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

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Introduction

C4 plants in contrast to their C3 plants counterparts, are usually located in warm-climate areas with high irradiance and some periods of water stress (tropical, sub-tropical and semi-desert areas) (Hatch, 1987). *P. dilatatum* is a C4-plant sub-type NADP-ME that forms dense prairies, resistant to mild frosts (Hacker et al.,1974) and to low temperatures (Bernardes da Silva, 1996) and although preferring wet soils, it can stand a high level of water stress as long as it has benefit from rain in part of the year (Pinto da Silva, 1969). In the C4 pathway Rubisco is involved in a secondary re-fixation of CO_2 in the bundle sheath cells, where C4 acid decarboxylation provides, at the level of Calvin cycle, a high CO_2 concentration. The effect of water stress on the enzyme is still a matter of controversy. It has been reported that slowly imposed water stress, in maize plants, leads to a sharp decrease in Rubisco activity whereas the Rubisco protein was affected to a lesser extent (Castrillo and Fernandez, 1990). However in sunflower it has been shown that the effect of water stress was mainly on the Rubisco-protein content (Turner,1981). Also, results from our laboratory, using the C4 gramineae *Setaria sphacelata* showed a decrease on Rubisco activity with water stress, whereas its content was kept relatively constant, oscillating around 8% of the total soluble protein (Marques da Silva, 1999). However when this plant was subjected to a rapid dehydration the maximal activity did not change with the stress whereas the inicial activity and Rubisco protein content increased. The aim of this work was the study of rapid dehydration effect on Rubisco activities and activation, using as a model the C4 gramineae *Paspalum dilatatum*.

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 monitered 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

Rubisco 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, 1mM

PMSF and 0.1mM NaF). The extract was centrifuged in a microfuge, at 14000 rpm for 20 s at room temperature and the supernatant directly assayed.

Enzyme assay

Rubisco activity was assayed by $^{14}CO_2$ incorporation into acid-stable products according to Keys and Parry (1990), immediately after extraction (initial activity-Vi) or after 5 min activation with saturating concentration of CO_2 an Mg^{2+} (total activity-Vt). Assays were started either with the extract (for vi) or with RUBP (for vt). Reactions were stopped after 30 sec by the addition of 2N HCl.^{14}C radioactivity was measured by liquid scintillation counting using a spectrometer Beckman LS 7800, USA.

Results and discussion

Total Rubisco activity increased 50% when RWC decreased from full water saturation to 60%. A further decrease in RWC down to 20% resulted in a decrease in Vt. Similar curves were obtained both when expressed on a dry weight and on a soluble protein bases (Figs 1 and 2).

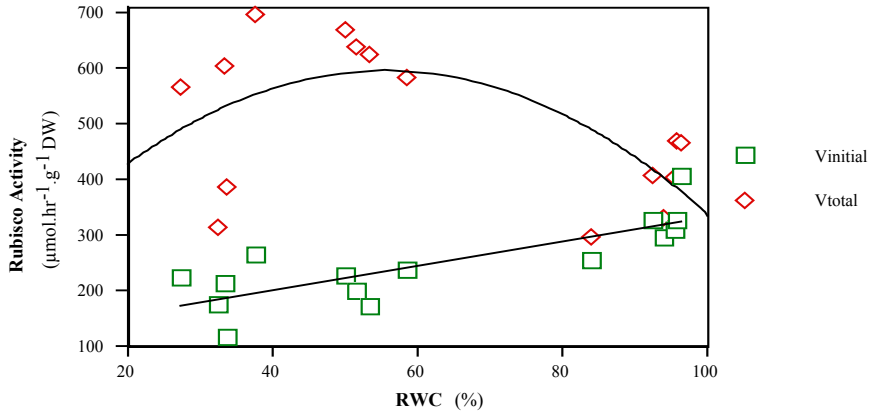

Fig. 1 . Rubisco activities (Vi and Vt) expressed on a dry weight basis at different levels of relative water content

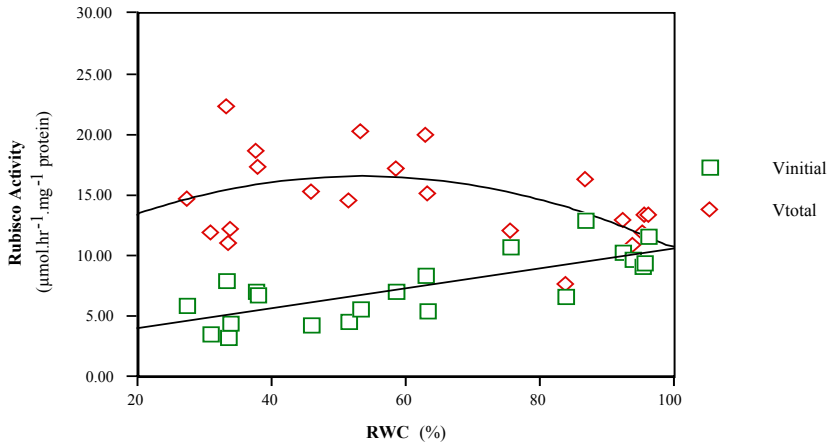

Fig.2 . Rubisco activities (Vi and Vt) expressed on a protein content basis at different levels of relative water content

On the contrary, initial activity decreased continuously with water deficit, down to 50% in severely stressed leaves. Accordingly, a sharp decrease in activation was observed under stress (Fig. 3).

Similar results were found in the C4 plants *Amaranthus cruentus* and *Zea mays* by Lal and Edwards, (1996), attaining high activation, of the order of 80 %, in control plants. At 100% RWC, the enzyme seems to be fully carbamylated, since the values of Vi and Vt are nearly the same.

The decrease in enzyme initial activity with water deficit may result from the susceptibility

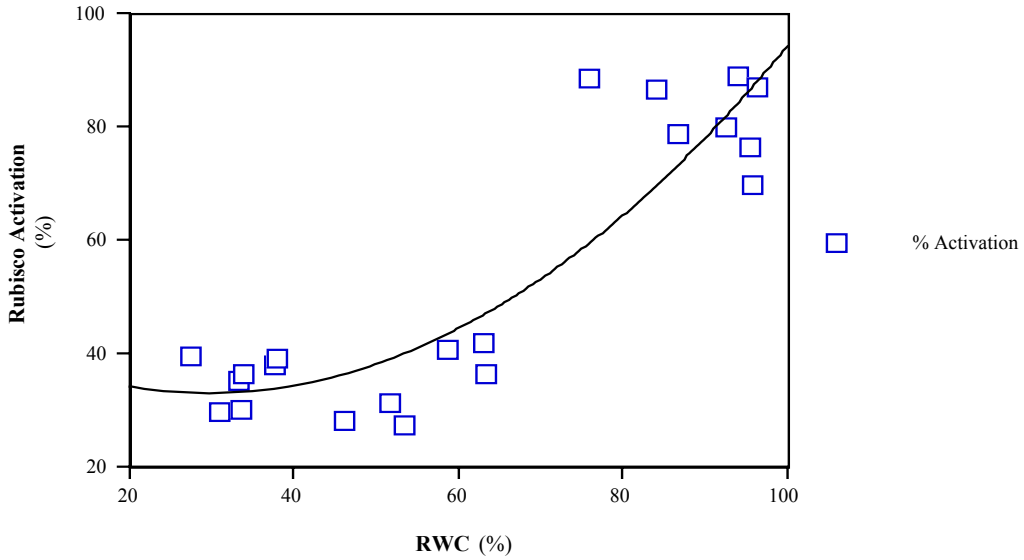

Fig. 3 . Dependence of the activation of Rubisco activity on the relativa water content

of Rubisco activase to a lack of ATP availability. Increases in the thylakoid proton gradient, associated with low levels of ATP, due to the deficient functioning of the ATP synthase have been reported (Boyer and Younis, 1984).

A decrease in ATP content in photosynthetic cells under stress was also observed by Lawlor and Khanna-Chopra (1984). The decrease on ATP content under stress conditions may be also the result of ADP and Pi unavailability since these compounds are not efficiently regenerated at the Calvin-Benson cycle. Also a decrease on RUBP pool was observed under stress (Tezara and Lawlor, 1995). The increase on Vmax with the stress from RWC 100% to 50% may be explained by an increase on Rubisco content. In fact in our laboratory studies in *Setaria sphacelata* performed by Marques da Silva, (1999), pointed to an increase on Rubisco-protein content with water stress, associated to a decrease on Rubisco specific activity.

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