Supplemental Material can be found at:
http://www.jbc.org/cgi/content/full/M606417200/DC1

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 12, pp. 8947–8958, March 23, 2007
© 2007 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

Contrasting Behavior of Higher Plant Photosystem I and II Antenna Systems during Acclimation*[S]

Received for publication, July 6, 2006, and in revised form, January 16, 2007 Published, JBC Papers in Press, January 17, 2007, DOI 10.1074/jbc.M606417200

Matteo Ballottari[‡], **Luca Dall'Osto**[‡], **Tomas Morosinotto**[§¶], **and Roberto Bassi**[‡§1]

From the [‡]*Dipartimento Scientifico e Tecnologico, Università di Verona, Strada Le Grazie, 15 37134 Verona, Italy,* [§]*Laboratoire de Génétique et Biophysique des Plantes, Université Aix-Marseille II, 163 Avenue de Luminy, 13009 Marseille, France, and* [¶]*Dipartimento di Biologia, Università di Padova, 35131 Padova, Italy*

In this work we analyzed the photosynthetic apparatus in *Arabidopsis thaliana* plants acclimated to different light intensity and temperature conditions. Plants showed the ability to acclimate into different environments and avoid photoinhibition. When grown in high light, plants had a faster activation rate for energy dissipation (qE). This ability was correlated to higher accumulation levels of a specific photosystem II subunit, *PsbS*. The photosystem II antenna size was also regulated according to light exposure; smaller antenna size was observed in high light-acclimated plants with respect to low light plants. Different antenna polypeptides did not behave similarly, and *Lhcb1*, *Lchb2*, and *Lhcb6* (CP24) are shown to undergo major levels of regulation, whereas *Lhcb4* and *Lhcb5* (CP29 and CP26) maintained their stoichiometry with respect to the reaction center in all growth conditions. The effect of acclimation on photosystem I antenna was different; in fact, the stoichiometry of any Lhca antenna proteins with respect to photosystem I core complex was not affected by growth conditions. Despite this stability in antenna stoichiometry, photosystem I light harvesting function was shown to be regulated through different mechanisms like the control of photosystem I to photosystem II ratio and the association or dissociation of *Lhcb* polypeptides to photosystem I.

Plants are exposed to an environment where light and temperature conditions are largely variable. Because they have no possibility of moving to a more favorable environment, plants have evolved several mechanisms of acclimation. Among all the parameters, light intensity has a major influence on plants life; it is a limiting growth factor at dusk or under dense canopy, but it can easily be in excess at midday or under full sun, thus leading to oxidative stress and photoinhibition (1, 2). Low temperature is known to play a synergistic role with excess illumination by limiting electron transport and carbon fixation rates. In these conditions, even a weak light can exceed energy utilization rate and become photoinhibitory (3).

Mechanisms of photo-protection can be classified according to the time scale of activation upon establishment of the stress. Illumination rapidly activates the dissipation of the excitation energy as heat, a process called nonphotochemical quenching (NPQ)[2] (4, 5). Its fastest component is called qE, and it is known to depend on the presence of the PSII subunit *PsbS* (6). NPQ also includes components activated on a slower time scale such as the synthesis of zeaxanthin from violaxanthin (7–9) with zeaxanthin also active in scavenging ROS (10). These mechanisms are effective in reducing light damage during fast changes in illumination. However, when excess light is experienced during long periods, plants activate other mechanisms of response, such as the modulation of the light harvesting apparatus and metabolic energy sinks. In fact, in high light, the stoichiometry of electron carriers and enzymes of the Calvin cycle increases (11–13), whereas the relative abundance of antenna proteins with respect to the reaction center complexes decreases (14, 15).

Here we have extended previous works on the acclimation changes of the model plant *Arabidopsis thaliana* upon growth in different light and temperature conditions. Functional analysis confirmed that plants were indeed acclimated as shown by the absence of PSII photoinhibition, the modulation of oxygen evolution, and the photochemical capacity in high light. Long term exposure to high light was also shown to induce the accumulation of *PsbS* and a correlated increase in the qE component of NPQ. Of particular interest is the regulation of the antenna system; the adaptation to different light and temperature conditions leads to extensive changes within the PSII antenna system. Each of the different Lhcb components is tuned in their relative amount with the exception of *Lhcb4* and *Lhcb5* whose stoichiometry with respect to RCII remained substantially constant. *Lhcb6* is peculiar because it undergoes the highest level of modulation, being virtually absent in HL conditions. On the contrary, in PSI no changes were detected in the stoichiometry of any of the Lhca proteins with respect to the reaction center. This suggests that PSI-LHCI behaves as the reference complex

* This work was supported by Ministero dell'Istruzione Università e Ricerca Progetti Fondo per gli Investimenti della Ricerca di Base RBAU01E3CX and GENEFUN (functional genetics) program. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.

[1] To whom correspondence should be addressed. Tel.: 390458027916; Fax: 390458027929; E-mail: bassi@sci.univr.it.

[2] The abbreviations used are: NPQ, nonphotochemical quenching; $\alpha(\beta)$-DM, *n*-dodecyl-$\alpha(\beta)$-D-maltoside; Chl, chlorophyll; CP, chlorophyll protein; Lhca (b), light harvesting complex of photosystem I (II); LHCI (LHCII), antenna complex of photosystem I (II); PSI (II), photosystem I (II); RC, reaction center; ROS, reactive oxygen species; μE, microeinstein; HPLC, high pressure liquid chromatography; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Rubisco, ribulose-bisphosphate carboxylase/oxygenase; qP, photochemical quenching; VDE, violaxanthin de-epoxidase; Ctrl, control; LL, low light; HL, high light; cLL, cold low light; cHL, cold high light; ZE, zeaxanthin epoxidase.

Downloaded from www.jbc.org at CNRS on May 20, 2008

with respect to which PSII-LHCII undergoes regulation to maintain chloroplast redox state and ensure photoprotection.

EXPERIMENTAL PROCEDURES

Plant Growth and Light Temperature Treatments—*A. thaliana* plants (Columbia ecotype) were grown for 4 weeks at 100 μE, 19 °C, 90% humidity, and 8 h of daylight. Afterward, they were moved under different light and temperature conditions for an additional 3 weeks. The conditions used are the following: control, 21 °C, 100 μE; low light (LL), 21 °C, 25 μE; high light (HL), 21 °C, 1600 μE; cold low light (cLL), 10 °C, 25 μE; and cold high light (cHL), 10 °C, 600 μE. In all measurements only fully expanded mature leaves were used, and they belonged to the 4th to the 7th leaf pair depending on the time and condition.

Chlorophyll Fluorescence and Photosynthetic Parameter Measurements—Chlorophyll fluorescence was measured at room temperature on intact leaves of acclimated plants with a PAM-101 fluorimeter with a saturating light at 4500 μE and actinic light at six different intensities as follows: 100, 360, 640, 1200, 1600, and 2000 μE. Before measurements, plants were dark-adapted for at least 30 min at room temperature. The same measurements were also performed with actinic light of 2000 μE but with an overnight dark adaptation. The parameters F_v/F_m, NPQ, and photochemical quenching (qP) were calculated as $(F_m - F_o)/F_o$, $(F_m - F_m')/F_m'$, and $(F_m' - F)/(F_m' - F_o)$ (16).

Chloroplast Isolation and Purifications of PSI-LHCI and LHCI—Chloroplasts were isolated from adapted plants by homogenizing leaves in a solution with 0.1 M Tricine/KOH, pH 7.8, 0.4 M sorbitol, 0.5% powder milk. The chloroplasts were then isolated by precipitation at 1500 × *g*. PSI-LHCI complexes were purified from all plants with the method described previously (17, 18).

Spectroscopy and Pigment Analysis—The absorption spectra were recorded using an SLM-Aminco DK2000 spectrophotometer in 5 mM Tricine, pH 7.8, 0.5 M sucrose, and 0.03% β-DM. HPLC analysis was performed according to Ref. 19. Chlorophyll to carotenoid ratio and Chl a/b ratio were independently measured by fitting the spectrum of acetone extracts with the spectra of individual purified pigments (18). CD spectra were measured at 10 °C on a Jasco 600 spectropolarimeter. Chloroplast fluorescence spectra at 77 K were measured with Varian Cary Eclipse and corrected for instrumental response. Samples were in glycerol 60% (v/v) and 10 mM HEPES, pH 7.5. Emission spectra were measured with a 475 nm excitation, whereas excitation spectra were measured with a 735 nm emission.

Short Term High Light Stress—Leaves from acclimated plants were transferred in glass tubes in N_2 atmosphere and illuminated with light at 1800 μE for 7, 15, and 30 min (20). Pigment extraction with 80% acetone was then performed on leaf disks frozen in liquid nitrogen.

SDS-PAGE Analysis and Coomassie Stain Quantification—PSI-LHCI complexes were analyzed with SDS-PAGE as described in Ref. 21 but using a acrylamide/bisacrylamide ratio of 75:1 and a total concentration of acrylamide + bisacrylamide of 4.5 and 15.5%, respectively, for the stacking and running gel, where 6 M urea was also incorporated (22). The staining for the densitometry was obtained with 0.05% Coomassie Blue R-250 in 25% isopropyl alcohol, 10% acetic acid to improve linearity of the stain with respect to protein amount (23). The protein amount was evaluated after SDS-PAGE by quantifying the stain bound to each band by colorimetry. We acquired the gel image using Bio-Rad GS710 scanner, and the picture was then analyzed with Gel-Pro Analyzer© software, which quantifies the staining of the bands as integrated optical density on the area of the band. At least five repetitions of each sample were analyzed to achieve sufficient accuracy. For the evaluation of Rubisco content, leaf disks were cut, and Chl content was determined with high accuracy. Then the amount of Rubisco, on a Chl basis, was determined by SDS-PAGE and Coomassie staining after identification of the band of Rubisco large subunit by Western blotting.

Immunoblot Assays and Western Blotting Quantifications—For the quantification of *PsbS*, VDE, ZE, *Lhcb3–6*, and CP47 in different acclimated plants, leaves were homogenized in liquid nitrogen with a solution with 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol. The chlorophyll content was then quantified by absorption spectra. For each sample 0.2, 0.4, 0.8, 1, and 1.2 μg of chlorophylls have been loaded on SDS-PAGE. Immunoblot assays with antibodies against different polypeptides were performed as described previously (24). To avoid any deviation between different immunoblots, samples were compared only when loaded in the same gel.

Deriphat PAGE Analysis—Nondenaturing Deriphat-PAGE was performed following the method described previously (25), but using 3.5% (w/v) acrylamide (38:1 acrylamide/bisacrylamide) in the stacking gel and in the resolving gel an acrylamide concentration gradient from 4.5 to 11.5% (w/v) stabilized by a glycerol gradient from 8 to 16%. Thylakoids concentrated at 1 mg/ml chlorophylls were solubilized with a final 0.8% β-DM, and 30 μg of chlorophylls were loaded. The gel images were then analyzed with Gel-Pro Analyzer©. The integrated optical density measured in each band was checked to linearly correlate to the chlorophyll amounts present in each complex.

Tocopherol Quantification—Tocopherols were quantified as described previously (26). After extraction with 100% methanol and separation by HPLC, tocopherol was detected with a fluorescence detector using an excitation wavelength at 295 nm and an emission wavelength was 340 nm. Tocopherol standards were obtained from Sigma.

RESULTS

Arabidopsis Plants Acclimate to Different Light Conditions and Avoid Photoinhibition—In this work we analyzed the photosynthetic apparatus of *Arabidopsis thaliana* plants grown in different light and temperature conditions. Plants grown for 4 weeks at 21 °C and 100 μE have been treated for a further 3 weeks at different conditions. Two different light intensities were combined with two temperatures, yielding a total of five different treatments, including the control condition: low light, high light, cold low light, and cold high light (growth conditions are detailed under "Experimental Procedures").

PSII quantum efficiency (F_v/F_m) gives an indication of the functionality of PSII reaction centers; F_v/F_m is normally around

Downloaded from www.jbc.org at CNRS on May 20, 2008

TABLE 1

Photosynthetic parameters of *Arabidopsis* plants grown in different light conditions

PSII quantum efficiency value (F_v/F_m) measured during the acclimation time, showing the initial photoinhibition and the successive recovery. Other fluorescence parameters reported are as follows: qE, the quenching relaxed after 100 s in the dark; and qI, the fraction of NPQ that is not relaxed after 19 min of darkness. Measurements were performed on plants at the end of the treatment with an actinic light of 2000 μE as in Fig. 1*A*.

	Ctrl	LL	HL	cLL	cHL
F_v/F_m (after 1 week)	0.86 ± 0.01	0.85 ± 0.02	0.76 ± 0.04	0.87 ± 0.01	0.76 ± 0.04
F_v/F_m (after 2 weeks)	0.85 ± 0.02	0.85 ± 0.01	0.82 ± 0.04	0.87 ± 0.02	0.82 ± 0.02
F_v/F_m (after 3 weeks)	0.86 ± 0.01	0.85 ± 0.01	0.84 ± 0.01	0.87 ± 0.01	0.84 ± 0.01
qE (after 3 weeks)	2.03 ± 0.09	2.00 ± 0.22	2.69 ± 0.12	2.11 ± 0.12	2.59 ± 0.25
qI (after 3 weeks)	0.83 ± 0.08	0.92 ± 0.03	0.20 ± 0.17	0.75 ± 0.12	0.12 ± 0.01

0.80, but when PSII is photo-inhibited this value decreases to 0.4 or less. We followed the PSII quantum efficiency, always measured in fully expanded mature leaves, during the light treatment as reported in Table 1. After the 1st week from the modification of light conditions, HL and cHL plants showed PSII photoinhibition, as evidenced by the reduction of F_v/F_m. In the following weeks, however, despite the prolongation of light treatment, plants recovered from photoinhibition (Table 1). At the moment of the harvesting, all plants showed the same PSII quantum efficiency, irrespective from growth conditions. Thus, after 3 weeks PSII reaction centers are not photo-inhibited in any treated plant, despite both HL and cHL conditions caused at first a photoinhibitory stress (Table 1). This behavior is consistent with the literature of the field that describes that an environmental perturbation first causes a stress response and later leads to a stable long term response defined acclimation (3).

Following treatment, plants exposed to low light conditions did not show an obvious phenotype apart from a slightly reduced growth at both temperatures. Plants grown in high light were also smaller and also showed accumulation of anthocyanins, especially at low temperature, probably the result of the stress perceived by these plants in the 1st week.

In conclusion, because PSII is known to be a major photoinhibition target, the F_v/F_m values measured suggest that plants analyzed in this work are able to respond to the different light conditions when they are exposed to strong illumination for a long enough time.

Plants Acclimated to High Light Increase Photosynthetic Electron Transport Rate—To confirm that our plants were indeed acclimated, we measured an additional photosynthetic parameter, qP. qP indicates the fraction of ^{1}Chl *a* excited states, detected from their fluorescence emission, which is quenched by the activity of photochemical reactions (27). The comparison of this parameter between the different plants thus gives an indication of their efficiency in using light for photochemistry (1, 4). Photochemical quenching values were measured with different light intensities (100, 360, 640, 1200, 1600, and 2000 μE). After 21 min of illumination, qP is clearly higher in plants adapted to HL with respect to both LL and Ctrl. This difference increases with the intensity of actinic light employed in the following measurement: at 1200 μE, qP values are 0.7, 0.35, and 0.14, respectively for HL, Ctrl, and LL (all values are reported in the supplemental figures). Thus, the growth in stronger light stimulates the ability to use energy for photosynthesis, in agreement with previous reports (12, 28) confirming that plants analyzed activate an acclimative response. To verify that qP values measured were indeed because of an increase of Calvin cycle enzymes content, we quantified one of them, the Rubisco, in the leaves acclimated to different conditions. With respect to Ctrl and cLL plants, LL plants contained less Rubisco (60%) whereas in HL plants its content is increased by 250% (200% in cHL). Thus, the observed increase in photochemical efficiency in high light plants was correlated with the presence of a higher content of Calvin cycle enzymes that utilize NADPH and ATP produced from light reactions, as observed previously in acclimation studies (12, 28, 29).

A further confirmation that plants analyzed are indeed acclimated comes from the analysis of oxygen evolution rates. Plants grown in high light showed a larger ability to evolve oxygen. In HL leaves it was around 2.5 times with respect to the control, but this activity reached four times the control in cHL plants, again consistent with previous data on *Arabidopsis* (28, 30).

We thus showed that the plants analyzed responded to different light conditions by modulating the size of their metabolic sinks, photosynthetic rates, and their ability to use light for photochemistry. All parameters analyzed are consistent with the present literature on acclimation. This deduction, together with the observed absence of PSII photoinhibition after 3 weeks of treatment, is a fundamental starting point for all the following analyses, because it demonstrates that we are observing the effects of acclimation to different light intensities rather than of photo-damage.

High Light Acclimated Plants Respond More Promptly to Illumination—A third fluorescence parameter, the NPQ, was also measured in all plants. Its value gives an indication on the fraction of fluorescence quenched nonphotochemically as heat (4). In Fig. 1*A*, NPQ kinetics measured with a 2000 μE illumination in differently acclimated plants are reported, showing a clearly distinct behavior; plants grown in high light are faster in their response, and NPQ reaches its maximum level within 6–7 min. In control and low light-grown plants, instead, NPQ amplitude continues to rise until the light was switched off after 21 min. These results are in agreement with previous reports of larger NPQ in HL acclimated plants with respect to control plants (31, 32). This faster response can be estimated by calculating qE, the fastest component of NPQ; values reported in Table 1 indicate how this component is clearly larger in HL and cHL plants. These plants are not only faster in responding to illumination but also in relaxing quenching after the light is switched off. The quenching not relaxed after 19 min of dark (qI (4)) is far lower in HL plants with respect to Ctrl and LL, as reported in Table 1. In this short dark period in fact, NPQ in HL plants almost relaxed to zero. When NPQ measurements were performed by using different intensities of actinic light, interesting differences were observed in their kinetics. In Fig. 1*B*,

Downloaded from www.jbc.org at CNRS on May 20, 2008

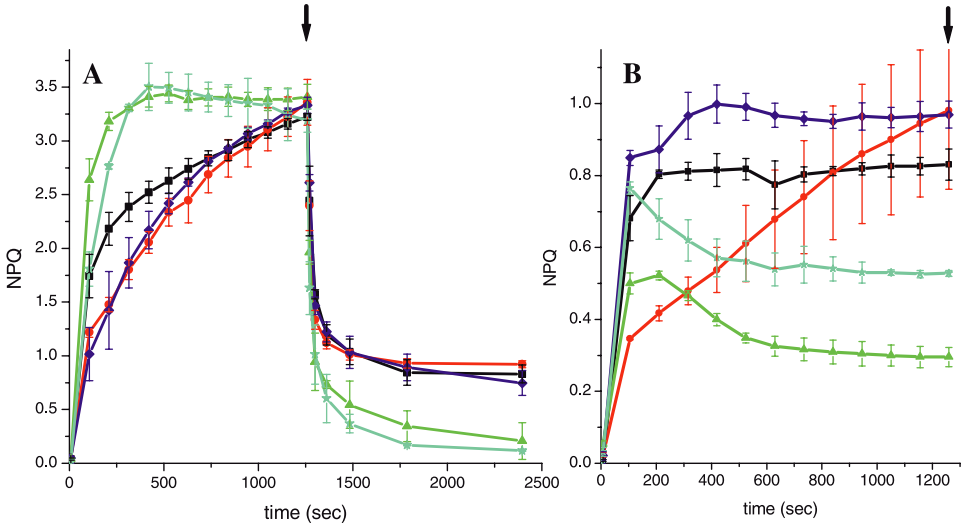

FIGURE 1. **Nonphotochemical quenching of plants acclimated to different conditions.** NPQ kinetics were measured at different light intensities of actinic light. Measurements performed with 2000 (*A*) and 360 μE (*B*) are reported, whereas measurements with 1200 and 640 μE can be found in the Supplemental Material. All data are the average of measurements of at least 5 leaves from different plants. *Arrows* indicate switching off of actinic light. In both panels kinetics curves are indicated as follows: *black squares,* Ctrl; *red circles,* LL; *green triangle*, HL; *blue diamonds,* cLL; and *cyan stars,* cHL.

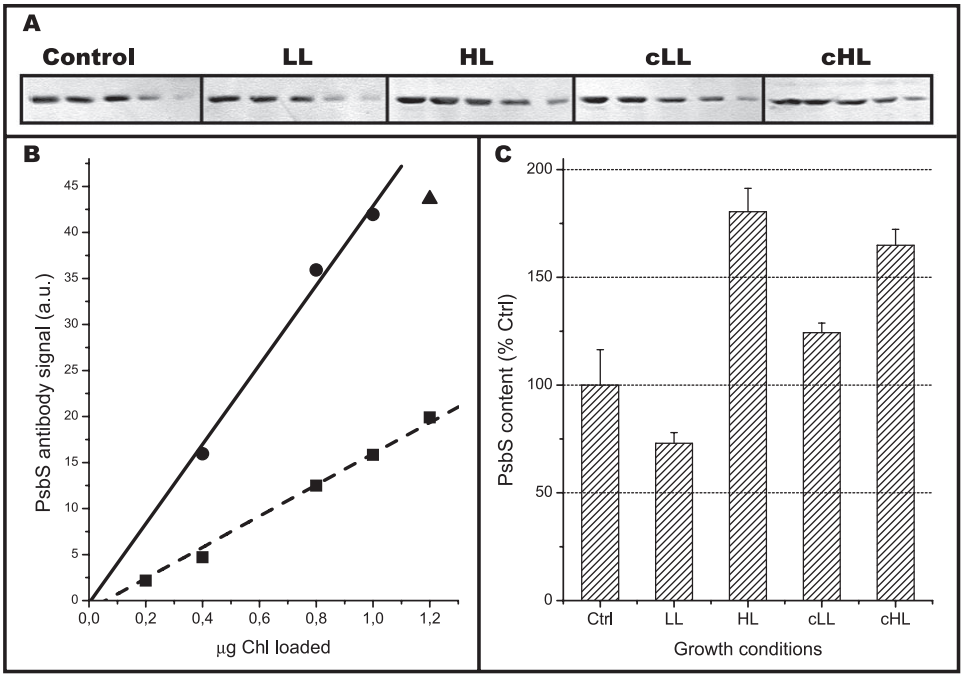

FIGURE 2. **Quantification of *PsbS* in plants acclimated to different light and temperature conditions.** *A,* example of one Western blotting used for the quantification. For each condition 1.2, 1.0, 0.8, 0.4, and 0.2 μg of Chls, respectively, were loaded, from *left to right*. All samples were loaded on a single SDS-PAGE slab. *B,* example of verification of signal linearity. In the case of one HL sample (*circles*), the last point (*triangle*) fell out from linearity (linear fit is shown as *solid line*). In the case of the LL sample, all points (*squares*) have a good linear fit (*dashed line*). *C, PsbS* amounts in different samples as quantified by Western blotting. Data were normalized to *PsbS* content in the control sample.

results obtained using the lowest light intensity (360 μE) are reported; the NPQ level continuously rises in plants adapted to LL, whereas it is saturated in a few minutes in cLL and control plants. In HL-adapted plants instead, NPQ is rapidly activated when plants are switched from dark to light but starts to decline after a few minutes, still under actinic light. This suggests that photochemical reactions, once activated, efficiently use absorbed light, whereas nonphotochemical quenching mechanisms are concomitantly inhibited.

NPQ kinetics thus suggest that HL plants are able to respond quicker to illumination by activating mechanisms for the dissipation of energy as heat. The fastest component of NPQ, defined as qE, or feedback de-excitation, was shown to be dependent on the PSII subunit *PsbS* (6). To verify if the increased response rate of HL plants was because of a different accumulation of this subunit, we measured the relative *PsbS* content by immunoblot titration. Five different dilutions of thylakoid membranes, corresponding to 0.2, 0.4, 0.8, 1, and 1.2 μg of chlorophyll, were loaded in the same SDS-polyacrylamide gel. Fig. 2*A* shows the results of detection with the antibody against *PsbS*, whose signal was quantified by densitometry. To avoid saturation, signal linearity through different dilutions was checked in all samples. In Fig. 2*B* two examples of the possible layout of linearity check is shown; in the case of an LL sample, all points can be fitted with a straight line with good accuracy. In the case of an HL sample, where *PsbS* content is higher, the last point clearly reaches saturation and stands out of the linear fit. Data points falling out of linearity, as in this case, were discarded. The results of the quantification, shown in Fig. 2*C*, demonstrate that *PsbS* indeed accumulates in HL plants with respect to control. Instead, in LL plants, *PsbS* content is slightly reduced. It is interesting to mention that the cLL sample, where *PsbS* content is close to control, also showed very similar NPQ kinetics. The content in *PsbS* thus strongly correlates with observed overall NPQ kinetics and amplitude. In the same samples we also quantified with the same method a PSII core subunit, CP47. If PsbS content is normalized to RCII content, differences between samples are not drastically changed, and the only significant difference with the data shown is that the difference between LL and control is smaller.

Carotenoid Biosynthesis Is Regulated during Acclimation—Fluorescence kinetic measurements reported above are a good tool for the evaluation of photosynthetic performances during

Downloaded from www.jbc.org at CNRS on May 20, 2008

TABLE 2

Pigment composition of plants treated with different conditions

Leaf pigment compositions of plants treated with different light/temperature conditions are reported. Data are normalized to 100 total Chl (a + b) molecules. Standard deviation is also reported.

Sample	Chl a/b	Neoxanthin	Violaxanthin	Antheraxanthin	Lutein	Zeaxanthin	β-Carotene	Total carotenoids
Control	3.0 ± 0.1	3.4 ± 0.1	2.9 ± 0.2		9.9 ± 0.3		6.7 ± 1.0	22.9 ± 1.5
LL	2.6 ± 0.1	2.9 ± 0.3	2.7 ± 0.4		9.7 ± 0.3		6.2 ± 0.8	21.6 ± 1.0
HL	3.5 ± 0.1	3.1 ± 0.3	3.5 ± 0.5	1.5 ± 0.3	10.7 ± 1.0	2.7 ± 0.3	8.3 ± 0.4	29.9 ± 1.9
cLL	2.7 ± 0.2	3.4 ± 0.3	3.5 ± 0.1		10.2 ± 1.3		6.1 ± 0.7	23.3 ± 2.3
Chl	3.6 ± 0.1	3.4 ± 0.5	4.9 ± 0.6	2.0 ± 0.4	12.3 ± 0.8	2.3 ± 0.4	7.6 ± 0.6	32.5 ± 3.4

TABLE 3

Xanthophyll cycle activation with strong high light treatment

Leaves from all plants were treated for 7, 15, and 30 min with 1600 μE in an N_2 atmosphere, inducing strong light excess and maximum activation of zeaxanthin biosynthesis. De-epoxidation index, calculated as $(Z + A/2)/(V + A + Z)$ is reported before and after light treatment. In HL and cHL cases, assuming that during the short treatment time synthesis of new xanthophyll molecules is negligible, the denominator is assumed constant, allowing for the difference calculation, which is shown in parentheses.

	De-epoxidation index			
	Before stress	7 min of stress (difference)	15 min of stress (difference)	30 min of stress (difference)
Control	0	0.46 ± 0.04	0.48 ± 0.06	0.48 ± 0.08
LL	0	0.32 ± 0.02	0.44 ± 0.06	0.45 ± 0.06
HL	0.30 ± 0.11	0.58 ± 0.03 (0.28)	0.57 ± 0.04 (0.27)	0.58 ± 0.03 (0.28)
cLL	0	0.43 ± 0.01	0.52 ± 0.08	0.60 ± 0.07
cHL	0.32 ± 0.1	0.56 ± 0.04 (0.24)	0.63 ± 0.09 (0.31)	0.63 ± 0.04 (0.31)

short light exposure. Long term modifications of photosynthetic apparatus can also be analyzed from changes in the thylakoid pigment compositions. As reported in Table 2, plants grown in distinct conditions showed a difference in their Chl a/b ratio as follows: increased in HL plants (both at 21 and 4 °C) and decreased in LL plants, irrespective to temperature conditions. Because Chl b is specifically bound to antenna proteins (Lhc), this modification in Chl a/b ratio suggests different accumulation levels of these polypeptides: higher in LL plants and lower HL, as shown previously (13, 28, 33–35). In HL acclimated plants the carotenoid content on a Chl basis is also 30% higher. It is worth noting that the increase in carotenoids occurs in conditions with a lower antenna size, suggesting extra carotenoids can be found free in the membrane. Not all carotenoid species, however, were increased to the same level; on a Chl basis, in fact neoxanthin and β-carotene levels are essentially unaffected. Violaxanthin and lutein content instead undergoes a significant increase in HL conditions together with the accumulation of significant amounts of antheraxanthin and zeaxanthin. Despite the lutein increase, however, components of the β-β branch of carotenoid biosynthetic pathway (β-carotene, zeaxanthin, violaxanthin, and neoxanthin) account for the highest part of the increase in carotenoid content with respect to the β-ε branch.

Zeaxanthin Production Rate Decreases in HL Acclimated Plants—Plants exposed to strong light convert violaxanthin into zeaxanthin, which increases their photoprotection ability (8). We evaluated the different capacity to produce zeaxanthin of acclimated plants by exposing leaves to strong light in N_2 atmosphere. This treatment drastically decreases photochemical quenching levels because of the absence of both CO_2 and O_2, the substrates of photosynthetic reductive and oxidative cycles, respectively. By consequence, electron transport rates decline, whereas lumen acidification increases, maximizing violaxanthin to zeaxanthin conversion (20). Thus, we can assume that in these extreme conditions zeaxanthin biosynthesis is stimulated to the maximum possible rate. During the 30 min of treatment, the de-epoxidation rate was similar in LL and control leaves, whereas it was higher in cLL. Surprisingly, the newly synthesized zeaxanthin and anteraxanthin were lower in HL and cHL acclimated plants (Table 3).

This result cannot be explained by the presence of some zeaxanthin molecules in HL plants before the treatment. In fact, despite zeaxanthin presence, violaxanthin content is still higher in HL with respect to control and LL-grown plants so the substrate of the reaction should not be limiting. On the contrary, it could be explained by two other hypotheses as follows: (i) the rate of zeaxanthin biosynthesis is regulated and reduced in HL-grown plants; (ii) stress treatment was less effective in HL plants than in Ctrl and LL. The latter hypothesis is unlikely because, as shown above, HL plants have a larger photosynthetic capacity, but in nitrogen atmosphere photosynthesis we expect the light excess to be very strong in all plants. To test the first hypothesis, we determined VDE content in all samples by quantitative Western blotting; on a Chl basis, VDE content was similar in Ctrl and LL acclimated plants and significantly decreased in cHL and HL plants (Fig. 3). In cLL plants, on the contrary, its content was higher. These results are substantially consistent with observed zeaxanthin production rate and thus support the first hypothesis.

Because the xanthophyll cycle depends on the activity of two enzymes catalyzing opposite reactions and the overall rate depends on the abundance of both enzymes, we also quantified ZE, which is responsible for the back-conversion of zeaxanthin to violaxanthin. With respect to control plants, LL had similar content; cLL content increased, and HL and cHL plants had smaller ZE content (Fig. 3). Thus, enzymes controlling zeaxanthin synthesis and back-conversion to violaxanthin are regulated similarly during acclimation. It must be emphasized, however, that because the quantification is based on Western blotting analyses and the two antibodies do not have the same dependence from protein concentration, it is not possible to evaluate if the stoichiometric ratio between the two proteins is modified.

Tocopherol Content Is Increased in HL—Besides carotenoids, other molecules like tocopherol have an established activity in

Downloaded from www.jbc.org at CNRS on May 20, 2008

protection from oxidative stress (36). To verify if the synthesis of such antioxidants molecules is also increased in HL, we thus evaluated the tocopherol content in thylakoids isolated from different plants. Consistent with previous reports (36, 37), we observed a strong increase of tocopherol in HL and cHL conditions that showed a tocopherol content 11 and 5.4 times higher, respectively, than in the control sample. In LL samples instead, tocopherol levels were similar to Ctrl plants.

PSII Antenna Size Is Modulated Depending on Growth Conditions—The modification of Chl a/b ratio observed in leaves suggests modulation of the number of Chl b-binding Lhc proteins per core complex (13, 33–35). We further detailed the level of acclimation by analyzing thylakoid membranes purified from plants acclimated to different conditions. Membranes were solubilized with 1% β-DM, a condition yielding a complete dissociation of antenna subunits from the PSII core complex, a necessary condition for an accurate evaluation of the antenna size. Pigment binding complexes were fractionated by nondenaturing gel electrophoresis. An example of the separation is shown in the Supplemental Material. Green bands were identified, and their pigment content was quantified. The results obtained (reported in the Supplemental Material) in HL plants showed a 52% reduction in the amount of Chl associated with photosystem II antenna proteins with respect to control. LL plants instead underwent a 46% increase. When light acclimation was accomplished in the cold, the effect was intermediate; the increase in cLL was 17% and the decrease in cHL was 32%. Thus, the PSII antenna size is confirmed to be extensively regulated according to illumination conditions, as shown previously (13, 28, 33–35). Although modulation of PSII antenna size has been reported before, the relative contribution of the different components, *Lhcb1–3* (LHCII), *Lhcb4* (CP29), *Lhcb5* (CP26), and *Lhcb6* (CP24), to this process in not completely clear. To clarify this point, *Lhcb1–2* polypeptide content was evaluated by densitometry after separation of thylakoid polypeptides by SDS-PAGE and staining with Coomassie. As shown in Fig. 4*A*, LHCII content is strongly increased in LL, whereas it is decreased in HL, cHL, and also cLL, in agreement with the observed regulation in overall PSII antenna size. *Lhcb3–6* polypeptides are less abundant in the thylakoid membrane and were thus quantified by immunotitration, normalizing their content to the core subunit CP47. Data reported in Fig. 4*B* show that *Lhcb3–6* undergo very different levels of regulation. *Lhcb3*, the third component of LHCII complex, is also affected by light treatment but to a smaller extent with respect to *Lhcb1–2*; it is decreased in HL but substantially unchanged in LL. *Lhcb4* (CP29) and *Lhcb5* (CP26), on the contrary, did not show any major change in any condition tested. The opposite behavior was observed in the third monomeric antenna, *Lhcb6* (CP24), which undergoes dramatic reduction in HL to less than 20% with respect to Ctrl conditions. In the cold, *Lhcb6* was reduced by 25 and 45%, respectively, in LL and HL conditions.

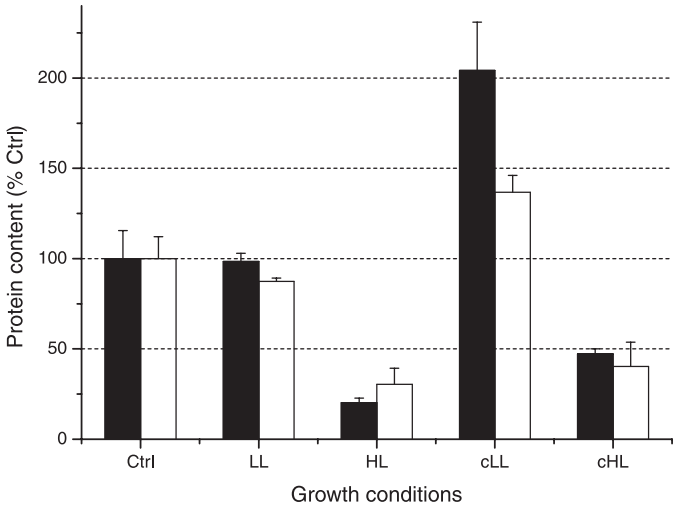

FIGURE 3. **Evaluation of xanthophyll cycle enzyme content.** The content in violaxanthin de-epoxidase (*black bars*) and zeaxanthin epoxidase (*white bars*) was evaluated in different acclimated plants by quantitative Western blotting as described above in the case of *PsbS*. Data are normalized to the protein content in Ctrl.

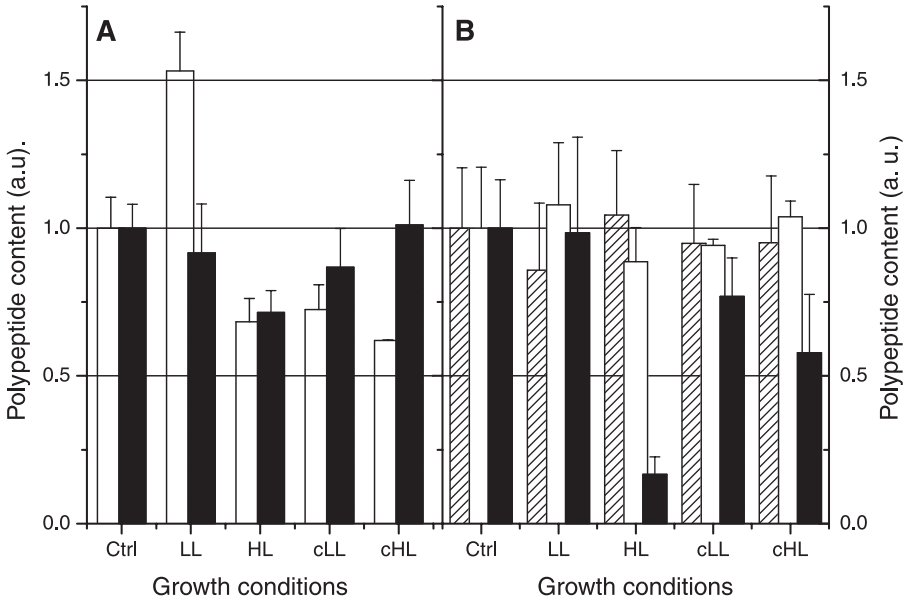

FIGURE 4. **Evaluation of *Lhcb* content in plants acclimated to different light and temperatures.** PSII antenna polypeptide content in different plants was evaluated by Coomassie staining (*Lhcb1* and *Lhcb2*) and immunoblot-titration (*Lhcb3–6*) and normalized to content in reaction center polypeptides, quantified by the same method. *A*, quantification of polypeptides composing the PSII major antenna (LHCII): *Lhcb1 + 2* (*white bars*) and *Lhcb3* (*black bars*). *B*, determination of monomeric antenna polypeptides content, *Lhcb4* (*gray bars*), *Lhcb5* (*white bars*), and *Lhcb6* (*black bars*) in differently acclimated plants.

PSI-LHCI Complex Stoichiometry Is Not Modified Depending on Growth Conditions—We then proceeded to the analysis of the changes in the stoichiometry of the PSI-LHCI complex depending on acclimation. The PSI-LHCI complex is stable upon detergent solubilization of thylakoid membranes, and therefore all PSI core subunits can be purified as a complex together with its antenna (22). We first compared

Downloaded from www.jbc.org at CNRS on May 20, 2008

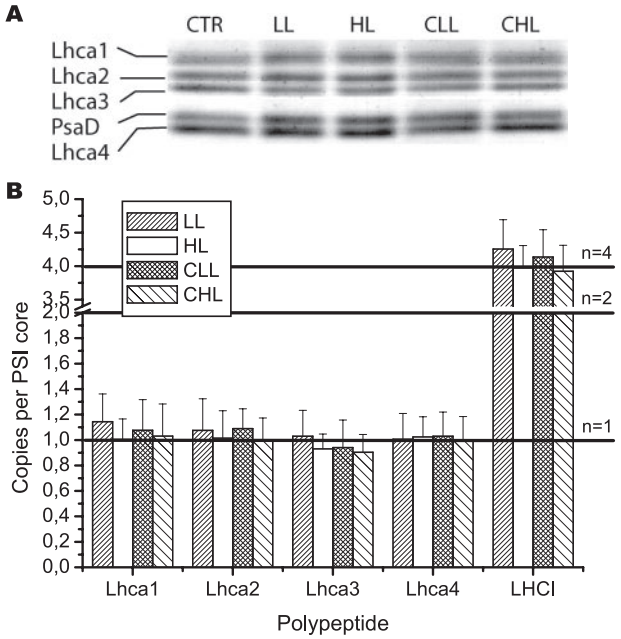

FIGURE 5. **Evaluation of *Lhca* content in plants acclimated to different light and temperatures.** *A,* SDS gel separation of PSI-LHCI purified from differently acclimated plants. The gel region where *Lhca1–4* and *PsaD* polypeptides migrate is shown. *B,* determination of *Lhca1–4* stoichiometry in different PSI samples. The total Lhca content is also indicated as LHCI.

absorption and fluorescence spectra of complex purified from different plants that, surprisingly, did not show significant differences as should be expected if the relative content of Chl b-containing antenna complex was modified (not shown).

A more detailed analysis was performed by measuring the stoichiometry of the *Lhca1–4* subunits following a procedure described previously (22). PSI-LHCI complexes were fractionated on an SDS-PAGE system optimized for the separation of all Lhca polypeptides from each other (Fig. 5*A*). Each polypeptide was then quantified by evaluating the amount of Coomassie dye bound to each PAGE band. As a reference, different PSI core polypeptides were quantified by the same method, namely PsaD and PsaF, allowing the calculation of the *Lhca1–4*/PSI core ratio in each sample. Previous work with PSI-LHCI from plants grown in the same control conditions assessed that *Lhca1–4* polypeptides are present in one copy per PSI core (22) in agreement with crystallographic studies (39). This information allowed normalization of our data. The results obtained for the stoichiometry of each Lhca polypeptide in all samples are reported in Fig. 5*B*. Surprisingly, growth conditions did not induce changes in the relative abundance of any specific Lhca polypeptide nor in the total number of antenna proteins with respect to PSI core subunits. It is worth mentioning that data have a standard deviation of ~20%, and therefore we cannot exclude that a change below this limit might occur. This accuracy, however, is sufficient to conclude that growing *Arabidopsis* plants in different light and temperature conditions did not have any major effect on *Lhca1–4* stoichiometry.

To verify if the PSI-LHCI particles isolated and analyzed were really representative of the PSI-LHCI in thylakoids, we checked that no Lhca polypeptide was lost during the PSI-LHCI purification by sucrose gradients. Antibodies against *Lhca1–4* did not detect any significant amount of Lhca polypeptides in fractions different from those containing the whole PSI-LHCI supercomplex. We therefore concluded that the PSI particles isolated are fully representative of the membrane composition of the thylakoids.

The number of LHCII units in the PSII antenna system was previously shown to respond to the zeaxanthin content of thylakoids. In mutants constitutively accumulating zeaxanthin, in fact the PSII antenna was shown to be reduced both in *Chlamydomonas reinhardtii* and in higher plants (40, 41). This result suggests that the stress responsible for zeaxanthin production is sensed by the PSII antenna and induces a destabilization on the proteins and a decrease in antenna size. To verify if the PSI antenna is similarly affected, we evaluated the stoichiometry of each *Lhca1–4* polypeptide, following the same method described above, in PSI particles purified from mutants accumulating different amounts of zeaxanthin *Npq2* and *Lut2Npq2* (41). Again, our results showed that PSI antenna size is not significantly affected by the constitutive accumulation of large amounts of zeaxanthin (not shown).

Does LHCII Contribute to PSI Antenna Size Regulation?—Because the biochemical result showing constancy of PSI antenna size was somehow surprising, we decided to extend our analysis to the distribution of Chl pigments between PSI and PSII by using both biochemical methods (nondenaturing PAGE) and a less invasive approach based on a spectroscopic method applied to the intact system.

Fig. 6*A* shows the ratio between PSI and PSII as determined biochemically by quantification of green bands from Deriphat-PAGE (shown in the Supplemental Material). In LL conditions PSI *versus* PSII ratio increased to 2.1, and in HL it shifted from 1.8 in the control to 1.5. These variations are consistent with values in the literature reporting a relative decrease of PSI content in HL conditions (28).

However, when data of total Chls associated with PSI-LHCI were considered against PSII core + *Lhcb* proteins, differences were smaller because the modifications in the reaction center content and variations in the PSII antenna size partially compensated each other. These somehow puzzling results could be reconciled by assuming that part of the Lhcb proteins were in fact connected to PSI rather than to PSII. To verify this possibility, we measured fluorescence excitation in chloroplasts purified from different plants detecting the specific PSI-LHCI emission at 735 nm. As references, we used the spectra taken from the purified PSI-LHCI complex, which has no LHCII contamination and also a preparation of stroma lamellae isolated by Yeda-press fractionation of dark-adapted control plants, a condition that yields very low levels of LHCII associated with PSI (42). In these spectra, Ctrl and LL-grown plants showed a higher Chl b contribution with respect to HL (Fig. 6*B*). The HL, cHL, and cLL spectra instead are very similar to the isolated PSI-LHCI. This result suggests that in the intact system a significant amount of energy is transferred from a Chl b-rich antenna to PSI, whose amplitude is modulated according to the acclimation conditions and in particular is decreased in HL conditions with respect to Ctrl and LL.

Downloaded from www.jbc.org at CNRS on May 20, 2008

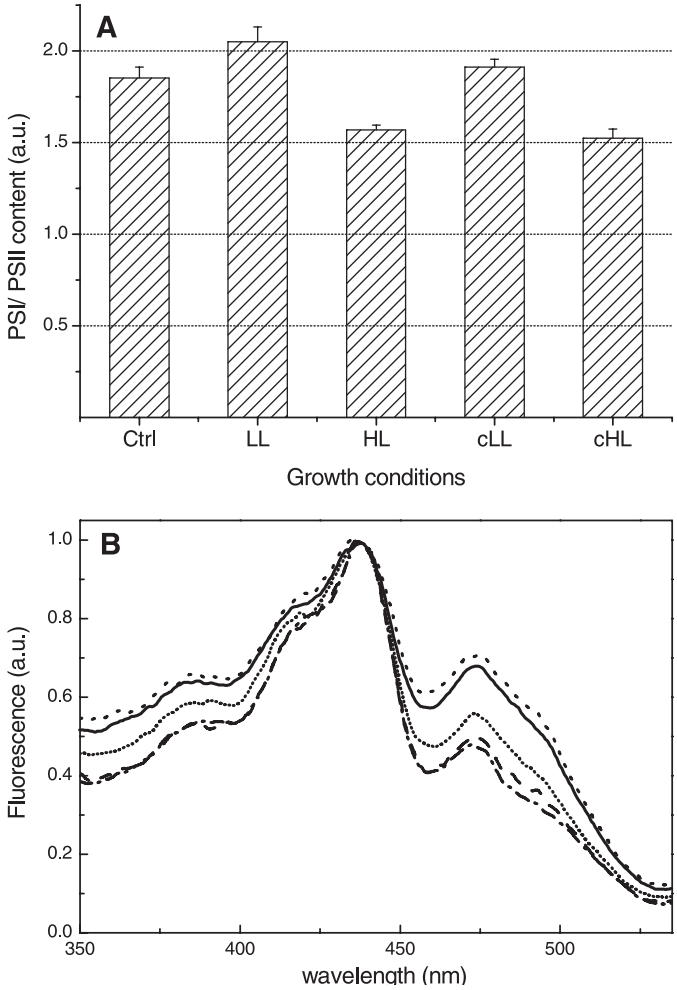

FIGURE 6. **Identification of other mechanisms for PSI antenna size regulation.** *A,* modification of the PSI/PSII ratio following different growth conditions is reported. PSI-LHCI and PSII core content were evaluated from nondenaturing gels, as shown in the Supplemental Material. *B,* low temperature fluorescence excitation spectra of chloroplasts isolated from different plants. The PSI-LHCI-specific fluorescence was detected at 735 nm. Ctrl (*solid line*), LL (*dotted line*), cHL (*short dotted line*), HL (*dashed line*), and PSI-LHCI (*dash-dotted line*) spectra are reported. cLL spectra were not reported for clarity as being very similar to LL and Ctrl.

DISCUSSION

In this work we analyzed the modifications in photosynthetic apparatus in plants grown in different light and temperature conditions. The evaluation of several parameters as photosynthetic capacity, Rubisco content, O_2 evolution rates, NPQ kinetics, Chl a/b ratios and PSI/PSII ratios, and tocopherol content demonstrated that these plants activated an acclimative response. High light treatment in this case was stronger that in previous analogous studies on *Arabidopsis* (28, 32); this was done to be sure that the observed stability in PSI antenna stoichiometry was not because of the mildness of the light treatment. Despite their exposure to an intense illumination, however, all plants did not shown any PSII photoinhibition at the end of the experiment.

In acclimated plants we characterize several photosynthetic properties as follows: the ability to activate photochemical and nonphotochemical quenching, regulation of carotenoid biosynthesis, adjustment of PSII and PSI antenna size, modification of PSI/PSII ratio, and LHCII association to PSI. The observation of all these parameters at the same time allowed us to obtain a global view of the acclimation of photosynthetic apparatus to variable light and temperature conditions.

Plants Acclimated to High Light Accumulate PsbS and Respond Quicker to Illumination—Plants adapted to high light showed a faster rate in activating NPQ after illumination, as shown in the kinetics reported in Fig. 1*A*. We also observed that *PsbS* content is significantly higher in HL-adapted plants with respect to control, whereas it is reduced upon LL acclimation. It should be mentioned that all previous estimations by Western blotting analysis failed to identify any significant variation of *PsbS* protein levels in plants adapted to different light intensities (43, 44), although *PsbS* mRNA levels were shown to be increased under strong illumination and low temperature conditions (44). The discrepancy with our results can be explained by considering the methods used for the quantification. In previous reports different sample dilutions have not been analyzed thus preventing a reliable evaluation of the antibody linearity range. Without this precaution, only major differences can be evidenced, and the 2-fold increase in HL with respect to control conditions we observed was hardly detectable.

According to our results, the increase in *PsbS* content correlates with the NPQ activation rate, because HL and cHL plants, where this protein is more abundant, showed the fastest kinetics. This correlation is even more remarkable by looking to cLL plants; in fact, *PsbS* content here is equivalent to Ctrl, and under all light conditions tested, NPQ kinetics are also very similar (see supplemental Fig. 2). The clearest example is shown in Fig. 1*B*; here, the amplitude of NPQ evaluated with a weak light is saturated in Ctrl and cLL, and it continues to grow for all measuring times in LL plants.

It should be pointed out that a portion, between 20 and 30%, of zeaxanthin and anteraxanthin is retained during the short dark adaptation period before NPQ measurements in HL and cHL plants. Because zeaxanthin is known to induce faster NPQ kinetics, its presence could in part explain our observations. Some considerations, however, suggest that the zeaxanthin effect, although probably present, is not sufficient to explain all the observed differences in kinetics. The first indication in this direction is that the correlation between *PsbS* content and NPQ is observed in all plants even in those without zeaxanthin; cLL showed kinetics more similar to control than LL even if zeaxanthin is absent in both. Second, the differences in kinetics are observed in all measurements even when the dark adaptation was prolonged overnight as data presented in Fig. 1*A*. Finally, some evidence in the literature suggests *PsbS* has a dose effect in the fastest component of NPQ, qE; and in fact, *Arabidopsis* plants overexpressing *PsbS* showed an increased qE amplitude, demonstrating that *PsbS* amount is limiting for NPQ (45–47), and its role is independent from zeaxanthin synthesis (48).

We thus support the idea that in high light conditions *PsbS* is overexpressed to increase the NPQ activation rate. On the contrary, when plants are grown in limiting light, PsbS level is decreased to prevent unnecessary energy dissipation. In fact, although it might appear that *PsbS*-dependent energy dissipation is inactive under low illumination, this is not the case

Downloaded from www.jbc.org at CNRS on May 20, 2008

because it was demonstrated previously that plants without *PsbS* grow faster than control plants in limiting light (9).

It could be asked if the *PsbS* effect on NPQ amplitude saturates or if a hypothetic massive accumulation would lead to a correspondingly large increase in fluorescence quenching. This question is interesting because it can provide clues on the *PsbS* mechanism of action, which is still obscure. Saturation would suggest that *PsbS* operates in strong connection to PSII by acting as a regulator of quenching ability elicited within pigment binding complexes of PSII core and/or Lhcb proteins. On the contrary, a proportional increase of *PsbS* and NPQ to very high levels would speak for an intrinsic quenching activity of *PsbS*. If the capacity for excess energy dissipation is proportional to *PsbS* accumulation, it could be expected that HL plants, which experienced a strong light excess, would accumulate *PsbS* accordingly, thus solving their photoprotection problems. However, the level of accumulation observed does not exceed a factor of 2 with respect to control, whereas HL plants show reduced growth and photoinhibition in the first days of the treatment. This indirectly suggests a saturation effect in *PsbS* quenching activity and thus for the presence of a limited number of sites within PSII where *PsbS* can exert its action. If this is true, then the *PsbS* mechanism of action is probably based in triggering quenching mechanisms resident in PSII core (49) and/or in LHCII (50) rather than in directly quenching excitation energy.

The Xanthophyll Cycle Is Slower in High Light—Plants respond to acclimation to different light conditions by regulating their carotenoid composition. In particular, in high light the synthesis of carotenoids from the β-β branch (products of two cyclization by lycopene β cyclase) is accumulated with a reduction of products from the β-ϵ branch, bearing one β and one ϵ ring. In LL, the molecules from the ϵ branch instead are more abundant. The enrichment in the products of the β branch increases the ability of the plants to synthesize zeaxanthin when needed, thus helping in the response to high light conditions (51).

Zeaxanthin is a carotenoid with a well documented role in photoprotection (8). It plays multiple roles, and in fact it was proposed to be active both free in the membrane and bound to antenna complexes and to *PsbS* (9, 10, 52). Zeaxanthin is synthesized from violaxanthin by VDE, an enzyme activated in high light conditions by the low luminal pH, which stimulated the enzymatic activity as well as the liberation of substrate in the membrane (53, 54). The opposite reaction is catalyzed by zeaxanthin epoxidase (ZE), whose enzymatic regulation instead is not very clear; most probably ZE has a constitutive activity, and when VDE is not active, it slowly converts all zeaxanthin to violaxanthin.

In this work we compared the efficiency of zeaxanthin formation in different acclimated plants, and surprisingly we observed that HL acclimated plants are less efficient in zeaxanthin synthesis. However, this result is consistent with the determination of VDE content in different plants, which in turn is correlated with the capacity for zeaxanthin synthesis.

The observed down-regulation of zeaxanthin synthesis in HL acclimated plants seems in contrast with the well proven role of this carotenoid in photoprotection. Two kinds of consideration could explain this finding. First, we showed that not only VDE but also ZE content is reduced in HL, suggesting that in these plants zeaxanthin back-conversion to violaxanthin is also slower. Thus, the rates of both steps of xanthophyll cycle are probably reduced in HL. As a result, zeaxanthin is produced to a lower rate in HL plants, but once present, it is maintained for a longer time. Moreover, it should be pointed out that in HL acclimated plants several additional protective mechanisms are activated. Among the ones we examined were the *PsbS* increase and the rise in tocopherol content. These protection mechanisms could reduce the need for a fast zeaxanthin synthesis, and its production could be more useful on a longer time scale. The capacity to synthesize zeaxanthin is instead maximized in plants adapted to LL and in Ctrl conditions where other photo-protective mechanisms are not activated, and thus its fast production is probably of crucial importance for excess light dissipation and ROS scavenging (9, 10, 55).

PSII Antenna Polypeptides Are Differently Regulated—The PSII antenna size is largely regulated following environmental conditions, as shown previously (13, 33–35) and confirmed in this work. However, we showed that individual *Lhcb* polypeptides behave differently. In fact, *Lhcb1* and *Lhcb2* undergo large variations with light/temperature conditions. Taking in account that these polypeptides are the most abundant in the thylakoids, we can thus conclude that modifications in LHCII content are the major responsibility for the PSII antenna size regulation. The third LHCII component, *Lhcb3*, has a distinct regulation, and its content is less variable, supporting the view that this polypeptide has a distinct role with respect to *Lhcb1–2* (56, 57).

Among the minor antennas, *Lhcb4* and *Lhcb5* undergo small modifications with environmental conditions, suggesting they constitute an inner part of the antenna system that is always present in all light conditions. This behavior is consistent with the position of these polypeptides in PSII-LHCII supercomplexes, closely associated with the reaction center (58). On the contrary, *Lhcb6* content is largely modified, especially in HL where its concentration is decreased five times. *Lhcb6* (CP24) is a minor antenna complex accounting for less than 5% of the total PSII chlorophyll, implying that the effects of these changes on the PSII antenna size are poorly relevant. However, because *Lhcb6* occupies a peripheral position in the PSII supercomplex (58), it might play a role in stabilizing the binding of LHCII-L (loosely bound) trimers to the PSII supercomplexes. We thus suggest that *Lhcb6* may be a regulator of PSII antenna size and its presence stabilizes the supplementary LHCII trimers accumulated in LL conditions (59).

LHCI Polypeptide Content Is Not Affected by Environmental Conditions—The modification of the antenna size is a well known mechanism for adaptation of the photosynthetic machinery to environmental conditions (33). This phenomenon, however, has been more thoroughly studied in PSII than in PSI. A first specific study suggested that the *Lhca* content responds to illumination regimes with *Lhca2* and *Lhca3* being accumulated at high irradiance more than *Lhca1* and *Lhca4*, yielding into a doubling of the LHCI content per PSI core with respect to low light (28). This study is not consistent with our observation that Lhca/PSI core stoichiometry remains unchanged

Downloaded from www.jbc.org at CNRS on May 20, 2008

upon adaptation to different light and temperature conditions (Fig. 4*B*). These contrasting results cannot be explained by differences in the plant growth conditions, as judged from the fact that PSII antenna size is consistently reported to undergo important changes in both studies. More likely, the reason for the different results may reside in the methods used for the quantification of the polypeptides. Bailey *et al.* (28) used Western blotting, whereas we quantified Coomassie staining for individual *Lhca* bands. The former method, in fact, although less sensitive, allows a much wider linearity range than immunodetection (23). In other cases, such as those of *PsbS*, VDE, and ZE, it has not been possible to identify a single band in SDS-polyacrylamide gels, and we also employed immunoblotting for quantification. However, to make certain that our determination was not undermined by technical limitations, we always analyzed several dilutions of each sample to check for linearity, and we chose as the most correct the signal obtained near the detection limit of the antibody.

We can thus confidently conclude that PSI-LHCI, differently from PSII-LHCII, is a very stable system where the number of Lhca is unaffected depending on the growth condition. In all conditions we analyzed, in fact one copy of each *Lhca1–4* polypeptide was found in the PSI-LHCI supercomplex. Further evidence for this stoichiometric stability of PSI antenna comes from the analysis of carotenoid mutants. It has been shown that accumulation of zeaxanthin in LHCII leads to monomerization and degradation of the major antenna complex (40, 41). Mutants with altered xanthophyll composition can thus mimic environmental conditions more extremely than those analyzed in this work. However, even in this case, PSI-LHCI stoichiometry was unaffected.

Based on the structural analysis of the interactions between subunits in the PSI-LHCI supercomplex, Ben-Shem *et al.* (60) concluded that LHCI assembly should be very flexible, because a low level of protein-protein interaction was detected between both LHCI subunits and between themselves and the core complex proteins. The discrepancy of the experimental data with respect to the expectations from protein structure can be reconciled by attributing a stronger relevance to gap pigments in mediating interactions between protein subunits and acting as a "green glue" in the stabilization of the PSI-LHCI complex, consistent with their role in the association of the antenna (61). Further support to a stable stoichiometry in PSI-LHCI comes from the analysis of Lhca-depleted plants, which shows that the lack of a single antenna subunit cannot be compensated by the overaccumulation of the remaining ones (61, 62). This is in striking contrast with PSII where, in the absence of *Lhcb1* and *Lhcb2*, *Lhcb3* and *Lhcb5* are overexpressed and form trimers, which replace the missing polypeptides and maintain the structural organization of PSII supercomplexes (63). The missing flexibility of Lhca subunits in the PSI supercomplex implies a specific binding site for each Lhca gene product making unlikely their replacement by other Lhc subunits in response to variable environmental conditions. Thus, although PSII regulates the size of its antenna system (14, 28), PSI cannot. This difference can be explained by the presence of gap pigments in PSI but not in PSII (22). Such an interfacial pigment pool is in fact incompatible with an antenna system that is changing extensively in size through association/dissociation of the component subunits; in a "gap" pigment-containing system, the dissociation of a subunit would release Chl molecules free in the membrane. These would be poorly photo-protected because of a lack of stable interactions with carotenoid molecules and thus be very prone to the formation of reactive oxygen species. This shows that PSI-LHCI behaves as a reference complex with respect to which PSII-LHCII undergoes regulation to maintain the chloroplast redox state and to ensure photoprotection.

PSI Antenna Size Is Regulated by Environmental Conditions—The results reported above show that the number of antenna proteins bound to PSI is not modified by the growth of *Arabidopsis* plants in different light/temperature conditions. It is worth asking if the stability of the PSI antenna size implies that PSI is not involved in any kind of regulation of light harvesting capacity. Such behavior would be unusual in a biological system, where regulation is vital. In fact, we showed that even if *Lhca* content is constant, different light and temperature conditions influenced the PSI light collecting ability by the modification of the PSI/PSII ratio and the association of LHCII subunits to PSI. In differently acclimated plants, the PSI/PSII ratio is modified according to light conditions, as shown previously (28). One additional regulation of PSI antenna function involves the different association of LHCII to PSI. LHCII populations are known to be able to migrate from PSII to PSI in response to light quality in a process named "state 1-state 2 transition" (64, 65). Here, we analyzed if LHCII association to PSI has a role in long term acclimation to light intensity as well by evaluating the LHCII content bound to PSI. It is also relevant to point out that we did not measure the ability of different plants to undergo state transition as in Ref. 32. Instead, by analyzing fluorescence excitation spectra, we evaluated the amount of LHCII polypeptides stably associated with PSI in conditions of steady-state illumination. Our results show that in HL plants the contribution of Chl b absorption to PSI emission is reduced with respect to control and LL plants. Because in the same plants Lhca content was proven to be constant, differences in Chl b contribution must be attributed to the reduction of LHCII polypeptides transferring energy to PSI. This result thus suggests that the functional PSI antenna size is indeed regulated, not by the modification of *Lhca* polypeptide content but by changing the amount of LHCII associated with PSI. This hypothesis is consistent with a recent report showing that plants unable to perform state transitions also have a reduced maximum rate of CO_2 fixation compared with wild type when grown at 60 μE (67). In fact, we showed that in LL and Ctrl *Arabidopsis* (grown at 25 and 100 μE, respectively) a significant amount of LHCII is associated with PSI and contributes to its light harvesting function. Thus, the regulation of antenna size in both photosystems is achieved by regulating the synthesis of the same *Lhcb* polypeptides. Work is in progress to identify which gene products are stably associated with PSI-LHCI in LL conditions.

Low Temperature Acts as a Multiplying Factor of High Light—Both light and temperature conditions are agents of abiotic stress for the plant (3). The transcriptional responses induced by these environmental factors partially overlap, although cold stress also induces the activation of specific genes (68, 69). On

Downloaded from www.jbc.org at CNRS on May 20, 2008

this basis it can be asked if low temperature conditions activate a specific regulation of antenna function. From all the parameters we have analyzed in this work, cold appears to act in strengthening the effect of light stress, and we never observed a qualitative difference in the regulation of antenna function in plants grown at different temperatures. This hypothesis is consistent with the observation that the increased tolerance to PSII photoinhibition measured in plants grown at low temperatures reflects the photosynthetic adjustment to high PSII excitation pressure rather than being a growth temperature effect *per se* (12).

Nevertheless, it is worth clarifying that this result does not exclude the presence of cold-dependent responses with a protective effect on photosynthetic apparatus. For instance, modulation of thylakoid lipid fluidity (38) or differences in the rate of cell development at low temperatures (66) might in part account for some of our results without involving specific alterations on antenna size.

Acknowledgments—We thank Giulia Bonente, Milena Mozzo, and Stefano Cazzaniga (Università di Verona) for help in antibody preparation and tocopherol quantifications. Stefano Caffarri (LGBP, Université de Marseille) is thanked for preliminary work.

REFERENCES

1. Demmig-Adams, B., and Adams, W. W. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **43,** 599–626
2. Barber, J., and Andersson, B. (1992) *Trends Biochem. Sci.* **17,** 61–66
3. Huner, N. P. A., Oquist, G., and Sarhan, F. (1998) *Trends Plant Sci.* **3,** 224–230
4. Van Kooten, O., and Snel, J. F. H. (1990) *Photosynth. Res.* **25,** 147–150
5. Niyogi, K. K. (1999) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50,** 333–359
6. Li, X. P., Bjorkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., and Niyogi, K. K. (2000) *Nature* **403,** 391–395
7. Niyogi, K. K., Grossman, A. R., and Björkman, O. (1998) *Plant Cell* **10,** 1121–1134
8. Demmig-Adams, B. (1990) *Biochim. Biophys. Acta* **1020,** 1–24
9. Dall'Osto, L., Caffarri, S., and Bassi, R. (2005) *Plant Cell* **17,** 1217–1232
10. Havaux, M., and Niyogi, K. K. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 8762–8767
11. Seeman, R. J., Sharkey, T. D., Wang, J., and Osmond, B. (1987) *Plant Physiol.* **84,** 796–802
12. Gray, G. R., Savitch, L. V., Ivanov, A. G., and Huner, N. (1996) *Plant Physiol.* **110,** 61–71
13. Walters, R. G., and Horton, P. (1994) *Planta* **195,** 248–256
14. Anderson, J. M., and Andersson, B. (1988) *Trends Biochem. Sci.* **13,** 351–355
15. Melis, A. (1991) *Biochim. Biophys. Acta* **1058,** 87–106
16. Demmig-Adams, B., Adams, W. W., Barker, D. H., Logan, B. A., Bowling, D. R., and Verhoeven, A. S. (1996) *Physiol. Plant.* **98,** 253–264
17. Croce, R., Zucchelli, G., Garlaschi, F. M., Bassi, R., and Jennings, R. C. (1996) *Biochemistry* **35,** 8572–8579
18. Croce, R., Morosinotto, T., Castelletti, S., Breton, J., and Bassi, R. (2002) *Biochim. Biophys. Acta* **1556,** 29–40
19. Gilmore, A. M., and Yamamoto, H. Y. (1991) *Plant Physiol.* **96,** 635–643
20. Dainese, P., Marquardt, J., Pineau, B., and Bassi, R. (1992) in *Research in Photosynthesis* (Murata, N., ed) Vol. I, pp. 287–290, Kluwer Academic Publishers Group, Dordrecht, Netherlands
21. Laemmli, U. K. (1970) *Nature* **227,** 680–685
22. Ballottari, M., Govoni, C., Caffarri, S., and Morosinotto, T. (2004) *Eur. J. Biochem.* **271,** 4659–4665
23. Ball, E. H. (1986) *Anal. Biochem.* **155,** 23–27
24. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 4350–4354
25. Peter, G. F., and Thornber, J. P. (1991) *J. Biol. Chem.* **266,** 16745–16754
26. Garcia-Plazaola, J. I., and Becerril, J. M. (1999) *Phytochem. Anal.* **10,** 307–313
27. Genty, B., Briantais, J.-M., and Baker, N. R. (1989) *Biochim. Biophys. Acta* **990,** 87–92
28. Bailey, S., Walters, R. G., Jansson, S., and Horton, P. (2001) *Planta* **213,** 794–801
29. Hurry, V. M., Malmberg, G., Gardestrom, P., and Oquist, G. (1994) *Plant Physiol.* **106,** 983–990
30. Oquist, G., Hurry, V. M., and Huner, N. (1993) *Plant Physiol.* **101,** 245–250
31. Demmig-Adams, B. (1998) *Plant Cell Physiol.* **39,** 474–482
32. Bailey, S., Horton, P., and Walters, R. G. (2004) *Planta* **218,** 793–802
33. Lichtenthaler, H. K., Kuhn, G., Prenzel, U., and Meier, D. (1982) *Physiol. Plant* **56,** 183–188
34. Leong, T.-Y., and Anderson, J. M. (1984) *Photosynth. Res.* **5,** 105–115
35. Maenpaa, P., and Andersson, B. (1989) *Z. Naturforsch.* **44,** 403–406
36. Havaux, M., Eymery, F., Porfirova, S., Rey, P., and Dormann, P. (2005) *Plant Cell* **17,** 3451–3469
37. Muller-Moule, P., Golan, T., and Niyogi, K. K. (2004) *Plant Physiol.* **134,** 1163–1172
38. Somerville, C. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 6215–6218
39. Ben Shem, A., Frolow, F., and Nelson, N. (2003) *Nature* **426,** 630–635
40. Polle, J. E., Niyogi, K. K., and Melis, A. (2001) *Plant Cell Physiol.* **42,** 482–491
41. Havaux, M., Dall'Osto, L., Cuine, S., Giuliano, G., and Bassi, R. (2004) *J. Biol. Chem.* **279,** 13878–13888
42. Bassi, R., Rigoni, F., Barbato, R., and Giacometti, G. M. (1988) *Biochim. Biophys. Acta* **936,** 29–38
43. Lindahl, M., Funk, C., Webster, J., Bingsmark, S., Adamska, I., and Andersson, B. (1997) *Photosynth. Res.* **54,** 227–236
44. Rorat, T., Havaux, M., Irzykowskia, W., Cuine, S., Becuwe, N., and Rey, P. (2001) *Physiol. Plant.* **113,** 72–78
45. Li, X. P., Muller-Moule, P., Gilmore, A. M., and Niyogi, K. K. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 15222–15227
46. Li, X. P., Gilmore, A. M., and Niyogi, K. K. (2002) *J. Biol. Chem.* **277,** 33590–33597
47. Li, X. P., Gilmore, A. M., Caffarri, S., Bassi, R., Golan, T., Kramer, D., and Niyogi, K. K. (2004) *J. Biol. Chem.* **279,** 22866–22874
48. Crouchman, S., Ruban, A., and Horton, P. (2006) *FEBS Lett.* **580,** 2053–2058
49. Finazzi, G., Johnson, G. N., Dallosto, L., Joliot, P., Wollman, F. A., and Bassi, R. (2004) *Proc. Natl. Acad. Sci. U. S. A.* **101,** 12375–12380
50. Bergantino, E., Segalla, A., Brunetta, A., Teardo, E., Rigoni, F., Giacometti, G. M., and Szabo, I. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 15265–15270
51. Davison, P. A., Hunter, C. N., and Horton, P. (2002) *Nature* **418,** 203–206
52. Aspinall-O'Dea, M., Wentworth, M., Pascal, A., Robert, B., Ruban, A. V., and Horton, P. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 16331–16335
53. Rockholm, D. C., and Yamamoto, H. Y. (1996) *Plant Physiol.* **110,** 697–703
54. Morosinotto, T., Baronio, R., and Bassi, R. (2002) *J. Biol. Chem.* **277,** 36913–36920
55. Niyogi, K. K., Björkman, O., and Grossman, A. R. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 14162–14167
56. Standfuss, J., and Kuhlbrandt, W. (2004) *J. Biol. Chem.* **279,** 36884–36891
57. Caffarri, S., Croce, R., Cattivelli, L., and Bassi, R. (2004) *Biochemistry* **43,** 9467–9476
58. Boekema, E. J., van Roon, H., Calkoen, F., Bassi, R., and Dekker, J. P. (1999) *Biochemistry* **38,** 2233–2239
59. Morosinotto, T., Bassi, R., Frigerio, S., Finazzi, G., Morris, E., and Barber, J. (2006) *FEBS J.* **273,** 4616–4630
60. Ben Shem, A., Frolow, F., and Nelson, N. (2004) *Photosynth. Res.* **81,** 239–250
61. Morosinotto, T., Ballottari, M., Klimmek, F., Jansson, S., and Bassi, R. (2005) *J. Biol. Chem.* **280,** 31050–31058
62. Klimmek, F., Ganeteg, U., Ihalainen, J. A., van Roon, H., Jensen, P. E., Scheller, H. V., Dekker, J. P., and Jansson, S. (2005) *Biochemistry* **44,**

Downloaded from www.jbc.org at CNRS on May 20, 2008

3065–3073
63. Ruban, A. V., Wentworth, M., Yakushevska, A. E., Andersson, J., Lee, P. J., Keegstra, W., Dekker, J. P., Boekema, E. J., Jansson, S., and Horton, P. (2003) *Nature* **421,** 648–652
64. Allen, J. F. (1992) *Trends Biochem. Sci.* **17,** 12–17
65. Wollman, F. A. (2001) *EMBO J.* **20,** 3623–3630
66. Pollock, C. J., and Eagles, C. F. (1988) *Symp. Soc. Exp. Biol.* **42,** 157–180
67. Bellafiore, S., Barneche, F., Peltier, G., and Rochaix, J. D. (2005) *Nature* **433,** 892–895
68. Thomashow, M. F. (2001) *Plant Physiol.* **125,** 89–93
69. Svensson, J. T., Crosatti, C., Campoli, C., Bassi, R., Stanca, A. M., Close, T. J., and Cattivelli, L. (2006) *Plant Physiol.* **141,** 257–270

The Journal of Biological Chemistry

Downloaded from www.jbc.org at CNRS on May 20, 2008