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Evaluation of pollen grains germination, viability and chemical composition of some date palm males

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ABSTRACT

This experiment was administrated in order to compare the four males morphologically and biometericaly of date palm (*Phoenix dactylifera* L.). Leave and pinnae morphological traits (leaf length, length of pinnae part, length of spinney part, pinnae length, pinnae numbers, spine length and spines number) were studied also and the differences were be statically significant in all parameters except spine length pollen grains of four date palm males were examined and photographed using Scanning Electron Microscopy (SEM) to identified and differentiates among males. The scanning electron analysis of pollen grains revealed specific variation in some morphological properties (length, width and number) and pore characteristics were varied between four males. Pore length (12.27-12.73 µm) pore width (6.14-6.82 µm) and pore numbers in (photomicrograph field 28.9 X 19.72 µm) ranged from (72-164). Morphological traits of pollen grains can help to identified and characterized date palm males. The chemical compositions of pollen grains (minerals and amino acids) were examined. Relationship between minerals (Mg, P, S, K, Ca, Cu, Zn, Fe and Se) was investigated by x-ray the analysis recorded the highest percentage ranged from 33.7 to 27.5% and 26 to 22.7% for potassium and phosphorus, respectively and the lowest values percentage were 2.5% to 0.6% and 1.1 to 0.1% for Magnesium and iron, respectively Viability of pollen grains for four males pollinators were tested and found the best male were M1 and M4 in the viability when tested before and after storage. Consequently, adiversity was observed due to the genetical dissimilarity between the male palms. This diversity is due to the fact that the majority of male palms are originated from seeds.

Key words: Date palm, male, pollen grains, morphology, amino acids, mineral composition.

Introduction

Date palm (*Phoenix dactylifera* L.) is a diploid (2n=36), perennial, monocotyledonous plant. Date palm has separate male and female individuals. Female trees are cultivated mainly for their nutritive fruits. Pollen grains play an important role in the size, shape, weight of fruit and time of ripening, as metaxenia phenomenon (Kavand 2014). Favorable fruit production is related to quality of pollen and its compatibility with a certain female variety. However, pollen of different males has been found to have different effects on the size of fruits and seeds as well as time of fruit ripening. The date palm is highly cross pollinated fruit tree. Due to its dioecious nature, about 5 to 10 male plants are capable for pollinating 100 female plants of date palm. To make pollination effective 2 to 3 strands of male flowers are inserted between the strands of female spath. Artificial pollination is considered to be the most important factor affecting fruit set and yield (Khushk *et al.*, 2009). Economic yield depends on higher percentage of normal fruit setting which further relies on pollination and its techniques efficiency (time), time of flowering of male/female palms, pollen source and quality, male-female compatibility, female flower receptivity and environmental factors like temperature, rain and wind. Many factors affect the pollination, one of them is pollen grain of different male types which affect fruit set, yield and fruit characteristics. Different researchers studied the effect of different male pollinizers on different cultivars of female palm (Shaheen *et al.* 1989, El-Hammady *et al.,* 1997; Aly 2008b; Iqbal *et al.,* 2009). Some other research workers investigated that pollen source affect the productivity, maturity and fruit quality of dates (Ibrahim and Shahid. 1994, Iqbal *et al.,* 2004; Jalal *et al.,* 2006 and Aly 2008b).

The effect of pollinator source can influence the physical and chemical characteristics of fruit set. The available number of date palm males is insufficient for efficient pollinating of increasing number of female plants. Most of available pollinating date palm males are mainly originated from

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seed propagation, resulting in many different local males that represent genetic diversity. Characterization and evaluation of available highly potent male palms is the first step to find superior ones to fertile female plants (Rizk *et al,* 2007).

Therefore, it is important to conduct experiments to evaluate male parents, in terms of vegetative and flower characteristics, determination of biodiversity, late pollination, and also monitoring the similarities and differences. Evaluation of physical and chemical quality of fruits, fertilized with different pollen males, is critical since source of pollen is one of the most important factors to improve production and fruits quality of date palm cultivars (Elshibli *et al*., 2007).

As to the phenotypic markers, the most common characters used to identify different cultivars in date palm are the morphology of leaves, spines and fruit characters. Such morphological features are sensitive to environmental factors (Sedra *et al.,* 1993 and 1996).

Pollen storage and viability are very important for pollination, breeding, biodiversity, biotechnology, conservation, and other biological and non-biological studies of the date palm. Optimizing procedures and duration of storage are important for effective and long-term date palm pollen storage and viability (Jaskani and Naqvi 2017).

So, the objectives of this investigation were to evaluate the surface morphology and ultrastructure of pollen grains from seedling date palm males used in the pollination of date palm cultivars in Alexandria region of Egypt and the best one for quality and yield pollinator (A) and (D) for Zaghloul and Samany cultivar, the recommendations concluded male (A) and (D) given the best viability and given best yield, fruit set percentage and fruit quality, therefore these males must be receive more attention and propagated by tissue culture to use them as certified and constant pollen grains source (Aly 2008b).

Materials and Methods

Present investigation was carried out on seedling date palm males in two successive seasons during 2011 and 2012 grown in El-Montazah Garden in Alexandria Governorate. Four male palms were chosen in this study. The experimental palms were healthy, uniform in growth, vigor and height. All cultural practices were carried out according to the normal schedule for experimental palms. The properties of the leaves were studied to illustrate the morphological differences among the male palms and study the physiological and chemical characteristics of pollen grain.

A. Leaves, pinnae and spines characteristics:

Five leaves were taken at March from each male palm as a target of study the morphological characteristics.

I. Leave characteristics:

1. Leaf length (Cm):
2. Length of pinnae part (Cm)
3. Length of spines part (Cm)

II. Pinnae characteristics:

1. Pinnae number
2. Pinnae length (Cm)
3. Part of leaf occupied with pinnae (cm).

III. Spines characteristics:

1. Spines number
2. Spines length (Cm)

B. Pollen grains characteristics:

Pollen samples were collected from flowers of four date palm males. Three spathes from various parts of each tree (three to each male) were isolated before anthesis with paper bags to avoid contamination with other pollen sources. The strands of each spathe were cut and left to dry at room temperature. Then, pollen grains were separated from the flowers using fine sieves (40 mesh) (Shaheen, 1986). Pollen was collected in small vials and stored in desiccators at 3-5 ◦C (Javady and Arzani, 2001) until used.

I. Microscopically Examination and Photography

Electron microscope was used for the comparison of the four samples Model: Joel, GSM-6360LA.Analytical Screening Electron Microscope, Analysis was done by Energy Dispersive spectroscopy {EDS} method. Pollen grains observed at 20 KV with a Stereoscan (360 SEM - FEI / Inspect S50 model) were photographed at 10000 x for whole grain.

II. Pollen grain minerals contents

X-ray fluorescence analysis (XRF) has previously been used in quantitative elemental analysis of a wide range of organic and inorganic samples (Arai 2006; West, *et al*., 2009 and West *et al*., 2010). The basis for the technique is that all elements emit secondary ('fluorescent') X-rays of characteristic energy when exposed to X-rays of appropriate higher energy, with energy and intensity of emitted X-rays used to determine elemental composition. X-Ray fluorescence (XRF) is a rapid technique for determining nutrient concentration in plant tissue (Kenneth *et al.,* 1992).

III. Amino acid analysis:

Amino acids composition was analyzed by automatic amino acids analyzer (AAA 400 INGOS Ltd. Czech Republic). The sample (10 gm) was hydrolyzed with 10 ml of 6 *M*HCl in a sealed tube at 110ºC in an oven for 24 h. After hydrolysis, the acid was evaporated in a vacuum evaporator under reduced pressure at 80ºC. The HCl free residue was dissolved in 2 ml of loading buffer (0.2 M, pH 2.2) to inject into the apparatus (Block, *et al.,* 1958).

C. Pollen grain variability before and after storage:

To discover favorable storage conditions for pollen viability of the collected pollen grains were placed in small glass vials with stoppers. The vials (3 replications) were stored at refrigerator (4ºC) and After 12 month pollens were subjected to viability tests using the technique based on the results of the first experiment (Mortazavi *et al.,* 2010).The collected pollen grains for four groups were tested according to the method described by Alpert (1930). Planted pollen grains dishes were inculpated at 30 ºC for 4 hours and the microscope investigation was done after staining with lacto-propionic orcein according to Dyer (1963).

Germinate percentage:

A little quantity of pollen grains was placed on sugar solution (at 8 % sucrose) in Petri-dish for 2-4 hours. After that, pollens were examined under Olympus compound microscope (Bx-51) to find pollen grains viability, which measured by ocular micrometer. The total number of pollen grains and number of germinated pollens was calculated as follows.

$$\text{Germinate \%} = \frac{\text{Germinate number of pollen grains}}{\text{Total number of pollen grains}} \text{X100}$$

Statistical analysis:

The experimental design was randomized complete block design (RCBD) with three replicates. The obtained data were statistically analyzed according to Senedecor and Cochran (1990). Duncan's Multiple Range test effect was used to compare between male palms (Duncan 1955).

Results and Discussion:

1. Leave Characteristics:
Leaf length (cm):
Length of pinnae part (cm)
Length of spinney part (cm)

The data presented in Table (1) showed that, leaf length and the length of spinney part varied from male to another the highest leaf length recorded for male (M2) for both characteristics and the lowest for (M3) for leaf length and (M1) for length of spinney part. While length of pinnae part (M4) recorded the highest length but (M3) was the lowest and the differences were statistically significant among all tested males in both seasons. These findings are harmony with those found by Shaheen *et al.,* (1986) Soliman *et al.,* (2013) and they found that leaf length ranged from 258 to 552 cm in different males, the leaves could be divided into 3 groups according to leave length and length of spinney part.

Table 1: Leaf, pinnae and spine morphological characteristics male seedling palms during 2011 and 2012 seasons

Male	Leaf length (Cm)	Length of pinnae part (Cm)	Length of spinney part (Cm)	Pinnae length (Cm)	Pinnae No.	Spine No.	Spine length (Cm)
2011							
M1	334.00c	290.33c	53.67d	49.67b	254a	32b	8.67a
M2	411.33a	297.67b	113.67a	45.33b	204c	24c	9.67a
M3	323.00d	266.00d	57.00c	50.00b	152d	24c	10.00a
M4	385.00b	321.00a	64.33b	56.67a	220b	34a	10.00a
2012							
M1	341.06c	287.89c	52.91d	48.92b	250a	32a	8.91a
M2	418.62a	297.99b	110.28a	46.21b	208c	26c	9.37a
M3	325.00d	268.41d	58.02c	49.68b	156d	24b	9.98a
M4	392.17b	324.02a	65.16b	57.27a	228b	32a	10.01a

2. Pinnae Characteristics:

Pinnae number and Pinnae length (cm):

The total number of pinnae per leaf in both seasons of study ranged from 152 to 254 the highest number recorded for male (M1) and the lowest number for (M3) and the differences were statistically significant among all tested males. The longest pinnae length recorded for male (M4) was 56.67 (cm), whereas the shortest one recorded for male (M2) was 45.33(cm) and the differences were statistically significant among all tested males as shown in Table (1). These findings are agreement with those found by Shaheen (1986) and Soliman *et al.* (2013).

3. Spine characteristics:

Spine number and spine length (cm):

Data presented in Table (1) clarified that, male (M4) recorded the highest spine number was 34 and 32 spinesfor first and second season, respectively, but male (M2) and (M3) recorded the lowest number were 24 and 26 spines for both seasons and the differences were statistically significant

among all tested males palm, in the other hand no significant differences found among all tested male for spine length.

B. Pollen grains characteristics:

I. Morphological characteristics of pollen grains

Pollen grain dimensions (µm)

A scanning electron microscope (SEM) study of the pollen grains of four date palm males indicated that all males had a monad, elliptical with one deep germinal furrow across the polar surface (Figure 1).

Statistical analysis for the morphological characteristics (length and width) of the pollen grains clearly indicates that average pollen grain length and width (dimensions) are significantly different among all date palm male under study. Date palm male (M2) showed longest pollen grain compared to other date palm males, while male (M3) showed the largest in width than other males, but on the other hand males (M1 and M4) no significant differences in length and width in both study seasons (Table 2). The pollen morphology is an expression that comes from genome and like any other characteristics to be it critical to understand differences of the genotypes. The macro-morphological properties may be useful in taxonomic studies of some species or plant groups. These results are in agreement with other authors on different date palm males (Laiadi *et al.,* 2013; Soliman and Al-Obeed, 2013 and Djerouni *et al.*, 2015).

Table 2: Pollen grain morphological characteristics of date palm males during 2011 and 2012 season

Males	Lengthµm	Widthµm	No. of pores
2011			
M1	12.39b	6.30b	72c
M2	12.73a	6.14c	144b
M3	12.27c	6.82a	164a
M4	12.40b	6.30b	76c
2012			
M1	12.40b	6.31b	76c
M2	12.76a	6.15c	141b
M3	12.30c	6.81a	159a
M4	12.41b	6.30b	74c

Number of pores

Concerning the number of pores in photomicrograph field dimension (28.90 X 19.72 µm) statistical analysis showed that was highest number in male (M3) and the lowest number found in males (M1 and M4)in both seasons of study and the differences were significant as shown in Figure (1). Number of pores differed significantly in male pollen grains and this is due to the different genotypes characteristics of each male to be taken to distinguish different males. In addition, these numbers of pores in the pollen grains could be used for the identification of such males. These results are in agreement with those found by (Tisserat and De Mason, 1982; Shaheen, 1983; Shaheen *et al.,* 1986).

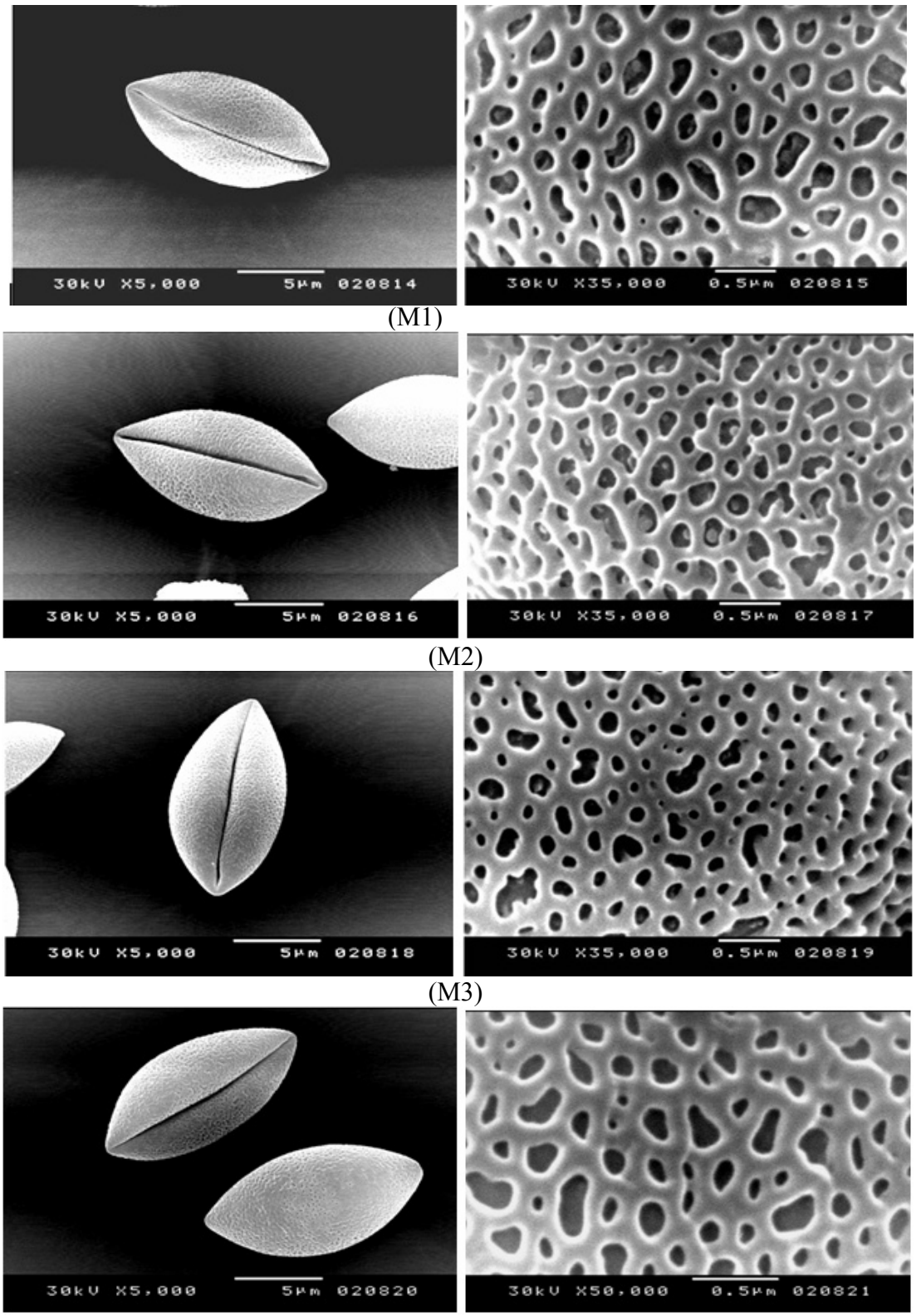


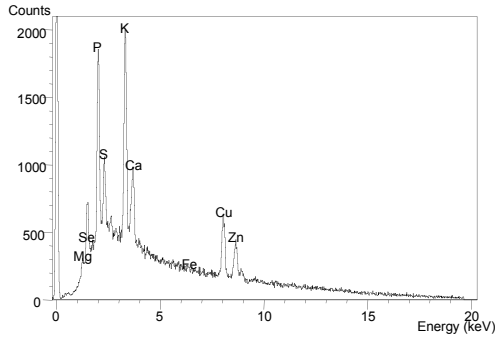
Fig. 1: Scanning electron microscopy (SEM) images of pollen grains morphology of date palm males

II. Minerals relationship percentage

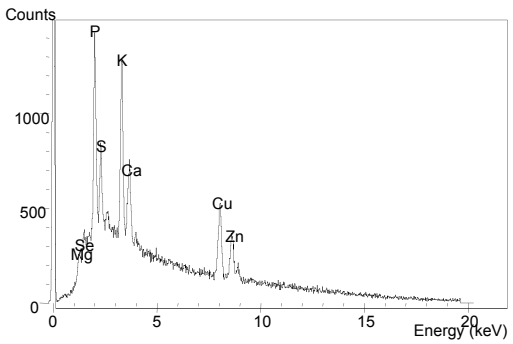
The mineral composition of palm pollen grains is shown in Table (3) and Figure (2). The obtained results revealed that palm pollen grains constitute a rich source of mineral elements. Mineral composition and relationship among them for palm pollen grains the predominant minerals were potassium and phosphorus the percentage for potassium varied between 33.7 to 27.5 % the highest percentage for male (M1) and the lowest for male (M2), while the highest percentage for phosphorus for male (M2) and the lowest for male (M3). Copper ranged from 14.7 to 10.0% male (M2) is greatest one and male (M4) is lowest, while sulpher male (M2) is the greatest one 11.6% but male (M1) is the lowest 9.5%. Calcium ranged from 10.9% for male (M4) to 8.7% for male (M2), whereas zinc ranged from 8.8% for male (M2) to 6.6% for male (M4). The lowest minerals for magnesium ranged from 2.5% for male (M4) and 1.5% for male (M1), Selenium ranged from 2.5% for male (M1) to 0.6% for male (M3) and iron ranged from 1.1% for male (M3) to 0.1 for male (M1) and (M2). These findings are agreed with Boughediri, (1991), he was found that the highest values for P, K and S for most cultivars under studies Bacha *et al.,* (1997) found that pollen grains of date palm contain the macronutrients, N was present in the highest concentrations, followed by Zn, Mn and Cu. Variations in the composition of palm pollen and other pollens reflect the differences in the floral origin of pollen and the plant growth conditions (Stanely, 1971). The capacity of the parent plant to accumulate salts in the pollen is also related to the species. Calcium plays an important role in plant growth and development cycle such as pollen tube growth and fertilization. Pollen germination requires calcium, and it is tube growth in pistil tissue depends on the presence of calcium. Calcium also plays a role in determining the direction of pollen tube growth. Calcium has an essential signaling, physiological, and regulatory role during sexual reproduction in flowering plants. Pollen tube elongation typically relies on external calcium stores in the pistil. Calcium establishes polarity of the pollen tube and forms a basis for growth (Ge, 2007 and Prajapati, 2010). Calcium is important for pollen germination in date palm. The concentration of this element is related to the kind of the date palm male cultivar (Kavand 2014).

Table 3: Minerals relationship percentage by X-ray (KeV)

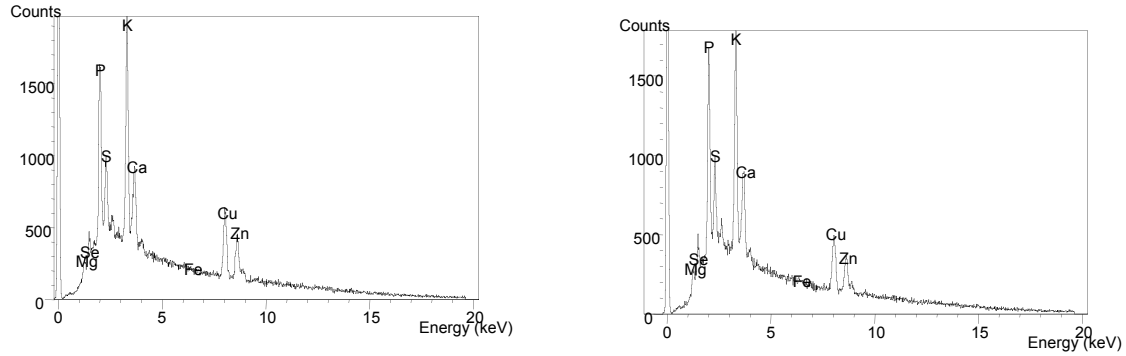
Males / Minerals	M1	M2	M3	M4
Mg	1.3	1.5	2.1	2.5
Se	2.5	1.1	0.6	0.8
P	24.0	26.0	22.7	24.7
S	9.5	11.6	10.8	11.1
K	33.7	27.5	33.6	33.2
Ca	9.3	8.7	10.0	10.9
Fe	0.1	0.1	1.1	0.2
Cu	12.3	14.7	12.2	10.0
Zn	7.3	8.8	6.9	6.6



Male (M1)



Male (M2)



Male (M3) Male (M4)

Fig. 2: Minerals relationship percentage by X-ray

III. Amino acids components

In the present study, palm pollen grains were analyzed for their contents of 16 of the most quantitatively important amino acids. Data obtained in Table (3) and Figure (3 and 4) showed that palm pollen grains contained seven nonessential amino acids and nine essential amino acids and. The amino acid contents in the pollens of the studied male types varied from one male to another, such variations could be attributed to the differences in the genetic of the different male types. The important nonessential amino acid for pollen germination Argenine were recorded from 17.41 to 11.80 mg/g dry matter for (M4) to (M2), respectively and Glycine were recorded from 81.92 to 44.02 mg/g dry matter for (M1) and (M2), respectively. Leucine and Phenylalanine were considered the main essential amino acids of palm pollen grains effective for germination, Leucine recorded 37.22 mg/g dry matter for (M1) to 19.94 mg/g dry matter for (M4), while Phenylalanine were recorded 18.90 mg/g dry matter for (M1) to 11.47 mg/g dry matter for (M3).Pollen contains all the essential amino acids but the amounts may vary between plant species (Roulston *et al.*, 2000).There was a positive correlation between pollen germination and the amount of glycine, luecine, xsyrosine, phenylalanine and arginine (Rashed *et al.,* 1995), whereas,increase in free lysine and valine results in decreased viability of pollen(Stanley and Linskens, 1974).

Table 3: Pollen Grain Amino Acids Content (mg/ g) in dry weight

Amino Acid	M1	M2	M3	M4
Nonessential amino acids				
Argenine*	15.91	11.80	12.49	17.41
Aspartic	46.98	37.32	42.16	32.31
Serine	19.16	13.22	17.68	7.25
Glutamin	47.09	39.14	44.45	39.28
Proline	22.83	17.31	20.72	18.14
Glycine*	81.92	44.02	46.97	61.49
Alanine	82.67	69.19	81.31	24.28
Essential amino acids				
Valine	22.01	26.81	28.04	12.41
Threonine	17.16	8.96	19.07	6.21
Methionine	6.88	4.90	4.60	8.02
Isoleucine	25.74	13.92	17.19	13.89
Leucine*	37.22	27.67	19.94	21.45
Tyrosine	15.35	9.14	12.98	3.67
Phenylalanine*	18.90	13.32	11.47	16.28
Histidine	18.43	12.44	13.96	8.17
Lysine	28.69	31.90	37.73	29.21

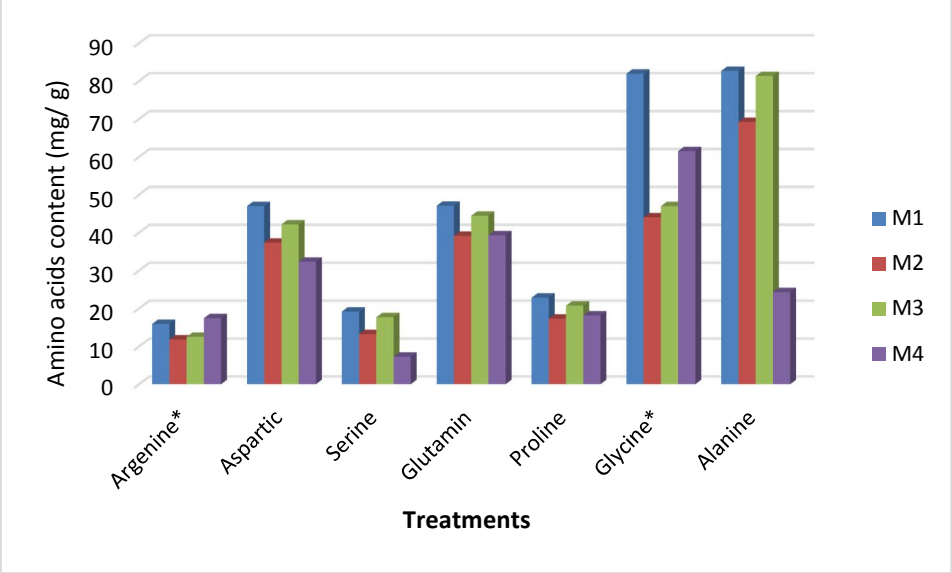


Fig. 3: Pollen grain nonessential amino acids content (mg/ g)

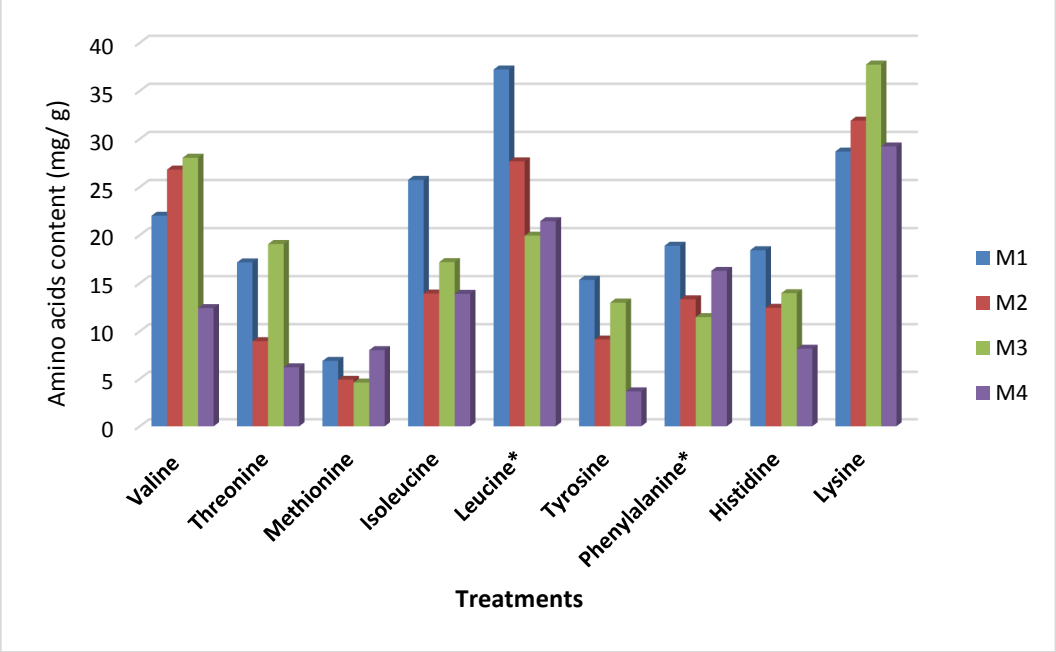


Fig. 4: Pollen grain essential amino acids content (mg/ g)

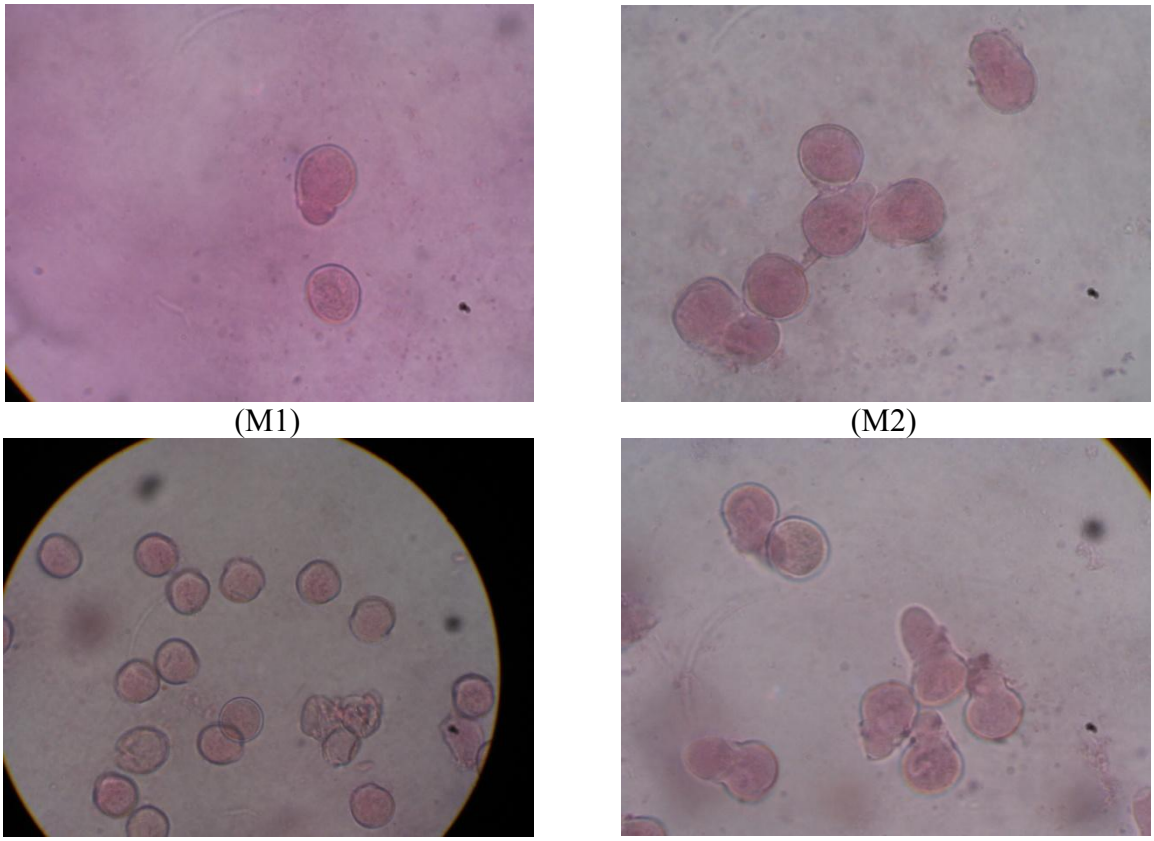
Specific importance of proline in aspects of plant reproduction as important as pollen development, pollination and pollen tube guidance, (Marco, *et al.,* 2015).The most abundant free amino acids reported previously in pollen include α–β - alanine, α-amino-n-butyric acid, arginine, aspartic acid, cysteine, ethanolamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tyrosine, valine (Stanley and Linskens 1974) and proline, cysteine, aspartic acid, histidine, glutamic acid, ornithine, amino-n-butyric acid and hydroxyl proline (Mondal *et al.,* 2009).

C. **Pollen grains viability and germination percentage before and after storage**:

Initial the viability testing showed that the pollen grains viability ranged from 82.% to 100% using the acetocarmine method Figure (5), whereas it ranged from 80.4% to 91.63% when using the germination, best viability were found in male pollinators (M1 and M4). These findings are in line by

those found by Shaheen (2004), Ibrahim, *et al.,* (2013) and Ismaiel (2014) they reported that germination percentage was significantly variable in different date palm males.

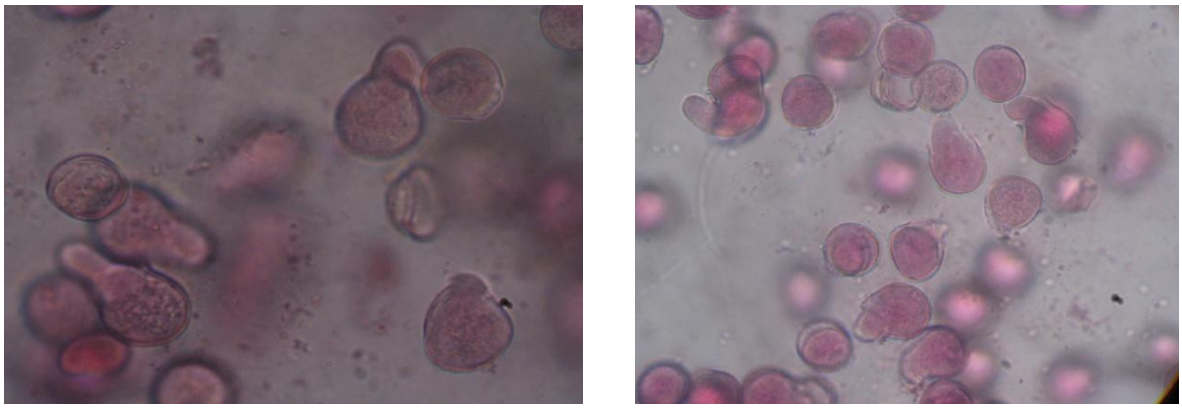
Germination percentage of pollen grain after storage of four date palm cultivars significantly differed when observed in germination (Figure 6). The highest mean pollen germination was observed in male (M4) (84.72%) followed by the male (M1) (76.30%) whereas male (M3 and M2) recorded the lowest percentage in germination after storage (54.12 and 51.7%), respectively. The differences in pollen germination among studied male could be due to the variability in their genetic back ground. These results coincided with those obtained by Djerouni (2015) and Jaskani, *et al*., (2015) who found that, Storage temperature, storage duration, incubation temperature and germination time had significant effect on long term date palm pollen viability.



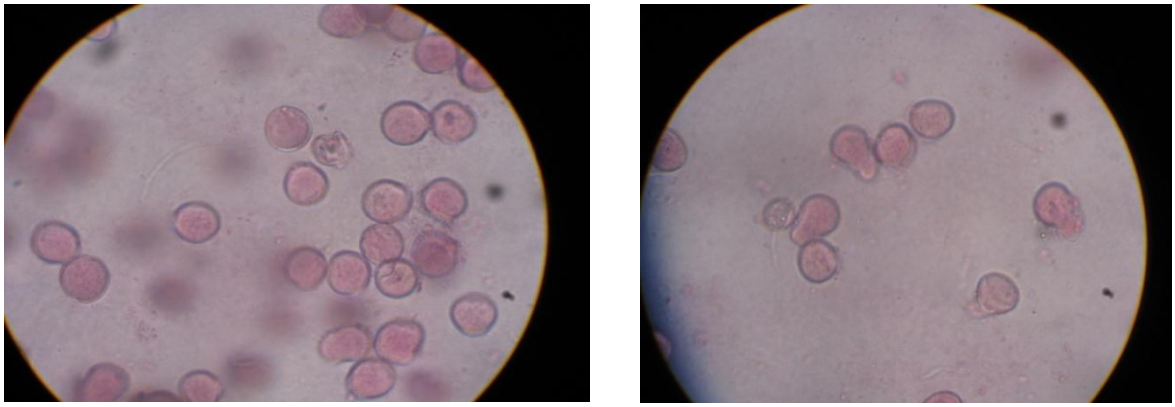
(M1) (M2)

(M3) (M4)

Fig. 5: Viability of pollen grains for four males pollinators by acetocarmin method before storage



(M1) (M2)



(M3) (M4)

Fig 6: Viability of pollen grains for four males pollinators by cetocarmin method after storage

Conclusion

Consequently, adversity was observed due to the genetically dissimilarity between the male palms. This diversity is due to the fact that the majority of male palms are originated from seeds.

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