S17-022

Effect of Rapid Dehydration on the Activity of PEPC from the C4 Grass *Paspalum dilatatum*

A. Bernardes da Silva, M.C.Arrabaça, J. Marques da Silva

Centro de Engenharia Biológica and Departamento de Biologia Vegetal, Faculdade de Ciências da Universidade de Lisboa, C2, Campo Grande, 1749-016, Lisboa, Portugal. Fax 351 21 7500048, email absilva@fc.ul.pt

Keywords: PEPC, water stress, C4 gramineae

Introduction

C4 species have usually a higher water use efficiency (WUE) than C3 plants and this seems to be directly related with primary CO_2 fixation by phosphoenolpyruvate carboxylase (PEPC, EC 4.1.31). This enzyme is fully saturated at a much lower CO_2 concentration than Rubisco and, consequently, is less sensitive to the decrease in stomatal conductance under water stress conditions (see Long, 1999). However, the effect of this stress on PEPC activity is still a matter of controversy. Previous work in our laboratory with the C4 graminea *Setaria sphacelata* showed that rapidly imposed water deficit decreased, while slowly imposed dehydration increased, PEPC activity (Marques da Silva, 1999). Also in maize plants under water stress, PEPC activity decreased (Castrillo *et al.*, 1998) or stabilized during drought (Saccardy *et al*., 1996; Foyer *et al*., 1998). The aim of the present work was to study the effects of different relative water contents (RWC), when imposed through rapid dehydration, on PEPC physiological and maximal activities in *Paspalum dilatatum* leaves.

Material and methods

Plants of *P. dilatatum* Poir. cv Raki were grown hydroponically in an environmental growth chamber under a PPFD of 600 μmol m^{-2} s^{-1}, a temperature of 25/18ºC (day/night) and a photoperiod of 16/8H (day/night). The first fully expanded leaf was excised and allowed to be rapidly dehydrated by exposure to the growth chamber atmosphere in light. Relative water content (RWC) was monitored according to Catsky (1960) in a parallel sample and leaves at different RWC were frozen in liquid nitrogen and later used for assays.

Enzyme extraction

PEPC was extracted in a chilled mortar containing 1% (w/v) Polyclar AT and ice-cold medium (50mM Hepes-KOH, pH 7.3, 20mM $MgCl_2$, 10mM DTT, 6% (w/v) PVP25, 1mMPMSF and 0.1mM NaF). The extract was centrifuged in a Microfuge at full speed for 20s, at room temperature, and the supernatant directly assayed.

Enzyme assay

PEPC activity was determined according to Bakrim *et al*. (1992), but using 10mM PEP to obtain maximal activity. Soluble protein content was determined according to Bradford (1976), using BSA as standard.

Results and discussion

In *Paspalum dilatatum* leaves submitted to water stress, maximal PEPC activity *per* dry weight increased 45% ($P<0.1$) with the decrease of RWC from full water saturation to 30% (Fig. 1A). However, PEPC activity measured under sub-optimal but physiological conditions doubled ($P<0.001$) at severe water stress (30% RWC).

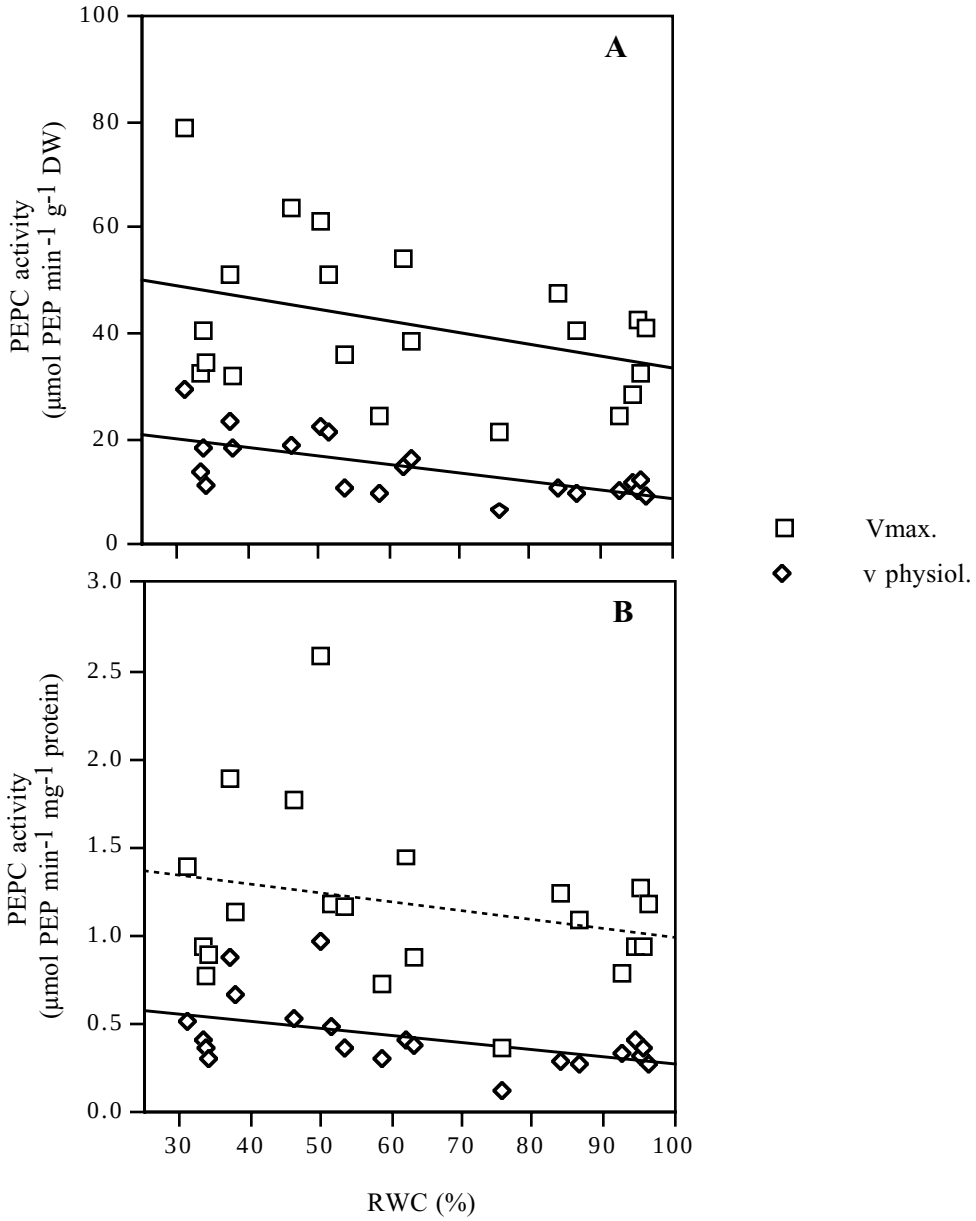

Fig. 1 - Maximal PEPC activity (Vmax) and physiological PEPC activity (v physiol.) in leaves of *Paspalum dilatatum* cv Raki at differents RWC, on a dry weight basis (**A**) and on a protein basis (**B**).

Most results with C4 plants indicated that PEPC activity decreased (Castrillo *et al*., 1998; Marques da Silva and Arrabaça, 2001) or was kept constant (Saccardy *et al.*, 1996; Foyer *et al*., 1998) with water stress. Increase on PEPC maximal activity was also found in *Setaria sphacelata,* when a slow dehydration was imposed (Marques da Silva, 1999). Ogawa *et al.* (1997) observed *in vitro* enzyme stabilization by osmoprotectors and suggested that the increase *in vivo* PEPC activity under stress, including water stress, may be due to this stabilization.

As shown in Fig. 1B, when PEPC activity was expressed on a soluble protein basis, a similar trend to that of Fig. 1A was obtained, but the increase on maximal PEPC activity was not significant. These results suggest that PEPC-protein may have been kept constant along the rapid dehydration of *Paspalum dilatatum*. A constant PEPC-protein content was also reported in maize plants during drought (Foyer *et al*., 1998).

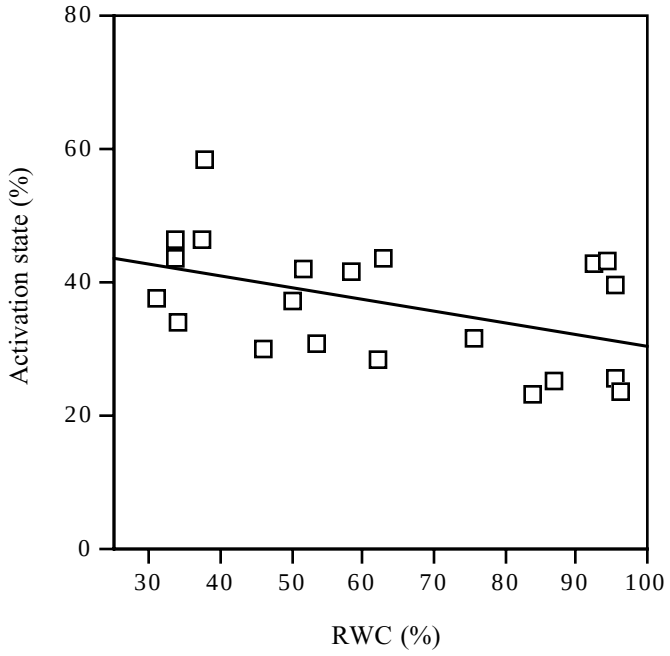

Fig. 2 - PEPC activation state in leaves of *Paspalum dilatatum* cv Raki at differents RWC.

PEPC physiological activity increases more than maximal activity of the enzyme with the decrease on RWC, leading to a higher activation state (Fig. 2). This may suggest a higher enzyme phosphorylation state and/or the presence of more positive (*e. g.* G-6-P) than negative (*e. g*. L-malate) effectors.

Acknowledgments

This work had the financial support of Fundação para a Ciência e a Tecnologia, Portugal, through Centro de Engenharia Biologica. The authors are indebted to Dr. J. A. Lancashire, Margot Forde Forage, Germplasm Centre, New Zealand, for the generous gift of *P. dilatatum* seeds. Fundação Calouste Gulbenkian, Portugal supported part of the travel expenses.

References

Bakrim, N., Echevarria, C., Cretin, C., Arrio-Dupont, M., Pierre, J. N., Vidal, J., Chollet, R. and Gadal, P. (1992) *European Journal of Biochemistry* **204**:821-830

Bradford, M. (1976) *Analytical Biochemistry* **72**:248-254

Castrillo, M., Fernandez, D., Calcagno, A. M. and Trujillo, I. (1998) in *Photosynthesis: Machanisms and Effects* (G. Garab ed.) **V**:3587-3590

Catsky, J. (1960) *Biologia Plantarum* **2**:76-77

Foyer, C., Valadier, M. H., Migge, A. and Becker, T. (1998) *Plant Physiology* **117**:283-292

Long, S. P. (1999) in *C4 Plant Biology* (R. F. Sage and R. K. Monson eds.) 215-250 Academic Press

Marques da Silva, J. M. (1999) *Ph.D. Thesis*, University of Lisbon

Marques da Silva, J. M. and Arrabaça, M. C. (2001*) Proceedings of the 12th International Congress on Photosynthesis, in press*

Ogawa, N., Yabuta, N., Ueno, Y and Izui, K. (1998) *Plant Cell Physiology* **39**:1010-1019

Saccardy, K., Cornic, G., Brulfert, J. and Reyss, A. (1996) *Planta* **199**:589-595