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Responses of ribulose-1,5-bisphosphate carboxylase, protein content, and stomatal conductance to water deficit in maize, tomato, and bean

M. CASTRILLO*, D. FERNANDEZ**, A.M. CALCAGNO***, I. TRUJILLO+, and L. GUENNI++

*Depto. Biologia de Organismos, Universidad Simon Bolivar, Apdo. Postal 89000, Caracas 1080, Venezuela**
*Dept. Biology, CUH Station University of Puerto Rico, Humacao, Puerto Rico 00791-4300, USA***
*Centro de Microbiologia, IVIC, Altos de Pipe Km 11, Carretera Panamericana, Apdo. Postal 21827, Caracas 1020 A, Venezuela****
Centro de Estudios de Agroecologia Tropical, Universidad Simon Rodriguez, Av. Mara, Sector El Cuji,
Altos de la Mariposa, Edo. Miranda, Venezuela+
Depto. Computo Cientifico y Estadistica, Universidad Simon Bolivar, Apdo. Postal 89000, Caracas 1080, Venezuela++

Abstract

We compared responses of maize, tomato, and bean plants to water stress. Maize reached a severe water deficit (leaf water potential −1.90 MPa) in a longer period of time as compared with tomato and bean plants. Maize stomatal conductance (g_s) decreased at mild water deficit. g_s of tomato and bean decreased gradually and did not reach values as low as in maize. The protein content was maintained in maize and decreased at low water potential (Ψ_w); in tomato it fluctuated and also decreased at low Ψ_w; in bean it gradually decreased. Ribulose-1,5-bisphosphate carboxylase/oxygenase activity remained high at mild and moderate stress in maize and tomato plants; in bean it remained high only at mild stress.

Additional key words: *Lycopersicum esculentum*; *Phaseolus vulgaris*; protein; water potential; *Zea mays*.

Introduction

The decrease in net photosynthetic rate (P_N) in plants induced by water deficit has often been reported. Mild water stress may decrease P_N by decreasing CO_2 diffusion principally due to stomatal closure, at severe water stress also by direct inhibition of biochemical reactions of photosynthesis (Cornic *et al.* 1989, Cornic and Briantais 1991, Brestic *et al.* 1995, Gimenez *et al.* 1992, Kanechi *et al.* 1995, Lawlor 1995, Tezara *et al.* 1999). Hence the decrease is attributed to both stomatal and non-stomatal limitations (Ben *et al.* 1987, Graan and Boyer 1990, Ort *et al.* 1994).

Tezara *et al.* (1999) showed that in leaves of sunflower, water stress decreased CO_2 uptake more than O_2 evolution and that the effects are not reversed by high concentration of CO_2. These authors found that water stress reduced the amounts of ATP and RuBP in leaves, but not the amount and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO). However, water stress decreases the RuBPCO activity (Mayoral *et al.* 1981, Vapaavouri and Valanne 1982, Vu *et al.* 1987, Martinez-Barajas *et al.* 1992, Parry *et al.* 1993). This decrease is associated with a decrease in osmotic potential (Mawson and Colman 1983, Kaiser *et al.* 1986) and/or in protein content (Castrillo and Calcagno 1989, Castrillo and Fernandez 1990, Castrillo and Trujillo 1994). However, some reports do not associate inhibited RuBPCO activity with its decreasing content and/or soluble protein content (Tezara and Lawlor 1995, Borland *et al.* 1998, Panković *et al.* 1999). According to Parry *et al.* (1993, 1999) RuBPC activity may be modulated in response to irradiance and concentrations of CO_2 or O_2 through the carbamylation of lysine 201 in the large subunit and/or by the binding of the inhibitor 2-carboxyarabinitol (2CA1P). The binding of such inhibitor, which reduces the number of active sites available to RuBP, is enhanced after extended drought stress. Changes in total RuBPCO activity reflect regulation by 2CA1P or a similar inhibitor. These authors

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*e-mail: mcastr@usb.ve

Abbreviations: RuBP, ribulose-1,5-bisphosphate; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase activity; Ψ_w, water potential.

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suggested that even under water stress RuBPCO activity is still regulated by inhibitors, and that a reduction in the inhibition of activity by tight-binding inhibitors permits the enzyme to achieve a higher specific activity than in irrigated plants. Moreover, the regulation of RuBPCO activity by inhibitors and carbamylation/decarbamylation responds to changes in water stress. The modulation of RuBPCO activity by inhibitors has been widely reported even in species that apparently do not produce 2CA1P and tight binding inhibitors may be produced during catalysis (Parry *et al.* 1993). One of these species is maize. Gustafson *et al.* (1987) reported that wheat and maize seedlings do not show the diurnal dependent effect and that in the regulation of RuBPCO not only the amount of active enzyme, but the amount of enzyme which the plant can activate upon demand is important. These authors reported that in maize the initial and total RuBPCO activities were high. Some evidences indicate that water deficit does not affect RuBPCO activation (Sharkey and Seemann 1989, Panković *et al.* 1999, Tezara, personal communication).

The aim of the present work was to compare the responses of RuBPCO activity (EC 4.1.1.39) and total soluble protein content under water deficit, and the relationship between RuBPCO reponses, g_s and protein content in maize, tomato, and bean.

Materials and methods

Plants: Seeds of maize *Zea mays* L. hybrid CPB8, tomato *Lycopersicum esculentum* Mill. cv. Pera Quibor, and bean *Phaseolus vulgaris* L. cv. Tacarigua were germinated on wet paper in plastic trays. After germination, the seedlings were transferred to plastic pots (one seedling per pot) and watered daily. The seedlings were maintained in a greenhouse. 50 cm^3 of Hoagland solution plus 0.25 mM $CaCl_2$ and commercial fertiliser (*POKON*, Bendien-Naarden, The Netherlands) was added twice per week. The general climatic conditions in the greenhouse were: average minimum/maximum temperatures 18±2.3/30±2.5 °C, average minimum/maximum relative humidity (RH) 53±8/97±2 %, average sun radiation 656 μmol m^{-2} s^{-1}. There was a difference in RH during the water deficit period, for maize and tomato the average minimum RH was 58 % while that for bean was 45 %. After the plants had been grown for 30 (maize), 39 (tomato), and 30-39 (bean) d, watering was withheld from fifty plants (plants under water deficit) and another fifty plants were watered and maintained as controls. Measurements were taken, in both groups of plants, at 2-d intervals during the water deficit period for 24 d in maize, 14 d in tomato, and 13 d in bean.

All measurements (four replicates, *i.e.*, 4 plants) were taken in young, fully expanded leaves, the second leaf from the apex in maize plants, and the third leaf in tomato and bean plants.

Water potential (Ψ_w) was measured in the morning between 05:30 and 06:30 h in leaf discs using a *HR-33T* Dew Point Microvoltmeter with *C-52* chambers (*Wescor*, Logan, USA); soil samples were measured simultaneously.

g_s was measured between 07:00 and 08:00 h with a porometer (*LI-65* with sensor *LI-25*, *Lambda*, Lincoln, USA). Abaxial and adaxial resistances were measured and the total stomatal conductance was calculated.

Extraction: Around noon, irradiated leaves (Servaites *et al.* 1986) without midvein were ground in a mortar (1:5, m/v). The extraction medium contained 0.2 M Tricine-NaOH, pH 8.1 (*Sigma*, St Louis, MO, USA), 10 mM $NaHCO_3$, 20 mM $MgCl_2$, 10 mM Na EDTA, 20 mM 2-mercaptoethanol (2-ME), and 16 kg m^{-3} polyvinylpolypyrrolidone (PVPP) (*Sigma*). The homogenate was filtered through four layers of muslin. The homogenate was centrifuged at 10 000×*g* during 30 s. The supernatant was desalted and concentrated using *Centricom 10* (*Amicon*, Beverly, MA, USA) tubes. The desalted and concentrated extracts were kept at 0 °C and used for assays and determinations.

RuBPCO assay: The reaction medium contained, in a final volume of 0.17 cm^3: 150 mM Tricine-NaOH, pH 8.1, 16 mM $NaH^{14}CO_3$ (3.7 GBq mol^{-1}) (*Amersham*, Amersham, England), 10 mM $MgCl_2$, 0.03 cm^3 of leaf extract, and 2 mM RuBP. The reactions took place at 27 °C and started by the addition of RuBP. All assays were stopped after 1 min by adding 0.2 cm^3 25 % acetic acid, evaporated to dryness, and the radioactivity incorporated was counted in a scintillation counter (*LKB Wallac 1217 Rackbeta*, Turku, Finland). All assays were performed within 5-15 min after extraction. As the extraction buffer contained $NaHCO_3$ and $MgCl_2$, the enzyme was exposed to CO_2 and Mg^{2+} from the extraction beginning. Therefore the enzyme was not pre-incubated before the assay. Calculations of the specific radioactivity were done for the $NaHCO_3$ included in the extraction buffer.

Protein content was estimated as described by Bradford (1976).

Statistical analysis: Three models of analysis of covariance were fitted to each set of data; in each of them, the response of variable (converted by using a logarithm transformation) was linearly related with the corresponding independent variable. In model 1, the response variable depends in the same way on the independent variable for the three species. Model 2 assumes equal slope but different intercepts in the equation. Model 3, the most complex of them, assumes different intercept and slopes for the three species by using an F-test of nested models; after the comparison model 3 was selected for each case.

Results and discussion

The decrease in soil Ψ_w produced a decrease in leaf Ψ_w. During water stress, maize leaf Ψ_w decreased slowly until the 9th d after which the decrease was steeper until 24th d, reaching a minimum value of −1.90 MPa. Tomato leaf Ψ_w decreased gradually reaching a minimum −2.50 MPa. Bean leaf Ψ_w decrease was more marked from the beginning, reaching a minimum of −1.70 MPa (Fig. 1). In maize, the leaf Ψ_w after 24-d stress was not as low as Ψ_w in tomato after 14 d and in bean after 13 d.

In maize, g_s decreased suddenly from the 5th to 8th d until 14th d and it had the lowest values till the end of stress. Tomato leaves showed a gradual g_s decrease during the stress. In bean leaf the g_s decrease was steeper until the 4th d and more gradual until the end of stress (Fig. 1). During the water deficit period the minimum average RH was 45 % in bean, while in maize and tomato it was 58 %. This could affect the g_s response in bean. g_s decrease started in maize at leaf Ψ_w of −0.40 MPa, in tomato at −0.70 MPa, and in bean at −0.30 MPa (Fig. 2). Maize leaves showed the lowest g_s and bean leaves the highest one.

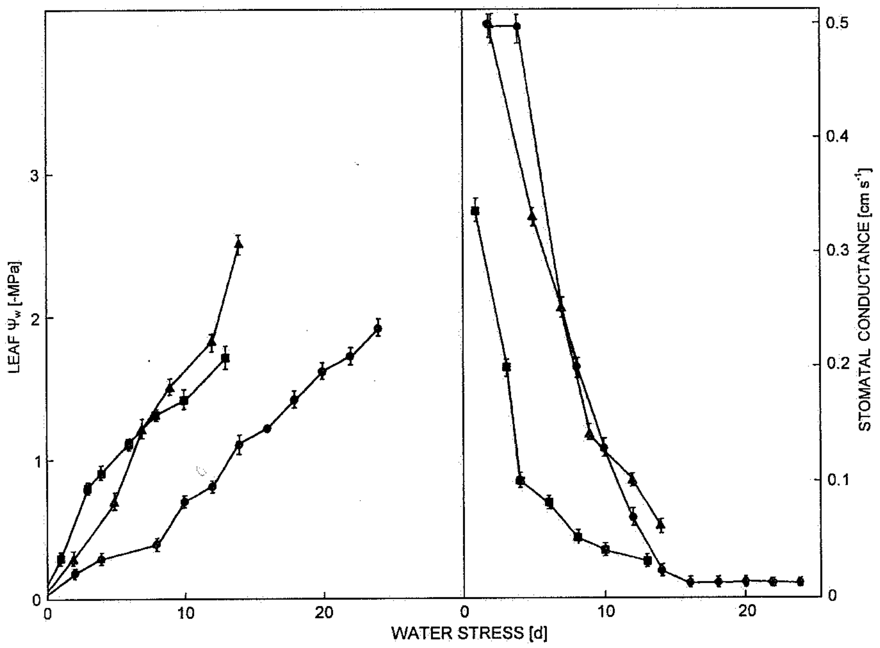

Fig. 1. Leaf Ψ_w [MPa] and stomatal conductance (g_s) during the water stress period in maize (●), tomato (▲), and bean (■) plants. Means ± SD, $n = 4$.

Maize protein content was fairly stable, it decreased at leaf Ψ_w of −1.70 MPa. Tomato protein content showed fluctuations at the beginning of stress period, at Ψ_w of −1.50 MPa it decreased and was low until Ψ_w of −2.50 MPa. Bean protein content decreased gradually until Ψ_w of −1.70 MPa (Fig. 2). Maize had the highest and bean the lowest protein content.

RuBPCO activity in maize showed some fluctuations at the beginning of stress period, the activity started to decrease at advanced Ψ_w of −1.70 MPa. Tomato RuBPCO activity was stable at the beginning, it decreased at Ψ_w of −1.70 MPa and was low until Ψ_w of −2.50 MPa. Bean showed a gradual decrease from −0.30 to −0.90 MPa, a steeper decrease at −1.40 MPa, and again a gradual decrease at the end of stress period.

The analysis of covariance revealed that the responses among species were significantly different.

We established three levels of water deficit: mild from −0.20 to −0.70 MPa, moderate from −0.80 to −1.10 MPa, and severe from −1.20 to −2.50 MPa. Maize reached a severe water deficit in a longer period of time as compared to tomato and bean. The rate of leaf Ψ_w decrease was lower in maize and higher in bean under relatively constant greenhouse climatic conditions (Fig. 1). The rate of g_s decrease was higher than the rate of leaf Ψ_w decrease in the studied species (Fig. 1). Maize plants showed a sudden decrease in g_s at mild water stress (at –0.40 MPa) while in tomato and bean g_s decreased gradually and did not reach as low values as in maize (Fig. 2). Cornic *et al.* (1989) mentioned that in C_4 plants (maize), the stomata components may be more sensitive to water stress than in C_3 plants. This is demonstrated in the present work. The leaf rolling initiation in maize took place at the 9th d of stress, when the leaf Ψ_w was –0.48±0.095 MPa and g_s 0.14±0.01 cm s^{-1}. The leaf rolling initiation associated with low leaf Ψ_s and high leaf temperature induces a reduction in leaf temperature (Fernandez and Castrillo 1999).

We found in the studied plant species a different pattern of protein content decrease during decreasing leaf Ψ_w. The majority of reports on the effect of water deficit on protein content agree with a reduction in protein content (Hanson and Hitz 1982). The protein degradation is associated with an increased hydrolytic activity (Kao 1981, Levy 1983). Some authors reported, however, an increase in protein content during water deficit (Tezara and Lawlor 1995).

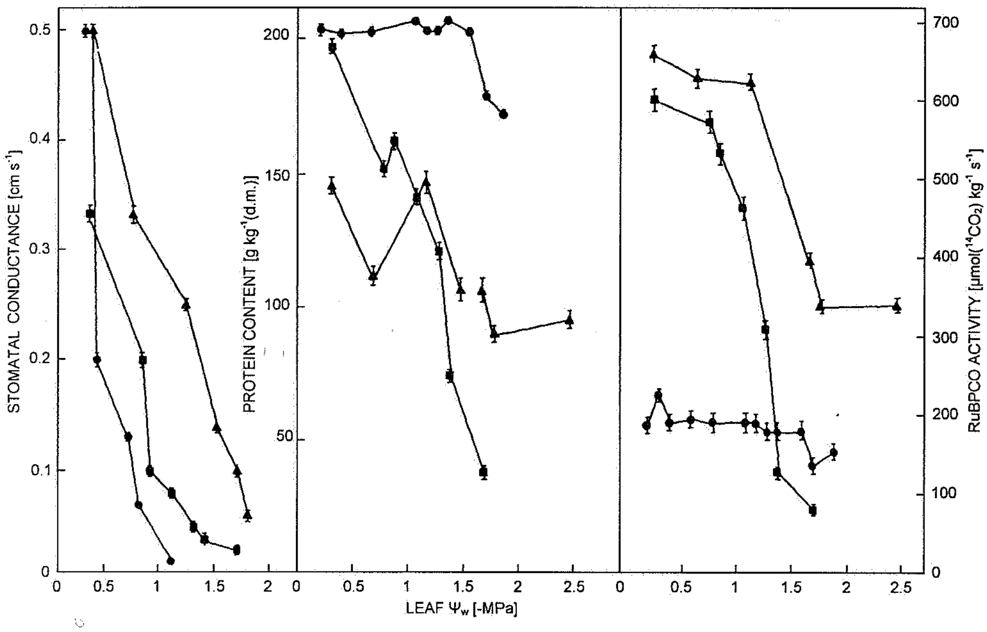

Fig. 2. Stomatal conductance (g_s), protein content, and RuBPCO activity during decreasing leaf Ψ_w [MPa] in maize (●), tomato (▲), and bean (■) plants under water deficit. Means ± SD, $n = 4$.

RuBPCO activity (Fig. 2) was maintained in tomato and bean at mild stress, but at moderate to severe stress it started to decrease. Maize plants have greater tendency to maintain high RuBPCO activity at mild and moderate water deficit stress than tomato and bean plants. At severe water deficit, RuBPCO activity decreased at leaf Ψ_w of –1.70 MPa in maize, –1.80 MPa in tomato, and –1.10 MPa in bean. The decrease was lower in maize. RuBPCO activity in maize did not show any relationship with g_s or protein content during water stress; in tomato it did not show any relationship at mild and moderate water deficit, at severe water deficit RuBPCO activity decreased with decreasing g_s or protein content. The relationship between RuBPCO activity and g_s or protein content in bean was independent only at mild water deficit, at moderate and severe water deficit the activity decreased with decreasing g_s or proteint content.

There are several reports about decrease in RuBPCO activity not associated with decreasing RuBPCO content and/or soluble protein content. Borland *et al.* (1998), working with three *Clusia* species, reported that drought stress did not affect the amount of RuBPCO protein in any of the species but RuBPCO activity was reduced. Similarly, Kanechi *et al.* (1996), working with coffee plants under water stress, reported that activity of RuBPCO decreased significantly while RuBPCO content was not affected. Tezara and Lawlor (1995) and Panković *et al.* (1999) found that RuBPCO activity in sunflower decreased at severe water deficit but RuBPCO content increased under prolonged drought.

Maize leaf g_s decreased suddenly and continued to decrease until minimum values were reached. In such conditions the leaf Ψ_w decreased slowly during water stress, reaching severe water deficit in a longer period of time. RuBPCO activity and protein content were directly affected at severe water deficit. The decrease in leaf Ψ_w

and associated change in g_s was gradual in tomato leaves. RuBPCO activity and protein content were affected faster at mild and/or moderate water deficit.

During the development of water deficit, g_s is first affected by decreasing leaf Ψ_w, the decrease in g_s is maintained during water stress period, and RuBPCO and protein contents are affected at prolonged water deficit, directly by lower leaf Ψ_w.

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