특강4 Low Temperature Photoinhibition and Protection of Photosynthesis in Higher Plants

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Introduction

Photoinhibition of photosynthesis results from an over-excitation of photochemical systems (e.g. Powles 1984). Low temperatures sensitize photosynthesis to photoinhibition so that even low light may induce photoinhibition. This is thought to occur primarily through temperature-imposed thermodynamic constraints on carbon metabolism that prevent excitation energy from being effectively utilized for CO2 assimilation and thus promote photoinhibition (Krause 1994, Huner et al. 1998).

Plants have developed a range of processes to balance the absorbed light energy with photosynthesis, thereby protecting the photosynthetic apparatus against photoinhibition (Anderson et al. 1997). According to Huner et al. (1993, 1998), there are species differences with respect to the mechanisms by which plants cope with low temperature-induced over-excitation. In several winter cereals grown at low temperature, the increased resistance to photoinhibition at chilling temperature has been ascribed to an increased capacity to keep QA, the primary stable electron acceptor of PSII, in an oxidized state through increase in capacity for photosynthesis (Huner et al. 1993). In cold-tolerant spinach (Boese and Huner 1990) and overwintering plant Scots pine (Krivosheeva et al. 1996), however, the increased resistance to photoinhibition was correlated with increases in carotenoids and antioxidant enzyme systems, rather than increases in photosynthesis. In cold-grown *Oxyria digyna* (Koroleva et al. 1994) and chilling-sensitive maize (Haldimann et al. 1996, Leipner et al. 1997), the increased resistance to a combination of chilling temperature and high light has been explained by increased capacity to dissipate excess light non-radiatively.

In this study, we are going to discuss on the protective mechanisms of crop

plants against low-temperature photoinhibition, especially focusing on the ways to dissipate excessive excitation energy absorbed by photosystems.

Materials and Methods

Plant material

Two rice (*Oryza sativa* L.) cultivars, a Japonica cultivar Dongjin-byeo and an Indica cultivar IR841, were grown in a growth chamber under a 14-h light period with a photosynthetic photon flux density (PPFD) of 100 μmol m-2 s-1 at a day/light temperature regime of 28/25℃. Barley (*Hordeum vulgare* L. cv. Albori) were grown at a PPFD of 50 μmol m-2 s-1 under a photoperiod of 14 h at 25℃ alternating with a 10-h dark period at 20℃. For all experiments, fully expanded leaves of 3-week-old plants were used.

Pigment analysis

Xanthophyll cycle components were determined according to Thayer and Björkman (1990) with some modifications. Leaves were frozen in liquid nitrogen and ground with a mortar and pestle in ice-cold 100% acetone. The pigment extracts were filtered through a 0.45 μm membrane filter. Pigment separation was performed in an HPLC system (HP 1100 series, Hewlett Packard, Waldbronn, Germany) on a Zorbax ODS-1 column protected by a guard column using a solvent mixture of acetonitrile: methanol (85:15, v/v) for 6 min followed by a 10-min linear gradient to methanol: ethyl acetate (66:34, v/v). All pigments were recovered from the column within about 30 min at a flow rate of 2 ml/min. The eluted pigments were monitored at 440 nm. Concentrations of the pigments were estimated by using the conversion factors for peak area to nanomoles as determined for this solvent mixture by Thayer and Björkman (1990).

Results and discussion

Photochemical and non-photochemical routes for excitation energy dissipation

Sensitivities to chilling-induced photoinhibition among two rice (*Oryza sativa* L.) cultivars (an Indica type cv. IR841 and a Japonica type cv. Dongjin-byeo) and a barley (*Hordeum vulgare* L. cv. Albori) were compared. When leaf segments were exposed to moderate illumination at 4℃, a sustained decrease in the photochemical efficiency of photosystem (PS) II measured as the ratio of variable to maximal fluorescence (Fv/Fm) were observed within several hours (Fig. 1). An analysis of fluorescence quenching revealed a sudden drop in

PSII-driven electron transport rate (ETR) (Fig. 2) and a rapid rise in the reduction state of primary electron acceptor QA upon exposure to chilling in moderate light (Xu et al. 1999). There was no appreciable difference in the level of non-photochemical quenching (NPQ) nor in the xanthophyll cycle activity between Japonica rice and barley (Fig. 3). However, barley was capable of sustaining a higher ETR thereby keeping a lower reduction state of QA throughout the chilling for 6 h. The Indica rice was characterized by the lowest ability to develop the xanthophyll cycle-associated NPQ (Fig. 3). It is concluded that the lower susceptibility of barley to chilling-induced photoinhibition than Japonica rice is attributable to its higher potential to dissipate excess light energy via photochemical mechanism, whereas Indica rice is more sensitive to photoinhibition at chilling temperature than Japonica rice due primary to its lower capacity to develop efficient non-photochemical quenching pathway.

We are investigating the possible electron accecptors which could keep a certain degree of ETR as shown in Fig. 2 in barley, because the activities in photosynthetic carbon reduction are expected to be reduced at low temperature.

Excess energy dissipation through phosphorylation of LHCII and CP-complexes

Photosynthetic apparatus exposed to high light is companied by phosphorylation of LHCII and CP-complexes, thereby reducing over-flow of excess light to core proteins and reaction centers. This functions as a reversible protective mechanism against degradation of core proteins caused by photoinhibition. It can be also induced under a low light coupled with a stress like low temperature, because photosynthetic electron transfer is greatly inhibited at low temperature as shown in Fig. 2.

Phosphorylation has been studied relating to the protective role against excess light and dephosphorylation of photosynthetic proteins known having an important role for the recovery of photosynthetic ability of plants exposed to stresses such as high light, low temperature and so on. In Fig. 5, the Fv/Fm decreased during chilling could be recovered rapidly within 20 min during dark-incubation at room temperature, and the recovery was significantly inhibited by the treatment of a phosphatase inhibitor, NaF. Similar results could be observed by phosphatase inhibitors including iodosobenzoate and iodoacetate (Kim et al. 1997). Difference in the rate of dephosphorylation was a possible reason for different chilling-tolerance of two rice varieties, a Japonica type and an Indica type.

Excess energy dissipation through zeaxanthin accumulation

Besides phosphorylation/dephosphorylation of thylakoid membrane proteins, xanthophylls cycle involves in protection against over-excitation of PSII as a

down-regulation mechanism of photosynthetic capacity. Build-up of delta pH by hight light or low temperature treatment limits photosynthetic electron transfer; thereby core proteins of PSII, D1/D2, can be inevitably damaged. Responding to such photoinhibtory damages, xanthophyll cycle pigments can reduce flow-in of excess light to core CP complexes and reaction centers by heat dissipation. The pigments responsible for this heat dissipation is zeaxanthin (Zea) that is formed from violaxanthin (Vio) through de-epoxidation and ensuing structural changes of PSII, contributing to reduce the antenna size of photosystems. Similar to the phosphorylation/ dephosphorylation mechanism, this cycle functions reversibly and leads to transform from Zea to Vio when plants escape from a stress or sub-optimal growth condition.

Interestingly by our experiments, some dephosphorylation pathway involves in epoxidation of Zea, which protect photosystems against excess light by heat dissipation and induction of conformation change in PSII. During chilling, the pretreatment of phosphatase inhibitors, NaF and Na2MoO4, caused slight acceleration of Vio de-epoxidation (Fig. 6). During dark-recovery, the Zea epoxidation was significantly blocked by the phosphatase inhibitors, which also blocked the recovery of Fv/Fm (Fig. 7). Furthermore, when leaves were preincubated with either phosphatase inhibitor or salicylaldoxine (SA), an inhibitor of epoxidase, an illumination of low light (60 μmol m-2 s-1) at room temperature for 90 min also caused changes in xanthophyll cycle pigments similar to those observed during light-chilling (Figs 8 and 9). The subsequent epoxidation of zeaxanthin in darkness was strikingly suppressed in the leaves pretreated with the chemicals. Based upon these results we speculate that the effects of phosphatase inhibitors on xanthophyll cycle activity occur via inhibition of epoxidase assuming that the epoxidase activity is controlled through reversible phosphorylation by thylakiod kinase/phosphatase enzyme system and the phosphorylated form of epoxidase. The epoxidase activity can also be controlled by phosphorylation of a CP complex, probably CP29, that may affect the epoxidase activity by changing the environment of the enzyme.

No causal relationship between Zea epoxidation and PSII reactivation after light-chilling

When leaves were allowed to recover at photosynthetic photon flux higher than 30 μmol m-2 s-1, both the decrease in the content of Zea and the increase in PSII efficiency were slowed down (Fig. 10). However, dithiothreitol (DTT) eliminated the retarding effect of light on the rate of Z decrease while exerting little effect on PSII recovery. When chilled leaves were incubated with an inhibitor of epoxidase, salicylaldoxime (SA), during recovery in darkness at 20 ℃, the rate of Zea decrease was greatly suppressed, but no significant effect

of SA on the kinetics of PSII recovery were observed (Fig. 11). These results led us to conclude that the recovery of PSII efficiency from low temperature photoinhibition is not mainly controlled by changes in the level of Zea in rice leaves.

The slow relaxing component of NPQ during dark recovery seemed to be well correlated both with the dephosphorylation kinetics of CP34 (phosphorylated form of CP29) and with the epoxidation kinetics of Z (Hwang et al. 1998). However, both the NPQ relaxation and the dephosphorylation of CP34 were not altered by SA, which could reduce the epoxidation rate significantly. Currently, the possible relationship of CP29 and the activity of Zea epoxidase and the effect of phosphorylation of these proteins are under investigation.

Acknowledgement

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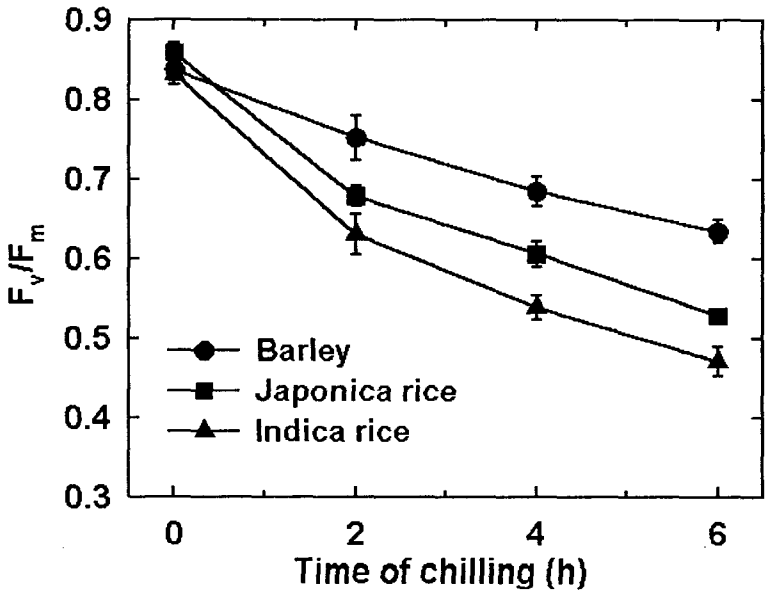

Figure 1. Time course of photoinhibition as determined by the changes in the photochemical efficiency of PSII (Fv/Fm). Leaves were pre-illuminated at 250 μmol m-2 s-1 and 25℃ for 30 min, and photoinhibition was induced by exposure to a PPFD of 250 μmol m-2 s-1 at 4℃. Chl fluorescence was measured after 20 min dark adaptation at 25℃. Error bars indicate the standard error (n = 4-6). Where not present, the errors were smaller than the symbol size.

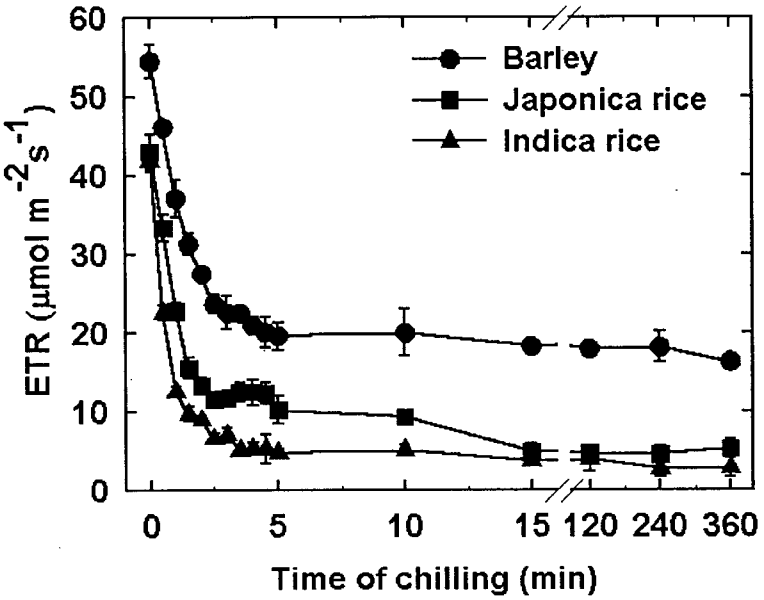

Figure 2. Time course of the changes in the in vivo PSII-driven electron transport rate (ETR) upon exposure to chilling in the light. Detached leaves were first illuminated at 250 μmol m-2 s-1 at 25℃ for 30 min and then transferred in the light with the same PPFD into a cold room at 4℃. After the transfer, the leaf temperature dropped to 4℃ within 100 s as monitored by a thermocouple sensor of PAM-2000. Error bars indicate the standard error (n = 5). Where not present, the errors were smaller than the symbol size.

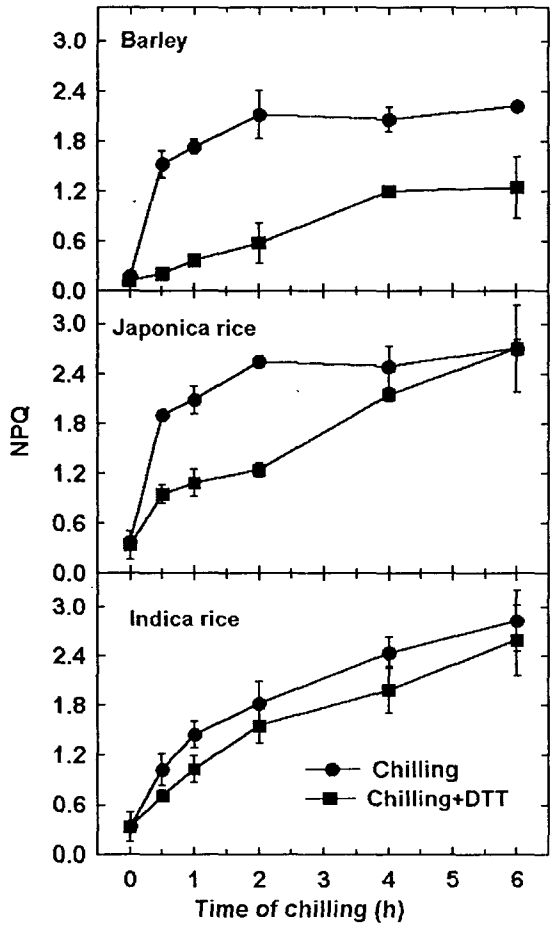

Figure 3. Time course of the development of non-photochemical fluorescence quenching (NPQ) in the absence and presence of DTT upon exposure to chilling in the light. The experimental conditions are the same as in Fig. 1. Fm at zero time of chilling was measured after the pre-illumination for 30 min, and Fm was measured after dark adaptation for 5 min before the onset of chilling. For DTT pretreatment, leaves were fed with 5 mM DTT at 30 μmol m-2 s-1 at 25℃ for 3 h. Error bars indicate the standard error (n = 4-6). Where not present, the errors were smaller than the symbol size.

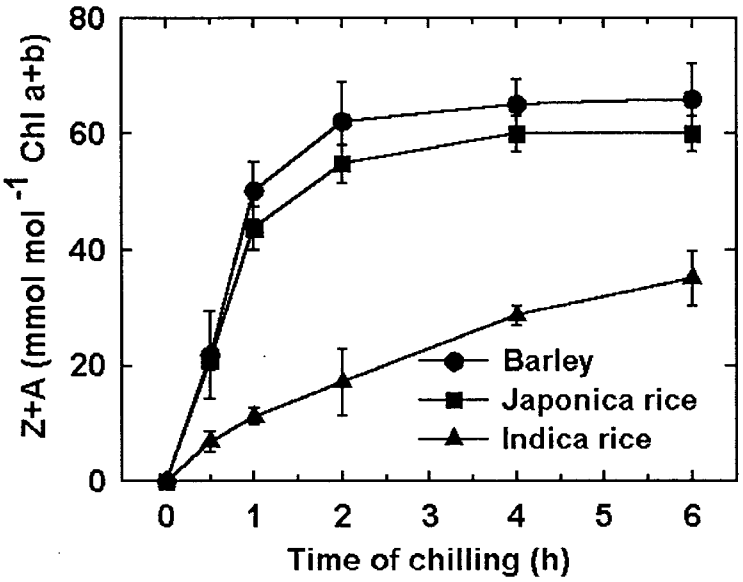

Figure 4. Time course of the formation of zeaxanthin and antheraxanthin upon exposure to chilling in the light. The experimental conditions are the same as in Fig. 1. Error bars indicate the standard error (n = 3).

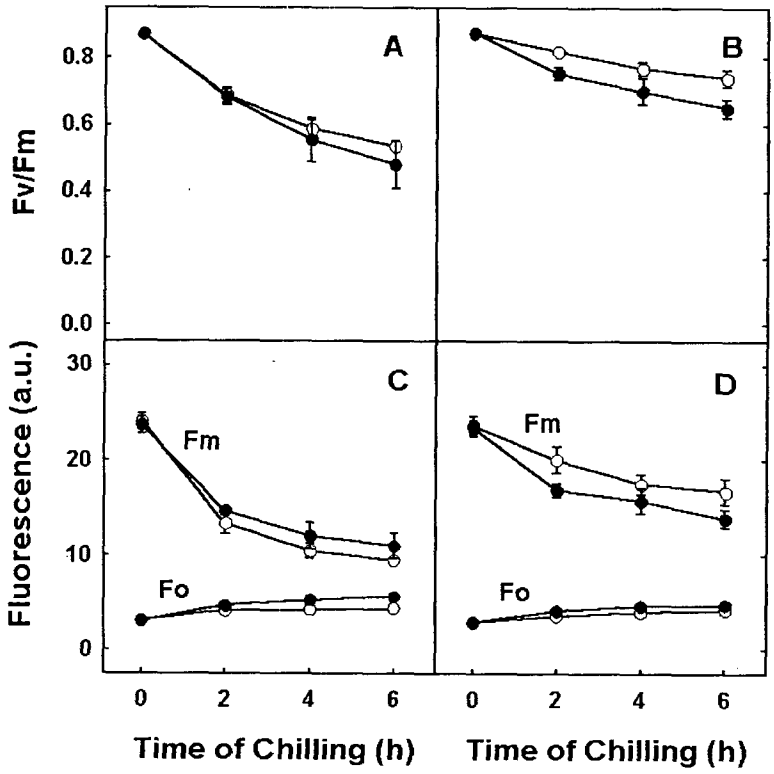

Figure 5. Effect of NaF on the changes in chlorophyll fluorescence parameters (Fv/Fm, Fm and Fo) during light-chilling and the subsequent dark recovery in Dongjin-byeo. Temperature for 20 min dark-adaptation and Chl fluorescence measurement was 4oC ((A) and (C)) or 25oC ((B) and (D)). Treatments: control (○) and 50 mM NaF (●). Results are means ±SD., n = 5.

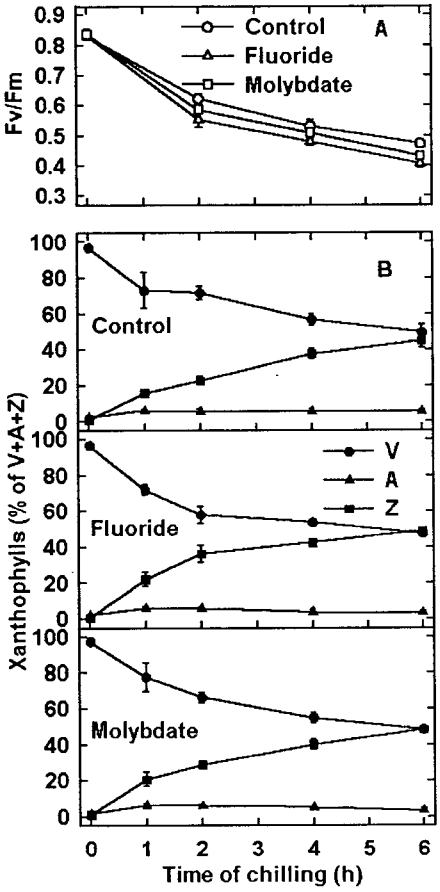

Figure 6. Time course of the changes in PSII photochemical efficiency (Fv/Fm) (A) and contents of the xanthophyll cycle pigments (B) during chilling in the light and the effects of phosphatase inhibitors. Leaves were preincubated with water or solutions containing 50 mM NaF or 50 mM Na2MoO4 in the dark for 3 h before chilling at 4℃ and 150 μmol m-2 per s. Prior to the measurement of Fv/Fm at a given measuring point, leaves were dark-adapted for 5 min at room temperature. Error bars represent the standard errors (n=3-5).

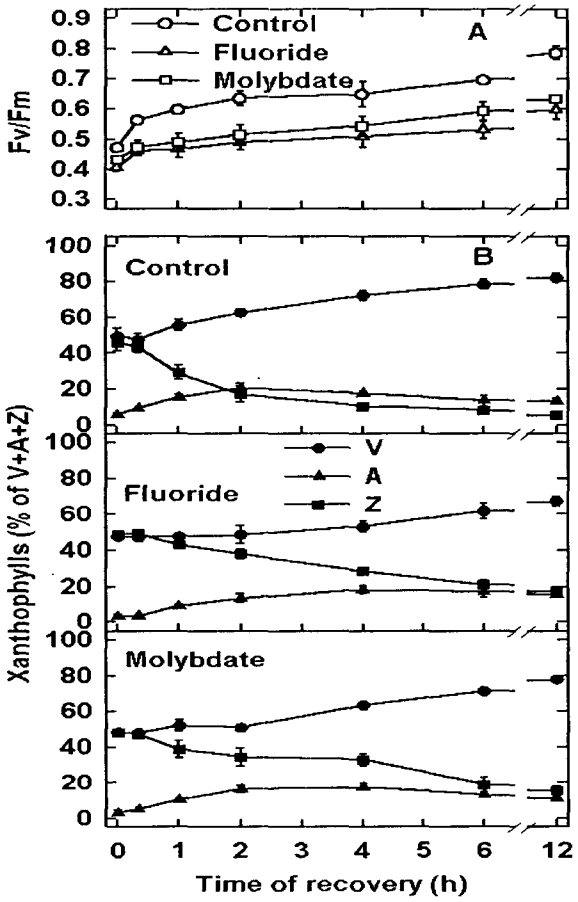

Figure 7. Time course of the changes in PSII photochemical efficiency (Fv/Fm) (A) and contents of the xanthophyll cycle pigments (B) during recovery in the dark at 22℃ after chilling in the light for 6 h and the effects of phosphatase inhibitors. Leaves were preincubated with water or solutions containing 50 mM NaF or 50 mM Na2MoO4 in the dark for 3 h before chilling at 4℃ and 150 μmol m-2 per s. Prior to the measurement of Fv/Fm at a given measuring point, leaves were dark-adapted for 5 min at room temperature. Error bars represent the standard errors (n=3-5).

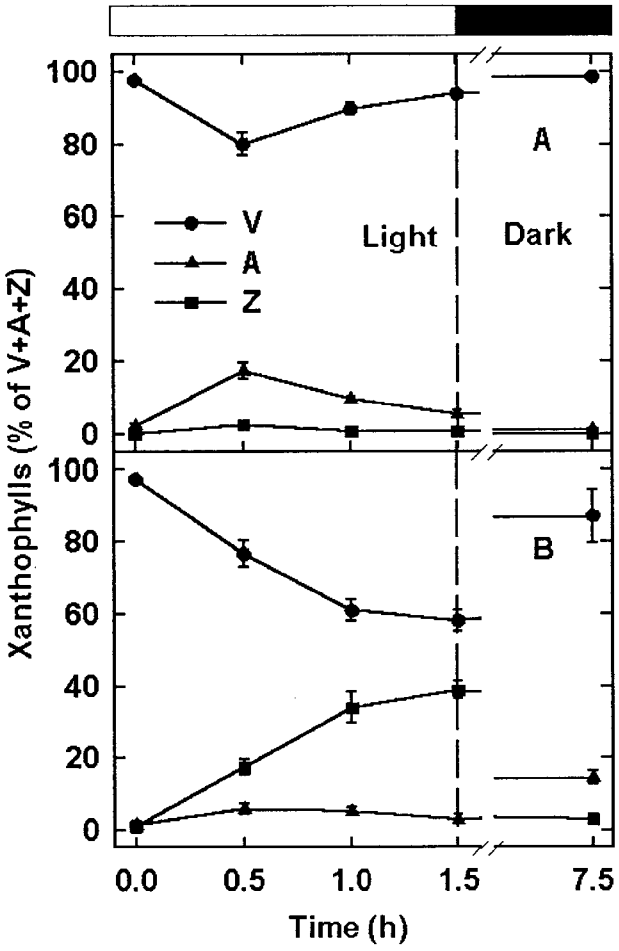

Figure 8. Time course of the conversion of xanthophyll cycle pigments in control (A) and NaF-treated (B) leaves. Leaves were preincubated with water or 50 mM NaF in the dark for 3 h and then exposed to 60 μmol m-2 per s at 2 2℃ for 1.5 h for induction of Z formation. The subsequent Z epoxidation was followed in the dark at 22℃ for 6 h. Error bars represent the standard errors (n=3).

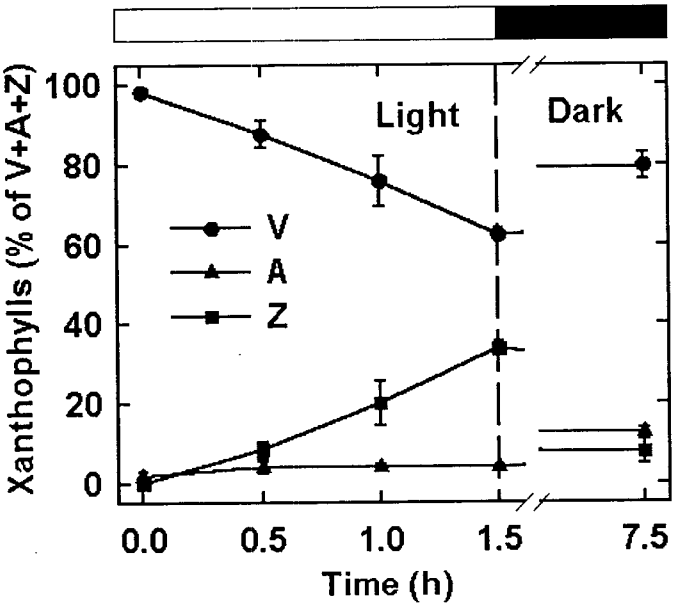

Figure 9. Time course of the conversion of xanthophyll cycle pigments. Leaves were preincubated with 5 mM salicylaldoxime in the dark for 3 h and then exposed to 60 μmol m-2 per s at 22℃ for 1.5 h for induction of Z formation. The subsequent Z epoxidation was followed in the dark at 22℃ for 6 h. Error bars represent the standard errors (n=3).

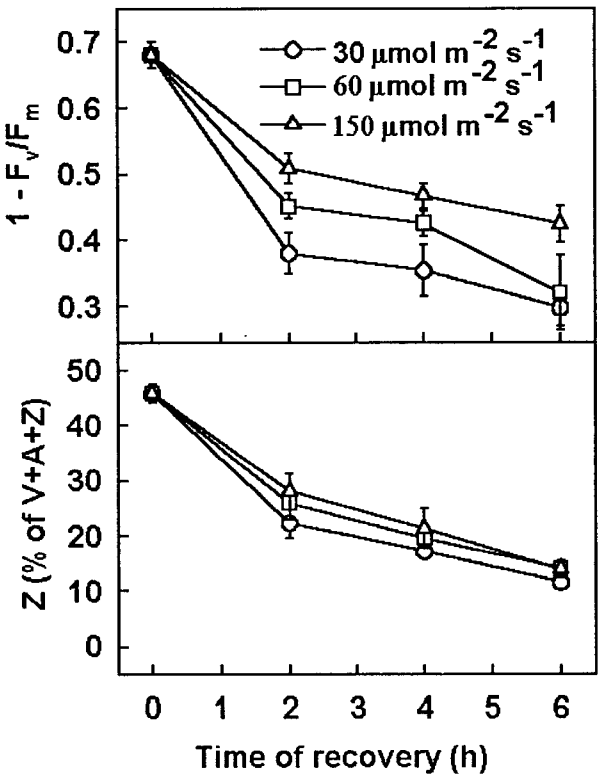

Figure 10. Effects of DTT on changes in the PSII photochemical efficiency (depicted as 1-Fv/Fm) and the level of zeaxanthin (Z) during recovery in the light at 20℃. Leaves were photoinhibited at a PPF of 500 μmol m-2 s-1 and 4℃ for 3 h. To inhibit de-epoxidase, leaves were vacuum infiltrated with 3 mmol/L DTT immediately after photoinhibitory treatment and were floated on the same solution during recovery. Error bars represent standard errors (n 3).

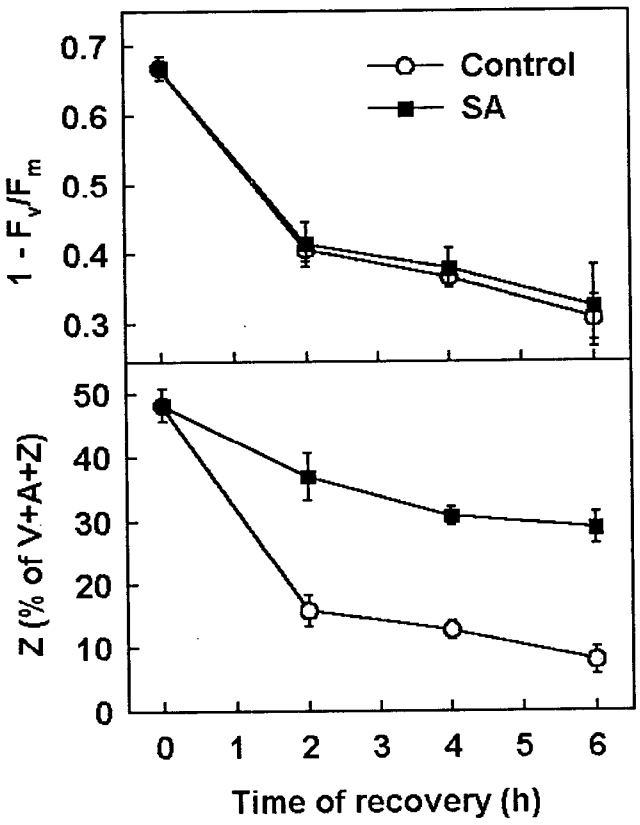

Figure 11. Effects of SA on changes in the PSII photochemical efficiency (depicted as 1-Fv/Fm) and the level of zeaxanthin (Z) during recovery in the dark at 20℃. Leaves were photoinhibited at a PPF of 500 μmol m-2 s-1 and 4℃ for 3 h. To inhibit depoxidase, leaves were vacuum infiltrated with 5 mmol/L SA immediately after photoinhibitory treatment and were floated on the same solution during recovery. Error bars represent standard errors (n 36).