

Conference Paper

Salinity Stress Alters Nutrient Uptake and Causes the Damage of Root and Leaf Anatomy in Maize

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Abstract

Salinity is one of major problems in agriculture especially in arid and semiarid area due to causes the damage of many aspects in plant growth and development. This study observed root and leaf anatomy and nutrient uptake in maize plants exposed to salinity stress. Maize seedlings were placed in the plantation room under same temperature, humidity and light intensity conditions and were treated with 0%, 1%, 2% and 3% NaCl for 5 d. Anatomy of root and leaf were observed using scanning electron microscopy (SEM). Nutrient uptake was estimated by the content of trace elements of leaves. Trace element were quantified using inductively coupled plasma-mass spectrometry (ICP-MS), but chlorine was determined by an atomic absorption flame spectrometer. The results showed that salinity slightly damaged roots anatomy. Epidermis cells and parenchyma cells of cortex and pith were shrinkage in 2% and 3% NaCl-treated plants. Leaf anatomy showed mesophyll and bundle sheath cells which slightly suppressed. Meanwhile, chloroplasts content inside those cells were dramatically decreased. Anatomical damage of roots and leaves was accompanied by altering uptake of some trace elements. The contents of aluminum, calcium, iron, magnesium, sodium, chlorine, in NaCl-treated plants were higher than control. Otherwise, boron, potassium and phosphor were lower in NaCl-treated plants. The rest of trace elements were in comparable concentration.

Keywords: maize; leaf; nutrient; root; salinity.

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1. Introduction

Here area of irrigated land in the world is only 230×10^6 ha (15%) of $1\,730 \times 10^6$ ha cultivated land. However, irrigated land play major role economically due to it supply one-third of world's food demand and its productivity is twice even more to rain fed lands productivity. Unfortunately, salinity has affected about 20% of irrigated land especially in arid and semi-arid regions [1, 2].

Salinity stress is caused by excessive uptake of toxic ions. Most commonly, ionic composition of saline soil is NaCl [3]. Although the mechanisms of salt stress are still complicated, it is considered that excessive accumulation of salt ions, mainly Na^+ and Cl^- in the plant tissues is a major contributory factor to the damage caused by

salinity [4, 5]. Salinity causes serious problems in cell metabolisms, because a high concentration of sodium is bad for the cells.

High salt content in the plant tissues can alter physiological and biochemical processes of the plants through two mechanisms: osmotic and ionic stresses [2, 6]. Osmotic stress occurs due to Na^+ and Cl^- uptake, which leads to a deficit of water in the plant tissues. Ion accumulation decreases water gradient between root and soil solution, making it more difficult for water to move through the root surface [7]. In turn, when water uptake decreases, and the osmotic effect spreads from the root surface to the internal tissues, the ion accumulation inside the plant alters the solute balances [8, 9]. Ionic stress due to high concentrations of toxic ions such as Na^+ and Cl^- reduces uptake of other mineral nutrients such as Ca and K, which causes metabolic disturbances [3, 6, 10].

Some studies about the effects of salinity on important metabolic processes in the chloroplasts have been well documented [11–14]. Ultrastructural studies on the chloroplast alteration in the plants exposed to salinity have also been recorded in some C3 plants such as rice and sweet potato [15–17]. Previously, we reported that salinity affected severe damage of chloroplast in some plants such as rice [18, 19]. In this study we examined the uptake of essential nutrient is needed by plant to growth and develop. We also observed anatomical structure of root and leaf on maize plants exposed to salinity stress.

2. Material and Method

2.1. Plant material

Three seeds were cultivated in a pot grown in the experimental room at 12-h photoperiod, temperature of (30/25)°C (light/dark), humidity of 70%, and light intensity of $600 \mu\text{m m}^{-2} \text{s}^{-1}$. Salinity treatment was started after the second leaf blades of the plants were fully developed by supplying 50 mL of 0 (control), 1%, 2% and 3% NaCl solutions every day at 9:00 a.m. Each treatment was replicated five times in which a pot was considered as one replicate. After salt treatments for 5 d, the plants were taken to the laboratory and the measurements of nutrients content and anatomy of leaf were prepared.

2.2. Anatomy of maize root and leaf

Anatomy of maize root and leaf were observed using scanning electron microscopy (SEM). The second leaf blades of control and 3% NaCl-treated plants were cut and immediately frozen in liquid nitrogen subsequently transferred to a freezing device (OKA Science Co.) overnight. The temperature was started from about of -75°C and gradually increased until the temperature of 25°C (room temperature) was reached. Then, the freeze-dried samples were taken from the freezing device and sliced free hand transversely with a razor blade. The sections were mounted on a stub and coated

with gold in a vacuum sputter coater. The coated specimens were analyzed in a Hitachi-4500 scanning electron microscopy.

2.3. Nutrient contents

The Nutrient uptake was estimated by the content of trace elements of leaves. A 0.1 g sample from second leaf blades of each replicated experiment plants were prepared. Trace element were quantified using inductively coupled plasma-mass spectrometry (ICP-MS). The data were expressed on a dry weight basis (mg g^{-1} dw).

2.4. Cl content

Leaf segments were cut from the middle portion of the second leaf blade. The segments were dried at 70°C for 48 h and weighed. Na and K were extracted with purified distilled water (Millipore SA 67120, Molshem, France) by shaking at room temperature for 70 h. Samples were centrifuged for 10 min. Cl content were estimated with an atomic absorption flame spectrometer (Shimadzu AA 6400F, Shimadzu Co. Ltd.). The data were expressed on a dry weight basis.

3. Results and Discussions

The results showed that salinity affected the damage roots anatomy (see Figure 1). Epidermis cells and parenchyma cells of cortex and pith were shrinkage in 2% and 3% NaCl-treated plants. Higher magnificent of SEM observation (Figure 1b, d) reveal the cells of root epidermal were suppressed and flattered in form. Meanwhile on the control plant, it cells were more arranged surrounding cortex and rounded shape. The cortical cell of control plant were arranged with relatively same in size. Those cells in salinity treated root plant were shrinkage caused the size of cells became uninform throughout the cortex area. There was not differences on the cells of vascular bundle between control and salinity treated plants root. Parenchyma cell of pith, the inner part of root show similar condition to those of cortex parenchyma.

Figure 2 shows SEM observation of leaf anatomy of control and salinity treated plants. Epidermal and mesophyll cell of the leaf clearly suppressed, but bundle sheath cells were not suppressed. The chloroplasts inside both mesophyll and bundle sheath cells of salinity treated plants were clearly reduced either in quantity or size. It shows that salinity caused the damage leaf anatomy especially the distortion to cells of leaf which have thin cell wall as epidermis and mesophyll cells. The damage of chloroplasts ultrastructure caused by salinity have been reported in rice [16] and maize [19]. In this study revealed that chloroplasts were reduced both in number and size.

The damage of root and leaf of the plants were exposed to salinity was caused by either osmotic effects or ionic toxicity due to Na accumulation in the plant tissues [3]. High salt concentration in soils lowers soil water potential. As a result, plants can no longer take up water. Ion toxicity due to excessive sodium ion causing the decrease of

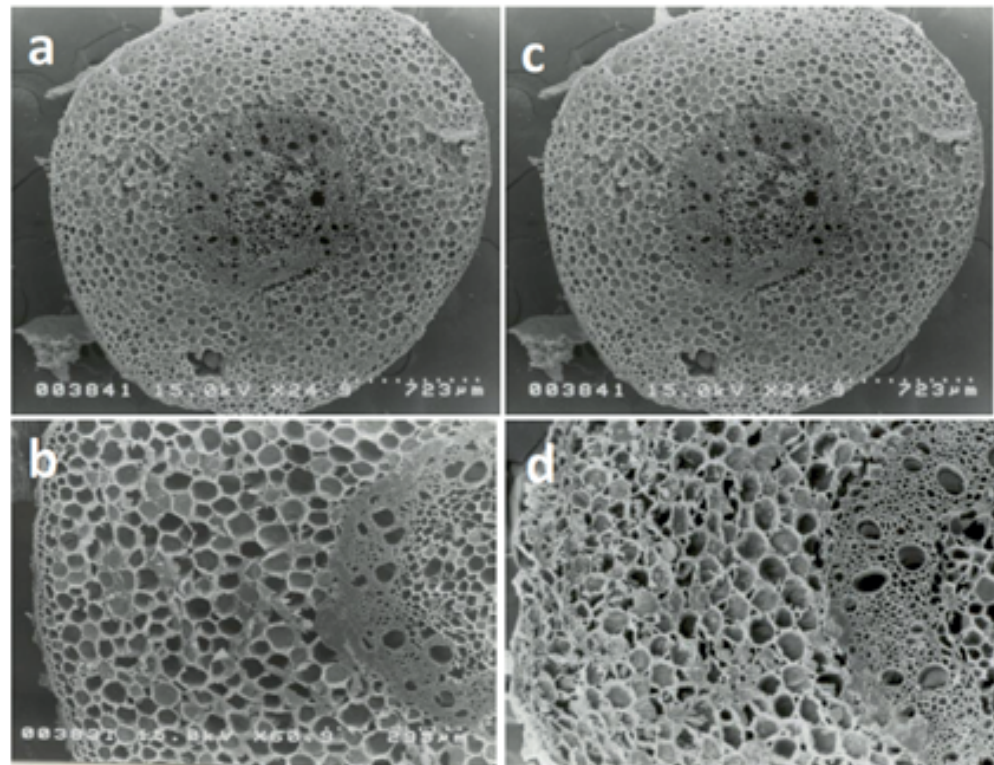


Figure 1: Root anatomy of control (a, b) and 3% NaCl-treated plants (c, d). Closer image showed the epidermis and cortical cells of salinity treated plants root were irregular and shrinkage.

ion acquisition, displaces calcium ion from the plasma membrane of root hairs, leading to a membrane leakage and inhibits many important enzymes [3] and causes nutrient imbalance in the tissues [9].

Anatomical damage of roots and leaves was accompanied by altering uptake of some trace elements (see Figure 3). The contents of aluminum, calcium, iron, magnesium, sodium, chlorine, in NaCl-treated plants were higher than control. Otherwise, boron, potassium and phosphorus were lower in NaCl-treated plants. The rest of trace elements were in comparable concentration. The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts. These concentrations fluctuate because of changes in water source, drainage, evapotranspiration, and solute availability [20]. Salt injury is due to Na accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize these ions in the vacuole. Ions then build up rapidly in the cytoplasm and inhibit enzyme activity, or they build up in the cell walls and dehydrate the cell [7, 21].

4. Conclusion

Salinity stress damaged the cells leaf and root which has thin cell wall such as epidermis and mesophyll cells of the leaf and epidermis and parenchyma cells of cortex and pith of the root. Chloroplasts content inside the leaf cells were dramatically decreased in number. Anatomical damage of roots and leaves was accompanied by

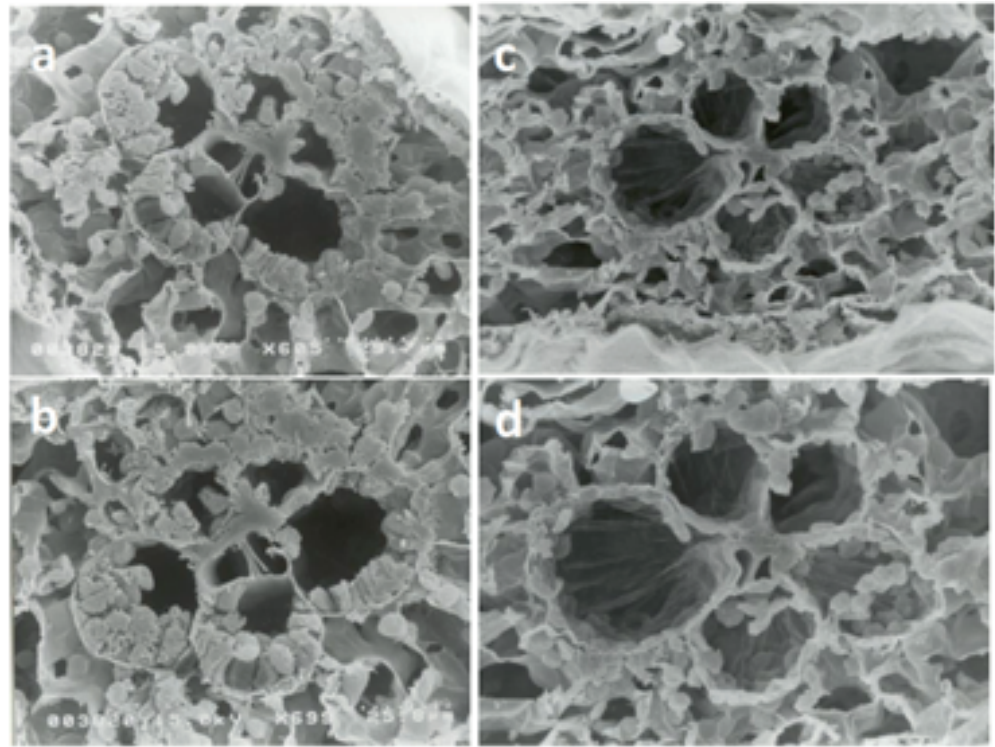


Figure 2: Cross section of leaf blades control (a, b) and 3% NaCl-treated plants (c,d). Cells of salinity treated plants were suppressed with less chloroplasts content inside compared to those of control.

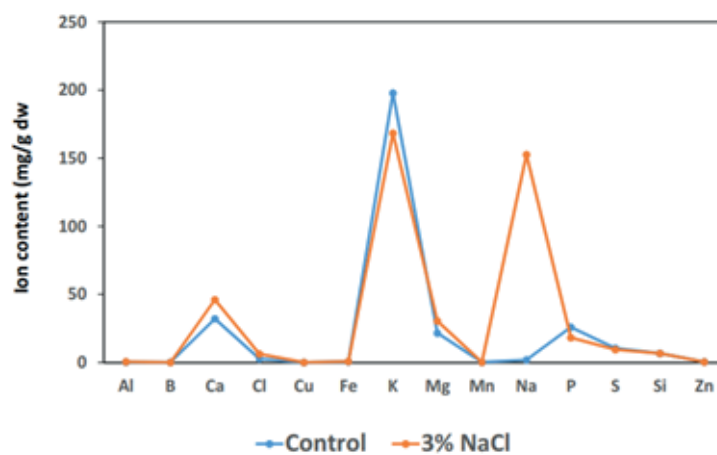


Figure 3: The content of elements in the leaf blade control and 3% NaCl-treated plants.

altering uptake of some trace elements due to ion toxicity caused by excessive sodium ion in tissue.

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References

- [1] FAO., Global network on integrated soil management for sustainable use of salt-affected soils. Land and plant nutrition management service; 2000. Accessed in 5 April 2014. Available on: <http://www.fao.org/ag/agl/agll/spush>.
- [2] R. Munns, "Genes and salt tolerance: Bringing them together," *New Phytologist*, vol. 167, no. 3, pp. 645–663, 2005.
- [3] R. Munns, "Comparative physiology of salt and water stress," *Plant, Cell and Environment*, vol. 25, no. 2, pp. 239–250, 2002.
- [4] M. A. Hajibagheri and T. J. Flowers, "X-ray microanalysis of ion distribution within root cortical cells of the halophyte Suaeda maritima (L.) Dum.," *Planta*, vol. 177, no. 1, pp. 131–134, 1989.
- [5] T. J. Flowers and M. A. Hajibagheri, "Salinity tolerance in *Hordeum vulgare*: Ion concentrations in root cells of cultivars differing in salt tolerance," *Plant and Soil*, vol. 231, no. 1, pp. 1–9, 2001.
- [6] P. M. Hasegawa, R. A. Bressan, J.-K. Zhu, and H. J. Bohnert, "Plant cellular and molecular responses to high salinity," *Annual Review of Plant Biology*, vol. 51, pp. 463–499, 2000.
- [7] T. J. Flowers and A. R. Yeo, "Ion relations of plants under drought and salinity," *Australian Journal of Plant Physiology*, vol. 13, no. 1, pp. 75–91, 1986.
- [8] H. Greenway and R. Munns, "Mechanisms of Salt Tolerance in Nonhalophytes," *Annual Review of Plant Physiology*, vol. 31, no. 1, pp. 149–190, 1980.
- [9] R. Munns and A. Termaat, "Whole-plant responses to salinity," *Australian Journal of Plant Physiology*, vol. 13, no. 1, pp. 143–160, 1986.
- [10] X. Niu, R. A. Bressan, P. M. Hasegawa, and J. M. Pardo, "Ion homeostasis in NaCl stress environments," *Plant Physiology*, vol. 109, no. 3, pp. 735–742, 1995.
- [11] J. A. Hernández, E. Olmos, F. J. Corpas, F. Sevilla, and L. A. del Río, "Salt-induced oxidative stress in chloroplasts of pea plants," *Plant Science*, vol. 105, no. 2, pp. 151–167, 1995.
- [12] D. A. Meloni, M. A. Oliva, C. A. Martinez, and J. Cambraia, "Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress," *Environmental and Experimental Botany*, vol. 49, no. 1, pp. 69–76, 2003.
- [13] R. K. Sairam and G. C. Srivastava, "Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress," *Plant Science*, vol. 162, no. 6, pp. 897–904, 2002.
- [14] J. M. Gómez, A. Jiménez, E. Olmos, and F. Sevilla, "Location and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) chloroplasts," *Journal of Experimental Botany*, vol. 55, no. 394, pp. 119–130, 2004.
- [15] S. Rahman, T. Matsumuro, H. Miyake, and Y. Takeoka, "Salinity-induced ultrastructural alterations in leaf cells of rice (*Oryza sativa* L.)," *Plant Production Science*, vol. 3, no. 4, pp. 422–429, 2000.

- [16] S. Mitsuya, M. Kawasaki, M. Taniguchi, and H. Miyake, "Light dependency of salinity-induced chloroplast degradation," *Plant Production Science*, vol. 6, no. 3, pp. 219–223, 2003.
- [17] K. Yamane, M. Kawasaki, M. Taniguchi, and H. Miyake, "Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings," *Journal of Plant Physiology*, vol. 160, no. 5, pp. 573–575, 2003.
- [18] R. Hasan, Y. Ohnuki, M. Kawasaki, M. Taniguchi, and H. Miyake, "Differential sensitivity of chloroplasts in mesophyll and bundle sheath cells in maize, an NADP-malic enzyme-type C₄ plant, to salinity stress," *Plant Production Science*, vol. 8, no. 5, pp. 567–577, 2005.
- [19] R. Hasan, M. Kawasaki, M. Taniguchi, and H. Miyake, "Salinity stress induces granal development in bundle sheath chloroplasts of maize, an NADP-malic enzyme-type C₄ plant," *Plant Production Science*, vol. 9, no. 3, pp. 256–265, 2006.
- [20] K. M. Volkmar, Y. Hu, and H. Steppuhn, "Physiological responses of plants to salinity: A review," *Canadian Journal of Plant Science*, vol. 78, no. 1, pp. 19–27, 1998.
- [21] T. J. Flowers and A. R. Yeo, "Ion relations of plant under drought and salinity," *Aust. J. Plant Physiol.*, vol. 13, pp. 75–91, 1986.