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Effect of Foliar Spraying With 5- Aminolevulinic Acid and Different Types Amino Acids on Growth of Date Palm of Plantlets after Acclimatization in the Green House

S. S. Darwesh Rasmia[1*], Abeer H. E. Abd-El Kareim[1] and H. M. Mona[1]

[1]Central Lab for Research and Development of Date Palm, Agriculture Research Center, Giza, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author SSDR designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AHAEK and HMM managed the analyses of the study. Author HMM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluation the effects of 5- aminolevulinic acid which is the precursor of chlorophyll, in addition to glutamine, asparagine, arginine, proline, tyrosine and tryptophan on the date palm plantlets (*Phoenix dactylifera L.*) which produced by tissue culture in the green house after acclimatization to increasing the growth parameters of this plantlets. Complete randomized block design was used. to conduct the experiment at three treatments 100,200 and 300mg/l with three replicates and control treatment (without spraying), these treatments was subjected as weekly spraying one time/week, all plantlets received 3g/l NPK with irrigation water one time/week, vegetative growth parameters of plantlets were estimated (plant height cm, leaves number/plantlet, stem thickness cm and fresh and dry weight of leaves (g), also chemical leaves contents as, Indoles, chlorophyll a and b, total amino acids and total sugars. Results showed the response of growth parameters increased with foliar application of all amino acids treatment, lowest mean value was found with tyrosine application, maximum response for growth parameters was observed at the level of 300 mg/l of all treatments of amino acids as compared to (control treatment) non-sprayed plants. Leaves contents of indoles mg/g f.w., chlorophyll a and b

**Corresponding author: E-mail: darweshrasmia@yahoo.com;*
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mg/g f.w., total amino acids mg/g d.w. and total sugars % were greater rising with spraying of different types of amino acids; the results showed that highest interaction was found between all types of amino acids with the concentration 300mg/l.So from results the application of 5- aminolevulinic acid and different types of amino acids was to be useful to promote growth of date palm plantlets in the greenhouse.

Keywords: 5- Aminolevulinic; amino acids; growth and foliar spraying.

1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the important fruits, so date palm micropropagation is an important tool of multiplication and produced plantlets require heavy agriculture inputs after shifting to soil and acclimatization stage in the greenhouse was needed for healthy growing in the short time. Therefore, foliar feeding is often the most effective and economical way to improve plant nutrient deficiency (Pradeep and Elamathi) [1].

Amino acids are organic nitrogenous compounds are play a role in the building blocks in the synthesis of proteins which formed by a process in which ribosome catalyze the polymerization of amino acids (Davies) [2]. Amino acids which have a high integrity with different metabolic pools in plants were used to promote plant growth (Coruzzi and Last) [3]. Effects of 5 aminolevulinic acid as a plant growth regulator and precursor of chlorophyll, vitamin B12 and other tetrapyrrole compounds in plants have been reported in relation to chlorophyll biosynthesis, photosynthesis activity also, increasing growth and enhancing agricultural productivity for many crops (Hotta et al. [4] and Yongin et al. [5] on Kidney, Barley, Potato and garlic). Applying amino acid, organic and biofertilizer may be useful to minimizing the amount of chemical fertilization (Abd El-Monem et al. [6] on grape vine).

commercially available amino acid stimulants can improve fertilizer assimilation, increase uptake of nutrients and water, enhance the photosynthetic rate and dry matter partitioning and hence increase crop yield, moreover, amino acids as commercial product (perfectose powder and liquid) at 0.45 to 0.27g/plant and peptone 5% (16% amino acids) increased plant height, number of branches and shoot dry matter of *Capsicum annum* L. (Dinnoo et al. [7]). Number of leaves and fresh weight of date palm cvsKhalas and Sukkariat- Yanbo were significant increased with 5- aminolevulinic acid from 200-250ppm (Al-Qurashi and Awad [8] on date palm and Aml et al. [9] on Olive seedling (*Oleaeuropaea* L.)). Glutamic acid from 100-200 as foliar application increased plant height, number of leaves, stem diameter and fresh and dry weights (Azza et al. [10] on *Cadiaeum variegatum* L.,). foliar spraying of diphenylamine and tryptophan at 50 or 100ppm significantly increased plant height, number of leaves/plant, stem diameter and fresh and dry weights of *Philodendron erubescens* (Abou Dahab and Abd El-Aziz [11]) foliar application of tyrosine, Phenylamine and tryptophan at 50-100 ppm and 5-10% free amino acids increased total soluble sugars, total free amino acids and chlorophyll a and b (Nahed and Laila [12] on *Salvia farinacea,* Nahed et al. [13] on *Anthirrhinum majus* and El-Kosary et al. [14] on *Mangifera indica* cvs Keitt and Ewais), recently, Xiaoqing et al. [15] they stated that, ALA increased chlorophyll content of *Solanum lycopersicum*. Therefore this work aimed to evaluate the effect of 5- aminolevulinic acid in addition to different types of amino acids with different application rates on the growth characters and chemical contents of acclimatized date palm plantlets in the green house.

2. MATERIALS AND METHODS

Pot experiment was conducted in the green house of Central Laboratory for Research and Development of Date Palm (ARC) during two successive seasons 2012- 2013 6 months for each season.

2.1 Plant Material

Date palm (*Phoenix dactylifera* L. cv. Bartamouda) derived *via* tissue culture technique after six months from acclimatization stage which cultured in peat moss + sand 2:1 in the plastic bags 40cm length and 25cm for width. Date palm plantlets (25-30cm in length and 5-6 leaves/plantlet) plantlets were irrigated for two times/week and fertilized with 3.0g/l NPK one time/week.

2.2 Treatments

Weekly foliar spraying of 5-aminolevulinic acid in in combination with six types of amino acids (glutamine, asparagine, arginine, proline, tyrosine and tryptophan) at three levels 100,200 and 300 mg/l.

2.3 All Chemicals from Merc

2.3.1 Procedure for production of date palm plantlets

2.3.1.1 Sterilization

Date palm offshoots were used as explant for culture initiation, older leaves were removed, apical meristem (shoot tip) was immersion for 20min in 7.0 and 3.0% and 1-3 rinses in sterilized water between them sodium hypochlorite solution with 2-3drops of Tween 20 followed by 4-5 rinses in sterilized water, Then the explant is immersed in 0.1% mercuric chloride solution for 5 minutes, followed by 4-5 rinses in sterilized distilled water.

2.3.1.2 Callus induction

outer 4-6 leaf primordia were removed to obtain shoot tip composed of apical meristem and cut longitudinally into four sections cultures were incubated at 27±2ºC under complete darkness, for eight months, the first subculture after 45 days, then cultures were with regular transfer to fresh medium of the same components every four weeks with medium consists of Murashige and Skooge (MS) + 2,4-D with change level from 100 to 10mg/l + 40mg/l adenine-sulfate + 3mg/l 2iP + 170 mg/l NaH_2PO_4 + 30g/l sucrose + inositol 100 mg/l + 2g/l AC +0.4mg/l thiamine HCl+ 5g/l agar.

2.3.1.3 Embruogenic callus

MS + 100mg/l glutamine+ 30 g/l sucrose+ 6g/l agar + 1.5 g/l AC + NAA 0.1mg/l + 1.0mg/l 2ip + 40 mg/l adenine-sulfate + inositol 100mg/l + 170mg/l NaH_2PO_4 + 0.4mg/l thiamine HCl + 0.4mg/l ABA

2.3.1.4 Germination of embryo

Cultures at light with 1/2 MS + 30 or 45g/l sucrose+ 1.5g/l AC + 170mg/l NaH_2PO + 0.1 mg/l NAA + 0.05 mg/l BA + + 100mg/l glutamine + 0.4 mg/l ABA for 2-3 subculture.

2.3.1.5 Elongation

1/2 MS + 30g/l sucrose + 1.5g/l AC + 0.1 NAA + 0.05mg/lBA+ 0.4mg/l Pbz or ABA + 0.5mg/l GA_3 for 2-3 subculture

2.3.1.6 Rooting

Cultures at light with 1/4 MS + 30g/l sucrose + 2mg/l NAA or IBA + 1g/l AC

2.3.1.7 Pre-acclimatization

Cultures at light with 1/4 MS + 2 mg/l NAA or IBA

2.4 Acclimatization

The plantlets were transferred to the greenhouse and washed under tap water, then plantlets were disinfecting two times with different fungicides 2g/l for 10 mint. Plantlets cultured with 2 peatmoss + 1 pearlite and irrigate 25 % Hogland's solution under plastic tunnels 90% humidity for three months with open the cover of tunnels for 5 mint every day (recommended) until new leaves were grown plantlets were exposed to the greenhouse conditions for further hardening.

2.5 Experimental Design

Complete randomized block design with three replicates and three plantlets for each one. Data were analyzed by analysis of variances (ANOVA) and the means were compared following t- test using L.s.d. values at 5% level (Snedecorand Chocran [16]). The following data were recorded.

2.6 Vegetative Growth Characters

Plant height (cm), number of leaves/plantlet, stem thickness (cm) and fresh and dry weight of leaves (g),

2.7 Chemical Constituents

- Indole content (mg/g f. w.) as described by Salim et al. [17]
- Chlorophyll a and b in leaves were determined as (mg/g f. w.) by Lichtentaler and Wellburn [18].
- Total amino acids contents in leaves (mg/g d.w.) were determined by using ninhydrin reagent according to Rosen [19].
- Total soluble sugars according to AOAC (1995) [20]

3. RESULTS AND DISCUSSION

3.1 Plant Height (Cm), Leaves Numbers/Plantlet and Stem Thickness (Cm) of Date Palm Plantlet

3.1.1Plant height (cm)

Present data shows that, in both seasons, date palm plantlet (*Phoenix dactylifera* L.); plant height cm, were significantly affected by all foliar spraying of amino acids types treatments at rates 100,200 and 300 mg/l. Since, the untreated plants (control) recorded the lowest results as compared with the treated plants. The average mean values of plant height (Table 1 and Fig. 1) exhibited significant increasing with foliar spraying of 5- aminolevulinic acid, glutamine, asparagine, arginine, proline and tryptophan were 72.1, 71.6, 69.3, 68.5, 72.2 and 71.9cm in the same order of amino acids treatments for both seasons without significant differs in between, tyrosine treatment had lowest mean value 63.5cm for both seasons, shortest plants were achieved with un treated plants 46.2 cm for both seasons. The rate of 300 mg/l scored significant tallest plant 85.0, 84.7, 81.2, 80.5, 85.5, 75.2 and 85.0 cm for both seasons in same order of 5- aminolevulinic acid, glutamine, asparagine, arginine, proline, tyrosine and tryptophan for both seasons as compared with untreated plants (control treatment), the rates of 200 and 100mg/l for all types of amino acids had the progressive results with significant variance in between for 1st and 2nd seasons.

3.1.2 Leaves numbers/plantlet

Mean values of leaves numbers (Table 1 and Fig. 1) exposed significant rising with all types of amino acids in both seasons at three levels of 100,200 and 300 mg/l, the results of leaves numbers showed significant elevation above untreated plants (control), the highest mean value in leaves numbers was 9.0, 9.0, 8.8 and 9.1 leaves/plant for both seasons in the same order of foliar spraying of amino acids treatments 5- aminolevulinic acid, glutamine, proline and tryptophan irrespective significant differences between them, fewer numbers of leaves associated with foliar spraying of asparagine arginine and tyrosine treatment 8.2, 8.2 and 7.4 leaves/plant in the same order of amino acids treatments for both seasons. The level 300 mg/l with foliar spraying of all tested amino acids types seems to be the preferable for greatest mean values of leaves numbers 10.2, 10.2, 9.0, 8.8, 10.0, 8.0 and 10.4 leaves/plant in the same order of 5- aminolevulinic acid, glutamine, asparagine, arginine, proline, tyrosine and tryptophan for both seasons. Significant variance was found between 100 and 200mg/l which produced the fewer numbers of leaves for both seasons. The significant increasing of plant height and number of leaves resulted from enhancing effect of different amino acids applications was previously reported by Awad [21] on date palm cv. Khalas stated that, plant length and number of leaves, grown diameter and fresh and dry weights were significantly increased with application of ALA at 0.02 to 0.08%, Aml et al. [9] stated that, spraying of amino acids at 0.5% increased plant height and number of leaves of olive, also these results supported by Yassen et al. [22] on anise plants (*Pimpinella anisum* L.), Shehata et al. [23] on *Cucumber hybrid* and Khalil Zadeh et al. [24] on *Vigna radiate*, which increased all growth parameters by application with tryptophan at 25 and 50ppm.

3.1.3 Stem thickness (cm)

Stem thickness of treated plantlets (Table 1 and Fig. 1) was significantly greatest under weekly spraying of all tested types of amino acids at 100,200 and 300mg/l as compared

with untreated plantlets (control treatment) in both seasons, the best treatment which caused the highest thickness of stem was weekly spraying of glutamine and asparagine 10.8 and 10.7cm as mean value for both seasons with insignificant differs in between, meanwhile, 5-aminolevulinic acid, arginine, proline and tryptophan produced 10.3, 10.2, 10.2 and 10..3cm in the same order of amino acids types regardless variance between them, lowest mean value of stem thickness were recorded under spraying of tyrosine treatment 9.1cm at both seasons. The level 300mg/l with all tested types of amino acids scored the greatest mean value of stem thickness which was 12.1, 12.9, 12.8, 11.9, 11.8, 10.4 and 12.2cm at both seasons in same order of 5 aminolevulinic acid, glutamine, asparagine, arginine, proline, tyrosine and tryptophan at both seasons, significant differs between levels 100 and 200mg/l and lowest mean values of stem thickness were resulted for both seasons. these previous increasing effects of amino acids treatments on the stem thickness were conformity with Hassan et al. [25] showed that stem diameter of plum trees increased with 20% amino acids, Nahed et al. [26] found stem diameter of *Thuja orientalis* significantly increased with 25,50 and 100ppm of tyrosine, thiamine and tryptophan. Mona et al. [27] they proved the stem diameter of *Dahlia pinnata* L. were greatest with 50,100 and150ppm putrescine.

Fig. 1. Effect of 5- aminolevulinic acid and different types of amino acids (at 300mg/l) on the growth of date palm plantlets

Con = (without treatments) 1= amino levulinic acid; 2= glutamine 3= asparagine 4= arginine 5= proline; 6= tyrosine 7= tryptophan

3.2 Fresh and Dry Weights (G)

Results presented in (Table 2) proved that, application of tested types of amino acids affected the mean values of leaves fresh and dry weights, it is obvious that weekly spraying of amino acids treatments resulted in a prominent increase in the fresh and dry weights as compared with their respective control. insignificant variance was scored of leaves fresh weights under treatments of 5- aminolevulinic acid, glutamine, asparagine, arginine, proline and tryptophan 8.4, 8.3, 8.2, 8.2, 8.7 and 8.5 (g) in the same order for types of amino acids as mean value for both seasons, lowest mean value of fresh weight were observed with

weekly spraying of tyrosine treatment 7.8 (g) as mean value for both seasons, moreover, mean values of leaves dry weights significant produced under all tested types of amino acids as compared with untreated plants (control), highest results were related with 5-aminolevulinic acid, proline, tryptophan 4.0,4.1 and 4.0 (g) with insignificant differs in between graduated by glutamine, asparagine, arginine was 3.9, 3.8, 3.8 (g) as mean value for both seasons in the order of amino acids types, lowest mean value was obtained under tyrosine treatment 3.6 (g) for both seasons. To regard the levels of amino acids 100,200 and 300 mg/l, highest significant results of leaves fresh and dry weights showed at the level of 300 mg/l with all tested types of amino acids followed by 200 and 100mg/l for both seasons, the level 300 mg/l with weekly spraying of proline treatment scored the highest mean value of fresh and dry weight 12.0 and 5.8 (g) in the same order for both seasons graduated by 300 mg/l with tryptophan, 5- aminolevulinic acid 11.6 and 11.5 (g) and 5.6, 5.6 (g) in the same order of fresh and dry weight and two types of amino acids without significant differs between them, lowest results of leaves fresh and dry weights were induced at 300mg/l with weekly spraying of tyrosine treatment 10.6 and 4.9 (g) as mean value for both seasons in the same order for fresh and dry weights, insignificant variance between level of 300mg/l with spraying of glutamine, asparagine and arginine on the leaves fresh and dry weights for both seasons. it may be the significant increasing of date palm leaves fresh and dry weights showed under spraying of different types of amino acids revert to the enhancing effects of amino acids for building the matters which used to increasing the plant growth, these effect of spraying amino acids was reported by. Russell [28] the increase in the fresh and dry weights as a result of the tryptophan treatments may be due to its conversion into IAA, El – Fawakhry and El- Tayeb [29] on *chrysanthemum* plants, Al-Khateeb et al. [30] they reported that, fresh weight of date palm cv.Khalas by spraying application of 5- aminolevulinic acid at 50- 100ppm, Attoa et al. [31] on *Iberisamara* L., Talaat et al. [32] on *Nigelia sativa* and Sudadi [33] on soybean stated that tryptophan at 0.001,0.1,1.0 and 10ppm increased fresh and dry weights of shoots.

3.3 Chemical Contents

3.3.1 Indole contents (mg/g f.w)

Indole contents (Table 3) showed that the highest contents of indoles were induced under all weekly spraying treatments of amino acids in comparable with the untreated plantlets which forming the lowest amount of indoles 2.0mg/g f.w. as the mean value for both seasons, under spraying glutamine, proline and tryptophan the greatest contents of indoles were scored 17.0, 17.0 and 17.0mg/g f. w. as mean value for both seasons sequenced by 5-aminolevulinic acid, aspragine, arginine with insignificant differences in between, meanwhile, lowest indole content was produced with weekly tyrosine spraying.

Table 1. Effect of 5- aminolevulinic acid and different amino acids on plant height (cm), leaves numbers and leaves stem diameter at 1st and 2nd seasons

Treatments		Plant height (cm)			Leaves numbers/plant			Stem diameter (cm)		
		1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control		30.3	62.0	46.2	5.3	8.0	6.7	5.8	8.3	7.1
ALA mg/l	100	32.3	78.7	55.5	5.7	9.3	7.5	7.3	8.4	7.9
	200	65.3	86.0	75.7	8.0	10.7	9.4	10.2	11.4	10.8
	300	75.3	94.7	85.0	8.7	11.7	10.2	11.4	12.8	12.1
	Mean			72.1			9.0			10.3
glutamine mg/l	100	32.0	78.3	55.2	5.7	9.3	7.5	8.0	8.7	8.4
	200	64.0	85.7	74.9	8.0	10.3	9.2	10.8	11.5	11.2
	300	75.0	94.3	84.7	8.7	11.7	10.2	12.6	13.2	12.9
	Mean			71.6			9.0			10.8
asparagine mg/l	100	31..6	77.0	54.3	6.0	9.0	7.5	7.9	8.4	8.2
	200	61.0	84.0	72.5	6.7	9.6	8.2	10.8	11.2	11.0
	300	72.3	90.0	81.2	7.3	10.7	9.0	12.0	13.5	12.8
	Mean			69.3			8.2			10.7
arginine mg/l	100	31.0	76.7	53.9	6.0	9.0	7.5	7.4	8.4	7.9
	200	59.3	83.0	71.2	6.3	10.0	8.2	10.1	11.3	10.7
	300	72.0	89.0	80.5	7.3	10.3	8.8	11.4	12.3	11.9
	Mean			68.5			8.2			10.2
proline mg/l	100	32.0	78.3	55.2	5.7	9.3	7.5	7.4	8.4	7.9
	200	64.7	87.0	75.9	7.3	10.7	9.0	10.4	11.5	11.0
	300	76.0	95.0	85.5	8.3	11.7	10.0	11.3	12.3	11.8
	Mean			72.2			8.8			10.2
tyrosine mg/l	100	30.7	64.3	47.5	5.3	8.0	6.7	5.9	8.3	7.1
	200	59.0	76.3	67.7	5.3	9.6	7.5	9.3	10.3	9.8
	300	61.3	89.0	75.2	6.0	10.0	8.0	10.0	10.7	10.4
	Mean			63.5			7.4			9.1
tryptophan mg/l	100	32.0	78.3	55.2	6.0	9.3	7.7	7.3	8.4	7.9
	200	65.0	86.0	75.5	8.0	10.3	9.2	10.2	11.3	10.8
	300	75.7	94.3	85.0	9.0	11.7	10.4	11.7	12.7	12.2
	Mean			71.9			9.1			10.3
L.s.d.		A= 2.9	B= 2.2	AB= 5.8	A= 0.6	B= 0.5	AB= 1.1	A= 0.6	B= 0.5	AB= 1.2

A= amino acids; B= levels; AB= A x B

Table 2. Effect of 5- aminolevulinic acid and different amino acids on leaves fresh and dry weights (g) and indoles content mg/g f.w. at 1st and 2nd seasons

Treatments		Leaves fresh weight (g)			Leaves dry weight (g)		
		1st season	2nd season	Mean	1st season	2nd season	Mean
Control		3.6	5.2	4.4	1.6	2.3	2.0
ALA mg/l	100	4.7	7.6	6.2	2.1	3.4	2.8
	200	6.5	8.4	7.5	3.1	3.9	3.5
	300	10.6	12.3	11.5	5.3	5.8	5.6
	mean			8.4			4.0
glutamine mg/l	100	4.7	7.6	6.2	2.2	3.4	2.8
	200	6.3	8.3	7.3	3.1	3.8	3.5
	300	10.4	12.2	11.3	5.0	5.8	5.4
	mean			8.3			3.9
asparagine mg/l	100	4.6	7.4	6.0	2.1	3.3	2.7
	200	6.1	8.2	7.2	2.8	3.9	3.4
	300	10.4	12.3	11.4	4.9	5.8	5.4
	mean			8.2			3.8
arginine mg/l	100	4.5	7.4	6.0	2.1	3.3	2.7
	200	6.2	8.4	7.3	2.8	3.9	3.4
	300	10.5	12.3	11.4	4.8	5.5	5.2
	mean			8.2			3.8
proline mg/l	100	4.6	7.9	6.3	2.2	3.6	2.9
	200	7.0	8.7	7.9	3.3	4.0	3.7
	300	11.3	12.6	12.0	5.5	6.1	5.8
	mean			8.7			4.1
tyrosine mg/l	100	4.3	7.4	5.9	2.0	3.3	2.7
	200	5.3	8.2	6.8	2.5	3.7	3.1
	300	9.4	11.8	10.6	4.4	5.4	4.9
	mean			7.8			3.6
tryptophan mg/l	100	4.7	7.8	6.3	2.2	3.6	2.9
	200	6.2	8.7	7.5	3.1	4.0	3.6
	300	10.6	12.6	11.6	5.1	6.1	5.6
	mean			8.5			4.0
L.s.d.		A= 0.6	B= 0.4	AB= 1.1	A= 0.3	B= 0.2	AB=0.5

A= amino acids; B= levels; AB= A x B

Grand contents of indoles were produced at 300mg/l with weekly spraying of all different types of amino acids, the level 300mg/l with glutamine, proline and tryptophan caused the greatest indole contents 21.3, 21.3 and 21.4 as mean value for both seasons in the same order of amino acids types, lowest content of indoles was obtained at 300mg/l with weekly spraying of tyrosine treatment, the levels 100 and 200mg/l with all types of amino acids produced lowest contents of indoles with significant variance. These finding agreed with those found by Tao et al. [34] pointed to the enhancement in plant growth as a result of amino acids` conversion to the growth promoter of IAA. Soad et al. [35] showed that total indoles increased with 250,500 and 1000ppm of peptone as foliar application on the *Helichrysum bracteatum,*

3.3.2 Chlorophyll a and b mg/g f.w

The highest contents of chlorophyll a and b observed in plantlets that sprayed with all types of amino acids (Table 3) un sprayed plantlets were amounted the lowest contents of chlorophyll a and bmg/g f.w. Greatest contents was found with spraying of 5- aminolevulinic acid, glutamine, proline and tryptophan which was 3.4, 3.4, 3.5 and 3.5mg/g f.w. for chlorophyll a and 2.5, 2.5, 2.6 and 2.6mg/g f.w. for chlorophyll b as mean value for both seasons in the same order with non-significant differs in between, lowest amounts was produced with spraying of tyrosine treatment for chlorophyll a and b 3.1 and 2.1mg/g f.w. as mean value for both season in the same order. Highest amounts of chlorophyll a and b induced by level 300 mg/l with all types of amino acids for both seasons as compared to un treated plantlets (control), greatest significant mean value of chlorophyll a and b was produced by proline and tryptophan with the level 300mg/l (4.3 and 2.6 respectively for chlorophyll a and b) with insignificant differs in between, followed by 5- amino levulinic acid, glutamine arginine and asparagine with insignificant differs in between. Fewer contents of chlorophyll a and b were associated with spraying of tyrosine at 300mg/l. in this respect Yongin et al. [36]. 5-aminolevulinic acid is a precursor of tetrapyrrole compounds such as chlorophyll, phycobilin, hem and vitamine B12. Nahedand Laila [37] total chlorophyll of *Salvia farinacea* which treated with foliar spraying of 50, 100ppm tyrosine, Sewedan et al. [38] on *Gladulus hybrid* cv. Sancerre which subjected with 100, 150 and 200ppm of free amino acids increased total chlorophyll. Abd El-Razek and Saleh [39] on Prince Peach which increased chlorophyll content by spraying application of amino acids at 0.25%.

3.4.1 Total amino acids mg/g d.w.

Significant increment of amino acids content (Table 4) were found with all weekly spraying of different amino acids treatments in comparison with significant little contents of amino acids in non-sprayed plantlets (control), greatest significant contents were obtained with 5-aminolevulinic acid, glutamine, proline and tryptophan for both seasons, on the other hand fewer contents of amino acids at weekly spraying of aspargine, arginine and tyrosine. The level 300mg/l scored the significant results with weekly spraying of 5- aminolevulinic acid, glutamine, proline and tryptophan in comparison with other levels. These results of increasing amino acids contents were found by Stasolla et al. [40] Pyrimidine derivatives, which are the building blocks for nucleic acid synthesis, energy sources, precursors for synthesis of sucrose, polysaccharides, and phospholipids, were found to have a role in cellular regulation and biosynthesis of some amino acids and secondary products in plants, Darwesh et al. [41] on *Phoenix canariensis*, El-Awadi and Hassan [42] on fennel (*Foeniculum vulgaris* Mill) and Talaat et al. [43] on *Catharanthus roseus* they showed that, free amino acids, methionine, putrescine and tryptophan at rates 100-500mg/l increased amino acids and total proteins.

Table 3. Effect of 5- aminolevulinic acid and different amino acids on indoles mg/g f.w. ch a and b mg/g f.w., at 1st and 2nd seasons

Treatments		Indoles mg/g f.w.			Chlorophyll a mg/g f.w.			Chlorophyll b mg/g f.w.		
		1st season	2nd season	Mean	1st season	2nd season	Mean	1st season	2nd season	Mean
Control		1.5	2.3	1.9	2.0	2.2	2.1	1.2	1.3	1.3
ALA mg/l	100	10.7	12.4	11.6	2.2	3.4	2.8	1.7	2.2	2.0
	200	17.3	19.0	18.2	2.8	3.9	3.4	2.2	2.9	2.6
	300	19.1	22.1	20.6	3.6	4.2	3.9	2.5	3.1	2.8
	mean			16.8			3.4			2.5
Glutamine	100	10.3	12.3	11.3	2.2	3.4	2.8	1.8	2.2	2.0
mg/l	200	17.8	19.0	18.4	2.7	4.1	3.4	2.2	2.9	2.6
	300	20.1	22.5	21.3	3.7	4.1	3.9	2.5	3.2	2.9
	mean			17.0			3.4			2.5
asparagine	100	10.7	12.4	11.6	2.2	3.3	2.8	1.6	2.1	1.9
mg/l	200	17.7	18.7	18.2	2.5	4.0	3.3	2.2	2.8	2.5
	300	19.0	21.3	20.2	3.5	4.1	3.8	2.4	3.1	2.8
	mean			16.7			3.3			2.4
Arginine mg/l	100	10.7	12.3	11.5	2.1	3.2	2.7	1.6	2.1	1.9
	200	17.7	18.3	18.0	2.4	4.2	3.3	2.1	2.8	2.5
	300	19.0	21.3	20.2	3.5	4.0	3.8	2.4	3.0	2.7
	mean			16.6			3.3			2.4
Proline mg/l	100	10.4	12.3	11.4	2.2	3.5	2.9	1.6	2.3	2.0
	200	17.7	19.0	18.4	2.6	4.0	3.4	2.4	3.0	2.7
	300	20.1	22.5	21.3	3.6	4.9	4.3	2.9	3.5	3.2
	mean			17.0			3.5			2.6
Tyrosine mg/l	100	9.7	10.7	10.2	2.0	3.1	2.6	1.4	2.0	1.7
	200	12.4	12.4	12.4	2.3	4.0	3.2	1.7	2.1	1.9
	300	16.5	17.8	17.7	3.2	4.0	3.6	2.2	3.0	2.6
	mean			13.4			3.1			2.1
	100	10.7	12.4	11.6	2.2	3.4	2.8	1.7	2.3	2.0
tryptophan	200	17.4	19.0	18.2	2.7	4.3	3.5	2.3	3.1	2.7
mg/l	300	20.1	22.5	21.4	3.7	4.7	4.3	2.7	3.7	3.2
	mean			17.0			3.5			2.6
L.s.d.		A= 1.1	B= 0.9	AB=2.3	A= 0.3	B= 0.2	AB=0.4	A= 0.2	B= 0.1	AB=0.3

A= amino acids; B= levels; AB= A x B

Table 4. Effect of 5- aminolevulinic acid and different selected amino acid son total amino acids mg/g d. w. and total sugars % contents at 1st and 2nd seasons

Treatments		Total amino acids mg/g d.w.			Total soluble sugar %		
		1st season	2nd season	Mean	1st season	2nd season	Mean
Control		4.0	4.3	4.2	11.2	11.9	11.6
ALA	100	7.3	9.6	8.5	24.6	25.6	25.1
	200	9.3	11.3	10.3	25.8	26.6	26.2
	300	11.3	12.9	12.1	28.6	29.2	28.9
	mean			10.3			26.7
glutamine	100	7.0	8.3	7.7	22.1	21.9	22.0
	200	11.0	10.7	10.9	30.2	31.2	30.7
	300	12.0	12.5	12.3	30.8	32.2	31.5
	mean			10.3			28.0
asparagine	100	7.0	8.7	7.9	24.5	25.6	25.1
	200	9.0	9.4	9.2	25.2	26.3	25.8
	300	11.0	10.7	10.9	26.3	27.5	26.9
	mean			9.3			25.9
arginine	100	6.4	8.3	7.4	24.6	25.8	25.2
	200	10.4	9.2	9.8	25.8	26.3	26.1
	300	11.4	10.7	11.1	25.7	27.5	26.6
	mean			9.4			25.9
proline	100	7.3	9.9	8.6	19.6	20.7	20.2
	200	9.2	10.9	10.1	28.5	30.6	29.6
	300	12.4	12.0	12.2	29.5	31.8	30.7
	mean			10.3			26.8
tyrosine	100	6.0	7.3	6.7	18.9	19.7	19.3
	200	8.0	8.9	8.5	19.8	20.3	20.1
	300	10.0	10.0	10.0	20.9	21.0	21.0
	mean			8.4			20.1
	100	7.3	8.7	8.0	20.1	21.0	20.6
tryptophan	200	9.0	11.7	10.4	27.5	30.8	29.2
	300	12.5	12.9	12.7	29.6	31.9	30.8
	mean			10.4			26.9
L.s.d.		A= 1.2	B= 0.9	AB=2.5			

A= amino acids; B= levels; AB= A x B

3.4.2 Total sugars%

The corresponded scored increased of total soluble sugars (Table 4) were related with weekly spraying of glutamine treatment 28.0% with significant variance between 5-aminolevulinic acid, proline, and tryptophan in the order of 26.7, 26.8 and 26.9% as mean value for both seasons, lowest contents of total soluble sugar was achieved by weekly spraying of tyrosine 20.1% as mean value for both seasons, meanwhile un sprayed plantlets contained the significant smallest amount of total sugars 11.6% as mean value for both seasons. In relation to the effect of different levels of types of amino acids 100,200 and 300 mg/l, biggest significant contents of total soluble sugars produced with 300mg/l with all types of amino acids, significant contents was 300 mg/l with spraying of glutamine 31.5% as mean value for both seasons followed by tryptophan and proline treatments 30.8 and 30.7% as mean value for both seasons in the same order with in significant variance in between. These results were in agreement with those of Talaat and Youssef [44] on basil plant (*Ocimum basilicum* L.), Feng et al. [45] Lysine, Ornithine and ALA at 100mg/l increased total soluble sugars of Kudzu (*Pueraria phaseoloides*), Azza et al. [10] on *Codiaeum variegatum* L. showed that glutamine at 100-200 ppm increased total soluble sugars, Ardebili [46] on *Aloe vera* L., and Ali and Hassan [47] on *Tagetes erecta*, they observed that, treatments of amino acids from 0.05 to 0.15% and from 500- 750ppm increased total soluble sugars.

4. CONCLUSION

From previous results, the fasting and healthy growth plantlets in the greenhouse after acclimatization is very important for possibility cultured date palm plantlets in the open field in the short time, therefore, it could be using different types of amino acids (5-aminolevulinic acid, glutamine, asparagine, arginine, proline, tyrosine and tryptophan as singly treatments or combinations in between) to greatest improving all vegetative growth of date palm in the greenhouse as height plants, greatest leaves numbers/plantlets, greatest thickness of stems, furthermore, the importance of highest contents of indoles, chlorophyll contents which they very importance for metabolism process in the plant cell.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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