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ARTÍCULO DE INVESTIGACIÓN

EFFECT OF FOLIAR APPLICATION OF AMINOACIDS ON PLANT YIELD AND SOME PHYSIOLOGICAL PARAMETERS IN FABA BEAN PLANTS IRRIGATED WITH SEAWATER

Efecto de la aplicación foliar de aminoácidos sobre el rendimiento y parámetros fisiológicos en plantas de haba irrigadas con agua de mar

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ABSTRACT

Salinity decreases yield in arid and semi-arid areas. With increasing demand for irrigation water, alternative sources are being sought. Seawater salinity was previously considered unusable for irrigation. However, this water can be used successfully to grow crops under certain conditions. Amino acids is well known biostimulant which has positive effects on plant growth and yield, and significantly mitigates the injuries caused by abiotic stresses. Therefore, in the present study, the effect of exogenously treatment amino acid on faba bean plant growing under seawater salt stress was investigated. Reduction of salinity damage in faba bean by using a mixture of amino acids to improve morphological and biochemical parameters, and thus raising the level of plant yield was tested. A pot experiment was conducted to alleviate the harmful effects of seawater salinity on faba bean cv. Giza 843 by foliar spraying of an amino acid mixture with different concentrations (0.0, 500, 1000 or 1500 mg L^{-1}). Irrigation of faba bean plants with seawater levels of 3.13 and 6.25 dS m^{-1} led to significant reductions in shoot length, number of leaves per plant, fresh and dry weight of shoots, photosynthetic pigments, total carbohydrates, polysaccharides, nucleic acid DNA and RNA contents of faba bean leaves. Seawater salinity induced higher contents of Na^+ and Cl^- and decreased contents of K^+, K^+:Na^+, Ca^{2+}, Mg^{2+} and P^{3+}. Irrigation of faba bean plant with different levels of seawater decreased seed yield and total dry weight per plant compared with those irrigated with tap water. Also, total carbohydrates and total protein contents in seeds were reduced by increased seawater salinity levels. Amino acid application as foliar spray significantly improved all the reduced parameters due to seawater stress. However, the highest level of amino acid of 1500 mg L^{-1} exerted the strongest effect in alleviating the harmful effect of seawater salinity stress.
Keywords: free amino acids, photosynthetic pigments, proline, salinity, *Vicia faba*.

RESUMEN

La salinidad disminuye el rendimiento en zonas áridas y semiáridas. Con el aumento de la demanda de agua de riego, se están buscando fuentes alternativas. El agua de mar se consideró previamente inutilizable para irrigación debido a su salinidad. Sin embargo, esta agua puede ser utilizada con éxito en cultivos bajo ciertas condiciones. Los aminoácidos son bioestimulantes bien conocidos por sus efectos positivos sobre el crecimiento y rendimiento, y por mitigar significativamente las lesiones causadas por estrés abióticos. Por lo tanto, en el presente studio se investigó el efecto del tratamiento exógeno con aminoácidos sobre plantas de haba que crecen bajo estrés salino por irrigación con agua de mar. Se evaluó la reducción de daños por salinidad en plantas de haba mediante el uso de una mezcla de aminoácidos para mejorar los parámetros morfológicos y bioquímicos, y por lo tanto elevar el nivel de rendimiento de la planta. Se desarrolló un experimento en macetas para paliar los efectos nocivos de la salinidad del agua de mar en el haba cv. Giza 843 por aspersion foliar de una mezcla de aminoácidos con diferentes concentraciones (0, 500, 1000 o

1500 mg L^{-1}). El riego de plantas de haba con niveles de agua de mar de 3.13 y 6.25 dS m^{-1} condujo a reducciones significativas en la altura de planta, número de hojas de la planta, peso fresco y seco de los brotes, y en el contenido foliar de pigmentos fotosintéticos, carbohidratos totales, polisacáridos y ácidos nucleicos (ADN y ARN). La salinidad del agua de mar indujo un mayor contenido de Na^+ y Cl^-, y una disminución del contenido de K^+, K^+: Na^+, Ca^{2+}, Mg^{2+} y P^{3+}. El riego de plantas de haba con diferentes niveles de agua de mar redujo el rendimiento de semillas y el peso seco total por planta en comparación con las regadas con agua corriente. Además, el contenido de carbohidratos y proteína total en las semillas disminuyeron con el aumento de los niveles de salinidad del agua de mar. La aplicación de aminoácidos por aspersion foliar increment significativamente todos los parámetros reducidos debido al estrés por agua de mar. Sin embargo, el más alto nivel de aminoácidos (1500 mg L^{-1}) ejerce el máximo efecto en el alivio de los efectos nocivos de estrés por salinidad del agua de mar.

Palabras clave: aminoácidos libres, pigmentos fotosintéticos, prolina, salinidad, *Vicia faba*.

INTRODUCTION

With increasing demand for irrigation water, alternative sources are being sought. Seawater (saline water) was previously considered unusable for irrigation. However, this water can be used successfully to grow crops under certain conditions (Zeid, 2011). Plants growing in saline stress face three main problems: High salt concentrations in the soil solution (that is, high osmotic pressure and correspondingly, low soil water potential "drought stress"), high concentrations of potentially toxic ions (such as Na^+ and Cl^-), and nutrient imbalance as a result of depressed uptake, impaired internal distribution and shoot transport of minerals (Hu and Schmidhalter, 2005). Foliar spraying treatment of plants with naturally occurring compounds in plant cells is an easy technique and an alternative approach used to overcome salinity problems.

Plants are continuously exposed to biotic and abiotic stresses. Salt stress is one of the most severe abiotic stresses limiting plant productivity. If excessive amounts of salt enter the plant, eventually rise to toxic levels in the older transpiring leaves, causing premature senescence, and reduces the photosynthetic leaf area of the plant to a level that cannot sustain growth (Erdal *et al.*, 2011). Salt stress that leads to both the decrease of the substrate osmotic potential and ion-specific toxicity affects almost every aspect of the physiology and biochemistry of plants (Cuartero *et al.,* 2006). Salinity reduces stomatal conductance greatly and consequently reduces photosynthetic rate (Munns and Tester, 2008). However, the inhibition of photosynthetic rate imposed by stomatal closure may promote an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis, leading to excess excitation and subsequent photoinhibitory damage of PSII reaction centers (Souza *et al.,* 2004). Differences in the accumulation patterns of Na^+ and K^+ were found under salinity stress. High K^+:Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ (Cuin *et al.*, 2003). Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel and Hirt, 2004). Chloroplasts are the major organelles producing reactive oxygen species (ROS) such as, the superoxide radical ($O2^{-}$), hydrogen peroxide (H_2O_2) and singlet oxygen (O^{-1}) during photosynthesis (Asada, 1992). Salt stress induces significant reduction in photosynthesis. This reduction depends on photosynthesizing tissue (leaf area) and photosynthetic pigments (Raza *et al.*, 2006).

Faba bean (*Vicia faba* L.) is considered the main leguminous crop grown in Egypt as its seeds are used for human consumption. Thus, many efforts have been consistly made to increase its productivity. As fresh water resources and the area allotted for this crop are not sufficient to meet the food demand of the increasing population (about 90 million) in Egypt, cultivation of marginal area together with the use of seawater may solve this problem.

Amino acids are considered as precursors and constituents of proteins (Rai, 2002), which are important for stimulation of cell growth. They contain both acid and basic groups and act as buffers, which help to maintain favorable pH value within the plant cell (Davies, 1982). Also, amino acids is a well known biostimulant which has positive effects on plant growth, yield and significantly mitigates the injuries caused by abiotic stresses (Kowalczyk and Zielony, 2008). Several hypotheses have been proposed to explain the role of amino acids in plant growth. Available evidence suggests several alternative routes of IAA synthesis in plants, all starting from amino acids (Hashimoto and Yamada, 1994). Amino acids can directly or indirectly influence the physiological activities in plant growth and development such as exogenous application of amino acids have been reported to modulate the growth, production and quality of tomato in plastic greenhouse (Boras *et al.*, 2011). Saeed *et al.*, (2005) on soybean found that treatments of amino acids significantly improved growth parameters of shoots and fresh weight as well as pod yield. Liu Xing-quan *et al.*, (2008) revealed that foliar application with the mixture of amino acids to radish plants increased N content of shoots. El-Zohiri and Asfour (2009) on potato found that spraying of amino acids at 0.25 ml l^{-1} significantly increased vegetative growth expressed as plant height and dry weight of plant. Abo Sedera *et al.,* (2010) revealed that spraying strawberry plants with amino acids (peptone) at 0.5 and 1.0 g l^{-1} significantly increased total nitrogen, phosphorus and potassium in plant foliage as well as total yield, weight, TSS, vitamin C and total sugars content of fruits compared with control treatment.

Thus, the present study was undertaken in a trial to alleviate the harmful effects of salinity on faba bean plant by using an amino acid mixture. It aiming to increase the salt tolerance of faba bean plant through its effects on growth, some physiological parameters, yield and some chemical constituents of the yielded seeds.

MATERIALS AND METHODS

Experimental procedures

This study was conducted at the wire-house of the National Research Centre, Dokki, Cairo, Egypt (30° 3'0" N / 31°15'0" E), during two successive winter seasons; 2011/12 and 2012/13. Daytime temperatures ranged from 14.5 to 30.2 °C with an average of 23.2 ± 3.8 °C whereas temperatures at night were 12.4 ± 1.8 °C, with minimum and maximum of 8.0 and 17.6 °C, respectively. Daily relative humidity averaged 57.7± 9.6 % in a range between from 38.1 to 78.7 %. Faba bean (*Vicia faba* L.) seeds variety cv. Giza 843 were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Faba bean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Ten uniform air dried faba bean seeds were sown along a centre row in each pot at 30-mm depth in plastic pots, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1(v:v), respectively in order to reduce compaction and improve drainage. Saline water was prepared by mixing fresh water (0.23 dS m^{-1}) with seawater (51.2 dS m^{-1}) to achieve salinity levels of 3.13 and 6.25 dS m^{-1}. Concentration of EC, pH, cations and anions of irrigation water and soil used in the pot experiment are shown in Table 1.

At sowing, a granular commercial rhizobia was incorporated into the top 30-mm of the soil in each pot with the seeds. Granular ammonium sulphate 20.5 % N at a rate of 40 kg N ha^{-1}, and single superphosphate (15% P_2O_5) at a rate of 60 kg P_2O_5 ha^{-1} were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing. Foliar application of the different concentrations of amino acids were done at 45 and 60 days after sowing (DAS). The commercial product "Amino total" was used as a source of amino acids. In the amino total, 17 different amino acids are present viz., Tiroanine (3.05-3.56 %), Aspartic acids (3.2- 3.45%), Serine (3.76-4.49%), Glutammic acids (7.24-9.12 %), Broline (2.23-3.5 %), Licyne (1.87- 2.45 %), Alanine (2.16-2.20), Cystine (1.87-2.45 %),Veline (2.8-3.1%), Methionine (0.23-0.3 %), Isoleucine (1.26-1.7 %), Leucine (1.98-2.8 %), Tyrosine (0.48- 1.02 %), Phenylalanine (1.03-1.78), lysine (1.39-2.3 %), Histidine (0.42-0.9 %.) and Arginine (5.2-6.2 %). Among them, Glutammic acids and Arginine are the most revelnt in the biostmulating activity. The experiment consisted of four levels of amino acids namely, 0, 500, 1000 or 1500 mg L^{-1} considered as AA0, AA1, AA2 or AA3, respectively, and irrigation water consisted of two concentrations of diluted seawater namely, 3.13 or 6.25 dS m^{-1} which were considered as SW1 and SW2, respectively, while control plants irrigated with tap water (0.23 dS m^{-1}) was considered as TW. Treatments were arranged at the wire-house benches in a factorial arrangement with five replicates for each treatment. Ten DAS, faba bean seedlings were thinned to four seedlings per pot and irrigated with equal volumes of tap water until maturity and final harvesting. Starting from 16th day, all pots were irrigated either with tap water or different diluted seawaters along the period of the experiment. Soil field capacity in the pots was estimated by saturating the soil in the pots with water and weighing them after they had drained for 48 h. Field water capacity was 0.36 g g^{-1}. Soil water content was maintained at about 90% of field water capacity. The level of soil moisture was controlled by weighing pots and daily loss of water was supplemented twice (morning and afternoon).

Table 1. EC, pH, and concentration of cations and anions of irrigation water and soil used in the pot experiment.

	EC	pH	Cations meq l^{-1}				Anions meq l^{-1}			
	dS m^{-1}		Ca^{2+}	Mg^{2+}	Na^+	K^+	HCO_3^-	CO_3^{2-}	SO_4^{2-}	Cl^-
					Water					
Tap water	0.23	7.35	1.00	0.50	2.40	0.20	0.10	0.00	1.30	2.70
Sea water	51.2	7.76	43.20	15.12	454.57	1.51	6.05	0.00	76.36	432.00
					Soil					
Sandy	0.14	8.11	2.60	2.40	1.31	0.21	1.13	0.00	4.22	0.70
Clay	1.40	7.59	5.60	1.90	5.90	0.37	1.50	0.00	6.77	5.50

Measurements

Plant samples were collected after 75 days from sowing for measurement of growth parameters shoot length, leaf number and fresh and dry weight of shoot/plant, photosynthetic pigments, polysaccharides, total carbohydrates, free amino acids, proline and mineral contents in leaves tissue. Chlorophyll a, chlorophyll b and carotenoids were determined using the spectrophotometric method described by Lichtenthaler and Buschmann (2001). Phenol-sulfuric acid method was used for the determination of total carbohydrates (TC) (Smith *et al*., 1956). Polysaccharides were determined according to Naguib (1963). DNA and RNA were extracted with 10% perchloric acid following the method of Schmidt and Thannhauser (1945) with some modifications as described by Morse and Carter (1949). Nucleic acids, which include DNA (deoxyribonucleic acid) was estimated by diphenylamine colour reaction described by Burton (1956), and RNA (ribonucleic acid) was estimated colorimetrically by the orcinol reaction (Dische, 1953). Total nitrogen (N) was determined using the Kjeldahl method, while, estimation of Sodium (Na) calcium (Ca), potassium (K) and chlorine (Cl) concentrations were done by the use of flame photometer. Also, magnesium (Mg) content was estimated using atomic absorption spectrophotometer. Phosphorus (P) was photometrically determined using the molybdate-vanadate method according to Jackson (1973). At maturity, measurement of yield and yield components were also recorded (seeds yield per plant and total dry weight per plant) and chemical constituents of the seed yield. The total carbohydrates percentage (TC %) was estimated on seed yield. Total crude protein percentage (TCP %) of the seed yield was determined according to the method described by Bradford (1976).

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) appropriate to the randomized complete block design applied after testing the homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). The significant differences between treatments were compared with the critical difference at 5% probability level by the Duncan's test.

RESULTS

Data in Table 2 clearly show that, growth parameters (shoot length, leaf number per plant, shoot fresh and dry weights) were reduced gradually and significantly with increasing salinity levels in faba bean plant in the two seasons SI and SII. Amino acid treatments caused stimulatory effects on such parameters under both saline and non-saline (control) conditions. Amino acid treatments alleviated the inhibitory effect of salt stress on the above mentioned parameters. Table 2 also shows an increased stimulation response of amino acid with increasing concentration in both seasons.

Seawater stress reduced gradually, chlorophyll *a*, chlorophyll *b*, carotenoids and total pigment contents of faba bean leaves (Table 3). The percentage of reduction reached 8.6, 30.0, 32.1, and 39.4 % for chlorophyll *a* in the first and second seasons respectively, and 23.9, 43.7, 9.0, and 23.6 % for chlorophyll *b* in the first and second

Table 2. Effect of amino acid (AA) supply on shoot length (cm), leaf number per plant, fresh weight (FW g plant^{-1}) and dry weight (DW g plant^{-1}) of faba bean plants grown at three seawater salinity (SW) levels in 2011/12 (SI) and in 2012/13 (SII)#

Treatments		Shoot length (cm)		leaf No. plant^{-1}		FW (g plant^{-1})		DW (g plant^{-1})	
SW	AA	SI	SII	SI	SII	SI	SII	SI	SII
TW	AA0	69.0b-d†	69.0d	10.7ef	16.3cd	10.1ef	10.8f	1.23f	1.49e
	AA1	70.3bc	71.0c	12.7bc	17.3bc	11.6c	11.9d	1.48d	1.79c
	AA2	73.0ab	73.0b	13.7b	18.3b	12.4b	13.7b	2.07b	2.26b
	AA3	77.0a	77.0a	15.0a	19.7a	13.1a	15.6a	2.54a	2.74a
SW1	AA0	63.0ef	63.0f	9.7fg	13.0gh	9.2g	9.8h	1.03g	1.28fg
	AA1	66.0c-e	66.0e	11.3de	14.0fg	10.3e	10.7f	1.29ef	1.47e
	AA2	69.0b-d	69.0d	12.8bc	15.3de	11.5c	11.9d	1.65c	1.70d
	AA3	73.0ab	73.0b	13.7b	16.3cd	12.1b	12.9c	2.05b	1.87c
SW2	AA0	56.0g	57.7h	8.3h	11.3i	8.4h	9.0i	0.82h	1.04h
	AA1	59.0fg	60.7g	9.0gh	12.7h	9.7f	10.1g	1.05g	1.22g
	AA2	61.0e-g	62.7f	10.8e	13.9fg	10.8d	11.0ef	1.24f	1.33f
	AA3	64.0d-f	64.0f	12.0cd	14.7ef	11.3c	11.3e	1.39de	1.45e

†Mean values (n=12) in the same column for each trait in each year with the same lower-case letter are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

#Measurements were made 75 d after sowing (DAS).

seasons, respectively, 6.8, 26.4, 15.4, and 25.3 % for carotenoids in the first and second seasons, respectively, and for total chlorophyll 12.4, 33.2, 25.0, and 34.3 % in the first and second seasons respectively, at 3.13 and 6.25 dS m^{-1} seawater salinity levels, respectively. Foliar spraying of faba bean plants with amino acid improved all fractions of photosynthetic pigments, especially in plants subjected to seawater stress in both seasons. However, amino acid treatments exerted stimulatory effects on photosynthetic pigments under both saline and non-saline (control) conditions. Amino acid treatments not only alleviated the inhibitory effect of salt stress but also in most cases induced an enhanced stimulating effect compared to the control plants.

Table 4 shows that seawater salinity at the two levels of 3.13 and 6.25 dS m^{-1} caused significant decreases in total carbohydrate and polysaccharides contents of faba bean shoots compared with control. These decreases reached its maximum at 6.25 dS m^{-1}. Application of amino acid with all concentrations increased significantly total carbohydrates, and polysaccharides compared with control. These increases were correlated positively with increasing its concentration. The interaction effect of salinity combined with amino acid was significant in total carbohydrates and polysaccharides. The changes in nucleic acid contents (DNA and RNA) in shoots extract of untreated and differently treated faba bean plant are presented in Table 4. Data illustrate that increasing salinity levels up to 6.25 dS m^{-1} significantly decreased RNA and DNA contents in faba bean shoots compared with control plant. Exogenous application of amino acids on faba bean plant grown under three levels of seawater could overcome the reduction in DNA and RNA (Table 4). Increases in DNA and RNA occurred gradually with the increases in amino acid concentrations, the highest values were recorded at 1500 mg L^{-1} amino acid treatment compared with the corresponding control and salinity levels.

The results in Table 5 show that mineral ion concentration including N, P, K^+, Ca^{2+}, and Mg in the leaves gradually decreased by increasing salinity levels to reach their lowest values at the greatest level of salinity. Although N, P, K^+, Ca^{+2}, and Mg were negatively affected by salinity, the complete reverse was true with Na^+ and Cl^-, thus, their concentration showed positive correlation with increasing salinity level to attain its greatest level over the non-stressed control plants. The K^+:Na^+ ratio of faba bean leaves gradually decreased by increasing salinity levels to reach their lowest values at the greatest level of salinity. Amino acid foliar application counteracted partially or completely the adverse effect of salinity as it increased the concentrations of Mg, N, P, K^+ and Ca^{2+}, in the same time it decreased the absorption of Na^+ and Cl^- in faba bean leaves compared with the corresponding salinity levels.

Table 6 revealed that, salinity stress significantly reduced seed yield and total dry matter per plant. Amino acid application as foliar spray significantly improved seed yield and total dry matter per plant irrigated either with tap water or with different saline water. The highest concentration of amino acid application (1500 mg L^{-1}) resulted in pronounced increase in seed yield and total dry matter per plant. Increasing salinity levels resulted in a significant reduction

Table 3. Effect of amino acid (AA) supply on chlorophyll *a*, chlorophyll *b*, caroteniods and total pigments (mg g^{-1} fresh weight) of faba bean plants grown at three seawater salinity (SW) levels in 2011/12 (SI) and in 2012/13 (SII)#

Treatments		Chlorophyll *a*		Chlorophyll *b*		Caroteniods		Total pigments	
SW	AA	SI	SII	SI	SII	SI	SII	SI	SII
TW	AA0	1.155d†	1.286d	0.503b	0.445b	0.235b	0.202b	1.893b	1.933b
	AA1	1.253c	1.340c	0.544b	0.462b	0.305a	0.215b	2.102a	2.018a
	AA2	1.345b	1.418b	0.574a	0.504a	0.312a	0.230a	2.231a	2.150a
	AA3	1.456a	1.499a	0.603a	0.525a	0.329a	0.249a	2.388a	2.272a
SW1	AA0	1.056e	0.873e	0.383a	0.405c	0.219b	0.171c	1.659a	1.449c
	AA1	1.147d	0.939d	0.451b	0.432b	0.245b	0.202b	1.843b	1.573c
	AA2	1.258c	0.982c	0.484b	0.449b	0.284a	0.225a	2.026a	1.656b
	AA3	1.385b	1.147b	0.509b	0.454b	0.292a	0.251a	2.186a	1.852b
SW2	AA0	0.809g	0.779g	0.283c	0.340d	0.173c	0.151c	1.265c	1.270d
	AA1	0.936f	0.812f	0.351c	0.369c	0.187c	0.176c	1.474c	1.358d
	AA2	1.049e	0.839e	0.485b	0.389c	0.202b	0.194b	1.737b	1.422c
	AA3	1.162d	0.919d	0.516b	0.394c	0.230b	0.208b	1.908b	1.521c

†Mean values (n=6) in the same column for each trait in each year with the same lower-case letter are not significantly different by Duncan's multiple range test at $p \leq 0.05$.
#Measurements were made 75 d after sowing (DAS).

in total carbohydrates and total protein contents. These reductions reached its highest values at 6.25 dS m^{-1} salinity levels compared with control plants. Exogenous application of amino acids either under control water or different salinity levels caused increases in total carbohydrates and protein contents compared with the corresponding salinity levels. These increases were gradually increased with increasing amino acid concentrations up to 1500 mg L^{-1} at different salinity levels.

DISCUSSION

As shown in Table 2, irrigation of faba bean plant with seawater levels at 3.13 and 6.25 dS m^{-1} resulted in significant reductions in growth parameters compared with tap water. These findings are in agreement with those obtained in wheat (El-Lethy *et al.,* 2013), maize (Awad *et al.,* 2012), common bean (Dawood *et al.,* 2014a), and faba bean (Bekheta *et al.,* 2009; Sadak *et al.,* 2010; Erdal *et al.,* 2011; Abdelhamid *et al.,* 2013; Dawood *et al.,* 2014b). Amino acid mixtures treatment had a pronounced ameliorative as well as growth promoting effect under both saline and non-saline conditions. This is in line with several reports supporting our obtained results, but obtained on different plant species (El-Zohiri and Asfour, 2009). The positive effect of amino acids on growth was stated by Goss (1973) who indicated that amino acids can serve as a source of carbon and energy when carbohydrates become deficient in the plant's releasing the ammonia and organic acid form which the amino acid was originally formed. The organic acids then enter Kerb's cycle, to be broken down to release energy through respiration. Thon *et al*., (1981) pointed out that amino acids provide plant cells with an immediately available source of nitrogen, which generally can be taken by the cells more rapidly than inorganic nitrogen. The ameliorative effect of amino acids might be linked to the observable increase in photosynthetic pigments (Table 3) as well as, leaf number (Table 2). Consequently the efficiency of the photosynthetic apparatus was increased due to amino acid treatments, which in turn considerably increased the biosynthesis of osmotic solutes under salinity stress. These osmolytes might increase the osmotic pressure of the cytoplasm and enhance water flow into different plant organs and tissues. This may indicate that amino acids might alleviate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells as reported by other investigators (on different plant species). These increases in the above mentioned data due to those amino acids can directly or indirectly influence the physiological activities of the plant. The regulatory effect of amino acids on growth could be explained by the notion that some amino acids e.g. phenylalanine, ornithine can affect plant growth and development through their influence on gibberellins biosynthesis (Walter and Nawacke, 1978). Also, amino total as a source of amino acids may play an important role in plant metabolism and protein assimilation which is necessary for cell formation and consequently increase in fresh and dry matter.

Photosynthetic pigments (Table 3) declined in both seasons as plants were subjected to salinity stress. The

Table 4. Effect of amino acid (AA) supply on polysaccharides (mg/g dry weight), total carbohydrates (mg/g dry weight), nucleic acid (RNA and DNA mg 100 g^{-1} dry weight) of faba bean plants grown at three seawater salinity (SW) levels in 2011/12 (SI) and in 2012/13 (SII)[#]

Treatments		Polysaccharide (mg g^{-1} DW)		Total carbohydrates (mg g^{-1} DW)		RNA (mg 100 g^{-1} DW)		DNA (mg 100 g^{-1} DW)	
SW	AA	SI	SII	SI	SII	SI	SII	SI	SII
TW	AA0	108.3d	98.9e	145.7e	129.8g	972c	523f	376de	227d
	AA1	115.8c	107.4c	155.8c	140.1de	1000bc	562e	416c	278c
	AA2	122.3b	116.5b	166.9b	152.5b	1076b	775b	499b	339b
	AA3	136.6a	125.4a	183.1a	164.0a	1266a	830a	582a	404a
SW1	AA0	96.4f	91.9gh	137.7f	124.7hi	807e	438g	260h	157f
	AA1	101.1e	94.6fg	146.8de	130.2g	880de	546ef	301g	190e
	AA2	108.6d	98.6e	159.3c	137.1ef	956cd	658d	341f	235d
	AA3	115.9c	103.5d	169.0b	143.7c	1030bc	715c	397cd	288c
SW2	AA0	79.6h	85.8i	129.5g	121.4i	555h	260j	201i	104h
	AA1	83.2h	90.5h	139.8f	127.7gh	580gh	303i	264h	134g
	AA2	87.4g	95.3f	149.8d	135.4f	639fg	381h	314g	177e
	AA3	93.1f	98.9e	158.5c	141.2cd	690f	457g	355ef	226

[†]Mean values (n=12) in the same column for each trait in each year with the same lower-case letter are not significantly different by Duncan's multiple range test at $p \leq 0.05$.
[#]Measurements were made 75 d after sowing (DAS).

Table 5. Effect of amino acid (AA) supply on nitrogen (N %), phosphorus (P %), potassium (K^+%), sodium (Na^+%), calcium (Ca^{2+}%), *magnesium (Mg^{2+}%)*, chloride (Cl^- ppm) and K:Na ratio of leaves of faba bean plants grown at three seawater salinity (SW) levels in 2011/12 (**SI**) and in 2012/13 (**SII**)#

Treatment		N (%)		P (%)		K^+ (%)		Na^+ (%)		Ca^{2+} (%)		Mg^{2+} (%)		Cl^- (ppm)		K:Na ratio	
SW	AA	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII	SI	SII
TW	AA0	1.83ef†	2.07f	0.30a	0.30a	2.33f	1.81f	0.13c	0.13c	2.27e	2.23e	0.43b	0.35a	2.92a	2.78f	17.5d	14.0f
	AA1	2.19cd	2.53c	0.32a	0.33a	2.48d	1.92d	0.11c	0.10c	2.46c	2.41cd	0.50a	0.42b	2.81ab	2.64g	23.0c	20.0d
	AA2	2.46b	2.76b	0.35a	0.36a	2.57b	2.19b	0.10c	0.10c	2.61b	2.58b	0.58a	0.48a	2.75b	2.62g	24.9b	22.0b
	AA3	2.73ᵃ	2.97a	0.40a	0.40a	2.64a	2.44a	0.10c	0.09c	2.83a	2.71a	0.61a	0.59a	2.62b	2.34h	25.6a	26.1a
SW1	AA0	1.72fg	1.69g	0.25b	0.24b	2.15g	1.60g	0.26b	0.24b	2.16f	2.05f	0.37c	0.24c	3.58c	3.31d	8.4g	6.8g
	AA1	2.08d	1.97d	0.27b	0.26b	2.36ef	1.83ef	0.22b	0.20b	2.26e	2.25e	0.42b	0.27c	3.50cd	3.28d	10.8f	9.3ef
	AA2	2.25c	2.18e	0.28b	0.28b	2.41c	2.01c	0.21b	0.16c	2.35d	2.37d	0.46b	0.34b	3.49cd	3.27d	11.7e	12.6c
	AA3	2.31c	2.32d	0.31a	0.31a	2.49b	2.20b	0.21b	0.14c	2.43cd	2.44c	0.53a	0.38b	3.45d	3.04e	11.8e	15.4b
SW2	AA0	1.61g	1.51h	0.22c	0.21c	2.05h	1.36h	0.39a	0.36a	2.06g	1.63i	0.32c	0.17d	3.94e	3.86a	5.3i	3.8h
	AA1	1.92e	1.75g	0.24b	0.23c	2.19g	1.60g	0.31a	0.29a	2.14fg	1.77h	0.35c	0.22c	3.86f	3.71b	7.1h	5.5g
	AA2	2.07d	1.98f	0.26b	0.25b	2.27f	1.75f	0.28a	0.26b	2.25e	1.92g	0.37c	0.26c	3.82f	3.61b	8.1g	6.8f
	AA3	2.09d	2.19e	0.28b	0.26b	2.32de	1.91de	0.32a	0.25b	2.39cd	2.08f	0.41b	0.32b	3.76g	3.49c	7.3h	7.6de

†Mean values (n=12) in the same column for each trait in each year with the same lower-case letter are not significantly different by Duncan's multiple range test at $p \leq 0.05$.

#Measurements was made 75 d after sowing (DAS).

decreasing effect of salinity is reflected in the biosynthesis of photosynthetically active pigments, which is consistent with the results of Azooz (2009). The inhibition of photosynthetic pigments of faba bean leaves irrigated with seawater may be attributed to the inhibition of assimilate translocation.. Amino acid foliar spraying of faba bean plant with different concentrations enhances photosynthetic pigments of plants irrigated either with tap water or saline water. This increase in chlorophyll contents might be due to the availability of higher levels of amino acids to the treated plants as amino acids help to increase the chlorophyll content and this may lead to the increase in different growth criteria (Awad *et al.,* 2007).

Table 4 shows that seawater levels of 3.13 and 6.25 dS m^{-1} caused significant decreases in total carbohydrate and polysaccharides content of faba bean leaves. The obtained results of total carbohydrates and polysaccharides are in good agreement with those obtained by Sadak *et al.,* (2010) and Taie *et al.,* (2013) on faba bean plants. This trend might be a result of reduction in photosynthetic activity and/or respiration in order to provide enough energy for water and nutrient absorption. Our results indicated that the application of amino acids as a foliar spray caused increases in the contents of total carbohydrates and polysaccharides of stressed and non -tressed plants. These results are in agreement with the finding of other studies on different plant species (Abdel Aziz *et al.,* 2010). There is positive correlation between photosynthesis rates and nitrogen contents in leaves. A high rate of photosynthesis due to a high nitrogen supply results in a higher biomass production (Neuberg *et al.,* 2010).

Data in Table 4 illustrate increasing salinity levels up to 6.25 dS m^{-1} significantly decreased DNA and RNA contents in faba bean plant compared with the control plant. The reduction in DNA and RNA in stressed plants may be attributed to the role of ROS which was released at salt stress in inducing DNAase activities, enhancement of DNA fragmentation. It was postulated that, the contents of DNA and RNA in tomato decreased by seawater due to its effect on the inhibition of synthesis and intensification of breakdown (Tsenov *et al.,* 1973). Also, salinization increases RNAase activity in barley, tomato and pea (Tal, 1977). Exogenous application of amino acid with different concentrations on faba bean plant grown under different levels of seawater salinity could overcome the decrease in DNA and RNA (Table 4). Similar promoting effects of amino acids were observed by other investigators who suggested that DNA and RNA contents were significantly higher in treated plants (Abd El-Monem, 2007). Foliar treatment of amino acids promoted the synthesis of DNA and RNA and/or prevented their degradation by nuclease enzymes. It was reported that amino acids reacting directly or indirectly with reactive oxygen species, thus contributed to maintain the integrity of cell structure such as proteins, lipids and nucleic acids from damage which was induced by salt stress (Cvetkovska *et al.,* 2005).

Table 6. Effect of amino acid (AA) supply on seed yield (g $plant^{-1}$), total dry weight (g $plant^{-1}$), seed total carbohydrates (seed TC%), and seed total crude protein (seed TCP%) of faba bean plants grown at three different seawater salinity (S) levels in 2011/12 (S**I**) and in 2012/13 (S**II**)[#]

Treatments		Seed yield (g $plant^{-1}$)		TDW (g $plant^{-1}$)		Seed TC (%)		Seed TCP (%)	
SW	AA	SI	SII	SI	SII	SI	SII	SI	SII
TW	AA0	6.55c[†]	7.58d	13.1de	12.4e	56.8e-f	56.7de	23.6d-f	23.0d
	AA1	7.69b	7.77c	15.4b	15.4c	58.3c-e	58.2b	26.2a-c	24.0c
	AA2	8.73a	8.44b	17.5a	17.5b	60.2b	59.5a	26.9ab	25.4b
	AA3	9.16a	9.17a	18.3a	19.3a	62.2a	60.1a	27.5a	26.5a
SW1	AA0	5.39e	4.22i	9.5h	8.8h	55.9fg	55.8ef	23.1ef	21.2f
	AA1	5.66de	4.84h	11.3fg	10.0g	57.4c-f	54.5g	25.3b-d	22.8de
	AA2	6.27cd	5.61f	14.6bc	11.6f	59.0bc	58.0bc	25.3b-d	24.0c
	AA3	7.28b	6.38e	14.9bc	13.8d	60.2b	59.5a	26.2a-c	24.9bc
SW2	AA0	3.94f	2.77k	7.9i	6.1j	54.9g	53.8g	21.9f	18.6h
	AA1	5.51e	3.47j	10.7g	7.6i	57.3d-f	54.8fg	23.8de	19.8g
	AA2	6.52c	4.23i	12.5ef	8.9h	58.6b-d	56.9c-e	24.6c-e	21.1f
	AA3	7.20b	5.05g	14.1cd	10.3g	58.8b-d	57.2b-d	25.0b-d	21.9ef

[†]Mean values (n=12) in the same column for each trait in each year with the same lower-case letter are not significantly different by Duncan's multiple range test at $p \leq 0.05$.
[#]Measurements were made 75 d after sowing (DAS)

Data presented in Table 5 revealed the response of N, P, K^+, Mg^{2+} and Ca^{2+}, Na^+ and Cl^- contents to different spraying concentrations of amino acid under normal and saline conditions. The lower concentrations of N, P, K, Mg and Ca were recorded for faba bean plant grown under seawater when compared with control plants grown under normal conditions (Table 5) on both seasons. The reduction was more pronounced at the higher salinity level. These results were in agreement with those reported by other researchers (Azooz, 2009; Abdelhamid *et al.,* 2010) on faba bean. K concentration was lower in leaves of faba bean plant grown under saline soil conditions than those grown in normal conditions. The exclusion of Na^+ ions and a higher K^+:Na^+ ratio in faba bean plants grown under saline conditions have been confirmed as important selection criteria for salt tolerance (Abdelhamid *et al.,* 2010). Na^+ is the main toxic ion in saline water for most plants and the influx and accumulation of Na^+ competes with K^+, and there is a decrease in K^+ uptake and an increase in Na^+ influx in plant cells during salt stress (Serrano and Rodriguez-Navarro, 2001). The reduction in Ca^{2+} and Mg^{2+} uptake under salt stress conditions might be due to the suppressive effect of Na^+ and K^+ on these cations or due to the reduced transport of Ca^{2+} and Mg^{2+} ions. In addition, salinity has an antagonistic effect on the uptake of Ca^{2+} and Mg^{2+} which was caused by displacing Ca^{2+} in membrane of root cells (Asik *et al.,* 2009) on wheat. The selectivity of a high K^+:Na^+ ratio in plants is an important control mechanism and a selection criterion for salt tolerance (Wenxue *et al.,* 2003). Cuin *et al.* (2003) concluded that high K^+:Na^+ ratio is more important for many species than simply maintaining a low Na^+ concentration. The damage caused by long-term salinity is the excessive accumulation of Na^+ and the main site of Na^+ toxicity is the leaf blade, where Na^+ is accumulated after being deposited in the transpiration stream. High Na^+ content in the leaf blade may disturb cellular ion homeostasis (Takahashi *et al.,* 2007). Potassium is an activator of many enzymes which are essential for metabolic reactions (Salisbury and Ross, 1992). Plant cells need to maintain high K^+ levels under salt stress to maintain normal metabolic reactions (Sairam and Tyagi, 2004), and K^+ and Na^+ homeostasis in plants is important for salt tolerance (Horie *et al.,* 2001). Spraying faba bean plants with amino acids at all investigated salinity levels significantly increased N, P, K, Mg, Ca content and the K^+: Na^+ ratio in the leaf tissues than control ones as well as the corresponding salinity levels, with clear superiority to the higher level of amino acid. The results are in agreement with those of Abo Sedera *et al.,* (2010). Sodium cocentration was higher in plants grown under different salinity levels, however amino acid application significantly reduced Na^+ concentration in faba bean leaves. Increased K^+ concentration and reduced Na^+ in leaves may be one of the possible mechanisms of increased salinity tolerance by amino acid application in faba bean plants. Amino acid has a chelating effect on micronutrient when applied, that make the absorption and transportation of micronutrients inside the plant easier due to its effect on cell membrane permeability (Marschner, 1995).

Data presented in Table 6 revealed that increasing seawater salinity stress induced gradual reduction in seed yield and total dry matter production compared with untreated plants. These results agree with those obtained by Sadak *et al.,* (2012) on sunflower plant. The depressive effect of salinity on yield may be attributed to the inhibitory effect of salinity on the vegetative growth (Table 2). In this connection, the reduction of faba bean seed yield per plant due to salinization might be due to the harmful effect of salt stress on growth, the disturbance in mineral uptake and/or enhancement of plant respiration. Moreover, Taffouo *et al.,* (2009) reported that, the significant decrease in yield was observed under salt stress in cowpea would be partly related to a significant reduction of foliar chlorophyll contents and K^+ concentration in saline medium. Amino acid application as foliar spray significantly improved yield (seed and dry matter) either in plants irrigated with tap water or ones irrigated with different saline water (3.13 and 6.25 dS m^{-1}). The overall improvement in plant yield due to application of amino acids may be due to providing a readily source of growing substances which form the constitutes of protein in the living tissues. Also, the positive effects of amino acids application may be brought about by its cell-internal function as osmo-regulatory (Treichel, 1975) can increase the concentration of cellular osmotic components. Increasing seawater salinity levels resulted in a significant reduction in total carbohydrates % and total protein % contents, and these reductions reached the highest values at 6.25 dS m^{-1} seawater levels compared with control plants. These results are confirmed by the results obtained by Sadak *et al.,* (2012) on sunflower plant. Exogenous application of amino acids either under control water or different salinity levels caused increases in total carbohydrates % and protein % compared with the corresponding salinity levels. Abd El-Monem (2007) concluded that, there is a close relationship between the effect of amino acids and the stimulation of the photosynthetic output (soluble sugars, polysaccharides and total carbohydrates) of faba bean plant. Thus, increases the efficiency of solar energy conversion which maximizes the growth ability of faba bean and consequently increases its productivity.

CONCLUSION

Irrigation of faba bean plants with diluted seawater (3.13 or 6.25 dS m^{-1}) led to significant reductions in shoot length, number of leaves per plant, fresh and dry weight of shoots, photosynthetic pigments, total carbohydrates, polysaccharides, nucleic acid DNA and RNA contents of faba bean leaves. Seawater salinity induced higher contents of Na^+ and Cl^- and decreased concentrations of K^+, K^+:Na^+,

Ca^{2+}, Mg^{2+} and P^{3+}. Irrigation of faba bean plant with different levels of diluted seawater decreased seed yield and total dry weight per plant compared with those irrigated with tap water. Also, total carbohydrates and total protein contents in seeds were reduced by increased seawater salinity levels. Application of amino acid mixture as foliar spray with different concentrations (500, 1000 or 1500 mg L^{-1}) significantly improved all the reduced parameters due to seawater stress. The highest level of amino acid of 1500 mg L^{-1} exerted the strongest effect in alleviating the harmful effect of seawater salinity stress.

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