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Effect of Rapid Dehydration on the activity of PEPC from the C_4 grass *Setaria sphacelata*

J Marques da Silva, MC Arrabaça

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) 217500048; email: mcca@fc.ul.pt

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Introduction

The most characteristics effects of water stress on plant growth are the decrease of leaf expansion and of photosynthetic rate. The decrease in photosynthesis is certainly due to a lower chloroplast CO_2 concentration, as a consequence of stomatal closure, and to alterations at the biochemical level. In C_4 plants, as it is the case of *Setaria sphacelata* var. *splendida* (Stapf) Clayton, Phospho *enol* pyruvate carboxylase (PEPC, E.C. 4.1.1.31) performs a primary fixation of carbon. The effects of water stress on the activity of photosynthetic enzymes are still matter of strong controversy (Berkowitz, 1998), with some authors reporting notorious decline of activity (*e.g.* Castrillo *et al.* 1990) and others claiming for no effects. This controversy is certainly due, at least in part, to the fact that very different stress situations are used in experiments, and some of these differences are not properly taken into account. Among these latter, the velocity of stress implement has been suggested to influence the results (Ogren, 1990), but these studies are still scarce and, as far as we know, absent in C_4 plants. Therefore, we aimed to understand the effect of rapidly imposed water stress on the activity of PEPC.

Materials and methods

Setaria sphacelata plants were grown one *per* pot, in a mixture of peat and soil, under a photosynthetic photon flux density (PPFD) of approximately 400 μmol m^{-2} s^{-1}, a photoperiod of 16 hours and a temperature of 25/18 ^{o}C (day/night). Plants were abundantly watered 3 times a week until they were 2 months old. Six leaf fragments with 2.5 cm length and one with 5 cm length were cut from the middle zone of young fully developed leaves. Excised fragments were dehydrated by exposure to the atmosphere of the growth chamber. A water deficit of 50% was reached in approximately 3 hours. The biggest fragment was periodically weighed and was used as an indicator of the relative water content (RWC) of the other fragments. These were sequentially frozen in liquid nitrogen and a sequence of leaf samples progressively more dehydrated was thereby obtained. The frozen fragments were kept in a freezer at -70 ^{o}C until extraction. The studied enzyme was extracted in a medium adapted from Edwards *et al.* (1988) composed by HEPES 50 mM pH 7.4, DTT 5 mM, $MgCl_2$ 5 mM, pyruvate 1 mM, 20% glycerol (Jiao *et al.*, 1991), PVP 5% (w/v) and Polyclar AT (2% w/v). Approximately 2 cm^2 of leaf were ground in 0.2 ml of medium during 30-40 s, being more 1 ml of medium added immediately after. The extract was centrifuged in a Microfuge at 14000 rpm during 30s. The supernatant was recovered and remained in ice until enzymatic assays. The maximal activity of PEPC was immediately determined from a modification of the method of Donkin & Martin (1980), in an assay medium composed by HEPES 25 mM pH

8.0, $MgCl_2$ 5mM, $NaHCO_3$ 10 mM, NADH 0.1 mM, PEP 12.5 mM and 8 units of malate dehydrogenase (MDH). The reaction was initiated with 100 µl of extract. The activity under physiological conditions was obtained with HEPES 25 mM pH 7.2 and PEP 2.5 mM, keeping all the other conditions unchanged. Soluble protein present in enzymatic extracts was determined according to Bradford (1976).

Results

Under stress conditions both the maximal and the physiological activities of PEPC sharply decreased with decreasing RWC. The maximal activity decreased from 150 µmol PEP h^{-1} mg^{-1} protein at full hydration to 15 µmol PEP h^{-1} mg^{-1} protein at RWC 60%. The physiological activity decreased from 55 µmol PEP h^{-1} mg^{-1} protein at full hydration to 7 µmol PEP h^{-1} mg^{-1} protein at RWC 60%. Both activities showed a trend to recover under extreme values of stress (Fig. 1). When expressed on a chlorophyll or a dry weight basis PEPC activities showed similar trends to that of Fig. 1(results not shown).

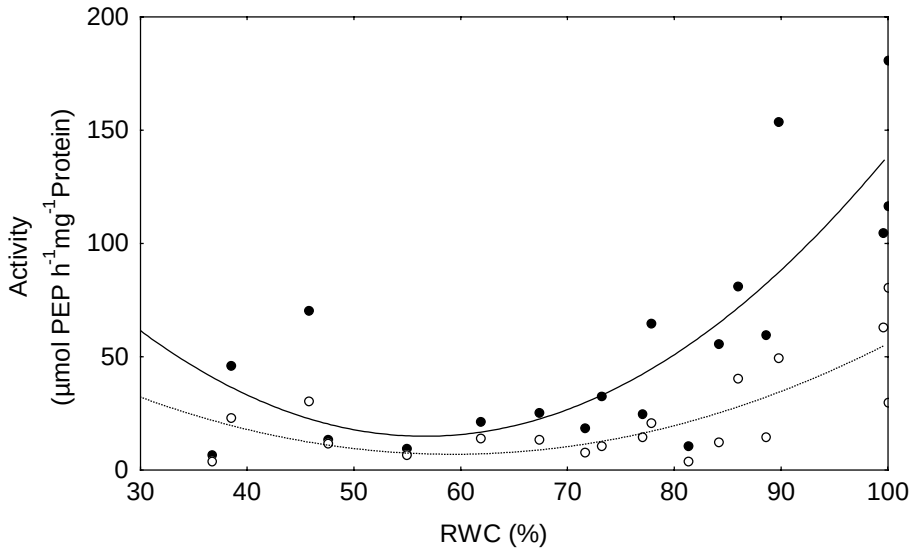

Fig. 1. Variation of maximal activity (●) and physiological activity (o) of PEPC, expressed by soluble protein, as a function of RWC. Each point corresponds to one measurement of activity. The line adjusted to maximal activity holds the following equation: $Y=225.94-7.45*X+6.58*10^{-2}*X^2$ ($r=0.823$, $p<0.001$). The line adjusted to physiological activity holds the following equation: $Y=110.40-3.49*X+2.94*10^{-2}*X^2$ ($r=0.765$, $p<0.001$).

The ratio between physiological activity and maximal activity increases slightly with decreasing RWC, from a value of approximately 35% in fully hydrated leaves to almost 70% at RWC 30% (Fig. 2).

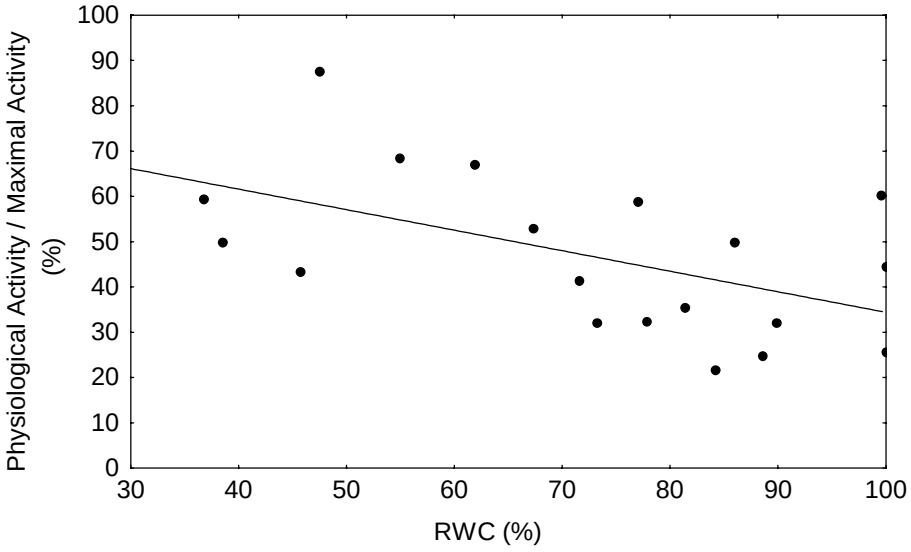

Fig. 2. Variation of the ratio between physiological and maximal activity of PEPC as a function of RWC. Data was obtained from Fig.1. The line adjusted to data holds the following equation: $Y=79.72-4.53*10^{-1}*X$ ($r=0.530$, $p<0.02$).

Discussion

PEPC activities decrease sharply under stress, showing a trend to increase under extreme stress values (Fig. 1). Saccardy *et al.* (1996) did not found in *Zea mays* any variation of the activity of PEPC under rapid stress conditions. Bernardes da Silva *et al.* (2001) found in *Paspalum dilatatum* an increase of maximal and physiological PEPC activities in rapidly stressed leaves. The linear increase on the ratio between physiological and maximal activities found under stress shows that the % of activation at severe stress (RWC 30%) is twice the control value. However, the increase is much lower than the total decrease of both activities, suggesting that phosphorylation / dephosphorylation processes and the regulation by metabolites may not play a main role in the variation of the activities under stress, being probably more relevant the processes related to the oligomerization state and / or the degradation and *de novo* synthesis of the enzyme (Fig. 2). Also Bernardes da Silva *et al.* (2001) found an increase on activation of *Paspalum dilatatum* PEPC under stress conditions. Saccardy *et al.* (1996) using the L-malate sensitivity test as an indicator of the phosphorylation state of *Zea mays* PEPC did not find any variation under stress condition. The increase on the % activation is certainly due to a higher phosphorylation state in the enzyme from stressed leaves, but an increase of positive effectors (*e.g.* G-6-P, F-6-P) (Wedding *et al.* 1989, Rajagopalan *et al.* 1994) and / or a decrease of inhibitors (*e.g.* L-malate) (Lepiniec *et al.*, 1994) are not to be excluded.

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