

## MINERAL STATUS AND LUPINE YIELD RESPONSES TO ASCORBIC ACID SPRAYING AND IRRIGATION BY DILUTED SEA WATER

### ABSTRACT

A pot experiment was conducted in the greenhouse of the National Research Centre at Dokki, Cairo Egypt during 2010 -2011 winter seasons to evaluate the effect of different salt stress degrees on the growth and yield characters. The salinity treatments were: Irrigation by three concentrations of diluted seawater (2000, 4000 and 6000 ppm) more than the control treatment (irrigated by tap water 250 ppm) and spraying ascorbic acid (AsA) with two concentrations (100 and 200 ppm). Salinity depressed the pods, straw, straw+ pods and seeds weight relative to the control plants but the depression of these traits showed its maximum values when plants subjected to the higher level of salinity (6000 ppm) markedly more than that with the other two levels of salinity. Slight differences in the mentioned characters of plants irrigated by solution contained 2000 or 4000 ppm. Gradual depressions in pods/straw, seeds/pods and seeds/straw ratios were detected with the increase in salt concentration in water of irrigation. Ascorbic acid application led to increase the pod, straw, total and seeds yields. The high increment in pods weight and seeds to straw ratio were shown by using 100 ppm ascorbic acid but the increment in straw, total seeds weight and seeds/pods and pods/straw ratios. Furthermore, the absorption rate of N, K, P, Ca, and Mg ions from the growth medium significantly inhibited as a result of treatment with diluted sea water. Meanwhile, significant increases in the uptake of these ions were obtained in response to ascorbic acid application.

**Keywords:** Lupine (*Lupinus termis* L)-Salinity-Ascorbic acid-Yield-Mineral status.

### INTERODUCTION

The genus *Lupinus* (Papilionaceae) is known to be a rich source for lupine alkaloids (Takamatsu, et al. 1990). *Lupinus termis* is cultivate in the Mediterranean region for its edible seeds (Tackholm, 1974). So, lupine is considered as one of the important plant from medical and nutritional points of view.

Adverse environmental condition plays an important role in lowering the food production and raised the poverty worldwide. High salinity and drought are common stress conditions that adversely affect plant growth and crop production. Under these both condition, the ability of plants to uptake water was reduced and this intern quickly causes reductions in growth rate along with cascade of metabolic changes (Costa, et al. 2007; Hussein and Zaki, 2013 and Hussein, et al. 2014c). Salinity stress significantly declined the photosynthetic rate (Hussein, et al. 2014 a&b and Hussein and Alva, 2014). This effect may result from stomata closure due to osmotic stress, or salt-induced damage to photosynthetic apparatus (Brugnoli and Lauteir, 1991). Products of altered chloroplast and mitochondrial metabolism during stress cause oxidative damage to different cellular compounds including membrane lipids, proteins and nucleic acids. Apse and Blumwald (2002) reported that one approach for inducing oxidative stress tolerance would be through increase the cellular of enzyme substrates such as ascorbic acid.

Ascorbic acid (AsA) is a small water soluble anti-oxidant molecule which acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide. Improved understanding of ascorbate in plant will lead to the possibility of increasing ascorbate concentration in plants by genetic manipulation. This will have benefits to tolerance of plants to oxidative stresses (Smimolf, 1995 and Beltagi, 2008). Salinity sensitivity varies between plant organs and between cell at different developmental stages in a single organ. The physiological and molecular bases for the differential responses are not known. It is well known that exposure of plants to salinity is induce formation of reactive oxygen species (ROS), which are involved in damage mechanisms but in addition in cell growth processes (Bernstein, et al. 2010). Nevertheless, Hafsi, et al. (2010) on halophyte *Hordeum maritimum* and Agati, et al. (2011) on *L. vulgare* concluded that the antioxidative response was enhanced by the low and moderate salinity levels.

Plant spraying or seed soaking with ascorbic acid affected the oxidative defense and mitigated the inhibitory effect of NaCl on the growth parameters (Talaat, 2003; Maggio, et al. 2006 and Abd El-Baky, et al. 2008).

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Therefore, the current research work was performed to investigate the effect of ascorbic acid application in ameliorate salt stress in lupine plants.

## MATERIAL AND METHODS

A pot experiment was conducted in the greenhouse of the National Research Centre at Dokki, Cairo Egypt during 2010-2011 winter seasons to evaluate the effect of different salt stress degrees on the growth and yield characters.

Table (1) Some physical and chemical properties of studied soil a, b and sea water c:

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### a. Soil mechanical analysis

Sand		Silt 20-2 $\mu$ %	Clay < 2 $\mu$ %	Soil Texture
Course >200 $\mu$ %	Fine 200-20 $\mu$ %			
7.20	14.25	48.33	30.22	Clay Loam

### b. Soil chemical analysis

pH 1:2.5	EC dSm <sup>-1</sup> 1:5	CaCO <sub>3</sub> %	CEC C mole Kg <sup>-1</sup>	OM %	Soluble cations and Anions meq/100 g soil							
					Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>2</sub> <sup>-</sup>
7.15	1.3	2.53	33.5	1.3	1.82	0.23	2.38	1.27	0.0	0.91	1.9	1.89
Available macro-nutrients %				Available micro-nutrients ppm								
N	P	K	Zn	Fe	Mn	Cu						
0.47	0.25	0.95	3.1	4.8	7.3	5.2						

### c- Chemical analysis of sea water.

pH	EC (dSm <sup>-1</sup> )	Cations (meq/L)				Anions (meq/L)			
		Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>2</sub> <sup>-</sup>
8.62	50.0	393.5	20.4	26.2	118.3	0.53	9.6	253.2	295.1

The treatments were as follows: 1 – Salinity: Irrigation by three concentrations of diluted seawater (2000 4000 and 6000 ppm) more than the control treatment (irrigated by tap water 300 ppm). 2 – Spraying ascorbic acid with two concentrations (100, and 200 ppm). The control plants sprayed by the same quantity of distilled water. The experiment included four levels of salinity in combination with three concentrations of ascorbic acid i.e. 12 treatments in 6 replicates. Metallic pots 35 cm. in diameter and 50 cm. in depth (The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal) were used. Every pot contained 30 kg of air dried clay loam soil with 2 kg of gravel (Particles about 2-3 cm in diameter) at the bottom of the pot. Some chemical and physical properties and nutrients concentration of the experimental soil are presented in Tables 1a and 1b. The irrigation water with different salt concentration was prepared by using the sea water as a source. The analysis of sea water is shown in Table 1c.

Seeds of lupine (*Lupinus termis L.*) c.v. Balady were sown in the first of December, 2010-2011 plants were thinned twice, the 1<sup>st</sup> days after sowing and the 2<sup>nd</sup> two weeks later to leave three plants/pot. Calcium super phosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) and potassium sulfate (48.5 % K<sub>2</sub>O) 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 was started 30 days after sowing.

Samples were taken from every sub-treatment, cleaned, dried in electrical oven at 70 C, ground in a stainless steel mill. Minerals determination was done as the methods was described by Cottenie *et al.*

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(1982).

Data collected were subjected to the proper statistical analysis with the methods described by **Snedecor and Cochran (1980)**.

## RESULTS AND DISCUSSION

### Salt stress

#### *a)- Yield*

Data presented in Table (2) showed that salinity depressed the pods, straw, straw+ pods and seeds weight relative to the control plants but the depression of these traits showed its maximum values when plants subjected to the higher level of salinity (6000 ppm) markedly more than that with the other two levels of salinity. Slight differences in the mentioned characters of plants irrigated by solution contained 2000 or 4000 ppm. Gradual depressions in pods/straw, seeds/pods and seeds/straw ratios were detected with the increase in salt concentration in water of irrigation. Several investigations reported the adverse effect of salinity on legumes growth and yield (**Beltagi, 2008, Hussein, et al. 2008 and Overland, et al. 2009**).

Salinity is generally detrimental to plant growth through its adverse effects on plant metabolism that induces important modifications in gene expression. Such modifications may intern lead to accumulation of depletion of certain metabolites resulting in an imbalance in the levels of a relatively mall set of cellular proteins, which could increase, decrease, appear or disappear after salt treatment (**Kong-nngem, et al. 2005, Beltagi, 2008 and Hussein and Abo Bakr, 2018**). The inhibitory effects of salt stress on yield of lupine may be attributed to the effect on growth traits such as number and area of green leaves, number of pods/plant and weight of seeds/pod which caused from the effect of one or more from these reasons: Protein formation (**Overland, et al. 2009 Khali, et al. 2012, and Hussein, et al. 2012**), Photosynthetic activity and carbohydrate accumulation (**Khan, et al., 2008 and Hayat, et al. 2010**). Hormonal balance (**Hare, et al. 1998 and Shakirova, et al. 2003 and Bekheta and Hussein, 2014**), enzymes activity and oxidative defense (**Hussein and Orabi. 2008, Misra and Saxena, 2009 and Orabi, et al. 2018**) Water adjustment (**Tavallali, et al. 2009**) and /or mineral absorption and distribution (**Hussein, et al. 2006; Hussein, et al. 2008, Daei, et al. 2009 and Hussein, et al. 2015**).

Table (2): Effect of salinity on yield of lupine plant.

Salinity	Pods	Straw	Total	Seeds	Pods/straw	Seeds/pods	Seeds/straw
T.W.	3.32	2.46	5.78	2.19	1.35	0.66	0.88
2000 ppm	2.44	2.04	4.48	1.48	1.20	0.61	0.73
4000 ppm	2.46	2.14	4.59	1.20	1.15	0.49	0.65
6000 ppm	1.44	1.14	2.59	0.57	0.79	0.40	0.54
LSD at 5 %	0.354	0.664	1.67	1.040	-----	-----	-----

T.W. = Tap water (250 ppm)

#### *b)-Mineral*

Data in Tables (3 and 4) revealed the fluctuated response in the P, K, and Ca concentration as well as Na: Ca and Ca (Na+ K) ratio as results of salt stress. Meanwhile, Na concentration and Na: K ratio increased parallel to the increase of salts concentration in diluted sea water used in irrigation.

Several studies have shown a close relationship between salt concentration and its effect on mineral concentration. **Li, et al. (2010)** noticed that the content of Na ion increased, while K ion decreased with increasing salinity and pH, and this suggesting competitive inhibition between absorptions of Na and K ions. **Ashraf and Ahmad (2000)** revealed that the salt-tolerant and salt-sensitive lines of cotton did not differ in leaf or root Na<sup>+</sup> concentrations. The salt-sensitive lines accumulated more chloride ions in the leaves than all the three salt-tolerant lines at the highest salt level. The salt-tolerant lines had higher concentrations of K, Ca<sup>2</sup> ions, K+/Na- ratio and nitrogen in the leaves than those of the salt-sensitive lines at the highest NaCl concentration, whereas no relationship could be drawn between salt tolerance and tissue

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phosphorus concentration. The seed oil content of salt-tolerant lines was slightly higher than that of the salt-sensitive lines.

Table (3): Effect of salinity on nutritional status of lupine straw.

Salinity ppm	Macro-nutrients %						Ratios			
	N	P	K	Mg	Ca	Na	Na:K	Na:Mg	Na:Ca	Ca:(Na+K)
T.W.	2.47	0.1093	1.35	0.62	2.20	0.91	0.67	1.47	0.41	0.97
2000	1.85	0.0837	1.13	0.41	1.73	0.99	0.88	2.42	0.57	0.82
4000	2.31	0.1066	1.18	0.56	2.13	1.09	0.92	1.95	0.51	0.94
6000	1.91	0.0082	1.76	0.43	2.16	2.08	1.18	4.83	0.96	0.56

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Table (4): Effect of salinity on nutritional status of lupine seeds.

Salinity ppm	Macro-nutrients %						Ratios			
	N	P	K	Mg	Ca	Na	Na:K	Na:Mg	Na:Ca	Ca:(Na+K)
T.W.	6.87	0.27	2.86	0.39	1.47	0.97	0.34	2.49	0.66	0.38
2000	6.63	0.29	2.50	0.35	1.44	0.51	0.20	1.46	0.35	0.48
4000	5.30	0.26	2.50	0.27	1.37	0.46	0.18	1.70	0.34	0.46
6000	5.13	0.20	2.40	0.26	1.15	0.43	0.18	1.65	0.37	0.41

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Low levels of potassium ions relieved sodium ions toxicity, but low levels of Na<sup>+</sup> enhanced K<sup>+</sup> toxicity. Tissue concentrations of Na<sup>+</sup> were reduced by Ca<sup>2+</sup> and K<sup>+</sup> in the rooting medium, and tissue concentrations of K<sup>+</sup> were enhanced by Ca<sup>2+</sup> and K<sup>+</sup> (Kinrade, 1999). The most significant response of lupine plants to excess of NaCl is the increase of leaves sucrose content, which is partially due to SS activity which increase under salinity; Fernandes, et al. (2004).

Adverse effects of salt stress may be due to the depression on photosynthetic rate and carbohydrate metabolism as found by Fernandez, et al. (2004) who showed the decrease in glucose content as salt increased up to 150 mMNaCl. While, Keutgen, et al. (2008) emphasized that salt stress affected mainly the protein building. Salinity affected the absorption, translocation, distribution and adjustments of water as detected by Maggio, et al. (2006) on eggplants. Antioxidants content and activity which affected the oxidative defense by the increase of salts in the zone plant (Athar, et al. 2008). Kinrade, et al. (1999) suggested that the depression on growth and yield may be due to the disturbance in mineral status. However, Hare, et al. (1997) and Hussein and El-Greatly (2007) related this phenomenon to the disturbance in growth hormones. These effects might be altered the dry matter accumulation rates and this intern affected the translocation of metabolites from source to sink which reflected in the weight of seeds (Salah, et al. 2009).

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#### Ascorbic acid

##### a)- Yield

Ascorbic acid application led to increase in the pod, straw, total and seeds yields. The high increment in pods weight and seeds to straw ratio were by using 100 ppm ascorbic acid but the increment in straw, total, seeds weight and seeds/pods and seeds/straw ratios, were obtained by application of 200 ppm as shown in Table (5). Shetewie (2007) indicated that plants sprayed with ascorbin and irrigated with water gave 174% fresh weight and 129% dry weight soybean of the control. Soybean plants sprayed with ascorbin yielded 159% as compared with non-sprayed plants. Foyer (1993) and Beltagy (2008) stated that Ascorbate in plants occurs in the cytosol, chloroplasts, vacuoles, mitochondria and cell wall. The concentration in chloroplast can be high (up to 50mM in spinach) and is probably related to its central role in photosynthesis. Abd El-Bakey, et al. (2008) on wheat concluded that spraying AsA improved growth and yield and enhancing the oxidative defense. Talaat, et al. (2003) on sweet pepper, mentioned that AS counteracted the suppressive effect of the high salinity levels on seedlings growth. Talaat, et al. (2003) mentioned that the interaction showed a marked decrease in concentration of Na, while, N, P and K percentages were increased.

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Table (5): Effect of Ascorbic acid on yield of lupine plant.

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Ascorbic acid	Pods	Straw	Total	Seeds	Pods/straw	Seeds/pods	Seeds/straw
T. W.	1.67	1.38	3.06	0.99	0.92	0.44	0.54
100 ppm	2.33	2.14	5.00	1.70	0.92	0.72	0.79
200 ppm	2.17	2.31	5.03	1.75	1.07	0.81	0.75
LSD at 5 %	0.49	0.199	N.S	0.135	-----	-----	-----

Tap Water 250 ppm

#### b)-Mineral

Data in Tables (6 and 7) for both straw and seeds) showed that foliar ascorbic application alleviated deleterious effects of salinity stress on growth and nutrient accumulation. The highest values when plants sprayed by 100 ppm AsA. The detrimental effects of salinity on nutrient accumulation in the stressed plants have been previously reported by several workers (Hammad, 1979; Nour, et al. 1989 and Mohamed, 2003). Exogenous supply of AsA enhanced the concentration of N, P and K in stressed plants in comparison with the control plants. These increases in N, P and K may be attributed to the positive effect of AsA on root growth which consequently increased the absorption of different nutrients and alleviating the harmful effects of salinity. Similar results were previously recorded by Hanafy-Ahmed et al., (1995) on wheat and faba bean plants, Talaat (1995) on spinach and lettuce plants, Tarraf, et al. (1999) on lemongrass plants; Singh, et al. (2001) on senna plants; and Neveen Shawky (2003) on sweet pepper plants. AsA is an important primary metabolite in plants that functions as an antioxidant, an enzyme cofactor and a cell signalling modulator in a wide array of crucial physio-logical processes, including biosynthesis of the cell wall, secondary metabolites and phytohormones, stress tolerance, photoprotection, cell division and growth (Wolucka, et al., 2005).

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Table (6): Effect of Ascorbic acid on nutritional status of lupine straw.

Ascorbic acid	Macro-nutrients %						Ratios			
	N	P	K	Mg	Ca	Na	Na:K	Na:Mg	Na:Ca	Ca:(Na+K)
T.W.	2.14	0.1103	1.23	0.40	2.04	1.27	1.03	3.18	0.62	0.82
100	2.13	0.0932	1.24	0.44	1.96	1.13	0.91	2.57	0.58	0.78
200	2.19	0.0800	1.15	0.55	2.08	1.40	1.22	2.55	0.67	0.82

Comment [F66]: Insert year and place of study.

Table (7): Effect of Ascorbic acid on nutritional status of lupine seeds.

Ascorbic acid	Macro-nutrients %						Ratios			
	N	P	K	Mg	Ca	Na	Na:K	Na:Mg	Na:Ca	Ca:(Na+K)
T.W.	5.93	0.25	2.62	0.35	1.39	0.65	0.25	1.86	0.47	0.43
100	5.95	0.27	2.62	0.32	1.37	0.60	0.23	1.88	0.44	0.43
200	6.08	0.25	2.45	0.28	1.32	0.53	0.22	1.89	0.40	0.44

### Salt stress X Ascorbic acid

#### a)- Yield

Data presented in Table (8) indicated that significant response of pods, straw and seeds yield to the interactive effect of AsA and salt stress. Control only revealed that total yield was not significant responded to this interaction. The highest values of straw, pods, seeds and total yield were observed when plants sprayed by 100 ppm AsA and irrigated regularly with fresh water but the lowest values on the opposite, when lupine plants irrigated by 6000 ppm. Shelling percentage increased as increase in concentration of AsA under different concentration rate of salts but the reverse was true under irrigation with water contains 6000 ppm salts. M'rah, et al. (2007) stated that the induced biosynthesis of the antioxidants ascorbic acid and  $\alpha$ -tocopherol (ATOF) appeared to be integrated into a network of reactions controlling the levels of reactive oxygen species which affected by salt stress. Xu, et al. (2008) noticed that, however, root applied AsA counteracted the adverse effects of salt stress on the growth of cv. S-24 only, particularly at

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100 mg L<sup>-1</sup>AsA level. AsA-induced enhancement in growth of salt-stressed plants of S-24 was associated with enhanced endogenous AsA level and CAT activity, and higher photosynthetic capacity, and accumulation of K<sup>+</sup> and Ca<sup>2+</sup> in the leaves of wheat.

Table (8): Effect of salinity and ascorbic acid on yield of lupine plants

Salinity ppm	Ascorbic acid ppm	Pods	Straw	Total	Seeds	Pods/straw	Seeds/pods	Seeds/straw
Tap Water	0	2.53	2.05	4.58	1.53	1.23	0.61	0.75
	100	3.97	2.76	6.73	2.68	1.44	0.65	0.94
	200	3.47	2.57	6.04	2.45	1.39	0.71	0.95
2000 ppm	0	2.09	1.53	3.62	1.20	1.37	0.57	0.78
	100	2.64	2.07	4.71	1.56	1.28	0.59	0.75
	200	2.60	2.51	5.11	1.67	1.04	0.64	0.67
4000 ppm	0	2.07	1.95	4.02	1.23	1.06	0.59	0.63
	100	2.71	2.18	4.89	1.24	0.80	0.46	0.57
	200	2.59	2.28	4.87	1.70	1.14	0.66	0.75
6000 ppm	0	1.23	1.21	2.44	1.01	1.02	0.82	0.84
	100	2.12	1.54	3.66	1.40	1.38	0.66	0.91
	200	2.21	1.89	4.10	1.17	1.17	0.52	0.61
LSD at 5 %		0.98	0.40	N.S	0.171	-----	-----	-----

Maggio, *et al.* (2006) reported that salinity decreased stomatal conductance, plant water use, leaf total and osmotic water potentials. In addition, reduced leaf area, plant dry mass yield, fruit yield and fruit size. AsA treatments reduced stomatal conductance, but had a negative effect on plant growth and yield regardless the irrigation treatment. Consistent with other reports, AsA reduced the stomatal conductance but did not seem to improve commercial yield and total dry matter in saline environment. Meanwhile, significant synergistic effect between NaCl (40 mM) and ascorbic acid treatment increased the contents of chlorophyll a and chlorophyll stability index in leaves of chick pea plants. The total number of protein bands/lane did not change under the low (20 mM) NaCl concentration but was dramatically reduced by the high (40 mM) NaCl treatment. Also, the sum of optical densities of protein bands was inhibited by the two levels of NaCl, but was induced by 10.68% by the added ascorbic acid at 20 mM NaCl and by 21.39% at 40 mM NaCl. Six different polypeptides of molecular weights 146.28, 117.98, 51.55, 49.6, 44.49 and 38.34 were completely disappeared under NaCl stress (40 mM). These bands reappeared in response to the application of ascorbic acid treatment. Moreover, the optical density of every individual protein band was induced by ascorbic acid under the low NaCl concentration. The results indicate synergistic interaction between salinity stress and ascorbic acid for the sake of salt resistance in chick pea plants (Beltagi, 2008). Nevertheless, a supply of exogenous AsA increased the nodule AsA+dehydro ascorbate (DHA) pool in comparable with water-stressed nodules without ASC, and significantly modulated the response to water stress of the unspecific guaiacol peroxidase in leaves and nodules.

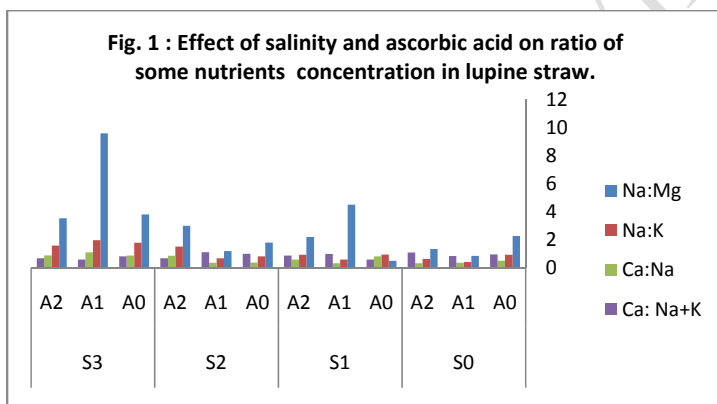
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Table (9): Effect of salinity and ascorbic acid on nutritional status of lupine straw

Salinity ppm	Ascorbic acid ppm	Macro-nutrients %					
		N	P	K	Mg	Ca	Na
Tap . W	0	2.38	0.098	1.504	0.611	2.734	1.398
	100	2.48	0.132	1.363	0.683	1.650	0.599
	200	2.56	0.098	1.175	0.558	2.213	0.751
2000 ppm	0	2.02	0.123	1.128	0.532	1.313	1.075
	100	1.69	0.119	1.178	0.155	1.875	0.699
	200	1.84	0.119	1.081	0.537	1.988	1.183
4000 ppm	0	1.98	0.111	1.222	0.419	1.988	0.753
	100	2.66	0.115	1.175	0.671	2.213	0.806
	200	2.52	0.094	1.128	0.574	1.980	1.720

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6000 ppm	0	2.20	0.082	1.081	0.463	2.138	1.881
	100	1.69	0.082	1.222	0.252	2.175	2.419
	200	1.84	0.082	1.222	0.546	2.175	1.935



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However, AsA 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 AsA+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 (Zabalza *et al.* 2008).

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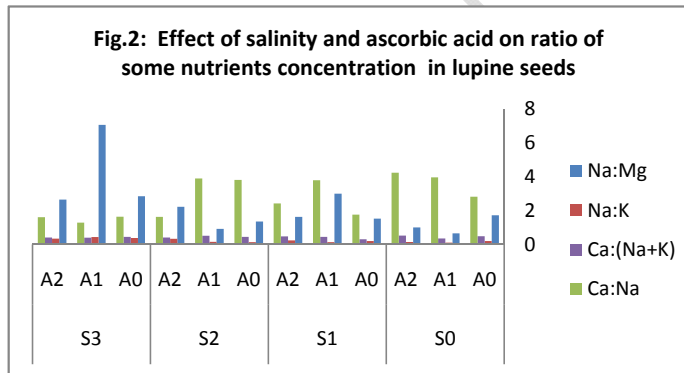
Table (10): Effect of salinity and ascorbic acid on nutritional status of lupine seeds.

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Salinity ppm	Ascorbic acid ppm	Macro-nutrients %					
		N	P	K	Mg	Ca	Na
Tap Water	0	6.6	0.24	3.2	0.38	1.83	0.65
	100	6.9	0.32	2.9	0.43	1.11	0.28
	200	7.1	0.24	2.5	0.35	1.48	0.35
300 ppm	0	5.6	0.30	2.4	0.33	0.88	0.50
	100	4.7	0.29	2.5	0.11	1.25	0.33
	200	5.1	0.29	2.3	0.34	1.33	0.55

4000 ppm	0	5.5	0.27	2.6	0.26	1.33	0.35
	100	7.4	0.28	2.5	0.42	1.48	0.38
	200	7.0	0.23	2.4	0.36	1.30	0.80
6000 ppm	0	6.1	0.20	2.3	0.31	1.43	0.88
	100	4.7	0.20	2.6	0.16	1.45	1.13
	200	5.1	0.20	2.6	0.34	1.45	0.90

**Comment [F74]:** Insert error bars on Fig. 2 to show significant differences between treatments.



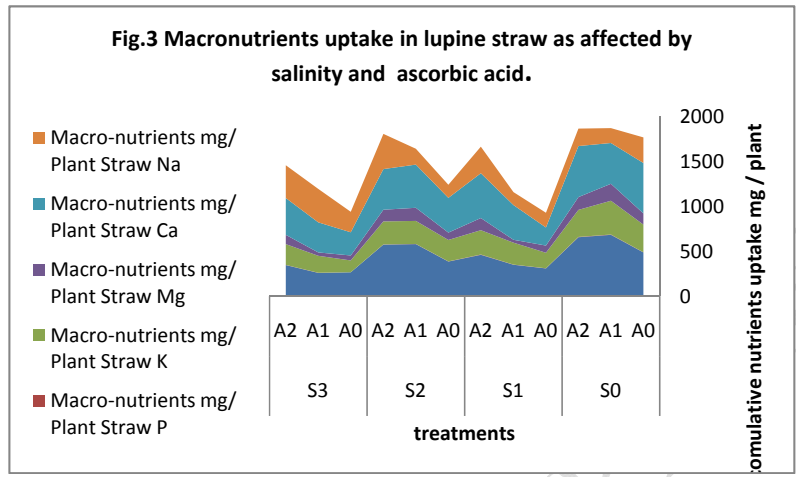
#### b)-Mineral

Both nutrient supply and nutrient balance are important factors for plant growth and development. Nutrient interactions consisting of vital influence on absorption, distribution and functioning exist. The interaction between nutrients can occur at the root surface or within the plant and might be due to: i) formation of precipitates and complexes between ions with different chemical properties, and ii) competition between ions with similar properties. Generally, increases N reduces cation uptake for both saline and non-saline treatments, Na/K, Na/Ca, Na/Mg and Ca/Na+K ratios were lowest in studied treatments as shown in Tables 9 and 10 as well as Fig. 1, 2, for concentration and Fig 3 and 4 for nutrients uptake in both straw and seeds. Slightly increase in Na, salinity was associated with an increase in straw N, and K with a decrease in P, Mg, and Ca, the same trend was noticed to the lupine seeds. Most interactions are complex i.e. a nutrient interacts simultaneously with more than one nutrient. (Robson and Pitman, 1983 and



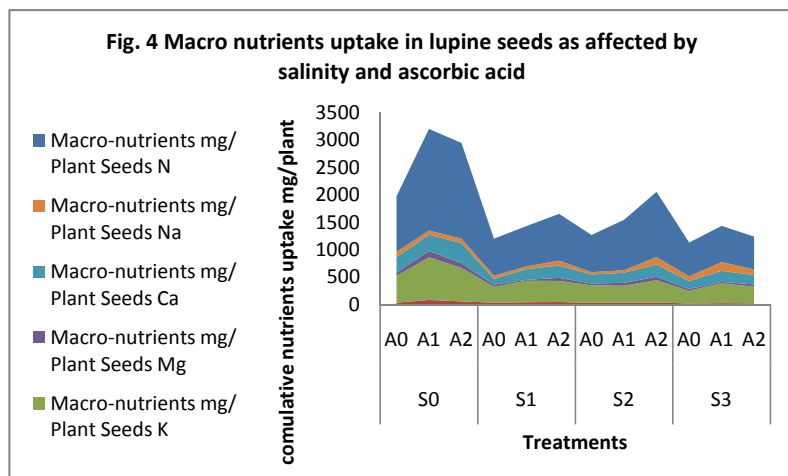
Fageria, 2001). These low ratios indicate that Ca, K and Mg transport was not impaired by Na under 2000 ppm 4000 ppm but impaired by Na under 6000 ppm and could disturb plant metabolism and reduce plant growth.

Comment [F75]: Use times new roman style.



Xu, *et al.* (2008) found that imposition of salt stress reduced the growth of both wheat cultivars by causing reduction in photosynthesis, and endogenous AsA level, and enhancing accumulation of Na<sup>+</sup> and Cl<sup>-</sup> coupled with a decrease in K<sup>+</sup> and Ca<sup>2+</sup> in the leaves and roots of both cultivars thereby decreasing tissue K<sup>+</sup>/Na<sup>+</sup> ratio. AsA-induced enhancement in growth of salt-stressed plants was associated with enhanced endogenous AsA level; higher photosynthetic capacity, and accumulation of K and Ca ions in the leaves. 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. They added that stress in both cultivars increased the antioxidant capacity, antioxidants pools (ascorbic acid, anthocyanins and superoxide dismutase) and selected minerals such as Na, Cl, K, N, P and Zn ions, as well as lipid peroxidation. Furthermore, salt stress increased the content 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.

Comment [F76]: Delete.



In salt-stressed fruits of cv. Elsanta, taste was significantly impaired. Athar, *et al.* (2008) Plant pre-conditioning with mild concentrations of ascorbic acid (AA) has been proved to be effective in protecting horticultural crops in hyperosmotic environment. The protective effect of AA seems to be associated to the control of stomatal aperture and, consequently, to a reduced water loss in response to hyperosmotic stress. They also added that salinity decreased stomatal conductance, plant water use, leaf total and osmotic water potentials. Salt stress increased dry matter content in all plant organs (leaf, root and fruit) and reduced leaf area, plant dry mass yield, fruit yield and fruit size. AA treatments reduced stomatal conductance, but had a negative effect on plant growth and yield regardless the irrigation treatment. Consistent with other reports, AA reduced the stomatal conductance but did not seem to improve total dry matter and yield matter in saline environment. Lu, *et al.* (2007).

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In conclusion, the obtained results in this study proved the beneficial effects of AsA on growth characters, N, K, and Ca counteracted the deleterious effects of salinity stress on the investigated parameters, help lupine plants to avoid Na toxicity and improved cell membrane stability and nutrient uptake under salinity stress and consequently the productivity of lupine plants under salinity stress conditions. These effects may be attributed to the protective role of AsA in plant cells from the oxidative stress induced by salinity. Therefore, we concluded that foliar application of AsA on lupine plants with 200 ppm at 30 and 60 days after sowing is the most effective treatment to enhance growth and yield of lupine plants under salinity stress conditions in comparison with the control plants. The addition of AsA could offer an economical and simple solution to problems in production of lupine plants in arid region soils caused by high salinity.

## REFERENCES

- Abd El-Baky, H.H.; Hussein, M.M. and Baroty, G.S. (2008). Algal extraction improve antioxidants defense Abilities and salt tolerance of wheat plant irrigated with sea water. *Electronic Journal of Environmental Agriculture and Food Chemistry*, 7(4): 281 –2832.
- Agati, G.; Biricolti, S.; Guidi, L.; Ferrini, F.; Fini, A. and Tattini, M. (2011). The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. *Original Research Article. Journal of Plant Physiology*, 168 Issue 3: 204-212.
- Ashraf, M. and Ahmad, S. (2000). Influence of sodium chloride on ions accumulation, yield components and fiber characteristics in salt-tolerant and salt-sensitive lines of cotton (*Gossypium hirsutum* L.). *Field Crops Research*, 66 Issue 2: 115-127.

Comment [F79]: Itallize all journal names.

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- Ashraf, M.; Apse, M.P. and Blumwald, E. (2002). Engineering salt tolerance in plants. *Curropin Biotechol.*, 13:145-150.
- Athar, H.R.; Khan, A. and Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environmental and Experimental Botany*, 63 Issues 1-3: 224-231.
- Beltagi, M.S. (2008). Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chick pea (*Cicer arietinum*L.) plants. *African Journal of Plant Science.*, 2 (10): 118-123.
- Bakheta, M.A. and Hussein, M.M. (2014). Uniconazole effect on endogenous hormones, proteins and proline contents of barley plants (*Hordium vulgare*) under salinity stress(NaCl). *Bioscience J.*, 6(1): 39-44.
- Bernstein, N.; Shores, M.; Xu, Y. and Huang, B. (2010). Involvement of the plant antioxidative response in the differential growth sensitivity to salinity of leaves vs roots during cell development. *Original Research Article. Free Radical Biology and Medicine*, 49 Issue 7: 1161-1171.
- Brugnoli, E. and Lauteri, M. (21991). Effects of Salinity on Stomatal Conductance, Photosynthetic Capacity, and Carbon Isotope Discrimination of Salt-Tolerant (*Gossypium hirsutum L.*) and Salt-Sensitive (*Phaseolus vulgaris L.*) C(3) Non-Halophytes. *Plant Physiol.*, 95(2):628-635.
- Costa, J.H.; Jolivet, Y.; Sauder, MP.; Orelleno, E.G.; Lima, MG.; Dizengremel, P. and Melo, D.F. (2007). Alternative oxidase regulation in root of *Vigna unguiculata* cultivars differing in drought/salt tolerance. *J. Plant Physiology*, 184: 718-727.
- Cottenie, A.; Verlo, M.; Kiekeus, L.; Velghe, G.; Camerlynck, (1982). R. *Chemical Analysis of plants and soils*. Laboratory of Analytical and Agrochemistry State University, Ghent-Belgium. 1982.
- Daei, G.; Ardekani, M.R.; Rejali, F.; Teimuri, S. and Miransari, M. (2009). Alleviation of salinity stress on wheat yield, yield componen. *Journal of Plant Physiology*, 166, Issue 6: 617-625.
- Fernandes F.M.; Arrabaça, M.C. and Carvalho L.M.M. (2004). Sucrose metabolism in *Lupinus albus L.* under salt stress. *Biologia Plantarum*, 48(2): 317-319.
- Foyer, C.H. (1993). Ascorbic acid: Alscher, R G.; Hess, J.L. eds. *Antioxidants in Higher Plants*. Boca Raton CRC press: 31-58.
- Hafsi, C.; Romero-Puertas, M.C.; Gupta, D.K.; Del Río, L.A.; Sandalio, L.M. and Abdelly, C. (2010). Moderate salinity enhances the antioxidative response in the halophyte *Hordeum aritimum L.* under potassium deficiency. *Original Research Article. Environmental and Experimental Botany*, 69 Issue 2: 129-136.
- Hayat, S.; Hasan, S.A.; Yusuf, M.; Hayat, Q. and Ahmad, A. (2010). Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiata*. *Environmental and Experimental Botany*, In Press, Corrected Proof, Available online 19 March
- Hussein, M.M.; Abo lilla. B. H.; Mohamed, S. and El-Liethy, S.. (2012). Effect of irrigation by salt water using Stroganov solution on anatomical structure of jatropha plants. *Australian J. of Basic and Applied Sci.* 8(1):491-496.
- Hussein, M.M. and Oraby, S.H. (2008). Growth and antioxidant enzymes activity in onion plants as affected by thiamine and salinity. *Plant Nutrition Management under Water Stress Conditions*. 17th Inter. Symposium of CIEC. Nov., 2008. NRC, Cairo, Vol. II: 260-278.
- Hussein, M.M.; Abd El-Hady, M.T.A. and El-Nagar, H.M. (2008a). Growth and biochemical changes in lupine plant grown under saline condition. *Egypt. J. of Agron.*, 30 (2): 295-308.
- Hussein, M.M.; Mehana, H.M.; Zaki, S. and Abd El-Hadi, N. (2014c). Influences of salt-stress and foliar fertilizers on growth chlorophyll and carotenoids of jojoba plants. *Middle East Journal of Agric.*, 3(2):Research.221-226.
- Hussein, M.M. and Safi -Naz S. Zaki, (2013). Influence of water stress on growth and photosynthetic pigments of some Fenugreek Varieties. *Original Articles. Journal of Applied Sciences Research*, 9(8): 5238-5245, 2013.
- Hussein, M.M.; Youssef, R.A. and Nesreen H. Abo-Bakr (2014). Influences of potassium foliar fertilization and irrigation by diluted seawater on growth and some chemical constituents of cotton. *IJSR*, 3(11):3127-3134.
- Hussein, M. M. and N.F. Abd El Hady (2014a). Growth and photosynthetic pigments responses of durum wheat varieties to rrigation by diluted sea water. *International Journal of Science and Research (IJSR)*, 3(12):1656-1663.

Hussein, M.M.; Bekheta, M.A. and Safi-naz S. Zaki (2014b). Influence of uniconazole on growth characters, photosynthetic pigments, total carbohydrates and total soluble sugars of *Hordium vulgare* L plants grown under salinity stress. *Intr. J. of Sci. and Res. (IJSR)*, 3 Issue 11:2208-2214.

Hussein, M.M., El-Saady, A.M. and Nesreen H. Abo-Talb (2015). Castor bean plants response to phosphorus sources under irrigation by diluted seawater. *Intr. J. of Chem. Tech. Res.*, 8(9): 261-271

Hussein, M.M.; Shaaban, M.M. and A.M. El-Saady, (2008). Response of cowpea plants grown under salinity stress to PK-foliar application. *American J. of Plant Physiol.*, 3(2): 81–88.

Hussein, M.M., El-Saady, A.M. and Nesreen H. Abo-Talb (2015). Castor bean plants response to phosphorus sources under irrigation by diluted seawater. *Intr. J. of Chem. Tech. Res.*, 8(9): 261-271.

Keutgen, A.J. and Pawelzik, E. (2008). Quality and nutritional value of strawberry fruit under long term salt stress. *Food Chemistry*, 107 Issue 4: 1413-1420.

Khalil. S.E, Hussein, M.M. and da Silva, J.T. (2012). Roles of antitranspirants in improving growth and water relations of *Jatropha curcas* L. grown under water stress conditions. *Plant Stress*, 6(1): 49-54.

Khan, W.; Prithiviraj, B. and Smith, D.L. (2008). Nod factor [Nod BjV (C<sub>18:1</sub>, MeFuc)] and lumichrome enhance photosynthesis and growth of corn and soybean. *Journal of Plant Physiology*, 165 Issue 13: 1342-1351.

Khodary, S.E.A. (2004). Effect of NaCl salinity on improvement of nitrogen metabolism and some ions uptake in lupine plants subjected to Gamma Irradiation. *International Journal of Agriculture & Biology*, 06 (1):1–4.

Kinraide, T.B. (1999). Interactions among Ca<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup> in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. *Journal Experimental Botany*, 50: 1495-1505.

Kong-ngem, K.; Dadurang, S.; WongKham, C.; Bunnag, S. and Koslttrakum, M. Three seedlings. *Sci. Asia*, 31: 403-498.

Li, R.; Shi, F. and Fukuda, K. (2010). Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). *Environmental and Experimental Botany*, 68 Issue 1: 66-74.

Maggio, G. Raimondi, A. Martino and Pascale, S. De. (2006). Effects of ascorbic acid applications on eggplants response to salinity. *ISHS Acta Horticulturae*, 747: VIII International Symposium on Protected Cultivation in Mild Winter Climates: Advances in Soil and Soilless Cultivation under Protected Environment 2006.

Misra, N. and Saxena, P. (2009). Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science*, 17 Issue 3:181-189.

M'rah, S.; Ouerghi, Z.; Eymery, F.; Rey, P.; Hajji, M.; Grignon, C. and Lachaâl, M. (2007). Efficiency of biochemical protection against toxic effects of accumulated salt differentiates *The llungiella halophila* from *Arabidopsis thaliana*. *Journal of Plant Physiology*, 164 Issue 4: 375-384.

Øverland, M.; Sørensen, M.; Storebakken, T.; Penn, M.; Krogdahl, A. and Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*) - Effect on growth performance, nutrient digestibility, carcass composition, guthealth, and physical feed quality. *Aquaculture*, 288 Issues 3-4: 305–311.

Salah, I.B.; Albacete, A.; Andújar, C.M.; Haouala, R.; Labidi, N.; Zribi, F.; Martinez, V.; Pérez-Alfocea, F. and Abdelly, C. (2009). Response of nitrogen fixation in relation to nodule carbohydrate metabolism in *Medicago ciliaris* lines subjected to salt stress, *Journal of Plant Physiology*, 166 Issue 5: 477-488.

Shakirova, F.M.; Sakhabutdinova, A.R.; Bezrukova, M.V.; Fatkhutdinova, R.A. and Fatkhutdinova, D.R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity *Plant Science*, 164 Issue 3: 317-322.

Shetewie, S.A. (2007). Improving growth and yield of salt stressed soybean by exogenous application of Jasmonic acid and ascorbin. *Intr. J. Agric. and Biol.*, 9(3): 473 – 478.

Simmoff, N. (1995). Antioxidant systems and plant responses to environment. In Simmoff, N. ed *Environment and Plant Metabolism: flexibility and acclimation*. Oxford Bios Scientific Publishers: 217-243.

Snedecor, G.W. and Cochran, W.G (1994). “Statistical Methods” 8<sup>th</sup> Ed. Iowa State Univ., Iowa, USA.

Tackholm, V. (1974). *Student Flora of Egypt*. 2nd Ed. P. 224. Cairo Univ. Press, Cairo, Egypt.

Takamatsu, S., Saito, K.; Sekine, T.; Ohmiya, S. and Kubo, H. (1990). Glycosidic alkaloids from *Lupin ushirsutus*. *Phytochem.*, 29: 3923–6.

- Talaat, N.B. (2003). Physiological studies on the effect of salinity, ascorbic acid and putresine of sweet pepper plant. Ph.D. Thesis, Fac. of Agric., Cairo Univ., Cairo, Egypt.
- Tavallali, V.; Rahemi, M.; Maftoun, M.; Panahi, B.; Karimi, S.; Ramezani, A. and Vaezpour, M. (2009). Zinc influence and salt stress on photosynthesis, water relations, and carbonic anhydrase activity in pistachio. *Scientia Horticulturae*, 123 Issue 2: 272-279.
- Xu, W.; Shi, W.; Ueda, A. and Takabe, T. (2008). Mechanisms of salt tolerance in transgenic *Arabidopsis thaliana* carrying a peroxisomal ascorbate peroxidase gene from barley. *Pedosphere*, 18 Issue: 486-495.
- Zabalza, A.; Gálvez, L.; Marino-D.; Royuela, M.; Arrese-Igor C and González, E.M. (2008). The application of ascorbate or its immediate precursor, galactono-1,4-lactone, does not affect the response of nitrogen-fixing pea nodules to water stress. *Journal of Plant Physiology*, 165 Issue 8: 805-812.

**Comment [F81]:** Use APA referencing style.  
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Write scientific names basing on Binomial system.

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