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Nickel in Higher Plants

FURTHER EVIDENCE FOR AN ESSENTIAL ROLE

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ABSTRACT

Soybeans (*Glycine max* [L.] Merr.) grown in Ni-deficient nutrient solutions accumulated toxic urea concentrations which resulted in necrosis of their leaflet tips, a characteristic of Ni deficiency. Estimates of the Ni requirement of a plant were made by using seeds produced with different initial Ni contents. When compared to soybeans grown from seeds containing 2.5 nanograms Ni, plants grown from seeds containing 13 nanograms Ni had a significantly reduced incidence of leaflet tip necrosis. Plants grown from seeds containing 160 nanograms Ni produced leaves with almost no leaflet tip necrosis symptoms. Neither Al, Cd, Sn, nor V were able to substitute for Ni.

In other experiments, a small excess of EDTA was included in the nutrient solution in addition to that needed to chelate micronutrient metals. Under these conditions, nodulated nitrogen-fixing soybeans had a high incidence of leaflet tip necrosis, even when 1 micromolar NiEDTA was supplied. However, in nutrient solutions containing inorganic sources of N, 1 micromolar NiEDTA almost completely prevented leaflet tip necrosis, although no significant increase in leaf urease activity was observed. Cowpeas (*Vigna unguiculata* [L.] Walp) grown in Ni-deficient nutrient solutions containing NO_3 and NH_4 also developed leaflet tip necrosis, which was analogous to that produced in soybeans, and 1 micromolar NiEDTA additions prevented these symptoms.

These findings further support our contention that Ni is an essential element for higher plants.

During the last decade the nutritional importance of Ni has become increasingly apparent. Ni has been shown to be essential for animals (9), several bacteria (17), and one strain of blue-green algae (18). The biological significance of Ni has been reviewed recently by Welch (19).

The enzyme urease from jack beans (2, 4) and soybean seed (12) is known to be a Ni-metalloenzyme. Several investigators have reported responses in plants to Ni additions when urea was supplied as their sole source of N. The growth of soybean, rice, and tobacco tissue cultures (10–12) and *Lemna paucicostata* (5) in media where N was supplied as urea was stimulated by Ni additions. Shimada *et al.* (13, 14) found that soybeans and tomatoes, when grown in nutrient solution with urea as the sole N source, accumulated urea in their leaves and developed necrotic lesions at their leaf tips. Addition of Ni increased leaf urease activity and prevented urea accumulation (14). Klucas *et al.* (7) also reported that Ni increased soybean leaf urease activity and that neither Cr, V, Sn, nor Pb was able to substitute for Ni. Recently Eskew *et al.* (3) demonstrated that soybeans grown in nutrient solutions containing NO_3 and NH_4 as sources of N developed necrotic lesions on their leaflet tips. These necrotic tips contained 2.5% urea (dry weight), and were more frequent and more extensive on plants dependent on N_2 fixation for their N. On this basis, they suggested that urea is produced during normal N metabolism in higher plants and that Ni, as a component of urease, is required to prevent urea accumulation to toxic levels.

In this paper we estimate the amount of Ni required by plants to prevent leaflet tip necrosis, show that neither Al, Cd, Sn, nor V will completely substitute for Ni, and extend our observations to cowpeas.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr.) cv Maple Presto and cowpea (*Vigna unguiculuta* [L.] Walp cv Vita 5) were used in these studies. Plant growth conditions and nutrient solution composition were previously described (3). Concentrated stock solutions of the macronutrient elements and Mes were purified by chelation chromatography on a 0.6 × 13 cm column of 8-hydroxyquinoline-controlled pore glass beads. Purification efficiency was monitored using ^{63}Ni as a radiotracer.

Seeds were germinated in rolls of acid-washed filter paper saturated with dilute nutrient solution (one-tenth of the concentration used previously). After 5 d, seedlings were transferred to 3.4 L polyethylene pots of more concentrated nutrient solution (one-fourth the previously described concentration). There were six pots per treatment and four seedlings per pot. Plants grown in nitrogen-free nutrient solution were inoculated at this time by immersing their radicles in a culture (yeast extract mannitol media) of *Rhizobium japonicum* USDA 110, for 30 s. After 1 week the nutrient solution was replaced with half-concentrated nutrient solution, and then after an additional week with full concentration nutrient solution. Nutrient solutions were changed weekly until the pod-filling growth stage when they were changed twice per week. At 30 d after seed imbibition, two of the four plants per pot were thinned.

All operations from purification of nutrient solutions to handling of plants were performed wearing unpowdered, polyvinyl chloride gloves (gloves provided by Scientific Products Co.[2] were found to have low surface Ni contamination and were used without further washing). All plastic and glassware used was detergent-washed, rinsed with deionized H_2O, soaked overnight in 10% HCl, and rinsed again with deionized H_2O having at least

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[2] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

10 Mohm resistance.

To investigate the effects of the Ni content of soybean seed on subsequent plant growth, plants were grown from seed produced by plants which had been grown in purified nutrient solutions supplied with either 0, 1, or 10 μg Ni l^{-1} with each change of nutrient solution. The conditions under which these seeds were produced, their Ni concentrations, and urease activities were reported earlier (3).

In studies designed to investigate the specificity of Ni in preventing leaf tip necrosis, 10 μg l^{-1} each of Al, Cd, Sn, and V, either with or without 10 μg l^{-1} Ni, were added with each change of nutrient solution. Commercial atomic absorption standard solutions (Fisher Scientific Co.) of these metals were diluted with 0.12 N HCl to 100 mg l^{-1} of each metal and 0.34 ml of this solution was added to each 3.4 l plastic pot.

In other experiments, a small excess of Na_2 EDTA was added in addition to the 50 μM of EDTA added as FeEDTA. The total concentration of EDTA was chosen to exceed the nutrient solution concentration of Fe, Zn, Cu, and Mn by 2 μM. The excess EDTA was expected to lower the activity of any Ni ions remaining in the purified nutrient solutions, thereby reducing the ionic Ni available for plant absorption (assuming ionic Ni^{2+} is the absorbed form). In some treatments, Ni was added as the EDTA complex at a level of 1 μM NiEDTA in solution.

Assays of leaf urease activity were as previously described (3).

RESULTS AND DISCUSSION

Previous experiments (3) showed that the addition of 1 or 100 μg Ni l^{-1} with each change of nutrient solution enhanced leaf and seed urease activity in soybeans and was sufficient to prevent both the accumulation of toxic urea levels and the resulting development of leaflet tip necrosis. The amount of Ni required by soybeans to prevent leaflet tip necrosis, however, was not determined. An estimate of this amount was obtained by using the seed, produced in the previously reported experiment, to grow a second generation of plants. These seeds contained ≤2.5, 13, or 160 ng Ni per seed when the parent plants were supplied 0, 1, or 10 μg Ni l^{-1}, respectively, in their nutrient solutions.

Results presented in Table I show that an initial seed content of 13 ng Ni was sufficient to significantly reduce the incidence of leaflet tip necrosis. Seeds with an initial Ni content of 160 ng produced plants with almost no injury symptoms, even when no additional Ni was added. Such a response suggests that whereas about 10 ng Ni in seed stores has a significant effect, this amount is not enough Ni for normal plant growth and that about 200 ng Ni in seeds would probably be sufficient. There are at least two assumptions in these suggestions. First, it must be assumed that no significant amounts of Ni were obtained from the nutrient solution or from other environmental sources, and second, that the metabolically active form of Ni stored in the seed is readily remobilized during germination and plant development. Although no direct information is available on remobilization of Ni during germination, Cataldo *et al.* (1) have shown that ^{63}Ni is readily remobilized to developing seeds from vegetative tissues. Thus, 200 ng Ni seems a reasonable approximation of the amount of Ni required by a soybean plant to prevent leaf tip necrosis from developing. Using this figure of 200 ng of Ni per plant and assuming a mature soybean plant weight between 50 and 100 g dry weight, the critical Ni concentration in soybean tissue can be calculated to be between about 2 and 4 ng of Ni g^{-1} dry weight (*i.e.* between 0.03 and 0.07 nmol · g^{-1}). Previously, of the 16 established essential elements for plant growth, Mo was required in the least amount. The critical nutrient concentration of Mo has been reported to be between 10 and 500 ng Mo g^{-1} dry weight (6), *i.e.* between 0.1 and 5.2 nmol·g^{-1}. Thus, Ni appears to be required for normal plant growth at a concentration that is much lower than that established for Mo (*i.e.* from about 1.3 to 30% of the critical Mo concentrations reported in nmol per g).

Seed yield tended to increase as the level of Ni stores in the seed increased while shoot dry weights tended to be higher. However, the variability in shoot and seed yield in this experiment was high and statistically significant differences between treatments were not obtained (Table I).

The second part of this experiment tested whether Al, Cd, Sn, or V could substitute for Ni in preventing leaf tip necrosis. These elements were chosen because the purification technique would be expected to remove not only Ni but also these elements (16), and because these elements have been found to have biological activity. Although a statistically significant reduction in the percentage of necrotic leaflet tips was found when 10 μg l^{-1} of all four elements was added, their additions failed to prevent the occurrence of the symptoms as had been observed with 1 μg Ni l^{-1} in the previous generation and with 10 μg Ni l^{-1} in the current experiment (Table I). Whether this finding was the result of partial substitution by one of these four elements for Ni (which seems doubtful) or the result of Ni contamination in the stock solutions of these elements (which is more likely) is not known.

Excess EDTA Experiments. In the research reported earlier (3), only Fe was added to the nutrient solutions as an EDTA chelate. However, as in most nutrient solutions containing Fe-EDTA, other metals may form their respective EDTA chelates by displacing some Fe from FeEDTA. These reactions may

Table I. *Effect of Total Ni Content of Seed, of Ni Supply in the Nutrient Solution, and of Al, Cd, Sn, and V (A-V) Supply in Nutrient Solution on Shoot Dry Weight at 56 Days, Seed Yield, and Occurrence of Leaflet Tip Necrosis in Soybeans*

Treatment (Ni supplied)		Shoot Dry Wt	Seed Yield	Leaflet Tip Necrosis
First generation	Second generation			
$\mu g\ Ni\ l^{-1}$	$\mu g\ l^{-1}$	$g\ plant^{-1}$		%
0 (2.5)[a]	0	27.0 a[b]	14.1 a	25.6 a
1 (13)	0	29.5 a	14.9 a	20.0 b
10 (160)	0	21.7 a	21.7 a	1.0 c
1	A-V	ND[c]	25.9 a	11.0 d
1	A-V + 10	ND	27.9 a	0.0 c
0	10	29.2 a	26.2 a	0.0 c
1	10	29.1 a	17.6 a	0.0 c
10	10	31.7 a	18.0 a	0.0 c

[a] Numbers in parentheses indicate the initial Ni content of the seed in ng Ni per seed.

[b] Values in the same column, followed by identical letters are not significantly different by Duncan's multiple range test.

[c] Not determined.

Table II. *Effects of Ni on Soybean Leaf Urease Activity, Per Cent Leaflet Tip Necrosis, and Plant Dry Weight at 56 Days for Soybeans Grown in Nutrient Solutions with 7 μM Na_2EDTA Added in Excess of That Added as FeEDTA*

Plants were either provided 13 mM NO_3 plus 2.5 mM NH_4 or were dependent on N_2 fixation for N. Nickel treatments were provided at 1 μM NiEDTA.

Nitrogen Source	Ni EDTA Supplied	Leaf Urease Activity	Leaflet Tip Necrosis	Plant[a] Dry Wt
	μM	$nmol\ mg^{-1}\ h^{-1}$	%	$g\ plant^{-1}$
N_2	0	7.9	55.8	18.4
N_2	1	8.0	55.1	17.8
NO_3^- and NH_4^+	0	8.6	51.6	42.8
NO_3^- and NH_4^+	1	10.8	2.6	43.7

[a] Ni supply had no statistically significant effect on dry wt of plants.

Table III. *Effects of Ni on Per Cent Leaflet Tip Necrosis and Cowpea Seed Yield in Cowpeas Grown in Nutrient Solutions*

The nutrient solutions contained 13 mM NO_3, 2.5 mM NH_4, and 7 μM Na_2EDTA excess above that supplied as FeEDTA. Nickel was supplied as 1 μM NiEDTA. There were two plants per replicate pot and three replicate pots per treatment.

NiEDTA	Leaflet Tip Necrosis	Seed[a] Yield
μM	%[b]	g plant^{-1}
0	63.8	24.5[c]
1	0	33.1[d]

[a] Differences between treatment means are not statistically significant.
[b] Mean of three replicates per treatment.
[c] Mean of two replicates; seeds from the third replicate were sent to a cooperator before seed weights were obtained.
[d] Mean of three replicates.

dramatically lower the concentration of the free cations of many micronutrient metals while increasing the concentration of Fe^{3+} until it becomes restricted by the precipitation of sparingly soluble compounds, presumably hydrous oxides and phosphates. Equilibrium calculations, using the computer program GEOCHEM (15) and equilibrium constants from Martell and Smith (8), show that Ni was relatively strongly chelated by EDTA in the nutrient solutions used (3), and that 90 to 100% of Ni was present as NiEDTA. These results and the work of others ([5]; Winkler and Polacco, personal communication, 1983) suggest that adding extra EDTA could suppress the free Ni^{2+} concentration even further and might enhance the development of Ni deficiency symptoms.

In these experiments, the concentration of Ni^{2+} was regulated by adding a small excess of EDTA (*i.e.* 2 μM) over the concentration of Fe, Zn, Cu, Mn, and Ni in two nutrient solutions. Solutions consisted of either 0 or 1 μM NiEDTA, 57 μM EDTA, 50 μM Fe, 2 μM Zn, 2 μM Mn, and 1 μM Cu, and had an initial pH of 5. Large excesses of EDTA, relative to nutrient cations, were avoided to reduce the potential for root damage and the likelihood of inducing multiple nutrient deficiencies. In these solutions, equilibrium calculations suggest that the initial concentration of Ni^{2+} was less than 10^{-15} M in the treatment to which no Ni was added (containing approximately 10^{-9} M total Ni [3]) and approximately 10^{-11} M in the 1 μM Ni treatment.

When soybeans were grown in these solutions and supplied with NO_3 and NH_4, 51.6% of leaflets showed tip necrosis on plants grown without added Ni, while only 2.6% showed this symptom when 1 μM NiEDTA was present (Table II). Similarly, cowpeas grown without added Ni displayed 63.8% leaflet tip necrosis, while no necrosis was evident when 1 μM NiEDTA was supplied (Table III). The necrotic portions of the cowpea leaves contained 3% urea (dry weight) which is similar to our earlier observations with soybeans (3). These results confirm that Ni alleviates leaflet tip necrosis but, contrary to expectations, the symptoms were not markedly more severe in the presence of excess EDTA than reported earlier (3).

When nodulated N_2-fixing soybeans were grown in these solutions without NO_3 and NH_4, leaflet tip necrosis was equally abundant whether NiEDTA was added or not (Table II). This finding supports the earlier contentions (3, 19) that a higher level of Ni is required by plants dependent on N_2 fixation and demonstrates that 1 μM Ni as NiEDTA was inadequate to alleviate symptoms. As in our previous studies, no differences in the dry weight yields of soybean plants or the seed yield of cowpeas were found as a result of Ni treatment.

In the presence of excess EDTA, the 1 μM concentration of NiEDTA in the nutrient solution may not have fully met the Ni requirements of soybeans even when provided with inorganic nitrogen. This possibility is suggested by the low and nearly identical activity of leaf urease, irrespective of Ni treatment, as well as by the appearance of some leaf tip necrosis at 1 μM level of added Ni (Table II). These results also suggest that the availability of Ni from solution was very low when present primarily as NiEDTA.

The characteristic of Ni deficiency symptom in cowpeas was identical to that observed in soybean plants. In both crops the onset of leaflet tip necrosis coincided closely with the onset of flowering. Although it took longer for the symptom to occur in cowpeas than soybeans, this delay could be related to the longer time needed for cowpeas to flower. It seems likely that the role of Ni in higher plants is of general importance because of the similarities in the responses of soybeans and cowpeas to Ni deficiency. However, further research is required to establish whether or not Ni is an essential element for all higher plants.

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