The Journal of Animal & Plant Sciences, 28(1): 2018, Page: 165-176
ISSN: 1018-7081

ALLEVIATION OF OXIDATIVE EFFECTS OF SALT STRESS IN WHITE LUPINE (*LUPINUS TERMIS* L.) PLANTS BY FOLIAR TREATMENT WITH L-ARGININE

S. A. Akladious and R. S. Hanafy

Biological and Geological Sciences Department, Faculty of Education, Ain Shams University, 1575 Cairo, Egypt
Corresponding Author E-mail: samiapola@yahoo.com

ABSTRACT

Salinization is one of the most important stress factors which reduce the growth and crop productivity of plants in various climatic regions. In this greenhouse study, the effects of 2.5 mM arginine as foliar spray on growth and some chemical constituents of lupine plants irrigated with three different levels of NaCl (75, 150 and 300 mM NaCl) were evaluated. The results indicated that the plants exposed to salinity stress exhibited a significant decline in growth parameters (shoot length, root length, number of leaves, fresh and dry weights of shoots and roots) and photosynthetic pigment content. On the other hand, salt stress caused significant increase in total free amino acids and total protein contents in comparison with control plants. In addition, proline content and lipid peroxidation increased with increasing concentrations of NaCl. Treatment with 2.5 mM arginine as foliar spray mitigated salt stress by inducing enzyme activities responsible for antioxidation, e.g., superoxide dismutase, ascorbate peroxidase, glutathione reductase, detoxification as well as improving all the above recorded parameters. Furthermore, treating the plants with 2.5 mM arginine alone or in combination with salt stress leads to differential expression of the genetic information in lupine plants, resulting in changes in gene products, including protein and isozymes profiles. These changes induced the synthesis of certain proteins and simultaneously decreased the expression of other protein sets. These findings confirm the effectiveness of spraying lupine plants with arginine on alleviating salinity stress in lupine plants.

Keywords: White lupine; Salinity; Arginine; Amino acids; Antioxidant enzymes.

INTRODUCTION

Salinity is one of the most serious problems that markedly affect growth and physiological attributes in crops that reduce crop production. Approximately, it was found that 6% of the world's land area and 20% of agricultural lands are already affected by salinity (FAO, 2009). Salinity disturbed some physiological and biochemical parameters in plants as it caused formation of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals that caused chlorophyll degradation, proteins injury, lipids of the membrane and nucleic acids which cause toxicity. Therefore, plants possess some strategies which scavenge ROS (Manai *et al*, 2014).

Arginine is an essential amino acid which is the precursor of polyamines synthesis including spermidine, spermine, and putrescine (Brauc *et al*, 2012). Polyamines (PAs) are polycationic nitrogenous found in some processes such as cell division, morphogenesis in phytochrome, plant hormone mediated action, growth and differentiation (Abd El-Monem, 2007). The treatment of arginine significantly increased the fresh and dry weights, certain growth regulators, chlorophylls and carotenoids contents in wheat (El-Bassiouny *et al*, 2008). Moreover, it was found that endogenous and exogenous arginine have important roles in different environmental stresses, such as salinization by overcoming oxidative damage through scavenging free radical species (Zhang *et al,* 2011).

White lupine (*Lupinus termis* L.) is one of the important medical plants and it is a source of nutrition according to some points of view. Lupine belongs to Fabaceae, its seeds contain a high level of protein (35-45%), oil content (10-15%) and minerals (Wiatr *et al,* 2007). Arginine was involved in the control of numerous cellular functions such as free radical scavenger and antioxidant activity and has been found to confer protection from numerous abiotic stresses (Zeid, 2004). According to role of polyamines in protection of stressed plants, the present study aimed to test the effect of arginine application, as precursor for Put. synthesis, in alleviation of salinity stress of lupine plants. Understanding the physiological and biochemical responses under different levels of salinity is imperative for efficient management of agronomic inputs (irrigation and nutrient).

MATERIALS AND METHODS

Plant Material: A pot experiment was carried out in the screen greenhouse of faculty of Education, Ain Shams University. Lupine seeds (*Lupinus termis* L.) were obtained from the Crop Institute, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Homogenous seeds were sterilized using 70% ethanol for

2 min. followed by 0.2% sodium hypochlorite (NaClO) for 3 min. then rinsed for 3 times with distilled H_2O_2. Seeds were sown in pots (25 cm in diameter) filled with 3.5 kg homogeneous loamy clay soil. Soil characteristics were sand 80 %; silt 15.5 %; clay 4.5 %; organic matter 0.45 with % pH 7.8; EC 0.4 dSm^{-1}. The experiment was carried out under environmental conditions (day length 12–14 hours, relative humidity (RH) 65- 70% and temperature 28–30°C). Each pot was planted with 10 seeds and thinned to 7 after one-week of sowing. The pots were divided into three groups; the first group (5 pots) was irrigated with tap water (holding capacity is 80%) as control. The 2nd group was irrigated with tap water until the 3-leaf stage appeared then they were divided into three sets (5 pots/set) and irrigated with sodium chloride concentrations (75, 150 and 300 mM). The 3rdgroup subdivided into four sets (5 pots/ set). The first set was irrigated with tap water and then sprayed with arginine (2.5 mM). The 2nd, 3rd and 4th sets of pots were irrigated with NaCl concentrations (75, 150, 300 mM) and then sprayed with 2.5 mM arginine. Every treatment consisted of five replicates distributed in a completely organized design. The pots were irrigated with equal volumes of the various salinity concentrations, 3 times with saline solutions and one with tap water. Spraying was carried out twice with two weeks interval. When the developed plants reached 11 weeks from sowing, plants were taken from each treatment and analyzed for certain morphological characters (shoot length, root length, number of leaves and fresh and dry weights of shoots and roots) in addition to certain measures.

Photosynthetic pigment contents: Chlorophyll a, Chlorophyll b and carotenoids contents were determined by using the methods of Lichtenthaler (1987). Fresh leaves were ground in 80% acetone. Extracts were centrifuged at 3000g for 5 minutes and the absorbance was measured at 663.2, 646.8 and 470 nm by using colourimeter VEB Carl Zeiss. The pigment contents were calculated as mg g^{-1}FW.

Total free amino acids: Total free amino acids were described by Moore and Stein (1954). Leaves were homogenized in 80% ethanol, boiling for 10 min. and then centrifuged at 2000g for 10 min. 0.05 ml of the supernatant, and 2 ml of ninhydrin were mixed and boiled. The mixture was cooled in room temperature and was made up to 10 ml using distilled water. The color developed read at 570 nm using pure glycine as a standard.

Estimation of total protein: Total protein was described by Lowry *et al.* (1951). Known weights of dry leaves (0.5 g) were ground using 10 ml distilled water then transferred to test tubes. 1 ml of trichloroacetic acid (TCA) (10%) was added, the tubes were placed in an ice bath for 10 minutes. The supernatant was centrifuged at 5000 g for 10 min at 4 °C. 20 ml sodium hydroxide (0.1 N) was added to the filtrate to dissolve the protein. 5 ml of freshly prepared copper solution (100 ml of (0.1 N) sodium chloride, 2 g of anhydrous sodium carbonates, 1 ml of (2.7%) sodium tartrate and 1 ml of (1%) copper sulphate) was added to 0.1 ml of the protein extract. The tubes were left for 15 min then measured in a spectrophotometer system at 570 nm. The protein value was measured and presented by using bovine serum albumin (BSA) as standard based on mg/g FW.

Determination of proline content: The proline content was described by Bates *et al.* (1973). 0.5 g of dry leaves was ground in 10 ml of 3% sulphosalicylic acid and centrifuged at 10.000g for 10 min. 2 ml of the supernatant was mixed with 2 ml of freshly prepared acid ninhydrin. The mixture was incubated in a water bath at 90ºC for 30 min. Reaction was terminated in cooled ice bath. The reaction was extracted by using 5 ml of toluene and vortex and the process was done for 15 seconds and lasted for 20 min in the dark at room temperature to separate the toluene and aqueous phases. The toluene phase was collected and the absorbance of the developed color was read at 520 nm using proline as standard and calculated to µg g^{-1} fresh weight.

Lipid peroxidation: Lipid peroxidation was determined by estimating malondialdehyde (MDA) production as described by Heath and Packer (1968). Leaves samples were homogenized in 0.1% trichloroacetic acid (TCA) and centrifuged at 10.000 g for 20 min. 1 ml of the supernatant was added to TCA (20%) mixed with (0.5 w/v) of TBA and 10 µl of butylated hydroxyl toluene (BHT) (4% in ethanol). The mixture was heated at 95ºC for 30 min. then cooled in ice bath and centrifuged at 10.000g for 15 min. The absorbance of the resulting supernatant was recorded at 532 and 600 nm. The nonspecific absorbance at 600 nm was subtracted from the 532 nm absorbance. The absorbance coefficient of TBARS was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Assay of enzymatic antioxidant: The shoots were ground in sodium phosphate buffer (pH 6.5) for SOD, APX and GR. The supernatant was used to measure the activity of the following enzymes:

Superoxide dismutase (**SOD; EC 1.15.1.1)** activity was assayed by monitoring the inhibition of photochemical reduction of nitrobluetetrazolium chloride (NBT). Absorbance was read at 560 nm according to Beauchamp and Fridovich (1971).

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was estimated according to the method of Nakano and Asada (1981). Enzyme activity was determined at the absorbance of ascorbate at 290 nm.

Glutathione reductase (GR; **EC 1.6.4.2)** activity was determined and based on the decrease in absorbance at 340nm due to the oxidation of NADPH to NADP according to the method of Foyer and Halliwell (1976).

Electrophoretic analysis of isozymes: The isozymes used were: peroxidase (POX) and polyphenol oxidase (PPO). Isozymes were described by Stegemann *et al.* (1985) and separated in 10% native-polyacrylamide gel electrophoresis. The staining gels were carried out according to Larsen and Benson (1970) for peroxidase and Sato and Hasegawa (1976) for polyphenol oxidase respectively. Gels were washed two or three times with tap water; fixed in ethanol: 20% glacial acetic acid (9:11 v/v) for 24 hours; and then photographed.

Protein electrophoresis: Electrophoretic protein profile of plant leaves were analyzed according to the method of Laemmli (1970) by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The molecular weights of protein were calculated by Gel-Pro analyzer program using the molecular weight marker.

Statistical analysis: The data was statistically analyzed using LSD at 5% level of probability according to SAS-Programme (1982).

RESULTS

Growth parameters: Data showed that the effect of salinity on lupine plant varied according to salinity level (Table 1). Irrigation of plants with the lowest concentration of NaCl (75 mM) caused significant increases in all growth parameters as compared with control plants. However, 150 and 300 mM salinity level caused significant reductions in the above mentioned parameters except dry weight of root in which the decrease was insignificant. The results also showed that, spraying lupine plants with arginine (2.5 mM) resulted in visible increases in all the above mentioned growth parameters as compared with control plants and the corresponding salinity levels. These increments were much more pronounced at the lowest concentration of salinity level (75 mM).

Changes in photosynthetic compounds: Irrigating lupine plants with the higher concentrations of saline water (150 & 300 mM) caused gradual and significant decreases in chlorophyll a, chlorophyll b and consequently total pigments contents as compared with control plants, while the lowest concentration of NaCl (75 mM) caused an opposite pattern of change (Table 2). However, 75 and 150 mM of saline water caused significant increases in carotenoid contents while 300 mM caused significant reduction as compared with control plants. The results in the same table also showed that, foliar spraying of lupine plants with arginine caused significant increases in chlorophyll a, chlorophyll b, carotenoids and consequently total pigments as compared with control and the corresponding salinity levels. The maximum increase in total pigments was observed in case of plants sprayed with arginine and grown under the lowest concentration of salinity stress.

Changes in osmolyte compounds:

Total free amino acids and protein contents: The obtained results showed that total amino acid contents increased significantly as compared to control plants (plants irrigated with non saline water) in response to irrigation with different salinity levels (Table 3). They increased gradually with increasing salinity levels. The percentages of increase were 108%, 117% and 127% as compared with control plant at 75, 150 and 300 mM NaCl, respectively. Meanwhile, data in the same table showed that, protein content of lupine shoots increased significantly with increasing salinity levels as compared with control plants. The magnitude of increase was reduced by increasing salinity level. The percentage of increases reached 125%, 113% and 101% at 75, 150 and 300 mM salinity level. Exogenous application of arginine under salinity levels caused significant increases in total free amino acids and protein contents as compared with control plants and the corresponding salinity levels.

Proline content: The proline content of leaves was significantly influenced by salinity levels. The free proline content was significantly enhanced in the stressed plants over control plants (Table 3). There was a considerable increase in free proline accumulation with increasing salinity levels as compared with control plants. A more pronounced increase was observed as less than 300 mM NaCl in comparison with the other treatments. Foliar spraying of lupine plants with arginine caused significant increases in proline content as compared with control plants and the corresponding salinity levels.

Changes in oxidative damage:

Lipid peroxidation: The oxidative damage was observed as malondialdehyde (MDA) content, which is a product of lipid peroxidation. The obtained results showed that different concentrations of NaCl caused gradual significant increases in lipid peroxidation of the leaves as compared with control plants (Table 3). These increases were gradual with corresponding salinity levels. It has been found in the present investigation that, arginine application significantly decreased the malondialdehyde contents under both normal and stress conditions.

Changes in enzymatic antioxidants: Changes in the activity of antioxidant enzymes are the consequence of oxidative stress. The effect of salt stress on SOD, APX and GR activities in leaves of lupine plant either without or with arginine treatment was assayed. The activities of

SOD, APX and GR of stressed plants increased at all concentrations of salinity stress when compared with those of the control plants (Fig. 1). The greatest activity of SOD was displayed at the highest level of NaCl (300 mM) as being compared with the unstressed control plants. Control plants had the minimum value for this enzyme. Arginine treatment caused a significant increase in SOD activity in contrast to salinity stress alone, indicating the higher efficiency of this antioxidant enzyme in the presence of arginine supplementation. The trends for APX and GR activities in the presence and absence of arginine in salt stressed plants were similar to those observed for SOD. Arginine treatment was more effective in increasing SOD activity than APX and GR activities.

Protein electrophoratic pattern: Seven protein bands with molecular weights ranging between 128.59 and 11.96 kDa were observed in lupine leaves (control). The total number of protein bands was increased from 7 bands in the control plants to 10, 11 and 13 bands in plants irrigated with 75, 150 and 300 mM NaCl, respectively (Table 4, Fig. 2).

Therefore, in the present study, salinity stress in general induced synthesis of a new set of protein bands (3 bands) at molecular weights 29.85, 11.28 and 7.66 KDa at all salinity levels, and at molecular mass 32.95, 26.34 and 9.22 KDa at the highest level of salinity (300 mM NaCl) only as compared with control plants. In addition, treating the plants with 150 mM NaCl caused the appearance of three new protein bands at molecular weights 56.33, 14.43 and 6.30 kDa, respectively. The results also showed the disappearance of protein bands at molecular weights 46.43 and 11.96 KDa at all salinity levels. These results indicate that the leaves of plants irrigated with different salinity levels characterized by appearance of certain new bands and the disappearance of other ones as compared with that of the untreated plants (Table 4).

Foliar spraying of lupine plants with arginine induced the appearance of two inducible protein bands at molecular weights 56.33 and 38.08 KDa and the disappearance of one protein band at molecular weight 46.43 as compared with control plants. Two protein bands with molecular weights 17.49 and 3.15 kDa were de novo synthesized in lupine leaves treated with arginine in combination with different levels of salinity. These bands disappeared in both control plants and plants exposed to salinity stress only.

Isoenzymes expression: The induction of new isozymes and the change in the isoenzymes profiles play an important role in the cellular defense against oxidative stress. Isoenzymes expression of lupine plants treated or non-treated with salt represented in (Tables 5, 6 and Figure 3).

Isoenzyme profiles of peroxidase in lupine leaves demonstrated five bands exhibited at Rf 0.47, 0.52, 0.62, 0.65 and 0.69 with different densities and intensities in untreated and salt treated plants. There were four polymorphic bands which present in some treatments and absent in the others. The band with Rf 0.52 was a unique band which characterizes the plants grown under the lowest salinity condition alone (75 mM NaCl). The band with Rf 0.62 was high in intensities and densities in salinized lupine leaves alone (300 mM NaCl) and in combination with arginine.

Polyphenol oxidase electrophoretic patterns are illustrated in Figure 3. Four bands with different intensities and densities were observed among the profiles of all treatments. One band was presented in all treatments (monomorphic band) at Rf 0.60. Two bands were presented in some treatments and absent in the others (polymorphic bands). The band which has Rf 0.50 was a unique band which characterize the plants grown under the lowest salinity condition alone (75 mM NaCl).

Table 1. Effect of foliar treatment of arginie on morphological criteria of lupine plants grown under control and salinity stress conditions.

Treatments	Shoot length (cm)	Root length (cm)	No. of leaves/ plant	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
Control	30.25	32.16	9.83	7.09	1.22	0.94	0.16
2.5 mM Arg	36.10[c]	40.23[c]	14.21[c]	9.24[c]	3.12[c]	1.24[c]	0.34[c]
75 mM NaCl	31.2[a]	33.46[a]	10.60[c]	7.44[a]	1.51[a]	1.07[c]	0.20[a]
150 mM NaCl	27.41[b]	29.32[b]	9.00[b]	6.34[b]	1.00[a]	0.83[b]	0.16[d]
300 mM NaCl	24.82[b]	28.51[b]	8.41[b]	4.81[b]	0.81[b]	0.58[b]	0.15[d]
75 mM NaCl + 2.5 mM Arg	38.61[c]	42.40[c]	14.82[c]	9.63[c]	4.16[c]	1.34[c]	0.53[c]
150 mM NaCl + 2.5 mM Arg	35.80[c]	39.83[c]	13.45[c]	8.01[c]	2.90[c]	1.14[c]	0.34[c]
300 mM NaCl + 2.5 mM Arg	33.43[c]	35.41[c]	12.23[c]	7.26[d]	1.78[c]	1.11[c]	0.22[c]
LSD at the 5 % level	0.88	0.99	0.46	0.29	0.22	0.05	0.03

[a]Significant [b]High significant decrease [c]High significant increase [d]Non-significant

Table 2. Effect of foliar treatment of arginine on the photosynthetic pigments (in milligrams per gram fresh weight) in leaves of lupine plants grown under control and salinity stress conditions.

Treatments	Chlorophyll a	Chlorophyll b	Chlorophyll (a+b)	Carotenoids	total pigments
Control	14.19	4.16	18.35	5.89	24.24
2.5 mM Arg	19.18[c]	6.33[c]	25.51[c]	8.18[c]	33.69[c]
75 mM NaCl	16.52[c]	4.85[c]	21.37[c]	7.62[c]	28.99[c]
150 mM NaCl	12.16[b]	3.58[b]	15.74[b]	6.58[c]	22.32[b]
300 mM NaCl	9.98[b]	2.39[b]	12.37[b]	4.94[b]	17.31[b]
75 mM NaCl + 2.5 mM Arg	22.09[c]	6.69[c]	28.78[c]	8.47[c]	37.25[c]
150 mM NaCl + 2.5 mM Arg	18.50[c]	5.81[c]	24.31[c]	8.16[c]	32.47[c]
300 mM NaCl + 2.5 mM Arg	17.04[c]	5.03[c]	22.07[c]	7.72[c]	29.79[c]
LSD at the 5 % level	0.75	0.27	1.02	0.24	1.24

[b]High significant decrease [c]High significant increase

Table 3. Effect of foliar treatment of arginine on total free amino acids, total protein, proline and lipid peroxidation contents of lupine plants grown under control and salinity stress conditions.

Treatments	Total free amino acids (mg/g FW)	Total protein (mg/g FW)	Proline (µg/ g FW)	Lipid peroxidation Total MDA (nmol/g FW)
Control	7.57	10.18	3.99	5.29
2.5 mM Arg	10.66[c]	13.20[c]	4.82[c]	4.15[b]
75 mM NaCl	8.18[a]	12.78[c]	5.20[c]	6.31[c]
150 mM NaCl	8.85[c]	11.47[c]	6.54[c]	7.84[c]
300 mM NaCl	9.61[c]	10.32[d]	8.19[c]	9.65[c]
75 mM NaCl + 2.5 mM Arg	15.54[c]	17.51[c]	9.92[c]	4.75[b]
150 mM NaCl + 2.5 mM Arg	11.03[c]	15.54[c]	12.99[c]	4.82[a]
300 mM NaCl + 2.5 mM Arg	9.84[c]	13.37[c]	15.18[c]	5.01[d]
LSD at the 5 % level	0.47	0.48	0.77	0.35

[a]Significant [b]High significant decrease [c]High significant increase [d]Non-significant

Table 4. Effect of foliar treatment of arginine on the protein patterns separated by SDS-PAGE of the leaves of lupine plants grown under control and salinity stress conditions.

Molecular weight (kDa)	Control	Arg (2.5 mM)	NaCl (mM)			NaCl (mM) +Arg (2.5 mM)		
			75	150	300	75+ Arg	150 + Arg	300 + Arg
128.59	+	+	+	+	+	+	--	+
114.4	+	+	+	+	+	+	+	+
76.34	+	+	+	--	+	+	+	+
70.68	--	--	--	+	+	+	+	--
65.51	+	+	+	+	+	+	--	+
56.33	--	+	--	+	--	--	--	--
52.15	--	--	+	--	+	+	+	+
46.43	+	--	--	--	--	--	--	--
38.08	--	+	+	--	+	+	+	+
32.95	--	--	--	--	+	+	+	+
29.85	--	--	+	+	+	+	+	--
26.34	--	--	--	--	+	--	--	+
19.52	+	+	+	+	--	+	+	--
17.49	--	--	--	--	--	+	+	+
14.43	--	--	--	+	--	+	+	+
11.96	+	+	--	--	--	--	+	+
11.28	--	--	+	+	+	+	+	--
9.22	--	--	--	--	+	+	+	--
7.66	--	--	+	+	+	+	--	+
6.30	--	--	--	+	--	--	--	--
3.15	--	--	--	--	--	+	+	+
Total no. of bands	**7**	**8**	**10**	**11**	**13**	**16**	**14**	**13**

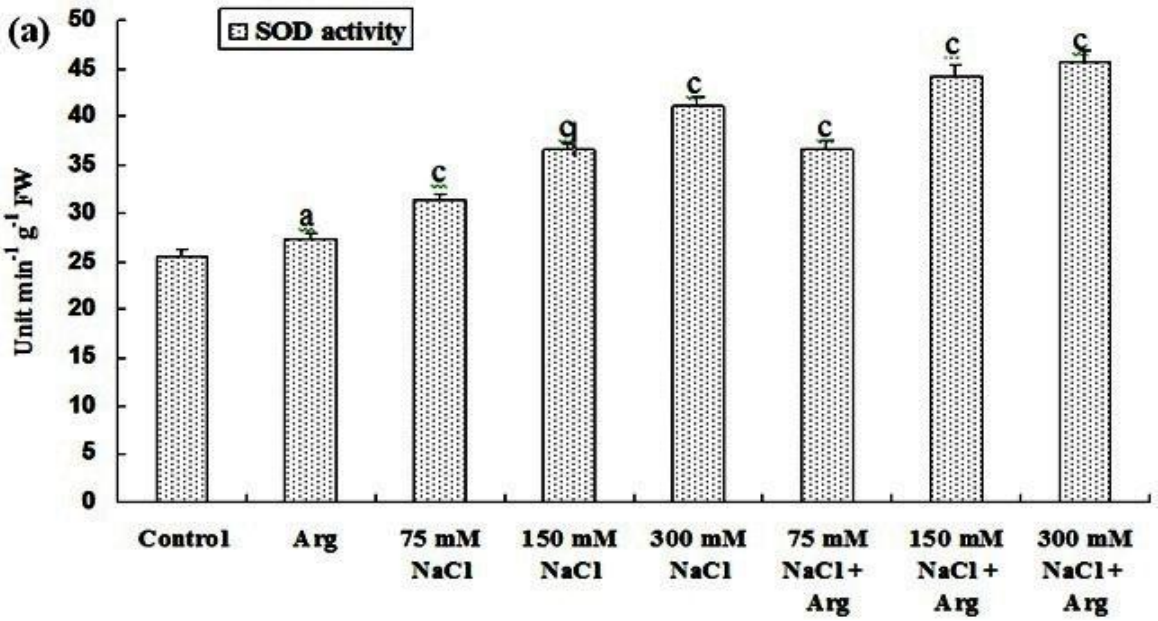
Table 5. Effect of foliar spraying of arginine on the intensity of two isozymes profiles, peroxidase (POX) and polyphenol oxidase (PPO) in lupine plants grown under salinity stress conditions.

Peroxidase (POX)								
Rf	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
0.47	0.38		0.21					
0.52			0.14					
0.62			0.11	0.16	2.11	0.89	1.95	2.39
0.65	0.99	2.39		0.12		0.63	2.32	
0.69	0.63		0.67					
Total	**3**	**1**	**4**	**2**	**1**	**2**	**2**	**1**
Polyphynol oxidase (PPO)								
0.44	0.53		0.35					
0.50			0.29					
0.60	0.92	2.18	0.26	0.31	1.30	0.84	2.24	1.41
0.64	0.72	1.26			0.97	0.69	1.77	1.22
Total	**3**	**2**	**3**	**1**	**2**	**2**	**2**	**2**

Lane 1 = 0 (control), Lane 2 = Arg (2.5 mM), Lane 3 = NaCl (75 mM), Lane 4 = NaCl (150 mM), Lane 5= NaCl (300 mM), Lane 6= 75 mM NaCl + 2.5 mM Arg, Lane (7) = 150 mM NaCl + 2.5 mM Arg, Lane (8) = 300 mM NaCl + 2.5 mM Arg

Table 6. Number and types of bands as well as the percentage of the total polymorphism generated by two isozymes, peroxidase (POX) and polyphenol oxidase (PPO).

Isozymes	Monomorphic Bands	Polymorphic		Total bands	Polymorphic %
		Unique	Non Unique		
POX	-	1	4	5	100%
PPO	1	1	2	4	75%



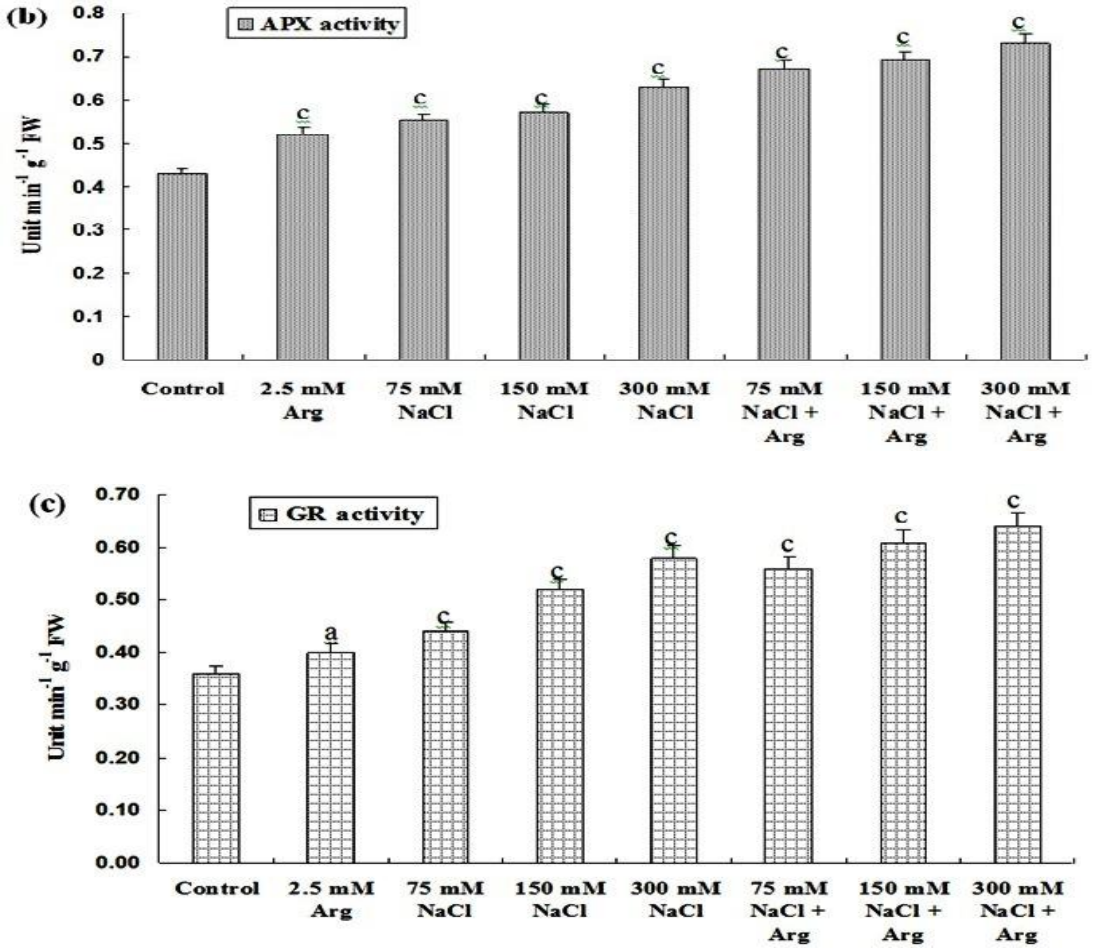


Fig. 1. Effect of foliar treatment of arginie on superoxide dismutase (a), ascorbate peroxidase (b), and glutathione reductase (c) activities of lupine plants grown under control and salinity stress conditions. Error bars represent the SE (n=3)

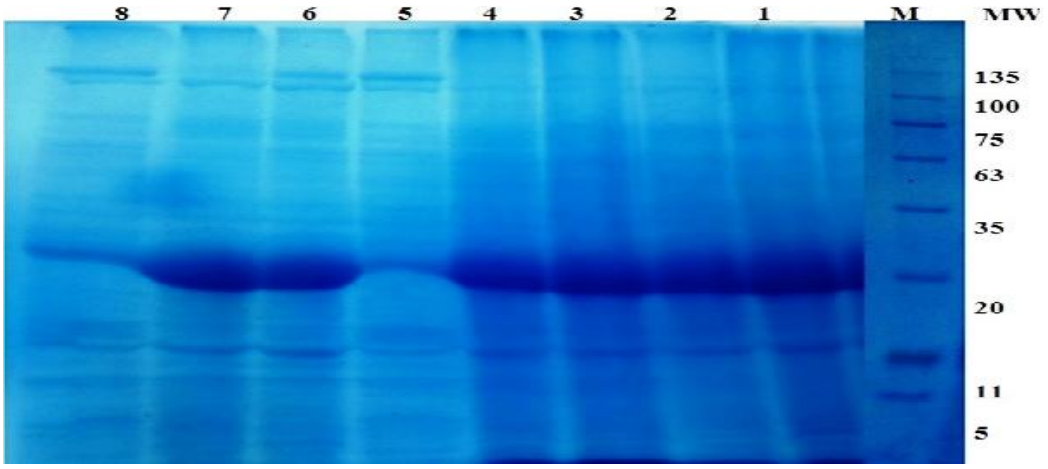


Fig. 2. Electrophoretic banding profiles of protein extracted from the leaves of lupine leaves in response to treatment with different concentrations of NaCl alone or in combination with arginine. M: Marker protein Lane 1 = (control), Lane 2 = Arg (2.5 mM), Lane 3 = NaCl (75 mM), Lane 4 = NaCl (150 mM), Lane 5= NaCl (300 mM), Lane 6= 75 mM NaCl + 2.5 mM Arg, Lane (7) = 150 mM NaCl + 2.5 mM Arg, Lane (8) = 300 mM NaCl + 2.5 mM Arg.

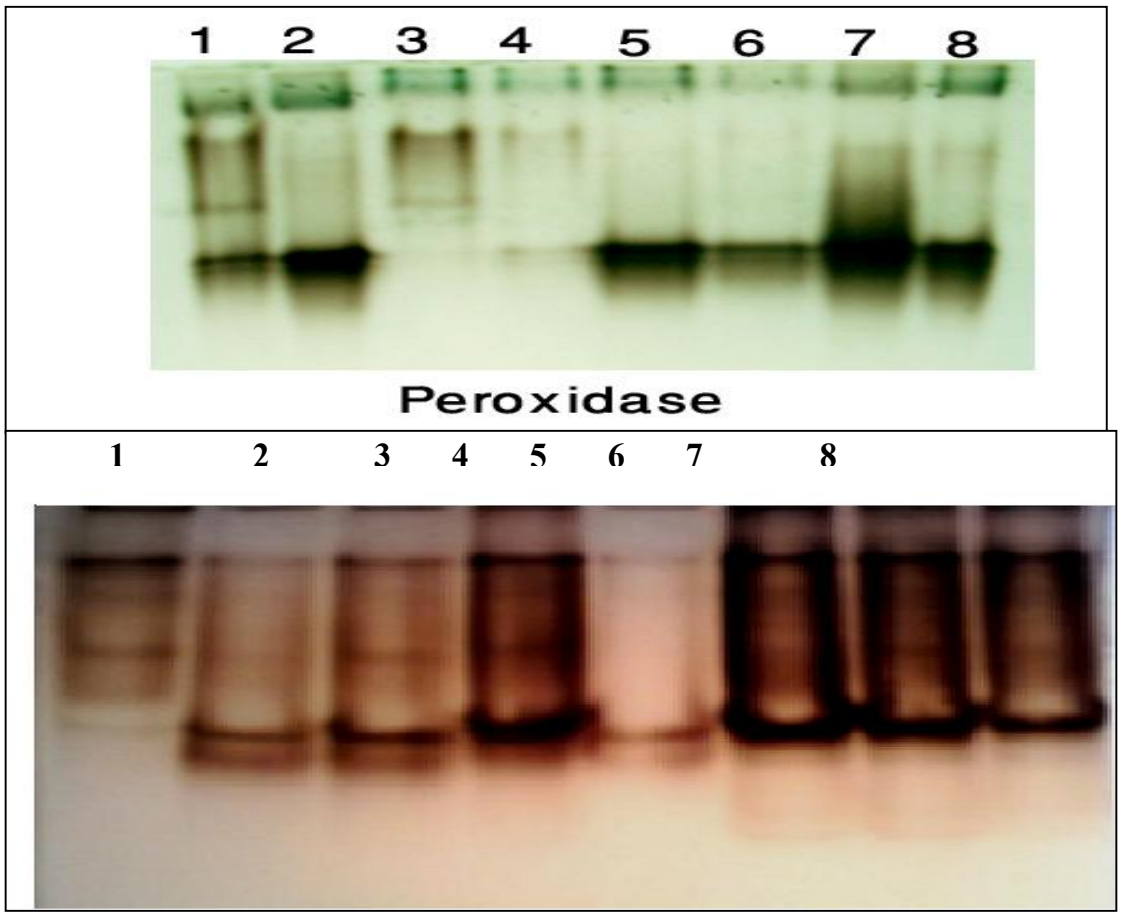


Fig. 3. Zymogram of two enzymes, peroxidase (POX) and polyphenol oxidase (PPO) of the leaves of lupine plants in response to treatment with different concentrations of NaCl alone or in combination with arginine. Lane 1 = (control), Lane 2 = Arg (2.5 mM), Lane 3 = NaCl (75 mM), Lane 4 = NaCl (150 mM), Lane 5= NaCl (300 mM), Lane 6= 75 mM NaCl + 2.5 mM Arg, Lane (7) = 150 mM NaCl + 2.5 mM Arg, Lane (8) = 300 mM NaCl + 2.5 mM Arg.

DISCUSSION

Recently, studies have addressed growth-regulating substances and nutrient mixes to be effective for inducing plant growth. Abiotic stresses such as drought, salinity, and temperature extremes are the most important factors which can reduce the crop yield and reduce productivity. Soil salinization enhanced reduction in all plant growth attributes might be a sign of changes in the endogenous hormonal balance.

Our obtained results showed that salinity caused significant decrease in growth aspects of lupine plants. Such reduction in plant growth due to salinity might be due to limitation in water absorption, decline in metabolic activities due to Na^+ and Cl^- toxicity, imbalance of nutrients caused by ionic interference depression, disturbance in mineral uptake, translocation and respiration enhancement. The decline in vegetative growth due to high salinity effect is in agreement with previous studies of Nasibi *et al.* (2014) on canola plants, Sadak *et al.* (2012) on sunflower and Nasri *et al*. (2017) on flax seedlings. The results also showed that, exogenous spraying lupine plants with arginine (2.5 mM) alleviate the harmful effect of salinity and resulted in obvious enhancement in growth parameters under normal and stress conditions. These results are in harmony with those obtained by Nejadalimoradi *et al.* (2014). Also Abd El-Monem (2007) found that, arginine at 2.5 mM was the optimum concentration in the alleviation of the adverse harmful effects of salt stress. In addition, Mostafa *et al*. (2010) concluded that application of arginine stimulates the growth and yield of wheat, endogenous contents of polyamines and amino acids and their translocation to the wheat grains. In support of these results, Coueen *et al*. (2004) reported that, effect of polyamines in the stimulation of plant growth may be due to flexibility of polyamine metabolism and the metabolic correlation between polyamine and ethylene synthesis. Thus,

polyamines play an important role in the plasticity of plant growth and development.

Increasing salinity levels from 75 to 300 mM resulted in gradual significant reductions of chlorophyll a, chlorophyll b, and consequently total pigment contents compared with control plants (Table 2). Meanwhile, in carotenoids contents salinity level (75 & 150 mM) caused significant increase while 300 mM NaCl caused significant reduction as compared with control plants. Limitation in the amount of photosynthetic pigments observed in our study is one of the various effects of salt stress in plants and it has been found in many crops (Saqib *et al*, 2012), the reduced chlorophylls in salinity stressed plants might be attributed to the increase in the activities of the chlorophyll-degrading enzymes, chlorophyllase and the decrease in carotenoid contents under salt stress leads to degradation of β-carotene. Carotenoids act as free radical scavengers are efficiently protect chlorophyll pigments from photo-oxidative destruction. Dolatabadian and Saleh Jouneghani (2009) reported that, salinity stresses induced free radical species and destructed chlorophyll molecules in chloroplasts and therefore, resulting in reduction of photosynthesis and growth. On the other hand, the increasing in chlorophylls content observed in our study due to treating the stressed plants by arginine which could be explained on the basis that arginine might retard chlorophylls loss or increase biosynthesis by changing the stability and permeability of the thylakoid membrane and prevents chloroplast from senescing resulting in retarding chlorophyll degradation. The obtained results of the present work indicated a specialized increase of carotenoids in the arginine in treated plants which accounts for the higher content of chlorophylls. The antioxidant carotenoids serve as a photodynamic effect which acts as a photoreceptor pigment. It has also been found in the present work that there is a positive correlation between the increase in growth level, assimilating leaf area and photosynthetic pigment contents in response to arginine treatment in lupine plants.

The obtained results in table (3) show that irrigation of lupine with various salinity levels increased significantly the total free amino acid and protein contents compared to control plants. These results agree with those observed by Rady *et al*. (2011). Free amino acids act as an osmotic protective solute leading to decline the osmotic potential in plants exposed to stress. Foliar spraying of lupine plants with arginine caused significant increases in total free amino acids and protein contents under saline and non saline irrigation. Our obtained results are confirmed with those obtained by Sadak *et al*. (2012) who indicated that arginine was one of the most effective compounds that induce total amino acid and protein synthesis of wheat plants and grains under normal or stressed conditions by increasing the uptake and translocation of N to be involved in various metabolic processes resulting in the formation of protective compounds such as amino acids.

Results presented in table (3) illustrate that proline content of lupine plants significantly increased gradually by increasing the salinity levels as compared with control treatment. These results demonstrated that, the physiological role of proline which accumulated in lupine under salinity acts as osmolyte and protectant and also has other roles related to stress. Proline content in lupine plants is in good agreement with those obtained by Hozayn *et al*. (2013) on mungbean. Several investigators suggested that proline is considered as a storage component of carbon and nitrogen, and as a protective agent of enzyme and membrane which cause rapid recovery, scavenge from free radical resulted in improving and adaptation ability for growth under stresses. In addition, proline under recovery of stress improves cytoplasmic and mitochondrial enzymes stability and induces expression of stress responsive genes. Moreover, exogenous application of proline is known to improve survival rate of plants under stress conditions.

In addition, proline content significantly increased in shoots of lupine plants when the plants were sprayed with arginine (2.5 mM) as compared with salt stressed plants and control plants. These results are in accordance with Abdul-Qados (2010) who found that arginine treatment caused significant increase in proline content in mung bean plants grown under salinity stress. Also, El-Bassiouny *et al*. (2008) reported that arginine was efficiently compound in increasing the levels of proline, protein and total amino acid of wheat plants and yielded grains under normal or stressed condition. Moreover, da Rocha *et al*. (2012) reported that treated plants with arginine or ornithine leading to elevate proline levels .The increase of proline content may be attributed to the increase in its synthesis associated with inhibition of its catabolism and/ or may be a mechanism for stress tolerance.

The present investigation showed that salinity stress caused a significant increase in lipid peroxidation, over the control plant (table 3). These results are in agreement with Astorga and Meléndez (2010) who observed that lipid peroxidation increased with 100, 150, and 200 mM of sodium chloride in *Paulownia imperialis*. The increase in MDA content is a sign of oxidative stresses such as salinity (Priya *et al*, 2015). Also, Mohammadkhani *et al*. (2016) reported that MDA content increased in roots and leaves of grape plants under salt stress. On the other hand, arginine application reduced significantly the malondialdehyde (MDA) contents under both normal and stress conditions. This result is in a good harmony with those obtained by Barand *et al*. (2015) who reported that, exogenous application of Arg. reduced the oxidative damage by decreasing the malondialdehyde content in response to

various stress conditions in pine and pistachio plants. Nasibi *et al*. (2014) reported that when canola plants pre-application with arginine, the content of MDA and H_2O_2 decline that cause effective plant adaptation and tolerance to salinity stress. Several investigators concluded that, the exogenous application of polyamines (PALs) reduced the auto-oxidation of lipids of membrane and stabilized the biological membrane in stressed plants (Khalil *et al*, 2009). This decrease might be resulting from the obvious increase in antioxidant enzymes activity (SOD, APX and GR) (Fig. 1) which in turn promoted the scavenging of the harmful free radicals and hydrogen peroxide.

The reactive oxygen species (ROS) increases as a response toward most abiotic stresses including salinity (Saqib *et al*, 2012). Under normal conditions, the formation of ROS in the plants is determined by the delicate balance between the multiple ROS producing pathways and the action of the enzymatic and non-enzymatic mechanism with them. Under stresses, ROS production is more than the plant abilities to get rid of it resulting in oxidative damages. In the present work it has been observed that, in lupine plants under salt stress (especially in 150 & 300 mM NaCl), SOD, APX, and GR activities were elevated over the controls (Fig. 1). Therefore, it is assumed that the plant antioxidant machinery might significantly struggle against oxidative damage. It was reported that the antioxidant enzymes have an effective role in imparting salt tolerance. Also, the increased antioxidant enzymes activity during increased salt stress has been reported in maize (Azevedo-Neto *et al,* 2006) and canola (Nasibi *et al*, 2014). Treatment of lupine plants with arginine significantly increased the SOD, APX and GR activities in plants which were under salt stress. These results are in line with those obtained by Nasibi *et al*. (2011) who reported that exogenous application of arginine increased the activity of SOD, APX, and glutathione reductase in tomato plants under drought stress which may induce the conversion of $O^{\cdot}_2$ into H_2O_2 and O_2 that protect cells from oxidative damage. Khalil *et al*. (2009) found that pre-treatment of wheat plants with the amino acids arginine and putrecin, induced the antioxidant defense enzymes (SOD and CAT) and limited the lipid peroxidation when plants were exposed to heat stress. Nasibi *et al*. (2014) also demonstrated that arginine alleviated serious effects of salinity on canola through promoting antioxidant enzymes activities. Arginine acts as a precursor for NO which has been found to alleviate salt stress in previous studies. For example, treatment with NO donors has an effective role in improving salinity tolerance in stressed plants through promoting of antioxidant enzyme activity in response to salt stress conditions (Manai *et al*, 2014).

The obtained results indicated that lupine plants irrigated with different salinity levels characterized by the appearance of new bands and disappearance of other ones as compared with that of the untreated plants. In this respect Bekheta and El-Bassiouny (2005) reported that stressed plants developed significant cross mechanisms of protection against salinity via the induction of de novo synthesis of new set protein.

Therefore, in the present study salinity stress, in general, induced synthesis of a set of new protein bands (3 bands) at molecular weights 29.85, 11.28 and 7.66 KDa at all salinity levels. Under salt stress, the increase of the new synthesized proteins may reflect the physiological reactions to a combination of ions in stress tolerance, osmotic adjustment and Na^+/K^+ homoeostasis. It was also revealed that salinity altered the patterns of protein synthesis, and this might due to the synthesis of several osmo-responsive genes that may be involved in adaptation to salinity. One of the most important responses of plants to environmental stresses is the activation of a number of genes involved in respective stress response (Rasul et al, 2017). Also, Lu *et al*. (2015) revealed that salt stress could enhance changes in modulating gene expression to adapt to the environmental stress condition. Foliar spraying of lupine plants with arginine induced the appearance of two inducible protein bands at molecular weights 56.33 and 38.08 KDa under non-stress conditions. In this respect, El-Bassiouny *et al*. (2008) and Khalil *et al*. (2009) indicated that arginine treatments induced the appearance of new protein bands at molecular weights 222.0, 214.6, 131.8, 93.1, 78.7, 50.7, 34.6 and 14.1 KDa in wheat plants. Protein profile of lupine leaves indicate that application of arginine modify the expression of salt-stress inducible proteins and also induced synthesis of de novo specific polypeptides, which are expected to play an important role in salt tolerance (Abdul-Qados, 2010).

Moreover, increase in number of protein bands in the leaves of lupine treated with arginine and grown under salinity stress is preceded by activation to transcribe nuclear DNA to RNA- dependent RNA polymerase. Pal *et al*. (2015) reported that polyamines (PALs) are considered as multifaceted compounds, which have a vital role in the regulation of stress tolerance through activating the expression of stress-responsive genes. In addition, polyamines can bind to various molecules of proteins stabilizing their structure leading to changes in their activities and functions, as well as to chromatin, causing change in the availability of genomic sites to DNA or RNA polymerases, resulting in altered DNA and RNA synthesis. On the other hand, the disappearance of some protein bands under salinity stress may be due to the suppression of the genes responsible for protein synthesis because of stress. Therefore, the developed tissue had lost their ability to synthesis these proteins under stress conditions (Akladious and Abbas, 2014).

The obtained results indicated that treatment with arginine alleviate the retarding effects of salt stress on lupine leaves by changing the levels of isoenzyme

profiles. These results are in agreement with El-Baz *et al*. (2003) who reported that the profile of peroxidase isoenzyme was altered throughout salinity conditions because of salinity tolerance or due to the effect of salt stress which cause shifting in gene expression. Also, Mohamed (2005) revealed that the alternation in the isoenzymes profiles play an effective role in the plant defense against oxidative stress, caused by salinity.

Conclusion: The exogenous application of arginine can be used to alleviate the harmful effect of salt stress. Therefore, the usage of arginine may help to solve the problems produced by salinity stress.

REFERENCES

Abd El-Monem, A.A. (2007). Polyamines as modulators of wheat growth, metabolism and reproductive development under high temperature stress. Ph.D. thesis. Ain Shams Univ., Cairo, Egypt.

Abdul-Qados, A.M.S. (2010). Effect of arginine on growth, nutrient composition, yield and nutritional value of mung bean plants grown under salinity stress. Natu. & Sci. 8(7): 30-42.

Akladious, S. A. and S. M. Abbas (2014). Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley (*Hordeum vulgare* L.) genotypes to drought and potassium nutrition. The J. Anim. Plant Sci. 24(2): 620-633.

Astorga, G. I. A., and L. A. Meléndez (2010). Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown in vitro. Electro. J. Biotech. 13(5): 1-15.

Azevedo-Neto, A.D., J.T. Prisco, J. Enéas-Filho, C.E.B. Abreu, and E. Gomes-Filho (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt sensitive maize genotypes. Environ. Exp. Bot. 56: 87–94.

Barand, A., F. Nasibi, and K.H. Manouchehri Kalantari (2015). The effect of arginine pretreatment in the increase of cold tolerance in *Pistacia vera* L. in vitro. Russ. Agric. Sci. 41(5): 340–346.

Bates, L.S., R.P.Waldren, and L.D. Teare (1973). Rapid determination of free proline for water – stress studies. Plant and Soil 39: 205–207.

Beauchamp, C., and I. Fridovich (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gel. Anal. Bio. Chem. 44: 276–287.

Bekheta, M.A., and H.M.S. El-Bassiouny (2005). Response of two wheat cultivars grown under salinity stress to putrescine treatment. J. Agric. Sci. Mansoura. Univ. 30(8): 4505-4521.

Brauc, S., E.D. Vooght, J.M.C. Geuns, M. Höfte, and G. Angenon (2012). Overexpression of arginase in *Arabidopsis thaliana* influences defense responses against Botrytis cinerea. Plant Biol. 14: 39–45.

Coueen, I., I. Hummel, C. Sulmon, G. Gouesbet, and A. El-Amrani (2004). Involvement of polyamines in root development. Netherlands 76(1): 1-10.

da Rocha, I.M.A., V.A. Vitorello, J.S. Silva, S.L. Ferreira-Silva, R.A. Viégas, and E.N. Silva (2012). Exogenous ornithine is an effective precursor and the δ-ornithine amino transferase pathway contributes to proline accumulation under high N recycling in salt-stressed cashew leaves. J. Plant. Physiol. 169: 41–49.

Dolatabadian, A., and R.J. Saleh Jouneghani (2009). Impact of exogenous ascorbic acid on antioxidant activity and some physiological traits of common bean subjected to salinity stress. Not. Bot. Hort. Agrobot. Cluj. 37(2): 165-172.

El-Bassiouny, H.M.S., H.A. Mostafa, S.A. El–Khawas, R.A. Hassanein, S.I. Khalil, and A.A. Abd El–Monem (2008). Physiological responses of wheat plant to foliar treatments with arginine or putrescine. Aust. J. Basic. Appl. Sci. 2(4): 1390-1403.

El-Baz, F. K., A. A. Mohamed, and A. A. Aly (2003). Development of biochemical markers for salt stress tolerance in cucumber plants. Pakistan J. Bio. Sci. 6(1): 16–22.

FAO (2009). Food and Agriculture Organization of United Nations, Agric Data FAO

Foyer, C.H., and B. Halliwell (1976). The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta 133: 21-25.

Heath, R.L., and L. Packer (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Bioch. Bioph. 125(1):189–198.

Hozayn, M., A.A. Abd El-Monem, M. Ebtihal, E.M. Abd El-hamid, and M.S.A. Amira (2013). Amelioration of salinity stress in mungbean (*Vigna radiate* L). plant by soaking in arginine. J. Appl. Sci. Res. 9(1): 393-401.

Khalil, S.I., H.M. El-Bassiouny, R.A. Hassanein, H.A. Mostafa, S.A. El-Khawas, and A.A. Abd El-Monem (2009). Antioxidant defense system in heat shocked wheat plants previously treated with arginine or putrescine. Aust. J. basic appl. Sci. 3(3): 1517-1526.

Laemmli, U.K. (1970). Cleavage of structural proteins during assembly of head bacteriophage T4. Nature 227: 680–685.

Larsen, A.L., and W.C. Benson (1970). Variety specific variants of oxidative enzymes from soybean seeds. Crop Sci. 10: 493–495.

Lichtenthaler, H.K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol 148: 350-382.

Lowry, O.H., N.J. Rosembrough, A.L. Farr and R.J. Randall (1951). Protein measurement with the Folin phenol reagent. J Biol. Chem. 193(1): 267-275.

Lu, X., X. Zhao, D. Wang, Z. Yin, J. Wang, W. Fan, S.H. Wang, T. Zhang, and W. Ye (2015). Whole-genome DNA methylation analysis in cotton (*Gossypium hirsutum* L.) under different salt stresses. Turk. J. Biol. 39: 396–406.

Manai, J., T. Kalai, H. Gouia, and F.J. Corpas (2014). Exogenous nitric oxide (NO) ameliorates salinity-induced oxidate stress in tomato (*Solanum lycopersicum*) plants. J Soil Sci and Plant Nutr 14(2): 433-446.

Mohamed, A. A. (2005). Two-dimensional electrophoresis of soluble proteins and profile of some isozymes isolated from maize plant in response to NaCl. Res. J. Agri. Bio. Sci. 1(1):38–44.

Mohammadkhani, N., R. Heidari, N. Abbaspour, and F. Rahmani (2016). Salinity effects on expression of some important genes in sensitive and tolerant grape genotypes. Turk. J. Biol. 40: 95-108.

Moore, S., and W.H. Stein (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211: 907-913.

Mostafa, H.A.M., R.A. Hassanein, S.I. Khalil, S.A. El-Khawas, H.M.S. El-Bassiouny, and A.A. Abd El-Monem (2010). Effect of arginine or putrescine on growth, yield and yield components of late sowing wheat. Inter. J. Appl. Sci. Res. 6(2):177-183.

Nakano, Y., and K. Asada (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol. 22: 867-880.

Nasibi, F., K.K.H. Manouchehri, and A. Barand (2014). Effects of seed pre-treatment with L-arginine on improvement of seedling growth and alleviation of oxidative damage in canola plants subjected to salt stress. IJPP 5(1): 1217-1224.

Nasibi, F., M. Yaghoobi, and K.H. Manouchehri (2011). Effect of exogenous arginine on alleviation of oxidative damage in tomato plant under water stress. J. Plant Interact. 6: 291-296.

Nasri, N., S. Maatallah, I. Saidi, and M. Lachaâl (2017). Influence of salinity on germination, seedling growth, ion content and acid phosphatase activities of *Linum usitatissimum* L. The J. Anim. Plant Sci. 27(2): 517-521.

Nejadalimoradi, H., F. Nasibi, K.M. Kalantari, and R. Zanganeh (2014). Effect of seed priming with L-arginine and sodium nitroprusside on some physiological parameters and antioxidant enzymes of sunflower plants exposed to salt stress. Agric. Communi. 2(1): 23-30.

Pal, M., G. Szalai, and T. Janda (2015). Polyamines are important in abiotic stress signaling. Plant Sci. 237: 16-23.

Priya, A. M., S. R. Krishnan, and M. Ramesh (2015). Ploidy stability of *Oryza sativa*. L cv IR64 transformed with the moth bean P5CS gene with significant tolerance against drought and salinity. Turk. J. Biol. 39: 407–416.

Rady, M.M., M. Sadak, H.M.S. El-Bassiouny, and A.A. Abd El-Monem (2011). Alleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α-tocopherol. Aust. J. Basic Appl. Sci. 5(10): 342-355.

Rasul, I., H. Nadeem, M.H. Siddique, R.M. Atif, M.A. Ali, A. Umer, F. Rashid, M. Afzal, M. Abid, and F. Azeem (2017). Plants sensory-response mechanisms for salinity and heat stress. The J. Anim. Plant Sci. 27(2): 490-502.

Sadak, M. S., A. A. Abd El-Monem, H. M. S. El-Bassiouny, and N. M. Badr (2012). Physiological response of sunflower (*Helianthus annuus* L.) to exogenous arginine and putrescine treatments under salinity stress. J. Appl. Sci. Res. 8(10): 4943-4957.

Saqib, Z. A., J. Akhtar, M. A. Ul-Haq, and I. Ahmad (2012). Salt induced changes in leaf phenology of wheat plants are regulated by accumulation and distribution pattern of Na^+ ion. Pakistan J. Agri. Sci. 49: 141-148.

SAS Programme (1982). SAS user's guide statistics (computer program). SAS Institute Inc Raleigh p 584.

Sato, M., M. Hasegawa (1976). The latency of spinach chloroplast phenolase. Phytochem. 15: 61-65.

Stegemann, H., A.M.R. Afifiy, and K.R.F. Hussein (1985). Cultivar Identification of dates (*Phoenix dectylifera*) by protein patterns. 2nd international Symposium of Biochemical Approaches to Identification of Cultivars. Braunschweig, West Germany 44 pp.

Wiatr, K., A. Dolata, and T. Mańczak, (2007). Concentration and variation of basic quality traits of seeds of lupine cultivars registered in Poland. Zesz. Prob. Post Nauk. Rol. 522: 75-85.

Zeid, M. (2004). Response of bean (*Phaseolus vulgaris*) to exogenous putrescine treatment under salinity stress. Pakistan J. Biol. Sci. 7: 215-229.

Zhang, S., H. Jiang, S. Peng, H. Korpelainen, and C. Li (2011). Sex-related differences in morphological, physiological and ultrastructural responses of *Populus cathayana* to chilling. J. Exp. Bot. 62: 675–686.