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Phenotypic and pomological characterization of a pomegranate (*Punica granatum* L.) germplasm collection and identification of the promising selections

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and has gained importance mainly because of its antioxidant properties. The present research was carried out on 100 pomegranate genotypes from Saveh part in Markazi province from Iran, to select the promising genotypes based on the morphological and pomological traits. A large variability was found among the pomegranate genotypes studied. The fruit ripening time in most of the studied genotypes was late. Fruit weight varied widely between 106.60 g and 496.91 g with an average of 272.78 g. Furthermore, 100 arils fresh weight ranged from 24.49 g to 51.32 g. Seven genotypes were determined as real soft-seed and are suitable in this important trait to be used for cultivation and/or in breeding programs for decreasing seed hardness in the commercial pomegranates. Fruit weight was positively correlated with fruit length, fruit width, fruit crown length, fruit crown diameter, fruit peel thickness, 100 arils fresh weight, and aril juice. Referring to the characters related to fruit quality, the genotypes ‘Saveh-69’, ‘Saveh-39’, ‘Saveh-36’, ‘Saveh-21’, ‘Saveh-41’, ‘Saveh-4’, ‘Saveh-83’, ‘Saveh-29’, ‘Saveh-2’, ‘Saveh-40’, ‘Saveh-35’, ‘Saveh-68’, ‘Saveh-15’, ‘Saveh-16’, ‘Saveh-71’, ‘Saveh-51’, ‘Saveh-50’, and ‘Saveh-58’ seem to be the most promising for fresh consumption since they are characterized by high fruit quality including soft seed with low acidity and high TSS contents.

1. Introduction

Pomegranate (*Punica granatum* L.) is a deciduous fruit tree, and its cultivation has been greatly expanded into several countries in recent years. The growing interest in this fruit is not only due to the fact that it is pleasant to eat, but 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 (Calani et al., 2013; Melgarejo-Sanchez 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 juice, jams, and extracts (Melgarejo-Sanchez et al., 2015).

The pomegranate's place of origin is the Central Asia, from where it has spread to the rest of the world (Mediterranean Basin, Southern Asia and several countries in North and South America). This is a temperate-climate species that requires high temperatures to mature the fruit 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 has led to its wide dispersion in this geographical area and to the appearance of a multitude of new, local individuals over time beginning with specific cultivars (Naeini et al., 2006; Martinez et al., 2006). Commercial plantations are concentrated in Iran which is considered as one of the biggest producers and exporters of pomegranate fruit in the world (Alighourchi and Barzegar, 2009).

The new pomegranate cultivars have been grouped under the same denomination; however, each one of them could have different agronomic characteristics as compared with their original progenitor. For instance, Melgarejo and Salazar (2003) observed that under the denomination of ‘Mollar de Elche’ (ME), there were cultivars with different agronomic characteristics. In order to better identify the genotypes, Verma et al. (2010) have mentioned the importance of agronomic-characterizing of 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 cultivars, as well as for improving agronomic characteristics of the crops.

Morphological characters are the first choice for describing and classifying the germplasm and are used for selection of parents in a

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targeted breeding scheme. Statistical methods including principal component and cluster analyses can be used for screening the genotypes. The usefulness of measuring morphological traits and chemical compounds in fruits has been revealed to determine the genetic diversity of pomegranate in different studies (Mars and Marrakchi, 1999; Mansour et al., 2011; Karimi and Mirdehghan, 2013; Khadivi-Khub et al., 2015).

Classifications have focused on the pomological characteristics such as color, shape, and other agronomical characteristics of the fruit (Ercan et al., 1992; Ozkan, 2005; Orhan et al., 2014). Aril color, seed hardness, maturity, juice content, acidity, sweetness, and astringency are considered among the most important traits (Stover and Mercure et al., 2007). For instance, ‘Hicaznar’ is the most popular cultivar in different regions of Turkey and has very appealing properties for consumers (Caliskan and Bayazit, 2013). In addition, ‘Wonderful’ is the most popular cultivar in USA and considered among the most deeply colored of pomegranates in both peel and juice, with rich flavor, and high juice yield. Furthermore, ‘Mollar de Elche’ is a cultivar grown in Spain with high yield, excellent internal fruit quality, large size, long harvest period, and great consumer acceptance (Costa and Melgarejo, 2000; Stover and Mercure et al., 2007; Caliskan and Bayazit, 2013). Large variability and sometimes unusual features such as spur-type growth habit, double flowers, and white flowers are observed in Chinese cultivars (Verma et al., 2010). Evergreen cultivars are also known and have been reported to be originated from India (Singh et al., 2015). Seedless fruit is a desirable economic trait for pomegranate that improves the consumptive qualities of fruits (Mars, 2000). Some of the new selections are found to be soft-seeded compared with old varieties (Levin, 1994), a trait often called “seedless” (Stover and Mercure et al., 2007).

Iran is one of the most suitable areas for diversity and cultivation of pomegranate in the world. Moreover, large areas of this country within the boundaries of the two deserts that occupy the central Iranian plateau have arid or semiarid conditions that make them suitable for pomegranate production. In the present work, morphometric compound measurements have been focused that will allow us to gain basic of a pomegranate germplasm collection grown under homogeneous conditions. The current results could lead us to further characterize the studied collection through genetic analysis. Therefore, the objective of the present work was to determine the phenotypic and pomological variability that exists among all the different genotypes of pomegranate, to understand the degree of variation in the morphometric characteristics among the genotypes, to establish the existing variability among the genotypes from the same condition and finally to select the promising selections.

2. Materials and methods

2.1. Plant material

The present research was carried out on the 100 pomegranate genotypes in a germplasm collection from Saveh part in Markazi province from Iran. Saveh region was selected because of its potential as one of the main regions of pomegranate production. Pomegranate trees were grown under homogeneous conditions in a loamy clay soil with the spacing of 5 × 4 m. The annual mean temperature in 2015 was 20 °C, with mild winters (5.70 °C in January) and hot summers (34 °C in August). A low annual precipitation of 84 mm was recorded, mostly falling in winter and autumn. In addition, the annual mean temperature in 2016 was 21 °C, with mild winters (5.90 °C in January) and hot summers (35 °C in August). A low annual precipitation of 95 mm was recorded, mostly falling in spring and autumn.

2.2. Morphological and pomological evaluation

The characters related to tree, flowers, leaves, fruit, and seeds were measured to have more complete information for determining the phenotypic and presumably genetic diversity among all the genotypes for two consecutive years. Morphometric measurements were carried out on samples from 20 mature fruits, 25 seeds, 50 leaves and 25 flowers from each genotype. At full boom stage, flowers were taken from tree representing each genotype and the petal color was recorded. For fruit characters, fruit samples per genotype were randomly harvested from fruitful trees for analysis. The present research work had the advantage that the data used were taken from the trees that were planted in the same field thereby avoiding any edaphoclimatic effect on the results.

Dimensions (length, width, diameter, and thickness) for leaf, fruit, aril, peel, crown and neck were measured with a digital caliper. The fruit weight, 100 aril fresh weight and aril weight were measured by an electronic balance with 0.01 g precision. The fruit juice was used for analyzing total soluble solids (TSS). The total soluble solids content (TSS) was determined on replicate juice samples per fruit using a temperature compensating hand-held Atago hand refractometer (ATAGO, Japan) in ∘Brix. The content of juice was determined by pressing 100 g of arils. In addition, some characteristics such as tree growth habit, petal color, fruit shape, fruit peel color, aril color and seed softness (soft, semi-soft, semi-hard and hard) were considered according on rating, panel test and coding based on the method developed by Mars and Marrakchi (1999).

2.3. Statistical analysis

The differences among the genotypes were evaluated by analyzing the different characters using SAS software (SAS® Procedures, 1990) via one-way ANOVA ($P < 0.01$). The method used to discriminate among means was Duncan’s test (Multiple Range Test). The following parameters were evaluated for the variables: mean, minimum value, maximum value, standard deviation (SD) and coefficient of variation (CV %). Coefficients of variation (CV%) were determined as indicators of variability. Correlations between the traits were determined using the Pearson correlation coefficients by SPSS software. Relationships among the genotypes were investigated by principal component analysis (PCA) using SPSS statistics software. Mean values were used to create a correlation matrix from which standardized principal component (PC) scores were extracted. To avoid the effects due to scaling differences, mean of each character was normalized prior to cluster analyses using Z scores. To better understand the patterns of variation among the genotypes, distance matrix generated from morphological data was used as input data for cluster analysis using PAST statistics software (Hammer et al. 2001). Cluster analysis was applied to standardized data for hierarchical associations employing Ward’s method for agglomeration and the squared Euclidean distance as dissimilarity measure. In addition, a scatter plot was created according to the PC1 and PC2 using PAST statistics software.

3. Results and discussion

3.1. Morphological description

The analysis of variance (ANOVA) showed a large variability among the genotypes studied. Minimum, maximum, average, standard deviations and coefficients of variation values calculated for the studied traits are presented in Table 1. Fruit cracking exhibited the highest coefficient of variation (CV = 369.50%); because most of the genotypes (90 out of 100 genotypes) did not show fruit cracking. Furthermore, the TSS showed the lowest CV (4.70%).

The range of CV for the characters related to the tree was from 24.47 to 65.41%. The characters including tree growth vigor, canopy density, shoot density and shoot branching were intermediate in most of the genotypes. Upright growth habit was predominant (88 genotypes) (Table 2). Only one genotype had one trunk, while most of them