Short-Term Responses of Photosystem II to Heat Stress in Cold-Acclimated Atrazine-Resistant and Susceptible Biotypes of *Erigeron canadensis* (L.)

Sándor Dulai[a,*], István Molnár[a], Evelin Péli[a] and Endre Lehoczki[a,b]

[a] Department of Plant Physiology, Eszterházy Teachers' Training College, H-3301 Eger, POB 43, Hungary. Fax: +36–36–520466. E-mail: ds@gemini.ektf.hu

[b] Department of Botany, Attila József University, H-6701 Szeged, Hungary

* Author for correspondence and reprint requests

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When leaves of atrazine-resistant (AR) and atrazine-sensitive (S) *Erigeron canadensis* (L.) plants grown at 5 °C were exposed to an elevated temperature (35 °C) for 30 min, the critical (T_c) and peak temperatures (T_p) of the F_0 vs. T curves were considerably higher for the leaves of the S biotype, but not for those of the AR biotype. The temperature dependences of F_v/F_m and $\Delta F/F_m'$ were not greatly different for the heat-treated cold-acclimated AR biotype, in contrast with the situation for the S plants. This short-term heat treatment resulted in a more significant shift in the optimal thermal interval of CO_2 fixation for the S than for the AR biotypes.

Introduction

It has been reported that there are differences in lipid/fatty acid composition between atrazine-resistant (AR) and atrazine-sensitive (S) plants (Pillai and St John, 1981; Burke *et al.,* 1982; Chapman *et al.,* 1985; Lehoczki *et al.,* 1985; Tremolieres *et al.,* 1988). The thylakoid fractions of AR plants rich in photosystem II (PS II) are characterized by higher concentrations of monogalactosyl-diacylglycerol (Lehoczki *et al.,* 1985; Tremolieres *et al.,* 1988) and the thylakoids of AR plants have been reported to contain more unsaturated fatty acids (Lehoczki *et al.,* 1985). Some authors consider that these differences in lipid/fatty acid composition of the thylakoid membranes may cause the enhanced heat sensitivity of AR plants (Havaux, 1989). As compared with S plants, this sensitivity is revealed at a moderately higher temperature by a generally decreased rate of O_2 evolution, an increased proportion of the quinone$_A^-$ (Q_A^-) level and reduced CO_2 fixation (Ducruet and Lemoine, 1985; Pölös *et al.,* 1986; Ducruet and Ort, 1988; Havaux, 1989; Dulai *et al.,* 1995, 1998). However, stress physiological research results seem to suggest that, besides having a decreased heat tolerance, the chloroplasts of AR *E. canadensis* plants (with modified lipid composition) lack a temperature adaptation mechanism, which is linked to the capacity to change the membrane fluidity. In accordance with the above-mentioned findings, the thermal optimum of CO_2 fixation was earlier found to be at low temperature for both cold and warm-acclimated AR plants (Dulai *et al.,* 1998). These features may play a role in the fact that the biomass productivity of AR plants grown under moderately low-temperature conditions is increased (Richroch *et al.,* 1987), and these biotypes are capable of efficient photosynthesis only in a relatively narrow, moderately cold temperature interval (Dulai *et al.,* 1998).

By investigating the effects of short-term temperature stress on the photosynthetic apparatus, some authors have recently shown that chloroplasts possess a rapid adaptive mechanism that senses a moderate temperature elevation and produces the fast conversion of PS II to a heat-resistant state. In parallel with this increased heat tolerance, the conversion of the xanthophyll cycle pigments begins (Havaux and Gruszeczki, 1993; Havaux and Tardy, 1995, 1996). However, it is known that, besides a decreased long-term temperature adaptation capacity arising from modification of the membrane fluidity, AR *E. canadensis* has a reduced xanthophyll cycle activity (Váradi *et al.,* 1994).

The present paper reports on a short-term heat treatment-induced response of the initial fluorescence (F_0), various fluorescence induction parameters associated with the PS II activity, and the

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CO_2 gas exchange in leaves of AR and S biotypes of *E. canadensis* grown under low-temperature conditions.

Materials and Methods

All main experiments were performed on intact leaves of cold-acclimated (3-month-old plants were transferred to 5 °C for 3 months) AR and S biotypes of *E. canadensis*. Both biotypes were grown under fluorescent illumination with a 16-h photoperiod of white light at a photon flux density of 100 μmol m^{-2} s^{-1}. Unless otherwise indicated, the fully expanded leaves of 6-month-old cold-acclimated plants, in the rosette stage, were used in all experiments before and after a short heat treatment (30 min at 35 °C under a 100 μmol m^{-2} s^{-1} photon flux density). Warm acclimated plants were grown at 25 °C for 3 months in the same light conditions as cold-acclimated plants. The breakpoints of F_0 vs. T curves of these plants are given only for comparison with the heat tolerance increase in cold-acclimated pre-heated plants.

The responses of the *in vivo* chlorophyll-a fluorescence of the AR and S *C. canadensis* biotypes to temperature change were measured in dark-adapted leaves with a pulse amplitude modulation fluorometer (PAM 101–102–103, Walz, Effeltrich, Germany) as described by Schreiber *et al.* (1986), and recorded with a potentiometric chart recorder (NE-244, EMG, Budapest, Hungary) and a computer as detailed in a previous paper (Dulai *et al.*, 1998). The variables and equations for quenching analysis were determined according to van Kooten and Snel (1990). The quantum efficiency of photochemistry was calculated as $\Delta F/F_m'$, as reported by Genty *et al.* (1989).

For determination of the critical and peak temperatures (T_c and T_p) for heat injury to the photosynthetic apparatus, the method of heat induction of fluorescence was applied as described by Schreiber and Berry (1977). The leaves were dark-adapted for 30 min, and then placed on the thermoelectric module. During heating from the growth temperature to 50 °C at a rate of 1 °C min^{-1}, the temperature was monitored by a thermocouple thermometer. F_0 was excited by a weak 650 nm light beam modulated at 1.6 kHz; PS I was maintained in a state of oxidation by low-intensity far-red background light. T_c and T_p were determined from the F_0 vs. T curves.

CO_2 assimilation was measured in normal air with an infrared gas analyser (LCA-2, Analytical Development Co. Ltd, Hoddesdon, UK) in an open gas-exchange system. The white light for the excitation of photosynthesis was provided by a Schott KL-1500 light source through a fiberoptic. The attached leaves were first exposed to the growth temperature in a thermostated leaf chamber (Analytical Development Co. Ltd, Hoddesdon, UK). After measurement of the CO_2 fixation rate at this temperature, the temperature was raised in 5 min to the next value (in the interval 5–50 °C). After stabilization of this new temperature (again a 5-min period), the CO_2 fixation rate was measured once more, and the temperature increase was continued. These measurements were made in the light-saturated state (800 μmol m^{-2} s^{-1} Photon flux density, PFD) of photosynthesis by using a temperature controller (Haake, F3, Berlin, Germany). The rates of net CO_2 fixation were calculated by using the equations of von Caemmerer and Farquhar (1981).

Results and Discussion

It is well documented that the critical (T_c) and peak (T_p) temperatures of F_0 vs. T curves are direct indices of the thermostability of chloroplasts, and can be used to estimate the thermotolerance of higher plants (Smillie and Nott, 1979; Bilger *et al.*, 1984). When the cold-acclimated plants were exposed to preheating (35 °C, 30 min), the temperature dependence of F_0 for the leaves of S *E. canadensis* displayed characteristic changes. There was a slow increase in F_0 for the preheated cold-acclimated AR biotype at 34.7 °C (T_1) and T_c was at 39.3 °C, in contrast with the S biotype, where the fluorescence level did not change up to 37.5 °C (T_1), with T_c at 40.5 °C (Table I). These short heat treatment-induced upward temperature shifts led to increases in F_0, indicating a higher thermal stability of PS II in the S biotype than in the AR biotype. The approximately 3.9 and 3.3 °C upward shifts in T_c and T_p in the F_0 vs. T curves as compared with the non-treated samples may be connected with the fact that, in parallel with the exposure to 35 °C, the thylakoids of the S biotype probably become significantly less fluid. Correspondingly, at higher temperatures the membrane is hyperfluidized and, in parallel with the preheat-

Table I. Breakpoints (T_1, T_c and T_p) of F_0 vs. T curves for intact leaves of preheated (30 min at 35 °C) and non-treated S and AR *E. canadensis* biotypes grown at 5 °C and of non-treated warm-acclimated plants grown at 25 °C.
The results are means of data from ten independent measurements on different leaves from different plants.

Susceptible biotype	T_1	T_c	T_p
Cold-acclimated	30.2±0.84	36.6±0.81	44.5±1.25
Cold-accl. heat-treated	37.5±0.91	40.5±1.08	47.8±1.15
Warm-acclimated	37.4±0.57	41.1±0.59	47.7±1.18
Atrazine resistant biotype			
Cold-acclimated	32.4±0.43	38.6±0.92	46.3±0.95
Cold-accl. heat-treated	34.7±1.18	39.3±1.05	47.1±1.56
Warm-acclimated	33.2±0.59	38.6±0.41	44.6±1.14

ing, the photosynthetic apparatus becomes tolerant to even moderate temperature stress.

The temperature responses of the optimal quantum yield of PS II photochemistry (F_v/F_m) (measured after a 30-min dark relaxation) for leaves of the S and AR biotypes grown at 5 °C differed in a wide range of heating temperature; the curve decreased sharply from 39 °C for the AR biotype and from 35 °C for the S biotype (Fig. 1). However, for the S biotype, after the brief exposure to 35 °C, the heating temperature dependence of F_v/F_m became tolerant to heat. In contrast with the non-treated leaves, in the preheated S plants F_v/F_m decreased sharply only above 40 °C; in the AR biotype, the change in the temperature depen-

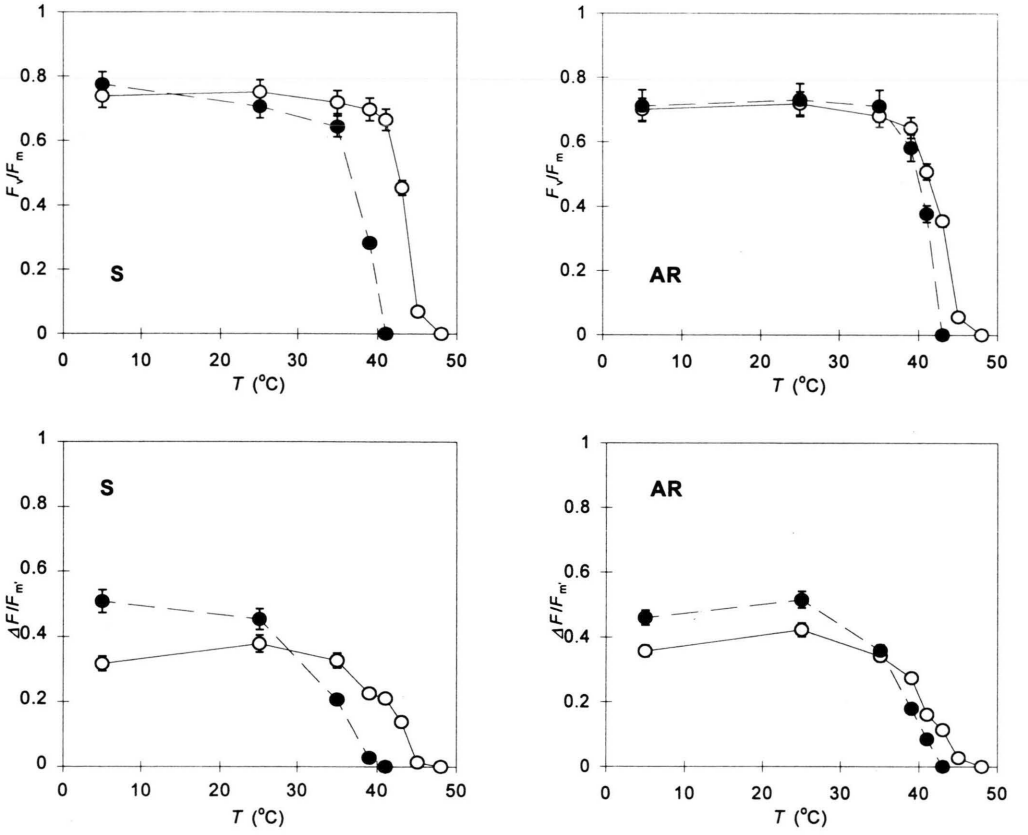

Fig. 1. Effects of heating on optimal (F_v/F_m, above) and effective quantum yields ($\Delta F/F_m'$, below) of PS II photochemistry for cold-acclimated (dashed line, filled circles) and pre-heated (30 min at 35 °C, continuous line, empty circles) intact leaves of S and AR. *E. canadensis* biotypes. The results are means ± SE of data from five independent measurements on different leaves from different plants.

dence of the optimal quantum yield was only less significant.

Figure 1 also shows the temperature dependence of the effective quantum yield of PS II associated with the linear electron transport activity, calculated at the end of a 15-min actinic light (AL) illumination of 100 μmol m^{-2} s^{-1}, expressed as $\Delta F/F_m'$ (Genty *et al.*, 1989). It can be seen that $\Delta F/F_m'$ was lower above 25 °C for the S biotype grown at 5 °C than that for the AR biotype. For the S biotype, $\Delta F/F_m'$ decreased continuously in parallel with heating from the growth temperature. For the AR biotype, the heating-induced decrease in $\Delta F/F_m'$ began only at 25 °C. The exposure to 35 °C caused a decrease in this parameter for both biotypes measured at all temperatures. The temperature dependence of $\Delta F/F_m'$ for the preheated S plants clearly demonstrates that the temperature optimum of linear electron transport in the S biotype shifts upward, while in the AR plants the shift is not considerable. Apart from the temperature-induced changes in F_v/F_m, and F/F_m', this increased thermostability is demonstrated by the significant upward shift in the optimal thermal range of the photosynthetic net carbon assimilation (Fig. 2). At the same time, the temperature dependences of the above parameters for the AR biotype are less sensitive to preliminary heat exposure: preheating did not markedly modify the heat-induced change in the photochemistry of PS II.

The heat response curves for CO_2 assimilation rates in leaves of cold-acclimated AR and S biotypes of *E. canadensis* are shown in Fig. 2. The temperature dependence of the net CO_2 fixation was influenced differently by the preheating for the cold-acclimated AR and S biotypes. For the heat-treated cold-acclimated S biotype, the optimal thermal interval of the assimilation rate was shifted significantly upwards as compared with that for the non-treated plants, but the maximal net CO_2 fixation rates measured below 25 °C were lower than the assimilation rates of non-treated S plants. Further, the thermal optima of the net CO_2 fixation measured at saturating light intensity are close to one another for the preheated and non-treated AR plants, which seems to suggest that the heat stress tolerance of the photosynthetic apparatus of AR *E. canadensis* is not greatly influenced by short-term heat treatment. This is reflected by

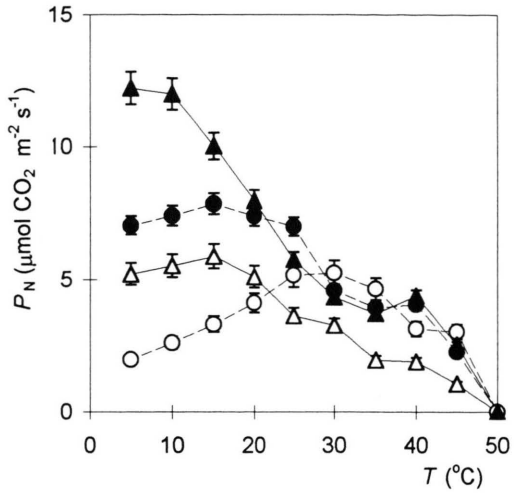

Fig. 2. Temperature dependence of net photosynthesis (at 800 μmol m^{-2} s^{-1} PFD) in cold-acclimated (filled symbols) and pre-heated (30 min at 35 °C, empty symbols) intact leaves of S (empty and filled circles) and AR (empty and filled triangles) *E. canadensis* biotypes. The results are means ± SE of data from five independent measurements on different leaves from different plants.

the fact that there was a smaller difference between T_c and T_p in the F_0 vs. T curves for the treated and non-treated AR biotype than for the S plants. Moreover, the thermal stability of PS II is known to be influenced by the fluidity of the thylakoid membrane (Berry and Björkman, 1980; Raison *et al.*, 1982), which is manifested in the temperature dependence of F_0. We therefore suggest that the chloroplasts of AR *E. canadensis* have a decreased short-term temperature adaptation capacity, which is probably linked to the capacity to rapid change in the membrane fluidity.

The above-mentioned findings are in accord with reports on the role of carotenoid pigments in the thermal tolerance of PS II (Havaux and Gruszeczki, 1993; Havaux and Tardy, 1995; Havaux and Tardy, 1996). Under certain conditions, these carotenoid changes are associated with an increased stability of PS II to heat stress. More precisely, elevated temperature in either light or darkness triggers the first step of the violaxanthin → zeaxanthin conversion. In agreement with this, a light-independent zeaxanthin synthesis was observed in potato leaves exposed to the elevated temperature of 35 °C (Havaux and Tardy, 1996), which was probably accompanied by a decreased membrane fluidity and an increased PS II thermal stability.

Nevertheless, it is also known that AR *E. canadensis* has a reduced xanthophyll-cycle activity (Váradi *et al.*, 1994; Darkó *et al.*, 1996), which might be connected with the fact that in this plant the short-term temperature adaptation capacity is limited. The background of the different short-term temperature capacities in the AR and S biotypes will be precisely elucidated in a future study.

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Berry J. and Björkman O. (1980), Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. **31,** 491–543.

Bilger, H. W., Schreiber U. and Lange O. L. (1984), Determination of leaf heat resistance: comparative investigation of chlorphyll fluorescence changes and tissue necrosis methods. Oecologia **63,** 256–262.

Burke J. J., Wilson F. and Wafford J. R. (1982), Characterisation of chloroplasts isolated from triazine-susceptible and triazine-resistant biotypes of *Brassica campestris* L. Plant Physiol. **70,** 24–29.

Chapman D. J., De-Felice J. and Barber J. (1985), Characteristics of chloroplast thylakoid lipid composition associated with resistance to triazine herbicide. Planta **166,** 280–285.

Darkó É., Váradi G., Dulai S. and Lehoczki E. (1996), Atrazine-resistant biotypes of *Conyza canadensis* have altered fluorescence quenching and xanthophyll cycle pattern. Plant Physiol. Biochem. **34,** 843–852.

Ducruet J. and Lemoine Y. (1985), Increased heat sensitivity of the photosynthetic apparatus in triazine-resistant biotypes from different plant species. Plant Cell Physiol. **26,** 419–429.

Ducruet J. and Ort D. R. (1988), Enhanced susceptibility of photosynthesis to high leaf temperature in triazine-resistant *Solamum nigrum* L.: evidence for photosystem II D_1 protein site of action. Plant Sci. **56,** 39–48.

Dulai S., Molnár I. and Lehoczki E. (1995), Heat-induced modulated chlorophyll *a* fluorescence changes in atrazine resistant and susceptible biotypes of *Conyza canadensis* (L.) Cronq. In: Photosynthesis: from Light to Biosphere (P. Mathis, ed.) Vol. IV, Chap. 18, Kluwer, Dordrecht, pp. 921–924.

Dulai S., Molnár I. and Lehoczki E. (1998), Effects of growth temperature of 5 and 25 °C on long-term responses of photosystem II to heat stress in atrazine-resistant and susceptible biotypes of *Erigeron canadensis* (L.) Cronq. Aust. J. Plant Physiol. **25,** 154–143.

Genty B., Briantais J. M. and Baker N. R. (1989), The relationship between the quantum yield of non-photochemical quenching of chlorophyll fluorescence and rate of photosystem 2 photochemistry in leaves. Biochim. Biophys. Acta **990,** 87–92.

Havaux M. (1989), Comparison of atrazine-resistant and -susceptible biotypes of *Senecio vulgaris* L.: Effect of high and low temperatures on the *in vivo* photosynthetic electron transfer in intact leaves. J. Exp. Bot. **40,** 849–854.

Havaux M. and Gruszeczki W. I. (1993), Heat- and light-induced chlorophyll a fluorescence changes in potato leaves containing high or low levels of the carotenoid zeaxanthin: indication of regulatory effect of zeaxanthin on thylakoid membrane fluidity. Photochem. Photobiol. **58,** 604–617.

Havaux M. and Tardy F. (1995), Short-term adaptive responses of photosynthesis to elevated temperatures and strong light. In: Photosynthesis: from Light to Biosphere (P. Mathis, ed.) Vol. IV, Chap. 18. Kluwer, Dordrecht, pp. 777–782.

Havaux M. and Tardy F. (1996), Temperature-dependent adjustment of the thermal stability of photosystem II *in vivo:* possible involvement of xanthophyll-cycle pigments. Planta **198,** 324–333.

Lehoczki E., Pölös E., Laskay G. and Farkas T. (1985), Chemical compositions and physical states of chloroplast lipids related to atrazine resistance in *Conyza canadensis* L. Plant Sci. **42,** 19–24.

Pillai P. and St John J. B. (1981), Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides. Plant Physiol. **68,** 585–587.

Pölös E., Laskay G., Szigeti Z., Pataki Sz. and Lehoczki E. (1986), Photosynthetic properties and cross-resistance to some urea herbicides of triazine-resistant *Conyza canadensis* Cronq. (L.). Z. Naturforsch. **42c,** 783–793.

Raison J. K., Roberts J. K. M. and Barry J. A. (1982), Correlations between the thermal stability of chloroplasts (thylakoid) membranes and the composition and fluidity of their polar lipids upon acclimation of higher plant *Nerium oleander* to growth temperature. Biochim. Biophys. Acta **688,** 218–228.

Ricroch A., Mousseau M., Darmency H. and Pernes J. (1987), Comparison of triazine-resistant and -susceptible cultivated *Setaria italica:* growth and photosynthetic capacity. Plant Physiol. Biochem. **25,** 29–34.

Schreiber U. and Berry J. (1977), Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. Planta **136,** 233–238.

Schreiber U., Sliwa U. and Bilger W. (1986), Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. **10,** 51–62.

Smillie R. M. and Nott R. (1979), Heat injury in leaves of alpine, temperate and tropical plants. Aust. J. Plant Physiol. **6,** 135–141.

Tremolieres A., Darmency H., Gasques J., Dron M. and Connan A. (1988), Variation of trans-hexadecanoic acid content in two triazine-resistant mutants of *Chenopodium album* and their susceptible progenitor. Plant Physiol. **86,** 967–970.

Van Kooten O. and Snel J. F. H. (1990), The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. **25,** 147–150.

Váradi Gy., Darkó É., Pölös E., Szigeti Z. and Lehoczki E. (1994), Xanthophyll cycle patterns and *in vivo* photoinhibition in herbicide-resistant biotypes of *Conyza canadensis.* J. Plant Physiol. **144,** 669–674.

von Caemmerer S. and Farquhar G. D. (1981), Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta **153,** 376–387.