

# Sodium Chloride Salt Stress Induced Changes in Thylakoid Pigment-Protein Complexes, Photosystem II Activity and Thermoluminescence Glow Peaks

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In the present study, mung bean (*Vigna radiata* L.) – a salt susceptible and Indian mustard (*Brassica juncea* L.) – a salt resistant crop was studied to find out the differences in stress responses of these crops. Seedlings were grown in water soaked cotton under continuous illumination of  $35 \mu\text{mole m}^{-2} \text{s}^{-1}$  at  $26 \pm 1^\circ\text{C}$ . Salinity treatment of 0, 0.5 and 1.0% (w/v) was given to the seedlings at 6 day. Photosynthetic pigment content and PS II electron transport activity was reduced under salinity in both mung bean and Indian mustard. The pigment protein pattern of both the crops were similar. Ratio analysis of B and Q thermoluminescence (TL) glow peaks suggested that  $\text{S}_2\text{Q}_\text{A}$ - charge recombination was relatively more affected than  $\text{S}_{2/3}\text{Q}_\text{B}$ - charge recombinations.

## Introduction

Salinity affects growth and metabolism of plants (Misra *et al.* 1995, 1996, 1997a, b). Chloroplasts are the most sensitive organelles affected by salt stress (Lapina and Popov, 1970). Any change in the structure and function of the chloroplast will affect the function of the organelle which in turn affect the ultimate yield of plants. Photosynthetic pigments are reported to change with the plant genotypes, the system used and the time period of stress imposed (Misra *et al.*, 1995, 1997a). Chlorophyll content was reported to decrease in salt susceptible crops like tomato (Lapina and Popov, 1970), potato (Abdullah and Ahmed, 1990), pea (Hamada and El-Enany, 1994) and *Phaseolus vulgaris* (Seemann and Critchley, 1985). But chloro-

phyll content was reported to increase in salt tolerant crops like pearl millet (Reddy and Vora, 1986), mustard (Singh *et al.*, 1990) and wheat (Kulashrestha *et al.*, 1987). Similarly the variability in the response of photosystem activities are reported.

Salinity affects the chloroplast ultrastructure and inhibits photochemical activity (Boyer 1976). This is sharp contrast to the report on the enhancement of PS I and PS II activity at lower NaCl concentrations followed by no significant effect on NaCl on barley seedlings (Sharma and Hall 1991). Halophytes and mangroves also showed an enhancement in the activity of PS II under NaCl salinity (Venkatesalu and Chellappan, 1993). These observations on the changes in chloroplasts also suggest that there is a genetic variation in the response of crop plants to NaCl salinity. This hypothesis is verified in this study by using a salt tolerant crop Indian mustard and a salt susceptible crop mung bean.

However, the site of action of NaCl on the alteration in chloroplast function is not well deciphered. The site of action of NaCl in changing PS II activity can be studied by using thermolumines-

**Abbreviations:** B band, thermoluminescence band at  $30^\circ\text{C}$ ; Chl, Chlorophyll; DCMU, 3-(3-4-dichlorophenyl)-1,1-dimethylurea; PS II, photosystem II;  $\text{Q}_\text{A}$ , the primary quinone electron acceptor in the reaction center of photosystem II; Q band, thermoluminescence band at  $10^\circ\text{C}$ ;  $\text{Q}_\text{B}$ , the secondary electron acceptor in the reaction center of photosystem II; TL, thermoluminescence.

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cence (TL) characteristics of chloroplasts (Misra *et al.*, 1997b). Thermoluminescence is a simple but very powerful tool for the investigation of photochemistry of PS II. It has been used extensively for characterising the mode of action of herbicides (Horvath, 1986) and the study of the alterations in the acceptor and donor side of PS II (Sane and Rutherford, 1986; Demeter and Govindjee, 1989; Vass and Inoue, 1992; Inoue, 1995). The TL glow curves have distinct temperature maxima representing characteristic charge pairs. The B band which is well characterised and is due to  $Q_{2/3}Q_B$ -charge recombinations appear at around +30 °C (Rutherford *et al.*, 1982; Demeter and Vass, 1984). However, treating thylakoids with DCMU abolishes the B band and generates a new band around +10 °C. This band is ascribed to  $S_2Q_A$ - and is known as Q band (Rutherford *et al.*, 1982; Demeter and Vass, 1984).

Changes in the photosynthetic pigment, Chl-protein contents, PS II activity and thermoluminescence glow peaks of thylakoids from stressed seedlings are studied to characterise the site of action of NaCl salinity on determining genetic variations in the response of mungbean and Indian mustard.

## Materials and methods

Mung bean (*Vigna radiata* L. Wilczek cv. Sujata) and Indian mustard (*Brassica juncea* Coss. cv. Pusa Bold) seedlings are grown in water soaked cotton under continuous illumination of 35  $\mu\text{mole m}^{-2} \text{s}^{-1}$  at  $26 \pm 1$  °C. Salinity treatment of 0, 0.5 and 1% (w/v) NaCl was given to the seedlings at 6 day. Photosynthetic pigment contents was measured as described by Misra *et al.* (1997 b).

Chloroplasts were isolated by grinding the leaf discs with a chilled mortar and pestle in ice chilled buffer containing Tricine 30 mM, pH 7.8, NaCl 0.3 M, and EDTA 1 mM. The homogenate was filtered through 8 layers of cheese cloth. The filtrate was centrifuged at  $500 \times g$  for 5 min. The chloroplast pellet was washed with MOPS 0.2 M, pH 7.2, containing sucrose 0.2 M and NaCl 20 mM. Chloroplast was suspended in media containing MOPS 20 mM, pH 7.2, NaCl 30 mM and BSA 0.2 mg. The electron transport ability of PS II was assayed using MOPS 20 mM, pH 7.2, NaCl 30 mM, chloroplast equivalent to 10 mg Chl, Gramicidin 2.5 mM

and  $K_3Fe(CN)_6$  400 mM. Oxygen evolution was measured using a Clark type oxygen electrode at 25 °C in a rate saturating red light.

Chloroplasts (equivalent to 20  $\mu\text{g}$  Chl) kept for in darkness (de-excited) were excited with sequence of 2 flashes (General Radio, Stroboslave) with a half-decay time of 3  $\mu\text{s}$  (0.5 J) white light and quickly frozen to 77K by dropping them into the Dewar containing liquid nitrogen. TL glow curves were recorded as described by Sane *et al.* (1977). To inhibit electron transfer between  $Q_A$  and  $Q_B$ , in the reaction center of PS II, 5  $\mu\text{l}$  of 10 mM DCMU was added to the chloroplast sample before illumination. The glow peaks arising at +30 °C and +10 °C are designated as B band and Q band, respectively (Inoue, 1995).

## Results and Discussion

Mung bean is a salt susceptible (Misra *et al.*, 1996) and Indian mustard is salt tolerant crop (Misra *et al.*, 1995). So it is assumed that their responses to salinity might vary. Chlorophyll content of both the plants decreased with salinity treatments, except that of an enhancement at 0.5% level at 8 day (Fig. 1). This type of enhancement in chlorophyll content at 0.5% salinity treatment in the field grown Indian mustard was reported (Misra *et al.* 1995). However, in the susceptible mungbean plant no enhancement in the pigment content was observed. A variation in the pigment content of rice genotypes under NaCl salinity is reported recently (Misra *et al.*, 1997b). Loss of photosynthetic pigment under salinity is suggested to be an effect of NaCl either on the retardation of synthesis and/or acceleration of pigment degradation. Salinity induced an enhancement in the chlorophyllase activity in pigeon pea and gingelly (Rao and Rao 1981).

All the pigment protein complexes of the thylakoids are present both in the control as well as in the salt stressed plants of mungbean and Indian mustard (Fig. 2). The pigment protein complexes of the salt stressed seedlings of mung bean showed a gradual increase in the polypeptide contents with an increase in the NaCl concentration. However, that of the *Brassica* decreased with an increase in salinity levels (Fig. 2). The differences in these responses could be due to the genotypic differences in the stability of Chl compared to that of thyla-

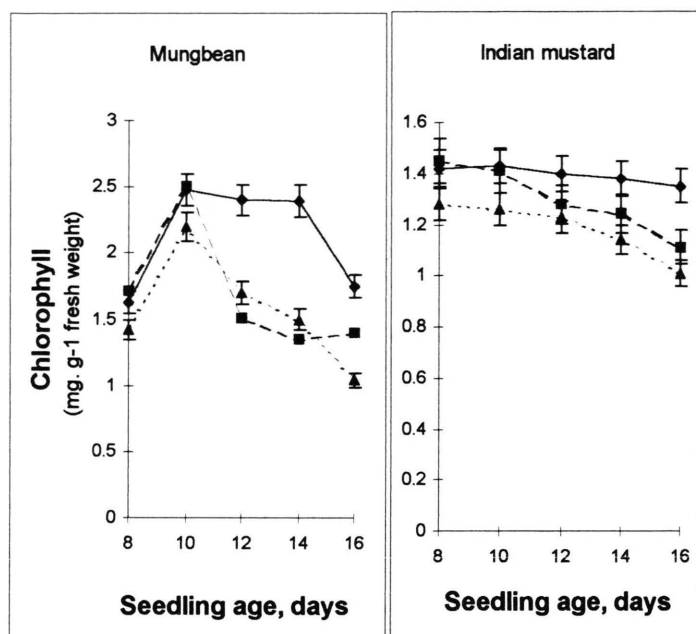


Fig. 1. Changes in chlorophyll (a+b) content of mung bean and Indian mustard seedling grown under NaCl salinity (control, 0% NaCl – circles; 0.5% NaCl – squares; 1.0% NaCl – triangles). The data are mean  $\pm$  S. E. of 5 separate experiments.

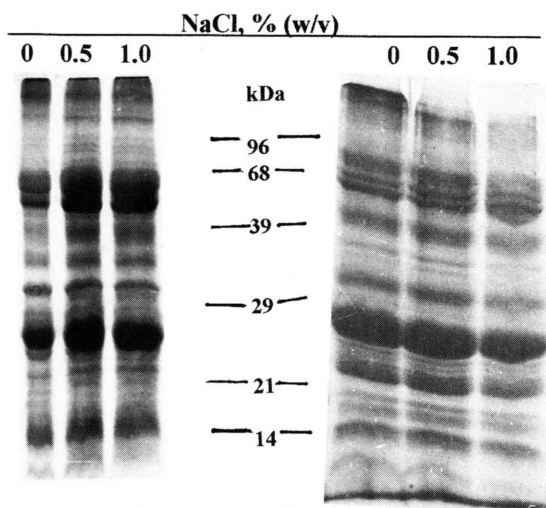


Fig. 2. SDS-PAGE of thylakoid membrane proteins from mung bean (A) and Indian mustard (B). Each lane is loaded with equal amount of thylakoid membranes as measured by equal amount of Chl for respective crops.

koid membrane proteins. As the lanes are loaded with SDS-solubilised thylakoid membranes on equal Chl basis, relatively faster rate of degradation of Chl in mung bean compared to that of *Brassica* (Fig. 1) could account for the increase in the Chl-

protein complexes in the gel (Fig. 2). A decrease in the polypeptide contents in SDS-PAGE of Indian mustard seedlings suggests salinity induced proteolysis of thylakoid membrane proteins (Misra *et al.*, 1995, 1997b).

Photoelectron transport activity of mung bean and Indian mustard decreased with age and salinity (Fig. 3). Aging induced decrease in PSII activity of chloroplasts is a well known phenomenon (Misra and Biswal, 1982; Misra and Misra, 1986). Salinity treatment of 0.5% had marginal effect on PSII activity of chloroplasts from 8 day old seedlings of mung bean and Indian mustard (Fig. 3). However, NaCl concentration of 1% (w/v) showed 29% inhibition in PS II activity of chloroplasts from Indian mustard and the inhibition was more than 46% in mung bean. The relative effect of NaCl stress on mung bean is more prominent compare to that of Indian mustard seedlings. This could be due to genetic variations between these two crop plants to salinity.

The decrease in PSII activity suggests that electron transport from water to ferricyanide is affected. However, it is not clear whether the site of damage is at the oxygen evolving complex, or at the reaction center of PSII itself, or a site beyond it. We have used thermoluminescence technique to

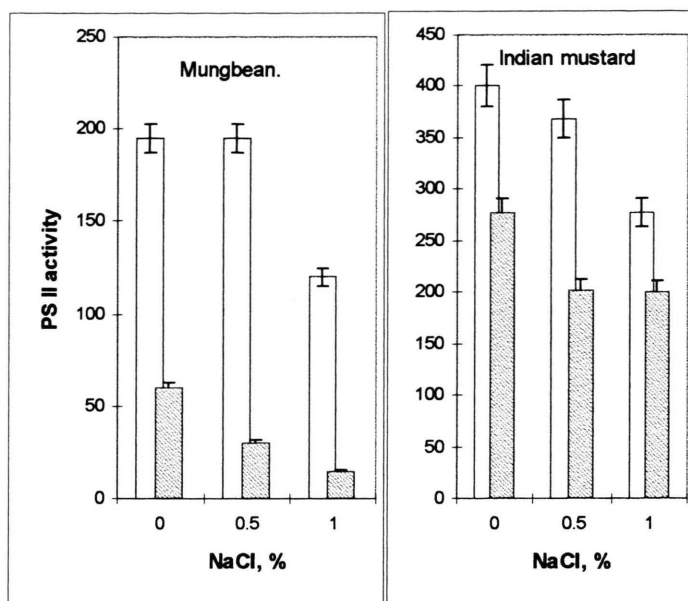


Fig. 3. Changes in PS II activity (measured from water to ferricyanide) of mung bean and Indian mustard chloroplasts isolated from leaves of NaCl salt stressed seedlings. Each data is mean  $\pm$  S. E. of 3 separate experiments. The open and hatched histograms represent 8 day and 14 day, respectively.

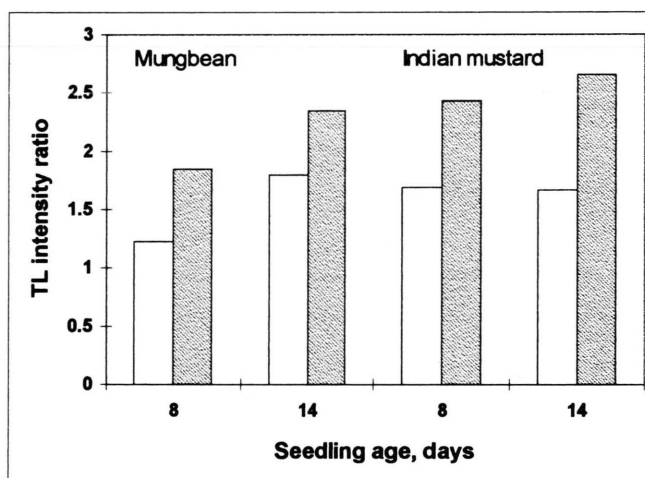


Fig. 4. Changes in the TL intensity ratio of  $Q_B$  (peak IV) and  $Q_A$  (peak II) of mung bean and Indian mustard chloroplasts isolated from leaves of NaCl salt stressed seedlings. Each data is an average of 2 separate experiments. The open histograms represent control and hatched histograms represent 1.0% NaCl salt stressed seedlings.

decipher the lesion in PSII (Misra *et al.*, 1997c). Salinity induced changes in the relative intensity ratio of TL bands B and Q is shown in Fig. 4. The TL intensity ratio gives information about the relative susceptibility of  $S_{2/3}Q_B$ - and  $S_2Q_A$ - charge recombinations in the PS II reaction center. Although, earlier studies utilised the TL peak intensities as a parameter to quantify the changes in the charge pair formation, here we are giving the ratio analysis for deciphering a subtle difference between the susceptibility of these charge pairs to abiotic stress. The major TL peak arising in DCMU untreated

chloroplasts at around  $+30^\circ\text{C}$  and at  $+10^\circ\text{C}$  is ascribed to  $S_{2/3}Q_B$ - and  $S_2Q_A$ - charge recombinations, respectively (Sane and Rutherford, 1986; Inoue, 1995). Ratio analysis of the TL intensities of thylakoids from 1% NaCl treated seedlings showed an increase over the control value. There was an enhancement in the TL intensity of B band, and a decrease in the intensity of Q band at 1% NaCl level (data not shown). This relative susceptibility of Q band suggests that  $S_2Q_A$ - charge recombination is affected by salinity in the seedlings of both salt susceptible and salt tolerant crops.



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