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Gas exchange and chlorophyll fluorescence parameters in four maize genotypes influenced by first phase of salt stress

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Abstract

Responses in photosynthesis, transpiration, stomatal conductance, chlorophyll fluorescence characteristics and chlorophyll content of four maize (*Zea mays* L.) genotypes were examined under first phase of salt stress. In the experiment four maize genotypes viz. indigenous yellow pure line, indigenous yellow, hybrid, and indigenous white were tested in two levels of salinity (control: without NaCl application; salinity: 12 dS m^{-1} by applying NaCl). The experiment was laid out following completely randomized design (CRD) with four replications in the net house of Department of Crop Botany, Bangladesh Agricultural University, Mymensingh. Plants were harvested on day 21 after 7 d application of full salt stress (12 dS m^{-1}). The reductions of shoot fresh masses under salinity were 70, 57, 55 and 61% in indigenous yellow pure line, indigenous yellow, hybrid, and indigenous white, respectively. Some core physiological parameters viz. net photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (gs), and the chlorophyll content decreased in all the maize genotypes except indigenous white under first phase of salt stress. In contrast, the ratio of variable fluorescence to maximal fluorescence (Fv/Fm) of photosystem II significantly decreased only in indigenous yellow pure line under salinity stress. Thus the first phase of salt stress seemed to be deleterious on its response to shoot fresh mass production in all the tested four maize genotypes with the concomitant decrease in rate of photosynthesis, rate of transpiration, stomatal conductance and chlorophyll content in all genotypes except indigenous white.

Key words: Photosynthesis, transpiration, stomatal conductance, Fv/Fm

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Introduction

Salinity has appeared as an important problem as regard to agricultural production in many parts of the world particularly in arid and semi-arid regions (Akça and Samsunlu, 2012). Salinity is a significant limiting factor in the agricultural productivity. More than 6% of the world's total land areas are affected by varying degree of salinity including both saline and sodic soils (FAO, 2008). Because of global climate change, salinity problem is predicted to turn into more extensive which results in reduction of growth and production of many important plant species (Ainsworth and Ort, 2010). Consequently, scientists throughout the world are challenged to produce 70% more food for an additional 2.3 billion people by 2050 (Kumar *et al.,* 2013). Hence, the development of crop plants suited to give reasonably better yield under salt stress is crucial to meet up future food demand.

Maize (*Zea mays* L.) is one of the staple cereal crops that serves as food and oil for human consumption and

as feed for livestock (Hussain *et al*., 2010). It is mostly grown in cool winter *Rabi* season in north-west and central part of Bangladesh, and its growing area is increasing at approximately 20% per year since early 1990s. Being a cross pollinated crop, salinity tolerance can in maize (Paterniani, 1990). In reality, maize is relatively salt-tolerant compared to other two important cereals, rice and wheat. Hence, the prospect of maize cultivation under salinity is higher than these two crops in agro-ecologically disadvantaged saline coastal zone of Bangladesh.

Vegetative growth of maize appears to be the most sensitive to first phase of salt stress (Khanoom et al., 2016, Uddin et al., 2013 & 2014; Hatzig et al., 2010; Pitan et al., 2009), while plants are much less affected at later stages (Cramer, 1994). Photosynthesis, stomatal conductance, transpiration, chlorophyll content, and the activity of photosystem II (PSII) (Abbasi et al., 2015) have been reported to be negatively affected due to salt stress. Contrarily, unaffected rate of transpiration in maize under salt stress has also been reported from hydroponic experiment (Schubert, 2009). More so, gas exchange parameters and chlorophyll fluorescence parameters have little been studied during the first phase of salt stress. Keeping this fact in mind the present study was conducted to investigate gas exchange parameters, chlorophyll fluorescence of photosystem II and chlorophyll content in four maize genotypes as influenced by first phase of salt stress.

Materials and Methods

***Experimental layout and salinity development*:** The pot experiment was conducted in net house in the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, followed by a completely randomized design (CRD) with four replicates of each treatment. Four maize genotypes namely indigenous yellow pure line (IYPL), indigenous yellow (IY), hybrid, and indigenous white (IW) were tested against 12 dS m^{-1} salinity. Plastic containers (10 L) were filled with 12 kg of air-dried subsoil fertilized with standard fertilizer doses and then water content was adjusted to 60% of total water holding capacity (WHC). Seeds of all maize genotypes were soaked in the aerated water overnight and then placed four seeds of each genotype to the plastic containers covering all replications. After germination only one healthy plant was kept in each container. To develop salinity, NaCl was applied to reach an EC value of 12 dS m^{-1}. One fourth of the total NaCl was applied on day 11 of seed sowing. On the following three consecutive days the rest amount of salt was applied, each time one fourth of the total NaCl. From day 14 until harvest (at 21 days after planting) the soil water was maintained at 60% of WHC.

***Chlorophyll measurement with SPAD*:** The chlorophyll content as SPAD value was measured with a chlorophyll meter (Minolta Co. Ltd., Japan). It is a relative measurement of leaf greenness. In each treatment data was recorded for 6th fully expanded leaf as an average of three measurements.

***Measurement of Fv/Fm ratio*:** The ratio of fluorescence to maximal fluorescence (Fv/Fm) of Chl-a, an indicator of the efficiency of the photosyntem II (PS II), was measured using Pocket PEA portable fluorometer (Hansatech Instruments, King's Lynn, Norfolk, UK). The same leaf from which chlorophyll was measured conveniently dark adapted for 30 minutes prior to measurement using the leaf-clips. Fluorescence was induced by saturating, red actinic light with energy of 3.500 µmol m^{-2} s^{-1}.

***Gas exchange parameters*:** In parallel to the Chl-a fluorescence measurement of 6th leaf, photosynthetic rate (A), transpiration rate (E) and stomatal conductance (Gs) were determined using a portable photosynthesis system (ADC Bio-scientific Ltd., UK) from the same leaf.

Results and Discussion

***Shoot growth*:** Salt stress significantly decreased shoot fresh weight in all the tested maize genotypes compared to their respective controls (Figure 1). The

reductions of shoot fresh masses under salinity were 70, 57, 55 and 61% in indigenous yellow pure line, indigenous yellow, hybrid, and indigenous white, respectively. Maize genotypes were exposed to full salinity (12 dS m^{-1}) for 7 d, and during this period no necrotic spots were observed in the lower leaves despite a huge shoot growth inhibition. Thus, it can be assumed that the salt treated plants were in the first phase of salt stress (Schubert, 2011). A number of research have revealed that salt stress causes a persistent decrease of the elongation rate of maize leaves (Chazen et al., 1995; Neumann, 1993), in that way reducing shoot growth (Khanoom et al., 2016; Sultana et al., 2016; Uddin et al., 2014; Uddin et al., 2013; Hatzig et al., 2010).

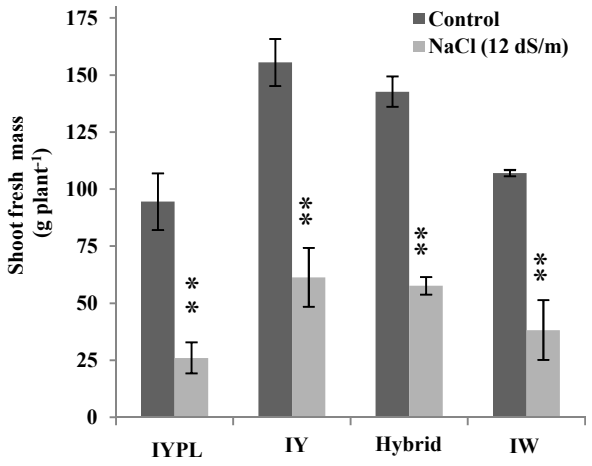

Figure 1. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on shoot fresh masses in four maize genotypes. Each data point is the average of four replicates±SEM.** indicates significantly different compared with control at P ≤ 1%.

***Chlorophyll content*:** Salt stress (12 dS m^{-1} NaCl) significantly ($p<0.05$) reduced chlorophyll content in IYPL and IY, while hybrid and IW were unaffected (Figure 2). The relative decreases in chlorophyll content under salt stress compared to control were 26.69, 33.39, 14.13 and 6.81% in IYPL, IY, hybrid and IW, respectively. This is similar with the results obtained by Tuna *et al*. (2008) who claimed that both chlorophyll a and b contents of maize plant decreased in response to salinity. The depressive effect of salt stress on chlorophyll biosynthesis may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater *et al.,* 1978) and /or damaging the photosynthetic apparatus (Singh *et al.,* 1981, Yasseen *et al.,* 1983). On the other hand, (Jaleel *et al*., 2007) suggested that the reduction in leaf chlorophyll under salinity could be ascribed to the destruction of the chlorophyll pigments and the instability of the pigment protein complex.

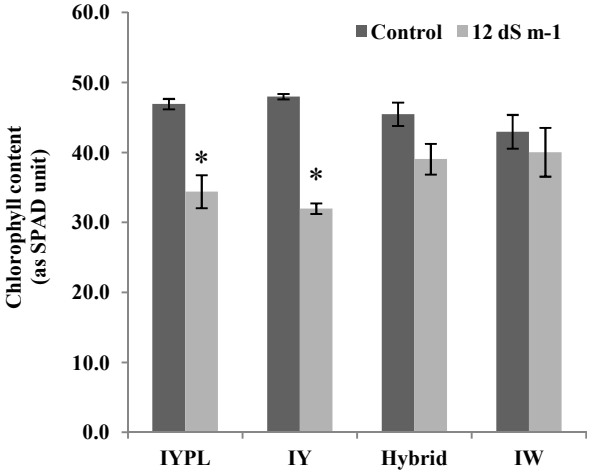

Figure 2. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on chlrorophyll content in four maize genotypes. Each data point is the average of four replicates±SEM. * denotes significantly different compared with control at P ≤ 5 %.

***Chlorophyll-a fluorescence ratio*:** Salt stress (12 dS m^{-1} NaCl) significantly ($p<0.05$) increased Fv/Fm ratio in IYPL while IY, hybrid and IW were unaffected (Figure 3). This is dissimilar with the results obtained by Abbasi *et al*. (2014) and Niu et al. (2012) who claimed that Fv/Fm ratio of plant decreased in response to salinity. However, it needs to be mentioned that they did not study plant in the first phase of salt stress. Our study clearly shows that the activity of PS II is not

limited in maize leaf under first phase of salt stress and thus light reaction of photosynthesis is also not limited under the same.

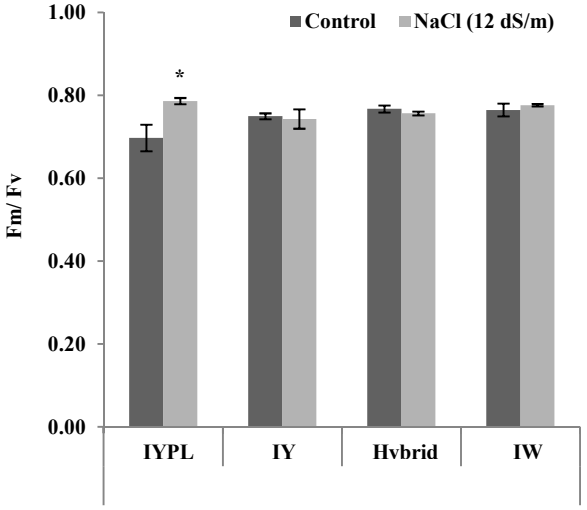

Figure 3. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on the ratio of variable fluorescence to maximal fluorescence (Fv/Fm) in four maize genotypes. Each data point is the average of four replicates±SEM. * denotes significantly different compared with control at P ≤ 5 %.

***Gas exchange parameters*:** The effects of NaCl on photosynthetic rate of four maize genotypes are described in Figure 4. Salt stress (100 mM NaCl) significantly ($p<0.05$) reduced photosynthetic rate in all the maize genotypes namely Indigenous yellow pure line, Indigenous yellow, Hybrid except Indigenous white compared to control. The Indigenous yellow and Hybrid decreased higher amount of photosynthesis (81.05 and 83.49%, respectively); whereas Indigenous yellow pure line and Indigenous white decreased the same by 59.55 and 63.57%, respectively under salt stress condition.

Figure **5** shows the effect of NaCl on transpiration. Salt stress (100 mM NaCl) significantly ($p<0.05$) reduced transpiration rate in IY and Hybrid compared to control.

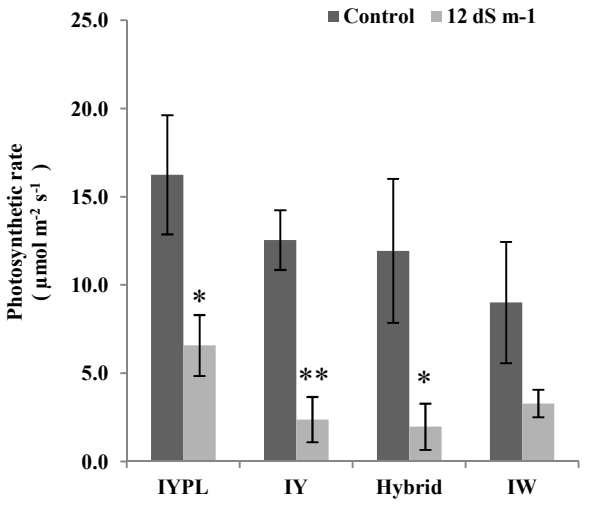

Figure 4. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on the photosynthtic rate in four maize genotypes. Each data point is the average of four replicates±SEM. * and ** denote significantly different compared with control with at P ≤ 5 % and P ≤ 1 %, respectively.

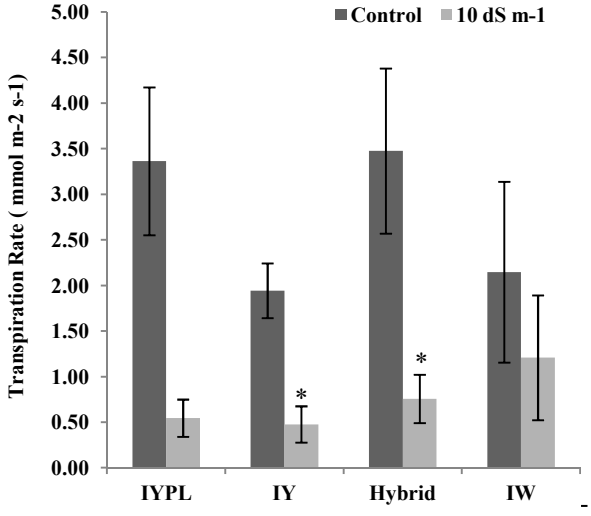

Figure 5. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on the transpiration rate in four maize genotypes. Each data point is the average of four replicates±SEM. * denotes significantly different compared with control at P ≤ 5 %.

The relative decrease in transpiration rate over control was 83.80, 75.47 and 78.20% in Indigenous yellow pure line, Indigenous yellow and Hybrid, respectively. But Indigenous white decreased lower amount (43.75%) than the previous three genotypes which mean it performed better under salt stress.

The effects of NaCl on Stomatal Conductance of four maize genotypes are shown in Figure **6.** Salt stress (100 mM NaCl) significantly ($p<0.05$) decreased Stomatal Conductance in IYPL and IY compared to control. The relative decrease in Stomatal Conductance under salt treated genotypes compared to control was 88.64, 80.85, 66.67 and 43.59% respectively.

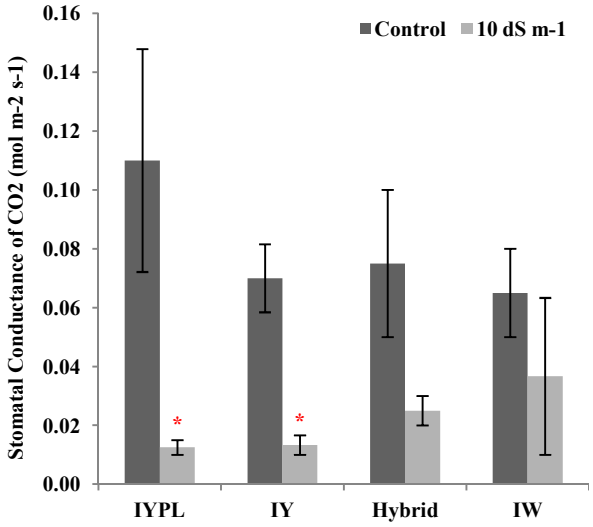

Figure 6. Effect of salinity in first phase of salt stress (12 dS m^{-1}) on the stomatal conductance in four maize genotypes. Each data point is the average of four replicates±SEM.* indicates significantly different compared with control at $P \leq 5$ %.

Higher amount of decrease of Stomatal Conductance was observed in Indigenous yellow pure line, Indigenous yellow and Hybrid. But the same of Indigenous white decreased less than the other three genotypes. This indicates that indigenous white performs relatively better under salt stress condition. Our result is matched with the result of (Niu *et al.*, 2012). He found negative relationship between gas exchange rate and salt stress. During a salt stress, the plant closes its stomata due to water loss (Chatrath*et al.,* 2000). In general, measurement of stomatal resistance provides effectual comparison for determining the degree of stress in plants. Salinity increased the stomatal resistance, which could be explained by inhibition of plant growth due to water stress (Chatrath *et al.,* 2000). There was found a strong negative correlation between stomatal resistance and NaCl stress (Turan *et al.,* 2007). Stomatal factors have also a more significant effect on photosynthesis (Wang et al., 1987, Uddin *et al.,* 2016). Possible mechanism in which photosynthesis is reduced under salt stress is not so clear. Salt stress disturbs the balance between production of reactive oxygen species (ROS) and antioxidant defense causing accumulation of reactive oxygen species, which induces oxidative stress (Uddin *et al.,* 2016, Uddin *et al.,* 2013). Increase in tissue Na^+ causes ion toxicity, which decreases the leaf growth, and results in early leaf abscission, which reduces the carboxylation. Salt stress limits photosynthesis due to a decline in activities of Rubisco, phosphoenolpyruvate carboxylase (PEPCase), and NADP-malic enzyme (NADP-ME). Moreover, noncyclic electron transport is down regulated to match the reduced requirements of reduced (nicotinamide adenine dinucleotide phosphate) production and thus reduces adenosine triphosphate synthesis (Farooq *et. al*., 2015).

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