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Reversible Photoinactivation of Photosystem II during Desiccation of Barley (*Hordeum vulgare* L. cv. Albori) Leaves in the Light

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We investigated the inherent protective mechanisms against stress and damage to photosystem II (PSII) in barley (*Hordeum vulgare* L. cv. Albori). Leaves were desiccated at 30% relative humidity, under either low or high light (photon flux densities of 100 or 300 μmol m^{-2} s^{-1}, respectively). During the treatment period, relative water content dropped to 35 to 45%, depending on light intensity. However, the photochemical efficiency of PSII (Fv/Fm) decreased only about 10%. This relatively stable response was due to the rapid, reversible increase in Fm (maximum fluorescence) during 20 min of dark-adaptation. During desiccation in the light, however, PSII was photo-inactivated by non-photochemical quenching (NPQ), with the excess excitation energy absorbed by the chlorophyll being dissipated as heat energy. This decline in NPQ in the first 2 min of treatment was caused by a relaxation in the energy-dependent quenching during dark-adaptation, but could be delayed significantly by a phosphatase inhibitor, NaF. In addition, the relaxation of other NPQ components related to state transition and phosphorylation of thylakoid phosphoproteins were blocked by NaF.

Keywords: barley, chlorophyll fluorescence, desiccation, phosphatase inhibitor, photosystem II

Under field conditions, plants are often exposed to various environmental stresses, such as desiccation, salinity, and low temperature. In particular, water deficit results in inhibited growth; accumulations of abscisic acid, proline, mannitol, and sorbitol; formation of radical scavenging compounds; stomatal closure; reduced transpiration rates; a decrease in the water potential of plant tissues; lower photosynthetic rates; and synthesis of new mRNA and proteins (Yordanov et al., 2000).

Photosystem II is highly resistant when desiccation is either given separately or applied with other environmental stresses, e.g., heat or high-intensity light (Havaux, 1992). At the same time, water deficit enhances the resistance of PSII to constraints, such as high temperatures and strong irradiance (Yordanov et al., 2000). In CO_2-enriched air, when Calvin cycle activity predominates, the quantum yield for PS II photochemistry in cereal crops is reduced only under drastic water deficits (Flagella et al., 1998). The influence of desiccation on PSII may be related to the combination of two factors (Cona et al., 1995). The first, the water deficit effect, is enhanced by irradiation, and leads to the disassembly of part of the PSII core. The second factor, mild dehydration, induces reorganizational processes that rebuild and maintain the remaining PSII function to counteract the depletion of that core. A decline in the quantum yield of photosynthesis during desiccation can also result from either damage to the photosynthetic apparatus or down-regulation of PSII (Calatayud et al., 1997). Nevertheless, Deltoro et al. (1998) have found that chlorophyll fluorescence parameters recover quickly when *Frullania dilatata* is re-hydrated, suggesting that the decrease in PSII efficiency is regulatory, and serves a photoprotective role.

Changes in chlorophyll fluorescence are good indicators of the effects of dehydration on lichens (Pol et al., 1999) and higher plants (Jensen et al., 1999). Fluorescence emitted by dark-adapted leaves can be used as a probe for the primary photochemistry of photosynthesis. Modulated measurements permit fluorescence quenching to be separated into photochemical (qP) and non-photochemical quenching (NPQ) components (Schreiber et al., 1986; Eickmeier

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Abbreviations: Fo, initial fluorescence; Fm, maximum fluorescence; Fv, variable fluorescence; Fv/Fm, photochemical efficiency of photosystem II; NPQ, non-photochemical quenching; qE, energy-dependent quenching; qI, photoinhibitory quenmching; qT, state1-state2 transition quenching; RWC, relative water content

et al., 1993). NPQ, an important protective phenomenon in plants under photoinhibitory conditions, dissipates the heat of excess light energy absorbed by antenna pigments, and generally consists of three major mechanisms (Krause and Weise, 1991): 1) energy-dependent quenching (qE), caused by intrathylakoid acidification during light-driven proton translocation across the membrane; 2) State 1-State 2 transition quenching (qT), regulated by the phosphorylation of light harvesting complexes of PSII (LHCII); and 3) photoinhibitory quenching (qI), which is related to the inactivation of photosynthesis. The first, qE, is thought to be linked with the xanthophyll cycle (Eickmeier et al., 1993). Evidence is increasing that photoinhibition, manifested as a decrease in the efficiency of photosynthetic energy conversion, might actually be attributable to the processes associated with the xanthophyll cycle, thereby playing a protective role in active down-regulation of photosynthesis (Demmig-Adams and Adams, 1992; Gilmore and Björkman, 1994).

The reversible phosphorylation of LHCII is a mechanism for regulating the distribution of excitation energy between PSI and PSII (Bennett, 1991), thereby protecting the photosynthetic apparatus against photoinhibitory damage (Horton and Lee, 1985; Kim et al., 1997; Hwang et al., 2003). NaF, as a phosphatase inhibitor, can alter this energy distribution *in vivo* (Karavaev et al., 1987) as well as suppress zeaxanthin epoxidation (Xu et al., 1999).

In the present study, we monitored the changes in various chlorophyll fluorescence parameters related to photosynthetic efficiency during the desiccation of barley leaves under low- or high-light intensities. Because phosphorylation and dephosphorylation are major controlling factors related to NPQ, we investigated the effect of a phosphatase inhibitor on those changes in parameters. We also tried to determine whether PSII resistance to desiccation in the light was caused primarily by reversible down-regulation or by photoinactivation.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Barley (*Hordeum vulgare* L. cv. Albori) seeds were sterilized in 1% sodium hypochloride solution for 30 min, then thoroughly washed in tap water more than 10 times. After the seeds were germinated in tap water in the dark at room temperature for 2 d, they were planted in pots containing a 1.0:1.0:0.5 (v:v:v) mixture of organic fertilizer, vermiculite, and peat moss. The seedlings were raised in a growth chamber at 28/23°C (day/night) and under a 14-h photoperiod (PPFD of 100 μmol m^{-2} s^{-1}). Expanded leaves of 9-d-old plants were used for all experiments.

Desiccation and NaF Treatments

Prior to the desiccation treatment, 3.5-cm-long leaf segments were floated for 2 h in the dark at room temperature in distilled water or a solution of 25 mM NaF. The latter was used to inhibit dephosphorylation. Afterward, desiccation was achieved, at 28 to 31°C, by exposing the tissues to an atmosphere of about 30% relative humidity. During this treatment period, light was provided from lamps with a PPFD of either 100 or 300 μmol m^{-2} s^{-1}.

Measurement of Relative Water Content

The extent of water loss was characterized by changes in the relative water content (RWC) of the leaf segments. RWC was calculated according to the ratio of (fresh weight dry weight) to (water saturated weight dry weight). The water-saturated weight was measured after floating the leaf segments on water for 2 h in the dark; dry weight was measured after oven-drying the segments at 80°C for 1 to 2 d. In this study, fresh weight referred to the weight of the segments after being desiccated for a specified period.

Measurement of Chlorophyll Fluorescence

The photochemical efficiency of PSII, expressed as the ratio of variable fluorescence (Fv) to maximum yield of fluorescence (Fm), was measured using a portable fluorometer (Plant Efficiency Analyzer, Hansatech, UK) after the barley tissues were dark-adapted for 20 min at 25°C. The maximum yield of fluorescence in light-acclimated leaves, Fm, was also measured without undergoing a dark-adaptation period, and the kinetics of the increase in Fm during 20 min of dark-adaptation was monitored to examine the relaxation of NPQ components.

RESULTS AND DISCUSSION

Changes in RWC and Chlorophyll Fluorescence during Desiccation in the Light

When leaf segments were exposed to desiccation in

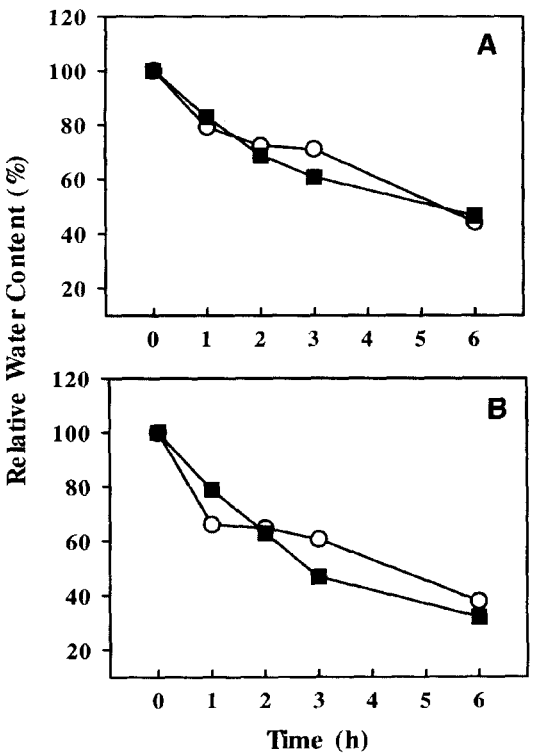

Figure 1. Changes in RWC of barley leaves and the effect of NaF during desiccation under **A,** 100 μmol m^{-2} s^{-1} (low light) or **B,** 300 μmol m^{-2} s^{-1} (high light). Leaf segments were pretreated either with water (○) or in the presence of 25 mM NaF (●).

the light, RWC after 3 h decreased to 70% of normal under low light (PPFD of 100 μmol m^{-2} s^{-1}) and to 60% under high light (300 μmol m^{-2} s^{-1}). When desiccation lasted for 6 h, RWC declined further, to 45% (low light) and 40% (high light) (Fig. 1). This decrease was most rapid in the first hour of the desiccation treatment, with the rate slowing thereafter. Newton and McBeath (1996) have also reported a two-stage decrease in RWC during the desiccation of tropical plants, with an initial gradual decline to 40 to 60%, followed by a steeper decrease to near 0%.

In contrast to our results described above, when leaf segments were treated with NaF, a phosphatase inhibitor, RWC kept decreasing without a noticeable phase transition (Fig. 1). This suggests that NaF disturbs one of the plant protective mechanisms against water loss. Integral membrane proteins serve as specific water channels, or aquaporins (Chrispeels and Maurel, 1994). These proteins occur in both the plasma membrane (Kammerloher et al., 1994) and the tonoplast (Höfte et al., 1992). Their water-channeling activities are regulated by phosphorylation and dephosphorylation, as has been demonstrated through the existence of multiple and consensus phosphorylation sites in many aquaporins (Reizer et al., 1993; Johansson et al., 2000). Therefore, we believe that the response observed with our NaF treatment might

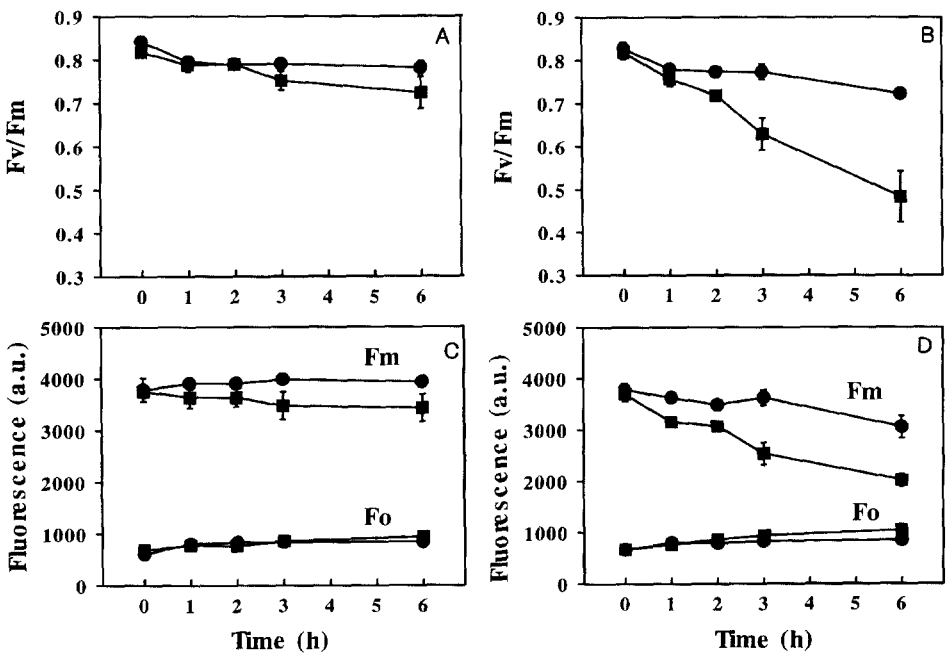

Figure 2. Changes in chlorophyll fluorescence parameters and the effect of NaF during desiccation of barley leaves under **A** and **C,** 100 μmol m^{-2} s^{-1} (low light) or **B** and **D,** 300 μmol m^{-2} s^{-1} (high light). Leaf segments were pretreated either with water (●) or in the presence of 25 mM NaF (■). Chlorophyll fluorescence parameters were measured after 20 min dark-adaptation at 25 °C. Error bars indicate SE (n=3).

have been caused by the inhibition of aquaporin dephosphorylation.

To investigate the effects of desiccation on photosynthesis, we measured initial fluorescence (Fo), maximal yield of fluorescence (Fm), and photochemical efficiency of PSII (Fv/Fm). After desiccation for 6 h under low light, Fv/Fm decreased only slightly (Fig. 2A), although RWC decreased to 45% (Fig. 1A). When the leaf segments were exposed to high-light desiccation for 6 h, RWC dropped to 40%, and the decrease in Fv/Fm was only 10% (Fig. 1B and 2B). A decrease in Fv/Fm generally becomes noticeable when RWC is less than 40% (Eickmeier et al., 1993), and photosynthetic efficiency can be drastically reduced when RWC is lower than 30% (Cornic et al., 1989; Brestic et al., 1995). Therefore, based on all these results, we would propose that the photosynthetic apparatus is rather tolerant of dehydration stress in the light, such that any decrease in Fv/Fm can be found only under a severe state of dehydration.

The effect of desiccation on Fm was dependent on light intensity (Fig. 2), and was not obvious under low-light conditions. However, Fm gradually decreased during high-light desiccation, although this shift was small (Fig. 2D). Under either light intensity, the change recorded within the first hour was due to an increase in Fo; its further decrease in high light primarily resulted from the reduction in Fm. Finally, Fo was only slightly increased during desiccation, indicating that this parameter was not dependent on light intensity.

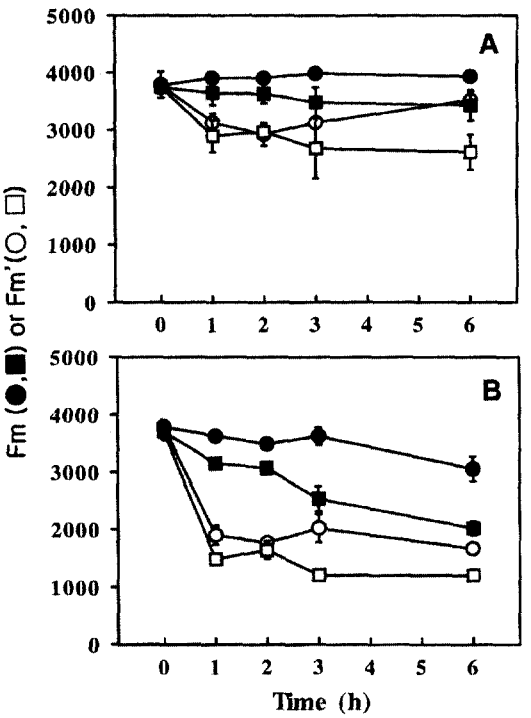

Figure 3. Changes in maximal yield of chlorophyll fluorescence and the effect of NaF during desiccation of barley leaves under **A,** 100 μmol m^{-2} s^{-1} (low light) or **B,** 300 μmol m^{-2} s^{-1} (high light). Yield measured with dark-adaptation for 0 min (open symbols) corresponds to value at light-acclimated state (Fm'); yield measured with dark-adaptation for 20 min at 25°C (closed symbols) corresponds to value at dark-adapted state (Fm). Leaf segments were pretreated either with water (circles) or in the presence of 25 mM NaF (squares). Error bars indicate SE (n=3).

Changes in Maximum Yield of Chlorophyll Fluorescence during Dark-Adaptation

Because we saw no significant decrease in Fm during desiccation, we measured Fm', which is the maximum yield of chlorophyll fluorescence in a light-adapted state without a dark-adaptation period. This parameter significantly declined after desiccation for 1 h, under either light intensity (Fig. 3). In contrast, its value rose during 20 min of dark-adaptation. These results imply that plant leaves can dissipate the excessive excitation energy that is absorbed by chlorophyll during desiccation in the light, resulting in a low Fm', but that this change can be reversed during the dark-adaptation period.

To further examine the components of NPQ developed during desiccation in the light, we monitored the relaxation of Fm' after leaves were dark-adapted for 0, 2, 6, 10, or 20 min following 3 or 6 h of desiccation (Fig. 4). Maximum chlorophyll yield relaxed very rapidly, to <50% of the down-regulated value, during the first 2 min of treatment (Fig. 4A and B). Because half-times for the relaxation of qE, qT, and qI are 2 to 5 min, 8 min, and >30 min, respectively (Walters and Horton, 1991), the component of NPQ that can be relaxed within 2 min is related mainly to energy dependent quenching, qE. Furthermore, the component of NPQ that can be relaxed between minutes 2 and 20 may be related to qT and to the dephosphorylation of some thylakoid phosphoproteins not related to state transition. Any NPQ that remains after 20 min of dark-adaptation is primarily qI, with some slowly dephosphorylating thylakoid phosphoproteins.

Effect of Phosphatase Inhibitor, NaF, on Changes in Maximum Yield of Chlorophyll Fluorescence during Dark-Adaptation

Because the development of qT depends on the

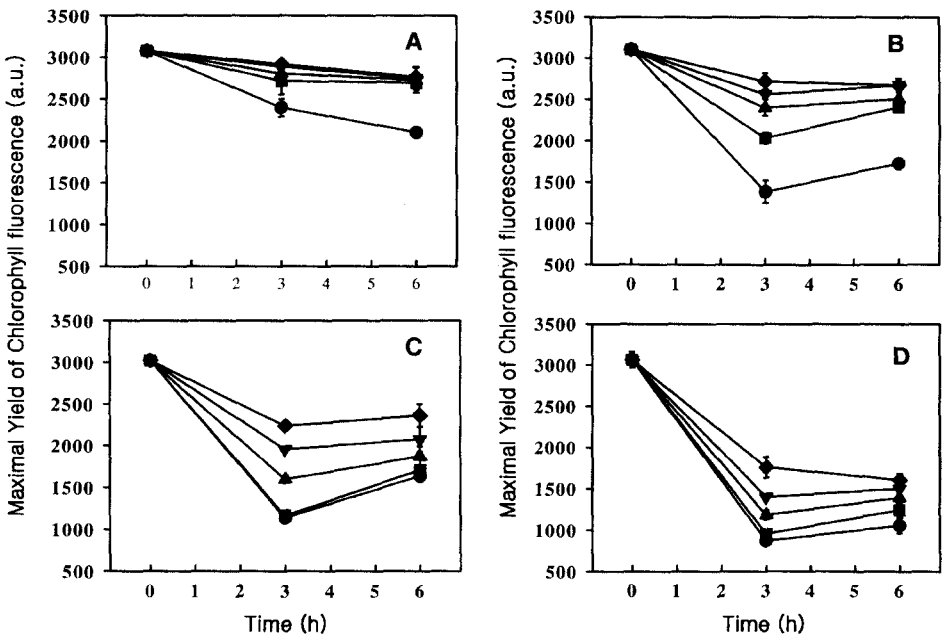

Figure 4. Relaxation kinetics of the maximal yield of chlorophyll fluorescence quenched and the effect of NaF during desiccation of barley leaves under **A** and **C,** 100 μmol m^{-2} s^{-1} (low light), or **B** and **D,** 300 μmol m^{-2} s^{-1} (high light). Yield was measured with dark-adaptation at 25°C for 0 (●), 6 (■), 10 (▲), 15 (▼), or 20 (◆) min. Yield measured with 0 min dark-adaptation corresponds to value at light-acclimated state (Fm'); yield measured with dark-adaptation for 20 min corresponds to value at dark-adapted state (Fm). Leaf segments were pretreated either with water (**A** and **B**) or in the presence of 25 mM NaF (**C** and **D**). Error bars indicate SE (n=3).

phosphorylation of LHCII (Krause and Weise, 1991), we investigated the effects of a phosphatase inhibitor, NaF, on changes in NPQ. During desiccation in the light, the chlorophyll fluorescence parameters were drastically altered in leaves treated with NaF compared with those in untreated controls (Fig. 2). Fv/Fm in NaF-treated leaves decreased by 7.8% after desiccation in low light (Fig. 2A), and by 33% for those leaves desiccated under high light (Fig. 2B). These changes were mainly due to modifications in Fm, so that the effects of NaF on Fo were not significant (Fig. 2C and D). Close examination of our data (Fig. 3) leads us to assume that qE relaxation is slowed considerably by NaF. Therefore, we predict that Fm', measured in the presence of NaF, should be close to the minimum Fm' that can be attained with NaF treatment. The slight rise in Fm', without either dark-adaptation or NaF, was probably due to the rapid relaxation of NPQ that occurred during the short period that Fm' was being measured. The level of maximal yield for Chl fluorescence that was reached after 20 min of dark-adaptation in NaF-treated leaf segments was very close to the value obtained after only 2 min of dark-adaptation in the absence of the phosphatase inhibitor (Fig. 4). We believe this conclusion is acceptable because NaF is known to block the relaxation of qT and the dephosphorylation of other thylakoid phosphoproteins. For example, dephosphorylation of LHCII and D1 is inhibited by treatment with NaF (Rintamäki et al., 1996; Kim et al., 1997). Therefore, the decreased maximal yield of Chl fluorescence by phosphorylation cannot be recovered in the presence of NaF. Likewise, because D1 turnover is suppressed, plant photosystems are easily damaged. However, it is questionable whether the slow relaxation of qE in the presence of NaF is a result of the inhibited dephosphorylation of a certain protein or whether it is an unknown side effect of NaF.

In summary, the photosynthetic apparatus of a plant seems to be rather tolerant of dehydration stress in the light. Therefore, we found that the decrease in Fv/Fm was not significant when RWC was above 40%. Under stress conditions, this apparatus was inactivated, or down-regulated, but then reactivated during the 20 min dark-adaptation period that was required for the measurement of Fv/Fm. The degree of down-regulation could be expressed as NPQ, and its component related to state transition and phosphorylation of the thylakoid phosphoproteins appeared to be inhibited by the phosphatase inhibitor NaF. Although the speed of the relaxation of another component, qE, was very slow in the presence of NaF, this

response must be further scrutinized. NaF also altered the kinetics of dehydration, possibly by phosphorylating the integral membrane proteins that serve as specific water channels, i.e., the aquaporins.

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