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Effect of zinc nutritional status on activities of superoxide radical and hydrogen peroxide scavenging enzymes in bean leaves

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Abstract

The effect of varied zinc (Zn) supply on the activities of superoxide dismutase (SOD), ascorbate (AsA) peroxidase, glutathione (GSSG) reductase, catalase and guaiacol peroxidase was studied in leaves of bean (*Phaseolus vulgaris*) plants grown for 15 days in nutrient solution. Zinc deficiency severely decreased plant growth and the leaf concentrations of soluble protein and chlorophyll. Resupply of Zn to deficient plants for up to 72h restored protein concentrations more rapidly than chlorophyll and plant growth. With the exception of guaiacol peroxidase, the activities of all enzymes were significantly decreased by Zn deficiency, in particular GSSG reductase and SOD. Within 72h of resupplying Zn to deficient plants, the enzyme activities reached the level of the Zn sufficient plants.The results indicate severe impairment in the ability of Zn-deficient leaves to enzymically scavenge O_2^- and H_2O_2. Consequences and reasons of this impairment are discussed in terms of photooxidation of chloroplast pigments and inhibition of the biosynthesis of the related scavenger enzyme proteins.

Introduction

Zinc-deficiency stress usually involves peroxidative damage of crucial components of biomembranes, enzymes and thylakoids (Marschner and Cakmak, 1989 ; Bray and Bettger, 1990) which are the major targets of toxic O_2radicals (Elstner, 1982). By binding sulfhydryl groups and phospholipids, Zn ions stabilize and protect cell membranes and enzyme proteins against oxidative attack of toxic O_2 species (Willson, 1989; Bray and Bettger,1990). Toxic O_2^- species such as the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) are produced in high amounts in various cell compartments, especially chloroplasts of higher plants (Elstner, 1982).

Chloroplasts are well equipped with defence enzymes against O_2^- and H_2O_2. Superoxide dismutases (SODs), predominantly CuZnSOD, detoxify O_2^- and play a key role against O_2^- derived cell toxicity (Fridovich, 1986; Bowler et al., 1992). Thus, Zn ions also participate in enzymic defence of cells against free radical damage. Dismutation of O_2^- by SOD or non-enzymatic reactions produce H_2O_2 which is also toxic and essential for the production of the powerful oxidant hydroxyl radical, OH. Detoxification of H_2O_2 can be mediated by catalase which is however absent in chloroplasts (Elstner, 1982). Chloroplasts have an efficient H_2O_2 scavenging system in which ascorbate (AsA) peroxidase and glutathione (GSSG) reductase are of particular importance

(Asada, 1992). AsA peroxidase detoxifies H_2O_2, and GSSG reductase is involved in regeneration of AsA as well as of $NADP^+$; the latter accepts electrons from ferredoxin and thus limits photoreduction of O_2 to O_2^-. In many instances, resistance against photooxidative damage to thylakoid constituents is correlated with elevated levels of these antioxidative enzymes (Bowler et al., 1992; Gressel and Galun, 1993).

As has been demonstrated previously, Zn deficient plants are highly susceptible to photooxidative damage; increases in light intensity rapidly induce leaf chlorosis and necrosis which reflect photooxidation of thylakoid constituents (Marschner and Cakmak, 1989). This light effect is attributed to enhanced formation of O_2^- at the expense of CO_2 reduction. In the present paper we studied the effect of varied Zn supply on the activities of O_2^- and H_2O_2 scavenging enzymes in leaves of bean plants grown under a high light intensity.

Material and methods

French bean (*Phaseolus vulgaris* L., cv. Prelude) plants were grown under controlled environmental conditions (light/dark regimes of 16/8h, temperature 24/20°C, and relative humidity 65/75%) at 520 μE m^{-2} sec^{-1}, provided by Osram HQI/T/2000/D bulbs.The composition of the nutrient solution and other growth conditions have been described elsewhere (Marschner and Cakmak, 1989). For control plants (Zn-sufficient plants, +Zn) Zn was supplied as $ZnSO_4$ at a concentration of 1×10^{-6} *M*; for Zn-deficient plants (-Zn) no Zn was added to the nutrient solution. Three additional treatments were imposed on 12, 13 and 14 day old Zn deficient plants by supplying their nutrient solutions with 3×10^{6}*M* Zn, thus providing plants recovering from Zn deficiency for 72, 48 and 24 h prior to harvest, respectively. After harvest, concentrations of Zn, chlorophyll and protein and the activities of SOD, AsA peroxidase, GSSG reductase, catalase and guaiacol peroxidase were measured in young leaves (expanding trifoliate leaves, second trifoliate from the tip). Protein and chlorophyll concentrations and enzyme activities were measured as described before (Cakmak and Marschner, 1992).

Results

The first visual symptom of zinc deficiency in bean plants is leaf epinasty, especially of young leaves. Thereafter, leaves develop interveinal chlorosis which is associated with stunted shoot growth. Within 15 days growth in nutrient solution, these visual symptoms became severe and dry matter production was depressed, especially of shoots (Table 1). Leaf concentrations of Zn and chlorophyll were much lower in deficient plants than in Zn-sufficient plants (Table 1). Within 72h after supplying Zn to deficient plants, enhancement in plant growth and chlorophyll concentration became significant, whereas leaf concentrations of Zn immediately increased and exceeded those of the Zn sufficient plants within 24h (Table 1).

Compared to Zn-sufficient plants, Zn deficiency decreased the concentration of extractable protein by a factor of 2.4 (Fig. 1). Following supply of Zn to deficient plants, protein concentration gradually rose and approached the levels of Zn sufficient plants after 72h. Zinc deficiency drastically decreased activities particularly of SOD and GSSG reductase, but also of AsA peroxidase and

Table 1. Effect of differing Zn supply on root and shoot dry matter production (g DW/3 plants) and leaf concentrations of Zn (mg Zn/g DW) and chlorophyll (mg Chl./g DW)in 15 day old bean plants

Zn supply	Dry Weight		Leaf Concentrations	
	Roots	Shoots	Zinc	Chlorophyll
+Zn($1x10^{-6}$)M	2.7	7.9	59	9.5
-Zn	1.6	3.1	10	4.6
-Zn, +24h Zna	1.8	3.1	109	4.6
-Zn, +48h Zn	1.6	3.5	124	4.8
-Zn, +72h Zn	1.7	3.8	142	6.4
s. e.m[b]	0.1	0.4	13	0.4

[a] Resupply of Zn to -Zn plants at a concentration of $3x10^{-6}$*M*.
[b] Standard errors of the means

catalase whereas guaiacol peroxidase was rarely affected. Using the cyanide sensitivity test, about 85% of the total SOD activity was ascribed to CuZnSOD. Supply of Zn to deficient plants markedly increased the activities of all enzymes except guaiacol peroxidase (Fig. 1). GSSG reductase showed the earliest and greatest response in its activity to Zn resupply. Following 72h resupply of Zn, increases in enzyme activities were 8-fold for GSSG reductase, 4.4-fold for SOD and 3-fold for AsA peroxidase and catalase.

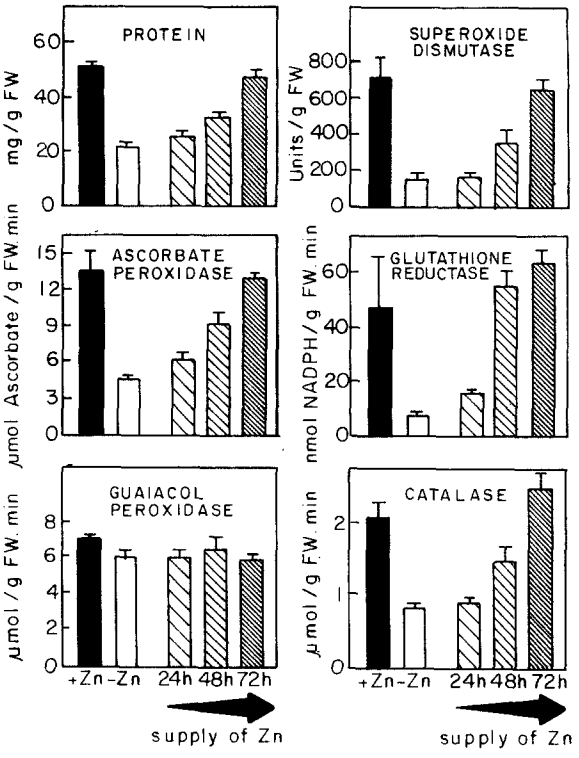

Fig. 1. Effect of Zn supply on concentrations of soluble protein and activities of superoxide dismutase, ascorbate peroxidase, glutathione reductase, guaiacol peroxidase and catalase. At 24, 48 and 72 h before harvest, Zn was supplied to 14, 13 and 12 day old Zn deficient plants to a concentration of 3×10^{-6} *M*.

Discussion

The most pronounced effect of Zn in cell metabolism is its involvement in protein metabolism. Zinc plays structural and regulatory roles in a large number of enzymes and proteins which directly affect replication and transcription processes and gene activation (Valle and Falchuk, 1993). Correspondingly, fairly high Zn concentrations are required in plant tissues with extensive synthesis of proteins like rice shoot meristems (Kitagishi and Obata, 1986) and pollen tubes (Ender et al., 1983). In agreement with these results, Zn-deficient leaves have lower protein concentrations, and respond to resupply of Zn with rapid increases in protein concentration (Fig. 1).

Under zinc deficiency the activities of SOD, catalase, AsA peroxidase and GSSG reductase were severely decreased. There was a rapid restoration in activities of these antioxidative enzymes following Zn supply to deficient plants (Fig. 1). By contrast, guaiacol peroxidase was not affected by Zn-deficiency (Fig. 1). This peroxidase is non-chloroplastic, localized mainly in cell walls and cytoplasm and less specific for ascorbate (Asada, 1992). In contrast to our results, Skoog (1940) showed high peroxidase activity in Zn deficient plants. The reason for this difference is not understood.

The depression in protein synthesis by Zn-deficiency (Fig. 1) suggests that the decreased activities of the antioxidative enzymes is a result of the inhibition of their biosynthesis. Decreases in SOD and catalase by Zn-deficiency are well documented (Vaughan et al., 1982; Cakmak and Marschner, 1988), but there are no reports in the literature on effects of Zn on AsA peroxidase and GSSG reductase. The changes in activities of the enzymes by varied Zn supply were not closely related to changes in protein levels. Zinc nutritional status of the plants affected enzyme activities more severely than the protein levels indicating further effects of Zn-deficiency. SOD is a Zn-containing enzyme and about 85% of the measured SOD activity is ascribed to CuZnSOD. It seems likely that in Zn-deficient tissues apoprotein of SOD is present and activated by Zn resupply. This suggestion supports results of Vaughan et al.(1982) who showed rapid restoration of SOD activity *in vitro* by addition of Zn. Reduction in activities of the antioxidative enzymes by Zn deficiency may also be a

consequence of either their inactivation or decreased production of their substrates, i.e. O_2^- and H_2O_2. Decreased production of the related substrates by Zn deficiency can be excluded because under Zn deficiency the production of these O_2 species is increased (Cakmak and Marschner, 1988; Marschner and Cakmak, 1989). Zinc ions are known to keep enzymes active by binding their sulfhydryl groups and thus protecting disulfide formation which leads to enzyme deactivation(Bray and Bettger, 1990).

Under conditions which enhance generation of O_2^- and H_2O_2 , SOD, AsA peroxidase and GSSG reductase are often induced, most probably by induced expression of related enzyme genes (Bowler et al., 1992; Gressel and Galun, 1993). Despite exposure of Zn-deficient cells to high levels of O_2^- and H_2O_2 such a gene expression can not be achieved in Zn-deficient cells because Zn itself is essential for this gene expression (Vallee and Falchuk, 1993).The severe loss in the ability of Zn-deficient leaf tissues to enzymically scavenge O_2^- and H_2O_2 would result in a peroxidative damage especially in chloroplsts under higher light intensities. This impaired detoxification of toxic O_2 species is the basis of the well-known susceptibility of Zn-deficient plants to high irradiation (Marschner and Çakmak, 1989).

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