PHOTOSYNTHETICA 37 (3): 493-497, 1999

BRIEF COMMUNICATION

Maize leaf rolling initiation

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Abstract

Maize plants of CPB2 and CPB8 hybrids were kept under water deficit for 22 d. In the CPB8 hybrid, leaf rolling initiated at the 9th d of water deficit period, while in CPB2 hybrid it was at the 15th d. Both hybrids showed leaf rolling initiation at the same leaf water potential, Ψ_w of -0.480±0.095 MPa. At leaf rolling initiation, the leaf osmotic potential, Ψ_s was -0.730±0.085 MPa in CPB8 and 0.630±0.110 MPa in CPB2. The leaf temperature and stomatal conductance were higher in CPB8 than in CPB2. Values of leaf Ψ_w, ribulose-1,5-bisphosphate carboxylase activity, chlorophyll content, and specific leaf area were similar in both hybrids. Phosphoenolpyruvate carboxylase activity and protein content were lower in the CPB2 hybrid than in CPB8. In both hybrids leaf rolling initiation was associated with: (*1*) higher leaf temperature, with leaf rolling effect related to leaf temperature reduction, and (*2*) lower leaf Ψ_s, related to osmotic adjustment as an additional component of drought-tolerance strategy.

Additional key words: chlorophyll; osmotic potential; PEPC; pressure potential; proteins; RuBPC; stomatal conduntance; water deficit; water potential; *Zea mays*.

Leaf rolling in grasses is a typical response to water deficits. It is used in selecting drought resistant rice cultivars (Ekanayake *et al.* 1993). O'Toole and Cruz (1980) mentioned that the initiation of leaf rolling in rice should not be associated with less

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Received 14 June 1999, *accepted* 30 August 1999.

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Abbreviations: Chl, chlorophyll; g_s, stomatal conductance; PEPC, phosphoenolpyruvate carboxylase; RuBPC, ribulose-1,5-bisphosphate carboxylase; RWC, relative water content; SLA, specific leaf area; Ψ_p, pressure potential; Ψ_s, osmotic potential; Ψ_w, water potential.

Acknowledgements: To CONICIT and the Research and Development Council, Universidad Simon Bolivar for financial support and to Dr. R. Higgs for critical revision.

bulk pressure potential, but only that of the specialised bulliform cells. Significant correlation among leaf stomatal response, rolling, and water potentials in rice have been reported (O'Toole *et al.* 1979, O'Toole and Cruz 1980). Leaf rolling decreases transpiration through changes in both leaf conductance (g_s) and "effective" (displayed or projected) leaf area (Begg 1980), though it has been argued that the most important effect of leaf rolling is reduction of leaf temperature *via* decreases in incident irradiation (Ripley and Redmann 1976). O'Toole and Cruz (1980) stated that in grasses which exhibit leaf rolling, the interaction with stomatal behaviour and effect on plant water balance may be significant. Premachandra *et al.* (1993) reported that the degree of maize leaf rolling was lowest in the cultivar which maintained the highest pressure potential *via* osmotic adjustment and it recovered from leaf rolling faster than the other cultivars. In the present study, the leaf water status components were measured along with activities of PEPC (EC 4.1.1.31) and RuBPC (EC 4.1.1.39), protein and chlorophyll (Chl) contents, g_s, and leaf temperature, to understand the relationship of these parameters at the onset of maize leaf rolling.

Seeds of two selected maize (*Zea mays* L.) hybrids CPB2 and CPB8 supplied by Venezuelan Fund for Agriculture and Husbandry, Maracay, Venezuela were germinated in trays on towel paper. After germination the seedlings were transferred to plastic pots containing a mixture of clay and sand (3 : 2). The plants were watered daily and maintained in a greenhouse under maximum average temperature 29.0± 2.5 °C, minimum average temperature 19.0±1.5 °C, maximum relative humidity (RH) 96 %, minimum RH 56 %, and average sun irradiance 656 μmol m^{-2} s^{-1}. After 30 d, watering was withheld from 10 plants and another 10 plants were watered and maintained as controls. The Ψ_w of leaf discs (four replicas) was measured in a thermocouple psychrometer (*Wescor HR-33T* Dew Point Microvoltmeter and *C-52* sample chamber, *Wescor*, Logan, USA). After measuring Ψ_w, the leaves were frozen in liquid nitrogen and the Ψ_s was determined in the expressed sap from thawed leaves in a thermocouple psychrometer (*Wescor HR-33T* Dew Point Microvoltmeter and *C-52* sample chamber, *Wescor*, Logan, USA). Ψ_p was calculated. The method of Turner (1981) was chosen for RWC determinations. All measurements were performed at predawn time. Leaf diffusive conductances, g_s (abaxial and adaxial) were measured between 07:30 and 08:30 h with an autoporometer (*Lambda LI-65*, with a sensor *LI 205*, Lincoln, USA). The total g_s was calculated. Leaf temperature (abaxial) was measured with a potentmeter (*Leeds & Northrop* model *8693-2*). The dry mass of 10 leaf discs was determined and mass/area ratio (SLA) was calculated. Leaf extractions and enzyme assays were performed as described in Castrillo and Fernandez (1990). Magnesium and bicarbonate were included in the extraction medium to obtain total RuBPC activity (Lorimer *et al.* 1976, Castrillo 1985). Irradiated leaves were used for extraction to obtain conditions appropriated for enzyme activity assays (Servaites *et al.* 1986). Chl and protein contents were estimated spectrophotometrically according to Bruinsma (1963) and Lowry *et al.* (1951), respectively. The second leaf from apex from four replicates of both control and stressed plants was chosen for measurements, extractions, and determinations. Leaf rolling initiation in the present

Table 1. Values of measured parameters at leaf rolling initiation in maize plants of CPB2 and CPB8 hybrids under water deficit (WD) or in control plants (C). Each value is a mean of four replicates ± s.d.

	CPB2		CPB8	
	WD	C	WD	C
leaf rolling [d]	15		9	
soil Ψ_w [MPa]	-0.280 ± 0.080	-0.050 ± 0.009	-0.330 ± 0.085	-0.050 ± 0.009
leaf Ψ_w [MPa]	-0.480 ± 0.094	-0.250 ± 0.080	-0.480 ± 0.096	-0.200 ± 0.070
leaf Ψ_s [MPa]	-0.630 ± 0.110	-0.500 ± 0.100	-0.730 ± 0.085	-0.550 ± 0.252
leaf Ψ_p [MPa]	0.150 ± 0.070	0.250 ± 0.085	0.250 ± 0.083	0.350 ± 0.093
RWC [%]	93.50 ± 2.00	98.00 ± 2.00	95.50 ± 2.00	98.00 ± 2.00
g_s [cm s^{-1}]	0.06 ± 0.01	0.50 ± 0.07	0.14 ± 0.01	0.50 ± 0.07
PEPC [μmol(CO_2) kg^{-1}(d.m.) s^{-1}]	390 ± 120	410 ± 120	440 ± 10	440 ± 10
RuBPC [μmol(CO_2) kg^{-1}(d.m.) s^{-1}]	190 ± 60	200 ± 50	200 ± 50	220 ± 50
Chlorophyll [g kg^{-1}]	18.5 ± 6.1	19.8 ± 6.5	19.0 ± 6.1	19.6 ± 6.5
Protein [g kg^{-1}]	182.0 ± 58.3	205.0 ± 65.6	210.0 ± 58.0	205.0 ± 50.0
Specific leaf area [m^2 kg^{-1}(d.m.)]	122.0 ± 40.0	130.0 ± 41.6	122.0 ± 40.0	140.0 ± 43.0
Leaf temperature [°C]	26.0 ± 1.5	25.0 ± 1.5	27.5 ± 1.5	26.5 ± 1.5
Air temperature [°C]	26.5 ± 1.5	26.5 ± 1.5	27.5 ± 1.5	27.5 ± 1.5

study is equivalent to the leaf rolling score of 2 reported by O'Toole and Cruz (1980).

Leaf rolling initiation was observed in both hybrids (Table 1), in the CPB8 hybrid at the 9th d of water deficit period, while in CPB2 hybrid at the 15th d. Similar values were found for soil Ψ_w, leaf Ψ_w, leaf Ψ_s, leaf Ψ_p, RWC, and SLA. PEPC and RuBPC activities were similar or slightly lower at this water deficit and Chl contents were similar to controls. However, the protein content in CPB2 hybrid was lower at leaf rolling initiation than in the control, whereas in CPB8 it was higher. At leaf rolling initiation, leaf Ψ_w, RuBPC activity, Chl content, and SLA had similar values in both hybrids (Table 1). In both hybrids leaf rolling initiated at the same leaf Ψ_w (-0.48± 0.095 MPa). The leaf temperature was higher in CPB8 than in CPB2, and g_s in CPB8 was higher than in CPB2. Early leaf rolling in CPB8 may be associated with lower leaf Ψ_s (osmotic adjustment) and higher leaf temperature. Leaf rolling initiation in CPB2 was delayed but similar to CPB8. However, the PEPC activity and protein content were lower in CPB2 than in CPB8 at leaf rolling initiation, probably due to a longer water deficit period (15 d) in CPB2, which could affect more the biochemical parameters.

Lower leaf Ψ_s is usually associated with osmotic adjustment (Turner 1975, Schmidhalter *et al.* 1998). Unlike Ekanayake *et al.* (1993) who reported that leaf resistance and rolling were linearly correlated with leaf Ψ_w, our results showed association of leaf rolling initiation with leaf temperature and leaf Ψ_s. Previous authors have mentioned that small decrease in transpiration rate, leaf temperature, and irradiance associated with leaf rolling may decrease the potential for photoinhibition, prolong physiological activity, and increase survival during growth (Heckathorn and DeLucia 1991). Yeo *et al.* (1997) reported that leaf rolling reduces water loss where stomatal closure is incomplete, reduces the thermal load on the leaf by reducing the surface area exposed, and that there are complex interdependencies between transpiration, photosynthesis, and leaf temperature such that leaf rolling can be of adaptive significance. These authors concluded that from an agronomic viewpoint, leaf rolling is generally viewed as an indication of drought-susceptibility in rice, though this may have more to do with its value as a symptom. White *et al.* (1992), working with turfgrass, reported that survival was associated with low basal Ψ_s before stress and osmotic adjustment, prolonged positive turgor maintenance, and delayed leaf rolling during stress. Our results support the association with low Ψ_s. Lilley *et al.* (1996) mentioned that while leaf rolling is a dehydration-avoidance mechanism, it is also an indication of leaf water status and, generally, lines with greater leaf rolling are classified as drought-sensitive because it indicates less efficient soil water extraction (drought avoidance). In rice lines which are able to adjust osmotically, the effects of poor water extraction capability on shoot water status would be reduced since osmotic adjustment defers turgor loss and hence leaf rolling (Turner *et al.* 1986). In terms of response to stress, osmotic adjustment is a component of the alternative drought-tolerance strategy (Lilley *et al.* 1996). Saneoka *et al.* (1996) reported that in maize plants under water deficit, the degree of leaf rolling was lower in drought-tolerant cultivars which maintained a higher osmotic adjustment under moderate and excessive water stress.

Our results for both hybrids show that leaf rolling initiation is associated with (*1*) high leaf temperature, with leaf rolling effect related to leaf temperature reduction, and (*2*) lower leaf Ψ_s, related to osmotic adjustment as an additional component of drought-tolerance strategy. The CPB8 hybrid initiated leaf rolling at higher g_s and lower leaf Ψ_s; this may indicate that both mechanisms, avoidance (leaf rolling) and tolerance (lower leaf Ψ_s, osmotic adjustment) are involved during the water deficit period. Our results support also the idea that leaf rolling reduces water loss where stomatal closure (in CPB8) is incomplete and will reduce the thermal load on the leaf by reducing the surface area exposed, prolonging physiological activity, further suggesting that leaf rolling is an adaptive mechanism.

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