Open Access
J. Glob. Innov. Agric. Sci., 2023, 11(4):649-655
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/23.1124
http://www.jgiass.com

Effect of Fluraton and male cultivar on embryonic development of flowers of date palm (*Phoenix dactylifera* L.) C.V.'Barhee'

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This study explores the influence of Fluoraton treatment and male cultivar selection on the embryonic development of offshoot flowers in tissue-cultured date palms (*Phoenix dactylifera* L.), specifically focusing on the 'Barhee' cultivar. Female offshoots, derived from tissue culture, were subjected to inoculation with two male cultivars, namely Al-Ghanamy Al-Akhdar and a male-seed strain. Concurrently, Fluoraton treatment, administered at concentrations of 0, 0.3, and 0.6 g L-1, was applied to the female inflorescences. Microscopic examinations unveiled significant impacts on pollen tube development, fertilization, and embryonic sac growth. These findings offer valuable insights into optimizing the pollination process, ultimately leading to enhanced fruit production. The study underscores the significance of employing Fluoraton treatment and selectively choosing certain male cultivars over others for the pollination of female cultivars, given their pivotal role in expediting the early ripening of fruits. Notably, the male was utilized in the pollination process independently of the female variety, as no discernible differences were observed between the flowers of the two female cultivars.
Keywords: Fluraton, male cultivar, embryonic development, flowers, date palm, Barhi.

INTRODUCTION

The date palm is one of the most important plant species in the Arecaceae family, which includes more than 200 genera and 2,500 species. It is also the most beneficial plant family for humans after the Gramineae family. The order Arecales, to which the date palm belongs, is one of the most important known plant orders, as many types of palms belong to it. The date palm belongs to the genus Phoenix and the species dactylifera (EL-Hadrami and EL-Hadrami, 2009; Jain *et al*., 2011). Since the date palm is dioecious and unisexual, the male flowers are borne on one tree and the female flowers on another; therefore, pollination must be done artificially to ensure good fruit production.

Pollen grains represent an important and fundamental role in setting and forming fruits, as the date palm is characterized by its high ability to respond to the source of pollen, not only for its stallion but also for other stallions that do not belong to its kind (dactylifera), but perhaps its response was greater (Derhab, 2004). This is what Al-Najjar, (2014) found in terms of significant differences in the degree of response of female cultivars to different male cultivars, represented by the time of fertilization, fruiting characteristics, and the studied productivity. The chemical and physiological changes that fruits go through during the stages of growth and development are considered among the scientific bases upon which it has relied to explain many of the physiological and chemical phenomena that accompany the development and growth of the fruit, as well as relied upon to determine the date of maturity and attainment of the fruits (Ibrahim, 2001).

When used in low concentrations, growth regulators are non-nutrient organic compounds that promote, inhibit, or modify physiological processes. They may occur naturally in plants or be synthesized in a laboratory. They also play an important role in the plant's response to external environmental factors. Auxins are the first and most important group of plant growth regulators, which increase growth irreversibly along the longitudinal axis when added in low concentrations (Trivellini *et al*., 2015). Auxins regulate a large number of growth and maturation processes, stimulate cell division and elongation, as well as stimulate rooting, enhance pollen germination, and pollen tube growth, improve fruit settling and growth, and the formation of maiden fruits in plants through the effect of auxins in increasing the transport of nutrients and vitality (Sotomayor *et al*., 2012).

Fluraton (fanphthyl acetamide, acetic acid) is a plant growth regulator that improves the effect of other plant hormones, stabilises flowers, and increases their knot and fruit

Mahmoudi, S.J.F.A., M.A.H. Alnajjar and W.F.F. Alpresem. 2023. Effect of Fluraton and male cultivar on embryonic development of flowers of date palm (*Phoenix dactylifera* L.) C.V.'Barhee'. Journal of Global Innovations in Agricultural Sciences 11:649-655.
[Received 12 Jun 2023; Accepted 23 Aug 2023; Published 22 Dec 2023]

formation, leading to abundant production. It also protects the plant from frost and high temperatures during pollination, fertilization and fruit set. It is an effective plant growth regulator for fruit trees, which lack nodes and fruit formation. It is a mixture of auxins. The active substance of Floraton is naphthalene acetate (NAA 4.5 g) and naphthalene acetamide (NAD 12.5 g), one of the commercial compounds of auxins.

***Aim of the study*:** This study was conducted to determine the physiological effect of fluraton on fruit development

MATERIALS AND METHODS

This study was conducted in one of the private orchards in the north of Basrah Governorate, and the laboratories of the College of Agriculture, University of Basrah, Department of Horticulture and Landscape Engineering, the study involved 18 female offshoots of 'Barhi' cultivar from tissue culture, inoculated with Al-Ghanamy Al-Akhdar and a male-seed strain. Fluoraton treatment at concentrations (0, 0.3, 0.6 g l-1) was applied to female inflorescences. Microscopic examinations were conducted at various time points (10, 24, 36, 48, 72, 96 hours) post-pollination to track pollen tube growth and embryonic sac development. Permanent anatomical sections were prepared for detailed analysis. Permanent anatomical sections of female flowers were prepared using paraffin technology according to the method presented in Al-Najjar *et al.* (2021) and Khafaji (2001). The serial slides were examined and photographed by a compound light microscope.

RESULTS AND DISCUSSION

It was found in this study and through the results of the microscopic examination of fixed serial slides in the female flowers of the Barhi cultivar pollinated using the pollen of Al-Ghanamy Al-Akhdar and the seeded male cultivar (prepared after 72, 48, 36, 24, 10, 96 hours) that the female flower is at the beginning of its early stages and before The fertilization process contains three ovaries (crabbles) with a chamber in the middle dedicated to the ovule, which is connected to the inner wall of the ovary using a cylindrical growth protruding from the placenta tissue known as the funicule. There was no trace of the pollen tube passage channel (Fig. 1-3).

Upon microscopic examination of the samples 10 hours after the pollination process, large numbers of swollen pollen grains were found (for the two male cultivars under study and all Fluraton treatments) on the surfaces of the stigmas of the flowers of the Barhi cultivar. It was also observed that some pollen tubes began to grow on the surface of the stigmas (for the pollen grains of the Al-Ghanamy Al-Akhdar cultivar). Moreover, Fluraton treatment with two concentrations (0.3 and 0.6 g l-1) and the pollen grains of the seed cultivar treated with Fluraton with a concentration of 0.6 g l-1) and there was no effect on the pollen tube passage channel, and the sizes of the three eggs did not change much and were very close to all treatments (Fig. 4-6).

Upon microscopic examination of the samples 24 hours after the pollination process, it was found that the pollen tube (for the two male cultivars under study and treated with Fluraton at a concentration of 0.6 g l-1) had developed in its growth and had penetrated most of the pen tissue towards the embryonic sac and left a clear passage channel starting from the apex of the stigma. (Figure 7) After 36 hours of fertilization, the microscopic examination of the slides revealed that the two male cultivars under study differed between them in terms of the development and growth of the pollen tube according to the treatment with Fluraton. Within 36 hours after pollination with the pollen of the male cultivar Al-Ghanamy Al-Akhdar and the male seed cultivar treated with Fluraton at a concentration of 0.6 g l-1, the pollen tube passage channel began to decay gradually, and the ovum began to grow and increase in size (Figure 8). As for the male seed cultivar, the pollen tube developed to a degree. It was large and was very close to the egg inside the embryonic sac when treated with Fluraton at a concentration of 0.3 g l-1 (Fig. 9).

After 48 hours of the pollination process, the microscopic examination of the slides showed that the male cultivar was green and the male seed cultivar treated with Fluraton at a concentration of 0.3 g L-1 caused fertilization in the female flowers, as the pollen tube passage channel disappeared. The fertilized egg developed, and the other two unfertilized eggs began to decay and decrease. Size is one of the signs of fertilization (Figure 10). Furthermore, after 72 hours of pollination, the microscopic examination of the slides showed that the male variety was green, without Fluraton treatment, which caused fertilization in some female flowers, where the pollen tube passage channel disappeared. The fertilized egg developed, and the other two unfertilized eggs began to decay and decrease in size, which is one indication of fertilization. While no anatomical indications indicated that fertilization occurred in other flowers, they were held virginally. After 96 hours of the pollination process, the microscopic examination of the slides showed that the seedless male cultivar without Fluraton treatment caused fertilization in some female flowers; as the pollen tube passage channel disappeared, the fertilized egg developed, and the other two unfertilized eggs began to shrink and decrease in size, and this is one of the indications for fertilization. When there were no anatomical indications of fertilization occurring in other flowers (Figure 11). The lack of fertilization in some female flowers pollinated with the two male cultivars under study without treatment with Floraton caused an increase in the sporangia of the fruit and the formation of a chip.

The microscopic examination of the slides showed that the flowers of the Barhi variety vaccinated with the pollen of the two male varieties under study and for all Fluraton concentrations had completed the fertilization process after

four days of pollination. Pollen tube (figure 12). This was confirmed by Al-Najjar (2014) that the reason for the growth and survival of one carpel is the occurrence of a node in one ovary, which leads to the abortion of the other two carpels. The two helpers guide the pollen tube towards the egg (Alnajjar *et al.,* 2020).

The current study showed, through microscopic examination of the longitudinal and oblique sections of the pistils, that the stigmas of female flowers pollinated by the two male varieties and treated with Fluraton witnessed the germination of large numbers of pollen grains. Their tubes grew through the stigma and pen in a shorter period than the rest of the treatments (non-Fluraton treatment), and they were crowded during the pen. It is directed towards the ovary, meaning that not a single pollen grain germinates, and not a single pollen tube grows and penetrates the pen, but many of them do.

The autopsy results of the female flowers of the Barhi cultivar pollinated with the pollen grains of the two male cultivars under study showed that the date of fertilization differed according to the male cultivar. The green Ghanami cultivar caused fertilization after 72 hours of the pollination process, and the male seed cultivar caused fertilization to occur after 96 hours of the pollination process with formation. A large percentage of the fruits are cysticulate, and this difference may be due to genetic reasons related to the male variety itself. At the same time, the treatment with Fluraton led to an early fertilization process for the female flowers of both male cultivars, according to the concentrations of the treatments, and this may be due to the role of Fluraton (auxins) in its effect on Pollen grains and female flowers treated with Fluraton increase their content of auxins, as pollen grains have an important role in providing the ovaries of female flowers of date palm with the hormones necessary for their growth and encouraging the ovaries to produce hormones. The second acts as a stimulant for auxin, originally present in pollen and female flowers (Haddad and Baerly, 2009). During its growth and development, the pollen tube feeds on the food stored in it and the nutrients it derives from the tissues of the stigma and stigma (Heywood *et al.,* 2007). Here, the effect of Fluraton treatment on the speed of pollen germination on the stigmas of female flowers and the growth and development of the pollen tube becomes clear, as Fluraton works to stimulate pollen germination and increase pollen tube length. Auxins, considered useful in the differentiation process, affect stamen length, angiosperm maturity, and pollen development and germination (Salinas *et al.,* 2018). Moreover, the levels of free auxin in pollen grains have a direct effect on the growth and elongation of the pollen tube, and thus its effect on the pollination process, the rate of fertilization of eggs, and the improvement of the percentage of fruit set, which affects the productivity of fruits (Ibrism, 2016, Jing *et al*., 2020).

The difference in the two male cultivars under study and the concentrations of Fluratone in the speed of growth and development of the pollen tube in the pollinated female flowers and the different timing of fertilization may be one of the factors affecting the difference in the percentage of fruit maturity (fruit ripening table) resulting from the use of the male cultivar and the treatment with Fluraton in the pollination process. The treatments that caused fertilization after 36 hours of the pollination process are the same treatments that recorded the highest rates for the percentage of fruit maturity, followed by the treatments that caused fertilization to occur after 48 hours of the pollination process, while the treatments that caused fertilization to occur after 72 hours of the pollination process are the same that recorded the lowest Averages for the percentage of fruit maturity.

Hence the importance of using the Fluraton treatment and the selection of some male cultivars over others to be used in the pollination of female cultivars due to their great role in the early ripening of the fruits. The male was used in the pollination process without the female variety, as there was no difference between the flowers of the two female varieties, Halawi and Al-Sayer, in the speed of growth and development of the pollen tube and the time of fertilization.

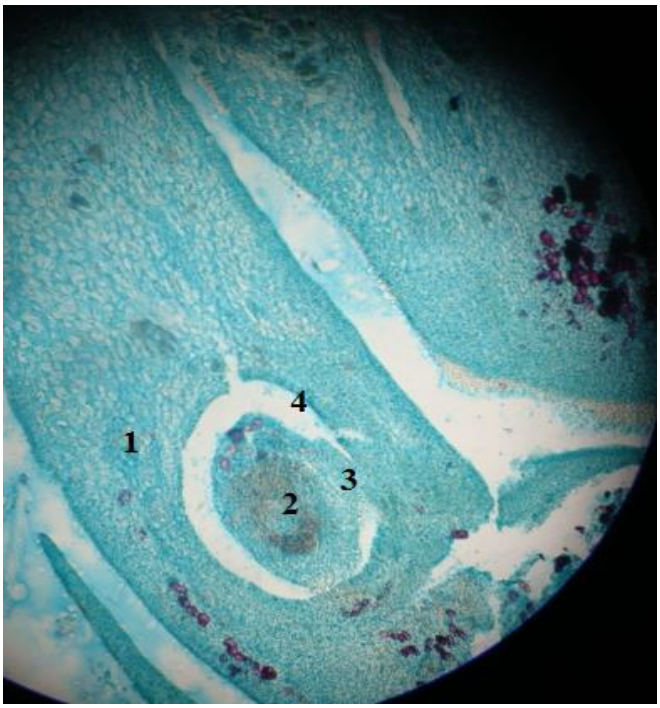


Figure 1. Longitudinal section of a female flower of date palm before pollination, showing 1- ovary 2- ovum 3- umbilical cord 4- placenta (x10)

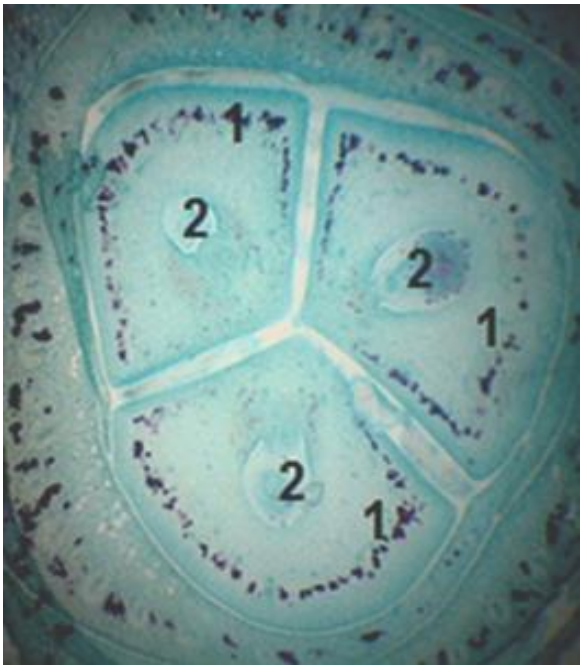


Figure 2. a cross section of a female flower of date palm before pollination process showing 1-ovary 2-ovule (x10)

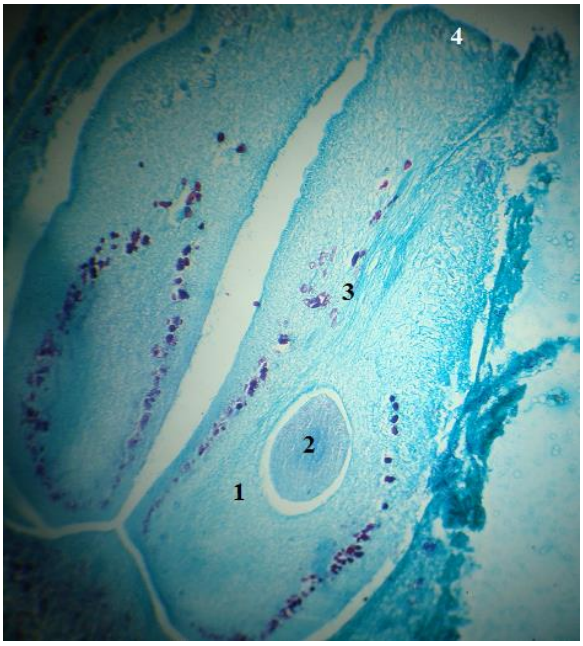


Figure 3. Longitudinal section of a female flower of date palm before pollination, showing 1- ovary 2-ovum 3- pen 4- stigma surface (x10).

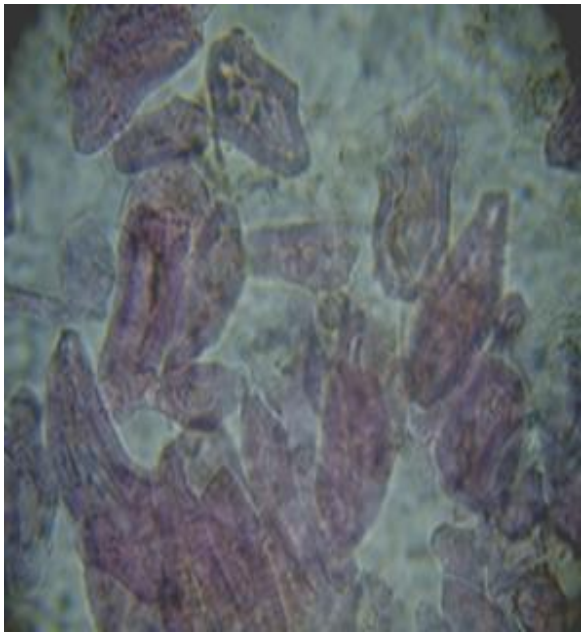


Figure 4. an oblique section of a female flower of date palm 10 hours after the pollination process, showing swelling and the beginning of the growth of pollen tubes on the surface of the stigma (x40)

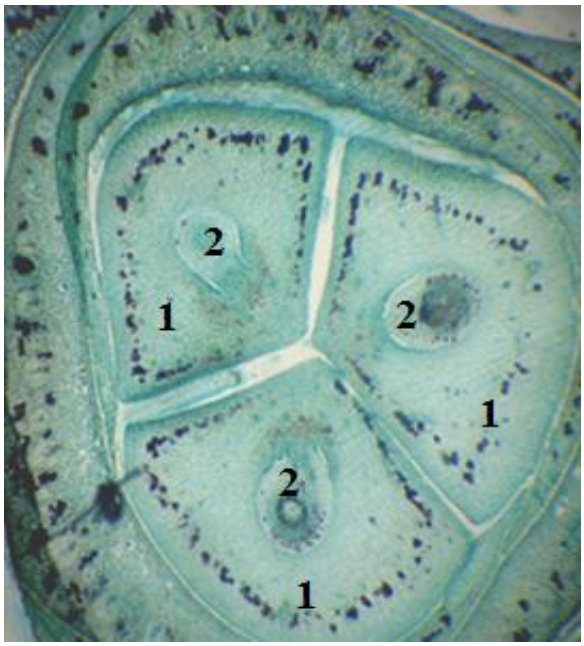


Figure 5- a cross section of a female flower of date palm 10 hours after the pollination process, showing that the sizes of the three ovaries and ovaries did not change much and were very close (x10).

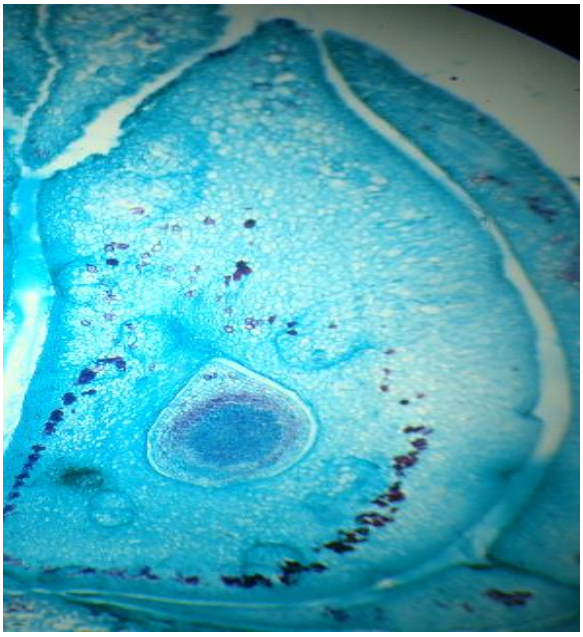


Figure 6. longitudinal section of a female flower of date palm ten hours after the pollination process, there was no trace of the pollen tube passage channel (x10).

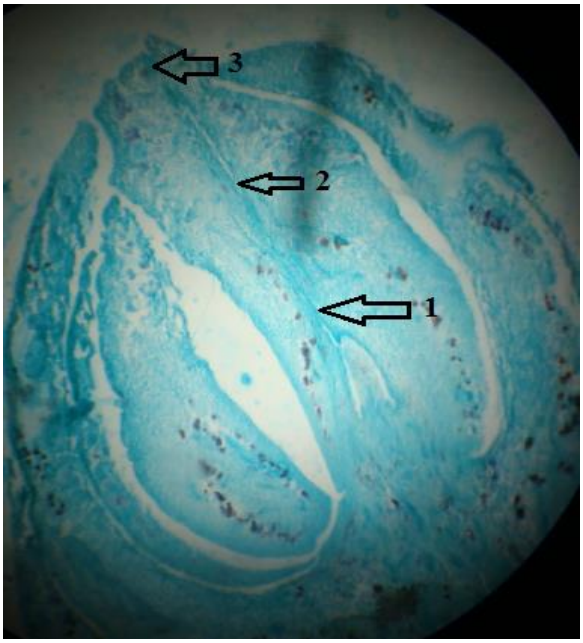


Figure 7. A longitudinal section of a female flower of a date palm 24 hours after the pollination process, showing the growth of 1- the pollen tube through 2- the pen starting from 3- the top of the stigma (x40).

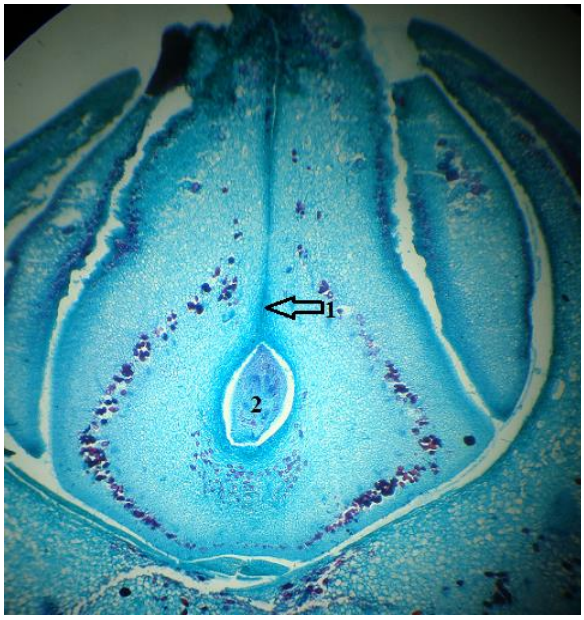


Figure 8. A longitudinal section of a female flower of date palm 36 hours after the pollination process with green galangal and treatment with Fluraton 0.6 mg L^{-1} showing the growth of the pollen tube through the pen and the embryonic sac up to the ovule and causing fertilization and the start of 1- the passage of the pollen tube to decay and start 2- The egg grows and increases in size (x10).

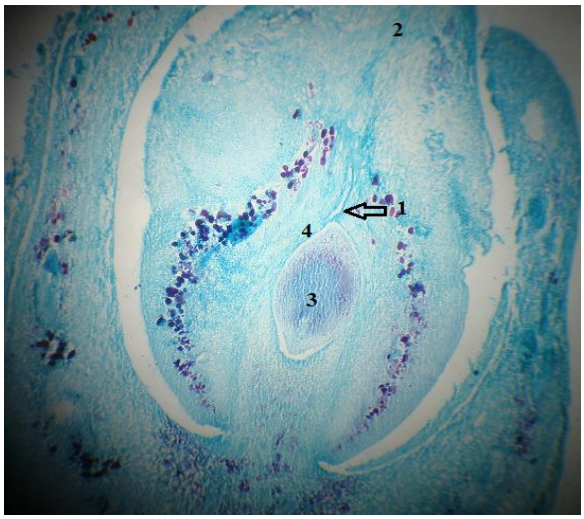
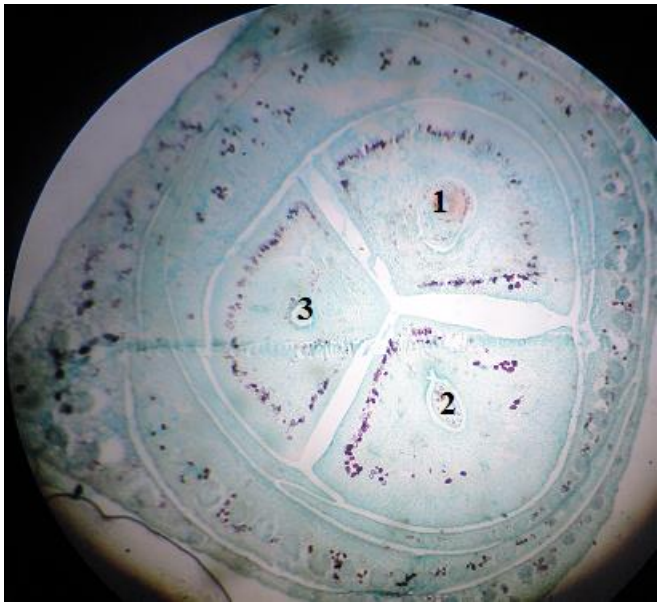


Figure 9. a longitudinal section of a female flower of date palm 36 hours after the pollination process with the seed variety and treatment with Fluraton 0.3 mg L^{-1} showing that fertilization did not occur and the development of the 1- pollen tube to a large extent through the 2- pistil and was very close to the 3- ovule inside 4 - embryonic sac (x10).



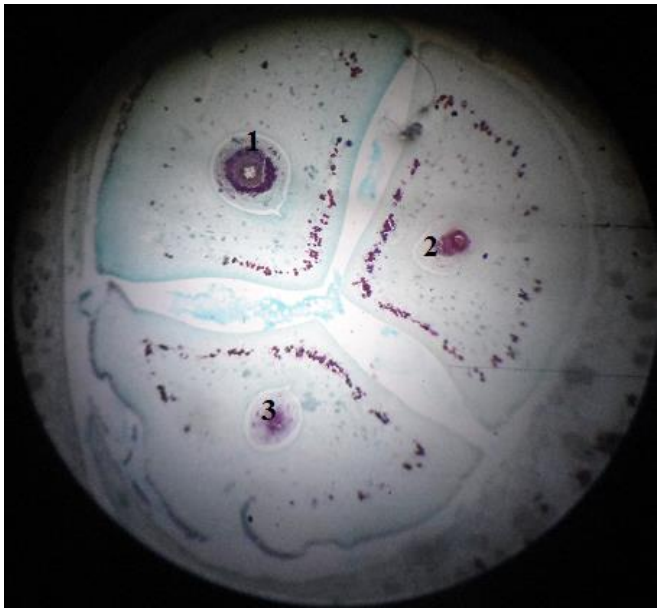


Figure 10. a section of a female flower of date palm 48 hours after the pollination process with the green galangal and the seed variety treated with Fluraton 0.3 mg L-1 showing the occurrence of fertilization in the female flowers and the development of 1- the fertilized egg and 2,3- the other two unfertilized eggs began to decay and decrease size (10x).

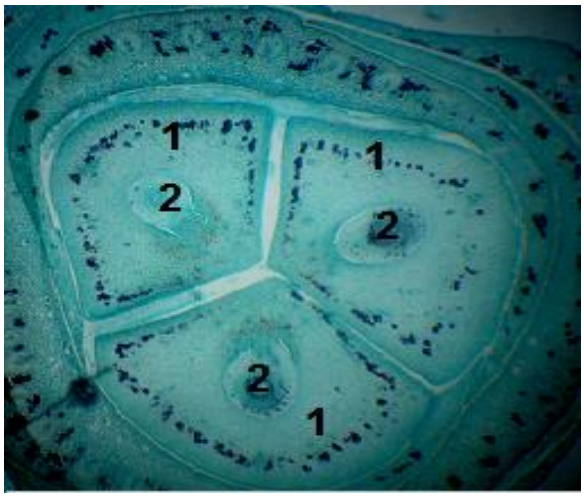


Figure 11. A section of a female flower of date palm 96 hours after the pollination process with the Gahnami Akhdar and the seed variety showing the occurrence of the virgin node in the female flowers and the absence of an increase in the size of 1- the ovary and the absence of a change in the size of 2- the three ovules (10x).

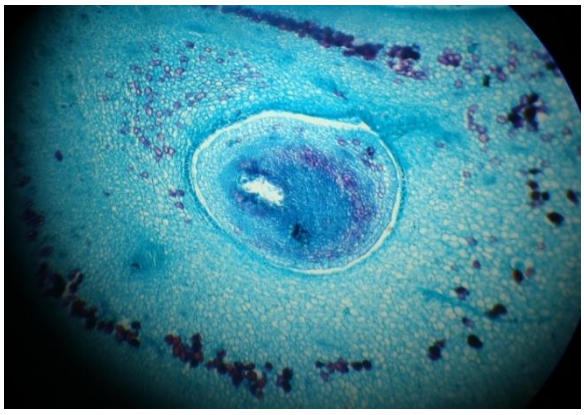


Figure 12. A longitudinal section of a female flower of date palm 96 hours after the pollination process, showing the completion of the fertilization process, and the fertilized egg has grown and developed clearly and large, and there was no trace of the pollen tube passage channel. (x10).

***Conclusion*:** Fluraton treatment and male cultivar selection significantly influence embryonic development and fertilization timing in date palm flowers. The findings underscore the importance of optimizing pollination practices for enhanced fruit maturity. Further research on the genetic factors affecting fertilization timing and fruit ripening is warranted for informed cultivar selection and cultivation practices.

***Authors' contributions*:** Authors' contributions: Mohammed Abdulameer Hasan Al-Najjar and wasen fawzi fadel Alpresem conceived and designed the experiments, Sorour J. F. Al-Mahmoudi.: performed the experiments, analyzed

***Funding*:** by Authors

***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere

***Code Availability*:** Not applicable

***Consent to participate*:** All authors are participating in this research study.

***Consent for publication*:** All authors are giving the consent to publish this research article in JGIAS

***Acknowledgements*:** This work is a part of the Research in date palm pollution. The authors extend their appreciation to the college of agriculture, Iraq, and Department of horticulture and land scape design, University of Basrsh, Iraq for hulping this work.

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