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BRIEF COMMUNICATION

Photosynthesis in rice under a salt stress

B.S. TIWARI*, A. BOSE and B. GHOSH

Department of Botany, Bose Institute,
93/1 Acharya Praffulla Chandra Road, Calcutta 700 009, India

Abstract

In four cultivars of *Oryza sativa* L., a gradual decrease in the activity of photosystems 1 and 2 as well as in chlorophyll (Chl) fluorescence transients and emission at 688 nm was observed with an increase in NaCl concentration. This decrease was more pronounced in salt-sensitive cultivars as compared to the tolerant ones. A drastic decrease in net photosynthetic rate was found in both cultivar types.

Additional key words: chlorophyll fluorescence; CO_2 assimilation; electron transport rate; *Oryza sativa* L.; photosystems 1 and 2.

Visible outcomes of salinity stress on plants are rather late manifestations of severe stress, and they are difficult to measure. Hence, physiological parameters are the most convenient and suitable mean to study the response(s)/mechanism(s) of salt tolerance. We studied the response of photosynthetic characteristics in two salt-tolerant and two salt-sensitive rice cultivars under NaCl stress. One of the problems observed during the NaCl treatment, especially in the polarographic measurement of photochemical reactions of photosynthesis, is ionic concentration: Na^+ and Cl^- present in the assay medium containing chloroplasts may interact with the charges present on the surface of the chloroplasts, causing the alteration of redox potential (Dominy *et al.* 1983). To confirm our observations made under NaCl stress, in a separate experiment 100, 200, 400, and 800 mM saccharose was added in chloroplast resuspending medium, and activities of photosystems were monitored (results not shown).

Four cultivars of rice (*Oryza sativa* L.), Nona Bokra and Pokkali (salt-tolerant) and IR 29 and IR 8 (salt-sensitive), were obtained and certified from the International

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*Fax: 91-033-350-6790; e-mail: bharati@boseinst.ernet.in
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Rice Research Institute, Phillipines. Seeds, surface sterilized in 0.1 % $HgCl_2$, germinated in Petri dishes in H_2O in the dark for 72 h at 35 °C. The resulting seedlings were grown in 1/2 strength Murashige and Skoog salt solution (pH 5.8) in a growth cabinet, with 14 h photoperiod, irradiance 100 μmol m^{-2} s^{-1}, temperature 28 ± 2 °C, and relative humidity 78 %. Salt treatments were effected by placing 12-d-old seedlings in NaCl solutions (0, 50, 100, 150, and 200 mM) for 6 h. After the treatment, leaves were harvested, chopped, frozen in liquid nitrogen, and stored at -70 °C for subsequent processing.

The net photosynthetic rate (P_N) was measured in excised leaves from salt-treated plants using an infra-red gas analyser (*Analytical Development Co.* model *225-MK 3*) under normal atmosphere [345 mmol(CO_2) m^{-3} and 21 % O_2], irradiance [300 μmol m^{-2} s^{-1}], leaf temperature [28±2 °C], and air humidity [78 %].

Chloroplasts were isolated from leaves following the method of Leegood and Malkin (1986), with slight modifications. Leaf samples (1 g) were ground in 50 mM Hepes-NaOH buffer (pH 7.5), 5 mM Na_2EDTA, 10 mM NaCl, 5 mM $MgCl_2$, and 0.5 M saccharose. Each homogenate was filtered through four layers of gauze, and filtrates were centrifuged at 400×*g* for 2 min. Supernatants were recentrifuged at 5000×*g* for 10 min. The pelleted chloroplasts were suspended in the isolation buffer. The entire procedure was done at 4 °C under a very weak irradiance. Photosynthetic electron transport activities, PS1 (ascorbate/2,6-dichlorophenolindophenol → methyl viologen) and PS2 (H_2O → *p*-benzoquinone/ferricyanide), were measured in the isolated chloroplasts as O_2 evolution/consumption following the method described by Allen and Holmes (1986). To assay the PS1 activity, the reaction mixture (2 cm^3) contained 10 mM Hepes-NaOH (*Sigma*), 5 mM sodium ascorbate (Asc), 1 mM methyl viologen (MV, *Sigma*), 5 μM 2,6-dichlorophenolindophenol (DCPIP, *Sigma*), 1 mM NaN_3, 2 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and isolated chloroplasts containing 30 g(Chl) m^{-3}. The PS2 activity was assayed using reaction mixture containing in a final volume of 2 cm^3 10 mM Hepes-NaOH (pH 7.5), 5 mM $MgCl_2$ (*Sigma*), 1 mM *p*-benzoquinone (BQ, *Sigma*), 1 mM potassium ferricyanide (FeCN), and chloroplasts containing 30 g(Chl) m^{-3}. All activities were measured at 25 °C with an oxygen electrode (*Hansatech*, Kings Lynn, Norfolk, U.K.).

Chl fluorescence emission spectra were determined on a *Perkin Elmer* spectrofluorimeter model *MPF 44 B*. The chloroplast suspension was excited at 468 nm, and emission was recorded between 500-800 nm. The variation in emission peak at 688 nm was taken into consideration. The excitation wavelength was selected by recording excitation spectrum of chloroplast suspension in the wavelength range of 400-650 nm. Chl fluorescence transient curves were measured in a *Hansatech DW 2/2* unit with an *FDP/2* detector probe. The initial fluorescence (F_0) was measured after at least 30 min dark adaptation of chloroplast suspension containing 3 g(Chl) m^{-3}, and maximum fluorescence (F_m) was obtained by exposing the chloroplast suspension to a red radiation pulse. Variable fluorescence (F_v) was calculated as $F_m - F_0$.

With increasing concentration of NaCl the activity of photosynthetic electron transport, *i.e.*, PS1 and PS2 in the chloroplasts from all four cultivars of rice decreased. This decrease was more pronounced in salt-sensitive cultivars as

compared to tolerant ones (Fig. 1). The reduction in PS2 activity was more severe than that of PS1 in both cultivar types.

Presence of high NaCl concentration in the chloroplasts from salt-stressed plants has been reported by Robinson *et al.* (1983), and the reduction in photochemical reactions of isolated chloroplasts by NaCl was consistent with the observations made by Baker (1978) and Wignarajah and Baker (1981). Nevertheless, Singh and Dubey (1995) did not find a decline in PS1 activity of rice chloroplasts, while the PS2 activity was much reduced. The greater sensitivity of PS2 activity upon environmental stresses has been reported by Havaux (1992, 1994). The target site of salt stress may be the dissociation of the 23 kDa polypeptide extrinsically bound to PS2 which plays major role in oxygen evolution (Kuwabara and Murata 1982, Miyao and Murata 1983, 1984, Murata *et al.* 1992). The decrease in activity of photochemical reactions may be due to dissociation of the 23 kDa polypeptide or to partial impairing of energy transfer in Chl/PS1 protein complex (Öquist *et al.* 1980, Valcke and Poucke 1983).

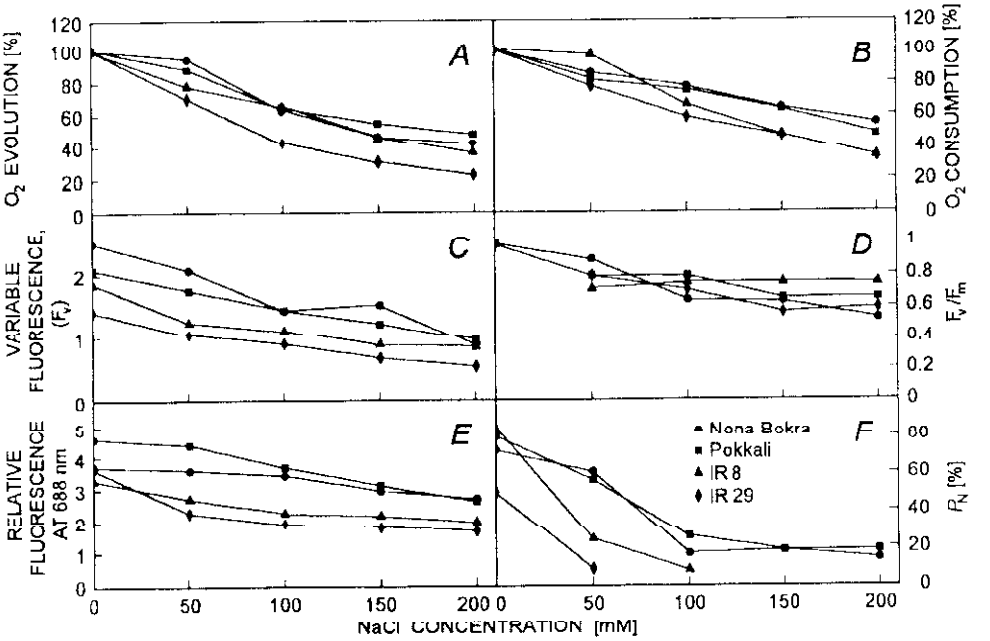

Fig. 1. Effects of salt stress on photosystem (PS) 2 activity (*A*), PS1 activity (*B*), variable chlorophyll fluorescence, F_V (*C*), F_V/F_m ratio (*D*), fluorescence emission peak at 688 nm (*E*) in isolated chloroplasts, and net photosynthetic rate, P_N (*F*) of excised leaves of 12-d-old rice seedlings treated with 50, 100, 150, and 200 mM NaCl solutions for 6 h. 100 % values for Nona Bokra, Pokkali, IR 8, and IR 29, respectively, were for PS2 = 72, 31, 30, 63 mol(O_2 evolved) kg^{-1}(Chl) s^{-1} (n = 3, SD ± 0.005), for PS1 activity = 182, 46, 39, 20 mol(O_2 consumed) kg^{-1}(Chl) s^{-1} (n = 3, SD ± 0.04-0.06), and for P_N = 0.018, 0.020, 0.022, 0.012 g(CO_2) kg^{-1}(Chl) (n = 3, SD ± 0.002).

All measured parameters of fluorescence decreased with NaCl stress and did so

more in sensitive than in tolerant cultivars (Fig. 1). This observation supports the above-mentioned findings of Öquist *et al.* (1980), Kuwabara and Murata (1982), Miyao and Murata (1983, 1984), Valcke and van Poucke (1983), Murata *et al.* (1992), and Singh and Dubey (1995). The P_N measured in excised leaves of salt-stressed plants decreased remarkably with increasing level of salinity (Fig. 1*F*). At 50 mM NaCl, a 70 and 80 % inhibition in P_N was observed in the sensitive cultivars IR 8 and IR 29. In the tolerant cultivars, P_N activity was measurable upto 200 mM NaCl.

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