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Effects of Molybdenum, Nickel, and Nitrogen Sources on the Mineral Nutrition and Growth of Rice Plants

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Abstract: Upland rice plants, cultivar 'IAC 202,' were grown in nutrient solution until full tillering. Treatments consisted of ammonium nitrate (AN) or urea (UR) as nitrogen (N) source plus molybdenum (Mo) and/or nickel (Ni): AN + Mo + Ni, AN + Mo − Ni, AN − Mo + Ni, UR + Mo + Ni, UR + Mo − Ni, and UR − Mo + Ni. The experiment was carried out to better understand the effect of these treatments on dry-matter yield, chlorophyll, net photosynthesis rate, nitrate (NO_3^--N), total N, in vitro activities of urease and nitrate reductase (NR), and Mo and Ni concentrations. In UR-grown plants, Mo and Ni addition increased yield of dry matter. Regardless of the N source, chlorophyll concentration and net photosynthesis rate were reduced when Mo or Ni were omitted, although not always significantly. The omission of either Mo or Ni led to a decrease in urease activity, independent of N source. Nitrate reductase activity increased in nutrient solutions without Mo, although NO_3^--N increased. There was not a consistent

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This article is dedicated to the memory of Prof. Eurípedes Malavolta; his example and passion for teaching will always be remembered.

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variation in total N concentration. Molybdenum and Ni concentration in roots and shoots were influenced by their supply in the nutrient solution. Molybdenum concentration was not influenced by N sources, whereas Ni content in both root and shoots was greater in ammonium nitrate–grown plants. In conclusion, it can be hypothesized that there is a relationship between Mo and Ni acting on photosynthesis, although is an indirect one. This is the first evidence for a beneficial effect of Mo and Ni interaction on plant growth.

Keywords: Chlorophyll, micronutrients, nitrate reductase, photosynthesis, plant nutrition, urease

INTRODUCTION

Molybdenum (Mo) was established to be essential in higher plants by Arnon and Stout (1939). Molybdenum is known as a constituent of enzymes such as nitrate reductase (EC 1.6.6.1), which reduces nitrate to nitrite, and the enzyme nitrogenase (EC 1.18.6.1), which reduces molecular nitrogen (N) to ammonia in all N-fixing organisms. Further details can be found elsewhere (Hewitt and Smith 1975; Epstein and Bloom 2005; Malavolta 2006).

Representative soils of Sao Paulo state, Brazil, analyzed by Bataglia, Furlani, and Valadares (1975), showed total Mo content between 0.11 and 3.73 $mg\,kg^{-1}$ and soluble content in ammonium oxalate extractant from 0.01 to 0.03 $mg\,kg^{-1}$. Symptoms of deficiency and amelioration by Mo addition have been observed in several crops, particularly in legumes.

Nickel (Ni) meets the direct (Dixon et al. 1975) and indirect (Eskew, Welch, and Cary 1983) criteria of essentiality. Urease (EC 3.5.1.5) is a ubiquitous metalloenzyme containing Ni, which splits urea hydrolytically into ammonia (NH_3) and carbon dioxide (CO_2). Ammonia ions released by urea hydrolysis are incorporated into glutamate (Gerendás, Zu, and Sattelmacher 1998). Wood, Reilly, and Nyczepir (2004) and Ruter (2005) diagnosed Ni deficiency under field conditions, in the United States, in pecan (*Carya illinoinensis*) and river birch (*Betula nigra*), respectively. Bertrand and DeWolff (1973) observed that soybean cultivated in a soil low in Ni had increased nodulation and grain yield resulting from Ni fertilization up to 40 $g\,ha^{-1}$. Leaf symptoms are characterized by dark spots and an anatomical deformation causing leaf rounding, known as "mouse ear" (Wood, Reilly, and Nyczepir 2004). Necrotic spots associated with Ni deficiency correspond to local accumulation of either urea (Shimada et al. 1980; Welch 1981) or oxalic and lactic acids (Bai, Reilly, and Wood 2006), indicating disturbance in N and carbon (C) metabolism.

Analyses of 38 samples of Brazilian soils from Sao Paulo state showed that total Ni varied in the range of less than 10 to 127 $mg\,kg^{-1}$.

Soluble diethylenetriaminepentaacetic acid (DTPA) Ni ranged from less than 0.5 to a maximum of 1.4 mg kg^{-1}, considered as low values (Rovers, Camargo, and Valadares 1983). Soils of pecan orchards in the United States, where Ni deficiency was observed and corrected through foliar spray of Ni sulfate, showed 0.4 to 1.4 kg ha^{-1} of Ni, equivalent to approximately 0.2 to 0.7 mg kg^{-1} (Wood, Reilly, and Nyczepir 2006).

It was observed that Ni stimulated the in vitro nitrate reductase (NR) activity of young grain sorghum and sudangrass leaf tissue as a result of reversion of cyanide inhibition (Maranville 1970). Nickel-deficient barley can accumulate more nitrate (NO_3^--N) than plants that have sufficient Ni (Brown, Welch, and Madison 1990). This effect is explained through the role of Ni in activation of L-Malate:NAD oxidoreductase (MDH) involved in nicotinamide adenine dinucleotide (NADH) production, which is required for nitrate reduction. Kevresan et al. (1998) grew sugar beet plants in water solution with cadmium (Cd), Mo, and Ni containing 0, 0.1, 10, or 1,000 μM of each element. Activities of nitrate reductase and glutamine synthetase, and protein content were reduced by Ni, whereas Mo stimulated these parameters. In a similar study, Kevresan et al. (2001) observed that Ni and Mo led to a reduction of nitrate content in shoots more than roots of pea plants. In low concentration, Ni increased dry matter of both shoot and roots.

Investigations on both Ni and urease and on Mo and nitrate reductase are plentiful (Mulder, Boxma, and Veen 1959; Eskew, Welch, and Norvell 1984; Martin, Saco, and Alvarez, 1995; Saco, Martin, and Alvarez 1995; Gerendás and Sattelmacher 1997a, 1997b; Gerendás, Zhu, and Sattelmacher 1998; Bai, Reilly, and Wood 2006). However, there are few works that relate these two micronutrients, direct or implicitly, with each of these two enzymes. The aims of the current work were to measure the effect of Mo and Ni on dry-matter yield of rice plants supplied with two N sources (ammonium nitrate and urea) and to evaluate the influence of Mo and Ni on variables related to dry-matter yield, such as activity of both urease and nitrate reductase, chlorophyll, net photosynthesis rate, total N, nitrate content, and Mo and Ni concentrations in roots and shoots..

MATERIAL AND METHODS

A greenhouse experiment was carried out at the Plant Nutrition Laboratory of the Center for Nuclear Energy in Agriculture (CENA), University of Sao Paulo (USP), Piracicaba, SP, Brazil. Seeds of upland rice (*Oryza sativa* L. cv. 'IAC 202') were germinated in vermiculite moistened with 0.1 mM calcium sulfate ($CaSO_4 \cdot 2H_2O$). Seedlings were transfered to 40-L plastic trays with a wooden perforated cover when they reached 5 cm high and were fixed with plastic foam around the

Table 1. Composition of the nutrient solutions[a]

Nutrient	Amount
N (NH_4NO_3 or urea) (mmol L^{-1})	6.00
K (mmol L^{-1})	2.00
P (mmol L^{-1})	0.25
Mg (mmol L^{-1})	0.50
Ca (mmol L^{-1})	2.00
Fe-EDTA (μmol L^{-1})	89.5
B (μmol L^{-1})	25.0
Mn (μmol L^{-1})	2.0
Zn (μmol L^{-1})	2.0
Cu (μmol L^{-1})	0.5
Mo (μmol L^{-1})	0.5
Ni (μmol L^{-1})	0.5

[a]Modified from Gerendás et al. (1998) and Epstein and Bloom (2005); Mo and Ni were omitted according to each treatment; NH_4NO_3, ammonium nitrate (AN).

bottom part of their culms. Plants were grown in aerated one-fifth-strength Johnson's solution (Johnson et al. 1957). After 2 weeks, two plants were put in 2-L plastic pots containing full-strength nutrient solution (Table 1), modified from Gerendás, Zhu, and Sattelmacher (1998) and Epstein and Bloom (2005). Nutrient solutions were kept under constant aeration, and their pH was adjusted to 5.8 whenever needed. Nutrient solutions were renewed every week. Analytical-grade reagents and deionized water from ion-exchange-resin treatment were used in this experiment. The treatments are shown in Table 2. Six replicates in a completely randomized design were used.

Five weeks after the start of treatments, two middle leaves from two plants in each treatment were collected to assay urease activity according to method described by Hogan, Swift, and Done (1983), with NH_3 determined as suggested by McCullough (1967). One week later, new leaf samples were taken using the same procedure to assay NR activity, according to a simplified technique (Mulder, Boxma, and Veen 1959).

Table 2. Distribution of treatments in the experiment

Pot number	Variables[a]
1–6	AN + Mo + Ni
7–12	AN + Mo − Ni
13–18	AN − Mo + Ni
19–24	UR + Mo + Ni
25–30	UR + Mo − Ni
31–36	UR − Mo + Ni

[a]AN: ammonium nitrate; UR, urea; + and −, with and without addition, respectively.

Nine weeks after start of treatments, the following determinations were made: Leaf sampling for chlorophyll analysis was carried out as described previously, and analyzed according to Arnon (1949). Indirect chlorophyll measurements were performed with a portable Minolta Soil–Plant Analysis Development (SPAD) 502 chlorophyll meter (Minolta Camera Co. Ltd., Tokyo, Japan), using the medium portion of top leaves but avoiding central ribbing; net photosynthesis rate was measured and calculated with an infrared gas analyzer (IRGA) Li-COR 6400 model (Li-COR, Inc., Lincoln, Neb.), with 1600 μmol photons $m^{-2} s^{-1}$. Shoots and roots were harvested, rinsed with distilled water, and oven dried at 65 °C to constant weight, and their weights were recorded. Plant materials were ground to pass a 1-mm sieve and digested, and total N, Ni, and Mo were analyzed according to Malavolta, Vitti, and Oliveira (1997). Soluble NO_3-N in shoots was determined as described by Bray (1948).

Statistical analyses were performed using Statistical Analysis System (SAS) software for Windows 6.11 (SAS 1996). Analysis of variance (F-test) was employed to evaluate significance of treatments. Tukey's test was used for means separation.

RESULTS AND DISCUSSION

Effects of treatments on dry-matter yield are shown in Table 3. Molybdenum and Ni caused different effects on plant growth depending on N sources. Regarding N sources, dry-matter yield was greater in urea-grown plants treated with Mo and Ni, likely because ammonia ions generated by hydrolysis of urea passively taken up by roots through

Table 3. Dry-matter yield of rice plants (g per pot)

Treatments[a]	Parts of plant		
	Root	Shoot	Total
AN + Mo + Ni	1.9b	5.6c	7.5d
AN + Mo − Ni	2.0ab	7.2bc	9.2bc
AN − Mo + Ni	1.8b	7.7b	9.5b
UR + Mo + Ni	2.3a	8.9a	11.2a
UR + Mo − Ni	2.3a	7.0c	9.3bc
UR − Mo + Ni	2.2ab	6.3d	8.5c
F-test	*	**	**
CV (%)	4.9	1.9	2.3

[a]AN, ammonium nitrate; UR, urea.

*, **Significant at 5 and 1% levels, respectively.

Note. The same letter in a given column indicates, nonsignificant differences at the 5% level by the Tukey test.

transmembrane channel are incorporated in organic compounds without prior reduction. On the other hand, in ammonium nitrate treatments, energy is required for active ammonium (NH_4^+-N) and NO_3^--N absorption by roots as well as carbon and protons consumed by the nitrate reduction process (Crawford et al. 2000).

Dry-matter yield was reduced in ammonium nitrate–grown plants supplied with Mo and Ni, compared with Mo- and Ni-deprived plants in the ammonium nitrate (AN or NH_4NO_3) treatment (Table 3). Because of the role of Ni as a component of the urease enzyme, which splits urea hydrolytically into NH_3 and CO_2, low Ni consumption in NH_4NO_3 treatments was expected. Therefore, in this case, Ni supply did not lead to a toxic accumulation, because there was greater Ni concentration in plants with no growth reduction. Molybdenum is necessary to activate nitrate reductase (Hewitt and Smith 1975), but sometimes Mo supplied in solution can interfere with absorption of micronutrients (Fargasova 1999). This may explain the greater dry-matter content in plants grown without Mo. Urea-grown plants need less Mo because urea is a reduced N source, but reduced dry-matter yield in Mo-deprived plants was observed. However, those plants did not show any Mo-deficiency symptoms, maybe because concentration of Mo in plant tissue was high enough for growth. A plausible explanation for this finding is still required.

Table 4 shows the treatment effect on the net photosynthesis rate and chlorophyll concentration in rice leaves. Chlorophyll index when indirectly evaluated (SPAD units) was not affected by either N sources or Mo deprivation; on the other hand, it was influenced by Ni deprivation. Data on total chlorophyll measurements presented the greatest values in urea-grown plants, which does not agree with the indirect measurement; chlorophyll concentration was weak in treatments with both Mo and Ni deprivation, independent of N sources.

Similar studies in the literature regarding the relationships of N sources, Ni, and Mo on plant nutrition were not found. Usually, information about Ni effects is related to urea as a N source. Wong and Chang (1991) observed significant increase of chlorophyll concentration in *Chlorella pyrenoidosa* (freshwater algae) when Ni concentration ranged from 0.1 to 1.0 mg L^{-1} in the culture medium. McIlveen and Negusanti (1994) noticed that among several organelles, chloroplasts contained the greatest Ni content, between 8.0 and 9.9% of the total content, while other organelles such as mitochondria and ribosomes showed from 0.32 to 2.85%. Nickel effects on chlorophyll concentration depend on doses and plant species; it can be negative for barley and tomato or positive for maize, oat, and potato.

In tomato plants, Tan, Ikeda, and Oda (2000) observed strong chlorophyll concentration when urea was amended with Ni, whereas AN

Table 4. Effect of N sources, Mo, and Ni on SPAD units, chlorophyll, and net photosynthesis rate

Treatments[a]	SPAD units	Chlorophyll a ($\mu g\,mL^{-1}$)	Chlorophyll b ($\mu g\,mL^{-1}$)	Total chlorophyll ($\mu g\,mL^{-1}$)	Net photosynthesis rate ($\mu mol\ CO_2\ m^{-2}\,s^{-1}$)
AN + Mo + Ni	40.05abc	1.52c	0.70a	2.22c	24.18ab
AN + Mo − Ni	36.82d	0.57d	0.31b	0.88d	20.27b
AN − Mo + Ni	39.62bc	1.00d	0.33b	1.33d	20.25b
UR + Mo + Ni	41.98a	3.73a	0.62ab	4.35a	28.46a
UR + Mo − Ni	37.98cd	1.92c	0.66a	2.58c	26.99a
UR − Mo + Ni	41.70ab	2.64b	0.58b	3.22b	21.55b
F-test	**	**	**	**	**
CV (%)	3.2	9.3	21.5	8.2	10.6

[a]AN, ammonium nitrate; UR, urea.

**Significant at the 1% level.

Note. The same letter in a given column indicates nonsignificant differences at the 5% level by Tukey test.

(with or without Ni) did not show any measurable effect. Results obtained by Gerendás and Sattelmacher (1997a) also showed that in nutrient solution containing urea without Ni, there was reduction of chlorophyll concentration in rye, wheat, soybean, rape, zucchini, and sunflower. Rahman et al. (2005) cultivated barley in Hoagland and Arnon nutrient solution and observed increases of chlorophyll index (SPAD units) with growing Ni doses, suggesting its addition in concentrations ranging from 1.0 to 10 μM for optimal plant growth. Based on the observation by Ilin, Kastori, and Malencic (2000), Ni can contribute to the avoidance of chlorophyll degradation by free radicals because of its effect on superoxide dismutase activity. Published information regarding effect of Mo on chlorophyll was not found, but the participation of this element in protein synthesis is well known as well as the fact that Mo deficiency inhibits chloroplast development (Römheld 2001). However, in the present work, Mo deprivation did not lead to chlorosis and net photosynthesis rate was not affected in either N treatment. Molybdenum or Ni deprivations seemed to reduce net photosynthesis rate (Table 4).

Results of urease and nitrate reductase activities, NO_3^--N, and total N are presented in Table 5. In general, there was no clear effect of N source on urease activity and total N. This is in agreement with observations by Gerendás and Sattelmacher (1999), whose experiment with *Brassica napus* showed similar urease activity in both urea- and ammonium-supplied plants. Urease activity in NH_4NO_3- supplied plants can be induced by endogenous urea, which controls the formation of this enzyme (Matsumoto et al. 1966).

Table 5. Treatment effects on urease and nitrate reductase activity, NO_3^--N, and total N

Treatments[a]	Urease (μmol NH_4^+-N g FW^{-1} h^{-1})	Nitrate reductase (μmol NO_2^--N g FW^{-1} h^{-1})	NO_3^--N (mg kg^{-1})	Total N (g kg^{-1})
AN + Mo + Ni	16.91a	12.27b	4,533a	43ab
AN + Mo − Ni	7.14cd	11.88b	4,452a	40ab
AN − Mo + Ni	9.57c	18.79a	5,089b	40ab
UR + Mo + Ni	17.11a	3.11c	1,325c	48ab
UR + Mo − Ni	6.14d	2.02c	1,373c	50a
UR − Mo + Ni	12.34b	1.24c	1,033c	39b
F-test	**	**	**	*
CV (%)	8.5	16.2	11.5	6.0

[a]AN, ammonium nitrate; UR, urea; N, nitrogen; FW, fresh weight.

**, *Significant at 1% and 5% levels, respectively.

Note. The same letter in a given column indicates nonsignificant differences at the 5% level by Tukey test.

Favorable Ni effect was expected and indeed observed for both N sources. Gerendás and Sattelmacher (1997b) observed reduction of urease activity in Ni-deprived plants growing with both urea and ammonium nitrate. However, urease activity was greater in urea-grown plants. In our work, Mo and Ni together led to an increased urease activity greater than each element supplied separately. It is possible that in urea-grown plants without Mo or Ni, urease activity was not greater, due to either its inhibition by NH_4^+-N excess produced during urea hydrolysis (Matsumoto et al. 1966) or excessive accumulation of urea in plant tissues. To our knowledge, Mo + Ni or Mo effects on urease activity have not been reported, and the results observed in this work do not allow a full explanation of the processes behind it.

Nitrate reductase activity was influenced by both N sources, and it was greater in ammonium nitrate–grown plants (Table 5). Nitrate ions induce NR activation, which needs Mo for its activity. An unexpected increase in NR was caused by Mo deprivation. Nickel-deprived plants grown with NH_4NO_3 presented reduction in this enzyme when compared to those grown with full Ni supply. In the available literature, no similar research was found. Nitrate reductase activity observed in urea treatments came from nitrate uptake, when young plants were supplied with diluted nutrient solution. Nickel effect on increasing NR activity is not in agreement with findings by Kevresan et al. (1998), which suggests no stimulating action by this micronutrient.

It may be possible that some Mo and Ni are present even in treatments in which no Mo or Ni were added. The amounts of Mo or Ni required by plants are very small, and there may have been some Mo and Ni contaminants from other components of the nutrient solution (Table 6).

Nitrate content was influenced by both N sources. Molybdenum-deprived plants growing with NH_4NO_3 plus Ni had their nitrate content increased, although nitrate reductase activity increased significantly. Studies of Ni effect on N metabolism have shown variable results, possibly in response to imposed experimental conditions. Brown, Welch, and Madison (1990) grew barley in nutrient solution containing ammonium and nitric N, and they observed an increase of nitrate content in Ni-deprived treatments; perhaps because Ni activates MDH, which produces the NADH required for nitrate reduction. According to Kevresan et al. (2001), young pea plants fertilized with Ni under soil conditions had less tissue nitrate. A clear relationship has not been established among Ni, NR activity, and nitrate content. In our experiment, total N in ammonium nitrate–grown plants was not affected by either Mo or Ni treatments. However, Mo-deprived plants grown with urea had a significant total N reduction, the least NR activity, and the weakest NO_3^--N concentration.

Table 6. Treatment effects on Mo and Ni concentrations

Treatments[a]	Molybdenum ($mg\,kg^{-1}$)		Nickel ($mg\,kg^{-1}$)	
	Root	Shoot	Root	Shoot
AN + Mo + Ni	4.6b	2.5a	12.6b	4.2ab
AN + Mo − Ni	8.3a	2.2ab	0.8c	1.6c
AN − Mo + Ni	0.7c	0.9c	20.5a	4.5a
UR + Mo + Ni	5.2b	2.0abc	3.2c	3.3abc
UR + Mo − Ni	4.7b	2.6a	2.8c	1.3c
UR − Mo + Ni	0.5c	1.0bc	4.0c	2.3bc
F-test	**	**	**	**
CV (%)	17.7	16.9	18.7	17.7

[a]AN, ammonium nitrate; UR, urea.

**, *Significant at 1% and 5% levels, respectively.

Note. The same letter in a given column indicates nonsignificant differences at the 5% level by Tukey test.

Molybdenum and Ni contents in roots and shoots are shown in Table 6. There was no effect of N sources on shoot Mo concentration, but Ni supply reduced root Mo concentration in ammonium nitrate–grown plants. On the other hand, N sources influenced Ni concentration, with ammonium nitrate–grown plants showing more Ni both in roots and shoots. Gerendás, Zhu, and Sattlemacher (1998) cultivated rice plants in nutrient solution with NH_4NO_3 or urea and also found greater shoot Ni concentration in ammonium nitrate–supplied plants. The greatest Ni concentration in ammonium nitrate–grown plants does not relate to greatest urease activity (Table 5). It is possible that this Ni increase is related to luxury consumption of Ni, because a dilution effect was not noticed. A relationship between shoot Mo concentration and NR activity was not observed (Table 5).

Molybdenum and Ni concentrations reported in the literature show a great range as a consequence of plant species and environmental growth conditions, which makes comparisons with our data difficult. At the tillering stage, Mo concentrations ranging from 0.5 to $2.0\,mg\,kg^{-1}$ are considered adequate in rice (Fageria 1984). In general, toxic Mo concentrations range between 10 and $50\,mg\,kg^{-1}$ (Kabata-Pendias and Pendias 2001). Therefore, our results for Mo and Ni concentrations cannot be considered toxic or excessive. High Ni doses can cause reduction of Mo concentration in barley and citrus, suggesting a possible antagonism between these two micronutrients (Sato 1969; Brune and Dietz 1995), although this behavior was not verified in the present study. In red clover cultivated in pot soil (Elmosly and Abdel-Sabour 1997), shoot Ni concentrations ranged from 0.5 to $1.1\,mg\,kg^{-1}$ in non-Ni-fertilized plants and from 7.5 to $14.0\,mg\,kg^{-1}$ in Ni-fertilized plants.

According to these authors, there was growth reduction at Ni concentration up to 7.5, 14.0, and 5.3 mg kg $^{-1}$, respectively, on alluvial soils with pH of 8.2 (silt loam), 7.9 (sandy loam), and 7.6 (sandy).

Critical levels for Ni in barley are between 11 and 19 mg kg^{-1} (McIlveen and Negusanti 1994), whereas toxic concentrations vary among species, reaching a maximum of 332 mg kg^{-1} in celery plants. Gupta, Ram-Kala, and Gupta (1996) analyzed several plant species grown in Ni-fertilized soil and found concentrations equivalent to 12, 11, and 21 mg kg^{-1} to be adequate for wheat, barley, and oat, respectively, and concentrations of 19–25, 18–26, and 25–50 mg kg^{-1} to be toxic for these plants. In general, Ni concentrations ranging from 0.05 to 5.0 mg kg $^{-1}$ are considered satisfactory for plant growth and excessive or toxic Ni concentrations can range from 25 to 50 mg kg $^{-1}$ (Malavolta and Moraes 2007). According to Kabata-Pendias and Pendias (2001), Ni concentrations ranging from 1 to 10 mg kg $^{-1}$ are acceptable to most cultivated plants. Kevresan et al. (2001) found toxic Mo and Ni concentrations in shoots of pea plants to be more than 357 to 813 mg kg $^{-1}$, respectively. In roots, the corresponding values were 480 and 2,262 mg kg $^{-1}$, respectively.

CONCLUSIONS

Molybdenum and Ni effects in rice growth depend on the N source. It is likely that urease activity is reduced as a consequence of both Mo and Ni omission. We hypothesize that an indirect relationship between Mo and Ni takes place in plant nutrition, perhaps by stimulating chlorophyll production and net photosynthesis rate.

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