

Research Article – Plant Science

Influence of NaCl salinity on plant growth and nutrient assimilation of *Zea mays* L.

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Abstract

Maize (*Zea mays* L.) is the world's leading edible oil and third largest important cereal. In addition to it is used as food for human consumption as well as food grain for livestock. High concentrations of NaCl in soils account for large decrease in the yield of a large variety of crops all over the globe. The objective of the present study was conducted to evaluate NaCl stress on growth and mineral nutrient composition of maize plants. Maize seeds were grown in plastic pots having fine sand. After 20 days of germination, the plants were subjected to seven different concentrations (Control, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) of NaCl. Plants were analyzed on 15th day after salt treatment. Factorial experiments in a completely randomized design (CRBD) with three replications were applied. The growth parameters and mineral contents Na, Ca, K and Cl were investigated from saline treated and non saline treated plants. Results indicated that the NaCl stress markedly reduced the shoot and root length fresh and dry masses. Moreover Na⁺, Cl⁻ content increased with increase in NaCl stress, while Ca²⁺ and K⁺ were decreased significantly.

Key words: Maize, Salinity, Morphological traits, Nutrients

Introduction

Saline soils are consist of two kind of ions that is cations are Na⁺, Ca²⁺, K⁺ and anions are Cl⁻, SO⁴²⁻ and carbonates (Rengasamy, 2010; Tavakkoli *et al.*, 2011). Salinity is one of the most detrimental factors responsible for decline in plant growth and yield (Shahbaz *et al.*, 2012). It is reported that plants are affected in three ways: reduced water potential in root zone causing water insufficiency, phytotoxicity of ions such as Na⁺ and Cl⁻ and nutrient disparity depressing uptake and transport of nutrients (Munns, 2002a). The shoot and root length are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot supply it to the rest of the plant, therefore root and shoot length provides an

important indication to the response of plants to salt stress (Jamil and Rha, 2004).

High concentration of salts in the root zone reduces soil water potential and the availability of water (Lloyd *et al.*, 1989). This scarcity in available water under saline condition causes dehydration at cellular level and eventually osmotic stress occurs (Hasegawa *et al.*, 2000). Salt stress disrupts in water potential and ion allocation by inducing restrain in the uptake of nutrients like K⁺, Ca²⁺ and NO³⁻ and accumulation of Na⁺ and Cl⁻ to potentially toxic levels within cells (Krouma, 2009).

The increased amount of sodium (Na⁺) and chloride (Cl⁻) in the environment affects the uptake of many crucial nutrients through competitive interactions and by disturbing the ion selectivity of membranes. For instance, external sodium negatively impacts intracellular potassium influx, attenuating the acquisition of this indispensable nutrient by cells. Moreover, sodium chloride influence transport of ions across plasma lemma of

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root cells through split of the cellular membranes (Alleva *et al.*, 2006). Finally, the interactions of salts with mineral nutrition may result in nutrient unevenness and shortage. The consequence of all these can eventually lead to plant death as a result of growth arrest and molecular damage. To achieve salt-tolerance, the chief task is either to prevent or alleviate the damage, or to re-establish homeostatic conditions in the new stressful environment (Pardossi *et al.*, 2004).

Plants are classified as glycophytes or halophytes according to their capacity to grow on high salt medium. Most plants are glycophytes and cannot tolerate salt-stress. Salinity causes severe ion toxicity in glycophyte, since Na^+ is not readily sequestered into vacuoles as in halophytes (Khan *et al.*, 2000). Maize (*Zea mays* L.) is one of the most important crops in the world (Kresovic *et al.*, 2014). It is the third most important cereal after wheat and rice in many countries, it is proudly called as "Queen of Cereals and "king of Fodder" and miracle crop (Rajurkar *et al.*, 2011). Maize is a genus of the family Graminae (Poaceae), commonly known as the grass family. It occupies a key position as one of the most important cereals both for human and animal consumption and grown under various conditions in different parts of the globe (Dowswell *et al.*, 1996). The aim of present study was to analyze the severe effects of salt stress on some morphological and physiological attributes of maize plant.

Materials and methods

Pot culture experiment

A pot experiment was conducted in the Botanical garden of the Annamalai University, Tamil Nadu under natural environmental condition. The seeds of maize (*Zea mays* L. Va. NK6240) were procured from Rasi Seed Company, Tamilnadu, India. The seeds were soaked for five minutes separately in 5 per cent sodium hypochlorite to prevent fungal infection and they were rinsed for about five minutes in running tap water. Equal sizes of seeds were sown in plastic pots. The pots with 22cm height and 26cm wide were chosen. The pots were filled with homogenous mixture of the garden soil containing red soil, sand along with farmyard

manure in the ratio of 1:1:1. In each pot, five seeds were sown and one plant was maintained finally. The pots were arranged in Completely Randomized Block Design (CRBD) and were irrigated with tap water. After 20days, the well established plants were selected for saline treatment. The saline treatment consisted of seven different concentrations (0, 25, 50, 75, 100, 125 and 150mM) of NaCl. Fifty plants were treated with each of the NaCl concentration. A control was maintained without any exogenous addition of salt. The samplings of these studies were collected on the 15th day after the salt treatment.

Morphological attributes

Shoot and Root length (cm/plant)

Plant height was recorded by measuring the height of the plant from the surface of the soil to the tip of the top most leaf. The root length was measured from the point of first cotyledonary node to the tip of longest root and expressed in cm plant⁻¹. This was recorded on 15th day after treatment.

Fresh and Dry weight

For the estimation of fresh weight, leaf, shoot and root portions were separated and weighed. They were dried in a hot air oven at 80°C for 24 hours. Then, the dry weight was taken by using an electronic balance.

Physiological attributes

Estimation of Sodium and Potassium

Sodium and Potassium determined by (Williams and Twine, 1960). Dried and ground tissues of 0.5g were digested in 100ml Kjeldahl flasks using 10ml of concentrated nitric acid, 0.5ml of 60 per cent perchloric acid and 0.5ml of concentrated sulphuric acid. Digestion was continued until the nitric acid and perchloric acid were driven off. The inorganic residue was cooled and diluted with 15ml of distilled water and filtered through Whatmann No.42 filter paper. The filtrate was made up to 50ml with distilled water. The filtrate were used for sodium and potassium estimation and a Flame Photometer (Systronics, India) was used for the purpose and standards were prepared with sodium chloride.

Calcium

The calcium content was estimated by the method of (Yoshida *et al.*, 1972). Two ml of the filtrate was mixed with 2ml of 5 per cent lanthanum oxide solution (58.65g LaO₃ dissolved in 100ml of distilled water and to that, 250ml of 6N Hydrochloric acid was added and diluted to 1000ml) and diluted with 10ml of 1N hydrochloric acid. The solution was fed into an Atomic Absorption Spectrophotometer (Perkin Elmer – 2280) at 211.9nm. Standard curve was prepared by using calcium chloride.

Estimation of Chloride

Chloride determined by (Krishnamurthy and Bhagwat, 1990). Five hundred mg of finely-ground dried plant material was extracted twice with 10ml boiled deionized water and the supernatants were collected by decanting. The residue was then extracted with 10ml of 25% nitric acid (v/v) for 10-15 minutes at 80°C. The suspension was cooled and centrifuged at 5000rpm for 15 minutes and the residue was washed twice with 10ml of 25% nitric acid and recentrifuged. The washings and nitric acid solutions were combined and made up to a total volume of 100ml with deionized water.

The aliquot of standard potassium chloride and 1ml of acid digested plant sample extract containing 5 to 150 μ moles chloride was taken in separate beakers and the final volume was made to 100ml. Five drops of diphenylcarbazone – bromophenol blue mixed indicators (prepared by dissolving 0.5g of crystalline diphenyl-carbozone and 0.05g of bromophenol blue in 75ml of 95% ethyl alcohol and diluted to 100ml with deionized water stored in brown bottle for maximum stability) were added and the sample was stirred. Thus, a blue-violet colour was developed and further, 0.5N nitric acid was added drop wise until it changed to yellow. Sometimes, if a deep orange colour was formed immediately on the addition of the indicator, the blue-violet colour was developed by adding 2N sodium hydroxide solution drop wise, followed by the acidification until the yellow colour developed. To the yellow acidified solution, 0.025N mercuric nitrate solution was titrated until a blue-violet colour persisted throughout the solution. The volume of

mercuric nitrate used up in the titration was recorded. The chloride present in unknown samples was calculated by comparing standard potassium chloride by the following equation: 1ml of 0.025 N mercuric nitrate titrated = 14.7 μ moles of chloride present in standard; chloride (m mole) /g dry weight of plant tissue = 1ml of 0.025N mercuric nitrate titrated X 2.94.

Statistical Analysis

Mean values were calculated for all the readings. Two-way analysis of variance (without replication) was calculated to know whether the mean values were significantly different or not. For percentage, the original values were transformed into arcsine values for the calculation of the ANOVA (Sokal and Rohlf, 1973).

Results

It is clear from the obtained data that applying NaCl levels resulted in a significant reduction in shoot and root length as well as fresh and dry weight of the shoot and root compared to the respective control plants.

Salinity treatment generally increased the sodium and chloride content in the three tissues. There was considerable increase in Na⁺ and Cl⁻ content with increasing concentration of NaCl salinity up to 150mM of NaCl. Of the three tissues the leaf accumulated more Na⁺ and Cl⁻ than the shoot and root. The K⁺ and Ca²⁺ content of all the three tissues were significantly decreased with rise of salinity levels from 25 to 150mM of NaCl.

DISCUSSION

Effect of NaCl on growth

Salinity stress is known to be one of the major abiotic stresses that limit plant growth and productivity (Sabir *et al.*, 2011). Salt-induced reduction in different growth attributes has been reported in a number of crop plants, for example proso millet (Sabir *et al.*, 2011) and sunflower (Akram and Ashraf, 2011). There are a number of believable explanations for this reduction in growth and productivity such as disturbed osmotic relations, nutritional and hormonal imbalance and oxidative stress (Hu *et al.*, 2012).

Fig. 1. Effect of NaCl on shoot and root length (cm plant⁻¹) of *Zea mays* on 15th day after salt treatment

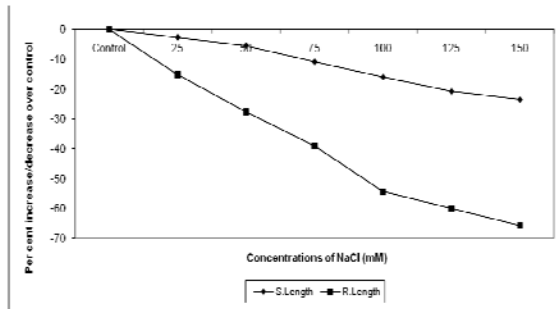


Fig. 2. Effect of NaCl on fresh weight of the shoot and root (g plant⁻¹) of *Zea mays* on 15th day after salt treatment

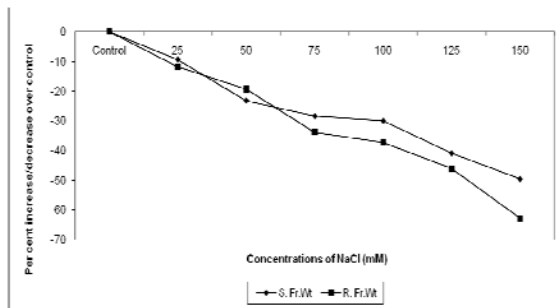


Fig. 3. Effect of NaCl on dry weight of the shoot and root (g plant⁻¹) of *Zea mays* on 15th day after salt treatment

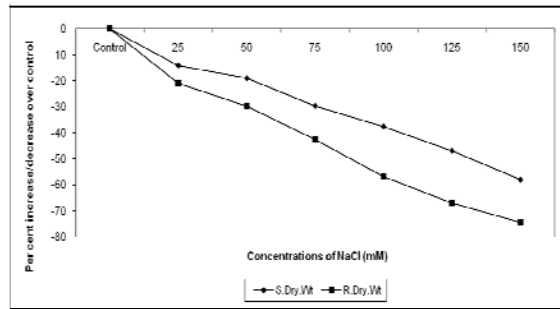


Fig. 4. Effect of NaCl on sodium content (mg/g dry wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment

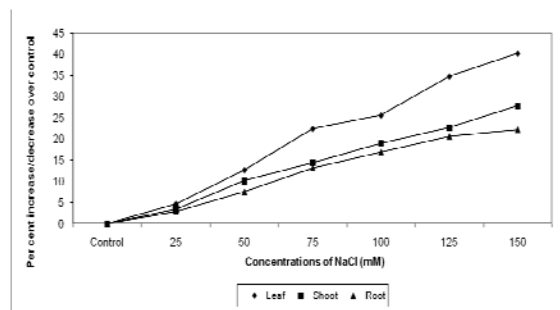


Fig. 5. Effect of NaCl on potassium content (mg/g dry wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment

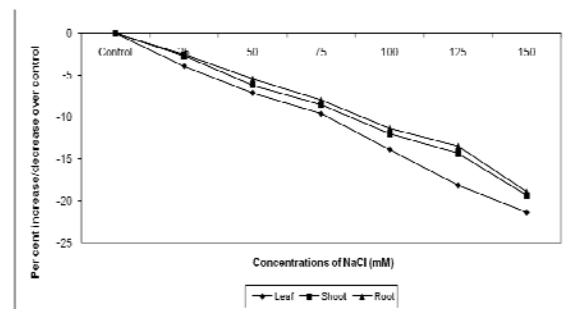


Fig. 6. Effect of NaCl on calcium content (mg/g dry wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment

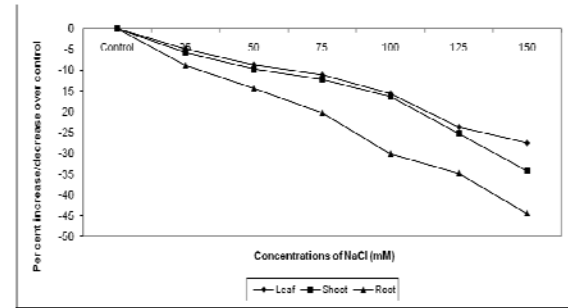
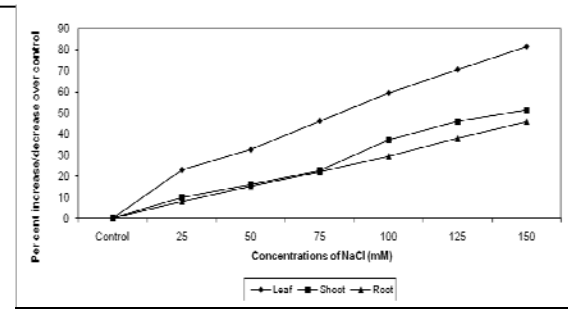


Fig. 7. Effect of NaCl on chloride content (mg/g dry wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment



Plants grown under increased salt concentration are not able to take up water and minerals like K⁺ and Ca²⁺ that finally leads to a reduction in growth (Parida and Das, 2005) and inhibition in cell elongation and cell division controlled by different auxins and its synthesis is controlled by salinity.

Shoot and root length

From the data of the present investigation, due to salinity the shoot and root length decreased (Figure1, 2) and this is because of reduced water content due to declined water

adsorption caused as a result of osmotic stress and drought stress induced. Under salt stress the suppression of shoot and root growth may either be due to osmotic reduction in water availability or to extreme accumulation of ions, known as specific ion effect (Marschner, 1995). Our findings are in line with the results of (Katerji *et al.*, 2004 and Mansour *et al.*, 2005).

Fresh Weight

Giaveno *et al.* (2007) reported that salt treatments affected root and shoot fresh weight. Tantawy *et al.* (2009) also noticed a decrease in fresh weight of tomato plant.

Dry Weight

Hasna *et al.* (2011) reported that reduction in biomass and growth can be due to higher Na^+ concentration in the shoots of *Arabidopsis thaliana* and ionic toxicity and decreases osmotic potential. Decrease of root dry weight is due to ion toxicity, imbalance of nutritional elements and disorder of osmotic regulation. Increased salinity reduced dry weight, indicating the loss of carbon gain, presumably from a shift in growth to combating the salt conditions (Netondo *et al.*, 2004).

Gulzar *et al.* (2003) also reported a significant effect of salinity on dry weight of roots and shoots of *Urochondra setulosa*. Decrease in dry weight of root and shoot was noticed by Khan *et al.* (2009) in three range grasses viz. *Panicum antidotale*, *Cenchrus ciliaris* and *Dichanthium annulatum* with the increasing levels of salinity.

Sodium chloride induced effect on sodium ions

In this study, Increase in Na^+ content of plants grown under salinity regimes has been reported by Tuncturk *et al.* (2011) and Babu *et al.* (2012). Horie *et al.* (2012) reported that the salt-tolerant cultivars avoid the inhibitory effects of toxic Na due to active exclusion at the plasma membrane or sequestration into the vacuoles. Moreover, ion accumulation ability of plants contributes towards their salinity tolerance. Salt tolerant plants accumulate lower levels of toxic Na as compared to that in salt-sensitive plants. Salt-induced increase in Na Contents has been reported in a number of crops, for instance, canola (Tuncturk *et al.*, 2011) and wheat (Tammam *et al.*, 2008).

Sodium chloride induced effect on calcium ions

Increasing concentrations of NaCl cause gradually greater dislocation of Ca^{2+} from cell membrane and disturb cellular calcium concentration (Cabanero *et al.*, 2006). Others reported a decrease in Ca concentrations due to NaCl stress in cotton (Meloni *et al.*, 2001). Ca^{2+} shown to play an important role in the salt tolerance of plants by maintaining the structural integrity and functions of membranes and cell walls (Marschner, 1995). Recent studies showed that increase Ca concentration in gourd and melon plants challenged with salinity stress could ameliorate the inhibitory effects of salinity stress on plant growth (Navarro *et al.*, 2003; Kaya *et al.*, 2003; Yetisir and Uygur, 2009).

Sodium chloride induced effect on potassium ions

The reduced intake of K^+ ions hamper protein synthesis as it plays a major role in binding tRNA to ribosomes (Blaha *et al.*, 2000). Flowers *et al.* (1991) reported that Na^+ transport from root to shoot is unidirectional and the resultant build up of Na^+ in leaves causes osmotic damage. Potassium content was found to be decreasing with increase in salt stress. These outcomes suggest that there was a competition between Na^+ and K^+ regarding their uptake. Similar findings were reported with green bean (Yasar *et al.*, 2006) and canola (Bandeh-Hagh *et al.*, 2008). Salinity-induced decrease in the accumulation of K^+ was recorded in barley (Tavakkoli *et al.*, 2011).

Sodium chloride induced effect on chloride ions

Chloride is an essential micronutrient, plays important roles in regulating the enzyme activities in cytoplasm, acts as a cofactor in photosynthesis, is involved in turgor and pH regulation (White and Broadley, 2001) and exerts toxic effects on plants at high concentrations (Moya *et al.*, 2003). High Cl^- tissue concentrations have been assumed responsible to cause an ion-specific toxicity in salt-treated plants, principally in those species with a capacity to exclude Na^+ from their shoots (Marschner, 1995). High Cl^- concentrations have been reported to cause membrane damage or enzyme inhibition negatively affecting photosynthetic processes (Seeman and Critchley,

1985). Salinity was found to increase Cl-accumulation in rice (Wang *et al.*, 2012).

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