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Evidence for the Contribution of the Mehler-Peroxidase Reaction in Dissipating Excess Electrons in Drought-Stressed Wheat[1]

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Gross O_2 evolution and uptake by attached, drought-stressed leaves of wheat (*Triticum aestivum*) were measured using a $^{16}O_2/^{18}O_2$ isotope technique and mass spectrometry. The activity of photosystem II, determined from the rate of $^{16}O_2$ evolution, is only slightly affected under drought conditions. During drought stress, net CO_2 uptake decreases due to stomatal closure, whereas the uptake of $^{18}O_2$ is stimulated. The main O_2-consuming reactions in the light are the Mehler-peroxidase (MP) reaction and the photorespiratory pathway. From measurements of the rate of carbon flux through the photorespiratory pathway, estimated by the analysis of the specific radioactivities of glycolate, we conclude that the rate of photorespiration is decreased with drought stress. Therefore, the O_2 taken up in the light appears to be preferentially used by the MP reaction. In stressed leaves, 29.1% of the photosynthetic electrons are consumed in the MP reaction and 18.4% drive the photorespiratory pathway. Thus, overreduction of the electron transport chain is avoided preferably by the MP reaction when drought stress restricts CO_2 reduction.

Drought stress restricts net CO_2 assimilation in wheat (*Triticum aestivum*), a C_3 plant. If leaves absorb the same amount of light, the rate of photosynthetically generated energy equivalents is in excess of what is required by the dark reactions of photosynthesis. In this way water deficit can lead to an overreduction of the photosynthetic electron transport chain, causing detrimental effects such as photoinhibition and photooxidation (Björkmann and Powles, 1984). The plant prevents this by decreasing photochemical PSII activity, thus decreasing the rate of electron transport. This down-regulation of PSII activity is accomplished by a reversible decrease in PSII efficiency as a result of increased conversion of absorbed light into heat. This thermal dissipation process occurs within the antennae (Demmig et al., 1988) or in the reaction center (Weis and Berry, 1987) of PSII. Aside from these nonphotochemical dissipation processes, another way the plant prevents overreduction is by draining off excess electrons by increasing consumption in pathways other than the Calvin cycle.

Several lines of evidence suggest that water deficit induces the allocation of photosynthetic electrons to O_2 (Smirnoff, 1993). The use of $^{18}O_2$ and MS allows the determination of Uo by intact leaves in the light. Canvin et al. (1980) showed that Uo is stimulated by a decreased CO_2 concentration surrounding the leaf, suggesting that O_2 can partially replace CO_2 as an electron acceptor. Renou et al. (1990) and Tourneux and Peltier (1995) showed that water deficit stimulates Uo in *T. aestivum* and *Solanum tuberosum*, respectively. Further evidence derived from fluorescense analysis suggests that an increased number of electrons are transferred to O_2, especially under conditions of drought stress (Stuhlfauth et al., 1988; Cornic and Briantais, 1991; Krall and Edwards, 1992).

O_2 can be consumed by two pathways associated with photosynthesis: photorespiration and the MP reaction. Photorespiration is initiated by the reaction of O_2 and RuBP, yielding one molecule each of phosphoglycerate and phosphoglycolate. The latter is metabolized by the glycolate pathway, forming phosphoglycerate and releasing CO_2. Under drought stress the rate of photorespiration increases relative to the rate of net photosynthesis, but not in absolute terms (Lawlor and Fock, 1975). Nevertheless, working with *Digitalis lanata*, Stuhlfauth et al. (1990) showed the importance of photorespiration and the reassimilation of the resultant CO_2 for energy dissipation. In the MP reaction O_2 can be reduced directly by components of the electron-transport chain yielding superoxide (O_2^-) (Mehler, 1951), which is then transformed to H_2O_2. Since chloroplasts lack catalase, H_2O_2 is scavenged by a peroxidase. This reaction sequence of generation and breakdown of H_2O_2 is called the MP reaction (Schreiber and Neubauer, 1990). In drought-stressed leaves the MP reaction is believed to consume surplus electrons and, therefore, prevents the accumulation of light-generated reductant. Another, more indirect function of the MP reaction under drought stress was suggested by Schreiber and co-workers (Schreiber and Neubauer, 1990; Neubauer and Yamamoto, 1992): the consumption of electrons maintains noncyclic electron transport under conditions of diminished CO_2 reduction, and this noncyclic electron transport establishes the decrease in pH in the thylakoid lumen, which in turn decreases the efficiency of PSII.

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Abbreviations: Eo, rate of gross O_2 evolution; MP, Mehler-peroxidase; Q_A, primary quinone acceptor of PSII; Q_N, coefficient of nonphotochemical quenching; Q_P, coefficient of photochemical quenching; P_N, rate of net CO_2 assimilation; RO_2, rate of refixed O_2; RuBP, ribulose-1,5-bisphosphate; Uo, rate of gross O_2 uptake.

Using isolated chloroplasts and fluorescence analysis, Wu at al. (1991) concluded that the MP reaction is less efficient in energy dissipation than is photorespiration. We have extended these studies by improving the methods. Since the MP reaction cannot be measured directly, we determined the rate of O_2 consumption in illuminated wheat leaves using $^{18}O_2$ and MS (Fig. 1). The resulting value comprises the MP reaction and photorespiration, so we can subtract the O_2 consumed by photorespiration (which was measured by flux studies using $^{14}CO_2$ and glycolate analysis) to obtain a value for O_2 consumed by the MP reaction. Taking Eo as a direct measure of noncyclic electron transport, we determined the allocation of photosynthetic electrons to CO_2 reduction, photorespiration, and the MP reaction in well-watered and in drought-stressed leaves. In this paper we present evidence that the MP reaction is increased in drought-stressed wheat leaves.

MATERIALS AND METHODS

Growth of Plants

Wheat (*Triticum aestivum*) seeds (Urban Winterweizen, Hofgut Stauffer, Obersülzen, Germany) were surface-sterilized in 0.5% hypochlorite and germinated on sterilized sand. After 1 week the plants were transferred into 2.5-L pots filled with sand and garden soil (10:90%). Plants were grown in normal air in a phytotron (BBC-YORK, Mannheim, Germany) at a light intensity of 200 μmol m^{-2} s^{-1} for 17 h at 23°C and 7 h in darkness at 19°C as described previously (Biehler and Fock, 1995). RH was held constant at 70%. Plants were watered daily with tap water and fertilized with a commercial nutrient solution. The youngest fully expanded leaves of 5-week-old plants were used for the experiments. Measurements were carried out at a light intensity of 90 or 850 μmol photons m^{-2} s^{-1} from a halogen lamp (Xenophot 12 V, Osram, Munich, Germany). Light intensity was altered by wire screens. Stress was induced by withholding watering and resulted in slow dehydration, which resembles natural drying conditions. After a period of about 14 d, a leaf water potential of −2.6 MPa (as measured with a laboratory-built pressure chamber) was obtained.

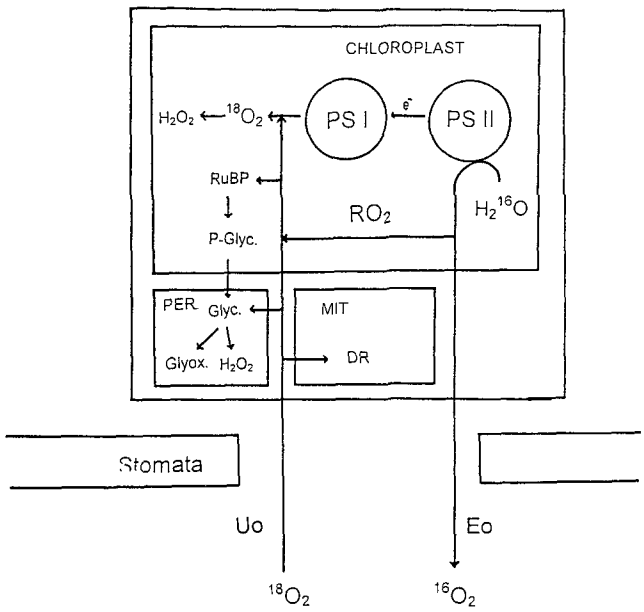

Figure 1. Scheme of O_2 fluxes in a photosynthesizing leaf. Per, Peroxisome; Mit, mitochondrium; DR, dark respiration; Glyox, glyoxylate.

Measurement of $^{16}O_2/^{18}O_2$ Gas Exchange

All experiments for measuring $^{16}O_2$ evolution and $^{18}O_2$ uptake by illuminated attached wheat leaves were carried out with a closed gas-exchange system (volume 54 ± 1 mL) as described previously (Biehler and Fock, 1995). The leaf was enclosed in a thermostated aluminum cuvette. Air (21% $^{18}O_2$, 10% Ar [as an indicator for gas losses through leaks], 69% N_2, and 0.035% CO_2) was circulated over the leaf by a gas tight pump (MB-21 E, Metal Bellows, Sharon, MA). Humidity was held constant at 70% during the measurements with a temperature-controlled condensor trap. The concentration of four gases, $^{18}O_2$, $^{16}O_2$, Ar, and CO_2, was continuously measured with a quadrupole mass spectrometer (3200 E, Finnigan, Bremen, Germany). In the light the CO_2 concentration of the system was kept constant by injecting CO_2 with a gas-tight, motor-driven 2.5-mL syringe. $^{18}O_2$ uptake and $^{16}O_2$ evolution were calculated using the method of Radmer and Ollinger (1980).

Net CO_2 Exchange and Transpiration

Net CO_2 uptake and transpiration of attached leaves were measured by connecting the leaf chamber to an open, IR gas analyzer-type gas-exchange system, as described by Stuhlfauth et al. (1988). A gas flow of 70 L h^{-1} air with 350 μL L^{-1} CO_2 was provided by three mass controllers for CO_2, N_2, and O_2. CO_2 uptake was measured by an IR gas analyzer (UNOR, Maihak, Hamburg, Germany) and H_2O by a humidity sensor (Vaisala HMP 125, Driesen and Kern, Tangstedt, Germany) during steady-state photosynthesis (after 30–45 min of illumination at 23°C). Dark respiratory CO_2 evolution was determined 30 min after illumination at 23°C.

Fluorescence Measurements

Fluorescence was measured with a pulse-amplitude modulation fluorometer (Walz, Effeltrich, Germany). Saturation light pulses (4600 μmol m^{-2} s^{-1}; 700 ms; KL 1500, Schott, Cologne, Germany) and measuring light were transferred fiberoptically to the upper side of the leaf at an angle of 45°. The fluorescence quenching parameters Q_N and Q_P were calculated as described by Schreiber et al. (1986) using the nomenclature of van Kooten and Snel (1990).

Glycolate Analysis

At steady-state photosynthesis, four leaf pieces, corresponding to 20 cm^2, were punched out of four attached leaves and quickly frozen in liquid nitrogen after 1 and 2

min of $^{14}CO_2$ assimilation in a special cuvette for rapid fixation (Bourquin and Fock, 1983). The leaf pieces were homogenized with a pestle in a mortar using sand and α-hydroxy-2-pyridine-methanesulfonic acid, an inhibitor of glycolate oxidase. The extraction of glycolate was carried out at −20°C with 50 mL of grinding medium (10 mM NaF and 90% HCOOH) as described by Krampitz and Fock (1984). Glyoxylate and other substances with oxofunctions were transformed to their corresponding phenylhydrazones using the method of Baker and Tolbert (1966). Combined ion-exchange chromatography on Dowex H^+ columns (Serva, Heidelburg, Germany) and Dowex formate resulted in fractions containing glycolate. For the analysis of glycolate, a phenacylester was formed and analyzed by HPLC at A_{254} (Durst et al., 1975) using a reverse-phase column (LiChrospher 100 RP-18, 5 μm, Merck, Darmstadt, Germany).

The carbon flux (*Fc*) through the photorespiratory pathway is estimated from the measurement of the inflow of ^{14}C and the specific radioactivity of glycolate (G^*/G) at steady-state photosynthesis. During the first 2 min of linear $^{14}CO_2$ uptake, as long as the outflow of ^{14}C from glycolate is negligible, the following equation can be applied (Reiner, 1953):

$$Fc(\mu\text{mol C m}^{-2}\text{ s}^{-1}) = \frac{\Delta G^*/\Delta t}{PG^*/PG} \quad (1)$$

where ΔG^* represents the incorporation of ^{14}C (Bq) during Δt and PG^*/PG is the specific radioactivity (Bq/μmol C) of P-glycolate (*PG*). Because of the small pool size of *PG*, the PG^*/PG ratio could not be determined in wheat leaves. Therefore, the average specific radioactivity of glycolate (G^*/G) during the first 2 min of $^{14}CO_2$ uptake was used as an approximation. Equation 1, then, is changed to:

$$Fc(\mu\text{mol C m}^{-2}\text{ s}^{-1}) = \frac{\Delta G^*/\Delta t}{G^*/G} \quad (2)$$

The validity of Equation 2 as an approximation of *Fc* was directly and indirectly confirmed by Krampitz and Fock (1984), Gerbaud and André (1987), Stuhlfauth (1989), and Stuhlfauth et al. (1990). From four carbon atoms traversing the photorespiratory pathway, one carbon atom is released as photorespiratory CO_2.

Electron Consumption by the Calvin Cycle and by Photorespiration

Four electrons are consumed for every CO_2 molecule reduced and four are consumed for every glycolate molecule metabolized (Stuhlfauth et al., 1990).

Calculation of Photosynthetic Electrons Consumed in the MP Reaction

The rate of photosynthetically generated electrons is derived from the rate of O_2 evolution (4 × Eo; see Fig. 3) measured by MS plus the rate of refixed O_2 (4 × RO_2; see Fig. 1). The difference between O_2 demand by photorespiration and Uo in well-watered controls is defined as the RO_2 (Fig. 1).

If the reduction of O_2 and the scavenging of H_2O_2 by the peroxidase system requires four electrons (Smirnoff, 1993), then the difference between the number of electrons generated by PSII (4 × Eo + 4 × RO_2) and the number consumed by CO_2 assimilation (see Fig. 6, control A) and by photorespiration (see Fig. 6, control B) comprises those electrons available for the MP reaction and other processes (see Fig. 6, control C + D). Since RO_2 has been defined by the O_2 metabolism of the controls, the fractions C and D cannot be separated in the controls.

Although in vivo RO_2 is higher in stressed than in control leaves, for calculation purposes the rates of RO_2 are assumed to be identical. The electrons formed by PSII activity in stressed leaves (see Fig. 6, stress A + B + C + D) are allocated to CO_2 assimilation (A) and to photorespiration (B) by the same method as in the controls. For the separation of electrons consumed in the MP reaction (see Fig. 6, stress C) and in other processes (see Fig. 6, stress D), the portion of electrons consumed in the MP reaction is calculated from O_2 metabolism (see Fig. 1). First, the Uo (see Fig. 5) by the stressed leaves is measured by MS. Second, for total consumption of O_2 by the leaves, RO_2 (3.0 μmol m^{-2} s^{-1}; see "O_2 Consumption by the Photorespiratory Pathway" in "Results") is added to Uo. Third, the rate of O_2 consumed by photorespiration is subtracted from the sum of Uo plus RO_2. Finally, the remaining rate of O_2 consumption is thought to be metabolized in the MP reaction. This rate of O_2 consumption is multiplied by 4 to yield the rate of electrons metabolized in the MP reaction of stressed leaves (see Fig. 6, stress C). The rate of electrons consumed by other processes (see Fig. 6, stress D) is given by:

$$D = Eo + RO_2 - A - B - C \quad (3)$$

RESULTS

Transpiration and Net CO_2 Uptake

With increasing water deficit, stomatal conductivity is restricted and the rate of transpiration decreases from 2.1 mmol H_2O m^{-2} s^{-1} in unstressed leaves to 0.4 mmol H_2O m^{-2} s^{-1} in stressed leaves (Fig. 2, left). Additionally, P_N decreases from 19.1 to 7.5 μmol CO_2 m^{-2} s^{-1} (Fig. 2, right). If light limits photosynthesis (90 μmol photons m^{-2} s^{-1}), the consequences of drought are less pronounced. Down to a leaf water potential of −2.6 MPa, the drought-stressed *T. aestivum* plants remain capable of recovering completely within 2 d after rewatering.

The Activity of PSII

PSII activity is measured by two different methods. The rate of Eo due to water splitting at PSII is used as a direct measure of PSII activity in an attached leaf (Fig. 1). In a well-watered leaf, 34.0 μmol O_2 m^{-2} s^{-1} are released at high light intensity (Fig. 3). Drought stress decreases PSII activity by 11.7%. Under low light conditions, drought

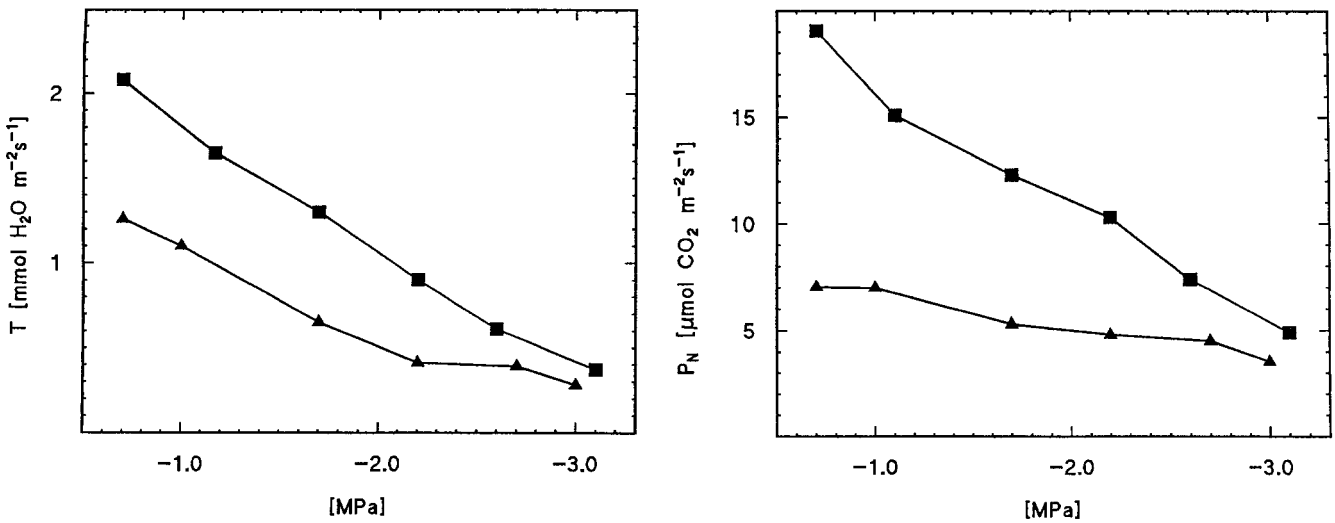

Figure 2. Transpiration (T, left) and steady-state net P_N (right) in relation to leaf water potential at 23°C and light intensities of 850 (■) and 90 (▲) μmol photons m^{-2} s^{-1}. Each point represents the mean of five individual measurements; SE < 5%.

stress does not affect Eo (Fig. 3). These data are confirmed by fluorescence analysis. With decreasing leaf water potential, Q_N shows a distinct increase of approximately 35% from 0.53 (−0.7 MPa) to 0.81 (−2.6 MPa; Fig. 4, right), which suggests that under stress conditions more absorbed light is dissipated as heat in drought-stressed plants than in the well-watered controls.

Water stress has a more pronounced influence on Q_N than on Eo. This discrepancy can be explained by the fact that fluorescence is emitted only from a thin layer of chloroplasts near the surface. The high rate of light absorption in this upper leaf layer leads to a lower efficiency of PSII. In contrast, the rate of Eo is averaged over the entire leaf, including areas with high and low light absorption and, therefore, different PSII efficiency.

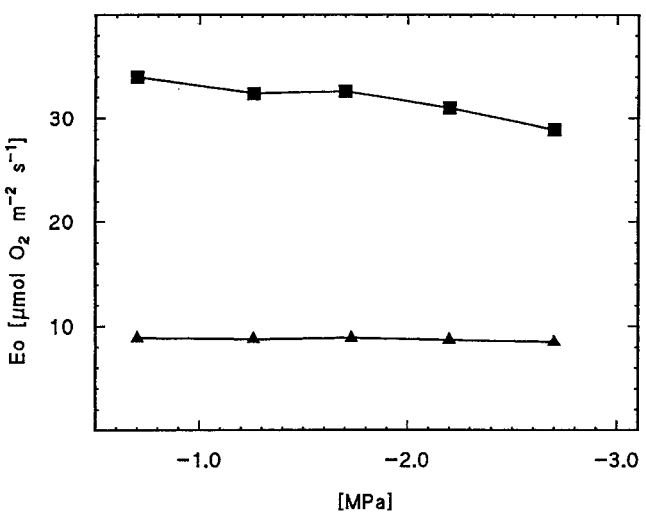

Figure 3. Steady-state Eo in relation to leaf water potential at 23°C and light intensities of 850 (■) and 90 (▲) μmol photons m^{-2} s^{-1}. Each point represents the mean of five individual measurements; SE < 10%.

The redox state of Q_A, which is reflected by the Q_P, is more affected by light intensity than by drought stress (Fig. 4, left). Since the rate of Q_A reduction, reflected by Eo (Fig. 3), is hardly affected by drought stress, the rate of Q_A oxidation by electron transport is also unaffected and does not depend on drought stress. It is evident that CO_2 reduction is reduced under stress (Fig. 2, right). Because of the high ratio of oxidized to reduced Q_A in stressed leaves, photosynthetic electrons must be consumed by acceptors other than CO_2.

It should be noted that PSII centers may occur in photochemically active and inactive populations. Inactive PSII centers may be inefficient in Q_A reoxidation, leading to an overestimation of Q_A^- and therefore to erroneously low Q_P values (Hormann et al., 1994). Furthermore, Q_P is also affected by nonphotochemical quenching processes (Havaux et al., 1991). Therefore, to characterize PSII activity in the entire leaf, we consider Eo to be a more precise indicator of electron transport rates than Q_P (Biehler and Fock, 1995).

O_2 Uptake

Figure 5 shows the response of Uo to leaf water deficit. Under low light conditions Uo is not affected by leaf water potential. At high irradiance 9.1 μmol O_2 m^{-2} s^{-1} are taken up in well-watered plants. With increasing water deficit (−2.6 MPa), Uo increases to 15.1 μmol O_2 m^{-2} s^{-1} at high illumination (Fig. 5). Similar responses of Uo in relation to irradiance and water deficit were found by Tourneux and Peltier (1995) in drought-stressed leaves of *S. tuberosum*, also using $^{18}O_2$ as a tracer. This increase in Uo suggests that O_2 can partially replace CO_2 as an electron acceptor. Under low light, drought stress has no significant effect on Uo.

O_2 Consumption by the Photorespiratory Pathway

At a leaf water potential of −0.7 MPa 8.1 μmol m^{-2} s^{-1} ± 0.5 SE glycolate are produced. Under drought stress

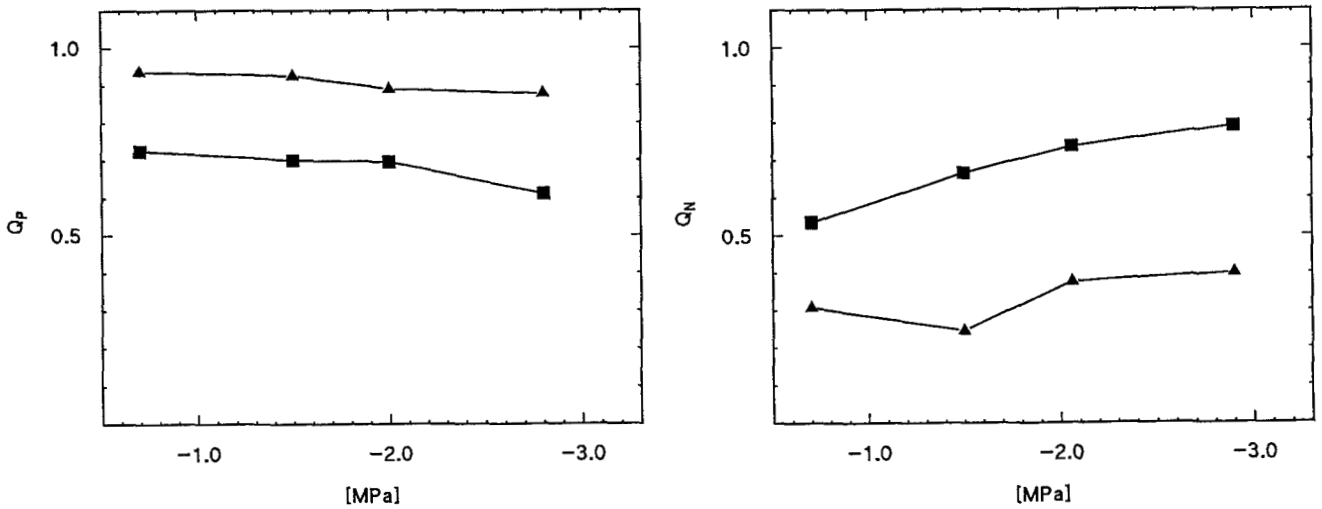

Figure 4. Q_P (left) and Q_N (right) in the steady state in relation to leaf water potential at light intensities of 850 (■) and 90 (▲) μmol photons m^{-2} s^{-1}. Each point represents the mean of five individual measurements; SE < 5%.

glycolate synthesis drops to approximately 5.9 μmol m^{-2} s^{-1} ± 0.6 SE. Taking into account that 1.5 molecules of O_2 are required for every molecule of glycolate that is synthesized by RuBP oxidation and transformation to glyoxylate by the glycolate oxidase, 12.1 μmol O_2 m^{-2} s^{-1} (8.1 × 1.5) are consumed in control leaves and approximately 8.8 μmol O_2 m^{-2} s^{-1} are consumed in stressed leaves in the photorespiratory pathway. In well-watered plants the O_2 consumption of the photorespiratory pathway exceeds the measured rate of Uo by 3.0 μmol O_2 m^{-2} s^{-1} (12.1 μmol O_2 m^{-2} s^{-1} consumed in the photorespiratory pathway minus 9.1 μmol O_2 m^{-2} s^{-1} uptake measured by MS; Fig. 5). The only source that can meet the additional demand for O_2 consumption is O_2 recycling within the leaf (RO_2; Fig. 1). Consequently, in control as well as in water-stressed leaves, the actual Eo by PSII is at least 3.0 μmol O_2 m^{-2} s^{-1} higher than the measured Eo. Because recycling of O_2 is negligible within the stomatal cavities (Gerbaud and André, 1987), it may take place in the mesophyll cells.

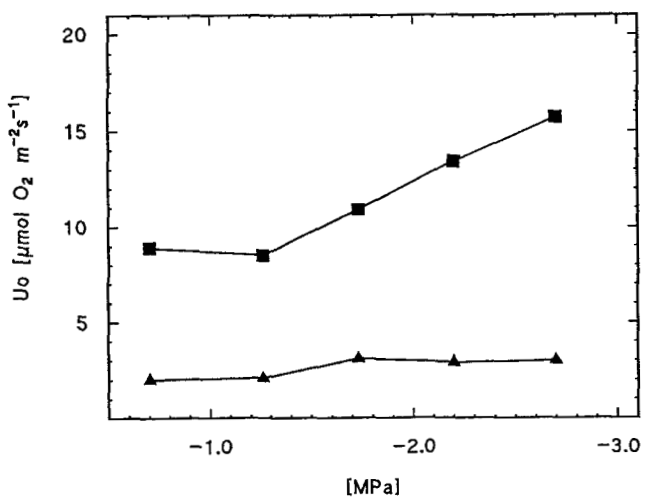

Figure 5. Steady-state Uo in relation to leaf water potential at light intensities of 850 (■) and 90 (▲) μmol photons m^{-2} s^{-1}. Each point represents the mean of five individual measurements; SE < 10%.

The Activity of the MP Reaction

From the consumption of O_2 in the photorespiratory pathway (approximately 8.8 μmol O_2 m^{-2} s^{-1}) and the total consumption of O_2 by stressed leaves (Uo from Fig. 5 plus RO_2), O_2 metabolized in the MP reaction can be determined. In stressed leaves and at high irradiance the total O_2 consumption is 18.1 μmol O_2 m^{-2} s^{-1} (15.1 by Uo [Fig. 5] plus 3.0 by RO_2). If approximately 8.8 μmol O_2 m^{-2} s^{-1} are used for photorespiration, 9.3 μmol O_2 m^{-2} s^{-1} (= 37.2 μmol electrons m^{-2} s^{-1} = 29.1%) are left to drive the MP reaction (Fig. 6, stress C).

The small rate of O_2 consumption by the MP reaction in the controls cannot be semiquantitatively determined, since the rate of recycled O_2 (RO_2 = 3.0 μmol O_2 m^{-2} s^{-1}), which is required for this estimation, has been defined by the O_2 metabolism of the controls.

DISCUSSION

In both low and high irradiance, Eo (Fig. 3) is not strongly influenced by decreasing leaf water potential. This is consistent with results from Tourneux and Peltier (1995) obtained from $^{18}O_2$ measurements on leaf discs of *S. tuberosum* and on data from fluorescence measurements suggesting that PSII activity is only slightly affected by drought stress. On the other hand, Uo strongly increases at high irradiance in relation to drought stress (Fig. 5). Eo is solely a measure of the water-splitting reaction at PSII, and thus reflects the rate of photosynthetically generated electrons. The interpretation of Uo is more complex than that of Eo because of the number of potential O_2-consuming reactions in the light: photorespiration, MP reaction, chlororespiration, and mitochondrial respiration. The rate of mitochondrial respiration determined by IR gas analysis is approximately 1.5 μmol CO_2 m^{-2} s^{-1} 30 min after illumi-

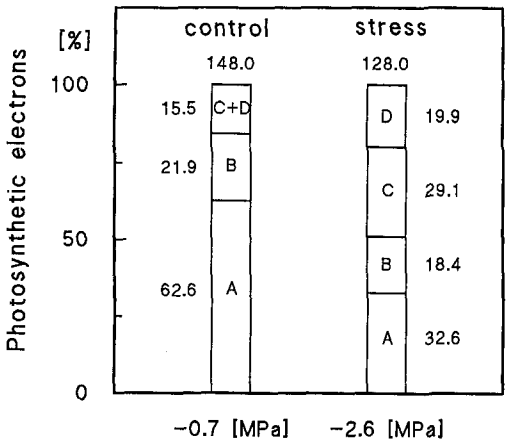

Figure 6. Percentage distribution of photosynthetic electrons in well-watered controls (−0.7 MPa, 148.0 μmol electrons m^{-2} s^{-1}) and drought-stressed (−2.6 MPa, 128.0 μmol electrons m^{-2} s^{-1}) leaves at 850 μmol photons m^{-2} s^{-1}. A, CO_2 reduction. Data are from Figure 2, right, plus photorespiratorily evolved CO_2 from the rate of glycolate metabolism. B, Photorespiration. Data are from "O_2 Consumption by the Photorespiratory Pathway" in "Results." C, MP reaction. Data are from "The Activity of the MP Reaction" in "Results." D, Additional electron-consuming reactions.

nation, whereas Uo reaches a maximum of 15.1 μmol O_2 m^{-2} s^{-1} (Fig. 5). Whether mitochondrial respiration continues in the light or not, O_2 consumption by this pathway is negligible. Peltier et al. (1987) concluded that chlororespiration does not exceed 20% of the mitochondrial respiration, so it appears that it does not significantly contribute to Uo.

According to our data, photorespiration appears to decrease with increasing water stress from 8.1 μmol m^{-2} s^{-1} glycolate in the controls to approximately 5.9 μmol m^{-2} s^{-1} under drought stress. Therefore, increasing Uo cannot be explained by photorespiration. Consequently, we conclude that the MP reaction is the main O_2-consuming reaction in stressed *T. aestivum*.

Indirect evidence for the increased rate of O_2 photoreduction during drought stress is derived from in vitro measurements of the activities of enzymes that are involved in the scavenging of O_2^-, the detrimental product of O_2 photoreduction. Because the chloroplast lacks catalase, H_2O_2, formed from O_2^- by superoxide dismutase, is scavenged by ascorbate peroxidase, forming H_2O and the ascorbate radical monodehydroascorbate. Monodehydroascorbate is then rereduced by an enzyme system that includes monodehydroascorbate reductase, dehydroascorbate, and glutathione reductase. Monodehydroascorbate reductase capacity readily increases in plants exposed to water deficit (Smirnoff and Colombe, 1988). It should be noted that another pathway for ascorbate regeneration in chloroplasts is direct photoreduction by the thylakoids (Miyake and Asada, 1992). However, the quantitative significance of the monodehydroascorbate reductase-catalyzed reaction in vivo is not clear. Pfeifer (1992) showed that the activity of ascorbate peroxidase and superoxide dismutase increases in *T. aestivum* leaves in relation to water stress. Stuhlfauth et al. (1988) found that water deficit increases the activity of glutathione reductase in *D. lanata*. Fluorescence studies have also shown that water stress may increase the rate of photosynthetic O_2 reduction (Stuhlfauth et al., 1988; 1990; Cornic et al., 1989; Cornic and Briantais, 1991).

To assess the allocation of photosynthetic electrons to the several processes that consume electrons, the rate of photosynthetically produced electrons is determined from Eo. For this the Eo measured by MS (Fig. 3) is considered, as well as the RO_2 (see "O_2 Consumption by the Photorespiratory Pathway" in "Results"). Therefore, the true PSII activity is 34.0 + 3.0 = 37.0 μmol O_2 m^{-2} s^{-1} in unstressed leaves and 29.0 + at least 3.0 = 32.0 μmol O_2 m^{-2} s^{-1} in stressed leaves. Consequently, 37 × 4 = 148.0 μmol electrons m^{-2} s^{-1} are transported via the photosystems in control leaves and 32 × 4 = 128.0 μmol electrons m^{-2} s^{-1} are transported in water-stressed leaves. We now discuss the distribution of the electrons generated by PSII to several electron acceptors (Fig. 6). In well-watered leaves 62.6% of the electrons transported over the electron chain are consumed in CO_2 reduction (Fig. 6, control A). The rate of CO_2 reduction is estimated assuming that the CO_2 evolved by photorespiration is totally refixed. In control leaves 21.9% of the photosynthetic electrons drive photorespiration (Fig. 6, control B); the remaining electrons (15.5%) are thought to support the reduction of sulfur, NO_3^-, NO_2^-, and NH_4^+ to organic metabolus (Fig. 6, control C + D). Furthermore, of this fraction (C + D), a small portion of electrons drives the MP reaction (Fig. 6, control C), by which the ATP/NADPH ratio may be modified to meet the demands of the Calvin cycle.

In stressed leaves net CO_2 assimilation is decreased by stomatal closure (Fig. 2, right). Therefore, only 32.6% of the photosynthetic electrons are used to drive the Calvin cycle (Fig. 6, stress A). Since the rate of photorespiration does not increase (Gerbaud and André, 1987; Stuhlfauth, 1989; Stuhlfauth et al., 1990), but appears to be diminished under stress, only 18.4% of the photosynthetic electrons are consumed by this pathway (Fig. 6, stress B). The relative contribution of photosynthetic electrons to the MP reaction is approximately 29.1% in stressed leaves (Fig. 6, stress C; see "The Activity of the MP Reaction" in "Results"). The remaining 19.9% of the photosynthetic electrons (Fig. 6, stress D) may be partially transported out of the chloroplast via the malate oxaloacetate translocator if an overreduction of the electron transport chain is pending during stress (Biehler et al., 1995). Furthermore, a small portion of photosynthetic electrons (Fig. 6, stress D) is consumed by zeaxanthin synthesis, since zeaxanthin is accumulated under stress conditions (Huber, 1994). Reduction of nitrogen and sulfur are negligible during water deficit because the growth of the leaf is severely restricted under stress.

There is now convincing evidence that electrons that cannot be used for carbon reduction under drought stress are transferred to O_2. At 25°C the O_2 concentration in chloroplasts has been experimentally estimated as 275 to 300 μM O_2 (Asada and Nakano, 1978). The K_m O_2 for oxygen reduction measured in intact chloroplasts and whole cells of *Scenedesmus* was 60 and 75 μM, respectively.

Therefore, in well-watered leaves O_2 reduction can be expected to proceed with maximal velocities. Because of that, another increase in O_2 reduction with drought stress seemed unlikely. However, the capacity for the MP reaction is not controlled only by the concentration of O_2, but also by the concentration of reduced Fd. The reduction of $NADP^+$ is saturated by 10 μM Fd, whereas the MP reaction is not saturated by 70 μM Fd (Hosler and Yocum, 1985). Measuring O_2 uptake in spinach thylakoids, Furbank and Badger (1983) found that the K_m of O_2 reduction increased with increasing concentration of reduced Fd. In the presence of 2 μM Fd, the K_m O_2 was less than 20 μM; at 25 μM Fd saturation of O_2 uptake occurred above 600 μM O_2 in the medium, with a K_m of approximately 60 μM O_2. Therefore, under normal conditions electrons are preferentially transferred to $NADP^+$. During stress, the concentration of reduced Fd increases due to a lack of $NADP^+$ that is only slowly reoxidized in the Calvin cycle. Consequently, the rate of the MP reaction increases and can be as high as the rate of $NADP^+$ reduction.

In summary, we conclude that O_2 may serve as a sink for surplus electrons when the reductant is in excess of the demand due to drought-stressed limitations of CO_2 assimilation.

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