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Research article

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, sulfur 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 $\mu g\ kg^{-1}$ 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 sulfur concentrations in the plant, even if these elements (Mo, S and Fe) play alike important roles in nitrogen metabolism. Furthermore, the physiological molybdenum level ($1\times$ Mo $= 0.01\ \mu M$) 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.

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).

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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 hemoglobin to methemoglobin in red blood cells by oxidizing the heme Fe^{2+} ion to Fe^{3+} (Bradberry, 2012; Wright et al., 1999). This oxidation prevents methemoglobin 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, sulfur) 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 (Figs. 1 and 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 and 75%, light/dark cycle was 16/8 h with a respective 25/20 °C

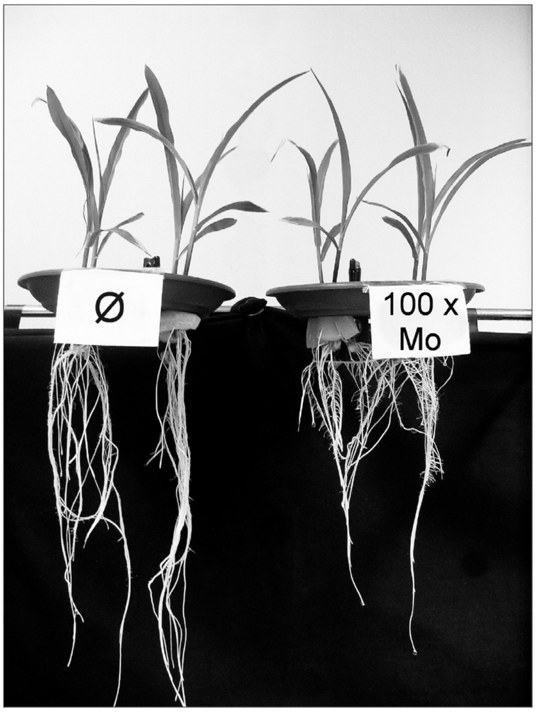

Fig. 1. Maize seedlings grown in nutrient solution (Ø = 0 mg dm^{-3} Mo, 100× Mo = 1 µM $(NH_4)_6Mo_7O_{24}$).