

Analyzing Phytohormone Levels in Fruits of Date Palm *Phoenix dactylifera* L. Derived from Tissue Culture under Different Pollination Treatments

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Abstract: This study evaluated three male date palm varieties, namely, Red-Ganami, Red-Ganami + Khakri Al-Adi and Red-Ganami + Al-Canary, in three age of spathes (early, late and middle) in a private orchard at Al-Seebah district, Basrah province. The plants were subjected to two pollination dates immediately after opening of female flowers and three days after opening of female flowers. The contents of auxin-like substances, gibberellin and abscisic acid in the flowers were determined. The second pollination date resulted in the highest contents of auxins, gibberellins and abscisic acid, with values of 24.305, 138.660 and 12.355 $\mu\text{g}\cdot\text{gm}^{-1}$, respectively. The mixture of Red-Ganami + canary cultivars had the highest concentrations of auxin 24.512 $\mu\text{g}\cdot\text{gm}^{-1}$ and gibberellins 136.842 $\mu\text{g}\cdot\text{gm}^{-1}$, whereas Red-Ganami had the highest concentration of abscisic acid 3.554 $\mu\text{g}\cdot\text{gm}^{-1}$. Early age of spathes showed significant differences in the concentrations of auxin and abscisic acid, with average values of 26.015 and 14.031 $\mu\text{g}\cdot\text{gm}^{-1}$, respectively. Late postmenopausal age recorded the highest concentration of gibberellins at 136.842 $\mu\text{g}\cdot\text{gm}^{-1}$. For the two- and three-way interactions, a statistically significant superiority was observed in most of the study coefficients. In conclusion, the contents of auxins, gibberellins and abscisic acid significantly differed with the pollination date and the pollin cultivar combinations showed the highest concentrations of these substances. The different ages of male puberty also affected pollination and fertilization.

Keywords: Abscisic Acid, Auxin, Date Palm, Gibberellin, Pollination.

Introduction

Date palm, *Phoenix dactylifera* L., which belongs to the Arecaceae family, is one of the most important fruit trees cultivated in Iraq and some regions of the Middle East (Ghnimi *et al.*, 2017; Alaida & Aldhebani, 2022). Fruit development is controlled by chemicals called plant hormones, which are non-food

organic compounds that naturally form in the plant in small quantities to regulate physiological processes. These hormones move from their sites of production to their sites of action, and their physiological effects occur within plant tissues; plant hormones are categorized based on their activity, namely,

growth promoters, such as auxins, gibberellins and cytokinins, and growth inhibitors, such as ethylene and abscisic acid (ABA) (Santner *et al.*, 2009; Suhim *et al.*, 2023). In flowering plants, the organ responsible for reproduction is the flower, in which pollen grain unites with the ovule inside the ovary. The transformation of the ovary into a fruit through several divisions in the tissue cells of its wall requires plant hormones, which are obtained from pollen grains (Fukuda, 2004). The hormones needed are fewer than those required for growth and development. Pollination and fertilization can stimulate the ovarian tissue to produce hormones necessary for fruit growth and development (Pandolfini, 2009). The seed is also a source of hormones necessary for cell division and embryonic cell formation (Angelovici *et al.*, 2010). The oxygen content increases as a result of the division of the endosperm of the seed (Farooq *et al.*, 2021). During flowering, in the absence of pollination and fertilization, the flower ovaries of virgin fruits contain high concentrations of phytohormones, which are sufficient for fruit setting, ovary growth, seedless fruit production, growth and development until the interstitial stage (Panoli *et al.*, 2015).

Determining the percentage of the crop, and this in turn depends on various factors, such as the source and quality of pollen, the period of pollination, the method of pollination and the compatibility between females and males (Salomón-Torres *et al.*, 2020). The physiological basis for the phenomenon of metazenia remains unknown and is the subject of many studies. Researchers reported that the differences in the concentrations of plant hormones auxins, gibberellins and cytokinins are due to different sources of pollen (Abd *et al.*, 2020).

There is some research that deals with changes in plant hormones. Cheruth *et al.* (2015) studied the levels of plant hormones in different periods of flowering for three UAE cultivars, Negal, Liloy and Khalas. The levels of phytohormones noticeably increased in the pre-flowering periods and decreased in the flowering and post-flowering periods. Ali-Dinar *et al.* (2021) showed that the levels of plant hormones (auxins and gibberellins) in the flowers of date palm pollinated by different methods of pollen increased until the 16th week after pollination; the levels then decreased at the end of the interstitial stage, and ABA increased until the 20th week after fertilization. The hormonal activities of date palms with different pollen appearance and reception periods have not been studied yet.

The present work aimed to evaluate the status of phytohormones in date palm flowers produced from tissue culture through pollination with different pollen cultivars and reception periods. The results will elucidate the nature of deformities that appear in date palm cultivars produced through tissue culture, such as the appearance of the partheno carpic set phenomenon. Specific hormonal imbalance or deficiency that triggers this problem will be identified.

Materials & Methods

The study was conducted in one of the private orchards of Al-Seebah district at Basrah province in the growing season of 2021–2022. 54 palm trees of the Barhi cultivar, homogeneous in age and growth, were selected. They were pollinated with three varieties of pollen grains, namely, Red-Ganami, Red-Ganami + Khakri Al-Adi and Red-Ganami + Al-Canary with three age of spathes early, middle and late ages by using cotton balls dipped in pollen of the same weight. The female flowers were immediately

covered to prevent pollen transfer from other treatments and maintain the temperature and humidity conditions required for successful fertilization. Nodes and fertilization occurred at the highest possible rate. Changes in plant hormones (auxins, gibberellins and ABA) were studied.

Quantitative assessment of plant hormones

Auxin-like substances

Estimation was conducted based on the method of Crozier *et al.* (1980) by using a spectrophotometer (UV- Visible, Shimadzu). Samples were measured at a wavelength of 280 nm, and concentrations were calculated based on a standard curve prepared with natural auxin (indole acetic acid, IAA, fig. 1).

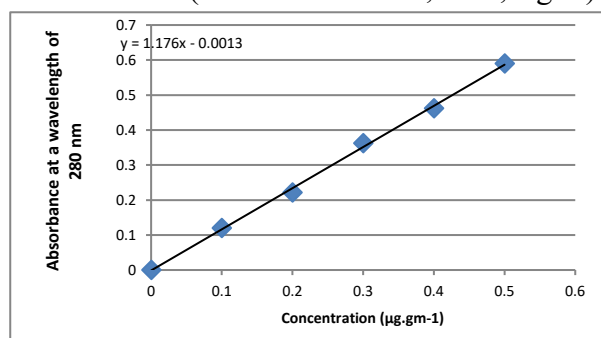


Fig. (1): Standard curve for auxin (IAA)

The results were expressed in microgram per kilogram fresh weight.

Gibberellin-like substances

The samples were read at a wavelength of 205 nm, and concentrations were calculated based on a standard curve with gibberellin acid (GA3, fig. 2). The results were expressed in microgram per kilogram fresh weight. Estimation was conducted in accordance with a previously reported method (Berríos *et al.*, 2004).

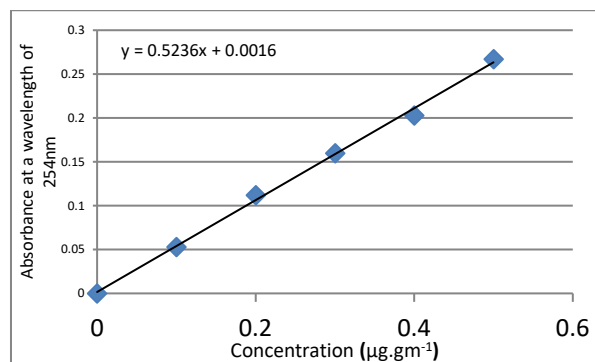


Fig. (2): Standard curve of gibberellin acid (GA3).

ABA-like substances

These substances were estimated based on a standard curve, in which ABA, (fig. 3) was used at a wavelength of 254 nm. The results were expressed as microgram per kilogram according to (Gómez-Cadenas *et al.*, 2002).

Statistical analysis

A factorial experiment was performed using a randomised complete block design with three factors (2×3×3) with three blocks: date of pollination (immediately and three days after opening the female flowers), type of pollen used (Red-Ganami, Red-Ganami + Khakri Al-Adi and Red-Ganami + Al-Canary) and age of male pollen (early, middle and late). The experiment was repeated three times. Data were analyzed statistically using a specific design and Gen Stat 2007 software. Mean values were compared using least-significant difference (LSD) test at a significant level of $p \leq 0.05$.

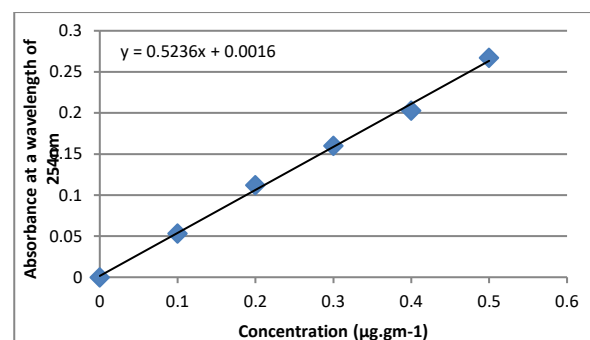


Fig. (3): The standard curve of abscisic acid (ABA).

Results

Auxin-like substances in flowers

As shown in table (1) the effect of pollination date, cultivar effect, pollen age and their interactions had an effect on the contents of auxin-like substances in the flowers of date palm of Barhi cultivar after pollination. Statistically significant differences were observed in the pollination date, where the first date (immediately after the female flower opened) showed higher levels than the second date (Three days after the female flower opened). After pollination, the highest average auxin concentration was recorded as $24.305 \mu\text{g}\cdot\text{gm}^{-1}$. The first date (immediately after the opening of the female flower) after pollination led to the lowest mean auxin concentration of $23.738 \mu\text{g}\cdot\text{gm}^{-1}$. The ghannamy red +canary cultivar was significantly superior, that is, it had the highest concentration of auxin $24.512 \mu\text{g}\cdot\text{gm}^{-1}$ over the Red-Ganami + Khakri Al-Adi and Red-Ganami cultivar 23.761 and $23.790 \mu\text{g}\cdot\text{gm}^{-1}$, respectively. In addition, the auxin concentration $26.015 \mu\text{g}\cdot\text{gm}^{-1}$ in the early age group significantly differed from those in the middle and late age groups, with values of 23.194 and $22.854 \mu\text{g}\cdot\text{gm}^{-1}$ for male pollocks, respectively. The overlap between the second date and the cultivar Red-Ganami + Al-Khkari Al-Adi was significantly distinguished in most of the interactions in terms of auxin content $26.525 \mu\text{g}\cdot\text{gm}^{-1}$ as well as in the interactions between the date and the age of male pollination. The highest average auxin content was the result of the overlap between the second date and the early age of the male pollen, with a significant difference from other interactions $27.517 \mu\text{g}\cdot\text{gm}^{-1}$. The interaction between the Red-Ganami + Canary cultivar and the early age of the male pollen, with a large difference, overcame

some of the interactions in terms of affecting the auxin concentration $27.942 \mu\text{g}\cdot\text{gm}^{-1}$. The results of the triple overlap showed that the two treatments of the second date with Red-Ganami+ Canary cultivar recorded the highest percentage of auxin after pollination 29.728 and $16.293 \mu\text{g}\cdot\text{gm}^{-1}$, respectively.

Gibberellins-like substances in flowers

Table (2) shows the changes in the concentration of gibberellin-like substances after pollination of date palm flowers of the Barhi cultivar. Statistically significant differences were found between the dates of opening of the female flowers. The second date showed a significant improvement in the contents of gibberellins after pollination $138.660 \mu\text{g}\cdot\text{gm}^{-1}$. The effect of different pollen varieties on the content of gibberellins in flowers was determined. The Red-Ganami + Canary cultivar was superior in providing the highest ratio of gibberellins $136.842 \mu\text{g}\cdot\text{gm}^{-1}$. Table (2) also shows the effect of the age of the male spawn on the same trait. The late male spawn was significantly superior, with an average gibberellin content of $144.737 \mu\text{g}\cdot\text{gm}^{-1}$. The second data (Three days after the opening of the female flowers) with Red-Ganami showed the highest concentration of gibberellin $143.822 \mu\text{g}\cdot\text{gm}^{-1}$. The first date (immediately after the opening of the female flowers) with the late male pollination recorded the highest content of $149.847 \mu\text{g}\cdot\text{gm}^{-1}$. Moreover, the overlapping treatments showed the superiority of the late Red-Ganami +Canary treatment in increasing the flowering content of gibberellin-like substances $155.606 \mu\text{g}\cdot\text{gm}^{-1}$, while the triple interactions showed significant differences in gibberellin-like concentrations. The first date (immediately after the female flowers opened) with Red-Ganami + Canary cultivar recorded the highest value of gibberellins $170.824 \mu\text{g}\cdot\text{gm}^{-1}$.

Table (1): Effect of cultivar, pollen age, and pollen reception date on the levels of auxins-like substances ($\mu\text{g}\cdot\text{gm}^{-1}$) in date palm Al-Barhi cultivar after pollination.

Dates	cultivar	age of spathes			dates average	cultivar average		age average	
		Early	middle	Late					
Immediately after opening	Red-Ganami	0.88 b*29.048±	0.18 b29.048±	0.22 116.293±	0.57B23.738±	Red-Ganami	0.66B 23.790±	early	0.43A 26.015±
	Red-Ganami + Al-Khkari	0.47 j18.333±	0.16 h22.075±	h0.87 22.585±					
	Red-Ganami +Al-canary	0.44 d26.156±	0.26 c26.667±	0.77 g23.435±		Red-Ganami + Al-Khkari	0.43B 23.761±	middle	0.21B 23.194±
Three days after opening	Red-Ganami	fg0.26 23.776±	0.56 i19.779±	0.41 e24.796±	0.67A24.305±	Red-Ganami +Al-canary	1.09A 24.512±	late	22.854±0.87 C
	Red-Ganami + Al-Khkari	0.76 b29.048±	0.38 f23.946±	0.45 cd26.582±					
	Red-Ganami +Al-canary	0.34 a29.728±	0.32i k17.653±	0.46 g23.435±					
dates *cultivar					dates*age				
Immediately after opening	Red-Ganami	0.12c24.796±			Immediately after opening	Early	0.11d24.512±		
	Red-Ganami + Al-Khkari	0.11f20.998±				Middle	0.36b25.930±		
	Red-Ganami + Al-canary	0.27b25.420±				Late	0.65e20.771±		
Three days after opening	Red-Ganami	0.34e22.783±			Three days after opening	Early	0.69a27.517±		
	Red-Ganami + Al-Khkari	0.21a26.525±				Middle	0.90f20.459±		
	Red-Ganami + Al-canary	0.48d23.605±				Late	0.51c24.938±		
cultivar *age									
		Early	Middle	Late					
	Red-Ganami	0.87b26.412±	0.22c24.413±	0.76g20.544±					
	Red-Ganami +Al-Khkari	23.690±0.76dc	0.47ed23.01±	0.29c24.583±					
	Red-Ganami Al-canary	0.52a27.942±	0.32f22.160±	1.23d23.435±					

*The same letters in each row represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

Table (2): Effect of cultivar, age of spathes, and date of pollen reception on the levels of gibberellins-like substances ($\mu\text{g}\cdot\text{gm}^{-1}$) in date palm cultivar Barhi after pollination.

Dates	Cultivar	age of spathes			dates average	cultivar average		age average	
		Early	Middle	Late					
Immediately after opening	Red-Ganami	103.089±0.80 n*	117.048±0.52 l	137.414±0.32 h	126.214±0.2 2B	Red-Ganami	131.503±0.39 B	early	125.648±0.28 C
	Red-Ganami + Al-Khkari	121.053±0.771 k	102.860±0.66 o	141.304±0.61 f		Red-Ganami + Al-Khkari	128.966±0.31 C	middle	126.926±0.84 B
	Red-Ganami + Al-canary	141.991±0.54 e	100.343±0.59 p	170.824±0.36 a		Red-Ganami + Al-canary	136.842±0.88 A	late	144.737±0.72 A
Three days after opening	Red-Ganami	153.661±0.65 c	127.803±0.93 j	150.000±0.54 d	138.660±0.7 4A	Red-Ganami	136.842±0.88 A	late	144.737±0.72 A
	Red-Ganami + Al-Khkari	117.506±0.28 l	162.586±0.22 b	128.490±1.25 i		Red-Ganami + Al-Khkari	128.966±0.31 C	middle	126.926±0.84 B
	Red-Ganami + Al-canary	116.590±0.33 m	150.915±0.21d	140.389±0.58 g		Red-Ganami + Al-canary	136.842±0.88 A	late	144.737±0.72 A
dates *cultivar					dates*age				
Immediately after opening	Red-Ganami	119.184±0.55e			Early	122.044±0.44e			
	Red-Ganami + Al-Khkari	121.739±0.32d			Immediately after opening	Middle	106.751±0.36f		
	Red-Ganami + Al-canary	137.719±0.28b			Immediately after opening	Late	149.847±0.27a		
Three days after opening	Red-Ganami	143.822±0.21a			Three days after opening	Early	129.25±0.54d		
	Red-Ganami + Al-Khkari	136.194±0.41c			Three days after opening	Middle	147.101±0.43b		
	Red-Ganami + Al-canary	135.965±0.48cd			Three days after opening	Late	139.626±0.21c		
cultivar *age									
		Early	middle	Late					
	Red-Ganami	128.375±0.31f	122.426±0.21h	143.707±0.14b					
	Red-Ganami + Al-Khkari	119.279±0.18i	132.723±0.53d	134.897±0.26c					
	Red-Ganami + Al-canary	129.291±1.02e	125.629±0.36g	155.606±0.68a					

*The same letter above bars represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

Table (3): Effect of cultivar, age of spathes, and date of pollen reception on the levels of abscisic acid-like substances ($\mu\text{g}\cdot\text{gm}^{-1}$) in date palm, cultivar Barhi, after pollination.

Dates	Cultivar	age of spathes			dates average	cultivar average	age average			
		Early	Middle	Late						
Immediately after opening	Red-Ganami	25.172±0.11 a*	3.782±0.54 h	12.758±0.42 d	11.209±0.48B	Red-Ganami	13.554±1.06A	Early	14.031±1.34A	
	Red-Ganami + Al-Khkari	2.636±0.34 i	16.387±0.43 c	12.567±0.78 d						
	Red-Ganami + Al-canary	4.163±0.21 h	6.837±0.55 g	16.578±0.80 c						
Three days after opening	Red-Ganami	20.970±0.54 b	8.365±0.89 f	10.275±0.38 e	12.355±0.76A	Red-Ganami + Al-Khkari	10.975±0.87B	Middle	10.530±0.25B	
	Red-Ganami + Al-Khkari	10.275±0.81 e	16.005±0.45 c	7.983±0.91 fg						
	Red-Ganami + Al-canary	20.970±0.16 b	11.803±0.33 d	4.545±0.44 h						
dates *cultivar					dates*age					
Immediately after opening	Red-Ganami	13.904±0.44a			early	10.657±0.38d				
	Red-Ganami + Al-Khkari	10.530±0.34e			middle	9.002±0.20e				
	Red-Ganami + Al-canary	9.193±0.37f			late	13.967±0.33b				
Three days after opening	Red-Ganami	13.203±0.27b			early	17.405±0.29a				
	Red-Ganami + Al-Khkari	11.421±0.12d			middle	12.058±0.81c				
	Red-Ganami + Al-canary	12.440±0.71c			late	7.601±0.32f				
cultivar *age										
		Early	middle	Late						
	Red-Ganami	23.071±0.62a	6.073±0.76g	11.516±0.21d						
	Red-Ganami + Al-Khkari	6.455±0.50g	16.196±0.85b	10.275±0.65ef						
	Red-Ganami + Al-canary	12.567±0.51c	9.320±0.71f	10.561±0.89e						

*The same letter above bars represents no significant difference between treatments, based on the range test at $p \leq 0.05$.

ABA-like substances in flowers

Table (3) showed the levels of ABA-like substances as affected by the three study factors. The abscisic acid content at the second appointment (Three days after the female flowers opened) was significantly affected compared to the first date (immediately after the female flowers opened), as the level was recorded at 12.355 micrograms.gm⁻¹. The Red-Ganami cultivar recorded the highest level of 13.554 µg.gm⁻¹ for the mentioned trait, while the early stage recorded the Early of 14.031 µg.gm⁻¹. The bidirectional interactions significantly affected the levels of auxin-like substances. The first date (immediately after the opening of the female flowers) with Red-Ganami variety showed a significant superiority, with ABA level of 13.904 µg.gm⁻¹.

The interaction between the second date and the early age of male flowers recorded the highest level of auxin-like substances 17.405 µg.gm⁻¹. For the last two-way interaction from the same table, the early Red-Ganami cultivar showed a significant superiority 23.071 µg.gm⁻¹ compared with the other treatment. In the triple interactions, the first date (immediately after the opening of the female flowers) with The Ghanami red cultivar and the early age of the male shoot led to the highest level of auxin-like substances 25.172 µg.gm⁻¹.

Discussion

Plant growth and development is controlled by hormones, which are chemical messengers or signals that regulate most of physiological processes within a plant (Fukuda, 2004). The results showed variations in the levels of plant hormones as a result of pollination with different pollen cultivars. This finding could be due to their different traits, including their chemical components, including proteins,

carbohydrates and antioxidants, as a result of their genetic origin and changing growth conditions (Altemimy *et al.*, 2019; Shahsavar & Shahhosseini, 2022). Pollen, which contains high amounts of carbohydrates and proteins, can fertilize and grow pollen tubes due to its role in the adhesion of the pollen grain. Simple sugars are used as an energy source to assist in the germination and development of pollen grains (Angelovici *et al.*, 2010). By contrast, in species whose pollen grains have low levels of protein and carbohydrates, the pollen tube growth is less (Shahsavar & Shahhosseini, 2022). The pollen cultivar and the period of pollen reception affect the levels of plant hormones in date palm. The appropriate pollination cultivar and date can enhance the secretion of plant hormones to stimulate the growth of date palm. Therefore, scholars should focus on the type of pollen used and the timing of pollen reception to improve phytohormones in date palm (Fattahi *et al.*, 2014; El-Kosary *et al.*, 2023). Auxin can serve as the primary pollination signal by the direct transfer of auxin present in the pollen grain to the female flowering stigmas (Karim *et al.*, 2022). In this study, the results showed the superiority of the early Canary + Red-Ganami pollen cultivar in increasing the levels of auxins in the female flowers three days after the spathes opened. Obtaining pollen grains early could increase the amount of gibberellin and auxin in plants, leading to increased production of dates. Additionally, studies have demonstrated that external treatment with gibberellin improved pollen germination and pollen tube growth and increased fertilization rate, thereby increasing the content of internal gibberellin, depending on variety and quantity (Hamzah, & Aubied, 2019). The source of pollen affects the setting of the fruits and their specific traits. This phenomenon is called mitazinia, and the mitazinic effect results

from the role of plant hormones produced directly or indirectly in pollen grains that are controlled by genetic factors. The levels of endogenous plant hormones increase after pollination and fertilization, which are necessary for plant growth and development, similar to the date of pollination (Qadir *et al.*, 2020). This increase was due to the increase in the content of amino acids in the flowers three days after the spathes opened, particularly the increase in tryptophan, which is the basic unit in building auxin. Thus, the effect is reflected in the percentage of fruit set (Pieck *et al.*, 2015). The study of plant hormones is an interesting topic to address the failure of pollination and fertilization in date palm cv. Barhi to understand the biology of reproduction. The results also provide solutions that improve the productivity of this important cultivar and address the challenges faced by palm farmers (low yield due to pollination and fertilization problems in tissue culture).

Conclusions

- 1- The second date increased the levels of auxins, gibberellins, and ABA.
- 2- The choice of pollen cultivar affected the levels of plant hormones. The Red-Ganami + Canary cultivar had the highest level of auxin and gibberellins, while the Red-Ganami cultivar had the highest level of ABA.
- 3- Early pollination led to increased levels of auxin-like substances and ABA but reduced the level of gibberellins.

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Conflicts of interest

The authors declare that they have no conflict of interests.

Contributions of authors

F.M.A.: Writing the research paper.

A.M.A.: Statistical analysis.

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تحليل مستويات الهرمونات النباتية في ثمار نخيل التمر *Phoenix dactylifera* L. الناتجة من زراعة الأنسجة تحت معاملات التلقيح المختلفة

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المستخلص: اجري البحث في احد البساتين الاهلية في محافظة البصرة، ناحية السببة لثلاثة اصناف ذكورية وهي الغنمى الاحمر والغنمى الاحمر+ الخكري العادي والغنمى الاحمر+ الكنارى ذات الاعمار المبكرة والمتأخرة والمتوسطة ولموعدين للتلقيح الأول بعد انشقاق الطلعة مباشرة والثاني بعد انشقاق الطلعة بثلاثة ايام، لدراسة محتوى الأزهار من المواد الشبيهة بالأوكسينات والجبرلينات وحامض الأبيسيك. أظهرت النتائج أن هناك فروقا معنوية في محتوى الأزهار من الأوكسينات والجبرلينات وحامض الأبيسيك، حيث اعطى الموعد الثاني للتلقيح أعلى محتوى للأزهار من المواد الشبيهة بالأوكسينات والجبرلينات وحامض الأبيسيك وبمعدل 24.305 و 138.660 و 12.355 مايكروغم.غم⁻¹ بالتتابع، وسجل صنف الغنمى الاحمر+ الكنارى أعلى القيم في تركيز الاوكسين والجبرلين وبمعدل 24.512، 136.842 مايكروغم.غم⁻¹ بالتتابع، في حين سجل صنف الغنمى الاحمر تفوقا في تركيز حامض الأبيسيك بلغ 13.554 مايكروغم.غم⁻¹، وسجل العمر المبكر للطلعة فرقا معنويا في تركيز الاوكسين وحامض الأبيسيك وبمعدل 26.015، 14.031 مايكروغم.غم⁻¹ بالتتابع في الوقت نفسه سجلت الطلعة المتأخرة أعلى معدل لتركيز الجبرلين بلغ 136.842 مايكروغم.غم⁻¹. اما بالنسبة للتدخلات الثنائية والثلاثية فقد اظهرت تفوقا معنويا في معظم معاملات الدراسة.

الكلمات المفتاحية: حامض الأبيسيك، الاوكسين، نخيل التمر، الجبرلين، التلقيح.