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Morphometric Characterization and Identification of Some Varieties of Pomegranate (Punica granatum) in the Wilaya of Tlemcen

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Abstract

The common pomegranate (Punica granatum) is a perennial tree, drought tolerant, able to grow in poor and alkaline soils. It has been considered as one of the oldest and most famous tree. Because of its richness in nutrients and versatile uses, it gained a huge economic interest. This fruit tree has been the subject of significant genetic improvement globally. However, in Algeria no such improvement program has been undertaken. In order to develop new varieties that match the needed criteria, and to preserve, restore and enhance the diversity of existing genetic material, this study was conducted for morphometric analysis for the identification and characterization of the common pomegranate in the wilaya of Tlemcen. The data were collected from farmers of five municipalities: Fellaoucen, Remchi, Nedroma, Ghazaouet and Ain Ghraba on 9 (nine) quantitative and 8 (eight) qualitative measurements (based on the recommendations of the Union for the Protection of new varieties of plants), from five fruis, and 100 arils of 65 pomegranates plants. The data were analyzed using R software following SPSS. The principal component analysis (PCA), hierarchical classification (CAH), Shannon and Weaver diversity index, and multiple correspondence analysis (MCA) were also made. This study revealed the existence of a large morphological diversity.

Keywords: Pomegranate, morphometric characterization, diversity
Introduction

Arboriculture has always been an essential part of the economy and social life of Algeria. This vast country has the privilege of cultivating several fruit species due to its geographical position and its various pedoclimatic conditions (Benettayeb, 1993). The pomegranate (*Punica granatum* L.) is one of the oldest cultivated fruit species in Algeria (Mars & Marrakchi, 2004). Although it is believed to be native to Persia and surrounding areas, areas of the Mediterranean are considered secondary center of the species diversification. The pomegranate; along with its seeds, bark and flowers is used for their medicinal and therapeutic properties in several regions, to treat gastrointestinal diseases and parasitic affections, it was then abandoned due to the toxicity of some of its active components. But it is still one of the richest products for antioxidants including soluble polyphenols, tannins and anthocyanins (Gil et al; 2000). In Algeria it was considered for a long time as a secondary species, but during the last decade, the pomegranate tree is thriving, and its cultivation has modified from traditional to commercial orchards (Haddioui; 2012). That’s probably due to the fact that this widely cultivated species is ubiquitous, given its great adaptive potential to environmental conditions (soils and climates). Pomegranate has been the subject of renewed interest over the past fifteen years, medically, pharmacologically and cosmetologically (Wald; 2009).

The description of the fruit varieties is usually based on botanical and agronomic characteristics. However, the genetic resources are one of the first sources used to develop new varieties that fit the required criteria of productivity, production quality and tolerance to biotic and abiotic stresses. Hence, in order to preserve, restore and enhance the diversity of available genetic material, it is necessary to study its genetic characteristics which are expressed externally by the phenotypic characteristics represented by morphology. In this context, the objective of this work is to focus on an important aspect of genetic diversity through the identification and morphometric characterization of varieties of pomegranate (*Punica granatum*) at the level of the Wilaya of Tlemcen.

Materials and Methods

Vegetal material and collection sites

The plant materials were obtained from a survey in the Wilaya of Tlemcen during the year 2019-2020 (Fig. 01).

Figure 01. The geographical map that shows the study areas

Three hundred and twenty-five pomegranates and six thousand five hundred arils were collected from sixty-five different pomegranate plants, 13 selected from each of the five localities: Fellaoucene (35°
2°17.412" N, 1° 35’ 30.192” W), Remchi (35° 8’ 29.616” N, 1° 25’ 30.324” W), Nedroma (35° 2’ 48.912” N, 1° 45’ 8.028” W), Ghazaouet (35° 4’ 13.08” N, 1° 50’ 18.708” W) and Ain Ghoraba (34° 45’ 30.06” N, 1° 26’ 6.216” W). The trees chosen are mature trees that have been in commercial production. Data on 17 parameters were measured; following a specific method with a specific material for each parameter. Nine of which are quantitative:

- Width of the tree (Lar.Ar) in centimeters (cm) was measured using a tape measure.
- The length of the fruit (Ln. Fr), fruits width (Lr.Fr), aril length (Ln.Ari), aril width (Lr.Ari), seed length (Ln.Grn), and seed width (Lr.Grn), were measured in centimeters (cm) using a caliper as shown in Figure 2. Each fruit was labeled before the measurements with the same label number as the parent tree to avoid confusion.
- The fruit weight (Pp.Fr) was measured in gram (g) using a scale.
- The number of trunk / tree (Nbr.Tr/Ar), have been identified with the naked eye. As were the six qualitative measurements: Color of the trunk (C.Tr), color of fruit peel (C.Ec), aril color (C. Ari), the hardness of the seed (Du.Gr), tree shape (F.Ar), and variety (Var).
- As for the sweet taste of the fruit (Go.S), fruit acidity (A.Fr), and also the hardness of the seed; a tasting was carried out (this tasting was carried out by ourselves).

![Figure 2. Measurements of the fruit, the aril, and the seed by the caliper based on the UPOV recommendations.](image)

These parameters were chosen based on the recommendations of the Union for the Protection of new varieties of plants (UPOV), to estimate the phylogenetic potential of pomegranate in the wilaya of Tlemcen.

**Statistical analyses**

Several statistical tests were performed using R software (version R 4.1.1) and SPSS software (version 25). The one-way ANOVA test was performed to study the effect of region and varieties. Pearson correlation (Correlation and linear regression) was used to measure the linear relationship between two random quantitative variables, 2 variables are dependent when the knowledge of one indicates the value of the other, which could inform us about an inaccessible trait based on a visible or measurable one, and therefore facilitates the selection process. A principal component analysis (PCA) was calculated to differentiate the plants according to quantitative measurements. Hierarchical classification (CHA) was carried out to classify the samples and obtain the optimal number of groups. The relative diversity index of S.W (Shannon and Weaver, 1948) was calculated to determine the phenotypic diversity of the studied pomegranate collection. And last but not the least the multiple correspondence analysis (MCA) was performed to detect and represent the underlying structures for nominal categorical data. The level of significance was assessed using Fisher test at $P < 0.05$. 

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Results

Quantitative characters

Descriptive analysis

The number of trunk/tree ranged from 1-6 with an average of 2.52 (Table 1). The tree width varied between 157 and 730 cm with an average of 374.26 cm. The minimum fruit length was 5 cm and the maximum was 12 cm which resulted with an average of 7.83 cm. As for the fruit width it varied between 4 and 9.5 cm with an average of 6.52 cm. The length of the aril ranged from 0.2 to 1.8 cm with an average of 0.90 cm. As for its width it ranged from 0.1 to 0.9 cm with an average of 0.61 cm. The seed minimum length and width varied from 0.1 and 0.01 cm respectively and the maximum from 0.8 and 0.3 cm respectively, which results with an average length of 0.49 cm and an average width of 0.11 cm. The fruit weight varied from 75 to 750 g with an average of 245.63 g.

Table 01. Minimum, maximum and average of the quantitative characteristics as influenced by the locations and varieties.

| Parameters | Fellaoucen | Remchi | Nedroma | Ghazaouet | Ain Ghraba | Sig |
|------------|------------|--------|---------|-----------|------------|-----|
| Nb.Tr/Ar   | 2.23±1.087 | 2.60±0.843 | 2.60±0.843 | 3.40±0.894 | 3.40±2.074 | 0.078 |
| Lar.Ar (cm) | 350.00±113.182 | 514.90±91.397 | 306.20±42.150 | 280.20±70.436 | 490.00±144.741 | 0.00 |
| Ln.Fr (cm) | 7.73±1.338 | 8.73±1.307 | 7.75±1.046 | 7.28±1.308 | 7.40±1.118 | 0.00 |
| Lr.Fr (cm) | 6.594±1.3377 | 6.910±1.0668 | 6.210±1.0256 | 6.120±1.2186 | 6.260±0.9908 | 0.00 |
| Ln.Ari (cm) | 0.892±0.1355 | 0.913±0.1462 | 0.919±0.1218 | 0.913±0.1166 | 0.899±0.1195 | 0.00 |
| Lr.Ari (cm) | 0.601±0.0925 | 0.620±0.0896 | 0.610±0.0728 | 0.622±0.0817 | 0.615±0.0833 | 0.00 |
| Ln.Gr (cm) | 0.496±0.0808 | 0.482±0.0755 | 0.490±0.0699 | 0.499±0.0862 | 0.482±0.0603 | 0.00 |
| Lr.Gr (cm) | 0.118±0.0439 | 0.101±0.0303 | 0.109±0.0377 | 0.111±0.0353 | 0.114±0.0382 | 0.00 |
| Po.Fr (g) | 233.30±122.390 | 357.00±134.964 | 212.80±56.750 | 203.20±73.865 | 216.80±61.760 | 0.00 |

Variations of individuals by region

All the traits differed significantly from one location to other except Nbr.Tr/Ar (Table 2). The number of Nbr.Tr/Arshowed no significant difference among the locations. This probably means that this trait is not under the influence of the environment. Width of the trees ranged from 280.20 cm at Ghazaouet to 514.90 cm at Remchi (Table 2).

Table 02. Character variation of individuals according to regions, values are expressed as means±standard deviation.

Note that the Remchi region has the greatest tree width and fruit’s length, width and weight with an average of 514.90, 8.73, 6.91 cm and 357.00 g respectively. The Ghazaouet region presents strong values in aril width and seed length with an average of 0.622 and 0.499 cm respectively. At the level of the Nedroma region the highest average was observed for the aril lengths 0.919 cm. The mean of the highest seed widths were reported at the level of the Fellaoucenic region 0.118 cm.

The width of the tree varies between 280 cm in the region of Ghazaouet to 514.90 cm in Remchi. The length and width of the fruit varies between 7.28 and 6.12 cm respectively in the region of Ghazaouet to 8.73 cm and 6.91 cm respectively in the region of Remchi as well. The aril’s length varied from

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0.892cm in the region of Fellaoucene to 0.919cm in the region of Nedroma, while the width ranged from 0.601cm in the region of Fellaoucene to 0.622cm in the region of Ghazaouet. However, the length of the seeds varied from 0.482 cm in both Remchi and Ain Ghoraba to 0.499 cm in the region of Ghazaouet. As for its width it varied from 0.101cm in the region of Remchi to 0.118cm in the region of Fellaoucene. The weight of the fruit varied from 203.2g in the region of Ghazaouet to 357g in the region of Remchi, which is slightly bigger than the average according to Martinez et al. 2006, and bigger than previous similar studies (Zoubida Kaci Meziane et al. 2016). Overall, there is a difference in the morphological characteristics between the individuals of the pomegranate at the level of the regions studied.

Variations of individuals according to the varieties

The analysis of variance by region shows that there are significant differences between varieties for the majority of traits (table 4). The number of trunks per tree presents a non-significant difference between the varieties; this suggests that this parameter probably has a similar genetic profile when it comes to this trait.

The Nemri variety presents strong values for the character width of the tree 499.50cm, length of the fruit 8.58cm, the width of the fruit 8.038cm, length of aril 0.925cm, the width of the rail 0.636cm, and weight of the average fruit 300.96g. As for the other varieties: Hamed has the highest averages in seed length and width 0.498 and 0.148cm respectively. Sefri variety has the highest average in the number of trunks.

The Hamed and Nemri varieties have the extreme values for all the studied parameters significantly. The width of the tree varied from 280.1cm corresponding to the variety Hamed to 499.5cm for the Nemri variety. The length and width of the fruit ranged from 7.3cm and 5.77cm respectively in the Hamed variety, to 8.58cm and 8.038cm respectively in the variety Nemri. The length and width of the aril ranged from 0.817cm and 0.558cm respectively in the variety Hamed, to 0.925cm and 0.636cm respectively in the variety of Nemri as well. However the length and width of the seeds varied between 0.481cm and 0.103cm respectively in the Nemri variety, to 0.498cm to 0.148cm in Hamed variety. Curiously enough, even though Nemri variety had the largest treewidth, length and width of the fruit, and length and width of the aril, it had the smallest seeds, and vice versa for the Hamed variety. And the weight of the fruit ranged from 300g in the variety Nemri to 215.9g in the Hamed variety.

Table 04. Variations of characters according to the varieties, values are expressed as means±standard deviation.

| The Parameters | Sefri          | Nemri          | Hamed          | Snanlaajel    | Sig  |
|----------------|----------------|----------------|----------------|---------------|------|
| Nbr.Tr/Ar      | 2.75±1.164     | 1.67±1.033     | 2.30±1.160     | 2.00±0.000    | 0.087|
| Lr.Ar (cm)     | 384.98±132.658 | 499.50±54.210  | 280.10±51.438  | 306.00±53.666 | 0.003|
| Ln.Fr (cm)     | 7.84±1.429     | 8.58±0.921     | 7.30±0.969     | 8.02±0.907    | 0.001|
| Lr.Fr (cm)     | 6.511±1.2470   | 8.03±0.6917    | 5.770±0.8343   | 6.54±0.8155   | 0.000|
| Ln.Ari (cm)    | 0.916±0.1245   | 0.925±0.1343   | 0.817±0.1408   | 0.918±0.1274  | 0.000|
| Lr.Ari (cm)    | 0.614±0.0793   | 0.636±0.1016   | 0.558±0.1016   | 0.625±0.0844  | 0.000|
| Ln.Gr (cm)     | 0.492±0.0748   | 0.481±0.0970   | 0.498±0.0917   | 0.487±0.0695  | 0.000|
| Lr.Gr (cm)     | 0.108±0.346    | 0.103±0.0296   | 0.148±0.0530   | 0.105±0.0346  | 0.000|
| Po.Fr (g)      | 247.38±132.942 | 300.96±78.575  | 215.90±86.959  | 232.00±46.993 | 0.028|

Post hoc test

The post hoc test was used to find out which groups are statistically different from others. The comparison was realized between region I, which is the variable to be explained and regions J, which are the explanatory variables (Table 5).
There were significant differences between the region (I) (Fellaoucen) and two of the regions (J) (Ghazaouet and Ain Ghraba) for number of trunk (Table 5), 3 of the regions for aril length, and between 3 of the region for aril width. There were also a significant differences between the region (I) (Remchi) and all the regions (J) in fruit length and fruit weight (Table 5), 3 of the regions (J) for tree width, and between 3 of the for fruit width. The significant differences between the region (I) (Nedroma) and regions (J) lies in the seed length in the regions (J) (Remchi, Fellaoucen and Ghazaouet), and seeds width in the regions (Remchi, Fellaoucen and Ghazaouet and Ain Ghraba).

Principal component analysis (PCA)

The principal component analysis (Figure 2) was used with the following variables: length (Ln. Ari), aril width (Lr. Ari), seed length (Ln. Grm), Seed width (Lr. Grm), number of trunk per tree (Nbr. Tr/Ar), aril width (Lr. Ari), fruit weight (Po.Fr), fruit length (Ln. Fr), and fruit width (Lr. Fr). The analysis showed that the first axe have 30.02% and the second one 15.01% of the total inertia. Significant (p ≤5%) relationships were observed between most studied parameters.
3 groups are formed; the first group contains three characters which are: aril length (Ln.Ari), aril width (Lr.Ari), seed length (Ln.Grn), they are positioned relatively close to each other, oriented in the same direction. Thus, they are positively correlated with each other. The second group also includes three characters: Seed width (Lr.Grn), number of trunk per tree (Nbr.Tr/Ar) and aril width (Lr.Ari), however; this grouping is not very reliable statistically, seen that the two characters; number of trunks per tree (Nbr.Tr/Ar) and Seed width (Lr. Grn) are positioned very close to the center of the circle. The third group contains the variables Fruit weight (Po.Fr), fruit length (Ln. Fr), fruit width (Lr. Fr). The fact that some traits are positively correlated is probably due to the fact that these traits are controlled by a number of genes in common. The first group and the other two groups are orthogonal, which means they not correlated. The third group and the character aril’s width (Lr. Ari) are positively correlated.

Hierarchical Ascendant Classification (HAC)

Mean according to regions

As it’s shown in figure 3, three groups are formed; Remchi and Ain Ghoraba have very similar profiles and form the first group. Fellaoucene, Nedroma, and Ghazaouet are fairly homogeneous, nonetheless Nedroma and Ghazaouet are closer, which means that they form the second group and Fellaoucene is the third group.

The average distance of the varieties

The Hierarchical Ascendant Classification based on varieties showed 3 classes, the first one is the variety of Nemri. Sefri, Hamed, and Snanlaajel seem to be homogeneous, but clearly, the varieties of Hamed and Snanlaajel are closer and therefore they form a group, and Sefri forms the third group.

Diversity relative index of the different characters depending on the regions

The Shannon and Weaver index was calculated using R software and Excel, the quantitative traits were put into 4 classes in order to perform the test, which was calculated from different characters in the studied regions (Fellaoucene, Remchi, Nedroma, Ghazaouet and Ain Ghoraba). The diversity index (H’) of all the studied varieties is 0.86 (Table 07). This index varies between 0.78 for Nedroma and 0.9 for Ghazaouet, and 0.95 for the number of trunks per tree and 0.42 for the width of the tree. As for the length of the fruit H’ = 0.96, the width of the fruit H’ = 0.60, and the weight of the fruit (H’ = 0.95). Concerning the aril length (H’ = 0.99), the aril width (H’ = 0.99), the seeds length (H’ = 0.99) and the seeds width (H’ = 0.89). The highest mean diversity index (H’≥ 0.90) is obtained for the accession of Ghazaouet, and the other values are obtained (0.78≤ H≤ 0.88) for the remaining accessions (Fellaoucene, Remchi, Nedroma). After consulting several databases, no similar work was found to compare the results.
Table 07. Comparison of the Shannon-Weaver diversity index between the five regions

| Region      | H' Nbr.Tr | H' Lar.Ar | H' Ln.Fr | H' Lr.Fr | H' P.Fr | H' Ln.Ari | H' Lr.Ari | H' Ln.Gr | H' Lr.Gr | Moy H' |
|-------------|-----------|-----------|----------|----------|---------|-----------|-----------|----------|----------|--------|
| Fellaoucen  | 0.99      | 0.43      | 0.99     | 0.38     | 0.99    | 0.99      | 0.99      | 0.99     | 0.99     | 0.86   |
| Remchi      | 0.98      | 0.42      | 0.95     | 0.57     | 0.99    | 0.99      | 0.99      | 0.99     | 0.99     | 0.87   |
| Nedroma     | 0.89      | 0.41      | 0.98     | 0.54     | 0.82    | 0.98      | 0.98      | 0.97     | 0.49     | 0.78   |
| Ghazaouet   | 0.98      | 0.42      | 0.92     | 0.79     | 0.99    | 0.99      | 0.99      | 0.99     | 0.99     | 0.90   |
| Ain Ghoraba | 0.90      | 0.42      | 0.97     | 0.70     | 0.97    | 0.99      | 0.99      | 0.99     | 0.99     | 0.88   |
| Moy H'      | 0.95      | 0.42      | 0.96     | 0.60     | 0.95    | 0.99      | 0.99      | 0.99     | 0.89     | 0.86   |

**Qualitative characteristics**

**Descriptive statistics**

Descriptive analysis of the qualitative parameters in the population (*Punica granatum*) studied shown in table 8.

**Multiple Correspondence Analysis (MCA)**

As it’s shown in the graph (figure 4); the MCA of qualitative parameters, corresponds to a positive correlation with the regions and the varieties studied. So these characters: tree shape (F. Ar), aril color (C. Ar), acidity (A.), seeds hardness (Du. Gr)....ect, are probably controlled by a number of genes in common, or they respond in the same way to environmental conditions. To exclude one or the other probability, we must have a situation where the same population evolves in different biotopes to see if the correlations change; if it doesn’t change it means that these characters are correlated by a certain number of genes in common.

As for the modalities, the majority of the trees of Fellaoucen are characterized by a slack shape, and they’re of the varieties of Sefri and Hamed, where the color of the fruit bark is red and orange, and the color of the arils is red. The majority of the trees grouped in the region of Nedroma are characterized by the varieties of Sefri and SnanLaajel, the sweet taste is medium, and the grain hardness is medium as well. The majority of Ghazaouet trees are characterized by a pink aril color, weeping tree forms, and the most common variety is Sefri. These characters are similar to the Remchi and Ain Ghoraba trees. So according to the modalities graph, we can summarize that Sefri is the common variety in all the regions but the populations of Ghazaouet, Remchi, and Ain Ghoraba are closer according to the statistics plan (figure 5).
Table 8. Descriptive analysis of the qualitative parameters in the population (Punica granatum) studied.

| Qualitative characters | Number | Percentage |
|------------------------|--------|------------|
| The color of the trunk | Gris   | 65         | 100        |
|                        | Jaune  | 169        | 52         |
|                        | Orange | 9          | 2.8        |
|                        | Rose   | 83         | 25.5       |
|                        | Rouge  | 64         | 19.7       |
| The color of the fruit rind | Rose   | 3930       | 60.5       |
|                        | Orange | 2500       | 38.5       |
|                        | Blanc  | 70         | 1          |
| The color of the Aril  | Rose   | 3930       | 60.5       |
|                        | Rouge  | 2500       | 38.5       |
|                        | Blanc  | 70         | 1          |
| The hardness of the seed | Moelle | 3500       | 53.85      |
|                        | Moyenne| 2000       | 30.77      |
|                        | Dure   | 1000       | 15.38      |
| The sweetness of the fruit | Faible | 500        | 7.69       |
|                        | Moyenne| 1000       | 15.38      |
|                        | Fort   | 5000       | 76.92      |
| The acidity of the fruit | Faible | 5000       | 76.92      |
|                        | Moyenne| 500        | 7.69       |
|                        | Fort   | 1000       | 15.38      |
| Shaftshape             | Etalé  | 42         | 64.62      |
|                        | Dressé | 14         | 21.54      |
|                        | Pleureur| 9         | 13.85      |
| Variety                | Sefri  | 4449       | 69.22      |
|                        | Nemri  | 501        | 7.71       |
|                        | Hamed  | 1000       | 15.38      |
|                        | Snanlaajel| 500    | 7.69       |

Figure 06. Graph of the qualitative parameters studied from the multiple correspondence analyses (MCA)
Figure 07. Multiple Correspondence Analysis (MCA) modalities graph

Ascending hierarchical classification of qualitative traits (CAH)

Hierarchical ascendant classification based on Multiple Correspondence Analysis using Ward’s method made it possible to determine three classes (figure 6), each group contains 3 subgroups.

Figure 08. Individual Ascending Hierarchical Classification (AHC)

Conclusion

At the end of our work which focused on the study of the morphometric characterization of pomegranate (*Punica granatum*) in the 5 regions (Nedroma, Ghazaouat, Ain Gheraba, Fellaoucen, and Remchi) in the Wilaya of Tlemcen, an important phenotypic variability for the studied variables, of a qualitative nature (color of the trunk, color of the bark of the fruit, color of the aril, the hardness of the seed, the sweet taste of the fruit, the acidity of the fruit, shape of the tree, and variety) and quantitative (Number of trunks per tree, treewidth, fruit length, fruit width, aril length, aril width, seed length, seed width, the weight of the fruit) was observed. Different tests were performed using the SPSS software and the R software; the large morphological diversity of this collection is reflected by the Shannon and Weaver index $H'$ (0.86). The results of the test of the variation of variables according to the region,
station and variety (ANOVA) showed that there are statistically significant differences for most of the characters, the test also showed that the number of trunks per tree is strongly linked to the region, which is an important asset for future breeding plans for this resource. The results of multiple correspondence analysis (MCA) and hierarchical classification (CAH) allowed us to distinguish 3 groups of mean according to regions and according to varities. Through the principal component analysis (PCA) we distinguished the formation of 3 groups of variables, which corresponds to a positive correlation between these parameters at the level of each group. This result is very important because it allows us in the event of selection to focus on a single trait per group (saving time and money for the agriculturers). In the light of these results and following the established remarks; it was noticed that there is a significant difference between the varieties and the regions studied.

For a more global view on this resource and more precise information; additional and broader prospecting work is therefore necessary. To deepen this study, it is important to increase the number of samples in order to study variations, notably botanical and genetic, and to measure other biometric parameters. A biochemical but also molecular analysis by DNA type markers and of great interest for any future aspect of management and improvement of this phylogenetic resource.

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