PHOTOSYNTHETICA 47 (2): 304-308, 2009

BRIEF COMMUNICATION

Effects of rapidly imposed water deficit on photosynthetic parameters of three C_4 grasses

A.S. SOARES-CORDEIRO[*,+], A.E. CARMO-SILVA[*,**], A. BERNARDES DA SILVA[*], J. MARQUES DA SILVA[*], A.J. KEYS[**], and M.C. ARRABAÇA[*]

Centro de Engenharia Biológica and Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal[*]
Department of Plant Sciences, Centre for Crop Genetic Improvement, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK[**]

Abstract

Water deficit, when rapidly imposed on three C_4 grasses of the different metabolic subtypes, *Paspalum dilatatum* Poiret (NADP-malic enzyme), *Cynodon dactylon* (L.) Pers (NAD-malic enzyme) and *Zoysia japonica* Steudel (phospho*enol*pyruvate carboxykinase), caused decreases in photosynthetic rates, in the quantum yield of PS II and photochemical quenching, and in the activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phospho*enol*pyruvate carboxylase (PEPC). The results provide evidence for non-stomatal limitations of photosynthesis differing in nature between the three species.

Additional key words: chlorophyll *a* fluorescence; drought stress; NAD-malic enzyme; NADP-malic enzyme; phospho*enol*pyruvate carboxykinase; phospho*enol*pyruvate carboxylase; photosynthetic capacity; Rubisco.

Many plants, in their natural environment, are subjected to cycles of water stress and recovery, which may become more severe with global climate change. C_4 plants are reputed to be better adapted to higher temperatures and drought than C_3 plants (Long 1999). Decreased net photosynthesis by C_4 plants under water deficit (Lal and Edwards 1996, Heckathorn *et al.* 1997, Ghannoum *et al.* 2003, Marques da Silva and Arrabaça 2004a, Carmo-Silva *et al.* 2007) may result from stomatal closure alone or together with non-stomatal factors. The photochemical apparatus of C_4 plants is resistant to moderate water deficit (Loreto *et al.* 1995, Ghannoum *et al.* 2003, Marques da Silva and Arrabaça 2004a) but may be impaired under severe drought. Studies of the effect of dehydration on carboxylating and decarboxylating enzyme activities in C_4 plants (Du *et al.* 1996, Saccardy *et al.* 1996, Foyer *et al.* 1998, Castrillo *et al.* 2001, Marques da Silva and Arrabaça 2004b,) have produced conflicting results concerning possible roles in the limitation of photosynthesis.

Photosynthetic rate measured by oxygen evolution at saturating CO_2 and high irradiance should be limited mainly by non-stomatal factors. We investigated the effects of water deficit on various photosynthetic parameters in three C_4 grasses belonging to different metabolic subtypes: *Paspalum dilatatum* Poiret (NADP-malic enzyme, NADP-ME), *Cynodon dactylon* (L.) Pers (NAD-malic enzyme; NAD-ME) and *Zoysia japonica* Steudel (phospho*enol*pyruvate carboxykinase; PEPCK). The main objectives were to compare the response of the

---

Received 23 October 2008, *accepted* 9 June 2009.

[+] Corresponding author; fax: 00351 21 750 00 48, e-mail: anascs@netcabo.pt

Abbreviations: DM – dry mass; NAD-ME – NAD-malic enzyme; NADP-ME – NADP-malic enzyme; NPQ – non-photochemical quenching; PEPC – phospho*enol*pyruvate carboxylase; PEPCK – phospho*enol*pyruvate carboxykinase; P_{max} – rate of photosynthesis at saturating CO_2 and high irradiance; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_P – photochemical quenching; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC – leaf relative water content; V_{max} – PEPC maximal activity; V_t – Rubisco total activity; Φ_{PSII} – effective quantum yield of PS II electron transport.

Acknowledgements: This work was partially supported by 'Programa de Desenvolvimento Educativo para Portugal' (PRODEP III) and by 'Federação Portuguesa de Golfe'. The authors thank Margot Forde Forage Germplasm Centre, New Zealand, and Dr. Daniel Ribeiro, Geodesenho, Portugal, for providing seeds of the C_4 grasses. The authors thank Ms. Manuela Lucas, Centro de Engenharia Biológica, Faculdade de Ciências da Universidade de Lisboa, Portugal, for technical assistance. A.S. Soares-Cordeiro and A.E. Carmo-Silva contributed in equal parts to the work presented.

three species to rapidly induced water deficit and to identify photochemical and/or metabolic processes causing decreased CO_2- and light-saturated photosynthesis.

Plants of the three species were grown hydroponically and subjected to a rapidly imposed water deficit with polyethylene glycol 4000, as previously described (Carmo-Silva *et al.* 2007). Measurements were made on the widest part of young fully expanded leaves, equidistant from the two ends, and started 20 to 28 h after water deficit induction and two hours after the beginning of the photoperiod. The leaf relative water content (RWC) was determined according to Čatský (1960). The rate of photosynthesis (P_{max}) was measured by O_2 evolution using a gas-phase oxygen electrode (*LD2*, *Hansatech Instruments Ltd*, King's Lynn, Norkfolk, UK) at saturating CO_2 (0.07 $m^3\ m^{-3}$) (Marques da Silva and Arrabaça 2004a), at 25 ºC and at a photosynthetic photon flux density (PPFD) of 1100 μmol(photon) $m^{-2}\ s^{-1}$ (*LS2 Light Source*, *Hansatech Instruments Ltd*, King's Lynn, Norkfolk, UK). The leaf fresh mass, dry mass (DM) and area (portable area meter *LI-3000*, *LI-COR Inc.*, Lincoln, Nebraska, USA) enclosed in the electrode chamber were determined. Chlorophyll *a* fluorescence parameters were measured at room temperature and at ambient atmospheric CO_2 using a pulsed amplitude modulation fluorometer (*PAM-210* with DA-TEACH 1.01 software, *Heinz Walz GmbH*, Effeltrich, Germany) as previously described (Carmo-Silva *et al.* 2007), but with an actinic

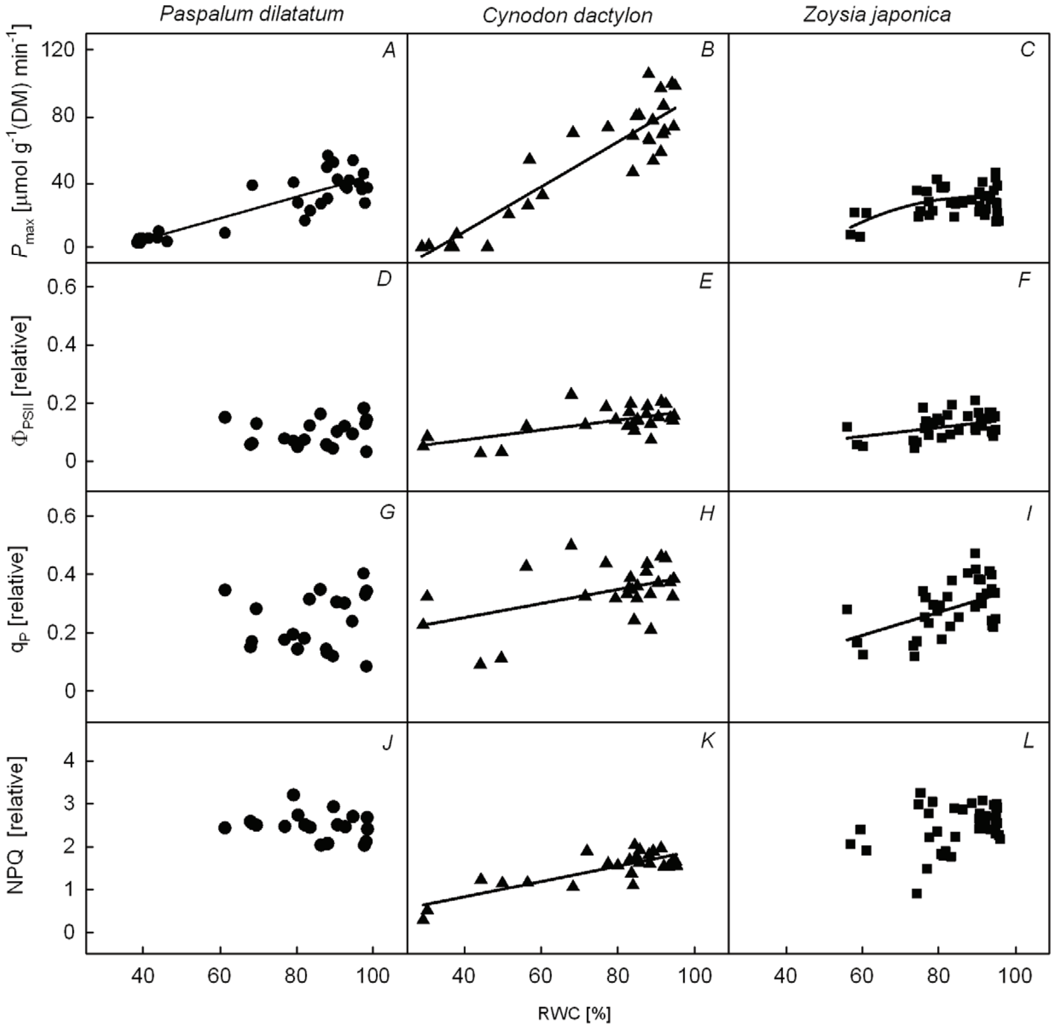

Fig. 1. Rate of photosynthesis at saturating CO_2 and high irradiance (P_{max}) (*A–C*), effective quantum yield of PSII electron transport (Φ_{PSII}) (*D–F*), photochemical quenching (q_P) (*G–I*) and non-photochemical quenching (NPQ) (*J–L*) at different leaf relative water contents (RWC) in the leaves of *Paspalum dilatatum* (*A*, $y = 0.65\ x - 21.7$, $r^2 = 0.73$; *D*; *G*; *J*), *Cynodon dactylon* (*B*, $y = 1.36\ x - 45.23$, $r^2 = 0.83$; *E*, $y = 0.0017\ x + 0.0057$, $r^2 = 0.40$; *H*, $y = 0.0024\ x + 0.156$, $r^2 = 0.22$; *K*, $y = 0.018\ x + 0.093$, $r^2 = 0.66$) and *Zoysia japonica* (*C*, $y = -0.018\ x^2 + 3.14\ x - 109.62$, $r^2 = 0.26$; *F*, $y = 0.0014\ x + 0.0036$, $r^2 = 0.14$; *I*, $y = 0.0039\ x - 0.049$, $r^2 = 0.23$; *L*). Gas-exchange measurements were performed at 25 ºC, 0.07 $m^3\ m^{-3}$ CO_2 and a PPFD of 1100 μmol(photon) $m^{-2}\ s^{-1}$. Chlorophyll *a* measurements were obtained under an actinic light intensity of 1850 μmol(photon) $m^{-2}\ s^{-1}$. Each data point represents a different sample.

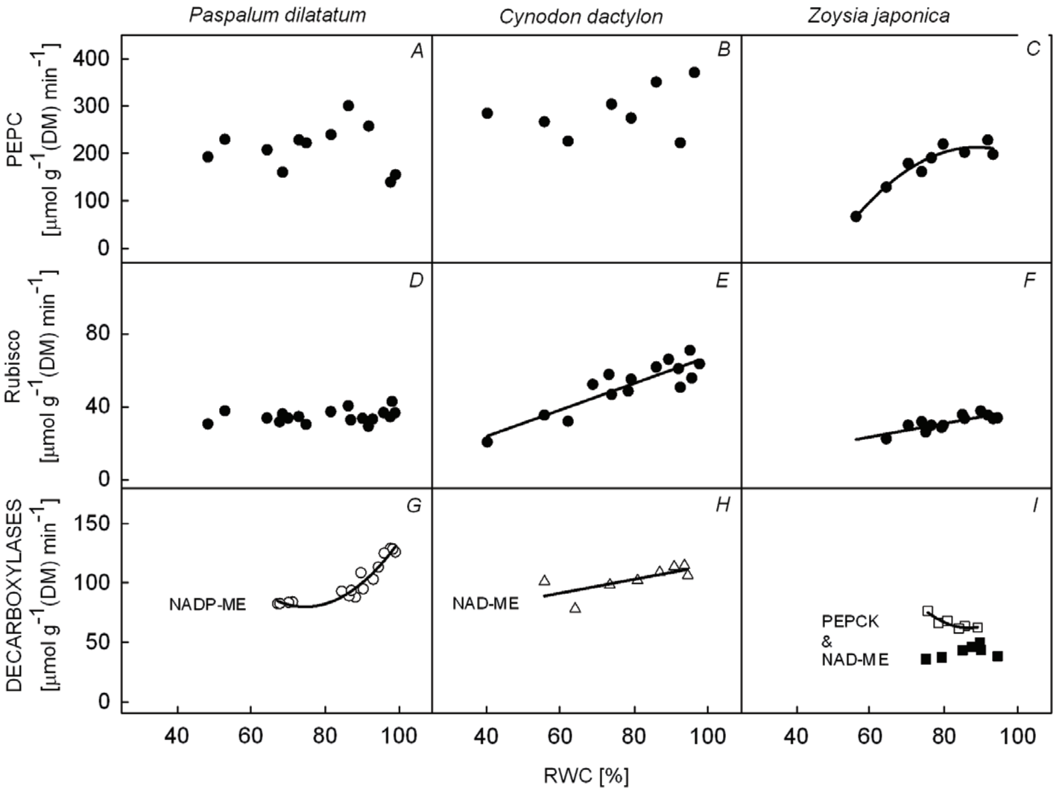

Fig. 2. Activities of PEPC (*A–C*), Rubisco (*D–F*) and of the decarboxylases NADP-ME (*open circles*), NAD-ME (*open triangles and closed squares*) and PEPCK (*open squares*) (*G–I*), at different leaf relative water contents (RWC) in the leaves of *Paspalum dilatatum* (*A*; *D; G*, y = 0.08 x^2 – 12.76 x + 556.32, r^2 = 0.93), *Cynodon dactylon* (*B*; *E*, y = 0.67 x – 4.86, r^2 = 0.78; *H*, y = 0.58 x + 57.10, r^2 = 0.51) and *Zoysia japonica* (*C*, y = –0.14 x^2 + 24.34 x – 866.0, r^2 = 0.93; *F*, y = 0.34 x + 1.36, r^2 = 0.34; *I*, y = 0.11 x^2 – 18.25 x + 852.75, r^2 = 0.87). Each data point represents a different sample.

light of 1850 µmol(photon) m^{-2} s^{-1}. Plants were dark-adapted for 4 min, then illuminated for 5 min before determining the steady-state value of fluorescence. A saturating light of 4000 µmol(photon) m^{-2} s^{-1} for 0.9 s was used for the measurements. Where necessary, leaves of *Z. japonica* were uncurled. Leaf samples for enzyme activity measurements were collected inside the growth chamber into liquid nitrogen and stored at –80 ºC. Crude extracts for measuring the activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39), phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31), NADP-ME (EC 1.1.1.40), NAD-ME (EC 1.1.1.39) and PEPCK (EC 4.1.1.49) were obtained as described previously by Carmo-Silva *et al.* (2007), except that, for PEPCK, 200 mM Bicine-KOH (pH 9.8) was used and 50 mM DTT was added, as proposed by Walker *et al.* (2002). Triplicate measurements of activities were made on each leaf extract. PEPC maximal activity (V_{max}) was measured spectrophotometrically at 340 nm and 25 ºC according to Bakrim *et al.* (1992) with some modifications (Carmo-Silva *et al.* 2007). Rubisco total activity (V_t) was assayed at 25 ºC by $^{14}CO_2$ incorporation into acid-stable products by the method of Parry *et al.* (1997). The decarboxylating enzymes activities were measured essentially as described by Ashton *et al.* (1990) for NADP-ME and NAD-ME and by Walker *et al.* (2002) for PEPCK. Regression analysis was applied to assess the changes induced by leaf dehydration on each measured parameter using the *Statistical Package for Social Sciences* (*SPSS*) 12.0, 2003 (*SPSS Inc.*, Chicago, Illinois, USA). Models that best explained the results were selected on the basis of an *F* test of probability ($p<0.05$).

Decreased P_{max} with water deficit in all three species (Fig. 1*A–C*) suggested the involvement of non-stomatal factors in the limitation of photosynthesis. In *P. dilatatum* and *C. dactylon*, P_{max} decreased linearly, as reported by Marques da Silva and Arrabaça (2004a), but in *Z. japonica* P_{max} decreased only when RWC fell below *ca.* 80%, as observed by Lal and Edwards (1996).

Chlorophyll *a* fluorescence indicated photochemical impairment at high irradiance in dehydrated leaves of *C. dactylon* and *Z. japonica*, but not in *P. dilatatum* (Fig. 1*D–L*). The decrease in the effective quantum yield of PS II electron transport (Φ_{PSII}) in *C. dactylon* and *Z. japonica* (Fig. 1*E,F*) may have contributed to the decrease in P_{max} with drought. Ghannoum *et al.* (2003) and Marques da Silva and Arrabaça (2004a) reported a linear decrease of Φ_{PSII} and photochemical quenching (q_P) with drought in C_4 plants, whereas Loreto *et al.* (1995) and Lal and Edwards (1996) found decreases only when

RWC decreased below 75 %. The constancy, or decrease of non-photochemical quenching (NPQ) under water deficit (Fig. 1*J–L*), together with decreased P_{max} (Fig. 1*A–C*), suggest that alternative electron sinks dissipate the excess excitation energy in the three species under drought. Since the major component of the NPQ is the energy-dependent quenching, a decrease in NPQ may reflect the incapacity to maintain the transthylakoidal proton gradient or the presence of proton leakage.

In the three C_4 grasses PEPC V_{max} (Fig. 2*A–C*) was always much higher than P_{max} (Fig. 1*A–C*) and was only affected by water deficit in *Z. japonica* when RWC decreased below 80 %. Thus PEPC activity could limit P_{max} only in *Z. japonica* at severe water deficit. Rubisco V_t was relatively unaffected in *P. dilatatum* but decreased linearly in *C. dactylon* with decreasing RWC (Fig. 2*D,E*) and may have contributed to limit photosynthetic CO_2 assimilation in this species. A less pronounced decrease of Rubisco V_t was observed in *Z. japonica* (Fig. 2*F*). Decreased Rubisco activity under water deficit suggests a decrease in amount of protein or down-regulation by tight-binding inhibitors (Parry *et al.* 1993, 2002). In the fully hydrated leaves of *P. dilatatum* and *C. dactylon*, Rubisco V_t (Fig. 2*D,E*) was lower than P_{max} (Fig. 1*A,B*), perhaps because tight-binding inhibitors were present under the growing conditions. Rubisco activase activity is enhanced by illumination (Lan *et al.* 1992), so that under the conditions used for P_{max} determination [1100 μmol (photon) m^{-2} s^{-1}] more inhibitors may be removed from the catalytic sites of Rubisco, allowing higher enzyme activities to match the photosynthetic rates. Decarboxylating enzyme activities (Fig. 2*G–I*) were higher than P_{max} (Fig. 1*A–C*) in all three species, suggesting that the rate of decarboxylation in the bundle sheath was not a limiting step for photosynthesis. However, NADP-ME and NAD-ME activities decreased with water deficit in *P. dilatatum* and *C. dactylon*, respectively, and may have contributed to the decrease in P_{max}. Drought-induced changes in the activities of PEPCK and NAD-ME in *Z. japonica* seem unlikely to have affected P_{max}.

Under severe drought, P_{max} decreased to almost zero (Fig. 1*A–C*) but PEPC and Rubisco retained considerable activity (Fig. 2*A–F*). Ghannoum (2009) reported a more consistent inhibition of C_3 than C_4 cycle enzymes under water deficit. While this was true for *C. dactylon*, in *Z. japonica* PEPC was more affected than Rubisco and in *P. dilatatum* the carboxylating enzymes were not significantly affected, suggesting the presence of additional limitations to photosynthesis under water deficit. Distinct differences exist between *P. dilatatum* (NADP-ME), *C. dactylon* (NAD-ME) and *Z. japonica* (PEPCK) in the behaviour of P_{max}, photochemical parameters and enzyme activities in response to rapid drought.

References

Ashton, A.R., Burnell, J.N., Furbank, R.T., Jenkins, C.L.D., Hatch, M.D.: Enzymes of C4 photosynthesis. – In: Lea, P.J. (ed.): Methods in Plant Biochemistry. Enzymes of Primary Metabolism. Vol. 3. Pp. 39-72. Academic Press, London 1990.

Bakrim, N., Echevarria, C., Cretin, C., Arrio-Dupont, M., Pierre, J.N. Vidal, J., Chollet, R., Gadal, P.: Regulatory phosphorylation of sorghum leaf phospho*enol*pyruvate carboxylase. Identification of the protein-serine kinase and some elements of the signal-transduction cascade. – Eur. J. Biochem. **204**: 821-830, 1992.

Carmo-Silva, A.E., Soares, A.S., Marques da Silva, J., Bernardes da Silva, A., Keys, AJ., Arrabaça, M.C.: Photosynthetic responses of three C4 grasses of different metabolic subtypes to water deficit. – Funct. Plant Biol. **34**: 204-213, 2007.

Castrillo, M., Fernandez, D., Calcagno, A.M., Trujillo, I., Guenni, L.: Responses of ribulose-1,5-bisphosphate carboxylase, protein content, and stomatal conductance to water deficit in maize, tomato, and bean. – Photosynthetica **39**: 221-226, 2001.

Čatský, J.: Determination of water deficit in discs cut out from leaf blades. – Biol. Plant. **2**: 76-77, 1960.

Du, Y.C., Kawamitsu, Y., Nose, A., Hiyane, S., Murayama, S., Wasano, K., Uchida, Y.: Effects of water stress on carbon exchange rate and activities of photosynthetic enzymes in leaves of sugarcane (*Saccharum* sp.). – Aust. J. Plant Physiol. **23**: 719-726, 1996.

Foyer, C.H., Valadier, M.-H., Migge, A., Becker, T.W.: Drought-induced effects on nitrate reductase activity and mRNA and on the coordination of nitrogen and carbon metabolism in maize leaves. – Plant Physiol. **117**: 283-292, 1998.

Ghannoum, O.: C4 photosynthesis and water stress. – Ann. Bot. **103:** 635-644, 2009.

Ghannoum, O., Conroy, J.P., Driscoll, S.P., Paul, M.J., Foyer, C.H., Lawlor, D.W.: Nonstomatal limitations are responsible for drought-induced photosynthetic inhibition in four C4 grasses. – New Phytol. **159**: 599-608, 2003.

Heckathorn, S.A., DeLucia, E.H., Zielinski, R.E.: The contribution of drought-related decreases in foliar nitrogen concentration to decreases in photosynthetic capacity during and after drought in prairie grasses. – Physiol. Plant. **101**: 173-182, 1997.

Lal, A., Edwards, E.: Analysis of inhibition of photosynthesis under water stress in the C4 species *Amaranthus cruentus* and *Zea mays*: electron transport, CO_2 fixation and carboxylation capacity. – Aust. J. Plant Physiol. **23**: 403-412, 1996.

Lan, Y., Woodrow, I.E., Mott, K.A: Light dependent changes in ribulose bisphosphate carboxylase activase activity in leaves. – Plant Physiol. **99**: 304-309, 1992.

Long, S.P.: Environmental Responses. – In: Sage, R.F., Monson, R.K. (ed.): C4 Plant Biology. Pp. 215-249. Academic Press, New York 1999.

Loreto, F., Tricoli, D., Di Marco, G.: On the relationship between electron transport rate and photosynthesis in leaves of the C4 plant *Sorghum bicolor* exposed to water stress, temperature changes and carbon metabolism inhibition. – Aust. J. Plant Physiol. **22**: 885-892, 1995.

Marques da Silva, J., Arrabaça, M.C.: Photosynthesis in the water-stressed C4 grass *Setaria sphacelata* is mainly limited by stomata with both rapidly and slowly imposed water deficits. – Physiol. Plant. **121**: 409-420, 2004a.

Marques da Silva, J., Arrabaça, M.C.: Photosynthetic enzymes of the C4 grass *Setaria sphacelata* under water stress: a comparison between rapidly and slowly imposed water deficit. – Photosynthetica **42**: 43-47, 2004b.

Parry, M.A.J., Andralojc, P.J., Khan, S., Lea, P.J., Keys, A.J.: Rubisco activity: Effects of drought stress. – Ann. Bot. **89**: 833-839, 2002.

Parry, M.A.J., Andralojc, P.J., Parmar, S., Keys, A.J., Habash, D., Paul, M.J., Alred, R., Quick, W.P., Servaites, J.C.: Regulation of Rubisco by inhibitors in the light. – Plant Cell Environ. **20**: 528-534, 1997.

Parry, M.A.J., Delgado, E., Vadell, J., Keys, A.J., Lawlor, D.W., Medrano, H.: Water stress and the diurnal activity of ribulose-1,5-bisphosphate carboxylase in field grown *Nicotiana tabacum* genotypes selected for survival at low CO_2 concentrations. – Plant Physiol. Biochem. **31**: 113-120, 1993.

Saccardy, K., Cornic, G., Brulfert, J., Reyss, A.: Effect of drought stress on net CO_2 uptake by *Zea* leaves. – Planta **199**: 589-595, 1996.

Walker, R.P., Chen, Z.-H., Acheson, R.M., Leegood, R.C.: Effects of phosphorylation on phospho*enol*pyruvate carboxykinase from the C4 plant guinea grass. – Plant Physiol. **128**: 165-172, 2002.