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Diversity of morpho-physicochemical traits in Iranian sour cherry genotypes using multivariate analysis

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Abstract: In this study, morpho-physicochemical characterization of sour cherry genotypes from Iran was investigated. Thirty-four morphological and eight physicochemical traits were recorded. Sour cherry genotypes had a high variability in traits related to fruit characters such as fruit weight, stone volume, total anthocyanin content and total soluble solid. As a result, sour cherry genotypes exhibit total phenolic content and antioxidant activity higher than "Ciganymeggy" and "Erdi botermo" cultivars. Principal component analysis (PCA) suggested that leaf dimensions, fruit weight, stone weight, and stone volume could be sufficient for identification of genotypes. Hierarchical cluster analysis classified sour cherry genotypes and "Ciganymeggy" and "Erdi botermo" cultivars into two main clusters. The first cluster was characterized by a upright tree vigour, depressed fruit pistil end, reniform shape of fruit, high sweetness, dark red juice, flower high length and diameter, fruit and stone weight and length and diameter, total soluble solid, low total phenolic content, high total flavonoid content and high total anthocyanin content.

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1. Introduction

Sour cherry, *Prunus cerasus* L., is known as tetraploid (2n =4x = 32), originated through natural hybridization of the large statured, cold sensitive sweet cherry (*P. avium* L., 2n = 2x = 16), and the low growing, cold tolerant ground cherry (*P. fruticosa* Pall., 2n = 4x = 32) (Olden and Nybom, 1968). This species originated around the Black and Caspian Seas and were cultivated in temperate and cold regions. Sour cherry spread slowly from its origin to other regions due to human and animal migrations (Pérez-Sánchez *et al.,* 2008). Sour cherry fruit is mostly used for industrial preserves (jams, purees, juices and concentrates), while only a small portion is assigned to fresh consumption. Sour cherry is also used as a sweet cherry rootstock. This rootstock is more resistant to soil wetness and cold

climate than wild sweet cherry and mahaleb forms.

In 2014, the total world production of sour cherry reached 1.1 million tons, being Turkey, the Russian Federation, Poland, Ukraine, Iran and Serbia the most important producing countries (Faostat, 2014). The main sour cherry producing areas in Iran are the Ardebil, Azerbaijan, Khorasan and Alborz provinces.

From the viewpoint of fruit quality, several studies for characterization of fruit traits have been accomplished recently. Sour cherry is a valuable source of vitamins (A, B1, B2, C, E, K, and Niacin), carotenoids like beta-carotene, minerals, fiber, various sugar like fructose, glucose, maltose, antioxidant agents such as caffeic acids, cyaniding-3-O-glucosylrutinoside and flavoids (Mulabagal *et al*., 2009; Ferretti *et al*., 2010). This products has positive effects on human health (Ataie-Jafari *et al*., 2008; Saric *et al.*, 2009; Kuehl *et al*., 2010). Analysis of flavonoids from *P. cerasus* identified kaempferol, quercetin, quercetin 3-O-glucoside, and isorhamnetin 3-O-rutinoside (Piccolella *et al.*, 2008). The main anthocyanins found in cherry are cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, cyanidin-3-O-glucosylrutinoside, cyanidin-3-O-sophoroside, pelargonidin-3-O-glucoside, peonidin-3-O-rutinoside and cyanidin-3-O-arabinosylrutinoside (Chaovanalikit and Wrolstad, 2004).

Much of the genetic diversity is available in the wild types and natives from the center of origin. Wild species are probable gene resources for the breeding objectives such as resistance to pests and diseases, more appropriate cultivars for table and industry, extending cherry season and developing new resistant and dwarfing rootstocks. Therefore, it is necessary to characterize and preserve these species (Demirsoy and Demirsoy, 2004; Aliyoun Nazari *et al*., 2012).

Description of the morphological characteristics is the usual methodology accepted from a legal point of view for patenting and registration of varieties. Several quantitative and qualitative evaluations showed a clear difference between sour cherry, with a more marked variability within the sour cherries group, probably due to the more intense domestication processes that have taken place. Morphological characterization continues to be the first step for germplasm description and classification, and the statistical method of factor analysis is a useful tool for screening the accessions of a collection (Badenes *et al*., 2000; Hajilou and Fakhimrezaei, 2011). Several morphological characterization studies have been carried on the sour cherry (Krahl *et al*., 1991; Rodrigues *et al*., 2008; Rakonjac *et al*., 2010; Najafzadeh *et al*., 2014).

As an origin of the subgenus *Cerasus*, Iran has a rich cherry germplasm. The area of this study is part of a large growing area in North West of Iran. Sour cherry has been cultivated in this area for many years. However, the conservation and characterization of local cultivars is important to avoid the loss of genetic variability and as a potential source of genetic variation for future sweet and sour cherry breeding programs. These genotypes show distinctive agronomic characters such as low susceptibility to fruit cracking, high levels of soluble solids and early fruit maturity. The objective of this study was to survey, identify and characterize sour cherry genotypes existing in the province of East Azerbaijan - Shabestar (Iran) for their later introduction into a germplasm bank.

2. Materials and Methods

Plant materials

The plant material was located on the Shabestar town in west side of the East Azerbaijan province, in north-west of Iran. A total of 15 sour cherry genotypes and two cultivars, “Ciganymeggy” and “Erdi botermo”, were used in this study.

Evaluation of morphological and physicochemical traits

Characterization of vegetal material and fruits was based on sour cherry descriptors developed by the International Union for the Protection of New Varieties of Plants - UPOV (UPOV, 2006). Thirty four morphological (16 qualitative and 18 quantitative) and eight physicochemical traits were recorded as described in Table 1 and 2. In this study, a total of 17 sour cherry genotypes, including 15 local sour cherry genotypes and two cultivars, “Ciganymeggy” and “Erdi botermo”, with three replicates for each genotype were evaluated. The evaluation for morphological characters was based on 30 measurements of each trait.

For the analysis of physicochemical traits, fruits were picked at the commercial maturity stage. All fruits were collected from a single plant, randomly from all cardinally oriented branches with different directions around the canopy. All samples were stored in a freezer at -20°C. The frozen fruit material (5 g) was homogenized with a polytron (2 min on ice) with 10 mL of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture

Table 1 - Sixteen qualitative traits and their states and codes studied of sour cherry genotypes

No.	Trait	1	2	3	4	5	6	7	9	11
1	Tree vigour	Very weak		weak		medium		strong	Very strong	
2	Tree habit	upright	Semi-upright	spreading	drooping					
3	Tree branching			weak		medium		strong		
4	Tree bud distribution	along entire branch	only on middle distal part of branch	only on distal part of branch						
5	Flower arrengment of petal	free	intermediate	overlapping						
6	Flower shape of petal	circular	medium obovate	broad obovate						
7	Flower arrangement	solitary	double	In clusters	irregular					
8	Starting bloom from April	9-11 day		11-13 day		13-15 day		15-17 day	17-19 day	›19 day
9	Fruit ripening time from June	5-10 day		10-15 day		15-20 day		20-25 day	25-30 day	› 30 day
10	Fruit pistil end	pointed	flat	depressed						
11	Stone shape	narrow elliptic	broad elliptic	circular						
12	Fruit shape	reniform	oblate	circular	elliptic					
13	Fruit color of skin	Orange red	Light red	Medium red	Dark red	Brown red	blackish			
14	Fruit color of flesh	yellowish	pink	Medium red	Dark red					
15	Fruit sweetness			low		medium		high		
16	Color of juice	Colorless	Light yellow	pink	Medium red	Dark red				

Table 2 - The range of 26 quantity variability in Sour cherry genotypes traits, mean and coefficient of variations (CV %)

No.	Trait	Unit	Min	Max	Mean	CV (%)
1	Flower diametr	Mm	23.5	39.5	28.1	12.4
2	Petal length	Mm	10.8	13.7	11.9	8.1
3	Petal width	Mm	9.6	14.0	11.6	11.9
4	Pestil length	Mm	11.4	13.6	12.4	5.1
5	Number of stamens	-	31.9	37.0	34.5	4.1
6	Fruit length	Mm	13.5	19.1	15.2	8.8
7	Fruit diameter	Mm	13.1	22.6	17.6	10.9
8	Fruit length/ diameter	-mm	0.8	1.0	0.9	6.1
9	Length of stalk	Mm	41.0	54.0	46.8	7.3
10	Fruit weight	Gr	12.4	54.3	21.4	55.7
11	Stone length	Mm	5.6	9.5	7.1	11.7
12	Stone diameter	Mm	5.5	9.0	7.4	13.2
13	Stone volume	cm^3	0.1	0.4	0.2	36.0
14	Stone weight	Gr	2.2	3.5	3.0	13.5
15	Leaf blade length	Mm	67.0	96.8	78.9	10.2
16	Leaf blade width	Mm	36.8	53.4	43.4	9.8
17	Leaf blade length/ blade width	-	1.7	2.0	1.8	4.0
18	Petiole length	mm	13.6	18.4	16.2	8.2
19	pH	-	2.0	3.6	3.3	13.9
20	Total soluble solid	%	12.1	23.3	16.8	20.9
21	Vitamin C	mg/100g FW	10.5	13.2	11.7	7.7
22	Titratable acidity	%	1.9	2.7	2.3	9.0
23	Total phenolic content	mg GAE/100 g FW	228.0	289.0	241.8	6.0
24	Total anthocyanin content	mg cyanidin 3-glucoside /100 g FW	60.1	130.3	98.0	21.5
25	Antioxidant activity	µg TE/ 100g FW	52.3	67.7	60.5	7.5
26	Total flavonoids content	mg QE/ 100 g FW	141.8	155.8	145.8	2.5

was incubated overnight at 4°C and then centrifuged for 20 min at 4°C and 20000 g. Supernatant was recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, anthocyanins, flavonoids, and antioxidant capacity assays (Cantin *et al.,* 2009). The content of phenolic compounds in methanol extracts was determined according to the Folin-Ciocalteu method (Waterhouse, 2001). Absorbance was measured at 725 nm using a spectrophotometer (UV-2100 SPECTROPHOTOMETER). Total anthocyanin content (TAC) was determined using the pH differential method (Giusti and

Wrolstad, 2001). The absorbances of the extracts at 510 and 700 nm were measured against a blank. TAC was calculated and expressed as mg cyanidin 3-glucoside equivalent/100 g of FW. Total flavonoid content of each extract was determined following colorimetric method (Chang *et al*., 2002). The antioxidant capacity was measured using the DPPH method adapted from Brand-Williams *et al.* (1995). Titratable acidity was established by titration with 0.1 N NaOH and sugar content was measured as total soluble solids (TSS) using digital refractometer (Atago PR 100, Japan). Vitamin C content was estimated according to the titration with 2, 6-Dichlorophenolindophenol method (AOAC, 2000).

Statistical analysis

Statistical analysis were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, maximum and minimum values, mean, and coefficients of variation (CV %) were calculated for each trait. Relationships among the species were investigated by principal component analysis (PCA). PCA was performed using SPSS statistics software. Scatter plot of the first two PCs and the cluster analysis were created by PAST statistics software (Hammer *et al*., 2001).

3. Results and Discussion

Characteristics of cultivars

Several researchers have reported the morphological variation between some *Prunus* subgenus *Cerasus* genotypes such as for sweet cherry (*P. avium*), sour cherry (*P. cerasus*), mahaleb (*P. mahaleb),* marmareh (*P. incana*) and tomentosa cherry (*P. tomentosa*) (Ganji-Moghadam and Khalighi 2007; Khadivi-Khub *et al*., 2008; Perez-Sanchez *et al.*, 2008; Zhang *et al*., 2008; Rakonjac *et al*., 2010; Aliyoun Nazari *et al*., 2012).

Morphological characteristics of the studied genotypes are resumed in the Table 1 and 2. Results showed that high variation among studied genotypes was found for fruit weight (CV=55.7%) and stone volume traits (CV=36%). This result is compatible with Zhang *et al.* (2008) report. They observed high morphological variation among populations, where the highest variations were in fruit weight, fruit width, and leaf width. Tree habits of the studied genotypes are different. Most of the genotypes have drooping, three have spreading and one has upright tree habit ("Erdi botermo").

TPC values ranged between 228 and 289 mg GAE/100 g FW of sour cherry genotypes, which is in good agreement with previously published results (Dragovic-Uzelac *et al.,* 2007; Khoo *et al.,* 2011; Alrgei *et al*., 2015). "Erdi botermo" had the lowest TPC among the studied genotypes and N02 had the highest, that is consistent with the results of Papp *et al.* (2010) that reported the "Erdi botermo" have lowest TPC among all tested genotypes. Behrangi *et al.* (2015) reported that TPC is versatile on the basis of fruit type, stage of growth, farm of landing, extraction method, component of TPC experiment and other factors. Therefore TPC decreased by transforming fruit from first stages of growth to fully ripe form that is compatible with our results.

As shown in Table 2, total antioxidant capacity (AC) of sour cherries was between 52.3 and 67.7 µg TE/100 g FW. The total AC of different sour cherry cultivars showed significant difference (Blando *et al.,* 2004; Bonerz *et al.,* 2007; Khoo *et al.,* 2011). The lowest TAC was found in N02 genotype (60.1 mg cyanidin 3-glucoside/100 g FW), while "Erdi botermo" had the highest TAC (130.3 mg cyanidin 3-glucoside/ 100 g FW). These differences in TAC showed that the plant growth region and the harvest period might have an impact on plant growth and metabolite concentration (Premier, 2002). Sour cherry is one of the richest source of flavonoid (Marinova *et al.,* 2005), that is consistent with our results.

Principals component analysis

Eighty percent of the variability observed was explained by seven components (Table 3). For each trait, a factor loading of more than 0.51 was considered as being significant. PC1 represents mainly fruit pistil end, fruit color of skin, fruit length, fruit diameter, fruit weight, stone length, stone diameter, stone volume, leaf blade length, leaf blade width and total flavonoids content with significant positive effects, also tree habit, flower shape of petal, fruit ripening time with negative effects and account for 29.65% of the variance. The second principals component with 13.4% of total variance included traits of the tree branching, vitamin C, titratable acidity with negative impacts and the trait of flower diameter, petal length, petal width and stone weight with positive impacts. High absolute values of the correlations between variables related to the growth, fruit and leaf size, and PC1 or PC2 were also established by Krahl *et al.* (1991) and Rakonjac *et al.* (2010) in sour cherry, by Lacis *et al.* (2010) and Rakonjac *et al.* (2014) in sweet cherry, Aliyoun Nazari *et al.* (2012) in

marmareh (*P. incana*) and by Khadivi-khub *et al.* (2012) in *Prunus* subgen. *Cerasus*. PC3 was correlated with starting bloom, stone shape, pistil length, fruit length/diameter, pH and length of stalk. The remaining components explain less variability.

Grouping of cultivars

Hierarchical cluster analysis classified native sour cherry genotypes and "Ciganymeggy" and "Erdi botermo" cultivars in two main clusters (Fig. 1). The first major cluster is divided into two subgroups; subgroups I consisted of Erdi botermo cultivar and and subgroup II contained Ciganymeggy and some of the genotypes, indicating that these sour cherry genotype had high similarity to Ciganymeggy cultivar. The second cluster included the native genotypes. The

Table 3 - Eigen values and cumulative variance for seven major factors obtained from principal component analysis (PCA) and traits within each factor for sour cherry genotypes

Trait	Factors						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Tree vigour	-0.10	0.32	0.12	-0.62**	0.02	0.30	-0.52
Tree habit	-0.87**	0.05	0.19	0.14	0.14	-0.15	0.00
Tree branching	0.14	-0.74**	0.21	0.21	0.02	-0.24	-0.08
Tree bud distribution	0.32	0.02	0.15	-0.60**	-0.04	-0.29	-0.39
Flower arrangement of petal	0.32	-0.34	0.35	-0.02	-0.14	0.39	0.32
Flower shape of petal	-0.90**	0.10	0.19	-0.03	-0.19	-0.20	-0.04
Flower arranement	-0.36	-0.39	0.18	-0.22	0.13	0.64**	-0.22
Starting bloom from April	0.02	-0.15	0.61**	-0.24	-0.48	0.14	0.27
Fruit ripening time from June	-0.87**	0.08	-0.10	0.15	0.20	0.02	-0.02
Fruit pistil end	0.81**	-0.13	-0.02	-0.29	-0.18	-0.13	-0.04
Stone shape	0.18	0.34	0.62**	0.50	0.02	0.26	0.07
Fruit shape	-0.83**	0.22	0.26	0.25	0.07	-0.05	-0.06
Fruit color of skin	0.80**	-0.12	-0.27	0.10	0.34	0.11	0.09
Fruit color of flesh	0.16	-0.50	0.14	0.26	-0.55**	0.26	-0.06
Fruit sweetness	0.63**	0.02	-0.51	-0.08	0.28	0.15	0.16
Color of juice	0.90**	-0.10	-0.19	0.03	0.19	0.20	0.04
Flower diameter	-0.28	0.71**	-0.15	0.17	0.05	0.15	0.39
Petal length	-0.10	0.77**	-0.14	0.32	-0.32	-0.03	0.15
Petal width	-0.10	0.70**	-0.12	0.14	-0.48	0.29	0.06
Pestil length	-0.18	0.08	0.70**	0.06	-0.36	0.02	-0.38
Number of stamens	-0.21	0.11	-0.46	0.46	0.40	-0.02	-0.19
Fruit length	0.90**	0.33	0.09	0.03	0.04	-0.11	-0.07
Fruit diameter	0.81**	0.23	-0.19	0.22	-0.13	-0.18	-0.15
Fruit length/diameter	-0.15	0.01	0.52**	-0.38	0.33	0.24	0.28
Length of stalk	0.01	-0.02	0.59**	-0.13	0.40	0.07	0.11
Fruit weight	0.86**	0.16	0.21	-0.14	0.28	-0.19	0.07
Stone length	0.89**	0.11	0.25	0.14	-0.10	-0.03	0.09
Stone diameter	0.60**	-0.20	0.47	-0.02	-0.23	-0.34	0.05
Stone volume	0.88**	-0.05	0.31	0.01	-0.17	-0.16	0.09
Stone weight	0.35	0.77**	0.26	-0.03	0.13	0.08	-0.05
Leaf blade length	0.64**	0.50	0.11	-0.17	0.01	0.17	-0.28
Leaf blade width	0.64**	0.41	0.03	-0.09	0.07	0.34	-0.49
Leaf blade length/leaf blade widht	0.05	0.49	0.21	-0.23	-0.13	-0.39	0.43
Petiol length	0.38	0.14	-0.39	-0.13	-0.12	0.18	0.54**
Total soluble solid	0.47	-0.17	0.31	0.45	0.45	-0.16	-0.13
pH	0.12	0.31	0.67**	0.59	0.09	0.25	0.01
Vitam C	-0.05	-0.56**	-0.13	-0.09	-0.15	0.07	0.19
Titratable acidity	0.21	-0.51**	0.47	0.04	0.39	0.21	0.31
Total phenol content	-0.10	0.14	0.40	0.12	0.27	-0.67**	-0.07
Total anthocyanin content	0.51	-0.20	-0.24	0.20	-0.59**	-0.12	0.00
Antioxidant activity	0.12	0.36	0.01	-0.42	0.34	-0.08	0.28
Total flavonoids content	0.61**	-0.32	-0.24	0.50	0.08	0.09	-0.25
Eigen value	12.45	5.63	4.69	3.32	2.42	1.43	1.18
Cumulative Variance (%)	29.65	43.06	54.24	62.16	69.30	75.11	80.54

first cluster were characterized by a upright tree vigour, depressed fruit pistil end, reniform shape of fruit, high sweetness, dark red juice, flower high length and diameter, fruit and stone weight and length and diameter, total soluble solid, low total phenolic content, high total flavonoid content and high total anthocyanin content. Perez-Sanchez *et al.* (2008) suggested that dendrogram gained from morphological characteristics clearly showed the relationships among the cultivars of sweet, sour and duke cherries. In addition, Khadivi-khub *et al.* (2012) reported that dendrogram obtained from morphological characteristics clearly separated some *Cerasus* genotypes. The second cluster were characterized by small fruit and stone, drooping or spreading tree habit.

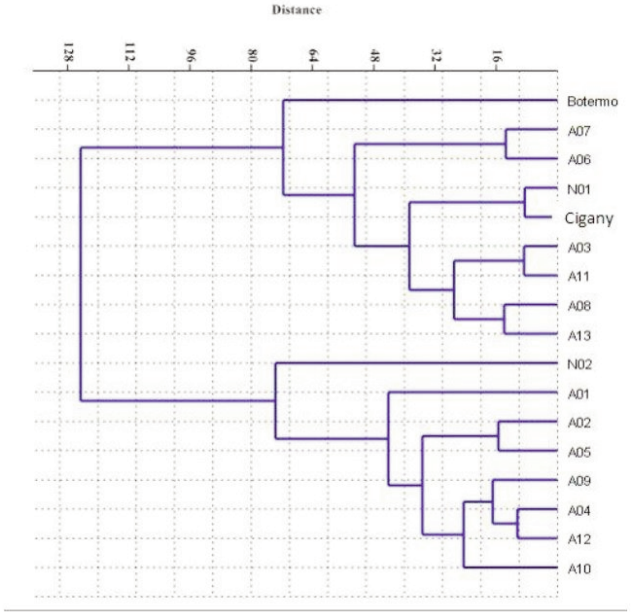

Fig. 1 - Dendrogram of 17 sour cherry genotypes based on morphological traits by PAST software.

Scatter plot was prepared according to the PC1 and PC2 by PAST software (Fig. 2). Starting from the positive to the negative values of PC1, these genotypes indicated a gradual decrease in fruit pistil end, fruit color of skin, fruit length, fruit diameter, fruit weight, stone length, stone diameter, stone volume, leaf blade length, leaf blade width and total flavonoids and an increase in tree habit, flower shape of petal, fruit ripening time. Starting from the negative towards the positive values of PC2, the genotypes indicated a gradual increase tree branching, vitamin C, titratable acidity and a decrease flower diameter, petal length, petal width and stone weight.

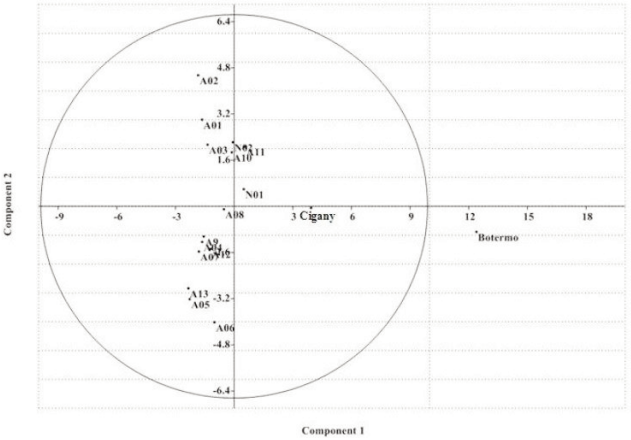

Fig. 2 - Factor scores for the first two principle components (PCs) for Sour cherry genotypes.

4. Conclusions

Morphological characterization continues to be the first step for the description and classification of germplasm and statistical methods like principal components analysis (PCA) are useful tools for screening the accessions of a collection (Cantini *et al.,* 1999; Badenes *et al.,* 2000). PCA is used for data reduction that transforms the original variables into a limited number of uncorrelated new variables. This technique, producing a smaller set of composite variables, account for much of the variance among the set of original variables and allows visualization of the differences among the individuals, identification of possible groups and finding relationships among individuals and variables (Martinez-Calvo *et al.,* 2008). High correlations were found between some traits and principal components, which could reduce the number of traits to be studied in sour cherry germplasm. For instance, measuring the traits of PC1 (such as FW, SL, SD. SV, FPE, and FCS) is suggested for future studied in sour cherry genotypes. Dependent on the trait, a certain number of genotypes were observed that showed lower or higher values than the commercially grown cultivars involved in this study. Especially in reference to the fruit weight, a high portion of genotypes were characterized by smaller fruits than that of “Ciganymeggy” and “Erdi botermo”. In addition, native genotypes showed higher values of total phenolic content and antioxidant activity traits than the commercial cultivars.

We conclude that this is the first study of sour cherry native genotypes, which deals with the morphological and physicochemical variation basis of genetic diversity. Although these accessions does not

represent the whole sour cherry germplasm in Iran, considerable genetic diversity observed in both morphological and physicochemical characteristics indicate rich and valuable plant material for sour cherry improvement.

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