Plant Physiol. (1992) 98, 1222–1227
0032-0889/92/98/1222/06/$01.00/0

Received for publication July 17, 1991
Accepted October 8, 1991

Magnesium Deficiency and High Light Intensity Enhance Activities of Superoxide Dismutase, Ascorbate Peroxidase, and Glutathione Reductase in Bean Leaves[1]

Ismail Cakmak and Horst Marschner*

Department of Soil Science and Plant Nutrition, University of Cukurova, Adana, Turkey (I.C.); and Institut für Pflanzenernährung, Universität Hohenheim, Postfach 70 05 62, W 7000 Stuttgart 70, Germany (H.M.)

ABSTRACT

The influence of varied Mg supply (10–1000 micromolar) and light intensity (100–580 microeinsteins per square meter per second) on the concentrations of ascorbate (AsA) and nonprotein SH-compounds and the activities of superoxide dismutase (SOD; EC 1.15.11) and the H_2O_2 scavenging enzymes, AsA peroxidase (EC 1.11.1.7), dehydroascorbate reductase (EC 1.8.5.1), and glutathione reductase (EC 1.6.4.2) were studied in bean (*Phaseolus vulgaris* L.) leaves over a 13-day period. The concentrations of AsA and SH-compounds and the activities of SOD and H_2O_2 scavenging enzymes increased with light intensity, in particular in Mg-deficient leaves. Over the 12-day period of growth for a given light intensity, the concentrations of AsA and SH-compounds and the activities of these enzymes remained more or less constant in Mg-sufficient leaves. In contrast, in Mg-deficient leaves, a progressive increase was recorded, particularly in concentrations of AsA and activities of AsA peroxidase and glutathione reductase, whereas the activities of guaiacol peroxidase and catalase were only slightly enhanced. Partial shading of Mg-deficient leaf blades for 4 days prevented chlorosis, and the activities of the $O_2^{\cdot-}$ and H_2O_2 scavenging enzymes remained at a low level. The results demonstrate the role of both light intensity and Mg nutritional status on the regulation of $O_2^{\cdot-}$ and H_2O_2 scavenging enzymes in chloroplasts.

Photoreduction of molecular O_2 in chloroplasts is unavoidable and leads to the production of superoxide anion radicals ($O_2^{\cdot-}$) and H_2O_2 (1, 29). In nonstressed chloroplasts, photoreduction of O_2 is estimated to be between 5 and 27% of the total electron flow (2, 3). Superoxide anion radicals, H_2O_2, and their derivatives hydroxyl radicals ($OH^{\cdot}$) and singlet oxygen ($^1O_2$) are highly toxic, resulting in destructive effects on the functional and structural integrity of chloroplasts (8, 17). As protection against these toxic O_2 species, chloroplasts are equipped with several antioxidants and defense enzymes.

SOD,[2] mostly localized in chloroplasts in leaves (19), catalyze the dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2, and the H_2O_2 is converted into O_2 and H_2O. As catalase is absent from chloroplasts (1, 17), the conversion of H_2O_2 in these organelles is mediated by a H_2O_2 scavenging system with the involvement of AsA peroxidase, DAsA reductase, and glutathione reductase (12, 14). This H_2O_2 detoxifying system is also present in the cytoplasm (21). Ascorbate and glutathione are particularly effective antioxidants against toxic O_2 species and are present in the chloroplast stroma at concentrations as high as 50 and 3 mM, respectively (17).

Photogeneration of toxic O_2 species can be intensified when plants grown at a high light intensity are exposed to an environmental stress that impairs the utilization of absorbed light energy for photosynthetic CO_2 fixation (2, 9, 18). Such conditions may lead to a limited $NADP^+$ pool for acceptance of electrons from PSI and, thereby, a higher potential for O_2 activation (18). Accordingly, in many instances, activities of SOD and enzymes of the H_2O_2 scavenging pathway are increased in response to environmental stress factors such as drought (33), chilling (30), and treatments with hyperbaric O_2 levels (11) and air pollutants (34).

Little is known about the effect of mineral nutrient deficiency as a stress factor that affects antioxidative mechanisms in leaves. In Mg-deficient leaves, CO_2 uptake is depressed with corresponding decreases in CO_2 fixation (35). CO_2 fixation may also be impaired by Mg deficiency as a result of inhibited sucrose export from leaves (10, I. Cakmak, H. Marschner, unpublished) or the Mg dependency of ribulose-1,5-bisphosphate carboxylase (31). Thus, in Mg-deficient chloroplasts, enhanced O_2 activation can be expected at the expense of CO_2 fixation. As has been demonstrated recently (25), Mg-deficient bean leaves are highly photosensitive; an increase in light intensity rapidly causes severe chlorosis without any significant change of the Mg concentration in the leaves. This light effect has been attributed to Mg deficiency-induced photooxidation of thylakoid constituents by photogenerated toxic O_2 species.

In the present paper, we have studied the responses of $O_2^{\cdot-}$ and H_2O_2 scavenging enzymes, together with the levels of ascorbate and nonprotein SH-compounds, in leaf extracts of bean plants subjected to different levels of Mg supply and light intensity.

MATERIALS AND METHODS

Plant Growth

French bean (*Phaseolus vulgaris* L. cv Prélude) plants were grown under controlled environmental conditions (light/dark

[1] This work was supported by the Deutsche Forschungsgemeinschaft.

[2] Abbreviations: SOD, superoxide dismutase; AsA, ascorbate; DAsA, dehydroascorbate; EPPS, *N*-2-hydroxethylpiperazine propane sulphonic acid.

regimen 16/8 h at 24/20°C, relative humidity 65–75%) with varied light intensities from 100 to 580 μE $m^{-2}s^{-1}$, provided by Osram HQI/T/2000/D bulbs.

Seeds were germinated in quartz sand moistened with saturated $CaSO_4$ solution. After 5 to 6 d, the seedlings were transferred to nutrient solutions with varied Mg supply, as indicated in the appropriate figures and tables. Light intensity in the growth chamber was varied by shading with muslin to obtain the values at canopy height as indicated in the text. Partial shading (80 μE $m^{-2}s^{-1}$) of leaf blades was carried out using filter paper. Composition of the nutrient solution used was as follows (M): 0.88×10^{-3} K_2SO_4; 2×10^{-3} $Ca(NO_3)_2$; 0.25×10^{-3} KH_2PO_4 0.1×10^{-3} KCl; 1×10^{-5} H_3BO_3; 4×10^{-5} FeEDTA; 1×10^{-6} $MnSO_4$; 1×10^{-6} $ZnSO_4$; 1×10^{-7} $CuSO_4$; 1×10^{-8} $(NH_4)_6MoO_{24}$.

Plants were harvested after 6 to 13 d growth in nutrient solution. At harvest, roots, primary leaves, and other shoot parts were separated and dried at 70°C for determination of dry weight and Mg concentration. For analysis of enzymes, primary leaves were harvested 11 to 12 h after the onset of the light period, frozen in liquid nitrogen, and stored at −25°C prior to analysis. Samples for Chl determinations were freeze-dried and ground after freezing in liquid nitrogen.

Enzyme Determinations

Usually, 1 g leaf material without the main midrib was homogenized in 10 mL 25 mM EPPS buffer (pH 7.8) containing 0.2 mM EDTA and 2% PVP. The homogenate was filtered through a nylon mesh and then centrifuged at 15,000*g* for 20 min. The supernatant was used for enzyme analysis. All operations (until analysis) were carried out at 3 to 5°C. With the exception of SOD, all enzyme activities were measured in a final volume of 1 mL using various aliquots of the supernatants (25–100 μL for AsA peroxidase; 200 μL for DAsA reductase; and 100 μL for glutathione reductase).

Activity of AsA peroxidase was measured according to Nakano and Asada (26) by monitoring the rate of ascorbate oxidation at 290 nm (E = 2.8 mM cm^{-1}). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 1.0 mM H_2O_2, 0.25 mM AsA, and the enzyme aliquot. No change in absorption was found in the absence of AsA in the test medium. The assay of DAsA reductase was carried out by measuring the increase in absorbance at 265 nm due to AsA formation (E = 14 mM cm^{-1}) (26). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 2.5 mM reduced GSH, 0.4 mM DAsA, and the enzyme aliquot. The reaction rate was corrected for the nonenzymatic reduction of DAsA by GSH. Glutathione reductase was assayed according to the method of Foyer and Halliwell (12) by following the decrease in absorbance at 340 nm due to NADPH oxidation (E = 6.2 mM cm^{-1}). The reaction mixture consisted of 25 mM EPPS buffer (pH 7.8), 0.5 mM GSSG, 0.12 mM NADPH, and the enzyme aliquot. Corrections were made for NADPH oxidation in the absence of GSSG. SOD was measured by the photochemical method as described by Giannopolitis and Ries (13). Assays were carried out on a rotating plate under illumination. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of *p*-nitro blue tetrazolium chloride reduction at 560 nm.

Catalase activity was assayed in a reaction mixture containing 25 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and enzyme. The decomposition of H_2O_2 was followed at 240 nm (E = 39.4 mM cm^{-1}). For the measurement of guaiacol peroxidase activity, the reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mM H_2O_2 and enzyme. Activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (E = 26.6 mM cm^{-1}).

Analytical Methods

AsA and DAsA were determined according to Law *et al.* (22) with some modifications. Usually, 1 g leaf tissue was extracted with 10 mL 5% *meta*-phosphoric acid and centrifuged at 22,000*g* for 15 min. Total ascorbate (Asa + DAsA) was measured after reduction of DAsA to AsA with DTT, and the concentrations of DAsA were estimated from the difference of total ascorbate (AsA + DAsA) and AsA. The reaction mixture for total ascorbate contained 0.2 mL aliquot of the 22,000*g* supernatant, 0.5 mL 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.1 mL 10 mM DTT. After 10 min at room temperature, 0.1 mL 0.5 *N*-ethylmaleimide was added to remove excess DTT. The reaction mixture for AsA contained 0.2 mL aliquot of the 22,000*g* supernatant, 0.5 mL 150 mM phosphate buffer (pH 7.4), and 0.2 mL water. In both reaction mixtures, the color was developed after addition of the following reagents: 0.4 mL 10% TCA, 0.4 mL 44% *ortho*-phosphoric acid, 0.4 mL 4% α'-dipyridyl in 70% ethylalcohol, and 0.2 mL 3% $FeCl_3$. The mixtures were then incubated at 40°C for 40 min and the color produced was read at 525 nm.

Using the method of Ellman (7), nonprotein SH-compounds were determined in the neutralized aliquots from the 22,000*g* supernatants obtained as above.

Concentrations of Chl (*a* + *b*) were measured as described by Lichtenthaler and Wellburn (23) after extraction with 80% acetone. The Mg concentrations were determined by atomic absorption spectrometry after ashing samples at 550°C and dissolving the ash in 3.3% HNO_3.

RESULTS

Plant Growth

Regardless of the Mg supply, increasing light intensity increased dry matter production, particularly after 6 d of growth (Table I). At low Mg supply, dry weight increments decreased with time, especially in the roots and at high light intensity. Compared with Mg-sufficient plants, the Mg concentrations in the leaves of the Mg-deficient plants were much lower (Table I) and clearly below the critical deficiency level (approximately 2 mg Mg g^{-1} dry weight). In the deficient plants, the Mg concentrations declined with leaf age, either as a consequence of retranslocation to young leaves, carbohydrate accumulation ("dilution effect"), or both. The concentrations of soluble carbohydrates increased in Mg-deficient leaves by a factor of 6 to 10 compared with those of Mg-sufficient leaves (data not shown). Table I also shows that Chl concentrations decreased with increased light intensity in both Mg-sufficient and Mg-deficient plants. This effect of light

Table I. *Root and Shoot Dry Weights and Concentrations of Mg and Chl in Primary Leaves of Bean Plants over 12 d of Growth in Nutrient Solutions with Sufficient (suff., 1000 μM Mg) and Deficient (def., 20 μM Mg) Mg Supply and Different Light Intensities*

Data points represent the means of three replicates.

Light Intensity	6 d		9 d		12 d	
	Mg suff.	Mg def.	Mg suff.	Mg def.	Mg suff.	Mg def.
$\mu E\ m^{-2}\ s^{-1}$			*root dry weight (g plant^{-1})*			
100	0.06	0.06	0.12	0.09	0.18	0.09
250	0.09	0.09	0.26	0.12	0.34	0.14
580	0.11	0.09	0.38	0.16	0.54	0.19
			shoot dry weight (g plant^{-1})			
100	0.35	0.38	0.78	0.60	1.02	0.90
250	0.49	0.48	1.10	0.95	1.66	1.43
580	0.59	0.59	1.55	1.42	2.59	1.91
			Mg concentrations (mg Mg g^{-1} dry wt)			
100	4.90	1.33	7.90	0.97	9.25	0.69
250	5.39	1.23	9.10	0.85	9.35	0.64
580	5.92	0.90	9.04	0.70	9.50	0.71
			Chl concentrations (mg g^{-1} dry wt)			
100	15.8	16.1	20.0	13.9	22.4	10.2[a]
250	14.7	13.1	16.5	9.4[b]	18.7	6.0[c]
580	12.0	8.9[a]	13.8	5.7[c]	13.7	4.2[d]

[a] Slight chlorosis. [b] Mild interveinal chlorosis. [c] Severe interveinal chlorosis. [d] Very severe interveinal chlorosis with some necrotic and reddish areas.

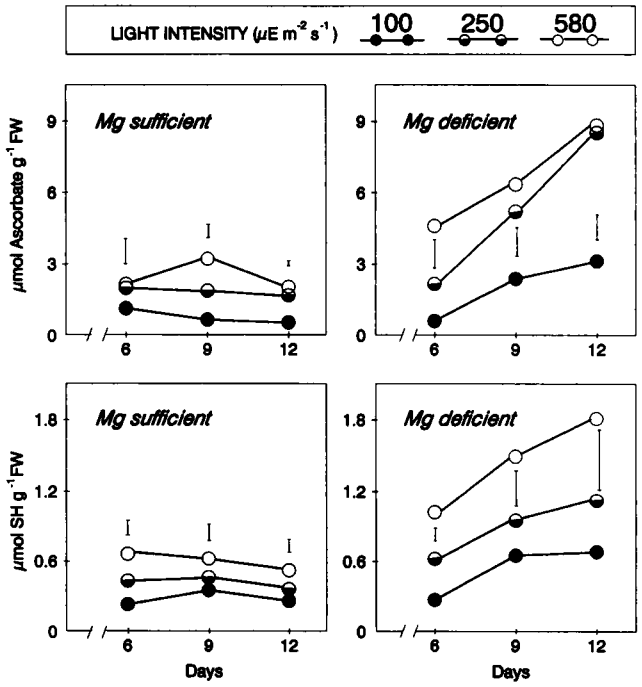

Figure 1. Concentrations of total ascorbate (AsA + DAsA) and nonprotein SH-compounds in primary leaves of bean plants over 12 d of growth in nutrient solution with different Mg supply (1000 μM, Mg-sufficient; 20 μM, Mg-deficient) and light intensity. Each data point represents the mean of three replicates. Bars indicate LSD 0.05.

became more distinct with leaf age, in particular under Mg deficiency.

Concentrations of AsA and DAsA and SH-Compounds

Increasing light intensity increased concentrations of total ascorbate (AsA + DAsA) and SH-compounds, especially in Mg-deficient leaves (Fig. 1). Over the 12-d period of growth for a given light intensity, levels of total ascorbate and SH-compounds remained at a similar level in Mg-sufficient leaves, but increased in Mg-deficient leaves, particularly at high light intensities. Increase in ascorbate and SH-compounds at low Mg supply was already evident in plants with no or with only slight depression of growth and Chl concentrations (Fig. 1; Table II). Of the total ascorbate, a higher proportion was in the reduced form (AsA), especially in Mg-deficient leaves, indicating a higher regeneration potential of oxidized ascorbic acid (DAsA) in the deficient leaves (Table II).

Activities of $O_2^{\cdot -}$ and H_2O_2 Scavenging Enzymes

Effects of light intensity and Mg supply on enzyme activities are shown in Figure 2. For a given harvest and Mg level, higher light intensities were correlated with higher enzyme activities, especially in Mg-deficient leaves after 6 d of growth. However, for a given light intensity, the Mg level had the most pronounced effect on the enzyme activities. With increase in time and, thus, severity of Mg deficiency, the activities of SOD and in particular of the AsA peroxidase and glutathione reductase increased. Even as early as day 6 at high light intensity, when first visual symptoms of chlorosis became evident (Table I), activities of AsA peroxidase and glutathione reductase were about 1.6 times higher in leaves of Mg-defi-

Table II. *Effect of Increasing Mg Supply on the Concentrations of Chl (a + b), AsA, DAsA, and Nonprotein SH-Compounds in Primary Leaves of 12-d-old Bean Plants Grown at 420 μE m^{-2} s^{-1}*

Data points represent the means of three replicates.

Mg Supply	Chl	(AsA)	(DAsA)	Total (AsA + DAsA)	SH-Compounds
μM	mg g^{-1} dry wt	μmol g^{-1} fresh wt			μmol g^{-1} fresh wt
10	3.8 ± 0.3	6.0 ± 0.2	1.6 ± 0.6	7.5 ± 0.5	2.4 ± 0.2
20	5.3 ± 0.8	6.2 ± 0.9	1.1 ± 0.4	7.2 ± 0.7	2.3 ± 0.4
50	7.4 ± 1.0	4.8 ± 0.5	0.6 ± 0.1	5.4 ± 0.5	1.4 ± 0.0
100	10.8 ± 0.2	1.8 ± 0.5	0.6 ± 0.4	2.4 ± 0.9	0.7 ± 0.1
1000	11.3 ± 0.5	0.9 ± 0.5	0.4 ± 0.1	1.3 ± 0.5	0.6 ± 0.1

cient compared with sufficient plants (Fig. 2). By day 12 in high light intensity, the activities of glutathione reductase, AsA peroxidase, and SOD were higher by factors of 7.0, 5.3, and 2.1, respectively, in Mg-deficient leaves than in Mg-sufficient leaves. Changes in the activity of the DAsA reductase were essentially the same as of the AsA peroxidase (data not shown).

Increases in SOD activity by Mg deficiency can be ascribed to CuZn-SOD, which is cyanide sensitive; addition of 2 mM KCN to the assay medium depressed SOD activity 88 and 94% in Mg-sufficient and -deficient leaves, respectively.

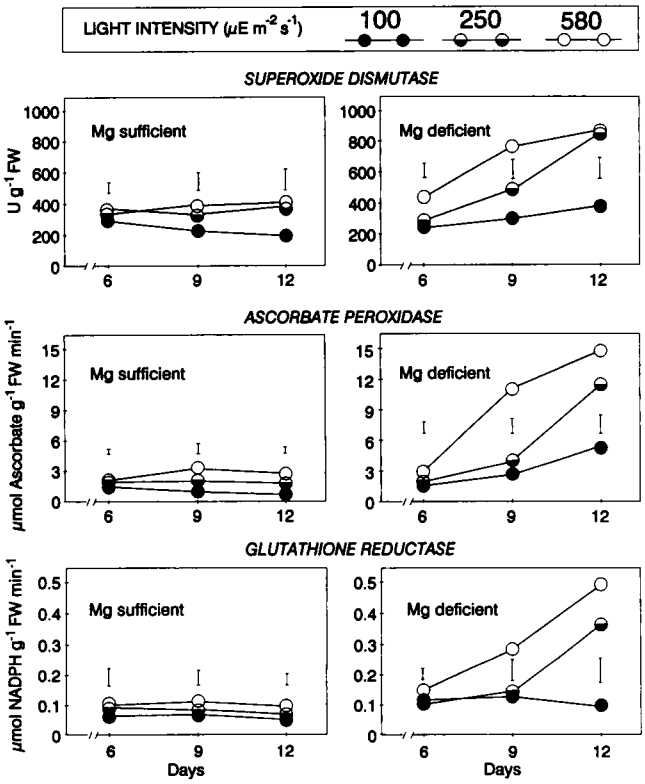

Figure 2. Activities of SOD, ascorbate peroxidase, and glutathione reductase in primary leaves of bean plants over 12 d of growth in nutrient solution with different Mg supply (1000 μM, Mg-sufficient; 20 μM, Mg-deficient) and light intensity. Each data point represents the mean of three replicates. Bars indicate LSD 0.05.

The relationships between Mg nutritional status, light intensity, and enzyme activities can also be demonstrated in the same leaf by partial shading (Fig. 3). Partial shading of the leaf blades for 4 d prevented development of interveinal chlorosis and decreased the activity of all four enzymes both in the Mg-deficient and -sufficient plants. However, the level of enzyme activity was again much higher in the Mg-deficient plants. As the protein concentrations were not much affected by Mg deficiency (data not shown), the relationships between Mg nutritional status and enzyme activities were similar on a fresh weight basis or on a protein basis (Table III). Compared with the strong enhancement effect on enzymes of the ascorbate-dependent H_2O_2 scavenging pathway, Mg deficiency only slightly increased the activities of guaiacol peroxidase and catalase. Under Mg deficiency, the activity of malate dehydrogenase was also slightly increased, whereas glycolate oxidase activity was not significantly affected (data not shown).

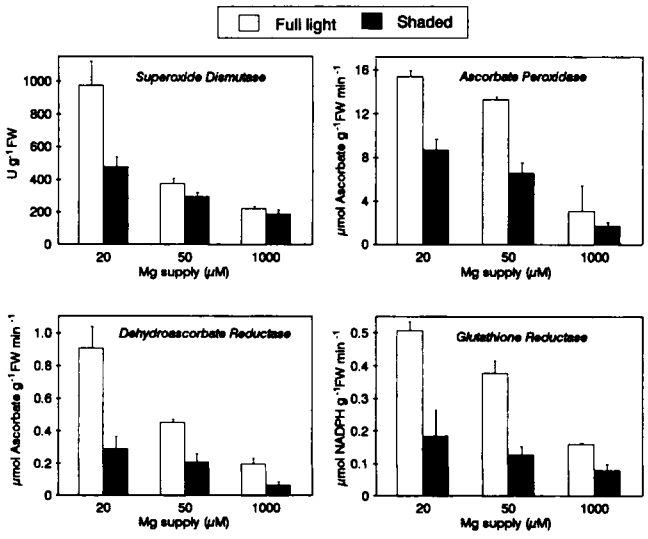

Figure 3. Activities of SOD, ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase in directly light-exposed (520 μE $m^{-2}s^{-1}$) and shaded (80 μE $m^{-2}s^{-1}$) areas of primary leaves of bean plants grown for 13 d in nutrient solution with different Mg supply. Shading using filter paper was started at 9 d of growth in nutrient solution. Vertical bars represent the mean ± SD of three or four replicates.

Table III. *Activities of Superoxide Dismutase, Ascorbate Peroxidase, Dehydroascorbate Reductase, Glutathione Reductase, Guaiacol Peroxidase, and Catalase in Primary Leaves of 13-d-old Mg-Sufficient (1000 μM) and -Deficient (20 μM) Plants*

Activities expressed either on fresh weight or protein basis. Data points represent the means of three replicates.

Enzyme Activity	Mg-Sufficient	Mg-Deficient	% Increase by Mg Deficiency
Superoxide dismutase			
U g^{-1} fresh wt	263 ± 5	601 ± 31	229
U mg^{-1} protein	18 ± 1	34 ± 2	189
Ascorbate peroxidase			
μmol g^{-1} fresh wt min^{-1}	2.00 ± 0.40	15.03 ± 0.51	752
μmol mg^{-1} protein min^{-1}	0.14 ± 0.02	0.84 ± 0.02	600
Dehydroascorbate reductase			
nmol g^{-1} fresh wt min^{-1}	102 ± 32	388 ± 95	380
nmol mg^{-1} protein min^{-1}	7 ± 3	22 ± 6	314
Glutathione reductase			
nmol g^{-1} fresh wt min^{-1}	97 ± 28	301 ± 18	310
nmol mg^{-1} protein min^{-1}	7 ± 2	17 ± 1	242
Guaiacol peroxidase			
μmol g^{-1} fresh wt min^{-1}	2.63 ± 0.06	4.57 ± 0.29	174
μmol mg^{-1} protein min^{-1}	0.18 ± 0.01	0.25 ± 0.02	139
Catalase			
μmol g^{-1} fresh wt min^{-1}	1.87 ± 0.21	2.87 ± 0.32	154
μmol mg^{-1} protein min^{-1}	0.13 ± 0.01	0.16 ± 0.02	123

DISCUSSION

With the onset of visual Mg deficiency symptoms, there is a rapid increase in antioxidative mechanisms against toxic O_2 species, especially under high light intensities. In Mg-deficient leaves, the concentration of ascorbate was approximately six times higher than in Mg-sufficient leaves (Fig. 1, Table II). Ascorbate is involved in nonenzymic detoxification of several toxic O_2 species and plays a key role in the enzymic AsA-GSH cycle in chloroplasts (14, 17). As for ascorbate, concentrations of nonprotein SH-compounds are also increased by Mg deficiency (Fig. 1, Table II). We assume that the major nonprotein SH-compound measured in bean leaves is glutathione, because in shoots of several plant species glutathione comprises the major fraction of the total nonprotein SH-compounds, for example 95% in spruce needles (16), and more than 80% in red clover, soybean, and bean leaves (24). Glutathione is involved in detoxification of H_2O_2 and protection of SH-containing enzymes against oxidation, especially in chloroplasts (17). Its concentrations are in the range of 20 μM in the vacuole, 60 μM in the cytosol, and 3.5 mM in chloroplasts (17, 28).

Increases in concentrations of ascorbate and SH-compounds in Mg-deficient leaves are accompanied by higher activities of SOD, AsA peroxidase, DAsA reductase, and glutathione reductase (Figs. 2 and 3). These increases in antioxidative capacity in Mg-deficient leaves occur before distinct depressions in plant growth and Chl concentrations (Tables I and II), indicating that elevated levels of antioxidative components, especially ascorbate, can be considered an early physiological response of plants to Mg deficiency.

The increase in antioxidative defense mechanisms indicates Mg deficiency-induced enhancement of production of toxic O_2 species, particularly $O_2^{\cdot -}$ and H_2O_2. It is well documented that such protective mechanisms are induced under stress conditions that stimulate production of toxic O_2 species (9, 27). The enhancements in activities of antioxidative enzymes by Mg deficiency most probably take place in chloroplasts where SOD (19) and the H_2O_2 detoxifying enzymic cycle (14, 26) are predominantly located. The hydrogen peroxide scavenging AsA-GSH cycle can, however, also operate in the cytoplasm, for example, of root nodules (6) and *Ricinus* endosperm (21). As discussed by Nakano and Asada (26) and Chen and Asada (5), the H_2O_2 scavenging peroxidase localized in chloroplasts is highly specific for ascorbate and has much less affinity for other electron donors such as guaiacol. By contrast, the nonchloroplastic peroxidases are less specific for ascorbate. We found that activities of AsA peroxidase and guaiacol peroxidase were not significantly different in Mg-sufficient leaves, whereas in Mg-deficient leaves ascorbate oxidation was nearly fourfold that of guaiacol (Table III). This supports the above suggestion that enhanced H_2O_2 scavenging peroxidase under Mg deficiency predominantly operates in chloroplasts, where enhanced photoproduction of $O_2^{\cdot -}$ and H_2O_2 occurs as a result of restricted consumption of photoreductants in CO_2 fixation (see Introduction).

In the literature, there are no available data on the relationships between Mg nutritional status and antioxidative defense mechanisms of plant cells. However, there are a number of environmental stress factors that, similar to Mg deficiency, strongly modify defense mechanisms of plant cells against toxic O_2 species. In agreement with our results, high light intensity (15), especially in combination with chilling treatment (30), increases the levels of ascorbate and activities of SOD and AsA peroxidase. Increased capacity for the detoxification of toxic O_2 species was also shown to increase tolerance or resistance of plants to related stress factors such as

chilling (20), ozone (34), and paraquat (32). Recently, we found that Mg-deficient leaves are much more resistant to paraquat-induced Chl degradation than Mg-sufficient leaves (I. Cakmak, H. Marschner, in preparation).

The results presented here together with others already published (25) suggest that photooxidation of thylakoid constituents are a major contributing factor in development of Mg deficiency chlorosis. Furthermore, the well-known differences between plant species in the expression of visual Mg deficiency symptoms or in critical deficiency concentrations of Mg in the leaf tissue (4) may be related to constitutive differences in generation or detoxification of harmful O_2 species in the chloroplasts.

ACKNOWLEDGMENTS

The authors are grateful to E.A. Kirkby and D. J. Pilbeam (University of Leeds, England) for correction of the English text.

LITERATURE CITED

1. **Asada K, Takahashi M** (1987) Production and scavenging of active oxygen in photosynthesis. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition. Elsevier, Amsterdam, pp 227–287
2. **Asada K, Takahashi M, Tanaka K, Nakano Y** (1977) Formation of active oxygen and its fate in chloroplasts. *In* O Hayaishi, K Asada, eds, Biochemical and Medical Aspects of Active Oxygen. Japan Scientific Societies Press, Tokyo, pp 45–63
3. **Badger MR** (1985) Photosynthetic oxygen exchange. Annu Rev Plant Physiol **36:** 27–53
4. **Bergmann W** (1988) Ernährungsstörungen bei Kulturpflanzen, Entstehung und Diagnose. Gustav Fischer Verlag, Jena, pp 166–178
5. **Chen G-X, Asada K** (1989) Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol **30:** 987–998
6. **Dalton DA, Russell SA, Hanus FJ, Pascoe GA, Evans HJ** (1986) Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. Proc Natl Acad Sci USA **83:** 3811–3815
7. **Ellman GL** (1959) Tissue sulphydryl groups. Arch Biochem Biophys **82:** 70–77
8. **Elstner EF** (1982) Oxygen activation and oxygen toxicity. Annu Rev Plant Physiol **33:** 73–96
9. **Elstner EF, Wagner GA, Schutz W** (1988) Activated oxygen in green plants in relation to stress situations. Curr Top Plant Biochem Physiol **7:** 159–187
10. **Fink S** (1991) Structural changes in conifer needles due to Mg and K deficiency. Fertilizer Res **27:** 23–27
11. **Foster JG, Hess JL** (1980) Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere of enriched oxygen. Plant Physiol **66:** 482–487
12. **Foyer CH, Halliwell B** (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta **133:** 21–25
13. **Giannopolitis N, Ries SK** (1977) Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol **59:** 309–314
14. **Gillham DJ, Dodge AD** (1986) Hydrogen-peroxide-scavenging systems within pea chloroplasts. A quantitative study. Planta **167:** 246–251
15. **Gillham DJ, Dodge AD** (1987) Chloroplast superoxide and hydrogen peroxide scavenging systems from pea leaves: seasonal changes. Plant Sci **50:** 105–109
16. **Grill D, Esterbauer H, Klösch U** (1979) Effect of sulphur dioxide on glutathione in leaves of plants. Environ Pollut **19:** 187–194
17. **Halliwell B** (1982) The toxic effects of oxygen on plant tissues. *In* LW Oberley, ed, Superoxide Dismutase, Vol I. CRC Press, Boca Raton, FL, pp 89–123
18. **Hodgson RAJ, Raison JK** (1991) Superoxide production by thylakoids during chilling and its implication in the susceptibility of plants to chilling-induced photo-inhibition. Planta **183:** 222–228
19. **Jackson C, Dench J, Moore AL, Halliwell B, Foyer C, Hall DO** (1978) Subcellular localization and identification of superoxide dismutase in leaves of higher plants. Eur J Biochem **91:** 339–344
20. **Jahnke LS, Hull MR, Long SP** (1991) Chilling stress and oxygen metabolizing enzymes in *Zea mays* and *Zea diploperennis*. Plant Cell Environ **14:** 97–104
21. **Klapheck S, Zimmer I, Cosse H** (1990) Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol **31:** 1005–1013
22. **Law MY, Charles SA, Halliwell B** (1983) Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts. The effect of hydrogen peroxide and paraquat. Biochem J **210:** 899–903
23. **Lichtenthaler HK, Wellburn AR** (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans **11:** 591–592
24. **Maas FM, DeKok LJ, Peters JL, Kuiper PJC** (1987) A comparative study on the effects of H_2S and SO_2 fumigation on the growth and accumulation of sulphate and sulphydryl compounds in *Trifolium pratense* L., *Glycine max* Merr. and *Phaseolus vulgaris* L. J Exp Bot **38:** 1459–1469
25. **Marschner H, Cakmak I** (1989) High light intensity enhances chlorosis and necrosis in leaves of zinc, potassium and magnesium deficient bean (*Phaseolus vulgaris*) plants. J Plant Physiol **134:** 308–315
26. **Nakano Y, Asada K** (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol **22:** 867–880
27. **Rabinowitch DH, Fridovich I** (1983) Superoxide radicals, superoxide dismutases and oxygen toxicity in plants. Photochem Photobiol **37:** 679–690
28. **Rennenberg H** (1982) Glutathione metabolism and possible biological roles in higher plants. Phytochemistry **21:** 2771–2781
29. **Robinson JM** (1988) Does O_2 photoreduction occur within chloroplasts in vivo? Physiol Plant **72:** 666–680
30. **Schöner S, Krause H** (1990) Protective systems against active oxygen species in spinach: response to cold acclimation in excess light. Planta **180:** 383–389
31. **Seftor REB, Bahr JT, Jensen RG** (1986) Measurement of the enzyme-CO_2-Mg^{2+} form of spinach ribulose 1,5-bisphosphate carboxylase/oxygenase. Plant Physiol **80:** 599–600
32. **Shaaltiel Y, Gressel J** (1986) Multienzyme oxygen radical detoxifying system correlated with paraquat resistance in *Conyza bonariensis*. Pestic Biochem Physiol **26:** 22–28
33. **Smirnoff N, Colombé SV** (1988) Drought influences the activity of enzymes of the chloroplast hydrogen peroxide scavenging system. J Exp Bot **39:** 1097–1108
34. **Tanaka K, Suda Y, Kondo N, Sugahara K** (1985) O_3 tolerance and the ascorbate-dependent H_2O_2 decomposing system in chloroplasts. Plant Cell Physiol **26:** 1425–1431
35. **Terry N, Ulrich A** (1974) Effects of magnesium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol **54:** 379–381