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EFFECT OF HIGH SALT STRESS ON PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES IN THE CYANOBACTERIUM, *SPIRULINA PLATENSIS*

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ABSTRACT
In this present study an attempt has been made to study the effect of high salt stress (0.8M NaCl) on the electron transport properties of the intact cells of *Spirulina platensis*. 9 h treatment of salt stress (0.65 M-0.8 M NaCl) caused significant inhibition in both whole chain electron transport and Photosystem (PS II) catalyzed electron transport assay. A 42% inhibition was noticed at 0.8M NaCl treatment. Similarly the loss in variable Chl *a* fluorescence was also observed. The light intensity measurements of PS II activity clearly demonstrated that the inhibition was more at light saturating conditions (400 Wm^{-2}) than that at light limiting conditions (50 Wm^{-2}). The possible reason for the loss of PS II activity could be alterations in light harvesting complex of the above cyanobacteria.

INTRODUCTION

Environmental stresses like temperature, high light, UV radiation and salt stress are known to affect the efficiency of photosynthesis (Stainer and Cohen –Bazire, 1977; Tandeau de Marsac and Houmard, 1993). Salt stress inhibits fundamental processes such as plant growth and productivity which are often associated with the decreased photosynthesis (Greenway and Munns,1980). A number of studies have been performed to identify the target of salt stress in the photosynthetic electron transport system. Ionic stress due to 0.5 M NaCl inactivated photosynthetic machinery in *Synechococcus* sp (Allakhverdiev *et al*., 2000). In *Synechocystis* sp 0.55 M NaCl decreased the PS II activity and stimulated respiratory electron transport activity (Jeanjean *et al*., 1993). The decrease in PS II activity in *Chlamidomaonas reinhardtii* has been found to be associated with state 2 transitions (Endo *et al*., 1995). Biswal *et al.,* (2002) have revealed that salt stress (0.5 M NaCl) caused inhibition in photosynthetic electron transport due to the destabilization of Q_A and Q_B (the primary and secondary electron acceptors of PS II respectively) in *Brassica junica*.L. In *Triticum aestivum* light enhanced the inhibitory effect of salt stress on PS II efficiency (Mishra *et al*.,1991).

The accumulation of intracellular sodium ions due to salt stress changes the ratio of K: Na seems to affect photosynthetic electron transport as well as PBPs, the major light harvesting complex in cyanobacteria (Verma and Mohanty, 2000b). PBPs have been found to be very susceptible to environmental stress conditions (Grossman *et al*.,1993). It has been demonstrated that PC is sensitive to salt stress and there by disturbs the energy transfer: the content of PC decreased in salt stressed *Synechocystis* (Schubert *et al*., 1993) and *Spirulina* cells (Lu and Vonshak, 2002). Lu and Vonshak (1999) found that the ratio of PC/Chl *a* was decreased in the salt adapted (0.8M NaCl) *Spirulina* cells.

Cyanobacteria have been used extensively for the studies on energy transfer and electron transport activities. Hence by using cyanobacterial system (*Spirulina platensis*), an attempt has been made to study the effect of high salt stress (0.65M and 0.8 M NaCl) at moderate light intensity (80μM photons m^{-2} s^{-1}) on whole chain electron transport activities.

MATERIALS AND METHODS

Spirulina platensis was grown in Zarrouk's medium (Zarrouk,1966) under continuous illumination at 25 ± 2^{o}C. The mid log phase cells were collected and exposed to 0.65 M-0.8 M NaCl under white light for 9 h. cells were collected by centrifugation and suspended in 25 mM HEPES - NaOH (pH 7.5) buffer and proceeded for both electron transport as well as spectral property measurement. Whole chain electron transport activity was measured in whole cells with

Table 1: Effect of salt stress (0.65 M and 0.8 M NaCl) on whole chain electron transport (WCE) activity of *Spirulina platensis*. 2 mLreaction mixture contains reaction buffer (25 mM HEPES – NaOH, (pH 7.5) containing 20 mM NaCl), 50 μM MV, 1 mM Na – azide and cells equivalent to 12 μg of Chl *a*

Assay	NaCl concentration	Activity μmol O_2 consumed (mg chl)$^{-1}$ h^{-1}	% inhibition
$H_2O \rightarrow MV$	0.00	281 ± 13	0
	0.65	237 ± 10	16
	0.85	64 ± 11	42

Table 2: Effect of salt stress (0.8 M NaCl) on DPC catalyzed electron transport activity of *Spirulina platensis*. 2 mLreaction mixture contains reaction buffer (25 mM HEPES – NaOH, (pH 7.5) containing 20 mM NaCl), 0.5 mM DPC, 50 μM MV and cells equivalent to 12 μg of Chl *a*

Assay	NaCl concentration	Activity μmol O_2 consumed (mg chl)$^{-1}$ h^{-1}	% inhibition
DPC→MV	0.00	303 ± 14	0
	0.65	260 ± 11	14
	0.85	174 ± 9	42

Table 3: Effect of salt stress (0.65 M and 0.8 M NaCl) on PS II electron transport activity of *Spirulina platensis*. 2 mL reaction mixture contains reaction buffer (25 mM HEPES – NaOH, (pH 7.5) containing 20 mM NaCl), 0.5 mM pBQ and cells equivalent to 12 μg of Chl *a*

Assay	NaCl concentration (M)	Activity μmol O_2 evolved (mg chl)$^{-1}$ h^{-1}	% inhibition
$H_2O \rightarrow pBQ$	0	0.00	415 ± 14
	16	0.65	349 ± 11
	42	0.85	239 ± 12

Table 4: Effect of illuminated light intensity on salt stress (0.8 M NaCl) induced inhibition of electron transport catalyzed by PS II. Other details were mentioned in Table 3

Light intensity Wm^{-2}	μmol O_2 evolved (mg Chl) $^{-1}$ h^{-1} $H_2O \rightarrow pBQ$		% inhibition
	Control	0.8 M NaCl	
400	415 ± 14	239 ± 12	42
300	334 ± 9	210 ± 11	37
100	180 ± 8	125 ± 6	31
50	115 ± 5	83 ± 5	29

methylviologen as terminal acceptor (Robinson *et al*., 1982). PS II catalyzed electron transport assay mixture contained intact cells, reaction buffer and 0.5 μM pBQ (Allen and Holmes, 1986). Induction of Chl *a* fluorescence was made with pulse amplitude modulated (PAM 101, Heinz walz GmbH, Effeltrich, Germany). The fluorescence original (F_o) and fluorescence maximum (Fm) were measured according to Campbell *et al.,* (1998). Fo was measured with a weak modulated light intensity (1 mWm^{-2}). The Fm level of fluorescence was recorded during saturating white light obtained from a halogen lamp.

RESULTS AND DISCUSSION

Several studies revealed that salt stress influences the photosynthetic electron transport activities in cyanobacteria.

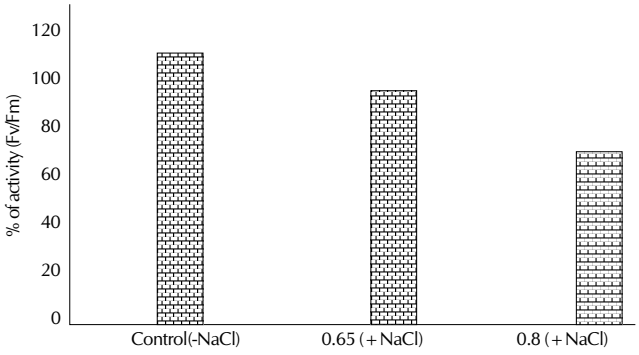

Figure 1:- Effect of salt stress on variable fluorescence (Fv) of intact cells of *Spirulina platensis*

(Jeanjean *et al*., 1993; Allakhverdiev *et al*., 2000). The increase in the salt concentration from 0.65 M to 0.8 M caused 42% inhibition in both whole chain ($H_2O \rightarrow MV$) and PS II catalyzed electron transport activities ($H_2O \rightarrow PBQ$) (Table 1 and 2). The possible reason for the above inhibition could be alterations at water oxidation complex. To verify the above proportion partial electron transport activity was measured from DPC→MV. DPC catalyzed electron transport activity is known to by-pass the oxidizing side of PS II. This measurement also clearly suggested that the extent of inhibition is similar to that of PS II catalyzed electron transport where H_2O acted as electron donor. These results are in agreement with the observations regarding *Synechococcus sp.* reported by Allakhverdiev *et al*., (2000).

To identify the target site in PS II catalyzed electron transport the Hill reaction of control and salt stressed samples have been measured at both light limiting and light saturating conditions (Table 4). The inhibition of PS II activity was more at light saturating conditions (400 Wm^{-2}) than at light limiting conditions (50 Wm^{-2}) in salt stressed samples. The possible reason for the inhibition at light limiting conditions could be alterations in light harvesting complex of PS II. The similar report has been reported in spirulina cells under mercury stress by Murthy *et al*., (1989). To relate the salt stressed inhibition of PS II activity, an attempt has been made to measure Chl *a* fluorescence kinetics using PAM kinetic fluorimeter. The ration of variable fluorescence to maximal fluorescence decreased from 100% to 60% as shown in Fig.1. This loss is due to the inhibition of PS II activity could be due to the damage of reaction centre of PS II (Campbell *et al*., 1998). Thus Chl *a* fluorescence kinetics can be used as an indication to know the status of PS II photochemistry under salt stress.

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