

## FRUIT CHARACTERISTICS BASED DIVERSITY IN POMEGRANATE ACCESSIONS OF AFGHANISTAN

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Pomegranate (*Punica granatum*) is a major fruit in Afghanistan, having a long history as wild and domesticated throughout the country. Best fruit accessions were evolved in the world through selection and utilization of genetic resources that were missing in the country. In our studies, various morphological (qualitative and quantitative) traits were recorded in fruits of 20 pomegranate accessions from Afghanistan. Principle component analysis (PCA) proved a high level of morphological diversity 93% in Afghan pomegranate germplasm with maximum variability covered in the first six components (84.03%) with the highest share of fruit and aril weight and wood portion index. The result of the first three components showed the variable PCA percentage 29.72%, 17.74% and 12.08%, respectively among pomegranate accessions based on the different quantitative traits. Our results concluded that there is a great diversity among the twenty pomegranate accessions from Afghanistan. Morphological diversity estimated in Afghan pomegranates makes it possible to improve genotypes. Hence these accessions can be taken for further breeding program by researchers.

**Keywords:** *Punica granatum*, diversity, germplasm, morphology, multivariate analysis.

### INTRODUCTION

Pomegranate (*Punica granatum* L.  $2n = 2x = 16, 18$ ) belongs to genus *Punica* of family Punaceae. It is a deciduous or ever green plant, native from Iran to the Himalayan belt (Stover and Mercure, 2007). Having high vitamin C, potassium, polyphenols, organic acid, fiber contents with low calories, and is a rich source of antioxidant. It is naturally adopted in a region with cool winter and hot summer. Semi-arid mild temperature to subtropical climate also favours to pomegranate (Ozgen, 2008). It is not only cultivated as a fruit tree but is appreciated as an ornamental plant with socio-economic and ecological benefits (Gong *et al.*, 2004).

It is among the fruit tree being used by humans for centuries. Its cultivation was started 4000 years before in Persia and Central Asian countries and then it was further cultivated to other arid and hot areas of Asia (McLean *et al.*, 2011). Now a day, it is being cultivated worldwide especially around the Mediterranean belt. The main areas for production and cultivation of pomegranate include Iran, Turkey, India, Afghanistan, Egypt, Spain, Middle East, and Morocco (Jbir *et al.*, 2008). However, Spain ranked first as an exporter since the major producers are Iran, India and USA (Jaime *et al.*, 2013).

Pomegranate is distributed throughout Afghanistan concentrated to Kandahar, Kapisa, Balkh, Farah, Herat and Nangarhar regions with a total area of cultivation of 8450 ha and production is 59150 tons. There are numerous unique accessions in Afghanistan with different skin, aril and taste i.e. Mahali-AFG0884, Sorkhak-AFG0859, Spin Trush, Kandahari-AFG6057, Sorkhak-AFG0878, Be dana, May Khosh Shink, Pastaki, Bedana-AFG6053, Sor Zod Ras etc. Pomegranate germplasm collection, characterization and conservation are the focused area of research worldwide (Stover and Mercure, 2007). Globally, there are 500 pomegranate accessions (IPGRI, 2001). Out of these, fifty are of commercial importance. Different countries have collected pomegranate germplasm, for example, 1157 accessions in Turkmenistan, 760 specimens, genotypes and cultivars in Iran (Behzadi and Shahrabaki, 1997), 40 accessions in Spain (Melgarejo *et al.*, 2000) and 238 in China (Fang *et al.*, 2006). However, Afghanistan has 48 so far described different pomegranate accessions (Samadi, 2011)

Physical traits provide basic information comparing different accessions for the further breeding program. Previously many researchers have characterized different fruit trees i.e. Pomegranate and Date Palm etc, genetic resource using different morphological tools (Hasnaoui *et al.*, 2011; Nafees

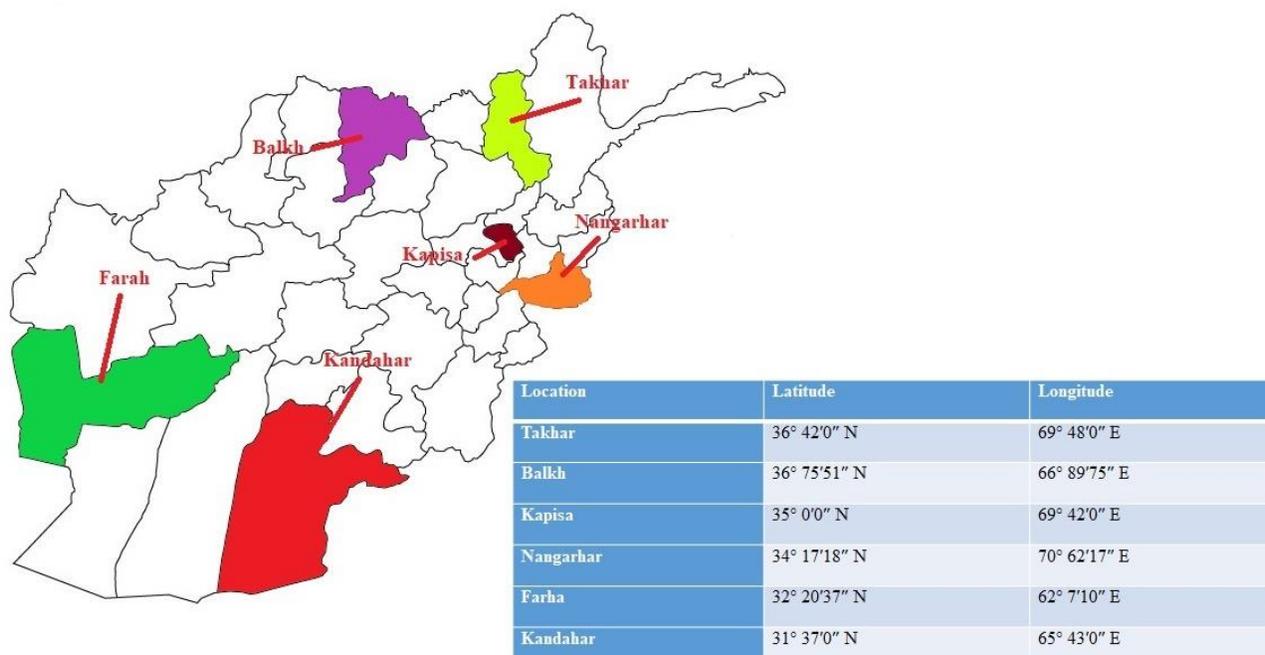
*et al.*, 2018; Naqvi *et al.*, 2015) and observed significant variation within accessions. To our knowledge, limited work has been done to characterize the genetic resources of Afghanistan. Many accessions are conserved at different research station need to be explored. Therefore, the objective of the present research work is to unveil the diversity in Afghani pomegranate accessions based on fruit

morphological traits so as to provide useful information for researchers and stakeholders to deliberate these accessions for future breeding program and economic benefits in Afghanistan.

**MATERIALS AND METHODS**

**Table 1. List of Pomegranate accessions along with site of collection and GPS coordinates.**

Serial No.	Clone Code	Accessions Name	Collection Site	Latitude (N)	Longitude (E)
1	AFG0762	Bedana-762	Kapisa	34° 48' 14"	69° 39' 49"
2	AFG0388	Spin Trush	Kandahar	31° 37' 11"	65° 35' 40"
3	AFG0860	Tashkurghani-860	Balkh	36° 42' 35"	67° 42' 8"
4	AFG6059	Kabuli	Nangarhar	34° 17' 18"	70° 62' 17"
5	AFG5021	Kabli	Kapisa	34° 50' 36"	69° 40' 1"
6	AFG4082	Baluch khani	Farah	27° 32' 12"	77°76' 09"
7	AFG0878	Sorkhak -878	Takhar	36° 34' 18"	69° 52' 2"
8	AFG0763	TuroshaShinki	Kapisa	34° 48' 14"	67° 42' 11"
9	AFG6062	Turosha Taki	Nangarhar	34° 27' 32"	70° 07' 42"
10	AFG6082	Sherinak -861	Balkh	36° 42' 35"	67° 42' 11"
11	AFG6064	Shina Danadar	Nangarhar	34° 27' 32"	70° 07' 42"
12	AFG0859	Sorkhak -859	Balkh	36° 42' 35"	67° 42' 13"
13	AFG0386	Spin khog -386	Kandahar	31° 37' 15"	65° 35' 40"
14	AFG0390	Tor	Kandahar	31° 37' 26"	65° 36' 11"
15	AFG0387	Bum	Kandahar	31° 40' 17"	65° 39' 45"
16	AFG0297	Kabutak	Balkh	63° 0' 48"	67° 41' 36"
17	AFG4081	Fakhri	Farah	27° 32' 12"	77° 76' 09"
18	AFG0345	Bedana-345	Kapisa	35° 2' 57"	59° 2' 57"
19	AFG6063	Tashkurghani_063	Nangarhar	34° 27' 32"	77° 07' 42"
20	AFG0383	Bedana-383	Kandahar	31° 37' 15"	65° 35' 35"



**Figure 1. Map of Afghanistan with collection sites of 20 pomegranate accessions from 6 different provinces. Colored areas indicate regions from where samples were collected.**

**Sampling:** A planned survey and collection mission of pomegranate fruit was carried out in the southern and eastern zone of Afghanistan. Fruits of twenty accessions were harvested at horticultural maturity stages (Table 1). The fruits were shifted to pomology laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad for further studies. Almost five fruits of each accession were harvested based on uniformity in color and size. In total, 13 different morphological characters were recorded according to pomegranate plant descriptor (UPOV, 2011) (Table 2).

**Table 2. Morphological traits of Pomegranate accessions studied**

Characters	Denominations	Unit
Fruit weight	FW	g
Fruit length	FL	cm
Fruit diameter	FD	mm
Crown length	CL	cm
Crown diameter	CD	mm
Rind thickness	RT	mm
Rind weight	RW	g
Seed weight	SW	g
Seed length	SL	cm
Seed width	SWI	mm
Aril length	AL	cm
Aril weight	AW	g
Aril width	AWI	mm

**Morphological traits evaluation:** The thirteen (13) quantitative traits including, fruit length (FL), fruit diameter (FD), fruit width (FW), crown diameter (CD), crown length (CL), skin thickness (ST), seed width (SW), seed length (SL), skin weight (SW), 100 seeds weight in grams, arils width (AW), arils length (L), and 100 arils weight in grams. All the geometric measurements were taken using digital vernier caliper while for weight determination digital weighing balance was used.

**Statistical Analysis:** Morphological data from the twenty accessions of pomegranate were subjected to multivariate (cluster) analysis using XLSTAT (2013) XLSTAT software (version 2013.1).

## RESULTS

**Descriptive statistics and correlation analysis of morphological traits of twenty pomegranate accessions from Afghanistan:** Thirteen morphological traits of pomegranate accessions were assessed. Minimum and maximum values, coefficient of variance (CV) standard deviation (SD) and means as well as outcomes of PCA analysis of morphological traits of 20 pomegranate accessions of Afghanistan is presented in Table 3. Result showed that a significant level of variability among 20 pomegranate accessions according to the different morphological traits. The maximum CV % observed for seed weight was 35.81% followed by seed width (24.97%), seed length (24.29%), fruit weight (22.49%), arils weight (20.36%) and calyx length (19.04%) while, minimum CV % was observed for arils width (9.87%) in all 20 selected pomegranate accessions of Afghanistan (Table 2). Maximum and minimum values of all the morphological parameters from twenty pomegranate accessions were observed. The results illustrated that fruit weight, fruit length and fruit width varied from 75.37 to 254.44 g, 56.11 to 91.15 mm and 54.38 to 95.28 mm, respectively. Diverse values of crown length (13.15-25.25 mm), crown diameter (12.96-21.08 mm), seed weight (0.00-4.51g), seed length (0.00-7.75mm), seed width (0.00-3.65 mm), arils weight (19.97-43.31 g), arils length (8.01-17.26 mm), arils width (5.96-3.39 mm), skin thickness (2.31-3.51 mm) and skin weight (28.11-77.66 g) were recorded in twenty different accessions.

**Correlation Matrix for qualitative variables of pomegranate accessions:** Correlation analysis of morphological traits in all domesticated and wild pomegranate accessions fruit revealed the maximum positive correlation (0.846) of fruits weight

**Table 3. Descriptive statistics of morphological traits in 20 Afghan pomegranate accessions.**

	N	Minimum	Maximum	Mean	SE	SD	CV%
<b>FW</b>	20	75.370	254.440	198.833	9.999	44.719	22.49
<b>FL</b>	20	56.114	91.150	75.331	2.274	10.169	13.50
<b>FD</b>	20	54.380	95.282	78.032	2.574	11.510	14.75
<b>CL</b>	20	13.158	25.250	20.245	0.862	3.855	19.04
<b>CD</b>	20	12.968	21.082	16.493	0.458	2.048	12.42
<b>RT</b>	20	2.314	3.518	2.758	0.082	0.365	13.23
<b>RW</b>	20	28.110	77.668	55.560	2.997	13.402	24.12
<b>SW</b>	20	0.000	4.510	2.512	0.198	0.884	35.19
<b>SL</b>	20	0.000	7.755	6.662	0.362	1.618	24.29
<b>SWI</b>	20	0.000	3.650	2.972	0.166	0.742	24.97
<b>AL</b>	20	8.018	17.261	10.679	0.412	1.842	17.25
<b>AW</b>	20	19.970	43.310	31.570	1.438	6.429	20.36
<b>AWI</b>	20	5.962	8.392	7.086	0.145	0.650	9.17

**Table 4. Correlation matrix for quantitative traits.**

Variables	FW	FL	FD	CL	CD	RT	RW	SW	SL	SWI	AL	AW
FL	<b>0.5951</b>											
FD	<b>0.7388</b>	<b>0.4724</b>										
CL	0.1372	0.3415	0.0713									
CD	0.1699	<b>0.4489</b>	0.1878	<b>0.5519</b>								
RT	0.2798	0.2849	-0.0074	0.3366	0.2200							
RW	<b>0.8455</b>	<b>0.7051</b>	<b>0.5563</b>	0.3276	0.3880	<b>0.5464</b>						
SW	<b>0.4909</b>	<b>0.5187</b>	0.3733	0.4159	<b>0.5092</b>	0.1420	<b>0.4674</b>					
SL	0.3024	<b>0.4444</b>	0.3098	<b>0.4576</b>	<b>0.4529</b>	0.1722	0.3479	<b>0.7329</b>				
SWI	0.4174	<b>0.4911</b>	<b>0.4505</b>	0.4086	<b>0.4562</b>	0.1600	<b>0.4811</b>	<b>0.6653</b>	<b>0.9194</b>			
AL	-0.3857	-0.0339	-0.1895	-0.2326	-0.2690	<b>-0.4490</b>	<b>-0.4642</b>	-0.2610	-0.1422	-0.2267		
AW	0.2981	-0.3346	<b>0.4950</b>	-0.2349	-0.2978	-0.3593	-0.0483	0.0794	-0.2040	-0.1185	-0.2079	
AWI	-0.4050	-0.4319	0.0022	<b>-0.4493</b>	-0.1277	<b>-0.5375</b>	<b>-0.5525</b>	-0.2054	-0.2393	-0.2366	0.2038	0.3369

Values in bold are different from 0 with a significance level alpha=0.05

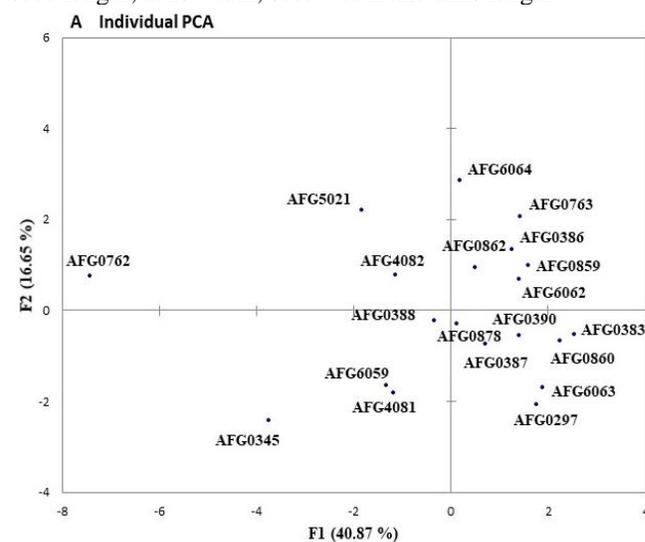
with rind weight followed by 0.739 fruit diameter and 0.595 for fruit length in all selected pomegranate accessions. However, arils length and arils width had a negative correlation with fruits weight while the least correlation (-0.137) was recorded between fruit weight and calyx length (Table 4). Fruit length had the highest correlation (0.705) with rind weight followed by 0.519 and 0.491 for seed weight and seed width respectively; however, negative correlation (-0.41) was observed for arils width. Fruit weight the highest correlation (0.556) with rind weight followed by arils weight (0.495) and arils width (0.51); but, the minimum negative correlation (-0.189) was observed for arils length. Calyx Length the highest correlation (0.556) with calyx diameter followed by 0.458 and 0.44 for seed length and arils width respectively; while, negative correlation (-0.235) was recorded for arils weight. Calyx diameter revealed highest correlation (0.509) with seed weight and negative correlation (-0.298) was observed for arils weight. Rind thickness showed the highest correlation (0.509) with rind weight but, the minimum negative correlation (-0.359) was observed for arils weight.

**PCA for qualitative variables of pomegranate accessions:**

All pomegranate accessions were investigated for 13 morphological traits in multivariate examination displayed in screen plot. Scree plot showed the cumulative variation and eigenvalue (Fig. 2). The eigenvalue is greater and equal to 0.05 that was taken as a level of significance for the calculation of PCA. PCA divided the data into six major components which showed the total variability of 84.03%. The result of the first three components showed the variable PCA percentage 29.72%, 17.74% and 12.08% respectively among pomegranate accessions based on the different quantitative traits.

As an outcome of PCA analysis, all morphological trait difference was enclosed in first six factors description 90.20% variability. The first factor added for 40.86% variation with maximum difference involvement by rind weight followed by seed width, fruit length, seed weight, seed length and calyx. The second factor described 16.5% diversity and observed the

maximum loading arils weight white arils followed by fruit diameter, fruit weight, arils width respectively. Third factor influenced 13.35% variability with the highest contribution of seed length, arils width, seed width and arils length.





AFG0390 and AFG0383 were predominantly large in size and may be selected as dominant parent in cultivar development program. Breeding of sweet and sour cultivar could develop sour and sweet breeding lines for fruit development. Soft seeded Afghan accessions were used for development of soft seeded commercial cultivars in other parts of the world. Our outcomes are in accordance with those of Riyahi *et al.* (2011) and Zamani *et al.* (2010) who stated that the pomegranate varieties with delicate and soft seeds are important choice for breeding program. Different acrid, semi sharp domesticated pomegranate accessions were created from wild pomegranate "Daru" in India with progressive Breeding of 'Ganesh' and "Daru". Number of seeds also cause variability in taste of pomegranate fruit as increase in number of seeds makes the taste of fruit bitter. Holland *et al.* (2009) stated that Indian pomegranate cultivars were improved via consecutive breeding of domesticated and wild pomegranates. Additionally, breeding of cultivars were made for less acidity and enhanced fruit quality, seed softening, disease resistance, fruit weight, aril color, juice production and yield (Samadia and Pareek, 2006; Jalikop *et al.*, 2005; Jalikop and Kumar, 1990; Wavhal and Choudhari, 1985; Karale *et al.*, 1979). Significant relationship of domesticated and wild pomegranate accessions was noted with particular region particularly for cultivar of AFG0388, AFG6064, AFG0383 and AFG0763. Our results are similar with the result of Holland *et al.* (2009) who reported that all genotypes of pomegranate were tamed separately worldwide with particular influence of agro-climatic conditions of the areas; however, subjective expose of pomegranate fruit might be assessed under various climatic conditions (Martinez *et al.*, 2006; Drogoudi *et al.*, 2005; Barone *et al.*, 2001). Quantitative and qualitative morphological traits of pomegranate fruits of different cultivar provide the root to associate genotypes for breeding and selection (Mars and Marrakchi, 1999). These results showed that there was a maximum level of variation in fruit, aril rind, wood portion index and seed weight in domestic and wild pomegranate accessions, respectively. These findings are similar with the work of previous researchers (Ozguven *et al.*, 1997; Ercisli *et al.*, 2007; Hasnaoui *et al.*, 2011; Martinez *et al.*, 2006), who proved maximum position of difference in rind weight, fruit size, color, aril weight, juice contents, seed hardness, acidity, sweetness and fruit maturity in domestic and wild accessions. Highest fruit weight in our pomegranate cultivar was 382.98 g whereas the maximum value (505 g) is described in Iranian accessions. On the other hand, fruit weight of Afghani pomegranate accessions ranged between 75.58 to 254.98 g, while fruit weight range of Iranian and Turkish pomegranate accessions have been reported from 103.38 to 505 g and 69.9 to 795.3 g, respectively (Caliskan and Bayazit, 2013; Akbarpour *et al.*, 2009). The difference in result may be due to different geographical origin, accession type and cultural practices (Caliskan and Bayazit, 2013).

PCA also proved maximum level of morphological diversity in Afghan pomegranate germplasm with maximum variability covered in the first six components with highest share of fruit and aril weight, and wood portion index. Our findings are similar with the finding of Caliskan and Bayazit (2013). AFG0859 AFG0386 AFG859, AFG086. AFG063 were similar for various morphological traits of fruits. In our finding, all Afghan pomegranate accessions (20) assembled in different groups irrespective of the growing conditions. Some accessions from Kandahar, Farah and Nangarhar were found morphologically similar and grouped in same cluster.

**Conclusion:** In conclusion, morphological traits are useful tools for the assessment of phenotypic diversity in pomegranate accessions. Morphological variation estimated in Afghanistan pomegranates creates it possible to develop genotypes with maximum fruit size, fruit and arils weights. Huge variation in important morphological traits of Afghani pomegranate could be helpful for further breeding research on pomegranate. However, accurate diversification of pomegranate accessions from Afghanistan requires a set of morphological, biochemical and molecular markers.

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