The Damage of Root, Leaf and Chloroplast Ultrastructure on Maize Seedlings Caused by Salinity Stress

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Abstract. Salinity is one of the major problems in agriculture especially in arid and semiarid area that causes the damage of many aspects in plant growth and development. This study observed root, leaf and chloroplast ultrastructure as well as nutrient uptake in maize plants exposed to salinity stress. Maize seedlings were treated with 0, 1, 2 and 3% NaCl for 5 days and placed in the growth chamber. Root, leaf, and chloroplast ultrastructure were observed by using SEM and TEM. Nutrient uptake was estimated from the content of trace elements of leaves that quantified by using ICP-MS, except chlorine by an atomic absorption flame spectrometer. The results showed that salinity slightly damaged roots anatomy. Epidermis and parenchyma cells of cortex and pith were shrinkage in 2 and 3% NaCl-treated plants. Leaf anatomy observation showed mesophyll and bundle sheath cells slightly suppressed. Chloroplasts content inside those cells were dramatically decreased. Ultrastructure observation showed the damage of chloroplasts inside mesophyll cells. Anatomical and ultrastructural damage of roots, leaves, and chloroplasts 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.

Keywords: chloroplast, leaf, maize, root, salinity stress

1. Introduction
As well as water deficit, salinity is a major environmental stress especially in arid and semi-arid regions that possess high salt content and low annual rainfall to leach salt excessive. Salinity severely restricts crop productivity and consequently has a negative effect on food availability [1,2]. It affected over 6% of the total land area around the globe, 19.5% of irrigated land and 2.1% dryland agriculture. Asia has the highest percentage agriculture land area that affected by salinity. In some countries salinity affected as high of 50% of the irrigated land. Salinity increasingly becomes a major threat in agriculture due to poor soil quality and mismanagement of irrigated that inadequate drainage facilities and land clearing for agricultural development and grazing [1,3-4].

Salinity stress affects negatively on plant growth and development as well as on agricultural yield throughout the world either for subsistence or economic gain. In response to salinity, the plant drives numerous processes that must function in coordination to alleviate both cellular hyperosmolarity and...
ion disequilibrium [5]. As the onset of salt stress, the earliest visible response of plant is usually a reduction and followed by a cessation of leaf blade expansion and early senescence of leaf as salinity intensified [6]. Several major processes such as photosynthesis, protein synthesis, and lipid metabolism may be impaired [6,7]. Growth inhibition in salinity stress has been linked to both water deficit and ion toxicity caused by excessive of salt uptake into plant cell that leads to mineral deficiency [8]. Furthermore, the lower photosynthetic rate in plants exposed to salinity stress might contribute to growth inhibition although still unclear whether as the direct or indirect effect of salt stress. In regard of stomatal conductance, photosynthesis decrease as a result of the stomatal closure. Salt stress provoke stomatal closure by limiting water uptake by plant root [5]. Salt accumulation in plant leaves leads to the damage of photosynthetic apparatus such as chlorophyll [9] and enzymes in photosystem [10].

A morphological feature of the plant caused by salinity stress is an expression of various physiological interaction as a response to salinity stress. Numerous studies showed the negative effect of salinity on plant growth, leaf area, length and number as the increase of salt concentration. The fresh and dry weight of root were also affected either negatively or positively by salinity in different concentration and plant type. Morphological deterioration of plant organs caused by salinity might be preceded by the damages of plant anatomy and ultrastructure. In this study, we want to reveal the effect of salinity stress on the damage of root, leaf and chloroplast ultrastructure on maize plant, as an important crop economically either in Indonesia or in most countries worldwide.

2. Material and methods

2.1. Plant materials

Three seeds were cultivated in a pot and grown in the growth room at 12-h photoperiod, the temperature of 30/25 °C (light/dark), humidity of 70%, and light intensity of 600 µm m⁻² 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 days, the plants were taken to the laboratory for preparing of parameters observation.

2.2. Scanning Electron microscopy (SEM)

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. Transmission electron microscopy (TEM)

For ultrastructure observation, the middle part of the second leaf blade of the plants was used. Sampling was done at 12:00, after six hours of illumination. Small portions (1x2 mm) of the leaf tissue were fixed in 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2), post-fixed in 2% osmium tetroxide in the same buffer, dehydrated with a graded acetone series and propylene oxide, embedded in Spurr’s resin and polymerized at 70 °C for 24 h. Chloroplast structure was evaluated on transverse ultrathin sections cut with a diamond knife on an Ultracut-N microtome (Reichert-Nissei, Austria). Sections were mounted on a 200 mesh grid, stained with uranyl acetate followed by lead citrate solution and examined with a transmission electron microscope (Hitachi H-7500, Tokyo, Japan) at 100 kV.
2.4. Nutrients contents

Nutrient uptake 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 elements were quantified using inductively coupled plasma-mass spectrometry (ICP-MS), except chlorine was estimated with an atomic absorption flame spectrometer (Shimadzu AA 6400F, Shimadzu Co. Ltd.). The data were expressed on a dry weight basis (mg g^{-1} dw).

3. Results

SEM observation revealed anatomical damage of root in maize seedling caused by salinity stress especially on the plant treated by 2 and 3% NaCl. Root cross-section of control plant contains intact tissues structure. Epidermal tissue consisted a layer of cells surrounding inner part of root with a complete circle in form. The cortex was filled with mostly intact parenchyma tissue. It consisted cells with round shape and almost uniform in size. Pith located in the central region of root has similar performance of parenchymal cells as those in the cortex. The stele was intact and consisted of pericycle, phloem, xylem tissues and surrounding pith at the inner part. It was separated from the cortex by endodermis at the outer part. Salinity treated plant root showed some differences with control plant as observed by SEM (figure 1). In cross-section, root shape was not completely circle due to some parenchymal cells composed the cortex was damaged at some location. In addition, parenchymal tissue possesses cells that were not uniform as in the cortex of control plant. The damage of parenchymal tissue was also visible in the pith of stele. However, there was no damage observed in the stele composed by permanent tissue such as xylem and pericycle salinity treated plant. Those tissue consisted cells possess thick cells wall made of lignin that made cell strength and stable.

Figure 1. Root cross-section of control (a) and 3% NaCl-treated maize plants (b). ep: epidermal, c: cortex, p: pith and s: stele.

Figure 2 show cross-section of the leaf in control and salinity treated plants. Entirely, the shape and thickness of leaf were not different between control and salinity treated plants due to it had fully developed when NaCl treatment was given. However, the cell composed mesophyll and bundle sheath contained different amount of chloroplasts inside. There were more chloroplasts visible in the leaf of the control plant, especially inside bundle sheath cells. The sample was collected at noon between 12:00 – 13:00 in which normally photosynthetic activity was high. Salinity stress could damage chloroplast or decreased photosynthetic activity through complex metabolism pathway.
Figure 2. Leaf cross-section of control (a) and 3% NaCl-treated maize plants (b). bsc: bundle sheath cells, ep: epidermal, mc: mesophyll cells.

Ultrastructure of leaf and chloroplast were observed with TEM (figure 3). In control plant, chloroplasts possess organized grana thylakoid, many starch granules, and few oil droplets. Mitochondrion was observed with clear cristae. In salinity treated plants, the damage of chloroplasts was visible. It possesses unorganized grana, swollen thylakoid in some parts and smaller starch if any. Although generally mitochondrion was still intact, cristae membrane was not preserved (figure 3f). Oil droplets more notably both in number and size.

4. Discussion
Salinity stress caused the damage to roots, leaves, and chloroplasts as well as the change of nutrient content in the shoot. High concentration of sodium and chlorine in the growth medium inhibited or proposed the uptake of other nutrients [11,14]. 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 comparable in content (table 1).

SEM observation shows the damage of root anatomy in the region consisted of parenchymatic tissues as indicated in the cortex and pith. Parenchymatic tissue made of cells typically by a thin cell wall and more cytoplasm fluid is a weak tissue of plant body. It is widely reported that salt stress directly inhibited plants grow on many plants [17–21]. The direct effect of salinity on the structural damage of root anatomy is still unclear, however, ion and osmotically stress caused by salt accumulation inside cells may contribute to cells distortion. High concentration of salt inside tissues caused either hyperosmotic and hyperionic stress that lead to an acceleration of cell death [11]. The image of SEM pointed to the decrease of chloroplasts number inside leaf cells (figure 2) was preceded by the damage of chloroplast apparatus. Salinity affects the severe damage of chloroplast ultrastructurally have been reported previously [16,18-19] as well as chlorophyll content declining [16-18]. The damaging steps of chloroplast ultrastructural visually with SEM on senescing broccoli florets was documented [20].

The damages of chloroplast ultrastructure, mitochondrion, and parenchymal tissue of root and the decrease of starch content inside chloroplast which may reflect the decrease of photosynthesis activity may involve the change of metabolic pathway [15]. As reported previously that salt stress induced the generation of reactive oxygen species (ROS) such as superoxide, peroxide and hydroxyl radical. ROS were harmful to the cell that can lead to damaging of some proteins and enzymes inside the cell. Salinity stress tolerant plants capable to generate superoxide dismutase (SOD), a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [11,16]. Therefore, they are an important antioxidant defense in nearly all cells exposed to oxygen. These ultrastructural
differences revealed that salinity stress caused the damage of chloroplast as photosynthetic apparatus. The damage of chloroplast can lead to restricting photosynthesis rate as reported in some study that measured photosynthesis activity on the plant exposed to salinity stress [15].

Figure 3. Ultrastructure of chloroplast and mitochondrion of control (a, b, c) and 3% NaCl-treated maize plants (b). Black arrow: swelling of thylakoid, white arrow: oil droplet, ch: chloroplast with starch, g: granum, mt: mitochondrion.

Table 1. The content of elements in the shoot of control and 3% NaCl-treated plants

| Element | Control | 3% NaCl | Percentage to control |
|---------|---------|---------|-----------------------|
| Al      | 0.281   | 0.539   | 192                   |
| B       | 0.225   | 0.123   | 55                    |
| Ca      | 32.057  | 46.063  | 144                   |
| Cl      | 2.765   | 6.276   | 227                   |
| Cu      | 0.047   | 0.043   | 91                    |
| Fe      | 0.611   | 0.826   | 135                   |
| K       | 197.900 | 168.400 | 85                    |
| Mg      | 21.600  | 30.427  | 141                   |
| Mn      | 0.199   | 0.217   | 109                   |
| Na      | 1.887   | 152.700 | 8,092                 |
| P       | 25.995  | 18.300  | 70                    |
| S       | 10.413  | 9.489   | 91                    |
| Si      | 6.740   | 6.670   | 99                    |
| Zn      | 0.398   | 0.425   | 107                   |
High concentration of salt in the plant tissues lead to altering physiological and biochemical processes through two mechanisms i.e. osmotic and ionic stresses [3,11]. Osmotic stress occurs due to Na$^+$ and Cl$^-$ uptake, which provokes water deficit inside the cell. Na$^+$ and Cl$^-$ accumulation decreases water gradient between root and soil solution, making it more difficult for water to move in the root surface [12]. 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 [13]. Ionic stress due to high accumulation of toxic ions such as Na$^+$ and Cl$^-$ directly reduces uptake of other mineral nutrients such as Ca and K, which causes metabolic disturbances [2,11,14].

5. Conclusion
Salinity stress damaged parenchymal cell composed cortex and pith of root, which has a thin cell wall, chloroplast ultrastructure and decreases starch content. Chloroplasts content inside the leaf cells were dramatically decreased in number. Anatomical damage of roots and leaves was accompanied by changing nutrient uptake of some trace elements due to ion toxicity caused by excessive sodium ion in tissue.

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