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Physiological and Chemical Responses of Tissue-Cultured Barhi and Hilali Date Fruits to Foliar Amino Acid Application.

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Abstract

This study was conducted in one of the private orchards in Nasiriyah district, Thi- Qar Governorate, Iraq, during the growing season of 2023, with the aim of knowing the effect of spraying tissue-propagated Barhi and Hilali date palm trees cultivars with a mixture of some amino acids to improve fruit set, reduce the percentage of parthinocarpic fruits, and some chemical characteristics of fruits in the Hababok stage. The mixture of protein amino acids consisted of (L-Mithionine, L- Lysine, L- Glutamic, L- Arginine, and L- Tryptophan) at two concentrations (25 and 50) mg L^{-1} for each of them, in addition to their interactions and the comparison treatment. The results of the study showed that spraying with the mixture of amino acids led to a significant increase in each of the percentage of fruit set and concentrations of amino acids, total soluble proteins, and total soluble carbohydrates, and the concentration treatment (50 ml L^{-1}) was significantly superior and achieved the highest averages of (72.83%), (6.95, 7.84, and 15.71) mg g^{-1}, respectively. The concentration treatment (50 ml L^{-1}) also achieved the lowest average of parthinocarpic fruits, reaching (24.41%). Regarding the cultivar effect, the Barhi cultivar significantly outperformed and achieved the highest averages for each of the percentage of fruit set and the fruit content of amino acids, total soluble proteins, and total soluble carbohydrates, reaching (68.98%), (5.42, 6.45, and 14.00) mg g^{-1}, respectively, the Barhi cultivar also achieved the lowest average, with a significant difference, for the percentage of parthinocarpic fruit, reaching (30.58%), compared to the Hilali cultivar. Interaction coefficients between the study factors were significantly superior to the interaction treatment between the Barhi cultivar and amino acids at a concentration of (50 ml L^{-1}), achieving the highest averages for each of the percentage of fruit set and its amino acid content, total soluble proteins and total soluble carbohydrates amounted to (74.68%) and (7.76, 8.63 and 18.47) mg g^{-1}, respectively. This interaction treatment also achieved the lowest average and significant difference in the percentage of parthinocarpic fruits, reaching (21.83%) compared to the rest of the interaction treatments.

Keywords: amino acids, variety, proteins, fruit set percentage.

I. .Introduction:

Date palms (Phoenix dactylifera L.) are dioecious plants, meaning that the male flowers are on one tree and the female flowers are on another. Pollination is cross-pollination (Cohen *et al.*, 2004), with male pollen being transferred to the stigmas of female flowers. This can occur naturally or artificially (Ibrahim, 2008). Failure in pollination and fertilization results in small, compressed seedless fruits due to their attachment to one point. These fruits typically do not reach maturity and are locally called "shees" or "parthinocarpic fruits" (Salomon-Torres, 2021).

The phenomenon of parthinocarpic fruits production in date palms has become widespread in young palms produced by tissue culture in many palm growing regions such as Saudi Arabia, the Emirates, Iran, South Africa, Namibia,

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Morocco and the United States of America (Mirani *et al*.,2019). This phenomenon may be caused by epigenetic or genetic differences (Mirani *et al*., 2020).

Salomon-Torres, (2017). in a study of this phenomenon, confirmed that fruiting abnormalities in date palms are caused by non-genetic differences and are more severe in the tissue-propagated Barhi variety, reaching up to 85%.

Ali-Dinar *et al*.,(2021) confirmed that the production of parthinocarpic fruits in young tissue-propagated date palms is mainly induced by hormonal imbalances and that young tissue-propagated date palms contain different levels of some plant hormones than vegetative propagated date palms.

Amino acids play vital and important roles in plants. They are the basic units for building proteins. Protein is a sequential chain of amino acids linked together by covalent bonds. Some amino acids are the starting compounds for some plant hormones and other growth substances. They also enter into the composition of nucleic acids. They also work to improve the efficiency of photosynthesis by increasing chlorophyll, such as glutamic acid and glycine, which are used in the formation of the chlorophyll molecule, thus increasing the rate of photosynthesis in the plant, enhancing its growth and increasing production. Amino acids also facilitate the absorption, transport and use of nutrients, as amino acids have a very small molecular weight. Therefore, when nutrients, especially trace elements, are bound to them, they act as a chelating substance that facilitates their entry into the plant (Pernison *et al*., 2011).

In general, amino acids are the basic building blocks of proteins and act as signaling molecules and stress-protective factors, they improve the absorption of essential nutrients, some amino acids are also precursors for the synthesis of some plant hormones, therefore, they contribute directly or indirectly to the effective growth of pollen tubes, thus increasing the fertilization rate (Rahman *et al*., 2024). In addition to their impact on the permeability of cell membranes and their role as a chelating substance for trace elements and facilitating their absorption by the plant (El-Desouky *et al*., 2011).

Given the lack of studies on the effect of amino acids on the phenomenon of early fruiting in date palm trees propagated by tissue culture, this study aimed to know the effect of spraying trees with a mixture of amino acids on some physiological and chemical characteristics of the fruits of the tissue-produced date palm cultivars Barhi and Hilali.

II. Materials and methods:

This experiment was conducted during the 2023 growing season in a private orchard in Nasiriyah district, the capital of Thi Qar Governorate, Iraq. To study the effect of spraying the vegetative system with a mixture of amino acids on some physiological characteristics such as fruit set percentage and parthinocarpic fruits percentage, and some chemical characteristics such as free amino acids, total soluble proteins and total soluble carbohydrates of tissue-propagated Barhi and Hilali cultivars fruits at the Hababok stage.

Eighteen date palm trees were selected, 9 trees of each cultivar suffering from the phenomenon of abnormal fruiting (parthinocarpic fruits production) based on data from the two previous seasons. The trees were homogeneous in vegetative growth and 8 years old, 8 inflorescences were left on each tree and the rest were removed and pollinated with green Ghanamy pollen after they opened naturally during the period from 25/3/2023 to 5/4/2023 and covered with paper bags to avoid mixing pollen grains until fruit set was complete. Leaf spray treatments were carried out with a mixture of L-Proteinous free amino acids, namely L-Mithionine, L-Lysine, L-Glutamic, L-Arginine and L-Tryptophan, at two concentrations (25 and 50) mg L^{-1} for each of them. On the two tissue-propagated Barhi and Hilali date palm cultivars, the experimental treatments were sprayed on tree leaves were sprayed for six times until completely wet, with the addition of Tween-20 to the solution to reduce the surface tension of the leaves, except for the control treatment which was sprayed with distilled water only. The first spray was at the beginning of November (flower bud formation) and the last spray was before flower buds opened. The period between sprays was 30 days.

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Fruit samples were collected during the hababok stage from each experimental unit to calculate and estimate the following traits:

Fruit set percentage (%):

The fruit set percentage was calculated for all treatments at the hababok stage 30 days after pollination according to the method described in (Omar *et al*., 2014). Five fruit spikes were randomly taken from each branch and from each tree (experimental unit) for both cultivars. The number of set flowers and the number of empty scars were counted, and the fruit set percentage was calculated based on the following equation:

$$\text{Percentage of fruits set (\%)} = \frac{\text{Number of flowers set}}{\text{Number of flowers set+Number of empty scars}} \times 100$$

Percentage of parthinocarpic fruits (%):

The percentage of parthinocarpic fruits that do not set was calculated using the following equation:

$$\text{Percentage of parthinocarpic fruits (\%)} = \frac{\text{Number of unset fruits}}{\text{Total number of fruits}} \times 100$$

Estimation of total free amino acids (mg g^{-1}):

Total free soluble amino acids were determined in date palm fruits at the Hababok stage for both cultivars according to the method described in (Lee and Takahashi, 1966). Spectrophotometers were used at a wavelength of 570 nm, the filtrate was replaced with ethanol to prepare the control sample, and the amino acid leucine was used to prepare the standard curve.

Estimation of Total Soluble Proteins (mg g^{-1}):

Total soluble proteins were extracted from fruit tissues of the two studied cultivars according to the method of (Bavi *et al*., 2011) and were estimated according to the method of (Bradford ,1976) using the Bradford reagent. A spectrophotometer was used at a wavelength of 595 nm, and the filtrate was replaced with distilled water to prepare the control sample, total soluble proteins were estimated using the albumin standard curve.

Estimation of total soluble carbohydrates (mg g^{-1}):

The method described in (Watanaba *et al*., 2000) was followed to estimate the amount of total soluble carbohydrates in the fruits using a spectrophotometer at a wavelength of 620 nm, the filtrate was replaced with distilled water to prepare the control sample, a standard glucose curve was used to estimate total soluble carbohydrates.

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Statistical Analysis:

The study was designed as a factorial experiment according to a Completely Randomized Block Design (C.R.B.D). The results were statistically analyzed according to the analysis of variance table, the least significant difference (L.S.D) test was used to compare means at a probability level of (0.05), the statistical program (SPSS) version (21) was used to analyze the data. The results represented the average of three replicates for each treatment.

III. Results and discussion:

Fruit set percentage (%):

The results in table (1) showed that the effect of spraying with a mixture of amino acids led to a significant increase in the percentage of fruit set compared to the control treatment. The spraying treatment at a concentration of (50 mg L^{-1}) achieved the highest average of (72.83 %), followed by the treatment at a concentration of (25 mg L^{-1}) with average of (66.43 %), compared to the control treatment, which recorded the lowest average of (57.81 %).

As for the effect of the cultivar, the Barhi cultivar was significantly superior and achieved the highest average of fruit set, reaching (68.98 %), compared to the Hilali cultivar, which recorded the lowest average, reaching (62.39 %).

Table (1) The effect of spraying with a mixture of some amino acids on the percentage of fruit set (%) for the tissue-propagated Barhi and Hilali cultivars.

Cultivar	Amino acids (mg L^{-1})			Cultivar average
	0	25	50	
Barhi	63.39	68.88	74.68	**68.98**
Hilali	52.22	63.97	70.97	**62.39**
Amino acids average	**57.81**	**66.43**	**72.83**	
L.S.D ≤ 0.05	**cultivar**	**Amino acids**	**interactions**	
	0.492	0.602	0.852	

The interaction coefficients between the study factors showed significant differences between them, and the interaction treatment between the Barhi cultivar and spraying with a mixture of amino acids at a concentration of (50 ml L^{-1}) was significantly superior and achieved the highest average of (74.68 %), followed by the interaction treatment between the Hilali cultivar and amino acids at a concentration of (50 ml L^{-1}) and recorded average of (70.97 %), while the control treatment for the Hilali cultivar recorded the lowest average with a significant difference from the rest of the interaction treatments, which amounted to (52.22 %).

Percentage of parthinocarpic fruits (%):

The results in Table (2) showed a significant decrease in the percentage of parthinocarpic fruits with increasing concentration of the amino acid mixture for the Barhi and Hilali cultivars. The spray treatment with a concentration of (50 ml L^{-1}) was significantly superior, recording the lowest average of parthinocarpic fruits, reaching (24.41 %),

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followed by the concentration treatment (25 ml L^{-1}) with average of (31.86 %), compared to the control treatment, which recorded the highest average, reaching (46.79 %).

As for the cultivar, the Barhi cultivar was significantly superior and recorded the lowest percentage of parthinocarpic fruits, reaching (30.58 %), compared to the Hilali cultivar, which recorded the highest average, reaching (38.12 %).

Table (2) The effect of spraying with a mixture of some amino acids on the percentage of parthinocarpic fruits (%) for the tissue-propagated Barhi and Hilali cultivars.

Cultivar	Amino acids (mg L^{-1})			Cultivar average
	0	25	50	
Barhi	40.54	29.37	21.83	**30.58**
Hilali	53.03	34.35	26.98	**38.12**
Amino acids average	**46.79**	**31.86**	**24.41**	
L.S.D ≤ 0.05	**cultivar**	**Amino acids**	**interactions**	
	4.358	5.338	7.549	

The interaction treatment between the Barhi cultivar and the amino acid mixture concentration (50 ml L^{-1}) was significantly superior and recorded the lowest average of parthinocarpic fruit percentage, reaching (21.83 %), while the control treatment of the Hilali cultivar recorded the highest average, reaching (53.03 %).

Fruit content of total soluble amino acids (mg g^{-1}):

It is noted from the results in table (3) that spraying date palm trees of the Barhi and Hilali cultivars with concentrations of the amino acid mixture resulted in significant differences in the fruit content of total soluble amino acids. The concentration treatment (50 ml L^{-1}) significantly outperformed, achieving the highest average of (6.95 mg g^{-1}), followed by the concentration treatment (25 ml L^{-1}) with average of (4.78 mg g^{-1}), compared to the control treatment, which recorded the lowest average of (2.96 mg g^{-1}).

As for cultivar, the Barhi cultivar significantly outperformed, recording the highest average of (5.42 mg g^{-1}), compared to the Hilali cultivar, which recorded the lowest average of (4.36 mg g^{-1}).

Table (3) The effect of spraying with a mixture of some amino acids on the Fruit content of total soluble amino acids (mg g^{-1}) for the tissue-propagated Barhi and Hilali cultivars.

Cultivar	Amino acids (mg L^{-1})			Cultivar average
	0	25	50	
Barhi	3.25	5.26	7.76	**5.42**

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Hilali	2.66	4.29	6.13	**4.36**
Amino acids average	**2.96**	**4.78**	**6.95**	
L.S.D ≤ 0.05	**cultivar**	**Amino acids**	**interactions**	
	0.1760	0.2156	0.3049	

The interaction coefficients between the study factors showed significant differences between them. The interaction treatment between the Barhi cultivar and amino acids at a concentration of (50 ml L^{-1}) achieved the highest average with a significant difference of (7.76 mg g^{-1}), while the control treatment for the Hilali cultivar recorded the lowest average of (2.66 mg g^{-1}).

Total soluble protein content of fruits (mg g^{-1}):

The results in table (4) showed significant differences in the total protein content of fruits as a result of spraying with amino acid concentrations. The concentration treatment (50 ml L^{-1}) achieved the highest average of (7.84 mg g^{-1}), followed by the concentration treatment (25 ml L^{-1}) with (5.71 mg g^{-1}), compared to the control treatment, which recorded the lowest average of (3.89 mg g^{-1}).

As for the effect of cultivar, the Barhi cultivar significantly outperformed, achieving the highest average of (6.45 mg g^{-1}), compared to the Hilali cultivar, which recorded the lowest average of (5.18 mg g^{-1}).

Table (4) the effect of spraying with a mixture of some amino acids on the Fruit content of total soluble protein (mg g^{-1}) for the tissue-propagated Barhi and Hilali cultivars.

Cultivar	**Amino acids (mg L^{-1})**			**Cultivar average**
	0	**25**	**50**	
Barhi	4.60	6.11	8.63	**6.45**
Hilali	3.18	5.31	7.05	**5.18**
Amino acids average	**3.89**	**5.71**	**7.84**	
L.S.D ≤ 0.05	**cultivar**	**Amino acids**	**interactions**	
	0.1357	0.1662	0.2350	

The interaction coefficients between the study factors differed significantly among themselves, and the interaction treatment between the Barhi cultivar and the amino acid concentration (50 ml L^{-1}) achieved the highest average of (8.63 mg g^{-1}), while the control treatment for the Hilali cultivar recorded the lowest average of (3.18 mg g^{-1}).

Fruit content of total soluble carbohydrates (mg g^{-1}):

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The results in table (5) showed that amino acid concentrations differed significantly among the fruit's total soluble carbohydrate content. The (50 mg L^{-1}) concentration achieved the highest average, reaching (15.71 mg g^{-1}), followed by the (25 ml L^{-1}) concentration, which reached (11.69 mg g^{-1}), compared to the control treatment, which recorded the lowest average, (8.72 mg g^{-1}).

The effect of the cultivar was significantly superior to the Barhi cultivar, which recorded the highest average, (14.00 mg g^{-1}), compared to the Hilali cultivar, which recorded the lowest average, (10.08 mg g^{-1}).

Table (5) the effect of spraying with a mixture of some amino acids on the Fruit content of total soluble carbohydrates (mg g^{-1}) for the tissue-propagated Barhi and Hilali cultivars.

Cultivar	Amino acids (mg L-1)			Cultivar average
	0	25	50	
Barhi	10.18	13.34	18.47	**14.00**
Hilali	7.25	10.04	12.94	**10.08**
Amino acids average	**8.72**	**11.69**	**15.71**	
L.S.D $\leq_{0.05}$	**cultivar**	**Amino acids**	**interactions**	
	2.299	2.816	3.982	

As for the effect of the interaction coefficients between the study factors, some of them were significant in the fruit content of total soluble carbohydrates, and the interaction treatment between the Barhi cultivar and amino acids at a concentration of (50 ml L^{-1}) achieved the highest average of (18.47 mg g^{-1}), while the control treatment for the Hilali cultivar recorded the lowest average of (7.25 mg g^{-1}).

The fruit-setting process is the first step in the growth and development of fruit. In angiosperms, this process depends on the successful completion of pollination and another equally important process called fertilization. This occurs when one of the male nuclei in the pollen grains unites with the female nucleus (egg cell) of the ovule to form the zygote, which then begins to grow and form the embryo (Kumar *et al*., 2014).

The significant increase in the percentage of fruit set and the significant decrease in the percentage of parthinocarpic fruits as a result of spraying Barhi and Hilali date palms with concentrations of a mixture of amino acids, as shown in tables (1 and 2), may be attributed to the important role amino acids play in improving hormonal balance within the plant, this helps in the formation of flower buds, regulating flowering, and increasing fruit set, they also increase the metabolism of various enzymes in the plant (Ibrahim, 2014). Improving the hormonal balance within the plant through amino acids may lead to an improvement in the level of plant hormones, especially auxins, gibberellins, and cytokinins, which play crucial and important roles in the pollination and fertilization processes in the plant, the onset of fruit set is regulated by the combined accumulation of these hormones, and auxin is the most important of these, especially since the amino acid tryptophan used in this experiment is considered the initiating compound for its formation. The effect of these hormones has been widely explained in previous studies that compared pollinated and parthinocarpic fruits, indicating a rapid and early increase in the expression of auxin genes and auxin-responsive genes during the fruit set process and an increase in the accumulation of gibberellins (1-3) days after pollination (Fenn and Giovannoni, 2021). Therefore, foliar spraying with a mixture of certain amino acids is an effective method for

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achieving a significant increase in the percentage of fruit set and productivity (Morales – Payan, 2015). Plants produce a wide range of amino acids, the levels of which vary depending on the plant and explant (Kawade *et al*., 2023). The results of this study, which indicated the significant effect of spraying with a mixture of amino acids on increasing the percentage of fruit set and a significant decrease in the percentage of parthinocarpic fruits, are consistent with the results of numerous studies, including (Ghailan *et al*., 2024) on tissue-propagated Barhi date palms, and with other studies conducted on other plants, such as (El-Badawy, 2019) on peach trees, (Almutairi *et al*., 2022) on guava trees, and (Mukhina *et al*., 2024) on apple trees.

The results of this study showed that increasing the levels of the amino acid mixture that treated the date palm trees of the Barhi and Hilali cultivars led to a significant increase in the content of the fruits in the Hababok stage of soluble amino acids, total soluble proteins and total soluble carbohydrates, as shown in tables (3, 4 and 5). The reason may be that increasing the levels of amino acids may lead to supplying the cells with total nutrients and thus increasing the concentrations of carbohydrates in them, and this may be reflected in the significant increase in the content of the fruits of amino acids, total soluble proteins and total soluble carbohydrates (Taha *et al*., 2001).

In addition, amino acids and plant hormones play complementary and complex roles in regulating the accumulation of amino acids, proteins, and carbohydrates, these roles include promoting growth and development, nutritional regulation, and stress response, as well as the mutual interaction between amino acids and plant hormones to ensure the plant's adaptation to the surrounding environment (Yang *et al*., 2022). Plant hormones can influence the concentration and distribution of amino acids, while amino acids can influence the sensitivity and responsiveness of cells to hormones, this interaction ensures a balanced physiological process and promotes harmonious growth and development (Mukherje *et al*., 2022). The results of this study are consistent with those of (Darwesh, 2013), which indicated that treating Portmoda date palms with free amino acids increased the levels of free amino acids in their leaves, it also enhanced growth by increasing the fresh and dry weights of the leaves. The results of this study are also consistent with those of (Abdullah *et al*., 2023), which indicated that external spraying with the amino acids arginine, glycine, and tryptophan increased the total soluble protein and total soluble carbohydrate content of date palm leaves.

The results of this study showed that the Barhi cultivar had a significant superiority in increasing the percentage of fruit set, decreasing the percentage of parthinocarpic fruits, and increasing the concentrations of free amino acids and total soluble proteins of the fruits in the Hababuk stage compared to the Hilali cultivar. This may be due to the genetic nature of the cultivar, as the genetic composition of the cultivar has a major role in determining the fruit characteristics of the cultivar due to the variation in morphological, physiological and chemical characteristics between the cultivars (Hussein, 2002).

The results of this study are consistent with the results of the study (Al-Ibrahimi and Al-Asadi, 2023), which indicated that the cultivars differ from each other in chemical properties, including total and reducing sugars and sucrose, during their study on date palm trees of the Shuwaithi and Sayer cultivars.

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