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Title: Effect of molybdenum treatment on molybdenum concentration and nitrate reduction in maize seedlings

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Abstract: Since 1940 molybdenum has been known as an essential trace element in plant nutrition and physiology. It has a central role in nitrogen metabolism, and its deficiency leads to nitrate accumulation in plants. In this study, we cultivated maize seedlings (Zea mays L. cv. Norma SC) in nutrient solution and soil (rhizoboxes) to investigate the effect of molybdenum treatment on the absorption of molybdenum, sulphur and iron. These elements have been previously shown to play important roles in nitrate reduction, because they are necessary for the function of the nitrate reductase enzyme. We also investigated the relationship between molybdenum treatments and different nitrogen forms in maize. Molybdenum treatments were 0, 0.96, 9.6 and 96 μg kg-1 in the nutrition solution experiments, and 0, 30, 90, 270 mg kg-1 in the rhizobox experiments.

On the basis of our results, the increased Mo level produced higher plant available Mo concentration in nutrient solution and in soil, which resulted increased concentration of Mo in shoots and roots of maize seedlings.

In addition it was observed that maize seedlings accumulated more molybdenum in their roots than in their shoots at all treatments. In contrast, molybdenum treatments did not affect significantly either iron or sulphur concentrations in the plant, even if these elements (Mo, S and Fe) play alike important roles in nitrogen metabolism. Furthermore, the physiological molybdenum level (1x Mo = 0.01 μM) reduced NO3-N and enhanced the NH4-N concentrations in seedlings, suggesting that nitrate reduction was more intense under a well-balanced molybdenum supply.

COVER LETTER

Motivation for the work and summarise the findings:

Molybdenum has a central role in nitrogen metabolism, and its deficiency leads to nitrate accumulation in plants, so in this study, we cultivated maize seedlings in nutrient solution and soil (rhizoboxes) to investigate the effect of molybdenum treatment on the absorption of molybdenum, sulphur and iron. These elements have been previously shown to play important roles in nitrate reduction, because they are necessary for the function of the nitrate reductase enzyme, moreover we also investigated the relationship between molybdenum treatments and different nitrogen forms in these plants. Our findings indicate that there is a strong correlation between molybdenum levels and nitrate reduction in maize seedlings, and nitrate content of these plants can be effectively reduced by supplying their physiological molybdenum demand.

***Response to Reviewers**

Dear Reviewer,

We accepted all instructions and modified our manuscript. Hereby I submit a smooth and final copy of manuscript in black fonts for further processing.

Yours sincerely,

Prof. Dr. Béla Kovács

Effect of molybdenum treatment on molybdenum concentration and nitrate reduction in maize seedlings

Highlights

- Application of molybdenum (Mo) improved the absorption of Mo in shoots and roots.
- Maize seedlings accumulated more molybdenum in their roots than in their shoots.
- The absence of Mo supply resulted the accumulation of nitrate in maize seedlings.
- Physiological Mo level (0.01 µM) reduced the NO_3-N and enhanced the NH_4-N contents.

Effect of molybdenum treatment on molybdenum concentration and nitrate reduction in maize seedlings

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Abstract

Since 1940 molybdenum has been known as an essential trace element in plant nutrition and physiology. It has a central role in nitrogen metabolism, and its deficiency leads to nitrate accumulation in plants. In this study, we cultivated maize seedlings (*Zea mays* L. cv. Norma SC) in nutrient solution and soil (rhizoboxes) to investigate the effect of molybdenum treatment on the absorption of molybdenum, sulphur and iron. These elements have been previously shown to play important roles in nitrate reduction, because they are necessary for the function of the nitrate reductase enzyme. We also investigated the relationship between molybdenum treatments and different nitrogen forms in maize. Molybdenum treatments were 0, 0.96, 9.6 and 96 μg kg^{-1} in the nutrition solution experiments, and 0, 30, 90, 270 mg kg^{-1} in the rhizobox experiments.

On the basis of our results, the increased Mo level produced higher plant available Mo concentration in nutrient solution and in soil, which resulted increased concentration of Mo in shoots and roots of maize seedlings.
In addition it was observed that maize seedlings accumulated more molybdenum in their roots than in their shoots at all treatments. In contrast, molybdenum treatments did not affect significantly either iron or sulphur concentrations in the plant, even if these elements (Mo, S and Fe) play alike important roles in nitrogen metabolism. Furthermore, the physiological molybdenum level (1x Mo = 0.01 μM) reduced NO_3-N and enhanced the NH_4-N concentrations in seedlings, suggesting that nitrate reduction was more intense under a well-balanced molybdenum supply.

Keywords: molybdenum, nitrate accumulation, nitrate reduction, nitrogen metabolism

1. Introduction

Molybdenum, a rare transition element, has for a long time been recognized as an essential micronutrient for higher plants (Bortels, 1930; Arnon and Stout, 1939). Though required only in small amounts, it has a large role within the plant system. As with other metals required for plant growth, molybdenum has been used by specific plant enzymes in the process of reduction and oxidative reactions (Mendel and Hänsch, 2002).

Molybdenum itself is not biologically active. It is, however, an integral part of an organic pterin complex called the molybdenum co-factor (Moco). Moco binds to the molybdoenzymes (enzymes which require molybdenum) found in most higher plants (Zimmer and Mendel, 1999; Kaiser et al., 2005; Mendel and Kruse, 2012; Bittner, 2014).

Molybdenum has been found as a cofactor in nitrate reductase, nitrogenase, xanthine oxidase and sulfite oxidase. In these enzymes molybdenum has both structural and catalytic functions as well as direct involvement in redox reactions. It has been found to play a vital role in the nitrogen metabolism of plants, including the processes of nitrogen fixation, nitrate reduction, and the transportation of nitrogen compounds (Srivastava, 1997; Mendel and Schwarz, 1999).

An essential aspect of molybdenum's crucial role as a plant nutrient is the part it plays in NO_3 reduction as a co-factor to nitrate reductase (NR) (Hamlin, 2007). Nitrate reductase is a homodimeric protein, as are other molybdenum enzymes in plants. Each identical subunit is able to operate in an independent way in nitrate reduction (Marschner, 1995), and each is made up of three functional domains: the N-terminal domain associated with a molybdenum

cofactor (Moco), the central heme domain (cytochrome b557), and the C-terminal FAD domain (Mendel and Schwarz, 1999). It acts as a catalyst in the first step of the NO_3^- reduction pathway, yielding NO_2^-, which in turn is further reduced to NH_4^+ (Campbell, 2001; Morozkina and Zvyagilskaya, 2007).

The induction of nitrate reductase in plants requires both nitrate and molybdenum: if either nutrient is deficient, the enzyme is either non-existent or less active. In deficient plants, the induction of enzyme activity by molybdenum has been found to be much faster than the induction of nitrate reductase activity by nitrate (Hamlin, 2007).

In fact, many studies have shown that application of Mo improves the absorption of Mo, the transformation of NO_3^--N to NH_4^+-N as well as free nitrogen to albuminous nitrogen in seeds, and it increases the nitrate reductase (Li-Ping et al., 2007).

Liu and Yang (1999) investigated the relationship between molybdenum and the nitrogen metabolism of three soybean varieties in each stage of growth. Five levels of molybdenum were studied. An increase in both nitrate reductase activity and total N content were found in leaves and a reduction of NO_3-N content was found with molybdenum application. In addition to this, according to Vieira et al. (1998) experiment, molybdenum foliar spray (40 g ha^{-1} of Mo) at 25 days after plant emergence significantly aided nitrate reductase activities, producing an increase of the total nitrogen accumulated in the plant shoots of common beans.

The nitrogen metabolism has been found to be affected by Mo-treatment in several studies: an increased nitrate reductase (NR), and a decreased NO_3^- content of the leaves was observed by Salcheva et al. (1979), an increase of Moco leaves and dry seeds was recorded by Vunkova-Radeva et al. (1988). This suggests that molybdenum directly affects the NR molecule because it contains a Moco pterine domain. This domain is common for all Mo-enzymes with the exception of nitrogenase (Campbell, 1988; Pelsy and Caboche, 1992). Since NR is the key enzyme in inorganic nitrogen assimilation, it may be assumed that the cryoprotective effect of molybdenum on NR activity is reflected in the nitrate assimilatory pathway.

On the other hand, Calonego et al. (2010) discovered that the absence of Mo foliar supply made for the accumulation of nitrate in common bean leaves: this as a result of the increased nitrogen availability in the soil, which indicated the inefficiency of nitrogen assimilation of plants in the absence of Mo. Srivastava (1997) came to a similar conclusion, stating that in molybdenum-deficient plants, nitrate-reductase activity is often reduced, which results in the buildup of a high concentration of NO_3^-.

Furthermore, a higher concentration of total nitrogen was recorded in Mo-deficient winter wheat, where Mo was seen to be the essential element for

nitrate reduction (Yu et al., 2010). Mo deficiency, therefore, resulted in an imbalanced nitrogen metabolism, evidenced by a much higher concentration of total nitrogen and nitrate (Hu et al., 2002; Yu et al., 2006). Thus, nitrogen metabolism was seen to be affected by the Mo status of a plant.

Nitrate accumulation in crop plants due to molybdenum deficiency might have serious consequences for human health. Excess nitrate consumption can increase the risk of cancer in adults and causes serious health damage especially in children. It can cause methaemoglobinaemia, a type of rare but potentially fatal haemoglobinopathy (Sanchez-Echaniz et al., 2001). In nitrate-induced methaemoglobinaemia, dietary nitrate is reduced to nitrite in the stomach, and the absorbed nitrite then converts haemoglobin to methaemoglobin in red blood cells by oxidising the heme Fe^{2+} ion to Fe^{3+} (Bradberry, 2012; Wright et al., 1999). This oxidation prevents methaemoglobin from binding oxygen and compromises oxygen delivery to peripheral tissues. Methaemoglobinaemia underlines the importance of optimal nitrate reduction in crop plants, which can be achieved by providing optimal molybdenum nutrition.

The present investigation deals with the treatment of maize seedlings with molybdenum and the effect of this treatment on element contents (molybdenum, iron, sulphur) and on endogenous concentrations of nitrate-, nitrite- and ammonium-nitrogen in shoots and roots. The main aim of the present study was to prove under laboratory circumstances that have a close relation between molybdenum supply and nitrate reduction: nitrate content of plants can be reduced by supporting their physiological Mo demand. To ensure adequate supply of Mo, nitrate content in the leaf and root vegetables can be reduced, to produce and consume healthier raw materials and foods, which are essential for human health aspects.

2. Materials and methods

2.1 General plant propagation

A maize (*Zea mays* L. cv Norma SC) as a monocotyledon was chosen for our research to study the contents of various elements (Mo, S, Fe) and nitrogen species in roots and shoots separately (*Figure 1-2*). Disinfected maize seeds were geotropically germinated between wet fluted filter papers at 22°C. Seedlings with 2.5-3.0 cm coleoptiles were placed into aerated nutrient solutions or rhizoboxes depending on experimental settings. Maize plants were grown in a climate room under strictly regulated environmental conditions. Relative humidity was maintained between 65-75%, light/dark cycle was 16/8 hrs with a respective 25/20ºC temperature periodicity, and light intensity was kept at a constant 220 $\mu E\ m^{-2}\ s^{-1}$ during daytime.

2.2 Plant growth in nutrient solution

The nutrient solution used for plant growth had the following composition: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 0.1 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$. Iron was supplied in the form of 10^{-4}M Fe-EDTA (Cakmak and Marschner, 1990).

Molybdenum was supplemented to the nutrient solution as $(NH_4)_6Mo_7O_{24}$ at four different concentrations as follows: Ø Mo, 1x Mo (0.96 µg kg^{-1}=0.01 µM), 10x Mo, 100x Mo, respectively. Nutrient solution was changed every other day, and evaporated water was replenished regularly. Since nitrate reduction in roots is dependent on respiration, we kept the temperature of the nutrient solution at a constant 20°C. The experiment ended 9 days after planting, when seedlings had approximately 12-12 cm long shoots and roots, respectively. Experiments were carried out in triplicates.

2.3 Plant growth in soil

Experiments in soil were carried out in rhizoboxes, which allowed us to easily monitor many aspects of root development, including overall growth, circadian rhythm of the growth as well as symptoms of phytotoxicity that might have been caused by increased concentrations of molybdenum. The experiments used calcareous chernozem soil obtained from the Látókép Experimental Station of our university. The parameters of this soil (Table 1) were essentially the same as previously described by Nagy et al. (2010). No additional PNK fertilization was carried out on this soil. Molybdenum was supplemented to the soil as an aqueous solution prepared with distilled water at four different concentrations: 0 (control), 30, 90, 270 mg kg^{-1}.

In order to ensure steady water uptake by plants, wet fluted filter papers were placed at the bottom of rhizoboxes before the soil was added. After planting the seedlings in the soil, the transparent side walls of rhizoboxes were covered with black foil. The plants were geotropically stimulated to force root growth along the transparent wall of the box, thus allowing convenient monitoring of the roots. The mass of rhizoboxes and the length of the roots were measured daily. Evaporated water was also replenished daily.

2.4 Sample preparation and analytical methods

At the end of the experiments, shoots were separated from roots. Plant parts were dried at 85°C until constant weight was achieved, then cooled to room temperature and weighed by an analytical scale (OHAUS). Dried samples (1± 0.01 g) were homogenized and decomposed by HNO_3-H_2O_2 treatment as previously described (Kovács et al., 1996). Briefly, samples were kept in 10 ml concentrated HNO_3 overnight, then heated to 60°C for 45 min in a LABOR

MIM OE 718/A block digestion apparatus. Following the first digestion step, 3 ml 30% H_2O_2 was added to the samples, and digestion was continued at 120^{o}C for another 90 min. After cooling the samples to room temperature, volume was adjusted to 50 ml with deionized water. Samples were then mixed by shaking and filtered through FILTRAK 388 filters.

Element analysis was carried out by inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer OPTIMA 3300 DV) and inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Elemental X7). In addition to molybdenum content, the concentrations of iron and sulfur were also determined since nitrate reductase also requires these elements for its function. Instrument settings and parameters were the same as described previously (Puskás-Preszner and Kovács, 2009).

To analyse the amounts of different nitrogen forms in maize seedlings, 0.1 g of dried and homogenized samples were weighed into centrifuge tubes. Then, 10 ml of 1 M KCl was added, and the test tubes were placed into an ultrasonic shaker for 60 min to release N-forms. NO_3-N, NO_2-N and NH_4-N concentrations of plant samples were determined by a FIAstar 5000 Analyzator.

2.5 Statistical analysis

Experimental data were analyzed by a bivariate general linear model (GLM), which is a combination of variance and linear regression analysis. Independent variables were molybdenum treatments and plant parts, while dependent variables were concentrations of elements in seedlings. R-squared values were calculated to assess how well independent variables predicted dependent variables. Statistical analysis was done by SPSS 22.0.

3. Results

3.1 Plant growth in nutrient solution

Molybdenum treatments in nutrient solutions resulted in only a slight decrease in the dry weights of shoots and roots compared to the control experiment (data not shown). Therefore, molybdenum did not appear to have any toxic effects even at its highest level used in our experiments.

When seedlings were cultivated in molybdenum-free nutrient solutions, molybdenum concentration of the plants was relatively low (Table 2), most likely corresponding to the original molybdenum concentration of the seeds. Molybdenum concentrations increased gradually with increasing molybdenum level in the nutrient solution. At 100x Mo treatment molybdenum concentration increased approximately 8-fold and 11-fold in shoots and roots, respectively, compared to baseline (Ø Mo) levels. Molybdenum supplementation of the

nutrient solution did not cause a significant increase in sulphur concentration of the seedlings. Iron concentration of shoots did not change significantly with increasing molybdenum level, but iron concentration of roots doubled at 1x and 10x molybdenum treatments. The highest (100x Mo) treatment reduced iron concentration in roots to baseline levels (Table 2). For all three elements, concentrations were consistently higher in roots than in shoots. Molybdenum supplementation resulted in comparable changes of these elements in shoots and roots, except for iron that appeared to be preferentially accumulated in roots. These data suggest that the three elements were present at optimal concentrations in leaves, and transport of these elements from roots was not activated at higher molybdenum levels.

Since molybdenum is important for nitrogen assimilation, we hypothesized that molybdenum levels significantly affected nitrate, nitrite an ammonium concentrations in maize plants. This effect is expected to be more prominent in seedlings which require increased protein synthesis and enzyme activities to support intensive vegetative growth. In order to test this hypothesis, we set out to investigate nitrate, nitrite and ammonium concentration in maize seedlings under different levels of molybdenum supplementation. Nitrate reduction can take place in two plant parts. In leaves, the needed reducing power is supplied by the light reaction of photosynthesis, while in roots, the active hydrogen ions are derived from respiration. The observed higher molybdenum and iron concentrations of roots (compare Table 2) suggests that nitrogen assimilation might be more intense in roots than in shoots.

Maize seedlings grown on molybdenum deficient nutrient solution have only endogenous molybdenum reserves, which would allow a baseline nitrogen reductase activity. Accordingly, NO_3-N concentration in shoots was relatively high, and NH_4-N concentration was relatively low under this condition (Table 3). When physiological concentration (1x Mo = 0.01 µM) of molybdenum was provided, NO_3-N concentration of shoots decreased, while NH_4-N concentration was much higher compared to the baseline (Ø Mo) condition. The 10x Mo treatment resulted in significant increase in the NO_3-N concentration, but the NH_4-N concentration also remained high. This seemingly counterintuitive observation can be explained by the enhanced uptake of nitrate. If NO_3-N is the only nitrogen source in the medium, a high affinity nitrate transporter system is activated, and this process is dependent on molybdenum. The resulting high concentration of nitrate in the plant in turn induces nitrate reductase, which leads to elevated NH_4-N concentrations. However, increasing the molybdenum level even further (100x Mo) decreased NH_4-N concentration of shoots to baseline level.

In roots, NO_3-N and NH_4-N concentrations were higher than in shoots even in molybdenum-free nutrient solution (Table 3). This observation could

indicate that in addition to the high affinity nitrate uptake mechanism, considerable amounts of nitrate were taken up by alternative mechanisms, such as ATP-dependent symport through the plasma membrane of root hair cells. The high NH_4-N concentrations in roots could be the consequence of higher molybdenum concentrations in roots compared to shoots. In fact, the rate of nitrogen assimilation (estimated from NH_4-N/NO_3-N ratios) was approximately 2-3 times higher in roots than in shoots regardless of the molybdenum concentration in the nutrient solution, and this difference correlated well with the difference in the molybdenum concentration of the plant parts (Table 2). Therefore, roots appear to be more active in nitrogen assimilation than shoots in maize seedlings.

Statistical analysis using the general linear model (Table 4 and 5) indicated that molybdenum treatment and plant part had 92-99% effect on the Mo, S and Fe concentrations of maize seedlings. Molybdenum treatment had a greater effect on molybdenum concentration than plant part had. For sulphur and iron, the effect of plant part was one order of magnitude higher than that of molybdenum treatment. Molybdenum treatment had no statistically significant effect on NO_3-N and NO_2-N concentrations of the seedlings, which could be the consequence of the relatively large standard deviation within the groups. The concentrations of these two nitrogen forms were primarily determined by the plant part. On the other hand, the concentration of NH_4-N was influenced by plant part and molybdenum treatment together, which accounted for 95.7% of this nitrogen form, although the effect of plant part was somewhat more dominant. We have to note, that during the statistical analysis of the NO_2-N concentration, the variance analysis model did not show significance (R^2 = 0.411).

3.2 Plant growth in soil

Dry weights of the shoot and root were 0.303 g and 0.244 g, respectively, when the maize seedling was grown in rhizobox without molybdenum treatment. Supplementation of the soil with 30 mg kg^{-1} molybdenum caused a significant increase in the dry weights of the shoot (175%) and root (202%). However, further increase in the soil's molybdenum level decreased the dry weights of both plant parts. The 90 and 270 mg kg^{-1} molybdenum treatments also decreased the dry weight ratio of shoots and roots compared to the control experiment, suggesting that these treatments inhibited the growth of the shoots more than the growth of the roots.

Maize seedling grown in rhizobox took up only a small amount of molybdenum from control soil. Molybdenum concentrations of the root and shoot were very low under these conditions, but increased gradually when the soil was supplemented with increasing levels of molybdenum (Figure 3).

Although we used only one seedling per molybdenum treatment in the experiment, the observed trend was very similar to that of the nutrient solution experiments (Table 2), supporting the validity of our results.

Molybdenum treatment also affected the concentrations of different nitrogen forms in maize seedlings grown in rhizoboxes. In shoots, NO_3-N concentration varied and was highest when the soil had the lowest and highest molybdenum content. NH_4-N concentration rose with increasing molybdenum level of the soil, indicating definite connection between molybdenum treatment and nitrogen assimilation in maize shoots. In roots, molybdenum treatments increased NH_4-N and reduced NO_3-N concentration, which suggested intensive nitrate reductase activity in this plant part (Table 6).

We have to note that both shoots and roots demonstrated similar ammonia concentrations in our rhizobox experiments, implying that nitrate reductase activities were comparable in these plant parts (Table 6).

4. Discussion

In this study we have found that molybdenum nutrition significantly affected molybdenum concentrations and the concentrations of different nitrogen forms in maize seedlings when they were cultivated either in nutrient solution or soil.

Seedlings grown in molybdenum-free solution contained very small amounts of molybdenum, most likely reflecting the molybdenum reserves derived from the seeds. Molybdenum treatments significantly increased molybdenum concentrations in both the shoots and roots of the seedlings, with roots having consistently higher concentrations than shoots at all treatments (including the molybdenum-free conditions). Molybdenum treatments did not affect either iron or sulphur concentrations significantly, indicating that molybdenum was not necessary for the absorption of these elements. Maize seedlings grown in rhizoboxes showed similar absorption trends of molybdenum than those grown in nutrient solution. Although molybdenum-free conditions could not be established in these experiments, due to endogenous amounts of this element in the soil, molybdenum uptake from the control soil was relatively low. Similar to the nutrition solution experiments, seedlings grown in rhizoboxes accumulated more molybdenum in their roots than in their shoots.

Kádár (1995) obtained similar data in his microelement-load experiments in calcareous chernozem soil obtained from Nagyhörcsök. According to his results, increased molybdenum load caused extremely high molybdenum accumulation in maize, but there were only small differences (with the exception of control soil) between molybdenum concentrations of shoots and roots. The ratios of molybdenum in roots vs shoots at different soil

molybdenum concentration were, 10, 1.30, 1.60 and 1.26, while in our experiments under the same conditions, they were 1.17, 2.98, 2.25 and 2.07, respectively (molybdenum treatments were the same in both sets of experiments). This comparison suggests that molybdenum transport from roots to shoots was somewhat less efficient in our experiments.

The consistently higher molybdenum concentration in roots could indicate that this plant part was more active in nitrogen assimilation, due to the fact that molybdenum is essential for the stability and activity of the nitrate reductase enzyme. Although we did not determine the activity of this enzyme directly, we assessed nitrogen assimilation in maize seedlings by measuring the concentrations of different nitrogen forms after molybdenum treatments. As expected, physiological molybdenum treatment (1x Mo = 0.01 μM) reduced NO_3-N and increased the NH_4-N concentrations in seedlings, suggesting that nitrate reduction was more intense under a well-balanced molybdenum supply. These data clearly suggest that there is a close relationship among molybdenum levels, nitrate absorption, nitrate reduction and overall nitrogen assimilation. Large proportion of the absorbed nitrate is reduced to ammonia which links to glutamate and subsequently, through transamination reactions, is utilized for the synthesis of other amino acids and proteins. Our measured ammonia concentrations most likely do not reflect the actual nitrate reductase activity due to fixation of ammonia by biochemical reactions mentioned above. Ammonia is cytotoxic, therefore its concentration must be tightly controlled. It acts as an uncoupling agent in thylakoid membranes and causes the depolarization of these membranes without the synthesis of ATP. Since our plants did not show toxic symptoms, we assume that sufficient amount of glutamate was available to fix ammonia. In addition, the citric acid cycle that provides the carbon skeleton of glutamate must be also active and efficient to support the glutamate demand of the plant. Therefore, our data suggest that molybdenum has an essential role in the intensive overall metabolism of maize seedlings. We have to note, however, that molybdenum treatment increased plant NH_4-N concentrations only up to the 10x Mo treatment. The concentration of this nitrogen form significantly dropped after 100x Mo treatments, implying that this molybdenum concentration might have inhibited nitrate reductase activity. These results differ from those of Kádár et al. (2000) who found a definite increase in shoot NO_3-N concentration after ammonium paramolybdenate load. They hypothesized, that the NH_4-N was nitrified by the end of their experiment, which contributed to the elevated concentrations on nitrate in shoots.

It was proved by our experiments, when Mo was added to nutrient solution the free nitrate concentration was decreased. In natural conditions, when the Mo is in low amount in soil, the nitrate accumulation can be effectively

minimized in the plants. Molybdenum has a physiological importance as the part of enzymes having outstanding role in nitrogen metabolism. Whereas the nitrogen is in reduced form in the organic chains, the reduction of nitrate needs lots of energy, which means high electric potential. Mo plays a role in the electron transport chain due to its different oxidation states, therefore it takes part in the nitrogen metabolism, moreover Mo can not be substituted by any other element in this physiological process.

Overall, our results demonstrate a correlation between molybdenum nutrition and nitrogen assimilation. In the absence of molybdenum, nitrate reduction slows down, which results in nitrate accumulation in plants. On the other hand, adequate molybdenum supply in soils can ensure a reduced nitrate content of leafy and root vegetables and in general, all fresh-cut agricultural products. However, it is important to note, that nitrate content of fresh vegetables is influenced by other environmental factors such as light conditions and the length of the daylight. Therefore, plants grown in greenhouses might have nitrate concentrations a magnitude higher than those grown in fields.

When planning molybdenum enrichment of soils, the potentially hazardous consequences of this treatment should also be taken into account. Although excessive molybdenum accumulation does not lead to metabolic problems or phytotoxic effects in plants, the same high molybdenum concentration can cause molybdenosis in animals. The risk of this disease significantly increases when molybdenum content is over 5 mg kg^{-1}, and this detrimental effect must be considered when feeding farm animals with plants grown on molybdenum enriched soils.

Contribution

Conceived and designed the experiments: Béla Kovács, Anita Puskás-Preszner, László Lévai and Éva Bódi. Performed the experiments: Béla Kovács, Anita Puskás-Preszner, László Lévai and Éva Bódi. Analyzed the data: Béla Kovács, László Huzsvai and Éva Bódi. Contributed reagents/materials/analysis tools: László Lévai and Béla Kovács. Wrote the paper: Béla Kovács, László Huzsvai and Éva Bódi.

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Effect of molybdenum treatment on maize seedlings

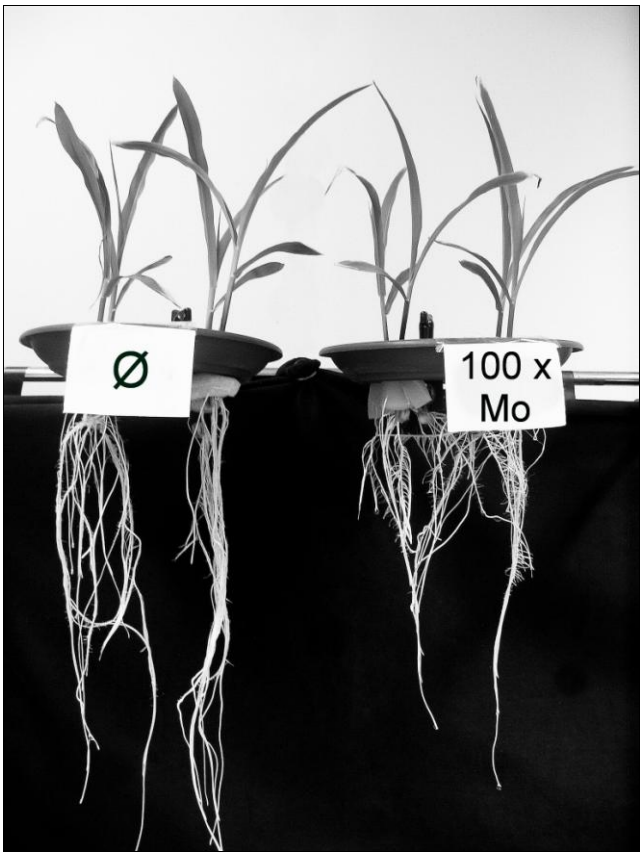


Fig. 1 Maize seedlings grown in nutrient solution (Ø=0 mg dm^{-3} Mo, 100x Mo=1 µM $(NH_4)_6Mo_7O_{24}$)

Effect of molybdenum treatment on maize seedlings

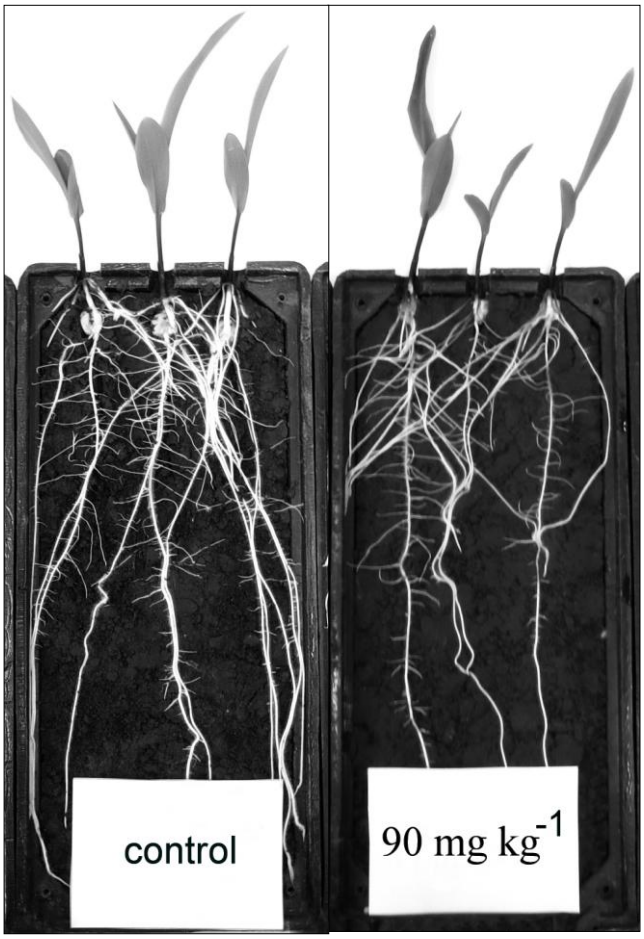
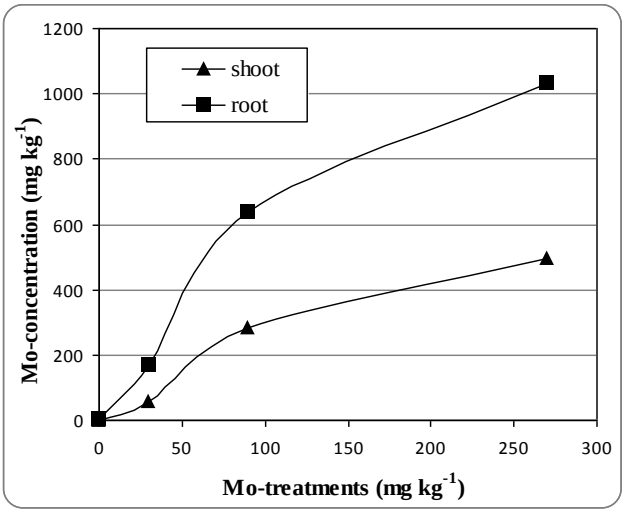


Fig. 2 Maize seedlings grown in rhizoboxes (control, 90 mg kg^{-1} Mo)

Effect of molybdenum treatment on maize seedlings

548



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550

551 **Fig. 3** Molybdenum concentration (mg kg^{-1} dry weight) of shoots and roots of maize seedlings

552 grown in rhizoboxes

553

554

Table 1 Parameters of soil applied in the experiments carried out in rhizoboxes

Depth	0-0.3 m
pH (KCl)	5.71
pH (H_2O)	6.58
Soil texture category	loamy clay
Total water-soluble salt	0.015 %
$CaCO_3$	0.202 %
Humus	3.54 %
KCl-soluble NO_3-N+NO_2-N	8.04
AL-soluble P_2O_5	199 mg kg^{-1}
AL-soluble K_2O	451 mg kg^{-1}
AL-soluble Na	332 mg kg^{-1}
KCl-soluble Mg	176 mg kg^{-1}
KCl-soluble SO_4^{2-}-S	6.04 mg kg^{-1}
KCl-EDTA-soluble Cu	5.79 mg kg^{-1}
KCl-EDTA-soluble Zn	7.9 mg kg^{-1}
KCl-EDTA-soluble Mn	262 mg kg^{-1}

574 **Table 2** Mo, S and Fe concentration (mg kg^{-1} dry weight) of shoots and roots of maize
575 seedlings grown in nutrient solution in case of Ø Mo (molybdenum free solution), 1x Mo (0.01
576 μM), 10x Mo and 100x Mo treatments
577

Plant part	Mo-treatment	Mo	S	Fe
Shoot	**Ø Mo**	2.52±0.17	2049±148	60.9±6.4
	1x Mo	3.06±0.08	2152±210	57.0±10.3
	10x Mo	6.08±0.40	2359±84	58.3±1.6
	100x Mo	20.6±0.50	2367±142	72.2±25.5
Root	**Ø Mo**	5.78±0.12	4644±145	167±14
	1x Mo	7.89±0.46	4970±425	303±64.5
	10x Mo	17.9±0.1	5197±634	331±38
	100x Mo	66.5±1.4	5076±439	187±26

578

579 **Table 3** Ammonium- (NH_4-N), nitrite- (NO_2-N), nitrate-nitrogen (NO_3-N) concentration (mg
580 kg^{-1} dry weight) of shoots and roots of maize seedlings grown in nutrient solution in case of Ø
581 Mo (molybdenum free solution), 1x Mo (0.01 µM), 10x Mo and 100x Mo treatments
582

Plant part	Mo-treatment	NH_4-N	NO_2-N	NO_3-N
Shoot	Ø Mo	111±55	0.59±0.16	2153±553
	1x Mo	199±142	1.06±0.29	1730±78
	10x Mo	160±39	1.42±1.20	3939±1427
	100x Mo	124±2	0.73±0.23	1772±1086
Root	Ø Mo	703±84	2.28±0.52	4407±180
	1x Mo	914±23	2.27±0.89	4611±221
	10x Mo	534±6	2.01±0.38	4934±820
	100x Mo	1075±125	2.00±1.73	5055±1109±

583

584 **Table 4** Variance analysis of maize seedlings grown in nutrient solution, dependent variables is
585 the concentration of Mo, S and Fe (mg kg^{-1} dry weight)
586

Dependent Variable	Source	df	Mean Square
Mo	Corrected Model	7	1368.147***
	Intercept	1	6369.618***
	Plant parts	1	1624.619***
	Mo-treatments	3	2051.483***
	Plant parts * Mo-treatments	3	599.320***
	Error	16	0.318
	Total	24	
	Corrected Total	23	
S	Corrected Model	7	6538590.167***
	Intercept	1	311371288.167***
	Plant parts	1	45040120.167***
	Mo-treatments	3	224434.500
	Plant parts * Mo-treatments	3	18902.500
	Error	16	111250.667
	Total	24	
	Corrected Total	23	
Fe	Corrected Model	7	38061.528***
	Intercept	1	573566.002***
	Plant parts	1	205313.002***
	Mo-treatments	3	9069.116***
	Plant parts * Mo-treatments	3	11303.449***
	Error	16	910.100
	Total	24	
	Corrected Total	23	

587

588 **Table 5** Variance analysis of maize seedlings grown in nutrient solution, dependent variables is
589 the concentration of ammonium-nitrogen (NH_4-N), nitrite- (NO_2-N) and nitrate- (NO_3-N) of
590 maize (mg kg^{-1} dry weight)
591

Dependent Variable	Source	df	Mean Square
NH_4-N	Corrected Model	7	445086.614***
	Intercept	1	5476796.106***
	Plant parts	1	2596505.005***
	Mo-treatments	3	86111.718***
	Plant parts * Mo-treatments	3	86922.045***
	Error	16	6031.260
	Total	24	
	Corrected Total	23	
NO_2-N	Corrected Model	7	1.411
	Intercept	1	57.645***
	Plant parts	1	8.431**
	Mo-treatments	3	0.174
	Plant parts * Mo-treatments	3	0.308
	Error	16	0.885
	Total	24	
	Corrected Total	23	
NO_3-N	Corrected Model	7	6262698.586***
	Intercept	1	306727900.091***
	Plant parts	1	33228744.261***
	Mo-treatments	3	2037619.581
	Plant parts * Mo-treatments	3	1499095.699
	Error	16	689026.543
	Total	24	
	Corrected Total	23	

592 **Table 6** Ammonium- (NH_4-N), nitrite- (NO_2-N), nitrate-nitrogen (NO_3-N) concentration (mg
593 kg^{-1} dry weight) of shoots and roots of maize seedlings grown in rhizoboxes in case of different
594 molybdenum treatments (mg kg^{-1})
595

Plant part	Mo-treatment	NH_4-N	NO_2-N	NO_3-N
Shoot	control	237	0.424	41.5
	30	331	0.681	0.736
	90	308	0.102	8.44
	270	401	1.51	214
Root	control	280	0.040	35.5
	30	334	0.040	15.8
	90	333	0.459	15.8
	270	200	2.84	134

596

Contribution

Conceived and designed the experiments: Béla Kovács, Anita Puskás-Preszner, László Lévai and Éva Bódi. Performed the experiments: Béla Kovács, Anita Puskás-Preszner, László Lévai and Éva Bódi. Analyzed the data: Béla Kovács, László Huzsvai and Éva Bódi. Contributed reagents/materials/analysis tools: László Lévai and Béla Kovács. Wrote the paper: Béla Kovács, László Huzsvai and Éva Bódi.