

Evaluation of Iranian Soft-seed Pomegranate Accessions by using Simple and Multivariate Analyses

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ABSTRACT

An important aspect of soft-seed pomegranates is their pleasant organoleptic character for breeding programs. In this study some quantitative and qualitative characters of 21 Iranian soft-seed pomegranate fruits and their seed components were recorded for categorizing the accessions. Analysis of variance showed that all of the characters in the examined accessions were significant, showing high variability. Results of bivariate simple correlation analysis showed the existence of significant positive and negative correlations among some important characters. Factor analysis showed that fruit length, fruit width and fruit juice, aril and seed characters composed the main factors. The most effective characters were categorized into 10 main factors (with an Eigen value ≥ 1) that contributed to 91.51% of total variance. For each factor, loading value of more than 0.5 was used as the significant threshold level. Cluster analysis was performed using these 10 factors and genotypes at a distance of 10 out of 25 were divided into 4 main clusters. These groups were mainly distinguished by their soft seededness, and aril and fruit size. Furthermore, by using three main factors, genotypes were plotted in 3 dimensions, in which accession Bihaste Shirin Khabre Baft with hard seeds was separated from the rest of the accessions.

Keywords: bivariate correlation, cluster analysis, factor analysis, *Punica granatum* L., quantitative and qualitative characters

INTRODUCTION

Punica granatum L., the pomegranate, belongs to the Puniceae family and has a long history of cultivation and consumption among edible fruits. This fruit is cultivated extensively in Iran, Afghanistan, India, Mediterranean countries (Tunisia, Turkey, Egypt, Spain and Morocco) and also extends to the U.S.A. (California), China, Japan and Russia (LaRue 1980; Mars 1994). Historical evidence revealed that its primary origin is Iran and then spread to other areas (Levin 1994). Some wild types of pomegranate grow in the North and West forests and in other districts of Iran. Some efforts have been made to collect about 760 genotypes, specimens and cultivars of pomegranate from different parts of the Iran, constituting the Yazd pomegranate collection (Behzadi Shahrabaki 1998). Among the collected pomegranates accessions, there are 21 soft-seed genotypes with almost no or little information about their germplasm potential. Pomegranate fruit characteristics such as shape, size, peel and aril color, water content, total soluble solids (TSS) and titrable acidity (TA), which display great differences among genotypes can be influenced by genetic and climate conditions, and harvesting time (Tous and Ferguson 1996). Some other important characteristics of pomegranate fruit include soft seededness, resistance to cracking, pests and diseases and its features for sorting and marketing. The soft-seed character, or seedlessness, is a desirable commercial index for establishing the quality of pomegranate fruit, with great differences among pomegranate genotypes being displayed. The severity of fruit cracking is influenced by environmental conditions, horticultural practices and management and also peel flexibility (Mars and Marrakchi 1998). The main pests with harmful effects on pomegranate fruit quality are *Spectrobates ceratoniae* and *Euzophera puniciella* to which sour and sweet-sour fruits are more tolerant than sweet ones (Tous and Ferguson 1996). Fruit transportation, shelf life and storing are also important for processing and consumers (Kahtani 1992). A modern objec-

tive in plant breeding may be achieved by evaluation of traits among genetic resources and combining those of interest in one cultivar (Fatahi *et al.* 2004). For this purpose precise determination and discrimination of the pomegranate genotypes is required to find those carrying useful traits. New methods for cultivar fingerprinting using molecular markers such as isozymes, Randomly Amplified Polymorphism DNA (RAPD), Simple Sequence Repeat (SSRs) and Amplified Fragment Length Polymorphism (AFLP) have proved to be useful in distinguishing varieties, but these methods are expensive and require well-equipped laboratories (Kumar 1999; Gupta and Rustgi 2004). On the other hand, morphological characterization is the first and basic step in description and classification of germplasm. Zamani (1990) characterized some pomegranates by using morphological traits. Diversity of pomegranate germplasm in Tunisia based on fruit characteristics was reported by Mars and Marrakchi (1999). Multivariate statistical analysis is also efficient and useful for germplasm evaluation by revealing obvious relationships between dependent and independent characters (Dennis and Adams 1979). Factor analysis is a powerful multivariate statistical method to detect biological relationships between characteristics, reducing many dependent characteristics to limited factors (Walton 1972; Guertin 1982; Johnson and Wichern 1988). Multivariate statistical methods have been used for separation and clustering some pomegranate genotypes (Sarkhosh *et al.* 2006), sour cherry (Karl *et al.* 1988), date (Jaradat and Zaid 2004) and other fruit tree genotypes (Koehler-Santos *et al.* 2003; Fatahi *et al.* 2004).

Due to the high economical importance of pomegranate in Iran, this study was performed to find out more about the relationships among characters of Iranian soft-seed pomegranate genotypes. To the best of our knowledge, this is the first report to evaluation the quantitative and qualitative characteristics of fruit and seed components in Iranian soft-seed pomegranate accessions.

MATERIALS AND METHODS

Plant material

Fruit samples at commercial maturity stage from 21 pomegranate accessions (**Table 1**) were collected from mature bearing trees growing in the Agricultural Research Center of Yazd, central Iran. Three trees per genotype as replications and five fruit samples per replication, i.e. altogether 15 mature fruits and 25 arils (seeds of pomegranate with fleshy edible outer layers) per replication for each accession were evaluated.

Fruit characteristics

Fruit characteristics were measured based on morphometric data and chemical analysis. Thirty six quantitative and qualitative characteristics were analyzed for each accession (**Table 2**) according to Zamani (1990) and Mars and Marrakchi (1999), as follows: fruit weight (g), fruit length (mm), fruit diameter (mm), juice pH, total soluble solids (by Refractometer Atago Co., Japan), and titrable acidity (measuring with 0.1 N NaOH until reaching the pH of 8.2 and calculating based on citric acid), fruit flavor index (TSS/TA), peel weight (g), peel thickness (mm), 100 aril fresh weight (100 Afw, g), 100 aril dry weight (100 Adw, g), aril dry weight percent (Adw%), 100 seed fresh weight (100 Sfw, g), 100 seed dry weight (100 Sdw, g), seed dry weight percent (Sdw%), peel thickness (mm), fruit crown length (mm), fruit neck diameter (mm), fruit crown diameter (mm), aril total weight (g), peel total weight (g), peel percent (%), aril percent (%), aril length and diameter (mm, 25 arils for each fruit), aril length/diameter, seed length and diameter (mm, 25 seeds/fruit), seed length/diameter.

Ascorbic acid content was measured according to the protocol of Redox titration using iodine solution (www.outreach.canterbury.ac.nz). For measuring the total phenol content, samples of arils and peels were air-dried at 55°C and homogenized. Then, dried sample (1 g) was transferred to a test tube containing 10 ml of extraction solution (50% methanol/H₂O) according to Vinson *et al.* (2001). The mixture was kept in the dark at 4°C for 24 h. The supernatant was collected and replaced with an equal quantity of extraction solution, then placed in the dark at 4°C for a further 48 h. The two supernatants were mixed and added to the extraction solution to get a final volume of 25 ml, which was then used to determine the phenol content.

The amount of total phenolic compounds was determined according to the procedure of Folin-Ciocalteu (Singleton and Rossi 1965), which is, briefly described next. Diluted extract (0.05 ml of extract and 0.45 ml of water) were added to 2.5 ml of 1:10 diluted Folin-Ciocalteu's phenol reagent, followed adding by 2 ml of

7.5% (w/v) sodium carbonate. After 5 min incubation at 50°C, absorbance was measured at 760 nm using a spectrophotometer (Perkin Elmer, Lambda EZ201, USA). Phenol content was estimated from a standard curve of gallic acid and results were expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ dry weight (dw).

Antioxidant activity of pomegranate juice (0.1 ml) was determined by the DPPH method described by Moon and Terao (1998). Pomegranate fresh juice was mixed with 0.9 ml of 100 mM Tris-HCl buffer (pH = 7.4) then 1 ml of DPPH (500 µM in ethanol) was added. The mixture was shaken gently and left for 30 min. Absorbance of the final solution was measured at 517 nm by a UV-Visible spectrophotometer (Perkin Elmer, Lambda EZ201, USA). The reaction mixture without DPPH was used as a background correction. The antioxidant activity (AA) was calculated using the following equation:

$$AA (\%) = (1 - A_{\text{sample}}(517 \text{ nm}) / A_{\text{control}}(517 \text{ nm})) \times 100.$$

For measuring anthocyanin, 1 ml of fruit juice was diluted in 3 ml water, and absorbance of the diluted solution was measured at 510 nm by the above mentioned spectrophotometer set.

For measuring the woody portion index (Wpi), 25 seeds were taken at random, individually weighed (Ws) and also their length and width were recorded. Testa were then separated and the interior part of the seeds was weighed (woody part, Wwp). The index, defined as $Wpi = (Wwp/Ws) \times 100$ (Melgarejo 1996) which is related to seed hardness and useful for measuring palatability was calculated.

Statistical analysis

The mean values of parameters were used to perform factor analysis and clustering of the genotypes. Analysis of variance for all traits was performed with SAS software. SPSS Ver. 10 software was used for factor analysis (Varimax rotation) and clustering of genotypes (Ward's method).

RESULTS AND DISCUSSION

Analysis of variance

Mean values of the studied morphometric characteristics showed large variations between genotypes for all traits. Mean values and ranges of variability for the different characteristics among accessions are presented in **Tables 2** and **3**. Characteristics showing a greater quantity range among accessions had a higher coefficient of variation (CV%), implying the existence of a higher range of selection for those characteristics. Aril length, aril length/diameter, seed dry weight, seed length and diameter, fruit flavor index and Wpi

Table 1 List of pomegranate accessions used in this study with their some specific characters.

No.	Genotypes*	Peel color	Taste	Seed
1	Bihaste Neiriz	Yellow	Sweet	Semi-soft
2	Bihaste Najaf Abad	Yellow	Sweet	Semi-soft
3	Bihaste Ladiz	Yellow	Sweet	Semi-soft
4	Bihaste Dane Sefide Ravar	Yellow	Sweet	Soft
5	Behaste Sistan va Balochestan	Yellow	Sweet	Semi-soft
6	Bihaste Porbar Shirin	Yellow	Sweet	Semi-soft
7	Shirin Bihaste Najaf Abad	Yellow	Sweet	Semi-soft
8	Bitolf Dane Ghermez	Red	Sweet-Sour	Semi-hard
9	Bihaste Khafre Jahrom	Red	Sour	Semi-soft
10	Bihaste Sangan	Yellow	Sweet	Soft
11	Bihaste Shirin Khabre Baft	Yellow	Sweet	Hard
12	Bidane Kashmar	Red	Sweet-Sour	Semi-hard
13	Bihaste Ghasrodasht	Yellow	Sweet	Semi-soft
14	Bihaste Shirin Kambar	Yellow	Sweet	Semi-soft
15	Bihaste Ardestan	Red	Sweet-sour	Semi-hard
16	Bitolf Dane Sefid	Red	Sour	Semi-hard
17	Bihaste Shirin Saravan	Yellow	Sweet	Soft
18	Bidane Darjazin	Yellow	Sweet	Semi-soft
19	Bihaste Chenche	Yellow	Sweet	Semi-soft
20	Bihaste Dane Ghermez Kerman	Yellow	Sweet	Semi-hard
21	Bihaste Hajiabad	Yellow	Sweet	Soft

*: Bihaste, Bidane and Bitolf are all soft-seeded pomegranate genotypes in Persian language (Farsi), depending on the names that have been given at the original locations where these cultivars are being cultivated

Table 2 Fruit characteristics, range of variability, means and coefficient of variability.

No.	Trait	Abbreviation	Unit	Min	Mean	Max	CV% ¹
1	100 seed dry weight	100 SeDW	g	2.85	3.18	3.70	25.07
2	Aril dry weight percent	ArDWP	%	15.36	18.35	24.90	24.46
3	Aril total weight	ArTW	g	91.82	157.69	234.55	36.30
4	Aril percent	Ar%	%	36.80	59.42	81.62	34.32
5	Peel total weight	PeTW	g	81.30	109.44	156.10	25.63
6	Peel percent	Pe%	%	27.53	41.65	62.01	26.52
7	100 aril fresh weight	100 ArFW	g	30.00	44.35	58.50	36.29
8	100 aril dry weight	100 ArDW	g	6.25	8.00	10.97	30.07
9	Seed dry weight percent	SdDW%	%	56.48	75.65	89.67	53.24
10	Peel thickness	PeT	mm	1.1	3.2	4.4	12.30
11	Aril length	ArL	mm	10	12.3	15.1	45.56
12	Aril diameter	ArD	mm	4.9	6.9	8.2	36.52
13	Aril length/diameter	ArL/ArD	Ratio	1.38	1.75	2.03	56.21
14	Seed length	SeL	mm	6.4	7.6	8.6	48.25
15	Seed diameter	SeD	mm	0.7	2.4	3.2	43.25
16	Seed length/diameter	SdL/SdD	Ratio	2.23	3.13	3.91	34.25
17	pH	pH	-	3.10	3.74	4.13	12.01
18	Electric conductivity	EC	mmoh/cm	0.30	2.25	3.40	20.13
19	100 seed fresh weight	100 SeFW	g	25.10	42.90	55.00	25.34
20	Anthocyanin absorbance OD _{510 nm}	AnA	OD _{510 nm}	0.83	1.21	1.94	13.26
21	Total soluble solids	TSS	%	11.36	13.87	16.20	9.47
22	Titration acidity	TA	%	0.15	0.26	0.94	18.06
23	Fruit flavor index	FrFI	Ratio	17.6	64.55	93.88	45.65
24	Fruit weight	FrW	g	164.89	271.08	375.76	35.46
25	Fruit length	FrL	mm	64	77.5	137.4	34.52
26	Fruit diameter	FrD	mm	68	78.8	86.9	37.51
27	Fruit length/diameter	FrL/FrD	Ratio	0.88	0.98	1.66	24.21
28	Fruit crown length	FrCL	mm	16.7	20.6	29.9	25.21
29	Fruit crown diameter	FrCD	mm	13.9	16.8	25	13.24
30	Fruit crown length/diameter	FrCL/FrCD	Ratio	0.91	1.24	1.59	14.62
31	Fruit neck diameter	FrND	mm	16.4	22.9	32.2	24.31
32	Ascorbic acid	AsA	mg/100 g fw	16.13	19.88	22.66	6.47
33	Gallic acid of aril	GaAA	mg/100 g dw	12.2	15.45	19.02	5.43
34	Gallic acid of peel	GaAP	mg/100 g dw	50.34	74.42	104.03	6.35
35	Antioxidant activity	AnAc	%	64.01	70.90	75.68	5.60
36	Woody portion index	Wpi	%	5.38	7.48	14.14	65.30

1: CV, coefficient of variation = (Standard deviation/Mean) * 100.

had considerable coefficients of variation.

Bivariate simple correlation

Bivariate correlation between two characters shows a relationship that is not considered as a kind of influence but makes it possible to indirectly measure the character (Johnson and Wichern 1988). When measuring a character that is expensive, complex or difficult, it is possible to record the characters that have a high correlation for indirectly measuring the corresponding character. The results of simple correlation analysis showed the existence of significant positive and negative correlations among characters (**Table 4**). Significant relationships among some fruit characteristics of pomegranate have been reported (Mars and Marrakchi 1998). In this research no significant correlation among size of the fruit and TSS, TA or EC of fruit juice were observed. A high negative correlation was found between Wpi and 100 aril fw. Also a significant correlation ($p \leq 0.01$) was found between TA and EC, TA and TSS but not between TSS and EC. A significant positive correlation existed between antioxidant activity and the amount of ascorbic acid ($r = +0.74$) and with gallic acid of arils ($r = +0.86$). Also a significant positive correlation was detected between the length of the aril and the weight of 100 arils. Zamani (1990) also reported a positive correlation between means of aril length and 100 arils weight. These correlations could prove to be good guidance for selection in a pomegranate breeding program.

Factor analysis

A simple and clear result by using ANOVA (variance analysis) that considers an extensive amount of data obtained

from the assessment of different morphological characters in a wide range of genotypes is virtually impossible. Thus, by using factor analysis, different characters can be grouped in factors in which each factor includes many correlated characters. In this way, the researcher is able to work on fewer factors than of the numerous characters that are initially available. **Table 5** shows the results of factor analysis. The amount of variance for each factor shows the importance of that factor in justifying total variance of the studied characters, which is explained as percentages. The first ten factors with an Eigen value of ≥ 1 accounted for 91.51% of the total variance. The characters with a threshold level of more than 0.5 (Johnson and Wichern 1988) were chosen to be significant for each factor (**Table 5**). The first factor accounted for 26.96% of the overall variance in which variables such as fruit length/diameter, fruit length, TA, Wpi, fruit crown length, 100 aril dry and fresh weights, 100 seed dry weight and pH had the highest factor loadings. Since these characters are related to fruit shape and fruit juice characters, first factor (F_1) could be characterized as a fruit shape, aril and juice factor. In the second factor (F_2), the largest scores were due to characters associated with the fruit size such as fruit weight, fruit diameter, aril total weight and peel percent. High loading characters on F_3 included seed and aril dry weight, peel thickness and aril length. According to **Table 4**, other factors could be characterized as aril factor (F_4), seed factor (F_5 and F_6), crown factor (F_7 and F_8), peel gallic acid and antioxidant factor (F_9), and peel factor (F_{10}). Factor analysis could decrease the 36 characters to 10 main factors, helping breeder to work with these fewer factors instead of all 36 initially measured characters.

Table 4 Bivariate simple correlation among studied characters. (Abbreviations listed in Table 2)

Characters	100 SeDW	100 ArDW	ArTW	Ar %	PeTW	Pe%	100 ArFW	ArDW %	SeDW %	PeT	ArL	ArD
100 SeDW	1											
ArDW	0.01	1										
ArTW	-0.30*	0.44*	1									
Ar%	-0.44*	-0.02	0.58**	1								
PeTW	0.29*	0.55**	0.39*	-0.03	1							
Pe%	0.12	0.01	-0.26*	0.33*	0.37*	1						
100 ArFW	-0.36*	0.78**	0.42*	0.21	0.27*	0.02	1					
ArDW%	0.58*	-0.22	-0.24*	-0.43*	0.18	-0.03	-0.76**	1				
SdDW%	0.13	-0.35*	0.07	0.22	-0.27*	-0.16	-0.02	-0.32*	1			
PeT	0.36*	0.23	-0.37*	-0.76**	0.18	-0.17	-0.10	0.44*	-0.31*	1		
ArL	-0.48*	0.38*	0.20	0.44*	-0.06	0.27*	0.63**	-0.59**	0.01	-0.28*	1	
ArD	-0.13	0.74**	0.21	0.04	0.31*	0.15	0.75**	-0.43*	-0.17	0.25*	0.66**	1
ArL/ArD	-0.36*	-0.42*	-0.11	0.37*	-0.48*	0.12	-0.19	-0.08	0.16	-0.35*	0.47*	-0.27*
SeL	-0.37*	0.31*	0.21	-0.06	0.14	-0.15	0.48*	-0.32*	-0.18	0.09	0.54**	0.46*
SeD	0.34*	0.32*	-0.29*	-0.40*	0.21	0.15	-0.06	0.40*	-0.44*	0.78**	-0.01	0.50**
SdL/SdD	-0.50**	-0.12	0.33*	0.29*	-0.18	-0.25*	0.37*	-0.61**	0.41*	-0.53**	0.40*	-0.05
pH	-0.35*	0.06	-0.17	0.39*	-0.24*	0.44*	0.40*	-0.62**	0.17	-0.27*	0.61**	0.35*
EC	-0.04	-0.04	0.06	0.05	-0.11	-0.10	-0.17	0.30*	0.13	0.07	0.11	-0.13
100 SeFW	0.37*	0.30	-0.26*	-0.46*	0.33*	0.16	-0.15	0.53**	-0.85**	0.46*	-0.24	0.12
An	0.42*	0.17	-0.29*	-0.51**	0.20	0.00	-0.14	0.46*	-0.37*	0.54**	-0.23	0.21
TSS	0.15	-0.12	0.13	-0.29*	0.04	-0.42*	-0.26*	0.34*	0.01	0.16	-0.23	-0.15
TA	0.40*	-0.21	-0.06	-0.40*	0.14	-0.25*	-0.53**	0.74**	-0.03	0.36*	-0.43*	-0.35*
FrFI	0.12	0.13	0.14	0.21	0.24	0.23	-0.14	0.06	0.08	-0.11	0.07	-0.18
FrW	0.10	0.40*	0.65**	-0.19	0.49*	-0.57**	0.14	0.22	-0.08	0.12	-0.29*	-0.02
FrL	0.41*	-0.23	-0.03	-0.45*	0.21	-0.30*	-0.51**	0.71**	0.00	0.38*	-0.49*	-0.41*
FrD	0.14	0.29*	0.52**	-0.32*	0.37*	-0.66**	0.04	0.27*	-0.07	0.17	-0.40*	-0.13
FrL/FrD	0.43*	-0.36*	-0.21	-0.40*	0.11	-0.12	-0.60**	0.73**	0.01	0.38*	-0.43*	-0.43*
FrCL	0.48*	-0.04	-0.07	-0.60**	0.33*	-0.29*	-0.37*	0.65**	-0.08	0.54**	-0.52**	-0.20
FrCD	0.51**	0.40*	0.30*	-0.20	0.39*	-0.21	0.08	0.28*	-0.16	0.13	-0.21	0.07
FrCL/FrCD	-0.14	-0.40*	-0.32*	-0.31*	-0.10	-0.07	-0.38*	0.28*	0.08	0.35*	-0.22	-0.21
FrND	-0.21	0.19	0.50**	0.18	0.22	-0.16	0.20	-0.11	-0.16	-0.09	0.06	0.16
AsA	-0.27*	-0.44*	-0.37*	-0.08	-0.54**	-0.14	-0.07	-0.29*	0.39*	-0.10	0.06	-0.31*
GaAA	-0.24*	0.26*	-0.18	-0.17	0.01	0.07	0.47*	-0.44*	-0.26*	0.32*	0.31*	0.38*
GaAP	-0.04	-0.02	-0.43*	-0.20	-0.09	0.20	0.16	-0.23	0.10	0.28*	0.11	0.16
AnAc	0.15	0.45*	0.33*	0.12	0.18	-0.10	0.42*	-0.18	-0.05	-0.20	0.16	0.27*
Wpi	0.63**	-0.60**	-0.42*	-0.39*	-0.03	0.01	-0.90**	0.85**	0.06	0.26*	-0.65**	-0.65**

** : Significant at 1%
* : Significant at 5%

Table 4 (Cont.)

Characters	ArL/ArD	SeL	SeD	SeL/SeD	pH	EC	100 SeFW	An	TSS	TA	FrW	FrL
ArL/ArD	1											
SeL	0.29*	1										
SeD	-0.28*	0.12	1									
SdL/SdD	0.45*	0.60**	-0.68**	1								
pH	0.30*	0.04	-0.06	0.14	1							
EC	0.39*	0.05	0.01	0.04	0.04	1						
100 SeFW	-0.35*	0.00	0.58**	-0.60**	-0.32*	-0.23	1					
An	-0.41*	-0.08	0.56**	-0.53**	-0.30*	0.19	0.55**	1				
TSS	0.02	0.31*	0.06	0.17	-0.62**	0.12	0.15	-0.04	1			
TA	0.05	0.02	0.17	-0.14	-0.76**	0.52**	0.18	0.46*	0.58**	1		
FrFI	0.22	0.13	-0.23	0.17	0.21	0.25*	-0.12	0.14	0.67**	-0.71**	1	
FrW	-0.45*	0.10	-0.16	0.09	-0.65**	0.01	0.10	0.05	0.41*	0.34*	0.46*	1
FrL	0.00	0.00	0.06	-0.09	-0.78**	0.35*	0.15	0.42*	0.48*	0.92**	0.97**	0.50**
FrD	-0.43*	0.08	-0.17	0.09	-0.68**	-0.01	0.12	0.04	0.45*	0.36*	0.19	0.95**
FrL/FrD	0.14	-0.04	0.15	-0.16	-0.65**	0.39*	0.15	0.46*	0.39*	0.92**	0.50**	0.89**
FrCL	-0.28*	0.01	0.25*	-0.22	-0.81**	0.22	0.26*	0.58**	0.39*	0.85**	0.61**	0.37*
FrCD	-0.42*	-0.18	0.04	-0.26*	-0.57**	-0.25*	0.41*	0.36*	0.12	0.27*	-0.15	0.40*
FrCL/FrCD	0.18	0.22	0.20	0.07	-0.16	0.46*	-0.19	0.15	0.24	0.49*	0.42*	0.13
FrND	-0.17	0.24	-0.06	0.14	-0.42*	-0.36*	0.07	-0.13	0.31*	0.05	-0.35*	-0.12
AsA	0.47*	0.07	-0.36*	0.44*	0.36*	0.16	-0.48*	-0.11	-0.16	-0.18	-0.24	-0.36*
GaAA	0.05	0.46*	0.23	0.15	0.43*	-0.36*	0.19	0.24	-0.18	-0.48*	-0.39*	-0.19
GaAP	0.07	0.05	0.21	-0.03	0.29*	-0.25*	-0.09	0.06	-0.28*	-0.25*	0.20	-0.17
AnAc	-0.19	0.22	-0.06	0.16	0.05	-0.11	0.16	0.07	0.02	-0.16	0.00	0.74**
Wpi	0.10	-0.37*	0.14	-0.37*	-0.53**	0.23	0.23	0.33*	0.33*	0.73**	0.00	0.74**

** : Significant at 1%
* : Significant at 5%

Table 4 (Cont.)

Characters	FrD	FrL/FrD	FrCL	FrCD	FrCL/FrCD	FrND	AsA	GaAA	GaAP	AnAc	Wpi
FrD	1										
FrL/FrD	0.22	1									
FrCL	0.54**	0.82**	1								
FrCD	0.57**	0.24	0.46*	1							
FrCL/FrCD	-0.07	0.46*	0.43*	-0.60**	1						
FrND	0.40*	0.00	0.17	0.33*	-0.11	1					
AsA	-0.28*	-0.05	-0.17	-0.51**	0.35*	-0.30*	1				
GaAA	-0.22	-0.34*	-0.36*	-0.17	-0.16	-0.04	0.59**	1			
GaAP	-0.43*	-0.07	-0.09	-0.22	0.12	-0.26*	-0.15	0.08	1		
AnAc	0.17	-0.25*	-0.22	0.32*	-0.52**	0.08	0.74**	0.86**	-0.15	1	
Wpi	0.09	0.82**	0.61**	0.15	0.35*	-0.20	-0.02	-0.44*	-0.13	-0.27*	1

** : Significant at 1%

* : Significant at 5%

Table 5 Eigen values accepted (≥ 1), Variance and Cumulative variance for ten factors resulted from factor analysis.

Factors	1	2	3	4	5	6	7	8	9	10	
Eigen value	10.25	6.43	4.84	3.48	2.68	2.26	1.41	1.35	1.17	1.00	
% of variance	26.96	16.68	12.74	9.17	7.05	5.95	3.70	3.56	3.07	2.64	
Cumulative variance%	26.96	43.65	56.36	65.55	72.60	78.55	82.25	85.80	88.87	91.51	
Characteristic	Units	Factor loading									
100 SeDW	g	0.51**	-0.22	0.26	0.41	-0.35	-0.15	0.47	-0.01	0.02	0.19
ArDWP	%	-0.47	0.09	0.71**	-0.12	0.38	-0.06	0.16	0.04	-0.02	0.21
ArTW	g	-0.21	0.67**	-0.15	-0.17	0.20	-0.15	0.13	0.11	0.43	0.23
Ar%	%	-0.42	-0.07	-0.37	-0.72**	0.02	-0.22	0.03	0.09	0.39	0.24
PeTW	g	0.11	0.32	0.17	0.15	0.06	0.13	0.12	0.07	0.06	0.86**
Pe%	%	-0.13	-0.60**	0.01	-0.26	-0.11	0.12	0.00	0.04	-0.04	0.71**
100 ArFW	g	-0.66**	0.35	0.20	-0.06	0.44	-0.11	0.24	-0.13	-0.18	0.24
100 ArDW	g	0.79**	-0.06	0.16	0.10	-0.32	0.35	-0.04	0.02	0.23	0.00
SdDW%	%	0.12	-0.25	0.87**	0.14	0.02	0.27	-0.08	0.10	0.02	0.06
PeT	mm	0.32	0.04	0.71**	0.36	0.02	0.22	-0.18	-0.02	-0.29	-0.06
ArL	mm	-0.46	-0.13	0.16	-0.58**	0.47	-0.08	0.13	-0.05	0.00	0.06
ArD	mm	-0.37	0.45	0.49	0.02	0.23	0.18	0.35	-0.12	0.03	0.33
ArL/ArD	Ratio	0.14	-0.42	-0.41	-0.71**	0.43	-0.02	-0.12	-0.11	0.01	-0.25
SeL	mm	-0.06	0.09	0.09	0.05	0.96**	0.09	-0.07	0.00	-0.03	0.03
SeD	mm	0.02	-0.07	-0.22	0.00	-0.07	-0.94**	0.02	-0.13	-0.05	-0.11
SdL/SdD	Ratio	-0.16	0.16	-0.45	-0.03	0.66**	-0.34	-0.03	-0.10	-0.07	-0.09
pH	-	-0.71**	-0.46	0.00	-0.27	0.05	-0.09	-0.04	-0.33	-0.14	0.04
EC	mmoh/cm	0.37	0.04	0.08	-0.30	0.13	-0.07	-0.23	-0.38	0.32	-0.08
100 SeFW	g	0.19	-0.08	0.34	0.26	-0.07	0.79**	0.27	0.17	0.03	0.11
AnA	O.D ₅₁₀	0.43	0.06	0.59**	0.02	-0.17	0.28	0.18	-0.24	-0.17	0.06
TSS	%	0.48	0.13	0.01	0.33	0.40	-0.08	-0.04	0.23	0.38	-0.23
TA	%	0.93**	0.16	0.12	0.03	0.08	0.00	-0.08	-0.12	0.17	-0.02
FrFI	Ratio	0.42	-0.21	0.22	-0.27	0.21	0.37	-0.24	-0.43	0.22	0.32
FrW	g	0.23	0.88**	-0.03	0.21	0.03	0.03	0.15	0.10	0.16	0.08
FrL	mm	0.94**	0.27	0.01	0.04	0.03	0.01	-0.04	0.00	-0.03	0.01
FrD	mm	0.28	0.84**	-0.07	0.31	0.01	0.05	0.11	0.11	0.15	-0.05
FrL/FrD	Ratio	0.97**	0.02	0.04	-0.05	0.01	0.00	-0.07	-0.03	-0.08	0.03
FrCL	mm	0.83**	0.37	0.24	0.20	-0.04	0.00	-0.09	0.02	-0.12	0.11
FrCD	mm	-0.20	0.11	-0.01	0.14	0.24	0.04	0.74**	-0.16	0.20	0.04
FrCL/FrCD	Ratio	0.42	-0.12	0.10	0.11	0.19	-0.10	-0.80**	-0.22	-0.03	-0.02
FrND	mm	0.01	0.44	0.00	-0.06	0.25	-0.02	-0.01	0.73**	0.14	0.09
AsA	mg/100 g fw	-0.06	-0.20	-0.37	0.01	0.13	-0.24	-0.32	-0.30	-0.55**	-0.34
GaAA	mg/100 g dw	-0.44	-0.18	0.12	0.23	0.44	0.29	0.06	0.05	-0.48	-0.03
GaAP	mg/100 g dw	-0.12	-0.28	0.16	-0.01	0.05	-0.13	-0.10	0.00	-0.81**	0.04
AnAc	%	0.30	0.48	0.14	0.05	-0.19	0.09	0.39	0.26	-0.05	0.10
Wpi	%	0.87**	-0.29	-0.09	0.14	-0.32	0.04	-0.03	0.04	0.07	-0.06

** Significant factor loading (Values above 0.50).

Cluster analysis

Cluster analysis based on all morphological characters, grouped the genotypes into four sub-clusters at distance of 10 out of 25 (Fig. 1). The first (A) sub-group included eight accessions: 'Bihaste Dane Sefide Ravar' (No. 4) and 'Bihaste Hajiabad' (No. 21) as soft-seed accessions, 'Bihaste Ardestan' (No. 15), 'Bitolf Dane Sefid' (No. 16), 'Bihaste Dane Ghermez Kerman' (No. 20) and 'Bidane Kashmar' (No. 12) as semi-hard seed accessions and 'Bihaste Ladiz' (No. 3) and 'Bihaste Porbar Shirin' (No. 6) as semi-soft seed accessions. 'Bihaste Dane Sefide Ravar' (No. 4) and 'Bihaste Hajiabad' (No. 21) with desirable soft-seed and

some similar characteristics to each other, as recorded in Table 3, were located in a separate sub-cluster in this group. These accessions are important for using as breeding plant materials for reducing seed hardness in progenies. The second group (B) consisted of two accessions including 'Bihaste Shirin Khabre Baft' (No. 11) with hard-seed and sour taste and 'Bihaste Shirin Kambar' (No. 14) as a semi-soft and sweet genotype. The third branch of the cluster (C) included two accessions, 'Bihaste Najaf Abad' (No. 2) and 'Bihaste Ghasrodasht' (No. 13), which had the same fruit characteristics such as taste, fruit weight, and semi-soft seed. The fourth group (D) included seven accessions, 'Bihaste Sangar' (No. 10) and 'Bihaste Shirin Saravan' (No. 17) as

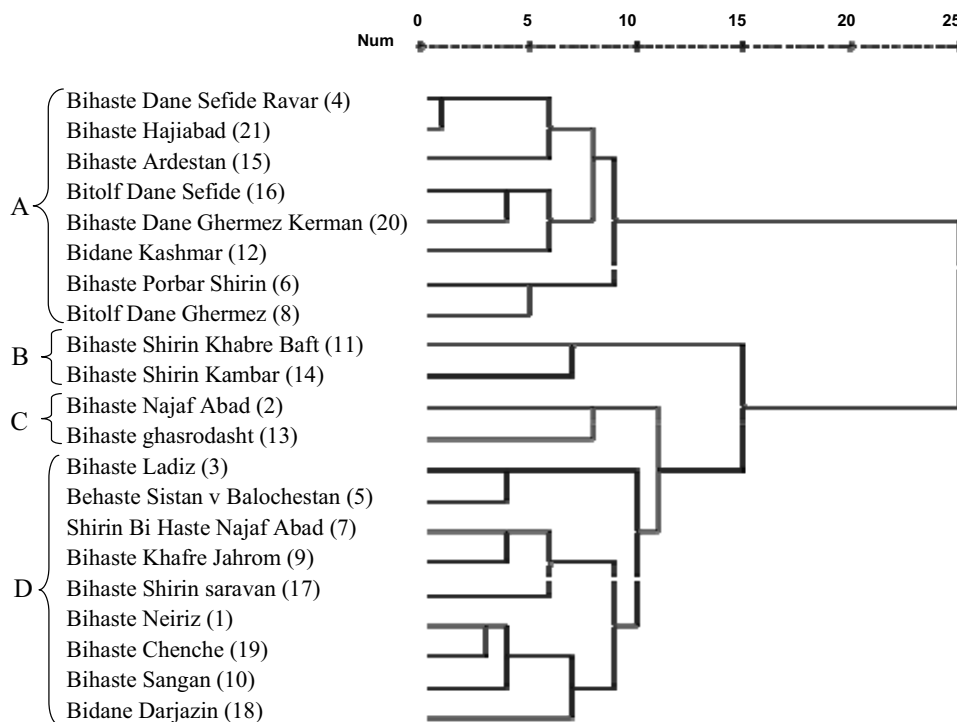


Fig. 1 Dendrogram of grouping 21 soft-seed pomegranate accessions based on ten main factors (Table 3) using Ward's method.

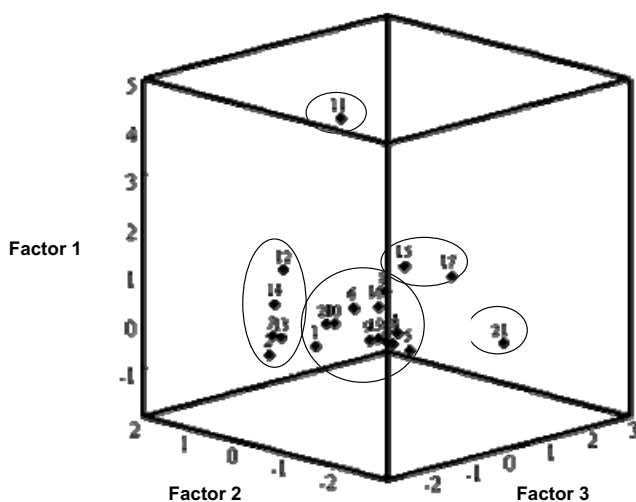


Fig. 2 Triplot analysis of 21 soft-seed pomegranate accessions based on three first main factors.

soft-seed and sweet accessions, ‘Shirin Bihaste Najaf Abad’ (No. 7), ‘Bihaste Khafre Jahrom’ (No. 9), ‘Bihaste Neiriz’ (No. 1), ‘Bihaste Chenche’ (No. 19) and ‘Bidane Darjazin’ (No. 18) as semi-soft and sweet accessions. Overall it seems that some characters such as taste, seed size, fruit shape and fruit size characters were more effecting the clustering of accessions.

Triplot analysis

Plot analysis creates two (Diplot) or three (Triplot) dimensional images that can subsequently be used as a main differential factor. Dispersion of genotypes within an area of two or three dimensions results to distinguishing differences among genotypes. In our study Triplot analysis was performed with three first main factors of factor analysis which explained 56.38% of total variance. By using Triplot analysis 21 Iranian soft-seed pomegranate accessions were located in five groups (Fig. 2). Nine characters such as fruit length/diameter, fruit length, TA, Wpi, fruit crown length, 100 aril dw, pH, 100 aril fw, and 100 seed dw were most effectively grouped by tri-plot analysis. ‘Bihaste Hajiabad’ (No. 21) as a soft-seed accession and ‘Bihaste Shirin Kha-

bre Baft’ (No. 11) as an extremely hard seed accession were clearly separated from other accessions and located individually (Fig. 2).

CONCLUSION

Pomegranate is one of the most important and widely grown fruit crops in Iran. Genetic studies and variety characterization of pomegranate for further uses in a breeding program focusing on introducing soft-seed character into commercial cultivars have great importance. Different methods based on morphological and molecular markers have been employed to differentiate genotypes. Evaluation of pomegranate genotypes is important task not only for genotyping but also to record and quantify their characteristics that might be useful in genetically improving this fruit in a modern breeding objective. Understanding the relationships among characters would help pomegranate breeders to quantify some hard-to-evaluate characters using correlated ones, saving time and money. Factor analysis summarized the initial 36 variables to 10 main factors of which the first, second and third factors accounted for more than 50% of the overall variance. Cluster and tri-plot analysis provided a useful and comprehensive tool to establish a first order of genotypes classification. Further data collected across the years will increase the genotyping precision of accessions. DNA markers could be also powerful means for categorizing of accessions since they are less affected by the environment than morphological characters. In this study, 21 Iranian soft-seed pomegranate accessions based on a taste panel were divided into four groups: 1) soft seed; 2) semi-soft seed; 3) semi-hard seed and 4) hard seed. This test indicated that all of these accessions are not potentially soft-seed (Table 1). Differences among accessions which have been commercially labeled as being seedless (soft seed) prove that environmental effects are at play or that some accessions have been mislabelled. The findings of our study provide a practical recommendation by extensive evaluation of morphological traits prior to choosing the ideal parent in a breeding program to combine soft seededness with some other important commercial traits in a single cultivar.

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