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BRIEF COMMUNICATION

Glutamine synthetase activity, relative water content and water potential in maize submitted to drought

L.O. MEDICI*, A.T. MACHADO*, R.A. AZEVEDO** and C. PIMENTEL***

*Departamento de Ciências Fisiológicas, BR 465, Km 7, Seropédica CEP 238990-000, RJ, Brasil**
*Departamento de Genética, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba CEP 13400-970, SP, Brasil***
*Embrapa Cerrados, Caixa Postal 08223, Planaltina CEP73301-970, DF, Brasil****

Abstract

Two maize (*Zea mays* L.) hybrids, BR 201 and P 6875, were submitted to drought by withholding irrigation at the vegetative or reproductive phases. During water deficit, in both phases, the relative water content (RWC) was higher and leaf water potential (Ψ_w) was lower in the hybrid P 6875 than in the hybrid BR201. This response indicated a better osmotic adjustment capacity of the hybrid P 6875 when compared to BR 201. Glutamine synthetase activity was not affected by drought even at a RWC of 60 % and Ψ_w of -1.2 MPa or during rehydration.

Additional key words: drought tolerance, osmotic adjustment, *Zea mays*.

Annual yield losses in the tropics due to water deficit between 10 to 50 % have been estimated (Duque-Vargas *et al.* 1994). Genotypic variation for water deficit adaptation in maize is very complex, involving many genetic trait and physiological mechanisms, which are not fully understood (Passioura 1997). Therefore, the selection for water deficit tolerance has a limited efficiency. The enzymes of the nitrogen metabolism play an important role in tolerance mechanisms to water deficiency (Hanson and Hitz 1982). Glutamine synthetase (GS) is an important enzyme in nitrogen assimilation in higher plants, being involved in the re-assimilation of NH_4^+ from photorespiration and proteolysis, processes which are increased by water deficiency (Lea *et al.* 1990, Bauer *et al.* 1997, Pimentel 1999, Tsai and Kao 2002, Chakraborty *et al.* 2002). Furthermore, GS activity is also related to osmotic adjustment, since the synthesis of proline depends on glutamine. The inhibition of the expression of GS1 isoform in *Nicotiana tabacum* led to a reduced synthesis of proline and higher sensitivity to salt stress (Brugiére *et al.* 1999). Therefore, GS activity may be considered for the characterization of water stress tolerant genotypes (Miranda-Ham and Loyola-Vargas 1994).

In this report, a study was carried out to evaluate the effect of water deficiency on the activity of GS, relative water content (RWC) and pre-dawn leaf water potential (Ψ_w) at the vegetative and reproductive stages of development of maize.

The two maize (*Zea mays* L.) double-hybrids (semi-dent) used in this study, P6875 and BR201, were kindly donated by Pioneer and Embrapa Milho e Sorgo. Maize plants were cultivated in a glasshouse in pots containing 11 kg of dry top soil (extracted from the first 20 cm of soil surface, pH 5.9) mixed with 3.5 g of ammonium sulphate and 10 g of phosphate. Two further doses of 5 g ammonium sulphate were applied at 56 and 63 days after planting (DAP).

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Abbreviations: DAP - days after planting; GS - glutamine synthetase; NADP-GDH - glutamate dehydrogenase; RWC - relative water content; Ψ_w - leaf water potential.
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Fax: (+55) 19 3433 6706, e-mail: lomedici@ufrrj.br

A first experiment was carried out to evaluate the behavior of the hybrids under drought. Both hybrids were submitted to drought by stopping irrigation at the vegetative stage (39 DAP) or at the beginning of flowering (68 DAP). During these two periods, three evaluations were carried out: after 2, 6 and 9 DAP of drought at the vegetative stage and after 2, 6 and 10 d of drought at the reproductive stage. The experimental design was a factorial of two hybrids versus two watering regimes (with and without drought) versus two phases and three evaluations with three replicates in a complete randomized block design. A second experiment was carried out to study the recovery of the hybrids after the water stress treatment. Plants in the reproductive stage 68 DAP were submitted to water stress for 11 d and 2 d of recovery, 13 d of water stress and control (continuously irrigated). The experimental design was a factorial of two hybrids versus three watering regimes (control, with drought and with re-watering after drought) and two replicates in a complete randomized block design. In both experiments the Ψ_w, RWC and GS activity in leaves were determined. At the vegetative stage the leaf analysed was the youngest with a visible collar, whereas at the reproductive stage the leaf analysed was the one above the first ear. Ψ_w was determined using a dew-point hygrometer (*Model HR-33T*, *Wescor*, Logan, USA) with a leaf chamber (*Model C-52*, *Wescor*) at pre-dawn between 04:30 and 06:00. The RWC was determined in leaf discs of 1 cm diameter harvested just after dawn, using the following formula: RWC=[(lm-drm)/(tm-drm)] × 100, where lm is the disc mass on removal, drm is the dry disc mass for 24 h at 60 °C and tm is the disc mass saturated with water after 12 h at 4 °C in a Petri dish containing distilled water. GS was extracted in a medium containing 50 mM Tris (pH 7.5) and 10 mM β-mercaptoethanol. The supernatant of the crude extract was used in enzyme assays following the method of Rhodes *et al.* (1975). GS assay was started with the addition of 0.3 cm^3 of enzyme extract to 1.7 cm^3 of a reaction mixture containing 100 µmol Tris/HCl, pH 7.8, 10 µmol β-mercaptoethanol, 40 µmol $MgSO_4$, 10 µmol hydroxylamine, 10 µmol ATP and 50 µmol L-glutamate. The reaction was incubated for 30 min at 30 °C and terminated by adding 1 cm^3 ferric chloride reagent. After centrifugation at 10 000 *g*, activity was determined by measuring the absorbance at 540 nm (spectrophotometer *Spectronic/Genesys 5*, Milton Roy, USA). Enzyme activity was expressed in µmol of γ-glutamylmonohydro-xamate g^{-1}(dry matter) h^{-1}.

The analysis of the results for RWC revealed that hybrid BR201 became dehydrated more rapidly than P6875 during drought at both stages. On the ninth day of drought during the vegetative stage, BR201 exhibited a significantly smaller RWC value than on the second day of drought, while RWC in P6875 was not significantly different between the first and the last day of stress

Table 1. Relative water content [%] in leaves of the maize hybrids BR201 and P6875 after 2, 6 and 9 d of drought initiated at 39 d after planting (vegetative stage), and after 2, 6 and 10 d of drought at 68 d after planting (reproductive stage). The control plants were continuously irrigated. Means with different letters, in the same stage, are significantly different ($P < 5$ %) according to the Newman Keuls test.

Stage	Hybrid		2 d	6 d	9 d
Veget.	BR201	control	94.7 ab	92.0 ab	97.1 ab
		drought	87.4 ab	96.2 ab	72.5 c
	P6875	control	92.6 ab	97.6 ab	99.6 a
		drought	96.0 ab	86.6 abc	79.0 bc
Reprod.	BR201	control	94.0 a	90.8 ab	96.4 a
		drought	95.0 a	79.4 bc	57.6 d
	P6875	control	97.2 a	94.9 a	99.9 a
		drought	91.3 a	77.3 c	67.1 cd

(Table 1). During the reproductive stage, a similar behaviour was observed so that during water stress the BR201 hybrid became more dehydrated than the P6875 hybrid. At this stage, on the tenth day of drought, BR201 exhibited a significantly smaller RWC than on the second and sixth days, whereas P6875 still exhibited a RWC value on the tenth day of drought similar to that observed on the sixth day of drought (Table 1). During the stress, RWC was more reduced in BR201 than P6875, but on the ninth day of stress, in both stage, both hybrids exhibited similar values (Table 1). However, in the second experiment, after 13 d of drought, a significant difference among the hybrids was observed, that is, a higher RWC value for P6875 (Table 2).

Table 2. Relative water content [%] in leaves of the maize hybrids BR201 and P6875 continuously irrigated (control), submitted to 13 d of drought at 68 d after planting and following 2 d of rehydration after 11 d of drought. Means with different letters are significantly different ($P < 5$ %) according to the Newman Keuls test.

Hybrid	Treatment	RWC
BR201	control	98.9 a
	drought	49.8 c
	rehydration	94.5 a
P6875	control	95.7 a
	drought	62.2 b
	rehydration	100.0 a

Under our experimental conditions, RWC was an efficient parameter capable of detecting differences between the hybrids submitted to the severe water deficit. Rossiello (1981) also observed that the maize cultivar with higher RWC during water deficit exhibited the highest dry matter accumulation in the root and in the leaves. Kramer and Boyer (1995) recommended the use of RWC instead of Ψ_w, as a parameter of the hydration degree of plants that perform osmotic adjustment, such as maize, since the osmotic adjustment and other mechanisms maintain a high RWC, which is therefore the result of adaptation mechanisms to drought (Neumann 1995).

Table 3. Water potential [-MPa] in leaves of hybrids BR 201 and P 6875 after 2, 6 e 9 d of drought initiated at 39 d after planting (vegetative stage) and after 2, 6 and 9 d of drought at 68 d after planting (reproductive stage). The control plants were continuously irrigated. Means with different letters are significantly different ($P < 5$ %) according to the Newman Keuls test.

Stage	Hybrid		2 d	6 d	9 d
Veget.	BR201	control	0.58 ab	0.55 ab	0.63 ab
		drought	0.58 ab	0.68 ab	0.73 ab
	P6875	control	0.40 a	0.71 ab	0.78 ab
		drought	0.41 a	0.81 ab	0.96 b
Reprod.	BR201	control	0.70 ab	0.56 a	0.70 ab
		drought	0.89 abcd	1.06 bcd	1.26 d
	P6875	control	0.61 a	0.55 a	0.68 ab
		drought	0.79 abc	1.13 cd	1.25 d

Similar to the results for RWC, the values of Ψ_w attained by the hybrid demonstrated that these were differently affected by water deficiency. At both stages tested, P6875 exhibited a Ψ_w value significantly lower at the end in relation to the beginning of the drought treatment, while BR201 exhibited values without significant difference during the drought treatment (Table 3). Therefore, hybrid P6875 promoted a stronger reduction in Ψ_w, accompanied by the maintenance of higher RWC, probably due to a higher osmotic adjustment, which is considered an adaptation mechanism to water deficiency in maize (Kramer and Boyer 1995). Quei and Wassom (1993) did not find a phenotypic and genotypic correlation between the osmotic adjustment and yield, suggesting that genetic gain is not expected during selection for osmotic adjustment. Bolaños and Edmeades (1991) also concluded that this trait has little adaptative value. However, these authors did not evaluate the cell wall elasticity adjustment as previously suggested by Neumann (1995). Probably, the hardening of the leaf cell wall in development may represent a mechanism of primary adjustment to the water deficit, preceding the slow osmotic adjustment, through active solute accumulation (Munns 1988). Thus, the evaluation of the water potential or the osmotic potential, as the only parameter indicative of tolerance to the water deficiency in plant breeding, without the evaluation of the cell wall elasticity or the RWC, does not correlate with yield in maize under water deficiency (Bolaños and Edmeades 1991). Two maize hybrids, contrasting for tolerance to drought, exhibited a similar reduction in RWC under drought, but the more tolerant hybrid maintained a higher Ψ_w, confirming the importance of the combined evaluation of RWC and Ψ_w (Pimentel 1999).

Table 4. Activity of glutamine synthetase [(μmol(γ-glutamyl-monohydroxamate) g^{-1}(d.m.) h^{-1}] in leaves of hybrids BR 201 and P 6875 after 2, 6 and 9 d of drought initiated at 39 d after planting (vegetative stage) and after 2, 6 and 10 d of drought at 68 d after planting (reproductive stage). The control plants were continuously irrigated. The control plants were continuously irrigated. Means with different letters are significantly different ($P < 5$ %) according to the Newman Keuls test.

Stage	Hybrid		2 d	6 d	9 d
Veget.	BR201	control	302 a	211 a	162 a
		drought	256 a	190 a	188 a
	P6875	control	243 a	188 a	155 a
		drought	239 a	214 a	161 a
Reprod.	BR201	control	118 a	110 a	144 a
		drought	116 a	102 a	129 a
	P6875	control	133 a	133 a	128 a
		drought	113 a	110 a	135 a

GS activity was not affected by the water deficit and a significant difference was not observed between the hybrids (Table 4). Miranda-Han and Loyola-Vargas (1994) reported that in maize plants water and salt stresses did not affect GS activity, whereas the glutamate dehydrogenase (NADH-GDH) activity was reduced, suggesting that the change in GS activity was not involved in the plant responses to water and salt stress. In tomato, Bauer *et al.* (1997) suggested that the increase in GS1 isoform under water stress may be involved in the transport of nitrogen, which is remobilized in the leaves under drought. It is important to point out that the enzymatic activity measured *in vitro* does not guarantee that the same activity is maintained *in vivo* (Plaut 1994).

In conclusion, the *in vitro* GS activity was not shown to be an efficient parameter to indicate water deficiency in maize since variation in activity was not detected for the two distinct maize hybrids used in this study. On the other hand, the evaluation of the relationship between RWC and Ψ_w, allowed a clear difference in response to water stress in maize plants and can be used as a parameter in breeding programs with the aim of selecting for improved tolerance to dehydration.

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