions, using 14 primers, and obtained 149 bands with 97.98% polymorphism, Shannon index of 0.42, *H* of 0.27; values close to the ones found in the present study.

FST values based on polymorphic loci also provide an additional perspective on population divergence. It was found that 98% (*FST* = 0.20) of the genetic variations at polymorphic loci was found with in *S. persica* populations thus indicating very little divergence.

The Shannon index may vary from 0 to 1, and lower genetic diversity is represented by values closer to zero [24]. Several studies on natural populations indicate the percentage of the polymorphic locus as an important measure of genetic diversity; however, despite being commonly used, a variation in these values is observed [10]. According to Nei [19], the proportion of polymorphic locus is not a significant measure of the genetic variation, and thus the parameter of genetic diversity (*He*) is more appropriate.

Values different from zero were expected in relation to the mean value of genetic diversity (0.15) and to the Shannon index (0.16), since under natural conditions, the Hardy-Weinberg equilibrium is not expected. This is because individuals are likely to incorporate new alleles using crosses, and lose alleles using genetic drift [25].

Knowledge on the genetic diversity of the selected individuals is of ultimate importance, since it contributes to the information on the species and allows the selection of genotypes to be included in future conservation programs. Thus, the most divergent genotypes can be selected, as well as the most similar, according to the research interest.

The maintenance of this level of genetic diversity should allow this species to maintain its ability to adapt to novel environmental changes. Finally, the great homogeneity of the diversity indices suggests that the species has sufficient capacity to oppose the natural loss of genetic variability by drift.

ISSR markers have demonstrated their efficiency in the study of genetic variability for several other species. Many studies have proved the effectiveness of this marker in articles on genetic diversity and characterization of accesses between and within populations, such as those with, *Capparis spinosa* L. [26], *Pitcairnia flammea* [27], *Erythrina velutina* [28] and *Croton tetradenius* [20].

The results of this study will help the research group to select plants for a collection of *S. persica* in the germplasm bank of medicinal and aromatic plants of the Hormozgan University of Iran.

5. Conclusions

The present study reveals that ISSR is informative marker for characterizing *S. persica* diversity at DNA level. The informative primers identified in our studies could be useful in genetic analysis of Iranian *S. persica* germplasm, seed bank management and utilization. Genetic variations among genotypes could be useful in selecting parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes. Further studies for sequencing the ISSR fragments may help the understanding of several genes regulation. Furthermore, it is useful to conduct comparative studies with different marker systems.

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