

Salinity types and level-based effects on the growth, physiology and nutrient contents of maize (*Zea mays*)

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Abstract

Salinity is a devastating problem of arid and semi-arid climatic regions with uneven salt accumulation which hinders growth and development of crops. The deleterious effects of salinity mainly depend on level and source of salinity. We hypothesized that types of sodium salt (NaCl and Na₂SO₄) might cause variable toxicity in maize (*Zea mays* L.) plants. The objective of the present study was to compare the effect of different types of sodium salt (NaCl and Na₂SO₄), each at EC 5 and 10 dS m⁻¹ on growth, physiology and nutrient contents of maize plant grown in earthen pots under wire house conditions. Results revealed toxic effects of salt stress on seed germination, root and shoot growth and biomass. Maize physiology in terms of sub-stomatal CO₂ index, chlorophyll and relative water contents, photosynthetic and transpiration rate also reduced under salt stress. Among the types of salt and levels of salinity, NaCl applied at the rate of 10 dS m⁻¹ caused the highest reduction in seed germination, growth and physiology due to high accumulation of Na and Cl ions whereas

low in K ion in maize plant tissues. Based on the findings, we do conclude that NaCl applied at the rate of 10 dS m⁻¹ has more negative impact on maize growth and nutrient acquisition than Na₂SO₄ at same level of salinity.

Introduction

Salinity is one of the major abiotic stresses, badly affecting crop growth, particularly in arid and semi-arid regions of the world (Ashrafi *et al.*, 2018). According to report 6.3 million ha in Pakistan and 800 million ha throughout the world has been affected by salinity (Akram *et al.*, 2010). Arable land and crop yield losses are attributed to salinity (Supper, 2003). Salinity impose stress in two ways *i.e.* high salt concentration in soil makes water extraction difficult for plant roots, can cause physiological drought and specific-ion toxicity (Zhu, 2001; Song *et al.*, 2005; Eraslan *et al.*, 2007), whereas higher contents within tissues can be toxic for plant metabolism and cause oxidative stress through the production of reactive oxygen species (ROS) for numerous biochemical processes of crop plants (Ditta, 2013; Patel and Saraf, 2013; Shrivastava and Kumar, 2015).

Germination of crop plants has been reduced significantly due to specific ion effect under salt stress (Tobe *et al.*, 2004; Aliu *et al.*, 2015; Gong *et al.*, 2018), which further reduces water availability to crops, hampers photosynthetic rate, vegetative growth and nutrient uptake (Erdal *et al.*, 2000; Gong *et al.*, 2018). Decreased photosynthesis is not only attributed to stomatal closure but also due to malfunctioning of photosynthetic enzymes involved, under high osmotic potential (Munns, 2002; Stepien and Klobus, 2006). Salinity creates hyperosmotic levels of Na⁺, Cl⁻ and SO₄²⁻ in soil, thereby reducing K⁺ and Ca²⁺ availability to the crop plants (Fricke *et al.*, 2004; Mansour *et al.*, 2005). Sodium ion accumulation in plant tissue results in cell damage (Munns, 2002).

Maize (*Zea mays* L.) is an important food and feed crop of the world and is often referred as *the king of grain crops*. It is vital cereal crop that provides fundamental foodstuff to millions of people in the world, providing more than 50% of the caloric energy consumption (Schnable, 2015). In order to fulfil such a demand for huge amount of maize, its productivity needs to be doubled by 2050, even under abiotic stress conditions like salinity stress which is one of the most common environmental stresses in optimum production of maize (Ray *et al.*, 2013; Farooq *et al.*, 2015).

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Its growth is severely affected under osmotic stress; depending on the level of salinity stress applied (Imran *et al.*, 2018).

Several studies have shown the effects of salt stress induced by NaCl on seed germination, growth and physiological parameters of various crops (Ambede *et al.*, 2012; Aliu *et al.*, 2015). Unfortunately, these studies could not provide enough information of real salinity stress *i.e.* prevalence of various salts stress under field conditions. In the present study, we have selected two types of salts under their multiple levels and used maize as a test crop. Limited information is available regarding the effect of different types and levels of salt stress on germination, growth and physiology of maize plant. We hypothesized that types of sodium salt (NaCl and Na₂SO₄) under different levels (EC: 5 and 10 dS m⁻¹) might cause variable toxicity to maize. The objective of the current study was to compare the effect of different levels and types of salt on growth, physiology and nutrient contents in maize.

Materials and methods

To explore the objectives as outlined above, a pot experiment was conducted in a wire house of Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. Before initiating experiment, EC, pH SAR, texture and saturation percentage of soil was measured following the methods of Ryan *et al.* (2001) (Table 1). Thereafter, soil was filled in earthen pots (15 kg soil capacity having 60 cm height and 30 cm diameter) and maize cultivar Faisalabad hybrid (FH-920) was sown as test crop on 27th of February 2016 under natural humidity, light and temperature conditions. Maize was sown at the rate of 10 seeds pot⁻¹ initially that were thinned to 3 uniform seedlings after 15 days of sowing. Soil was treated with calculated amounts of NaCl and Na₂SO₄ to create artificial salinity at 5 and 10 dS m⁻¹ (Abbreviated as SC₅ (T₂) and SC₁₀ (T₃) for NaCl treated pots and SS₅ (T₄) and SS₁₀ (T₅) for Na₂SO₄ treated pots) and natural soil (CTRL, T₁) having 1.25 dS m⁻¹ salinity level was considered control. Experiment was conducted using completely randomized design (CRD) with three replications of each treatment. N, P and K were applied at 175:160:90 kg ha⁻¹ using urea, single super phosphate (SSP) and sulphate of potash (SOP) as source fertilizers, respectively. P and K are applied as basal dose while N was applied in three splits *i.e.* at sowing, at time of 1st and 2nd irrigation.

Germination percentage (%) was recorded after seven days of sowing, whereas shoot length (cm), root length (cm), shoot and root dry weights (g pot⁻¹) were recorded at harvesting stage (on 3rd of May, 2016). Chlorophyll contents (µg L⁻¹) were measured at the time of flowering from the flag leaf with Chlorophyll meter (Minolta SPAD-502 DL meter Japan). Plant physiological parameters photosynthetic rate (A) (µmol m⁻²sec⁻¹) transpiration rate (E) (mmol m⁻²sec⁻¹) and sub-stomatal CO₂ (Ci) (µmol mol⁻¹) were recorded using Infra-Red Gas Analyzer (IRGA-CI-340) at physiological maturity (Neto *et al.*, 2004). Younger leaves after harvesting were washed with deionized (DI) water and frozen. Frozen leaf samples were thawed and crushed using a stainless-steel rod with tapered end and sap was extracted. The sap was removed by a micropipette, stored in Eppendorf tubes and centrifuged at 6500 rpm for 5 minutes. The supernatant sap was collected and used for ionic analysis (Abdolzadeh *et al.*, 2008). The sap was diluted as required with distilled water. Sodium and potassium were determined by using Flame Photometer (Sherwood 410) and chloride via titration method.

The data obtained on all parameters was analysed statistically for analysis of variance (ANOVA) technique using Statistix 8.1[®] (Analytical Software, Tallahassee, USA). Treatment means were compared by using Tukey's test at 5% probability (Montgomery, 2013).

Results and discussion

Effect of source and salinity levels on physical characteristics of maize

Germination rate

Germination rate of maize decreased significantly in the presence of the salts as compared to control. Maximum seed germination was found in control (100%), followed by 62-81% in Na₂SO₄ (SS) and 57-76% in NaCl (SC) treated soils (Figure 1A). The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects whereas types of salt (SS and SC) did not have significant effects on maize seed germination rate. The possible reason for this decrease with increasing salinity levels might be due to increased accumulation of Na⁺ and/or Cl⁻ ions in germinating seeds, therefore, any salt stress could reduce seed germination because it accelerates breakdown of stored food in embryo (Atak *et al.*, 2006). In addition, sodium is a constituent of both salts, which causes dispersion of soil particles with least water holding and compaction restricts radical development resulting into least germination while chloride ion is toxic for plant tissue causing more degradation as compared to sulphate (Kaymakanova and Stoeva, 2008; Stoeva and Kaymakanova, 2008). Similar to our results, Aliu *et al.* (2015) reported negative effects of NaCl and CaCl₂ on seed germination of maize when applied at 400 and 200 mM, respectively. Farooq *et al.* (2015) reported seed germination is the sensitive stage to salt stress. Several other studies have also reported the inhibitory effect of salinity on germination attributes (Elouaer and Hannachi, 2012; Afzal *et al.*, 2012; Hussain *et al.*, 2013). These inhibitory effects may have been due to osmotic pressure (Moud and Maghsoudi, 2008) and toxicity of salts (Saboor and Kiarostami, 2006) or due to the effect of added Cl⁻ ion causing abrupt raise in the osmotic stress (Almodares *et al.*, 2007).

Shoot and root dry weight

The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects on shoot dry weight, whereas source of salt (SS and SC) were differed significantly only at EC 5 dS m⁻¹ (Figure 1B). Inter-compar-

Table 1. Physico-chemical characteristics of experimental soil prior to experiment.

Parameters	Unit	Concentration
Sand	%	40
Silt	%	37.5
Clay	%	22.5
Textural class	Loam	
SAR		7.86
EC _e	dS m ⁻¹	1.45
pH		7.9
Saturation percentage	%	35.5

ison of salinity levels produced by different salts produced 13 and 6 g shoot dry weight in treatments received SC₅ and SC₁₀, 30 and 1.5% less, respectively as compared to treatments SS₅ and SS₁₀. Similar trend was observed in root dry weight (Figure 1C). Inter-comparison of same EC levels generated by different salts showed significant variation as (SS₅ and SS₁₀) produced 64 and 178%, higher root dry biomass as compared to SC₅ and SC₁₀ respectively. Similar responses were reported in Bambara groundnut (Ambede *et al.*, 2012). Na⁺ occupies exchange sites on clay particles causing particle separation. These dispersed particles have lesser ability to hold water and nutrients due to lack of structural aggregates. Least porous medium developed that also decreases the aeration necessary for root respiration (Zhu, 2002; Tester and Davenport, 2003; Mahajan and Tuteja, 2005). Low nutrients, water and aeration

results in declined biomass production with low shoot dry weights (Attia *et al.*, 2008). Salt accumulation in root zone causes the development of an osmotic stress and disrupts cell ion homeostasis by inducing uptake of essential nutrients and accumulation of Na⁺ and Cl⁻ to potentially toxic levels within cells (Zhu, 2001; Wang *et al.*, 2009). The causes of growth suppression under saline conditions may include shrinkage of cell contents, reduced development and differentiation of tissues, unbalanced nutritional status and damage to membrane integrity (Akhtar *et al.*, 2005; Sobhanian *et al.*, 2010). Reduction in root dry weights could also be found to decrease water potential of rooting medium due to high ion concentrations as initial growth inhibition in saline environment is related to osmotic effect (Mahajan and Tuteja, 2005).

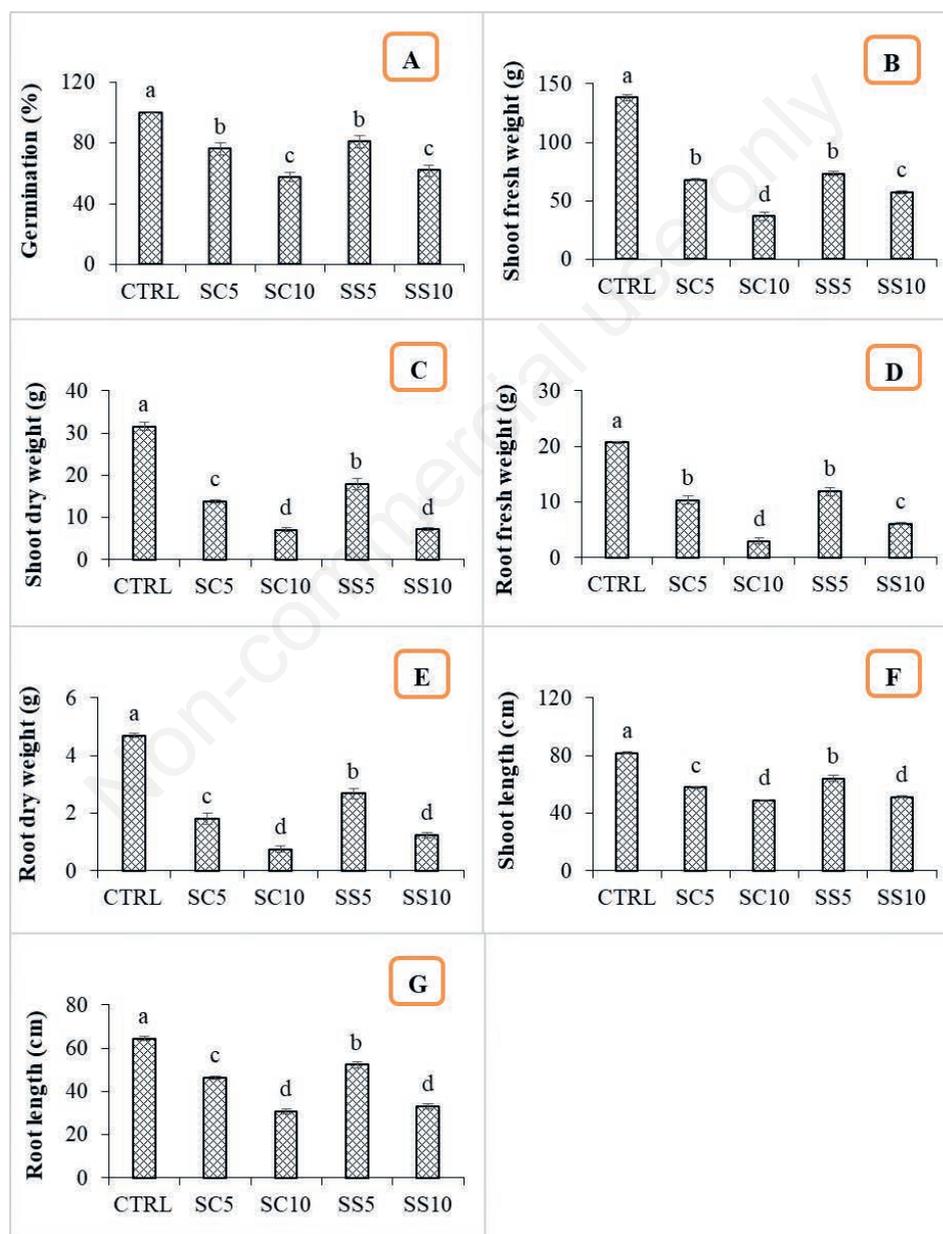


Figure 1. Effect of type and level of salinity [SC₅, SC₁₀: NaCl at EC 5 and 10 dS m⁻¹], [SS₅, SS₁₀: Na₂SO₄ at EC 5 and 10 dS m⁻¹] on maize agronomic parameters (A) Germination rate, (B) Shoot dry weight, (C) Root dry weight, (D) Shoot length and (E) Root length.

Shoot and root length

Addition of salts showed significant reduction in shoot (Figure 1D) and root length (Figure 1E) with salt type and salinity level effects. Figure 1D shows a very close dependency of maize shoot length upon the soil ionic concentration. The minimum shoot length (49 cm) was found in SC₁₀ while maximum shoot length (82 cm) was recorded in control. The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects on shoot length, whereas source of salt (SS and SC) were differed significantly only at EC 5 dS m⁻¹ (Figure 1D). Figure 1E clearly indicates a significant decline in root length with increasing soil salinity; however, toxicity depends upon source and level of salinity. The treatments SC₁₀, SS₁₀, SC₅ and SS₅ produced root length 110, 95, 39 and 23%, respectively lesser than control treatment. Inter-comparison of salts at same EC level showed 13 and 8% lesser root length under SC₅ and SC₁₀ than SS₅ and SS₁₀, respectively.

Salt stress has severe devastating impact on plant growth (Zhang and Shi, 2013) and reduces crop yield remarkably (Ray *et al.*, 2013; Qadir *et al.*, 2014; Gholizadeh *et al.*, 2014). External hypertonic solution insists plant to evacuate more water from root cells for osmotic adjustments with lesser nutrient uptake which hampers plant growth (Wang *et al.*, 2009; Zhang *et al.*, 2010). Root growth also gets hampered because of lesser aggregation. Accumulation of excessive salts in cell wall customized the metabolic pathway; limit the cell wall elasticity and ultimately the shoot length (Mahajan and Tuteja, 2005). Further, the secondary cells appear earlier and cell wall becomes rigid and inflexible. Resultantly turgor pressure efficiency in cell enlargement declines. These processes may cause the shoot to remain smaller (Saqib *et al.*, 2002; Shabala *et al.*, 2014). Root length of maize was indication of impact of salt stress on plant growth. It is attributed that as the salt content enhanced there is observable reduction in root growth. Hence, Na⁺ and Cl⁻ toxicity affects integrity and root permeability by displacing Ca²⁺ from the plasma lemma that ultimately restrains root growth and length (Slama *et al.*, 2015).

Effect of source and level of salinity on maize physiology

Sub-stomatal CO₂ concentration (μmol mol⁻¹)

Sub-stomatal CO₂ concentration (C_i) is an indication of plant metabolism. Significant variation in sub-stomatal CO₂ concentration of maize was revealed with changes in EC of soil from 5 to 10 dS m⁻¹ (Figure 2A). It indicates a degrading impact of increasing salt content on plant metabolism. Maximum C_i (345 μmol mol⁻¹) was recorded in control treatment while minimum C_i (250 μmol mol⁻¹) was recorded in SC₁₀ treatment in which salinity level was maintained at 10 dS m⁻¹ using NaCl salt. The level of salt (EC: 5 and 10 dS m⁻¹) and source of salt (SS and SC) had significant effects on C_i when compared to each other at same level of salinity (Figure 2A). Same salinity levels produced by NaCl and Na₂SO₄ were compared with each other, and 6 and 19% higher values were observed in case of SS₅ and SS₁₀ than SC₅ and SC₁₀ pots, respectively. Osmotic imbalance inhibits the entry of water into roots reducing transpiration and forcing plant to close stomata with significant reduction in CO₂ intake resulting in decreased C_i. This primary stress induces the generation of reactive oxygen species (Melloni *et al.*, 2003; Ashraf and Harris, 2004) that, causes hormonal changes (Munns, 2002; Gupta and Huang, 2014), alter carbohydrate metabolism (Khelil *et al.*, 2007), reduce the activity of certain enzymes (Munns and Tester, 2008; Ozgur *et al.*, 2013) and impair photosynthesis (Uzilday *et al.*, 2015).

Photosynthetic rate (μM m⁻² s⁻¹)

Higher rate of photosynthesis results in greater growth and vice versa. Maximum photosynthetic rate (0.5 μM m⁻² s⁻¹) was recorded in control treatment that was significantly differed (at P value 5%) from all the other treatments having photosynthetic rates of 0.26, 0.23, 0.11 and 0.04 μM m⁻² s⁻¹ under SS₅, SC₅, SS₁₀ and SC₁₀ treatments, respectively (Figure 2B). The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects on photosynthetic rate, whereas type of salt (SS and SC) were differed significantly only at EC 10 dS m⁻¹ (Figure 2B). Results revealed that SS₅ and SS₁₀ caused 16 and 175% more photosynthetic rate than same salinity level caused by NaCl in the case of SC₅ and SC₁₀ respectively. Osmotic stress compels plant tissue to ooze out water against the gradient hampering transpiration with stomatal closure short supplying water and CO₂ for photosynthesis (Gong *et al.*, 2011).

Transpiration rate (μM m⁻² s⁻¹)

Transpiration rate had significantly reduced with the addition of salt. Salinity stressed treatments SS₅, SC₅, SS₁₀ and SC₁₀ resulted 0.073, 0.067, 0.057 and 0.047 μM m⁻² s⁻¹ transpiration rates, respectively, which were significantly lower than control treatment (0.143 μM m⁻² s⁻¹). None of the salt treatment either level (EC: 5 or 10 dS m⁻¹) and source of salt (SS and SC) had significant effects on transpiration rate when compared to each other (Figure 2C). Transpiration is said to be necessary evil for plant to continue life sustaining processes *i.e.* photosynthesis and respiration. Tissue shrinkage due to outflow of water in saline environment and thereby cause significant reduction in transpiration to preserve CO₂ and water for metabolic processes (Gong *et al.*, 2011). Growth suppression in the form of reduced leaf area, number of leaves and stomata are the factors that affect transpiration under saline environments (Akhtar *et al.*, 2005).

Relative water content (%)

Figure 2D shows the relative water contents (RWC) in maize tissues under various sources of salt applied at different levels. RWC had reduced significantly in the presence of salt as compared to control (at P value 5%). The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects on RWC, whereas source of salt (SS and SC) were differed significantly only at SC₁₀ and SS₅ when compared to each other (Figure 2D). Osmotic stress considerably reduced water uptake because of water outflow from roots under hypertonic environments resulting into closure of stomata, hence here with reduction in transpiration and photosynthetic activities. Transpiration also removes water from plant tissues to maintain the plant temperature for regulation of metabolic processes at appreciable rates. The first measurable effect of water deficiency is growth reduction that is caused by the declining in the cellular expansion (Khodarahmpour *et al.*, 2012). Growth suppression under saline conditions get hindered due to shrinkage of cell contents, hampered tissue, unbalanced nutrition and damaged membrane integrity (Akhtar *et al.*, 2005).

Chlorophyll content (μg L⁻¹)

Salinity stress reduced the chlorophyll content in the plant leaves significantly (Figure 2E). Least chlorophyll content (8.27 and 10.6 μg L⁻¹) was measured in SC₁₀ and SS₁₀ that were significantly lower than (15.97 and 18.46 μg L⁻¹) of SC₅ and SS₅ (at P value 5%). Chlorophyll contents did not vary significantly in the treatments having same level of EC developed from both salts (NaCl and Na₂SO₄). Osmotic imbalance reduces entry of water into roots, decreases nutrient uptake including Mg (central constituent

of chlorophyll molecule) that resulted low chlorophyll contents in plant tissues. It may also be due to reduction in net photosynthesis under salt stress which results in decreased production of photosynthetic pigments like chlorophyll (a and b) and carotenoids (El Sayed, 2011; Qu *et al.*, 2012). Similarly, a linear decrease in

chlorophyll contents was observed with increasing salinity in maize plant (Cha-um and Kirdmanee, 2009). Exchange site occupation by Na^+ cause soil compaction that resist root penetration and reduces the uptake of nutrients (Mg and N) results into less chlorophyll contents in plant tissues.

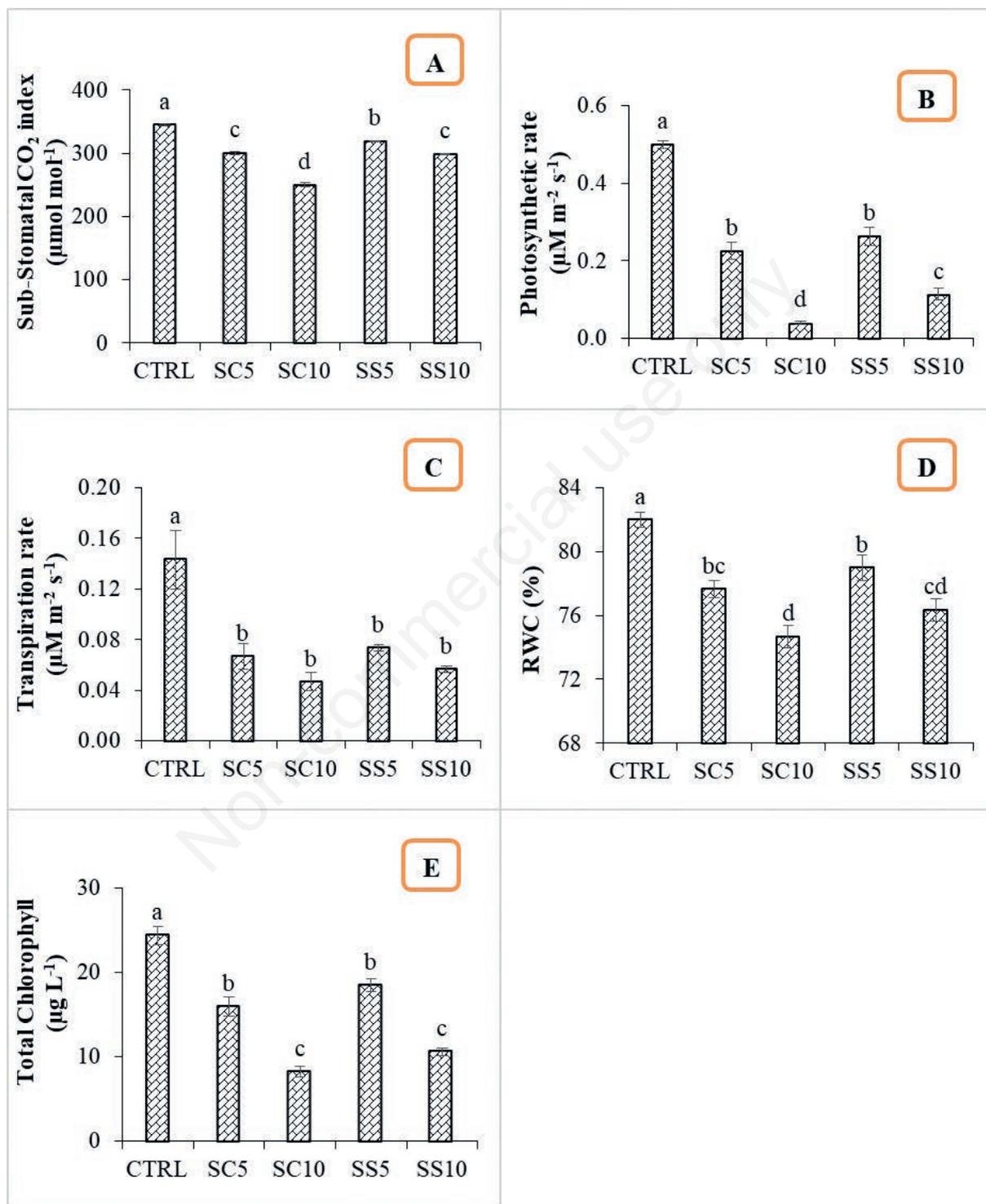


Figure 2. Effect of type and level of salinity [SC5, SC10: NaCl at EC 5 and 10 dS m^{-1}], [SS5, SS10: Na_2SO_4 at EC 5 and 10 dS m^{-1}] on maize physiological parameters (A) Sub-stomatal CO_2 index, (B) Photosynthetic rate, (C) Transpiration rate, (D) Relative water contents and (E) Total chlorophyll contents.

Effect of type and level of salinity on maize tissue Na⁺, Cl⁻ and K⁺ concentration

Shoot and root Na content

Accumulation of Na⁺ in shoot tissues of maize plant increases with increase in exogenous salt concentration (Figure 3A). Data revealed significant accumulation of shoot Na⁺ in SC₁₀ and SS₁₀

treatments as compared to control. The level of salt (EC: 5 and 10 dS m⁻¹) had significant effects whereas source of salt at either level did not show any significant effects on shoot Na⁺ (at P value 5%). Figure 3B shows a gradual increase of Na⁺ in the root tissue with increase in soil salt contents. Accumulation of Na⁺ was recorded highest in SC₁₀ treatment (0.51%) that was statistically at par with SS₁₀. In the sequence, a similar behaviour was observed at SC₅ and

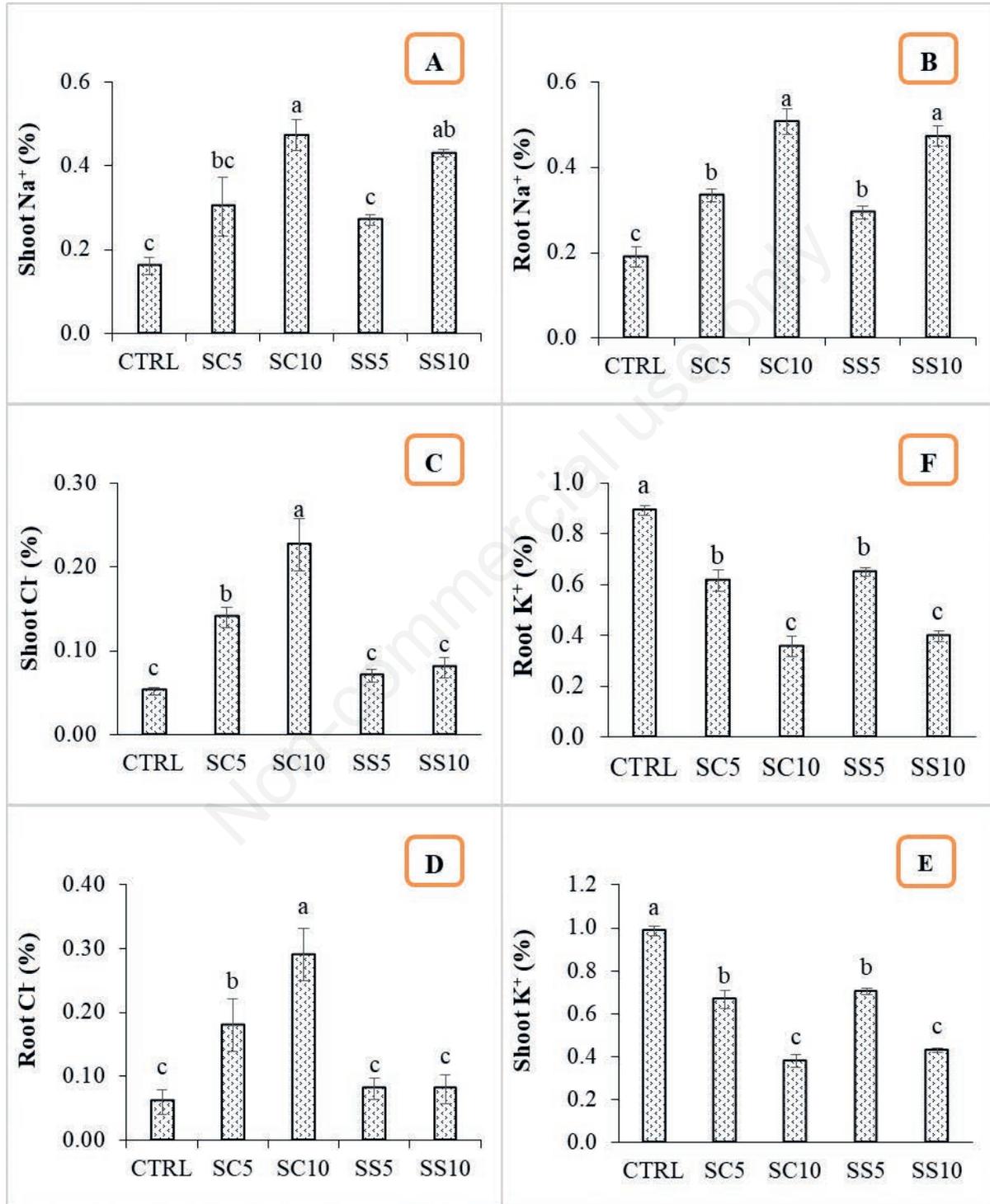


Figure 3. Effect of type and level of salinity [SC5, SC10: NaCl at EC 5 and 10 dS m⁻¹], [SS5, SS10: Na₂SO₄ at EC 5 and 10 dS m⁻¹] on maize shoot and root ionic contents: (A) Shoot Na⁺, (B) Root Na⁺, (C) Shoot Cl⁻, (D) Root Cl⁻, (E) Shoot K⁺ and (F) Root K⁺.

SS₅ treated plants. The control treatment had the least content of root Na⁺ (0.19%) that was significantly lower than all of the treated plants (at P value 5%). There was less content of Na⁺ inside the tissue, thus its concentration was found higher inside plant due to concentration gradient. Plants maintain internal hypertonic environment by up-taking more sodium ion to tolerate osmotic stress. Efficient Na⁺ exclusion is a good selection criterion for salt tolerance in cereals and glycophytes (Nawaz *et al.*, 2002). The increase in Na⁺ contents in leaves with increasing salinity was attributed to the increased concentration of Na⁺ in rooting medium, passive Na⁺ diffusion through damaged membranes and decreased efficiency of exclusion mechanism (Fricke *et al.*, 2004; Qu *et al.*, 2012). Besides, this increase in Na⁺ concentration could be due to greater uptake of sodium ion to build osmotic pressure. Sodium being a monovalent is very effective for osmotic adjustment (Akhtar *et al.*, 2005).

Shoot and root Cl content

Data revealed that highest significant accumulation of chloride in shoot and root was found only in the SC₅ and SC₁₀ treated plants, however rest of the treatments (SS₅, SS₁₀ and control) showed similar effects in this regard (Figure 3C and D). Chloride is attributed to be toxic agent and reduces plant growth. The addition of NaCl in soil increase the availability of Cl⁻ ion to plants that results its accumulation in plant tissues, which is attributed mainly due to either passive movement of Cl⁻ ion inside, or its high-water solubility and mobility. Salt tolerant crop plants maintain less Cl⁻ concentration in the tissues at high salinity level mainly through exclusion mechanism (Ebrahimi *et al.*, 2011). The increase in chloride contents in plant tissues with increasing salinity was attributed to the increased concentration of chloride ions in rooting medium, passive Cl⁻ uptake through damaged membranes and decreased efficiency of exclusion mechanism (Nawaz *et al.*, 2002; Qu *et al.*, 2012).

Shoot and root K content

Maximum accumulation of K⁺ ions in shoot and root parts of maize plants was observed in control, followed by SS₅, SC₅, SS₁₀ and SC₁₀ treatments (Figure 3E and F). Increased level of EC by either source showed significant reduction in shoot/root K contents; however, source of salt at same level of EC (SC₅ vs. SS₅, SC₁₀ vs. SS₁₀) did not show any significant effects on shoot/root K⁺ contents. Potassium is the most mobile and abundant cation in mica dominated soils. The intrinsic ability of soil either parent material or canal irrigation built up high amount of native K in soil that might reduce due to hyperosmotic stress leading to its accumulation in plant tissues. Same is the case in present condition that salt concentration in soil hampers amount of water necessarily required for the release of K from mica minerals, and thus limited amount of K⁺ available for plant uptake. Our results corroborated the results of Qu *et al.* (2012) who reported a significant reduction of K in maize with increasing salinity. There is a debate that K⁺ influx could be used as an index to salinity tolerance (Nawaz *et al.*, 2002).

Conclusions

Results revealed deleterious effects of salinity on these indices; however, the highest salinity level (10 dS m⁻¹) which was induced with NaCl salt had caused the most devastating impacts on maize plant growth. The same treatments caused significant reduction in

chlorophyll contents, transpiration and photosynthetic rate. These toxic effects are attributed to accumulation of Na⁺ and Cl⁻ ions in maize plant tissues that caused reduction in various growth and physiological parameters. Based on these facts, we concluded that NaCl was more toxic than Na₂SO₄ whereas higher level of both salts showed toxicity to maize seedlings. We, in this study, report that salt stress varies according to their types and doses under field conditions and that our results are important to determine the selection of plant species resistant to specific salt and salt level in order to maximize the marginal land utilisation for enhanced crop production.

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