

## Uptake, transport and distribution of molybdenum in two oilseed rape (*Brassica napus* L.) cultivars under different nitrate/ammonium ratios\*

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**Abstract:** Objectives: To investigate the effects of different nitrate sources on the uptake, transport, and distribution of molybdenum (Mo) between two oilseed rape (*Brassica napus* L.) cultivars, L0917 and ZS11. Methods: A hydroponic culture experiment was conducted with four nitrate/ammonium ( $\text{NO}_3^-:\text{NH}_4^+$ ) ratios (14:1, 9:6, 7.5:7.5, and 1:14) at a constant nitrogen concentration of 15 mmol/L. We examined Mo concentrations in roots, shoots, xylem and phloem sap, and subcellular fractions of leaves to contrast Mo uptake, transport, and subcellular distribution between ZS11 and L0917. Results: Both the cultivars showed maximum biomass and Mo accumulation at the 7.5:7.5 ratio of  $\text{NO}_3^-:\text{NH}_4^+$  while those were decreased by the 14:1 and 1:14 treatments. However, the percentages of root Mo (14.8% and 15.0% for L0917 and ZS11, respectively) were low under the 7.5:7.5 treatment, suggesting that the equal  $\text{NO}_3^-:\text{NH}_4^+$  ratio promoted Mo transportation from root to shoot. The xylem sap Mo concentration and phloem sap Mo accumulation of L0917 were lower than those of ZS11 under the 1:14 treatment, which suggests that higher  $\text{NO}_3^-:\text{NH}_4^+$  ratio was more beneficial for L0917. On the contrary, a lower  $\text{NO}_3^-:\text{NH}_4^+$  ratio was more beneficial for ZS11 to transport and remobilize Mo. Furthermore, the Mo concentrations of both the cultivars' leaf organelles were increased but the Mo accumulations of the cell wall and soluble fraction were reduced significantly under the 14:1 treatment, meaning that more Mo was accumulated in organelles under the highest  $\text{NO}_3^-:\text{NH}_4^+$  ratio. Conclusions: This investigation demonstrated that the capacities of Mo absorption, transportation and subcellular distribution play an important role in genotype-dependent differences in Mo accumulation under low or high  $\text{NO}_3^-:\text{NH}_4^+$  ratio conditions.

**Key words:** *Brassica napus* L.; Nitrogen source; Transport; Subcellular distribution; Xylem; Phloem  
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### 1 Introduction

Molybdenum (Mo) is an essential micronutrient for plant growth and it plays an important role in carbon, sulfur, and nitrogen metabolism, which is mainly via metalloenzymes in plants (Havemeyer

et al., 2011; Hille et al., 2011). Generally in China, soils are acidic and poor availability of Mo in soils is widespread (Ye et al., 2011; Cao et al., 2012; Liu et al., 2012), especially in Southeast China (Shi et al., 2006). However, oilseed rape (*Brassica napus* L.), an important oil crop, is predominantly grown in Mo-deficient soils (Yin et al., 2009). Moreover, oilseed rape requires plenty of nitrogen (N) fertilizer for growth and yield (Behrens et al., 2002; Barló and Grzebisz, 2004a; 2004b; Rathke et al., 2006; Leiva-Candia et al., 2013) and Mo deficiency often

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decreases the activity of nitrate reductase which leads to N deficiency in plants (Chatterjee and Nautiyal, 2001; Ide *et al.*, 2011), poor seedling growth and lower grain yield (Yang *et al.*, 2009; Liu *et al.*, 2010). Furthermore, winter wheat subjected to higher rate of N-fertilization is prone to Mo deficiency (Wang *et al.*, 1999) while supplying  $\text{NH}_4\text{NO}_3$  to plants stimulates the remobilization of Mo in black gram (Jongruaysup *et al.*, 1997). Mo plays an important role in the N metabolism of plants, including nitrogen fixation and the transformation of nitrate ( $\text{NO}_3^-$ )-N to ammonium ( $\text{NH}_4^+$ )-N (Kovács *et al.*, 2015). However, little information is available about the Mo uptake, transport, or distribution under different N sources.

$\text{NH}_4^+$  and  $\text{NO}_3^-$  are two forms of inorganic N available for plant uptake. Studies on  $\text{NH}_4^+$  stress showed that a low  $\text{NO}_3^-:\text{NH}_4^+$  ratio inhibits plant growth and changes ion balance in the plant (Liu *et al.*, 2014; Babalar *et al.*, 2015; Sokri *et al.*, 2015). Esteban *et al.* (2016) showed that complementation with nitrate at low doses, which has long been practised in agriculture, can counterbalance the ammonium toxicity in the laboratory. Zhu *et al.* (2015) reported that the Mo content of Chinese cabbage declined significantly with a decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio. Moreover, the molybdate transporter 1 (CrMOT1) of *Chlamydomonas reinhardtii* is activated by nitrate but not by molybdate (Tejada-Jiménez *et al.*, 2007), while molybdate transporter 2 (CrMOT2) is activated by molybdate, but not by the N source (Tejada-Jiménez *et al.*, 2011). The responses of plants particularly of different cultivars to variable  $\text{NO}_3^-:\text{NH}_4^+$  ratios are still not well known. In this study, we evaluated the variations in Mo uptake, transport, and distribution between two *B. napus* genotypes treated with different  $\text{NO}_3^-:\text{NH}_4^+$  ratios under hydroponic culture, mainly by tissue Mo, sap flow and subcellular Mo concentrations.

## 2 Materials and methods

### 2.1 Plants and growth conditions

Seeds of oilseed rape cultivars ZS11 (high Mo accumulating cultivar) and L0917 (low Mo accumulating cultivar) were sterilized with 10% (v/v) NaClO for 30 min and washed by deionized water for 5–10 min. The sterilized seeds were cold-treated at 4 °C

for 2 d and then were germinated on moistened gauze that was fixed to a black plastic tray filled with deionized water at room temperature. After one week, 15 uniform seedlings were transferred to black polyethylene boxes containing 10 L 1/2-strength modified Hoagland solution for 6 d and subsequently full-strength solution. The modified Hoagland solution contains 4 mmol/L  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 6 mmol/L  $\text{KNO}_3$ , 1 mmol/L  $\text{NH}_4\text{H}_2\text{PO}_4$ , 2 mmol/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 46.2  $\mu\text{mol/L}$   $\text{H}_3\text{BO}_3$ , 9.1  $\mu\text{mol/L}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.8  $\mu\text{mol/L}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.3  $\mu\text{mol/L}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 100  $\mu\text{mol/L}$  EDTA-Fe, and 1  $\mu\text{mol/L}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ . After 20 d, the seedlings were treated with four different  $\text{NO}_3^-:\text{NH}_4^+$  ratios with four replications. Plants were cultured in primary solution ( $\text{NO}_3^-:\text{NH}_4^+$  14:1) or in primary solution with  $\text{NH}_4\text{Cl}$  ( $\text{NO}_3^-:\text{NH}_4^+$  9:6, 7.5:7.5, 1:14) according to Nimptsch and Pflugmacher (2007). The pH of solutions was adjusted to  $6.5 \pm 0.2$  with 1 mol/L NaOH. The solutions were renewed every 4 d during the 35-d experiment. At the end of the experiment, plants were harvested and separated into fresh samples (leaves immediately frozen in dry ice and kept frozen until use) and dry samples (roots, stems and leaves were dried at 80 °C until a constant weight was achieved). Meanwhile, xylem sap and phloem sap were collected.

### 2.2 Exudation techniques

The xylem sap was collected by the method described by Ueno *et al.* (2011) and Wu *et al.* (2015). Briefly, plant shoots (two plants) were cut at 3 cm above the roots and the xylem sap was collected from the cut surface by the root pressure method (the initial 1–2  $\mu\text{l}$  of exudates were discarded). The volume of xylem sap was recorded and 4 ml xylem sap was placed in a 50-ml volumetric flask and dried on a hot plate for Mo determination. The phloem sap was collected as described by Tetyuk *et al.* (2013). Shoots were incubated in a conical flask filled with 65 ml 25 mmol/L EDTA- $\text{Na}_2$  in a growth chamber for 24 h at 20 °C and 95% humidity in dark. The collected solution was dried on a hot plate for Mo determination.

### 2.3 Cell wall, organelle and vacuole isolation

The frozen plant fresh leaves (1 g) were homogenized and the subcellular fractions were separated according to Su *et al.* (2014) and Li *et al.* (2016). Each plant sample was homogenized in 12-ml

precooled extraction buffer containing 250 mmol/L sugar, 50 mmol/L Tris-HCl (pH 7.5), 1 mmol/L  $\text{MgCl}_2$ , and 10 mmol/L cysteine using a chilled mortar and pestle. The mixture was centrifuged at 2057g for 10 min with a refrigerated centrifuge (Eppendorf 5810 R, Germany), and the precipitate was designated as the cell wall fraction mainly consisting of cell walls and cell wall debris. The supernatant was re-centrifuged at 12 857g for 50 min, and the precipitate was designated as the organelle fraction which consisted of membrane and organelle components, and the resultant supernatant solution is referred to as the soluble fraction which mainly includes vacuoles and cytoplasm. All the above mentioned steps were performed at 4 °C.

#### 2.4 Mo determination

Plant materials and dried xylem and phloem sap samples were digested with  $\text{HNO}_3/\text{HClO}_4$  (4:1, v/v) mixture at 190 °C for 2 h and then at 205 °C. The samples were then dissolved in 10 ml water, and Mo concentration ( $C_{\text{Mo}}$ ) was determined using a graphite furnace atomic absorption spectrometer (Z-2000 series, Hitachi, Japan) method (Nie *et al.*, 2014). The translocation factor (TF) is calculated by  $C_{\text{Mo}}$  in leaf/ $C_{\text{Mo}}$  in stalk or  $C_{\text{Mo}}$  in stalk/ $C_{\text{Mo}}$  in root, and Mo accumulation is calculated by  $C_{\text{Mo}} \times \text{weight of dry matter}$ .

#### 2.5 Statistical analysis

Data were statistically analyzed using SPSS 13.0. One-way analysis of variance (ANOVA) with a least significance difference (LSD) test was performed to analyze the significant differences between treatments ( $P < 0.05$ ). Data were presented as mean  $\pm$  standard error (SE) and Sigma Plot v12.0 was used for the graphs.

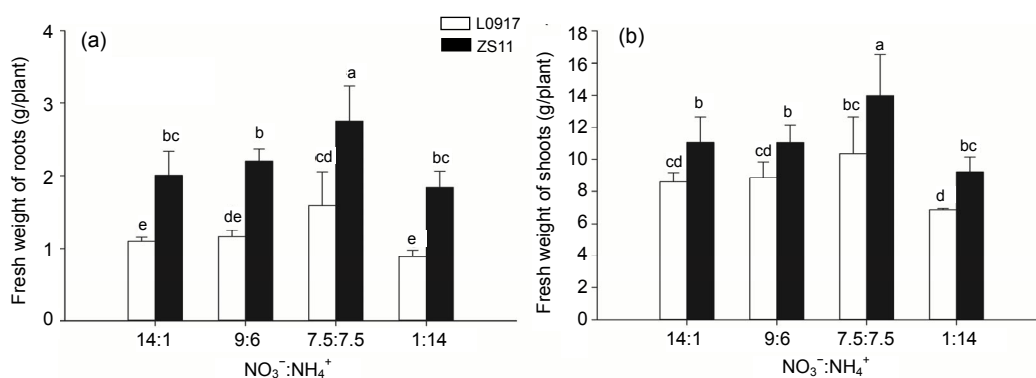
### 3 Results

#### 3.1 Biomass

All the biomass (fresh weight (FW)) of roots and shoots of the two rape cultivars reached a maximum at the 7.5:7.5 ratio, but they declined to a minimum at the ratio of 1:14 (Fig. 1). The biomass decreased by 43.8% and 33.9% for cultivar L0917 and by 33.5% and 34.0% for cultivar ZS11 for roots and shoots, respectively, by changing the ratio from 7.5:7.5 to 1:14. The average biomass of cultivar ZS11 roots and shoots was 1.86 and 1.31 times higher than that of cultivar L0917, respectively. This suggests that the two rape cultivars produce the highest biomass under the same concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , but a higher proportion of  $\text{NH}_4^+$  results in significant diminution of the biomass of both cultivars particularly the root biomass of L0917 ( $P < 0.05$ ). The high Mo accumulator ZS11 not only had the higher biomass but also had less growth inhibition at the 1:14 ratio than the low Mo accumulator L0917.

#### 3.2 Mo distribution in tissues

Mo accumulation by both cultivars reached a maximum at the 7.5:7.5 ratio, while the high Mo accumulator ZS11 exhibited a 24.8% ( $P < 0.05$ ) decrease in Mo accumulation at the 1:14 ratio (Fig. 2a), suggesting that high  $\text{NH}_4^+$  inhibited Mo accumulation in the plant. The accumulation percentages of Mo by L0917 were 12.4%, 66.6%, and 21.0% in roots, stalks and leaves, respectively, while these were respectively 14.2%, 36.8%, and 49.0% by ZS11 (Fig. 2b), suggesting that the majority of Mo occurs in the stalks of L0917 and the leaves of ZS11. The proportion of Mo in leaves of both cultivars was increased with



**Fig. 1** Fresh weights of roots (a) and shoots (b) at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture  
Data were presented as mean  $\pm$  SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P < 0.05$  by LSD-test

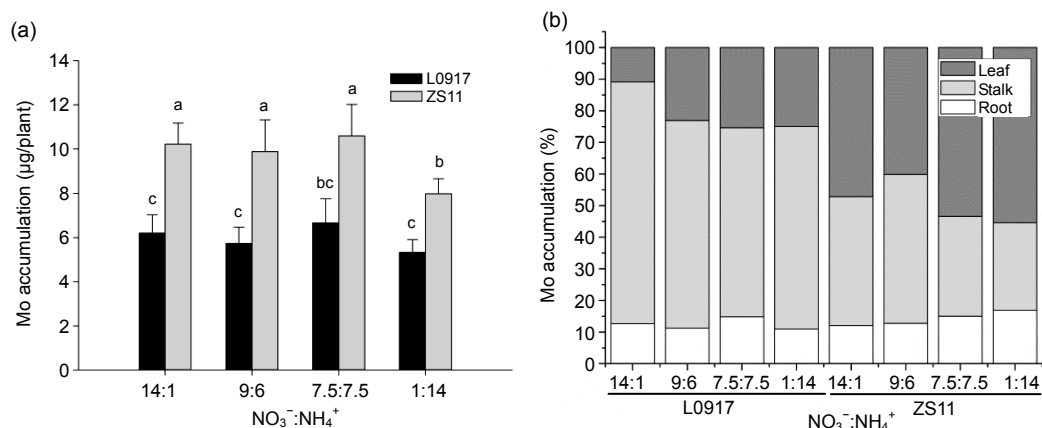
the decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio, but the proportion of Mo in stalks decreased under the same conditions.

The root Mo concentrations of both the cultivars treated with the 7.5:7.5 ratio were higher than those under the other ratios (Fig. 3a). However, Mo concentrations of L0917 shoots showed no significant difference among the  $\text{NO}_3^-:\text{NH}_4^+$  ratios, while Mo concentrations of ZS11 significantly decreased with decreasing  $\text{NO}_3^-:\text{NH}_4^+$  (Fig. 3b). Root and shoot Mo concentrations were higher in cultivar ZS11 than in cultivar L0917 at all treatments. The shoot/root Mo TFs of cultivar L0917 initially declined with the decreasing  $\text{NO}_3^-:\text{NH}_4^+$  but then increased to the highest value under the 1:14 treatment. The TFs of cultivar ZS11 decreased with the decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio (Fig. 4). The results indicated that Mo concentrations

of the roots of both cultivars have the same absorption tendency treated with  $\text{NO}_3^-:\text{NH}_4^+$  ratios, while Mo transportation from roots to shoots was inhibited particularly in cultivar ZS11.

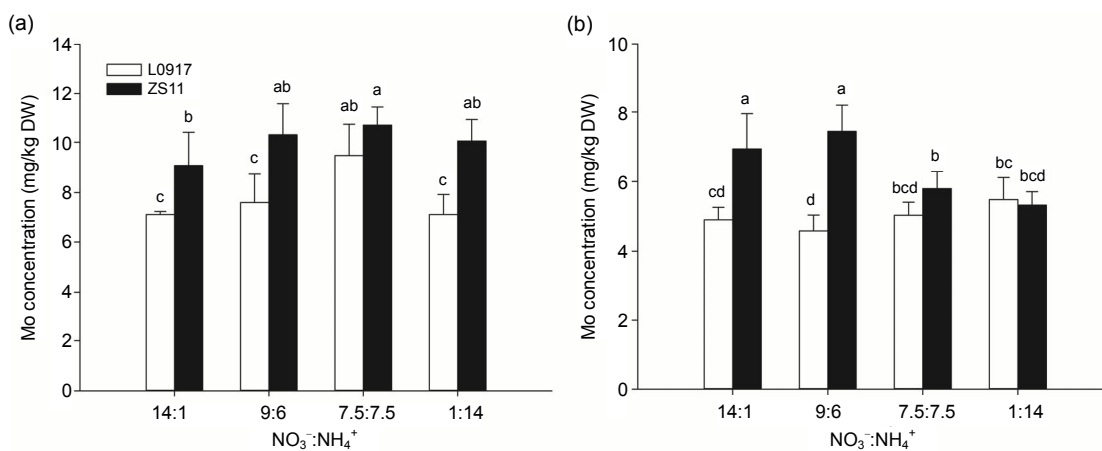
### 3.3 Mo transport in xylem and phloem

Mo contents of xylem and phloem sap were measured to further study the effects of  $\text{NO}_3^-:\text{NH}_4^+$  ratio on Mo transport in the oilseed rape (Fig. 5). The Mo concentration in xylem sap of L0917 decreased by 23.40% ( $P<0.05$ ) but that of ZS11 increased by 49.65% ( $P<0.05$ ) at the ratio of 1:14 as compared to 14:1. Similarly, Mo accumulation in phloem sap of cultivar L0917 decreased by 59.48% ( $P<0.05$ ) when the  $\text{NO}_3^-:\text{NH}_4^+$  ratio was decreased from 14:1 to 1:14. However, phloem sap Mo accumulation in ZS11



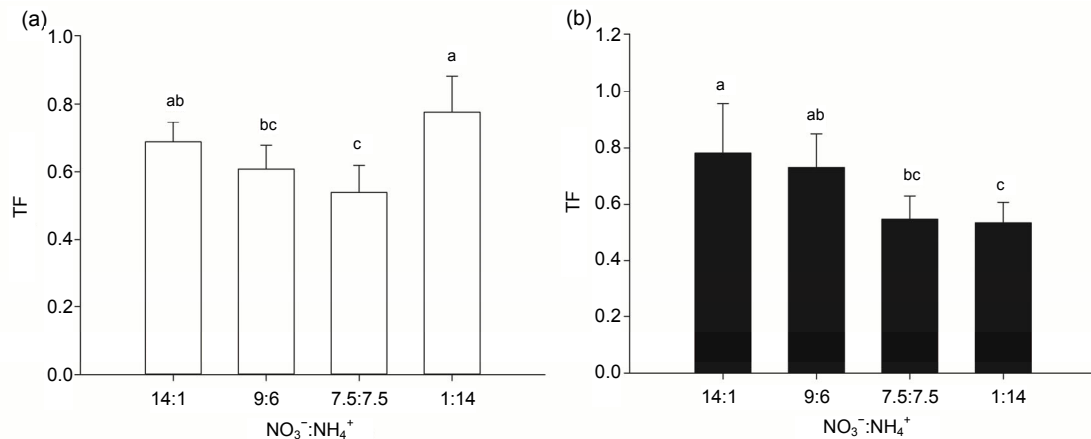
**Fig. 2 Total plant Mo accumulations (a) and tissue Mo accumulation percentages (b) at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture**

Data were presented as mean $\pm$ SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P<0.05$  by LSD-test



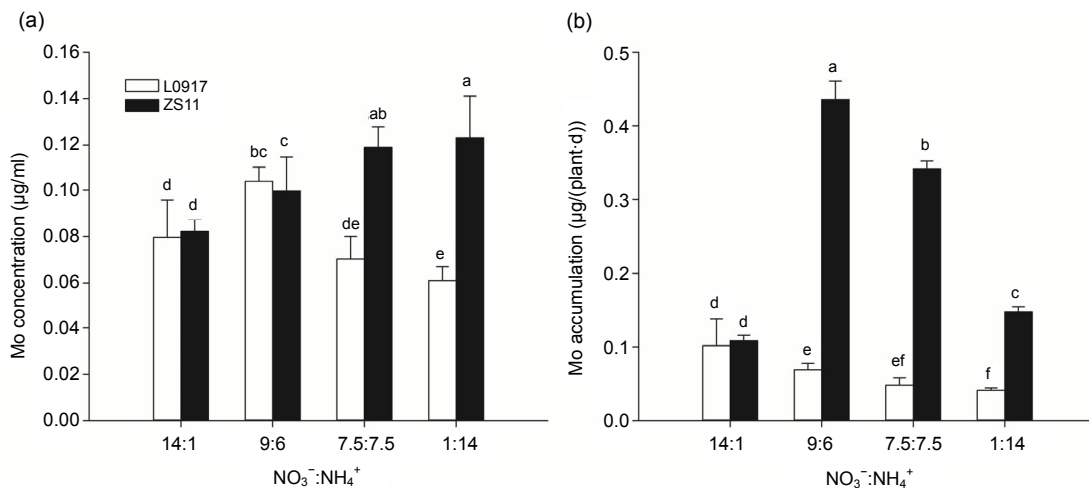
**Fig. 3 Mo concentrations in roots (a) and shoots (b) of oilseed rape grown at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture**

Data were presented as mean $\pm$ SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P<0.05$  by LSD-test



**Fig. 4** Mo translocation factors (TFs) for shoot/root of L0917 (a) and ZS11 (b) at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture

Data were presented as mean $\pm$ SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P<0.05$  by LSD-test



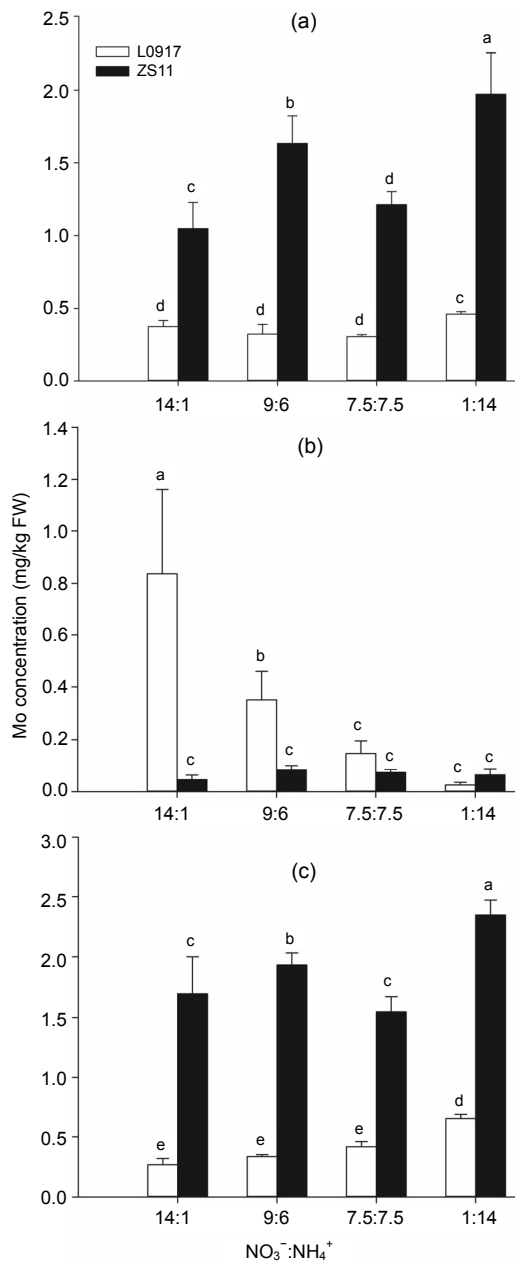
**Fig. 5** Mo concentration in xylem sap (a) and Mo accumulation in phloem sap (b) of oilseed rape grown at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture

Data were presented as mean $\pm$ SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P<0.05$  by LSD-test

increased to a maximum at the ratio of 9:6 before decreasing again with a further reduction in the  $\text{NO}_3^-:\text{NH}_4^+$  ratio. At the ratio of 1:14, the phloem sap Mo accumulation was 36.78% ( $P<0.05$ ) higher than that at 14:1. The Mo concentrations and accumulations in xylem and phloem sap of ZS11 were 2.02- and 3.59-fold ( $P<0.05$ ) higher than those of L0917, respectively. These results showed that ZS11 exhibits notably higher Mo loads in the xylem and phloem transport systems than L0917. In particular, Mo transport in xylem sap of L0917 was inhibited while that of ZS11 was considerably promoted by a low  $\text{NO}_3^-:\text{NH}_4^+$  ratio or higher  $\text{NH}_4^+$  concentration.

### 3.4 Subcellular Mo distribution in leaves

Mo concentrations and Mo percentages in subcellular fractions of leaves under different treatments are shown in Figs. 6 and 7. Mo concentrations in cell wall fractions and soluble fractions of both the cultivars under the 1:14 ratio were higher than those under other ratios (Figs. 6a and 6c). In contrast, the Mo concentration in the organelle fraction of L0917 under the 14:1 ratio was 33 times higher than that under the 1:14 ratio ( $P<0.05$ ), while there was no significant difference in the organelle Mo concentrations of the ZS11 cultivar among the treatments (Fig. 6b). Overall,



**Fig. 6** Mo concentrations in cell wall (a), organelle (b), soluble fractions (c) of oilseed rape grown at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture

Data were presented as mean $\pm$ SE ( $n=4$ ). Different letters above the bar mean significant differences at  $P < 0.05$  by LSD-test

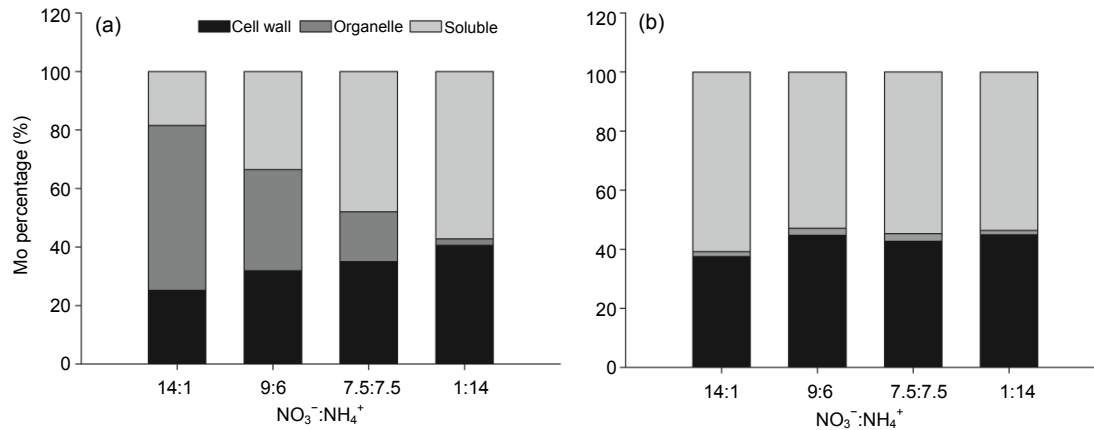
the average Mo concentrations of both cultivars in the cell wall, organelle, and soluble fractions were 0.915, 0.204, and 1.151 mg/kg, respectively. The percentages of Mo in the organelle fraction of both cultivars were at a minimum with the 1:14 ratio of  $\text{NO}_3^-:\text{NH}_4^+$

with 2.2% and 1.5% in cultivars L0917 and ZS11, respectively (Fig. 7). The Mo concentrations in the cell wall fractions of leaves of ZS11 were 2.80, 5.05, 3.94, and 4.28 times higher than those of L0917 under the 14:1, 9:6, 7.5:7.5, and 1:14 treatments, respectively. The Mo concentrations in the soluble fractions of leaves of ZS11 were 6.21, 5.67, 3.70, and 3.63 times higher than those of L0917 cultivar under the 14:1, 9:6, 7.5:7.5, and 1:14 treatments, respectively. While the Mo concentration in the organelle fractions of leaves of L0917 were 18.08, 4.05, 2.01, and 0.39 folds higher than those of ZS11 under the 14:1, 9:6, 7.5:7.5, and 1:14 treatments, respectively. These results clearly indicated that more Mo was distributed in cell wall and soluble fractions of ZS11, while Mo distribution in L0917 was much greater in the organelle fractions particularly under high  $\text{NO}_3^-:\text{NH}_4^+$  ratios. High  $\text{NO}_3^-:\text{NH}_4^+$  ratios increased the Mo distribution in organelle fractions of L0917, but low  $\text{NO}_3^-:\text{NH}_4^+$  ratios increased the Mo distribution in cell wall fractions and soluble fractions of both the cultivars.

#### 4 Discussion

In recent years, the understanding with regard to plant responses to the  $\text{NO}_3^-:\text{NH}_4^+$  ratio has increased, whereas the effects of the ratio on Mo status are still unknown. The significant symptom of higher  $\text{NH}_4^+$  concentrations is the suppression of plant growth (Jampeetong and Brix, 2009). In this work, we found that oilseed rape grows better under a  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 7.5:7.5 than under 14:1, 9:6 or 1:14. Similar results were reported by Lu *et al.* (2009) in tomato and Tabatabaei *et al.* (2008) in strawberry plants, and their results indicated that combined application of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  significantly enhanced the biomass as compared to sole application of either form of N. Compared with the 7.5:7.5 ratio, the decrease in biomass of L0917 cultivar at the 1:14 ratio was greater than that of ZS11 cultivar, indicating that the high Mo accumulating cultivar ZS11 had a better resistance to  $\text{NH}_4^+$  stress. Therefore, combined and particularly equal applications of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  were the most beneficial to both oilseed rape cultivars and the low Mo accumulator cultivar L0917 was more sensitive to higher concentration of  $\text{NH}_4^+$ .





**Fig. 7** Mo percentages in subcellular fractions for L0917 (a) and ZS11 (b) at different  $\text{NO}_3^-:\text{NH}_4^+$  ratios in hydroponic culture

Although the uptake mechanisms underlying ammonium inhibition are not fully understood, previous studies have found that the uptake of many inorganic cations ( $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) is reduced under  $\text{NH}_4^+$  nutrition and consequent changes in plant ion balance (Britto and Kronzucker, 2002; Dickson *et al.*, 2016). There is evidence that the effects of  $\text{NH}_4^+$  nutrition are predominantly upon root growth and compete with or pass through the same protein channels (Li *et al.*, 2010; Zheng *et al.*, 2015). In the present study, on two oilseed rape cultivars, Mo accumulation was only decreased under the  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 1:14 (Fig. 2), suggesting that higher  $\text{NH}_4^+$  concentrations restrain Mo absorption in oilseed rape. Similarly, Zhu *et al.* (2015) reported that shoot Mo contents were decreased significantly with a decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio at 15 mmol/L N on Chinese cabbage. The percentages of Mo accumulated in the roots of both oilseed rape cultivars increased, whereas the shoot Mo concentrations and the shoot/root TFs for cultivar ZS11 decreased with decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio, suggesting that Mo transport from root to shoot was inhibited by decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratio. The Mo accumulation of ZS11 was respectively 64.9%, 72.2%, 59.2%, and 49.6% higher than that of L0917 at the 14:1, 9:6, 7.5:7.5, and 1:14 treatments. Therefore, it is probable that efficient xylem loadings play an important role in shoot mineral accumulation (Ueno *et al.*, 2011; Wu *et al.*, 2015) and phloem is a good indicator for the nutritional conditions in leaves (Peuke, 2010). Yu *et al.* (2002) also reported that higher phloem mobility was a key factor for leaf

Mo accumulation. The present study suggested that at low  $\text{NO}_3^-:\text{NH}_4^+$  ratios Mo concentrations in xylem and phloem sap of ZS11 were all higher than those of L0917. These results also suggested that ZS11 exhibited a higher Mo transport in xylem, as well as an enhanced ability for remobilization of Mo through phloem transport. Jongruaysup *et al.* (1994; 1997) also explained that variability of Mo mobility in phloem was found in Mo-adequate plants and  $\text{NH}_4\text{NO}_3$  stimulated the remobilization of Mo in the tissues. Therefore, the high Mo concentrations in xylem and phloem sap indicate good uptake in the root and well-supplied leaves.

Compartmentalization of Mo is vital for Mo homeostasis in plant cells. The average Mo concentrations in the cell wall, organelle and soluble fractions of leaves of the two rapeseed cultivars across the four treatments were 0.366, 0.204, and 1.151 mg/kg respectively (Fig. 6). The percentages of Mo in cell wall and solution fractions for L0917 were increased but in the organelle fraction were decreased with decreasing  $\text{NO}_3^-:\text{NH}_4^+$  ratios. It also appears that Mo was mostly accumulated firstly by the soluble fraction and then by the cell wall, which could be a good reason why the high  $\text{NO}_3^-:\text{NH}_4^+$  ratio was toxic to organelle and cell wall fractions (Küpper *et al.*, 1999; Hale *et al.*, 2001; Britto and Kronzucker, 2002; Zhao *et al.*, 2015). Plant cell wall binding and soluble fractions are the important mechanisms for metal accumulation (Li *et al.*, 2006; Fu *et al.*, 2011). We showed that the higher Mo accumulator ZS11 distributed more Mo firstly in the soluble fractions and

secondly in the cell wall than cultivar L0917. It could be accordingly concluded that the soluble fraction in the high accumulation cultivar is more inclined to retain Mo than that in the low accumulation cultivar. Low  $\text{NO}_3^-:\text{NH}_4^+$  ratio sharply decreased the Mo distribution in the organelle fractions of L0917, indicating that organelle damage could be the integrated effect of  $\text{NH}_4^+$  excess. This could also be associated with the biosynthesis of the Mo cofactor which is localized in the cytosol (Mendel, 2011).

In conclusion, this study indicated that the two rapeseed cultivars produced more biomass and accumulated more Mo while the nitrogen was supplied at the  $\text{NO}_3^-:\text{NH}_4^+$  ratio of 7.5:7.5, and the Mo uptake and Mo transport from roots to shoots were inhibited by low  $\text{NO}_3^-:\text{NH}_4^+$  ratios or higher  $\text{NH}_4^+$ . The high Mo accumulator ZS11 not only exhibited higher Mo concentrations in all the tissues, cell-organs, xylem and phloem sap, but also accumulated more Mo firstly in the soluble fraction and secondly in the cell wall than L0917.

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### Compliance with ethics guidelines

Shi-yu QIN, Xue-cheng SUN, Cheng-xiao HU, Qi-ling TAN, and Xiao-hu ZHAO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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## 中文概要

**题目:** 不同  $\text{NO}_3^-:\text{NH}_4^+$  比对两种甘蓝型油菜钼吸收、转运和分布的影响

**目的:** 采用不同  $\text{NO}_3^-:\text{NH}_4^+$  比的营养液, 探索不同氮源对钼元素在两种甘蓝型油菜 (L0917 和 ZS11) 中的吸收、转运和分布的影响及品种间的差异。

**创新点:** 在不同氮源条件下, 从组织分布、汁液运输、亚细胞分布以及品种等方面研究了甘蓝型油菜的钼营养状况。

**方法:** 将甘蓝型油菜 L0917 和 ZS11 的幼苗在正常营养液培养 20 天后, 分别转移至 4 种  $\text{NO}_3^-:\text{NH}_4^+$  比 (14:1、9:6、7.5:7.5、1:14) 且总氮为 15 mmol/L 的营养液中培养 15 天后收获。采用原子吸收分光光度计-石墨炉法测定根、茎和叶不同部位钼含量, 木质部和韧皮部液钼含量, 叶肉细胞细胞壁组分、细胞器组分和可溶性组分钼含量。

**结论:** 在高或低  $\text{NO}_3^-:\text{NH}_4^+$  比条件下, 高的钼吸收能力、木质部转运、韧皮部再迁移以及叶片亚细胞钼储存在甘蓝型油菜钼积累上扮演重要角色。

**关键词:** 甘蓝型油菜; 氮源; 运输; 亚细胞分布; 木质部; 韧皮部