Plant Physiol. (1987) 83, 438–441
0032-0889/87/83/0438/04$01.00/0

The Susceptibility of Photosynthesis to Photoinhibition and the Capacity of Recovery in High and Low Light Grown Cyanobacteria, *Anacystis nidulans*

Received for publication April 18, 1986 and in revised form August 28, 1986

GÖRAN SAMUELSSON*, ANDERS LÖNNEBORG, PETTER GUSTAFSSON, AND GUNNAR ÖQUIST
Department of Plant Physiology (G.S., A.L., G.Ö.), *and Institute of Applied Cell and Molecular Biology* (P.G.), *University of Umeå, S-901 87, Umeå, Sweden*

ABSTRACT

The susceptibility of photosynthesis to photoinhibition and the rate of its recovery were studied in the cyanobacterium *Anacystis nidulans* grown at a low (10 micromoles per square meter per second) and a high (120 micromoles per square meter per second) photosynthetically active radiation. The rate of light limited photosynthetic O_2 evolution was measured to determine levels of photoinhibition and rates of recovery. Studies of photoinhibition and recovery with and without the translation inhibitor streptomycin demonstrated the importance of a recovery process for the susceptibility of photosynthesis to photoinhibition. We concluded that the approximately 3 times lower susceptibility to photoinhibition of high light than of low light grown cells, significantly depended on high light grown cells having an approximately 3 times higher recovery capacity than low light grown cells. It is suggested that these differences in susceptibility to photoinhibition and recovery depends on high light grown cells having a higher turnover rate of photosystem II protein(s) that is(are) the primary site(s) of photodamage, than have low light grown cells. Furthermore, we demonstrated that photoinhibition of *A. nidulans* may occur under physiological light conditions without visible harm to the growth of the cell culture. The results give support for the hypotheses that the net photoinhibitory damage of photosystem II results from the balance between the photoinhibitory process and the operation of a recovery process; the capacity of the latter determining significant differences in the susceptibility of photosynthesis to photoinhibition of high and low light grown *A. nidulans*.

Photoinhibition of photosynthesis may occur when plants are exposed to excessive light (9). We showed in an earlier work (11) that the cyanobacterium *Anacystis nidulans* responded to photoinhibitory treatments with changes in the function of the photosynthetic apparatus that are typical for higher plants (6, 9) and algae (7, 10); *i.e.* inhibition at, or close to, the reaction center of PSII. Although there are evidences that one site of inhibition is at the Q_B-protein (4, 7) there is still no agreement on the molecular mechanism of photoinhibition.

By studying photoinhibition of photosynthesis and its recovery in *A. nidulans* in the presence and absence of transcription and translation inhibitors, we (11) and others (3, 4, 7) have given evidence for the existence of a repairing process that restores efficient photosynthesis in dim light. From the results of our own work (11) we suggested that the extent of photoinhibitory damage observed is the net result of a balance between the photodamage and the operation of a repairing process.

It is well documented that the susceptibility to photoinhibition is higher in shade adapted than in sun adapated plants (1, 2, 9). These different susceptibilities have been ascribed to sun plants having higher rates of light saturated photosynthesis than shade plants, thereby giving the sun plants a higher capacity to deexcitate excited Chl in strong lights. However, accepting the hypotheses that the capacity of a recovery process also is important for net photoinhibition to occur (11), it was of interest to investigate if high light acclimated cells have a higher capacity of the recovery process than have low light acclimated plants.

For this purpose we have grown *A. nidulans* at two different light levels and assessed the capacity of the recovery process by adding streptomycin to the two algal cultures during photoinhibition and recovery. We show that high light acclimated *A. nidulans* have a much higher rate of recovery of photosynthesis after photoinhibition than have low light acclimated *A. nidulans*. We concluded that the difference in the susceptibility to photoinhibition of low and high light grown *A. nidulans* to a significant extent is determined by different capacities of a recovery process operating under excessive light exposure.

MATERIALS AND METHODS

Culture Conditions. The cyanobacterium *Anacystis nidulans* 625 (*Synechococcus* 6301) was grown in batch cultures in an inorganic medium (12) as described earlier (8). The culture was in equilibrium with air which gave cells adapted to low carbon. The temperature was kept at 38°C and the cultures were exposed to continuous PAR of a low (10 μmol $m^{-2}s^{-1}$) and a relatively high (120 μmol $m^{-2}s^{-1}$) photon flux density, respectively (Li-Cor quantum radiometer; Lambda Instruments, Lincoln, NE). Light sources were incandescent lamps (Philips PAR 38 150"/220V). The cultures used for experiments were always in the logarithmic phase of growth. The generation times of the cells were 4.5 and 24 h for low (10 μmol $m^{-2}s^{-1}$) and high (120 μmol $m^{-2}s^{-1}$) light grown *A. nidulans*, respectively. Cell densities, Chl *a*, and phycocyanin concentrations were measured with a single beam spectrophotometer and the extinction coefficients used were those of (13). Cell densities and growth were determined from the *A* at 750 nm (5).

Photoinhibitory Treatment and Reactivation. The photoinhibitory treatment was done in a glass water bath, at 38°C. Photoinhibitory light was supplied by a halogen lamp (Osram, power star, HQI-IS 400 W, Berlin, FRG). Cells were kept in tubes (10 ml) and the photon flux density was regulated by placing the lamp at different distances from the samples. Care was taken not to change the temperature of the cells during transfer from the culturing to the photoinhibiting conditions. The cell concentration never exceeded 2×10^9 cells/ml and the Chl concentration was approximately 1 to 2 μg/ml. The experimental conditions

used during reactivation of photosynthesis after photoinhibition are described in the figure legends.

Photosynthetic Measurements. Photosynthesis was measured using a Clark type O_2 electrode (Hansatech, Ltd., Norfold, UK) and N_2 was bubbled through the cuvette to decrease the partial pressure of O_2 before the measurements. The electrode was calibrated to the absolute mode by using dithionite and air saturated water at 38°C. Prior to measurements, 10 mM $NaHCO_3$ was added to the solution to avoid CO_2 deficiency at high light. The signal from the electrode was registered on a chart pen recorder and, when stable rates were obtained, photosynthesis was calculated as μmol O_2 evolved mg^{-1}Chl h^{-1}. The extent of photoinhibition was determined by calculating the rate of light limited photosynthesis, expressed as percent of a control, measured immediately before photoinhibitory treatment. Rates of light limited photosynthesis were assayed at 25 and 80 μmol $m^{-2}s^{-1}$ for cells grown at 10 and 120 μmol $m^{-2}s^{-1}$, respectively. Light source was an Atlas projector lamp (A1,215 24V/150 W).

RESULTS

Figure 1 shows the light dependence of the photosynthetic O_2 evolution of *A. nidulans* cultivated under low and high PAR. Photosynthesis of low light grown cells saturated at approximately 3 times lower PAR than did high light grown cells; 100 and 300 μmol $m^{-2}s^{-1}$, respectively.

When samples of *A. nidulans* from the two light conditions were treated for 90 min in a gradient of PAR, ranging from 10 to 600 μmol $m^{-2}s^{-1}$, low light grown cells became photoinhibited at much lower light levels than did high light grown cells (Fig. 2A). Photoinhibition became apparent above 25 and 125 μmol $m^{-2}s^{-1}$ for low and high light grown cells, respectively. High light grown cells furthermore differed from the low light grown cells by (a) showing an increased rate of photosynthesis when incubated at a PAR below 100 μmol $m^{-2}s^{-1}$ and (b) by the extent of photoinhibition reaching a plateau between 150 and 200 μmol $m^{-2}s^{-1}$ (rate of photosynthesis approximately 80% of the control). When a similar experiment was performed with the translation inhibitor streptomycin present during the incubation for photoinhibition, a different pattern appeared (Fig. 2B); both low and high light grown cells became severely inhibited at relative low PAR. The light dependent patterns of inhibition of photosynthesis were very similar in the presence of streptomycin for the two types of cells.

The observation that high light grown cells were much more resistant to photoinhibition than low light grown cells (Fig. 2A) and the finding that the susceptibilities to photoinhibition became approximately equal for the two types of cells after the addition of the translation inhibitor streptomycin are in agreement with the hypothesis that different capacities of the recovery processes determine the different susceptibilities to photoinhibition of photosynthesis of the two types of cells.

To further test this hypotheses both low and high light grown cells were photoinhibited to show a photosynthetic rate that was 60% of the control. This was achieved by exposing low and high light acclimated *A. nidulans* to 130 and 450 μmol $m^{-2}s^{-1}$ for 90 min, respectively. It was found that high light grown cells recovered initially 2.6 times faster than the low light grown cells (Fig. 3). Furthermore, the high light grown cells recovered to photosynthetic rates higher than that of the control, whereas the low light grown cells were not able to recover fully. The light conditions during recovery were 5 and 25 μmol $m^{-2}s^{-1}$ for low and high light grown cells. This was because the optimal rates of recovery were obtained at these PAR values. Poor recoveries were obtained in the dark and the rates of recovery slowly decreased at PAR above the levels that were optimal (data not shown).

To further demonstrate the different rates of recovery of the photoinhibitory damage in photosynthesis of low and high light grown *A. nidulans*, we photoinhibited the two cell types so they retained 60 to 70% of their photosynthetic capacity. This was again achieved by exposing low and high light grown cells to 130 and 450 μmol $m^{-2}s^{-1}$ for 90 min, respectively. Streptomycin was then added, after which addition the rates of photoinhibition became initially 3 times faster for the high light grown cells than for the low light grown cells (Fig. 4).

DISCUSSION

We have shown earlier that the recovery after photoinhibition of photosynthesis of *A. nidulans* is insensitive to the transcription

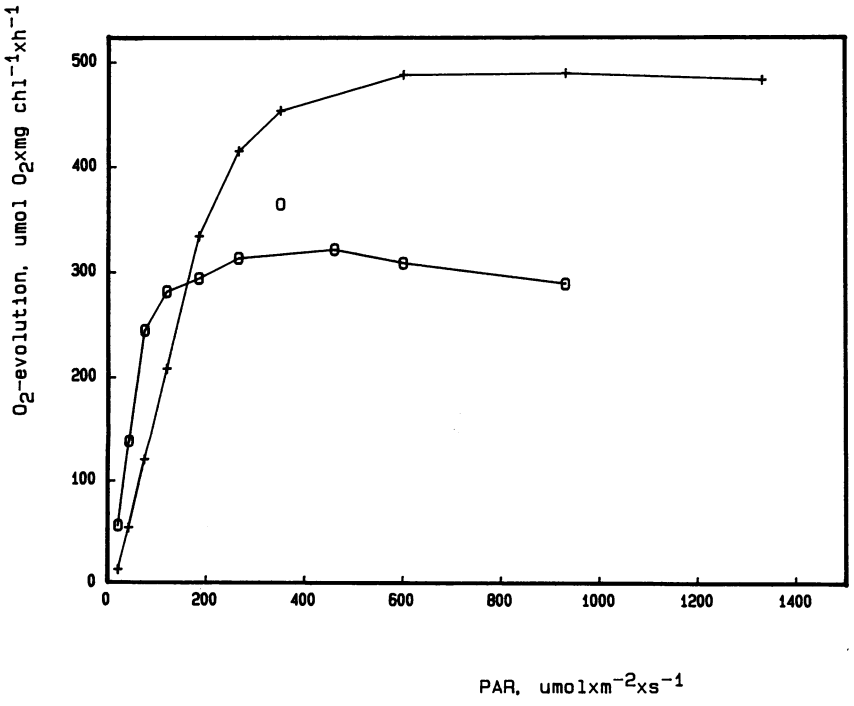

FIG. 1. Photosynthetic O_2 evolution as a function of PAR of *A. nidulans* grown under low (10 μmol $m^{-2}s^{-1}$) and high (120 μmol $m^{-2}s^{-1}$) PAR. (□) low light grown cells; (+) high light grown cells.

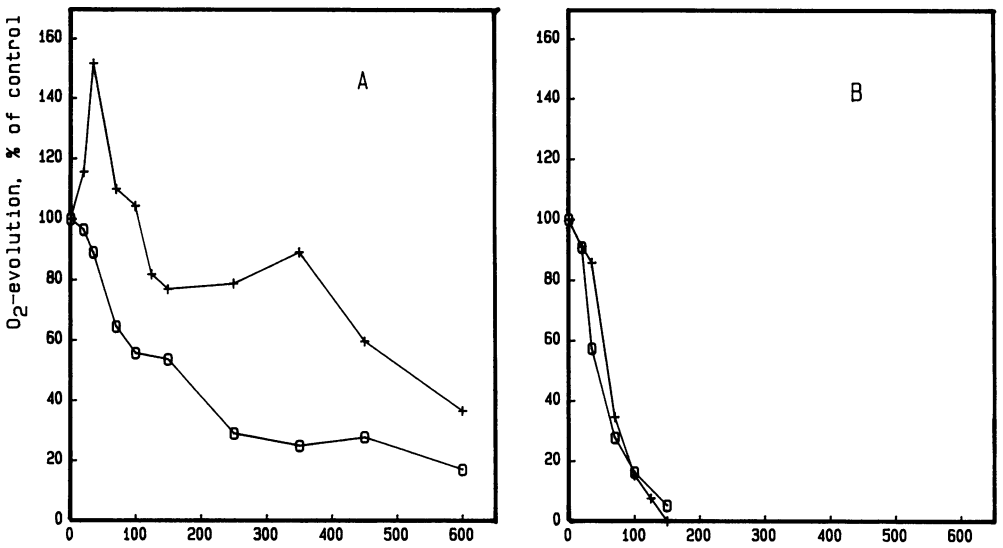

FIG. 2. Photosynthetic O_2 evolution (percent of control) after photoinhibition treatment, for 90 min at various PAR, of *A. nidulans* grown at low (10 μmol $m^{-2}s^{-1}$) and high (120 μmol $m^{-2}s^{-1}$) PAR: A, without and B, with the translation inhibitor streptomycin (250 μg/ml). (□) Low light grown cells; (+) high light grown cells.

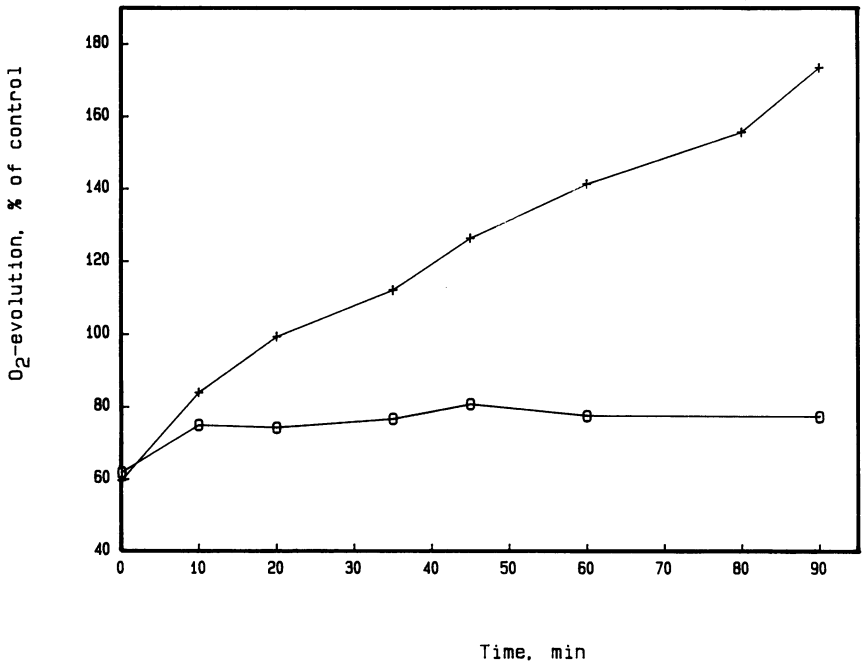

FIG. 3. Recovery of photosynthetic O_2 evolution (percent of control) after partial photoinhibition (about 40%) of *A. nidulans* grown at low (10 μmol $m^{-2}s^{-1}$) and high (120 μmol $m^{-2}s^{-1}$) PAR. The optimal PAR for recovery were 5 and 25 μmol $m^{-2}s^{-1}$ for low and high light grown cells, respectively. (□) Low light grown cells; (+) high light grown cells.

inhibitor rifampicin but completely inhibited by translation inhibitors such as streptomycin and kanamycin (11). The finding that recovery occurs despite inhibition of transcription implies that the necessary protein synthesis occurs on relatively stable mRNA or that the synthesis of this transcript is insensitive to rifampicin.

Although it is known that photoinhibition occurs at, or near, the reaction center of PSII, the molecular mechanisms of photoinhibition and recovery are not known. However, the Q_B-protein has been shown to be damaged during photoinhibition of photosynthesis in *Chlamydomonas reinhardii* and replaced because of a high turnover rate during recovery of photosynthesis (7). The fact that the recovery was incomplete and occurred slowly with a long lag phase makes us believe that the photoinhibitory damage to the Q_B-protein in C *reinhardii* is secondary; in *A. nidulans* there was a complete recovery with maximal rate immediately after termination of photoinhibitory treatment (11). Based on these observations it is tempting to believe that the potential of *A. nidulans* to recover after photoinhibition of photosynthesis is dependent on the rate of turnover of the protein(s) that is (are) the primary site(s) of photodamage.

By the use of the translation inhibitor streptomycin we show in this work that the capacity to recover after about 40% photoinhibition of photosynthesis was approximately 3 times higher in high light than in low light grown *A. nidulans* (Fig. 3). When, in addition, approximately 3 times more light was needed to reach about 40% photoinhibition of high light than of low light grown cells (Fig. 2A), and since the rate of photoinhibition was approximately 3 times faster in high light grown than in low light grown cells after the addition of streptomycin (Fig. 4), we conclude: (a) high light grown cells have a higher capacity to recover after photoinhibition than have low light grown cells, (b) this recovery process is operative during, as well as after, the photoinhibitory treatment, and (c) a significant factor for making high light grown cells more resistant to photoinhibition than low light grown cells is the higher capacity of the recovery process of the

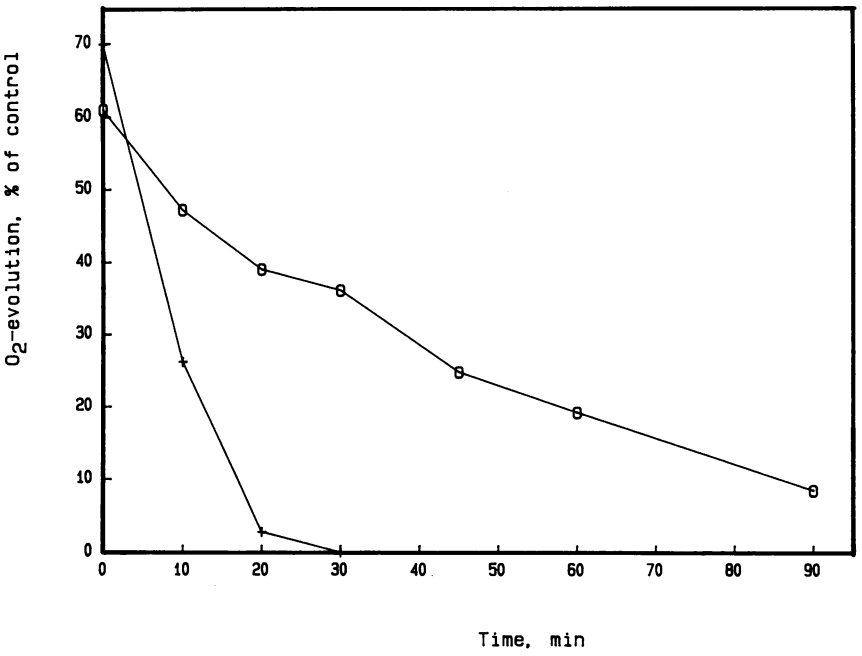

FIG. 4. The time dependence of photoinhibition of photosynthesis (percent of control) after the addition of streptomycin (250 μg/ml) to *A. nidulans* grown at low (10 μmol $m^{-2}s^{-1}$) and high (120 μmol $m^{-2}s^{-1}$) PAR. To obtain approximately similar levels of photoinhibition at the starting point (time zero), low and high light grown cells were exposed to 130 and 450 μmol $m^{-2}s^{-1}$ during 90 min, respectively, prior to the addition of streptomycin, and these light levels were maintained during the following course of inhibition. (□) Low light grown cells; (+) high light grown cells.

former cells. We tentatively believe that the different susceptibilities to, and the different rates of recovery after photoinhibition of photosynthesis, depend on different turnover rates of target protein(s) in PSII. It is interesting to note that when protein synthesis was inhibited both high and low light grown cells showed about similar susceptibilities to photoinhibition (Fig. 2B). This implies that the intrinsic susceptibility of the PSII complex to excessive light is similar for the two types of cells and that the net photoinhibition observed in the absence of streptomycin (Fig. 2A) first of all depends on the turnover rate of target protein(s).

One problem when using translation inhibitors like streptomycin is that they have no specificity but inhibit all protein synthesis of the cells. However, streptomycin had no inhibitory effect on photosynthetic O_2 evolution of *A. nidulans* when incubated for 90 min in darkness (11). Furthermore the type of photoinhibition of photosynthesis occurring in *A. nidulans* with or without streptomycin was mechanistically equal as deduced from similar effects on variable fluorescence of PSII (data not shown). We therefore conclude that our experiments with streptomycin are relevant with respect to our conclusions of the importance of the recovery process for net photoinhibition to occur.

Interestingly, exposing high light grown *A. nidulans* to various PAR for 90 min increased the photosynthetic capacity at PAR lower than 100 μmol $m^{-2}s^{-1}$ when measured at 80 μmol $m^{-2}s^{-1}$ (Fig. 2A). Furthermore, high light grown cells recovered to more than 100% of the control, when incubated at a PAR of 25 μmol $m^{-2}s^{-1}$ (Fig. 3), the PAR optimal for recovery of high light cells. Our interpretation to these observations is that *A. nidulans* grown at 120 μmol $m^{-2}s^{-1}$ in fact is partially photoinhibited during steady state growth conditions. Finally, the observation that photoinhibition becomes visible at relatively moderate light levels, particularly for the low light adapted cells, and the finding that cells grown at 120 μmol $m^{-2}s^{-1}$ show signs of partial photoinhibition, clearly demonstrate that photoinhibition of photosynthesis not only is of academic interest occurring in extremely bright light. Instead it may occur under physiological light conditions and at least *A. nidulans* can grow with a high generation time of 4.5 h despite signs of net photoinhibition of photosynthesis. The strategy by which *A. nidulans* has managed to overcome cell death due to excessive light might be by increasing the turnover of the recovery process.

Acknowledgments—We are grateful to Mrs. Gunilla Malmberg for skillful technical assistance and to Mrs. Ingrid Forsmark for typing the manuscript.

LITERATURE CITED

1. BJÖRKMAN O, P HOLMGREN 1963 Adaptability of the photosynthetic apparatus of light intensity in ecotypes from exposed and shaded habitats. Physiol Plant 16: 889–914
2. BJÖRKMAN O, SB POWLES 1981 Leaf movement in the shade species *Oxalis oregana*. Response to light level and light quality. Carnegie Inst Wash Year Book 80: 59–62
3. GREER DH, JA BERRY, O BJÖRKMAN 1986. Photoinhibition of photosynthesis in intact bean leaves: environmental responses of recovery. Planta. In press
4. KYLE DJ, I OHAD, CJ ARNTZEN 1984 Membrane protein damage and repair: Selective loss of a quinone-proteine function in chloroplast membranes. Proc Natl Acad Sci USA 81: 4070–4074
5. LÖNNEBORG A, LK LIND, SR KALLA, P GUSTAFSSON, G ÖQUIST 1985 Acclimation processes in the light harvesting system of the cyanobacterium *Anacystis nidulans* following a light shift from white to red light. Plant Physiol 78: 110–114
6. ÖGREN E, G ÖQUIST 1984 Photoinhibtion of photosynthesis in *Lemna gibba* as induced by the interaction between light and temperature. II. Chlorophyll fluorescence at 77 K. Physiol Plant 62: 193–200
7. OHAD I, DJ KYLE, CJ ARNTZEN 1984 Membrane protein damage and repair: removal and replacement of inactivated 32 kilodalton polypeptides in chloroplast membranes. J Cell Biol 99: 481–485
8. ÖQUIST G 1969 Adaptation in pigment composition and photosynthesis by far red radiation in *Chlorella pyrenoidosa*. Physiol Plant 22: 516–528
9. POWLES SB 1984 Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol 35: 15–44
10. SAMUELSSON G, K RICHARDSON 1982 Photoinhibition at low quantum flux densities in a marine dino-flagellate (*Amphidinium carterae*) Mar Biól 70: 21–26
11. SAMUELSSON G, A LÖNNEBORG, E ROSENKVIST, P GUSTAFSSON, G ÖQUIST 1985 Photoinhibition and reactivation of photosynthesis in the cyanobacteria *Anacystis nidulans*. Plant Physiol 79: 992–995
12. SIVA B, K RAO, JJ BRAND, J MYERS 1977 Cold shock syndrome in *Anacystis nidulans*. Plant Physiol 59: 965–969
13. STEVENS CLR, J MYERS 1976 Characterization of pigment mutants in a blue green alga *Anacystis nidulans*. J Phycol 12: 99–105