Changes in Root Water Uptake and Chlorophyll Fluorescence of Rice (*Oryza sativa* L. cv. Dongjin) Seedling under NaCl Stress

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The physiological and photochemical responses of rice seedling to NaCl stress were investigated through measuring leaf relative water content (RWC), root water uptake and chlorophyll fluorescence. When plants were exposed to increased salinity stress, the visual symptoms of injury were significant at ≥500 mM NaCl concentration for 4 and 5 day stress periods. The differences in Fv/Fm between control treatment and plants treated with 500 mM and 1,000 mM NaCl were evident after 5 day and 4 day, respectively, whereas in root water uptake its effect was observed at 500 mM and 1,000 mM NaCl at 2 day of salt-stressed periods. Leaf RWC in salt-stressed plants decreased gradually with increasing salinity in exogenous solution and duration of salt stress, and these decrease showed leaf RWC of 58-68% atduration over 2 day stress of 1,000 mM NaCl treatment and 88% at 1 day stress. NaCl stress led to a significant inhibition of the light-induced greening in etiolated rice plants, especially in 4 and 5 day salt-stressed plants, which linearly decreased with NaCl concentration (R^2=0.812 and 0.918, respectively). The effects of NaCl stress in rice seedlings indicate that water uptake in root is more sensitive to increasing NaCl concentration and stress duration than Fv/Fm in leaves compared with the same NaCl concentration.

Key words : NaCl stress, *Oryza sativa*, root water uptake, chlorophyll fluorescence, leaf RWC

Introduction

Most of the salt stress is due to sodium chloride (NaCl) salts in nature [13]. Salinity related with NaCl can damage the plant through its osmotic effect, specific toxic effect of ions and by disturbing the uptake of essential nutrients [20,34]. The high Na^+ concentration of a sodic soil not only injures plants directly but also degrades the soil structure, decreasing porosity and water permeability [7,18].

Salinization is a major cause of soil degradation and affects 19.5% of irrigated land and 2.1% of dryland agriculture at global level [8,29]. Man is a major cause of soil salinization, which is about 78% of the global human-induced salt-affected soils [8]. Intensive agriculture and improper water management practices have caused and continue to cause substantial salinization of crop lands by adding an annual application of irrigation water in addition to salt accumulation in soil [28]. Natural processes or mismanagement in irrigated agriculture results in inhibition of plant growth and crop yield [7]. When irrigation water contains a high concentration of solutes and when there is no opportunity to flush out accumulated salts to a drainage system, salts can quickly reach levels that are injurious to salt-sensitive species, in particular, rice, lettuce and bean [25,28].

Rice (*Oryza sativa* L.), a crop of great importance in Asia, especially in Korea, is glycophyte and greatly affected by salt stress. Therefore, it is of great necessity and significance to increase rice productivity through taking all sorts of effective measures to enhance its salt tolerance [5,6, 11,12,26,31].

Although many studies have shown that expression of specific proteins [19,24,33] and metabolic alterations based on growth, gas exchange and antioxidant enzymes, such as superoxide dismutase, peroxidase and polyphenol oxidase [4,5,30], are induced by salt stress, limited success has been achieved in revealing the underlying mechanisms of salt affecting in plants.

Hence, to observe the responsiveness of plants to salt stress, physiological approaches that enlighten the functions of intricate systems involved in abiotic factors need further research.

In this study, at the physiological level the effect of salt stress against NaCl treatment in rice seedlings was studied in view of root water uptake, chlorophyll fluorescence, leaf RWC and the light-induced greening of etiolated rice

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plants approach and obtained results are discussed the concentration adjusted to NaCl toxic effects, tolerance to exclude the potentially toxic Na^+ and Cl^- ions and the response of plant to salt stress.

Materials and Methods

Plant culture and growth conditions

Seeds of rice (*Oryza sativa* L. cv. Dongjin) were used after harvesting for experimental purpose in 2005 at the general farm of Jinju National University and were surface-sterilized for 30 min with 1% Sodium hypochlorite (NaOCl) solution, then washed with tap water several times to remove the reagent from the seed surface. They were germinated in 2 l distilled water cylinder bottle with soaking about 1 l grains for 3 day. New distilled water was freshly exchanged daily for soaking. Germination in a growth chamber has taken at 25°C under a 16 hr daylight period and 120 μmol m^{-2} s^{-1} photon flux. A total number of 150-170 seeds were planted at 7 cm depth in a conical pot of the Φ10×Φ7.3×8.8 cm size containing moistened TOSHILI soil, which used as a nursery bed soils and for seedling periods, and then cultivated in a growth chamber for 9 day. Composition of TOSHILI were pH 5.5-7.0, EC 0.6±0.1 dS m^{-1}, NH_4-N≥150 mg l^{-1}, NO_3-N≥300 mg l^{-1}, P_2O_5≥300 mg l^{-1}, CEC 20±5 cmol l^{-1}, K^+ 2.0±0.5 cmol l^{-1}, Mg^{2+} 1.2±0.2 cmol l^{-1} and Na^+≤1.6 cmol l^{-1}.

The environmental conditions in the chamber were a temperature of 25±1°C, a relative humidity of 85% and a light intensity of 400 μmol m^{-2} s^{-1} provided by a combination of fluorescent tubes (Philips TLD 36W) over a 16 hr photoperiod. 9 day-old plants of the second leaves were used as the experimental materials.

Treatment conditions

To investigate the physiological effects of the salinity, the treatment conditions were exogenously adjusted to 0, 100, 200, 300, 500 and 1,000 mM l^{-1} NaCl. Afterward, each pots were transferred in 500 ml beaker tanks containing the different NaCl concentrations. The conical pot was well surmounted withbeaker opening and remained in situ. Salinity was treated to plants into a hole of pot from beaker NaCl solution as increased an osmotic potential for 1-5 day. For tolerance to salt stress, they were replaced on the beakers with distilled water for 4-8 day recovery duration after 1, 2, 3, 4 and 5 day of stress periods. The control material was defined as the 18 day-old plants with the second leaves that were not exposed to salt stress. Treatments were identified them of duration stress for 1-5 day and recovery period for 4-8 day.

Measurement of root water uptake

In the growth chamber, water loss of culture solution was induced by the evaporation as well as plant transpiration and leaf guttation, and this water loss concentrates salts. The water uptake of plant was calculated (ml unit) by adding the culture solution at 24 hr interval after starting the salt treatment. Root water uptake was obtained from the following formula:

$$\text{Root Water Uptake (\%)} = \frac{\text{Daily Uptake after Treated Plant}}{\text{Daily Uptake before Treated Plant}} \times 100$$

Chlorophyll fluorescence

After 20 min dark period in ambient conditions in the laboratory (Fig. 3), the emission of chlorophyll fluorescence from the upper surfaces of the leaves was measured using a Plant Efficiency Analyzer (Handy PEA; Hansatech, UK) as described previously Chun et al. [3]. Measurements of minimal (Fo) and maximal (Fm) fluorescence yields allowed determination of the optimal quantum yield (Fv/Fm) being used to calculate the maximal potential efficiency of PS II of dark adapted leaves. The maximum variable fluorescence (F_v) was calculated as the difference between F_m and F_o at a specific time. For the condition of the optimal control in chlorophyll fluorescence, 9 day-old plants were used to measure chlorophyll fluorescence at the beginning, and the optimal period of dark-adaption was determined over 20 min of the adaptation, which maintained a steady state during the measurements of chlorophyll fluorescence (Fig. 1).

Leaf relative water content

Leaf relative water content (RWC) was calculated based on the methods from Whetherley [32]. About 3 g fresh leaf samples were 90 mm length, and then weighed to obtain fresh mass (FM). In order to determine the turgid mass (TM), leaves were floated on distilled water. Maximum turgidity was determined by weighing leaves (after gently wiping the water from the leaf surface with tissue paper) until no further weight increase occurred. At the end of the imbibitions period, leaf samples were placed in a pre-heated oven at 70°C for 48 hr, in order to obtain dry

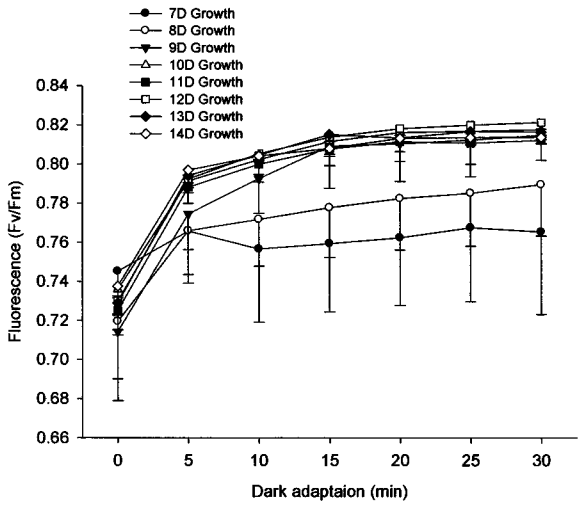

Fig. 1. Chlorophyll fluorescence (Fv/Fm) in the plant leaves after 0-30 min of dark adaptation at the different growth. Each point represents the average of five measurements on different individual plant. Standard errors are shown as vertical bars. After 9-day-old leaves and in the dark adaptation for 20 min, the fluorescence kinetics maintained the steady state. Hence, these conditions were applied for research that investigates the quantum yields of PS II (Fv/Fm).

mass (DM). All mass measurements were made using an analytical scale, with a precision of 0.0001 g. Values of FM, TM and DM were used to calculate leaf RWC using the equation below:

$$LRWC(\%) = \frac{FM - DM}{TM - DM} \times 100$$

Chlorophyll determination

Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Chlorophyll extraction was carried out on fresh fully expanded leaf material. 150 mg leaf sample was kept in test tubes with 10 ml of 80% acetone, at 4°C in the dark, for 24 hr. After that, the supernatant was decanted in assay tubes and this procedure was repeated three times until the green color of the pellet disappeared [9]. The absorbance was measured with a UV/visible spectrophotometer (SHIMADIU UV-165PC, JAPAN) and chlorophyll concentrations were calculated using the equation proposed by Arnon [1].

Chl a (mg ml^{-1})=11.64×(A663)−2.16×(A645)

Chl b (mg ml^{-1})=20.97×(A645)−3.94×(A663)

Where, (A663) and (A645) represent absorbance values read at 663 and 645 nm wavelengths, respectively.

Statistical analysis

The six concentrations of NaCl were arranged in a randomized complete block design with five replications. The data for all parameters were statistically analyzed to work out two-way analysis of variance using the SPSS computer package for Windows. Actual data were used for leaf relative water content (RWC) for which actual data were transformed into percentages before analysis was applied, and were likewise transformed into percentage for root water uptake. Statistically different groups were compared using an LSD test ($P<0.05$).

Results

Visual symptoms

The injury caused by NaCl, after 4 day applications of over 500 mM concentrations, begin with leaf tip chlorosis, then spreading in the middle of the leaves, which finally showed necrosis at 1,000 mM concentrations. By contrast, after 4 day of below 300 mM concentrations leaves did not show any symptoms of necrosis. The symptoms were more obvious at 100 mM, 200 mM and 300 mM NaCl concentrations for 1 to 3 day treatments (Fig. 2). However, the symptoms of injury were significant at ≥500 mM NaCl of exogenous solution for 4 and 5 day stress periods.

Chlorophyll fluorescence

Maximal quantum yield of PS II (Fv/Fm) showed no significant difference between control plants and salt-treated plant seedlings for 1-4 day at 100, 200, 300 and 500 mM NaCl (Fig. 3A, B, C and D) and the values were closed to 0.80. This ratio decreased slightly when plant was in the presence of 1,000 mM (0.724 and 0.71) for 5 day stress periods (Fig. 3E).

For 6 and 7 day recovery after 2 and 3 day stress periods, Fv/Fm decreased slightly at only 1,000 mM NaCl treatment (0.724 and 0.64) (Fig. 3B and C), but decreased largely at 1,000 mM (0.558) after only 4 day, and did at 500 mM (0.565) and 1,000 mM (0.285) after 5 day stress periods, respectively (Fig. 3D and E). Fv/Fm in relation to NaCl concentrations and stress periods showed clear difference salt-stressed and control plants at 1,000 mM treatment for 4 and 5 day.

Root water uptake

Under salt stress, the root water uptake was significantly inhibited in the exogenous solution with different NaCl

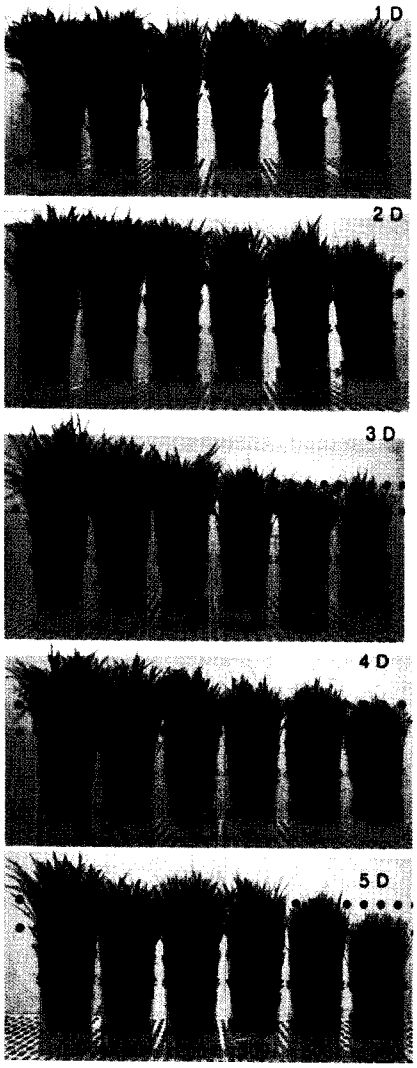

Fig. 2. Plant photographs of visual symptoms after 1-5 day NaCl treatments and 4-8 day recovery. For one example, when treatment period was 1 day, recovery periods were 8 day, and the sum of these of treatment and recovery were always 9 day. All of experimental days were finally 18 day.

concentrations compared to the control. This inhibition showed no clear difference in salt-stressed plant for only 1 day (Fig. 4A). Plants in saline conditions showed clear difference to 95% of leaf RWC in the presence of 1,000 mM NaCl compared to 129% of untreated plants to 100% of the control at 2 day salt-stressed plants (Fig. 4B) and were also reduced the water consumption in pure water solution during recovery (Fig. 4C, D and E).

In root water uptake, the effects of salt tress showed highly difference between plants treated and plants untreated with NaCl, and showed gradually higher inhibition

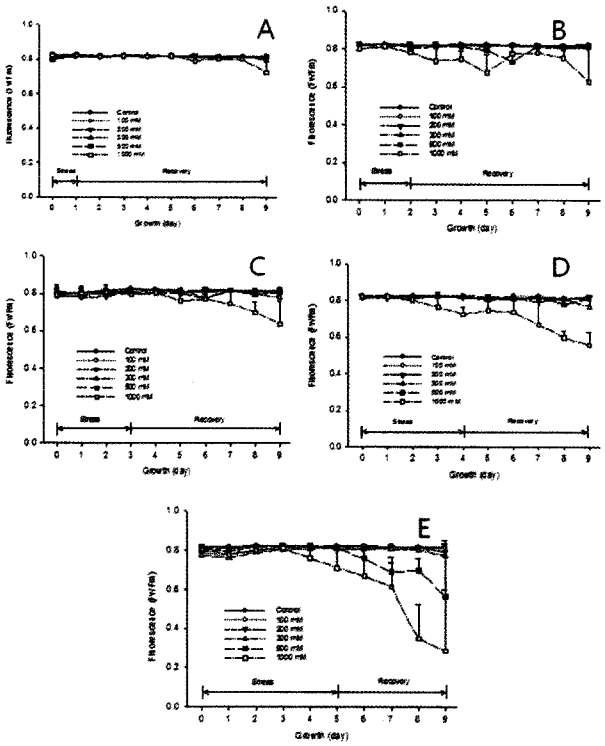

Fig. 3. The effects of external NaCl on leaf chlorophyll fluorescence (Fv/Fm) in the rice plants after 8-4 day recovery with distilled water following 1-5 day exposure to NaCl. Plants were first grown for 9 day. After that, they were placed in culture solution treated with 0, 100, 200, 300, 500 and 1,000 mM NaCl for 1-5 day, and then were replaced in pure water solution for 8-4 day for the recovery. Each point represents the average of five measurements on different individual plant. Standard errors are shown as vertical bars. A, 1+8; B, 2+7; C, 3+6; D, 4+5; E, 5+4 treatment.

with increasing salinity and duration of salt stress similarly to growth observed in the visual symptoms (Fig. 2).

Leaf relative water content (RWC)

The differences between RWC before stress initiation and 1-5 day exposure to NaCl were not clear within 300 mM NaCl, but became clearer at 500 mM and 1,000 mM NaCl. Leaf RWC decreased gradually with increasing salinity in exogenous solution and duration of salt stress, and showed lower decrease for recovery at 1,000 mM NaCl over 2 day than for 1 day. In this experiment, control treatment showed nearly 90% of leaf RWC. Increased salinity was reduced by values between 70% and 80% at 500 mM and between 58% and 67% at 1000 mM from 2 to 5 day salt-stressed plants, respectively. In relation between NaCl concentration and salinity application duration, salinized

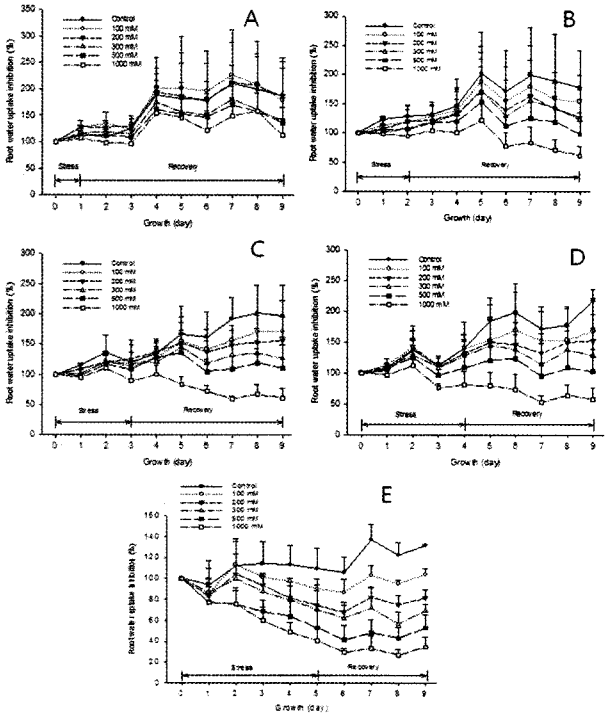

Fig. 4. Effects of increasing NaCl concentration in exogenous culture solution on root water uptake after 1-5 day exposure to NaCl and remained for 8-4 day recovery with distilled water. Plants were first grown for 9 day. After that, they were placed in culture solution treated with 0, 100, 200, 300, 500 and 1,000 mM NaCl for 1-5 day, and then were replaced in pure water solution for 8 day for the recovery. Each point represents the average of five measurements on different individual plant. Standard errors are shown as vertical bars. A, 1+8; B, 2+7; C, 3+6; D, 4+5; E, 5+4 treatment.

seedlings of rice showed a small decrease in RWC with 500 mM NaCl for 3 day compared with 300 mM NaCl for 4 day (Fig. 5)

The light-induced greening of etiolated seedlings

Chlorophyll content obtained at the end of treatments and following recovery duration, NaCl stress led to a significant inhibition of the light-induced greening in etiolated rice seedlings.

Chlorophyll content was irregularly reduced at all of NaCl levels from 1 to 2 day of salt stress duration, but was gradually reduced with increasing NaCl concentrations from 3 to 5 day (Fig. 6). At 4 and 5 day salt stress, the total chlorophyll content linearly decreased with NaCl concentration as a sharp slope change. The coefficient of determination (R^2), between total chlorophyll content and NaCl concentrations at 1, 2, 3, 4 and 5 day stress were in order

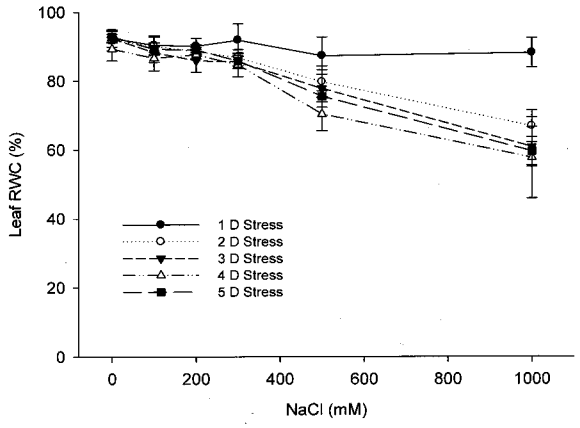

Fig. 5. The effects of different NaCl concentrations on leaf relative water content (RWC) in the rice seedlings; 1D stress, 1+8 (1 day salt-stressed and 8 day recovery); 2D, 2+7; 3D, 3+6; 4D, 4+5; 5D, 5+4 treatments. Plants were first grown for 9 day. After that, they were placed in culture solution treated with 0, 100, 200, 300, 500 and 1,000 mM NaCl for 1, 2, 3, 4 and 5 day each, and then were replaced in pure water solution during 8-4 day for the recovery. Finally, experimental leaves were grown for 18 day old in all treatments.

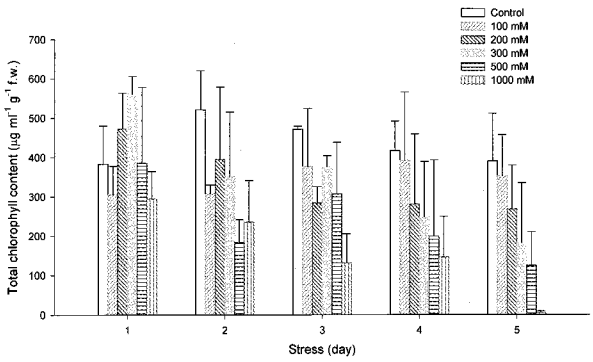

Fig. 6. The effects of different NaCl concentration on the light-induced greening of etiolated plants following salt stress durations between 1 and 5 day and remained in distilled water solution further until they were grown for 9 day. The etiolated plants were grown first in dark boxes for 9 day. After that, for the light-induced greening under salt stress, they were placed in culture solution treated with 0, 100, 200, 300, 500 and 1,000 mM NaCl by 1-5 day durations under 400 μmol m^{-1} s^{-1} photons, and then were replaced in pure water solution for 4-8 day by each stress treatment for the recovery.

0.107, 0.512, 0.824, 0.812 and 0.918. Both treatments combined with 500 mM NaCl and 5 day stress and with 1,000 mM NaCl and 4 day stress appear to be critical condition for triggering necrosis due to inhibition of chlorophyll synthesis. Moreover, treatment combined with 1,000 mM

NaCl and 5 day stress was severely occurred cell death in leaves in response to osmotic and salt stress.

Discussion

Rice is generally considered to be sensitive to salinity. Salinity causes water deficit (osmotic stress), ion toxicity and nutrient deficiency even if exposed to moderate salt during seedling establishment [24]. The physiological and photochemical responses of rice seedling to salinity were examined in this research. Experimental design of NaCl treatments was made to treat the osmotic stress with different NaCl concentrations into a pot containing the bed soil from external NaCl solution, and plants were grown in the growth chamber. Therefore, a NaCl concentration in these numerals of figures does not mean the NaCl in pot soil, or culture solution (Fig. 2), but means the NaCl concentration in external solution.

When plants are exposed to increased salinity by adding different NaCl concentration, the visual symptoms of injury were significant at $\geq$500 mM NaCl of exogenous solution for 4 and 5 day stress periods. The difference, in Fv/Fm, between control plants and plants treated with 500 mM NaCl and 1,000 mM was evident after 5 and 4 day, respectively, whereas in root water uptake it was evident at 500 mM and 1,000 mM NaCl even after 2 day. Similar result was reported in *Phaseolus* species that stated that the toxic effects of high concentrations (80 mM NaCl) of Na^+ and Cl^- in plant tissue and the saline-induced changes in mineral nutrient uptake likewise contributed to the reduction of plant growth [2]. The leaf injury could be a result of the accumulation of toxic levels of Cl^- and Na^+, ion imbalance, nutrient deficiencies and water stress. The transport of Cl ions occurs mainly in the transpiration stream, which explains the high concentration of these ions in leaves and the occurrence of salt injury [20], and higher concentration of NaCl in plant tissues seems to be the cause of reduction of leaf water and osmotic potentials as stress intensified [2]. This result showed that the root water uptake was decreased at lower salt concentration, whereas Neocleous and Vasilakakis [20] proposed that plant water consumption was reduced because of stomatal closure by water stress in the root zone, or salt toxicity in the plant tissue at higher salt concentration.

Leaf RWC, in this experiment, decreased gradually with increasing salinity in exogenous solution and duration of salt stress, and in addition salt-stressed plants in 1,000 mM NaCl showed lower decrease following recovery of duration over 2 day stress compared to 1 day stress (Fig. 5). RWC in leaves reflects the metabolic activity in tissues as an alternative measure of plant water status [30]. Decrease in RWC indicated a loss of turgor that resulted in limited water availability because of lower water availability under stress conditions, or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface [16,17]. Similar reports have been made for many plant species under salinity stress condition [2,20,27,30]. Of course, the reduction in leaf cellular turgor may not be the main cause for the reduction of stomatal conductance in saline conditions [15] and is not simply depended on stomatal conductance [20], however, some reports lead to the suggestion that salt stress induces changes in membrane permeability, as well as water relations [20,30].

Sultana et al. [27] proposed that NaCl drastically reduced leaf relative water content, which resulted in loss of turgor, which led to reduced photosynthetic rate, and this occurred in present experiment as well. NaCl stress led to a significant inhibition of the light-induced greening in etiolated rice plants, especially at 4 and 5 day salt-stressed plants, which linearly decreased with NaCl concentration (R^2=0.812 and 0.918, respectively) (Fig. 6). In addition, maximal quantum yield of PS II (Fv/Fm) showed significant difference for control plants and salt-treated seedlings for 4 day and 5 day stress duration at 1,000 mM NaCl concentration (Fig. 3). The diminished capacity for PS II photochemistry was the result of an increased reduction state of the efficiency of energy transfer by open reaction centers in the light (Fv/Fm) [14,22], which was correlated with an increase in non-photochemical quenching (NPQ) suggesting that salt stress induced dissipation of damaging excess energy [11]. These results are consistent with the behavior of the chlorophyll contents in NaCl-treated plants because of poor chlorophyll development from etiolated chloroplasts in rice plant seedlings. The decrease of chlorophyll content in salt-stressed plant may appear due to the formation of proteolytic enzyme, such as chlorophyllase, which is responsible for damaging to the photosynthetic apparatus [30], or the disassembly of photoactive NADPH:protochlorophyllide oxidoreductase (POR), which is a key enzyme for the light-induced greening of etiolated angiosperm plants [23]. In the physiological level, the multitude

of effects of salt stress indicates that uptake and transport of high NaCl lead to limiting for photosynthetic rate and plant growth due to the reduction in stomatal conductance, and K^+ deficiency resulting from competition with the uptake of other nutrient ions [12,13,34].

Although salt-stressed plants were replaced under high osmotic potential with distilled water, this current work suggests that the plant is able to escape stress when duration of salinity is short. An understanding of the mechanisms by which salinity affects water uptake and photosynthesis would aid the improvement of growth conditions and crop yield and would be useful tools for future breeding for salt tolerance in plants [21].

In addition to this research, the effects of chlorophyll degradation under saline and the increase of resistance to salinity by adding the bioactive compounds such as terpenoids, glycinebetaine and proline etc. need more for physiological approaches. These experimental works may be applied to check up the responsiveness of salinity for the screening of newly breeding cultivars and also, the biological responsiveness in cultivars collected from native rice cultivated for a long time.

Acknowledgement

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초록 : NaCl 스트레스에 따른 벼 유식물의 뿌리 수분흡수와 엽록소형광의 변화

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염분에 대한 벼 유식물의 생리학적 광화학적 반응을 잎의 상대수분함량, 엽록소 형광 및 뿌리의 수분흡수를 통하여 연구하였으며, 벼 유식물이 농도가 다른 NaCl에 노출되었을 경우, 500 mM 이상의 농도와 4일, 5일간 스트레스를 준 처리구에서 식물체의 외관상 심각한 장해 징후가 나타났다. 500 mM에서는 5일간, 1,000 mM 에서는 4일간 스트레스를 준 처리구와 NaCl를 처리하지 않은 대조구 간의 광합성 Fv/Fm에서 유의성이 있는 차이가 나타났으며, 그러나 뿌리 수분흡수에서는 Fv/Fm에 비해 스트레스 기간이 짧은 2일에서도 수분흡수의 차이가 나타나기 시작했다. NaCl에 노출된 식물에서 잎의 상대수분함량은 외부 염분의 농도가 증가하고, 스트레스 기간이 길어짐에 따라 점차 감소하였다. 잎의 상대수분함량 결과에서 1,000 mM농도로 1일간 처리된 경우(88%)와 비교했어 2일 이상 NaCl를 처리한 경우들(58-67%)에서 보다 낮은 수분함량을 보였다. NaCl 스트레스는 4일과 5일간 처리한 경우 etiolate된 벼 유식물의 광 유도 녹화과정에서 NaCl농도가 증가함에 따라 직선적으로 심하게 억제하였다(각각의 R^2=0.812 과 0.918). 염분 스트레스 기간과 NaCl농도가 증가되었을 때, NaCl의 농도가 같음에도 잎의 Fv/Fm보다는 뿌리의 수분흡수가 더 민감하게 반응하는 것으로 보아 잎에서의 장해보다는 뿌리에서의 반응이 먼저 일어나는 것으로 보인다.