Effects of Salinity Stress on the Seminal Root Tip Ultrastructures of Rice Seedlings (*Oryza sativa* L.)

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Abstract: Growth and structural changes in the seminal root tip of rice seedlings (*Oryza sativa* L. cv. Nipponbare) in response to NaCl salinity were studied. Seedlings were grown in agar medium with 0, 0.1, 0.3, 1.0, 2.0 and 3.0% NaCl (agar culture), and in water with 0, 0.01, 0.03, 0.06 and 0.1% NaCl (water culture). Seedling growth was significantly suppressed by higher concentrations of NaCl. The effect of NaCl appeared faster in water culture than in agar culture. In both agar and water cultures, root growth was markedly suppressed over shoot growth. Under saline conditions, epidermis, cortex and root cap cells appear to be damaged to a greater extent than the meristem and stelar cells. The most notable ultrastructural change in response to salinity was the development and increment of vacuoles, which seem to provide a space for accumulation of excess ions. Many electron dense deposits were observed in the larger vacuoles of the epidermal and cortical cells. Under saline conditions, cell wall thickening of the epidermis, an increase in endoplasmic reticulum, myelin figures, less compact Golgi bodies and inhibited production of Golgi vesicles were also observed.

Keywords: Growth, *Oryza sativa* L., Root tip, Salinity, Transmission electron microscopy (TEM), Ultrastructure, Vacuole.

Soil salinity is an important agricultural problem, especially in farmlands dependent on irrigation. The problem is compounded by the relatively low salt tolerance of most crop plants. About 7% of the land surface and about 5% of cultivated land are affected by soil salinity. Most importantly, about 20% of irrigated land has suffered from secondary salinization and 50% of irrigation schemes are affected by salinity (Flowers et al., 1997). Responses to salinity stress at the whole plant level are variable and depend on a number of factors such as plant age, tissue organization and distribution of ions throughout the plant body, and the kinds of salt ions in the external environment (Yeo and Flowers, 1982). The first reaction of plants to a saline environment is the uptake of salt ions and their accumulation in plant cells at a high concentration (Greenway and Munns, 1980; Flowers et al., 1987; Rodriguez et al., 1997). Adaptation of plants to saline environments is a complex process. Through exclusion, excetration or dilution mechanism, the cells have to prevent themselves from large accumulation of ions in their cytoplasm (Hamza, 1980; Ben Rais et al., 1993). Thus, the specific morphological and biochemical modification that occurs in each species might reflect the adaptive capacity of the plants (Ben Rais et al., 1993). Salt injury in plants is caused mostly by altered water absorption and by Cl− or Na+ toxicity (Matsubara and Tasaka, 1987).

Rice is an important cereal crop grown worldwide and highly sensitive to salinity especially at the seedling stage (Yeo et al., 1990). Employing tolerant rice cultivars in a particular environmental stress condition is the best way to cope with the stress condition as far as successful rice culture is concerned. It is obviously important to elucidate the salt tolerance mechanisms in rice, in order to breed salt-tolerant cultivars and to establish a system of cultivating rice in saline environment. However, in spite of persistent efforts, genetic improvement of rice for tolerance to abiotic stress is yet to be fully realized (Grover et al., 1996). The lack of full understanding of how plants, in general, cope with such environmental factors is considered to be the main reason for this failure (Grover et al., 1993).

Most physiological and morphological studies on the effect of salinity have been devoted to shoots; however it is the root that is directly exposed to the soil salinity and this should be the plant organ where the effects of this stress should be more pronounced (Hajibagheri et al., 1985). The salt tolerance of the plant depends decisively on the tolerance of the roots (Jeschke and Wolf, 1988). Recent research has been focused on changes in cell structure, which can indicate adaptive mechanisms under conditions of mineral stress. Ultrastructural alterations of root cells in response to salt stress have been found in several species such as bean, barley, maize, wheat, sorghum, *Agrostis stolonifera* and *Suada maritima* (Udovenko et al., 1970; Nassery and Jones, 1976; Yeo et al., 1977; Smith et al., 1982; Werker et al., 1983; Hajibagheri et al., 1985; Huang and Van Steveninck, 1990;
Cachorro et al., 1995; Koyro, 1997; El-Banna and Attia, 1999). However, only a few reports have been found concerning the NaCl effect on internal characteristics of rice roots, which included only the cortical cell death and cell proliferation (Kawai et al., 1998; Samarajeeva et al., 1999). Accordingly, we carried out light and electron microscopic studies to elucidate the cellular alterations and the adaptive response of rice plants to salt stress using the root tip which acts as a finely-tuned sensor for different kinds of stress (Colmer et al., 1994).

**Materials and Methods**

1. **Plant materials**

Seeds of rice (*Oryza sativa* L. cv. Nipponbare) were surface sterilized with a 5% sodium hypochlorite solution for 5 minutes. After washing several times with distilled water, seeds were imbibed in petridishes containing distilled water in a culture room at 24±2°C until the appearance of the white tip of the coleoptile. Then, the seedlings were cultured using the following two methods.

(1) **Agar culture**

Agar culture method was used in this experiment, as it is easier to separate the root with intact root tip/root cap from agar than from soil.

Seedlings were grown in 300 mL tall beakers containing 100 mL agar medium {0.5% (w/v)} without or with NaCl of different concentrations (0.1, 0.3, 1.0, 2.0 and 3.0%). Agar medium was autoclaved at 100°C for 5 minutes. Fifteen well-germinated seeds were placed into each beaker containing the agar medium. The beakers were placed in a culture room for 7 days at 25°C and the seedlings were exposed to the light from fluorescent lamps, which provided 40 µmol m⁻²s⁻¹ at the plant level. Then the root and shoot lengths of 15 seedlings grown in each beaker were measured at 7 days after seeding.

(2) **Water culture**

After imbibition the seeds were sown on plastic nets placed on the surface of 300 mL NaCl solution at different concentrations (0.01, 0.03, 0.06 and 0.1%) and on distilled water (as control) in tall beakers (500 mL).

Seedlings were grown in a culture room for 7 days at 25°C and they were exposed to light from fluorescent lamps, which provided 40 µmol m⁻²s⁻¹ at the plant level. Then the root and shoot lengths of 15 seedlings per treatment were measured at 7 days after seeding.

Data obtained from the experiment were statistically analysed according to Duncan’s Multiple Range Test.

2. **Light and electron microscopic studies**

Microscopic studies were made using the seminal root tip of 5-day-old seedlings grown on agar medium containing 0, 0.3 and 1.0% NaCl, and in water containing 0, 0.01, 0.03 and 0.06% NaCl. To prevent the entry of air bubbles the root tip sections (1–2 mm) were cut with a sharp blade keeping them immersed in 5% glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. Then the sections were fixed overnight in 5% glutaraldehyde at 4°C and subsequently rinsed with 0.05 M phosphate buffer six times at 30-minute intervals. Tissues were then post fixed in 2% OsO₄ in the same buffer at 4°C for 12 hours and dehydrated with a graded series of acetone (30, 50, 70, 90, 99 and 100%) and finally acetone was replaced by propylene oxide. Samples were then embedded in Spurr resin (Spurr, 1969) and polymerized at 70°C for 24 hours.

Semithin sections (1 µm in thickness) were cut with glass knives and stained with 0.5% toluidine blue and 1.5% Na₂CO₃ on a heating plate (60°C). Then, the sections were observed under a light microscope (Nikon OPTIPHOT-2). Ultrathin sections (70–90 nm in thickness) were cut with a diamond knife and picked up on 200 mesh copper grids. The grids were stained with 2% uranyl acetate for 20 minutes followed by lead citrate for 5 minutes. Then the sections were viewed at 100 kV on a Hitachi H600 transmission electron microscope.

The changes in ultrastructures were examined in the longitudinal sections of several positions in the root tip (epidermis, cortex, meristem and stelar cells) and in the root cap (central and peripheral cells).
Explanations of figures

Fig. 3. Light micrographs of the longitudinal sections of root tip cells of rice grown in agar culture (Bars=3 μm). A : control; B : 0.5% NaCl and C : 1% NaCl. Arrows indicate the boundary between root cap and root proper.

Fig. 4. Transmission electron micrographs of the longitudinal sections of root tip epidermal cells of rice grown in agar culture (Bars=3 μm). A : control; B : 1% NaCl. Arrows indicate thickening of cell wall.

Fig. 5. Transmission electron micrographs of the longitudinal sections of root tip cortical cells of rice grown in agar culture. A : control (Bar=3 μm); (B-C) : 1% NaCl (B. Bar=2 μm and C. Bar=2 μm). Arrowheads showing the electron dense deposits inside the vacuole.

Fig. 6. Transmission electron micrographs of the longitudinal sections of root tip meristem (A and B) and stelar cells (C and D) of rice grown in agar culture. A : control (Bar=2 μm); B : 1% NaCl (Bar=3 μm). C : control (Bar=3 μm); D : 1% NaCl (Bar=3 μm).

Fig. 7. Transmission electron micrographs of the longitudinal sections of root cap central (A and B) and peripheral cells (C and D) of rice grown in agar culture. A : control cells (Bar=3 μm) showing starch containing amyloplasts; B : 1% NaCl (Bar=3 μm). C : control cells (Bar=1 μm) showing well developed Golgi bodies. Arrows show vesicles secreted from Golgi bodies; D : 1% NaCl treated cells (Bar=1 μm) showing less compact Golgi bodies with inhibited production of vesicles in their vicinity.

Abbreviations on figures

A, amyloplast; GW, cell wall; ER, endoplasmic reticulum; GB, Golgi body; M, myelin figure; Mt, mitochondria; N, nucleus; Nu, nucleolus; Pp, proplastid; V, vacuole.

Results

1. Effects of salinity on plant growth

In agar culture, seedling growth was suppressed with increasing concentrations of NaCl (Fig. 1). Root growth was more strongly suppressed than shoot growth at all concentrations of NaCl. A significant decrease in the length of both root and shoot was observed at 0.3% and higher concentrations of NaCl. At ≥ 2.0% NaCl, the root did not emerge but the shoot did although the growth was very poor (11% of the control). At 1.0% NaCl, the lengths of root and shoot were 51.1 and 27% of the control, respectively.

In water culture, the effect of NaCl was stronger than in agar culture. The lengths of root and shoot were significantly increased by 0.01% of NaCl (Fig. 2), but reduced with increasing NaCl concentrations. A significant decrease was observed at 0.06% NaCl and at a higher concentration, but the decreasing magnitude was higher in the root. At the highest concentration of NaCl (0.1%), the length of root and shoot was 6.8 and 52.9% of the control, respectively.

2. Effects of salinity on ultrastructures

The observation made by light microscopy showed a marked difference between the control and NaCl-treated plants. Fig. 3 shows the junction of root tip and root cap cells in the control and NaCl-treated plants. In control (Fig. 3A) and 0.3% NaCl-treated plants (Fig. 3B), the junction is clearly distinguishable, but in 1% NaCl-treated plants (Fig. 3C) it is not clearly distinguishable. Cells in this region of the control and 0.3% NaCl-treated plants were small with prominent nuclei. In peripheral cells of the control root cap, a few small vacuoles were visible in the cytoplasm while root tip cells had no vacuoles. However, in 1% NaCl-treated plants, the cells
were more elongated, swollen and characterised by the presence of numerous vacuoles in the root tip epidermal and cortical cells and in most cells of the root cap.

The observations made by transmission electron microscopy did not show any marked differences between the control and NaCl-treated plants in the nucleus, plastids or mitochondria of the root tip and root cap cells. However, in the cells of control roots, the stroma of the plastids, the matrix of the mitochondria, the nucleoplasm and the cytoplasm all appeared to have a comparable high density.

Under saline conditions, the epidermal, cortical and root cap cells appeared to be damaged to a greater extent than the meristem and stele cells. In control roots, the cell wall of the epidermal cells was thin (Fig. 4A), but in salt-treated roots it was thick (Fig. 4B). Most of the epidermal cells of the salt-treated roots were vacuolated while the control cells had no vacuole. A granular electron dense deposit was observed in the large vacuoles of the NaCl-treated epidermal cells. Nucleolus and nuclear chromatin were present and found intact in the epidermal cells of the control roots (Fig. 4A) but in most cells of the NaCl-treated roots, they were vanished or absent and a magnitude of small to large vacuoles were observed inside the cytoplasm (Fig. 4B).

Rice plants grown in the absence of NaCl possess more electron dense cytoplasm and did not exhibit any vacuolation in root tip cortical cells (Fig. 5A). Moreover, the plants grown in the presence of 0.3% NaCl also did not demonstrate any vacuolation (photograph is not presented). However, the corresponding cells of the plants treated with 1.0% NaCl demonstrated more frequent production of smaller to larger vacuoles in the root tissues (Figs. 5B-C). The coalescence of small vacuoles into larger units were observed (Fig. 5B). Many vacuoles had electron dense inclusions and granular substances and some showed myelin figures inside them (Fig. 5B). Myelin figures were also observed developing from the endoplasmic reticulum (Fig. 5C). There was no sharp difference between the control and salt treated plants in the structure of nucleolus, nuclear chromatin, proplastids and mitochondria.

Many vacuoles were also observed in the meristem (Fig. 6B) and stele cells (Fig. 6D) of the root tip of NaCl-treated plants, but they were smaller in size than those of the epidermal, cortical and root cap cells. No other remarkable difference in the structure of the organelles was observed between the control (Figs. 6A and C) and salt-treated plants.

In NaCl-treated plants, root cap cells were swollen and enlarged. The greater part of the root cap cells was occupied by large vacuoles. In control cells, there were many starch-containing amyloplasts in central root cap cells and they were arranged sequentially in a line (Fig. 7A). In NaCl-treated cells, the amyloplasts were randomly distributed in the cytoplasm (Fig. 7B). In peripheral root cap cells of control plants, a large number of Golgi bodies with well developed cisternae and many secretory vesicles in their vicinity were observed (Fig. 7C); but in NaCl-treated cells, the Golgi bodies were less compact and a few secretory vesicles were observed in their vicinity (Fig. 7D).

In response to salinity, the number of endoplasmic reticula increased and importantly, the endoplasmic reticulum membranes were localized around the cell periphery (Fig. 6D). Some endoplasmic reticula were observed with the occasional presence of Golgi bodies in the cells of salt-treated plants (Fig. 7B).

In water culture, root cells were found vacuolated even in control plants but in saline conditions the degree of vacuolation was greatly increased. Large vacuoles contained more electron dense granular deposits than those in agar culture.

**Discussion**

In both the agar and water culture, root was more sensitive than the shoot to salt. This is in agreement with the findings of Asana and Kale (1965) and Ansari et al. (1980) who reported that NaCl and CaCl2 are more inhibitory to root growth than shoot growth in wheat plants. Samarakajewa et al. (1999) also suggested that NaCl inhibits the cell number increase and cell death of cortical tissues, which retards the growth significantly in the primary roots of rice seedling.

In water culture, the presence of 0.01% NaCl, the length of root and shoot increased and the magnitude was higher in the roots. This might be due to the low osmotic potential and/or low ionic concentration in the control plants (Munns et al., 1995). Salama et al. (1994) have reported the stimulated growth in wheat at low concentrations of NaCl (25 mM). Anju and Verma (1992) have also observed an increase in height of wheat plants at a lower level of salinity (6.0 mmhos cm−1 EC) especially during early stages of growth.

In our experiment, each tissue in the root tip region showed different sensitivities to salt treatment. The epidermis, cortex and the root cap were more sensitive than the meristem and stele. The most frequently observed ultrastructural alteration due to NaCl treatment was the formation of many small to large vacuoles in the root tip and root cap cells.

The appearance of vacuoles in cells after exposure to NaCl observed in our experiment is in agreement with the results reported for other species (Nassery and Jones, 1976). It has also been suggested for other plant species that the capacity to withstand NaCl salinity be in relation to the degree of compartmentation of toxic ions within the cell. Matoh et al. (1987) suggested that vacuolation is an adaptive response to compartmentalize sodium ions away from the cytosol. Hajibagheri et al. (1985) pointed out that earlier commencement of vacuolation is advantageous under saline conditions since it protects the expanding cells particularly when they are vulnerable to high ion-concentrations in the
cytoplasm. It also provides an extended vacuolar storage capacity for ions in the roots and the capacity to respond to very large, short term, or rapid changes in external ion concentrations. Koyro (1997) pointed out that a multiplicity of small vacuoles possesses a larger surface than one big vacuole, hence a higher exchange capacity (Na versus K). This system enables a plant cell to avoid ion toxicity, imbalance, and interactions between substances in the cytoplasm.

Vacuoles, where ions could accumulate, account for a large proportion of the cell volume (Huang and Van Steveninck, 1988). Wiencke and Läuchli (1980) observed the development of the vacuolar system in Porphyra umbilicalis under hyperosmotic stress and suggested that the appearance of the vacuoles might play a role in accumulating inorganic ions.

Early vacuolation of cells in response to salinity may be a way of protecting the cytoplasm from toxic levels of ions by storing ions in the vacuole (Nassery and Jones, 1976; Gorham and Wyn Jones, 1983; Hajibagheri et al., 1985). This process may be accelerated by salinity treatment. Huang and Van Steveninck (1988) suggested that a stable potassium concentration is a priority requirement for active meristematic cells in the root tip, and this requirement may be facilitated by early vacuolation and reduction of cytoplasmic volume relative to the total volume of the cell.

An increase in the thickness of root tip epidermal cell wall in salt-treated plants as observed in our experiment was previously observed in salt-tolerant tobacco cells (Bressan et al., 1990). This alteration would act as a mechanism to preserve the water potential of the cell against an external high-salinity medium (Singh et al., 1989).

Electron dense inclusions and granular deposits were observed in the epidermis and cortex vacuoles of the NaCl-treated plants grown in both agar and water culture. It has also been observed in maize roots subjected to oxygen deficiency (Campbell and Drew, 1983) and is thought to be caused by the loss of tonoplast integrity and entry of phenolics in the vacuole.

Myelin figures as observed in our experiment are considered as artifacts due to double fixation with glutaraldehyde and osmium tetroxide (Bowers and Masler, 1988). These artifacts were absent or rarely observed in the control plants but they were frequently observed in the NaCl-treated plants and thus were considered as to reflect the membrane changes due to the NaCl treatment.

An increase in endoplasmic reticulum was observed in our experiment. Endoplasmic reticulum constitutes a compartment through which substances might be transported symplastically from one cell to another via desmotubule, the narrow central tubule of plasmodesmata, without passing the cytosol, and endoplasmic reticulum cisternae may then fuse with tonoplast, releasing their contents into vacuole (Kramer, 1979). The presumed function of endoplasmic reticulum suggests that the root tip cells of rice seedlings in an early stage of salt stress start vacuolation and development of endoplasmic reticulum for transportation and compartmentalization of sodium ions into vacuole.

The present results showed that root cells of rice plants vacuolate at an early stage of expansion under saline conditions and the degree of vacuolation was higher in the epidermis, cortex and root cap than in the meristem and stelle. The ultrastructural changes demonstrated have a potential to provide a highly valuable means of detecting early stages of NaCl stress, and may also provide opportunities for screening different varieties for their adaptation to salinity.

References


*In French with English summary.
**In Japanese with English summary.