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Carbonic Anhydrase Activity and CO_2-Transfer Resistance in Zn-Deficient Rice Leaves[1]

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It has been reported that carbonic anhydrase (CA) activity in plant leaves is decreased by Zn deficiency. We examined the effects of Zn deficiency on the activity of CA and on photosynthesis by leaves in rice plants (*Oryza sativa* L.). Zn deficiency increased the transfer resistance from the stomatal cavity to the site of CO_2 fixation 2.3-fold and, consequently, the value of the transfer resistance relative to the total resistance in the CO_2-assimilation process increased from 10% to 21%. This change led to a reduced CO_2 concentration at the site of CO_2 fixation, resulting in an increased gradient of CO_2 between the stomatal cavity and this site. The present findings support the hypothesis that CA functions to facilitate the supply of CO_2 from the stomatal cavity to the site of CO_2 fixation. We also showed that the level of mRNA for CA decreased to 13% of the control level during Zn deficiency. This decrease resembled the decrease in CA activity, suggesting the possible involvement of the CA mRNA level in the regulation of CA activity.

CA (EC 4.2.1.1) catalyzes the reversible conversion of CO_2 to HCO_3, which can be dissolved more easily, and has been recognized as an important enzyme that is closely associated with photosynthesis. However, the application of inhibitors of CA to intact chloroplasts did not lower the rate of photosynthesis (Swader and Jacobson, 1972; Jacobson et al., 1975), suggesting that CA is not essential for the photosynthetic assimilation of CO_2. It has also been reported that inactivation of CA mRNA by the introduction of antisense RNA had no significant negative effect on the photosynthetic assimilation of CO_2 by tobacco leaves (Majeau et al., 1994; Price et al., 1994; Williams et al., 1996). In spite of experimental data showing the absence of an association of CA with photosynthesis, researchers showed that CA increased the CO_2 concentration at the site of CO_2 fixation in chloroplasts by 15 to 20 μL L^{-1}, and that CA played a role in facilitating CO_2 diffusion through the use of ^{13}C and ^{18}O in CO_2 (Price et al., 1994; Williams et al., 1996).

In a previous study we tried to separate the r_m into the r_r and the r_c by measuring the $\delta^{13}C$, and we succeeded in estimating the magnitude of the r_r from the intercellular space of mesophyll cells to the site of CO_2 fixation in the chloroplasts (Sasaki et al., 1996). The purpose of the present study was to elucidate the relationship between the activity of CA and each component of mesophyll resistance in an attempt to determine the mechanism that explains why CA activity is not related to photosynthetic performance.

CA is a Zn-containing enzyme, so Zn is essential for its catalytic activity (Bar-Akiva and Lavon, 1969; Silverman, 1991). Guliev et al. (1992) reported that removal of Zn from CA in vitro resulted in the irreversible loss of catalytic activity. Several authors have also reported that CA activity can be specifically inhibited by Zn deficiency in some plants without any significant reduction in the rate of photosynthesis (Edwards and Mohamed, 1973; Randall and Bouma, 1973; Ohki, 1976, 1978). Therefore, the second goal of this study was to elucidate the mechanism of the specific inhibition of CA activity by Zn deficiency at the level of expression of CA mRNA.

MATERIALS AND METHODS

Cultivation of Plants

Seeds of rice (*Oryza sativa* L. cv Hatsunishiki) were sown on wet quartz sand. Four weeks later, seedlings were transplanted to 40-L polyethylene containers filled with culture solution prepared as described by Yoshida et al. (1976) at a density of 56 plants per container. The solution contained 1.425 mM NH_4NO_3, 0.323 mM NaH_2PO_4, 0.513 mM K_2SO_4, 0.998 mM $CaCl_2$, 0.998 mM $MgSO_4$, 0.009 mM $MnCl_2$, 0.075 μM $(NH_4)_6Mo_7O_{24}$, 0.019 mM H_3BO_3, 0.155 μM $CuSO_4$, and 0.036 mM $FeCl_3$. The plants were treated with 150 nM $ZnCl_2$ from 4 weeks after germination (+Zn), with 15 nM $ZnCl_2$ from 11 weeks after germination (±Zn), and without Zn (−Zn). Three containers were prepared for each treatment. The plants were grown in a naturally illuminated greenhouse that was air conditioned to give a day temperature of 30°C and a night temperature of 25°C.

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Abbreviations: CA, carbonic anhydrase; $[CO_2]_{atm}$, CO_2 concentration in the atmospheric air; $[CO_2]_{cht}$, CO_2 concentration at the site of CO_2 fixation in the chloroplast; $[CO_2]_{stc}$, CO_2 concentration in the stomatal cavity; Δ, carbon isotope discrimination by the plant; δ_a, relative concentration of ^{13}C in the atmospheric air; δ_p, relative concentration of ^{13}C in the photosynthetic products; $\delta^{13}C$, relative concentration of ^{13}C in total carbon atoms; r_c, CO_2-fixation resistance; r_m, mesophyll CO_2 resistance; r_r, CO_2-transfer resistance; r_s, stomatal CO_2 resistance.

Determination of Zn Content

The leaf area of the uppermost fully expanded leaf on the main stem was measured for 6 plants from each container (18 plants for each treatment) 13 weeks after germination, and these leaves were dried in an electric dryer. The dry weight was determined and the specific leaf weight was calculated. Samples of 0.1 g dry weight were digested overnight in 5.0 mL of concentrated HNO_3 and 0.3 mL of 40% (w/v) HF in a 50-mL Teflon beaker, and wet ashed on the heater the following day. The Zn content was determined with an inductively coupled plasma atomic spectrometer (SPS-7000A, Seiko Instruments, Tokyo, Japan).

Measurement of the Gas-Exchange Rate

Leaf photosynthesis and transpiration were measured simultaneously for the uppermost fully expanded leaf on the main stem for 6 plants from each container (18 plants for each treatment) 13 weeks after germination using a handmade gas-exchange system with an IR gas analyzer (ZAP-AZ012, Fuji Electric Co. Ltd., Tokyo, Japan) and a humidity sensor (HMP111Y, Visala, Helsinki, Finland). A leaf (approximately 8–12 cm^2) was clamped in the leaf chamber with a water jacket providing background cooling, and maintained under controlled conditions of temperature, light, humidity, and CO_2 concentration. The measurements were made at a CO_2 concentration of 340 μL L^{-1}, an irradiance greater than 1400 μmol m^{-2} s^{-1} photon flux density under artificial light, and a leaf temperature of 30°C ± 3°C, after preillumination for 60 min to obtain steady-state readings of photosynthesis and transpiration.

Measurement of ^{13}C Values of Soluble Sugars

Ten plants in each container were placed in darkness at 12 PM to starve the leaves of photosynthetic products. The next morning the leaves were exposed to artificial light at an irradiance greater than 1400 μmol m^{-2} s^{-1} photon flux density from 9 AM to 12 PM in a 72-m^3 laboratory ventilated at 2160 m^3 h^{-1}, and were then cut off from the plant. No one entered the laboratory during the exposure and there was no difference between the values of the isotopic composition of CO_2 in the air in this room and outside. The accumulation of soluble sugars was conducted for three treatments at the same time. The soluble sugars were extracted and ^{13}C was determined with a mass spectrometer (MAT-252, Finnigan MAT, San Jose, CA) as reported previously (Sasaki et al., 1996).

^{13}C Determination

^{13}C of soluble sugars was determined with a mass spectrometer according to the method of Sasaki et al. (1996). Δ can be expressed by the following equation (Hubick et al., 1986):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_a} \quad (1)$$

where δ_a and δ_p are the relative concentrations of ^{13}C in the atmospheric air and in the photosynthetic products, respectively. The δ_a was determined as −8.0‰ ± 0.1‰ from the actual measurement of the air in our laboratory, and δ_p was determined by the mass-spectrometric method with soluble sugars extracted from the leaves. The Δ obtained was inserted into Equation 2 (Sasaki et al., 1996):

$$[CO_2]_{cht} = \frac{\left\{\Delta \times 10^3 - 4.4 + (4.4 - 1.8) \times \frac{[CO_2]_{stc}}{[CO_2]_{atm}}\right\} \times [CO_2]_{atm}}{(29 - 1.8)} \quad (2)$$

where 4.4 is the discrimination coefficient in the CO_2-diffusion process through stomata in C_3 plants, 1.1 is the discrimination coefficient in the CO_2-dissolution process in water at 25°C, 0.7 is the discrimination coefficient in the CO_2-diffusion process in the liquid phase at 25°C, and 29 is the discrimination coefficient in the CO_2-carboxylation process by Rubisco. Carbon discrimination also occurred in the processes of dark respiration and photorespiration; however, we assumed the value of these factors to be zero because according to published reports the extent is almost negligible (Farquhar et al., 1982; Evans et al., 1986). $[CO_2]_{atm}$ was maintained at about 340 μL L^{-1} in this experiment and $[CO_2]_{stc}$ was obtained from the measurement of photosynthesis and transpiration. Therefore, $[CO_2]_{cht}$ can be obtained from Equation 2 after inserting the value of Δ determined from Equation 1.

Calculation of Stomatal Transfer and Fixation Resistances

The r_s and the r_m were calculated using the following equations:

$$r_s = 1.56 \times \frac{e_i - e_a}{Tr} \left(= \frac{[CO_2]_{atm} - [CO_2]_{stc}}{LPS}\right) \quad (3)$$

$$r_m = \frac{[CO_2]_{stc} - \Gamma}{LPS} \quad (4)$$

where e_i and e_a are the intercellular and atmospheric vapor pressures, respectively, Tr is transpiration, LPS is leaf photosynthesis, and Γ is the CO_2-compensation point. The factor 1.56 is the ratio of the diffusivities of water vapor and CO_2 in air. If $[CO_2]_{cht}$ is obtained from Equation 2, the r_r from the stomatal cavity to the site of CO_2 fixation and the r_c can be obtained using the following equations:

$$r_r = \frac{[CO_2]_{stc} - [CO_2]_{cht}}{LPS} \quad (5)$$

$$r_c = \frac{[CO_2]_{cht} - \Gamma}{LPS} \quad (6)$$

Determination of CA Activity

CA activity was measured in the uppermost fully expanded leaf on the main stem for 6 plants from each

container (18 plants for each treatment). The detached leaves, which had been illuminated for 1 h at 1400 μmol m^{-2} s^{-1} photon flux density, were ground with a buffered solution (pH 8.3) that contained 50 mM barbital-H_2SO_4, 5 mM DTT, and 0.2% (w/v) PVP. The homogenate was centrifuged at 12,000*g* for 2 min, and the supernatant was used for the determination of CA activity, according to the method of Sasaki et al. (1996).

Determination of Rubisco Content and Activity

The content of Rubisco was determined in the uppermost fully expanded leaf on the main stem for six plants from each container (18 plants for each treatment). The leaves were excised immediately after the measurement of leaf photosynthesis and stored at −80°C in a freezer before the determinations. The content of Rubisco was determined by the method of Sasaki et al. (1996). The activity of Rubisco was measured in terms of the initial activity at 30°C according to the method described by Usuda (1985) for the uppermost fully expanded leaf on the main stem for 6 plants from each container (18 plants for each treatment) after illumination for 1 h at 1400 μmol m^{-2} s^{-1} photon flux density.

Quantitation of Soluble Protein

The amount of soluble protein in the supernatant prepared for the measurement of the content of Rubisco was determined as described by Lowry et al. (1951) using BSA as the standard.

Quantitation of Chlorophyll

The amount of chlorophyll in the homogenate prepared for the measurement of Rubisco was determined for 6 plants from each container (18 plants for each treatment) as described by Schmid (1971).

Isolation of RNA and Northern-Blot Hybridization

Total RNA was isolated from 0.2 g of frozen sample as described by Fromm et al. (1985). Total RNA (20 μg per lane) was fractionated on a 1.15% agarose gel that contained 1.85% (v/v) formaldehyde, transferred to a nylon membrane (Hybond-N, Amersham), and allowed to hybridize with radiolabeled DNA probes. After hybridization the blot was washed twice in 2× SSC (SSC contains 0.15 M NaCl and 0.015 M trisodium citrate) containing 0.1% SDS at 42°C for 5 min, and then washed again in 0.2× SSC containing 0.1% SDS at 45°C for 1 h. The signals attributable to the radiolabeled probe were visualized and quantified with a Bio-Imaging Analyzer (BAS 2000, Fujix, Tokyo, Japan). A 0.64-kb fragment of cDNA for rice chloroplastic CA (S. Suzuki and J.N. Burnell, unpublished data; accession no. U08404) and a full-length clone of the rice Rubisco small subunit (Matsuoka et al., 1988; accession no. D00644) were used as the probes.

RESULTS

Levels of Zn and Enzymes and Rates of Photosynthesis

The Zn contents per unit leaf area of the −Zn and ±Zn plants were as low as 0.19 and 0.23 mg m^{-2}, respectively, compared with 0.51 mg m^{-2} in the +Zn plants (Table I). In spite of the great reduction in the Zn content of leaves, we observed no change in specific leaf weight or in chlorophyll content, which is considered to be a critical indicator of Zn deficiency. Therefore, −Zn and ±Zn plants were only moderately stressed. In contrast, although the CA activity in −Zn plants decreased dramatically to as little as 14% of that in +Zn plants, the Rubisco activity decreased only to 89% of that in +Zn plants. The levels of soluble protein and Rubisco increased slightly. Moreover, little change in the rate of photosynthesis was observed in the leaves with a reduced Zn content. These results indicated that Zn deficiency resulted in the specific inhibition of CA activity.

Stomatal Transfer and Fixation Resistance

To clarify the contribution of CA activity to the assimilation process, we calculated the r_c and the r_r by measuring leaf photosynthesis and ^{13}C in the soluble sugars extracted from the leaves (Table II). Although no significant difference was found in r_c and r_s in leaves of +Zn, −Zn, and ±Zn plants, we observed a 2.3-fold increase in r_r, which increased from 1.2 mol^{-1} CO_2 m^2 s in +Zn plants to 2.7

Table I. *Effects of Zn deficiency on Zn content, photosynthesis, $\delta^{13}C$ value, chlorophyll content, and levels of CA and Rubisco in fully expanded leaves*

Values are expressed as means ± SE; $n = 18$ except for the $\delta^{13}C$ value ($n = 3$). Numbers in parentheses are percentages relative to values for +Zn plants.

Parameter	+Zn	±Zn	−Zn
Zn content (mg m^{-2})	0.51 ± 0.05	0.23 ± 0.03 (45)	0.19 ± 0.04 (37)
Specific leaf wt (g m^{-2})	19.3 ± 2.0	20.2 ± 2.6 (105)	19.9 ± 3.6 (103)
Chlorophyll content (g m^{-2})	0.59 ± 0.09	0.49 ± 0.15 (82)	0.45 ± 0.14 (78)
Photosynthesis (μmol CO_2 m^{-2} s^{-1})	22.9 ± 1.7	19.7 ± 3.6 (86)	20.4 ± 2.3 (89)
Soluble protein (g m^{-2})	6.5 ± 0.3	7.7 ± 0.3 (118)	7.5 ± 0.2 (116)
CA activity (× 10^3 units m^{-2})	473 ± 57	126 ± 23 (27)	68 ± 36 (14)
Rubisco content (g m^{-2})	3.5 ± 0.3	4.3 ± 0.3 (126)	4.8 ± 0.4 (139)
Rubisco activity (μmol CO_2 m^{-2} s^{-1})	29.4 ± 2.6	23.5 ± 4.3 (80)	26.3 ± 5.2 (89)
$\delta^{13}C$ value (‰)	−25.83 ± 0.24	−24.05 ± 1.27	−23.55 ± 1.91

Table II. *Effects of Zn deficiency on CO^2-diffusion resistance*

Values are expressed as means ± SE; n = 18 for stomatal and mesophyll resistances and n = 3 for CO_2-transfer and CO_2-fixation resistances. Numbers in parentheses are percentages relative to the total resistance.

Resistance	+Zn	±Zn	−Zn
		mol^{-1} CO_2 m^2 s	
Stomatal	4.7 ± 0.5 (40)	5.7 ± 0.6 (42)	5.2 ± 0.5 (40)
Mesophyll	7.0 ± 0.5 (60)	8.0 ± 1.6 (58)	8.0 ± 1.2 (60)
CO_2 transfer	1.2 ± 0.3 (10)	2.2 ± 0.9 (16)	2.7 ± 0.7 (21)
CO_2 fixation	5.9 ± 0.4 (50)	5.8 ± 0.8 (42)	5.3 ± 0.6 (40)

mol^{-1} CO_2 m^2 s in −Zn plants. This corresponded to an increase from 10% to 21% when r_r was calculated as a percentage of total resistance. This indicates that the Zn deficiency affected only the CO_2-transfer step in the CO_2-assimilation process.

The Concentration of CO_2 in Leaves

In all leaves examined $[CO_2]_{stc}$ was maintained at about 220 μL L^{-1}. In contrast, $[CO_2]_{cht}$ in +Zn leaves was 195 ± 4 μL L^{-1}, and $[CO_2]_{cht}$ decreased with a reduction in the Zn content of leaves to 171 ± 6 and 166 ± 5 μL L^{-1} in ±Zn and −Zn leaves, respectively. The gradient of the CO_2 concentration between the stomatal cavity and the site of CO_2 fixation increased from 29 to 59 μL L^{-1} (Fig. 1). The resistance in CO_2 flux with Zn deficiency and our findings support the hypothesis that CA plays a role in facilitating the supply of CO_2 to the sites of carboxylation.

Northern-Blot Analysis

The results of northern-blot analysis of mRNAs for CA and Rubisco are shown in Figure 2. The level of the mRNA for CA decreased with the reduction in Zn content of the leaf, decreasing to 26% (±Zn) and 13% (−Zn) of that in control plants (+Zn). In contrast, the level of the mRNA for the small subunit of Rubisco showed no consistent trend. It seems likely that the reduction in CA activity was caused not by deactivation of the enzyme but, rather, by a decrease in the expression of the CA mRNA caused by Zn deficiency.

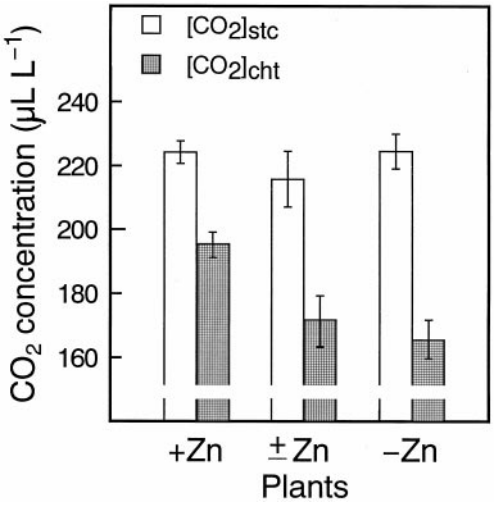

Figure 1. $[CO_2]_{stc}$ and $[CO_2]_{cht}$ in plants with three different levels of Zn. Values are expressed as means ± SE. See text for details.

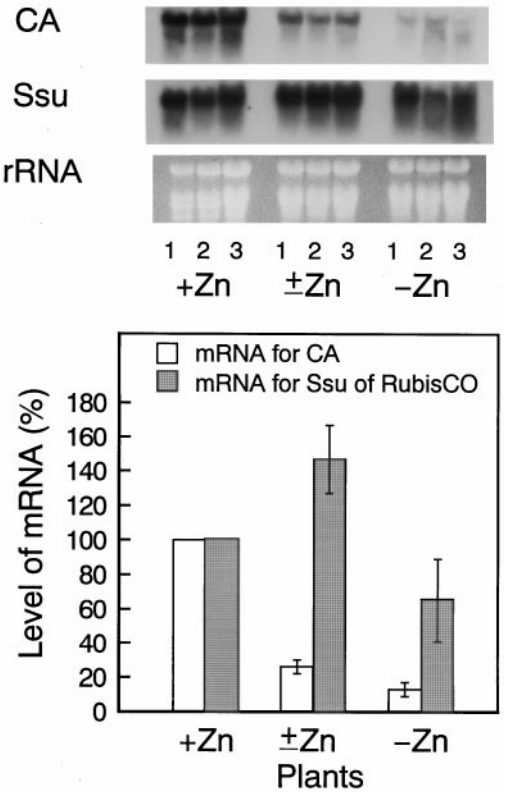

Figure 2. Northern-blot analysis of the expression of mRNAs for CA and the small subunit of Rubisco in plants with three different levels of Zn. Total RNA, 20 μg per lane, was separated on 1.5% agarose containing formaldehyde, transferred to a nylon membrane, and hybridized to radiolabeled DNA probes. Each level of Zn included three lanes and each lane derived from one container. Levels of mRNAs in +Zn plants are set at 100%. Values are expressed as means ± SE. See text for details. Ssu, Small subunit of Rubisco.

DISCUSSION

CA has been recognized as an important enzyme that is closely associated with photosynthesis. In C_3 plants CA activity is located mainly in the mesophyll chloroplast, with much smaller activity in the cytosol. Chloroplastic CA activity was reported as 87% of total cellular activity in potato leaves (Rumeau et al., 1996), and from 86% to 95% in wheat, spinach, *Moricandia arvensis*, and *Mesembryanthemum crystallinum* (Tsuzuki et al., 1985). Recently, cytosolic and chloroplastic CA isoenzymes were isolated in Arabidopsis and potato, and a polyclonal antibody hybridized to two kinds of CA (Fett and Coleman, 1994; Rumeau et al., 1996). The hypothesis that an alternative processing occurred during transit-peptide removal has been proposed by Johansson and Forsman (1992). Furthermore, two Arabidopsis cDNA clones encoding putative cytosolic and

chloroplastic CAs were isolated, and comparison of the predicted amino acid sequences indicated very similar genes (Fett and Coleman, 1994). In our experiment CA activity in the crude extract was measured and cDNA for rice chloroplastic CA was used as a probe for the northern-blot analysis. Considering the localization, percentage, and homology, although the CA activity and mRNA expression for CA in our study could not be distinguished from those for cytosolic and chloroplastic CA, they were considered to consist mainly of chloroplastic activity and mRNA expression for chloroplastic CA, respectively.

The main purpose of this study was to analyze the relationship between the activity of CA and each component of mesophyll resistance by using plants with reduced CA activity as a consequence of Zn deficiency. Major reductions in CA activity to 27% and 14% in ±Zn and −Zn plants, respectively, of that in +Zn plants were observed without any change in r_s or r_c. Therefore, we were able to detect a correlation between the transfer of CO_2 from the stomatal cavity to the site of CO_2 fixation and CA activity. It appeared that r_r increased with the reduction in CA activity (Tables I and II). Although $[CO_2]_{stc}$ was maintained at about 220 μL L^{-1} during photosynthesis, $[CO_2]_{cht}$ decreased with the reduction in CA activity, and the gradient of the concentration of CO_2 between the stomatal cavity and the site of CO_2 fixation increased from 29 to 59 μL L^{-1} with the reduction in CA activity (Fig. 1). With antisense RNA for CA a great reduction in the levels of CA to as little as 2% of wild-type levels caused an increase in the gradient of the CO_2 concentration of about 15 μmol mol^{-1} (Price et al., 1994; Williams et al., 1996), which is similar to our results. Consequently, we can conclude that a reduction in CA activity tampers with the transfer of CO_2 from the stomatal cavity to the site of Rubisco and causes a low $[CO_2]_{cht}$. CA is mostly localized in chloroplasts in C_3 plants. Therefore, our findings support the hypothesis that CA has a role in facilitating the supply of CO_2 to the sites of carboxylation within the chloroplast.

The considerable reduction in CA activity was accompanied by the reduction in Zn content, but we observed no major change in the rate of photosynthesis. In the antisense analysis, rates of CO_2 assimilation were also unaffected by low levels of CA in chloroplasts in transgenic tobacco plants (Majeau et al., 1994; Price et al., 1994). Such observations might be explained by the smaller contribution of the CO_2-transfer process than the stomatal or CO_2-fixation processes (Sasaki et al., 1996). Although $[CO_2]_{cht}$ decreased with a reduction in the activity of CA from 195 to 166 μL L^{-1}, we could not observe any marked decrease in the rate of photosynthesis as in $[CO_2]_{cht}$. If the CO_2-compensation point and other factors were constant, it is possible that a 29 μL L^{-1} difference in $[CO_2]_{cht}$ would cause an 18% to 20% decline in the rate of photosynthesis. We found that the rate of photosynthesis decreased only to 89% of that in +Zn plants. Although the initial activity of Rubisco was similar to the rate of photosynthesis, the Rubisco content unexpectedly increased slightly, from 3.5 to 4.8 g m^{-2}, with a reduction in the Zn content of leaves (the increase of Rubisco content has not been shown in other experiments on Zn deficiency). We suggest that the absence of any additional decrease in photosynthesis might be explained by the increase in Rubisco content.

Zn is necessary for catalysis by CA (Bar-Akiva and Lavon, 1969; Silverman, 1991). In this study expression of the mRNA for CA decreased to 34% (±Zn) and 13% (−Zn) of that in control plants. This decrease resembled that in the CA activity in these plants with the reduction in the Zn content of leaves (Table I; Fig. 2). Therefore, we suggest that the reduction in CA activity attributable to Zn deficiency was a result not of failure to activate CA but of a decrease in the level of CA. Although modification of transcript abundance does not always indicate transcriptional regulation, it is possible that a feedback system balances the amount of CA synthesized with the available Zn in the cell.

Some studies on the levels of CA in intact leaves have suggested that coordinated regulation of the expression of CA and Rubisco might occur under some growth conditions, e.g. with changes in the supply of nitrogen (Makino et al., 1992) and in the level of CO_2 (Porter and Grodzinski, 1984; Peet et al., 1986). Previously, we suggested that CA activity, most of which is found in the chloroplast, changes in association with changes in Rubisco activity in the chloroplast (Sasaki et al., 1996). Hudson et al. (1992) reported that CA activity changed in association with Rubisco activity in transgenic tobacco to which antisense Rubisco mRNA had been introduced. The activity ratio of CA to Rubisco is reportedly maintained in spite of differences in the levels of Rubisco and CA among cultivars and among leaf nitrogen contents in pea and wheat (Makino et al., 1992; Majeau and Coleman, 1994). Although such observations suggest the coordinated regulation of levels of CA and Rubisco, this relationship can easily be disrupted in low-CA plants (which are unusual), such as those in this study (Table I), which were induced by antisense RNA (Price et al., 1994). It seems likely that the level of CA is influenced by the level of Rubisco, but not vice versa, and although many studies show coordinated changes in CA and Rubisco, it appears that CA plays a role in facilitating the supply of CO_2 to sites of carboxylation.

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LITERATURE CITED

Bar-Akiva A, Lavon R (1969) Carbonic anhydrase activity as an indicator of zinc deficiency in citrus leaves. J Hortic Sci **44:** 359–362

Edwards GE, Mohamed AK (1973) Reduction in carbonic anhydrase activity in zinc-deficient leaves of *Phaseolus vulgaris* L. Crop Sci **13:** 351–354

Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol **13:** 281–292

Farquhar GD, Ball MC, von Caemmerer S, Roksandic Z (1982) Effect of salinity and humidity on ^{13}C values of halophytes: evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressure of CO_2 under different environmental conditions. Oecologia **52:** 121–124

Fett JP, Coleman JR (1994) Characterization and expression of two cDNAs encoding carbon anhydrase in *Arabidopsis thaliana*. Plant Physiol **105:** 707–713

Fromm H, Devic M, Fluhr R, Edelman M (1985) Control of *psbA* gene expression: in mature *Spirodela oligorrhiza* chloroplasts light regulation of 32 kD protein synthesis is independent of transcript level. EMBO J **4:** 291–295

Guliev NM, Briramov SM, Aliev DA (1992) Functional organization of carbonic anhydrase in higher plants. Sov Plant Physiol **39:** 537–544

Hubick KT, Farquhar GD, Shorter R (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. Aust J Plant Physiol **13:** 803–816

Hudson GS, Evans JR, von Caemmerer S, Arvidsson YBC, Andrews TJ (1992) Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants. Plant Physiol **98:** 294–302

Jacobson BS, Fong F, Heath RL (1975) Carbonic anhydrase of spinach. Studies on its location, inhibition and physiological function. Plant Physiol **55:** 468–474

Johansson IM, Forsman C (1992) Processing of chloroplast transit peptide of pea carbonic anhydrase in chloroplasts and in *Escherichia coli*: identification of two cleavage sites. FEBS Lett **314:** 232–236

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem **193:** 265–275

Majeau N, Arnold MA, Coleman JR (1994) Modification of carbonic anhydrase activity by antisense and over-expression constructs in transgenic tobacco. Plant Mol Biol **25:** 377–385

Majeau N, Coleman JR (1994) Correlation of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. Plant Physiol **104:** 1393–1399

Makino A, Sakashita H, Hidema J, Mae T, Ojima K, Osmond B (1992) Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2-transfer resistance. Plant Physiol **100:** 1737–1743

Matsuoka M, Kano-Murakami Y, Tanaka Y, Ozeki Y, Yamamoto N (1988) Classification and nucleotide sequence of cDNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase from rice. Plant Cell Physiol **29:** 1015–1022

Ohki K (1976) Effect of zinc nutrition on photosynthesis and carbonic anhydrase activity in cotton. Plant Physiol **38:** 300–304

Ohki K (1978) Zinc concentration in soybean as related to growth, photosynthesis, and carbonic anhydrase activity. Crop Sci **18:** 79–82

Peet MM, Huber SC, Patterson DT (1986) Acclimation to high CO_2 in monoecious cucumber. Carbon exchange rates, enzyme activities, and starch and nutrient conditions. Plant Physiol **80:** 63–67

Porter MA, Grodzinski B (1984) Assimilation to high CO_2 in bean: carbonic anhydrase and ribulose bisphosphate carboxylase. Plant Physiol **74:** 413–416

Price GD, von Caemmerer S, Evans JR, Yu J-W, Lloyd J, Oja V, Kell P, Harrison K, Gallagher A, Badger MR (1994) Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in transgenic tobacco plants has a minor effect on photosynthetic CO_2 assimilation. Planta **193:** 331–340

Randall PJ, Bouma D (1973) Zinc deficiency, carbonic anhydrase, and photosynthesis in leaves of spinach. Plant Physiol **52:** 229–232

Rumeau D, Cuiné S, Fina L, Gault N, Nicole M, Peltier G (1996) Subcellular distribution of carbonic anhydrase in *Solanum tuberosum* L. leaves. Planta **199:** 79–88

Sasaki H, Samejima M, Ishii R (1996) ^{13}C measurement on mechanism of cultivar difference in leaf photosynthesis of rice (*Oryza sativa* L.). Plant Cell Physiol **37:** 1161–1166

Schmid GH (1971) Origin and properties of mutant plants: yellow tobacco. Methods Enzymol **23:** 171–194

Silverman DN (1991) The catalytic mechanism of carbonic anhydrase. Can J Bot **69:** 1070–1078

Swader JA, Jacobson BS (1972) Acetazolamide inhibition of photosystem in isolated spinach chloroplasts. Phytochemistry **11:** 65–70

Tsuzuki M, Miyachi S, Edwards GE (1985) Localization of carbonic anhydrase in mesophyll cells of terrestrial C3 plants in relation to CO_2 assimilation. Plant Cell Physiol **26:** 881–891

Usuda H (1985) The activation state of ribulose-1,5-bisphosphate carboxylase in maize (*Zea mays*) leaves in dark and light. Plant Cell Physiol **26:** 1455–1464

Williams TG, Flanagan LB, Coleman JR (1996) Photosynthetic gas exchange and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in tobacco plants modified by an antisense construct to have low chloroplastic carbonic anhydrase. Plant Physiol **112:** 319–326

Yoshida S, Forno DA, Cock JH, Gomes KA (1976) Routine procedure for growing rice plants in culture solution. *In* Laboratory Manual for Physiological Studies of Rice. The International Rice Research Institute, Los Vanõs, Philippines, pp 61–66