

The pattern of changes in photosynthetic apparatus in response to cold acclimation and de-acclimation in two contrasting cultivars of oilseed rape

M. RAPACZ and K. HURA

Department of Plant Physiology, Faculty of Agriculture, Agricultural University of Kraków,
Podłużna 3, 30-239 Kraków, Poland

Abstract

In spring and winter cultivars of oilseed rape (*Brassica napus* var. *oleifera*), acclimation of photosynthetic apparatus to cold was connected with the increase in activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and sucrose-phosphate synthase (SPS). Conversely, cold de-acclimation entailed the decline of RuBPCO and SPS activities. The rate of this photosynthetic de-acclimation might depend on day temperature. On the other hand, temperature rise during de-acclimation (identical during the day and night) resulted in the improvement of photosynthetic activity measured by means of chlorophyll fluorescence. An increase in SPS activity (and even transitory increase in RuBPCO activity) was observed when the elongation growth rate (EGR) accelerated during de-acclimation. Throughout re-acclimation, plants with high EGR were unable to maintain or recover higher photosynthetic capacity, despite the fact that SPS activity remained high or even increased during re-acclimation.

Additional key words: *Brassica napus* var. *oleifera*; carbon metabolism; chlorophyll fluorescence; cultivar differences; elongation growth rate; low temperature; photosynthesis; ribulose-1,5-bisphosphate carboxylase/oxygenase; sucrose-phosphate synthase.

Introduction

When warm-grown plants are transferred to chilling temperature, photosynthesis is inhibited mainly because low temperature inhibits sucrose synthesis (Strand *et al.* 1999). After a longer period of low temperature, growth and phloem export both slow down and soluble sugars accumulate in source leaves (Guy *et al.* 1992, Strand *et al.* 1999). During cold acclimation of cold-tolerant herbaceous plants photosynthetic capacity should be high despite the accumulation of large, soluble saccharide pools. Thus in cold hardy herbaceous species, photosynthesis recovers in leaves that develop at low temperature (Öquist *et al.* 1992, Hurry *et al.* 1995). The recovery is connected with photosynthetic carbon metabolism. General increases in the activities of Calvin cycle metabolism, such as RuBPCO, and a selective increase in the activity of enzymes for sucrose synthesis, including SPS,

contribute to this effect (Savitch *et al.* 1997, Strand *et al.* 1999). The triggering stimuli for photosynthetic acclimation may be an imbalance between photons absorbed through photochemistry *versus* the energy utilised through metabolism (increased PS2 excitation pressure) (Huner *et al.* 1998). Thus the dependence of stimulation of photosynthetic activity on the day temperature during acclimation was observed (Rapacz 1998, Rapacz and Janowiak 1998).

The acclimation of photosynthetic apparatus to cold was attributed to winter- but not spring-type plants (Hurry *et al.* 1995), which were considered as unable of cold-acclimation. According to Rapacz (1999), cultivars of spring oilseed rape have some potential to cold acclimate and may be acclimated to the level characteristic of winter plants. The cessation of elongation growth during

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Fax: 4812-4253202, e-mail: rrapacz@cyf-kr.edu.pl

Abbreviations: Chl – chlorophyll; EGR – elongation growth rate; F_0 – chlorophyll *a* fluorescence when all PS2 reaction centres are open in dark-acclimated leaves; F_m , F'_m – fluorescence when all PS2 reaction centres are closed in dark- and light-acclimated leaves, respectively; F_s – steady state fluorescence in light acclimated leaves, F_v/F_m – apparent quantum yield of PS2, where $F_v = F_m - F_0$; PPFD – photosynthetic photon flux density; PS2 – photosystem 2; q_{NP} – non-photochemical quenching of fluorescence; q_P – photochemical quenching of fluorescence; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SPS – sucrose-phosphate synthase; SS – sucrose synthase; ϕ_{PS2} – quantum efficiency (current photochemical efficiency) of PS2.

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cold acclimation is a prerequisite for this phenomenon. Inhibition of elongation growth and acclimation of photosynthesis to cold occur simultaneously and are triggered by high PS2 excitation pressure (Huner *et al.* 1998, Rapacz 1998). In oilseed rape such effect was observed during pre-hardening, *i.e.* at the moderately low day temperature (10–15 °C) before cold-acclimation (Rapacz 1998). Spring plants are unable to maintain growth cessation during prolonged low-temperature periods and promotion of stem elongation is accompanied by the loss of both frost resistance and photosynthetic acclimation (Rapacz and Chilmonik 2000, Rapacz *et al.* 2001). The recent experiments also showed that the changes in frost resistance, photosynthetic capacity, and elongation

growth rate (EGR) during de-acclimation and re-acclimation are interrelated and depend also on the interaction of temperature and irradiance (Rapacz 2002).

The aim of the present work was to determine the pattern of changes in photosynthetic apparatus (with special attention to RuBPCO and SPS activities) during cold-acclimation and de-acclimation under various temperature regimes, and re-acclimation to cold. Since alternating growth rate during these periods may also affect photosynthetic capacity, the experiment was performed on spring and winter cultivars of oilseed rape, which are characterised by different patterns of EGR under such growth conditions.

Material and methods

Plants: Seedlings of spring oilseed rape (*Brassica napus* L. var. *oleifera* f. *annua* cv. Star; DLF Trifolium, Denmark) and winter oilseed rape (*Brassica napus* L. var. *oleifera* f. *bienae* cv. Górczanski; HBP, Poland) were grown in groups of 8 plants per pot (20 cm in diameter) containing loam soil, sand, peat 1 : 1 : 1 (v:v:v). Seeds were germinated under controlled-environment conditions: a PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (sodium lamps Agro, Philips), temperature of 20 °C, and a photoperiod of 12 h. When about 50 % of plants emerged, the temperature was reduced to 12 °C (conditions of pre-hardening). After 5 weeks the plants were subjected to cold acclimation at 2 °C. After next 4 weeks, the plants were subjected for 4 weeks to de-acclimation under various day/night temperature regimes: 20/20, 20/12, 12/20, 12/12, 12/2, 2/12, and 2/2 °C (as the control). PPFD was controlled like in the previous stages of the experiment. After de-acclimation, the plants were transferred back to the cold-acclimating conditions in order to re-acclimate. Water was supplied during the day as required and plants were fertilised once a week with a half-strength Hoagland's solution.

All values are averages of 2 independent experiments (series) comprising 5 pots per each experimental treatment.

Chlorophyll (Chl) *a* fluorescence was measured in the youngest fully expanded leaf (usually in 10 replications,

5 for every series) with a pulse modulated portable fluorometer FMS2 (Hansatech Instruments, King's Lynn, UK) at +12 °C (leaf temperature) in the dark (dark adapted leaf) and next after 15 min of irradiation at a PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (light adapted leaf). q_P and q_{NP} were calculated according to Havaux *et al.* (1991): $q_P = (F'_m - F_s)/(F'_m - F'_0)$, $q_N = (F'_m - F_m)/(F_m - F'_0)$. Quantum efficiency of PS2 was calculated according to Genty *et al.* (1989): $\phi_{PS2} = (F'_m - F_s)/F'_m$. During de-acclimation measurements were made about 1 h after the +12 °C-shift.

Enzyme activities were monitored spectrophotometrically on the youngest, but fully expanded leaves. Leaves of known fresh mass and area were harvested in the light, frozen, and stored in liquid nitrogen. Analyses were performed in 4 biological (2 leaves for every series) and 2 instrumental replications. RuBPCO activity was determined according to Sharkey *et al.* (1991). SPS activity was evaluated by measuring fructose-6-phosphate dependent sucrose formation from UDP-G (Kalt-Torres *et al.* 1987). The reaction was run at 5 and 20 °C to show possible changes in SPS activity specific to low temperature.

Other analyses: Data concerning frost resistance (electrolyte leakage test) and EGR were measured as described in Rapacz (1999) and Rapacz *et al.* (2001).

Results

After shift to 2 °C a progressive acclimation of photosynthetic apparatus to cold was observed in both cultivars. It was reflected in an increasing ϕ_{PS2} (Fig. 1B,D) and q_P (Fig. 2A,C) and in decreasing q_{NP} (Fig. 2B,D). During prolonged stay in cold (plants grown at 2/2 °C during de-acclimation) no further changes in ϕ_{PS2} , a gradual increase in q_{NP} (to the level similar to that observed in the week 0) as well as transitory increase in q_P was ob-

served in the winter cultivar (Figs. 1B and 2A,B). In spring cultivar a partial reverse of photosynthetic acclimation to cold was observed. At the end of the experiment, ϕ_{PS2} , q_P , and q_{NP} were similar to those after 2 weeks of acclimation (Figs. 1D and 2C,D).

The pattern of changes in apparent quantum yield of PS2 (F_v/F_m) was similar in both cultivars (Fig. 1A,B). In the winter cultivar Górczanski, F_v/F_m was maximum

reduced by over 25 % two weeks after low temperature shift and in the spring cultivar Star by over 20 % after three weeks of cold-treatment. Then an increase in F_v/F_m

was recorded in both cultivars. During prolonged stay at low temperature (plants grown at 2/2 °C during de-acclimation) this recovery reached over 90 % of initial values

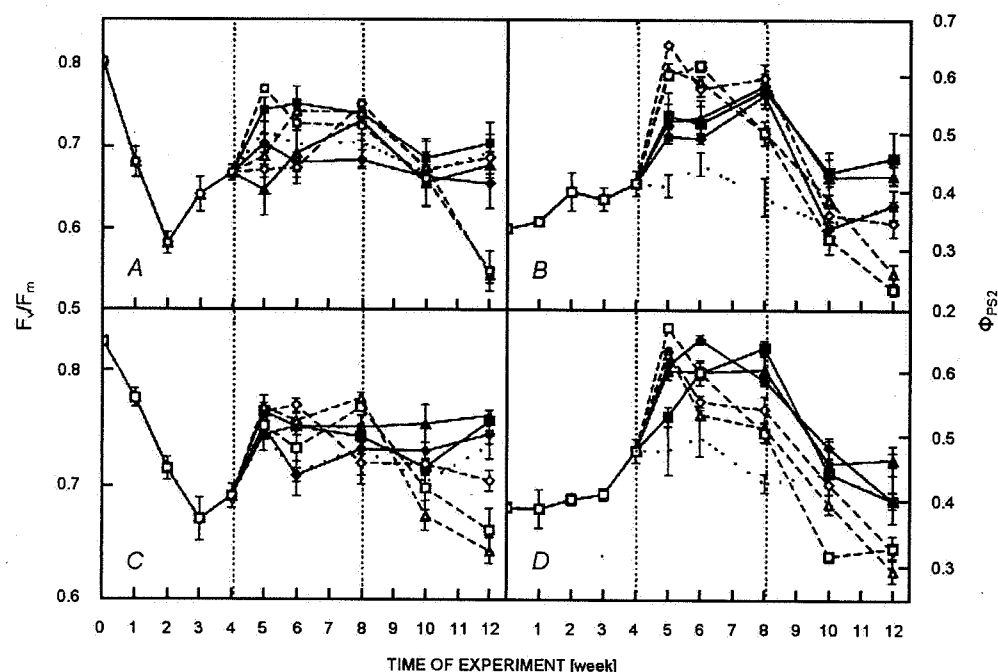


Fig. 1. Changes in apparent (F_v/F_m ; A, C) and current (Φ_{PS2} ; B, D) quantum yield of PS2 in winter (A, B) and spring (C, D) cultivars of oilseed rape affected by growth temperature. Weeks 0-4 cold-acclimation at +2 °C; weeks 4-8 de-acclimation at: 2/2 °C (day/night) – unmarked dotted line, 2/12 – ■, 12/2 – ▲, 12/12 – ◆, 12/20 – ◇, 20/12 – △, 20/20 – □; weeks 8-12 re-acclimation at 2 °C. Means of 10 replications (on different leaves) $\pm S_{EM}$. Measurements were made at 12 °C, Φ_{PS2} was determined at the PPFD of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

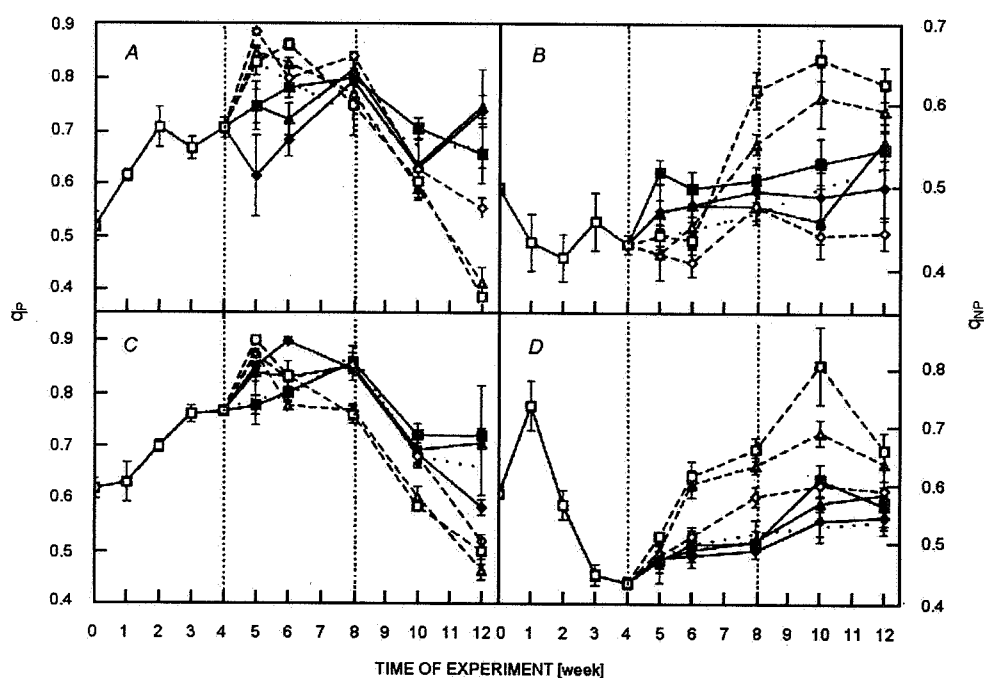


Fig. 2. Changes in photochemical (q_p ; A, C) and non-photochemical (q_{NP} ; B, D) chlorophyll fluorescence quenching coefficients in winter (A, B) and spring (C, D) cultivars of oilseed rape affected by growth temperature. Weeks 0-4 cold-acclimation at +2 °C; weeks 4-8 de-acclimation at: 2/2 °C (day/night) – unmarked dotted line, 2/12 – ■, 12/2 – ▲, 12/12 – ◆, 12/20 – ◇, 20/12 – △, 20/20 – □; weeks 8-12 re-acclimation at 2 °C. Means of 10 replications (on different leaves) $\pm S_{EM}$. Measurements were made at 12 °C and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD.

and apparent quantum yield of PS2 remained constant up to the end of the experiment.

During cold-acclimation an increase in both initial and total activities of RuBPCO was recorded in both cultivars (Fig. 3). The level of the activity after accli-

mation was similar for both cultivars although smaller increase was noticed for the spring one. The SPS activity also increased more in the winter cultivar than in the spring cultivar (Fig. 4). Additionally, in the winter cultivar a similar increase was noted when SPS activity was

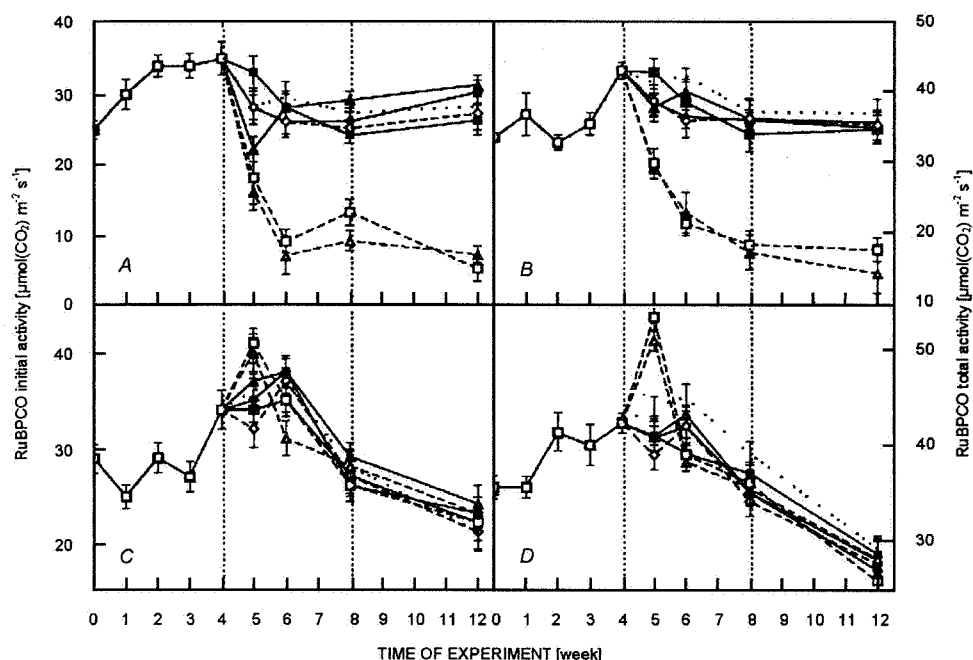


Fig. 3. Changes in RuBPCO initial (A, C) and total activities (B, D) in winter (A, B) and spring (C, D) cultivars of oilseed rape affected by growth temperature. Weeks 0-4 cold-acclimation at $+2^\circ\text{C}$; weeks 4-8 de-acclimation at: $2/2^\circ\text{C}$ (day/night) – unmarked dotted line, $2/12^\circ\text{C}$ – ■, $12/2^\circ\text{C}$ – ▲, $12/12^\circ\text{C}$ – ◆, $12/20^\circ\text{C}$ – ◇, $20/12^\circ\text{C}$ – △, $20/20^\circ\text{C}$ – □; weeks 8-12 re-acclimation at 2°C . Means of 4 biological (leaves) and 8 instrumental (measurements) replication $\pm S_{\text{EM}}$.

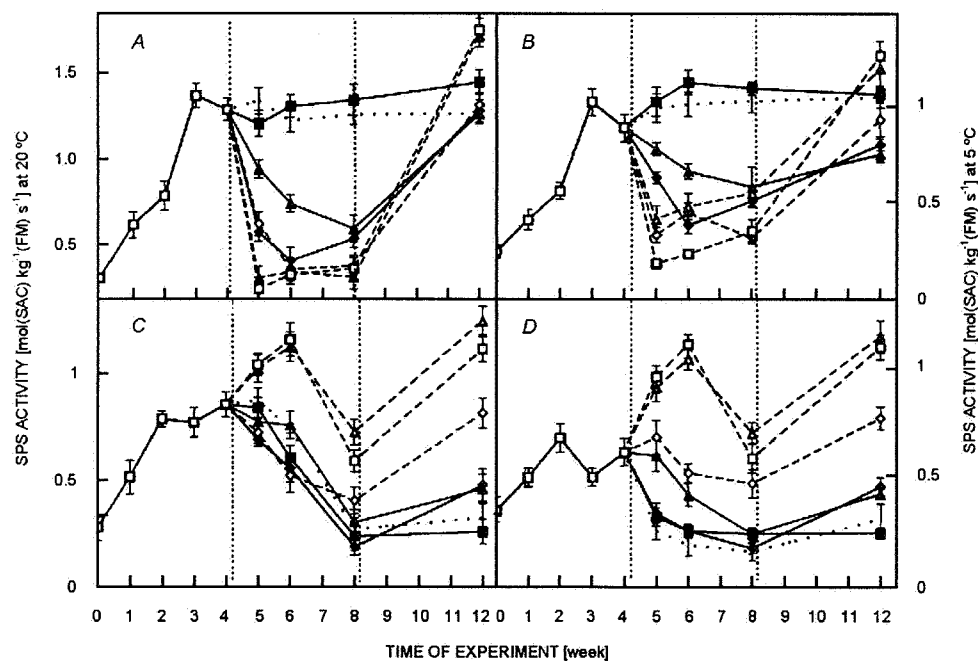


Fig. 4. Changes in SPS activity measured at 20°C (A, C) and 5°C (B, D) in winter (A, B) and spring (C, D) cultivars of oilseed rape affected by growth temperature. Weeks 0-4 cold-acclimation at $+2^\circ\text{C}$; weeks 4-8 de-acclimation at: $2/2^\circ\text{C}$ (day/night) – unmarked dotted line, $2/12^\circ\text{C}$ – ■, $12/2^\circ\text{C}$ – ▲, $12/12^\circ\text{C}$ – ◆, $12/20^\circ\text{C}$ – ◇, $20/12^\circ\text{C}$ – △, $20/20^\circ\text{C}$ – □; weeks 8-12 re-acclimation at 2°C . Means of 4 biological (leaves) and 8 instrumental (measurements) replication $\pm S_{\text{EM}}$.

assayed at 20 or 5 °C (Fig. 4A,B). In the spring cultivar the increase in SPS activity observed during acclimation was stronger when the activity was measured at 20 °C. No further changes in RuBPCO and SPS activities were observed during prolonged low-temperature stay in the Górczanski plants (control 2/2 plants, Figs. 3A,B and 4A,B). In the cultivar Star the prolonged chilling was accompanied also by a decrease in RuBPCO and SPS activities (Figs. 3C,D and 4C,D).

Cold de-acclimation triggered changes of photosynthetic activity. Both a loss of acquired photosynthetic acclimation to cold and an improvement of photosynthetic activity were observed due to the growth in warm. The observed reaction of photosynthetic apparatus to de-acclimation was strongly differentiated between cultivars. In cv. Górczanski the loss of the acclimation of photosynthetic apparatus to cold, which found expression in the decrease in RuBPCO and SPS activities, was documented (Figs. 3A,B and 4A,B). The rate of this decrease depended on the day temperature during de-acclimation and was the fastest at 20/20 and 20/12 °C. The decline in RuBPCO activity was hardly visible for other conditions of de-acclimation, whereas SPS activity did not decrease only at 2/12 °C (and in the control). De-acclimation of winter cultivar also resulted in an improvement of photosynthetic activity measured by means of Chl fluorescence. As distinct from changes in enzyme activities described above, the rate of this recovery seems to depend on the

mean temperature of de-acclimation. The increase in ϕ_{PS2} and q_P was faster in 20/12, 20/20, and 12/20 °C de-acclimated plants, but at the end of de-acclimation both parameters were similar under all conditions of de-acclimation except of 20/12 and 20/20 °C where the lowest values were found (Figs. 1B and 2A). It may be due to the decrease in photosynthetic enzyme activities observed in 20/12 and 20/20 °C de-acclimated plants (Figs. 3 and 4). The increase in q_{NP} was observed after 4 weeks of de-acclimation at 20/12 and 20/20 °C (Fig. 2B). An increase in F_v/F_m was observed in all plants with exception of those de-acclimated at 12/12 °C (Fig. 1A).

During de-acclimation of the spring cultivar Star a decrease in RuBPCO total and initial activities was observed (Fig. 3C,D). The decrease was preceded, in most cases, with the temporary increase in the activities. It was observed shortly after one week of de-acclimation in the 20/20 and 20/12 de-acclimated plants whereas in the 12/20, 12/12, and 12/2 °C de-acclimated plants it was visible one week later. This increase in activity may be related to the intense elongation growth that started some time later (Table 1). SPS activity also seemed to increase markedly at that time (Fig. 4C,D, Table 1). SPS activity was higher in plants, which elongated stems to the greater extent, *i.e.* in 20/12, 20/20, and 12/20 °C de-acclimated plants (Fig. 4C,D; Table 1). The pattern of changes in Chl fluorescence parameters was in general similar to those observed in the Górczanski plants (Figs. 1C,D and 2C,D).

Table 1. Changes in elongation growth rate (shoots and leaves) during cold-de-acclimation (week 5-8) and re-acclimation (week 9-12) and the ability to frost resistance recovery during re-acclimation of winter cultivar Górczanski and spring cultivar Star of oilseed-rape.

De-acclimation [°C: day/night]	Promotion of elongation growth [week of the experiment]		Frost resistance recovery during re-acclimation [% of resistance acquired after cold-acclimation]	
	Górczanski	Star	Górczanski	Star
2/2	—	12	112	92
2/12	—	12	108	91
12/2	—	8	105	92
12/12	—	8	99	91
12/20	—	8	102	91
20/12	8	6	82	65
20/20	8	6	80	56

It indicates that the reaction of photosynthetic apparatus to warming is similar in both cultivars. Like in the winter cultivar an increase in q_{NP} was observed at the moment of elongation-growth induction and after decline of RuBPCO activity (at 20/20 and 20/12 °C – week 6, 12/20 °C – week 8) (Figs. 2D and 3C,D, Table 1). During cold re-acclimation a recovery of photosynthesis in cold was observed only in plants that were not characterised by intense EGR and were able to recover frost resistance to level similar to that observed after cold acclimation (Table 1). In plants with an intense EGR (20/20, 20/12 °C de-acclimated plants of both cultivars and 12/20-de-acclimated spring plants) an increased susceptibility to pho-

toinhibition, which occurred at the low-temperature stay, was observed. It was visible as the decrease in F_v/F_m (Fig. 1A,C). The deterioration in photosynthetic capacity was visible also in the decrease in ϕ_{PS2} and q_P , whereas q_{NP} originally increased and then declined (Figs. 1B,D and 2). In the other spring plants, which started to elongate irrespective of de-acclimation conditions, limited abilities to frost resistance recovery (Table 1) were accompanied with decrease in ϕ_{PS2} and q_P if compared to the values after cold acclimation (Figs. 1D and 2C). The abilities of photosynthetic apparatus to re-acclimate to cold were probably limited by the low activity of RuBPCO, which dropped down during re-acclimation (Fig. 3). In plants

not affected by intense EGR, RuBPCO activity after re-acclimation was similar to that recorded after initial cold acclimation. During re-acclimation an increase in SPS activity was observed irrespective of cultivar and conditions of de-acclimation (Fig. 4). This increase seems to accompany both the acclimation of photosynthesis to cold and the elongation growth. In the winter cultivar SPS activity recovered to the level noticed after cold-acclimation, but plants in which elongation growth had been stimulated during de-acclimation (20/20, 20/12 °C) were characterised by the highest SPS activity, particularly when measured at 20 °C (Fig. 4A,B). In the spring plants

Discussion

We showed that both the spring and winter oilseed rapes acclimated their photosynthesis to cold, if the EGR was ceased during pre-hardening preceding cold acclimation. The increase in photosynthetic capacity after low-temperature shift was connected with the increase in RuBPCO and SPS activities similarly as reported for winter-type plants (Hurry *et al.* 1995). A sustained reduction in the maximum photosynthetic efficiency of PS2 measured as F_v/F_m and observed during low temperature stay of both the spring and winter rape should be interpreted as protection of PS2 reaction centres from overreduction *via* accumulation of zeaxanthin (Adams *et al.* 1995).

In contrast, plants characterised with intense EGR cannot recover photosynthetic capacity. It was observed during re-acclimation and reflected in progressive decrease in RuBPCO activity, photochemical quenching, and photochemical efficiency of PS2. After the low-temperature shift an increase in non-photochemical quenching was observed which might be considered as a reaction, which protects photosynthetic apparatus from excess radiant energy (Demmig-Adams and Adams 1992). The further low-temperature stay results in the decrease in non-photochemical quenching as a result of an increase in photochemical quenching noticed during low-temperature acclimation of photosynthesis (Huner *et al.* 1993, Savitch *et al.* 2000). In plants, which are characterised with progressive loss of photosynthetic capacity when grown in the cold, an increase in q_{NP} accompanied with a decline in q_P may be a consequence of photoinhibition (Huner *et al.* 1993). In our experiment similar effect was observed also during de-acclimation after the induction of elongation growth and decrease in RuBPCO activity. During re-acclimation of plants characterised with particular high rate of elongation growth a simultaneous decrease in both types of quenching and F_v/F_m was noticed. It may indicate severe damages to photosynthesis observed during re-acclimation in plants with low photosynthetic capacity. Similar results were obtained by Hurry *et al.* (1995) for spring oilseed rape. In the present work no decrease in F_v/F_m was observed during re-acclimation in other spring plants. According to Adams *et al.* (1995), it may be inter-

preted as a decrease in flexibility of response to cold treatment with a minimal risk of photoinhibition. As a consequence, if these plants had been treated longer with cold, the damages in photosynthetic apparatus would have been observed, as in the case of plants, which earlier started the intense elongation growth.

Temperature rise during de-acclimation (identical during the day and at night) resulted in the improvement of photosynthetic capacity due undoubtedly to acceleration of plant metabolism including repair systems for potential photodamages and decrease in assimilate content in leaves which formerly resulted in feedback inhibition of photosynthesis. On the other hand, cold-de-acclimation triggered the decrease in RuBPCO and SPS activities and the rate of it depended on the temperature of the day during de-acclimation. It may suggest that mechanisms responsible for acclimation of photosynthetic apparatus to cold (Hurry *et al.* 2000) may be also responsible for the de-acclimation.

In cabbage seedlings the activity of sucrose synthase (SS) and SPS increases during cold acclimation, but decreases to the activity before cold acclimation during de-acclimation (Sasaki *et al.* 2001). Hence SS and SPS are probably regulated by cold acclimation and de-acclimation and play important roles in sugar accumulation and acquisition of freezing tolerance in the leaves. In the present study, changes in SPS activity resulting from changes of the day temperature during de-acclimation and re-acclimation reflect not only changes in the level of acclimation of photosynthesis to cold and changes in frost resistance connected with sugar accumulation in leaves (values not shown) but also reflect changes in EGR. In fast growing plants an increase in SPS activity was always observed. Conditions of high day-temperature were favourable for the promotion of elongation growth (Rapacz 1998). But growth under such conditions triggers also a de-acclimation of photosynthetic apparatus (Rapacz 2002) which may be connected with decreasing SPS activity. During promotion of elongation growth, an increase in assimilate translocation should be expected. SPS is the key enzyme controlling transport pool size (Kalt-Torres *et al.* 1987) and the increase in SPS activity

was observed with increasing growth rate and gibberellin contents (Zamski and Schaffer 1996). In the present study the increase in SPS activity accompanied the increase in EGR. Also the transient increase in RuBPCO activity was observed in plants close to the start of elongation growth. Thus, increase in the activity of photosynthetic enzymes during cold acclimation/de-acclimation seems to be connected not always with acclimation of photosynthetic apparatus to cold but sometimes may accompany the decreasing ability to cold-acclimate caused by increasing

rate of elongation growth.

According to suggestion of Levitt (1972), high EGR may interfere with cold acclimation mainly as a result of competition for photosynthates between growth and acclimation (when assimilates accumulate to reduce osmotic potential). Our results suggest that the other possible reason of reduced cold-acclimation abilities in fast growing plants may be a disorder in acclimation of photosynthesis to cold.

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