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SUB-CELLULAR DISTRIBUTION OF NUTRIENT ELEMENTS AND PHOTOSYNTHESIS PERFORMANCE IN *ORYZA SATIVA* L. SEEDLINGS UNDER SALT STRESS

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Abstract

In our study we focus on effects of multiple concentrations of NaCl (25, 50, 100, and 150 mM) on rice seedlings. The results showed that significant oxidation stress was caused, as indicated by the content of malondialdehyde (MDA). Na accumulation increased, and the vast majority of the ions were laied in the cell wall and these nutrient ions in the cell wall fraction were elevated, while those stored in the soluble or organelle fraction were declined. Meanwhile, the levels of photosynthetic pigments in leaves of 812HS decreased markedly. These findings were confirmed by the reduced photosynthesis. At 150 mM salt concentrations resulted in a significant damage to chloroplast structure. The data from the protein immunoblot assay revealed that salt stress affected the stability of protein. The major subunits of LHC II were more sensitive than reaction center proteins of PSII under solt stress. All together, these results showed that high salt concentrations disordered the ions homeostasis, stimulated reactive oxygen accumulation and lipid peroxidation in rice, leading to seriously damage to chloroplast ultra-structure, pigments and protein. Thereby, photosynthesis was inevitably blocked. This study is significant for comprehending the physiological and biochemical process under salt stress, and is important for devoting to organic agriculture in soil.

Key words: Salt stress; Sub-cellular distribution; Photosynthesis; Ultra-structure.

Introduction

Salinization is a worldwide issue and an important factor limiting crops growth and development, due to plants' sensitivity to salt stress (Wu *et al*., 2012; Messedi *et al.*, 2016). The detrimental effects of high salinity on plants can be observed at the whole-plant level (Parida *et al.*, 2003). The stress can cause water deficit, ion toxicity, and nutrient deficiency leading to molecular damage and even plant death (Maggio *et al.*, 2010). Due to the disorders in ions homeostasis caused by adversity stress, absorbing ion and distribution become important for plant growth, especially for plant development under salt stress (Adams III *et al.*, 1992). Therefore, a suitable amendment should be determined to capitalize on the interaction or competitive relationship between NaCl and other nutrients. Meanwhile, despite the existence of many supporting evidences on the impact exerted by salt stress to PS II (Parida *et al.*, 2003), the response mechanisms through which the photosystem reacts to the salt stress remain unclear to date. Additionally, the effects of salt stress on PS I have been also very rarely reported. Ribulose 1,5-bisphosphate (RuBP) carboxylase/ oxygenase (RuBisCO) can increase the endurance to CO_2 spread from the mesophyll wall to chloroplast (Makino *et al.*, 1994). While, these results about influence of salt stress on RuBisCO are extremely scarce. The assessment of the effects of high salt concentrations on chloroplast organization is also essential, as it contributes substantially to comprehend physiological and biochemical changes of plants under stress.

Rice (*Oryza sativa* L.) a semi-aquatic crop, occupies a large part of the world agricultural lands. However, its growing is often threatened by environmental stress factors such as salt contamination (Wu *et al.*, 2012). More than 20% of all irrigated land on earth is affected by salinization (Wu *et al.*, 2012). This work focus on resolving ultra-structural, physiological and biochemical changes about photosynthetic organ exposed to diverse salt concentrations. We investigated the changes in MDA content and the sub-cellular distribution of Na and other nutrients, and examined the NaCl-induced degradation of thylakoid protein complexes. The changes in the ultra-structure of chloroplasts were also examined, as well as those in the content of chlorophyll and the rates of photochemical activities

Material and Methods

Materials and salt stress treatment: Plant materials were cultivated in hydroponics in the greenhouse of Nanjing Normal University. Seeds of the super high-yield hybrid rice LYPJ were obtained from Jiangsu Academy of Agricultural Sciences. 0.5% H_2O_2 was used to sterilize these seeds for 20 min. After washed with distilled water, these seeds were germinated on three layers of sterilized paper at 25°C under dark condition. Uniformly germinated seeds were choosed and set them into pots with the Kimura B nutrient solution. Thees seedlings were dealt with NaCl solution (0, 25, 50, 100, and 150 mM) after 7 days. These NaCl concentrations selected in our work were according to pre-experimental examinations. These plant were then grew in a chamber under the above conditions for 7 days. All reagents were changed every two days, each experiment repeated three times.

Separation, measurement of Na and nutrient elements in sub-cellular component: The methods of measurement were conducted by Liu *et al.* (2012).

Lipid peroxidation: MDA content was estimated by the thiobarbituric acid (TBA) (Singh *et al*., 2002). Leaves (0.5 g) were grinded with 10% (w/v) trichloroacetic acid (TCA) buffer and 5000×g centrifugation for 10 min. Then equal volume 0.5% (w/v) TBA solution in 20% (w/v) TCA was edded into the supernatant and heated 30 min at 100ºC. The OD value was recorded at 532 nm and corrected by nonspecific OD value at 600 nm after quickly cooled on ice. The concentration of MDA was calculated by using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as mmol g^{-1} fresh weight (FW).

Pigment content determination: The chlorophyll (Chl) a, Chl b, and carotenoids (Car) were extracted with 80 % acetone, and a UV-754 spectrophotometer (Shanghai Institute of Plant Physiology, China) was used to mearsure the absorbance at 470, 647, and 663 nm. The concentrations of Chl a, Chl b, and Car were estimated according to the procedure proposed by Qiao *et al*. (2014).

Chloroplast isolation, ATP content and activities of Ca^{2+}-ATPase and Mg^{2+}-ATPase: Chloroplast isolationwas measured according to Howitz & Maccarty. (1982) with slight modifications. Plants were kept in the dark for 2 days prior to organelle isolations so as to reduce the level of internal starch. Plant material (50 g) was minced using a Waring blender with extraction solution (0.5 mM Tricine, pH 6.5, 10 µM $MgCl_2$, 330 mM sorbital, 20 µM EDTA, and 0.1 mg/mg bovine serum albumin, BSA). The homogenate was filtered through four layers of muslin and a fraction containing chloroplasts was centrifuged at 1,000 × g for 2 min. The chloroplasts were further purified by gradient centrifugation in extraction buffer containing 40% Percoll (Sigma) at 13,300×g for 15 min. These pellets were washed twice in resuspension buffer in order to get chloroplasts, which were kept on ice under dark. ATP content was measured by the bioluminescence method as described by Ma *et al.* (2016). For Mg^{2+}-ATPase activity assessment, chloroplast suspension was added into activate the solution and then activated by illumination (50 µmol m^{-2} s^{-1}) after 1 min. Then, the solution was mixed with the reaction buffer (0.5 ml of 20% TCA) and incubated for a few minutes. The process was terminated by TCA. After centrifuged, the supernatants could be used to measure Pi. Ca^{2+}-ATPase activity was measured essentially as above method except that $MgC1_2$ was instead of $CaCl_2$.

Chloroplast ultra-structural observation: The method of observation chloroplast ultra-structural was according to Ma *et al.* (2016). Leaves were cut into small pieces (0.1 ×0.5 cm) and fixed into 2.5% (v/v) glutaraldehyde in 0.1 M PBS (pH 7.3) and 5% (w/v) aqueous osmium tetroxide for 2 h at 4ºC, respectively. An ascending ethanol gradient was used for dehydrating and finally these samples were embedded in Epon 812 resin. Ultrathin samples were got on an LKB-V ultramicrotome (LKB Ultrascan XL, Bromma, Sweden). After stained, these samples were observed by a Hitachi H-7650 transmission electron microscope.

Western blot analysis: Immunodetection of the peptide composition of the thylakoid membrane complexes was as discript by Rintamäki *et al.* (2,000). The protein content was determined by spectrophotometry. After 20 mg proteins were dealt with loading buffer, the solution was boiled for 5 min. Then samples were separated by SDS - PAGE and transferred to PVDF membranes. These membranes were blocked with Tris-HCl buffer and probed with primary antibodies. After these membranes were incubated with secondary antibody (Sigma) (1:20,000), signals were obtained by BCIP/NBT (Roche, Switzerland) (Lindahl *et al*., 1996).

Statistical analysis: Datas were presented as mean ± SD from at least three repeated tests. These datas were assessed by one-way analysis of variance (ANOVA) and T-test by using SPSS 17.0. $p<0.05$ was considered statistically significant.

Results and Discussion

Salinization is a most important factor affecting plant growth and development (Wu *et al.*, 2012). It is known that salt stress causes oxidation destroy attributed to generation of reactive oxygen (Wu *et al.*, 2012). MDA is a outcome of the decomposition of polyunsaturated fatty acids (PUFA). It can act as biomarker for lipid peroxidation(Mittler, 2002). In our work, MDA contents rose sharply with the rise of salt concentrations, and the maximum value of 279.93% was reached by treatment with 150 mM NaCl (Fig. 1), showing that at 150 mM NaCl membranes suffered a great deal of peroxidation, and the structure and function of membranes were effected, and finally inhibited growth of rice seedlings.

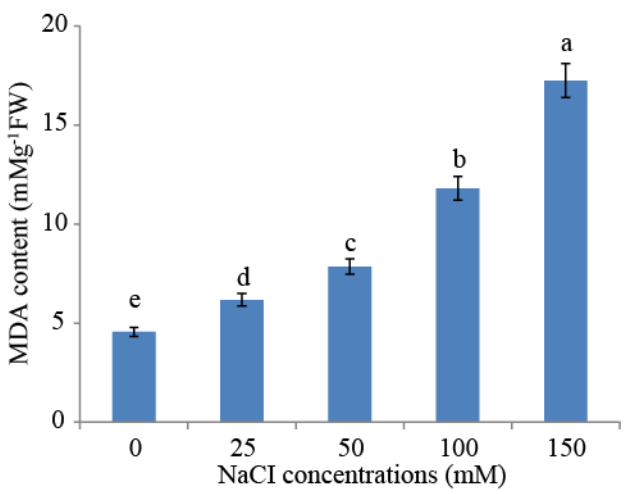

Fig. 1. Effect of salt on MDA content in the leaves of rice seedlings. The data are expressed as mean ± SD of triplicates. The value designated over the bars in different letters indicated significant difference at $p<0.05$.

Table 1. Na distribution in the cell wall, organelles, and soluble fraction in leaf cells of rice, grown in the different concentrations Na for 7 days. Data are expressed as mean ± SD of triplicates. The values followed by different small letters within a row are significantly different at *p<0.05*, determined by using the T-test

Na content (µg g^{-1} FW)	Na concentration (mM)				
	0	**25**	**50**	**100**	**150**
Total	5700 ± 467^{e} (100%)	5998 ± 567^{d} (100%)	6303 ± 371^{c} (100%)	7215 ± 756^{b} (100%)	8963 ± 378^{a} (100%)
Cell wall (% of total)	2316 ± 321^{e} (40%)	3116 ± 313^{d} (52%)	3946 ± 198^{c} (58%)	4534 ± 175^{b} (63%)	6610 ± 417^{a} (74%)
Soluble fraction (% of total)	3228 ± 423^{a} (57%)	2729 ± 322^{b} (45%)	2681 ± 348^{c} (39%)	2477 ± 270^{d} (34%)	2017 ± 245^{e} (22%)
Organelles fraction (% of total)	156 ± 25^{d} (3%)	155 ± 25^{d} (3%)	177 ± 26^{c} (3%)	204 ± 12^{b} (3%)	338 ± 15^{a} (4%)

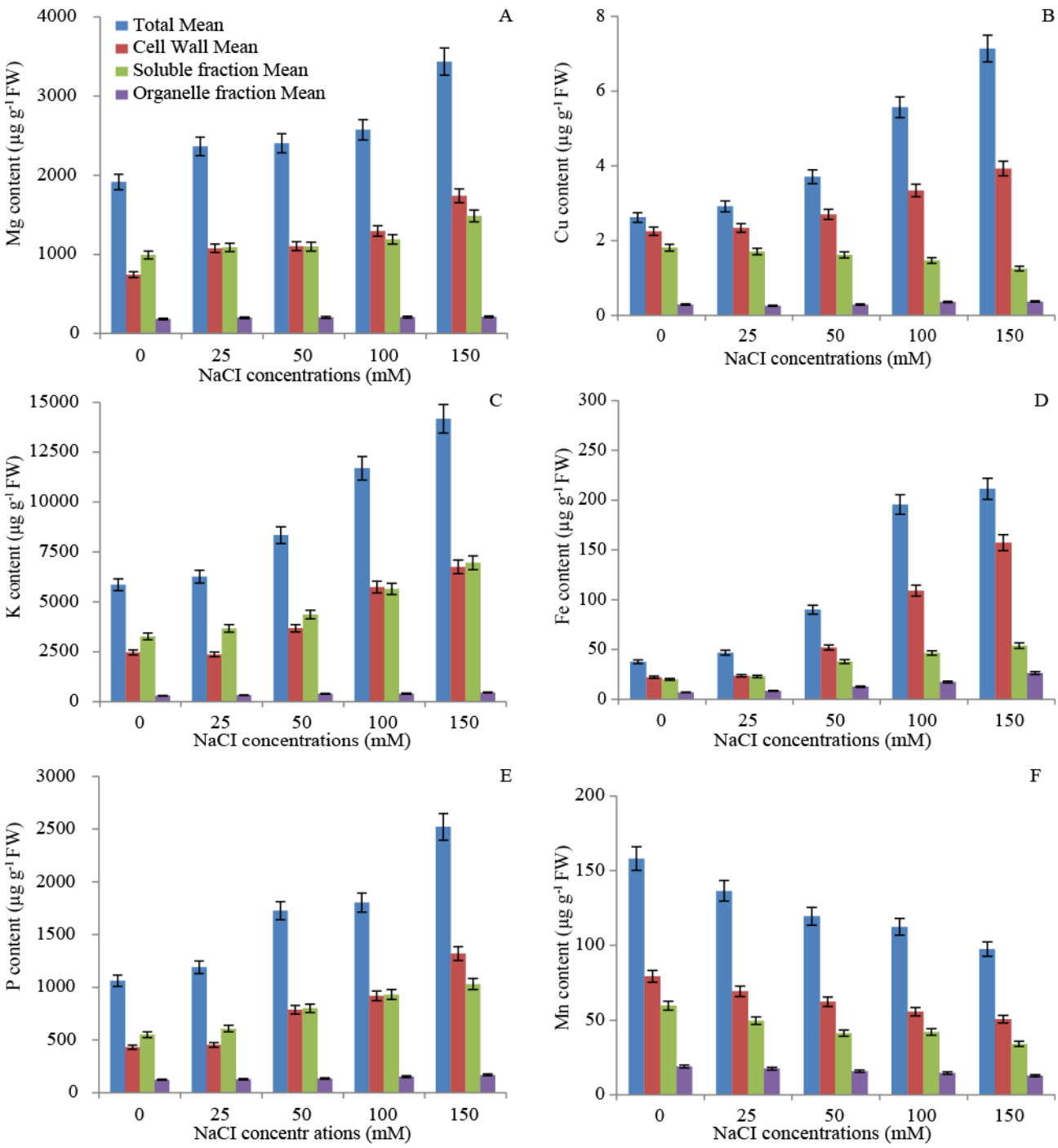

Fig. 2. Effect of salt on nutrient element contents in the leaves of rice seedlings. The data are expressed as mean ± SD of triplicates. The value designated over the bars in different letter refers to significant difference at *p<0.05*

As displayed in Table 1, lots of Na was accumulated in rice (Table 1; r = 0.8754, p<0.05) and 33138.86 μg g^{-1} FW was accumulated when seedlings were treated with 150 mM NaCl. Meanwhile, cell walls have proteins and polysaccharides which can act as ligands to bind metals, and as the first shelter prevent metal from entrancing into cells (Maine *et al.*, 2001). These results of the analysis at the subcellular level showed that lots of the Na was located at cell wall (40–74%), followed at soluble (57–22%), and organelles (3–4%). These results demonstrated that the cell wall could function as the first obstacle prevent organelles from NaCl stress (Maine *et al.*, 2001). When ions in the cell walls are saturated, excess ions are entered into cytoplasm or organelles (Maine *et al.*, 2001). Thus, at 150 mM NaCl the content of Na in the cell wall was decreased and it was accumulated in the soluble and organelles (Fig. 1). In addition, the rise in the salt concentration of the solution led to a obviously change of absorption of other nutrients. These findings of present study revealed that Na accumulation in the leaves was related to declined of Mg absorption (r_{Mg} = -0.9867, P < 0.01) (Fig. 2A), this phenomenon whould result in enhancing penetration of membranes to stress and decreased chlorophyll synthesis rates, respectively (Greenway & Munns, 1980). And we measured a decline of content of Chl a (55.54%) and Chl b (44.58%). The most considerable decrease of chlorophyll amount appeared 7 days after the treatment with 150 mM NaCl. In addition, K content was also decrease in present study (r_K = -0.9941, p<0.01) (Fig. 2C). Its reduce is typical for plants grown at saline condition (Baker & Long, 1986) and can be detrimental to the photosynthetic apparatus (Bilger & Schreiber, 1990). Fe is present in the redox center of proteins which are essential for photosynthesis and cellular respiration (Gross *et al.*, 2003). From our study, the Fe content was greater under salt stress (r_{Fe} = 0.9912, p<0.01) (Fig. 2D), indicating that the growth of the tops were restricted or membrane permeability was abrupt change (Bhivare & Nimbalkar, 1984). In addition, P content was also increased (r_P = 0.9337, p<0.01) (Fig. 2E). Previous reports also indicated that salinity had effects of stimulatory and inhibitory on P content (Sameni *et al.*, 1980). Mn (r_{Mn}= 0.9884, p<0.01) quantity increased in the salt-treated plants (Fig. 2F). A similar trend was also established by other researchers in tomato, soybean, and squash (Mass *et al.*, 1972; Mckimmie & Dobrenz, 1991). Such an influence possibly because of restricting growth of the tops (Bhivare & Nimbalkar, 1984) or membrane disorganization (Greenway & Munns, 1980). The excessive accumulation of Mn may cause toxic effects (Mass *et al.*, 1972) (Fig. 2B).

The nutrient shortage might due to the reduction of energy (ATP), which was associated with function of membrane transport (Fig. 3) (Xu *et al.*, 2010) and cell metabolism. The reduction of ATP amount is a characteristic response to salt stress (Xu *et al.*, 2010) and a prospective inference to the general disorder for cell metabolism (Xu *et al.*, 2010). In our result, salt stress led to 62.22% decrease of ATP content in the leaves of rice seedlings under the highest NaCl concentration condition (Fig. 3C), which is possibly caused by the absence of a developed detoxification system. This assumption was confirmed by the drastic decline of Ca^{2+}-ATPase and Mg^{2+}-ATPase activities (Fig. 3A, B), this reduction reached 27.28% and 25.01%, respectively. The decreased photosynthesis performance of salt-stressed seedlings possibly offered an explanation that a diminished exploit of energy towards sustaining photochemical reactions (Wang *et al.*, 2014). Moreover, obvious impairment in the ultra-structure of chloroplasts in leaves (Fig. 5) would influence the oxidative- and photo-phosphorylation and lead to ATP content decline (Fig. 3C) (Xu et *al*., 2010).

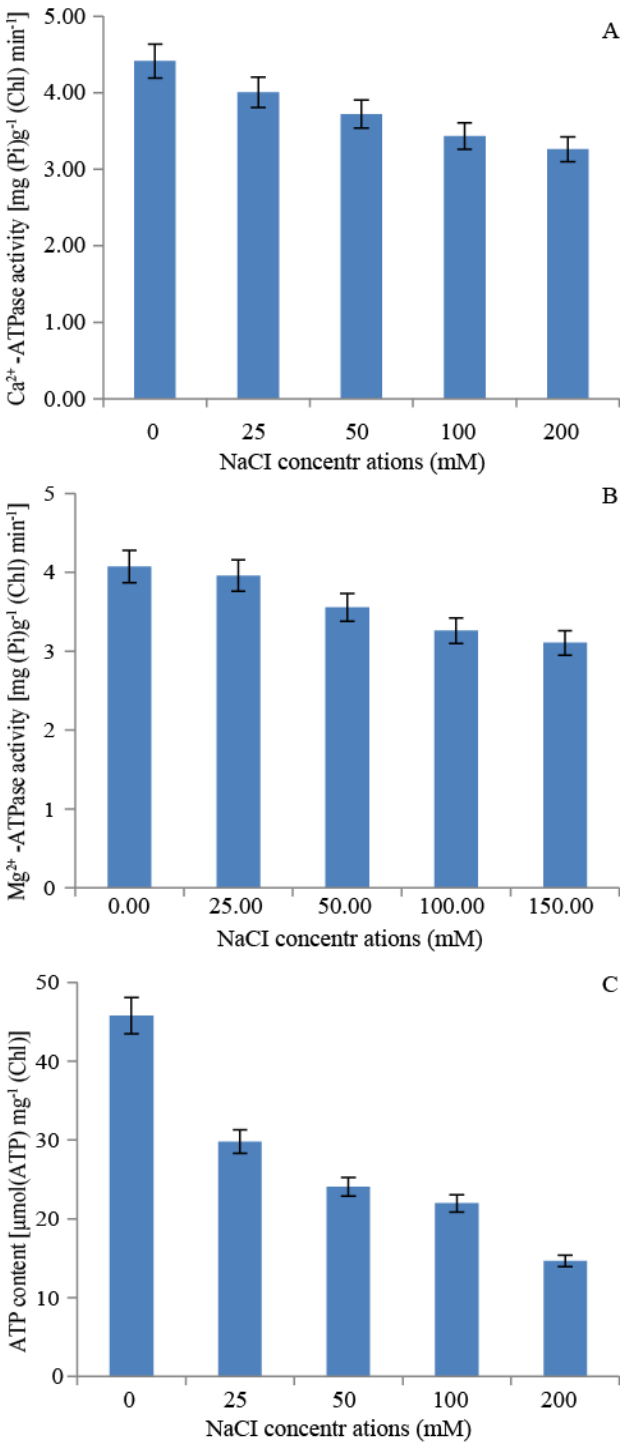

Fig. 3. Ca^{2+}-ATPase, Mg^{2+}-ATPase activities, and ATP content in the leaves of rice seedlings in response to various levels of salt stress. The data are expressed as mean ± SD of triplicates. The value designated over the bars in different letters indicates significant difference at p<0.05.

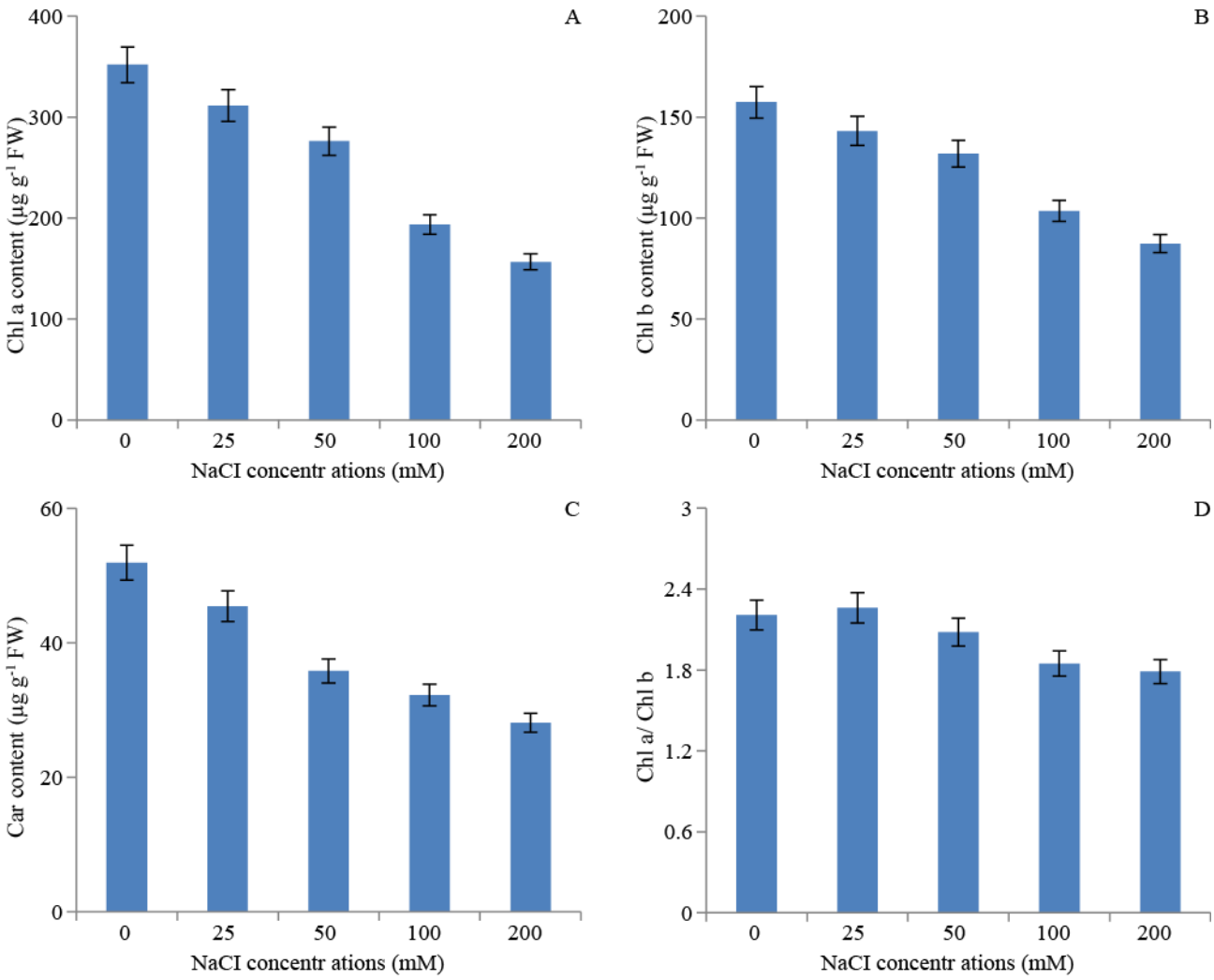

Fig. 4. Chl a, Chl b, and Car content and the ratio Chl a/ b in the leaves of rice seedlings in response to various levels of salt stress. The data are expressed as mean ± SD of triplicates. The value designated over the bars in different letters denotes significant difference at $p<0.05$.

Chlorophyll content is used as reply response to the action of an stress factor or a combination of multiple stress factors, and it is also a biomarker of biotic and anthropogenic stress (Ferrat *et al.*, 2003). In the present investigation, compared with the controls, a sharp reduction in the amounts of Chl a, Chl b, Car, and in the value of the ratio Chl a/Chl b was registered when these leaves were treated with 150 mM NaCl (55.54%, 44.58%, 44.59%, and 20.17%, respectively) (Fig. 4). The diminished chlorophyll level induced by salt might be due to inhibition of the enzymes involved in chlorophyll biosynthesis and the damage to chloroplast ultra-structure (Ferrat *et al.*, 2003). By using TEM, we compared the ultra-structure of chloroplast in the rice seedlings under normal conditions and different NaCl concentrations conditions to investigate the impact of NaCl on chloroplast development. In the control plants, these chloroplasts were lens-shaped and grana and stroma thylakoids were orderly arranged. The grana thylakoid appeared to be connected by intergranal lamella (Fig. 5A). However, the exposure to NaCl had obvious changes on the ultra-structure of chloroplasts. Thylakoid grana was swelling (Fig. 5B) after 25 mM NaCl treated. When treated with 50 mM NaCl, the the outer membrane of some chloroplasts was occurred breakage and inner structure was also disorganized (Fig. 5C, E); The swollen grana was disconnected and disorganized with the chloroplast stroma, as well as vacuoles appeared in the chloroplast (Fig. 5E). These results evidenced that the thylakoid structure of the chloroplasts became disorganized; the cell membranes were distorted and wrinkled (Fig. 5E). Thereby, when the complete structure of chloroplast was damaged, photosynthesis was inevitably inhibited.

To analyze changes of protein composition about photosynthetic under different NaCl concentration, by immunoblot analysis, we examined the abundance of thylakoid membrane complexes subunits by using antibodies such as RuBisCO, PS core complexes and LHC protein (Fig. 6). The content of core antenna proteins CP47 associated with PSII reaction center was reduced (Fig. 6), which was consistent with the results obtained by Parida *et al.* (2003). They found that a 30% decline in CP47 content when treated with 400 mM NaCl in *Bruguiera parviflora*, which suppressed electron transport activity because of oxidative damage

of NaCl-induced. Only Chl a not Chl b existS in CP47 (Minagawa & Takahashi, 2004), which can as a reason for the decline of Chl a/b. Apart from the reduction in the amounts of CP47, the D1 and D2 reaction center proteins of PSII were also diminished, which revealed that energy distribution imbalance between QA and QB (Neelam & Subramanyam, 2013). The content of light harvesting complex proteins (LHCb 1, 2) was also reduced (Fig. 6), which was evidenced by the reduction of pigments in rice seedlings. Additionally, the RuBisCO and PSI complexes subunit also showed instability, each submit including Rbc l, Psa A, LHCa 1, and LHCa 2 which indicated their reduced levels, especially by the significant degradation in the Psa A, which indicated that the oxidative damage of rice was serious (Neelam & Subramanyam, 2013). Recent reports of investigation in the green alga *Dunaliella salina* had revealed that the generation of reactive oxygen caused supression of PSII activities with ascendant salt concentrations (Liu *et al.*, 2012).

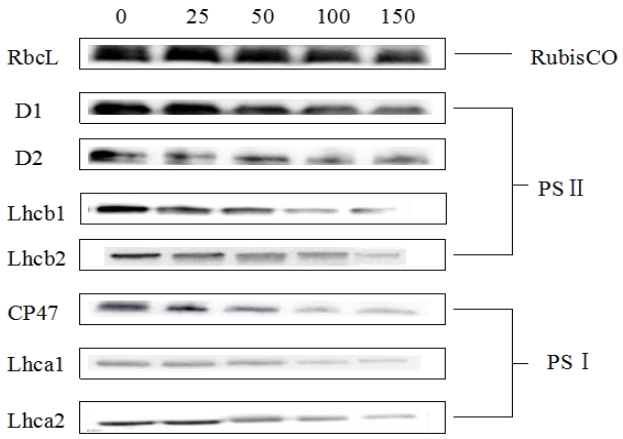

Fig. 6. Immunodetection of the peptide composition of the thylakoid membrane complexes isolated from rice seedlings after 7 days of exposure to 25, 50, 100, and 150 mM NaCl. Each lane was loaded with same amount of proteins in our experimental conditions

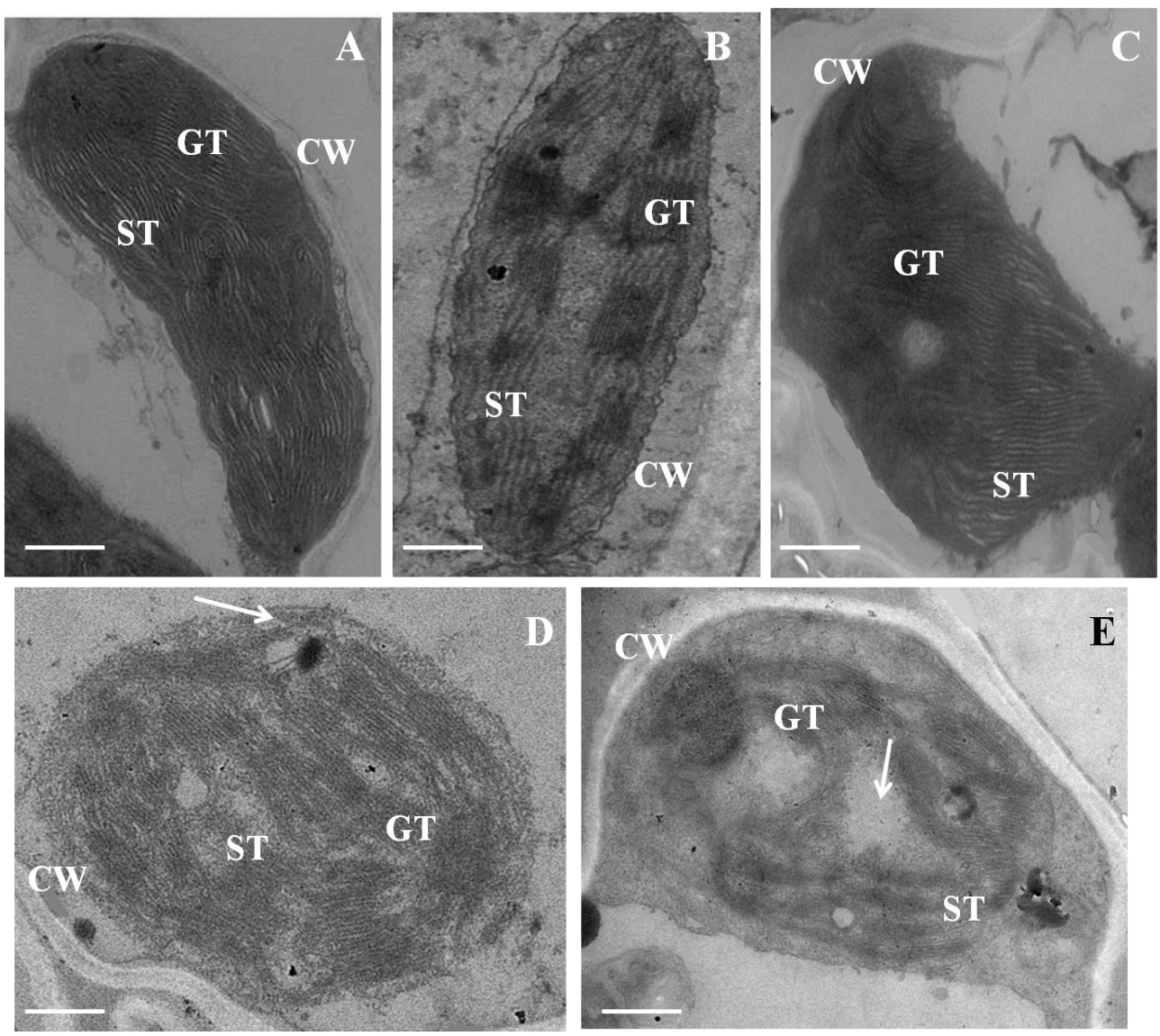

Fig. 5. Effects of 7-day salt exposure on the ultrastructure of chloroplasts. A. chloroplast with an orderly arrangement of grana and stroma thylakoids in the control leaf cells. B. Leaf cells treated with 25 mM salt. C. Leaf cells treated with 100 mM salt. D. Leaf cells treated with 100 mM salt. E. Leaf cells treated with 150 mM salt (Bar = 50 μm).

Conclusions

The ions homeostasis were disordered under high salt concentrations, which stimulated the generation of reactive oxygen and accelerate membrane lipid oxidation in rice, leading to degradation of chlorophyll and protein, By using TEM, we found that the thylakoid structure of the chloroplasts became disorganized; the cell membranes were distorted and wrinkled. Thereby, the complete structure of chloroplast was damaged, photosynthesis was inevitably inhibited. These results are significant for comprehending the physiological and biochemical process under salt stress, and is important for devoting to organic agriculture in soil.

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Reference

Adams III, W.W., M. Volk, A. Hoehn and B. Demming-Adams. 1992. Leaf orientation and the response of xanthophyll cycle to incident light. *Oecologia*, 90: 404-410.

Baker, N.R. and S.P. Long. 1986. *Photosynthesis in contrasting environments.* (Ed) Elsevier, Sole distributors for the USA and Canada, Elsevier Science Pub. Co.

Bhivare, V.N. and J.D. Nimbalkar. 1984. Salt stress effects on growth and mineral nutrition of French beans. *Plant Soil*, 80: 91-98.

Bilger, W. and U. Schreiber. 1990. Chlorophyll luminescence as an indicator of stress-induced damage to the photosynthetic apparatus. Effects of heat-stress in isolate chloroplasts. *Photosynth. Res.*, 25: 161-171.

Ferrat, L., C. Pergent-Martini and M. Roméo. 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. *Aquat. Toxicol*., 65: 187-204.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Biol.*, 31: 149-190.

Gross, J., R.J. Stein, A.G. Fett-Neto and J.P. Fett. 2003. Iron homeostasis related genes in rice. *Genet. Mol. Biol.*, 26: 477-497.

Howitz, K.T. and R.E. Mccarty. 1982. Ph dependence and kinetics of glycolate uptake by intact pea chloroplasts. *Plant Physiol.*, 70: 949-52.

Lindahl, M., S. Tabak, L. Cseke, E. Pichersky, B. Andersson and Z. Adam. 1996. Identification, characterization, and molecular cloning of a homologue of the bacterial FtsH protease in chloroplasts of higher plants. *J. Biol. Chem.*, 271: 29329-29334.

Liu, W.H., Y. Ming, P. Li and Z.W. Huang. 2012. Inhibitory effects of hypo-osmotic stress on extracellular carbonic anhydrase and photosynthetic efficiency of green alga *Dunaliella salina* possibly through reactive oxygen species formation. *Plant Physiol. Bioch.*, 54: 43-48.

Ma, J., C.F. Lv, M.L. Xu, G.X. Chen, C.G. Lv and Z.P. Gao. 2016. Photosynthesis performance, antioxidant enzymes, and ultrastructural analyses of rice seedlings under chromium stress. *Environ. Sci. Pollut. R.*, 23: 1768-1778.

Maggio, A., G. Barbieri, G.. Raimondi and S.D. Pascale. 2010. Contrasting effects of GA3 treatments on tomato plants exposed to increasing salinity. *J. Plant Growth Regul.*, 29: 63-72.

Makino, A., H. Nakano and T. Mae. 1994. Responses of ribulose-l,5-bisphosphate carboxylase, cytochrome f, and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. *Plant Physiol*., 105: 173-179.

Maine, M.A., M.V. Duarte and N.L. Suñé. 2001. Cadmium uptake by floating macrophytes. *Water Res.*, 35: 2629-2634.

Mass, E.V., G. Ogata and M.J. Garber. 1972. Influence of salinity on Fe, Mn, and Zn uptake by plants[1]. *Agron. J.*, 64: 793-795.

Messedi, D., F. Farhani, K.B. Hamed, N. Trabelsi and R. Ksouri. 2016. Highlighting the mechanisms by which proline can confer tolerance to salt stress in cakile maritima. *Pak. J. Bot.*, 48: 417-427.

Mckimmie, T. and A.K. Dobrenz. 1991. Ionic concentrations and water relations of alfalfa seedlings differing in salt tolerance. *Agron. J.*, 83: 363-367.

Minagawa, J. and Y. Takahashi. 2004. Structure, function and assembly of Photosystem II and its light-harvesting proteins. *Photosynth. Res.*, 82: 241-263.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*., 7: 405-410.

Neelam, S. and R. Subramanyam. 2013. Alteration of photochemistry and protein degradation of photosystem II from *Chlamydomonas reinhardtii* under high salt grown cells. *J. Photoch. Photobio. B.*, 124: 63-70.

Parida, A.K., A.B. Das and B. Mittra. 2003. Effects of NaCl stress on the structure, pigment complex composition, and photosynthetic activity of mangrove *Bruguiera parviflora* chloroplasts. *Photosynthetica*, 41: 191-200.

Qiao, X.Q., G.X. Shi, Z.Z. Zheng, M. Huang and H.Y. Yang. 2014. Photochemical performance of thylakoid membrane in lead-treated *Nymphoides peltatum*. *B. Environ. Contam. Tox.*, 93: 251-255.

Rintamäki, E., P. Martinsuo, S. Pursiheimo and E.M. Aro. 2000. Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. *P. Natl. Acad. Sci. USA.*, 97: 11644-11649.

Sameni, A.M., M. Maftoun, A. Bassiri and A.R. Sepaskhah. 1980. Growth and chemical composition of dry beans as affected by soil salinity and N fertilization. *Plant Soil*, 54: 217-222.

Singh, B.K., A. Walker and D.J. Wright. 2002. Persistence of chlorpyrifos, fenamiphos, chlorothalonil, and pendimethalin in soil and their effects on soil microbial characteristics. *B. Environ. Contam. Tox.*, 69: 181-188.

Wang, Y.W., X.H. Jiang, K. Li, M. Wu, R.F. Zhang, L. Zhang and G.X. Chen. 2014. Photosynthetic responses of *Oryza sativa* L. seedlings to cadmium stress: Physiological, biochemical and ultrastructural analyses. *Biometals*, 27: 389-401.

Wu, H.F., X.L. Liu, L.P. You, L.B. Zhang, D. Zhou, J.H. Feng, J.M. Zhao and J.B. Yu. 2012. Effects of salinity on metabolic profiles, gene expressions, and antioxidant enzymes in halophyte *suaeda salsa*. *J. Plant Growth Regul.*, 31: 332-341.

Xu, Q.S., J.Z. Hu, K.B. Xie, H.Y. Yang, K.H. Du and G.X. Shi. 2010. Accumulation and acute toxicity of silver in *Potamogeton crispus* L. *J. Hazard. Mater.*, 173: 186-193.

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