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Short Note

Selected pomegranate germplasm from Afghanistan: morphological variability and relationship among collected accessions

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Abstract: During the period 2008-09 and in the frame of PHDP 58 Afghan and 18 foreign pomegranate accessions have been collected and grown under homogeneous environmental and cultivation practice conditions in *ex situ* collections. Standardized procedures were adopted to describe mature trees, leaves, flowers and fruits for a total of 30 phenotypic traits. Within the National Collection of Pomegranate of Afghanistan, coefficients of variability ranged from 8.8 to 31.7% for fruit diameter and weight of non edible part, respectively. Principal component analysis revealed the absence of correlated variables among different organs. The whole set of accessions resulted discriminated on the basis of the studied morphological parameters and all the accessions were grouped into 3 sub-sets by hierarchical cluster analysis. Local accessions resulted distributed in the 3 clusters, nevertheless the largest one held all the foreign varieties while the second one included all the accessions collected under the putative name of 'Bedana'. The adopted morphological studies allowed to identify one true-to-type 'Bedana' accession, considered the best Afghan variety for fresh consume due to its very soft seed, and to solve the cases of homonymy. Analogously, various accessions originally collected from different regions of Afghanistan under the name of 'Kandahari' were identified and renamed.

1. Introduction

The market of pomegranate fruits is noticeably expanding worldwide. The increasing interest towards this photophilous and moderately xerophytic species relies essentially on it nutritional and nutraceutical properties, being considered a functional product of relevant benefit for the prevention of human diseases (Martinez *et al.*, 2012; Kotsiou and Tesseromatis, 2015). *Punica granatum* L. is native to Iran (Teixeira da Silva *et al.*, 2013), nevertheless a western area (which includes part of Anatolia, the Caucasus and Iran) and an eastern area (southeastern Turkmenistan, southern Tajikistan, Afghanistan, northern Pakistan, Kashmir, northern India and Nepal) are generally recognized (Alam, 2011). Due to its hardiness pomegranate naturalizes quite easily and many local landraces and varieties can be found in the area of the Mediterranean basin, in Middle east and in the Arabic peninsula (Al-Sadi *et al.*, 2015). Iran embrace a rich germplasm of pomegranate, with 760 specimens, 24 genotypes and various commercially important cultivars listed in different studies, nevertheless also India, Israel, Egypt, Tunisia, Morocco, Spain and Italy hold valuable genetic resources of *Punica granatum* (Holland *et al.*, 2009).

More than 20 varieties of pomegranate (locally called 'anar'), among which 'Bedana' (soft seeded), 'Khog Dandar', 'Tarwah', 'Boocha', 'Pastakai', and 'Soor', were grown in Afghanistan during the second half of the XX century (Alam, 2011). More recently, 48 cultivars of pomegranate from various provinces of Afghanistan were described and characterized in relation to fruit colour, flavour and seed hardiness

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(Samadi, 2008); among them, 'Kandahari' and 'Bedana' were considered excellent cultivars (Glozer and Fergusson, 2008).

In the frame of the Perennial Horticulture Development Project (PHDP - Phase I), several pomegranate producing areas of Afghanistan were surveyed during the period 2007-2008 and a set of the most valuable varieties was collected (Giordani *et al.*, 2014). The *in situ* pomegranate collection resulted comprehensive of 59 local accessions which were propagated by cuttings. The obtained saplings were planted in *ex situ* collections, together with 19 imported cultivars in Kandahar and Jalalabad, thus representing the National Collection of Fruits and Nut of Afghanistan (Saeedi *et al.*, 2012).

Morphological characterisation of native germplasm accessions is the first step for their rational introduction in modern and efficient production chains and a standardized description of phenotypic traits based on the adoption of specific descriptor lists is a pre-requisite for this purpose (Frankel, 1970).

The present study aims at describing and analysing the pomegranate accessions belonging to the National Collection of Varieties of Fruits and Nuts of Afghanistan.

2. Materials and Methods

Plant material

Data were collected during 2013 and 2014 from self-rooted 4 years old trees grown in the repository of the National Collection of Fruits and Nuts of Afghanistan located in Kandahar (latitude 31°36' N and longitude 65°42' E; altitude 1009 m a.s.l.). For each accession flowers (10 samples), fully expanded leaves (10 samples) and fruits (20 samples) were randomly collected for two consecutive years (2013-2014) from trees (6 samples) grown following standardized cultural practices.

Data collection

Traits were described by rating and coding procedures based on the EC 29 GENRES Project Pomegranate descriptor list and guidelines (GENRES, 1998; Bellini *et al*., 2008). Quantitative variables were measured and weighed adopting a manual calliper and a precision (0.01 g) electronic balance, respectively. Colour parameters were visually determined using a specifically designed colour chart; other qualitative characteristics were attributed by using illustrated charts. Data of 13 qualitative characteristics (tree habit, vigour and suckering tendency; shoot thorniness; leaf apex shape; number of flowering periods; flower calyx and corolla colour; colour change of calyx after petal fall; fruit juiciness and fruit skin colour and thickness; seed colour) and 17 biometrical variables (Table 1) were collected for all the studied accessions.

Table 1 - Mean values, ranges and coefficients of variation for 17 quantitative traits of pomegranate accessions of the National Fruit and Nuts Collection of Afghanistan

Variable	Mean	Range	CV (%)	Standard deviation
Leaf - Total length (mm)	68.4	52.6-86.2	11.5	7.9
Leaf - Blade length (mm)	63.2	48.0-78.5	11.5	7.3
Leaf - Blade width (mm)	20.7	16.2-26.7	11.7	2.4
Flower - Petal length (mm)	26.1	19.2-30.8	9.1	2.4
Flower - Petal width (mm)	19.9	14.3-30.2	13.4	2.7
Flower - Length of calyx (mm)	38.5	29.5-46.7	10.2	3.9
Flower- Width of calyx (mm)	13.8	9.1-24.3	14.4	2.0
Fruit - Equatorial diameter (mm)	84.5	64.9-99.2	8.1	6.8
Fruit - Diameter of calyx (mm)	21.1	11.6-35.3	19.6	4.1
Fruit - Height without calyx (mm)	76.4	56.7-94.0	8.8	6.7
Fruit - Calyx height (mm)	23.2	14.8-34.6	18.6	4.3
Fruit - Total weight (g)	309.3	134.8-490.1	24.3	75.1
Fruit - Weight of non edible part (g)	144.6	76.6-323.1	31.7	45.8
Fruit - Edible/Total weight (%)	53.8	18.7-70.8	13.5	7.3
Seed - Weight (g)	0.3	0.2-0.4	18.8	0.1
Seed - Length (g)	10.3	8.0-13.6	8.9	0.9
Seed - Width (mm)	6.8	4.9-8.4	12.2	0.8

Data processing and statistical analysis

Average and mode were calculated for all qualitative and quantitative parameters respectively; such values were used for the attribution of each accession to one class, regardless the year of data collection. Classes and relative notes were defined for each quantitative measured trait. After average value standardization, Multivariate Principal Component Analysis and Hierarchical Cluster Analysis (Euclidean distance and Ward's agglomeration method) were performed on XLS Stat software.

3. Results and Discussion

Studied accessions

The survey allowed to collect 58 Afghan accessions (Fig. 1) in stands represented by orchards and home gardens distributed in the Eastern (Kapisa, Nangarhar; 29 accessions), Southern (Kandahar and Farah; 16 accessions) and Northern (Takahr, Balkh and Sar-e-Pul; 13 accessions) areas, at altitudes ranging from 422 m a.s.l. to 1513 m a.s.l. Such accessions

were grown together with 19 imported varieties including the international reference cultivar 'Wonderful'.

Morphologic and phenological trait variation

Descriptive statistical values for continuous biometric data of the whole set of 77 accessions are reported in Table 1. Leaf size showed a relatively low variation (CV% ≈ 11.5 for total leaf length, length and width blade), as well as flower biometric traits, while higher values of variations were observed in fruit and seed traits. Namely, minimum and maximum mean of fresh fruit weight (≈ 134-490 g) fall within the range of analogous values observed by Okatan *et al*. (2015), Caliskan and Bayazit (2013) in pomegranate collections from Turkey and by Mars and Marrakchi (1999) in a set of 16 accessions collected in Tunisia. On the other hand, Orhan *et al*. (2014), Mondragòn Jacobo (2012), Bartual *et al.* (2012), Ferrara *et al*. (2012), Russo *et al*. (2012), and Legua *et al*. (2012) observed higher mean values of fruit weight in accessions collected in Coruh Valley (Turkey), in Mexico, in Elche (Spain), in Apulia (Italy) and in Morocco, respectively. A narrower range of variability for this parameter (204-288 g) was observed in a study on Iranian accessions (Theranifar *et al*., 2010). Seed (aril) fresh weight (mean 0.33 g; range 0.17-0.33 g), resulted higher than the values reported by Okatan *et al*. (2015) (0.28 g; range 0.14-0.41 g) for Turkish accessions and by Mondragòn Jacobo (2012)(0.20 g; range 0.12-0.26 g) for Mexican accessions, but lower than those found out by Caliskan and Bayazit (2013) in Turkish pomegranate accessions (0.42 g; range 17-67 g) and by Ferrara *et al*. (2012) (0.38 g; range 0.27-0.51) in Apulian pomegranates in Italy. The ratio between the edible part of the fruit (arils) and the total fresh fruit weight ranged from 18.7% to 70.8%, with an average of 53.8%, lower than the value (55.6%) found by Legua *et al*. (2012) in accessions from Morocco.

The beginning of flowering time ranged from 104 to 115 days from the beginning of the year while day 253 resulted the earliest date of the beginning of ripening time (Fig.1 A, B). The range of flowering and ripening time of the investigated accessions resulted of 11 and 44 days, with a discontinuous frequency for ripening time between days 269 and 284 (Fig. 1 B). The ripening time period (end of September-October) resulted similar to the Spanish and Turkish one; no significant correlation has been found between fruit size and ripening time, a pattern observed by different authors in the germplasm of other areas (Holland *et al*., 2008; Martínez *et al*., 2012; Caliskan and Bayazit, 2013).

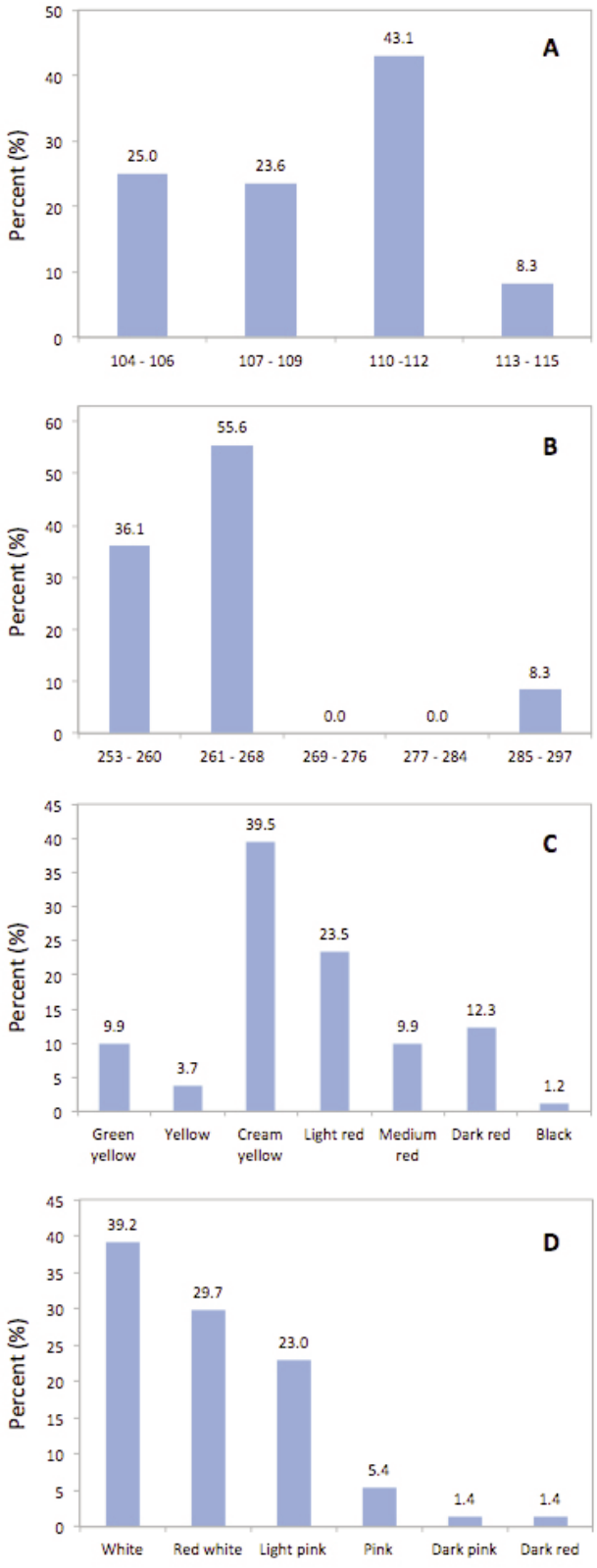

Fig. 1 - Distribution (%) of the collected pomegranate accessions in relation to: a) beginning of flowering time (day of the year); b) beginning of ripening time (day of the year); c) fruit skin color and d) fleshy part of seed (aril) color.

Attaining to colour fruit traits, which are important quality attributes in pomegranate marketing (Mena *et al.*, 2011), most of accessions presented fruits with yellowish skin (53.1%), followed by reddish colouration (45.7%), and only 1.2% of accessions showed totally black skin colour. Fleshy part of seeds was in most cases white (39.2% of accessions), while pinkish and red-white colour shared the same percentage (≈30%) of accessions and only 1.4 % of varieties showed dark red arils (Fig. 1 C, D).

Relationship between the described accessions

Principal Component Analysis based on the averages of the discrete scores of the morphological qualitative and quantitative variables showed a low correlation between the utilized variables, with 12 principal components with eigenvalue higher than 1, all together accounting only for 76.8% of the variability. Taking into account the sub-set of 19 biometric continuous variables regarding leaves, fruits, and seeds, the 6 principal components with eigenvalue>1 accounted for 78% of the whole variability, confirming a very low correlation among variables for the same set of accessions. Higher levels of correlation among the biometric variables have been reported for different groups of Turkish (Caliskan and Bayazit, 2013; Okatan *et al.*, 2015) and Tunisian (Mars and Marrakchi, 1999) local varieties.

The average Euclidean distance of the generated proximity matrix based on the 17 biometric variables resulted equal to 5.96±1.21, ranging from 1.99 observed in the pair 'Bedana Samashkeli - Jan Mohammadi' up to 11.67 for the couple 'Sorkhak-Sakerdze'. The dendrogram generated by the Cluster Analysis is reported in figure 2, where three main significant clusters (C1, C2 and C3) can be observed. Cluster C1 resulted the largest with 35 accessions, 19 of which represented by foreign non Afghan accessions, including the international cultivar 'Wonderful'.

Cluster C2 was constituted by 19 accessions, 6 of which were originally collected as 'Bedana', a typical Afghan variety appreciated by the presence of very soft seeds. Albeit this easily appreciable and discriminating trait, the standardized characterisation of the presumed 'Bedana' accessions showed that only one accession could be identified as belonging to the true 'Bedana' type. The accession collected in Kandahar and entered in the collection as 'Bedana-AFG0383' showed heavy and hard seeds and other relevant differences from the 'Bedana' type, such as big calyx, pale skin colour and low juiciness, and it has been

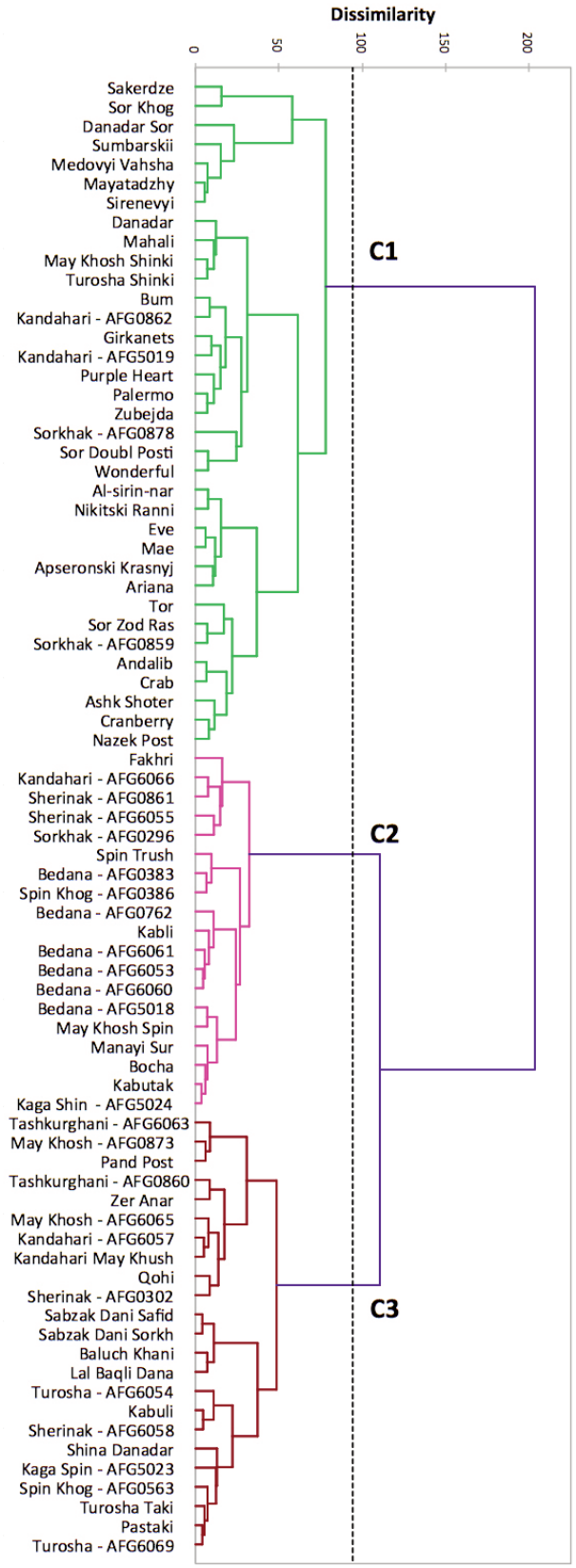

Fig. 2 - Dendrogram (Euclidean distance; Ward's agglomerating method) obtained by analyzing 30 multi-state discrete morphological variables of 58 pomegranate accessions of Afghan origin and 19 imported varieties belonging to the National Collection of Fruits and Nuts of Afghanistan (bold: imported cultivars; bold cursive: putative 'Bedana' type; cursive: putative 'Kandahari' type).

identified as 'Mirwais Khani' (Fig. 2). Similarly the accession originally denominated 'Bedana-AFG0762', brought medium hard seeds, white-pink arils, and was renamed as 'Abasi'. Also 'Bedana-AFG5018', collected in Tabag district of Kapisa Province, and 'Bedana - AFG6061', originated from Sherzad district of Jalalabad Province, did not show the typical characteristics of the true-to-type 'Bedana', and were renamed as 'Ghani Kheli' and 'Jan Mohammadi' respectively. Finally, the presumed 'Bedana' type 'AFG6053', originated in the district of Jalalabad, notwithstanding its high morphological similarity with the true-to-type 'Bedana' (soft seeds, juicy and good taste), showed low productivity and was denominated 'Bedana Samaskheli'.

Cluster C3, holding 23 local accessions, is not evidencing any special relationship among previously known cultivars.

During the survey, a part from 'Bedana' name, another recurrent denomination attributed by farmers to local accessions was 'Kandahari' (from Kandahar). Indeed 'Kandahari' accessions were collected from different provinces of Afghanistan and they showed different and unique phenotypic traits during the characterisation hence they have been considered as different varieties. For instance, 'Kandahari - AFG6066' originated from Sher Zad district of Nangarhar Province, had thin fruit skin and hard seeds, and it was renamed as 'Rahmani'; 'Saifi' was the accepted name for the former 'Kandahari - AFG5019', collected in Tagab district (Kapisa province) producing fruits with hard seeds, reddish arils and low juiciness. 'Mukhtari' was originally 'Kandahari - AFG0862' from Khulam district (Balkh Province) with medium hard seeds and white arils. Actually 'Kandahari' name was kept only for the accession collected as 'Kandahari - AFG6057' originated from Tagab district (Kapisa Province) and producing large fruits with medium hard skin, medium hard seeds and red arils.

4. Conclusions

This study was the first of its kind carried on for pomegranate in Afghanistan in terms of standardized methodology; comparative analysis with previous data collected from local Afghan varieties of pomegranate trees grown in different environmental conditions and as unique individuals resulted not completely reliable. The results revealed a wide variability of morphological traits within the Afghan pomegranate collected accessions; this finding is consistent with similar researches carried on local pomegranate accessions in countries where *P. granatum* is present since ancient time. Such variation can be considered as a result of the combined effect of sexual reproduction and asexual propagation. On the other hand, many cases of homonyms have been observed, hence confirming a common trend in the denomination of varieties by farmers of countries where perennial species are grown traditionally.

More specific morphological studies associated to the evaluation of tree productivity and fruit biochemical and commercial traits would be the next step in order to better classify the Afghan pomegranate accessions in relation to their main suitability for different uses (fresh fruit consume, juice production, food supplements, etc.).

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