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Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought

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Abstract

A gradual reduction in leaf water potential (Ψ_{leaf}), net photosynthetic rate (P_N), stomatal conductance, and transpiration rate was observed in two drought tolerant (C 306 and K 8027) and two susceptible (RW 893 and 899) genotypes subjected to water stress. The extent of reduction was lower in K 8027 and C 306 and higher in RW 893 and RW 899. Rewatering the plants after 5 d of stress restored P_N and other gas exchange traits in all four cultivars. Water stress had no significant effect on variable to maximum fluorescence ratio (F_v/F_m) indicating that water stress had no effect on primary photochemistry of photosystem 2 (PS2). However, water stress reduced the efficiency of excitation energy transfer (F'_v/F'_m) and the quantum yield of electron transport (Φ_{PS2}). The reduction was more pronounced in susceptible cultivars. Water stress had no significant effect on photochemical quenching, however, the non-photochemical quenching increased by water stress.

Additional key words: chlorophyll fluorescence; net photosynthetic rate; stomatal conductance; transpiration rate; *Triticum aestivum*; water potential.

Introduction

Water availability mostly affects growth of leaves and roots, stomatal conductance (g_s), photosynthesis, and dry matter accumulation (Blum 1996). The reduction in photosynthesis as a result of water stress can be attributed to both stomatal and non-stomatal limitations (Graan and Boyer 1990, Shangguan *et al.* 1999). Non-stomatal reduction in photosynthesis is attributed to reduction in RuBP carboxylation efficiency, reduction in RuBP regeneration, or reduction in the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) (Kanechi *et al.* 1995, Tezara and Lawlor 1995). Generally, water stress damages oxygen-evolving complex of photosystem 2 (PS2) (Canaani *et al.* 1986, Toivonen and Vidaver 1988) and PS2 reaction centres (Havaux *et al.* 1988, He *et al.* 1995). However, there are several studies concluding that PS2 photochemistry is not affected by water stress (Cornic and Briantais 1991, Cornic 1994, Liang *et al.* 1997). The PS2 is well protected under water stress (Cornic *et al.* 1989, Jefferies 1994) by increased non-radiative energy dissipation and by increased photorespiration (Heber *et al.* 1996). The kinetics of chlorophyll (Chl) fluorescence, a tool that monitors the function of the photosynthetic apparatus, changes in response to water stress and salinity (Bongi and Loreto 1989, Monneveux *et al.* 1989). At whole plant level, the effect of water stress was perceived as reduction in photosynthesis and growth (Cornic and Massacci 1996, Mwanamwenge *et al.* 1999). We examined the effect of water stress in four wheat cultivars differing in drought tolerance using leaf gas-exchange and Chl fluorescence measurements.

Materials and methods

Plants and water stress treatment: Two wheat (*Triticum aestivum* L.) cultivars, which are more sensitive to drought, and two cultivars, which are less sensitive to drought, were grown in earthen pots containing a mixture

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Abbreviations: Chl = chlorophyll; ETR = apparent photosynthetic electron transport rate ETR; F_m, F_m' = maximum fluorescence of dark- and light-adapted leaves, respectively; F_v, F_v' = minimal fluorescence of dark- and light-adapted state, respectively; P_N = net photosynthetic rate; PPFD = photosynthetic photon flux density; PS2 = photosystem 2; q_P, q_N = coefficients of photochemical and non-photochemical quenching of variable fluorescence, respectively; RuBPCO = ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC = relative water content; Φ_e = efficiency of excitation energy capture by PS2 reaction centre; Φ_{PS2} = *in vivo* quantum yield of PS2 photochemistry; Ψ_{leaf} = leaf water potential.

of soil collected from the farm and sand (2 : 1, v/v) during the normal wheat growing period in greenhouse. Five days after germination, the plants were thinned to 2 plants per pot. Starting from 45 d of sowing, two watering treatments were applied: one group of plants, involving 4 pots per cultivar, were provided with optimal irrigation (control) and the second group, involving 4 pots per cultivar, was subjected to water stress treatment by withholding irrigation. After 5 d of water stress, water stressed plants were re-irrigated and the recovery was studied.

Leaf gas exchange was measured using a *LI-6400* portable photosynthesis measuring system (*LICOR*, USA) with a *6400-02B* LED source providing a photosynthetic photon flux density PPFD of 1 300 µmol m^{-2} s^{-1}. Temperature was maintained at 25 °C, relative humidity at 70 %, and CO_2 concentration at 350 g m^{-3}.

Chl fluorescence was recorded with a pulse amplitude modulation fluorometer (*PAM 2000*, *Walz*, Effeltrich, Germany) at room temperature. The instrument was connected to a leaf-clip holder (*2030-B*, *Walz*) and to a computer with data acquisition software (*DA-2000*, *Walz*). The minimal fluorescence level (F_0) and maximal fluorescence level (F_m) were recorded on attached leaf, predarkened for 20 min, on which the gas exchange was measured. The plants were then exposed to natural sunlight in the greenhouse for approximately 6 min for induction of photosynthesis. The minimal fluorescence level in irradiated state (F'_0) was determined by irradiating the leaf with far-red radiation. By using fluorescence parameters determined on both light- and dark-adapted leaves, the following parameters were calculated: the maximal quantum yield of PS2 photochemistry F_v/F_m, the photochemical quenching coefficient $q_P = (F_m' - F_t)/(F'_m - F'_0)$, non-photochemical quenching coefficient $q_N = 1 - (F'_m - F'_0)/(F_m - F_0)$, the efficiency of excitation capture by open PS2 centres $\Phi_e = F'_v/F'_m$, *in vivo* quantum yield of PS2 photochemistry $\Phi_{PS2} = (F'_v/F'_m \times q_P)$, and apparent photosynthetic electron transport rate ETR = yield × PAR × 0.5 × 0.84, where yield represents the overall photochemical quantum yield [$Y = (F'_m - F'_t)/F'_m$], PAR corresponds to the flux density of incident PPFD [µmol(quantum) m^{-2} s^{-1}], transport of one electron requires absorption of two quanta, as two photosystems are involved (factor 0.5). It is assumed that 84 % of the incident quanta are absorbed by the leaf (factor 0.84).

Leaf water potential was measured using digital pressure chamber (model *1003*, *PMS Instrument Co*, USA).

Results and discussion

Withholding irrigation resulted in gradual reduction of leaf water potential (Ψ_{leaf}) in all the four cultivars studied. However, the reduction was more pronounced in RW 893 and RW 899, which are more sensitive to water stress (Fig. 1). Although Ψ_{leaf} recovered in all the four cultivars tested, the recovery is more pronounced in C 306 and K 8027, which are less sensitive to water stress. However, the recovery in Ψ_{leaf} was only partial in all the four cultivars tested.

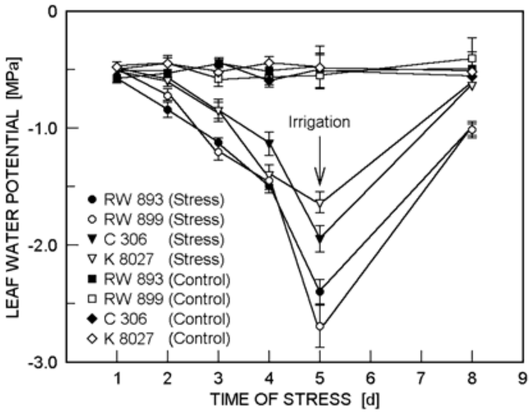

Fig. 1. Effect of water stress on water potential of wheat leaves. Plants were subject to water stress by withholding irrigation. Leaf water potential was determined every day up to 5 d of stress after which the pots were re-irrigated to study recovery and water potential was again measured after 3 d of recovery. Means of four replications ± SE.

The photosynthetic efficiency, transpiration rate (*E*), and g_s were measured during water stress treatment and 3 d after re-watering both in control and stressed plants. Leaf photosynthetic traits did not change appreciably in well watered control plants during the experimental period. In control treatment, significant variation in leaf net photosynthetic rate (P_N) was observed among the four wheat cultivars. P_N was highest in RW 893 [41.5 µmol(CO_2) m^{-2} s^{-1}] and lowest in RW 899 [31.2 µmol(CO_2) m^{-2} s^{-1}].

A gradual reduction in P_N was observed in all four cultivars (Fig. 2*A*). The difference in the reduction in P_N between relatively tolerant and susceptible cultivars was much more apparent under moderate stress condition. The extent of reduction in P_N after 5 d of stress was lower in tolerant cultivars K 8027 (62 %) and C 306 (59 %) and more pronounced in relatively susceptible RW 893 (68 %) and RW 899 (71 %). Re-watering the plants, after 5 d of irrigation deprivation resulted in partial recovery of P_N in all four cultivars. The P_N recovered from 14.7 to 22.9 µmol(CO_2) m^{-2} s^{-1} in K 8027 and in C 306 it recovered from 14.6 to 24.4 µmol(CO_2) m^{-2} s^{-1}. In RW 893 the P_N recovered from 13.2 to 16.8 µmol(CO_2) m^{-2} s^{-1} and in RW 899 from 9.0 to 16.4 µmol(CO_2) m^{-2} s^{-1}. Similar pattern was observed in *E* (Fig. 2*B*) and g_s (Fig. 2*C*).

The Chl fluorescence parameters were measured 4 d after imposition of water stress. Water stress had no significant effect on F_v/F_m (Fig. 3*A*), but it reduced the efficiency of excitation energy transfer Φ_e and the quantum yield of electron transport Φ_{PS2} (Fig. 3*B,C*). The reduction was more pronounced in susceptible cultivars. Imposition of water stress had no significant effect on q_P (Fig. 3*D*). However, non-photochemical quenching (q_N) was increased by water stress (Fig. 3*E*). Apparent electron transport rate (ETR) (Fig. 3*F*) was significantly reduced by water stress. The effect was more pronounced in drought susceptible cultivars than in the less susceptible ones.

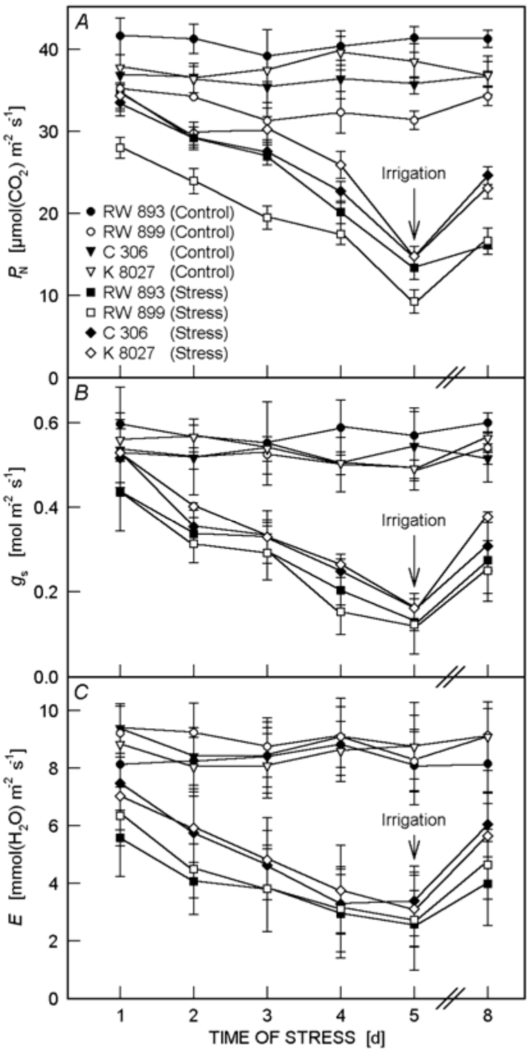

Fig. 2. Effect of water stress on net leaf photosynthetic rate, P_N (*A*), transpiration rate, E (*B*), and stomatal conductance, g_s (*C*). Leaf gas-exchange characteristics were measured every day during water stress treatment and 3 d after re-irrigation to study the recovery. Means of four replications ± SE.

Leaf gas exchange parameters indicate that under progressively increasing soil moisture stress P_N, E, and g_s declined in all cultivars tested. Drought rapidly reduces the expansion of leaves and g_s and may eventually impact primary events in the photosynthetic apparatus (Passioura 1994). Stomatal closure under water stress is one of the most important factors affecting photosynthesis. However, under severe stress, photosynthesis may be controlled by chloroplast capacity to fix CO_2 rather than by increased diffusive resistance (Herppich and Peckmann 1997). Non-stomatal reduction in photosynthesis is attributed to reduction in RuBP carboxylation efficiency, reduction in RuBP regeneration, or reduction in the amount of RuBPCO (Kanechi *et al.* 1995, Tezara and Lawlor 1995). Genotypic differences related to drought tolerant traits were reported in wheat (Labhilli *et al.* 1995). Loggini *et al.* (1999) reported that photosynthesis of drought sensitive and drought tolerant wheat cultivars responds differently to water stress imposed. Our data on leaf gas exchange indicate that the inhibition in photosynthesis and other related traits was more pronounced in relatively tolerant cultivars than in the relatively susceptible ones.

Chl fluorescence method is one of the important tools in stress physiology and environmental research (Krause and Weis 1991). Imposition of water stress did not result in discernible changes in dark adapted fluorescence parameters indicating that water stress had not influenced the primary photochemistry of PS2 and energy distribution within the light-harvesting complex (Flagella *et al.* 1994, Lu and Zhang 1999). However, in leaves adapted to irradiance, water stress imposition resulted in reduced Φ_{PS2} and Φ_e. Since water stress did not affect q_P, the reduction in Φ_{PS2} was mainly due to the reduction in Φ_e (F'_v/F'_m). The reduction in F'_v/F'_m may reflect the light-induced non-photochemical quenching. Reduction in F'_v/F'_m could be interpreted as increase in thermal energy dissipation (Schindler and Lichtenthaler 1996). We found that q_P showed very little reduction under stress. Conflicting results were reported in the literature regarding the effect of water stress on q_P. A linear reduction in q_P with decreasing relative water content (RWC) was reported by Marques da Silva and Arrabaca (2004). Loreto *et al.* (1995) observed a decrease in q_P under water stress in *Sorghum bicolor* leaves. Havaux *et al.* (1988) observed a significant reduction in q_P and this reduction was attributed to high actinic irradiance adopted in their experiment (Flagella *et al.* 1994). However, Campos (1998) reported no effect of water stress on q_P in *Vigna glabrescens.* Similar results were reported by Flagella *et al.* (1994) and Lu and Zhang (1999) in wheat. In our experiments q_N increased with water stress. An increase in q_N induced by water stress was reported in wheat (Lu and Zhang 1999). Gulías *et al.* (2002) reported an increase in NPQ and reduction in ETR in *Rhamnus ludovici-salvatoris* and other Mediterranean tree species. A sharp increase in q_N under moderate stress was reported by Marques da Silva and Arrabaca (1993). Furthermore, Schreiber and Bilger (1987) found in *Arbutus unedo* an increment of q_N at 60 % RWC but a decrease at 36 %

RWC. The increased q_N could dissipate some excitation energy at the expense of photochemical utilization (Brestic *et al.* 1995). The non-photochemical quenching of fluorescence related to photoinhibition (q_I) may be excluded since the plants were grown in moderate irradiance. Thus the differences in q_N reflect what happened in its main component, q_E. The high-energy state quenching (q_E) is probably the most important mechanism of non-photochemical energy dissipation (Horton *et al.* 1988) being related with the increase of proton concentration in the interior of the thylakoids (Quick and Stitt 1989). When the rate of photosynthesis is low, due to imposed water stress, the turnover and regeneration of ATP are slow, because the proton concentration in the lumen of thylakoids is high. This increases the heat dissipation in PS2. The increase in non-photochemical quenching of variable fluorescence was due to an increased rate constant of thermal dissipation of excitation energy and this increase represents a mechanism to down regulate photosynthetic electron transport and match utilization of NADPH and ATP under reduced photosynthesis (Lu and Zhang 1999, Subrahmanyam and Rathore 2000).

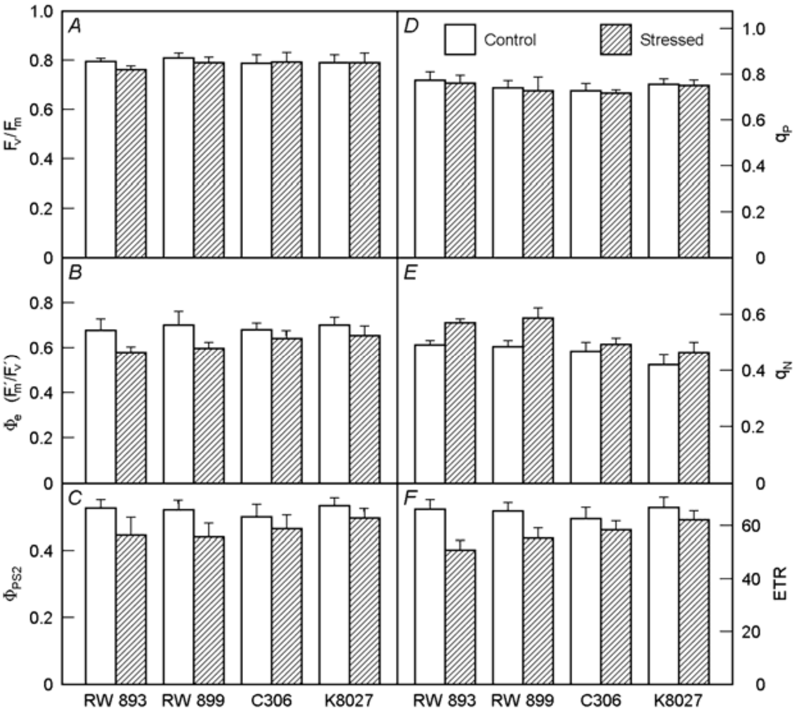

Fig. 3. Effect of water stress on (*A*) the maximal quantum yield of photosystem 2 (PS2) photochemistry F_v/F_m, (*B*) the efficiency of excitation capture by open PS2 centres Φ_e (F'_v/F'_m), (*C*) *in vivo* quantum yield of PS2 photochemicstry Φ_{PS2} ($F'_v/F'_m \times q_P$), (*D*) coefficient of photochemical quenching (q_P), (*E*) coefficient of non-photochemical quenching (q_N), and (*F*) apparent electron transport rate (ETR). Fluorescence parameters were measured 4 d after imposition of water stress. Means of four replications ± SE.

In conclusion, our results indicate that the relative tolerance to water stress in genotypes C 306 and K 8027 could be due to their ability to maintain high leaf water potential, high leaf P_N, and due to their ability to recover substantially more than the susceptible RW 893 and RW 899 which incidentally had more rapid reduction of P_N.

References

Blum, A.: Crop response to drought and the interpretation of adaptation. – Plant Growth Regul. **20**: 135-148, 1996.

Bongi, G., Loreto, F.: Gas-exchange properties of salt-stressed olive (*Olea europea* L.) leaves. – Plant Physiol. **90**: 1408-1416, 1989.

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.

Campos, P.: Effects of Water Stress on Photosynthetic Performance and Membrane Integrity in *Vigna* spp. – the Role of Membrane Lipids in Drought Tolerance. – Ph.D. Thesis. University of Lisbon, Lisbon 1998.

Canaani, C., Havaux, M., Malkin, S.: Hydroxylamine, hydraine and methylamine donate electrons to photooxidizing side of PSII in leaves inhibited in oxygen evolutioin due to water stress. – Biochim. biophys. Acta **851**: 151-155, 1986.

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

Cornic, G., Briantais, J.-M.: Partioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. – Planta **183**: 178-184, 1991.

Cornic, G., le Gouallec, J.-L., Briantais, J.-M., Hodges, M.: Effect of dehydration and high light on photosynthesis of two C_3 plants (*Phaseolus vulgaris* L. and *Elatostema repens* (Lour) Hall f.). – Planta **177**: 84-90, 1989.

Cornic, G., Massacci, A.: Leaf photosynthesis under drought stress. – In: Baker, N.R. (ed.): Photosynthesis and Environment. Pp. 347-366. Kluwer Academic Publ., Dordrecht – Boston – London 1996.

Flagella, Z., Pastore, D., Campanile, R.G., Di Fonzo, N.: Photochemical quenching of chlorophyll fluorescence and drought tolerance in different durum wheat (*Triticum durum*) cultivars. – J. agr. Sci. **122**: 183-192, 1994.

Graan, T., Boyer, J.S.: Very high CO_2 partially restores photosynthesis in sunflower at low leaf water potentials. – Planta **181**: 378-384, 1990.

Gulías, J., Flexas, J., Abadía, A., Medrano, H.: Photosynthetic response to water deficit in six Mediterranean sclerophyll species: possible factors explaining the declining distribution of *Rhamnus ludovici-salvatoris,* an endemic Balearic species. – Tree Physiol. **22**: 687-697, 2002.

Havaux, M., Ernez, M., Lannoye, R.: Correlation between heat tolerance and drought tolerance in cereals demonstrated by rapid chlorophyll fluorescence tests. – J. Plant Physiol. **133**: 555-560, 1988.

He, J.X., Wang, J., Liang, H.G.: Effects of water stress on photochemical function and protein metabolism of photosystem II in wheat leaves. – Physiol. Plant. **93**: 771-777, 1995.

Heber, U., Bligny, R., Streb, P., Douce, R.: Photorespiration is essential for the protechion of the photosynthetic apparatus of C3 plants against photoinhibition under sunlight. – Bot. Acta **109**: 307-315, 1996.

Herppich, W.B., Peckmann, K.: Responses of gas exchange, photosynthesis, nocturnal acid accumulation and water relations of *Aptenia cordifolia* to short-term drought and re-watering. – J. Plant Physiol. **150**: 467-474, 1997.

Horton, P., Oxborough, K., Rees, D., Scholes, J.D.: Regulation of the photochemical efficiency of photosystem II; consequences for the light response of field photosynthesis. – Plant Physiol. Biochem. **26**: 453-460, 1988.

Jefferies, R.A.: Drought and chlorophyll fluorescence in field-grown potato (*Solanum tuberosum*). – Physiol. Plant. **90**: 93-97, 1994.

Kanechi, M., Kunitomo, E., Inagaki, N., Maekawa, S.: Water stress effects on ribulose-1,5-bisphosphate carboxylase and its relationship to photosynthesis in sunflower leaves. – In: Mathis, P. (ed.): Photosyntheis: from Light to Biosphere. Vol. IV. Pp. 597-600. Kluwer Academic Publishers, Dordrecht – Boston – London 1995.

Krause, G.H., Weis, F.: Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Lauer, M.J., Boyer, J.S.: Internal CO_2 measured directly in leaves. Abscisic acid and low leaf water potential cause opposing effects. – Plant Physiol. **98**: 1310-1316, 1992.

Labhilli, M., Jouchier, P., Gautier, M.F.: Characterization of cDNA encoding *Triticum duram* dehydrins and their expression patterns in cultivars that differ in drought tolerance. – Plant Sci. **112**: 219-230, 1995.

Liang, J., Zhang, J., Wong, M.H.: Can stomatal closure caused by xylem ABA explain the inhibition of leaf photosynthesis under soil drying? – Photosynth. Res. **51**: 149-159, 1997.

Loggini, B., Scartazza, A., Brugnoli, E., Navari-Izzo, F.: Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought stress. – Plant Physiol. **119**: 1091-1099, 1999.

Loreto, F., Tricoli, D., Di Marco, G.: On the relationship between electron transport rate and photosynthesis in leaves of the C_4 plant *Sorghum bicolor* exposed to water stress, temperature changes and carbon metabolism inhibition. – Aust. J. Plant Physiol. **22**: 885-892, 1995.

Lu, C., Zhang, J.: Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. – J. exp. Bot. **50**: 1199-1206, 1999.

Marques da Silva, J., Arrabaca, M.C.: Effects of rapid dehydration on modulated fluorescence emission by leaves of *Setaria sphacelata*. – In: Sanchez Diaz, M., Pereira, J., Medrano, H. (ed.): Actas del 1st Simposium Hispano-Portugues Relaciones Hidricas en las Plantas. Pp. 181-184. UIB/CSIC, Palma de Mallorca 1993.

Marques da Silva, J., Arrabaca, M.C.: Photosynthesis in the water-stressed C_4 grass *Setaria sphacelata* is mainly limited by stomata with both rapidly and slowly imposed water deficits. – Physiol. Plant. **121**: 409-420, 2004.

Monneveux, P., Monneveux, P., Damania, A.B.: Chlorophyll fluorescence as a predictive test for salt tolerance in cereals: preliminary results on durum wheat. – Rachis **8**: 16-19, 1989.

Mwanamwenge, J., Loss, S.P., Siddique, K.H.M., Cocks, P.S.: Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L.). – Eur. J. Agron. **11**: 1-11, 1999.

Passioura, J.B.: The yield of crops in relation to drought. – In: Boote, K.J., Bennett, J.M., Sinclair, T.R., Paulsen, G.M. (ed.): Physiology and Determination of Crop Yield. Pp. 365-389. ASA, CSSA, SSSA, Madison 1994.

Quick, W.P., Stitt, M.: An examination of factors contributing to non-photochemical quenching of chlorophyll fluorescence in barley leaves. – Biochim. biophys. Acta **977**: 287-296, 1989.

Schindler, C., Lichtenthaler, H.K.: Photosynthetic CO_2-assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and a cloudy day. – J. Plant Physiol. **148**: 399-412, 1996.

Schreiber, U., Bilger, W.: Rapid assessment of stress effects on plant leaves by chlorophyll fluorescence measurements. – In: Tenhunen, J.D., Catarino, F.M., Lange, O.L., Oechel, W.C. (ed.): Plant Response to Stress. Pp. 27-53. Springer-Verlag, Berlin – Heidelberg – New York – London – Paris – Tokyo 1987.

Shangguan, Z., Shao, M., Dyckmans, J.: Interaction of osmotic adjustment and photosynthesis in winter wheat under soil drought. – J. Plant Physiol. **154**: 753-758, 1999.

Subrahmanyam, D., Rathore, V.S.: Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. – Photosynthetica **38**: 449-453, 2000.

Tezara, W., Lawlor, D.W.: Effects of water stress on the biochemistry and physiology of photosynthesis in sunflower. – In: Mathis, P. (ed.): Photosynthesis: from Light to Biosphere. Vol. IV. Pp. 625-628. Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Toivonen, P., Vidaver, W.: Variable chlorophyll *a* fluorescence and CO_2 uptake in water stressed white spruce seedlings. – Plant Physiol. **86**: 744-748, 1988.