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Growth and Biochemical Changes in Lupine Plant Grown under Saline Condition

M.M. Hussein, M.S. Abdel-Hady* and Hoda M.H. EL-Naggar*,
*Water Relations & Irrigation Dept. and * Botany Dept., Agric. Div., National Research Centre, Cairo, Egypt.*

A POT experiment was conducted in the greenhouse of the National Research Centre at Dokki, Cairo Egypt during 2007/2008 winter season to evaluate the effect of different salt stress levels on some physiological and biochemical parameters. Lupine plants were irrigated by three concentrations of diluted seawater (2000, 4000 and 6000 ppm separately) higher than the control treatment (irrigated by tap water 250 ppm) and sprayed with ascorbic acid at two levels (100 and 200 ppm). The control plants received the same quantity of distilled water. Obtained results approved a negative relationship between salt concentration in the root media and vegetative growth parameters, *i.e.* plant height, root length, number of leaves and number of branches of lupine, on one hand. Also dry weight of the different plant parts decreased as the concentration of salt increased in water of irrigation. On the other hand, the top to root ratio decreased as the concentration of salt increased. Plant height, root length and number of branches gave its higher values when plants sprayed by 200 AsA. Root fresh weight showed the same response when plants spriayed by 200ppm of ASA. Fresh weight of stem, top and whole plants increased as the concentration of (Ascorbic acid) AsA increased up to the highest level used. Dry weight of different parts of lupine plant increased parallel to the increase in AsA level. The AsA treatments improved the plant height and number of green leaves. This was more clear under the highest salinity and ascorbic acid levels. Generally, root length or number of branches was slightly affected except for root length under the higher level of salinity which showed the same response of plant height and number of leaves. Negative relationship between the increase of salt concentration in water of irrigation by diluted seawater and the dry mass of different lupine plants and whole plant dry weight markedly depressed by salt stress but the AsA supply enhancing the resistance against this abiotic stress. The electrophoratic patterns (SDS-PAGE) for water soluble proteins of lupine cultivar under salt-stress and antioxidant (ascorbic acid) activity conditions showed that electrophoratic bands could be a useful tool for identification and characterization of biochemical genetic marker that are related to salinity tolerance also antioxidant activity which was used to protection from salinity conditions.

Keywords: Lupine (*lupinus termis* L.), Salinity, Diluted seawater, Ascorbic acid (AsA), Growth, Protein, Electrophoratic patterns.

Sweet lupines are now widely accepted as a supplement for ruminants because they are high in available energy and protein and have advantages in handling,

E-mail: mmoursyhus@gmail.com

storing and feeding. They are particularly used to feed sheep in times of pasture shortage. They are also widely used in pig and poultry nutrition where they are valued for their consistent quality and low content of anti-nutritional factors. In Egypt lupine (*Lupinus termis* L.) used in human nutrition and its cultivated area was more in Upper Egypt than Delta but characterized by its higher phenolic compounds and bitter taste which needs to soaked in water before use.

Salt stress growth and yield of plants through its effect on the metabolic processes inside the plants (Mousa *et al.*, 1985; El-Zeiny, 1990; Abd El- Aziz *et al.*, 2006; Wilson *et al.*, 2006 and Beltagi, 2008). photosynthesis and shoot respiration were unaffected by NaCl concentrations up to 150 mol m^{-3}. However, nitrogenase activity decreased with increased NaCl concentration up to 100 mol m^{-3}, whilst the O_2 diffusion resistance increased with 100 mol m^{-3} NaCl, but showed no further change when 150 mol m^{-3} NaCl was applied for 6 days . Increases in NaCl concentration decreased nodular starch content while increasing sucrose content, suggesting an osmotic regulation. These changes were associated with a 77% decrease in sucrose syntheses activity (Fernansez-Pascual *et al.*, 1995). Nandwal *et al.* (2007) suggested that ethylene in relation to water status and lipid peroxidation and along with other metabolic processes has an important role in induced nodules senescence under salinity. The activated enzymes could not overcome the accumulation of H_2O_2 in nodules. Ascorbic acid content declined in the range from 20 to 38%, whereas Na^+/K^+ ratio and Cl^- content were significantly enhanced.

Salt stress (50 and 150 mM NaCl) effects on sucrose metabolism was determined in *Lupinus albus* L. Sucrose syntheses (SS) activity increased under salt stress and sucrose phosphate syntheses activity decreased. Acid invertase activity was higher at 50 mM NaCl and decreased to control levels at 150 mM NaCl. Alkaline invertase activity increased with the salt stress. Glucose content decreased with salt stress, sucrose content was almost three times higher in plants treated with 150 mM NaCl and fructose content did not change significantly. The most significant response of lupin plants to NaCl excess is the increase of sucrose content in leaves, which is partially due to SS activity increase under salinity (Fernandes *et al.*, 2004).

Application of ascorbic acid induced some physiological effects as found by: Asard *et al.* (1995), Zaki & Tarraf (1999) and Noctor & Foyer (1998). The maintaining effect of ascorbic acid on salinity stress were studied by many authors as Shalata & Neumann (2001), Al-Hakmi (2001) and Athar (2008). Ascorbate peroxidases (APX), localized in the cytosol, peroxisome, mitochondria, and chloroplasts of plant cells, catalyze the reduction of H_2O_2 to water by using ascorbic acid as the specific electron donor. Peroxisomal type ascorbate peroxidase (APX), an antioxidant enzyme play a role in protection against salt-induced oxidative stress (Xu *et al.*, 2008).

Besides the expected up regulation of pathways related to the biosynthesis of compatible solutes (raffinose family oligosaccharides and certain amino acids), we observed a down regulation of numerous genes putatively localized to and possibly

involved in the reorganization of cell walls, an association that had not been recognized previously. The induced biosynthesis of the antioxidants ascorbic acid and α-tocopherol appeared to be integrated into a network of reactions controlling the levels of reactive oxygen species (Ghassemian *et al.*, 2008).

Although ascorbic acid (AsA) is one of the most important and abundantly occurring water soluble antioxidants in plants, relatively little is known about its role in counteracting the adverse effects of salt stress on plant growth (Athar *et al.*, 2008).

Electrophoretic techniques for protein polymorphism have been used as identification method which provide correlation between the altered expression of specific genes and changes in the environment . These changes in expression of genes would be involved in adaptation and could be used as biochemical genetic markers for salt stress (Abdel-Tawb *et al.*, 2003 and Rashed *et al.*, 2004)

This work designed to investigate the effect of exogenous ascorbic acid in elevation of lupine plants versus salt stress through study the response of growth and protein to the irrigation by diluted seawater.

Materal and Methods

A pot experiment was conducted in the greenhouse of the National Research Centre at Dokki, Cairo, Egypt during 2007/2008 winter season to evaluate the effect of different salt stress levels on growth and yield characters. The treatments were as follows:

1 – Salinity: salt treatment was started after three weeks from sowing by three concentrations of diluted seawater (2000, 4000 and 6000 ppm) higher than the control treatment (tap water of 250 ppm).

2 –Spraying plants with ascorbic acid (AsA) two weeks latter after sowing at the rate of (100, and 200 ppm). The control plants received the same quantity of distilled water.

The experiment included 4 levels of salinity in combination with three concentrations of ascorbic acid, *i.e.* 12 treatments in 6 replicates. Metallic ten pots 35 cm. in diameter and 50 cm in depth were used. Every pot contained 30 kg of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2 kg of gravel (Particles about 2-3 cm in diameter) were presenr, so the movement of water from the base upward.

Seeds of lupine (*Lupinus termis* L.) c.v. Balady were sown in the first of December, 2007 plants were thinned twice, the 1^{st} days after sowing and the 2^{nd} two weeks latter to leave three plants / pot. Calcium super phosphate (15.5 % P_2O_5) and potassium sulfate (48.5 % K_2O) in the rate of 6.0 and 3.0 g/pot were added before sowing. Ammonium sulfate (20.5 % N) in the rate of 3.43 g / pot

was added. Irrigation with diluted seawater in different concentrations were started 30 days after sowing (One irrigation by salt water and the next was by fresh water alternatively) till before harvest.

SDS-protein electrophoresis

Sodium dedecylsulfate polyaccrlamide gel electrophoresis SDS-PAGE was used to study the genetic background for the studied cultivar under control, salt stress and antioxidant activity. Water soluble proteins fractions were prepared according to the method of Laemmli (1970) as modified by Studier (1973). The SDS protein gel was scanned and analyzed using Gel Doc prgreames.

Collected data were subjected to the proper statistical analysis with the methods described by Snedecor & Cochran (1990).

Results and Discussion

Growth

Effect of salinity

A negative relationship was detected between salt concentration in the root media and vegetative growth parameters, *i.e.* plant height, root length, No. of leaves and No. of branches of lupine. Dry weight of the different plant parts decreased as the concentration of salt increased in the irrigation solution (Table 1). Also top to root ratio decreased as the concentration of salt increased. Gaballah & Moursy (2004), Khalil (2006), Costa *et al.* (2007) and Treeby *et al.* (2008) showed the same response. This adverse effect may be due to one ore more from the following reasons: interference in plant absorption and distribution and / or water adjustment in plant tissues (Mansour, 1998 and Treeby *et al.*, 2008), accumulation and toxicity of Na and Cl ions (Kinraide, 1999), carbohydrate and protein building (Khodary, 2004; Fernandes *et al.*, 2004 and Pinheiro *et al.*, 2008), enzymes activity (Abd El-Baky *et al.*, 2003 and Ashraf, 2008) and oxidative defense (Mittova *et al.*, 2004 and Costa *et al.*, 2007).

The protein, amino acid and nucleic acid contents of lupine from seeds not exposed to gamma irradiation decreased with increasing concentrations of NaCl and increased with increasing NaCl concentrations in lupine derived from seeds exposed to gamma radiation. Treatment with NaCl inhibited the NO_3, K and P ion uptake of lupines from the growth medium. However, exposure to gamma irradiation increased the uptake of these ions by the plant (Khodary, 2004). However Keutgen & Pawelzik (2008) noticed that while mean fruit weight decreased, dry matter and contents of total soluble carbohydrates, as well as sweetness index of fruits, remained constant, but salt stress in both cultivars increased the antioxidant capacity, antioxidants pools (ascorbic acid, anthocyanins, superoxide dismutase) and selected minerals such as Na^+, Cl^-, K^+, N, P and Zn^{2+}, as well as lipid peroxidation. Furthermore, salt stress used increased the contents of free and essential amino acids, especially in cv. Elsanta. The more tolerant cv. Korona was characterized by an increase of reduced glutathione and a better fruit taste. In salt-stressed fruits of cv. Elsanta, taste was significantly impaired.

TABLE 1. Growth of lupine plants as affected by salinity.

Salinity	Plant height cm	Root length cm	Leaves No.	Branches No.	Fresh weight (gm)					Dry weight (gm)					
					Root	Stem	Leaves	Top	whole	Root	Stem	Leaves	Top	Whole	Top/root ratio
TW	41.6	11.7	33.1	3.1	2.07	7.70	8.89	17.50	19.57	0.41	1.95	1.91	3.86	4.27	9.42
S1	40.7	9.7	32.4	3.0	2.13	7.25	8.22	16.63	18.56	0.36	1.86	1.50	3.36	3.72	9.33
S2	36.0	10.5	33.1	3.2	1.43	7.23	9.24	16.47	18.83	0.32	1.30	1.51	2.81	3.13	8.78
S3	34.6	8.1	24.2	2.9	1.25	5.50	5.60	11.10	12.84	0.20	0.86	0.73	1.59	1.79	7.95
LSD at 5%	0.59	N.S	6.11	N.S	N.S	1.80	2.61	4.22	5.39	0.21	0.97	0.60	1.79	1.09	------

TW: Tap water.
S1: 2000 ppm Salt.
S2: 4000 ppm Salt.
S3:6000ppm Salt.

Effect of ascorbic acid (AsA)

Plant height, root length and number of branches gave its higher values when plants sprayed by ppm. AsA. Root fresh weight showed the same response. Fresh weight of stem, top and whole plants increased as the concentration of AsA increased up to the highest level used. Dry weight of different parts of lupine plant increased parallel to the increase in AsA level (Table 2). Ross *et al.* (1999) observed that ascorbate and ascorbate peroxidase are important antioxidants that are abundant in N_2-fixing legume root nodules. Antioxidants are especially critical in root nodules because leghemoglobin, which is present at high concentrations in nodules, is prone to autoxidation and production of activated oxygen species such as $O_2\sqrt{}^-$ and H_2O_2. Al-Hakimi (2001) emphasized that soaking of wheat grains before sowing in AsA synergistically improved the photosynthetic rate which in turn on growth of plants. Abd El-Aziz *et al.* (2006) demonstrated that treatment of *Khaya senegalensis* with AsA up to 400 ppm led to improvement of all plant organs. Ghassemian *et al.* (2008) mentioned that the induced biosynthesis of the antioxidants ascorbic acid and α-tocopherol appeared to be integrated into a network of reactions controlling the levels of reactive oxygen species.

Interaction between sailinty leves and ascorbic acid

The interaction between salinity leves and ascorbic acid spraying (Table 3 and Fig. 1 & 2) caused increasing on plant height, root length and number of branches gave its higher values when plants sprayed by 200 AsA. Root fresh weight showed the same response when plants spriayed by 200ppm of AsA. Fresh weight of stem, top and whole plants increased as the concentration of (Ascorbic acid) AsA increased up to the highest level used. Dry weight of different parts of lupine plant increased parallel to the increase in AsA level. The AsA treatments improved the plant height and number of green leaves.

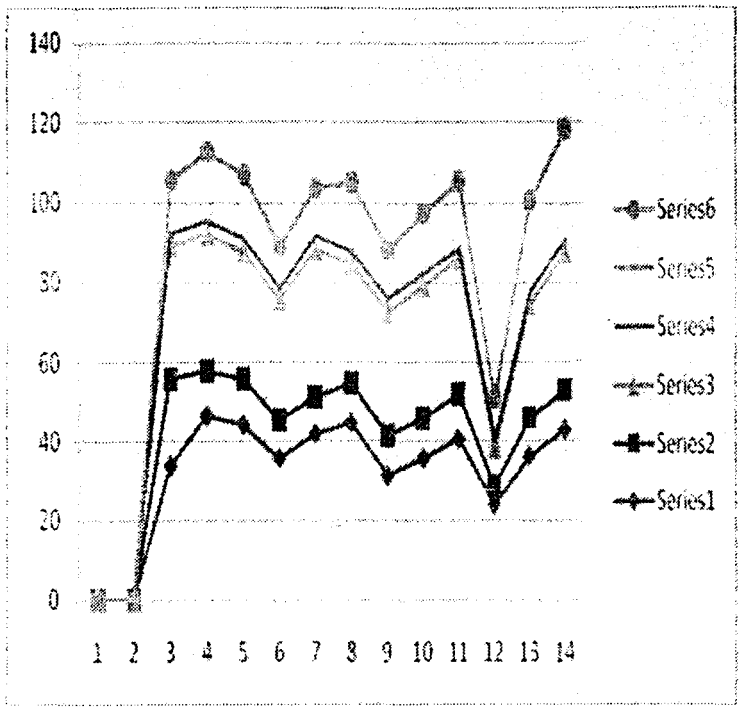
TABLE 2. Growth of lupine plants as affected by ascorbic acid.

AsA ppm	Plant height cm	Root length cm	Leaves No.	Branches No.	Fresh weight (gm)					Dry weight (gm)					
					Root	Stem	Leaves	Top	Whole	Root	Stem	Leaves	Top	Whole	Top/root ratio
O	31.1	9.18	21.3	2.8	1.38	5.37	6.91	12.28	13.36	0.26	1.44	1.14	2.58	2.84	9.90
100	40.2	10.08	33.3	3.1	1.97	7.16	7.96	15.12	17.81	0.34	1.54	1.48	3.02	3.36	8.88
200	38.3	10.75	32.7	3.0	1.81	8.26	9.50	17.76	20.57	0.37	1.86	1.63	3.49	3.86	9.43
LSD at 5 %	0.19	N.S	4.48	N.S	N.S	1.25	1.32	4.01	5.97	0.28	0.33	0.31	1.25	0.46	-----

TABLE 3. Growth of lupine plants as affected by ascorbic acid spraying and irrigated by diluted seawater.

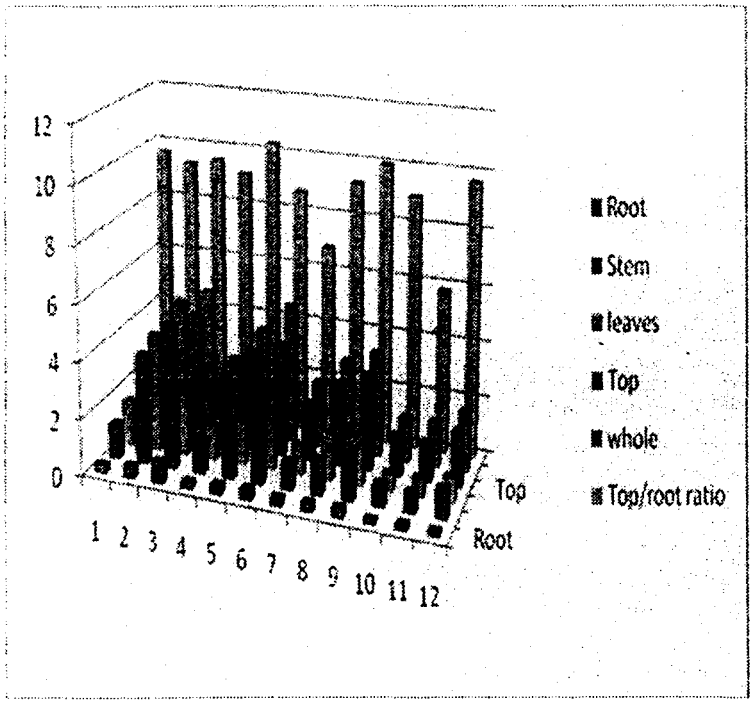
Salinity	AsA ppm	Plant height	Root length	No.of leaves	No of branches	Fresh weight (gm)					Dry weight (gm)					
						Root	Stem	Leaves	Top	Whole	Root	Stem	Leaves	Top	Whole	Top/root ratio
TW	0	33.9	11.0	33.3	3.0	1.61	7.81	7.99	18.64	18.05	0.30	1.29	1.61	2.90	3.20	9.67
	100	46.7	11.3	34.3	3.0	2.20	7.57	8.14	17.58	19.74	0.44	2.12	1.98	4.10	4.54	9.32
	200	44.3	11.7	31.7	3.3	2.40	7.63	10.01	18.70	21.10	0.78	2.45	2.15	4.60	5.08	9.56
S1	0	35.7	9.7	30.3	2.7	1.39	4.94	6.57	12.28	13.67	0.27	1.22	1.25	2.47	2.74	9.14
	100	42.0	9.3	37.0	3.3	3.29	7.79	7.99	16.59	19.88	0.35	2.03	1.58	3.61	3.96	10.34
	200	44.7	10.0	30.0	3.0	1.72	9.16	10.11	20.15	22.17	0.42	2.33	1.68	4.01	4.97	8.72
S2	0	31.2	10.3	31.3	3.3	1.31	6.47	8.55	15.6	16.97	0.30	1.00	1.06	2.06	2.36	6.87
	100	35.7	10.0	33.7	3.0	1.77	6.81	8.10	16.24	17.51	0.32	1.26	1.70	2.96	3.28	9.25
	200	40.7	11.3	34.3	2.3	1.70	8.40	10.26	19.97	21.67	0.34	1.63	1.77	3.40	3.74	10.00
S3	0	24.0	4.7	9.7	2.3	1.19	2.26	2.15	4.41	5.88	0.16	0.81	0.63	1.44	1.60	9.00
	100	36.3	9.7	28.7	3.0	1.13	6.39	7.10	14.49	15.31	0.24	0.73	0.67	1.40	1.64	5.83
	200	43.0	10.0	34.7	3.3	1.43	7.86	7.56	15.42	17.85	0.20	1.04	0.90	1.94	2.14	9.70
LSD at 5%		0.39	3.01	9.95	N.S	N.S	N.S	2.65	7.04	10.76	N.S	N.S	N.S	N.S	N.S	------

TW: Tap water. S1: 2000 ppm Salt. S2: 4000 ppm Salt. S3: 6000 ppm Salt.



3: tap water
4: tap water- 100 ppm ASA
5: tap water- 100 ppm ASA
6: 2000 ppm salt-tap water.
7: 2000 ppm salt-100 ppm ASA.
8: 2000 ppm salt-200 ppm ASA.
9: 4000 ppm salt-tap water .
10: 4000 ppm salt-100 ppm ASA.
11: 4000 ppm salt-200 ppm ASA.
12. 6000 ppm salt-tap water.
13: 6000 ppm salt-100 ppm ASA.
14: 6000 ppm salt-200 ppm ASA.

Fig. 1. Vegetative growth of lupine plants as affected by ascorbic acid and irrigated by diluted seawater.



1: tap water
2: tap water- 100 ppm ASA
3: tap water- 100 ppm ASA
4: 2000 ppm salt-tap water.
5: 2000 ppm salt-100 ppm ASA.
6: 2000 ppm salt-200 ppm ASA.
7: 4000 ppm salt-tap water .
8: 4000 ppm salt-100 ppm ASA.
9: 4000 ppm salt-200 ppm ASA.
10. 6000 ppm salt-tap water.
11: 6000 ppm salt-100 ppm ASA.
12: 6000 ppm salt-200 ppm ASA.

Fig. 2. Dry matter of lupine plants as affected by spraying ascorbic acid and irrigated by diluted seawater.

Imposition of salt stress reduced the growth of both wheat cultivars by causing reduction in photosynthesis, endogenous AsA level, and enhancing accumulation of Na^+ and Cl^- coupled with a decrease in K^+ and Ca^{2+} in the leaves and roots of both cultivars thereby decreasing tissue K^+/Na^+ ratio. However, root where AsA was applied it counteracted the adverse effects of salt stress on the growth of cv. S-24 only, particularly at 100 mg l^{-1} AsA level. AsA-induced enhancement in growth of salt-stressed plants of S-24 was associated with enhanced endogenous AsA level and catalase (CAT) activity, and higher photosynthetic capacity, and accumulation of K^+ and Ca^{2+} in the leaves. Although applied AsA to root did not improve the growth of salt-stressed plants of MH-97, it enhanced endogenous level of AsA, CAT activity, photosynthetic capacity, and leaf K^+ and Ca^{2+}. These findings led us to conclude that root applied AsA counteracts the adverse effects of salt stress on growth of wheat by improving photosynthetic capacity of wheat plants against salt-induced oxidative stress and maintaining ion homeostasis, however, these effects were cultivar specific (Athar *et al.*, 2008). Ascorbate peroxidases (APX), localized in the cytosol, peroxisome, mitochondria, and chloroplasts of plant cells, catalyze the reduction of H_2O_2 to water by using ascorbic acid as the specific electron donor. Peroxisomal type ascorbate peroxidase (APX), an antioxidant enzyme play a role in protection against salt-induced oxidative stress (Xu *et al.*, 2008).

Beltagi (2008) indicated that the added AsA (4mM) improved the stem and root fresh and dry weights of stressed plants. Consistent findings reported on the beneficial effects of the exogenous application of AsA in partially mitigating the adverse effect of salt stress on growth like cell division and cell enlargement (Mozafar & Oertli, 1992 and Ahmed-Hamad & Monsaly, 1998). Moreover, in tomato seedlings, the exogenous supply of AsA increased tissue levels and percentage age for surviving the toxic effects on NaCl (Shalata & Neumann, 2001). However, Zabalza *et al.* (2008) stated that nitrogen fixation in legumes is dramatically inhibited by abiotic stresses, and this reduction is often associated with oxidative damage. Although ascorbate (AsC) has been firmly associated with antioxidant defense, recent studies have suggested that the functions of AsC are related primarily to developmental processes. A supply of exogenous AsC increased the nodule AsC+dehydroascorbate (DHA) pool compared to water-stressed nodules without AsC, and significantly modulated the response to water stress of the unspecific guaiacol peroxidase (EC 1.11.1.7) in leaves and nodules. However, AsC supply did not produce recovery from water stress in other nodule antioxidant enzymes, nodule carbon and nitrogen enzymes, or nitrogen fixation. The supply of the immediate AsC precursor, galactono-1,4-lactone (GL), increased the nodule ASC+DHA pool, but also failed to prevent the decline of nitrogen fixation and the reduction of carbon flux in nodules. These results suggest that AsC has a limited role in preventing the negative effects of water stress on nodule metabolism and nitrogen fixate. Also, Abd El-Baky *et al.* (2008) revealed that application of bio-growth regulators, ascorbic acid and benzyl adenine had a slight effect on growth, activity and antioxidant enzymes activity in the seawater irrigated plants compare with that in unstressed wheat plants.

SDS-protein electrophoresis

From the SDS-PAGE analysis, thirteen band were detected with different molecular weights (MW) ranging from 97.3 to 14.5 KD_a. In lupine cultivars under control, salt stress and (AsA) treatments for water soluble proteins as shown in Table 4 and Fig. 3.

Six bands (-3, 6, 7, 9, 11 and 12) were commonly presented in all treatments, with molecular weights 77.2, 44.6, 35.5, 22.3, 18.7 and 16.9 KD_a, respectively. These bands were considered as marker bands for the lupine genotype. Substantial differences among the different treatments in these molecular weights were recorded. These treatments were discriminated from each other by some unique bands. Some other bands were absent in the most treatments compared with the control like bands numbers 1, 2 , and 5 with molecular weights 97.3, 83.6 and 65.7 KD_a, respectively, which could be attributed to the effect of salt stress. Four present bands at numbers 4, 8, 10 and 13 with molecular weights 70.6, 30.3, 20.7 and 14.5 KD_a, respectively, which may be synthesized to protect lupine plants from salt stress, as the result of using (AsA since these bands were absent under control conditions). Antioxidant treatments were considered highly correlated with the activation of gene expression or proteins synthesis. Generaly, salinity (Sulphate, chloride and carbonate salinity) inhibited protein, and nucleic acids content in lupine (Mousa *et al.*, 1985; El-Zeiny, 1990; Pastori & Foyer, 2002 and Beltagi, 2008).

TABLE 4. Densitomtric analysis for water soluble protein profiles of the lupine cultivar under control, salt stress and antioxidant (AsA) treatments showing number of bands (B.No) and molecular weights (Mw).

Bands	MW	A			B			C			D		
No.	kDa	1	2	3	4	5	6	7	8	9	10	11	12
1	97.3	1	0	0	0	0	0	0	0	0	0	0	0
2	83.6	1	0	1	0	0	0	0	0	1	0	0	0
3	77.2	1	1	1	1	1	1	1	1	1	1	1	1
4	70.6	0	0	0	0	0	1	1	1	1	1	1	0
5	65.7	1	1	0	0	0	0	0	0	0	0	0	0
6	44.6	1	1	1	1	1	1	1	1	1	1	1	1
7	36.5	1	1	1	1	1	1	1	1	1	1	1	1
8	30.3	0	0	0	0	0	0	0	0	1	0	1	1
9	22.3	1	1	1	1	1	1	1	1	1	1	1	1
10	20.7	0	1	0	0	0	0	0	0	1	1	1	1
11	18.7	1	1	1	1	1	1	1	1	1	1	1	1
12	16.9	1	1	1	1	1	1	1	1	1	1	1	1
13	14.5	0	1	0	0	0	0	1	1	1	1	1	1

MW: molecular weight
(1): present (0) : absent
A : irrigated by tap water
B : irrigated by 2000 ppm salt
C : irrigated by 4000 ppm salt.
O: Absent band.
1: Present band.
D: irrigated by 6000 ppm salt
1, 4, 7, 10 supplied with tap water
2, 5, 8, 11 supplied 100 ppm ascarbic acid
3, 6, 9, 12 supplied 200 ppm ascarbic acid

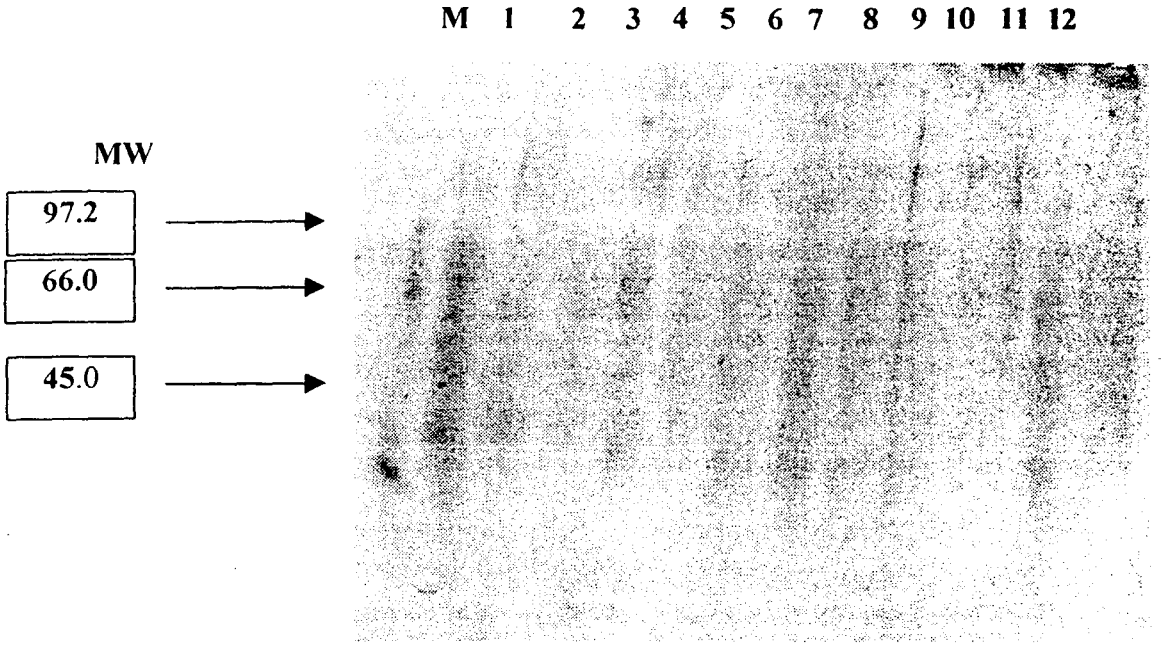


Fig. 3. SDS-PAGE profiles of lupine leaves protein (water soluble fraction) under control , salt stress and antioxidant ascorbic acid treatment .

M: Molecular Marker MW: Molecular weight.
1: tap water 2: tap water- 100 ppm ASA
3: tap water- 100ppm ASA 4: 2000 ppm salt-tap water
5: 2000 ppm salt-100 ppm ASA. 6: 2000 ppm salt-200 ppm ASA
7: 4000 ppm salt-tap water. 8: 4000 ppm salt-100 ppm ASA.
9: 4000 ppm salt-200 ppm ASA. 10: 6000ppm salt-tab water.
11: 6000 ppm salt-100 ppm ASA. 12: 6000 salt-200 ppm ASA

The results were in agreement with those of Abd El-Tawab *et al.* (1997) who found that there was specific protein markers associated with the effect of salt stress in inbred lines of maize. The highly expressed proteins in present study can be considered in a similar manner to that published by Al-Shabi (2002) who detected some specific protein bands associated with salt stress in sorghum cultivars. However, Beltagi (2008) on chickpea reported that the total number of protein bands /lane did not change under the low (20 MM NaCl) concentration but was dramatically reduced by the high (40mM) NaCl treatment. Some of optical densities of protein bands was inhibited by both levels of NaCl , but was induced by 10.68 % by added ascorbic acid at 20mM NaCl and by 21.39 % at 40mM NaCl. Six different polypeptides of molecular weights 146.28, 117.98, 51.55, 49.60, 44.49 and 38.34 were completely disappeared under NaCl stress. These bands reappeared in response to the added ascorbic acid treatment. Moreover, the optical density of every individual protein band was induced by ascorbic acid under the low NaCl concentration.

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النمو والتغيرات الحيوية لنبات الترمس النامى تحت الظروف الملحية

محمد مرسى حسين، محمد ثروت عبد الهادى* و هدى محمد حسن النجار*
قسم العلاقات المائية والرى و*قسم النبات – شعبة البحوث الزراعية والبيولوجية-
المركز القومى للبحوث- القاهرة – مصر .

أجريت تجربة أصص بصوبة المركز القومى للبحوث بالجيزة خلال الموسم الشتوى ٢٠٠٧ / ٢٠٠٨ وذلك لتقييم تأثير مستويات مختلفة من الملوحة على بعض القياسات الفسيولوجية والبيوكيميائية حيث رويت نباتات الترمس بثلاث تركيزات من ماء البحر المخففة (تستخدم منفصلة) وهى ٢٠٠٠ ، ٤٠٠٠ ، ٦٠٠٠ جزء فى المليون بالإضافة لمعاملة المقارنة التى رويت بماء الصنبور ٢٥٠ جزء فى المليون بالإضافة إلى الرش بحامض الأسكوربيك على مستويين ١٠٠ ، ٢٠٠ جزء فى المليون علاوة على معاملة المقارنة. أوضحت النتائج المتحصل عليها وجود علاقة عكسية بين تركيز الملح والصفات الخضرية وأيضاً الوزن الجاف للأجزاء المختلفة للنبات بينما أعطت الصفات الخضرية والوزن الجاف قيم مرتفعة عند الرش بحامض الأسكوربيك بالمستوى الثانى ٢٠٠ جزء فى المليون. وهذا يعنى أن حامض الأسكوربيك يزيد من مقاومة النبات لتحمل الملوحة.

كما أوضحت نتائج التفريد الكهربى للبروتينات الذائبة لنبات الترمس تحت تأثير مستويات الملوحة واستخدام مضادات الأكسدة (حامض الاسكوربيك) أنها أعطت دليل بيوكيميائى وراثى مرتبط بالتحمل للملوحة واستخدام مضاد الأكسدة الذى يساعد النباتات على تحمل الظروف الملحية.