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Effect of long-term photoinhibition on growth and photosynthesis of cold-hardened spring and winter wheat

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Abstract. The effect of repeated exposure to high light (1200 μmol · m^{-2} · s^{-1} photosynthetic photon flux density, PPFD) at 5° C was examined in attached leaves of cold-grown spring (cv. Katepwa) and winter (cv. Kharkov) wheat (*Triticum aestivum* L.) over an eight-week period. Under these conditions, Kharkov winter wheat exhibited a daily reduction of 24% in F_V/F_M (the ratio of variable to maximal fluorescence in the dark-adapted state), in contrast to 41% for cold-grown Katepwa spring wheat. Both cultivars were able to recover from this daily suppression of F_V/F_M such that the leaves exhibited an average morning F_V/F_M of 0.651 ± 0.004. Fluorescence measurements made under steady-state conditions as a function of irradiance from 60 to 2000 μmol · m^{-2} · s^{-1} indicated that the yield of photosystem II (PSII) electron transport under light-saturating conditions was the same for photoinhibited and control cold-grown plants, regardless of cultivar. Repeated daily exposure to high light at low temperature did not increase resistance to short-term photoinhibition, although zeaxanthin levels increased by three- to fourfold. In addition, both cultivars increased the rate of dry-matter accumulation, relative to control plants maintained at 5° C and 250 μmol · m^{-2} · s^{-1} PPFD (10% and 28% for Katepwa and Kharkov, respectively), despite exhibiting suppressed F_V/F_M and reduced photon yields for O_2 evolution following daily high-light treatments. Thus, although photosynthetic efficiency is suppressed by a long-term, photoinhibitory treatment, light-saturated rates of photosynthesis are sufficiently high during the high-light treatment to offset any reduction in photochemical efficiency of PSII. We suggest that in these cold-tolerant plants, photoinhibition of PSII may represent a long-term, stable, down-regulation of photochemistry to match the overall photosynthetic demand for ATP and reducing equivalents.

Key words: Carbon gain – Cold hardening – Photoinhibition – Photosystem II (down-regulation) – *Triticum* (photoinhibition) – Zeaxanthin

Introduction

Photoinhibition of photosynthesis is typically manifested as a light-dependent decrease in the quantum yield of photosystem II (PSII) photochemistry. This occurs whenever light is absorbed in excess of the capacity of the chloroplast to utilise the energy of absorbed photons for electron transport and carbon fixation. The decrease in efficiency of PSII photochemistry may reflect either damage to PSII (Kyle 1987) or the release of energy through dissipative, photoprotective mechanisms such as thermal de-excitation (Henley et al. 1991; Krause and Weis 1991). Because the yield of PSII photochemistry is similar in control plants and plants exposed to high-light (HL) conditions on the time scale of minutes or hours, it has been suggested that photoinhibition of PSII may represent a long-term adjustment of the photochemical yield to the prevailing light conditions (Öquist et al. 1991). Although the factor that determines what level of irradiance is excessive has not been elucidated unequivocally, it appears to be associated with the redox state of Q_A, the primary stable electron acceptor of PSII (Ögren 1991; Öquist et al. 1991). Thus, environmental factors such as unfavourable low temperatures, that reduce the rate of photosynthetic electron transport through reduced CO_2

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Abbreviations and symbols: Chl = chlorophyll; HL = high light; PPFD, photosynthetic photon flux density; F_O = minimum fluorescence in the dark-adapted state; F_M = maximum fluorescence in the dark-adapted state; F_V = maximum variable fluorescence in the dark-adapted state (F_M–F_O); F_V/F_V = photosynthetic efficiency of the dark-adapted state; F_V'/F_M' = photosynthetic efficiency of the light-adapted steady state; q_P = photochemical quenching parameter; q_N = non-photochemical quenching parameter; Φ_e = yield of electron transport and equals $q_P \cdot F_V'/F_M'$; $1-q_O = F_O$ quenching parameter; Φ_{app} = apparent photon yield.

fixation, also potentially increase the excitation pressure at any given irradiance, and consequently increase the sensitivity of a leaf to photoinhibition.

Reduced susceptibility to low-temperature-induced photoinhibition has been reported for cold-hardened leaves of winter rye (Öquist and Huner 1991) and spinach (Somersalo and Krause 1990; Boese and Huner 1990). Regardless of the plant species, we have shown that the capacity to exhibit a reduced susceptibility to photoinhibition is dependent upon growth and development at low temperature (Boese and Huner 1990; Öquist and Huner 1991). Leaves fully expanded at warm temperatures (20° C) do not exhibit a reduced susceptibility to photoinhibition after being shifted to low temperature (5° C) for three to four weeks (Boese and Huner 1990; Öquist and Huner 1991). Thus, the developmental history of a leaf determines its susceptibility to low-temperature-induced photoinhibition.

The role of the xanthophyll cycle in protection from photoinhibition has received much recent attention (Demmig-Adams 1990). Schöner and Krause (1990) have reported that cold hardening of spinach results in increased levels of the xanthophylls lutein, zeaxanthin and violaxanthin. The authors suggested a protective role for the increased xanthophylls during exposure to light and low temperature. However, the importance of this cycle to low-temperature-induced photoinhibition during cold acclimation has not been evaluated in detail.

It has been shown that the photon yield of PSII photochemistry reflects both the photon yield of O_2 evolution (Björkman and Demmig 1987; Hurry and Huner 1991) and CO_2 exchange (Baker et al. 1989; Genty et al. 1989; Baker 1991). It has long been supposed in the literature that reductions in the photon yield for PSII photochemistry lead to reductions in plant productivity and crop yields. Baker and co-workers (Baker et al. 1989) found a correlation between quantum efficiency for CO_2 exchange and 'conversion efficiency', a measure at the plant-canopy level of the ratio of intercepted photosynthetically active radiation to dry-matter accumulation, for a *Brassica napus* crop during the fall and winter. In addition, Ögren and Sjöström (1990) reported that about 10% of the potential carbon gain can be lost due to photoinhibition of peripheral willow shoots, even during exposure to optimal temperature conditions. Thus, there is some evidence for a relation between photon yield for PSII photochemistry and plant productivity, but it remains to be established unequivocally.

The objectives of the present study were: (i) to determine the impact of long-term, repetitive exposures to photoinhibition on net carbon gain and the capacity for growth and development in cold-hardened spring and winter wheat cultivars; (ii) to determine whether daily, repetitive exposure to HL results in an enhancement of resistance to photoinhibition; and (iii) to assess the role of zeaxanthin in the development of resistance to photoinhibition.

Materials and methods

Plant material. Two cultivars of wheat (*Triticum aestivum* L.), one winter (cv. Kharkov) and one spring (cv. Katepwa) were grown in coarse vermiculite in 7-cm-diameter plastic pots at a density of five plants per pot. Water and nutrients were supplied as required in the form of a modified Hoagland's solution as described previously (Krol et al. 1984). Seeds were germinated under controlled-environment conditions with a day/night temperature regime of 20/16° C, at a photosynthetic photon flux density (PPFD) of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 16-h photoperiod. This irradiance was approx. 80% saturating for both cultivars. After 7 d, some winter and spring seedlings were cold-hardened for 21 d by transfer to a growth temperature regime of 5/5° C with photoperiod and irradiance the same as controls. At this stage the third leaf was beginning to expand.

Photoinhibition treatments. After 21 d, half of the cold-hardened seedlings were exposed to a standard daily 12-h photoinhibitory treatment. These plants, subsequently referred to as 'high-light' (HL) plants, received 2 h of standard 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD light in the morning prior to the HL treatment and 2 h at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD in the evening following treatment. High-light treatments were carried out in a 4° C cold-room with light supplied through a 10-cm-deep continuous-flow water bath by a bank of three Lucalux LU-400 (Canada GE, Mississauga, Canada) high-pressure sodium lamps. The photon flux density incident at mid-canopy was 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Two oscilllating fans maintained leaf temperatures at between 4° C, at the beginning of the high-light treatment, to 7° C at the end of the treatment. The temperature of the upper leaf surface was measured with a thermocouple attached to an infrared gas analyser (6200; LiCor, Lincoln, Neb., USA). This treatment regimen was continued for 56 consecutive days. Control cold-hardened plants remained at 5° C and 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD with a 16-h photoperiod for the entire experiment.

Sensitivity to short-term photoinhibition was determined using fully expanded fourth leaves of 84-d-old HL and 84-d-old, control, cold-hardened Kharkov winter wheat and Katepwa spring wheat, detached prior to the daily HL treatment. Leaf segments (10 cm long) were placed on moist filter paper with the adaxial side face up and the cut ends of the leaf segments covered with moist filter paper. Paper and segments were placed in trays in a 4° C cold-room under the same light and temperature regime described above. The ratio F_V/F_M (maximum variable fluorescence/maximum fluorescence in the dark-adapted state) was used to quantify photoinhibition for both the daily HL treatments and short-term photoinhibition experiments.

Measurements of room-temperature chlorophyll a fluorescence. Room-temperature chlorophyll (Chl) *a* fluorescence at 695 nm was measured with a PSM Chlorophyll fluorometer (Biomonitor S.C.I AB, Umeå, Sweden). Instantaneous F_O (minimum fluorescence in the dark-adapted state), F_V and F_M were determined and the F_V/F_M ratio, expressing the maximum photochemical yield of PSII, was calculated. The actinic light source had a photon flux of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, with a peak wavelength of 500 nm. Exposure was for 2 s. Prior to fluorescence measurements all leaves were dark-adapted for 30 min at room temperature.

To determine steady-state quenching parameters, modulated fluorescence (PAM Chlorophyll Fluorometer; H. Walz, Effeltrich, FRG) was used with the PAM 103 accessory and two Schott lamps: one to provide saturating flashes and a second to provide actinic illumination (Schreiber et al. 1986). The protocol followed was similar to that of Genty et al. (1989) as modified by Öquist et al. (1992). Exposed, fully expanded fourth and fifth leaves of 80- to 84-d-old HL plants were detached after the daily high-light treatment and their quenching characteristics were compared with those of fully expanded fourth and fifth leaves of 80- to 84-d-old non-photoinhibited, control, cold-hardened plants. Prior to fluorescence measurements, all leaves were dark-adapted for 30 min at room

temperature. To reduce boundary-layer resistance to CO_2 uptake during fluorescence induction, a stream of air was allowed to pass over the leaf samples.

Growth kinetics. Aerial portions of seedlings from one pot exposed to daily photoinhibitory conditions and from one control put exposed to cold-hardening conditions were harvested every 10 d. After plant height was measured, leaves counted and leaf area measured, shoot tissues were dried at 105° C to constant weight. Growth coefficients (k_1') were calculated from the slope of *ln* of total shoot dry weight and leaf number versus time (Macdowall 1974).

Pigment analysis. Bulk pigments were extracted from fresh fourth and fifth leaves of 84-d-old HL and 84-d-old, control, cold-hardened leaves harvested prior to the daily HL treatment. Total Chl content and Chl *a*/*b* ratios were measured in acetone according to the methods of Arnon (1949) and Porra et al. (1989). Leaf absorptance was estimated from the following equation in Öquist et al. (1992):

$$\text{absorptance} = \frac{0.96X}{X+0.047}$$

where X = mmol Chl · m^{-2}

Carotenoids were extracted in petroleum benzene according to the protocol of Diaz et al. (1990). Separation of the carotenoids was achieved using thin-layer chromatography, the plates for which were prepared using the protocol of Diaz et al. (1990). The separation medium was petroleum benzene/acetone/chloroform (25/25/20, by vol.). The separate bands were redissolved in 1 or 2 ml of ethanol for spectroscopic identification and quantitative analysis.

Measurement of O_2 evolution. Oxygen evolution was measured with a leaf-disc electrode (Model LD2, Hansatech; King's Lynn, Norfolk, UK) at 20° C with an initial gas mixture 5% CO_2:5% O_2:90% N_2. Additional CO_2 was supplied in the form of sodium-bicarbonate (0.6 M $NaHCO_3$)-soaked capillary mats to ensure CO_2 saturation. Light was provided by a set of photodiodes (Hansatech; λ_{max} 660 nm). Measurements were made on leaf discs punched from the mid region of fully expanded fourth leaves of 80-d-old HL, 80-d-old control cold-hardened and 25-d-old non-hardened winter (Kharkov) and spring (Katepwa) wheat. Irradiance-response curves were measured over a PPFD range of 0–950 μmol · m^{-2} · s^{-1}. Dark respiration rates and the light compensation points were calculated from these data. The apparent photon yield (Φ_{app}) for O_2 evolution was calculated from the plot of the rate of O_2 evolution versus PPFD in the light-limited (1–60 μmol · m^{-2} · s^{-1}) range of the irradiance-response curve. Photon yield for O_2 evolution (Φ_{corr}) based on absorbed photons was estimated by correcting for leaf absorptance using the protocol of Öquist et al. (1992).

$$\Phi_{corr} = \frac{\Phi_{app}}{\text{absorptance}}$$

Results

Effect of daily high light exposure on F_V/F_M. Throughout the experimental period the maximal yield of PSII photochemistry was measured as F_V/F_M after a 30-min dark adaptation at the beginning and end of the 12-h treatment period to determine the extent of the daily photoinhibition and the ability of the plants to recover at 5° C (Table 1). The winter wheat Kharkov experienced an average daily reduction in F_V/F_M of 24% compared with 41% for the spring wheat Katepwa. Both cultivars were able to recover from this photoinhibition during the 12 h (4 h at 250 μmol · m^{-2} · s^{-1} PPFD + 8 h dark) between treatments. Both cultivars also established the same base-line F_V/F_M levels during this HL experiment (F_V/F_M = 0.651) which was lower than that typically found for control cold-hardened plants (F_V/F_M = 0.730) (Table 1). In contrast to the lower baseline F_V/F_M levels maintained by both cultivars during the HL treatment, F_O levels remained the same as controls throughout the experiment (Hurry 1991). Hence the reduction in F_V/F_M was a consequence of quenched F_V.

Table 1. Summary of daily percentage loss in photosynthetic efficiency (F_V/F_M) of plants exposed to high photon fluxes at 5° C. Measurements were made on five randomly selected mature leaves. These values were averaged to give a single daily value for either the control or treated leaves. The treatment consisted of moving pots from control conditions (5° C and 250 μmol · m^{-2} · s^{-1} PPFD) to HL conditions (5° C and 1200 μmol · m^{-2} · s^{-1} PPFD) 2 h after the beginning of the photoperiod for a period of 12 h. At the end of the treatment period, plants were returned to control conditions where they received 2 h light prior to the 8 h dark period. Treatments were repeated daily for 56 d. Data represent mean ± SE of all daily measurements. n = 130

Cultivar	Control F_V/F_M	HL Treated		Daily reduction in F_V/F_M (%)
		F_V/F_M Before	F_V/F_M After	
Winter (Kharkov)	0.728 ±0.005	0.651 ±0.007	0.495 ±0.018	24
Spring (Katepwa)	0.732 ±0.004	0.651 ±0.006	0.387 ±0.015	41

Evolution of O_2. Growth under the HL regimen led to an 8% reduction in the photon yield for O_2 evolution, measured prior to the daily HL treatment, relative to cold-

Table 2. Effect of long-term photoinhibition at low temperature on photosynthetic efficiency ((F_V/F_M) and φ for O_2 evolution) and dark respiration (R_{dark}) of spring and winter wheat leaves. Measurements were made at 20° C on leaf discs punched from the mid region of fully expanded fourth leaves from 25-d-old non-hardened, 80-d-old control cold-hardened and 80-d-old HL plants. All leaves were harvested 2 h after lights-on in the morning. The HL leaves were harvested prior to daily HL exposure. Data represent the mean ± SE, n = 4

Cultivar	Regime	F_V/F_M	φ_{corr}[a] (mol O_2 · (mol photons)$^{-1}$)	R_{dark} (μmol O_2 · m^{-2} · s^{-1})
Winter (Kharkov)	20° C	0.780 ±0.011	0.0920 ±0.0005	−0.82 ±0.13
	5° C	0.736 ±0.006	0.0882 ±0.0028	−2.86 ±0.01
	5° C HL	0.675 ±0.013	0.0823 ±0.0070	−2.46 ±0.35
Spring (Katepwa)	20° C	0.777 ±0.016	0.0888 ±0.0055	−1.16 ±0.22
	5° C	0.731 ±0.008	0.0830 ±0.0043	−1.94 ±0.08
	5° C HL	0.662 ±0.012	0.0765 ±0.0084	−2.32 ±0.04

[a] Corrected photon yield = φ_{app} corrected for leaf absorptance; hence units are mol O_2 evolved per mol photons absorbed

hardened controls of both cultivars (Table 2). Thus, the O_2 data are consistent with the Chl *a*-fluorescence data which indicate that growth of both cultivars under the HL regimen leads to a sustained reduction in PSII efficiency relative to cold-hardened controls.

Growth at 5° C stimulated the rates of dark respiration in both the winter and spring cultivars (Table 2). However, there was no significant difference in the rate of dark respiration in Kharkov and Katepwa after prolonged exposure to the HL treatment.

Growth kinetics. The relative growth rates of both Kharkov winter wheat and Katepwa spring wheat grown under control cold-hardening and HL conditions are shown in Fig. 1. Kharkov winter wheat grown under conditions that reduced photochemical efficiency by an average of 24% each day for a 56-d period were able to increase their growth rate by 25% relative to control cold-hardened plants growing at a PPFD of 250 μmol · m^{-2} · s^{-1}. The more susceptible spring wheat

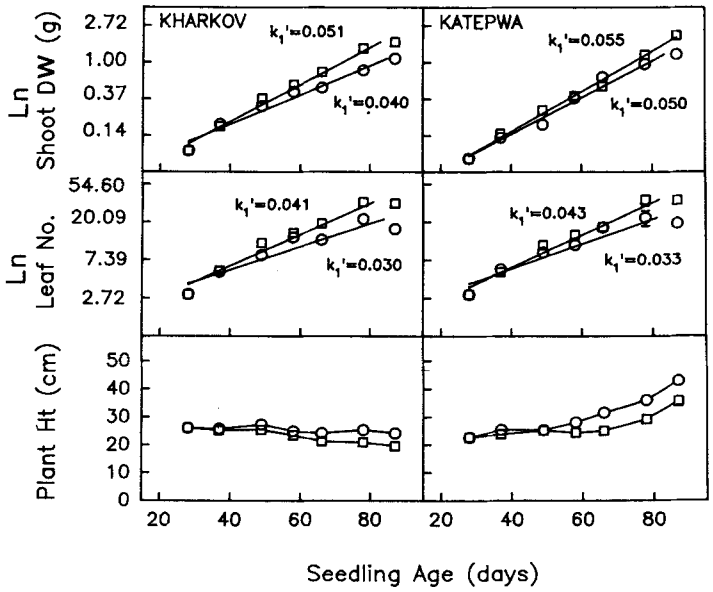

Fig. 1. Relative growth rates of the winter wheat Kharkov and the spring wheat Katepwa under control cold-hardening and HL photoinhibitory conditions. Growth coefficients (k_1') were calculated from the slope of *ln* of total shoot dry weight and leaf number versus time. ○, control cold-hardened; □, HL-grown plants. Error bars represent SE and are equal to or smaller than the symbol size, $n = 5$

Katepwa (average daily reduction in F_v/F_M of 41%) increased growth by 10% under the HL regimen. These increases in growth rate yielded plants at 75 d that were, on the basis of shoot dry weight, 2.1 and 1.7 times the size of control cold-hardened plants for the winter cultivar Kharkov and the spring cultivar Katepwa, respectively. In addition, repeated exposure to HL at low temperature favoured a 9% and 31% increase in the partitioning of dry matter to the shoot relative to the root for Kharkov and Katepwa, respectively (Hurry 1991). Both cultivars showed an increase in the rate of leaf initiation by 35% and 30% for Kharkov and Katepwa, respectively. The HL regimen had no effect on plant height of Kharkov, which developed a prostrate growth habit at both irradiances, nor did it substantially alter the erect growth habit of the spring cultivar Katepwa (Fig. 1).

Pigment content. The effects of the three growth regimes employed on the Chl content, leaf absorptance and Chl *a*/*b* ratio of Kharkov winter wheat and Katepwa spring wheat are summarised in Table 3. Growth under control cold-hardening conditions produced an increase in total Chl without any significant change in the Chl *a*/*b* ratio. However, growth under the HL regimen led to a twofold decrease in Chl content, and to a 25–40% increase in Chl *a*/*b* ratios in both wheat cultivars. These large changes in total leaf Chl are reflected in only minor changes in leaf absorptance (Table 3).

Growth under control cold-hardening conditions led to a general increase in xanthophyll levels of both wheat cultivars, particularly pigments of the xanthophyll cycle, violaxanthin (V) and zeaxanthin (Z) (Table 4). Growth under the HL regimen led to further increases in the xanthophylls, particularly zeaxanthin, which increased threefold in the winter wheat Kharkov and fourfold in the spring wheat Katepwa, relative to control cold-hardened plants. Similar large increases in xanthophyll-cycle pigments have been reported for plants growing under HL regimes (Bilger and Björkman 1990; Somersalo and Krause 1990), and have been associated with increased resistance against photoinhibition of PSII. In the current work, the large increases in xanthophyll-cycle pigments (Z+V) following cold-hardening resulted in the (Z+V): Chl *a*+*b* ratio (mmol · mol^{-1}) increasing from 13–16 in non-hardened leaves to 45–48 in control cold-hardened leaves of both cultivars. This ratio increased further to 110 and 166 following growth of Khar-

Table 3. The effect of three different growth regimes on the chlorophyll content of winter and spring wheat. Fourth and fifth leaves from 25-d-old non-hardened, 84-d-old control cold-hardened and 84-d-old HL leaves were harvested 2 h after lights-on in the morning. Leaves from HL plants were harvested prior to commencement of the daily HL treatment. Data represent the mean ± SE, $n = 5$

Cultivar	Regime	mmol Chl · m^{-2}	Absorptance[a]	Chl *a*/*b*[b]	Chl *a*/*b*[c]
Winter (Kharkov)	20° C	0.457 ± 0.016	0.870	5.0 ± 0.2	3.4 ± 0.1
	5° C	0.665 ± 0.025	0.897	5.5 ± 0.2	3.6 ± 0.2
	5° C HL	0.333 ± 0.033	0.841	7.9 ± 0.2	4.5 ± 0.1
Spring (Katepwa)	20° C	0.449 ± 0.035	0.869	4.9 ± 0.2	3.4 ± 0.2
	5° C	0.533 ± 0.033	0.882	4.2 ± 0.1	3.2 ± 0.2
	5° C HL	0.291 ± 0.008	0.826	7.1 ± 0.6	4.4 ± 0.4

[a] Calculated after Öquist et al. (1991a)
[b] Chl *a*/*b* ratio measured according to Porra et al. (1989)
[c] Chl *a*/*b* ratio measured according to Arnon (1949)

Table 4. Effect of long-term photoinhibition at low temperature on the level of xanthophyll-cycle pigments in spring and winter wheat leaves. Fourth and fifth leaves from 25-d-old non-hardened, 84-d-old control cold-hardened and 84-d-old HL leaves were harvested 2 h after lights-on in the morning. Leaves from HL plants were harvested prior to the daily HL treatment. Values are from one isolation of pigments from 2 g of fresh leaf tissue

Cultivar	Regime	Violaxanthin (V)	Zeaxanthin (Z)	Reduction in F_V/F_M[a] (%)	Z: Chl $a+b$	Z+V: Chl $a+b$
		nmol · (g leaf FW)$^{-1}$			mmol · mol^{-1}	
Winter (Kharkov)	20° C	34.8	T.A.	62	–	16.3
	5° C	95.2	17.6	28	7.1	45.4
	5° C HL	81.6	51.9	22	42.5	109.2
Spring (Katepwa)	20° C	30.5	T.A.	62	–	12.9
	5° C	100.0	23.9	41	9.2	47.7
	5° C HL	113.0	93.0	37	74.8	165.6

[a] Reduction in F_V/F_M due to photoinhibition following a 12-h exposure of intact leaves to an irradiance of 1200 μmol · m^{-2} · s^{-1} at 5° C, expressed as a percent of initial value
T.A. = trace amounts

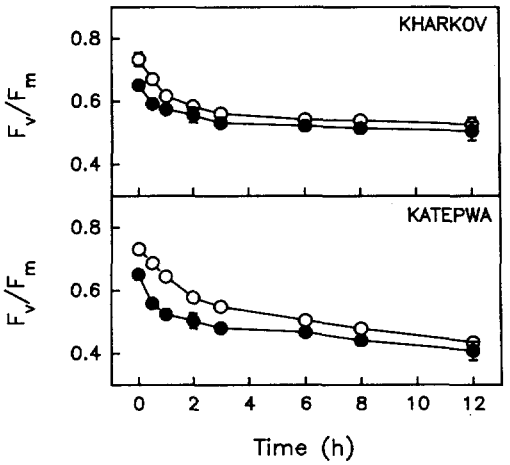

Fig. 2. Effect of the HL regimen on sensitivity to photoinhibition of intact cold-hardened winter (Kharkov) and spring (Katepwa) wheat leaves. Photoinhibition treatments consisted of a constant photon flux of 1200 μmol · m^{-2} · s^{-1} incident on the leaf at 5° C, ○, control cold-hardened leaves; ●, HL leaves. Each point is the mean of six leaves. Bars represent SE. Where absent, error bars are equal to or smaller than the symbol size

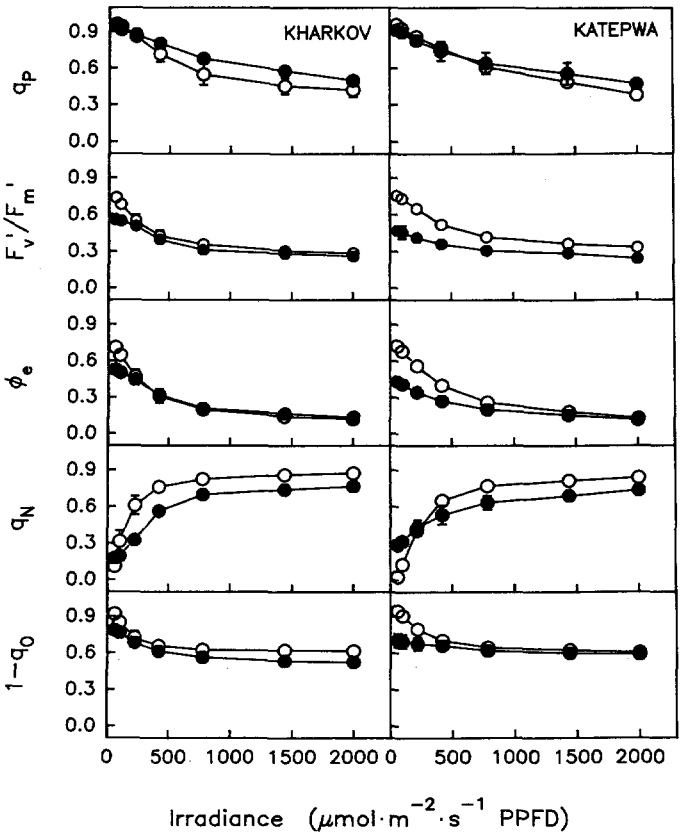

Fig. 3. Effect of photoinhibition on the steady-state fluorescence-quenching characteristics, the steady-state yield of electron transport and the steady-state tapping efficiency of HL leaves compared with non-photoinhibited, control, cold-hardened leaves. ○, control cold-hardened; ●, HL leaves treated with 1200 μmol · m^{-2} · s^{-1} PPFD at 5° C for 12 h. Each point is the mean of four to six leaves. Bars represent SE

kov and Katepwa, respectively, under the HL regimen (Table 4) with little effect on the susceptibility of the HL leaves to short-term photoinhibition relative to cold-hardened controls (Fig. 2, Table 4). Thus, the increase in the xanthophyll: Chl ratio does not appear to be correlated with altered susceptibility to photoinhibition in these wheat cultivars. It has been shown that accumulation of xanthophyll-cycle pigments in iron-deficient sugar beet does not alter its susceptibility to photoinhibition (Morales et al. 1990).

Susceptibility to short-term photoinhibition. Figure 2 shows the response of detached control cold-hardened and HL leaves of Kharkov winter wheat and Katepwa spring wheat to standard photoinhibitory conditions. The repeated daily photoinhibitory treatments experienced by the HL plants of both cultivars lowered their initial F_V/F_M. However, after 2 and 3 h, respectively, both control cold-hardened and HL leaves of the winter cultivar Kharkov and the spring cultivar Katepwa had similar F_V/F_M ratios which remained constant throughout the rest of the 12-h treatment period. Clearly, repeated daily exposure of these cultivars to photoinhibitory conditions did not increase resistance to photoinhibition as assessed by F_V/F_M. Both the winter and spring cultivars responded similarly, although the spring cultivar showed

greater susceptibility to photoinhibition than the winter cultivar even after the 56-d HL treatment (Fig. 2).

Steady-state fluorescence characteristics. The HL cold-hardened leaves taken after the daily 12-h HL-treatment period exhibited significantly lower yields of PSII electron transport ($\varphi_e = F_V'/F_M' \cdot q_P$) under light-limiting conditions than control cold-hardened leaves taken directly from the growth cabinet 2 h after lights-on in the morning (Fig. 3). However, light-limiting levels of φ_e of HL leaves from the spring cultivar (Katepwa) appeared to be more sensitive to the prolonged photoinhibitory treatment than those of the winter cultivar (Kharkov). This could be accounted for by a decreased steady-state trapping efficiency (F_V'/F_M') rather than any significant difference in the response of q_P as a function of irradiance.

In contrast, under light-saturating conditions, both Kharkov and Katepwa leaves have trapping efficiencies and q_P levels similar to non-photoinhibited, control, cold-hardened plants, and consequently similar yields of electron transport (Fig. 3). Thus, at the irradiance used during the daily photoinhibition treatment (1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD), HL-treated plants of both cultivars are able to utilise the available light just as effectively as non-photoinhibited controls.

In addition to a decreased trapping efficiency under light-limiting conditions, HL-treated Katepwa exhibited higher levels of non-photochemical quenching (q_N) than control plants. This was associated with higher levels of F_O quenching ($1-q_O$) under light-limiting conditions (Fig. 3). This response was not evident in the winter wheat cultivar. The HL leaves of both cultivars maintained slightly lower saturating levels of q_N after 12 h at 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD.

Discussion

Both wheat cultivars were able to increase their growth rates under conditions that substantially reduce the efficiency of PSII. The winter wheat Kharkov, which was photoinhibited on average by 24% each day, increased its growth rate under the HL regimen by 25%, relative to control cold-hardened plants grown at 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD. The spring wheat Katepwa, which was more sensitive to photoinhibition (average daily loss in F_V/F_M of 41%), increased its growth rate by 10% relative to cold-hardened controls. Both cultivars appear to have been able to achieve this because the HL treatment did not reduce the yield of electron transport at saturating irradiance relative to control cold-hardened leaves. For example, φ_e calculated at an irradiance of 250 $\mu mol \cdot {}^{-2} \cdot s^{-1}$ (Fig. 3) declined in Katepwa from approx. 0.50 to about 0.30 when control plants are compared to HL plants. However, the estimated flux of electrons ($\varphi_e \cdot I$) increased from approx. 125 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for control cold-hardened wheat exposed to a photon flux of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to 210 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the HL plants that were exposed to a photoinhibitory photon flux of 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When evaluating the effects of photoinhibition on photosynthetic performance and growth it is important to compare the flux rather than the efficiency; a flux parameter integrates both efficiency and the available light whereas an efficiency parameter does not. Under HL conditions, Katepwa clearly requires a greater than 40% reduction in PSII efficiency before photosynthetic efficiency begins to impact on the flux of electrons through PSII and the growth rate. Thus, these data indicate that for cold-hardened wheat, where growth is predominantly controlled by the low temperature, the imposition of a reduction in photosynthetic efficiency has little additional effect on growth rate. Since growth is already controlled by temperature, we suggest that these wheat plants deal with the excess light by down-regulating PSII photochemistry through photoinhibition, thus, adjusting the photosynthetic efficiency to reflect the demand for ATP and reducing equivalents under the prevailing HL environment. This means that the loss of photosynthetic efficiency may not necessarily result in lower productivity, that is decreased carbon gain, for cold-grown plants exposed to high photon fluxes at low temperatures. Light-saturated rates of photosynthesis are sufficiently high during the photoinhibitory treatment to offset the reduction in photosynthetic efficiency.

Growth of winter and spring wheat plants at 5° C under an HL regimen had two main effects on their fluorescence characteristics. *First,* exposure to HL led to a *daily* decline in the efficiency of PSII photochemistry for both cultivars (24% and 41% reductions in F_V/F_M for Kharkow winter wheat and Katepwa spring wheat, respectively). However, both cultivars showed the capacity to recover from this lost efficiency each day. Thus, the daily quenching and recovery in F_V/F_M is rapidly reversible at 5° C. Furthermore, the leaves do not appear to accumulate photoinhibitory damage with repeated exposure to HL as they recover to stable, baseline levels of F_V/F_M (0.651), each morning (Table 1). Thus, the daily cycling of the efficiency of PSII photochemistry observed in the HL leaves is probably not a consequence of irreversible damage to PSII polypeptides and their subsequent replacement through protein synthesis, but may be due instead to a rapidly reversible, photoinhibitory "down-regulation" of PSII efficiency. This may occur as a consequence of PSII conformational changes which lead to the formation of reversibly modified PSII quenching centres. Such rapidly reversible PSIIα quenching centres have been proposed by Krause and Weis (1991).

Second, in addition to the daily quenching of PSII efficiency, measured as F_V/F_M, both cultivars maintained a stable, *sustained* level of F_V/F_M (0.651) (Table 1) after recovery from the HL treatment that was lower than control cold-hardened leaves. This was due to quenching of F_V, not to significant changes in the level of F_O. Associated with this sustained quenching of F_V/F_M is an increase in carotenoid levels, particularly of the xanthophyll, zeaxanthin (Table 3). This pigment increased three- and fourfold between control cold-hardened and HL leaves of Kharkov and Katepwa, respectively. A similar relation between sustained fluorescence quenching and increased zeaxanthin levels has been shown in *Nerium oleander* following prolonged exposure to a combination of HL and water stress (Demmig et al. 1988). Demmig and co-workers (1988) postulated that this increased level of zeaxanthin lead to an increase in

k_D and thus increased thermal dissipation in the antenna. As expected this was associated with F_O quenching. However, in the present study the increase in zeaxanthin was not associated with any detectable change in F_O. Thus, the mechanistic basis for the sustained quenching of variable fluorescence observed in these HL wheat leaves remains unclear.

In the current study, the accumulation of large amounts of zeaxanthin did not confer reduced susceptibility to photoinhibition, measured as either reductions in F_V/F_M or φ for O_2 evolution. This is contrary to the suggestion of a positive effect of increased levels of xanthophylls on susceptibility to low-temperature-induced photoinhibition in spinach (Schöner and Krause 1990). The further increase in xanthophylls seen in the HL leaves of wheat may be simply a consequence of the growth regimen.

In conclusion, both cultivars were strongly photoinhibited daily for eight weeks without any further increase in resistance to photoinhibition. The spring wheat cultivar remained more sensitive than the winter cultivar. Leaves of both cultivars rapidly recovered from photoinhibition at 5° C such that the leaves returned to the same F_V/F_M each morning and no accumulation of apparent damage occurred. Concomitantly, both cultivars responded to exposure to high photon fluxes at 5° C by increasing overall growth. This indicates that the photoinhibition observed in these leaves represents a light-induced, reversible, down-regulation PSII efficiency rather than damage to PSII. The photosynthetic data coupled with growth kinetics strengthen considerably the notion that photoinhibition may be considered a protective, acclimation response for the long-term regulation of PSII under HL conditions.

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