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Low-Temperature Effects on Photosynthesis and Correlation with Freezing Tolerance in Spring and Winter Cultivars of Wheat and Rye[1]

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Winter cultivars of rye (*Secale cereale* L., cv Musketeer) and wheat (*Triticum aestivum* L. cvs Kharkov and Monopol), but not a spring cultivar of wheat (Glenlea), grown at cold-hardening temperatures showed, at high irradiances, a higher proportion of oxidized to reduced primary, stable quinone receptor (Q_A) than did the same cultivars grown under nonhardening conditions. In addition, there was a positive correlation between the effects of low-growth temperature on this increased proportion of oxidized Q_A, and a concomitant increase in the capacity for photosynthesis, and LT_{50}, the temperature at which 50% of the seedlings are killed, in cultivars showing different freezing tolerances. This suggests that low-temperature modulation of the photosynthetic apparatus may be an important factor during the induction of freezing resistance in cereals. Finally, the control of photosystem II photochemistry by nonphotochemical quenching of excitation energy was identical for nonhardened and cold-hardened winter rye. However, examination of measuring temperature effects per se revealed that, irrespective of growth temperature, nonphotochemical quenching exerted a stronger control on photosystem II photochemistry at 10°C rather than at 20°C.

Growth and development of winter cultivars of cereals such as rye (*Secale cerale* L.) and wheat (*Triticum aestivum* L.) at low, nonfreezing temperatures induces freezing resistance (Levitt, 1980; Krol et al., 1984). However, it is now established that growth at low temperature not only induces freezing resistance of winter cereals, but also induces an increased resistance to low-temperature-induced photoinhibition of photosynthesis. In contrast, spring cereals exhibit limited ability to acquire an increased resistance to photoinhibition during prolonged growth at low, nonfreezing temperatures (Öquist and Huner, 1991; Hurry and Huner, 1992). We have hypothesized that the increased resistance to photoinhibition of winter cereals upon cold-hardening reflects modulation of the photosynthetic apparatus to optimize photosynthesis under the suboptimal growth conditions experienced during the cold autumn months, thus providing the energy necessary for growth and establishment of seedlings with high freezing tolerance (Öquist and Huner, 1991). Furthermore, winter rye acquires the increased resistance to photoinhibition of photosynthesis during growth at cold-hardening temperature due to an increased capacity to maintain a greater fraction of the PSII reaction centers in an open configuration under given light and temperature conditions (Öquist and Huner, 1992). It was also shown that the greater capacity of cold-hardened rather than NH rye to keep Q_A oxidized could be largely accounted for by an increased capacity for light-saturated photosynthesis, thus providing the leaves with an increased resistance to photoinhibition of photosynthesis.

In this report, we ask how this increase in the capacity for photosynthesis during growth and development at low temperatures is related to the induction of freezing tolerance. This question is based on the hypothesis that photosynthesis provides the energy necessary for the induction of freezing tolerance in plants (Tumarow, 1931; Dexter, 1933; Andersson, 1944). We provide support for this hypothesis by demonstrating that spring and winter wheat cultivars as well as rye show a positive correlation between the effect of cold hardening on the proportion of oxidized Q_A, the rate of light-saturated photosynthesis, and freezing tolerance.

Furthermore, to understand and distinguish the modulation of photosynthesis as a consequence of low-temperature growth from direct effects of low-measuring temperature on photosynthetic functions, we have studied the fluorescence quenching parameters and photosynthetic oxygen evolution in NH and H winter rye at 10 and 20°C. We demonstrate that H and NH leaves exhibit identical low-measuring temperature effects on the dynamic regulation of PSII photochemistry. Thus, responses to low-measuring temperatures can be distinguished clearly from responses associated with long-term exposure to low-growth temperature.

Abbreviations: F_m and F'_m, fluorescence when all PSII reaction centers are closed in dark- and light-acclimated leaves, respectively; F_o and F'_o, fluorescence when all PSII reaction centers are open in dark- and light-acclimated (energized) leaves, respectively; F_s, steady-state fluorescence in light; F_v and F'_v, variable fluorescence after dark acclimation ($F_m - F_o$) and under light-acclimated conditions ($F'_m - F'_o$), respectively; H, grown at cold-hardening temperatures; LT_{50}, the temperature at which 50% of the seedlings are killed; NH, grown at nonhardening temperatures; P_{max}, rate of light-saturated photosynthesis; Φ_e, quantum yield of PSII electron transport; Φ_{O_2}/q_P, quantum yield of open PSII reaction centers; Q_A, the primary, stable quinone acceptor of PSII; q_N, nonphotochemical quenching of fluorescence; q_P, photochemical quenching of fluorescence.

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MATERIALS AND METHODS

Plant Material

Two cultivars of winter wheat (*Triticum aestivum* L., cvs Kharkov and Monopol), one cultivar of spring wheat (*Triticum aestivum* L., Glenlea), and one cultivar of winter rye (*Secale cereale* L., Musketeer) were grown in coarse vermiculite and fertilized with a modified Hoagland solution as described previously (Krol et al., 1984). Seeds were germinated under controlled environment conditions (PPFD 250 μmol m^{-2} s^{-1}, day/night temperature regime 20/16°C, photoperiod 16 h). After 7 d, when the primary leaf had fully expanded, the winter and spring varieties of rye and wheat were transferred to H conditions (PPFD 250 μmol m^{-2} s^{-1}, day/night temperature regime 5/5°C, photoperiod 16 h). NH control plants remained in the temperature regime of 20/16°C. Fully expanded, second leaves developed under either NH or H conditions were used. To reach maturity, the second leaves of NH and H plants required approximately 10 and 30 d, respectively. This means that the second leaves of NH and H plants were of different chronological age, but in the same developmental state. The varieties were selected depending on their freezing resistance as determined by the LT_{50}, based on regrowth (Hurry, 1991).

Measurements of Fluorescence

To determine fluorescence quenching parameters, modulated fluorescence (PAM Chlorophyll Fluorimeter, H. Walz, Effeltrich, Germany) was used with a PAM 103 and two Schott lamps (KL 1500; Schott Glaswerke, Mainz, Germany) providing saturating flashes (FL103) and actinic illumination for photosynthesis. The experimental protocol of Genty et al. (1989) was followed. A detailed description of these measurements is presented elsewhere (Öquist and Huner, 1992). Fluorescence induction kinetics were monitored under ambient air conditions at 5 and 20°C and at different PPFDs ranging from 0 to 2000 μmol m^{-2} s^{-1}. To minimize boundary layer resistance to CO_2 diffusion, a stream of air was blown over the leaf surface. The fluorescence characteristics were evaluated when F_s was reached. q_P, q_N, and other fluorescence parameters were calculated according to van Kooten and Snel (1990), and F_o quenching was accounted for in the calculations according to Bilger and Schreiber (1986).

Measurements of Photosynthetic O_2 Evolution

Photosynthetic oxygen evolution of leaf discs (15.4×10^{-5} m^2) was meaured in a leaf disc electrode (Hansatech, King's Lynn, UK) according to Delieu and Walker (1983). The measurements were performed at 10 and 20°C and in air containing 21% O_2 and 5% CO_2 as obtained from a 1 M carbonate/bicarbonate buffer at pH 9 in equilibrium with air. The top of the electrode was modified to fit the fiber optic light guide of the PAM fluorimeter so that fluorescence and oxygen evolution could be measured simultaneously. The Schott lamp used for the measurement of modulated fluorescence was used to provide photosynthetic light. Quantum yield determinations were based on absorbed photons as calculated from the incident PPFD times leaf absorptance. Details are given elsewhere (Öquist and Huner, 1992).

RESULTS

Effects of Cold-Hardening on the Redox State of Q_A and the Relation to Freezing Tolerance

q_P was determined as a function of irradiance at 5°C in ambient air for H and NH winter and spring wheat cultivars (Table I). Data for winter rye published previously (Öquist and Huner, 1992) are included for comparison. H winter varieties of wheat and rye showed higher values of q_P, that is, a higher proportion of oxidized-to-reduced Q_A under a given irradiance at 5°C, than did NH plants. In contrast, the spring wheat, Glenlea, did not change its response of q_P to irradiance when developed under H conditions. Although less apparent, the same trends were observed when q_P was assayed at 20°C as a function of irradiance (data not shown). The relative changes in q_P expressed by q_PH/q_PNH as a function of irradiance are summarized in Figure 1. Clearly, at high irradiance, q_PH/q_PNH at 5°C was greatest for winter rye and lowest for the spring wheat cultivar Glenlea. The two winter wheat cultivars, Monopol and Kharkov, were

Table I. *The dependence of q_p on the PPFD at 5°C in NH and H leaves of different cultivars of wheat and rye*

Winter cultivars of wheat, Khrakov and Monopol; spring wheat cultivar, Glenlea; winter cultivar of rye, Musketeer. The data for Musketeer are from Öquist and Huner (1992). Mean values of two replicates are shown except for mean values (±SD) for Kharkov (H), Monopol (H), and Musketeer, which are based on six, three, and four replicates, respectively.

PPFD	q_P							
	Kharkov		Monopol		Glenlea		Musketeer	
	NH	H	NH	H	NH	H	NH	H
μmol m^{-2} s^{-1}								
60	0.955	0.964 ± 0.008	0.942	0.956 ± 0.011	0.960	0.963	0.924 ± 0.018	0.969 ± 0.008
100	0.906	0.946 ± 0.010	0.893	0.925 ± 0.018	0.922	0.933	0.848 ± 0.031	0.932 ± 0.016
220	0.778	0.860 ± 0.023	0.757	0.853 ± 0.065	0.824	0.864	0.660 ± 0.063	0.861 ± 0.024
430	0.574	0.719 ± 0.065	0.521	0.684 ± 0.107	0.635	0.667	0.413 ± 0.065	0.719 ± 0.050
780	0.360	0.549 ± 0.087	0.370	0.512 ± 0.090	0.438	0.461	0.274 ± 0.052	0.568 ± 0.066
1440	0.269	0.454 ± 0.066	0.291	0.429 ± 0.099	0.312	0.323	0.194 ± 0.060	0.386 ± 0.048
2000	0.212	0.422 ± 0.056	0.256	0.380 ± 0.056	0.261	0.303	0.164 ± 0.060	0.370 ± 0.060

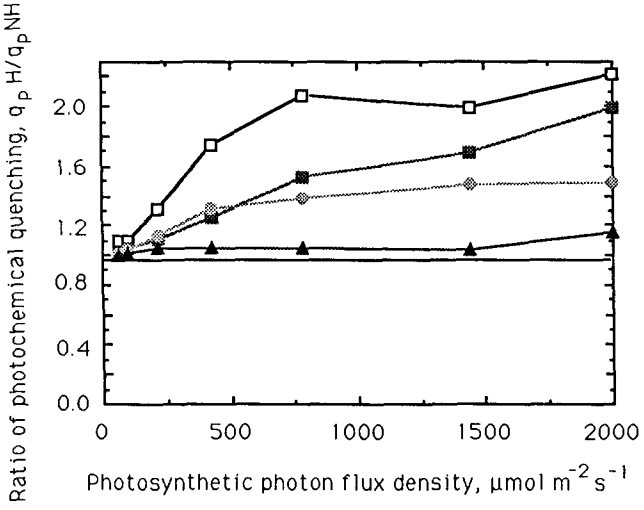

Figure 1. The ratios of q_p between H and NH leaves as a function of PPFD measured at 5°C. The presentation is based on data from Table I. Musketeer (□), Kharkow (▩), Monopol (◉), Glenlea (▲).

intermediate. Thus, the data show that the spring wheat was least able to adjust the redox state of Q_A as a function of irradiance upon cold-hardening, whereas winter rye exhibits the greatest capacity to adjust the redox state of Q_A, with the winter wheat cultivars exhibiting an intermediate capacity.

According to Genty et al. (1989), the yield of electron transport over PSII is given by $\Phi_e = q_p \times F'_v/F'_m$. The results in Figure 2 illustrate that trends observed in q_pH/q_pNH (Fig. 1) are mimicked for Φ_eH/Φ_eNH at 5°C. This means that the different yields of PSII electron transport observed in hardy and nonhardy winter cultivars of wheat and rye (Fig. 2) are largely controlled by the redox state of Q_A under a given irradiance.

We wished to see more directly to what extent the capacity to keep Q_A oxidized after cold-hardening (q_pH/q_pNH) was related to the LT_{50} of different cultivars of wheat and rye. The result in Figure 3A illustrates that freezing tolerance was positively correlated to an increased capacity to keep Q_A oxidized at 5°C under high, saturating light conditions (r^2 = 0.956).

An effective means of increasing the proportion of open PSII reaction centers during growth at H temperatures would be to increase the capacity for photosynthesis during growth under suboptimal temperature conditions. Thus, we compared the capacity of these cultivars to modulate the maximum light saturated rates of photosynthesis (P_{max}H/P_{max}NH) as a function of cold-hardiness (Table II). In Table II, we have also included photosynthesis data from Hurry (1991) for three additional wheat cultivars. Musketeer winter rye exhibited almost a 3-fold increase of P_{max} in the H state relative to the NH state when measured at 20°C. P_{max}H/P_{max}NH was 2.42 for rye when measured at 10°C. In contrast, the spring wheats Glenlea, Marguis, and Katepwa exhibited an approximately 50% decrease in P_{max} in the H state relative to the NH state. The winter cultivars of wheat (Kharkov, Monopol, and Augusta) were intermediate between the winter rye and the spring wheat cultivars. The results in Figure 3B indicate that the capacity to modulate the maximum rate of photosynthesis in the H state is positively correlated with LT_{50} of the studied cultivars (r^2 = 0.933).

Of the studied fluorescence quenching parameters, it was only q_p that could be related to growth and development at low temperature and to different degrees of induced freezing tolerance (Figs. 1 and 3A). q_N and q_o were somewhat higher for cold- than for warm-grown plants at saturating PPFDs;

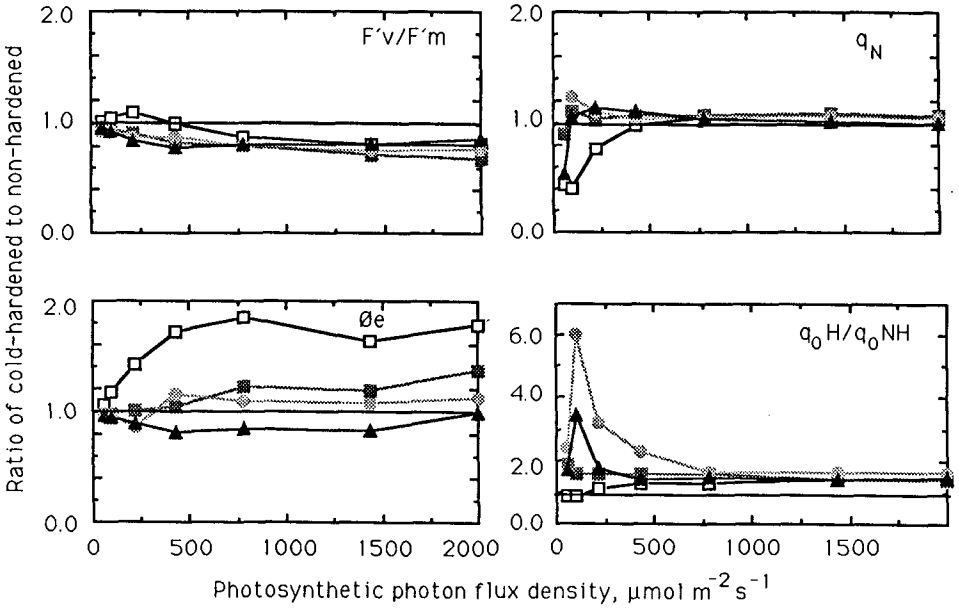

Figure 2. The ratios of F'_v/F'_m, $\Phi_e = (q_P \times F'_v/F'_m)$, q_N and q_o between H and NH leaves as a function of PPFD measured at 5°C. Musketeer (□), Kharkow (▩), Monopol (◉), Glenlea (▲).

i.e. $q_N H/q_N NH$ and $q_o H/q_o NH > 1$ (Fig. 2). However, this was a general trend induced by cold-acclimation and not related to the degree of cold-hardiness induced during growth and development at low temperature; spring and winter cultivars showed quantitatively similar responses. The somewhat decreased ratio of F'_v/F'_m in plants grown under H conditions was also a general response to growth and development at low temperature, not related to the induced degree of cold-hardiness.

Energy-Dependent Regulation of PSII

The maximal quantum yield of O_2 evolution for winter rye based on absorbed photons was unaffected by cold-hardening whether measured at 10°C (NH = 0.102 ± 0.005; H = 0.101 ± 0.013) or 20°C (NH = 0.094 ± 0.010; H = 0.088 ± 0.011) (Öquist and Huner, 1992). Concomitant determinations of q_P and q_N allowed us to investigate how long-term exposure to cold-hardening on one hand, and exposure to low measuring temperature on the other hand, influenced the way by which the yield of PSII photochemistry was regulated by q_P and q_N. Clearly, in both NH and H winter rye, the quantum yield of oxygen evolution depended similarly on q_P at a given temperature (Fig. 4A). However, at 10°C and q_P values above 0.6, the quantum yield for oxygen evolution was somewhat higher than at 20°C. This temperature dependence of the properties of PSII, independent of the state of temperature acclimation, was also demonstrated by plotting the yield of open PSII reaction centers (Φ_{O_2}/q_P) as a function of q_N according to Weis and Berry (1987) (Fig. 4B). The well-known largely linear relation between (Φ_{O_2}/q_P) and q_N implies that the energy-dependent quenching exerted by the ΔpH gradient across the thylakoids regulates the yield of open PSII reaction centers similarly in NH and H winter rye. However, at given values of q_N, the yields of open PSII reaction centers were generally somewhat higher at 10 than at 20°C.

DISCUSSION

Photosynthesis and Freezing Tolerance

We show, for the first time, a strong positive correlation between the cold-hardening-induced increase in the capacity to maintain a high proportion of oxidized-to-reduced Q_A under high light at 5°C and freezing tolerance (Fig. 3A). This ability was coupled to a similar correlation between the capacity to increase light-saturated rates of photosynthesis upon cold-hardening and freezing tolerance (Fig. 3B). As seen in Figure 3, A and B, the winter rye cultivar Musketeer exhibited the greatest capacity to increase its light-saturated rate of photosynthesis after cold-hardening and exhibited the highest freezing tolerance, whereas spring wheats, which exhibited the lowest freezing tolerance, in fact decreased their rates of light-saturated photosynthesis upon cold-hardening. The winter wheats exhibited intermediate capacities to increase their photosynthesis rates and exhibited also an intermediate range of freezing tolerance among cereals. We emphasize that freezing tolerance is correlated with the capacity to increase rates of photosynthesis upon cold-hardening, but is not necessarily correlated with the absolute rate of photosynthesis. Thus, we suggest that the capacity to *change* the light-saturated rate of photosynthesis, as also reflected in the

Table II. *Modulation of maximum rates of photosynthesis in NH and H winter and spring cultivars of wheat and rye*

Data for winter rye represent light-saturated (absorbed photons 1500 μmol m^{-2} s^{-1}) rates of O_2 evolution (P_{max}) measured in a leaf disc O_2 electrode in 5% CO_2 in air as μmol O_2 evolved m^{-2} s^{-1}. Data for the wheat cultivars were obtained from Hurry (1991) and represent light-saturated (incident photons 1000 μmol m^{-2} s^{-1}) rates of CO_2 uptake in ambient air expressed as μmol m^{-2} s^{-1}. The growth conditions for all cultivars were the same as specified in "Materials and Methods." All data were measured at 20°C and represent means ± SD with n = 4 to 6. Data for LT_{50} are from Hurry (1991).

Cultivars	P_{max}		$P_{max}H/P_{max}NH$	LT_{50}
	NH	H		
				°C
Musketeer	12.1 ± 1.2	34.7 ± 2.9	2.87	−32
Kharkov	13.8 ± 1.1	18.1 ± 0.8	1.31	−21
Augusta	13.0 ± 0.8	16.0 ± 0.8	1.23	−13
Monopol	15.4 ± 1.0	15.8 ± 0.5	1.03	−12
Katepwa	15.5 ± 0.8	12.0 ± 0.8	0.77	−9
Glenlea	17.3 ± 0.8	9.5 ± 0.9	0.55	−8
Marguis	15.5 ± 0.9	7.1 ± 1.0	0.46	−7

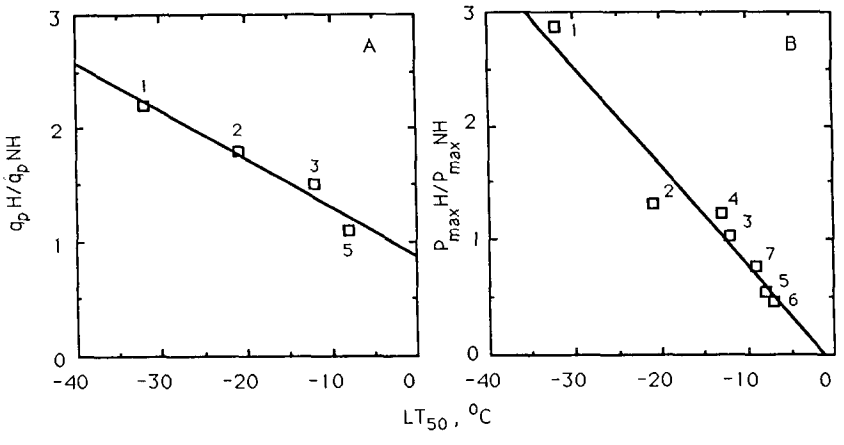

Figure 3. A, The ratios of q_P measured at 5°C, and B, the ratios of P_{max} measured at 20°C, between H and NH cereals. Winter rye cultivar: 1, Musketeer; Winter wheat cultivars: 2, Kharkov; 3, Monopol; 4, Augusta; spring wheat cultivars: 5, Glenlea; 6, Marquis; 7, Katepwa. The ratios of q_pH/q_pNH are from Table I (2000 μmol m^{-2} s^{-1}) and the ratios of $P_{max}H/P_{max}NH$ are from Table II. The LT_{50} data are from Hurry (1991).

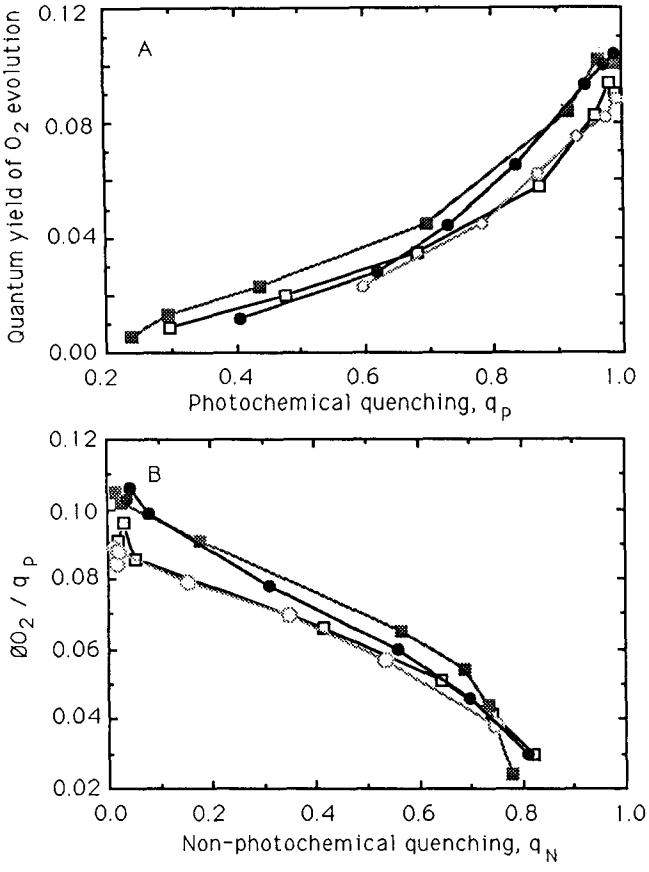

Figure 4. A, The quantum yield of oxygen evolution based on absorbed photons as a function of q_P as induced by different PPFDs and B, the quantum yield of open PSII reaction centers (Φ_{O_2}/q_P) as a function of q_N. Plants were NH and H winter rye cultivar Musketeer, and measurements were performed at two temperatures, 10 and 20°C. □, NH leaves measured at 20°C; ▩, NH leaves measured at 10°C; ○, H leaves measured at 20°C; ●, H leaves measured at 10°C. Means of four experiments are shown.

redox state of Q_A, represents a specific photosynthetic acclimation to growth at cold-hardening temperatures.

Why is there such a strong correlation between photosynthetic capacity and freezing tolerance in these cereals? We do not believe that the observed correlation proves any direct mechanistic link between photosynthesis and frost hardening. We rather favor the view that the good correlation results from photosynthesis providing the energy necessary for the cellular changes that are required for the induction of frost hardiness. The observation that increased sucrose levels are strongly correlated with freezing tolerance has been known for some time (Tumarow, 1931; Dexter, 1933; Andersson, 1944; Levitt, 1980). We suggest that plant breeders will select the greenest, most robust plants that survive an overwintering season. Thus, it is possible that breeders have inadvertently selected for plants with high capacities for photosynthesis at low temperatures while selecting plants that exhibit the greatest winter survival. Increased sucrose levels as well as other carbohydrates reflect an increased capacity for photosynthesis. This, in turn, represents an increased energy supply for all the other metabolic and biosynthetic processes that occur during the cold-hardening process, which results in increased freezing tolerance. The role of low mol wt carbohydrates in stabilizing cellular membranes upon freeze-dehydration has also been emphasized (Santarius, 1982). Based on the fact that the fluorescence characteristic q_P exhibits such a strong, positive correlation with freezing tolerance (Fig. 3A), we suggest that this specific Chl *a* fluorescence characteristic may be exploited as an alternative to photosynthetic measurements to screen cereals for increased freezing tolerance.

Regulation of PSII

It is well established that cold acclimation per se does not significantly lower the quantum yield of photosynthesis, neither for pine (Öquist and Strand, 1988) nor for cereals (Huner, 1985; Huner et al., 1986). This insensitivity of the quantum yield of photosynthesis to cold-hardening was corroborated by the observations that in both NH and H winter rye, the quantum yield of oxygen evolution and of open PSII reaction centers depended similarly on the redox state of Q_A (Fig. 4A) and on q_N (Fig. 4B), respectively. However, we noted that lowering the measuring temperature from 20 to 10°C increased the control exerted by q_N (Fig. 4B), independent of the acclimation state of the leaves. The mechanism behind this increase of q_N control of the photochemical yield of open PSII reaction centers is not known, but might be related to temperature effects on the permeability of thylakoids to protons. Further work is required to substantiate this.

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