

MORPHO-PHYSIOLOGICAL RESPONSE OF HYBRID MAIZE (*Zea mays* L.) TO SALT STRESS IN COMBINATION WITH EXOGENOUS CALCIUM APPLICATIONS

KHAN, A.^{1*} – AHMAD, R.¹ – AFSAR, S.¹ – ZAFFAR, H.² – BAIG, A.¹ – MUGHEES, M.¹ – SHAH, M. M.¹

¹Department of Biotechnology, COMSATS University Islamabad, Abbottabad Campus, 22010 Abbottabad, Pakistan

²Department of Environmental Sciences, COMSATS University Islamabad, Abbottabad Campus, 22010 Abbottabad, Pakistan

*Corresponding author
e-mail: asma_quaidian@yahoo.com

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Abstract. Maize, the third most important cereal crop around the globe, is hampered by devastating abiotic factors including salt stress. Calcium is known to increase plant tolerance to biotic and abiotic stresses by influencing physiological processes. The current study was carried out to investigate the genetic variation of maize hybrids against salt stress in combination with exogenous application of calcium (Ca^{2+}) in a hydroponic culture. Three levels of salinity, each with NaCl (0 mM, 75 mM, and 150 mM) and Ca^{2+} (0 mM, 2.5 mM, 5 mM) were applied to maize hybrids in three replications and data were recorded for key morpho-physiological traits. The results showed that all concentrations of NaCl significantly affect the maize hybrids with Sarhad White Azad Jammu Kashmir (SWAJ 6,7) and Syngenta 8441 being the least affected by both 75 mM and 150 mM NaCl concentrations. The exogenous application of 2.5 mM Ca^{2+} with 75 mM salt concentration showed significant effects on all morpho-physiological traits of maize hybrids. Better growth performance, maintenance of nutrient contents, lower accumulation of toxic sodium ions and lower Na^+/K^+ in SWAJ6,7 and Syngenta 8441 indicate that these maize hybrids are more tolerant to salinity stress than the others under study.

Keywords: hydroponic, ion contents, *Zea mays* L.), NaCl, proline

Introduction

Soil salinity is one of the key environmental constraints that limit the production of crop throughout the world (Jiang et al., 2018). In arid and semiarid regions, the soil salinity more severely affects crop production due to global climate changes and as consequences of irrigation practices (Farooq et al., 2015). In Pakistan around 6.8 million hectares of land is affected by salinity. Therefore, major agricultural area remains uncultivated (Chaudhary et al., 2017). Saline soils have high pH and electrical conductivity ($\text{EC} > 4.0 \text{ dS/m}$) and are usually rich in sodium, chloride and sulfate ions, with high absorption rate of sodium (Flowers and Flowers, 2005). In the rhizosphere, osmotic pressure due to high saline condition reduces mineral and water uptake by the plant, which ultimately affect primary and secondary metabolism of the plant (Hendawy and Khalid, 2005). The cumulative response of meristematic and expanding cells to salt stress is the inhibition of shoot growth which is observed primarily under salinity stress (Shoresh et al., 2011). Salt stress causes various morphological and metabolic modifications in plants by creating osmotic stress and ionic imbalance thus disrupts the vital cellular functions (Taylor et al., 2004; Yildirim et al., 2006). Osmotic stress leads to the reduced water absorption by roots and increase water loss from leaves. This

mechanism is called hyperosmotic stress and it leads to various physiological modifications in plants such as nutrient imbalance, decreased photosynthetic activity, decreased stomatal apertures, membranes interruptions, modifications in antioxidant enzymes and failure to detoxify reactive oxygen species (ROS) (Soliman and El-Shaieny, 2014). The formation of ROS due to salt stress interrupts vital cellular functions by causing damage to various cell components such as DNA, protein and lipids (Gupta and Huang, 2014). Plant tissues exposed to saline soil accumulate more Na^+ and Cl^- which is one of the most detrimental effect as it causes severe ionic imbalance in plants by decreasing uptake of important mineral ions which are essential for the growth and development of plant (James et al., 2011). Reduced water permeability and aquaporin abundance of membrane under salinity stress is restored by calcium treatment (Martínez-Ballesta et al., 2008).

Calcium, an essential plant nutrient is responsible for the maintenance of structure and functions of plant membranes. It also increases the stability of cell wall structures, selectivity and transport of ions and improves the metabolic activities in plants. Calcium as second messenger plays a crucial role in signal transduction in response to developmental and environmental signals (Guo et al., 2019). Almost half of the cellular Ca^{2+} is bound mainly to carboxyl groups of pectins in the cell wall (Cramer, 2002). Previous studies indicated that salt induced inhibition of growth is improved by high content of Ca^{2+} bound to pectin on cell wall (Madea et al., 2005). Increased Na^+ ions due salinity stress reduces calcium availability and its transport to growing regions of the plant (Shoresh et al., 2011). The reduction of Ca^{2+} concentration in salinized plants severely effects the physiological function. The addition of supplemental Ca^{2+} provide protection by stabilizing on the cell-wall components (Tuna et al., 2007).

Maize (*Zea mays* L.), an important cereal crop is grown under wide range of soil and climatic conditions. It is an important C4 plant belongs to the grass family Poaceae (Farooq et al., 2015). About 21% of the total maize grain produced is consumed as food throughout the world (Chaudhary et al., 2017). The larger producer of maize is the United States with annual yield of about 4096190 thousand million tons. In Pakistan average yield of maize is 4268 kg/ha (Economic Survey of Pakistan, 2012-13). Various biotic and abiotic stresses extremely affect the yield potential of maize crop. Among abiotic stresses, salinity reduces the maize yields by causing numerous biochemical and physiological changes in plants (Zafar-ul-Hye et al., 2014). Maize is found to be moderately sensitive to salt stress. However, extensive genetic variation exists intra-specifically in maize for salt resistance (Mansour et al., 2005). The current study was aimed to explore the genetic variation of five different maize hybrids against salt stress in combination with exogenous application of Ca^{2+} .

Materials and Methods

In order to investigate the influence of exogenously applied CaSO_4 as calcium source on five maize hybrids under salt stress, an experiment was conducted in 2018 under hydroponic conditions in the greenhouse of COMSATS University Islamabad, Abbottabad Campus, Pakistan. Maize hybrids used in current study were: COMSATS Random Mating 8 (CTRM 8), Pirsabak Experimental Variety 2 (PSEV 2), Sarhad White Azad Jammu Kashmir 6, 7 (SWAJ 6, 7) and Syngenta 8441.

Plant growth and experimental treatments

The seeds of five maize hybrids (CTRM 8, PSEV 2, SWAJ 6-7, Syngenta 8441 and XPW3) were sterilized with 1% HOCl for 10 minutes and rinsed with distilled water, the process being repeated 3 times. The seeds were germinated on moist thin sheets of foam at 25°C in an incubator. After germination, uniform sized seedlings were transferred in constantly aerated pots containing 1/3rd strength Hoagland's solution. Plants were adapted to full strength nutrient solution in 25% increments every second day in similar way as described by Richter et al. (2015). The experiment was carried out in a completely randomized design (CRD). Plants were subjected to different concentrations of NaCl along with application of Ca²⁺ as foliar at four leaf stage. Seven different treatment combinations with three replicates per treatment were used. Treatment combinations were as follow:

T1: Control (Hoagland solution alone); T2: Salt Stress (75 mM NaCl); T3: Salt stress and foliar (75 mM NaCl + 2.5 mM Ca²⁺); T4: Salt stress and foliar (75 mM NaCl + 5 mM Ca²⁺); T5: Salt Stress (150 mM NaCl); T6: Salt stress and foliar (150 mM NaCl + 2.5 mM Ca²⁺); T7: Salt stress and foliar (150 mM NaCl + 5 mM Ca²⁺).

Growth parameters

Growth parameters were scored at seventh day of treatments application by measuring leaf number, leaf area, shoot length and maximum root length. Fresh weights of plants were taken immediately after harvesting. For dry weight, plant organs were placed in an incubator at 75°C for 72 hrs (Sun et al., 2018). Leaf area was calculated according to Equation 1:

$$A = LL * W * K \quad (\text{Eq.1})$$

where,
LL represents leaf length,
W is the maximum width and
K is a shape factor with value 0.75.

Chlorophyll content

Chlorophyll extraction was done by grinding 0.1 g of fresh leaves in 15 ml of acetone (80%). This mixture was then subjected to centrifugation at 5000 rpm for 10 minutes at 4°C. Supernatant from each sample was collected until the residue was turned into colorless mixture. The chlorophyll content was measured using spectrophotometer at wavelength of 645 nm and 663 nm (Nayyar et al., 2005).

Relative water content

Relative Water content (RWC) was determined according to Equation 2 as described by Loutfy et al. (2012).

$$RWC(\%) = [(FW - DW)/(TW - DW)] * 100 \quad (\text{Eq.2})$$

where,
FW represents fresh weight,
TW is Turgid weight and
DW is Dry weight.

Electrolyte leakage

For determination of electrolyte leakage (EL), 10 leaf discs of about 10 mm diameter from expanded leaves of all maize hybrids were taken and rinsed with distilled water to get rid of electrolytes adhered to the surface. These leaf discs were incubated at 10°C after placing them in glass tubes filled with 10 ml of distilled water for 24 hr. The initial electrical conductivity (EC1) of the solution was measured using conductometer. To release all electrolytes, these tubes were heated at 95°C in water bath for 20 min followed by cooling at room temperature. After that final electrical conductivity (EC2) was determined.

EL was calculated using *Equation 3* (Yildirim et al., 2009).

$$EL = \left(\frac{EC1}{EC2} \right) * 100 \quad (\text{Eq.3})$$

Plant mineral ion contents

For determination of mineral ions, 100 mg of dried plant samples (roots and leaves) were heated for about 5 hrs in furnace at 520°C for ash formation. The ash of each plant sample was then solubilized in nitric-perchloric acid mixture (5:1) and volume was raised to 15 ml using distilled water. Afterwards filtered suspensions were used to determine the concentrations of Na⁺, K⁺, Ca⁺² and Mg⁺² using atomic absorption spectrometry. For standard curves, different concentrations of Ca⁺², K⁺, Na⁺ and Mg⁺² were prepared by diluting stock solution of CaSO₄, KCl, NaCl and MgSO₄. The standard curve was used to determine the content of each element in the plant organs (roots and leaf) and expressed in mg g⁻¹ dw (Qadir, 2016).

Proline contents

Leaf proline content was extracted and analyzed by the method of Bates et al. (1973). 0.1 g of fresh leaf of all maize hybrids were ground using liquid nitrogen in a mortar. 1 ml of sulfosalicylic acid (3% w/v) was added to the resulted homogenate powder of leaves and then filtered. The filtrate was reacted with 1 ml of glacial acetic acid (GAA) and 1 ml of ninhydrin reagent (1.25 mg Ninhydrin in 30 ml of GAA and 20 ml 6 M H₃PO₄) and placed in an incubator for 1 hr at 95°C. To terminate the reaction, reaction mixture containing tubes were kept in ice bath for 5 min followed by vigorous mixing with 2 ml toluene. After warming at 25°C, the chromophore was measured at 520 nm. L-proline was used as a standard.

Statistical analysis

For statistical analysis, results of all experiments were expressed as mean ± standard deviation (SD) of three replicates. Data were subjected to one-way analysis of variance (ANOVA) and LSD multiple comparison test (*P* < 0.05) using the SPSS statistical package. Significant differences are indicated by alphabets in figures and tables.

Results

Growth parameters of maize under salt stress and Ca²⁺ treatments

The growth parameters of maize seedlings were evaluated by measuring leaf area, root length and shoot length of plants under salt stress. Effect of salt stress and calcium on

growth parameters of five maize hybrids (CTRM8, PSEV2, SWAJ 6, 7, Syngenta 8441 and XPW3) are listed in *Table 1*. Under salt stress all five Maize hybrids react differently. Salt addition showed significant effect on growth parameters like leaf area, root length and shoot length of plants. The maximum decrease in these parameters was observed in CTRM 8 and XPW 3 whereas maximum shoot length, maximum root length and leaf area was exhibited by SWAJ 6, 7 and Syngenta 8441 which showed improved growth traits. However, effects of salinity were significantly alleviated in all five maize hybrids by foliar application of 2.5 mM Ca^{2+} . Although application of 5 mM Ca^{2+} also improved plant growth but the effect was not significant as compare to 2.5 mM Ca^{2+} (*Table 1*).

Table 1. Shoot length (cm), Root length (cm), and Leaf area (m^2) concentration in leaves of all five Maize hybrids in response to salinity and Ca^{2+} concentrations

Maize hybrid	Treatment	Shoot length	Root length	Leaf area
CTRM8	T1	31.00 CD	21.33 G	21.85 IJ
	T2	22.68 I	17.00 IH	13.79 L
	T3	32.50 C	19.83 H	27.55 D
	T4	30.33 D	15.67 I	25.72 F
	T5	15.68 KL	13.33 J	13.34 L
	T6	30.17 D	17.17 IH	24.04 FG
	T7	24.00 G	11.83	17.23 K
PSEV2	T1	33.50 BC	18.17 H	24.38 FG
	T2	22.67H	16.17 I	18.55 IK
	T3	33.33 BC	21.50 G	26.58 E
	T4	30.00 E	18.33 H	25.09 F
	T5	18.50 K	12.67 J	18.77 IK
	T6	32.00 D	16.17 I	25.96 EF
	T7	27.00 EF	8.17 M	18.19 IK
SWAJ 6, 7	T1	34.67 C	33.50 A	26.46 E
	T2	28.17 F	16.83 I	23.04 G
	T3	33.17 BC	32.50 B	31.64 B
	T4	31.00 CD	25.67 E	26.83 E
	T5	20.50 J	17.67 H	18.86 IK
	T6	33.17 BC	27.00 D	25.24 F
	T7	31.67 D	16.17 I	25.08 F
Syngenta 8441	T1	38.17 A	33.83 A	28.88 CD
	T2	27.00EF	25.57 E	26.06 E
	T3	37.00 AB	32.67 B	33.26 A
	T4	33.50 BC	23.33 F	30.14 BC
	T5	23.83 GH	26.67 DE	23.63 G
	T6	33.67 CD	21.00 G	29.67 C
	T7	36.17 B	28.83 C	27.75 D
XPW3	T1	27.00 EF	26.50 DE	21.85 H
	T2	24.83 H	14.50	17.08 K
	T3	29.33 DE	18.67 H	22.74 GH
	T4	25.33 FG	14.17 K	22.35 GH
	T5	15.83 KL	13.00 K	14.93 KL
	T6	26.67 G	14.33 K	20.13 IJ
	T7	24.17 H	12.67 L	19.55 I

Mean values followed by different letters within each column differ significantly at $P \leq 0.05$. T1: Control (non- saline); T2: 75 mM NaCl; T3: 75 mM NaCl + 2.5 mM Ca^{2+} ; T4: 75 mM NaCl + 5 mM Ca^{2+} ; T5: 150 mM NaCl; T6: 150 mM NaCl + 2.5 mM Ca^{2+} ; T7: 150 mM NaCl + 2.5 mM Ca^{2+}

Leaf chlorophyll content in maize under salt stress and Ca^{2+} treatments

Salt stress showed significant effect on total leaf chlorophyll contents of maize hybrids. Increase in salt stress resulted decrease in chlorophyll contents of maize leaves. It is found from the results that the high levels of salinity (150 mM NaCl) induced reduction in the total chlorophyll contents of leaves significantly in comparison to leaves of control plants. However, Ca^{2+} application on plants under salt stress increased the leaf chlorophyll contents (Table 2). Importantly, 2.5 mM Ca^{2+} treatments showed significantly higher total chlorophyll contents compared to that of 5 mM Ca^{2+} treatments.

Table 2. Total leaf chlorophyll contents (mg g^{-1} FW), RWC (%), and Electrolyte leakage (%) concentration in leaves of all five Maize hybrids in response to salinity and Ca^{2+} concentrations

Maize hybrid	Treatment	Total chlorophyll (mg g^{-1} FW)	Relative water (%)	Electrolyte leakage (%)
CTRM8	T1	15.19 C	77.09 I	22.61 R
	T2	9.42 F	72.32 J	61.67 D
	T3	13.58 D	86.60 D	33.93 N
	T4	11.80 E	80.56 H	29.61 P
	T5	6.79 G	60.14	68.63 A
	T6	10.88 EF	78.73 I	65.26 B
	T7	10.35 EF	59.60 N	63.15 C
PSEV2	T1	17.73 B	84.34 E	25.33 Q
	T2	12.01 DE	70.14 K	58.54 E
	T3	15.79 C	96.34 A	36.33 M
	T4	13.55 D	79.25 H	32.33 N
	T5	8.51 F	61.26 M	63.33 C
	T6	13.43 D	77.16 I	61.33 D
	T7	14.06 CD	65.67 L	59.33 E
SWAJ 6, 7	T1	19.38 A	90.85 C	20.00 S
	T2	13.16 D	73.05 J	46.39 K
	T3	16.26 BC	93.31 B	31.00 O
	T4	14.45 CD	85.85 E	27.53 Q
	T5	10.56 EF	61.81 M	56.27 F
	T6	15.10 C	81.84 G	49.38 I
	T7	13.59 D	72.01 J	46.05 K
Syngenta 8441	T1	19.60 A	93.68 B	19.32 S
	T2	13.68 D	80.27 H	45.21 L
	T3	16.63 BC	96.76 A	30.32 P
	T4	14.99 CD	86.05 D	26.42 Q
	T5	11.75 E	68.34 L	55.15 G
	T6	14.40 CD	81.66 G	48.00 J
	T7	15.40 C	67.06 L	45.00 L
XPW3	T1	17.46 B	85.25 D	23.08 R
	T2	10.51 EF	65.67 L	59.68 E
	T3	14.97 C	89.66 C	33.74 N
	T4	14.42 CD	85.29 D	30.26 P
	T5	6.53 G	49.77 P	69.83 A
	T6	9.93 F	83.82 F	51.43 H
	T7	8.38 F	54.29 O	59.80 E

Mean values followed by different letters within each column differ significantly at $P \leq 0.05$. T1: Control (non-saline); T2: 75 mM NaCl; T3: 75 mM NaCl + 2.5 mM Ca^{2+} ; T4: 75 mM NaCl + 5mM Ca^{2+} ; T5: 150 mM NaCl; T6: 150 mM NaCl + 2.5 mM Ca^{2+} ; T7: 150 mM NaCl + 2.5 mM Ca^{2+}

RWC of maize under salt stress and Ca^{2+} treatments

Salt stress significantly reduced RWC in all maize hybrids with more prominent effect in CTRM8 and XPW3 in comparison with the control conditions. Salt-induced water imbalance resulted in decrease RWC. However, foliar application of 2.5 mM and 5 mM Ca^{2+} on salt-stressed maize plants increased RWC significantly in comparison to plants under salt stress only (Table 2).

Electrolyte leakage under salt stress and Ca^{2+} treatments

The increasing salt concentrations enhanced EL value of all maize hybrids (Table 2). The highest EL was observed in CTRM 8 and XPW3 in comparison to SWAJ 6, 7 and Syngenta 8441 under all stress conditions. However, supplemented Ca^{2+} reduced MP of salt stressed plants compared to plants without Ca^{2+} under salt stress. The application of Ca^{2+} significantly improved MP by decreasing the electrolyte leakage under salt stress.

Na, K⁺, Ca and Mg contents of leaves and roots under salt stress and Ca^{2+} treatments

Salinity has very clear effects on ionic composition of maize. Maize genotype SWAJ 6, 7 and Syngenta 8441 showed better chemical attributes at all levels of salt stress. In all five maize hybrids, Na^+ accumulation in leaves and roots was proportional to the NaCl concentration in the medium applied exogenously. Significant increase of Na^+ levels was found in roots and leaves of all maize hybrids under treatments. This increase was more obvious in the leaves of XPW 3 than that in SWAJ 6, 7 and Syngenta 8441 hybrids (Fig. 1a). The highest concentration of Na^+ were observed in the roots of SWAJ 6, 7 and Syngenta 8441 at 150 mM NaCl treatment in combination with 2.5 and 5 mM foliar Ca^{2+} applications (Fig. 1b). Sodium accumulation in roots and leaves of maize hybrids under combined treatment of NaCl + Ca^{2+} was found less than sodium in plants under treatment of NaCl alone. Ca^{2+} application thus alleviates the hyperaccumulation of Na^+ ions in plants under salt stress (Fig. 1a,b).

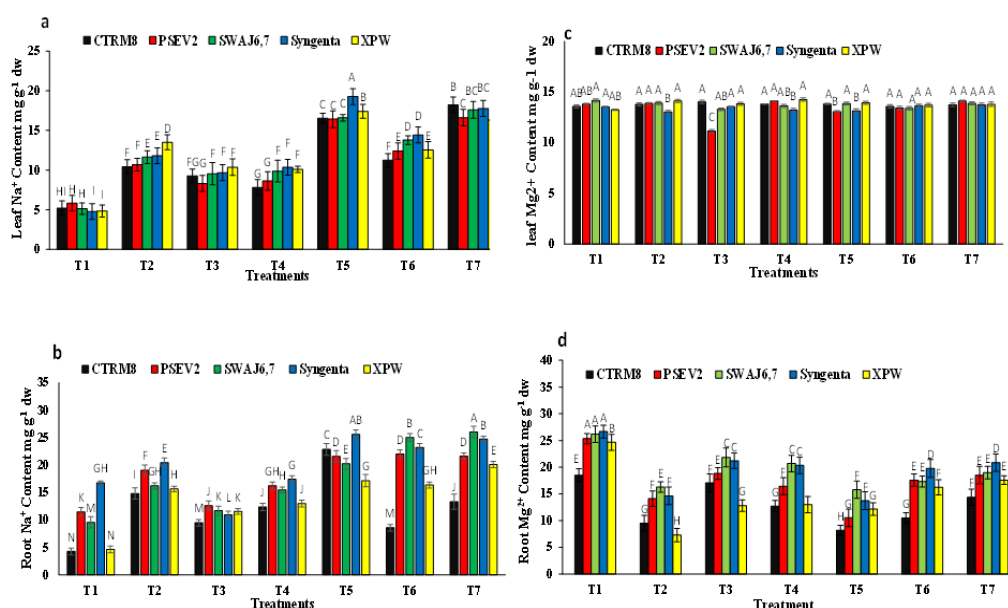


Figure 1. The Na concentration of leaf (a) and root (b) Mg concentration of leaf (c) and root (d) of five maize hybrids (CTRM8, PSEV2, SWAJ 6, 7, Syngenta 8441, XPE3) in response to salinity and Ca^{2+} supplement. Values are means $\pm$ SE of three replicates

Mg²⁺ content of leaf showed no significant effect under salt stress and foliar application of Ca²⁺ under salinity did not change the Mg²⁺ contents in leaf (Fig. 1c). However, salinity showed negative effect on root Mg²⁺ contents. Foliar Ca²⁺ application enhanced concentration of Mg in roots of all maize hybrids under salt stress (Fig. 1d).

Salt stress had induced noticeable variations in Ca²⁺ and K⁺ contents in leaves and roots of all maize hybrids. In comparison to that of control plants, all five maize hybrids showed decline in K⁺ and Ca²⁺ contents of roots and leaves under salt stress. This decrease in K⁺ and Ca²⁺ was more pronounced in CTRM8 and XPW3 as compared to SWAJ 6, 7 and Syngenta 8441 maize hybrids. Supplemental Ca²⁺ enhanced the K⁺ and Ca²⁺ concentrations of leaves and roots of maize plants under salt stress. At all salinity levels, maintenance of high level of K⁺ was better in SWAJ 6, 7 and Syngenta 8441 in comparison to CTRM8 and XPW3 (Fig. 2).

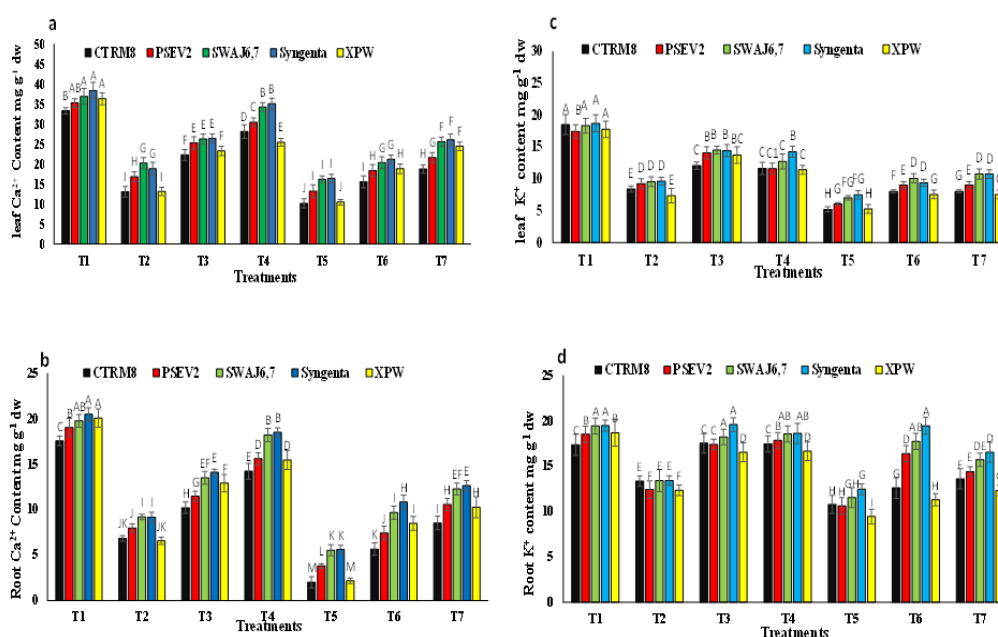


Figure 2. The Ca concentration of leaf (a) and root (b) K concentration of leaf (c) and root (d) of five maize hybrids (CTRM8, PSEV2, SWAJ 6, 7, Syngenta 8441, XPE3) in response to salinity and Ca²⁺ supplement. Values are means $\pm$ SE of three replicates

For all five maize hybrids, Na⁺/K⁺ ratio increased dramatically under salt stress with more notable effect in leaves of CTRM8 and XPW3 (Fig. 3). Supplemental Ca²⁺ resulted significant decline in Na⁺/K⁺ ratio of maize leaves under salt stress.

Proline contents of maize leaves under salt stress and Ca²⁺ treatments

Analysis of proline contents of maize leaves indicated that application of salt resulted in increased proline contents in all maize hybrids with more pronounced effect in CTRM8 and XPW3 as compare to SWAJ 6, 7 and Syngenta 8441. Under salt stress, proline contents in maize enhanced significantly (Fig. 4). However, proline concentration was markedly decreased in plants with exogenous application of 2.5 mM and 5mM concentrations of Ca²⁺ under salt stress. The less proline contents were found in plants treated with 2.5 mM Ca²⁺.

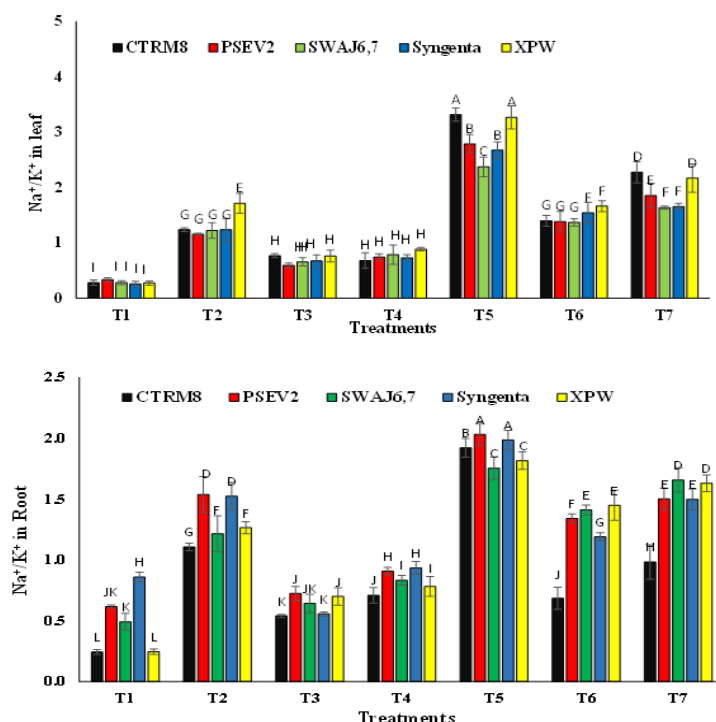


Figure 3. The Na/K Ratio in roots (a) and leaf (b) of leaf of five maize hybrids (CTRM8, PSEV2, SWAJ 6, 7, Syngenta 8441, XPE3) in response to salinity and Ca²⁺ supplement. Values are means $\pm$ SE of three replicates

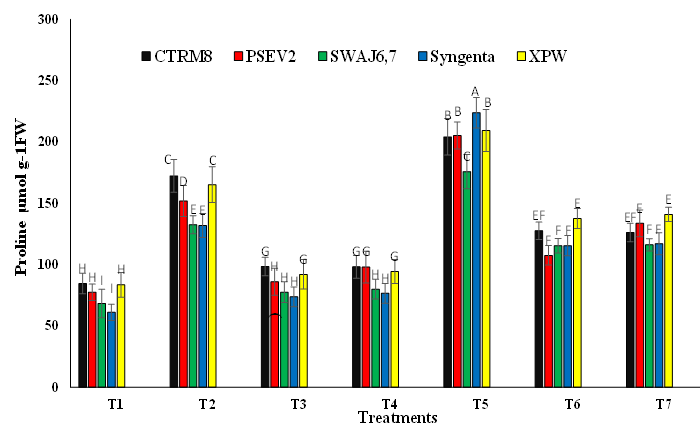


Figure 4. Proline content of of five maize hybrids (CTRM8, PSEV2, SWAJ 6, 7, Syngenta 8441, XPE3) in response to salinity and Ca²⁺ supplement. Values are means $\pm$ SE of three replicates

Discussion

The current study was carried out to investigate the genetic variation of five maize hybrids against salt stress in combination with exogenous application of calcium (Ca²⁺). The decrease in plant growth parameters such as shoot length, root length, leaf area, plant weight (fresh and dry) due to salt stress, confirming many prior findings (Mulholland, 2002; Yurtseven et al., 2003; Agong et al., 2004; Ahmad, 2012). Excessive Na⁺ accumulation in presence of NaCl resulted in nutritional and metabolic imbalances due

to ion toxicity which led to reduction in growth parameters of maize hybrids. It was reported that higher accumulation of Na^+ ions is the main cause of decreased plant growth and metabolism (Zhu, 2002). The genotypes with less concentration of Na^+ produced more biomass as confirmed by Munns and James (2003).

Ca^{2+} ion shows ameliorating effect on growth parameters of plants under salt stress may be by competing with Na^+ ion for binding with membranes. Ca^{2+} application minimized the harmful effects of NaCl and enhanced plant growth of all five maize hybrids. Previous studies also reported that exogenous Ca^{2+} application increased plant growth and tolerance to salt stress in *Cassia angustifolia* (Senna) (Arshi et al., 2005), *Eleusine coracana* (finger millet), *Thinopyrum ponticum* (wheatgrass) and *Lolium perenne* (ryegrass) (Sima et al., 2009), *Cakile maritime* (Sea Rocket) (Amor et al., 2010), *Zea mays* (Maize) (Shores et al., 2011), *Cunninghamia Lanceolata* (Chinese fir) (Liu et al., 2014), *Lycopersicon esculentum* (Tomato) (Parvin et al., 2015) and *Calligonum mongolicum* (Xu et al., 2017). Ameliorative effect of calcium by enhancing the vegetative growth and total dry weight of plants under salt stress has also been confirmed by Manivannan et al. (2007). Improved growth performance of SWAJ 6, 7 and Syngenta 8441 under salt stress indicate that these maize hybrids are more tolerant to salinity stress than CTRM8, PSEV2 and XPW3.

Salt stress showed significant effect on total leaf chlorophyll contents of maize hybrids. Increase in salt stress resulted decrease in chlorophyll contents of maize leaves. It is found from the results that the high levels of salinity (150 mM NaCl) induced reduction in the total chlorophyll contents of leaves significantly in comparison to leaves of control plants. Netondo et al. (2004), Amini and Ehsanpour (2006) and Naher (2014) also reported similar findings. Moreover, chlorophyll contents were less affected in SWAJ 6, 7 and Syngenta 8441 than CTRM8 and XPW3 indicated that they were able to maintain photosynthetic capacity under salt stress. However, Ca^{2+} application on plants under salt stress increased the leaf chlorophyll contents (Table 2). A range of plant species responses from positive to negative effects to supplemental Ca^{2+} have been described in previous studies (Cramer, 2002; Cabot et al., 2009). Nevertheless, total leaf chlorophyll significantly increased by supplemental Ca^{2+} under salt stress (Table 2). Similar responses were found in other plants such as *Cucumis sativus* L. (Cucumber), *Fragaria ananassa* (strawberry) and *Vigna radiate* (Mung bean) (Kaya and Higgs, 2002; Kaya et al., 2002; Manivannan et al., 2007). Importantly, 2.5 mM Ca^{2+} treatments showed significantly higher total chlorophyll contents compared to that of 5 mM Ca^{2+} treatments.

Reduction in RWC in salt stressed plant is a common phenomenon. In present study all five maize hybrids showed decreased RWC under salt stress with less significant effect under mild stress of 75 mM NaCl in Syngenta 8441 and SWAJ 6, 7. Similar water shortage induced by salt was observed in *Oryza sativa* and *Zea mays* affected with salt (Cicek and Cakirlar, 2002; Tuna et al., 2008). Plants supplemented with Ca^{2+} showed improved RWC may be due to water retention under salt stress. Similar results were reported by Tahjib-Ul-Arif et al., (2018).

Membrane permeability (MP) can be determined by measuring electrolyte leakage (EL). Plasma membranes are primarily affected by ion-specific salt injury (Mansour et al., 2005). Hence, for the identification of salt tolerant plants, determination of electrolyte leakage from plasma membranes is an important criterion (Ashraf and Ali, 2008). In present study, all maize hybrids showed increase in EL under salt stress which is an indication of membrane dysfunction. The increased permeability of cellular membranes for electrolytes and ions is the clear indication of membrane dysfunction caused by salt

stress and it can be measured by the electrolytes and efflux (Qadir, 2016). The highest EL was observed in CTRM 8 and XPW3 in comparison to SWAJ 6, 7 and Syngenta 8441. Demidchik et al. (2014) stated that efflux of K^+ is mainly related to electrolyte leakage. However, supplemented Ca^{2+} reduced MP of salt stressed plants compared to plants without Ca^{2+} under salt stress. The application of Ca^{2+} significantly improved MP by decreasing the electrolyte leakage under salt stress.

Salinity has very clear effects on ionic composition of maize. Significant increase of Na^+ levels was found in roots and leaves of all maize hybrids under treatments. This increase was more obvious in the leaves of XPW 3 than that in SWAJ 6, 7 and Syngenta 8441 hybrids (Fig. 1a). The highest concentration of Na^+ were observed in the roots of SWAJ 6, 7 and Syngenta 8441 at 150 mM NaCl treatment (Fig. 1b). Similar response was also reported in other plants such as *Malus* species, *Myrtus Communis* L. (Myrtle), Citrus and *Pistacia vera* (Pistachio) (Liu et al., 2012; Acosta-Motos et al., 2015; Martínez-Alcántara et al., 2015; Rahnesan et al., 2018).

Salt stress had induced noticeable variations in Ca^{2+} and K^+ contents in leaves and roots of all maize hybrids. In comparison to that of control plants, all five maize hybrids showed decline in K^+ and Ca^{2+} contents of roots and leaves under salt stress. This decrease in K^+ and Ca^{2+} was more pronounced in CTRM8 and XPW3 as compared to SWAJ 6, 7 and Syngenta 8441 maize hybrids. Supplemental Ca^{2+} enhanced the K^+ and Ca^{2+} concentrations of leaves and roots of maize plants under salt stress, similar to findings in other plant species (Tuna et al., 2007; Nadjimi and Daoud, 2009; Cabot et al., 2009; Kwon et al., 2009). At all salinity levels, maintenance of high level of K^+ was better in SWAJ 6, 7 and Syngenta 8441 in comparison to CTRM8 and XPW3 (Figure 2).

Increase in salinity levels lead to increased uptake of Na^+ which dramatically increased Na^+/K^+ ratio in roots and leaves of all maize hybrids. For all five maize hybrids, Na^+/K^+ ratio increased dramatically under salt stress with more notable effect in leaves of CTRM8 and XPW3 (Fig. 3). Supplemental Ca^{2+} resulted significant decline in Na^+/K^+ ratio of maize leaves under salt stress. It is documented that in salt-stressed plants, Ca^{2+} sustains K^+/Na^+ selectivity and K^+ transport at the plasma membrane. Exogenous Ca^{2+} can reduce K^+ loss and enhance its uptake in plants under salt stress by affecting different channels as demonstrated by several previous studies (Shabala and Newman, 2000; Maathuis and Sanders, 2001; Shabala et al., 2003, 2005, 2006). This indicates that Ca^{2+} amelioration of salt stress is a critical process in K^+ transport regulation that may induce growth in plants. Better chemical attributes of SWAJ 6, 7 and Syngenta 8441 at all levels of salt stress could be the reason of their tolerance to salt stress.

Proline is considered as a source of nitrogen and carbon for rapid recovery of plants under salt stress. It acts as free radical scavenger and not only adjust the osmotic pressure of plant but also found to involve in stabilization of membranes and some macromolecules in plant cells. Less accumulation of proline contents in salt stressed maize seedlings treated with calcium shows protective role of calcium in plants by reducing osmotic pressure caused by salt stress (Hoque et al., 2007). Therefore, plants accumulated less proline contents under salt stress with application of calcium. Accumulation of proline is the primary defense response in many plants under salt stress to adjust the osmotic potential as reported in various salt sensitive/ tolerant cultivars such as *Zea mays* (Maize), *Vigna radiate* (Mung bean), *Sesamum indicum* (sesame), *Setaria italica* L. (foxtail millet) and *Pistacia vera* (Pistachio) (Mansour et al., 2005; Misra and Gupta, 2005; Koca et al., 2007; Veeranagamallaiah et al., 2007; Chelli-Chaabouni et al., 2010). Some studies showed that under salt stress, tolerant

cultivars accumulate more proline than the salt sensitive cultivars (Misra and Gupta, 2005; Rahneshan et al., 2018). However, proline accumulation is also controversial with reference to tolerance and osmotic adjustment in plants exposed to undesirable environmental conditions (Ghars et al., 2008) as indicated by some studies where more proline accumulated in salt sensitive cultivars than the salt tolerant cultivar (Claussen, 2005). In current study proline accumulation was significantly higher in maize hybrids evaluated as salt sensitive (CTRM8 and XPW3) than in salt tolerant (SWAJ 6, 7 and Syngenta 8441). These results suggest that loss of cell homeostasis is responsible for excessive proline accumulation in salt sensitive hybrids. Similar results have been reported in previous studies where salt sensitive genotype of *Sorghum bicolor* L. (*Sorghum kaya*) (Wheat Arta) (Akbari et al., 2016) accumulated more proline under salt stress. These findings indicate that more proline accumulation is due to loss of cell homeostasis. Moreover, these results suggest that salt tolerance is not fully reflected by proline accumulation itself in plants; however, proline accumulation could be an indicator of stress.

Conclusion

The overall results indicate that the growth parameters of all five maize hybrids were affected significantly by different salt stresses. However, better growth performance, maintenance of nutrient contents, less accumulation of toxic sodium ions and lower Na^+/K^+ in SWAJ 6, 7 and Syngenta 8441 indicate that these maize hybrids are more tolerant to salinity stress than all others. It is also concluded that the foliar application of Ca^{2+} against salt stresses improved the growth parameters of hybrids. Therefore, it can be concluded that the salt stress can be mitigated by the exogenous application of Ca^{2+} in crop plants. Further, the experiment might be repeated in different locations with different maize hybrids for making concrete recommendations.

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