

Full Length Research Paper

Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant

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In this study, effect of applied NaCl on growth and stomatal resistance, proline and total chlorophyll concentrations of maize plant (*Zea mays* L. cv: RX 947) was investigated. The experiment was arranged in a completely randomized design with four replications under the greenhouse condition. The experimental soil was salinized with NaCl at the rates of 0 and 100 mM NaCl. Growth of the maize plants was inhibited by salinity. Applied NaCl significantly decreased dry mass of maize plants. Stomatal resistance and proline concentrations were increased by high salinity, while total chlorophyll concentration was decreased. NaCl caused to increase Na and Cl concentrations of plant.

Key words: *Zea mays*, salt stress, stomatal resistance, proline, sodium chloride.

INTRODUCTION

Salinity, due to over-accumulation of NaCl, is usually of great concern and the most injurious factor in arid and semi arid regions. Saline soils are widespread on Earth. More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area (FAO, 2008). Their genesis may be natural or accelerated by the extension of irrigated agriculture, the intensive use of water combined with high evaporation rates and human activity (Lambers, 2003).

Despite the essentiality of chloride as a micronutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some C₄ species, salt accumulation may convert agricultural areas in unfavorable environments, reduce local biodiversity, limit growth and reproduction of plants, and may lead to toxicity in non-salt-tolerant plants, known as glycophytes. Most of the cultivated plants are sensitive to salt-stress, in which NaCl-salinity causes reduction in carbohydrates that are needed for cell growth. Carbohydrates are supplied mainly through the process of photosynthesis and

photosynthesis rates are usually lower in plants exposed to salinity and especially to NaCl (Ashraf and Harris, 2004; Parida and Das, 2005), and this would furthermore lead to restriction in water availability and imbalance in nutrient uptake by plants (Pessarakli and Tucker, 1988; Katerji et al., 2004; Arzani, 2008) with inhibition in seed germination due to ionic disturbance, osmotic and toxic effects.

Plants have improved complex mechanisms systems for adaptation to osmotic and ionic stress caused by high salinity, under the salt stress. The adaptation is generally associated with osmoregulation adjustment by using some osmotic regulators, such as potassium, soluble sugar, proline and betaine (Munns, 2005; Hong-Bo et al., 2006). One of the mechanisms is proline accumulation into cell. The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Kavi et al., 2005; Ashraf and Foolad, 2007). However, the significance of proline accumulation in osmotic adjustment is still debated and varies according to the species (Lutts et al., 1996; Rodriguez et al., 1997).

The present study investigated the response of maize plant to a high NaCl treatment (e.g., 100 mM) at dry matter, stomatal resistance, proline, total chlorophyll and

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Table 1. Effect of NaCl on the dry weights (g pot⁻¹) of the maize plants.

NaCl, mM	Dry weight	Range, (%)
0	25.36 ± 2.15 a	-
100	13.83 ± 0.98 b	45.46
Treatments	***	

*** Significant at $P < 0.001$ level. Means followed by the same letter in column are not significantly different (Duncan's Multiple Range test, $P < 0.01$).

concentrations of Na, Cl and K.

MATERIALS AND METHODS

Soil material

The experimental soil taken from Aridisol great soil group was non-calcareous (0.58 % CaCO₃), clay in texture, slightly alkaline (pH: 7.42 and EC: 0.148 dS m⁻¹; both in 1:2.5 water extract). The soil sample had 82.9 mg kg⁻¹ exchangeable Na and water extractable Cl was 9.37 mg kg⁻¹.

Pot experiment

The experiment was conducted under greenhouse conditions (humidity 65 - 75%, air temperature 25 - 30°C and neutral light intensity 340 - 450 μmol m⁻² s⁻¹). The soil (3000 g) was placed into pots and was salinised with NaCl at the rates of 0 and 100 mM NaCl. For basal fertilizers, 100 mg N kg⁻¹ as ammonium nitrate and 80 mg P kg⁻¹ as triple super phosphate were applied to the pots. Five maize (*Zea mays* L. cv: RX947) seeds were sown into each pot which were thinned to three after emergence. Six weeks after germination, the vegetative growth was harvested.

Chemical analysis

After weighting and washing the fresh shoot material, plants were sampled taking 5.0 g of fresh plant material for total chlorophyll and total proline determinations. The remaining plant samples were dried at 65°C and were digested with HNO₃:HClO₄ acid mixture (4:1) in order to determine dry matter and the concentrations of Na.

Stomatal resistance was determined during the span of time (14h00 and 15h00) before harvest by use of a "steady-state" porometer (EA 540 - 026 AP4 model) attached to the abaxial side of leaves. The readings were taken on six fully-expanded leaves, situated at the different position of the canopy. Proline was determined by the ninhydrin method described by Bates et al. (1973). In this method, proline was extracted from 0.5 g of fresh leaf tissue into 10 ml of 3% sulfosalicylic acid and filtered through Whatman No. 42 filter papers and determined in Shimadzu UV-1201 model spectrophotometer. Total chlorophyll (chlorophyll a + b) was extracted in 80% (v/v) aqueous acetone and absorption measured in a Shimadzu UV-1201 model spectrophotometer at 645 and 663 nm (Arnon, 1949).

Sodium and potassium concentration were determined by using Eppendorf Elex 6361 model flame photometry described by Miller (1998). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

Statistical analysis

The pot experiment was arranged in a completely randomized design with two salt concentrations and four replicates. Analysis of variance of data for all variables was computed using MINITAB computer package (Minitab Release 10.51). MSTAT package program (Version 3.00) was used to compare treatment means by Duncan's Multiple Range test.

RESULTS

Dry weights of maize plants

The effects of salinity on the growth of maize were summarized in Table 1. Dry weight results indicated that growth was negatively correlated to the substrate concentration of NaCl ($p < 0.001$). Maize plants grown at the low levels of NaCl (0 mM) reached relatively higher dry weights and did not imply toxicity symptoms, however, the plant dry weight was significantly reduced at higher levels of salinity (100 mM) indicating the symptoms of salt toxicity as growth depression. The concentration of NaCl that significantly reduced dry weight was 100 mM by 45.46% in comparison to the control.

Stomatal resistance, proline and total chlorophyll concentrations of maize plants

Stomatal resistance, proline and total chlorophyll (chlorophyll a + b) concentration of maize plants was significantly influenced by salinity ($P < 0.001$, Table 2). While NaCl treatment decreased total chlorophyll concentration, increased stomatal resistance and proline concentration of maize plants.

Ion distribution and accumulation

The concentrations of Na and Cl ions significantly increased in parallel to amount of NaCl ($p < 0.01$, Table 3). NaCl treatments caused to decrease K concentrations and K/Na ratio of maize plants (Table 3).

DISCUSSION

Dry weights of maize plants

The results of biomass indicated that applied NaCl inhibited the growth of maize plant, and led to a decrease in biomass. This may be related to the effect of salt-stress which resulted in the limitation of water absorption and biochemical processes (Cusido et al., 1987; Parida and Das, 2005). In addition, a decline in the rates of net photosynthesis occurs, due to adverse affect on CO₂ assimilation, which leads to a decrease in nutrient uptake and finally growth of plants (Seeman and Sharkey, 1986;

Table 2. Effects of NaCl on stomatal resistance, proline and total chlorophyll (chlorophyll a + b) concentration of the maize plants.

NaCl, mM	Stomatal resistance (s cm ⁻¹)	Proline (μ mol g ⁻¹) fresh weight	Total chlorophyll (mg g ⁻¹) fresh weight
0	0.95 ± 0.02 a	0.806 ± 0.08 a	6.13 ± 1.72 a
100	3.25 ± 0.10 b	5.812 ± 1.08 b	2.43 ± 0.97 b
Treatments	***	***	***

*** Significant at P < 0.001 level. Means followed by the same letter are not significantly different (Duncan's Multiple Range test, P < 0.01).

Table 3. Effects of NaCl on sodium, chloride and potassium concentrations and K/Na ratio of the maize plants.

NaCl (mM)	Na (g kg ⁻¹)	Cl (g kg ⁻¹)	K (g kg ⁻¹)	K/Na ratio
0	0.32 ± 0.02 a	1.86 ± 0.96 a	27.17 ± 2.62 a	84.91
100	4.46 ± 1.03 b	44.16 ± 5.75 b	17.93 ± 1.35 b	4.02
Treatments	***	***	***	

** Significant at P < 0.001. Means followed by the same letter in column are not significantly different (Duncan's Multiple Range test, P < 0.001).

and finally growth of plants (Seeman and Sharkey, 1986; Cha-Um and Kirdmanee, 2009).

The suppression of plant growth under salt-stress may either be due to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect (Marschner, 1995). There are many reports on osmotic stress and ionic toxicity resulted from salt stress in maize plants (Katerji et al., 2004; Mansour et al., 2005; Eker et al., 2006).

Stomatal resistance, proline and total chlorophyll concentrations of maize plants

During a salt stress, the plant has to close their 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). It was found to be a strong negative correlation between stomatal resistance and NaCl (Turan et al., 2007a). Stomatal factors have also a more significant effect on photosynthesis (Wang et al., 1987).

One of the most important mechanisms by higher plants under salt-stress is the accumulation of compatible solutes such as proline. Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Several reports show a significant role of proline in osmotic adjustment, protecting cell structure and its function in plants in salt-tolerant and salt-sensitive cultivars of many crops (Desingh and Kanagaraj, 2007; Koca et al., 2007; Veeranagamallaiah et al., 2007; Turan et al., 2007a).

On the other hand, a positive correlation was determined

between proline and tissue-Na concentrations under salt stress (Bajji et al., 2001). The present study shows that the salt treatments induced an increase in proline concentration in maize plants. Similar result has been reported by Cha-Um and Kirdmanee (2009).

The total chlorophyll concentration of maize leaves was reduced by increased level of NaCl treatment. According to Cha-Um and Kirdmanee (2009), salinity decreased the total chlorophyll concentration of two maize varieties. The reduction in photon yield in the salt stressed seedlings of maize was positively correlated to net photosynthetic rate (P_n); in which the significant drop in P_n of salt stressed seedlings resulted in considerable growth reduction. Similar findings were reported by Petolino and Leone (1980) for *Phaseolus vulgaris*. Many scientists have suggested a positive correlation between decrease in chlorophyll content and salt-induced weakening of protein-pigment-lipid complex (Strogonov et al., 1970) or increased chlorophyllase (EC: 3.1.1.14) enzyme activity (Stivsev et al., 1973).

Ion distribution and accumulation

Increasing levels of NaCl induced a progressive absorption of Na and Cl in plant, agreeing with Chavan and Karadge (1986), Taban et al., (1999) and Turan et al. (2007a, b). Excessive Na concentration in the plant tissue hinders nutrient balance, osmotic regulation and causes toxicity (Bernstein, 1963). Accumulation of Cl in the root tissue is disruptive to membrane uptake mechanisms, and these results in increased translocation of Cl to the shoots (Yousif et al., 1972).

When NaCl was applied to the soil, the levels of K in plant were reduced in accordance with the antagonism between Na and K (Alberico and Cramer, 1993; Azevedo

and Tabosa, 2000). Cramer et al. (1985) showed that excess NaCl leads to the loss of potassium due to membrane depolarization by sodium ions.

The present study showed that soil salinity inhibited plant growth and caused a decrease in biomass of maize plant. On the other hand, while stomatal resistance and proline concentration of plant were increased by salinity, total chlorophyll concentration was decreased. This suggests the critical importance of need to unravel the cellular mechanisms such as the correlation between chlorophyll content or proline accumulation and sodium intake so as to give a meaning to salt stress studies. Despite a number of studies on salinity effects on plants, neither the metabolic sites at which salt stress damages plants nor the adaptive components of salt tolerance are fully understood. Distinguishing the cellular metabolisms appears to play an important role in the acclimation of crops to salt stress.

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