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Characterization of pomegranate (*Punica granatum* L.) hybrids and their potential use in further breeding

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Abstract: Pomegranate is an important fruit species, with increasing consumption and production in recent years throughout the world. In Turkey, Hicaznar, which was originally developed through selective breeding, is the most highly produced and exported cultivar. To isolate lines with improved productivity and quality, Hicaznar was selfed, open-pollinated, or crossed with the sweet pomegranate cultivars Ernar and Fellahyemez. Arising from the crosses, 3118 lines were developed, and 67 were considered for important traits. These 67 pomegranate genotypes, from different crosses, were compared with their parents, which are commercially important pomegranate genotypes regarding tree and fruit traits. Twenty-one morphological characteristics were evaluated to cover the present genetic variations within the investigated materials using principal component analysis (PCA). Simple correlation analyses showed significant positive and negative correlations for certain important characteristics. Factorial and PC analyses revealed that large variation exists regarding the evaluated tree and fruit characteristics.

Key words: Correlation analysis, genotype, pomegranate, fruit breeding

1. Introduction

Pomegranate (*Punica granatum* L.), a plant indigenous to Turkey and surrounding countries, is a valuable fruit species that has been used as human food and for human health since ancient times. Pomegranate has recently attracted more research attention, leading to important developments in cultivation techniques, food applications, and storage and transport technologies. These innovations have led to steadily increased production, consumption, and trade value of the fruit over the last decades both in Turkey and throughout the world (Gündoğdu et al., 2010; Yazıcı, 2014).

Parallel to the incremental increases in production and consumption of pomegranate, there is increasing interest in new pomegranate cultivars. Most new pomegranate cultivars were developed through selective breeding from local pomegranate cultivars based on different traits (Mısra et al., 1983; Liang and Cheng, 1991; Bist et al., 1994; Mars and Marrakchi, 1999; Barone et al., 2001; Kafyrova, 2003; Martinez et al., 2012). To this day, hybridization studies on pomegranate (Manivannan and Rengasamy, 1999; Mars and Marrakchi, 1999; Nageswari et al., 1999; Xian and Xian, 1999; Karale and Desai, 2000; Bartual et al., 2012) remain rather limited. Therefore, very few cultivars are of hybrid origin.

In separate studies, various characteristics of different pomegranate genotypes have been investigated (Levin, 1990; Manivannan and Rengasamy, 1999; Nageswari et al., 1999; Xian and Xian, 1999; Jalikop, 2009). A few pomegranate selection studies carried out in the Mediterranean (Onur and Kaşka, 1985), Aegean, and Southeast Anatolia (Boz, 1988) regions of Turkey identified individual plants that possessed the majority of the requested characteristics and determined standard cultivars for the regions based on adaptation trials (Özbek, 1977; Dokuzoğuz and Mendilcioğlu, 1978; Onur and Tibet, 1993; Özgüven, 1998; Gündoğdu and Yılmaz, 2012). These selection studies revealed that seedless fruits occurred only in sweet pomegranate cultivars, whereas desirable skin colors were obtained in low-sugar ones. In pomegranate cultivars with light fruit and aril color, the fruit size, aril mass, aril yield, fruit juice yield, and water-soluble crude matter were insufficient. In sourish pomegranates, lines with the desired skin and seed colors had hard seeds, smaller fruit size, and lower aril yield and fruit juice yield compared with sweet pomegranates. Furthermore, the early-sourish pomegranate genotypes were not investigated during the selection studies (Onur et al., 1999).

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Even though 47 released pomegranate cultivars were developed through various selection and adaptation studies in Turkey, the only cultivar with broad production and export capacity is Hicaznar. This cultivar has a red skin color, dark red arils, a sour taste, and a high yield and is especially suitable for transport and storage. Its sourish taste and hard (firm) seed seem to be the only disadvantages of this particular cultivar. Therefore, hybrid breeding studies were initiated among several pomegranate cultivars in order to develop sweet and juicy genotypes with soft, large, red arils, as well as dark red skin color (Onur et al., 1999).

In the first hybridization work in Turkey, Hicaznar was used as the female parent and the cultivars Ernar (early-sweet) and Fellahyemez (late-sweet) were used as pollen sources (Onur et al., 1999). In the work reported here, 67 pomegranate genotypes from the crosses Hicaznar × Hicaznar (H×H), Hicazanar × Fellahyemez (H×F), Hicaznar × Ernar (H×E), and open-pollinated Hicaznar (OPH) were compared with their parents regarding tree and fruit characteristics. Their similarities and differences are presented and the respective correlations are calculated. Further, desirable fruit and tree characteristics were tracked in the progeny. The potential usefulness of the obtained results in further pomegranate breeding is discussed.

2. Materials and methods

The phenotypic analysis was carried out between the years of 2006 and 2009 at the West Mediterranean Agricultural Institute in Antalya. The climate of the area is typically subtemperate. The annual rainfall ranges between 800 and 1300 mm. The orchard soil was sandy in texture with pH 8.10, 397 µhos/cm electrical conductivity (EC), and 2.85% organic carbon content. From the H×H, H×F, and H×E crosses and OPH, which were done in 1998, 67 individual plants were preselected in 2006 and were used as research material. Twenty-six were from OPH, 18 from H×F, 13 from H×E, and 10 from H×H.

The characteristics of the parental materials used for crossing are as follows: Ernar - thin, pink skin, hard seed, small fruits, small arils, early cultivar, less suckering tendency, and high yielding capacity; Hicaznar - dark red skin and aril, intermediately hard seed, sourish, late season cultivar, high suckering tendency, and high yield; Fellahyemez - light pink skin and arils, soft seed, large fruits and arils, late cultivar, high suckering tendency, and medium yield.

Six plants from each of the mentioned 67 genotypes, totaling 402 plants, were evaluated at their bearing stage (2006–2009) for the following traits: trunk cross-sectional area, tree height, tree canopy width, plant vigor, suckering density, branching, thorniness, fruit yield, fruit weight, fruit diameter, fruit length, calyx length, peel thickness, 100-aril weight, aril ratio, total soluble solids content, total acidity, taste, skin color, aril color, and seed hardness. These traits were compared with those of their parents.

The tree characteristics, such as suckering tendency, trunk cross-sectional area, developmental stage, thorniness, and habit were determined according to the method developed by Tibet and Onur (1999). The diameter measured 15 cm above the ground was converted to trunk cross-sectional area (cm^2). Tree height and canopy width were measured using a tape line. Vigor was rated as very weak (1), weak (2), medium (3), vigorous (4), or very vigorous (5). Suckering tendency was rated as absent (0), extremely low (1), low (2), medium (3), high (4), or very high (5). Branching and thorniness were rated as dense (3), medium (2), or rare (1).

Fruit characteristics were evaluated for twenty fruit samples from each tree harvested in July and August. Fruit weight (g) was determined for each fruit by a 0.01-g precision scale. Fruit diameter at the most bulged point; fruit length (mm), which was the length between the connection of the fruit and fruit stalk and the beginning point of the calyx; and fruit calyx length (mm) were measured by compasses. Skin color was rated using a color scale (Munsell Color Book) as 1: light pink, 2: pink, or 3: red. Aril color was rated using the color scale (Munsell Color Book) as 1: white, 2: light pink, 3: pink, 4: red, or 5: dark red. Rind thickness (mm) was determined by measuring rind pieces at three different points by compasses. 100-Aril weight (g) was determined by weighing 100 arils with 5 replications. Aril ratio (%) was determined by weighing the whole fruit and then separating and weighing the arils. The soluble solids content of the arils was measured by a Carl-Zeiss Abbe refractometer. Titratable acidity was determined by titration of 5 mL of fruit juice with 0.1 N NaOH and expressed as citric acid content (Tibet and Onur, 1999). The pH of the juice was measured with a pH meter. Samples of 10 fruits were used in sensory analyses, which were conducted by 15 panelists. Taste was the mean of given points using a 100-point scale. Seed hardness was determined as soft (1.0–4.5), medium (5.0–6.0), or firm (6.5–10.0) (Tibet and Onur, 1999).

Simple correlations, factor and cluster analyses, and scatter plots (Backhaus et al., 1989) were prepared by using SPSS 20.0 for Windows. Factor analysis was performed by using the Varimax factor rotating method, where each variable was used to calculate relationships between variable and investigated factors. A dendrogram of the genetic similarities between the genotypes was compiled using the Ward method.

3. Results

Sixty-seven individual genotypes from different crosses were compared with their parents regarding tree and fruit characteristics. Important differences were also determined between individuals from the same crosses and between different crosses and parents.

3.1. Tree characteristics

Principal component analysis (PCA) was used to assess the variation between the tree characteristics of the pomegranate genotypes. The first 7 axes accounted for 100% of the variability among 67 genotypes (Table 1). The 1st PC axis accounted for 40.38% of the variation, while the 2nd, 3rd, and 4th axes accounted for 24.78%, 10.66%, and 8.80%, respectively. For each factor, a factor loading of more than 0.55 was considered significant. The first axis was mainly related to trunk cross-sectional area, tree height, plant vigor, branching, and thorniness. The second axis was mainly related to tree canopy width and suckering density. The remaining 5 axes were related to other tree characteristics (Table 1).

The morphological differences between the progeny of different crosses and the parents are given in Table 2. In the H×H and H×E combinations, the trunks' cross-sectional areas were 17.76 cm^2 and 16.45 cm^2, respectively. On the other hand, it was 18.75 cm^2 for Hicaznar. The largest average tree height (3.08 m) resulted from open pollination (OPH). Plant vigor was the same as that of the parents and the crossed progeny. Suckering density was the highest in the parents Hicaznar and Fellahyemez and their progeny from H×H and H×F. Branching was the highest in Fellahyemez and H×H. Thorniness was the greatest in Fellahyemez and in H×F.

Correlation between pairs of the 7 tree characteristics was analyzed (Table 3). Some interesting correlations were found: trunk cross-sectional area was positively correlated with tree height (0.642), suckering density (0.311) and tree canopy width (0.284); tree height was positively correlated with tree canopy width (0.354); tree canopy width was positively correlated with suckering density (0.334); suckering density was positively correlated with

Table 1. Eigen values and proportions of variance described by 7 principal components.

	PC axis						
	1	2	3	4	5	6	7
Eigen values	3.23	1.98	0.85	0.70	0.55	0.41	0.26
Explained proportion of variation (%)	40.38	24.78	10.66	8.80	6.93	5.16	3.31
Cumulative proportion of variation (%)	40.38	65.15	75.81	84.61	91.54	96.70	100.00
Characteristic	Eigen vectors						
Trunk cross-sectional area (cm^2)	0.72	0.46	0.11	0.33	–0.01	0.07	–0.38
Tree height (m)	0.74	0.33	0.29	–0.05	–0.16	0.43	0.22
Tree canopy width (m)	0.25	0.66	0.06	–0.67	0.19	–0.10	–0.09
Plant vigor	–0.55	0.47	–0.42	0.16	0.44	0.29	0.03
Suckering density	0.09	0.71	–0.55	0.07	–0.43	–0.15	0.09
Branching	–0.79	0.47	0.38	0.11	–0.07	–0.05	0.02
Thorniness	–0.79	0.47	0.38	0.11	–0.07	–0.05	0.02

Table 2. Morphological characteristics of pomegranate cultivars and their hybrids.

Tree characteristic	Hicaznar	Fellahyemez	Ernar	OPH	H×F	H×E	H×H
Trunk cross-sectional area (cm^2)	18.75 ± 3.07	14.25 ± 0.65	16.34 ± 0.31	15.27 ± 8.96	12.84 ± 2.43	16.45 ± 1.96	17.76 ± 3.20
Tree height (m)	2.35 ± 0.38	2.13 ± 0.33	2.45 ± 0.37	3.08 ± 0.35	2.16 ± 0.27	2.30 ± 0.18	2.30 ± 0.37
Tree canopy width (m)	2.25 ± 0.37	2.37 ± 0.32	2.65 ± 0.20	2.47 ± 0.26	2.33 ± 0.15	2.52 ± 0.19	2.27 ± 0.27
Tree vigor	3	3	3	3	3	3	3
Suckering tendency	5	5	1	3	4	2	4
Branching	3	2	1	2	2	2	3
Thorniness	2	4	1	2	3	2	2

Table 3. Correlation coefficients between some tree traits among different pomegranate genotypes.

	TCSA	TH	TCW	PV	SD	B	T
TCSA	1.000						
TH	0.642	1.000					
TCW	0.284	0.354	1.000				
PV	–0.168	–0.320	0.094	1.000			
SD	0.311	0.164	0.334	0.293	1.000		
B	–0.286	–0.334	0.053	0.469	0.111	1.000	
T	–0.286	–0.334	0.053	0.469	0.111	1.000	1.000

TCSA: trunk cross-sectional area; TH: tree height; TCW: tree canopy width; PV: plant vigor; SD: suckering density; B: branching; T: thorniness

branching (0.111) and thorniness (0.111); and plant vigor was positively correlated with branching (0.469) and thorniness (0.469).

Using the morphological measurements, the selected pomegranate genotypes could be clustered into 2 main groups and into 5 subgroups (Figure 1; A–E), with 1 outlier from the H×F cross. The hierarchical clustering of the different pomegranate genotypes and the standard cultivars was based on the similarity of their tree characteristics (Figure 1). Different progeny resulting from the same cross did not necessarily group together. These identified groups and their subgroups can be considered distinct pomegranate germplasm pools for future selection and breeding projects (Figure 1).

The parental cultivars Ernar, Fellahyemez, and Hicaznar clustered in groups A, C, and D, respectively. The genotypes arising from the cross between H×E did group in the same cluster with the Ernar parent, Group A, which contained only one genotype from the cross H×F. Progeny from the OPH were mostly clustered in Group B. Most of the genotypes from the cross H×F clustered in Group C. On the other hand, Group D consisted of genotypes from the crosses H×H and the open-pollinated parent, OPH. From all the crossed progenies, only one genotype, from the H×F cross, was clearly distinctive from all other investigated genotypes. Because the genotypes from the OPH may have resulted from pollen from all the parental genotypes because of open pollination, they are distributed across the 5 distinct subgroups. Since the paternal genotypes grouped with most of the progeny derived from their corresponding cross (in groups A, C, and D), it can be concluded that the paternal genotype has a large effect on the phenotypic variation in the arising progeny. However, a different situation exists in Group B. Different genotypes, from the crosses OPH, H×H, H×F, and H×E, were clustered in the same group. This may reflect a dominate influence of the parental cultivar Hicaznar. It is interesting to note that these genotypes from different combinations displayed remarkable variations.

The 67 progenies and their parents were analyzed using a 3-D scatter plot based on the comparison of investigated traits (Figure 2). The first three principal components were plotted on the axes X, Y, and Z. The scatter plot revealed a high level of total variance. Each hybridization and the parents were plotted according to its principal component score (the cumulative proportion of variance) for each of the first 2 axes (Figure 2).

The distribution of each genotype in the plot showed the relative influence of each of the 3 principal components.

The PCA revealed that the cultivars Hicaznar, Fellahyemez, and Ernar were very different regarding the investigated tree properties. While the genotypes from the crosses H×F and H×E showed a distribution between their parents, the combinations H×H and OPH displayed higher variation in the investigated traits.

3.2. Fruit characteristics

PCA was used to assess the variation between the fruit characteristics of the pomegranate genotypes. The first 7 axes accounted for 81.35% of the variability among the 67 genotypes (Table 4). The 1st PC axis accounted for 22.36% of the variation, while the 2nd, 3rd, and 4th axes accounted for 18.58%, 11.15%, and 9.84%, respectively. For each factor, a factor loading of more than 0.55 was considered significant. The first axis was mainly related to fruit weight, fruit diameter, fruit length, and 100-aril weight. The second axis was mainly related to fruit yield, rind thickness, and total acidity. The third axis was mainly related to skin color, aril color, and taste. The remaining 4 axes were related to other fruit characteristics (Table 4).

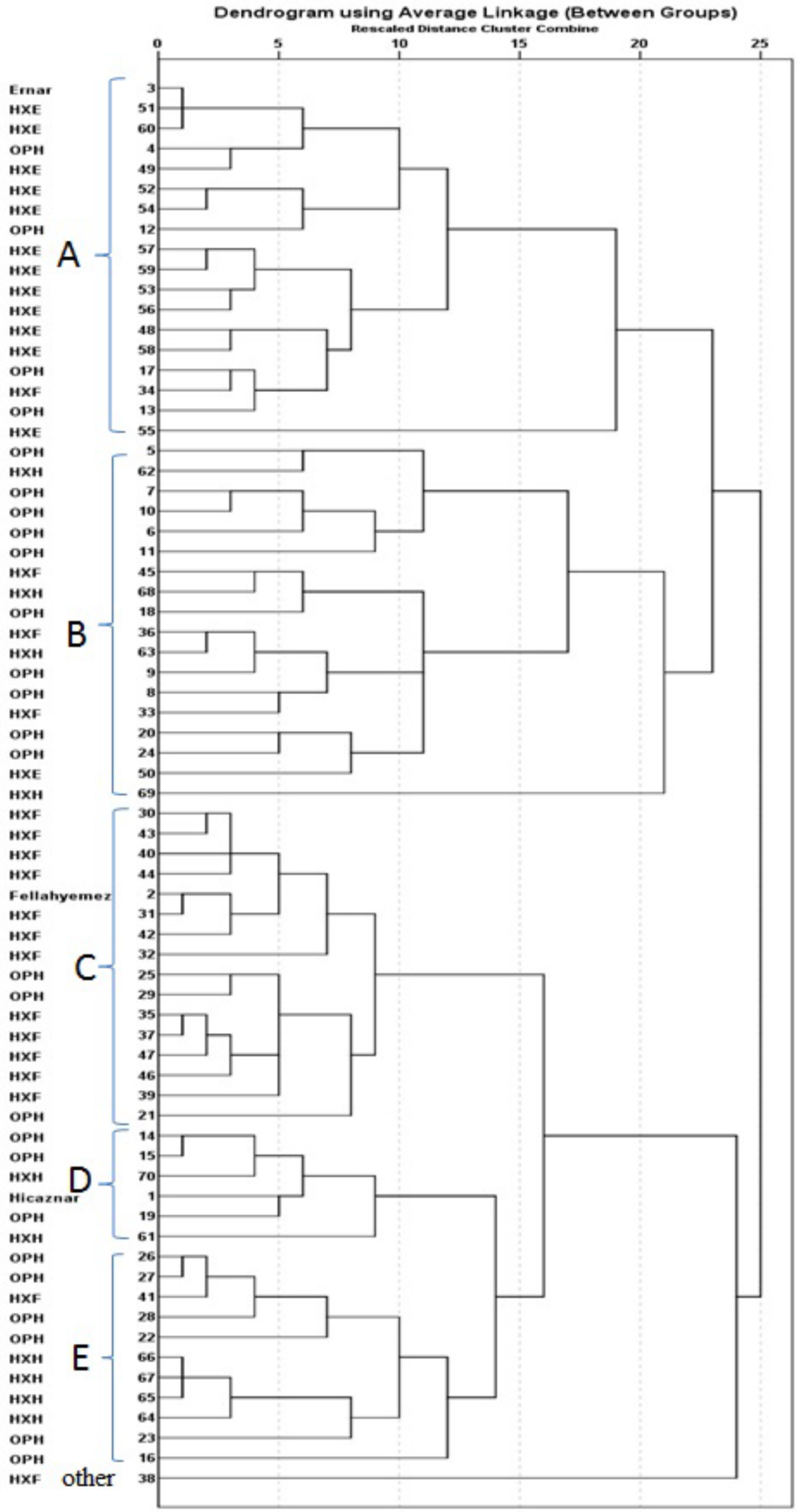

Figure 1. Cluster analysis for 67 genotypes and parental standard cultivars based on morphological data of tree properties.

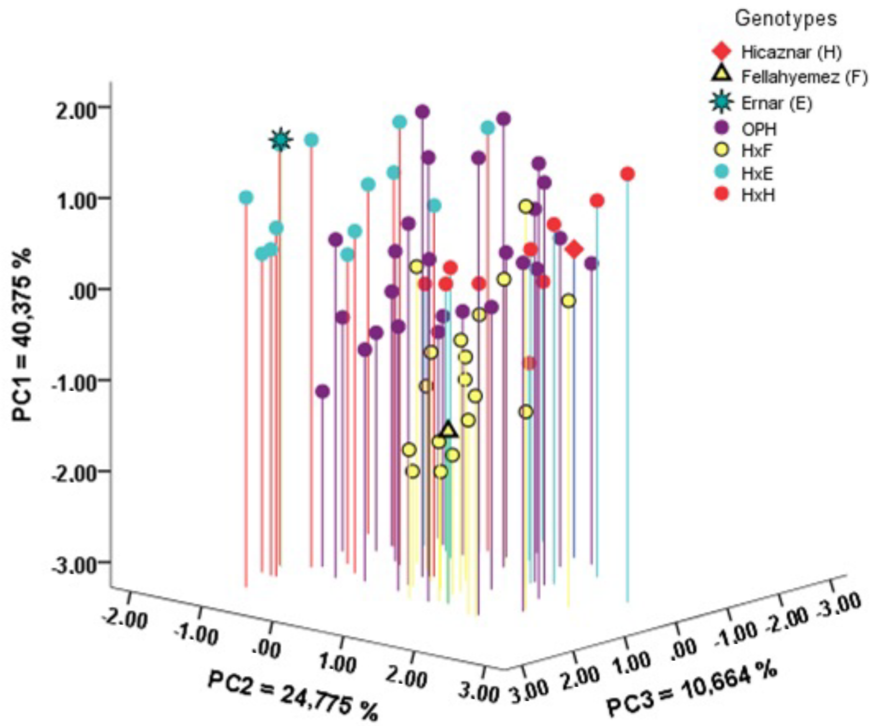

Figure 2. 3D scatter diagrams of the relationships among the genotypes and their parents (based on tree properties).

Table 4. Eigen values and proportions of variance described by 7 principal components.

	PC axis						
	1	2	3	4	5	6	7
Eigen values	3.13	2.60	1.56	1.38	1.07	0.86	0.79
Explained proportion of variation (%)	22.36	18.58	11.15	9.84	7.66	6.14	5.61
Cumulative proportion of variation (%)	22.36	40.95	52.10	61.93	69.59	75.74	81.35
Characteristic	Eigen vectors						
Fruit yield (kg/plant)	0.19	0.70	0.24	0.09	–0.16	–0.17	0.10
Fruit weight (g)	0.77	0.40	–0.13	0.01	0.06	0.19	0.01
Fruit diameter (mm)	0.82	0.36	–0.07	0.10	–0.01	0.08	0.01
Fruit length (mm)	0.84	0.21	–0.11	–0.03	0.06	–0.24	–0.02
Fruit calyx length(mm)	–0.12	0.50	–0.19	0.63	0.13	0.07	–0.28
Rind thickness (mm)	–0.16	0.60	–0.18	–0.50	–0.26	0.24	–0.07
100-Aril weight (g)	0.71	–0.35	0.02	–0.09	0.01	0.16	0.19
Aril ratio (%)	0.35	–0.45	0.47	0.41	0.11	–0.29	0.16
Total soluble solids content (%)	0.20	0.24	0.33	–0.55	0.52	–0.01	0.15
Total acidity (%)	–0.21	0.71	–0.14	0.12	0.17	–0.45	0.01
Skin color	–0.01	0.32	0.60	0.38	–0.16	0.51	0.11
Aril color	–0.40	0.39	0.55	–0.13	–0.26	–0.20	0.29
Seed hardness	–0.43	0.20	–0.01	0.11	0.73	0.25	0.14
Taste	0.15	–0.05	0.63	–0.21	0.11	–0.05	–0.70

Fruit characteristics were measured for the 67 crossed progeny and the parental cultivars (Table 5). The highest fruit yield, fruit weight, fruit diameter, fruit length, and 100 aril weight were in individuals resulting from the crosses H×H or H×F. The peel was thickest in individuals from the H×H and OPH crosses. The cross H×H yielded the highest values for total soluble solids content and taste. Skin color and aril color values scored the highest for the H×H and H×E crosses. The aril ratio was the highest in progeny from the cross H×F.

The correlation coefficients revealed interesting relationships between several of the 14 fruit characteristics (Table 6). Fruit yield was positively correlated with fruit weight (0.29), fruit diameter (0.32), fruit length (0.25), fruit calyx length (0.23), and rind thickness (0.22), but was negatively correlated with 100-aril weight (–0.08) and aril

Table 5. Fruit characteristics of pomegranate genotypes and individuals from different cross combinations.

	Hicaznar	Fellahyemez	Ernar	OPH	H×F	H×E	H×H
Fruit yield (kg/plant)	41.89 ± 3.07	24.39 ± 0.65	28.48 ± 0.31	26.91 ± 8.96	28.48 ± 8.27	24.39 ± 9.19	41.89 ± 8.25
Fruit weight (g)	438.90 ± 4.47	454.22 ± 5.00	350.21 ± 4.99	366.31 ± 4.91	437.44 ± 4.88	354.10 ± 3.68	432.86 ± 6.58
Fruit diameter (mm)	95.50 ± 0.57	99.40 ± 0.53	87.50 ± 0.87	89.35 ± 4.27	95.05 ± 3.31	87.95 ± 1.20	95.21 ± 2.58
Fruit length (mm)	84.20 ± 1.06	90.20 ± 1.51	69.90 ± 0.56	79.63 ± 5.71	86.52 ± 2.85	75.29 ± 1.70	86.08 ± 3.93
Fruit calyx length (mm)	19.22 ± 0.23	21.55 ± 0.67	22.98 ± 0.50	20.95 ± 2.63	21.56 ± 2.45	21.32 ± 1.21	20.83 ± 1.24
Rind thick.(mm)	3.30 ± 0.15	3.00 ± 0.33	1.90 ± 0.11	4.81 ± 0.97	3.86 ± 0.54	3.77 ± 0.68	4.66 ± 0.90
100-Aril weight (g)	36.73 ± 0.53	43.1 ± 0.96	28.01 ± 0.80	32.90 ± 4.55	37.57 ± 3.87	32.79 ± 4.61	34.19 ± 3.60
Aril ratio (%)	50.56 ± 0.50	52.21 ± 0.77	45.65 ± 0.45	47.54 ± 6.98	52.89 ± 4.05	50.61 ± 2.96	49.94 ± 5.29
Tot. sol. solids cont.(%)	17.30 ± 0.11	15.50 ± 0.30	14.40 ± 0.21	14.91 ± 1.02	14.34 ± 1.00	14.80 ± 1.08	15.54 ± 0.50
Total acidity (%)	1.83 ± 0.02	0.39 ± 0.04	0.43 ± 0.02	1.46 ± 0.95	1.31 ± 0.84	1.58 ± 1.00	1.80 ± 0.36
Taste	69.88	73.43	70.32	65.01	61.28	61.96	70.49
Skin color	4	2	3	2	3	4	4
Aril color	5	2	3	4	3	4	4
Seed hardness	5	3	7	5	4	7	6

Table 6. Correlation coefficients between some of the fruit traits of different pomegranate genotypes.

	FY	FW	FD	FL	FCL	RT	AW	AR	TSSC	TA	SC	AC	SH	T
FY	1.000													
FW	0.288	1.000												
FD	0.326	0.791	1.000											
FL	0.255	0.638	0.717	1.000										
FCL	0.227	0.082	0.119	0.003	1.000									
RT	0.226	0.133	0.059	0.010	0.100	1.000								
AW	–0.089	0.374	0.361	0.447	–0.270	–0.234	1.000							
AR	–0.119	0.002	0.127	0.227	–0.110	–0.556	0.321	1.000						
TTSC	0.165	0.162	0.151	0.198	–0.171	0.155	0.124	–0.034	1.000					
TA	0.383	0.094	0.036	0.056	0.405	0.254	–0.375	–0.292	0.090	1.000				
SC	0.306	0.103	0.116	–0.121	0.225	0.022	–0.046	0.109	0.031	0.017	1.000			
AC	0.263	–0.199	–0.196	–0.256	–0.015	0.291	–0.354	–0.027	0.113	0.286	0.310	1.000		
SH	–0.048	–0.132	–0.239	–0.291	0.204	0.033	–0.312	–0.139	0.142	0.223	0.072	0.088	1.000	
T	0.049	0.028	0.032	0.078	–0.126	–0.054	0.056	0.184	0.227	–0.117	0.153	0.094	–0.080	1.000

FY: fruit yield; FW: fruit weight; FD: fruit diameter; FL fruit length; FCL: calyx length; RT: peel thickness; AW: 100-aril weight; AR: aril ratio; TSSC: total soluble solids content; TA: total acidity; T: taste; SC: skin color; AC: aril color; SH: seed hardness.

ratio (–0.11). Fruit weight was positively correlated with fruit diameter (0.79), fruit length (0.63), and aril weight (0.37). Fruit diameter was positively correlated with fruit length (0.71), while fruit length was positively correlated with 100-aril weight (0.45). 100-Aril weight was positively correlated with aril ratio (0.32). The total soluble solids content was positively correlated with taste (0.22). Skin color was positively correlated with aril color (0.31). Seed hardness was positively correlated with total acidity (0.22). On the other hand, taste was negatively correlated with rind thickness (–0.54), total acidity (–0.117), and seed hardness (–0.08).

The fruit characteristics of the 67 genotypes were subjected to cluster analysis, resulting in three main groups and 6 subgroups (Figure 3, A–F). The parental genotypes Ernar, Hicaznar, and Fellahyemez were sorted into the groups B, D, and F, respectively. Genotypes arising from the open pollination of the Hicaznar parent (OPH) were distributed all over the dendrogram and also composed an outlying subgroup consisting of 4 different genotypes. The H×E progeny were either in the same group with Ernar or in Group D, which consisted mostly of genotypes arising from H×H. Genotypes developed from the cross H×F were clustered in groups E and F. As in the investigation of the tree characteristics, genotypes arising from different crosses combining Hicaznar displayed remarkable variation and are distributed throughout the obtained clusters.

A 3-D scatter plot of the correlation data of the fruit characteristics revealed a high level of total variance as described by the 3 axes representing the 3 principal components (Figure 4). Each genotype was plotted according to the score of its principal components (the cumulative proportion of variance) for each of the first 3 axes (Figure 4). Based on the fruit properties, progeny derived from crosses H×F and H×E displayed a distribution between their parents (Figure 4). Again the combination H×H and OPH showed a distribution around the genotype Hicaznar.

4. Discussion

Pomegranate breeding studies, conducted all over the world, have been aimed at obtaining good vegetative growth, sufficient flowering and fruit set, high yield, large fruit, red skin and red seeds, soft arils, and better taste. In the present study, the most important cultivar in Turkey, Hicaznar, was pollinated with different genotypes, selfed, or open-pollinated to generate new genetic stock. These genotypes were differentiated and sorted by cluster analysis according to their tree and fruit phenotypes. The morphological characteristics investigated in this study are traits normally selected for pomegranate breeding studies (Nath and Randhawa, 1959; Hussein and Hussein, 1972; Zahir-ud-din et al., 1974; Brooks and Olmo, 1978; Misra et al., 1983; Mansour et al., 2011; Bartual et al., 2012; Karimi and Mirdehghan, 2013).

Morphological characterizations of different pomegranate cultivars and genotypes have been aided using PCA and cluster analysis in different countries. For example, Karimi and Mirdehghan (2013) in Iran characterized economically important local pomegranate cultivars in respect to their fruit diameter, fruit weight, fruit length, peel weight, peel thickness, seed length, seed diameter, seed firmness, and calyx diameter. Fruit size was shown to have the highest distinguishing value in the tested cultivars; therefore this trait can be used for the separation and selection of pomegranate genotypes. These analyses revealed genetic relationships among pomegranate genotypes that can be used for selection of parents in future breeding programs.

The use of PCA and cluster analysis revealed that remarkable phenotypic and genotypic variations exist in local pomegranate genotypes in Tunisia (Mars and Marrakchi, 1999). Sarkhos et al. (2006) investigated the relationships between qualitative and quantitative fruit traits of different pomegranate genotypes and, using simple correlation analysis, determined that multivariate analysis could be a useful method for the discrimination of pomegranate genotypes. Martinez et al. (2012) used cluster analysis on local pomegranate germplasm in Spain and found considerable phenotypic and genetic diversity. Our study differs in the way that the variation was created—through hybridization breeding—but the use of PCA and cluster analysis enabled determination of the correlation between the investigated morphological traits.

Estimation of the correlation between morphological characters could provide information that would aid breeders in determining the most efficient design for genotype evaluations (Tancred et al., 1995). Estimations of correlation also allow comparison of indirect selection with direct selection, computation of a correlated response in a second trait if selection pressure is applied to the first, and establishment of a selection strategy for hard-to-select traits (Falconer and Mackay, 1996). In this respect, the correlation coefficients for some parameters of pomegranate fruit have been reported, such as peel thickness positively correlating with diameter of calyx and fruit weight with fresh and dry aril weight (Zamani et al., 2006). In our study, peel thickness was positively correlated with calyx length and fruit weight. Sarkhos et al. (2006) reported that the anthocyanin content of arils was negatively correlated with fruit size in some Iranian pomegranate genotypes. Karimi and Mirdehghan (2013) also postulated that fruit juice, aril, and seed characteristics are the main factors that separate the studied pomegranate genotypes.

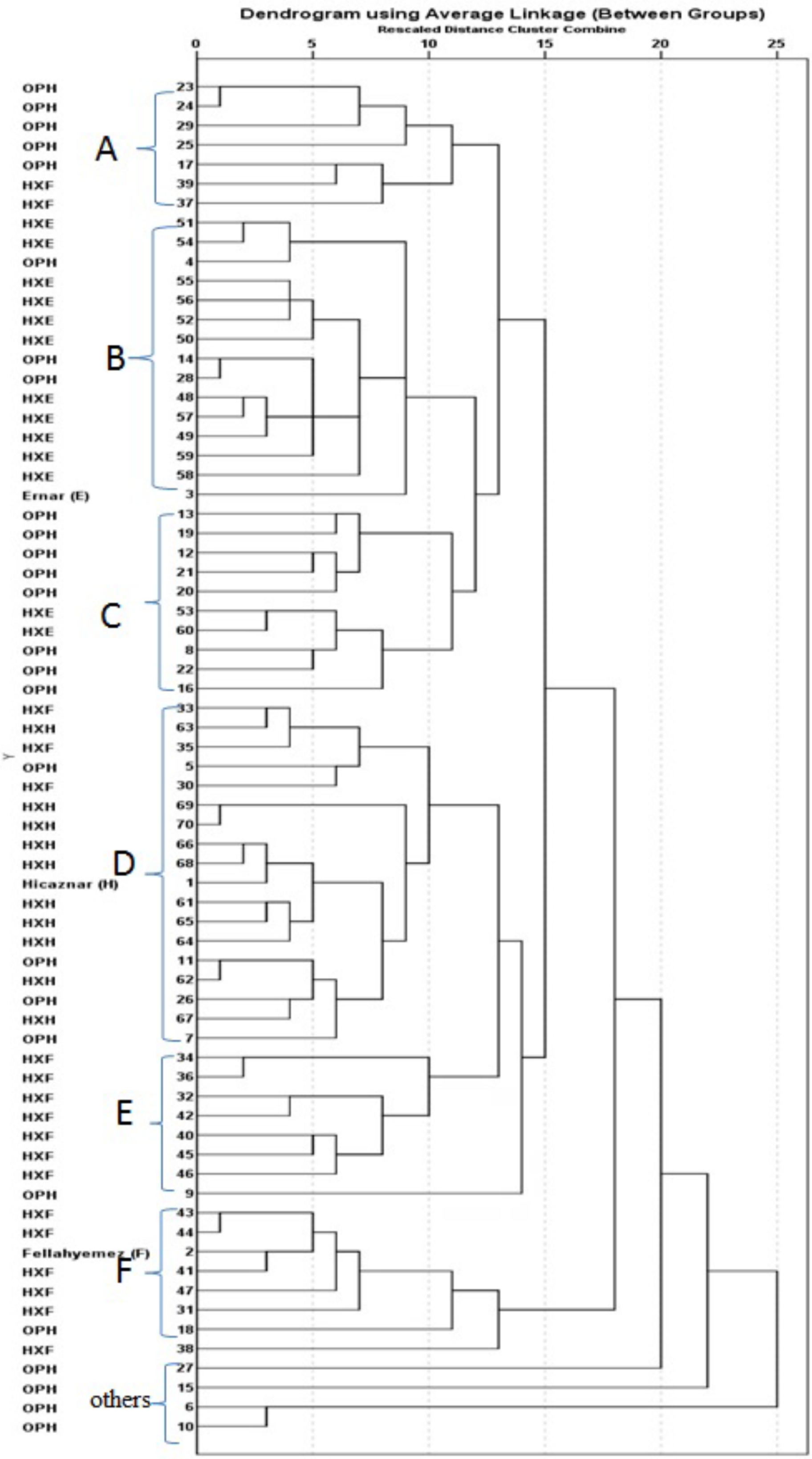

Figure 3. Cluster analysis for 67 genotypes and the parental standard cultivars based on morphological data of fruit properties.

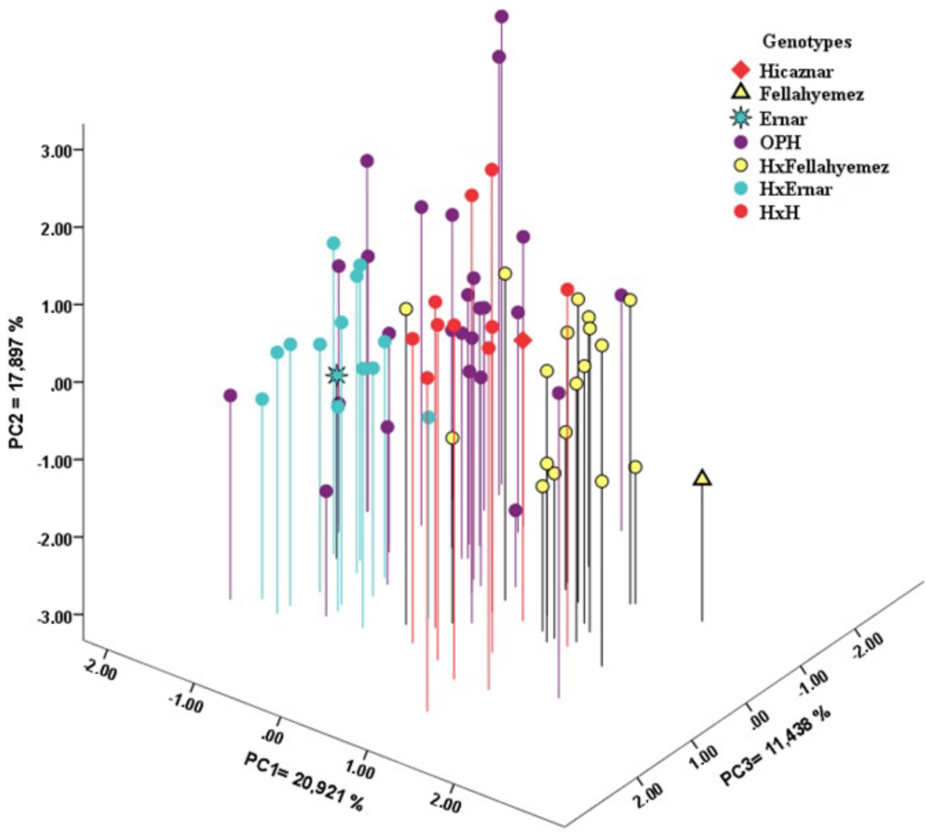

Figure 4. 3D scatter diagrams of the relationships among the genotypes and their parents (based on fruit properties).

In another study, the heterosis values for fruit traits in 53 hybrid individuals were no higher than the values of their parents (Karale and Desai, 2000). However, in our study, hybrid types were superior to their parents for the properties of soft seed hardness, red skin color, fruit size, and taste. In a similar study comparing taste, rind color, aril color, and seed hardness in 2487 types and their parents, the chance of obtaining a hybrid with red skin was higher if the genotype with red skin color was used as the female parent rather than as the male parent (Ataseven and Çelik, 2006). Furthermore, the highest rate of soft seed genotypes was obtained from crosses where the hard seed type was used as the female parent and the soft seed type was used as the male. Similarly, in our study, hybrids with red skin color were obtained more frequently from crosses where the red-skinned cultivar Hicaznar was used as the female parent and those with soft seeds were obtained more frequently from crosses where the soft-seeded Fellahyemez was used as the male parent.

In pomegranates with soft seeds, the testa width is thinner, the seed and testa density are lower, and the ratio of testa weight to total seed yield is lower (Prohit, 1985). How soft seededness is inherited is not well known, but it is known that testa hardness increases if a soft-seeded type is crossed with a hard-seeded or soft-seeded type and that testa hardness decreases if a hard-seeded type is crossed with a soft-seeded type (Prohit, 1987). There are similarities between those results and our results, because we observed that seed hardness decreased if the hard-seeded Hicaznar was crossed with the soft-seeded Fellahyemez.

Morphological characters are the first choice for describing and classifying the germplasm and are used for selection of parents in a targeted breeding scheme. Statistical methods, including principle component or cluster analyses, can be used for screening accessions. Additionally, some difficult-to-distinguish morphological characteristics, such as seed softness and dark red skin color, have been used for evaluation and therefore may be useful as markers in breeding programs (Mars and Marackhi, 1999; Karimi et al., 2009; Mansour et al., 2011; Karimi and Mirdehghan, 2013). As described in our results, remarkable variation could be seen regarding tree and fruit characteristics. These valuable data will be useful in future molecular characterization studies of pomegranate.

In conclusion, these data showed that the pollinator genotype may have a direct influence on the hybrids obtained. The present study revealed a genetic relationship among pomegranate genotypes based on tree and fruit characteristics that can be used for selection of parents in breeding programs. The obtained results will further inform crossbreeding work and can be used in the selection of male and female parents in crossbreeding programs.

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References

Ataseven E, Çelik M (2006). Similarities between pomegranate parents and crosses regarding some fruit characteristic. 1st International Symposium on Pomegranate and Minor Mediterranean Fruits, 16–19 October 2006; Adana, Turkey, pp. 229-232.

Backhaus K, Erichson B, Plinke W, Weiber R (1989). Multivariate Analysis Methods. Heidelberg, Germany: Springer Verlag.

Barone E, Caruso T, Marra FP, Sottile F (2001). Preliminary observations on some Sicilian pomegranate (*Punica granatum* L.) varieties. J Amer Pomolog Soc 55: 4-7.

Bartual J, Valders G, Andreu J, Lozoya A, Garcia J, Badenes ML (2012). Pomegranate improvement through clonal selection and hybridization in Elche. II. International Symposium on the Pomegranate, Options Mediterraneans: Serie A. Seminaires Mediterraneens 103: 71-74.

Bist HS, Srivastava R, Sharma G (1994). Variation in some promising selections of wild pomegranate (*Punica granatum* L.). Hort J 7: 67-70.

Boz Y (1988). Research on the morphological and pomological characteristics of some important pomegranate varieties grown in Şanlıurfa. Master's thesis, Ondokuz Mayıs University, Institute of Science and Technology, Samsun, Turkey.

Brooks RM, Olmo HP (1978) Register of new fruit and nut varieties. HortScience 13: 522-532.

Dokuzoğuz M, Mendilcioğlu K (1978). Pomological studies on pomegranate varieties in Aegean Sea Region. Journal of Agricultural Faculty of Ege University 15: 133-159.

Falconer DS, Mackay TFC (1996). Introduction to Quantitative Genetics, 4th ed. London, UK: Addison-Wesley Longman Ltd.

Gündoğdu M, Yılmaz H, Şensoy RIG, Gündoğdu Ö (2010). Pomological traits of pomegranate grown in Sirvan (Siirt) Region. Yuzuncu Yil University Journal of Agricultural Sciences 20: 138-143 (article in Turkish with an abstract in English).

Gündoğdu M, Yılmaz H (2012) Organic acid, phenolic profile and antioxidant capacities of pomegranate (*Punica granatum* L.) cultivars and selected genotypes. Sci Hortic 143: 38-42.

Hussein MAH, Hussein MAS (1972). Suitability of pomegranate varieties for processing. Assuit J Ag Sci 3: 303-307.

Jalikop SH (2009). Breeding of pomegranate and annonaceous fruits. II. International Symposium on Pomegranate and Minor Mediterranean Fruits, 23–27 June 2009; University of Agricultural Science, Daharwad, India.

Kafyrova NM (2003). Introduced pomegranate varieties in southern Dagestan. Sadovodstvoi Vinogradarstvo 2: 23-28.

Karale AR, Desai UT (2000). Study of heterosis for fruit characters in inter cultivar crosses of pomegranate (*Punica granatum* L.). Indian J Genet Pl Br 60: 191-196.

Karimi HR, Zamani Z, Ebadi A, Fatahi MR (2009). Morphological diversity of Pistacia species in Iran. Genet Resour Crop Ev 56: 561-571.

Karimi HR, Mirdehghan SH (2013). Correlation between the morphological characters of pomegranate (*Punica granatum* L.) traits and their implications for breeding. Turk J Bot 37: 355-362.

Levin GM (1990). Problems of methodological breeding in pomegranate. Subtropicheskie-Kul'tury 6: 70-73.

Liang CC, Cheng YD (1991). Selections of elite pomegranate. J Fruit Sci 8: 59-60.

Manivannan K, Rengasamy P (1999). Genetic evaluation of certain parents and hybrids in pomegranate (*Punica granatum* L.). J Andaman Sci Assoc 15: 64-68.

Mansour E, Khaled AB, Haddad M, Mabrouka A, Backar K, Ferchichi A (2011). Selection of pomegranate (*Punica granatum* L.) in South-eastern Tunisia. Afr J Biotecnol 10: 9352-9361.

Mars M, Marrakchi M (1999). Diversity of pomegranate (*Punica granatum* L.) germplasm in Tunisia. Genet Resour Crop Ev 46: 461-467.

Martinez JJ, Malgarejo P, Legua R, Martinez R, Hernandez F (2012). Diversity of (*Punica Granatum* L.) germplasm in Spain. II. International Symposium on the Pomegranate, Options Mediterraneennes: Serie A. Seminaires Mediterraneens. 103: 53-66.

Mısra RS, Srıvastava RP, Kuksal RP (1983). Evaluation of some pomegranate cultivars for valley areas of Garhwal Hills. Prog Hort 15: 24-26.

Nageswari K, Manivannan K, Thamburaj S, Balakrishnamoorthy G (1999). A note on the performance of hybrid progenies of pomegranate. South Indian Hort 47: 139-140.

Nath N, Randhawa GS (1959). Studies on floral biology in pomegranate (*Punica granatum* L.) I. Flowering habit, flowering season, bud development and sex ratio flowers. Indian Jour Hort 16: 61-68.

Onur C, Kaska N (1985). Selection of pomegranate (*Punica granatum* L.) of Mediterranean region. Turk J Agric For 9: 25-33.

Onur C, Tibet H (1993). Adaptation of pomegranate varieties in Antalya. Derim 10: 3-18.

Onur C, Tibet H, Ataseven Işık E (1999). Pomegranate breeding through hybridization. proceedings of Turkey III. National Horticulture Conference, 14–17 September 1999; Ankara, Turkey, pp. 58-61.

Ozgüven AI (1998). World production status of pomegranate (*Punica granatum* L). 2nd MESFIN Meeting on Plant Genetic Resources, 5-8 August 1998; Madeira, Portugal, pp. 247-264.

Özbek S (1977). General Fruit Growing. Publication number: 111. Çukurova University, Adana, Turkey: Faculty of Agriculture Academic Press.

Prohit AG (1985). Soft-seedness of commercial pomegranate varieties. Indian J Agric Sci 55: 367-368.

Prohit AG (1987). Effect of pollen parent on seed hardness in pomegranate. Indian J Agric Sci 57: 753-755.

Sarkhos A, Zamani Z, Fatahi R, Tabatabaei SZ, Akrami MR (2006). Study on relationship among quantitative and qualitative characteristics of fruit components of pomegranate genotypes. I. International Symposium on Pomegranate and Minor Mediterranean Fruits, 16–19 October 2006; Adana, Turkey.

Tancred SJ, Zeppa AG, Cooper M, Stringer JK (1995). Heritability and patterns of inheritance of the ripening date of apples. Hortic Sci 30: 325-328.

Tibet, H, Onur C (1999). Adaptation of pomegranate (*Punica granatum* L.) cultivar in Antalya ecological conditions (III). III. National Horticulture Conference, 14–17 September 1999; Ankara, Turkey, pp. 31-35.

Xian K, Xian KZ (1999). Daluzi, a sport of Qingpiruanzi pomegranate variety. South China Fruits 28: 41.

Yazıcı K (2014). Breeding studies on pomegranate. Turkey Seed Growers Association Journal 9: 24-29.

Zahir-ud-din A, Idris A, Arshad MM, Raja MR, Rahman A (1974). Quality of some deciduous fruits (apple, grape and pomegranate) of Baluchistan. J Agric Res 12: 43-52.

Zamani Z, Sarkhosh A, Fatahi R, Ebadi A (2006). Genetic relationships among pomegranate genotypes studied by fruit characteristics and RAPD markers. J Hortic Sci Biotech 82: 11-18.