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 µg.gm\(^{-1}\), respectively. The mixture of Red- Ganami + canary cultivars had the highest concentrations of auxin 24.512 µg.gm\(^{-1}\) and gibberellins 136.842 µg.gm\(^{-1}\), whereas Red-Ganami had the highest concentration of abscisic acid 3.554 µg.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 µg.gm\(^{-1}\), respectively. Late postmenopausal age recorded the highest concentration of gibberellins at 136.842 µg.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 Areccaceae family, is one of the most important fruit trees cultivated in Iraq and some regions of the Middle East (Ghnimi *et al.*, 2017; Alaida & Aldhebiani, 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)](image1)

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 (Berrios et al., 2004).

![Fig. (2): Standard curve of gibberellin acid (GA3).](image2)

**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 \((2 \times 3 \times 3)\) 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).](image3)
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 μg.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 μg.gm⁻¹. The ghannamy red +canary cultivar was significantly superior, that is, it had the highest concentration of auxin 24.512 μg.gm⁻¹ over the Red-Ganami + Khakri Al-Adi and Red-Ganami cultivar 23.761 and 23.790 μg.gm⁻¹, respectively. In addition, the auxin concentration 26.015 μg.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 μg.gm⁻¹ for male pollocks, respectively. The overlap between the second date and the cultivar Red-Ganami + Al-Khakari Al-Adi was significantly distinguished in most of the interactions in terms of auxin content 26.525 μg.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 μg.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 μg.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 μg.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 μg.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 μg.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 μg.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 μg.gm⁻¹. The first date (immediately after the opening of the female flowers) with Red-Ganami + Canary cultivar recorded the highest value of gibberellins 170.824 μg.gm⁻¹.
Table (1): Effect of cultivar, pollen age, and pollen reception date on the levels of auxins-like substances (μg.gm⁻¹) in date palm Al-Barhi cultivar after pollination.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Cultivar</th>
<th>Cultivar average</th>
<th>Age average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately after opening</td>
<td>Red-Ganami</td>
<td>0.66B ±23.790</td>
<td>early 0.43A ±26.015</td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>0.43B ±23.761</td>
<td>middle 0.21B ±23.194</td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-canary</td>
<td>1.09A ±24.512</td>
<td>late 22.854±0.87C</td>
</tr>
<tr>
<td>Three days after opening</td>
<td>Red-Ganami</td>
<td>0.67A ±24.305</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>0.67A ±24.305</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-canary</td>
<td>0.67A ±24.305</td>
<td></td>
</tr>
</tbody>
</table>

*The same letters in each row represents no significant difference between treatments, based on the range test at p ≤ 0.05.
Table (2): Effect of cultivar, age of spathes, and date of pollen reception on the levels of gibberellins-like substances (μg.gm⁻¹) in date palm cultivar Barhi after pollination.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Cultivar</th>
<th>age of spathes</th>
<th>dates</th>
<th>cultivar average</th>
<th>age average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early</td>
<td>Middle</td>
<td>Late</td>
<td></td>
</tr>
<tr>
<td>Immediately after opening</td>
<td>Red-Ganami</td>
<td>103.089±0.80 a*</td>
<td>117.048±0.52 l</td>
<td>137.414±0.32 h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami</td>
<td>121.053±0.771 k</td>
<td>102.860±0.66 o</td>
<td>141.304±0.61 f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>141.991±0.54 e</td>
<td>100.343±0.59 p</td>
<td>170.824±0.36 a</td>
<td></td>
</tr>
<tr>
<td>Three days after opening</td>
<td>Red-Ganami</td>
<td>153.661±0.65 c</td>
<td>127.803±0.93 j</td>
<td>150.00±0.54 d</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>117.506±0.28 l</td>
<td>162.586±0.22 b</td>
<td>128.490±1.25 i</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-canary</td>
<td>116.590±0.33 m</td>
<td>150.915±0.21 d</td>
<td>140.389±0.58 g</td>
<td></td>
</tr>
</tbody>
</table>

*The same letter above bars represents no significant difference between treatments, based on the range test at p ≤ 0.05.
Table (3): Effect of cultivar, age of spathes, and date of pollen reception on the levels of abscisic acid-like substances (μg·gm⁻¹) in date palm, cultivar Barhi, after pollination.

**Dates** | **Cultivar** | **Age of spathes** | **Dates** | **Cultivar** | **Age average**<sup>1</sup> |
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early</td>
<td>Middle</td>
<td>Late</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red-Ganami</td>
<td>25.17±0.11 a*</td>
<td>3.782±0.54 h</td>
<td>12.758±0.42 d</td>
<td>11.209±0.48B</td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>2.636±0.34 i</td>
<td>16.387±0.43 c</td>
<td>12.567±0.78 d</td>
<td>13.554±1.06A</td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-canary</td>
<td>4.163±0.21 h</td>
<td>6.837±0.55 g</td>
<td>16.578±0.80 c</td>
<td>10.975±0.87B</td>
</tr>
<tr>
<td>Immediately after opening</td>
<td>Red-Ganami</td>
<td>20.970±0.54 b</td>
<td>8.365±0.89 f</td>
<td>10.275±0.38 e</td>
<td>12.355±0.76A</td>
</tr>
<tr>
<td></td>
<td>Red-Ganami + Al-Khkari</td>
<td>10.275±0.81 e</td>
<td>16.005±0.45 c</td>
<td>7.983±0.91 fg</td>
<td>Red-Ganami + Al-canary</td>
</tr>
<tr>
<td>Three days after opening</td>
<td>Red-Ganami + Al-canary</td>
<td>20.970±0.16 b</td>
<td>11.803±0.33 d</td>
<td>4.545±0.44 h</td>
<td>Middle</td>
</tr>
</tbody>
</table>

**Notes:**

- *The same letter above bars represents no significant difference between treatments, based on the range test at p ≤ 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; Shahsav & 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 (Shahsav & 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 spathe 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 spathe 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.

**Acknowledgement**

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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.
M.A.A: Reviewing the research paper.

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تحليل مستويات الهرمونات النباتية في ثمار نخيل التمر

الناتجة من زراعة Phoenix dactylifera L.

الأنسجة تحت معاملات التلقيح المختلفة

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المستخلص: اجري البحث في أحد البساتين الاهلية في محافظة البصرة، ناحية السبيبة لثلاثة اصناف تركية وهي الغنامي الأحمر والغنامي الأحمر الاصلي والغنامي الأحمر الاصلي. الاضرار المبكرة والمتأخرة والمنشقة ويليام ابتدأ التلقيح الأول بعد اتفاق الطلاءة في الثاني بعد اتفاق الطلاءة بثلاثة ايام، لدراسة متغيرات الأزهار من المواد الشهية بالأوكسينات والجبريلينات وحمض الأسيك، حيث اعتناء الغنامي الأحمر في التلقيح على مستوى الأزهار من المواد الشهية بالأوكسينات والجبريلينات وحمض الأسيك، وسجل صفت الغنامي الأحمر الصافي على القيم في تركيز الأوكسين والجبريلين وسجل 136.842 مايكروغرم، غرام بالتنبيه، في حين سجل صفت الغنامي الأحمر تركيز في تركيز حمض الأسيك بلغ 13.554 مايكروغرام، غرام بالتنبيه، وسجل العامل المبكر للطاقة تركيز في تركيز الأوكسين وحمض الأسيك وسجل 14.031 مايكروغرام، غرام بالتنبيه، في الوقت نفسه سجلت الطلاءة المتأخرة أعلى معدل لتركزيح الجبريلين بلغ 136.842 مايكروغرام، غرام بالتنبيه، أما بالنسبة للداخلات الثلاثية والثلاثية فقد أظهرت نتائج معنوية في معظم معاملات الدراسة.

الكلمات المفتاحية: حمض الأسيك، الأوكسين، نخيل التمر، الجبريلين، التلقيح.