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INTERACTIVE EFFECTS OF SALINITY STRESS AND NICOTINAMIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF FABA BEAN PLANT

Efectos combinados del estrés por salinidad y la nicotinamida sobre parámetros bioquímicos y fisiológicos en plantas de haba

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ABSTRACT

A possible survival strategy for plants under saline conditions is to use some compounds that could alleviate the salt stress effect. One of these compounds is nicotinamide (vitamin B3/niacin). The effect of exogenous application of nicotinamide with different concentrations (0, 200 or 400 mg l^{-1}) on faba bean (*Vicia faba* L.) plant grown at different NaCl levels (0, 50 or 100 mM) was investigated in the wire house of the National Research Centre, Cairo, Egypt. Salinity stress significantly reduced the photosynthetic pigments, polysaccharides, total carbohydrates, total-N contents of shoot, plant height, leaves number, fresh and dry weights of shoot, seed yield, total carbohydrates and total crude protein of the yielded seeds compared with those of the control plants. In contrast, salinity induced marked increases in sucrose, total soluble sugars, total free amino acids, proline, lipid peroxidation product (MDA) and some oxidative enzymes (polyphenol-oxidase and peroxidase). Also, salinity stress increased Na^+ contents with the decreases of other macro and micro elements contents (P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) of shoots and the yielded seeds of faba bean. Foliar spraying of nicotinamide alleviated the adverse effects of salinity stress through increased the photosynthetic pigments, polysaccharides, total carbohydrates, total N concentration of shoot, plant height, leaves number, fresh and dry weights of shoot, and seed yield as well as, sucrose, total soluble sugars, total free amino acids and proline, compared with those of the corresponding salinity levels, while decreased lipid peroxidation product as malondialdehyde (MDA) and the oxidative enzymes (polyphenol oxidase and peroxidase enzymes). Nicotinamide inhibited the uptake of Na^+ and accelerated the accumulation of P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} concentrations in the shoots of salt stressed plants and enhanced total carbohydrate and total crude protein percentage and solutes concentrations in seeds of salinity treated plants. Nicotinamide, not only neutralized the effect of salinity stress but resulted in a significant improvement in physiological and biochemical parameters as well as the concentrations of soluble sugars, proline, amino acids, and total N and other mineral contents.

Keywords: growth, salt stress, sodium chloride, *Vicia faba*, vitamin B3.

RESUMEN

Una posible estrategia de supervivencia para plantas que se desarrollan bajo condiciones de salinidad es emplear algunos compuestos que les permitan disminuir el estrés salino. Uno de estos compuestos es la nicotinamida (vitamina B3/ niacina). Se investigó el efecto de la aplicación exógena de nicotinamida en diferentes concentraciones (0, 200 o 400 mg l^{-1}) sobre plantas de haba (*Vicia faba* L.) creciendo a diferentes niveles de NaCl (0, 50 o 100 mM) en los terrenos del *National Research Centre*, Cairo, Egipto. El estrés por salinidad reduce significativamente los contenidos de pigmentos fotosintéticos, polisacáridos, carbohidratos totales, nitrógeno total, peso de las plantas, número de hojas, pesos fresco y seco de tallos, rendimiento de semillas, y contenido de carbohidratos y proteina cruda total en semillas comparado con plantas control. En contraste, la salinidad induce incrementos marcados en sacarosa, azúcares solubles totales, aminoácidos libres totales, prolina, productos de peroxidación de lípidos como el malondialdehido (MDA) y algunas enzimas oxidativas (polifenol oxidasa y peroxidasa). También, el estrés por salinidad incrementa el contenido de Na^+ y genera disminución de otros macronutrientes y micronutrientes (P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} y Cu^{2+}) de los tallos y el rendimiento de semillas en plantas de haba. La aspersión foliar de nicotinamida disminuye los efectos adversos del estrés salino incrementando los pigmentos fotosintéticos, polisacáridos, carbohidratos totales, concentración total de nitrógeno en tallos, peso de las plantas, número de hojas, pesos frescos y secos de tallos, y rendimiento de semillas; así como los niveles de sacarosa, azúcares solubles totales, aminoácidos libres totales y prolina, comparado con aquellos correspondientes al estrés por salinidad, mientras disminuyeron los productos de peroxidación de lípidos (MDA) y las enzimas oxidativas (polifenol oxidasas y peroxidasas). La nicotinamida inhibe la toma de Na^+ y acelera la acumulación de P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} y Cu^{2+} en los tallos de plantas bajo estrés salino, y aumenta el porcentaje total de carbohidratos y proteína cruda y la concentración de solutos en semillas obtenidas de plantas tratadas contra el estrés salino. La nicotinamida no solo neutraliza el efecto del estrés salino, sino que mejora significativamente parámetros fisiológicos y bioquímicos tales como la concentración de azúcares solubles, prolina, aminoácidos y contenido total de nitrógeno y otros minerales.

Palabras clave: crecimiento, estrés salino, cloruro de sodio, *Vicia faba*, vitamina B3.

INTRODUCTION

Faba bean (*Vicia faba* L.) is one of the most important winter crops of high nutritive value in the world as well as in Egypt. Mature seeds of faba bean are good sources of protein (about 25 % in dried seeds), starch, cellulose, vitamin C and minerals (Hamilton, 2005). Consumed as fresh faba bean pods, seeds, conservative faba bean, and as a dried seeds. Moreover, they have been used as a drug for kidney stones, liver malfunctioning and eye diseases. At the same time they are used for animal food, broken seeds are mixed into animal diet and the vegetative parts of the plants are used as the animal fodder (Akcin, 1988).

Salinity has become more and more important to the scientific and political agenda. Over 6 % of the world's total land area and 20 % of irrigated land are salt-affected (FAO, 2008). Salinity problems are particularly relevant for arid and semiarid areas like Egypt. Approximately 33 % of the cultivated land and most extension agricultural land in Egypt is already salinized (Ghassemi *et al.*, 1995). The reduction in yield of different crops due to salinity in most of these areas is about 60 % when compared with normal soil. Exploiting and increasing production in these areas is necessary to bridge the gap between production and consumption of many crops like faba bean.

Salinity could be caused by (1) poor irrigation water which contains considerable amounts of salts, (2) accumulation of salts in the top layer of the soil due to over-irrigation, (3) proximity to the sea, and (4) the capillarity rise of salts from underground water into the root zone due to excessive evaporation. Also, low rainfall, high evaporation rate and poor water management could cause salinity related problems in these areas (along the Nile Valley, including Egypt). Salinity reduces the ability of plants to utilize water and causes reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002). Plants growing under saline conditions are stressed basically in three ways: (1) reduced water potential in the root zone causing water deficit, (2) phytotoxicity of ions such as Na^+ and Cl^- and (3) nutrient imbalance by depression in uptake and/or shoot transport. This is attributed to the fact that Na^+ competes with K^+ for binding sites essential for cellular function (Tester and Davenport, 2003). This role makes K^+ an important element as more than 50 enzymes are activated by K^+, and Na^+ cannot substitute in this role. On one hand, the latter implication of these two macronutrients in salinity is thought to be one of the factors responsible for reduction in the biomass and yield components. On the other hand, however, the reduction in growth is generally the consequences of several physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency and carbon allocation, and utilization (Gama *et al.*, 2007: Bekheta *et al.*, 2009; Abdelhamid *et al.*, 2010; Taie *et al.*, 2013). Also, the deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution, nutritional imbalance, specific ion effect, hormonal imbalance and induction of oxidative stress, or a combination of these factors (Rahnama *et al.*, 2010; Abdelhamid *et al.*, 2013).

Amelioration of the adverse effects of sodium chloride (NaCl) salinity by vitamin treatments has been reported (Bassouny *et al.*, 2008; Sadak *et al.*, 2010). Vitamins are required in trace

amount to maintain normal growth and proper development of all organisms. These compounds act as coenzyme systems and thus take essential part in the regulation of metabolism. Vitamins, can be limiting factors in the development of plant (Bassouny *et al.*, 2008). Nicotinamide is a water-soluble vitamin and is part of the vitamin B group. Nicotinamide, also known as niacinamide and nicotinic acid amide, is the amide of nicotinic acid (vitamin B3/niacin). Nicotinamide is a well-characterized constituent of the pyridine dinucleotide coenzymes NADH & NADPH, which are involved in many enzymatic oxidations - reductions reactions in living cells. In addition, nicotinamide is a stress-associated compound that induces and regulates secondary metabolic accumulation and/or the manifestation of defense metabolism in plants (Berglund, 1994).

This study aimed to measure the potential roles of nicotinamide in alleviating the deleterious effects of salinity on some physiological and biochemical traits of faba bean plants.

MATERIALS AND METHODS

Plant material and growth conditions

A pot experiment was conducted in a wire-house at the National Research Centre, Dokki, Cairo, Egypt (30°20' N; 31°53' E) from 21 November 2011 to 23 April 2012. During this period, daily temperature ranged from 12,3 - 28,6 °C, with an average of 17,4 ± 2,6 °C. Daily relative humidity averaged 58 ± 11,7 %, and ranged from 21 - 87 %.

Seeds of faba bean (*Vicia faba* L.; cv. Giza 843) were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Healthy faba bean seeds (n = 10) were selected for uniformity by choosing those of equal size and of the same colour. The selected seeds were washed in distilled water, sterilized in 1 % (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again in distilled water, and left to dry at room temperature (25 °C) for approx. 1 h. Ten, uniform, air-dried faba bean seeds were sown along a centre row in each plastic pot (30 cm diameter) at a depth of 30 mm, in approx. 7,0 kg of clay soil. To reduce compaction and improve drainage, the soil was mixed with yellow sand in a proportion of 3:1(v:v). Some characteristics of the soil used in the experiment before cultivation are pH 8,52, the cations were Ca^{2+} 7,31, Mg^{2+} 3,00, Na^{+} 8,26 and K^{+} 1,80 meq L^{1-} while anions were HCO_3^{-} 4,8, Cl^{-} 7,2, SO_4^{2-} 8,36 meq L^{1-}.

A granular commercial *Rhizobium leguminosarum* (obtained from the Biofertilizer Inoculum production Unit, Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Centre, Giza, Egypt) was incorporated into the top 30 mm of soil in each pot with the seeds at the time of sowing. Granular ammonium sulphate [20,5 (w/w) % N] was applied at a rate of 40 kg N ha^{-1}, and single superphosphate [15 % P_2O_5) was added at a rate of 60 kg P_2O_5 ha^{-1} to each pot. These N and P fertilizers were mixed into the soil in each pot immediately before sowing.

The experiment was arranged in a factorial arrangement with three levels of sodium chloride (NaCl) and three levels of nicotinamide. Four replicates were used. Ten days after sowing (DAS), faba bean seedlings were thinned to four seedlings per pot and irrigated with equal volumes of tap water until 21 DAS. To induce salt stress, NaCl was dissolved in fresh water and the plants were watered with an equal volume of 0, 50 or 100 mM (S0, S1, or S2), starting from the 22nd DAS along the rest period of the experiment, until final harvesting on 23 April 2012. Saline water was prepared by mixing fresh water (0.21 dS/m) with NaCl to achieve salinity levels of 50 and 100 mM. Starting from 22nd day, plants were sprayed three times, seven days interval between each spraying and the other, with three levels of nicotinamide (0, 200 or 400 mg l^{-1}) considered as N0, N1 and N2, respectively. The control treatment was sprayed with fresh water to exclude the effect of spraying and irrigated either with tap water (0.23 dS/m). The soil water capacity was estimated by saturating the soil in each pot with water and weighing it after it had drained for 48 h. The water capacity of the soil in each pot was 0.36. Soil water contents were maintained at approx. 90 % of pot water capacity. The level of soil moisture was controlled by weighing each pot and any loss of water was supplemented daily.

Plant growth samples were taken at 65 days after sowing (DAS) to estimate photosynthetic pigments contents, carbohydrates fractions, proline, total free amino acids, lipid peroxidation and oxidative enzymes (polyphenol oxidase, peroxidase enzyme) and some mineral contents of shoots (total N, P, K^{+}, Mg^{2+}, Na^{+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}). The above-ground portion of each plant was carefully removed from each pot and separated into leaves, stems, and pods. The shoot fresh weight (FW) was recorded. Various plant organs (leaves, stems, and pods) were oven-dried for 72 h at 70 °C, and their dry weights (DW) were recorded. The dried leaves were ground to a powder and kept in a desicator to determine their concentrations of phenolic compounds, total free amino acids, proline, total soluble carbohydrates, total carbohydrates, total nitrogen (N), phosphorus (P), potassium (K^{+}) , calcium (Ca^{2+}), sodium (Na^{+}), magnesium (Mg^{2+}), iron (Fe^{2+}) manganese (Mn^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}). Two fresh leaves per plant were washed with distilled water to remove any surface dust and used to determine the concentration of photosynthetic pigments and the activities of three anti-oxidant enzymes [polyphenol-oxidase (PPO) and peroxidase (POX), lipid-peroxidation (MDA)]. At harvesting in 23 April 2012, seed yield and the chemical constituents of the yielded seeds [(total carbohydrates %, total crude protein %, and some mineral concentrations (P, K^{+}, Mg^{2+}, Na^{+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+})] were determined.

Chemical Analysis

Chlorophyll *a*, chlorophyll *b* and carotenoids concentrations in fresh leaves were estimated using the method of Lichtenthaler and Buschmann (2001). Fresh tissues were ground in a mortar and pestles using 80 % acetone. The optical density

(OD) of the solution was recorded for chlorophyll *a* and *b* and carotenoids at 662 and 645 and 470 nm, respectively using a spectrophotometer (Shimadzu UV-1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/g FW. Sucrose and polysaccharides concentrations were determined according to Herbert *et al.* (1971). Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80 % (v/v) ethanol at 25 °C with periodic shaking, and centrifuged at 600 g. The supernatant was evaporated till completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme *et al.*, 1992). TSS were analyzed by reacting of 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72 % H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples at 625 nm using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany (Yemm and Willis, 1954). Determination of total carbohydrates was carried out according to Herbert *et al.*, (1971). A known weight of 0.5 g dried tissue was placed in a test tube, and then 10 ml of sulphuric acid (1N) was added. The tube was sealed and placed overnight in an oven at 100 °C. The solution was then filtered into a measuring flask (100 ml) and completed to the mark with distilled water. The total sugars were determined colorimetrically according to Smith *et al.* (1956) as follow: an aliquot of 1ml of sugar solution was transferred into test tube and treated with 1ml of 5 % aqueous phenol solution followed by 5 ml of concentrated sulphuric acid. The tubes were thoroughly shaken for ten minutes then placed in a water bath at 23 - 30 °C for 20 min. The optical density of the developed color was measured at 490 nm using Shimadzu spectrophotometer model UV 1201. Free amino acid and proline concentration were extracted according to Vartanain *et al.* (1992). Hot water extracts were prepared by boiling ground dry leaves in 10 mL of distilled water for 1 h. Free amino acid was determined with the ninhydrin reagent method (Yemm and Cocking, 1955). 1 ml acetate buffer (pH 5.4) and 1 ml chromogenic agent were added to 1 ml free amino acid extraction. The mixture was heated in boiling water bath for 15 min. after cooled in tap water, 3 ml ethanol (60 % v/v) was added. The absorbance at 570 nm was then monitored using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany). Proline was assayed according to Bates *et al.* (1973) briefly: 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany). A standard curve was obtained using a known concentration of authentic proline. The level of lipid peroxidation was measured by determining the malonadialdehyde (MDA) concentration. Malonadialdehyde is the product of lipid peroxidation and that assayed by thiobarbaturic acid reactive substance (TBARS) contents (Stewart and Bewley, 1980). Extracting the enzymes was done according to MuKherjee and Choudhuri (1983). Polyphenoloxidase (PPO, EC 1.10.3.1) activity assayed using the method of Kar and Mishra (1976). Peroxidase (POX, EC 1.11.1.7) activity assayed according to Bergmeyer (1974). The enzyme activities were calculated by methods described by Kong *et al.* (1999). The enzyme activities were assayed by a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany). Macro and microelement contents of faba bean shoots and the yielded seeds were determined according to Chapmen and Pratt (1978). Total N was determined by using micro-Kjeldahl method as described in AOAC (1970). P^+ was determined using a Spekol spectrocolorimeter (VEB Carl Zeiss; Jena, Germany, while, estimation of Ca^{2+}, K^+ and Na^+ contents were done using a flame photometer. Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+} contents were estimated using atomic absorption spectrophotometer.

Statistical Analysis of the Data

All data were subjected to analysis of variance (ANOVA) for a randomized complete block design, after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Statistically significant differences between means were compared at $p \leq 0.05$ using Duncan's multiple range test.

RESULTS

Photosynthetic Pigments Concentrations

Table 1 shows clearly that, increasing salinity level from 0,0 to 50 to 100 mM of NaCl resulted in significant reductions in chlorophyll *a*, chlorophyll *b*, carotenoids and total pigments of faba bean leaves. Maximum reduction was obtained at 100 mM NaCl salt. Foliar treatment of nicotinamide on faba bean plant with different concentrations (200 and 400 mg l^{-1}) significantly increased chlorophyll *a*, chlorophyll *b*, carotenoids and total pigments compared with the control plants and corresponding salinity levels (Table 1) except at 400 mg/l it caused significant decrease in carotenoid content.

Carbohydrates Concentrations

Changes in TSS, polysaccharides, total carbohydrates and sucrose concentrations in faba bean shoot plant due to foliar application of nicotinamide grown under NaCl salinity are presented in Table 2. Increasing salinity level from 0.0 to 50 to 100 mM NaCl caused significant increases in sucrose and TSS, while significant gradual reduction in polysaccharides and total carbohydrates concentrations in faba shoots. Meanwhile, foliar treatment caused significant increases in polysaccharides and total carbohydrates of shoots of faba bean plants compared with the untreated controls and the corresponding salinity levels.

Proline and Free Amino Acids

Tables 3 and 4 show that salt stress induced decreases in total N while increased accumulation of proline and free

Table 1. Effect of nicotinamide (***N***) on photosynthetic pigments (mg/g FW) in the leaves of faba bean grown under NaCl-salinity.

NaCl	Nicotinamide	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Total pigments
(S)	(*N*)		(mg g^{-1} FW)		
S0	N0	0.943c†	0.242c	0.269ab	1.454d
	N1	1.007a	0.320b	0.278a	1.605b
	N2	1.016a	0.358a	0.260b	1.634a
S1	N0	0.930c	0.229d	0.246c	1.405e
	N1	0.972b	0.246c	0.264b	1.481c
	N2	0.978b	0.248c	0.237cd	1.463d
S2	N0	0.787e	0.189f	0.235d	1.211h
	N1	0.798e	0.190f	0.240cd	1.227g
	N2	0.816d	0.200e	0.230d	1.245f

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

Table 2. Effect of nicotinamide (***N***) on total soluble sugar, polysaccharide, total carbohydrates, and sucrose in the shoots of faba bean grown under NaCl-salinity.

NaCl	Nicotinamide	Total soluble sugar	Polysaccharide	Total carbohydrates	Sucrose
(S)	(N)	(%)			
S0	N0	5.54c†	26.34b	31.88b	2.75d
	N1	6.19b	28.41a	34.60a	3.02cd
	N2	5.63a	29.85a	35.48a	3.50c
S1	N0	6.50b	21.31d	27.81c	4.48b
	N1	6.46b	24.50c	30.96b	5.13a
	N2	6.90a	24.72c	31.62b	4.64ab
S2	N0	6.63a	14.86f	21.49f	3.58c
	N1	6.25b	17.25e	23.50e	3.34cd
	N2	6.69a	18.38e	25.07d	3.45c

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

Table 3. Effect of nicotinamide (***N***) on proline, free amino acids, peroxidase, polyphenol-oxidase and lipid peroxidation enzymes in the shoots of faba bean grown under NaCl-salinity

NaCl	Nicotinamide	Proline	Free amino acid	Peroxidase	Polyphenol Oxidase	Lipid-Peroxidation (MDA)
(S)	(*N*)	(mg/g DW)		unit min-1 g^{-1} FW		(nmol/g FW)
S0	N0	0.43d†	6.21f	11.8d	9.9d	10.7e
	N1	0.46c	8.43d	9.2e	6.6f	8.8f
	N2	0.46c	9.03d	8.7e	6.5f	8.18f
S1	N0	0.46c	7.14e	17.7b	11.8c	16.5b
	N1	0.55b	8.85d	15.2c	10.2e	14.9e
	N2	0.55b	12.69c	12.8d	8.6d	10.9c
S2	N0	0.58b	11.64c	20.8a	20.8a	19.9a
	N1	0.61a	13.02b	17.2b	17.1c	15.5d
	N2	0.65a	15.33a	12.2d	12.2b	12.6bc

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

Table 4. Effect of nicotinamide (***N***) on total nitrogen (N), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), sodium (Na^+), magnesium (Mg^{2+}), iron (Fe^{2+}) manganese (Mn^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}). In the shoots of faba bean grown under NaCl-salinity.

NaCl	Nicotin-amide	N	P	K^+	Mg^{2+}	Na^+	Ca^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}
(S)	(*N*)	(%)						ppm			
S0	N0	2.81c†	0.33a	2.35c	0.34a	0.08c	1.12c	308b	163a	81b	10.3a
	N1	3.05b	0.34a	2.56b	0.42a	0.08c	1.49b	316b	174a	97a	10.3a
	N2	3.56a	0.35a	2.78a	0.43a	0.08c	1.89a	411a	168a	92a	10.7a
S1	N0	1.95e	0.33a	1.84e	0.22a	0.14b	0.72e	256d	142b	73b	9.0b
	N1	2.17d	0.34a	2.03d	0.31a	0.12b	0.88d	254d	155a	98a	9.7b
	N2	2.23d	0.34a	2.17c	0.34a	0.12b	1.09c	275c	156a	86b	9.7b
S2	N0	1.39g	0.29a	1.34h	0.18a	0.25a	0.32g	208e	82e	60d	8.7b
	N1	1.42g	0.27a	1.79g	0.25a	0.22a	0.62f	213e	102d	67c	9.0b
	N2	1.64f	0.34a	2.03f	0.28a	0.19a	0.90e	240d	121c	71c	9.7b

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

amino acids with increasing the salinity level. Foliar application of nicotinamide resulted in significant increases in the concentrations of total N, proline and free amino acids contents. In addition, these marked increases in nitrogen contents in nicotinamide treated plants were over those of untreated salinized plants and non-salinized control plants.

Lipid Peroxidation, Peroxidase and Polyphenol-Oxidase Activities

Table 3 shows effect of nicotinamide on, peroxidase, polyphenol-oxidase, and lipid peroxidation enzymes in the shoots of faba bean grown under NaCl-Salinity. MDA is one of the end products which are produced as a result of lipid peroxidation damage by free radicals. Lipid peroxidation level as indicated by accumulated MDA. MDA increased significantly under salt stress, and the effect was more pronounced at 100 mM NaCl salinity level. Foliar spraying of nicotinamide caused marked decreases in enzyme activities as compared under all salinity levels.

Mineral Concentrations

Table 4 shows influence of nicotinamide on N, P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} in the shoots of faba bean grown under NaCl-Salinity. NaCl-Salinity stress significantly increased Na^+ concentration, while decreased P, K^+, Mg^{2+} and Ca^{2+} ions concentrations compared to non-salinized plants. Foliar application of nicotinamide (200 and 400 mg/l) under various levels of salinity (0.50 and 100 mM NaCl) caused reduction of Na^+ accumulation and increases in the contents of P, K^+, Mg^{2+} and Ca^{2+} Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} compared to non-salinized plants and the corresponding salinity levels.

Faba Bean Plant Growth

Effect of nicotinamide on plant height (cm), leaf number per $plant^{-1}$, shoot FW, and shoot DW of faba bean grown under NaCl-Salinity (Table 5). Increasing salinity level resulted in significant gradual reductions in plant height and shoot DW (g) compared to control plants. Results also show that, foliar spraying of faba bean plant with nicotinamide increased significantly plant height and Shoot DW under normal and salinity stress conditions, these increases were gradually increased with increasing nicotinamide concentrations.

Seed Yield and Seed Chemical Composition

Table 6 shows faba bean seed yield and the chemical constituents of the yielded seeds [(total carbohydrates %, total crude protein %, and some mineral concentrations (P, K^+, Mg^{2+}, Na^+, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+})] in response to nicotinamide under NaCl-Salinity. Increasing salinity level induced significant decreases in seed yield per plant of *Vicia faba* plant compared to control plants. Also, different salinity levels significantly decreased percentage of total carbohydrates and total N % compared to control. External applications of different concentrations of nicotinamide (200 or 400 mg l^{-1}) induced significant increases in seed yield and on the quality of the yielded seeds (total carbohydrates % and total crude protein %). In general, the maximum increase was recorded in plants sprayed with 400 mg l^{-1} nicotinamide under all salinity levels.

DISCUSSION

An average reduction of 16,54 % in chlorophyll a, 21,90 % in chlorophyll b, 12,64 % in carotenoids and 16,71 % in total pigments contents were observed in faba bean plant compared to control plants (Table 1). These results are in good agreements with those obtained by Desingh and Kanagaraj (2007) in cotton, Atlassi Pak *et al.* (2009) in rape, Dolatabadian and Saleh Jouneghani (2009) in bean and El-Khallal *et al.* (2009) in maize plant, Bekheta *et al.* (2009) and Taie *et al.* (2013) in faba bean, and Bahari *et al.* (2013) in wheat plant. The inhi-

Table 5. Effect of nicotinamide (***N***) on plant height (cm), Leaf number per plant^{-1}, shoot FW, and shoot DW of faba bean grown under NaCl-salinity.

NaCl (S)	Nicotinamide (*N*)	Plant height (cm)	Leaf number plant^{-1}	Shoot FW (g plant^{-1})	Shoot DW (g plant^{-1})
S0	N0	52.5b†	11.7b	14.96b	2.25b
	N1	56.0a	13.0a	15.63a	2.35a
	N2	59.0a	12.3a	16.43a	2.47a
S1	N0	43.0d	10.5b	9.31d	1.85c
	N1	46.0c	11.3b	14.56b	2.09b
	N2	48.0c	11.3b	15.16a	2.19b
S2	N0	40.5e	9.3c	7.71e	1.16e
	N1	42.7d	11.0b	10.64c	1.50d
	N2	45.0d	11.3b	12.30c	1.65d

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

Table 6. Effect of nicotinamide (***N***) on seed yield and some chemical constituents of the yielded seeds [(total carbohydrates %, total crude protein%, and some mineral concentrations (P, K^+, Mg^{2+}, Na^+, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+})] of faba bean grown under NaCl-salinity.

NaCl (S)	Nicotinamide (*N*)	Seeds yield (plant /g)	TC	TCP	P$^+$	K$^+$	Mg^{2+}	Na^+	Ca^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}	Cu^{2+}
						(%)					(ppm)		
S0	N0	10.52b	49.1b†	20.1	0.26b	1.21b	0.07a	0.08b	0.02a	52.0a	8.3a	50.7a	8.3a
	N1	15.03a	61.7a	24.4	0.29a	1.29a	0.09a	0.07b	0.02a	56.7a	13.7a	72.3a	13.3a
	N2	15.84a	62.2a	20.9	0.31a	1.34a	0.08a	0.07b	0.03a	56.2 a	8.7a	72.7a	12.0a
S1	N0	8.02d	44.2c	19.6	0.22c	1.17b	0.05a	0.09b	0.02a	50.2a	11.3a	66.0a	13.0a
	N1	9.54c	53.1b	20.6	0.25b	1.20b	0.06a	0.09b	0.02a	54.0a	11.7a	71.3a	13.3a
	N2	10.92b	53.3b	21.0	0.27b	1.22b	0.08a	0.09b	0.01a	56.3a	15.3a	65.7a	13.7a
S2	N0	2.44f	32.7f	21.0	0.21c	1.07c	0.05a	0.13a	0.02a	55.7a	12.7a	70.3a	13.0a
	N1	2.83f	36.0e	21.3	0.23c	1.17b	0.08a	0.12a	0.02a	55.3a	14.0a	64.3a	10.7a
	N2	3.43e	40.5d	21.8	0.24c	1.19b	0.09a	0.14a	0.02a	57.2a	10.0a	69.7a	9.7a

† Mean values (n = 4) in the same column for each trait followed by the same lower-case letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$. Measurements were made 65 d after sowing (DAS). S0 (0.23 dS/m); S1 (50 mM NaCl); S2 (100 mM NaCl); N0 (0 nicotinamide); N1 (200 mg l^{-1} nicotinamide); N2 (400 mg l^{-1} nicotinamide).

bitory effect of salinity stress on the photosynthetic pigments may be due to the effect of salinity on the activities of photosynthetic enzymes and this may be a secondary effect mediated by the reduced CO_2 partial pressure in the leaves caused by stomatal closure (DeRidder and Salvucci, 2007). Also, Desingh and Kanagaraj (2007) presume that salinity stress might affect the biochemistry of photosynthesis by causing disorientation of the lamellar system of chloroplasts and loss of chloroplast integrity leading to a decrease in the activities of photosystems. The higher concentration of 400 mg l^{-1} of nicotinamide was more effective than 200 mg l^{-1}. These results are in agreement with those obtained by Hassanein *et al.* (2009) in *Zea mays* and Sadak *et al.* (2010) in sunflower plant. Nicotinamide may interfere with the protection of chloroplast and their membrane against salt toxicity and the maintaining their integrity (Hassanein *et al.*, 2009) or vitamins protect chloroplast from oxidative damage (Munne-Bosch *et al.*, 2001). In addition, nicotinamide has a role in activation of enzymes that regulate photosynthetic carbon reduction (Taylor *et al.*, 1982).

The obtained results shown in Table 2 of changes in TSS, polysaccharides, total carbohydrates and sucrose concentrations are in harmony with those obtained by Hassanein *et al.*, (2009), Sadak *et al.* (2010) and Sadak *et al.* (2012). Marschner (1995) reported that the organic acid especially sugars are the main solutes involved in osmotic adjustment in some plants submitted to osmotic and saline stress. Moreover, Bartels and Sunkar (2005) reported that the increase in TSS under salinity stress was considered protective and adaptive functions of soluble carbohydrates under salinity stress. The reduction in total carbohydrates of salt stressed faba bean plant concomitantly with the reduction in the leaf photosynthetic pigments led to the conclusion that salinity may inhibit photosynthetic activity and/or increased partial

utilization of carbohydrates into other metabolic pathways (Hassanein *et al.*, 2009). De Ridder and Salvucci (2007) reported that the reduction of total carbohydrates under salinity stress is probably due to higher sensitivity of photosystem II, decrease of CO_2 in intercellular spaces of stomata, reduction in photochemical quantum efficiency of CO_2 uptake, low level of O_2 evolution and low level of 3-phosphoglycerate. These circumstances may lead to the reduction in carbon allocation to new leaves and furthermore to potential photosynthetic capacity resulted in reduction of photon yield of CO_2 assimilation and consequently minimize starch synthesis under salinity stress. Nicotinamide application resulted in significant increases in sucrose and TSS compared with control plants. In the meantime, it caused variable effects in salinized plants as compared with the corresponding salinity levels. These results are in agreement with those obtained by Hassanein *et al.* (2009) in maize and Sadak *et al.* (2010) in sunflower plant. The increases in total soluble sugars and sucrose in faba bean plants may indicate more stimulation in the enzymes of sugar hydrolysis. In addition, Srivastava *et al.* (1995) stated that accumulations of carbohydrate play a key role in alleviating salinity stress either via osmotic adjustment or by conferring desiccation resistance to plant cells.

Proline accumulation is one of the most frequently reported modifications induced by water and salt stress in plants and is often considered to be involved in stress resistance mechanisms. It is possible roles have been attributed to stabilizing the structure of macromolecules and organelles through stabilizing proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes (Munns, 2002). Salt stress induced decreases in total N while increased accumulation of proline and free amino acids with increasing the salinity level (Tables 3 and 4). These findings are in good agreement with those obtained by Khattab (2007), Amirjani (2010), Sadak *et al.* (2010), and Taie *et al.* (2013). Reduction in total N can be attributed to the decrease in protein synthesis and/or to the increase in its degradation. Compatible osmolytes (class of small molecules such as proline, glycinebetaine, polyamines, and TSS) are potent osmoprotectants that play a role in counteracting the effects of osmotic stress. These results are in agreement with the result observed by Bassouny *et al.* (2008). Thus, it can be concluded that nicotinamide vitamins treatments not only alleviated the inhibitory effect of salinity stress, via osmotic adjustment or by conferring some desiccation resistance to plant cell, but also stimulated the accumulation of nitrogen constituents over those in the non-salinized plants. Moreover, vitamins might act as activators of protein synthesis via significant alteration in the enzymes related to protein metabolism (Kodandaramaiah, 1983).

MDA is one of the end products which are produced as a result of lipid peroxidation damage by free radicals. Salt stress significantly increased MDA, and the 100 mM NaCl was more pronounced. These results are in good agreement with those of Khattab (2007) on canola and Bekheta *et al.* (2009), and Sadak *et al.* (2010) on faba bean. This suggested that, salt stress induced membrane injury, which may be due to changes in the membrane lipids or protein or both (Scandalios, 1993). The increase in lipid peroxidation might be due to incapability of endogenous antioxidants to scavenge all ROS resulted from salt stress. Application of nicotinamide could attenuate the effect of salinity, by decreasing MDA level, compared to the corresponding salinity level. The most effective treatment was 400 mg l^{-1} nicotinamide. Therefore, treatment with vitamin PP alleviated the adverse effect of salinity on growth and metabolic activities through decreasing the build-up of active oxygen species and thereby increasing resistance to salt stress (Hassanein *et al.*, 2009). Salt treatments induced increases in the activity of peroxidase (EC.1.11.1.7) and Polyphenol-oxidase (EC.1.10.3.1) in faba bean. This was confirmed by the results of Khattab (2007), Bekheta *et al.* (2009), Sadak *et al.* (2010), Bahari *et al.* (2013) and Zhang *et al.* (2013). These increases could be considered as an indicative of the increased production of ROS and a build-up of a protective mechanism to reduce oxidative damage triggered by stress experienced by plants (Dolatabadian and Jouneghani, 2009). The most effective treatment was 400 mg l^{-1} and these increases were concurrently with increasing protein levels indicating that vitamins could alleviate the inhibitory effects of salt stress by enhancing protein synthesis where vitamins might act as activators for protein synthesis (Kodandaramaiah, 1983).

NaCl-Salinity stress significantly increased Na^+ concentration, while decreased P, K^+, Mg^{2+} and Ca^{2+}. The obtained results are in agreement with those obtained by Bassouny *et al.*, (2008), Asik *et al.*, (2009), Atlassi Pak *et al.*, (2009), Abdelhamid *et al.* (2010), Amirjani (2010), Rady *et al.*, (2011) and Sadak and Abd Elhamid (2013). It was suggested that increased accumulation of sodium ions in the tissues inhibits biochemical processes related to photosynthesis through direct toxicity. Enhancing of Na^+ uptake by salinity was accompanied by a corresponding decline in K^+ concentration, showing an apparent antagonism between K^+ and Na^+ (Erdei *et al.*, 1996). Thus, higher concentration of Na+ should affected intercellular K^+ accumulation. Presumably by competing for sites through which influx of both cations occurs (Jeschke, 1984) or affecting membrane integrity and causing leakage of K^+ (Haro *et al.*, 1993). 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 reduced transport of Ca^{2+} and Mg^{2+} ions (Varsheny *et al.*, 1998). Increasing salinity level caused gradual decreases in Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} concentrations in faba bean shoots. Under salinity stress, solubility of micronutrient (Cu^{2+}, Mn^{2+}, Fe^{2+} and Zn^{2+}) is particularly low and plants grown in these soils often show deficiencies of this element (Page *et al.*, 1990). Those results were confirmed by El-Bassiouny (2005), Sadak *et al.* (2010), Abdelhamid *et al.* (2010), and Taie *et al.* (2013).

Nicotinamide led to increase in the concentrations of ions in shoots of the stressed faba bean plants through their role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients from soil solution (Buschmann and Lichtenthaler, 1979). Moreover, nicotinamide caused slight increases in the above microelement compared to the corresponding salinity level.

Significant gradual reductions in plant height and shoot DW (g) with increasing salinity level were found (Table 5). These decreases has been reported by Asik *et al*. (2009), Abdelhamid *et al*. (2010), El-Khallal *et al*. (2009) and Sadak *et al*. (2010), and Taie *et al*. (2013) in different plant species. The decrease in plant height and shoot DW by increasing salinity level could be ascribed to the decrease in photosynthetic output as indicated by the significant decrease in chlorophylls and total carbohydrates in saline stressed plants (Tables 1 and 2) (Chaparzadeh *et al*., 2004). Similar results were obtained by Hassanein *et al*. (2009) in *Zea mays* plant and Sadak *et al*. (2010) in sunflower. This alleviation (partially or completely) of the inhibitory effects of high levels of salinity is probably by increasing the efficiency of water uptake and utilization as well as protecting of the photosynthetic apparatus. Nicotinamide may act as growth stimulants, which can play a role in mitigating the adverse effect of salt on metabolic activities relevant to growth.

Faba bean seed yield per plant was significantly reduced with increasing salinity level. These results agree with those obtained by Ragab *et al*. (2008), Sadak *et al*. (2010) and Sadak and Abd Elhamid (2013) on different crops. Significant decreases of seed yield observed under salt stress in faba bean plant would be partly related to the significant reduction of the leaf chlorophyll contents and K^+ concentration in saline medium or might be due to the harmful effect of salt stress on growth, the disturbance in mineral uptake and/or enhancement of plant respiration (Taffouo *et al*., 2009). These reductions may be attributed to the reduction in concentration in saline medium, which plays important roles in proteins synthesis, translocation of assimilates as well as increasing growth and yield of plant Zadeh and Naeini (2007). Moreover, these reductions may be attributed also to the weakening of salinity to protein - pigment - lipid complex or enzyme activities, which resulted in, decrease the carbohydrates and total N percent of the produced seeds. These results are in harmony with those obtained by Sadak *et al*. (2010) on sunflower plant. Nicotinamide vitamin appear either to form sink mobilizing the different nutrients, which are involved in building new tissues in faba bean plants and/or enhance photosynthesis. Also, it can be concluded that the increments of seed yield per plant in response to nicotinamide treatments is mainly due to the effect of nicotinamide on enhancing protein synthesis and delaying senescence (Sahu *et al*., 1993). Inorganic constituents of the yielded seeds macronutrients (N, P, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} and some micronutrients (Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+}) of faba bean seeds were reduced steadily with increasing salinity levels meanwhile, Na^+ increased with the increased of salinity levels.

CONCLUSION

It was concluded that salinity stress adversely affected growth, biochemical parameters and yield components compared to control faba bean plants. Nicotinamide (Vitamin B3) not only neutralized the effect of salinity stress, but resulted in a significant improvement of physiological and biochemical parameters as well as the concentrations of soluble sugars, proline, amino acids, and total N and other mineral contents.

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