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Optimization temperature and Boron modulate Phytohormnes content to improve fruit set for Barhee date palm (*Phoenix dactylifera* L.) cultivar derived from tissue culture

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Abstract

Temperature is one of the crucial factors that can affect the success of pollination in date palm. The present investigated the impact of bagging treatments and boron spraying on temperature, hormone levels, and fruit set in Barhee date palms cultivar derived from tissue culture. The study also measured the levels of plant hormones, including indole acetic acid, gerbilline acid, kinetin-like compound, and abscisic acid, before and after pollination under different treatments. The results showed that bagging treatments significantly affected the temperature inside the bags, with higher temperatures observed in the white paper treatment. The results also showed that the levels of these hormones varied depending on the treatment and stage of fruit development. the high levels of IAA and KC show in the pollination treatments involving brown paper and white paper, the highest levels of GA_3 were found in bagging with brown paper without pollination. The temperature has been shown to significantly affect the levels of GAs and ABA in flowers, leading to Parthenocarpic fruit. Treatment with spraying boron and bagging with brown paper showed the highest percentage of seeded fruit set while bagging with brown paper without pollination resulted in the highest percentage of seedless fruit set. The study highlights the importance of carefully managing bagging treatments for successful pollination and fruit set in date palm cultivation. Maintaining optimal temperature conditions is crucial for successful pollination in date palms.

Keywords: Abscisic acid, Bagging, Fruit set, Indole acetic acid, Gerbilline acid, Boric acid, Pollination.

Introduction

Date palm (*Phoenix dactylifera* L.) is a dioecious fruit tree (male and female plants) endemic to the world's hot, dry regions and primarily grown in the Middle East and North Africa. Due to germplasm exchange, date palm cultivation has spread to Australia, South Africa, South America, Mexico, and the United States (Al-najm *et al*., 2021). Barhee is a mid-season cultivar widely grown in the gulf region. Its fruit is marketed and consumed fresh in the fully ripe yellow stage (Khalal) as a crunchy, apple-like fruit due to its low soluble tannin contents, unlike wide other cultivars (AL-khayri *et al*., 2017). Recently, the cultivation of date palms from tissue culture has increased in various regions, especially those cultivated with the Barhee cultivar.

Despite the typical pollination method, it has been regularly observed in tissue culture-derived date palm populations that, in addition to the female carpels, the stamens also grow to create a group of 3-9 little fruit-like structures without a single parenchyma inside (Hadi *et al.*, 2015). In tissue culture-derived date palm plantations, this aberrant and complex development results in depressingly poor yields. The number of unpollinated fruits (seedless or parthenocarpic, locally known as Shees) rose, resulting in a decrease in the amount of pollinated fruits (economic fruits or seeded fruits) and a severe financial concern (Hamza *et al*., 2016). Gibberellins and endogenous auxins are found in higher concentrations in naturally occurring parthenocarpic. (Zhang *et al*., 2021). The role of the phytohormone in fruit date palm formation is well documented (Cheruth *et al*. 2015; Shareef and AL-khayri 2020; Elbar *et al*. 2023). Abscisic acid (ABA) has been predicted as a factor in the process of development in plants. In date palm fruit development, ABA is generally associated with indole acetic acid (IAA) and gerbilline acid (GA_3) (Torahi *et al*., 2021). ABA and zeatin concentrations were significantly greater during the pre-flowering stage but gradually reduced during the flowering period and subsequently increased following the flowering stage (Cheruth *et al*. 2015). Closed paper bags provide higher temperatures inside the bag, which helps produce seed fruits, and this high temperature helps adjust hormone levels (Shareef and AL-khayri, 2020). Temperature is an important environmental factor affecting flower development, pollination, and fruit set. High temperatures can inhibit flower bud formation and development, reduce pollen viability and germination, and increase flower and fruit drop (Yang *et al*., 2019). Nutrients such as boron have been used to obtain high set in many date palms (Harhash and Abdel-nasser 2010; Shareef 2016; AL-hajjaj and Ayad 2018; Abd-elhaleem *et al*. 2019; Jabbar and Hassan 2020). Spraying boron onto the flowers is more effective and cost-efficient than soil application (Abd-elhaleem *et al*. 2019). Boron (B) influences several plant activities, including hormone transport, salt absorption, blooming and fruiting, pollen germination, and pollen tube growth direction. It also involves protein synthesis, sugar transport, and glucose metabolism (Shireen *et al*., 2018).

Based on the information provided, this study aims to assess the potential use of boron and closed paper bags to stimulate the level of hormones in flowering and a management strategy for improving Barhee date palm's pollination through tissue culture to produce normal fruit.

Materials and Methods

Plant Material and Pollination Conditions

The investigation was carried out during the growing season of 2022 in a private orchard on palm trees of the Barhee cultivar obtained from tissue culture and at the age of four years in Al-Hartha region - Basrah (30°44'43.4"N 47°39'40.9" E). 28 palm trees were selected. The trees were treated with the same pruning, irrigation, and fertilization field services. Five inflorescences are left on each tree. The experiment was designed using randomized complete blocks. It was distributed among seven treatments, each treatment having four replicates. Boron spray treatments (Boric acid 2 gm l^{-1}) were sprayed according to the treatments at the beginning of flowering. The treatments were distributed as follows: 1- Control pollination without bagging and spraying (T1), 2- Bagging with brown paper without Pollination (T2), 3- Pollination with brown paper (T3), 4- Pollination with white paper (T4), 5- Pollination with spraying Boron (T5), 6- Pollination with spraying Boron + brown paper (T6), 7- Pollination with spraying Boron + white paper (T7). Environmental conditions change, as shown in Table 1.

Table 1. The change in daily light intensity and temperature during the ten days of April 2022

Type of bag	Light intensity, (cd)	Light intensity under bag, (cd)	Air temperature, (ºC)	Temperature under bag, (ºC)	Relative humidity (%)	Relative humidity under a bag (%)
brown paper	0.976	0.000	28.57	33.16	27.54	32.56
white paper	0.976	0.105	28.57	33.73	27.54	30.49

Collection of Samples

Four-year-old tissue culture-derived plants of cultivars 'Barhee' known that pollination was unsuccessful or that the fruits (non-seed) were selected for sample collection based on their fruiting record were collected. Spathes chosen for the experiment were covered with paper bags before being opened to prevent outcrossing and to manage pollination accurately. On April 1st, after the natural opening of the spathe, the flowers in the inflorescence were pollinated artificially. The pollen grains were collected from the male clones Al-Ghannami Al-Akhdar, and the inflorescences were wrapped in white and brown paper bags (45 x 35cm) according to the treatments. After 15 days, seeded and non-seed fruit'shees' could be easily separated, and samples were taken. Before hormone extraction, the samples were packed in bags inside an ice box, transported to the laboratory, and stored at -20ºC. The temperature and relative humidity under the bag were recorded every day at 11.00 h with a hygro-thermometer to record the right air temperature in circulation. Every day at 11.00 a.m., a hygro-thermometer records the right air temperature in circulation.

Fruit set (%). After 15 days, the fruit set was determined using the following equation: Fruit set (%) = (number of retained fruits on the strand/number of retained fruits plus the number of floral scars on the same strand) ×100

Fig. 1 The fruits of the date palm cv. Barhee, after 40 days of pollination was affected by the treatments. Brown bags (BB), White bags (WB). (T1) control pollination without bagging and spraying, (T2) Bagging with brown paper without pollination, (T3) Pollination with brown paper, (T4) Pollination with white paper, (T5) Pollination with spraying boron, (T6) Pollination with spraying boron + brown paper, (T7) Pollination with spraying boron + white paper.

Analyze Hormones

To ensure levels of indoleacetic acid (IAA), abscisic acid (ABA), gibberellic acid (GA_3), and Kinetin-like compound (KC) were determined using the same tissue extracts. Date fruit samples were washed, surface dried with a paper towel, immediately deposited in liquid nitrogen, and stored at -20°C. One gram of fresh mass (FM) samples was crushed in liquid nitrogen and medium-term extracted with 30 ml of 80% cold methanol at 4 °C. The concentrate was centrifuged for 15 minutes at 2000 xg and 4 °C, and the supernatant was collected. At that point, additional cold methanol was used to fill the remaining, which had been extracted four times using the abovementioned methods. The all-out methanolic separation was dried in a rotary evaporator and divided into 10 mL aliquots of methanol. According to Tang *et al*. (2011), IAA, ABA, GA_3, and CK were determined by infusing the concentrate into a turnaround stage HPLC on a switch stage C18 section (250 4.60 mm, 5 microns) in an isocratic elution mode utilizing a portable stage comprised of acetonitrile: water (26:74) with 30mM phosphoric acid.

Statistical Analysis

A randomized block design was used in the experiment, which included three factors (bagging) at two levels (brown and white paper), sampling time (before and after pollination), and Boron spraying. Each of the seven treatments had four replications. Analysis of variance (ANOVA) was performed on the data using SPSS variant 21.0 (SPSS, Chicago, IL), and the means were separated using the Duncan test at the 0.05 significant level.

Results

Phytohormones level in flowers before and after pollination

The levels of IAA, GA_3, and kinetin-like compound (KC) were measured both before pollination and after pollination under different treatments (Table 2). The results showed that the highest levels of IAA were found in T6 (3.63 µg g^{-1}) and T7 (3.46 µg g^{-1}), while the lowest levels were found in T1 (1.52 µg g^{-1}) after pollination. The highest levels of GA_3 were found in T2 after pollination, and the lowest levels were found in T4 (11.10 µg g^{-1}) after pollination. The highest levels of KC were found in T5 (54.23 µg g^{-1}) after pollination, while the lowest levels were found in T4 (20.05 µg g^{-1}) before pollination. Figure 2 shows the abscisic acid (ABA) levels in developing seeded and seedless fruits of the Barhee cultivar before and after pollination under different treatments. The results indicate that the levels of ABA varied depending on the treatment and the stage of fruit development. Before pollination, the levels of ABA ranged from 0.352 µg g^{-1} in T2 to 0.44 µg g^{-1} in T6. After pollination, the levels of ABA increased in some treatments, ranging from 0.3 µg g^{-1} in T6 to 0.92 µg g^{-1} in T1. The highest levels of ABA before pollination were found in T6 (0.44 µg g^{-1}) and the lowest in T2 (0.352 µg g^{-1}). After pollination, the highest levels of ABA were found in T1 (0.92 µg g^{-1}), and the lowest levels were found in T6 (0.3 µg g^{-1}) in seeded fruits.

Table 2 Phytohormone level (µg g[-1]) in developing seeded and seedless fruits of Barhee cultivar before and after pollination

Treatments	IAA		GA_3		Kinetin-like compound (KC)	
	BP	AP	BP	AP	BP	AP
T1*	1.80±0.06i	1.52±0.01 j	24.31± 0.58c	14.38±0.59f	20.11±0.84i	24.19±1.24 h
T2	1.80±0.03i	2.30±0.01g	24.65±0.65c	30.380±5.24a	20.15±1.11i	40.62±0.61 g
T3	1.80±0.03i	3.14±0.04c	24.64±0.57c	12.13±0.50g	20.28±1.11i	54.23±1.17 b
T4	1.81±0.03i	2.82±0.02d	24.02±0.44c	11.10±0.68g	20.05±0.94i	45.82±0.83e
T5	2.07±0.03h	2.57±0.02f	28.36±0.85b	18.58±0.83e	43.61±0.93f	61.06±1.39 a
T6	2.68±0.03e	3.63± 0.01a	28.30± 1.16b	21.22±1.08d	43.62±1.42f	52.11±1.54 c
T7	2.66±0.02e	3.46±0.02b	28.35± 1.17b	17.61±0.73e	43.47±0.98f	49.78±1.05 d

*(T1) control pollination without bagging and spraying, (T2) bagging with brown paper without Pollination, (T3) Pollination with brown paper, (T4) Pollination with white paper, (T5) Pollination with spraying boron, (T6) Pollination with spraying boron + brown paper, (T7) Pollination with spraying boron + white paper, (BP) before pollination, (AP) after pollination (n= 4; means±SE). Values with different letters within columns are significantly different (P < 0.05).

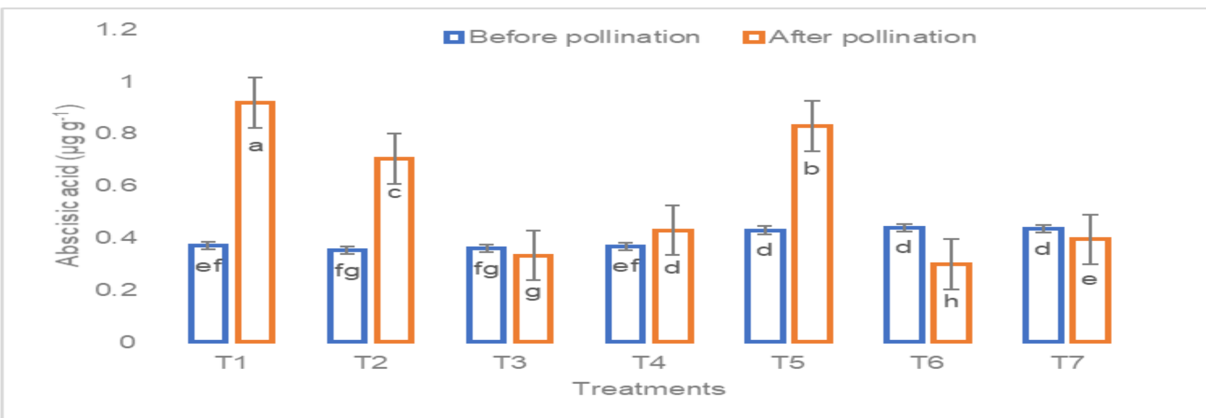

Fig. 2 Abscisic acid level (µg g-1) in developing seeded and seedless fruits of Barhee cultivar, (T1) control pollination without bagging and spraying, (T2) bagging with brown paper without pollination, (T3) Pollination with brown paper, (T4) Pollination with white paper, (T5) Pollination with spraying boron, (T6) Pollination with spraying boron + brown paper, (T7) Pollination with spraying boron + white paper, (n= 4; means±SE). Means denoted by different letters differ significantly at P ≤ 0.05.

Seeded and seedless set percentage after pollination

Figure 3 shows the percentage of seeded fruit set of Barhee cultivar in different treatments. The results indicate that the percentage of seeded fruit set varied considerably among the treatments. In more detail, Treatment T6 (pollination with spraying Boron + brown paper) had the highest percentage of seeded fruit set (83.25%), while treatment T2 (bagging with brown paper without pollination) had the lowest percentage of seeded fruit set (1.5%). Treatment T5 (pollination with spraying Boron) had an average percentage of seeded fruit set (52.75%), while treatment T1 (control pollination without bagging and spraying) had a meager percentage of seeded fruit set (3.5%).

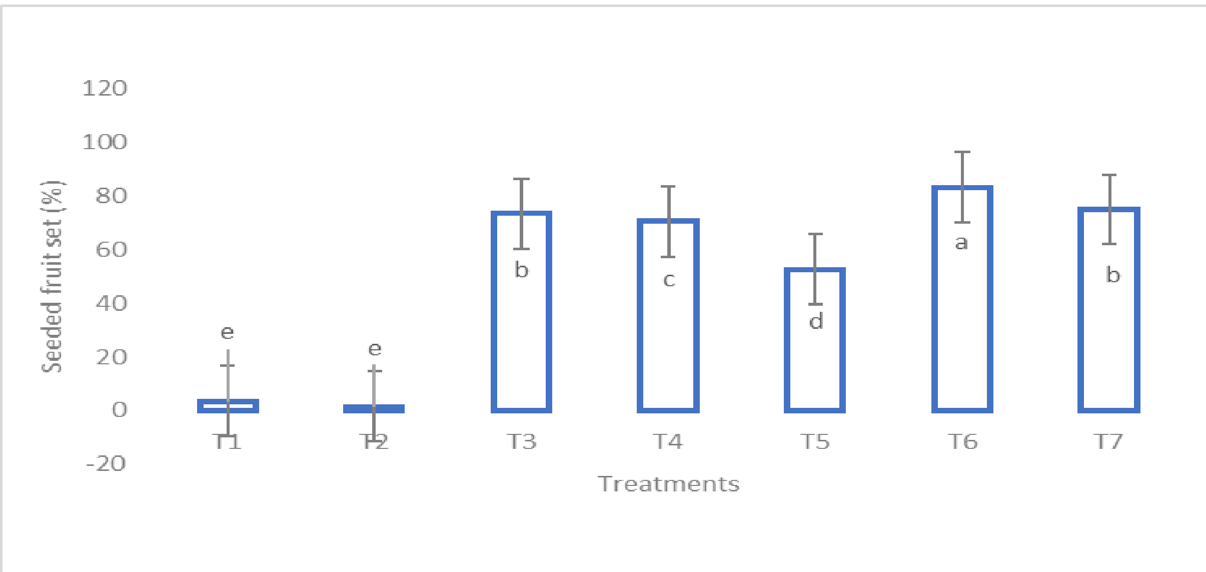

Fig. 3 Seeded fruit set (%) of Barhee cultivar by (T1) control pollination without bagging and spraying, (T2) bagging with brown paper without pollination, (T3) pollination with brown paper, (T4) Pollination with white paper, (T5) Pollination with spraying Boron, (T6) Pollination with spraying boron + brown paper, (T7) Pollination with spraying boron + white paper, (n= 4; means±SE). Means denoted by different letters differ significantly at $P \leq 0.05$.

Figure 4 shows the percentage of seedless fruit set for Barhee cultivar under different pollination treatments. The data show that the highest percentage of seedless fruit set was observed in T2, which involved bagging with brown paper without pollination, while the control treatment (T1) showed the lowest percentage of seedless fruit set.

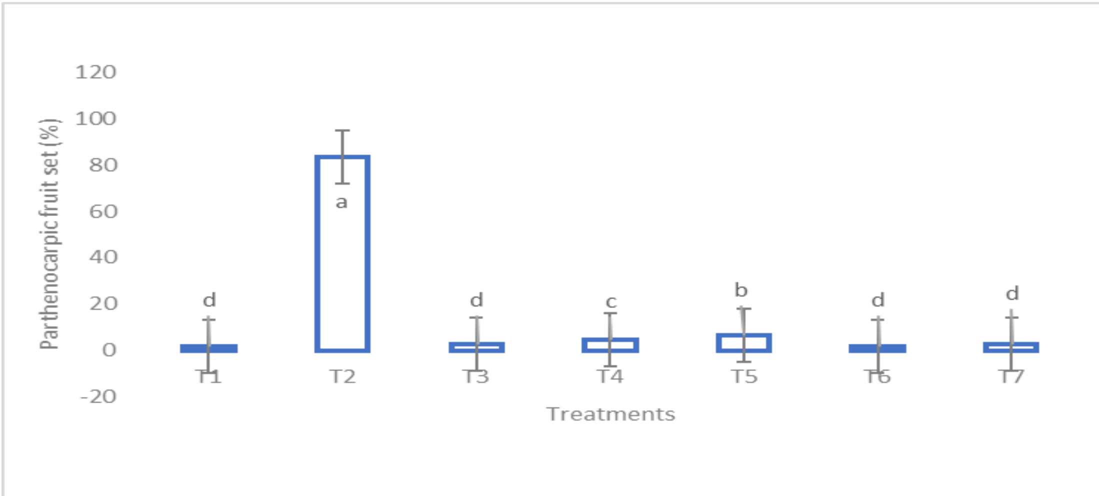

Fig. 4 Seedless fruit set (%) of Barhee cultivar by (T1) control pollination without bagging and spraying, (T2) bagging with brown paper without pollination, (T3) Pollination with brown paper, (T4) Pollination with white paper, (T5) Pollination with spraying boron, (T6) Pollination with spraying boron + brown paper, (T7) Pollination with spraying boron + white paper, (n= 4; means±SE). Means denoted by different letters differ significantly at $P \leq 0.05$.

Discussion

The levels of hormones were different in flowering and affected by the type of fruits and treatments (Table 1). The presence of hormones in both types of flowers/fruits suggests that the abnormal development of the seedless fruit may be attributable to a temperature difference or the absence or addition of some compounds, such as the element boron. Many plant fruits also observed higher levels of IAA and GA_3 (Serrani *et al*. 2007; Tiwari *et al*. 2012). Elevated amounts of these hormones in date palm tissue culture flowers are likely among the factors responsible for forming Parthenocarpic fruit (PF) 'shees'. Several studies investigated the phytohormones content of date palm fruits in early stages; for example, Cheruth *et al*. (2015) found that GA levels appear to be much more significant in early flowering types, while higher amounts of ABA may lead to late flowering in mid and late varieties. Shareef and AL-khayri (2020) found when compared to the fruit bagging treatment, direct sunshine lowered the content of phytohormones such as indoleacetic acid, gibberellin, and zeatin while increasing abscisic acid accumulation. Elbar *et al*. (2023) found that endogenous ABA levels in date fruit pericarp have been demonstrated to rise at the fruit green-to-yellow transition and increase consistently throughout fruit ripening. Auxins, gibberellic acids, and cytokinins are major players in fruit set induction. Furthermore, the synergistic and antagonistic interplay between these hormones is critical in determining the fate of the fruit set (Sharif *et al*., 2022). GA has been shown to promote the initiation and development of flowers and to enhance pollen germination and pollen tube growth (Singh *et al*., 2002). On the other hand, CKs have been shown to promote cell division and differentiation and enhance flower induction and development (Park *et al*., 2021). In particular, the high levels of IAA and KC observed in the pollination treatments involving brown paper and white paper suggest that these plant hormones could contribute to the enhanced fruit set followed in these treatments (Table 2). The results also indicate that the application of boron may have further improved the levels of these hormones and the fruit set (Fig. 3). The highest levels of GA_3 were found in T2 after pollination (Table 2), the highest percentage of seedless fruits was recorded in same the treatment (Fig. 4). Variation in Parthenocarpic fruit development rates caused by exogenous hormone treatment is related with changes in Boron concentration in floral tissues and regulation of VvBOR3 and VvBOR4 expression levels (Alva *et al*., 2015). The application of boron has been shown to enhance the levels of various plant hormones, including auxins, gibberellins, and cytokinins, which are essential regulators of flower development, pollination, and fruit set. Boron stimulates the synthesis and transport of auxins (Table 2), which promote cell division and elongation, and enhance flower development and pollination. Moreover, boron has been shown to increase the levels of cytokinins, which promote cell division and differentiation, and improve flower induction and development. After pollination, spraying boron and covering the inflorescences boosted the percentage of fruit set, productivity, and fruit quality of the Barhee date palm (Abd-elhaleem *et al*., 2019). The mechanisms by which boron enhances hormone synthesis and activity are not fully understood. Nonetheless, it is thought that boron may interact with enzymes involved in hormone synthesis and metabolism and control the expression of genes involved in hormone biosynthesis (Shireen *et al*. 2018). Boron may also affect the transport of hormones within the plant by regulating the permeability of cell membranes or by modifying the activity of transport proteins (Miwa and Fujiwara, 2010). The results presented in the manuscript suggest that the application of boron, and using brown paper or white paper during pollination, may enhance the levels of plant hormones and improve fruit set in Barhee cultivar. To my knowledge, no studies specifically investigate the combined effect of bagging and Boron on the levels of plant hormones and flower set and pollination. However, maybe the application of boron during bagging could enhance the levels of plant hormones and improve flower development and pollination, mainly if bagging is done in a way that excessively reduces light and airflow to the flowers. Moreover, the temperature inside the bags was observed to be higher than the temperature outside the bags in both the brown paper and white paper treatments (Table 1), which in turn can affect the success of pollination in Barhee cultivar. The 25-30°C temperature range during the day and 17-22°C at night is generally considered optimal for flower development and pollination in date palms (Salomón-torres *et al*., 2021). High temperatures can cause heat stress to the developing pollen grains and inhibit their germination and tube growth. In contrast, low temperatures can reduce the metabolic activity of the pollen grains, leading to reduced pollen viability and germination (Çetinbaş-genç *et al*., 2019). Therefore, maintaining optimal temperature conditions is crucial for successful pollination in date palm. The temperature has been shown to significantly affect the levels of GAs and ABA in flowers, leading to Parthenocarpic fruit (Table 2), two crucial plant hormones involved in flower development and pollination (LI *et al*., 2021). Gibberellins (GAs) are plant hormones that promote stem elongation, cell division, and flower development. GA levels are typically higher in warm temperatures and lower in cold temperatures. The high-temperature treatments can increase GA levels, increasing flower bud initiation and development (Gupta and Chakrabarty, 2013). ABA is a hormone that regulates plant responses to stress, including drought, high salinity, and cold temperatures. ABA levels are typically higher in cold temperatures and lower in warm temperatures (Sah *et al*., 2016). Other results have shown that cold temperature treatments can increase ABA levels in plants, leading to reduced flower bud initiation and development (Vishwakarma *et al*., 2017). Finally, the study found that bagging can affect the temperature inside the bags, affecting the success of pollination in Barhee cultivar. Inside the bags, the temperature was higher than outside, with the increase in temperature being more pronounced in the white paper treatment. High temperatures inside the bags can cause heat stress to the developing pollen grains and inhibit their germination and tube growth. In contrast, low temperatures can reduce the metabolic activity of the pollen grains, leading to reduced pollen viability and germination (Rieu *et al*., 2017). As a result, maintaining optimal temperature conditions is crucial for successful pollination in date palms.

Conclusion

Overall, the results suggest that understanding the specific temperature requirements of different plant species and the effects of temperature on hormone levels can help optimize plant growth and improve pollination. Bagging without pollination leads to parthenocarpic fruit. The data also indicate that the level of phytohormones in the developing fruits varies significantly among the treatments, which may be related to the observed differences in the fruit set. These findings may have practical implications for enhancing fruit production in Barhee and other similar cultivars. However, additional research is required to examine the underlying mechanisms and validate the findings in diverse growing circumstances and locales.

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درجة الحرارة المثالية والبورون تنظم محتوى الهرمونات النباتية لتحسين عقد ثمار صنف نخيل التمر (*Phoenix dactylifera* L.) البرحي الناتج من زراعة الأنسجة

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الخلاصة

تعتبر درجة الحرارة أحد العوامل الحاسمة التي يمكن أن تؤثر على نجاح التلقيح في نخيل التمر. تناول البحث تأثير معاملات التكييس والرش بالبورون على درجة الحرارة ومستويات الهرمونات وعقد الثمار في صنف نخيل التمر البرحي المشتق من زراعة الأنسجة. كما قامت الدراسة بقياس مستويات الهرمونات النباتية ومنها حامض اندول الخليك وحامض الجربلين والمركب الشبيه بالكينيتين وحامض الأبسيسيك قبل وبعد التلقيح تحت معاملات مختلفة. وأظهرت النتائج أن معاملات التكييس أثرت بشكل كبير على درجة الحرارة داخل الأكياس، مع ملاحظة ان درجات حرارة أعلى في معالجة الورق الأبيض. كما أظهرت النتائج أن مستويات هذه الهرمونات تختلف باختلاف المعاملة ومرحلة نمو الثمرة. تظهر المستويات العالية من الاوكسين والسايتوكاينين في معاملات التلقيح التي تتضمن الورق البني والورق الأبيض، وتم العثور على أعلى مستويات للجبرلين في التكييس بالورقالاسمر بدون تلقيح. ثبت أن درجة الحرارة تؤثر بشكل كبير على مستوياتالجبرلين وحامض الابسيسك في الازهار، مما يؤدي إلى ظهور ثمار عذرية. أظهرت المعاملة برش البورون والتكييس بالورق الاسمر أعلى نسبة لعقد الثمار البذرية بينما أدى التكييس بالورق الاسمر بدون تلقيح إلى أعلى نسبة لعقد الثمار بدون بذور. تسلط الدراسة الضوء على أهمية إدارة معاملات التكييس بعناية من أجل نجاح التلقيح وتكوين الثمار في زراعة نخيل التمر. ويعد الحفاظ على ظروف درجة الحرارة المثلى أمرًا بالغ الأهمية لنجاح التلقيح في أشجار النخيل.

الكلمات المفتاحية: حامض الأبسيسك، التكييس، عقد الثمار، إندول حامض الخليك، حامض الجربلين، حامض البوريك، التلقيح.