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Genetic diversity of pomegranate germplasm collection from Spain determined by fruit, seed, leaf and flower characteristics

Juan José Martínez, Pablo Melgarejo, Pilar Legua, Francisco García, Francisca Hernández

Background. The objective of this research was to determine the genetic variability that exists among all the different genotypes, to understand the degree of polymorphism of the morphometric characteristics among varieties, and to establish the existing variability that exists among accessions from the same family. **Methods.** Fifty-three pomegranate (*Punica granatum* L.) accessions were studied in order to determine their degree of polymorphism and to detect similarities in their genotypes. Thirty-one morphometric characteristics were measured in fruits, arils, seeds, leaves and flowers, as well as juice content, its pH, titratable acidity, total soluble solids and maturity index. ANOVA, principal component analysis and cluster analysis showed that there is a considerable phenotypic and genetic diversity in the local pomegranate germplasm. **Results.** The cluster analysis produced a dendogram with four main clusters. The dissimilarity level ranged from 1 to 25, indicating that there were varieties that were either very similar to each other or very different from the others, with varieties from the same geographical areas being more closely related. Some polyclonal varieties were identified. Within each varietal group, different degrees of similarity were found, although there were no accessions that were identical. These results highlight the crop's great genetic diversity, which can be explained not only by their different geographical origin, but also to the fact that these are indigenous plants have not come from genetic improvement programs. The geographic origin was a determinant criterion for cultivar clustering. Parameters with high discriminating values were those related to fruit and seed size, as well as juice characteristics. **Conclusions.** As a result of the present study, we can conclude that among all the parameters analyzed, those related to the size of the fruit and the seeds and the acidity and pH of the juice were the ones that had a high power of discrimination, and were therefore the most useful for genetic characterization studies of pomegranate germplasm banks. This is opposed to leaf and flower characteristics, which had a low power of discrimination. This germplasm bank, more specifically, was characterized by its considerable phenotypic (and presumably genetic) diversity among pomegranate accessions, with a greater proximity existing among the varieties from the same

geographical area, suggesting that over time, there has not been an exchange of plant material among the different cultivation areas. Also, within the same varietal group, a great variability was found, as no identical accessions were found. In general, knowledge on the extent of the genetic diversity of the collection is essential for germplasm management.

Genetic diversity of pomegranate germplasm collection from Spain determined by fruit, seed, leaf and flower characteristics

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Short Title: Genetic diversity of pomegranate

Abstract

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Methods. Fifty-three pomegranate (*Punica granatum* L.) accessions were studied in order to determine their degree of polymorphism and to detect similarities in their genotypes. Thirty-one morphometric characteristics were measured in fruits, arils, seeds, leaves and flowers, as well as juice content, its pH, titratable acidity, total soluble solids and maturity index. ANOVA, principal component analysis and cluster analysis showed that there is a considerable phenotypic and genetic diversity in the local pomegranate germplasm.

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Conclusions. As a result of the present study, we can conclude that among all the parameters analyzed, those related to the size of the fruit and the seeds and the acidity and pH of the juice

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Keywords: accession, characterization, biodiversity, fruit, pomegranate, *Punica*.

Introduction

Pomegranate is a deciduous fruit tree, and its cultivation has been greatly expanded into several countries in recent years, especially those with a Mediterranean-like climate. In Spain, for example, the total acreage used today for its cultivation is about 2,791 ha, with an annual production of about 43,324 metric tons (MAGRAMA, 2014). The growing interest in this fruit is not only due to the fact that it is pleasant to eat, but it is also because it is considered to be a functional product that has been shown to be beneficial to human health, as it contains several types of substances that are useful in disease prevention (Melgarejo and Artés, 2000; Melgarejo and Salazar, 2002; Cam et al., 2009; Legua et al., 2012; Zaouay et al., 2012; Calani et al., 2013 and Melgarejo-Sánchez et al., 2015). Therefore, the demand for this fruit has increased in the last 10 years, as it has been used in industrial processing to obtain pomegranate juice, jams, vegetable extracts, etc. (Melgarejo-Sánchez et al., 2015).

The pomegranate's place of origin is considered to be Central Asia, from where it has spread to the rest of the world (Mediterranean Basin, Southern Asia and several countries of North and South America). It is a temperate-climate species that requires high temperatures to mature properly, but it is also easily spread in arid and semi-arid areas of the world, as it is tolerant to salinity and water scarcity, factors that usually limit the growth of other agronomical crops in these areas. Its successful adaptation to abiotic stress conditions, which characterize the Spanish Mediterranean climate, has led to its wide dispersion in this geographical area and to the appearance of a multitude of new, local individuals through time starting with specific varieties. These new varieties have been grouped under the same denomination, however, each one of them could have different agronomic characteristics as compared to their original progenitor. For example, Melgarejo and Salazar (2003) observed that under the denomination "Mollar de

Elche" (ME) there were varieties with different agronomic characteristics. In order to better identify the fruit, Verma et al. (2010) have mentioned the importance of agronomically characterizing the varieties of a specific cultivar from the place where they originated to the areas where they disseminated, as being useful for understanding the evolution of the cultivar and for maintaining the biodiversity of the varieties, as well as for the improvement of agronomic characteristics of the crops.

In 1992, the Miguel Hernandez University created a germplasm bank of the varieties of pomegranate found in Southeastern Spain in order to preserve the crop's wide genetic diversity. Since the start, many local types have been inventoried, described and planted in the experimental farm at EPSO (Escuela Politécnica Superior de Orihuela, Santomera, Alicante). Currently, the collection contains 59 accessions that have been collected from different growing areas from Spain, representing about 16 local denominations (Melgarejo, 1993). Once this collection was established, the next step was to determine its genetic biodiversity, and to classify the germplasm bank according to their agronomic characteristics rather than only from a botanical point of view, as pomegranate consumption is important in both the fresh-consumption market and the processing industry. In order to do this, the evaluation of the different morphometric and fruit characteristics was necessary, as this would lead to a better description and comparison of the genetic diversity of this germplasm collection. Mars and Marrakchi (1999) revealed the usefulness of measuring morphometric and chemical fruit variables such as weight, length, diameter, external color, seed number, length and diameter of the calyx, juice's volume, color, pH, total soluble solids TSS (g/l) and total acidity TA (g/l), in order to determine the genetic diversity of a pomegranate germplasm bank in Tunisia, composed of thirty pomegranate (*Punica granatum* L.) accessions. In our study, aside from the parameters mentioned above, parameters related to seeds, leaves and flowers were also measured, in order to have more complete information for determining the genetic diversity among all the accessions. Therefore, the objective of this research was to determine the genetic variability that existed among all the different genotypes, to understand the degree of polymorphism of the morphometric characteristics among varieties, and to establish the existing variability among accessions from the same family. Also, this research work had the advantage that the data used were taken on three consecutive years from trees that were planted in the same type of soil and climatic conditions, thereby avoiding any environmental influences.

Material and methods

Plant material

The areas prospected and the germplasm collecting procedures were as reported in Melgarejo (1992). Fifty-three accessions, representing 16 denominations, were included in the present study (Fig. 1, Table 1). They were represented by adult trees maintained within the same collection in Alicante in the Southeast region of Spain (Melgarejo, 1993).

Pomegranate trees were grown under homogeneous conditions in a loamy clay soil with a spacing of 5 × 4 m. A drip irrigation system was used for fertigation purposes. The collection was located in the experimental orchards belonging to the Miguel Hernández University, located in the province of Alicante, Spain (latitude: 38º 03′ 50″ N, longitude: 02º 03′ 50″ W and an altitude of 26 m above sea level). According to Papadakis' classification (Papadakis, 1966), the experimental plot had a subtropical Mediterranean climate. The annual mean temperature was 19 ºC, with mild winters (11 ºC in January) and hot summers (28 ºC in August). A scarce annual precipitation of 300 mm was recorded, mostly falling in spring and autumn.

Characters studied

The studies were based on measuring the characteristics of fruits, seeds, leaves and flowers. Morphometric measurements and chemical analyses were carried out on samples from 20 mature fruits, 25 seeds, 50 leaves and 25 flowers from each variety per tree. The study was conducted over three consecutive years, and the following variables were measured:

Fruits. Fruit weight (FW), expressed in g; equatorial diameter (FD1), expressed in mm; calyx diameter (FD2), expressed in mm; fruit height without calyx (FL1), expressed in mm; total fruit height (FL2), expressed in mm; calyx height (FL3), expressed in mm; number of carpels (Nc) counted on the equatorial section; rind weight plus weight of carpellary membranes (PcMc), expressed in g; skin thickness (Ec), expressed in mm (measurements were performed on two opposite sides in the equatorial zone); aril yield calculated as (Rs) = [FW-(PcMc)/FW]x100 (%).

Diameters, fruit height and skin thickness were measured with an electronic digital slide gauge (Mitutoyo), accurate to 0.01 mm. Fruit weights and Rind weight plus weight of carpellary membranes were measured with a digital scale (Sartorius Model BL-600) accurate to 0.1 g.

Arils. After extracting the seeds by hand, 25 of them were randomly chosen from a homogenized sample in every sampling year. The following seed characteristics were studied (Martínez, et al. 2006): maximum width (Sw) and length (SL), measured with a digital caliper (Mitutoyo) accurate to 0.01 mm; aril weight (SW), determined with a precision weighing device (Mettler AJ50) accurate to 0.0001 g; juice volume (JV), using an electric extractor and a seed sample of 100 g; total soluble solids (TSS) (ºBrix), determined with an Atago N-20 refractometer at 20 ºC; acidity, expressed as citric acid (A), determined with an acid–base potentiometer and pH; and maturity index (MI = TSS/A).

The most current classification that has been established for Spanish varieties (Melgarejo, 1993) were used: Sweet varieties: MI = 31–98; Sour-sweet varieties: MI = 17–24; Sour varieties: MI = 5–7. Three repetitions per clone and year were carried out.

Seeds: The parameters measured in the seeds (woody portion) were: maximum width (w) and length (l), measured with the same digital caliper as above; weight of the woody portion (wpw) of each seed using the above-mentioned precision balance; woody portion index (wpi), determined from the wpw/SW ratio 100 (%);

Leaves: The leaves studied were collected in September, by choosing 50 adult leaves per tree, normal and leaves that sprouted in the spring. This sampling was done in the four cardinal directions of the tree. The length were measured with a digital caliper (Mitutoyo) accurate to 0.01 mm. The leaf surface area was determined with an image analyzer "Digital Image Analysis System" Delta-T model. The measured variables were: LW, leaf width (mm); Ll, blade length (mm); Lt, total length of the leaf (mm); Lp, petiole length (mm); LS, leaf surface area (mm^2).

Flower. The flowers were randomly sampled during the flowering period in the month of May, taking a total of 25 flowers per tree. This sampling was done in the four cardinal directions of the tree. Length measurements were performed using a digital caliper (Mitutoyo) accurate to 0.01 mm. The measured variables were: FD, flower diameter (mm); FL, flower length (mm); NP, number of petals; NS, number of sepals; LP, petal length (mm); AP, petal width (mm); LE, style length (mm); NE, number of stamens.

Statistical analysis

The results were analyzed using the SPSS 22.0 software program for Windows (SPSS Science, Chicago, IL, USA). The differences between cultivars ($P < 0.05$) found after analyzing the different parameters studied were evaluated by analysis of variance (ANOVA). The mean values measured for each parameter were used to perform: a) a principal component analysis (PCA) and b) a clustering of cultivars into similarity groups using Ward's method for agglomeration and the squared Euclidean distance as a measurement of dissimilarity.

Results and discussion

The pomegranate genotype grouping results after the PCA were mainly based on the first three PCs, which accounted for 53.75% of the variability observed, i.e. for 27.77%, 17.49% and 8.49% respectively (Fig.2). The most important variables integrated by PC1 were fruit weight (FW), lengths (FL1, FL2, FL3), diameters (FD1, FD2) and both aril (SW, Sw, SL) and seed weight, length and width (Wpw, W, L) (Table 2). The correlations between PC1 and leaf and flowers characteristics were less important.

PC1 mainly separated the cultivars by the shape and size of their fruits and arils, with the groups composed by the cultivars PTO, CRO, PTB1 and ADO being the ones that had the largest fruits and arils (Figure 3, 4th quadrant, bottom right), with the accession group ME being the one with the smallest sizes (Figure 3, 3rd quadrant, bottom left). Radunic et al., (2015), in a characterization study of 8 pomegranate accessions from Croatia also observed that the main differences among them were due to the weight of the fruits.

PC2 was mainly correlated with the rind weight plus the weight of carpellary membranes (and therefore with the yield of arils), the woody portion index of the arils (seeds), leaf size, and juice acidity. But overall, this component differentiated the varieties by the acidity of their juice as well as their woody portion index. Figure 3 shows how the varietals BO1 and BA1, which have a sourer flavor, were grouped on the upper part of the first quadrant of the figure.

Likewise, the varieties found in the first and second quadrant have a greater index of woody tissue.

PC3 integrated characters related with the shape and size of the flowers, leaf shape, skin thickness and the maturity index (Table 2), although this component was less significant than PC1 and PC2. The other flower and leaf characteristics were not as important in the present study.

The cluster analysis produced a dendogram with four main clusters (Figure 4). The dissimilarity level (d) ranged from 1 to 25, revealing that there was a great degree of similarity/dissimilarity among varieties. The first cluster (I) included the ME group's cultivars (21 accessions), as well as the variety MO2, which was more similar to varieties from the ME group than to its own varietal group (MO). All of these fruits were medium sized (275.9-356.1 g), had a low-acidity juice, and high maturity indices in general (Table 4). As previously shown, the varieties from these groups were placed on the 3rd quadrant in the PC1 and PC2 principal component analysis graphic shown in figure 3.

The second cluster (II) grouped cultivars BA1 and BO1, which were characterized by having medium-large fruit, and high juice acidity and woody portion index (Tables 3 and 4). The dendogram showed that these varieties were very similar, even though they came from different locations. These results can be found on the upper right part of Figure 3 on the first quadrant.

The third cluster (III) was the most-heterogeneous group, as it was composed by 16 varieties from various locations, with fruits that were medium-large in size (331.5-436.5 g), and sweet juice (Tables 3 and 4). The dendogram shows that there was a high degree of similarity between these 16 varieties, but at the same time, among these groups, this similarity was greater between those that came from the same location or geographical area. These were mostly located in the second quadrant on Figure 3.

The last group (IV) was composed by 12 varieties, all of them from the same geographical area. As a whole, the varieties in this cluster were more similar among themselves than the varieties from clusters I and III. The cluster IV varieties were characterized by their heavier fruit (358.8-464.2 g/fruit), and their large seeds (0.4-0.7 g/seed) (Table 3). Most of the varieties from this group were placed in the fourth quadrant in the principal component analysis results shown in Figure 3.

This principal component and cluster analysis revealed three important issues. First, in this pomegranate germplasm collection from Southeastern Spanish, there was a considerable variability among ascensions that may be due, mainly, to recombination (resulting from outcrossing) combined with sexual and vegetative propagation that occurred over a long period of time, as well as uncontrolled spread of plant material (Mars, 1996), as pomegranate is partially cross-pollinated (Jalikop and Sampath, 1990 and Martínez et al. 2009). Second, within the group of cultivars 'ME', 'MO', 'MA', 'PTO' or 'ADO', a high degree of heterogeneity was observed.

It is therefore possible to think of these groups as "variety-population" (Boulouha et al., 1992; Tous et al., 1995; Mars and Marrakchi, 1999). It is also interesting to point out that within the varieties analyzed, the four groups obtained in the cluster analysis (Figure 4) coincided almost completely with their geographical origin, with the origin becoming a determinant criterion for the grouping of the varieties, except for MO2, BA1, BO1, PG and PTO5 (Figure 4, Table 1). This is in agreement with results reported for other fruit species (Barbagollo et al., 1997) but also contradicts the grouping criteria obtained by Mars and Marrakchi (1999), in a study performed on pomegranate diversity in Tunisia, where the geographical origin was not a determining factor for their grouping. These last authors have suggested that in Tunisia, the geographical origin was not a determinant factor because over time, there had been an exchange of plant material between the different growing areas of this species. Zhao et al. (2013), in a study performed on 46 pomegranate cultivars, indicated that cultivars were not clustered according to their morphological traits, agronomic traits, or geographic origin. According to a previous study, several causes for these inconsistencies included: (1) the reproducibility of gene mutations caused the same mutation to emerge repeatedly in the distantly-related individuals from different areas (Zhu, 2002); (2) the amplified polymorphic loci were not parts of the genes responsible for these morphological or the agronomic traits (Jbir et al., 2008 and Ebrahimi et al., 2010;) and (3) the quantitative traits were significantly influenced by the environment (Zhu, 2002)

In this germplasm bank, no identical accessions were found within a single group, as shown in the ANOVA results on table 5, as significant differences were found between the accessions belonging to a single group in most of the parameters analyzed, except for the juice characteristics, where differences in pH, TSS, A and MI were observed only in the PTO group among its 7 accessions. Among all the groups analyzed, ME was notable, as there were significant differences in all the physical parameters measured in the fruits among its accessions. This evidences the great genetic diversity that exists even within a single group, which can be explained not only by its different geographical origin, but also due to the fact that it is native material that has been developing for many years, and has not suffered recombination with native material from other geographical areas. The data from this experiment also further confirmed the results from a previous study performed by Melgarejo et al (2009) which evaluated the genetic diversity of pomegranate cultivars based on Restriction Fragment Length

Polymorphisms (RFLP) and Polymerase Chain Reaction (PCR) techniques. Ten pomegranate accessions from the varietal groups Mollar de Elche (ME3.1, ME14, ME15, ME16 and ME17), Mollar de Albatera (MA5), Mollar de Orihuela (MO3), Valencianas (VA3 and VA4) and Bordes (BO1) were evaluated, resulting in different genetic profiles for the different groups as well as for the accessions within a single group. A more recent work performed by Ferrara et al. (2014) with simple sequence repeats (SSR) markers on Italian and Israeli pomegranates has also confirmed the high genetic variability of this crop.

Conclusions

As a result of the present study, we can conclude that among all the parameters analyzed, those related to fruit and seed size and the juice's acidity and pH were the ones that had the highest power of discrimination, and were therefore the most useful for genetic characterization studies of pomegranate germplasm banks. This is opposed to leaf and flower characteristics, which had a low power of discrimination. This germplasm bank, more specifically, was characterized by its considerable phenotypic (and presumably genetic) diversity among pomegranate accessions, with a greater phenotypic proximity existing among the varieties from the same geographical area, suggesting that over time, there has not been an exchange of plant material among the different cultivation areas. Also, within the same varietal group, a great variability was found, as no identical accessions were found. In general, knowledge on the extent of the genetic diversity found in the collection is essential for germplasm management. In this study, these data may help in the developing of strategies for pomegranate germplasm management and may allow for more efficient use of this germplasm in future breeding programs for this species.

Acknowledgments

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Figure Legends

Figure 1. Location of the areas of origin of the accessions that composed the germplasm collection studied. The name of each accession according to the codes used can be found in Table 1.

Figure 2. Principal components screen plot.

Figure 3. Biplot of the two principal components PC1 and PC2 showing dispersion of Spanish pomegranates, based on morphological characteristics of the fruit and leaves, and the pH and acidity of the juice.

Figure 4. Cluster analysis grouping of 53 Spanish pomegranate cultivars. See Table 1 for cultivars names abbreviations.

Blanca AB1, SFB1, PB1	**Albatera** BA1, MA1, MA2, MA3, MA4, MA5
Crevillente MC1, VA1	**Ojos:** ADO2, ADO3, BO1, CRO1, CRO2, PDO2, PTO2, PTO3, PTO4, PTO5, PTO6, PTO7, PTO8
Elche ME1, ME2, ME3, ME3.1, ME4, ME5, ME6, ME7, ME8, ME9, ME10, ME11, ME12, ME13, ME14, ME15, ME16, ME17, ME18, ME19, ME20, ME21	**Orihuela** MO2, MO3, MO4, MO5, MO6

Figure 1

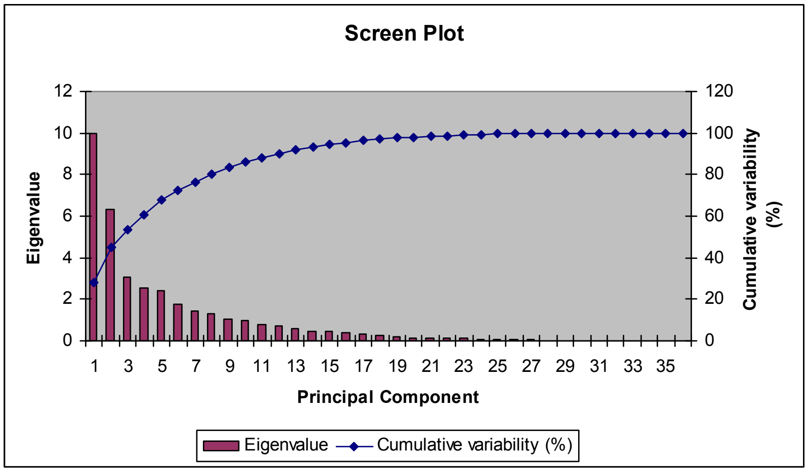

Screen Plot
Eigenvalue
Cumulative variability (%)
Principal Component
Eigenvalue
Cumulative variability (%)

Figure 2

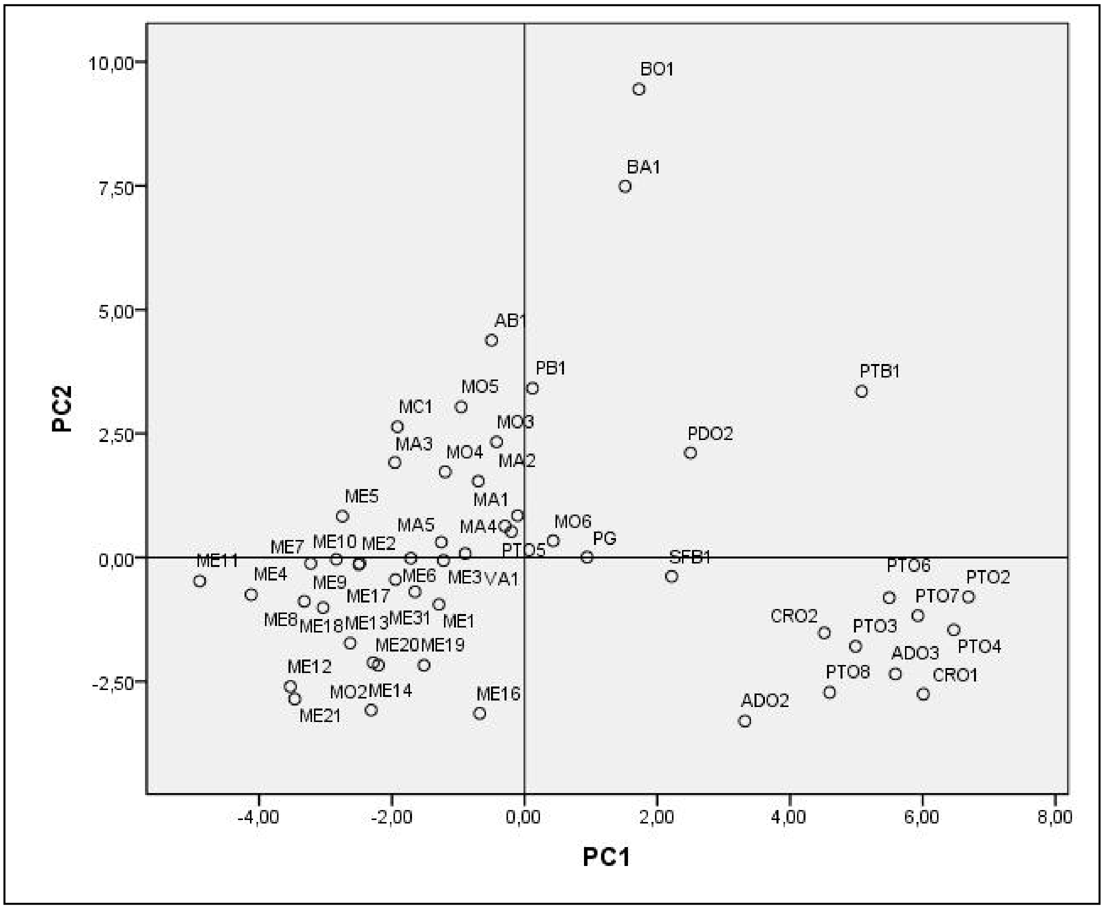
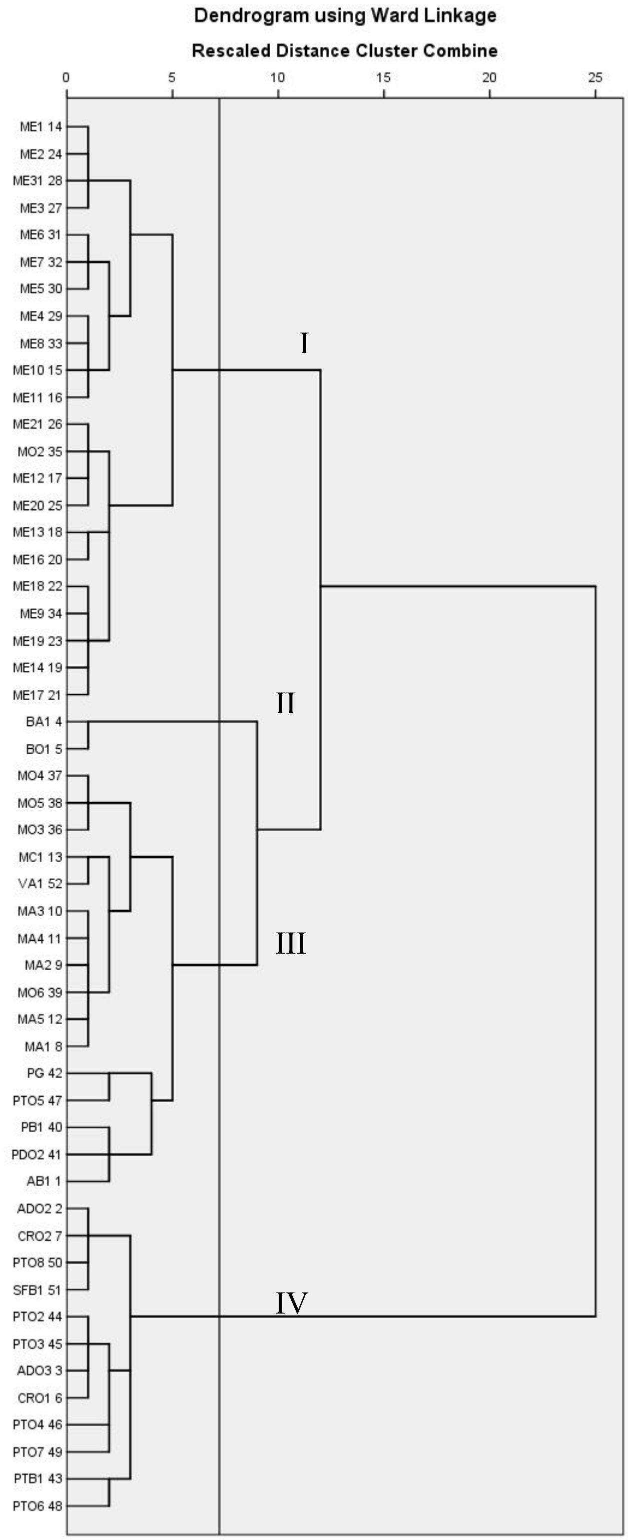

PC2
PC1
10,00
7,50
5,00
2,50
0,00
-2,50
-4,00
-2,00
0,00
2,00
4,00
6,00
8,00
BO1
BA1
AB1
PB1
PTB1
MO5
MC1
MO3
PDO2
MA3
MO4
MA2
ME5
MA1
MA5
MA4
MO6
PG
ME7
ME10
ME2
PTO5
SFB1
ME11
ME4
ME6
ME3
VA1
PTO6
PTO2
ME9
ME17
PTO7
CRO2
ME8
ME18
ME13
ME31
ME1
PTO3
ME20
ME19
ADO3
PTO4
ME12
PTO8
CRO1
MO2
ME14
ME16
ADO2
ME21

Figure 3



Dendrogram using Ward Linkage
Rescaled Distance Cluster Combine
0 5 10 15 20 25
ME1 14
ME2 24
ME31 28
ME3 27
ME6 31
ME7 32
ME5 30
ME4 29
ME8 33
ME10 15
ME11 16
ME21 26
MO2 35
ME12 17
ME20 25
ME13 18
ME16 20
ME18 22
ME9 34
ME19 23
ME14 19
ME17 21
BA1 4
BO1 5
MO4 37
MO5 38
MO3 36
MC1 13
VA1 52
MA3 10
MA4 11
MA2 9
MO6 39
MA5 12
MA1 8
PG 42
PTO5 47
PB1 40
PDO2 41
AB1 1
ADO2 2
CRO2 7
PTO8 50
SFB1 51
PTO2 44
PTO3 45
ADO3 3
CRO1 6
PTO4 46
PTO7 49
PTB1 43
PTO6 48
I
II
III
IV

Figure 4

Table 1. Names, abbreviations and origin of pomegranate accessions evaluated.

Code	**Accession**	**Location**	**Code**	**Accession**	**Location**
AB1	Albar de Blanca 1	Blanca (Murcia)	ME13	Mollar de Elche 13	Elche (Alicante)
ADO2	Agridulce de Ojós 2	Ojos (Murcia)	ME14	Mollar de Elche 14	Elche (Alicante)
ADO3	Agridulce de Ojós 3	Ojos (Murcia)	ME16	Mollar de Elche 16	Elche (Alicante)
BA1	Borde de Albatera 1	Albatera (Alicante)	ME17	Mollar de Elche 17	Elche (Alicante)
BO1	Borde de Ojós 1	Ojos (Murcia)	ME18	Mollar de Elche 18	Elche (Alicante)
CRO1	Casta del Reino 1	Ojos (Murcia)	ME19	Mollar de Elche 19	Elche (Alicante)
CRO2	Casta del Reino 2	Ojos (Murcia)	ME20	Mollar de Elche 20	Elche (Alicante)
MA1	Mollar de Albatera 1	Albatera (Alicante)	ME21	Mollar de Elche 21	Elche (Alicante)
MA2	Mollar de Albatera 2	Albatera (Alicante)	MO2	Mollar de Orihuela 2	Orihuela (Alicante)
MA3	Mollar de Albatera 3	Albatera (Alicante)	MO3	Mollar de Orihuela 3	Orihuela (Alicante)
MA4	Mollar de Albatera 4	Albatera (Alicante)	MO4	Mollar de Orihuela 4	Orihuela (Alicante)
MA5	Mollar de Albatera 5	Albatera (Alicante)	MO5	Mollar de Orihuela 5	Orihuela (Alicante)
MC1	Molar de Crevillente	Crevillente (Alicante)	MO6	Mollar de Orihuela 6	Orihuela (Alicante)
ME1	Mollar de Elche 1	Elche (Alicante)	PB1	Piñonenca de Blanca 1	Blanca (Murcia)
ME2	Mollar de Elche 2	Elche (Alicante)	PDO2	Piñón duro de Ojós 2	Ojos (Murcia)
ME3	Mollar de Elche 3	Elche (Alicante)	PG	Puente Genil	Puente Genil (Córdoba)
ME3.1	Mollar de Elche 3.1	Elche (Alicante)	PTB1	Piñón tierno de Blanca 1	Blanca (Murcia)
ME4	Mollar de Elche 4	Elche (Alicante)	PTO2	Piñón tierno de Ojós 2	Ojos (Murcia)
ME5	Mollar de Elche 5	Elche (Alicante)	PTO3	Piñón tierno de Ojós 3	Ojos (Murcia)
ME6	Mollar de Elche 6	Elche (Alicante)	PTO4	Piñón tierno de Ojós 4	Ojos (Murcia)
ME7	Mollar de Elche 7	Elche (Alicante)	PTO5	Piñón tierno de Ojós 5	Ojos (Murcia)
ME8	Mollar de Elche 8	Elche (Alicante)	PTO6	Piñón tierno de Ojós 6	Ojos (Murcia)
ME9	Mollar de Elche 9	Elche (Alicante)	PTO7	Piñón tierno de Ojós 7	Ojos (Murcia)
ME10	Mollar de Elche 10	Elche (Alicante)	PTO8	Piñón tierno de Ojós 8	Ojos (Murcia)
ME11	Mollar de Elche 11	Elche (Alicante)	SFB1	San Felipe de Blanca 1	Blanca (Murcia)
ME12	Mollar de Elche 12	Elche (Alicante)	VA1	Valenciana de Albatera 1	Albatera (Alicante)

Table 2. Eigenvalues, proportion of variation and eigenvectors associated with three axes of the PCA in pomegranate germplasm.

Principal components (axes)	1	2	3
Cumulated proportion of variation	27.77	45.26	53.75
Characters		Eigenvectors	
FW	0.28	0.06	-0.01
FD1	0.28	0.07	-0.01
FD2	-0.15	0.07	-0.19
FL1	0.26	0.09	-0.03
FL2	0.27	0.09	0.01
FL3	0.11	0.03	0.11
Nc	-0.12	0.02	0.15
PcMc	0.11	0.26	-0.18
Ec	-0.16	0.08	-0.24
Rs	0.22	-0.17	0.17
SW	0.25	-0.15	0.07
SL	0.27	-0.15	0.05
Sw	0.19	-0.17	0.09
l	0.25	-0.02	0.05
w	0.11	0.07	0.13
wpw	0.13	0.12	0.11
wpi	-0.13	0.25	0.01
LW	-0.14	0.17	0.31
Ll	0.02	0.28	-0.16
Lt	0.01	0.28	-0.15
Lp	-0.10	0.18	0.03
Ll/LA	0.12	0.07	-0.38
LS	-0.01	0.22	0.15
FD	-0.19	0.11	0.20
FL	0.17	0.21	0.23
Np	0.16	0.17	0.20
Lp	-0.10	0.24	0.12
Wp	-0.11	0.18	0.25
Ns	0.16	0.18	0.20
LS	-0.01	0.29	0.14
NS	0.16	0.11	-0.06
JV	0.12	-0.01	0.15
pH	-0.10	-0.22	0.22
TSS	-0.10	0.08	0.14
A	0.08	0.25	-0.20
MI	-0.19	-0.12	0.23

For explanation of character symbols, see Material and methods

Table 3. Mean values of fruit characters of pomegranate accessions

Variety	FW	FD1	FD2	FL1	FL2	FL3	Nc	PcMc	Ec	Rs
AB1	361.4	89.6	18.5	77.2	94.0	16.9	7.0	165.4	3.5	53.7
ADO2	361.1	90.1	19.6	76.0	95.3	19.3	6.4	137.2	3.2	61.4
ADO3	458.1	96.3	18.9	81.3	98.6	17.2	6.6	150.6	3.1	66.1
BA1	400.1	92.1	22.4	78.4	96.2	17.8	6.4	186.6	3.9	52.8
BO1	395.7	92.4	23.0	80.1	98.4	18.3	6.4	192.1	4.4	51.4
CRO1	456.4	98.4	19.5	82.4	99.6	17.1	6.5	163.0	3.2	63.6
CRO2	371.1	92.8	20.4	78.3	96.8	18.5	6.7	146.6	3.0	60.2
MA1	362.9	90.4	19.8	77.1	91.3	14.3	6.5	165.3	4.5	53.7
MA2	362.0	89.7	21.2	78.1	94.3	16.1	6.7	161.2	3.6	55.0
MA3	354.3	88.6	22.0	75.6	90.8	15.1	6.7	154.9	4.1	55.7
MA4	367.0	89.5	18.8	77.6	93.3	15.8	6.9	153.5	4.0	57.8
MA5	343.5	87.7	21.7	75.8	90.1	14.3	6.5	137.5	4.1	59.4
MC1	344.8	87.9	21.9	78.3	94.4	16.1	6.1	167.4	4.3	51.4
ME1	321.4	85.8	19.2	73.3	91.6	18.3	6.7	134.4	3.0	58.2
ME10	322.1	86.3	21.2	75.7	91.2	15.5	6.5	142.2	3.8	55.1
ME11	275.9	82.3	21.7	71.1	86.7	15.7	6.9	130.3	3.9	52.1
ME12	340.9	87.2	20.4	73.4	89.5	16.1	7.3	140.3	2.9	57.7
ME13	332.7	85.0	19.6	71.9	88.7	16.7	6.3	141.5	4.4	57.9
ME14	333.5	88.3	23.8	74.0	90.7	16.7	6.5	156.8	4.6	52.7
ME16	350.0	87.9	20.6	77.8	92.9	15.1	6.3	149.3	4.1	57.0
ME17	350.7	89.8	24.5	75.8	91.6	15.8	6.6	166.1	4.6	52.1
ME18	335.1	85.3	21.4	77.8	93.1	15.2	6.4	148.1	4.2	55.5
ME19	347.2	89.4	21.3	77.2	92.0	14.7	6.7	142.0	4.0	58.7
ME2	301.3	84.3	20.8	72.9	90.7	17.8	6.6	134.0	3.7	55.7
ME20	346.6	88.1	20.8	74.9	90.6	15.8	7.1	139.8	3.3	58.9
ME21	309.5	82.4	19.1	71.6	88.0	16.4	6.7	142.8	3.5	53.5
ME3	356.1	88.7	20.2	76.5	92.9	16.4	6.2	137.3	3.6	61.2
ME31	329.0	87.6	19.4	75.0	92.9	17.8	6.8	138.3	3.1	57.8
ME4	278.4	83.6	21.1	71.8	88.4	16.6	6.8	124.5	4.0	54.7
ME5	312.3	85.9	21.4	73.5	90.5	17.0	6.8	139.4	4.6	54.9
ME6	348.3	88.1	21.6	77.4	93.3	15.9	6.7	148.1	4.4	57.1
ME7	317.0	83.6	20.5	73.4	88.6	15.2	6.5	138.3	4.0	55.6
ME8	300.6	82.9	20.2	72.3	88.0	15.6	6.5	141.9	4.1	52.7
ME9	328.5	87.3	22.5	75.7	92.6	16.9	6.5	151.9	4.4	52.8
MO2	350.7	87.0	19.4	75.6	90.7	15.1	6.6	145.7	3.4	57.1
MO3	369.7	90.9	20.5	78.9	95.4	16.5	6.6	154.9	3.4	57.5
MO4	352.1	88.1	21.2	75.7	91.8	16.2	6.6	145.8	3.8	57.7
MO5	375.9	90.4	21.1	77.0	91.8	14.8	6.9	150.5	3.1	59.9
MO6	379.6	91.8	20.6	76.6	93.0	16.4	6.6	155.0	3.5	58.7
PB1	366.6	90.8	19.7	75.5	92.8	17.3	7.0	168.4	3.6	53.9
PDO2	436.5	95.8	18.0	82.8	96.8	14.0	6.8	179.5	3.2	58.8
PG	331.5	87.8	17.9	75.2	90.3	15.1	6.3	146.3	3.7	55.3
PTB1	436.0	97.0	17.3	84.7	101.7	17.1	6.6	192.2	3.0	55.8
PTO2	428.5	97.8	19.5	83.8	102.3	18.5	6.4	166.7	3.6	60.5
PTO3	428.4	96.1	19.3	82.0	98.6	16.6	6.4	163.5	3.7	61.3
PTO4	425.4	95.8	19.5	80.7	95.8	15.1	6.2	144.3	3.3	65.8
PTO5	339.4	86.3	18.2	76.8	90.0	13.2	6.1	140.4	4.1	58.3
PTO6	412.7	94.7	19.7	80.0	96.3	16.3	6.4	139.9	3.4	65.9
PTO7	464.2	95.9	19.1	82.4	98.9	16.5	6.4	153.9	3.2	65.9
PTO8	369.5	86.7	17.8	75.3	93.9	18.6	6.3	129.3	2.6	64.1
SFB1	358.8	89.0	19.4	78.1	95.2	17.2	6.1	136.4	3.2	61.0
VA1	341.7	87.2	22.0	76.6	91.7	15.1	6.3	164.6	3.8	51.7

For explanation of character symbols, see Material and methods

Table 4. Mean values of aril, seed and juice characteristics of pomegranate accessions

Variety	SW	SL	Sw	L	W	Wpw	Wpi	JV	pH	TSS	A	MI
AB1	0.4	10.4	6.8	7.0	2.6	0.1	13.5	49.7	4.1	14.3	0.2	79.0
ADO2	0.6	13.2	7.0	7.3	1.7	0.0	6.8	53.0	4.1	12.8	0.3	47.0
ADO3	0.7	13.0	8.3	6.7	2.3	0.0	6.5	57.3	4.0	14.1	0.3	49.0
BA1	0.4	10.0	6.0	6.6	2.1	0.0	13.8	48.5	2.8	14.9	1.8	8.1
BO1	0.3	9.8	5.5	6.4	1.9	0.0	12.2	55.7	2.8	14.1	2.3	6.3
CRO1	0.6	13.0	7.7	7.5	2.1	0.1	8.2	58.7	4.0	13.1	0.3	45.8
CRO2	0.6	12.3	7.6	7.2	2.3	0.0	7.7	60.3	3.9	12.2	0.3	37.3
MA1	0.4	10.4	6.8	6.3	2.2	0.0	10.6	48.5	3.8	15.3	0.2	62.2
MA2	0.4	10.3	6.4	6.2	2.0	0.0	10.0	49.3	4.1	15.7	0.2	73.7
MA3	0.4	9.3	6.4	5.8	2.1	0.0	11.6	58.7	3.9	15.0	0.3	50.1
MA4	0.4	10.7	6.9	6.6	2.3	0.0	9.1	60.0	4.1	15.5	0.2	72.1
MA5	0.4	10.6	6.6	7.1	2.5	0.0	9.8	46.8	4.1	15.0	0.2	69.7
MC1	0.4	9.9	6.1	6.1	1.6	0.0	10.3	45.3	3.9	14.0	0.3	56.2
ME1	0.4	10.6	6.7	6.2	2.0	0.0	9.4	58.0	4.1	14.7	0.2	70.4
ME10	0.4	9.9	6.3	5.9	1.9	0.1	13.0	55.5	4.1	14.2	0.2	71.9
ME11	0.4	9.2	5.5	6.0	2.2	0.1	12.8	46.7	4.1	15.2	0.2	66.6
ME12	0.4	9.6	5.6	5.6	1.3	0.0	9.7	44.3	3.9	14.3	0.2	70.5
ME13	0.4	10.4	5.8	6.1	1.6	0.0	9.4	49.3	4.0	14.3	0.2	72.3
ME14	0.4	11.0	7.0	5.9	1.6	0.0	8.0	48.7	4.1	13.5	0.2	55.3
ME16	0.4	10.6	7.1	5.8	2.0	0.0	7.3	46.0	3.9	14.8	0.2	61.3
ME17	0.4	10.8	6.7	6.2	1.7	0.0	9.0	52.7	4.1	14.1	0.2	73.8
ME18	0.3	10.0	5.7	6.1	2.0	0.0	10.5	52.7	4.0	15.2	0.2	66.5
ME19	0.4	10.4	6.6	6.6	2.0	0.0	9.7	48.5	4.0	14.6	0.3	47.5
ME2	0.4	10.4	6.6	5.9	2.1	0.0	9.6	54.7	4.1	14.5	0.2	71.7
ME20	0.4	9.9	6.7	5.7	1.6	0.0	10.0	51.7	4.0	14.2	0.2	63.8
ME21	0.4	9.8	6.6	5.8	1.9	0.0	8.6	50.7	4.0	15.5	0.2	69.0
ME3	0.4	10.5	6.3	6.3	2.0	0.0	10.6	56.5	4.2	15.3	0.2	73.7
ME31	0.4	10.4	6.7	6.1	2.2	0.0	9.2	57.8	4.1	15.2	0.2	73.5
ME4	0.4	9.5	6.0	5.7	1.9	0.0	11.9	58.3	4.0	13.3	0.2	64.4
ME5	0.4	11.1	7.0	6.8	2.3	0.0	11.9	56.0	4.2	15.7	0.2	69.8
ME6	0.4	10.7	7.4	6.4	2.5	0.0	10.7	47.7	4.0	14.6	0.2	69.0
ME7	0.4	10.3	6.9	6.3	2.2	0.0	11.2	50.3	4.1	15.0	0.2	62.8
ME8	0.4	10.3	6.5	6.2	2.1	0.0	9.9	53.7	4.1	13.5	0.2	74.0
ME9	0.4	10.2	6.5	6.6	2.0	0.0	10.3	49.3	3.8	14.6	0.2	71.8
MO2	0.4	10.4	6.3	6.0	1.8	0.0	8.9	48.0	4.0	15.5	0.2	71.2
MO3	0.4	10.2	5.9	5.8	1.5	0.0	10.1	47.5	4.0	14.2	0.2	75.3
MO4	0.4	10.3	6.6	5.9	1.6	0.0	9.2	49.5	4.1	13.7	0.2	67.8
MO5	0.4	10.2	6.1	5.9	1.7	0.0	10.6	48.7	4.0	14.5	0.2	74.0
MO6	0.4	10.9	6.7	6.5	2.0	0.0	11.6	53.7	4.0	15.6	0.2	74.0
PB1	0.3	10.0	6.1	7.0	2.4	0.0	14.1	56.0	3.4	14.7	0.3	43.7
PDO2	0.4	10.8	7.2	7.4	2.9	0.1	13.8	54.0	3.9	14.3	0.3	55.0
PG	0.5	10.2	6.5	6.3	1.9	0.1	11.2	56.3	3.4	13.6	0.7	19.8
PTB1	0.5	11.5	6.5	7.3	2.3	0.0	9.8	50.0	4.0	15.3	0.3	45.5
PTO2	0.6	13.2	7.5	8.3	2.3	0.1	8.2	60.1	4.0	13.9	0.3	46.6
PTO3	0.6	12.7	7.0	8.3	2.2	0.0	7.6	52.0	3.9	14.1	0.3	43.2
PTO4	0.6	13.7	7.3	9.6	2.5	0.1	9.0	53.0	3.8	13.5	0.3	46.7
PTO5	0.3	9.3	5.9	6.6	2.0	0.0	13.6	62.3	3.6	14.4	0.5	27.5
PTO6	0.6	12.7	7.1	7.3	1.9	0.0	6.7	61.0	3.9	13.7	0.3	49.7
PTO7	0.5	11.9	7.0	7.1	2.2	0.0	8.2	54.3	3.3	15.5	1.0	16.2
PTO8	0.6	12.9	8.3	7.1	2.1	0.0	6.9	52.0	3.9	14.3	0.3	49.5
SFB1	0.6	11.7	7.3	6.3	1.6	0.0	7.1	56.0	3.8	13.7	0.3	46.0
VA1	0.4	10.1	6.9	5.8	1.7	0.0	8.3	56.7	3.9	13.8	0.2	60.5

For explanation of character symbols, see Material and methods

Table 5. Mean values of leaf and flowers characteristics of pomegranate accessions

	Leafs						Flowers							
Variety	LW	Ll	Lt	Lp	Ll/LW	LS	FD	FL	Np	Lp	Wp	Ns	LS	NS
AB1	20.8	61.2	68.0	6.8	3.0	8.7	15.5	33.6	6.2	26.5	20.0	6.2	21.3	375.0
ADO2	19.2	51.5	57.0	5.5	2.7	7.3	10.3	31.4	6.3	19.9	15.5	6.3	12.4	368.6
ADO3	20.6	55.0	60.3	5.3	2.7	7.7	10.4	30.5	6.2	19.8	15.7	6.2	13.6	340.6
BA1	22.8	60.3	65.6	5.2	2.7	8.3	16.3	34.7	7.7	24.9	17.0	7.7	20.7	312.6
BO1	21.2	62.2	68.7	6.4	3.0	9.5	16.5	37.5	7.8	24.7	17.9	7.8	23.0	364.0
CRO1	19.1	49.6	54.7	5.2	2.6	7.1	10.3	30.4	6.1	20.6	15.8	6.1	11.8	325.8
CRO2	19.1	53.0	58.4	5.4	2.8	7.6	11.0	33.0	6.6	21.9	16.3	6.6	13.1	339.4
MA1	21.3	57.3	62.3	5.0	2.7	8.2	10.5	28.0	6.8	23.1	16.4	6.8	9.4	349.3
MA2	22.0	54.0	60.1	6.1	2.5	8.2	12.7	30.5	7.3	22.3	17.4	7.5	17.1	281.6
MA3	23.1	53.9	59.9	6.0	2.4	8.0	13.8	32.3	6.5	22.8	16.9	6.5	20.9	335.6
MA4	22.4	52.9	58.8	6.0	2.4	8.3	13.1	30.9	6.6	22.5	17.4	6.6	19.3	365.6
MA5	22.0	55.9	61.9	6.0	2.6	8.5	12.9	30.7	6.3	21.0	16.4	6.3	18.8	339.0
MC1	22.4	56.8	63.2	6.4	2.6	8.7	13.4	30.8	6.4	23.0	17.8	6.4	19.8	314.9
ME1	21.2	50.3	55.6	5.3	2.4	7.5	16.0	33.9	7.0	23.4	18.1	7.0	16.0	232.1
ME10	21.7	52.3	58.3	6.0	2.4	7.6	14.8	23.5	6.4	22.8	18.4	6.4	13.9	238.0
ME11	21.4	52.5	58.6	6.0	2.5	6.9	15.4	26.7	6.2	21.6	17.0	6.2	11.5	231.7
ME12	20.2	47.9	53.4	5.5	2.4	7.0	14.6	23.8	6.0	23.4	16.5	6.0	9.6	247.4
ME13	20.2	56.6	62.7	6.1	2.8	7.5	13.3	22.9	6.1	19.9	15.1	6.1	10.3	245.4
ME14	20.9	49.2	54.1	4.9	2.4	7.7	12.8	20.1	6.2	20.3	14.9	6.2	11.0	224.9
ME16	18.3	52.4	57.9	5.5	3.0	6.7	13.5	22.6	6.0	20.5	14.3	6.0	9.1	251.7
ME17	20.4	54.6	59.3	4.7	2.7	8.4	15.1	26.6	6.0	22.1	17.2	6.0	13.0	290.4
ME18	20.4	48.9	54.6	5.6	2.4	8.1	15.2	23.4	6.1	22.8	18.2	6.1	9.8	250.9
ME19	20.1	47.8	52.1	4.3	2.4	7.7	15.2	27.0	6.2	22.4	18.9	6.2	9.8	248.8
ME2	22.2	50.6	56.2	5.6	2.3	8.9	16.1	35.1	7.0	23.1	17.7	7.0	15.3	209.7
ME20	20.5	54.6	59.4	4.9	2.7	8.5	14.4	21.2	6.1	20.9	15.6	6.1	7.5	246.1
ME21	21.2	47.2	52.4	5.2	2.3	7.5	15.1	25.6	6.4	20.6	17.1	6.3	10.6	244.6
ME3	23.2	49.6	55.5	5.9	2.1	7.8	17.4	31.7	7.4	23.2	18.7	7.4	12.5	202.4
ME31	22.6	53.1	58.8	5.7	2.4	8.3	17.1	31.6	6.6	21.2	17.8	6.6	10.9	208.3
ME4	22.8	48.8	54.3	5.6	2.2	8.2	13.5	27.2	6.4	22.6	17.7	6.4	18.4	228.5
ME5	22.9	54.0	60.4	6.3	2.4	8.4	16.7	28.6	6.3	22.1	18.7	6.2	15.2	280.9
ME6	21.8	51.5	57.6	6.2	2.4	7.8	17.8	29.3	6.1	22.2	17.6	6.1	15.0	299.2
ME7	21.9	52.4	58.4	6.0	2.4	7.8	16.7	29.3	6.2	23.8	18.6	6.2	13.5	250.1
ME8	22.3	52.2	57.7	5.5	2.4	8.0	14.6	25.8	6.2	22.4	16.8	6.2	18.4	251.4
ME9	20.8	50.0	55.6	5.6	2.4	7.5	15.8	29.3	6.2	22.8	18.7	6.2	14.8	250.1
MO2	21.7	48.8	54.1	5.4	2.3	9.0	14.6	22.2	6.4	20.7	17.1	6.3	8.0	255.8
MO3	22.5	56.1	62.2	6.1	2.5	8.5	16.8	30.9	7.8	22.5	17.1	7.8	17.7	364.4
MO4	23.3	54.9	60.8	5.9	2.4	8.5	14.2	31.4	7.5	24.6	18.3	7.5	19.2	356.0
MO5	23.0	56.2	62.6	6.4	2.5	8.8	15.2	34.4	7.5	24.3	18.8	7.5	21.5	383.0
MO6	21.7	54.3	60.1	5.7	2.5	8.1	11.8	30.6	6.7	20.6	15.8	6.7	15.5	328.3
PB1	21.7	61.0	66.1	5.1	2.9	8.6	10.6	33.9	6.7	22.5	18.5	6.7	14.3	212.8
PDO2	19.9	52.6	58.2	5.6	2.7	8.0	11.8	34.1	6.8	25.7	20.5	6.8	14.1	206.2
PG	17.5	51.3	57.5	6.1	3.0	7.0	11.9	34.7	6.6	19.6	17.5	6.6	15.9	240.4
PTB1	20.8	60.2	66.1	5.8	2.9	9.1	13.1	35.3	7.6	19.9	15.7	7.6	16.4	324.8
PTO2	19.2	52.5	57.6	5.2	2.7	7.4	12.0	31.8	7.1	21.5	15.9	7.1	11.9	365.5
PTO3	20.1	49.3	54.2	4.9	2.5	7.6	11.6	29.6	6.8	21.2	16.2	6.8	10.0	380.2
PTO4	18.5	49.1	54.6	5.5	2.7	7.0	12.0	35.6	7.8	20.9	16.5	7.8	13.4	312.7
PTO5	18.8	47.2	52.4	5.2	2.6	6.8	11.8	35.1	7.1	25.6	18.3	7.1	11.8	422.2
PTO6	21.2	54.2	58.5	4.3	2.6	10.0	12.9	35.6	8.4	18.7	13.6	8.4	14.4	327.4
PTO7	20.0	47.1	52.2	5.1	2.4	6.9	10.4	31.4	7.7	19.7	15.0	7.7	8.9	364.0
PTO8	19.5	52.5	57.9	5.3	2.7	7.6	12.5	34.2	7.6	19.1	14.3	7.6	15.4	297.8
SFB1	20.7	55.3	60.7	5.4	2.7	8.0	14.4	36.2	7.0	24.0	18.8	7.0	12.4	289.6
VA1	20.5	57.9	63.9	5.9	2.8	7.9	11.9	28.4	6.2	20.3	15.7	6.2	14.5	356.6

For explanation of character symbols, see Material and methods

Table 6. Analysis of variance of each variable analyzed within each group of varieties studied

Fruit characteristics										
	FW	FD1	FD2	FL1	FL2	FL3	Nc	PcMc	Ec	Rs
ADO	**	*	ns	*	ns	*	ns	ns	ns	**
CRO	**	*	ns	ns	ns	ns	ns	ns	ns	*
MA	ns	ns	***	ns	ns	ns	ns	ns	**	**
ME	***	***	***	***	***	**	***	**	***	***
MO	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
PTO	**	***	**	**	***	**	ns	*	**	***

Aril, seed and juice characteristics												
	SW	SL	Sw	l	w	wpw	wpi	JV	pH	TSS	A	MI
ADO	***	***	***	*	***	**	***	*	ns	ns	ns	*
CRO	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	**
MA	***	***	*	***	***	***	***	ns	ns	ns	ns	ns
ME	***	***	***	***	***	***	***	ns	ns	ns	ns	**
MO	ns	**	**	***	*	***	***	ns	ns	ns	ns	ns
PTO	***	***	***	***	***	***	***	ns	***	**	***	***

	Leafs						Flowers							
	LW	Ll	Lt	Lp	Ll/LW	LS	FD	FL	Np	Lp	Wp	Ns	LS	NS
ADO	***	***	**	ns	ns	ns	***	***	ns	ns	***	ns	ns	ns
CRO	ns	***	***	ns	**	*	ns	ns	**	**	ns	**	ns	ns
MA	**	**	**	*	***	ns	ns	***	***	***	***	***	ns	***
ME	***	***	***	***	***	***	***	***	***	***	***	***	***	***
MO	***	***	***	***	***	*	***	***	***	***	***	***	***	***
PTO	***	***	***	***	***	***	***	***	***	***	***	***	***	***

*, **, *** and 'ns' indicate significant differences at P<0.05, P<0.01, P<0.001 levels as well as non-significant, respectively.
For explanation of character symbols, see Material and methods