Thermostability and Photostability of Photosystem II of the Resurrection Plant *Haberlea rhodopensis* Studied by Chlorophyll Fluorescence

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The stability of PSII in leaves of the resurrection plant *Haberlea rhodopensis* to high temperature and high light intensities was studied by means of chlorophyll fluorescence measurements. The photochemical efficiency of PSII in well-hydrated *Haberlea* leaves was not significantly influenced by temperatures up to 40 °C. F_0 reached a maximum at 50 °C, which is connected with blocking of electron transport in reaction center II. The intrinsic efficiency of PSII photochemistry, monitored as F_v/F_m was less vulnerable to heat stress than the quantum yield of PSII electron transport under illumination (ΦPSII). The reduction of ΦPSII values was mainly due to a decrease in the proportion of open PSII centers (qP). *Haberlea rhodopensis* was very sensitive to photoinhibition. The light intensity of 120 μmol m^{-2} s^{-1} sharply decreased the quantum yield of PSII photochemistry and it was almost fully inhibited at 350 μmol m^{-2} s^{-1}. As could be expected decreased photochemical efficiency of PSII was accompanied by increased proportion of thermal energy dissipation, which is considered as a protective effect regulating the light energy distribution in PSII. When differentiating between the three components of qN it was evident that the energy-dependent quenching, qE, was prevailing over photoinhibitory quenching, qI, and the quenching related to state 1-state 2 transitions, qT, at all light intensities at 25 °C. However, the qE values declined with increasing temperature and light intensities. The qI was higher than qE at 40 °C and it was the major part of qN at 45 °C, indicating a progressing photoinhibition of the photosynthetic apparatus.

Key words: PSII Photochemistry, Chlorophyll Fluorescence, High Temperature Stress, Photoinhibition

Introduction

The study of light-induced *in vivo* chlorophyll fluorescence of green plant tissue provides basic information on the function of the photosynthetic apparatus and on the capacity and performance of photosynthesis (Krause and Weis, 1991; Govindjee, 1995). Under optimum conditions of photosynthesis the proportion of absorbed light energy emitted as heat or as chlorophyll (Chl) fluorescence is rather low. Various stress conditions, however, can reduce the rate of photosynthesis and disturb or block the light-driven photosynthetic electron transport (Lichtenthaler and Rinderle, 1988). This results in an increased de-excitation of the absorbed light energy via heat emission and Chl fluorescence. The inverse relationship between *in vivo* Chl fluorescence and photosynthetic activity can be used to study functional changes of the photosynthetic apparatus under different environmental stresses.

Temperature is one of the main factors controlling the formation and functional activity of the photosynthetic apparatus. Among all cell functions, the photosynthetic activity of chloroplasts is believed to be one of the most heat sensitive processes and can be inhibited long before other symptoms of the heat stress are detected (Berry and Björkman, 1980). High temperature treatment causes inhibition of various photochemical reactions, including light energy capture and utilization by photosystems, the photosystem II (PSII) and photosystem I (PSI) mediated electron transfer

Abbreviations: Chl, chlorophyll; F_0, F_m, minimum and maximum dark-adapted fluorescence yield; F'_m, maximum light adapted fluorescence yield; F_v/F_m, quantum yield of photosystem II photochemistry in the dark-adapted state; F'_v/F'_m, efficiency of excitation capture by open photosystem II reaction centers; qP, photochemical quenching; qN, non-photochemical quenching; qE, energy-dependent quenching; qT, quenching related to state 1-state 2 transitions; qI, photoinhibitory quenching; ΦPSII, quantum yield of photosystem II photochemistry in the light-adapted state; PSI, photosystem I; PSII, photosystem II.

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and energy transduction processes (Vani *et al.,* 2001; Georgieva and Brugnoli, 2002). Many investigations have shown that PSI activity is much more heat-resistant than PSII (Sayed *et al.,* 1989; Boucher and Carpentier, 1993).

High radiation is often associated with high temperatures, which have a strong impact on photosynthesis (Ludlow, 1987; Havaux and Tardy, 1996; Königer *et al.,* 1998). Exposing plants to a combination of high light intensities and high temperatures leads to a more severe and sustained photoinhibition than exposure to either of the stress conditions alone (Ludlow, 1987; Gamon and Pearcy, 1990). Photoinhibition adversely affects the function of PSII in chloroplasts. The photoinactivation and impairment of electron transport occurs at the donor and acceptor sides of PSII (Eckert *et al.,* 1991; Aro *et al.,* 1993). The susceptibility of plants to photoinhibition depends on the species and growth light environment (Osmond, 1994). In general, shade plants are more vulnerable to photoinhibition than sun plants.

Haberlea rhodopensis Friv. (Gesneriaceae) is a smaller sized rock poikilohydric plant, forming dense tufts of leaves. It prefers the shady mostly northward slopes of limestone ridges in creek gorges and mountain zones with higher humidity. It is considered as a homoiochlorophyllous desiccation tolerant plant, since it preserves its chlorophyll content during dehydration. The adult rosettes can dehydrate to a water content as low as 10% and remain in this viable but desiccated state for a considerable time.

One of the objectives of this study was testing the sensitivity of *Haberlea* to heat stress. We wanted to find out if this plant which is able to withstand drought is also tolerant to high temperature stress since in natural conditions drought stress is usually accompanied by high temperatures. The sensitivity to increasing light intensities was also studied.

Material and Methods

Plant material

Well-hydrated *Haberlea rhodopensis* plants were collected from their natural habitat (the vicinity of Asenovgrad, Bulgaria) at the period of flowering in May–June. Young, fully expanded leaves from the middle of rosettes with similar size and appearance were used in order to obtain reproducible results.

Chlorophyll fluorescence

Chlorophyll fluorescence emission from the upper leaf surface was measured with a pulse amplitude modulation fluorometer (PAM 101–103, Walz, Effeltrich, Germany) as described by Schreiber *et al.* (1986). The initial fluorescence yield in weak modulated measuring light (0.075 μmol m^{-2} s^{-1} PPFD), F_0, and maximum total fluorescence yield induced by a saturating white light pulse (1 s, over 3500 μmol m^{-2} s^{-1} PPFD, by a Schott KL 1500 light source), F_m, were determined. The leaf disc (10 mm diameter) was then illuminated with continuous red light (actinic light). Simultaneously with the onset of actinic light illumination, the modulation frequency was switched from 1.6 to 100 kHz. After 15 min actinic light the short saturating light pulse was used to obtain the fluorescence intensity F'_m with all PSII reaction centers closed. Induction kinetics were registered and analyzed with the program FIP 4.3, written by Tyystjärvi and Karunen (1990).

Thermotolerance

The chlorophyll fluorescence was measured in leaf disks as a function of rising temperature. A new leaf disk was used for each temperature treatment. The parameters of Chl fluorescence were registered following 5 min treatment at the respective temperature, as previously described (Georgieva and Yordanov, 1993). Photosynthetic apparatus thermosensitivity of *Haberlea rhodopensis* was investigated in the temperature range 25–60 °C.

Resistance to high light intensity

The photostability of *H. rhodopensis* was tested by applying actinic light with different intensities – in the range 50–1100 μmol m^{-2} s^{-1}. The resistance of *Haberlea* leaves to high light intensity was investigated at different temperatures (25–45 °C) in order to study the combined effect of both stress factors.

Dark relaxation measurements

The relative proportions of the components of non-photochemical quenching, qN, were determined via dark relaxation measurements when the actinic light was turned off. For this purpose saturating light flashes were applied every minute after turning off actinic light. With the saturation flashes

applied, *e.g.* within 1 min and after 5 or 20 min of the dark period, F'_m1, F'_m5 and F'_m20 were obtained for calculation of qN and its constituents: the fast (1–5 min) relaxing component qE (the energy-dependent quenching), the intermediate (5–20 min) relaxing component qT (quenching related to state 1-state 2 transitions) and the very slow relaxing photoinhibitory quenching qI (> 20 min up to several hours or 1 or 2 d). Following formulas, introduced by Lichtenthaler *et al.* (2004), were used:

$$qE = (NF1 - NF5)/F'_v$$
$$qT = (NF5 - NF20)/F'_v$$
$$qI = NF20/F_v,$$

where $NF = F_m - F'_m$ 1 min (NF1), 5 min (NF5) and 20 min (NF20) after turning off the actinic light, respectively.

Statistics

The data presented are means of at least six replications analyzed by the Student's *t*-test.

Results and Discussion

Effect of high temperature treatment

The changes in the intensity of Chl fluorescence upon gradually heating have been extensively studied and used to compare the thermosensitivity of different plants. The temperature at which PSII denaturates corresponds to the temperature at which ground Chl fluorescence F_0 starts to increase (Havaux and Tardy, 1996). In a native photosynthetic object this parameter reflects the state of antenna Chl and is a measure for the initial dis-

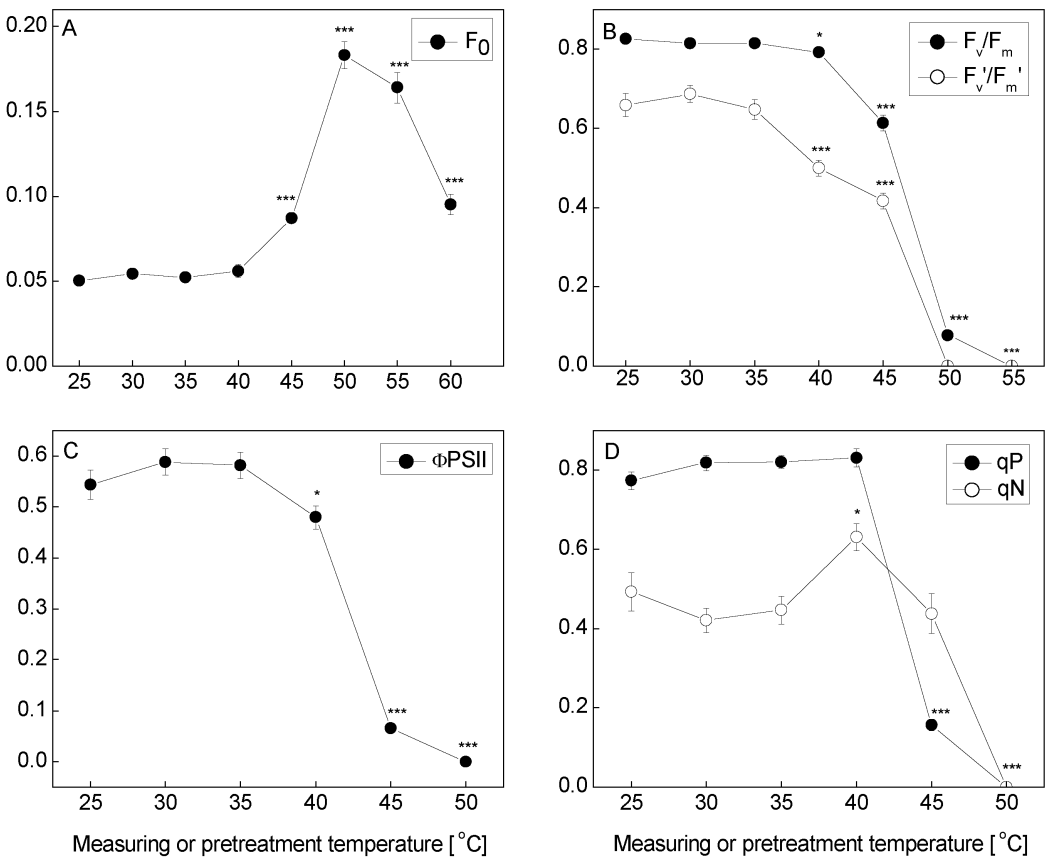

Fig. 1. Temperature dependence of (A) the ground Chl fluorescence F_0, (B) the maximum photochemical efficiency of PSII, F_v/F_m, and the efficiency of excitation capture by open PSII reaction centers, F'_v/F'_m, (C) the quantum yield of PSII electron transport in the light-adapted state, ΦPSII, and (D) the photochemical qP and non-photochemical qN fluorescence quenching in *Haberlea* leaves. Chl fluorescence was measured following 5 min treatment at the respective temperature. Each point is the mean from 6 replications. Significant deviation as compared to the control at 25 °C is indicated by: * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

tribution of energy to PSII and the effectiveness of excitation capture in P680. Our data showed that F_0 significantly increased at temperatures higher than 40 °C (Fig. 1A) and reached a maximum at 50 °C, which is connected with blocking of electron transport in reaction center II (Yamane *et al.,* 1997). The values of the maximum Chl fluorescence, F_m, were reduced by 23% after 5 min treatment at 45 °C and fully inhibited at 55 °C (data not shown). No significant reduction in the maximum efficiency of PSII, measured by the ratio F_v/F_m, was found in *Haberlea* leaves treated at temperatures lower than 40 °C (Fig. 1B). The further increase in temperature reduced the F_v/F_m ratio and it declined by 22% at 45 °C and was only 9% of the control level at 50 °C. The 50% inhibition temperature of this parameter was 47 °C.

The actual quantum yield of PSII electron transport in the light-adapted state (ΦPSII) was much more influenced by high temperatures than the F_v/F_m ratio (Fig. 1C). This parameter was equal to the product of photochemical quenching (qP) and the efficiency of excitation capture by open PSII reaction centers (F'_v/F'_m) (Genty *et al.,* 1989). ΦPSII started to decrease at 40 °C and was strongly reduced at 45 °C. The inhibition of the quantum yield of PSII electron transport at 45 °C was mainly due to significant reduction in the proportion of open PSII centers (qP), whereas the value of F'_v/F'_m was less influenced by treatment at 45 °C (Fig. 1). The 50% inhibition temperatures of ΦPSII, qP and F'_v/F'_m were 42.5 °C, 43.5 °C and 46.5 °C, respectively.

The protective effect of excessive energy dissipation by non-photochemical quenching, qN, was evident up to 40 °C (Fig. 1D), while the proportion of thermal energy dissipation in the antenna, estimated by the parameter 1 – (F'_v/F'_m) (Demmig-Adams *et al.,* 1996), further increased with rising temperature (data not shown). According to Pastenes and Horton (1996) a decline in qN at high temperature would arise either from an increased ATP consumption or from a failure to maintain a proton gradient due to increased H^+ permeability of the thylakoid membrane.

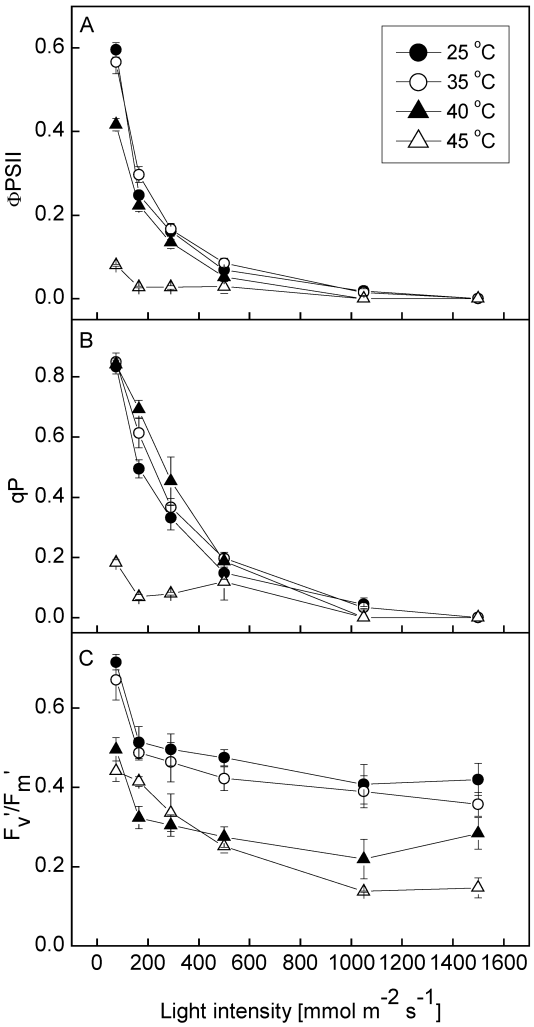

Fig. 2. Effect of increasing light intensity on (A) the quantum yield of PSII electron transport in the light-adapted state, ΦPSII, (B) the photochemical qP and non-photochemical qN fluorescence quenching and (C) the efficiency of excitation capture by open PSII reaction centers, F'_v/F'_m, measured at different temperatures. Chl fluorescence was registered following 5 min treatment at the respective temperature. The duration of actinic light was 15 min.

Effect of high light intensity

Increasing the light intensity from 50 to 120 μmol m^{-2} s^{-1} sharply decreased the quantum yield of PSII photochemistry (ΦPSII), measured in *Haberlea* leaves at 25 °C (Fig. 2A). The values of ΦPSII gradually decreased with increasing the light intensity and they were almost 90% reduced after exposure to 350 μmol m^{-2} s^{-1}. The photochemical quenching, qP, was also very sensitive to rising the light intensity. qP declined by 40% at 120 μmol m^{-2} s^{-1}, 80% at 350 μmol m^{-2} s^{-1} and was almost fully inhibited at 800 μmol m^{-2} s^{-1} (Fig. 2B). The efficiency of excitation capture by open PSII reaction centers (F'_v/F'_m) seemed to be

more resistant to high light intensity. It was reduced by 28% at 120 μmol m^{-2} s^{-1}, 34% at 350 μmol m^{-2} s^{-1} and 42% at 1100 μmol m^{-2} s^{-1} (Fig. 2C). The decline of F'_v/F'_m with increasing light intensity was due to a decrease in the maximum Chl fluorescence yield in the light-adapted state (F'_m), whereas the minimum Chl fluorescence yield in the light-adapted state (F'_0) was not significantly influenced (data not shown).

The reduced quantum yield of PSII photochemistry as a result of increasing light intensity from 50 to 120 μmol m^{-2} s^{-1} was accompanied by a sharp enhancement in the non-photochemical fluorescence quenching qN, the proportion of thermal energy dissipation in the antenna ($1 - F'_v/F'_m$) and the fraction of absorbed light energy which was not used for photochemistry [LNU = $1 - (\Delta F/F'_m)/(F_v/F_m)$] (Fig. 3). Further rise in light intensity slightly increased the values of qN and ($1 - F'_v/F'_m$) – by about 30% at 1100 μmol m^{-2} s^{-1} (Figs. 3A and B). LNU gradually enhanced with increasing the light intensity and after exposure to 1100 μmol m^{-2} s^{-1} it was 274% higher than that at 50 μmol m^{-2} s^{-1} (Fig. 3C).

Effect of high light intensity and high temperature

The combined effect of high light intensity and high temperature on *Haberlea* leaves was studied by measuring the light dependence of Chl fluorescence parameters at different temperatures. The results showed that the temperatures up to 40 °C did not influence significantly the shape of light curves of ΦPSII, qP and F'_v/F'_m (Fig. 2). Moreover, the treatment at 35 °C did not change considerably the value of these parameters. Exposure of *Haberlea* leaves to 40 °C at light intensities over 350 μmol m^{-2} s^{-1} reduced much stronger the quantum yield of PSII photochemistry (ΦPSII) and photochemical quenching (qP) than the efficiency of excitation capture by open PSII reaction centers (F'_v/F'_m). Treatment at 45 °C strongly inhibited ΦPSII and qP and there was no additional effect of increasing light intensities. The values of F'_v/F'_m, measured at 45 °C, gradually decreased with increasing the light intensity (Fig. 2).

As it was shown by data on temperature dependence of non-photochemical quenching, qN, its value significantly increased at 40 °C (Fig. 1). Increasing the light intensity up to 120 μmol m^{-2} s^{-1} enhanced qN by 30%, but its value did not change significantly with further rising the light intensity (Fig. 3). Treatment at 45 °C declined the non-photochemical

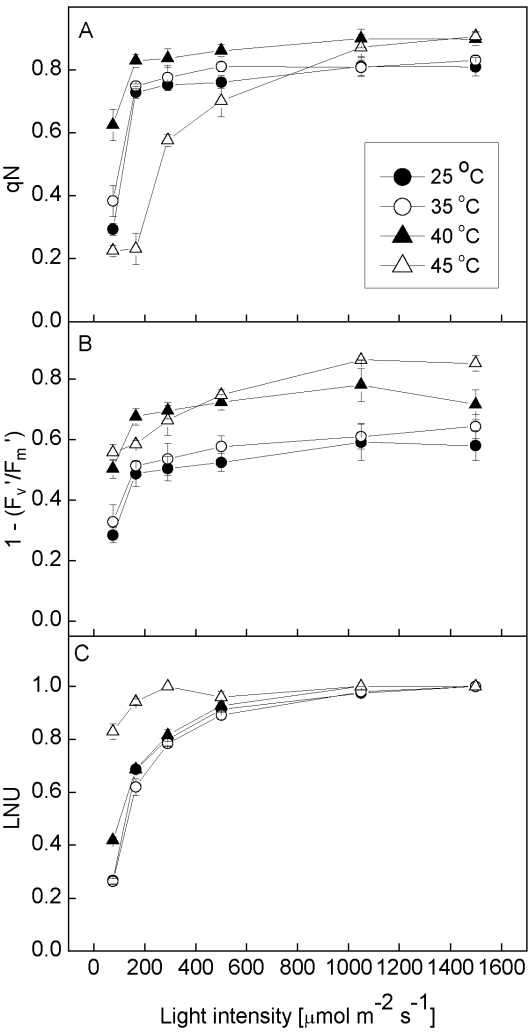

Fig. 3. Effect of increasing light intensity on (A) the non-photochemical fluorescence quenching, qN, (B) the relative proportion of excitation energy dissipated as heat in the PSII antennae and (C) the proportion of light not used for photochemistry, measured at different temperatures. Chl fluorescence was registered following 5 min treatment at the respective temperature. The duration of actinic light was 15 min.

fluorescence quenching when it was measured at 50 and 120 μmol m^{-2} s^{-1}. However, it sharply increased with increasing the light intensity and at 1100 μmol m^{-2} s^{-1} it was 300% higher than that at 50 μmol m^{-2} s^{-1} (Fig. 3). Increasing light intensity enhanced the amount of light not used for photochemistry but there was no additional effect of temperatures up to 40 °C (Fig. 3). Treatment of *Haberlea* leaves at 45 °C at low light intensity sharply in-

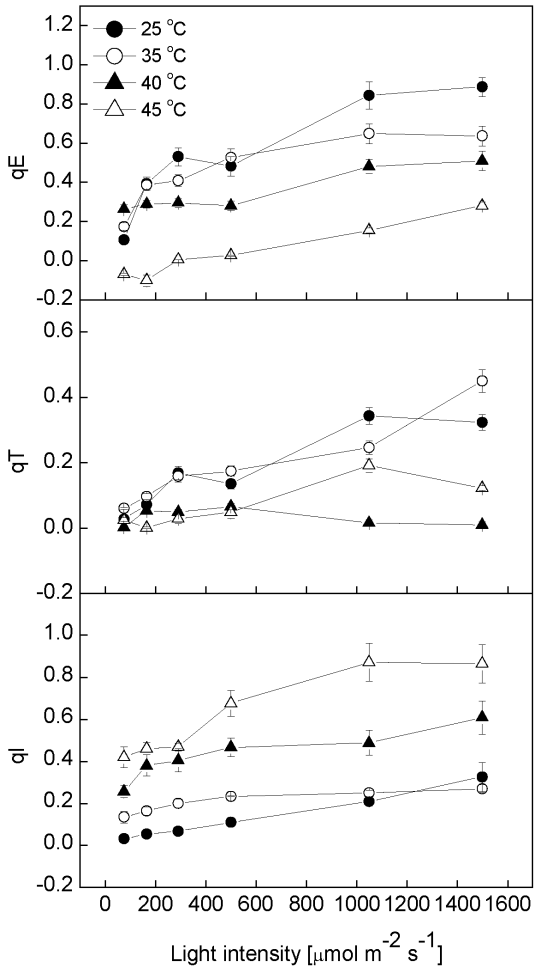

Fig. 4. The relative proportions of the components of non-photochemical quenching: (A) the fast (1–5 min) relaxing component qE (the energy-dependent quenching), (B) the intermediate (5–20 min) relaxing component qT (quenching related to state 1-state 2 transitions) and (C) the very slow relaxing photoinhibitory quenching qI (> 20 min), determined via dark relaxation measurements when the actinic light was turned off. The saturating light flashes were applied every minute up to 30 min of dark.

creased the value of LNU and it was only 20% higher at 1100 μmol m^{-2} s^{-1}.

Dark relaxation kinetics

The individual components of qN, such as the energy-dependent quenching qE, the photoinhibitory quenching qI, and the quenching component qT related to state transitions of the photosynthetic apparatus were determined by dark relaxation kinetics (Fig. 4). The values of qE, qT and qI strongly increased with increasing actinic light intensity from 50 to 1100 μmol m^{-2} s^{-1} when Chl fluorescence was recorded at 25 °C. Elevated temperatures enhanced the values of qN components at a low light intensity (50 μmol m^{-2} s^{-1}) and the effect of high temperature with increasing irradiance was less expressed (Fig. 4). When actinic light of 50 μmol m^{-2} s^{-1} was applied qE reached a maximum at 40 °C and decreased at 45 °C, whereas qI further increased with rising temperature and the qI values were almost 13 times higher at 45 °C than at 25 °C. The qE values declined with increasing light intensity and temperature. However, qI increased with increasing temperature also at higher light intensities. It was found that qE prevailing over qT and qI at 25 °C and 35 °C at all light intensities. qI was a little higher than qE at 40 °C and it was the major part of qN at 45 °C, indicating a progressing photoinhibition of the photosynthetic apparatus.

The ability of *Haberlea* leaves to recover from high light treatment was estimated by measuring the ratio F_v/F_m 30 min after switching off the actinic light (Fig. 5). The primary photochemical activity of PSII in leaves exposed to light intensities in the range 50–1100 μmol m^{-2} s^{-1} at 25 °C and 35 °C was closed to that measured in the dark-adapted state. The recovery of F_v/F_m was less expressed when it was carried out at 40 °C and espe-

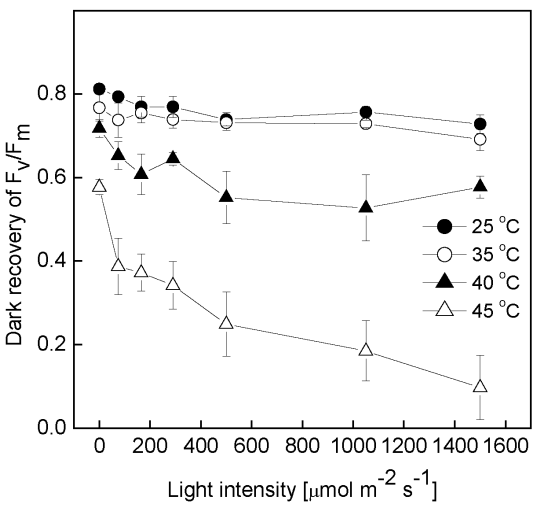

Fig. 5. The recovery of the maximum photochemical efficiency of PSII, F_v/F_m, from high light treatment at different temperature, measured 30 min after switching off the actinic light.

cially at 45 °C. The ratio F_v/F_m was 20% and 83% reduced after 30 min of recovery from treatment at 1100 μmol m^{-2} s^{-1} at 40 °C and 45 °C (Fig. 5). The results showed that treatment at 45 °C at increasing light intensities caused irreversible damage of the PSII function in *Haberlea* leaves.

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