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Oxygen Exchange in Relation to Carbon Assimilation in Water-stressed Leaves During Photosynthesis

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In a study on metabolic consumption of photosynthetic electrons and dissipation of excess light energy under water stress, O_2 and CO_2 gas exchange was measured by mass spectrometry in tomato plants using $^{18}O_2$ and $^{13}CO_2$. Under water stress, gross O_2 evolution (E_O), gross O_2 uptake (U_O), net CO_2 uptake (P_N), gross CO_2 uptake (TPS), and gross CO_2 evolution (E_C) declined. The ratio P_N/E_O fell during stress, while the ratios U_O/E_O and E_C/TPS rose. Mitochondrial respiration in the light, which can be measured directly by $^{12}CO_2$ evolution during $^{13}CO_2$ uptake at 3000 µl l^{-1} $^{13}CO_2$, is small in relation to gross CO_2 evolution and CO_2 release from the glycolate pathway. It is concluded that PSII, the Calvin cycle and mitochondrial respiration are down-regulated under water stress. The percentages of photosynthetic electrons dissipated by CO_2 assimilation, photorespiration and the Mehler reaction were calculated: in control leaves more than 50 % of the electrons were consumed in CO_2 assimilation, 23 % in photorespiration and 13 % in the Mehler reaction. Under severe stress the percentages of electrons dissipated by CO_2 assimilation and the Mehler reaction declined while the percentage of electrons used in photorespiration doubled. The consumption of electrons in photorespiration may reduce the likelihood of damage during water deficit.

Key words: *Lycopersicon esculentum*, tomato, high-pigment mutant, water stress, oxygen exchange, CO_2 assimilation, photorespiration, mitochondrial respiration in the light, Mehler reaction, mass spectrometry.

INTRODUCTION

We investigated the effects of water stress on the light reactions of photosynthesis and on the primary metabolism of wild-type tomato (*Lycopersicon esculentum* Mill. 'Moneymaker') and a high-pigment *hp-1* mutant with respect to their use of photosynthetic electrons and the consumption of surplus electrons in primary leaf metabolism.

Net CO_2 uptake (P_N) is the result of CO_2 fixation (TPS), CO_2 evolution in the glycolate pathway (PR) and CO_2 evolution from mitochondrial respiration in the light (R_C). The rates of net O_2 evolution, which are almost identical to P_N, are composed of O_2 evolution in the Hill reaction of photosystem II (PSII), O_2 uptake by the Mehler reaction and the glycolate pathway and O_2 uptake in mitochondrial respiration in the light.

We analysed the contribution of these reactions and pathways to the energy balance in water-stressed tomato plants by feeding leaves with $^{18}O_2$ and $^{13}CO_2$ during steady state photosynthesis. By combining data from CO_2 and O_2 gas exchange measurements it is possible to determine the rates of the electron-consuming reactions: CO_2 assimilation (including intercellular reassimilation); photorespiration (taking account of mitochondrial CO_2 release from dissimilation/respiration); and also the Mehler reaction. Earlier results and theoretical considerations of these mass spectrometric $^{16}O_2/^{18}O_2$ and $^{12}CO_2/^{13}CO_2$ gas exchange studies have been published elsewhere (Haupt-Herting and Fock, 2000; Haupt-Herting *et al*., 2001).

Compared with wild-type tomato plants, the *hp-1* mutant has darker green foliage and higher contents of chlorophyll, carotenoid and anthocyanin (Adamse *et al*., 1989) because of an increased sensitivity to far-red light and activated phytochrome (Kerckhoffs *et al*., 1997). The *hp-1* gene product, which is less expressed in the mutant, may be an inhibitor of the signal cascade of the phytochrome answer (Peters *et al*., 1992). As a consequence, the chlorophyll content of mutant leaves is 30 % higher than in the wild type, and light absorption and net photosynthesis are increased (Nieuwhof and van de Dijk, 1988; Peters *et al*., 1992; Haupt-Herting, 2000). Enhanced light absorption in the mutant could lead to higher rates of photosynthetic electron transport and therefore necessitate the dissipation of surplus electrons under high light and water deficit. As a consequence, the metabolic reactions that are involved in con-sumption of surplus electrons may be more distinct in the mutant than in the wild type.

MATERIALS AND METHODS

Plant growth and stress application

Seeds of wild-type tomato (*Lycopersicon esculentum* Mill. 'MoneyMaker'; Hild, Marbach, Germany) and a high-pigment mutant (*hp-1*^{w}; Dr D. Kendrick, Institute for Plant Breeding, Wageningen, The Netherlands) were sown individually in small pots of compost (ED 73, Einheitserdenwerk, Hameln, Germany) and then transferred

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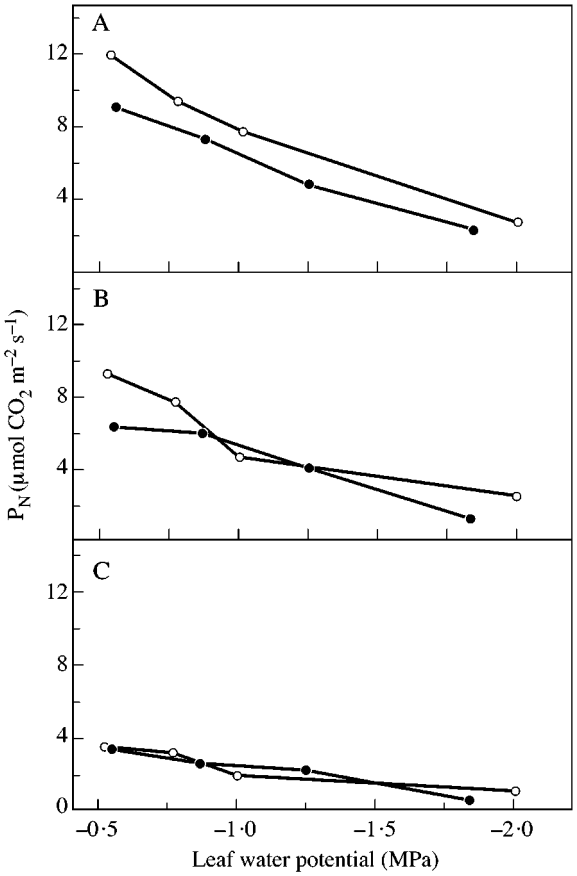

FIG. 1. Net CO_2 uptake (P_N) by attached leaves of wild-type tomato (closed circles) and the *hp-1* mutant (open circles) during steady state photosynthesis at three different light intensities [850 (A), 400 (B) and 90 (C) μmol photons m^{-2} s^{-1}] in relation to leaf water potential. Measurements were made in an open gas exchange system using an infrared gas analyser. Leaves (23 cm^2) were supplied with air (gas flow 50 l h^{-1}) containing 350 μl l^{-1} CO_2 and 210 ml l^{-1} O_2 at 70 % relative humidity and 23 °C. Points are means of six replicates (= independent plants); s.e. ⩽ 10 %.

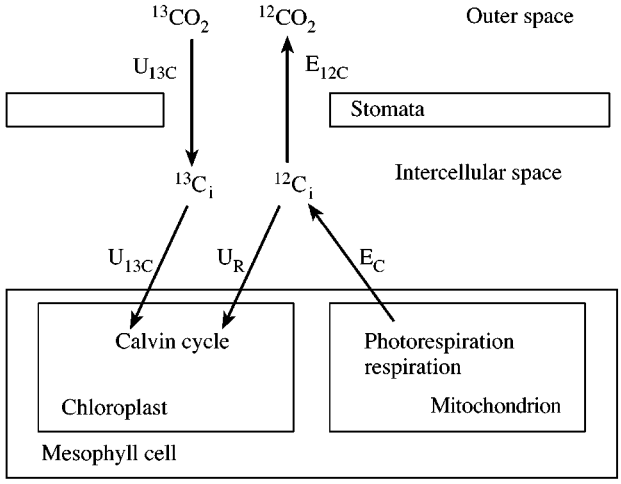

FIG. 2. Scheme of CO_2 fluxes into and out of an illuminated leaf provided with $^{13}CO_2$ in the atmosphere for 1 min. The measurable fluxes of $^{12}CO_2$ and $^{13}CO_2$ (E_{12C}, $^{12}CO_2$ evolution; U_{13C}, gross $^{13}CO_2$ uptake) outside the leaf and the assumed fluxes inside the leaf (E_C, gross $^{12}CO_2$ evolution; U_R, $^{12}CO_2$ reassimilation) are shown.

7 d after germination to 2·5 l pots containing a mixture of 10 % sand in potting compost. Plants were grown in a growth chamber under moderate light intensity (200 μmol m^{-2} s^{-1}, fluorescent lamps; Osram, München, Germany) with a 16/8 h light/dark period, temperatures of 23 °C (light)/17 °C (dark) and a constant relative air humidity of 70 %. Plants were watered daily and were regularly supplied with a commercial nutrient solution (Flori 3; Planta Düngemittel, Regenstauf, Germany). The youngest, fully expanded leaf (normally the fifth leaf from the top) of 5-week-old plants was used in experiments. Leaves of well-watered plants had a leaf water potential of –0·6 MPa, measured according to Scholander *et al.* (1965) using a pressure bomb (Metallwerkstätten der Universität, Kaiserslautern, Germany) just after photosynthetic measurements had been made. To induce an almost natural, reversible water stress allowing the plant enough time to acclimate, irrigation was stopped 2, 5 or 8 d before measurements were taken. These treatments resulted in weak (leaf water potential –0·9 MPa), moderate (–1·3 MPa) or severe (–1·8 MPa) water stress. Even severely stressed plants showed complete recovery of leaf water potential, transpiration and net photosynthesis within 2 d after rewatering.

Gas exchange measurements

Rates of gross O_2 evolution (E_O) and gross O_2 uptake (U_O) were determined on attached leaves at photosynthetic steady state in a closed gas exchange system coupled to a mass spectrometer (5970 Series Mass Selective Detector; Hewlett-Packard, Waldbronn, Germany) using $^{18}O_2$ (Chemotrade, Leipzig, Germany) in the atmosphere (210 ml l^{-1} $^{18}O_2$ in N_2, 350 μl l^{-1} CO_2) as described previously (Canvin *et al.*, 1980; Biehler *et al.*, 1997; Haupt-Herting and Fock, 2000). In addition, a humidifier, water vapour sensor and a gas phase Clark oxygen electrode (MSIPO; Biolytik, Bochum, Germany) were integrated into the system.

To determine rates of net CO_2 uptake, gross CO_2 assimilation, gross CO_2 evolution (E_C), photorespiration and mitochondrial respiration in the light, attached leaves were provided with $^{13}CO_2$ in an open gas exchange system coupled to a mass spectrometer as described by Haupt-Herting *et al.* (2001).

RESULTS AND DISCUSSION

The $^{12}CO_2$/$^{13}CO_2$ isotope technique

In order to study ^{12}C/^{13}C carbon fluxes, a new gas exchange system was established and its validity tested (Haupt-Herting *et al.*, 2001). Net CO_2 uptake was first determined in relation to light intensity and water stress by conventional $^{12}CO_2$ gas analysis in an open system with an infrared gas analyser (Fig. 1). As is well documented, P_N values increase with increasing light intensities and decline as water stress

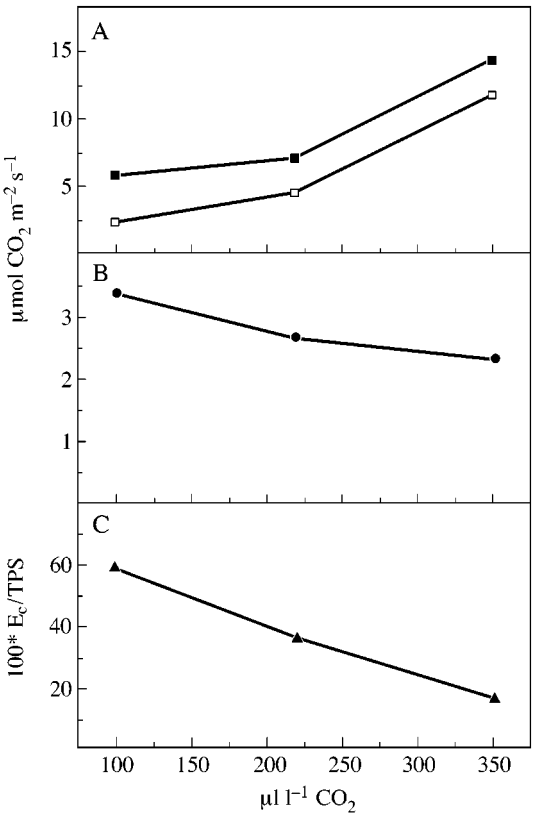

FIG. 3. Rates of gross CO_2 uptake (TPS, closed squares), net CO_2 uptake (P_N, open squares) (A) and gross CO_2 evolution in the light (E_C) (B) by attached leaves of wild-type tomato during steady state photosynthesis at saturating light intensity (850 µmol photons m^{-2} s^{-1}) in relation to ambient CO_2. C, Ratio of E_C/TPS. Measurements were made in an open gas exchange system using a $^{12}CO_2$ /$^{13}CO_2$ isotope technique. Leaves (23 cm^2) were supplied with air (gas flow 50 l h^{-1}) containing 210 ml l^{-1} O_2 and 350 µl l^{-1} $^{12}CO_2$, or 250 µl l^{-1} $^{12}CO_2$ or 100 µl l^{-1} $^{12}CO_2$ at 70 % relative humidity and 23 °C. When steady state photosynthesis was reached $^{12}CO_2$ uptake was measured. Then 350 µl l^{-1} $^{13}CO_2$ (or 250 µl l^{-1} $^{13}CO_2$ or 100 µl l^{-1} $^{13}CO_2$) in 21 % O_2 was supplied for 1 min and $^{13}CO_2$ uptake as well as $^{12}CO_2$ evolution were determined. From mass spectrometer readings, TPS, P_N, E_C and the ratio of E_C/TPS were calculated. Points are means of six replicates; s.e. ⩽ 10 %.

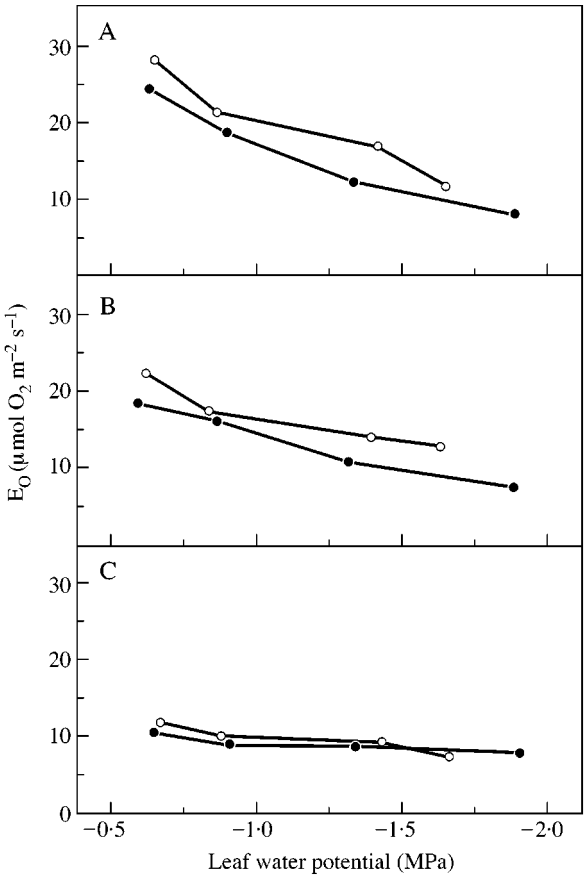

FIG. 4. Gross oxygen evolution (E_O) of attached leaves of wild-type tomato (closed circles) and the *hp-1* mutant (open circles) during steady state photosynthesis at three different light intensities [850 (A), 400 (B) and 90 (C) µmol photons m^{-2} s^{-1}] in relation to leaf water potential. Measurements were made in a closed gas exchange system using a mass spectrometric $^{16}O_2$/$^{18}O_2$ isotope technique. Leaves (23 cm^2) were supplied with air (gas flow 50 l h^{-1}) containing 350 µl l^{-1} CO_2 and 210 ml l^{-1} $^{18}O_2$ in N_2 at 70 % relative humidity and 23 °C. Points are means of six replicates; s.e. ⩽ 10 %.

increases (Lawlor, 1995). Differences in P_N between the wild type and the *hp-1* mutant of tomato are significant only in unstressed control plants at saturating light intensities. Higher rates of P_N in the mutant may be correlated to the higher chlorophyll content (Nieuwhof and van de Dijk, 1988; Peters *et al*., 1992).

Mass spectrometric measurements of P_N, determined as net $^{12}CO_2$ uptake, show the same rates as conventional measurements with an infrared gas analyser. When 350 µl l^{-1} $^{12}CO_2$ is replaced by 350 µl l^{-1} $^{13}CO_2$ in long-term experiments during steady state photosynthesis, the same rates are obtained. Furthermore, when $^{12}CO_2$ is replaced by $^{13}CO_2$ for 1 min, the uptake of $^{13}CO_2$ into (U_{13C}) and the evolution of $^{12}CO_2$ (E_{12C}) out of an illuminated leaf are measured (Fig. 2). If reassimilation of evolved CO_2 (U_R) is considered, gross CO_2 uptake (TPS = U_{13C} + U_R), net CO_2 uptake and gross CO_2 evolution (E_C = TPS – P_N = E_{12C} + U_R) can be determined (Haupt-Herting *et al*., 2001).

Thus, TPS, P_N and E_C of attached wild-type tomato leaves were studied in relation to the CO_2 concentration around the leaves (Fig. 3). At 350 µl l^{-1} CO_2 and 850 µmol photons m^{-2} s^{-1}, the rate of net CO_2 uptake was 12·4 µmol m^{-2} s^{-1} and was similar to P_N measured in Fig. 1. Under these conditions, 14·8 µmol CO_2 m^{-2} s^{-1} were fixed in TPS and 2·4 µmol CO_2 m^{-2} s^{-1} evolved in gross CO_2 evolution. With decreasing CO_2 partial pressures from 350 to 220 to 100 µl l^{-1} CO_2, oxygenation was favoured over carboxylation (Lawlor, 1995) and TPS and P_N declined. Correspondingly, E_C and the ratio of E_C/TPS increased. These results are in the same order of magnitude and agree with those reported by Gerbaud and André (1987) for sunflower and bean leaves and those obtained by Stuhlfauth *et al*. (1990) for *Digitalis*. The rates of gross CO_2 uptake, net CO_2 uptake and gross CO_2 evolution in the light measured with the new mass spectrometric $^{12}CO_2$/$^{13}CO_2$ isotope technique changed typically in relation to ambient CO_2 (Fig. 3) and O_2 (not shown) partial pressures. This is convincing evidence for the validity of the new method.

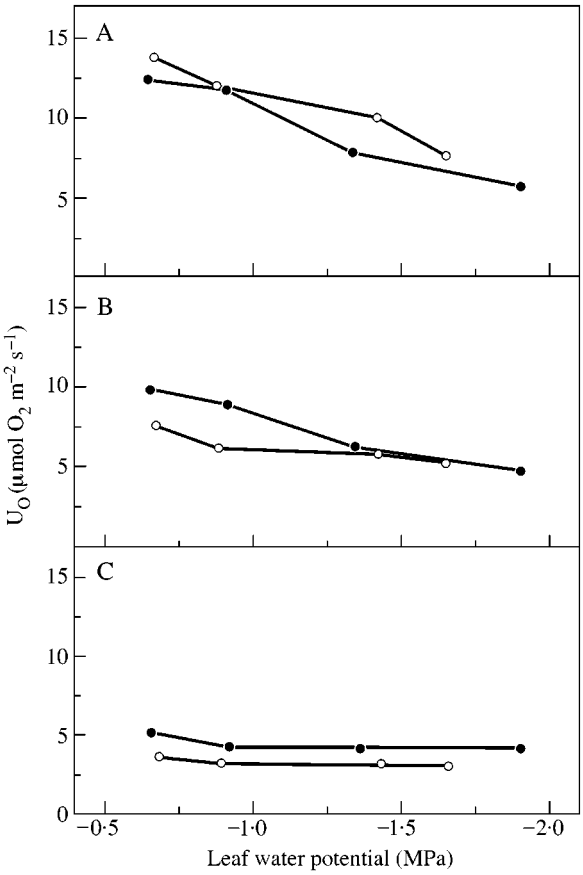

FIG. 5. Gross oxygen uptake (U_O) of attached leaves of wild-type tomato (closed circles) and the *hp-1* mutant (open circles) during steady state photosynthesis at three different light intensities [850 (A), 400 (B) and 90 (C) μmol photons m^{-2} s^{-1}] in relation to leaf water potential. Measurements were made in a closed gas exchange system using a mass spectrometric $^{16}O_2/^{18}O_2$ isotope technique. For further details see Fig. 4.

Gross O_2 evolution and gross O_2 uptake

The rate of gross O_2 evolution by PSII is used as a measure of the rate of photosynthetically generated electrons, as four electrons traversing the photosystems equal one O_2 released. In our experiments leaves were fed $^{18}O_2$ in ambient air and E_O was determined with a mass spectrometer in a closed gas exchange system. Simultaneously, gross oxygen uptake was determined. Net O_2 evolution calculated as $E_O - U_O$ was almost identical to net O_2 evolution as measured with a Clark electrode at the same time, and net O_2 evolution fitted net CO_2 uptake determined under the same conditions (data not shown).

In accordance with earlier experiments on wheat (Gerbaud and André, 1980; Biehler and Fock, 1996), *Hirschfeldia* and bean leaves (Canvin *et al*., 1980), E_O rose with increasing light intensity (Fig. 4). From low light at 90 μmol photons m^{-2} s^{-1} to high light at 850 μmol photons m^{-2} s^{-1}, E_O changed from 10·9 to 24·3 μmol O_2 m^{-2} s^{-1} in wild-type controls. E_O values of the *hp-1* mutant were slightly higher than those of the wild type in control plants at moderate and high light intensities because of enhanced light absorption. The stimulation of E_O by decreasing light, with increasing water deficit, or under saturating light and severe stress (8·1 μmol O_2 m^{-2} s^{-1}) was only a fraction of E_O in the controls (24·3 μmol O_2 m^{-2} s^{-1}). In stressed plants differences between the two plant types were not significant.

The decline of E_O and thus the declining flux of photosynthetic electrons in relation to increasing water deficit was also observed on potato leaf discs (Tourneux and Peltier, 1995) and soybean leaves (Thomas and André, 1982). Therefore, the flux of photosynthetic electrons under severe stress is already saturated at low light intensity and charge separation at photosystem II is strongly reduced under these conditions.

The ratios of P_N to E_O (Haupt-Herting and Fock, 2000) and TPS to E_O (Figs 4 and 6) declined with increasing water deficit. Therefore, a smaller proportion of photosynthetic electrons was consumed by CO_2 assimilation in water-stressed leaves compared with control leaves. Although E_O declines under conditions of water deficit with increasing light intensities, there are still excess electrons that must be used up by acceptors other than CO_2.

An important alternative acceptor for excess photosynthetic electrons is oxygen. In our experiments, gross oxygen uptake rose with increasing light intensity from 3·8 to 13·8 μmol O_2 m^{-2} s^{-1} in the controls and from 3·7 to 5·1 μmol O_2 m^{-2} s^{-1} in severely stressed plants (Fig. 5). Differences in U_O between the wild type and the *hp-1* mutant are not significant. The increase in U_O with increasing light intensity may be caused by light activation of Rubisco and by elevated rates of ribulose-bisphosphate oxygenase and glycolate oxidase (Lawlor, 1995; Badger *et al*., 2000). Oxygenase activity would also be stimulated by a lower intercellular CO_2 concentration. In tomato leaves, the intercellular CO_2 concentration (C_i) declines with rising light intensities (Haupt-Herting and Fock, 2000). Alternatively, photosynthetic electrons may be diverted directly to oxygen in the Mehler reaction.

In the two tomato genotypes tested, U_O declined with increasing water stress (Fig. 5). At high light intensity U_O was reduced from 13·8 μmol O_2 m^{-2} s^{-1} in the well-watered *hp-1* mutant to 7·5 μmol O_2 m^{-2} s^{-1} in the stressed plants, and from 12·3 to 5·6 μmol O_2 m^{-2} s^{-1} in the wild type. Although E_O and U_O declined with increasing water stress (Figs 4 and 5), the ratio of U_O/E_O rose. This result, which is in agreement with reports from several laboratories [for a review, see Haupt-Herting and Fock (2000)], means that a greater proportion of photosynthetic electrons flows to oxygen rather than to CO_2 in stressed tomato plants as compared with the controls.

Gross CO_2 uptake, net CO_2 uptake and gross CO_2 evolution in the light

The rates of gross CO_2 uptake, net CO_2 uptake and gross CO_2 evolution were determined at photosynthetic steady state on well-watered and stressed leaves of the two tomato genotypes by feeding leaves with $^{13}CO_2$. While data on wild-type tomatoes have been published elsewhere (Haupt-Herting *et al*., 2001), results for the *hp-1* mutant are presented for the first time and discussed below.

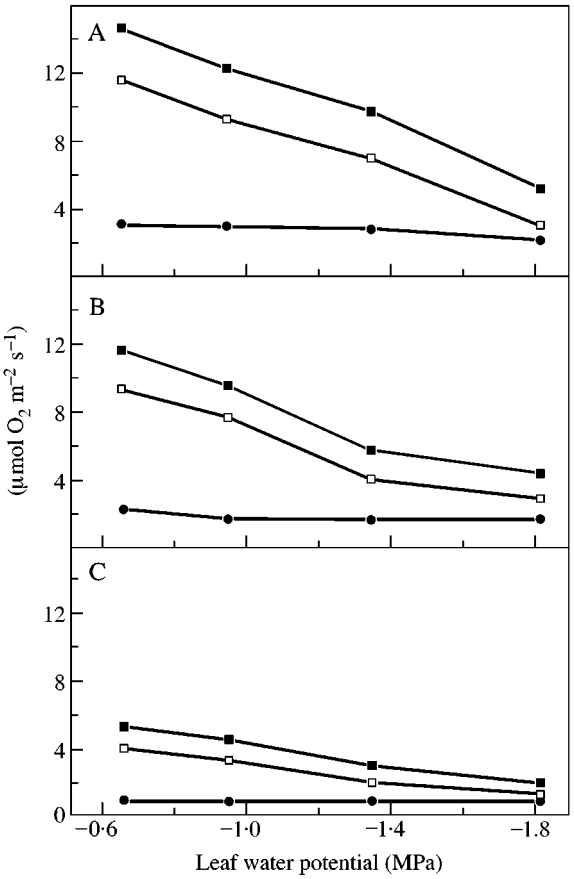

FIG. 6. Rates of steady state net CO_2 uptake (P_N, open squares), gross CO_2 uptake (TPS, closed squares) and gross CO_2 evolution (E_C, circles) of attached leaves of tomato *hp-1* mutant during steady state photosynthesis at three different light intensities [850 (A), 400 (B) and 90 (C) μmol photons m^{-2} s^{-1}] in relation to leaf water potential. Measurements were made in an open gas exchange system using a $^{12}CO_2/^{13}CO_2$ isotope technique. Leaves (23 cm^2) were supplied with air (gas flow 50 l h^{-1}) containing 210 ml l^{-1} O_2 and 350 μl l^{-1} $^{12}CO_2$ or $^{13}CO_2$, respectively, at 70 % relative humidity and 23 °C. Points are means of six replicates; s.e. ⩽ 10 %.

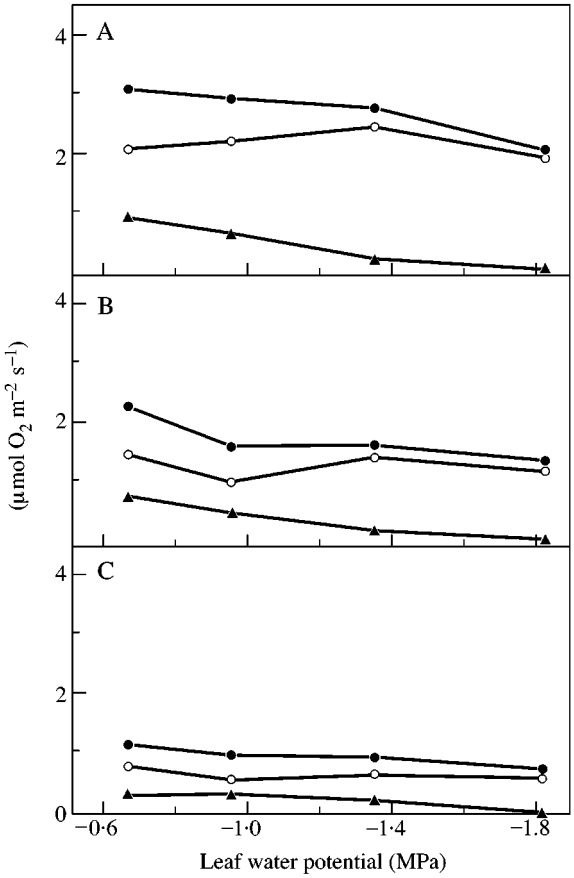

FIG. 7. Rates of steady state gross CO_2 evolution (E_C, closed circles), photorespiration (PR, open circles) and mitochondrial respiration in the light (R_C, triangles) of attached leaves of tomato *hp-1* mutant during steady state photosynthesis at three different light intensities [850 (A), 400 (B) and 90 (C) μmol photons m^{-2} s^{-1}] in relation to leaf water potential. Measurements were made in an open gas exchange system using a $^{12}CO_2$ /$^{13}CO_2$ isotope technique. Leaves (23 cm^2) were supplied with air (gas flow 50 l h^{-1}) containing 210 ml l^{-1} O_2 at 70 % relative humidity and 23 °C. The CO_2 concentration was 350 μl l^{-1} $^{12}CO_2$ or $^{13}CO_2$, respectively, for determination of E_C, and 3000 μl l^{-1} $^{12}CO_2$ or $^{13}CO_2$, respectively, for measurement of R_C. PR is calculated from E_C and U_R. Points are means of six replicates; s.e. ⩽ 10 %.

With increasing light intensity, TPS in well-watered controls rose from 5·3 μmol CO_2 m^{-2} s^{-1} at low light to 14·7 μmol CO_2 m^{-2} s^{-1} at light saturation, while P_N changed from 4·0 to 11·9 μmol CO_2 m^{-2} s^{-1} (Fig. 6). In saturating light, P_N of mutant controls was 32 % higher than that of wild-type plants. Enhanced CO_2 uptake in the mutant could be caused by higher activities of Rubisco (Haupt-Herting, 2000) and increased electron transport rates (Fig. 4). While differences between the two genotypes were clearly seen in P_N, they were less obvious in gross CO_2 assimilation: TPS of the mutant was only 15 % higher than TPS of the wild type. This indicates that the ratio of CO_2 evolution (e.g. from photorespiration) to CO_2 assimilation in the mutant is smaller than that in the wild type. Lowering the leaf water potential from –0·6 MPa in the controls to –1·8 MPa in severely stressed plants led to a significant decrease in leaf conductance (not shown) and internal CO_2 (not shown) as well as in TPS and P_N. At saturating light and during severe stress, TPS and P_N were reduced by approx. 70 and 80 %, respectively; TPS and P_N of severely stressed leaves were only slightly stimulated by high light intensity. Consequently, CO_2 fixation under water stress is not limited by light but may be impaired by CO_2 deficiency because of stomatal closure and/or by inhibition of ATP synthase and photosystem II efficiency or Rubisco activity (Lawlor, 1995; Haupt-Herting, 2000).

CO_2 evolution in the light, which is the sum of CO_2 evolved by photorespiration and CO_2 released by mitochondrial respiration in the light, was enhanced by rising light intensities from 1·3 μmol CO_2 m^{-2} s^{-1} at low light to 3·4 μmol CO_2 m^{-2} s^{-1} at saturating light in well-watered controls (–0·6 MPa) (Fig. 6). Under conditions of severe stress (–1·8 MPa) and light saturation, E_C was reduced from 3·4 to 2·0 μmol CO_2 m^{-2} s^{-1}.

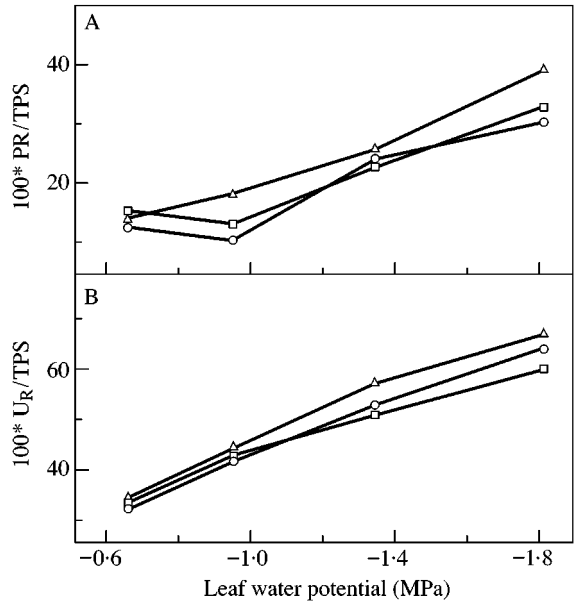

FIG. 8. Ratio of PR/TPS (A) and U_R/TPS (B) of attached leaves of tomato *hp-1* mutant during steady state photosynthesis in relation to leaf water potential at 90 (squares), 400 (circles) and 850 (triangles) µmol photons m^{-2} s^{-1}. Measurements were made in an open gas exchange system using a $^{12}CO_2/^{13}CO_2$ isotope technique. Values are from Figs 6 and 7.

Mitochondrial respiration in the light, photorespiration and reassimilation of (photo)respiratory CO_2

Gross CO_2 evolution in the light (Fig. 6) can be separated into photorespiration and mitochondrial respiration in the light. As PR was inhibited at 3000 µl l^{-1} CO_2, R_C was measured as $^{12}CO_2$ released at 3000 µl l^{-1} ambient $^{13}CO_2$ around an attached leaf (Fig. 7) (Haupt-Herting *et al*., 2001).

Assuming that R_C is not affected by CO_2 between 350 and 3500 µl l^{-1} CO_2, measured rates of mitochondrial respiration in the light at 3000 µl l^{-1} CO_2, estimated RC, and estimated PR at 350 µl l^{-1} CO_2 are shown in Fig. 7. R_C was correlated with light intensity (0·3 µmol CO_2 m^{-2} s^{-1} at low and 0·9 µmol CO_2 m^{-1} s^{-1} at saturating light in the controls) and fell within the range of dark respiratory rates (0·8 µmol CO_2 m^{-2} s^{-1}). Mitochondrial respiration in the light (Fig. 7) as well as in the dark (data not shown) was affected by water deficit; at saturating light R_C responded to leaf water potential by decreasing from 0·9 µmol CO_2 m^{-2} s^{-1} in the controls to 0·1 µmol CO_2 m^{-2} s^{-1} under severe stress (Fig. 7A). The question of whether R_C at 3000 µl l^{-1} CO_2 is similar to R_C at 350 µl l^{-1} CO_2 has been discussed thoroughly elsewhere (Haupt-Herting and Fock, 2001). From this investigation it appears that R_C at 350 µl l^{-1} CO_2 is much smaller than PR (Fig. 7).

The rate of photorespiration represents the major part of gross CO_2 evolution in the light. PR was affected by light intensity (0·8 µmol CO_2 m^{-2} s^{-1} under low light and 2·1 µmol CO_2 m^{-2} s^{-1} under light saturation in well-watered controls) but not by water deficit (2·1 µmol CO_2 m^{-2} s^{-1} at –0·6 MPa and 2·0 µmol CO_2 m^{-2} s^{-1} at –1·8 MPa leaf water potential and light saturation). In saturating light PR was slightly lower in the *hp1* mutant (2.1 µmol CO_2 m^{-2} s^{-1}) than in the wild type (2.8 µmol CO_2 m^{-2} s^{-1}) and the ratio of PR to TPS was markedly smaller in the mutant (Fig. 8) than in the wild type (Haupt-Herting *et al*., 2001). Whether this is caused by differences in CO_2 and O_2 concentrations around Rubisco or altered Rubisco characteristics is unclear and should be investigated further.

It is interesting that PR does not increase with water deficit as it does when the ambient CO_2 concentration is decreased (Fig. 3). This suggests that the inhibition of photosynthesis observed during water deficit can only partly be explained by stomatal limitations. Both non-stomatal inhibition of P_N and the missing increase of PR could be caused by inhibition of Rubisco activity (Lawlor, 1995; Haupt-Herting, 2000) or a deficiency of ribulose-1,5-bisphosphate (Gimenez *et al*., 1992; Gunasekera and Berkowitz, 1993; Tezara *et al*., 1999), and, therefore, reduced flux through the glycolate pathway. As PR is less reduced by water deficit than TPS, the ratio of PR to TPS rises with decreasing leaf water potential (Fig. 8A). This was observed under all light intensities studied and indicates that the flux of carbon through the glycolate pathway is stimulated under water deficit relative to the flux of carbon in the Calvin cycle.

The CO_2 released by the glycolate pathway and by mitochondrial respiration in the light (Fig. 2) is mixed with CO_2 from the atmosphere in the intercellular space. Before leaving the leaf, E_C is partly reassimilated (Fig. 2). Reassimilation of (photo)respiratory $^{12}CO_2$ in relation to gross CO_2 assimilation (TPS = U_{13C} + U_R) is slightly increased by light intensity and strongly enhanced by water deficit (Fig. 8B). Reassimilation of (photo)respiratory CO_2 consumes additional ATP and reducing equivalents. Therefore, increased rates of reassimilation under water stress can be interpreted as contributing to dissipation of excess electrons (Fock *et al*., 1992). In addition, reassimilation maintains carbon flux and enzymatic substrate turnover, which enables the plant to recover rapidly after rewatering (Stuhlfauth *et al*., 1990).

Heterogeneity of leaf photosynthesis would influence the calculations of reassimilation, but in tomato leaves no patchiness occurs under water stress (Haupt-Herting, 2000).

Oxygen consumption in the Mehler reaction

The rate of net oxygen consumption in the Mehler reaction can be calculated by subtracting the rates of oxygen uptake in photorespiration (rate of photorespiratory CO_2 evolution in Fig. 7 multiplied by three) and mitochondrial respiration in the light (R_C value in Fig. 7, assuming that the rate of CO_2 evolution equals the rate of O_2 uptake; for discussion of possible deviations from this assumption, see Haupt-Herting *et al*., 2001) from gross O_2 uptake (Fig. 5), according to Biehler and Fock (1996). These rates must be corrected for oxygen formation by superoxide dismutase in the Halliwell–Asada cycle (Asada, 1999) to obtain the rate of photoreduction of O_2 (MR). In our experiments, the rate of photoreduction of O_2 rises with increasing light intensity from 1·4 µmol O_2 m^{-2} s^{-1} at low light to 7·3 µmol O_2 m^{-2} s^{-1} at high light in wild-type control plants (data not shown).

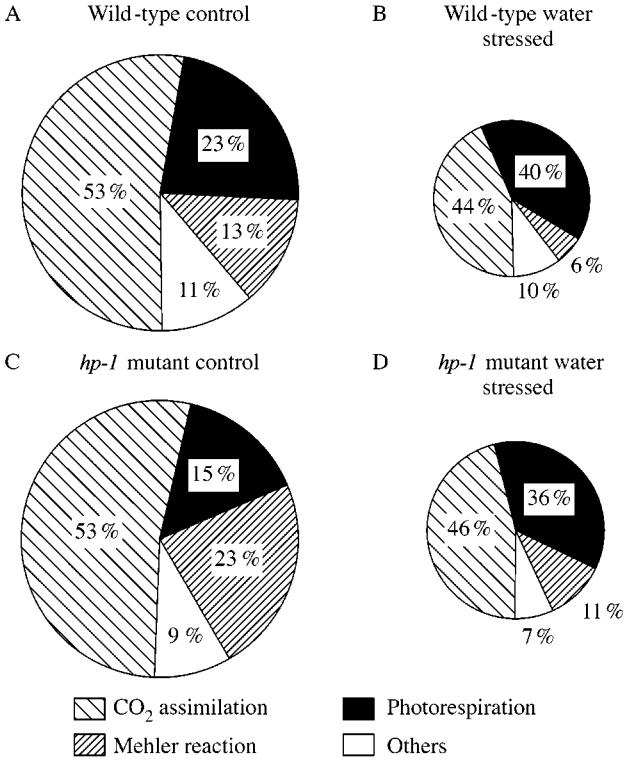

FIG. 9. Photosynthetic electrons (%) consumed/dissipated by CO_2 assimilation (TPS), photorespiration (PR), the Mehler reaction and other reactions at 850 µmol photons m^{-2} s^{-1} in controls (–0·6 MPa) and water-stressed (–1·8 MPa) wild type tomato (A, B) and the *hp-1* mutant (C, D). The area of the circles represents 100 % of photosynthetic electrons formed; these are 97 (A) and 32 (B) µmol e^- m^{-1} s^{-1} in wild-type leaves, and 112 (C) and 45 (D) µmol e^- m^{-1} s^{-1} in leaves of the *hp-1* mutant (see Fig. 4). The fractions of electrons (%) that are consumed/dissipated by different reactions are derived from Fig. 6 and from Haupt-Herting *et al.* (2001) for CO_2 assimilation (TPS), and from Figs 5 and 7 for photorespiration (PR) and the Mehler reaction (MR). Electron consumption that cannot be allocated to specific reactions is termed 'others'.

Differences between the wild type and the *hp-1* mutant are significant only in control plants under high light (13·0 µmol O_2 m^{-2} s^{-1} for *hp-1* mutant at 850 µmol photons m^{-2} s^{-1}). These faster rates of the Mehler reaction accompany a nearly doubled activity of O_2 scavenging enzymes such as superoxide dismutase, monodehydroascorbate reductase and dehydroascorbate reductase in the mutant compared with the wild type (Haupt-Herting, 2000).

With increasing water deficit, MR declines in both plant types, especially under saturating light intensities (1·8 µmol O_2 m^{-2} s^{-1} at severe stress). The rate of photoreduction might depend mainly on the electron transport rate so that MR follows the course of photosystem II activity represented by E_O (Fig. 4) in relation to light intensity and water deficit.

Balance between formation and consumption/dissipation of photosynthetic electrons

To analyse the contribution of photosynthetic electrons to the processes that consume/dissipate reducing power, electron formation by PSII on the one hand and electron consumption/dissipation by the Calvin cycle, the glycolate pathway and the Mehler reaction on the other are considered. The rate of photosynthetically generated electrons is derived from Fig. 4 (electrons formed = $E_O \times 4$). At saturating light approx. $28 \times 4 = 112$ µmol electrons m^{-2} s^{-1} are transported through the photosystems in control leaves of the *hp-1* mutant (= 100 % in Fig. 9C). In these calculations refixation of photosynthetic oxygen (Biehler and Fock, 1996), which may increase total flux of electrons to some extent, has been neglected.

We then discuss the substrates and pathways that consume/dissipate photosynthetic electrons. The main electron consumer in photosynthesis is CO_2 (in TPS). The stochiometry of electron consumption to CO_2 assimilation is still controversial in the current literature; we use a stochiometry of four electrons consumed per CO_2 fixed as an approximation. Higher ratios of electron consumption to gross CO_2 uptake, as found by Flexas *et al.* (1999), would result in a higher percentage of electrons used up in CO_2 fixation and a smaller percentage left for so-called 'other' reactions (see below). The amounts of electrons consumed in photorespiration or in the Mehler reaction are calculated from O_2 measurements and are not influenced by the electron-per-CO_2 ratio used. At light saturation, 14·7 µmol CO_2 m^{-2} s^{-1} were fixed in the controls of the *hp-1* mutant (Fig. 6, TPS); this is equivalent to $14{\cdot}7 \times 4 = 58{\cdot}8$ µmol electrons m^{-2} s^{-1} or 53 % of photosynthetic electrons being consumed by CO_2 assimilation in the Calvin cycle (Fig. 9C). Photorespiration equalled 2·1 µmol CO_2 m^{-2} s^{-1} in the controls. Consequently, $2{\cdot}1 \times 8 = 16{\cdot}8$ µmol electrons m^{-2} s^{-1} or 15 % of photosynthetic electrons are dissipated in the photorespiratory pathway (including ammonium reassimilation).

In the Mehler reaction two O_2 molecules are reduced by two photosynthetic electrons to form two superoxide radical anions, which are converted into $O_2 + H_2O_2$ by superoxide dismutase. Scavenging of H_2O_2 in the Halliwell–Asada cycle leads to the formation of two molecules of monodehydroascorbate that are reduced to ascorbate by monodehydroascorbate reductase under consumption of two electrons (Asada, 1999). Therefore, a stochiometry of two electrons per O_2 reduced can be assumed. In the case that monodehydroascorbate reductase uses NADH as an electron donor instead of NADPH, the consumption of photosynthetic electrons would be smaller. The rate of electron consumption resulting from photoreduction of O_2 is calculated to be $13{\cdot}0 \times 2 = 26$ µmol electrons m^{-2} s^{-1} or 23 % of photosynthetic electrons. The remaining 9 % of the photosynthetic electrons which cannot be allocated to CO_2 assimilation, photorespiration or the Mehler reaction are summarized as 'others' (Fig. 9C).

Under conditions of severe stress and light saturation, the distribution of photosynthetic electrons is as follows: in leaves of the *hp-1* mutant $11{\cdot}2 \times 4 = 44{\cdot}8$ µmol electrons m^{-2} s^{-1} are generated by photosystem II (= 100 % in Fig. 9D). Of these, 46 % support CO_2 assimilation, 36 % are used in photorespiration and 11 % are dissipated by the Mehler reaction (Fig. 9D).

The allocation of photosynthetic electrons to primary metabolism in leaves of wild-type tomato is shown in

Fig. 9A and B. The values for this presentation are derived from Figs 4 and 5 and from Haupt-Herting *et al*. (2001).

It follows from Fig. 9 that more than half of the photosynthetic electrons are consumed by CO_2 assimilation (TPS; including reassimilation) in control leaves of the wild type as well as the *hp-1* mutant. A minor proportion of the photosynthetic electrons is used in photorespiration. A small fraction of electrons is consumed in other reactions and the rest are transported to O_2 in the Mehler reaction. Electron consumption in 'other' reactions might include nitrate and sulfate assimilation, synthesis of fatty acids and reduction of glutathione and ascorbate.

Under conditions of severe stress the flux of photosynthetic electrons is strongly reduced in both genotypes ($8{\cdot}1 \times 4 = 32{\cdot}4$ μmol electrons m^{-2} s^{-1} in the wild type and $11{\cdot}2 \times 4 = 44{\cdot}8$ μmol electrons m^{-2} s^{-1} in the *hp-1* mutant). Because of water stress limitations, the proportion of electrons that is required for CO_2 assimilation declines with increasing water deficit. This fraction would be even smaller if reassimilation of (photo)respiratory CO_2 was not enhanced in stressed plants. Consequently, the flux of electrons to the photorespiratory pathway (40 % in stressed wild-type leaves and 36 % in stressed leaves of the *hp-1* mutant; Fig. 9B and D) is increased in relation to TPS. The proportion of electrons dissipated in the Mehler reaction declines under stress in both plant types while the fraction used up in other reactions remains unchanged.

The activity of the Mehler reaction in tomato leaves is higher than expected from earlier publications (Badger *et al*., 2000). Experiments involving transgenic tobacco with reduced Rubisco capacity show no extra electron transport to alternative acceptors such as the Mehler reaction (Ruuska *et al*., 2000) and various studies on electron transport and O_2 uptake under different stresses and CO_2 conditions indicate that the Mehler reaction is only a minor component of O_2 uptake compared with photorespiration (Badger *et al*., 2000). On the other hand, the percentages of electrons consumed in the Mehler reaction in tomato leaves shown here are in the same order of magnitude as those published for water-stressed *Triticum aestivum* by Biehler and Fock (1996). In our study, MR values are calculated from given O_2 consumption by mitochondrial respiration in the light (measured as CO_2 evolution). If respiratory O_2 consumption does not equal CO_2 release, e.g. because of significant malate-valve activity, MR could be slightly overestimated. Another explanation for the unexpectedly high rates of the Mehler reaction could lie in the growth conditions that were similar for *Triticum* (Biehler and Fock, 1996) and tomato but which differed from those of tobacco (higher light intensities, fluctuations of temperature and relative humidity) in Ruuska *et al*. (2000). The values for non-photochemical quenching in severely stressed tomato plants presented in Haupt-Herting and Fock (2000) indicate a low capacity for thermal dissipation. It is possible that our plants have to dissipate more energy through PR and MR than other plants, having developed a higher capacity for thermal dissipation because of more severe growth conditions.

However, our experiments show that in water-stressed tomato leaves the photorespiratory pathway plays the major role in the use of photosynthetic electrons that are not consumed by CO_2 assimilation. In the mutant, in particular, which, under control conditions dissipates only a minor proportion of electrons in photorespiration compared with the Mehler reaction, photorespiration becomes more important than the Mehler reaction during water deficit. This is in accordance with earlier postulations that photorespiration is essential and more effective than the Mehler reaction in protecting plants against photodamage under excessive light intensities or water deficit (Wu *et al*., 1991; Heber *et al*., 1996; Badger *et al*., 2000). On the other hand, our results partly contradict the findings of Biehler and Fock (1996) who reported that oxygen in the Mehler reaction was an important sink for reducing power in stressed wheat leaves when photosynthetic electrons were in excess of demand.

CONCLUSIONS

It is concluded that photosystem II, the Calvin cycle and mitochondrial respiration are down-regulated under water stress in both wild-type tomato and the *hp1*-mutant. In stressed leaves, photodamage may be avoided by dissipating excess photosynthetic electrons, especially in the photosynthetic oxidation cycle rather than in the Mehler reaction.

The reasons for differences in the rates of photorespiration and the Mehler reaction between control plants of the wild type and *hp-1* mutant, especially in terms of Rubisco characteristics and O_2 scavenging enzymes, should be investigated further.

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