BRIEF COMMUNICATION

Effects of NaCl stress on photochemical activity and thylakoid membrane polypeptide composition of a salt-tolerant and a salt-sensitive rice cultivar

R.L. WANG*, C. HUA**, F. ZHOU**, and Q.-C. ZHOU**

*Jiangsu Food Science College; Huaian 223003, P. R. China**
*Life Science Department of Nanjing Xiaozhuang College; Nanjing 211171, P. R. China***

Abstract

NaCl stress (200 mM) inhibited the electron transport activity of photosystem 2 (PS2) more than that of PS1. The degree of electron transport activity inhibition was lower in the salt-tolerant cultivar Pokkali than in the salt-sensitive cultivar Peta. The polypeptide composition of the thylakoid membrane and PS2 particles did not change after NaCl treatment but there was a difference in polypeptide compositions of thylakoid membrane and PS2 particles between the two cultivars. PS2 particles of cv. Pokkali contained more 33-kDa and 43-kDa polypeptides than cv. Peta. Additionally, PS2 particles after NaCl treatment showed deficiency of 23-kDa outside polypeptides of PS2.

Additional key words: electron transport activity; *Oryza*; polypeptides; photosystems 1 and 2.

The effects of salt stress on the composition and function of thylakoids (Maslenkova 1993, Vazquez-Duhalt and Arredondo-Vega 1991, Jeanjean and Matthijs 1993, Diao *et al.* 1997, Parida *et al.* 2005) differ because of different materials and treatment methods. There exists discussion whether or not the oxygen evolution complexes in PS2 are sensitive to NaCl (Andersson *et al.* 1984, Ball *et al.* 1985) and whether salt stress can affect photosynthetic electron transport (Gilmour *et al.* 1985, Fry *et al.* 1986). This is why we studied the photosynthetic electron transport activities and polypeptide compositions of thylakoid membranes from two rice cultivars of different salt-tolerance.

Oryza sativa L. salt-tolerant cv. Pokkali and salt-sensitive cv. Pata were selected from the International Rice Research Institute (IRRI) (Yan and Tan 1991). Rice seeds were surface-sterilized in 0.1 % $HgCl_2$ for 10 min, flushed well with running water, and immersed in distilled water for 24 h. After germinating, seeds were sown in plastic pots with thin sand and planted outdoors. The seedlings were planted outdoor with a photoperiod of 12 light and 12 darkness from May to June, a relative humidity of 60/80%, and a proton flux density of 600–1400 μmol m^{-2} s^{-1}. Rice seedlings at the three leaves and one spindle stage were cultivated with Kimura solution B [48.2 mM $(NH_4)_2SO_4$, 65.9 mM $MgSO_4$, 18.5 mM KNO_3, 24.8 mM KH_2PO_4, 59.9 mM $Ca(NO_3)_2$, and 15.9 mM K_2SO_4] in small turnover boxes and exposed to natural irradiation. Temperature was 30±2/24±2 °C (day/night). Culture solution was renewed every 4 d. After the sixth leaf fully expanded, the seedlings were cultivated in solution B containing 0 and 200 mM NaCl. All solutions were renewed and ventilated for roughly 0.5 h everyday during the treatment. After treatment for 8 d, the leaves of seedlings were sampled and tested.

PS2 particles were prepared according to Berthold *et al.* (1981) and Dunahay *et al.* (1984). About 50−100 g fresh rice leaves were cut, washed, and dried, then irradiated for 20 min, and finally homogenized in 250 cm^3 ice cold buffer B_1 [0.4 M sucrose, 2 mM $MgCl_2$, 0.3 % bovine serum albumin (BSA), 20 mM *Tricine*, pH 8.0]. After the samples were filtered through 8 layers of gauze and centrifuged for 2 min at 300×*g*, the supernatant was centrifuged (10 min, 4 000×*g*) and chloroplasts were deposited, then the pellet was lysed and re-suspended in buffer solution B_2 (0.15 M sucrose, 5 mM $MgCl_2$,

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*Corresponding author; fax: +0086 517 87088009; e-mail: wrl3501988@yahoo.com.cn
Abbreviations: Chl – chlorophyll; DCMU − 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP − dichlorophenol indophenol; MV − methyl viologen; PS − photosystem; SDS-PAGE − sodium dodecyl sulphate polyacrylamide gel electrophoresis.
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Table 1. Effects of NaCl treatment (+) for 8 d on electron transport activities of PS2 and PS1 [mmol kg^{-1}(Chl) s^{-1}] of salt-tolerant cv. Pokkali and salt-sensitive cv. Peta. Means ±SE of three replications.

Cv.	Pokkali		Peta	
NaCl	−	+	−	+
PS2	39.77±3.06	16.83±2.49	32.10±2.40	10.21±2.81
PS1	23.42±2.18	26.11±2.83	46.12±3.15	20.75±2.69

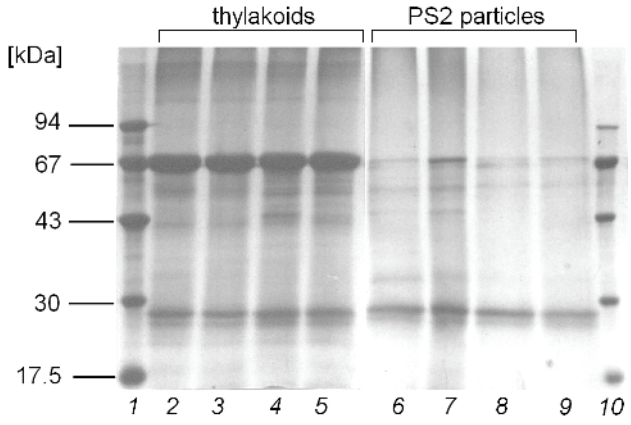

Fig. 1. Effect of NaCl treatment on membrane polypeptides of thylakoids (lanes *2*−*5*) and PS2 particles (lanes *6*−*9*) of rice leaves of cv. Peta without NaCl (lanes *2* and *9*) and with NaCl (lines *3* and *8*) and cv. Pokkali without NaCl (lines *4* and *7*) and with NaCl (lines *5* and *6*). Lanes *1* and *10* are markers.

0.2 % BSA, 20 mM Tricine, pH 8.0) and the thylakoids were deposited by centrifugation. Finally, thylakoids were re-suspended in buffer solution B_3 (15 mM NaCl, 5 mM $MgCl_2$, 20 mM *MES*, pH 6.5). The chlorophyll (Chl) content was 2 kg m^{-3}. Thylakoids were treated with 20 % *Triton X-100* using Chl : *Triton* ratio of 1 : 20 (m/m), and then ice cooled, blended for 30 min, and centrifuged (10 min, 44 000×*g*). The supernatants were centrifuged for 30 min at 123 000×*g*, and pellets (PS2-rich membranes) were re-suspended in the buffer solution B_4 (B_3+0.4 M sucrose, pH 6.5). PS2 particles were then purified from the PS2-rich membrane preparation according to Ghanotakis *et al.* (1987). Samples were frozen in liquid nitrogen and stored at −80 °C. The activity of PS1 and PS2 were measured using thylakoids suspension.

According to the method of Coombs *et al.* (1985), PS1 and PS2 activities were measured with a Clark-type electrode (*Hansatech Instruments*, Norfolk, UK). The measuring solution for PS1 contained 27 g m^{-3} Chl, 0.5 mM sucrose, 50 mM *Tricine*-NaOH (pH 7.6), 5 mM $MgCl_2$, 0.5 mM methyl viologen (MV), 5 mM NH_4Cl, 2 mM NaN_3, 0.5 mM dichlorophenol indophenol (DCPIP), and 2 mM 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU). The PS2 reaction solution contained 27 g m^{-3} Chl, 0.5 mM sucrose, 50 mM *Tricine*-NaOH (pH 7.6), 5 mM $MgCl_2$, 5 mM $K_3Fe(CN)_6$, and 1 mM *p*-phenylenediamine (Li *et al.* 1987).

According to the method of Laemmli (1970), the polypeptide composition of the membranes was analyzed by the SDS-PAGE with a 12.5 %-acrylamide separating gel and a 5 %-acrylamide stacking gel containing 6 M urea. The proteins were visualized by staining with Coomassie brilliant blue G250.

Salt stress damages plant cell membranes (Koca *et al.* 2007), with chloroplasts being the most sensitive organelle to salt stress (Demetriou *et al.* 2007). Electron transport activity of PS2 from the salt-tolerant cv. Pokkali was higher than that of the salt-sensitive cv. Peta in both control and NaCl treatments (Table 1). After 8 d of NaCl treatment, the electron transport activity of PS2 in cv. Pokkali and cv. Peta decreased by 57.6 and 68.2 %, respectively, while that of PS1 decreased by 48.8 and 55.0 %, respectively. These results are in accordance with those of Tiwari *et al.* (1997) and Murata *et al.* (2007), but differ from the report of Fry (1986). Singh and Dubey (1995) found no PS1 activity decline in rice seedlings while PS2 activity decreased significantly. The absorption and fluorescence emission spectrum at room temperature of thylakoid membranes was reduced under NaCl stress (data not shown). This confirms that salt stress might change the construction of thylakoids, destroying the PS2 reaction centre and combination state of Chl molecules in light-harvesting 2 complex (LHC2) (Liu and Shen 2004, 2005).

Although NaCl stress damaged the construction of thylakoids and then affected their photochemical activities, it might just change the space construction of protein complexes and not result in the degradation of peptide components. There was no obvious difference between peptide components of thylakoids and PS2 particles in both cultivars after the NaCl treatment (Fig. 1). The isolated thylakoid membranes contained Psa A/B, CP47, CP43, and 23 kDa proteins (Fig. 1). Contents of these thylakoid proteins did not significantly vary. The electrophoresis result of peptide composition in PS2 particles was similar to that of thylakoids (Fig. 1). There was no significant difference between control and NaCl treatment. The polypeptide composition of PS2 of cvs. Pokkali and Peta was different. PS2 particles of cv. Pokkali contained 33- and 43-kDa peptides compared with cv. Peta. Additionally, NaCl treatment of PS2 particles could result in the deficiency of 23 kDa outside peptides of PS2, which are the key to oxygen evolution (Murata *et al.* 1992). The deficiency of 23 kDa protein or effect of energy transformation in light-harvesting complex 1 (LHC1) is a result of the decrease in photochemical activity (Oquist *et al.* 1980). However, in thylakoids and PS2 particles from rice leaves stressed with NaCl, ion contents of Na^+ and Cl^- were lower than those after treatment *in vitro* (Wang *et al.* 2002). Therefore, we did not find the deficiency of 23 kDa peptides in our experiment (Fig. 1). Such difference may be related to NaCl stress.

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