J. Phycol. **44,** 1071–1079 (2008)

DOI: 10.1111/j.1529-8817.2008.00546.x

ACCLIMATION TO LOW TEMPERATURE OF TWO *ARTHROSPIRA PLATENSIS* (CYANOBACTERIA) STRAINS INVOLVES DOWN-REGULATION OF PSII AND IMPROVED RESISTANCE TO PHOTOINHIBITION[1]

Avigad Vonshak[2] *and Nurit Novoplansky*

Microalgal Biotechnology Laboratory, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boker Campus, 84990 Israel

This study aimed to compare the ability of two *Arthrospira platensis* (Nordst.) Gomont strains, M2 and Kenya, isolated from two different habitats, to acclimate to low temperature (15°C). Both strains had similar growth rates at 30°C, but once acclimated to low temperature, M2 showed a greater decline in growth (59% vs. 41% in the Kenya strain). We suggest that the Kenya strain acclimated better to low temperature by down-regulating its photosynthetic activity through (i) decreasing antenna size and thus reducing energy flux into the photosystems; (ii) decreasing reaction center density (RC/CS_X) and the performance index, thus decreasing the trapping probability and electron transport rate while maintaining electron transport probability for electron transport beyond Q_A^- unchanged; (iii) increasing the energy dissipation flux. In contrast, the M2 strain showed no difference in antenna size and exhibited a much lower decrease in RC/CS_X and a lower dissipation rate. Hence, the Kenya strain minimized potential damage on the acceptor side of PSII compared to the M2 cells. Furthermore, acclimation to low temperature was accompanied by an improved mechanism for handling excess energy resulting in an enhanced ability of the Kenya strain to rapidly repair damaged PSII RCs and withstand a high photon flux density (HPFD) stress; this finding might be defined as a cross-adaptation phenomenon. This study may provide a tool to identify strains suitable for outdoor mass-production in different regions characterized by different climate conditions.

Key index words: Arthrospira; **cyanobacteria; fluorescence; OJIP test; outdoor production; photosynthesis; photosystems I and II**

Abbreviations: **chl_0, chl at time 0; DCMU, 3-(3,4-dichloro-phenyl)-1,1dimethhylurea; dwt, dry weight; F_o, F_v, F_m, minimal, variable, and maximal fluorescence yields of dark-adapted cultures; F_v/F_m, maximum quantum yield of primary photochemistry; HPFD, high photon flux density; k_{pi}, rate constant of photoinactivation; k_{rec}, rate constant of recovery; Pc, phycocyanin; $PI_{(ABS)}$, performance index on an absorption basis; PQ, plastoquinone; Q_A, primary quinine electron acceptor of PSII; Q_B, secondary quinine electron acceptor of PSII; RC, reaction center; RC/CS_X, reaction center density; TMPD, *N′N′N′N′*-tetramethyl-*p*-phenylenediamine; φ_{Eo}, quantum yield of electron transport beyond Q_A^-; φ_{Po}, maximum quantum yield for primary photochemistry; ψ_o, electron transport probability for electron transport beyond Q_A^-**

In nature, cyanobacteria are exposed to daily and seasonal fluctuations in temperature and light, which are the major factors determining photosynthetic and growth rates (Huner et al. 1998, Raven and Geider 2003). Low temperatures or high light intensities may disrupt the balance between the absorption of energy through photosynthesis and the ability to utilize this energy. Such conditions may cause a higher excitation pressure on PSII that results in damage to the photosynthetic apparatus (Huner et al. 1998, Yamamoto 2001, Wilson et al. 2006).

Several major regulatory mechanisms may be involved in the protection of the PSII apparatus from photodamage under stress conditions: photochemical processes related to electron transport, nonphotochemical processes by which excess energy is dissipated as heat and fluorescence or transferred to other systems, and modification in D1 protein turnover under excess energy stress (Prasil et al. 1992, Campbell et al. 1998, Huner et al. 1998, Melis 1999, Adams et al. 2001, Yamamoto 2001, Tsonev and Hikosaka 2003).

Polyphasic rise in chl *a* fluorescence (OJIP test) has been used as a tool to evaluate modifications in PSII photochemistry in a wide range of studies, not only in higher plants (Tomek et al. 2001, Ban Dar and Leu 2003, Force et al. 2003, Zhu et al. 2005, Strauss et al. 2006) but also in algae (Hill et al. 2004, Hill and Ralph 2005, Kruskopf and Flynn 2006) and cyanobacteria (Strasser et al. 1995, 2004, Lu and Vonshak 1999, Lu et al. 1999, Qiu et al. 2004, Lazár 2006). This tool has been used in a variety of studies, including structure and function

[1]Received 12 October 2007. Accepted 13 February 2008.
[2]Author for correspondence: e-mail avigad@bgu.ac.il.

of the photosynthetic apparatus, characterization of vitality and physiological condition, and selection of species tolerant to stress conditions (Hermans et al. 2003, Goncalves and Santos 2005).

In many cases, exposure of photosynthetic cells to extreme environmental conditions results in a decrease in the maximum quantum yield of primary photochemistry (F_v/F_m) and is often interpreted as photodamage (Powles 1984, Torzillo et al. 1998, Maxwell and Johnson 2000). Nevertheless, this decrease is often identified as an adaptive acclimation process of down-regulation of PSII, a protective mechanism that helps dissipate excess energy from the photosynthetic apparatus (Allen and Ort 2001, Tsonev et al. 2003, Hill et al. 2004, Kramer et al. 2004, Hill and Ralph 2005).

Sonoike et al. (2001) demonstrated the importance of down-regulation in the acclimation process to high light stress by comparing the response of wildtype and a pmgA mutant of *Synechocystis* PCC 6803. The wildtype down-regulated PSII activity faster than the mutant and demonstrated a greater decrease in photochemical efficiency that resulted in lower electron flow and higher nonphotochemical quenching. In spite of the fact that the mutant cells grew faster during the first 3 d of exposure to high light, the failure to down-regulate the flow of electron transport finally led to cell death.

Mass production of *Arthrospira* is carried out mainly in large open ponds where light and temperature fluctuations vary from early morning (15°C and 1,000 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) to midday (35°C and 2,200 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) and are difficult to control (Vonshak et al. 1982, Vonshak 1997). Such conditions may cause a significant decrease in photosynthetic activity and productivity (Lu and Vonshak 1999, Vonshak et al. 2001).

Any attempt to improve productivity to increase the economic feasibility of algal mass culturing requires selection of strains that are adapted to the local climate fluctuations. Very little is known about attempts to isolate and use different strains that are suitable for specific climatic conditions. Many of the studies related to selection of strains compare the growth and productivity of the cultures under optimal conditions. In this study, we emphasize the importance of screening and selecting strains on the basis of their ability to withstand and acclimate to environmental stress. The strains selected in this study grow at a similar rate under optimal conditions but respond differently once exposed to environmental stress.

MATERIALS AND METHODS

Growth conditions and acclimation. Two strains of the filamentous cyanobacterium *A. platensis*—the strain marked as M2 isolated from Lake Chad, and the Kenya strain isolated from Lake Nakuru in Kenya—were obtained from the culture collection of the Centro di Studio dei Microrganismi Autotrofi in Florence, Italy, and were grown in batch-cultures in Zarrouk's medium containing 0.2 M NaCO2 (Vonshak et al. 1982). Cultures were incubated in a gyratory shaker agitated (Innova 4340, New Brunswick Scientific, Edison, NJ, USA) continuously at 120 rpm with an enriched atmosphere of 1% CO_2 and illuminated continuously at a photon flux density of 70–75 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. Constant temperature was maintained, either at the optimal temperature of 30°C or at a low-temperature treatment of 15°C. Cultures were grown and periodically diluted to chl concentration of 5 μg $\cdot$ mL^{-1} to maintain a logarithmic phase of growth. All measurements were performed on logarithmic phase cultures.

Measurement of pigments. Pigments were assayed spectrophotometrically; chl *a* was measured at 666 nm according to Bennet and Bogorad (1973) (OD_{666} = 13.9 μg $\cdot$ mL^{-1}). Phycocyanin was measured at 620 nm (Boussiba and Richmond 1979; OD_{620} = 125 μg $\cdot$ mL^{-1}).

Growth parameters. Dry weight (dwt) of cell biomass was determined by filtering culture samples through a GF5/2 glass fiber paper (Schleicher & Schuell Inc., Keen, NH, USA) and drying for 2 h at 105°C. Specific growth rate (μ) was calculated from the logarithmic phase growth curve, using the formula

$$\mu = (\ln X_2 - \ln X_1)(t_2 - t_1)^{-1} \quad (1)$$

where X_1 and X_2 are the chl concentrations at times t_1 and t_2, respectively, and units of μ are the reciprocal of time.

Chl fluorescence measurement. The polyphasic rise in chl fluorescence transients was recorded using a portable fluorometer (Plant Efficiency Analyzer, Hansatech Instruments Ltd., King's Lynn, UK) equipped with a liquid sample holder. Cultures grown at the logarithmic phase at either the optimal temperature treatment or the low temperature treatment were diluted to a final chl concentration of 5 μg $\cdot$ mL^{-1} and dark adapted for 10 min at room temperature. A polyphasic rise in fluorescence transients (OJIP test) was recorded and plotted on a logarithmic timescale from 50 μs to 1 s. The following parameters: F_0 (50 μs), $F_{300\ \mu s}$, F_J (2 ms), F_I (30 ms), and F_m (tF_{MAX}) were analyzed according to the OJIP-test procedure and used for calculation of energy fluxes based on the model of energy fluxes in the membranes (Strasser and Strasser 1995, Force et al. 2003, Strasser et al. 2004). The energy flux ratios in the photosynthetic apparatus are defined in the following equations and enable evaluation of the absorbed flux (ABS), the trapping flux (TR), the electron transport flux (ET), and the dissipation flux (DI).

PSII and PSI activity measurements. Cells were harvested, washed, and resuspended to a final concentration of 5 μg $\cdot$ mL^{-1} chl with 20 mM Bis Tris Propane buffer pH 6.8 with 0.5 M mannitol. Permeabilization of the cell walls was achieved by incubating the cells in the same buffer containing 9 mM *p*-benzoquinone for 3 min in the dark.

Electron transport activity through PSI was measured after adding a buffer with an assay mix containing 0.9 mM *p*-benzoquinone, 2 mM sodium azide (NaN_3) (inhibiting respiration), 10 μM DCMU (inhibiting PSII activity), 0.1 mM TMPD (*N*′*N*′*N*′-tetramethyl-*p*-phenylenediamine; electron donor), 5 mM sodium ascorbate (reducing TMPD), and 0.1 mM methyl viologen (electron acceptor) (all chemicals are from Sigma Inc., St. Louis, MO, USA). O_2 uptake in the dark and light was measured polarographically with a Clark's-type oxygen electrode at 30°C under a rate-saturating light intensity of 400 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. PSI activity was determined as net O_2 consumption in the light. According to Robinson et al. (1982) intact trichomes of *A. platensis* are permeable to the acceptor and donor used in our measurements.

Electron transport activity through PSII was measured using 0.9 mM *p*-benzoquinone as an electron acceptor under a rate-saturating light intensity of 400 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} and determined by O_2 consumption in the light (Satoh et al.1992).

Photoinhibitory treatments (exposure to HPFD) and photoinactivation of PSII. Cultures at the log-phase of growth were harvested and resuspended in fresh growth medium to yield a chl concentration of 25 $\mu g \cdot mL^{-1}$. The cultures were placed in a thermoregulated, double-jacket cylindrical glass vessel and maintained at various temperatures (15, 25, 30, or 35°C), in the presence and absence of 10 $\mu g \cdot mL^{-1}$ chloramphenicol (Sigma Inc.). Cultures were illuminated at an HPFD of 1,000 μmol photons $\cdot m^{-2} \cdot s^{-1}$ using a high-intensity halogen lamp (230 V, 1,000 W; Osram, München, Germany). At given intervals, samples were withdrawn for determination of maximum quantum yield of primary photochemistry (F_v/F_m).

Analysis of the fraction of active PSII was performed according to the Tsonev and Hikosaka (2003) model. They assumed that the reduction in the fraction of active PSII during the exposure to HPFD follows a model of two opposing reactions with different rate constants: (i) photoinactivation of PSII (k_{pi}) and (ii) recovery of inactivated PSII (k_{rec}). Furthermore, they assumed that the rate constants are proportional to the concentrations of active and inactive PSII. The fraction of active PSII (a) is calculated from the differences in the rate constants of inactivation of PSII during the exposure to HPFD in the absence and presence of a protein synthesis inhibitor and expressed as

$$a = \{k_{rec} + k_{pi}\exp[-(k_{pi}+k_{rec})t]\}(k_{pi} + k_{rec})^{-1} \quad (2)$$

where t is illumination time. In the presence of 10 $\mu g \cdot mL^{-1}$ chloramphenicol, k_{rec} becomes zero and the fraction of active PSII (a) is expressed as

$$a = \exp(-k_{pi}t) \quad (3)$$

RESULTS

Growth and biomass composition. The growth of two *Arthrospira* strains, M2 and Kenya, at 30°C and 15°C is depicted in Figure 1. While no significant difference could be observed in the growth rate of the two strains at 30°C, a significant decline was observed when cultures were grown at 15°C. It is worth noting that the decline in the growth of the Kenya strain was less pronounced than that of the M2 strain (41% and 59%, respectively; Table 1).

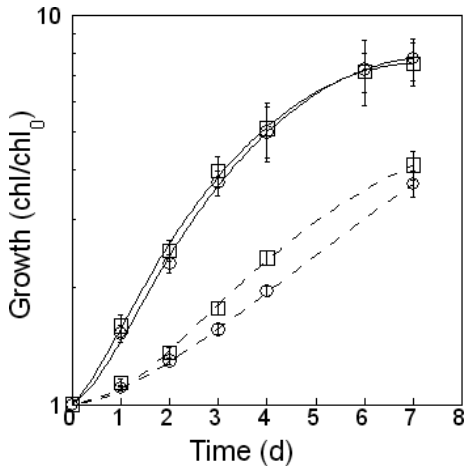

FIG. 1. Growth of M2 (○) and Kenya (□) strains grown at 30°C (control temperature, solid line) and 15°C (low temperature, broken line). Values represent mean ± SE of five (30°C) and seven (15°C) independent replication experiments per treatment.

The two strains grown under low temperature revealed a decline in cell content of chl and phycocyanin. While the decline in chl was almost the same (60%) in both, phycocyanin content decreased more in the Kenya strain than in the M2 strain (68% and 61%, respectively). Thus, the ratio of phycocyanin to chl in M2 was the same in cells grown at both optimal and low temperatures, while in the Kenya strain, a significant decrease in the ratio was observed between the two treatments (Table 1).

PSII photochemistry. To assess the modifications that took place in the photosynthetic machinery of the two *Arthrospira* strains acclimated to low temperature, the polyphasic rise in chl *a* fluorescence transient, including the O, J, I, and P steps, was measured and analyzed in control and acclimated cells (Force et al. 2003, Strasser et al. 2004). The OJIP transient expresses the successive reduction in the electron acceptor pool of PSII (Govindjee 2004) and as such provides a useful tool for comparing and evaluating modifications induced by environmental stress on PSII (Force et al. 2003, Zhu et al. 2005, Lazár 2006).

Although both strains demonstrated a similar pattern of polyphasic rise in fluorescence when grown at 30°C (Fig. 2a), the decrease in the fluorescence yield at the OJIP phases in cultures grown at low temperature revealed significant differences between the two strains. In M2, the effect on the J step was minor, and most of the decline appeared at the P step; in the Kenya strain, a decline in both J and P steps was observed (Fig. 2, a and b). The curves of the polyphasic rise in chl *a* fluorescence were used to extract specific parameters, such as yields and RC/CSX (Table 2).

It is worth noting that in the Kenya strain, the decreases in maximum quantum yield for primary photochemistry (φ_{Po}; −39%), quantum yield of electron transport beyond Q_A^- (φ_{Eo}; −42%), and RC/CS_X (−35%) were almost the same, while electron transport probability for electron transport beyond Q_A^- (ψ_o) decreased only slightly (6%). A different pattern was observed in M2, where the decreases in φ_{Po} (−13%), ψ_o (−17%), and density of RC/CS_X (−16%) were almost the same, with a greater decline in φ_{Eo} (−28%) (Table 2).

Comparing the performance index (PI_{ABS}; energy bifurcation in PSII on absorption basis) of the two strains grown at low temperature indicates that the decrease was greater in the Kenya strain. This finding suggests a greater ability of the strain to down-regulate its PSII activity compared with M2 (Table 2).

PSI and PSII activity. To examine whether the decrease in yield for primary photochemistry (φ_{Po}) and in RC/CS_X was generated by damage to PSII or

TABLE 1. Growth rate parameters and pigment composition in the M2 and Kenya strains grown at control and low temperatures.

	Control		Low temperature	
	M2	Kenya	M2	Kenya
Specific growth rate (μ) (d^{-1})	0.447 ± 0.013 (100)	0.448 ± 0.013 (100)	0.202 ± 0.010 (45)	0.265 ± 0.011 (59)
Chl/dwt	1.46 ± 0.07 (100)	1.61 ± 0.09 (100)	0.59 ± 0.08 (40)	0.70 ± 0.08 (43)
Pc/dwt	7.68 ± 0.03 (100)	9.14 ± 0.41 (100)	3.00 ± 0.235 (39)	2.905 ± 0.325 (32)
Pc/chl	4.90 ± 0.18 (100)	5.49 ± 0.11 (100)	4.87 ± 0.18 (99)	4.2 ± 0.14 (67)

Values are mean ± SE of five (Pc/chl) and two (chl/dwt, Pc/dwt) independent replication experiments. Numbers in parentheses are percentages of the control value. dwt, dry weight; Pc, phycocyanin.

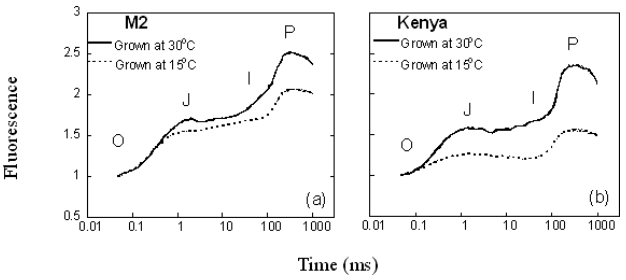

FIG. 2. Polyphasic chl *a* fluorescence transients (OJIP) in M2 (a) and Kenya (b) strains, grown at 30°C (○) and 15°C (□).

by down-regulation of PSII, the activity of PSI and PSII of the cultures acclimated to low temperature was measured. In both strains grown at low temperature, a lower PSII activity was observed, but the decrease in activity was higher in M2 than in the Kenya strain (45% and 26%, respectively; Table 3). In contrast, a marked increase in PSI activity was observed in both strains, with a much higher activity in the Kenya strain than in M2 (691% and 270%, respectively; Table 3).

Photoinactivation and recovery of PSII under HPFD. Acclimation to low temperature requires the development of protective mechanisms to deal with excess energy absorbed. We would therefore expect the low-temperature-acclimated cells to demonstrate better ability to handle high light stress. To explore this idea, we exposed cultures grown at low temperature to high light conditions and evaluated the quantitative contribution of photoprotective mechanisms in the two strains. According to Tsonev and Hikosaka (2003), rate constants that reflect photoinactivation and recovery in light-stressed cells can be calculated following the rate of decline in F_v/F_m in cultures exposed to HPFD in the presence and absence of an inhibitor of protein synthesis (for more details, see Materials and Methods). The changes in F_v/F_m in cultures grown at 30°C or 15°C and exposed to HPFD under different incubation temperatures in the absence and presence of 10 $\mu g \cdot mL^{-1}$ chloramphenicol were measured. No significant differences were observed between the two strains grown at 30°C. The only difference in the 30°C grown cells was between the protein inhibited and noninhibited cells incubated at temperatures above 15°C (data not shown).

A more evident difference between the two strains was observed when cultures grown at 15°C were exposed to HPFD with and without the protein synthesis inhibitor. While in the Kenya strain, the difference between the protein-synthesis-inhibited cells and the noninhibited cells was evident at incubation temperatures above 15°C (Fig. 3, e–f), in the M2 strain the difference was observed only in cells exposed to HPFD at 35°C (Fig. 3d).

TABLE 2. Yields at $t = 0$, density of reaction center (RC/CS_X) and performance indexes (PI_{ABS}), calculated from the OJIP-test in the M2 and Kenya strains grown at control and low temperatures.

	Control		Low temperature	
	M2	Kenya	M2	Kenya
Maximum quantum yield for primary photochemistry (φ_{Po})(TR_0/ABS)	0.63 ± 0.0032 (100)	0.60 ± 0.0068 (100)	0.55 ± 0.0049 (87)	0.37 ± 0.0019 (61)
Quantum yield for electron transport (φ_{Eo}; ET_0/ABS)	0.35 ± 0.004 (100)	0.35 ± 0.003 (100)	0.25 ± 0.004 (72)	0.20 ± 0.002 (58)
Quantum yield for energy dissipation (φ_{Do}; $1 - [F_v/F_m]$)	0.37 ± 0.003 (100)	0.40 ± 0.007 (100)	0.45 ± 0.005 (122)	0.63 ± 0.002 (159)
Electron transport probability (ψ_o; ET_0/TR_0)	0.55 ± 0.005 (100)	0.58 ± 0.005 (100)	0.46 ± 0.005 (83)	0.54 ± 0.005 (94)
Density of RC (RC/CS_X)	24.4 ± 0.34 (100)	18.2 ± 0.59 (100)	20.6 ± 0.39 (84)	11.9 ± 0.24 (65)
Performance index on an absorption basis, $PI_{(ABS)}$	0.70 ± 0.017 (100)	0.59 ± 0.015 (100)	0.25 ± 0.009 (36)	0.11 ± 0.003 (18)

Values represent mean ± SE of two independent replication experiments per treatment with four repetitions for each. Numbers in parentheses are percentages of the control value.

TABLE 3. PSI activity (μmol O_2 uptake · mg chl $a^{-1} \cdot h^{-1}$) and PSII activity (μmol O_2 evolved · mg chl $a^{-1} \cdot h^{-1}$) of M2 and Kenya strains grown at control and low temperatures.

	Control		Low temperature	
	M2	Kenya	M2	Kenya
PSI activity	52.3 ± 2.6 (100)	52.6 ± 1.4 (100)	141.3 ± 8.3 (270.1)	363.5 ± 28.8 (691.3)
PSII activity	248.5 ± 8.1 (100)	229.4 ± 7.7 (100)	137.7 ± 6.6 (55.4)	169.0 ± 10.5 (73.7)

Values represent means ± SE of three replications. Numbers in parentheses are percentages of the control value.

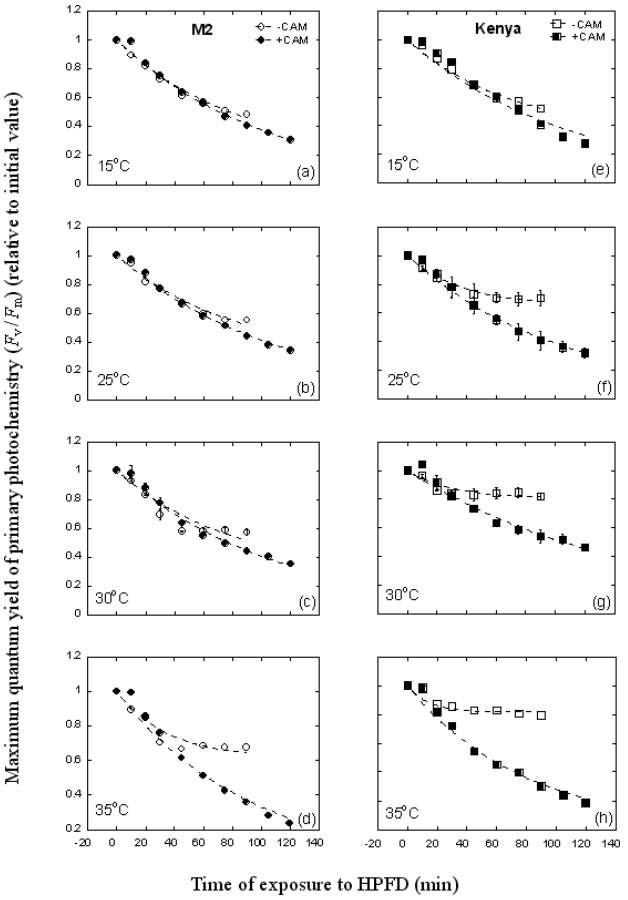

FIG. 3. Photoinactivation of PSII, indicated by relative reduction in maximal photochemical efficiency of PSII, in M2 (a–d) and Kenya (e–h) strains, grown at 15°C, and exposed to high photon flux density (HPFD) of 1,000 μmol photons · $m^{-2} \cdot s^{-1}$ at 15°C (a, e), 25°C (b, f), 30°C (c, g), and 35°C (g, h) in the presence (closed symbols) and absence (open symbols) of 10 μg · mL^{-1} of chloramphenicol (CAM). Results are means of four replicates in 1–4 independent experiments. Cells were incubated at each temperature for 5 min at dim light.

Summarizing the data (Fig. 4) revealed a major difference between the strains. The M2 strain grown at 30°C demonstrated a greater resistance to light stress. While an opposite pattern was observed in cultures grown at 15°C, the Kenya strain demonstrated a better capacity to withstand the light stress.

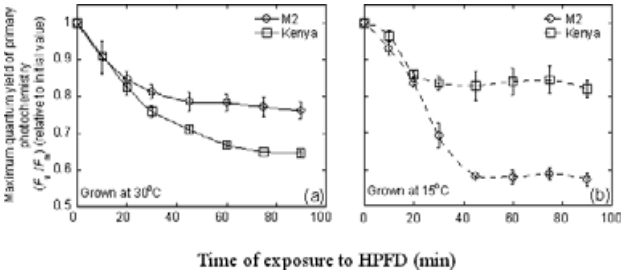

FIG. 4. Maximal photochemical efficiency of PSII in M2 (○) and Kenya (□) strains, grown at 30°C (a) and at 15°C (b), exposed to high photon flux density (HPFD) of 1,000 μmol · $m^{-2} \cdot s^{-1}$ at 30°C. Values were calculated from four replicates in 2–4 independent experiments. (Data extracted from Fig. 3.)

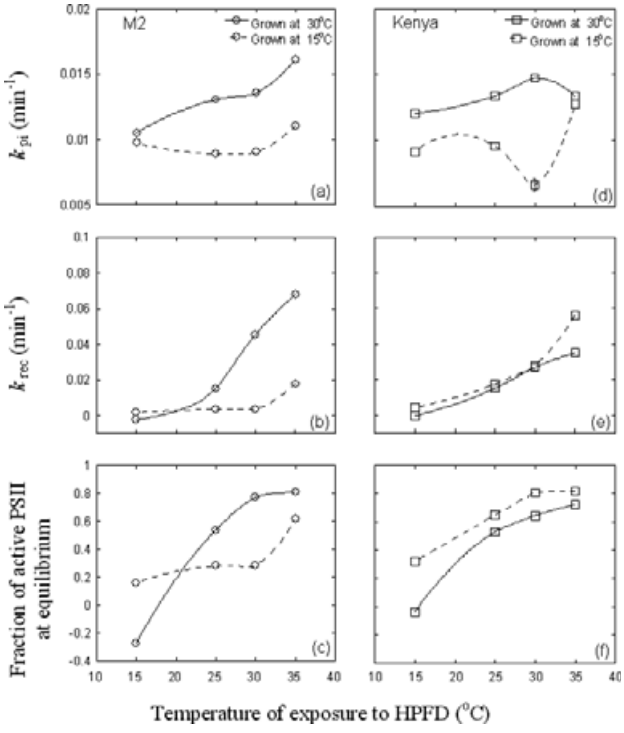

FIG. 5. The effect of temperature on the rate constants of photoinactivation, k_{pi} (a, d), the rate constants for recovery, k_{rec} (b, e), and the fraction of active PSII at equilibrium (c, f) in M2 (a–c) and Kenya (d–e) strains, at control (solid line) and low temperatures (broken line). Values were calculated from four replicates in 1–4 independent experiments. (Data were calculated from Fig. 3.)

The changes in the rate constant of photoinactivation (k_{pi}; extracted from Fig. 3) as a function of incubation temperature are shown in Figure 5, a and d. In M2 grown at 30°C, an increase in k_{pi} was observed with the increase in incubation temperature. Such an increase was not observed in the low-temperature culture (Fig. 5a). A somewhat different response was observed in the Kenya strain, which showed only a moderate increase in the culture grown at 30°C and a marked decline in the low-temperature culture (Fig. 5d).

The major difference was revealed in the rate constant of recovery (k_{rec}) of the two strains. While the Kenya strain maintained its recovery ability even in the low-temperature treatment (Fig. 5e), the M2 strain grown at 15°C demonstrated a low recovery constant that increased only when exposed to HPFD at 35°C (Fig. 5b).

Calculating the fraction of active PSII in the two strains revealed yet another difference between the strains: the fraction of active PSII in the Kenya strain responded similarly to incubation temperatures regardless of growth temperature (Fig. 5f). On the other hand, in the M2 strain grown at 15°C, the fraction of active PSII did not increase with incubation temperature; it did increase in the culture incubated at 35°C (Fig. 5c).

DISCUSSION

The growth of cyanobacteria, like that of many other photoautotrophs, is highly dependent on the ability of the organisms to correlate tightly the process of light energy harvested by the photosynthetic antenna and energy consumption by the photosynthetic process. Thus, any change in environmental conditions requires the development of acclimation mechanisms that will adjust the energy flow from the antenna through the two photosystems to the Calvin cycle (Huner et al. 1998, Ensminger et al. 2006, Wilson et al. 2006).

The ability of the two *Arthrospira* strains to acclimate to low temperature was compared to improve our understanding of the mechanisms involved in the photosynthetic apparatus. Moreover, the comparison may provide a better insight into the question of cross-adaptation in cyanobacteria. The ability of the Kenya strain to better acclimate to low temperature is first indicated in its ability to maintain a higher growth rate than the M2 strain when grown at the low temperature. Another indication of the difference is reflected in the changes in the antenna size. Downsizing the antenna in the Kenya strain results in less energy being transferred to the photosynthetic RCs (Table 1).

The effect of low temperature on the polyphasic fluorescence transient and analysis of photosynthetic parameters. The chl *a* fluorescence transient (OJIP test) is frequently used to analyze changes induced in the photosynthetic apparatus in response to environmental stress in algae as well as in cyanobacteria (Force et al. 2003, Strasser et al. 2004). The reduction in the J step (Fig. 2) observed mainly in the Kenya strain grown at low temperature may indicate a decrease in the accumulation of reduced Q_A (Govindjee 2004, Zhu et al. 2005, Lazár 2006) as well as a decrease in the concentration of active PSII RC (RC/CS_X; Strasser and Strasser 1995), as shown in Table 2. The decline in the P step (Fig. 2), which was steeper in the Kenya strain, reflects a bigger decline in the concentration of Q_A^-, Q_B^{2-}, and plastoquinol (PQH_2) (Govindjee 2004, Zhu et al. 2005, Lazár 2006). This outcome may be the result of a decrease in the accumulation of reduced Q_A and a smaller concentration of active PSII RC (RC/CS_X) in the Kenya strain. These results are in line with other reports on the response of algae to other stresses, such as bleaching (Hill et al. 2004), photoinhibition (Lu and Vonshak 1999), and high salt (Lu et al. 1999).

In the Kenya strain grown at low temperature, the decrease in the concentration of active PSII RC (RC/CSx; Table 2) resulted in a similar decrease in φ_{Po}, followed by a similar decrease in φ_{Eo}. In contrast, no significant change was observed in ψ_o, meaning there was no effect on the capacity of Q_A^- reoxidation. A somewhat different response was observed in M2 grown at low temperature. A higher concentration of active PSII RC (RC/CSx) was maintained, which reflected maintenance of a similar level of φ_{Po}. Thus, it is worth noting that ψ_o was probably affected due to the impairment in the acceptor side of PSII that decreased the capacity of Q_A^- reoxidation and resulted in a significant decrease in φ_{Eo}. Similarly, *Arthrospira* cells exposed to salt stress exhibited an inhibition in both the acceptor and the donor sides of PSII (Lu and Vonshak 2002).

In conclusion, we suggest that the Kenya strain acclimates better to low temperature through down-regulating its photosynthetic activity by (i) decreasing the antenna size and thus reducing the energy flux into the photosystems; (ii) decreasing RC/CSx and performance index on cross-section basis (PI_{CS}), thereby lowering the trapping probability and the electron transport rate, but keeping ψ_o unchanged; and (iii) increasing the dissipation flux, which reflects the energy that either dissipates or is transferred to other electron transport pathways. In contrast, in the M2 strain, the antenna size stays constant (Table 1) and shows a much lower decrease in the density of active RC, as well as a lower dissipation rate (Table 2). Thus, the Kenya strain minimizes the potential damage on the acceptor side of PSII, while M2 cells exhibit damage on the acceptor side reflected in a decrease in the ψ_o.

PSI and PSII activity. Dissipation of energy not utilized in the photochemical process may take place by alternative electron transport pathways such as respiration or state transitions between PSII and PSI (Campbell et al. 1998). State transition has been suggested to be a part of the acclimation mechanisms to environmental stress in cyanobacteria, including *A. platensis*, which adjusts the distribution of excitation energy between the two photosystems and is regulated by the redox state of the electron transport (Campbell et al. 1998, El Bissati et al. 2000, McConnell et al. 2002, Allen and Mullineaux 2004, Li et al. 2004, Mullineaux and Emlyn-Jones 2005). Furthermore, Rakhimberdieva et al. (2001) determined that in *A. platensis*, PSII contains only

5% of the chl and binds to only 20% of the phycobilisomes. Thus, PSI is the massive fraction that is connected to most of the phycobilisomes, and thus the species can absorb energy not only through PSII.

It is worth noting that the larger increase in PSI activity in the Kenya strain acclimated to low temperature is in line with the increase in dissipation flux. This concordance indicates the potential involvement of state transition as a photoprotective mechanism against excess energy, resulting in down-regulation of PSII efficiency (Campbell et al. 1998). This idea is further supported by the higher PSII activity in the Kenya strain grown at the low temperature (Table 3), suggesting that state transition is a control mechanism that provides protection to PSII from excitation energy (Wollman 2001) and previously suggested in *A. platensis* under salt stress (Lu et al. 1999).

The effect of low temperature on the response to light stress. Acclimation to low temperature, similar to the acclimation to high light, is tightly connected with the ability to utilize and regulate excess energy (Huner et al. 1996, Miskiewicz et al. 2002, Wilson et al. 2006). Sonoike et al. (2001) demonstrated that under acclimation to high light stress the adjustment of photosynthesis is not by maintaining efficient photosynthesis but by down-regulating electron transport. In the current work, when acclimating to low temperature, another environmental stress, the Kenya strain down-regulated the electron transport rate. The similarity in the adjustment in response to different stresses suggests that the strain acclimated to low-temperature stress may acquire a better ability to withstand an HPFD stress.

Exposing cells to HPFD in the presence and absence of an inhibitor of protein synthesis helps to distinguish between the rate of damage and the impact of the recovery process on the photoinhibitory response. Once grown at 15°C, the M2 cells appear to lose a significant part of their ability to recover from the HPFD stress (Fig. 3). The difference between the strains in relation to their acclimation ability is more evident when comparing the response of the two strains grown at 30°C or 15°C and exposed to HPFD at 30°C (Fig. 4). While M2 cells grown at 30°C are more resistant to HPFD (Fig. 4a), an inversion of the response is observed in cells grown at 15°C (Fig. 4b). Thus, it seems that the acclimation to low temperature in the Kenya cells provided them with an improved ability to tolerate the HPFD stress. Part of this ability is enabled through a better recovery capacity of the Kenya strain (Fig. 5e), resulting in a higher fraction of active PSII (Fig. 5f). This finding is further supported by the suggestion of Nishiyama et al. (2006) that in *Synechocystis* low temperature affects mainly the rate of recovery and not the rate of induced damage in PSII.

CONCLUSION

This study demonstrates that the acclimation process associated with the photosynthetic apparatus requires the ability to down-regulate photosynthetic activity, which is achieved by modifying the flow into and through, and dissipation of energy from the photosynthetic apparatus. The ability of the Kenya strain to acquire all of the protective mechanisms results in a better acclimation capacity. Furthermore, the acclimation process to low temperature was accompanied by an improved mechanism for handling excess energy, resulting in a greater ability of the Kenya strain to withstand an HPFD stress—a finding that may be defined as a cross-adaptation phenomenon (Bowler and Fluhr 2000, Savitch et al. 2000, Wilson and Huner 2000, Pocock et al. 2001, Pastori and Foyer 2002, Wang et al. 2004). Although both strains used in this study are identified as *A. platensis*, it is interesting to note that they have developed somewhat different strategies to cope with the fluctuations and changes in temperature.

The authors would like to thank Prof. R. J. Strasser for his valuable comments and helpful advice.

Adams, W. W., III, Dommig–Adams, B., Rosenstiel, T. N., Ebbert, V., Brightwell, A. K., Barker, D. H. & Zarter, C. 2001. Photosynthesis, xanthophyll, and D1 phosphorylation under winter stress. *In PS2001 Proceedings: 12th International Congress on Photosynthesis.* CSIRO publishing, Melbourne, Australia. Available at http://www.publish.csiro.au/ps_2001.

Allen, J. F. & Mullineaux, C. W. 2004. Probing the mechanism of state transition in oxygenic photosynthesis by chlorophyll fluorescence spectroscopy, kinetics and imaging. *In* Papageorgiou, G. C. & Govindjee [Eds.] *Chlorophyll a Fluorescence: A Signature of Photosynthesis.* Springer, Dordrecht, the Netherlands, pp. 447–61.

Allen, D. J. & Ort, D. R. 2001. Impacts of chilling temperatures on photosynthesis in warm-climate plants. *Trends Plant Sci.* 6:36–42.

Ban Dar, H. & Leu, K.-L. 2003. A possible origin of the middle phase of polyphasic chlorophyll fluorescence transient. *Funct. Plant Biol.* 30:571–6.

Bennet, J. & Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue–green alga. *J. Cell Biol.* 58:419–35.

Boussiba, S. & Richmond, A. 1979. Isolation and purification of phycocyanins from the blue–green alga *Spirulina platensis. Arch. Microbiol.* 120:155–9.

Bowler, C. & Fluhr, R. 2000. The role of calcium and activated oxygens as signals for controlling cross-tolerance. *Trends Plant Sci.* 5:241–6.

Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P. & Öquist, G. 1998. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.* 62:667–83.

El Bissati, K., Delphin, E., Murata, N., Etienne, A.-L. & Kirilovsky, D. 2000. Photosystem II fluorescence quenching in the cyanobacterium *Synechocystis* PCC 6803: involvement of two different mechanisms. *Biochim. Biophys. Acta* 1457:229–42.

Ensminger, I., Busch, F. & Huner, N. P. A. 2006. Photostasis and cold acclimation: sensing low temperature through photosynthesis. *Physiol. Plant.* 126:28–44.

Force, L., Critchley, C. & van Rensen, J. J. S. 2003. New fluorescence parameters for monitoring photosynthesis in plants. 1. The effect of illumination on the fluorescence parameters of the JIP-test. *Photosynth. Res.* 78:17–33.

Goncalves, J. F. C. & Santos, U. M. 2005. Fluorescence technique as a tool for selecting tolerant species to environments of high irradiance. *Brazil. J. Plant Physiol.* 17:307–13.

Govindjee 2004. Chlorophyll a fluorescence: a bit of basics and history. *In* Papageorgiou, G. C. & Govindjee [Eds.] *Chlorophyll a Fluorescence: A Signature of Photosynthesis.* Springer, Dordrecht, the Netherlands, pp. 321–62.

Hermans, C., Smeyers, M., Rodriguez, R. M., Eyletters, M., Strasser, R. J. & Delhaye, J.-P. 2003. Quality assessment of urban trees: a comparative study of physiological characterization, airborne imaging and on site fluorescence monitoring by the OJIP-test. *J. Plant Physiol.* 160:81–90.

Hill, R., Larkum, A. W. D., Frankart, C., Kühl, M. & Ralph, P. J. 2004. Loss of functional photosystem II reaction centers in zooxanthellae of corals exposed to bleaching conditions: using fluorescence rise kinetics. *Photosynth. Res.* 82:59–72.

Hill, R. & Ralph, P. J. 2005. Diel and seasonal changes in fluorescence rise kinetics of three scleractinian corals. *Funct. Plant Biol.* 32:549–59.

Huner, N. P. A., Maxwell, D. P., Gray, G. R., Savitch, L. V., Krol, M., Ivanov, A. G. & Falk, S. 1996. Sensing environmental temperature change through imbalances between energy supply and energy consumption: redox state of photosystem II. *Physiol. Plant.* 98:358–64.

Huner, N. P. A., Öquist, G. & Sarhan, F. 1998. Energy balance and acclimation to light and cold. *Trends Plant Sci.* 3:224–30.

Kramer, D. M., Avenson, T. J., Kanazawa, A., Cruz, J. A., Ivanov, B. & Edwards, G. E. 2004. The relationship between photosynthetic electron transport and its regulation. *In* Papageorgiou, G. C. & Govindjee [Eds.] *Chlorophyll a Fluorescence: A Signature of Photosynthesis.* Springer, Dordrecht, the Netherlands, pp. 251–78.

Kruskopf, M. & Flynn, K. J. 2006. Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. *New Phytol.* 169:525–36.

Lazár, D. 2006. The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Funct. Plant Biol.* 33:9–30.

Li, D., Xie, J., Zhao, J., Xia, A., Li, D. & Gong, Y. 2004. Light induced excitation energy redistribution in *Spirulina platensis* cells: ''spillover'' or ''mobile PBSs''? *Biochim. Biophys. Acta* 1608:114–21.

Lu, C.-M., Torzillo, G. & Vonshak, A. 1999. Kinetic response of photosystem II photochemistry in cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. *Aust. J. Plant Physiol.* 26:283–92.

Lu, C.-M. & Vonshak, A. 1999. Photoinhibition in outdoor *Spirulina platensis* cultures assessed by polyphasic chlorophyll fluorescence transients. *J. Appl. Phycol.* 11:355–9.

Lu, C.-M. & Vonshak, A. 2002. Effect of salinity stress on photosystem II function in cyanobacterial *Spirulina platensis* cells. *Physiol. Plant.* 114:405–13.

Maxwell, K. & Johnson, G. N. 2000. Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* 51:659–68.

McConnell, M. D., Koop, R., Vasil'ev, S. & Bruce, D. 2002. Regulation of the distribution of chlorophyll and phycobilin-absorbed excitation energy in cyanobacteria. A structure-based model for the light state transition. *Plant Physiol.* 130:1201–12.

Melis, A. 1999. Photosystem-II damage and repair cycle in chloroplasts: what modulates the rate of photodamage in vivo? *Trends Plant Sci.* 4:130–5.

Miskiewicz, E., Ivanov, A. G. & Huner, N. P. A. 2002. Stoichiometry of the photosynthetic apparatus and phycobilisome structure of the cyanobacterium *Plectonema boryanum* UTEX 485 are regulated by both light and temperature. *Plant Physiol.* 130:1414–25.

Mullineaux, C. W. & Emlyn-Jones, D. 2005. State transitions: an example of acclimation to low-light stress. *J. Exp. Bot.* 56: 389–93.

Nishiyama, Y., Allakhverdiev, S. I. & Murata, N. 2006. Regulation by environmental conditions of the repair of photosystem II incyanobacteria. *In* Demmig-Adams, B., Adams, W. W., III & Mattoo, A. K. [Eds.] *Photoprotection, Photoinhibition, Gene Regulation, and Environment. Advances in Photosynthesis and Respiration.* Vol. 21. Springer, Dordrecht, the Netherlands, pp. 193–203.

Pastori, G. M. & Foyer, C. H. 2002. Common components, networks, and pathways of cross-tolerance to stress. The central role of ''redox'' and abscisic-acid-mediated controls. *Plant Physiol.* 129:460–8.

Pocock, T. H., Hurry, V., Savitch, L. V. & Huner, N. P. A. 2001. Susceptibility to low-temperature photoinhibition and the acquisition of freezing tolerance in winter and spring wheat: the role of growth temperature and irradiance. *Physiol. Plant.* 113:499–506.

Powles, S. B. 1984. Photoinhibition of photosynthesis induced by visible light. *Annu. Rev. Plant Physiol.* 35:15–44.

Prasil, O., Adir, N. & Ohad, I. 1992. Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. *In* Barber, J. [Ed.] *The Photosystems: Structure, Function and Molecular Biology.* Elsevier Science Publishers, Amsterdam, the Netherlands, pp. 295–348.

Qiu, B., Zhang, A., Zhou, W., Wei, J., Dong, H. & Liu, Z. 2004. Effects of potassium on the photosynthetic recovery of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae) during rehydration. *J. Phycol.* 40:323–32.

Rakhimberdieva, M. G., Biochenco, A. V., Karapetyan, N. V. & Stadnichuk, I. N. 2001. Interaction of phycobilisomes with photosystem II dimers and photosystem I monomers and trimers in the cyanobacterium *Spirulina platensis. Biochemistry* 40:15780–8.

Raven, J. A. & Geider, R. J. 2003. Adaptation, acclimation and regulation in algal photosynthesis. *In* Larkum, A. W. D., Douglas, E. & Raven, J. A. [Eds.] *Advances in Photosynthesis and Respiration: Photosynthesis in Algae.* Vol. 14. Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 385–412.

Robinson, S. J., DeRoo, C. S. & Yocum, C. F. 1982. Photosynthetic electron transfer in preparations of the cyanobacterium *Spirulina plantensis. Plant Physiol.* 70:154–61.

Satoh, K., Koike, H., Ichimura, T. & Katoh, S. 1992. Binding affinities of benzoquinones to the Q_B site of photosystem II in *Synechococcus* oxygen-evolving preparation. *Biochim. Biophys. Acta* 1120:45–52.

Savitch, L. V., Massacci, A., Gray, G. R. & Huner, N. P. A. 2000. Acclimation to low temperature or high light mitigates sensitivity to photoinhibition: roles of the Calvin cycle and the Mehler reaction. *Aust. J. Plant Physiol.* 27:253–64.

Sonoike, K., Hihara, Y. & Ikeuchi, M. 2001. Physiological significance of the regulation of photosystem stoichiometry upon high light acclimation of *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* 42:379–84.

Strasser, R. J., Srivastava, A. & Govindjee 1995. Polyphasic chlorophyll-alpha fluorescence transient in plant and cyanobacteria. *Photochem. Photobiol.* 61:32–42.

Strasser, B. J. & Strasser, R. J. 1995. Measuring fast fluorescence transients to address environmental questions: the JIP-test. *In* Mathis, P. [Ed.] *Photosynthesis: From Light to Biosphere.* Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 977–80.

Strasser, R. J., Tsimilli-Michael, M. & Srivastava, A. 2004. Analysis of the chlorophyll a fluorescence transient. *In* Papageorgiou, G. C. & Govindjee [Eds.] *Chlorophyll a Fluorescence: A Signature of Photosynthesis.* Springer, Dordrecht, the Netherlands, pp. 321–62.

Strauss, A. J., Krüger, G. H. J., Strasser, R. J. & Van Heerden, P. D. R. 2006. Ranking of dark chilling tolerance in soybean genotypes probed by the chlorophyll a fluorescence transient O-J-I-P. *Environ. Exp. Bot.* 56:147–57.

Tomek, P., Lazár, D., Ilik, P. & Naus, J. 2001. On the intermediate steps between of the O and P steps in chlorophyll a fluorescence rise measured at different intensities of exciting light. *Aust. J. Plant Physiol.* 28:1151–60.

Torzillo, G., Bernardini, P. & Masojidek, J. 1998. On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina plantensis* (cyanobacteria). *J. Phycol.* 34:504–10.

Tsonev, T. D. & Hikosaka, K. 2003. Contribution of photosynthetic electron transport, heat dissipation, and recovery of

photoinactivated photosystem II to photoprotection at different temperatures in *Chenopodium album* leaves. *Plant Cell Physiol.* 44:828–35.

Tsonev, T., Velikova, V., Georgieva, K., Hyde, P. F. & Jones, H. G. 2003. Low temperature enhances photosynthetic downregulation in French bean (*Phaseolus vulgaris* L.) plants. *Ann. Bot.* 91:343–52.

Vonshak, A. 1997. Outdoor mass production of *Spirulina*: the basic concept. *In* Vonshak, A. [Ed.] Spirulina platensis *(*Arthrospira*): Physiology, Cell-Biology and Biotechnology*. Taylor & Francis, London, UK, pp. 79–99.

Vonshak, A., Abeliovich, A., Boussiba, S. & Richmond, A. 1982. Production of *Spirulina* biomass: effect of environmental factors and population density. *Biomass* 2:175–85.

Vonshak, A., Torzillo, G., Masojidek, J. & Boussiba, S. 2001. Sub-optimal morning temperature induces photoinhibition in dense outdoor cultures of the alga *Monoduse subterraneus* (Eustigmatophyta). *Plant Cell Environ.* 24:1113–8.

Wang, L.-J., Huang, W.-D., Li, J.-Y., Liu, Y.-F. & Shi, Y.-L. 2004. Peroxidation of membrane lipid and Ca^{2+} homeostasis in grape mesophyll cells during the process of cross-adaptation to temperature stresses. *Plant Sci.* 167:71–7.

Wilson, K. E. & Huner, N. P. A. 2000. The role of growth rate, redox-state of the plastoquinone pool and the trans-thylakoid ΔpH in photoacclimation of *Chlorella vulgaris* to growth irradiance and temperature. *Planta* 212:93–102.

Wilson, K. E., Ivanov, A. G., Öquist, G., Grodzinski, B., Sarhan, F. & Huner, N. P. A. 2006. Energy balance, organellar redox status, and acclimation to environmental stress. *Can. J. Bot.* 84:1355–70.

Wollman, F. A. 2001. State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO (Eur. Mol. Biol. Organ.) J.* 20:3623–30.

Yamamoto, Y. 2001. Quality control of photosystem II. *Plant Cell Physiol.* 42:121–8.

Zhu, X.-G., Govindjee, Baker, N. R., deSturler, E., Ort, D. R. & Long, S. P. 2005. Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with photosystem II. *Planta* 223:114–33.