Chilling Tolerance of Photosynthesis in Plants is Dependent on the Capacity to Enhance the Levels of the Xanthophyll Cycle Pigments in Response to Cold Stress

Hyun-Ju Kim[1], In-Soon Kang[1], Chin Bum Lee[2], Choon-Hwan Lee[3], Sung Ho Cho[4] and Byoung Yong Moon[1]*

[1]Department of Biotechnology & Biomedical Sciences, Inje University, Gimhae 621-749, Korea
[2]Department of Biology, Dong-eui University, Busan 614-714, Korea
[3]Department of Molecular Biology, Pusan National University, Busan 609-735, Korea
[4]Department of Biological Sciences, Inha University, Incheon 402-751, Korea

Plants possess the ability to dissipate the excitation energy for the protection of photosynthetic apparatus from absorbed excess light. Heat dissipation is regulated by xanthophyll cycle in thylakoid membranes of chloroplasts. We investigated the mechanistic aspects of xanthophyll cycle-dependent photoprotection against low-temperature photoinhibition in plants. Using barley and rice as chilling-resistant species and sensitive ones, respectively, chilling-induced chlorophyll fluorescence quenching, composition of xanthophyll cycle pigments and mRNA expression of the zeaxanthin epoxidase were examined. Chilled barley plants exhibited little changes in chlorophyll fluorescence quenching either of photochemical or non-photochemical nature and in the photosynthetic electron transport, indicating low reduction state of PS II primary electron acceptor. In contrast to the barley, chilled rice showed a marked decline in those parameters mentioned above, indicating the increased reduction state of PS II primary electron acceptor. In addition, barley plants were shown to have a higher capacity to elevate the pool size of xanthophyll cycle pigments in response to cold stress compared to rice plants. Such species-dependent regulation of xanthophyll cycle activity was correlated with the gene expression level of cold-induced zeaxanthin epoxidase. Chilled rice plants depressed the gene expression of zeaxanthin epoxidase, whereas barley increased its expression in response to cold stress. We suggest that chilling-induced alterations in the pool size of xanthophyll cycle pigments related to its capacity would play an important role in regulating plant's sensitivity to chilling stress.

key words: chilling sensitivity, xanthophyll cycle, non-photochemical quenching, chlorophyll fluorescence, zeaxanthin epoxidase, photoprotection

INTRODUCTION

In the natural ecosystem, when plants are exposed to the incident light intensity higher than that required to be utilized in photosynthesis, excess light energy can damage the photosynthetic machinery and cause the reduction in the photosynthetic performance, a phenomenon termed photoinhibition [1]. Even the moderate light intensity, when combined with cold stress, can induce photoinhibition and photooxidation in higher plants.

When light energy absorbed by plants exceeds the capacity of photosynthesis, the primary photoprotective process is the non-radiative dissipation of energy absorbed in excess [2]. The phenomenon can be easily demonstrated as non-photochemical quenching of Chl fluorescence (NPQ). It is known that several processes contribute to NPQ. The major component is energization-dependent quenching, namely qE. It is rapidly reversible and associated with acidification of the

thylakoid lumen [3]. Acidification of thylakoid lumen induces protonation of specific LHC amino acid residues [4] that is proposed to cause conformational changes in antenna organization, leading to facilitate qE. Moreover, zeaxanthin production by the xanthopyll cycle in response to high-light stress has been correlated with NPQ [5, 6] When plants are exposed to strong light, they change their carotenoid composition of the chloroplast membranes with zeaxanthin (Z) being rapidly formed by converting violaxanthin (V) via the intermediate antheraxanthin (A) [5, 6, 7]. In low light or darkness, Z is reconverted to V. Such a pigment interconversion is called xanthophyll cycle or violaxanthin cycle. Regarding changes in pigments, violaxanthin de-epoxidase (VDE) is activated by low pH and converts V into Z. On the other hand, zeaxanthin epoxidase (ZE) catalyzes the reverse reactions and is inhibited by low pH. Therefore, the result of the ΔpH generated in high light is the formation of Z (+A) by the xanthophyll cycle. The combination of protonated LHCs and interaction of Z (+A) with LHCs are the major factors that cooperate in promoting qE [8, 9]. Recently it has been demonstrated that xanthophyll-dependent energy dissipation may also require PsbS and PsbZ proteins [10, 11] and involve

a structural change of the PS II core, possibly related to D1 phosphorylation [12].

Carotenoids can protect photosynthetic organisms against toxic effects of light because they quench ^3Chl and $^1O_2$ [13, 14]. It has also been suggested that Z could not only quench the singlet state of chlorophylls (^1Chl) but also stimulate proton-induced aggregation of the LHCs of PS II leading to energy dissipation. An increase in thermal deactivation of ^1Chl is helpful in protecting the PS II reaction centers from photoinhibition. Furthermore, it can reduce the possibility of ^3Chl and $^1O_2$ formation in the LHCs [15]. Previously, the photoprotective function of carotenoids had been related entirely to these reactions. It is now well established that function of carotenoids is not restricted to these mechanisms. Xanthophyll pigments including zeaxanthin and antheraxanthin have a specific function in the photoprotection of the photosynthetic system.

When plants are illuminated with strong light, plants grown under different light conditions show the marked increase in the sum of the xanthophylls cycle pigments, namely zeaxanthin, antheraxanthin, and violaxanthin. Thayer and Björkman [16] compared the pigment composition of shade and sun leaves for many species growing in shade or full sun and they found very large pool sizes of the xanthophyll cycle in the sun-grown leaves. The underlying mechanisms responsible for increased carotenoid synthesis in excessive light are not fully elucidated. On these perspectives, we speculated that the genetic background of the different chilling sensitivity of plants might involve formation of different pool sizes of the xanthophyll cycle affected by exposure to light during cold stress.

We propose in this work that chilling-resistant plants can tolerate low-temperature photoinhibition of the photosynthetic machinery, at least in part, by regulating the pool size of the xanthophyll cycle in response to cold stress.

MATERIALS AND METHODS

Plant materials and growth conditions.

Barley (*Hordeum vulgare* L. cv. Albori) and rice (*Oryza sativa* L. cv. Dongjin) plants were grown from seeds in a soil mixture (1:1) composed of vermiculite and a commercial soil (Bio-soil, Heung-nong Co., Korea). The seeds were germinated in darkness at 25°C and seedlings were grown in controlled growth room with temperature of 25°C under a 14 h photoperiod. The plants were illuminated by white fluorescent light at an intensity of 170 $\mu mol\ m^{-2}\ s^{-1}$.

Chilling treatment

For chilling treatment of plants, control plants were kept under the same growth condition, whereas other seedlings were transferred to chilling temperature (4°C) with the same light regime.

Extraction of pigments

Leaf segments with 3 cm in length were ground in liquid N_2 and solubilized in 2 ml of 100% acetone under dim light at 0-4°C. The homogenate was divided into two tubes and spun with a microcentrifuge at 4°C for 3 min at a speed of 12,000 rpm. The supernatant was removed and the pellets were extracted again with 1.25 ml of 100% acetone at room temperature for 5 min with occasional vortex mixing before spinning for 3 min. The supernatant was pooled and filtered through 0.2 μm syringe filters (Millex-FG, Millipore). The pigment extracts were immediately analyzed by HPLC.

Liquid chromatography

To measure xanthophyll cycle components, HPLC analysis was performed with HP 1100 Series Liquid Chromatograph (Hewlett Packard, Waldbronn, Germany) equipped with a Spherisorb ODS C_{18} column (5 μm-particle size, 4.6 x 250 mm, Alltech, USA) according to the modified procedure of Gilmore and Yamamoto [17]. The column was equilibrated at 25°C with acetonitrile:methanol:0.1 M Tris-HCl buffer (pH 8.0) (72:12:7, solvent A) for 10 min at a flow rate of 2 ml min^{-1}, followed by sample injection of 20 μl. Then the separation was carried out by eluting with solvent A for 3 min, followed by elution for 9 min with 6:4 mixture of solvent A and B (methanol:n-hexane = 4:1). Then a linear gradient of 0 to 100 of solvent B was applied for 1 min and finally eluted with 100% of solvent B until 25 min at the same flow rate as mentioned above. Detective wavelength was 440 nm and O.D. values were modulated with the range of 0-0.05.

RT-PCR

Total RNA was isolated using Tri-Reagent (Sigma) according to the manufacturer. Three micrograms of total RNA was used to synthesize the first strand of cDNAs, which were provided to reverse transcription for the detection of mRNA expression of zeaxanthin epoxidase. The product of reverse transcription was subjected to PCR with primers designed from the GenBank database (AB050884).

Measurement of chlorophyll fluorescence

Chlorophyll *a* fluorescence was measured at room temperature by a low-intensity modulated (1.6 kHz) beam from light-emitting diodes, with the excitation wavelength at 655 nm, and detected above 710 nm using the pulse amplitude modulation fluorometer PAM 101-103 (H. Walz, Effeltrich, Germany) [18]. Briefly, maximum F_m and minimum F_o fluorescence yields were determined at set time intervals after 15 min of dark adaptation. F_o was reached by illuminating leaf segments with measuring red light at an intensity of less than 0.1 $\mu mol\ m^{-2}\ s^{-1}$. F_m was reached by exposing leaf segments to saturated white light pulse at an intensity of 3,000 $\mu mol\ m^{-2}\ s^{-1}$. After measuring F_o and F_m, leaves were illuminated with 655 nm non-modulated actinic light (~112 $\mu mol\ m^{-2}\ s^{-1}$) superimposed by a series of 0.8 s pulses of

super-saturating white light (~4,000 μmol m^{-2} s^{-1}) fired at 30 s intervals. Fluorescence measurements were preceded by a 15-min period of darkness to allow for relaxation of qE quenching. The variable fluorescence, F_v, was given by the difference between F_m and F_o. The maximum PS II quantum yield was calculated as $F_v/F_m = (F_m - F_o)/F_m$. ETR, namely the relative electron transport rate, is the product of the effective quantum yield of PS II and was calculated as $(1-F_t/F_m') \times 0.5 \times$ PPFD (photosynthetic flux density) x leaf absorptance (given as about 0.85) [19]. Fluorescence quenching coefficients were calculated according to Schreiber et al [18]. F_t represents the steady-state fluorescence and F_m' the maximum fluorescence that were measured in the light, respectively. F_m' value was taken after applying saturated light pulses when all PS II reaction centers were closed. Non-photochemical quenching (NPQ) was calculated as $(F_m-F_m')/F_m'$ [20]. The nomenclature of fluorescence parameters was followed as described by van Kooten and Snel [21].

RESULTS

Cold-affected chlorophyll fluorescence induction

In order to examine how plants having different sensitivity to chilling respond to cold stress in terms of photochemical efficiency, we measured values of F_o and F_m as well as F_v/F_m that had been manifested, during growth at 4°C, by intact barley (chilling-resistant species) and rice seedlings (chilling-sensitive species). For chilling treatment, plants that had been grown at 25°C with a photoperiod of 14 L:10 D at an intensity of 170 μmol m^{-2} s^{-1} were exposed to 4°C with continuous light of the same intensity. Then leaves were detached and subjected to dark adaptation for 15 min at room temperature, followed by the induction of chlorophyll fluorescence. Fig.

1A and Fig. 1B show changes in F_v/F_m and in F_m or F_o in the course of light-chilling, respectively. Barley maintained F_v/F_m values in the range of 0.8 to 0.76 during growth for 5 days under cold stress, indicating that PS II photochemical efficiency was little affected. Contrary to barley plants, rice plants showed marked decline in F_v/F_m falling down to 0.31 from the original control value of 0.81, indicating that they severely suffered from cold stress. When grown under favorable temperature, namely at 25°C, rice plants showed normal F_v/F_m values in the range of 0.80 to 0.81during the same experimental period. It is noteworthy that F_o in rice remained relatively constant during cold stress in contrast to F_m showing rapid decline, suggesting that decrease in F_m was responsible for the cold-affected reduction in F_v/F_m (Fig. 1B).

Photochemical quenching (qP) and relative electron transport rate (ETR) are two other parameters of chlorophyll fluorescence, representing PS II photochemical efficiency in cooperation with F_v/F_m. Figure 2A showed cold stress-affected alterations of 1-qP in barley and rice leaves. Barley maintained a relatively constant low level of 1-qP in the course of cold treatment, in contrast to rice plants showing a sharp increase. Since 1-qP represents the relative amount of reduced Q_A, which is a primary electron acceptor of PS II, the cold-affected increase in 1-qP as observed in rice suggested that electron transport was slowed down as a result of inhibited Calvin cycle by cold temperature. The observation is consistent with the results shown in Fig. 2B, in which ETR of rice was markedly depressed by cold stress. Fig. 2B showed changes in ETR of rice and barley when affected by cold stress for 5 days. ETR of barley was maintained high quite constantly, as compared to rice showing a large decrease, falling down to about 50% of the original level after 3 days of growth at 4°C.
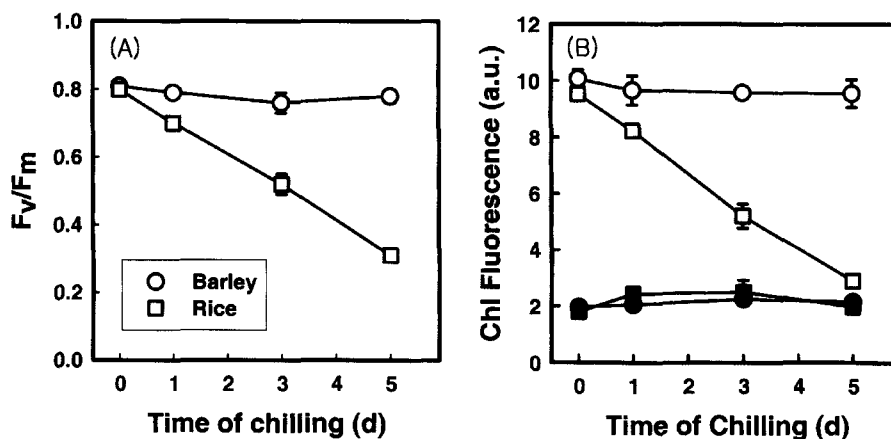

Figure 1. (A) Changes in F_v/F_m from intact leaves of barley (○) and rice (□) under light-chilling. (B) Changes in values of F_m (open symbol) and F_o (closed symbol) from intact leaves of barley (○) and rice (□) under light-chilling. For chilling treatment, plants that had been grown at 25°C for 5 days in the light at an intensity of 170 μmol m^{-2} s^{-1} were exposed to 4°C at the same PPFD for the designated period. Chlorophyll fluorescence was measured after 15 min of dark adaptation at room temperature to allow for relaxation of qE quenching. Each data point represents the mean ± SD of at least three separate experiments. If error bars are not shown, they are smaller than the size of the symbols.
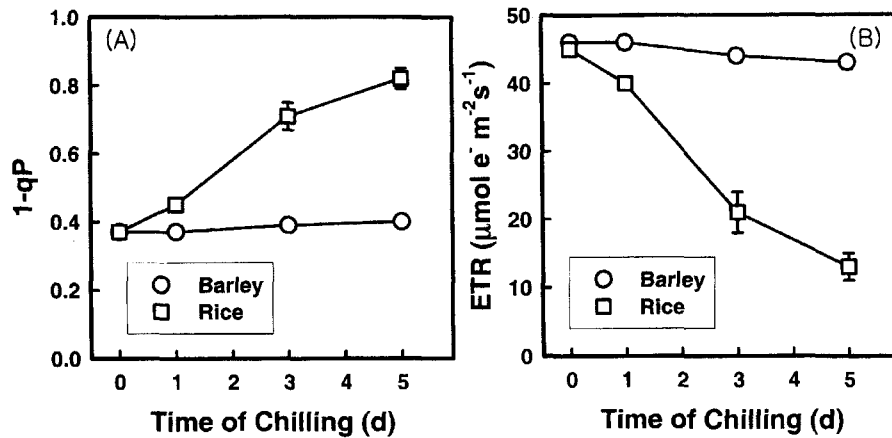
Figure 2. (A) Changes in the reduction state of Q_A, in terms of 1-qP, from intact leaves of barley (O) and rice (□) under light-chilling. (B) Changes in the electron transport rate (ETR) from intact leaves of barley (O) and rice (□) under light-chilling. Other remarks are the same as described in Fig. 1. Each data point represents the mean ± SD of at least three separate experiments. If error bars are not shown, they are smaller than the size of the symbols.
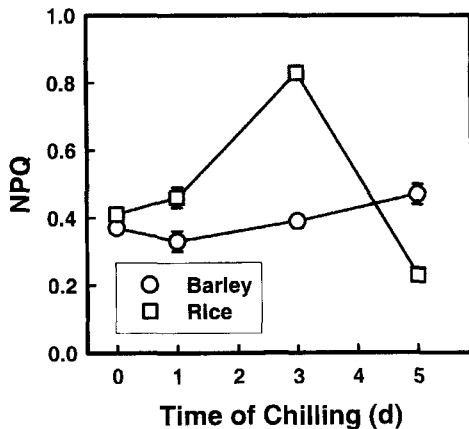

Figure 3. Changes in non-photochemical quenching (NPQ) obtained from the quenching analysis of chlorophyll fluorescence from intact leaves of barley (O) and rice (□) under light-chilling. Other remarks are the same as described in Fig. 1. Each data point represents the mean ± SD of at least three separate experiments.

Xanthophyll cycle during cold stress

On the basis of these observations, we examined whether barley and rice plants have different mechanisms of excessive energy dissipation during light-chilling. In an attempt to explain the observed discrepancy in the extent of low-temperature photoinhibition, we compared the two types of plants in terms of xanthophyll cycle activity. Among several mechanisms that allow excess excitation energy to be dissipated in order to prevent damage to the photosynthetic apparatus, the process of non-photochemical quenching (NPQ) visualizes important regulatory adjustments in the photosynthetic membrane, including the operation of xanthophyll cycle. Fig. 3 showed changes in NPQ of barley and rice plants in the course of light-chilling. Barley showed a gradual, but very limited increase in NPQ during cold stress, with the value range from 0.33 to 0.47. Contrary to barley

plants, rice plants showed quite a marked increase about two times as much as the original level after 3 days of chilling, followed by a sharp decline to a level corresponding to 50% of the original value after 5 day-growth at 4°C.

With regard to the role of zeaxanthin-related NPQ in the photoprotection of PS II under photoinhibitory conditions, we attempted to examine whether there exist any differences in the operation of xanthophyll cycle between the two types of plants having different sensitivity to chilling. For this purpose, we measured cold-affected changes in relative levels of component pigments of xanthophyll cycle, which shows light-dependent interconversion between zeaxanthin (Z) + antheraxanthin (A) and violaxanthin (V). Cold-affected barley rarely showed the formation of Z+A, in contrast to rice plants showing marked formation of Z+A with contradictory decrease in V in the course of light-chilling. In order to demonstrate light-induced formation of Z+A in a more understandable form, de-epoxidation state (DEPS) was calculated as (0.5A+Z)/(V+A+Z). Fig. 4 showed that cold-affected rice markedly enhanced the DEPS of xanthophyll cycle, converting 47%, 56% and 63% of V into A+Z after 1, 3 and 5 days of light-chilling at 4°C, respectively, as compared to barley which rarely showed the conversion of the pigments. Based on the idea that the capacity of plants for the dissipation of excessive excitation energy is correlated with amounts of carotenoids, we investigated the pool sizes of the xanthophyll cycle in the two types of plants and displayed the results as shown in Fig. 5. It is noteworthy that cold-affected barley largely enhanced the pool size of xanthophylls in contrast to rice which slightly depressed it (Fig. 5). To address the different regulation of xanthophyll cycle activity that had been observed in between two types of plants, we examined whether there exist any difference in mRNA expression levels of zeaxanthin epoxidase (ZE), an enzyme involved in the reversion of Z to V in the light. The results showed that barley
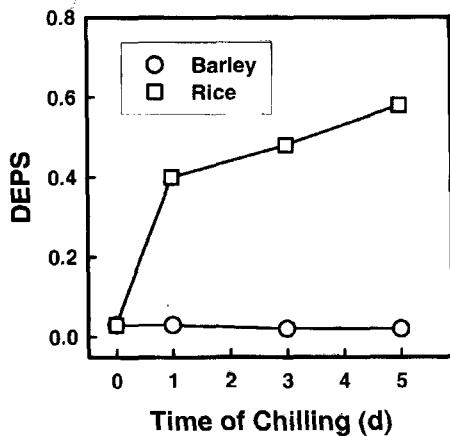
Figure 4. Changes in the de-epoxidation state (DEPS) of xanthophyll cycle pigments in intact leaves of barley (○) and rice (□) under light-chilling. DEPS was calculated as $(Z+0.5A)/(V+A+Z)$, where A, V and Z stand for antheraxanthin, violaxanthin and zeaxanthin, respectively. Each data point represents the typical value obtained from at least three separate experiments.
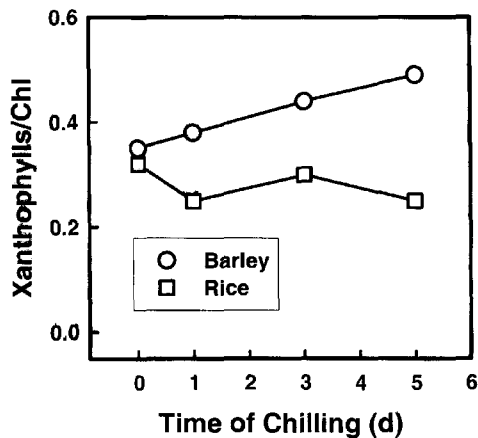

Figure 5. Changes in the pool size of xanthophyll pigments $(V+A+Z)$ in intact leaves of barley (○) and rice (□) under light-chilling. The values were normalized with respect to the total chlorophyll content of the same leaves. Each data point represents the typical value obtained from at least three separate experiments.

expressed increasingly higher level of ZE mRNA during light-chilling compared to rice in which mRNA change was very minor or even decreased (Fig. 6).

DISCUSSION

Differential influence of cold stress on the chlorophyll fluorescence characteristics

When the light energy absorbed by plant leaves exceed the photosynthetic utilization, photoinhibition may occur. Even the moderate intensity of light that is combined with low temperature could lead to photodamage to the photosynthetic machinery, namely low-temperature photoinhibition. Low temperature slows down energy-consuming carbon metabolism
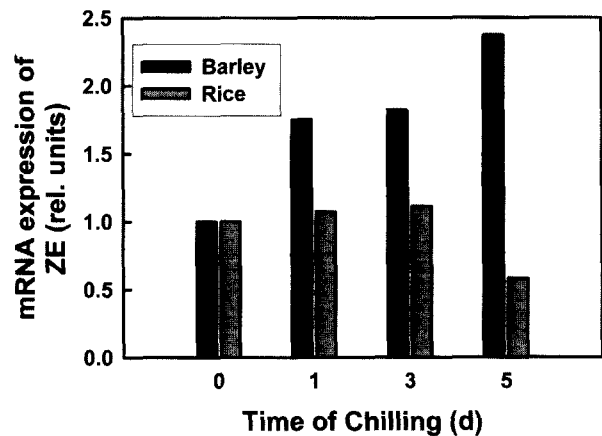

Figure 6. mRNA expression of zeaxanthin epoxidase gene in the intact leaves of barley (black bar) and rice (gray bar) that had been exposed to light-chilling for the designated period. Three micrograms of total RNA were reversely transcribed and PCR-amplified with 25 cycles. Gene expression for β-tubulin was used as control. Density values of RT-PCR products were expressed as relative units on the basis of unchilled control plants (1.0). Plants were exposed to chilling in the light at an intensity of 170 μmol m^{-2} s^{-1} at 4°C.

and repair processes in the chloroplasts, and thus it imposes conditions of excess light [22]. On these perspectives, we explored the mechanism of plant cold sensitivity between two types of plants having different genetic background of resistance against low temperature.

In chilling-sensitive species such as rice (Fig. 1), cold stress may slow down the enzymes of Calvin cycle, thus leading to the accumulation of the reduced Q_A. As a result, the incoming light energy channeled into the electron transport chain becomes more excessive and it may lead to low-temperature photoinhibition, which is known to be manifested as light-induced damage to D1 protein of PS II along with inhibition of electron transport. Contrarily, chilling-resistant species such as barley was shown to maintain the level of reduced Q_A at constant and low level (Fig. 2A), suggesting that the redox state of the electron transport carriers is balanced so that induction of low-temperature photoinhibition is prevented (Fig. 2B). Therefore, it is evident that barley is advantageous to rice during cold stress in having the capacity to sustain Calvin cycle activity as well as ETR, which might be benefited from the higher degree of fatty acid unsatutration of thylakoid membrane lipids and furthermore, other energy-dissipating mechanisms such as xanthophyll cycle.

Differential influence of cold stress on the development of xanthophyll cycle

During cold stress, the enzymes of the Calvin cycle are slowed down so that the incoming light energy becomes more excessive. This leads to the generation of reactive oxygen species in the chloroplasts such as singlet oxygen ($^1O_2$) and superoxide radicals which induce the photodamage to PS II.

Non-photochemical quenching (NPQ) is believed to be a primary protective measure by which light energy received in excess by PS II is dissipated as heat. NPQ reduces energy delivery to PS II by deactivating excited chlorophylls and thereby it minimizes generation of $^1O_2$ in PS II. In this way NPQ can protect PS II from the photodamage [23].

In the present study, NPQ in barley exposed to light-chilling was shown to remain almost constant (Fig. 3). It suggests that barley carries out normal photosynthetic electron flow even in the absence of NPQ mechanism. Moreover, the observation is consistent with the result that barley plants almost did not show chilling stress-induced symptoms in terms of the reduced Q_A (Fig. 2A). Contrarily, rice plants showed a marked increase in NPQ in response to light-chilling (Fig. 3) in parallel with the accumulation of the reduced Q_A. The decline of NPQ, which had been observed after 5 days of light-chilling, occurred in company with leaf chlorosis associated with chilling injury. Some workers [24] hypothesized that plant fitness under fluctuating light situation, is dependent upon thermal energy dissipation by PsbS protein, which belongs to the family of light-harvesting proteins. Although the precise mechanism of its function remains unknown, the PsbS is assumed to sense the presence of excessive light and trigger the switch from conversion of absorbed light by photosynthesis to thermal energy dissipation. In view of these suggestions, chilling-sensitive plants and chilling-resistant ones may have discrepancy in the inheritable characteristics with respect to PsbS gene expression during exposure to cold stress.

Zeaxanthin epoxidase is an enzyme which is associated with reversible conversion of Z to V, via the intermediate A, in the weak light condition, in opposite to the action of violaxanthin de-epoxidase (VDE), which rapidly and reversibly converts V to Z for the protection of PS II under excess light [6]. As shown in Fig. 6, cold-affected barley showed an increased expression of ZE in contrast to rice which depressed its expression. The results suggest that long-term cold stress stimulated active cycling of LHC pigment changes in barley in contrast to rice where it induced malfunctioning of xanthophyll cycle. Xanthophyll cycle has been suggested to provide two photoprotective effects: (1) it protects thylakoid membrane lipids specifically against photooxidation [25], (2) it converts PS II to a state of high energy dissipation by altering xanthophyll/Chl antenna interactions, which leads to decreasing excitation delivery to the reaction centers [26]. When rice plants had lower rates of photosynthesis during cold stress (Fig. 2B), they exhibited more de-epoxidation (they formed more zeaxanthin) leading to an increase in thermal energy dissipation (Fig. 4). Moreover, they showed decreasing rate of ZE expression (Fig. 6). Taken together, these observations suggest that rice plants, as compared with barley, lack the dynamic interconversion of the xanthophyll cycle pigments during cold stress. There are other reports that tolerance to photoinhibition or Chl fluorescence quenching is not associated with high Z levels [26, 27].

On the other hand, barley and rice under light-chilling showed a contradictory trend with respect to the pool size of xanthophyll pigments (Fig. 5). Barley under cold stress elevated the pool size of xanthophyll pigments, whereas rice depressed it. An increase in the carotenoid content relative to chlorophyll content is a potentially protective change and this pigment change was also observed in various plant species exposed in the long term to excess light energy [28]. The size of the xanthophyll cycle pool (V+A+Z) undergoes a marked acclimation to the light environment [16, 29, 30]. It has been suggested that the stimulation of the synthesis of the xanthophylls cycle components occurs in response to the degree of excessive light exposure. It may be also possible that a large xanthophyll cycle pool is associated with a high rate of photosynthesis and high metabolic rate, which in turn can support high rate of carotenoid biosynthesis. Therefore, the pool size of xanthophylls is assumed to be closely associated with capacity of plants to tolerate chilling. Carotenoids are assumed to play a key role in the protection of photosynthetic organism against the toxic effects of reactive oxygen species generated from the over-reduction of photosynthetic electron carriers during light-chilling. On the basis of these observations, we propose that the genetic ability of plants to elevate the level of LHC carotenoids seems to be crucial in surviving the low-temperature photoinhibition.

ACKNOWLEDGMENTS

REFERENCES

1. Aro, E.-M., I. Virgin, and B. Andersson (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim. Biophys. Acta 1143, 113-134.
2. Niyogi, K. K. (1999) Photoprotection revisited: genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 333-359.
3. Horton, P, A.V. Ruban, and R.G. Walters (1994) Regulation of light harvesting in green plants. Plant Physiol. 106, 415-420.
4. Gilmore, A.M. (1997) Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. Physiol. Plant. 99, 197-209.
5. Demmig-Adams, B. (1990) Carotenoids and photoprotection in plants: A role for the xanthophyll zeaxanthin. Biochim. Biophys. Acta 1020, 1-24.
6. Pfündel, E. and W. Bilger (1994) Regulation and possible function of the violaxanthin cycle. Photosyn. Res. 42, 89-109.
7. Hager, A. (1980) The reversible, light-induced conversion of

xanthophylls in the chloroplasts. *In*, F.-C. Czygan (ed.), *Pigments in Plants*, Fischer, Stuttgart, pp. 57-79.

8. Rees, D., A. Young, G. Noctor, G. Britton, and P. Horton (1989) Enhancement of the pH-dependent dissipation of excitation energy in spinach chloroplasts by light activation: correlation with the synthesis of zeaxanthin. *FEBS Lett.* **256**, 85-90.

9. Härtel, H., H. Lokstein, B. Grimm, and B. Rank (1996) Kinetic studies on the xanthophyll cycle in barley leaves (Influence of antenna size and relations to nonphotochemical chlorophyll fluorescence quenching). *Plant Physiol.* **110**, 471-482.

10. Li, X.-P., O. Björkman, C. Shih, A.R. Grossman, M. Rosenqusit, S. Jansson, and K.K. Niyogi (2000) A pigment binding protein essential for regulation of photosynthetic light harvesting. *Nature* **403**, 391-395.

11. Swiatek, M., R. Kuras, A. Sokolenko, D. Higgs, J. Olive, G. Cinque, B. Mueller, L. A. Eichacker, D. B. Stern, R. Bassi, R. G. Herrmann, and F.-A. Wollman (2001) The chloroplast gene ycf9 encodes a photosystem II core subunit, PsbZ, that participates in photosystem II supramolecular architecture. *Plant Cell* **13**, 1347-1367.

12. Ebbert, V., B. Demmig-Adams, W. W. Adams, K. E. Mueh, and L. A. Staehelin (2001) Correlation between persistent forms of zeaxanthin-dependent energy dissipation and thylakoid protein phosphorylation. *Photosyn. Res.* **67**, 63-78.

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

14. Frank, H. A. and R. J. Cogdell (1996) Carotenoids in photosynthesis. *Photochem. Photobiol.* **63**, 257-264.

15. Horton, P., A.V. Ruban, and R.G. Walters (1996) Regulation of light harvesting in green plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 655-684.

16. Thayer, S.S. and O. Björkman (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. *Photosyn. Res.* **23**, 331-343.

17. Gilmore, A.M. and H.Y. Yamamoto (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C-18 high-performance liquid chromatographic column. *J. Chromatogr.* **543**, 137-145.

18. Schreiber, U., U. Schliwa, and B. Bilger (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosyn. Res.* **10**, 51-62.

19. Genty, B., J.-M. Briantais, and N.R. Baker (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* **990**, 87-92.

20. Bilger, W. and O. Björkman(1990) Role of the xanthophylls cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosyn. Res.* **25**, 173-186.

21. van Kooten, O. and J.F.H. Snel (1990) The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosyn. Res.* **25**, 147-154.

22. Huner, N.P.A, G. quist, V.M. Hurry, M. Krol, S. Falk, and M. Griffith. (1993) Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosyn. Res.* **37**, 19-39.

23. Demmig-Adams, B. and W.W. III Adams (2000) Harvesting sunlight safely. *Nature* **403**, 371-374.

24. Külheim, C., J. Ågren and S. Jansson (2002) Rapid regulation of light harvesting and plant fitness in the field. *Science* **297**, 91-93.

25. 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.

26. Tardy, F. and M. Havaux (1996) Photosynthesis, chlorophyll fluorescence, light-harvesting system and photoinhibition resistance of a zeaxanthin-accumulating mutant of *Arabidopsis thaliana*. *J. Photochem. Photobiol. B: Biology* **34**, 87-94.

27. Hurry, V., J.M. Anderson, W.S. Chow, and C.B. Osmond (1997) Accumulation of zeaxanthin in abscisic acid-deficient mutants of *Arabidopsis* does not affect chlorophyll fluorescence quenching or sensitivity to photoinhibition *in vivo*. *Plant. Physiol.* **113**, 639-648.

28. Adams, W.W. III, A. Hoehn, and B. Demmig-Adams (1995) Chilling temperatures and the xanthophyll cycle. A comparison of warm-grown and overwintering spinach. *Aust. J. Plant Physiol.* **22**, 75-85.

29. Demmig-Adams, B. and W.W. III Adams (1992) Carotenoid composition in sun and shade leaves of plants with different life forms. *Plant Cell Environ.* **15**, 411-419.

30. Demmig-Adams, B., D.L. Moeller, B.A. Logan, and W.W. III Adams (1998) Positive correlation between levels of retained zeaxanthin+antheraxanthin and degree of photoinhibition in shade leaves of *Schefflera arboricola* (Hayata) Merrill. *Planta* **205**, 367-374.