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RECOVERY OF THE PHOTOSYNTHETIC APPARATUS IN BEAN PLANTS AFTER HIGH- AND LOW-TEMPERATURE INDUCED PHOTOINHIBITION *

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Summary. The recovery after photoinhibition in 20-day-old bean plants (*Phaseolus vulgaris* L.) induced by 4 h treatment with low (12 °C), normal (24 °C) and high (42 °C) temperatures under high (1000 μmol.m^{-2}.s^{-1}) and low (100 μmol.m^{-2}.s^{-1}) photon flux density (PFD) was studied. The changes in the photosynthetic apparatus were analysed using chlorophyll fluorescence measurements. The ratios F_v/F_m measured by PAM fluorimeter were used as characteristics of quantum efficiency of photosystem II. Data showed that both high and low temperatures enhance the photoinhibition manifested as F_v/F_m decrease. At all temperatures investigated this decrease was due in greater extent to F_m decrease while the increases in F_o were insignificant. Different pattern of photosynthetic apparatus recovery after photoinhibition at various temperatures was observed, suggesting that different mechanisms of photoinhibitory injury predominate in these cases.

Key words: chlorophyll fluorescence, high and low temperature stress, *Phaseolus vulgaris* L., photoinhibition, recovery

Abbreviations: F_o, F_v and F_m – initial, variable and maximal chlorophyll fluorescence; PSI – photosystem I; PSII – photosystem II; Q_A – primary electron acceptor of PSII; PFD – photon flux density

Introduction

The capability of plants to recover after stress is a major characteristics determining the possibility for a given plant species to survive under unfavourable conditions. It

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depends on the degree of the plant adaptation to the imposed stress and to what extent the eventual injuries are reversible. The injuries in the photosynthetic apparatus provoked by high light are connected mainly with photosystem II (PS II) (Krause, 1988) and lead to inactivation of the electron transport and following oxidative injuries in the reaction centres of PS II (especially D_1 protein). A hypothesis suggesting that D_1 protein degradation regulates the recovery cycle of PSII at photoinhibitory conditions was announced and was confirmed by experiments with higher plants (Aro et al., 1993). The proteolysis of D_1 protein and its replacement with new synthesized protein is possibly the main result of PS II inactivation as a result of excess of light energy. It is very likely that these processes have different velocities at different temperatures.

There are evidences that environmental stresses such as low and high temperatures further limit the ability of the plants to utilize light energy and enhance the photoinhibitory response (Hurry and Huner, 1992; Krause 1994; Stefanov et al., 1996). At such conditions photoinhibition can occur even under illumination with weak light and it proceeds via mechanisms different from those under normal conditions (Sonoike, 1996). Under both high and low temperatures the extent of photoinhibition is determined by inactivation of PS II reaction centres but the mechanisms and causes of this inactivation are different. One of the most predominant factors in photoinhibition at low temperature is in the decrease of the activity of the enzymes in carbon metabolism leading to increased proportion of excess light energy available to PS II (Sonoike et al., 1995). The repair via D_1 protein turnover and formation of zeaxanthin and energy-dependent quenching are also delayed at low temperature (Krause, 1988). On the other hand, high temperature leads to inhibition of water-splitting system (Katoh and San Pietro, 1967), destruction of PS II reaction centre P680, release of light harvesting chlorophyll *a*/*b* proteins (LHC II) from PS II core complexes, inhibition of Q_A reduction and inhibition of electron flow from Q_A^- to Q_B (Yordanov, 1992; Yamane et al., 1995).

Taking into account the differences in the mechanisms of high- and low-temperature provoked photoinhibition, it is very possible that the pattern of recovery from photoinhibition after placing the plants at optimal temperature and low light would be different. In this study we compare the effects of high and low temperature on photoinhibition (manifested as F_v/F_m decrease) of bean plants and the time course of the recovery of their photosynthetic apparatus.

Materials and Methods

Bean plants (*Phaseolus vulgaris*, cv. Cheren starozagorski) were grown for 20 days in a chamber at light (120 $\mu mol.m^{-2}.s^{-1}$ photon flux density, PFD) to dark cycle 13/11 hours under constant temperature of 25 ± 1^oC and $60 \pm 5\%$ relative air humidity.

The plants were treated for 4 h with different combinations of temperature (12, 24 and 42 °C) and light intensity (100 and 1000 μmol.m^{-2}.s^{-1} PFD) in climatic chamber with controlled light, temperature, air humidity and CO_2 concentration. The recovery of the photoinhibited plants was realized at 120 μmol.m^{-2}.s^{-1} PFD and temperature 25 °C.

The induction kinetics of chlorophyll fluorescence was recorded at room temperature using a pulse modulation chlorophyll fluorometer PAM 101 (H. Walz, Germany). After 5 min dark adaptation, the initial fluorescence yield, F_o, in weak modulated light (0.075 μmol.m^{-2}.s^{-1} PFD, modulation frequency 1.6 kHz), and maximum fluorescence yield, F_m, emitted during a saturating light pulse (1 s, > 3000 μmol.m^{-2}.s^{-1} PFD) were measured. Two independent experiments were carried out giving 6 replications for all parameters measured.

Results

The changes in ratio F_v/F_m characterizing the PSII state are presented in Fig.1. More essential differences between photoinhibited and non-photoinhibited plants were observed immediately after treatment. The F_v/F_m ratio, representing the potential quantum yield of PSII photochemistry is a very stable parameter and its decrease is a reliable evidence that plants are subjected to stress. The insignificant changes in F_v/F_m at low light and 12 and 42 °C shows that 4-h-treatment with these temperatures does not lead to plant injuries. However, 4-h-treatment with high light leads to a decrease of F_v/F_m ratio by about 10–15% evidently due to photoinhibition. After 2 h recovery the ratio F_v/F_m was increased and after 4 h it reached about 96–98% of the full recovery. It is evident that the course of recovery is different at the various treatment temperatures. After low-temperature treatment the increase was linear, while in plants subjected to photoinhibition at the background of high temperature an increase in F_v/F_m was observed after 2 h and later, after 4 h, it dropped. Obviously here, besides photoinhibitory injuries, there are also high-temperature injuries appearing with some delay. This suggestion is confirmed by the course of recovery in the variant treated with high temperature at low light intensity.

The changes in F_v/F_m ratio could be due to changes in both initial and maximal chlorophyll fluorescence. To determine which effect is prevailing we plotted the changes in F_o and F_m in the course of plant recovery (Fig. 2 and 3). These data show that the changes in F_m are higher and its effect on the F_v/F_m ratio is stronger, while at high and low temperature the photoinhibitory effect on F_o level is low. It can also be seen that the effect of light reflects mainly on maximal fluorescence while the temperature influences predominantly initial fluorescence.

To distinguish the effects of photoinhibition from high- and low-temperature response we normalized the curves representing the course of recovery of F_v/F_m by us-

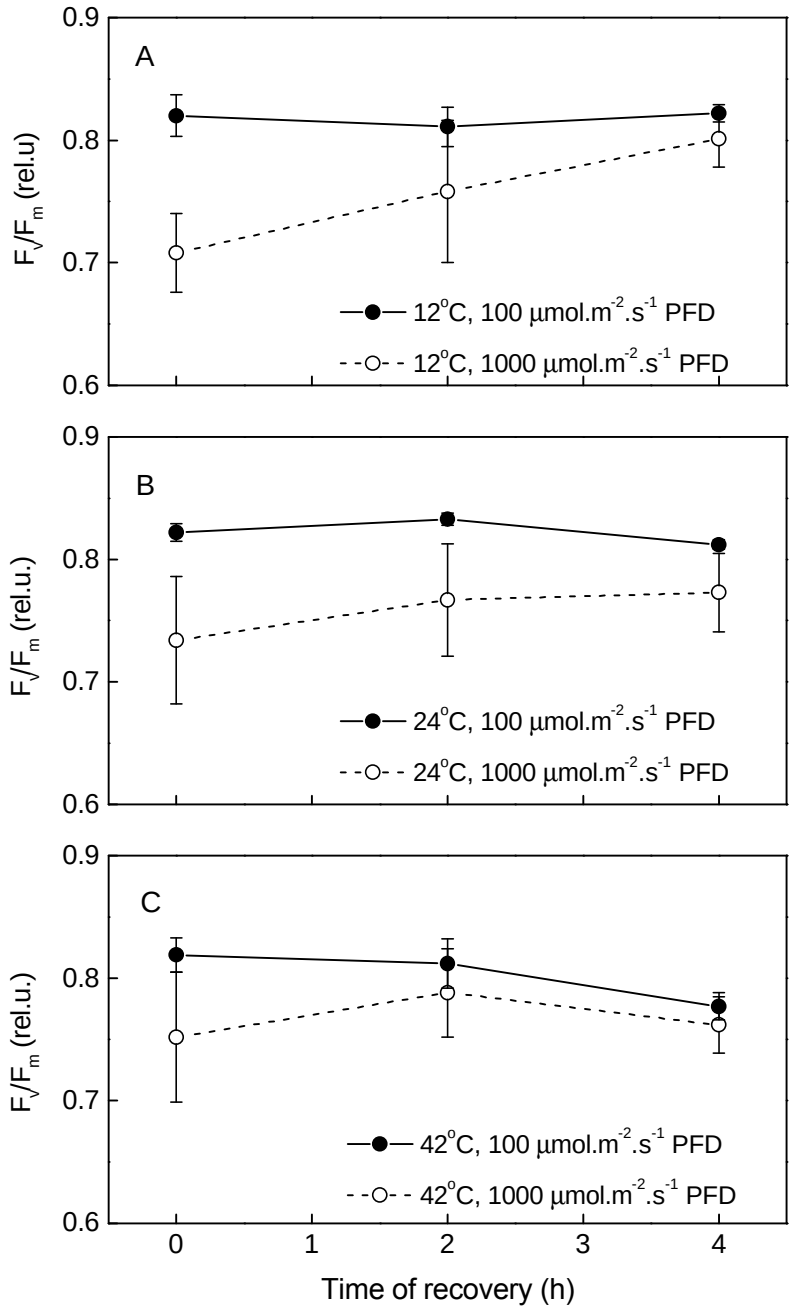

Fig. 1. Changes in variable to maximal fluorescence ratio (F_v/F_m) during the course of plant recovery after 4 hours photoinhibition at 12 °C (A), 24 °C (B) and 42 °C (C) air temperature.

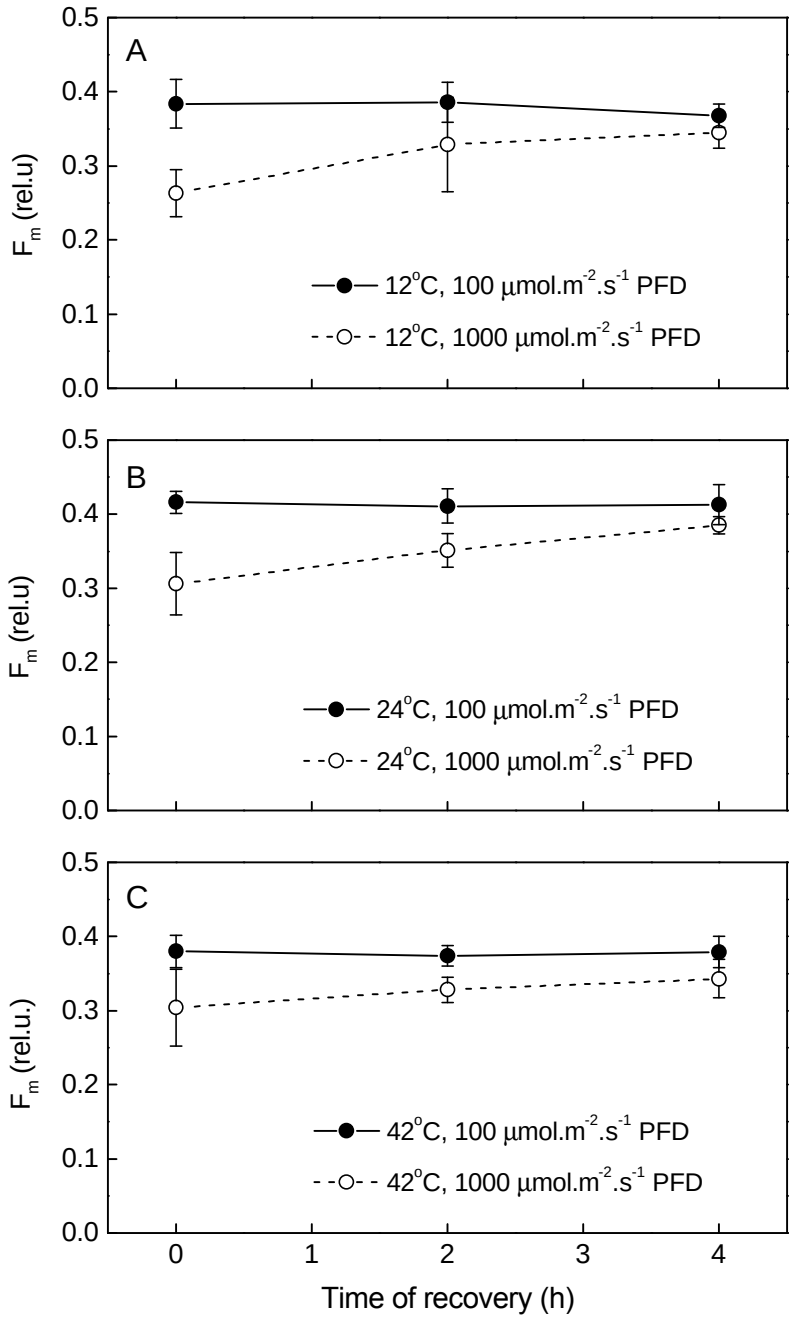

Fig. 2. Changes in the maximal chlorophyll fluorescence (F_m) during the course of plant recovery after 4 hours photoinhibition at 12 °C (A), 24 °C (B) and 42 °C (C) air temperature.

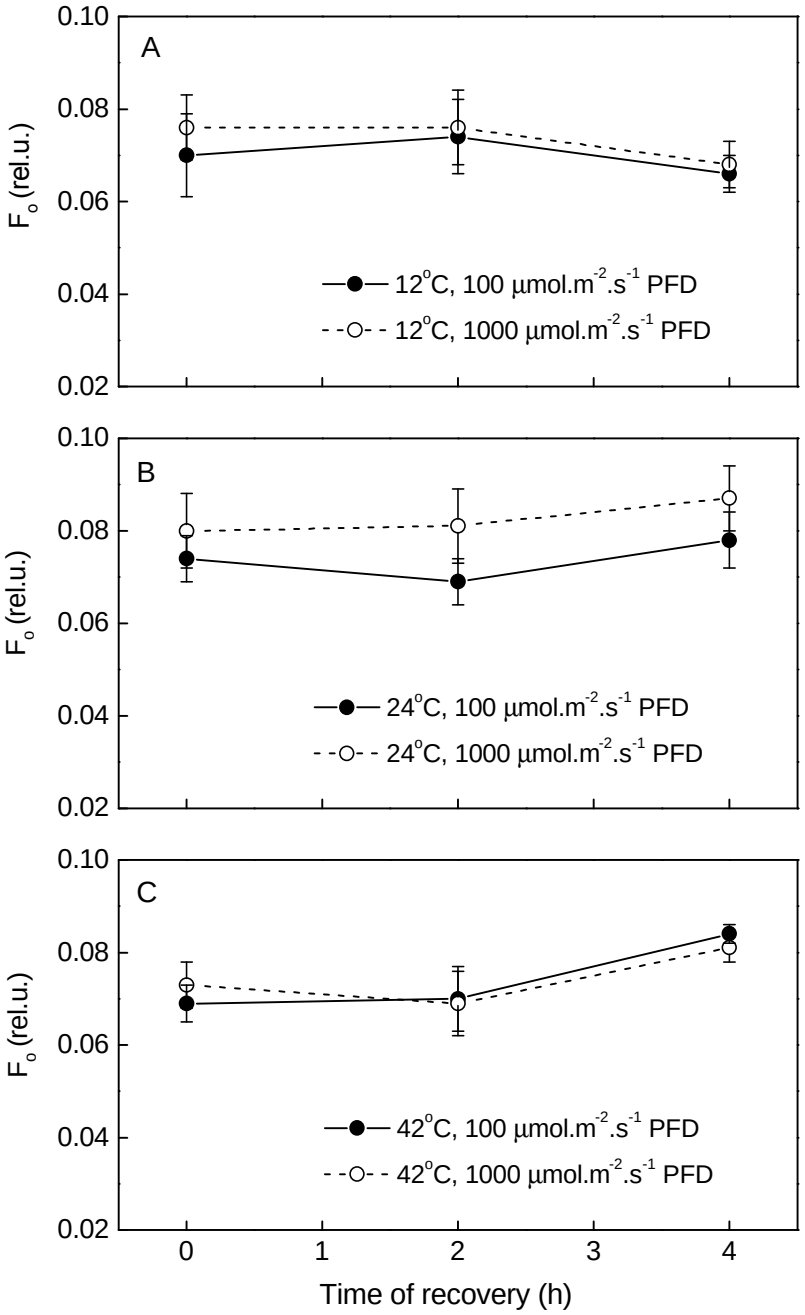

Fig. 3. Changes in the initial chlorophyll fluorescence (F_o) during the course of plant recovery after 4 hours photoinhibition at 12 °C (A), 24 °C (B) and 42 °C (C) air temperature.

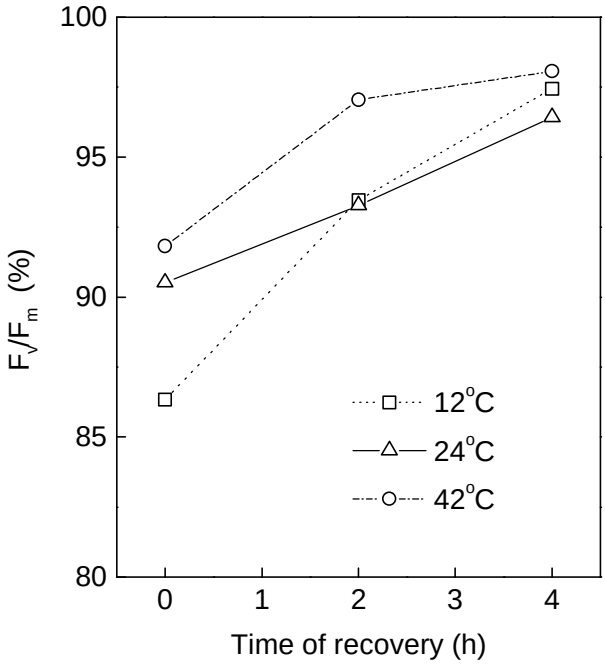

Fig. 4. Changes in the normalized values of variable to maximal fluorescence ratio (F_v/F_m in high-light treated plants as percent of F_v/F_m in low-light treated plants) during the course of plant recovery after 4 hours photoinhibition at different air temperatures: 12 °C (A), 24 °C (B) and 42 °C (C).

ing the percent of F_v/F_m in high-light treated plants from F_v/F_m in low-light treated plants (Fig.4). It can be seen that the recovery in plants treated with high light at normal temperature (24 °C) was linear. At the same time the course of recovery in plants photoinhibited at high and low temperature was nonlinear and has two phases – faster recovery in the first hours and slower in the next hours. It is also evidently from Fig. 4 that the rate of photoinhibition is higher in low-temperature treated plants.

Discussion

Our data showed that both high and low temperatures enhance the photoinhibition manifested as F_v/F_m decrease. At all temperatures investigated this decrease was due in greater extent to F_m decrease while the increases in F_o were insignificant. The F_m decrease can be related to inhibition of oxygen evolution (Katoh and San Pietro, 1967) and inactivation of reaction centres of PSII. The chlorophyll fluorescence at open PS II centres, F_o, depends on several processes sometimes changing it in opposite directions (Demmig-Adams, 1990) and as a result the net changes in F_o could be small. A de-

crease in F_o may be due to energy dissipation processes within the chlorophyll pigment bed. On the other hand, a F_o increase could be attributed to irreversible detachment of light-harvesting chlorophyll *a*/*b* protein complexes from reaction centre complexes of PSII, to partly reversible inactivation of PSII (Schreiber and Armond, 1978; Yamane et al., 1997), and to dark reduction of Q_A (Havaux, 1996).

We observed a different pattern of photosynthetic apparatus recovery after photoinhibition at various temperatures, proving that different mechanisms of photoinhibitory injury predominate in these cases. In low- and high-temperature treated plants we observed faster increase of F_v/F_m ratio during the first 2 h of recovery and slower for the next 2 h (Fig. 4). This biphasic kinetics of PSII efficiency recovery after photoinhibition is in agreement with the results reported by Krause and Weis (1991), Hurry and Huner (1992) etc. The first phase is completed usually within the first hour of recovery and because of its fast kinetics and occurrence at low temperature is possibly connected with a direct reactivation of PSII without D_1 degradation (Krause, 1994). According to Thiele et al. (1997) the fast phase is closely correlated with epoxidation of zeaxanthin, which mediates formation of an energy dissipating state of PSII that functions to diminish D_1 protein inactivation. During the second (slow) phase of recovery the photoinhibited reaction centre is repaired by replacing the damaged D_1 protein with its newly synthesized copy (Prasil et al., 1992). A PSII repair cycle hypothesized by Melis (1991) probably takes part in the slow phase. According to it a reassembly of the PSII reaction centre in the stroma lamellae leads first to the not fully functional Q_B-nonreducing PSII that subsequently becomes activated, migrates to the grana and is transformed to $PSII_\alpha$ by attachment of the peripheral light-harvesting complex.

Our results showed that photoinhibition occurring at low and high temperatures is reversible under low light and optimal growth temperature. The relatively fast recovery of the photosynthetic apparatus after high- and low-temperature provoked photoinhibition to 96–98% of the control supports the suggestion (Krause, 1994) that photoinhibition provides a mechanism for dynamic down-regulation of PSII, rather than causing damage and destruction of PSII.

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