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BRIEF COMMUNICATION

Response of carbonic anhydrase to polyethylene glycol-mediated water stress in wheat

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Abstract

Carbonic anhydrase (CA) activity in wheat leaves changed upon leaf dehydration: it decreased at mild stress (relative water content, RWC, 81 %), but increased at severe water stress (RWC 74 %). Phosphoenolpyruvate carboxylase activity was not significantly affected by these stresses.

Additional key words: leaf dehydration; phosphoenolpyruvate carboxylase; *Triticum aestivum*.

Reduction of photosynthetic carbon assimilation under water deficit is attributed to both stomatal closure and altered biochemical/photochemical reactions (for reviews see Kaiser 1987, Chaves 1991). We have recently distinguished between relative stomatal and nonstomatal limitations of wheat photosynthesis upon PEG-mediated water stress (Kicheva *et al.* 1994). It has been suggested that besides stomatal limitation a decreased mesophyll conductance to CO_2 transfer (g_m) may limit photosynthesis when mild leaf dehydration is manifested. Some other results (Renou *et al.* 1990, Caemmerer and Evans 1991) point to the decreased g_m causing considerably lower chloroplast CO_2 concentration in comparison to the intercellular CO_2 concentration (C_i) in dehydrated wheat leaves.

Since the CO_2 transfer conductance depends on CA activity at first place (Makino *et al.* 1992) and this activity might become limiting to photosynthesis under water stress (CA activity is unusually low in wheat), we have determined soluble CA activity in dehydrated wheat leaves.

Experiments were carried out on second fully expanded leaves of 21 d old wheat (*Triticum aestivum* L. cv. Trakia) plants grown on nutrient solution in a growth

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chamber (irradiance 160 μmol m^{-2} s^{-1}, photoperiod 12 h, temperature 25±2 °C, relative humidity 60±5 %) (Kicheva *et al.* 1994). Leaf dehydration was achieved after submerging plant roots into 15 % (m/v) polyethylene glycol (*PEG 6000*) solution. The 6 and 24 h treatments caused decline in leaf RWC of about 10 % (mild water stress) and 20 % (severe water stress), respectively. Soluble CA activity was determined in leaf extracts by measuring the pH decrease at 2 °C with a pH electrode as described by Popova *et al.* (1996). Enzyme activity was defined as 1 unit = $10(t_0-t)/t$, in which t and t_0 represent the time at 2 °C for a pH decrease from 8.3 to 7.8, with and without the enzyme, respectively. Phosphoenolpyruvate carboxylase (PEPC) activity was determined radiometrically (Popova *et al.* 1988).

The CA activity declined in response to mild leaf dehydration (Table 1). If CA activity in wheat is not present in great excess over what is required to maximise photosynthesis, then the decreased CA activity of about 18 % might affect the CO_2 diffusion towards carboxylation sites in the chloroplast. Therefore the chloroplast CO_2 concentration in the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) vicinity would be lowered. This suggestion is supported by the fact that both the net photosynthetic rate and CA activity are decreased while C_i remains constant in mildly dehydrated wheat leaves (this study and Kicheva *et al.* 1994).

Under severe leaf dehydration the CA activity increased by 67 % (Table 1). We hypothesise that CA could be involved in an alternative process of CO_2 fixation driven by PEPC. The finding that RuBPCO carboxylation activity is considerably decreased (Kicheva *et al.* 1994) while PEPC activity is not altered (Table 1) in severely dehydrated wheat leaves supports this interpretation (cf. also Popova *et al.* 1996). There is a strong evidence that cytosolic CA isozymes exist in C_3 plants (Fett and Coleman 1993, Sultemeyer *et al.* 1993). The function of these isozymes can hardly be seen as facilitating CO_2 diffusion to the chloroplast because of the short distance between cell wall and chloroplast envelope and low concentration of HCO_3^- species in the nearly neutral cytoplasm (Cowan 1986).

Table 1. Carbonic anhydrase (CA) and phosphoenolpyruvate carboxylase (PEPC) activities in wheat leaves after plant treatment with 15 % polyethylene glycol (PEG) solution. Means ±SE of 4 independent experiments. $^*p<0.05$, $^{**}p<0.01$.

Stress conditions	CA	[%]	PEPC
	[10^6 (unit) kg^{-1}(protein)]		[μmol(CO_2) kg^{-1}(protein) s^{-1}]
Control	108.5 ± 4.9	100	567 ± 62
15 % PEG, 6 h	88.7 ± 0.6*	82	567 ± 34
15 % PEG, 24 h	181.1 ± 11.5**	167	533 ± 80

All these results make the role of CA in water-stressed leaves an intriguing problem for further investigation. We believe that some additional pathway for inorganic carbon utilisation in C_3 leaves is potentially useful for plant survival under severe leaf dehydration when CO_2 input through the stomata is limited. Studies on CA protein expression in water-stressed wheat plants are in progress.

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