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ORIGINAL ARTICLE

Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and *Pseudomonas fluorescens*

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Abstract This study was executed to evaluate the impact of endomycorrhizae and *Pseudomonas fluorescens* on growth parameters, yield and some biochemical constituents as well as spontaneous nodulation of Rhizobium-uninoculated cowpea (*Vigna unguiculata* L.) under salt stress. Results revealed that mycorrhizal infection percentage decreased with increasing the water salinity. Cowpea plants irrigated with tap water and inoculated with endomycorrhizae and *P. fluorescens* gave the highest significant increment in fresh and dry weights of the 4th leaf, pod length, number of seeds per pod and total protein being 3.560, 0.543 g, 13 cm, 9 seed/pod and 31.8 mg/g f.wt., respectively. The highest significant increment in the weight of 100 seed was recorded with plants irrigated with 6000 ppm NaCl solution and inoculated with *P. fluorescence* being 19.71 g. The highest significant increment in carotenoides was recorded with plants irrigated with 6000 ppm NaCl solution and inoculated with endomycorrhizae + *P. fluorescens* being 0.449 mg/g f.wt. The highest significant activity of Superoxide dismutase (SOD) was recorded with uninoculated plants irrigated with 3000 ppm NaCl solution, being 7.25 unit/mg protein. The highest significant concentrations of proline and total soluble sugars were recorded in plants irrigated with 6000 ppm NaCl solution and inoculated with endomycorrhizae being 1001.62 μg/g f.wt. and 2.5684 mg/g f.wt. respectively.

Introduction

Cowpea (*Vigna unguiculata* L.) is an important seed legume crop. It is cultivated to obtain seeds and pods for human consumption, and as a source of green manure and organic material.

Salinity is a problem of great concern, at which, about one third of the world's irrigated land is not in use (Giri and Mukerji, 2004) and about ten million hectares of irrigated agricultural land are abandoned annually. This problem restricts crop yield on almost 40 million hectares of irrigated lands. Due to increasing world's population and food needs, it is recommended to use all available soil and water resources. Many

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studies confirmed the inhibitory effects of salinity on the germination, vegetative growth, and yield of cowpea as well as other crops (Murillo-Amador et al., 2001), while others referred that reduction of protein content as well as photosynthetic pigments (Taffouo et al., 2010).

Several microorganisms are known to have the ability to tolerate high salt concentrations and survive under a wide salinity range, among these microorganisms are mycorrhizal fungi. Most plants in natural conditions form a symbiosis with arbuscular mycorrhizal fungi (AM) which has distinguishing importance due to great capability to increase plant growth and yield under different conditions (Zayed et al., 2013; Eissa et al., 2015). Many studies have demonstrated that arbuscular mycorrhizal (AM) symbiosis is often alleged to improve plant resistance to water salinity stress through the alteration of plant physiology and expression of some plant genes, help in water regulation of plants by extending their hyphae toward the available moisture zones for continuous water absorption and translocate it to plants (Giri et al., 2004). Also, AM association can affect the host plants in terms of stomatal movement and photosynthesis of leaves and increase the chlorophyll concentration. Different studies have indicated that AM plants are frequently more tolerant to salt stresses than non-AM plants (Al-Karaki, 2006), with only few exceptions (Ouziad et al., 2006).

Pseudomonas fluorescens is plant growth promoting rhizobacterium (PGPR) and lives in the plant rhizosphere. More specifically, *P.fluorescens* have received particular attention throughout the global science because of excellent root colonizing ability and its capacity to produce a wide range of enzymes and metabolites that help plant to withstand under varied biotic and abiotic stress conditions (Mayak et al., 2004). Also, some reports have shown that PGPR has a strong stimulatory impact on the mycorrhizal establishment, growth and function (Artursson et al., 2006; Vázquez et al., 2000).

The aim of this study was to evaluate the impact of VAM and *Pseudomonas fluorescens* on growth, productivity and some biochemical constituents of cowpea grown under salt stress.

Materials and methods

Mycorrhizal inoculant

Vesicular arbuscular mycorrhizal spores (VAM) were originally extracted from soil around roots of maize, grown in the experimental field of Fac. Agric., Ain Shams Univ., Shobra El-Kheima, Cairo, Egypt, using the wet sieving and decanting technique described by Gerdemann and Nicolson (1963). Five ml from mycorrhizal spore suspension containing about 50 spores/1 ml was used as standard inocula.

Pseudomonas inoculant

P. fluorescens was obtained from the Unit of Biofertilizers, Fac., Agric., Ain Shams University. Microbial inoculant was maintained in King's medium (King et al., 1954). The microbial densities were adjusted to be 10^8 cfu/ml and examined for zeatin, indole acetic acid and gibberellic acid using high-performance liquid chromatography (HPLC) according to the method described by Tien et al. (1979).

Pot experiment

The present study was carried out in greenhouse facilities of the Microbial Inoculant Activity Fac. Agric., Ain Shams Univ, Cairo, Egypt, as a pot experiment from May to July 2014. Cowpea plants were grown in 5.5 kg air-dried sand soil distributed in circular earthen pots (40 cm depth and 30 cm diameter). Cowpea seeds (*V. unguiculata* (L.) Kareem 7) were sterilized with 10% NaClO for 1 min and washed with distilled water. Six seeds were planted in each pot. After 2 weeks three homogenous seedlings showing the strongest growth were selected and left to grow. Plants were irrigated every 2–3 days and fertilization program depended on the program recommended for the legume cultivation by the Egyptian Ministry of Agriculture (150 kg superphosphate, 50 kg sulfate potassium and 50 kg sulfate ammonium/Feddan).

The experiment comprised three levels of salinity (tap water, 3000 and 6000 ppm NaCl solution). Seedlings of 7 day old were inoculated with 5 ml of *Pseudomonas fluorescence* and/or 2.5 ml of mycorrhizal spores which were pipetted into holes and covered with soil.

The applied treatments were divided into three groups according to the type of irrigation water as follows: group (1) irrigated with tap water, group (2) irrigated with saline solution containing 3000 ppm NaCl solution and group (3) irrigated with saline solution containing 6000 NaCl solution, Each group was divided into four treatments as follows: control, mycorrhizae, *P. fluorescence* and mycorrhizae + *P. fluorescens*.

Growth parameters

The following parameters were measured: fresh and dry weights of the fully expanded fourth leaf from the top of plants (g), area of the fourth leaf as measured by an Image J software (mm^2), pod length (cm), pod weight (g), number of pods per plant, number of seeds per pod and weight of 100 seed (g). The parameters were measured after 50 of planting. Six plants were taken from each treatment for different determinations.

Mycorrhizal development

The percentage of mycorrhizal root infection was estimated by visual observation of fungal colonization according to Phillips and Hayman (1970). The percentage of mycorrhizal colonization was calculated according to the gridline intersect method (Giovannetti and Mosse, 1980).

Biochemical analysis

All biochemical analyses were determined in 0.5 g of the fully expanded fourth leaf from the top of the plant for each analysis. Determinations of chlorophyll (a&b), total chlorophylls, carotenoides, proline, superoxide dismutase activity, total protein and total soluble sugars were assessed at 50 days after sowing.

Total chlorophylls and carotenoides

Chlorophyll (a&b), total chlorophyll and carotenoides were extracted and estimated according to the methods described

by Moran (1982). Formula and extinction coefficients were used as described by Lichtenthaler and Wellburn (1983) and expressed as mg/g fresh weight.

Proline concentration

Proline concentration was determined using a ninhydrin colorimetric method of Troll and Lindsley (1955) as modified by Peters et al. (1997). The proline concentration was calculated from the standard curve of L-proline and expressed as µg proline/g f.wt.

Total protein

Protein concentration was determined according to the method of Bradford (1976) and calculated using the standard curve of bovine serum albumin (BSA) and expressed as mg protein/g f.wt.

Superoxide dismutase activity

The activity of superoxide dismutase (SOD) (EC 1.15.1.1) was assayed by the method of Beyer and Fridovich (1987), and expressed as unit/mg protein.

Total soluble sugars

Total soluble sugars (TSS) were extracted according to the method described by Horwitz (1965) and measured according to the method recorded by Dubois et al. (1956) and expressed as mg/g f.wt.

Statistical analysis

The experiment was conducted in a complete randomized design with three replicates, and each replicate included 9 plants. Plant growth parameters and chemical analyses were statistically analyzed using One-way ANOVA and Post hoc-LSD tests (the least significant difference) using SAS (Institute, 2003) at 0.05 level of probability.

Results

Assessment of some metabolic activities of ***P. fluorescens***

P. fluorescens' supernatant was able to produce gibberellins (GAs), zeatin (ZE) and indole-3-acetic acid (IAA), with high concentrations being 40.5, 150.8 and 12.020 µg/ml respectively in the King's medium.

Mycorrhizal colonization percentage

Mycorrhizal colonization rates (Table 1) showed that mycorrhizal infection percentage decreased by increasing the water salinity. Plants of uninoculated treatments were not colonized by VAM. Also, the authors perceived decrease in the number of vesicles and arbuscles by increasing the salinity level.

Growth and yield parameters

Data presented in Table 2 showed that uninoculated plant exhibited decreases in fresh and dry weights of the 4th leaf when irrigated with 3000 ppm NaCl solution, though they exhibit increase in fresh and dry weights of the 4th leaf when irrigated with 6000 ppm NaCl solution when compared to those irrigated with tap water. In addition, plants exhibit decrease in leaf area by increasing salinity.

Cowpea plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens* gave the highest significant increments in fresh and dry weights of the 4th leaf when compared to all treatments being 3.560 and 0.543 g respectively, followed by plants irrigated with tap water and inoculated by *Pseudomonas fluorescens* being 3.03 and 0.47 g, respectively.

The highest significant leaf areas were recorded in plants irrigated with tap water either inoculated by endomycorrhizae and *P. fluorescens* or *P. fluorescens* only being 127.41 and 128.53 mm^2 respectively. On the other hand, the lowest fresh and dry weights as well the leaf area of the 4th leaf were recorded in cowpea plants irrigated with 6000 ppm NaCl solution either inoculated by endomycorrhizae + *P. fluorescens* or *P. fluorescens* only being 1.746, 1.803, 0.276 & 0.270 g and 52.0 & 50.16 mm^2 in respective order.

By comparing between the treatments in each group separately, cowpea plants irrigated with 3000 ppm NaCl solution and inoculated by *P. fluorescens* recorded the highest significant increment in both fresh and dry weights of the 4th leaf being, 2.88 and 0.4 g in respective order while the highest significant leaf area was recorded in plants inoculated by endomycorrhizae + *P. fluorescens* being 83.75 mm^2 when compared to all the treatments within the same group. Plants treated with 6000 ppm NaCl solution without inoculation recorded the highest significant increment in both fresh and dry weights of the 4th leaf being 2.99 and 0.363 g while the highest significant leaf areas were recorded in plants inoculated by endomycorrhizae and those uninoculated (control) being

Table 1 Effect of interaction between endomycorrhizae and *Pseudomonas fluorescence* on roots infection percentages with VAM under salt stress.

Treatments	VAM%
Tap water	
Control	–
Mycorrhizae	90
Pseudomonas fluorescence	–
Mycorrhizae + *P. fluorescens*	95
NaCl (3000 ppm)	
Control	–
Mycorrhizae	75
Pseudomonas fluorescence	–
Mycorrhizae + *P. fluorescens*	78
NaCl (6000 ppm)	
Control	–
Mycorrhizae	35
Pseudomonas fluorescence	–
Mycorrhizae + *P. fluorescens*	45

67.85 and 67.41 mm^2 when compared to the treatments within the same group in respective order.

Concerning yield parameters, the data could be categorized into three groups in accordance with the type of their irrigation water. The first group includes plants irrigated with tap water which exhibited the highest records in most parameters (pod length, number of seed/pod and weight of 100 seed) especially those inoculated by endomycorrhizae + *P. fluorescens* when compared to other two groups. The second group includes plants irrigated with 3000 ppm NaCl solution which exhibited clear variations between treatments. The third group includes plants irrigated with 6000 ppm NaCl solution which display ceases in plant flowering in all treatments except those inoculated by *P. fluorescens*.

In general, uninoculated plant exhibited decreases in pod length, pod weight, number of pods/plants, number of seeds per pod and the weight of 100 seed as a result of increasing sodium chloride concentration.

Concerning pod length (cm), results revealed that cowpea plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens* gave the highest significant pod length being 13 cm compared to all other treatments either irrigated with tap water or NaCl. In plants irrigated with 3000 ppm NaCl solution, the uninoculated plants recorded the highest significant pod length being 9.33 cm. On the contrary, plants inoculated by endomycorrhizae and *P. fluorescens* recorded the lowest significant value of pod length being 7 cm when compared to all treatments within the same group.

Plants irrigated with tap water and uninoculated revealed the highest significant increment in pod weight being 2.66 g/pod when compared to all other treatments, while the lowest pod weight was recorded within plants irrigated with 3000 ppm NaCl solution only being 0.58 g/pod. In the second group at which plants irrigated with 3000 ppm NaCl solution the highest significant increment was recorded with plants inoculated by endomycorrhizae being 1.633 g/pod when compared to all treatments within the same group.

Regarding number of pods/plant, it is clear that all the treatments in the first group as well as plants irrigated with 3000 ppm NaCl solution and inoculated by endomycorrhizae showed the highest significant increment in the number of pods per plant being 3.66, 3.33, 2.66, 2.66 and 3.33 pods/plants respectively, followed by those inoculated with *P. fluorescens* in both second and third groups being 2 pod/plant for both of them, while the lowest number was recorded within plants irrigated with 3000 ppm NaCl solution only being 1 pod/plant.

Concerning number of seeds per pod, the highest significant numbers were recorded with plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens* followed by those irrigated with 3000 ppm NaCl solution and inoculated by endomycorrhizae compared to all treatments being 9 and 7 seed/pod in respective order, while the lowest value was recorded within plants irrigated with 6000 ppm NaCl solution and inoculated by *P. fluorescens* being 1.66 seed/pod.

Regarding the weight of 100 seed, the highest significant value was recorded with plants irrigated with 6000 ppm NaCl solution and inoculated by *P. fluorescence* and those irrigated with 3000 ppm NaCl solution and inoculated by endomycorrhizae within when compared to all treatments being 19.71 and 19.2 g respectively. On the other hand, the lowest weight of 100 seed was recorded within the treatment irrigated with 3000 ppm NaCl solution and not inoculated being 12.8 g.

Table 2 Growth and yield parameters of cowpea as affected by AMF and/or *P. fluorescence* inoculation under different salt stress levels.

Treatments	Growth parameters			Yield parameters				
	Fresh weight (g)	Dry weight (g)	Leaf area mm^2	Pod length (cm)	Pod d.w. (g)	Number of pods/plant	Number of seeds/pod	Weight of 100 seed
Tap water								
Control	2.683	0.390	110.67	11.00	2.66	3.66	6.00	16.53
Mycorrhizae	2.363	0.300	101.79	8.66	1.536	3.33	6.00	17.56
Pseudomonas fluorescens	3.030	0.470	128.53	10.33	2.423	2.66	5.66	18.60
Mycorrhizae + *P. fluorescens*	3.560	0.543	127.41	13.00	1.810	2.66	9.00	17.00
3000 ppm NaCl								
Control	2.317	0.343	79.95	9.33	0.580	1.00	3.66	12.80
Mycorrhizae	2.283	0.303	64.95	8.00	1.633	3.33	7.00	19.20
Pseudomonas fluorescens	2.88	0.400	60.61	8.33	1.160	2.00	5.00	15.20
Mycorrhizae + *P. fluorescens*	2.763	0.350	83.75	7.00	0.980	1.33	3.66	14.50
6000 ppm NaCl								
Control	2.99	0.363	67.41	0.00	0.000	0.00	0.00	0.00
Mycorrhizae	2.566	0.336	67.85	0.00	0.000	0.00	0.00	0.00
Pseudomonas fluorescens	1.803	0.270	50.16	7.66	0.836	2.00	1.66	19.71
Mycorrhizae + *P. fluorescens*	1.746	0.276	52.00	0.00	0.000	0.00	0.00	0.00
LSD	0.2626	0.0464	17.26	1.256	0.1789	0.688	8.858	0.5749

Biochemical constituents

Data recorded in Table 3 showed the effect of different microbial inoculants under salt stress on total chlorophylls, carotenoids as well some biochemical contents in cowpea plants. In general, uninoculated plants exhibited increases in chlorophyll (a&b), carotenoides, superoxide dismutase (SOD), proline, protein and total soluble sugars (TSS) as a result of increasing sodium chloride concentrations in irrigation water.

In accordance with photosynthetic pigments, chlorophyll (a&b), cowpea plants irrigated with 6000 solution NaCl and inoculated by endomycorrhizae and *P. fluorescens* recorded the highest significant content being 1.3697 and 0.51815 mg/g f.wt. respectively compared to all other treatments. On the contrary, the lowest chlorophyll (a&b) contents were recorded within plants irrigated with tap water, of which chlorophyll (a) was recorded within plants inoculated by endomycorrhizae, and chlorophyll (b) was recorded within uninoculated plants being 0.947 and 0.3214 mg/g f.wt. respectively. No significant difference was recorded between treatments in plants irrigated with tap water for both chlorophyll (a&b). While in plants irrigated with 3000 ppm NaCl solution, the highest significant concentrations of chlorophyll (a&b) were recorded within plants inoculated by *P. fluorescens* when compared to the control within the same group being 1.1371 and 0.45294 mg/g f.wt. respectively.

Concerning total chlorophyll, the highest significant concentration was recorded within plants inoculated by *P. fluorescens* either irrigated with 3000 or 6000 ppm NaCl solution being 1.9281 mg/g fresh weight for both of them. Plants irrigated with tap water and uninoculated recorded the lowest concentration of total chlorophyll being 1.4362 mg/g f.wt.

Regarding the concentration of carotenoids, the highest significant level was recorded within plants irrigated with 6000 ppm NaCl solution and inoculated by endomycorrhizae and *P. fluorescens* being 0.449 mg/g f.wt. compared to all treatments. No significant differences were recorded between the treatments.

Superoxide dismutase (SOD) activity recorded increment in uninoculated plants by increasing salinity from 4.7467 unit/mg protein when irrigated with tap water to 7.25 unit/mg protein when irrigated with 3000 ppm NaCl solution, which exhibits the highest significant activity in SOD when compared to all treatments, while plants irrigated with 6000 ppm NaCl solution recorded more activity of SOD being 5.5133 unit/mg protein which is higher than those irrigated with tap water and lower than those irrigated within 3000 ppm NaCl solution. The lowest SOD (3.53 unit/mg protein) activity was recorded within plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens*.

For plants inoculated by endomycorrhizae no significant difference was recorded between plants in SOD irrigated with either tap water or 3000 ppm NaCl solution, as well it recorded significant increment in plants irrigated with 6000 ppm NaCl solution when compared to both of them. While in plants inoculated by *P. fluorescens* and those inoculated by endomycorrhizae and *P. fluorescens* the highest significant increase in (SOD) activity was recorded in plants irrigated with 3000 ppm NaCl solution when compared to those irrigated with tap water and 6000 ppm NaCl solution.

The highest significant concentration of proline was recorded with plants irrigated with 6000 ppm NaCl solution and inoculated by endomycorrhizae, followed by those inoculated by endomycorrhizae and *P. fluorescens* within the same group being 1001.62 and 810.18 µg/g f.wt, while the lowest concentration was recorded within plants irrigated with tap water and uninoculated being 322.63 µg/g f.wt.

Concerning the proline in plants irrigated with tap water, all the treatments showed significant increase in proline concentration when compared to control and no significant differences were recorded between the treatments, while plants irrigated with 3000 ppm NaCl solution showed no significant difference between all the treatments comparing with control.

Concerning total protein, the highest significant level was recorded within plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens* being 31.8 mg/g fresh weight. No significant differences were found between all other treatments except the treatment irrigated with 3000 ppm NaCl solution and inoculated by endomycorrhizae and *P. fluorescens* which recorded the lowest protein concentration being 23.253 mg/g f.wt.

Regarding total soluble sugars (TSS), plants irrigated with 6000 ppm NaCl solution and inoculated with endomycorrhizae showed the highest significant increment in TSS when compared to all treatments being 2.5684 mg/g f.wt, while the lowest concentration was recorded within plants irrigated with tap water and inoculated by endomycorrhizae and *P. fluorescens* being 0.7647 mg/g f.wt. Plants irrigated with tap water or 3000 ppm NaCl solution recorded the highest significant level of (TSS) compared to other treatments within the same group for each one of them.

In the absence of Rhizobium, cowpea plants either inoculated by the applied microbiota or not, showed no ability to produce spontaneous nodules on its root system.

Discussion

Salt stress imposes a major environmental threat to agriculture by limiting plant growth and reducing crop yield, since it reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolism (Munns, 2002). Plants growing under saline conditions are stressed basically in three ways: (1) reduction in water potential in the root zone causing water deficit, (2) phytotoxicity of ions such as Na^+ and Cl^-, and (3) nutrient imbalance by depression in uptake and/or shoot transport which is attributed to the fact that Na^+ competes with K^+ for essential binding sites for cellular function (Tester and Davenport, 2003).

A relatively high mycorrhizal root colonization percentage occurred after inoculation and plants grown in control soil had higher AM colonization than those treated with NaCl. These results are in agreement with those obtained by (Huang et al., 2010). Previous research has shown that salinity reduced AM colonization which may be caused by inhibiting the germination of spores (Hildebrandt et al., 2001), inhibiting growth of hyphae in soil and hyphal spreading after initiating the infection (McMillen et al., 1998), as well reducing the number of arbuscles (Pfetffer and Bloss, 1988). The present results showed similar trend as the AM colonization of plants reduced by increasing the concentration of sodium chloride.

Table 3 Effect of AMF and/or *P. fluorescence* inoculation under different salt stress levels on biochemical constituents of cowpea.

Treatments	Photosynthetic pigments				Biochemical properties			
	Chl a mg/g f.wt.	Chl b mg/g f.wt.	Total Chl mg/g f.wt.	Carot mg/g f.wt.	SOD unit/mg protein	Proline µg/g f.wt.	Protein mg/g f.wt.	TSS mg/g f.wt.
Tap water								
Control	0.959	0.321	1.436	0.263	4.747	322.630	29.280	1.002
Mycorrhizae	0.947	0.366	1.595	0.282	4.550	442.850	29.920	0.926
Pseudomonas fluorescence	1.040	0.375	1.724	0.326	3.977	462.060	30.427	0.856
Mycorrhizae + *P. fluorescence*	1.022	0.376	1.702	0.311	3.530	461.240	31.800	0.765
3000 ppm NaCl								
Control	1.033	0.407	1.747	0.315	7.250	598.190	26.220	1.996
Mycorrhizae	0.982	0.383	1.657	0.306	4.483	556.690	28.213	1.092
Pseudomonas fluorescence	1.137	0.453	1.928	0.342	6.630	536.270	29.667	1.781
Mycorrhizae + *P. fluorescence*	1.046	0.415	1.772	0.336	7.187	540.190	23.253	1.335
6000 ppm NaCl								
Control	1.100	0.456	1.747	0.325	5.513	716.570	29.067	2.001
Mycorrhizae	0.974	0.425	1.657	0.277	6.120	1001.620	26.880	2.568
Pseudomonas fluorescence	1.078	0.475	1.928	0.301	5.730	690.450	26.720	2.345
Mycorrhizae + *P. fluorescence*	1.370	0.518	1.772	0.449	6.273	810.180	26.720	1.917
LSD	0.276	0.092	0.409	0.095	0.591	94.022	5.072	0.0004

Chl = Chlorophyll.
Carot = Carotenoid.
TSS = Total soluble sugar.
SOD = Superoxide dismutase.

Many studies have shown that the fresh and dry weights of shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present, or type of plant species (Cha-um et al., 2013; Memon et al., 2010). The increase in fresh and dry weights of the shoot system in the third group may be due to the ability of the plant to increase the size of its sap vacuoles, which allow plants to collect a lot of water, and this in turn dissolves salt ions that have accumulated which leads to the subsequent increase in fresh weight (Munns, 2002).

The notable decrease in leaf area found in this study could be attributed to increasing the concentrations of sodium chloride, might be explained as negative effect of salt on photosynthesis which leads to reduction in plant growth, leaf growth, and chlorophyll concentration. Numerous studies showed the affection of leaf area negatively by using different concentrations of NaCl (Yilmaz and Kina, 2008).

In the present work, it was verified that continuous application of saline water, particularly during initial growth stages, and flowering reduced the pod production, dry biomass of pods, seeds, the number of pods, the numbers of seeds per pod and the weight of 100 seeds.

The effect of salinity on photosynthesis can be gauged from the effect on the photosynthetic pigments. The obtained results reveal increases in chlorophyll (a&b) and carotenoids concentration by increasing salt concentrations, which are in agreement with earlier studies reported by Misra et al. (1997) on rice seedlings and Lacerda et al. (2006) on cowpea.

The present results revealed increases in proline and total soluble sugars concentration by increasing salt level. Previous studies have documented that proline (Taffouo et al., 2010) and sugar levels (Praxedes et al., 2011), are increased in salt stressed legumes and play a key role as osmolytes to maintain the water relation in plants. Therefore, proline and total soluble sugars concentration is good indicator to identify the salt tolerant abilities in leguminous species. In addition, proline protects membranes and proteins against the adverse effects of high concentration of inorganic ions. It also functions as a hydroxyl radical scavenger (Cha-um et al., 2013).

Protein concentration can be affected negatively or positively, by salt stress. Our results demonstrated a decrease in protein concentrations in plants treated with 3000 ppm NaCl solution, and increases, in protein concentrations in plants treated with 6000 ppm NaCl solution. These results are in agreement with those reported by Kapoor and Srivastava (2010) as they observed an increase in protein concentration with increasing salt concentration and those obtained by Khosravinejad et al. (2009) as they reported that treatment with sodium chloride reduced protein concentration in the plant seedlings.

Most of the environmental stresses stimulate plants to produce reactive oxygen species (ROS), such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Foyer and Noctor, 2003) which are extremely cytotoxic and can seriously disrupt normal metabolism through oxidative damage to lipids (Alscher et al., 2002), nucleic acids and proteins (Herbette et al., 2002). In order to avoid the damage caused by ROS compounds, plants evolve molecular defense systems that both limit the formation of ROS and promote its removal (Alscher et al., 2002). The plant enzymatic defenses include antioxidant enzymes such as superoxide dismutase (SOD) which considered the first line of defense against ROS and promote the scavenging of ROS by catalyzing the dismutation of O_2^- to H_2O_2 and molecular oxygen (Cavalcanti et al., 2004). Under salt stress, many research papers proved that activity of antioxidant enzyme (SOD) becomes higher to eliminate more active oxygen (Lee et al., 2001).

The obtained results demonstrated increases in SOD activities in plants treated with 3000 ppm NaCl solution, and decreases in SOD activities in plants treated with 6000 ppm NaCl solution. We suggest bad effects of high salinity

concentration, which makes the plants to be deprived of the ability to control their metabolites. Similar increases in the activities of these enzymes have been reported by Rajguru et al. (1999).

The AM fungi protected the host plant against the detrimental effects of salinity and increase the resilience of host plants which lead to increasing their growth (Giri and Mukerji, 2004). Since inoculated plants accumulated greater amounts of P and K, and decreased the Na uptake by increasing the salinity which helps in chlorophyll synthesis (Giri and Mukerji, 1999). In addition, increased total chlorophyll, leaf sugars, and carbohydrate concentrations subsequently increased the plant resistance to salinity and lead to increasing plant growth (Ezz and Nawar(1994). Also, mycorrhizal colonization significantly improved leaf area in the salt-stressed plants and enhanced activities of superoxide dismutases (SOD) in leaves, resulting in improved osmotic adjustment of mycorrhizal seedlings (Huang et al., 2010).

The reduced effect of AMF on SOD activity after long salt stress might be a result of inhibition AM colonization by salt stress, which had been reported in previous literatures (Huang et al., 2010).

The AM fungi inoculation decreased the leaf content of soluble protein and enhanced activities of superoxide dismutases (SOD), resulting in improved osmotic adjustment to mycorrhizal seedlings which leads to improving all growth, yield and biochemical parameters (Wu and Xia, 2005).

The present results revealed increases in most growth, yield and biochemical constituents which could be interpreted by the ability of *Pseudomonas* spp. to impart some developing mechanisms as stress tolerance degree to plants toward a biotic stresses like salinity. Production of indole acetic acid, gibberellins and some unknown determinants by PGPR, leads to increasing root length, root surface area and number of root tips, which give rise to enhance the uptake of nutrients and improve plant fresh and dry weights under stress conditions (Egamberdieva and Kucharova, 2009). Plant growth promoting bacteria especially *Pseudomonas* spp. has been found to improve growth of tomato, pepper, canola, bean and lettuce under saline conditions (Barassi et al., 2006).

References

Al-Karaki, G.N., 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci. Hortic. 109 (1), 1–7.

Alscher, R.G., Erturk, N., Heath, L.S., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53 (372), 1331–1341.

Artursson, V., Finlay, R.D., Jansson, J.K., 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. 8 (1), 1–10.

Barassi, C., Ayrault, G., Creus, C., Sueldo, R., Sobrero, M., 2006. Seed inoculation with Azospirillum mitigates NaCl effects on lettuce. Sci. Hortic. 109 (1), 8–14.

Beyer, W.F., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem. 161 (2), 559–566.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1), 248–254.

Cavalcanti, F.R., Oliveira, J.T.A., Martins-Miranda, A.S., Viégas, R. A., Silveira, J.A.G., 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol. 163 (3), 563–571.

Cha-um, S., Batin, C.B., Samphumphung, T., Kidmance, C., 2013. Physio-morphological changes of cowpea ('Vigna unguiculata' Walp.) and jack bean ('Canavalia ensiformis' (L.) DC.) in responses to soil salinity. Aust. J. Crop Sci. 7 (13), 2128–2135.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356.

Egamberdieva, D., Kucharova, Z., 2009. Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol. Fertil. Soils 45 (6), 563–571.

Eissa, N.H., Zayed, M.S., Abdallah, M., 2015. Effect of Biofertilization and Soil Solarization on Pepper Quality, first ed. LAP LAMBERT Academic Publishing, 82 pp. (in press).

Ezz, T., Nawar, A., 1994. Salinity and mycorrhizal association in relation to carbohydrate status, leaf chlorophyll and activity of peroxidase and polyphenol oxidase enzymes in sour orange seedlings. Alexand. J. Agricult. Res. 39, 263-263.

Foyer, C.H., Noctor, G., 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol. Plant. 119 (3), 355–364.

Gerdemann, J., Nicolson, T.H., 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46 (2), 235–244.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84 (3), 489–500.

Giri, B., Kapoor, R., Agarwal, L., Mukerji, K., 2004. Preinoculation with arbuscular mycorrhizae helps Acacia auriculiformis grow in degraded Indian wasteland soil. Commun. Soil Sci. Plant Anal. 35 (1–2), 193–204.

Giri, B., Mukerji, K., 1999. Improved growth and productivity of Sesbania grandiflora Pers. under salinity stress through mycorrhizal technology. J. Phytol. Res. 12, 35–38.

Giri, B., Mukerji, K., 2004. Mycorrhizal inoculant alleviates salt stress in Sesbania aegyptiaca and Sesbania grandiflora under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14 (5), 307–312.

Herbette, S., Lenne, C., Leblanc, N., Julien, J.L., Drevet, J.R., Roeckel-Drevet, P., 2002. Two GPX-like proteins from Lycopersicon esculentum and Helianthus annuus are antioxidant enzymes with phospholipid hydroperoxide glutathione peroxidase and thioredoxin peroxidase activities. Eur. J. Biochem. 269 (9), 2414–2420.

Hildebrandt, U., Janetta, K., Ouziad, F., Renne, B., Nawrath, K., Bothe, H., 2001. Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza 10 (4), 175–183.

Horwitz, W., 1965. Official Methods of Analysis. Association of Official Agricultural Chemists, Washington, DC, 957 pp.

Huang, Z., He, C.-X., He, Z.-Q., Zou, Z.-R., Zhang, Z.-B., 2010. The effects of arbuscular mycorrhizal fungi on reactive oxyradical scavenging system of tomato under salt tolerance. Agricult. Sci. China 9 (8), 1150–1159.

Institute, S., 2003. SAS System Version 9.1 for Windows. SAS Institute Cary^ eNC NC.

Kapoor, K., Srivastava, A., 2010. Assessment of salinity tolerance of Vinga mungo var. Pu-19 using ex vitro and in vitro methods. Asian J. Biotechnol. 2, 73–85.

Khosravinejad, F., Heydari, R., Farboodnia, T., 2009. Effect of salinity on organic solutes contents in barley. Pak. J. Biol. Sci.: PJBS 12 (2), 158–162.

King, E.O., Ward, M.K.a., Raney, D.E., 1954. Two simple media for the demonstration of pyocianin and fluorescin. J. Lab. Clin. Med. 44, 301–307.

Lacerda, C.F., Assis Júnior, J.O., Lemos Filho, L.C., Oliveira, T.S.d., Guimarães, F.V., Gomes-Filho, E., Prisco, J.T., Bezerra, M.A., 2006. Morpho-physiological responses of cowpea leaves to salt stress. Brazil. J. Plant Physiol. 18 (4), 455–465.

Lee, D.H., Kim, Y.S., Lee, C.B., 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). J. Plant Physiol. 158 (6), 737–745.

Lichtenthaler, H., Wellburn, A., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.

Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci. 166 (2), 525–530.

McMillen, B.G., Juniper, S., Abbott, L., 1998. Inhibition of hyphal growth of a vesicular–arbuscular mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol. Biochem. 30 (13), 1639–1646.

Memon, S.A., XiLin, H., LiangJu, W., 2010. Morphological analysis of salt stress response of pak choi. Electron. J. Environ., Agricult. Food Chem. 9 (1), 248–254.

Misra, A., Sahu, S., Misra, M., Singh, P., Meera, I., Das, N., Kar, M., Sahu, P., 1997. Sodium chloride induced changes in leaf growth, and pigment and protein contents in two rice cultivars. Biol. Plant. 39 (2), 257–262.

Moran, R., 1982. Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol. 69 (6), 1376–1381.

Munns, R., 2002. Comparative physiology of salt and water stress. Plant, Cell Environ. 25 (2), 239–250.

Murillo-Amador, B., Troyo-Diéguez, E., López-Cortés, A., Jones, H., Ayala-Chairez, F., Tinoco-Ojanguren, C., 2001. Salt tolerance of cowpea genotypes in the emergence stage. Anim. Product. Sci. 41 (1), 81–88.

Ouziad, F., Wilde, P., Schmelzer, E., Hildebrandt, U., Bothe, H., 2006. Analysis of expression of aquaporins and Na+/H+ transporters in tomato colonized by arbuscular mycorrhizal fungi and affected by salt stress. Environ. Exp. Bot. 57 (1), 177–186.

Peters, W., Beek, E., Piepenbrock, M., Lenz, B., Schmitt, J.M., 1997. Cytokinin as a negative effector of phosphoenolpyruvate carboxylase induction in Mesembryanthemum crystallinum. J. Plant Physiol. 151 (3), 362–367.

Pfeiffer, C., Bloss, H., 1988. Growth and nutrition of guayule (Parthenium argentatum) in a saline soil as influenced by vesicular–arbuscular mycorrhiza and phosphorus fertilization. New Phytol. 108 (3), 315–321.

Phillips, J., Hayman, D., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55 (1), 158-IN118.

Praxedes, S.C., de Lacerda, C.F., Ferreira, T.M., Prisco, J.T., DaMatta, F.M., Gomes-Filho, E., 2011. Salt tolerance is unrelated to carbohydrate metabolism in cowpea cultivars. Acta Physiol. Plant. 33 (3), 887–896.

Rajguru, N., Banks, S.W., Gossett, D.R., Lucas, M.C., Fowler Jr., T. E., Millhollon, E.P., 1999. Antioxidant response to salt stress during fiber development in cotton ovules. J. Cotton. Sci 3, 11–18.

Taffouo, V., Wamba, O., Youmbi, E., Nono, G., Akoa, A., 2010. Growth, yield, water status and ionic distribution response of three Bambara groundnut (Vigna subterranea (L.) Verdc.) landraces grown under saline conditions. Int. J. Bot.

Tester, M., Davenport, R., 2003. Na+ tolerance and Na+ transport in higher plants. Ann. Bot. 91 (5), 503–527.

Tien, T.M., Gaskins, M.H., Hubbell, D.H., 1979. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37, 1016–1024.

Troll, W., Lindsley, J., 1955. A photometric method for the determination of proline. J. Biol. Chem. 215 (2), 655–660.

Vázquez, M.M., César, S., Azcón, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl. Soil Ecol. 15 (3), 261–272.

Wu, Q., Xia, R., 2005. Effects of AM fungi on drought tolerance of citrus grafting seedling trifoliate orange/cara cara. Ying yong sheng tai xue bao = J. Appl. Ecol./Zhongguo sheng tai xue xue hui, Zhongguo ke xue yuan Shenyang ying yong sheng tai yan jiu suo zhu ban 16 (5), 865–869.

Yilmaz, H., Kina, A., 2008. The influence of NaCl salinity on some vegetative and chemical changes of strawberries (Fragaria × ananssa L.). Afr. J. Biotechnol. 7 (18), 3299–3305.

Zayed, M.S., Hassanein, M., Esa, N.H., Abdallah, M., 2013. Productivity of pepper crop (*Capsicum annuum* L.) as affected by organic fertilizer, soil solarization, and endomycorrhizae. Ann. Agricult. Sci. 58 (2), 131–137.