# INTERACTIVE EFFECTS OF NACL INDUCED SALINITY, CALCIUM AND POTASSIUM ON PHYSIOMORPHOLOGICAL TRAITS OF SORGHUM (*SORGHUM BICOLOR* L.)

## MUHAMMAD HASSAN SHARIAT JAFARI[1], MOHAMMAD KAFI[1*] AND ALIREZA ASTARAIE[2]

[1]*Department of Agronomy, Ferdowsi University of Mashhad, Iran*
[2]*Department of Soil Sciences, Ferdowsi University of Mashhad, Iran.*
[*]*Correspondence author E-mail: m.kafi@um.ac.ir; Phone: 098 915 306 6269*

**Abstract**

The interactive effects of salinity, calcium and potassium on physiomorphological traits of sorghum (*Sorghum biclolor* L.) were studied in a green-house experiment. Treatments included 4 levels of NaCl (0, 80, 160, and 240 *mM* NaCl), 2 levels of CaCl$_2$ (0 and 20 m*M*), and 2 levels of KCl (0 and 20 m*M*). Salinity substantially reduced the plant growth as reflected by a decrease in the plant height, shoot and root weight, and percent of mortality and delay in the reproductive stage, but the relative water content as well as CO$_2$ assimilation rate and transpiration rate were negatively affected only at severe salinity. Root/shoot weight and leaf blade/total plant weight ratios were increased under saline conditions, which led sorghum to tolerate in the presence of salinity. Application of supplemental calcium resulted in partially restoring the adverse effects of high salinity on plant growth, whilst application of additional potassium had a negative interaction on plant growth characteristics. Combined application of Ca$^{2+}$ and K$^+$ also resulted into an ameliorative effect on sorghum growth and morphological traits under salinity stress.

## Introduction

Many plant species suffer a decline in growth while exposed to salinity stress. The inhibition of growth in long-term exposure to salt stress may result from osmotic effects on water availability, reduction in net assimilation, specific ion effects, or ion imbalance due to interference with uptake of essential ions or a combination of any of those adverse factors (Bernstein *et al*., 1993; Noreen *et al*., 2007; Ashraf, 2009). Many studies denote the interaction between NaCl and the elevated Ca$^{2+}$ and K$^+$ concentrations on plants (Reid & Smith, 2000; Banuls *et al*., 2004). Calcium has many roles in plants and is required in different levels depending on the process in which it is involved from micro amounts in regulating some aspects of cytosolic metabolism to macro amounts in cell wall structure (Rubio *et al*., 2004; Ashraf *et al.,* 2008). The interaction of calcium with other elements under NaCl stress is proven (Cramer *et al*., 1986; Lynch & Lauchli, 1988; Sabir & Ashraf, 2007). In these circumstances plant growth may relate more to the protective effect of high Ca$^{2+}$ concentrations than to the amounts needed for incorporation into physical structure such as cell walls (Ashraf *et al.,* 2008). The protective effect of Ca$^{2+}$ in salinized plants is probably due to its role in maintaining membrane integrity, because one of the primary effects of salinity is a disruption of membrane integrity caused by displacement of Ca$^{2+}$ from the cell surface by Na$^+$ (Cramer *et al*., 1986; Lynch & Lauchli, 1988; Ashraf, 2004; Mahmood, 2009).

K$^+$ has been also considered often to play a role in osmotic stress and salt toxicity remediation and some studies show inhibition of K$^+$ influx by NaCl (Shirazi *et al*., 2005; Abdul Majid *et al.,* 2007). K$^+$ uptake occurs through different transport mechanisms depending on the external K$^+$ concentrations. Carriers that exhibit a high affinity for K$^+$ and some K$^+$ inward-rectifying (KIR) channels work at low K$^+$, whereas different types of channels such as KIR channels or non-selective cation channels (NSCCs), are involved

when external $K^+$ concentration is in the milimolar range (Malhorta & Class, 1995; Rubio *et al*., 2004). In the cytosol, $K^+$ is an essential activator for some enzymes and $Na^+$ can rarely substitute for these biochemical functions. $Na^+$ can compete directly for $K^+$-binding sites on enzymes, suggesting that the cytosolic $K^+$ to $Na^+$ ratio, rather than the absolute $Na^+$ concentration, is critical for tolerance (Carden *et al*., 2003; Ashraf *et al.,* 2008). It is known that $Ca^{2+}$ sustains $K^+$ transport and $K^+/Na^+$ selectivity at the plasma membrane in $Na^+$- challenged plants (Shabala *et al*., 2006).

Sorghum is moderately tolerant to salinity and is widely grown in semi-arid areas on soils prone to salinity. Netondo *et al*. (2004) studied response of sorghum to salinity and found that growth of sorghum varieties was sensitive to salt due to increased metabolic energy cost and reduced carbon gain. Sodium was the major cation that accumulated in roots and stems as salinity increased. Considering the fact that few researches have got through studying the interaction of $Ca^{2+}$ and $K^+$ with salinity stress on sorghum plant, this study has been carried out to understand the interactive effects of these two macro nutrients with salt stress on sorghum, a crop planted mostly in arid saline soils.

## Materials and Methods

**Plant growth and treatments:** The seeds of line M5 of sorghum (*Sorghum bicolor* L.) were grown in well-washed sand in the 60 x 40 x 32 cm plastic boxes, at the Research Greenhouse of Ferdowsi University of Mashhad, Iran in July 2008. Sixteen seeds were planted in each box and thinned to 8 per box keeping equal distance between the seedlings. Plants were irrigated using an automated dripping system in which the pumps in the drums supplied nutritional solution as well as salinity, calcium and potassium treatments for 3 boxes in a group (standing for 3 replications of each treatment). The sown seeds were irrigated by tap water till emergence and thereafter. Hoagland nutrient solution (Hoagland & Arnon, 1950) was supplied instead of water. Plants were watered daily and irrigated 15-20 minutes whenever desired provide at least 50% leaching requirement in order to avoid accumulation of salts in the growth medium. The evapotranspirated water in the containers was replaced daily by adding water to the desired level. The nutrient solutions were renewed every other week to maintain the nutrients and salts in proper level. Natural illumination, average maximum and minimum temperature of 18 and 30 $^O$C, respectively and average relative humidity of 60% were supplied.

Salt as well as calcium and potassium treatments started at the growth stage 1 (described by Vanderlip, 1993). Sodium chloride was added in 4 concentrations of 0, 80, 160 and 240 m*M* to the nutrient solution. To avoid osmotic shock of salinity, saline treatment was imposed incrementally by increasing the concentration of 80 m*M* every other day until the final concentration reached. Calcium treatment in two levels (0 and 20 m*M* $CaCl_2$) and potassium treatment in two levels (0 and 20 m*M* KCl) were also imposed along with the NaCl treatment.

**Measurement of plant growth:** The number of died plants was counted, and the growth stage of mortality was considered in each treatment. Average height, phenological stage (Varderlip, 1993), number of expanded, under-expanded and died leaves were also monitored in two individuals in each box. Two plants were cut at the sand surface and after measuring the fresh weight of shoots; they were separated into leaf blades, sheaths, stems and inflorescences precisely. After measurement of leaf blades area (by Leaf Area Meter; Model LI- 31 DOC, LI-COR), the samples were oven dried at 75$^o$C for 4 days and then weighed. Plants were harvested 90 day after germination and all analytical measurements were conducted at this stage. Roots were also harvested together, carefully washed, oven dried as mentioned and weighed.

**Relative water content:** Relative water content (RWC) was determined from 60 leaf discs with a diameter of 6 mm, excluding the midrib, from the youngest fully expanded leaf. Discs were weighed and then immediately floated in double distilled water in Petri dishes. In this way, leaf discs were saturated with water for 24h in the dark at $4^{o}C$. Turgid weights of leaf discs were obtained after removing superficially adhering droplets. Dry weights of discs were then measured after drying at $70^{o}C$ for 48h. Relative water content of the discs was calculated as RWC= (f.wt- d.wt)/ (t.wt- d.wt) (Netondo *et al*., 2004) where f.wt, d.wt, and t.wt are the fresh, oven dry and turgid weights, respectively.

**Photosynthesis:** Gas exchange and related parameters were measured between 11 am to 1 pm using a portable ADC infra-red gas analyzer (model: LCA4), at PPFD>950 µmol m$^{-2}$ s$^{-1}$; leaf chamber temperature at $33\pm2^{o}C$. All measurements were carried out on the intact youngest fully developed leaf, keeping the chamber constantly vertical to solar radiation, till stabilizing gas exchange in the leaf chamber. At least, 10 records were obtained for each leaf after reaching steady state. Total chlorophyll content of the leaves was representatively estimated by a SPAD instrument (Minolta, Japan) and SPAD numbers were recorded for three points in the youngest fully expanded leaves of two plants.

**Statistical analysis:** The experiment was designed and analyzed as factorial based on a completely randomized design with salinity treatment (in 4 levels), supplemental calcium (in 2 levels), and supplemental potassium (in 2 levels), with 3 replications. Statistical significance, where indicated, is at 5% level as determined by analysis of variance and Fisher's LSD test.

**Results**

**Morphological traits:** Salinity significantly reduced the plant height as shown in Fig. 1. This reduction was partially prevented using supplemental $Ca^{2+}$ and $K^{+}$ at 160 m*M*. In the low salinity stress, application of additional $Ca^{2+}$ or $K^{+}$ caused a decrease in height, especially when applied calcium. Supplemental $Ca^{2+}$ increased plant height in general, however, not significantly, but supplemental $K^{+}$ lowered this parameter. Therefore, application of 20 m*M* $CaCl_2$ as well as 20 m*M* KCl to the root medium ameliorated salinity effects on sorghum, especially at moderate and high stress.

Number of leaves was significantly affected by NaCl, while elevated calcium promoted total number of leaves, particularly at high levels of NaCl (Table 1). The interaction effect of $Ca^{2+}$ and $K^{+}$ improve leaf generation only at 160 m*M* NaCl. Salt stress, increased mortality of leaves exposed to salinity. Application of $Ca^{2+}$ and $K^{+}$ didn't make up the adverse effects of salinity, in comparison to control. Number of fully expanded leaves followed the same pattern of the total leaf number (Table 1). The phenological growth stage at which the growth of died plants ceased, as indicated as the number of leaves in died plants, has been shown in Table 1. Increase in the number of leaves represents delay in mortality, therefore, supplemental $Ca^{2+}$ and $K^{+}$ led to delay in cessation of growth. This ameliorative effect was evident when $Ca^{2+}$ and $K^{+}$ were applied in combination. Mortality of sorghum plants increased strongly, by adding NaCl to the root medium. Simultaneous application of calcium and potassium restored mortality under saline conditions and elevated $Ca^{2+}$ also decreased the number of died plants at the highest level of NaCl.
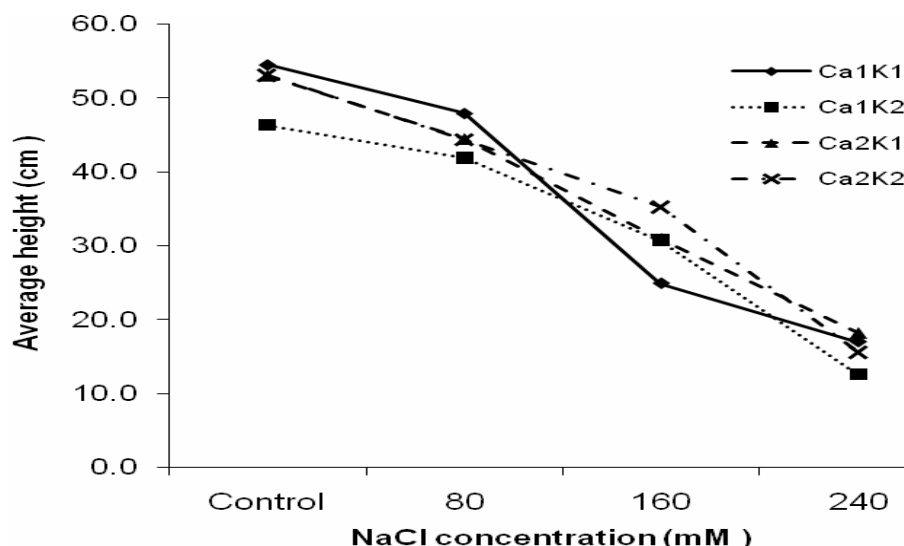
Fig. 1. Interactive effects of salt stress, calcium and potassium on the height of sorghum plants (cm) grown in the greenhouse. The treatments included salinity as NaCl in 4 levels (control, 80, 160, and 240 m*M*), calcium as $CaCl_2$ in two levels (control (Ca1) and 20 m*M* (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m*M* (K2)).

**Table 1. Interactive effects of salinity, calcium and potassium on some morphological traits of sorghum including total leaf number, died leaves/total leaf number, fully expanded leaves (in alive plants) and leaf number in died plants.**

| | Total number of leaves | | | | Died leaves/total leaf number | | | |
|---|---|---|---|---|---|---|---|---|
| $CaCl_2$ (m*M*) | **0** | **0** | **20** | **20** | **0** | **0** | **20** | **20** |
| KCl (m*M*) | **0** | **20** | **0** | **20** | **0** | **20** | **0** | **20** |
| NaCl (m*M*) | | | | | | | | |
| 0 | 16.3 | 14.7 | 16.2 | 15.5 | 0.44 | 0.41 | 0.38 | 0.40 |
| 80 | 14.8 | 14.5 | 13.7 | 14.5 | 0.45 | 0.49 | 0.43 | 0.38 |
| 160 | 12.7 | 12.7 | 14.3 | 14.7 | 0.54 | 0.46 | 0.45 | 0.43 |
| 240 | 11.7 | 10.8 | 12.8 | 10.5 | 0.59 | 0.59 | 0.62 | 0.57 |
| | LSD=1.50 | | | | LSD=0.091 | | | |
| NaCl (m*M*) | **Leaf number** (in alive plants) | | | | **No. of leaves in died plants** | | | |
| 0 | 12.7 | 11.5 | 12.8 | 12.2 | - | - | - | - |
| 80 | 12.2 | 11.5 | 10.3 | 11.0 | 4.0 | 7.0 | 6.5 | - |
| 160 | 10.5 | 10.7 | 11.2 | 11.3 | 5.0 | 5.8 | 7.0 | - |
| 240 | 10.0 | 9.7 | 11.2 | 9.2 | 4.7 | 6.3 | 6.8 | 6.4 |
| | LSD=1.22 | | | | | | | |

LSD = Least significant differences at 5%

**Plant growth:** Exposure of sorghum plants to salt stress strongly prevented growth and accumulation of dry matter. Average shoot fresh weight declined from 88.81g plant$^{-1}$ in control to 6.77 g plant$^{-1}$ at 240 m*M* NaCl. Application of 20 m*M* $CaCl_2$ caused an increase from 41. 99 g to 47.71 g in fresh weight, while in contrast, application of 20 m*M* KCl decreased fresh weight from 48.64 to 40.57 g plant$^{-1}$ (Fig. 3).

Although no application of $Ca^{2+}$ kept the fresh weight higher in low stress, application of $Ca^{2+}$ or both $Ca^{2+}$ and $K^+$ ameliorated partially the adverse effects of high levels of salinity. Shoot dry weight also showed the same trend (Fig. 3). Additional 20 m*M* $K^+$ reduced growth at all NaCl concentrations, indicating that this amount of potassium not only promoted growth reduction in saline conditions, but also imposed adverse effects on accumulation of dry matter, accompanying with salinity, or even in the

absence of NaCl. Any supplemental $Ca^{2+}$ or $K^+$ also caused reduction in growth in control plants (Mahmood, 2009).

Although inflorescence dry weight as shown in Table 2, was significantly affected by the addition of 240 m$M$ NaCl, but it performed differently at low levels of NaCl. This parameter reduced from 0.436 to 0.408 and 0.032 in 0, 80 and 160 m$M$ NaCl, respectively, but was 0.039, 0.394, and 0.128 for elevated potassium, respectively. These results show that, although, salinity delayed reproductive growth, application of $K^+$ in the root medium promoted reproductive stage by enhanced investment on reproductive organs. The interaction of $Ca^{2+}$ and $K^+$ also led to faster development.

Leaf blade to shoot dry weight ratio also was affected by NaCl stress and increased from 0.592 in salt free to 0.668 in 240 m$M$ NaCl (significant at 0.01). Any additional $Ca^{2+}$ or $K^+$ mostly elevated this ratio, particularly for Ca plus K treatment which showed the maximum amount on the high levels of salinity (Fig. 3). This indicates that the leaf blade increased under saline conditions and ameliorative effects of $Ca^{2+}$ and $Ca^{2+}$ plus $K^+$ application was most effective in leaf development under salinity stress. $K^+$ applied plants had the highest root weight in normal conditions. Total biomass (shoot + root) also behaved similar to shoot dry weight, as explained earlier (Fig. 3)

Root to shoot ratio, as an important factor in osmotic stress condition, was also influenced by salinity, $Ca^{2+}$ or $K^+$. The root/shoot ratio decreased at low salinity, but increased substantially at 240 $mM$ NaCl. This indicates the fact that sorghum plants, most probably, invested more on partitioning assimilates to root, rather than to shoot, under high salinity stress and supplemental $Ca^{2+}$ or $Ca^{2+}$ plus $K^+$, assisted this partitioning. Application of $K^+$ failed to increase root/ shoot weight ratio. The proportional weight of each module has been indicated in Figure 4.

**Leaf area:** Measured leaf area was affected strongly by salinity and decreased sharply by increasing NaCl. The beneficial effects of supplemental $Ca^{2+}$ and $Ca^{2+}$ plus $K^+$ were evident in higher levels of salt (Fig. 5). $K^+$ treatments produced always lower leaf area, which denotes the adverse effect of 20 $mM$ potassium on leaf development of sorghum plants. Specific leaf weight increased at high levels of salinity, from 40.78 to 51.45 gm$^{-2}$ in control and 240 $mM$ NaCl, respectively (Fig. 5). The elevated $Ca^{2+}$ intensified this effect more, while $K^+$ application resulted in lower SLW (Fig. 5).

**Relative water content:** RWC, was not affected by salinity significantly, with the exception of the highest salinity level (240 m$M$ NaCl). Although all treatments resulted in RWC increase, $Ca^{2+}$ application accelerated RWC at 240 m$M$ NaCl (Fig. 6).

**Gas exchange characteristics:** $CO_2$ assimilation rate decreased generally at the highest level of salinity. Elevated $Ca^{2+}$ prompted photosynthesis at 80 m$M$ NaCl dramatically, from 17.5 to 27.4 µmol $CO_2$ m$^{-2}$ s$^{-1}$, whilst additional K resulted in decrease in photosynthesis at 80 m$M$ NaCl, from 26.3 to 21.7 µmol $CO_2$ m$^{-2}$ s$^{-1}$ (Table 3).

Transpiration rate decreased against high salinity, mostly by supplemental K. Application of $Ca^{2+}$ accelerated transpiration, at 80 m$M$ NaCl from 2.77 to 3.42 mmol $H_2O$ m$^{-2}$ s$^{-1}$, but addition of K imposed adverse effect on transpiration. SPAD number, as represents total leaf chlorophyll content, declined significantly at 240 m$M$ NaCl especially for additional K, indicating decrease in chlorophyll content of leaves. Combined application of Ca and K restored chlorophyll contents at the highest salinity. Substomatal $CO_2$ didn't show any particular pattern, however, it increased relatively, especially at severe salt stress (Table 3).
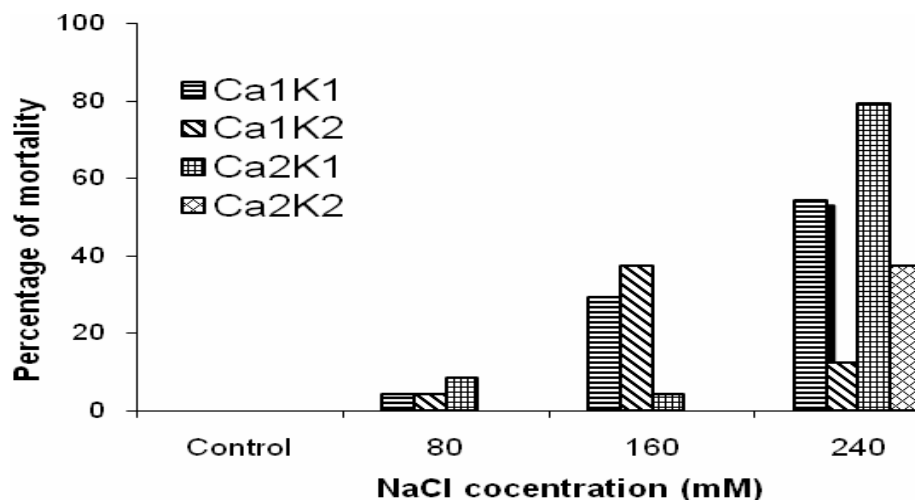
Fig. 2. Percent of plant mortality of sorghum plants as affected by interactive effects of salt stress, calcium and potassium. The treatments included salinity as NaCl in 4 levels (control, 80, 160, and 240 m*M*), calcium as CaCl$_2$ in two levels (control (Ca1) and 20 m*M* (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m*M* (K2)).

**Table 2. Interactive effects of salt stress, calcium and potassium on leaf blade, sheath, inflorescence and stem dry weight.**

| | Leaf blade dry weight (g plant$^{-1}$) | | | | Sheath dry weight (g plant$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| CaCl$_2$ (m*M*) | 0 | 0 | 20 | 20 | 0 | 0 | 20 | 20 |
| KCl (m*M*) | 0 | 20 | 0 | 20 | 0 | 20 | 0 | 20 |
| NaCl (m*M*) | | | | | | | | |
| 0 | 9.342 | 6.672 | 8.802 | 7.922 | 4.89 | 3.22 | 4.43 | 4.00 |
| 80 | 7.675 | 4.703 | 5.878 | 6.168 | 3.89 | 2.28 | 2.79 | 3.24 |
| 160 | 1.983 | 2.029 | 3.875 | 4.172 | 0.93 | 1.04 | 1.88 | 1.93 |
| 240 | 0.915 | 0.713 | 0.610 | 0.703 | 0.41 | 0.35 | 0.30 | 0.26 |
| | LSD=1.755 | | | | LSD=1.07 | | | |
| NaCl (m*M*) | Inflorescence dry weight (g plant$^{-1}$) | | | | Stem dry weight (g plant$^{-1}$) | | | |
| 0 | 0.436 | 0.039 | 0.223 | 0.259 | 1.912 | 0.810 | 1.628 | 1.303 |
| 80 | 0.408 | 0.394 | 0.202 | 0.355 | 1.257 | 0.699 | 0.867 | 1.011 |
| 160 | 0.032 | 0.128 | 0.020 | 0.159 | 0.207 | 0.254 | 0.447 | 0.509 |
| 240 | 0.000 | 0.000 | 0.013 | 0.000 | 0.053 | 0.060 | 0.059 | 0.031 |
| | LSD=0.361 | | | | LSD=0.526 | | | |

LSD = Least significant differences at 5%

**Table 3. Interactive effects of salinity, calcium and potassium on assimilation rate, substomatal CO$_2$ concentration, transpiration rate, and SPAD count.**

| | Assimilation rate (µmol CO$_2$ m$^{-2}$s$^{-1}$) | | | | Substomatal CO$_2$ concentration (vpm) | | | |
|---|---|---|---|---|---|---|---|---|
| CaCl$_2$ (m*M*) | 0 | 0 | 20 | 20 | 0 | 0 | 20 | 20 |
| KCl (m*M*) | 0 | 20 | 0 | 20 | 0 | 20 | 0 | 20 |
| NaCl (m*M*) | | | | | | | | |
| 0 | 17.5 | 26.3 | 17.5 | 17.6 | 385.0 | 396.3 | 390.7 | 360.7 |
| 80 | 22.8 | 21.7 | 27.4 | 17.7 | 358.3 | 342.0 | 373.0 | 395.7 |
| 160 | 21.7 | 15.3 | 17.4 | 13.3 | 412.0 | 387.1 | 360.6 | 377.0 |
| 240 | 14.1 | 9.1 | 17.1 | 13.6 | 396.6 | 354.7 | 391.1 | 387.7 |
| NaCl (m*M*) | Transpiration rate (mmol H$_2$O m$^{-2}$s$^{-1}$) | | | | SPAD | | | |
| 0 | 2.40 | 3.00 | 2.77 | 2.24 | 35.4 | 43.4 | 41.5 | 38.3 |
| 80 | 2.67 | 2.70 | 3.42 | 2.16 | 41.5 | 39.2 | 39.6 | 35.2 |
| 160 | 2.46 | 1.41 | 2.39 | 2.17 | 41.8 | 41.1 | 41.8 | 39.2 |
| 240 | 1.69 | 0.93 | 1.81 | 1.34 | 31.3 | 18.3 | 27.5 | 38.6 |
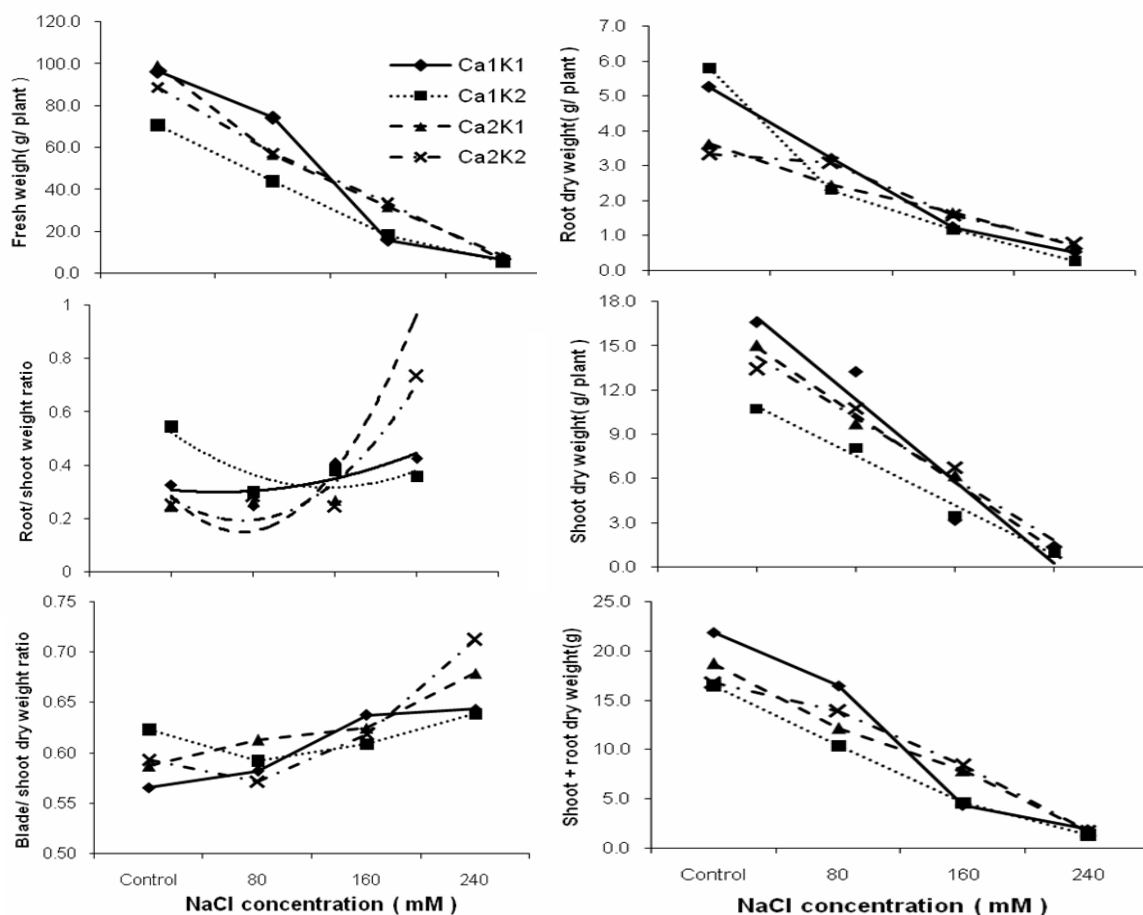
Fig 3. Interactive effects of salt stress, calcium and potassium on shoot fresh weight, shoot dry weight, root dry weight, shoot + root dry weight (biomass production), root/ shoot weight ratio, and leaf blade/ shoot dry weight ratio. The treatments included salinity as NaCl in 4 levels (control, 80, 160, and 240 m$M$), calcium as CaCl$_2$ in two levels (control (Ca1) and 20 m$M$ (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m$M$ (K2)).

## Discussion

Growth of sorghum plant was strongly inhibited by high levels of salinity. Reduction in dry weight reflects the increased metabolic energy cost and reduced carbon gain. It also reflects salt impacts on tissues (Karimi *et al*., 2005) reduction in photosynthetic rates (ZisKa *et al.*, 1990; Ashraf, 2004) and attainment of maximum salt concentration tolerated by the fully expanded leaves (Hu *et al*., 2000).

These findings are in agreement with data shown by Bernstein *et al.,* (1993) and Netondo *et al*., (2004) on sorghum. According to Netondo *et al*., (2004) sodium was the major cation that accumulated in roots and stems of sorghum as salinity increased, but salinity inhibited the accumulation of K$^+$ and Ca$^{2+}$ in roots and stems. Ca$^{2+}$ application partially resulted in amelioration of adverse effects of high NaCl in sorghum growth, despite the fact that supplemental effect of Ca$^{2+}$ increased osmolality, in a study on salinity-calcium interactions on corn (Cramer, 1992). Davenport *et al*., (2005) studying sodium-calcium interaction in two wheat species showed that the rate of sodium uptake into the roots did not vary between the salt sensitive and salt-tolerant species, except at low calcium when the rate of uptake was higher in the tolerant species and more sensitive to inhibition by additional Calcium.
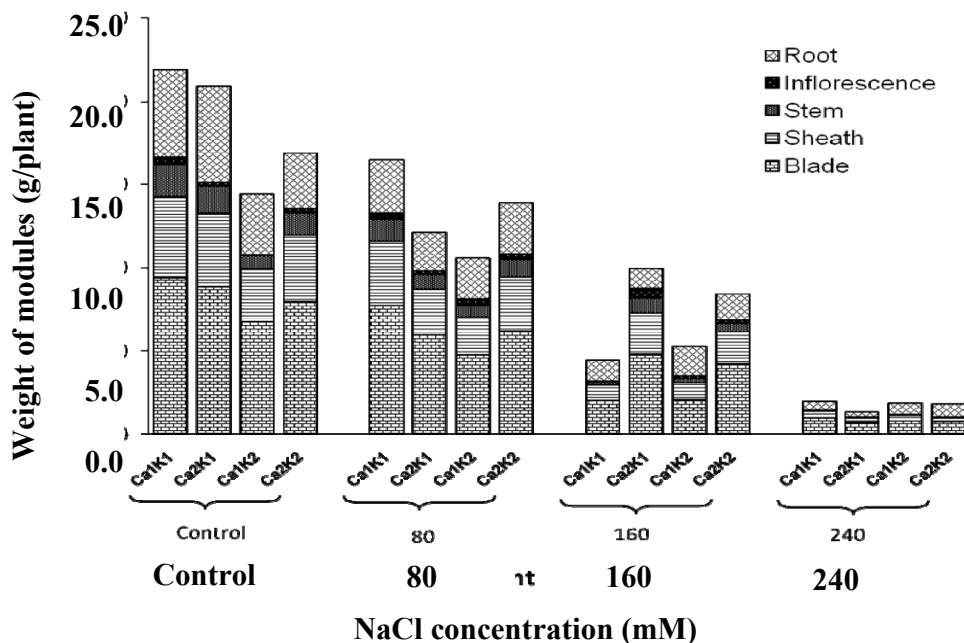
Fig. 4. Proportional dry weight (gr) of different plant organs including root, inflorescence, stem, leaf sheath, and blade of sorghum plants as affected by interactive effects of salt stress, calcium and potassium on sorghum plants. The treatments included salinity as NaCl in 4 levels (control, 80, 160, and 240 m*M*), calcium as $CaCl_2$ in two levels (control (Ca1) and 20 m*M* (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m*M* (K2)).
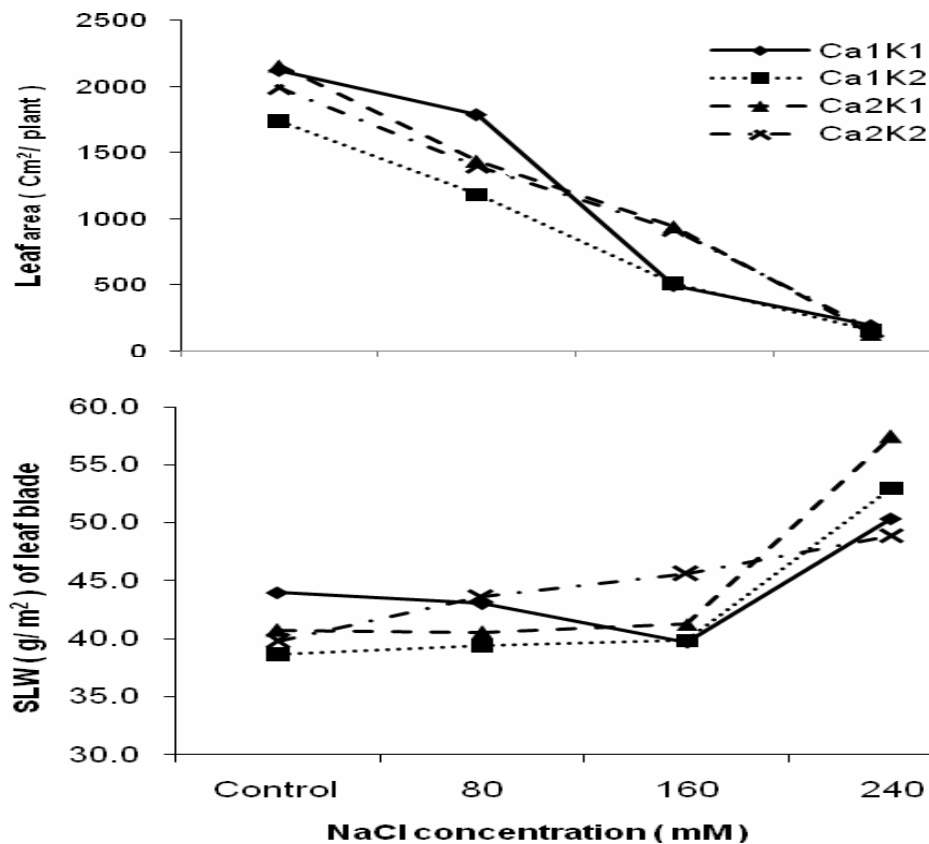


Fig 5. Leaf area and specific leaf weight of sorghum plants as affected by interactive effects of salt stress, calcium and potassium. The treatments included salinity as NaCl in 4 levels (control, 80, 160, and 240 m*M*), calcium as $CaCl_2$ in two levels (control (Ca1) and 20 m*M* (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m*M* (K2)).
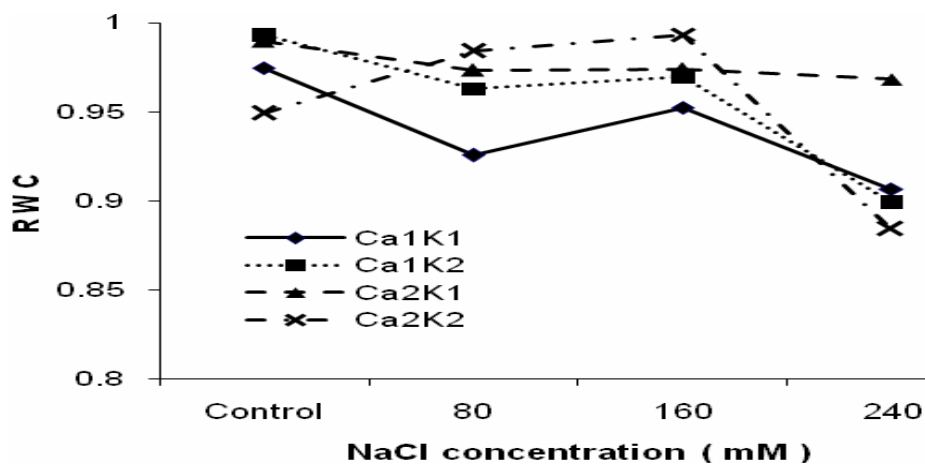
Fig. 6. Relative water content of leaves of sorghum plants as affected by combination of salt stress, calcium and potassium. The treatments included $CaCl_2$ in two levels (control (Ca1) and 20 m$M$ (Ca2)), and potassium as KCl in two levels (control (K1) and 20 m$M$ (K2)).

Our findings showed that $K^+$ application not only stimulated the negative effects of salinity on growth, but also reduced dry matter accumulation particularly at low and medium stress. This is in contrast with the data of some authors who have reported ameliorative effect of $K^+$ on salinity tolerance (Shirazi *et al*., 2005; Ottow *et al*., 2005). In plants, $K^+$ plays an essential role as an osmoticum and charge carrier (Ashraf *et al*., 2008). The capacity of plants to maintain a high cytosolic $K^+/Na^+$ ratio is likely to be one of the key determinants of salt tolerance. The main cause of these results was that the $K^+$ content of the soil in the site of experimentation was 340 ppm which is quit enough for plant nutrition and additional $K^+$ could not bring extra benefit for stressed plants.

Cramer *et al*., (1987) investigated the cation influx in salt stressed cotton and found that increasing concentrations of NaCl significantly inhibited $K^+$ influx. Potassium influx in salt stressed roots was, however, unaffected by the $Ca^{2+}$ concentrations. Leaf blade to shoot weight ratio of sorghum plants increased at high levels of salinity. This indicates the mechanism of increasing leaf area for tolerating salt. Interactive effects of $Ca^{2+}$ and $K^+$ caused this ratio to increase, denoting accelerated investment of plant in increasing leaf blade for better performance under saline conditions (Ashraf *et al*., 2008). Root to shoot ratio also increased sharply in high salinity, which helps water uptake under high osmotic stress.

The adverse effects of $K^+$ in our study, probably reveals the high concentrations of $K^+$ applied (i.e. 20 m$M$) that may have had osmotic effects on plant growth, or even ion toxicity by influence through or on non-active $K^+$ channels (Ashraf *et al*., 2008).

Specific leaf weight was increased by adding NaCl. Our data support the previous findings of accumulation of ions in leaves for salinity tolerance, in order to osmotic adjustment. Some plant species use accumulation of $Na^+$, $Cl^-$, $Ca^+$, $K^+$, and other compatible solutes to decrease increase osmotic potential and in turn to reduce water potential, which leads to increase of SLW (Cramer *et al*., 1987; Gagneul *et al*., 2007).

Leaf area substantially decreased by the application of NaCl to the root medium. Bernstein *et al*., (1993) studied dynamics of sorghum leaf development at various Na/$Ca^{2+}$ salinities. In their study, salinity (100 *mM* NaCl) significantly reduced leaf growth and shoot development. The reduction was partially prevented when elevated concentrations of $Ca^{2+}$ were supplied to root medium. Under saline conditions, the solute potential in the external medium is low, and root pressure is reduced, the supply of $Ca^{2+}$ to the enclosed leaves is also reduced. Elevated $Ca^{2+}$ concentration in the root medium

may result in higher $Ca^{2+}$ concentration, and higher growth speed, especially in young leaves (Mutlu & Bozcuk, 2007).

The relative water content of leaves decreased at the highest level of salinity. Netondo *et al.,* (2004) studied water relations of sorghum plants under salinity and found that sodium chloride significantly reduced leaf water, osmotic, and pressure potentials and RWC. These reductions implied that there was reduction of turgor that plants suffer from restricted water availability to cells. RWC of elevated $Ca^{2+}$ concentration showed almost constant at high salinity, probably due to an advanced ability for osmotic adjustment.

Net $CO_2$ assimilation of sorghum plants decreased at high levels of salinity, after an elevation at low stress, for NaCl or NaCl plus $CaCl_2$ treatments, and was in conformity with changes in transpiration rate. This relationship indicates that the changes in photosynthesis of sorghum under salinity stress are probably due to stomatal, rather than non-stomatal components. Study of photosynthetic and stomatal response of two mangrove species to salinity (Ball & Farquhar, 1984) also showed decline in photosynthetic capacity, with increasing salinity. Those data showed that the stomatal conductance and photosynthetic capacity changed in the same sense and therefore co-limited the assimilation rate. In the present investigation, SPAD data, representatively chlorophyll amount, declined significantly at the higher levels of salinity, most probably caused part of decrease in photosynthesis. $CO_2$ assimilation rate increased at low salt stress, in consistence with an increase in transpiration rate. This issue possibly gives a fuzzy picture of the role of some aspects of drought resistance of sorghum, as a crop equipped with several mechanisms of drought tolerance (Abdul Majid *et al.*, 2007) i.e., stimulated transpiration rather than expected decrease, which assists better performance in stomatal conductance and hence accelerated carbon gain at mild salinity stress.

**References**

Abdul Majid, S., R. Asghar and G. Murtaza. 2007. Potassium- calcium interrelationship linked to drought tolerance in wheat (*Triticum aestivum* L.). *Pak. J. Bot.,* 39(5): 1609- 1631.
Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora,* 199: 361-376.
Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech. Adv.,* 27: 84-93.
Ashraf, M., M. Ozturk and H.R. Athar. 2008. Salinity and Water Stress (Eds). Series: tasks for vegetation science series 44. *Improving Crop Efficiency*. Springer-Verlag, Netherland. P246.
Ball, M. C. and G. D. Farquhar. 1984. Photosynthetic and stomatal responses of two mangrove species, *Aegiceras corniculantum* and *Avecinnia marina*, to long term salinity and humidity conditions. *Plant Physiol*., 74: 1- 6.
Banuls, J., F. legaz and E. Primo-Milo. 1991. Salinity- calcium interactions on growth and ionic concentration of citrus plants. *Plant Soil*, 133: 39-46.
Bernstein, N., A. Lauchli and W. K. Silk. 1993. Kinematics and dynamics of sorghum (*Sorghum bicolor* L.) leaf development at various Na/ $Ca^{2+}$ salinities. *Plant Physiol*., 103: 1107- 1114.
Carden, D. E., D. J. Walker, T. J. Flowers and A. J. Miller. 2003. Single- cell measurements of the contributions of cytosolic $Na^+$ and $K^+$ to salt tolerance. *Plant Physiol*., 131: 676- 683.
Cramer, G. R. 1992. Kinetics of maize leaf elnogation. II. Response of a Na-excluding cultivar and a Na-including cultivar to varying Na/Ca salinities. *J. Exp. Bot.,* 43: 857-864.
Cramer, G. R., J. Lynch, A. Lauchli and E. Epstein. 1986. Effects of NaCl and $CaCl_2$ on ion activities in complex nutrient solutions and root growth of cotton. *Plant Physiol*., 81: 792-797.

Cramer, G. R., J. Lynch, A. Lauchli and E. Epstein. 1987. Influx of $Na^+$, $K^+$ and $Ca^{2+}$ into roots of salt stressed cotton seedlings. *Plant Physiol.*, 83: 510- 516.

Davenport, R. J., R. A. James, A. Zakrisson-Plogander, M. A. Tester and R. E. Munns. 2005. Control of sodium transport in durum wheat. *Plant Physiol.,* 137: 807-818.

Gagneul, D., A. Aïnouche, C. Duhazé, R. Lugan F. R. Larher and A. Bouchereau. 2007. A reassessment of the function of the so-called compatible solutes in the halophytic plumbaginaceae *Limonium latifolium. Plant Physiol.*, 144: 1598-611.

Hoagland, D. R. and D. I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agric. Exp. Sta.* Circ., 347: 313-320.

Hu, Y., H. Schnyder and U. Schmidhalter. 2000. Carbohydrate deposition and partitioning in elongation leaves of wheat under saline soil conditions. *Aust. J. Plant Physiol.*, 27: 363- 370.

Karimi, J., M. Ghorbanli, H. Heidari, R. A. Khavari Nejad and M.H. Assareh. 2005. The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrata. Biol. Planta,* 49: 301- 304.

Lynch, J. and A. Lauchli. 1988. Salinity affess intracellular calcium in corn root protoplasts. *Plant Physiol.*, 87: 351-356.

Mahmood, A. 2009. A new rapid and simple method of screening wheat plants at early stage of growth for salinity tolerance. *Pak. J. Bot.*, 41(1): 255-262.

Malhotra, B. and A. D. M. Glass. 1995. Potassium fluxes in *Chlamydomonas reinhardtii. Plant Physiol.*, 108: 1527- 1536.

Mutlu, F. and S. Bozcuk. 2007. Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots differing in salt tolerance. *Pak. J. Bot.*, 39(4): 1097-1102.

Netonda, G. W., J. C. Onyango and E. Beck. 2004. Sorghum and salinity: I. Responses of growth, water relations, and ion accumulation to NaCl salinity. *Crop Sci.*, 44: 797- 805.

Netonda, G. W., J. C. Onyango and E. Beck. 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci.*, 44: 806-811.

Noreen, Z., M. Ashraf and M. U. Hassan. 2007. Inter-accessional variation for salt tolerance in pea (*Pisum sativum* L.) at germination and seedling stage. *Pak. J. Bot.*, 39(6): 275-285.

Ottow E. A. and A. Polle. 2005. *Populus euphoratica* displays apoplastic sodium accumulation, osmotic adjustment by decrease in calcium and soluble carbohydrates and develops leaf succulence under salt stress. *Plant Physiol.*, 139: 1762- 1772.

Reid R. J. and F. A. Smith. 2000. The limits of sodium/calcium interactions in plant growth. *Aust. J. Plant Physiol.,* 27**:** 709-715.

Rubio, L. and M. A. Botella. 2004. Regulation of $K^+$ transport in tomato roots by the *TSS1* locus; implications in salt tolerance. *Plant Physiol.*, 134: 452- 459.

Sabir, P. and M. Ashraf. 2007. Screening of local accessions of *Panicum miliaceum* L., for salt tolerance at the seedling stage using biomass production and ion accumulation as selection criteria. *Pak. J. Bot.,* 39(5): 1655-1661.

Shabala S., V. Demidchik, L. Shabala, T. A. Cuin, S. J. Smith, A. J. Miller, J. M. Davies and I. A. Newman. 2006. Extracellular $Ca^{2+}$ ameliorates NaCl-induced $K^+$ loss from *Arabidopsis* root and leaf cells by controlling plasma membrane K+-permeable channels. *Plant Physiol.,* 141: 1653-1665.

Shirazi, M. U., M. Y. Ashraf, M. A. Khan and M. H. Naqvi. 2005. Potassium induced salinity tolerance in wheat. *Int. J. Environ. Sci. Tech.,* 2: 233- 236.

Vandrelip, R. L. 1993. How a sorghum plant develops. URL: http://www.oznet.ksu.edu/library/crps12/samplers/s3.asp; verified 3Dec, 2008.

Ziska, L. H., J. R. Seemann and T. M. Dejong. 1990. Salinity induced limitation on photosynthesis in *Prunus salicina*, a deciduous tree species. *Plant Physiol.*, 93: 864- 870.