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STUDY OF EMBRYOGENIC DEVELOPMENT IN SOFT, DRY AND SEMI-DRY CULTIVARS OF DATE PALM (*Phoenix dactylifera* L.)

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ABSTRACT

This study was conducted to investigate the difference in embryogenic development through the anatomical study of the flowers of three cultivars of dry ('Derry'), semi-dry ('Zahdy') and soft ('Barhee') date palms, were cultivated in Basrah Governorate, Southern of Iraq, and all were pollenated with the male cultivar 'Ghanami Akdher'. Female flower samples were collected after several periods of pollination (6, 12, 24, 36, 48, 72, and 96 hours). The growth of the pollen tube from the stigma to the embryonic sac and the development of the three carpels in these flowers were traced through anatomical sections. The results showed that the female flower at the beginning of its early stages and before the fertilization process contains three ovaries in the middle of each of them a chamber dedicated to the ovule. This ovule is connected to the inner wall of the ovary by a cylindrical growth that protrudes from the placenta tissue called the funiculus. After 6 hours of pollination, some pollen tubes began to grow on the surface of the stigma, and there was no trace of the pollen tube passage channel. The 12 hours after the pollination process, the pollen tube passage channel appeared for all the female cultivars under study. The samples after 24 hours of pollination process, found that the pollen tube for all the female cultivars under study had developed in its growth to the style ovary tissue penetrated towards the embryonic sac. The cultivars differed from each other in terms of the growth and development of the pollen tube after 36 hours of pollination. The pollen tube developed and grew through the style ovary and embryonic sac to reach the ovule and caused double fertilization to occur for the 'Barhee' cultivar.48 hours after the pollination process, the results indicated that double fertilization took place in the female flowers of the 'Zahdy' cultivar. The 72 hours after pollination, fertilization was found in the female flowers of the 'Derry' cultivar, where the passage channel of the pollen tube disappeared, the fertilized egg developed, and the other two unfertilized ovules began to decay and decrease in size. The flowers of the three cultivars had completed the fertilization process before the 96 hours after

the pollination process, and the fertilized ovules had grown and developed clearly and significantly, and the unfertilized ovules continued to decay, and there was no trace of the pollen tube passage channel.

Keywords: Anatomy section; double fertilization; embryo sac; funiculus; pollination; stigma.

INTRODUCTION

The date palm is the first tree in Iraq, and it is one of the evergreen fruit trees belonging to the Arecaceae family, one of the monocotyledons. The southern Arabian Gulf is the most widespread area of palm trees in the world as it spread from it to other areas with a suitable atmosphere [1]. Dates were divided into three groups, soft, dry and semi-dry, depending on their sucrose content and the firmness of the fruits. The dates of the first group are characterized by the softness of their fruits and the high percentage of water content in them, ranging between 25-35%, including the 'Khedrawi', 'Barhee', 'Hillawi' and 'Sayer' cultivar. The second group, the percentage of water content in its dates ranges between 15-25%, and it is characterized by the fact that its fruits are firmer than the soft cultivars, including 'Zahdy', 'Khalas' and 'Maktoum'. As for the third group, their water content is less than 15%, and characterized by the firmness of the fruits at the stage of ripening, as its fruits reach the date stage without going through the rutab stage, including 'Derry' and 'Asherisi' [2]. Al-Najjar and Al-Hamad [3] conducted a comparative anatomical study of the leaves of soft, dry and semi-dry cultivars of date palms to investigate the anatomical differences of the leaves of three cultivars of date palms from dry 'Derry', semi-dry 'Zahdy' and soft 'Hillawi' developing in Basrah Governorate, Southern of Iraq. This study showed the possibility of adopting anatomical features of the leaves as a classification index to distinguish between the different cultivars of date palm as first indicators in botanical taxonomy. The chemical and physiological changes that fruits undergo during the stages of growth and development are considered one of the scientific foundations on which to explain the physiological and chemical phenomena that accompany the development and growth of the fruit, as well as depend on them to determine the date of maturation and ripening [4]. To ensure seed fruit production, pollen grains must be transferred from male flowers to female tree inflorescences. In the female flower has three compact carpels together surrounded by six stamens. Each carpel made up of the ovary chamber and an ovule and the ovary ends at the top with a style and stigma. The flower is closed before pollination, and three stigmas and a part of the style emerge from its top. When the pollination and double fertilization processes occur in the female flowers, one carpel remains in which the ovary grows and gives one fruit, and two carpels fall due to the growth of a fertilized ovary from among the three ovaries in the flower, which increases its size to five times what it was before pollination [5]. Abbas [6] showed in his anatomical study of the embryogenic development of some cultivars of date palms, that the passage channel of the pollen tube appeared 6 hours after pollination and that the fertilization process took place 48 hours after pollination. The pollination effectiveness is in the period during which pollen grains can fertilize the ovules in the female flowers of date palms, which depends on the speed of pollen tube growth in addition to the age of the ovule or the duration of its fertility [7]. Whereas Al-Najjar [8] explained that the date of fertilization has changed according to the male cultivar, some of which are the reason for fertilization to take place 36 hours, or 48 hours and some may take even 72 hours for fertilization. Many anatomical studies of date palms have been doing, but no comparison has been made between these cultivars specifically. Since there is no study on the embryogenic development of these cultivars to compare them, this anatomical study of the pollinated flowers of 'Barhee', 'Zahdy' and 'Derry' cultivars due to finding out the differences in the embryogenic development of those cultivars.

MATERIALS AND METHODS

This study was conducted to investigate the difference in embryogenic development through the anatomical study of the flowers of three cultivars of soft 'Barhee', semi-dry 'Zahdy' and dry 'Derry' date palms growing in Basrah Governorate, Southern of Iraq. The 3 palms were

selected for each cultivar, which is uniform in length, growth strength and age as much as possible. Six pistillate inflorescences left on each female palm, and all of them were pollinated with the male cultivar 'Ghanami Akdher'.

The flower anatomical sections were prepared according to the method of Khafaji [9]. Female flower samples collected after 0, 6, 12, 24, 48, 96 hours periods of pollination. The pollen tube from the stigma to the embryogenic sac and the three carpels development traced in those flowers, and a fixation process was performed in Formalin-acetic acid-alcohol (F.A.A) solution for 48 hours.

The explant samples were passed with increasing concentrations of ethyl alcohol and then the samples were embedded with paraffin wax at a temperature of 58°C. After that, these samples were cut with a rotary microtome with a thickness of 10 µm. Then the anatomical sections were put on glass slides and dyed with Safranin dye, then put in Fastgreen dye. Then the anatomical sections were covered with a few drops ofDi-butylphthalate Polystyrene Xylene(DPX)which used to preserve the anatomical sections, and covered with coverslides. Then it is assessed and measurement in the micrometer (µm) by an ocular micrometer in an Olympus optical microscope equipped with a camera connected to the computer.

The results were analyzed using the variance analysis of the anatomical structure of cultivars using the SPSS statistical program (Ver. 25). The means of treatments were compared between them according to the R-LSD at a probability level of 0.05 [10].

RESULTS AND DISCUSSION

The results of microscopic test of the fixed sequences in the flowers that pollinated with the pollen of the male 'Ghanami Akdher' cultivar that were prepared after 0, 6, 12, 24, 48 and 96 hours showed at Tables 1 to 3. The female flower at the beginning of its early stages and before the pollination process is composed of three ovaries (carpels) in the middle of each ovary dedicated to the ovule, which is connected to the inner wall of the ovary by a cylindrical growth that protrudes from the placenta tissue known as the funiculus. The ovule is semi-anatropous, meaning that the micropyle opening is towards the placenta and the nuchal end is far from the placenta. There was no trace of the pollen tube passage channel (Figs. 1, 2, and 3). The studied cultivars differed in terms of ovary length, reaching 1280.50, 1199.40 and 1183.60 µm in ‘Barhee’, ‘Zahdy’ and ‘Derry’ cultivars, respectively. The highest means of ovary width and ovule diameter for ‘Barhee’ cultivar was 1080 and 310.50 µm, respectively, while ‘Derry’ cultivar recorded the lowest means. On microscopic test of the samples 6 hours after the pollination process, large number of pollen grains were found on the stigma surfaces of the flowers of the three female cultivars. It was also observed that some pollen tubes began to grow on the surface of the stigma, and there was no trace of the pollen tube passage channel. The studied cultivars differed in terms of ovarian length, reaching1780.60, 1649.34 and 1596.00 µm in the ‘Barhee’, ‘Zahdy’ and ‘Derry’ cultivars, respectively. The highest mean ovary width and ovule diameter for the ‘Barhee’ cultivar were 1680 and 441.20 µm, respectively. While the ‘Derry’ cultivar recorded the lowest values compared other two cultivars. The ovule sizes of the three cultivars did not change much in value (Figs. 4 and 5).

After 12 hours of pollination, the pollen tube passage channel appeared for all the female cultivars under study, starting from the top of the stigma through the beginning of the style (Fig. 6). The cultivars under study differed in terms of ovary length, reaching1944.90; 1893.30 and 1734.50 µm for the ‘Barhee’, ‘Zahdy’ and ‘Derry’ cultivars, respectively. The highest mean of ovary width and ovule diameter for ‘Barhee’ cultivar was 1861 and 526.40 µm, respectively, while ‘Derry’ cultivar recorded the lowest values. The results related with Simpson [11], who indicated that the pollen tube penetrates the stigma and the mucous. The hollow stigma structure helps it in that, and then penetrates the style tissue. Usually this is done either through the intracellular spaces of the style cells or by secreting enzymes that dissolve the cells. Its means the pollen tube penetrates the style by secreting enzymes that dissolve the cells into the embryogenic sac. The evidence for this is that the pollen tube, as it passed through the style, which led to the

formation of a clear passage channel. Also, the current study found that large numbers of pollen tubes penetrate the tissue of the style towards the embryogenic sac and not a single tube (Fig. 7).

Table 1. The effect of the time period after pollination on the ovary length (µm) of the flowers for the three female cultivars

Female cultivar	The time period after pollination (hours)					
	0	6	12	24	48	96
'Barhee'	1280.50	1780.60	1944.90	2489.00	3187.70	4100.10
'Zahdy'	1199.40	1649.34	1893.30	2367.50	2845.50	3734.10
'Derry'	1183.60	1596.00	1734.50	2222.70	2732.00	3399.00
R-LSD 5%	11.28	74.43	40.48	63.46	77.34	98.86

Table 2. The effect of the time period after pollination on the ovary width (µm) of the flowers for the three female cultivars

Female cultivar	The time period after pollination (hours)					
	0	6	12	24	48	96
'Barhee'	1080	1680	1861	2232	2875	4041
'Zahdy'	1047	1551	1793	1930	2157	3587
'Derry'	1031	1317	1551	1718	1922	3178
R-LSD 5%	10.16	95.34	112.11	98.77	78.65	123.23

Table 3. The effect of the time period after pollination on the ovule diameter (µm) of the flower for the three female cultivars

Female cultivar	The time period after pollination (hours)					
	0	6	12	24	48	96
'Barhee'	310.50	441.20	526.40	691.10	801.20	870.70
'Zahdy'	230.20	420.20	496.20	596.00	586.10	688.00
'Derry'	205.60	414.80	484.30	541.50	570.10	591.30
R-LSD 5%	22.13	4.43	7.45	32.54	8.34	26.57

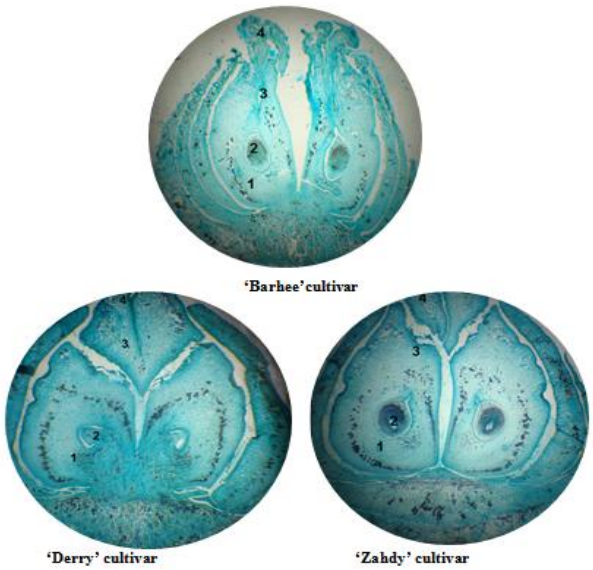

Fig. 1. A longitudinal section of a female flower of a date palm before pollination. Ovary (1); Ovule (2); Style (3); Stigma surface (4); magnification power(x10)

Fig. 2. A cross section of a female flower of a date palm before pollination. Ovary (1); Ovule (2); magnification power (x10)

Fig. 3. A longitudinal section in a female flower of a date palm before pollination. Ovary (1); Ovule (2), Funiculus (3); Placenta (4); magnification power (x10)

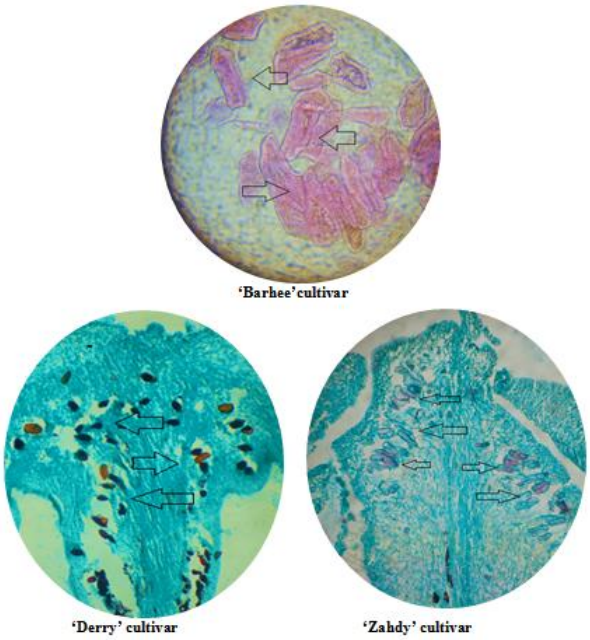

Fig. 4. An oblique section of a female flower of the date palm after pollination, showing swelling and the start of pollen tubes on the stigma surface; Magnification power (x40)

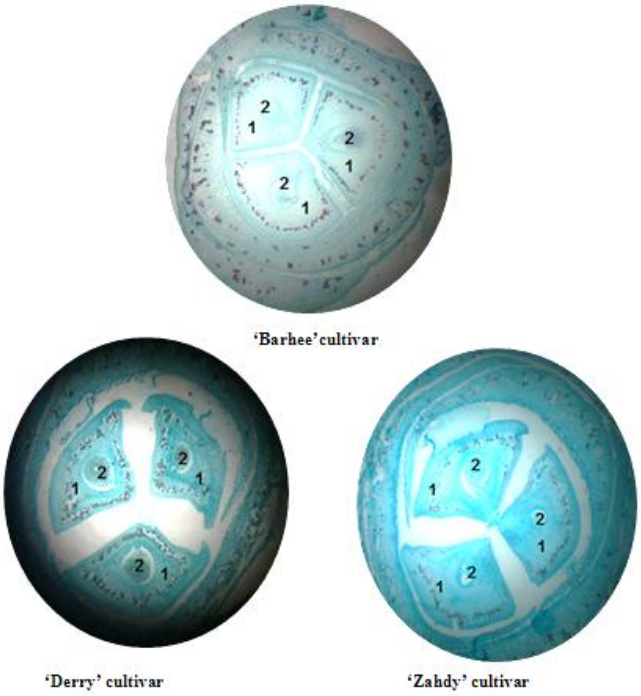

Fig. 5. A cross section of a female flower of a date palm after pollination. Ovary (1); Ovule (2); Magnification power (x10)

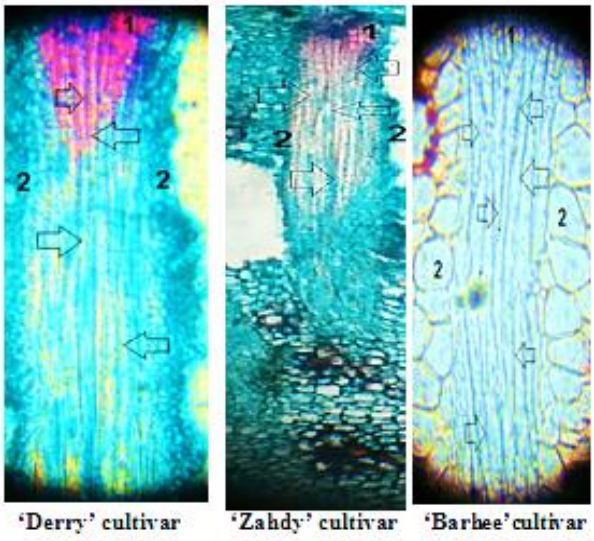

Fig. 6. A longitudinal section in a female flower of a date palm after pollination. A group of pollen tube channels appears through the style (1); Starting at the top of the stigma (2); towards the embryogenic sac (3); magnification power (x10)

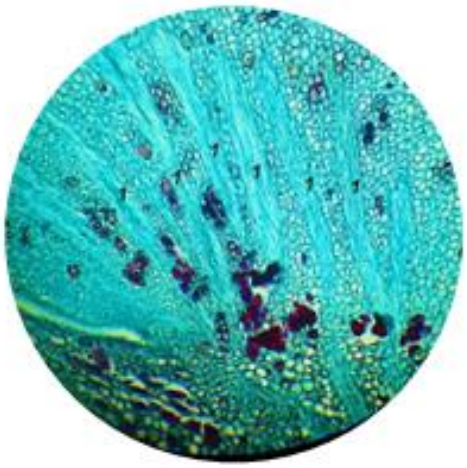

Fig. 7. A longitudinal section of a female flower of a date palm after pollination. A large numbers of pollen tubes penetrate the style tissue towards the embryogenic sac and not a single tube

Upon microscopic test of the samples after 24 hours of the pollination process, it was found that the pollen tube for all the female cultivars under study had developed in its growth and penetrated most of the style tissue towards the embryogenic sac, leaving a clear passage channel starting from the top of the stigma (Fig. 8). The ovary length reached 2489.00, 2367.50 and 2222.70 µm for the 'Barhee', 'Zahdy' and 'Derry' cultivar, respectively. The highest values of ovarian width and ovule diameter for the 'Barhee' cultivar were 2232 and 691.10 µm respectively, while the 'Derry' cultivar recorded the lowest values.

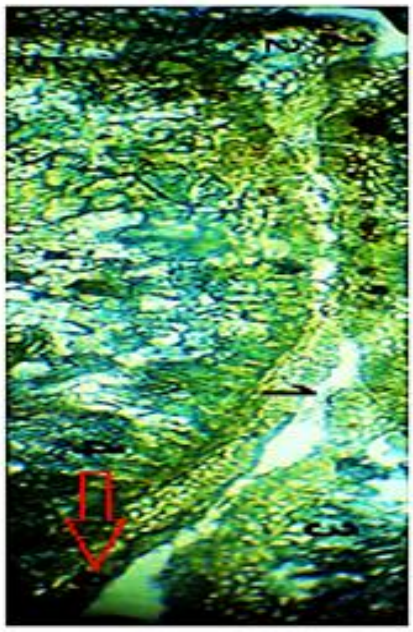

Fig. 8. A longitudinal section in a female flower of a date palm after pollination. The pollen tube has developed in its growth and penetrated most of the style tissue towards the embryonic sac, and leaving a clear passage channel starting from the top of the stigma; magnification power (x40)

After 36 hours of the pollination process, the microscopic test of the slides showed that the cultivars under study differed among them in terms of the development and growth of the pollen tube. The pollen tube developed and grew through the style and embryogenic sac to reach the ovule and caused double fertilization to take place in the 'Barhee' cultivar, as the pollen tube passage channel began to decay gradually as the ovule began to grow and increase its size. 48 hours after the pollination process of the microscopic test of the slides, fertilization took place in the female flowers of the 'Zahdy' cultivar, as the passage channel of the pollen tube disappeared and the fertilized ovule developed. The cultivars under study differed in terms of ovary length, reaching 3187.70, 2845.50 and 2732.00µm for the 'Barhee', 'Zahdy' and 'Derry' cultivars, respectively. The highest values of ovary width and ovule diameter for the 'Barhee' cultivar were 2875 and 801.20 µm respectively, while the 'Derry' cultivar recorded the lowest values.

Upon microscopic test of the samples 72 hours after the process of pollination, double fertilization occurred in the female flowers of the 'Derry' variety, where the passage channel of the pollen tube disappeared, the fertilized ovule developed, and the other two unfertilized ovules began to decay and decrease in size. This may indicate double fertilization in these flowers (Fig. 9).

After the 96 hours after the pollination process, the microscopic test of the slides showed that the flowers of the three female cultivars had completed the fertilization process before this period. The fertilized ovules had grown and developed clearly and significantly, and the unfertilized ovules continued to decay. The studied cultivars differed in terms of ovary length, reaching 4100.10, 3734.10, and 3399.00 µm for the 'Barhee', 'Zahdy' and 'Derry' cultivars, respectively. The highest average ovary width and ovule diameter for the 'Barhee' cultivar were 4041.00 and 870.70 µm respectively, while the 'Derry' cultivar recorded the lowest values, and there was no trace of the pollen tube passage channel (Fig. 10). This is confirmed by Matar [12] that the reason for the growth and survival of one carpel is the occurrence of a double fertilization in

one of ovaries, which leads to the miscarriage of the other two carpels. Heywood et al. [13] have shown that the pollen tube during its growth and development is consuming the nutrients in it as well as the nutrients that it takes from the tissues of the style and stigma. It is believed that determining the direction of pollen tube growth penetrating the tissues of the stigma, the style and then the ovary is due to the chemotropism. It is also believed that the two synergid nuclei play a role in guiding the pollen tube towards the ovule.

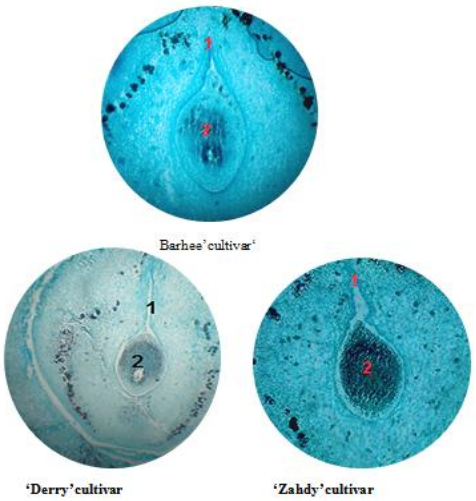

Fig. 9. A longitudinal section in a female flower of the date palm after pollination. The pollen tube growth appears through the style and the embryogenic sac to reach the ovule and causes double fertilization to take place and the start of the pollen tube passage channel with decay (1); and the start of the ovule to grow and increase its size (2); magnification power (x10)

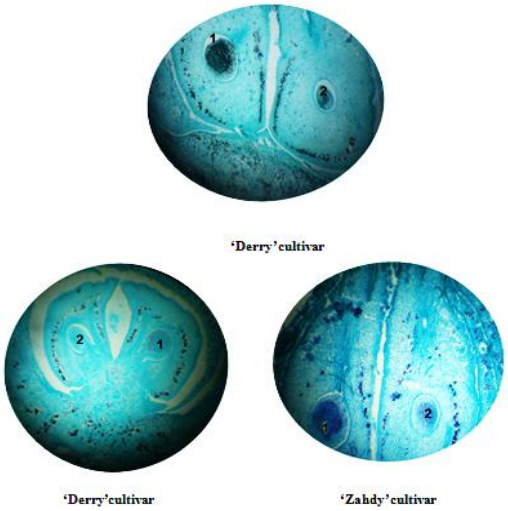

Fig. 10. A longitudinal section in a female flower of a date palm after pollination, showing the completion of the double fertilization process: The fertilized ovule and its clear increase in size and continuation in growth and development (1); the decay unfertilized ovules (2); Magnification power (x10)

The results of this study arein line with the findings consistent with the results of Al-Bajlani [14], Al-Attar [15] and Abbas [6] in the formation of the pollen tube passage channel, but they differ in the time of their appearance after the pollination process, as the first researcher indicated that they appeared three hours after the pollination process, while the second researcher indicated that it clearly appeared 48 hours after the pollination process. The last researcher indicated that it appeared six hours after the pollination process. The results of the anatomical female flowers under study indicated that the date of fertilization differed according to the female cultivar, and the difference may be due to genetic reasons related to the cultivar. The difference in the cultivars under study in the speed of pollen tube growth and development in the pollinated female flowers and the difference in the date of fertilization may be one of the factors affecting the difference in the percentage and time of fruit ripening and their hardness.

CONCLUSION

The flowers of the 'Barhee', 'Zahdy' and 'Derry' cultivars had completed the double fertilization process before the 96 hours of the pollination process, and the fertilized ovules had grown and developed clearly and significantly, and the unfertilized ovules continued to decay, and there was no trace of the passage channel for pollen tube. The anatomical female flowers under study indicated that the time of fertilization differed according to the female cultivar, and due to genetic reasons related to the cultivar.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Al-Jubouri HJ. The Importance of Date Palm (*Phoenix dactylifera* L.) trees in the State of Qatar. The National Teaching Course on Applications of Plant Tissue Culture in Improving Plant Production. 2002;1-25.
2. Ibrahim AO. Date palm tree of life. The Arab Center for the Studies of Arid Zones and Lands (ACSAD). League of Arab States, Damascus, Syrian Arab Republic. 2008;199-217.
3. Al-Najjar MAH, Al-Hamad ADS. A comparative anatomical study of the leaves of the date palm (*Phoenix dactylifera* L.) in dry, semi-dry and soft cultivars. Jordan Journal of Agricultural Science. 2016; 12(4):1325-1331.
4. Ibrahim AO, Khalaf AN, Abdul-Wahid MS. Certain physico-chemical changes during growth and maturity of fruits of two date palm (*Phoenix dactylifera* L.) cultivars 2. Chemical Characteristics. Basrah Date Palm Res. J. 2001;1:11-19.
5. DeMason DA, Stolte KW, Tisserat B. Floral development in *Phoenix dactylifera* L. Proceeding of the first symposium on the date palm, March23-25, King Faisal Univ, Al-Hasaa, Saudi Arabia. 1982; 46-59.
6. Abbas KI. Chromosomal, anatomical and phenotypic study of some cultivars of date palm. PhD Thesis, College of Science, University of Basrah, Iraq. 2000; 165.
7. Zaid A, deWet PF. Date palm cultivation. FAO Plant production and protection, United Nations. 2002;167.
8. Al-Najjar MAH. An evaluation and taxonomic study of the date palms (*Phoenix dactylifera* L.) males developing in the central and southern regions of Iraq. Ph.D. Thesis, College of Agriculture, University of Basra, Iraq; 2014.
9. Khafaji MA Microtechnics of botanicals, College of Agriculture, Mansoura University, Egypt. 2001;312.
10. Bashir SZ. Your Guide to the SPSS Statistical Program. The 10th Edition, Arab Institute for Training and Statistical Research. Iraq. 2003;262.
11. Simpson MG. Plant Systematic. 3rd Edition. Elsevier Academic Press, California, USA. 2019;774.
12. Matar AM. Palm cultivation and production. Dar Al-Hikmah Press. University of Basrah, Iraq. 1991;240.

13. Heywood VH, Brummitt RK, Culham A, Seberg O. Flowering Plant Families of the World. Richmond Hill, Ontario, Canada: Firefly Books; 2007.
ISBN: 1-55407-206-9.
14. Al-Bajlani ANR. Study the growth and embryogenic development of the 'Sukari' cultivar date palm flowers, and some factors affecting fruit set. M.Sc. Thesis, College of Agriculture, University of Baghdad, Iraq. 1985;89.
15. Al-Attar AA. A study of the embryogenic development of the flowers and fruits of the date palm 'Khedrawi' cultivar and its relationship to the time of pollination and the split of the spadix of pistillate inflorescence. Date Palm Journal. 1986; 4(2):19-36.