PHOTOSYNTHETICA 41 (2): 209-219, 2003

Response of photosynthetic apparatus of spring barley (*Hordeum vulgare* L.) to combined effect of elevated CO_2 concentration and different growth irradiance

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Abstract

The response of barley (*Hordeum vulgare* L. cv. Akcent) to various photosynthetic photon flux densities (PPFDs) and elevated [CO_2] [700 μmol(CO_2) mol^{-1}; EC] was studied by gas exchange, chlorophyll (Chl) *a* fluorescence, and pigment analysis. In comparison with barley grown under ambient [CO_2] [350 μmol(CO_2) mol^{-1}; AC] the EC acclimation resulted in a decrease in photosynthetic capacity, reduced stomatal conductance, and decreased total Chl content. The extent of acclimation depression of photosynthesis, the most pronounced for the plants grown at 730 μmol m^{-2} s^{-1} ($PPFD_{730}$), may be related to the degree of sink-limitation. The increased non-radiative dissipation of absorbed photon energy for all EC plants corresponded to the higher de-epoxidation state of xanthophylls only for $PPFD_{730}$ barley. Further, a pronounced decrease in photosystem 2 (PS2) photochemical efficiency (given as F_V/F_M) for EC plants grown at 730 and 1 200 μmol m^{-2} s^{-1} in comparison with AC barley was related to the reduced epoxidation of antheraxanthin and zeaxanthin back to violaxanthin in darkness. Thus the EC conditions sensitise the photosynthetic apparatus of high-irradiance acclimated barley plants (particularly $PPFD_{730}$) to the photoinactivation of PS2.

Keywords: ambient and elevated CO_2 concentration; chlorophyll *a* fluorescence; high irradiance; photosynthesis; xanthophyll cycle.

Introduction

The impact of elevated [CO_2] on the photosynthetic apparatus of various plant species is one of the relevant topics in contemporary ecophysiology. The short-term elevation of [CO_2] stimulates net photosynthesis rate (P_N) in C_3 plants due to insufficient ambient [CO_2] [about 360 μmol(CO_2) mol^{-1}] to saturate ribulose-1,5-*bis*phosphate carboxylase/oxygenase (RuBPCO) activity and to inhibit photorespiration (Bowes 1991, Stitt 1991, Makino and Mae 1999). However, the stimulation of CO_2 assimilation may disappear after several days of exposure to elevated [CO_2] (Sage *et al.* 1989, Stitt 1991, Sicher *et al.* 1994, Osborne *et al.* 1998, Makino and Mae 1999). Effects of long-term (weeks to years) CO_2 elevation on photosynthesis are variable and mechanisms of down-regulation of the photosynthetic activity are still under debate. One hypothesis suggests the sink-limitation, when increased saccharide production associated with elevated [CO_2] exceeds the capacity to use and store saccharides (sink) (Stitt 1991, Sicher *et al.* 1994, Paul and Foyer 2001). Such loss of photosynthetic activity (so called

Received 5 February 2003, *accepted* 2 June 2003.
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Abbreviations: A – antheraxanthin; AC and EC – ambient and elevated CO_2 concentration [350 and 700 μmol(CO_2) mol^{-1}]; β-car – β-carotene; Car – carotenoid; Chl – chlorophyll; [CO_2] – CO_2 concentration; DEPS – de-epoxidation state of xanthophyll cycle pigments; FACE – free-air carbon enrichment; F_M, F_0, F_V – maximal, minimal, and variable fluorescence in dark-adapted state ($F_V = F_M - F_0$); F_V/F_M – maximal photochemical efficiency of photosystem 2; g_S – stomatal conductance; L – lutein; LHCs – the light-harvesting complexes; N – neoxanthin; NPQ – non-photochemical fluorescence quenching; NRD – non-radiative dissipation of absorbed excitation energy; PPFD(s) – photosynthetic photon flux density(ies); P_N – the steady-state rate of CO_2 assimilation; PS1 and PS2 – photosystems 1 and 2; RuBPCO – ribulose-1,5-*bis*phosphate carboxylase/oxygenase; SV_0 – non-photochemical fluorescence quenching originating in light-harvesting complexes; V – violaxanthin; VAZ – xanthophyll cycle pool – violaxanthin+antheraxanthin+zeaxanthin; Z – zeaxanthin.
Acknowledgements: We thank V. Kerlínová and J. Rychtárová for help with some measurements. This research was supported by the Grant Agency of the Czech Republic (522/00/1381) and by The Ministry of Education of the Czech Republic (LN00A141).

a cclimation depression of photosynthesis) has been associated with decreased activity and/or content of RuBPCO (Sage *et al.* 1989, Webber *et al.* 1994, Osborne *et al.* 1998), redistribution of nitrogen (Makino and Mae 1999), inhibition of photosynthetic genes (Stitt 1991, Osborne *et al.* 1998), *etc*.

Rather contradictory results of prolonged exposure to elevated [CO_2] were reported partially due to different experimental devices for cultivation (cabinets with controlled environment, branch bags, open-top chambers, and free-air carbon enrichment – FACE), various microclimatic conditions, or different growth stages of plants (Garcia *et al.* 1998, Hymus *et al.* 1999). The up-regulation of photosynthetic activity accompanied with increased photochemical energy usage mitigating photoinhibition of PS2 have been shown in a study on winter wheat grown for six weeks in elevated [CO_2] in controlled environment (Habash *et al.* 1995). The field study using FACE also confirmed prolonged stimulation of photosynthetic activity of spring wheat grown under 550 μmol(CO_2) mol^{-1} during two month (Garcia *et al.* 1998). On the other hand, in other field studies on cereals a marked acclimation depression of photosynthesis was reported (Tuba *et al.* 1994, Sicher and Bunce 1997). Sicher and Bunce (1997) observed a loss in photosynthetic activity in barley and winter wheat grown in open-top chambers at 700 μmol(CO_2) mol^{-1} within a two-year study. The acclimation depression of photosynthesis under elevated [CO_2] has been associated with decreased PS2 photochemical efficiency (Peterson 1991, Van Oosten and Besford 1995, Marek and Kalina 1996, Kalina *et al.* 2000) and enhanced PS2 sensitivity to high irradiance (Špunda *et al.* 1998, Hymus *et al.* 1999). These results are in agreement with findings of Hymus *et al*. (2001) that elevated [CO_2] can either increase or decrease the sensitivity of PS2 photochemical activity to photoinhibition depending on whether up- or down-regulation of CO_2 assimilation occurs. The opposite response of sun and shade acclimated plants to elevated [CO_2] regarding the depression or stimulation of photosynthetic activity was shown for Norway spruce (Kalina *et al.* 2001). The utilisation of photon energy within PS2 and PS1 occurs in the dynamic balance with ATP and NADPH uptake in Calvin cycle. Therefore, the acclimation of photosynthetic apparatus to elevated [CO_2] may strongly depend on irradiation. The plant acclimation to high PPFD itself lies in increased amount of components of electron transport chain and/or Calvin cycle enzymes resulting in higher photosynthetic activity and in reduction of light-harvesting function (Anderson 1986, Gray *et al.* 1996, Logan *et al.* 1996, Melis 1998). The additional response to high PPFD is the increased ability to harmlessly dissipate absorbed excitation energy within the light-harvesting complexes (LHCs) in a process involving the xanthophyll cycle. The increased capacity of non-radiative dissipation (NRD) of absorbed excitation energy is associated with higher amount of xanthophyll cycle pigments and enhanced convertibility of violaxanthin to zeaxanthin and antheraxanthin (Thayer and Björkman 1990, Demmig-Adams and Adams 1996, Adams *et al.* 1999).

Despite the extensive literature on the response of plants either to elevated [CO_2] or different PPFDs alone, little is known about the concurrent effect of CO_2 enrichment and various PPFDs on plants under controlled environment (growth chamber). Hence, in the present study we focused on response of barley photosynthetic apparatus to elevated and ambient [CO_2] together with different growth PPFDs. The main aims of our study were as follows. (*1*) To establish if the acclimation depression of photosynthesis will occur for barley after a week of cultivation under elevated [CO_2] inside the growth chamber. (*2*) To explain the expected different responses to elevated [CO_2] for the barley grown under the different PPFDs. (*3*) To characterise effect of elevated [CO_2] on pigment content, non-radiative dissipation of absorbed excitation energy, and its relationship with the xanthophyll cycle. (*4*) Finally, to support or refute the hypothesis that a direct synergetic effect of high PPFD and elevated [CO_2] enhances PS2 susceptibility to photoinactivation.

Materials and methods

Plants: The seeds of spring barley (*Hordeum vulgare* L. cv. Akcent) were planted in 4 500 cm^3 pots (100 seeds per pot) in soil substrate (*AGRO CS*, Czech Republic) with defined mineral nutrition (each pot contained 1.2 g of ammonia and nitrate nitrogen, 1.1 g P_2O_5, and 1.9 g K_2O). Plants were grown under controlled environment inside commercially delivered growth chamber (*HB 1014 Bioline-Heraeus*, Germany) at constant temperature of 20 °C, constant 65 % relative humidity, and 16/8 h day/night regime. They were daily watered with de-ionised water. The measurements were carried out on barley grown under a low (100 μmol m^{-2} s^{-1}), high (730 μmol m^{-2} s^{-1}), and extremely high (1 200 μmol m^{-2} s^{-1}) PPFD, *i.e*. $PPFD_{100}$, $PPFD_{730}$, $PPFD_{1200}$, respectively. PPFD was estimated at pot height. The required irradiance with the same shape of spectra for low, high, and extremely high PPFD was achieved by combination of xenon (*Powerstar HQI-T 400 W/D daylight*; *Osram*, Germany) and krypton (*Krypton 100 W LO*; Hungary) lamps mounted inside growth chamber. Each PPFD treatment was applied on barley plants divided into seven pots placed in the chamber for 9 d from seed sowing. The ambient [350 μmol(CO_2) mol^{-1}; AC] or elevated [700 μmol(CO_2) mol^{-1}; EC] CO_2 concentration inside the growth chamber during the plant cultivation was controlled by infrared CO_2/H_2O analyser (*LI–6262*, *LI-COR*, USA). The primary leaves of 8-9 d old plants were used for all measurements. The plants were randomly selected for measurements of gas

exchange, chlorophyll (Chl) *a* fluorescence, and pigment analysis. By the end of the experiment the roots had not fully explored the entire soil volume, and thus influence of root restriction was not considered in this study.

Gas exchange: The measurements of CO_2 assimilation were carried out with an open portable photosynthetic system (*CIRAS-1*, *PP Systems*, UK) with infrared gas analyser. The steady-state values of P_N [μmol(CO_2) m^{-2} s^{-1}] were determined on barley leaf segments (3 cm^2) at PPFD and [CO_2] used for plant growth. The irradiance-saturated rates of CO_2 assimilation (P_{Nmax}) were established at PPFD of 1 500 μmol m^{-2} s^{-1} and given growth [CO_2]. The maximal CO_2 assimilation capacity (P_{Nsat}) was measured at both saturating [CO_2] [1 300 μmol(CO_2) mol^{-1}] and saturating PPFD [1 500 μmol m^{-2} s^{-1}]. The ability of this PPFD and [CO_2] to saturate CO_2 assimilation capacity was tested in a previous experiment (values not shown). The steady-state CO_2 assimilation rates were recorded at maximal stomatal conductivity approximately after 8-10 min of exposure to a given PPFD. Corresponding stomatal conductances [g_S, g_{Smax}, and g_{Ssat}; mmol(CO_2) m^{-2} s^{-1}] were calculated from leaf transpiration estimated as the rate of water vapour changes. The microclimatic conditions inside the assimilation chamber were kept constant during all the measurements (temperature of leaves 23±2.2 °C, relative air humidity 55±3 %).

Chlorophyll (Chl) fluorescence at room temperature was measured using a *PAM 101, 103* fluorometer (*H. Walz*, Effeltrich, Germany) on barley leaf segment (1.5 cm^2). The estimation of steady-state fluorescence parameters was performed under AC as well as saturating [CO_2], both at growth PPFD and high PPFD (1 900 μmol m^{-2} s^{-1}). The initial (F_0) and maximal (F_M) fluorescence levels were measured on the dark-adapted (1–8 h) leaf segments and the PS2 photochemical efficiency was calculated as $F_V/F_M = (F_M - F_0)/F_M$. Then, the given actinic PPFD was applied for 10 min. The saturation pulses of 1 s duration and incident PPFD approx. 5 000 μmol m^{-2} s^{-1} were optimal to induce the F_M' level. To estimate the value of F_0' the actinic PPFD was switched off for 3 s and the lowest fluorescence level during this period was regarded as F_0'. The following Chl *a* fluorescence parameters were calculated from F_0' and F_M' values measured in steady state at given PPFD (8–10 min): non-photochemical quenching of F_M ($NPQ = F_M/F_M' - 1$) and F_0 ($SV_0 = F_0/F_0' - 1$) (Bilger and Björkman 1990).

Pigment analysis: Plant pigments from frozen (at liquid nitrogen) dark-adapted (1-h) barley leaf segments (3 cm^2) were extracted with 80 % acetone and a small amount of $MgCO_3$. The spectrophotometric (*UV/VIS 550*, *Unicam*, England) estimation of the pigment contents (Chl *a*, Chl *b*, and total carotenoids, Cars) according to Lichtenthaler (1987) and isocratic reversed-phase HPLC quantification of individual Cars were described previously (Kurasová *et al.* 2002). The de-epoxidation state of xanthophyll cycle pigments was estimated on 1-h dark-adapted leaf segments ($DEPS_{dark}$), on segments exposed for 10 min to actual growth PPFD ($DEPS_{act}$), and on segments exposed for 10 min to high PPFD of 1 900 μmol m^{-2} s^{-1} ($DEPS_{sat}$), respectively. The de-epoxidation state at mentioned experimental conditions was calculated according to Adams *et al*. (1999) as DEPS = [Z+A]/[V+A+Z], where Z, A, and V indicate zeaxanthin, antheraxanthin, and violaxanthin, respectively.

Statistical analysis: To test the effect of growth AC and EC on pigment content, Chl *a* fluorescence, and gas exchange parameters a two-sample F-test for variances followed by a *t*-test were used. Based on the result of the F-test a *t*-test was performed assuming either equal variances or unequal variances. A sample size of 8-10 per treatment was used for statistical analyses. The levels of significance 0.05, 0.01, and 0.001 were indicated as *, **, and ***. All statistical tests were performed using analysis tools from *Microsoft® Excel 97 SR-2*.

Results

Chl and Car contents: The decline of Chl *a*+*b* and Car *x*+*c* amounts estimated per dry matter was observed with increasing growth PPFD in AC conditions (Table 1). The AC barley plants grown under 1 200 PPFD exhibited reduced Chl *a*+*b* content by 62 % as compared with plants grown under 100 PPFD, and similar reduction (57 %) was observed for EC barley. Some studies concerning pigment analysis under EC have shown a decrease of the total Chl content (DeLucia *et al.* 1985, Sage *et al.* 1989, Špunda *et al.* 1998, Kalina *et al.* 2001). In agreement, the barley acclimation to EC conditions resulted in reduction of total Chls and Cars expressed both per dry matter (Table 1) and leaf area (values not shown) as compared with AC plants under corresponding PPFDs. The mentioned reduction of Chl *a*+*b* content per dry matter amounted 21 % ($PPFD_{100}$), 50 % ($PPFD_{730}$), and 12 % ($PPFD_{1200}$). In addition, if compared with AC, the EC barley exhibited also lower Chl *a*+*b* to Car *x*+*c* ratio at all growth PPFDs (Table 1). The extent of Chl *a*/*b* increase observed both under AC and EC conditions corresponded with typical acclimation response of barley to increasing irradiances (Čajánek *et al.* 1999, Kurasová *et al.* 2000, 2002).

Car composition: No effect of EC conditions on contents of neoxanthin (N) and xanthophyll cycle pigments (VAZ)

was observed for $PPFD_{100}$ plants, whereas content of lutein (L) was slightly increased and content of β-carotene was 68 % higher in comparison with AC plants (Table 2). The acclimation of AC barley to increasing growth PPFD evoked no pronounced differences in N and L (Kurasová *et al.* 2002). On the contrary, EC cultivation led to slight decline of N in plants with increasing growth PPFD, whereas L significantly increased and its content was by 6, 11, and 33 % higher if compared with AC plants grown under 100, 730, and 1 200 PPFD, respectively (Table 2). We observed more than threefold increase in VAZ pool for AC plants grown under 1 200 PPFD in comparison with those cultivated under 100 PPFD, and the same quantitative change was also observed for EC plants (Table 2). Thus, HPLC analysis confirmed that increased relative content of Cars to Chls for EC barley plants (Table 1) was related particularly to the accumulation of β-carotene (at low PPFD) and L (at high PPFD).

Table 1. The pigment contents in barley plants grown under different PPFDs (100, 730, and 1 200 μmol m^{-2} s^{-1}) and ambient [350 μmol(CO_2) mol^{-1}; AC] or elevated [700 μmol(CO_2) mol^{-1}; EC] CO_2 concentration. The total chlorophyll (Chl *a*+*b*) and carotenoid (Car *x*+*c*) contents per dry matter, Chl *a*/*b*, and Chl *a*+*b*/Car *x*+*c* ratios were estimated on dark-adapted plants. The mean values (*n*) from ten measurements ± standard deviations (SD) are given. The statistical differences between conditions of AC and EC at corresponding growth PPFD are presented by symbols *, **, ***, and ns (in upper index) which indicate differences at 0.05, 0.01, 0.001 levels of significance and non-significant difference, respectively.

		Growth PPFD [μmol m^{-2} s^{-1}]		
		100	730	1 200
Chl *a*+*b* [g kg^{-1}]	AC	$17.25 \pm 0.93^{**}$	$11.14 \pm 0.88^{***}$	$6.58 \pm 0.29^{*}$
	EC	13.65 ± 1.55	5.59 ± 0.44	5.81 ± 1.11
Car *x*+*c* [g kg^{-1}]	AC	$3.14 \pm 0.29^{*}$	$2.41 \pm 0.17^{***}$	1.98 ± 0.12^{ns}
	EC	2.76 ± 0.32	1.51 ± 0.58	1.86 ± 0.32
Chl *a*+*b*/Car *x*+*c*	AC	$5.52 \pm 0.27^{***}$	$4.63 \pm 0.38^{***}$	$3.33 \pm 0.23^{*}$
	EC	4.96 ± 0.11	3.69 ± 0.09	3.12 ± 0.13
Chl *a*/*b*	AC	3.14 ± 0.03^{ns}	3.43 ± 0.06^{ns}	$3.97 \pm 0.10^{*}$
	EC	3.17 ± 0.04	3.40 ± 0.07	3.72 ± 0.21

Table 2. The composition of carotenoids in barley plants grown under different PPFDs (100, 730, and 1 200 μmol m^{-2} s^{-1}) and ambient (AC) or elevated (EC) CO_2 concentration. The carotenoid contents [mmol mol^{-1}(Chl)] expressed on total chlorophyll (Chl *a*+*b*) basis were estimated by HPLC analysis on dark-adapted plants. N – neoxanthin, L – lutein, β-car – β-carotene, and VAZ – sum of violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z). The statistical differences are presented by the same way as in Table 1. $n = 10 \pm$ SD.

		Growth PPFD [μmol m^{-2} s^{-1}]		
		100	730	1 200
N	AC	47.5 ± 1.3^{ns}	$48.3 \pm 2.6^{***}$	39.0 ± 5.3^{ns}
	EC	46.3 ± 1.0	39.6 ± 2.8	37.9 ± 1.7
L	AC	$176.8 \pm 2.8^{***}$	$189.5 \pm 5.1^{**}$	$181.4 \pm 9.1^{***}$
	EC	187.3 ± 2.9	211.9 ± 12.7	240.8 ± 12.9
β-car	AC	$78.3 \pm 13.7^{***}$	149.3 ± 12.8^{ns}	139.5 ± 33.8^{ns}
	EC	131.3 ± 9.4	143.2 ± 11.0	170.7 ± 6.6
VAZ	AC	59.9 ± 2.5^{ns}	$97.1 \pm 7.3^{**}$	183.7 ± 18.0^{ns}
	EC	60.3 ± 4.6	116.6 ± 11.8	188.5 ± 17.1

De-epoxidation of the xanthophyll cycle pigments: A characteristic enhancement of de-epoxidation of xanthophylls (estimated on barley leaf segments 10 min exposed to actual growth PPFD – $DEPS_{act}$), with increasing growth PPFD is shown in Fig. 1*A*. The lowest extent of V de-epoxidation (only 25 %) was established for AC plants grown under 100 PPFD, whereas 730 and 1 200 PPFD induced almost 2.5 and 3 times higher $DEPS_{act}$. The EC plants revealed a quantitatively similar increase of $DEPS_{act}$, but extent of de-epoxidation was slightly higher in comparison with AC treatment at corresponding PPFDs (Fig. 1*A*). The maximal convertibility of V was estimated on plants shortly (10 min) exposed to PPFD of 1 900 μmol m^{-2} s^{-1} ($DEPS_{sat}$; Fig. 1*B*) that was sufficient to saturate V de-epoxidation (values not shown). Under both EC and AC conditions the lowest $DEPS_{sat}$ values were observed for the $PPFD_{100}$ plants, whereas the highest V convertibility ($DEPS_{sat}$ = 0.80 and 0.83) was achieved in $PPFD_{1200}$ plants. A significantly increased $DEPS_{sat}$ under EC conditions was established only for $PPFD_{730}$ plants (Fig. 1*B*), in agreement with the fact that the EC cultivation induced an enlargement of VAZ pool in comparison with AC plants only at this growth PPFD (Table 2). If the plants grow under optimal PPFD almost all Z+A is readily epoxidised back to V under the following period of darkness. However, high-irradiance stress may considerably slow-down the epoxidation to hours or even days (Jahns and Miehe 1996, Demmig-Adams *et al.* 1998). As shown in Fig. 1*C*, an enhancement of Z+A amount remaining after 1-h of darkness ($DEPS_{dark}$) upon increasing growth PPFD was observed both under AC and EC conditions. The $PPFD_{100}$ barley was characterised by only negligible residual Z+A amount in darkness ($DEPS_{dark}$ = 0.02 under AC, and 0.04 under EC). On the contrary, extremely high $DEPS_{dark}$ (0.59) was obtained

for EC barley grown under 1 200 PPFD (Fig. 1*C*). Further, the strikingly slowed-down epoxidation of Z+A during the dark period was observed mainly for EC barley. The most pronounced enhancement of $DEPS_{dark}$ occurred again for $PPFD_{730}$ plants, when a 3.5-fold higher de-epoxidation state was evoked by EC compared to AC conditions (Fig. 1*C*).

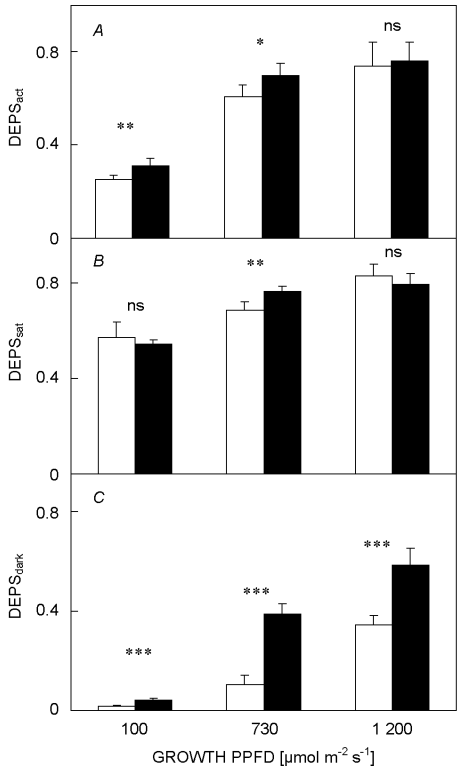

Fig. 1. The de-epoxidation state of the xanthophylls (DEPS) expressed as [Z+A]/[V+A+Z] for barley grown under different PPFDs (100, 730, and 1 200 µmol m^{-2} s^{-1}) and ambient [350 µmol(CO_2) mol^{-1}; *empty bars*] or elevated [700 µmol(CO_2) mol^{-1}; *full bars*] CO_2 concentration. The DEPS was estimated on barley plants exposed for 10 min to actual growth PPFD ($DEPS_{act}$; *A*), for 10 min to high PPFD of 1 900 µmol m^{-2} s^{-1} ($DEPS_{sat}$; *B*), and on dark-adapted (1-h) barley ($DEPS_{dark}$; *C*). The statistical differences between conditions of ambient and elevated [CO_2] at corresponding growth PPFD are marked *, **, ***, and ns for 0.05, 0.01, 0.001 levels of significance and non-significant difference, respectively. $n = 10 \pm$ SD.

The CO_2 assimilation and stomatal conductance: The net CO_2 assimilation per leaf area (P_N) was determined at actual growth PPFD and actual growth [CO_2] (Fig. 2*A*). Under AC the highest P_N value was observed at 730 PPFD, whereas it was reduced by 32 % at 1 200 PPFD as a result of photoinhibition (Kurasová *et al.* 2002). P_N values were slightly lower in EC plants grown under low (by 14 %) and extremely high (by 9 %) PPFD compared to AC plants. On the contrary, elevation of growth [CO_2] caused P_N suppression by 58 % in comparison with the AC conditions for cultivation at 730 PPFD. Similarly to the results of P_N, 50 % reduction of irradiance-saturated rate of CO_2 assimilation (P_{Nmax}) occurred at 730 PPFD (Fig. 2*B*). The $PPFD_{730}$ and $PPFD_{1200}$ AC plants revealed assimilation capacity (P_{Nsat}) higher by 77 or 65 % compared to those grown under 100 PPFD (Fig. 2*C*) as a typical acclimation response to high PPFD. On the contrary, the EC plants lost the ability to significantly enhance assimilation capacity at high growth PPFDs (Fig. 2*C*). Moreover, the EC conditions induced pronounced depression of P_{Nsat} as compared with AC plants at all PPFDs, but the most pronounced effect of long-term EC characterised by almost three-time lower P_{Nsat} values was observed again in $PPFD_{730}$ barley.

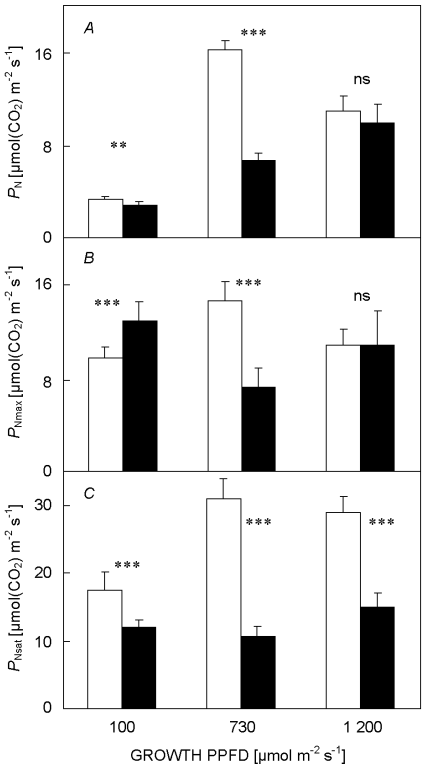

Fig. 2. The rates of CO_2 assimilation (P_N) expressed per leaf area on barley grown under 100, 730, and 1 200 PPFD and ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. *A*: The actual P_N at corresponding growth PPFD and growth [CO_2]. *B*: The rates of irradiance-saturated photosynthesis, P_{Nmax} (1 500 µmol m^{-2} s^{-1}) and actual growth [CO_2]. *C*: The capacity of photosynthesis at saturating irradiance and saturating [CO_2] [1 300 µmol(CO_2) mol^{-1}], P_{Nsat}. The statistical differences are presented by the same way as in Fig. 1. $n = 8 \pm$ SD.

The different extent of reduction of photosynthesis under EC (Fig. 2) is supported by g_S measurements (Fig. 3). A typical response of g_S to growth PPFDs was observed under AC cultivation, where the highest g_S was observed at 730 PPFD (Fig. 3*A*). Significantly lower g_S by 65 and 45 % at 100 and 1 200 PPFD corresponded to low growth irradiance and to photoinhibition, respectively, in agreement with the P_N changes (Fig. 2*A*). The

saturating PPFD evoked very similar values of g_{Smax} for AC plants grown under 100 and 730 PPFD (Fig. 3*B*). By contrast, markedly lower g_{Smax} was estimated for the highest growth PPFD. The EC treatment pronouncedly reduced g_S at all growth PPFDs, but the most pronounced reduction was again observed under 730 PPFD (Fig. 3). The reduction of g_S and g_{Smax} (Fig. 3*B,C*) for EC plants was 86 % compared to the AC barley grown under 730 PPFD. The AC plants sensed the exposure to saturating irradiance and [CO_2] reducing g_S by 48 % ($PPFD_{100}$, $PPFD_{730}$) and 43 % ($PPFD_{1200}$) if we compare g_{Ssat} and g_{Smax} values (Fig. 3*B,C*). Under EC the response of stomata to saturating [CO_2] was not considerably changed at $PPFD_{100}$ in comparison with AC conditions (g_{Ssat} was by 51 % lower than g_{Smax}). On the contrary, for $PPFD_{730}$ plants the g_{Ssat} was only by 16 % lower than g_{Smax} that indicated the most pronounced reduction of stomata reactivity.

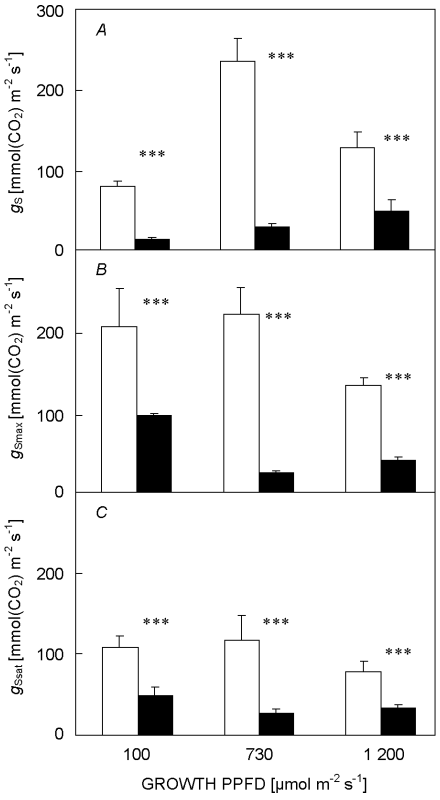

Fig. 3. The stomatal conductance for barley grown under 100, 730, and 1 200 PPFD and ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. *A*: The actual stomatal conductance (g_S) at corresponding growth PPFD and growth [CO_2]. *B*: g_{Smax} was estimated under saturating PPFD (1 500 µmol m^{-2} s^{-1}) and actual growth [CO_2]. *C*: Stomatal conductance at saturating irradiance and saturating [CO_2], g_{Ssat}. The differences between stomatal conductances at ambient and elevated [CO_2] at corresponding growth PPFD were significant at level of 0.001 in all cases. $n = 8 \pm$ SD.

Photochemical efficiency of PS2 and non-radiative energy dissipation: The concurrent effect of high PPFDs (730 or 1 200 µmol m^{-2} s^{-1}) and EC treatment caused pronounced decrease of PS2 photochemical efficiency measured on dark-adapted plants (F_V/F_M; Fig. 4). As shown, non-significant difference between F_V/F_M values was observed for barley grown under AC and EC at 100 PPFD. In addition, these values of F_V/F_M (0.818 for AC and 0.816 for EC) were close to optimum (0.831±0.004) that was estimated for many healthy plant species

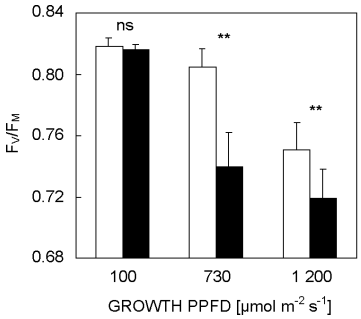

Fig. 4. The dependence of the photochemical efficiency of PS2 (F_V/F_M) of dark-adapted barley on growth PPFD. The barley plants were grown under ambient (*empty bars*) and elevated (*full bars*) CO_2 concentration. The statistical differences are presented by the same way as in Fig. 1. $n = 20 \pm$ SD.

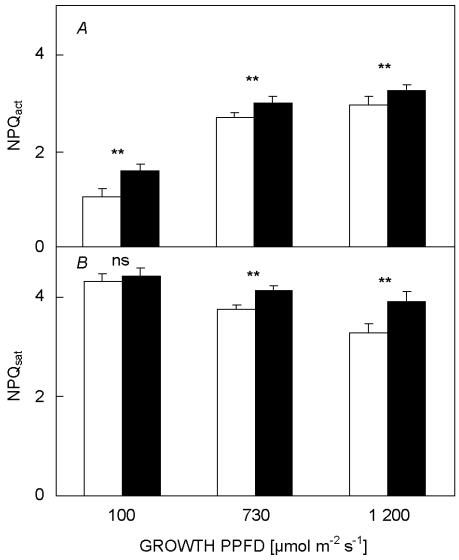

Fig. 5. The non-photochemical quenching of maximal (F_M) chlorophyll *a* fluorescence characterising extent of non-radiative dissipation of absorbed excitation energy in photosynthetic apparatus of barley grown under ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. *A*: NPQ_{act} was estimated on barley leaves shortly (10 min) exposed to actual growth PPFD. *B*: NPQ_{sat} estimated after 10-min exposure to high PPFD (1 900 µmol m^{-2} s^{-1}). The statistical differences are presented by the same way as in Fig. 1. $n = 9 \pm$ SD.

growing under physiological conditions (Björkman and Demmig 1987). The plants grown under high PPFD were characterised by gradual suppression of their photochemical efficiency. However, in EC plants the F_V/F_M was significantly lowered in comparison with AC plants, particularly at 730 PPFD (Fig. 4). Due to fact that stable F_V/F_M values were estimated during 1-8 h of dark adaptation, the observed F_V/F_M decrease under EC and 730 or 1 200 PPFD was associated with mostly irreversible PS2 photoinactivation. Thus, the acclimation to EC sensitises photosynthetic apparatus of barley plants grown at high PPFD to PS2 photoinactivation.

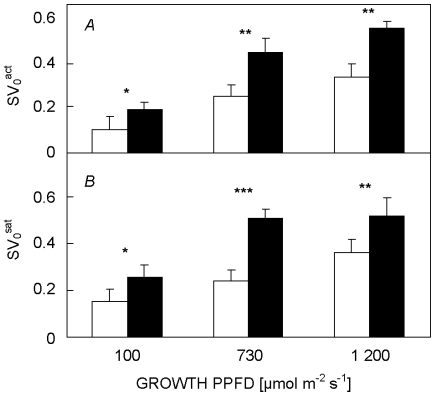

Fig. 6. The non-photochemical quenching of minimal (F_0) chlorophyll *a* fluorescence characterising extent of non-radiative dissipation of absorbed excitation energy originating in light-harvesting complexes and measured in barley grown under ambient (*empty bars*) or elevated (*full bars*) CO_2 concentration. *A*: SV_0^{act} was estimated on barley leaves shortly (10 min) exposed to actual growth PPFD. *B*: SV_0^{sat} was estimated after 10-min exposure to high PPFD (1 900 µmol m^{-2} s^{-1}). The statistical differences are presented by the same way as in Fig. 1. $n = 9 \pm$ SD.

The decreased utilisation of absorbed photons in PS2 photochemical reactions (Fig. 4) associated with acclimation depression of photosynthesis detected for EC plants (Fig. 2) induced enhancement of total non-radiative dissipation (NRD) of absorbed energy (determined as NPQ; Fig. 5) and that originating within LHCs (SV_0; Fig. 6). If NRD was estimated at actual growth PPFDs (NPQ_{act}; Fig. 5*A*) a typical NPQ increase in dependence on growth PPFD was observed for AC and EC plants, too. These growth conditions already caused significantly greater extent of NRD in EC barley compared with AC plants. To invoke a maximal NRD the plants were shortly exposed to PPFD of 1 900 µmol m^{-2} s^{-1} (NPQ_{sat}; Fig. 5*B*). The high PPFD induced again a larger NRD in EC than AC plants, but now only for 730 and 1 200 PPFDs. As presented at Fig. 5*B*, the decline of NPQ_{sat} was observed with increasing growth PPFD in both AC and EC plants. Thus in plants acclimated to low PPFD the fluorescence quenching induced within LHCs (SV_0) contributes only restrictively to total NRD (compare Figs. 5*B* and 6*B*), whereas the growth under high PPFDs is accompanied with significant increase of SV_0 (Fig. 6*A*).

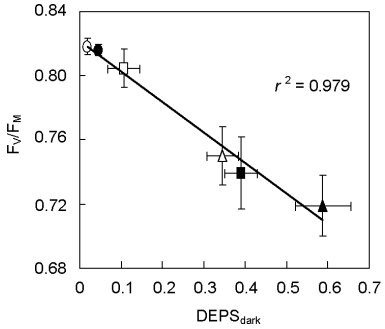

Fig. 7. The correlation between photochemical efficiency of PS2 of dark-adapted barley leaf segments (F_V/F_M) and conversion-state of xanthophylls estimated after 1-h of darkness ($DEPS_{dark}$). Values were obtained from Figs. 4 and 1*C* for barley grown under 100 (*circles*), 730 (*squares*), and 1 200 (*triangles*) PPFD together with ambient (*empty symbols*) or elevated (*full symbols*) CO_2 concentration. The linear regression of mean values is presented.

The higher extent of NRD originating within LHCs for EC plants was estimated from increased F_0 quenching both under actual growth PPFDs (SV_0^{act}; Fig. 6*A*) and high PPFD (SV_0^{sat}; Fig. 6*B*) for all barley plants. The most pronounced difference was observed for EC barley grown under 730 PPFD for which SV_0^{act} and SV_0^{sat} were higher by 69 and 120 % than for AC plants.

Discussion

The extent of acclimation depression of photosynthesis under EC: A suppression of photosynthetic activity often occurs after initial stimulation by CO_2 enrichment if there is long-term exposure of plants to elevated [CO_2] (Bowes 1991, Stitt 1991, Sicher *et al.* 1994, Osborne *et al.* 1998, Makino and Mae 1999). The comparative studies have shown that down-regulation of photosynthesis varies with plant species as well as environmental aspects (Sage *et al.* 1989, Luo *et al.* 1994). Recently, we found that sun exposed needles of Norway spruce showed a slight acclimation depression of photosynthesis after three seasons of cultivation under EC, whereas shade needles in the lower crown layer maintained stimulation of CO_2 assimilation (Kalina *et al.* 2001). The qualitatively same results were obtained in continuing experiments during the next vegetation season (Marek *et al.* 2002). We suppose that the acclimation depression of sun type assimilatory apparatus may be caused either by direct synergetic effect of high PPFD and EC or by different degree of sink-limitation. In the present paper, the stimulation of

CO_2 assimilation did not occur for barley even under low growth PPFD (100 μmol m^{-2} s^{-1}) and EC. These plants showed significantly lower P_N (Fig. 2*A*) and CO_2 assimilation capacity lowered by 31 % (P_{Nsat}; Fig. 2*C*) in comparison with AC plants. A similar effect of EC cultivation on P_{Nsat} was found at 1 200 PPFD, but P_{Nsat} was almost twice reduced in comparison with AC plants. Surprisingly, the most pronounced acclimation depression of photosynthesis was observed for the EC barley cultivated under 730 PPFD, wherein P_{Nsat} was almost three times lower compared with AC plants. Thus the extent of acclimation depression of photosynthesis for EC barley is not directly connected with synergetic effect of high PPFD and EC, but is rather proportional to the degree of sink-limitation. Acclimation pattern of assimilation/ intercellular CO_2 response curves (P_N/C_i; values not shown) supported strictly sink-limited photosynthesis (Webber *et al.* 1994) for all growth PPFDs and EC. The highest decrease of RuBPCO activity and ribulose-1,5-*bis*phosphate regeneration under EC cultivation occurred at $PPFD_{730}$ as judged from suppressed maximal rates of carboxylation and electron transport (calculated according to Farquhar *et al.* 1980; values not shown).

Based on these results, we can formulate the following hypothesis for strictly sink-limited species: The strongest down-regulation of photosynthesis develops in plants characterised by maximal assimilate production under AC conditions. For these plants the exposure to EC quickly depletes the sink capacity. In present study, the AC plants grown under 730 PPFD exhibited the highest P_N, P_{Nmax}, and P_{Nsat} values if compared with $PPFD_{100}$ or $PPFD_{1200}$ plants (Fig. 2). Thus, a suggested dependence of sink-limitation on the CO_2 assimilation activity of plants grown under AC explains a significantly more pronounced depression of photosynthesis under CO_2 enrichment for the plants grown at $PPFD_{730}$ than $PPFD_{1200}$.

Whereas short-term CO_2 elevation results in a prompt reversible reduction of g_S (Jarvis *et al.* 1999), the long-term excess of CO_2 supply over that used in Calvin cycle may induce changes in stomata number and/or size associated with permanent g_S reduction (Morison 1998, Bunce 2000). The observed g_S decline was much more pronounced than corresponds to short-term elevation of [CO_2] (Fig. 3). Further, the reduction of g_{Smax} and g_{Ssat} was the most marked for $PPFD_{730}$, less pronounced for $PPFD_{1200}$ plants with the least effect under the lowest PPFD. That is in direct relation to degree of acclimation depression of photosynthetic capacity (P_{Nsat}; Fig. 2*C*) and supports the different degree of missing sink for intercellular carbon for the plants cultivated under EC and different PPFDs.

In agreement with the fact that the extent of acclimation depression of photosynthesis is dependent on the plant ability to use additional saccharides (Arp 1991, Luo *et al.* 1994), some plant species, *e.g.* spring barley, bean (Sage *et al.* 1989), or cotton (DeLucia *et al.* 1985), accumulate a great deal of saccharides in the leaves and incline to down-regulation of photosynthesis. The suggested reason of acclimation depression of photosynthesis is in agreement with results of Hibberd *et al.* (1996) who reported a greater accumulation of saccharides in barley leaves grown under elevated than ambient [CO_2] accompanied with decline of the carboxylation efficiency. In our experiment the greater accumulation of assimilates for EC plants was indirectly confirmed from reduction of total Chl content per dry matter in comparison with AC plants (Table 1) that was the strongest under 730 PPFD. This decrease of Chl content was not due to diminution of LHC size as judged from the same Chl *a*/*b* ratio for EC and AC plants at corresponding growth PPFDs (Table 1). Therefore, the reduction of Chl content confirmed that the amount of excessive accumulation of assimilates in EC barley leaves was related to sink depletion rate. The performed growth analysis also proved earlier growth and expansion of second and third barley leaf under EC and high PPFD treatment relative to AC plants. The extremely rapid appearance of the third leaf for EC barley grown under 730 PPFD already 6-7 d after sowing may be related to maintaining sink strength to use photosynthates. CO_2 enrichment (Makino and Mae 1999) and thus saccharide accumulation (Paul and Foyer 2001) in leaves can lead to accelerated leaf senescence. Hence, both the reduced Chl content and increased relative amount of Cars (Table 1), particularly of β-carotene and L (Table 2), seem to be in accordance with earlier ageing of primary leaves under EC conditions.

The response of barley to EC revealed more pronounced manifestation of acclimation depression of photosynthesis in our study than in field studies on cereals (Sicher and Bunce 1997) and Norway spruce (Marek and Kalina 1996, Kalina *et al.* 2000) or in a FACE study with wheat (Garcia *et al.* 1998). This fact can be explained by a higher daily dose of photosynthetically active radiation for barley plants inside growth chamber, where a given PPFD is applied for the duration of 16 h. By contrast, in field conditions with diurnal PPFD changes the exposure of plants to high incident PPFD occurs maximally during a few hours at noon.

The feedback induced alteration of PS2 function under EC: The utilisation of PPFD in photochemical processes occurs in a dynamic equilibrium with consumption of products of the primary photochemical reactions in Calvin cycle (Melis 1998). Hence, the decline of photosynthetic activity reduces utilisation of PPFD in photochemical reactions and enhances demand on the other de-excitation processes, particularly on non-radiative dissipation of absorbed radiant energy (NRD). The increased NRD capacity is one of the general features of acclimation to high PPFD and close relationship between NRD and de-epoxidation of the xanthophylls and/or VAZ pool is usually observed (Thayer and Björkman 1990, Demmig-Adams and Adams 1996, Logan *et al.* 1996,

Melis 1998, Adams *et al.* 1999). Accordingly, the increase of growth PPFD evoked a typical enhancement of de-epoxidation state of the xanthophylls and corresponding enlargement of NRD within PS2 estimated as NPQ_{act} and SV_0^{act} (compare Figs. 1*A*, 5*A*, and 6*A*). The enhanced demand on NRD for EC barley at all growth PPFDs in comparison with AC grown plants (Figs. 5*A* and 6*A*) is in qualitative agreement with decreased CO_2 assimilation (Fig. 2*A*) and enhanced de-epoxidation of xanthophylls (Fig. 1*A*). The significantly higher extent of the irradiance-saturated de-epoxidation of V (V convertibility) for EC barley in comparison with AC plants observed only at 730 PPFD (Fig. 1*B*) corresponds to significant enlargement of VAZ pool under EC conditions again only for 730 PPFD (Table 2). As mentioned, the greatest acclimation depression of photosynthesis under EC conditions occurred under this growth PPFD (Fig. 2). The strongly decreased photochemical utilisation of photon energy in this case resulted in selective photoprotective response of xanthophylls closely connected with significantly enhanced capacity of NRD within LHCs (Fig. 6*B*).

The AC and EC barley responded to high growth PPFD with threefold higher VAZ pool (Table 2) and significantly increased amount of convertible V from 55 % (at 100 PPFD) to the 80 % at the highest growth PPFD (Fig. 1*B*). However, the positive correlation between V convertibility (Fig. 1*B*) and NPQ_{sat} (Fig. 5*B*) was not observed. According to Kurasová *et al.* (2002) the high NPQ for barley acclimated to low PPFD is related to the domination of Z+A-independent NRD originating in photoinactivated PS2 reaction centres. On the contrary, the increased capacity of NRD within LHCs (SV_0^{sat}) for both AC and EC plants grown under high PPFD (Fig. 6*B*) corresponds partially to the V convertibility (Fig. 1*B*). Therefore, for barley and those plant species, which are susceptible to rapid PS2 inactivation upon short-term exposure to PPFD saturating V de-epoxidation, NPQ is not a suitable probe for the Z+A-dependent NRD within LHCs, but rather SV_0 should be used.

In agreement with Jahns and Miehe (1996) and Demmig-Adams *et al.* (1998) the markedly slowed epoxidation of Z and A during the dark period was observed upon increased growth PPFD (Fig. 1*C*). However, a considerably higher $DEPS_{dark}$ was estimated under high growth PPFDs and EC conditions. For EC plants grown under 730 PPFD, 44 % of Z+A accumulated under the actual growth conditions was subjected to conversion back to V during 1-h of darkness. On the contrary, the AC barley showed epoxidation of 82 % of Z+A amount back to V after the same adaptation to darkness. The marked retention of Z+A for EC barley also closely correlates with permanent depression of PS2 efficiency (Fig. 4) and thus corresponds to enhanced PS2 sensitivity to high PPFD (730 and 1 200 μmol m^{-2} s^{-1}). In agreement with results of Demmig-Adams and Adams (1996) and Verhoeven *et al.* (1997) the negative linear relationship between the sustained de-epoxidation state of xanthophylls ($DEPS_{dark}$) and PS2 photochemical efficiency (F_V/F_M) was found for the barley grown under different PPFDs and both CO_2 concentrations (Fig. 7). As indicated, under EC conditions the permanent PS2 down-regulation depends on reduced epoxidation of xanthophylls in a quantitatively similar way as observed for the plants cultivated in ambient [CO_2].

We conclude that acclimation of spring barley to 700 μmol(CO_2) mol^{-1} sensitises the photosynthetic apparatus to high growth PPFDs. However, the direct synergetic effect of high growth PPFD and EC was not proved as the significantly greater influence on PS2 function was observed at 730 than 1 200 PPFD. We suggest that changes of the xanthophyll cycle and PS2 function under EC resulted from the feed-back limitation of the primary photochemical reactions that was proportional to the degree of acclimation depression of photosynthesis. The consistent response of barley to EC on the CO_2 assimilation efficiency, pigment composition, and PS2 function revealed more pronounced manifestation of acclimation depression of photosynthesis compared to that found previously (Marek and Kalina 1996, Sicher and Bunce 1997, Špunda *et al.* 1998). This supports the fact that even under optimal mineral nutrition, the photosynthesis of primary barley leaves can be strongly sink-limited and unable to use an additional carbon supply after a week of exposure to EC. The present study was not aimed at prediction of the long-term response of barley to EC during the whole vegetation period, but rather to better understand the range and nature of relatively fast response (after a week) of photosynthetic apparatus to different irradiance conditions.

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