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DOES LUTEIN PLAY A KEY ROLE IN THE PROTECTION OF PHOTOSYNTHETIC APPARATUS IN *ARABIDOPSIS* UNDER SEVERE OXIDATIVE STRESS?

HONG-YING HUANG[1, 2, §], QIANG ZHANG[1, §], LI-PING ZHAO[2], JI-NAN FENG[2] AND CHANG-LIAN PENG[1]*

[1]*Key Laboratory of Ecology and Environmental Science in Guangdong Higher Education, College of Life Sciences, South China Normal University, Guangzhou 510631, China*
[2]*Department of Chemistry and Life Science, Xiangnan College, Chenzhou, Hunan 423000, China*
**Corresponding author: E-mail: pengchl@scib.ac.cn; Tel: + 86-20-85217612, Fax: + 86-20-85217612,*
[§] *These authors contributed equally to this work*

Abstract

Many environmental stresses result in increased generation of reactive oxygen species (ROS), such as superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH), in plant cells. A mild or moderate stress induces a significant increase in the generation of $\cdot O_2^-$ and H_2O_2 and a severe stress induces a significant increase in the generation of ·OH. The three ROS: $\cdot O_2^-$, H_2O_2 and ·OH were used to treat leaf discs of the wild type (WT), the *npq1* mutant lacking zeaxanthin and the *lut2* mutant lacking lutein in *Arabidopsis* by chlorophyll fluorescence imaging to test our previous hypothesis that lutein might play an important photoprotective role under severe stress. During the $\cdot O_2^-$ and H_2O_2-treatment under light, F_v/F_m, Φ_{PSII}, ETR, qP and NPQ exhibited the sequence of sensitivity to ROS in the leaves of the three phenotypes: *npq1* > *lut2* > WT. However, during the ·OH-treatment under light, these chlorophyll parameters exhibited another different sequence: *lut2* > *npq1* > WT. Thus, it can be concluded that xanthophyll cycle plays a key role under mild and moderate stress but lutein plays a key role under severe stress. These results provided more experimental evidence to support our previous hypothesis.

Abbreviations: $\cdot O_2^-$– superoxide; ·OH– hydroxyl radical; ETR– apparent electron rate; F_m– maximum fluorescence yield of a dark-adapted leaf disc; F_m'– maximum fluorescence yield of a light-adapted leaf disc; F_o– minimum fluorescence yield of a dark-adapted leaf disc; F_v/F_m– maximal PSII quantum yield; H_2O_2– hydrogen peroxide; NPQ– nonphotochemical quenching; qE– $\triangle$pH-dependent component of nonphotochemical quenching; qP– coefficient of photochemical quenching; ROS– reactive oxygen species; Φ_{PSII}– effective PSII quantum yield.

Introduction

Plants functioning in an aerobic environment are often subjected to continuous threat from molecular oxygen which is due to toxic ROS like $\cdot O_2^-$, H_2O_2 and ·OH (Khan & Panda, 2002; Panda, 2002). Most environmental stresses (e.g., cold, drought or high light intensity) have an effect on the production of ROS in plants, causing oxidative stress (Vranová *et al*., 2002). Even during normal metabolism, ROS are generated as a side product in electron transport processes, such as photosynthesis and respiration. The plant will suffer oxidative stress once the balance between the production of ROS and the quenching activity of antioxidant is upset, resulting oxidative damage (Elstner *et al*., 1988; Smirnoff, 1993). Plants respond to these day-to-day or long-term stress exposures by particular stress-induced responses and have evolved a number of mechanisms to protect

themselves from ROS-mediated damage (Lichtenthaler, 1996, 1998; Rentel & Knight 2004). The xanthophyll cycle can be considered to be an important protective mechanism that helps to minimize oxidative damage to the photosynthetic apparatus (Muller *et al*., 2001; Niyogi *et al*., 2001). When a plant absorbs incident light energy that exceeds its capacity for CO_2 fixation, excess light energy leads to oxidative stress, resulting in increased generation of ROS causing photo-oxidative damage (Asada, 1996). The xanthophyll cycle consists of the enzymatic interconversions of the three carotenoid, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). The pigment Z is formed from V via the intermediate A when leaves are exposed to high light (Verhoeven *et al*., 2001) and both Z and A are involved in a process that facilitates the increased thermal dissipation of the excess absorbed excitation energy in the light-harvesting antennae of photosystem II (PSII) (Demmig *et al*., 1988; Gilmore & Yamamoto, 1993; Havaux & Niyogi, 1999).Therefore, the plants can avoid the oxidative damage by the xanthophyll cycle. More than one mechanism to scavenge ROS protects plants from oxidative damage (Havaux & Niyogi, 1999). Despite the function of xanthophyll cycle has been well understood, the physiological function of lutein, which is the major component of carotenoid and constitutes approximately 50% of the total carotenoid content, is still not clear up to now.

In the last decades, with the development of genetic and molecular research tools, new ways to study light stress and photosynthesis have been opened (Niyogi, 1999). The *Arabidopsis npq1* mutant, being defective in the violaxanthin de-epoxidase gene, is unable to synthesize zeaxanthin *via* the xanthophyll cycle. Characterization of *npq1* plants have provided molecular genetic evidence that zeaxanthin is necessary for most the qE component of NPQ (Niyogi *et al*., 1998) and that zeaxanthin has an additional role in prevention of lipid peroxidation (Havaux & Niyogi, 1999). The *lut2* mutant affects the lycopene □-cyclase gene of *Arabidopsis* and is, therefore, unable to synthesize either lutein or *α*-carotene. Instead, flux through the carotenoid biosynthesis pathway is directed only into the *β*-carotene branch of the pathway, and the absence of lutein is compensated by increased levels of xanthophylls derived from *β*-carotene (Pogson *et al*., 1996). Our earlier work implied that lutein might play a key role in photoprotection as a secondary barrier (Peng & Gilmore, 2003). To continue our analysis of the roles of lutein in photoprotection, we used *npq1* mutant and *lut2* mutant of *Arabidopsis* that is unable to accumulated zeaxanthin (*via* the xanthophyll cycle) and lutein, respectively. The present study would provide more experimental evidence to advance our understanding of the physiological functions of lutein and contribute to the elucidation of the mechanisms that protect the photosynthetic apparatus in plants. In addition, under mild and moderate stress, most of increased ROS are $\cdot O_2^-$ and H_2O_2 (e.g., Jiang, 1999; Jiang & Zhang, 2002; Sharkey, 2005) and under severe stress, most of increased ROS is ·OH (e.g., Babbs *et al*., 1989; Jiang, 1999; Alvarez-Peral *et al*., 2002). Therefore, the present study was conducted to characterize the role played by lutein under oxidative stress of varying levels.

Materials and Methods

Plant materials and growth conditions: Seeds of *Arabidopsis thaliana* wild-type (WT), *npq1* mutant and *lut2* mutant were provided by the *Arabidopsis* Biological Resource Center, Columbus, OH, USA. The *npq1* mutant and the *lut2* mutant of *Arabidopsis* are unable to accumulate zeaxanthin and lutein, respectively. Seeds were imbibed for 2 days at 4°C in the dark to synchronise germination, and then sown on sterilised compost. Plants were grown routinely in a controlled growth chamber at 20-22°C with a 16-h photoperiod (80μmol m^{-2} s^{-1}) and a relative humidity of 80%. The third and fourth mature leaves from 25-day-old plants were used for the present study.

Three reactive forms of oxygen treatment: The leaf discs were exposed to 10 μmol/L MV solutions ($\cdot O_2^-$ -treatment), 100 mmol/L H_2O_2 solutions (H_2O_2-treatment) and a combination of 50 mmol/L H_2O_2 and 10 mmol/L $FeSO_4$ (·OH-treatment), respectively. The leaf discs (diameter: 8 mm) which were soaked in the three different solutions were placed in a controlled growth chamber at 25 °C with illumination (90μmol m^{-2} s^{-1}) and a relative humidity of 80% for the stress treatment.

Chlorophyll fluorescence imaging measurements: Chlorophyll fluorescence measurements were carried out with an IMAGING-PAM chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) as described by Siebke & Weis (1995) and Rascher *et al*., (2001). Fluorescence was measured with relative weak measuring light pulses (0.5 μmol m^{-2} s^{-1}) at a low frequency (1Hz) for measurement of F_o. F_m was measured during an 800-ms exposure to a PPFD of approximately 2700 μmol m^{-2} s^{-1}. The light intensity of continuous actinic illumination was adjusted to 185 μmolm^{-2} s^{-1}. All fluorescence measurements were started after an additional 10-min dark adaptation. After an area of interest (AOI) was selected in the leaf disc, Values of the chlorophyll fluorescence parameters F_o, F_m, F_m', F_v/F_m, Φ_{PSII}, NPQ and qP were the average of the AOI. In addition, their images were simultaneously obtained from the IMAGING-PAM software. The definition and calculation of ETR were performed according to Shao *et al*., (2008).

Statistical analysis: All of the data were from three to four measurements. Differences between different treatments were tested by one-way ANOVA. Statistical analyses were performed with SPSS11.5 (SPSS, Chicago, IL).

Results

Effects of ROS-treatment on F_v/F_m in the leaves of three *Arabidopsis* phenotypes: Chlorophyll fluorescence parameters are indicative of several important processes in the photosynthetic apparatus. During normal cellular functioning, F_v/F_m in plants is very stable; however, this parameter decreases significantly when plants are exposed to photoinhibition. Therefore, F_v/F_m is a sensitive parameter to probe the degree of photoinhibition in plants (Xu *et al*., 1992). Figure 1 shows an obvious decreasing trend in F_v/F_m in *Arabidopsis* leaves over 0-240 min ROS-treatment under illumination, which indicated that the sensitivity of PSII in the leaves of three *Arabidopsis* phenotypes was significantly different and that the WT type had a higher tolerance to the ROS-treatment under illumination than the two mutants. The WT type, the *lut2* mutant and the *npq1* mutant exhibited a different pattern in the response to the three ROS-treatments. During the $\cdot O_2^-$-treatment and the H_2O_2-treatment under illumination, the *npq1* mutant was the most sensitive and F_v/F_m declined by 53.9% and 80.2% after treatment for 240 min., respectively. Simultaneously, during the $\cdot O_2^-$-treatment and the H_2O_2-treatment under light, the WT type showed the highest capacity of tolerance and F_v/F_m only declined by 31.0% and 41.9% after treatment for 240 min, respectively. Among the three *Arabidopsis* phenotypes, it was evident that the capacities of tolerance to the two ROS treatment exhibited the sequence WT □ *lut2* □ *npq1* (Fig. 1A & B) ($p<0.05$). However, during the ·OH-treatment under light, the values of F_v/F_m in the leaves of the WT type, *npq1* mutant and *lut2* mutant decreased by 55.4%□84.1% and 100% after treatment for 240 min., respectively. The *lut2* mutant showed a higher sensitivity to ·OH-treatment under light compared to the *npq1* mutant and the capacities of tolerance to the ROS-treatment

displayed the sequence WT □ *npq1* □*lut2* (Fig. 1C) ($p<0.01$), which markedly differed from that in the $\cdot O_2^-$-treatment and the H_2O_2-treatment.

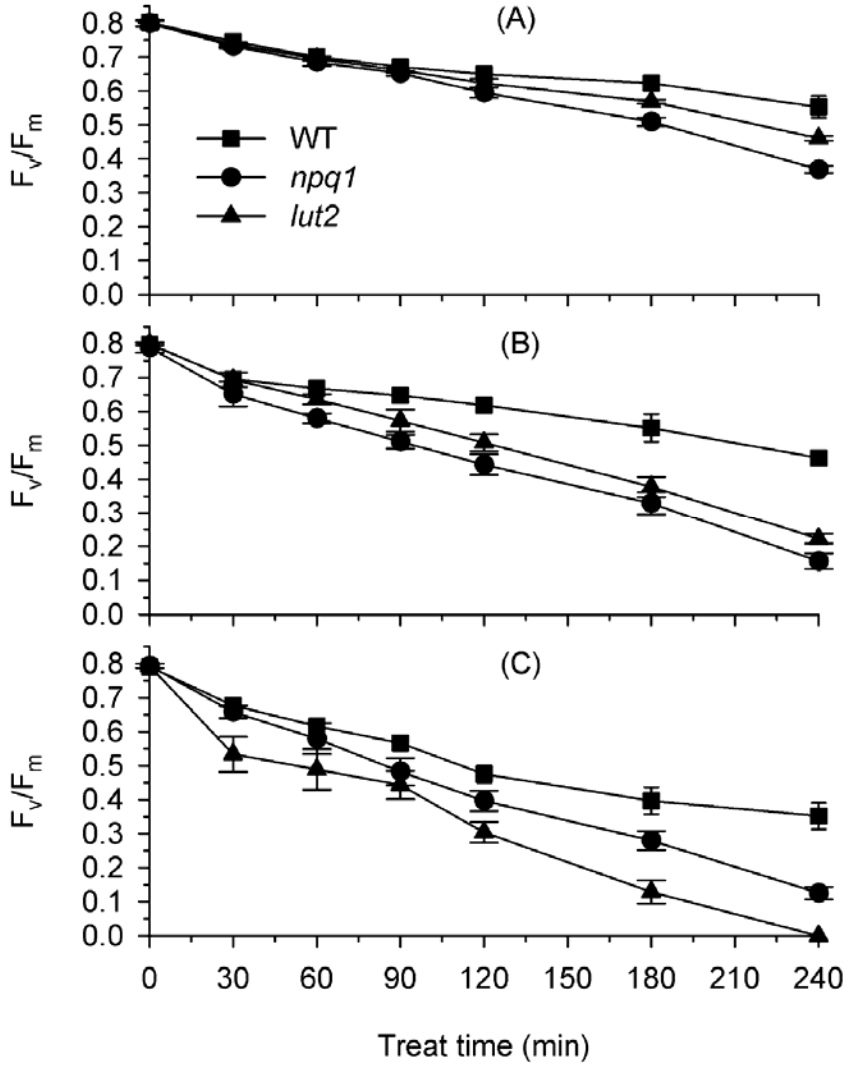

Fig. 1. Effects of different ROS treatment on the maximum PSII quantum yield (F_v/F_m) in the leaves of three *Arabidopsis* phenotypes (A. $\cdot O_2^-$-treatment; B. H_2O_2-treatment C. $\cdot$OH-treatment). Data are the mean ± standard error (*n*=3-4).

Images of F_v/F_m in the leaves of *Arabidopsis* during different exogenous ROS-treatment under light exhibited remarkable differences (Fig. 2). After $\cdot O_2^-$-treatment and H_2O_2-treatment under light for 240 min., the imaging colour of F_v/F_m in the leaves of *Arabidopsis* changes from blue ($F_v/F_m = 0.8$) to mélange of red and yellow ($F_v/F_m = 0.2 \sim 0.3$). It was evident that the quantum efficiency of light energy transfer in PSII was near to 0.2. However, after ·OH-treatment under light for 240 min, the quantum efficiency of light energy transfer in PSII in the leaves of the *lut2* mutant was almost zero.

Effects of ROS-treatment on Φ_{PSII} and ETR in the leaves of three *Arabidopsis* phenotypes: The quantum efficiency and electron transport rate of non-cyclic electron transport are indicated by Φ_{PSII} and ETR. Φ_{PSII} measures the proportion of the light absorbed by chlorophyll associated with PSII that is used in photochemistry (Krall & Edward, 1992; Maxwell & Johnson, 2000), and ETR is a reflection of PSII activity (Genty *et al*., 1989). Over the 0-240 min., $\cdot O_2^-$-treatment and H_2O_2-treatment under light, the values of both Φ_{PSII} and ETR in the leaves of *Arabidopsis* decreased progressively, which

illustrated that the *npq1* mutant decreased at the highest rate and the WT type declined at the lowest rate. The capacities of tolerance to the two ROS-treatments in *Arabidopsis* exhibited the sequence WT □ *lut2* □*npq1* (Figs. 3 & 4) ($p<0.05$) However, during the ·OH-treatment under light for 240 min., the leaves of the three *Arabidopsis* phenotypes showed a different response compared to that during the $\cdot O_2^-$-treatment and H_2O_2-treatment and the *lut2* mutant were the most sensitive. The capacities of tolerance to the ·OH-treatment in the three *Arabidopsis* phenotypes displayed the sequence WT □*npq1* □ *lut2* ($p<0.05$).

Fig. 2 also showed the images of Φ_{PSII} in the leaves of *Arabidopsis* under the three ROS-treatments under illumination. After 240 min $\cdot O_2^-$-treatment and H_2O_2-treatment, the imaging colour of Φ_{PSII} in the leaves of *Arabidopsis* changes from green ($\Phi_{PSII} = 0.5$) to mélange of red and yellow ($\Phi_{PSII} = 0.1 \sim 0.2$), and meanwhile an area of black ($\Phi_{PSII} = 0$) was also observed, which means that the quantum efficiency of non-cyclic electron transport was close to 0.1. However, after 240 min ·OH-treatment under light, the quantum efficiency of non-cyclic electron transport in the leaves of the *lut2* mutant was zero.

Effects of ROS-treatment on qP and NPQ in the leaves of three *Arabidopsis* phenotypes: NPQ are indicative of the capacity of harmlessly dissipate excess excitation energy as heat in plants (Muller *et al.*, 2001). As shown in Fig. 4, over 0-240 min ROS-treatment, NPQ showed a tendency to decrease in the three *Arabidopsis* phenotypes. NPQ was inhibited greatly in the *npq1* mutant lack of Z during the ROS treatment, which implied that the capacity of thermal dissipation of excess absorbed light energy obviously reduced because of lack of Z, consistent with previous study on *Chlamydomonas reinhartii* mutant lack of Z (Havaux & Niyogi, 1999), which demonstrated the NPQ strictly depending on the xanthophyll cycle pigment pool. Especially during the ·OH-treatment, the values of NPQ was almost zero in the leaves of the three *Arabidopsis* phenotypes (Fig. 5C) within 30 min., which indicated that their photosynthetic apparatus were completely damaged because of their lost capacity of thermal dissipation of excess absorbed light energy as heat.

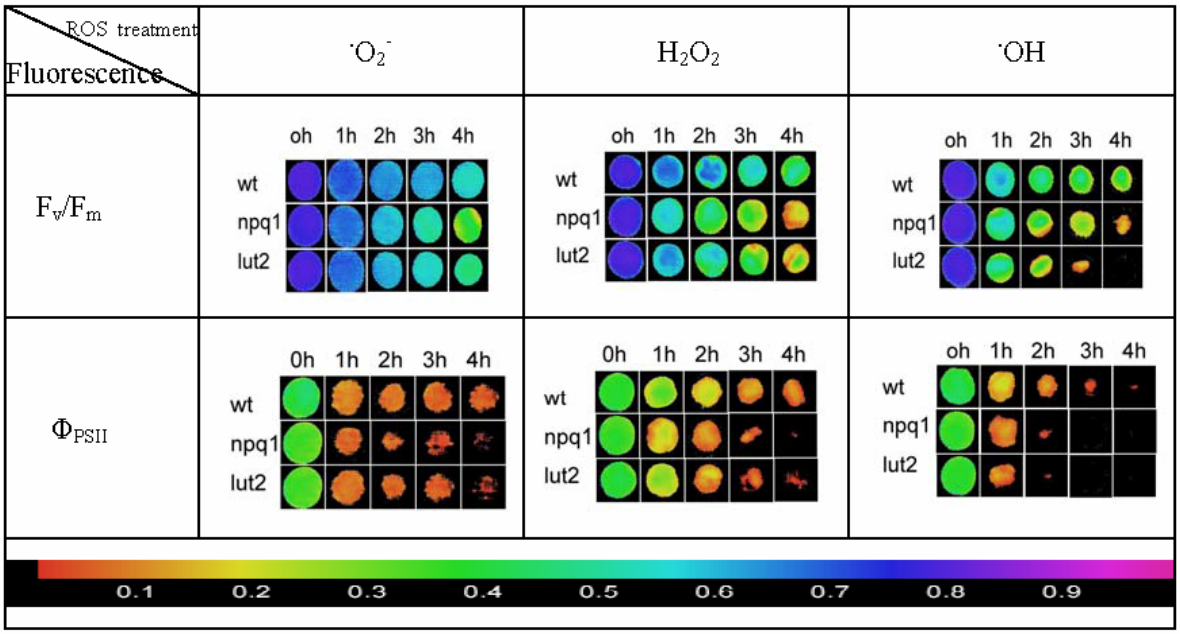

Fig. 2. Changes in the fluorescence images of the maximum quantum yield (F_v/F_m) and the effective PSII quantum yield (Φ_{PSII}) in the leaves of three *Arabidopsis* phenotypes under different ROS treatment ((A. $\cdot O_2^-$-treatment; B. H_2O_2-treatment C. ·OH-treatment). Fluorescence images are

indicated by the false colour code at the bottom. The code ranges from black via red, orange, yellow, green, blue and violet to purple, and these colours code for numbers between 0 and 1.

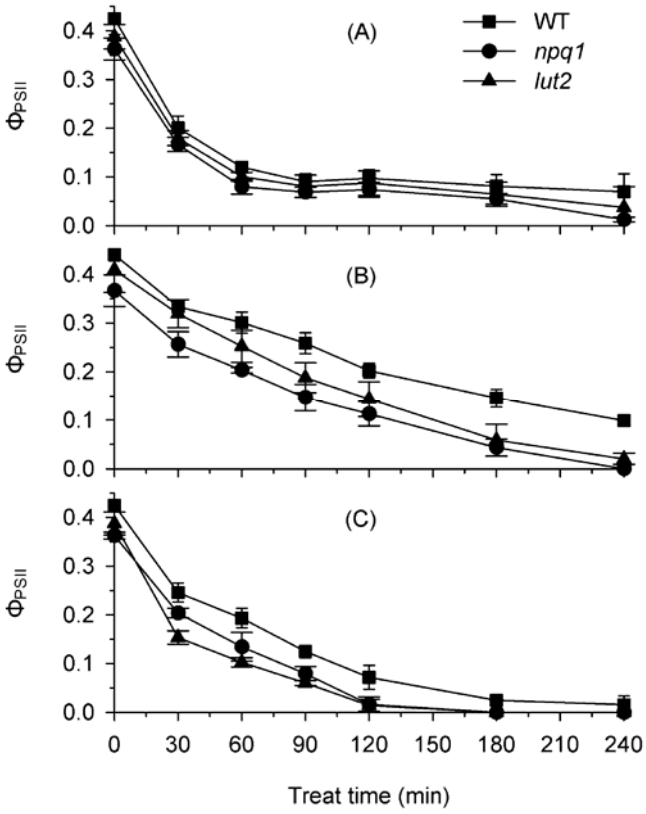

Fig. 3. Effects of different ROS treatment on the effective PSII quantum yield (Φ_{PSII}) in the leaves of three *Arabidopsis* phenotypes (A. $^{\cdot}O_2^-$-treatment; B. H_2O_2-treatment C. $^{\cdot}OH$-treatment). Data are the mean ± standard deviation (n=3-4).

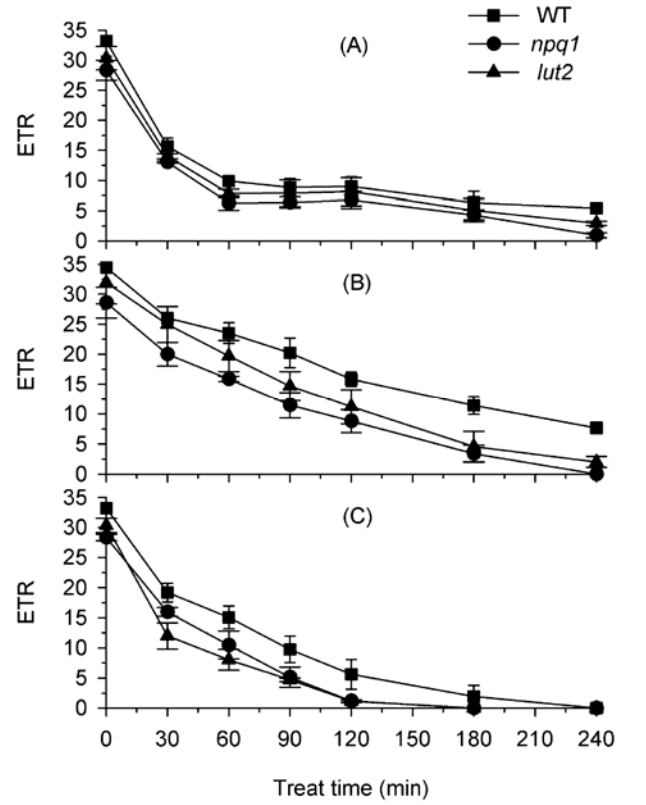

Fig. 4. Effects of different ROS treatment on the electron transport rate (ETR) in the leaves of three *Arabidopsis* phenotypes (A. $\cdot O_2^-$-treatment; B. H_2O_2-treatment C. $\cdot$OH-treatment). Data are the mean ± standard deviation (n=3-4).

The proportion of open reaction centers of PSII is indicated by qP, which is somewhat different from Φ_{PSII} (Maxwell & John, 2000). As shown in Fig. 6, a significant decrease in qP in the leaves of the three *Arabidopsis* phenotypes was observed during the ROS treatment. After the $\cdot O_2^-$-treatment and H_2O_2-treatment for 2h, the values of qP in the leaves of the *npq1* mutant declined by 71.1% and 47.1%, respectively; and that of the *lut2* mutant declined by 65.9% and 41.2%, respectively, which demonstrated that the *npq1* mutant has a higher sensitivity of the two ROS-treatment compared to the *lut2* mutant. However, after ·OH-treatment for 1h, the values of qP in the leaves of the WT type, the *npq1* mutant and *lut2* mutant decreased by 40.9%, 51.1% and 62.1%, respectively, which illustrated that the *lut2* mutant is the most sensitive. After ·OH-treatment for 2 h, the values of qP in the leaves of two mutants were zero, suggesting the ·OH-treatment causing the complete injury of Photosystem II.

Discussion

Many environmental stress conditions limit the ability of a plant to utilize light energy through photosynthesis so that excessive excitation of the photosystems can occur even at moderate light intensities (Demmig-Adams & Adams, 1992). When the absorption of light energy exceeds the capacity of photosynthesis and the photoprotective mechanisms are overwhelmed, ROS, such as$\cdot O_2^-$, H_2O_2, and ·OH, can be produced during illumination of chloroplast (Havaux & Niyogi, 1999), thereby resulting in oxidative stress. The productions of ·OH are implications for severe stress in plants (Jiang, 1999). Plants have evolved a number of mechanisms to protect themselves from ROS-mediated damage. Carotenoids play a key role in the protection of photosynthetic organisms against the toxic effects of light. In the last decades, lots of attentions were paid to the functions of xanthophyll cycle, however, little attention was paid to the research of lutein, which is the major component of carotenoid and constitutes approximately 50% of the total carotenoid content. In our previous work, we assumed that luteins might play a key role as a secondary barrier under severe stress (Peng & Gilmore, 2003), which has been proved by the present work. One importance of the present study is to confirm that the xanthophyll cycle plays a key role in the protecting *Arabidopsis* from the damages of $\cdot O_2^-$ and H_2O_2. During these two ROS treatments, the WT type and the *lut2* mutant showed higher capacities of tolerance than the *npq1* mutant, which implicated that the xanthophyll cycle plays a key role to protect plants from the damage of $\cdot O_2^-$ and H_2O_2. By the chlorophyll fluorescence measurement, F_v/F_m, Φ_{PSII} and qP demonstrated a decrease at lower rate in both the WT type and the *lut2* mutant compared to the *npq1* mutant during the two ROS treatments, consistent with our earlier study (Huang *et al.*, 2008). It is interesting that a significant increase in NPQ was observed in the WT type and the *lut2* mutant within the first 30 min $\cdot O_2^-$ treatment, consisted with our previous study, which was caused by the presence of MV enhancing the Mehler reaction with O_2 acting as an electron acceptor (photoreduction pathway of O_2) (Shao *et al.*, 2008). Thus, the xanthophyll cycle plays a key role in the protecting *Arabidopsis* under mild and moderate stress.

Another importance of the present study is to confirm that the lutein plays a key role in the protecting *Arabidopsis* from the damages of ·OH. A combination of iron accessibility and H_2O_2 results in the formation of the very reactive ·OH *via* the Fenton reaction, which can cause DNA damage (Nunoshiba *et al.*, 1999). In general, the production of ·OH implicated that plant suffered a severe stress. During the ·OH treatment, both the WT type and the *npq1* mutant showed a higher capacity of tolerance compared to the *lut2* mutant,

suggesting that the major component of carotenoids-lutein plays a more important functional role in the protection of photosynthetic apparatus in the plants under severe stress conditions.

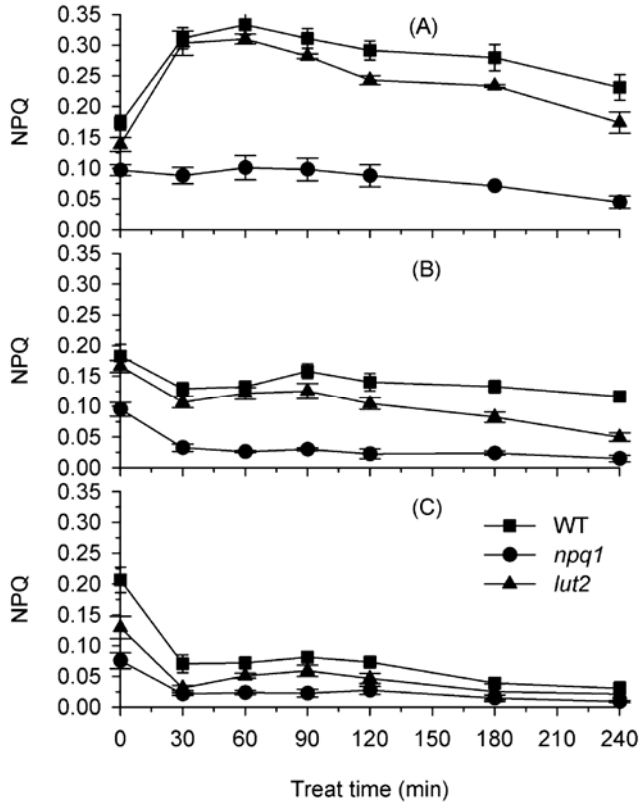

Fig. 5. Effects of different ROS treatment on the non-photochemical quenching (NPQ) in the leaves of three *Arabidopsis* phenotypes (A. $^{\cdot}O_2^-$-treatment; B. H_2O_2-treatment C. $^{\cdot}OH$-treatment). Data are the mean ± standard deviation (*n*=3-4).

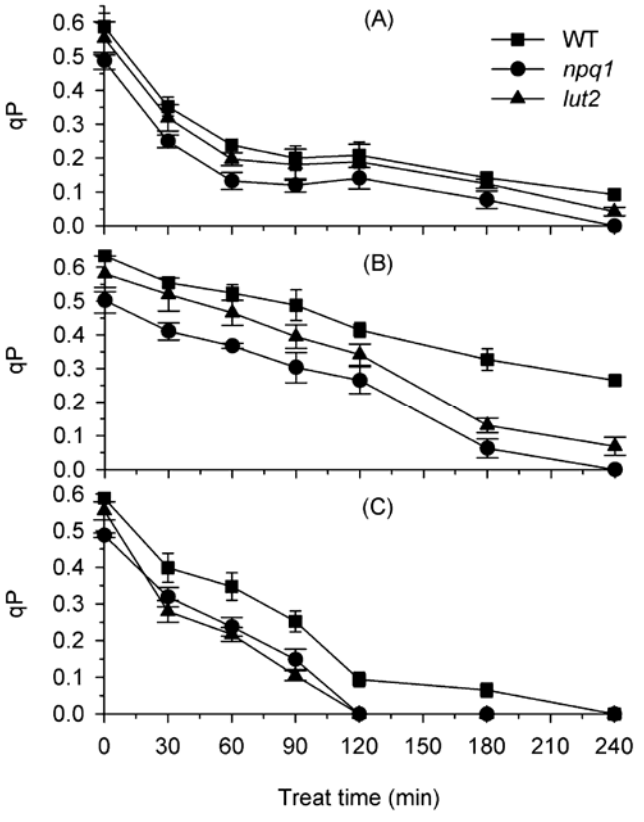

Fig. 6. Effects of different ROS treatment on the coefficient of photochemical quenching (qP) in the leaves of three *Arabidopsis* phenotypes (A. $\cdot O_2^-$-treatment; B. H_2O_2-treatment C. $\cdot OH$-treatment). Data are the mean ± standard deviation (n=3-4).

In conclusion, the present study not only confirmed the role played by xanthophyll cycle in the protection of photosynthetic organisms during mild stress and moderate stress, but also confirmed that lutein plays a key role in plants under severe stress. The present environmental evidence also confirmed our earlier hypothesis. Therefore, it can be concluded that lutein plays a key role as a secondary barrier in the protecting plants from severe oxidative stress.

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References

Alvarez-Peral, F.J., O. Zaragoza, Y. Pedreno and J-C. Argüelles. 2002. Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. *Microbiology*, 148: 2599-2606.

Asada, K. 1996. Radical production and scavenging in the chloroplasts. In: *Photosynthesis and the Environments*. (Ed.): N.R. Baker. Kluwer Academic Publishers, Dordrecht, pp. 123-150.

Babbs, C.F., J.A. Pham and R.C. Coolbaugh. 1989. Lethal hydroxyl radical production in paraquat-treated plants. *Plant Physiol*., 90: 1267-1270.

Demmig, B., K. Winter, A. Kruger and F-C. Czygan. 1988. Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. *Plant Physiol*., 87: 17-24.

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

Elstner, E.F., G.A. Wagner and W. Schutz. 1988. Activated oxygen in green plants in relation to stress situations. *Curr. Topics Plant Biochem. Physiol.*, 7: 159-187.

Genty, B., J.M. Briantals and N.R. Baker. 1989. Relationship between the quantum yield of photosynthetic electron transport and the quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta*, 990: 87-92.

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

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

Huang, H.Y., B. Deng, L.J. Su, D.G. He and C.L. Peng. 2008. Comparison on capacities of scavenging for O_2^- and ·OH by lutein and β-carotene. *Plant Physiol. Commun.*, 44: 445-448.

Jiang, M.Y and J.H. Zhang. 2002. Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. *Free Radical Res.*, 36: 1001-1015.

Jiang, M.Y. 1999. Generation of hydroxyl radicals and its relation to cellular oxidative damage in plants subjected to water stress. *Acta Bot. Sin.*, 41(3): 229-234.

Khan, M.H and S.K. Panda. 2002. Induction of oxidative stress in roots *Oryza sativa* L. in response to salt stress. *Biol. Plant*, 45: 625-627.

Krall, J.P and G.E. Edward. 1992. Relationship between photosystem II activity and CO_2 fixation in leaves. *Physiol. Plant.* 86: 180-187.

Lichtenthaler, H.K. 1996. Vegetation stress: an introduction to the stress concept in plants. *J. Plant Physiol.*, 148: 4-14.

Lichtenthaler, H.K. 1998. The stress concept in plants: an introduction. In: *Stress of Life: from Molecules to Man*. (Eds.): P. Csermely. Annals of New York Academy Sciences, pp. 187-198.

Maxwell, K and G.N. Johnson. 2000. Chlorophyll fluorescence–a practical guide. *J. Exp. Bot.*, 51: 659-668.

Muller, P., X.P. Li and K.K. Niyogi. 2001. Non-photochemical quenching. A response to excess high energy. *Plant Physiol.*, 125(4): 1558-1566.

Niyogi, K.K. 1999. Photoprotection revisited: Genetic and molecular approaches. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 50: 333-359.

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

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

Nunoshiba, T., F. Obata, A.C. Boss, S. Oikawa, T. Mori, S. Kawanishi and K. Yamamoto. 1999. Role of iron and superoxide for generation of hydroxyl radical, oxidative DNA lesions and mutagenesis in *Escherichia coli*. *J. Biol. Chem.*, 274: 34832-34837.

Panda, S.K. 2002. The biology of oxidative stress in green cells: A review. *In: Advances in Stress Physiology of Plants*. (Ed.): S.K. Panda. Scientific Publishers, India, pp. 1-13.

Peng, C.L and A.M. Gilmore. 2003. Contrasting changes of photosystem efficiency in *Arabidopsis* xanthophyll mutants at room or low temperature under high irradiance stress. *Photosynthetica*, 41(2): 233-239.

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

Rascher, U., M.T. Hütt, K. Siebke., B. Osmond, F. Beck and U. Lüttge. 2001. Spatiotemporal variation of metabolism in a plant circadian rhythm: The biological clock as an assembly of coupled individual oscillators. *Proc. Natl Acad. Sci. USA.*, 98: 11801-11805.

Rentel, M.C and M.R. Knight. 2004. Oxidative stress-induced calcium signaling in *Arabidopsis*. *Plant Physiol.*, 135: 1471-1479.

Shao, L., Z. Shu, S.L. Sun, C.L. Peng, Z.F. Lin and C.W. Yang. 2008. Enhanced sensitivity of *Arabidopsis* anthocyanin mutants to photooxidation: a study with fluorescence imaging. *Funct. Plant Biol.*, 35: 714-724.

Sharkey, T.D. 2005. Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant Cell Environ.*, 28: 269-277.

Siebke, K and E. Weis. 1995. Assimilation images of leaves of *Glechoma hederacea* Analysis of nonsynchronous stomata related oscillations. *Planta*, 196: 155-165.

Smirnoff, N. 1993. The role of active oxygen in the responses of plants to water deficit and desiccation. *New Phytol*., 125: 27-58.

Verhoeven, A.S., R.C. Bugos and B.H. Yamamoto. 2001. Transgenic tobacco with suppressed zeaxanthin formation is susceptible to stress-induced photoinhibition. *Photosynth. Res.*, 67: 27-39.

Vranová, E and D. Inzé. 2002. Van Breusegem F. Signal transduction during oxidative stress. *J. Exp. Bot.*, 53: 1227-1236.

Xu, D.Q., Y.Z. Zhang and R.X. Zhang. 1992. Photoinhibition of photosynthesis in plants. *Plant Physiol. Commun.*, 28: 237-243.

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