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Contrasting changes of photosystem 2 efficiency in *Arabidopsis* xanthophyll mutants at room or low temperature under high irradiance stress*

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Abstract

We compared the responses of wild type (WT) and three mutants including *npq*1 (lutein-replete and violaxanthin deepoxidase-deficient), *lut*2 (lutein-deficient), and *lut*2-*npq*1 (double mutant) to high irradiance (HI, 2 000 μmol m^{-2} s^{-1}) at both low (LT, 5 °C) and room (25 °C) temperature. Xanthophyll-dependent energy dissipation was highest in the WT, followed by the *lut*2, *npq*1, and *npq*1-*lut*2. At 25 °C the relative stress tolerance expressed by F_v/F_m was consistent with the energy dissipation capacity for the first 2 h of treatment. After 3-4 h, the F_v/F_m levels in *lut2* and *npq1* converged. Under combined LT and HI the relative tolerance sequence was in contrast to the energy dissipation capacity being WT > *npq*1> *lut*2 > *lut*2-*npq*1. There were little or no significant change in the contents of xanthophylls and carotenes or the chlorophyll (Chl) *a/b* ratio in any of the materials. Thus lutein (L) substitution possibly alters the conformation/organisation of L binding proteins to enhance damage susceptibility under HI at LT. The enhanced vulnerability is not compensated for the energy dissipation capacity in the *lut*2 background at LT.

Additional key words: antheraxanthin; β-carotene; chlorophyll; fluorescence; light-harvesting complex; lutein; neoxanthin; violaxanthin; zeaxanthin.

Introduction

Plants often absorb more photons than they are able to use for photosynthesis. In addition, many environmental stresses, such as drought, extremes of temperature, or nutrient deprivation can further limit the ability of a plant to use photon energy (Demmig-Adams and Adams 1992, Demmig-Adams *et al.* 1996). Absorbed excess radiant energy can lead to sustained depression in photosynthetic efficiency (photoinhibition), often due to oxidative damage to the photosynthetic apparatus (Powles 1984, Kyle *et al.* 1987, Barber and Anderson 1992, Long *et al.* 1994, Niyogi *et al*. 1998). Photosynthetic organisms have evolved multiple mechanisms to cope with the absorption of excessive photons and its consequence. The harmless heat dissipation of excess absorbed radiant energy that depends on the xanthophyll cycle is believed to play a key role in regulating light harvesting and electron transport and is important for the prevention of photooxidative damage to the photosynthetic apparatus (Gilmore 1997, 1998, Niyogi 2000, Müller *et al*. 2001).

Recent breakthroughs in applied molecular biology have generated several important mutations that strongly influence the content and composition of the main oxygen containing carotenoids (xanthophylls) in the photosynthetic apparatus of *Arabidopsis thaliana* L. (Pogson *et al*. 1996, 1998, Niyogi *et al*. 1998, Li *et al*. 2000). The new mutants provide a unique opportunity to investigate

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Abbreviations: A – antheraxanthin; Chl – chlorophyll; L – lutein; LHC2b – main light-harvesting pigment-protein complex of PS2; *lut*2 – lutein-deficient mutant; *lut*2-*npq*1 – lutein and violaxanthin de-epoxidase deficient mutant; *npq*1 – lutein-replete violaxanthin de-epoxidase-deficient mutant; NPQ – non-photochemical quenching; PS1 – photosystem 1; V – violaxanthin; [VAZ] – summed concentration of pigments of the xanthophyll cycle; WT – wild phenotype; Z – zeaxanthin.
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the putative photosynthetic roles and physiological significance of the major higher plant xanthophylls, namely, lutein (L) and the xanthophyll cycle carotenoids, violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z) (Gilmore 2001, Niyogi *et al.* 2001, Rissler and Pogson 2001, Lokstein *et al*. 2002). The *Arabidopsis npq*1 mutant, which is defective in the V de-epoxidase gene, is unable to synthesise Z *via* the xanthophyll cycle. Characterisation of *npq*1 plants has provided molecular genetic evidence that Z is necessary for NPQ (Niyogi *et al.* 1998) and that Z and V de-epoxidase activity have an additional role in prevention of lipid peroxidation (Havaux and Niyogi 1999).

The *lut*2 mutant affects the lycopene ε-cyclase gene of *Arabidopsis* (Pogson *et al*. 1996) and is therefore unable to synthesise either L or α-carotene. L is the most abundant xanthophyll in the thylakiod (Niyogi *et al*. 1997). Gilmore and Yamamoto (1990) first hypothesised that L, like its structural isomer Z, may be directly involved in heat dissipation. The hypothetical structural requirement of xanthophylls involved in the heat dissipation mechanism was subsequently expanded to include several molecules with an epoxide-free 3-hydoxy β-ionone endgroup, *i.e*., including Z, A, diatoxanthin, L, and possibly β-cryptoxanthin (Gilmore and Yamamoto 1993, Gilmore 1997). One main role of L in particular is commonly believed to be an accessory light-harvesting pigment, collecting photon energy to be transferred to chlorophyll (Chl) for use in photosynthesis (Siefermann-Harms 1987). Furthermore, Kühlbrandt *et al.* (1994) suggested that L is an important structural component of the main LHC2b protein. Recent studies (Niyogi *et al*. 1998, Pogson *et al*. 1998, Gilmore 2001) have confirmed that L has either an indirect structural influence and/or a direct role in ΔpH-dependent PS2 heat dissipation. This study focuses on the kinetic response of changes in the PS2 efficiency of *Arabidopsis* xanthophyll mutants at low temperature (LT) and room temperature under high irradiance (HI) stress. The purpose of the present study is to understand the different responses of these mutants to environmental stresses and to clarify and separate the effects of L and the xanthophyll cycle components on the stress tolerance of the photosynthetic apparatus.

Materials and methods

Plants: Nuclear gene mutations, *npq*1, *lut*2, and the double *lut*2-*npq*1 were obtained from *Arabidopsis thaliana* cv. Columbia with the courtesy of the authors of Pogson *et al.* (1996) and Niyogi *et al.* (1998). Plants were grown from seed for 4 to 5 weeks in a growth chamber under a PPFD of 230 μmol m^{-2} s^{-1} with a 16 h photoperiod. Relative humidity was 90 % and day/night temperature was 22/17 °C.

LT treatment: The detached leaves were floated, adaxial side up, on distilled water in a container kept at a water bath (5 °C). Irradiation in normal air was provided at 2 000 μmol(photon) m^{-2} s^{-1} by an *HMI* Universal Spotlight (model *HMI 575W/GS*; *Osram*) behind a heat filter (*Schott 115*, *Tempax*) and a piece of glass. The HI-exposed leaves were then used for determinations of F_v/F_m, pigment contents, and 77 K fluorescence emission spectra as described below. Room temperature treatment was conducted on detached leaves with the water bath at 25 °C and all other conditions were the same as for the 5 °C experiments. After F_v/F_m was measured at each of the different time points during the HI-treatments, the treated leaves were allowed to recover at low irradiance (20 μmol m^{-2} s^{-1}) for 2 h at room temperature (25 °C) and the time course of the recovery of F_v/F_m was monitored.

PS2 Chl *a* fluorescence was measured with a *Walz PAM 101* fluorometer (*H. Walz*, Effeltrich, Germany). The detached leaves were dark-adapted (<10 μmol m^{-2} s^{-1}) for 20 min prior to *PAM* fluorescence determinations. The F_0 level of fluorescence was determined with the low irradiance of modulated beam (1.6 kHz, < 0.15 μmol m^{-2} s^{-1}, 440 nm) and the emission was monitored with a filter passing all radiation F>660 nm. The initial F_m level was monitored with a saturating 2 s pulse of "white light" (> 10 000 μmol m^{-2} s^{-1}) passed through an infrared heat filter (*DT Cyan*, *Walz*), while the *PAM* modulated measuring beam automatically switched to 100 kHz. The "white actinic light" (2 000 μmol m^{-2} s^{-1}), passed through a heat filter (*DT Cyan*, *Walz*), lasted for different time (10, 20, 30, 60, 90, 120, and 180 min) at each min of which F'_m was determined during 2 s saturating pulse under LT (5 °C). The final F_m was determined 15 min after the end of the actinic radiation treatment with a third saturating pulse under room temperature (25 °C). All fluorescence intensity parameters were used as defined by Van Kooten and Snel (1990). The irradiance-limited PS2 quantum efficiency was calculated as the ratio F_v/F_m, where the variable fluorescence, $F_v = F_m - F_0$.

The 77 K Chl fluorescence spectra of leaves were performed with an *SLM 8100* spectrofluorometer (*Spectronic Inst.*, USA) in photon counting mode (Gilmore and Ball 2000). Excitation was at 435 nm (peak Chl *a* absorbance) through an 8 mm slit width (16 nm bandpass) and emission was monitored with a slit width of 2 mm (4 nm bandpass) using a double-monochromator emission at a slow rate of 1 nm per s and a 1 s integration time. The instrument-corrected fluorescence emission spectrum between 650–770 nm was recorded. The relative

fluorescence yields at 685 nm (F_{685}) and 735 nm (F_{735}) were used to describe the energy emission from PS2 and PS1.

Pigments were extracted with acetone and assayed by HPLC as described by Gilmore and Yamamoto (1991). Absorption at 440 nm was measured with a *Waters 490* (*Waters*, Milford, USA) variable wavelength detector.

Results

Steady state *PAM* fluorescence intensity parameters of lutein-replete wild-type and *npq*1 mutant and the lutein-deficient *lut*2 and *lut*2-*npq*1 mutants under LT (5 °C): The fluorescence parameters (Fig. 1) were the mean values of different time in leaves of mutants and wild type (WT) exposed to saturating irradiance (2 000 µmol m^{-2} s^{-1}) under 5 °C. Fig. 1*A* shows that prior to irradiation, the PS2 quantum efficiency measured as the initial F_v/F_m ratio was nearly equal for each mutant and WT. Fig. 1*B* compares the final dark-adapted F_m values, measured after the respective experimental irradiation treatments, to the F_m measured prior to irradiation by plotting the ratio of the final F_m/initial F_m. The result indicated that the mean value of F_m in *npq*1-*lut*2, *npq*1, *lut*2, and WT recovered to 17.6, 36.9, 24.4, and 45.3 %, respec-tively. More recovery was found in *npq*1 than *lut*2 at chilling temperature.

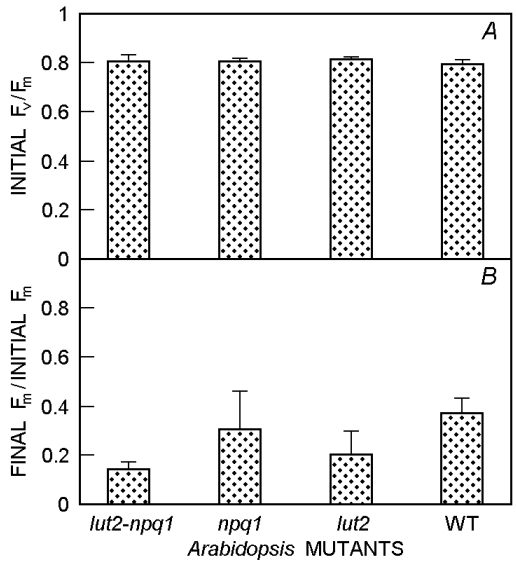

Fig. 1. Comparison of the effect of saturating irradiance (2 000 µmol m^{-2} s^{-1}) treatment on key photosystem 2 chlorophyll *a* fluorescence parameters in leaves of *Arabidopsis* mutants and wild-type at chilling temperature (5 °C). *A*: Initial F_v/F_m ratios prior to irradiation. *B*: Final F_m measured after irradiation to the initial F_m measured prior to irradiation. Means of fluorescence parameter in detached leaves exposed to saturating irradiance for different time (10, 20, 30, 60, 90, 120, and 180 min).

Effect of LT on the time course of decreasing F_v/F_m under HI: When detached leaves of the *npq1*, *lut2,* and *npq1-lut2* mutants and WT were exposed to 2 000 µmol m^{-2} s^{-1} at 5 °C for the times (shown in Fig. 2), the F_v/F_m values gradually decreased. During the initial 10 to 120 min of the treatment period, *lut*2 and *lut*2-*npq*1 were more sensitive to HI than WT and *npq*1. Further, there was little difference either between *lut*2 and *lut*2-*npq*1 or between WT and *npq*1 in the 10 to 120 min time frames. After prolonged exposure times from 180 to 360 min, the relative differences in the response to HI at LT were resolved among the four *Arabidopsis* strains. Compared to the dark control, the F_v/F_m decreased, respectively, by 59 (WT), 69 (*npq*1), 79 (*lut*2), and 85 (*npq*1-*lut*2) % at the end of exposure (360 min), respectively. The sequence of tolerance to HI and LT among the strains was WT>*npq*1>*lut*2>*lut*2-*npq*1. It was clear the *npq*1 mutant maintained higher maximal photochemical efficiency of PS2 than either of the L-deficient mutants.

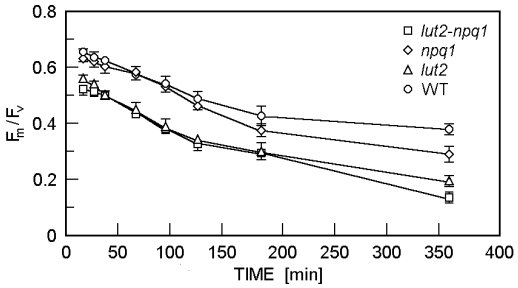

Fig. 2. Effect of low temperature (5 °C) on F_v/F_m in detached leaves of *Arabidopsis* mutants and wild type under high irradiance (2 000 µmol m^{-2} s^{-1}). Means of at least three separate samples. The *bars* are the standard deviations.

Comparison of changes in 77 K fluorescence parameter, the ratio of F_{685}/F_{735}, in leaves of *Arabidopsis* mutants and WT treated with HI at LT: The fluorescence bands at approximately 680 and 690 nm are attributed to PS2, whereas the 730 nm band is due to the PS1 LHC (Bassi *et al*. 1990, Krause and Weis 1991). The ratio of the Chl fluorescence emission at 685 nm to the Chl fluorescence emission at 735 nm (F_{685}/F_{735}) in *lut*2-*npq*1 and *lut*2 decreased more significantly than in *npq*1 and WT after HI treated for 1.5 or 6.0 h under chilling temperature compared to their dark control (Fig. 3*A*). *lut*2 and *lut*2-*npq*1 had a slightly different distribution of radiant energy between PS2 and PS1, in favour of PS1. The trend of decreasing F_{685}/F_{735} was similar to that of decreasing F_v/F_m. The sequence of tolerance to HI at LT among four *Arabidopsis* strains was also WT > *npq*1 > *lut*2 > *lut*2-*npq*1. This confirms that *lut*2 was more sensitive to photoinhibition than *npq*1 under chilling temperature.

The extent of F_{685}/F_{695} is plotted against F_v/F_m for all mutants and WT exposed to HI for 1.5 and 6.0 h at chilling temperature (Fig. 3*B*). A near-linear correlation was obtained. The change of F_{685}/F_{695} is strongly positive relative to that of F_v/F_m. We think that the decrease in F_{685} is most likely due to photoinhibition of PS2 lowering its yield because state transitions rarely exceed 15 % of F_m (Lunde *et al.* 2000). The results demonstrated that *lut*2 was more sensitive to photoinhibition than *npq*$_1$ under LT.

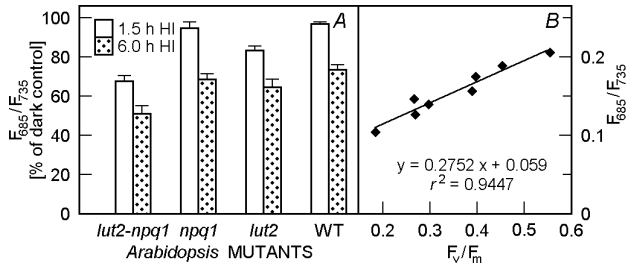

Fig. 3. Changes of low temperature (77 K) fluorescence parameter F_{685}/F_{735} (*A*) and relationship between F_{685}/F_{735} and F_v/F_m (*B*) in leaves of *Arabidopsis* mutants and wild type treated with low temperature (5 °C) under high irradiance (2 000 μmol m^{-2} s^{-1}) for 1.5 and 6.0 h.

Changes of photosynthetic pigments in leaves of *Arabidopsis* strains treated with LT and HI: Table 1 shows the results of pigment analysis for the WT and three mutants. The sizes of the xanthophyll-cycle pool (V+Z+A) in L-deficient lines (*lut*2 and *npq*1-*lut*2) were significantly larger than in L-replete lines (WT and *npq*1). There were no obvious changes in Chl *a/b* ratio and β-carotene content. The ratio of L to total carotenoids also was not altered by 6 h of stress treatment in either the WT or *npq*1 mutant. The light-driven de-epoxidation of V resulting in an accumulation of Z *via* A in the thylakoid, was characterised using the de-epoxidation state (DES), where DES = (Z+A)/(V+Z+A) (Gilmore and Yamamoto 2001). The DES increased from 0.532 to 0.736 in WT exposed to HI for 10 to 360 min, whereas little changes were exhibited in *lut*2 following a 10-min treatment.

Effect of room temperature on the time course of decreasing F_v/F_m under HI: As shown in Fig. 4, the F_v/F_m values decreased with increasing exposure time in detached leaves of the WT and mutant *Aradopisis* strains treated with irradiance of 2 000 μmol m^{-2} s^{-1} at room temperature (25 °C). In comparison to the dark control after 2 h, the F_v/F_m was reduced by 43 (WT),

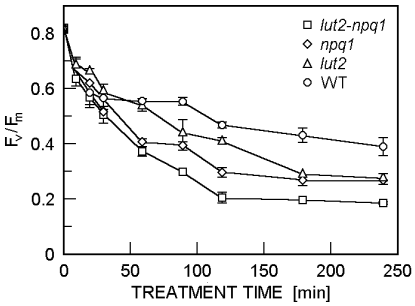

Fig. 4. Effect of high irradiance (2 000 μmol m^{-2} s^{-1}) on F_v/F_m in detached leaves of *Arabidopsis* mutants and wild-type under room temperature (25 °C). Means of at least three separate samples.

Table 1. Changes of pigment composition in leaves of *Arabidopsis* strains treated with low temperature (5 °C) and high irradiance (2 000 μmol m^{-2} s^{-1}).

Strain	Treatment [min]	Neoxanthin	V+A+Z	β-carotene	Lutein	Chl *a/b*	[Z+A] [VAZ]$^{-1}$	[L] [Car]$^{-1}$
lut2-npq1	0	24.75±2.10	108.26± 8.70	78.55±6.7	ND	3.46±0.06	0.015±0.001	ND
	10	26.76±1.60	111.85± 5.30	77.71±3.5	ND	3.38±0.03	0.016±0.002	ND
	90	32.78±1.30	112.47±10.20	74.36±6.8	ND	3.31±0.09	0.024±0.003	ND
	360	30.48±2.90	115.26±11.80	75.24±5.3	ND	3.21±0.02	0.020±0.001	ND
npq1	0	22.29±1.00	23.00± 1.80	57.66±4.2	103.3±8.1	3.26±0.05	0.012±0.003	0.50±0.01
	10	22.73±1.50	28.30± 2.50	59.67±3.9	104.9±5.7	3.35±0.03	0.015±0.003	0.49±0.01
	90	24.66±0.90	26.20± 1.30	57.36±5.1	116.4±6.9	3.19±0.04	0.021±0.008	0.52±0.03
	360	27.13±1.70	27.80± 1.50	62.83±4.5	126.7±6.5	3.17±0.01	0.014±0.005	0.52±0.03
lut2	0	19.89±1.40	110.07± 7.70	78.2±6.2	ND	3.56±0.02	0.171±0.030	ND
	10	20.25±1.00	107.73± 6.53	75.9±3.5	ND	3.46±0.01	0.412±0.020	ND
	90	21.44±0.80	107.53± 4.60	73.5±2.9	ND	3.35±0.03	0.420±0.007	ND
	360	21.33±2.00	108.52± 9.20	73.0±3.0	ND	3.25±0.04	0.416±0.010	ND
Wild type	0	26.76±0.90	38.35± 2.90	64.6±2.8	112.9±4.5	3.20±0.01	0.242±0.007	0.47±0.01
	10	25.50±2.00	35.58± 3.20	62.2±3.4	111.7±2.9	3.15±0.03	0.532±0.009	0.48±0.02
	90	27.44±2.50	46.49± 3.90	61.3±4.9	124.7±7.8	3.17±0.05	0.642±0.010	0.48±0.03
	360	28.07±1.90	52.86± 4.30	60.8±2.1	112.9±4.3	3.13±0.02	0.736±0.020	0.48±0.02

50 (*lut*2), 63.4 (*npq*1), and 75.3 (*npq*1-*lut*2) %, respectively. The relative tolerances of the room temperature HI treatment were consistent with the xanthophyll-dependent energy dissipation capacity (WT > *lut*2 > *npq*1 > *lut*2-*npq*1) and in contrast to the data in Fig. 2 at low temperature. The result demonstrated that *lut*2 was better able to acclimate to HI at room temperature than at LT.

After HI treatment at room temperature, the detached leaves were submitted to a 2-h room temperature recovery at a PPFD of 20 μmol m^{-2} s^{-1}. As shown in Fig. 5, the inhibition of F_v/F_m could be completely recovered as leaves exposed to HI from 10 to 30 min in all four strains, however, it was irreversible in mutants exposed to HI for longer time (2–4 h). The sequence of recovery was WT > *lut*2 > *npq*1 > *lut*2-*npq*1, similar to F_v/F_m during treatment.

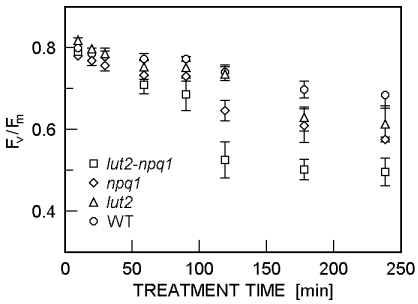

Fig. 5. F_v/F_m recovery under low irradiance for 2 h. After high irradiance (HI) treatment for different time at room temperature, the detached leaves were submitted to a 2 h recovery in PPFD of 20 μmol m^{-2} s^{-1}. The time scale thus refers to the length of time the samples were exposed to HI in Fig. 4; all samples underwent the same 2 h recovery period. Means ± S.D. of three replicates.

Discussion

When leaves of three *A. thaliana* xanthophyll mutants were exposed to HI at room temperature, the extent of photoinhibition and recovery were, as expected, consistent with the observed variations in the photoprotective energy dissipation capacity (Figs. 4 and 5). The double mutant *lut*2-*npq*1 that lacks both L and V de-epoxidase had the lowest photoprotective capacity and was the most susceptible. The most tolerant to excess photon energy was the WT followed by the *lut*2 and *npq*1 in order of the measured photoprotective capacity. These results were consistent with Niyogi *et al*. (1997), who reported that a double mutant of green alga *Chlamydomonas reinhardtii* lacking almost NPQ was extremely sensitive to HI. Niyogi *et al.* (1998) reported that de-epoxidation of V to A and Z is necessary for most of the rapidly NPQ reversible *Arabidopsis* mutants. The same conclusion can be drawn from our previous data (Gilmore 2001). Induction of NPQ under HI was strongly reduced both in *lut*2-*npq*1 and *npq*1 because of inhibition of the formation of Z and A, whereas *lut*2 only showed slightly less NPQ than the WT, which was also consistent with the report by Pogson *et al.* (1998).

When the three mutant strains and the WT were subjected to the same HI at chilling, as opposed to room temperature, the stress tolerance levels revealed an unanticipated pattern that was not consistent with the xanthophyll dependent photoprotection capacity. The tolerance sequence was altered at chilling mainly between the *npq*1 and *lut*2: *lut2* was more sensitive to photoinhibition than *npq*1. We put forth that this is not surprising because L is the predominant oxygenated carotenoid in plants where it functions as a central, but apparently inessential structural component of the main LHC2b and other Chl *a* and *b* binding proteins (Kuhlbrandt *et al.* 1994) and is apparently positioned to efficiently scavenge ^{3}Chl and $^1O_2$. L may also have more direct role in heat dissipation (Gilmore and Yamamoto 1990, Niyogi *et al*. 1997). Since *npq*1 lacked Z formation under strong irradiance (Havaux and Niyogi 1999), it is likely that the higher tolerance to photoinhibition at chilling temperature in *npq*1*,* compared to *lut*2, was mostly dependent on the WT xanthophyll composition of the antenna. Hence *npq*1 exhibits about 50 % of the xanthophyll cycle-dependent NPQ capacity of the WT (Gilmore 2001).

Taken together, our results demonstrated that the role of xanthophyll composition in the photoprotection was considerably complicated under these contrasting irradiance and temperature stress conditions. It is generally believed that the intensified photoinhibition and photooxidation by LT is in relation to the altered stabilities of membrane and pigment-protein complex (Saxton *et al.* 1979, Van Hasselt and Van Berlo 1980, Venema *et al.* 2000). The main function of L at LT is probably due to its structural/conformational influence in the LHCs and or antioxidant properties. Perhaps key structural elements of the antenna are compromised when the xanthophyll cycle analogues V, Z, or A are substituted for L as earlier described by Gilmore (2001) and Pogson *et al.* (1998). The *lut*2 lines exhibit strongly reduced NPQ *versus* Z+A efficiency compared to the L replete lines which indicates that L substitution alters the structural and/or conformational integrity of antenna proteins. There is particularly high and potentially lethal excess radiant energy sensitivity when all L is replaced with all Z for instance (Pogson *et al.* 1998). We thus conclude that other structural and/or antioxidant roles may be more important than excess radiant energy dissipation at LT (compared to room temperature) when the native pool of L is substituted with xanthophyll cycle components. There is a need for further investigation into the molecular mechanism(s) responsible for the different sensitivities to irradiance-temperature combinations between these and related xanthophyll cycle and L-deficient mutants of *Arabidopsis*.

References

Barber, J., Andersson, B.: Too much of a good thing: light can be bad for photosynthesis. – Trends biochem. Sci. **17**: 61-66, 1992.

Bassi, R.L., Rigoni, F., Giacometti, G.M.: Chlorophyll binding proteins with antenna function in higher plants and green algae. – Photochem. Photobiol. **52**: 1187-1206, 1990.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Demmig-Adams, B., Gilmore, A.M., Adams, W.W., III: *In vivo* functions of carotenoids in plants. – FASEB J. **10**: 403-412, 1996.

Demmig-Adams, B., Winter, K., Krüger, A., Czygan, F.-C.: Zeaxanthin synthesis, energy dissipation, and photoprotection of photosystem II at chilling temperature. – Plant Physiol. **90**: 894-898, 1989.

Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. – Physiol. Plant. **99**: 197-209, 1997.

Gilmore, A.M.: Xanthophyll cycle-dependent nonphotochemical quenching in photosystem II: Mechanistic insights gained from *Arabidopsis thaliana* L. mutants that lack violaxanthin deepoxidase activity and/or lutein. – Photosynth. Res. **67**: 89-101, 2001.

Gilmore, A.M., Ball, M.C.: Protection and storage of chlorophyll in overwintering evergreens. – Proc. nat. Acad. Sci. USA **97**: 11098-11101, 2000.

Gilmore, A.M., Shinkarev, V.P., Hazlett, T.L., Govindjee: Quantitative analysis of the effects of intrathylakoid pH and xanthophyll cycle pigments on chlorophyll *a* fluorescence lifetime distributions and intensity in thylakoids. – Biochemistry **37**: 13582-13593, 1998.

Gilmore, A.M., Yamamoto, H.Y.: Zeaxanthin formation in q_E-inhibited chloroplasts. – In: Baltscheffsky, M. (ed.): Current Research in Photosynthesis. Vol. II. Pp. 495-498. Kluwer Academic Publishers, Dordrecht – Boston – London 1990.

Gilmore, A.M., Yamamoto, H.Y.: Resolutions of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C_{18} high-performance liquid chromatographic column. – J. Chromatogr. **543**: 137-145, 1991.

Gilmore, A.M., Yamamoto, H.Y.: Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. – Photosynth. Res. **35**: 67-78, 1993.

Gilmore, A.M., Yamamoto, H.Y.: Time-resolution of the antheraxanthin and ΔpH-dependent chlorophyll *a* fluorescence components associated with photosystem II energy dissipation in *Mantoniella squamata*. – Photochem. Photobiol. **74**: 291-302, 2001.

Havaux, M., Niyogi, K.K.: The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. – Proc. nat. Acad. Sci. USA **96**: 8762-8767, 1999.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Kühlbrandt, W., Wang, D.N., Fujiyoshi, Y.: Atomic model of plant light-harvesting complex by electronic biology crystallography. – Nature **367**: 614-621, 1994.

Kyle, D.J., Osmond, C.B., Arntzen, C.J. (ed.): Photoinhibition. – Elsevier, Amsterdam – New York – Oxford 1987.

Li, X.P., Björkman, O., Shih, C., Grossman, A., Rosenquist, M., Jansson, S., Niyogi, K.K.: A pigment-binding protein essential for regulation of photosynthetic light harvesting. – Nature **403**: 391-395, 2000.

Lokstein, H., Tian, L., Polle, J.E.W., DellaPenna, D.: Xanthophyll biosynthetic mutants of *Arabidopsis thaliana*: altered nonphotochemical quenching of chlorophyll fluorescence is due to changes in Photosystem II antenna size and stability. – Biochim. biophys. Acta **1553**: 309-319, 2002.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. – Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Lunde, C., Jensen, P.E., Naldrup, A., Knoetzel, J., Scheller, H.V.: The PS I-H subunit of photosystem I is essential for state transitions in plant photosynthesis. – Nature **408**: 613-615, 2000.

Moll, B.A., Steinback, K.E.: Chilling sensitivity in *Oryza sativa*: the role of protein phosphorylation in protection against photoinhibition. – Plant Physiol. **80**: 420-423, 1986.

Müller, P., Li, X.P., Niyogi, K.K.: Non-photochemical quenching. A response to excess light energy. – Plant Physiol. **125**: 1558-1566, 2001.

Niyogi, K.K.: Safety valves for photosynthesis. – Curr. Opin. Plant Biol. **3**: 455-460, 2000.

Niyogi, K.K., Björkman, O., Grossman, A.R.: The roles of specific xanthophylls in photoprotection. – Proc. nat. Acad. Sci. USA **94**: 14162-14167, 1997.

Niyogi, K.K., Grossman, A.R., Björkman, O.: *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. – Plant Cell **10**: 1121-1134, 1998.

Niyogi, K.K., Shih, C., Chow, W.S., Pogson, B.J., Della Penna, D., Björkman, O.: Photoprotection in a zeaxanthin- and lutein-deficient double mutant of *Arabidopsis*. – Photosynth. Res. **67**: 139-145, 2001.

Pogson, B.J., McDonald, K., Truong, M., Britton, G., Dellapenna, D.: *Arabidopsis* carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants. – Plant Cell **8**:1627-1639, 1996.

Pogson, B.J., Niyogi, K.K., Björkman, O., Della Penna, D.: Altered xanthophyll compositions adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. – Proc. nat. Acad. Sci. USA **95**: 13324-13329, 1998.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Rissler, H.M., Pogson, B.J.: Antisense inhibition of the beta-carotene hydroxylase enzyme in *Arabidopsis* and the implications for carotenoid accumulation, photoprotection and antenna assembly. – Photosynth. Res. **67**: 127-137, 2001.

Saxton, M.J., Breidenbach, R.W., Lyons, J.M.: Membrane dynamics: Effect of environmental stress. – Basic Life Sci. **14**: 203-233, 1979.

Siefermann-Harms, D.: The light-harvesting and protective functions of carotenoids in photosynthetic membranes. – Physiol. Plant. **69**: 561-568, 1987.

Van Hasselt, P.R., Van Berlo, H.A.C.: Photooxidation damage to the photosynthetic apparatus during chilling. – Physiol. Plant. **50**: 52-56, 1980.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Venema, J.H., Villerius, L., Van Hasselt, P.R.: Effect of acclimation to suboptimal temperature on chilling-induced photodamage: Comparison between a domestic and a high-altitude wild *Lycopersicon* species. – Plant Sci. **152**: 153-163, 2000.