CHAPTER III

Results
The artificial electron acceptor MV which accepts electron at the reducing side of PS I has been shown to have a free-access to the thylakoid membranes in the case of intact cells of *Spirulina* (Robinson et al., 1982). Therefore, we have studied the effect of cold stress on the whole chain electron transport activity (H₂O→MV) in intact cells of *Spirulina platensis* (Table 5). Control cells showed a high rate of O₂ consumption involving whole chain electron transport chain (252 μmole O₂ consumed mg Chl⁻¹ h⁻¹). Exposure of cells to temperatures for 45 min caused temperature dependent inhibition in the whole chain electron transport activity. 56 % inhibition in whole chain electron transport activity was noticed at 10°C low temperature exposure. The inhibition in whole chain electron transport activity could be due to two reasons (a) changes at PS II complex (or) (b) changes at PS I complex. Since the cold stress treatment inhibited the whole chain electron transport, an investigation has been made on the effect of cold stress on PS II catalysed pBQ Hill reaction in intact cells. pBQ is an artificial electron acceptor and it accepts electrons from PQ pool (Warburg and Luthgens, 1944; Trebest, 1974). pBQ being lipophilic in nature it enters easily into intact cells of *Spirulina*. Control cells exhibited a high rate of PS II dependent O₂ evolution activity (361 μmoles O₂ mg Chl⁻¹ h⁻¹) (Table 6). The gradual decrease in the temperature from (25 - 10°C) caused inhibition in Hill activity. After 10°C low temperature treatment 65 % loss in PS II catalysed electron transport activity was observed. The inhibition in PS II catalysed electron transport activity by cold stress could be most probably due to alterations at the oxidizing side of PS II. Table 7
Table 5: Effect of low temperature (25-10°C) on whole chain electron transport (H₂O → MV) of the cyanobacterium, *Spirulina platensis*. 3 ml of reaction mixture contains reaction buffer (25 mM Hepes-NaOH (pH 7.5) containing 20 mM NaCl), 0.5 mM MV, 1 mM Na-azide and cells equivalent to 15 µg of Chl. Other details were given in Material and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Whole chain electron transport activity (H₂O → MV) µ moles of O₂ ↓ mg Chl⁻¹ h⁻¹</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>252 ± 21</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>214 ± 19</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>170 ± 16</td>
<td>29</td>
</tr>
<tr>
<td>10</td>
<td>111 ± 10</td>
<td>56</td>
</tr>
</tbody>
</table>

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Table 6: Low temperature induced alteration in PS II catalysed electron transport activity (H₂O → p-BQ) of the intact cells of the cyanobacterium *Spirulina platensis*. Reaction mixture (3ml) for this assay contained reaction buffer (25 mM Hepes-NaOH (pH 7.5) containing 20 mM NaCl), 0.5 mM pBQ, and cells equivalent to 15 µg of Chl. Other details were given in Material and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>PS II electron transport activity (H₂O → p-BQ) µ moles of O₂/mg Chl⁻¹ h⁻¹</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>361 ± 33</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>282 ± 26</td>
<td>22</td>
</tr>
<tr>
<td>15</td>
<td>209 ± 19</td>
<td>42</td>
</tr>
<tr>
<td>10</td>
<td>126 ± 11</td>
<td>65</td>
</tr>
</tbody>
</table>
Table 7: Time dependent effect of low temperature (10°C) induced alteration in PS II catalysed electron transport of the intact cells of the cyanobacterium *Spirulina platensis*. Reaction mixture (3ml) for this assay contained reaction buffer (25 mM Hepes-NaOH (pH 7.5) containing 20 mM NaCl), 0.5 mM pBQ and cells equivalent to 15 µg of Chl. Other details were given in Materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Duration of exposure (min)</th>
<th>PS II electron transport activity (H₂O → p-BQ) μ moles of O₂↑ mg Chl⁻¹ h⁻¹</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>357 ± 34</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>314 ± 29</td>
<td>12</td>
</tr>
<tr>
<td>30</td>
<td>264 ± 23</td>
<td>26</td>
</tr>
<tr>
<td>45</td>
<td>185 ± 15</td>
<td>48</td>
</tr>
<tr>
<td>60</td>
<td>128 ± 9</td>
<td>63</td>
</tr>
</tbody>
</table>
shows the time dependent effect of cold stress (10°C) on PS II catalysed electron transport activity. These results clearly demonstrated that there is a time dependent effect in low temperature induced inhibition and only after 45 min of exposure 48% loss was observed. Therefore all experiments were carried out by maintaining 10°C along with 45 min of exposure. Unlike the situation with the whole chain electron transport and PS II catalysed electron transport, PS I catalysed reaction could not be assayed in intact cells of *Spirulina* as reduced DCIP/ TMPD/ DAD did not readily enter into intact cells. Therefore, thylakoid membrane fragments were prepared to study the effect of cold stress on PS I catalysed electron transport. These membrane fragments were more or less free from PC as seen from the absorption spectrum (Fig 15). As shown in Fig 15, these membrane fragments exhibited two majors peaks and two shoulders in the absorption spectra. The peak at 679 nm is due to the absorption of Chl *a* and peak at 438 nm is due to soret band of Chl *a*. The shoulder at 629 nm is due to residual PC and hump at 482 nm is mostly due to the carotenoid absorption. These fragments did not evolve O₂ with pBQ as a Hill acceptor. Hence we have characterized them by assaying PS I activity with DCPIP/ TMPD/DAD donors as shown in the Table 8. Reduced DCPIP/ TMPD/DAD as donors in mediating the MV photoreduction by PS I. The rates were matching with the rates of chloroplast thylakoid membrane, with reduced DCPIP as donor. Therefore we have selected DCPIPH₂ as electron donor system and studied the effect of cold stress on PS I catalyzed electron transport activity and only 17% loss was noticed after 10°C of low temperature.
Fig 15: The absorption spectrum of thylakoid membranes isolated from *Spirulina platensis*. Other details were mentioned in the Materials and methods.
Table 8: Different electron donors supported photosystem I catalysed electron transport activities in thylakoid fragments of *Spirulina*. The reaction mixture (3 ml) for PS I catalysed electron transport assay contained reaction buffer and different electron donors, acceptors and inhibitors as mentioned in Materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Assay system</th>
<th>PS I activity (μ moles O₂ consumed mg Chl⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMPD + Asc → MV</td>
<td>551 ± 56</td>
</tr>
<tr>
<td>DAD + Asc → MV</td>
<td>495 ± 47</td>
</tr>
<tr>
<td>DCPIP + Asc → MV</td>
<td>452 ± 43</td>
</tr>
</tbody>
</table>
exposure for 45 min (Table 9). The possible reason for the inhibition of PS I catalysed electron transport activity could be inactivation of intersystem electron transport carriers in the thylakoid of *Spirulina*. From the PS II catalysed electron transport activity measurements it is clear that there could be alteration in the light harvesting complex which is responsible for the altered photochemistry. To examine whether the inhibition in Hill activity by cold stress is linked to the alterations of light harvesting complex (LHC) or not, the PS II activity was measured both at light saturating and light limiting conditions. Table 10 shows that the inhibition in PS II activity by cold stress was more at light saturating conditions than that at light limiting conditions. These results indicates that the possible reason for loss of PS II activity under cold stress could be the alteration in the light harvesting complex of the cyanobacterium, *Spirulina platensis*. Phycobilisomes are the major light harvesting pigments proteins complex in PS II of cyanobacteria. Therefore to exactly pinpoint the target for cold stress both absorption and spectral measurements of phycobilisomes have been made using intact cells (Fig 16, 17, 18). Fig 16 shows the absorption characteristics of control cells. The peak at 679 nm is due to the absorption of Chl a; peak at 630 nm is due to the absorption of PC of PBsomes; a hump at 484 nm is due to the absorption of carotenoids; and a peak at 437 nm is due to soret band of Chl a (Fork and Mohanty, 1986). After low temperature treatment (10°C) there was a drastic decrease in the absorption at 630 nm with a 2 nm red shift. There was no change in absorption capacities of Chl a and carotenoid and xanthophyll. Since
Table 9: Low temperature induced alterations in PS I catalysed electron transport activities of the (DCPIPH$_2$ → MV) intact cells of the cyanobacterium, *Spirulina platensis*. Thylakoid fragments were isolated from the cells treated under different temperature for 45 min. Other details were given in Materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>PS I electron transport activity</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^0$C</td>
<td>(DCPIPH$_2$ → MV) μ moles of O$_2$ ↓ mg Chl$^{-1}$ h$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>429 ± 41</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>403 ± 38</td>
<td>6</td>
</tr>
<tr>
<td>15</td>
<td>382 ± 37</td>
<td>11</td>
</tr>
<tr>
<td>10</td>
<td>356 ± 33</td>
<td>17</td>
</tr>
</tbody>
</table>
Table 10: Illuminated light intensity dependent effect on the low temperature induced inhibition of PS II catalysed electron transport activity (H₂O→pBQ) in the cyanobacterium, *Spirulina platensis*. Reaction mixture (3ml) for this assay contained reaction buffer, 0.5 mM pBQ and intact cells equivalent to 15 µg of Chl a. Other details were given in Materials and methods. The values are average of 3 separate experiments. The SD is not more than 10%.

<table>
<thead>
<tr>
<th>Light intensity (Wm⁻²)</th>
<th>PS II catalysed electron transport activity µ moles of O₂↑ mg Chl⁻¹ h⁻¹</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (25⁰C)</td>
<td>Treated (10⁰C)</td>
</tr>
<tr>
<td>12</td>
<td>44 ± 3</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>125</td>
<td>125 ± 11</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>262</td>
<td>256 ± 24</td>
<td>154 ± 14</td>
</tr>
<tr>
<td>446</td>
<td>358 ± 33</td>
<td>201 ± 19</td>
</tr>
</tbody>
</table>
Fig 16: Effect of cold stress on the absorption spectra of the intact cells of the cyanobacterium, *Spirulina platensis*. Cells were treated at 10°C for 45 min before measurements were made. Intact cells equivalent of 6 μg of Chl were suspended in 3 ml of reaction buffer for measuring spectral properties. Other details were given in Materials and methods.
Fig 17: Effect of cold stress (10°C) on PC fluorescence emission spectra of intact cells of *Spirulina*. Cells equivalent to 5 µg of Chl a were suspended in 3 ml reaction buffer for measuring spectral characteristics. Cells were excited with 545 nm light beam (Slit width for both excitation and emission was 5 nm).
Fig 18: Effect of cold stress (10°C) on 77 K fluorescence emission spectra of intact cells of *Spirulina*. Cells were excited with 440nm. Cells equivalent to 5 µg of Chl a were used (Slit width for both excitation and emission was 5 nm).
cold stress affected the PC absorbance quite extensively, a measurement of room temperature PC florescence emission of *Spirulina* was made (Fig 17). In control cells, excited at 545 nm, an emission peak at 647 nm emanating from PC was prominent in the spectrum (Singhal et al., 1981; Fork and Mohanty, 1986). Cold stress (10°C) caused decrease in the fluorescent intensity and induced 5 nm blue shift. The decrease in the fluorescence intensity indicates the uncoupling of energy transfer and blue shift shows the structural alterations in the pigment proteins of phycoecyanin. To examine the specific target of cold stress in PS II, the Chl fluorescence emission spectra at low temperature (77K) was measured by exciting with 440 nm light beam. In control, three prominent peaks were observed as shown in Fig 18; emission band 645 nm is due to PC; 695 nm band is due to PS II reaction center Chl; 735 nm band is due to PS I reaction center Chl and the hump at 665 nm is due to APC. Low temperature treatment (10°C) mainly caused the suppression of the 695 nm peak with 3 nm blue shift. In addition the ratio of fluorescence intensity between control and low temperature treated samples (F695/F735) decreased indicating the change in the spillover of energy between the two photosystems. After studying the functional aspects of pigment proteins an attempt has been made to determine the affect of cold stress on polypeptide profile of *Spirulina* cell total protein by using SDS-PAGE analysis. To achieve this, cells were incubated at 10°C for 12 h and then the polypeptide analysis has been made. SDS-PAGE analysis of total protein of untreated *Spirulina* resolved its components in the molecular range of 97 to 13.5 kDa (Fig 19). The phycobiliproteins of intact cells were in
Fig 19: SDS-PAGE polypeptide profile of total soluble thylakoid proteins of control *Spirulina* cells and treated (10°C) for 12 h. Other details were given under Materials and methods. Lane 1 indicates the molecular masses of standard protein markers, Lane 2 and 3 indicates the polypeptide composition of Control and Treated samples respectively.
the range of 16 to 22 kDa. These proteins are chromophore linked and they can be seen on the gel prior to staining with Coomassie Blue –R-250. Fig 19 shows the polypeptide profile of Spirulina control and cold stress treated (10°C) cells. The low temperature caused the decrease mainly in 37 kDa and 97.1 kDa polypeptides. There is no change in the chromophore bearing polypeptide which are in the range from 16-22 kDa. These two polypeptides (97.1 and 37 kDa) according to literature are related to anchor and linker polypeptides respectively. In addition it also caused the appearance of a new polypeptide around 47 kDa which could be stress responsive protein. After studying the effect of cold stress on intact cells it is clear that PBsomes are the main target for cold stress. Therefore an attempt has been made to isolate PBsomes form control as well as low temperature treated Spirulina cells. Fig 20 shows the presence of PBsomes in the sucrose density gradient centrifugation. A thick blue colour band was observed at 1 M region of sucrose after ultracentrifugation. In cold stress treated samples exhibited a thick blue layer at 1 M region in addition to another light blue band at 0.5 M sucrose gradient. This clearly demonstrated that cold stress caused the dissociation of some of the pigment proteins from intact PBsomes. To determine the functional integrity of PBsomes, spectral properties of phycobilisomes were determined. Fig 21 shows the spectral characteristics of the isolated PBsomes. The absorption spectrum of PBsomes exhibited main peak at 613 nm due to PC and a pronounced shoulder at 650 nm due to APC as shown in Fig 21. The PBsomes isolated from cold stressed samples caused decrease in the absorption
Fig 20: Sucrose density gradient sedimentation profile of intact PBSomes isolated from Control and Treated (12h cold stressed) samples of *Spirulina platensis* cells.
Fig 21: Absorption spectrum of isolated PBsomes from Control and Treated Spirulina cells. PBsomes equivalent of 15 µg of protein were suspended in 3 ml reaction buffer containing 0.75 M PO₄ (pH 7.0) for measuring spectral characteristics.
and shifted the peak towards blue region of the spectrum by 3 nm indicating the structural alterations in PBsomes. Since fluorescence and energy transfer is related to absorption and fluorescence emission, spectra of PBsomes was measured by exciting the PBsomes with 545 nm light beam showed the characteristics emission peak at 669 nm emanating from the longer wavelength absorbing species (Fig 22). This indicates that the energy transfer in the isolated PBsomes is intact and it is getting transferred from PC---APC. These spectral characteristics are in agreement with the observation of Gantt et al. (1979). The low temperature treatment (10°C) caused the decrease in the fluorescence emission and shifted the peak by 4 nm towards the blue region indicating the uncoupling of energy transfer from PC---APC B. To identify the specific alterations in phycobilisomes, polypeptide and chromophore protein interaction, the CD spectra of control and cold stress (10°C) treated samples was measured. As shown in the Fig 23 the control phycobilisomes CD spectrum exhibited a trough at 350 nm and a positive band in the visible region of the CD spectrum. The sharp positive CD band due to strong that no proteolysis of this polypeptide occurred during the isolation procedure. The anchor polypeptide has been demonstrated to be very susceptible for proteolytic degradation and hence utmost care is necessary for preventing the degradation during the isolation procedures (Gantt et al., 1979). The PBsomes isolated from cold stressed samples exhibited the loss in the content of linker polypeptides in the range of 32-37 kDa particularly low temperature induced the degradation of 35 kDa which links PC to APC. Thus low temperature
Fig 22: Fluorescence emission spectrum of isolated PBsomes from control and treated (45 min) *Spirulina* cells. PBsomes equivalent of 15 µg of protein were suspended in 3 ml reaction buffer containing 0.75 M K-PO$_4$ (pH 7.0) for measuring spectral characteristics. Cells were excited with 545 nm light beam (Slit width for both emission was 5 nm).
Fig 23: Effect of cold stress on Circular dichroism spectra of isolated phycobilisomes.
treatment affected the energy transfer process during short term (45 min) incubation and degradation of linker polypeptide rotational strength was observed at 598 nm in control phycobilisomes spectrum. The cold stress (10°C) treatment caused shift in the peak only 45 min of exposure towards the blue region. This exposure in addition caused the suppression of trough observed at 350 nm almost by 100%. These drastic changes in the CD spectrum seem to rise because of the dissociation of phycobilisomes and also possible due to change in the chromophore protein interaction. These results are in agreement with the observations of Sah et al. (1998). From the analysis of spectral measurement, it is clear that phycobiliproteins are the major targets for cold stress. Hence, to identify the structural organization, the PBsomes have been isolated from 12 h cold stress (10°C) cells. The electrophoretogram showed the polypeptide profile of control and cold stress PBsomes of Spirulina (Fig 24). Besides the bilin-carrying subunits of phycobiliproteins, five non-pigmented polypeptides were clearly seen (lane 2), associated with PBsomes. The polypeptide at 94 kDa in the electrophoretogram is the anchor polypeptide which links the PBsomes to the thylakoid membrane. The polypeptides between 32 and 37 kDa are linkers which help in the attachment of PC-PC rods (Yamanaka and Glazer, 1980). The intense bands, 16 to 22 kDa are the subunits of phycobiliproteins (both PC and APC). The presence of single band of the anchor polypeptide indicated after long term incubation (12 h). To analyze whether the cold stress mediated alterations in electron transport is related to lipid alterations or not, lipid peroxidation in control and
Fig 24: Effect of cold stress (10°C) on the polypeptide composition of phycobilisomes isolated from 12 h stressed samples of *Spirulina* intact cells. C-control and T-treated (<10°C).
cold stress treated thylakoid membranes has been measured in terms of MDA formation. For this purpose the cells were given cold treatment (10-25°C) for 45 min and lipid peroxidation was measured in terms of MDA formed. In control cells 36 n mole MDA/ mg protein was observed (Table 11). The fall in the temperature caused gradual increase in the lipid peroxidation and at 10°C, 80% enhancement was noticed. To study the comparative effect of cold stress (10°C) on PS II catalysed electron transport in relation with lipid peroxidation of thylakoid membrane an attempt was made. For this study the cells were treated with different temperatures and in the same samples both PS II electron transport as well as lipid peroxidation was measured (Table 12). The increase in the temperature gradually inhibited the PS II activity. The inhibition of PS II activity is very much related with the increase of lipid peroxidation up on temperature fall. In this way there was an inverse relationship between electron transport activity and lipid peroxidation. To analyse the possible scavenging mechanism of lipid peroxides a study has been made using ascorbate (2 mM) as a protectant. For this study cells were incubated at 10°C for 45 min both in the presence and absence of ascorbic acid. In the absence of ascorbate at 10°C there was 65% increase in lipid peroxidation. But when the cells were incubated with ascorbate in the reaction mixture even at 10°C, almost the lipid peroxidation was negligible (Table 13). In the presence of ascorbate the lipid peroxidation even at these low temperatures is almost equal to that of control cells. Thus ascorbate protects the peroxidation of lipids under low temperature treatment. Most probably due to temperature stress there is a raise in the toxic
Table 11: Effect of cold stress on thylakoid membrane lipid peoxidation (MDA formation) in the cyanobacterium *Spirulina platensis*. Thylakoid membranes equivalent to 15 μg/ ml of Chl a were used for the estimation of MDA levels. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Lipid peroxidation (n moles MDA mg⁻¹ protein)</th>
<th>Percentage increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>36 ± 3.2</td>
<td>0</td>
</tr>
<tr>
<td>20°C</td>
<td>49 ± 3.9</td>
<td>36</td>
</tr>
<tr>
<td>15°C</td>
<td>61 ± 4.2</td>
<td>69</td>
</tr>
<tr>
<td>10°C</td>
<td>65 ± 4.5</td>
<td>80</td>
</tr>
</tbody>
</table>
Table 12: Comparative effect of cold stress on PS II catalyzed electron transport (H$_2$O→pBQ) and lipid peroxidation of thylakoid membranes. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>PS II catalyzed electron transport</th>
<th>Lipid peroxidation (nmole MDA mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (25°C)</td>
<td>361±33</td>
<td>36±3.2</td>
</tr>
<tr>
<td>20</td>
<td>282±26</td>
<td>48±3.8</td>
</tr>
<tr>
<td>15</td>
<td>209±19</td>
<td>60±5.7</td>
</tr>
<tr>
<td>10</td>
<td>126±11</td>
<td>66±5.1</td>
</tr>
</tbody>
</table>
Table 13: Ascorbate (2 mM) mediated protection of thylakoid membrane lipid peroxidation under cold stress. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature  °C</th>
<th>Lipid peroxidation (n mol MDA mg(^{-1})protein)</th>
<th>Percentage increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (25°C)</td>
<td>38±3.1</td>
<td>0</td>
</tr>
<tr>
<td>10°C (-Ascorbate)</td>
<td>63±5.8</td>
<td>65</td>
</tr>
<tr>
<td>10°C (+Ascorbate)</td>
<td>39±3.2</td>
<td>2</td>
</tr>
</tbody>
</table>
oxyradicals which can in turn affect the PS II polypeptide with altered membrane lipid interaction. Stress is a 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 cold stress. Control cells showed a high rate of O_2 consumption involving whole chain electron transport (241 μmole 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. Where as in combination the inhibition was brought to 62 %. This clearly demonstrates the additive effect of white light in bringing the inhibition of cold stressed samples (Table 5 and 14). 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

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Table 14: Effect of low intensity white light (100 Wm\(^{-2}\)) on low temperature induced inhibition of whole chain electron transport in the intact cells of cyanobacterium, *Spirulina platensis*. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Whole chain electron transport activity (H(_2)O → MV) μ moles of O(_2) ↓ mg Chl(^{-1}) h(^{-1})</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>241 ± 23</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>202 ± 21</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>152 ± 17</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>92 ± 10</td>
<td>62</td>
</tr>
</tbody>
</table>
of white light in the electron transport activities. Control cells exhibited a high rate of PS II dependent O₂ evolution activity (354 μmole O₂ evolution mg Chl⁻¹ h⁻¹). The application of cold stress independently caused loss in PS II photochemistry by 65 %. When the low temperature stress is combined with white light and applied there is an increase in the inhibition upto 71 % (Table 6 and Table15). The possible reason for the inhibition of PS II activity could be alterations at water oxidation complex. To verify the susceptible nature of PS I the electron transport activity has been measured using DCPIP as donor. Control activity is equal to (431 μmole O₂ consumption mg Chl⁻¹ h⁻¹) application of cold stress along with white light brought the inhibition in PS I activity from 17 to 26 %. This shows clearly the susceptible nature of PS I towards low temperature stress (Table 7 and 16).
Table 15: Effect of low intensity white light (100 Wm⁻²) on low temperature induced inhibition of PS II catalysed electron transport of intact cells of the cyanobacterium, *Spirulina platensis*. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>PS II electron transport activity (H₂O → p-BQ) μ moles of O₂↑mg Chl⁻¹ h⁻¹</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>354 ± 34</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>265 ± 27</td>
<td>25</td>
</tr>
<tr>
<td>15</td>
<td>196 ± 18</td>
<td>45</td>
</tr>
<tr>
<td>10</td>
<td>105 ± 10</td>
<td>71</td>
</tr>
</tbody>
</table>
Table 16: Effect of low intensity white light (100 Wm$^{-2}$) on low temperature induced inhibition of PS I catalysed electron transport intact cells of the cyanobacterium, *Spirulina platensis*. Other details were given in Materials and methods.

<table>
<thead>
<tr>
<th>Temperature $^\circ$C</th>
<th>PS I electron transport activity (DCPIPH$_2$ $\rightarrow$ MV) $\mu$ moles of O$_2$ $\downarrow$mg Chl$^{-1}$ h$^{-1}$</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>435 $\pm$ 42</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>401 $\pm$ 39</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>360 $\pm$ 35</td>
<td>18</td>
</tr>
<tr>
<td>10</td>
<td>325 $\pm$ 31</td>
<td>26</td>
</tr>
</tbody>
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