EFFECT OF SOME VITAMINS ON THE IONIC BALANCE AND PROTEIN PROFILES OF SALT- STRESSED FLAX SEEDLINGS

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ABSTRACT

The adverse effects of salinity on some physiological and biochemical responses of flax (*Linum usitatissimum*) seedlings grown at 100, 200, and 300 mM NaCl were studied. Salinity induced marked decreases percentage of germination, reduction in nucleic acids, as well as membrane stability index (MSI) of salinized flax seedlings. Data also revealed that salinized flax seedlings accumulated high levels of Na, Cl, P and Mg while K and Ca ions were significantly decreased. Application of some vitamins (folic acid, ascorbic acid and cobalamin) counteracted the adverse effects of salinity. The mechanism of these vitamins on alleviating salt stress hazards might be mediated by stimulating the *de novo* synthesis of new set of low molecular weight proteins, reduction in some ions (Na and Cl) coupled with increases in the accumulation of others (K, Ca, P and Mg), consequently vitamins maintain high percentage of MSI throughout the experiment, thereby enhanced the capacity for germination under salt stress conditions.

Key words: flax; salinity; vitamins; protein profile; membrane stability index.

INTRODUCTION

Salt stress, like other abiotic stresses, can lead to oxidative stress through the increase in ROS which can damage essential membrane lipid as well as protein and nucleic acids **(Noctor and Foyer, 1998)**. Maintenance of cellular ion homeostasis is fundamental to physiological activities in plants. The germination of the seed is an appropriate stage in the life of plants at which seeds are particularly sensitive to saline environment; in fact it has been found that the sensitivity to salt may be greater during germination than during seedling growth **(Howell, 1998)**. A decline in seed germination percentage with increasing salinity was also reported in *Atriplex spp* **(Ungar, 1996)** and in *Beta vulgaris* **(Ghoulam and Fares, 2001**). Moreover, salinity stress slows down the rate of germination or inhibits it completely as recorded by **(Radi *et al*., 1988; Nuran and Hüsnü, 2002)**.

The deleterious effect of salinity was suggested as a result of water stress, ion toxicity, ion imbalance, or combination of these factors. Mineral contents of many plants were found to be altered under salinity stress conditions particularly during seed germination **(Izzo *et al*., 1991; Lutts *et al*., 1996)**. Indeed, salinity causes nutrient imbalances, consequently maintaining a suitable K/Na ratio **(Lacerda *et al*., 2003)** results in protection against the formation of ROS.

Relative accumulation of sodium in plant cell may induce some adaptation to high osmotic potential but excess sodium may be toxic as indicated by **Lechno *et al*. (1996); Cicek and Cakirlar, (2002); Faheed *et al*. (2004) and Yildirim *et al*.(2006).**

ROS may also damage macromolecules such as DNA and proteins **(Pastori and Foyer, 2002)**. Regulation of cellular ion homeostasis under salt stress is controlled by

various kinds of membrane protein, such as channels or pumps **(Serrano and Rodrigguez-Navarro, 2001)**. Several investigations have shown the synthesis of new proteins in many species when subjected to salinity stress **(Hurkman and Tanaka, 1987; Singh *et al*., 1987)**. The synthesis of several proteins located in either membrane fraction **(Hurkman *et al.*, 1989)**, cytosol **(Zhao and Herrmann, 1992; Reviron *et al.*, 1992)**, chloroplasts **(Winicov and Button, 1991)** or intercellular spaces **(Esaka *et al.*, 1992)** was found to be either up- or down- regulated by osmotic stress. These specifically synthesized proteins under salt stress appear to have a role in providing tolerance or adaptation to the plants. However, the overall mechanism of how these proteins could provide adaptation is not yet clear. Ericson and **Alfinito (1984)** reported the accumulation of 26 and 32-kDa proteins in salinized tobacco cells. The 26-kDa protein (osmotin) is specifically synthesized and accumulated in cells undergoing osmotic adjustment to salt or desiccation stress, It is believed that osmotin provids osmotic adjustment to the cells either by inducing the accumulation of solutes or by providing certain metabolic alterations in the cell, which may be helpful in osmotic adjustment **(Singh *et al*., 1987)**.

Hong-Bo *et al*. (2005) reported that, some salt induced proteins called late embryogenesis abundant (LEA) proteins (M.wt: 10-30 kDa) which may act as osmoprotectants and / or antioxidants against severe salt stress.

Amelioration of the adverse effects of NaCl salinity by addition of some vitamins have been reported by **Li and Wang (1991)** on *Zea mays*; **Shalata and Neumann (2001)** on *Lycopersicum esculentum*; **Khattab (2001)** on *Oryza sativa*; **Ali (2002)** on *Ricinus communis*; **El-Bassiouny *et al*. (2005)** on *Vicia faba*; **Hamad and Hamada (2005)** on *Triticum aestivum*; **El-Tohamy and El-Gready (2007)** on snap bean plants and **Azooz (2009)** on *Hibiscus sabdariffa*.

The role of ascorbic acid in alleviating hazards caused by salinity has been reported by many investigators **(Mozafar and Oertli, 1992; Zhang and Kirkham, 1996; Khattab, 2001; Hamada and Hamada, 2005; Bassuony *et al*., 2008)**. However, the effect of exogenous application of folic acid and cobalamin as active oxygen scavengers have not been investigated to present date.

The objective of this study is to asses the possible roles of vitamins in the defense against ROS under salt stress conditions. Moreover, vitamins play an essential role in ion homeostasis and osmoregulation under salt stress; this may provide further information on repairing the injurious effects of salinity by vitamin applications.

MATERIALS AND METHODS

Pure strain of flax seeds (*Linum usitatissimum* L) cultivar, Sakha 2 was obtained from the Agriculture Research Center, Fiber Crops Research Section, Giza, Egypt. Preliminary experiments were done to test the salt sensitivity of flax seeds as well as to choose the proper concentrations of folic acid, ascorbic acid and cobalamin. Three different concentrations of sodium chloride were chosen (100, 200, and 300 mM NaCl in ¼ strength Hogland's solution). The proper concentrations of folic acid (vitamin B9), ascorbic acid (vitamin C) and cobalamin (vitamin B12) were 20 μM, 0.5 mM, and 2 μM respectively. The seeds were surface sterilized by dipping in 1% sodium hypochlorite solution for 5 minutes, then rinsed thoroughly with distilled water and germinated in Petri dishes on filter paper (Whatman No.1) saturated with 10 ml water, vitamins and / or NaCl solutions. The solutions were replaced every 2-3 days.

Seedlings were exposed to normal day length and natural temperature (about 22/13 ±2˚C and 11 h photoperiod). The number of germinated seeds was recorded daily through the experimental period. Seedlings were collected after 12 days at the end of the experiment for measuring some growth parameters in terms of fresh and dry weights of flax seedlings, mineral ion contents (Na, K, Ca, Cl, Mg and P), DNA and RNA, protein profile, as well as membrane stability index (MSI).

Protein electrophoresis

The total soluble proteins were separated on SDS-polyacrylamide gel and visualized by Coomassie blue stain to estimate the changes in protein profiles induced by NaCl stress in the absence and presence of vitamins. Electrophoretic determination of total protein was estimated according to their molecular weights by denatured sodium dodcyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method described by **Laemmli (1970)** and modified by **Studier (1973)**.

Determination of membrane stability index (MSI).

MSI was measured as described by **Sairam *et al*. (1997)**.

Quantitative estimation of nucleic acids

The method suggested by **Ogur and Rosen (1950)** for the extraction of nucleic acids was adopted in the present investigation.

Determination of certain elements

The method of extraction used in this investigation was essentially that of **Chapman and Pratt (1961)**, sodium, potassium, and calcium were estimated according to the method described by **Ranganna (1977)** using atomic absorption spectrophotometer (Pekrin Elmer USA 3100). Phosphorous was estimated according to the method described by **Humphries (1956)**. Magnesium can be calculated by multiplying the values of phosphorus by the factor: 0.0784 according to the method described by **Word and Johnston (1962)**. Chloride ion concentration was measured by silver nitrate titration method as described by **Jackson and Thomas (1960)**.

Statistical Analysis

Analysis of variance was conducted using ANOVA one way variance test using Microsoft Excel 2000. Statistical probability values were calculated to quantify the levels of significance for each treatment. The values of analysis of seedlings grown under 100, 200 and 300 mM NaCl were used as a reference controls for vitamin treated stressed ones, as well as they compared also, with the untreated control. Each treatment is an average of three different measurements.

RESULTS

Changes in percentage of germination

It is clearly shown that NaCl brought about a marked depressive effect on seed germination. Salinity stress delayed seed germination and reduced germination percentage particularly at high salinity levels (200 mM NaCl) as compared with non salinized control. Lower concentration of NaCl (100 mM) reduced the percentage of germination to 47%. This reduction was positively related to NaCl concentrations (fig.1). The germination was

completely inhibited in response to the higher concentration of NaCl (300 mM); non of these seeds could germinate during the incubation period. Flax seeds that fail to germinate at 300 mM NaCl, responded markedly to folic acid, ascorbic acid or cobalamin treatment which caused 67%, 34% and 33% respectively increase in germination percentage as compared with their corresponding control grown under sever salt stress (300 mM NaCl). Salinized seeds treated with any of the vitamins used in the present work germinated by the end of incubation period (Plates 1&2).

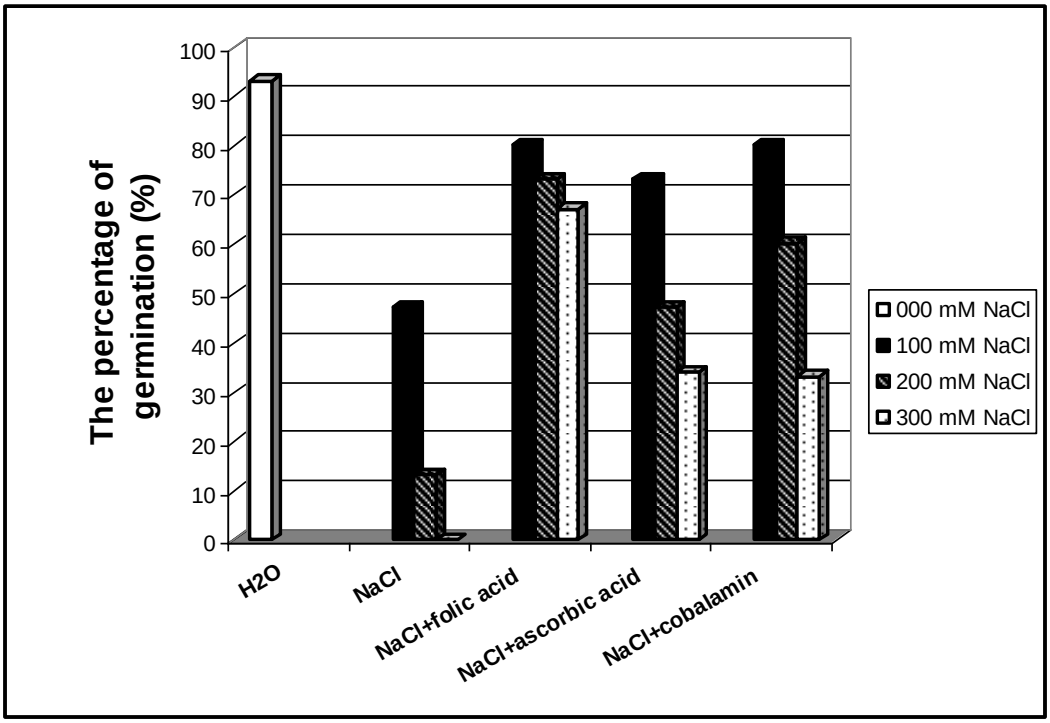

***Figure* (1):** Effects of folic acid, ascorbic acid or cobalamin on the percentage of germination of *Linum usitatissimum* seeds grown under salt stress conditions.

H_2O ***300 mM NaCl***

***Plate* (1):** Germination and growth responses pattern of *Linum usitatissimum* seeds grown under sever concentration of NaCl (300 mM).

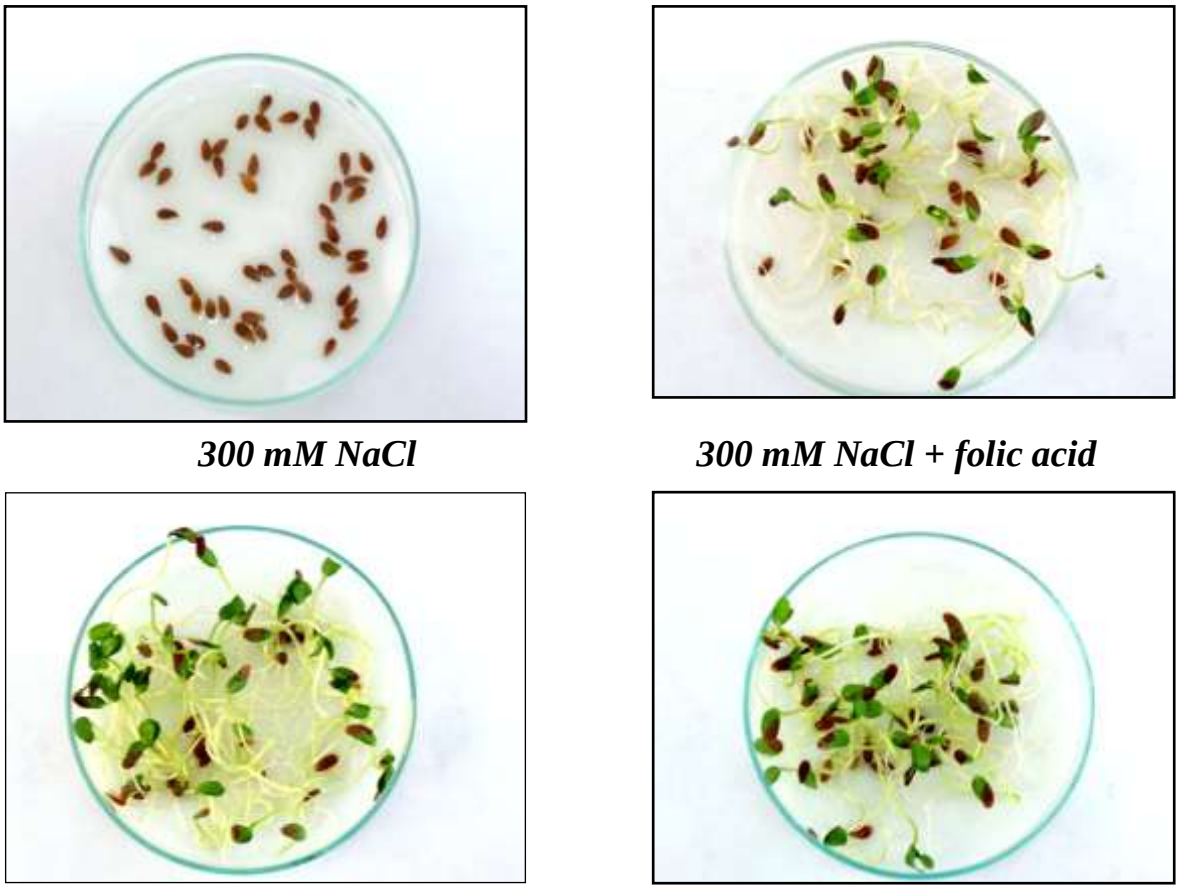

***Plate* (2):** Germination and growth responses pattern of *Linum usitatissimum* seeds grown under sever salt stress conditions (300 mM NaCl) in the absence and presence of folic acid, ascorbic acid or cobalamin.

Changes in biomass and membrane stability index (MSI)

Data in table (1) revealed that salt stress induced significant reduction in both fresh and dry masses of salinized flax seedlings particularly at the high salinity levels. At 200 mM NaCl the reduction in fresh and dry matter yields of salinized flax seedlings was 68.7% and 45.3% respectively below the control value.

***Table* 1** Effects of folic acid, ascorbic acid or cobalamin on some growth parameters of *Linum usitatissimum* seedlings grown under salt stress conditions. Each value is a mean of ten replicates ±SE

Treatments	***NaCl (mM)***	***Fresh weight (g/seedling)***	***Dry weight (g/seedling)***	***(MSI)*** ***Membrane stability index (%)***
NaCl	000	1.387 ± 0.6	0.075 ± 0.02	79.5 ± 2.4
	100	0.649 ± 0.2^{c}	0.064 ± 0.01	25.0 ± 1.3^{c}
	200	0.434 ± 0.1^{c}	0.041 ± 0.01^{a}	21.4 ± 1.1^{c}
	300	0.299 ± 0.1^{c}	0.010 ± 0.01^{c}	05.1 ± 0.4^{c}
NaCl + folic acid	100	0.780 ± 0.3	0.080 ± 0.03	66.6 ± 3.5^{b}
	200	0.847 ± 0.2^{a}	0.130 ± 0.04^{b}	50.0 ± 2.1^{c}
	300	0.380 ± 0.1	0.068 ± 0.01^{a}	18.0 ± 0.9^{c}
NaCl + ascorbic acid	100	0.815 ± 0.3	0.115 ± 0.03^{a}	52.6 ± 2.7^{b}
	200	0.896 ± 0.4^{b}	0.103 ± 0.02^{b}	33.3 ± 1.8^{b}
	300	0.332 ± 0.1	0.061 ± 0.01^{a}	15.0 ± 0.7^{c}
NaCl + cobalamin	100	0.856 ± 0.2	0.136 ± 0.04^{b}	50.0 ± 2.2^{c}
	200	0.786 ± 0.3^{a}	0.121 ± 0.03^{b}	40.2 ± 1.9^{b}
	300	0.341 ± 0.1	0.062 ± 0.01^{a}	11.1 ± 0.7^{c}

Values with a superscript are significant different from the control. Letter a =* at P>0.05, b =** at P<0.01, c =*** at P <0.001, and absence of letter = non significant.

Vitamin treatments greatly reduce the inhibitory effects of salinity on growth of flax seedlings. This stimulatory effect was more pronounced in cobalamin-treated flax seeds, such effect reaches about 31.8% and112.5% increase in fresh and dry weights at 100 mM NCl compared to the reference control.

Membrane stability index (MSI) was estimated as electrolyte leakage. The results revealed that, membrane stability index significantly decreased in salinized flax seedlings. Lower dose of NaCl (100 mM) reduced the percentage of MSI by 68.5% below the control value. This reduction was positively related to the concentrations NaCl. Vitamin treatments significantly increased the MSI percentage particularly in folic acid treated salinized seedlings.

Changes in Protein banding Pattern

Scanning of the gel revealed the appearance of a number of polypeptide bands ranging from 4-14 polypeptide bands (Plate 3 & Table 2).

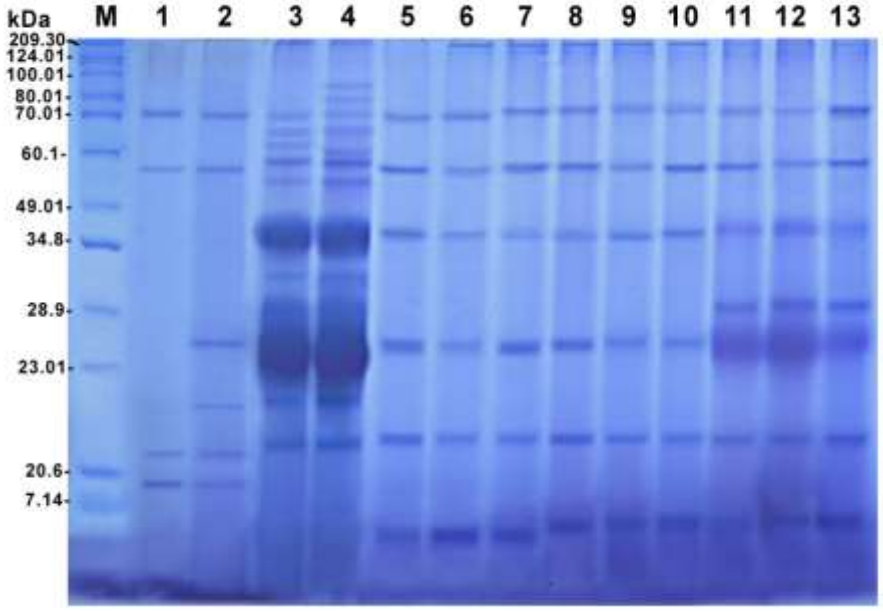

***Plate* (3):** Electrograph SDS-PAGE of total soluble protein extracted from *Linum usitatissimum* seedlings grown under different concentrations of NaCl in the absence and presence of folic acid, ascorbic acid or cobalamin.

M : Marker
Lane 1 : 000 mM NaCl
Lane 2 : 100 mM NaCl
Lane 3 : 200 mM NaCl
Lane 4 : 300 mM NaCl
Lane 5 : 100 mM NaCl + ascorbic acid
Lane 6 : 100 mM NaCl + cobalamin
Lane 7 : 100 mM NaCl + folic acid
Lane 8 : 200 mM NaCl + ascorbic acid
Lane 9 : 200 mM NaCl + cobalamin
Lane 10 : 200 mM NaCl + folic acid
Lane 11 : 300 mM NaCl + ascorbic acid
Lane 12 : 300 mM NaCl + cobalamin
Lane 13 : 300 mM NaCl + folic acid

***Table* (2):** Relative concentration (band %), molecular weight (M.wt) and mobility rate (Rm) of the SDS-PAGE of total soluble protein extracted from *Linum usitatissimum* seedlings treated with folic acid, ascorbic acid or cobalamin and exposed to different concentrations of NaCl.

Band No	Treatment and Band %													Rm	M.wt. kDa
	1	2	3	4	5	6	7	8	9	10	11	12	13		
1	-	-	4.40	3.01	-	20.07	11.70	10.08	20.03	20.03	3.02	4.80	6.72	0.02	209.3
2	-	-	-	3.38	-	-	-	-	-	-	-	-	-	0.07	97.7
3	-	-	-	3.07	-	-	-	-	-	-	-	-	-	0.09	80.4
4	30.24	20.35	3.33	3.16	20.31	18.62	10.39	13.72	13.11	15.07	8.02	3.84	5.82	0.12	75.9
5	-	-	3.18	3.07	-	-	-	-	-	-	-	-	-	0.15	68.3
6	-	-	2.91	2.82	-	-	-	-	-	-	-	-	-	0.18	65.4
7	19.34	39.05	22.92	8.52	10.19	19.68	12.93	9.72	14.68	20.49	7.07	4.50	5.41	0.23	58.2
8	-	-	5.47	3.09	-	-	-	-	-	-	-	-	-	0.24	55.4
9	-	-	2.90	3.10	10.04	8.12	8.34	10.06	16.14	10.09	13.00	8.18	9.36	0.27	45.0
10	-	-	34.54	31.59	-	-	-	-	-	-	-	-	-	0.32	32.0
11	-	-	3.54	3.38	-	-	-	-	-	-	10.15	9.39	7.29	0.39	28.5
12	-	13.03	3.40	12.59	2.24	6.95	16.50	8.07	7.54	8.04	36.39	34.72	38.97	0.40	26.3
13	-	9.30	6.00	9.00	-	-	-	-	-	-	-	-	-	0.49	23.7
14	19.69	10.96	7.41	10.04	39.17	12.70	26.14	35.55	20.21	14.14	13.27	26.52	14.82	0.54	20.9
15	30.73	7.00	-	-	-	-	-	-	-	-	-	-	-	0.69	15.0
16	-	-	-	-	18.05	13.86	14.00	12.80	8.23	12.12	9.08	8.00	11.61	0.74	6.2
No. of Bands/lane	4	6	12	14	6	7	7	7	7	7	8	8	8		

Three common protein bands with M.wt: 75.9, 58.2 and 20.9 kDa were detected in the control and in response to the treatments with the three applied concentrations of NaCl in the absence and presence of vitamins.

It is clearly shown from table (2) that, the low salinity level resulted in the appearance of two new protein bands (M.wt: 26.3 and 23.7 kDa), the moderate salt concentration (200 mM NaCl) resulted in the appearance of 9 polypeptides bands (M.wt: 209.3, 68.3, 65.4, 55.4, 45.0, 32.0, 28.5, 26.3 and 23.7 kDa), six of which disappeared in response to vitamin treatments (M.wt: 68.3, 65.4, 55.4, 32.0, 28.5 and 23.7 kDa). At high salinity level (300 mM NaCl), 11 polypeptide bands were detected (M.wt: 209.3, 97.7, 80.4, 68.3, 65.4, 55.4, 45.0, 32.0, 28.5, 26.3 and 23.7 kDa), seven of which disappeared in response to vitamin treatments (M. wt: 97.7, 80.4, 68.3, 65.4, 55.4, 32.0 and 23.7 kDa).

Moreover the electrophoretic pattern confirms the specific accumulation of low molecular weight protein (M.wt: 6.2 kDa) in salinized flax seedlings treated with folic acid, ascorbic acid or cobalamin.

Changes in mineral composition

The results revealed that salinity is capable of inducing a general increase in Na, Cl, P and Mg ions while K and Ca ions were significantly decreased as salinity levels increased. Seed treatment with any of the applied vitamins was generally of depressive effect on the accumulation of Na as well as Cl ions. However, they have stimulatory effects on the accumulation of K, Ca, Mg and P ions as well as K/Na, Ca/Na, Mg/Na and P/Na ratios.

The of reduction in Na ions level due to folic acid treatment in salinized flax seedlings was from 44.0% to 65.1%, while in case of ascorbic acid treatment it was from 41.3% to 50.4% below their corresponding controls. With respect to cobalamin treatment, the decrease in Na ions level below the control values was 61.7%, 56.7% and 51.6% at different concentrations of NaCl (Table 3).

***Table* 3** Effects of folic acid, ascorbic acid or cobalamin on mineral composition of *Linum usitatissimum* seedlings grown under salt stress conditions. Values are listed are expressed as mg / g dry weight. Each value is a mean of three replicates ±SD

Treatments	***NaCl (mM)***	***Na***	***K***	***Ca***	***Mg***	***P***	***Cl***
NaCl	000	16.1±0.9	72.7±6.9	62.3±5.7	0.68±0.1	8.7±1.0	6.3±0.4
	100	58.2±2.8^{c}	70.2±6.5	51.8±3.7	1.27±0.2^{a}	16.2±1.1^{a}	37.9±2.9^{c}
	200	81.3±7.5^{c}	60.2±4.3^{a}	49.2±3.1^{b}	1.38±0.4^{b}	17.6±1.3^{b}	48.5±4.6^{c}
	300	106.2±9.8^{c}	38.7±2.1^{c}	46.1±3.1^{b}	1.52±0.5^{c}	19.3±1.5^{c}	64.5±7.6^{c}
NaCl+ folic acid	100	20.3±1.2^{b}	128.1±9.9^{b}	59.1±4.2	1.67±0.4	21.3±2.1	30.8±2.4^{a}
	200	45.5±2.1^{b}	99.9±8.8^{b}	81.5±7.8^{b}	1.61±0.5	20.5±1.8	43.0±4.2
	300	52.1±2.4^{c}	99.1±9.8^{c}	62.1±4.9^{b}	1.54±0.4	19.6±1.6	44.8±4.3^{b}
NaCl+ ascorbic acid	100	31.5±1.7^{b}	171.1±12.0^{c}	50.1±5.2	2.26±0.9^{a}	28.8±1.9^{a}	9.0±1.2^{c}
	200	40.3±2.4^{c}	139.2±10.2^{c}	54.2±4.9	1.59±0.4	20.3±1.7	9.5±1.3^{c}
	300	62.3±4.6^{c}	85.0±7.8^{c}	53.4±6.0	1.47±0.3	18.8±1.8	18.6±2.0^{c}
NaCl+ cobalamin	100	22.3±1.4^{b}	87.2±9.4^{a}	49.3±3.2	1.73±0.4^{a}	22.1±1.3^{a}	9.5±1.1^{c}
	200	35.2±1.4^{c}	90.5±8.5^{b}	82.3±6.9^{c}	1.59±0.3	20.4±1.5	16.6±1.4^{c}
	300	51.4±3.6^{c}	96.0±9.3^{c}	61.2±5.4^{b}	1.58±0.2	20.1±1.2	43.2±3.6^{b}

Values with a superscript are significant different from the control. Letter a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, and absence of letter = non significant.

In addition, the reduction in the Cl ion concentrations due to vitamin treatments was more pronounced in ascorbic acid treatment where it reached about 71.2% below its corresponding control (300 mM NaCl).

It is worthy to note that, the maximum increase of K ion levels measured in salinized flax seedlings treated with ascorbic acid was 131.2% compared to its corresponding control (200 mM NaCl). However, at the same level the increase in K in response to folic acid or cobalamin treatment was 65.9% and 50.0% respectively compared with their corresponding control.

Changes in nucleic acid contents

Salinity induced a significant reduction in DNA and RNA contents of flax seedlings (Table 5). This reduction was salt concentration dependant. A non- significant reduction in RNA content was observed in flax seedlings exposed to 100 mM NaCl, The decreases in RNA levels were about 6.4%, 19.0% and 25.3% at 100, 200 and 300 mM NaCl respectively below the control value.

Similarly the decrease in DNA content was about 38.4%, 41.1% and 52.8% respectively at the same salinity levels. The application of folic acid, ascorbic acid or cobalamin alleviated the inhibitory effects of NaCl on RNA synthesis but significantly reduced DNA level as being compared with the corresponding control.

DISCUSSION

The present study suggests that, flax seeds tolerated NaCl salinity up to 200 mM NaCl; the germination was completely inhibited at 300 mM NaCl. Salinity stress inhibits seed germination either due to accumulation of toxic ions **(Ramagopal, 1988)**; or through osmotic stress which reduce the uptake of water **(Sharma, 1990)**. This is the case in our study it was clearly shown that under salinity stress Na was sharply accumulated in salinized flax seedlings while K concentration as well as K/Na, Ca/Na, Mg/Na and P/Na ratios were significantly decreased as salinity levels increased (Tables 3 and 4). On the other hand, the completely non germinated flax seeds (at 300 mM NaCl) showing an amazing capacity for recovery and germination when exposed to folic acid, ascorbic acid or cobalamin treatments (Plates1 & 2). It is interesting to note here that vitamin treatments greatly nullify the inhibitory effects of salinity on growth of flax seedlings (Table 1). Salt stress leads to changes in growth, morphology and physiology of roots that would in turn change water and ion uptake **(Alpaslan and Gunes, 2001; Alves da costa *et al*., 2005; Salter *et al*., 2007)**. Reduced rate of new cell production may make additional contributions to inhibition of growth **(Boscaiu *et al*., 2005)**. In this respect ascorbic acid is implicated in regulation of root elongation and cell wall expansion of many plant species **(Noctor and Foyer, 1998)**. Folic acid and cobalamin are known to be necessary for cell **division (Andrew *et al*., 2000; Smith *et al*., 2007)**.

Table 4 Effects of folic acid, ascorbic acid or cobalamin on K/Na, Ca/Na, Mg/Na and P/Na ratios of *Linum usitatissimum* seedlings grown under salt stress conditions. Values are listed are expressed as mg / g dry weight.

Treatments	***NaCl (mM)***	***K/Na***	***Ca/Na***	***Mg/Na***	***P/Na***
NaCl	000	4.52	3.87	0.04	0.54
	100	1.21	0.89	0.02	0.27
	200	0.74	0.61	0.01	0.21
	300	0.36	0.43	0.01	0.18
NaCl + folic acid	100	6.31	2.91	0.08	1.05
	200	2.19	1.79	0.03	0.45
	300	1.90	1.19	0.02	0.37
NaCl + ascorbic acid	100	5.43	1.59	0.05	0.91
	200	3.45	1.34	0.03	0.55
	300	1.36	0.86	0.02	0.30
NaCl + cobalamin	100	3.91	2.21	0.07	0.94
	200	2.57	2.21	0.04	0.58
	300	1.86	1.19	0.03	0.39

Membrane stability index has been used to assess tolerance of various plant species **(Sudhakar *et al*., 2001; Eraslan *et al*., 2007: Azooz, 2009)**.

The membrane injury in salinized flax seedlings was concomitant in most cases with sharp decrease in membrane stability index (MSI). MSI was subjected to a significant increase in salinized flax seedlings exposed to folic acid, ascorbic acid or cobalamin (Table 1). The maintenance of high percentage of MSI throughout the experiment show that vitamin–treated salinized flax seedlings can overcome hazards caused by salt stress.

One approach to understanding the ability of flax seedlings to tolerate salt stress has been to identify stress-induced changes of individual proteins under the assumption that stress adaptation results from alterations in gene expression **(Natarajan *et al*. 1996)**.

The observed changes in protein profile and consequently the physiological responses suggested that the changes in protein pattern might play a critical role in the response of flax seedling to salt stress (Table 3). The present data revealed the presence of three common protein bands (M.wt: 75.9, 58.2 and 20.9 kDa) detected in the control and in response to the various concentrations of NaCl in the absence and presence of the vitamins which might be specific for flax seedlings irrespective of the treatments. In addition sever salt stress (300 mM NaCl) induced the *de novo* synthesis of salt specific polypeptides (M.wt: 209.3, 97.7, 80.4, 68.3, 65.4, 55.4, 45.0, 32.0, 28.5, 26.3 and 23.7 kDa). These salt specific proteins might be involved in salt tolerance and / or a member of LEA family which are acting as antioxidants; membrane and protein stabilizers **(De Abreu and Mazzafera, 2005)**. It is suggested that not all proteins produced in saline condition are correlate with stress tolerance, which might be the case in our study. In spite of the detection of 26 and 32-kDa proteins under salt stress (200 and 300 mM NaCl), the membrane injury in salinized flax seedlings was concomitant with sharp decrease in membrane stability index (MSI).

Consequently, these polypeptides may not have a function in the process of salt adaptation in flax seedlings grown under sever salt stress conditions.

The new protein bands which accumulated in response to salt stress and disappeared in the vitamin treated seedlings (M.wt: 97.7, 80.4, 68.3, 65.4, 55.4, 32.0 and 23.7 kDa) might be responsible for the observed decrease in MSI under sever salt stress. The disappearance of some high molecular weight proteins in response to all the previous treatments might be attributed to the increase of the synthesis of other proteins **(Robinson *et al*., 1990)**. The greatest increase in the concentration (measured as band intensity) of the low molecular weight protein (M.wt: 6.2 kDa) in salinized flax seedlings treated with any of the vitamins was concomitant with the better performance of flax seedlings grown under saline conditions (Table 2). The most characteristic feature of vitamin treatments is their role on stimulating the biosynthesis of new set of low molecular weight proteins in addition to the disappearance of others. These proteins might be enzymes and / or phytohormone receptors **(Napeir and Venis, 1990; Dunn, 1993)**.

The accumulation of ions in different plant species facilitate the osmotic adjustment and increase the tolerance of these plants to saline environment. In the present study, it was clearly shown that under salinity stress Na was sharply accumulated in salinized flax seedlings while K concentration as well as K/Na, Ca/Na, Mg/Na and P/Na ratios were significantly decreased as salinity levels increased (Tables 3 and 4). These results are in confirmatory with the results obtained by many authors who found that salt stress was found to alter the mineral content of many plants particularly during germination. Salinity stress frequently induces an increase in Na and Cl as well as a decrease in K and Ca concentrations **(Izzo *et al*., 1991; Lutts *et al*., 1996; Alpasian and Gunes 2001; Inal, 2002).** It was suggested that, the effect of salinity on mineral ions was due to decrease in leaf water potential, relative water content, and water retention capacity concurrently with increased water saturation deficit **(Kabir *et al*., 2004)**. The observed increase in Na might be attributed to the fact that, under salinity stress the uptake of Na ions was increased, while the contrary was observed with respect to K ions. Also, the competition between potassium and sodium has been reported **(Yildirim *et al*., 2006; Roussos *et al*., 2007; López *et al*., 2008)**. The antagonistic relation between Na and K ions indicated that, the high levels of Na ions generated a kind of competition on the level of sites of K ions absorption and thus limited the absorption of K **(Rejili *et al*., 2007)**. The high content of Na could disrupt the nutrient balance, thereby causing specific ions toxicity despite disturbing osmotic regulation.While the reduction in K concentration could inhibit growth by reducing the capacity for osmotic adjustment and turgor maintenance or by adversely affecting metabolic functions **(Ashraf and Harris, 2004; López *et al*., 2008; Inal *et al*., 2009)**.

There was a beneficial effect of vitamin treatments which was reflected in the reduction in Na and Cl and increases in K, Ca, Mg, and P contents. Consequently K/Na, Ca/Na, Mg/Na and P/Na ratios (Tables 3 and 4). This may represent a tool exerted by the vitamin-treated plants to partially overcome the toxic effect of NaCl during salt stress.

There are many reports indicating the importance of adequate levels of Ca in alleviating the deleterious effects of salinity on plant growth **(Sivritepe *et al*., 2003)**. Moreover, calcium [functions] to limit intercellular Na ions accumulation by regulating processes that restrict influx and enhance efflux of these cations across the plasma membrane **(Pardo *et al*., 1998)**. On the other hand, one of the mechanisms of damage by salt is through displacement of Ca ions from functional sites as reported by **Jeschke *et al*. (1986)**.

Under salt stress, protecting the DNA was a priority which is confirmed by the data reported by **Hasegawa and Bressan (2000) Hamed (2004)**. The results observed in the

present work and those obtained by many other investigators show that, salinity disturbed nucleic acid metabolism (Table 5). Both DNA and RNA contents were markedly decreased with the increase in the applied NaCl doses. The reduction of both DNA and RNA at injurious levels of NaCl might be due to its effects on the inhibition of synthesis and intensification of break down **(ABo-Kasssem, 2006)**. The results obtained in the present work showed that folic acid, ascorbic acid or cobalamin alleviated the inhibitory effects of NaCl on RNA production but did not evoke the same response in case of DNA. Enhanced ascorbate content was not itself sufficient to stimulate DNA production. Similarly this is the case in folic acid and cobalamin treated seedlings (Table 5). Consequently, one can say that vitamins regulate DNA synthesis at the genome level **(Noctor and Foyer, 1998)**.

***Table* 5** Effects of folic acid, ascorbic acid or cobalamin on nucleic acid contents of *Linum usitatissimum* seedlings grown under salt stress conditions. Values are listed are expressed as mg / g dry weight.

Each value is a mean of three replicates ±SE

Treatments	***NaCl (mM)***	***RNA***	***DNA***
NaCl	000	5780± 14.0	280.7 ± 5.5
	100	5410± 12.4	172.7 ± 9.5^{b}
	200	4680± 10.1^{b}	165.4 ± 8.4^{c}
	300	4320 ± 10.3^{b}	132.4 ± 7.3^{b}
NaCl + folic acid	100	6910 ± 11.6^{c}	132.1 ± 7.2^{a}
	200	6510 ± 14.9^{c}	107.3 ± 8.4^{b}
	300	5120 ± 12.8^{b}	101.4 ± 7.0^{b}
NaCl + ascorbic acid	100	6201 ± 18.9^{b}	145.1 ± 6.4^{a}
	200	6108 ± 16.4^{b}	118.5 ± 7.8^{b}
	300	5822 ± 13.2^{b}	115.2 ± 8.2^{b}
NaCl + cobalamin	100	6690 ± 14.5^{c}	130.1 ± 7.9^{b}
	200	6652 ± 18.2^{c}	113.3 ± 7.7^{b}
	300	5690 ± 12.2^{b}	110.1 ± 7.3^{b}

Values with a superscript are significant different from the control. Letter a =* at P > 0.05, b =** at P < 0.01, c =*** at P < 0.001, and absence of letter = non significant.

In conclusion, salinized flax seedlings were thus ill equipped to face salt stress. The mechanism of these vitamins on alleviating salt stress hazards of flax seedlings might be mediated by stimulating the *de novo* synthesis of new set of low molecular weight proteins, reduction in some ions (Na and Cl) coupled with increases in the accumulation of others (K, Ca, P and Mg) and consequently the maintenance of high percentage of MSI throughout the experiment.

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تأثير بعض الفيتامينات على الاتزان الايونى والنمط البروتينى لبادرات نبات الكتان تحت ظروف الاجهاد الملحى

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تهدف هذه الدراسه الى تقييم الدور الذى تلعبه بعض الفيتامينات مثل حمض الفوليك (فيتامين ب٩) و حمض الاسكوربيك (فيتامين ج) او الكوبلامين (فيتامين ب١٢) فى زيادة مقاومة نبات الكتان للاجهاد الملحى عند تركيزات (١٠٠، ٢٠٠ و ٣٠٠ مللى مولار) و كذلك تأثير هذه الفيتامينات على انبات و نمو وايضا بعض التغيرات الايضيه المصاحبه لذلك.

كما تهدف الدراسه ايضا الى دراسه التأثير المشترك للملوحه و بعض الفيتامينات على انبات و نمو وكذلك بعض التغيرات الايضيه المصاحبه لنمو نبات الكتان و هو الامر الذى قد يلقى مزيدا من الضوء فى مجال معاملة البذور و تهيئتها للانبات و النمو تحت ظروف غير ملائمه و كذلك محاولة تحفيز المقاومه للاجهاد الملحى فى نبات الكتان باستخدام الفيتامينات السابق ذكرها.

و لقد اسفرت هذه الدراسه عن نتائج يمكن ايجازها فيما يلى:

١- أظهر الملح المستخدم (كلوريد الصوديوم) فعاليه عاليه فى تثبيط انبات بذور نبات الكتان و خصوصا عند التركيزات العاليه من الملح التى بدورها أدت الى منع انبات بذور الكتان عند تركيز (٣٠٠ مللى مولار).

٢- اختزال الى حد كبير فى نمو البادرات خاصه عند المستويات العاليه من الملح.

اسفرت معامله بذور نبات الكتان تحت ظروف الاجهاد الملحى بكل من حمض الفوليك (فيتامين ب٩)، حمض الاسكوربيك (فيتامين ج) او الكوبلامين (فيتامين ب١٢) عن ايقاف التأثير الضار للملوحه و نمو البادرات عند مستويات الملوحه العاليه و كانت هذه الاستجابه مصحوبه بزيادة محتوى البادرات من أيونات الصوديوم و الكلور و الفوسفور والماغنسيوم و نقص فى كل من البوتاسيوم والكالسيوم. علاوة على ذلك فان معامله بذور الكتان بكل من الفيتامينات السابق ذكرها أدى الى نقص ملحوظ فى محتوى البادرات من أيونات الصوديوم و الكلور مع زيادة واضحه فى كل من أيونات البوتاسيوم و الكالسيوم و الفسفور والماغنسيوم.

٣- كما لوحظ ان كل الفيتامينات المستخدمه قد ازالت الاثار المثبطه للملح على تخليق الحمض النووى RNA وعلى الجانب الاخر لم يستجب الحمض النووى DNA للمعامله باى من الفيتامينات المستخدمه فى هذا البحث الامر الذي قد يلعب دورا في ازاله الاثار الضاره للاجهاد الملحى وكان هذا ممثلا بزيادة واضحه فى (MSI).

٤- باستخدام طريقه التفريد الكهربى للبروتين امكن فصل البروتينات المستخلصه من بادرات نبات الكتان تحت تأثير الاجهاد الملحى و كذلك البادرات الغير معامله و المعامله ببعض الفيتامينات السابق ذكرها وقد اسفرت النتائج عن ظهور حزم بروتينيه جديدة نتيجه للمعامله بالفيتامينات المذكورة و هذا يوضح الفكرة بأن هذه البروتينات قد تكون مسئوله عن تكيف البذرة مع الملوحه و كذا استحثاث انبات و نمو بذور الكتان تحت ظروف الاجهاد الملحى.