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BIOCHEMICAL AND POMOLOGICAL CHARACTERIZATION OF POMEGRANATE ACCESSIONS IN FARS PROVINCE OF IRAN

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SUMMARY

In this study, 27 biochemical and pomological attributes were used to assess 50 pomegranate (*Punica granatum* L.) accessions from five leading regions in Fars province of Iran for two consecutive years. Studied accessions exhibited significant diversity in the fruit characteristics in particular fruit color, seed hardness, anthocyanin, TA, and vitamin C. PCA analysis were carried out and seven principal components were identified; the most important factors were seed hardness, aril size, and color-related characters. Biochemical attributes of fruit juice including TA, antioxidant capacity, total phenolic, and vitamin C were also important for characterization. There were high positive and negative correlations between some of the studied variables; in particular, seed hardness was positively correlated with 100 arils fresh weight, woody portion percent, and aril length/diameter. Fruit color was positively correlated with aril color, anthocyanin, seed hardness and vitamin C, while it was negatively correlated with aril length. Cluster analysis confirmed the results of tri-plot and divided the accessions mostly according to the fruit and aril color and seed hardness as well as fruit taste. Among different samples we used in our study, seven accessions were determined as real soft-seeded and five accessions as semi soft-seeded, which are suitable in this important trait to be used for cultivation and breeding programs for decreasing of seed hardness in commercial pomegranates. Also by multiple regression analysis, some attributes were identified to be important to be considered in breeding programs with the aim of reducing seed hardness. Altogether, there is high diversity in pomegranate accessions in Fars province and this represents genetic resources for future conservation and breeding work.

Key words: *Punica granatu*, correlation, accession, seed hardness, genetic diversity

Key findings: High level of variation was found in different studied attributes indicating wide range of diversity in pomegranates from Fars. Some promising accessions were identified that have potential to be used in pomegranate breeding programs for different aspects especially seed softness characters.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is considered as one of the oldest edible fruit trees that have been cultivated from ancient times (Damania, 2005). This fruit tree is moderately tolerant to unfavorable climatic and soil conditions and is an attractive crop for cultivation and land validation in arid and semi-arid regions with high salinity in soil and water resources; where most of the other fruit trees cannot be commercially grown. Most of researchers believe that pomegranate is native to Persia and some surrounding areas and then diversified to other regions (Levin, 1994; Mars and Marrakchi, 1999; Zamani *et al.*, 2007; Stover and Mercure, 2007). Iran has numerous types of cultivated and wild forms of this fruit tree and is very rich in gene pool of pomegranate. This country is considered as the center of origin and diversity for this fruit tree and is one of the richest gene pool of pomegranate in the world that can be utilized in the breeding programs (Zamani *et al.*, 2007).

Assessment of morphological traits among genetic resources is one of the main steps in the breeding programs. In fact, collecting information about morphological attributes would be of great importance for describing and classifying different germplasm and can result in identification of accessions as donor parents in breeding programs. Morphological attributes of different pomegranate accessions have been evaluated in different regions (Mars and Marrakchi, 1999; Martinez *et al.*, 2006; Karimi and Mirdehghan, 2013; Zarei *et al.*, 2013; Khadivi-Khob *et al.,* 2015). According to available reports, pomegranate accessions exhibit a high degree of pomological diversity and have a wide spectrum of fruit related attributes that could be used in breeding programs to improve available cultivars. However, it is reported that leaf and flower characteristics have low power of discrimination among pomegranate genotypes (Martinez-Nicolas *et al.*, 2016).

Fars province is the leading area for pomegranate production in Iran (22,480 ha under cultivation). This province, with varied eco-geographical regions, is one of the major centers for pomegranate diversity, and pomegranate populations are widely scattered in this region (Anonymous, 2013). All pomegranate producers of Iran are using local pomegranate accessions; hence, there is a wide range of phenotypically different pomegranate accessions that have not been subject to breeding programs. Therefore, the present study was carried out on two consecutive years to evaluate the biochemical and morphological variation and for selection of promising accessions from 50 local pomegranate accessions of Fars region. These findings are expected to be valuable contribution to pomegranate future breeding programs.

MATERIALS AND METHODS

Fruit traits

Fifty pomegranate accessions were selected from five leading pomegranate cultivation regions of Fars Province, Iran (Table 1). Over two consecutive years, ten fruits from each accession were collected for recording some qualitative and quantitative characters that previously reported to be important in pomegranate evaluation (Sarkhosh *et al.*, 2005; Martinez *et al.*, 2006; Zamani *et al.*, 2010; Zarei *et al.*, 2013) (Table 2). These traits were evaluated according to the national guideline for the conduct of tests for distinctness, uniformity and stability in pomegranate (UPOV, 2012). Crown and neck dimensions were measured by a digital caliper. An electronic balance with 0.01 g precision was used to measure weight related traits. The fruit juice was used for analysis of total soluble solids (TSS), titratable acidity (TA) and pH. TSS were determined by refractometer (pocket PAL-1 ATAGO Corporation, Tokyo, Japan), in Brix. TA was measured by neutralization to pH 8.10 with 0.10 N NaOH, data are given as g/l of citric acid. The pH values were measured by using a pH meter (PHS-3D-01, Sanxine Corporation, Shanghai, China). Fruit taste index was calculated by dividing TSS value by the TA value. According to the Martinez *et al.* (2006), this index is expressed as follow: sweet genotypes 31-98, sweet-sour genotypes 17-24 and sour genotypes 5-7. Woody portion percent was computed from the percentage of fresh seed weight relative to fresh aril weight from 10 replications. Seed hardness was evaluated both qualitatively (taste panel) and quantitatively using a texture analysis instrument and expressed as newton (N) required to seed breakage (Zarei *et al.*, 2013). For this purpose, seeds (after removing fleshy part of arils) of pomegranate were placed on a stationary platform of a texture analyzer instrument, then the moving platform of this instrument crush the seed and measured the force needed for seed breakage. At least 10 seeds were analyzed by this method for each accession.

Table 1. Local name, geographical origin and some morphological traits of 50 pomegranate genotypes studied in this research.

Genotype ID	Genotype name	City of sampling	Locality	Fruit color	Aril color	Taste	Seed hardness
SHNN	Shirine-Kambar-Neyriz	Neyriz	N28°94’ E54°82’	Brown	Red	Sweet-Sour	Hard
RPGN	Rababe-Poost-Ghermez	Neyriz	N28°89’ E54°87’	Brown	Red	Sweet-Sour	Hard
BPGN	Berit-Poost-Ghermez	Neyriz	N28°91’ E54°75’	Red	Red	Sweet-Sour	Semi-Soft
AYN	Anare-Yazdi	Neyriz	N29°04’ E54°32’	Brown	Red	Sour	Hard
ZPKN	Zardnare-Poost-Koloft	Neyriz	N29°15’ E54°33’	Pink	White	Sour	Hard
KSN	Kalaghi-Shirin	Neyriz	N29°20’ E54°32’	Yellow	White	Sweet	Hard
BNN	Bihaste-Neyriz	Neyriz	N29°12’ E54°36’	Yellow	White	Sweet	Soft
GBN	Galoo-Barik	Neyriz	N28°99’ E54°52’	Red	Red	Sweet-Sour	Hard
TNN	Torsh-Nar	Neyriz	N28°97’ E54°72’	Brown	Red	Sour	Hard
RPNN	Rabab-Poost-Nazok	Neyriz	N29°09’ E54°38’	Brown	Red	Sweet-Sour	Hard
BGM	Bitolf-Ghasredasht	Marvdasht	N29°97’ E52°95’	Yellow	White	Sweet	Soft
SSM	Sheitoni-Seydan	Marvdasht	N29°99’ E52°97’	Pink	Pink	Sweet-Sour	Hard
PSSM1	Poost-Sefide-Shirin	Marvdasht	N29°97’ E52°99’	Yellow	White	Sweet	Hard
BSM	Bidane-Sefid	Marvdasht	N29°88’ E53°05	Yellow	White	Sweet	Soft
MFM	Meykhosh-Farogh	Marvdasht	N29°88’ E52°99’	Brown	Pink	Sweet-Sour	Hard
TSM	Tafti-Shirin	Marvdasht	N29°79’ E52°90’	Pink	White	Sweet	Hard
SM	Sakaly	Marvdasht	N29°85’ E52°83’	Brown	Red	Sour	Hard
SPK	Shahvare-Poost-Koloft	Marvdasht	N29°78’ E52°80’	Red	Red	Sweet-Sour	Hard
BSM	Bitolf-Shirin	Marvdasht	N29°75’ E52°85’	Yellow	Pink	Sweet	Semi-Soft
PSSM2	Poost-Sefide-Seydan	Marvdasht	N29°97’ E53°08’	Yellow	Red	Sweet	Hard
PSKJ	Poost-Siyah-Khafr	Jahrom	N28°93’ E53°26’	Black	Black	Sweet	Hard
AKJ	Atabaki-Khafr	Jahrom	N28°94’ E53°25’	Brown	Red	Sweet-Sour	Hard
GFJ	Golnar-Farsi	Jahrom	N28°95’ E53°23’	Brown	Red	Sweet-Sour	Hard
OPGJ	Oude-Poost-Ghermez	Jahrom	N28°95’ E53°26’	Red	Red	Sweet-Sour	Hard
SHJ	Shirin-Hastedar	Jahrom	N28°96’ E53°22’	Pink	Red	Sweet	Hard
BKJ1	Bihaste-Khafr	Jahrom	N28°96’ E53°18’	Yellow	White	Sweet	Soft
BKJ2	Bipiye-Kambar	Jahrom	N28°94’ E53°13’	Yellow	White	Sweet	Soft
NSJ	Nare-Siyah	Jahrom	N28°97’ E53°17’	Black	Black	Sweet	Hard
MSJ	Malase-Shahvare	Jahrom	N28°95’ E53°08’	Red	Pink	Sweet-Sour	Hard
GBJ	Galo-Barik	Jahrom	N28°99’ E53°11’	Brown	Red	Sweet-Sour	Hard
MPD	Malase-Porbar	Darab	N28°82’ E54°61’	Brown	Red	Sweet-Sour	Hard
PGED	Poost-Ghermez-Eij	Darab	N28°79’ E54°58’	Red	Red	Sweet-Sour	Hard
PRLD	Pish-Rase-Layzangan	Darab	N28°75’ E54°56’	Red	Pink	Sweet-Sour	Semi-Soft
TDD	Torshe-Dirras	Darab	N28°78’ E54°53’	Brown	Red	Sour	Hard
APSD	Atabaki-Poost-Sefid	Darab	N28°80’ E54°53’	Yellow	White	Sweet	Hard
TED	Tashto-Eij	Darab	N28°76’ E54°60’	Pink	White	Sour	Hard
SSD	Shirin-Shahvar	Darab	N28°75’ E54°58’	Yellow	Pink	Sweet-Sour	Semi-Soft
MMD	Meykhosh-Mamoli	Darab	N28°77’ E54°56’	Brown	Red	Sweet-Sour	Hard
MHD	Malase-Hajiabad	Darab	N28°75’ E54°53’	Red	Red	Sweet-Sour	Hard
PSSD	Poost-Siyahe-Shirin	Darab	N28°79’ E54°57’	Black	Black	Sweet	Hard
KTK	Khani-Torsh	Kazeron	N29°62’ E51°65’	Yellow	White	Sweet-Sour	Hard
KBK	Khani-Bipiye	Kazeron	N29°61’ E51°60’	Yellow	White	Sweet	Soft
ZSK	Zaghe-Shirin	Kazeron	N29°59’ E51°72’	Black	Black	Sweet	Hard
MPK	Malase-Porbar	Kazeron	N29°59’ E51°69’	Red	Red	Sweet-Sour	Hard
KSK	Kampiye-Sefid	Kazeron	N29°60’ E51°70’	Yellow	Pink	Sweet	Semi-Soft
APSK	Atabaki-Poost-Sefid	Kazeron	N29°62’ E51°63’	Yellow	Red	Sweet	Hard
KPNK	Kadro-Poost-Nazok	Kazeron	N29°64’ E51°65’	Brown	Pink	Sweet-Sour	Hard
MPGK	Meykhosh-Poost-Ghermez	Kazeron	N29°67’ E51°74’	Red	Red	Sweet-Sour	Hard
PSK	Poost-Siyah	Kazeron	N29°68’ E51°76’	Black	Black	Sweet	Hard
TSK	Torshe-Sabz	Kazeron	N29°65’ E51°73’	Pink	White	Sweet-Sour	Hard

Table 2. Descriptive statistics for measured traits among *Punica granatum* samples.

Characters	Abbreviation	Unit	Min.	Max.	Mean	SD	CV%
Fruit weight	FrWe	G	154.86	315.75	250.49	41	16.37
100 aril fresh weight	100 ArFrW	G	28.64	48.27	37.32	4.65	12.46
Seed hardness	SeHa	N	118.94	512.04	405.69	129.84	32
100 aril dry weight	100 ArDrW	G	14.37	24.27	20.53	2.66	12.96
Woody portion percent	WPP	%	9.04	25.69	19.17	5.06	26.38
Total soluble solids	TSS	%	12.78	17.45	15.65	1.28	8.21
Titratable acidity	TA	% Citric acid	0.29	1.25	0.66	0.28	42.37
Taste index	TI	Code	13.85	49.49	27.95	10.69	38.27
Taste	Ta	Code	1.00	5.00	2.44	1.34	55.02
Anthocyanin	Anth	%	0.14	1.34	0.66	0.34	51.37
Peel diameter	PeDi	Mm	1.70	4.00	3.08	0.59	19.11
Peel percent	PePe	%	32.42	56.35	45.81	5.21	11.37
Peel dry percent	PDP	%	23.42	40.31	32.84	4.62	14.08
Crown length	CrLe	Mm	15.50	35.00	24.12	4.51	18.69
Crown diameter	CrDi	Mm	10.50	24.50	16.05	3.07	19.15
Crown length/diameter	CrLe/Di	Ratio	0.84	2.73	1.54	0.37	23.75
Fruit color	FrCo	Code	1.00	9.00	4.44	2.65	59.71
Aril color	ArCo	Code	3.00	9.00	5.80	1.98	34.12
Vitamin C	VitC	Mg/100 g fw	2.93	25.63	11.26	5.88	52.26
Total phenolic	ToPh	Mg/100 g GAE	60.23	77.17	68.79	4.38	6.37
Antioxidant capacity	AnCa	Ratio	35.43	49.86	42.34	3.43	8.10
Aril length	ArLe	Mm	8.80	14.00	11.00	1.4	12.71
Aril diameter	ArDi	Mm	7.00	9.40	8.13	0.63	7.72
Aril length/diameter	ArLe/Di	Ratio	1.18	1.71	1.35	0.12	8.75
Seed length	SeLe	Mm	6.00	10.00	7.46	0.81	10.89
Seed diameter	SeDi	Mm	3.00	4.20	3.60	0.35	9.78
Seed length/diameter	SeLe/Di	Ratio	1.50	2.73	2.08	0.26	12.40

The content of total phenols was determined by using the Folin–Ciocalteu colorimetric method based on the method described by Velioglu *et al.* (1998). To do this, 5 ml of 80% methanol was added to 500 mg of grounded aril. The mixture was placed on a shaker in dark conditions for 24 h and 150 µl of filtered mixture was added to the 1500 µl Folin–Ciocalteu reagent. After 1 min incubation, 1500 µl of 6% Sodium carbonate was added. Followed by 1 h of incubation at room condition, total phenols were measured at 725 nm. In addition, a standard curve was drawn by different concentration of gallic acid and the total phenolics were expressed as mg/100 g gallic acid equivalents (GAE). Iodometric titration was used to measure vitamin C of fruit juice (Nweze *et al.*, 2015). To do so, 10 drops of 1% starch solution were added to 25 ml fruit juice and then titrated against iodine solution (potassium iodide, potassium iodate and sulfuric acid in distilled water) until blue-black color was observed. Vitamin C standard solution was prepared by dissolving 250 mg ascorbic acid in 250 ml distilled water. Antioxidant activity was measured using DPPH radical as described by Moon and Terao (1998). For this purpose, 900 µl Tris-HCl buffer (pH 7.4) and 1 ml of 500 µM DPPH were mixed with100 µl of aril juice. After incubation at 25 °C for 30 min, optical density of mixture was measured at 517 nm. The optical density of each sample without DPPH solution was used as control and antioxidant capacity was calculated according to the formula described by Zarei *et al.* (2016b). All of the photometry analysis was performed using the UV-spectrophotometer (Perkin Elmer, Lambda EZ201, U.S.A). The average values of data from two consecutive years were used for statistical analysis.

Statistical analysis

The mean values for each parameter of a given accession were used to perform statistical analysis of morphological traits. Relationships among accessions were investigated by multivariate analysis of variance (principal component analysis, PCA) using SPSS statistics software ver. 19 (SPSS Inc., Chicago, United States, Norusis, 1998). Tri-pot was depicted by three main PCs using SPSS software. NTSYS 2.2 software was used to perform cluster analysis using Unweighted Pair Group Method with Arithmetic mean (UPGMA) based on Euclidean distance coefficient for pairs of accessions (Rohlf, 1998).

RESULTS AND DISCUSSION

Characters variation

Mean values, standard deviation (SD), and coefficients of variation (CV %) for different characters of accessions are presented in Table 2. The range of the mean values of each studied accessions exhibited a significant variation in the fruit characteristics (Table 2). Coefficient of variation was the lowest for total phenolics (CV = 6.37%), while it was the highest for fruit color (CV = 59.71 %). Anthocyanin (CV = 51.37%) and fruit taste (55.03%) were among the traits that showed high variation in the studied accessions. Although pomegranate cultivars are divided into sweet, sour and sweet-sour types according to their taste, there is less variation between their TSS (CV = 8.21%). In fact, the amount of fruit acidity (CV = 42.37%) is more important and determines the final taste of pomegranate fruit. On the other hand, pomegranate accessions are highly variable for their color and have a wide range of aril color (from white to completely dark), which indicates that they have different types of anthocyanin that participate in the health properties of this fruit. Vitamin C (CV = 52.26%) and seed hardness (CV = 32.00%) were also among the highly variable traits. Similarly, woody portion percent, which was significantly different among soft- and hard-seeded accessions, was highly variable. In this study, coefficient of variation for 12 characters were more that 19%, indicating high variation for fruit traits in the studied accessions, and represent high potential for these traits for selection and utilization in breeding programs.

PCA was used with the purpose of reducing the numbers of variables and explaining the same amount of variance with fewer variables and to recognize the correlated traits. This analysis revealed that there is high variability among different varietal groups and among the accessions within each group. For each factor, a principal component loading with more than 0.50 was considered as being significant. According to our results, seven components explained 82.91% of the total variance (Table 3). The values of first three PCs, which accounted for 56.11% of the observed variability, were 28.29%, 16.90%, and 10.92%, respectively. Respective tri-plot based on these three factors (PC1, PC2 and PC3) scattered the accessions mainly by seed hardness, aril color and taste index, and separated hard-seeded accessions from soft-seeded ones (Figure 1).

Based on PCA, color and seed hardness related traits such as fruit and aril color, seed hardness, woody portion percent, aril dry weight, and aril length/diameter accounted for 28.29 % of the variance (eigenvalue, 7.64) as the first main factor, indicating that these attributes have the highest variation among accessions and have the greatest impact on separation of the accessions. Taste and weight related traits; including TSS, TA, fruit weight and 100 arils weight as well as anthocyanin were significant for the second factors with 16.90% of overall variance (eigenvalue, 4.56). The third factor with 10.92% of the overall variance (eigenvalue, 2.95) featured the biochemical characteristics such as total phenolic, antioxidant capacity, and vitamin C. While fruit peel related traits, including peel percent, diameter, and dry percent as well as crown length and diameter, were significant in the fourth component with explaining 9.70% of total variance. The three remaining components explained 17.09% of variance and included the remaining variables.

Associations between characteristics revealed by PCA analysis may represent the genetic linkage between loci controlling traits or a pleiotropic effect (Iezzoni and Pritts, 1991). These results in some cases were in agreement with the results reported by others in pomegranate studies (Zamani *et al.,* 2010; Karimi and Mirdehghan, 2013).

Table 3. Eigenvalues, proportion of total variability and correlation between the original characters and seven principal components in the studied pomegranate accessions.

Traits	Components						
	1	2	3	4	5	6	7
Fruit color	0.65 [a]	-0.01	0.42	0.12	0.07	-0.27	0.29
Aril Color	0.64 [a]	-0.07	0.59 [a]	0.18	0.03	-0.18	0.33
Total soluble solids	0.47	0.65 [a]	-0.29	-0.21	0.23	-0.16	-0.01
Titratable acidity	0.55 [a]	0.66 [a]	-0.21	-0.24	0.29	-0.15	0.00
Taste index	-0.64 [a]	-0.60 [a]	0.20	0.24	-0.26	0.16	-0.02
Taste	0.53	0.65 [a]	-0.23	-0.24	0.27	-0.14	-0.10
Fruit weight	0.37	0.52 [a]	-0.27	-0.25	-0.19	0.15	0.19
100 aril fresh weight	-0.71 [a]	0.53 [a]	0.03	-0.08	0.08	0.04	0.04
Seed hardness	0.92 [a]	-0.18	-0.08	-0.05	-0.09	0.18	0.00
Aril dry weight%	0.79 [a]	-0.16	-0.16	0.01	-0.19	0.16	0.30
Woody portion%	0.86 [a]	-0.14	-0.10	-0.10	-0.17	0.22	0.09
Anthocyanin	0.64 [a]	-0.08	0.59	0.21	0.07	-0.21	0.29
Peel diameter	0.16	0.37	-0.06	0.83 [a]	0.00	-0.12	-0.12
Peel dry percent	-0.31	-0.21	0.05	-0.54 [a]	0.15	0.32	0.10
Crown length	-0.08	0.01	0.19	0.57 [a]	0.56 [a]	0.25	0.10
Crown diameter	0.05	0.26	-0.21	0.65 [a]	-0.41	-0.06	-0.03
Crown length/diameter	-0.06	-0.15	0.35	-0.08	0.83 [a]	0.26	0.06
Peel percent	-0.03	0.40	-0.23	0.54 [a]	0.14	0.00	-0.15
Total phenolic	0.27	0.54 [a]	0.51 [a]	-0.10	-0.18	-0.06	-0.33
Antioxidant capacity	-0.04	0.29	0.71 [a]	-0.18	-0.34	0.10	-0.27
Vitamin C	0.33	0.36	0.68 [a]	-0.20	-0.16	0.08	-0.33
Aril length	-0.72	0.59 [a]	0.02	0.05	-0.04	0.03	0.27
Aril diameter	-0.34	0.57 [a]	-0.15	-0.04	-0.38	0.18	0.43
Aril length/diameter	-0.74 [a]	0.36	0.17	0.10	0.32	-0.09	0.02
Seed length	-0.56 [a]	0.51 [a]	0.38	-0.05	-0.15	0.11	0.27
Seed diameter	0.22	0.53 [a]	0.17	0.24	-0.03	0.70 [a]	0.02
Seed length/diameter	-0.69	0.03	0.18	-0.23	-0.12	-0.47 [a]	0.22
Eigenvalue	7.64	4.56	2.95	2.62	2.08	1.40	1.14
Variance%	28.29	16.90	10.92	9.70	7.69	5.17	4.23
Cumulative %	28.29	45.19	56.11	65.81	73.51	78.68	82.91

[a] Eigenvalues are significant ≥ 0.50

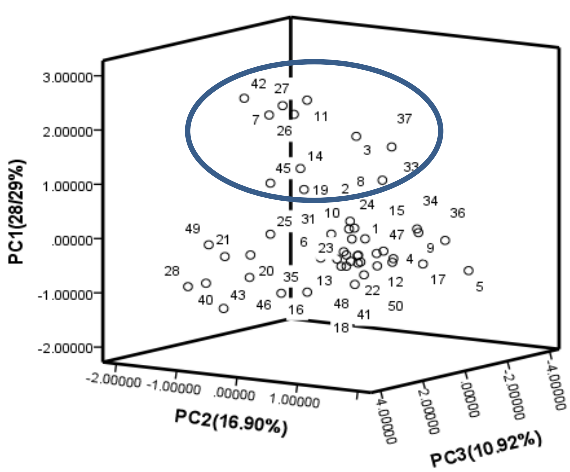

Figure 1. Tri-plot obtained from three main PCs reflecting relationship among accessions in terms of phenotypic resemblance and fruit's biochemical attributes. Numbers are based on Table 1. Blue circle shows individuals having similar characteristics including soft- and semi soft-seeded, light color for fruit and aril, and sweet taste.

Karimi and Mirdehghan (2013) reported seed firmness and fruit weight had the highest loading value for the first component among the traits. Zamani *et al.*, (2010) indicated that taste and seed hardness were two main traits affecting the pomegranate grouping. According to Durgac *et al.* (2008), fruit characteristics in pomegranate have the highest loading values for the first component in PCA analysis. In our study, 100 arils fresh weight, aril dry weight and aril length/diameter were important in PC1. Mars and Marrakchi (1999) suggested that size related traits of fruit are important factors in differentiating of Tunisian pomegranate. Other more recent and similar reports from different pomegranate producer countries indicated that the weight and size related traits of fruits and arils were among the most important variables integrated by PC1 that differentiate the pomegranate genotypes; as found in Turkey (Caliskan and Bayazit, 2013) Croatia (Radunic *et al.*, 2015) and Spain (Martinez-Nicolas *et al.*, 2016).

According to this study, seed hardness, color and taste related traits showed the highest variation and can be used for study of pomegranate accessions. It is noteworthy that seed hardness was evaluated qualitatively in all of the previous reports; therefore, importance of this trait for differentiation of various accessions in our study may be the result of precise quantification of this trait. Accessions in this study showed a wide range of variation in seed hardness. Eight truly soft-seeded and five semi soft-seeded accessions were analyzed in our experiment. Seed softness is a desirable attribute in pomegranate and numerous investigations have been devoted to different aspects of this important trait in the recent years (Lu *et al.*, 2006; Sarkhosh *et al.*, 2011; Zamani *et al.*, 2010; Zarei *et al.*, 2013, 2016a). Soft-seeded genotypes are more acceptable and pleasant for the consumers, if other characters such as taste and color are also at average acceptance. Therefore, high differentiations between seed hardness and color in this study make them useful tools for pomegranate characterization and utilization in breeding programs.

Cluster analysis

Grouping of individuals based on fruit traits divided them into two main clusters, mostly according to the seed hardness, fruit and aril color as well as fruit taste, reflecting a high morphological variability in the collected accessions for these variables (Figure 2).

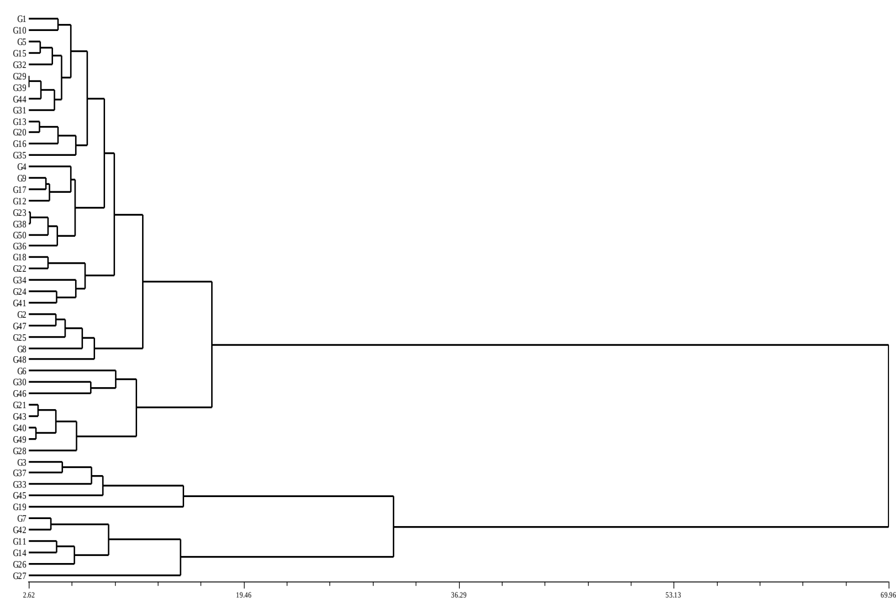

Figure 2. Cluster analysis grouping 50 pomegranate accessions from Fars province of Iran based on pomological and biochemical characteristics.

Results of UPGMA clustering supported the results of tri-plot analysis. In the first group, 39 accessions were clearly distinguished mainly by traits such as darker color of their fruit and aril, higher anthocyanin, seed hardness and higher woody portion percent. There was not any soft-seeded accession in this cluster and all of the samples in this group needed a force more than 450 N for their seed breakage (mean = 465.33 N, SE = 13.11). Woody portion percent, another seed hardness related factor, was among affecting traits on sub-group formation in this cluster and all of the members of the first cluster had woody portion percent higher than 18 (mean = 21.55, SE = 2.45), while this value for the members of second group was lower than 12 (mean = 10.15, SE= 1.15). Most of the members in this cluster had sour and sweet-sour taste. Members of this group were divided into two sub-clusters mainly according to their taste as well as fruit and aril color. In addition, fruit weight had major effects on groupings. For example, all of the five accessions denominated by black color of fruit and aril, high anthocyanin, and sweet taste were grouped together. Moreover, all of the members in this sub-group had high value for anthocyanin with a mean value of 1.22 (SE = 0.10). On the other hand, these five accessions had sweet taste (average of TSS/TA = 38.07, SE = 1.23) and low fruit weight (mean = 194.73 g, SE = 10.64).

The second main cluster included 11 accessions. Soft-seeded or semi soft-seeded fruits, light color for fruit and aril, low anthocyanin, and sweet taste were among the main characteristics of the accessions resident in this cluster. This cluster was further divided into two sub-clusters; one included all of the semi soft-seeded accessions that required 200-250 N forces for their seed breakage, while the other one included soft-seeded accessions that required less than 200 N forces for their seed rupture. There are several reports indicating that fruit taste and seed hardness as well as color have prominent role in pomegranate grouping (Zamani *et al.*, 2007; Zarei *et al.*, 2013; Martinez-Nicolas *et al.*, 2016). In fact, high variation among TA, seed hardness and fruit and aril color in different pomegranate accessions make these traits very important in pomegranate classification.

Simple correlation among traits

The correlation between each pair of traits was calculated (Table 4). The correlation coefficient can provide valuable information on the characteristics that are most important in assessing genotypes (Norman *et al.,* 2011). By inferring traits that are significantly correlated, it will be possible to predict one from other; that could facilitate selection of appropriate genotypes. Some of the studied attributes showed significant correlation with each other that can be used for breeding programs. It was found that seed hardness had significant correlation with color related characteristics including fruit color (r = +0.47), aril color (r = +0.49) and anthocyanin (+0.48). Also seed hardness showed high negative correlation with length/diameter of aril (r = -0.83) and seed (r = -0.65). High negative correlation between seed hardness with aril and seed length were also reported in the comparative study of soft-seeded and hard-seeded genotypes (Zarei *et al.,* 2013), but Lu *et al.* (2006) reported no strong correlation between seed softness and aril shape. However, due to the significantly higher content of juice and bigger size in the aril of Indian soft-seeded pomegranate genotypes, Jalikop and Kumar (1998) recommended the soft-seeded genotypes to be used as parents for developing cultivars containing high level of juice.

Seed hardness was positively correlated with TA (r = +0.36) and negatively with taste index (r = -0.45) agreeing with finding of Khadivi-khob *et al.* (2015). All of the soft-seeded pomegranates of Iran have sweet taste and there is high correlation between taste and seed hardness. In fact, incorporation of seed-softness into commercially sweet-sour genotypes is one of the main objectives in Iranian pomegranate breeding (Zamani *et al.*, 2010; Sarkhosh *et al.,* 2011; Zarei *et al.,* 2016a) programme. In addition, positive correlation was found between vitamin C and color related attributes such as fruit color, aril color and anthocyanin. Fruit weight was positively correlated with total phenol, and both were significantly correlated with taste related attributes. On the other hand, sour or sweet-sour fruits are heavier and have higher TSS, TA and total phenolics than sweet genotypes.

Table 4. Bivariate correlations among the measured fruit traits in the studied *P. granatum* samples.

Traits	FrCo	ArCo	TSS	TA	TI	Ta	FrWe	100 ArFrW	SeHa	100 ArDrW	WPP	Anth	PeDi	PDP
FrCo	1.00													
ArCo	0.77[a]	1.00												
TSS	0.19	0.08	1.00											
TA	0.27	0.17	0.87[a]	1.00										
TI	-0.37	-0.24	-0.85[a]	-0.95[a]	1.00									
Ta	0.23	0.11	0.86[a]	0.97[a]	-0.92[a]	1.00								
FrWe	0.10	0.04	0.54[a]	0.50[a]	-0.59[a]	0.47[a]	1.00							
100 ArFrW	-0.42	-0.48[a]	0.01	-0.03	0.13	-0.04	0.03	1.00						
SeHa	0.47[a]	0.49[a]	0.30[b]	0.36[b]	-0.45[a]	0.35[b]	0.30[b]	-0.75[a]	1.00					
100 ArDrW	0.44[a]	0.46[a]	0.24	0.27	-0.35[b]	0.23	0.34[b]	-0.63[a]	0.82[a]	1.00				
WPP	0.42[a]	0.46[a]	0.29[b]	0.34[b]	-0.42[a]	0.33[b]	0.30[b]	-0.63[a]	0.93[a]	0.85[a]	1.00			
Anth	0.80[a]	0.98[a]	0.08	0.18	-0.25	0.12	0.03	-0.48[a]	0.48[a]	0.45[a]	0.43 [a]	1.00		
PeDi	0.15	0.17	0.18	0.18	-0.15	0.18	0.02	-0.01	0.01	0.05	-0.02	0.20	1.00	
PDP	-0.24	-0.23	-0.11	-0.18	0.20	-0.16	-0.15	0.07	-0.22	-0.22	-0.18	-0.25	-0.62[a]	1.00
CrLe	0.03	0.12	-0.07	-0.07	0.09	-0.09	-0.28	-0.01	-0.07	-0.14	-0.16	0.15	0.38[a]	-0.05
CrDi	-0.03	-0.02	0.08	0.03	-0.01	0.05	0.03	-0.06	0.02	0.05	0.01	-0.01	0.61[a]	-0.32[b]
CrLe/Di	0.06	0.13	-0.07	0.01	0.02	-0.02	-0.24	0.05	-0.06	-0.15	-0.12	0.15	-0.19	0.21
PePe	-0.06	-0.10	0.25	0.13	-0.13	0.16	0.11	0.18	-0.11	-0.07	-0.11	-0.09	0.56[a]	-0.31[b]
ToPh	0.27	0.28[b]	0.32[b]	0.35[b]	-0.37[a]	0.35[b]	0.28[b]	0.04	0.12	0.02	0.08	0.30[b]	0.17	-0.12
AnCa	0.12	0.22	-0.06	-0.03	0.07	-0.01	0.04	0.10	-0.08	-0.22	-0.11	0.20	-0.07	0.02
VitC	0.32[b]	0.43[a]	0.18	0.27	-0.28	0.28[b]	0.10	0.04	0.23	0.07	0.27	0.41[a]	0.01	-0.10
ArLe	-0.39[a]	-0.39[a]	0.00	-0.02	0.15	-0.05	0.07	0.81[a]	-0.76[a]	-0.55[a]	-0.67[a]	-0.42[a]	0.09	0.07
ArDi	-0.25	-0.28[b]	0.14	0.12	-0.03	0.10	0.32[b]	0.48[a]	-0.34[b]	-0.13	-0.22	-0.32[b]	0.06	0.02
ArLe/Di	-0.35[b]	-0.31[b]	-0.12	-0.14	0.23	-0.15	-0.18	0.79[a]	-0.83[a]	-0.69[a]	-0.79[a]	-0.32[b]	0.08	0.10
SeLe	-0.24	-0.13	-0.07	-0.09	0.15	-0.11	0.02	0.68[a]	-0.55[a]	-0.45[a]	-0.44[a]	-0.17	0.00	0.17
SeDi	0.10	0.15	0.20	0.27	-0.26	0.26	0.33[b]	0.17	0.18	0.15	0.21	0.13	0.34[b]	-0.08
SeLe/Di	-0.30[b]	-0.25	-0.23	-0.31[b]	0.36[b]	-0.32[b]	-0.27	0.47[a]	-0.65[a]	-0.54[a]	-0.57[a]	-0.28	-0.27	0.21

[a] Correlation is significant at the 0.01 level
[b] Correlation is significant at the 0.05 level

(cont'd)

Table 4. Bivariate correlations among the measured fruit traits in the studied *P. granatum* samples.

Traits	CrLe	CrDi	CrLe/Di	PePe	ToPh	AnCa	VitC	ArLe	ArDi	ArLe/Di	SeLe	SeDi	SeLe/Di
FrCo													
ArCo													
TSS													
TA													
TI													
Ta													
FrWe													
100													
ArFrW													
SeHa													
100													
ArDrW													
WPP													
Anth													
PeDi													
PDP													
CrLe	1.00												
CrDi	0.27	1.00											
CrLe/Di	0.60[a]	-0.58[a]	1.00										
PePe	0.20	0.28 [b]	-0.07	1.00									
ToPh	-0.04	0.09	-0.08	0.08	1.00								
AnCa	-0.08	-0.04	-0.02	-0.19	0.61[a]	1.00							
VitC	-0.13	-0.11	0.05	-0.06	0.70[a]	0.70[a]	1.00						
ArLe	0.08	0.12	-0.04	0.23	0.04	0.17	-0.10	1.00					
ArDi	-0.09	0.26	-0.30[b]	0.14	0.08	0.20	-0.07	0.76[a]	1.00				
ArLe/Di	0.20	-0.07	0.23	0.22	-0.02	0.08	-0.06	0.79[a]	0.20	1.00			
SeLe	0.09	0.07	0.02	0.07	0.25	0.37[a]	0.24	0.70[a]	0.54[a]	0.56[a]	1.00		
SeDi	0.20	0.21	0.04	0.26	0.30[b]	0.24	0.37[a]	0.18	0.27	0.06	0.3[b]	1.00	
SeLe/Di	-0.09	-0.09	-0.04	-0.14	-0.04	0.14	-0.11	0.50[a]	0.29[b]	0.46[a]	0.62[a]	-0.55[a]	1.00

[a] Correlation is significant at the 0.01 level
[b] Correlation is significant at the 0.05 level

In fact, sour or sweet-sour fruits not only have higher TSS but also have higher TA than sweet ones, and the latter is more effective than the former and gives rise to the final taste of the fruit. Consistent with report of Martinez *et al.* (2006) woody portion percent was negatively correlated with seed length (r = -0.44). Martinez *et al.* (2006) reported that Spanish soft-seeded genotypes, in spite of having the highest seed and aril lengths, showed the lowest woody portion percent values. Zarei *et al.* (2013) also reported similar observation in Iranian pomegranate accessions. All of the accessions in this study had higher woody portion percent compared with reported Spanish genotypes (Martinez *et al.*, 2006). However, similar to those reports, soft-seeded accessions showed a lower woody portion. In fact, soft-seeded fruits not only had a thinner seed but also had a thicker fleshy part of aril than hard-seeded ones (Zarei *et al.*, 2013).

In addition, high positive correlation was observed between fruit color with seed hardness, aril color, woody portion percent and anthocyanin, while fruit color was negatively correlated to the aril length as well as seed and aril length/diameter. Aril color was positively correlated with seed hardness, woody portion percent, anthocyanin and vitamin C, while negatively correlated with aril length and 100 arils fresh weight. This information would be of great importance for selecting genotypes for industrial processing such as juicing. Close relationship between attributes could facilitate or prevent gene introgression since strong selection for a desirable character could favor the presence of another desirable character from germplasm (Khadivi-Khub, 2014a).

Multiple regression analysis

Due to the importance of seed hardness, this character was considered as dependent variable and then the effect of each independent variable on this trait was calculated using multiple regression analysis. Results revealed that seed hardness is associated with woody portion percent, 100 aril weight, taste index and seed length/diameter. An understanding of association between these traits can help breeders for selection and crosses (Khadivi-Khob, 2014b). Therefore, these four traits were the main variables accounting for seed hardness and need to be considered together in breeding programs for reducing seed hardness. In the simple correlation analysis, these four traits showed to be significantly correlated with seed hardness. On the other hand, multiple regression analysis compliments the results of simple correlation among traits.

Pomegranates are produced for both fresh consumption and industrialization, mainly juice manufacturing. The fruits with larger dimensions of arils and lower seed hardness are more acceptable for fresh consumption and those with high woody portion percent and harder seeds are used for industrialization (Nuncio-Jáuregui *et al.*, 2015). Also recently, industrial companies are willing to use soft-seeded or semi soft-seeded fruits for producing compote and other canned products containing fruit pulp. Therefore, our data suggest that some of the soft-seed and semi soft-seed accessions including BKJ2, BPGN, and SSD that have high fruit weight and aril dimensions are merit to be considered in breeding programs for fresh consumption as well as in industrialization for producing fruit pulp products.

CONCLUSION

Various morphological attributes were analyzed on fruits of fifty pomegranate accessions from five different regions of Fars province in Iran. Results indicated high level of variability in the studied attributes. Seed hardness, fruit and aril color as well as fruit taste and the juice's acidity had the highest power of discrimination, and were, therefore, the most useful for genetic characterization of pomegranate accessions in Fars province. These characters also had prominent role in clustering. Several soft- and semi soft-seeded accessions were identified, which are suitable for cultivation and breeding programs for decreasing of seed hardness in commercial pomegranates. Multiple regression analysis revealed that seed hardness is associated with woody portion percent, 100 arils weight, taste index and seed length/diameter. These traits may be considered in breeding programs in order to lessen seed hardness in commercial cultivars.

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