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Phenotypic characterisation of almond accessions collected in Afghanistan

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Abstract: Almond [*Prunus dulcis* (Mill.) D.A. Webb] accessions of Afghanistan have been surveyed, propagated, and grown in *ex situ* collections. Trees, leaves, flowers, and fruits were characterised following standardized procedures taking into account 48 phenotypic traits. The National Collection of Varieties of Almonds of Afghanistan showed a significant variability in terms of morphological traits, with a predominance of early flowering time accessions. Among the 56 accessions, Sattarbai, a unique group of cultivar typology characterized by crescent dry fruit, soft and thin "paper shell" and high kernel/dry fruit weight ratio (>0.65) was clearly represented by Cluster Analysis. Other accessions resulted closer to the international cultivars Lauranne, Carmel, Ferraduel and Ferragnes, considered as reference.

1. Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] presumably originated in mountain and desert areas of Central Asia and the Middle East (Martínez-Gómez *et al*., 2007; Gradziel, 2011; Zeinalabedini *et al*., 2012). After its domestication, which possibly occurred during the third millenium BC, it spread towards western areas via seeds carried by caravans along the old Silk Route (Fernandez i Martì *et al*., 2015). In Afghanistan, a geographical area criss-crossed by ancient trade routes and a cultural bridge between East and West, almond is called by its Persian name ''bādām'', similarly to Iran, India, Kashmir, Pakistan, Tajikistan, Tibet, and Turkey, or "badam-e-shirin". Almond is considered a species belonging to Afghan flora (Alam, 2011) and its cultivation has spread widely from ancient times. The presence of bitter tasting seeds is considered a relic of the domestication process (Rigoldi *et al*., 2015), hence the ancient presence of almond in Afghanistan can be corroborated by the occurrence of many bitter tasting seed specimens scattered throughout the Afghan territory. Furthermore, recent studies on almond evolution showed the existence of an unambiguous gene flow of wild almonds from the centre of origin to Iran (Fernandez i Martì *et al*., 2015), neighbouring to Afghanistan. On the other hand, *Amygdalus* species, among which *A. zabulica*, growing spontaneously in a restricted area of Afghanistan, was not considered by Ladizinsky (1999) as a possible almond progenitor because of its fleshy fruits, as well as *A. kuramica*, another species found only in Afghanistan and North West Pakistan; nevertheless Kester *et al*. (1991) reported that this species may bring sweet and "paper shell" fruit types, the latter trait being quite common in Afghan almond germplasm.

Although it has a cold, harsh continental climate, with hot dry summers and cold winters, Afghanistan is an important almond producing and exporting country; about 53,000 t of almonds in shell have been produced yearly in the last five years, representing about 1.8% of total world production (FAOSTAT, 2016). Almond kernels are an important source of nutritional and nutraceutical compounds for local populations, as well as a significant income for the

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country since a substantial percentage of almond nuts are exported to India and other countries of the region (Kaska *et al*., 2006; AAIDO, 2015).

The main production areas of export quality almonds are located in the regions of Samangan, Kunduz, Takhar, and Balkh (AAIDO, personal communication), and almonds are also traditionally grown in the "bagh" (home garden) and cultivated in orchards in almost all the valleys and flatlands of the country. The specific orography of the country, rich in narrow and isolated valleys, the multi-ethnic composition of the Afghan population, together with a primitive traditional agriculture, based in ancient times mostly on sexual reproduction of trees which is a suitable method of propagation for a species with a short juvenile period like almond, were factors that fostered the occurrence of a rich and differentiated local almond germplasm. Furthermore, almond is considered the most diverse species belonging to the genus *Prunus* (Socias i Company and Felipe, 1992) due to its self-incompatibility system (Socias i Company, 1990), the use of open pollinated seedlings in traditional fruitculture, and its adaptability to more diverse microclimates (Fernandez i Martì *et al*., 2015).

Within the framework of the Perennial Horticulture Development Project (PHDP - Phase I), several almond producing areas of the country were surveyed during the period 2007-2008 in order to collect the most valuable varieties (Giordani *et al*., 2014). This was achieved by following the indications of local fruit growers, scientists, and agronomists. The *in situ* collection was comprehensive of 82 local accessions which were propagated by budding. The obtained saplings were planted in *ex situ* replicated repositories in Kunduz and Mazar-i-Sharif, representing the National Collection of Fruits and Nut of Afghanistan.

Usually the primary evaluation of germplasm accessions of fruit tree species, including almond, is based on the observation and standardized description of phenotypic traits of the tree and its main organs, with the adoption of specific descriptor lists (Gülcan, 1985; Talhouk *et al*., 2000; De Giorgio *et al*., 2007; Chalak *et al*., 2006; UPOV, 2011; Rigoldi *et al*., 2015) and suitable uni- and multivariate statistical analyses to study the morphological variability, the relationships among the studied accessions and the correlation between the described traits (Lansari *et al*., 1994; Khadivi-Khub and Etemadi-Khah, 2015). Such approach is essential for germplasm characterisation and protection against bio-piracy, and it is a suitable method for the phenotypic distinction of collected varieties, a basic step for the further development of the almond industry given the high occurrence of synonyms and homonyms among the accessions indicated for commercial propagation and production purposes.

The present study aims to describe and analyse the morphological variability of a set of 56 accessions of almond described in the National Collection of Varieties of Fruits and Nuts of Afghanistan - Almond (Ministry of Agriculture, Irrigation and Livestock of Afghanistan, 2014), in relation to the results achieved by similar approaches on other almond gene pools.

2. Materials and Methods

Plant material

Flowers (n. 10), fully expanded leaves (n. 10), and fruits (n. 20) were randomly collected for two consecutive years from trees (n. 6) of the same accessions grown following traditional cultural practices in the orchards of the National Collection of Fruits and Nuts located in Kunduz (latitude 36.70893 N; longitude 68.86145 E; altitude 405 m a.s.l.) and Mazar-el-Shariff (latitude 36.73232 N; longitude 67.02216 E; altitude 353 m a.s.l.) in Afghanistan. Trees, grafted on almond seedlings, were four to five years old, productive and free of harmful viral diseases; off-type trees observed in the six planting plots were not included in the trial.

Data collection

Morphological and pomological characterisation of genotypes were performed following the Perennial Horticulture Development Project procedures, based on DUS-UPOV and IPGRI guidelines for almond (Gülcan, 1985; UPOV, 2011). Data related to tree characteristics were collected in the field; measurements and descriptions of the other organs were performed in the laboratory. Descriptive traits were determined based on rating and coding according to the adopted almond descriptor. Data were entered in Microsoft Excel® sheets, designed for the purpose with multiple choice notes for qualitative traits or open fields depending on the descriptor typology. 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 such as organ shape were attributed by using illustrated charts.

Data processing and statistical analysis

Average and mode were calculated for all quantitative and qualitative parameters respectively; such values were used for the attribution of each accession to one class, regardless of the year and place of data collection. Classes and relative notes were defined for each measured quantitative trait. After mean values 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 56 studied accessions (Table 1) were initially collected in stands (orchards or scattered plants in home gardens) located in different areas distributed throughout the country, within latitudes 31° 36' 42.8400'' N - 36° 43' 42.9276'' and longitudes 65° 40' 51.9600'' E - 71° 9' 10.0008'' E. Altitudes ranged from 287 m to 1022 m a.s.l. Most of the accessions (>70%) were collected in various districts of northern provinces of Kunduz, Samangan and Balkh, considered the typical areas of almond production, although all accessions were susceptible in varying degrees to the risk of frost damage during the flowering time common in these locations. There were no late flowering varieties equivalent in flowering time to cultivars such as Lauranne, Ferragnes and Ferraduel.

Morphologic and phenological trait variation

Minimum, maximum, mean and standard deviation values for each analysed trait for the whole set of studied accessions is reported in Table 2. Among 140 possible notes attributable to 46 different internationally agreed morphological descriptors related to tree, leaf, flower, shell and kernel, only 17 (≈12 %) of them showed no cases, indicating a wide variability for almost all traits. The sole descriptor showing no variation is related to the number of pistils (always 1 in 100% of cases); colour of tip of flower, sepals and petals showed low variability as well. Among fruit traits, a special note, not present in the international standardised descriptor lists, was included to describe the "crescent" shape of green fruits and kernels, which showed a frequency of 1% and 16% of cases respectively for the whole collection.

The set of described accessions was characterised mainly by vigorous, open trees with dense foliage; medium size, mostly dark green leaves with short petiole and crenate margin. Flowers very frequently showed red brown sepals, broad elliptic white petals, stigmas above the anthers, and reddish stamen filaments. The predominant dry fruit traits were elongated and crescent shape, pointed apex, thick but medium-low resistant to cracking endocarps. Kernels were characterised by elliptic-narrow elliptic and crescent shapes, red brown colour, medium-weak rugosity and small-medium size. Kernel weight (1.1±0.4 g) was lower than the mean value (2.4 g) reported for a germplasm collection of Iranian, European and North American accessions, and similar to those observed in different almond collections of the Persian and Middle-East areas (Talhouk *et al*., 2000; Chalak *et al*., 2006; Zeinalabedini *et al*., 2012; Khadivi-Khub and Etemadi-Khah, 2015) and Afghanistan (Kaska *et al*., 2006). A distinctive trait of Afghan almonds is related to shell cracking resistance, a characteristic with relevant implications in value-added products (Ledbetter, 2008), which was found to be very low or low for 30% of accessions, indicating a consistent occurrence of "paper shell" and soft dry fruits. Conversely, a similar percentage of accessions with hard and very hard shell (28%) was observed. Kernel weight/dry fruit (nut + shell) weight ratio, sometimes indicated as shelling percentage or kernel yield, is another important commercial trait. Afghan accessions in the present study showed higher mean value (0.51±0.12) for this characteristic than those found by other authors on almond germplasm of the same geographic area (between 0.31 and 0.35 for the Persian area) (Sorkheh *et al*., 2010; Zeinalabedini *et al*., 2012; Khadivi-Khub and Etemadi-Khah, 2015), from Lebanon (from 0.30 to 0.41)(Talhouk *et al*., 2000; Chalak *et al*., 2006), and similar to the mean value (0.50±0.11) found in a previous study carried out on 17 local varieties of northern Afghanistan (Kaska *et al*., 2006). Lower mean values of kernel/dry fruit ratio were observed also on Moroccan almond germplasm (0.38)(Lansari *et al*., 1994), on European germplasm collections (Godini, 1984; Cordeiro *et al*., 2001; De Giorgio and Polignano, 2001), and on newly released cultivars (Vargas *et al*., 2008).

With regard to other fruit morphological traits linked to market value, the percentage of double kernel, a negative trait for international standardised trade but not very influent on domestic and subregional commerce, resulted absent or very low (0-2%

Table 1 - List of the described almond [*Prunus dulcis* (Mill.) D.A. Webb] accessions from Afghanistan, area of *in situ* collection, altitude and remarks on their commercial value

National Collection Code	Cultivar name	Area of collection (Province/Locality)	Remarks
AFG0153	Abdul Wahidi	Balkh - Khulm	Good quality
AFG0144	Belabai	Balkh - Khulm	Slight bitterness
AFG0167	Carmel	Kunduz - Char Darah (I)	International cultivar, later flowering than any native Afghan almond
AFG0527	Changaki	Kandahar - Kandahar	Low market value
AFG1008	Du Maghza Kulula	Kunduz - Char Darah	Low market value
AFG1007	Du Maghza Spin	Kunduz - Char Darah	-
AFG6308	Ferraduel	Kunar - Asad Bad (I)	International cultivar
AFG6206	Ferragnes	Nangarhar - Jalalabad (I)	International cultivar
AFG0793	Kaf	Samangan - Aybak	Medium market value
AFG0773	Kafmal	Kunduz - Char Darah	-
AFG4048	Kaghazi Du Posta	Herat - Guzara	Low market value
AFG4016	Kaghazi Gerd	Herat - Guzara	Medium market value
AFG0739	Kaghazi Herati	Herat - Enjil	Low market value (exceptionally large kernel)
AFG0532	Kaghazi Kalan	Kandahar - Kandahar	Valuable for kernel size (AFG0739 has 15% bigger kernels and half the percentage of doubles
AFG6040	Kaghazi Maida	Nangarhar - Muhmand Dara	Low market value; small kernel
AFG0535	Kaghazi Sia Dana	Kandahar - Kandahar	Good quality
AFG0847	Kajak Samangan	Samangan - Aybak	Good market quality
AFG0778	Kelk Arus	Kunduz - Char Darah	-
AFG0146	Khairodini	Balkh - Khulm	Moderately hard shell-
AFG6309	Lauranne	Kunar - Asad Bad (I)	International cultivar
AFG0173	Mahali Kunduz	Kunduz - Char Darah	Low market value; hard shell
AFG0147	Majidi Balkh	Balkh - Khulm	Small kernel
AFG2010	Majidi Samangan	Samangan - Aybak	Small kernel
AFG0166	Marawaja Doum	Kunduz - Char Darah	Low market value
AFG0165	Marawaja Du Maghdza	Kunduz - Char Darah	-
AFG0775	Marawaja Maida Dana	Kunduz - Char Darah	Low market value
AFG0534	Marawaja Safid Post	kandahar - Kandahar	Hard shell
AFG0151	Pista Badam	Balkh - Khulm	Low market value
AFG0160	Qaharbai	Samangan - Aybak	-
AFG1003	Qaharbai Allah Mir	Kunduz - Char Darah	-
AFG1006	Qaharbai Aykhanum	Kunduz - Char Darah	-
AFG0780	Qaharbai Hazratan	Kunduz - Char Darah	-
AFG0143	Qambari	Balkh - Khulm	-
AFG0142	Qambari 142	Kunduz - Char Darah	-
AFG0772	Qambari Kunduz	Kunduz - Char Darah	-
AFG0158	Sangak Dahum	Samangan - Aybak	Low market value
AFG0740	Sangak Haftum	Herat - Enjil	Low market value
AFG0519	Sangak Hashtum	Kandahar - Kandahar	Low market value
AFG0530	Sangak Nohum	Kandahar - Kandahar	Low market value
AFG0531	Sangak Shashum	Kandahar - Kandahar	Low market value
AFG0380	Sangi Du Maghza Kalan	Kandahar - Kandahar	Low market in shell
AFG0774	Satarbai Yaqubi	Kunduz - Char Darah	-
AFG0159	Sattarbai Bakhmali	Samangan - Aybak	High market value
AFG1002	Sattarbai Doum	Kunduz - Char Darah	High market value
AFG0157	Sattarbai Guldar	Samangan - Aybak	High market value
AFG0168	Sattarbai Mumtaz	Kunduz - Char Darah	Highest market value, intrinsically low yielding
AFG0154	Sattarbai No.4	Balkh - Khulm	High market value-
AFG2011	Sattarbai Sais Aybak	Samangan - Aybak	High market value
AFG0164	Sattarbai Sais Kunduz	Kunduz - Char Darah	High market value
AFG0156	Sattarbai Sais Talkhak	Samangan - Aybak	-
AFG0777	Sattarbai Sais Zuhrabi	Samangan - Aybak	High market value
AFG0145	Sattarbai Sufi	Balkh - Khulm	-
AFG1005	Shakh-i-Buz	Kunduz - Char Darah	-
AFG0155	Shakh-i-Buz Safid	Balkh - Khulm	-
AFG0162	Shokorbai	Samangan - Aybak	Acceptable market value
AFG0148	Zang Kafter	Balkh - Khulm	-

Table 2 - List of phenotypic traits, codes, states, classes and descriptive statistics adopted to describe the set of 56 accessions from the National Collection of Almond from Afghanistan. In brackets the percentage of cases observed for each note/descriptor

No.	Trait	Classes (% of cases)	Min.	Max.	Mean	SD
1	Juvenile tree vigour	1 - Weak (0); 2 - Medium (45); 3 - Strong (55)	2	3	2.55	0.81
2	Tree - Habit	1 - Up-right (3); 2 - Slightly open (19); 3 - Open (52); 4 - Spreading (24); 5 - Drooping (2)	1	5	3.00	0.63
3	Foliage - Density	1 - Loose (4); 2 - Medium (48); 3 - Dense (48)	1	3	2.41	0.29
4	Leaf blade - Length	1 - Short (12); 2 - Medium (88); 3 - Long (0)	1	2	1.91	0.42
5	Leaf blade - Width	1 - Narrow (9); 2 - Medium (91); 3 - Broad (0)	1	2	1.84	0.60
6	Leaf blade – Ratio length/width ratio	1 - Small (17); 2 - Medium (81); 3 - Large (2)	1	2	2.54	0.45
7	Leaf blade - Colour	1 - Light green (5); 2 - Medium green (36); 3 - Dark green (59)	1	3	1.73	0.47
8	Leaf blade - Incisions of margin	1 - Serrate (26); 2 - Crenate (74)	1	2	1.32	0.50
9	Leaf - Petiole length	1 - Short (67); 2 - Medium (33); 3 - Long (0)	1	2	1.41	0.36
10	Flower buds: - Shape	1 - Conical (59); 2 - Ovoid (41); 3 - Rounded (0)	1	2	2.02	0.69
11	Flower bud - Colour of tip of petals	1 - White (3); 2 - Pink white (93); 3 - Pale pink (2); 4 - Pink (2); 5 - Carmine (0); 6 - White, with carmine tip (0)	1	4	2.82	0.86
12	Flower bud - Colour of sepals	1 - Green (0); 2 - Brown green (34); 3 - Red brown (50); 4 - Dark red (16)	2	4	2.34	0.64
13	Flower bud - Hairiness of sepals	1 - Absent or very weak (24); 2 - Weak (22); 3 - Medium (52); 4 - Strong (2); 5 - Very strong (0)	1	4	1.80	0.82
14	Flower - Size	1 - Small (31); 2 - Medium (57); 3 - Large (12)	1	3	2.27	0.40
15	Flower - Shape of petals	1 - Narrow elliptic (24); 2 - Elliptic (26); 3 - Broad elliptic (50)	1	3	1.20	0.65
16	Flower - Colour of petals	1 - White (83); 2 - Pink white (17); 3 - Pink (0); 4 - Dark pink (0)	1	3	1.18	0.39
17	Flower - Number of stamens	1 - Few (31); 2 - Medium (55); 3 - Many (14)	1	3	1.84	0.53
18	Flower - Number of pistils	1 - Always 1 (100); 2 - Sometimes 2 (0); 3 - Frequently two (0); 4 - Few-medium (0); 5 - Medium many (0)	1	1	1.00	0.00
19	Flower - Position of stigmas compared to anthers	1 - Below (2); 2 - Same level (36); 3 - Above (62)	1	3	2.61	0.41
20	Stamen - Anthocyanin colouration of filaments	1 - Absent (22); 2 - Present (78)	1	2	1.79	0.81
21	Stigma - Size	1 - Small (26); 2 - Medium (65); 3 - Large (9)	1	3	1.73	0.63
22	Green fruit - Length	1 - Small (9); 2 - Medium (72); 3 - Large (19)	1	3	2.11	0.53
23	Green fruit - Shape	1 - Rounded (2); 2 - Ovate (9); 3 - Elliptic (2); 4 - Pointed (74); 5 - Crescent (1)	1	5	3.84	0.78
33	Green fruit - Pubescence	1 - Slight (29); 2 - Medium (59); 3 - Much (12)	1	3	1.82	0.64
34	Dry fruit - Shape	1 - Type 1 (5); 2 - Type 2 (0); 3 - Type 3 (48); 4 - Type 4 (28); 5 - Type 5 (19);	1	5	3.59	0.99
35	Dry fruit - Shape of apex	1 - Flat (7); 2 - Rounded (9); 3 - Pointed (84)	1	3	2.77	0.57
36	Dry fruit - Thickness of endocarp	1 - Thin (28); 2 - Medium (34); 3 - Thick (38)	1	3	2.09	0.82
37	Dry fruit - Resistance to cracking	1 - Very low (9); 2 - Low (21); 3 - Medium (42); 4 - High (19); 5 - Very high (9)	1	5	3.00	1.08
38	Dry fruit - Keel development	1 - Absent or very weak (9); 2 - Weak (28); 3 - Medium (37); 4 - Strong (24); 5 - Very strong (2)	1	5	2.79	0.97
39	Kernel - Shape	1 - Crescent (16); 2 - Narrow elliptic (31); 3 - Elliptic (34); 4 - Broad elliptic (17); 5 - Very broad elliptic (2)	1	5	2.57	1.02
40	Kernel - Size	1 - Small (64); 2 - Medium (36); 3 - Large (0)	1	3	1.36	0.48
41	Kernel - Thickness	1 - Thin (24); 2 - Medium (71); 3 - Thick (5)	1	3	1.82	0.51
42	Kernel - Main colour	1 - Yellow (2); 2 - Yellow brown (26); 3 - Light brown (16); 4 - Red brown (49); 5 - Dark chestnut brown (7)	1	5	3.32	1.01
43	Kernel - Intensity of colour	1 - Light (9); 2 .- Medium (74); 3 - Dark (17)	1	3	2.07	0.50
44	Kernel - Rugosity	1 - Very weak (3); 2 - Weak (34); 3 - Medium (58); 4 - Strong (5)	1	4	2.63	0.65
45	Kernel - Percentage of double kernel	1 - Absent/very low (23); 2 - Low (17); 3 - Medium (30); 4 - High (22); 5 - Very high (8)	1	4	2.66	1.21
46	Kernel/Dry fruit weight percentage	1 - Very low (0); 2 - Low (8); 3 - Medium-low (15); 4 - Medium (23); 5 - Medium-high (33); 6 - High (21); 7 - Very high (0)	1	6	4.48	1.19
47	Time of flowering	1 - Very early (48); 2 - Early (31); 3 - Medium (14); 4 - Late (5); 5 - Very late (2)	1	5	1.88	1.03
48	Ripening time (harvesting)	1 - Very early (0); 2 - Early (62); 3 - Medium (33); 4 - Late (5); 5 - Very late (0)	1	4	2.38	0.49

of fruit with double kernel) and low (3-5% of fruits with double kernel) in 29% and 20.5% of cases, respectively, with a general average of 4% of fruits with double kernels. This value is similar to the average value (4.5%) found by Sorkheh and colleagues (2010) in a germplasm collection composed of Iranian and European and North American accessions, but much lower than the findings of Kaska (Kaska *et al*., 2006) on a set of almond fruit collected mainly in northern provinces of Afghanistan (≈ 46 %) and those observed on Persian (39%)(Khadivi-Khub and Etemadi-Khah, 2015) and Lebanese (≈ 43%) germplasm (Chalak *et al*., 2006).

Time of flowering showed a high variation, with cultivars flowering from very early (before February 26; 49% of cases, among which many "Sattarbai" and "Sangak" type accessions) to very late (after March 12; ≈ 2% of cases, represented by the international cultivar Lauranne), which clearly indicates a prevalence of early flowering accessions in the almond germplasm from Afghanistan. Conversely, the National Collection of Almond lacks very early (before July 14) and very late (after 29 August) ripening accessions. The range of phenological and measured morphologic traits are shown in Table 3.

Relationship between the described accessions

Individual descriptive sheets were published in 2014 as The National Collection of Varieties of Fruits and Nuts of Afghanistan - Almond and are available online on the Internet (http://afghanistanhorticulture.org/pages/Germplasm.aspx), while the results of multivariate PCA and Cluster Analysis are reported in Table 4 and Figure 1. Principal Component Analysis

Table 3 - Scales defined for quantitative and phenological traits observed on the almond accessions from Afghanistan

Traits	Scales
Leaf blade	Length (mm): short (< 58); medium (58-83); long (>83)
Leaf blade	Width (mm): narrow (<20); medium (20-29); broad (>29)
Leaf blade	Ratio length/width ratio: small (<2.5); medium (2.5-3.5); large (>3.5)
Leaf	Petiole length (mm): short (<11); medium short (11-19); medium (>19-28); medium long (>28-36); long (>36)
Flower	Size (mm): small (<3); medium (3-4); large (>4)
Green Fruit	Length (mm): small (<32); medium (32-50); large (>50)
Kernel	Size (g): small (<0.7); medium (0.7-1.9); large (>1.9)
Kernel	Thickness (mm): thin (<5.5); medium (5.5-10.5); thick (>10.5)
Double kernel (%)	Very low (<15); low (15-27); medium-low (28-39; medium (40-51); medium-high (52-63); high (64-75); very high (>75)
Time of flowering	Very early (before February 26); early (February 27 - March 2); medium (March 3-6); late (March 7-11); very late (after March 11)
Time of maturity	Very early (before July 14); early (July 14-29); medium (July 30 - August 13); late (August 14-28); very late (after August 28)

Table 4 - Eigenvalues, expressed variability and correlations of the first seven Principal Components (accounting for 51.3% of variability) obtained from the whole accession/variable data set. The highest absolute correlation of each variable is in bold

Principal component	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	5.3	3.0	2.6	2.4	2.2	2.0	1.7
Variability (%)	14.0	7.9	6.8	6.5	5.9	5.3	4.6
Cumulative variability %	14.0	21.9	28.8	35.3	41.3	46.6	51.3
Variable correlation							
Juvenile tree - Vigour	0.19	0.13	**-0.27**	0.05	0.12	0.12	0.25
Tree - Habit	-0.28	-0.01	-0.03	0.01	-0.50	-0.11	**-0.43**
Foliage - Density	-0.01	-0.34	0.07	0.04	**0.55**	0.16	-0.07
Leaf blade - Length	-0.01	0.44	**0.47**	0.19	0.28	0.25	0.09
Leaf blade - Width	0.07	0.30	**-0.57**	0.03	0.16	-0.31	-0.21
Leaf blade - Ratio length/width	0.07	0.36	**0.62**	0.19	-0.03	0.19	0.25
Leaf blade - Colour	0.01	0.07	0.14	**0.50**	-0.23	-0.13	-0.03
Leaf blade - Incisions margin	-0.03	0.27	**0.41**	0.34	-0.15	0.41	-0.13
Leaf - Petiole length	0.03	0.42	0.26	**-0.36**	0.10	-0.33	-0.17
Flower bud - Shape	0.27	0.21	-0.22	-0.17	**0.31**	-0.20	0.26
Flower bud - Colour of tip of petals	0.21	0.17	-0.13	**0.46**	-0.26	-0.25	0.16
Flower bud - Colour of sepals	0.23	0.10	0.36	0.06	0.08	-0.26	-0.13
Flower bud - Hairiness of sepals	-0.04	**0.51**	0.05	-0.37	-0.08	0.06	-0.16
Time of beginning of flowering	-0.08	**0.39**	-0.33	0.21	-0.07	0.38	-0.07
Flower - Size	0.19	0.11	**0.49**	-0.38	0.13	-0.19	-0.10
Flower - Shape of petals	0.24	0.05	**0.28**	-0.24	0.22	0.11	0.13
Flower - Colour of petals	0.11	-0.23	0.14	0.16	**0.34**	-0.10	-0.29
Flower - Number of stamens	0.08	**0.55**	-0.13	-0.30	0.03	-0.11	-0.09
Flower - Position of stigmas compared to anthers	-0.31	0.32	-0.23	0.19	0.39	0.06	**-0.43**
Stamen - Anthocyanin colouration of filaments	-0.09	0.09	0.12	-0.38	-0.07	**0.48**	-0.18
Stigma - Size	0.23	**0.36**	0.11	-0.14	0.33	-0.18	0.29
Green Fruit - Size	**-0.66**	-0.15	-0.10	0.19	0.25	-0.08	0.15
Green Fruit - Shape	**-0.62**	-0.01	0.26	0.34	0.07	-0.39	0.08
Green Fruit - Pubescence	-0.04	0.12	-0.28	0.09	0.23	**0.47**	0.34
Time of Maturity	0.26	0.26	0.01	0.20	**-0.38**	0.09	-0.10
Dry Fruit - Shape	**-0.76**	-0.06	0.26	0.09	0.10	-0.31	0.04
Dry Fruit - Shape of Apex	**-0.50**	0.18	0.01	0.10	-0.17	0.09	0.48
Dry Fruit - Thickness of Endocarp	**0.71**	-0.16	0.14	0.23	0.03	-0.03	0.04
Dry Fruit - Resistance to Cracking	**0.81**	-0.38	0.08	0.09	0.09	-0.10	0.04
Dry Fruit - Keel Development	-0.27	**0.45**	-0.06	-0.05	-0.07	-0.14	0.21
Fruit - Percentage of Double kernels	-0.07	-0.30	-0.01	**-0.36**	0.19	0.01	-0.12
Kernel - Shape	**0.74**	0.07	-0.11	0.09	-0.07	0.25	-0.25
Kernel - Size	-0.09	0.01	**-0.44**	0.18	0.28	-0.01	0.06
Kernel - Thickness	**0.63**	0.24	-0.20	-0.26	-0.06	-0.10	0.26
Kernel - Main Colour	**0.35**	0.31	-0.01	0.32	0.20	-0.27	0.01
Kernel - Intensity of Colour	0.28	0.41	-0.03	**0.46**	0.31	-0.05	-0.35
Kernel - Rugosity	-0.16	-0.13	0.11	0.16	**0.48**	0.32	-0.11
Kernel Dry/Fruit ratio	**-0.73**	0.31	-0.09	-0.21	0.08	0.07	-0.14

based on the averages of the discrete scores of the 48 morphological and phenological variables for each accession indicated a low correlation between the utilized variables, with 15 PCAs with eigenvalue higher than 1, accounting for 75.8% of variability within the whole set. Such result can be attributed to the adoption of a multi-state discrete scoring methodology but also to a generalized low correlation between variables.

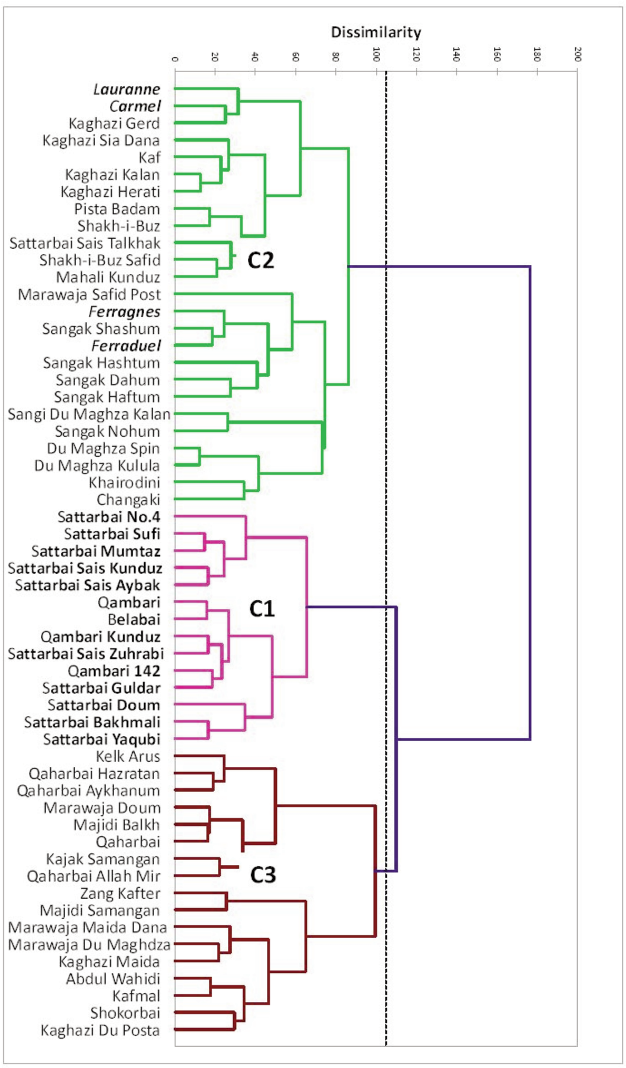

Fig. 1 - Dendrogram (Euclidean distance; Ward's agglomerating method) obtained by analyzing 48 multi-state, discrete morphological variables of 56 almond accessions belonging to the National Collection of Fruits and Nuts of Afghanistan (bold cursive: International cultivars; bold: accessions belonging to C1 cluster "Sattarbai group").

The average Euclidean distance of the generated proximity matrix between the studied accessions resulted equal to 8.46±1.21, ranging from 4.94 (minimum value) observed in the pair “Kaghazi Kalan - Kaghazi Herati” (collected from Kandahar - Dehe Kochi Village and Herat Province, Enjil District, Kahdestan village, respectively) up to 12.59 (maximum value) for the couple “Sattarbai N.4 - Marawaja Safid Post”. The dendrogram generated by Cluster Analysis is reported in Figure 1, where three main significant clusters (C1, C2 and C3) can be observed. The first cluster C1 (7.35±0.92 average distance; 5.43 minimum distance and 9.53 maximum distance for pairs “Sattarbai Sufi - Sattarbai Mumtaz” and “Sattarbai N.4 - Sattarbai Doum”, respectively) is almost completely composed by “Sattarbai” denominated accessions (10 of 14 accessions), while only one “Sattarbai” type (“Sattarbai Sais Talkhak”) belongs to a different cluster (C1). Table 5 reports the representative characteristics of the “Sattarbai” almond type, considered the best in terms of fruit quality and market value, as resulted from the analysis of the 48 morphological and phenological variables of the 10 “Sattarbai” accessions included in cluster C1. Even if a certain degree of variation within single traits of leaves (colour), flowers (petal shape), dry fruits (shape, thickness of endocarp, keel development and percentage of double kernel) and kernels (shape and main colour) was observed, as a whole, these accessions are very closely related and they constitute a clearly defined special subset within the Afghan almond germplasm (Fig. 1). The accession Sattarbai Sais Talkhak, the only “Sattarbai” named cultivar outside cluster C1, showed six characteristics (namely leaf colour, flower size, shell resistance to cracking, dry fruit shape and kernel main colour) with significant differences and 16 traits slightly differing from the “Sattarbai” standard type; this fact substantiates its location in a different cluster. On the other hand, Qambari, Qambari Kunduz, Qambari 142 are varieties often confused with “Sattarbai” type also by expert traders, which confirms the high phenotypic similitude between “Qambari” and “Sattarbai” groups and justifies their presence in cluster C1. Conversely, Belabai, albeit showing many traits of the “Qambari/Qaharbai” type, differentiates for the distinctive and sometimes bitter flavour of the kernel which detracts from its otherwise medium value.

Cluster C2 is formed by the four international cultivars (Lauranne, Carmel, Ferragnes and Ferraduel) belonging to the National Collection and by 21 local accessions, among which all “Sangak” (*sangak* meaning “stone” in local languages) accessions, a part of “Kaghazi” types and other unique varieties. Broad elliptic sepals, Type 3 dry fruit shape, thick endocarp,

Table 5 - Morphological and phenological characteristics of "Sattarbai" almond type

No.	Trait	Note (mode)	Range
1	Juvenile tree - Vigour	Medium	Medium-Strong
2	Tree - Habit	Open	Open-Spreading
3	Foliage - Density	Dense	Medium-Dense
4	Leaf blade - Length	Medium	Medium-Long
5	Leaf blade - Width	Medium	Medium
6	Leaf blade - Ratio length/width	Medium	Small - Medium
7	Leaf blade - Colour	Medium green	Medium green - Dark green
8	Leaf blade - Incisions margin	Crenate	Crenate - Serrate
9	Leaf - Petiole length	Short	Short-Medium
10	Flower bud - Shape	Conical	Conical - Ovoid
11	Flower bud - Colour of tip of petals	Pink white	Pink white
12	Flower bud - Colour of sepals	Red brown	Red brown - Brown green
13	Flower bud - Hairiness of sepals	Medium	Medium - Weak
14	Flower - Size	Small	Small - Medium
15	Flower - Shape of petals	Narrow elliptic	Narrow elliptic - Elliptic
16	Flower - Colour of petals	White	White
17	Flower - Number of stamens	Few	Few - Medium
18	Number of pistils	Always 1	Always 1
19	Flower - Position of stigmas compared to anthers	Above	Above - Same level
20	Stamen - Anthocyanin colouration of filaments	Present	Present - Ansent
21	Stigma - Size	Small	Small - Medium
22	Green Fruit - Size	Large	Large - Medium
23	Green Fruit - Shape	Pointed	Pointed - Crescent
33	Green Fruit - Pubescence	Medium	Medium - Slight
34	Dry Fruit - Shape	Type 5 - Crescent	Type 5 - Type 4
35	Dry Fruit - Shape of Apex	Pointed	Pointed
36	Dry Fruit - Thickness of Endocarp	Thin	Thin - Medium
37	Dry Fruit - Resistance to Cracking	Very low	Very low - Low
38	Dry Fruit - Keel Development	Strong	Medium - Strong
39	Kernel - Shape	Narrow elliptic	Narrow elliptic - Crescent
40	Kernel - Size	Small	Small - Medium
41	Kernel - Thickness	Thin	Thin - Medium
42	Kernel - Main Colour	Yellow brown	Yellow brown - Light brown
43	Kernel - Intensity of Colour	Medium	Medium - Light
44	Kernel - Rugosity	Medium	Medium - Weak
45	Fruit - Percentage of Double kernels	Medium high	Absent - very low- High
46	Kernel - Dry/Fruit ratio	High (0.64)	High (0.58-0.72)
47	Time of beginning of flowering	Very early	Very early - Early
48	Ripening time (harvesting)	Early	Early - Medium

medium resistance to cracking, red brown kernel, medium percentage of double kernels and medium kernel/dry fruit weight ratio (average 0.45, about 30% less that C1 mean value) were the traits differentiating this group of varieties from those of cluster C1. The last cluster (C3) held 17 local accessions mostly belonging to “Qaharbai”, “Marawaja” and “Majidi” types and other unique varieties; such accessions resulted characterised by medium size flowers, dry fruits with weak keel development, high percentage of double kernels, and medium high kernel/dry fruit weight ratio (average 0.53).

4. Conclusions

This first comprehensive study of morphological and phenological traits of almond accessions from Afghanistan clearly reveals an important level of variation among traits of the local germplasm. Analogous findings were reported for Iranian and Lebanese germplasm (Talhouk *et al*., 2000; Chalak *et al*., 2006; Zeinalabedini *et al*., 2012; Khadivi-Khub and Etemadi-Khan, 2015). Furthermore, a special subset of local varieties, to the best of our knowledge not yet reported by scientific international literature and unique in its kind because of dry fruit and kernel crescent shape and very soft "paper shell" endocarp, denominated "Sattarbai" type, has been clearly highlighted. The best Sattarbai types are extremely crescent shaped, with very narrow, elongated kernels and a very clear fissure in the "paper shell" along its length. The Qambari types have less exaggerated length and crescent shape nuts, but clearly have "paper shell". Qaharbai types are less elongated and tend to have more thickening of the semi "paper shells". Shakhe Buz (="Goat's horn") has an exaggerated "S" shape. Other Kaghazi or "paper shell" types may be downgraded because of untypical shape, but the accession Kaghazi Herati AFG0739 has kernels some 40-50% heavier than the typical Sattarbai or Qambari kernel. The Sattarbai, Qambari, Qaharbai and some other types of almond were found exclusively in the north of Afghanistan, indicating a probable connection to the central Asian germplasm, particularly to the famous almond growing areas of the Fergana Valley.

Nevertheless, a very early flowering time represents a limit to the expansion of these cultivars, and breeding programs are already being developed to overcome this obstacle. Specific morphological studies associated to DNA fingerprinting would be the next step in order to better clarify the identity of individual local accessions, to reveal the relationship of the Afghan almond germplasm with *Prunus dulcis* genetic resources of other areas, and to study the role of Afghanistan, an intersectionbetween East and West, in the flow of almond around the world.

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