PHOTOSYNTHETICA 40 (3): 363-368, 2002

Changes in chlorophyll fluorescence during the course of photoperiod and in response to drought in *Casuarina equisetifolia* Forst. and Forst.

R. MARTÍNEZ-CARRASCO, J. SÁNCHEZ-RODRIGUEZ, and P. PÉREZ

Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC, Apdo. 257, 37071 Salamanca, Spain

Abstract

The effects of drought and the diurnal changes in photosynthetic electron transport were studied in non-nodulated plants of *Casuarina equisetifolia*. The induction of fluorescence showed a slightly higher I step in water-stressed than control plants, and the time from the start of irradiation to the P step of induction was significantly shortened by drought. The quantum efficiency of photosystem 2 (PS2) in the dark-adapted state (F_v/F_m) was generally not affected by drought, whereas it decreased during the central hours of the day. The decrease in quantum yield of PS2 electron transport (Φ_2) in water-stressed plants was associated with decreases in the photochemical efficiency of open (oxidised) PS2 centres (F_v'/F_m') and increases in non-photochemical quenching (q_N) rather than with increased closure of PS2 centres (lowered photochemical quenching, q_P). In contrast, the changes in quantum yield of electron transport during the day were related to changes in q_P rather than in F_v'/F_m'. When chlorophyll fluorescence was measured at the same irradiance during the day, a greater q_N was observed at the end of the drying cycle than after watering, and early and late in the photoperiod than in the central hours of the day. The greater q_N at the beginning and end of the day did not prevent an increase in energy not used photochemically nor dissipated non-photochemically. Drought did not affect this excess of photon energy.

Additional key words: energy dissipation; photochemical and non-photochemical quenching; photosystem 2; quantum yield; water stress.

Introduction

Decreased photosynthetic rate under water stress may be the result of stomatal closure. This conclusion is based on estimates of intercellular CO_2 concentration from measurements of ^{18}O exchange (Tourneux and Peltier 1995) or chlorophyll (Chl) fluorescence (Dai *et al.* 1992, Sánchez-Rodríguez *et al.* 1999), as well as on studies in which stomatal limitations were overcome by high CO_2 concentrations (Quick *et al.* 1992, Brestic *et al.* 1995, Tourneux and Peltier 1995). Low availability of terminal acceptors (particularly CO_2) as a consequence of drought potentially damages the photosynthetic apparatus (Lawlor and Cornic 2002) although the decrease in photochemical efficiency of PS2 at low leaf water contents (Giardi *et al.* 1996, Calatayud *et al.* 2000) may also be caused by a down-regulation of the capacity of PS2 electron transport (Osmond 1994). Most of the decrease in photon energy use for photochemistry during drought can be explained in terms of reductions in the efficiency of electron capture by open PS2 centres (Cornic 1994), involving increases in the non-photochemical dissipation of excess excitation energy (Osmond 1994). In contrast, the relative concentration of open PS2 centres (q_P) is not decreased by drought (Lawlor and Cornic 2002). Non-photochemical dissipation of excess energy requires the presence of a trans-thylakoid pH gradient as well as the de-epoxidised forms of the xanthophyll cycle, zeaxanthin and antheraxanthin (Horton *et al.* 1994, Demmig-Adams and Adams 1996).

Under natural conditions, plants often undergo periods when they receive more radiation than they can use in photosynthetic electron transport. Coping with this excess involves the capacity for safe dissipation of non-photo-

Received 4 April 2002, *accepted* 22 July 2002.
Fax: +34923219606; e-mail: rafaelmc@usal.es.
Abbreviations: Chl – chlorophyll; F_0, F_m – minimal and maximal fluorescence levels in the dark adapted state, respectively; F_0', F_m', F_s – minimal, maximal, and steady-state fluorescence in the light adapted state, respectively; Φ_2 – quantum yield of photosystem 2 electron transport; PS2 – photosystem 2; q_P, q_N – photochemical and non-photochemical quenching coefficients, respectively; RuBP – ribulose-1,5-bisphosphate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase.
Acknowledgments: This work was funded by the European Union (TS3*-ct91-0027). J.S.-R. was the recipient of a CSIC grant. The technical assistance of Mr. A. Verdejo is acknowledged.

chemical excitation energy as heat (Horton *et al.* 1994, Demmig-Adams *et al.* 1996). The decrease in the quantum yield of electron transport under excess irradiation does not result from high levels of reduction (closure) of PS2 centres, but rather from decreases in the efficiency of open PS2 centres, associated with increased heat dissipation (Demmig-Adams and Adams 1996). Thus, two different environmental stresses, drought and excess photons, may cause similar changes in photosynthetic electron transport.

Changes in enzyme amount and activity and in contents of metabolites of the Calvin cycle (Azcón-Bieto 1983, Kobza and Edwards 1987, Kobza and Seemann 1989) during the day affect the rate of carbon assimilation and can consequently alter the rate of photosynthetic electron transport and the fraction of absorbed photons that is dissipated non-photochemically. On the other hand, the amount of assimilates available for export from chloroplast to cytosol diminishes with decreasing water contents, as does sucrose synthesis (Sánchez-Rodríguez *et al.* 1999, Lawlor and Cornic 2002). Thus, it is very unlikely that the accumulation of assimilates under drought would result in feedback inhibition of carbon assimilation, unlike the build-up of saccharides in leaves during the course of the light period.

We measured Chl fluorescence to examine the effect of drought on photosynthetic electron transport in *Casuarina equisetifolia*, a nitrogen-fixing tree from arid regions. Trees were grown in pots and subjected to long-term drought stress. The contribution of PS2 photochemistry to the decreased quantum yield of electron transport was assessed by measuring fluorescence at constant high irradiance during the day and at two stages of drying. We also compared the down-regulation of electron transport caused by drought with the variations in electron transport rate during the light period, which are probably caused by changes in the contents of enzymes and metabolites involved in carbon assimilation.

Materials and methods

Seeds of *C. equisetifolia* Forst. & Forst. germinated in trays containing perlite under 200 μmol m^{-2} s^{-1} irradiation at 25 °C. The seedlings were transferred to hydroponic culture and then planted individually in 2 000 cm^3 pots containing 300 g of perlite and placed in an unheated glasshouse with minimum and maximum temperatures of 12 and 28 °C. The plants were supplied with a nutrient solution containing 4 mM $CaCl_2$, 1.5 mM $MgSO_4$, 0.4 mM Na_2HPO_4, 0.94 mM NaH_2PO_4, 10.1 mM KNO_3, 0.1 mM iron citrate, and micro-nutrients (Hewitt 1966). After a 30-d period of adaptation, half of the pots were allowed to dry to 50 % field capacity. The remaining plants were kept at field capacity as controls. The water lost by evapotranspiration was estimated by weighing the pots. The plants were watered weekly with water and nutrient solution.

The induction of Chl fluorescence was measured soon after the plants had reached the set point of drought. Recently matured branchlets were darkened for 20 min and then the measuring radiation of a fluorimeter (*PAM-2000*, *Walz*, Germany) was switched on for 0.5 s prior to turning on a red actinic LED providing 50 μmol m^{-2} s^{-1} irradiation for 2 s. Twelve plants distributed into two blocks were measured for both control and water-stressed plants.

Following the procedure described by Genty *et al.* (1989), Chl fluorescence was measured at 2-h intervals during the light period at the glasshouse temperature on days 1 (18 and 25 October) and 7 (24 October) of the drying cycle. After darkening the branchlets for 20 min, F_0 was recorded and a saturating pulse of radiation (2 500 μmol m^{-2} s^{-1}) was given for 0.8 s to determine F_m. F_0 and F_m represent, respectively, the minimal and maximal fluorescence in the dark adapted state. Then, the branchlet was irradiated (880 μmol m^{-2} s^{-1}) with "white actinic light" (halogen) and saturating pulses were given every 20 s. Recordings were continued for at least 10 min to reach steady-state. Following this, the branchlet was rapidly covered with a black cloth, the actinic radiation was turned off, and a far-red radiation was turned on for a period of 3 s to rapidly re-oxidise the PS2 centres and measure F_0', the minimum fluorescence in the dark with a non-photochemical quenching similar to that found at steady-state under irradiation. We calculated the maximal photochemical efficiency in the dark-adapted state [$F_v/F_m = (F_m - F_0)/F_0$], the photochemical [$q_P = (F'_m - F_s)/(F'_m - F'_o)$] and non-photochemical [$q_N = (F_m - F'_m)/(F_m - F'_o)$] quenching coefficients, the photochemical efficiency of open PS2 centres [$F'_v/F'_m = (F'_m - F'_o)/F'_m$], and the quantum yield of PS2 electron transport [$\Phi_2 = (F'_m - F_s)/F'_m$], where F_m' is the maximal fluorescence under irradiation and F_s is the steady-state fluorescence in the light. Excess photon energy which does not go into photosynthetic electron transport or thermal dissipation was estimated as F_v'/F_m' $(1 - q_P)$ (Demmig-Adams *et al.* 1996). Three replicate samples were measured for both water-stressed and control plants.

The dark relaxation kinetics of q_N was determined on 3rd and 6th day of the weekly cycle of desiccation. After steady state in the light had been reached, the actinic irradiation was turned off and saturating pulses were applied at increasing time intervals for a period of 15 min in order to record the fluorescence quenching. The *SIMFIT* (W.G. Bardsley, University of Manchester) package was used to fit a function to the quenching *vs.* time data, a sum of two exponential functions showing the best fit and allowing the resolution of two different types of non-photochemical quenching (Sánchez-Rodriguez *et al.* 1997). Four replicate measurements were made. All values were subjected to analyses of variance to test for significant differences between drought-stress and control plants and between hours in a day.

Results and discussion

Fig. 1 shows the kinetics of fluorescence induction of water-stressed and control plants. There was no significant effect of water stress on F_0. Hence step I (or flat PL) in the induction curve was slightly higher in drought-stressed plants. Under the relatively low irradiance used in the measurements (Lázar 1999), the step I represents the closure of Q_B non-reducing PS2 reaction centres (Guenther *et al.* 1990) or inactive PS2 centres (Graan and Ort 1986). Thus, drought tended to increase the proportion of Q_B-non-reducing centres. Although the maximum P was not significantly different with drought as compared to the control (Fig. 1), the time from the start of irradiation to this step was significantly shortened by drought (1.85 and 1.60 s for control and water-stressed branchlets, respectively, $p<0.04$). A decrease in this time may imply an increase in the reducing activity of PS2 (Lázar 1999) or greater antenna size (Öquist and Wass 1988). Therefore, our fluorescence induction results are consistent with our previous finding (Sánchez-Rodriguez *et al.* 1997) that an increase in the efficiency of excitation capture by open PS2 centres occurs at the start of a drought period. Giardi *et al.* (1996) also found that, in addition to a depletion of the PS2 core, drought causes a reorganisation of the PS2 complex, with increases in the light harvesting complex/reaction centre ratio.

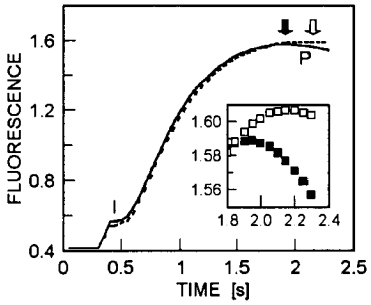

Fig. 1. Induction of fluorescence in well-watered (*broken line*) and water-stressed (*solid line*) plants of *Casuarina equisetifolia* after a 20-min dark adaptation period. Plants were irradiated at 50 μmol m^{-2} s^{-1} for 2 s with red LED's. Letters represent the steps I and P of the fluorescence curve. The time from start of irradiation when maximal fluorescence was reached is *marked with arrows*. *Insert*: final part of curves at an extended scale. *Open symbols* represent well-watered plants and *closed symbols* water-stressed plants.

Maximal photochemical efficiency (F_v/F_m, Fig. 2*A-C*) decreased during the central hours of the day and, soon after re-watering (18 and 25 October) it showed a recovery in the evening. In turn, drought only decreased F_v/F_m significantly on 18 October. At the end of the previous drying cycle (16 October, values not shown), the quantum yield of electron transport showed the lowest values in association with a high fraction of closed PS2 centres (values not shown), indicating a more severe water deficit. The values of F_m thus obtained may not correspond to a maximal F_v/F_m and therefore may not represent the true maximal F_m, due to retention in the dark of high levels of zeaxanthin and antheraxanthin that remain engaged in a state primed for energy dissipation (Adams and Demmig-Adams 1995). However, decrease in q_N in the central hours of the day makes it unlikely that an increased capacity for non-photochemical energy dissipation persisted upon darkening to cause a decrease in F_v/F_m.

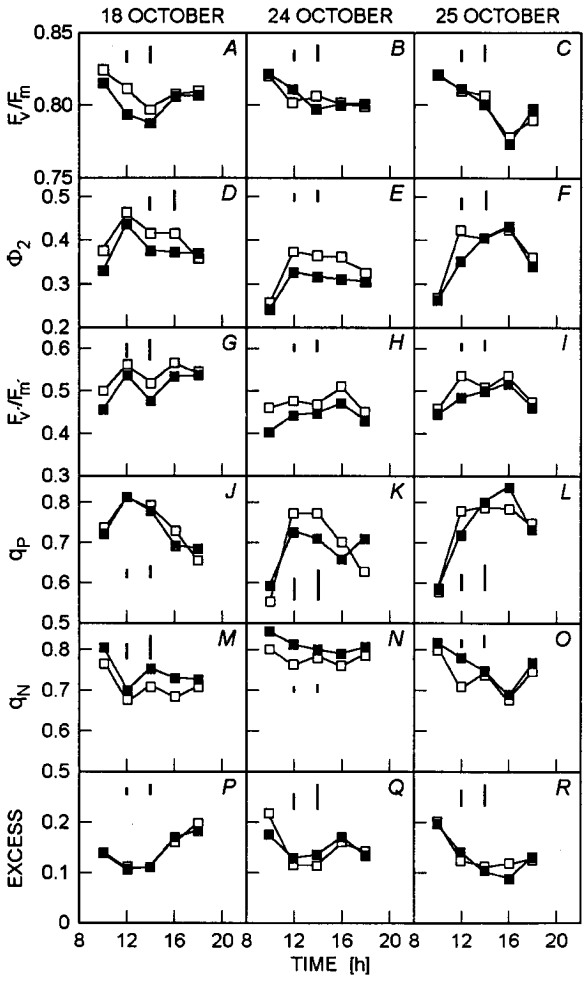

Fig. 2. Diurnal changes in chlorophyll fluorescence parameters of well-watered (*open squares*) and water-stressed (*closed squares*) *Casuarina equisetifolia* plants 1 (18 October) and 7 (24 October) d after watering. *Vertical bars* represent least significant differences ($p<0.05$) between control and drought treatments (*left bar*) and between times of measurement (*right bar*). Branchlets were irradiated with a "white halogen light" providing 880 μmol m^{-2} s^{-1} photon flux density.

To further explore the relation between decreased F_v/F_m and retention in the dark of a high capacity for energy dissipation, we examined the kinetics of dark relaxation of q_N after a period of irradiation on days 3 and

6 after watering (Fig. 3). A greater q_N at higher water deficits (day 6) was correlated to increased values of the fast-relaxing component of non-photochemical quenching, while the slowly relaxing component was similar to that in plants under lower water deficits (day 3). This suggests that in our experiments the capacity for energy dissipation remaining in the dark was not greater at higher water deficits. Thus, at midday and also upon rehydration after a comparatively high water deficit (18 October), the decreased F_v/F_m indicates that plants were unable to harmlessly dissipate the excess of energy and they likely experienced photoinhibition. Conversely, when an adequate capacity for thermal disipation of excitation energy had developed, water stress did not cause chronic photoinhibition (Osmond 1994, Sánchez Rodriguez *et al.* 1997, Lawlor and Cornic 2002) in agreement with previous conclusions that the photosynthetic apparatus is very resistant to drought (Sharkey and Seemann 1989, Cornic 1994, Giardi *et al.* 1996).

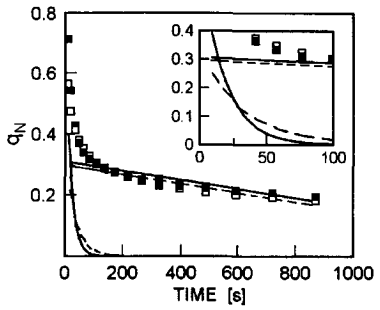

Fig. 3. Dark relaxation kinetics of non-photochemical quenching of chlorophyll fluorescence in *Casuarina equisetifolia* plants 3 (*open squares, broken lines*) and 6 (*closed squares, solid lines*) d after watering. A sum of two exponential functions was fitted to each set of 4 replicate measurements and the curves are shown along with the two resolved components.

The equations are:

for 3 d,

(2.46 E – 01 ± 1.98 E – 02) exp[–(2.77 E – 02 ± 5.15 E – 03) t] + (2.94 E – 01 ± 1.53 E – 02) exp[–(6.39 E – 04 ± 1.24 E – 04) t]

for 6 d,

(3.93 E – 01 ± 1.19 E – 02) exp[–(5.15 E – 02 ± 3.66 E – 03) t] + (3.04 E – 01 ± 6.84 E – 03) exp[–(6.12 E – 04 ± 6.08 E – 05) t].

The first and second exponential terms correspond to the fast- and slow-relaxing components of quenching, respectively. *Insert*: initial part of curves at an extended scale.

Measurements of Chl fluorescence throughout the light period (Demmig-Adams *et al.* 1996, Hymus *et al.* 2001, Barker *et al.* 2002) normally reveal a decrease in PS2 photochemistry with increasing irradiance. Here we observed changes in photochemistry during the day at constant irradiance, pointing to variations in the photochemical capacity. Contrary to the pattern described above, the capacity for the photochemical use of photon energy was higher in the middle of the day than early and late in the day (Fig. 2*D-F*). A lower photochemical capacity about 2 h after dawn probably resulted from insufficient photosynthetic induction in this species. The activation of Calvin cycle enzymes such as RuBPCO (Kobza and Seemann 1989, Pearcy and Seemann 1990) and the contents of photosynthetic intermediates (Kobza and Edwards 1987) may be low at the start of the day and may limit photosynthesis. In turn, at the end of the day, RuBPCO deactivation (Kobza and Seemann 1989) and saccharide accumulation (Azcón-Bieto 1983) could account for the decreased photochemical capacity. Since this pattern was hardly altered under drought, it does not seem to depend on water availability. Thus, although the PS2 photochemistry was minimal at midday, the photochemical capacity was maximal during these hours and decreased at the beginning and end of the photoperiod.

Drought decreased the quantum yield of electron transport (Φ_2, Fig. 2*D-F*). After watering the control and water-stressed plants to field capacity and to half of this value, respectively, the Φ_2 in stressed plants progressively became equal to the values of the control plants (25 October), or, following a more severe water stress on the preceding drying cycle (18 October), Φ_2 remained lower than in the controls. This suggests that a limitation to electron transport persisting after re-watering was overcome only slowly. The decrease in quantum yield of electron transport under drought was due to a lowered efficiency of excitation energy capture by open PS2 centres (F_v'/F_m') on all dates (Fig. 2*G-I*) and, only with increased drought at the end of the drying cycle, also to a tendency of the fraction of open PS2 centres to decrease (q_P, Fig. 2*K*). This effect, however, was not significant. This indicates that the decrease in Φ_2 under drought was primarily due to a reduction in PS2 photochemical efficiency, which presumably reflects an increased thermal dissipation of excess excitation energy before it reaches the reaction centres (Barker *et al.* 2002). Closure of PS2 centres would occur under more extreme drought. In contrast to drought, the changes in quantum yield during the day were more dependent on changes in q_P than in F_v'/F_m'. We speculate this different effect of drought and

time of day on PS2 reaction centre closure may be related to a differential availability of electron transport acceptors. Thus, while under water stress ribulose-1,5-bisphosphate (RuBP) oxygenation can compensate for the decreased use of the products of electron transport in carboxylation (Brestic *et al.* 1995), with increasing accumulation of phosphorylated intermediates and saccharides to a maximum at midday or at the end of the photoperiod, depending on the compound (Cheng *et al.* 1998, Geiger *et al.* 1999), a limitation by triose-phosphate utilisation is likely to restrict both the carboxylation and oxygenation of RuBP, maintaining the electron transport chain and PS2 in a more reduced state.

Non-photochemical dissipation of energy generally increased at the beginning and end of the photoperiod, *i.e.* during the hours of lowest photochemical capacity, hence playing a role in the protection of the photosynthetic apparatus. Greater q_N values were observed at the end of the drying cycle than after re-watering, pointing to an adjustment of thermal dissipation to the use of energy in carbon assimilation. Drought increased q_N (Fig. 2*M-O*). A restricted CO_2 supply through closed stomata will limit ATP and NADPH consumption in the Calvin cycle, causing an increase in the pH gradient through the thylakoids. Increased acidification of the thylakoid lumen is associated with the development of non-photochemical quenching (Ruban and Horton 1999).

Despite the higher non-photochemical energy dissipation at the beginning and end of the photoperiod, the excess energy that is not used in photochemistry nor dissipated non-photochemically (Demmig-Adams *et al.* 1996) showed greater values during these hours (Fig. 2*P-R*). We suggest this could result from an incapacity to increase thermal dissipation of energy to sufficiently high levels. In contrast with this temporal difference, dróught did not significantly alter the excess of unused energy, showing that there was an efficient compensation of the decrease in the photochemical use of photons with in-creases in the thermal dissipation of energy in water-stressed plants.

In conclusion, the comparison of changes in Chl fluorescence during the course of the photoperiod with those caused by drought shows that maximal photochemical efficiency was not decreased by water stress, except under severe drought, while it decreased at midday relative to values at the beginning and end of the light period. Both drought and the progress of time during the day affected the quantum yield of electron transport, the former through changes in the efficiency of excitation energy capture by open PS2 centres and the latter mainly through changes in the fraction of open PS2 centres. Finally, drought did not increase the excess radiant energy not used photochemically nor dissipated non-photochemically, while this excess was greater at the beginning and end of the day than at midday.

References

Adams, W.W., III, Demmig-Adams, B.: The xanthophyll cycle and sustained thermal energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. – Plant Cell Environ. **18**: 117-127, 1995.

Azcón-Bieto, J.: Inhibition of photosynthesis by carbohydrates in wheat leaves. – Plant Physiol. **73**: 681-686, 1983.

Barker, D.H., Adams, W.W., Demmig-Adams, B., Logan, B.A., Verhoeven, A.S., Smith, S.D.: Nocturnally retained zeaxanthin does not remain engaged in a state primed for energy dissipation during the summer in two Yucca species growing in the Mojave Desert. – Plant Cell Environ. **25**: 95-103, 2002.

Brestic, M., Cornic, G., Fryer, M.J., Baker, N.R.: Does photorespiration protect the photosynthetic apparatus in French bean leaves from photoinhibition during drought stress? – Planta **196**: 450-457, 1995.

Calatayud, P.-A., Llovera, E., Bois, J.F., Lamaze, T.: Photosynthesis in drought-adapted cassava. – Photosynthetica **38**: 97-104, 2000.

Cheng, S.H., Moore, B.D., Seemann, J.R.: Effects of short- and long-term elevated CO_2 on the expression of ribulose-1,5-bisphosphate carboxylase/oxygenase genes and carbohydrate accumulation in leaves of *Arabidopsis thaliana* (L.) Heynh. – Plant Physiol. **116**: 715-723, 1998.

Cornic, G.: Drought stress and high light effects on leaf photosynthesis. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis. From Molecular Mechanisms to the Field. Pp. 297-313. Bios Scientific Publ., Oxford 1994.

Dai, Z., Edwards, G.E., Ku, M.S.B.: Control of photosynthesis and stomatal conductance in *Ricinus communis* L. (castor bean) by leaf to air vapor pressure deficit. – Plant Physiol. **99**: 1426-1434, 1992.

Demmig-Adams, B., Adams, W.W., III: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. – Planta **198**: 460-470, 1996.

Demmig-Adams, B., Adams, W.W., III, Barker, D.H., Logan, B.A., Bowling, D.R., Verhoeven, A.S.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plant. **98**: 253-264, 1996.

Geiger, M., Haake, V., Ludewig, F., Sonnewald, U., Stitt, M.: The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. – Plant Cell Environ. **22**: 1177-1199, 1999.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **990**: 87-92, 1989.

Giardi, M.T., Cona, A., Geiken, B., Kucera, T., Masojídek, J., Mattoo, A.K.: Long-term drought stress induces structural and functional reorganization of photosystem II. – Planta **199**: 118-125, 1996.

Graan, T., Ort, D.R.: Detection of oxygen-evolving Photosystem II centers inactive in plastoquinone reduction. – Biochim. biophys. Acta **852**: 320-330, 1986.

Guenther, J.E., Nemson, J.A., Melis, A.: Development of photosystem II in dark grown *Chlamydomonas reinhardtii*. A light-

dependent conversion of PS II_β, Q_B-nonreducing centers to the PS II_α, Q_B-reducing form. – Photosynth. Res. **24**: 35-46, 1990.

Hewitt, E.J.: Sand and water culture methods used in the study of plant nutrition. – Tech. Comm. 22. 2nd Ed. Pp. 430-472. Commonwealth Agricultural Bureau, Farnham Royal 1966.

Horton, P., Ruban, A., Walters, R.G.: Regulation of light harvesting in green plants. Indication of nonphotochemical quenching of chlorophyll fluorescence. – Plant Physiol. **106**: 415-420, 1994.

Hymus, G.J., Dijkstra, P., Baker, N.R., Drake, B.G., Long, S.P.: Will rising CO_2 protect plants from the midday sun? A study of photoinhibition of *Quercus myrtifolia* in a scrub-oak community in two seasons. – Plant Cell Environ. **24**: 1361-1368, 2001.

Kobza, J., Edwards, G.E.: The photosynthetic induction response in wheat leaves: net CO_2 uptake, enzyme activation, and leaf metabolites. – Planta **171**: 549-559, 1987.

Kobza, J., Seemann, J.R.: Regulation of ribulose-1,5-bisphosphate carboxylase activity in response to diurnal changes in irradiance. – Plant Physiol. **89**: 918-924, 1989.

Lawlor, D.W., Cornic, G.: Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. – Plant Cell Environ. **25**: 275-294, 2002.

Lazár, D.: Chlorophyll *a* fluorescence induction. – Biochim. biophys. Acta **1412**: 1-28, 1999.

Öquist, G., Wass, R.: A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. – Physiol. Plant. **73**: 211-217, 1988.

Osmond, C.B.: What is photoinhibition? Some insights from comparisons of shade and sun plants. – In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis. From Molecular Mechanisms to the Field. Pp. 1-24. Bios Scientific Publ., Oxford 1994.

Pearcy, R.W., Seemann, J.R.: Photosynthetic induction state of leaves in a soybean canopy in relation to light regulation of ribulose-1,5-bisphosphate carboxylase and stomatal conductance. – Plant Physiol. **94**: 628-633, 1990.

Quick, W.P., Chaves, M.M., Wendler, R., David, M., Rodrigues, M.L., Passaharinho, J.A., Pereira, J.S., Adcock, M.D., Leegood, R.C., Stitt, M.: The effect of water stress on photosynthetic carbon metabolism in four species grown under field conditions. – Plant Cell Environ. **15**: 25-35, 1992.

Ruban, A.V., Horton, P.: The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach. – Plant Physiol. **119**: 531–542, 1999.

Sánchez-Rodriguez, J., Martínez-Carrasco, R., Pérez, P.: Photosynthetic electron transport and carbon-reduction-cycle enzyme activities under long-term drought stress in *Casuarina equisetifolia* Forst. & Forst. – Photosynth. Res. **52**: 255-262, 1997.

Sánchez-Rodríguez, J., Pérez, P., Martínez-Carrasco, R.: Photosynthesis, carbohydrate levels and chlorophyll fluorescence-estimated intercellular CO_2 in water-stressed *Casuarina equisetifolia* Forst. & Forst. – Plant Cell Environ. **22**: 867-873, 1999.

Sharkey, T.D., Seemann, J.R.: Mild water stress effects on carbon-reduction-cycle intermediates, ribulose bisphosphate carboxylase activity, and spatial homogeneity of photosynthesis in intact leaves. – Plant Physiol. **89**: 1060-1065, 1989.

Tourneux, C., Peltier, G.: Effect of water deficit on photosynthetic oxygen exchange measured using $^{18}O_2$ and mass spectrometry in *Solanum tuberosum* L. leaf discs. – Planta **195**: 570-577, 1995.