Effect of heat stress on the photosynthetic apparatus in maize (Zea mays L.) grown at control or high temperature

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Abstract

The effects of short exposure to heat stress in the dark on maize seedlings grown at 25 °C or 41 °C were investigated by means of chlorophyll fluorescence and oxygen evolution. Exposure of plants grown at 25 °C to 35 °C for 20 min in the dark led to a transient inhibition (by about 50%) of the rate of photosynthesis, which recovered within 4 h. Treatments at temperatures above 45 °C led to permanent damage; the plants did not recover within 96 h. In contrast, no damage occurred to plants grown at 41 °C after exposure to temperatures up to 50 °C. Improved thermo-tolerance as a result of high growth temperature was not related to the presence of zeaxanthin, since it did not accumulate in heat-stressed leaves in the dark. In plants grown at 25 °C, measurements of the quantum yield of electron transport at PSII in leaves infiltrated with methylviologen (MV) indicated the presence of a heat-sensitive component of the photosynthetic apparatus, located downstream of photosystem II (PSII) and before the carbon cycle.

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1. Introduction

The photosynthetic apparatus has long been recognized as one of the plant components most sensitive to high temperature stress (Berry and Bjorkman, 1980). Inhibition of photosynthesis has been observed after short exposure (15–60 min) to moderately high temperature (35–40 °C) in various plant species (Havaux, 1993a; Law and Crafts-Brandner, 1999; Crafts-Brandner and Salvucci, 2000) including maize (Crafts-Brandner and Salvucci, 2002). Following heat stress, the loss of photosynthetic electron transport in potato leaves was attributed to the thermolability of photosystem II (PSII) and water-splitting was the most heat-sensitive component (Havaux, 1993a). More recently, inhibition of the activation of ribulose-1,5-biphosphate carboxylase/oxygenase has been identified as one of the most heat-sensitive components of the photosynthetic apparatus (Law and Crafts-Brandner, 1999; Crafts-Brandner and Salvucci, 2000, 2002). Although the photosynthetic apparatus is relatively heat-sensitive, improved thermo-tolerance can be achieved by exposure to moderately high temperature. The exposure of potato plants to 35 °C for

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20 min significantly increased the stability of PSII to heat stress (Havaux, 1993b). This rapid acclimation was attributed to the accumulation of the xanthophyll zeaxanthin in the leaves (Havaux and Tardy, 1996), which stabilizes the lipid phase of the thylakoid membrane (Havaux, 1998). Additionally, unsaturation of membrane lipids, as shown in the cyanobacterium Synechocystis (Gombos et al., 1994), and the accumulation of heat-shock proteins, which was observed in Chenopodium album and Lycopersicum esculentum (Downs et al., 1999), are thought to play a role in protecting the photosynthetic apparatus from heat damage. Information concerning the ability of maize to acclimate to high temperature, however, is scanty. It was demonstrated that further heat tolerance of maize plants was induced by moderate heat shock (Gong et al., 2001) or gradually increased temperature. The latter was associated with the appearance of a new activase polypeptide (Crafts-Brandner and Salvucci, 2002).

To get new insights into the mode of acclimation to heat stress, we addressed the question whether the primary target of heat-induced perturbation of photosynthesis is identical in heat-acclimated and non-acclimated maize plants. To elucidate this, the effect of high growth temperatures for 20 min in a dark growth chamber: 25, 30, 35, 40, 45, or 50 °C and were then transferred to their previous growth conditions for a given period of time (0, 20, or 40 min or 1, 2, 4, 24, 48, or 96 h). For the measurements of photosynthetic parameters, segments from the middle part of the third leaf were placed with the upper surface exposed in the leaf chamber of a Hansatech leaf disc O₂ electrode (Type LD2, Hansatech Ltd., Norfolk, UK) that was connected to a water bath at a constant temperature of 25 °C. The chlorophyll fluorescence parameters were measured simultaneously using a portable chlorophyll fluorometer (PAM-2000, H. Walz, Effeltrich, Germany). The tip of the optic fiber was inserted into the top water jacket of the LD2 chamber. The maximum quantum efficiency of PSII ($F_{m}$/$F_{o}$) and respiration were measured after 20 min of dark adaptation in the LD2 chamber. After these measurements in the dark, actinic light (Björkman lamp) was turned on (300 µmol m⁻² s⁻¹ PPFD); after 15 min of adaptation to the light, fluorescence characteristics and net photosynthetic O₂ evolution were determined in 5% CO₂. The definitions of chlorophyll fluorescence characteristics are according to Van Kooten and Snel (1990). The photochemical quenching factor [$qP = 1 - (F_{I} - F_{o})/(F_{m} - F_{o})$] and the efficiency of excitation energy captured by open PSII reaction centers [$F_{o}'/F_{m}' = (F_{o}' - F_{o}'')(F_{m}' - F_{o}''')/(F_{m}' - F_{o}''') = (F_{o}'' - F_{o}''')(F_{m}' - F_{o}''')/(F_{m}' - F_{o}''')$] according to Oxboough and Baker (1997). This method was preferred to the usual $F_{o}$ determination after exposure to far red light, because the latter is based on the assumption that electron transport between PSII
and PSI is functional, which is not the case after heat stress (see results). The non-photochemical quenching (NPQ), which represents the dissipation of energy as heat, was obtained by means of the Stern–Volmer equation: NPQ = (Fm′ − F0)/Fm′. The quantum yield of electron transport (ΦPSII) was calculated as (Fm′ − Fv)/Fm′ (Genty et al., 1989). The results are averages of two independent experiments with three plants.

2.3. Response to methylviologen

Segments of the third leaf (about 2 cm long) from plants grown at 25 °C were immersed in 0.1 M of methylviologen (MV) or distilled water (control) at room temperature. This concentration of MV was chosen to provide a sufficient pool of MV in the chloroplasts to substitute to the carbon cycle as electron acceptor after PSI. The leaf segments were then sealed in plastic tubes and subjected to a temperature treatment (25 °C) in a water bath for another 20 min in the dark. Each piece of leaf was then placed in the leaf chamber at 25 °C. ΦPSII was measured at 1 min intervals after turning on the actinic illumination at 150 μmol m−2 s−1. This relatively low intensity was chosen to minimize oxidative damage to the chloroplasts due to the presence of MV. The results are averages of three individual plants.

2.4. Pigments analysis

Leaf discs of 1 cm diameter were punched from the third leaf right after the temperature treatment (25 °C or 35 °C) in water bath until analysis. Pigments were analyzed by HPLC according to the method of Gilmore and Yamamoto (1991) and the modifications described by Leipner et al. (2000).

3. Results

The effects of a short exposure (20 min) to high temperature in the dark on the functioning of the photosynthetic apparatus were studied in maize plants grown at 25 and 41 °C (Fig. 1). Heat treatments similarly affected photosynthetic O2 evolution and ΦPSII in leaves developed at 25 and 41 °C. A regression analysis of the pooled data of both leaf types (Fig. 1) revealed an r2 of 0.963 for the linear relationship between the rate of photosynthesis and ΦPSII. The photosynthesis of leaves grown at optimal temperature decreased considerably when exposed to 35 °C or higher. This was associated with a reduction in ΦPSII and photochemical quenching factor (qP), while the efficiency of open reaction centers (Fv′/Fm′) decreased only slightly between 35 and 45 °C. A substantial decrease in the maximum quantum efficiency of PSII (Fv′/Fm) of these leaves was observed only at the highest temperature (50 °C). Heat stress induced an increase in NPQ between 35 and 40 °C, which disappeared at higher temperatures in leaves grown at 25 °C. In contrast, leaves grown at high temperature were remarkably more tolerant to heat stress. A significant decrease in the photosynthesis and ΦPSII in leaves of plants grown at 25 and 41 °C did not recover significantly within 48 h. Leaves treated at 40 °C after 48 h. Leaves treated at 45 °C or 50 °C did not recover significantly within 96 h. Treatment at 40 °C, which did not affect Fv′/Fm′
Fig. 1. The effects of 20 min heat stress in the dark on photosynthetic parameters of maize leaves grown at 25 °C (open symbols) and 41 °C (filled symbols). Photosynthetic parameters were determined at 25 °C and, with the exception of $F_v/F_m$, at 300 μmol m$^{-2}$ s$^{-1}$. Values are mean ± S.D. of six replications.

Table 1
Xanthophyll cycle pigments in maize leaves (μmol m$^{-2}$) grown at 25 and 41 °C after treatments at different temperatures without (dark) and with (dark + light) subsequent illumination for 20 min at 300 μmol m$^{-2}$ s$^{-1}$.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Growth</th>
<th>Dark</th>
<th>Dark + light</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Viola xanthin</td>
<td>Antheroxanthin</td>
</tr>
<tr>
<td>25</td>
<td>25</td>
<td>27.6 ± 4.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>35</td>
<td>35</td>
<td>31.8 ± 5.8</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>40</td>
<td>40</td>
<td>28.1 ± 4.7</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
<td>34.4 ± 6.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>29.0 ± 5.3</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>41</td>
<td>25</td>
<td>24.6 ± 2.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>22.8 ± 2.4</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>22.4 ± 4.3</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>24.6 ± 6.1</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21.2 ± 2.8</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

Values are mean ± S.D. of three replications; NS, not significant.

$^*$ Statistical significance in comparison to the 25 °C treatment is given by the t-test ($P = 0.05$).

$^{* *}$ Statistical significance in comparison to the 25 °C treatment is given by the t-test ($P = 0.01$).
immediately after the high-temperature treatment, led to a progressive decrease in this parameter upon the return of the plants to previous growth conditions for 4 h, after which it recovered. The increase in NPQ induced at 35 °C also recovered within 4 h as the rate of photosynthesis increased.

In leaves developed at 41 °C, heat treatment below 50 °C did not induce changes in any of the parameters right after the treatment. However, treatment at 45 °C induced a progressive decrease in the rate of photosynthesis and $F_v/F_m$ for 4 h which recovered after 24 h as it was observed at 40 °C for leaves grown at 25 °C. The immediate decrease in the photosynthetic rate of leaves grown at 41 °C and treated at 50 °C was reversed, reaching its previous level within 48 h. This indicates that there was no permanent damage to these leaves.

The involvement of the intactness of the photosynthetic electron transport chain in the heat-induced inhibition of photosynthesis, which was observed in leaves grown at 25 °C right after treatment at 35 °C (Fig. 1), was investigated by means of measurements of $\Phi_{PSII}$ in leaves infiltrated with the artificial electron acceptor MV (Fig. 3). In control leaves that were treated at 25 °C, $\Phi_{PSII}$ reached steady state after 10–15 min of illumination. Similar steady state values were found directly after start of illumination in MV-treated leaves indicating that MV functioned as electron acceptor when carbon fixation was not fully active. After high-temperature treatment (35 °C), $\Phi_{PSII}$ decreased slightly after transition from dark to light and reached a steady state of about 0.4. The presence of MV did not affect the inhibition of $\Phi_{PSII}$ that was observed in leaves treated at 35 °C.
Fig. 3. The effect of methylviologen (MV) treatment on the kinetics of the quantum yield of electron transport at PSII ($\Phi_{PSII}$) upon actinic illumination in maize leaves grown at 25°C. Open symbols, leaves treated at 25°C; filled symbols, leaves treated at 35°C; circles, leaves treated with water; squares, leaves treated with MV. Values are mean ± S.D. of three replications.

4. Discussion

The linear relationship between the rate of photosynthesis and $\Phi_{PSII}$ suggests that the proportion of electron transport used for photosynthesis remained constant, regardless of growth temperature and heat treatments. A similar relationship was found for maize grown under cold conditions in the field (Leipner et al., 1999).

According to Genty et al. (1989), the quantum yield of electron transfer at PSII ($\Phi_{PSII}$) is the product of the efficiency of the open PSII reaction centers ($F_v'/F_m'$) and the photochemical quenching factor ($q_P$). Therefore, the decrease in net photosynthetic O$_2$ evolution and $\Phi_{PSII}$ in leaves grown at 25°C after a heat stress up to 45°C (Fig. 1) is due primarily to the decrease in $q_P$. This implies that inhibition of electron transport might have occurred after PSII, since $q_P$ is a proxy for the redox state of QA, the primary electron acceptor after PSII. This is also consistent with the increase in energy dissipation revealed by the increase in NPQ at 35–40°C and by the observation that $F_v/F_m$ was hardly affected at 45°C or below. It is also confirmed by the progressive decrease in $F_v/F_m$ observed in leaves grown at 25°C and treated at 40°C during the recovery phase (Fig. 2) which can also be interpreted as photoinhibition induced by a temporary inhibition of electron transport downstream of PSII. Therefore, the most sensitive component of the photosynthetic apparatus to heat stress in the dark is unlikely to be PSII as found for potato (Havaux, 1993a).

From 45°C and above, the maize plants grown at 25°C failed to recover fully (Fig. 2) and failed to increase NPQ as found after treatments at 35°C or 40°C. This suggests additional irreversible damage, perhaps due to a loss of integrity of the thylakoid membrane, which would have prevented the establishment of the proton gradient required for the development of NPQ and for the de-epoxidation of violaxanthin. $F_v/F_m$ in 25°C grown leaves stressed at 50°C (Fig. 1) is probably underestimated because the formula used to calculate $F_v$ is not valid under photoinhibitory conditions (Maxwell and Johnson, 2000).

Besides its protective effect on Rubisco activase in heat-stressed plants (Crafts-Brandner and Salvucci, 2000), MV can act as an electron acceptor after PSI. It was effective in leaves not treated at high temperature because it eliminated the typical induction phase required to reach a steady state of electron transport (Fig. 3). After 20 min illumination, $\Phi_{PSII}$ was similar in MV-treated and control leaves, indicating that no oxidative damage occurred in leaves treated with MV. A similar MV treatment was effective in restoring high $\Phi_{PSII}$ values in maize leaves at low temperature (Fracheboud and Leipner, 2003). However, MV did not restore electron transport in leaves treated at 35°C to values seen in leaves treated at 25°C, suggesting that the component which was responsible for the decrease in photosynthesis after heat stress in the dark is located upstream of PSI and is not Rubisco activation as found for heat-stressed maize in the light (Crafts-Brandner and Salvucci, 2002).

Growth at high temperature considerably increased the tolerance of the photosynthetic apparatus to heat stress in the dark. Even after treatment at 50°C, complete recovery occurred within 2 days (Fig. 2). The acclimation of potato to heat stress is induced by a short exposure to moderate high temperature in the light (Havaux, 1993b). This phenomenon was attributed to increased membrane stability due to the accumulation of zeaxanthin in the leaves during acclimation (Havaux and Gruszecki, 1993; Havaux and Tardy, 1996). This was not the case in maize developed at high temperature, since zeaxanthin was not found in these leaves even after exposure to heat stress in the dark (Table 1) unless they were subsequently exposed to light. Leaves developed at high
temperature might be resistant to heat stress due to the presence of certain heat-shock proteins known to protect the photosynthetic apparatus (Downs et al., 1999) and/or changes in the thermostability of membranes due to changes in the composition of membrane lipids (Gombos et al., 1994, and references therein).

In summary, the results suggest the presence of an additional heat-sensitive component in the photosynthetic electron transport chain, located downstream of PSII, which had not been identified before. Improved thermo-tolerance, as a result of high growth temperature, was not related to the presence of zeaxanthin.

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