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Changes in nitrogen metabolism enzyme activities of *Vicia faba* in response to aluminum and cadmium

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Abstract

Nodules of faba bean (*Vicia faba* L. cv. Giza 3) plants grown in pots containing clay-loam soil for 90 d have an active nitrate reductase (NR), while the leaves did not show detectable activity. Spraying the plant with increasing concentrations of Al^{3+} or Cd^{2+} (0 - 1000μM) significantly inhibited the nodules NR activity, the decline being more pronounced in Cd^{2+} treatment. The specific activity of glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) were more prominent in the 60- than in 90-d-old plants; GOT was always higher than GPT. Furthermore, GOT was more sensitive to Al^{3+} and Cd^{2+} treatments and its activity was significantly decreased when the metal concentration increased. Also, Cd^{2+} proved to be more effective than Al^{3+} in suppressing the GOT activity in the nodules, with less significant effect observed in the leaves. In contrast, GPT was hardly affected by the various metal treatments, particulary in the leaves.

Key words: faba bean, glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, heavy metals, nitrate reductase, nodules.

Introduction

Aluminum is not generally regarded as an essential element for plant growth, but under some conditions, it can increase growth and produce other desirable effects (Mullette 1975, Foy 1984, Kinraide and Parker 1987). The toxic level of Al^{3+} generally reduced the root growth and the uptake of several essential nutrients, as well as it could interfere with metabolism through the inactivation of enzymes or displacement of other metals from functional sites (Foy *et al.* 1978, Roy *et al.* 1988, Drady *et al.* 1993).

Cadmium, a non-essential element, enters the environment through various industrial processes and its accumulation in plants principally causes growth inhibition and even death (Clyjsters and Van-Assche 1985). Studies concerning the

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phytotoxicity of Cd^{2+} have been focused on nutritional aspects, water relations, enzyme activities and photosynthesis (Mathys 1975, Naguib *et al.* 1983, Poschenrieder *et al.* 1989, Somashekaraiah *et al.* 1992).

Nitrogen fixation in the nodulated plants is usually initiated about 15 - 30 d after planting, thus the initial nitrogen requirements for growth are derived from remoblization of those stored within the seeds or nitrogen absorbed from soil. Nitrate is considered the primary source of soil nitrogen, the extent of its uptake and subsequently its reduction by nitrate reductase in root and shoot depends on plant species and on various environmental factors (Guerrero *et al.* 1981). Thus, the temperate legume species *e.g. Lupinus, Pisum* and *Vicia faba* carry out most of their nitrate assimilation in their nodules (Andrews 1986). Generally, N_2-fixation and nitrate reduction contributed nitrogen at defined stages of plant growth (Randall *et al.* 1978, Lahiri *et al.* 1993). The ammonia produced from both processes is incorporated into amino acids in the nodules prior to translocation (Guerrero *et al.* 1981). Glutamine is the first product of ammonia assimilation, which is frequently converted to asparagine before export from the nodules, particularly in temperate legumes (Reynolds *et al.* 1982). Thus, the formation of aspartate in a transamination reaction between glutamate and oxaloacetate by the action of GOT plays an important role in asparagine production in amide-transporting legumes.

The effect of heavy metals on the enzymes concerned with nitrogen metabolism in legumes has received little interests. Huang *et al.* (1974) revealed that Pd^{2+} and Cd^{2+} inhibited the nodulation and nitrogenase activity in soybean nodules. A similar suppression was observed in *Vicia faba* nodules exposed to Al^{3+} and Cd^{2+} (Shalaby and Saleh 1991). It has been proposed that heavy metals can interact with a variety of enzymes causing growth reductions (Mathys 1975, Foy *et al.* 1978, Yandow and Klein 1986).

The present investigation was conducted to study the effect of Al^{3+} and Cd^{2+} on the activities of nitrate reductase, GOT and GPT in *Vicia faba* nodules and leaves to clarify the possible role of such heavy metals in either alteration the synthesis or function of these enzymes.

Materials and methods

Faba bean (*Vicia faba* L. cv. Giza 3) seeds were inoculated with a commercial strain of *Rhizobium leguminosarum* biovar *viceae*, and sown in porous pots, each containing 10 kg of clay-loamy soil with superphosphate fertilizer. The pots were watered to field capacity for the duration of the experiment and kept in a greenhouse under natural conditions. After emergence, the seedlings were thinned to four per each pot. Twenty days after sowing the plants were sprayed daily, for other 30 d, with 10 cm^3 per pot of either $AlCl_3$ or $CdCl_2$ solution to give final concentrations of Al^{3+} or Cd^{2+} 0, 50, 100, 500 and 1000 μM. The pots were arranged in a randomized complete block design with 8 replications for each treatment.

The plants from each treatment were harvested, early in the morning, 60 and 90 d after sowing, then thoroughly washed with distilled water. Nodules and leaves were

separated and immediately kept at 2 °C. Nitrate reductase was extracted from a known amount of frozen tissue with 0.025 M Tris-HCl buffer, pH 7.5, containing 0.025 M cysteine. The enzyme was assayed *in vitro* using the method described by Harper and Hageman (1972), following the conversion of nitrate to nitrite. The GOT and GPT were assayed in the clear enzyme extract according to the methods used by Reitman and Frankel (1957). The rate of pyruvate production after 30 and 60 min incubation periods is taken as a measured activity of GPT and GOT, respectively. Each enzyme determination was conducted three times for each experimental conditions. The least significant differences between treatment means was determined by analysis of variance.

Results and discussion

The nitrate reductase activity of 90-d-old *Vicia faba* plants was restricted completely to the nodules, whereas the activity in the leaves was very low (Table 1). The negligible leaf NR activity suggests a process of reduction in enzyme synthesis at this growth stage rather than an inability to synthesize this enzyme in this plant part. Therefore, it is concluded that faba bean is nearly or completely dependent upon its nodules for nitrate utilization when the leaf NR activity is minimal. The important role of nodule NR in nitrate reduction during reproductive stage was also reported by Randall *et al.* (1978), declining activity in the leaves by Harper and Hageman (1972) and Thibodeau and Jaworski (1975) and reduction of the nodule nitrogenase activity by Shalaby and Saleh (1991) and Lahiri *et al.* (1993).

Table 1. Effect of Al^{3+} and Cd^{2+} on nitrate reductase (NR) activity in *Vicia faba* nodules after 90 d from planting.

Treatment	NR activity [10^{-4} × μmol(NO_2^-) g^{-1}(f.m.) s^{-1}] metal concentration [μM]				
	0	50	100	500	1000
Aluminum	38.61	28.05	20.75	23.14	15.64
Cadmium	38.61	30.56	10.47	11.31	7.58

L.S.D. at 5 % level of treatment = 0.521 and of concentration = 0.823.

Al^{3+} and Cd^{2+} significantly suppressed the nodules NR activity, a phenomenon that was increased with raising the metals concentration. The activity was reduced to 40% and 20% of the control at 1000μM concentration of Al^{3+} and Cd^{2+}, respectively (Table 1). Both metals could act as a repressor for the enzyme synthesis by interacting with sulfhydryl (-SH) groups on the NR-protein, which in turn affect the enzyme function. Cd^{2+} was found to reduce the NR activity due to its interference with -SH sites (Mathys 1975). Meanwhile, the decrease of NR activity by Al^{3+}-treatment confirms the finding of Santoro *et al.* (1984) and Yandow and Klein (1986). This supperssion may be attributed to the effect of Al^{3+} on cell membranes

causing a reduction in nitrate uptake (Santoro *et al.* 1984, Jarvis and Hatch 1986) and to the ability of Al^{3+} to form coordination complexes with carboxyl and -SH groups of the enzyme protein (Foy 1984).

The present results show that the specific activities of GOT and GPT declined with the progress of age in both nodules and leaves, irrespective of the metals used (Table 2 and 3). In addition, the nodules had higher levels of both enzymes than the leaves, whereas the GOT activity was always greater than that of GPT in both organs, particularly in the ·n-treated plants. Thus, the higher level of GOT could be a contributing factor for the rease of aspartate and asparagine formation in this amide-transporting plant. This result is consistent with that reported by Reynolds and Farnden (1979) and Reynolds *et al.* (1982) who explained the higher aspartate level in lupin nodules by a more active GOT (aspartate amino-transferase).

Table 2. Effect of Al^{3+} and Cd^{2+} on GOT activity in *Vicia faba* nodules and leaves after 60 and 90 d from planting.

Treatment		GOT activity [10^{-2} × μmol(pyruvate) g^{-1}(f.m.) s^{-1}] metal concentration [μM]				
		0	50	100	500	1000
Aluminum	60-d-old nodules	28.05	23.12	24.32	25.72	24.05
Cadmium	60-d-old nodules	28.05	25.45	20.83	19.74	20.10
Aluminum	90-d-old nodules	14.55	13.22	10.78	10.67	10.05
Cadmium	90-d-old nodules	14.55	11.50	8.12	8.38	7.28
Aluminum	60-d-old leaves	16.12	13.67	12.22	12.67	11.72
Cadmium	60-d-old leaves	16.12	14.38	12.64	12.50	11.03
Aluminum	90-d-old leaves	10.88	9.67	7.88	8.45	7.33
Cadmium	90-d-old leaves	10.88	8.62	6.55	5.32	5.12

L.S.D. at 5 % level of treatment and concentration = 0.249 and 0.393 (60-d-old nodules), 0.142 and 0.224 (90-d-old nodules), n.s. and 0.258 (60-d-old leaves), 0.239 and 0.378 (90-d-old leaves), respectively.

The activities of GOT and GPT were variably affected by application of Al^{3+} and Cd^{2+}, where the GOT is more sensitive than GPT. As the level of each metal increased a significant inhibition of GOT activity was observed in both organs (Table 2). Moreover, the GPT activity was stimulated in the nodules and only slightly affected in the leaves (Table 3). Accumulation of amino acids has been found in Al^{3+}-treated plant (Cambraia *et al.* 1983, Santoro *et al.* 1984) and in Cd^{2+}-treated plants (Naguib *et al.* 1983), possibly due to the increase in protein breakdown. Moreover, Bauer *et al.* (1977) reported that the percentage of glutamine increased while asparagine decreased in plant treated with Al^{3+}. These observations indicate that both metals may interfer with the synthesis of enzymes responsible for the production of glutamic and aspartic acids, especially transaminases. Interaction of Al^{3+} and Cd^{2+} with the synthesis of GOT induced a reduction in its activity which could be associated with accumulation of glutamate and decrease in aspartate level in both nodule and leaf tissue. In the meantime, GPT activity was almost unaffected as

the level of Al^{3+} or Cd^{2+} varied, particularly in the leaves, indicating that both metals can not alter either the synthesis or function of this enzyme.

Table 3. Effect of Al^{3+} and Cd^{2+} on GTP activity in *Vicia faba* nodules and leaves after 60 and 90 d from planting.

Treatment		GTP activity [10^{-2} × μmol(pyruvate) g^{-1}(f.m.) s^{-1}] metal concentration [μM]				
		0	50	100	500	1000
Aluminum	60-d-old nodules	23.95	26.17	28.07	30.00	31.22
Cadmium	60-d-old nodules	23.95	25.00	26.00	27.38	28.31
Aluminum	90-d-old nodules	13.22	13.91	16.32	16.22	16.84
Cadmium	90-d-old nodules	13.22	14.00	14.38	11.45	12.83
Aluminum	60-d-old leaves	11.05	9.83	10.28	9.38	10.12
Cadmium	60-d-old leaves	11.05	12.31	11.50	10.24	9.95
Aluminum	90-d-old leaves	3.38	4.25	3.72	3.45	2.74
Cadmium	90-d-old leaves	3.38	3.28	3.67	2.83	1.72

L.S.D. at 5 % level of treatment and concentration = 0.403 and 0.637 (60-d-old nodules), 0.224 and 0.354 (90-d-old nodules), 0.233 and 0.368 (60-d-old leaves), 0.042 and 0.066 (90-d-old leaves), respectively.

In conclusion, the present study demonstrates that *Vicia faba* plant is sensitive to the foliar application of Al^{3+} and Cd^{2+} due to the inhibition of NR and GOT which are the key enzymes in nitrogen metabolism, while GPT appears to be less sensitive to both metals.

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