Plant Physiology and Biochemistry 115 (2017) 200–211

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Effect of exogenously applied molybdenum on its absorption and nitrate metabolism in strawberry seedlings

Li Liu [1], Wei Xiao [1], Ling Li, Dong-Mei Li, Dong-Sheng Gao, Cui-ying Zhu*, Xi-Ling Fu**

State Key Laboratory of Crop Biology, College of Horticulture Science and Engineering, Shandong Agricultural University, Taian, China

ARTICLE INFO

Article history:
Received 16 December 2016
Received in revised form
22 March 2017
Accepted 22 March 2017
Available online 23 March 2017

Keywords:
Molybdenum
NUE
Nitrate metabolism
MOT1
Strawberry

ABSTRACT

Molybdenum (Mo)—an essential element of plants—is involved in nitrogen (N) metabolism. Plants tend to accumulate more nitrate and show lower nitrogen use efficiency (NUE) under Mo-deficient conditions. Improving NUE in fruits reduces the negative effect of large applications of chemical fertilizer, but the mechanisms underlying how Mo enhances NUE remain unclear. We cultivated strawberry seedlings sprayed with 0, 67.5, 135, 168.75, or 202.5 g Mo·ha^{-1} in a non-soil culture system. The Mo concentration in every plant tissue analyzed increased gradually as Mo application level rose. Mo application affected iron, copper, and selenium adsorption in roots. Seedlings sprayed with 135 g Mo·ha^{-1} had a higher [^{15}N] shoot:root (S:R) ratio, and ^{15}NUE, and produced higher molybdate transporter type 1 (*MOT1*) expression levels in the roots and leaves. Seedlings sprayed with 135 g Mo·ha^{-1} also had relatively high nitrogen metabolic enzyme activities and up-regulated transcript levels of nitrate uptake genes (*NRT1.1*; *NRT2.1*) and nitrate-responsive genes. Furthermore, there was a significantly lower NO_3^- concentration in the leaves and roots, a higher NH_4^+ concentration in leaves, and a higher glutamine/glutamate (Gln/Glu) concentration at 135 g Mo·ha^{-1}. Seedlings sprayed with 202.5 g Mo·ha^{-1} showed the opposite trend. Taken together, these results suggest that a 135 g Mo·ha^{-1} application was optimal because it enhanced NO_3^- transport from the roots to the shoots and increased NUE by mediating nitrogen metabolic enzyme activities, nitrate transport, and nitrate assimilation gene activities.

1. Introduction

Molybdenum (Mo), a transitional element, is essential for plant growth and productivity (Arnon and Stout, 1939; Kaiser et al., 2005). Mo can be found in several oxidation states ranging from zero to VI (Kaiser et al., 2005). Its role is to catalyze an oxo-transfer reaction that is coupled to the electron-transfer between a substrate and other cofactors, such as iron-sulfur (Fe—S) centers, hemes, or flavins (Schwarz et al., 2009). MoO_4^{2-} is the main form for plants taking up from soil and Mo availability is strongly dependent on soil pH, the adsorbing oxides and organic compound concentrations in the soil colloids (Kaiser et al., 2005). In Shandong Province, China, excessive applications of nitrogen (N) and phosphate fertilizer, along with insufficient applications of organic fertilizer and trace elements has led to soil acidification in orchards (Li et al., 2011). The negative agronomic impact of plant Mo nutritional deficiency frequently occurs in acidic soils. When the soil pH is lower than 5.5, Mo tends to form a sediment with binding free iron (Fe), manganese (Mg), and aluminum (Al) (Gong et al., 1998), which decreases the bioavailability of MoO_4^{2-}. Approximately 4 million ha of Mo-deficient arable land exists in China (Wang et al., 2002), so it is desirable to address this deficiency. Mo deficiency can lead to low N fertilizer use efficiency, limit the growth and production of crops and fruits, and cause Mo deficiency symptoms, such as abnormal leaves and stunted plant growth. Gao et al. (2016) reported that *Lotus japonicus* grown on media without Mo for 45 days exhibited Mo-deficient phenotypes, such as yellowing leaves and stunted growth of the taproot and lateral roots. However, the Mo-deficient phenotypes could be reversed by supplying Mo.

Abbreviations: Al, aluminum; AO, aldehyde oxidase; Cu, copper; GOGAT, glutamate synthase; GS, glutamine synthetase; ICP-MS, inductively coupled plasma mass spectrometry; Fe, iron; Mg, manganese; mARC, mitochondrial amidoxime reducing component; Mo, molybdenum; Moco, molybdenum cofactors; MOT1, molybdate transporter type 1; NR, nitrate reductase; N, nitrogen; NUE, nitrogen use efficiency; Se, selenium; SO, sulfite oxidase; XDH, xanthine dehydrogenase.

* Corresponding author.
** Corresponding author.
E-mail addresses: chunying196217@163.com (C.-y. Zhu), xilingfu@sdau.edu.cn (X.-L. Fu).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plaphy.2017.03.015
0981-9428/

Mo itself is biologically inactive unless it is incorporated by complex biosynthetic mechanisms into Mo cofactor (Moco). Moco binds to Mo-requiring enzymes and form the active site of Mo-requiring enzymes. In plants, the most prominent Mo-requiring enzymes are nitrate reductase (NR), xanthine dehydrogenase (XDH), aldehyde oxidase (AO), sulfite oxidase (SO), and the mitochondrial amidoxime reducing component (mARC). Mo plays a central role in N metabolism, such as, N fixation, nitrate reduction, and N assimilation (Mendel and Schwarz, 2011), and its deficiency may induce nitrate accumulation in plants.

N is critical for plant growth and productivity. Most farmers rely on the excessive application of N fertilizers to obtain higher productivities, but this can cause serious problems, such as low N use efficiency (NUE), soil acidification, and eutrophication. A low NUE leads to a waste of N resources, which can threaten the environment and human health (Chen et al., 2014; Xu et al., 2012). Nitrate is the main N source for plants in aerobic soils. After uptake, it is reduced to ammonium before N assimilation and incorporation into organic compounds. Ammonium is assimilated via the glutamine synthetase (GS)/glutamate synthase (GOGAT) cycle. NR plays a key role in catalyzing the first and rate-limiting step of nitrate assimilation, which reduces nitrate to nitrite. It is an inductive enzyme, and its activity is regulated by various environmental and endogenous factors, such as, Mo, nitrate, and light. During nitrate reduction, NR uses NAD(P)H as an electron donor that provides two electrons to the FAD cofactor, which are then transferred to the heme group and finally reduce the Mo center of the enzyme.

The relationship between Mo and the N metabolism has been reported in several papers. Adequate Mo levels could affect NR and GS activity, enhance nitrate uptake and transformation into ammonium, and promote ammonium transformation into organic N (Men and Li, 2005; Yu et al., 2010; Kovács et al., 2015). In addition, Liu et al. (2015) reported that appropriate Mo nutrition could enhance biomass, root activity, and the NUE of apple rootstock *Malus hupehesis Rehd*. Seedlings. Moraes et al. (2009) demonstrated that Mo addition increased the dry matter yields of rice plants cultured in a nutrient solution containing urea as the N source, but chlorophyll concentration and net photosynthesis rate decreased when Mo was omitted from the nutrient solution, regardless of the N source (Moraes et al., 2009). Taken together, these results suggest that Mo can affect NR and GS activity and may be involved in N metabolism. It may also affect nitrate and ammonium concentrations, and photosynthesis. However, it is not clear how exogenous Mo is transported into plants and how it then affects nitrate uptake, transport, and assimilation to improve NUE. The Mo doses chosen in this study were selected from previous experiments as shown in Fig. S1. Past results suggested that 168.75 g $Mo \cdot ha^{-1}$ was the optimal application rate, but plants sprayed with 675 g $Mo \cdot ha^{-1}$ and 1350 g $Mo \cdot ha^{-1}$ showed symptoms of poisoning. Our previous results showed that sodium molybdate (Na_2MoO_4) foliar sprays can have an effect on key N metabolic enzyme activities, but strawberry seedlings sprayed with a high concentration of Na_2MoO_4 had withered leaves (Supplementary Fig. 1) (Liu et al., 2016). Therefore, the optimum Mo concentration, Mo homeostasis, and the effect of Mo applications on each N metabolism step need to be identified. The molybdate transporter type 1 (*MOT1*) family has been reported to be involved in the uptake, translocation, and intra-cellular distribution of MoO_4^{2-} (Baxter et al., 2008; Gasber et al., 2011; Tomatsu et al., 2007). The genes responsible for nitrate transport and assimilation include some members of the NITRATE TRANSPORT (NRT) gene families (*NRT1.1*, *NRT2.1*), and the genes for nitrate and nitrite reductase (*NIA* and *NiR*) (Wang et al., 2007). Over the past 50 years, the large amount of N fertilizer applied has led to diminishing returns and deleterious impacts on environment. Therefore, improving NUE is crucial for the development of sustainable agriculture. The results presented here provide strong evidence that the N assimilating enzymes, the transcript expression of representative genes responsible for N assimilation, and the concentrations of different N forms involved in N assimilation are affected by Mo metabolism. We also investigated how Mo applications affect the absorption, distribution, and utilization of nitrate N using the ^{15}N technique tracer, which can be used to highlight the direction and distribution of N fertilizer in plants. We then propose physiological mechanisms that may control how Mo is involved in NO_3^- use. These experimental results will offer a scientific basis for enhancing NUE in fruits.

2. Materials and methods

2.1. Plant material

Strawberry (*Fragaria* × *ananassa* Duch. cv. Akihime) seedlings (n = 200) were grown in a greenhouse under natural daylight, 25–28 °C (day) 5–10 °C (night), and an RH of 55%–65%. The seedlings were cultivated in a non-soil system using elevated horizontal troughs with a coir substrate and were irrigated with Hoagland's nutrient solution without Mo. The modified Hoagland's nutrient solution consisted of 5 mM KNO_3, 5 mM Ca ($^{15}NO_3)_2$, 2 mM $MgSO_4$, and 1 mM KH_2PO_4, 100 μM EDTA-Fe, 37 μM H_3BO_4, 9 μM $MnCl_2 \cdot 4H_2O$, 0.3 μM $CuSO_4 \cdot 5H_2O$ and 0.76 μM $ZnSO_4 \cdot 7H_2O$. When the strawberry seedlings had 10–15 true leaves, they were subjected to N starvation for 5 days and then cultured in 1/4 or 1/2 modified Hoagland's nutrient solution for 7 days. Then they were finally cultured with modified Hoagland's nutrient solution for 7 days before the Na_2MoO_4 treatments were applied.

Mo was sprayed onto the plants as Na_2MoO_4 at five different treatment levels (0 (control, T1), 67.5 (T2), 135 (T3), 168.75 (T4), and 202.5 g $Mo \cdot ha^{-1}$ (T5)). The Na_2MoO_4 treatment was applied at 7 day intervals from October 19, 2015 to November 2, 2015. The solutions were irrigated every two days. All solutions were prepared with deionized water and there were three replicates per treatment. Strawberry seedlings were harvested at 5, 10, and 15 days after treatment for the key enzymes and related genes determinations, whereas the seedlings were sampled at 25 days after treatment for minerals (Mo, Fe, copper (Cu), and selenium (Se)), and ^{15}N absorption, distribution, and utilization analyses.

2.2. Mineral element measurements using inductively coupled plasma mass spectrometry (ICP-MS)

Trace minerals (Mo, Cu, Fe, and Se) were determined using the NexION™ 300 ICP-MS System (Perkin Elmer, Waltham, Massachusetts, USA) as described by Filipiak-Szok et al. (2014). The ground and dried strawberry fruit samples (0.3 g/replication) were transferred to a digestion tank containing 5 mL nitric acid (65% v/v) for mineralization using a MARS6 (CEM, Matthews, North Carolina, USA) high-throughput closed microwave digestion system. The ashes were dissolved and filtered overnight. Then 2 mL of hydrogen peroxide (H_2O_2, 30% v/v) was added to each digestion tank. The final solution was transferred to a 50 mL volumetric flask and then the volume was made up to 50 mL with ultrapure water (in triplicate).

2.3. Growth parameter assays

The strawberry seedlings were harvested 25 days after the different Mo concentration treatments had been applied. The seedlings were divided into their roots, stems and leaves. Then they were heated at 105 °C for 30 min and dried at 80 °C for 5 days. The dry weight was recorded as the biomass.