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Research Paper

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

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ABSTRACT

The effect of pollen on the maturity and the quality of the fruits of the variety "Deglet Nour" was evaluated in this study. The results showed that the duration of maturation and the quality of fruit obtained vary depending on pollinator. P10 is the most efficient and this thanks to its ameliorative effect on fruit quality and advancement of maturation. The "Bisser" stage was reached 180 days after pollination and lasted from 3 to 4 weeks depending on the pollinator. The "Tamar "stage started after 130 days greater or lesser seven days. The stage lasted 4 to 6 weeks. Selection for maturation earliness was already possible at the "Bisser" stage. Molecular typing of these 10 pollinators was performed using microsatellite or SSR analysis. A total of 22 alleles corresponding to 6 targeted microsatellite loci were generated, which provides evidence for a significant DNA diversity among Tunisian date- palms. Using those microsatellite markers not only highlighted the intra cultivar homogeneity, but allowed for genetic fingerprinting of the ecotypes. The data suggests that there is a specific consensus SSR profile for all pollinators. Moreover, by using multiloci genotyping with only 3 microsatellites, a varietal identification key was created allowing for perfect discrimination. These 6 new co-dominant markers will be a starting point for researchers making use of the markers for genetic mapping and diversity analysis of date palm.

Key words*: Phoenix dactylifera* L., pollinator, metaxenic effect, SSR markers, MDSCALE.

INTRODUCTION

In Tunisia, the date palm plays a strategic role in the socio-economic stability of the oasis agro-ecosystem. Indeed, it is the main axis of the agriculture in desert regions and provides principal financial of oasis.

However, with the economic and social development of the country, palm groves of Jerid and Nefzaoua reorganized to satisfy a growing demand for high-quality dates like "Deglet Nour" cultivar. This reorganization was followed by improvement of the date's quality, while forgetting the pollen participation in the maturation dates. In 1926, Nixon highlights on the date palm the term metaxenia resulting in the direct impact of pollen on the quality and dates repining period. Bchini (2006) also confirmed that some pollinators can be inducers of earliness or lateness of dates ripening; the difference is subject to annual fluctuations and the difference in cumulative heat. Interspecific pollinations showed that over the crossing is made with distant species of *Phoenix dactylifera L*. more fruit set is poor and more fruits and kernels are small, and the time of ripening is later (Lakhouah, 1966) . Despite all that, the pollen contribution, although known for years, is not taken into consideration. With the direction of domestic and international markets to

the qualitative aspect and concurrency of dates" Deglet Nour", the adoption of a technology package consisting of a set of techniques and sources to improve fruit quality is crucial. As to the date of maturity dates, pollen seems until then the only factor acting on the earliness or lateness of maturation.

Under characterization of different varieties of date palm, many studies referred to identify varieties of Tunisian date palm using either morphological traits or enzymatic markers (Ahmed and Al-Qaradawi, 2009). But morphological criteria become insufficient to correctly estimate the diversity (Elhoumaizi et al., 2002) . Therefore, marker technology for DNA fingerprinting has become increasingly important to discriminate among closely related cultivars. Several marker systems have been used to study the genetic diversity of date palm. In short, Randomly Amplified Polymorphic DNA (RAPD) fingerprints have been used to identify date palm accessions (Ameer et al., 2014; Sedra et al., 1998; Trifi, 2001; Trifi et al., 2000). Amplified Fragment Length polymorphic (AFLP) markers have been used to study the genetic diversity of date palm cultivars (Adawy et al., 2005; El- Assar et al., 2005). Microsatellite or simple sequence repeat SSR markers has been used in plant diversity analysis; the popularity of these markers is due to their ease of amplification by polymerase chain reaction (PCR), their co-dominant nature and their typically high levels of allelic diversity at different loci. SSR molecular markers are also used by Zehdi et al. (2004), Hamza et al. (2011) and Zehdi et al. (2012). Recent studies allowed the identification of microsatellite markers to discriminate the sex of the plant that would be a great contribution in the breeding work of the date palm (Cherif et al., 2012). In the date palm, 16 dinucleotide microsatellite markers (GA) n, were generated from a DNA bank (Billotte et al., 2004). These markers have been validated for their accuracy in distinguishing different cultivars of Tunisia (Elshibili and Korpelainen, 2007) and Sudan (Zehdi et al., 2004).

The present work aims to identify genetically a number of pollinators and study their relationship and their genetic diversity. In another part, we will study the metaxenic effect of these pollinators on the term of dates maturity of the variety Deglet- Nour, the pomological characters (size, dry matter) and biochemical effect (reducing sugar content).

MATERIALS AND METHODS

Molecular analysis

Plant material and DNA extraction

The study included 10 date palm pollinators called P6,P7,P10,P11,P12,P17,P45,P173,P196 and PY which 4 pollinators belong to Tozeur oasis, 5 pollinators from the oasis experimental of the centre regional CRRAO and one individual from a farmer. These pollinators were chosen for their good quality; total cellular DNA was extracted from frozen young leaves using DNAeasy Plant Mini Kit (Qiagen S.A., The quality of DNA was assessed by running on 0.8% agarose gels in 1 × TAE buffer).

Polymerase Chain Reaction (PCR) Analysis

We have tested 6 primers developed by Billote et al. (2004): DP157F, DP160F, DP171F, DP172F, MPdCIR050 and MPdCIR078. PCR reactions were performed in a total reaction mixture of 25 µl containing: 2 µl (30-40 ng) of total DNA, 12.5 µl of readymix 1X (Promega), 1.5 µl (10 pmol/µl) for each primer (forward and reverse) and 7.5 µl of nuclease free water.

Amplifications were performed in a Biorad-Thermocycler (Icycler, Biorad France) with the following conditions: a denaturation step of 5 min at 95°C followed by 35 cycles of 30 s at 95°C, 1 min at 52°C and 1 min at 72°C, and a final extension step at 72°C for 7 min. The PCR products were separated on 3% agarose gels in 1 × TAE buffer stained with Syber Safe DNA stain (Invitrogen).

Physiological and biochemical analysis of pollen

Viability test

For determining pollen grains viability, a small amount of pollen grains was placed on a slide and 1-2 drops of 1% acetocarmine solution was added. The slides were placed for few minutes on a hot plate. The viability of the pollen grains was examined with the microscope at 40× magnification power. Two slides were prepared for each male and 4 fields were tested from each slide. Pollen grains that stained red with considered viable, whereas, the colorless pollen grain were considered non-viable.

Germination test

The culture medium used is called medium of Brewbaker and KWATCH (1963). A small amount of pollen grains was added to the media in Petri dishes, the dishes were placed in an incubator at 28°C for 24 h. A square piece of the media of about 1 cm length was taken and placed on a slide for testing under the microscope. An initiation of a pollen tube growth was considered as evidence of germination. Germination counts were taken from 3 fields for each slide.

Metaxenia

Three female date palm trees from Deglet Nour variety were selected for this study, these trees were located in

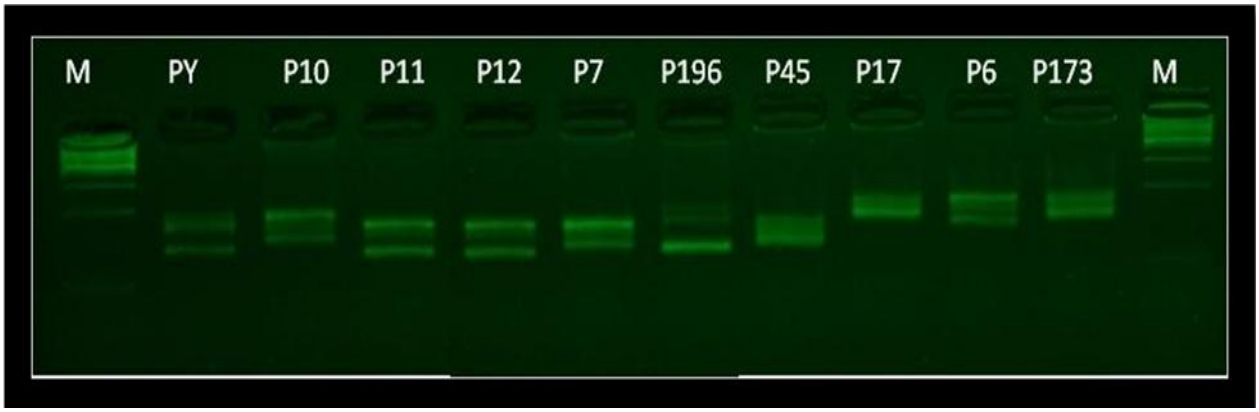

Figure 1. Example of an amplification profile obtained by the primer NPdCIR050. Legend: M: molecular weight markers: Lambda DNA /Hind III; numbered wells correspond to the studied accessions.

three oases regions namely: Tozeur, Deguech and Kebili.

The cultivars were selected based on relative better yield performance. Three spathess are used for each female three. Each spathe is divided into ten groups of five spikelets. These were covered with a paper bags to prevent contamination by foreign pollen from the atmosphere. The day of pollination were put three male spikelets in each group, then the bags were closed. Each group is marked with a label containing the name of pollinator used.

Thirty days (30) after the pollination, bags were removed. The fruit evolution was followed until maturity.

Counting of fruits is done after distinction of parthenocarpic fruit. When the first fruits reach the stage "Bisser", characterized by change color from green to yellow, counting is done weekly and the percentage of "Bisser" is noted. At the stage "Tamar", characterized by change color from yellow to brown, the percentage of "Bisser" and "Tamar" were monitored weekly until harvest. When to harvest, each specific group of pollen is deposited in packaging bearing the reference group; three fruits of each group are selected at random for determining caliber using a caliper. The sugar contained in the different fruits is also determined by a kit called ENZYTEC.

Data analysis

Microsatellite bands were precisely measured by gel documentation system Geldoc Biorad View software V.3.0.0.0 and scored for each genotype. Each polymorphic DNA at particular position on the gel was treated as a separate character and scored as allele size. Data were scored manually '1' for present,'0' for absent and '9' for missing data. These result were used to generate binary data on excel spread sheet. These data was then used for the construction of the genetic similarity matrix using the Dice coefficient (Dice, 1972). The phylogenetic relationship among the genotypes on the basis of genetic distance was drawn by the mean of UPGMA (Unweighted Pair Group of Arithmetic Means) using NTSys software version 2.02. To understand the structure of the polymorphism, the similarity matrix (obtained by means of the coefficient SM) generated by primer, and the dendrogram corresponding were treated by means of the analysis MDSCALE (Multidimensional scaling) by the NTSYS version 2.02 software. Finally, a three-dimensional spatial representation was obtained describing the distribution of individuals in the space of three axes (X, Y and Z). Statistical analysis of physiological, biochemical and pomological data were made with the STATISTICA software version 10.0. The comparison of means and the establishment of order classes (homogeneous classes) are made by the Newman-Keuls test at 5% probability threshold.

RESULTS AND DISCUSSION

Level of Polymorphism

The six primers used to estimate genetic relationships among the selected 10 date palm pollinators produced several polymorphic bands and the result is given in. A total of 22 alleles were scored with an average of 4 alleles per locus (Figure 1). The sizes of the bands obtained vary between 650 bp and 1 kb. The number of alleles varied among the markers and across the genotype. The numbers of polymorphic alleles are 21 with an average of 5.83 polymorphic alleles per locus. The average percentage of polymorphism is 95 which indicate a high genetic diversity between these pollinators. PIC values fluctuate to 0.831 for the most polymorphic locus DP171F to 0.378 for DP157F, with an average of 0.601. This value reflects the efficiency and power of these SSRs markers used for the study of genetic diversity among palm pollinator date palm cultivars selected. The numbers of alleles per locus detected in this study were less than those graded by Zehdi et al. (2004) who recognized 7.14 alleles per locus; it may be due to using more number of microsatellite loci (14 microsatellite

Table 1. Primer sequences, repeat motifs and expected sizes of microsatellite loci of SSR of date palm.

Primer name	Primer Séquence (5'-3')	Tm (°)	Expected size(pb)	Motif repeat
DP172F	TTGCTGGTTGAAATGGTGTT	51.95°C	199-235	(AGG)11
DP172R	GCAACAGATGCTCTTGCTCA	54.53°C		
DP157F	TGGACAATGACACCCCTTTT	52.77°C	108-244	(TC)19
DP157R	GCCCACACAACAACCTCTCT	55.78°C		
DP160F	AAGAGCGACAATCATGACCA	52.87°C	180-136	(GAAA)5
DP160R	GGAAATTGAAGGGCATCTTG	50.59°C		
DP171F	GTGGGAGTAGCGAGGTATGG	54.98°C	197-218	(TTC)10
DP171R	GTCCGGCACTTTAGGAAGTT	53.47°C		
MPdCIR050	CTGCCATTTCTTCTGAC CACCATGCACAAAAATG	48.5°C	568	(GA)21
MPdCIR078	TGGATTTCCATTGTGAG CCCGAAGAGACGCTATT	49.6°C	260	(GA)13

loci).

Genetic similarity

To estimate the genetic similarities between different pollinators studied, we used the data on the array using the formula of Nei and Li (1979) on the Dice coefficient. In applying NTSYS software (version 2.0) -pc to all of the binary matrix resulting molecular data, genetic similarity matrix was obtained.

The analysis of this matrix shows that genetic similarity coefficients ranged from 0363 to 0909 and with an average of 0.636. This shows that the studied pollinators are genetically independent groups since the majority of genetic similarity indices fluctuate between genetic distances between 0.5 and 0.6 (Table 2).

Indeed, the highest similarity was observed in the combination (P196, P173) by a similarity index of 0.909, which shows the high molecular similarity between these two pollinators. For cons, the combination with the lowest similarity to 0363 is (P11, PY) implying that this couple have the maximum divergence at microsatellite loci are.

Phylogenetic relationships

The dendogram showed the genetic relationship among the 10 studied pollinators (Figure 2). It consists of two main clusters A and B, the clusters A is divided in two sub clusters. The first includes P11 and P7, the second includes the pollinator P6. These pollinators do not have the same effect on the rate of fruit maturation. However, fruits from these pollinators share some characters which are fruit setting rate and water activity.

The cluster B is divided in two subgroups. The subgroup B1 contains two sets, the first contains the pollinators P10, P17 and P45 who's the common characters is viability rate. The second set includes P12, P173 and P196 which are retarders' maturation.

The subgroup B2 has only PY, it appears somewhat detached from the subgroup B1. Indeed, the pollinator does not belong to the center oasis.

This study shows that these pollinators are almost genetically distant; represent a gene pool, implying that their effects on the maturing time and the quality of dates will be different

Study of the spatial representation of pollinators

The MDSCALE analysis reflects the diversity between different pollinators studied based on genetic distance. The graph shows that the majority of pollinators are more or less scattered. In going from the bottom up we can distinguish four groups: A, B, C, D and E (Figure 3).

Group A is formed only by the pollinator PY which is followed by Group B that carries 3 pollinators P12, P173 and P196. It may be noted that the 3 pollinators share some characters between them as the rate of the viability and activity of water. Group C consists of the P10 pollinators, P45 and P17 P10 and P45 which are two drivers of fruit ripening. In addition to a D group that is formed by the P6 and P7 which have a fruit set rates converge This group is followed in Group E which includes only the P11 which is characterized by low levels of sustainability, a small size

Table 2. similarity matrix of the 10 date palm pollinators calculated on the basis of SSR marker data based on Dice coefficient.

	P6	P7	P10	P11	P12	P17	P45	P173	P196	PY
P6	1.0000000									
P7	0.6363636	1.0000000								
P10	0.5000000	0.5000000	1.0000000							
P11	0.5454545	0.6363636	0.5909091	1.0000000						
P12	0.6818182	0.5909091	0.5454545	0.5909091	1.0000000					
P17	0.5454545	0.5454545	0.7727273	0.6363636	0.5000000	1.0000000				
P45	0.5909091	0.4090909	0.7272727	0.5909091	0.4545455	0.8636364	1.0000000			
P173	0.4545455	0.6363636	0.6818182	0.5454545	0.6818182	0.7272727	0.6818182	1.0000000		
P196	0.4545455	0.6363636	0.5909091	0.6363636	0.6818182	0.7272727	0.5909091	0.9090909	1.0000000	
PY	0.5454545	0.5454545	0.5000000	0.3636364	0.5909091	0.5454545	0.5909091	0.6363636	0.5454545	1.0000000

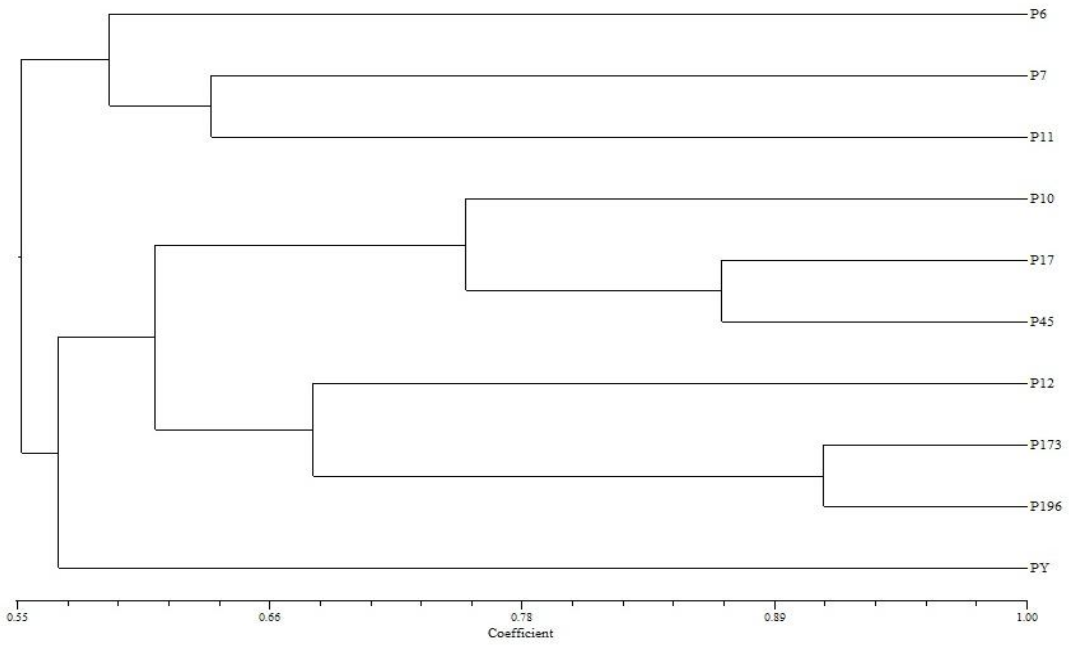

Figure 2. Dendrogram derived from a UPMGA Analysis using Dice Coefficient based on 22 Polymorphic SSR bands Showing Relationship among 10 pollinators.

and a sucrose and reducing sugar levels almost identical.

Viability of pollen grains

The viability test was performed on fresh pollen, the viability rate varies with pollen (Figure 4) Indeed, pollen grains viability ranged from 68.70 to 95.79% in the acetocarmine test. The pollinator P12 gave the highest rate, on the other side P11 gave the lowest viability rate. These results are in agreement with those found by Asif et al. (1983) who consider that the viability of the pollen levels in

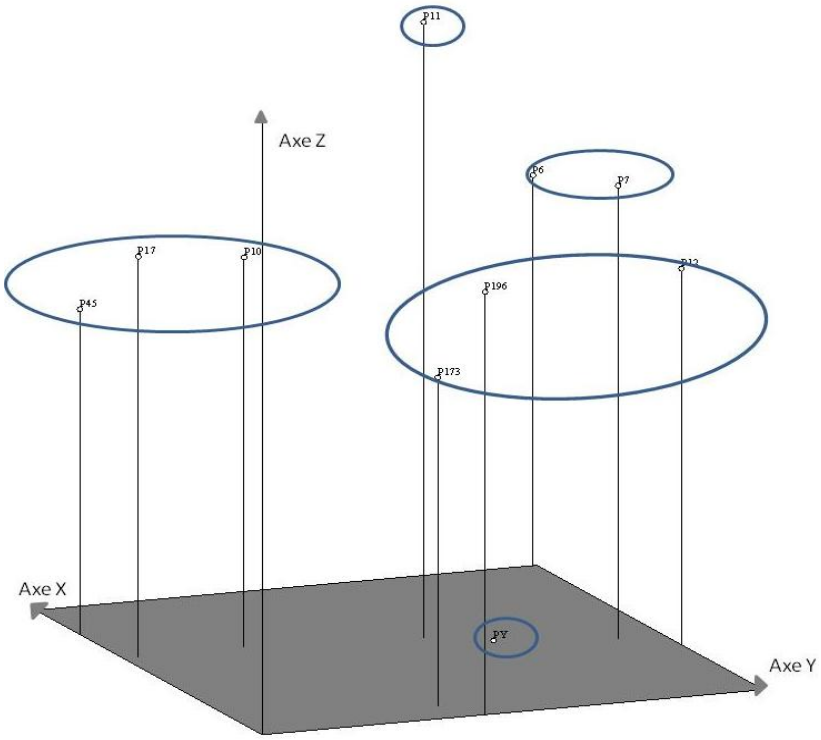

Figure 3: Graphical representation of the projection of male pollinators in space by a MDSCALE analysis using the coefficient SM and binary matrix.

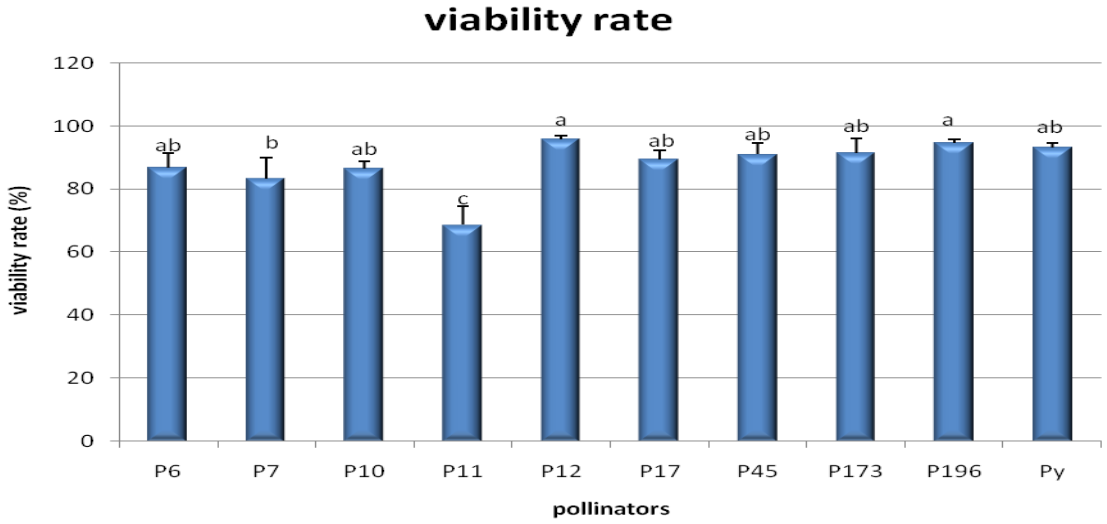

Figure 4. Rate of pollen grain viability based on ten pollinators.

the date palm is between 40 and 100%. These results show that the viability rate represents a selection character for the pollen.

Germination of pollen grains

This test allows us to compare the quality of different pollinators so it may be a basic criterion for the comparison of ten pollinators especially when the Newman Keuls test applied revealed a difference between these percentages (Figure 5). The result shows that the difference between the pollinators is significantly. The highest germination rates were obtained with the P10 pollinators (80.58%) followed by P45 (77.05) and the lowest rate is obtained with the pollinator P7 (53.25%). Asif et al. (1983) found

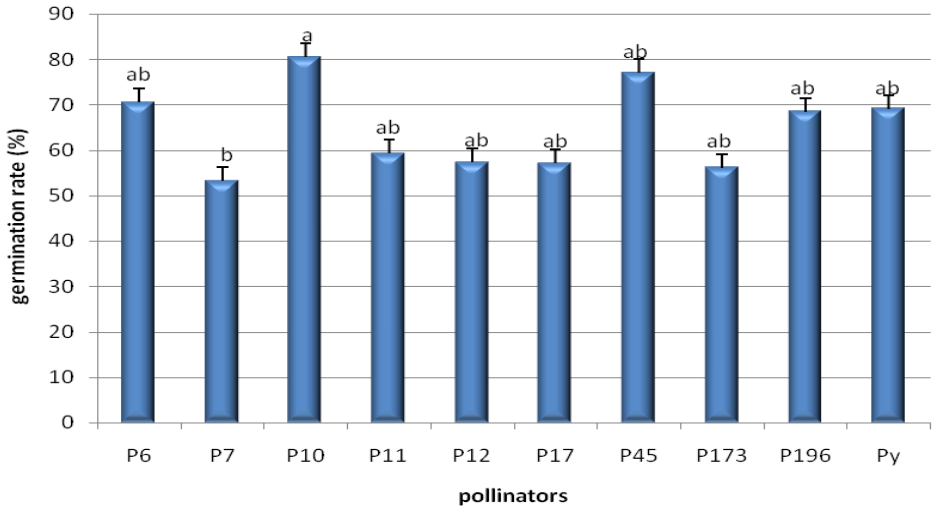

Figure 5. Pollen germination rate based on ten pollinators studied.

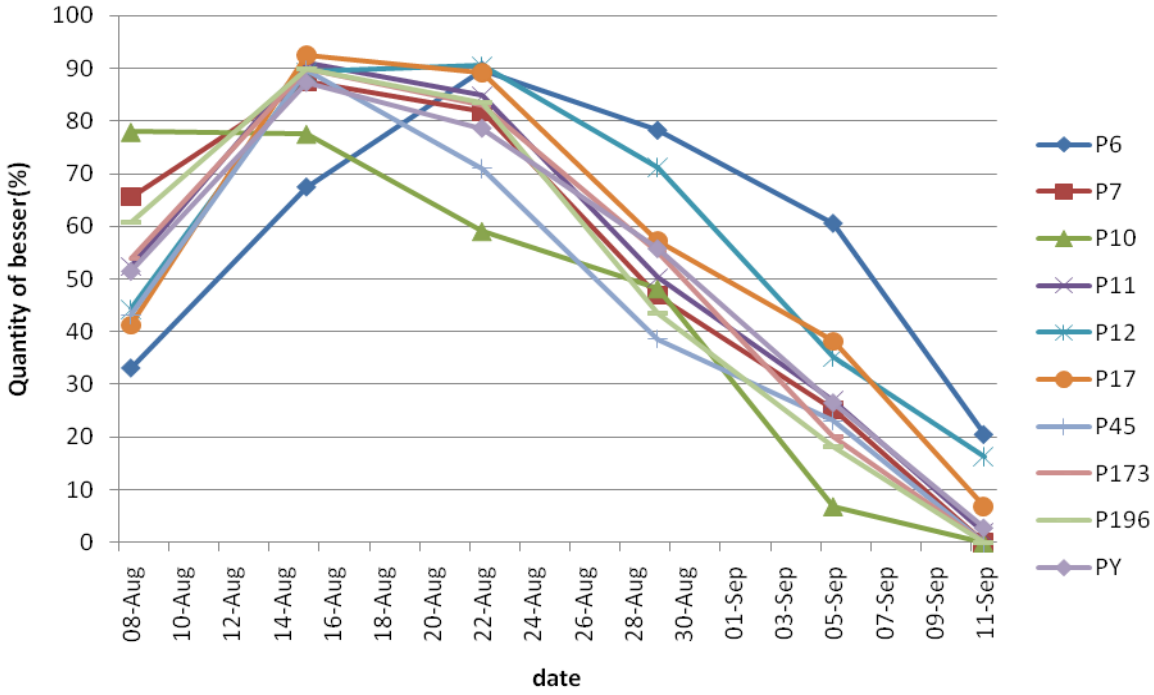

Figure 6. Changes in the percentage of the fruit at the stage "Bisser" according pollinators in the region of Tozeur.

that the germination rate at the date palm is between 2 and 93.9%. Abdallah (2000) considers that pollen must germinate over 50% for a good fruit set rate of *in vivo*. So the results show that these pollinators can be used for pollination since the germination rate of all pollinators exceeded 50%.

Effect of pollen on the maturation dates

Dates "Deglet Nour" go through three different colors during their maturation (Dowson and Aten, 1963). Fruits are called "Blah" as long as they remain green. Turn to yellow indicates the evolution of fruits to the stage "Bisser" (Figure 6).

During this stage the fruits are very sensitive to rain, the speed with which the fruits beyond this stage to reach the stage "Tamar" with brown color is interesting on two levels:

Firstly dodging the first rains of autumn and early harvest, secondly availability dates on the national and international

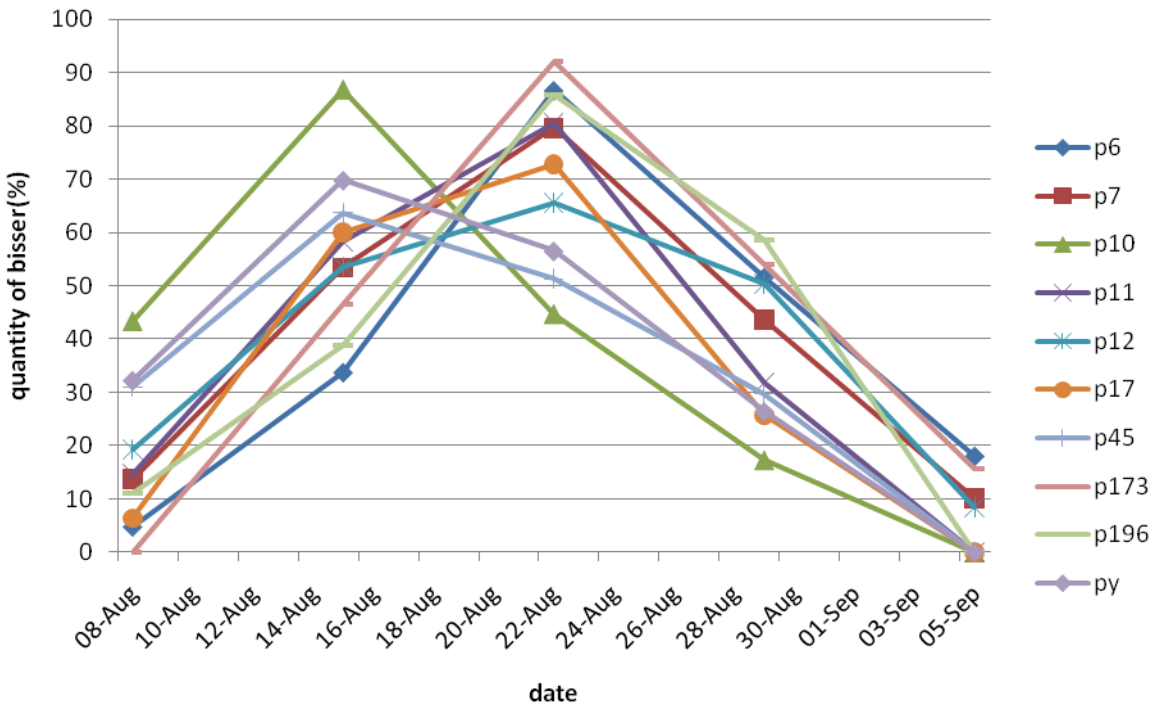

Figure 7. Changes in the percentage of the fruit at the stage "Bisser" according pollinators in the region of Dguech.

market.

Changes of fruits to the stage "Bisser"

This stage began early in the month of August up to October for all pollinated fruits reach the stage "Bisser". The change of dates to the stage "Bisser" is fast in the three regions. This increase in the amount of "Bisser" was followed by a rapid decrease with a speed varies depending the pollinator and Site, Indeed, in the city of Tozeur, the amount of "Bisser" contained in the group pollinated by P6 and P12 arrives to maximum towards the end of August while other pollinators. While other pollinators, except P10 which gave a maximum amount of "Bisser" from the beginning of August, give a maximum amount to half of the month. From 23 August the amount of "Bisser" begins to decrease with a non-constant speed, and around 11 September, this percentage is zero for pollinators P7, P10, P11, P45, P173 and P196, this is to say that the entire amount of "Bisser" is moving towards the stadium "Tamar". On other side pollinators P6, P12, P17 and PY each one gave a percentage of "Bisser" equal respectively to 20.46, 16.28, 6.73 and 2.83%, at the same date. For Deguech region the fruits pollinated by P10, P45 and PY have given a maximum amount of "Bisser" to August 15 while the other to 23 August.

Figure 7 show that the development speed to the stadium "Bisser" varies according the pollinator and the region. Indeed the percentage of "Bisser" of the fruits pollinated by P10 is equal to 43.34% to 5 August. By against P6 gave a percentage of "Bisser" equal to 4.67%.

In Kebili (Figure 8), fruits pollinated by P10 and P45 have completed their stage at 20 October and those pollinated by P7, P11, P17, P173, P196 and PY have completed this stage at 30 October. While fruits pollinated by P6 and P12 each one gave, at the same date, a percentage of "Bisser" equal respectively to 8.61 and 8% respectively.

These results show that the effect of pollinators on the fruits ripening period is expressed in the three sites. Some pollinators seem always inductors on early stage "Bisser" as P10 and P45, the lateness while others are inductors of the lateness maturation such as P6 and P12.

The effects of other pollinators (P17, P7, PY and P173) on the ripening fruit are influenced by the regions (climate, soil type etc).

Evolution of fruits to the stage "Tamar"

This stage begins, according pollinators and sites, 131 days after pollination and lasts for four to six weeks to reach full maturity, two weeks more compared to the stadium "Bisser". The difference of two weeks compared to stage "Bisser" may be the result of climate disruption fall.

Figure 9 shows that the maturity of fruits pollinated by P7, P10, P45, P173 and P196 has been completed for four weeks against the use of pollinators P6, P11, P12, and P17 PY makes changes of fruits to the stadium "Tamar "very late.

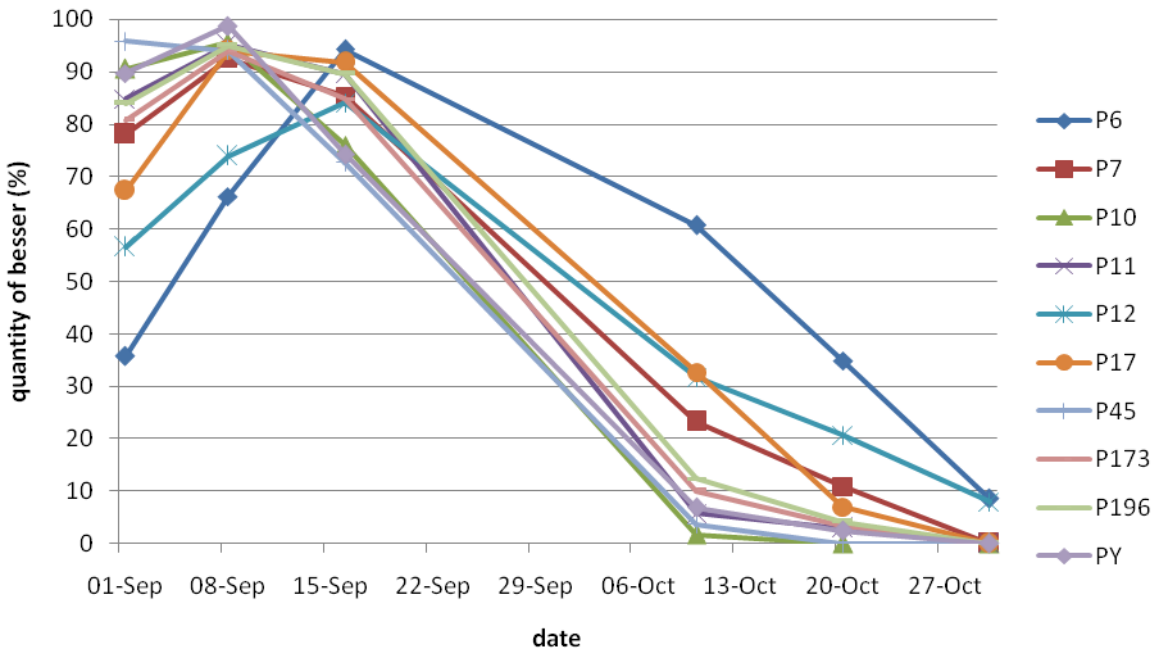

Figure 8. Changes in the percentage of the fruit at the stage "Bisser" according pollinators in the region of Kebili.

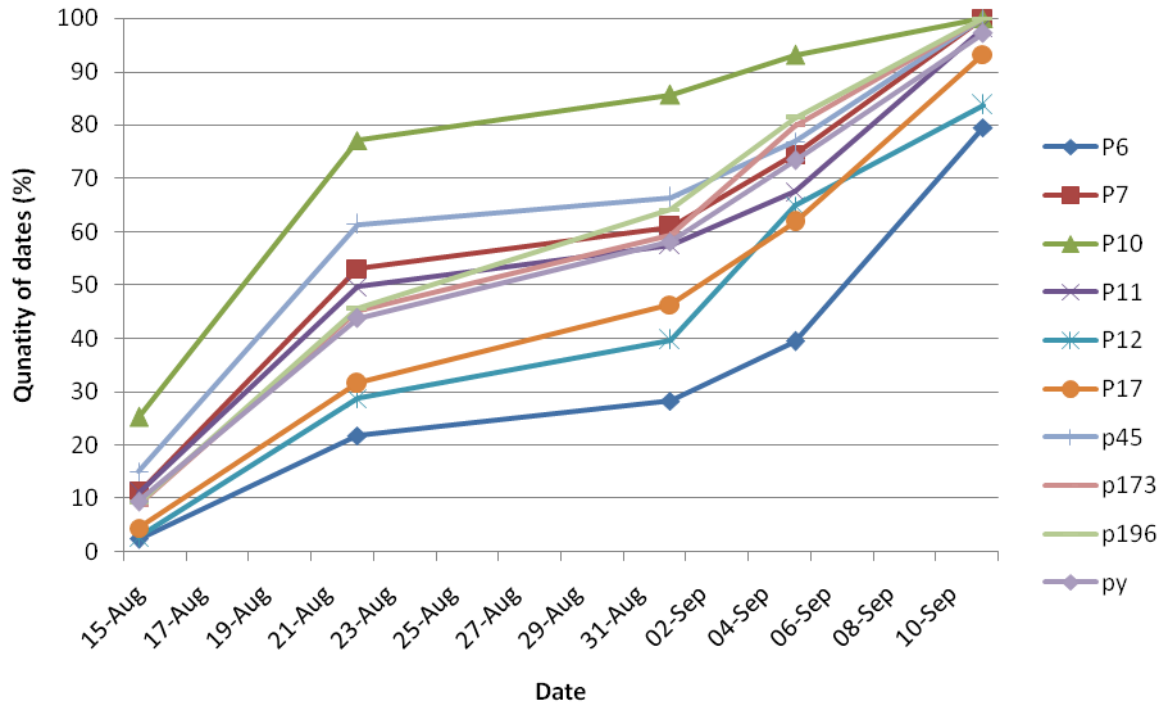

Figure 9. Evolution of the percentage of the fruit at the stage "Tamar" according pollinators in the Tozeur region.

We can see that pollinators that induced early entry dates to the stadium "Bisser" are those who have held the top spot at the stadium "Tamar", it would be possible to predict time to maturity from the stage "Bisser".

In Deguech (Figure 10), only fruits pollinated by P10, P45 and P11 have completed the maturation stage. The fruits pollinated by P17, P196 and PY have not completed their maturation despite having induces early stage "Bisser".

Late effect of maturation dates from these pollinators is expressed to the stadium "Tamar".

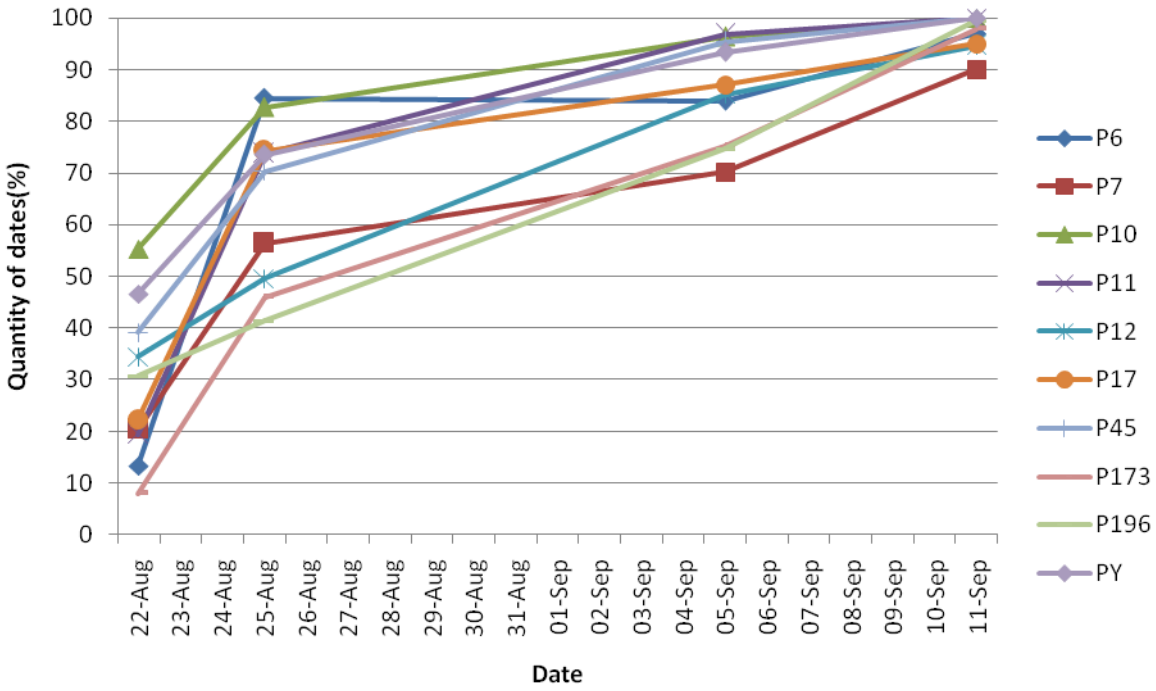

Figure 10. Evolution of the percentage of the fruit at the stage "Tamar" according pollinators in the region of Dguech.

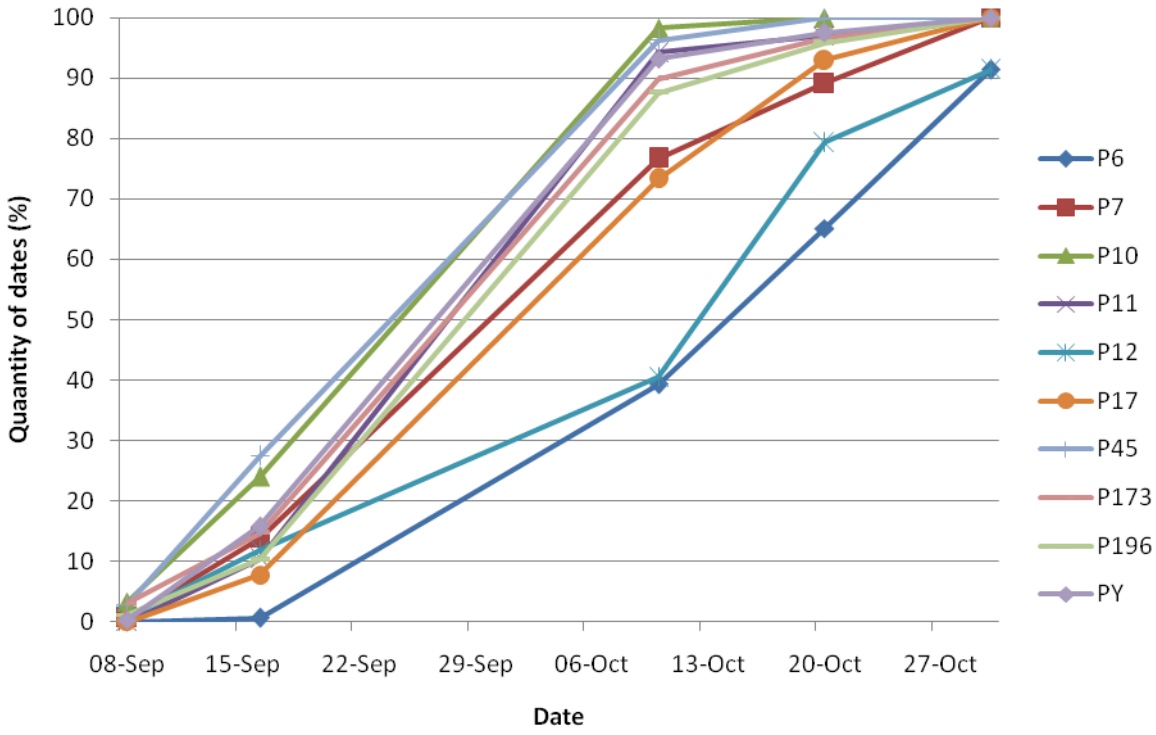

Figure 11. Evolution of the percentage of the fruit at the stage "Tamar" according pollinators in the region of Kebili.

The same for the Kebili region (Figure 11), where the fruits pollinated by P10 and P45 have completed the stage of maturation to 20 October, while those pollinated by P7, P17, P173, P196 and PY have completed their maturation to 30th October. The fruits pollinated by pollinators P6 and P12 fail to complete the stage of maturation. Indeed, these two pollinators give a percentage of dates lower to 100% (91.38%), at 30 October.

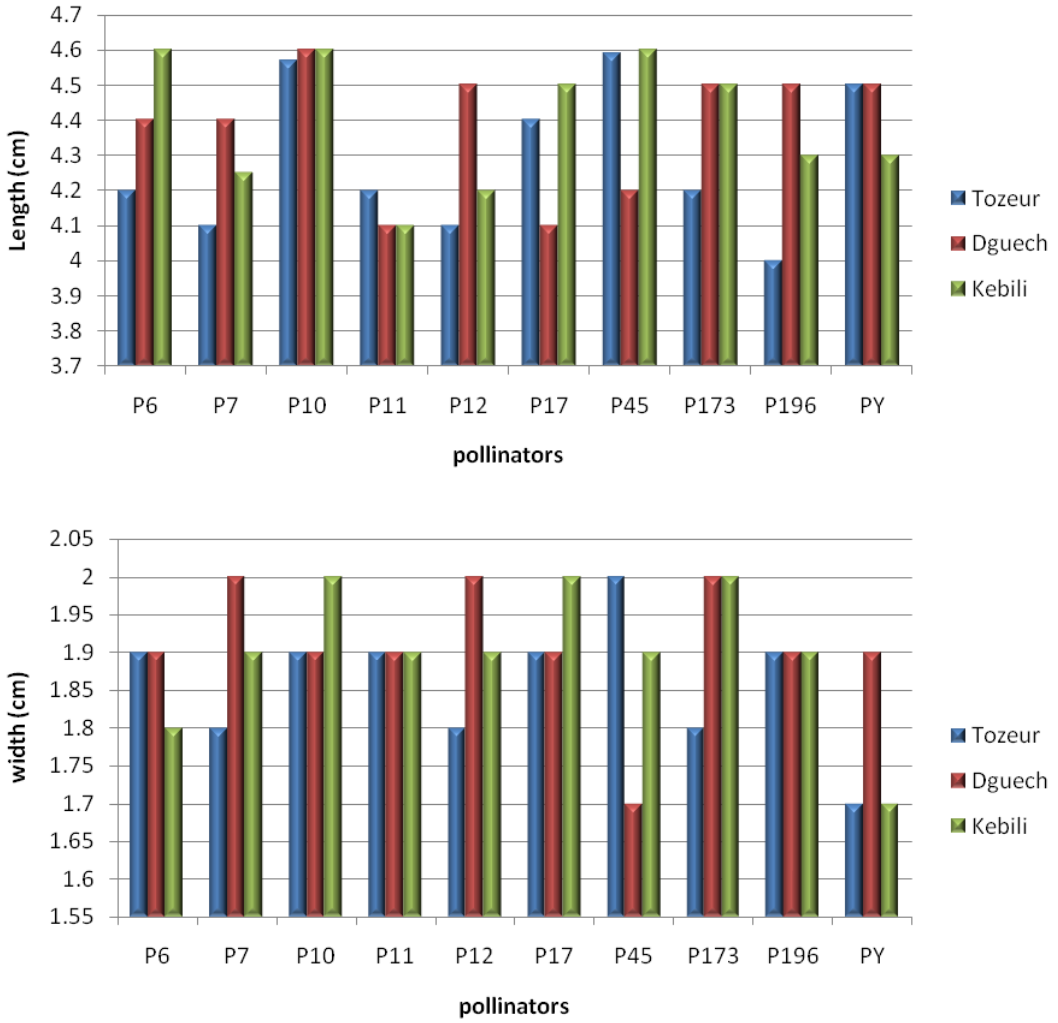

Figure 12. Variation of the length and width of fruit depending on pollinators and sites.

The P10 and P45 have kept the top positions at both stages "Bisser" and "Tamar" in the three regions. It seems that these pollinators induce earliness to maturity dates, since the fruits pollinated by these pollinators reach full maturity to 180 days after pollination, on the other side, P6 and P12 are two inducers of lateness maturation since the maturity dates from these pollinators is not completed. As for other pollinators, they showed instability in their effects on the maturation dates, hastening or delaying maturity depending on the site. That is the case of P7 who presented an early effect in Tozeur and a retarding effect in two other sites.

Effect of pollen on the quality of dates

Effect of pollen on fruit size

The negative correlation between early maturation and fruit size was reported by Nixon. In 1931, Nixon found that fruit "Deglet Nour" which ripen early to the first October are of lesser quality than those that ripen in October and November

The results (Figure 12) show that fruit size is influenced by the type of pollen and site. The average size of the fruits varied from 4 to 4.6 cm in length and from 1.7 to 2 cm of width. The pollinator P10 gave the largest fruit in the regions of Kebili, Deguech and Tozeur. By against, P11 gave small fruits also in the 3 regions, noting that P10 is an inducer of early maturation, while the pollinator P6, which is an inducer of lateness maturation, gave medium fruit size in all three sites. The smaller size has been obtained with the dates pollinated by P12, in the region of Tozeur and those pollinated by P45 in Deguech and PY in Kebili.

It has been demonstrated that fruits inducers to precocity maturation reduce fruit size while reducing kernel size. Moreover, the early-inducing pollinator (P45) shows a reduction of the caliber of fruit associated with decreased core caliber (Figure 13). Only P10 is detached of these results and provides large fruits while reducing kernel size.

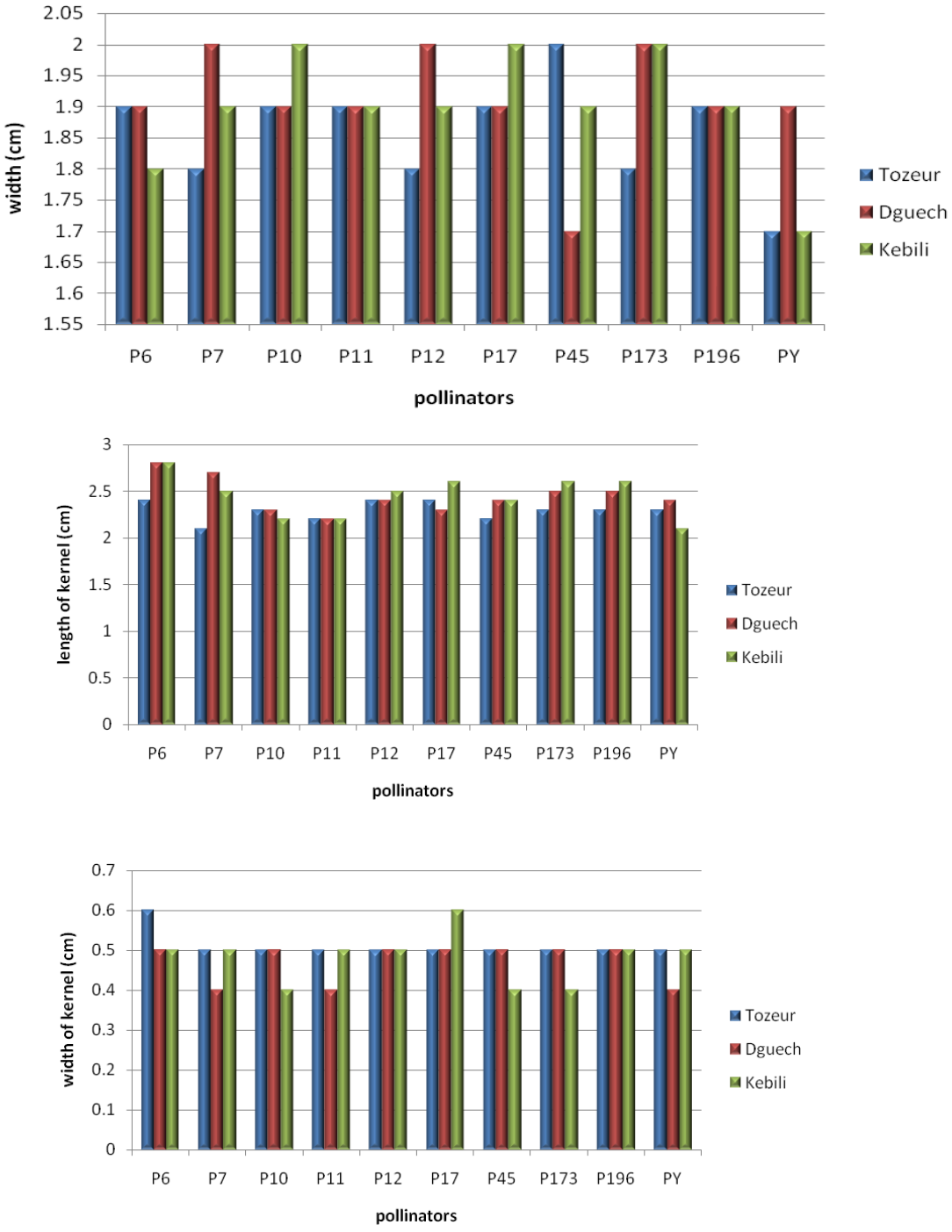

Figure 13. Variation of the length and width of the core of the fruit according to pollinators and sites.

The result shows that the use of this pollinator drives a gain in the pulpit dates.

Effect of pollen on the sugar contained in fruits

Dates are considered fruits rich in sugars, this excise in two forms: sucrose and reducing sugars, the main reducing sugars are fructose and glucose but dates contain other sugars such as galactose, arabinose (Al Khouli et al., 1998; Manoel et al., 2014). The results (Figure 14), show that the total sugars varies from one pollinator to another, which implies that pollen has a metaxenic effect on the sugar content in fruits.

The composition analysis of sugar dates identified three distinct groups of pollinators:

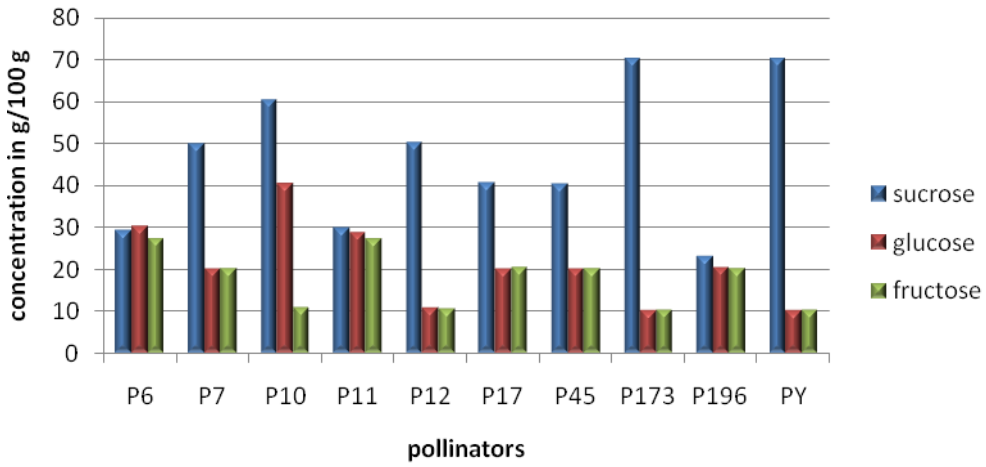

Figure14. Variation of sugars rate according to pollinators.

(i) The first group includes the P6, P11 and P196. Indeed, fruits pollinated by these pollinators show a sucrose rate almost equal to their rate of glucose and fructose.
(ii) The second group is made up of P7, P12, P17, P45, P173 and PY, they gave an almost equimolar concentration of glucose and fructose when sucrose shows a very high rate compared to reducing sugars.
(iii) The third group comprises only P10 which gave a higher glucose levels compared to other pollinators.

Jemni et al. (2014) said that the better quality fruits are those that have high levels of sugar; she also added that the good dates have a high content of reducing sugars (fructose and glucose), because they represent the largest constituents in terms of energy calories available, easily digestible and hydrolysable. So for this study fruits pollinated by P10 appear to be better followed by those pollinated by P11, P6, P17 and P45.

Conclusion

This work is a contribution to research reliable criteria for the selection and evaluation of pollinators have high added values in the date palm cv Deglet Nour. Indeed, the choice of pollinator is a key step in the production dates.

The use of SSR markers in the date palm for the polymorphism analysis has proven effective.

Six (6) selected from 10 primers tested generated 21 polymorphic bungs can be useful markers to distinguish the studied pollinators. The analysis of the similarity matrix showed a greater or lesser genetic diversity with genetic distances which varies between 0.363 and 0.909. This study allowed us to group the pollinators of date palm in 4 groups, with (P6, PY) is the farthest pair against by (P173, P196) is the most related couple. However, this technique has certain limitations; the major problem is that concerning the need to have information concerning the sequences of primers flanking the repeating units and obtaining this information is time consuming and has a high cost. Often it is necessary to review the gene library to identify and characterize microsatellite loci of the species studied.

Study of the metaxenic effect of these pollinators on fruit repining date has allowed dividing pollinators into three groups:

(i) Pollinators hastening the ripening of fruit: P10 and P45.
(ii) Pollinators retardant fruit ripening: P6 and P12.
(iii) Pollinators have unstable effect on ripening: P7 and P17.

These results conform to those found by Bchini (2006) concerning the effect of early induction attributed to P10.

The study of the effect of pollen on to maturity was complemented by a qualitative study of fruits to compare their qualities, the analysis of the results allowed to classify fruits into three groups:

(i) Fruits having good pomological and biochemical characteristics such as P10 and P6 that showed high levels of reducing sugars.
(ii) Fruits with good biochemical properties (pH, sugar content ...) but they have a small caliber as the P11, P45 and P196.
(iii) Fruits with a quality which varies depending on the site such as P7, P17, P173, P12 and PY.

The identification of a pollinator that induces precocity of ripening fruit with good pomological and biochemical characters is of great interest to producers.

This work has helped to highlight the P10 pollinator is the most efficient pollinators among the 10 studied. Indeed, the P10 is ranked in first position on its ameliorative effect on the quality and the progress of fruit maturation, Furthermore it is the least influenced by the surrounding conditions.

Weather conditions play an important role while influencing these traits studied (maturation date, size, water content.), generally ancient oasis are the least affected by these conditions for their cropping system in three floor representing a microclimate that attenuates the effect of environmental conditions (heat, sand storms, sirocco wind).

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