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Changes in amino acid levels and their effects on parthenocarpic fruit formation in young Barhi date palms *Phoenix dactylifera* L. derived from tissue culture

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Abstract

This research aims to analyse the quantities of non-essential amino acids (histidine, arginine, alanine, cysteine and tyrosine) and essential amino acids (aspartic, glutamine, serine, glycine, phenylalanine, threonine, valine, isoleucine, leucine, methionine and lysine) in the leaves of three different date palm phenotypes. These phenotypes are derived through tissue culture from and parthenocarpic fruits (shees) produces, normal fruits (normal) produce and propagated by offshoots of normal fruits (vegetal) produces. This study focuses on three distinct stages: pre-flowering, flowering and fruiting. The amino acid levels were determined using HPLC. Results of this study indicate that the shees phenotype has the lowest levels of amino acids compared with the normal and vegetal phenotypes. Most amino acids exhibit a consistent trend throughout the examined stages, with levels declining from the pre-flowering stage to the flowering stage, but show an increase in the fruiting stage compared with the preceding stage. In addition, the results reveal that methionine is absent in the shees phenotype during all stages of the study but present at high levels in the vegetal and normal phenotypes. These results indicate that the metabolism of amino acids varies among different phenotypes of date palms. This variation directly or indirectly affects the development of parthenocarpic fruits in date palms of the Barhi cultivar derived from tissue culture during the juvenile period.

Keywords

essential amino acids; HPLC analysis; flowering; phenotypes; methionine

Introduction

Date palms (*Phoenix dactylifera* L.) show great importance as a fruit crop because of its highly nutritious fruit and beneficial by-products, and it serves as a primary food source for humans and livestock in arid regions worldwide (1). Date palms are traditionally propagated through seeds or offshoots (2). However, seed propagation is not preferred because of the heterozygosity it brings. The resulting plantlets from seeds are not identical to the mother plant and are of lower quality. Moreover, they are approximately 50% male (3). On the contrary, propagation through offshoots is considered a superior method. However, the number of offshoots produced from each tree during the palm's lifespan is insufficient (4). Tissue culture, an effective technique for the rapid mass production of

plants, is a relatively recent development for date palms. Although the methods for *in vitro* propagation of date palms through tissue culture were established over 44 years ago (5), the establishment of commercial tissue culture-based orchards in Iraq only gained widespread popularity in the past 10-15 years (6). During tissue culture, all offspring plants must maintain phenotypic and genetic similarity to their parent plants. However, tissue culture can lead to the emergence of 'off-types', which are plants that exhibit visual differences compared with the original cultivar. This occurrence, which is referred to as somaclonal variation, results from genetic and epigenetic changes that arise during the in vitro process (7). Several distinct off-type phenotypes have been identified among date palms that are produced through tissue culture. These phenotypes include trees that yield seedless parthenocarpic fruits (8). The date flower consists of three carpels. After pollination, only one carpel undergoes development into a fruit, while the remaining two carpels deteriorate. However, in the case of unsuccessful pollination, triple parthenocarpic fruits are produced, wherein all three unfertilised carpels develop (9). Previous research has primarily focused on investigating the potential factors influencing this occurrence by examining the anatomical and hormonal aspects of the tissue culture trees involved (10,11). To the best of our understanding, none of the prior studies have explored the alteration in amino acid metabolism as a means to identify the potential reasons for this abnormality in fruit development. Thus, this study aims to investigate the potential alteration in amino acid metabolism as a new perspective to understand the formation of parthenocarpy in Barhi date palm cultivar derived from tissue culture in the juvenile period.

Materials and Methods

Plant selection and sample collection

In the present study, tissue culture–derived Barhi cultivar plants that were carefully selected for their distinct fruiting traits were used. Plants that displayed typical fruiting behaviour at 10 years old, which are referred to as the normal phenotype, and those that exhibited abnormal fruiting behaviour (parthenocarpic fruits) at 7 years old, known as the Shees (local name) phenotype, were specifically selected for sample collection. These selections were based on a thorough review of their documented fruiting records from the previous two seasons. Furthermore, this study included plants that were propagated through the conventional offshoot method at 7 years old, representing the vegetal phenotype. Leaf samples were collected from all phenotypes examined from date palm orchards located in the Al-Midaina district within the Basrah governorate, situated in the southern part of Iraq. Date palm leaf sampling was performed at three distinct time points. The first sampling occurred in January 2022, prior to spathe emergence, and represented the pre-flowering stage. The second sampling took place in March 2022, following spathe opening, and represented the flowering stage. The third sampling occurred 40 days after pollination, during the Kimri fruit stage, and represented the fruiting stage. All samples were dried under controlled conditions and subsequently transported to the Ministry of Science and Technology's laboratories for digestion and free amino acid analysis.

Free amino acid extraction and analysis

The content of leaves from three different phenotypes of female date palm was analysed for free amino acids using the Automatic Free Amino Acid Analyzer from Korea. Analysis was conducted at the laboratories of the Ministry of Science and Technology in Baghdad, following a standard method (12). To prepare the samples, 0.2 g of fully dried date palm leaves was precisely weighed using an analytical balance and then ground into fine components using a grinding machine. The resulting components were carefully stored in airtight polyethylene bags to prevent any damage until they could be further analysed. The samples were placed into vacuum test tubes containing 12 mL of 6M HCL and capped immediately. The tubes containing the samples and HCL were placed in an oven at 110 °C for about 24 h to complete the hydrolysis of the samples. Then, the samples were taken out of the oven, and the mixture was filtered through a 0.8 µm filter paper and washed two times with 50 mL of deionised distilled water. The filtrate and washings were concentrated to dryness in a rotary evaporator at 50 °C. A total of 10 mL of distilled water was added and evaporated to dryness in the rotary evaporator again at 50 °C. Samples were reconstituted again in 3.5 mL of 0.02 M HCL. After acid hydrolysis, 30 mL of citrate buffer (pH 2.2) was added, and the pH was adjusted between 0.5 and 1 with 7.5 M NaOH and 2.2 with 1 M NaOH. A total of 100 µL of filtered symbol was collected from the mixture that consists of 200 µL of O-Phthalaldehyde 5% and 1 mL of extracted sample with shaking for 2 min mixed with the mobile phase, which consists of methanol, acetonitrile and 5% formic acid at a ratio of 20, 60 and 20 with a flow ratio of 1 mL/min for 25 min, and injected into the Auto-Free Amino Acids Analyzer.

Results

Non-essential amino acids

The results presented in Table 1 indicate that all non-essential amino acids initially increased and then decreased during pre-flowering, flowering and fruiting stages. In general, the concentration of these amino acids exhibited a decline from the pre-flowering stage to the flowering stage, followed by an increase in the fruiting stage, with the exception of alanine acid, which showed a gradual increase from the pre-flowering stage to the flowering stage, and then further increased in the fruiting stage. The results indicate that the concentration of all non-essential amino acids studied was highest in the vegetal phenotype, followed by the normal phenotype, and lowest in the shees phenotype with the exception of alanine and tyrosine acids, which exhibited the highest concentration in the shees phenotype compared with the normal phenotype.

- The concentration of each non-essential amino acid for

each phenotype studied across the pre-flowering, flowering and fruiting stages is presented below.

• Histidine: The concentration of histidine acid initially decreases from 497.43 mg in the pre-flowering stage to 89.46 mg in the flowering stage and then increases to 232.64 mg in the fruiting stage. The vegetal phenotype has the highest histidine acid concentration (485.29 mg), followed by the normal phenotype (244.86 mg) and the shees phenotype (89.38 mg). Notably, the presence of this acid is undetectable in the shees phenotype during the flowering and fruiting stages.

• Arginine: The concentration of arginine acid initially decreases from 236.74 mg in the pre-flowering stage to 62.39 mg in the flowering stage and then increases to 129.11 mg in the fruiting stage. The vegetal phenotype has the highest arginine acid concentration (185.21 mg), followed by the normal phenotype (122.95 mg) and the shees phenotype (120.09 mg).

• Alanine: The level of alanine acid gradually rises from 24.79 mg during the pre-flowering stage to 67.09 mg during the flowering stage and then further increases to 171.00 mg during the fruiting stage. Among the different phenotypes, the vegetal phenotype exhibits the highest concentration of alanine acid (187.50 mg), followed by the shees phenotype (46.74 mg) and the normal phenotype (28.65 mg). As shown in Table 1, this acid was undetectable in the vegetal phenotype at the pre-flowering stage, in the normal phenotype at the pre-flowering and flowering stages and in the shees phenotype at the fruiting stage.

• Cysteine: The concentration of cysteine acid initially decreases from 392.85 mg in the pre-flowering stage to 159.46 mg in the flowering stage and then increases to 314.49 mg in the fruiting stage. The vegetal phenotype contains the highest cysteine acid concentration (428.18 mg), followed by the normal phenotype (208.47 mg), and the shees phenotype has the lowest cysteine acid concentration (176.19 mg). This acid is undetectable in the shees phenotype during the flowering stage.

• Tyrosine: The concentration of tyrosine acid initially decreases from 299.38 mg in the pre-flowering stage to 93.45 mg in the flowering stage and then increases in the fruiting stage to 255.11 mg. The vegetal phenotype has the highest tyrosine acid concentration (332.91 mg), followed by the Shees phenotype (169.38 mg), and the normal phenotype has the lowest tyrosine acid concentration (135.56 mg).

Essential amino acids

The findings displayed in Table 2 demonstrate a consistent trend among essential amino acids throughout the examined stages. The levels of these amino acids declined from the pre-flowering stage to the flowering stage but increased in the fruiting stage compared with the preceding stage, except for valine, isoleucine, methionine and lysine. These particular amino acids experienced a decrease from the pre-flowering stage to the flowering stage and continued to decline in the fruiting stage. The results also indicated that the concentration of essential amino acids varied across the studied phenotypes. The vegetal phenotype exhibited the highest concentration of essential amino acids, except for valine and leucine, which were higher in the normal phenotype. On the contrary, the shees phenotype displayed the lowest levels of glutamine, phenylalanine, threonine, valine, methionine and lysine. The normal phenotype had the lowest concentrations of aspartic acid, serine, glycine and isoleucine. Only leucine acid demonstrated the lowest concentration in the vegetal phenotype.

The concentrations of each essential amino acid in the Barhi date palm across the stages of growth, development and phenotypes were as follows:

Table 1. Non-essential amino acid composition of Barhi date palm phenotypes during the pre-flowering, flowering and fruiting stages.

Amino acid	Phenotype	Stage			Mean of phenotype
		Pre-flowering	Flowering	Fruiting	
Histidine	Vegetal	640.99	216.27	598.62	485.29
	Normal	583.15	52.13	99.31	244.86
	Shees	268.15	0	0	89.38
Mean		497.43	89.47	232.64	
Arginine	Vegetal	259.27	142.85	153.51	185.21
	Normal	269.02	44.33	55.51	122.95
	Shees	181.94	0.01	178.33	120.09
Mean		236.74	62.40	129.12	
Alanine	Vegetal	0	135.45	427.07	187.51
	Normal	0	0	85.95	28.65
	Shees	74.39	65.84	0	46.74
Mean		273.73	73.52	178.19	
Cysteine	Vegetal	649.69	311.24	485.61	482.18
	Normal	313.32	167.16	144.95	208.48
	Shees	215.66	0	312.92	176.19
Mean		297.57	90.71	205.45	
Tyrosine	Vegetal	453.47	162.56	382.71	332.91
	Normal	225.95	87.45	93.56	125.65
	Shees	188.73	30.36	289.06	169.38
Mean		296.27	89.57	213.29	

Table 2. Essential amino acid composition of Barhi date palm phenotypes during the pre-flowering, flowering and fruiting stages.

Amino acid	Phenotype	Stage			Mean of phenotype
		Pre-flowering	Flowering	Fruiting	
Aspartic	Vegetal	105.89	18.69	20.36	48.31
	Normal	64.17	0	18.23	27.47
	Shees	46.25	0.92	43.39	30.19
Mean		72.10	6.54	27.33	
Glutamine	Vegetal	615.16	583.00	403.70	533.95
	Normal	587.59	151.84	385.84	375.09
	Shees	392.18	23.79	0	138.66
Mean		531.64	252.88	263.18	
Serine	Vegetal	427.33	577.1	581.33	528.59
	Normal	434.25	0	287.88	240.71
	Shees	354.54	0	744.33	366.29
Mean		405.37	192.37	537.85	
Glycine	Vegetal	459.29	287.84	0	249.04
	Normal	220.68	34.85	64.10	106.54
	Shees	0	0	393.58	131.19
Mean		226.66	107.56	152.56	
Phenylalanine	Vegetal	739.34	94.92	270.34	368.20
	Normal	434.65	76.97	72.93	194.85
	Shees	152.61	6.80	281.33	146.91
Mean		329.98	127.16	239.38	
Threonine	Vegetal	513.86	144.28	469.76	375.97
	Normal	244.65	30.07	52.98	109.23
	Shees	283.84	0.69	0	94.84
Mean		347.45	58.35	174.25	
Valine	Vegetal	513.66	256.67	0	256.78
	Normal	394.56	166.70	212.03	257.76
	Shees	256.65	1.76	0	86.14
Mean		388.29	141.71	70.68	
isoleucine	Vegetal	401.40	106.21	195.00	234.20
	Normal	0	41.92	0	13.97
	Shees	266.02	59.39	0	108.47
Mean		222.47	69.17	65.00	
Leucine	Vegetal	343.33	0	0	114.44
	Normal	336.30	31.18	118.31	161.93
	Shees	161.59	0.56	193.62	118.59
Mean		280.41	10.58	103.98	
Methionine	Vegetal	876.15	202.05	0	359.40
	Normal	246.85	168.14	184.99	199.99
	Shees	0	0	0	0.00
Mean		374.33	123.40	61.66	
Lysine	Vegetal	0	179.00	0	59.67
	Normal	458.00	0	131.50	196.50
	Shees	0	12.70	0	4.23
Mean		152.67	63.90	43.83	

•Aspartic: The concentration of aspartic acid initially decreases from 72.10 mg in the pre-flowering stage to 6.87 mg in the flowering stage and then increases to 27.33 mg in the fruiting stage. The vegetal phenotype has the highest aspartic acid concentration (48.32 mg), followed by the shees phenotype (30.19 mg) and the normal phenotype (27.47 mg). Notably, this acid is undetectable in the normal phenotype during the flowering stage.

• Glutamic: The concentration of glutamic acid initially decreases from 531.64 mg in the pre-flowering stage to 252.87 mg in the flowering stage and then increases to 263.18 mg in the fruiting stage. The vegetal phenotype has the highest glutamic acid concentration (533.95 mg), followed by the normal phenotype (375.09 mg) and the shees phenotype (138.65 mg). The concentration of this acid was below detectable levels in the shees phenotype during the fruiting stage.

• Serine: The concentration of serine acid initially decreases from 405.37 mg in the pre-flowering stage to 192.37 mg in the flowering stage and then increases to 537.84 mg in the fruiting stage. The vegetal phenotype has the highest serine acid concentration (528.59 mg), followed by the shees phenotype (266.29 mg) and the normal phenotype (241.71 mg). This acid was undetectable in the shees and normal phenotypes during the flowering stage.

• Glycine: The concentration of glycine acid initially decreases from 226.65 mg in the pre-flowering stage to 107.56 mg in the flowering stage and then increases to 152.26 mg in the fruiting stage. The highest glycine acid concentration was found in the vegetal phenotype (533.95 mg), followed by the normal phenotype (375.09 mg) and and the shees phenotype (138.65 mg). The concentration of this acid was below detectable levels in the shees

phenotype during the pre-flowering and flowering stages, as well as in the vegetal phenotype during the fruiting stage.

• Phenylalanine: The concentration of phenylalanine acid initially decreases from 442.20 mg in the pre-flowering stage to 59.56 mg in the flowering stage and then increases to 208.20 mg in the fruiting stage. The vegetal phenotype has the highest phenylalanine acid concentration (368.20 mg), followed by the normal phenotype (194.85 mg) and the shees phenotype (146.91 mg). The concentration of this acid was below detectable levels in the shees phenotype during the fruiting stage.

• Threonine: The concentration of threonine acid initially decreases from 375.96 mg in the pre-flowering stage to 58.34 mg in the flowering stage and then increases to 174.24 mg in the fruiting stage. The vegetal phenotype has the highest threonine acid concentration (375.96 mg), followed by the normal phenotype (102.56 mg) and the shees phenotype (94.84 mg). The concentration of this acid was below detectable levels in the shees phenotype during the fruiting stage.

• Valine: The concentration of valine acid initially decreases from 388.29 mg in the pre-flowering stage to 141.71 mg in the flowering stage and then increases to 70.67 mg in the fruiting stage. The normal phenotype has the highest valine acid concentration (257.76 mg), followed by the vegetal phenotype (256.77 mg) and the shees phenotype (86.13 mg). The concentration of this acid was below detectable levels in normal and shees phenotypes during the fruiting stage.

• Isoleucine: The concentration of isoleucine acid initially decreases from 222.47 mg in the pre-flowering stage to 68.84 mg in the flowering stage and then increases to 65.00 mg in the fruiting stage. The vegetal phenotype has the highest isoleucine acid concentration (234.20 mg), followed by the shees phenotype (108.13 mg) and the normal phenotype (13.97 mg). The concentration of this acid was below detectable levels in the normal phenotype during the pre-flowering and fruiting stages, as well as in the shees phenotype during the fruiting stage.

• Leucine: The concentration of leucine acid initially decreases from 280.40 mg in the pre-flowering stage to 10.58 mg in the flowering stage and then increases to 93.79 mg in the fruiting stage. The vegetal phenotype has the highest leucine acid concentration (114.44 mg), followed by the normal phenotype (161.93 mg) and the shees phenotype (128.59 mg). The concentration of this acid was below detectable levels in the vegetal phenotype during the flowering and fruiting stages.

• Methionine: The concentration of methionine acid initially decreases from 374.33 mg in the pre-flowering stage to 123.39 mg in the flowering stage and then continuously decreases in the fruiting stage (61.66). The vegetal phenotype has the highest methionine acid concentration (359.40 mg), followed by the normal phenotype (199.99 mg). However, this acid was below detectable levels in the shees phenotype during all the stages. It was also below detectable levels in the vegetal phenotype during the fruiting stage.

• Lysine: The concentration of lysine acid initially decreases from 152.66 mg in the pre-flowering stage to 63.90 mg in the flowering stage and then further decreases to 43.83 mg in the fruiting stage. The vegetal phenotype has the highest lysine acid concentration (59.66 mg), followed by the normal phenotype (43.83 mg) and the shees phenotype (4.23 mg). The concentration of this acid was below detectable levels in the vegetal and shees phenotypes during the flowering and fruiting stages, as well as in the normal phenotype during the flowering stage.

Discussion

Off-type date palm trees produced through tissue culture often exhibit reduced fruit set and abnormal flower development. Most flowers on these trees develop into parthenocarpic fruitlets (seedless fruits) with three carpels. However, the underlying cause of these abnormalities remains unclear. The abnormal fruiting in young tissue-cultured trees might be due to a combination of various factors, with the slow growth of the pollen tubes during the initial phases of fruit development being an important contributor (13). According to the reports, the abnormality in the fruit of date palm plants grown through tissue culture is attributed to the disturbance in the hormone profiles of flowers and fruits (6,11). This finding is supported by the increased levels of IAA and GA3, as well as the decreased levels of BHH (a kinetin-like compound) and an unidentified compound. It was reported that the hormonal balance in date palms plays a critical role in fruit set (8). Young tissue-cultured date palms (6 years old) have high ABA contents at early fruit growth that can temporarily inhibit the effects of IAA and GA3, which eventually result in poor fruit set. However, this inhibitory effect decreases with increasing age of the date palms, because of changes in the environment, metabolic activities and hormonal homeostasis. In plants, amino acids have various major functions. Besides their involvement in the making of proteins, they also act as indispensable constituents of some other biosynthetic pathways and as significant components involved in signaling and plant stress resistance. There are four amino acids that are important for phytohormones production such as tryptophane, methionine, phenylalanine and arginine for example auxins, melatonin, ethylene, salicylic acid and polyamines (14, 15). A number of amino acids play critical roles in pollen tube development, which can be affected by various influencing factors such as germination, elongation and tip integrity (16). Valine and threonine, which are necessary for protein synthesis and auxin metabolism, contribute to pollen tube elongation and directionality, whereas arginine promotes development through protein and energy metabolism (17, 18). Leucine promotes pollen tube formation by increasing protein synthesis and affecting cell signalling pathways (19). However, some amino acids, such as histidine and methionine, have inhibitory effects because of their high concentrations, which may lead to competition with other amino acids, thereby affecting the function and structure of proteins, as well as plant physiological processes (20).

The specific effects of these amino acids vary depending on the plant species, developmental stage of the pollen tube and environmental conditions.

Plants depend on amino acids as their major nitrogen reserves because nitrogen is essential for growth and reproduction. They get nitrogen from inorganic sources such as ammonium and nitrate, as well as organic ones such as amino acids (21). Among the 21 proteinogenic amino acids, arginine stands out because of its high nitrogen-to-carbon ratio. It serves as an important form of organic nitrogen storage and transportation in plants. In addition, arginine plays multiple roles, including being an amino acid for protein synthesis, a precursor for polyamines and nitric oxide and an essential metabolite for various cellular and developmental processes (18).

The present study revealed that all the examined amino acids were more concentrated in normal phenotypes and vegetal as compared to shees phenotype. Different plants have different amounts of amino acids due to metabolic processes (13). This shows that there is a defect in factors controlling amino acid metabolism thus compromising their direct and indirect functions in fertilization and insemination. An interesting discovery from this research is that none of the samples from shees phenotypic had methionine as an amino acid. Lysine, methionine, and glutamic acid are some examples of such amino acids as they have been shown to increase pollen germination rate as well as development of pollen tube through pollination (22). Shees phenotype did not contain any measureable glycine levels during pre-flowering and flowering stages compared to vegetal phenotype. However, during fruiting stage, a significant increase in glycine content has been noticed. Glycine activates photosynthesis (23) increasing its efficiency by increasing chlorophyll synthesis besides promoting vegetative growth while it also plays role in pollination and fruit formation.

Conclusion

The outcomes of this research concluded that amino acid levels differed between the phenotypes of Barhi cultivar and growth and developmental stages. The difference in acid levels results from how effectively metabolism is done in each phenotype. Reducing of amino acid concentrations may have some impact on parthenocarpic fruit formation in shees phenotype compared to other phenotypes investigated. The findings proved that there was no methionine amino acid in shees such that proteins needed for fertilization and pollination failed to be synthesized. This research's findings give an account on amino acids' metabolism in the juvenile phase derived from tissue culture date palm Barhi cultivar and its comparison with vegetatively propagated date palm.

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Authors' contributions

SAG contributed to the collection of samples and carried out laboratory analyses. AAS Contributed to the development of the research idea, supervision, and data analysis. KMA Contributed to the interpretation of the results and the writing of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors declared there was no conflict of interest

Ethical issues: None

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