IMPROVING THE TOLERANCE OF *VICIA FABA* AGAINST ENVIRONMENTAL SALINITY RESULTED FROM THE IRRIGATION WITH SEA WATER BY USING KNO_3 AND $(NH_4)_2SO_4$ AS CHEMICAL OSMOREGULATORS.

Mejoramiento de la tolerancia de *Vicia faba* a salinidad ocasionada por irrigación con agua de mar usando KNO_3 y $(NH_4)_2SO_4$ como osmoreguladores químicos

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ABSTRACT

The familiar solutes, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}, SO_4^{2-}, soluble carbohydrates, amino acids and soluble proteins, which play a role in osmotic adjustment were estimated to investigate the role of potassium nitrate and ammonium sulphate as osmoregulators and their effects on the solutes composition. *Vicia faba* L. was cultivated and irrigated with 5, 10, 15 and 20 % (v:v) sea water. The plants were divided to three groups. The first was irrigated with sea water only. The second was treated with 5 mM KNO_3 while the third was treated with 5mM $(NH_4)_2 SO_4$. The plants were left to grow until flowering stage. The results indicated that the non- treated group increased the soluble carbohydrates in the roots to avoid the influx of sodium. The treatment with KNO_3 decreased the sodicity (SAR) while $(NH_4)_2 SO_4$ treatment decreased the $S_{K:Na}$ value in the shoots at higher salinity. The availability of nitrogen as nitrate or ammonium ions enhances the accumulation of soluble carbohydrates in shoots. The plants of all groups were depended on Ca^{2+}, as compatible solute more than Na^+, and K^+.

Key words: Osmoregulation, irrigation, salinity, sea water, *Vicia faba*.

RESUMEN

Los solutos Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, PO_4^{3-}, SO_4^{2-}, carbohidratos y proteínas solubles, así como amino ácidos son importantes en ajuste osmótico y fueron estimados para determinar el papel de nitrato de potasio y sulfato de amonio como osmoreguladores y su efecto en la composición de solutos. *Vicia faba* L. fue cultivada e irrigada con 5, 10, 15 y 20 % (v:v) de agua de mar permitiendo crecimiento hasta el estado de floración. Las plantas fueron divididas en tres grupos. El primero fue irrigado con agua de mar sola-mente. El segundo fue tratado con 5 mM KNO_3, mientras que el tercer grupo fue expuesto a 5mM $(NH_4)_2 SO_4$. Los resultados indican que las plantas del primer grupo

(no tratado) incrementan carbohidratos solubles en sus raíces para evitar influjo de sodio. El tratamiento con KNO_3 disminuye riqueza de sodio (SAR) mientras que la exposición a $(NH_4)_2 SO_4$ diminuye la de $S_{K:Na}$ en brotes a salinidad alta. La disponibilidad de nitrato o iones amonio aumenta acumulación en las raíces de carbohidratos solubles. Las plantas de todos los grupos dependen de Ca^{2+} como soluto compatible más que de Na^+ o K^+.

Palabras clave: osmoregulación, irrigación, salinidad, agua de mar, *Vicia faba*.

INTRODUCTION

The world now faces serious problems. The global warm is the greatest one; it will affect the rate and locations of rainfall (Xie *et al.*, 2010). The population increasing is great problem too. Certainly, the arid and semiarid region will suffer strongly from both water deficit and soil salinity (Huq *et al.*, 1999). Thus, the plants and crop production will be affected with drought and salinity (Ashraf *et al.*, 2008; Kamal Uddin *et al.*, 2009; Kamal Uddin *et al.*, 2011). Therefore, several researchers have studied their effects on crop production (Mahajan and Tuteja, 2005; Jaleel *et al.*, 2007) and the responses of plants to overcome these environmental stresses (Wyn Jones and Pritchard, 1989; Koyro, 2006; Heidari *et al.*, 2008; Keshavarzi, 2012). One of the conclusions is that sea water or brackish water and salt tolerant plants should be considered for research (Breckle, 2009). Two main mechanisms are used by plants to overcome the drought and salinity stresses. The first is the quickly response through the re-osmotic adjustment depending upon inorganic solutes (Jacoby, 1999; Rubinigg *et al.*, 2003; Neocleous and Vasilakakis, 2007; Hajlaouia *et al.*, 2010; Aldesuquy *et al.*, 2012). The solute particle number is the main reason which causes stress. The second way to overcome stress is depended mainly upon organic solutes (Wyn Jones and Pritchard, 1989). The later way need long time for compound synthesis and transformations to compatible solutes as proline, glycinebetaine, etc.

The most familiar inorganic solutes in nature are Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-. These solutes when be available in excess in the medium will cause salt stress. The most harmful ions are Na^+ and Cl^- (Bergmann, 1992). The competition with K^+ may decrease the high accumulation of Na^+ (Kamel 2002; Kamel and El-Tayeb 2004). Calcium can also ameliorate sodium injury (Caines and Shennan, 1999; Hasegawa *et al.*, 2000; Shabala *et al.*, 2003; Arshi *et al.*, 2005; Renault, 2005). NO_{3-} ion can compete against Cl^- and may lead to avoid chloride toxicity. Several physiological and eco-physiological studies were carried to study the effect of salinity on plants. Flowers and Flowers, 2005, suggested that domestication of halophytes is the best alternative to produce economical plants that could be grown in saline deserts. This suggestion can be useful in certain places around the world, where the saline deserts are found. But in the dry countries especially in North Africa, the agricultural lands suffer extremely from salinity as a result of high evaporation. Most of the crop plants are sensitive to moderately tolerant for salinity. Thus, it is necessary to find a suitable solution to avoid the salinity problems. Depending o the compatibility some ions as K^+, Ca^{2+} and NO_{3-}, etc., these ions cab be used as osmoregulators.

The current investigation is a complementary work to form chemical osmo-regulators. They can be used as fertilizers and as osmo-regulators which can help the plants to

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overcome the soil salinity under the effect of irrigation with saline waters.

MATERIALS AND METHODS

Faba beans (*Vicia faba* L. cv. Giza 40) seeds were cultivated in 39 pots, eight seeds in each pot. Each pot (15 cm width × 12 cm height) contained 2 kg of sand: clay soil (2:1 respectively). They divided to four groups. First three groups were divided into four subgroups (3 replicates each) and irrigated with mixture of tap and sea water at 5, 10, 15 and 20 % sea water (v:v) respectively. Additionally to sea water treatments, the third group was treated with 5 mM KNO_3 while the fourth group was treated with 5mM $(NH_4) SO_4$. The fourth group was a control irrigated with tap water, and contained three pots. The plants were grown till flowering stage in greenhouse. The temperature ranged between 33 °C and 5 °C day/night, humidity range 25 - 85 % and average day length about 13 h. The total dissolved salts in the soil solution were 215 mg.L^{-1} and electrical conductivity was 364 µS at 24 °C. At harvest, the plants were separated into roots and shoots. Roots were washed in the solution used for their irrigation and dried with a filter paper. All plant parts were dried at 70 °C for 48 h. The dried parts were ground and the solutes were extracted in distilled water according to El-Sharkawi and Michel, 1975. Sodium and potassium were measured by flame photometry (flame photometer, corning M410, UK) according to Williams and Twine, 1960. A volumetrical estimation of Cl^-, Ca^{2+} and Mg^{+2} was performed according to Jackson, 1958 and Johnson and Ulrich, 1959. Sulphate, phosphate, soluble sugars, amino acids and soluble proteins were measured colorimetrically (spectrophotometer Jenway M6405 UK) according to Black *et al.*, 1965, Woods and Mellon, 1941, Dubois *et al.*, 1956, Lee and Takahashi, 1966 and Lawry *et al.*, 1951, respectively. Potassium:sodium selectivity in the shoots was calculated as:

$$S_{K:Na} (Shoot) = (K_{sh} * Na_r) / (Na_{sh} * K_r) \qquad \text{Equation 1}$$

Where the subscripts r and sh indicate the ion concentration in the roots and shoots.
All experiments were performed with three replicates. Data were subjected to one way ANOVA, and significant differences between means were determined using the Statistical Package for Social Sciences (SPSS) for Windows (version 20.0). Results were expressed as means and their standard deviations. Significance was tested at the 5 % level.

RESULTS AND DISCUSSION

There were no significant differences in water content in the plants whether irrigated with sea water only or treated potassium nitrate or ammonium sulphate (Fig. 1). Certain crops may benefit from selection pressures, which improve their capacity to adjust osmotically or maintain more favorable water relations under salt stress (Tal and Gardi 1976; Shannon *et al.*, 1987). This reflects the tendency of plants to maintain their water content at different stresses. The plants were depended on osmotic readjustment to maintain cell turgidity.

Sodium chloride is the most important constituent of sea water. Chloride represents only 55.03 % of the total salinity of sea water (Castro and Huber, 2003).
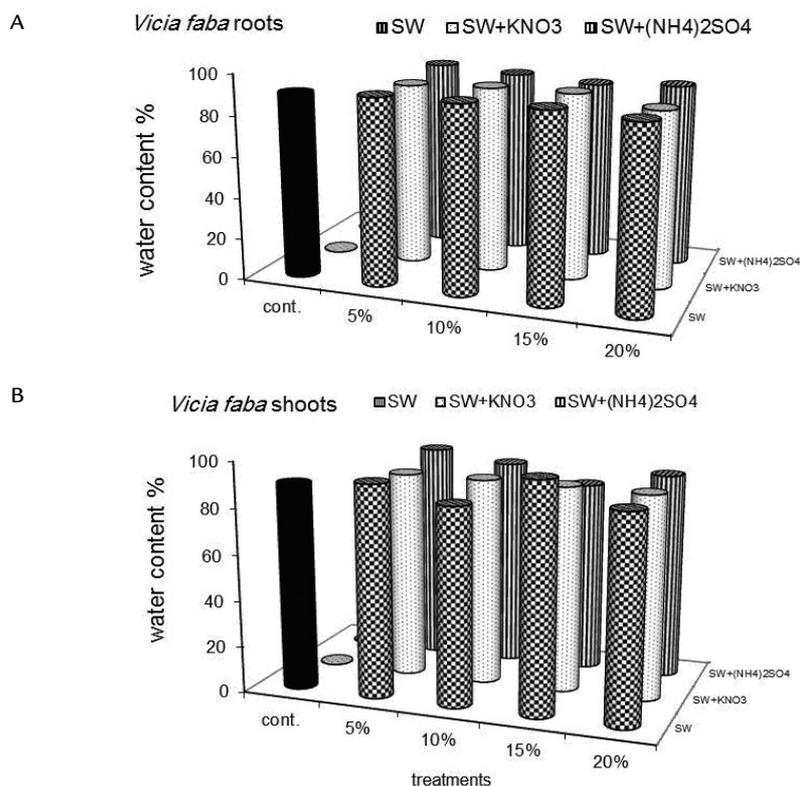
Figure 1. The percent of water content in *Vicia faba* roots and shoots irrigated with 5, 10, 15 and 20 % sea water (SW), with adding 5 mM KNO$_3$ or 5mM (NH$_4$)$_2$ SO$_4$. Data were analyzed with Post Hoc, ANOVA procedure and the mean and least significant difference (LSD at P< 0.05) against control plants were calculated. The results in roots and shoots were insignificant in all groups, at the level of significance (P < 0.05), only the water content in the shoots which irrigared with 15 % seawater showed a significant difference at P < 0.05.

Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na$^+$ or Cl$^-$ exclusion, and the tolerance of tissue to accumulated Na$^+$ or Cl$^-$ (Munns and Tester, 2008). Thus, plants irrigated with sea water increased their contents of chloride, sodium, potassium, calcium and magnesium (Fig. 2; Fig. 3) in their shoots gradually, to overcome the increase in external salinity. The osmotic readjustment is quickly induced by the changes in ion fluxes than the synthesis of compatible solutes (Wyn Jones and Pritchard, 1989; Lew, 1996). Generally, chloride content in the shoots of the plants irrigated with sea water was greater compared with the shoots of control plants. While chloride content was 33.7 mg.g^{-1} dry wt. in the control plants; was 38 mg.g^{-1} dry wt. in the plants it irrigated with 5 % sea water (Fig. 2). The shoots of all treated groups tended to increase their chloride concentration paralleled with the increasing of external salinity. Chloride is the micronutrient to be confirmed as generally most recent essential for the growth of higher plants (Broyer *et al.*, 1954). All plants seem to be able to accumulate Cl$^-$ in the vacuoles of their cells, whereas many are deficient in the Na$^+$/H$^+$antiporter needed for Na$^+$ occlusion
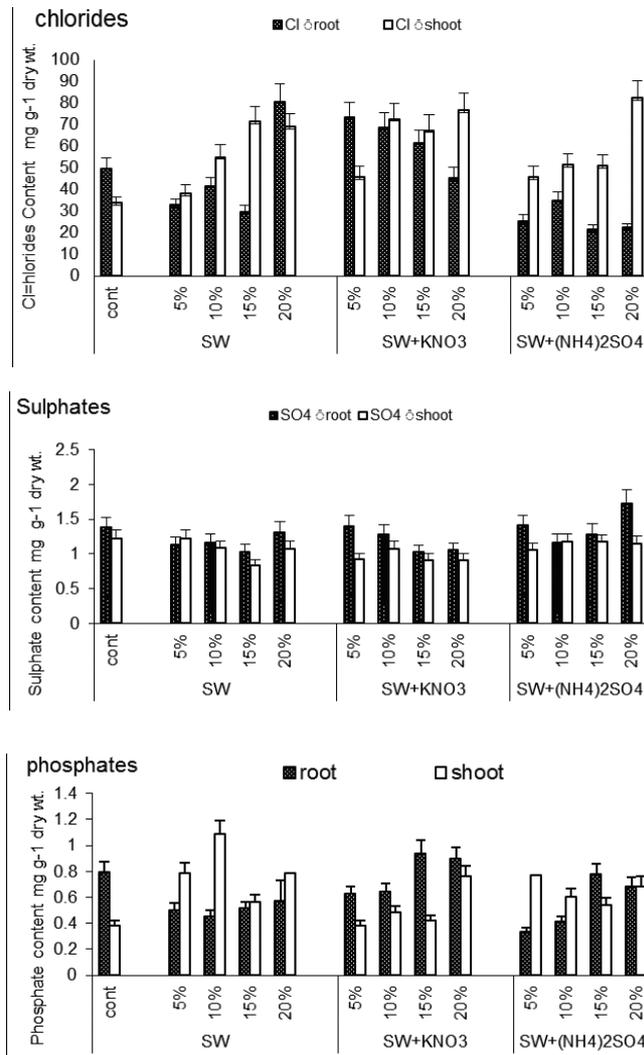
Figure 2. Chlorides, sulphates and phosphates contents mg.g^{-1} dry wt. in roots and shoots of the treated plants. Chlorides in the roots and shoot showed significance at most treatments at P < 0.01, but the roots irrigated with 20 % SW + KNO$_3$ showed significance at P < 0.05. Sulphate was significant in the shoots of the first (SW) and second (SW + KNO$_3$) groups at all levels of salinity but was insignificant in the third group [SW + (NH$_4$)$_2$ SO$_4$]. Phosphates showed significance in both roots and shoots in the different groups.

in the vacuoles (Mennen *et al.*, 1990). The minimal requirement of Cl$^-$ for crop growth of 1 g.kg^{-1} dry weight has been suggested (Marschner, 1995). The gradual increase of Cl$^-$ in the shoots will increase chloride toxicity. High tissue Cl^{-1} concentrations can be toxic to crop plants (Xu *et al.*, 2000). Generally Cl$^-$ content was lower in roots than shoots (Fig. 2). Several species accumulate high concentrations of Cl$^-$ and not Na$^+$ in leaves, such as

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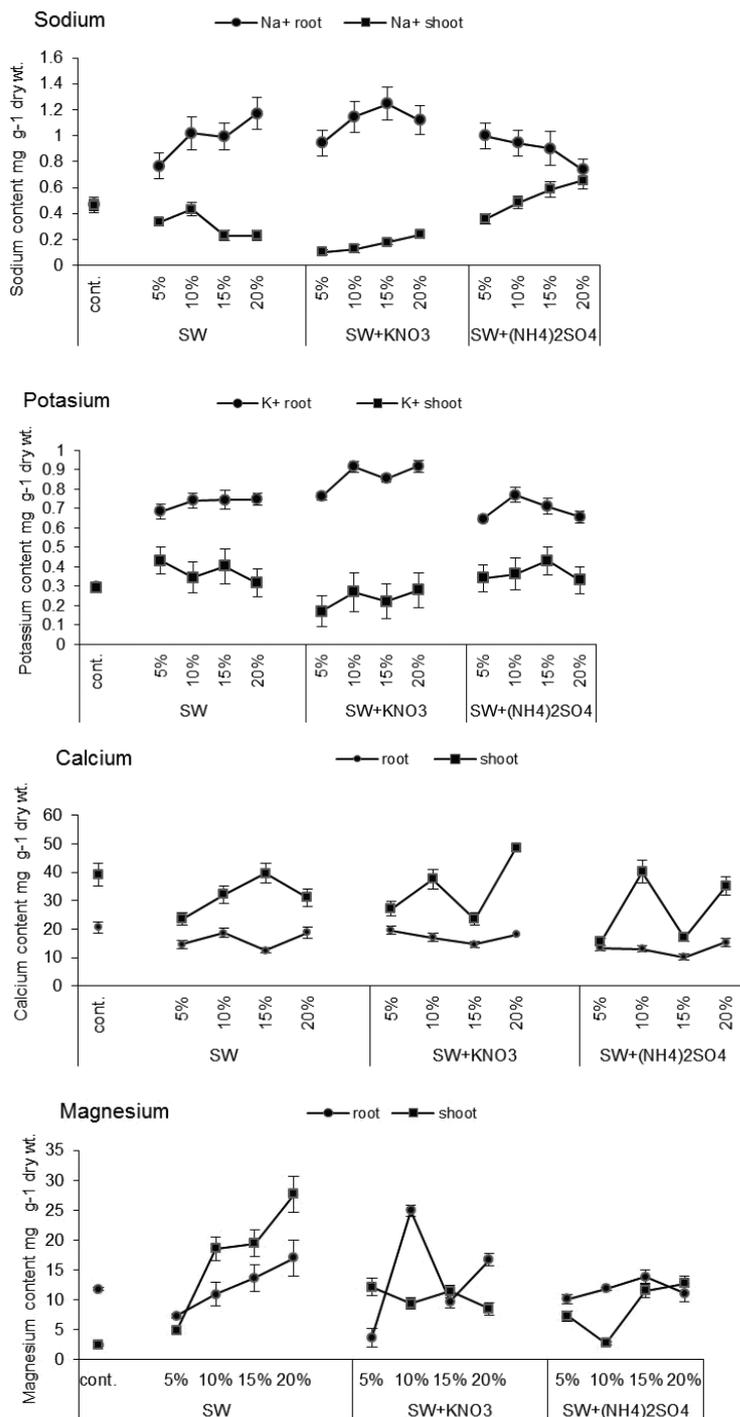   usando KNO₃ y (NH₄)₂SO₄ como osmoreguladores químicos. Kamel.*

Figure 3. Sodium, potassium, calcium and magnesium contents mg.g^{-1} dry wt. in roots and shoots of the treated plants. Na$^+$ content was significant at P < 0.01 at all levels of the different groups. Potassium showed insignificance at the second group. Treated with KNO₃. Ca^{2+} and Mg^{2+} showed significance at the level of significance (P < 0.05) in both roots and shoots, the exception was with Mg^{2+} at the higher salinity in the roots of the third group.

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soybean, woody perennials such as avocado (Munns and Tester, 2008). The low content of Cl^- in roots may due to the exclusion of Cl^- from roots. In most plants, Na^+ and Cl^- are effectively excluded by roots while water is taken up from the soil (Munns, 2005), or may due to the high rate of transport of chlorides to shoots to maintain turgidity. The treatment with 5 mM $(NH_4)_2 SO_4$ enhanced the accumulation of Cl^- in the shoots compared with roots which tended to decrease their content of Cl^-. Martinez and Cerda, 1989, found that Cl^- uptake was enhanced in cucumber when half the NO_{3-} in the solution was replaced by NH_4.

Plants tend to accumulate cations to eliminate the toxic effect resulted from chloride accumulation. Chloride is the prevalent anion accompanying Na^+ and K^+, hence its concentration in vacuoles, as well as cytoplasm, is usually in the same range as the sum of Na^+ and K^+. This concurrence of Na^+ and Cl^- complicates the evaluation of Cl^- specific toxicity (Pessarakli, 1999). As shown in figure 3, plants accumulated Na^+ and K^+ in roots more than shoots. The increase of external salinity increased the root content of sodium and potassium gradually in the plants which irrigated with seawater only or treated with KNO_3. Shoots of first group, irrigated with seawater only, decreased their content of Na^+ and K^+. The treatment with KNO_3 decreased Na^+ content in the shoots compared with the control plants, except at 20 % seawater with KNO_3. The low accumulation of Na^+ in the shoots reflects the avoidance of sodium toxicity. It has long been known that NaCl toxicity is largely attributable to the effects of Na^+, and only rarely those of Cl^- (Tester and Davenport, 2003). This explains the high accumulation of Cl^- in shoots compared with Na^+. In most plants, Na^+ and Cl^- are effectively excluded by roots while water is taken up from the soil (Munns, 2005). Therefore, the increase of Na^+ content in the shoots of the first group was opposite to the decrease in the roots. The treatment with KNO_3 enhanced the transport of Na^+ from roots to shoots. The treatment with $((NH_4)_2 SO_4$ enhanced the transport of $Na+$ and K^+ from roots to shoots.

At the low salinity (5 %), the roots content of Na^+ was the highest than control and treated plants. The Na^+ content decreased in the roots with increased external salinity, while shoots content of Na^+ increased. The treatment with $(NH_4)_2 SO_4$ increased the NH^{4+} concentration and consequently NH^{4+}/NO_{3-} ratio which increased the accumulation of Na^+ in the leaves (Feigin, 1990). The availability of Ca^{+2} and Mg^{+2} in sea water enhanced the uptake of both (Fig. 3). The plants in the three groups accumulated Ca^{+2} more than Mg^{+2}. Calcium has been extensively used to alleviate salt toxicity because of its crucial role in the maintenance of membrane processes, modulation of enzyme activities, and buffering of Na^+-toxicity (Eschie 1995; Kurth *et al.*, 1986; Bose *et al.*, 1982). Magnesium was affected with external conditions. In group (1) plants, the roots content of Mg^{+2} decreased than control but increased gradually with increase of the external salinity to exceed the control at 20 % seawater with 5 mM $(NH_4)_2 SO_4$. The shoots of second group increased their content of Mg^{+2} more than control and tended to decrease their content with increase of external salinity under the effect of KNO_3 (Slavko *et al.*, 1994). The salinized plant with the addition of 5 mM $(NH_4)_2 SO_4$ increased their content of Mg^{+2} more than control; the increase was continued with the increased external salinity. The roots decreased Mg^{+2} content compared with control and increased gradually with external salinity (Alston, 1966).

The transport of inorganic solutes inside the plants which survived under salt stress must be controlled to ensure the osmotic readjustment. So, some ratios of the main

estimated ions were computed in table 1. K^+/Na^+ ratio was higher in the shoots of the first and second groups. The higher $K^+/N+$ a ratio in shoots of barley cultivars compared with that in root medium solution indicated selective uptake of K^+, which seems to be among the processes involved in tolerance of cultivars to salinity stress (Niazi *et al.*, 1992). In the third group, treated with 5 mM $(NH_4)_2$ SO_4, the ratio was lower than other groups. This may due to the high selectivity of potassium in the first and second groups, while the presence of $(NH_4)_2$ SO_4 decreased the $S_{K:Na}$ value (Alder and Wilcox, 1995). The high accumulation of Ca^{+2} and Mg^{+2} decreased SAR ratio (Table 1). This ratio was high in roots due to the high accumulation of Na^+, excluded from shoots (Drew and Lauchli, 1985) and selected K^+. Potassium sodium selectivity highest values were recorded at the highest salinity in the first group (15 and 20 % seawater) and the lowest salinity in the second group (5, 10 and 15 % sea water with 5 mM KNO_3). All plants avoided the accumulation of Na^+ and K^+ in the shoots to avoid the sodium toxicity. So, SAR values in shoots were lower than in roots (table 1), this may due to the avoidance the transport of Na^+ and K^+ from roots to shoots. The first group plants tended to stabilize the sodicity in the roots, but in the same time the sodicity in the shoots decreased gradually with the increasing external salinity. This may due to the transport of Na^+ and K^+ from shoots to roots or decreasing the transport roots to shoots (Zepeda Jazo *et al.*, 2008). The treatment with KNO_3 helps the plants to stabilize the sodicity in the shoots. The treatment with ammonium sulphate in the third group affected the potassium sodium selectivity in the shoots. Potassium sodium selectivity decreased with increasing the external salinity. Sodicity values in the third group were the highest compared with the other two groups and control. The high accumulation of sulphates enhanced the accumulation of sodium more to neutralize the acidity resulted from SO_4^{-2} in the plants.

Treatment	% Sea Water	K/Na (root)	K/Na (shoot)	SAR (root)	SAR (shoot)	S$_{K:Na}$ (shoot)	Cl/Cations (root)	Cl/Cations (shoot)
Control	00	0.366	0.375	0.021	0.019	1.026	1.353	0.858
	05	0.527	0.768*	0.050	0.018	1.456*	1.287	1.315**
	10	0.428	0.469	0.048	0.012**	1.095	1.195	0.966
	15	0.441	1.038**	0.049	0.006**	2.352**	0.885**	1.104**
Sea water	20	0.376	0.814**	0.043	0.005**	2.167**	1.821**	1.001
Sea water	05	0.477	0.980**	0.064	0.004**	2.058**	2.939**	1.079*
+	10	0.470	1.251**	0.034*	0.004**	2.663**	1.259	1.523**
	15	0.403	0.727**	0.071	0.007**	1.803**	2.056**	1.760**
5mM KNO$_3$	20	0.482	0.698**	0.042	0.007**	1.448*	1.044	1.361**
Sea water	05	0.382	0.564	0.058	0.022**	1.475*	0.876**	1.796**
+	10	0.482	0.441	0.050	0.019	0.916	1.121	1.263**
	15	0.464	0.432	0.047	0.028**	0.931	0.680**	1.522**
5mM(NH$_4$)$_2$ SO$_4$	20	0.522	0.296	0.038	0.020*	0.567**	0.707**	1.607**

Table 1. Sodium adsorption ratio (SAR) in root and shoot systems, potassium selectivity in shoot system (S$_{K:Na}$) and Cl/Cations (Na, K, Ca and Mg) ratio. **Significant at P = 0.01. *Significant at P = 0.05.

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Cl/Cations ratio was higher in the roots of control plants than in shoot. In the first group, the ratio was 1.29 and 1.32 for the roots and shoots respectively. Except the ratio computed for roots of 20 % seawater irrigated plants; the plants tended to decrease and adjust the value of the ratio around one (Bear and Prince, 1945). The treatment with 5 mM KNO_3 enhanced the accumulation of Cl^- in the plants compared with control (Fig. 2). On another side, under the effect of external salinity, the chlorides decreased in root and this may due to the transport of Cl^- to shoots (Maas and Ogata, 1972). So, the values of Cl/Cations were irregular, but the highest computed values were recorded in the roots of the plants irrigated with 5 % seawater with 5 mM KNO_3 which reached up near three. The irrigation of plants with seawater and 5 mM $(NH_4)_2$ SO_4 affected the distribution of chlorides inside plants. There was a tendency to decrease the Cl^- in the roots may due to the low absorption of Cl^- or the high rate of Cl^- transport from roots to shoot, while chlorides increased gradually in the shoots. Thus, the Cl/Cations ratio was higher in all the levels of salinity in the shoots than roots.

Sodicity is the presence of a high proportion of sodium (Na^+) ions relative to calcium (Ca^{+2}) and magnesium (Mg^{+2}) ions. The shoot in general decreased the sodicity (Table 1) inside plants by decreasing the Na^+ concentration, avoiding the sodium toxicity. The studied plants tended to accumulate K^+ more than Na^+. So, the K/Na ratio was higher than that computed for roots of control, first and second groups. In the third group, the NH4+ decreased the accumulation on K^+ in the shoots (Szczerba et al., 2008). Therefore, the K/Na ratio was lower than that computed for other groups (Table 1). With the high preference to accumulate K^+, the plants tended to accumulate Ca^{2+} and Mg^{2+} in the shoots to ameliorate the toxic effect of sodium (Aldesuquy et al., 2012). Then, the $Na^+K/\sqrt{Ca^+Mg}$ ratio was clearly lower than in roots. The computed potassium sodium selectivity ($S_{K:Na}$) showed that the plants avoided the sodium toxicity by increasing their content of potassium at the highest salinity in the first group (Table 1). The treatment of plants with 5 mM KNO_3 increased the K^+ in the medium, so, the K^+ compete the Na^+ at the lower levels of salinity (Ashraf and Naqvi, 1991). The availability of NH4+ affect the absorption of potassium, so, the $S_{K:Na}$ decreased with increasing the external salinity. The preference of potassium accumulation in the shoots decreased the hazard producing from sodium accumulation. The high tendency of plants to accumulate calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions decreased the sodicity (SAR) strongly in the shoots compared with roots. SAR decreased gradually with the increasing of salinity. This reflects the increased selectivity of divalent cations by plants (Obermeyer and Tyerman, 2005), with the increasing salinity. The availability of potassium ions with the accumulation of Ca^{2+} and Mg^{2+} is very helpful for plants to avoid the sodium toxicity. The presence of $(NH_4)_2$ SO_4 in the external medium for third group enhanced the transport of Na^+ and K^+ from roots to shoots (Fig. 3), so, the SAR values were the highest in the shoots of third group (Table 1).

The low SAR in the shoots especially the plants that treated with KNO_3 decreased the sodium toxicity and the increased potassium:sodium selectivity reflects the preference of K^+ which plays important role in protein synthesis (Marschner, 1995). This decreased the concentration of amino acids and peptides (Fig. 4) and their role in osmotic adjustment, compared with non-treated plants. In another way, the treatment with potassium nitrate or ammonium sulphate enhanced the synthesis of carbohydrates which played the main role for osmotic adjustment in shoots to prevent the accumulation of inorganic solutes

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Soluble sugars
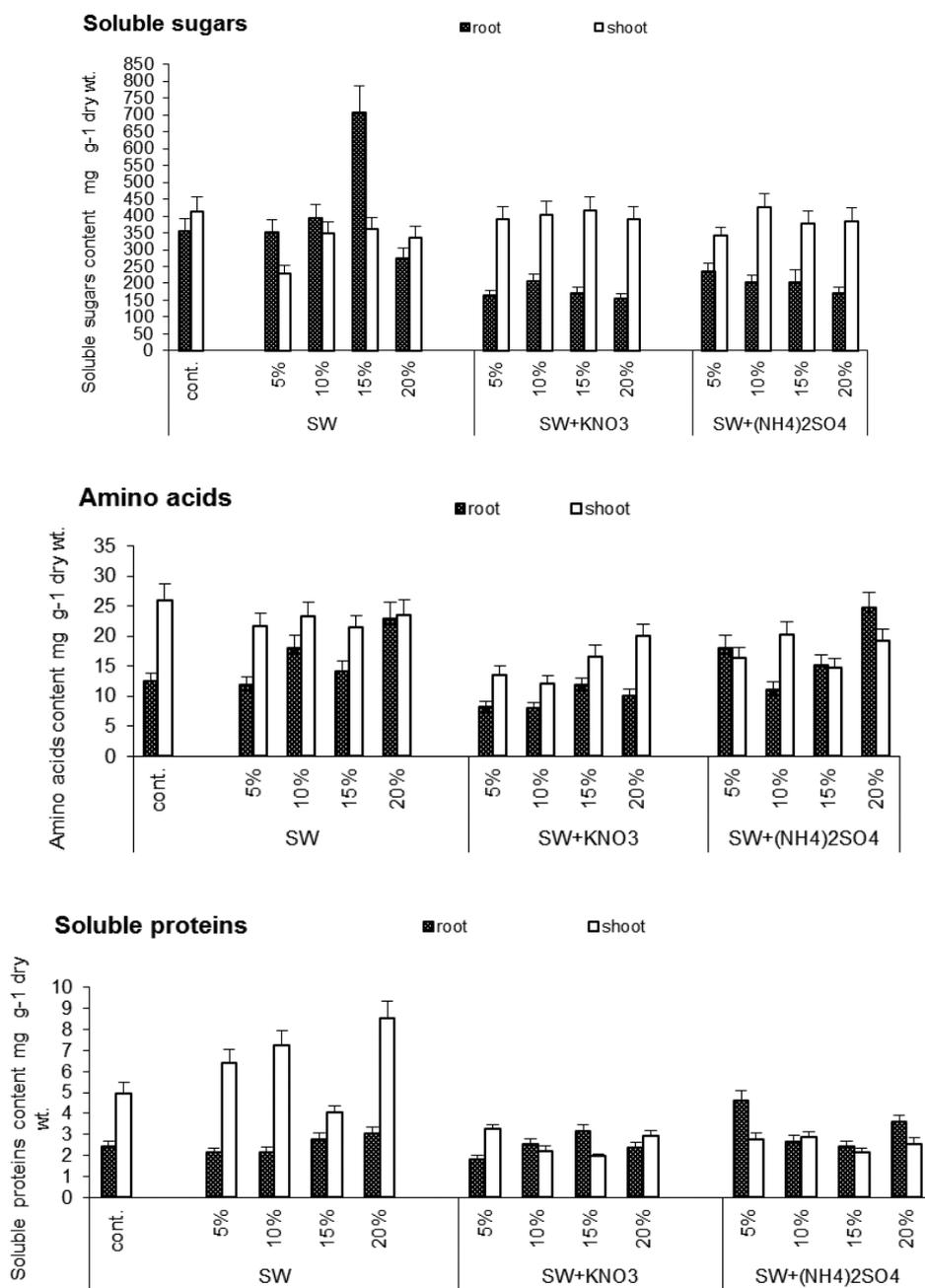

Amino acids

Soluble proteins

Figure 4. Soluble sugars, amino acids and soluble proteins contents mg.g^{-1} dry wt. in roots and shoots of the treated plants. Estimated aminoacids showed significance at the level of significance (P < 0.05) in both roots and shoots. Both soluble sugars and soluble proteins showed insignificant differences in the roots of 2nd and 3rd groups.

especially Na^{+} avoiding to injury. The plant treated with ammonium sulphate increased their content of amino acids and soluble proteins, where ammonium and sulphates plays a role in nitrogen assimilation in plants (Obermeyer and Tyerman, 2005). On the other hand, the non-treated plants accumulated more amounts of amino acids and soluble proteins to overcome the salinity hazard. This decreases the anabolism and growth (Munns 1988; Rodríguez *et al.*, 1997). It was remarkable that the non-treated plants accumulated more soluble sugars in the roots (Marian *et al.*, 2000) to increase the roots osmotic pressure, to prevent the influx of inorganic ions especially sodium. The high accumulation of soluble proteins in the non-treated plants shoots may be considered as a strategy to maintain water as bound water in the shoots (Frolow *et al.*, 1996).

It can be concluded that the treatment with chemical compounds which contain compatible ions as K^{+}, Ca^{2+} can be used as osmoregulators in the arid and semi-arid region where the salinity is high. The usage of potassium ions which can compete against the high concentration of Na^{+} in soil solution lead to decrease the effect of sodicity. Calcium also can ameliorate the harmful effects of salinity. Ammonium and nitrate, as a nitrogen sources, can be used as osmoregulators. The manufacturing of chemical osmoregulators is very useful to overcome the salinity in arid and semi-arid regions or to facilitate the usage of sea water in agriculture.

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