PHOTOSYNTHETICA 46 (1): 127-134, 2008

Partitioning of photosynthetic electron flow between CO_2 assimilation and O_2 reduction in sunflower plants under water deficit

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Abstract

In sunflower (*Helianthus annuus* L.) grown under controlled conditions and subjected to drought by withholding watering, net photosynthetic rate (P_N) and stomatal conductance (g_s) of attached leaves decreased as leaf water potential (Ψ_w) declined from –0.3 to –2.9 MPa. Although g_s decreased over the whole range of Ψ_w, nearly constant values in the intercellular CO_2 concentrations (C_i) were observed as Ψ_w decreased to –1.8 MPa, but C_i increased as Ψ_w decreased further. Relative quantum yield, photochemical quenching, and the apparent quantum yield of photosynthesis decreased with water deficit, whereas non-photochemical quenching (q_{NP}) increased progressively. A highly significant negative relationship between q_{NP} and ATP content was observed. Water deficit did not alter the pyridine nucleotide concentration but decreased ATP content suggesting metabolic impairment. At a photon flux density of 550 µmol m^{-2} s^{-1}, the allocation of electrons from photosystem (PS) 2 to O_2 reduction was increased by 51 %, while the allocation to CO_2 assimilation was diminished by 32 %, as Ψ_w declined from –0.3 to –2.9 MPa. A significant linear relationship between mean P_N and the rate of total linear electron transport was observed in well watered plants, the correlation becoming curvilinear when water deficit increased. The maximum quantum yield of PS2 was not affected by water deficit, whereas q_P declined only at very severe stress and the excess photon energy was dissipated by increasing q_{NP} indicating that a greater proportion of the energy was thermally dissipated. This accounted for the apparent down-regulation of PS2 and supported the protective role of q_{NP} against photoinhibition in sunflower.

Additional key words: chlorophyll fluorescence; electron transport; fluorescence quenching; *Helianthus annuus*; intercellular CO_2 concentration; net photosynthetic rate; stomatal conductance.

Introduction

Water deficit is the main factor limiting plant growth and yield in the world. There is a big controversy as to whether water deficit mainly limits photosynthesis by stomatal closure (Cornic 2000, Flexas *et al.* 2006) or metabolic damage (Lawlor 1995, Lawlor and Cornic 2002).

Water deficit induces stomatal closure diminishing net CO_2 assimilation and growth (Lawlor 1995). However, production capacity of reducing power is little affected by drought. There is evidence that the pools of reduced pyridine nucleotides are little affected (NADPH) or increased (NADH) in stressed leaves; $NADP^+$ increases and then decreases, while NAD^+ decreases with electrons for the reduction of acceptors and supply of progressive stress. This suggests that both supply of oxidized nucleotides are adequate and the process is efficient even under severe stress (Lawlor and Khanna-Chopra 1984, Stuhlfauth *et al.* 1991). However, the available data for NADPH are scarce. Electron transport is, therefore, considered to continue at a significant rate even in severely stressed tissues, and the electrons are passed on to the normal physiological acceptors $NADP^+$ and ferredoxin and to alternative acceptors, such as oxygen in the Mehler reaction (Biehler and Fock 1996, Heber 2002, Kitao *et al.* 2003), and eventually used in

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Received 26 July 2007, *accepted* 20 September 2007.

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Abbreviations: C_a – ambient CO_2 concentration; C_i – intercellular CO_2 concentration; F_m, F'_m – maximum fluorescence after 1 h dark adaptation and under steady-state irradiation; F_s – fluorescence at steady state in the light; F_v – variable fluorescence after 1 h dark adaptation; F_v/F_m – maximum quantum yield of photosystem 2; g_s – stomatal conductance; J_C – electron flow to CO_2 fixation; J_O – electron flow to O_2; J_T – total electron transport; P_N – net photosynthetic rate; PFD – photon flux density; PS – photosystem; q_P, q_{NP} – coefficients for photochemical and non-photochemical quenching of fluorescence; RWC – relative water content; Ψ_w – leaf water potential; Φ_{CO2} – apparent quantum yield of CO_2 assimilation; Φ_{PS2} – relative quantum yield of PS2 electron transport.

Acknowledgements: This work was financed by a BID-CONICIT (Venezuela) scholarship to WT and a contribution fellowship from Rothamsted International. We thank A. Herrera, A. Pieters, and R. Urich for critical reading of the manuscript.

photorespiration (Lawlor 1995, Lawlor and Cornic 2002). The absorption of excess photon energy by leaves, which potentially occurs when stomatal closure reduces CO_2 assimilation, is a source of damage in water-stressed plants (Björkman and Powles 1984, Björkman and Demmig-Adams 1994). The ability of a plant species to efficiently use its capabilities for quantum energy dissipation provides the investigator with a measure of water deficit tolerance (Scheuermann *et al.* 1991).

During water deficit, restricted CO_2 availability due to stomatal closure may lead to increased susceptibility to photodamage (Powles 1984). Sometimes such damage does not occur under natural and greenhouse conditions (Epron and Dryer 1992, Tezara *et al.* 1999, Lawlor and Cornic 2002), suggesting that the mechanisms of protection against an excess of absorbed excitation energy are efficient. The major process involved in protection against photodamage is probably the increase in non-photochemical quenching (q_{NP}), which reduces the quantum yield of photosystem 2 (Φ_{PS2}) in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism (Weis and Berry 1987, Krause and Weis 1991). Furthermore, photorespiration in C_3 plants may be an alternative sink for light-induced electron flow and is often presented as a process that may help to consume a considerable proportion of the electron flow during periods of high irradiance and restricted CO_2 availability in the chloroplasts (Stuhlfauth *et al.* 1990). Considerable oxygen uptake occurs during photorespiration of C_3 plants (Heber 2002).

There is good evidence that a large proportion of the electron flux is diverted from CO_2 assimilation to O_2 reduction under stress at low intercellular CO_2 concentration (C_i) (Osmond 1981). Maintenance of a high capacity for photosynthetic and photorespiratory carbon metabolism is the primary means of protection against photoinhibition when stomata close (Osmond 1981, Powles 1984). Cornic and Briantais (1991) concluded from measurements of fluorescence parameters and calculation of the quantum yield of electron flow that the allocation of electrons to O_2 reduction increased, particularly at the more severe stress (Cornic 1994), as a consequence of the decrease in C_i and stomatal conductance (g_s).

In some studies water deficit did not inhibit O_2 evolution at very high CO_2 (Chaves 1991, Quick *et al.* 1992), leading to the effects of stress on CO_2 assimilation being ascribed to stomatal limitation. There is little evidence supporting that water splitting is inhibited by water deficit within the physiological range. No inhibition of O_2 evolution and PS2 activity in mildly stressed leaves of sunflower was observed (Tezara *et al.* 1999). However, rates of O_2 evolution by stressed leaves have been measured with oxygen electrodes under elevated CO_2 and in some cases a decrease has been found (Lawlor and Khanna-Chopra 1984, Havaux *et al.* 1987).

Our main objective was to examine the effects of water deficit on the partitioning of photosynthetic electron flow between CO_2 assimilation and O_2 reduction by simultaneous measurements of gas exchange and fluorescence in attached leaves of water-stressed sunflower plants. ATP and pyridine nucleotide content were also determined.

Materials and methods

Plants: Sixty sunflower plants (*Helianthus annuus* L. cv. Avante) were grown in a glasshouse in pots containing 5 000 cm^3 of organic soil under controlled environmental conditions: temperatures 20/18 ºC day/night, 360 μmol(CO_2) mol^{-1}, 500 μmol m^{-2} s^{-1} photon flux density (PFD, 400–700 nm), and 50–70 % relative humidity. When irradiance was lower than 300 μmol m^{-2} s^{-1}, additional irradiation provided at the top of the canopy by lamps gave a maximum PFD of 700 μmol m^{-2} s^{-1}. Plants were watered daily and supplied weekly with 100 cm^3 of a complete nutrient solution (*Vitax* 4 : 3 : 1). All measurements were performed on the third pair of leaves. Water deficit was induced in one-month-old plants by withholding irrigation over 12 d.

Leaf water status: Relative water content (RWC) of leaf discs was determined as RWC = (FM – DM)/(TM – DM), where FM is the fresh mass, TM is water saturated mass obtained after re-hydrating the disc for 6 h in distilled water at 25 ºC and DM is the dry mass after oven-drying for 24 h at 80 ºC. Predawn leaf water potential (Ψ_w) was measured on three individual leaves using a custom-made pressure chamber (Rothamsted, U.K.).

Gas exchange was measured on fully expanded leaves, using a six-chamber computerized infra-red open gas-exchange system (*WA-225-MK3*; *Analytical Development Co.*, Hoddesdon, UK) as described by Jacob and Lawlor (1991). Areas of 10 cm^2 of leaves attached to the plant were sealed into the chambers. During measurements incident PFD was 1 100 μmol(photon) m^{-2} s^{-1}, leaf temperature 25 ºC, O_2 concentration 0.21 mol mol^{-1}, and ambient CO_2 concentration (C_a) 350 μmol mol^{-1}. Vapour pressure deficit between the leaf and chamber was 1.0–1.4 kPa.

Photosynthetic O_2 evolution was measured with a leaf-disc oxygen electrode (*Hansatech*, Norfolk, U.K.) at 10 mmol mol^{-1} of CO_2. Leaf discs of 10 cm^2 were irradiated at 1 100 μmol m^{-2} s^{-1} PFD from a slide projector bulb; temperature was controlled at 25 ºC.

Irradiance-response curves (P_N/PFD) at constant C_a (350 μmol mol^{-1}) and a leaf temperature of 25 ºC were done by irradiating the leaf at different PFD until P_N was constant. The magnitude of PFD was modified using neutral density filters (*Lee Filters*; *A.C. Lighting*, Bucks., UK). Measurements of gas exchange were taken during steady state photosynthesis after a period of 1-h adjustment by the leaf to the chamber conditions. Calculations of P_N, g_s, and C_i were done after Farquhar and Sharkey (1982). Leaf absorbance was determined using an integrating sphere (*Applied Physics*), as described by Rackham and Wilson (1968). The apparent quantum yield of CO_2 (Φ_{CO2}) was calculated as the slope of the linear portion of the P_N/PFD curve at 0–150 µmol m^{-2} s^{-1} PFD.

Chlorophyll *a* fluorescence of PS 2 and gas exchange were measured simultaneously at room temperature on intact attached leaves in a temperature-controlled metal chamber with a glass window, as described by Habash *et al.* (1995). Fluorescence was measured using a modulated fluorometer (*MSMF*; *Hansatech*, Kings Lynn, UK) providing a low-intensity modulated beam of less than 0.5 µmol m^{-2} s^{-1} PFD set at 580 nm using a narrow-band pass filter *585DF 44* (*Omega Optical*, Brattleboro, VT, USA) and selectively measuring the resulting fluorescence at 695 nm using a band pass filter *695 DF 30* (*Omega Optical*). Actinic irradiation was provided by metal-halide lamps used to drive photosynthesis for both gas-exchange and fluorescence measurements. To fully reduce Q_A a 2-s saturating flash of 7 000 µmol m^{-2} s^{-1} PFD of 400–635 nm was shone through a short-pass filter from a pulse source *PLS2* (*Hansatech*, Kings Lynn, UK). Far-red radiation of 15 W m^{-2} was supplied by the *PLS2* through a far-red filter *RG 715* (*Schott*, Mainz, Germany) for the determination of minimum fluorescence (F'_0) at the steady state photosynthesis. The protocol for fluorescence measurements was similar to that described by Genty *et al.* (1989), except that the measurements were done on attached leaves. The relative quantum yield of PS2 at steady state photosynthesis is defined as $\Phi_{PS2} = (F'_m - F_s)/F'_m$ according to Genty *et al.* (1989), where F_s and F'_m are fluorescence signal at steady state photosynthesis and maximum fluorescence under irradiation, respectively.

Determination of ATP and pyridine nucleotides: The amount of ATP was determined by an enzymatic method of Stitt *et al.* (1989). Oxidized (NAD^+, $NADP^+$) and reduced (NADH, NADPH) pyridine nucleotides were determined by the method of Carrier and Neve (1979).

Electron-transport rate in leaves: Whole chain electron transport rate in the leaves (J_T) was estimated by the method of Krall and Edwards (1992) from the equation:

$$J_T = \Phi_{PS2}\ \text{PFD}\ a\ f$$

where *a* is the fraction of incident PFD absorbed by the leaf, and *f* the absorption of PS2 divided by the absorption of PS1+PS2. It is assumed that the two photosystems are equally involved in linear electron transport, so a value of 0.5 was used for *f*.

Electron transport allocation to CO_2 and O_2 was calculated as described by Peterson (1989). Assuming that the linear electron flow is uniquely devoted to carboxylation and oxygenation by ribulose-1,5-bisphosphate carboxylase/oxygenase, *i.e.* all the other processes consuming electrons are either negligible or at least constant (Cornic and Briantais 1991), partitioning of electron flow was calculated as

$$J_T = J_C + J_O$$

where J_C and J_O are the electron flows attributable to carboxylation and oxygenation reactions, respectively. Assuming that four electrons are consumed per CO_2 molecule fixed, J_C was estimated as

$$J_C = 1/3\ [J_T + 8\ (P_N + R_D)]$$
$$J_O = 2/3\ [J_T - 4\ (P_N + R_D)]$$

where R_D (day respiration) is the rate of CO_2 evolution in the light from processes other than photorespiration. R_D was determined as described by Jacob and Lawlor (1993).

Statistics: The statistical analyses were done using the statistical packages *Statistica* and *Sigmaplot*. All linear single regressions, correlations, and one way ANOVA were tested for significance at $p<0.05$. The data shown are the results of at least three different experiments. Results are presented as means of a minimum of five or maximum of ten measurements plus one standard.

Results

Leaf water status: Decreasing water supply decreased RWC and Ψ_w of sunflower leaves. There was a strong linear positive relationship between RWC and Ψ_w (RWC = 18.9 Ψ_w + 95.4; $r^2 = 0.90$; $p<0.05$). A decrease of Ψ_w to –3.0 MPa with a 40 % reduction in RWC was observed.

CO_2 exchange: P_N and g_s decreased with water deficit by 98 and 97 %, respectively, as Ψ_w declined from –0.3 to –3.0 MPa (Fig. 1*A,B*). Although g_s decreased over the whole range of Ψ_w, the C_i/C_a ratio showed little response to Ψ_w, remaining at an average value of 0.8 as Ψ_w decreased to –2.0 MPa, but C_i/C_a increased as Ψ_w decreased further (Fig. 1*C*).

Response of P_N and fluorescence to PFD: Under well-watered conditions, P_N was not saturated by photon energy at a PFD of 1 200 µmol m^{-2} s^{-1}. However, as Ψ_w decreased to –2.9 MPa, energy saturation was evident and maximum P_{Nmax} progressively decreased (Fig. 2). Φ_{CO2} also declined from 0.035 to 0.004 as Ψ_w decreased from –0.3 to –2.9 MPa. The fraction of PFD absorbed by the leaf was 0.89±0.15, and did not change with Ψ_w.

The Φ_{CO2} was more affected by moderate stress than Φ_{PS2}, which decreased only at severe water deficit (Fig. 3*A*). There was a strong positive, curvilinear relationship between Φ_{CO2} and Φ_{PS2} ($r^2 = 0.80$, $p<0.05$). The q_p followed a similar trend as $\Phi_{PS2,}$ decreasing only at very severe water deficit (Fig. 3*B*), whereas q_{NP} increased progressively in a linear fashion with declining Ψ_w ($r^2 = 0.91$; $p<0.05$) (Fig. 3*B*). F_v/F_m was not affected by decreasing Ψ_w (Fig. 3*C*).

ATP content decreased significantly (Table 1), while q_{NP} increased as Ψ_w decreased. A non-linear negative relationship between q_{NP} and ATP was observed ($r^2 = 0.64$; $p<0.05$; Fig. 4).

Water deficit did not alter pyridine nucleotide contents (Table 1). The NADPH and NADH contents were maintained, so that the ratio of total reduced to oxidised nucleotides was unaffected by water deficit.

Table 1. ATP [µmol m^{-2}] and pyridine nucleotide contents [µmol m^{-2}] of fully expanded leaves of sunflower measured at control and water deficit.

Ψ_w [MPa]	ATP	NADH	NADPH	NAD^+	$NADP^+$
–0.36±0.09	18.20±1.50 a	0.78±0.12 a	1.40±0.30 a	6.50±0.70 a	8.14±1.01 a
–1.96±0.15	8.30±1.20 b	0.76±0.05 a	1.10±0.30 a	6.10±0.20 a	8.21±2.70 a

Total electron transport rate in watered and droughted leaves: The calculated rates of total electron flow (J_T) decreased by 71 % with decreasing Ψ_w (Fig. 5). Water deficit had a stronger effect on J_C than on J_O. At a PFD of 550 µmol m^{-2} s^{-1} J_C decreased by 32 % with a decline of Ψ_w from –0.3 to –2.9 MPa, whilst J_O increased by 51 % in the same range of Ψ_w (Fig. 5). There was a positive linear relationship between J_T and P_N in control plants ($r^2 = 0.99$; $p<0.05$), the relationship became curvilinear as Ψ_w declined from –0.3 to –2.9 MPa (Fig. 6*A*). When P_N was completely suppressed at severe stress, a relatively high J_T [50 µmol(e^-) m^{-2} s^{-1}] was still observed. A strong linear relationship between J_T and O_2 evolution (measured at an external CO_2 concentration of 10 mmol mol^{-1}) ($r^2 = 0.97$; $p<0.05$) was observed during the development of water deficit (Fig. 6*B*). The values of O_2 evolution measured at 10 mmol(CO_2) mol^{-1} at low Ψ_w were significantly and substantially lower than in control leaves (Fig. 6*B*).

Discussion

The parameters characterizing plant water status (RWC, Ψ_w) decreased with increasing water deficit in the sunflower plants, in agreement with earlier reports (Tezara *et al.* 1999, 2002). Water deficit provoked a reduction in RWC and Ψ_w, which markedly diminished P_N and g_s (Lawlor and Cornic 2002).

The decline in P_N with decreasing Ψ_w is correlated with a reduction in g_s (Cornic 1994, 2000) which reduces diffusion of CO_2 into the leaf (C_i decreases). However, in the present study, C_i remained nearly constant up to –3.0 MPa, in spite of decreased g_s. Moreover, severe water deficit increased C_i possibly due to metabolic causes: respiratory metabolism dominates, and therefore C_i and compensation CO_2 concentration (Γ) rises (Lawlor and Cornic 2002). The ratio C_i/C_a associated with maximum P_N did not decrease during drought in sunflower suggesting metabolic impairment in accordance with an earlier report (Tezara *et al.* 1999). Similar effects of water deficit on C_i/C_a have been reported (Lawlor 1995) whereas a reduction in C_i/C_a was observed in *Prunus avium* (Centritto 2005). Precaution must be taken on the conclusions relating C_i/C_a ratio with stomatal and metabolic limitations of P_N, which in some cases may be misleading due to the erroneous calculation of C_i because of stomatal patchiness (Terashima *et al.* 1988, Buckley *et al.* 1997) or to an increase in cuticular conductance to water vapour but not to CO_2 (Boyer *et al.* 1997). In addition, water deficit may cause changes in the mesophyll conductance of CO_2 (Flexas *et al.* 2002, 2006, Centritto *et al.* 2003, Centritto 2005).

Both apparent quantum yield and photon energy-saturated P_N decreased with increasing water deficit, suggesting that either energy harvesting and/or linear electron transport were affected by water deficit or that enzymatic processes were responsible for decreasing CO_2 fixation or all factors functioned together. Similar results were reported in the desert shrub *Encelia farinosa* (Ehleringer and Cook 1984) and in *Lycium nodosum* (Tezara *et al.* 2003). With decreasing Ψ_w, Φ_{CO2} decreased more than Φ_{PS2}, which decreased only at severe stress and F_v/F_m was not affected by water deficit, in agreement with other studies (Lawlor and Cornic 2002, Centritto 2005), suggesting that PS2 activity is resistant to water deficit.

The q_P under photorespiratory conditions followed Φ_{PS2}, decreasing only at very severe stress which indicates a higher reduction state of primary acceptors (the Q_A pool) in stressed *vs.* control plants. In contrast, q_P was not affected by water deficit in sunflower (Scheuermann *et al.* 1991) and in wheat (Biehler and Fock 1993, 1996).

Water deficit increased q_{NP} in plants of sunflower, indicating that a greater proportion of the energy was thermally dissipated, thus accounting for the apparent down-regulation of PS2 and supporting the protective role of the non-photochemical quenching against photoinhibition. In contrast, q_{NP} was unaffected by water deficit in wheat (Biehler and Fock 1993) and increased slightly in sunflower (Scheuermann *et al.* 1991).

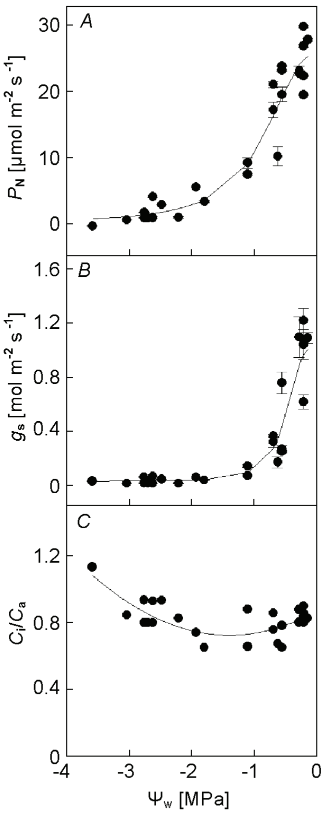

Fig. 1. Effect of leaf water potential (Ψ$_w$) on (*A*) net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), and (*C*) ratio of intercellular and ambient CO_2 concentrations (C_i/C_a) measured at a photon flux density (PFD) of 1 100 μmol m^{-2} s^{-1}, leaf temperature of 25 ºC, O_2 concentration of 0.21 mol mol^{-1}, and C_a of 350 μmol mol^{-1}. Means±SE, *n* = 10.

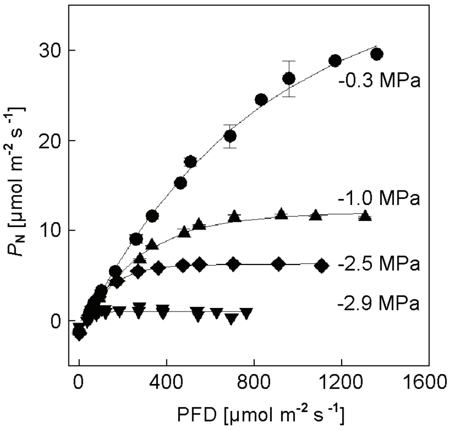

Fig. 2. Photosynthetic responses (P_N) to photon flux density (PFD) of attached sunflower leaves at different leaf water potential (Ψ$_w$). Measurements at leaf temperature of 25 ºC, O_2 of 0.21 mol mol^{-1}, and ambient CO_2 concentration (C_a) of 350 μmol mol^{-1}. Means±SE, *n* = 6.

A negative relationship between q_{NP} and ATP content in sunflower suggests that increased thylakoid membrane energization was a consequence of the inhibition of either ATP generation or ATP consuming reactions, in agreement with earlier reports (Stuhlfauth *et al.* 1988, Tezara *et al.* 1999). In the intact leaf, q_{NP} consists mainly of the "energy quenching" which is related to the proton gradient across the thylakoid membrane, and affects PS2 *via* membrane energization (Schreiber *et al.* 1986, Krause *et al.* 1988). In this study, ATP content decreased in sunflower subjected to water deficit, supporting the hypothesis of impaired metabolism and confirming an earlier report of Tezara *et al.* (1999).

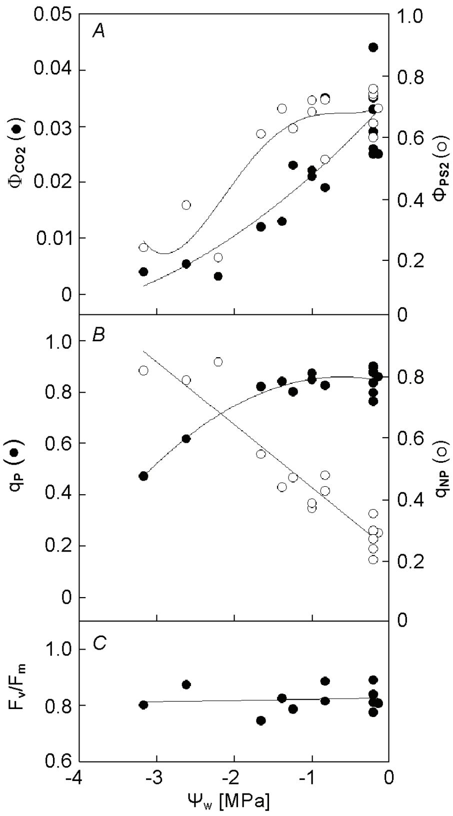

Fig. 3. Changes of fluorescence parameters with leaf water potential (Ψ$_w$) measured at 550 μmol m^{-2} s^{-1} of PFD: (*A*) the relative quantum yield of photosystem 2, Φ$_{PS2}$ (○) and the apparent quantum yield of CO_2, Φ$_{CO2}$ (●); (*B*) the photochemical (q_P) (●) and non-photochemical quenching coefficients (q_{NP}) (○); and (*C*) the maximum quantum yield of PS2 (F_v/F_m). Means±SE, *n* = 6.

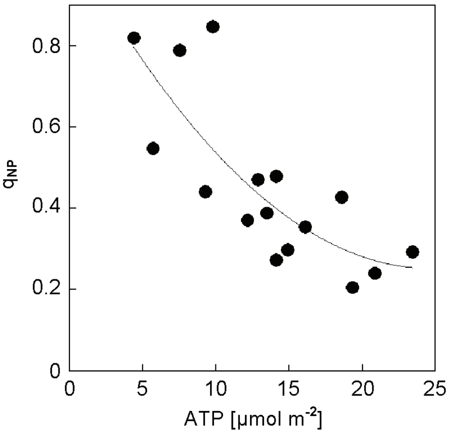

Fig. 4. Relationship between the non-photochemical quenching coefficients (q_{NP}) measured at photon flux density (PFD) of 550 μmol m^{-2} s^{-1} and ATP content. Means±SE, *n* = 4.

Our results confirm that in sunflower plants, when P_N is inhibited due to water deficit, there is a fraction of PS2 centres which stays open and is able to perform photochemical work. Horton *et al.* (1994) suggested that a certain fraction of PS2 centres stays open when P_N is very low and the Φ_{PS2} is greatly reduced by, for instance, stressful environmental conditions.

Water deficit caused a decline of J_T in sunflower plants. Lower saturated J_T and Φ_{PS2} during water deficit were observed, suggesting that the photochemical system was down-regulated by changes in leaf water status, and that PS2 activity was resistant to water deficit and not sensitive to photoinhibition. Similarly, J_T was reduced by approximately 40 % in maize leaves subjected to water deficit (Scheuermann *et al.* 1991). The reduction in Φ_{PS2} and J_T due to water deficit was lower than the decrease in P_N, possibly due to higher photorespiration at low Ψ_w, as suggested by Lawlor and Cornic (2002).

The observed reductions in Φ_{PS2}, J_T, and q_P may partly contribute with the metabolic limitations of P_N in sunflower under water deficit (Tezara *et al.* 1999, 2002). The decrease in J_T of sunflower may have contributed to the increase in metabolic limitations through a reduction in ATP and/or RuBP contents (Tezara *et al.* 1999, Lawlor and Cornic 2002).

In sunflower leaves J_C was more affected than J_O suggesting greater effect on electron flux to oxygenation and possible photorespiration increase (Tezara *et al.* 1999), which supports the protective effect of q_{NP} against over-reduction and photoinhibition in sunflower under water deficit. Similar results were reported in sunflower and bean plants subjected to water deficit (Scheuermann *et al.* 1991). In contrast, the rate of photorespiration decreased with water deficit in wheat and therefore the O_2 taken up in the light might be preferentially used by the Mehler-peroxidase reaction (Biehler and Fock 1996).

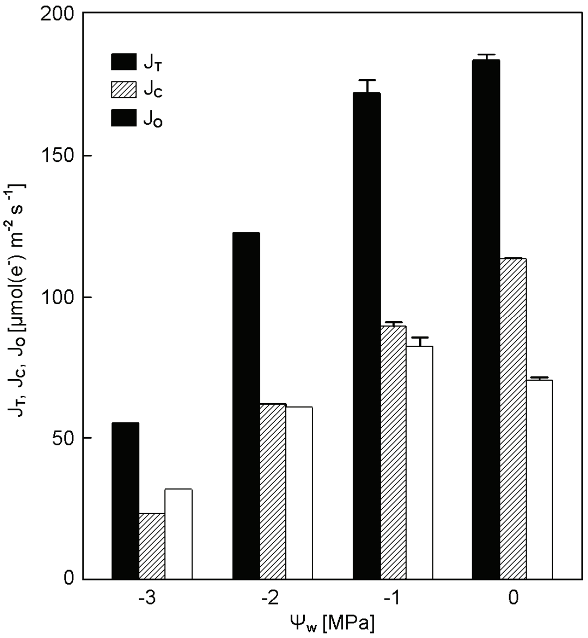

Fig. 5. Effects of leaf water potential (Ψ_w) on the rate of total linear electron transport (J_T), electron transports allocated to carboxylation (J_C) or to oxygenation (J_O) in attached leaves of sunflower. Measurements were done at 550 µmol m^{-2} s^{-1} of PFD, leaf temperature of 25 ºC, O_2 of 0.21 mol mol^{-1}, and ambient CO_2 concentration (C_a) of 350 µmol mol^{-1}. Means±SE, $n = 6$.

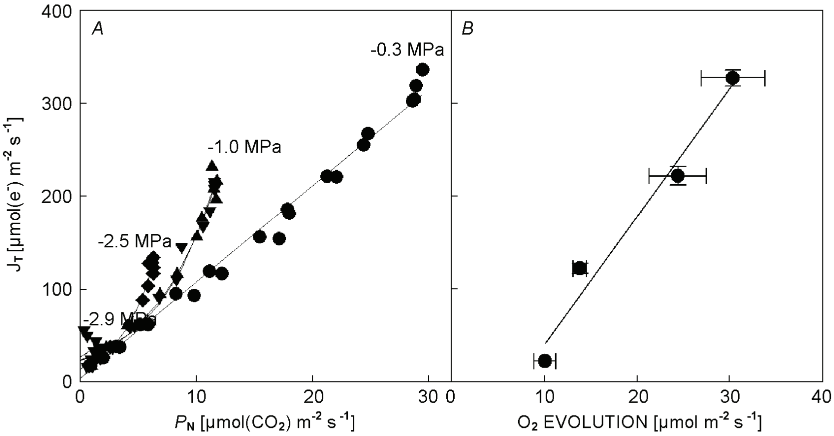

Fig. 6. Relationship between the rate of total linear electron transport (J_T) and (*A*) net photosynthetic rate (P_N) in attached leaves of sunflower at different leaf water potential and (*B*) O_2 evolution of disc (measured at 1 mmol mol^{-1} of CO_2, PFD of 1 100 µmol m^{-2} s^{-1}, and 25 ºC. Means±SE, $n = 5$.

When P_N was totally inhibited at low Ψ_w a residual electron transport of 50 µmol(e^-) m^{-2} s^{-1} could be measured. This has also been observed in water stressed plants of sorghum (Loreto *et al.* 1995). The positive linear relationship between J_T and P_N observed in control plants became curvilinear as Ψ_w declined, indicating that P_N was more affected by water deficit than J_T. Alternative electron sinks may develop when P_N is very low.

Our results show that the reduction state of the pyridine nucleotides in stressed leaves was not significantly altered as water deficit increased while ATP/NADPH dropped substantially, indicating that carbon metabolism was more affected by ATP content than by NADPH. Similarly, NADPH content decreased only slightly during water deficit (Lawlor and Khanna-Chopra 1984, Stuhlfaulth *et al.* 1991).

Measurements of O_2 evolution, in which diffusion limitations were negligible, showed that P_N was not substantially increased by high CO_2 concentrations (10 mmol mol^{-1}) as Ψ_w declined, supporting that dehydrated leaves suffered from more than stomatal limitation. In other plant species, 50–150 mmol(CO_2) mol^{-1} may be required to fully saturate the photosynthetic machinery (Chaves 1991, Quick *et al.* 1992, Cornic 1994).

We conclude that the declines in P_N, g_s, and ATP content associated with a nearly constant C_i/C_a ratio indicated metabolic impairment in sunflower. During severe water deficit, the increase in q_{NP} allowed a greater proportion of the energy to be thermally dissipated. The lower saturated J_T and Φ_{PS2} suggested that the photochemical system was down-regulated, and that leaves were not sensitive to photoinhibition. Changes observed in photochemical activity of PS2 can partly contribute to the metabolic limitations of P_N in sunflower under water deficit.

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