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

Hanan A.A. Taie, Magdi T. Abdelhamid, Mona G. Dawood, Rania M.A. Nassar

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 (TW P0). 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).
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 (Poustiti 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 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 1 h.

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

<table>
<thead>
<tr>
<th>Soil</th>
<th>Water</th>
<th>EC (dS/m)</th>
<th>pH</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺</th>
<th>HCO₃⁻</th>
<th>CO₂⁻</th>
<th>SO₄²⁻</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy</td>
<td>Tap water</td>
<td>0.23</td>
<td>7.35</td>
<td>1.00</td>
<td>0.50</td>
<td>2.40</td>
<td>0.20</td>
<td>0.10</td>
<td>0.00</td>
<td>1.30</td>
<td>2.70</td>
</tr>
<tr>
<td>Sandy</td>
<td>Sea water</td>
<td>51.2</td>
<td>7.76</td>
<td>43.20</td>
<td>15.12</td>
<td>454.57</td>
<td>1.51</td>
<td>6.05</td>
<td>0.00</td>
<td>76.36</td>
<td>432.00</td>
</tr>
<tr>
<td>Clay</td>
<td>Tap water</td>
<td>0.14</td>
<td>8.11</td>
<td>2.60</td>
<td>2.40</td>
<td>1.31</td>
<td>0.21</td>
<td>1.13</td>
<td>0.00</td>
<td>4.22</td>
<td>0.70</td>
</tr>
<tr>
<td>Clay</td>
<td>Sea water</td>
<td>1.40</td>
<td>7.59</td>
<td>5.60</td>
<td>1.90</td>
<td>5.90</td>
<td>0.37</td>
<td>1.50</td>
<td>0.00</td>
<td>6.77</td>
<td>5.50</td>
</tr>
</tbody>
</table>

At sowing, a granular commercial rhizobia was incorporated into the top 30-mm of the soil in each pot with the seeds. Granular ammonium sulphate 20.5% N at a rate of 40 kg N ha⁻¹, and single superphosphate (15% P₂O₅) at a rate of 60 kg P₂O₅ 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 16th 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, Ca2+, and Na+ were measured using a Dr. Lang M8D flame photometer. Nitrogen, P, K+, Ca2+, 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°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 (Bulus 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.

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

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 (TW0P). 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 O₂ (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 (TW0P). 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 \(b\), carotenoids, 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 (SW1P1 and SW1P2) showed significant increase in free amino acids relative to (SW1P0). Meanwhile, those irrigated with 6.25 sea water (SW2P1 and 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.](image)

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 CO2 stomata conductance, CO2 concentration in leaf tissues, electron transport system, CO2 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.](image)

**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 Ca²⁺ 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, Ca²⁺, 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.

<table>
<thead>
<tr>
<th>Sea Water</th>
<th>Proline</th>
<th>N%</th>
<th>P%</th>
<th>Ca%</th>
<th>K%</th>
<th>Na%</th>
<th>K/Na ratio</th>
<th>Cl(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW</td>
<td>P0</td>
<td>3.93e</td>
<td>0.37c</td>
<td>2.72b</td>
<td>2.23b</td>
<td>0.17c</td>
<td>13.4b</td>
<td>2.85d</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>4.14d</td>
<td>0.42b</td>
<td>2.84a</td>
<td>2.36a</td>
<td>0.15c</td>
<td>16.6a</td>
<td>2.74e</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>4.33c</td>
<td>0.47a</td>
<td>2.95a</td>
<td>2.46a</td>
<td>0.14c</td>
<td>17.6a</td>
<td>2.67f</td>
</tr>
<tr>
<td>SW1</td>
<td>P0</td>
<td>4.17d</td>
<td>0.34c</td>
<td>2.42d</td>
<td>2.16c</td>
<td>0.26b</td>
<td>8.4d</td>
<td>3.96c</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>4.34c</td>
<td>0.37c</td>
<td>2.52c</td>
<td>2.22b</td>
<td>0.24b</td>
<td>9.3c</td>
<td>3.87d</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>4.52b</td>
<td>0.40b</td>
<td>2.64b</td>
<td>2.30b</td>
<td>0.22b</td>
<td>10.5c</td>
<td>3.76c</td>
</tr>
<tr>
<td>SW2</td>
<td>P0</td>
<td>4.28c</td>
<td>0.29d</td>
<td>2.28e</td>
<td>2.04d</td>
<td>0.35a</td>
<td>5.8f</td>
<td>4.19a</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>4.50b</td>
<td>0.33c</td>
<td>2.38d</td>
<td>2.10d</td>
<td>0.33a</td>
<td>6.4e</td>
<td>4.08b</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>4.68a</td>
<td>0.36c</td>
<td>2.45d</td>
<td>2.16c</td>
<td>0.31a</td>
<td>7.0e</td>
<td>3.96c</td>
</tr>
</tbody>
</table>

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).

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 new 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 5 mM (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 5 or 10 mM of proline may be due to de novo synthesis of new protein. In support of present results, (Khedr et al., 2003) on Pancratium maritimum 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 these organs. 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

<table>
<thead>
<tr>
<th>Histological characters</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TW</td>
</tr>
<tr>
<td>Stem diameter</td>
<td>4650</td>
</tr>
<tr>
<td>Stem wall thickness</td>
<td>982</td>
</tr>
<tr>
<td>Epidermis thickness</td>
<td>25</td>
</tr>
<tr>
<td>Cortex thickness</td>
<td>116</td>
</tr>
<tr>
<td>Fiber tissue thickness</td>
<td>97</td>
</tr>
<tr>
<td>Phloem tissue thickness</td>
<td>87</td>
</tr>
<tr>
<td>Xylem tissue thickness</td>
<td>231</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>47</td>
</tr>
<tr>
<td>Parenchymatous Pith thickness</td>
<td>429</td>
</tr>
<tr>
<td>Hollow pith diameter</td>
<td>2684</td>
</tr>
</tbody>
</table>

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. 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/midvein 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.

<table>
<thead>
<tr>
<th>Histological characters</th>
<th>Treatments</th>
<th>TW</th>
<th>SW2</th>
<th>% to TW</th>
<th>TW+P2</th>
<th>% to TW</th>
<th>SW2+P2</th>
<th>% to TW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of midvein</td>
<td>TW</td>
<td>862</td>
<td>718</td>
<td>16.7</td>
<td>934</td>
<td>+8.4</td>
<td>792</td>
<td>−8.1</td>
</tr>
<tr>
<td></td>
<td>SW2</td>
<td>427</td>
<td>362</td>
<td>−16.7</td>
<td>498</td>
<td>+16.6</td>
<td>468</td>
<td>+9.6</td>
</tr>
<tr>
<td></td>
<td>TW+P2</td>
<td>375</td>
<td>287</td>
<td>−24.1</td>
<td>412</td>
<td>+22.6</td>
<td>296</td>
<td>−11.9</td>
</tr>
<tr>
<td></td>
<td>SW2+P2</td>
<td>29</td>
<td>22</td>
<td>−8.8</td>
<td>32</td>
<td>+10.3</td>
<td>30</td>
<td>+3.8</td>
</tr>
<tr>
<td>Dimensions of midvein bundle:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>TW</td>
<td>336</td>
<td>287</td>
<td>−14.6</td>
<td>412</td>
<td>+22.6</td>
<td>296</td>
<td>−11.9</td>
</tr>
<tr>
<td></td>
<td>SW2</td>
<td>171</td>
<td>144</td>
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<td>223</td>
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<td>31</td>
<td>8.8</td>
<td>38</td>
<td>+11.8</td>
<td>29</td>
<td>−14.7</td>
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TW= Tap water (0.23 dS/m); SW2=Sea water (6.25 dS/m); P2=10 mM Proline

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References


