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ORIGINAL ARTICLES

Pre-sowing Seed Treatment with Proline Improves some Physiological, Biochemical and Anatomical Attributes of Faba Bean Plants under Sea Water Stress

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ABSTRACT

Irrigation with diluted seawater can act as an alternate water resource and thus plays an important role in saving fresh water resources as well as promoting agriculture. Salinity stress is considered as one of the major abiotic stresses which strongly reduced crop productivity. A pot experiment was conducted at wire house of the National Research Centre, Dokki, Cairo, Egypt to elucidate the effect of pre-sowing seed treatment with proline (0, 5, and 10 mM as P0, P1 and P2, respectively) on some physiological, biochemical and anatomical attributes of faba bean (*Vicia faba* L.) plants under seawater stress (0.23, 3.13, 6.25 dS/m as tap water TW, SW1 and SW2, respectively). The irrigation with sea water was applied 16 days after sowing and lasted for 50 days. Plant samples were collected after 65 days from sowing. Results showed that increasing sea water concentration induced reduction in all growth parameters (plant height, number of leaves and shoot dry weights/plant), photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids), total carbohydrate, contents of P, Ca^{++}, K^{+} and K:Na ratio of faba bean leaves compared with those of the untreated unstressed plants(TWP0). Increasing sea water stress led to increases in total phenolics, free amino acids, proline and soluble carbohydrate as well as values of N, Na^{+}, and Cl^{-}. Special attention was paid to the effect of proline treatments on the salt stressed faba bean that stimulates plant salt tolerance via improving growth parameters, photosynthetic pigments, soluble carbohydrate and total carbohydrate meanwhile phenolic content, proline, Na^{+}, Cl^{-} were decreased relative to their corresponding salinity controls. Sea water stress and proline treatments induced over expression for new protein bands with high density. The effect of salinity stress and/or proline on anatomical structure of vegetative organs was under consideration. From these results, pre-sowing faba bean seed treatment with proline seem to enhance faba bean salt tolerance by amelioration of photosynthetic pigments, ion accumulations, and anatomical structure of vegetative organs, hence improved plant growth and the preservation of a suitable plant water status under salinity conditions.

Key words: Anatomical structure, ion accumulation, proline pre-sowing, protein pattern, salt stress, *Vicia faba*.

Introduction

With increasing demand for irrigation water, alternative sources are being sought. Saline water was previously considered unusable for irrigation. However, this water can be used successfully to grow crops under certain conditions (Zeid, 2011). Saline water has been used in different crops including food, fuel and fodder crops (Abazarian *et al.*, 2011).

Salinity stress in nature is mainly due to excess of sodium salts; particularly sodium chloride (NaCl). There is a general agreement that salinity stress at certain critical stages in plant growth causes more injuries arising from high accumulation of salts (Abdelhamid *et al.*, 2010). Salt stress can affect several physiological processes, from seed germination to plant development. The complexity of the plant response to salt stress can be partially explained by the fact that salinity imposes both an ionic and an osmotic stress (Jahari *et al.*,2010).

Numerous attempts have been made to improve the salt tolerance of crops by traditional breeding programmes, but commercial success has been very limited (Santa- Cruz *et al.,* 2002). Pre-sowing seed treatment or seed priming is an easy technique and an alternative approach recently used to overcome salinity problems. Priming (osmo-conditioning) is one of the physiological methods, which improves seed performance and provides faster and synchronized germination (Ashraf and Foolad, 2005). Pre-soaking or priming seeds of a number of crops has improved germination, seedling establishment and, in some cases, stimulated vegetative growth and hence crop yield (Kaur *et al.,* 1998). Seed priming enhance many of the metabolic processes involved with the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions (Desai *et al.*, 1997).

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Plants under salinity stress accumulate a number of metabolites, which are termed compatible solutes because they do not interfere with the plant's metabolism even at molar concentrations (Alonso *et al.,* 2001). Compatible solutes, such as proline, accumulate under salt stress in many crops acting as one of the osmoprotectants (Poustini *et al.,* 2007). It is evident from different reports that exogenous application of proline induces abiotic stress tolerance in plants (Claussen, 2005; Ali *et al.,* 2007). Its further role in salinity appears to involve the induction of salt responsive genes, with the resultant formation of new proteins which may improve the adaptation to salinity stress (Khedr *et al.,* 2003). Moreover, proline may be having a role in stabilization of cellular proteins and membranes in presence of high concentrations of osmotic stress. Proline accumulation in plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism (Jahari *et al.,* 2010). In addition, Vendruscolo *et al.,* (2007) reported that proline is involved in tolerance mechanisms against oxidative stress and this is the main strategy of plants to avoid detrimental effects of water stress. Proline plays an important role as a sink for energy to regulate redox potentials (Simiroff, and Cumbes, 1989), alleviates salt stress-induced by enhancement in oxygenase and carboxylase activities of Rubisco (Sivakumar *et al.,* 2000), and protects plants from free radical that induced damage by quenching of singlet oxygen (Matysik *et al.,* 2002). Several functions are proposed for the accumulation of proline in tissues exposed to salinity stress: osmotic adjustment (Voetberg and Sharp, 1991), C and N reserves for growth after stress relief (Hellmann *et al.,* 2000), detoxification of excess ammonia (Skopelitis *et al.,* 2006), stabilisation of proteins and membranes (Mansour, 1998), protection of macromolecules from denaturation (Hamilton and Heckathorn, 2001), osmoprotection (Kishor *et al.,* 1995), free radical scavenging (Chen and Dickman, 2005) antioxidation (Hoque *et al.,* 2007), and regulation of cytosolic acidity (Sivakumar *et al.,* 2000).

Faba bean (*Vicia faba* L.) is an important food crop in Egypt grown in winter season. It is a good source of protein for human food, and animal feeding which contains most of the necessary amino acids for human and animal nutrition and low sulphur amino acids concentrations.

Therefore, the present study was carried out to examine the effect of pre-sowing seed treatment with proline (0, 5, and 10 mM) on some physiological, biochemical and anatomical attributes of faba bean plants under diluted seawater irrigation (0.4, 6.1, and 12.2%).

Materials and Methods

Experimental procedures:

This study was conducted at the wire-house of the National Research Centre, Dokki, Cairo, Egypt (30° 3' 0" N / 31° 15' 0" E), from 6 December 2010 to 10 February 2011. Daytime temperatures ranged from 14.5 to 30.2◦C with an average of 23.2 ± 3.8◦C whereas temperatures at night were 12.4 ± 1.8◦C, with minimum and maximum of 8.0 and 17.6◦C, respectively. Daily relative humidity averaged 57.7± 9.6% in a range between from 38.1 to 78.7%.

Faba bean (*Vicia faba* L. cv. Giza-461) seeds were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Faba bean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. The seeds were divided into three groups, the first group soaked with distilled water, while second and third groups were soaked with two concentrations of proline 5& 10 mM, respectively for 12 hours then allowed to dry at room temperature (25◦C) for about 1h.

Ten uniform air dried faba bean seeds were sown along a centre row in each pot at 30-mm depth in plastic pots, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1(v:v), respectively in order to reduce compaction and improve drainage. Saline water was prepared by mixing fresh water (0.23 dS/m) with sea water (51.2 dS/m) to achieve salinity levels of 3.13 and 6.25 dS/m. Concentration of EC, pH, cations and anions of irrigation water and soils used on the pot experiment are shown on Table 1.

Table 1: EC,pH, concentrations of cations and anions of irrigation water and soils used on the pot experiment.

	EC	pH	Cations (meq/L)				Anions (meq/L)			
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^-	CO_3^{2-}	SO_4^{2-}	Cl^-
Water										
Tap water	0.23	7.35	1.00	0.50	2.40	0.20	0.10	0.00	1.30	2.70
Sea water	51.2	7.76	43.20	15.12	454.57	1.51	6.05	0.00	76.36	432.00
Soil										
Sandy	0.14	8.11	2.60	2.40	1.31	0.21	1.13	0.00	4.22	0.70
Clay	1.40	7.59	5.60	1.90	5.90	0.37	1.50	0.00	6.77	5.50

At sowing, a granular commercial rhizobia was incorporated into the top 30-mm of the soil in each pot with the seeds. Granular ammonium sulfate 20.5% N at a rate of 40 kg N ha^{-1}, and single superphosphate (15% P_2O_5) at a rate of 60 kg P_2O_5 ha^{-1} were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

The experiment consisted of three levels of proline, namely, 0, 5 & 10 mM considered as P0, P1 and P2, respectively, and irrigation water consisted of two concentrations of sea water namely, 3.13 and 6.25 dS/m which considered as SW1 and SW2, respectively, while control plants irrigated with tap water (0.23 dS/m) was considered as TW. Treatments were arranged at the wire-house benches in a factorial arrangement with five replicates for each treatment. Ten days after sowing (DAS), faba bean seedling were thinned to four seedlings per pot and irrigated with equal volumes of tap water until 15 DAS. Starting from 16^{th} day, all pots were irrigated either with tap water or different diluted sea waters along the period of the experiment (65 days).

Soil field capacity in the pots was estimated by saturating the soil in the pots with water and weighing them after they had drained for 48 h. Field water capacity was 0.36. Soil water content was maintained at about 90% of field water capacity. The level of soil moisture was controlled by weighing pots and daily loss of water was supplemented twice (morning and afternoon).

Measurements:

Plant samples were collected after 65 days from sowing for measurement of some growth parameters (i.e. plant height, leaves number and dry weights of shoot/plant), photosynthetic pigments, proline, total free amino acids, total phenolics, soluble carbohydrate, total carbohydrates and mineral contents in leaves tissue. Chlorophyll a, chlorophyll b and carotenoids were determined using spectrophotometric method described by Metzner *et al.*, (1965). Proline was estimated according to Bates *et al.*, (1973) and total free amino acids were determined according to Muting and Kaiser, (1963). The amount of total phenolics was determined using the Folin–Ciocalteu method Zhang and Wang, (2001). A calibration curve of Gallic acid was prepared, and the results were expressed as mg GAE (gallic acid). The phenol-sulfuric acid method was used for the determination of total carbohydrates (TC) (Smith *et al.*, 1956). Total soluble carbohydrates were determined according to (Yemm and Willis, 1954). Total Nitrogen was determined using the Kjeldahl method and P was photometrically determined using the molybdate-vanadate method. Potassium, Ca^{2+}, and Na^{+} were measured using a Dr. Lang M8D flame photometer. Nitrogen, P, K^{+}, Ca^{2+}, Na^{+} and Cl^{-} were measured in oven-dried faba bean leaves for 70◦C for 72 h according to (Jackson, 1973).

Electrophoretic determination of protein bands:

Faba bean samples were subjected to protein analysis according to their molecular weights by denatured sodium dodecyl sulphate (SDS)–PAGE as described by Laemmli, (1970). Protein bands were visualized by the naked eye and the data were recorded on photographs.

Anatomical studies:

A comparative microscopical examination was performed on plant material for treatments which showed remarkable response. Tested material included the main stem at its median portion and lamina of the first leaflet blade of the compound leaf developed on the median portion of the main stem of normal faba bean plants and those of plants grown under salinity stress of 6.25 dS/m as well as of those affected by seed soaking with 10 mM proline and of those received combined treatment of salinity and proline. Specimens were taken from plants aged 65 days, killed and fixed for at least 48 hrs. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56^{o}C, sectioned to a thickness of 20 microns, double stained with safranin-light green, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of noticeable responses resulted from mentioned treatments and photomicrographed

Statistical analysis:

The data were subjected to the analysis of variance (ANOVA) appropriate to the randomized complete block design applied after testing the homogeneity of error variances according to the procedure outlined by (Gomez and Gomez, 1984). The significant differences between treatments were compared with the critical difference at 5% probability level by the Duncan's test.

Results and Discussion

Faba bean growth parameters:

Salt stress, like many abiotic factors, reduces the ability of plants to take up water, leading to growth reduction as well as metabolic changes and upset nutritional balance of plant. Meanwhile, the effectiveness of

proline application on plants depends on the type of species, plant developmental stage, time of application and concentration. Moreover, under adverse environmental conditions, the effect of proline application is species specific (Ashraf and Foolad, 2007). Data presented in Table 2 indicate that increasing sea water concentration (SW1P0 and SW2P0 as 3.13 and 6.25 dS/m, respectively) caused significant decreases in plant height and shoot dry weight of faba bean plant and improvement on these traits were coincided with the combined SW with proline (P1 and P2). Similar data were recorded by (Kharadi *et al.,* 2011; Khalil and El-Noemani, 2012; Heidari, 2012). The reduction in growth parameters of faba bean plants under salinity stress might be attributed to the reduction in cell division, cell elongation and meristematic activity (Bolus *et al.,* 1972) or due to the reduction in water absorption, reduced metabolic activities due to Na^+ and Cl^- toxicity and nutrient deficiency caused by ionic interference (De Lacerda *et al.,* 2003). It is clear that sea water stress reduced plant height, leaves number and shoot dry weight while improved when combined with proline (SW1P1, SW1P2, SW2P1 and SW2P2).The increases in growth characters caused by low and high proline concentrations might be due to the role of proline in protecting enzymes, 3D structures of proteins and organelle membranes and also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque *et al.,* 2007; Ashraf and Foolad, 2007).

Table 2: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on plant height, number of leaves and shoot dry weight of faba bean plant at 65 days after sowing.

Sea water	Proline	Plant height (cm)	Number of leaves/plant	Shoot dry weight (g/plant)
TW	P0	61.0a[1]	10.5b	1.92bc
	P1	62.3a	10.9b	2.08b
	P2	64.3a	11.8a	2.69a
SW1	P0	48.7c	10.3b	1.56cd
	P1	56.7ab	10.8b	1.81bc
	P2	59.0ab	11.0a	1.87bc
SW2	P0	44.3d	10.0b	1.28d
	P1	51.7c	10.2b	1.55cd
	P2	56.0ab	10.4b	1.82bc

[1]Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$).

Photosynthetic pigments:

The chlorophyll content reflects the photosynthesis rate of plant, which strongly influenced by environmental factors (Qiu and Guo, 2007). Data in (Fig. 1A, B, C, and D) showed that chlorophyll a,b, carotenoids, and total pigments were negatively affected by application of sea water irrigation. These results are similar with those findings by (Stoeva and Kaymakanova, 2008; Heidari, 2012). Reduction in chlorophyll content under salinity can be attributed to a salt-induced weakening of protein-pigment-lipid complex or inhibition synthesis of chlorophyll or accelerating its degradation via increased chlorophyllase enzyme activity (Stivesev *et al.,* 1973). On the other hand, application of proline increased chlorophyll a, b, carotenoids, and total pigments under saline and non-saline conditions. Fig. 1 demonstrates that SW1P1, SW1P2, SW2P1 and SW2P2 caused significant increases in chlorophyll a, b and total photosynthetic pigments relative to SW1P0 and SW2P0. The highest values of chlorophyll a, b, carotenoids and total pigments were scored in plant leaves treated with SW0P2 and the lowest values resulted from SW2P0. Proline-treatments had the ability to alleviate the adverse effects of salinity on photosynthetic pigments. Yan *et al.,* (2011) mentioned that proline not only functioned as a nutrient but also possessed some defensive mechanisms for damaged plants under salt stress. These mechanisms were, promoting photosynthesis, maintaining enzyme activity and scavenging ROS. Ali *et al.,* (2007) explained the beneficial effect of proline applied was due to its promotive effects on photosynthetic capacity by overcoming stomata limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from water stress-induced degradation.

Total phenolics, free amino acids, and proline contents:

Results in (Fig. 2-A) showed that total phenolics (mg/g) increased significantly with increasing sea water concentration (SW1P0 and SW2P0) relative to control (TWP0). These data are in good agreement with those obtained by (Mohamed and Aly, 2008) on onion plant and El Hariri *et al.,* (2010) on flax plant. It is well known that, phenolic compounds play a key role as protective components of plant cells. The potential activity of phenolics to act as an antioxidant is mainly due to their properties to act as hydrogen donators, reducing agents and quenchers of singlet O_2 (Zhang and Wang, 2001). The synthesis of phenolics is generally affected in response to different biotic/abiotic stresses including salinity (Parida *et al.,* 2004). Two proline doses (5 and 10 mM) caused increases in phenolic contents in faba bean plants irrigated with tap water (0.23 dS/m) relative to control plant (TWP0). Proline treatments (P1 and P2) caused marked decrease in phenolic content in plants irrigated with two levels of sea water (SW1 and SW2) as compared to corresponding salinity control (SW1P0 and SW2P0). These decreases were significant in all cases except that from SW1P2 was non significant.

Moreover, it was noted that proline at 5 mM showed more pronounced effect in decreasing phenolic content than proline at 10 mM under salinity stress.

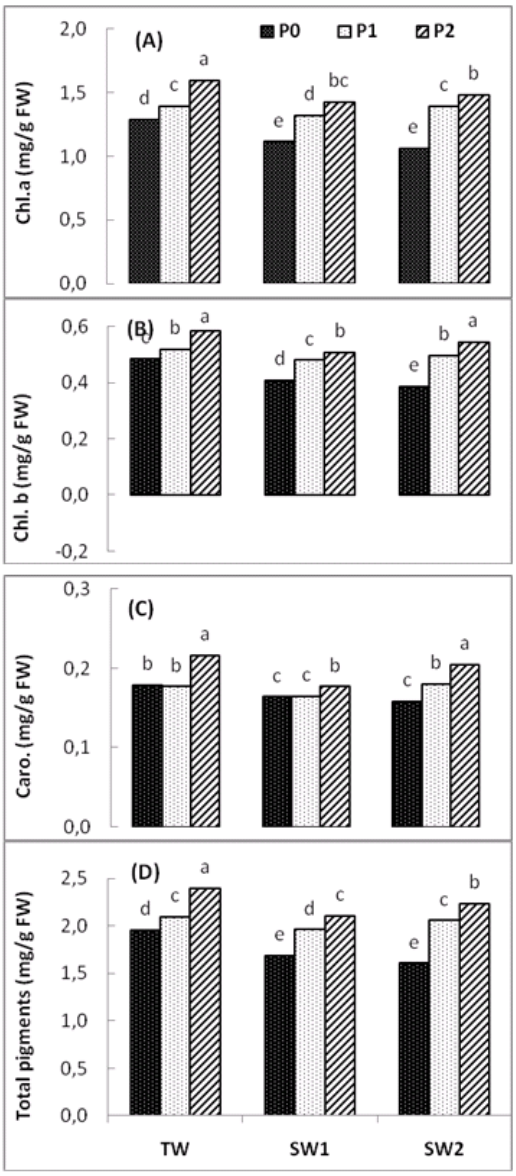


Fig. 1: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on chlorophyll *a*, chlorophyll *b*, caroteniods, and total photosynthetic pigments of faba bean plant at 65 days after sowing.

Fig. 2-B shows that either salinity (SW1P0 and SW2P0) or proline treatments (TWP1 and TWP2) caused significant increases in free amino acids (mg/g) relative to control plants (TWP0). These results are in agreement with those reported by Rao *et al.,* (2009) and Kala and Godara, (2011). It was noted that plants treated with two proline doses and irrigated with 3.13 dS/m of sea water (SW1P1and SW1P2) showed significant increase in free amino acids relative to (SW1P0). Meanwhile, those irrigated with 6.25 sea water (SW2P1and SW2P2) showed significant decreases relative to (SW2P0).

Fig. 2-C illustrated that proline accumulation in faba bean plants increased significantly and gradually with increasing sea water concentration relative to TWP0 treatment. The increase in proline levels due to salinity was also demonstrated by Poustini *et al.,* (2007). The higher accumulation of proline under salinity stress could be due to enhanced activities of ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P-5-CR), the enzyme involved in proline biosynthesis Giridara *et al.,* (2003), as well as due to inhibition of proline oxidase and proline dehydrogenase (PDH), the proline catabolizing enzymes Kandpal *et al.,* (1981). Pronounced accumulation of organic solutes (proline, saccharides, protein and total amino acids) for osmotic adjustment was reported by Abd El-Samad *et al.,* (2011). In view of some earlier reports who suggested that exogenously

proline applied might have enhanced endogenous proline accumulation under water stress conditions which not only protects enzymes, 3D structures of proteins and organelle membranes, but it also supplies energy for growth and survival thereby helping the plant to tolerate stress (Hoque *et al.,* 2007; Ashraf and Foolad, 2007). Fig. 2-C illustrates that proline treatments at 5 and 10 mM caused significant increases in proline contents(mg/g) in leaves of faba bean plants that irrigated with tap water (TWP1 and TWP2) or those irrigated with sea water at two levels (SW1P1, SW1P2, SW2P1 and SW2P2) relative to control (TWP0). Special attention must be paid to that faba bean plants treated with proline at two doses and irrigated with sea water at two levels showed significant reduction in proline content than corresponding controls (SW1P0 and SW2P0).

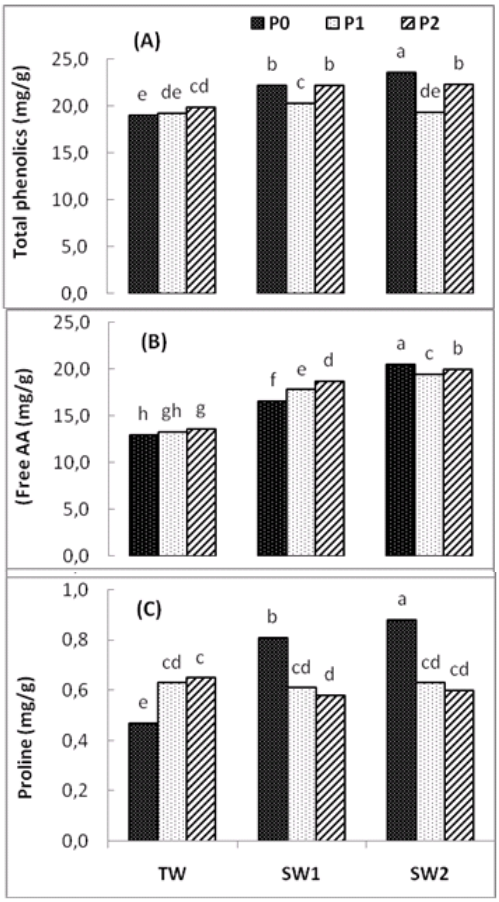


Fig. 2: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on total phenolics, free amino acids and proline of faba bean plant at 65 days after sowing.

Total carbohydrates and soluble carbohydrates:

Fig. 3-A shows that total carbohydrate in faba bean leaves was negatively affected by salinity stress. The second level of salinity (SW2P0) recorded the highest decrease in total carbohydrate. This is may be due to inhibition of photosynthesis which is associated with decline in total pigments content (Fig. 1-D), while total soluble carbohydrate (Fig. 3-B) showed opposite trend, since it was increased significantly and gradually with increasing salinity levels. These results are in harmony with those reported by Maria *et al.,* (2000) who mentioned that salinity stress caused an increase in soluble sugar content with increasing salinity levels while an opposite trend was obtained with respect to polysaccharide concentration. The increase in soluble sugars may be attributed to certain chemical stimulus (mostly ABA) through xylem vessels to leaves of stressed plants which led to stomata closure, reduction of each CO_2 stomata conductance, CO_2 concentration in leaf tissues, electron transport system, CO_2 fixation, rate of photosynthesis and eventually quantity of photosynthesis, thus decline in growth rates (Abdalla and El-Khoshiban,2007). Treated plants with low and high proline concentrations and irrigated with tap water exhibited significant increase in both total carbohydrates and total soluble carbohydrate compared with control treatment (TWP0). Meanwhile, Fig. 3-A illustrates that SW1P1 and SW1P2 as well as SW2P1 and SW2P2 showed significant increase in total carbohydrate % relative to SW1P0 and SW2P0. Salinity and / or proline treatments caused increase in soluble carbohydrates (Fig. 3-B) relative to corresponding

control plants. The previous data are in contrast with those obtained by Tarraf, (1999) who reported that application of proline decreased soluble sugar and carbohydrate contents of lupine plant.

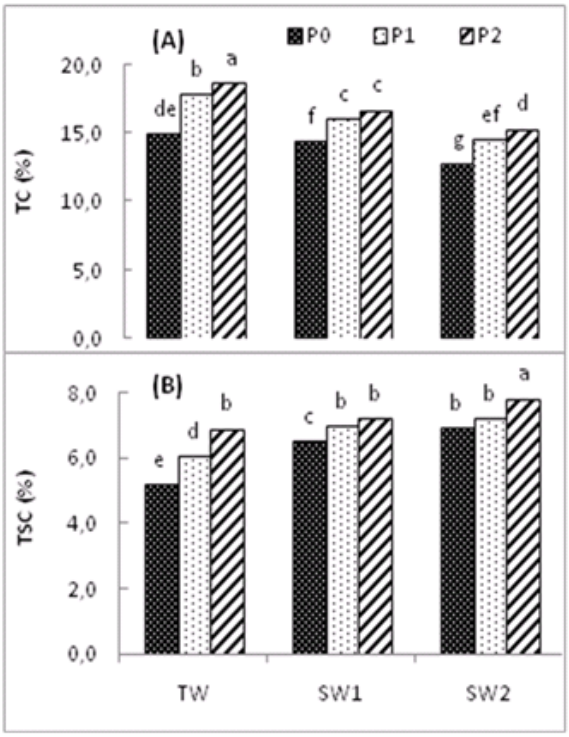


Fig. 3: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on total carbohydrate (TC%) and total soluble carbohydrate (TSC%) of faba bean plant at 65 days after sowing.

Minerals content:

Salinity stress caused significant increase in N, Na^+, Cl^- contents, and significant decrease in phosphorous, calcium, and potassium contents of faba bean plants (Table 3) relative to control (TWP0). The K: Na ratio decreased significantly and gradually in faba bean plants with increasing salinity levels. Our results are in agreement with Gadallah, (1999). In this connection, Kiarostami *et al.,* (2010) suggested that increased accumulation of sodium (Na^+) and (Cl^-) ions in the tissues inhibits biochemical processes related to photosynthesis through direct toxicity and led to low water potential. The promotion of Na^+ uptake by salinity was accompanied by corresponding decline in K^+ concentration, showing an apparent antagonism between K^+ and Na^+ (Cuin *et al.,* 2009). The decrease of K^+ concentration with increasing soil salinity suggests that Na^+ inhibited the K^+ uptake. Such increase in Na^+ values in response to stress is considered as one of the defense mechanism which stressed plants can lead in order to control osmotic pressure of stressed cells and tissues so as to raise their ability of water and solute uptake from soil (Rodriguez *et al.,* 1996). On the other hand, the decreased levels of each of K^+, P, and Ca^{++} in response to stress were ascertained by the work of each of (Bie *et al.,* 2004; Koyro, 2006; Wu and Xia, 2006). Such reductions in the contents of these elements in leaf tissues were attributed primarily to soil water deficiency which markedly reduces the flow rates of elements in soil, their absorption by stressed root cells and also its ability to translocation through the different organs and tissues. Application of proline (5 mM &10 mM) exhibited significant enhancement in N, P, Ca^{++}, K^+ content, and K:Na ratio and non significant decrease in Na^+, but significantly decreased Cl^- in plants leaves. Same trend appears as a result of proline treatments (P1 and P2) combined with salinity (SW1 and SW2) as compared to their corresponding salinity control. Cuin and Shabala, (2007) reported that solutes like glycinebetaine or proline significantly reduced K^+ efflux from the cell and maintains cytosolic K^+ homeostasis possibly through the enhanced activity of H^+ATPase. This is in turn controls voltage-dependent outward-rectifying K^+ channels and created the electrochemical gradient necessary for secondary ion transport processes (Cuin and Shabala, 2005).

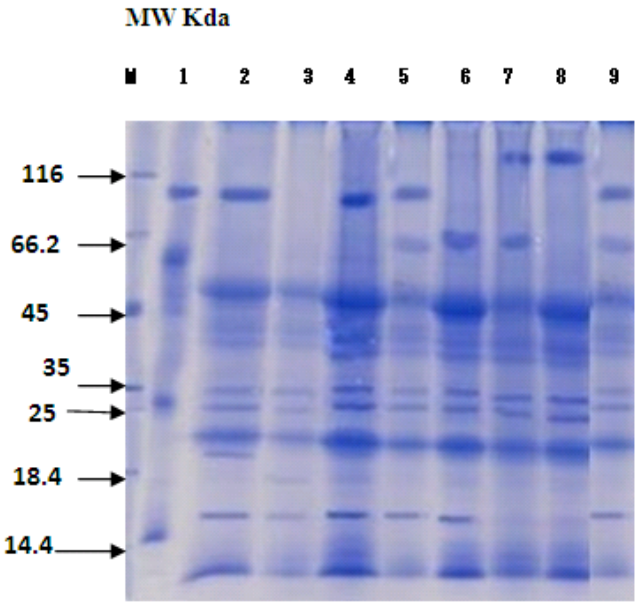
Table 3: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on N%, P%, Ca%, K%, Na% ,Cl(ppm), and K/Na ratio of leaves of faba bean plant at 65 days after sowing.

Sea Water	Proline	N%	P%	Ca%	K%	Na%	K/Na ratio	Cl(ppm)
TW	P0	3.93e[1]	0.37c	2.72b	2.23b	0.17c	13.4b	2.85d
	P1	4.14d	0.42b	2.84a	2.36a	0.15c	16.6a	2.74e
	P2	4.33c	0.47a	2.95a	2.46a	0.14c	17.6a	2.67f
SW1	P0	4.17d	0.34c	2.42d	2.16c	0.26b	8.4d	3.96c
	P1	4.34c	0.37c	2.52c	2.22b	0.24b	9.3c	3.87d
	P2	4.52b	0.40b	2.64b	2.30b	0.22b	10.5c	3.76e
SW2	P0	4.28c	0.29d	2.26e	2.04d	0.35a	5.8f	4.19a
	P1	4.50b	0.33c	2.38d	2.10d	0.33a	6.4e	4.08b
	P2	4.68a	0.36c	2.45d	2.16c	0.31a	7.0e	3.96c

[1]Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($P < 0.05$)
TW= Tap water (0.23 dS/m); SW1=Sea water (3.13 dS/m); SW2=Sea water (6.25 dS/m)
P0= 0 Proline; P1=5 mM Proline; P2=10 mM Proline

Protein pattern:

Salinity stress induced changes in the protein profile of faba bean plant .Quantitative and qualitative differences were obtained when faba bean seeds soaked with different concentrations of proline (5&10mM) in the presence or absence of sea water (Fig.4).



1: Control (tap water without proline)
2: Tap water + Proline (5 mM)
3: Tap water +Proline (10 mM)
4: Sea water (3.13 dS/m)
5: Sea water 3.13 dS/m + Proline 5 mM
6: Sea water 3.13 dS/m + Proline 10 mM
7: Sea water (6.25 dS/m)
8: Sea water 6.25 dS/m + Proline 5 mM
9: Sea water 6.25 dS/m + Proline 10 mM

Fig. 4: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on SDS-PAGE protein patterns of faba bean plant at 65 days after sowing.

As shown in figure 4, seven bands were scored in the protein profile of the leaves extract for faba bean under control treatment (TW). Salinity stress (SW1) induced over expression for nine bands with high density.

When plants under control conditions subjected to proline (P1) eight bands were detected at (105, 60, 52, 40, 32, 19, and 15 Kda). Whereas with P2 one band was disappeared at (91Kda). Soaking of faba bean seeds with P1 caused disappearance of two bands with molecular weight (61, and 48 Kda). Whereas (P2) in the presence of salinity (SW1) showed disappearance of one band with (95 Kda) was observed. Sea water (SW2) induced induction of new two bands at (122, and 63Kda) compared to Lane four (SW1) sea water. Consistent disappearance for one band at (60 Kda) with sea water at 6.25 dS/m combined with proline 5mM (SW2P1). In contrast when seeds soaked with 10 mM proline in the presence of 6.25 dS/m sea water (SW2P2) three new bands were detected at 89, 61, and 17Kda compared to SW2P1. The changes in protein profile may be due to adaptation of faba bean plants to sea water stress. The new and disappearance bands of proteins with salinity or in combination with 5or10 mM of proline may be due to *de novo* synthesis of new protein. In support of present results, (Khedr *et al.,* 2003) on *Pancratium maritimim* L., Bahrman *et al.,* (2003) on wheat and Chourey *et al.,* (2003), on rice and Muayed *et al.,* (2012) on *Citrus sinensis* L. They demonstrated that osmotic stresses were able to trigger the accumulation of several major stress proteins. They also stated that the accumulation levels of these proteins correlated with stress tolerance in the various plant species, suggesting protective roles under osmotic stress, and that recovery from salt stress was consistently accompanied by degradation of the salt-stress induced proteins. The new bands and the significant increase in the intensity of faba bean as well as the original bands appearing in the control indicate that proline has stimulatory effect on the protein biosynthesis, which might be linked with the improvement of growth. Therefore, it can be suggested that the new proteins which appeared in plants grown under salinity stress alone or combined with proline did not appear in untreated plants (control), may play an inductive role in triggering a special system helping plants to tolerate salinity stress and increase their ability to grow.

Anatomical studies:

As inferred earlier throughout the investigations on vegetative growth, increasing salinity level (0.23, 3.13 and 6.25 dS/m) decreased all the studied growth parameters (plant height, number of leaves, and dry weights of faba bean plant). In direct contrast, seed soaking with 5 and 10 mM proline increased all investigated growth parameters. At the same time, proline treatment counteracted the harmful effect of salinity on vegetative growth characters under investigation. This may justify a further study on the internal structure of the main stem and the leaves of normal faba bean plants and of those grown under salinity stress as well as of those obtained from seeds soaked in proline either grown under tap water irrigation or under salinity stress. Microscopical characters were examined through specimens of the median internode of the main stem and its corresponding leaf from plants aged 65 days. This surely highlights the effect of studied treatments on microscopically characters of these organs.

Anatomy of the main stem:

Microscopical measurements of certain histological characters in transverse sections through the median internode of the main stem of faba bean grown under salinity stress of 6.25 dS/m and affected by seed soaking with 10 mM proline are given in Table 4. Likewise, microphotographs illustrating these treatments as well as the untreated plants are shown in transverse sections in Fig. 5. As to the effect of salinity stress on stem anatomy of faba bean, it is clear that the concentration of 6.25 dS/m decreased the internodes diameter by 25% below the control. This decrease in internodes diameter was mainly due to a decrease in thickness of the stem wall as well as the diameter of hollow pith. The decrements below the control were 26.2 and 23.9%, respectively. It is realized that the decrease observed in stem wall thickness as a result of salinity stress could be attributed to the decrements induced in all included tissues. The thickness of epidermis, cortex, fiber tissue, phloem tissue, xylem tissue and parenchymatous area of the pith were decreased in treated plants below the control by 4.0, 19.8, 16.5, 16.1, 30.3 and 31.9%, respectively. Likewise, the mean value of vessel diameter in treated plants was decreased below the control by 23.4%. In conclusion, salinity stress (at 6.25 dS/m) caused considerable thinned stems of faba bean by decreasing internode diameter due to decrease in thickness of the stem wall as well as the diameter of hollow pith. The decrease in stem wall thickness was accompanied by decrements in all included tissues. These results are generally in harmony with those reported by Reda *et al.,* (2000) on leucaena plants and by Reda, (2007) on coffee senna plants. It is obvious that seed soaking with 10 mM proline induced prominent increase in internode diameter by 17.3% over the control. This increment in internode diameter was mainly due to the prominent increase in the thickness of stem wall and in the diameter of hollow pith by 13.7 and 19.8% over the control; respectively. It is clear that the increase which was observed in stem wall thickness could be attributed to the increments induced in all included tissues except that of parenchymatous area of the pith which was decreased by 15.4% below the control. The increments due to proline effect were 28.0, 47.5, 53.6, 40.2 and 18.6% over the control for thickness of epidermis, cortex, fiber tissue, phloem tissue and xylem tissue;

respectively. A worthy to mention that, the mean value of vessel diameter was also increased by 10.6% over the control.

Table 4: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on measurements in microns of certain histological characters in transverse sections through the middle part of the main stem of faba bean plant at 65 days after sowing

Histological characters	Treatments						
	TW	SW2	± % to TW	TW + P2	± % to TW	SW2+P2	± % to TW
Stem diameter	4650	3487	–25.0	5452	+17.3	4139	– 11.0
Stem wall thickness	982	725	– 26.2	1116	+13.7	907	– 7.6
Epidermis thickness	25	24	– 4.0	32	+28.0	30	+20.0
Cortex thickness	116	93	– 19.8	171	+47.5	128	+10.4
Fiber tissue thickness	97	81	– 16.5	149	+53.6	105	+8.3
Phloem tissue thickness	87	73	– 16.1	122	+40.2	82	– 5.8
Xylem tissue thickness	231	161	– 30.3	274	+18.6	192	– 16.9
Vessel diameter	47	36	– 23.4	52	+10.6	49	+4.3
Parenchymatous Pith thickness	429	292	– 31.9	363	-15.4	374	– 12.8
Hollow pith diameter	2684	2042	– 23.9	3216	+19.8	2316	– 13.7

TW= Tap water (0.23 dS/m); SW2=Sea water (6.25 dS/m); P2=10 mM Proline

Data presented in Table 4 and microphotographs shown in Fig. 5 reveal that proline treatment enhanced all histological characters of salinity stressed stems of faba bean and this means that proline treatment counteracted the harmful effect of salinity on stem anatomy of faba bean. Stem diameter was decreased by 11.0% below the control. Such decrement in stem diameter could be attributed to the decrease induced in stem wall thickness and hollow pith diameter by 7.6 and 13.7 % below the control, respectively. The decrease in stem wall thickness could be attributed mainly to the decrements induced in thickness of phloem tissue, xylem tissue and parenchymatous area of the pith by 5.8, 16.9 and 12.8% below the control; respectively. Other included tissues showed increments in this respect. Thickness of epidermis, cortex and fiber tissue were increased over the control by 20.0, 10.4 and 8.3%; respectively. Also, mean diameter of vessel was increased by 4.3% over the control. Information about the effect of seed soaking with proline on stem anatomy of normal and stressed plants of faba bean is not available.

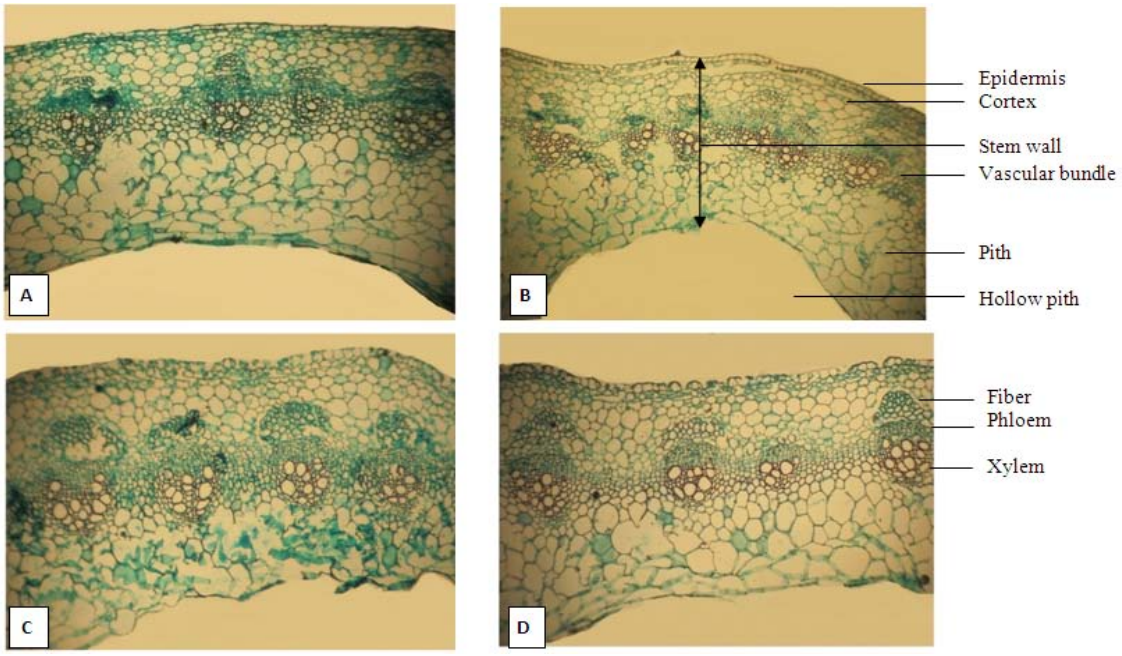


Fig. 5: Effect of pre-sowing seed treatment with proline on cross sections of stem anatomy of faba bean plants grown under sea water stress. (x 68). (A: tap water without proline; B: 6.25 dS/m sea water; C: Tap water +10 mM proline; D: 6.25 dS/m sea water + 10 mM proline).

Anatomy of the leaf:

Certain microscopical characters in transverse sections of the first leaflet blade of the compound leaf developed on the median portion of the main stem of faba bean grown under salinity stress and affected by seed

soaking with proline were followed up in form of counts and measurements being given in Table 5. These characters in control and treated plants are further shown as microphotographs illustrated in Fig. 6. It is realized that salinity stress at the level of 6.25 dS/m reduced the thickness of both midvein and lamina of leaflet blade by 16.7 and 17.6% less than those of control, respectively. The thinner leaflets induced by salinity stress could be attributed to the decrease induced in thickness of both palisade and spongy tissues as well as in the dimensions of midvein bundle. The decrements below the control were 30.3, 13.6, 14.6 and 15.8% for palisade tissue, spongy tissue, and length of midvein bundle and width of midvein bundles, respectively. Also, the number of vessels/midvein bundle was decreased less than the control by 24.1%. Moreover, the mean diameter of vessel for leaves of stressed plants was decreased by 8.8% less than the control. The obtained results are in agreement with those reported by Wignarajak *et al.*, (1975) on beans as well as by Reda *et al.*, (2000) on leucaena and by Boghdady, (2009) on mung bean. As to the action of seed soaking with 10 mM proline, it is clear that such treatment increased thickness of both midvein and lamina of leaflet blades of faba bean plant by 8.4 and 16.6% more than the control, respectively. It is obvious that the increase in lamina thickness was accompanied with 7.0 and 29.0% increments in thickness of palisade tissue and spongy tissue compared with the control, respectively. Likewise, the main vascular bundle of the midvein was increased in size as a result of proline treatment. The increment was mainly due to the increase in length by 22.6% and in width by 30.4% more than the control. Moreover, average number of vessels/medvein bundle was increased by 10.3% over the control. Likewise, xylem vessels had wider cavities, being 11.8% more than the control, which amounted to more total active conducting area to cope with the vigorous growth resulting form seed soaking with 10 mM proline.

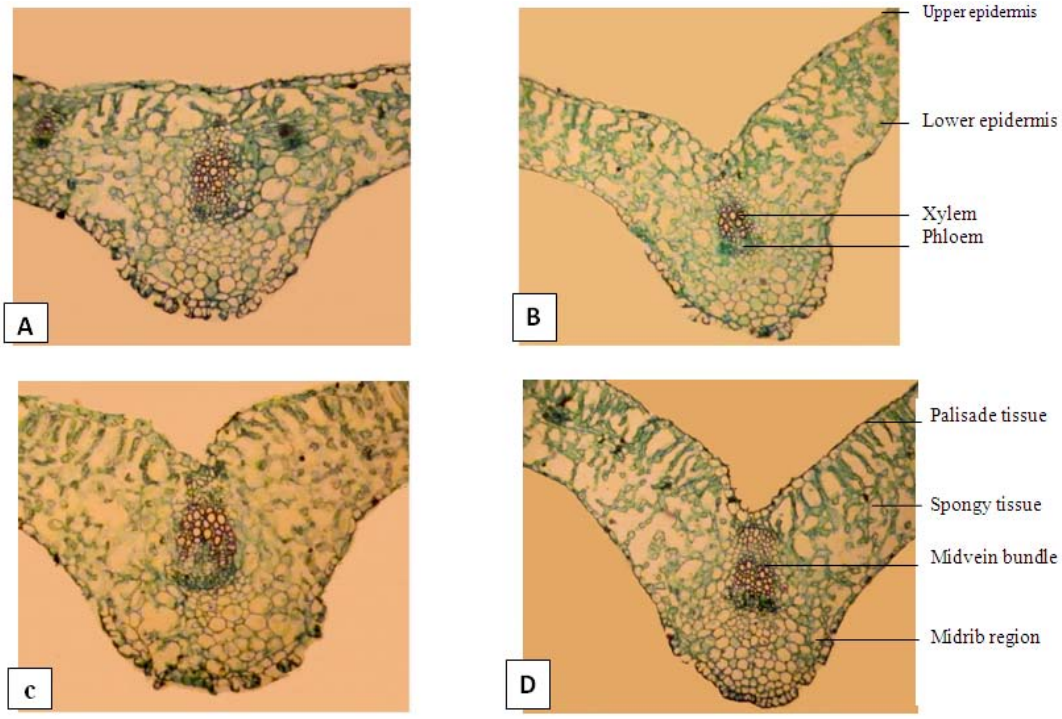


Fig. 6: Effect of pre-sowing seed treatment with proline on transverse section of leaflet blade of faba bean plants grown under sea water stress. (x 68). (A: tap water without proline; B: 6.25 dS/m sea water; C: Tap water +10 mM proline; D: 6.25 dS/m sea water + 10 mM proline).

Results also indicated that proline treatment enhanced most of the histological characters of leaflets of stressed plants and this means that seed soaking with 10 mM proline had the ability to minimize the deleterious effect of salinity on anatomical structure of faba bean leaves. It is clear that midvein thickness was decreased by 8.1% less than the control. Likewise, the dimensions of midvein bundle were decreased in length by 11.9% and in width by 3.5% below the control although an increase of 3.8% in number of vessels/midvein bundle was observed over the control and the mean vessel diameter was decreased below the control by 14.7%. At the same time thickness of lamina was increased by 9.6% over the control due mainly to the increase observed in thickness of palisade tissue by 23.2% and in thickness of spongy tissue by 7.9% over the control. Information about the effect of seed soaking with proline on leaf anatomy of normal and stressed plants of faba bean is not available.

Table 5: Interactive effects of pre-sowing seed treatment with different concentrations of proline (P) and different levels of sea water stress (SW) on counts and measurements in microns of certain histological characters in transverse sections through the leaflet blade of the compound leaf developed on the median portion of the main stem of faba bean plant at 65 days after sowing.

Histological characters	Treatments						
	TW	SW2	± % to TW	TW+P2	± % to TW	SW2+P2	± % to TW
Thickness of midvein	862	718	– 16.7	934	+8.4	792	–8.1
Thickness of lamina	427	362	– 17.6	498	+16.6	468	+9.6
Thickness of palisade tissue	142	99	– 30.3	152	+7.0	175	+23.2
Thickness of spongy tissue	214	185	– 13.6	276	+29.0	231	+7.9
Dimensions of midvein bundle:							
Length	336	287	– 14.6	412	+22.6	296	–11.9
Width	171	144	– 15.8	223	+30.4	165	– 3.5
No. of vessels/midvein bundle	29	22	–24.1	32	+10.3	30	+3.8
Vessel diameter	34	31	– 8.8	38	+11.8	29	– 14.7

TW= Tap water (0.23 dS/m); SW2=Sea water (6.25 dS/m); P2=10 mM Proline

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References

Abazarian, R., M.R. Yazdani, K. Khosroyar, P. Arvin, 2011. Effects of different levels of salinity on germination of four components of lentil cultivars," Afri. J. of Agric. Res., 6: 2761-2766.

Abdalla, M.M., N.H. El-Khoshiban, 2007. The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* cultuivars. J. of Appl. Sci. Res., 3: 2062-2074.

Abdelhamid, M.T., M. Shokr, M.A. Bekheta, 2010. Growth, root characteristics, and leaf nutrients accumulation of four faba bean (*Vicia faba* L.) cultivars differing in their broomrape tolerance and the soil properties in relation to salinity. Comm. in Soil Sci. and Plant Anal., 41: 2713-2728.

Abd El-Samad, H.M., M.A.K. Shaddad, N. Barakat, 2011. Improvement of plants salt tolerance by exogenous application of amino acids. J.of Med. Pla. Res., 5: 5692-5699.

Ali, Q., M. Ashraf, H.R. Athar, 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pakistan J. of Bot., 39: 1133-1144.

Alonso, R., S. Elvira, F.J. Catillo, B.S. Gimeno, 2001. Interactive effects of ozone and drought stress on pigments and activities of antioxidative enzymes in *Pinis halpensis*. Plant Cell Envi., 24: 905-916.

Ashraf, M., M.R. Foolad, 2005. Pre-sowing seed treatment-a shotgun approach to improve germination growth and crop yield under saline and non-saline conditions. Adva. Agro., 88: 223-271.

Ashraf, M., M.R. Foolad, 2007. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. Envir. and Exp. Bot., 59: 206-216.

Bahrman, N., J. Le Gouis, L. Negroui, L. Amilhat, P. Leory, A. Laine, O. Jaminon, 2003. Differential protein expression assessed by electrophoresis for two wheat varieties grown at four nitrogen levels. Proteomics, 4: 709-719.

Bates, L.S., R.P. Waldan, L.D. Teare, 1973. Rapid determination of free proline under water stress studies. Plant and Soil, 39: 205-207.

Bie, Z., T. Ito, Y. Shinohara, 2004. Effects of sodium sulphate and sodium bicarbonate on the growth, gas exchange and mineral composition of lettuce .Scientia Hortic., 99: 215-224.

Boghdady, M.S., 2009. Physiological and anatomical studies on mung bean plant under salinity conditions. Ph.D. Thesis, Faculty of Agri., Zagazig University, 222.

Bolus, S.T., M.N. El-Shourbary, N.L. Missak, 1972. Studies on the effect of salintty on the epidermis and mesophyll tissues of some *Ricinus communis*, L. varieties. Desert Insti. Bull., 22: 421-432.

Chen, C., M.B. Dickman, 2005. Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. Proc. Nati. Acad.Sci. USA, 102: 3459-3464.

Chourey, K., S. Ramani, S.K. Apte, 2003. Accumulation of LEA proteins in salt (NaCl) stressed young seedlings of rice (*Oryza sativa* L.) cultivar Bura Rata and their degradation during recovery from salinity stress. J. Plant Physio., 160: 1165-1174.

Claussen, W., 2005. Proline as a measure of stress in tomato plants. Plant Sci., 168: 241-246.

Cuin, T.A., S. Shabala, 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. Plants Cell Physio., 46: 1924-1933.

Cuin, T.A., S. Shabala, 2007. Compatible solutes reduce ROS-induced potassium efflux in cultivars (*Cucumis me*lo L.) under NaCl stress. Afri. J. Biotech., 10: 18381-18390.

Cuin, T.A., Y. Tian, S.A. Betts, R. Chalmandrier, S. Shabala, 2009. Ionic relations and osmotic adjustment in durum and bread wheat under saline conditions. Func. Plant Biol., 36: 1110-1119.

De Lacerda, C.F., J. Cambraia, M.A. Oliva, H.A. Ruiz, 2003. Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress. Braz. J. Plant Physiol., 15: 113-118.

Desai, B.B., P.M. Kotecha, D.K. Salunkhe, 1997. Seeds Handbook. Marcel Dekker, Inc., New York.

El Hariri, D.M., M.Sh. Sadak, H.M.S. El-Bassiouny, 2010. Response of flax cultivars to ascorbic acid and α-tocopherol under salinity stress conditions. Inter.J.Acad. Res., 2: 101-109.

Gadallah, M.A.A., 1999. Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress, Plant Biol., 42: 249-257.

Giridara, K.S., A. Matta Reddy, C. Sudhakar, 2003. NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus Alba* L.) with contrasting salt tolerance. Plant Sci., 165: 1245-1251.

Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons Inc., Singapore, 680.

Hamilton, E.W., S.A. Heckathorn, 2001. Mitochondrial adaptations to NaCl. Complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. Plant Physio., 126: 1266-1274.

Heidari, M., 2012. Effects of salinity stress on growth, chlorophyll content and osmotic components of two basil (*Ocimum basilicum* L.) genotypes. Afri. J. Biotec., 11: 379-384.

Hellmann, H., D. Funk, D. Rentsch, W.B. Frommer, 2000. Hypersensitivity of an Arabidopsis sugar signaling mutant toward exogenous proline application. Plant Physio., 122: 357-368.

Hoque, M.A., M.N. Banu, E. Okuma, K. Amako, Y. Nakamura, Y. Shimoishi, Y. Murata, 2007. Exogenous proline and glycinebetaine increase NaCl-induced ascorbate-glutathione cycle enzyme activities, and proline improve salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. J. Plant Physio., 164: 1457-1468.

Jackson, M.L., 1973. Soil chemical analysis. 1st Edition. Prentice Hall of India Pvt. Ltd., New Delhi, India, 61-73.

Jahari, M.P., N. Qasimov, H. Maralian, 2010. Effect of soil water stress on yield and proline content of four wheat lines. Afri.J. Biotec., 9: 036-040.

Kala, S., A.K. Godar, 2011.Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of *ziziphus mauritiana*. J. Sci. Res., 55: 65-69.

Kandpal, R.P., C.S. Vaidyanathan, M. Udaykumar, K.S. Krishnassastry, N. Appaji-Rao, 1981. Alteration in the activities of the enzyme of praline metabolism in ragi (*Eleusine coracane*) leaves during water stress, J. Biosci., 3: 361-369.

Kaur, S., A.K. Gupta, N. Kaur, 1998. Gibberellic acid and kinetin partially reverse the effect of water stress on germination and seedling growth. Plant. Growth Regu., 25: 29-33.

Khalil, S.E., A.A. El-Noemani, 2012. Effect of irrigation intervals and exogenous proline application in improving tolerance of garden cress plant (*Lepidium sativum* L.) to water stress. J. App. Sci. Res., 8: 157-167.

Kharadi, R., S.D. Upadhyaya, A. Upadhyay and P.S. Nayak, 2011. Differential responses of plumbagin content in *Plumbago zeylanica* L. (Chitrak) under controlled water stress treatments. J. Stress. Physi. & Bioch., 7: 113-121.

Khedr, A.A., M.A. Abbas, A.A. Abdel Wahid, W. Paul Quick, G.M. Abogadallah, 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt stress. J. Exp. Bot., 54: 2553-2562.

Kiarostami, Kh., R. Mohseni, A. Saboora, 2010. Biochemical changes of *Rosmarinus officinalis* under salt stress. J. Stress. Physi. & Bioche, 6: 114-122.

Kishor, P.B.K., Z. Hong, G.H. Miao, C.A.A. Hu, D.P.S. Verma, 1995. Overexpression of 1- pyrroline-5-carboxylate synthetase increase proline production and confer osmotolerance in transgenic plants. Plant Physio., 108: 1387-1394.

Koyro, H.W., 2006. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte (*Plantago coronopus* L.). J.Envir. and Exp. Bot., 56: 136-146.

Lammeli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Mansour, M.M.F., 1998. Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress", Pla. Physio. and Bioche., 36: 767-772.

Maria, E.B., D.A. José, C.B. Maria, P.A. Francisco, 2000. Carbon partitioning and sucrose metabolism in tomato plants growing under salinity. Physiol. Planta., 110: 503-111.

Matysik, J., B.A. Bhalu, P. Mohanty, 2002. Molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci., 82: 525-532.

Metzner, H., H. Rau, H. Senger, 1965. Determination of photosynthetic pigment. Mangel Mutanten von Chorella. Planta, 65: 186-191.

Mohamed, A.A., A. Aly, 2008. Alterations of some secondary metabolites and enzymes activity by using exogenous antioxidant compound in onion plants grown under seawater salt stress. Amer. Eura.J. Sci. Res., 3: 139-146.

Muayed, F., A. Abbas, M. Jasim, H.A. Al-Taha, 2012. Effect of exogenous proline on protein pattern changes in *Citrus sinensis* (L.) Osbeck under in vitro salt stress. Advances in Agriculture & Botanics. Inte. J. Bioflux Soci., 4: 36-41.

Muting, D., E. Kaiser, 1963. Spectrophotometric methods of determining of amino- N in biological materials by means of the ninhydrin reactions. Hoppe Seylers Z Physiol Chem., 332: 276-281.

Nassar, M.A., K.F. El-Sahhar, 1998. Botanical Preparations and Microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, 219 (In Arabic).

Parida, A., A.B. Das, Y. Sanda, P. Mohanty, 2004. Effect of salinity on biochemical components of the mangrove *Aegiceras corniculatum*. Aquatic Bot., 80: 77-87.

Poustini, K., A. Siosemardeh, M. Ranjbar, 2007. Proline accumulation as a response to salt stress in 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance, Genet. Resour. Crop. Evil., 54: 925-934.

Qiu, D., P. Lin, S.Z. Guo, 2007. Effects of salinity on leaf characteristics and CO2/H2O exchange of *Kandelia candel* (L.) Druce seedlings. J. Forest Sci., 53: 13-19.

Rao, V.K., A.C. Rathor, H.K. Singh, 2009. Screening of aonla *(Emblica officinalis gaertn*.) cultivars for leaf chlorophyll and amino acid under different sodicity and salinity levels. Ind. J. Soil Conse., 37: 193-196.

Reda, Faten, M., 2007. Morphological, anatomical and physiological studies on *Senna occidentalis* (L.) Link plants grown under stress of different levels of salinity in irrigation water. J. Agri. Sci. Mansoura University, 32: 8301-8314.

Reda, Faten, M., S.L. Maximous, O.S.M. El-Kobisy, 2000. Morphological and anatomical studies on leucaena (*Leucaena leucocephala*) plants grown under stress of different levels of salinity in irrigation water. Bull. Faculty of Agri. Cairo University, 51: 309-330.

Rodriguez, D., J. Goudriaan, M. Oyarzabal, M.C. Pomor, 1996. Phosphorus nutrition and water stress tolerance in wheat plants. J. Plant Nutr., 19: 29-39.

Santa-Cruz, A., M.M. Martinez-Rodriguez, F. Perez-Alfocea, R. Romero-Arandana, M.C. Bolarin, 2002. The rootstock effect on the tomato salinity response depends on the shoot genotype. Plant Sci., 162: 825-831.

Simiroff, N., Q.J. Cumbes, 1989. Hydroxyl radical scavenging activity of compatible solutes, Phytoche, 28: 1057-1060.

Sivakumar, P., P. Sharmila, P.P. Saradhi, 2000. Proline alleviates salt-stress-induced enhancement in Ribulose-1, 5-bisphosphate oxygenase activity. Bioch. and Biophy.Res. Comm., 279: 512-515.

Skopelitis, D.S., N.V. Paranychianakis, K.A. Paschalidis, E.D. Pliakonis, I.D. Delis, D.I. Yakoumakis, A. Kouvarakis, A.K. Papadakis, E.G. Stephanou, K.A. Roubelakis-Angelakis, 2006. Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. Plant Cell., 18: 2767-2781.

Smith, F., M.A. Gilles, J.K. Hamilton, P.A. Godees, 1956. Colorimetric method for determination of sugar related substances. Anal. Chem., 28: 350-356.

Stivesev, M.V., S.A. Ponnamoreva, E.A. Kuzenstova, 1973. Effect of salinization and herbicides on chlorophyllase activity in tomato leaves. Fiziol. Rast, 20: 62-65.

Stoeva, N., M. Kaymakanova, 2008. Effect of salt stress on the growth, photosynthetic rate of bean plants (*Phaseolus vulgaris* L.). J. Cent. Euro. Agric., 9: 385-392.

Tarraf, S., 1999. Biochemical and physiological aspects of lupine plant under the effect of kinetin and some amino acids. Egy. J. App. Sci., 14: 59-73.

Vendruscolo, A.C.G., I. Schuster, M. Pileggi, C.A. Scapim, H.B.C. Molinari, C.J. Marur, L.G.C. Vieira, 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. J. Plant Physio., 164: 1367-1370.

Voetberg, G.S., R.E. Sharp, 1991. Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physio., 96: 1125-1130.

Wignarajak, D., H. Jennings, J.F. Handely, 1975. The effect of salinity on growth of *Phaseolus vulgaris* L. Anatomical changes in the first trifoliate leaf. Ann. Bot., 39: 1029-1038.

Wu, Q., R. Xia, 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physio., 163: 417-425.

Yan, Z., G. Shirong, S. Sheng, S. Jin, T. Takafumi, 2011. Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. Afri. J. Biote., 10: 18381-18390.

Yemm, E.W., A.J. Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J., 57: 508-514.
Zeid, I.M., 2011. Alleviation of seawater stress during ger-mination and early growth of barley. Inter. J. Agric. Res. Rev., 1: 59-67.
Zhang, W., S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agri. and Food Chem., 49: 5165-5170.