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Original article

Genetic diversity, heritability and inter-relationships of fruit quality and taste attributes among Iranian pomegranate (*Punica granatum* L.) cultivars using multivariate statistical analysis

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Summary

Introduction – **Little information is published about estimating genetic parameters of fruit traits in Iranian pomegranate cultivars. A two-year study was conducted using 20 cultivars to determine the extent of the phenotypic and genotypic variability, calculate heritability and related genetic parameters, search for useful correlations, and classify Iranian pomegranate cultivars based on their differences in fruit quality attributes.** *Materials and methods* – **Fruits and arils traits, as well as pH, titratable acidity (TA), total soluble solids (TSS) and antioxidant activity were measured. Analysis of variance, genetic parameters estimation, principal component analysis (PCA), and cluster analysis were performed.** *Results and discussion* – **Both simple descriptive statistics and ANOVA showed significant differences among cultivars. The highest genotypic (96.16%) and phenotypic (93.68%) coefficients of variation were observed for the TA, the lowest variations were recorded for TSS (phenotypic coefficient = 19.67% and genotypic coefficient = 14.44%). Highest broad sense heritability was achieved in TA (H = 0.95, whilst, antioxidant activity showed the lowest broad sense heritability (H = 0.36). High phenotypic correlations were found between aril width and aril length (r = 0.92), skin weight and fruit weight (r = 0.88), and b* and L* fruit color (r = 0.88). Similarly, most genotypic correlations were high including aril width and fruit weight (r = 0.98), fruit weight and aril weight (r = 0.98), antioxidant activity and pH (r = 0.97). Cluster analysis elicited four main clusters for the cultivars studied here. In the PCA, the first two components accounted for 65% of the total variation, while the first two factors from the factor analysis accounted for 88% of the total variation.** *Conclusion* – **High levels of genotypic (14.44–96.16%) and phenotypic (19.67–93.68%) variations and high to very high broad sense heritability (0.46–0.95) which were calculated for fruit quality attributes are suggesting the possibility of genetic improvement for pomegranate fruit quality through conventional breeding. Illustrated relationships among cultivars and correlations between pairs of fruit quality traits are useful information which could be utilized in future breeding programs. Further investigation on the studied cultivars is recommended, especially those promising for productivity and drought resistance.**

Significance of this study

What is already known on this subject?

- Morphological and molecular methods confirmed the wide range of variations in fruiting, yield and fruit quality characteristics among Iranian pomegranate cultivars and landraces.
- Iran is potentially a rich source of germplasm for pomegranate genetic improvement.

What are the new findings?

- Very high levels of variation as well as moderate to high amount of broad sense heritability were recorded among 20 cultivars evaluated for morphological and biochemical fruit characteristics.
- Strong phenotypic and genotypic correlations were detected between some traits which could be utilized in efficient characterization of breeding populations.
- Four clusters were distinguished among the cultivars, which could be considered as parent materials in future breeding programs.

What is the expected impact on horticulture?

- The generated information will help breeders to plan further pomegranate breeding programs especially in selecting parent cultivars, traits of interest, as well as efficient characterization of breeding populations.

Keywords
Iran, pomegranate, *Punica granatum*, genetic resources management, phenotypic characterization, cluster analysis

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Introduction

Pomegranate (*Punica granatum* L.) is one of the most important fruit crops in the tropical and subtropical region, with low maintenance cost and acceptable yields in areas under low soil and water condition and other limiting factors. According to historical documents, as well as genetic studies, Iran is the primary center of diversity for pomegranate germplasm (Harlan, 1975; Levin, 1994; Verma *et al*., 2010). A plausible reason for this claim is the natural distribution of a wide range of wild pomegranate and its gene pools in the northern and to some extent western forests of Iran (Karimi and Mirdehghan, 2013). Nowadays, pomegranate is widely grown commercially in Iran, India, Syria, Afghanistan, Turkmenistan, Pakistan, Spain, Morocco, China, Japan, Russia, and to some extent in the United States (arid parts of California and Arizona) (Fadavi *et al*., 2006). Currently, Iran is the world's largest producer of pomegranate, with an annual production of 600,000 t spread over 65,000 ha of cultivation area (Holland and Bar-Ya'akov, 2008). There is a wide range of variation in fruiting, yield, fruit quality characteristics, as well as morphological parameters among Iranian pomegranate cultivars and landraces (about 762 native accessions were documented), mainly due to sustained sexual reproduction over centuries of production in Iran (Behzadi Shahrbabaki, 1998; Zamani *et al*., 2007). Thus, potentially Iran has a rich source of germplasm for genetic improvement and breeding. Availability of data and information about genetic diversity in the accessible collections of germplasm of plant species facilitates reliable classification of genotypes and identification of different subsets, for use in crop improvement programs (Fear *et al*., 1985). Successful breeding programs rely on the availability of genetic variability to increase productivity and quality of commercial cultivars (Cilas *et al*., 2003). There are different methods for determining plant genetic variability, including morphological, bio-chemical, and molecular characterization. Unlike well-known crops such as wheat and maize, evaluation of genetic diversity based on differences in morphological characters of trees and fruit crops is in its infancy in the description and characterization of germplasm collections (Tibbits *et al*., 1991). Basic details such as the value of genotypic and phenotypic variability in line with the heritability of yield and yield related traits, as well as the correlations and associations between traits can simplify improvement of original cultivars and aid development of appropriate breeding procedures (Hummel *et al*., 1982). Cultivars evaluated in different years might have significant fluctuations in yield and other traits due to variable responses to different environmental factors (Falconer, 1975). According to Machikowa *et al*. (2011), measuring genotype × environment interactions is very important in determining the optimum breeding strategy for developing and releasing cultivars with adequate adaptation to targeted environments. Moreover, since heritability is the degree of phenotypic and observable variation accounted for by phenotypic and genotypic values, and indicates the influence ratio of the genetic background on the traits, the assessment of heritability is required to develop effective breeding programs (Sprague and Tatum, 1942). To study the genetic diversity of a germplasm collection of pomegranate, morphometric traits and fruit chemical compounds, microsatellite markers (Singh *et al*., 2015) and single nucleotide polymorphism (SNP) markers (Ophir *et al*., 2014) have also been used previously.

While there are a few reports on the genetic diversity of Iranian pomegranate germplasm based on DNA markers (Nemati *et al*., 2012; Noormohammadi *et al*., 2012; Sarkhosh *et al*., 2009), there is still no report on genetic diversity of fruit quality and taste attributes of Iranian pomegranate cultivars. More so, there is little published information about estimating heritability and genetic gain for fruit traits in pomegranate to be used by breeders. In order to facilitate the development of pomegranate breeding programs, a two-year study was conducted to evaluate 20 Iranian cultivars for fruit quality attributes including quantitative and biochemical characteristics by using multivariate statistical methods. The objectives of this research were: 1) to determine the extent of the phenotypic and genotypic variability in fruit quality attributes among a diverse set of Iranian pomegranate cultivars; 2) to calculate heritability and related genetic parameters for fruit quality characteristics; 3) to search for useful correlations between fruit quality traits to be used for indirect selection; and 4) to classify these cultivars based on differences of their trait performance.

Materials and methods

Plant materials

Twenty pomegranate cultivars originating from different regions of Iran (Table 1) were evaluated for fruit biochemical and morphological characteristics during two growing seasons (2015 and 2016) at the Agriculture and Natural Resources Research Centre (ANRRC), Yazd, Yazd Province. Excluding 'Rabab Poost Ghermez Neyriz' and 'Malas Yazdi' cultivars which are among the dominant commercial cultivars nationally, other cultivars are locally important in different provinces. Healthy and productive 26-year-old trees planted in a randomized complete block design were chosen for the experiment. All experimental trees received uniform cultural practices, including pruning, irrigation, and fertilization, according to the standard practices of the area. Irrigation was done using bubbler systems with an irrigation interval of 5 days. The climate of experimental site is dry with the average annual rainfall of 60.5 mm. The annual average temperature reaches 18.9 °C.

Measurement of fruit physical properties

Fruit harvest was performed at commercial maturity for each cultivar (Table 1) and transferred to the pomology laboratory in the Department of Horticultural Sciences, Shiraz University, Shiraz, Iran. Morphometric measurements and chemical analyses were carried out on samples of 10 mature fruits from each tree, using a total of three trees (one tree as replicate) per cultivar ($10 \times 3 = 30$ fruits cultivar^{-1}). The fruits were weighed using a digital scale (Mettler AJ50, Hong Kong) with an accuracy of 0.001 g. Husks were carefully cut at the equatorial zone with a sharp knife and the arils were manually extracted. To measure the physical properties of aril, the peel and arils were carefully separated from the fruit. Then the arils of each fruit and skin per fruit were weighed again on the same scale.

The maximum width and length of the aril (20 arils fruit^{-1} and 6 fruits cultivar^{-1}) were measured using a digital caliper (Mitutoyo, USA) accurate to 0.01 mm. The edible portion of the fruit was determined using the following formula:

$$Edible\ portion\ of\ fruit\ (\%) = \frac{[Fruit\ weight] - [Skin\ weight] - [Carpillary\ membranes]}{Fruit\ weight} \times 100$$

Fruit and aril colors (indicated by L*, a* and b*) were measured using a chromatometer (Chroma Meter CR-400,

Table 1. Name, origin, fruit and tree characteristics of the Iranian pomegranate cultivars used in this study.

Cultivars	Province	City	Fruit and tree characteristics
Anar Siah	Isfahan	Isfahan	Black skin and aril, sweet taste, non-commercial, medicinal properties, *mid ripening date
Bihaste Khafr Jahrom	Fars	Jahrom	Soft seed, yellow skin, white aril, medium fruit size, sweet taste, locally important, *early ripening date
Bihaste Ravar	Kerman	Ravar	Soft seed, yellow-pink skin, white aril, sweet taste, early ripening date, locally important
Bihaste Sangan Khash	Sistan Baluchistan	Khash	Soft seed, white skin and aril, sweet taste, early ripening date, locally important
Jangali Poost Ghermez Roodbar	Guilan	Roodbar	Big fruit, red skin and aril, sweet-sour taste, *late ripening date, locally important
Khajei Ghasrodasht Fars	Fars	Shiraz	Big fruit, pink skin, white aril, sweet taste, early ripening date, locally important
Malas Pishva Varamin	Tehran	Varamin	Medium fruit, yellow skin, white aril, sweet taste, early ripening date, locally important
Malas Yazdi	Yazd	Yazd	Big fruit, red skin and aril, sweet-sour taste, mid ripening date, commercial cultivar
Makhmal Malas Shahreza	Isfahan	Shahreza	Medium fruit, red skin and aril, sweet-sour taste, locally important
Malas No. 1 Saravan	Sistan Baluchistan	Saravan	Small fruit, yellow-white skin, white aril, sweet-sour taste, dwarf, mid ripening date, locally important
Poost Nazok Torosh Abarkuh	Yazd	Abarkuh	Medium fruit, red skin and aril, sweet-sour taste, late ripening date, locally important
Poost Sefid Dezfoul	Khuzestan	Dezfoul	Small fruit, yellow-white skin, sweet-sour taste, white aril, late ripening date, locally important
Rabab Poost Ghermez Neyriz	Fars	Neyriz	Big fruit, red skin and aril, sweet-sour taste, mid ripening date, commercial cultivar
Rabab Poost Ghermez Kazeroon	Fars	Kazeroon	Big fruit, red skin, pink aril, sweet-sour taste, mid ripening date, locally important
Sefid Biardal Borujen	Chahar Mahal-Bakhtiari	Borujen	Medium fruit, yellow-white skin, pink aril, sour taste, late ripening date, locally important
Shirin Jangal Sisangan	Mazandaran	Sisangan	Big fruit, red-yellow skin, pink aril, sweet-sour taste, mid ripening date, locally important
Shirin Semnan	Semnan	Semnan	Big fruit, green-yellow skin, white aril, sweet taste, early ripening date, locally important
Shahsavar Seydan Marvdasht	Fars	Marvdasht	Big fruit, white-yellow skin, white aril, sweet taste, strong tree, early ripening date, locally important
Torosh Goli Naz Behshahr	Mazandaran	Behshahr	Big fruit, white-yellow skin, white aril, sour taste, late ripening date, locally important
Torosh Nar Riz Zirab	Fars	Darab	Small fruit, green-yellow skin, white aril, barbate stem, very sour taste, late ripening date, wild accession

* Cultivars with early, mid and late ripening date had harvest date between 15–30 September, 1–15 October or 15–31 October, respectively.

Konica Minolta, Japan). The color parameters represent whiteness or brightness/darkness (L*), redness/greenness (a*) and yellowness/blueness (b*) (Gharaghani *et al.*, 2017).

Fruit biochemical analyses

Fruit juice was directly used to measure total soluble solids (TSS), total acidity (TA) and pH from 3 fruits per cultivar per replicate. Sampled arils were juiced manually for each cultivar and replication. TSS were measured using a hand refractometer (Atago NI, Japan) and expressed as °Brix at 20 °C. TA was determined by titrating aliquots of juice samples (5 mL) to an endpoint pH of 8.2 with 0.1 N NaOH and expressed as a percentage of citric acid (g 100 mL^{-1}) (AOAC, 1980). The pH of the juice was measured using a pH meter (WTW 526, Germany), which had been previously standardized to a pH of 4 and 7.

Gallic acid of pomegranate juice was estimated using the Folin-Ciocalteu (Folin-C) colorimetric method as described by Singleton and Rossi (1965), and expressed as the mean (mg) of Gallic acid equivalents (GAE) mL^{-1} juice. Gallic acid equivalents were determined spectrophotometrically at 750 nm by adding Folin-Ciocalteu reagent to the juice sample.

Antioxidant activity was also assessed by the commercially available free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Moon and Terao, 1998). Briefly, 0.1 mL of pomegranate juice was mixed with 0.9 mL of 100 mM Tris-HCl buffer (pH = 7.4) to which 1 mL of DPPH (500 µM in ethanol) was added. The control sample was prepared in a similar manner by adding 0.1 mL of distilled water instead of pomegranate juice. The mixtures were shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. The reaction mixture without DPPH was used for background correction. The DPPH radical scavenging was calculated in terms of percent inhibition of DPPH by antioxidant percent

in the samples using the following equation:

$$Antioxidant\ activity\ (\%) = \left[1 - \frac{A\ sample\ (517\ nm)}{A\ control\ (517\ nm)}\right] \times 100$$

where *A sample* is the absorbance of sample after 30 min, and *A control* is the absorbance of sample at time 30 min.

Statistical analysis

Descriptive statistics such as means and standard deviations in addition to analysis of variance (ANOVA) and estimated genetic parameters were calculated through the software SAS v.9.3 (SAS Institute, 2003). Multivariate statistical methods including cluster analysis, principal component analysis (PCA), and factor analysis (FA), were performed through the software Minitab v.18 (Minitab Inc., State College, PA, USA). The expected values of mean squares for genotypic variance calculation were estimated using the following formula in SAS system using proc IML written by the authors (Formula 1).

Formula 1.

Sources	Mean squares	Expected values	Estimated variances
Year (Y)	MS_Y	$\sigma^2_{e1} + r g \sigma^2_y$	$Vg = (MS_G - MS_{y\times r})/_{ry}$
Repeat (Y)	MS_{E1}	σ^2_{e1}	$V_E = MS_{e2}$
Genotype (G)	MS_G	$\sigma^2_{e2} + ry\sigma^2_g + r\sigma^2_{yg}$	$V_P = V_E + V_g$
Y × G	$MS_{Y\times A}$	$\sigma^2_{e2} + r\sigma^2_{yg}$	$H = (V_g/V_P)$
Error	MS_{E2}	σ^2_{e2}	

where r, e, E, Y, y, G, g, and P represent replicate, error, environmental effect, year, year effect, genotype, genotypic effect and phenotypic effect terms in the expected values of mean squares, respectively, and *H* represents the broad sense heritability.

Response to selection (*RS*) was calculated based on the formula (Montgomery, 2008):

$$RS = 2.06 \times \sqrt{VP} \times (H/100)$$

Genotypic (GCV) and phenotypic (PCV) coefficients of variation were calculated according to the following formulae, where μx is the grand mean:

$$GCV = (\sqrt{VG}/\mu x) \times 100$$
$$PCV = (\sqrt{VP}/\mu x) \times 100$$

Results and discussion

Simple statistics and analysis of variance

The minimum and maximum values, as well as the means, showed a wide range of variability among the cultivars for most morphological and biochemical characters (Table 2). This will provide the breeder with an interesting range for genetic combinations to obtain superior pomegranate cultivars. Singh *et al.* (2015) suggested that morphological characterization is an essential step in the development of breeding programs as it allows performance characterization and the selection of the best cultivars according to the desired traits.

The physical and biochemical measurements also expressed performance differences among the pomegranate cultivars (Table 2). The yield components such as the aril and fruit weights were between 16–210 g and 35–365 g, respectively. Varasteh *et al.* (2006) evaluated the pomological characteristics of five commercial pomegranate cultivars in Iran growth conditions (dry climate, traditional cultivation method, multi-branch training system and minimum usage of agrochemicals including fertilizers and pesticides). They reported that 'Malas-e-yazdi' had the highest fruit weight, volume, length, diameter, aril percentage and juice content as well. Yildiz *et al.* (2003) also reported fruit weights between 192.3 and 388.3 g among some pomegranate populations in Turkey. The average fruit weight of the studied pomegranates cultivars are less than that reported from Spanish (Martinez-Nicolas *et al*., 2016), Turkish (Caliskan and Bayazit, 2013) and Moroccan (Martínez *et al*., 2012a) cultivars. This difference could arise from different climatic conditions (probably drier climate in Iran) as well as a higher diversity of the explored germplasm (plant materials including wild, local and commercial cultivars in this study compared with mainly commercial cultivars in these reports).

Moreover, the fruit juice biochemical characteristics including TSS, pH, TA, antioxidant activity and GAE varied from 12–20 °Brix, 2.74–4.56, 0.17–8.55%, 13.32–89.25%, and 0.68–6.81 mg L^{-1}, respectively. The obtained range of TSS is quite similar to the values reported for Spanish varieties (Martinez-Nicolas *et al*., 2016), although lower than those reported for Turkish 'Eksi' (Caliskan and Bayazit, 2013). However, the high number of cultivars investigated and the different climatic conditions should let consider cautiously any comparisons. In previous studies, acidity values were in the range of 2.1–2.4% for genotypes from Greece (Drogoudi *et al*., 2005), 0.4–2.5% from Italy (Cristofori *et al*., 2011), 0.3–2.4% from Iran (Tehranifar *et al*., 2010), 0.3–1.0% from Spain (Martinez *et al*., 2006), and 0.2–1.9% from Tunisia (Zaouay and Mars, 2011). The maximum acidity values of our accessions were recorded for 'Torosh Nar Riz Zirab' which is a non-commercial semi-wild pomegranate and is famous for its strong sour taste. However, the Turkish varieties are reported to have less acidity (average acidity = 1.4%) than the materials of this study (Caliskan and Bayazit, 2013).

The chemical composition of pomegranate fruit differs depending on the cultivar, growing region, climate, maturity stage, cultivation practice, and storage conditions (Caliskan and Bayazit, 2013). Fruit and aril color is one of the main fruit characteristics that determine pomegranate fruit quality and is an important criterion in consumer decisions. Fruit color in this study varied from green, red, yellow, yellow-green, yellow-red and black (Figure 1). Aril colors included white, yellow, yellow-pink, pink, dark pink, red and black (Figure 1). Fruit color traits (L*, a* and b* values) recorded in pomegranate cultivars in Turkey were 58.71, 32.72 and 28.97, respectively (Gozlekci *et al*., 2011). Drogoudi, Tsipouridis and Michailidis (2005) reported the aril color range of Turkish pomegranate genotypes including L* value (lightness) from 28.06 to 111.51; a* value from 4.97 to 49.84, and b* value from 12.79 to 43.35.

Effect of year from a combined analysis of variance (ANOVA) (Table 3) was significant ($P < 0.05$) for most traits, indi-

Table 2. Descriptive statistics of fruit traits in 20 pomegranate cultivars over 2 years. Data are values from 3 replicates ($n = 120$). SD: Standard deviation.

Variables	Means	SD	Sums	Minimum	Maximum
L* (aril)	31.17	12.86	3,549	7.30	57.67
a* (aril)	6.33	4.97	741	0.23	20.88
b* (aril)	10.67	4.21	1,230	3.43	20.95
L* (fruit)	52.62	15.47	5,970	22.87	72.28
a* (fruit)	19.04	12.60	2,249	0.45	43.92
b* (fruit)	25.22	11.57	2,847	0.46	42.87
pH (juice)	3.60	0.72	420.76	2.74	4.56
Total soluble solid (in °Brix)	15.89	2.93	1,864	12.00	20.00
Total acidity (in %)	2.00	1.92	240	0.17	8.55
DPPH (antioxidant activity, in %)	60.18	25.54	7,160	13.32	89.25
GAE (Gallic acid equivalents, in mg mL^{-1})	3.58	1.50	426.27	0.68	6.81
Skin weight (in g)	66.50	32.92	7,923	19.00	188.00
Aril weight (in g)	89.53	40.71	10,695	16.00	210.00
Fruit weight (in g)	156.74	66.28	18,704	35.00	365.00
Edible portion (in %)	57.37	12.68	6,748	24.32	73.54
Aril length (in mm)	10.20	3.12	1,134	7.00	13.50
Aril width (in mm)	7.16	2.20	816.8	5.00	11.50

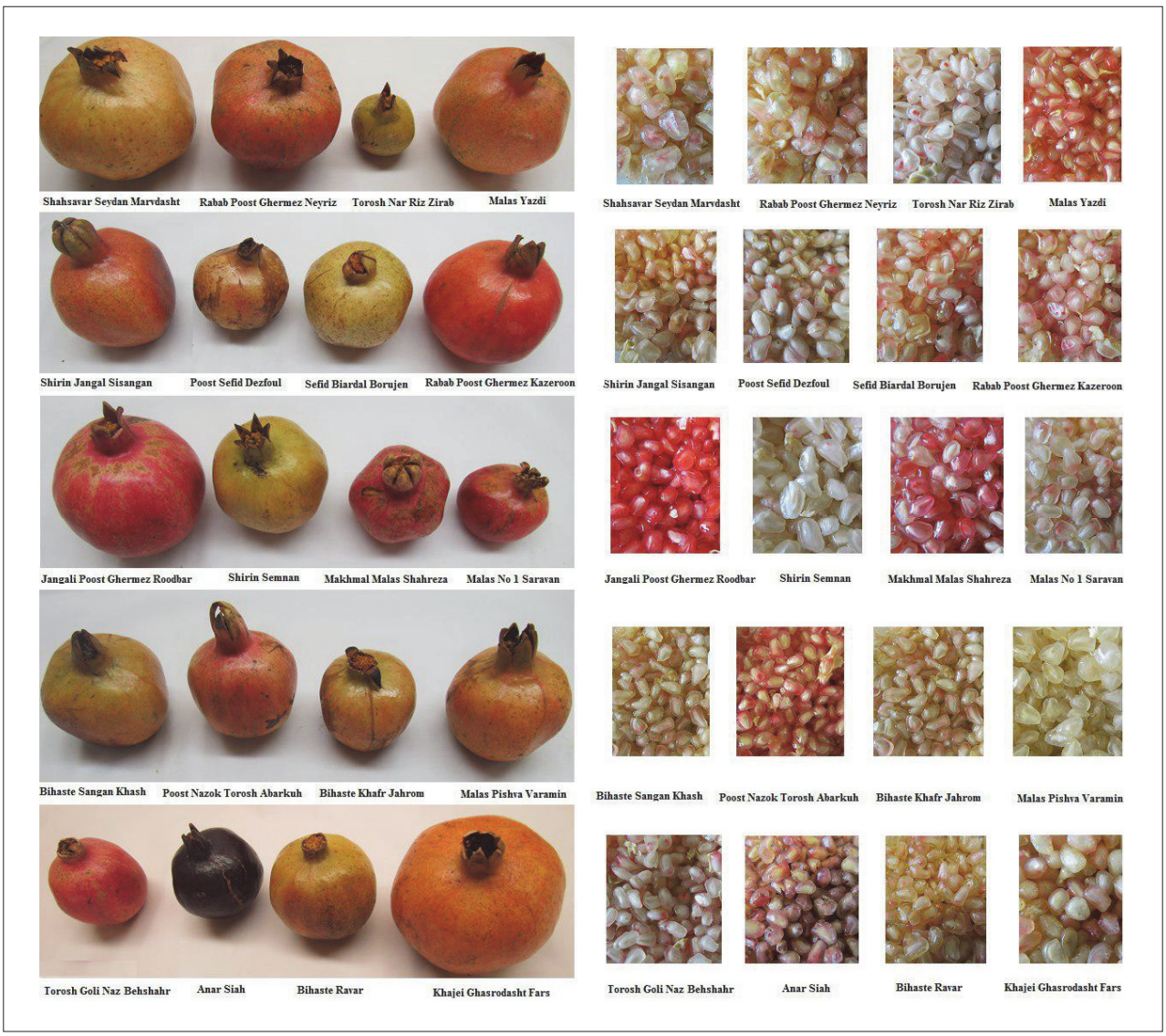

Figure 1. Fruit and aril colors of twenty Iranian pomegranate cultivars.

TABLE 3. Combined analysis of variance (ANOVA) for fruit traits in 20 pomegranate cultivars. Data are values from 3 replicates ($n = 120$). DF: Degree of freedom; CV: Coefficient of variation; Rep: Replicate; Gen: Genotype (cultivar); TSS: Total soluble solids; TA: Total acidity; GAE: Gallic acid equivalents.

Sources of variability	DF	Mean squares								
		L* (aril)	a* (aril)	b* (aril)	L* (fruit)	a* (fruit)	b* (fruit)	TSS	pH	TA
Year	1	233.58**	9.30**	565.26**	2671.92**	0.20^{ns}	5385.46**	4.52**	4.99**	0.98*
Rep (Year)	4	2.30	0.78	1.69	7.31	8.48	0.87	0.02	0.02	0.12
Gen	19	707.88**	114.28**	44.44**	1069.08**	910.01**	410.85**	32.89**	1.51**	21.75**
Year × Gen	18	286.96**	37.46**	16.18**	66.62**	61.58**	42.18**	2.7**	0.06**	0.63**
Error	77	13.76	1.3	1.77	9.07	4.83	2.69	4.51	0.31	0.13
CV		12.54	18.48	12.97	6.05	11.73	6.91	13.66	15.96	18.28

		Antioxidant activity		Aril		Fruit		Aril	
		DDPH	GAE	Skin weight	Weight	Weight	Edible weight	Length	Width
Year	1	39,808.16**	705,271.65**	3,482.13*	0.01^{ns}	2,596.79**	552.86*	1.82^{ns}	0.04^{ns}
Rep (Year)	4	10.95	1637.19	262.71	463.91	100.55	51.04	1.34	0.32
Gen	19	856.13**	72,019.23**	4,434.12**	6,075.24**	18,910.04**	552.32**	43.33**	23.19**
Year × Gen	18	571.78**	14,665.99**	1,256.04**	2,846.21**	5,904.88**	108.96**	12.96**	5.92**
Error	77	23.94	1211.4	264.6	346.92	729.89	62.23	1.11	0.23

cating the influence of yearly fluctuations of the climate. The cultivar effect was highly significant ($P < 0.05$), indicating significant genotypic differences among the cultivars. Interaction between year and cultivar was additionally significant ($P < 0.05$) for all traits, thus suggesting that the cultivars had varying response to the weather. The combined ANOVA results indicate that the cultivars used in this study provide the variation that is necessary for use in genetic improvement through breeding. Identification of the variance components is necessary to facilitate the determination of the genetic control of traits and selection potential (Cristofori *et al*., 2011). Tehranifar *et al.* (2010) suggested that experimental variation coefficients, which provides an indication of experimental precision when carried out in the field, may be considered low when they are less than 10%, medium if 10 to 20%, high if 20 to 30%, and very high if greater than 30%. Therefore, the experimental variation coefficients obtained in this study for fruit color characteristics (L* and b*), fruit biochemical characteristics (antioxidant activity via DDPH and GAE) and aril width were considered to be low, since they have values less than 10%, whereas taste traits (TA, pH, TSS), aril color, fruit weight, edible portion and aril length were medium. The exception is skin weight and aril weight, which showed a coefficient of variation (CV) of ~25 and 21%, respectively.

Genotypic parameters

Some correlation coefficients (R^2) were found not significant in phenotypic assessments while these are significant in genotypic assessments and vice versa (Table 4). Moreover, as most of the R^2 in both genotypic and phenotypic assessment are significant, the degree of association between variables are different. In some cases, the sign of the R^2 is altered between phenotypic and genotypic correlations. The results of genotypic and phenotypic correlations indicated that different sources of plant material or genetic background affected the associations between parameters. Therefore, in deploying indirect selection or even direct selection for genetic improvement, the genetic background and source of plant material should be clearly assessed. Since it enables the screening and development of superior genotypes, genetic variance is crucial in plant breeding programs (Martinez *et al*., 2006). More so, the knowledge of genetic variation is also very important for a breeding program, since it indicates the genetic variation amplitude of a character in view of improvement possibility (Zaouay and Mars, 2011). The overall phenotypic correlation was relatively low. However, high phenotypic correlations were also observed in some traits including between aril width and aril length (0.92), skin weight and fruit weight (0.88), and b* fruit color and L* fruit color (0.88). On the contrary, most genotypic correlations were high including aril width with aril length (0.98), aril width with fruit weight (0.98), fruit weight with aril weight (0.98), and antioxidant activity with pH (0.97). Aril width also showed high genotypic correlations with fruit weight (0.92), aril weight (0.98), and antioxidant activity (0.95). Genetic parameters including genotypic, environmental, and phenotypic variances (Table 5) showed that genetic background had a significant effect on trait performance. Since the variances are affected by the dimension and unit of the variables, standard coefficients such as genotypic and phenotypic coefficient of variation could be used for comparing different measured parameters. The highest genotypic (96.16%) and phenotypic (93.68%) coefficients of variation were observed in total acidity (TA), while the lowest coefficients were observed in TSS (phenotypic coefficient = 19.67% and genotypic coeffi-

Table 4. Phenotypic correlation (above the diagonal) and genotypic correlation (below the diagonal) of fruit traits in 20 pomegranate cultivars. X1: L* (aril); X2: a* (aril), X3: b* (aril), X4: L* (fruit); X5: a* (fruit); X6: b* (fruit); X7: total soluble solids; X8: pH; X9: total acidity; X10: antioxidant activity (DDPH); X11: antioxidant activity (GAE); X12: skin weight; X13: aril weight; X14: fruit weight; X15: edible weight; X16: aril length; X17: aril width.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17
X1	1	0.09^{ns}	0.62**	0.51**	0.28**	0.35**	0.32**	0.20^{ns}	0.09^{ns}	0.27**	0.16^{ns}	0.38**	0.57**	0.54**	0.47**	0.41**	0.41**
X2	0.36**	1	0.09^{ns}	0.12^{ns}	0.54**	-0.09^{ns}	0.42**	0.05^{ns}	0.03^{ns}	0.00^{ns}	0.16^{ns}	0.12^{ns}	0.15^{ns}	0.15^{ns}	0.18^{ns}	0.24*	0.30**
X3	0.88**	0.2**	1	0.68**	0.18^{ns}	0.68**	0.30**	0.45**	0.03^{ns}	0.67**	0.48**	0.22*	0.50**	0.43**	0.58**	0.41**	0.39**
X4	0.95**	0.12**	0.95**	1	0.05^{ns}	0.88**	0.47**	0.37**	0.30**	0.61**	0.42**	0.12^{ns}	0.38**	0.30**	0.64**	0.47**	0.49**
X5	0.29**	0.87**	0.18**	0.05^{ns}	1	-0.05^{ns}	0.41**	0.01^{ns}	0.11^{ns}	0.14^{ns}	0.29**	0.42**	0.39**	0.43**	0.16^{ns}	0.23*	0.30**
X6	0.95**	-0.20**	0.86**	0.88**	-0.07^{ns}	1	0.26*	0.33**	0.23*	0.69**	0.39**	0.03^{ns}	0.28*	0.20^{ns}	0.52**	0.29**	0.32**
X7	0.65**	0.54**	0.64**	0.47**	0.53**	0.48**	1	0.47**	0.36**	0.25**	0.48**	0.17^{ns}	0.29*	0.25*	0.69**	0.39**	0.42**
X8	0.39**	0.22**	0.59**	0.37**	-0.08	0.19**	0.26**	1	-0.30**	0.55**	0.20^{ns}	0.35**	0.35**	0.40**	0.54**	0.41**	0.41**
X9	0.05^{ns}	0.02^{ns}	-0.15**	0.30**	0.15**	0.24**	0.49**	-0.52**	1	-0.01^{ns}	0.53**	-0.27*	-0.20^{ns}	-0.26*	0.20^{ns}	-0.06^{ns}	-0.04^{ns}
X10	1.51^{ns}	0.48**	1.31^{ns}	0.61**	0.25**	0.65**	0.80**	0.97**	-0.45**	1	0.53**	0.18^{ns}	0.29**	0.28*	0.40**	0.23*	0.28*
X11	0.11*	0.46**	0.02^{ns}	0.42**	0.39**	0.02^{ns}	0.79**	-0.29**	0.73**	0.25**	1	-0.06^{ns}	0.03^{ns}	-0.02^{ns}	0.36**	0.11^{ns}	0.13^{ns}
X12	0.78**	0.42**	0.82**	0.12^{ns}	0.51**	0.20**	0.3**	0.58**	-0.35**	0.85**	-0.17**	1	0.62**	0.88**	-0.05^{ns}	0.33**	0.31**
X13	0.87**	0.80**	1.15^{ns}	0.38**	0.44**	0.63**	0.59**	0.55**	-0.35**	0.94**	-0.16**	0.90**	1	0.9**	0.51**	0.32**	0.33**
X14	0.85**	0.62**	1.02^{ns}	0.30**	0.45**	0.42**	0.43**	0.58**	-0.36**	0.90**	-0.20**	0.96**	0.98**	1	0.30**	0.36**	0.36**
X15	0.70**	0.53**	0.89**	0.64**	0.17**	0.76**	0.86**	0.39**	0.23**	0.80**	0.39**	0.27**	0.73**	0.51**	1	0.38**	0.43**
X16	0.74**	0.39**	0.80**	0.47**	0.41**	0.40**	0.70**	0.85**	-0.12**	1.51^{ns}	0.03^{ns}	0.90**	1.10^{ns}	1.01^{ns}	0.80**	1	0.92**
X17	0.74**	0.49**	0.78**	0.49**	0.46**	0.35**	0.74**	0.73**	-0.06^{ns}	1.25^{ns}	0.04^{ns}	0.84**	0.98**	0.92**	0.74**	0.98**	1

**, *, ns indicate significant correlation at 1%, 5%, and lack of significance, respectively.

Table 5. Genetic parameters of fruit traits in 20 pomegranate cultivars. X1: L* (aril); X2: a* (aril), X3: b* (aril), X4: L* (fruit); X5: a* (fruit); X6: b* (fruit); X7: total soluble solids; X8: pH; X9: total acidity; X10: antioxidant activity (DDPH); X11: antioxidant activity (GAE); X12: skin weight; X13: aril weight; X14: fruit weight; X15: edible weight; X16: aril length; X17: aril width. GV: Genetic variance; EV: Environmental variance; PhV: Phenotypic variance; *H*: Broad sense heritability; PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; RS: Response to selection; NGM: Next generation mean.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17
GV	70.15	12.80	4.71	167.08	141.40	61.44	5.03	0.24	3.52	47.39	9,558.87	529.68	538.17	2,167.53	73.89	5.06	2.88
EV	44.11	5.32	3.37	15.46	11.14	7.08	4.30	0.28	0.19	84.81	2,706.35	374.76	624.62	1,304.89	67.42	2.43	0.86
PhV	114.27	18.12	8.08	182.54	152.54	68.52	9.34	0.53	3.71	132.20	12,265.23	904.44	1,162.79	3,472.41	141.31	7.49	3.74
H	0.61	0.71	0.58	0.92	0.93	0.90	0.54	0.46	0.95	0.36	0.78	0.59	0.46	0.62	0.52	0.68	0.77
PCV	36.15	68.96	27.73	27.16	65.90	34.89	19.67	20.70	96.16	19.27	31.18	45.55	38.26	37.81	21.14	28.97	28.41
GCV	28.32	57.96	21.17	25.98	63.45	33.04	14.44	14.04	93.68	11.54	27.52	34.86	26.03	29.87	15.29	23.81	24.92
RS	13.52	6.20	3.41	25.47	23.59	15.29	3.39	0.69	3.76	8.49	177.80	36.28	32.51	75.77	12.80	3.81	3.07
Mean	29.57	6.17	10.25	49.75	18.74	23.73	15.53	3.51	2.00	59.67	355.22	66.03	89.13	155.86	56.23	9.45	6.81
NGM	43.09	12.37	13.66	75.22	42.33	39.02	18.93	4.19	5.77	68.16	533.02	102.31	121.64	231.64	69.03	13.26	9.87

cient = 14.44%). The higher the genetic variation in a trait, the higher the probability for improving this trait through breeding programs in the next generations. The high experimental precision observed in the study of Luby (1991) for most of the characters resulted in high genotypic determination coefficients, an equivalent of broad-sense heritability. The VCg estimates obtained were high, indicating the existence of a large genetic variability among genotypes, useful for the improvement of these cultivars to obtain superior genotypes with more attractive fruit characteristics. Remarkable phenotypic and genotypic variations exist in local pomegranate genotypes in Tunisia (Mars and Marrakchi, 1991) estimates of R^2 allow comparison of indirect with direct selection, computation of correlated response in a second trait if selection pressure is applied to the first trait, and establishment of selection strategy (Watkins and Spangelo, 1970). Fadavi *et al.* (2006) studied the relationships among fruit quantitative and qualitative characteristics of Iranian pomegranate genotypes and reported that the anthocyanin content of arils negatively correlated with fruit size. They also postulated that fruit juice, aril and seed characteristics were the main factors for separation of studied pomegranate genotypes.

Nemati *et al.* (2012) investigated the relationships between qualitative and quantitative fruit traits of different pomegranate genotypes and determined (using simple correlation analysis) that multivariate analysis could be a useful method for discrimination of pomegranate genotypes. The R^2 for different parameters of pomegranate fruit were reported by Zamani *et al.* (2007). The authors observed that fruit characteristics such as peel thickness positively correlated with the diameter of calyx and fruit weight with fresh and dry aril weights.

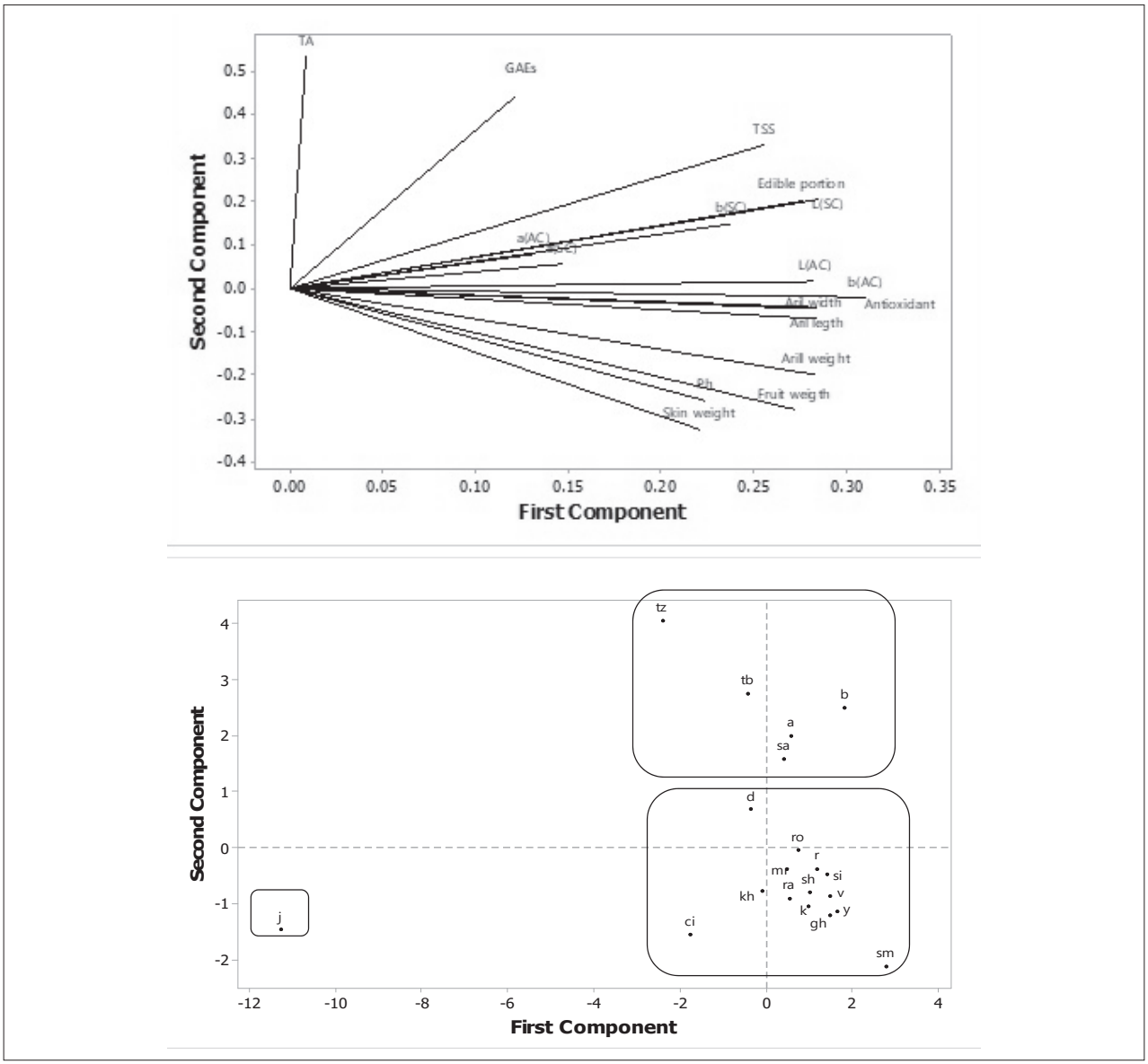

Figure 2. Loading plot (upper) and Score plot (lower) of the first two components based on principal component analysis (PCA) of Iranian pomegranate cultivars: k: 'Rabab Poost Ghermez Kazeroon'; si: 'Shirin Jangal Sisangan'; r: 'Rabab Poost Ghermez Neyriz'; y: 'Malas Yazdi'; gh: 'Khajei Ghasrodasht Fars'; mr: 'Makhmal Malas Shahreza'; ro: 'Jangali Poost Ghermez Roodbar'; ci: 'Anar Siah'; d: 'Poost Sefid Dezfoul'; kh: 'Bihaste Sangan Khash'; ra: 'Bihaste Ravar'; v: 'Malas Pishva Varamin'; sm: 'Shahsavar Seydan Marvdasht'; sh: 'Shirin Semnan'; b: 'Sefid Biardal Borujen'; tb: 'Torosh Goli Naz Behshahr'; sa: 'Malas No. 1 Saravan'; a: 'Poost Nazok Torosh Abarkuh'; tz: 'Torosh Nar Riz Zirab'; j: 'Bihaste Khafr Jahrom' (AC: aril color, SC; skin color).

The highest broad sense heritability was observed in total acidity (H = 0.95) along with a* fruit color (H = 0.93) and L* fruit color (H = 0.92). Overall, the broad sense heritability of all measured traits were generally high suggesting the possibility of genetic improvement through conventional breeding. The lowest broad sense heritability was observed in antioxidant activity (H = 0.36), which is moderate in respect of a direct goal for breeding. Previous studies suggested that TSS and TA were moderately to highly heritable in peach (de Souza *et al*., 1998), strawberry (Shaw, 1990), and kiwifruit (Daoyu *et al*., 2002). However, the magnitude of inheritance of many quantitatively inherited horticultural traits of pomegranate appears unclear. Since very little is known about the heritability of desirable traits in pomegranate, experiments were conducted to study the inheritance of some important features, such as acidity (TA), seed hardness, and aril color. From crosses between 'Daru' and 'Ganesh' progenies, it has been found that high acidity was always dominant over low acidity, pink aril color was dominant over white color, and hard-seededness was dominant over soft-seededness (Jalikop *et al*., 2005). The broad sense heritability estimates determined for fruit traits in this study were generally high and similar to the results in mango (Brettell *et al*., 2004), apple (Durel *et al*., 1998), apricot (Couranjou, 1995), and peach (de Souza *et al*., 1998), suggesting that the manipulation of these traits by breeding would be an excellent proposition.

Multivariate statistics

ANOVA and genetic parameter results suggest significant variation among cultivars. Determining the similarity and/or genetic distance among cultivars and grouping them into genetic groups would be a complementary approach to identify cultivars suitable for breeding for genetic improvement. Principal component analysis and factor analysis were also carried out and a two-dimensional plot for both cultivars and variables was obtained. Principal component and cluster analyses revealed considerable variability that may be due mainly to recombination (resulting from outcrossing) including sexual reproduction combined with vegetative propagation for a long time, and uncontrolled spread of plant material (Fuhrman and Aviram, 2006).

The number of principal components as well as the proportion of variability each accounted for were determined. The first two components accounted for 66.6% of the total variation. The depiction of the first two components *vs*. genotypes (Score plot) and variables (Loading plot) are presented in Figure 2. The first two components accounted for 65% of the total variation, while the first two factors from factor analysis accounted for 88% of the total variation, using Varimax rotation and the maximum likelihood method in Minitab v.16 (Figures 4A, B and 5A, B). In selecting cultivars that can be used in crossing programs to obtain heterosis and achieve genetic gain on a trait it would be necessary to use cultivars from different groups than within groups due to a higher genetic distance between cultivars from different groups. Previously, principal component analysis (PCA) was used to evaluate germplasm of different *Prunus* species, including peach (Nikolić *et al*., 2010), apricot (Ruiz and Egea, 2008), mahaleb (Moghadam and Khalighi, 2007), cherry plum (Horvath *et al*., 2008), and cherry (Hillig and Iezzoni, 1988). Mars and Marrakchi (1999) used fruit size and color and juice characteristics to discriminate among 30 Tunisian varieties using PCA. PCA and cluster analysis were also used to differentiate Tunisian from Chinese pomegranate

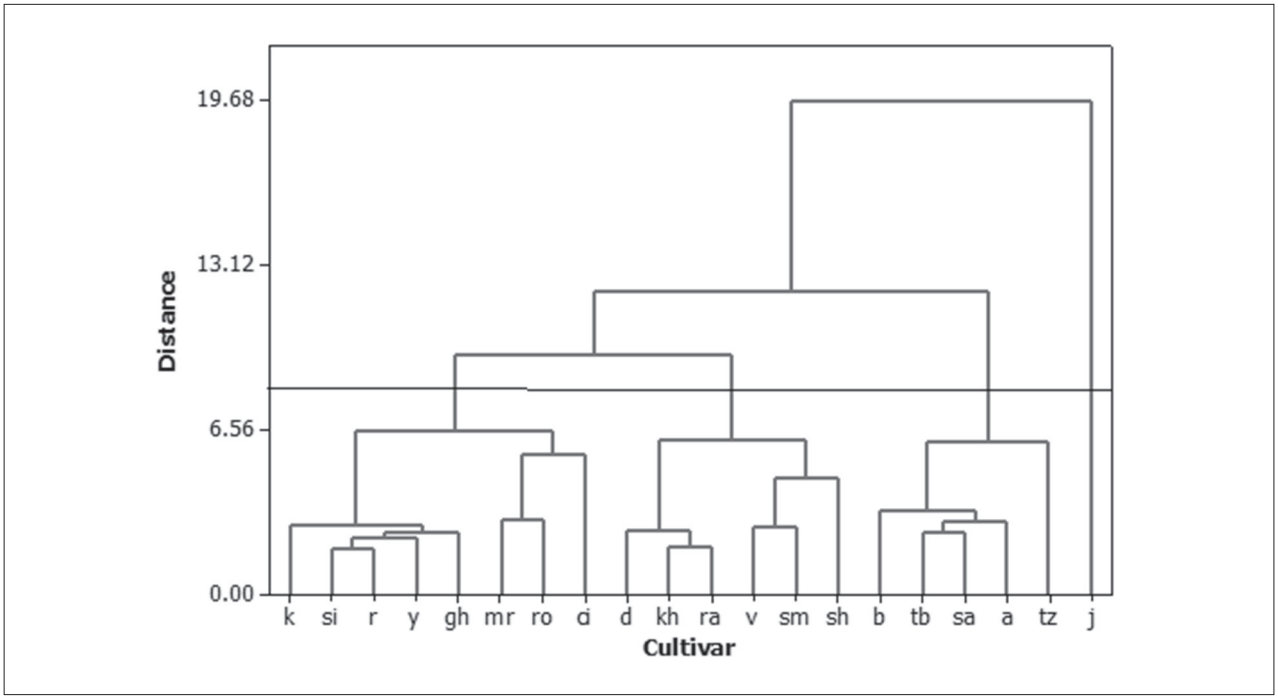

Figure 3. Phenogram depicting relationships of the Iranian pomegranate cultivars based on all measured traits. The phenogram is dendrogram of dissimilitude with standardized Euclidean Distances representing the closest accessions in homogeneous groups, where: k: 'Rabab Poost Ghermez Kazeroon'; si: 'Shirin Jangal Sisangan'; r: 'Rabab Poost Ghermez Neyriz'; y: 'Malas Yazdi'; gh: 'Khajei Ghasrodasht Fars'; mr: 'Makhmal Malas Shahreza'; ro: 'Jangali Poost Ghermez Roodbar'; ci: 'Anar Siah'; d: 'Poost Sefid Dezfoul'; kh: 'Bihaste Sangan Khash'; ra: 'Bihaste Ravar'; v: 'Malas Pishva Varamin'; sm: 'Shahsavar Seydan Marvdasht'; sh: 'Shirin Semnan'; b: 'Sefid Biardal Borujen'; tb: 'Torosh Goli Naz Behshahr'; sa: 'Malas No. 1 Saravan'; a: 'Poost Nazok Torosh Abarkuh'; tz: 'Torosh Nar Riz Zirab'; j: 'Bihaste Khafr Jahrom'.

cultivars, using storage protein and amino acid contents (Elfalleh *et al*., 2012). Stone *et al.* (1993) studied 18 genotypes representing soft, semi-soft and hard-seeded pomegranate cultivars for 11 fruit attributes. Significant variations were observed within soft-seeded types for 100-aril weight, within semi-soft varieties for 100-aril weight, seed mellowness and aril weight 100 g^{-1} fruit, and within hard-seeded entries for rind thickness and aril weight 100 g^{-1} fruit. Factor analysis showed that the characteristics of the fruit provided the main factors that determined 34% total variance, suggesting that these traits must be taken into consideration when differentiating among pomegranate genotypes. According to Couranjou (1995), fruit characteristics in pomegranate have the highest factor loading for the first component in principal component analysis.

Furthermore, the means for genotypes separately in each year and also in combination of both years along with cluster means for each measured variable are presented in supplementary materials (Supplementary Tables 1–3). Cluster 1 contained only 'Bihaste Khafr Jahrom', had small fruit weight, and low antioxidant and GAE. 'Torosh Nar Riz Zirab', 'Poost Nazok Torosh Abarkuh', 'Malas No. 1 Saravan', 'Torosh Goli Naz Behshahr', and 'Sefid Biardal Borujen' were grouped in cluster 2. The cultivars in this group had similar L* fruit color, medium fruit weight, low pH and high acidity, Tss and GAE. The third cluster grouped together 'Shirin Semnan', 'Shahsavar Seydan Marvdasht', 'Bihaste Ravar', 'Malas Pishva Varamin', 'Bihaste Sangan Khash', and 'Poost Sefid Dezfoul', that all had similar pH, edible weight, b* and L* fruit color. Cluster 4 included 8 cultivars of which 'Rabab Poost Ghermez Kazeroon', 'Shirin Jangal Sisangan', 'Rabab Poost Ghermez Neyriz', 'Malas Yazdi', and 'Khajei Ghasrodasht Fars' showed very similar fruit characters.

The cluster analysis revealed considerable variability that may be due to recombination (resulting from out-crossing) combined with vegetative propagation, for a long time and uncontrolled spread of plant material (Fuhrman and Aviram, 2006). Martínez *et al.* (2012b) used cluster analysis to group local pomegranate germplasm in Spain and found considerable phenotypic and genetic diversity. Sarkhosh *et al.* (2009) also suggested that more morphological and perhaps phenological traits of leaf, flower and fruit might be needed to get more reliable results in pomegranate genotypes. Mutations and other genetic changes in characteristics, such as fruit color and shape, tree size, shape and branching habit (which are easily recognizable phenotypically) may not be detectable by the application of some molecular markers such as RAPD (Gupta and Rustgi, 2004; Kumar, 1999).

Conclusion

Both simple descriptive statistics and ANOVA showed that there were high levels of genotypic (14.44–96.16%) and phenotypic (19.67–93.68%) variations available among the evaluated pomegranate cultivars for many of morphological and biochemical fruit attributes, indicating the value of Iranian genetic resources. On the other hand, moderate to high and even very high broad sense heritability (0.46–0.95) calculated for fruit quality attributes is suggesting the possibility of genetic improvement for many pomegranate fruit quality traits through conventional breeding. In this regard

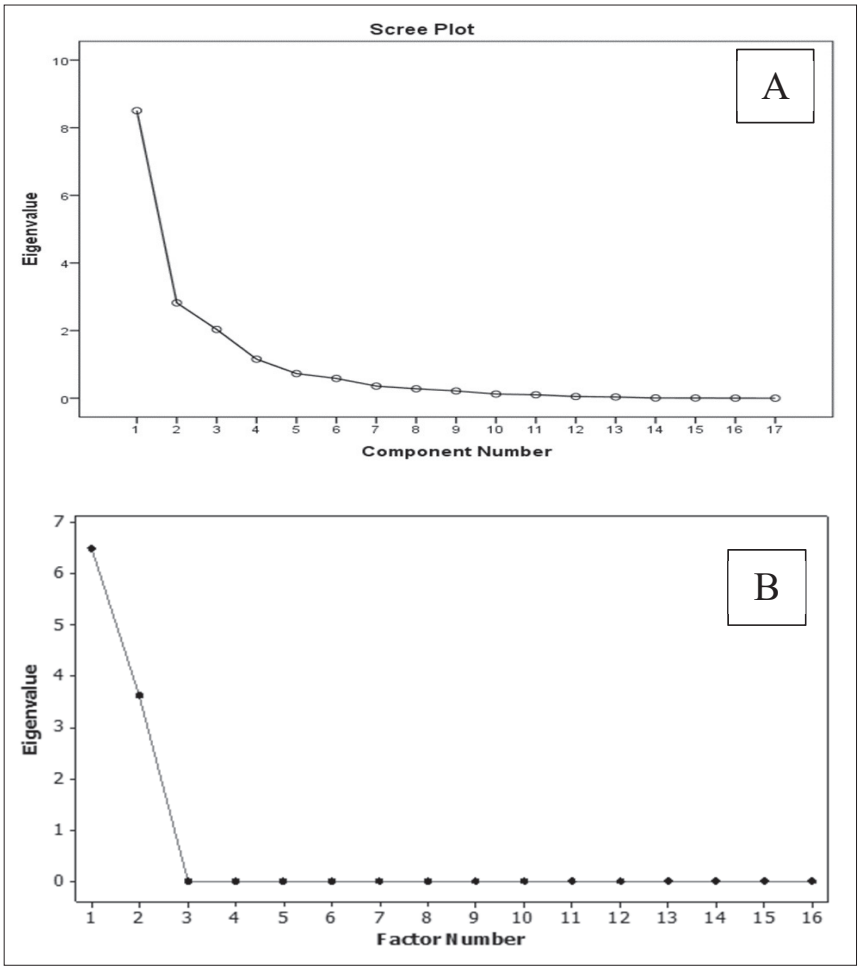

Figure 4. Screen plots of eigenvalues versus components extracted from (A) principal component analysis, and (B) factor analysis.

fruit and aril color, fruit size and some of fruit chemicals such as TA and GAE have the highest potentials for improvement. Cultivars from different clusters could be used in crossing programs to obtain higher variations of fruit quality attributes in the next generations. For this purpose, crossing each of the two commercial cultivars in cluster 1 ('Malas Yazdi' and 'Rabab Poost Ghermez Neyriz') with promising locally important cultivars from the other clusters (such as soft-seeded cultivars including 'Bihaste Sangan Khash' and 'Bihaste Ravar' in cluster 2, or 'Poost Nazok Torosh Abarkuh' in cluster 3, could yield diverse seedling populations to be screened for superior cultivars with high-quality fruit attributes. The morpho-chemical characterization of pomegranate cultivars, as established in this study, would enable future research by using molecular marker analysis to illustrate the high diversity among cultivars and so far, to search for useful trait-marker associations to be used in marker-assisted selection. Further investigation on the studied cultivars is recommended, especially those promising in terms of fruit quality and chemical properties, for productivity and drought resistance.

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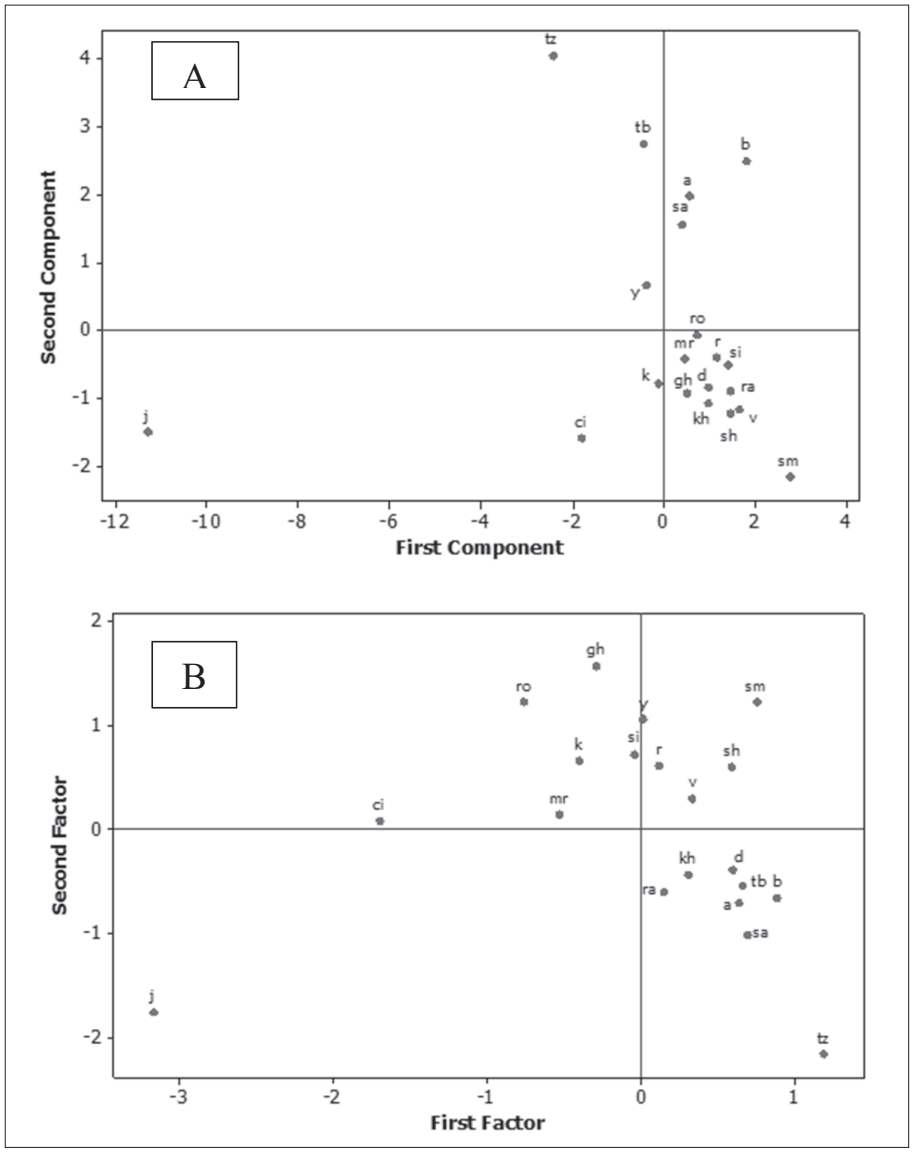

Figure 5. Score plots of the first two components based on (A) principal component analysis, and (B) factor analysis.

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Supplementary Tables

Supplementary Table 1. Mean of measured traits for 20 pomegranate in first year.

Cultivar*	Aril color			Skin color			TSS	Ph	TA	Antioxidant	GAEs	Skin weight	Aril weight	Fruit weight	Edible portion	Aril length	Aril width
	L(AC)	a(AC)	b(AC)	L(SC)	a(SC)	b(SC)											
k	27.01	7.02	7.61	43.78	37.35	16.1	15.43	3.37	1.38	71.96	2.92	125.67	58	183.67	31.16	10	7.67
d	30.55	4.92	9.32	56.3	3.74	24.21	17.12	3.73	1.96	51.54	3.74	55	79	134	59.02	9.83	6.17
b	52.5	6.65	13.14	56.99	13.02	23.79	18.1	2.89	3.84	66.85	4.31	35.67	65	100.67	64.62	10.67	7.67
mr	18.91	14.84	5.41	41.16	31.28	11.54	16.3	3.85	0.63	29.92	1.74	68	99	167	59.51	10.5	7.67
si	43.77	15.17	11.65	45.95	25.8	17.4	16.77	3.22	2.26	28.75	2.12	74.33	105.67	180	58.97	11.83	8
tb	34.73	7.15	8.15	38.37	33.78	12.45	18.07	2.79	6.25	26.1	5.16	52	71.67	123.67	57.74	8.47	6.33
v	47.21	1.48	10.68	49.2	22.28	23.28	15.2	3.48	0.88	33.3	1.49	84.33	123.33	207.67	61.07	12.33	8.83
tz	13.6	1.58	3.81	55.14	3.14	24.08	15.97	2.76	7	16.77	4.05	25.67	23.67	49.33	47.83	8.5	5.67
ro	19.35	9.72	10.37	39.9	26.19	12.24	16.97	2.98	2.08	23.95	3.97	80.33	114.67	195	58.78	10.83	7.83
kh	11.7	2.13	4.85	46.44	5.88	20.8	14.75	3.54	0.69	43.24	1.33	31	39.67	70.67	55.87	12.83	8
y	43.29	0.59	7.7	44.52	35.8	16.99	15.75	3.62	0.9	75.68	2.61	118.33	170	288.33	59.35	11	7.57
r	48.03	6.55	11.25	47.85	24.92	16.57	15.03	3.37	1.78	46.29	2.93	98.33	116.67	215	54.51	10.37	7.4
sa	34.29	6.21	8.94	44.64	31.42	15.9	17.72	2.92	2.36	16.65	3.34	48.33	103	151.33	68.16	8.67	6.33
gh	36.08	6.2	8.17	43.23	28	17.23	16.42	3.29	1.98	26.84	2.91	146.33	104.67	251	41.59	10.5	6.5
sm	49.46	9.97	13.82	61.58	7.45	25.03	14.43	3.87	0.44	49.25	7.2	94.67	170	264.67	64.18	12.5	9
ra	32.82	4.09	5.28	53.82	3.04	23.39	15.33	3.79	0.46	54.64	1.71	38	61	99	61.48	11.83	11
ci	11.57	4.3	3.66	23.13	6.19	0.69	14.22	4	0.59	49.95	3.46	96.67	61.67	158.33	38.46	10.83	7.67
a	29.81	18.76	7.93	55.8	31.38	17.71	18.68	2.85	2.17	43.45	4.18	50	68.33	118.33	57.61	9.87	7.17
sh	33.98	1.54	10.96	55.15	5	24.53	14.98	3.87	0.55	72.85	2.57	93.33	139.33	232.67	59.97	0	0
j	0	0	0	0	0	0	14.43	3.91	0.19	20.6	1.50	19.11	16.11	35.22	45.74	0	0

* Pomegranate cultivars (k: 'Rabab Poost Ghermez Kazeroon'; si: 'Shirin Jangal Sisangan'; r: 'Rabab Poost Ghermez Neyriz'; y: 'Malas Yazdi'; gh: 'Khajei Ghasrodasht Fars'; mr: 'Makhmal Malas Shahreza'; ro: 'Jangali Poost Ghermez Roodbar'; ci: 'Anar Siah'; d: 'Poost Sefid Dezfoul'; kh: 'Bihaste Sangan Khash'; ra: 'Bihaste Ravar'; v: 'Malas Pishva Varamin'; sm: 'Shahsavar Seydan Marvdasht'; sh: 'Shirin Semnan'; b: 'Sefid Biardal Borujen'; tb: 'Torosh Goli Naz Behshahr'; sa: 'Malas No. 1 Saravan'; a: 'Poost Nazok Torosh Abarkuh'; tz: 'Torosh Nar Riz Zirab'; j: 'Bihaste Khafr Jahrom').

Supplementary Table 2. Mean of measured traits for 20 pomegranate in second year.

Cultivar*	Aril color			Skin color			TSS	Ph	TA	Antioxidant	GAEs	Skin weight	Aril weight	Fruit weight	Edible portion	Aril length	Aril width
	L(AC)	a(AC)	b(AC)	L(SC)	a(SC)	b(SC)											
k	43.95	3.41	15.05	48.28	34.59	31.08	15.67	3.84	1.55	81.6	4.50	86.33	102.67	189	54.16	10.67	7.5
d	10.51	3.29	10.92	56.85	2.46	33.6	16.5	4.17	1.06	69.51	3.93	37	48.87	87.5	57.87	9.6	7.57
b	32.54	6.21	13.6	63.6	17.7	38.77	18.67	3.28	3.45	86.69	5.98	46	118	164	71.99	9.27	7.13
mr	26.1	18.26	12.53	44.16	40.9	20.72	18.67	4.21	0.64	83.6	4.59	58.66	86.97	145.62	60.32	9.67	7.23
si	31.25	7.67	11.71	56.46	30.23	30.63	15.33	4.05	1.35	83.22	4.64	93	116.33	209.33	55.11	7.9	7.67
tb	24.09	7.35	9.08	61.86	30.77	33.48	17	3.15	5.48	83.94	4.31	45.31	82.53	127.85	64.67	7.43	6.23
v	27.64	1.69	14.97	61.33	16.11	35.76	15.67	3.89	1.41	85.78	3.04	47	99	146	67.81	11.27	7.43
tz	29.31	3.81	10.97	65.35	3.95	36.01	17.17	3.14	8.48	72.07	5.47	24	37.67	61.67	60.78	7.2	5.2
ro	15.89	12.63	10.77	45.28	33.8	21.95	18.33	3.49	2.18	76.91	4.35	87.33	141.67	229	61.88	9.2	7.37
kh	24.57	4.31	15.29	59.11	13.51	34.31	14	4.38	0.5	80.43	4.54	80	122.67	202.67	60.4	10.67	8.03
y	21.43	15.35	10.32	61.41	23.14	34.62	16	3.7	1.47	82.77	3.85	69.14	69.51	138.65	49.82	10.47	7.47
r	31.31	5.9	12	58.48	15.4	30.32	14.67	3.53	2.57	84.66	4.95	72.13	94.12	166.25	56.62	10.77	7.2
sa	32.01	1.74	15.1	69.39	23.74	37.8	15.33	3.34	3.3	87.44	6.01	52	83.33	135.33	61.52	9.13	6.2
gh	35.81	2.69	15.13	57.25	26.92	40.99	14.67	3.65	2.51	83.65	3.93	90.67	132.67	223.33	59.53	10.07	7.9
sm	36.75	4.51	16.1	66.24	5.72	39.4	13	4.16	0.61	77.64	2.44	73.96	99.67	200.33	68.76	11.23	7.63
ra	31.1	2.28	11.96	61.08	8.77	37.03	15	4.07	0.55	81.79	2.51	78	94	172	54.3	12.13	7.67
ci	29.22	4.11	13.22	28.89	14.68	1.22	13.5	4.44	0.7	74.7	5.02	54	61.67	115.67	53.32	10.03	6.8
a	38.63	10.45	12.41	57.43	28.12	26.48	15.5	3.5	3.22	81.15	6.34	50.07	84	134.07	62.66	8.8	6.5
sh	42.15	2.38	16.18	64.47	3.5	40.95	15	4.16	0.7	80.54	4.76	61.38	99.32	160.7	62.04	11.07	7.07
j	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Pomegranate cultivars (k: 'Rabab Poost Ghermez Kazeroon'; s: 'Shirin Jangal Sisangan'; r: 'Rabab Poost Ghermez Neyriz'; y: 'Malas Yazdi'; gh: 'Khajei Ghasrodasht Fars'; mr: 'Makhmal Malas Shahreza'; ro: 'Jangali Poost Ghermez Roodbar'; ci: 'Anar Siah'; d: 'Poost Sefid Dezfoul'; kh: 'Bihaste Sangan Khash'; ra: 'Bihaste Ravar'; v: 'Malas Pishva Varamin'; sm: 'Shahsavar Seydan Marvdasht'; sh: 'Shirin Semnan'; b: 'Sefid Biardal Borujen'; tb: 'Torosh Goli Naz Behshahr'; sa: 'Malas No. 1 Saravan'; a: 'Poost Nazok Torosh Abarkuh'; tz: 'Torosh Nar Riz Zirab'; j: 'Bihaste Khafr Jahrom').

Supplementary Table 3. Mean of measured traits for 20 pomegranate and defined clusters in both years.

Cultivar*	Aril color			Skin color			TSS	pH	TA	Antioxidant	GAEs	Skin weight	Aril weight	Fruit weight	Edible portion	Aril length	Aril width
	L(AC)	a(AC)	b(AC)	L(SC)	a(SC)	b(SC)											
k	35.48	5.22	11.33	46.03	35.97	23.59	15.55	3.61	1.46	76.78	3.71	106	80.33	186.33	42.66	10.33	7.58
d	20.53	4.11	10.12	56.58	3.1	28.91	16.81	3.95	1.51	60.53	3.83	46	63.93	110.75	58.44	9.72	6.87
b	42.52	6.43	13.37	60.29	15.36	31.28	18.38	3.09	3.65	76.77	5.14	40.83	91.5	132.33	68.3	9.97	7.4
mr	22.51	16.55	8.97	42.66	36.09	16.13	17.48	4.03	0.63	56.76	3.17	63.33	92.98	156.31	59.92	10.08	7.45
si	37.51	11.42	11.68	51.2	28.02	24.02	16.05	3.63	1.8	55.99	3.38	83.67	111	194.67	57.04	9.87	7.83
tb	29.41	7.25	8.61	50.12	32.28	22.97	17.53	2.97	5.86	55.02	4.73	48.66	77.1	125.76	61.21	7.95	6.28
v	37.43	1.59	12.83	55.27	19.19	29.52	15.43	3.69	1.15	59.54	2.27	65.67	111.17	176.83	64.44	11.8	8.13
tz	21.45	2.7	7.39	60.25	3.54	30.04	16.57	2.95	7.74	44.42	4.76	24.83	30.67	55.5	54.3	7.85	5.43
ro	17.62	11.17	10.57	42.59	30	17.09	17.65	3.24	2.13	50.43	4.16	83.83	128.17	212	60.33	10.02	7.6
kh	18.14	3.22	10.07	52.78	9.69	27.56	14.38	3.96	0.6	61.84	2.93	55.5	81.17	136.67	58.14	11.75	8.02
y	32.36	7.97	9.01	52.96	29.47	25.8	15.88	3.66	1.19	79.22	3.23	93.74	119.76	213.49	54.59	10.73	7.52
r	39.67	6.23	11.62	53.16	20.16	23.45	14.85	3.45	2.17	65.47	3.94	85.23	105.39	190.63	55.57	10.57	7.3
sa	33.15	3.97	12.02	57.02	27.58	26.85	16.53	3.13	2.83	52.05	4.67	50.17	93.17	143.33	64.84	8.9	6.27
gh	35.95	4.45	11.65	50.24	27.46	29.11	15.54	3.47	2.25	55.25	3.42	118.5	118.67	237.17	50.56	10.28	7.2
sm	43.1	7.24	14.96	63.91	6.59	32.22	13.72	4.02	0.52	63.44	1.58	84.32	134.83	232.5	66.47	11.87	8.32
ra	31.96	3.19	8.62	57.45	5.91	30.21	15.17	3.93	0.5	68.22	2.11	58	77.5	135.5	57.89	11.98	9.33
ci	20.4	4.2	8.44	26.01	10.44	0.96	13.86	4.22	0.65	62.33	4.24	75.33	61.67	137	45.89	10.43	7.23
a	34.22	14.6	10.17	56.62	29.75	22.09	17.09	3.17	2.7	62.3	5.26	50.03	76.17	126.2	60.13	9.33	6.83
sh	38.07	1.96	13.57	59.81	4.25	32.74	14.99	4.02	0.63	76.7	3.66	77.36	119.33	196.68	61	5.53	3.53
j	0	0	0	0	0	0	7.22	1.96	0.09	10.3	0.75	9.56	8.06	17.61	22.87	0	0
Cluster																	
1	31.59	9	10.69	48.41	29.6	22.74	16.14	3.58	1.66	62.84	3.57	90.61	108.04	198.66	54.38	10.27	7.5
2	29.95	3.64	11.23	53.12	8.45	26.02	14.91	3.97	0.79	64.66	2.95	66.03	92.8	160.85	58.9	10.44	7.35
3	32.15	6.99	10.31	56.86	21.7	26.65	17.22	3.06	4.56	58.11	4.91	42.9	73.72	116.62	61.76	8.8	6.44
4	0	0	0	0	0	0	7.22	1.96	0.09	10.3	0.75	9.56	8.06	17.61	22.87	0	0

* Pomegranate cultivars (k: 'Rabab Poost Ghermez Kazeroon'; si: 'Shirin Jangal Sisangan'; r: 'Rabab Poost Ghermez Neyriz'; y: 'Malas Yazdi'; gh: 'Khajei Ghasrodasht Fars'; mr: 'Makhmal Malas Shahreza'; ro: 'Jangali Poost Ghermez Roodbar'; ci: 'Anar Siah'; d: 'Poost Sefid Dezfoul'; kh: 'Bihaste Sangan Khash'; ra: 'Bihaste Ravar'; v: 'Malas Pishva Varamin'; sm: 'Shahsavar Seydan Marvdasht'; sh: 'Shirin Semnan'; b: 'Sefid Biardal Borujen'; tb: 'Torosh Goli Naz Behshahr'; sa: 'Malas No. 1 Saravan'; a: 'Poost Nazok Torosh Abarkuh'; tz: 'Torosh Nar Riz Zirab'; j: 'Bihaste Khafr Jahrom').