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Sodium in the leaf apoplast does not affect growth of maize (*Zea mays* L.) under saline field conditions

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Summary

Studies dealing with leaf apoplastic Na^+ concentration of monocots, such as maize, under actual saline soils are scarce. Therefore, the current study was aimed to investigate the growth, total ions and leaf apoplastic Na^+ concentration of salt sensitive maize plants growing in saline soils. Plants were subjected to salt stress with an electrical conductivity (EC) of 3, 8 10 and 14 dS m^{-1} using completely randomized design (CRD) for 3 weeks. Shoot fresh weight, plant height, leaf area and leaf length of maize plants drastically decreased when plants were exposed to increasing salt stress. We found that maize could display a steep increase in Na^+ concentration in the total shoot biomass with maximum 82.3 µmol g^{-1} FW, when plants were subjected to highest soil salinity at 14 dS m^{-1}. As expected, other cations *i.e.*, K^+, Ca^{2+} and Mg^{2+} decreased with increasing EC of the soil compared to Na^+. Surprisingly, a maximum of 17 mM Na^+ were found in the leaf apoplast of maize grown under very high soil salinity at EC 14 dS m^{-1}. Considering this lower leaf apoplastic Na^+ concentration at such a high EC level in maize plants, current study does not corroborate that surplus sodium in the leaf apoplast can result in dehydration and cell death under salt stress.

Keywords: Soil salinity, Sodium, Apoplast, Growth, *Zea mays* L.

Introduction

Salinity is considered as the major environmental hazard especially for the agricultural crops like maize in which rapid growth reduction has been observed already in the initial phase of salinity stress (Zörb et al., 2015). Maize is the third most important cereal crop after wheat and rice that is utilized as a staple food in many parts of the world. In Pakistan maize was cultivated on about 1.13 million hectares during the year 2010-2015 (GoP, 2015). In the year 2013-17, average maize production in Pakistan was 5.476 million tons (FAO, 2019). However, maize yield in Pakistan is considered still very low compared to the remaining maize producing countries. The reduced production of maize is a result of high soil pH, soil salinity and shortage of good quality of water for irrigation purposes.

Plant growth is generally affected by salt stress in three different ways *i.e.* osmotic stress, ionic imbalance in cells and ultimately ionic toxicity. Moreover, soluble salt concentration and duration of exposure to salt stress decides the severity of plant growth reduction (Tavakkoli et al., 2011). Reduction in the growth is characterized by the rapid response due to decreased soil water potential. Wide-range metabolic and osmotic problems of plants can be triggered by the high absorption of Na^+ ions in shoots under salt stress. The accumulation of Na^+ ions in shoots is greater than in roots; therefore, shoots are more susceptible to Na^+ (Munns and Tester, 2008). As already shown by Fortmeier and Schubert (1995), Na^+ ions do significantly affect maize growth compared to Cl^- ions. Sodium ions get stored in higher amounts in shoots in contrast to roots, for this reason leaves are much susceptible towards sodium ions (Tester and Devenport, 2003). Therefore, current study focuses on the investigation of Na^+ concentration in the leaf apoplast of maize plants. Oertli (1968) proposed that high level of Na^+ accumulation in the apoplast leads to turgor loss, dehydration and finally death of leaves. Flowers et al., (1991) and Speer and Kaiser (1991) measured 600 mM and 87 mM Na^+ concentrations in the leaf apoplast of rice and salt-sensitive pea, respectively under salt stress, whereas later study also found low apoplastic Na^+ concentration (7 mM) in salt-resistant spinach. In contrast, Mühling and Läuchli (2002) found extremely low Na^+ accumulation in the leaf apoplast of maize in a hydroponic experiment under salinity. The aim of the current investigation was to study growth reduction of salt-sensitive maize plants as a result of Na^+ accumulation in the leaf apoplast under actual saline environment, as studies under saline field conditions are missing to clarify whether Na^+ in the leaf apoplast is causing a decline in leaf growth.

Materials and methods

Soil analysis and maize cultivation

Saline soils were collected from four different areas of district Faisalabad, from up to 0-6 and 6-12 cm depths with the help of auger. There were total twenty soil samples which were dried in the air and sieved to remove any stone and sand particles and further treated for soil analysis *i.e.*, electrical conductivity (EC), pH, saturation percentage (SP) and textural class which are given in Tab. 1. According to international soil classification system soil textural class was analyzed with the help of hydrometer method as mentioned by Moodie et al. (1959).

To determine the saturation percentage the soil paste was dried to a constant weight at 105 °C and was later calculated with the help of following formula.

$$\text{Saturation } \% = \frac{\text{Mass of wet soil} - \text{Mass of oven dry soil}}{\text{Mass of oven dry soil}} \times 100$$

pH meter was used to measure pH of the saturated soil paste. Electrical conductivity was measured by using a digital conductivity meter. For this purpose, an extract of saturated soil paste was used for the analysis.

Maize seeds of RMW8 * PSEV3-157.5.4.2 were sown in saline soils in seed lab in COMSATS, Abbottabad with controlled environment under optimum light energy. At first six seeds were sown per pot, which were later reduced to four plants for further experimentation. Deionised water was used for irrigation throughout the growing period. Maize plants were harvested during the vegetative growth without salt injury symptoms after three weeks.

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Tab. 1: Location, depth, EC, pH, SP% and textural class of the collected soil samples from district Faisalabad, Pakistan (n = 5).

Sample Name	Location	Depth	EC(dS m^{-1})	pH	SP %	Textural class
Soil 1	(Chak No. 26 GB) 31°15'36.4"N 73°17'40.1"E	6-12 cm	3	7.88	41.95%	Loamy sand
Soil 2	(Chak No. 73 GB) 31°18'19.6"N 73°11'41.6"E	6-12 cm	8	7.9	40.19%	Loamy sand
Soil 3	(Chak No. 73 GB) 31°18'19.6"N 73°11'41.6"E	0-6 cm	10	8.42	31.96%	Loamy sand
Soil 4	(Chak No. 33 GB) 31°14'12.2"N 73°09'23.5"E	0-6 cm	14	7.89	35.10%	Sandy clay loam

Collection of apoplastic washing fluid

Infiltration – centrifugation technique was used for the collection of apoplastic washing fluid (LOHAUS et al., 2001; MÜHLING and SATTELMACHER, 1995). Leaves were removed from plants with the help of a razor blade and were cautiously washed with deionised water. Later leaves were cut in segments of about 5.5 cm and with the help of tissue paper, the leaf segments were carefully dried and weighed before infiltration. The leaf segments were then put in empty plastic syringes (60 ml). The syringes were then filled with deionized water up to 40 ml. Tip of the syringe was covered with silicon cork and a pressure of almost 20 kPa was produced on the leaf segments by pulling plunger of the syringe. Then plunger was released and pushed so that leaves get infiltrated (LOHAUS et al., 2001). After that, the infiltrated leaf segments were dried carefully with tissue paper and weighed again to get approximate volume of infiltrated water. Leaf segments were placed in a plastic vessel of 10 ml, which was then placed in a 50-ml falcon tube. The falcon tube containing intact leaves was placed in a centrifugation tube, which was adjusted at 100 × g at 5 °C for 5 minutes. Samples of apoplastic washing fluid were pipetted into 1.5 ml Eppendorf tube and stored at -20 °C for further analysis.

Growth parameters

Fresh weight of the plants was obtained soon after the harvest with the help of an analytical balance having precision of 0.1 mg. Later, plant shoot was placed in an oven at 60 ºC for three days to obtain the dry weight. Digital images of the plants and leaves were taken after harvesting. Later on, area and length of each leaf and shoot height of the harvested plants was calculated with the help of ImageJ 1.49 software by setting a scale against specific number of pixels. Leaf area and length were demarcated, thereby obtaining leaf area and length measurements.

Determination of total ion absorption in shoot of maize plants

The dried leaves samples were crushed manually with the help of ceramic mortar and pestle. Crushed samples were weighed, then, put in ceramic pots to be placed in an oven at 520 °C for about 4 hours to obtain ash of the samples. Cations were investigated in this ash of dried plant matter. 2 ml of 4 M HNO_3 was added to each ash sample and was stirred gently after every half an hour up to a total of three hours to get suspension. Then 8 ml distilled water was added to make 10 ml final volume. Whatman filter paper No. 21 was used to filter the suspension into vials. After that, ions concentration was determined in the filtrate for Na^+, Ca^{2+}, K^+ and Mg^{2+} through AAS i.e atomic absorption spectrophotometer (AAnalyst 700, Perkin Elmer, USA). The unit 'µmol g^{-1} FW' was used for the calculation of concentration of the ions in total shoot samples.

Determination of total ion concentration in apoplastic washing fluid

Collected apoplastic washing fluid of each sample was first dried in an oven at 80 °C for 4 hours. 3 ml of 4N HNO_3 was added and was stirred gently after every half an hour up to a total of three hours to get suspension. Later 7 ml deionised water was added and a final volume of 10 ml was achieved. Atomic absorption spectrometer was used to analyse the Na^+, K^+, Ca^{2+} and Mg^{2+}. The unit 'mM' was used to demonstrate the amount of cations in leaf apoplast of maize plants.

Statistical analysis

Data were normally distributed and significant variances at $P \leq 0.05$ among treatments were determined by means of the general linear model with a Tukey test using SPSS statistics 17.0 (Statistical Product and Service Solutions, Chicago, IL, USA). The significant differences were indicated with the help of small alphabets at top of each bar. The mean standard error (S.E.) was indicated by error bars on the bars of figures.

Results

Agronomic traits affected under salt stress

Fresh shoot biomass of maize plants was maximum (6.1 g) when treated with soil of EC 3 dS m^{-1}. In contrast to plants treated with EC 3 dS m^{-1}, maize plants growing in soil with an EC of 14 dS m^{-1} showed significant damaging effect with 68% decreased shoot biomass. An inverse relation was observed between plant growth and EC of the soil samples, as with increasing concentration of salt in the soil *i.e.,* EC 8 dS m^{-1}, 10 dS m^{-1} and 14 dS m^{-1} resulted in shoot biomass of 2.9 g, 2.3 g, and 2.0 g, respectively (Fig. 1a). When considering the dry shoot biomass maximum value was 0.61 g when treated with Soil 1 however, this dry weight was significantly reduced to 0.17 g when plants were treated with Soil 4 *i.e.,* EC of 14 dS m^{-1}. In comparison to EC 3 dS m^{-1} (Soil 1) dry shoot biomass was reduced by 56%, 67%, and 72% when plants were treated with soil having an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3), and 14 dS m^{-1}(Soil 4), respectively.

Maximum shoot height of maize plant was 37 cm when EC of soil was 3 dS m^{-1} *i.e.,* Soil 1. The shoot height of maize plants was significantly reduced to 12 cm when treated with Soil 4 having an EC of 14 dS m^{-1}, which is 67% reduction when compare to the shoot height in Soil 1. With an increase in soil salinity shoot height was reduced *i.e.,* 23 cm and 19 cm, with an EC of 8 dS m^{-1} (Soil 2) and 10 dS m^{-1} (Soil 3), respectively. Leaf length was 28 cm when EC of the collected soil sample was 3 dS m^{-1} (Soil 1) and at highest EC level *i.e.,* 14 dS m^{-1} (Soil 4) the leaf length was (7.18 cm) significantly reduced by 74%. The findings related to leaf length under salt stress further revealed that it was considerably decreased with increasing EC levels, as with an EC of 8 dS m^{-1} (Soil 2) and 10 dS m^{-1} (Soil 3)

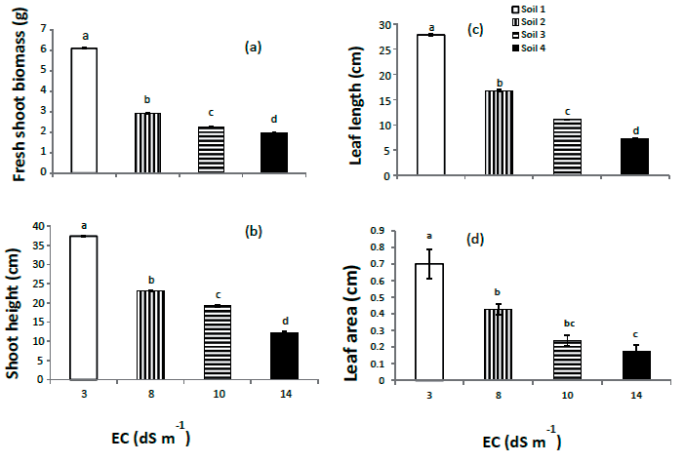

Fig. 1: Fresh shoot biomass g (a), shoot height cm (b), leaf length cm (c) and leaf area cm^2 (d) of maize plants (on Y axis) as affected by varying EC level of the soil samples (on X axis). Different letters indicate significant differences between the EC levels at $P < 0.05$ ($n \geq 3$).

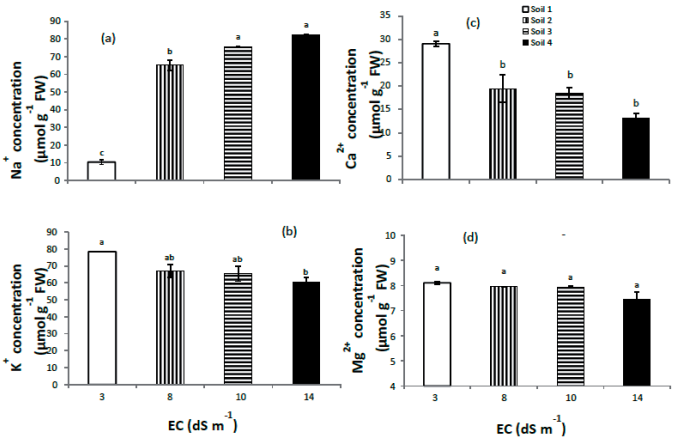

Fig. 2: Effect of various EC level of the soil samples (on X axis) on Na^+ concentration µmol g^{-1} FW (a), K^+ concentration µmol g^{-1} FW (b), Ca^{2+} concentration µmol g^{-1} FW (c), and Mg^{2+} concentration µmol g^{-1} FW (d), in the total shoot of maize plants. Different letters indicate significant differences between the treatments at $P < 0.05$ ($n \geq 3$).

the leaf length of maize plant was 16 cm and 11 cm, respectively. Fig. 1(d) displays the relationship of leaf area of maize plants with respect to EC of the soil. In Soil 1 the leaf area of maize plant was 0.701 cm^2. In contrast, a significant reduction of 75% in the leaf area (0.174 cm^2) of the maize plants was observed in Soil 4. Similar to above mentioned growth parameters leaf area of the maize plants was found to be decreased with an increase in the soil salinity.

Salt stress induced significant ionic changes in total shoot of the maize plant

Maximum Na^+ concentration in the total shoot of maize plants was 82 µmol g^{-1} FW when they were subjected to 14 dS m^{-1} (Soil 4), while minimum was 10 µmol g^{-1} FW when treated with 3 dS m^{-1} (Soil 1). An absolute increase in the Na^+ concentration was observed starting from EC 8 dS m^{-1} (Soil 2) to 14 dS m^{-1} (Soil 4). However, when compared to EC 3 dS m^{-1} (Soil 1), increase in Na^+ concentration in the total shoot of maize plants was 523%, 631%, and 695% when treated with soil samples having an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3), and 14 dS m^{-1} (Soil 4), respectively (Fig. 2a). An inverse relation was observed between K^+ concentration and EC of the soil samples. With an increase in soil salinity level *i.e.,* EC 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3) and 14 dS m^{-1} (Soil 4), K^+ concentrations in maize shoots were decreased by 15%, 17%, and 23%, respectively, when compared to K^+ concentration in Soil 1. Maximum K^+ concentration in the total shoot of maize plant was 78 µmol g^{-1} FW, when they were subjected to Soil 1, while minimum was 60 µmol g^{-1} FW when treated Soil 4 (Fig. 2b).

Decrease in the Ca^{2+} concentration was observed starting from EC 3 dS m^{-1} to 14 dS m^{-1}. Maximum Ca^{2+} concentration was 29 µmol g^{-1} FW, when EC of soil was 3 dS m^{-1} (Soil 1). Ca^{+2} concentration was reduced to 13 µmol g^{-1} FW, when plants were treated with Soil 4. Similarly, when soil had an EC of 8 dS m^{-1} (Soil 2) and 10 dS m^{-1} (Soil 3), the Ca^{2+} concentration was reduced by 33% and 36%, respectively when compared to EC 3 dS m^{-1} (Soil 1) (Fig. 2c). Maximum Mg^{2+} concentration was 8.09 µmol g^{-1} FW in Soil 1. However, Mg^{2+} concentration was 7.43 µmol g^{-1} FW in Soil 4 which was 8% reduction compared to the Mg^{2+} concentration in Soil 1. Similarly, Mg^{2+} concentration was, 7.95 µmol g^{-1} FW, and 7.93 µmol g^{-1} FW when maize plants were treated with soil that had an EC of 8 dS m^{-1} (Soil 2), and 10 dS m^{-1} (Soil 3), respectively (Fig. 2d).

Ionic pattern of the extracted leaf apoplastic washing fluid in maize under salt stress

An increase in the Na^+ concentration was noticed starting from EC 3 dS m^{-1} (Soil 1) to 14 dS m^{-1} (Soil 4). Maximum Na^+ concentration in the extracted apoplastic washing fluid of maize leaves was 17 mM when they were subjected to Soil 4, while minimum was 5 mM, when treated with Soil 1. However, noticeably when compared to EC 3 dS m^{-1} (Soil 1), increase in Na^+ concentration in the extracted apoplastic washing fluid of maize leaves was 64%, 164%, and 229% when treated with soil samples having an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3), and 14 dS m^{-1} (Soil 4), respectively (Fig. 3a). K^+ concentration in the extracted apoplastic washing fluid of maize leaves was 4.5 mM when treated with soil having an EC of 3 dS m^{-1} (Soil 1) however, this K^+ concentration was significantly reduced to 0.1 mM when plants were treated with Soil 4 with an EC of 14 dS m^{-1}. In comparison to EC 3 dS m^{-1} (Soil 1), K^+ concentration was reduced by 36%, 66%, and 97% when plants were treated with soil having an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3), and 14 dS m^{-1} (Soil 4), respectively (Fig. 3b).

Ca^{2+} concentration in the extracted apoplastic washing fluid of the maize leaves was 5.24 mM when treated with Soil 1. Ca^{2+} concentration was 1 mM, when EC of the soil was 14 dS m^{-1} (Soil 4). Decrease in the Ca^{2+}concentration was observed starting from EC 3 dS m^{-1} (Soil 1) to 14 dS m^{-1} (Soil 4). However, compared to EC 3 dS m^{-1} (Soil 1), when treated soil had an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3) and 14 dS m^{-1} (Soil 4) then Ca^{2+} concentration was significantly reduced by 49%, 58%, and 81%, respectively (Fig. 3c). Mg^{2+} concentration was found maximum with 26 mM in Soil 1 (*i.e.,* EC 3 dS m^{-1}). This Mg^{2+} concentration was 3 mM when EC of the soil was 14 dS m^{-1} (Soil 4), which is 89% reduction when compared to the Mg^{2+} concentration at EC 3 dS m^{-1} (Soil 1). In comparison to Mg^{2+} concentration of EC 3 dS m^{-1} (Soil 1), soil samples with an EC of 8 dS m^{-1} (Soil 2), 10 dS m^{-1} (Soil 3), and 14 dS m^{-1} (Soil 4) showed a Mg^{2+} concentration of 19 mM, 11 mM, and 3 mM, respectively (Fig. 3d).

The ratios between K^+ and Na^+, Ca^{2+} and Na^+ and Mg^{2+} and Na^+ decreased in total shoot and leaf apoplast of maize plants under salt stress. Maximum ratios between K^+:Na^+, Ca^{2+}:Na^+ and Mg^{2+}:Na^+ were found at EC 3 dS m^{-1} (Soil 1) in both total shoot and in the leaf apoplast of maize plants. However, minimum ratios were found at EC 14 dS m^{-1} (Soil 1) (Tab. 2).

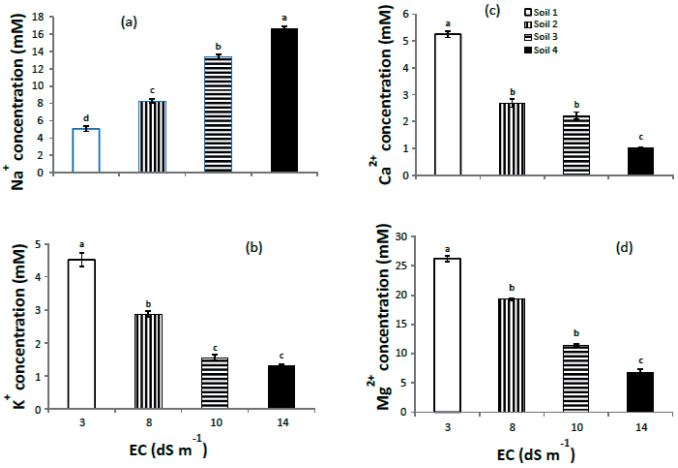

Fig. 3: Na^+ concentration mM (a), K^+ concentration mM (b), Ca^{2+} concentration mM (c), and Mg^{2+} concentration mM (d) as influenced by the increasing EC level of the soil samples, Different letters indicate significant differences between the treatments at $P < 0.05$ ($n \geq 3$).

Discussion

Effect of soil salinity on cation composition in leaves of maize

Salinity is the major environmental problem damaging crop plants by their growth restriction, ultimately reducing agricultural yield throughout the world (MANIVANNAN et al., 2007; SHRIVASTAVA 2015). Numerous crops e.g. barley wheat, rice, cotton, bean and maize have been mentioned for their yield reduction under salt stress (DEMIRAL et al., 2005; JALEEL et al., 2008; BASAL 2010; SHAHZAD et al., 2012). In current study, when compared to soil with an EC 3 dS m^{-1}, maize growing in soil possessing an EC of 14 dS m^{-1} resulted in significant damaging influence with 68% decreased shoot biomass during salt stress (Fig. 1a). Increased shoot and root osmolality can be resulted through increased ion concentration in plant tissues under salinity stress. However, sudden reduction in the growth of the roots and leaves is an immediate consequence of the osmotic effect (MUNNS, 2002; MUNNS and TESTER, 2008; WANG et al., 2012). Reduced growth under higher saline conditions have been attributed to the inefficient plants capability to osmotic balance which can be a result of saturated solute uptake, or increased energy demands of the system (MUNNS, 1988; GALE and ZERONI, 1984). According to SLABU et al. (2009) and TESTER and DAVENPORT (2003) and FORTMEIER and SCHUBERT (1995) Na^+ causes more damage in saline conditions as compared to any other ion e.g Cl^-. SÜMER et al. (2004) mentioned that Na^+ toxicity can also occur in the initial phase of salinity stress. In the present investigation, decrease in fresh biomass of plant shoots and leaf area (Fig. 1a and 1d) was found inversely proportional to increase in salinity level. In contrast to plants subjected to soil with EC 3 dS m^{-1}, results related to leaf area showed a significant decline (75%) in maize growing in soil with an EC of 14 dS m^{-1}. It has been reported that continuous exposure to increased level of salinity in the rooting medium progressively reduces the size of the leaf over time (MUNNS et al., 1988). When compared to the shoot height at EC 3 dS m^{-1}, 67% reduction was noticed when plants were treated with soil having an EC of 14 dS m^{-1}. Moreover, in the present study, in comparison to EC 3 dS m^{-1}, leaf length was also significantly reduced by 74% when treated with soil having an EC of 14 dS m^{-1}, which is in line with previous findings where reduced growth of the younger leaves compared to the older leaves suggested that leaf elongation is restricted immediately when the salt is applied to the maize roots (CRAMER, 1992; SHAHZAD et al., 2012).

According to TESTER and DAVENPORT (2003) K^+ is involved in the activation of numerous enzymes in plants however, higher Na^+ contents compete with K^+ for binding sites as a consequence leads to enzymes inactivation which disturbs the important cellular functions. In the current study compared to EC 3 dS m^{-1}, increase in Na^+ concentration in the total shoot of maize plant was 695% when treated with soil samples having an EC of 14 dS m^{-1} (Fig. 2a). Moreover, an inverse relation was observed between K^+ concentration and EC of the soil samples as increase in soil salinity resulted in decreased K^+ concentrations (Fig. 2b). This is in conformity with our result as the K^+ content significantly decreased by 23% in the presence of EC 14 dS m^{-1} (Fig. 2b). Low K^+:Na^+, Ca^{2+}:Na^+ and Mg^{2+}:Na^+ ratios under salinity stress (Tab. 1 and 2) disturb plant expansion and eventually prove to be damaging (SCHACHTMAN and LIU, 1999). Ca^{2+} may also get replaced from the membranes of root cells due to high concentration of Na^+, thus leading to a decrease in the K^+:Na^+ selectivity (MURATA et al., 2000). Enhancement in Ca^{2+} regulated membrane stability, consistently results in decline of K^+ loss from the root zone and a more suitable root K^+ proportion (CACHORRO et al., 1994; NAVARI-IZZO et al., 1993). Ca^{2+} normally plays a regulatory role in the metabolic processes. Ca^{2+} provide shield to cell membrane against harmful saline impact, as Ca^{2+} compete with Na^+ for membrane binding spots (ZIDAN et al., 1990; ABDEL, 2011). It has been observed that Ca^{2+} contents declined in tomato, wheat and barley leaf by raising NaCl content in the nutrient solution (NAVARRO et al., 2000; CUARTERO et al., 2006; EHRET et al., 1990). EBERT et al. (2002) stated that cationic associations in the shoot cells such as Ca^{2+}:Na^+ exhibit a major impact on salt adaptability than entire sodium contents. In the present study Ca^{2+} concentration was reduced to 55% at EC 14 d Sm^{-1} in comparison to EC 3 d Sm^{-1}. Our finding confirms the decline in Ca^{2+} uptake in maize under NaCl application (CRAMER, 2002; HU et al., 2007).

High salt deposition is a probable reason for selectivity of nutrient absorbance because excessive Na^+ and Cl^- may obstruct the uptake of other ions (K^+, Ca^{2+} and Mg^{2+}) in the root and their passage through the xylem into the aerial parts, ultimately leading to nutritional scarcity in the tissue (MURILLO-AMADOR et al., 2006). When compared to EC 3 dS m^{-1} Mg^{2+} was reduced by 8% when treated with soil possessing an EC of 14 dS m^{-1}. The proportions of Na^+:Ca^{2+}, Na^+:K^+ and Na^+:Mg^{2+} under saline conditions noticed in the total plant revealed that K^+, Ca^{2+} and Mg^{2+} transfer is hindered by Na^+ under salt treatment and may disrupt plant metabolic processes and affect plant development.

Tab. 2: Influence of different EC levels on the K^+/Na^+, Ca^{2+}/Na^+ and Mg^{2+}/Na^+ ratios in total shoot and apoplast of maize plants. Different letters indicate significant differences between the treatments at $P < 0.05$ ($n \geq 3$), ±SE.

EC (dS m^{-1})	Total shoot K^+/Na^+	Apoplast K^+/Na^+	Total shoot Ca^{2+}/Na^+	Apoplast Ca^{2+}/Na^+	Total shoot Mg^{2+}/Na^+	Apoplast Mg^{2+}/Na^+
3	7.80 ± (0.95)a	0.89 ± (0.01)a	2.90 ± (0.40)a	1.04 ± (0.04)a	0.81 ± (0.10)a	5.21 ± (0.20)a
8	1.05 ± (0.10)b	0.35 ± (0.01)b	0.30 ± (0.03)b	0.32 ± (0.01)b	0.12 ± (0.01)b	2.34 ± (0.03)b
10	0.86 ± (0.06)b	0.12 ± (0.004)c	0.24 ± (0.02)b	0.17 ± (0.01)c	0.10 ± (0.001)b	0.87 ± (0.01)c
14	0.73 ± (0.04)b	0.08 ± (0.005)d	0.16 ± (0.01)b	0.06 ± (0.002)d	0.09 ± (0.003)b	0.41 ± (0.04)c

Does the apoplastic Na^+ concentration affect leaf growth under saline field conditions?

Studies dealing within the leaf apoplast of monocots, such as maize, to determine the cations and anions under actual saline soils are scarce. Many of the deleterious effects of Na^+ seem to be related to the structural and functional integrity of membranes (Kurth et al., 1986). Volkmar et al. (1998) suggested that when plants are continuously exposed to salinity stress, Na^+ can get accumulated either in the cytoplasm or in the apoplast of cell. Earlier studies suggested that reduction in shoot growth can possibly be attributed to dehydration of leaf cell, when xylem Na^+ entered the leaf apoplast that induces osmotic stress (Flowers et al., 1991; Oertli, 1968). We are reporting here for the first time the apoplastic Na^+ (Fig. 3a) accumulation under the influence of saline soils with various EC levels that were collected from district Faisalabad. Our results showed that when maize plants are treated with soil having an EC of 14 dS m^{-1}, it results in (3.3 fold) increase of apoplastic Na^+ concentration compared to EC 3 dS m^{-1}. However, maximum Na^+ concentration in apoplastic fluids (AWF) remained too low (17 mM) under short term salt stress when compared to absolute Na^+ concentration in total shoot of maize plants (Fig. 3a). These findings do not support Oertli's hypothesis that leaves dehydration and turgor loss can be a consequence of high levels of salt accumulation in the apoplast of salt-sensitive plants. In addition, we confirm the earlier study with maize by Mühling and Läuchli (2002a,b) which were performed in hydroponics. Possible mechanism of low apoplastic Na^+ concentration in leaf tissue during continuous exposure to excessive salt could be through Na^+ transport via H^+/Na^+ antiporter into leaf vacuoles (Pitann et al., 2009).

Conclusions

Maize growth was significantly reduced under increased EC salinity levels ranged from 3 dS m^{-1} to 14 dS m^{-1} (slightly to highly saline soils). Na^+ concentration in total shoot significantly increased with higher EC levels, which has been suggested by numerous scientists as a reason for decreased maize growth at high salinity levels. However, in this study low Na^+ concentration in the subcellular compartment *i.e.,* leaf apoplast of maize was found under the influence of actual saline soil, which has not been reported earlier.

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Conflict of interest

The authors declare that they have no conflict of interest.

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