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Xanthophyll cycle activity in detached barley leaves senescing under dark and light

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Abstract

Senescence-induced changes in the xanthophyll cycle activity and chlorophyll (Chl) fluorescence parameters were compared in detached barley (*Hordeum vulgare* L.) leaf segments kept for 6 d in darkness or under continuous "white light" (90 μmol m^{-2} s^{-1}). Before detachment of the leaf segments, the plants were grown at periodic regime [12 h light (90 μmol m^{-2} s^{-1})/12 h dark]. The de-epoxidation state of the xanthophyll cycle pigments (DEPS) in the leaf samples was determined immediately (the actual DEPS), after 1 h of dark-adaptation (the residual DEPS), and during 14 min of a high-irradiance (HI) exposure (500 μmol m^{-2} s^{-1}) (HI-induced DEPS). In the light-senescing segments, senescence was delayed pronouncedly compared to dark-senescing ones as the Chl content, the photosystem 2 photochemistry, and electron transport processes were highly maintained. Further, the actual DEPS increased, probably due to the increased mean photon dose. The HI-induced increase in the DEPS was stimulated in the light-senescing segments, whereas it was slowed down in the dark-senescing ones. However, after the 14 min HI-exposure of the dark-senescing segments the HI-induced DEPS was not markedly lower than in the mature leaves, which indicated the maintenance of the xanthophyll cycle operation.

Additional key words: ageing; carotenoids; chlorophyll fluorescence induction; de-epoxidation state; *Hordeum vulgare*; photosystem 2.

Introduction

Senescence of plant leaves is their final ontogenetic phase (Šesták 1985), comprising many physiological, biochemical, and molecular events that occur in a highly controlled manner (for reviews, see *e.g.* Grover and Mohanty 1993, Smart 1994, Matile 2001). Leaf senescence is characterized by a decrease in the rate of photosynthesis. This decrease is caused mainly by a progressive loss of chlorophyll (Chl). However, it is also associated with a decline in activities of photosystems and dark reactions of the Calvin cycle. The senescence-induced inhibition of the dark reactions (caused mainly by a decline in amounts and activities of stromal enzymes, especially ribulose-1,5-bisphosphate carboxylase/oxygenase) usually precedes a decrease in function of pigment-protein complexes in thylakoid membranes (Camp *et al.* 1982, Grover 1993). Especially, the photosystem 2 (PS2) photochemistry seems to be diminished rather slightly during senescence of intact leaves (Lu and Zhang 1998a,b, Špundová *et al.* 2003).

Senescence of plants grown under natural conditions is significantly affected by many environmental factors, especially by irradiance, temperature, nutrition, and water supply, and by the periodic circadian irradiation. Compared to the progressive senescence proceeding in darkness, the moderate irradiance usually retards the degradation of photosynthetic components (Thimann *et al.* 1977, Kar *et al.* 1993, Klerk *et al.* 1993, Chang and Kao 1998) and supports conservation of the photosynthetic activity (Kar *et al.* 1993, Špundová *et al.* 2003). However, when irradiance exceeds some critical level, the senescence-induced changes are accelerated (Hidema *et al.* 1992, Kar *et al.* 1993, Biswal 1995), most probably due to an

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Abbreviations: A, antheraxanthin; Car, sum of carotenoids; Chl, chlorophyll; DEPS, de-epoxidation state of the xanthophyll cycle pigments; HI, high irradiance; MGDG, monogalactosyldiacylglycerol; NPQ, non-photochemical chlorophyll fluorescence quenching; PAR, photosynthetically active radiation; PS2, photosystem 2; V, violaxanthin; Z, zeaxanthin.

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increasing photo-oxidative damage to the photosynthetic apparatus caused by over-excitation (Munné-Bosch and Allegre 2002). Under field conditions, the protective and deleterious effects of irradiation may appear successively in a periodic manner.

One of the protective mechanisms of plants against the over-excitation is the xanthophyll cycle, the reversible conversion of violaxanthin (V) to antheraxanthin (A) and zeaxanthin (Z) (Demmig-Adams and Adams 1992, Horton *et al.* 1996, Choudhury and Behera 2001). The extent of this conversion is usually expressed as the de-epoxidation state (DEPS) of the xanthophyll cycle pigments. Numerous studies have shown a close positive correlation between the DEPS and the non-radiative dissipation of absorbed photon energy reflected in the non-photochemical quenching (NPQ) of Chl fluorescence (*e.g.* Demmig-Adams 1990, Demmig-Adams and Adams 1996).

Senescence-induced changes in the xanthophyll cycle have not been yet fully elucidated. Biswal (1995) has implied a senescence-induced inhibition of the xanthophyll cycle. But recent studies have shown an increase in the DEPS during senescence under field conditions, especially in the leaves exposed to high irradiance (HI) (Murchie *et al.* 1999, García-Plazaola and Becerill 2001, Lu *et al.* 2001a,b, 2003, Yang *et al.* 2001, Jiao *et al.* 2003). Their results indicate that the xanthophyll cycle plays a role in photoprotection of the photosynthetic apparatus in senescing leaves through the thermal dissipation of excess photon energy (García-Plazaola and Becerill 2001, Lu *et al.* 2001a,b). All mentioned studies investigated only the "actual" DEPS in the leaves exposed to particular irradiance. Changes in the DEPS during HI-exposure of dark-adapted leaves reflecting the activation of the xanthophyll cycle have not been yet studied during senescence.

Further, the leaves attached to plants were used in all the mentioned studies. The connection of the leaves with the plant enables the source-sink relations, the transport of nutrients from the senescing leaves to the rest of plant, and the signal regulation of some processes in these leaves (Matile 2001). In the present study we used barley leaves detached from plants, *i.e.* a system without a possibility of translocation of degradation products and without any influence of the rest of plant. The natural light/dark circadian changes were eliminated by keeping the barley leaf segments in darkness or under continuous irradiation (90 μmol m^{-2} s^{-1}) for 6 d at constant temperature and water supply. We have followed senescence-induced changes in the actual DEPS as well as changes in the DEPS during HI-exposure (500 μmol m^{-2} s^{-1}, 14 min) of dark-adapted leaf samples in order to determine changes in the activity of the xanthophyll cycle during senescence. The kinetics of the HI-induced V de-epoxidation has been compared with the induction kinetics of non-photochemical Chl fluorescence quenching (NPQ) and $1 - q_P$ parameter.

Materials and methods

Plants and senescence conditions: Plants of spring barley (*Hordeum vulgare* L.) were grown in a growth chamber at 23 °C on an artificial medium composed of perlite and Knop's solution. The irradiation regime was 12 h dark/12 h light (fluorescent radiation) with a PAR irradiance of 90 μmol m^{-2} s^{-1}. The mature primary leaves of the plants in the growth phase of two leaves (the growth phase 1.2 according to Feekes 1941) were used for measurements. Leaf segments were cut off from the primary leaf blades (1.5 cm from the tip of the leaf, length of 5.0 cm) and placed into dishes with distilled water during the first two hours of the dark period. The leaf segments floated on the water surface. The segments were kept under dark or light (continuous PAR, irradiance 90 μmol m^{-2} s^{-1}) at an air temperature of 23 °C.

Pigment analyses: Leaf segments were frozen in liquid nitrogen. Pigments were extracted from the segments with 80 % acetone and a small amount of $MgCO_3$. The extract was centrifuged (3 600×*g*, 5 min) and the filtered supernatant was used for spectrophotometric (*Specord M40*, *Carl Zeiss*, Jena, Germany) estimation of Chl and total carotenoid (Car) contents (Lichtenthaler 1987), and for HPLC determination of relative changes in individual pigments of the xanthophyll cycle. The isocratic reverse-phase HPLC was performed according to a slightly modified procedure of Färber and Jahns (1998). The HPLC system consisted of a *ProStar 230* Delivery Module, *ProStar 32*0 UV-VIS detector *(Varian Analytical Instruments*, Walnut Creek, USA), and a reverse phase column (5 μm particle size, *250/4 RP18*, *Lichrocart*, Germany). The pigments were eluted isocratically for 13 min with acetonitrile : methanol : 0.1 M Tris (87 : 10 : 3, v/v) followed by a 12-min isocratic elution with methanol : ethyl acetate (34 : 16, v/v) at a flow rate of 33.3 mm^3 s^{-1}. Absorbance was measured at 440 nm. The de-epoxidation state of the xanthophyll cycle pigments (DEPS) was calculated as $DEPS = (Z + A)/(V + A + Z)$ (Gilmore and Björkman 1994), where Z is zeaxanthin, A is antheraxanthin, and V is violaxanthin. The actual DEPS, residual DEPS after 1 h of dark-adaptation (for control leaves and light-senescing segments), and "HI-induced DEPS" in three time points (4, 9, and 14 min) of a HI-exposure ("white light", 500 μmol m^{-2} s^{-1}) were evaluated. A difference between the HI-induced DEPS and residual DEPS was estimated and presented as ΔDEPS.

Chl fluorescence was measured at room temperature (23 °C) with a modulation fluorometer *PAM 2000* (*Walz*, Effeltrich, Germany). The $F_V/F_M = (F_M - F_0)/F_M$ was determined from the minimal Chl fluorescence intensity (F_0) and maximal fluorescence intensity (F_M) measured on the adaxial side of the barley leaves or leaf segments. The control (attached) leaves were measured during the fifth and sixth hours of the dark period, the light-senescing segments were measured after 1 h of dark-adaptation. F_0 was obtained upon excitation of the leaf segment with a weak measuring beam of 0.2 µmol m^{-2} s^{-1}, F_M was measured by means of a 0.8-s saturating pulse (≈6 000 µmol m^{-2} s^{-1}).

After the F_V/F_M measurement, the induction kinetics of Chl fluorescence parameters was recorded during 14 min of HI-exposition of the leaf samples ("white light", 500 µmol m^{-2} s^{-1}). During fluorescence induction the saturating pulses (0.8 s, 6 000 µmol m^{-2} s^{-1}) were applied in order to determine F_M' (= maximal Chl fluorescence intensity during the actinic irradiation). The first two pulses were applied 2 and 22 s after switching on the HI, following 9 pulses in 40 s intervals, and the last 6 pulses in 80 s intervals. After each pulse, HI was switched off and far-red radiation was applied for 3 s in order to measure F_0' (= minimal Chl fluorescence intensity during the actinic irradiation). The induction kinetics was measured each day within 6 d after detachment (except the first day after detachment when no significant changes in the studied parameters were expected). For better readability only the kinetics measured in the control leaves and 2, 4, and 6 d after detachment is presented (Fig. 4).

The excitation pressure on PS2 was calculated from a coefficient of photochemical fluorescence quenching q_P (van Kooten and Snell 1990) as $1 - q_P = 1 - (F_M' - F_t)/(F_M' - F_0')$, where F_t was the fluorescence intensity at time t. Non-photochemical fluorescence quenching (NPQ) was calculated as $F_M/F_M' - 1$ according to Bilger and Björkman (1990). A general statistical description (medians and quartiles) was used in the case of Chl fluorescence parameters (Lazár and Nauš 1998).

Results

The content of Chls and total Cars did not change significantly in barley leaf segments kept under continuous irradiation (Table 1). The ratios of contents of Chl/Car and Chl/xanthophyll cycle pigments (V+A+Z) slightly decreased after the detachment (Table 1).

In the dark-senescing segments of barley leaves a gradual decrease in Chl and Car contents was found together with a decrease in Chl/Car, indicating a relatively more rapid decrease in the Chl content in comparison with Cars (Table 1). The Chl/(V + A + Z) ratio decreased, too. On the 6th d after detachment it was reduced by about 50 % compared with the control leaves and segments senescing under continuous irradiation. The pronounced decrease in the relative amount of Chls was caused by the decrease in their content (Table 1) rather than by an increase in the xanthophyll cycle pigment pool.

Changes in the F_V/F_M ratio in the light-senescing leaf segments were almost negligible; the values above 0.80 within 6 d after detachment indicated a nearly unchanged maximal efficiency of PS2 photochemistry (Fig. 1). In the dark-senescing segments, F_V/F_M decreased to about 0.65.

The actual de-epoxidation state of the xanthophyll cycle pigments (DEPS, estimated as a ratio (A + Z)/(A + Z + V) in the control (attached) leaves was about 0.06

Table 1. Changes in contents of chlorophyll (Chl) and carotenoids (Car) and ratios Chl/Car, Chl *a*/*b*, and Chl/xanthophyll cycle pool pigments (V+A+Z) (V, violaxanthin; A, antheraxanthin; Z, zeaxanthin) in senescing segments of barley leaves. The last ratio was evaluated from HPLC results ($n = 3$), the other parameters from spectrophotometric measurements ($n = 5$). The segments were kept under continuous irradiation (90 µmol m^{-2} s^{-1}) or in the dark. Means and SD are shown. Statistically significant differences from control values are indicated by the letter a, and between the segments kept under light and dark conditions for corresponding day after detachment by the letter b. $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$.

	Time after detachment [d]	Chl *a*+*b* [g m^{-2}]	Car *x*+*c* [g m^{-2}]	Chl/Car	Chl *a*/*b*	Chl/(V+A+Z)
Control	0	23 ± 3	4.4 ± 0.5	5.3 ± 0.1	3.6 ± 0.2	7.9 ± 0.3
Light	2	20 ± 4	4.4 ± 0.8	4.7 ± 0.2 a***	3.5 ± 0.2	6.7 ± 0.6 a**
	3	24 ± 3	4.6 ± 0.7	5.2 ± 0.2	3.1 ± 0.2 a**	7.0 ± 0.2 a***
	4	27 ± 4	5.4 ± 0.8	4.9 ± 0.1 a**	3.3 ± 0.1 a*	6.9 ± 0.2 a***
	5	20 ± 5	4.2 ± 1.0	4.7 ± 0.2 a***	3.2 ± 0.1 a*	7.4 ± 0.6
	6	18 ± 6	4.3 ± 1.3	4.2 ± 0.2 a***	3.5 ± 0.1	7.2 ± 0.2 a**
Dark	2	18 ± 5	4.0 ± 1.1	4.5 ± 0.2 a***	3.5 ± 0.1	6.4 ± 0.4 a***
	3	14 ± 3 a***, b***	3.5 ± 0.6 a**, b**	4.0 ± 0.3 a***, b***	3.3 ± 0.1 a*	5.4 ± 0.6 a***, b*
	4	10 ± 3 a***, b***	3.3 ± 0.3 a**, b***	2.9 ± 0.6 a***, b***	3.2 ± 0.1 a*	5.8 ± 1.2 a**
	5	7 ± 2 a***, b***	2.7 ± 0.4 a***, b***	2.6 ± 0.7 a***, b***	3.1 ± 0.1 a**	5.5 ± 0.2 a***, b**
	6	6 ± 2 a***, b**	2.6 ± 0.4 a***, b***	2.2 ± 0.7 a***, b***	3.2 ± 0.3	4.0 ± 0.8 a***, b**

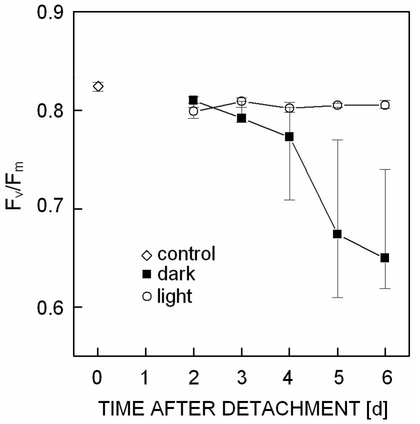

Fig. 1. The maximal efficiency of photosystem 2 photochemistry (F_V/F_M) in the control (attached) barley leaves (◇) and in the leaf segments senescing in darkness (■) and under continuous light (○). Medians and quartiles are shown; *n* = 10–12.

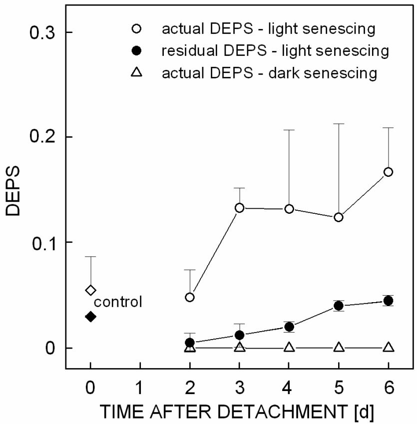

Fig. 2. The actual (*open symbols*) and residual (*solid symbols*) de-epoxidation state of the xanthophyll cycle pigments (estimated as the amount of A and Z related to amounts of A, Z, and V) in the control (attached) barley leaves (*diamonds*) and in the light senescing (*circles*) and dark senescing (*triangles*) barley leaf segments. The residual DEPS was determined after 1 h of dark adaptation. Means and SD, *n* = 3.

at the end of the light period (Fig. 2). The residual DEPS (*i.e.* the DEPS after 1 h of dark-adaptation) was about 0.03 as a part of Z was converted back to V. There was a pronounced increase in the actual DEPS in the light-senescing segments from the 3rd day after detachment. The residual DEPS slightly decreased on the 2nd d after detachment in comparison with the control leaves, and then a slight increase was found. The actual DEPS in the dark-senescing segments was zero as neither Z nor A were detectable from the 2nd d after detachment (Fig. 2).

The HI-exposure of the control leaves and senescing segments was aimed to reveal the rate and extent of conversion of V into A and Z during HI treatment. This exposure caused an increase in the DEPS designated as ΔDEPS (ΔDEPS = "HI-induced DEPS" – residual DEPS, Fig. 3). In the control leaves the ΔDEPS reached a relatively low plateau after 4 min of HI. In the light-senescing segments the HI-exposure caused a more pronounced increase in the ΔDEPS, especially after 9 and 14 min. The de-epoxidation of V was strongly delayed in the dark-senescing segments as an increase in the ΔDEPS was gradually slowed down. However, the ΔDEPS after 14 min of the HI-exposure was even slightly higher on the 2nd and 4th d or non-significantly lower on the 6th d after detachment in comparison with the control leaves (Fig. 3).

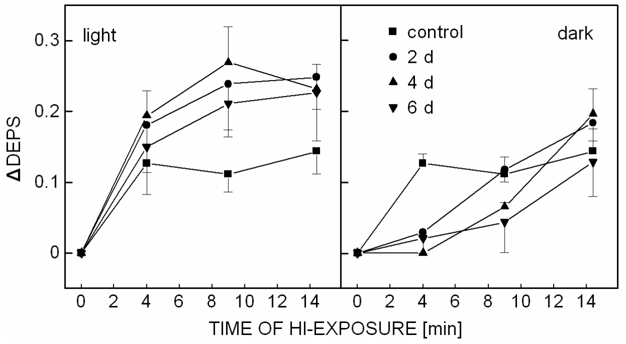

Fig. 3. The high-irradiance (HI) induced de-epoxidation state of the xanthophyll cycle pigments (estimated as ΔDEPS = actual DEPS – residual DEPS) during the HI (500 µmol m^{-2} s^{-1}) exposure of the control (attached) barley leaves and leaf segments senescing in darkness (dark) and under continuous light (light) for 2, 4, and 6 d after detachment. Means and SD, *n* = 3.

The induction kinetics of Chl fluorescence parameters (NPQ and 1 – q_P) was measured during 14 min of the HI-exposure in the same leaf samples for which the ΔDEPS was followed. In the case of control leaves and light-senescing segments, NPQ should be also understood as an increment (*i.e.* ΔNPQ) as in the case of the ΔDEPS, because the F_M value estimated after 1 h of dark-adaptation of these samples might have been lowered by the residual DEPS (Fig. 2). In the control leaves, the induction kinetics of NPQ was characterized by a gradual increase to a steady-state value (of about 2.3) (Fig. 4*A*). The increase in NPQ in the light-senescing segments during the first 4 min of HI was almost the same as in the control leaves (except a slight acceleration observed in the segments on the 2nd d after detachment). However, there was a suppression of the subsequent NPQ increase and steady-state values. The steady-state value of NPQ decreased on the 2nd d after detachment to about 1.3 and remained near this value (Fig. 4*A*). Interesting changes were found in the NPQ induction kinetics of the dark-senescing segments. The increase of NPQ induced by HI became bi-phasic: after an initial increase within the first 4 min there was a plateau and subsequent more rapid increase even without reaching saturation. The NPQ increase was gradually suppressed in the dark-senescing segments and the NPQ value after 14 min of HI decreased significantly on the 6th d after detachment (Fig. 4*A*).

The senescence-induced changes in the 1 – q_P

induction kinetics were analogous to those in the NPQ kinetics. The decrease in value of $1 - q_P$ during the HI-exposure was accelerated in the segments senescing under continuous irradiation in comparison with control leaves (Fig. 4*B*). In the dark-senescing segments this decrease was gradually inhibited within 6 d after detachment.

Discussion

The continuous exposure of the senescing barley leaf segments to the cultivation irradiance (90 µmol m^{-2} s^{-1}) markedly retarded the Chl degradation in comparison with the situation in darkness (Table 1) and maintained the maximal efficiency of PS2 photochemistry (F_V/F_M; Fig. 1). Induction kinetics of NPQ and $1 - q_P$ was even accelerated compared to the control leaves (Fig. 4). However, in the control leaves the kinetics was measured within the fifth and sixth hours of the dark period so that the activation of the photosynthetic apparatus could be slowed down in comparison with the light-senescing segments that were dark-adapted for 1 h.

Moderate irradiance retards the senescence-induced symptoms such as a decrease in contents of photosynthetic pigments (*e.g.* Thimann *et al.* 1977, Kar *et al.* 1993, Meir and Philosoph-Hadas 1995) and proteins (*e.g.* Klerk *et al.* 1993, Chang and Kao 1998), in PS2 photochemistry (*e.g.* Kar *et al.* 1993, Špundová *et al.* 2003), and in some other functions. However, there is an increasing risk of photo-oxidative damage to the photosynthetic apparatus when senescing leaves are irradiated (Munné-Bosch and Allegre 2002). A supply of $NADP^+$ as a final acceptor of electrons in the photosynthetic transport chain decreases due to an inhibition of stromal reactions so that probability of the electron flow to molecular oxygen, accumulation of the reactive oxygen species, and photodamage to the chloroplast components increase. One of the protective mechanisms against photodamage is the pH-dependent thermal dissipation of excited states occurring in the PS2 antenna pigment bed. This process involves the de-epoxidation of V through A to Z which is triggered by the acidification of thylakoid lumen (Demmig-Adams and Adams 1992, Horton *et al.* 1996). A higher de-epoxidation has been recently found in the leaves of the plants senescing under natural conditions, which indicated that the xanthophyll cycle played a role in the photoprotection of the photosynthetic apparatus in senescing leaves. The increase in the actual DEPS during natural senescence was observed in the attached leaves of field-grown wheat (Lu *et al.* 2001a,b, 2003) and rice (Murchie *et al.* 1999, Yang *et al.* 2001), and in leaves of beech (García-Plazaola and Becerill 2001). The senescence-induced increase in the actual DEPS was more pronounced in the leaves exposed to higher irradiance (at midday in comparison with the morning: Murchie *et al.* 1999, Lu *et al.* 2001a,b, 2003, Yang *et al.* 2001, Jiao *et al.* 2003, or in the sun leaves compared to the shade ones: García-Plazaola and Becerill 2001). These results indicate that a long lasting exposition of senescing leaves to HI leads to a higher DEPS.

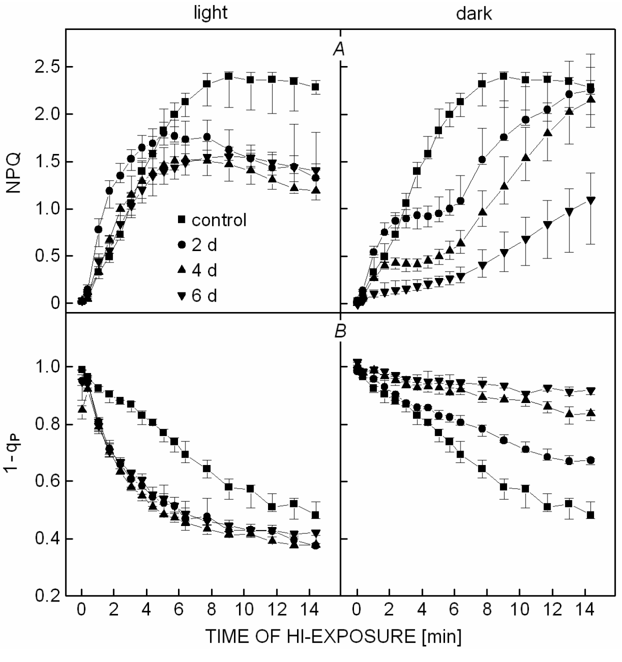

Fig. 4. Induction kinetics of NPQ (non-photochemical quenching of chlorophyll fluorescence) (*A*) and $1 - q_P$ (*B*) in the control (attached) leaves of barley and leaf segments senescing in darkness (dark) and under continuous irradiation (light) for 2, 4, and 6 d after detachment during their high-irradiance exposure (500 µmol m^{-2} s^{-1}). Medians and quartiles are shown; *n* = 6.

In our case, the actual DEPS was also increased in the light-senescing leaf segments (Fig. 2) even if they were exposed to a relatively low irradiance (90 µmol m^{-2} s^{-1}). However, a long-time photon dose was doubled compared to that before the detachment, because the plants were growing under a periodic irradiation (12 h light/12 h dark) whereas the segments were kept under continuous irradiation. Afitlhile *et al.* (1993) have reported a decrease in the actual DEPS in detached barley leaves senescing under continuous irradiation. However, they used lower irradiance (46 µmol m^{-2} s^{-1}) and the photon dose was not increased after detachment as in our case.

The increased actual DEPS might contribute to the maintenance of Chl content and PS2 photochemistry in the light-senescing segments (Fig. 2) because the protective effects of Z and A in thylakoid membranes include a regulation of the thylakoid membrane fluidity (Gruszecki and Strzalka 1991, Strzalka and Gruszecki 1997, Tardy and Havaux 1997) and inhibition of membrane lipid peroxidation (Havaux *et al.* 2000). The slightly increased residual DEPS in the light-senescing segments (Fig. 2) indicated the slowed-down epoxidation of Z and A back to V.

In HI-treated (500 µmol m^{-2} s^{-1}) dark-adapted leaf samples, the xanthophyll cycle was activated, and the level of de-epoxidation (ΔDEPS) increased. The rate and extent of conversion of V into A and Z was stimulated in the light-senescing segments, which was indicated by the more rapid increase in the ΔDEPS and by the higher ΔDEPS values after 14 min of HI-exposure in comparison with the control leaves (Fig. 3). An increase in the DEPS should be accompanied by a corresponding increase in NPQ (Demmig-Adams 1990, Demmig-Adams and Adams 1996). Such changes were found during senescence in the field (Murchie *et al.* 1999, Lu *et al.* 2001a,b, 2003, Jiao *et al.* 2003). In our case such correlation might be valid in the initial phases of the induction kinetics (compare Figs. 3 and 4*A*). However, the steady-state values of NPQ in the light-senescing segments were lower (despite of the higher ΔDEPS) in comparison with the control leaves. Similar results were reported by Kurasová *et al.* (2002) for barley plants acclimated to HI: the lowest NPQ was observed in the leaves with the highest DEPS. Kurasová *et al.* (2002) suggested a specific HI-acclimation of barley without a pronounced increase in non-radiative dissipation (see also Čajánek *et al.* 1999). In our case, the decrease was observed also in steady-state values of SV_0 in the light-senescing segments (data not shown). SV_0 is the quenching of minimal Chl fluorescence and it might be related to the part of non-radiative dissipation localized within light-harvesting complexes (Pospíšil 1997, Špunda *et al.* 1997). Therefore this quenching should better correspond to the DEPS. However it was not our case. A reason for the lower non-radiative dissipation in the light-senescing segments with the higher DEPS remains misty.

During senescence in darkness, similar changes in the induction kinetics of both ΔDEPS and NPQ were observed (Figs. 3 and 4*A*). The increase in the ΔDEPS and NPQ was gradually slowed down and the steady state could not be reached within the 14 min of HI-exposure. The gradual retardation of V de-epoxidation and slowing down of the NPQ increase might be connected with a partial inhibition of lumen acidification and impairment of the electron transport processes in thylakoid membranes, which was indicated by the gradual inhibition of $1 - q_P$ kinetics (Fig. 4*B*). The slowing down of the $1 - q_P$ decrease during the HI-exposure can be interpreted as the decrease in the electron withdrawing from PS2 reaction centres (Björkman and Demmig-Adams 1995). The inhibition of the linear electron transport was reported for the senescing leaves (Grover and Mohanty 1993 and references therein).

Other explanation of the delayed conversion of V into A and Z might be connected with changes in the lipid composition of thylakoid membranes during senescence in darkness. According to a model of Latowski *et al.* (2002) the activity of violaxanthin de-epoxidase is associated with monogalactosyldiacylglycerol (MGDG) domains in the thylakoid membranes. The authors suggest that V has to diffuse into such MGDG-enriched domains to be converted into A and Z. During dark-induced senescence a degradation of galactolipids (preferentially MGDG) precedes the degradation of other membrane lipids (Gut and Matile 1989, Meir and Philosoph-Hadas 1995). Wanner *et al.* (1991) have shown for dark-senescing barley leaves that the carbon of acyl residues originating from galactolipids is utilized for gluconeogenesis. So we can speculate that the inhibited kinetics of the V de-epoxidation could be associated with an extension of a diffusion path of the V to the MGDG-enriched domains caused by a decrease in a relative content of MGDG in the thylakoid membranes of the dark-senescing segments.

Summing up, the increased actual DEPS in the barley leaf segments senescing under continuous cultivation irradiance indicated that the xanthophyll cycle participated in the protection of the photosynthetic apparatus from over-excitation similarly as in case of the attached leaves senescing under field conditions and exposed to the increased irradiance. The increased level of V de-epoxidation was induced by the increase in the mean photon dose. The conversion of V into A and Z induced by the HI-exposure was stimulated in the light-senescing segments. This process was slowed down in the dark-senescing segments (probably due to the slowing down of the thylakoid lumen acidification), but it was still functional for 6 d after detachment.

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