Research Article ISSN 2250-0480 Vol 2/Issue 2/Apr-Jun 2012

International Journal of Life science & Pharma Research

EFFECT OF NUTRIENT CATIONS TO IMPROVING SALINITY-TOLERANCE RESPONSES IN *SORGHUM BICOLOR* L.

OMAR ABDUALLAH AL-AMOUDI* AND AFAF ABDUALLAH RASHED

Biology Department, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.

ABSTRACT

Seedling of *Sorghum bicolor* L. were grown hydroponically in growth units, filled with continuously aerated half strength of Hogland-nutrient solution. Different treatments were manipulated to determine the combined effect of three NaCl levels (30, 60 and 90 mM) with combined levels of Ca^{2+} : K^{+} : Mg^{2+} (10:12:3 mM) on seedling growth. Seedling length, leaf sap osmolality, total chlorophyll, soluble protein, proline, phosphpenolpyruvate carboxylase (PEPC) andsucrose phosphate synthase (SPS) were used as reaction criteria. Several ions (Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}) were analysed in seedling material. Saline treatments decreased the growth, which was partly restored with the cation ratio treatment. A significant relation was observed between the electric conductivity of the nutrient solution and the osmolality of leaves using independent treatment (salinity or cation ratio). Salinity at all concentrations used in this work were reduced the total chlorophyll and soluble protein yield compared with that obtained at 0 mMNaCl. However, there was an increase, of previous criteria, as a consequence of the application of cation ratio treatments in each saline concentration. Higher concentrations of proline in shoot were obtained with increasing salinity concentrations. PEPC enzyme activity in roots was considerably higher than shoot under salt medium as well as with cation treatments. Both shoot and root SPS activities were slightly higher in root than in shoot subjected to salt and cation treatments. At high NaCl concentration, the ratio Na/Ca and Na/Mg decreased as a consequence of cation treatments. Therefore, the cation combination treatments were applied in the nutrient solution could be important factors in the hydroponic culture of sorghum grown under saline conditions.

Key words: Sorghum, salinity, Ca^{2+}, K^{+}, and Mg^{2+}cations, relative shoot growth rate, salt stress, total chlorophyll

INTRODUCTION

Salinity is a major problem in sea-irrigated agriculture. Millions of tons of salt are annually dumped onto the soil from the sea-water irrigation (Hasegawa et al*.,* 2000 and Zhu, 2001). The growth of plants may be reduced under salt stress because of (a) an osmotic stress due to a lowering of the external water potential, or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition (Carvajal et al*.,* 2000). Moreover, ion regulation and osmoregulation are subjects of intensive research into possible mechanisms of salt tolerance (Karley et al*.,* 2000 and Vera-Estrella et al*.,* 2004). In grain Sorghum (*Sorghum bicolor* L.) and in various organs of other species, the primary effects of salt stress are to reduce growth rate, leaf emergence rate, and overall shoot development (Neves-Piestun and Bernstein, 2001). In many, but not all, salt–sensitive species, elevated calcium (Ca^{2+}) concentrations in the root media ameliorate part of the growth reduction caused by the stress. Several investigations have been carried out to study the possible role of various kinds of chemical treatment to improve the irrigation medium and growth in saline environments (Carvajal et al.*,* 2000). In literature, there are conflicting reports concerning the effects of calcium (Ca^{2+}), potassium (K^+) and magnesium (Mg^{2+}) on salt tolerance in plants (Lopez and Satti, 1996). In contrast, addition of calcium to salinized media has been reported to effectively enhance salt tolerance in bean (Younis et al.*,* 1993), citrus (Banuls et al., 1991), rice (Shah et al.*,* 1987) and mung bean (Nakamara et al.*,* 1990). In plant cells, maintaining cytosolic potassium (K^+) in an environment with a high sodium (Na^+) concentration is a key factor in determining the ability to tolerate salinity (Maathuis and Amtmann, 1999). Typically involved in these responses are sugars, sugar alcohols, and many others molecules such as amino acids, organic acids, or inorganic ions (Munns, 2005). The effect of soil salinity on biomass and grain yield in many varieties of *Sorghum* has been tested (Begdullayeva et al.*,* 2007). It should be noted that the principal of measuring the activates of enzymes due to that been reported (Huber and Huber, 1991), under salt stress could regulate the activity of sucrose-phosphate synthase (SPS), whereas phosphoenolpyruvatecarboxylase (PEPC) could be sensitive to inorganic salts (Osmond, 1972). In previous studies (Rashed, 2008) three levels of combined cations Ca^{2+} K^+ Mg^{2+} (in mM ratio of 4:6:1, 7:9:2 and 10:12:3) been tested on sorghum exposed to salinity, and growth yield exhibited a positive results. In the same way, the cation ratio Ca^{2+} : K^+ : Mg^{2+} (10:12:3 mM) gave increase in growth biomass and greater production in saline treated. In the present study we, therefore, re-evaluate previous findings by performing a full assessment of the effect of salinity and cation ratio Ca^{2+} : K^+ : Mg^{2+} (10:12:3 mM) into Sorghum, under controlled salinity conditions. In addition to comparing these parameters as relative osmolality of leaf cell sap, plant length, root/shoot ratio, total chlorophyll concentration, soluble protein and proline contents. The activities of both enzymes (PEPC) and (SPS), as well as the concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} within and across treatments were analysed in seedling material.

MATERIALS AND METHODS

Growth conditions and experimental design

Grains of (*Sorghumbicolor* L.), were obtained from Fiffa east of Gazan, south west of Saudi Arabia. Grains were sterilized by 10% sodium hypochlorite solution for 10 min and rinsed several time with distilled water and soaked in water for 24 h, then transferred to rectangular plates covered by two layers of sheath cloth. The grains were also covered with a layer of wetted sheath cloth, irrigated with distilled water and left at growth room temperature (30 °C) for germination. Seedling of one week (shoot lengths reached about 2 cm) old were transferred to the growth units and subjected to different salinity levels : 0, 30, 60 and 90 mMNaCl, which correspond to 1.98, 4.82, 7.82 and 12.2 dSm^{-1} salinity levels. Different levels of cations Ca^{2+} : K^+ : Mg^{2+} (10:12:3 mM) were added to the nutrient solution, which correspond to 6.74, 9.21 and 14.8 dSm^{-1} salinity levels. Seedlings were grown for 3 weeks hydroponically in growth units consists of 4 polyethylene tubes with dimensions: 5 cm diameter, 51 cm length. Each tube has an out and inlet in order to pore the nutrient solution. The capacity of each tube is 1500 ml with 31 pores (8 mm) were made in each tube distributed in two alternation lines, where the seedlings should be settled. The plastic tips of micropipettes (Eppendorf), were used to support the seedlings during growth and when they were harvested. An air pump terminal with a pressure (200 ml/min), which allows a continues aeration to the nutrient solution, were used. The growth units were placed in a growth room with photosynthetic photon flux density was 800 $\mu mol\ m^{-2}\ s^{-1}$ with day/night temperature (32/28 °C), and the surface water temperature reaches to about 30 °C, with relative humidity means value was 65%.

Measurement of plant length and root and shoot ratio

Plant length of each plant was measured with a ruler prior to harvest, with a precision of ~ 1 mm. After harvesting, shoots and roots were frozen in liquid nitrogen, lyophilized, weighed, ground to a powder, and kept in a freezer (-20 °C) for further analyses. The rest of the plant material was weighed after drying in a forced air circulation oven at 80°C for 72 h. Roots and shoots ratio were recorded according to Kingsbury et al. (1984).

Soluble protein and proline analysis

Soluble proteins were extracted from lyophilized shoot of treated seedlings. Sample of 0.20 g powder was homogenized in 3 ml Tris-HCl buffer (0.1 M, pH 7.5) containing 1m Mphenylmethanesulfonylfloride (PMSF), at 4°C. The homogenate was centrifuged at 10000 x g for 30 min. Protein in the supernatant was measured by the method of Lowry et al., (1951) using bovine albumin to establish a standard curve.Extraction and determination of proline was measured spectrophotometrically according to Bates et al. (1973).

Chlorophyll content

Chlorophyll content was measured from Lyophilized shoot powder. Sample of 0.1 g was extracted with 5ml of 80% (v/v) acetone and filtered. Absorbencies of spectrophotometer were calculated at 645 and 663 nm. Chlorophyll content (mg g-1 fw leaf) was estimated by equations of Lichtenthaler (1987).

Cell sap osmolality

Samples of shoot sap were obtained using a Scholander pressure chamber according to the method of Jachetta et al. (1986). Osmolality of cell Sap (mOsmol/kg) was directly measured, according to manual standard method (Osmette A, S^{TM} model 2004).

Determination of phosphpenolpyruvate carboxylase (pepc)

PEPC was assayed in extracts of lyophilized material. Shoot and root powders were homogenized in a morter and pestle with 4ml of ice-cold extraction buffer (0.1 M Tris/HCl buffer, pH 7.8, 1 mM $MgSO_4$ and 0.5 mM EDTA). The extract was centrifuged in a cooling centrifuge (Beckman– centrifuge, Model J2–21) for 25 min at 20000 rpm. The enzyme activity was assayed at 30 °C in a total of one ml as final volume of the following reaction medium (50 µM HEPES/NaOH (pH 8.57), 10 µM $MgCl_2$, 10 µM $NaHCO_3$), and 50 µl PEP, 50 µl NADH, 10 µl MDH**.** The reaction rate was measured by the decrease in absorbance at 340 nm of NADH (oxidation of NADH) using a Shimatzu multipurpose recording Spectrophotometer (Cecil CE 7200 split – beam spectrophotometer) according to Tietz and Wild (1991).

Determination of sucrose phosphate synthase (sps)

SPS was assayed in extracts of lyophilized material. Shoot and root powders were homogenized in a morter and pestle with 4ml of ice-cold extraction buffer (50 mM HEPES / KOH buffer, pH 7.4), and absorbance was measured at 620 nm using a spectrophotometer (model UV–200–RS), and flow the method described by Vassey (1988).

Ion analysis

For each sample analysed, 10 mg of dry seedling material was digested with 3ml of concentratedultrapure $HNO_3$and 2ml of H_2SO_4. The samples were placed overnight in microwave at 90°C. After digestion at 270 °C for 6 hours according to the method described by Netondo et al., (2004). The amount of Na^+, K^+, Ca^{2+} and Mg^{2+} in the sample was then determined with Atomic Absorption Spectrophotometer (Model do dlame). Ion concentrations were expressed as mmol per g of dry weight.

Statistics

Statistical analyses were done with a ANOVA by SigmaStat.

RESULTS AND DISCUSSION

The Effect of treatments on cell sap osmolality and growth parameters

S*orghum bicolor* L. was exposed for 3 weeks to nutrient medium supplemented with different concentration of NaCl levels : 0, 30, 60 and 90 mMNaCl, which correspond to 1.98, 4.82, 7.82 and 12.2 dSm^{-1} salinity levels and in combination with cation ratio of $Ca^{2+} : K^+ : Mg^{2+}$ (10:12:3 mM). Song and Fujyiama, (1996) state that cation ratio support Na^+ action. Positive linear relationship is established between electric conductivity (EC) and cation-Na^+ combination in the nutrient solution (Table1). Plant grown in saline media accumulates high levels of salt (Munns, 2002).An osmotic adjustment is needed to keep root water potential lower than the external medium. Such osmotic adjustment may lead to positively related to growth reduction (Ottow et al., 2005). As the responses to salinity cell sap osmolality in this work was increased by salt stress. Stress effects were based on several mechanisms such as a driven uptake of water, which wasnecessary for the cell enlargement (Fricke and Peters 2002). The difference between saline treatment only and saline with cation was significant, except at the concentration of 30 mM /21 day. We found that salt stress also affected cell sap osmolality (Table 2). Cell sap osmolality was higher in concentration of 90mM/21 day with cation treatment than in salt (90 mM) only. Various cation have been reported to contribute to the osmotic adjustment, and as mechanism of maintenance of the cell under saline conditions (Saneoka et al., 2001). Many important physiological and morphological processes, such as leaf enlargement stomatal opening (Al-Shintinawy, 2000 and Netondo et al., 2004), and associated leaf photosynthesis are directly affected by the reduction of leaf turgor (Maas et al., 1986; Boursier and Läuchli, 1990), which accompanies the loss of water from leaf tissue (Jones and Turner, 1978). They reported that although relative water content was decreased, leaf osmolality increased the slow development of water deficits resulted not only in osmotic adjustment, but also in a decrease in leaf tissue elasticity. Similar trends could be seen in the results of other (Çiçek andÇakirlar 2002). Hamada et al. (1992) supposed that an increase of ion concentration in tissues caused an increase in osmolality. Thus, shoot and root cell-sap osmolality increased by an increase in salinity concentrations, reflecting enhanced ion concentration in the tissues.

A significant decrease was observed in seedling length (cm plant^{-1}) when the NaCl concentration was increased from 0 to 90 mM (Table 2). Meanwhile, germination seeds at all salinity levels with cation ratio were higher than that of salinity ones (result not shown).Although seeds had better efficiency for water absorption from growing media which is obviously due to metabolic activities during germination process. A growth reduction in seedling length of approximately (45%) was found over a period (21d) of experiment at 90 mM salt only (Table 2). It may either be due to reduction in cell size or to inhibition of the mitotic activity (Achakzai et al., (2009). No significant changes were observed within the two levels (30 and 60mM) of NaCl. Evidence that salinity (>30mM) inhibited the growth of shoot and root (Amazallag et al., 1990; Bernstein et al., 1993; Flowers 2004). In many crops, growth is progressively inhibited by increasing NaCl-treated (Erdei and Taleisnik, 1993; Fricke and Peters 2002). Carvajal et al. (2000) found that the growth of tomato was reduced at high salt concentration. Salinity directly affects the cell wall as well as membranes (Shalhevet and Hsiao, 1986; Hasegawa et al., 2000). In the same way, the decreases which appeared in seedling length was partial when elevated concentration of cation was supplied to the medium. Furthermore, inhibition of plant growth by salinity was associated with the appearance of leaf chlorosis or necrosis (Tavakkoli et al. 2011). However, in the current work, the seedling remained healthy for the duration of experiment and the shoots showed no sign of senescence or salt injury. When shoot and root dry weight as relative shoot and root growth rate (RGR) was calculated, it increased when salinity was applied with cation (result not shown).Results also revealed that root /shoot ratios are significantly increased by receiving high concentration of applied salts (Table 2). A maximum increase for root shoot ratio (0.50) is recorded in the highest level of salt (90 mM) a combining with cation ratio. The results and data presented in this investigation confirm this view other earlier reports did (e.g. Bengum et al., 1992; Mühling and Läuchli, 2002 and Begdullayevaet al., 2007). Growth reduction reflects the increased metabolic energy cost and reduced carbon gain, which are associated with salt adaptation (Netondo et al., 2004). It is known that roots allow a plant to absorb nutrients from the surrounding medium, and a healthy root system is a key to a healthy plant. Plant grown with various ways by which plants can keep endogenous levels of ions low has been reported (Zhu, 2001). An increase in root**:** shoot ratio could be an indication of a healthier plant, provided that the increase comes from a greater root size and not from a decrease in shoot weight (Aishahet al., 2011).

Table 1. ***Electric conductivity of cation treatment at each saline level***

NaCl	Ca : K : Mg 10 : 12 : 3	EC (dS m^{-1})
0 mM	-	1.98
	+	3.89
30 mM	-	4.82
	+	6.74
60 mM	-	7.82
	+	9.21
90 mM	-	12.2
	+	14.8

Table 2. ***Effect of NaCl concentration (0, 30, 60 and 90 mM) and cation (Ca^{2+}: K^{+}: Mg^{2+}) ratio (10:13:3) on cell sap osmolality (mOsmol Kg^{-1}), plant length (cm), and root/shoot ratio of Sorghum.***

Cation ratio	NaCl (mM)	Growth parameter/ plant age								
*		7			14			21		
		Cell Sap Osmolality	Plant length	Root/ Shoot	Cell Sap osmolality	Plant length	Root/ Shoot	Cell Sap osmolality	Plant length	Root/ Shoot
-	0	1570±110 a	19.9±0.9 b	0.46 a	1430±70 a	27.7±1.2 a	0.42 a	1356±66 b	32.7±1.6 a	0.31 b
	30	1225±120 a	22.0±0.9 a	0.32 b	2450±75 a	28.3±2.7 a	0.53 a	1910±112 a	35.4±0.6 a	0.25 c
	60	1870±80 a	18.9±2.2 b	0.43 a	2466±170 a	22.1±0.6 b	0.50 a	3180±220 a	25.1±2.2 a	0.39 a
	90	2711±92 a	20.7±2.4 a	0.46 a	3625±290 a	17.6±1.6 c	0.47 a	2452±22 a	11.3±1.2 c	0.41 a
+	0	1460±90 a	26.4±1.7 a	0.37 b	1520±120 a	29.6±1.1 a	0.36 b	1688±130 a	32.4±0.8 a	0.30 b
	30	1890±190 a	21.6±1.7 a	0.36 b	1850±150 a	26.4±0.8 a	0.26 c	20900±95 a	32.2±4.5 a	0.45 a
	60	2350±130 a	21.8±1.7 a	0.45 a	2100±99 a	23.5±0.7 b	0.35 b	2260±165 a	27.9±3.0 a	0.37 b
	90	3120±160 a	19.2±0.3 b	0.50 a	3560±220 a	22.6±2.4 b	0.50 a	3730±140 a	29.3±0.7 a	0.47 a

* Cation ratio =(Ca2+ K+ Mg2+; 10:12:3 in mM). Data are mean ±SE (4=n). Different letters indicate significantly different values at a probability levels of *P* =0.05.

Relationship between chlorophyll content across treatments

Salinity caused decreases in chlorophyll content and affect the synthesis of chlorophyll (Netondo et al., 2004), or accelerate its degradation (Reddy and Vora, 1986). Total chlorophyll content declined significantly during the whole experimental period with increased salinity (Table 3). It is more relevant at high salinity inhibiting photosynthesis and biomass production (Netondo, et al., 2004). The strongest effect of salt occurred about (74%) as loss in chlorophyll content, with plants grown under 90mM NaCl(21d). Treated with cation ratio, it recovered the total chlorophyll contents and loss could be accounted by about 13% only through the experimental period. A small drop occurred in the chlorophyll total (20%) at 60mM NaCl (14d) with cation correction, but then it was restored. However, the drop in the chlorophyll total was nearly linear. The reduction in the chlorophyll total content is expected to be under stress conditions. Its stability depends on membrane stability, which - under saline condition – hardly remains intact (Carpici et al., 2009). The chlorophyll total of plants may be taken as an indicator of improving new genotypes for salt stress depending on the present or other findings.

Relationship between soluble protein and proline content across treatments

Salinity caused decreases in soluble protein. Salt stress reduces protein synthesis, increases protein hydrolytic enzyme activity, decreases amino acid synthesis and interferes with enzyme structures leading to decreases in soluble protein content (Bavei et al., 2011). However, as one result of this study, salinity stress with cation did not detect that decreases in protein content of leaves. Perhaps this is related to gene expression (Bavei et al., 2011). The measurement of proline accumulation is also an important criterion for determining plant tolerance to salt stress (Çiçek and Çakirlar 2002). However, there were great differences in the increased proline contents in salt stress medium (Table 3). It is generally assumed that proline is acting as a compatible solute in osmotic adjustment and the increase of proline in salt stress is more apparent in sensitive genotypes than in tolerant ones (Khan et al., 2009). Furthermore, it was shown that the capability of a number of crop plants to accumulate proline in response to salt or other stresses was highly variable within species (Ashraf et al., 2004).

Table 3. ***Effect of NaCl concentration (0, 30, 60 and 90 mM) and cation (Ca^{2+}: K^{+}: Mg^{2+}) ratio (10:13:3) on plant chlorophyll content (mg g^{-1} leaf fresh weight), soluble protein (mg g^{-1}) and proline content (µmol g^{-1}) of Sorghum.***

Cation ratio added to medium *	NaCl (mM)	Growth parameter/ plant age								
		7			14			21		
		Chlo. content	Soluble protein	Proline content	Chlo. content	Soluble protein	Proline content	Chlo. content	Soluble protein	Proline content
-	0	1.72±0.17 a	10±1.4 b	0.069±0.01 c	1.94±0.72 a	8±1.5 b	1.2±0.02 a	2.28±0.81 a	9±0.8 b	2.1±0.1 a
	30	1.84±0.48 a	8.5±0.8 b	1.3±0.3 a	1.49±0.9 a	6.5±0.6 b	0.86±0.2 a	2.11±0.42 a	11±1.2 a	0.75±0. 1 b
	60	1.6±0.41 a	9±1.1 b	0.56±0.02 a	1.7±0.30 a	12±1.4 a	0.77±0.1 a	2.1.9±0.7 a	9.5±1.6 b	0.82±0.03 b
	90	1. 92±0.42 a	8±0.7 b	0.63±0.05 a	1.8±0.33 a	12±1.9 a	0.68±0.04 a	0.49±0.04 b	6.2±0.5 b	0.91±0.07 b
+	0	1.51±0.2 b	14.2±2.6 a	0.88±0.01 a	1.49±0.8 a	15.6±1.9 a	1.6±0.3 a	1.81±0.1 a	10.5±1.3 b	1.5±0.2 a
	30	1.54±0.03 b	13.3±1.2 a	0.9±0.2 a	1.53±0.23 b	16.5±8.6 a	0.34±0.1 b	1.84±0.13	12.6±2.3 a	0.45±0. 02 a
	60	1.53±0.3 b	15.5±1.9 a	0.41±0.1 a	1.23±0.1 b	13.5±1.7 a	0.58±0.02 a	1.70±0.07 a	12.4±1.7 a	0.52±0.04 a
	90	1.66±0.09 a	12.3±2.2 a	0.42±0.04 a	1.72±0.02 b	12.5±2.2 a	0.45±0.07 a	1.45±0.3 a	10.5±1.1 b	0.65±0.04 a

* Cation ratio =(Ca^{2+} K^{+} Mg^{2+}; 10:12:3 in mM).Data are mean ±SE (4=n). Different letters indicate significantly different values at a probability levels of *P* =0.05.

Relationship between PEPC andSPS activities across treatments: The effects of salt concentration and cation ratio on the contribution of PEPC and SPS activities were analyzed in both shoot and root seedling. PEPC enzyme activity in roots were considerably higher than shoot under salt medium as well as with cation treatments. Both shoot and root SPS was least affected under salinity treatments, however, the activities were slightly higher in root than in shoot subjected to salt and cation treatments (Table 4). The finding for both enzymes can be interesting because the observations on salinity with cation has not been recognized by other works. Obviously, the results confirm that both enzymes root was highly affected by salinity levels than shoot, and this might be considered to play an important role in such treatments. The induction of PEPCase under saline treatment only was reported in leaves plant (Yen et al., 1995). Guerrier (1988) also reported that the levels of PEPCase activity could be used as biochemical indicator of salt tolerance. On the other hand, Notton and Blanke (1992) recorded an increase in apparent Km of PEPCase activity in Avocado fruit under salinity. In general Vernon et al., (1988) recorded a *de novo* synthesis of PEPCase in stressed *Mesembryanthemumcrystallinum*. PEPCase has unusual properties when compared to PEPC from C_4 plants (Jiao et al., 1991). Tietz and Wild (1991) reported that PEPCase activity could be taken as a biochemical indicator of damage tissues, and this could be reflect the effect of salt by disrupting lipid-protein associations.

PEPCase is expected to contribute the control of carbon flux in the photosynthetic pathway under slain condition (Soussiet al., 1999). Based on the data reported by Garcia-Mauriňo et al. (2003), it can be hypothesized that both enzymes PEPCase and SPS may have positive impact on osmotic adjustment. Different lines of evidence suggest that PEPCase enzyme contribute to the regulation of atmospheric CO_2 uptake, whereas SPS enzyme contribute to the control of sucrose production in leaves. There were some evidence that these enzymes changes may be related to the degree of many factors such as cell composition, the pattern of cation distribution, growth condition and certainly the age of the tissue is likely to be an important factor, although the photoperiod, temperature, salinity and nutritional status were widely appreciated (Huber and Huber, 1992). In this investigation, the activities of both enzymes in salt stressed seedlings were approximately agreed with the data from other varieties of sorghum (Huber and Huber , 1992).

Table 4. ***Effect of NaCl concentration (0, 30, 60 and 90 mM) and cation (Ca^{2+}: K^+: Mg^{2+}) ratio (10:13:3) on the contribution of PEPC andSPS activities in Sorghum.***

Cation ratio added to medium *	NaCl (mM)	Phosphphenolpyruvate carboxylase activity (μmol NADH g^{-1} h^{-1})		Sucrose phosphate synthase activity (mg sucrose g^{-1} h^{-1})	
		shoot	root	shoot	root
-	0	0.36 ± 0.08 b	0.44 ±0.06 a	0.049 ±0.007 b	0.29 ±0.06 a
-	30	0.61 ±0.11 a	0.75 ±0.07 a	0.056 ±0.01 b	0.42 ±0.03 a
-	60	0.88 ±0.06 a	1.3 ±0.51 a	0.083 ±0.002 b	0.62 ±0.10 a
-	90	1.0 ±0.09 a	2.6 ±0.6 a	0.088 ±0.002 b	0.86 ±0.07 a
+	0	0.42 ±0.03 a	0.55 ±0.07 a	0.026 ±0.003 c	0.04 ±0.008 b
+	30	0.42 ±0.02 a	0.62 ±0.05 a	0.034 ±0.002 b	0.044 ±0.002 b
+	60	0.71 ±0.01 a	0.76 ±0.08 a	0.042 ±0.006 b	0.072 ±0.03 b
+	90	0.84 ±0.01 a	0.92 ±0.11 a	0.056 ±0.005 b	0.086 ±0.003 b

* Cation ratio =(Ca^{2+} K^+ Mg^{2+}; 10:12:3 in mM).Data are mean ±SE (4=n). Different letters indicate significantly different values at a probability levels of *P* =0.05.

Na^+ among cation of K^+,Ca^+, and Mg^{2+}

Chemical composition was analyzed in the seedling. Apart from the changes in the concentration of Na^+ and Cl^- , the only differences produced by the treatments were in the concentrations of K^+,Ca^+, and Mg^{2+} . As the concentration of Na increased, a higher concentration of NaCl was added to the nutrient solution (Figure 1 A) as the toxic effect of Na^+ or $Cl^-$was still present (Carvajal et al., 2000). However, at both levels of NaCl (60 and 90 mM), and when the cation balance was increased, the concentration of Na decreased significantly. Potassium (Figure 1 B) was decreased after the application of the concentrations of NaCl, but an increase was observed at 0 mMNaCl when the cation balance was increased. Concentrations of both Mg and Ca (Figure 1 C and D) did not change with cation treatments at 0 mMNaCl, but a significant decrease occurred at 30, 60 and 90 mMNaCl, which was partly restored by cation application. Moreover, in the study by Kamal Uddin et al. (2011) shoot K: Na ratio reduced in different salt stress among species *Paspalumvaginatum.* The concentration of Cl^- was always increased proportionally after increasing the NaCl concentration in the nutrient solution (data not shown).

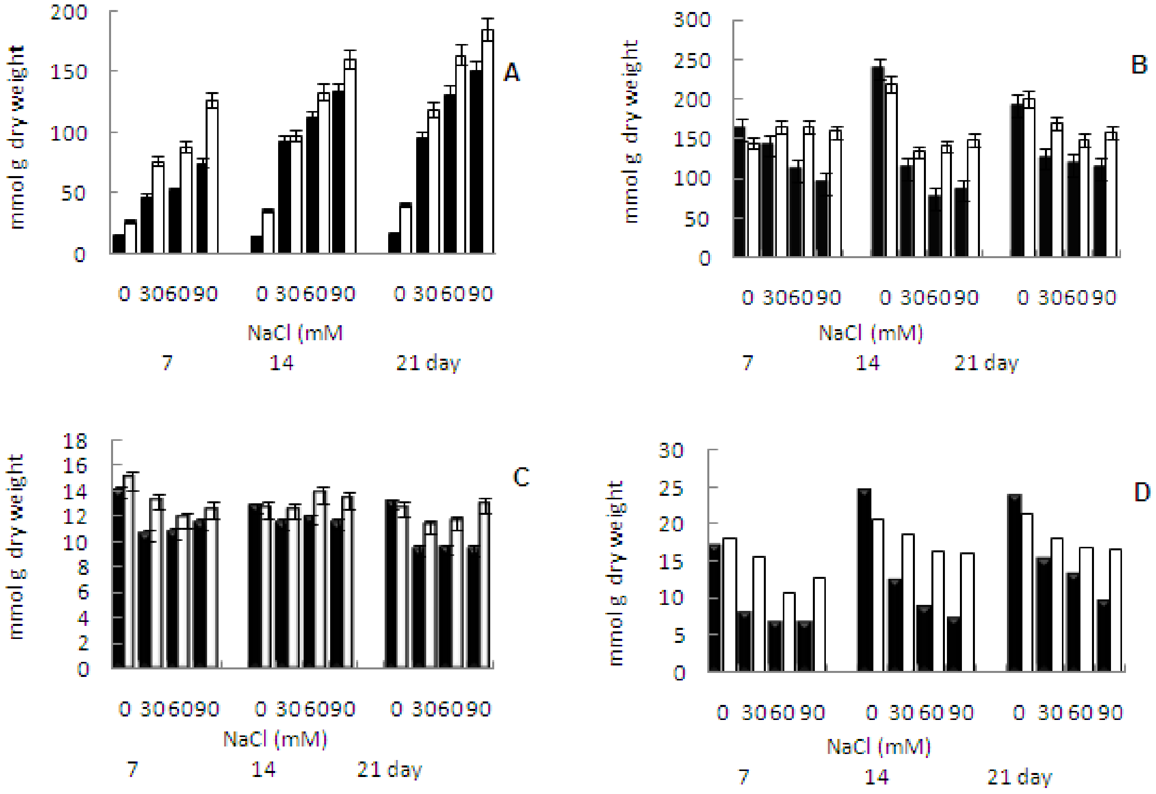

Figure 1. ***Cation concentration of sodium =(A); potassium =(B); magnesium = (C) and calcium = (D) in mmol per g of dry weight of seedling treated with cation ratio (Ca^{2+} K^+ Mg^{2+} : 10:12:3 mM) and three saline levels (30,60 and 90 mM). Treated (■), saline only (□).Data are mean ±SE (4=n).***

The ratio among cation was calculated in seedling plant. It can be noticed (see Figure 1 A-D) that at high NaCl concentration, the ratios Na/Ca and Na/Mg decreased as a consequence of cation treatments. Large amounts of NaCl decreased the concentrations of K^+, Ca^+, and Mg^{2+} , giving high values of the ratios of Na^+/ Ca^+ and Na^+/ Mg^{2+} (Zimmermann 1978). However, in our work, the ratio Na/K did not change much with the treatment.

CONCLUSION

To summarize, we can conclude that the concentration of cation in plants grown under saline conditions could be an important factor in sorghum production using hydroponic systems. When saline is available, with concentrations of the ratio Ca^{2+} : K^+ : Mg^{2+} (10:12:3 mM) increased in a number of growth parameters, although the toxic effect of Na^+ or Cl^- was still present. Further improvement of sorghum yield will require further research into the effect of plant mineral nutrition on the regulation of seeds number and size as well as the regulation of water relations of the plants and enzymes involved in nitrogen metabolism and transport within sorghum plants.

ACKNOWLEDGEMENT

This research waspartly supported by theKing Abdulaziz City for Science and Technology (KACST) and by Umm Al-Qura University, which made the studies possible.We are grateful to Dr. Ian Dodd for his comments.We also acknowledge the assistance of LEC technician staff member at Lancaster University.

REFERENCES

1. AchakzaiAK ,Kayani SA and Hanif A. Root and shoot growth response of sunflowerunder salt stress. Caderno de PesquisaSér Bio. 2009; 21 (1): 22- 41.
2. Aishah S, Saberi HAR, Halim RA and Zaharah AR. Yield responses of forage sorghums to salinity and irrigation frequency. Afr JBiotechnol. 2011; 10 (20): 4114 - 4120.
3. Al-Shintinawy F. Photosynthesis in two wheat cultivars on differing in salt susceptibility. Photosynthetic. 2000; 38: 615 - 620.
4. Amazallag GN, Lerner HR andPoljakoff-Mayber A. Induction of increased salt tolerance in Sorghum bicolor by NaCl pretreatment. J Exp Bot. 1990; 41: 29 - 34.
5. Ashraf M, Mukhtar N, Rehman S, Rha ES. Salt-induced changes in photosynthetic activityand growth in a potential plant bishops weed (*Ammoleimajus*L.). Photosynt. 2004; 42: 543-550.
6. Banuls J, Legaz F and Primo-Millo E. Salinity calcium interactionson growth and ionic concentration of citrus plants. Plant and Soil. 1991; 133: 39–46.
7. Bates LS, Waldren RP and Teare ID. Rapid determination of proline for water stress studies. Plant and Soil. 1973; 39: 205–207.
8. Bavei V, Shiran B, Khodambashi M and Ranjbar A. Protein electrophoretic profiles and physiochemical indicators of salinity tolerance in *Sorghum bicolor*L.Afr J Biotechnol. 2011; 10 (14): 2683-2697.
9. Begdullayeva T, Kienzler K, Kan E, Ibragimov N and Lamers J. Response of *Sorghum* (*Bicolor* varieties) to soil salinity and feed production in Karakalpakstan, Uzbekistan. Irri Drain Sys. 2007; 21: 237-250.
10. Bengum F, Karmoker J L, Fattah Q A and Maniruzzaman AF. The effect of salinity on germination and its correlation with K, Na, Cl
11. accumulation in germinating seeds of *Triticmaestivum* L. Cv. Akbar. Plant Cell Physiol. 1992; 33: 1009-1014.
12. Bernstein N, Läuchli A and Silk WK. Kinetics and dynamics of sorghum (*Sorghum bicolor* L.) leaf development at various Na/Ca salinities: I. Elongation growth. Plant Physiol. 1993; 103: 1107-1114.
13. BoursierP andLäuchli A. Growth responses and mineral nutrient relations of salt – stressed Sorghum. Crop Sci. 1990; 30: 1226-1233.
14. Carpici EB, Celik N, BayramG . Effects of salt stress on germination of some maize (*Zea mays* L.) Cultivars. Afr JBiotechnol. 2009; 8 (19): 4918-4922.
15. Carvajal M, Cerdá A and Martinez V. Modification of the response of saline stressed tomato plants by the correction of cation disorders. Plant Growth Regulation, 2000; 30: 37–47.
16. Çiçek N, Çakirlar H. The effect of salinity on some physiological Parameters in TwoMaize Cultivars. Bulg J Plant Physiol. 2002; 28 (1-2): 66-74.
17. ErdeiL andTaleisnik E. Changes in water relation parameters under osmotic and salt stress in maize and sorghum. Physiol Plant. 1993; 89: 381-387.
18. Flowers T J. Improving crop salt tolerance. J Exp Bot. 2004; 55 (396): 307-319.

19. Fricke W and Peters W S. The Biophysics of leaf growth in salt-stressed barley. Plant Physiol. 2002; 129 (1): 374–388.
20. 20. Garcia-Mauriňo S, Monreal J, Alvarez R, Vidal J and Echevarria C. Characterization of salt stress-enhanced phosphoenolpyruvate carboxylase kinase activity in leaves of *Sorghum vulgare*: independence from osmotic stress, involvement of ion toxicity and significance of dark phosphorylation. Planta, 2003; 216 (4): 648–655.
21. Guerrier G. CapacitésPEPCaseet MDH extraites des plantulesgermées en milieu salé: des paramétresbiochimiques de l'écophysiologie de la plante. Seed Sci Tech. 1988; 16: 571-578.
22. Hamada EAM, Hamoud MA, El-Sayed MA, Kirkwood RC, El–Sayed H.Studies on the adaptation of selected species of the family Gramineae A. JUSS. to salinization. FeddesRepertorium, 1992; 103: 87–98.
23. Hasegawa A, Bressan RA, Zhu JK and Bohnert H J. Plant culture and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol. 2000; 51: 463-499.
24. Huber SC and Huber JL . Salt activation of sucrose-phosphate synthase from darkened leaves of maize and other C-4 plants. Plant Cell Physiol. 1991; 32 (3): 327-333.
25. Huber SC and Huber JL. Role of sucrose – phosphate synthase in sucrose metabolism in leaves. Plant Physiol. 1992; 99: 1275-1278.
26. Jachetta JJ, Appleby AP and Boersma L. Use of the pressure vessel to measure concentrations of solutes in apoplastic and membrane-filtered symplasticsap in sunflower leaves. Plant Physiol. 1986; 82: 995-999.
27. Jiao J, Vidal J, Echevarria C and Chollet R. In vivo regulatory phosphorylation site in C4-leaf phosphoenolpyruvate carboxylase from maize and sorghum. Plant Physiol. 1991; 96: 297-301.
28. Jones MMN and Turner C. Osmotic adjustment in leaves of *Sorghum* in response to water deficits. Plant Physiol. 1978; 61: 122–126.
29. Kamal Uddin MD, Juraimi A, Mohd RMD, Othman AR and Abdul Rahim A.Effect of salinity stress on nutrient uptake and chlorophyll content of tropical turfgrass. Aust J Crop Sci. 2011; 5(6): 620-629.
30. Karley A, Leigh R and Sanders D. Differential ion accumulation and ion fluxes in the mesophyll and epidermis of barley. Plant Physiol. 2000; 122: 835-844.
31. Khan MA, Shirazi MU, Ali Khan M, Mujtaba SM, Islam E, Mumtaz S, Shereen A, Ansarı RU, Yasin Ashraf M. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*TriticumaestivumL.*) Pak J Bot. 2009; 41(2): 633- 638.
32. Kingsbury RW, Epstein E and Pearcy RW. Physiological responses to salinity in selected lines of wheat. Plant Physiol. 1984; 74: 417- 423.
33. Lichtenthaler HK. Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods Enzymol. 1987; 148: 350–382.
34. Lopez MV and Satti SME. Calcium and potassium – enhanced growth and yield of tomato under sodium chloride stress. Plant Sci. 1996; 114: 19-27.
35. Lowry OH, Rosebough NJ, Farr AL and Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951; 193: 265–275.
36. Maas EV, Poss JA and Hoffman G J. Salinity sensitivity on sorghum at three growth stages. Irrig Sci. 1986; 7: 1-11.
37. Maathuis FJM and Amtmann A. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. Ann Bot. 1999; 84: 123–133.
38. Mühling KH and Läuchli A. Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. Plant Physiol. 2002; 159: 137-146.
39. Munns R. Comparative physiology of salt and water stress. Plant Cell Environ. 2002; 25: 239-250.
40. Munns R. Genes and salt tolerance: bringing them together. New Phytol. 2005; 167: 645–663.
41. Nakamara Y, Tanaka K, Ohta E and Sakarta M. Protective effect of external Ca^{2+} on elongation and intracellular concentration of K^+ in intact mung bean roots under high NaCl stress. Plant Cell Physiol. 1990; 31: 815–821.
42. Netondo G, Onyango J and Beck E. Sorghum and salinity: I. Responses of growth, water relations, and ion accumulation to NaCl salinity. Crop Sci. 2004; 44: 797–805.
43. Neves-Piestun B and Bernstein N. Salinity-induced inhibition of leaf elongation in maize is not mediated by changes in cell wall acidification capacity. Plant Physiol. 2001; 125: 1419-1428.

44. Notton BA and Blanke MM . Contribution of phosphoenolpyruvate carboxylase to the carbon economy of cv. fuerte avocado fruit categorization of photosynthesis and effects of simulated salinity, CO_2 shock and ca-storage. Proc. 2nd World Avocado Congress. 1992; 449-455.
45. Osmond CB. Salt responses of carboxylation enzymes from species differing in salt tolerance. Plant Physiol. 1972; 49: 260-263.
46. Ottow E A, Brinker M, Teichmann T, Fritz E , Kaiser W , Brosché M, KangasjärviJ, Jiang X and Polle A. *Populuseuphratica* displays apoplastic sodium accumulation,osmotic adjustment by decreases in calcium and soluble carbohydrates and develops leaf succulence under salt Stress. Plant Physiol. 2005; 139: 1762–1772.
47. Rashed AA. Modification of the response of saline stressed *Sorghum bicolor* L. by cation correction of nutrient. The Msc thesis, Faculty of Applied Sciences, Umm Al-Qura University, Makkah, Saudi Arabia. 2008.
48. Reddy M P and Vora A B . Changes in pigment composition, Hill reaction activity and saccharides metabolism in Bajra (*Pennisetumtyphoides* S & H) leaves under NaCl salinity. Photosynthetica. 1986; 20: 50-55.
49. Saneoka H, Ishiguro S and Moghaieb R E A. Effect of salinity and abscisic acid on accumulation of glycinebetaine and betaine aldehyde dehydrogenase mRNA in sorghum leaves (*sorghum bicolor*). J Plant Physiol. 2001; 158: 853-859.
50. Shah M, Akbar M andNeue HV. Effect of Na/Ca and Na/K ratio in saline culture solution on the growth and mineral nutrition of rice (*Oryza sativa* L.). Plant and Soil. 1987; 104: 57– 62.
51. Shalhevet J and Hsiao TC . Salinity and drought. A comparison of their effects on osmotic adjustment, assimilation, transport and growth. Irrig Sci. 1986; 7: 249-264.
52. Song JQ and Fujyiama H. Differece in response of rice and tomato subjected to sodium salinization to the addition of calcium. Soil Sci Plant Nutrition. 1996; 42: 503-510.
53. Soussi M, Liuch C and Ocaňa A. Comparative study of nitrogen fixation and carbon metabolism in two chick-pea (*Cicerarietinum*L.) cultivars under salt stress. J Exp Bot. 1999; 50: 1701-1708.
54. Tavakkoli E, Fatehi F, Coventry S, Rengasamy P and McDonald G K. Additive effects of Na+ and Cl– ions on barley growth under salinity stress. J Exp Bot. 2011 ;62 (6): 2189–2203.
55. Tietz S and Wild A. Phosphoenolpyruvate carboxylase activity and malate content of spruce needles of healthy and damaged trees at three mountain sites. BiochemPhysiolPflanzen. 1991; 187: 273–282.
56. Vassey T. Phytochrome mediated regulation of sucrose phosphate synthase activity in maize. Plant Physiol. 1988; 88: 540–542.
57. 57. Vera-Estrella R, Barkla B, Bohnert H and Pantoja O. Novel regulation of aquaporins during osmotic stress. Plant Physiol. 2004; 135: 2318-2329.
58. Vernon D M, Ostrem J A, Schmitt J M and Bohnert H J. PEPCase transcript levels in *Mesembryanthemumcrystallinum* decline rapidly upon relif from salt stress. Plant Physiol. 1988; 86: 1002-1004.
59. Yen H E, Grimes HD and Edwards G E. The effects of high salinity, water – deficit, and abscisic acid on phosphoenolpyruvate carboxylase activity and proline accumulation in *Mesembryanthemumcrystallinum* cell cultures. Plant Physiol. 1995; 145: 557-564.
60. Younis M E, Abbas M A and Shukry WM. Effects of salinity on growth and metabolism of *Phaseolus vulgaris*. Biol Plant. 1993; 35: 417–424.
61. Zhu J K. Plant salt tolerance. Trends in Plant Sci. 2001; 6 (2): 66-71.
62. Zimmermann U. Physics of turgor and osmo-regulation. Ann. Rev. Plant Physiol. 1978; 29: 121-148.