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

Sándor Dulai*, István Molnár*, Evelyn Péli* and Endre Lehoczki*ab

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

**Department of Botany, Attila József University, H-6701 Szeged, Hungary

*Author for correspondence and reprint requests

Z. Naturforsch. 54c, 665–670 (1999); received November 2, 1998/February 19, 1999

*Erigeron canadensis*, Heat Stress, Chlorophyll Fluorescence, Photosystem II, Photosynthesis

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 

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

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 $Q_A^-$ ($Q_A^{-}$) 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 a 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...
CO₂ 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 breaks-points of F₀ 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 ΔF/Fₘ', as reported by Genty *et al.* (1989).

For determination of the critical and peak temperatures (Tₜ and Tₚ) 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 thermostatically controlled module. During heating from the growth temperature to 50 °C at a rate of 1 °C min⁻¹, the temperature was monitored by a thermosteppar thermocouple. F₀ 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. Tₜ and Tₚ were determined from the F₀ vs. T curves.

CO₂ 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 CO₂ 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 CO₂ 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 CO₂ fixation were calculated by using the equations of von Caemmerer and Farquhar (1981).

**Results and Discussion**

It is well documented that the critical (Tₜ) and peak (Tₚ) temperatures of F₀ vs. T curves are direct indices of the thermotolerance 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 F₀ for the leaves of S *E. canadensis* displayed characteristic changes. There was a slow increase in F₀ for the preheated cold-acclimated AR biotype at 34.7 °C (Tₜ) and Tₚ was at 39.3 °C, in contrast with the S biotype, where the fluorescence level did not change up to 37.5 °C (Tₜ), with Tₚ at 40.5 °C (Table I). These short heat treatment-induced upward temperature shifts led to increases in F₀, 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 Tₜ and Tₚ in the F₀ 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.

<table>
<thead>
<tr>
<th></th>
<th>$T_1$</th>
<th>$T_c$</th>
<th>$T_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold-acclimated</td>
<td>30.2 ± 0.84</td>
<td>36.6 ± 0.81</td>
<td>44.5 ± 1.25</td>
</tr>
<tr>
<td>Cold-accl. heat-treated</td>
<td>37.5 ± 0.91</td>
<td>40.5 ± 1.08</td>
<td>47.8 ± 1.15</td>
</tr>
<tr>
<td>Warm-acclimated</td>
<td>37.4 ± 0.57</td>
<td>41.1 ± 0.59</td>
<td>47.7 ± 1.18</td>
</tr>
</tbody>
</table>

Atrazine resistant biotype

<table>
<thead>
<tr>
<th></th>
<th>$T_1$</th>
<th>$T_c$</th>
<th>$T_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold-acclimated</td>
<td>32.4 ± 0.43</td>
<td>38.6 ± 0.92</td>
<td>46.3 ± 0.95</td>
</tr>
<tr>
<td>Cold-accl. heat-treated</td>
<td>34.7 ± 1.18</td>
<td>39.3 ± 1.05</td>
<td>47.1 ± 1.56</td>
</tr>
<tr>
<td>Warm-acclimated</td>
<td>33.2 ± 0.59</td>
<td>38.6 ± 0.41</td>
<td>44.6 ± 1.14</td>
</tr>
</tbody>
</table>

The temperature responses of the optimal quantum yield of PS II photochemistry ($F_{\text{v}}/F_{\text{m}}$) (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 $F_{\text{v}}/F_{\text{m}}$ became tolerant to heat. In contrast with the non-treated leaves, in the preheated S plants $F_{\text{v}}/F_{\text{m}}$ decreased sharply only above 40 °C; in the AR biotype, the change in the temperature depen-

Fig. 1. Effects of heating on optimal ($F_{\text{v}}/F_{\text{m}}$, above) and effective quantum yields ($\Delta F/F_{\text{m}}'$, 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 ΔF'/Fm' (Genty et al., 1989). It can be seen that ΔF'/Fm' was lower above 25 °C for the S biotype grown at 5 °C than that for the AR biotype. For the S biotype, ΔF'/Fm' decreased continuously in parallel with heating from the growth temperature. For the AR biotype, the heating-induced decrease in ΔF'/Fm' 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 ΔF'/Fm' 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 Fv/Fm and F'/Fm', 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 CO₂ 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 CO₂ 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 CO₂ fixation rates measured below 25 °C were lower than the assimilation rates of non-treated S plants. Further, the thermal optima of the net CO₂ 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 the fact that there was a smaller difference between Tc and Tp in the F₀ 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 F₀. 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 Gruszczki, 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.

**Acknowledgements**

This work was supported by a grant from the Department of the Sciences of Hungarian Ministry of Education (pfp 5113/1997) to S. D. and by an OTKA research grant (T016455) to E. L.


