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Salinity in the First Phase of Salt Stress Alters Photo-physiology, Water Use Efficiency and Total Soluble Phenolics of Maize Genotypes

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ABSTRACT

Salinity is one of the most important abiotic stresses that adversely affects plant growth and development around the world. In order to elucidate the growth and physiological responses of maize genotypes under the first phase of salt stress (12 dS m^{-1} for two weeks), we investigated some growth and physiological traits at vegetative stage (28 d old plant) of four maize genotypes, namely indigenous yellow pure line, indigenous yellow, hybrid, and indigenous white. Salt stress significantly reduced shoot height and stem diameter in almost all genotypes. Under salt stress, instantaneous water use efficiency was highly increased in indigenous yellow pure line (285.5%), in contrast it was decreased significantly in hybrid (16.99 %) genotype compared to their respective control. Photosynthesis rate (70–87%), transpiration rate (81–91%), and stomatal conductance (80–92%) were significantly reduced due to salinity in all the tested genotypes. In the younger shoot, total phenolics content increased significantly in the young shoots of hybrid (42.26 %) and indigenous yellow pure line (40.03 %) genotypes under the first phase of salt stress. In contrast, there was no significant influence of salinity on total phenolics content of older shoot fraction in any genotype tested. Apparently, the growth and physiological traits were hampered in the first phase of salt stress in all tested genotypes. However, deposition of soluble phenolics under salt stress was genotype and leaf-region specific. As the most traits studied were highly influenced by the salinity in the first phase of salt-stress among genotypes at vegetative stage, breeders can potentially use these traits further in breeding program for the development of maize genotypes tolerant to the salt-stress at the vegetative stage of growth.

Introduction

Maize (*Zea mays* L.) occupies one of the important cereal crops all over the world (Hassan *et al.,* 2018). It is used as a source of food, feed, oil and fuel throughout the world (Khatoon *et al.,* 2010; Ullah *et al.,* 2010). Maize is also a very popular crop in Bangladesh where it is usually grown in cool winter Rabi season in North-West and central part of Bangladesh, and its growing areas are increased at nearly 20% per year since early 1990s (Banik *et al.,* 2010). Furthermore, due to the rising poultry industry in Bangladesh, the need for maize is increasing very sharply as maize is an important component of poultry feed (Pandey and Koirala, 2017; Hassan *et al.,* 2018). In Bangladesh, total land area and production of maize are 395500 ha and 279500 m tons, respectively (AIS, 2017).

However, the production of maize now a day seriously hampered due to different abiotic stresses around the world including Bangladesh (Waqas *et al.,* 2019). Among different abiotic stresses, salinity is one of the most frequently occurring environmental threats that alters various cellular functions (Niu *et al.,* 2012) and reduced vegetative growth of maize (Uddin *et al.,* 2013, 2014, 2019). Salt intrusion is also a huge concern for the coastal area in southern part of Bangladesh which deteriorates the soil health and fertility status resulting low agricultural production thus threatens food security (Ahmed and Haider, 2014). In Bangladesh context, about 1.056 million ha of arable lands are affected by varying degrees of salinity which adversely impairs crop ecosystem (SRDI, 2010).

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Salt stress affects all growth stages of maize plants; particularly vegetative growth stage is known to be more sensitive (Uddin *et al.*, 2013, 2014, 2019; Pitan *et al.*, 2009). Maize is considered as moderately salt-resistant though the salt resistance level varies with the growth stages (Ouda *et al.,* 2008; Carpici *et al.,* 2010). Salinity alters plant growth at two phases; firstly, salinity exerts osmotic stress in the root zone that responds to the huge growth retardation both in shoot and root (Munns, 2005). Further growth inhibition occurs due to the accumulation of excess salt ions in the plant cells that result in alterations of various metabolic processes. The disruption of intracellular ionic balance and osmotic gradients inhibits a number of vital physiological functions that ultimately respond to the malfunctioning of plant physiological traits (Islam, 2012; Dawood *et al.,* 2014; Semida and Rady, 2014; Sadak and Abdelhamid, 2015). As a consequence of these primary effects of salt stress, the growth and development of plants as well as productivity are severely reduced, and, in extreme cases cause plant death (Krasensky and Jonak, 2012).

Cell-wall bound phenolics have been reported to be increased in response to the first phase of salt stress in the salt sensitive maize genotype (Uddin *et al.*, 2014). It was demonstrated previously that the rate of transpirations of maize plants that were grown hydroponically by imposing salt stress, are not necessarily reduced compared to control plants (Schubert, 2009). In this study, salt-stress response of four maize genotypes to moderate salinity level at early vegetative growth stage was investigated in terms of growth traits, gas exchange parameters, instantaneous water use efficiency (IWUE) and total soluble phenolics (TSP) contents, whether they were influenced or not.

Materials and Methods

Plant materials and experimental layout

Four maize genotypes namely indigenous yellow pure line (IYPL), indigenous yellow (IY), hybrid, and indigenous white (IW) were used as plant materials in this study. The pot experiment was conducted in net house of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, followed by a completely randomized design (CRD) with four replications. Two levels of salinity (control: without addition of NaCl and salinity: addition of NaCl to reach EC 12 dS m^{-1}) were tested against each genotype.

Plant cultures and salinity stress imposition

Maize caryopses were sterilized with Vitavax 0.2% followed by proper washing with water. Afterwards, caryopses were soaked in the aerated water overnight. Four seeds of each genotype were placed into the 10 L plastic container that was filled with 12 kg air-dried sub-soil fertilized with standard fertilizer doses and water content inside the pot was adjusted to 60% of total water holding capacity (WHC) by adding water. After 4 days of germination, only one healthy seedling was kept in each pot. After 10 days of seedling establishment, the salinity treatment was applied by means of adding NaCl in four consecutive days employing one fourth amount of NaCl (EC- 3 dS m^{-1}) in each time to reach a final EC of 12 dS m^{-1}. The first and the last salt-stress imposition took place on the day 11th and 14th, respectively after seeding. Afterwards, plants were grown in this saline environment for next 14 d without further addition of salt. The control plants were grown without the addition of NaCl.

Determination of photophysiological parameters and water use efficiency

Net photosynthetic rate (A), transpiration rate (E) and stomatal conductance (g_s) were recorded from the fully expanded 8th leaf of 27 days old maize plant using a portable photosynthesis system (LCi-SD System, ADC Bioscientific Ltd., Hoddesdon, UK) (Abbasi *et al.,* 2015). Afterwards, IWUE was calculated by dividing the net photosynthetic rate (A) with the transpiration rate (E) following the method of Dias *et al.* (2017).

Determination of phenolic content

To analyse the phenolics content of shoot, 8th and above order leaves were separated from the rest. The basal 10 cm of these leaves were separated being younger shoot and the rest above parts of these leaves without basal 10 cm were kept being older shoot. Fresh leaf tissue (50 mg) from each fraction of leaves was homogenized with methanol for 2 min by OV-5 homogenizer [Modified from Albano and Miguel (2011)]. Then, some extracts were centrifuged at 14,000 *g* in a vial for 5 min and afterwards, extracted liquid samples mixed with Folin-Ciocalteau's phenol reagent in a test tube. Then, 10% sodium bicarbonate solution ($NaHCO_3$) were added and allowed 30 min in dark condition. Finally, the absorbance was recorded at 760 nm by using DR-6000 spectrophotometer. Gallic acid standard was prepared by diluting appropriate amount of analytical grade gallic acid to the methanol and results were expressed as mg phenol as gallic acid equivalent/100 g of leaf fresh mass.

Data analyses

Data were subjected to t-test in Microsoft Excel 10 to realize any difference between the salt stress and the control treatments. Data presented in the graphs were processed in GraphPad Prism 8.

Results and Discussion

Shoot length

In the present study, the significant reduction of shoot length was observed in all of the studied maize genotypes when the seedlings were exposed to salinity (P ≤ 0.1 %) (Fig. 2a). At 3 days of salt stress, shoot length of indigenous yellow pure line decreased 21.84 %, which finally decreased 10.98 % when it was exposed full strength of salt stress after 12 days of full salt-stress. In case of indigenous yellow genotype, on day 3 of salt stress there was no noticeable change in shoot length although on day 12 after salinization, the shoot length was reduced by 16.96 %. Although there was little genotypic differences for salt stress during early stage, it became distinct during later stages (12 days of salt application) with the significantly decreased maximum reduction of shoot length in indigenous white (23.82 %) followed by indigenous yellow (16.96 %) maize genotypes compared to controlled plants. In contrast, shoot length of IYPL and hybrid genotypes declined to a lesser extent but more or less on the same magnitude (10.98 % and 11.67 %, respectively) under full strength of salinity stress (Fig. 2a).

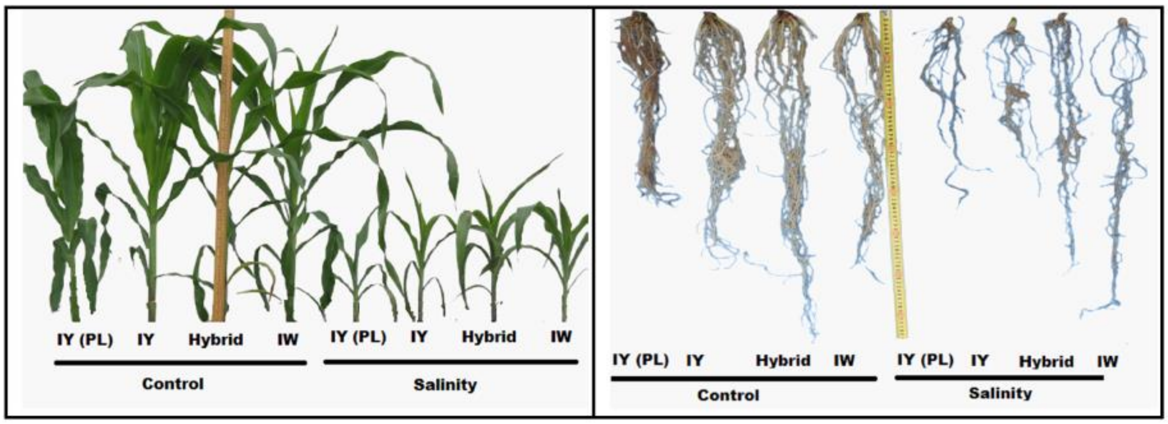

Figure 1. Shoot and root growth of four maize genotypes viz., indigenous yellow pure line (IY PL), indigenous yellow (IY), hybrid, and indigenous white (IW) as influenced by the first phase of salt stress (12 dS m^{-1}).

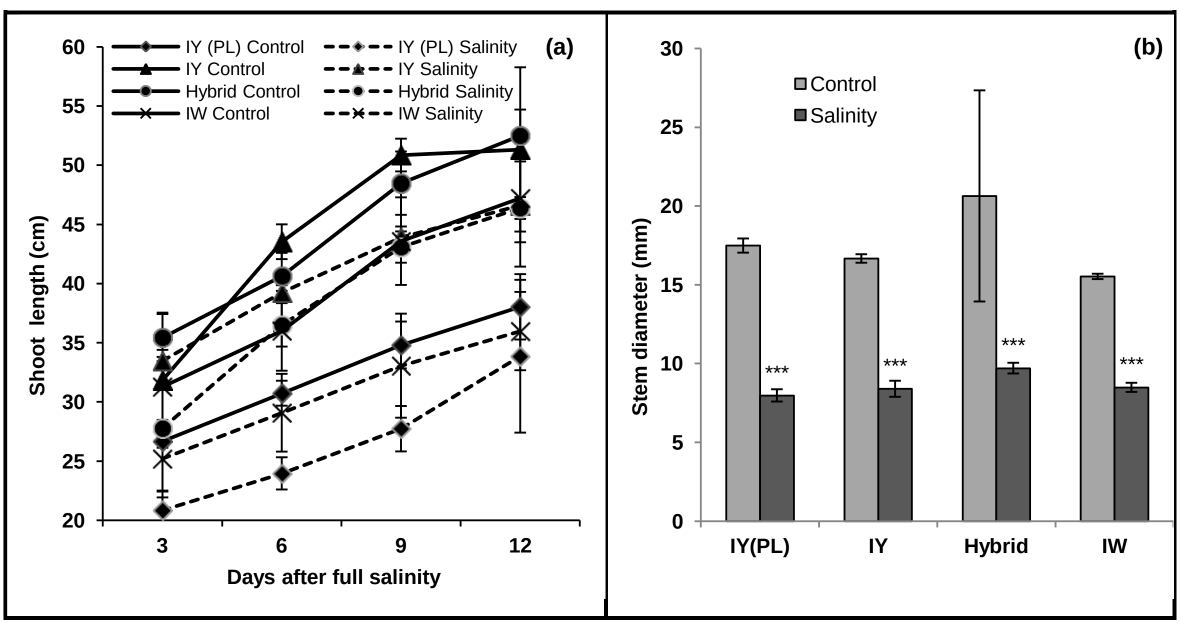

Figure 2. Trend of [a] shoot lengths of four maize genotypes namely, indegonous yellow pure line (IYPL), indigenous yellow (IW), hybrid and indigenous white (IW) after different days of full salt-stress (12 dS m^{-1}) imposition. Stem diameter recorded at final harvest [b]. Each data point is the mean ± standard error of means of four replicates. *** denotes significantly different compared with control at P ≤ 0.1.

Hassan *et al.* (2018) reported that the maize genotypes Barnali, BARI Maize 5, and Mohor exhibited more than 40% reduction in plant height at high salt stress (200 mM NaCl). Salinity causes a persistent reduction of the elongation rate of maize leaves (Chazen *et al*., 1995; Neumann, 1993), and thereby reducing shoot length (Khanoom *et al*., 2016; Uddin *et al*., 2014; Uddin *et al*., 2013; Hatzig *et al*., 2010). We did not notice any marked visible necrosis in the older shoot of salt-stressed plants even after two weeks of full salinity treatment. Thus it can be assumed that plants were predominantly in the first phase of salt stress and growth reduction at this stage was mainly because of the osmotic effect rather than the ion toxicity.

Stem diameter

Stem diameters were significantly reduced in all tested genotypes due to the salinity (Fig. 2b). In our study, minimum stem diameter reduction had been displayed by indigenous white (45.4 %), and indigenous yellow (49.6 %) under salt stress. In contrast, other two genotypes viz., indigenous yellow pure line and hybrid demonstrated a bit higher reduction of stem diameter when exposed to 12 dS m^{-1} salinity level compared to their respective controls. Salt stress induced growth inhibition in maize plant such as stem diameter (Khan *et al.,* 2001; Zhao *et al.,* 2006, Azoz *et al.,* 2004; Asha and Dhingra, 2007). In low osmotic pressure, stem cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Anjum *et al.,* 2011). Salinity caused impaired mitosis; cell elongation and expansion resulted in reduced stem diameter traits (Hussain *et al.,* 2008). In our reports, all tested genotypes exhibited more than 40% reduction in stem base diameter. This fits with the hypothesis proposed by the Munns (1993) that the magnitude of growth reduction in the first phase of salt stress is more or less similar in the salt-resistant and salt-sensitive genotypes.

Photophysiology as influenced by salinity

Rate of Photosynthesis

In our present study, salt stress decreased the rate of photosynthesis in four maize genotypes. Salt stress decreased maximum 87.21%, 83.58 %, and 83.02 % photosynthesis rate in Hybrid, indigenous yellow, indigenous white genotypes, respectively (Fig. 3**a**) compared to their respective controls (P ≤ 0.1%). IYPL genotype exhibited reduction rate of photosynthesis under 12 dS m^{-1} salinity (Fig. 3a). This is consistent with the previous study (Niu *et al.,* 2012, Abbasi *et al.,* 2015). Salinity stress severely hampered the gas exchange parameters of plants and this could be due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in structure of pigments and proteins (Menconi *et al.,* 1995).

Transpiration and stomatal conductance

Our present study reveals that, the rate of transpiration under salt stress declined by 90.77%, 81.44%, 81.60% and, 82.8% in IYPL, IY, hybrid, and IW, respectively, compared to control (P ≤ 0.1%) (Fig. 3**b)**. Likely wise the rate of transpiration, stomatal conductance of CO_2 were greatly inhibited by salt stress in all tested genotypes (Fig. 3c). Both IYPL and IY genotypes showed maximum degree of reduction in leaf stomatal conductance (92.30% and 92.31%, respectively). Other two genotypes i.e. hybrid and IW showed reduction of the same by 85.71% and 80%, respectively, when they were exposed to the salt stress (Fig. 3**c**). Our results are in line with that reported by Anjum *et al.* (2011), who indicated that salt stress in maize led to considerable decline in net photosynthesis (33.22%), transpiration rate (37.84%) and stomatal conductance (25.54%) as compared to well watered control. Also, reduced photosynthesis in couple with consistent decreases in stomatal conductance (g_s) has been reported in both Aristo and Arper maize varieties under salt stress (Hichem *et al.,* 2009).

Many studies have shown the decreased photosynthetic activity due to stomatal or non-stomatal mechanisms (Del Blanco *et al.,* 2000). Stomata are the route of H_2O exit and CO_2 entrance, and stomatal closure is one of the first responses to salt stress which results in declined rate of photosynthesis. Stomatal closure deprives the leaves from CO_2 and thus photosynthetic carbon assimilation is decreased. The transpiration rate can be used as an indirect measure for CO_2 assimilation (James *et al.,* 2008, Rahnama *et al.,* 2010), because as long as the stomata are open and the plants transpire, CO_2 can enter the leaves for being assimilated. The stomata are unselective openings for CO_2 entry and the release of water and O_2 (Tiessen *et al.,* 2006).

Instantaneous water use efficiency

It is generally known that reduced photosynthetic rate leads to reduced plant growth in most plants. Salinity can decrease root water uptake through its osmotic effect, and then induce water stress. The results of the current study showed that four maize genotypes displayed differential regulation in instantaneous water use efficiency (IWUE) in response of salinity stress (Fig. 3d). Among the genotypes, instantaneous WUE was found significantly increased in IYPL (228.98 %) followed by IY (40.25 %) (Fig. 3d). In contrast, instantaneous WUE, in hybrid genotype reduced by 16.99 % under salinity.

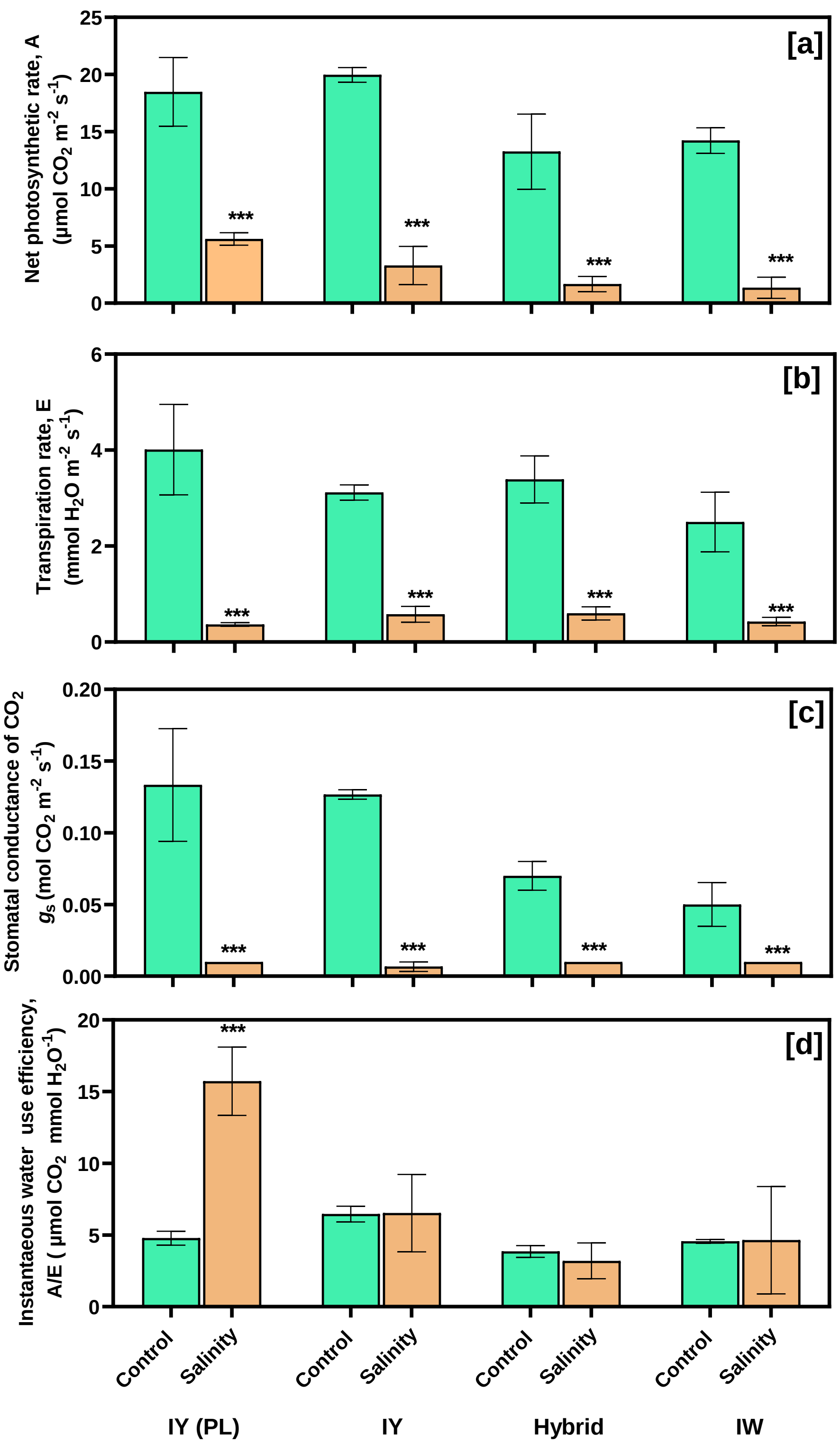

Figure 3. Effect of salinity in the first phase of salt stress (12 dS m^{-1}) on [a] rate of photosynthesis [b] rate of transpiration [c] stomatal conductance and [d] instantaneous water use efficiency in four maize genotypes viz. indigenous yellow pure line (IYPL), indigenous yellow (IY), hybrid, and indigenous white (IW). Each data point is the average of four replicates ± SEM. *** denotes significantly different compared with control at $P \leq 0.1$ %.

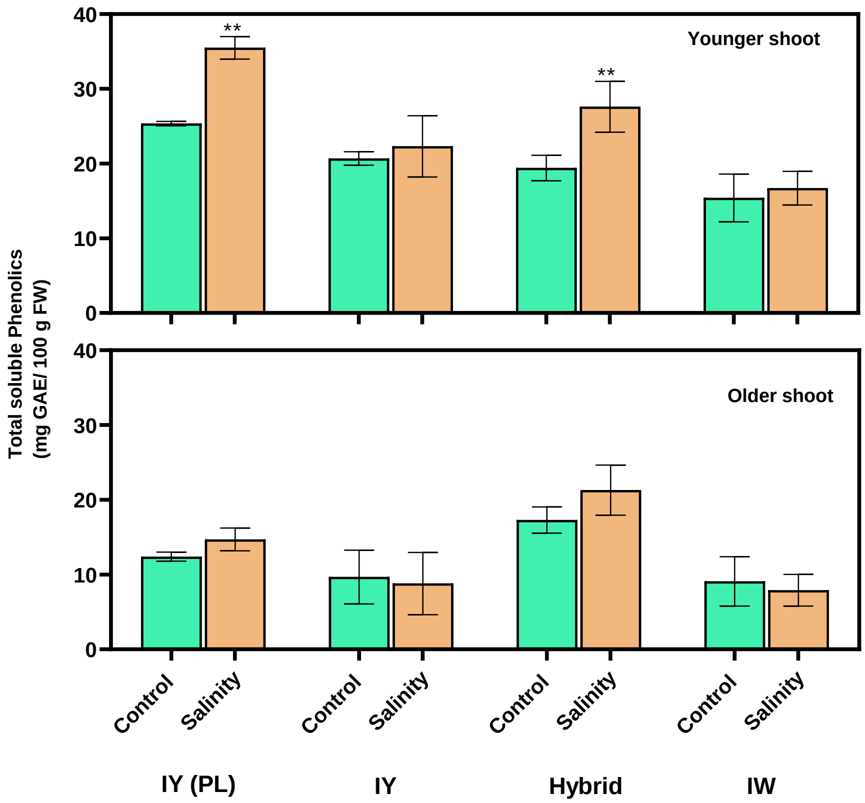

Figure 4. Effect of salinity in the first phase of salt stress (12 dS m^{-1}) on total soluble phenolics in the [a] younger and [b] older shoots of four maize genotypes. The varieties include indigenous yellow pure line (IYPL), indigenous yellow (IY), hybrid, and indigenous white (IW). Each data point is the average of four replicates ± SEM. * denotes significantly different compared with control at P ≤ 5 %.

The reduction of water use efficiency under salt stress was previously reported by another group of researchers in tomato (Romero-Aranda *et al.,* 2001). On the other hand, salt induced stress remarkably enhanced instantaneous WUE in IYPL genotype in compared to the control treated plants (Fig. 3d). IYPL maintained relatively higher rate of photosynthesis and lower rate of transpiration under salinity as compared to the other genotypes. This accounted for higher instantaneous WUE of these genotypes under salt stress. Some previous investigations reported increased water use efficiency in different plant species (Reddy *et al.,* 2003, Yin C *et al.,* 2005) under stress condition.

Total soluble phenolics in the shoot

Generally, salinity stress induces phenolic compound in maize genotypes. Salinity resulted in changes of total soluble phenolic content in young shoot of maize varieties, as shown in (Fig. 4a). Under salinity stress, there was a significant and highest increase of total soluble phenolic content in the young shoots of hybrid (42.26 %) and indigenous yellow pure line (40.03 %) genotypes. Similarly, salt stress also led insignificant increment of total soluble phenolic content in the younger shoots of indigenous yellow (7.88 %) and indigenous white (8.44) genotypes (Fig. 6a). In our present study phenolic content increased differentially among the genotypes which are consistent Uddin *et al*. (2014) who reported salinity induced augmentation of cell-wall bound phenolics in Pioneer 3906 maize genotype. Salinity induced disturbances of the metabolic process leading to an increase in phenolic compounds have been reported by Radi *et al.* (2013). Phenolic compounds such as phenolic acids, flavonoids play an important role in scavenging free radicals (Ksouri *et al.,* 2007, Huang *et al.,* 2005). Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (Huang *et al*., 2005). In our study, salt stress did not induce any significant change in total soluble phenolic content in the older shoot of all tested genotypes compared to their control. However, the younger shoot fraction, there was a tendency in the increase of soluble phenolics in the indigenous yellow pure line (18.5%) and hybrid genotype (23.12%) under salinity.

Conclusion

All growth parameters viz., shoot length and stem diameter of maize plants were negatively influenced by salinity in the present study. The parameters mentioned before were declined more or less in all maize genotypes. Gas exchanges parameters like net photosynthesis rate, rate of transpiration and stomatal conductance of CO_2 were decreased in all the tested maize genotypes after exposing to full strength of NaCl stress for 14 days. But salinity caused increment in instantaneous water use efficiency, in indigenous yellow pure line. In response to the salinity, total alcohol soluble phenolics were increased significantly in indigenous yellow pure line and hybrid genotypes but only in the younger shoot fraction (basal 10 cm of 8th and above order leaves) In contrast, older shoot (8th and above order leaves without basal 10 cm) did not show any significant change in total soluble phenolics due to salinity. Thus, it is be concluded that all the parameters studied here were greatly influenced under first phase of salt stress and can be used for further screening purposes to identify salt resistance genotype for our future demand of food supply.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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