Study of genetic diversity in pomegranate germplasm of Yazd province of Iran

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Abstract

A total of 117 pomegranate genotypes collected from different areas of Yazd province of Iran were studied for genetic variation by evaluating 23 morphological traits according to the international descriptor. Similar diversity pattern of the measured characteristics was observed in three types of sweet, sweet-sour and sour varieties. The traits shape of fruit base, suckering tendency, vigor of tree, fruit shape and aril color had the highest power of discrimination. The results of evaluation of genetic distances revealed discrepancies in the pairs of genotypes with similar denominations. The sites of Taft and Yazd were located in the same group in the dendrogram of origins of the germplasm, while Bafq and Ardakan together were placed in another group separated from group of Ashkezar and Mehriz. Abarhuk and Khatam regions also made single-membered groups. The results of discriminant analysis revealed that a large number of genotypes were assigned to their original collecting locations with low probabilities indicating a high degree of genetic exchanges. The genotypes were separated in 11 different groups by K means clustering method with defined characteristics. The supplement of characterization of the germplasm was suggested through evaluating commercial traits and by molecular markers.

Key words: Gene Bank, Gene Pool, Genetic Resources, Genetic Variation.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a member of *Punicaceae* family and is considered for its edible fruits, medicinal properties and application in food industry (Martinez-Nicolas *et al.,* 2016). Pomegranate is originated from Iran and neighboring countries (Stover and Mercure, 2007). The importance of this product in non-oil exports made it to be considered as an export commodity in the agricultural sector (Anonymous, 2009). Iran is one of the largest pomegranate producers in the world (Sarkhosh *et al.,* 2006). About 5% of the production of horticultural products and about 15% of semi-tropical fruits production of Iran are allocated to pomegranates. Approximately 10% of the cultivation area of the subtropical fruits of Iran is dedicated to pomegranate (Anonymous, 2009).

Pomegranate is able to grow in a wide range of climatic conditions and is suitable for a variety of soils. However, it is mainly cultivated in the marginal areas of the desert, which is not suitable for the economic cultivation of many fruit trees (Sarkhosh *et al.,* 2006). Considering that the marginal areas of the desert cover the vast surface of Iran, pomegranate cultivation is very important in this unfavorable situation (Mirjalili, 2003). Yazd province is located in the central plain of Iran. This province is ranked fifth in terms of cultivation area and production of pomegranates after the provinces of Fars, Markazi, Khorasan Razavi and Isfahan (Anonymous, 2009). Yazd province is characterized by the distribution of its pomegranate orchards in most of the provinces so that it can be said that most of the agricultural land is suitable for the production of this horticultural crop (Behzadi Sharebabaki, 1991). Pomegranate cultivation in the province faces restrictions such as cold weather in winter and early spring. The land of pomegranate orchards in this province is subjected to destruction and erosion. Many of the old orchards are a mixture of different varieties of high quality and poor quality with different characteristics, and some orchards do not have enough fruiting, and therefore, it is necessary to take systematic steps to improve and replace these orchards (Behzadi Sharebabaki, 1991; Vazifeshenas, *et al.,* 2009).

Identifying genetic diversity and classifying genetic resources is a fundamental issue for the successful design of breeding programs and has a significant role in facilitating the management of conservation of genetic collections (Martinez-Nicolas *et al.,* 2016). Iran has a rich pomegranate germplasm. More than 760 pomegranate genotypes from different provinces of Iran have been collected in Yazd collection. Due to the long history of cultivation and diversity, pomegranate cultivars have similarity of names or similar genotypes in different regions. Studies have been carried out to identify pomegranate genotypes, properties and diversity in some regions of Iran with limited morphological characteristics (Behzadi Sharebabaki, 1991) as well as molecular features in the past (Moslemi *et al.,* 2010; Zeinalabedini *et al.,* 2012; Nemati *et al.,* 2012; Kazemi Alamuti *et al.,* 2013). Properties such as geometric mean diameter, volume, true density and packaging coefficient values, projected and surface areas, terminal velocity, dropping time and drag and buoyancy forces which are necessary to the design of transportation, grading and processing equipment of the pomegranate varieties have been investigated in commercial pomegranate varieties (Dadashi *et al.,* 2001). Investigation on genetic relations among some closely related Iranian pomegranate genotypes with similar names, from Yazd and Isfahan areas showed that despite the low level of detected polymorphism by AFLP marker, differences existed among genotypes in one cultivar as well as between the two geographical regions (Rahimi *et al.,* 2005). Investigation of physicochemical properties of ten varieties of Yazd pomegranate seeds showed that the highest quantities of minerals belonged to Mg, K and Na (Samadloiy *et al.,* 2006). In this research, the quantities of ash and protein in pomegranate seeds were 1.81-2.35% and 6.63- 12.95%, respectively. By studying some of the major physical and chemical properties of six pomegranate cultivars at the ripening stage, Zarei and Azizi (2010) concluded that Faroogh cultivar had more considerable advantages than the other evaluated cultivars and was recommend to be used as either for export and internal consumption or as processed forms in fruit juice factories. This cultivar had the highest aril and content of reducing sugar and lowest fruit weight and peel percentage. Evaluation of 26 quantitative and qualitative fruit traits in eleven commercial pomegranate cultivars showed that they were significantly different for all evaluated traits, except peel and aril percentage (Tatari *et al.,* 2011). The results of diversity analysis of 49 wild and commercial pomegranate genotypes by 26 qualitative and quantitative traits of fruits and leaves showed that besides commercial genotypes, wild genotypes also had desired traits, so the authors insisted on preventing these genotypes from extinction and also on using them for pomegranate improvement and establishment of collections as a basic step for the preservation purpose (Adabi Firouz Jaei *et al.,* 2013).

This research was conducted to investigate the genetic diversity of pomegranate genotypes in Yazd province and to identify distinctive traits.

MATERIALS AND METHODS

Plant materials of this research included pomegranate germplasms previously collected from different regions within Yazd province of Iran (Table 1). These comprised 117 out of more than 760 pomegranate genotypes which are maintained at Yazd pomegranate collection in Agriculture and Natural Resources Research Centre of Yazd (35°31′N, 51°67′E, 918 m alt.). The studied trees were 25 years old and planted in four replicates. A total of 23 traits were evaluated according to international descriptor of International Union for the Protection of New Varieties of Plants (2011) by obtaining thirty samples from each replicate in the 2011-2012 growing season. These traits included fruit shape, shape of fruit base, shape of fruit apex, fruit symmetry, length of fruit crown, skin color, aril color, petiole color, apical nectar gland, leaf length to width ratio, relative length of petiole to leaf middle vein, position of flowers, bearing habit, regularity of flowering, growth habit, vigor of tree, suckering tendency, wood surface, thorness, color of one-year-old shoot, shape of leaf blade, shape of leaf apex and intensity of leaf green color. Descriptive statistics including minimum, maximum, median and mode were calculated. Shannon index (Shannon, 1948) was used as the criterion for the variability of the evaluated traits within each group of sweet, sweet-sour and sour

Table 1. Pomegranate germplasm of Yazd province used for the study of genetic diversity.

No.	Genotype	Origin	No.	Genotype	Origin	No.	Genotype	Origin
1	Shekari Marvast	Khatam	20	Sahourehi Taft	Taft	39	Shirin Poust Koloft Harat	Khatam
2	Zagh Yazdi	Yazd	21	Gl Mamouli Taft	Taft	40	Gl Gazi Torsh Harabarjan	Khatam
3	Shahvar Shirin Yazdi	Yazd	22	Nabati Ardakan	Ardakan	41	Galou Barik Harat Mehriz	Mehriz
4	Malas Yazdi	Yazd	23	Gl Gabri Taft	Taft	42	Nimouli Riz Harabarjan	Khatam
5	Bazri Marvast Mehriz	Mehriz	24	Aminah Khatouni Taft	Taft	43	Khani Daneh Sefid Harabarjan	Khatam
6	Goroch Shahvar Yazdi	Yazd	25	Meykhosh Aghdaei Ardakan	Ardakan	44	Gl Gazi Torsh Hararbajan	Khatam
7	Poust Siah Abrand-Abad	Yazd	26	Poust Siah Ardakan	Ardakan	45	Nimouli Kadouei Harabarjan	Khatam
8	Togh Gardan Yazdi	Yazd	27	Souski Daneh Siah Taft	Taft	46	Dadashi Poust Koloft	Ashkezar
9	Tab-o-Larz Mehrmahi	Yazd	28	Zagh Saryazd Mehriz	Mehriz	47	Koutji Poust Nazok Bafq	Bafq
10	Khormaei Marvast Mehriz	Khatam	29	Gl Dabbehi Taft	Taft	48	Meykhosh Poust Koloft Abarkuh	Abarkuh
11	Meykhosh Marvast Mehriz	Khatam	30	Gl Peivandi Taft	Taft	49	Torsh Poust Sefid Abarkuh	Abarkuh
12	Daneh Ghermez Harati Mehriz	Mehriz	31	Khatouni Shirin Aghda	Ardakan	50	Malas Shahvar Behabad	Bafq
13	Torsh Poust Sefid Abrand-Abad	Yazd	32	Khajeh Attar Mehriz	Mehriz	51	Gardan Golabi Abarkuh	Abarkuh
14	Aminah Khatouni Abrand-Abad	Yazd	33	Shahvar Aghda Ardakan	Ardakan	52	Shahvar Dadashi Darajeh 2	Ashkezar
15	Zagh Marvast Mehriz	Mehriz	34	Hasibi Saryazd Mehriz	Mehriz	53	Sefideh Poust Khoshk Bafq	Bafq
16	Abnabati Mehriz	Mehriz	35	Mehrmahi Mehriz	Mehriz	54	Gl Magasi Taft	Taft
17	Gabri Ardakan	Ardakan	36	Tab-o-Larz Abanmahi	Yazd	55	Shirin Poust Nazok Abarkuh	Abarkuh
18	Souski Daneh Ghermez Mehriz	Mehriz	37	Zagh Dorosht Harabarjan Mehriz	Khatam	56	Dadashi Goroch Poust Nazok	Ashkezar
19	Souski Daneh Ghermez Taft	Taft	38	Torsh Poust Ghermez Harabarjan	Khatam	57	Kermani Torsh Bafq	Bafq

Continue.

58	Meykhosh Zaghi Abarkuh	Abarkuh	78	Narak Torsh Ashkezar	Ashkezar	98	Shirin Poust Nazok Saghand	Ardakan
59	Goroch Shahvar Darajeh 2	Yazd	79	Malas Gereh Abarkuh	Abarkuh	99	Shirin Malas Marvast Mahriz	Khatam
60	Malas Shahvar Post Koloft Bafq	Bafq	80	Narak Torsh Bafq	Bafq	100	Torsh Poust Koloft Saghand	Ardakan
61	Torsh Poust Nazok Abarkuh	Abarkuh	81	Dadashi Peivandi Ashkezar	Ashkezar	101	Shirin Poust Koloft Saghand	Ardakan
62	Redki Daneh Ghermez Bafq	Bafq	82	Sefideh Shireh Shirin	Bafq	102	Torsh Tafti Marvast Mehriz	Mehriz
63	Koutji Porbar Bafq	Bafq	83	Nabati Poust Sefid Ashkezar	Ashkezar	103	Gabri Sourati Abrandabad	Yazd
64	Zagh Poust Sefid Ashkezar	Ashkezar	84	Koutji Zaghi Bafq	Bafq	104	Shirin Tah Sorkh Darajeh 2 Saghand	Ardakan
65	Ghors Galou Koutah Bafq	Bafq	85	Malas Gardan Boland Ashkezar	Ashkezar	105	Malas Daneh Ghermez Saghand	Ardakan
66	Malas Peivandi Ashkezar	Ashkezar	86	Redki Daneh Sefid Bafq	Bafq	106	Bafti Poust Koloft Saghand Torsh	Ardakan
67	Narak Shirin Ashkezar	Ashkezar	87	Meykhosh Poust Nazok Abarkuh	Abarkuh	107	Bafti Poust Nazok Saghand	Ardakan
68	Shirin Poust Koloft Abarkuh	Abarkuh	88	Shahvar Dadashi Darajeh 1	Yazd	108	Torsh Poust Koloft Saghand	Ardakan
69	Malas Shahvar Post Koloft Behabad	Bafq	89	Tokhm Moushi Taft	Taft	109	Shour Post Koloft Saghand	Ardakan
70	Shirin Shahvar Ashkezar	Ashkezar	90	Shirin Poust Nazok Marvast	Khatam	110	Gabri Saih Abrandabad	Yazd
71	Post Ghermez Ashkezar	Ashkezar	91	Shour Poust Nazok Saghand	Ardakan	111	Malas Shahvar Poust Nazok Bafq	Bafq
72	Goroch Darajeh 1 Ashkezar	Ashkezar	92	Shrin Taneh Sorkh Saghand	Ardakan	112	Seh Anboli Taft	Taft
73	Malas Daneh Siah Bafq	Bafq	93	Abanmahi Abrandabad	Yazd	113	Goroch Dadashi Poust Nazok	Ashkezar
74	Post Sefid Khoshk Bafq	Bafq	94	Shirin Shahvar Darajeh 1 Saghand	Ardakan	114	Torsh Poust Sefid Chak Chak Ardakan	Ardakan
75	Gl Torsh Mamouli Taft	Taft	95	Poust Ghermeze Chak Chak Ardakan	Ardakan	115	Meykhosh Mamouli Saghand	Ardakan
76	Malas Post Ghermez Meybodi	Ashkezar	96	Shirin Poust Koloft Behabad	Bafq	116	Zagh Poust Ghermez Saghand	Ardakan
77	Ghors Galou Boland	Bafq	97	Torsh Poust Koloft	Ardakan	117	Torsh Poust Nazok	Ardakan

Table 2. Descriptive statistics of the studied traits in the pomegranate germplasm of Yazd province, Iran.

Trait	Minimum	Maximum	Median	Mode	Shannon
Fruit shape	1	3	2	1	1.05
Shape of fruit base	1	4	2	1	1.28
Shape of fruit apex	1	2	2	2	0.69
Fruit symmetry	2	5	5	5	0.99
Length of fruit crown	1	3	1	1	0.83
Skin color	3	5	3	3	0.93
Aril color	3	7	5	3	1.03
Petiole color	1	5	1	1	0.67
Apical nectar gland	1	3	1	1	0.40
Leaf length to width ratio	5	7	5	5	0.69
Relative length of petiole to leaf middle vein	1	3	2	2	0.92
Position of flowers	1	3	1	1	0.93
Bearing habit	1	3	1	1	0.62
Regularity of flowering	1	3	1	1	0.69
Growth habit	1	3	3	3	0.57
Vigour of tree	1	3	2	3	1.07
Suckering tendency	3	9	3	3	1.09
Wood surface	1	3	3	3	0.66
Thorness	1	7	1	1	0.79
Color of one-year-old shoot	2	3	2	2	0.16
Shape of leaf blade	1	3	1	1	0.09
Shape of leaf apex	1	3	3	3	0.29
Intensity of leaf green color	3	5	3	3	0.67

varieties as well as in total germplasm. The score of genotypes for each class within the assayed traits were converted to 0 or 1, representing the absence or presence of the related characteristics, respectively, for the statistical analyses. Rogers method (Rogers, 1992) was used for estimating the genetic similarity and genetic distances among genotypes. Variability of distances of origins of the studied germplasm was displayed by boxplot. Dendrogram of germplasm origins was constructed based on genetic distances. Discriminant analysis of principal components (Jombart *et al.,* 2010) was performed to investigate assignment degree of the genotypes to their original collecting site. In order to make a better distinction within the germplasm population, K means clustering approach was conducted based on principal components. In this procedure, the dimension of data is reduced by a principal component analysis (PCA) and the transformed data is subjected to successive K-means analyses with an increasing number of clusters (k). For each model, Bayesian information criterion (BIC) is computed as a statistical measure of goodness of fit, which allows to choose the optimal k (Jombart *et al.,* 2010). Features of the clusters were described by examining frequent characteristics through mode statistics. Statistical analysis and drawing the plots were performed by R software 3.2.2.

RESULTS

The distribution mode of the studied traits within the germplasm was examined by descriptive statistics (Table 2). The values of mode indicated that the traits spheroid fruit shape (1), truncate shape of fruit base (1), regular shape of fruit apex (2), non-angular symmetrical shape of fruits (5), short fruit crown (1), reddish yellow skin of fruit (3), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), regular habit of flowering (1), upright growth habit of tree (3), strong vigor of tree (3), low suckering tendency (3), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3) were the most prevalent characteristics among the investigated genotypes.

The traits shape of fruit base and shape of leaf blade had the highest and lowest diversities based on Shannon index, respectively (Table 2). Three groups of sweet, sour and sweet-sour genotypes showed a similar trend in diversity pattern of the traits based on Shannon index (Figure 1). Sweet genotypes had higher Shannon values for the traits fruit shape, shape of fruit base, fruit symmetry, fruit skin color, frequency of apical nectar glands, position of flowers, wood surface than sour and sweet-sour genotypes. Diversity of the traits length of crown, suckering tendency, leaf apex shape and intensity of leaf green color were highest in sweet-sour genotypes. Sour genotypes possessed the highest amount of Shannon index for the traits shape of fruit apex, aril color, petiole color, leaf length to width ratio, relative length of petiole to leaf middle vein, regularity of flowering, growth habit, vigor of tree, thorness, and color of one-year-old shoots as well as shape of leaf blade.

Based on genetic distances, genotypes 'Dadashi Poust Koloft' (46) and 'Khatouni Shirin Aghda' (31), and 'Narak Shirin Ashkezar' (67) and 'Shirin Poust Koloft Behabad' (96) had in pairs the highest similarity and genotypes 'Shirin Poust Nazok Marvast' (90) and 'Nimouli Kadouei Harabarjan' (45), and 'Shirin Poust Nazok Marvast' (90) and 'Shirin Poust Koloft Abarkuh' (68) had in pairs the highest distance within sweet germplasm (Figure 2). Genotypes with highest similarity in sweet-sour germplasm were in pairs 'Malas Shahvar Behabad' (50) and 'Zagh Dorosht Harabarjan Mehriz' (37), and 'Malas Shahvar Behabad' (50) and 'Gardan Golabi Abarkuh' (51), and 'Malas Gardan Boland Ashkezar' (85) and 'Malas Shahvar Behabad' (50) (Figure 2).

The highest distance in sweet-sour germplasm belonged in pairs to genotypes 'Gabri Sourati Abrandabad' (103) and 'Zagh Yazdi' (2), and 'Gabri Sourati Abrandabad' (103) and 'Nabati Poust Sefid Ashkezar' (83) (Figure 2). Genotypes 'Narak Torsh Ashkezar' (78) and 'Bazri Marvast Mehriz' (5), 'Redki Daneh Ghermez Bafq' (62) and 'Gl Gabri Taft' (23), 'Gl Torsh Mamouli Taft' (75) and 'Nimouli Riz Harabarjan' (42), 'Redki Daneh Sefid Baq' (86) and 'Nimouli Riz Harabarjan' (42), 'Torsh Poust Sefid Chak Chak Ardakan' (114) and 'Gl Gazi Torsh Harabajan' (44), 'Torsh Poust Koloft Saghand' (100) and 'Gl Magasi Taft' (54), and 'Torsh Poost Koloft Saghand' (97) and 'Gl Torsh Mamouli Taft' (75) were in pairs the most similar and genotypes 'Koutji Poust Nazok Bafq' (47) and 'Torsh Poust Ghermez Harabarjan' (38), 'Shour Poust Nazok Saghand' (91) and 'Torsh Poust Ghermez Harabarjan' (38), 'Torsh Poust Ghermez Harabarjan' (38) and 'Koutji Poust Nazok Bafq' (47), 'Kermani Torsh Bafq' (57) and 'Koutji Poust Nazok Bafq' (47), 'Shour Poust Nazok Saghand' (91) and 'Kermani Torsh Bafq' (57), 'Shour Poust Nazok Saghand' (91) and 'Goroch Darajeh 1 Ashkezar' (72) were in pairs

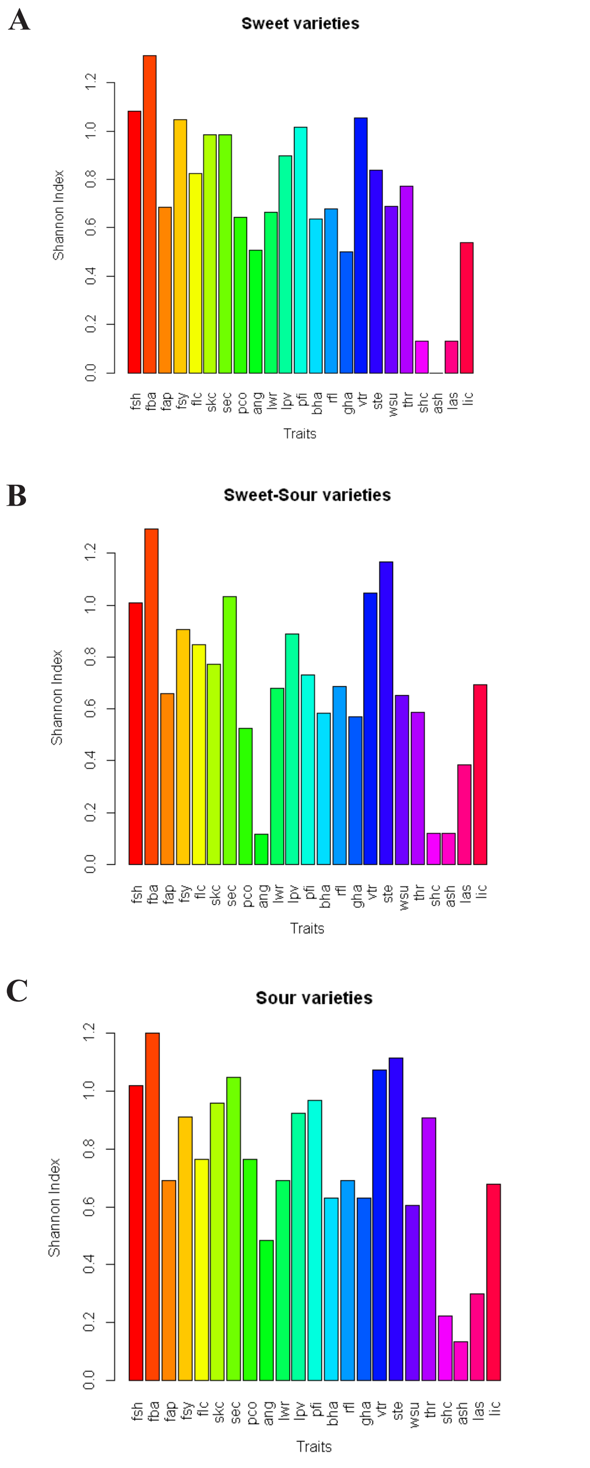

Figure 1. Barplot of Shannon index for the studied traits of Yazd pomegranate germplasm in each of **A:** sweet, **B:** sweet-sour and **C:** sour varieties.
fsh: fruit shape, fba: shape of fruit base, fap: shape of fruit apex, fsy: fruit symmetry, flc: length of fruit crown, skc: skin color, sec: aril color, pco: petiole color, ang: apical nectar gland, lwr: leaf length to width ratio, lpv: relative length of petiole to leaf middle vein, pfi: position of flowers, bha: bearing habit, rfl: regularity of flowering, gha: growth habit, vtr: vigour of tree, ste: suckering tendency, wsu: wood surface, thr: thorness, shc: color of one-year-old shoot, ash: shape of leaf blade, las: shape of leaf apex, lic: intensity of leaf green color.

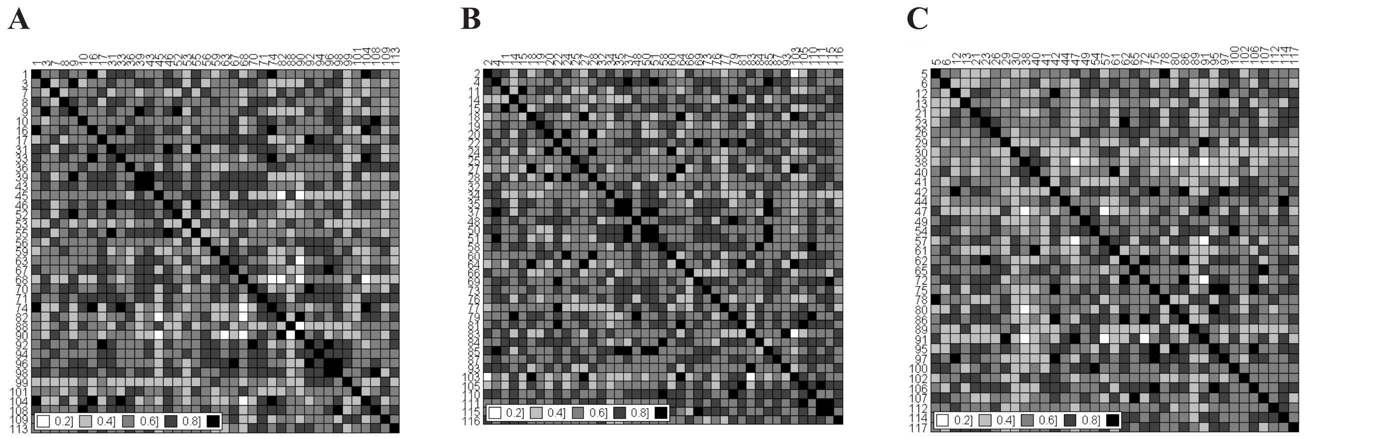

Figure 2. Gray-scaled heat map of similarity matrices between pomegranate genotypes of Yazd (Iran) in each of **A:** sweet, **B:** sweet-sour and **C:** sour classes.

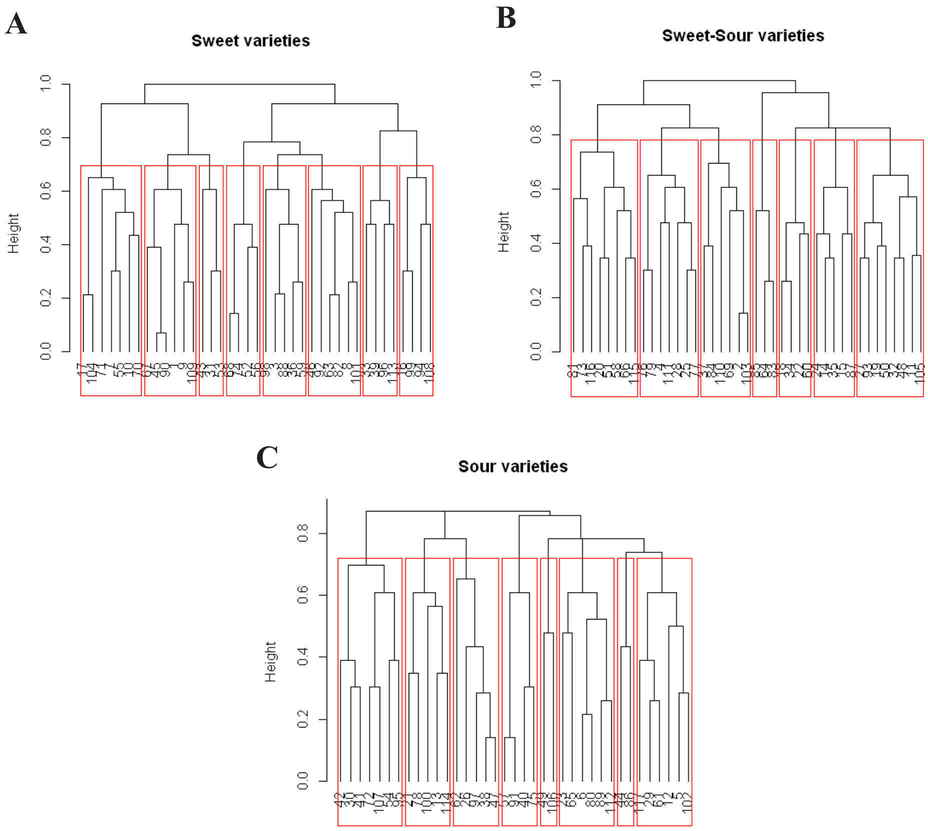

Figure 3. Dendrogram of cluster analysis of pomegranate **A:** sweet, **B:** sweet-sour and **C:** sour varieties of Yazd, Iran, through complete linkage method based on Rogers (1992) distances.

the least similar in sour germplasm. The genotypes were divided into eight, seven and eight groups by dendrogram of cluster analysis within each of sweet, sweet-sour and sour germplasm, respectively (Figure 3).

Genetic distances of the studied germplasms were also investigated based on their origin and the results were shown by boxplot (Figure 4). The boxes of Taft and Abarkuh were located higher in the boxplot indicating higher distances to other origins. These regions along with Yazd had the shortest length of boxes showing lower variation in the distances of these regions to other places. Ardakan had the highest box length which indicates that this region had varied distances with other origins being very close to some

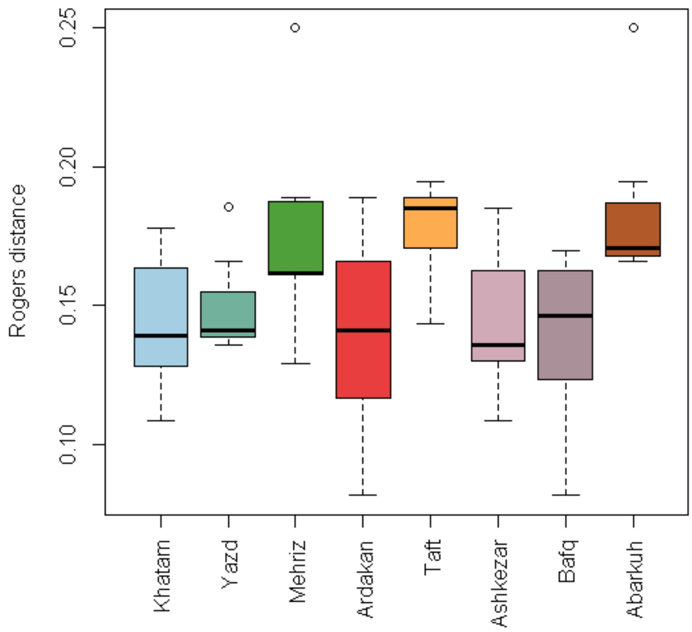

Figure 4. Genetic distances of pomegranate genotypes with Yazd origin.

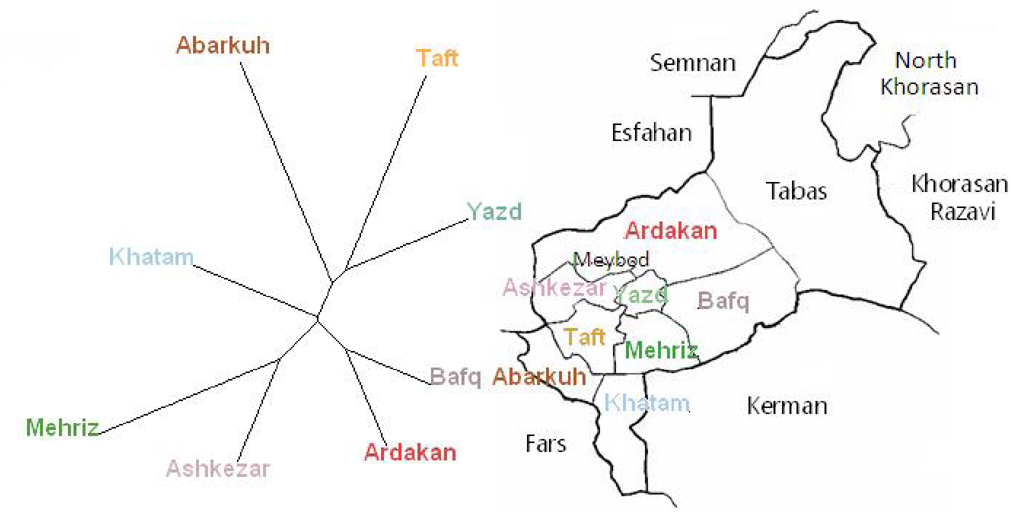

Figure 5. Comparison of dendrogram and geographical origins of pomegranate genotypes of Yazd, Iran.

of them while far from the others. Dendrogram of the origins of the studied germplasm located Taft and Yazd regions in the same group while Bafq and Ardakan together were placed in another group distinct from group of Ashkezar and Mehriz. Each of Abarhuk and Khatam regions also made single-membered groups. (Figure 5).

Discriminant analysis was performed to study the origin relationship of the genotypes more precisely. Three regions of Abarkuh, Taft and Mehriz were differentiated from five other origins in the distribution plot of individuals based on the first two discriminant functions (Figure 6). Discrimination of Taft was mainly based on the first discriminant function, with the highest contribution of the traits thorness (thr.5 and thr.7), petiole color other than red and yellow (pco.3), shape of leaf blade (ash.1 and ash.3), very short length of petiole relative to leaf middle vein (lpv.1) and lateral position of flowers (pfi.1) (Figure 7).

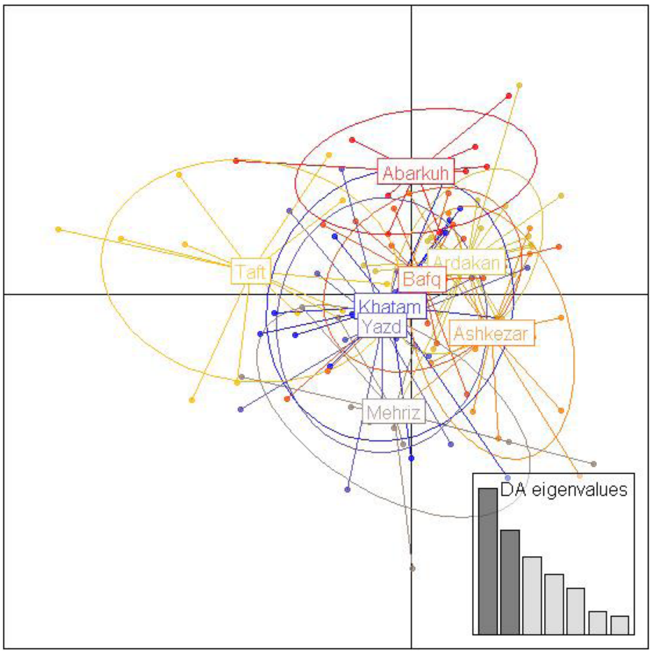

Figure 6. Distribution of pomegranate germplasm of Yazd (Iran) in biplot of the first two discriminant functions.

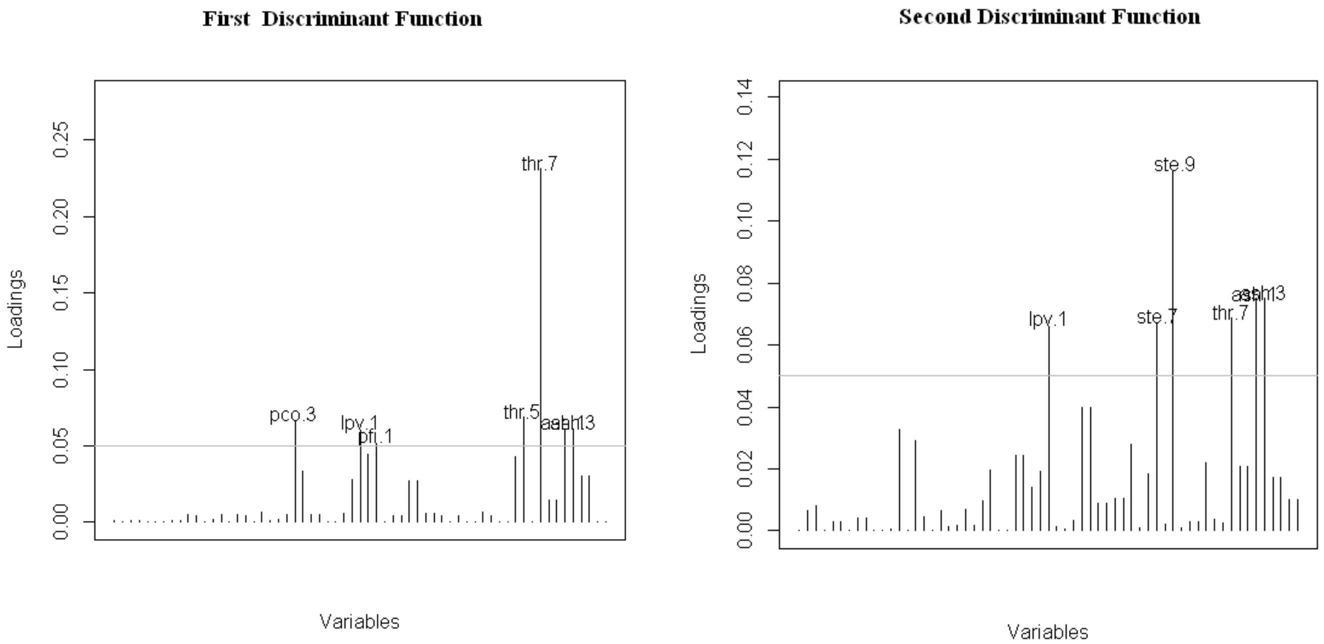

Figure 7. Presentation loadings of the traits in the first two discriminant functions in the pomegranate germplasm of Yazd, Iran. thr: thorness, ste: suckering tendency, pco: petiole color, ash: shape of leaf blade, lpv: relative length of petiole to leaf middle vein, pfi: position of flowers.

The other regions were differentiated along with axis of the second discriminant function. The loading plot revealed that the traits very high and high suckering tendency (ste.7 and ste.9), shape of leaf blade (ash.1 and ash.3), very high thorness (thr.7), and very short length of petiole relative to leaf middle vein (lpv.1) had the main contribution in variation of second discriminant function.

Membership probability of assignment of the individuals to the eight germplasm origin was plotted

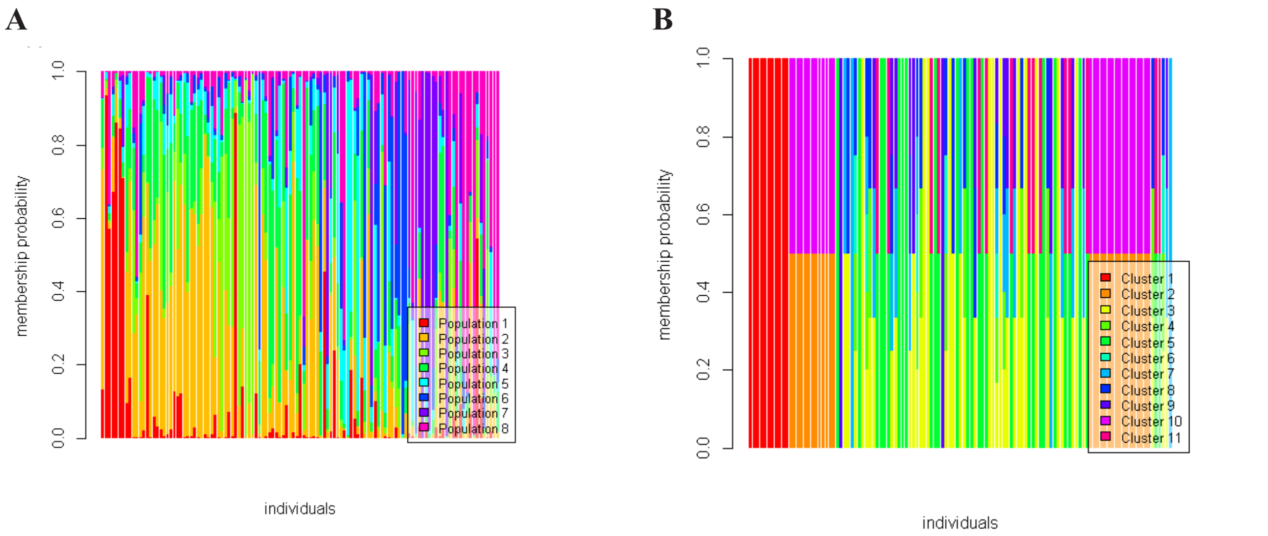

Figure 8. Membership probability of assignment of individuals to **A:** the germplasm origins and **B:** groups developed through K means clustering approach after performing discriminant function analysis on pomegranate genotypes of Yazd, Iran. Population 1 to eight are Abarkuh, Ardakan, Ahkezar, Bafq, Khatam, Mehriz, Taft and Yazd, respectively.

(Figure 8). The results revealed a high level of admixture in the studied germplasm. K means clustering approach was performed to detect the structures in the population with the highest discrimination and to compare the resulting differentiation pattern with that resulted from origins of the genotypes. The values of BIC showed that with 11 clusters in the population, the highest discrimination is attainable (Figure 9). Plotting the number of cluster members against that of individuals related to their origin revealed that some clusters comprised individuals of certain origins exclusively (Figure 10). Cluster 11 made the most exclusive group comprising only individuals belonging to Ardakan, Taft and Bafq while clusters 4 and 10 generally included individuals from all origins. Clusters 5, 7 and 9 comprised mostly of sweet varieties. Sweet-sour varieties were mostly included in clusters 1, 3, 4, 6, 8 and 10, and sour varieties were mainly lodged in clusters 2 and 11. Clusters 2 and 10 had the highest similarity while clusters 7 and 10 had the maximum distance (Table 3).

Mode statistic was used to determine the features of each cluster (Table 4). With this approach the traits ovoid fruit shape (3), opened shape of fruit base (4), long fruit apex (1), symmetrical pentagon shape of fruits (2), short fruit crown (1), reddish yellow skin of fruit (3), dark red aril (sec), red petiole (1), absence of apical nectar gland (1), high ratio of leaf length to width ratio (7), medium length of petiole relative to leaf middle vein (3), lateral position of flowers or inflorescence (1), predominant distribution of flower

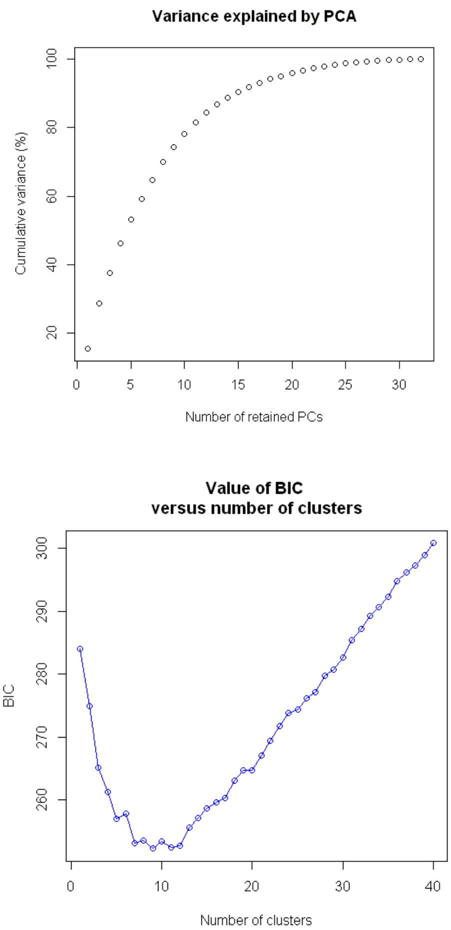

Figure 9. Variance values explained by principal components and the trend of Bayesian information criterion (BIC) on selecting different number of clusters through K means clustering approach in the study of pomegranate genotypes of Yazd, Iran.

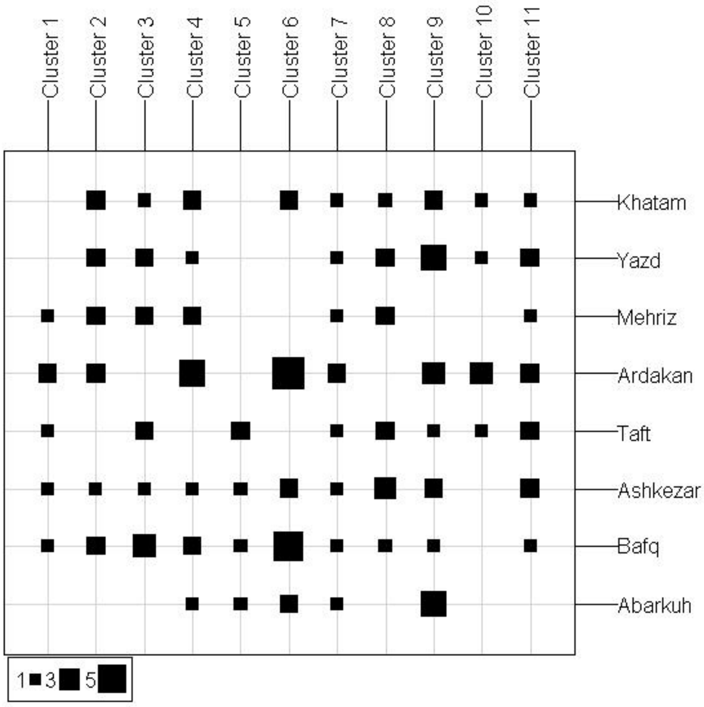

Figure 10. Membership distribution of pomegranate genotypes of Yazd (Iran) with different origins within groups developed by K means clustering approach.

Table 3. Distance matrix of different groups within pomegranate germplasm of Yazd (Iran) developed by K means clustering approach.

Clusters	2	3	4	5	6	7	8	9	10	11
1	0.334	0.292	0.475	0.311	0.285	0.440	0.468	0.378	0.402	0.397
2		0.359	0.434	0.384	0.341	0.427	0.410	0.412	0.209	0.381
3			0.326	0.341	0.260	0.429	0.440	0.279	0.348	0.342
4				0.385	0.346	0.447	0.264	0.391	0.288	0.287
5					0.309	0.415	0.453	0.411	0.382	0.423
6						0.329	0.380	0.368	0.425	0.310
7							0.439	0.465	0.498	0.39
8								0.376	0.409	0.282
9									0.377	0.383
10										0.487

buds on young shoots (1), irregular habit of flowering (rfl), upright growth habit of tree (gha), strong vigor of tree(vtr), low suckering tendency (3), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low or medium intensity of leaf green color (3-5) were known as the major features of cluster 1.

Cluster 2 was characterized with the traits ellipsoid fruit shape (2), truncate shape of fruit base (1), long fruit apex (1), non-angular symmetrical shape of fruits (5), short fruit crown (1), red skin of fruit (4), light red aril (5), red petiole (1), absence of apical nectar gland (1), high ratio of leaf length to width ratio (7), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), irregular habit of flowering (3), upright growth habit of tree (3), strong vigor of tree (3), low suckering tendency (3), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape

Table 4. Features of the groups in pomegranate germplasm of Yazd (Iran) developed by K means clustering approach based on the values of mode statistics.

Trait	Cluster										
	1	2	3	4	5	6	7	8	9	10	11
tst	2	3	2	2	1	2	1	2	1	2	3
fsh	3	2	2	1	3	1	1	1	2	2	1
fba	4	1	1	3	2	2	2	3	1	1	3
fap	1	1	2	2	1	2	2	2	2	1	2
fsy	2	5	2	5	3	3	3	5	2	5	5
flc	1	1	1	2	1	1	1	3	1	1	3
skc	3	4	3	4	5	3	3	3	3	4	3
sec	7	5	3	3	3	7	3	3	3	5	3
pco	1	1	1	1	1	1	1	3	1	1	1
ang	1	1	1	1	1	1	3	1	1	1	1
lwr	7	7	5	5	5	7	7	5	5	5	7
lpv	3	2	2	2	2	2	1	2	2	2	3
pfi	1	1	1	3	1	1	2	1	3	3	1
bha	1	1	1	1	1	1	3	3	1	1	1
rfl	3	3	1	1	3	3	3	1	1	1	3
gha	3	3	3	3	3	3	3	3	1	3	1
vtr	3	3	3	2	2	3	2	1	1	2	3
ste	3	3	3	3	3	3	7	3	7	3	7
wsu	3	3	1	1	1	1	1	3	3	3	1
thr	1	1	1	1	1	1	1	1	1	1	1
shc	2	2	2	2	2	2	2	2	2	2	2
ash	1	1	1	1	1	1	1	1	1	1	1
las	3	3	3	3	3	3	3	3	3	3	3
lic	*3-5	3	3	3	3	3	3	3	5	3	*3-5
	18	4	22	2	7	6	19	3	10	1	54
	26	12	44	11	13	14	30	5	36	8	84
	27	21	67	15	25	17	38	9	49	16	89
	59	23	77	29	41	43	40	20	69	33	100
	64	32	81	31	56	48	45	24	107	34	101
	71	42	87	36	82	58	53	28	108	35	116
	73	57	96	47	90	65	61	39	111	37	
	99	62	98	55	94	70	63	52	113	50	
Members	103	72	105	66	102	79	68	60	115	51	
	109	86	114	91		92		78		74	
	112	88				110		83		75	
		97						93		76	
		117								80	
										85	
										95	
										104	
										106	

*The case of multi-modes

tst: type of varieties based on taste, fsh: fruit shape, fba: shape of fruit base, fap: shape of fruit apex, fsy: fruit symmetry, flc: length of fruit crown, skc: skin color, sec: aril color, pco: petiole color, ang: apical nectar gland, lwr: leaf length to width ratio, lpv: relative length of petiole to leaf middle vein, pfi: position of flowers, bha: bearing habit, rfl: regularity of flowering, gha: growth habit, vtr: vigour of tree, ste: suckering tendency, wsu: wood surface, thr: thorness, shc: color of one-year-old shoot, ash: shape of leaf blade, las: shape of leaf apex, lic: intensity of leaf green color.

of leaf apex (3) and low intensity of leaf green color (3).

Major characteristics of Cluster 3 included ellipsoid fruit shape (2), truncate shape of fruit base (1), regular size of fruit apex (2), symmetrical pentagon shape of fruits (2), short fruit crown (1), reddish yellow skin of fruit (3), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), regular habit of flowering (1), upright growth habit of tree (3), strong vigor of tree (3), low suckering tendency (3), smooth wood surface (1), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (ash) (1), acute shape of leaf apex (3) and low intensity of leaf green color (3).

Cluster 4 could be recognized mainly with the traits spheroid fruit shape (1), covex shape of fruit base (3), regular size of fruit apex (2), non-angular symmetrical shape of fruits (5), medium fruit crown (2), red skin of fruit (4), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), terminal position of flowers or inflorescence (3), predominant distribution of flower buds on young shoots (1), regular habit of flowering (1), upright growth habit of tree (3), medium vigor of tree (2), low suckering tendency (3), smooth wood surface (1), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3).

Cluster 5 had the features of ovoid fruit shape (3), covex shape of fruit base (2), long fruit apex (1), asymmetrical shape of fruits (3), short fruit crown (1), dark red skin of fruit (skc) (5), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), irregular habit of flowering (3), upright growth habit of tree (3), medium vigor of tree (2), low suckering tendency (3), smooth wood surface (1), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3).

The traits spheroid fruit shape (1), covex shape of fruit base (2), regular size of fruit apex (2), asymmetrical shape of fruits (3), short fruit crown (1), reddish yellow skin of fruit (3), dark red aril (7), red petiole (1), absence of apical nectar gland (1), high ratio of leaf length to width ratio (7), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), irregular habit of flowering (3), upright growth habit of tree (3), strong vigor of tree (3), low suckering tendency (3), smooth wood surface (1), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3) were majorly assigned to the cluster 6.

Characteristics spheroid fruit shape (1), covex shape of fruit base (2), regular size of fruit apex (2), asymmetrical shape of fruits (3), short fruit crown (1), reddish yellow skin of fruit (3), light pink aril (3), red petiole (1), moderately developed apical nectar gland (3), high ratio of leaf length to width ratio (7), very short length of petiole relative to leaf middle vein (1), both lateral and terminal position of flowers or inflorescence (pfi) (2), predominant distribution of flower buds on old shoots (bha) (3), irregular habit of flowering (rfl) (3), upright growth habit of tree (gha) (3), medium vigor of tree(vtr) (2), low suckering tendency (ste) (7), smooth wood surface (wsu) (1), absence of thorns (thr) (1), green color of one-year-old shoot with pink stripes (shc) (2), obtuse shape of leaf blade (ash) (1), acute shape of leaf apex (las) (3) and low intensity of leaf green color (lic) (3) were major in Cluster 7

Cluster 8 was characterized with the traits spheroid fruit shape (1), angular shape of fruit base (3), regular size of fruit apex (2), non-angular symmetrical shape of fruits (5), long fruit crown (3), reddish yellow skin of fruit (3), light pink aril color (3), petiole color other than red or yellow (3), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on old shoots (3), regular habit of flowering (1), upright growth habit of tree (3), weak vigor of tree (1), low suckering tendency (3), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3).

The traits ellipsoid fruit shape (2), truncate shape of fruit base (1), regular size of fruit apex (2), symmetrical pentagon shape of fruits (2), short fruit crown (1), reddish yellow skin of fruit (3), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), terminal position of flowers or inflorescence (3), predominant distribution of flower buds on young shoots (1), regular habit of flowering (1), spreading growth habit of tree (1), weak vigor of tree (1), high suckering tendency (7), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and medium intensity of leaf green color (5) were known as the major features of cluster 9.

Cluster 10 could be recognized mainly with the traits ellipsoid fruit shape (2), truncate shape of fruit base (1), long fruit apex (1), non-angular symmetrical shape of fruits (5), short fruit crown (1), red skin of fruit (4), light red aril color (5), red petiole (1), absence of apical nectar gland (1), medium ratio of leaf length to width ratio (5), short length of petiole relative to leaf middle vein (2), terminal position of flowers or inflorescence (3), predominant distribution of flower buds on young shoots (1), regular habit of flowering (1), upright growth habit of tree (3), strong vigor of tree (2), high suckering tendency (3), coarse wood surface (3), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low intensity of leaf green color (3).

Members of mainly express the traits of Cluster 11 spheroid fruit shape (1), angular shape of fruit base (3), regular size of fruit apex (2), non-angular symmetrical shape of fruits (5), long fruit crown (3), reddish yellow skin of fruit (3), light pink aril color (3), red petiole (1), absence of apical nectar gland (1), high ratio of leaf length to width ratio (7), medium length of petiole relative to leaf middle vein (3), lateral position of flowers or inflorescence (1), predominant distribution of flower buds on young shoots (1), irregular habit of flowering (3), spreading growth habit of tree (1), strong vigor of tree (3), high suckering tendency (7), smooth wood surface (1), absence of thorns (1), green color of one-year-old shoot with pink stripes (2), obtuse shape of leaf blade (1), acute shape of leaf apex (3) and low or medium intensity of leaf green color (lic) (3-5).

DISCUSSION

The population under study contained a few pairs of genotypes with similar nominations. Two genotypes of 40 and 44 had the same name of 'Gl Gazi Torsh Harabarjan', however they had similarity value of 0.48 which was lower than the third quartile of similarity values (0.64), but also very close to the first quartile (0.44). In other words, despite their names being the same, these two genotypes did not show close resemblance. Genotype 97 and 108 also had the same names as 'Torsh Poust Koloft Saghand'. Similarity value for these two genotypes was estimated 0.57 which is lower than the third quartile of similarity values (0.64) and close to the median (0.52). Therefore these two genotypes also appeared to be different despite the same designation. There were two genotypes (14 and 24) with the name of 'Aminah Khatouni', which one of them was originated form Taft and the other from Abrand-Abad. Based on the value of similarity (0.39) these two genotypes also had low resemblance. Genotypes 60 and 69 (from Bafq and Bahabad, respectively) both were named 'Malas Shahvar Post Koloft' and with a similarity value (0.61) not higher than the third quartile. Genotypes 78 and 80 with common name of 'Narak Torsh' and originated from Ashkezar and Bafq, respectively, also appeared to have low resemblance with the similarity value of 0.39. Two genotypes 68 and 96 shared the name of 'Shirin Poust Koloft' and were from Abarkuh and Bahabad, respectively, and had medium similarity of 0.52. There were also two genotypes (55 and 90) with the name of 'Shirin Poust Nazok', collected from Abarkuh and Marvast, respectively, with a low similarity value of 0.42. The name of 'Souski Daneh Ghermez' was assigned to genotypes 18 from Mehriz and 19 from Taft, while these two genotypes had a medium similarity value of 0.61. Two genotypes 13 (from abarkuh) and 49 (from Abrand-Abad) with the common name of 'Torsh Poust Sefid' showed a low similariy value (0.43). Therefore, as it is evident, the pairs of genotypes which shared the common names within the studied germplasm did not appear to have similar phenotypic features. Grouping varieties with different agronomic characteristics under the same designation have been reported before. Melgarejo and Salazar (2003) observed that there were varieties with different agronomic characteristics under common name of 'Mollar de Elche''.

Identification of the distinctive traits is necessary for better characterization and analysis of genetic diversity of germplasm collection (Martinez-Nicolas *et al.,* 2016). Shannon index for the traits shape of fruit base, suckering tendency, vigor of tree, fruit shape and aril color was higher than one. So these traits had the capability of making higher distinction in the studied germplasm. Traits fruit symmetry, position of flowers or inflorescence, skin color and relative length of petiole to leaf middle vein also had a high value of Shannon index (higher than 0.9). Therefore, these traits could be considered as differentiating characteristics of pomegranate germplasm in the second order. Martinez-Nicolas *et al.* (2016) suggested parameters related to fruit and seed size as well as the juice's acidity and pH as the most useful for pomegranate genetic characterization since they had the highest power of discrimination which was opposed to leaf and flower characteristics with low power of differentiation.

High level of admixture in the studied germplasm revealed by the results of discriminant analysis suggests a high exchange rate in plant materials among the different cultivation regions over time. These results are consistent with observations of Kazemi Alamuti *et al.* (2013) for Iranian sour pomegranate germplasm by using microsatellite markers and in conflict with the findings of Martinez-Nicolas *et al.* (2016) based on morphological characterization. Unlike these observations of total admixture in the studied germplasm, the results of dendrogram of population origins revealed a slight conformity with geographical distribution in that some neighboring regions were located in the same group as for Yazd and Taft. Using amplified fragment length polymorphism (AFLP) to detect intra- and inter-population genetic diversity of pomegranate, Moslemi *et al.* (2010) declared that in some cases, accessions from the same region were grouped together but in most cases, there was gene exchange.

Membership of genotypes to defined groups was improved by implementation of K means clustering approach as evident by the figure 8. The resulting clusters were well separated from each other indicating suitable genetic variation. The results of clustering based on fruit characteristics by Durgaç *et al.* (2008) indicated that some local cultivars of Turkey were similar to each other and they were separated from rest of the cultivars.

CONCLUSION

The results of analyses performed in this research made a well separation and differentiation in Iranian pomegranate germplasm with Yazd origin. The studied genotypes were grouped in distinguished clusters with defined features which could be utilized for breeding purposes. The variation among the clusters in terms of the evaluated traits indicates suitable diversity in the genetic material under study. It was found that the traits shape of fruit base, suckering tendency, vigor of tree, fruit shape and aril color had the highest degrees of discrimination and are suggested for the characterization of pomegranate germplasm collections. Similarity of phenotypic characteristics in genotypes with common denomination was investigated and the results indicated high discrepancies. It is suggested that characterization of the germplasm is continued by supplementing commercial traits and molecular marker evaluations.

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