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EFFECT OF COLD HARDENING ON SOME PHOTOSYNTHETIC CHARACTERISTICS OF PEA (*Pisum sativum* L., CV. RAN 1) PLANTS

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Summary. The effect of 96 h cold acclimation of pea (*Pisum sativum* L., cv. Ran 1) plants on their photosynthetic capacity was investigated. It was found that pea plants were able to modulate their photosynthetic rate during growth at low temperature and adjust it to needs for survival. The advantages of hardening to low temperature were very clearly expressed when the effects on O_2 evolution and on the relative part of Q_B non-reducing centres were analysed. Analysis of photosynthetic rates and fluorescence parameters showed that these processes were more resistant to freezing in cold hardened than in non-hardened pea plants. It was proposed that for pea plants the maintenance of active photosynthesis at low overzero temperatures is also very important for survival, because in early spring such unfavourable conditions are not rare.

***Key words*:** acclimation, chlorophyll fluorescence, low temperature, photochemical activity, photosynthesis

***Abbreviations*:** LT – low temperature; PS_{maxCO_2} – maximum photosynthetic CO_2 uptake; PS_{maxO_2} – maximum photosynthetic O_2 evolution; H – hardened; NH – non-hardened; CH – cold hardened; PFD – photon flux density; IK – induction kinetics; PS – photosystem; F_0, F_v and F_m – initial, variable and maximal fluorescence, respectively; RC – reaction centre(s); Q_A and Q_B – the primary and secondary electron acceptors of PS2.

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Introduction

The ability of plants to acclimatize their photosynthetic apparatus to unfavourable temperature changes and to increase its tolerance is well documented (Yordanov, 1979; Berry and Björkman, 1980; Levitt, 1980; Havaux, 1992) but the acclimation mechanisms are sill not fully elucidated.

Cold hardening is the development of tolerance to subzero temperature which would provide lethal temperature for unhardened plants. Light and CO_2 needed for growth and development are required during exposure of winter herbaceous crops to low temperature (LT) in order to obtain maximum cold hardening (Tumanow, 1931; Dexter, 1933; Lawrence et al., 1973; Andrews et al., 1974; Hurry and Huner, 1991). It is hypothesized that photosynthesis provides the energy for this growth and development.

It was shown that photosynthetic CO_2 uptake, O_2 evolution and chlorophyll fluorescence in cold hardened winter cereals and spinach were more resistant to photoinhibition than in non-hardened ones (Somersalo and Krause, 1989, 1990; Hurry and Huner, 1991; Öquist and Huner, 1993b). This phenomenon was also demonstrated by means of *in vivo* measure of chlorophyll *a* fluorescence (Lapointe et al., 1991). Winter rye and wheat plants grown at 5°C are able to maintain light and saturated rates equivalent to or higher than plants grown at 20°C (Huner et al., 1986). Similar results were obtained in some other plants – monocotyledonous (Pollock et al., 1984) and dicotyledonous winter annuals (Regher and Bazzaz, 1976; Boese and Huner, 1990) and algae (Maxwell et al., 1994). In contrast to winter cereals on the other hand cold grown spinach did not exhibit any significant changes in PS_{maxCO_2} and PS_{maxO_2} compared to physiologically equivalent warm grown spinach, i.e. $PS_{max}(H)/PS_{max}(NH)=1$. In contrast to winter cereals when pines are exposed to cold hardening conditions they become photosynthetically inhibited (Öquist and Martin, 1986; Öquist and Malmberg, 1989). According to Falk et al. (1993) both strategies – improved photosynthetic performance in winter cereals or reduced photosynthetic performance in pines at LT hardening are successful in ensuring the survival of the plant species during adverse environmental conditions. In view of the fact that at low growth temperature the reaction of spring cereals is different from that of winter ones (Hurry and Huner, 1991) it was considered interesting to characterize the response of photosynthesis in belonging to spring type, but very early pea plants (cv. Ran 1) to low growth temperature leading to their cold hardening.

Materials and Methods

Room temperature chlorophyll fluorescence was measured with PAM fluorimeter as described by Schreiber and Bilger, 1975.

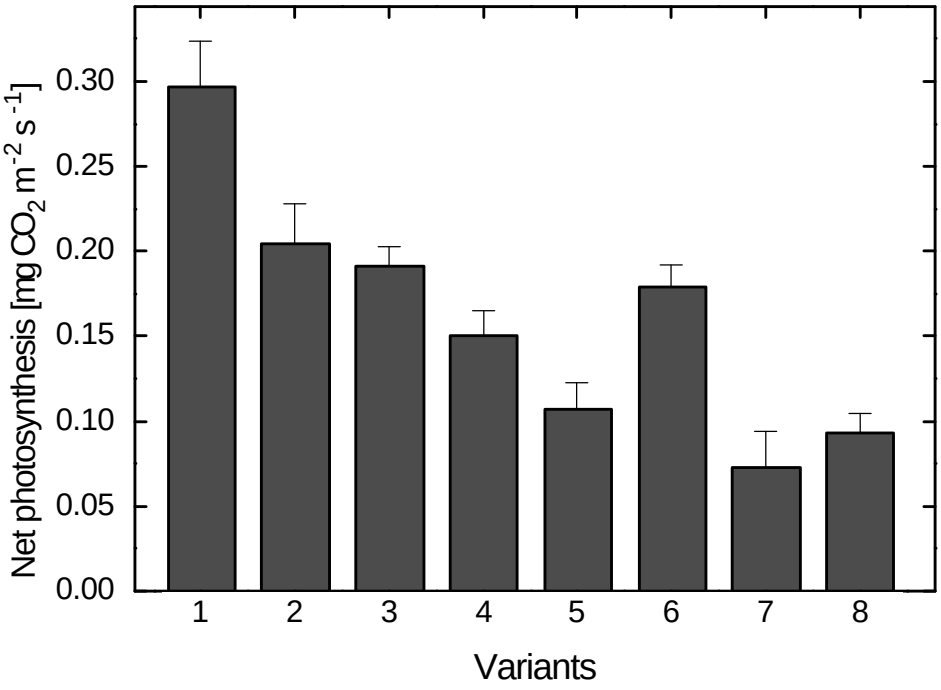

Fig. 1. Net photosynthetic rate of 2nd pea leaf after acclimation to low temperature, followed by freezing of different time. Pea plants were grown at temperature 23–25 °C and irradiance 110 μmol.m^{-2}.s^{-1} for 7 d. Than half of them were acclimated 96 h at low temperature (5°C). Thus following variants were formed: 1. Control, 25°C; 2. Hardened at 5 °C, 4 d; 3. Control 25 °C transferred (→) to –8°C, 1 h; 4. Hardened at 5°C → –8 °C, 1 h; 5. Control 25 °C → –8 °C, 3 h; 6. Hardened at 5 °C → –8 °C, 3 h; 7. Control 25°C → –8 °C, 5 h; 8. Hardened at 5 °C → –8 °C, 5 h

The experiments were carried out with 12 d old pea (*Pisum sativum* L. cv. Ran 1) plants grown in climatic chamber at 23–25°C, 110 μmol.m^{-2}.s^{-1} photon flux density (PFD) and 12 h photoperiod. After 8 d growth at 23–26 °C half of plants were transferred to hardening conditions (PFD 110 μmol.m^{-2}.s^{-1}, day/night temperature regime 5 °C, photoperiod 12 h for 4 days (96 h). Non-hardened control plants remained in the temperature regime of 23–25 °C. Thus the following variants were formed: 1. Control, 25 °C ; 2. Hardened at 5°C, 4 d; 3. Control 25 °C transferred (→) to –8 °C, 1 h; 4. Hardened at 5 °C → –8 °C, 1 h; 5. Control 25 °C → –8 °C, 3 h; 6. Hardened at 5 °C → –8 °C, 3 h; 7. Control 25 °C → –8 °C, 5 h; 8. Hardened at 5 °C → –8 °C, 5 h.

After above mentioned treatments CO_2 uptake and O_2 evolution as well as chlorophyll *a* fluorescence induction kinetics (IK) parameters were measured. Fully expanded second leaves developed under either non-hardening (NH) or hardening (H) conditions were used.

Oxygen evolution was measured by leaf-disk oxygen electrode LD 2/2 (Hansatech, UK) at temperature about 25 °C and irradiance 900 μmol.m^{-2}.s^{-1} PFD.

Parameters of chlorophyll fluorescence IK were determined by Pulse modulated fluorimeter PAM 101-103 (H. Walz, Germany) using two pulses sequence of exciting light. The first IK was registered at low actinic light (60 μmol.m^{-2}.s^{-1}, 5 s duration). The second pulse with saturating light (over 3000 μmol.m^{-2}.s^{-1}, 2 s duration) was done after additional 2 min dark period (for details see Yordanov et al., 1997a). The measuring light was 0.75 μmol.m^{-2}.s^{-1}.

Results and Discussion

Effect of growth temperature on the rate of CO_2 uptake and O_2 evolution

On Fig. 1 are shown the changes in photosynthetic rate in NH and H to low temperature plants depending on freezing treatment and its duration. It can be seen that the photosynthetic rate of hardened non-freezed at –8 °C pea plants is lower than that of control (25 °C) plants. After 1 h freezing the photosynthetic rate of NH plants is also a little higher (var. 3) than that of hardened ones (var. 4). But when NH and H plants were freezed 3 h at –8 °C the situation is changed – the H at LT plants (var. 6) photosynthesized with a higher rate than NH ones (var. 5). These differences between NH and H plants practically disappeared after 5 h freezing (var. 7 and 8). According to Hurry and Huner (1991) spring cultivars grown at 5 °C showed 35% lower apparent photon yield for CO_2 exchange and 25% lower light saturated rates of CO_2 exchange compared to 20 °C grown control. In our experiments the advantage of LT hardening become apparent only after 3 h freezing at –8 °C of both NH and H pea plants.

The advantages of hardening to LT is very clearly expressed when the effect of growth temperature and freezing on O_2 evolution is analysed (Fig. 2). In this case the rate of O_2 evolution in non-freezed plants grown at both 5 and 25 °C is practically equal with the tendency to be a little higher in H plants. It is also interesting to mention that the hardening effect increased with the duration of freezing at –8 °C of NH and H plants (see e.g. var. 3 and 4 on the one hand and var. 7 and 8 on the other hand). These results are similar to the data of Huner's group received with winter cereals. After exposure to growth and development at 5 °C cold hardened winter rye and wheat exhibited PS_{maxO_2} or PS_{maxCO_2} that were greater than the same cultivars grown at 20 °C (NH) regardless of measuring temperature between 5 and 20 °C (Huner at al., 1986; Hurry and Huner, 1991; Öquist and Huner, 1993a; Öquist et al.,1993). It was concluded that these cereals are able to modulate the photosynthetic rate during growth at LT so that their photosynthetic capacity is increased [PS_{max}(H)/PS_{max}(NH) > 1] with no changes in photosynthetic efficiency.

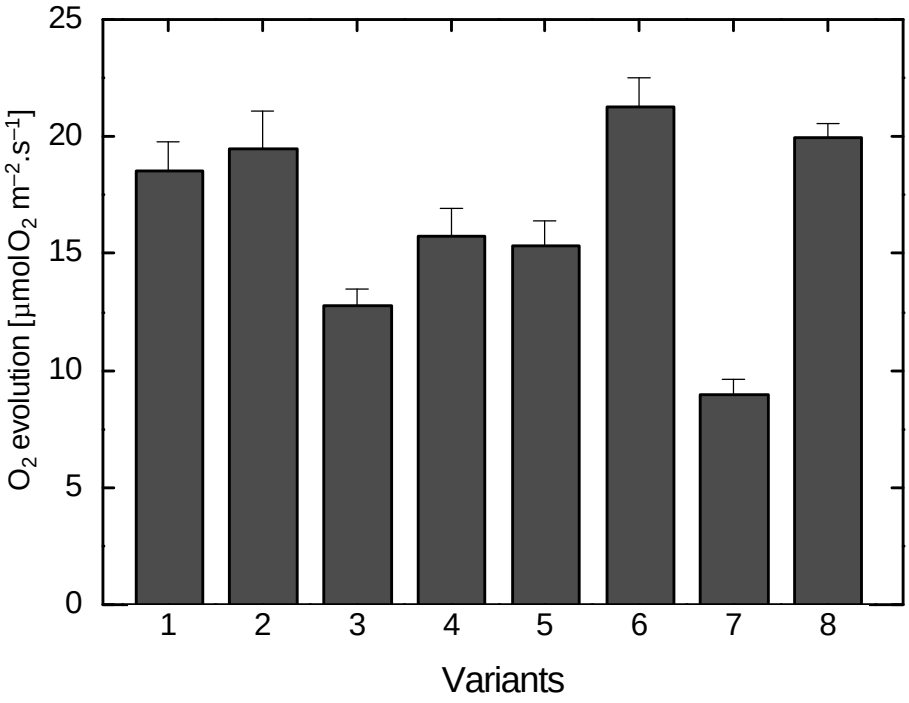

Fig. 2. Dynamics of oxygen evolution in leaf disks from acclimated and non-acclimated plants. The variants are as in Fig. 1

Effect of growth temperature and freezing on photochemical activity

The effect of growth temperature and freezing of pea plants was evaluated on the basis of changes in chlorophyll *a* fluorescence IK. At low irradiance the IK shows well-pronounced OID phase (value F_1) correlated with fluorescence of closed Q_B non-reducing centres. Registered after additional high light pulse the maximum fluorescence (F_m) corresponds to the maximal one of all PS2 centres in the sample. Thus we can use the ratio $(F_i–F_0)/(F_{im}–F_{i0})$ as a criterion of relative concentration of Q_B non-reducing centres. On the other hand, we used the relative F_v during high light pulse $(F_{im}–F_{i0})/F_{i0}$ as a measure of photochemical activity (for details see Yordanov et al.,1997b).

Data shown on Fig. 3 illustrate the dependence of photochemical activity, evaluated from the $(F_{im}–F_{i0})/F_{i0}$ ratio, on the growth temperature followed by different time of freezing at –8 °C. As seen all plants grown 4 days at LT have photochemical activity higher than the control plants, regardless of freezing duration. The differences between plants grown all the time at 25 °C and plants grown 96 h at 5 °C were higher after their 5 h freezing at –8 °C (variants 7 and 8) and lower after 1 h freezing (variants 3 and 4).

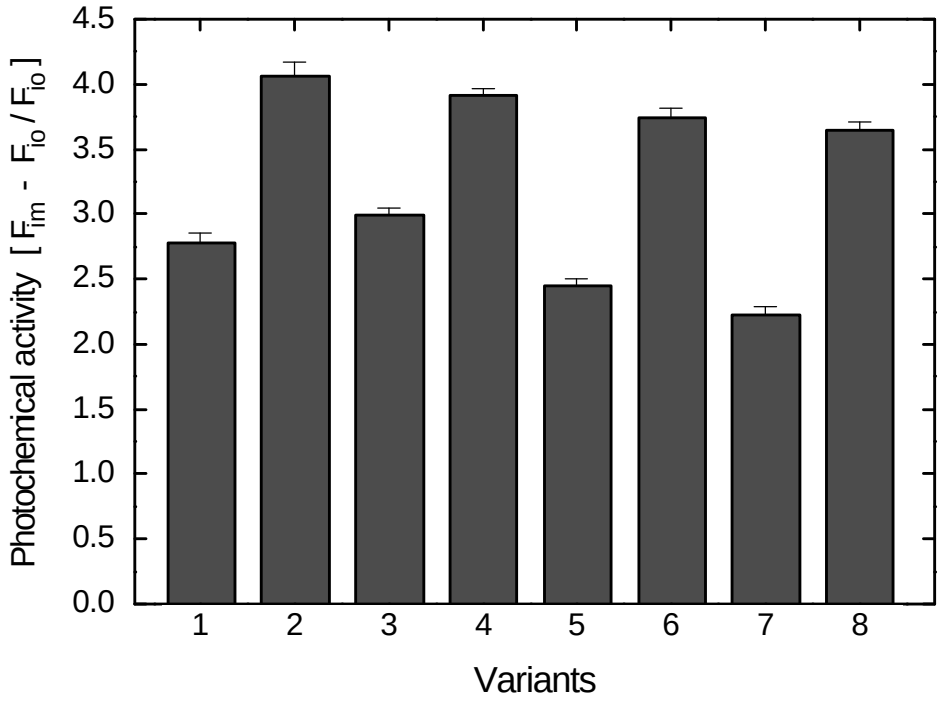

Fig. 3. Low temperature influence on photochemical activity [calculated from the ratio $(F_{im}–F_{i0})/F_{i0}$] of 2nd pea leaf. Induction kinetics (IK) of chlorophyll fluorescence was excited by two light pulses. After 3 min dark adaptation the initial fluorescence (F_0) was measured by weak light beam (0.75 μmol.m^{-2}.s^{-1} PFD). The first IK was excited by 5 s low light pulse (60 μmol.m^{-2}.s^{-1} PFD), followed by 2 min dark adaptation and illumination by saturating light pulse (> 3000 μmol.m^{-2}.s^{-1} PFD) for registering of the second IK. The variants are as in Fig. 1

An interesting question is the structural and functional heterogeneity of reaction centres (RC) in stress conditions. As known one of the processes reducing F_v in stress conditions is the reduction of chlorophyll antenna size (transition of $PS2_\alpha \rightarrow PS2_\beta$) or the efficiency of excitation energy migration to RC (Sundby et al., 1986; Cao and Govinjee, 1990; Goltsev et al., 1994). Our results (Fig. 4) showed that the relative part of Q_B non-reducing $PS2_\beta$ centres is higher both in non-freezing (var. 2) and freezing for a different time (variants 3, 5 and 7) control plants compared with the plants grown 96 h at 5°C and then experiencing 1, 3 or 5 h freezing.

Similarly to photochemical activity, the differences between control plants (25°C) and plants grown 96 h at 5 °C were highest after 5 h freezing at –8°C. These data are analogous with results received from Öquist and Huner (1993b). They showed that the leaves of hardened winter cereals were able to keep a larger fraction of PS2 RC in an open configuration, i.e. a larger ratio of oxidized/reduced Q_A, than the leaves of the

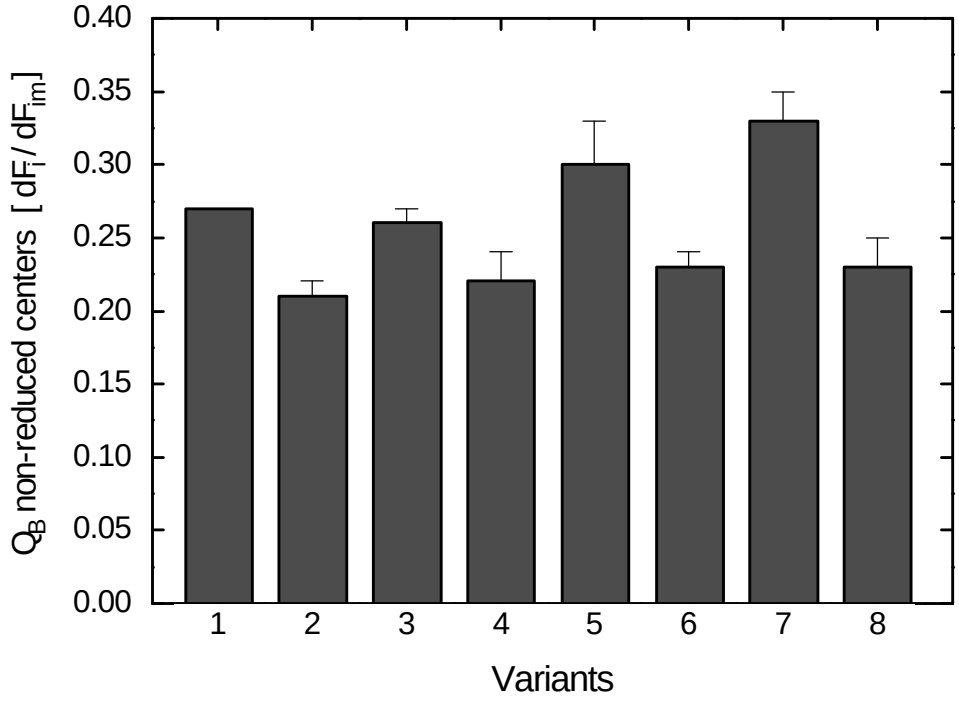

Fig. 4. Changes in relative concentration of Q_B non-reducing PS2 centres [calculated from the ratio $(F_I - F_0)/(F_{im} - F_{i0})$] after low temperature acclimation of plants, followed by freezing of different time. The IK parameters were registered as in Fig. 3. Other details are as in Fig. 1

non-hardened ones. Moreover, in order to establish the same proportion of oxidised to reduced Q_A a threefold higher PFD was required for cold hardened leaves than for the non-hardened ones which accounts for their higher resistance to photoinhibition.

Therefore, similar to hardened winter cereals pea plants are able to adjust their rate of photosynthesis and photosynthetic parameters during growth at LT so that photosynthetic capacity is increased. For pea plants the maintenance of active photosynthesis at low overzero temperatures is also very important for survival, because in early spring such unfavourable conditions are not rare. Analysis of photosynthetic rates and fluorescence parameters showed that these processes were more resistant to freezing in cold hardened than in non-hardened pea plants.

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