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Genetic diversity study on common Tunisia date palm (*Phoenix dactylifera* L.) pollinators revealed by morphological, physiological, and molecular (RAPD) markers.

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Abstract -Due to its geographical location and favorable climatic conditions, Tunisia is considered a very diversified environment for growing date palm.The goal of this study is to investigate the genetic diversity in the most commonly used male pollinators of date palm in southern Tunisia. This analysis was carried out based on morphological, physiological features, and molecular markers of the Random Amplified Polymorphic DNA type (RAPD). The results showed that the analysis of genetic diversity within the population, using morph-physiological markers, revealed that the genetic similarities are varied between 0.291 and 0.479, with an average of 0.385. These values reflect a high rate of polymorphism within the population analyzed. The physiological study resulted in 62.5% to 77.63% variation in the germination of the pollens and 83.3% to 67.1% in their coloration. Analysis of the molecular polymorphism showed that the 28 used primers were effectively generating 303 reproducible markers, of which 241 are polymorphic, with a polymorphism rate of 79%. The study of the genetic diversity demonstrated very high similarities (≥ 60%) between the populations analyzed, whereas small differences between these individuals occur. This study will pave the road to conduct the same work in other regions of the country

Keywords: allele, germination, genetic diversity, morphological descriptors, polymorphism

1. Introduction

The date palm (*Phoenix dactylifera* L.) is a dioecious monocotyledonous species widely cultivated in arid regions (Bendahou et al. 2007). This plant is considered as the framework of the oasis ecosystem due to its great capacities in the maintenance in extreme ecological conditions, in the production of dates, the creation of a microclimate favorable to the life of man and animals as well as to the development of the underlying cultures (Rhouma 1996). In the Maghreb, many problems arise in the cultivation of the date palm (*Phoenix dactylifera* L.). This dioecious and heterozygous species has a slow mode of reproduction, calling on the rejections, which does not allow to effectively multiply the efficient and/or endangered female genotypes and the male genotypes with high added value (Salah 2015). The development of phoeniciculture depends on the removal of several constraints, the main ones being: the predominance of the elite variety Deglet Nour over dates with high commercial value (Abdelmajid 2005; Reynes et al. 1994), the threat and the spread of certain fatal diseases such as the disease of *Bayoud*(Fernandez et al. 1995; El Hadrami et al. 1998; Zouine and El Hadrami 2007), brittle leaf syndrome (Namsi et al. 2006), as well as the difficulty of propagating certain cultivars (Rhouma 2005). The selection of high-quality varieties with resistance to the disease using only conventional generic methods is very slow. Besides, difficulties of the recognition of existing cultivars (for lack of reliable discriminating criteria) and for the assistance and evaluation of the directed crossbreeding programs started in recent years in the three Maghreb countries. Male palms are frequently called *Dokkars* or pollinators. These latter seedlings were initially used selectively. However, farmers have been able to realize the value of certain pollinators in terms of the quality of the pollen produced and its fertility. Pollination is, therefore a very important technique with a direct effect on the quantity and quality of the date production (Sedra 2003). In this perspective, it is necessary to select a certain number

of pollinators with higher quality genotypes. The choice of such quality pollinators is based essentially on morpho-physiological criteria including the size of spathes, their mode of development, the pollen viability rate, its pollination power materialized by good germination power, and, abundant production of pollen (Bchini 2006). But also, the selection is based on molecular criteria such as the degree of kinship between the different pollinators and the similarity between them. Indeed, these date palm pollinators (*Phoenix dactylifera* L.), although they play an important role in the production cycle of dates, are currently threatened by serious genetic erosion, especially the haphazard cuts by some farmers to get the juice palm tree. Therefore, it is imperative to develop a strategy aimed at the evaluation of genetic diversity and the preservation of genetic material of this genetic heritage of Tunisian palm trees. In this context, numerous studies have been carried out to identify the female varieties of Tunisian date palms using either morphological traits (Hammadi et al. 2009; Hamza et al. 2011) or molecular markers (Kadri et al. 2017; Karim et al. 2010; Trifi 2001; Zehdi et al. 2002 ; Hamza et al. 2011, 2012). However, the evaluation of pollinators and more specifically, their morphological and molecular characterization are very rare in the literature(Kadri et al. 2017). Only fragmentary studies relating to the analysis of metaxenic effects have been reported (Karim et al. 2015). In this context is included this work which aims to analyze the genetic diversity existing in some pollinators in the region of Djerid (southwest of Tunisia). This study focused on the characterization of these pollinators based on morphological characterization, physiological evaluationof pollenquality andmolecular polymorphism of pollinators using RAPD markers (Random amplified polymorphic DNA).

2. Material and methods

2.1. Plant materials

Six preselected pollinators (P6, P7, P10, P12, P45and P138), belonging to the Djerid region (Figure 1), were used to study certain morpho-physiological and molecular parameters. The selection of the pollinators is linked to a survey that confirmed their popularity and is commonly used as the best pollinators in Djerid region and also based on their metaxenic effects (Bchini 2006). The pollinators are part of the varietal collection of the botanical garden of the Regional Research Center for Oasis Agriculture, four of which are collected from Deguche (P6; P7; P10; P12) and two from Tozeur (P45; P138).

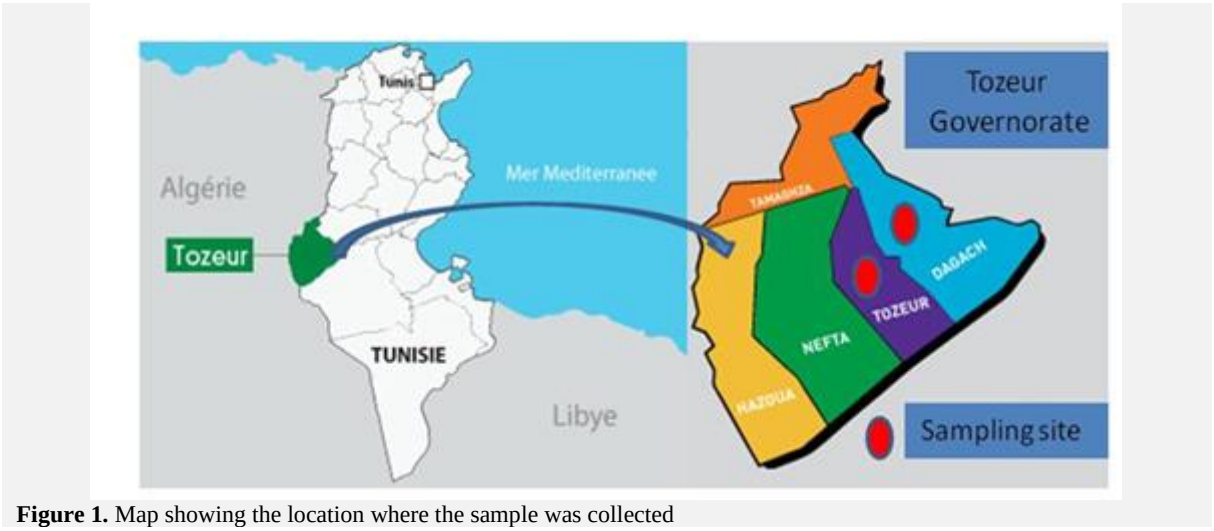

Figure 1. Map showing the location where the sample was collected

2.2. Morphological study

The morphological measurements have been taken from healthy,pruned, and same-age trees (15 years). The morphological parameters studied are described by (Bchini 2006) and the descriptor list (Supplementary file 1) of the International Plant Genetic Resources Institute (IPGRI). Following a set of 43 plant descriptors such as growth, palm, inflorescence, and pollen have been investigated in the present study.

2.2. Physiological study

Pollen harvest: The gradual maturation of spathes on the male foot was followed up for three to fourth months. The inflorescence was harvested just when the spathes burst to avoid mixing the pollen and left to dry in the open air for a few minutes. The spikelets were cut at their insertion point and then were

spread on Kraft paper and placed in a ventilated place at room temperature (Figure 2). Spikelets were monitored daily to avoid the development of mold.

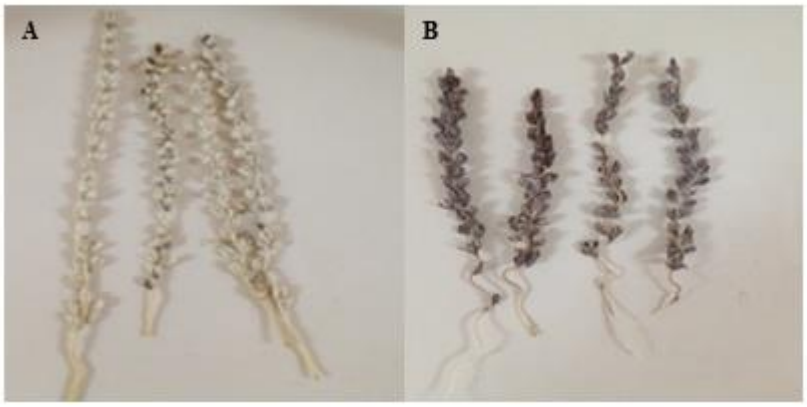

Figure 2. Photos of (A) fresh and (B) dry date palm spikelets

Viability and germination test: The visualization of the nucleus and chromatins of the pollen for each pollinator was proceeded using a solution of acetic carmine (Zhou et al. 2011). Since its double fixing and coloring action, Semichon acetic carmine is used for the observation of nuclei and counting of chromosomes which are strongly and finely colored. Iron acetate was used to change the intense red coloration to blackish. A drop of acetic carmine and a few mg of pollen placed between blade and coverslip; allowed to dry in 15 minutes, and then observed under an optical microscope. The germination test was conducted on liquid media described by (Brewbaker and Kwack 1963). The pollen culture was incubated at 27°C for 24 hours, and after that, the number of germinated cells was counted using Mallassez cells (Marin et al. 2015). The percentage of germinated pollen wascounted in 20 pots for each pollinator.

2.3. Molecular study

The genomic DNA was extracted fromyoung leaves harvested from the heart of pollinator using DNAeasy Plant Mini Kit (Qiagen) according to the manufacture protocol. The DNA polymorphisms were detected by PCR using 28 primers (Table 1). The final RAPD reaction adjusted to a volume of 25 µl containing 2 µl of genomic DNA (50ng / µl), 5 µl of the 5x concentrated polymerization enzyme buffer (0.1M Tris HCl, pH 9; 1.5 mM MgCl2; 0.5 M KCl), 3 µl of primer (300 nM), 1 µl of each dNTPs (200 µM), 2.5 µl of MgCl2 (25 mM), 0.2 µl (1 U) of Taq polymerase (5 U / µl) and 8.5 µl of nuclease-free water. The PCR cycles programmed to the following steps: 94 ° C for 3 min, followed by 40 cycles of 94 ° c for 45 s, 34 ° C for 45 s, 72 ° C for 2 min, and 72 ° C for 10 min for the final extension. The amplified DNA was visualized using 1.8% agarose gel under UV light.

2.4. Data analysis

The DNA gel image analysis has been proposed based on the presence and absence of the band (1 assigned to present and 0 assigned to absent).Only well-amplified fragments were considered. The obtained data has been transformed into a binary matrix. The matrix of the morphological data obtained is based on quantitative values and universal codes for qualitative data. The genetic/morphological similarities between the pollinators were calculated according to the Dice coefficient (Dice 1945). A dendrogram of the genetic/morphological relatedness among six pollinators was produced with the Unweighted Pair Group Method with Arithmetic Average (UPGMA) analysis using the NTSYS software, package version 1.70 (Rohlf 1993). Analysis of variance (ANOVA) was conducted, and the Fisher test was applied to evaluate the level of significance between the pollinators.

Table 1. Selected RAPD primers in terms of their sequence, number of amplified and polymorphic fragments.

Primers	Sequence	Amplified bands	Polymorphic bands	Polymorphic rate %
OPA-01	CAGGCCCTTC	6	5	83.3
OPA-04	AATCGGGCTG	10	9	90
OPA-05	AGGGGTCTTG	6	5	83.3
OPA-10	GTGATCGCAG	9	6	66.7
OPA-12	TCGGCGATAG	10	7	70
OPA-10	GTGATCGCAG	8	7	87.5
OPA-15	TTCCGAACCC	13	11	84.6
OPA-16	AGCCAGCGAA	11	11	100
OPA-19	CAAACGTCGG	8	8	100
OPB-01	GTTTCGCTCC	15	9	60
OPB-04	GGACTGGAGT	13	12	92.3
OPB-05	TGCGCCCTTC	11	9	81.8
OPB-12	CCTTGACGCA	15	12	80
OPB-14	TCCGCTCTGG	12	11	91.7
OPB-15	GGAGGGTGTT	12	12	100
OPB-16	TTTGCCCGGA	8	4	50
OPD-02	GGACCCAACC	9	7	77.8
OPD-10	GGTCTACACC	12	8	66.7
OPD-16	AGGGCGTAAG	8	8	100
OPE-12	TTATCGCCCC	16	12	75
OPG-12	CAGCTCACGA	17	16	94.1
OPH-13	GACGCCACAC	10	7	70
OPJ-10	AAGCCGAGG	17	11	64.7
OPJ-12	GTCCCGTGGT	8	7	87.5
OPL-12	GGGCGGTACT	12	12	100
OPM-01	GTTGGTGGCT	12	11	91.7
OPM-05	GGGAACGTGT	12	11	91.7
OPK-15	CTCCTGCCAA	14	11	78.8
Total		**314**	**259**	**83.5**

3. Results and discussions

3.1. Morphological characterization of the vegetative part

Evaluation of qualitative traits

The qualitative vegetative results of the six pollinators showed differences in vigor, habit, shape of the trunk, and presence of leaf scars (Table 2). The results have also demonstrated a similarity in some characteristics, such as the presence of high offshoot, fluff mane, and the capacity to produce basal offshoot. Most of the pollinators have also shown a similarity in terms of the following palm descriptors such as angle of curvature, rotation of the palm, petiole color, the color of the pinnae, and disposition of pinnae. However, the pollinator P6 has greater flexibility of the pinnae than the other pollinators. In addition, the angle of terminal pinnae in P12 was presented as medium type comparing to the rest pollinators where the angle was in low type form. The two pollinators (P6 and P7) have rigid spines, whereas the other pollinators have flexible spines. The inflorescence characteristics showed that the pollinator P138 has a later flowering date, in comparison to the other pollinators. Also, two of the pollinators (P10 and P12) have yellow pollen while the other ones are shaving whitish color. The evaluation of the quantitative characteristics showed that only six traits (length at base of petiole, maximum width of the pinnae, maximum width of spathe, number of spikelets per spathe, number of flowers per spathe, and pollen weight per spathe) appeared to have significant differences ($P= 0,05$) (Table 3).

Table 2. Results of the qualitative descriptors from the six pollinators evaluated in this study

	List of descriptors	Pollinators					
		P6	**P7**	**P10**	**P12**	**P45**	**P138**
GD	Vigor	Strong	Medium	strong	small	medium	medium
	Port	erected	Spherical	falls	erected	falls	erected
	Appearance of the crown	medium	Medium	medium	airy	dense	airy
	Shape of the trunk	conical	Conical	cylindrical	cylindrical	cylindrical	conical
	Persistence of leaf scars	No	Yes	yes	yes	no	no
	Presence high offshoot	No	No	no	no	no	no
	Presence of fluff mane	Much	Little	little	little	little	little
	Capacity to produce basal offshoot	Little	Little	little	little	little	little
	Curvature in the palm	in mid	at 1/3 of the palme	at 2/3 of the palme	in mid	at 1/3 of the palme	at 2/3 of the palme
	Angle of curvature	accentuated	Accentuated	accentuated	accentuated	accentuated	accentuated
	Rotation of the palm	Yes	Yes	yes	yes	yes	yes
	Petiole color	Yellow	Yellow	yellow	yellow	yellow	yellow
	Stiffness of spine	Rigid	Rigid	flexible	flexible	flexible	flexible
	Colour of the pinnae	green yellow	green yellow	green yellow	green yellow	green yellow	green yellow
	Grouping of pinnae (single, double, three, four)	by 3	by 3	by 2	by 2	by 3	by 4
	Disposition of pinnae	external	External	external	external	external	external
	Flexibility of pinnae	Hard	Slight	slight	slight	slight	slight
	Angle divergence of terminal pinnae	Low	Low	low	medium	low	low
	Spathe shape	inflated	Fusiform	fusiform	fusiform	lanceolate	lanceolate
	Flowering spathe	Early	Early	early	early	early	late
	Spikelet density	medium	Compact	compact	medium	cowardly	medium
PND	Pollen smell	medium	Medium	strong	strong	medium	strong
	Pollen color	whitish	Whitish	yellowish	yellowish	whitish	whitish

GD - growth descriptors; **PND** – pollen descriptors

Table 3. Results of quantitative descriptors from the six pollinators evaluated in this study

	List of descriptors	Pollinators evaluated						
		P6	P7	P10	P12	P45	P138	*P-value*
PD	Maximum palm width (cm)	45	60	65	46	40	45	0.127
	Total palm length (m)	3.5	4.5	5.5	4.5	3	3.2	0.425
	Rachis thickness	7	12	10	15	7	8	0.564
	Length at base of petiole (cm)	5	6	6	8	6	5	**0.001**
	Number of spine	36	24	28	32	25	30	0.425
	Maximum thickness of the spine (mm)	3	3	3	3	3	3	0.002
	Maximum spine length (cm)	14	12	7	15	8	9	0.235
	Maximum width of the pinnae[1]	4	4.6	3.1	3.4	2.9	3.8	**0.007**
	Maximum length of the pinnae[1]	62.6	45.3	37.8	50.5	40.9	45.7	0.327
	Length of apical pinnae (cm)	13	28.4	23	25	18.5	30	0.429
	Maximum apical pinnae width (cm)	1.2	1.5	2.3	1	1.5	2.2	0.635
	Total length of the spathe (cm)	114.5	164	126	153.2	133	64.5	0.387
	Maximum width of spathe (cm)	95	37	24.7	26.5	30.66	11.75	**0.003**
	Number of spikelets per spathe	285	254	241	250	177	150	**0.006**
	Length of longest spikelet (cm)	34.25	27.5	39	29	29	17	0.528
	Length of the shortest spikelet (cm)	13.25	7.5	9	12.6	8	9	0.258
	Number of flowers per spathe	195717	160000	149000	108936	74000	99232	**0.009**
	Number of flowers in the longest spikelet	117	82	96	100	70	82	0.635
ID	Number of flowers in the shortest spikelet	40	19	19	19	11	11	0.325
PND	Pollen weight per spathe (g)	17	37	10.7	12.1	27	12.7	**0.008**

[1]the measurements were performed in the middle of the palm (cm); PD – palm descriptors; ID - Inflorescence descriptors; PND – pollen descriptors

Morphological similarity

The genetic similarities between the different pollinators based on the morphological characteristics, determined between 0.291 and 0.479similarity coefficients with an average of 0.385 (Table 4). The lowest similarity level has been observed in the combination of P6 and P12 (0.291) and that attributed to obvious morphological differences between the pollinators in tree vigor, the shape of the stipe, and pollen color. At the same time, the combination (P45 and P138) and (P10and P12) recorded the highest similarity (0.479). This morphological relationship between the pollinators illustrated by the dendrogram with the highest similarity coefficients (0.41) based on UPGMA analysis (Figure 3).Similar morphological characteristics have been used by several authors (Azeqour et al. 2002; Belguedj 2002; Rhouma 1994; Salem et al. 2008) to study morphological diversity in date palm. The results obtained showed great variability in the pollinators studied. Similar results have been reported by Elhoumaizi et al. (2002) indicated a high degree of diversity in 26 Moroccan cultivars according to26 morphological vegetative traits, with usefulness for the traits considered before the fruiting period. Salem et al. (2008) have used 18 morphological traits to characterize Mauritanian date palm cultivars, and among the 18 traits, only 14 showed strong discriminating power. Hammadi et al. (2009) have used 30 morphological markers to study diversity within 26 Tunisian date palm cultivars. In conclusion, a high level of polymorphism has been observed within the individuals studied. Indeed, 65% dissimilarity is recorded in this collection of six pollinators confirmed a great genetic divergence within this group. The results obtained showed the effectiveness of these morphological traits or in the selection and conservation of the various male date palms with superior characteristics and in future selection programs.

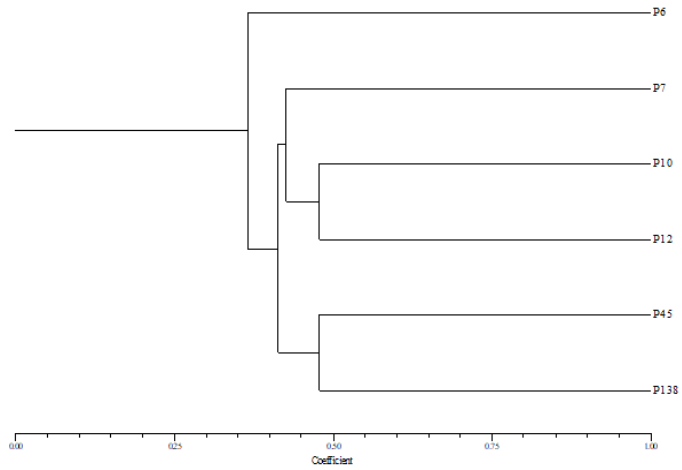

Figure 3. UPGMA dendrogram showing genetic relationships among the six date palm pollinators based on morphological data.

Combine Figure 3 and 4 : it's clear that molecular date reflect the morphological one, please discuss and how this finding will be useful??

Table 4. Genetic similarities between the six date palm pollinators based on morphological markers.

	P6	**P7**	**P10**	**P12**	**P45**	**P138**
P6	1.00					
P7	0.375	1.000				
P10	0.312	0.437	1.000			
P12	**0.292**	0.412	0.479	1.000		
P45	0.396	0.458	0.437	0.396	1.000	
P138	0.396	0.395	0.375	0.396	**0.479**	1.000

3.2. Physiological study

Several physiological criteria make it possible to distinguish between pollinators, but the most important to take into account is corresponding to the viability and germination rates.

Germination test

The pollen tubes investigated in this study demonstrate clear vigorous in appearance. Thus, some tubes have several curvatures, while the others are straight. However, they have several stages of development, which can be explained by the inequitable absorption of the solution from the germination medium. This is reflected in the lengthening of the pollen tube in different stages of development. The germination rate in this study was varied from one pollinator to another. The total percentage of the in vitro germination of the six cultivars were varied from 62.5% to 77.63%. These results showed that the rate of pollen germination is a variable indicator that discriminates between the pollinator. The highest rate was obtained with P138 pollinator (77.63%), and the lowest rate was recorded with P7 pollinator 62.50% (Table 5). The analysis of variance showed that there are significant differences (*P=0.005*) between the six pollinators. The germination percentages obtained in this study are higher than Peyron's standard (Peyron 2000) which considers that pollen must germinate in vitro more than 60% to ensure good fruit set, putting into consideration that pollen tubes appear after 24 hours of incubation in the germination media. Thus, it is considered in the range of the high fertilization capacity (Chaibi et al. 2002).

Pollinators P7, P10, P45, and P12 are classified among the middle class (60-70%), and the other two pollinators P6 and P138 among the significant germination rate class (70-90%). The results obtained in this study are matching with what (Kadri et al. 2017; Karim et al. 2015) found.

Table 5. Variation in viability and germination rate of the pollen among the six pollinators

Pollinators	P6	P7	P10	P12	P45	P138	P-value
Germination rate %	73.1	62.5	68.1	67.6	68.4	77.6	**0.003**
Viability rate %	83.3	67.1	80.5	70.5	72.6	74.8	0.42

Viability test

The pollinators P6 and P10 exhibited a high percentage of coloration (83.3%), and the pollinator P7 recorded the lowest percentage (67.1%). Analysis of variance shows that there is no significant difference in vitality between the pollinators (Table 5).

According to (Halimi 2004), the pollens tested in the fresh state are viable from 47 to 99%, which contributes to the estimation of the quality of fresh pollen by determining the rate of empty pollen. The pollens tested in the fresh state by (Kadri et al. 2017) were viable from 90.50 to 95.80%, which is higher comparing to this result. Thus, this study highlighted that there is a high homogeneity between the pollens. However, the phenomenon of germination and emission of pollen tubes could be attributed to the genotypic control and the environmental influence (Laiadi et al. 2018). Several studies have shown that the rate of vitality and the percentage of germination are intrinsic characteristics that differ from one male to another (Al-Helal 1994 and Shaheen et al. 1989). Furthermore, (Soliman and Al-Obeed 2013) recorded significant biometric differences in pollen grain in 11 varieties of the date palm. Other studies showed that other parameters such as storage temperature (Ateyyeh 2012; Kadri et al. 2017) and composition of the culture media (Ismail 2014) can influence germination rate and viability. In conclusion, we observed that the pollinators P6 and P10 showed a very high rate of viability comparing to other pollinators. On the other hand, the results of the germination rate of pollinators (P6; P10) are not compatible with their viability rates. P138 and P6 have the highest germination rate. Therefore, the outcome of this test showed that the viability rate is not proportional to the germination rate. (not every viable cell is fully capable of germinating).

3.3. Molecular study

Twenty-eight primers were used to analyze the genetic differences among the six pollinators. A total of 314 reproducible bands were obtained during amplification. Only 259 bands were polymorphic (83.54%) with an average of 40.16%.Moreover, the size of the amplified fragments was varied among primers and ranging from 300 to 1700bp. The result of this study is similar to what has been found by (Hela et al. 1999) which reported a fragment size ranged from 200 to 1600 bp, while (Adawy et al. 2004) found fragment sizes ranged from 310 to 2800 bp using ten primers of RAPD analysis in five date palm cultivars. Statistical analysis of the polymorphic bands showed that the rate obtained among the six pollinators (83.54%) reflects a significant polymorphism allowing us to consider these (Table 1) pollinators have a rich and divergent genetic heritage. The analysis of the amplified and polymorphic fragments (Table 1) shows that the number of amplified fragments varies depending on the primer and genotype, the primers OPG12 and OPJ10 recorded the highest number of amplifying locus with 17 bands. The primers OPA16, OPA19, OPB15, and OPD16 recorded polymorphism rates of 100%. Similar results were recorded by (Trifi 2001) showing that the primers OPD-16 and OPE16 were more efficient, with reproducible products, both for varietal identification (will allow distinguishing different multilocus profiles) more than for the differentiation of hybrid descendants.

Genetic similarities

To estimate the genetic similarities between the different pollinators, we used the data relating to the binary matrix generated by the twenty-eight RAPD primers. The analysis of matrix showed that the genetic similarity coefficients varied between 0.548 and 0.791, with an average of 0.670. Moreover, the highest similarities were observed between the following combinations (P7 and P12 and 0.791), (P10 and P12: 0.773), and (P10-P45: 0.782) (Table 6). The genetic relationship between the six pollinators is distinguished in a threshold of 60% similarity level, which is demonstrated by dendrogram based on

UPGMA analysis (Figure 4). Furthermore, this result suggests that these pollinators constitute closely related groups since they represent the maximum similarity.

Table 6. Genetic similarities between the six date palm pollinators based on the RAPD markers.

	P6	P7	P10	P12	P45	P138
P6	1.000					
P7	0.636	1.000				
P10	0.579	0.752	1.000			
P12	0.630	**0.791**	0.774	1.000		
P45	0.555	0.714	0.783	0.768	1.000	
P138	**0.549**	0.703	0.701	0.704	0.700	1.000

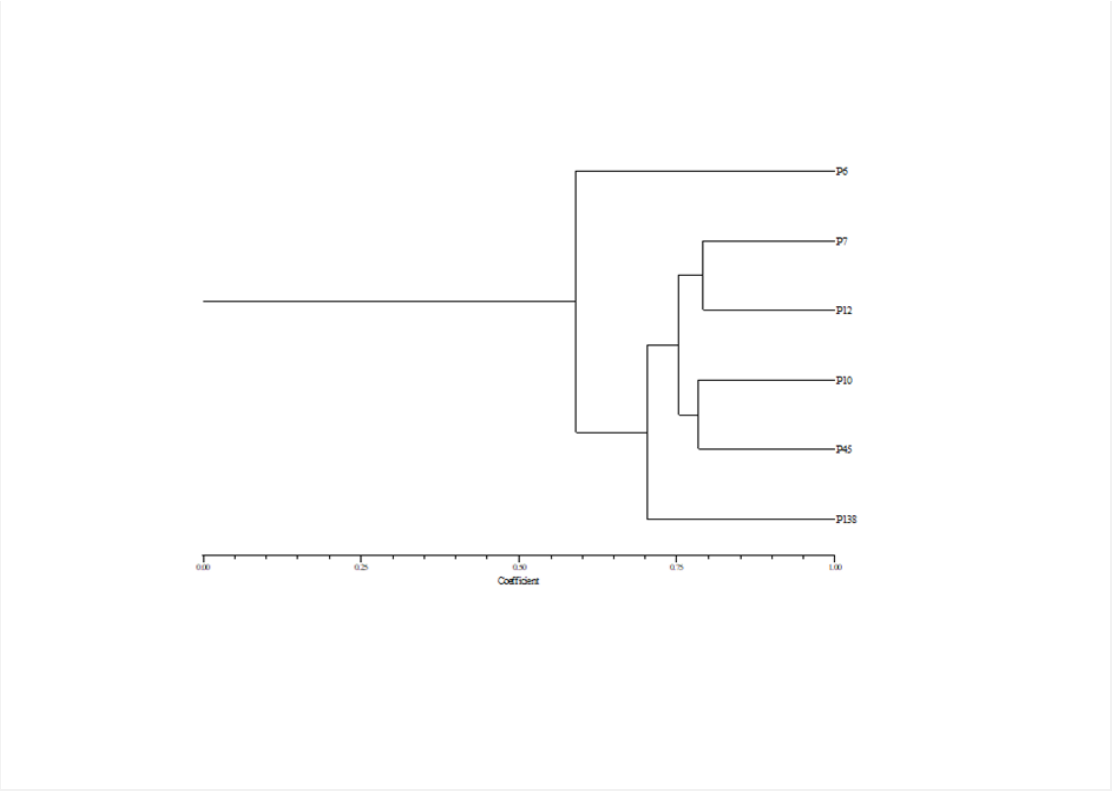

Figure 4. UPGMA dendrogram showing genetic relationships among the six date palm pollinators based on the RAPD data.

This similarity is attributed to the closeness of their localities of origin, in addition to the mode of multiplication by offshoot. On the other hand, the lowest coefficients are obtained with the combinations (P6 and P138: 0.548) and (P6and P45: 0.554), thus reflecting a large divergence between these pollinators, this divergence can be attributed to seedling propagation followed by the farmers in the area which led to untrue to type plant. Concerning the other pollinators, they presented intermediate similarity coefficients, which can be grouped with varying degrees of closeness.

On the other hand, (Elshibli and Korpelainen 2011) in their study, exhibited complex genetic relationships between some of the populations, particularly when geographic remoteness was considered. Also, they reported that there is a great diversity of date palm genetic material. This study points out the role of the biological nature of the tree, the environmental effects, and isolation by distance on enriching this diversity. Indeed, these remarks were consistent with the results recorded in our studies. The results of the molecular analysis are thus consistent with agronomic findings in the field and show the effectiveness of the RAPD technique in this type of study. The results generated four clusters consists of P6, P7 and P12, P10 and P 45, and P138 (Fig 4). However, it represents the lowest coefficient (0.61), andaccording to our results, the low polymorphism was observed in the individuals studied which, up to the 60% similarity threshold, all the varieties form a single group. This allows us to suggest the hypothesis that date palm pollinators represent a narrow genetic base because they come from the same

phoenicultural region, these results also clearly show that random amplification of polymorphic DNA constitutes an informative approach.
Several studies have been carried out in Tunisia on female varieties of date palm using the RAPD technique (Ben Abdallah et al. 2000; Trifi 2001). However, this study is the first attempt to use RAPD markers for the analysis of genetic diversity in male date palm cultivars in Tunisia. The results confirmed that RAPD markers are acceptable tools for the detection of genetic diversity. The mastery and knowledge of genetic polymorphisms have direct implications for programs for the selection and conservation of genetic resources. Indeed, the analysis of genetic diversity within a population can be estimated using various approaches, including genealogical information, morphological and molecular markers.RAPD markers have been used in many plant species for studies of molecular polymorphism (Xuemei et al. 2012). Another study by(Abdulla and Gamal 2010) have applied this technique for the identification of DNA fingerprints from the date palm.
In this study, the results of molecular polymorphism revealed a very weak intra-varietal polymorphism (> 60% genetic similarity) testifying to an important genetic convergence in this collection of cultivars. Our results are similar to those of (Mostafa et al. 2018) who used the RAPD technique for the analysis of genetic diversity, in four female and five male date palm cultivars. However, (Adawy et al. 2002) stated that RAPD did not detect intra varietal variations among five date palm cultivars from the Aswan region of southern Egypt. These results also revealed a low level of inter-varietal polymorphism among the six cultivars studied, suggesting a narrow genetic background for these cultivars. This relatively low diversity can be explained by the small number of pollinators studied and their belonging to the same phoenicultural region. Furthermore, this work preferably targeted the common pollinators used by farmers, which militates in favor of a possible mode of multiplication by discharges practiced within these pollinators. (Guettouchi et al. 2017) used the RAPD and ISSR (Inter Specific Sequence Repeat) techniques for the analysis of genetic diversity in twenty Algerian date palm cultivars. The results obtained from the two markers showed a great variability of specific and random sites within the genome of the date palm. The study of correlation of markers with the distance matrix of the combined data showed a stronger correlation of RAPD with the combined data, these results suggest that the RAPD technique presents a stronger grouping model than the ISSR markers. (Srivashtav et al. 2013) suggested that RAPD markers were more effective markers than ISSR for assessing the genetic variation of date palms in the Kutch region of India. This study also showed the limits of the morphological data in the structuring of genetic diversity since the rate of polymorphism obtained with the morphological data revealed a significant divergence between these pollinators, however, the molecular study showed a very great similarity on the genomic plan, hence the interest of the RAPD technique in the study of phylogenetic relationships between the cultivars studied. These research results are confirmed by (Sonboli 2011; Xuemei et al. 2012) that the RAPD marker system reveals high levels of polymorphism among species, which indicates its effectiveness in assessing Intra and interspecific genetic diversity in the genus. Regarding the phylogenetic distribution of date palm cultivars, the results of this study were confirmed by (Cipriani et al. 1996; Hormaza 2000) who stated that the RAPD technique provides genetic markers that have been widely used in many different applications.

4. Conclusion

Based on this study, we can conclude that there are no major morphological differences between the six most commonly used male pollinators' date palm in the Djirid Region-Tunisia. The physiological markers exhibited clear differences between the pollens germination and their coloration. The use of the molecular analysis (RAPD) revealed hight genetic similarities between the investigated male pollinators

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