

Morphological variability of the fruits of seven pomegranate (*Punica granatum* L.) cultivars grown in Messaad region in central Algeria

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Description of the subject. In the Messaad region, in central Algeria, pomegranate (*Punica granatum* L.) is one of the main fruit crops in agricultural systems after apricot.

Objectives. To explore cultivar diversity, a study of the morphological variability of pomegranate fruits and seeds was carried out on the fruits of seven pomegranate cultivars: Khadraye KH1, Hamraye HM1, Senin Alouj SL1 from Messaad orchards, AM2 (Amourah) from Amourah orchards, Khadraye KH3, Mezabi MZ3, Senin Alouj SL3 from Zaccar orchards.

Method. Analysis of variance (ANOVA), a principal Component Analysis (PCA) followed by an Ascending Hierarchical Classification (AHC) based on quantitative morphological parameters are used.

Results. The results show similarities between classes according to which four main classes were grouped. The first class C1 consists of two cultivars (KH1 and SL1 of Messaad), while the second class C2 consists of one cultivar, HM1 of Messaad. The third class C3 consists of the very characteristic Romane Amourah (AM2) and the fourth and last class C4 includes all cultivars of the third station of Zaccar. The evaluation of the qualitative characteristics of color and taste of the fruit by a panel of amateur tasters reveals significant dissimilarities and similarities.

Conclusions. The results presented in this work clearly justify the variability and its interest in the preservation, exploitation and valorization of this genetic material, and for improving cultivated varieties of pomegranate that show appreciable economic performances.

Keywords. Aril, antioxidant activity, functional foods, Punicaceae, cultivar selection.

Variabilité morphologique des fruits de sept cultivars de grenadier (*Punica granatum* L.) cultivés dans la région de Messaad, au centre de l'Algérie

Description du sujet. Dans la région de Messaad, au centre de l'Algérie, le grenadier (*Punica granatum* L.) est l'une des principales cultures fruitières des systèmes agricoles après l'abricot.

Objectifs. Pour explorer la diversité des cultivars, une étude de la variabilité morphologique des fruits et des graines du grenadier a été réalisée sur les fruits de sept cultivars de grenade : Khadraye KH1, Hamraye HM1, Senin Alouj SL1 des vergers de Messaad, AM2 (Amourah) des vergers d'Amourah, Khadraye KH3, Mezabi MZ3, Senin Alouj SL3 des vergers de Zaccar.

Méthode. Une analyse de la variance (ANOVA), une Analyse en Composantes Principales (ACP) suivie d'une Classification Ascendante Hiérarchique (CAH) basée sur des paramètres morphologiques quantitatifs sont utilisées.

Résultats. Les résultats montrent des similitudes entre les classes, selon lesquelles quatre classes principales ont été regroupées. La première classe C1 est constituée de deux cultivars (KH1 et SL1 de Messaad), tandis que la seconde classe C2 est constituée d'un cultivar, HM1 de Messaad. La troisième classe C3 est constituée d'un grenadier très caractéristique, Romane Amourah (AM2) et la quatrième et dernière classe C4 comprend tous les cultivars de la troisième station de Zaccar. L'évaluation des caractéristiques qualitatives de la couleur et du goût du fruit par un panel de dégustateurs amateurs révèle des dissemblances et des similitudes significatives.

Conclusions. Les résultats présentés dans ce travail justifient clairement la variabilité et son intérêt pour la préservation, l'exploitation et la valorisation de ce matériel génétique, ainsi que pour l'amélioration des variétés cultivées de grenadier qui présentent des performances économiques appréciables.

Mots-clés. Arille, activité antioxydante, aliment fonctionnel, Punicaceae, sélection de cultivars.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is from the Punicaceae (L.) or the Lythraceae (APG II, 2003) botanical family which includes only one genus and two species, the other one, little-known, being *P. protopunica* Balf. peculiar and endemic to Socotra Island (Yemen). The plant is drought tolerant, winter hardy and can thrive well under desert conditions (Morton, 1987; Aseri et al., 2008; Chandra et al., 2010). It is a native tree from Iran, Afghanistan, and south east Turkey. The species is a fruit tree with deciduous leaves which, in recent years, has seen great expansion in several countries, especially those with Mediterranean-like climates, where fruits of excellent quality can be produced. The pomegranate tree is well adapted to many different climates and soils; very often it grows in poor soils (Martinez et al., 2006; Soriano et al., 2011; Crivellaro et al., 2013; Hernández et al., 2014).

Pomegranate had a rich symbolic role in the art, literature, and religion of numerous cultures. In nearly every part of the globe where the pomegranate grew, it came to represent fundamental dualities: life and death, inside and out, many and one (Ruis, 2015).

Recently, pomegranate has established itself as a functional food of increasing interest, both economically and within the scientific community, which has generated a significant increase in publications focused mainly on its characteristics, benefits, and nutritional composition (Melgarejo et al., 2020). Indeed, pomegranate is a species of great importance for not only the food, pharmaceutical and cosmetic industries but also for the traditional medicine, it is regarded as “a Pharmacy unto itself” in Ayurveda which specifically uses it to treat infections, inflammations and fungal diseases. Many pomegranate cultivars can be considered as a source of fiber and natural antioxidants to develop or enhance functional foods (Alcaraz-Mármola et al., 2017; Khwairakpam et al., 2018; Souza et al., 2018).

Its therapeutic properties have been used to treat different conditions (cardiovascular, neurological, diabetes and cancer) for hundreds of years. Numerous studies have recently shown the antioxidant, anti-inflammatory and anti-tumoral properties of pomegranate as cited by Khwairakpam et al. (2018) and Sreekumar et al. (2014). As well, inhibition of tumor invasion has been proven in several experimental models of urological tumors such as in prostate cancer (Chéchile-Toniolo, 2012). It has always been allocated for fresh consumption, but recently there is a huge demand for industrial processing to obtain pomegranate juice, jams, etc. (Martinez et al., 2006).

Worldwide *P. granatum* production has expanded greatly due to recent evidence on the fruit health

attributes (Borochoy-Neori et al., 2011). Current global production estimates for pomegranate are unavailable (Stover & Mercure, 2007). From the data provided by different researchers and associations, the total world area dedicated to pomegranate cultivation is well above 300,000 ha, of which more than 76% is found in five countries (India, Iran, China, Turkey and the USA), the estimation of world pomegranate production is more than 3 million tons. The data provided does not, therefore, correspond to an exact year and constitutes an estimation based on different sources (Melgarejo-Sánchez et al., 2013).

In the 2013/2014 crop year, Algeria's location in the Mediterranean basin makes it one of the most productive pomegranate countries with 81,390 tons, where the cultivated area was estimated at 9,439 ha with a yield of 8,620 kg·ha⁻¹ (MADR, 2016). Consequently, the country occupies a significant position among pomegranate-producing countries despite the technical and economic difficulties, such as exportation encountered in this sector.

With a pomegranate area of 1,094 ha, *i.e.* 11.59% of the total cultivated area in Algeria, a production of 10,704 tons, *i.e.* more than 13.15% of the national production of pomegranate, a yield of 9,784 kg·ha⁻¹ and the quality of its fruits, the region of Messaad, which traditionally includes the areas of Messaad, Ain El Ibel, Zaccar, Moudjebara, Amourah and Faid El Botma, in central Algeria, has become a promising area for this activity (DSA, 2017). In addition, *P. granatum* varieties from Messaad, some of which are subject to severe erosion, adapt well to aridosols despite the limiting conditions such as physical, chemical and climatic characteristics. The development of these soils, their management and the development of these crops is of paramount importance given the size of this land in Algeria (Lahouel, 2014).

The objective of this work was to determine the phenotypic variability among several *P. granatum* cultivars grown in these regions and to highlight its economic interest. A morphological study is conducted by the examination of the characteristics of the fruits and the seeds of seven cultivars from three different locations.

2. MATERIALS AND METHODS

2.1. Study location and environmental conditions

The study took place in the region located south of Djelfa district in central Algeria. Three different locations: Messaad, Amourah and Zaccar sites were retained.

The evolution of rainfall and temperatures reflects the recent climate upheaval experienced in recent years

in North Africa and the Great Maghreb (Nouaceur et al., 2013). During 16 years (2002-2017), it is mentioned that July is the hottest month for the three stations Messaad, Amourah and Zaccar with respectively average values of 29.23 °C, 28.04 °C and 27.28 °C, the coldest month is January with respectively 7.27 °C, 6.08 °C and 5.32 °C. During the same period, precipitation is low with a large inter-monthly and inter-annual variability and it is particularly concentrated in winter and autumn and is lower in summer (June-July) (**Figure 1**) (ONM, 2020).

The inter-annual variability of precipitation is significant and is 182.80 mm for the year 2002 and 146.5 mm for the year 2017 with a standard deviation of 63.37 mm in Messaad. It is 202.80 mm for the year 2002 and 166.5 mm for the year 2017 with a standard

deviation of 63.67 mm in Amourah and about of 212.80 mm for the year 2002 and 176.5 mm for the year 2017 with a standard deviation of 67 mm in Zaccar. The dry period in Messaad and Amourah is longer than in Zaccar station (**Figure 1**). The main characteristic of the prevailing winds in the region is the frequency in summer of the sirocco, of desertic origin, hot and dry, whose duration may vary from 20 to 30 days per year (**Figure 1**) (ONM, 2020). Due to the altitude effect (**Table 1**), the sites Messaad and Amourah are located in the bioclimatic arid stage with a cool winter while the station Zaccar is located in arid bioclimate with cold winter on the Emberger's climatogram (data not shown).

In the absence of soil maps on the region, except for that of Pouget (1976), our description will take into account this former document. The gradient related to soil categories shows their paucity and fragility as a general comment. The soil profile of the three sites is classified as calcimagnesian xeric soil with a calcareous crust (limestone), which means that the soils are calcareous from the surface; this results in an alkaline pH, sometimes close to neutrality and saturated absorbent complexes. These characteristics therefore play only a very limited role in soil differentiation, but also as ecological factors responsible for some ecological determinism (edaphic ecological group: psamophytes, halophytes, gypsophytes; indicator species for edaphic factors: sand, salt, gypsum, etc.). Their richness in nitrogen, especially the rhizospheric area, is the result of the root activity and the microbial biomass in the soil/root interface (Pouget, 1980; Lahouel et al., 2016).

2.2. Plant material

Our study was conducted during the *P. granatum* fruiting season of 2018 in Messaad region. The selected plant material (KH1, HM1, SL1, AM2, KH3, MZ3 and SL3) is among the main cultivars existing in the central region of Algeria (**Table 1**).

The consecutive fruit's ripening of cultivars SL1 of Messaad, MZ3 of Zaccar, KH3 and SL3 of Zaccar at end of September occurs with more or less high temperatures (between 20-23 °C). During the month of October, the temperature declines to less than 18 °C, the cultivars HM1 of Messaad, AM2 of Amourah and KH1 (Messaad) reach respectively maturity and can be harvested.

According to the phenological stages of pomegranate (**Table 2**), fruit setting and growth coincide with high temperatures, low rainfall and sirocco winds, which occur during the summer period. This situation pushes the farmers to practice regular irrigation in order to maintain their crops, formative and fruit tree pruning and organic manuring in the objective to increase production.

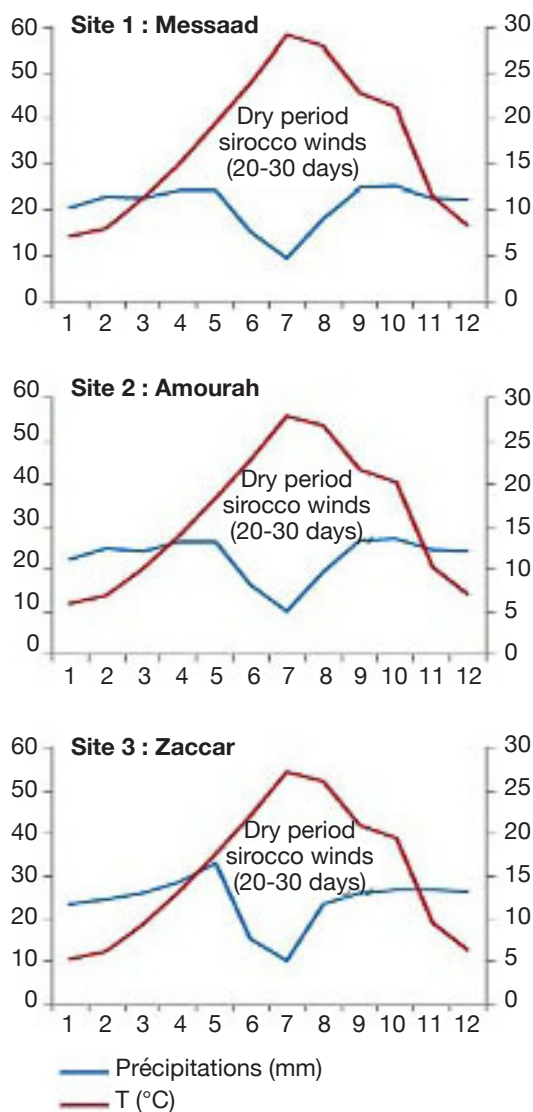


Figure 1. Umbrothermal diagram of the Messaad area (2002-2017) — *Diagramme ombrothermique de la zone de Messaad (2002-2017).*

Table 1. Geographical origin (G.o), age, harvest day, density and yield of the studied pomegranate cultivars grown in the studied area (Borehole B, Natural water source Nws) — *Origine géographique (G.o), âge, date de récolte, densité et rendement des cultivars de grenadier dans la zone étudiée (Forage B, Source d'eau naturelle Nws)..*

Cultivar	Code	G.o	D.M.S	Altitude	Irrigation	Age (year)	Harvest date	Area (ha)	Density (tree·ha ⁻¹)	Yield (kg·tree ⁻¹)
Khadraye of Messaad	KH1	Messaad	34° 15' 28" N 3° 23' 07" E	829 m	B	14	10/10/2018	2.5	200	35
Hamraye of Messaad	HM1	Messaad	34° 10' 00" N 3° 31' 06" E	751 m	B	17	02/10/2018	2.0	250	40
Senin Aloudj of Messaad	SL1	Messaad	34° 09' 47" N 3° 29' 59" E	754 m	B	15	20/09/2018	3.0	300	45
Romane Amourah	AM2	Amourah	34° 21' 21" N 3° 52' 25" E	1,022 m	Nws	25	05/10/2018	2.5	150	20
Khadraye of Zaccar	KH3	Zaccar	34° 25' 38" N 3° 19' 58" E	1,076 m	Nws	18	25/09/2018	1.5	200	25
Mezabi of Zaccar	MZ3	Zaccar	34° 25' 38" N 3° 19' 58" E	1,076 m	Nws	18	20/09/2018	1.5	200	20
Senin Aloudj of Zaccar	SL3	Zaccar	34° 25' 38" N 3° 19' 58" E	1,076 m	Nws	18	25/09/2018	1.5	200	30

2.3. Morphological parameters studied

The study was conducted on seven cultivars. Thirty trees were randomly selected from each orchard, 10 fruits were randomly picked from each tree and 20 seeds were manually extracted randomly from each fruit. The morphological parameters studied were measured on 300 fruits (300 repetitions) and 6,000 seeds (6,000 repetitions) for each cultivar (**Figure 2**).

Quantitative parameters. The diameter and the length of the fruit, the length of the crown, the length and the width of the seeds, the length and the width of the tegmen (woody portion) were measured, using a digital caliper with an accuracy of 0.01 mm. Fruit and rind weights were measured with a scale with an accuracy of 0.1 g (**Table 3**).

Qualitative parameters. For the external fruit shape coded by Fs and the presence of the nipple on the fruit coded by Pn; these two parameters are measured with the naked eye and classified according to the UPOV (2012) descriptor (**Table 4**).

Taste and color. To measure the sensory characteristics for the taste and the color of the *P. granatum* fruit, a test centered on consumers was used with a panel of inexperienced amateur tasters (Watts et al., 1991) of thirty people at the different stations. The amateur taster panels (pilot consumer panels usually consisting of 30 to 50 inexperienced people) are selected from the workers who are employed (non-trained tasters) in the orchard. The focus should be on selecting a group of tasters comparable to the target consumer population that uses the product (representative of consumers). Thirty people were chosen for each site in order to discriminate between the three tastes: sweet, sour-sweet, sour for each cultivar. To record the results, our method consists of asking subjects to rate the perceived degree of each of the above mentioned sensory characteristics of the fruit. The rating is held as one point for each selected taste.

Expert taster panels are used for product-oriented testing. These panels are usually small and made up of 5 to 15 tasters who have been chosen for their sensory acuity and have received special training for the task (Bacle et al., 2009). Unfortunately, there are no expert tasters in our district who have received special training to taste the fruit.

2.4. Statistical analysis

Statistical analyses have been performed using XLSTAT software (version 2014 for Windows). A basic descriptive statistical analysis was followed by an Analysis of variance and a Principal Component Analysis (PCA). An Ascending Hierarchical Classification (AHC) was

Table 2. Phenological stages of the pomegranate in the Messaad region — *Stades phénologiques du grenadier dans la région de Messaad.*

Month	Nov	Dec	Jan	Feb	March	April	May	June	July	Ag	Sep	Oct
Phenological stage	Leaf fall and vegetative rest				Bud burst	Flowering		Fruit setting			Fruit ripening	
						Leaf growth		Fruit growth				

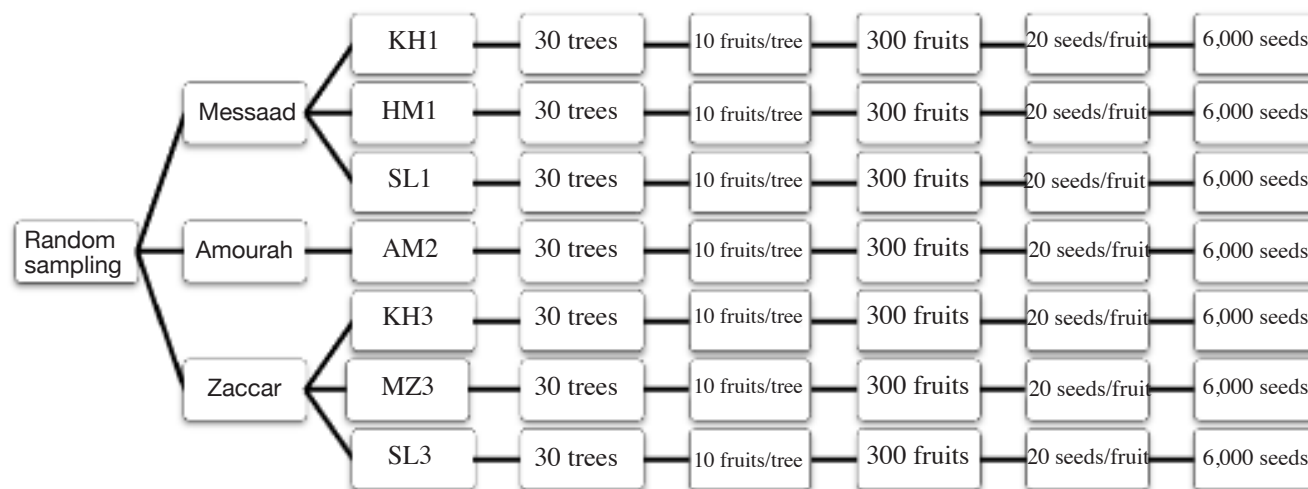


Figure 2. Sampling experimental design — *Protocole expérimental de l'échantillonnage.*

Table 3. Main studied quantitative morphological parameters for the fruits and the seeds — *Principaux paramètres morphologiques quantitatifs étudiés pour les fruits et les graines.*

Parameter	Fruit length without crown (mm)	Fruit diameter (mm)	Fruit weight (g)	Crown length (mm)	Rind weight + carpellary membranes (g)	Seeds yield	Seed length (mm)	Seed width (mm)
Code*	L1	D1	Fw	L2	Rw+Cm	Sy	Ls	Ws
Parameter	Tegmen length (mm)	Tegmen width (mm)	Fruit form index	Fruit calyx index				
Code*	Lt	Wt	If	Ic				

* Sy= [Fw-(Rw+Cm)/Fw]×100 (%), If= (D1/ L1) × 100 (%), Ic= [L2/ (L1+L2)] × 100 (%).

also performed. This analysis has been applied to the data for hierarchical associations using the Ward's method.

3. RESULTS

3.1. Quantitative parameters

Showing a very highly significant effect, all cultivar lengths and diameters L1 and D1 are more than 80 mm, excepted for AM2 (Amourah) for which it was registered a length of 75.21 mm and a diameter close to 70 mm. Our samples diameters show values between

nearly 70 mm for AM2 (Amourah) and 91.95 mm for SL3 (Zaccar). At the same time, L1 and D1 are positively correlated. Fruits with the longest lengths have the largest diameters such as HM1 of Messaad, SL3 and KH3 of Zaccar (Table 5).

For the fruit weight (Fw), this agronomically interesting variable, signification is very high between cultivars. The average Fw varies between a maximum value of 317.39 g for HM1 (Messaad) and 135.85 g for AM2 (Amourah) as a minimum value. Cultivars KH3, MZ3 and SL3 of Zaccar have average weights of 271.25 g, 287.78 g and 276.21 g respectively, which are, relatively, close to each other. Another agronomically valuable variable, seed yield, shows a

Table 4. Qualitative variables measured for the fruits and the seeds (adapted from UPOV, 2012) — *Variables qualitatives mesurées pour les fruits et les grains (adapté de UPOV, 2012).*

Variable	Code	Value
Fruit shape	Fs	1, 3, 5, 7
Presence of nipple	Pn	1, 9
Fruit color	Fc	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Seed color	Sc	1, 2, 3, 4, 5, 6, 7
Taste	Ts	Sour; sour-sweet; sweet

Fs: 1, round — *rond*; 3, ovate — *ovoïde*; 5, oval — *ovale*; 7, elliptical — *elliptique*; Pn: 1, absent on fruit — *absent sur le fruit*; 9, present on fruit — *présent sur le fruit*; Fc: 1, orange — *orange*; 2, orange red — *orange rouge*; 3, pink — *rose*; 4, pink red — *rose rouge*; 5, medium red — *rouge moyen*; 6, red purple — *rouge pourpre*; 7, purple — *pourpre*; 8, dark purple — *pourpre foncé*; 9, light green — *vert clair*; 10, yellow red — *jaune rouge*; Sc: 1, white — *blanc*; 2, light pink — *rose clair*; 3, medium pink — *rose moyen*; 4, dark pink — *rose foncé*; 5, light red — *rouge clair*; 6, medium red — *rouge moyen*; 7, dark red — *rouge foncé*.

very high significant effect. HM1 (Messaad) records the highest value with 73.33%, and in the same site, SL1 (Messaad) has the lowest value with 62.03%. Although the Fw of SL3 (Zaccar) is higher than that of KH3 (Zaccar), the seed yield of KH3 with 66.32% is higher than that of SL3 (Zaccar) with 65.11%. Statistically, Fw is positively correlated with the rind and carpellary membranes weight Rw+Cm, consequently they have almost the same quantity of the edible part (Tables 5 and 6).

Highly significantly differences among cultivars are recorded, the crown length is between 17 mm and 23 mm, this is a mean value for all cultivars. It is correlated positively with L1 and D1 which present the size of the fruit, thus the fruits of HM1 of Messaad and SL3 of Zaccar have the longest crowns (Tables 5 and 6). Despite the non-significance in values, the Ic records are between 18.57% for KH3 (Zaccar) and 19.17% for HM1 (Messaad). The If of the seven studied cultivars is always less or near to 100, indicating that the equatorial diameter in all fruits is smaller than the fruit length without calyx (between 89.98% for SL1-Messaad and 96.94% for KH3-Zaccar).

The values of the quantitative parameters for the seeds show a statistically very high significance between cultivars excepted for the tegmen length. *Ls* is between 9.35 mm for AM2 (Amourah) and 11.66 mm for KH1 (Messaad); a *Ws* between 6.21 mm for AM2 (Amourah) and 7.53 mm for SL1 (Messaad). For the woody part, *Lt* is between 6.97 mm for AM2 (Amourah) and 7.85 mm for KH1 (Messaad), *Wt* is

between 2.93 mm for AM2 (Amourah) and 3.22 mm for KH1 (Messaad). Cultivar AM2 (Amourah) shows the lowest values for all the studied parameters. In addition, the correlation matrix shows a positive dependence between *Ls* and *Lt* with a coefficient of 0.94 and between *Ws* and *Wt* with a coefficient of 0.88 (Tables 5 and 6).

The Pearson squared coefficient (R^2) gives an idea on the proportion of variability in one variable which may be explained by the other. Through our statistical analyses (Table 5), the quantitative fruit characteristics (L1, D1, Fw, L2, Rw+Cm and Sy) and the quantitative seed parameters (*Ls*, *Ws* and *Wt*) and their differences can be strongly related to cultivar types, these results are confirmed by the ANOVA which shows a very high significance for all these parameters (dependent variables) and which is explained statistically by the "cultivar" effect (independent factor).

3.2. Qualitative parameters

Most cultivars have a round to ovate fruit shape without nipple, results are confirmed by the If values (Table 5) which are mostly near 100%.

Cultivars show variations in fruit color from light to dark color. SL1 (Messaad) and SL3 (Zaccar) have a yellow red fruit color, KH1 (Messaad) and KH3 (Zaccar) have the same fruit color light green; AM2 (Amourah) has a red purple fruit color (Figure 3).

Cultivars KH1 (Messaad), KH3 and MZ3 (Zaccar) have the same seed color: dark red. HM1 (Messaad), SL1 (Messaad) and SL3 (Zaccar) have medium to light seed color (Table 7, Figure 3). For the pomegranate flavor, our sample is divided into two groups; the first one: KH1, SL1 (Messaad), AM2 (Amourah) and SL3 (Zaccar) have a sweet taste while the second one: HM1 (Messaad), KH3 and MZ3 (Zaccar) is characterized by a sour-sweet taste (Table 7).

3.3. Principal Component Analysis (PCA) and Ascending Hierarchical Classification (AHC)

In general, all the traits studied are positively correlated with each other except Sy but the intensity of the binding differs from each other. The parameters L1, D1, L2, Rw+Cm and *Ws* are positively correlated with Fw, with highly significant values (close to value 1). Tegmen length and width *Lt*, *Wt* have a highly significant correlation with *Ls* and *Ws* (Table 6). The seed yield is negatively correlated with Rw+Cm with a value of -0.5, indicating the influence of the peel weight on the fruit edible part.

The first three principal components explain more than 94% of the total variation. Almost 85.97% of the observed variability (Figure 4) is explained by the first two components concerning morphological

Table 5. Mean values for the studied quantitative morphological parameters of pomegranate fruits and seeds (ANOVA test) — Valeurs moyennes des paramètres morphologiques quantitatives étudiées des fruits et graines de grenadier (Test d'ANOVA).

Cultivar	Parameter	L1***	D1***	Fw***	L2***	Rw+Cm***	Sy***	LS***	WS***	Lt ^{ns}	Wt***	If***	Ic ^{ns}
	R ²	0,75	0,75	0,78	0,41	0,81	0,96	0,92	0,81	0,04	0,60	0,35	0,04
	Pr > F	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	< 0,0001	0,25	< 0,0001	< 0,0001	0,17
KH1 Messaad		87,04 ± 5,40	82,69 ± 6,25	243,4 ± 28,0	19,90 ± 2,69	71,2 ± 8,0	70,74 ± 0,20	11,66 ± 0,11	7,24 ± 0,07	7,85 ± 0,05	3,22 ± 0,04	94,99 ± 3,42	18,61 ± 1,34
HM1 Messaad		96,34 ± 3,30	90,65 ± 2,65	317,4 ± 33,7	22,85 ± 0,90	84,6 ± 8,6	73,33 ± 0,60	10,31 ± 0,19	7,37 ± 0,21	7,09 ± 0,15	3,15 ± 0,08	94,09 ± 2,42	19,17 ± 0,56
SL1 Messaad		89,89 ± 5,43	80,89 ± 5,24	228,6 ± 41,8	21,30 ± 1,92	86,8 ± 15,1	62,03 ± 0,72	10,98 ± 0,23	7,53 ± 0,34	7,53 ± 0,38	3,21 ± 0,12	89,98 ± 3,95	19,16 ± 1,09
AM2 Amourah		75,21 ± 3,87	69,99 ± 5,26	135,9 ± 25,3	17,29 ± 2,17	38,8 ± 7,4	71,43 ± 0,70	9,35 ± 0,25	6,21 ± 0,24	6,97 ± 0,18	2,93 ± 0,07	93,06 ± 3,57	18,69 ± 1,74
KH3 Zaccar		90,27 ± 3,89	87,51 ± 3,41	271,2 ± 19,1	20,58 ± 1,18	91,4 ± 5,7	66,32 ± 0,94	10,39 ± 0,22	7,39 ± 0,13	7,36 ± 6,73	3,16 ± 0,11	96,94 ± 1,94	18,57 ± 0,82
MZ3 Zaccar		93,75 ± 2,05	90,12 ± 1,85	287,8 ± 25,7	21,45 ± 0,87	87,8 ± 6,9	69,48 ± 0,62	11,02 ± 0,09	7,16 ± 0,09	7,48 ± 0,12	3,04 ± 0,06	96,13 ± 1,46	18,62 ± 0,69
SL3 Zaccar		97,72 ± 3,26	91,95 ± 2,79	276,2 ± 25,5	22,40 ± 3,68	96,4 ± 8,8	65,11 ± 1,00	11,08 ± 0,30	7,26 ± 0,18	7,75 ± 0,17	3,19 ± 0,09	94,09 ± 2,35	18,65 ± 2,24

L1, D1, Fw, L2, Rw+Cm, Sy, LS, WS, Lt, Wt, If, Ic: see table 3 — voir tableau 3; ***: very highly significant — très hautement significatif; **: highly significant — hautement significatif; *: significant — significatif; ns: non-significant — non significatif.

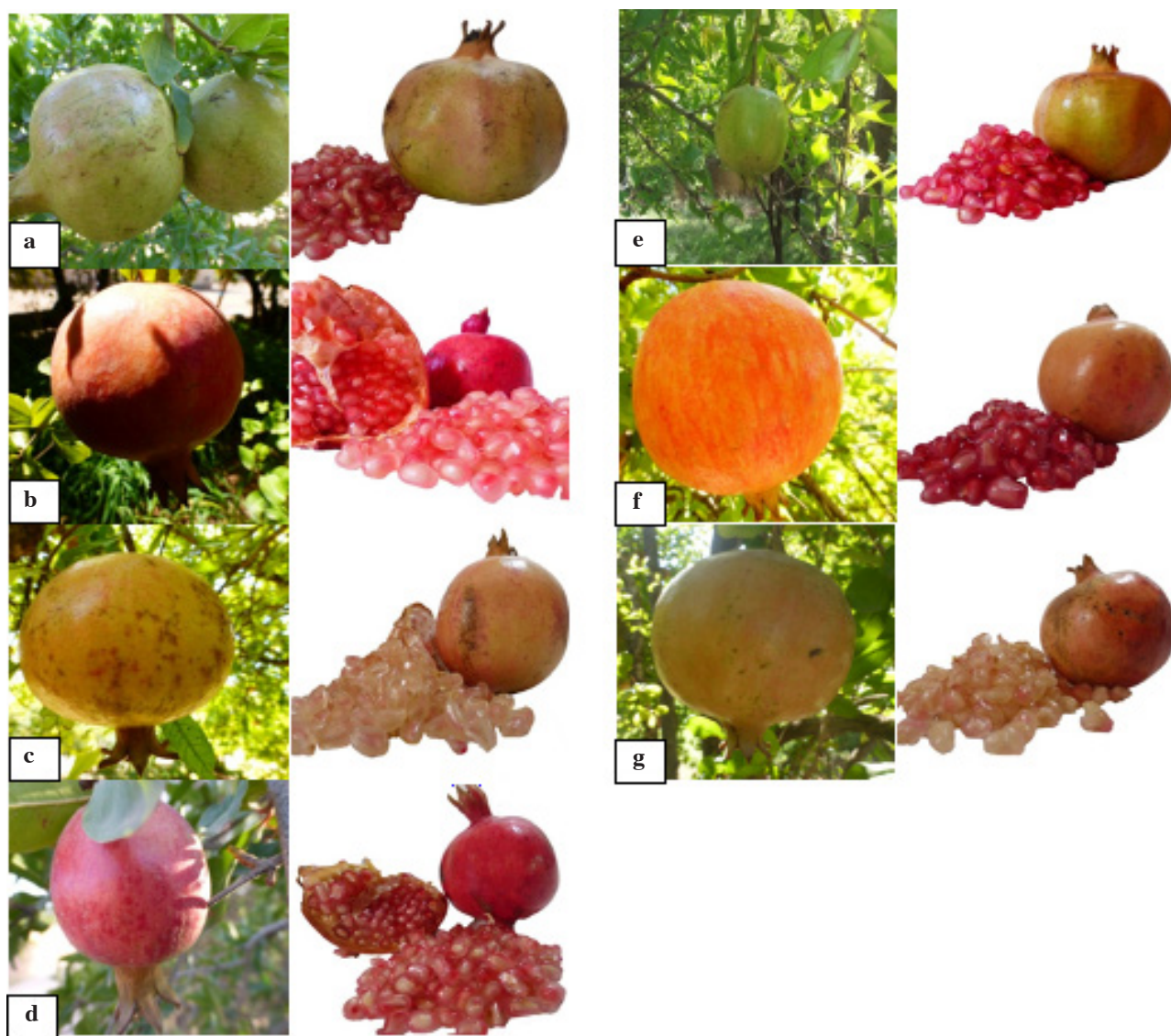
parameters (F1 and F2 axes). The first component, F1, which counts 68.59% of the total variance, is positively related to fruit size (L1, D1, Fw, L2 and Rw+Cm) and seed size (LS, WS, Lt and Wt) (Figure 4), while F2 counts 17.38% of the total variance. The F2 axis is positively correlated to the biometric measurements of the seed (LS, WS, Lt and Wt), while inversely correlated to those of the fruit (L1, D1, Fw, L2 and Sy) (Figure 4).

Messaad's Hamraye (HM1) cultivar registers a more negative result on the Y-axis of the second principal component and thus confirms its differentiation from other cultivars which is explained essentially by maximum values recorded for parameters like the fruits weight. Some cultivars such as KH1 and SL1 of Messaad are relatively close to each other on the X-axis (F1). Ecotypes KH3, MZ3 and SL3 of Zaccar scored positively on the X axis (F1) and separated from the others on the Y axis (F2); these two groups are characterized by large fruit size compared to the others. AM2 (Amourah) is negatively correlated with F1 and this is related to its small fruit and seed size (Figure 4).

The results obtained from AHC, using the agglomerative method based only on the quantitative morphological characteristics of fruits and seeds, are presented as dendrograms (Figure 5), in which four main classes were grouped together. The first class C1 consists of two cultivars: KH1 (Messaad) and SL1 (Messaad), while the second class C2 includes only one particular cultivar: HM1 (Messaad). The third class C3 consists of the very characteristic Romane Amourah cultivar (AM2) and the fourth and last class C4 includes the cultivar of the third station of Zaccar, with morphologically similar characteristics.

Table 6. Correlation matrix (Pearson [n]) — *Matrice de corrélation (Pearson [n])*.

Variables	L1	D1	Fw	L2	Rw+Cm	Sy	Ls	Ws	Lt	Wt
L1	1	0,97	0,94	0,98	0,93	-0,23	0,55	0,80	0,41	0,61
D1	0,97	1	0,96	0,91	0,90	-0,10	0,52	0,74	0,39	0,53
Fw	0,94	0,96	1	0,91	0,86	0,01	0,50	0,79	0,29	0,56
L2	0,98	0,91	0,91	1	0,89	-0,22	0,50	0,82	0,32	0,62
Rw+Cm	0,93	0,90	0,86	0,89	1	-0,50	0,60	0,90	0,50	0,70
Sy	-0,23	-0,10	0,01	-0,22	-0,50	1	-0,33	-0,46	-0,46	-0,45
Ls	0,55	0,52	0,50	0,50	0,60	-0,33	1	0,69	0,94	0,76
Ws	0,80	0,74	0,79	0,82	0,90	-0,46	0,69	1	0,53	0,88
Lt	0,41	0,39	0,29	0,32	0,50	-0,46	0,94	0,53	1	0,70
Wt	0,61	0,53	0,56	0,62	0,70	-0,45	0,76	0,88	0,70	1

**Figure 3.** Fruits and seeds — *Fruits et graines*.

a: KH1 (Messaad); b: HM1 (Messaad); c: SL1 (Messaad); d: AM2 (Amourah); e: KH3 (Zaccar); f: MZ3 (Zaccar); g: SL3 (Zaccar).

Table 7. Mean values registered for the studied qualitative morphological parameters of pomegranate fruits and seeds — *Valeurs moyennes des paramètres morphologiques qualitatifs étudiés des fruits et graines de grenadier.*

Fruit	Fruit shape (frequency %)		Presence of nipple (frequency %)		Fruit color	Seed color	Taste
	Round	Ovate	Absent	Present			
KH1 Messaad	64.00	36.00	100.00	0	Light green	Dark red	Sweet
HM1 Messaad	62.33	37.67	100.00	0	Orange red	Medium pink	Sour-sweet
SL1 Messaad	79.00	21.00	100.00	0	Yellow red	White	Sweet
AM2 Amourah	53.33	46.67	100.00	0	Red purple	Light red	Sweet
KH3 Zaccar	42.67	57.33	100.00	0	Light green	Dark red	Sour-sweet
MZ3 Zaccar	61.33	38.67	100.00	0	Orange red	Dark red	Sour-sweet
SL3 Zaccar	84.67	15.33	100.00	0	Yellow red	White	Sweet

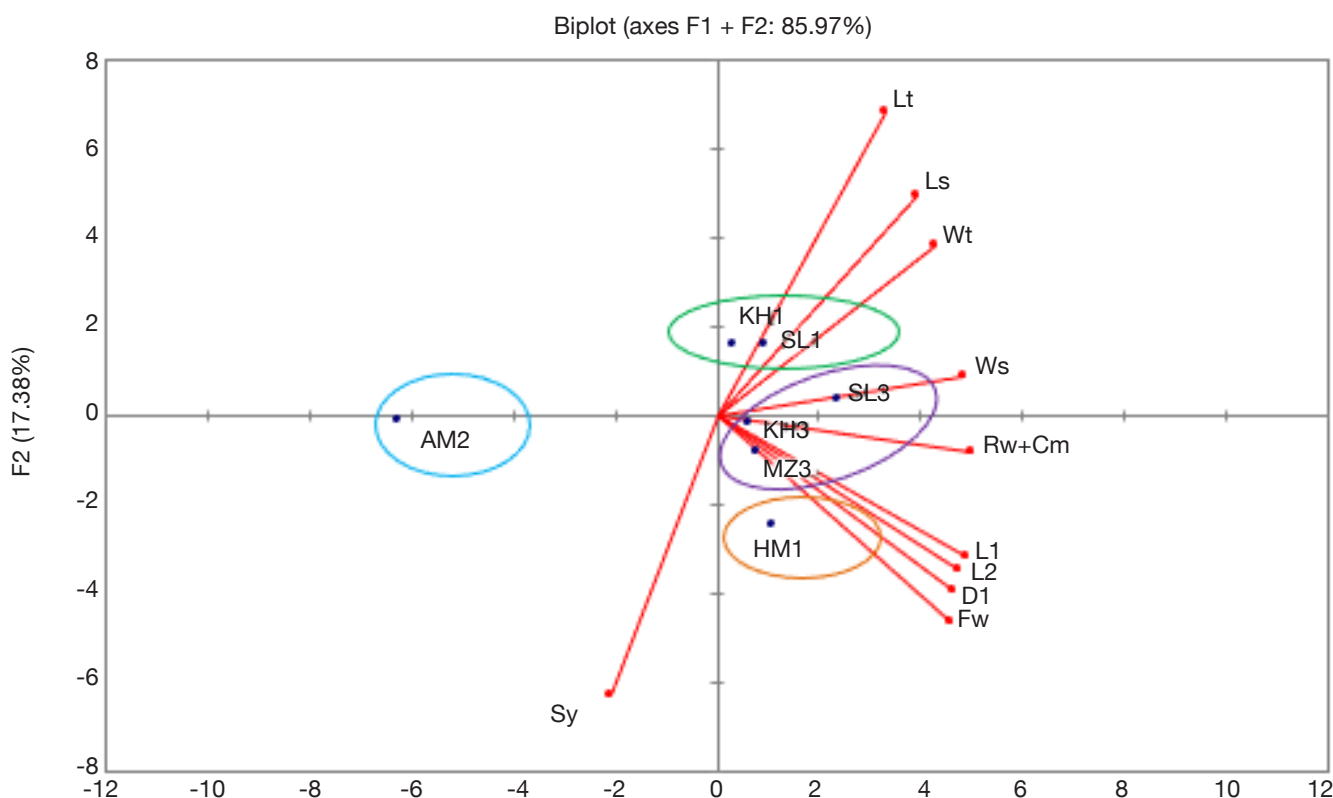


Figure 4. Principal Component Analysis (PCA) Biplot — *Analyse en Composantes Principales (ACP) Biplot.*

4. DISCUSSION

4.1. Quantitative parameters

In Mars & Sayadi (1992) study, the fruits with the greatest height are those of Zehri (8.2 cm) and Kalaii (8.1 cm) varieties. The fruits with the highest equatorial diameter are found on Kalaii (8.9 cm) and Zehri (8.8 cm). The smallest pomegranates are those of the Spanish variety (7.9 cm) while Tapiia-Campos et al. (2021) recorded 8.92 cm as a mean value for

polar diameter, 8.14 cm as a mean value for equatorial diameter in 16 pomegranate genotypes studied.

For our samples, all cultivar lengths are classified as long, except for AM2 (Amourah) which is considered as medium. Comparing to a Tunisian study (Mars & Marrakchi, 1999) for 30 accessions, lengths recorded show a great variability between short, medium and long sizes. For diameters, this result ranks all cultivars as large referring to the *P. granatum* descriptor (NRCP, 2005).

For the fruit weight, our samples are practically in the same range, comparing to results recorded by

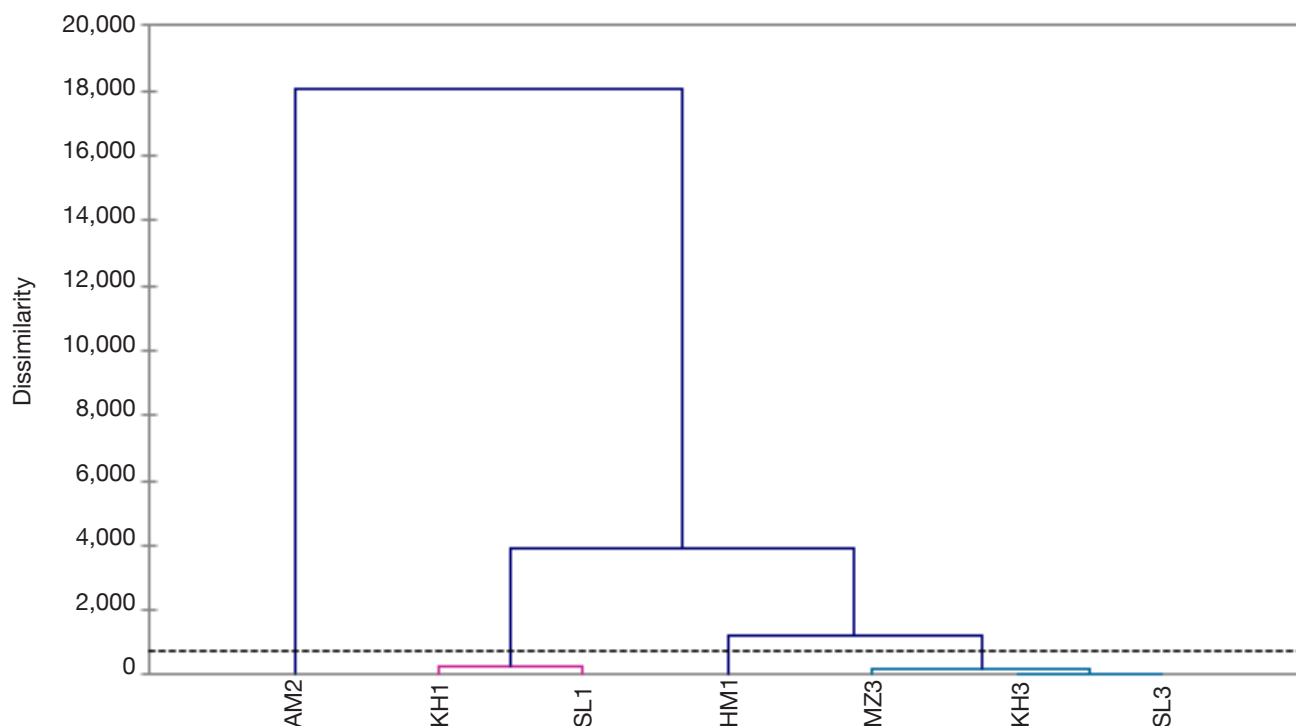


Figure 5. Ascending Hierarchical Classification (AHC) based on the quantitative morphological characteristics of the fruits and the seeds — *Classification Ascendante Hiérarchique (CAH) basée sur les caractéristiques morphologiques quantitatives des fruits et des graines.*

Martinez et al. (2006) which reported between 251.05 g as minimum and 421.10 g as maximum values in the characterization of five Spanish *P. granatum*. For Mars & Sayadi (1992), values between 292 g as minimum and 400 g as maximum were recorded for the study of five pomegranate varieties. Fruit size, rind weight, total aril weight, 100-aril weight, juice weight and aril width were found to have a strong positive correlation ($p < 0.01$) with fruit weight (Chandra et al., 2012). Kumari et al. (2019) specified that a highest critical difference at 5% were 13.21 for fruit weight. Fruit weight was positively correlated with fruit peel thickness ($r = + 0.11$). In Tapia-Campos et al. (2021) study, authors reported that the polar diameter, equatorial diameter and fruit weight can explain more than 90% of the variation using a PCA test in first factor PC1.

In our case, fruit length, fruit diameter, crown length and rind weight are highly correlated with fruit weight. These results are confirmed by Mars & Sayadi (1992) where the heaviest fruits have the highest measured fruit parameters.

For the seed yield, Hernández et al. (2014) recorded for the Spanish *P. granatum* accessions values varying between 52.67% and 65.87%, while for Martinez et al. (2012) the values are between 53.4% and 61.2% for the Moroccan cultivars. For a selection of Tunisian

pomegranate (Mansour et al., 2011), the percentage of seeds is between 57.72% and 78.90%. According to these studies, our samples have considerable seed yields.

According to Martinez et al. (2012), the morphometric variables measured in the fruits have economic interests, and not just because of their acceptance or rejection by the consumer, but additionally because of their influence upon industrial manipulation of this fruit; for instance, cultivars with a high calyx index are more sensitive to calyx breakage, and therefore their external aspect becoming negatively affected.

Al-Yahyai et al. (2009) claim that the variability in fruit shape during the period of commercial harvest, fruit harvesting based solely on size and shape for all cultivars are not sufficient to ensure optimum maturity for fruit utilization and performance during postharvest handling and storage. Other indicators such as the changes in physico-chemical fruit attributes at maturity may be taken into account when assessing readiness to harvest to ensure optimum income returns to growers.

For the seed parameters (L_s , W_s , L_t and W_t), all are positively correlated. L_s and W_s have a high value of correlation coefficient. Martinez et al. (2006) showed a positive correlation between these parameters excepted between W_s and L_t which is negative.

4.2. Qualitative parameters

For the taste, all cultivars are suitable for fresh consumption. Kumari et al. (2019) confirmed that *P. granatum* is quite popular among consumers for its striking, sweet acidic taste and refreshing arils. According to Boussaa et al. (2018), the consumers showed significant differences in their overall liking and in their satisfaction degree for all studied sensory attributes. The descriptive panel was not able to find differences in the intensity of the individual sensory attributes under study (sourness, sweetness, and astringency) as affected by the microclimates, the consumers scored better their satisfaction degrees for both aroma and taste.

Messaad, Amourah and Zaccar cultivars showed variations in fruit color from yellow red, light green to red purple. Dafny-Yalin et al. (2010) indicated that the skin color of the peel is the first trait affecting consumer choices; however, the color of the peels cannot predict the appropriate day of harvest and aril quality. Color varies significantly between cultivars, from white-yellow through orange-pink, to intense red and purple.

The color of the fruit and the seeds showed significant dissimilarities and similarities from white to dark red. All cultivars are presenting appropriate organoleptic characteristics for fresh consumption. These dissimilarities are very useful for breeding; an interesting research work conducted by Jalikop et al. (2010) revealed that selecting genotypes with vividly colored arils is useful in breeding varieties free from aril browning. Other authors (Boročov-Neori et al., 2011) revealed on the basis of RP-HPLC analysis of the arils' anthocyanins under climate effects, that anthocyanin accumulation, which conferred fruit's red color, changed inversely to the season's temperatures. Cyanidins were generally more abundant but delphinidin accumulation was enhanced in cooler season. Monoglucosylated anthocyanins prevailed at cooler temperatures and subsided during seasonal warming with a concomitant increase in diglucoside proportion. The findings can benefit breeding and agricultural efforts to enhance pomegranate quality, especially in the face of "global warming".

According to Boussaa et al. (2018), descriptive sensory analysis revealed differences between oasis and regular orchard with full sun exposure in the aril color intensity. Arils from fruits cultivated under full shade oasis system were characterized by high intensity of the red color (trained panel) which made them well appreciated by the consumer panel.

4.3. Principal Component Analysis (PCA) and Ascending Hierarchical Classification (AHC)

Whereas statistically significant differences were observed through ANOVA in the evaluated morphological

parameters (both in the fruit size and in the whole seed), the PCA and AHC analysis show similarities in many cultivars and discriminate them in four classes. Analysis revealed variability related to morphological parameters, supported by several other parameters.

The first class (KH1-Messaad and SL1-Messaad) is characterized by a sweet and acceptable taste, a high yield, 35 kg-tree⁻¹ for KH1 (Messaad) classified as medium production and 45 kg-tree⁻¹ for SL1 (Messaad) (**Table 1**) classified as high production referring to Melgarejo (1993) reported by Martinez et al. (2006). The second class (HM1 of Messaad) which shows differences with the remaining cultivars by the size, has an acceptable taste and a high production of 40 kg-tree⁻¹ (**Table 1**). These three less aged cultivars receive permanent care through agricultural practices, including regular irrigation in order to sustain crops, formative and fruit tree pruning and organic manuring in the objective to increase production. The third class is formed by AM2 (Amourah), the oldest cultivar characterized by smaller size, a sweet and acceptable taste and a low yield of 20 kg-tree⁻¹ (**Table 1**). Even though these low records, yet, this cultivar remains a resource to be preserved and valorized. The fourth class (Zaccar cultivars: KH3, MZ3 and SL3) older than Messaad's is marked by similar morphological aspects with a large fruit size, but with low to medium productions (20-30 kg-tree⁻¹) (**Table 1**) according to the levels established by Melgarejo (1993) reported by Martinez et al. (2006).

The climatic gradient and cultural practices, especially the type of irrigation between drip irrigation from a borehole and traditional channel irrigation from a natural water source, contribute significantly to this variability among cultivars. Singh (2004) noted highly significant inter-varietal differences in vegetative growth, yield, fruit size, fruit cracking, number and weight of arils in arid to semi-arid ecosystems and interesting results were recorded in terms of performance for the desired traits. In addition, Mir et al. (2012) stipulate that even though pomegranate grows well in low fertility soils, production can be increased by application of manures and fertilizers. Galindo et al. (2017) and Mellisho et al. (2012) added that regular and strategic drip irrigation during the fruit ripening period (under water stress) improves the fruit size and its chemical characteristics linked to taste and color.

The cultivar variability allows choices for growers according to their different technical, climatic and edaphic conditions, the discrimination can be pronounced by other punctual researches like molecular markers. Studies as of Jbir et al. (2014) showed that cultivars from southern Tunisia represent a considerable common genetic base despite their phenotypic differences and misnames: synonymy and/or homonymy reported. Naming problems may

explain all the results obtained. They reported that pomegranate cultivars are locally named according to their geographical origins or according to the agronomic characteristics of the fruit as reported by Mars & Marrakchi (1999). While the results reported by Ajal et al. (2014) showed clearly that the ISSR molecular markers used during the study proved to be efficient in terms of highlighting molecular polymorphism in pomegranate as well as exploring the genetic relationships between the cultivars involved, the analysis of these markers revealed a great diversity of the accessions studied.

The description of pomegranate germplasm is based mainly on pomological and agronomic criteria and genetic studies are rare. The use of other biochemical and molecular markers such as isozymes and RAPD fingerprints could supply complementary useful information as for many other fruit species (Khadari et al., 1995; Ouazzani et al., 1995; Trifi et al., 1998; Mars & Marrakchi, 1999; Mars, 2000).

5. CONCLUSIONS

In conclusion, we can orient the choice for growers to a ranking of our cultivars according to several considerations. Referring specifically to the economic point of view of high yield, acceptable taste quality and lastly size, it is necessary to affirm that the cultivars of Messaad KH1, HM1 and SL1 are the most interesting for the agricultural and industrial use. According to their performances of parameters linked to the fruit and the seed, the cultivars of the Zaccar site (KH3, MZ3 and SL3) are the most vigorous and still a phylogenetic resource to be valorized by improving the cultural practices in the orchard especially the type of irrigation used. As for the adaptation to the different gradients related to the climatic and edaphic conditions, the cultivars of Messaad show an interesting vigor and give acceptable yields due essentially to the correct cultural practices; those of Zaccar and Amourah give a negative response by reducing their yield under traditional irrigation by channel in these conditions.

Based on these different findings, the results reveal the strengths of our experimental device to differentiate the seven cultivars from the point of view of performance and economic interest and its limitations to highlight the chemical differences and variability at the level of molecular polymorphism that can guide growers to make the right choices. It is essential in the future to complete this work by a chemical analysis and a mineral composition of the fruit. A completed study of the molecular polymorphism of the cultivars in our case using genetic markers such as Random amplification of polymorphic DNA (RAPD), simple sequence repeats (SSRs), or amplified fragment length

polymorphism (AFLP) to highlight the molecular diversity is more than necessary.

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