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Research Article

Genetic Diversity Assessment and Identification of New Sour Cherry Genotypes Using Intersimple Sequence Repeat Markers

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Iran is one of the chief origins of subgenus *Cerasus* germplasm. In this study, the genetic variation of new Iranian sour cherries (which had such superior growth characteristics and fruit quality as to be considered for the introduction of new cultivars) was investigated and identified using 23 intersimple sequence repeat (ISSR) markers. Results indicated a high level of polymorphism of the genotypes based on these markers. According to these results, primers tested in this study specially ISSR-4, ISSR-6, ISSR-13, ISSR-14, ISSR-16, and ISSR-19 produced good and various levels of amplifications which can be effectively used in genetic studies of the sour cherry. The genetic similarity among genotypes showed a high diversity among the genotypes. Cluster analysis separated improved cultivars from promising Iranian genotypes, and the PCoA supported the cluster analysis results. Since the Iranian genotypes were superior to the improved cultivars and were separated from them in most groups, these genotypes can be considered as distinct genotypes for further evaluations in the framework of breeding programs and new cultivar identification in cherries. Results also confirmed that ISSR is a reliable DNA marker that can be used for exact genetic studies and in sour cherry breeding programs.

1. Introduction

The sour cherry belongs to the family of Rosaceae, subfamily Prunoideae, genus *Prunus,* and subgenus *Cerasus* [1]. It is an allotetraploid species ($2n = 4x = 32$) resulting from a natural hybridization between *Prunus avium* L. (Sweet Cherry) and *Prunus fruticosa* Pall. (Ground Cherry) [2]. This species is reported to have originated from the area that comprises Asia Minor, Iran, Iraq, and Syria [3] and has been used as rootstock and also in breeding programs for developing new commercial cultivars, dwarf, and resistant rootstocks [4–6]. According to the FAO database, Iran ranked third worldwide in 2011 for cherry production after Turkey and the USA with a total of 241 thousand tons produced [7].

Genetic variability is a prerequisite for any plant breeding program [8]. As an origin of the subgenus *Cerasus*, Iran has rich cherry germplasm resources. Using diverse *Cerasus* subgenus resources to broaden the genetic base of cherry cultivars and rootstocks and improving them for development of the cherry industry are important goals for cherry breeders in Iran [9, 10]. Therefore, it is necessary to characterize and preserve these genotypes and cultivars [11]. DNA markers are very useful in distinguishing between accessions and in investigations of genetic diversity or relatedness [12]. Different DNA markers have been broadly used to analyze genetic variations in *Prunus*, such as RAPD [13], AFLP [14], and SSR [15]. The major limitations of these methods are low reproducibility of RAPD, high cost of AFLP, and the need to know the flanking sequences to develop species specific primers for SSR polymorphism [16]. In comparison, intersimple sequence repeats (ISSRs) have been developed that overcome most limitations [17]. These markers involve

Table 1: Accessions of genotypes used in this study and their origins.

Number	Accession	Origin
1	KaThLa1SSGe21	Lavasan
2	Hamedan	Hamedan
3	KaTaJo2Ge9	Taleghan
4	KaThMe3Ge19	Chalus
5	KaThLa8Ge31	Lavasan
6	KrRIV4C20	Kerman
7	EsASC1V1SS1	Esfahan
8	KaThLa3Ge23	Lavasan
9	Bulgar	Bulgaria
10	Montmorency	France
11	Erdi Jubileum	Hungary
12	Erdi Botermo	Hungary

PCR amplification of DNA by a single, 16–18 bp long primer composed of a repeated sequence [18]. ISSR gives multilocus patterns which are very reproducible, abundant, and polymorphic in plant genomes [19]. It is useful in areas of cultivar identification, germplasm characterization, phylogenetic relationship analysis, and genetic linkage mapping in a wide range of plant species [20, 21], including cherries [20–24].

In the present study, ISSR analysis was used to evaluate the genetic variation new Iranian sour cherries, with the aim of using the ISSR technique to these genotypes for the use them in cherries breeding programs as well as for conservation management of subgenus Cerasus germplasm in Iran. It is hoped that with supplementary tests and analyses we can identify and introduce new sour cherry cultivars to the fruit industry.

2. Materials and Methods

2.1. Plant Materials. During the breeding programs, collection, and evaluation of local sour cherry germplasms from different regions in Iran in order to achieve proper cultivars and rootstocks, after five years visual observations, it was found that some of the genotypes had quite superior growth characteristics and fruit quality that they can be considered for the introduction of new cultivars [25]. These superior genotypes were chance seedlings, so they were selected according to the 5-year visual observations and grafted onto "Mahlab" rootstocks which were available in the fruit research collection of the Seed and Plant Improvement Institute in Kamal Abad, Karaj, Iran. Then 2-year determination of the genotypes also approved it and showed that these selected genotypes had such superior growth characteristics and fruit quality [26]. In this study, these selected genotypes (see Table 1) and 4 improved cultivars (Bulgar, Montmorency, Erdi Jubileum, and Erdi Botermo) were analyzed. These genotypes were 5 years old and had been planted at 4 × 5 m. The young leaves of these genotypes were collected during May 2012. The characterization of the accessions is shown in Table 1.

2.2. DNA Extraction and PCR Amplification. Total genomic DNA was extracted from the young leaves using a CTAB (hexadecyl trimethyl ammonium bromide) method, according to the protocol described in Saunders et al. [27]. Then, the DNA extract was suspended in 50 μL 1X TE buffer (1 M pH 8.0 Tris-HCl; 0.5 M pH 8.0 EDTA) and kept at –20°C. The quality and concentration of each DNA sample was determined using a NanoDrop spectrophotometer at 260, 280 nm (ND-1000, Co, USA) and running 3 μL DNA in 0.7% (w/v) agarose gels in 0.5X TAE buffer. 30 ISSR primers which were selected by Agricultural Biotechnology Research Institute Laboratory, Esfahan, Iran, according to [21, 22, 24, 28, 29] studies and synthesized by Metabion Co. (Germany) were used. These synthesized ISSR primers were initially screened and finally 23 primers were selected to be used in this study. The list of primers and their information are presented in Table 2. For PCR analysis, approximately 25 ng of genomic DNA was used in a 25 mL reaction containing 1X PCR reaction buffer, 2 mM $MgCl_2$, 0.8 mM dNTPs, 5 pmol of each primer, 1U Taq DNA polymerase (Fermentas, Lithuania), and DNA-free water. Amplifications were performed in a thermocycler (Applied Biosystems, Veriti, USA) programmed for a first denaturation step of 3 min at 94°C, followed by 40 cycles of 30 s at 94°C for denaturation, 30 s at 30–57°C (varied for each primer according to Table 2) for annealing, 1 min at 72°C for elongation, and final extension at 72°C for 5 min. They were then held at 4°C until the tubes were removed. Amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels at constant voltage (95 V) in 1X TAE buffer for approximately 90 min, stained with gelred, and photographed with UV light (Figure 1). The size of produced fragments was defined according to size marker (GeneRuler 1 kb DNA ladder, SM0311, Fermentas).

2.3. Data Analysis. Only reproducible and well-defined alleles were considered potential polymorphic markers. The alleles scored as present (1) or absent (0) with Phoretix Pro, ver. 10.4 software. In order to increase the accuracy of the scoring, each gel electrophoresis was scored in three replications. The cophenetic correlation coefficient (CCC) was calculated and the similarity matrix was used for the cluster analysis and construction of dendrogram using the Simple Coefficient and Unweighted Pair-Group Method with Arithmetic average (UPGMA) [30] and for genetic relationships among subgenera and sections, Principal Coordinate Analysis (PCoA) and three-dimensional projection of genotypes (3D) [31] were used using the NTSYS software ver. 2.02 [32]. The following parameters were calculated for each primer: number of total alleles per locus, number of polymorphic alleles, polymorphism percentage, Polymorphism Information Content (PIC), average gene diversity (Hi), and fragment size. Polymorphism percentage was calculated using the ratio of number of polymorphic alleles to total alleles [33]. PIC was calculated as described by Warburton and Crossa [34] (PIC $= 1 - \sum (P_i/P_k)^2$), where P_i is the proportion of the population carrying the ith allele calculated for each microsatellite locus and P_k is total alleles. Average gene diversity was calculated by direct counts for the putative locus, identified by each primer described by the IPGRI and Cornell University [33] method ($H_i = 1 - p2 - q2$), where p and q are the frequency of the ith allele.

Table 2: 23 ISSR primers used in this study and their results.

Number	Primers	Sequence (5′-3′)	TA[1] (°C)	Total number of alleles (a)	Number of polymorphic alleles (b)	% Polymorphism $(b/a) \times 100$	PIC[2]	Average gene diversity (Hi)	Band size range (bp)
1	ISSR-1	GTGGTGGTGGC	30	16	16	100	0.90	0.45	600–1500
2	ISSR-2	GAGAGAGAGAGAGAGAT	48	21	21	100	0.94	0.40	400–1500
3	ISSR-3	CTCTCTCTCTCTCTCTG	47	25	24	96.00	0.94	0.43	700–2500
4	ISSR-4	GAGAGAGAGAGAGAGATG	52	26	26	100	0.94	0.37	400–1400
5	ISSR-5	AGAGAGAGAGAGAGAGTT	52	21	21	100	0.94	0.36	400–2100
6	ISSR-6	CTCTCTCTCTCTG	39	29	29	100	0.94	0.40	400–2200
7	ISSR-7	CTCTCTCTCTCTCTCTTG	47	18	17	94.44	0.93	0.37	700–1700
8	ISSR-8	CACACACACACAAC	39	25	24	96.00	0.95	0.40	700–2300
9	ISSR-9	CACACACACACAGT	39	18	18	100	0.89	0.39	700–2800
10	ISSR-10	CACACACACACAGG	36	21	21	100	0.94	0.37	400–1700
11	ISSR-11	CACACACACACAAG	30	23	23	100	0.94	0.43	700–3000
12	ISSR-12	CACACACACACACACAGG	47	15	14	93.33	0.88	0.43	500–2100
13	ISSR-13	CTCTCTCTCTCTCTCTRG	53	29	29	100	0.96	0.34	400–2800
14	ISSR-14	DBDACACACACACACAC	55	29	29	100	0.95	0.42	400–2800
15	ISSR-15	HVHTCCTCCTCCTCCTCCTCCTCC	57	9	7	77.77	0.85	0.45	600–1800
16	ISSR-16	GAGAGAGAGAGAGAGAC	53	25	25	100	0.95	0.45	400–3200
17	ISSR-17	ACACACACACACACACC	53	17	17	100	0.90	0.44	600–2200
18	ISSR-18	GAGAGAGAGAGAGAGAYC	47	15	14	93.33	0.90	0.45	600–1800
19	ISSR-19	AGAGAGAGAGAGAGAGYT	52	29	29	100	0.95	0.41	280–1600
20	ISSR-20	ACACACACACACACACYG	53	16	16	100	0.90	0.43	400–2800
21	ISSR-21	CACACACACACACACART	48	24	24	100	0.94	0.39	600–1700
22	ISSR-22	GAGAGAGAGAGAGAGAYG	47	20	20	100	0.93	0.40	400–2200
23	ISSR-23	GAGAGAGAGAGAGAGACG	55	18	18	100	0.94	0.42	400–3200
	Mean	—	—	21.26	20.95	98.45	0.93	0.41	—
	Total	—	—	489	482	—	—	—	—

Note: D = (G, A, T), B = (G, T, C), H = (A, T, C), V = (G, A, C), R = (A, G), Y = (C, T), and N = (A, T, G, C).
[1]Annealing temperature. [2] Polymorphism Information Content.

3. Results

The results of ISSR fingerprinting of 12 sour cherry genotypes using 23 primers are given in Table 2. Results showed that 489 alleles were generated at 23 ISSR loci, 482 of which were polymorphic. The number of total alleles per locus varied from 9 (ISSR-15 loci) to 29 (ISSR-6, ISSR-13, ISSR-14, and ISSR-19 loci) alleles with an average of 21.26 across the genotypes. Other primers with a high number of alleles per locus were ISSR-4 (26 alleles) followed by ISSR-3, ISSR-8, and ISSR-16 (25 alleles). The average polymorphic alleles per primer were 20.95, and of the 23 loci, ISSR-6, ISSR-13, ISSR-14, and ISSR-19 loci had the highest polymorphic alleles (29 alleles), followed by ISSR-4 (26 alleles), ISSR-16 (25 alleles), and ISSR-3 and ISSR-8 (24 alleles). The polymorphism percentage for primers ranged from 77.77% to 100.0% with an average of 98.45% (Table 2).

PIC values ranged from 0.85 to 0.96 with an average of 0.93. The highest PIC, that is, an indicator of effectiveness of primers used in genetic diversity studies, was ISSR-13 (0.96) with 29 alleles, followed by ISSR-8, ISSR-14, ISSR-16, and ISSR-19 (0.95). The lowest PIC value belonged to ISSR-15 (0.85) with (7 alleles) (Table 2). Average gene diversity ranged from 0.34 to 0.45 with a mean of 0.41. Among the loci, the highest H_i value belonged to the ISSR-1, ISSR-15, ISSR-16, and ISSR-18 locus (0.45), while the lowest values belonged to the ISSR-13 loci (0.34) (Table 2). Fragment size of the 23 primers ranged from 280 to 3200 bp. The lowest ranges were those of ISSR-19 (280–1600) and ISSR-4 (400–1400), and the highest range was related to ISSR-16 and ISSR-23 (400–3200) bp (Table 2).

The highest cophenetic correlation coefficient based on ISSR data with a simple similarity coefficient was ($r = 0.91$) (Table 3). The high value of this coefficient indicates the

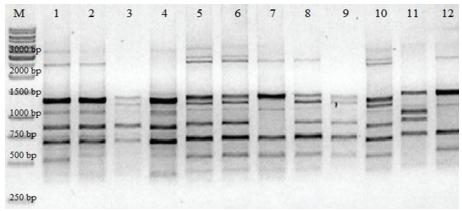

Figure 1: ISSR band profiles generated by the primer ISSR-16. Genotype number: see Table 1. M: weight marker: 1 kb DNA.

Table 3: Comparison of different methods for constructing similarity matrices and dendrograms.

Similarity coefficient	Cluster algorithm		
	UPGMA	UPGMC	Complete linkage
D	$r = 0.72$	$r = 0.42$	$r = 0.71$
J	$r = 0.74$	$r = 0.47$	$r = 0.73$
SM	$r = 0.91$	$r = 0.24$	$r = 0.79$

D: Dic [36]; J: Jaccard [37]; SM: Simple Matching [30].
UPGMA: Unweighted Pair-Group Method with Arithmetic average; UPGMC: Unweighted Pair-Group Method using Centroids.

suitability of the grouping method. The CCC is considered to be a good representation of the data matrix in the dendrogram if it is $0.80 \leq$ CCC [35]. Thus, the similarity matrix was used for the cluster analysis and construction of dendrogram using the simple coefficient and UPGMA.

Genetic similarity between genotypes was estimated using the simple similarity coefficient. The genetic similarity ranged from 0.56 to 0.77 with an average of 0.72. It showed a high diversity among genotypes. The EsASC1V1SS1 and Bulgar showed the lowest similarity (0.56), and the KaThMe3Ge19 and KaThLa8Ge31 showed a high similarity (0.77). Other genotypes such as Bulgar with KaTaJo2Ge9 (0.58), KrRIV4C20 (0.59), and Erdi Botermo (0.59) had low similarity. Moreover, KaThLa8Ge31 with KrRIV4C20 (0.75), Erdi Jubileum (0.75) and KaThLa3Ge23 (0.76), KaTaJo2Ge9 with KrRIV4C20 (0.76), and Ka ThMe3Ge19 with Erdi Botermo (0.76) had high similarity (Table 4).

A dendrogram based on the simple similarity coefficient and UPGMA analysis is presented in Figure 2. According to the dendrogram, genotypes were separated into two main clusters. Promising Iranian genotypes were separated from the improved cultivars and were further divided into eight subclusters with a genetic similarity of 0.72. One (I) included the KaThLa1SSGe21 genotype (from Lavasan), two (II) included Hamedan (Hamedan), and subcluster three (III) contained KaTaJo2Ge9 (Taleghan) and KrRIV4C20 (Kerman). These two genotypes were similar to the matrix 0.76, so it seemed that they had the same genetic origin. Subcluster 4 (IV) which was the biggest subcluster included KaThMe3Ge19 (Chalus), KaThLa8Ge31 (Lavasan), KaThLa3Ge23 (Lavasan), and Erdi Botermo (Hungary). Five (V) included the Erdi Jubileum cultivar (Hungary); six (VI) included EsASC1V1SS1 (Esfahan); seven (VII) included the Bulgar (Bulgaria); eight (III) included Montmorency cultivar

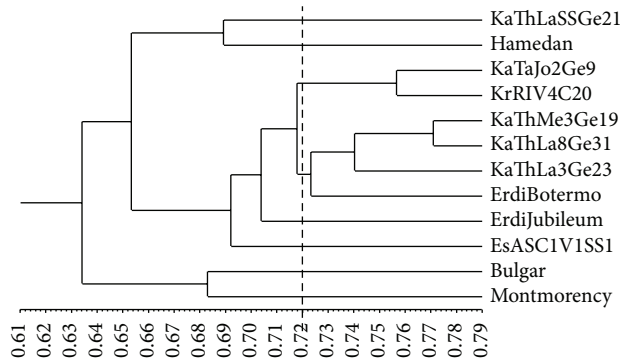

Figure 2: Dendrogram of ISSR analysis on sour cherry genotypes used in this study.

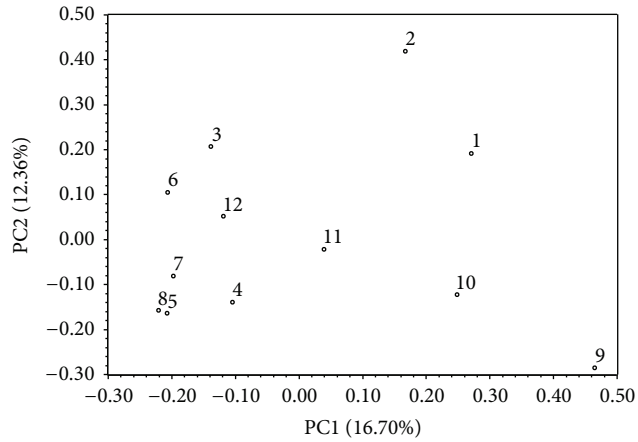

Figure 3: Principle coordinate analysis (PCoA) for 23 ISSR primers applied on sour cherry genotypes. Numbers represent the genotypes according to Table 1.

(France). According to these results, it seems that the genetic diversity of the sour cherry genotypes was not entirely a function of geographical variation; thus, the genotypes of the 4th subgroup with different distribution centers were placed in adjacent genetic groups.

The genetic relationship between these genotypes was also visualized by performing PCoA that showed two significant axes, which explained 16.70% and 12.36% of the total variance, respectively. The first two eigenvalues accounted for 29.06% of the variation observed in the genotypes (Table 5).

The two-dimensional plot generated from PCoA also supported the clustering pattern of the UPGMA dendrogram (Figure 3). This reflected a higher genetic diversity in the studied collection, which was confirmed by a principle component analysis of the genotype data. Results of this analysis showed a wider genetic distribution of genotypes in the studied collection.

In the three-dimensional PCoA plot, generally, similar groupings with the UPGMA dendrogram and additional information were also revealed. The first three principal axes accounted for 16.70%, 12.36%, and 10.52% of the total variation, respectively, indicating the complex multidimensional nature of ISSR variation (Figure 4).

Table 4: The simple similarity matrix for sour cherry genotypes based on ISSR data.

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.00											
2	0.69	1.00										
3	0.67	0.70	1.00									
4	0.67	0.64	0.71	1.00								
5	0.64	0.64	0.72	0.77	1.00							
6	0.63	0.67	0.76	0.70	0.75	1.00						
7	0.64	0.63	0.66	0.69	0.72	0.69	1.00					
8	0.63	0.63	0.73	0.72	0.76	0.71	0.73	1.00				
9	0.64	0.60	0.58	0.64	0.61	0.59	0.56	0.60	1.00			
10	0.66	0.66	0.66	0.66	0.69	0.64	0.64	0.66	0.68	1.00		
11	0.70	0.68	0.70	0.70	0.75	0.70	0.68	0.70	0.64	0.70	1.00	
12	0.65	0.67	0.73	0.76	0.72	0.71	0.67	0.69	0.59	0.67	0.68	1.00

Genotype number: see Table 1.

Table 5: Eigen values, percentage, and cumulative proportions for 11 principal coordinate axes, derived from ISSR markers application on sour cherry genotypes.

Axes	Eigen value	Percent	Cumulative
1	1.15	16.70	16.70
2	0.94	12.36	29.06
3	0.90	10.52	39.59
4	0.88	9.84	49.43
5	0.82	8.98	58.42
6	0.77	8.60	67.02
7	0.75	8.00	75.03
8	0.72	7.35	82.39
9	0.66	6.56	88.95
10	0.63	5.99	94.94
11	0.61	5.05	100.00

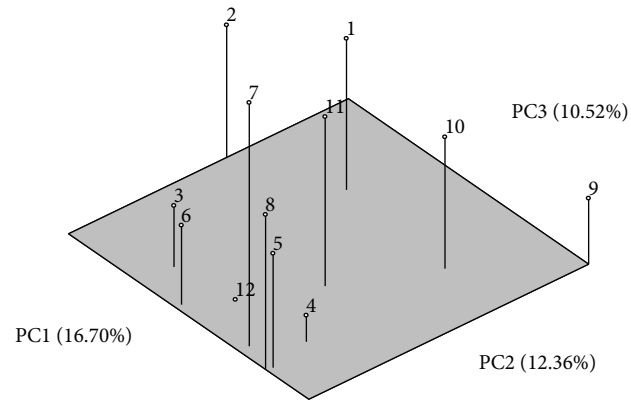

Figure 4: 3D plot for 23 ISSR primers applied on sour cherry genotypes. Numbers represent the genotypes according to Table 1.

4. Discussion

Iran is accepted as an origin and diversity center for cherries. In this study, we report for the first time the use of ISSR markers to assess the genetic characterization and to determine genetic relationships between promising Iranian sour cherry genotypes selected from different regions of Iran and improved cultivars. In the present study, 23 ISSR loci in sour cherry genotypes were assayed. The results obtained showed that ISSR primers can be effectively used for genetic diversity studies as well as genetic identification of sour cherries, which was also found in other investigations of cherries [20–24]. In fact, primers tested in this study produced good and various levels of amplifications.

A total of 489 amplified products were obtained using 23 ISSR primers. The average number of total alleles per locus identified in this study (21.26) was higher than the number identified in other studies of cherries. Average polymorphism percentage across all genotypes was 98.45% indicating a high level of polymorphism. Among the 23 loci, ISSR-6, ISSR-13, ISSR-14, and ISSR-19 had the highest polymorphic alleles. Shahi-Gharahlar et al. [24] in their study of 12 ISSR primers tested on 39 accessions of Iranian wild *Prunus* sub-*cerasus* reported that these ISSR primers generated 156 alleles and that the number of alleles per locus ranged from 9 to 19 with an average of 13 alleles, and the polymorphism percentage was 81.80–100% with an average of 96.46%. Ganopoulos et al. [20] studied 10 ISSR primers on 19 Greek traditional sweet cherries and two international cultivars. They reported that these ISSR primers generated 91 alleles and that the number of alleles per locus ranged from 2 to 10 with an average of 9.1 alleles, and the polymorphism percentage was 25–75% with an average of 57.7%. Moreover, Li et al. [22] studied 18 ISSR primers on 10 Chinese sour cherries and reported that these ISSR primers generated 150 alleles and that the number of alleles per locus ranged from 4 to 13 with an average of 8.33 alleles, and the polymorphism percentage was 1.33–32% with an average of 18.67%. The high number of generated alleles in our study may be due to the use of several different genotypes that had high genetic diversity.

The PIC values ranged from 0.85 to 0.96 with a mean value of 0.93. The high value of PIC represents the larger number of alleles and polymorphism [18]. Yilmaz et al. [21] in their study of 20 ISSR primers tested on 16 genotypes from

genus *Prunus* reported that PIC ranged from 0.35 to 0.93 with an average of 0.74. The average gene diversity ranged from 0.34 to 0.45 with a mean of 0.41. Ganopoulos et al. [20] reported that gene diversity ranged from 0.29 to 0.48 with an average of 0.36. This particular average gene diversity value (0.41) was higher than that (0.36) identified in another survey [20]. The high h_i showed a high diversity among sour cherry genotypes. Fragment size of the 23 primers ranged from 280 to 3200 bp. Band size ranges using ISSR markers were also found in other investigations of cherries with the range of 530–3100 bp [20], 231–1986 bp [22], 400–1950 bp [23], and 200–2100 bp [24].

According to these results, primers tested in our study produced good and various levels of amplifications as compared to other studies. For example, Shahi-Gharahlar et al. [24] in their study on subgenus *Cerasus* used ISSR-2 primer and reported that this primer produced 12 total alleles with polymorphism percentage of 91.70% and fragment size of 250 to 1200 bp, while in our study this primer produced 21 total alleles with polymorphism percentage of 100% and size range of 400–1500 bp. Also Li et al. [22] used ISSR-20 primer in sour cherries and reported that this primer produced 7 total alleles with size range of 281–1458 bp, while this primer produced 16 total alleles with polymorphism percentage of 100% and size range of 400–2800 bp in our study. Moreover, Yilmaz et al. [21] tested ISSR-3 and ISSR-18 primers in genus *Prunus* and reported that these primers produced 13 and 9 total alleles with polymorphism percentage of 100 and 89%, respectively, while we found that these primers produced 25 and 15 total alleles with polymorphism percentages of 96 and 93%, respectively. Also these primers have been tested by other researchers in various species. For example, Sofalian et al. [28] studied ISSR-2, ISSR-3, and ISSR-5 primers on wheat accessions. They reported that these primers produced 3, 15, and 10 total alleles, respectively, while the total alleles produced by these primers in our study were higher than their study. Moreover, Acharya and Sharma [29] in their study on genus *Papaver* tested these primers and reported that ISSR-1, ISSR-6, ISSR-8, ISSR-10, ISSR-11, and ISSR-19 did not produce any band, while in our study these primers produced good and various levels of amplifications. So ISSR-6 and ISSR-19 had the highest number of alleles (29 alleles). The highest number of alleles in their study related to ISSR-2 (4 alleles) and ISSR-3 (6 alleles). Our results indicated a high level of polymorphism of the genotypes based on these markers specially primers with high numbers of polymorphic alleles, polymorphism percentage, PIC values, and gene diversity were to ISSR-4, ISSR-6, ISSR-13, ISSR-14, ISSR-16, and ISSR-19, which can be used effectively in genetic diversity studies of the sour cherry.

Autochthonous varieties, cultivars, and wild genotypes are rich resources for genes for breeding objectives [10, 11]. The mean number of alleles produced by Iranian genotypes was higher than the number of alleles produced by foreign genotypes. These results indicated that since the Iranian genotypes were not selected for breeding programs, they were more likely to have a more diverse genetic background, and they can be used to select different genotypes in order to produce new cultivars. The high average gene diversity with a mean of 0.41 and the high number of alleles observed with a mean of 21.26 for all loci showed that these ISSR markers are highly polymorphic and can be useful in the study of genetic diversity.

Genetic similarity between genotypes ranged from 0.56 to 0.77 with an average of 0.72, which showed a high diversity among the genotypes. Cluster analysis with a genetic similarity of 0.72 divided the genotypes into eight distinct groups that separated Iranian genotypes from improved cultivars, and PCoA supported the cluster analysis results. These genotypes grouped within the same cluster in the dendrogram also occupied the same positions in two-dimensional scaling. Shahi-Gharahlar et al. [24] reported that the genetic similarity measured within 39 accessions of *subcerasus* ranged from 0.04 to 0.85 with an average of 0.28. A dendrogram constructed according to ISSR data of these genotypes divided them into 11 subclusters in which improved cherry cultivars were separated from wild genotypes, and this is consistent with the results of our study.

5. Conclusions

In summary, the good discrimination efficiency and high reproducibility of ISSR markers make them particularly suitable to identify the closely related and unknown sour cherry genotypes. In addition, the high genetic diversity observed within superior Iranian sour cherry genotypes and improved cultivars reflects the necessity for the conservation of this germplasm. Since the Iranian genotypes were superior to the improved cultivars and were separated from them in most of the groups, these genotypes can be considered as distinct genotypes for further evaluations in the framework of breeding programs and new cultivar identification in cherries. Hence, it is expected that the results of this study will assist current sour cherrybreeding efforts in Iran and will maintain the genetic integrity of the genetic resources. It is hoped that with supplementary tests and analyses we can identify and introduce new sour cherry cultivars to the fruit industry.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] N. E. Looney and A. D. Webster, *Cherries: Crop Physiology, Production and Uses*, CAB International Press, Oxfordshire, UK, 1996.

[2] A. F. Lezzoni, H. Schmidt, and A. Albertini, "Cherries," in *Genetic Resources of Temperate Fruit and Nut Crops*, J. N. Moore

and J. R. Ballington, Eds., pp. 109–175, Society for Horticultural Science, Wageningen, The Netherland, 1991.

[3] N. I. Vavilov, *The Origin, Variation, Immunity and Breeding of Cultivated Plants*, Ronald Press, New York, NY, USA, 1951.

[4] G. Charlot, M. Edin, F. Flochlay, P. Soing, and C. Boland, "Tabel Edabriz: a dwarf rootstock for intensive cherry orchards," *Acta Horticulturae*, vol. 667, no. 1, pp. 217–222, 2005.

[5] K. Hrotko, L. Magyar, and M. Gyeviki, "Evaluation of native hybrids of *Prunus fruticosa* Pall. As cherry interstocks," *Acta Agriculturae Serbica*, vol. 13, pp. 41–45, 2008.

[6] L. Magyar and K. Hrotkó, "*Prunus cerasus* and prunus fruticosa as interstocks for sweet cherry trees," *Acta Horticulturae*, vol. 795, pp. 287–292, 2008.

[7] FAOSTAT, FAOSTAT, FAO Statistical Databases (United Nations), FAO, 2013, http://faostat.fao.org/.

[8] G. S. Khush, "Molecular genetics-plant breeder's perspective," in *Molecular Techniques in Crop Improvement*, S. M. Jain, D. S. Brar, and B. S. Ahloowalia, Eds., pp. 1–8, Kluwer Academic, Dordrecht, The Netherlands, 2002.

[9] E. Ganji-Moghadam and A. Khalighi, "Relationship between vigor of Iranian *Prunus mahaleb* L. selected dwarf rootstocks and some morphological characters," *Scientia Horticulturae*, vol. 111, no. 3, pp. 209–212, 2007.

[10] A. Shahi-Gharahlar, Z. Zamani, M. R. Fatahi, and N. Bouzari, "Assessment of morphological variation between some Iranian wild *Cerasus* sub-genus genotypes," *Horticulture, Environment and Biotechnology*, vol. 51, no. 4, pp. 308–318, 2010.

[11] H. Demirsoy and L. Demirsoy, "Characteristics of some local sweet cherry cultivars from Homeland," *Journal of Agronomy*, vol. 3, no. 2, pp. 88–89, 2004.

[12] G. Lacis, I. Rashal, S. Ruisa, V. Trajkovski, and A. F. Iezzoni, "Assessment of genetic diversity of Latvian and Swedish sweet cherry (*Prunus avium* L.) genetic resources collections by using SSR (microsatellite) markers," *Scientia Horticulturae*, vol. 121, no. 4, pp. 451–457, 2009.

[13] Y. L. Cai, D. W. Cao, and G. F. Zhao, "Studies on genetic variation in cherry germplasm using RAPD analysis," *Scientia Horticulturae*, vol. 111, no. 3, pp. 248–254, 2007.

[14] D. Struss, R. Ahmad, S. M. Southwick, and M. Boritzki, "Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers," *Journal of the American Society for Horticultural Science*, vol. 128, no. 6, pp. 904–909, 2003.

[15] M. Bouhadida, A. M. Casas, M. J. Gonzalo, P. Arús, M. Á. Moreno, and Y. Gogorcena, "Molecular characterization and genetic diversity of Prunus rootstocks," *Scientia Horticulturae*, vol. 120, no. 2, pp. 237–245, 2009.

[16] A. Belaj, Z. Satovic, G. Cipriani et al., "Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive," *Theoretical and Applied Genetics*, vol. 107, no. 4, pp. 736–744, 2003.

[17] K. Wu, R. Jones, L. Danneberger, and P. A. Scolnik, "Detection of microsatellite polymorphisms without cloning," *Nucleic Acids Research*, vol. 22, no. 15, pp. 3257–3258, 1994.

[18] W. Powell, G. C. Machray, and J. Proven, "Polymorphism revealed by simple sequence repeats," *Trends in Plant Science*, vol. 1, no. 7, pp. 215–222, 1996.

[19] B. Bornet and M. Branchard, "Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting," *Plant Molecular Biology Reporter*, vol. 19, no. 3, pp. 209–215, 2001.

[20] I. V. Ganopoulos, K. Kazantzis, I. Chatzicharisis, I. Karayiannis, and A. S. Tsaftaris, "Genetic diversity, structure and fruit trait associations in Greek sweet cherry cultivars using microsatellite based (SSR/ISSR) and morpho-physiological markers," *Euphytica*, vol. 181, no. 2, pp. 237–251, 2011.

[21] K. U. Yilmaz, S. Ercişli, B. M. Asma, Y. Doğan, and S. Kafkas, "Genetic relatedness in prunus genus revealed by inter-simple sequence repeat markers," *HortScience*, vol. 44, no. 2, pp. 293–297, 2009.

[22] M. M. Li, Y. L. Cai, Z. Q. Qian, and G. F. Zhao, "Genetic diversity and differentiation in Chinese sour cherry *Prunus pseudocerasus* Lindl., and its implications for conservation," *Genetic Resources and Crop Evolution*, vol. 56, no. 4, pp. 455–464, 2009.

[23] A. Lisek and E. Rozpara, "Identification and genetic diversity assessment of cherry cultivars and rootstocks using ISSR-PCR technique," *Journal of Fruit and Ornamental Plant Research*, vol. 17, no. 2, pp. 95–106, 2009.

[24] A. Shahi-Gharahlar, Z. Zamani, R. Fatahi, and N. Bouzari, "Estimation of genetic diversity in some Iranian wild *Prunus* subgenus *Cerasus* accessions using inter-simple sequence repeat (ISSR) markers," *Biochemical Systematics and Ecology*, vol. 39, no. 4–6, pp. 826–833, 2011.

[25] N. Bouzari, E. Ganji-Moghadam, F. Karami et al., *National Project Collection and Evaluation of Local Sour Cherry Germplasms in Order To Achieve Proper rootsTock and Cultivars, Project No. 0-100-120000-04-0000-84104*, Seed and Plant Improvement Institute, Horticultural Section, Karaj, Iran; Agricultural Research and Educatin Organization Press, Tehran, Iran, 2010.

[26] N. Najafzadeh, K. Arzani, and N. Bouzari, "Assesment of morphological and pomological variation of some selected Iranian sour cherry (*Prunus cerasus* L.) genotypes," *Seed and Plant*, vol. 1, no. 2, pp. 123–137, 2014.

[27] J. A. Saunders, M. J. Pedroni, L. D. J. Penrose, and A. J. Fist, "AFLP analysis of opium poppy," *Crop Science*, vol. 41, no. 5, pp. 1596–1601, 2001.

[28] O. Sofalian, N. Chaparzadeh, A. Javanmard, and M. S. Hejazi, "Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers," *International Journal of Agriculture and Biology*, vol. 10, no. 4, pp. 466–468, 2008.

[29] H. S. Acharya and V. Sharma, "Molecular characterization of opium poppy (*Papaver somniferum*) germplasm," *American Journal of Infectious Diseases*, vol. 5, no. 2, pp. 148–153, 2009.

[30] P. H. A. Sneath and R. R. Sokal, *Numerical Taxonomy, the Principles and Practice of Numerical Classification*, Freeman WH, San Francisco, Calif, USA, 1973.

[31] S. A. Mohammadi and B. M. Prasanna, "Analysis of genetic diversity in crop plants-salient statistical tools and considerations," *Crop Science*, vol. 43, no. 4, pp. 1235–1248, 2003.

[32] F. G. Rohlf, *NTsys-Pc Numerical Taxonomy and Multivariate System Version 2.0 Applied*, vol. 12, Biostatistics, New York, NY, USA, 2000.

[33] IPGRI and Cornell University, *Genetic Diversity Analysis With Molecular Marker Data: Learning Module, Measures of Genetic Diversity*, IPGRI, Maccarese, Rome; Cornell University, Ithaca, NY, USA, 2003.

[34] M. Warburton and J. Crossa, *Data Analysis in the CIMMYT. Applied Biotechnology Center for Fingerprinting and Genetic Diversity Studies*, CIMMYT, Mexico City, Mexico, 2002.

[35] H. C. Romesburg, *Cluster Analysis for Researchers*, Krieger, Malabar, Fla, USA, 1990, Reprint of 1984 edition.

[36] N. M. Nei and W. Li, "Mathematical model for studying genetic variation in terms of restriction endonucleases," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 76, no. 10, pp. 5269–5273, 1979.

[37] P. Jaccard, "Nouvelles recherches sur la distribution florale," *Bulletin de la Societe Vaudoise des Sciences Naturelles*, vol. 44, pp. 223–270, 1908.