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Effect of the Stimulative Solutions on Pollen Grain Germination, Fruit set, Anatomical development of Flower, Yield and Fruit Quality in Hayany, Orabi and Zaghlol Date Palm Cultivars

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Abstract:

This study was designed to evaluate the effect of Brewbaker and Kwack's (BK) and Murashige and Skoog (MS) stimulating solutions followed by dusting pollination technique of mixed pollen grains on improving the germination ability of Date palm pollen and elongation of pollen tube through the flower styles on female date palm trees (Phoenix dactylifera L.) of Hayany, Orabi and Zaghlol cultivars. Fruit set, fruit drop, yield/ palm, physical and chemical characteristics of fruits were estimated. The results revealed that the studied traits were influenced by spraying these two stimulating solutions. The highest germination percentage was obtained by applying BK stimulating solution (92.1 %) with 24 hr. incubation period. The longitudinal and transversal anatomical sections of the flowers 72 hr. after pollination showed evolution of one carpel after fertilization and noticed enlargement in size with condensation of tannin cells around embryo sac. Applying of BK stimulating solution with Orabi date palm cultivar was significantly increased fruit set percentage over the other treatments and also recorded the lowest percentage of fruit drop and achieved the highest yield. The highest values of fruit weight, length, size and diameter were coupled with spraying BK solution on the Zaghlol cultivar Spathes. Also, the highest percentage of total sugars was in the Zaghlol and Hayany cultivars when using the spraying treatment with the BK stimulating solution. The same treatment also achieved the highest value in the TSS percentage in the Zaghlol and Orabi cultivars. Moreover, spraying either BK or MS solutions exhibited the significant increases in the anthocyanin content compared to the control with all cultivars. On the other hand, the control treatment recorded the highest average of tannins content of Orabi cultivar fruit. Generally, the application of BK and MS on Date Palm spathes enhanced the fruit set, fruit quality, shortening the germination and elongation time of pollen grain tubes led to maximizing palm yield and overcoming the unfavorable environmental conditions during pollination and fruit set periods.

INTRODUCTION:

Date palm (*Phoenix dactyifera* L.) is one of the most important fruit trees and distributed at large scale in the world especially Arabic region. The fruits are consumed in several stages after maturity, either fresh or dried to various degrees. Hayany, Orabi, Zaghlol, the most economically important date palm cultivars grown in Egypt. Date palm is a diploid (2n=36), perennial, monocotyledonous diecious plant and belongs *Arecaceae* family which comprises of 183 genera and 2600 species **(Dransfield *et al.*, 2008).** The most famous these the genus Phoenix, which includes 14 species native to the tropical and subtropical regions of Southern Asia or East and North Africa **(Shengji *et al.*, 2010)**. It is a leading fruit tree in many countries their fruits play important role in the nutrition's pattern of many people as a source of food, sugar, nutrients and antioxidants as well as a strategic crops food and biochemical industries **(Al-Shahib and Marshall 2003; Khayyat *et al.*, 2007).** Chemically, dates are considered rich source of sugars (mainly reducing) and protein contents as compared to other fruits **(Amira *et al.*, 2011 and Rastegar *et al.*, 2012).** It

becomes essential for the majority of date palm crops' fruiting and fruit set to be successful., although it is an expensive practice due to the palm tree's rhythm of blossoming and repeated climbing (**Kotb, 1993).** The effectiveness of artificial pollination is influenced by numerous factors (**Attalla *et al.*, 1998).** According to numerous researchers, pollination period is thought to be a significant factor impacting fruit output in date palms **(Daud and Ahmed, 2008).** The opportunity for pollen to fertilize the ovule is where efficient pollination occurs. Both the length of the ovule and the rate of pollen tube elongation are factors. Environmental factors and stigma receptivity are intimately related to pollen grains' ability to germinate **(Ruther and Crawford, 1964).** As a result of the metaxenia phenomenon, pollen grains have a significant impact on the size, shape, weight, and ripening period of fruit (**Kavand, 2014).** The size of the fruits and seeds, as well as the timing of fruit ripening, have been observed to be affected differently by the pollen of various males **(Aly, 2018).** Mixing pollen grains with macro and micronutrients and minerals were effective in enhancing the mechanical pollination, increasing yield/ palm and fruit quality **(Ragab *et al.*, 2004; El-Sharabasy *et al.*, 2020 and El-Salhy *et al.*, 2021).** Stimulating solution as Brewbaker and Kwack's (BK) and Murashige and Skoog (MS) stimulating solutions contain nutritional elements such as micro, macro elements, multi vitamins and amino acids. Using modified BK medium were able to obtain a high percentage of germination and growth in pollen tube length due to the transport of inorganic ions such Ca_2^+, B^+ and K^+ which are present in the medium across the plasma membrane of the pollen **(Boughediri and Bounaga, 1987).** Also**, (Soumitra and Subrata, 2016)** showed how various nutrients, including sucrose, boric acid, and certain salts, including calcium nitrate, potassium nitrate, and magnesium sulphate, affected the in vitro germination of Jacquinia ruscifolia pollen. It is needed to find the best pollination technique that may be easiest and most convenient for improving fruiting of date palms **(Iqbal *et al.*, 2010).** During the flowering period, date palm trees are exposed to unsuitable weather conditions, such as rainfall and hot winds, and this leads to the failure of the pollination and fertilization process and thus produces uneconomical fruits. Therefore, stimulating the process of pollen germination leads to a rapid completion of the fertilization process and thus overcoming this problem. Therefor the aim of this study is stimulating and accelerating the pollen grain germination *in vitro* and in the field by applying stimulating solutions, as well as studying their effects on the anatomical development of flowers and on fruit set, yield, and fruit quality.

Materials and Methods:

The present work was accomplished during three successive seasons 2019, 2020 and 2021, respectively on 27 female date palms (*Phoenix dactylifera*, L.) of Hayany, Orabi and Zaghlol date palm cultivars. Palms were 30-year-old cultivated in research orchard of Mansoura University at Kalabshou region, Dakahlia governorate, Egypt. The tested 27 trees were chosen similar in height and growth vigor and subjected to the normal schedule of cultural practices in this region. The leaf/ bunch ratio was maintained at 7:1 in Hayany and Zaghlol according to **Awad (2010) and El-Dengawy (2017)** or at 9:1 in Orabi according to **Hegazi *et al*. (2008).**

Pollen grains extraction

The ripe male spathes on palms were harvested in March when the sheath surrounding the inflorescence cracks. The sepals were cut from the male palm and brought to a temperature-controlled room at 22 ± 2 ˚C to continue drying for several days. The drying process should begin in a room with low humidity (45% R.). As the inflorescence's moisture decreases, its flowers begin to open and release the pollen grains. After that, the pollen grains were extracted and collected from the inflorescence**(Zaid and Arias-Jimenez, 2002).**

Pollen germination test

One liter of the stimulating solution of modified **Brewbaker and Kwack's (BK) (1963)** medium was used. It consisted of (0.1g K_2SO_4, 0.2 g $MgCl_2$, 0.3g $Ca(NO_3)2$, and 0.1g H_3BO_3 as well as vitamin b1, b6 and b12 at 200, 50, and 1.0 mg/l, respectively) and **Murashige and Skoog (MS) (1962)** medium were prepared *in vitro* from (1650 mg NH_4NO_3, 1900 mg KNO_3, 440 mg $CaCl_2.2H_2O$, 370 mg $MgSO_4.7H_2O$, 170 mg KH_2PO_4, 22.30 mg $MnSO_4.4H_2O$, 8.60 mg $ZnSO_4.4H_2O$, 6.20 mg H_3BO_3, 0.83 mg KI, 0.25 mg $NaMoO_4.2H_2O$, 0.025 mg $CuSO_4.5H_2Om$, 0.025 mg $CoCl_2.6H_2O$, 37.25 mg Na_2EDTA, 27.85 mg $FeSO_4.7H_2O$, 0.5 mg Nicotinic acid, 0.5 mg Pyridoxine-HCl, 0.1 mg Thiamine-HCl, 100.0 mg Myo-inositol and 2.0 mg/l Glycine). Sucrose was added to both medium with concentration of 12 %.The pH was optimized to 5.7 for date palm pollen germination **(Al-Helal, 1989).** Then, the prepared media were autoclaved for 20 min at 121 °C and pressure of 1.5 bars for sterilization. To perform pollen germination assays and to track the

development of pollen tube growth, 5 cm diameter Petri dishes were used, in which 10 ml sterile stimulating solution of either BK or MS medium was placed in each Petri dish. Then a small amount (about 0.001g) of pollen grains which were previously collected was dusted by a camel hairbrush onto the medium in each petri dish. Then they were incubated at a temperature of 25-27°C for periods of 3, 6, 12 and 24 hours. After incubation, samples of 0.2 ml were transferred from pollen suspension of each incubation period separately to a slide and examined under a light microscope for determining pollen grain germination percentage and pollen grain tube length was measured by the micrometric slide **Fig. 1** which contains a gradient of 1 millimeter in length, and the gradient is divided into 100 segments, each segment 10 micrometers. Such step was repeated 5 times and the average of the obtained results was calculated. The emerging of the pollen grain tube was observed in 4 microscopic fields of view by two magnification power 100x as evidence of pollen grain germination according to **Shaheen (2004).**The percentage of pollen grains germination was calculated as follow:

Pollen grains germination % =(Number of germinated pollens)/(Total number of pollens grains) ×100

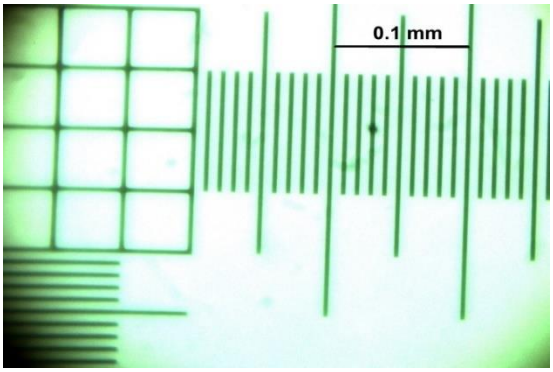


Figure 1: Microscopic slide (100X)

The procedure of pollination techniques

Eight female inflorescences on each palm were lapelled and subjected to one of the following pollination processes. Pollination process was done through two steps, the first one is spraying 10 ml of BK or MS stimulating solution on each chosen female inflorescence, just after cracking spathe, then with a manual duster, a mixture of pollen grains and flour (1:9 v/v) was twice dusted on the same female inflorescence. The Pollination process was carried out at 10-12 am. The female trees were pollinated at three times (1 week in between) starting from the last week of March. All tested spathes were bagged by a large paper bag and remained covered after pollination for three weeks to protect it against the strange pollen grains.

The pollination treatments in the field were accomplished as follows:

T1= Dusting a pollen grains mixture (PGM) on Hayany (control)

T2= Spraying BK stimulating solution + dusting PGM on Hayany

T3= Spraying MS stimulating solution + dusting PGM on Hayany

T4= Dusting a pollen grains mixture (PGM) on Orabi (control)

T5 = Spraying BK stimulating solution + dusting PGM on Orabi

T6= Spraying MS stimulating solution + dusting PGM on Orabi

T7= Dusting a pollen grains mixture (PGM) on Zaghlol (control)

T8= Spraying BK stimulating solution + dusting PGM on Zaghlol

T9= Spraying MS stimulating solution + dusting PGM on Zaghlol

Anatomical studies of the female flowers

Seventy-two hours after pollination, 3 strands of female flowers were taken from each treatment, and female flowers were carefully obtained. Whole female flowers samples were killed and fixed for at least 48 hr. in FAA solution (10 ml formalin, 5 ml glacial acetic, 35 ml distilled water and 50 ml ethyl alcohol 95%) and used for anatomical study of longitudinal and cross sections in date palm female flowers according to **Nassar and El-Sahhar (1998)**.

Fruit set, total drop percentages and yield/palm measurement

Twenty strands were labelled in each replicate (one palm). On the marked strands, number of true setting fruits (TSF), number of abnormal setting fruits (AF) and number of flower scars (FS) were counted after 3 weeks from pollination **(El-Dengawy, 2017)**. The fruits setting % was calculated by the following equation:

Fruits setting %=(No. of TSF after 3 weeks from pollination)/(Total number of TSF+ AF+ FS)×100

Total drop percentage was calculated from the following equation:

Total drop % =(No. of floral scars-(No. of seedless fruit after 2 months+ No. of retained fruits at harvest/strand))/(No. of floral scars/strand)×100

At the harvest time which was in September, October and November for Zaghlol, Hayany and Orabi cvs. respectively, 3 bunches of each palm

were picked, weighed then the average weight of bunch multiplied by the number of bunches in each replicate (8) to obtain the yield/ palm (kg).

Fruit physical and chemical characteristics

Samples of 3 kg ripe fruits were collected from the marked strands from each treatment (1 kg/replicate) at the end of the khalal stage. The collected fruits were cleaned with paper towels and kept in polyethylene bags under ice-cooled conditions in a container and transferred to the Horticulture laboratory at the Faculty of Agriculture, Damietta University. Where the fruit physical and chemical characteristics were measured. **e.g.** fruit weight (g), fruit length and diameter (cm), fruit size (cm^3), total sugars content was analyzed in flesh **(Hedge and Hofreiter, 1962)** and the results were computed on a dry weight basis. Total Soluble Solids (TSS%) was determined in fruit juice directly extracted from date fruits with a hand Refractometer according to **A.O.A.C. (2014)**. Anthocyanin was determined in fruit peel according to the method of **Rabino *et al.*, (1977)**, Taninns content was extracted in flesh and determined according to the method of **Schanderl, (1970).**

Increase/ decrease comparing to control % (I/C or D/C %): in several measures was calculated using the following formula:

I/C or D/C% =(control average value-treatment average value)/(control average value)×100

Statistical Analysis

Twenty seven date palm trees were used in this study and three replications with one tree per each of the nine treatments were used. To analyze the data, a randomized complete block design was employed. **Duncan's, (1965)** multiple range tests were used for determining the statistical significance of the means ($p < 0.05$). **SPSS** software was used for the data analysis.

Result and Discussion

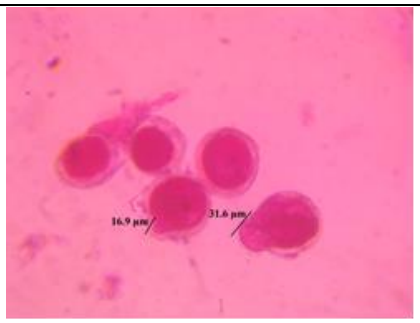
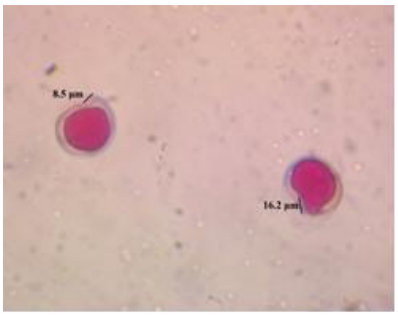
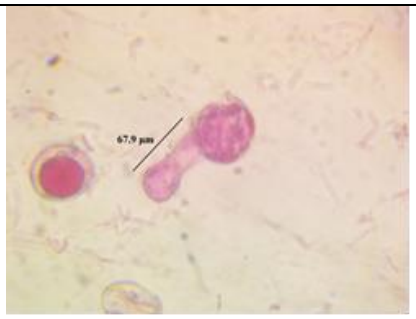
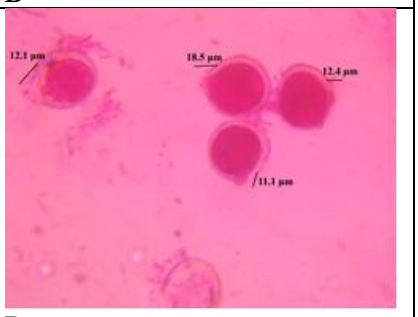
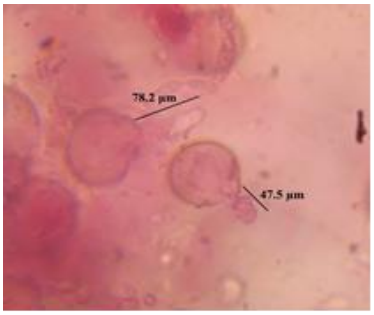
1. Pollen germination percentage and the length of pollen tube

The results in **Table 1** showed that BK stimulating solution gave the highest germination percent (92.1%) followed by MS stimulating solution which gave 89.4% in 24 hr. incubation period and the difference between them was not significant. While the lowest germination percentage 56.6% was recorded with using MS stimulating solution 3 hr. after incubation. On the other hand, the data of the average length of pollen tube in (µm) showed that the length of PG tube was increased with longer period of incubation in both stimulating solution as shown in **Fig 2**. The highest average length of pollen tube was found with stimulating solution BK and it reached to 294.64 µm 24 hr. after incubation period as appeared in **Fig. G.** These results are in the line with those obtained by **Lin *et al.*, (2017)** where they found that Murashige and Skoog (MS) and sucrose (0.05% MS + 0.01 g/mL sucrose) played a catalytic role in palm pollen germination and pollen tube growth. **Rosell *et al.*, (1999)** indicated that pollen needs to be pre-hydrated before it can germinate in a test tube, and that sucrose at a concentration of 5–10%, adequate boron, and calcium are required for this to happen. These results were proved by **Kavand *et al.*, (2014)** that germination and the development of pollen tubes depend on the presence of boron and calcium in the media. **Wang *et al.*, (2003)** also showed that pollen germination was three times higher in boron-efficient medium than it was in boron-deficient medium. The involvement of boron in numerous physiological processes, including as cell wall construction and maintenance, sugar transport, membrane integrity, and RNA metabolism, may be the cause of boron's regulatory beneficial effect in pollen germination and pollen tube growth **(Camacho-Cristobal *et al.*, 2008 and Acar *et al.*, 2010).** Moreover, pollen germination and pollen tube growth are also regulated by transferring inorganic Ca_2^+ and K^+ ions across the plasma membrane **(Taylor and Hepler, 1997).** The addition of sucrose enhances pollen tube growth much more effectively as proven by **Okusaka and Hiratsuka, (2009).** According to their findings, Japanese pears' pollen did not germinate effectively on agar medium containing fructose, but did so on media with 10% sucrose or glucose. Our results were in the line with **Fei and Nelson, (2003)** they stated that supplementation of sucrose in a culture medium is vital because it serves as a main nutrient for the germination of the pollen and the formation pollen tube's cell walls. Likewise, sucrose can serve as a carbon-based energy reserve that is required for active metabolism and membrane transfer during germination. Along with preserving the osmotic pressure of the surrounding environment **(Huang *et al.*, 2004 and Youmbi *et al.*, 2015).** Other factors that can significantly contribute towards optimum germination and growth of pollen tube, includes ideal time and temperature of incubation **(Yao *et al.*, 2005)** and that agreed with our finding as by increasing the incubation period, the length of pollen tube was increased. **Melagarejo *et al.*, (2000)** reported that the germination percentage was slightly higher at 27° C.

Table 1: Effect of stimulating solution (BK, MS) on pollen germination percentage and the average length of pollen tube (based on micrometer) in different incubation times

Treatments	Average Pollen germination (%)		The average length of pollen tube (µm)	
	BK	MS	BK	MS
3 hrs.	75.2 bc	56.6 d	20.21 e	9.37 ef
6 hrs.	81.8 b	70.5 c	56.88 d	19.29 e
12 hrs.	87.5 ab	77.9 b	142.26 c	75.98 d
24 hrs.	92.1 a	89.4 a	294.64 a	257.67 b

Values within each column followed with the same letter are not significantly different at 5% level

Treatments	BK medium	MS medium
3 hrs.	 **A**	 **B**
6 hrs.	 **C**	 **D**
12 hrs.	 **E**	 **F**

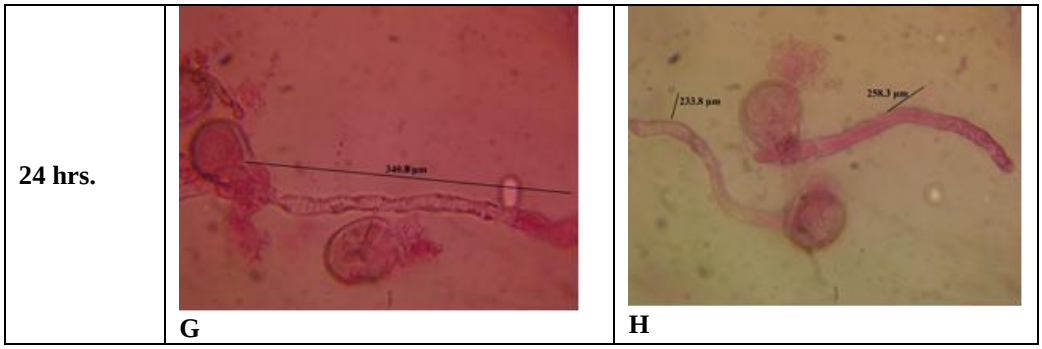


Figure 2: The development of pollen tube length under the effect of BK and MS medium and different incubation periods

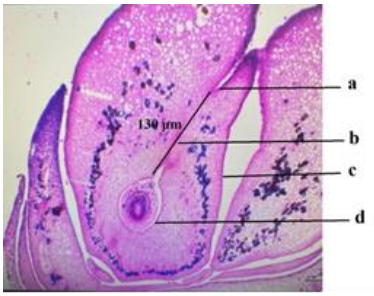
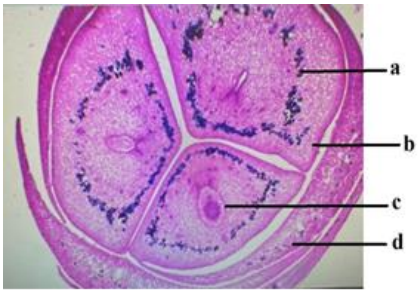
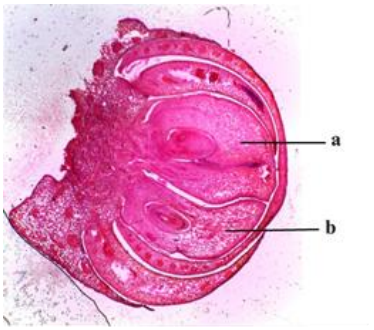
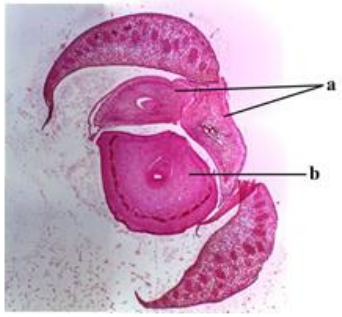
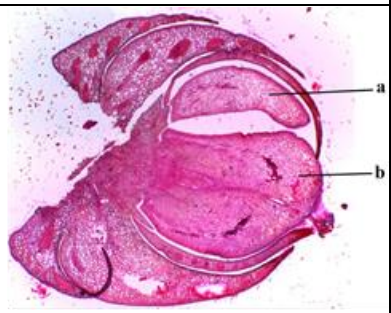
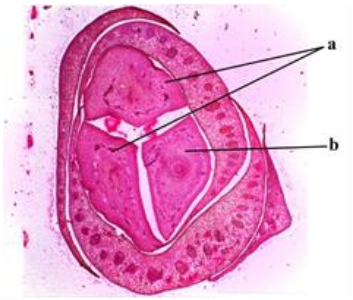
2. Anatomical studies of the female flowers

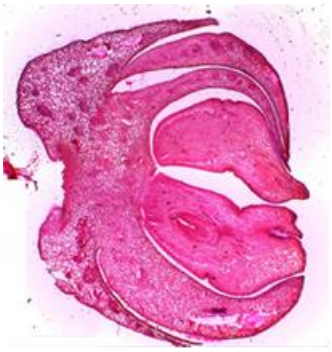
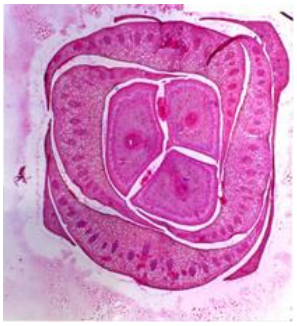
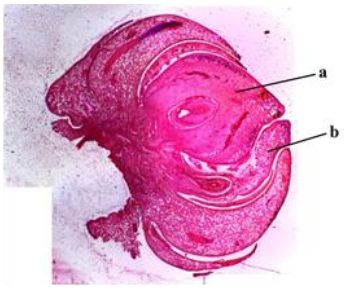
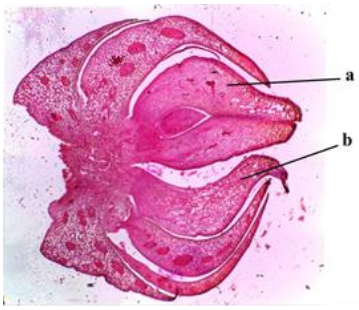
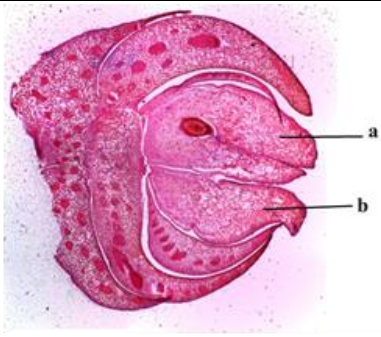
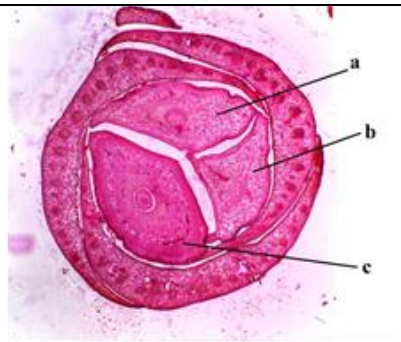
The results of anatomical sections shown in the **Table 2** can be illustrated as following:

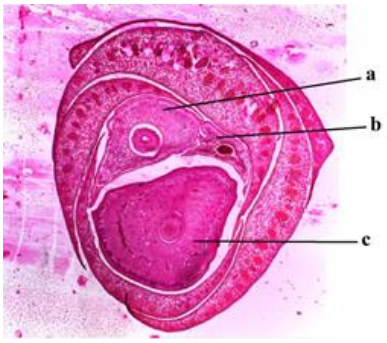
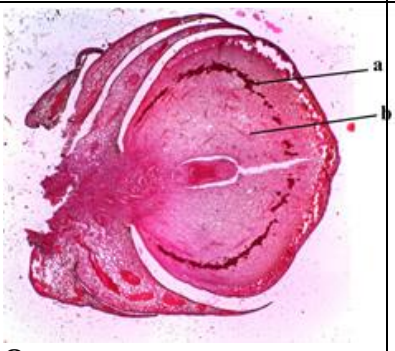
For control treatment in Hayany date palm cultivar (T_1) In **Fig. (A)** the longitudinal section of the normal flower showed. a) Stigma. b) Style (duct of pollen tube). c) Carpel. d) Ovary. While cross section in **Fig. (B)** showed a) Tannin cells. b) Carpel. c) Ovule. d) Petals. While the anatomical dissections for (T_2) in Hayany date palm cultivar **Fig. (C)** showed the longitudinal section with a) Developed carpel after fertilization and enlarged in size. b) Unfertilized carpel and in **Fig. (D)** showed the cross section with a) Unfertilized 2 carpels. b) Fertilized and enlarged carpel with condensation of tannin cells around embryo sac. For (T_3) which involve the Hayany date palm cultivar treated with MS stimulating solution before dusting **Fig. (E)** showed the longitudinal section of flower with. a) Unfertilized and decreased in size carpel and absence of ovule. b) Fertilized carpel with condensation of tannin cells around embryo sac. In the other hand the cross section of the flower in **Fig. (F)** showed a) unfertilized carpel with absence of ovule. b) Fertilized carpel with enlarged size and clear embryo sac and tannin cells. For anatomical sections of Orabi date palm cultivar flowers, **Fig. (G)** showed the longitudinal section of flower after 72 hr. of control treatment (T_4). While the cross section with three normal carpels with no developmental changes appeared in **Fig. (H)**. The flowers after 72 hr. of spraying BK stimulating solution and dusting with pollen grain mixture in Orabi cultivar (T_5) showed in longitudinal section the **Fig. (I)** a) fertilized carpel with embryo sac inside surrounded with tannin cells. b) Unfertilized carpel and began to be atrophied. In cross section in **Fig. (J)** showed the 3 carpels appeared. a) the developed and fertilized carpel. b) Two atrophied and unfertilized carpels. The longitudinal section of Orabi date palm flower after 72 hr. spraying with MS solution then dusting pollination (T_6) showing in **Fig. (K)** a) Fertilized carpel with clear duct of pollen tube. b) Smaller unfertilized carpel. Also in **Fig. (L)** cross section there were a) two normal carpals with ovules inside and tannin cells around. b) Carpal with absence of ovule. For anatomical dissections of control treatment of Zaghlol date palm cultivar flower (T_7), the longitudinal section in **Fig. (M)** showed a) Fertilized carpel with embryo sac inside and appearance of duct of pollen tube. b) Smaller carpel with atrophy of ovule. in **Fig. (N)** showed the cross section showed 3 carpels. a) Small carpel with no tannin cells. b) Atrophied and undeveloped carpel with no ovule. c) Developed and fertilized and bigger in size carpel with embryo sac and tannin cells in comparison with the two other carpels. In Zaghlol date palm flowers sprayed with BK solution and dusted with pollen grain mixture **(T_8),** the longitudinal section in **Fig. (O)** showed a) Smaller undeveloped carpel. b) Developed and bigger in size carpel with embryo sac. While in cross section appeared in **Fig. (P)** showed 3 carpels. a) Unfertilized carpel with ovule inside. b) Atrophied carpel. c) Fertilized and developed carpel with condensed tannin cells and embryo sac inside. The longitudinal section of Zaghlol date palm flower after 72 hrs. of spraying MS solution then dusting (T_9) in **Fig. (Q)** showing 1 carpel. a) Condensed tannin cells. b) Enlarged carpel with embryo sac and duct of pollen tube and the cross section in **Fig. (R)** showing 3 carpels. a) Developed and fertilized carpel with embryo sac. b) Atrophied carpel. c) Normal unfertilized carpel with ovule inside. The date palm flower consists of three carpels. Every carpel consists of an ovary chamber and inside it an ovule. After one day of pollination, It is noticed that there were an increase in the length and width of ovary and the ovule. This may be due to presence of pollen grain hormones or increase in cells divisions **(Abbas *et al.*, 1995). Wassan *et al*, (2015)** reported that fertilization occurred after two days of pollination in the date palm female flowers pollinated with the green Ghanamy pollen grain

cultivar. Although **Castanol, *et al.*, (2014)** mentioned that fertilization occurred after 36 hours of pollination in the flowers of Al-Halawi Date palm cultivar. The difference may be due to the date of fertilization or the effect of the male cultivar, which may be related to genetic reasons or presence of difference in hormonal content in pollen, and its role in stimulating the ovarian wall to produce necessary hormones **(Abbas *et al.*, 2012).** Also, **Damankeshan and Panahi, (2013)** agreed with our findings because they looked at the very early phases of date palm fruit development. They discovered that the fertilized carpel grows and develops more quickly than the other two carpels, and that it began to grow in size at the end of the third week following pollination.

Table 2: Effect of different treatments on the anatomical development of the date palm flowers of Hayany, Orabi and Zaghlol cultivars

Treatment		Longitudinal section	Cross section
T1	**Dusting PGM on Hayany (con.)**	 **A**	 **B**
T2	**Spraying BK stimulating solution + dusting PGM on Hayany**	 **C**	 **D**
T3	**Spraying MS stimulating solution + dusting PGM on Hayany**	 **E**	 **F**

T4	**Dusting a pollen grains mixture (PGM) on Orabi (con.)**	 **G**	 **H**
T5	**Spraying BK stimulating solution + dusting PGM on Orabi**	 **I**	 **J**
T6	**Spraying MS stimulating solution + dusting PGM on orabi**	 **K**	 **L**
T7	**Dusting a pollen grains mixture (PGM) on Zaghlol (con.)**	 **M**	 **N**

T8	**Spraying BK stimulating solution + dusting PGM on Zaghlol**	 **O**	 **P**
T9	**Spraying MS stimulating solution + dusting PGM on Zaghlol**	 **Q**	 **R**

3. Fruit set and fruit drop percentages and yield/palm

Results in **Table 3** indicated that spraying stimulating solution BK in Orabi cultivar (T5) produced the highest fruit set percentage (76.4%) and yield/palm (145.4 kg) while, spraying BK in Zaghlol cultivar (T_8) exhibited the highest values than other treatments on fruit set and yield as increases over control % (114.4, 48.5 percentages, respectively). While, the lowest fruit set (29.5, 26.9%) were coupled with control treatments of Zaghlol and Hayany cultivars (T_7, T_1) respectively. Consequently, the lowest fruit set over control % (21.3) was found on spraying Orabi cultivar with MS stimulating solution (T_6). The lowest yield per palm was obtained with control treatment of Zaghlol cultivar (T_7) (84.2 kg) and the increase over control percentage of yield /palm was found when applying MS on Orabi cultivar (T_6). The total drop percentage was decreased with application of BK stimulating solution in Orabi cultivar (T_5) 45.3%, it also gave the lowest value when compared to control (- 27.5). The highest value of total drop % was obtained from control treatment of Hayany cultivar (T_1) (74.9 %). On the other hand, total drop percentage in Hayany cultivar sprayed with MS (T_3) decreased than control with (- 5.6 %). These results were strengthened by that found by Soliman and Al-Obeed, (2011). They mentioned that the application of spray pollination and spray liquid sugar increased fruit retained on different cultivars of date palm. It also regulates tree bearing without thinning process. The pollen grains did not need to release from male strands in the case of the strands placement method or need a mode of pollen dispersion (wind), which minimizes the fruit set time when the female flower bud is mature to receive pollen, which may account for the higher percentage of fruit set by dusting and pollen suspension methods (Attalla et al., 1998). Also, our results agreed with that reported by El- Salhy et al., (2007). Adding sucrose solution to stimulating solution BK and MS sprayed before dusting pollen grains helped in improving fruit set percentage as it led to increasing moisture absorption around the flower, then the pollen grains can be easily directed towards the flowers and drift is minimized. Boron also encouraged the germination and growth of pollen grains as well as movement of sugars and fungi control. Alwusaibai et al., (2012) indicated that hand application of pollens resulted in the highest fruit set and the lowest overall drop percentage. However, when dusting pollination technique was applied, yield per bunch was considerably higher, which was consistent with our findings when employing hand dusting technique. That result disagrees with El-Kassas and Mahmoud, (1986).

Table 3: Effect of spraying flowers before pollination with two stimulators on fruit set %, fruit drop % and yield/ palm in three date palm cultivars (average values of 2019, 2020 and 2021 seasons)

Treatments			Fruit Set %		Total drop %		Yield/ Palm	
NO.	Cultivars	Stimulators	Percentage	Over Control %	Percentage	+/- Comparing to Control %	Kg	Over Control %
T_1	Hayany	Control	26.9 e	-----	74.9 a	----	98.2 g	-----
T_2	Hayany	BK	50.1 c	86.24	64.2 c	- 14.2	110.6 e	12.5
T_3	Hayany	MS	36.7 d	36.1	70.6 b	- 5.6	103.6 f	5.4
T_4	Orabi	Control	53.2 c	-----	62.4 d	----	123.1 d	-----
T_5	Orabi	BK	76.4 a	43.6	45.3 f	- 27.5	145.4 a	18.1
T_6	Orabi	MS	64.5 b	21.3	56.1 e	- 10.1	127.1 b	3.3
T_7	Zaghlol	Control	29.5 e	-----	71.7 b	----	84.2 h	-----
T_8	Zaghlol	BK	63.3 b	114.4	56.3 e	- 21.5	125.1 c	48.5
T_9	Zaghlol	MS	45.6 c	54.6	65.2 c	- 9.0	98.8 g	17.4

Values within each column followed with the same letter are not significantly different at 5% level

Control = Traditional pollination, BK= Brewbaker and Kwack's medium with some modifications, MS= Murashige and Skoog medium

They did not observe any effect of mechanical and manual pollination methods on fruit qualities of Dates. (Albert, 1930 and Nasr et al., 1988) also found that for maximum fruit set, pollination should not be delayed longer than 3-4 days. However, flower remains receptive for 24 hr. only and its receptivity decreases with time but not later than 120 hr. **Iqbal *et al*., (2010)** demonstrated that the fruit setting and other quality factors had been greatly impacted by various pollination procedures. Concerning fruit set percentage, **El-Mardi *et al*., (2007)** mentioned that the amount of pollen grains used had a great effect on producing fruits of good quality after pollination. This may be attributed to effectively pollen powder reaches the female flowers using either of the methods. It is a likely that the male inflorescence strands remain in the female bunch for longer time in manually pollinated strands and therefore increases the chances of pollination for longer duration. Similarly, the findings were reported by **Khan and Ghafoor, (1993)** demonstrated using the placement approach to pollinate the cv. Dhakki date led to the maximum fruit set and documented the fruit set with the lowest yield in control treatment. Pollination time is also considered one of the factors that improve fruit setting%. There was an increase in the percentage of fruit set by earlier pollination in our results. This may be explained as the pollen grain tube can easily germinate and elongate to penetrate the stigma and style of the female flower. The moderate temperature is conjunction with more humidity in such period that resulting in better fertilization and fruit set **(Shaheen, 1986)**. Many fruiting plants face a major economic danger from fruit drop, and under varied pressures, approximately 50% of the flowers and immature fruits dropped during growth **(Iqbal *et al*., 2009)**. Major factors that contribute to fruit drop include genetic, physiological, and environmental events. **(Robinson *et al*., 2010 and Zhu *et al*., 2010).** Numerous factors, such as drought, hot weather, and nutrient shortages or imbalances, can cause excessive fruit drop, impair fruit quality, and eventually lower yield **(Racskó *et al*., 2007)**. One of the most significant physiological elements that contribute to this cultivar's increased fruit drop is nutritional deficit or inequality. According to our results using stimulating solution BK and MS before pollination was found to decrease total drop %. That result agreed with those found by **Elsabagh, (2012)**. The use of K_2SO_4 at 2% with or without urea resulted in improved fruit size and weight, lower fruit drop, greater weight/bunch over both years, and increased fruit retention. Also, K_2SO_4 according to **Yadav *et al*., (2014)** resulted in dates' fruit length and diameter (mm) increasing significantly. Fruit number, size, weight, and other relevant variables all work together to determine yield **(Al- Khateeb *et al*., 2001).** Also, **Jat and Laxmidas, (2014)** mentioned that K helped in increasing the average weight/ bunch and that were in the line with our results as shown in **Table 3** noticed increase in the yield/palm. Also, their combined activity accelerated photosynthesis and metabolic processes, increasing fruit size and weight and fruit yield.

Potassium (K) found in both two stimulating solution BK, MS complements reducing sugars in a way that causes the turgor potential necessary for cell expansion as reported by **(Guardia and Benlloch, 1980).**

4. Physical characteristics of fruit quality

The results in **Table 4** concerning with the effect of spraying BK and MS stimulating solution on improving the fruit physical characteristics showed that fruit weight, fruit length, fruit diameter and fruit size reached to the highest value (27.6 g, 7.1cm, 2.8cm and 30.4 cm^3), respectively with spraying BK on Zaghlol cultivar (T8). Also, this treatment (T_8) scored the superior rank for fruit weight, fruit diameter and fruit size when comparing to control (16.7 %, 31.3% and 33.1%), respectively.

On contrary, the lowest values of fruit weight, fruit length, fruit diameter and fruit size were related with control treatment of Orabi cultivar (T_4) their values were (17.9 g, 3.4 cm, 2.2 cm and 17.8 cm^3), respectively. While the lowest values comparing to control were obtained from spraying Hayany cultivar with MS stimulating solution (T_3) (2.8%, 10.2%, 5.3% and 11.3%), respectively. Increasing fruit physical characteristics may be attributed to the improvement of fruit growth and uptake of nutrients that accelerate metabolic processes. Similar finding was reported by **(Khayyat *et al.*, 2007 and Desouky *et al.*, 2007)** on Shahany and Barhee date palm cultivars. Characteristics like fruit length, weight and flesh thickness, seed weight, seed length and diameter are of importance in differentiation between the cultivars **(Abdullah and Salah, 1999)**. Zinc is used to increase the amount of fruits. Additionally, applying Zn improves citrus fruit productivity and fruit quality metrics **(Yasin *et al.*, 2012).** That results agreed with ours concerning fruit quality characteristics. There was an increase in fruit weight, fruit length and fruit size in **Table 4** with application of stimulating solution BK. These results could be attributed to its contents of B, Ca, K and vitamin B complex that increase cell division and elongation rate. These increases are required to provide the optimal growth distances for setting fruits, which will reduce fruit falling and enhance fruit quality. Nearly every aspect of plant development involves the use of calcium, including the maintenance of membrane integrity and cell wall formation **(Harper *et al.*, 2004).** It also helped in determining the quality of fruit **(Rizzi and Abruzzese, 1990).**

Table 4: Effect of spraying flowers before pollination with two stimulators on fruit weight, fruit length, fruit diameter and fruit size of three date palm cultivars during 2019, 2020 and 2021 seasons

Treatments			Fruit Weight (g)		Fruit Length (cm)		Fruit Diameter (cm)		Fruit Size (cm^3)	
NO.	Cultivars	Stimulators	Average	Over control %	Average	Over control %	Average	Over control %	Average	Over control %
T_1	Hayany	Control	20.9 d	----	4.9 cd	----	2.4 c	----	21.1 e	----
T_2	Hayany	BK	22.9 c	9.6	5.8 b	19.9	2.8 ab	14.7	25.3 c	20.1
T_3	Hayany	MS	21.5 d	2.8	5.4 bc	10.2	2.6 bc	5.3	23.5 d	11.3
T_4	Orabi	Control	17.9 e	----	3.4 f	----	2.2 d	----	17.8 f	----
T_5	Orabi	BK	20.8 d	16.1	4.4 de	30.2	2.6 bc	15.5	23.3 d	30.7
T_6	Orabi	MS	19.9 d	11.3	3.8 ef	12.9	2.5 c	10.2	21.1 e	18.3
T_7	Zaghlol	Control	23.6 c	----	5.6 bc	----	2.2 d	----	22.8 d	----
T_8	Zaghlol	BK	27.6 a	16.7	7.1 a	26.6	2.8 a	31.3	30.4 a	33.1
T_9	Zaghlol	MS	24.9 b	5.6	6.2 b	10.3	2.7 ab	25.3	27.2 b	19.5

Values within each column followed with the same letter are not significantly different at 5% level

Control = Traditional pollination, BK= Brewbaker and Kwack's medium with some modifications, MS= Murashige and Skoog medium

The activation of photosynthesis, synthesis of total carbohydrates, and protein synthesis by nutrients such as potassium, calcium, and a moderate amount of nitrogen and phosphorus found in stimulating solution was found to enhance cell division and enlargement, increasing fruit weight and size **(Gomezy and Rodriguez, 2000; Sajid *et al.*, 2014).** The presence of $CaCl_2$ in the MS stimulating solution has been demonstrated to delay physiological processes like respiration, climacteric onset, fruit ripening, and senescence, which increases fruit weight as reported by **Hussain *et al.*, (2012).**

5. Chemical characteristics of fruit quality

The results in **Table 5** showed that the highest values of total sugars % in flesh, TSS % in juice and anthocyanin in fruit peel were obtained from spraying Zaghlol cultivar with BK solution (T_8) which gave 31.4%, 34.9% and 32.5mg/100gm, respectively. Moreover, the same treatment also gave the lowest value of tannins content (162.4 mg/100g), consequently. Also Spraying BK stimulating solution + dusting PGM on Zaghlol (T_8)

produced the highest results of TSS% and anthocyanin increase when compared to control, as it gave 11.3% and 23.2mg/100g, respectively. and the lowest value of tannins content (- 14.9%) decreases than control. The lowest value of total sugars (18.6%) was obtained with control treatment of Orabi cultivar (T4) where the lowest one when compared to the control was obtained from Orabi cultivar sprayed with MS solution (T_6). While the lowest values of TSS and anthocyanin were recorded with control treatment of Hayany cultivar. However, in the same **Table 5** it appeared that, the highest values of tannins content were found on Orabi cultivar in control treatment (T_4) and spraying with MS solution (T_6) and their values were (229.9 and 227.5 mg/100g Fw), respectively.Date fruit quality is thought to be significantly influenced by the quantity of reducing sugars present in the fruit. In the majority of date palms, glucose and fructose are the reducing sugar types that are most prevalent (**Kulkarni *et al.*, 2008).** All date cultivars see a rise in sugar content as the fruit develops, peaking at fruit maturity (**Hasnaoui *et al.*, 2011).** Our results showed noticed increase in the total sugars % and TSS% with applying BK stimulating solution, that may be due to presence of K that plays an important role in improving chemical constituents in fruits as it is involved in the transport of sugars to other parts of the plant resulting in improved fruit quality and increase TSS% as reported by **Ashour *et al.*, (2004).** On date palm, all boron spraying treatments improved physical and chemical characteristics **(Attalla *et al.*, 2007).** In addition to playing a crucial part in the synthesis of starch and glucose, phosphorus was also shown to be involved in the production of the UTP molecule, which is required for the synthesis of sucrose and glucose **(Awad, 2006).** It was also claimed that fructose, one of the sugars in the fruit, contains phosphorus. For the growth and fruiting of date palm trees, where dates accumulate with a high sugar content of between 44 and 88% depending on the cultivar, stage of maturity, and environmental circumstances, potassium is a crucial and vital element **(Al-Shahib and Marshall, 2003; Awad *et al.*, 2011).** Potassium is a crucial ingredient for proteins, lipids, carbohydrates, and chlorophyll to form and function properly, as well as to keep the balance of salts and water in plant cells, **(Marschner, 1995).** Numerous physiological processes that occur in plants, such as maintaining cell structure, hydration, and permeability, are also activated by it. It stimulates numerous enzyme systems involved in the production of proteins and carbohydrates **(Abdel-Rahman, 2010).** The role of K in converting complex compounds into simple ones to speed up the metabolic activity of fruit may be the cause of the increased total sugars **(Osman, 2010; Jat and Laxmidas, 2014 and Khan, 2015).** Also**, Hansch and Mendel, (2009)** discovered that boron plays a key role in several activities, particularly the transfer of sugars and the metabolism of carbohydrates. Our results agreed with that as BK contains 0.1g H_3BO_3 and MS contains 6.20 mg H_3BO_3. When K is used in any way, the TSS of date palm cultivars is substantially enhanced according to research of **Al-Obeed *et al.*, (2013).** These results were in the line of ours as stimulating mediums BK and MS contain potassium, boron, phosphorus. The breakdown of polysaccharides and concentrated juice content brought on by dryness with the passage of storage time is likely what caused the increase in TSS **(Akhtar *et al.*, 2010).** Carotenoids, which include polyphenols, anthocyanins, and flavonoids, are low-molecular-weight antioxidant substances that effectively combat free radicals to shield intracellular components from their harmful effects **(Basu *et al.*, 2010**). Ripened dates carotenoids content gradually reduced, while anthocyanins were only present in unripe dates (**Hong, *et al.*, 2006)**. Due to the high concentrations of chlorophyll a and b present during the kimri stage of date palm growth, carotenoids did not have the opportunity to appear. Our results were parallel to that found by **Boudries *et al.*, (2007**). The antioxidant activity of the date fruits had a favorable connection with total phenolic compounds (TPC) levels from khalal through tamar stages and decreased with fruit ripeness **(Al-Turki *et al.*, 2010 and Awad *et al.*, 2011a).** TPC's decline is brought on by polyphenol oxidase oxidizing it **(Amiot *et al.*, 1995)** and decline in tannins as the matured dates **(Myhara *et al.*, 1999)**.The tannins content decreased in harvested fruits as shown in our results and gave the lowest value with using BK stimulating solution. These results proved by **Hammouda *et al.*, (2013).** The utility of date fruits throughout the khalal or later phases of fruit growth is significantly influenced by changes in tannin compounds that occur during the development of date fruits. From the kimri to the khalal, rutab, and tamar phases, the amount of fruit tannin reduced. Due to their hydroxyl groups, tannins can form reversible interactions with polysaccharides, proteins, and alkaloids. The balance between soluble and insoluble tannins is crucial in deciding whether date fruit is edible since dates with high tannin concentrations may not be as tasty (**Al-Redhaiman, 2004).** This might be brought on by the enzymatic breakdown of chlorophyll as the fruit ripens.

Table 5: Effect of spraying flowers before pollination with two stimulators on Total sugars %, TSS %, Anthocyanin and Taninns content in three dates palm cultivars (average values of 2019, 2020 and 2021 seasons)

Treatments			Total sugars % in flesh		TSS % in Juice		Anthocyanin (mg/100g) in fruit peel		Taninns content (mg/100g Fw) in flesh	
NO.	Cultivars	Stimulators	Average	Over control %	Average	Over control %	Average	Over control %	Average	+/- Comparing to Control %
T_1	Hayany	Control	22.6 d	----	29.1 d	----	24.3 ef	----	218.7 b	----
T_2	Hayany	BK	30.2 ab	33.5	32.4 bc	11.4	29.2 bc	19.9	205.3 c	- 6.1
T_3	Hayany	MS	26.3 c	15.9	30.8 c	5.9	25.7 de	5.8	214.4 b	- 1.9
T_4	Orabi	Control	18.6 e	----	31.4 c	----	25.1 de	----	229.9 a	----
T_5	Orabi	BK	21.9 d	17.8	33.6 ab	6.9	30.6 b	21.7	215.4 b	- 6.3
T_6	Orabi	MS	19.7 e	5.8	31.5 c	0.4	27.2 cd	8.1	227.5 a	- 1.1
T_7	Zaghlol	Control	25.2 c	----	31.3 c	----	26.4 de	----	190.8 c	----
T_8	Zaghlol	BK	31.4 a	24.6	34.9 a	11.3	32.5 a	23.2	162.4 e	- 14.9
T_9	Zaghlol	MS	28.8 b	14.3	32.1 bc	2.4	28.2 cd	9.8	175.9 d	- 7.8

Values within each column followed with the same letter are not significantly different at 5% level

Control = Traditional pollination, BK= Brewbaker and Kwack's medium with some modifications, MS= Murashige and Skoog medium

CONCULUSION: Considering the results, it could be concluded that applying BK and MS stimulating solutions on female flowers and dusting (PGM) at % for Hayany, Orabi and Zaghlol date palm cultivars stimulates and accelerates germination pollen grain of date palm. Thence, it improved the fruit set percentage, decreased fruit drop and consequently increased yield/date palm. Moreover, these pollination techniques succeeded to increasing the physical and chemical characteristics values of fruit qualities as weight, size, and contents of total sugars, TSS and anthocyanins as well as reducing acidity and total tannins percentage. Generally, it could be recommended spraying BK and MS stimulating solutions and dusting 10% (PGM) as an improved pollination technique. It kept the required pollen grains compared with applying 10 % (PGM) only. The combination of mixing pollen grains with BK and MS stimulating solutions is useful for establishing mechanical pollination and getting an economical yield with good fruit quality.

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AUTHOR CONTRIBUTIONS

El-Dengawy, E. F. A.; Eliwa, G. I. and Gawish, M. S suggested, established and designed the research idea. Gawish, M. S and Attia, A. A Methodology, sample collection, and lab analysis, El-Dengawy, E. F and Attia, A. A writing the original draft preparation, El-Dengawy, E. F. A.; Eliwa, G. I. and Gawish review and editing final manuscript. All authors have read and agreed to the published version of the manuscript.

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الملخص العربى

تأثير الرش بالمحاليل المحفزة على إنبات حبوب اللقاح وعقد الثمار والتطور التشريحي للزهرة والمحصول وجودة الثمار في أصناف نخيل البلح الحياني والعرابي والزغلول

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صممت هذه الدراسة لتقييم تأثير المحاليل المحفزة Brewbaker and Kwack (BK) وMurashige and Skoog (MS) متبوعة بتقنية التلقيح بالتعفير لحبوب اللقاح المختلطة من أجل تحسين كفاءة وقدرة إنبات حبوب لقاح نخيل البلح واستطالة أنبوب اللقاح خلال قلم الزهرة على إناث نخيل البلح (*Phoenix dactylifera* L.) من أصناف الحياني والعرابي والزغلول. تم تقدير عقد وتساقط الثمار وكمية المحصول/النخلة بالإضافة إلى الخصائص الفيزيائية والكيميائية للثمار. أظهرت النتائج أن الصفات المدروسة تأثرت برش هذين المحلولين المحفزين، وكانت أعلى نسبة إنبات لحبوب اللقاح تم الحصول عليها 92.1% وذلك عند تطبيق المحلول المحفز BK لمدة 24 ساعة. أظهرت نتيجة الدراسة التشريحيه للقطاعات الطوليه والعرضيه للأزهار في المعاملات المدروسه و التي تم الحصول عليها بعد 72 ساعة من التلقيح تطور كربلة واحدة بعد الإخصاب مع تضاعف حجمها بخلاف الباقي، مع ازدياد تكثف الخلايا التانينية حول الكيس الجنيني. كما أظهرت النتائج زيادة نسبة عقد الثمار عند تطبيق المحلول المحفز BK مع صنف عرابي مقارنة بالمعاملات الأخرى، كما سجلت أقل نسبة تساقط كلي للثمار، وكذلك حققت أعلى متوسط لكمية المحصول/ نخلة. أما بالنسبة إلى وزن الثمرة، وطولها، وحجمها، وقطرها فكانت أعلى قيم لها في صنف زغلول، وذلك عند رشه بالمحلول المحفز BK. كما أن أعلى نسبة للسكريات الكلية كانت في الصنفين الزغلول والحياني عند استخدام معاملة الرش بالمحلول المحفز BK. كما حققت نفس المعاملة أعلى قيمة في نسبة المواد الصلبة الذائبة في صنفي الزغلول والعرابي. علاوة على ذلك، أظهر الرش بالمحاليل المحفزة BK أو MS زيادات كبيرة في محتوى الأنثوسيانين عند مقارنتها مع معاملة التعفير بحبوب اللقاح فقط في جميع الأصناف، من ناحية أخرى سجلت معاملة التعفير بحبوب اللقاح فقط أعلى متوسط لمحتوى المواد التانينية وذلك في صنف عرابي، بشكل عام اثبتت الدراسه أن استخدام المحاليل المحفزة (BK) أو (MS) رشًا على النورات المؤنثه لاصناف نخيل البلح التي تم دراستها أدى إلى تحسين عقد الثمار، وجودة الثمار، وتقصير زمن إنبات، وزيادة استطالة أنابيب حبوب اللقاح مما أنعكس أثره على زيادة ملحوظة في المحصول والإنتاج، مما ساعد في التغلب على الظروف البيئية الغير مواتيه أثناء التلقيح وعقد الثمار.