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Analysis of qualitative contribution of assimilatory and non-assimilatory de-excitation processes to adaptation of photosynthetic apparatus of barley plants to high irradiance

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Abstract

The adaptation of barley (*Hordeum vulgare* L. cv. Akcent) plants to low (LI, 50 μmol m^{-2} s^{-1}) and high (HI, 1000 μmol m^{-2} s^{-1}) growth irradiances was studied using the simultaneous measurements of the photosynthetic oxygen evolution and chlorophyll *a* (Chl *a*) fluorescence at room temperature. If measured under ambient CO_2 concentration, neither increase of the oxygen evolution rate (*P*) nor enhancement of non-radiative dissipation of the absorbed excitation energy within photosystem 2 (PS2) (determined as non-photochemical quenching of Chl *a* fluorescence, NPQ) were observed for HI plants compared with LI plants. Nevertheless, the HI plants exhibited a significantly higher proportion of Q_A in oxidised state (estimated from photochemical quenching of Chl *a* fluorescence, q_P), by 49-102 % at irradiances above 200 μmol m^{-2} s^{-1} and an about 1.5 fold increase of irradiance-saturated PS2 electron transport rate (ETR) as compared to LI plants. At high CO_2 concentration the degree of *P* stimulation was approximately three times higher for HI than for LI plants, and the irradiance-saturated *P* values at irradiances of 2 440 and 2 900 μmol m^{-2} s^{-1} were by 130 and 150 % higher for HI plants than for LI plants. We suggest that non-assimilatory electron transport dominates in the adaptation of the photosynthetic apparatus of barley grown at high irradiances under ambient CO_2 rather than an increased NPQ or an enhancement of irradiance-saturated photosynthesis.

Additional key words: chlorophyll fluorescence; *Hordeum vulgare*; non-radiative dissipation; non-assimilatory electron transports; photosynthetic oxygen evolution.

Introduction

Higher plants possess several protective and regulatory mechanisms to avoid the irreversible photooxidative damage of their photosynthetic apparatus under excess irradiances. Photosystem 2 (PS2) is the target of photodamage at optimal temperatures (Chow 1994, Barber 1995, Melis 1999). Therefore a prompt enhancement of non-radiative dissipation (NRD) of absorbed excitation energy within light-harvesting complexes (LHC) and/or PS2 reaction centres (PS2 RC's) is of crucial importance (Demmig-Adams 1990, Demmig-Adams and Adams 1996, Gilmore 1997, Špunda *et al.* 1998). The protective role of increased NRD (determined as non-photochemical quenching of the maximum Chl *a* fluorescence, NPQ) lies in partial reduction of excess excitation pressure on PS2 RC's. This is given as approximate estimation of reduced Q_A expressed from photochemical quenching of Chl *a* fluorescence as $1 - q_p$ (Demmig-Adams *et al.* 1990, Gray *et al.* 1996, Špunda *et al.* 1998). A similar protective reduction of PS2 excitation pressure is related to the processes accelerating the photochemical de-excitation of PS2 RC's. In case of limitation of linear electron transport, the enhancement of photosynthetic CO_2 assimilation can avoid over-reduction of components of electron transport chain (Genty and Harbinson 1997). Additional role in photoprotection was attributed to the stimulation of cyclic electron transport

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Abbreviations: Chl, chlorophyll; ETR, electron transport rate through photosystem 2 estimated from chlorophyll *a* fluorescence; FIC, slow phase of fluorescence induction curve; NPQ, non-photochemical quenching of chlorophyll *a* fluorescence; NRD, non-radiative dissipation; *P*, rate of photosynthetic oxygen evolution; PS, photosystem; q_p, photochemical quenching of chlorophyll *a* fluorescence.
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around PS2 and PS1 (Lapointe *et al.* 1993, Niyogi 1999) and to oxygen-dependent electron transports such as Mehler reaction (Park *et al.* 1996, Polle 1996, Asada 1999) and photorespiration (Wu *et al.* 1991, Osmond and Grace 1995, Heber *et al.* 1996). Among the above mentioned processes preventing the over-reduction of the electron transport chain components, just the Mehler reaction is associated with increased NRD due to the generation of a ΔpH across the thylakoid membrane (Schreiber and Neubauer 1990, Biehler and Fock 1996, Park *et al.* 1996, Asada 1999).

The adaptation of plants to high growth irradiances can generally increase the efficiency of all the above mentioned protective processes. However, different plant species reveal a partly different strategy of adaptation regarding the effects on photosynthetic activities and/or dissipative processes (Huner *et al.* 1993). Reduction of LHC amount and enhancement of photosynthetic activity belong to the main and general aspects of plant adaptation to high irradiances. The mentioned LHC reduction is connected with changes of photosynthetic pigment composition such as higher Chl *a* to Chl *b* ratio, larger xanthophyll pool, and higher convertibility of violaxanthin to zeaxanthin. It usually results in increased capacity of NRD (Horton *et al.* 1994, 1996, Maxwell *et al.* 1995, Demmig-Adams and Adams 1996, Špunda *et al.* 1998). On the contrary, adaptation of cereals to high excitation pressure results almost entirely in elevated photosynthetic capacity, whereas the NRD enhancement is insignificant (Gray *et al.* 1996, Huner *et al.* 1998, Ivanov *et al.* 1998).

In the present report we attempted to characterise the adaptation of the photosynthetic apparatus of barley to low and high irradiances on the base of simultaneous measurements of photosynthetic oxygen evolution and Chl *a* fluorescence under both ambient and high CO_2 concentration. First of all, we confirmed the general hypothesis on adaptation of photosynthetic apparatus of cereals for the barley grown at high irradiance of 1 000 μmol m^{-2} s^{-1}. Further, the attention was focussed on the qualitative estimation of contribution of assimilatory and non-assimilatory de-excitation to the reduction of PS2 excitation pressure for the barley grown under high irradiances.

Materials and methods

Plants: Seeds of spring barley (*Hordeum vulgare* L. cv. Akcent) were germinated in regularly watered soil substrate under controlled climate (growth chamber *HB 1014 Bioline-Heraeus*, Germany) at 20 °C, relative humidity 65 %, and 16/8 h day/night regime. Plants were grown at low (50 μmol m^{-2} s^{-1} - LI_{50}) and high (1 000 μmol m^{-2} s^{-1} - HI_{1000}) irradiances. All measurements were carried out on the primary leaves of 8-d-old plants.

Chlorophyll *a* fluorescence: The measurements of the pulse amplitude modulated Chl *a* fluorescence at room temperature were performed using a *PAM 101, 103* fluorometer (*H. Walz*, Effeltrich, Germany) as described in Čajánek *et al.* (1999). The fluorescence was measured simultaneously with the oxygen evolution. The two sets of fluorescence experiments were performed:

(*1*) **Steady state Chl *a* fluorescence parameters against irradiance**. Their dependencies on irradiance ("light curves") for LI_{50} and HI_{1000} plants were measured at ambient CO_2 concentration. Individual irradiances of leaf segment surface were as follows: 36, 51, 77, 115, 263, 557, 805, and 1 560 μmol m^{-2} s^{-1}. The irradiance of the modulated measuring beam was adjusted to the level that did not induce any fluorescence induction related to the reduction of PS2 acceptors and ΔpH formation. Initial (F_0) and maximal (F_M) fluorescences were measured of dark-adapted leaf segments. In order to estimate the true F_M and F_M' values, the saturation pulses of 1-s duration and an incident irradiance of approximately 5 000 μmol m^{-2} s^{-1} were optimal. The F_0' value was estimated as the lowest fluorescence level during 3 s of darkness after the irradiation period at each irradiance. The following Chl *a* fluorescence parameters were calculated from F_0', F_M', and F_S values measured in the steady state at each irradiance: non-photochemical quenching of F_M [NPQ = $F_M/F_M' - 1$] (Gilmore and Yamamoto 1993) and photochemical quenching $q_P = (F_M' - F_S)/(F_M' - F_0')$ (Bilger and Schreiber 1986). The indirect estimation of electron transport rate (ETR) through PS2 was obtained as: $ETR = (F_M' - F_S/F_M') \times PhAR \times 0.8 \times 0.5$ (modified according to Genty *et al.* 1989), where PhAR is incident irradiance, 0.8 is an assumed leaf absorptance, and 0.5 is the equal excitation energy distribution between PS2 and PS1.

(*2*) **Chl *a* fluorescence induction at room temperature**. Again F_0 and F_M were measured of dark-adapted leaf segments. Then the slow phase of fluorescence induction curve (dependence of relative Chl *a* fluorescence on time, FIC) was recorded during 10 min at actinic irradiance of 850 μmol m^{-2} s^{-1}. During FIC the saturation pulses were applied in 1-min intervals in order to monitor the development of NPQ. The measurements were performed both under ambient and high CO_2 concentrations.

Oxygen evolution: The rate of the photosynthetic O_2 evolution normalised per unit leaf area [*P*; μmol(O_2) m^{-2} s^{-1}] was measured with leaf segments (1.5 cm^2) at room temperature (21-23 °C) using system with leaf-disc O_2

electrode (*LD2/2 Hansatech Instruments*, King's Lynn, U.K.). The measurements of P for LI_{50} and HI_{1000} plants were performed under high incident irradiances: 1 660, 2 440, and 2 900 μmol m^{-2} s^{-1} (irradiance-saturated P). The P values were determined at steady-state conditions within 7-10 min at each irradiance. Each leaf segment was exposed only to one of the above mentioned irradiances. All measurements were performed also at high CO_2 concentration induced by addition of 1 M bicarbonate solution into the sample chamber before the measurement of each leaf segment. According to Delieu and Walker (1983) this concentration of bicarbonate is sufficient to suppress photorespiration, avoid CO_2 limitation, and saturate the capacity of photosynthetic CO_2 assimilation.

Pigment analysis: Contents of Chl *a*, Chl *b*, and total carotenoids were estimated spectrophotometrically in pigment extracts in 80 % acetone with addition of a small amount of $MgCO_3$ according to Lichtenthaler (1987).

Statistical analysis: The experimental values were tested for significance by F-test (a two-sample test for variances) followed by a *t*-test. Based on the result of the F-test the *t*-test was used assuming either equal variances or unequal variances. The levels of significance α = 0.05, 0.01, and 0.001 were indicated as *, **, and ***. All statistical tests were performed using *Microsoft® Excel 97 SR-2*.

Results

Pigment analysis: For HI_{1000} plants the Chl *a* and Chl *b* contents per leaf area were 1.7 and 1.5 fold higher than for LI_{50} plants, respectively, whereas the total carotenoid content (Car x+c) was 1.8 fold higher (Table 1). Higher pigment contents per leaf area for HI_{1000} plants were particularly due to a considerably greater leaf thickness (values not shown), because per dry matter there was a typical decrease of Chl *a* and Chl *b* contents (by 20 and 27 %) as compared to the LI_{50} plants (values not shown). The significantly higher Chl *a*/*b* ratio by 9.6 % was found for HI_{1000} plants, whereas the ratio of Chl (*a*+*b*) to total carotenoids (Chl *a*+*b*/Car x+c) was by 8.9 % lower. These changes in pigment contents and composition were typical adaptation of barley plants to high irradiances similar to those observed for barley by Čajánek *et al.* (1999) and by Gray *et al.* (1996) for other cereal species. The changes in pigment contents and particularly the increase of Chl *a*/*b* ratio and the decrease of Chl *a*+*b*/Car x+c are related to the reduction of LHC amount (Lichtenthaler *et al.* 1981, 1982, Logan *et al.* 1996, Melis 1998).

Table 1. Total chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid (Car x+c) contents per leaf area, and the ratios Chl *a*/*b* and Chl *a*+*b*/Car x+c in barley plants grown at high (1 000 μmol m^{-2} s^{-1}; HI_{1000}) and low (50 μmol m^{-2} s^{-1}; LI_{50}) irradiances. Means from six measurements ± standard deviations are given. The differences in pigment content and pigment ratios between HI_{1000} and LI_{50} are all significant at a level of significance α = 0.001.

Sample	HI_{1000}	LI_{50}
Chl *a* [mg m^{-2}]	329 ± 27	198 ± 9
Chl *b* [mg m^{-2}]	100 ± 8	66 ± 4
Car x+c [mg m^{-2}]	82 ± 5	46 ± 3
Chl *a*/*b*	3.29 ± 0.03	3.00 ± 0.09
Chl *a*+*b*/Car x+c	5.23 ± 0.15	5.74 ± 0.10

Oxygen evolution: From the dependence of P on irradiance under ambient CO_2 we estimated that the irradiance of 1 660 μmol m^{-2} s^{-1} is sufficient to saturate photosynthesis for both HI_{1000} and LI_{50} plants (values not shown). We also measured the irradiance-saturated P for HI_{1000} and LI_{50} plants both at ambient (marked as LI_{50} and HI_{1000}) and high (marked as $^{+}LI_{50}$ and $^{+}HI_{1000}$) CO_2 concentrations (Fig. 1). The significantly lower P was

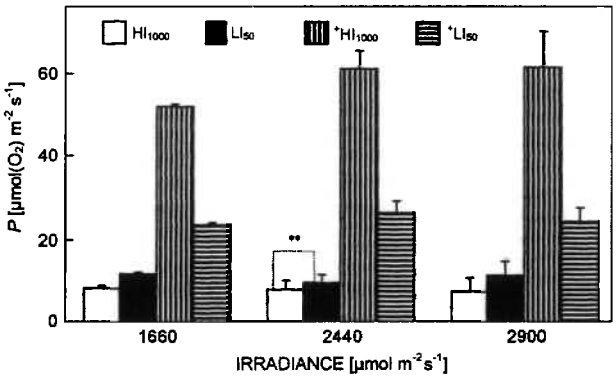

Fig. 1. The photosynthetic O_2 evolution rate, P [μmol(O_2) m^{-2} s^{-1}] at high irradiances measured both under high (marked as $^{+}$) and ambient CO_2 concentrations and estimated at steady-state conditions. The measurements were carried out on leaf segments (1.5 cm^2) of barley (*Hordeum vulgare* L. cv. Akcent) grown under high (1000 μmol m^{-2} s^{-1}; HI_{1000}) and low (50 μmol m^{-2} s^{-1}; LI_{50}) irradiances. The mean values from six measured samples (5-10 steady-state values for each leaf segment) and standard deviations are presented. If not indicated otherwise, the differences between individual variants are significant at level of significance α = 0.001.

observed for HI_{1000} than LI_{50} under ambient CO_2: the differences were 30, 19, and 35 % for irradiances of 1 660, 2 440, and 2 900 μmol m^{-2} s^{-1}, respectively. A more pronounced decrease of P per Chl (*a*+*b*) for HI_{1000} in comparison with LI_{50} plants was estimated under

ambient CO_2 and above mentioned irradiances (by 57, 50, and 60 %). If measured under high CO_2, *P* was significantly (α = 0.001) higher for both HI_{1000} and LI_{50} plants compared to those measured under ambient CO_2 concentrations (Fig. 1) and the *P*-saturating irradiance was higher (2 440 μmol m^{-2} s^{-1}). Moreover, this *P* enhancement at high CO_2 and high irradiances was much more pronounced for HI_{1000} then LI_{50} plants. *P* of $^{+}HI_{1000}$ plants was five-fold to eight-fold higher then values for HI_{1000} plants for the individual high irradiances, whereas for the $^{+}LI_{50}$ the stimulation of *P* resulted only in 2.0-2.7-fold higher *P* as compared with LI_{50}. Hence, due to the strongly high CO_2-induced *P* stimulation, *P* under high CO_2 was by 130 and 150 % higher for $^{+}HI_{1000}$ than for $^{+}LI_{50}$ at 2 440 and 2 900 μmol m^{-2} s^{-1}.

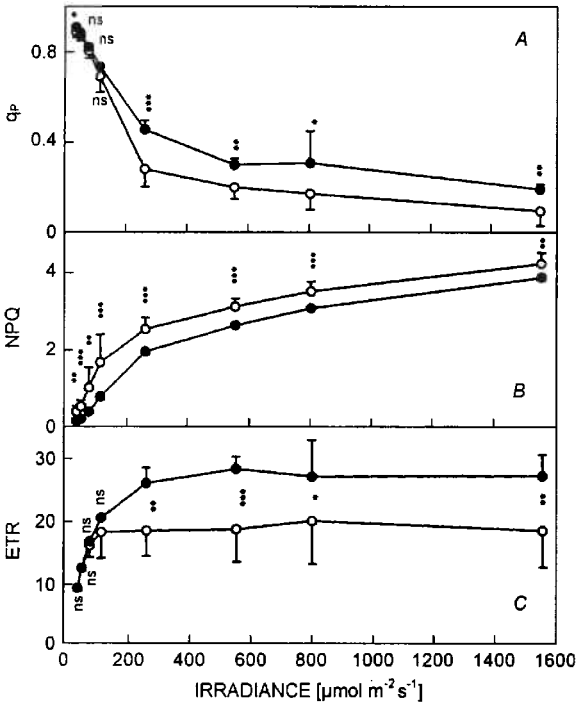

Fig. 2. The irradiance-response curves of photochemical, q_P (*A*) and nonphotochemical quenching, NPQ (*B*) of chlorophyll *a* fluorescence at room temperature and electron transport rate through PS2, ETR (*C*) measured under ambient CO_2 concentration. The full and empty circles represent mean values of q_P, NPQ, and ETR from six measurements for barley (*Hordeum vulgare* L. cv. Akcent) grown under high (1 000 μmol m^{-2} s^{-1}; HI_{1000}) and low (50 μmol m^{-2} s^{-1}; LI_{50}) irradiances. The standard deviations and levels of significance are presented.

Chl *a* fluorescence: The typical decrease of q_P upon gradual increase of irradiance monitoring the progressive Q_A reduction occurred for both HI_{1000} and LI_{50} variants (Fig. 2*A*). At low irradiances (up to 115 μmol m^{-2} s^{-1}) we did not observe any significant difference in the q_P levels between the HI_{1000} and LI_{50} barley. Starting from 263 μmol m^{-2} s^{-1} the q_P was significantly higher by 49-102 % for HI_{1000} as compared to LI_{50} plants. The so-called excitation pressure on PS2 expressed as the proportion of reduced primary stable acceptor Q_A ($1 - q_P$) is a marker estimating the quantity of absorbed photons in respect to the capacity of photon utilisation in PS2 photochemical reactions. According to Demmig-Adams (1990) the irradiance resulting in an increase of $1 - q_P$ above 0.6 value is excess irradiance. From the irradiance response of q_P the excitation pressure corresponding to the growth irradiance can be roughly determined. Thus, in our case the HI_{1000} plants were grown under excess excitation pressure ($1 - q_P > 0.6$), whereas the LI_{50} plants grew under low excitation pressure on PS2 ($1 - q_P$ around 0.1).

The dependence of non-photochemical quenching of F_M (NPQ) on irradiance is a useful tool for estimating the relative increase of non-radiative dissipation of absorbed excitation energy (NRD). Surprisingly, significantly higher NPQ values were observed for LI_{50} than for HI_{1000} plants within the whole range of applied irradiances (Fig. 2*B*). Hence, NPQ did not reach iradiance-saturation at

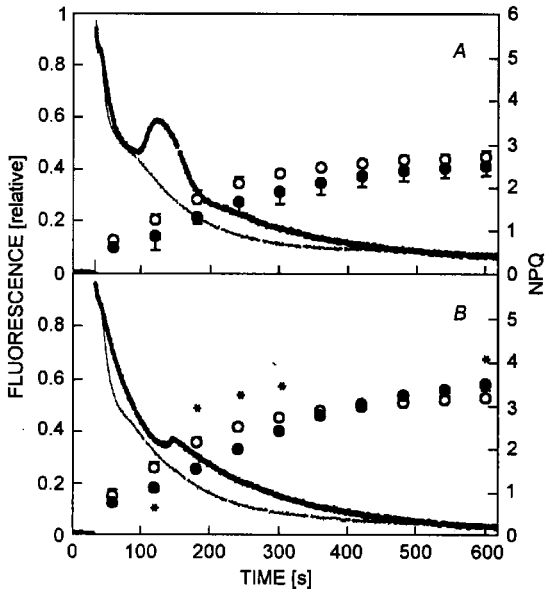

Fig. 3. A slow phase of the chlorophyll *a* fluorescence induction curves (FIC, *lines*) and time dependence of non-photochemical quenching of chlorophyll *a* fluorescence (NPQ; *circles*) for HI_{1000} (*A*) and LI_{50} (*B*) plants measured under ambient (*empty circles, thin line*) and high (*full circles, broad line*) CO_2 concentrations and at irradiance of 850 μmol m^{-2} s^{-1}. For better legibility of changes in FIC, the chlorophyll *a* fluorescence intensity was normalised to 0 at F_0 level and to 1 at F_M level. Representative FIC and mean values of NPQ from three measurements and standard deviations are presented. If not indicated otherwise, the differences between variants measured under ambient and high CO_2 are insignificant.

about 1 600 μmol m^{-2} s^{-1} for both variants. The significantly lower NPQ values for HI_{1000} were also obtained

from supplementary measurements at high irradiances (850-2 900 μmol m^{-2} s^{-1}) under ambient and high CO_2 concentration compared to LI_{50} plants (values not shown). Even these high irradiances still induced a moderate increase of NPQ for both LI_{50} and HI_{1000} barley plants. Hence, in contrast to other plant species such as Norway spruce (Špunda *et al.* 1998) the irradiance-saturated NPQ can not be obtained for barley (Čajánek *et al.* 1999).

In Fig. 2*C* the dependencies of electron transport rate through PS2, ETR [μmol(electron) m^{-2} s^{-1}] on irradiance under ambient CO_2 concentration are shown for HI_{1000} and LI_{50} plants. ETR was irradiance-saturated at 115 and 557 μmol m^{-2} s^{-1} for LI_{50} and HI_{1000}, respectively. The significantly higher ETR was observed at irradiance of 263 μmol m^{-2} s^{-1} and higher for HI_{1000} plants as compared to LI_{50} barley plants (by 42, 52, 36, and 49 % higher at 263, 557, 805, and 1 560 μmol m^{-2} s^{-1}, respectively).

The time dependencies of NPQ and fluorescence (slow phase of FIC) for HI_{1000} and LI_{50} plants measured under ambient and high CO_2 are shown for the selected irradiances (Fig. 3). The pronounced M-peak appeared in FIC for both LI and HI plants under high CO_2. According to Sivak and Walker (1985), the M-peak is the result of ATP consumption related to the induction of CO_2 assimilation in Calvin cycle reactions causing partial relaxation of ΔpH-dependent part of NPQ. The appearance of M-peak depends on CO_2 concentration, irradiance, duration of dark interval, *etc.*, but generally a pronounced M-peak is usually observed only at low irradiances. This is consistent with our finding that at high irradiance of 850 μmol m^{-2} s^{-1} the M-peak was practically absent in FIC under ambient CO_2 for both HI and LI plants (Fig. 3*A,B*). This indicates that not only for LI plants but also for HI plants the effect of the induction of Calvin cycle reactions under ambient CO_2 was not sufficient to induce the partial relaxation of NPQ at high irradiances. In our case, the more pronounced M-peak appeared for $^{+}HI_{1000}$ plants measured under high CO_2 as compared to $^{+}LI_{50}$ at 850 μmol m^{-2} s^{-1} (Fig. 3*A,B*). The time dependent changes of NPQ were measured simultaneously with recording of the FIC (Fig. 3). These measurements confirmed the idea that the manifestation of M-peak in FIC measured at 850 μmol m^{-2} s^{-1} under high CO_2 is related to the diminution of NPQ as compared to the NPQ at ambient CO_2. The above mentioned results indicate that high CO_2 induces dramatically larger stimulation of assimilatory electron transport for HI barley as compared to LI plants. However, the steady-state q_P and the irradiance-saturated ETR values were not significantly increased under high CO_2 for both LI and HI plants (values not shown). Therefore, the total capacity of photochemical de-excitation of PS2 in the electron transport chain is probably not significantly enhanced under high CO_2.

Discussion

The adaptation of plants grown under high irradiances involves a decreased capacity to absorb incident radiation as a consequence of reduced concentrations of LHC2 (Lichtenthaler *et al.* 1981, 1982) and increased capacity for non-radiative dissipation of excess excitation energy (Demmig-Adams 1990, Maxwell *et al.* 1995, Logan *et al.* 1996, Park *et al.* 1997). The mentioned down-regulation of the light-harvesting proteins is connected with an increased capacity to maintain Q_A in oxidised state. The 1.1 times higher Chl *a*/*b* ratio observed for HI_{1000} barley grown under high excitation pressure on PS2 ($1 - q_P > 0.6$) as compared to LI_{50} barley grown under low excitation pressure ($1 - q_P$ about 0.1) indeed indicated a moderately reduced amount of LHC2. However, we did not observe any enhancement of non-radiative dissipation of absorbed excitation energy (NPQ) for HI_{1000} plants (Figs. 2*B* and 3). The fact that increased level of NPQ does not belong to the features of adaptation of the barley photosynthetic apparatus to high irradiance was shown recently by Čajánek *et al.* (1999). This agrees with the ideas of Huner *et al.* (1993) suggesting that the increased resistance of cereals grown at high irradiance against photoinhibition is a consequence of stimulation of photon utilisation in electron transport rather than of increased capacity for non-radiative dissipation of excitation energy.

The increased capacity of photochemical de-excitation was supported by the finding that the irradiance-saturated ETR values were approximately 1.5-fold higher for HI_{1000} than LI_{50} barley plants both under ambient (Fig. 2*C*) and high CO_2 concentrations. However, the estimation of ETR based on Chl *a* fluorescence excited mainly from the uppermost leaf layer provides only a qualitative assessment of total electron transport within the whole leaf. The measurements of irradiance-saturated *P* under photorespiratory and non-photorespiratory conditions were used in order to reveal how was the above mentioned increase of ETR for HI_{1000} plants related to assimilatory and/or non-assimilatory processes. Under non-photorespiratory conditions the irradiance-saturated *P* was 2.5-fold higher for HI barley than for the LI one (Fig. 1). Hence, a similar extent of stimulation of photosynthetic capacity measured under high CO_2 concentration was observed as for spring and winter wheat adapted to high irradiances (Gray *et al.* 1996). However, under ambient CO_2 the rates of oxygen evolution, expressed both per leaf area (Fig. 1) and total Chl content (values not shown), were significantly lower

for HI than LI plants within the whole range of applied irradiances (Fig. 1). This indicates that the adaptation of barley grown at ambient CO_2 to high irradiance cannot be simply attributed to the increased capacity of photochemical de-excitation associated with elevated photosynthetic CO_2 assimilation. Thus, we suppose that reactions resulting in O_2 uptake are much more effective in HI plants.

Sivak and Walker (1985) showed that under moderate irradiance the pronounced uptake of ATP during induction of photosynthetic CO_2 assimilation induced a partial diminution of ΔpH-dependent NPQ resulting in appearance of pronounced M-peak in FIC. Hence, due to a stronger uptake of ATP connected to CO_2 assimilation, the appearance of the M-peak should be more pronounced under non-photorespiratory conditions. The M-peak appeared in FIC measured under high CO_2 for both LI and HI plants and its manifestation was sharply pronounced for HI plants (Fig. 3). This indicates that ATP consumption was more stimulated for HI than LI plants and corresponds also to an enormous increase of oxygen evolution in HI plants exposed to high CO_2 as compared to P measured under ambient CO_2 concentration (Fig. 1). However, the ETR and q_P measured under non-photorespiratory conditions did not significantly differ from those obtained under ambient CO_2. Thus, we suppose that for the barley growing under ambient CO_2 the strongly increased efficiency of non-assimilatory electron transport pathways may be the dominant feature of functional adaptation of photosynthetic apparatus to extremely high irradiance. We are aware that the more detailed analysis of contribution of photorespiration and/or other processes consuming oxygen is needed in order to get precise information about the efficiency of this electron sinks for HI adapted barley.

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