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Molybdenum, nickel and nitrogen sources on the mineral nutrition 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 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 nitrogen (N), in vitro activities of urease and nitrate reductase (NR), Mo and Ni concentration. In urea-grown plants, Mo and Ni addition increased yield of dry matter. Regardless of the nitrogen 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, independently of N source. Nitrate reductase activity increased in nutrient solutions without Mo, although NO3--N increased. There was not a consistent variation in total N concentration. Molybdenum and Ni concentration in roots and shoots were influenced by their supply in the nutrient solution. Mo concentration was not influenced by N sources, whereas Ni content in both root and shoots was higher 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: Plant nutrition; micronutrients, urease; nitrate reductase; chlorophyll; photosynthesis.

Introduction

Essentiality of molybdenum (Mo) in higher plants was established by Arnon and Stout [1]. Mo is known as a constituent of enzymes such as nitrate reductase (EC 1.6.6.1) that reduces nitrate to nitrite, and the enzyme nitrogenase (EC 1.18.6.1), that reduces molecular nitrogen to ammonia in all nitrogen-fixing organisms [2, 3, 4].

Nickel (Ni) meets the direct [5] and indirect [6] criteria of essentiality. Urease (EC 3.5.1.5) is a ubiquitous metalloenzyme containing nickel, which splits urea hydrolytically into ammonia (NH_3) and carbon dioxide (CO_2). Ammonia ions released by urea hydrolysis are incorporated into glutamate [7]. Wood et al. [8] and Ruter [9] diagnosed Ni deficiency under field conditions, in the United States, in pecan (*Carya illinoinensis*) and river birch (*Betula nigra*), respectively. Leaf symptoms are characterized by dark spots and an anatomical deformation causing leaf rounding, known as "Mouse-ear" [8].

Investigations on both nickel and urease and on molybdenum and nitrate reductase are plentiful [7, 10, 11, 12]. However, there are few works which relate these two micronutrients, direct or implicitly, with each one of these two enzymes.

The aims of the current work were to measure the effect of molybdenum and nickel on dry matter yield of rice plants supplied with two nitrogen (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 concentration in roots and shoots.

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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 sulphate ($CaSO_4.2H_2O$). Seedlings were transfered to 40-L plastic trays with a wooden perforated cover when they reached 5-cm high, and fixed with plastic foam around the bottom part of their culms. Plants were grown in aerated one-fifth strength Johnson's solution [13]. After two weeks, two plants each were put in 2-L plastic pots containing full strength nutrient solution (Table 1), modified from Gerendás et al. [7] and Epstein and Bloom [3]. 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. Treatments consisted of ammonium nitrate or urea as nitrogen source plus molybdenum and/or nickel: AN+Mo+Ni, AN+Mo–Ni, AN–Mo+Ni, UR+Mo+Ni, UR+Mo–Ni, and UR–Mo+Ni. Six replicates in a completely randomized design were used.

Five weeks after start of treatments, two middle leaves from two plants in each treatment were collected to assay urease activity according to method described by Hogan et al. [14], NH_3 being determined as suggested by McCullough [15]. One week later, new leaf samples were taken using the same procedure to assay NR activity, according to a simplified technique [10].

Nine weeks after start of treatments the following determinations were made: Leaf sampling for chlorophyll analysis was carried out as described above, and analyzed according to Arnon [1]. 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 IRGA (Infrared Gas Analyzer) Li-COR 6400 model, with 1600 µmol photons m-2 s-1. Shoots and roots were harvested, rinsed with distilled water, 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 et al. [16]. Soluble NO_3^--N in shoots was determined as described by Bray [17].

Statistical analyses were performed using Statistical Analysis System (SAS) software for Windows 6.11 [18]. Analysis of variance (F-Test) were employed to evaluate significance of treatments. Tukey's test was used for means separation.

Resultados e Discussão

Mo and Ni caused different effects on plant growth depending on nitrogen sources (Table 2). Regarding N sources, dry matter yield was higher in urea-grown plants treated with Mo and Ni, likely because ammonia ions generated by hydrolysis of urea passively taken up by roots through 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 [19].

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 2).

Chlorophyll index when indirectly evaluated (SPAD units) was not affected by either nitrogen sources or Mo-deprivation; on the other hand, it was influenced by Ni-deprivation (Table 3). Data on total chlorophyll measurements presented highest values in urea-grown plants, which does not agree with the indirect measurement; chlorophyll concentration was low in treatments with both Mo and Ni deprivation, independently of N sources. Similar studies in the literature regarding relationship among N sources, Ni and Mo on plant nutrition were not found. Usually, information about Ni effects is related to urea as a N source.

Gerendás and Sattelmacher [12] showed that in nutrient solution containing urea without Ni, there was reduction of chlorophyll concentration in rye, wheat, soybean, rape, zucchini and sunflower.

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 [20]. However, in the present work, Mo deprivation did not lead to a chlorosis and net photosynthesis rate was not affected in either N treatment. Mo or Ni deprivations seemed to reduce net photosynthesis rate (Table 3).

In general, there was no clear effect of N source in urease activity and total N (Data not showed). Favorable Ni effect was expected and indeed observed for both N sources. In our work, Mo and Ni together led to an increased urease activity greater than each element supplied separately.

Nitrate reductase activity was influenced by both N sources, and it was higher in ammonium nitrate-grown plants (Data not showed). Nitrate ions induce NR activation, which needs Mo for its activity.

Nitrate content was influenced by both N sources (Data not showed). Mo-deprived plants growing with NH_4NO_3 plus Ni had their nitrate content increased, although nitrate reductase activity increased significantly.

There was no effect of N sources on shoot Mo concentration, but Ni supply reduced root Mo concentration in ammonium nitrate-grown plants (Table 4). On the other hand, N sources influenced Ni concentration, with ammonium nitrate-grown plants showing higher Ni both in roots and shoots.

Our results for Mo and Ni concentrations can not be considered toxic or excessive. Data 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 tillering stage Mo concentrations ranging from 0.5 to 2.0 mg kg^{-1} are considered adequate in rice [21]. In general, toxic Mo concentrations range between 10 and 50 mg kg^{-1} [22]. 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} [23].

Conclusions

Molybdenum and nickel effects in rice growth depend on the nitrogen source. It is likely that urease activity is reduced as a consequence of both molybdenum and nickel 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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Table 1. Composition of the nutrient solutions[a].

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

[a]Modified from Gerendás et al. [7], and Epstein and Bloom [3]; Mo and Ni were omitted according to each treatment; NH_4NO_3: ammonium nitrate (AN).

Table 2. Dry matter yield of rice plants.

Treatments[a]	Parts of plant		
	Root	Shoot	Total
	g per pot		
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	*	**	**
C.V., %	4.9	1.9	2.3

[a]AN: ammonium nitrate, UR: urea. *, ** significant at 5 and 1% level, respectively. Same letter in a given column indicate nonsignificant differences at the 5% level by Tukey test.

Table 3. Effect of N sources, Mo and Ni on SPAD units, chlorophyll, and net photosynthesis rate.

Treatements[a]	SPAD	Chlorophyll a	Chlorophyll b	Total chlorophyll	Net photosynthesis rate
	units	µg mL^{-1}			µmol 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	**	**	**	**	**
C.V., %	3.2	9.3	21.5	8.2	10.6

[a]AN: ammonium nitrate, UR: urea. ** significant at 1% level. Same letter in a given column indicate nonsignificant differences at the 5% level by Tukey test.

Table 4. Treatment effects on Mo and Ni concentrations.

Treatments[a]	Molybdenum		Nickel	
	Root	Shoot	Root	Shoot
	mg kg^{-1}			
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	**	**	**	**
C.V., %	17.7	16.9	18.7	17.7

[a]AN: ammonium nitrate, UR: urea. **, * significant at 1% e 5% level, respectively. Same letter in a given column indicate nonsignificant differences at the 5% level by Tukey test.