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Light utilization in photosynthesis after cold acclimation of plants representing two different seasonal growth strategies

LV Savitch^{2,4}, ED Leonardos³, M Krol², S Jansson¹, B Grodzinski³, NPA Huner², G Öquist¹

¹Umeå Plant Science Center, Department of Plant Physiology, Umeå University, S-901 87 Umeå, Sweden, Stefan.Jansson@plantphys.umu.se;

Gunnar.Oquist@plantphys.umu.se; Fax: (+46) 90 786 66 76

²Department of Plant Sciences, The University of Western Ontario, London, ON, N6A 5B7 Canada, mkrol@uwo.ca; nhuner@uwo.ca; Fax: (519) 661-3935

³Department of Plant Agriculture, University of Guelph, Guelph, ON, N1G 2W1 Canada, dleonard@uoguelph.ca; bgrodzin@uoguelph.ca; Fax: (519) 767-0755

⁴Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Center, Ottawa, ON, K1A 0C6 Canada, savitchl@em.agr.ca; Fax: (613) 759-6566

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Introduction

Photosynthesis provides the energy required for cold acclimation of plants during the autumn, making them resistant to subfreezing winter temperatures (Levitt 1980). However, the combined exposure of plants to low temperatures and light increases the probability that plants will succumb to partial inactivation of photosynthesis due to photoinhibition (Öquist et al. 2001). In fact, evergreen species such as Scots pine may suffer substantial photoinhibition of photosynthesis during the autumn months (Ottander et al. 1995). In contrast, however, herbaceous winter annuals such as winter cereals are much more resistant to low temperature induced photoinhibition of photosynthesis (Öquist and Huner 1993). We hypothesized (Huner et al. 1993) that these different autumnal responses of photosynthesis in conifers and cereals is related to their different growth and developmental strategies. In a comparative study of Lodgepole pine and winter wheat, we tested this hypothesis and demonstrate that the different growth and developmental strategies exhibited by these two species during cold acclimation is indeed correlated with two totally different strategies to utilise the photosynthetically absorbed light.

Materials and methods

Winter wheat (*Triticum aestivum* L., cv. Monopol) plants were grown at a photon flux density of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a 16 h photoperiod under a temperature regime of 20/16°C (day/night), for control, and 5/5°C for cold acclimated plants. Cold acclimated and dark adapted one-year-old seedlings of Lodgepole pine (*Pinus contorta* L.) were transferred to the temperature regime of 25/15°C (day/night), a PFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a 17 h photoperiod, to initiate the second year growth. After a period of six weeks at 25°C/250 PFD, the second-year needles were fully developed and considered as “summer” pine. At this stage, plants were transferred to a temperature regime of 15/10°C (day/night), a PFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and an 8 h photoperiod. After a period

of six weeks at 15°C/250 PFD, plants were considered to be partially cold acclimated and defined as “autumn” pine. For further cold acclimation, “autumn” pine was transferred to a temperature regime of 5/5°C (day/night), a PFD of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a maintained 8 h photoperiod. After a period of six weeks these seedlings were defined as “winter” pine. The whole-plant net carbon exchange rates (NCER) were measured as described by Leonardos et al. (1994) and daily carbon gain was estimated according to Dutton et al. (1988). All Chl *a* fluorescence measurements were made as described in detail by Savitch et al. (2000). All fluorescence parameters were calculated according to Schreiber et al. (1994).

Results

When comparing the effects of cold stress on whole plant net carbon exchange rates (NCER), both pine and wheat plants responded similarly by decreasing the maximum rates of photosynthesis (Fig. 1B, H). However, after cold acclimation, pine still exhibited an inhibition of NCER (Fig. 1C, E), particularly when measured under high light and elevated CO_2 conditions.

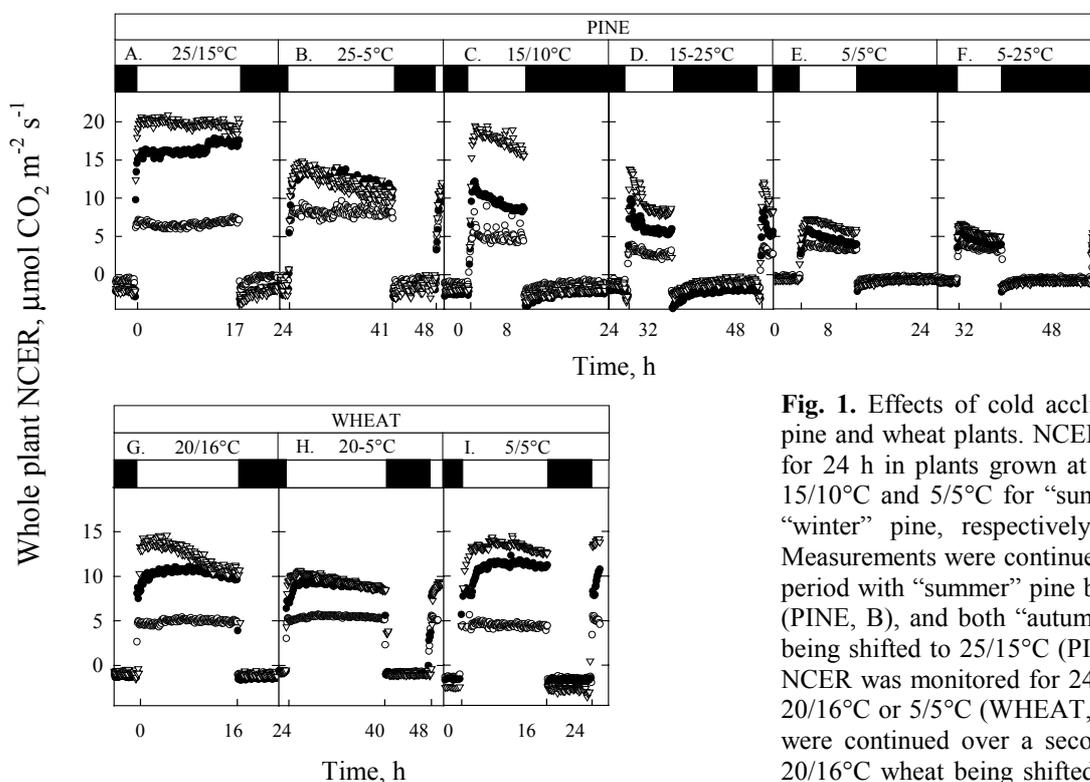


Fig. 1. Effects of cold acclimation on NCER in pine and wheat plants. NCER was monitored first for 24 h in plants grown at 25/15°C (day/night), 15/10°C and 5/5°C for “summer”, “autumn” and “winter” pine, respectively (PINE, A, C, E). Measurements were continued over a second 24 h period with “summer” pine being shifted to 5/5°C (PINE, B), and both “autumn” and “winter” pine being shifted to 25/15°C (PINE, D, F). Similarly, NCER was monitored for 24 h in wheat grown at 20/16°C or 5/5°C (WHEAT, G, I). Measurements were continued over a second 24 h period with 20/16°C wheat being shifted to 5/5°C (WHEAT, H). NCER measurements for pine and wheat were performed at the growth light irradiance of 250 PFD and $p(\text{CO}_2)$ of 35 Pa (open circles), at 1000 PFD and $p(\text{CO}_2)$ of 35 Pa (filled circles), and at 1000 PFD and $p(\text{CO}_2)$ of 100 Pa (open triangles). All data are averages of three independent experiments.

Simply shifting 25/15 grown “summer” pine plants to a measuring temperature of 5/5 °C (Fig. 1B) caused much less inhibition of NCER during the day than observed after cold acclimation (Fig. 1E). Likewise, shifting “autumn” and “winter” cold acclimated pine to 25/15°C did not affect NCER (Fig. 1D, F). In contrast, wheat did not respond to cold acclimation with any inhibition of NCER (Fig. 1G, I). NCER under high light with or without elevated CO_2 , showed that cold acclimation of wheat increased its capacity for photosynthesis. As a result, the net carbon gain over a 24 h light/dark cycle for cold acclimated “autumn” and “winter” pine was only about

20% of that observed in the control "summer" pine grown at 25/15°C. In contrast, the daily carbon gain of wheat was unaffected by cold acclimation (Fig. 2). In accordance with the gas exchange measurements, cold acclimation of pine resulted in strong reductions of both the efficiencies of open PSII reaction centers and the photochemical yields of PSII electron transport at all PFDs studied, while the effects by cold acclimation on PSII in wheat were minor (Fig.3). In pine, this occurred with loss of PSII reaction centers as indicated by decreased levels of D1 protein (Fig. 4). No similar loss of D1 protein was observed in cold acclimated wheat (Fig. 4).

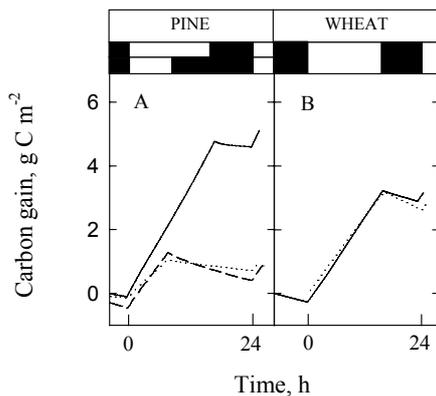


Fig. 2. Effects of cold acclimation on the carbon gain of pine and wheat plants. Presented data reflect the carbon gain of (A) 25/15°C (solid line), 15/10°C (dash line) and 5/5°C (dotted line) grown pine, and (B) 20/16°C (solid line) and 5/5°C (dotted line) grown wheat over a 24 h period. The growth photon flux density of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a CO_2 partial pressure of 35 Pa was used.

Cold acclimation reduced the Chl content of pine needles, but had no significant effect on the Chl content of winter wheat leaves (Fig.4), with only minor changes in the Chl a/b ratio in both species (data not shown). In addition, EPS decreased strongly in the pine needles during cold acclimation (Fig.4). In contrast, similarly high EPS values were observed in both cold acclimated and non-acclimated wheat leaves. The decreased EPS ratio and the sustained high capacity for non-photochemical quenching at limiting irradiances observed in cold acclimated "autumn" and "winter" pine (Fig. 3) correlated with a strong induction of the PsbS protein (Fig. 4), while the level of PsbS was unchanged in cold acclimated winter wheat.

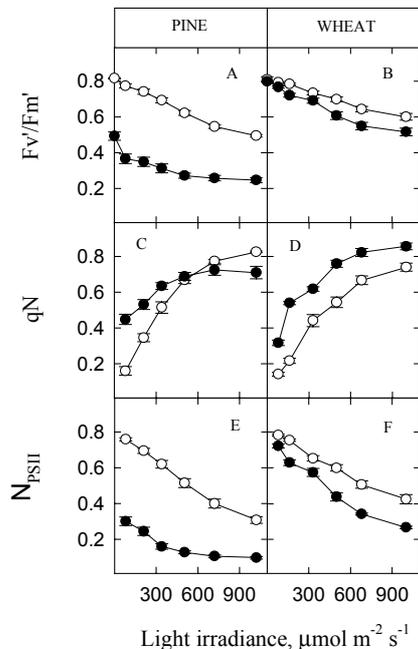


Fig. 3. Effects of cold acclimation on Chl fluorescence parameters in needles and leaves of pine (A, C, E) and wheat (B, D, F). The light response curves of F_v'/F_m' , q_N and N_{PSII} were measured at a $p(\text{CO}_2)$ of 35 Pa and the growth temperatures of 25°C and 20°C for 25/15°C pine and 20/16°C wheat, respectively (open circles), and at 5°C for 5/5°C cold acclimated pine and wheat (filled circles). Data represent mean \pm SE for $n=5$.

Discussion

Conifers must enter a short day dependent dormant growth state in the autumn in order to attain maximum freezing tolerance. In contrast, cereals require growth and development at low temperatures to exhibit maximum freezing tolerance. In concert with these different demands for photosynthetic assimilates during the autumn, we show that Lodgepole pine decreases both its capacity (Fig. 1E) and efficiency (Fig. 3A, E) of photosynthesis after cold acclimation, with an associated partial loss of photosystem II reaction centers. This is accompanied by a low daily carbon gain, and the development of a high and sustained capacity for non-photochemical quenching of absorbed light. This sustained dissipation of absorbed

light as heat correlates with an increased de-epoxidation of the xanthophyll cycle pigments and an induction of the PsbS protein (Fig. 4) - the site of zeaxanthin-dependent quenching of excitation energy (Li et al. 2000). In contrast, winter wheat with a high sink capacity due to active growth and development maintains both its capacity (Fig. 1I) and efficiency (Fig. 3B, F) of photosynthesis, shows no loss of chlorophyll content per leaf area, and shows a high daily carbon gain and a minimal non-photochemical quenching after cold acclimation. In accordance, cold acclimation of wheat neither increases the de-epoxidation of the xanthophylls nor the content of the PsbS protein.

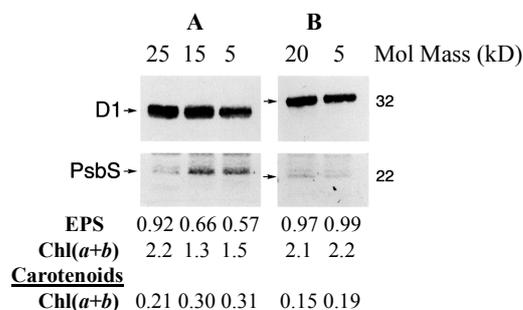


Fig. 4. Western blots of D1 and the Chl-binding protein PsbS from pine (A) acclimated to 25/15°C, 15/10°C and 5/5°C, and from wheat (B) acclimated to 20/16°C and 5/5°C. Lanes of SDS-PAGE were loaded on an equal Chl basis. Chl content and carotenoid/Chl ratio were expressed in mg g⁻¹ fresh wt and w/w, respectively.

The increased capacity for non-photochemical dissipation of absorbed light energy in pine, as photosynthesis becomes inhibited by a limited sink capacity, and eventually by freezing temperatures, is viewed as an important acclimative strategy for avoiding photo-oxidative damage of the needle foliage during autumn and winter. In winter cereals, on the other hand, with its highly efficient photosynthesis at chilling temperatures throughout the autumn, the prevailing high yield of photochemistry can efficiently dissipate absorbed light, and little sustained non-photochemical dissipation of absorbed light is required.

Acknowledgments

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