

A hormonal study of the phenomenon of parthenocarpic fruits in the date palm *Phoenix dactylifera* L. of the Barhi cultivar derived from tissue culture

Salah A. Ghailan^{1,3*}, Aqeel A. Suhim¹, Khairullah M. Awad²

¹ Department of Horticulture and Landscape, College of Agriculture, University of Basrah, Iraq.

² Date palm Research Centre, University of Basrah, Iraq

³ Department of Plant Protection, College of Agriculture, University of Misan, Iraq

*Email: salah.ghilan@uomisan.edu.iq

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Abstract

Phytohormones play a pivotal role in the growth and development of plants, including date palms. This study investigates the dynamics of four key hormones—indole-3-acetic acid (IAA), gibberellic acid (GA3), abscisic acid (ABA), and kinetin (Kin) across various developmental stages (pre-flowering, flowering, and fruiting) in three date palm phenotypes: phenotype derived from tissue culture and producing abnormal fruits (shees), phenotype derived from tissue culture and producing normal fruits (normal), and phenotype propagated by offshoots and producing normal fruits (vegetal). The results showed that IAA and GA3 levels in leaves exhibited a significant decline in the shees phenotype, while the normal and vegetal phenotypes displayed decreases from the pre-flowering to flowering stages, followed by increases in the fruiting stage. Interestingly, the vegetal phenotype showed a unique rise in IAA and GA3 levels during the fruiting stage. In flowers and fruits, the normal phenotype consistently exhibited the highest IAA and GA3 levels, while the shees phenotype displayed the lowest. Conversely, ABA levels showed distinct patterns among the phenotypes. The shees phenotype exhibited a sharp increase from pre-flowering to flowering, followed by a slight decrease in the fruiting stage. The normal phenotype displayed a significant decrease from pre-flowering to flowering, with a slight increase in the fruiting stage. The vegetal phenotype maintained relatively constant ABA levels. Kinetin levels displayed variations, with the normal phenotype consistently showing the highest

concentrations. Leaves had the highest Kin levels during the pre-flowering stage, while fruits exhibited the lowest levels.

Keywords: ABA, GA3, Hormonal interactions, IAA, Kin, Parthenocarpic fruits

Introduction

The Phoenix dactylifera L., commonly known as the date palm, holds significant importance in the arid regions of the Middle East and North Africa. It serves as a staple fruit tree with far-reaching impacts on various aspects, including social, economic, environmental, and agricultural. In Iraq, the cultivation of date palms plays a crucial role in the fabric of society and the economy (Suhim et al., 2023). The conventional propagation techniques that rely on offshoots are confronted with practical limitations in terms of commercial viability. This traditional approach is impeded by various factors, such as the limited number of offshoots generated during the lifespan of a palm, low rates of planting survival, and the potential spread of diseases and pests (Hasan et al., 2022). The utilization of tissue culture propagation techniques has brought about a significant transformation in the cultivation of date palms, enabling swift and extensive propagation on a larger scale. Among the various methods currently employed globally, organogenesis and somatic embryogenesis have emerged as the primary techniques for the mass-propagation of date palms (Naik & Al-Khayri, 2016). The capacity to produce genetically homogeneous and enduring date palms in vitro is a crucial objective. However, this progress has been accompanied by the emergence of undesirable plant off-types resulting from somaclonal variations, a phenomenon that is particularly widespread in date palms (Mirani et al., 2019). In tissue culture-generated date palm trees, off-types frequently manifest a reduced ability to set fruit. Typically, the flowers on such trees undergo parthenocarpic fruit development, characterized by the presence of three carpels. In more severe cases, supernumerary carpels emerge, accompanied by distortions of carpels and stigmas. Notably, these anomalies tend to ameliorate as the trees age, with roughly 50% of affected trees returning to normal fruiting within ten years following transplantation (Cohen et al., 2004). Although this phenomenon has been observed in other cultivars of date palm, its severity in the 'Barhi' cultivar is particularly noteworthy, with prevalence rates ranging from 59% to 86% (Al Khateeb, 2008). Several researchers have worked over the years to determine the nature and probable explanations of this phenomenon. (H. M. Ali-Dinar & Alkhateeb, 2005) posited that the absence of regular fruiting in juvenile tissue culture-derived date palm, specially the 'Barhi' cultivar, may be ascribed to a

series of interconnected occurrences that culminate in tardy pollen tube growth during the initial phases of fruit maturation. This tardiness may be exacerbated by heightened levels of abscisic acid (ABA) during this crucial period. Moreover, Bouhouche et al. (2007) conducted a comprehensive assessment of the fertilization failure phenotype in elite date cultivars, 'Khllass' and 'Barhi,' with a focus on different propagation techniques. While organogenesis proved successful, yielding highly productive trees with fertilization percentages of 94-98% for both cultivars, somatic embryogenesis was associated with abnormal fruit set. Interestingly, direct and indirect embryogenesis both led to high fruit set failure in Khllass and Barhi cultivars. However, only plants propagated through indirect embryogenesis exhibited heightened susceptibility to this abnormality, with most anomalous parthenocarpic fruits featuring 2-5 carpels. Furthermore, (Hadi et al., 2015) delved into the hormonal intricacies underlying date palm flower and early fruit development, aiming to identify the hormones linked to shees fruit formation. Their analysis of hormone levels in young flowers/fruits of Barhi and Nabtet-Saif cultivars revealed that, among the seven hormones detected by high-performance liquid chromatography (HPLC), levels of indole-3-acetic acid (IAA) and gibberellic acid (GA3) were significantly elevated in shees flowers/fruits. Conversely, levels of a kinetin-like compound and an unknown compound were significantly diminished in shees counterparts compared to normal ones.

Recently, a notable phenomenon, locally named 'shees,' has emerged in tissue culture-derived 'Barhi' date palms within the Basrah Governorate-Iraq.

The aim of this study is to determine the specific hormonal changes that occur during different stages of flower development, pollination, and fruit set in both 'shees' affected and healthy date palms.

Materials and Methods

Sample Collection

Samples of flowers, fruits, and leaves were systematically collected from date palm orchards located in the Al-Midaina district within the Basrah governorate, situated in the southern region of Iraq.

Plant Selection

For this study, tissue culture-derived plants belonging to the 'Barhi' cultivar were chosen based on their distinctive fruiting behaviors. Specifically, plants known for their normal fruiting behavior at 10 years of age and those exhibiting shees production at 7 years of age were

identified for sample collection. These selections were made based on their documented fruiting records over the course of the two preceding seasons. Additionally, plants propagated through the conventional method of offshoots at 7 years of age were also included in the study.

Pollination practice

To ensure precise control over the pollination process and to prevent unintended out-crossing, the spathes chosen for the experiment were securely covered with paper bags prior to their natural opening. Once the spathes had naturally opened, a controlled pollination process was carried out using a specific male cultivar, namely Ghanami ahmar during the period from 28/3/2022 to 1/4/2022. Following this, the inflorescences were once again securely covered with paper bags to maintain a controlled pollination environment.

Samples collection and Sampling Time Points

Sampling was conducted at three distinct time points. Initially, prior to the emergence of spathes, only leaves were collected during January 2022, denoted as the pre-flowering stage. The second sampling took place subsequent to the natural opening of spathes. At this juncture, three rachillae bearing flowers were meticulously collected from the central region of the inflorescences, immediately before pollination. Simultaneously, leaf samples were obtained, constituting the flowering stage. The third sampling event entailed the collection of fruit samples at the Kimri stage,' along with leaf samples, precisely 30 days post-pollination, marking the fruiting stage. All collected samples were transported to the laboratory and stored at a temperature of -20°C for a period of 10 to 30 days, preparatory to the subsequent hormone extraction process

Phytohormones extraction

samples weighing ten grams each were homogenized in 70% (v/v) methanol and allowed to stir overnight at 4°C. Subsequently, the extract underwent filtration using a Whatman filter, with the methanol being evaporated under vacuum conditions. The resulting aqueous phase was then adjusted to a pH of 8.5 using a 0.1 M phosphate buffer, after which it was subjected to partitioning with ethyl acetate three times. Following the removal of the ethyl acetate phase, the pH of the aqueous solution was subsequently lowered to 2.5 through the addition of 1 N HCl. Further partitioning ensued, involving the aqueous solution being treated with diethyl ether three times. The solution was then passed through anhydrous sodium sulfate for purification. The diethyl ether phase was subsequently evaporated under vacuum, leaving behind a dry residue containing the targeted hormones. To ensure optimal storage and stability, the dry hormone-

containing residue was dissolved in 2.0 mL of methanol and securely stored in vials at a temperature of 4°C (Kelen et al., 2004).

HPLC analysis

HPLC analysis was performed on high performance liquid chromatography HPLC model (SYKAM) Germany, the mobile phases used were acetonitrile-water (26:74 % v/v). The separation was column C18-ODS (25 cm * 4.6 mm) was equilibrated for mobile phase condition. The separation was carried out by isocratic elution with a flow rate of 0.8 mL/min. The detector was UV - VIS at 280 nm. The analysis was conducted in the Ministry of Science and Technology laboratories in Baghdad-Iraq.

Statistical analysis

Experiments that generated quantifiable results were performed with three replicates, and the resulting data underwent analysis through an F test utilizing SPSS version 22 software (SPSS Inc., Chicago, IL, USA). With a significance level set at <0.05, the LSD test was employed to least significant difference among the experimental means.

Statistical analyses were conducted to compare the levels of the investigated phytohormones in leaves sample at examined developmental stages. Additionally, separate statistical comparisons were performed between flowers and fruits at flowering and fruiting stages.

Results

Utilizing HPLC techniques, four hormones (IAA, GA3, ABA, and Kin) within samples extracted from leaves, flowers, and fruits of the three distinct date palm types at various developmental stages (pre-flowering, flowering, and fruiting). were detected. The results for each hormone are detailed as follows.

IAA levels in the leaves

The results of Table 1 show the analysis of the concentration of the indole-3-acetic acid (IAA) hormone in the leaves of three types of date palms—Shees, Normal, and Vegetal—across three stages of growth: pre-flowering, flowering, and fruiting. For the shees type, the IAA levels exhibited a trend of decrease from the pre-flowering stage (17.8 ppm) to the flowering stage (8.99 ppm) and then further decreased in the fruiting stage (6.58 ppm). Similarly, the normal type showed a pattern of decrease from the pre-flowering stage (18.68 ppm) to the flowering stage (16.55 ppm), but then experienced a substantial increase in the fruiting stage (22.89 ppm). The vegetal type, Interestingly, the vegetal type initially experienced a decrease in IAA from the

pre-flowering stage (16.77 ppm) to the flowering stage (15.89 ppm), but exhibit a notable increase in the fruiting stage (22.84 ppm). When examining the means of types, the normal type displayed the highest average IAA content across all stages (20.37 ppm), which is significantly different from the other types. The vegetal type exhibited the second highest mean (18.50 ppm), while the shees type had the lowest mean (11.12 ppm). Observing the stage means, it becomes evident that the fruiting stage stands out with the highest average IAA content across all types (18.43 ppm), showing a notable distinction from the other stages. Subsequently, the pre-flowering stage presents the second-highest mean (17.75 ppm), followed by the flowering stage with the lowest mean (13.81 ppm). In terms of the interaction between type and stage, the highest IAA value was observed in the normal type during the fruiting stage (22.89 ppm), indicating its potential influence on fruit development. On the other hand, the lowest IAA value was found in the shees type during the fruiting stage (6.58 ppm), underscoring its distinct behavior in terms of IAA concentration.

IAA levels in the flowers and fruits

The data in table 2 presents the results of IAA levels in both flowers and fruits during the flowering and fruiting stages, revealing significant differences. Notably, in the flowering stage, normal flowers exhibited the highest IAA content at 21.48 ppm, significantly surpassing the levels observed in shees flowers (10.25 ppm) and vegetal flowers (18.59 ppm). In the fruiting stage, the pattern continued, with normal fruits showing the highest IAA levels at 21.25 ppm, followed by vegetal fruits at 19.55 ppm, while shees fruits had the lowest IAA content at 9.25 ppm. These results highlight the presence of significant differences in IAA concentrations between the different plant parts and stages of development. Additionally, considering the mean IAA levels across each type, normal had the highest average IAA content of 21.36 ppm, which was significantly different from the averages of vegetal (19.07 ppm) and shees (9.75 ppm). Similarly, when analyzing the mean IAA levels across both plant parts, the flower part displayed a mean of 16.77 ppm, significantly differing from the slightly lower average of 16.68 ppm observed in the fruit part.

Table 1. IAA concentrations in Date Palm Leaves Across Various Growth Stages and Phenotypes

Type	Stage			Mean of type
	Pre-flowering	Flowering	Fruiting	
Shees	17.80	8.99	6.58	11.12c

Normal	18.68	16.55	22.89	20.37a
Vegetal	16.77	15.89	22.84	18.50b
Mean of stage	17.75b	13.81c	18.43a	

Table 2. IAA Concentrations in Flowers and Fruits Across Flowering and Fruiting Stages in Various Date Palm Phenotypes

Type	part		Mean of type
	Flower	fruit	
Shees	10.25	9.25	9.75c
Normal	21.48	21.25	21.36a
Vegetal	18.59	19.55	19.07b
Mean of part	16.77a	16.68b	

GA3 levels in the leaves

Table 3 provides an analysis of the gibberellic acid (GA3) hormone concentration in the leaves of three distinct types of date palms—shees, normal, and vegetal—across three critical stages of growth: pre-flowering, flowering, and fruiting. The shees type exhibits a notable decrease in GA3 levels from the pre-flowering stage (27.60 ppm) to the flowering stage (10.28 ppm), followed by a further decline in the fruiting stage (7.44 ppm). Meanwhile, the normal type experiences a modest, non-significant increase from the pre-flowering stage (25.13 ppm) to the flowering stage (25.50 ppm), but subsequently registers a decrease in the fruiting stage (22.57 ppm). The vegetal type, however, demonstrates an initial marginal decrease in GA3 from the pre-flowering stage (24.92 ppm) to the flowering stage (22.84 ppm), which persists into the fruiting stage (19.89 ppm). When focusing on the type means, the normal type takes the lead with the highest average GA3 content across all stages (24.40 ppm), marking a significant difference from the other types. Following this, the vegetal type showcases the second highest mean (22.43 ppm), while the shees type records the lowest mean (15.10 ppm). Turning to the stage means, it becomes apparent that the pre-flowering stage claims the spotlight with the highest average GA3 content across all types (25.88 ppm), a distinct contrast from the other stages. Subsequently, the flowering stage holds the second-highest mean (19.42 ppm), with the fruiting stage displaying the lowest mean (16.63 ppm). In terms of the interaction between type and stage, the normal type reaches the peak GA3 value during the flowering stage (25.50 ppm), indicating the highest point of this hormone concentration. On the other hand, the shees type

reaches its lowest GA3 value during the fruiting stage (7.44 ppm), marking the lowest point in this hormone's concentration for this particular type and stage.

GA3 levels in the flowers and fruits

Table 4 shows the data on GA3 levels found in both flowers and fruits during the flowering and fruiting stages and shows significant differences. Particularly during the flowering stage, normal type flowers have the highest GA3 content at an impressive 33.59 ppm, which is a significant different to vegetal type flowers at 27.44 ppm and shees type flowers at 17.58 ppm. This trend continues into the fruiting stage, with normal type fruits remaining at the top with the highest GA3 content of 14.55 ppm, followed by vegetal type fruits at 10.78 ppm. In contrast, shees type fruits have the lowest GA3 content at just 5.1 ppm. Additionally, when the average GA3 content of each type is analyzed, the normal type has the highest average GA3 content of 24.07 ppm, which is significantly higher than the averages of the vegetal (19.11 ppm) and shees (11.35 ppm). Furthermore, when mean GA3 levels in both plant parts are examined, the flower part has a mean of 26.0 ppm, which is significantly higher than the lower average of 10.15 ppm observed in the fruit part.

Table 3. GA3 concentrations in Date Palm Leaves Across Various Growth Stages and Phenotypes

Type	Stage			Mean of type
	Pre-flowering	Flowering	Fruiting	
Shees	27.60	10.28	7.44	15.10c
Normal	25.13	25.50	22.57	24.40a
Vegetal	24.92	22.84	19.89	22.43b
Mean of stage	25.88a	19.42b	16.63c	

Table 4. GA3 Concentrations in Flowers and Fruits Across Flowering and Fruiting Stages in Various Date Palm Phenotypes

Type	part		Mean of type
	Flower	fruit	
Shees	17.58	5.12	11.35c
Normal	33.59	14.55	24.07a
Vegetal	27.44	10.78	19.11b
Mean of part	26.20a	10.15b	

ABA levels in the leaves

Table 5 presents an analysis of the concentration of the abscisic acid (ABA) hormone in the leaves of three distinct types of date palms, namely shees, normal, and vegetal, during three critical stages of growth, namely pre-flowering, flowering, and fruiting. The shees type exhibits a significant increase in ABA levels from the pre-flowering stage (8.08 ppm) to the flowering stage (33.25 ppm), followed by a slight decrease in the fruiting stage (30.89 ppm). In contrast, the normal type experiences a sharp decline from the pre-flowering stage (33.08 ppm) to the flowering stage (8.55 ppm), but subsequently experiences a slight increase in the fruiting stage (9.55 ppm). The vegetal type, however, shows a consistent level of ABA during the three stages, without significant differences, which were 10.12, 10.25, and 10.89 ppm for pre-flowering, flowering, and fruiting stage respectively. When considering the type means, the shees type has the highest average ABA content across all stages (24.07 ppm), marking a significant difference from the other types. Following this, the normal type showcases the second-highest mean (16.06 ppm), while the vegetal type records the lowest mean (10.42 ppm). Regarding the stage means, it was clear that the flowering stage had the highest mean ABA content among all the stages (17.35 ppm). Next, the fruiting stage had the second highest average (17.11 ppm), while the pre-flowering stage had the lowest average (16.09 ppm). In terms of the interaction between type and stage, the highest value of ABA has been observed in the shees type during the flowering stage (33.25 ppm). Also, the shees type shows the lowest ABA value during the pre-flowering stage (8.08 ppm).

ABA levels in the flowers and fruits

Table 6 presents noteworthy variations in the levels of abscisic acid (ABA) detected in both flowers and fruits during the flowering and fruiting stages. The results indicate that shees type flowers exhibited the highest concentration of ABA throughout the flowering stage, with a remarkable value of 41.88 parts per million (ppm), which is significantly greater than the ABA levels observed in vegetal type flowers (15.15 ppm) and normal type flowers (12.05 ppm). A similar trend was observed at the fruiting stage, where shees type fruits displayed the highest ABA level (22.25 ppm), followed by vegetal type fruits (10.45 ppm). In contrast, normal-type fruits exhibited the lowest ABA level (8.55 ppm). Furthermore, when the average ABA content of each type is considered, the shees type has the highest average ABA content of 32.06 ppm, which is significant from the averages of the vegetal (12.80 ppm) and normal (10.30 ppm). In addition, when mean ABA levels in both plant parts are examined, the flower part has a mean of

23.02 ppm, which is significantly higher than the lower average of 13.75 ppm found in the fruit part.

Table 5. ABA concentrations in Date Palm Leaves Across Various Growth Stages and Phenotypes

Type	Stage			Mean of type
	Pre-flowering	Flowering	Fruiting	
Shees	8.08	33.25	30.89	24.07a
Normal	33.08	8.55	9.55	16.06b
Vegetal	10.12	10.25	10.89	10.42c
Mean of stage	16.09c	17.35a	17.11b	

Table 6. ABA Concentrations in Flowers and Fruits Across Flowering and Fruiting Stages in Various Date Palm Phenotypes

Type	part		Mean of type
	Flower	fruit	
Shees	41.88	22.25	32.06a
Normal	12.05	8.55	10.30c
Vegetal	15.15	10.45	12.80b
Mean of part	23.02a	13.75b	

Kin levels in the leaves

The present study investigated the levels of Kinetin (Kin) hormone in date palm leaves (Frag et al., 2012), as presented in Table 7. The results revealed a significant decrease in Kin concentration in shees type leaves, from 25.43 ppm at the pre-flowering stage to 15.58 ppm at the flowering stage, which further declined to 11.58 ppm at the fruiting stage. A similar trend was observed in normal type leaves, with Kin levels of 27.08, 23.69, and 21.00 ppm at the pre-flowering, flowering, and fruiting stages, respectively. In contrast, vegetal type leaves exhibited a different pattern, with Kin content decreasing from 25.91 ppm at the pre-flowering stage to 10.25 ppm at the flowering stage, but subsequently increasing to 19.21 ppm at the fruiting stage. According to the results of the type means, it is revealed that the normal type displays the highest mean Kin content across all stages, with a value of 23.92 ppm. This observation highlights a significant differentiation from the other types. Subsequently, the vegetal type exhibits the second-highest average Kin content, with a value of 18.45 ppm, while the shees type records the lowest mean Kin content, with a value of 17.53 ppm. In relation to the stages, it was evident that the pre-flowering stage exhibited the greatest mean concentration of Kin in comparison to all other stages (26.14 ppm). Subsequently, the fruiting stage demonstrated the second highest mean

concentration (17.26 ppm), whereas the flowering stage displayed the lowest mean concentration (16.50 ppm). Regarding the interaction between type and stage, it has been observed that the normal type exhibits the highest Kin value during the pre-flowering stage, with a recorded value of 27.08 ppm. At the same time, the normal type displays the lowest Kin value during the flowering stage, with a recorded value of 10.25 ppm.

Kin levels in the flowers and fruits

Table 8 presents the Kin levels data obtained from flowers and fruits during the flowering and fruiting stages. The results indicate that normal type flowers exhibited the highest Kin content during the flowering stage, with a remarkable concentration of 30.12 ppm. This value was significantly different from the Kin content of vegetal type flowers, which measured 25.44 ppm, and shees type flowers, which measured 17.44 ppm. This trend persisted into the fruiting stage, with normal type fruits recorded the highest Kin content at 17.89 ppm, followed by vegetal type fruits at 15.74 ppm. While, shees type fruits displayed the lowest Kin content, measuring only 10.25 ppm. Moreover, upon conducting an analysis of the average Kin content of each type, it was observed that the normal type exhibited the highest average Kin content of 24.00 ppm. This value was found to be significantly greater than the means of the vegetal (18.45 ppm) and shees (13.84 ppm) types. Additionally, upon examining the mean Kin levels in both plant parts, it was observed that the flower part exhibited a mean of 24.33 ppm, which was significantly higher than the lower average of 14.62 ppm observed in the fruit part.

Table 7. Kin concentrations in Date Palm Leaves Across Various Growth Stages and Phenotypes

Type	Stage			Mean of type
	Pre-flowering	Flowering	Fruiting	
Shees	25.43	15.58	11.58	17.53c
Normal	27.08	23.69	21.00	23.92a
Vegetal	25.91	10.25	19.21	18.45b
Mean of stage	26.14a	16.50c	17.26b	

Table 8. Kin Concentrations in Flowers and Fruits Across Flowering and Fruiting Stages in Various Date Palm Phenotypes

Type	part		Mean of type
	Flower	fruit	
Shees	17.44	10.25	13.84c
Normal	30.12	17.89	24.00a
Vegetal	25.44	15.74	20.59b

Mean of part	24.33a	14.62b	
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Discussion

The importance of synchronized hormonal processes in fruit formation has been widely acknowledged in various plant species (Fenn & Giovannoni, 2021). The results from this study suggest that various hormones, such as IAA, GA3, Kin (growth-promoting hormones), and ABA (a growth-inhibiting hormone), play significant roles in the development of date palm fruit. Notably, our findings indicate that IAA, GA3, and Kin levels were notably lower in date palms of the shees phenotype compared to the normal and vegetal phenotypes. This trend was observed in samples from both flowers and fruits, as well as in leaf samples, particularly during the flowering and fruiting stages. On the contrary, ABA exhibited contrasting patterns. The results of this study are consistent with the observations made by (H. M. Ali-Dinar & Alkhateeb, 2005), who reported higher levels of abscisic acid (ABA) in un-pollinated fruits compared to their pollinated counterparts. The authors attributed the failure of pollination and fertilization to the delayed growth of pollen tubes during the initial stages of fruit development, which was linked to the elevated levels of ABA. The current study's observations align with (Setha et al., 2004) findings, which highlighted the complex antagonistic interplay between the plant hormone ABA and hormones IAA and GA3 throughout different fruit development stages. Additionally, the results regarding Kin in this study are consistent with this observed pattern. The results of the present investigation were incongruent with the study conducted by (Hadi et al., 2015), which reported that un-pollinated of date palm fruits exhibit heightened concentrations of IAA and GA3 in comparison to their pollinated counterparts. Nonetheless, their study lacks data on ABA concentration. (Shahsavari & Shahhosseini, 2021) have reported that auxins are involved in directing the growth of pollen tubes towards the egg cell within the pistil. A dearth or disproportion of auxin concentrations may lead to unsuccessful pollination, ultimately leading to the production of seedless fruits (shees). Moreover, it has been established that GA3 plays a crucial role in the process of pollination. This assertion is supported by the observation of mutants that lack GA3 or exhibit insensitivity to it, which provides compelling evidence for the significance of GA synthesis and its associated signaling pathways in the initiation of flowers and the development of pollen tubes (Chhun et al., 2007). Despite the considerable research attention given to date palms, the main focus has largely been on studying hormone levels during

various stages of fruit development, from early growth to full maturation. In contrast, there is a notable lack of knowledge when it comes to understanding hormone concentrations in leaves during the crucial flowering and fruiting stages. This current study addresses this gap in knowledge by examining the levels of phytohormones in date palm leaves at three distinct phases: pre-flowering, flowering, and fruiting. Investigating leaves is particularly significant because they play a central role in secondary metabolism, including the synthesis of hormones. The findings of this study suggest that there are no statistically significant variations in the levels of IAA and GA3 during the pre-flowering stage among all phenotypes. Nevertheless, during the flowering stage, both hormones exhibited a round twofold increase, while during the fruiting stage, they demonstrated a round threefold increase in both natural and vegetal phenotypes, compared to the shees phenotype. The present study has uncovered that the concentration of abscisic acid (ABA) in leaves of normal phenotype during the pre-flowering stage was four times higher than that in leaves of shees phenotype. However, as the transition to the flowering phase occurred, this pattern was entirely reversed. The concentration of ABA in leaves of shees phenotype increased, while it decreased in leaves of normal phenotype, ultimately reaching only a quarter of the concentration observed in the shees phenotype. This trend persisted into the fruiting stage. Notably, the findings also demonstrated that the ABA concentration in leaves of vegetal phenotype remained relatively stable across all three phases investigated. Regarding the Kin results, it's noteworthy that during the pre-flowering stage, the concentration was consistent across the leaves of all three phenotypes being studied. However, as the transition to the flowering stage occurred, there was a decrease in Kin concentration in all phenotypes. Notably, the decrease in the normal phenotype was lower than shees and vegetal phenotypes. Upon reaching the fruiting stage, the concentration of Kin continued to decline in the normal and shees phenotypes, while in contrast, it increased in the vegetal phenotype. The present study's findings are consistent with those of (Cheruth et al., 2015), who conducted a study on three distinct cultivars of date palms, namely early, medium, and late flowering, to investigate the levels of hormones such as indole-3-acetic acid (IAA), gibberellic acid (GA3), zeatin, and abscisic acid (ABA) in their leaves. Their research revealed that the concentration of ABA exhibited stage-dependent changes, with higher levels during the pre-flowering stage, a gradual decrease during flowering, and subsequent increases after flowering. Furthermore, IAA concentration was notably elevated during the flowering induction period across all date palm varieties, while GA

concentration was significantly elevated in the early flowering varieties of date palms. The observed fluctuations in hormone levels within the leaves, flowers, and fruits of the date palm tree are suggestive of underlying physiological and metabolic changes. These changes are likely to be influenced by environmental factors and the specific phenotype of the tree. The modulation of hormone levels is believed to exert a significant influence on fruit production, potentially leading to both anomalous and typical fruiting outcomes (H. Ali-Dinar et al., 2021). According to Setha et al. (2004), the ABA hormone was found to have a negligible impact on the growth stage of the fruit. Its sudden decrease, however, facilitates the promotion of effective early fruit growth and development. In general, the role of hormones in the process of fruit-set is substantiated by significant evidence, including the observation that exogenous applications of hormones are capable of initiating fruit development. Additionally, parthenocarpic mutants, which are typically impaired in hormone biosynthesis and/or signaling, further support the involvement of hormones in fruit-set. Specifically, gibberellins (GAs), auxins, and, in certain species, cytokinins have been demonstrated to be highly effective in stimulating fruit growth (Dorcey et al., 2009).

Conclusion

In conclusion, the present study emphasizes the dynamic interplay of phytohormones in the leaves, flowers, and fruits of the date palm tree during critical developmental stages. Notably, the hormones IAA, GA3, and Kin exhibited stage-dependent variations, displaying distinct behaviours in different phenotypes. Conversely, ABA demonstrated contrasting patterns, particularly in the shees phenotype. These findings provide insight into the hormonal orchestration underlying date palm fruit development and the parthenocarpic phenomenon in plants derived from tissue culture. The research highlights the necessity for further investigations into the intricate hormonal mechanisms governing this process, with potential implications for enhancing fruit production and quality.

References

- Al Khateeb, A. A. (2008). The problems facing the use of tissue culture technique in date palm (*Phoenix dactylifera* L.). *Sci J King Faisal Univ*, 9, 85–104.
- Ali-Dinar, H. M., & Alkhateeb, A. A. (2005). Barhee” fruit setting problems at Kingdom of Saudi Arabia: Research approaches to understand the physiological and physical events of the phenomenon. *Proceeding the International Workshop on True-to-Typeness of Date Palm Tissue Culture-Derived Plants*, 121–127.
- Ali-Dinar, H., Mohammed, M., & Munir, M. (2021). Effects of pollination interventions, plant age and

- source on hormonal patterns and fruit set of date palm (*Phoenix dactylifera* L.). *Horticulturae*, 7(11), 427.
- Cheruth, A. J., Kurup, S. S., & Subramaniam, S. (2015). Variations in hormones and antioxidant status in relation to flowering in early, mid, and late varieties of date palm (*Phoenix dactylifera*) of United Arab Emirates. *The Scientific World Journal*, 2015.
- Chhun, T., Aya, K., Asano, K., Yamamoto, E., Morinaka, Y., Watanabe, M., Kitano, H., Ashikari, M., Matsuoka, M., & Ueguchi-Tanaka, M. (2007). Gibberellin regulates pollen viability and pollen tube growth in rice. *The Plant Cell*, 19(12), 3876–3888.
- Cohen, Y., Korchinsky, R., & Tripler, E. (2004). Flower abnormalities cause abnormal fruit setting in tissue culture-propagated date palm (*Phoenix dactylifera* L.). *The Journal of Horticultural Science and Biotechnology*, 79(6), 1007–1013.
- Dorcey, E., Urbez, C., Blázquez, M. A., Carbonell, J., & Perez-Amador, M. A. (2009). Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in *Arabidopsis*. *The Plant Journal*, 58(2), 318–332.
- Farag, K. M., Elsabagh, A. S., & ElAshry, H. A. (2012). Phytohormonal changes in fruits of 'Zaghloul' date palm in relation to metaxenic influences of used pollinators. *American-Eurasian Journal of Agricultural and Environmental Science*, 12(7), 862–871.
- Fenn, M. A., & Giovannoni, J. J. (2021). Phytohormones in fruit development and maturation. *The Plant Journal*, 105(2), 446–458.
- Hadi, S., Al-Khalifah, N. S., & Moslem, M. A. (2015). Hormonal basis of shees' fruit abnormality in tissue culture derived plants of date palm. *International Journal of Agriculture and Biology*, 17(3).
- Hasan, M., Abdullah, H. M., Hasibuzzaman, A. S. M., & Ali, M. A. (2022). Date Palm Genetic Resources for Breeding. *Cash Crops: Genetic Diversity, Erosion, Conservation and Utilization*, 479–503.
- Kelen, M., Demiralay, E. C., ŞEN, S., & ALSANCAK, G. Ö. (2004). Separation of abscisic acid, indole-3-acetic acid, gibberellic acid in 99 R (*Vitis berlandieri* x *Vitis rupestris*) and rose oil (*Rosa damascena* Mill.) by reversed phase liquid chromatography. *Turkish Journal of Chemistry*, 28(5), 603–610.
- Mirani, A. A., Teo, C. H., Abul-Soad, A. A., Markhand, G. S., Jatt, T., Mirbahar, A. A., & Solangi, N. (2019). Phenotypic reversion of somaclonal variants derived from inflorescence of date palm (*Phoenix dactylifera* L.) in the open field trials. *Sarhad J. Agric*, 35(3), 719–726.
- Naik, P. M., & Al-Khayri, J. M. (2016). Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) through cell suspension culture. *Protocols for In Vitro Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants, Second Edition*, 357–366.
- Setha, S., Kondo, S., Hirai, N., & Ohigashi, H. (2004). Xanthoxin, abscisic acid and its metabolite levels associated with apple fruit development. *Plant Science*, 166(2), 493–499.
- Shahsavari, A. R., & Shahhosseini, A. (2021). Pollen grain hormones of date palm pollinator cultivars and their relationship with hormones of different stages of 'Piarom' date fruit growth. *Scientia Horticulturae*, 288, 110389.
- Suhim, A. A., Awad, K. M., Jaffer, O. N., & Abass, M. H. (2023). The Impact of Salicylic and Jasmonic Acid in Mitigating Salinity Stress on Date Palm *Phoenix dactylifera* L. Barhi Cv. *Basrah Journal of Agricultural Sciences*, 36(1), 120–130.