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The Genetic Diversity Analysis of Tunisian Male Date Palm Cultivars (*Phoneix dactylifera* L.) Revealed by Phenotypic and Molecular Markers

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ABSTRACT

Tunisian oases reveal an important genetic heritage of date palm cultivars, with various qualities of use. Since the beginning of the century, this heritage has evolved to a selective orientation based on the monoculture of "Deglet Nour," this orientation risks causing the loss of many cultivars. The male of the date palm is part of this heritage and so far remains marginalized. Although they are important for the date palm production cycle, it is in this context that lies our work to study the genetic diversity of a collection of male date palm pollinators from southern Tunisia. The morphological study of the 20 date palm pollinators using 45 IPGRI (International Plant Genetic Resources Institute) descriptors showed significant discrimination, with a similarity index ranging from 0.207 to 0.457, divided them into five similarity groups. The use of 7 ISSR (Inter Simple Sequences Repeat) primers resulted in 64 reproducible bands, of which 57 were 90% polymorphic, and statistical analysis showed a more or less significant genetic diversity with genetic distances 0.491 to 0.873. According to the Mentel test, a non-significant weak correlation (r = 0.015) was noted between the molecular and morphological data. However, the processing of molecular data by various methods generated very significant correlations. Indeed, the correlation between the SM (Simple matching) coefficient and the DICE coefficient showed an important correlation with r = 0.748, which confirms the discriminating power of the ISSR markers in studying the genetic diversity of date palm pollinators.

Keywords: Date palm, ISSR markers, genetic diversity, morphological traits, mentel correlation, pollinators.

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I. Introduction

The oases constitute a particular and unique ecosystem of their kind in the world. The date palm (*Phoenix dactylifera* L.) has emerged from a tremendous natural evolution process and farmer's patient work. For millennia, the date palm has not ceased to disperse in a wide variety of environments, far beyond its center of origin. Thus, the date palm evolves and gradually adapts to a wide variety of oasis environments, which has led to the formation and expression of significant genetic diversity in oases worldwide (Trifi *et al*., 2000). The constantly growing interest in date palm exceeds its cultivation area and sometimes even its centers of origin (Alfarsi *et al*., 2008). This plant, long considered as a "providential tree" only for the populations of arid zones, is currently becoming strategic and even attracts the attention of the countries located in the north of the Mediterranean who have so far considered it as an exotic plant with only an ornamental and tourist role (Saadi, 1998).

In Tunisia, the date palm occupies an area of around 40,976 ha, for a total population of 5.5 million plants, of which 66% (3.66 million plants) is Deglet Nour (ONAGRI). Tunisian palm groves cover 1.9% of total tree areas. They are mainly located in Kébili (58%), Tozeur (21%), Gabès (16%) and Gafsa (5%). The date palm contributes to the income of around 60,000 direct and indirect operators (ONAGRI). In 2019, the exports of Tunisian dates recorded a record with 120,000 tones contributing to revenue of 871 million Tunisian dinars (ONAGRI). These values indicate the important role played by the palm sector in the Tunisian economy. Moreover, the fast development of biotechnology allowed rapid multiplication and large scale of the best genotypes by in vitro culture (Hammami *et al.,* 2009) and the discovery in most pre-Saharan and Saharan areas of deep aquifers allowing the irrigation and development of large desert areas, thus creating a market potential of date palm plants (Ferchichi *et al*., 2008). Genetic resources are an essential component of biodiversity and represent a major challenge for research and development. The study of the date palm genetic resources, the identification of cultivars, their evaluation, and their conservation remain a research priority (Ben Abdallah *et al*., 2000). In Tunisia, date palm cultivation is the backbone of agricultural activity in the South West regions, particularly in the Djerid and Nefzaoua areas (Ben Abdallah *et al*., 2000). It is the pillar of the oases, which are the only sources of greenery and life in the middle of the

desert (Rhouma, 2005). The covering, ensured by the palm tree's foliage, creates a favorable climate for installing several plant species, multiple sources for human and animal nutrition. So we are still used to talking about oasis cultures by floor. Thus, phoeniculture occupies an agro-economic place of choice in the stability of the oasis agro-system. It then constitutes a pillar that influences economic growth and farmer's added value (Kadri *et al*., 2015). Considerable efforts have been made to improve the production of date palm by extending the plantation to irrigated areas and encouraging young farmers in the region to create modern oases (Hamza *et al*., 2009). Also, there is a massive planting of the variety "Deglet Nour," which remains the most demanded on the national and international date market and reached 65% of total cultivation (Rhouma 2005). Consequently, that will erode the diversity and dominating mono-varietal plantation. In Tunisia, the research was carried out for several years on the national territory scale and was mainly oriented towards female palm trees rather than male palm trees. Ben Abdallah (1990) suggests considering the two other genetic resources: endanger male pollinators and several varieties risk for being lost due to uncontrolled slaughter to extract palm juice. Besides, date palm growers have neglected the importance of pollen on the quantity and quality of date palm production (Kadri *et al*., 2017). The characterization of this marginalized cultivation heritage becomes a priority for conserving the genetic resources of the oasis. The use of morphological parameters is one of the standard methods implemented to identify the date palm variation and diversity level (Kadri *et al*., 2016). However, this characterization needs a comprehensive set of phenotypic traits that are sometimes problematic to measure due to environmental influences (Rao, 2004) or vary in plant vegetative stages. Many traits related to fruit parameters are useful for date palm characterization (Elhoumaizi *et al*., 2002). Many studies have highlighted this concern and used the fruits parameters as informative description, phenotypic diversity, and phylogenic relationship among date palm. Climate change, genetic erosion, conversion of agricultural land, pest, and diseases are the leading cause of date palm genetic erosion. Many studies have exhibited the effectiveness of using molecular markers in date palm (Rhouma- Chatti *et al*., 2011; Kadri *et al*., 2015; Karim *et al*., 2015). These markers were ideal in cultivar identification, genomic diversity, evolution, and sex-specific markers (Echrif *et al*., 2013). Thus; these tools can be utilized in marker-assisted selection for date palm breeding programs. This study aims to use morphological descriptors and Inter Simple Sequence Repeat marker (ISSR) to quantify genetic diversity and establish phylogenetic relationships within a collection of 20 Tunisian date palm pollinators. In addition, the practical goal of this diversity study is to identify the most genetically distant cultivars to prepare crosses between these pollinators and female plants of the Deglet Nour variety for the improvement of fruit qualities.

II. Materials and Methods

A. Plant Material

The study was carried out using a collection of 20 date palm pollinators cultivated in the experimental plot in the Regional Research Center for Oasis Agriculture (CRRAO)-Tozeur, southwest of Tunisia (Fig. 1). The cultivars were selected according to their fertility criteria which are mainly based on their pollen quality (Table I). The cultivars in this study coded as follows: 1: PL1, 2: PL4, 3: PL5, 4: PL6, 5: PL7, 6: PL13, 7: PL26, 8: PL67, 9: PL70, 10: PL73, 11: PL83, 12: PL85, 13: PL108, 14: PL117, 15: PL133, 16: PL136, 17: PL154, 18: PL155, 19: PL166, and 20: PL181.

Fig. 1. Regional Research Center on Oasis Agriculture, experimental plot sampling area in Tozeur, southwest of Tunisia.

TABLE I: Morph-Physiological Characteristics of the Selected Pollinators

Pollinators	Viability (%)	Germination (%)	Spathe weight (g)	Pollen productivity
PN1	93	88	1800	High
PN4	92	82	1350	High
PN5	95	85	870	High
PN6	88	79	790	Medium
PN7	98	90	710	High
PN13	91	88	1480	High
PN26	92	85	1630	Medium
PN67	93	77	1260	Little
PN70	97	84	1560	High
PN73	90	91	1540	High
PN83	92	89	1720	High
PN85	86	80	1470	Medium
PN108	88	82	2040	High
PN117	87	81	1650	High
PN133	93	84	1140	High
PN136	95	86	610	High
PN154	91	87	1940	High
PN155	88	83	1650	Medium
PN166	86	81	1520	Little
PN181	90	79	960	High

B. Difference between Male and Female Feet in the Date Palm

The date palm is dioecious. Therefore, it is necessary to wait for 6 to 8 years to induce the first flowerings in order to identify the plant sex. The morphological differences between flowers are exceptionally early since it is already marked when the inflorescence is only 10 mm long, even before the sexual differentiation occurs. The difference between male and female feet could be noticed morphologically.

1) Female Inflorescence

The female inflorescences show significant elongation of the peduncle as well as bilateralization. The inflorescences and spikelets are longer related to their position on the spine (Fig. 2A).

Fig. 2. Female inflorescence (a) and male inflorescence (b) (pinterest.fr).

2) Male Inflorescence

The male inflorescence has a conical shape, and the number of floral meristems is higher on the spikelets. The latter's length is independent in males and relative to the spine position (Fig. 2B).

C. Morphological Study

The morphological traits were determined based on the date palm descriptor developed by (IPGRI. 2005). These descriptors are developed in a participatory manner between researchers and Magrebian farmers to facilitate the choice of male and female cultivars to plant, the recognition of date palm cultivars (female), the in situ and in vitro conservation of cultivars in danger of disappearance. Four types of descriptors were used depending on the target plant parts (Fig. 3).

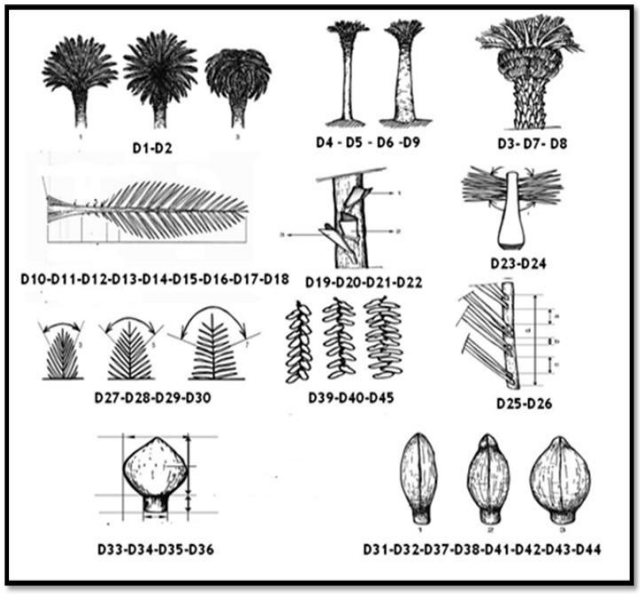

Fig. 3. Descriptors used in the analysis of the morphological diversity of pollinators (IPGRI, 2005).

1) Growth Descriptors

Visual observations must be made on healthy plants of the same age and not less than 5 years of production. These morphological traits are qualitative, the measured characteristics were: vigor (D1), the port (D2), the appearance of the crown (D3), the shape of the trunk (D4), Persistence of leaf scars (D5), the presence of high off-shoot (D6), the presence of fluff mane (D7) and the capacity to produce basal off-shoot (D8).

2) Palm Descriptors

Three replicates (3 palms) represent each cultivar in this study, these morphological traits are quantitative. The phenotypes measured were: the level of curvature (D9), the rotation (D10), the total length (D11, m), the maximum width (D12, cm), length of the part thorny (D13, cm), the thickness of the spine (D14, cm), the color of the petiole (D15), the width of the palm at the base of the petiole (D16, cm), the average number of thorns (D17), the rigidity of the thorns (D18), the maximum thickness of the central spine (D19), the number of spines by type of grouping (D20), the maximum thickness of the central spine (D21, mm), maximum length of the thorn (D22, cm), color of leaf (D23), flexibility of leaf (D24); leaf grouping (D25), angle divergence of terminal leaflet (D26); maximum width of middle leaflet (D27, cm), maximum length of middle leaflet (D28, cm), length of terminal leaflet (D29, cm) and width of terminal leaflet (D30, cm), (Fig. 3).

3) Inflorescence Descriptors

The following characteristics were taken on three replicates spathes in each pollinator: the shape (D31), the mode of development of the spathe (D32), the total length (D33, cm), the maximum width of the spathe (D34, cm); the density of the spikelet in the spathe (D35); the number of spikelet per spathe (D36); the length of the longest spikelet (D37, cm); length of shortest spikelet (D38, cm), number of flowers per longest spikelet (D39), number of flowers per shortest spikelet (D40), (Fig. 3).

4) Pollen Descriptors

The measurements were conducted based on pollen productivity per spathe (D41), pollen odor (D42), pollen color (D43), weight of the spathe (D44, kg), and spikelet shape (D45).

D. Molecular Study

1) Sampling for the Study of Genomic Polymorphism

For each male foot studied, we took the basal part of whitish color located at the base of the trunk's apical meristem; this can minimize the contamination by polyphenols that are found with high contents in the green leaves of the date palm.

2) Liquid Nitrogen Grinding Technique

One gram of the plant material ground with 100 ml of liquid nitrogen for quantification using a mortar and pestle until obtaining an excellent powder. The samples were ground to a satisfactory powder level and placed in 2 ml tubes containing 100 mg of plant material and then stored in the freezer at -80 ° C.

3) DNA Extraction

DNA extracted according to DNeasy Plant Mini Kit (QIAGEN) manufacture protocol. The DNAs obtained run on 0.8% agarose gel to verify the quality of DNA. DNA assay was carried out using QubitR 2.0 fluorometer (Invitrogen) and stored at 4 °C.

4) ISSR Amplification

The PCR conducted in a final volume of 20 µl containing: 10 µl of PCR Master Mix 2X buffer (Promega), 2 µl of DNA (15 ng/µl), 2µl of primers (2 pmol/µl), (invitrogen), and 6 µl of nuclease-free water (Promega). The reaction mix loaded in

the PCR tubes and placed in a thermocycler (BioradIcycler) programmed as follows: initial denaturation at 94 °C for 60 seconds, followed by 35 cycles each comprising a denaturation step at 94 °C for 30 seconds, annealing phase for 45 seconds, and a step of elongation at 72 °C for 120 seconds. The last step of the amplification reaction consists of a final elongation at 72 °C for 7 min. PCR products loaded on 1.8% agarose gel and bands visualized under UV using the GelDoc imaging system (Biorad).

5) PIC (Polymorphism Information Content) Calculation

This parameter inquiries about the ability of a given primer to generate polymorphism between the different provenances studied. It is referred to as PIC. It is calculated according to Smith *et al*. (2000) by the following formula: PIC = 1 - Σ (Pij) 2, where Pij is the frequency of the i^{th} band revealed by the j^{th} primer, P (ij) is summed across all the bands revealed by the primers. The mean value of the PIC highlights the most polymorphic loci for all the individuals studied.

E. Statistical Analysis Methods

The different morphological variables of date palm cultivars calculated with specific basic statistical parameters such as maximum, minimum, arithmetic mean, and standard deviation. The obtained morphological data was processed using variance with a classification factor (ANOVA): the analysis of variance test with classification factor consists of comparing more than two means of several individuals from simple and independent random sample data (Dagnalle *et al.*, 2006). NEWMEN was used to compare the means two by two, and KEULS (SNK) test at the 5% threshold was used to classify the cultivars studied for each character.

1) Genetic Similarities

The data matrix (presence/absence of bands) was submitted to the SIMQUAL program (Similarity for qualitative data program) of NTSYS-pc software (The numerical Taxonomy and Multivariate Analysis System for personal computer) version 1.70 (Sorensen and Foottit, 1992) to generate the similarity matrix between pollinators. Similarity coefficients were used in the UPGMA analysis sub-program "Unweighted Pair-Group Method using Arithmetic" from the NTSYS software to establish a dendrogram group of different pollinators (Nei *et al*., 1979).

2) Principal Component Analysis (PCA)

Multivariate analysis based on correlations applied to transform the initial variables (in this case, the morphological markers) into synthetic variables or axes. That constitutes the main components defined by a set of eigenvalues, reflecting the initial proportion of variables. Furthermore, the XLSTAT 2014 program was used for morphological markers. Besides, Mdscale program in NTSYS-pc software version 2.02 for molecular markers.

3) Study of the Correlation

In the genetic, morphological, and geographical distance matrix, a statistical analysis was performed to establish the correlation between the various matrices in pairs estimated by the Mantel test (1970) MxComp program (Rohlf, 1968) of the NTSYSpc 2.02j software.

III. Results

A. Study of Morphological Data

Morphological characters are taken into account by breeders and farmers to select date palm pollinating trees, which are mainly based on the inflorescence quality. The present study targets detection of the probable phenotypic polymorphism in the 20 pollinators tested. Concerning the growth descriptors (D1 to D8), the results showed that these traits are very similar for the majority of the characters with a variety "Deglet Nour" in particular the exact shape of the stipe (Cylindrical), Persistence of leaf scars, and the presence of fluff mane (Supplementary Data). Only PL6, PL73, and PL83 represent an erected port. However, the rest of the pollinators represent a spherical port. The results showed that the PL1 pollinator has a compact density of spikelets with many numbers per bunch. However, PL4, PL117, and PL166 have an average density of spikelets and a high number per bunch. The vastest number of flowers per spikelet has significant agronomic value. The pollinators with the vastest number of flowers per spikelet are PL1, PL26, PL70, PL73, PL85, PL117, ranging from 85 to 115 flowers/spikelet (Table II. supplementary data). Early flowering pollinators: PL1, PL4, PL26, PL67, PL117, PL133, PL154, and PL181, have a low pollen production capacity comparing with the medium flowering period: PL6, PL70, PL73, and PL136. PL83 and PL108, which are late flowering, are favorable criteria for farmers with a significant pollen production. The analysis of variance results showed a highly significant relationship for the traits: the total length (D11), the average number of thorns (D17), angle divergence of terminal leaflet (D26), maximum length of middle leaflet (D28) and length of terminal leaflet (D29). Newman and Keuls test at the 5% threshold highlight three homogeneous groups A, B, and C, for these descriptors. A highly significant relationship between the two characters is the maximum width of the spathe (D34) and weight of the spathe (D44). Comparison of means by Newman and Keuls test at the 5% threshold allows us to classify the cultivars for these characteristics in 2 homogeneous groups.

B. Genetic Similarity

The matrix obtained disclosed similarity coefficients varying from 0.207 to 0.457, with an average of 0.332. These values express divergent groups in the plant material studied. Thus, we noticed that the pair "PL7-PL13" recorded a similarity coefficient equal to 0.207. However, these two pollinators exhibited significant discrimination distance. The differences were mainly noticed on the following morphological characteristics: length of the part thorny (D13), the color of the petiole (D15), the width of the palm at the base of the petiole (D16), the rigidity of the thorns (D18), the number of spines by type of grouping (D20), flexibility of leaf (D24), and angle divergence of terminal leaflet (D26). Moreover, pollinators tested for the following characteristics: the density of the spikelet in the spathe (D35), pollen productivity per spathe (D41), pollen odor (D42), pollen color (D43). Accordingly, the highest similarities (0.457) were observed between pairs of: "PL26, PL117"; "PL117, PL166"; "PL117, PL154" and "PL155, PL26," which are sharing several numbers of characters. For exemple PL26 and PL117 were similar for the following characters the color of the petiole (D15), the rigidity of the thorns (D18), leaf

grouping (D25), maximum width of middle leaflet (D27), number of flowers per longest spikelet (D39) and number of flowers per shortest spikelet (D40).

C. Principal Component Analysis (PCA)

The morphological parameters examined using the principal component analysis (PCA) using the software SPSS. We choose axis1 (F1) and axis2 (F2), which have absorbed the maximum of existing variability between the date palm pollinators. They showed successively 17.91% and 13.45%. According to the results, the axis F1 representing a variability of 17.91% (Fig. 4), is defined by the following characters: the total length (D11), the thickness of the spine (D14), the rigidity of the thorns (D18), the maximum thickness of the central spine (D19), flexibility of leaf (D24), (the total length (D33), the maximum width of the spathe (D34); the density of the spikelet in the spathe (D35); the number of spikelet per spathe (D36); the length of the longest spikelet (D37), number of flowers per longest spikelet (D39) and weight of the spathe (D44).While the axis F2 is defined by the maximum width (D12), the average number of thorns (D17), the number of spines by type of grouping (D20), the maximum thickness of the central spine (D21), maximum length of the thorn (D22), angle divergence of terminal leaflet (D26), maximum length of middle leaflet (D28) and length of terminal leaflet(D29).

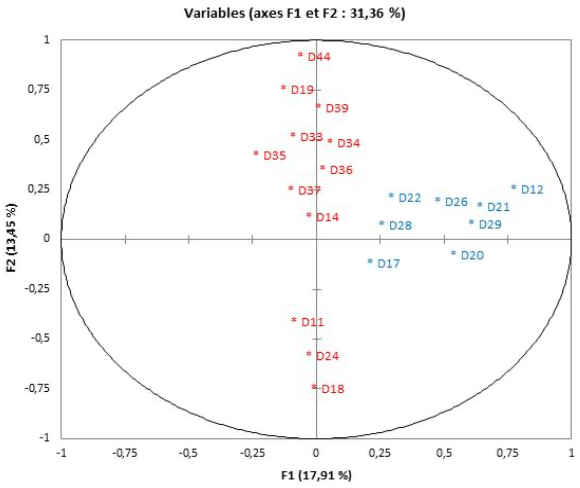

Fig. 4. Principal component analysis of the correlation matrix between variables and factors (F1 and F2).

1) Dispersion of Individuals in the PCA F1-F2 Plan

The projection of pollinators in the plane defined by the two axes F1-F2 representing the inertia of 31.36%, shows that the pollinators form 5 different groups A, B, C, D, and E (Figure 5). Group A is formed by two pollinators PL26, PL117 which are very far from other groups and have almost exact coordinates, and this group is independent of the positive side of the F1 axis and the negative side of the F2 axis. Thus, they discriminate from others by defining the axis F1 as the average number of thorns, the maximum palm width. Group B is represented by the pollinators PL7, PL5, PL6, PL136, PL67, PL4, and PL181, which correlate negatively with F1; therefore, they gathered by characters defining the latter distinguished from others by the same characters. Group C consists of pollinators: PL133, PL166, PL154, PL108, and PL155. Group D, which correlates positively with F2, comprises the pollinators PL1, PL83, PL73, PL85, and PL13. Group E only carries the pollinator PL70, which is highly correlated with F2; therefore, this pollinator differs from the others by the characters defining this axis: the average number of thorns, number of thorns by type of grouping, maximum thickness of the middle thorn.

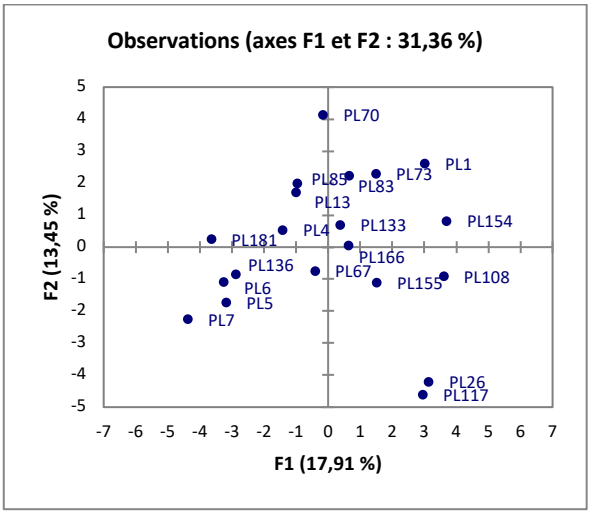

Fig. 5. Dispersion of the 20 pollinators of date palm in the plane defined by the first two components 1 and 2 of the PCA which absorb 31.36% of the variability based on the morphological markers.

D. Phylogenetic Dendrogram

The similarity coefficients were used by the WPGMA analysis sub-program of the NTSYS software to obtain a dendrogram that presents the different pollinators studied (Fig. 6). For the 20 pollinators studied, five groups were distinguished from a similarity threshold of 46%. Group A includes the pollinators PL1, PL13, and PL181, which are divided into two subgroups; the first one includes PL 1 and PL13, the second is the pollinator PL181; these three pollinators have several common characteristics such as the maximum length of the palm (ranging from 3.7 to 3.9m), thickness of the rachis, width of the palm at the base of the petiole, average stiffness of the spines, and olive green color and the grouping in 2 of the leaflet. These three pollinators have early flowering and have the same pollen characteristics (high productivity, yellowish color, strong odor). Group B is composed of PL4, PL6, PL70, and PL136 divided into two subgroups, the first subgroup comprises PL6 and PL70, and the second comprises PL136. Their common characteristics are the mode of development (medium period), the average density of the spikelets, and the high pollen productivity. Group C groups PL5, PL26, PL117, and PL155, are divided into 2 subgroups, one is composed of PL5, and the other subgroup includes PL115, PL26, PL117, the last two pollinators are almost identical. This group has a yellowish-colored petiole, almost the exact width of the palm at the base of the petiole, medium to flexible stiffness of the spines, almost the same number leaflet, the same maximum length of the thorn, the same color of the leaflet (yellowish-green), flexibility and apical divergence of the leaflet, spindle-shaped of the spathe, early mode of development of the spathe, medium density of spikelets in the spathe. The subgroups PL26, PL117, and PL155 have the same pollens properties (high productivity, whitish color, weak odor). Group D includes PL67, PL166, PL108, PL154, PL73, PL83, PL85, and PL133, which are divided into two subgroups, a and b.

The subgroup comprises PL108, PL154, PL67, and PL166, and subgroup b is formed by PL73, PL83, PL85, and PL133. The first subgroup has almost the same descriptors for the palm, inflorescence, and pollens. By comparing the two sub-groups, we find several common points between them such as the olive green color of leaflet, maximum thickness of the middle spine (5 cm), the grouping of leaflet (in 3), length of the most spikelet long (100 cm), weak odor of pollen. Group E is defined only by the pollinator PL7, which has low pollen productivity.

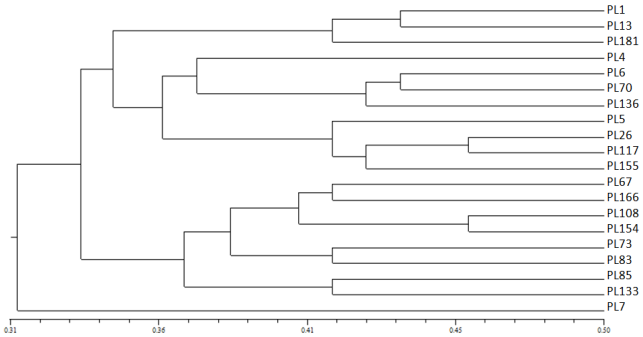

Fig. 6. Dendrogram grouping the 20 date palm pollinators established by the WPGMA method from the matrix of genetic similarities determined on morphological data.

E. Molecular Study

1) ISSR Amplification

Ten primers were used to study 20 date palm pollinators, with only seven primers succeeded in reproducing and polymorphic bands. The results proved the efficiency of those primers in exploring molecular polymorphism in date palm. A total of 64 reproducible bands were obtained during amplification, of which 57 bands are polymorphic with a polymorphism rate of 90%. Three primers demonstrated a polymorphism rate of 100% generating bands ranging from 6 to 9. The average of bands per primer was 9.14 for all the bands and 8.1 for the polymorphic bands. The average of bands by genotype was 3.2. The sizes of the bands obtained varied between 250bp and 1000bp. The Polymorphism Information Content (PIC) allows us to measure each marker polymorphism level. The PIC values fluctuated 0.897 to 0.717 in most polymorphic locus ISSR15 andISSR10, with an average of 0.846, revealing a high level of polymorphism for all loci (Table II). This height value of the PIC can be due to the dioecious nature of the date palm germoplasm (Arabnezhad *et al*., 2012). In addition, this value demonstrates the efficiency and power of ISSR markers used to study the genetic diversity of selected date palm pollinators.

1) Genetic Similarity and Structure

The estimation of genetic similarities among the 20 pollinators was conducted by engaging the related data matrix in Nei and Li (1979) formula related to the coefficient of Dice. Applying the software NTSYS (version 2.0) -pc to the entire binary matrix (resulted from the molecular data) revealed to obtain the genetic similarity matrix. Analysis of the matrix showed that the genetic similarity coefficients varied from 0.491 to 0.873 and with an average of 0.682; this exhibited that the pollinators studied constitute genetically linked groups since most of the genetic similarity indices fluctuate between genetic distances ranging from 0.5 to 0.6. Furthermore, the highest similarity was observed in the combination (PL5, PL6) with a similarity index of 0.873, which showed the solid molecular resemblance between the two pollinators. On the other hand, the combination of lowest similarity with 0.491 was (PL13, PL85). According to Dice, the similarity coefficients were used in UPGMA analysis sub-program of the NTSYS software (version of 2.02) to generate the dendrogram for grouping investigated pollinators. Considering the 20 pollinators studied, five groups: A, B, C, D, and E, were distinguished from a threshold of 55% similarity (Fig. 7). Group A has two sub-groups, a and b; subgroup a is composed of 2 pollinators PL1 and PL181, the second subgroup is further divided into two subgroups, subgroup n1 is composed of pollinators PL4 and PL73, the second subgroup is divided into two other subgroups, the first is formed by PL5 and PL6 and the second by PL7. Group B consisted of 2 pollinators PL70 and PL117. Group C is the largest group composed of 2 subgroups A and B. Subgroup A is composed of 2 subgroups, and the first subgroup, a1, is divided into two other subgroups a1.1 and a1.2, a1.1 is formed of 2 subgroups a1.1.1 composed of P13 and a112, which P67 and P108 form, a1.2 is formed by a single pollinator PL83. The sub-group a2 includes three pollinators PL26, PL133, and PL136. A single PL166 pollinator forms subgroup b. Group D only carries two pollinators PL154 and PL155. Group E is represented only by the pollinator PL85.

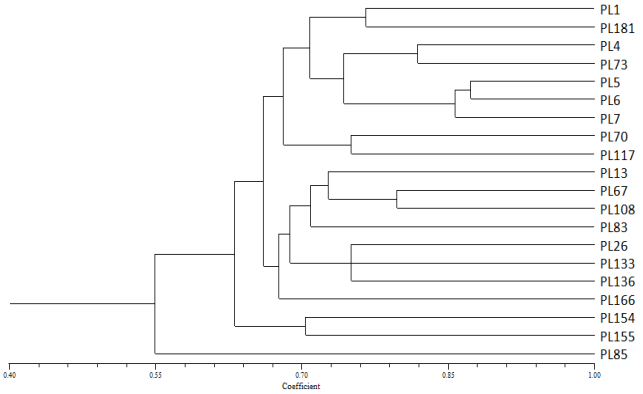

Fig. 7. Dendrogram grouping the 20 date palm pollinators established by the UPGMA method from the genetic similarities (DICE coefficient) matrix determined on ISSR data.

TABLE I: NUMBER OF TOTAL BANDS, THE NUMBER OF POLYMORPHIC BANDS, RATE OF POLYMORPHISM, AND PIC OF THE BANDS GENERATED BY ISSRS MARKER ON DATE PALM POLLINATORS

Primers	Tm (°C)	Coded	Numbers of amplified bands	Numbers of polymorphic bands	Polymorphism rate %	PIC value
(CT)10G	49	ISSR8	11	10	91%	0,8725
(GA)CC	35	ISSR10	8	7	88%	0,7179
(AG)10C	57	ISSR11	9	9	100%	0,8881
(GT)10(AGC)2ACT	52	ISSR12	7	7	100%	0,7792
(CA)7(GCT)2AGT	50	ISSR13	9	9	100%	0,8856
(TC)10C	57	ISSR14	9	6	67%	0,8820
(TC)10G	60	ISSR15	11	9	82%	0,8974
Total / mean		-	64	57	90%	0,8461

2) Spatial Structure of Pollinators

In order to understand the structuring of polymorphism, the binary matrix (absence and presence of bands) generated by primers, similarity matrix (obtained using the similarity coefficient) was employed in MDSCALE analysis. (Multidimensional scaling) using NTYS-pc version 2 .02 software. The MDSCALE Analysis reflects the diversity among the different pollinators studied based on genetic distance. The graphical representation shows that the majority of pollinators are more or less dispersed. Going from bottom to top, we can distinguish seven groups (Fig. 8). Group A is formed only by the pollinator PL155, which is followed by group B, which carries two pollinators PL85 and PL154.

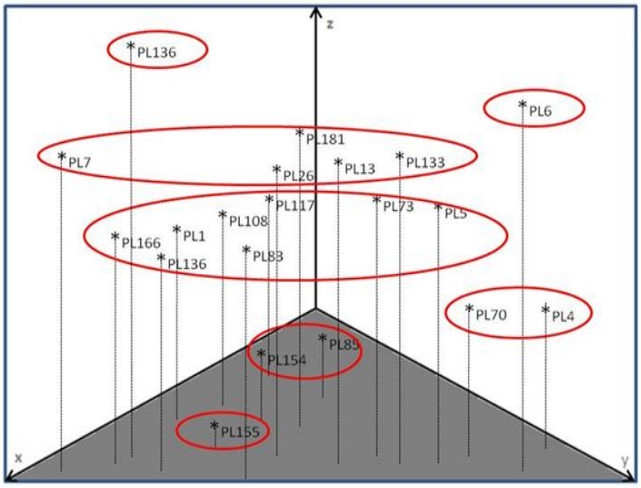

Fig. 8. Graphic representation of the projection of male pollinators in space by an MDSCALE (UPGMA) analysis using the coefficient SM and the binary matrix.

The two pollinators PL155 and PL154 share several morphological characters, such as the thickness of the spine, the number of pinnae, and the spikelets density. Group C consists of the pollinators PL70 and PL4. Group D is subdivided into two subgroups, a and b, subgroup a carries two pollinators PL73 and PL5, and sub-group b is formed by PL166, PL67, PL1, PL108, PL83, and PL117. This group is followed by group E, which contained two subgroups, a and b. Subgroup a is formed by the pollinator PL7, which is the pollinator that differs from the others by its low pollen productivity, and subgroup b carries the pollinators PL13, PL181, PL26, PL133. The pollinators PL26 and PL117, belonging to two successive groups D and E, are similar to each other by several morphological markers such as the palm width, the average number of thorns, and divergence apex of the pinnae. Group F and Gare formed only by PL6 and PL136, respectively.

3) Mantel's correlation tests

The correlation between the genetic similarity matrix (SM) and the morphological traits taken two by two is processed through the Mantel test (1970) and resulted in a non-significant weak correlation (r = 0.01573, p = 0.4066 with n = 1000 permutation) between the molecular and morphological data (Fig. 9).

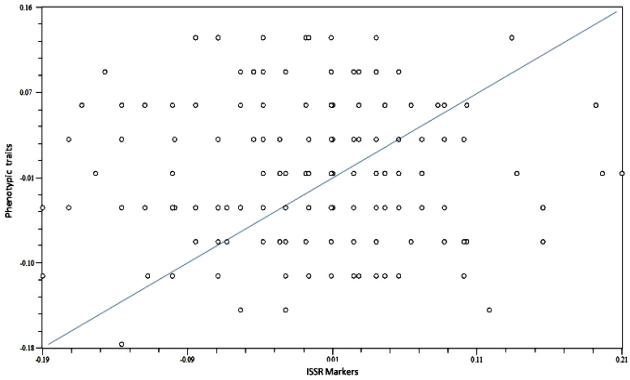

Fig. 9. Correlation matrix between molecular markers (ISSR) and morphological traits.

The processing of molecular data by various methods generated very significant correlations. However, the correlation between the coefficient SM and the coefficient DICE showed an important correlation (figure 10) with r = 0.74854 (0.2 <r <0.9) and p = 0.002 (p≤0.005). On the other hand, for morphological markers, the correlation between the SM coefficient and the DICE coefficient showed a poor correlation between the markers with r = 0.003 and p = 0.02.

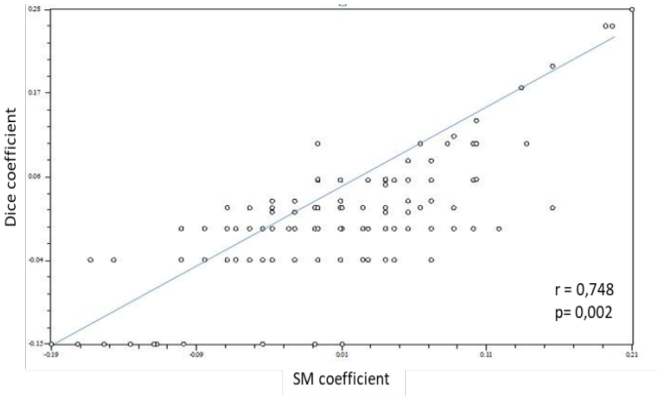

Fig. 10. Correlation matrix between the coefficient SM and the coefficient DICE for ISSR markers.

IV. Discussions

Phenotypic characteristics are essential elements in the definitive classification of living organisms. We tried to set up an experiment based on morphological tools during this study, allowing the groups of pollinators to characterize the most discriminative one. Consequently, we assume that the feet studied are characterized by a higher level of genetic diversity, strongly supported by the pollinators' projection in the main PCA plan and the UPGMA clusters' analysis. The results obtained showed, on the one hand, a similarity between the date palm pollinators studied, for the following morphological characters: The same shape of the stipe (Cylindrical), The Persistence of leaf scars, the absence of aerial and mane releases of fluff, the angle of the palm, the rotation of the palm and the flexibility of the leaflet. These results are consistent with a one found with date palm cultivars (Kadri *et al*., 2016; Kadri *et al*., 2015), including the stipe's exact shape (Cylindrical), Persistence of cornafs, the absence of air, and mane fluff releases. Naqvi *et al.* (2015) and Abul-Soad *et al*. (2013) investigated some morphological features in date palm cultivars and revealed morphological variability among them related to heterogeneity within

cultivars from the same origin.

On the other hand, Hammadi *et al*. (2012) and Ahmad *et al.,* (2011) reported significant variability, especially concerning palm descriptors; this can generate a wide range of diversity within genotypes. The most discriminating characteristics according to our principal component analysis are (D11), (D14), (D18), (D19), (D24), (D33), (D34), (D35), (D36), (D37), (D39), (D44), (D12), (D17), (D20), (D21), (D22), (D26), (D28), and (D29). Our results were generally matching with Ould Mohamed Salem *et al.,* (2008), reported for 11 cultivars and the male foot of Mauritanian date palm, conducted based on 18 phenotypic markers. The principal component analysis resulted in discriminating 6 characters: width of the leaf, length of the petiole, thickness of the spine, number of pinnae, length of the palm, and length of the thorny part. (Naviq *et al*., 2015) analyzed 20 date palm cultivars and reported the effectiveness of specific palm descriptors in discriminating cultivars; among them, we have used the following: the length of the penne in the middle of the palm, the ventral angle of the middle pin, the width of the middle pin, the apical angle of the palm, the length of the apex pin and the width of terminal leaflet. Also, Elhoumaizi *et al.* (2002) studied 26 Moroccan date palm cultivars by 26 morphological characters and noticed a tremendous morphological variation, in particular for the width of the leaves, number of pinnae, width of the pinna in the middle, the length of the pinnae at the bottom, and the width of the apical leaf. Elshibli and Korpelainen (2009) According to morphological criteria, observed with studding 15 cultivars of date palm, significant differentiation of cultivars concerning tree height, and number and length of pinnae spines.

Moreover, Elsafy *et al.* (2015) explored the phenotypic diversity with fifty Sudanese date palm cultivars. It revealed that fourteen out of the sixteen quantitative and qualitative traits investigated showed a vital discriminating factor suggesting their possible use the initiation of date palm morphological descriptor list. Analysis of variance and classification of homogeneous groups show that each character has a part to contribute to variability. This variability between cultivars is probably due to genotypic origin (most from semi) and associated with the environmental condition because the cultivars are established on the same farm. El Kadri *et al.* (2019) studied the morphological and genetic diversity of 24 male date palm genotypes from Southern Tunisia. The results revealed that fourteen of the fifteen quantitative characters studied showed a high discriminating power to distinguish male date palm trees.

The second part of this study aimed to contribute to identifying cultivars and their relationships using molecular markers. We evaluated the polymorphism of 7 ISSR markers in 20 male date palm cultivars. ISSR markers have been widely used to assess molecular diversity in plant species, including date palm (Karim *et al*., 2010; Hamza *et al*., 2012). The results obtained indicate a significant level of polymorphism between the cultivars tested with the morphological markers used, which allowed us to quantify genetic variation and analyze phylogenetic relationships inside this date palm male's collection. The results are comparable to those employing the same marker systems. Comparable results of alleles and percentages of polymorphisms have been reported by (Haider *et al*., 2012) identified Syrian date palm cultivars using 23 accessions, and 15 ISSR primers resulted in a high level of polymorphism. The same result was recorded in Algeria (Guettouchi *et al*., 2017), Iran (Marsafari and Mehrabi, 2013), and Tunisia (Hamza *et al*., 2012). In Tunisia, the polymorphism level obtained in this study on male feet is more significant than that obtained with the same technique for female feet. However, Karim *et al*. (2010), using 7 ISSR primers on ten female varieties of date palm in Tunisia, found a level of polymorphism of 82%.

Similarly, Hamza *et al.* (2012) on 26 female cultivars of the Tunisian date palm with 7 ISSR primers, found a polymorphism rate of 78%. These results show that the amount of genetic diversity also depends on gender. This difference can also be explained by the fact that the male palmis propagated by seedling, which is the primary source of heterozygosity in date palm.

In contrast, the standard propagation of female date palm is by off-shoot, which results in a true-to-type progeny homozygous or identical to the mother plant. The study of the correlation between morphological and molecular markers using Mentel's test indicates a low linkage ($r= 0.0157$; $p =0.4066$). That attributed to the environmental effect on morphological traits or an eco-physiological adaptation of the cultivars studied associated with these conditions. However, the molecular markers are independent of a bioclimatic condition (Ahmed *et al*., 2010). The results were agreed with by Hamza *et al*., (2011) with weak correlations ($r = -0.027$, $p= 0.382$) between SSR markers and morphological criteria. Studies on other species have found weak correlations between molecular and morphological markers (Lewontin, 1984; Lynch, 1996; Reed and Frankham, 2001).

On the other hand, a significant correlation was recorded with molecular data between the similarity matrix (SM coefficient) and the dendrogram (DICE coefficient), which showed a strong correlation with $r = 0.74854$ ($0.2 <r <0.9$) and $p = 0.002$ ($p\leq0.005$). The same results were obtained by Guettouchi *et al*., 2017), who analyzed the correlation between the genetic distance and the genetic similarity matrix for the ISSR markers ($r = 0.360$; $p < 0.006$), the same result was shown with Jamel *et al*. (2014) by analyzing the genetic diversity of ten date palm cultivars from Saudi Arabia with AFLP and ISSR markers. The independence of the molecular markers can explain this with the environmental conditions; indeed, we can differentiate according to the morphological markers between two individuals, but not on the genotype level, which raises the problem of synonymy of individuals but with different names. In this study, the couple PL7 and PL13 is distant on the phenotypic level with the lowest similarity coefficient of (0.207); however, they lay at the same group E according to the spatial representation (MDscale). Therefore, this suggests that ISSRs markers are more efficient and stable than morphological markers to study such a significant genetic diversity. In conclusion, the molecular marker could be the most discriminatory criterion for distinguishing date palm cultivars from morphological traits. Thus, specific morphological criteria distinguish the closely related date palm male cultivars before the fruiting stage.

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APPENDIX

TABLE II: LIST OF MEASURED QUANTITATIVE AND QUALITATIVE MORPHOLOGICAL CHARACTERS

Pollinators Descriptors	PN1	PN4	PN5	PN6	PN7	PN13	PN26	PN67	PN70	PN73	PN83	PN85	PN108	PN117	PN133	PN136	PN154	PN155	PN166	PN181
D1	Hard	Med*	Med*	Med*	Med*	Hard	Hard	Med*	Med*	Med*	Hard	Hard	Hard	Med*	Hard	Med*	Hard	Hard	Hard	Med*
D2	Sph*	Drop	Sph*	Erect	Sph*	Sph*	Sph*	Sph*	Sph*	Erect	Erect	Sph*	Sph*	Sph*	Sph*	Sph*	Sph*	Sph*	Sph*	Sph*
D3	Airy	Airy	Des*	Airy	Airy	Med*	Des*	Airy	Airy	Med*	Des*	Med*	Airy	Des*	Med*	Airy	Airy	Airy	Airy	Airy
D4	Coc*	Coc*	Cyl*	Cyl*	Coc*	Cyl*	Cyl*	Cyl*	Cyl*	Coc*	Coc*	Coc*	Cyl*	Cyl*	Coc*	Cyl*	Coc*	Cyl*	Coc*	Coc*
D5	Yes	Yes	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	Yes
D6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
D7	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
D8	Low	Low	Med*	Low	Med*	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low	Low
D9	2/3*	1/2*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	2/3*	1/3*	2/3*	2/3*	2/3*	1/3*	1/3*
D10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
D11 (m)	3.72	3.21	3.43	3.05	3.06	3.9	4.45	3.68	3.81	3.61	3.27	3.38	4.40	4.11	3.52	3.77	4.17	4.03	3.93	3.85
D12 (cm)	70.60	61.83	61.16	64.16	51.60	49.30	44.50	49.30	80.66	82.50	61.66	50.33	64.66	39.33	58.28	50.60	50.33	41.43	54.50	50.00
D13 (cm)	73.00	72.60	55.30	61.50	62.83	102.0	77.00	139.0	80.93	74.75	93.83	74.16	60.83	84.33	68.60	70.83	107.1	114.5	77	71.50
D14 (cm)	2.30	2.06	2.10	2.20	2.26	2.16	2.85	1.85	2.30	2.50	2.26	2.10	3.00	3.00	3.00	2.15	2.56	2.36	2.15	2.30
D15	Yg*	Yg*	Yg*	Br*	Br*	Yg*	Yg*	Yg*	Br*	Yg*	Yg*	Gr*	Yg*	Mr*	Yg*	Yg*	Yg*	Yg*	Yg*	Wh*
D16 (cm)	7.90	6.50	8.00	7.50	9.06	8.60	9.40	6.00	12.16	8.80	7.63	7.25	9.26	7.50	7.20	6.50	7.36	7.90	7.70	8.00
D17	26	28	22	22	23	41	19	37	34	35	34	49	34	26	18	26	27	48	46	13
D18	Med*	Med*	Med*	Med*	Rig*	Med*	Flex*	Med*	Med*	Med*	Med*	Med*	Rig*	Flex*	Med*	Rig*	Med*	Flex*	Med*	Med*
D19 (mm)	15.10	15.40	17.10	17.00	15.90	18.80	20.80	19.00	18.50	18.30	17.80	17.40	20.50	21.50	18.40	17.20	20310	19.50	20.50	16.00
D20	Tg2*	Tg2*	Tg1*	Tg1*	Tg2*	Tg3*	Tg1*	Tg2*	Tg2*	Tg1*	Tg3*	Tg2*	Tg2*	Tg1*	Tg2*	Tg2*	Tg2*	Tg2*	Tg1*	Tg2*
D21 (mm)	7.30	6.00	6.00	4.00	4.20	6.60	4.75	5.20	6.60	7.50	7.00	6.00	5.00	4.30	7.00	4.10	5.20	4.00	5.00	4.50
D22 (cm)	17.90	16.50	21.00	26.50	28.50	20.40	22.50	20.60	18.20	20.50	15.80	17.80	27.00	30.00	16.50	16.00	19.90	21.90	11.70	17.50
D23	Og*	Og*	Yg*	Yg*	Yg*	Og*	Og*	Yg*	Yg*	Og*	Og*	Yg*	Yg*	Og*	Yg*	Og*	Yg*	Yg*	Og*	Og*
D24	Med*	Med*	Med*	hard	Med*	Hard	Med*	hard	Med*	Med*	Med*	Med*	Low	Med*	Med*	Hard	Med*	Med*	Hard	Hard
D25	Lg*2	Lg*2	Lg*2	Lg*3	Lg*3	Lg*2	Lg*3	Lg*3	Lg*2	Lg*3	Lg*3	Lg*3	Lg*3	Lg*2	Lg*2	Lg*2	Lg*3	Lg*3	Lg*3	Lg*2
D26	Med*	Med*	Low	Med*	Low	Low	Low	Med*	Med*	Med*	Med*	Hard	Low	Low	Med*	Low	Med*	Low	Low	Low
D27 (cm)	2.70	3.50	3.40	3.60	3.30	4.15	4.55	2.80	2.55	3.10	2.90	2.50	3.50	2.50	3.30	2.40	3.5	2.40	3.00	4.20
D28 (cm)	55.30	44.30	39.30	43.30	41.83	43.60	41.50	35.80	57.50	52.75	47.50	53.33	55.60	47.00	42.20	43.30	46.20	49.30	46.60	43.50
D29 (cm)	23.30	21.10	20.60	18.30	17.15	29.00	12.50	19.00	37.30	29.25	18.90	37.60	20.30	19.25	22.60	24.00	28.80	28.60	29.00	27.50
D30 (mm)	12.00	11.00	12.00	14.00	13.00	21.60	13.50	13.00	18.60	17.00	15.30	15.00	12.00	7.30	18.00	18.20	17.30	17.45	18.30	26.50
D31	Lct*	Fus*	Fus*	Fus*	Fus*	Fus*	Fus*	Lct*	Fus*	Fus*	Fus*	Fus*	Fus*	Fus*	Inf*	Lct*	Fus*	Fus*	Fus*	Fus*
D32	Prc*	Prc*	Prc*	Med*	Med*	Prc*	Prc*	Prc*	Med*	Med*	Prc*	Late	Prc*	Late	Late	Med*	Late	Late	Late	Late
D33 (cm)	126	61.50	53.30	70.00	58.50	135	78.00	100	49.00	107	100	75.00	105	80.00	92.50	62.00	125	90.00	108	69.00
D34 (cm)	20.00	14.00	9.00	10.50	9.20	12.00	19.00	11.00	13.50	11.50	13.40	14.00	14.30	17.00	18.50	12.00	14.00	15.00	14.50	13.00
D35	Cp*	Med*	Low	Med*	Low	Low	Med*	Med*	Med*	Med*	Med*	Low	Med*	Med*	Med*	Med*	Cp*	Med*	Med*	Low
D36	639	301	135	115	57	320	225	271	29	229	274	225	272	343	272	156	250	213	433	202
D37 (cm)	30.50	22.00	18.00	18.50	19.00	17.00	33.20	25.50	29.00	27.70	23.50	23.60	29.50	37.00	25.00	24.00	25.20	31.50	21.00	19.00
D38 (cm)	4.50	4.00	4.10	5.00	5.10	3.00	2.60	5.00	4.50	2.00	7.50	2.50	7.00	3.40	3.00	4.00	3.00	3.70	4.00	3.00
D39	101	63	39	56	39	54	115	86	95	90	57	103	59	92	68	60	84	88	83	24
D40	11	9	6	5	4	11	5	9	17	4	16	4	15	14	11	7	8	6	17	7
D41	Hgt*	Hgt*	Lht*	Hgt*	Lht*	Med*	Hgt*	Hgt*	Hgt*	Hgt*	Hgt*	Med*	Hgt*	Hgt*	Hgt*	Hgt*	Hgt*	Med*	Lht*	Hgt*
D42	Stg*	Stg*	Lht*	Stg*	Lht*	Stg*	Lht*	Lht*	Stg*	Lht*	Lht*	Lht*	Lht*	Lht*	Lht*	Stg*	Lht*	Lht*	Lht*	Stg*
D43	Ylw*	Ylw*	Wht*	Wht*	Ylw*	Ylw*	Wht*	Wht*	Ylw*	Wht*	Wht*	Wht*	Wht*	Wht*	Wht*	Wht*	Ylw*	Ylw*	Wht*	Wht*
D44 (g)	1800	1350	870	790	710	1480	1630	1260	1560	1540	1720	1470	2040	1655	1140	610	1940	1630	1520	960
D45	Sin*	Sin*	Sin*	Sin*	Sin*	Sin*	Sin*	Sin*	Sin*	Vsi*	Sin*	Sin*	Sin*	Sin*	Sin*	Sin*	Vsi*	Sin*	Sin*	Sin*

A. Supplementary Data: List of Measured Quantitative and Qualitative Morphological Characters

vigor (D1), the port (D2), the appearance of the crown (D3), the shape of the trunk (D4), Persistence of leaf scars (D5), the presence of high off-shoot (D6), the presence of fluff mane (D7) and the capacity to produce basal off-shoot (D8), the level of curvature (D9), the rotation (D10), the total length (D11, m), the maximum width (D12, cm), length of the part thorny (D13, cm), the thickness of the spine (D14, cm), the color of the petiole (D15), the width of the palm at the base of the petiole (D16, cm), the average number of thorns (D17), the rigidity of the thorns (D18), the maximum thickness of the central spine (D19), the number of spines by type of grouping (D20), the maximum thickness of the central spine (D21, mm), maximum length of the thorn (D22, cm), color of leaf (D23), flexibility of leaf (D24); leaf grouping (D25), angle divergence of terminal leaflet (D26); maximum width of middle leaflet (D27, cm), maximum length of middle leaflet (D28, cm), length of terminal leaflet (D29, cm) and width of terminal leaflet (D30, cm), the shape (D31), the mode of development of the spathe (D32), the total length (D33, cm), the maximum width of the spathe (D34, cm); the density of the spikelet in the spathe (D35); the number of spikelet per spathe (D36); the length of the longest spikelet (D37, cm); length of shortest spikelet (D38, cm), number of flowers per longest spikelet (D39), number of flowers per shortest spikelet (D40) pollen productivity per spathe (D41), pollen odor (D42), pollen color (D43), weight of the spathe (D44, kg), and spikelet shape (D45).

Med* : Medium ; Sph* : Spheric ; Des* : Dense ; Coc*: Conical; Cyl*: Cylindric; 2/3*: curvature at 2/3 of the palm; 1/3*: curvature at 1/3 of the palm; 1/2* : curvature at 1/2 of the palm; Yg* : yellowish green color; Gr* : Green; Br* : Brown; Mr*: Marbled; Wh*: Whitish; Rig* : Rigid; Flex* : Flexible; Tg1* : Thorns grouping by 1; Tg2*: Thorns grouping by 2; Tg3*: Thorns grouping by 3; Og* : Olive green; Bg*; Yg* : Yellowish green; Lg*2 : Leaf grouping by 2. Lg*3: Leaf grouping by 3; Lct* : Lonceolate; Fus* : Fusiform; Inf* : Inflated; Prc*: precocious; Lte* : Late; Cp* : Compact; Hgt* : High; Lht* : Light* : Much; Stg* : Strong; Ylw* : Yellowish. Wht*: Whitish; Sin*: Sinuous, Vsi*: Very sinuous.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

Abdelmajid, R. (2005), *Le Palmier Dattier en Tunisie: I. Le patrimoine génétique-Volume 2*, International Plant Gentic Resources, Bioversity International. Rome Italy. French.

Arabnezhad H., Bahar M., Mohammadi H.R., Latifan M. (2012). Development characterization and use of microsatellite markers for germplasm analysis in date palm (*Phoenix dactylifera* L.). *Scientia Horticulturae.* 134, 150–156.

Abulsoad A. A., Jatoi M. A., Markahand G. S. (2013). Performance of three Saudi Arabian date palm varieties under the agro-climatic conditions of Khairpur. *Pakistan Journal of Agricultural Sciences*. 2013, 50, 571-576.

Ahmed, T. (2010). *Genetic and environmental variation among Qatari date palm cultivars assessed by DNA markers*. Qatar Foundation Annual Research, Hamad bin Khalifa University Press (HBKU Press), EEP8, 20(1).

Ahmed, T. A., Alsamaraee, S. A., Zaidan, H. Z., Elmeer, K. (2012). Inter-simple sequence repeat (ISSR) Analysis of somaclonal variation in date palm plantlets regenerated from callus. *Second International Conference on Environment and Industrial Innovation, IPCBEE*. 126-130.

Alfarsi M. A., & Lee, C. Y. (2012). Nutritional and functional properties of dates: a review. *Critical Reviews in Food Science and Nutrition.* 48 (2), 877-887.

Ben Abdallah A., Stiti K., Dujardin P., Lepôivre P. A. (2000). Identification de cultivars de palmier dattier (*Phoenix dactylifera* L.) par l'amplification aléatoire d'ADN (RAPD). *Cah Étud Recher Francoph/Agric*. 9, 103-107.

Ben Abdallah, A. (1990). Les systèmes Agricole Oasiens. *La phoeniciculture Options Méditerranéennes*, Série A. N° 11.: 105-120. French.

Bezato, T. (2013). *Les palmiers dattiers «Phoenix dactylifera* L*» à Toliara: Étude De La Filière, Utilisation Et Diversité Variétale*. Mémoire d'étude approfondie en biodiversité et environnement. Université de Toliara des sciences Madagascare. French.

Cherif E., Zehdi S., Castello K., Chabrillange N., Abdoulkader S., Pintaud J. C., et al. (2013). Male-specific DNA markers provide genetic evidence of an XY chromosome system, a recombination arrest and allow the tracing of paternal lineages in date palm. *New Phytologist*. 197, 409-415.

Dagnall N., Parker A., Munley G. J. (2007). Paranormal belief and reasoning. *Personality and Individual Differences.* 43 (6),1406-1415

Elkadri N., Mimoun M. B., Hormoza J. H. (2019). Genetic diversity of Tunisian male date palm (*Phoenix dactylifera* L.) genotypes using morphological descriptors and molecular markers. *Scientia Horticulturae*. 253, 24-34.

Elhoumaizi M. A., Saaidi M., Oihabi A., Cilas C.J. (2022). Phenotypic diversity of date-palm cultivars (*Phoenix dactylifera* L.) from Morocco. *Genetic Resources and Crop Evolution*. 49, 483-490.

Elsafy M., Gustavasson L., Mujaju C. (2015). Phenotypic diversity of date palm cultivars (*Phoenix dactylifera* L.) from Sudan estimated by vegetative and fruit characteristics. *International Journal of Biodiversity.* 7, 325-332.

Elshibli, S. & Korpelainen, H.R. (2009). Biodiversity of date palms (*Phoenix dactylifera* L.) in Sudan: chemical, morphological and DNA polymorphisms of selected cultivars. *Plant Genetic Resources.* 7, 194-203.

Ferchichi, A. & Hamza, H. (2008). *Le patrimoine génétique phoenicicole des oasis continentales tunisiennes*, Institut des régions arides. Mednin, Tunisia. French.

Guettouchi A., Elshibli S., Haider N., Nabulsi I., Ykhlef N. M. (2017). Molecular diversity in date palm (*Phoenix dactylifera* L.) cultivars from Algeria indicated by RAPD and ISSR polymorphisms. *Plant Cell Biotechnology and Molecular Biology.* 18(1), 76-89.

Haider N., Nabulsi I., Mirali N.R. (2012). Phylogenetic relationships among date palm (*Phoenix dactylifera* L.) cultivars in Syria using RAPD and ISSR markers. *Journal of Plant Biology Research*. 1(2), 12-24.

Hammadi H., Mokhtar R., Mokhtar E., Ali F. (2009). New approach for the morphological identification of date palm (*Phoenix dactylifera* L.) cultivars from Tunisia. *Pakistan Journal of Botany*. 41, 2771-2681.

Hamza H., Elbekkay M., Benabderrahim M., Ferchichi A. (2011). Molecular and morphological analyses of date palm (*Phoenix dactylifera* L.) subpopulations in southern Tunisia. *Spanish Journal of Agricultual Resources.* 9, 484-493.

Hamza H., Ben Abderrahim M. A., Elbekkey M., Ferdous G., Triki T., Ferchichi A. (2012). Investigation of genetic variation in Tunisian date palm (*Phoenix dactylifera* L.) cultivars using ISSR marker systems and their relation with fruit characteristics. *Turkish Journal of Biology*. 36, 449-458.

Hussein E. H., Adawy S. S., Ismail S., Elitriby H. (2005). Molecular characterisation of some Egyptian date palm germplasm using RAPD and ISSR markers. *Arab Journal of Biotechnology*. 1, 83-98.

IPGRI (2005). Descripteurs du Palmier dattier (Phoenix dactylifera L*.). Institut international des ressources phytogénétiques,* Rome, Italie*. French.*

Kadri K., Baba W., Golem Z., Makhlouf S., Barrani H. (2016). Analysis of morpho-physiological diversity of some Tunisian date palm pollinators and study of their whorl pollen grain on fruit set, the time of ripening and yield of date. *International Journal of Current Agricultural Sciences (IJCAS*). 6(5), 34-43.

Kadri K., Kods B., Souhayla M. (2017). Physiological characterisation of some male pollinators in Tunisia and study of the effect of conditioning temperature on the viability and germination of pollen. *Journal of New Sciences*. (48), 2907-2920.

Kadri K., Raddaoui I., Makhlouf S., Hcini K. (2015a). Morphological and molecular evaluation of the genetic diversity of Tunisian local date palm pollinators. *Academia Journal of Biotechnology*. 3(2), 26-34.

Kadri K., Halima B., Khaled C., Nabila K., Foued., B. (2015b). Genetic

diversity analysis of a local date palm pollinators collection (*Phoenix dactylifera* L.) using SSR markers and study of their metaxenic effects on the maturation and quality of dates obtained. *Academia Journal of Agricultural Research*. 3(12), 381-394.

Lewontin, R. (1984). Detecting population differences in quantitative characters as opposed to gene frequencies. *American Naturalist.* 123, 115-124.

Lynch, M. (1995), A quantitative-genetic perspective on conservation issues. In: Conservation genetics: case histories from nature. Avise J.C., Hamrick J.L., eds. Chapman and Hall, NY, USA.

Mantel, N. (1967). The detection of disease clustering and a generalised regression approach. *Cancer Research.* 27, 377-394.

Marsafari, M., & Mehrabi, A. (2013). Molecular identification and genetic diversity of iranian date palm (*Phoenix dactylifera* L.) Cultivars using ISSR and RAPD markers. *Australian Journal of Crop Science*. 7, 1160-1166.

Naqvi S. A., Khan I. A., Pintaud J. C., Jaskani M. J., Ali A. (2015). Morphological characterisation of Pakistani date palm (*Phoenix dactylifera* L.) genotypes. *Pakistan Journal of Agricultural Science*. 52(3), 645-650.

Nei, M., & Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of National Academy of Sciences.* 76, 2569-5273.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *PANS.* 70 (12), 3321-3323.

ONAGRI. *Observatoire National de l'agriculture* May (2018). http://www.agridata.tn/dataset?tags=production+dattes.

Reed, D. H., & Frankham, R. J. (2001). How closely correlated are molecular and quantitative measures of genetic variation? A metaanalysis. *Evolution*. 56, 1095-1103.

Rohlf, F.J., & Fisher, D. (1968). Tests for hierarchical structure in random data sets. *Systematic Zool*ogy. 17, 407-412.

Sorensen, J.T., Foottit, R.G. (1992). *Ordinations in the study of morphology, evolution and systematics of insects: applications and quantitative genetic rationals*. Amsterdam: Elsevier.

Sabir J. S., Aboaba S., Bafeel S., Zari T. A., Edris S., Shokry A. M., *et al.* (2014). Characterisation of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. *Comptes Rendus Biologies.* 337 (1), 6-18.

Salem O.M., Rhuma S., Zehdi S., Marrakchi M., Trifi M. (2008). Morphological variability of Mauritanian date-palm (*Phoenix dactylifera* L.) cultivars as revealed by vegetative traits. *Acta Botanica Croatica*. 67 (1), 81-90.

Trifi M., Rhouma A., Marrakchi M. (2000). Phylogenetic relationships in Tunisian date-palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. *Agronomy.* 20, 665-671.