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Combination stress mediated alterations in the photosynthetic electron transport activities of Cyanobacterium; *Spirulina platensis*

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ABSTRACT

Exposure of intact cells of the Cyanobacterium; Spirulina platensis to low temperature (10°C-25°C) and low intensity white light stress (100 Wm^{-2}) caused inhibition in various partial photochemical reactions. When the stress is applied in combination there is an additional loss of 6-8% activity in the electron transport measurements. Thus PS II seems to be more susceptible to these combination stresses when compared to PS I .Thus low temperature in combination with low white light intensity exhibited synergistic effect in the electron transport activity of Spirulina platensis.

Key words: Electron transport activities, Low temperature stress, *Spirulina platensis*, white light stress.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes whose photochemical functions are identical to those of the higher plant [1] ; they both perform oxygenic photosynthesis. The prokaryotic nature of the Cyanobacteria makes them an attractive system for studying the molecular organization of photosynthetic apparatus [2-3]. In Cyanobacteria the phycobiliproteins constitute the major light harvesting pigments which are attached to the outer surface of the thyalokid membranes in aggregated complex called phycobilisomes (PBsomes) [4-5] are connected to the Photosystem (PS) II and PS I [6-7]. Environmental factors such as temperature, UV-light, dought, salinity, hight light are known to affect the photosynthesis in cyanobacteria and plants. The intact filaments of *Spirulina platensis* have been used as a test system to characterize the effects of various stresses [8-11]. But in the environment the stress factors may work either alone or in combination and affect the photosynthetic electron transport activities. Several reports on various stress factors on the basic electron transport activities on *Spirulina platensis* were reported earlier but the stress factors in combination were scanty. So in this investigation we have made an attempt to study the low temperature stress and low intensity white light stress in combination on the photosynthetic electron transport activities of the cyanobacterium ; *Spirulina platensis*.

MATERIALS AND METHODS

Spirulina platensis was grown axenically in the medium of Zarrouk (1966) [12] at 25 ± 2 ^{0}C. Under continuous irradiance of 40 µ mol (photon) m^{-2} S^{-1}. Cells from the late log grown cultures were harvested by centrifuging at 6,000 Xg for 10 min. The collected cells were suspended in 25 mM HEPES-NaOH buffer (pH 7.5) at a Chl concentration of 200 µg ml^{-1}. Samples were incubated at low temperature (10-25 ^{0}C) for 45-60 min in the Petri dishes under constant stirring. The reaction mixure used for the assay of whole chain electron transfer (H_2O → methyl vilogen(MV) contained reaction buffer 25 mM HEPES-NaOH buffer, (pH 7.5), 0.5 mM MV and 1 mM Na-azide [13]. The reaction mixture for PS II catalysed electron transfer (H_2O → *p*-benzoquinone(pBQ) contained the above mentioned reaction buffer and 0.5 mM pBQ [9,14]. Thylakoid membranes were prepared according to the method of Rajagopal (1999) [15]. The reaction mixture of PS I catalysed electron transfer ($DCPIPH_2$ → MV) contained reaction buffer, 0.1 mM DCPIP, 5 mM ascorbate, 1 mM azide, 10 µM DCMU, and 0.5 mM MV. In all assay cells equivalent to 15 µg of Chl were used. All the photochemical activities were measured at saturated light intensity of white light (410 Wm -2) under continuous stirring. Low light intensity when required was provided by passing the light through calibrated neutral density filters. Chl content was estimated by following the methods of Mackinney (1941) [16] by using methanol extraction.

Cold stress and low intensity white light treatment:
Cells from the late log grown cultures were harvested by centrifuging at 6,000 X g for 10 min. The collected cells were suspended in 25mM HEPES-NaOH buffer (pH 7.5) at a Chl conc of 200 µg mL-1. Samples were incubated at low temperature (10-25^{o}C) and exposed to low intensity white light (100Wm^{-2}) for short term (15-60 min) in conical flasks under constant stirring and controlled experimental conditions.

RESULTS AND DISCUSSION

Stress is an unfavorable condition in which environmental factors are working together or alone to influence the physiological activities of the plants where the plant productivity will be less. This level of alteration in the plants growth depends on the intensity, duration of stress and its combination with others. These different stressors influence the plant growth and other physiological activities like photosynthesis. Since photosynthesis is a fundamental and essential process there is a need to study the effect of stress either alone or in combination. Up to now the studies are being made in higher plants and some of selected cyanobacterial cells. Therefore there is a need to study the effect of stress in combination to have an integrated approach of the problem. Hence we have studied the effect of low white light (100 Wm^{-2}) in combination with low temperature stress. Control cells showed a high rate of O_2 consumption involving whole chain electron transport (241µmole O_2 consumption mg Chl^{-1} h^{-1}). Exposure of cells to different low temperatures in combination with white light (100 Wm^{-2}) caused strong inhibition in the electron transport activity. When compared to that of cells exposed to only low temperature stress. At 10°C of low temperature alone caused 56 % inhibition in whole chain electron transport activity (Table1). Where as in combination the inhibition was brought to 62 % (Table 2). This clearly demonstrates the additive effect of white light in bringing the inhibition of low temperature stressed samples (Table 1 and 2). Since whole chain electron transport activity is contributed by both PS II and PS I an attempt have been made to study the additive effect of white light in the electron transport activities. Control cells exhibited a high rate of PS II dependent O_2 evolution activity (354 µmole O_2 evolution mg Chl^{-1} h^{-1}). The application of low temperature stress independently caused loss in PS II photochemistry by 65 % (Table 3). When the low temperature stress is combined with white light and applied there is an increase in the inhibition up to 71 % (Table 4).The possible reason for the inhibition of PS II activity could be alterations at water oxidation complex (WOC). To verify the susceptible nature of PS I the electron transport activity has been measured using $DCPIPH_2$ as donor. Control activity is equal to (431µmole O_2 consumption mg Chl^{-1} h^{-1}) application of low temperature stress (Table 5) along with white light brought the inhibition in PS I activity from 17 to 26 % (Table 6) . This shows clearly the susceptible nature of PS I towards low temperature stress and white light stress. (Table 5 and 6). When the stress is applied in combination and there is an addition loss of 6-8 % activity was noticed in the above electron transport measurements when compared to the application of low temperature stress alone. Thus low temperature stress exhibits synergistic effect when it is combined with white light in the electron transport activity in the intact cells of *Spirulina platensis*.

RESULTS

Table 1: Effect of low temperature (25-10°C) on whole chain electron transport ($H_2O \rightarrow MV$) of the cyanobacterium, *Spirulina platensis*

Temperature ^{0}C	Whole chain electron transport activity ($H_2O \rightarrow MV$) µ moles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}	Percentage inhibition
25	252 ± 21	0
20	214 ± 19	15
15	170 ± 16	29
10	111± 10	56

Table 2: Effect of low intensity white light (100 Wm^{-2}) and low temperature induced inhibition of whole chain electron transport in the intact cells of cyanobacterium, *Spirulina platensis*.

Temperature °C	Whole chain electron transport activity ($H_2O \rightarrow MV$) µ moles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}	Percentage inhibition
25	241 ± 23	0
20	202 ± 21	17
15	152 ± 17	37
10	92± 10	62

Table 3: Low temperature induced alteration in PS II catalysed electron transport activity ($H_2O \rightarrow$ *p*-BQ) of the intact cells of the cyanobacterium *Spirulina platensis*

Temperature ^{0}C	PS II electron transport activity ($H_2O \rightarrow$ *p*-BQ) µ moles of $O_2\uparrow$mg Chl^{-1} h^{-1}	Percentage inhibition
25	361 ± 33	0
20	282 ± 26	22
15	209 ± 19	42
10	126 ± 11	65

Table 4: Effect of low intensity white light (100 Wm^{-2}) and low temperature induced Inhibition of PS II catalysed electron transport of intact cells of the cyanobacterium, *Spirulina platensis*

Temperature ^{0}C	PS II electron transport activity ($H_2O \rightarrow$ *p*-BQ) µ moles of $O_2\uparrow$mg Chl^{-1} h^{-1}	Percentage inhibition
25	354 ± 34	0
20	265 ± 27	25
15	196 ± 18	45
10	105 ± 10	71

Table 5: Low temperature induced alterations in PS I catalysed electron transport activities of the ($DCPIPH_2 \rightarrow MV$) intact cells of the cyanobacterium, *Spirulina platensis.*

Temperature ^{0}C	PS I electron transport activity ($DCPIPH_2 \rightarrow MV$) µ moles of $O_2 \downarrow$ mg Chl^{-1} h^{-1}	Percentage inhibition
25	429 ± 41	0
20	403 ± 38	6
15	382 ± 37	11
10	356 ± 33	17

Table 6: Effect of low intensity white light (100 Wm^{-2}) and low temperature induced inhibition of PS I catalysed electron transport intact cells of the cyanobacterium, *Spirulina platensis*

Temperature ^{0}C	PS I electron transport activity ($DCPIPH_2 \rightarrow MV$) µ moles of $O_2 \downarrow$mg Chl^{-1} h^{-1}	Percentage inhibition
25	435 ± 42	0
20	401 ± 39	8
15	360 ± 35	18
10	325 ± 31	26

CONCLUSION

Low temperature is one of the most important abiotic factors which limit the growth, productivity and distribution of plants. The low temperature (10°C) is able to cause alterations in the PS II catalysed electron transport activity in the thylakoid membranes of intact cells of *Spirulina*. Cold stress causes alterations in the energy transfer of Phycobilisomes under *in vivo* and *in virto* conditions [17]. Low temperature stress in combination with low white light (100 Wm^{-2}) exerts additional inhibition in the electron transport catalysed activities than its individual effect in the electron transport activities.

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