GENETIC DIVERSITY OF SOME IRANIAN SWEET CHERRY (*PRUNUS AVIUM*) CULTIVARS USING MICROSATELLITE MARKERS AND MORPHOLOGICAL TRAITS

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The aim of this study was to characterize 23 important Iranian sweet cherry (Prunus avium) cultivars collected from different provinces of Iran and 1 foreign cultivar, which was used as control, considered for breeding programs by using 21 microsatellite markers and 27 morphological traits. In sweet cherry (Prunus avium) accessions, leaf, fruit, and stone morphological characters were evaluated during two consecutive years. The study revealed a high variability in the set of evaluated sweet cherry accessions. The majority of important correlations were determined among variables representing fruit and leaf size and variables related to color. Cluster analysis distinguished sweet cherry accessions into two distinct groups. Principal component analysis (PCA) of qualitative and quantitative morphological parameters explained over 86.59 % of total variability in the first seven axes. In PCA, leaf traits such as leaf length and width, and fruit traits such as length, width, and weight, and fruit flesh and juice color were predominant in the first two components, indicating that they were useful for the assessment of sweet cherry germplasm characterization. Out of 21 SSR markers, 16 were polymorphic, producing 177 alleles that varied from 4 to 16 alleles (9.35 on average) with a mean heterozygosity value of 0.82 that produced successful amplifications and revealed DNA polymorphisms. Allele size varied from 95 to 290 bp. Cluster analyses showed that the studied sweet cherry genotypes were classified into five main groups based mainly on their species characteristics and SSR data. In general, our results did not show a clear structuring of genetic variability within the Iranian diffusion area of sweet cherry, so it was not possible to draw any indications on regions of provenance delimitation. The results of this study contribute to a better understanding of sweet cherry genetic variations in Iran, thus making for more efficient programs aimed at preserving biodiversity and more rational planning of the management of reproductive material.

Key words: *genetic relationship; microsatellite; informative markers.*

Introduction. The sweet cherry (*Prunus avium* L.) is a perennial plant, propagated vegetatively, and a high level of heterozygosity is specific to it. Heterozygosity of genome is increased by gametophytic self-incompatibility, which is controlled by multi-allele S-locus. Sweet cherry belongs to the Rosaceae family, Prunoideae subfamily, Prunus genus, and Cerasus subgenus [1]. *Prunus avium* originated in the area between the Black and Caspian seas of Asia Minor [2, 3]. Knowledge of genetic variation as well as information on mating systems and pollen and seed dispersal is of utmost importance. Great morphological variation exists among *P. avium* accessions grown naturally in Iran. Prominent characteristics among cherry accessions such as fruit weight, length, and width, fruit juice color, and resistance to pests and diseases should be considered as traits important for assessment [4]. Since morphological characterization continues to be the first step for the description and classification of germplasm, numerous sweet cherry genotypes have been evaluated and characterized for various traits, including diversity [5–9]. Since most types of cherries are cross-compatible, they show a higher rate of diversity in comparison with self-compatible ones. In this relation, various molecular markers were utilized to investigate the genetic diversity of sweet cherry [10–12]. Microsatellite markers were introduced as efficient and appropriate markers for the assessment of sweet cherry. Microsatellite markers were designed by Dirlewanger et al. [13] to study the genotypes of peach. SSR markers, originally designed for investigating the genotypes of peach, were proved efficient in identifying the genotypes of sour and sweet cherry by Schueler et al. [14]. Research has included genetic diversity analysis [9, 13, 15–19], cultivar identification and fingerprinting [10, 15, 20–25], self-incompatibility and population genetic structure evaluation and recruitment [26–30], and mapping genetic linkage [31]. SSR markers are more transferable between species of the same genus, or between closely related genera, than between distant genera of the same family [32–35]. Previously, SSR (Simple Sequence Repeats) markers have been successfully used on genotypes belonging to different Prunus genus in diversity studies [16, 18, 25, 36, 37].

Therefore, it is worthwhile to study genetic diversity among Iranian sweet cherry, considering the

specific geographical location of this product. Iran is one of the most important genetic sources for cherries in the world, and it provides an important source of variation for plant breeding. On the basis of these prerequisites, the main objectives of the current study were: 1) to quantify and characterize the variability of morphological traits within a set of 23 sweet cherry accessions from different provinces of Iran; 2) to calculate the coefficients of correlation between the studied characteristics; 3) to detect relationships between genotypes; 4) to identify the most useful traits for discriminating among genotypes. The outcome of such research can be used to plan sweet cherry amplification projects, the production of commercially suitable types, and the expansion of sweet cherry orchards.

Materials and methods. *Plant materials.* Sampling was conducted on young leaves of 23 Iranian sweet cherry cultivars and 1 foreign cultivar as a control (Table 1), collected from different provinces which belong to special geographical region of Iran. These genotypes were quite different based on fruit characteristics. The age of the trees was 13 years, and they were in the full productivity stage.

Phenotypic analysis. Accessions were evaluated using a set of 27 traits: fruit weight (g), stone weight (g), fruit length (mm), fruit width (mm), fruit diameter (mm), fruit stalk length (mm), fruit stalk weight (g), stone shape (code), stone size (code), skin cracking (code), fruit shape (code), fruit size (code), fruit doubled (code), titratable acidity (%), fruit juice color (code), fruit skin color (code), fruit flesh firmness (code), fruit stalk leaves (code), stone type (code), eating quality (code), pH, total soluble solids (%), anthocyanin index (O.D. 510 nm), fruit flesh color (code), leaf length (mm), leaf width

Table 1. **Sweet cherry cultivars used in this study and some of their main morphological traits**

Number	Cultivar	Origin	Geographic region	Fruit		
				color	size	hape
1	Mojtahedi	Karaj	Central North	Reddish Yellow	Small	Heart
2	Ghazvin	Ghazvin	Central North	Blackish Red	Large	Heart
3	Ghermeze Rezaeie	Uromiye	Western North	Reddish Yellow	Medium	Heart
4	Zarde Daneshkede	Karaj	Central North	Reddish Yellow	Large	Heart
5	Gilase Shomare 46	Karaj	Central North	Reddish Yellow	Medium	Globular
6	Sefid va Ghermeze Baghe	Karaj	Central North	Reddish Yellow	Medium	Globular
7	Mahali Karaj	Karaj	Central North	Reddish Yellow	Small	Globular
8	Dorage Shomare Yeke Karaj	Karaj	Central North	Blackish Red	Large	Heart
9	Arak	Arak	Central North	Reddish Yellow	Small	Globular
10	Gilase Shomare 28	Karaj	Central North	Reddish Yellow	Large	Heart
11	Shoao Saltane	Karaj	Central North	Reddish Yellow	Small	Globular
12	Siahe Daneshkade	Karaj	Central North	Blackish Red	Large	Heart
13	Mashhad	Mashhad	Eastern North	Blackish Red	Small	Globular
14	Abardeh	Mashhad	Eastern North	Blackish Red	Medium	Heart
15	Sefide Rezaeie	Uromiye	Western North	Cream	Large	Globular
16	Meshkinshahr	Azarbayejn	Western North	Black	Small	Globular
17	Soorati Lavasan	Lavasan	Central North	Reddish Yellow	Medium	Heart
18	Rafat	Lavasan	Central North	Reddish Yellow	Large	Heart
19	Haj Yosefi	Karaj	Central North	Reddish Yellow	Large	Globular
20	Siahe Mashhad	Mashhad	Eastern North	Blackish Red	Large	Heart
21	Hamedan	Hamedan	Central North	Reddish Yellow	Small	Globular
22	Ghermeze Baghe Nou	Karaj	Central North	Reddish Yellow	Medium	Globular
23	Siahe Zoodres	Mashhad	Eastern North	Blackish Red	Large	Globular
24	Italyia Late	Italy	International	Blackish Red	Large	Heart

Note. Fruit size based on fruit dimension (length, width and diameter) and fruit weight.

(mm), and leaf area (mm^2), based on standards of the Food and Agriculture Organization (FAO). The phenotypic traits were measured in two consecutive years (2007 and 2008). Fruit was randomly harvested from different parts of the tree according to the Dever approach [51]. Statistical analysis was performed using average values from two-year measurements. To show the relationships among the traits, Spearman rank coefficients of correlation were performed. Cluster analysis was applied and performed using Ward's method with Euclidean distances to evaluate relationships among accessions. A principal component analysis (PCA) was performed to summarize the manifold data in the first principal component containing the highest possible variability of the data. The eigenvalues of the 7 PCs were compared for each trait. Data analysis was conducted using 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

DNA extraction. Total genomic DNA was extracted from young leaf tissue according to the method described by Doyle and Doyle [55]. Subsequently, an RNAse treatment was performed on the eluted DNA samples. Purity and concentration of the DNA were checked both on 1 % (w/v) agarose gels and by NanoDrop® ND-1000 Spectrophotometer.

Table 2. **Eigenvalues, proportion of total variability and correlation between the original variables and the first seven principal components for sweet cherry accessions**

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Fruit weight	−0.76	−0.49	−0.24	−0.26	−0.16	−0.09	0.03
Stone weight	−0.79	−0.02	−0.12	0.12	−0.04	0.04	−0.20
Fruit length	−0.67	−0.37	−0.54	−0.05	−0.19	0.12	0.16
Fruit width	−0.85	−0.38	0.00	−0.29	−0.05	−0.12	−0.10
Fruit diameter	0.14	0.30	0.15	−0.46	0.16	0.06	0.33
Fruit stalk length	−0.35	0.10	−0.49	−0.07	0. 50	0.17	−0.30
Fruit stalk weight	−0.22	−0.58	−0.14	−0.49	−0.26	−0.10	0.31
Stone shape	0.12	0.37	0.18	−0.53	0.12	0.08	0.38
Stone size	−0.20	0.18	−0.34	0.44	0.58	−0.20	0.06
Skin cracking	−0.13	−0.55	−0.17	−0.39	−0.24	−0.11	0.32
Fruit shape	0.33	0.05	−0.78	0.27	−0.25	0.29	0.18
Fruit size	0.05	0.32	−0.42	−0.69	0.35	0.21	−0.01
Fruit doubled	0.43	0.06	−0.69	0.37	−0.17	0.25	0.28
Titratable acidity	−0.24	−0.48	−0.12	−0.39	−0.36	−0.11	0.34
Fruit juice color	0.42	−0.76	0.19	0.04	0.26	0.19	0.01
Fruit skin color	0.27	−0.74	0.21	0.13	0.40	0.20	0.06
Fruit flesh firmness	−0.17	0.16	−0.24	0.38	0.34	−0.38	0.16
Fruit stalk leaves	0.13	0.39	0.17	−0.56	0.14	0.09	0.37
Stone type	−0.16	0.17	−0.26	0.36	0.31	−0.36	0.17
Eating quality	−0.22	0.15	−0.44	0.34	0.54	−0.30	0.03
pH	−0.27	0.14	−0.34	0.36	0.44	−0.35	0.08
Total soluble solids	−0.09	−0.20	−0.29	0.33	0.29	−0.65	0.28
Anthocyanin Index	−0.10	0.32	0.23	−0.11	0.14	−0.14	0.68
Fruit flesh color	0.51	−0.77	0.02	−0.06	0.22	0.15	0.13
Leaf length	−0.77	0.23	0.07	−0.01	0.35	0.36	0.16
Leaf width	−0.71	−0.06	0.38	0.51	−0.02	0.12	0.15
Leaf area	−0.60	0.11	0.37	0.37	0.01	0.40	0.22
Eigenvalue	4.89	3.08	2.60	2.08	1.40	1.21	1.17
% Variance	25.78	16.23	13.72	10.93	7.40	6.37	6.15
% Cumulative	25.78	42.02	55.74	66.67	74.07	80.44	86.59

SSR analysis. 21 simple sequence repeat (SSR) markers (Table 2) were isolated from peach and sweet cherry and used in this study [9, 13, 26]. Because three groups of microsatellite markers were selected, three different protocols were employed to carry out PCR reaction. Regarding markers of the BPPCT group, PCR reaction was conducted at a volume of 15 microliters in accordance with the Dirlewanger et al. [13] protocol. Amplification conditions consisted of an initial denaturation step of 1 min at 94 °C, followed by 35 cycles of 45 sec at 94 °C, 45 sec at 57 °C, and 2 min at 72 °C with a final extension at 72 °C for 4 min. The PCR products were first separated on a 3 % (w/v) agarose gel run at 80 V for 2 hrs. The gel was then stained with ethidium bromide at a concentration of 10 mg/ml. For further determination of polymorphisms, the amplification reaction products were separated on a 6 % (w/v) denaturing polyacrylamide gel using a Sequi-Gen GT Sequencing Cell 30 cm gel apparatus (BioRad Laboratories Inc., Hercules, CA, USA). The amplified fragments were detected using the silver staining method as described by Bassam et al. [56]. The analyses were repeated at least twice to ensure reproducibility of results. Allele sizes were determined for each SSR locus using the Beckman CEQTM fragment analysis software.

Data analysis. The genetic analysis program «IDENTITY» 1.0 [57] was used based on Paetkau et al. [31] to calculate number of alleles, allele frequency, expected and observed heterozygosity, estimated frequency of null alleles, and probability of identity per locus. Genetic dissimilarity was determined with the program «MICROSAT» (version 1.5) using proportion of shared alleles, which was calculated by using «ps (option 1 − (ps))», as described by Bowcock et al. [58]. The results were then converted to a similarity matrix, and a dendrogram was constructed using (UPGMA) the unweighted pair group method with arithmetic averages [59]. This was achieved by employing sequential, agglomerative, hierarchical, and nested clustering (SAHN) using the numerical taxonomy and multivariate analysis system (NTSYS-PC), version 2.00. Observed heterozygosity (Ho) and expected heterozygosity (He) were calculated using POPGENE, version 1.32 [60]. The degree of polymorphism was quantified using the polymorphic information content (PIC). Probability of identity (PI) was estimated according to Paetkau et al. [31]. The number of effective alleles was calculated as $Ne = 1/(1 - He)$. Analysis of molecular variance (AMOVA) was performed using the Arlequin version 2.00 [14] to determine genetic variations [61]. Average value of the Shannon index was also measured [50]. Informative markers were determined by stepwise regression using the SPSS software version 10.0 for Windows (SPSS Inc., Chicago, IL).

Structure analysis of the cultivars. A clustering approach was designed in order to study how geographic-based groups (Table 1) represent inter-cultivar similarity in genetically homogeneous populations. For that purpose, the STRUCTURE v.2.2 software was used [44, 54, 62, 63]. This software places cultivars in K clusters that have distinct marker frequencies, where K is chosen as a priority and can be varied across different runs. Cultivars can have memberships in several clusters, with membership coefficients equaling 1 across clusters. Cultivars were divided into genetic clusters using the STRUCTURE software package. SSR data were analyzed by treating each class of cultivars as being, effectively, diploid alleles, according to the software documentation. A no-admixture ancestry model was used and allele frequencies were correlated, with a burn-in length of 30,000 followed by 100,000 runs at each K. Five STRUCTURE runs produced nearly identical membership coefficients at each K (data not shown).

Result and discussion. *Morphological trait analysis.* Sweet cherry genotypes from different provinces of Iran were characterized using a set of 27 traits (Table 3). Leaf area (LA), as an essential component to estimate plant growth, ranged from 2654.17 (Mojtahedi) to 6627.95 mm^2 (Siahe Daneshkade). Fruit characterization included several parameters of interest from the commercial growing viewpoint. Fruit stalk length varied from 35.23 (Mashhad) to 59.44 mm (Sefide Rezaeie), which was similar to the findings of Perez-Sanchez et al. [32]. A uniform fruit shape for the sweet cherry genotypes was not observed, but rather a frequent tendency for 'flat around' to 'elongated' fruit shapes (38.40 and 25.27 %, respectively) was observed, sometimes tending more towards 'round' or 'kidney shaped' fruit shapes (22.11 and 11.10 %, respectively). In this study, fruit skin color ranged from `yellow` to `black`, which was in accordance with Usenik et al. [26] who stated that fruit skin color was a widely varying characteristic among cherry culti-

vars. The majority of the studied genotypes had reddish-yellow skin color (21.21 %), dark red flesh color (36.36 %), and red juice color (27.27 %). Among the qualitative characteristics, the largest variabilities corresponded to external and internal colors of the fruit (including skin, flesh, and juice color). For quantitative traits, indicated by the value of coefficient of variation (Table 3), the highest variations were detected for fruit stalk length and fruit width (36 and 28 %, respectively). Mean, maximum, minimum, and percentage of coefficient of variation (CV%) of 27 morphological characters are shown in Table 3. A large diversity was observed in the characters, indicating a high level of variation in the studied plant materials.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among sweet cherry accessions. The obtained dendrogram had two main clusters (Fig. 1). The first cluster (I) included the majority (18) of accessions studied with different distances, and six sweet cherry genotypes were placed in the second cluster (II). Cluster analysis of sweet cherry accession clusters showed that leaf traits were the most important for grouping. The accessions represented in Cluster I were characterized by a larger leaf width ranging from 5.7 mm (Ghermeze Baghe Nou) to 6.3 mm (Ghazvin) and higher leaf area: 4494.63 mm^2 (Ghermeze Rezaeie) to 6627.95 mm^2 (Dorage Shomare Yeke Karaj). This cluster was split off into five distinct subgroups, defined as clusters IA, IB, IC, ID, and IE, respectively. Subclustering was further done according to fruit traits such as shape, weight, and color properties.

Table 3. **Morphological characters of fruits and leaves scored for one foreign and 23 Iranian cultivars**

Trait	Unit	Mean	Min	Max	(CV%)
Fruit weight	g	5.10	2.65	5.85	13
Stone weight	g	0.36	0.24	0.50	17
Fruit length	mm	18.74	14.78	21.33	31
Fruit width	mm	19.22	7.15	21.49	28
Fruit diameter	mm	16.72	12.36	19.43	26
Fruit stalk length	mm	41.55	35.23	59.44	36
Fruit stalk weight	g	0.09	0.06	0.14	10
Stone shape	Code	5.26	3.00	7.00	22
Stone size	Code	5.10	3.00	7.00	21
Skin cracking	Code	4.28	3.00	5.00	20
Fruit shape	Code	2.36	1.00	5.00	18
Fruit size	Code	5.62	1.00	9.00	23
Fruit doubled	Code	3.26	1.00	7.00	22
Titratable acidity	%	0.29	0.21	0.44	11
Fruit juice color	Code	3.82	1.00	6.00	22
Fruit skin color	Code	4.21	1.00	6.00	21
Fruit flesh firmness	Code	5.10	3.00	7.00	20
Fruit stalk leaves	Code	5.21	3.00	7.00	21
Stone type	Code	2.03	1.00	4.00	19
Eating quality	Code	6.46	4.00	9.00	28
pH	-	3.84	3.44	4.22	22
Total soluble solids	%	12.57	6.5	21.00	26
Anthocyanin Index	O.D.	0.28	0.024	1.88	11
Fruit flesh color	Code	2.69	1.00	7.00	21
leaf length	mm	9.3	7.4	11.9	27
leaf width	mm	5.2	4.2	6.3	20
Leaf area	mm^2	4494.63	2654.17	6627.95	3241

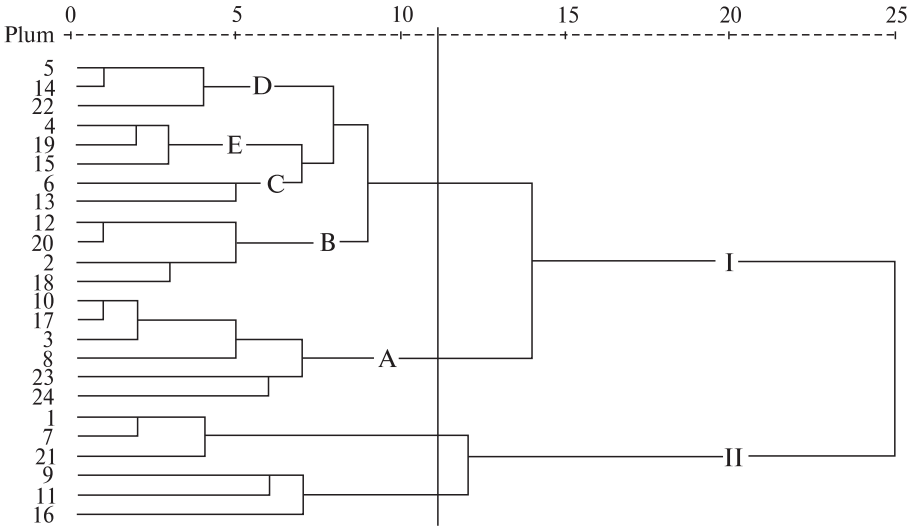

Fig. 1. Dendrogram of 23 Iranian and a foreign sweet cherry cultivars based on morphological data with Ward method. Numbers represent cultivars according to Table 1

Accessions in Cluster II (1, 7, 21, 9, 11, and 16) showed many similarities in fruit shape and size and the lowest values of leaf traits among all studied accessions. No other trait could be used to separate those accessions from the others since genotypes had different tree vigor, all kinds of cropping efficiency, from yellow to black fruit skin color, and from cream-white to black red fruit flesh color. The overall organization of sweet cherry accessions on the dendrogram suggests that there is considerable phenotypic variability in natural populations from different provinces of Iran. The high level of diversity can be explained by the fact that the sweet cherry genotypes were highly diverse because of outcrossing or seed dispersal from other populations. No evidence of relationships was found for most of the accessions according to their geographic location, contrary to the results of Moreno and Trujillo [38] for sweet cherry cultivars and Li et al. [39] for the Chinese sour cherry (*Prunus pseudocerasus*). Our results are not unexpected considering the fact that sweet cherry as an open-pollinated species is characterized by a high degree of heterozygosity; therefore, in one locality, different genotypes can be found. Two previous studies [40, 41] based on isozymes also indicated a very low level of differentiation among populations and a comparatively high level of genetic diversity.

The principal component analysis (PCA) used to identify the most significant variables in the data set produced seven principal components with eigenvalues greater than one (Table 2). These components are enough to explain 86.59 % of the total observed variability. The value of the extracted information is adequate considering the number of involved variables and the study's purpose. The percentage of variation explained by the first four components was only 66 %. According to Reim et al. [42], this result reveals a great morphological variation indicating a high genetic diversity within the sweet cherry population and suggests that evaluation of different morphological characteristics remains necessary for a meaningful characterization. The distribution of cultivars based on the PC-1 and PC-2 shows the phenotypic variations among the cultivars and how widely dispersed they are along both axes (Fig. 2). The first component presented 25.78 % of the variation, and variables with higher scores on PC1 (over 0.70 absolute value) were related to leaf (length and width), fruit size (length, width, and weight), and stone weight. The second component explained 16.23 % of the total variation and featured fruit skin color, flesh color, and juice color. The third component accounted for 13.72 % of the variation in which fruit shape was dominant. Finally, PC4 explained 10.93 % of the variation which had high loading for leaf length/width ratio (Table 2). The rest of the components varied to a lesser extent of about 35 % of total variance. High absolute values of the correlations

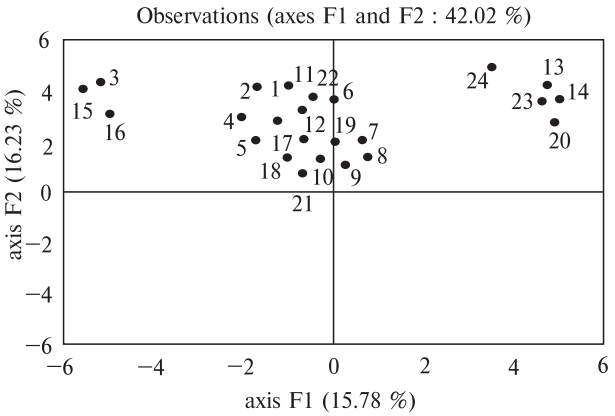

Fig. 2. PCA with phenotypic data from the 24 sweet cherry cultivars

between variables related to fruit and leaf size and PC1 or PC2 were also established by Krahl et al. [43] and Rakonjac et al. [44] in sour cherry, by Hjalmarsson and Ortiz [45] and Lacis et al. [18, 46] in sweet cherry, and by Khadivi-Khub et al. [47] in several *Prunus* subgen. *cerasus* species. This indicates that these traits could be sufficient for reliable germplasm characterization. These characteristics are also important in agricultural practices and breeding. In this study, in PC1, which explained the largest proportion of variability, as many as 6 traits showed a high loading. The summarization of these traits in one component reflected a strong correlation between leaf length and leaf width as well as a correlation between fruit length, fruit width, fruit weight, and stone weight reciprocally. Similarly in PC2, three traits (skin color, flesh color, and juice color) that had the most considerable loading were significantly correlated with each other. This result suggested that a reduction of these nine traits to three main characters, namely, leaf size, fruit size, and fruit color, could be sufficient.

Multivariate analysis assumes inclusion of genotypes with maximum genetic divergence [48, 49]. Variability was observed in all 27 measured characteristics, suggesting the presence of a high degree of phenotypic polymorphism among the cultivated cultivars of sweet cherry in Iran. This indicates the presence of diverse morphotypes at the individual genotype level, pointing to ample possibilities for obtaining desirable trait combinations in specific cultivars. This would be crucial in a breeding program for meeting the diverse demands of farmers, researchers, and consumers of this tree crop.

SSR marker analysis. The results of this study showed the cross amplification ability of microsatellite markers among the studied sweet cherry genotypes. In total, 177 alleles were detected for the 16 microsatellites evaluated (Table 4). Ganopoulos et al. [24] genotyped 21 sweet cherry cultivars using 15 SSR markers, and Sneath et al. [50] evaluated the genetic diversity of sweet cherry cultivars using 13 previously published SSR primer pairs. Struss et al. [30] used 15 SSR markers to identify 15 sweet cherry cultivars. The number of alleles per locus in the 24 sweet cherry cultivars ranged from 4.00 to 16.00, with a mean of 9.35 (5.80 for the effective number of alleles, Ne) for the 16 polymorphic microsatellites scored. The average number of alleles per locus identified in this study was bigger than the number identified in other studies of sweet cherry. For example, Dirlewanger et al. [13] genotyped 21 sweet cherry cultivars from all over the world using 33 SSR primer pairs and identified an average of 2.80 alleles per locus. The expected heterozygosity in the present study ranged from 0.018 to 0.930 with an average of 0.684; in three earlier studies on sweet cherry, the expected heterozygosity averaged 0.600 [13], 0.460 [51], and 0.600 [9]. The observed heterozygosity ranged from 0.033 to 0.863 (mean 0.611). The mean values for He (0.684) and Ho (0.611) were high and very similar, because self-incompatibility in sweet cherry prevents selfing and reduces inbreeding. The average value of the Shannon index was 1.69, which varied from 0.29 in EMPaS01 to 2.70 in EMPA015. PCR products and the length polymorphisms of BPPCT002 marker on agarose gel among 24 genotypes showed in Fig. 3.

PIC values ranged from 0.113 to 0.960, averaging 0.643. This particular PIC value (0.643) was similar to that (0.650) identified in another survey. PI values ranged from 0.130 to 0.980 with an average of 0.654. According to the PIC and PI values, the most informative markers were UCD-CH39 and EMPA015 (Table 4). The least informative marker was EMPA005 with a PI of 0.240 and a PIC of 0.140. Based on sampling sites, average He was 0.682, and the largest heterozygosity was observed for cultivars from Karaj (0.731). Based on SSR data, the studied sweet cherry genotypes were classified into eight main groups with a similarity limit of 0.57 (Fig. 4) as the 13 cultivars from different provinces were gathered into one group. A high

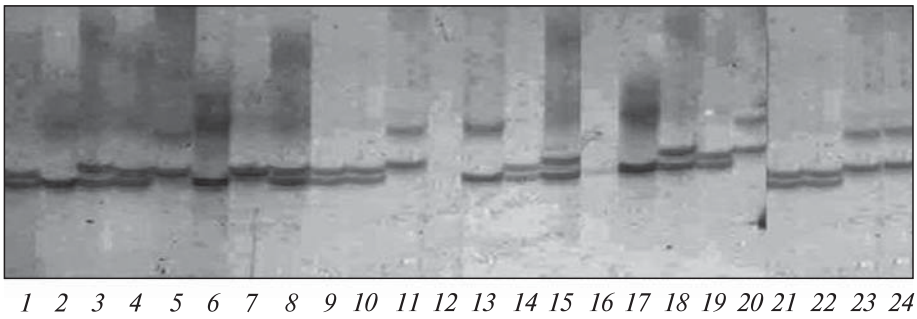

Fig. 3. PCR products and their length polymorphisms of BPPCT002 marker on agarose gel among 24 genotypes (see Table 1 for the codes of genotypes)

level of heterozygosity for all loci (0.682) can be attributed to cross-pollination and the self-incompatible nature of the sweet cherry. The high values of polymorphic loci (71 %), average number of alleles per locus (9.35), He (0.775), average polymorphism information content (0.472), and PI (0.256) observed in this study indicate that SSR markers are able to identify genetic variation among the studied sweet cherry genotypes. According to PI and PIC values, EMPA015, BPPCT039, EMPA018, UCD-CH12, BPPCT040, EMPA004, UCD-CH36, and BPPCT034 are the best markers for further studies of sweet cherry genetic diversity. The percentage of polymorphic SSR loci (71%) in this study was much higher than that estimated for RFLPs (23.9 %), suggesting that SSRs can act as better systems for sweet cherry cultivar identification [52]. In this investigation, cluster analyses showed that most Iranian landraces are well separated from the foreign genotype (Italyia Late), which was used as a control, indicating that they are native to Iran and the foreign genotype originated from a different geographical region. Ac-

Table 4. **Characterized useful SSR markers amplified from sweet cherry (*Prunus avium*)**

Locus name	Average Size	Na	Ne	Shannon Index	Ho	He	PIC	PI
BPPCT002	175	9	3.196	1.79	0.175	0.058	0.96	0.170
BPPCT004	172	8	3.514	1.47	0.376	0.018	0.83	0.342
BPPCT034	156	7	4.455	1.65	0.053	0.473	0.113	0.914
BPPCT039	157	8	4.570	2.22	0.371	0.888	0.856	0.450
BPPCT040	199	7	2.665	2.01	0.777	0.068	0.545	0.201
EMPA004	162	9	5.098	1.58	0.066	0.438	0.944	0.512
EMPA005	94	7	3.484	2.05	0.175	0.367	0.140	0.240
EMPA015	160	16	8.674	2.07	0.057	0.139	0.607	0.340
EMPA018	163	7	4.410	2.61	0.096	0.368	0.626	0.311
EMPaS01	174	6	4.388	0.29	0.863	0.664	0.581	0.655
EMPaS02	172	5	3.500	1.30	0.735	0.526	0.851	0.790
UCD-CH11	175	6	1.508	2.06	0.128	0.768	0.750	0.130
UCD-CH12	165	5	3.279	1.13	0.237	0.298	0.665	0.780
UCD-CH31	180	7	3.965	2.70	0.047	0.378	0.550	0.710
UCD-CH36	114	4	2.223	0.65	0.033	0.563	0.213	0.714
UCD-CH39	160	12	6.513	2.03	0.637	0.178	0.075	0.980
Mean		7.69	5.800	1.97	0.611	0.684	0.643	0.654

Note. Na, Ne, Ho, He, PIC, and PI are number of alleles per locus, effective number of locus alleles per locus, observed heterozygosity, expected heterozygosity, polymorphic information content and probability of identity, respectively.

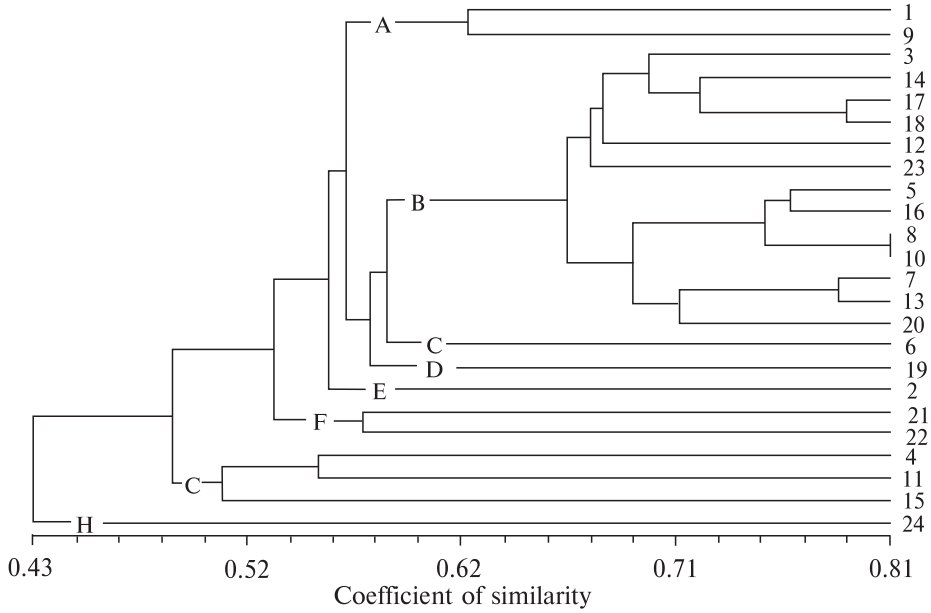

Fig. 4. Dendrogram showing the relationships between 23 Iranian and a foreign sweet cherry accessions using simple matching index and unweighted pair group method whit arithmetic mean (UPGMA). Numbers represent cultivars according to Table 1

cording to the results of this study, SSR data failed to separate genotypes based on their sampling sites. Germplasm migration or insufficient SSR markers can explain this incomplete separation. The results showed that cultivars including Dorage Shomare Yeke Karaj and Gilase Shomare 28 were gathered in one group expressing a great similarity rate. One possible explanation is that they might carry a common genetic background from the same parents. It might occur because of a possible mistake in their nomination. Informative markers are most applicable for breeding purposes. These markers have previously been used in the identification of peach and nectarine varieties [53]. The results provide guidance for the future efficient use of these molecular methods in the genetic analysis of sweet cherry. The collection of primers used in this study gave a reasonable number of amplification products for genetic diversity analysis. Based on the results, the genetic diversity among 24 cultivars is discussed. This study reveals the great importance of ensuring the differentiation of sweet cherry cultivars and their application for certification purposes.

Phylogenetic comparisons of morphological analysis and SSR markers. In morphological analysis, the dendrogram generated from the hierarchical cluster analysis (Fig. 1) based on the Ward method classified the 24 sweet cherry cultivars into two main groups (I and II) and some subgroups. Some sweet cherry cultivars originating from the same geographic location were found to be closely clustered. The pair cultivars namely (5 and 14), (12 and 20), and (10 and 17) showed the closest relationships with each other. In this analysis, the foreign cultivar (24) was not separated from Iranian cultivars.

SSR markers distinguished 22 sweet cherry cultivars with unique fingerprints, while 2 cultivars (8 and 10) fell into one non-distinguishable pair, indicating possible homonymy (Fig. 4). The dendrogram displaying the molecular relationships among the 24 tested sweet cherry cultivars separates them into eight main groups from A to H and several subgroups. In Group A, we can clearly distinguish two cultivars (1 and 9). Cultivars (17 and 18), (5 and 16), and (7 and 13) were found to be closely related and were grouped together. The close genetic relationship of these cultivars has been confirmed in this study; however, one is not a clone of the other in a strictly genetic sense. These cultivars differ in one of the 16 polymorphic loci tested. The foreign cultivar (24) classified in distinct group H showed the greatest genetic distance compared with the remaining cultivars. Based on the dendrogram of results, the SSR markers failed to separate genotypes according to their geographical origins

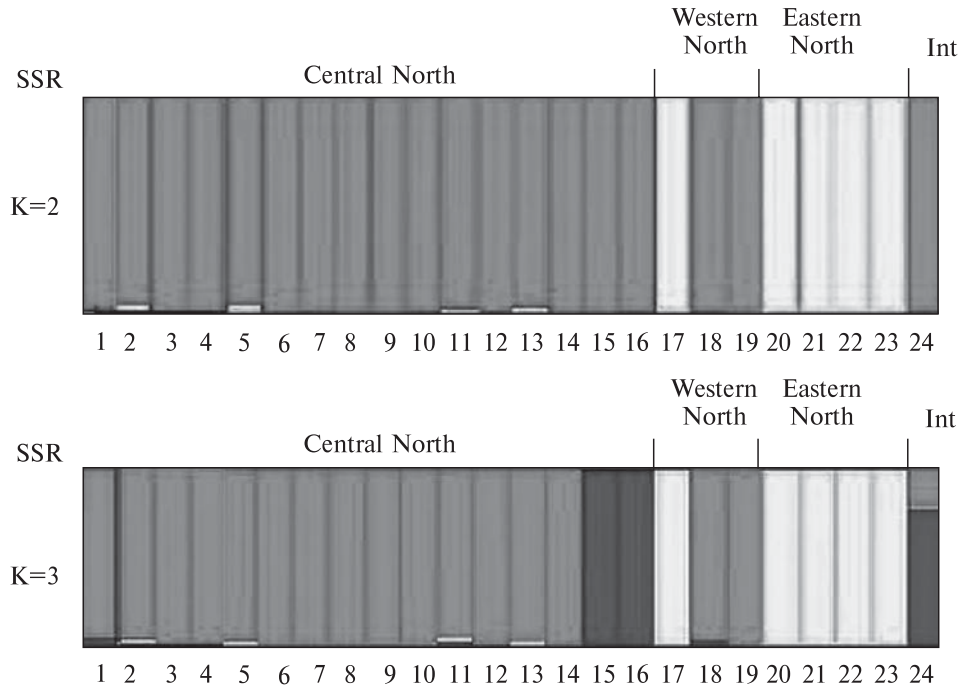

Fig. 5. Estimated geographic group structure for 24 sweet cherry cultivars with SSR markers (Table 1). Each individual is represented by a vertical line, which is partitioned into K segments that represent the individual's estimated membership fractions in K clusters. Different greyscale colors indicate different geographic groups (Central North, Western North, Eastern North and International = Int). Long black lines indicate the separation among a priori assigned groups

as the 13 cultivars from different provinces like Karaj, Mashhad, Uromiye, Azarbayejan, and Lavasan were gathered into one group.

Using the SSR method, all cultivars were distinguished, in contrast to the previously applied morphological technology. Therefore, the use of SSRs may reflect a greater degree of genetic resolution than the morphological method, as was shown in the case of the Italyia Late cultivar. This cultivar was indistinguishable by the morphological analysis, whereas the application of SSRs showed that it was indeed different. SSR markers, which were originally devised for discriminating among closely related plant cultivars, were proven in our study with sweet cherries to be effective in distinguishing closely related cultivars that would otherwise remain indistinguishable with the conventional morphological traits method.

For studies of cultivar identification, SSR experiments have a significant advantage over morphological analysis in that they are free of environmental effects. The prevailing opinion over the past few years has been that microsatellite-based approaches are complementary methods to provide the most complete coverage of the genome. Hence, in the pedigree analysis, the SSR analysis was found to be the most accurate system for identifying cultivars from the same parental source.

Structure analysis of the cultivars with SSR. Structure analysis with SSRs (Fig. 5) showed that at K = = 2 sweet cherry cultivars from north central Iran (Table 1) were grouped together with the one international cultivar included in this study and two cultivars from northwestern Iran (Sefide, Rezaeie, and Meshkinshahr), while the remaining five from eastern and northwestern Iran were grouped separately. In contrast to what was expected for sweet cherries, not all cultivars clustered according to geographical origin. At K = 3, the north central group was separated well enough from the northeastern sweet cherry cultivars. Cultivar «Italyia Late» indicated a commonality in origin with the two cultivars from north central Iran. At higher K values, the new groups were composed of individuals belonging to different clusters, making it difficult to iden-

tify the underlying classification criterion. Only a part of the cultivars clustered clearly according to geographic origin, while others showed a partial or even predominant membership in populations of other regions, for example «Italyia Late». This finding is probably due to humans moving cultivars to different sites during the past several thousand years of sweet cherry cultivation. These results suggest that traditional cultivars of Iran which correspond to pre-defined regional groups belong partly to genetically distinct groups. The structure analysis methodology has been previously used to investigate genetic structure in wild and cultivated olives from the Mediterranean region by Baldoni et al. [54].

Conclusions. High variability was found in the set of evaluated sweet cherry genotypes with regard to the characteristics studied. Although these accessions do not represent the whole sweet cherry germplasm in Iran, the considerable genetic diversity observed in both quantitative and qualitative characteristics indicates rich and valuable plant material for cherry improvement. The importance of the utilization of morphological and DNA markers in the management of the sweet cherry cultivars was established in this study, improving the conservation and management of relevant genetic resources. Cluster analysis distinguished sweet cherry accessions into two distinct groups, where the first one was split off into five subgroups. In principal component analysis, fruit and leaf traits such as leaf length and width, fruit length, width, and weight, and skin, flesh, and juice color were predominant in the first two components, indicating that they are not only useful for the assessment of genetic diversity but also for sweet cherry germplasm characterization. In order to keep a valuable sweet cherry natural germplasm resource, the following measures are proposed. First, different occurrences of the *P. avium* should be protected in situ as gene conservation stands. Second, ex situ conservation should be a good complement to in situ measures, where material should be tested to verify disease infection status. Third, an appropriate number of seeds should be sampled from all these populations and stored in a gene bank. Finally, research into intraspecific variation and genetic structure by molecular markers must be intensified. A combination of molecular and morphological data is the best choice for finding informative markers. In summary, results of the present study reveal that microsatellite markers can be successfully used to assay genetic diversity among Iranian sweet cherry landraces/cultivars and to identify informative markers for the breeding of important traits.

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ИЗУЧЕНИЕ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ НЕКОТОРЫХ ИРАНСКИХ СОРТОВ ЧЕРЕШНИ (*PRUNUS AVIUM*) С ПОМОЩЬЮ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ И МОРФОЛОГИЧЕСКИХ ПРИЗНАКОВ

A. Farsad, M. Esna-Ashari

Целью работы было изучение 23 наиболее важных иранских сортов черешни (*Prunus avium*), собранных в различных провинциях Ирана, и одного иностранного сорта в качестве контроля для использования в селекционных программах, с помощью 21 микросателлитного маркера и по 27 морфологическим признакам. Морфологические признаки образцов листьев, плодов и косточек оценивались на протяжении двух лет подряд. Результаты показали высокую вариабельность исследованных образцов. Большинство важных корреляций затрагивали признаки величины плодов и листьев, а также окраски. Кластерный анализ позволил разделить образцы на две различные группы. Метод главных компонент (Principal component analysis, PCA) качественных и количественных морфологических параметров объяснил более 86.59 % общей вариабельности в первых семи осях. Согласно PCA такие признаки листьев, как длина и ширина, и такие признаки плодов, как длина, ширина и вес, цвет мякоти плода и сока, были предоминантными в первых двух компонентах, что свидетельствует об их важности для определения характеристик зародышевой плазмы черешни. Из 21 SSR маркера 16 были полиморфными, давая 177 аллелей, что варьировало от 4 до 16 аллелей (в среднем 9.35) со средним уровнем гетерозиготности 0.82. Это давало успешные амплификации и выявило полиморфизм ДНК. Размер аллелей варьировал от 95 до 290 п.н. Кластерный анализ показал, что изученные генотипы черешни классифицируются в пять главных групп, основываясь главным образом на характеристиках вида и данных SSR. В целом наши результаты не показали ясной структуры генетической изменчивости внутри иранской области распространения черешни, так что невозможно сделать выводов об определении границ областей происхождения. Результаты исследования вносят вклад в лучшее понимание генетической изменчивости че-

решни в Иране, создание более эффективных программ сохранения биоразнообразия и более рациональное планирование работы с репродуктивным материалом.

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