BRIEF COMMUNICATION

Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit

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Abstract

In mature and young leaves of sunflower (*Helianthus annuus* L. cv. Catissol-01) plants grown in the greenhouse, photosynthetic rate, stomatal conductance, and transpiration rate declined during water stress independently of leaf age and recovered after 24-h rehydration. The intercellular CO_2 concentration, chlorophyll (Chl) content, and photochemical activity were not affected by water stress. However, non-photochemical quenching increased in mature stressed leaves. Rehydration recovered the levels of non-photochemical quenching and increased the F_v/F_m in young leaves. Drought did not alter the total Chl content. However, the accumulation of proline under drought was dependent on leaf age: higher content of proline was found in young leaves. After 24 h of rehydration the content of proline returned to the same contents as in control plants.

Additional key words: chlorophyll; drought; intercellular CO_2 concentration; leaf water potential; non-photochemical quenching; photochemical activity; rehydration; stomatal conductance to water vapour; transpiration rate.

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The leaf water status and carbon uptake is under stomata control and stomata closure is one of the earliest responses to drought, resulting in protection of the plants against harmful dehydration but also inevitably results in reduction of CO_2 uptake for photosynthesis. The stomata control is important only under mild stress (Cornic 2000). However, metabolic inhibition of photosynthesis also takes place at mild water stress (Tezara *et al.* 1999) and it becomes more important as the water stress intensifies. Most of the studies in this area were done on mature leaves and the few available data comparing the effects of water stress on mature and young leaves indicate that the photosynthetic responses to water stress are strongly dependent on leaf age (David *et al.* 1998). In addition, there are indications that water stress accelerates leaf senescence (Olsson 1995).

The accumulation of osmolyte compounds in the cells as a result of water stress is often associated with a possible mechanism to tolerate the harmful effect of water shortage. The contribution of sugars as an osmotic solute in expanded and partly expanded sunflower leaves was studied by Jones and Turner (1980). They found that contents of sugars did not change in fully expanded leaves. In opposition, the contents of soluble sugars in partly expanded leaves were reduced. In addition to sugars, some plants also accumulate other low molecular mass compounds, such as proline (Gzik 1996, Bajji *et al.* 2001). These osmolyte accumulations in plant cells might contribute, *via* lowering the cell osmotic potential, to maintaining several physiological processes, such as photosynthesis, stomatal conductance, and leaf expansion even under stressed conditions. The present experiment was designed to study the responses of photosynthesis to water stress in fully expanded and expanding leaves of sunflower plants. Additionally, the change in proline content and the ability of plants to recover from the water stress were also evaluated.

The experiment was conducted in a greenhouse under natural photoperiod with maximum day and night temperatures close to 30 and 18 °C, respectively. The sunflower (*Helianthus annuus* L. cv. Catissol-01) plants were grown in 8 000 cm^3 pots filled with a 47 : 13 : 40 % pinus bark, vermiculite, and peat mixture. The plants were supplied with 300 cm^3 of a 50 % of full strength Long Ashton solution (Hewitt 1966) per pot, twice a week, and with tap water on the other days. The substrate

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Received 20 June 2005, *accepted* 31 August 2005.
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Acknowledgement: IC, SCR, and VCO thank FAPESP—Fundação de Amparo à Pesquisa do Estado de Sao Paulo, Brazil—for financial support.

moisture in the pots was maintained close to field capacity until 32 d after sowing. Irrigation was then partially suspended in a separate set of pots for a period of 9 d, and terminated by rehydrating the pots again. In order to obtain a slow rate of stress, the pots of stressed plants were covered with plastic to avoid evaporation from the substrate and received 150 cm^3 of 50 % Long Ashton solution twice a week and the same amount of tap water on the other days. Control (irrigated) plants were maintained at field capacity throughout the experiment and also received the same amount of Long Ashton solution as the stressed plants.

Leaf gas exchange, chlorophyll (Chl) fluorescence, leaf water potential, and Chl and proline contents were determined in mature leaves (7 and 8, numbered acropetally) and in young leaves which had a length between 5–6 cm at the time of stress imposition. The mature leaves were fully expanded at the beginning of the stress treatment and their size did not vary anymore for the subsequent period.

A portable infra-red gas analyzer (*LCpro*, *ADC*, Hoddesdon, UK) was used for measurements of net photosynthetic rate (P_N), stomatal conductance to water vapour (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i). Measurements were made inside the greenhouse and photosynthetically active radiation (PAR) of 1 800 μmol m^{-2} s^{-1} was supplied by a light unit mounted on the top of leaf chamber. Chl *a* fluorescence was measured with a portable fluorometer (*PAM-2000*, *Walz*, Effeltrich, Germany). F_v/F_m which represents a measure of the potential efficiency of photosystem 2 (PS2) in darkness was obtained early in the morning after 10 min of dark adaptation. The steady-state fluorescence signal was measured under sunlight conditions inside the greenhouse early in the morning (PAR around 500 μmol m^{-2} s^{-1}). The quantum efficiency of PS2 photochemistry (Φ_{PS2}) was then calculated as $(F_m' - F_s)/F_m'$ (Genty *et al.* 1989). The apparent linear electron transport rate (ETR) was calculated as $\Phi_{PS2}\times PAR\times 0.5\times 0.84$. PAR corresponds to the flux density of incident radiation, 0.5 is a factor that accounts for the partitioning of energy between the two photosystems, and 0.84 is the most common percentage of irradiance that is absorbed by the leaf. Following the saturation pulses, the leaves were transiently covered with a dark cloth in the presence of far-red radiation in order to estimate the zero level fluorescence (F_0') in the light-adapted leaves. Photochemical (q_P) and total non-photochemical (q_N) quenching coefficients were calculated as $(F_m' - F_s)/(F_m' - F_0')$ and $(F_m - F_m')/(F_m - F_0')$, respectively.

Leaf water potential (Ψ_{leaf}) was determined in the same leaf used for photosynthesis and Chl fluorescence measurements by using a Scholander pressure bomb (model *3005*, *Soilmoisture Equipment Corp.*, Santa Barbara, USA). These leaves were then oven dried for 48 h at 70 °C for posterior dry mass and proline determinations. Chl content was measured on leaf discs of known area from leaf opposite that used for gas exchange, Chl fluorescence, and Ψ_{leaf}. Chl was extracted in 80 % aqueous acetone (Arnon 1949). The content of proline was determined on the same leaf used for gas exchange, Chl fluorescence, and Ψ_{leaf} according to Torrelo and Rice (1986), and expressed on a dry mass (DM) basis.

All measurements were made on 5 individual plants per treatment. The data were submitted to analyses of variance (F test) followed by a multiple comparison test (Tukey test) at the 5 % level by using *SPSS/PC for Windows*.

Water stress did not affect the DM of mature leaves but the young leaves grown under water shortage had lower DM compared to control plants (data not shown). Growing tissues, the most sensitive to changes in environmental factors and cellular division and elongation, are influenced by water deficit (Heckenberger *et al.* 1998). The lower DM in young stressed leaves could also be a result of an increase in proline synthesis (see below) since it is involved in inhibition of growth of callus cultures of sunflower (Carceller and Dambrogio 1994). Although the young stressed leaves had lower Ψ_{leaf} than the control leaves, their Ψ_{leaf} was higher than that of the mature stressed leaves (Table 1). Young leaves were not able to recovery Ψ_{leaf} to the same level as the control leaves within 24 h of rehydration.

The recognition of differential effects of dehydration and rehydration on different leaf ages is of great importance to understand responses to carbon dioxide assimilation. David *et al.* (1998) found that dehydration reduced Chl content in older leaves and that the incapacity of photosynthetic apparatus of those leaves to recover after stress relief was associated with a further accentuation of decline in Chl content. In sunflower plants, neither water stress nor rewatering affected the photosynthetic pigments, irrespective of leaf age (Table 1). Therefore, the results obtained in the present work indicate that the content of photosynthetic pigments was not limiting carbon dioxide assimilation.

After 9 d of drought, E decreased by about 61 and 47 % in mature and young leaves, respectively (Table 1). When the water-stressed plants were rewatered for 24 h, E of both mature and young leaves recovered to the same control level. Drought depressed g_s by about 70 % irrespective of leaf age, but leaf g_s fully recovered after 24 h rehydration (Table 1). The C_i of stressed leaves showed similar values as C_i of the control leaves but they had lower P_N (Table 1). The reduction in P_N was more marked in mature than young leaves. Our results suggest that in sunflower plants stomatal closure is not fully responsible for reduction in P_N. By removing the lower epidermis of sunflower leaves, Tang *et al.* (2002) were able to demonstrate that CO_2 depletion was responsible for reduction in photosynthesis only in the early phases of water stress and that water stress acted directly on mesophyll metabolism as leaf water potential became

Table 1. Leaf water potential (Ψ_{leaf}), chlorophyll (Chl) content [g m^{-2}], proline content [mmol kg^{-1}(DM)], intercellular CO_2 concentration, C_i [μmol mol^{-1}], stomatal conductance, g_s [mol m^{-2} s^{-1}], transpiration rate, E [mmol m^{-2} s^{-1}], net photosynthetic rate, P_N [μmol m^{-2} s^{-1}], and Chl *a* fluorescence parameters in sunflower plants under 9 d of drought and after 24 h of rehydration. Means±SE of 5 plants. Values sharing the same letters are not significantly different at $p<0.05$.

	Mature leaf Control	Stressed	Rehydrated	Young leaf Control	Stressed	Rehydrated
Ψ_{leaf}	-0.54±0.08a	-1.86±0.08b	-0.74±0.06a	-0.66±0.02a	-1.66±0.03b	-0.84±0.04c
Chl *a+b*	0.49±0.05a	0.50±0.01a	0.47±0.02a	0.54±0.03a	0.57±0.03a	0.48±0.02a
Proline	9.59±1.66a	43.51±9.49b	18.91±4.47a	9.80±2.00a	71.79±5.97b	12.08±1.32a
C_i	263.2±10.2a	281.4±10.5a	249.8±7.4a	217.0±9.5ab	200.4±7.7a	234.8±7.6b
g_s	0.43±0.04a	0.11±0.05b	0.35±0.10ab	0.58±0.03a	0.17±0.04b	0.70±0.05a
E	5.17±0.24a	2.02±0.66b	3.89±0.64ab	5.78±0.14a	3.05±0.46b	5.58±0.19a
P_N	16.96±1.68a	4.94±1.58b	13.31±1.84a	27.36±1.72a	14.18±2.51b	23.61±1.24a
F_v/F_m	0.842±0.005a	0.835±0.009a	0.856±0.002a	0.866±0.001a	0.838±0.003a	0.877±0.001b
q_P	0.498±0.076a	0.482±0.059a	0.608±0.043a	0.789±0.016a	0.789±0.023a	0.862±0.022a
q_N	0.787±0.022a	0.876±0.016b	0.720±0.018a	0.794±0.014a	0.784±0.013a	0.771±0.006a
Φ_{PS2}	0.340±0.048ab	0.279±0.040b	0.449±0.036a	0.565±0.009a	0.577±0.019a	0.657±0.016b
ETR	72.98±10.69a	60.02±8.81a	95.82±8.13a	119.54±1.93a	124.42±4.07a	140.32±3.20b

more negative. Flexas and Medrano (2002b) explain that the metabolism gets impaired as a consequence of decreased synthesis of ribulose bisphosphate and ATP in the early phases of water stress when g_s is still higher than 0.15 mol m^{-2} s^{-1} in most cases. Only under severe stress decreased ribulose-1,5-bisphosphate carboxylase/ oxygenase activity and photochemistry are reported. David *et al.* (1998) showed that the ability of photosynthetic activity to recover after relief of water stress is dependent on leaf age. However, this is not the case in the present study where both mature and young leaves fully recovered the rates of photosynthesis after rehydration, indicating that damage to the photosynthetic apparatus did not occur under this level of water stress.

Mature leaves of both well-watered and water-stressed plants had lower F_v/F_m, q_P, Φ_{PS2}, and ETR values than young leaves (Table 1). F_v/F_m is affected by treatments that interfere directly with the state of PS2 reaction centre polypeptides. The lower PS2 activity in old leaves was attributed to loss of polypeptides (Bertamini and Nedunchezhian 2002). The water stress did not affect the PS2 photochemistry of dark adapted leaves (Table 1). Panković *et al.* (1999) also found no changes in F_v/F_m in water-stressed sunflower plants in spite of 50 % reduction in photosynthesis. However, after rehydration the young leaves had higher F_v/F_m values than the control leaves.

The evaluation of PS2 photochemistry during steady-state of photosynthesis, in light-adapted leaves, shows that neither Φ_{PS2} nor the apparent ETR was affected by water deficit in both mature and young leaves (Table 1). This is confirmed by other studies which report that reductions in photosynthesis are not accompanied by parallel decrease in Φ_{PS2} or electron transport rates (Flexas *et al.* 1999). Thus, electron transport was not limiting photosynthesis of sunflower plants under these water stress conditions. The maintenance of electron flux is probably diverted to other sinks such as photorespiration (Flexas and Medrano 2002a, Haupt-Herting and Fock 2002), thus avoiding photodamage by the excess electron transport when photosynthesis is inhibited. Photochemical quenching in both mature and young leaves remained unchanged under water stress. Increase in total q_N as a safety dissipation of excess excitation energy in order to protect the photosynthetic apparatus was obseved only in mature leaves and they fully recovered after rehydration (Table 1).

The osmotic adjustment includes accumulation of a variety of compounds which are species and leaf age dependent. One of the most studied solutes is the amino acid proline and high proline content in plants under water stress is frequently observed in several species (Clifford *et al.* 1998, Bajji *et al.* 2001). Proline may act as a regulatory or signaling molecule to activate multiple responses that are part of the adaptation process (Maggio *et al.* 2002). Also, proline is a reliable indicator of the environmental stress imposed to plants (Claussen 2005). In this experiment, both mature and young leaves of control plants had similar proline content (Table 1). The evaluation of changes in proline content shows that a great and significant increase in proline content was observed in both mature and young stressed leaves. However, the young stressed leaves synthesized nearly seven times more proline than non-stressed leaves while the mature stressed leaves synthetized it only four times more. After rewatering, the synthesis of proline in both young and mature leaves returned to the initial content. These findings support a positive role of proline as an osmoregulator, particularly in young leaves, which seems to act as a survival mechanism for the plants under water stress.

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