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INTERACTION OF MOLYBDENUM AND PHOSPHORUS SUPPLY ON UPTAKE AND TRANSLOCATION OF PHOSPHORUS AND MOLYBDENUM BY *BRASSICA NAPUS*

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INTERACTION OF MOLYBDENUM AND PHOSPHORUS SUPPLY ON UPTAKE AND TRANSLOCATION OF PHOSPHORUS AND MOLYBDENUM BY *BRASSICA NAPUS*

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□ A hydroponic trial was conducted to assess interaction of molybdenum (Mo) and phosphorus (P) on uptake and translocation of P and Mo by *Brassica napus*. Molybdenum was applied at four rates (0, 0.01, 0.1 and 1 mg L⁻¹) and P at three rates (1, 30, and 90 mg L⁻¹) in nutrient solution. The results indicated that P increased shoot growth and 0.01 mg L⁻¹ Mo improved the growth of shoots and roots. Molybdenum increased shoot P uptake and root P concentration and uptake when higher P was provided, and had a stimulating effect on P translocation from shoots to roots. P increased shoot Mo concentration and uptake, decreased those in roots, and enhanced Mo transport from roots to shoots. These results implied that both Mo and P had beneficial effects on Mo and P absorption and translocation and co-application of them were necessary to promote growth and utilization of Mo and P for *Brassica napus*.

Keywords: interaction, molybdenum, phosphorus, uptake, translocation, *Brassica napus*

INTRODUCTION

There have been several research studies about the interactions of molybdenum (Mo) and phosphorus (P) on the absorption of nutrients by tomato (Stout and Meagher, 1948; Stout et al., 1951), soybean (Singh and Kumar, 1979), rice (Basak et al., 1982), wheat (Modi, 2002), etc. However, the results in this regard are conflicting so far. Some researchers believe that there is a

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synergistic interaction of Mo and P on their uptakes; for example, Mo presence could enhance P uptake by plants and vice versa (Stout and Meagher, 1948; Singh and Kumar, 1979; Basak et al., 1982). However, others held that there might be antagonistic effects between them. Kumar and Singh (1980) reported that Mo significantly reduced P concentration of berseem and attributed this to the fact that both phosphates and molybdates were absorbed in anionic forms, and they might compete with each other for the absorption sites. Heuwinkel et al. (1992) also found that P deficiency enhanced markedly Mo uptake by tomato plants and implied that molybdate was taken up by a phosphate transporter. Therefore, further studies on interactions of Mo and P in relation to nutrients absorption by plants are worth to conducting.

Brassica napus is an important edible oil crop throughout the world (Li et al., 2004). It is highly sensitive to Mo or P deficiency at seedling stage and Mo and P deficiency in soils often resulted in poor growth of *Brassica napus* seedling and affected its grain yield (Hewitt and Bolle-Jones, 1952; Wang, 1973; Duan et al., 2002; Cao and Liang, 2004). Until now there was also little fundamental information on the interactions of Mo and P with respect to growth and nutrients uptake of *Brassica napus*.

The main objective of this study is to investigate effects of Mo and P supply on growth, uptake of P and Mo and nutrients translocation between roots and shoots of *Brassica napus* seedlings under a hydroponic condition. The results from this study may be used to provide not only more insights into the interactive effects of Mo and P in higher plants but also some fertilizer instructions for rapeseed production.

MATERIALS AND METHODS

Culture Description

The seeds of *Brassica napus* "Zhongyouza No. 2" were sterilized by rinsing in 0.5% (v/v) sodium hypochlorite (NaClO) solution for 30 min and germinated in deionized water at 25°C for seven days. After germination, similar-sized seedlings were selected and transferred to modified Hoagland nutrient solutions held in plastic containers of 4 L, each growing 24 plants. One-quarter strength and a half-strength nutrient solution were used in the first and second week, respectively, and then a full-strength solution was used.

Molybdenum was added to the nutrient solution as ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ at four rates: 0, 0.01, 0.1, and 1.0 mg L^{-1} and P as sodium phosphate ($\text{NaH}_2\text{PO}_4\cdot 2\text{H}_2\text{O}$) at three rates: 1, 30, and 90 mg L^{-1} . Each treatment was replicated three times with a randomized block design. The composition of the full-strength nutrient solution was as follows: 945 mg L^{-1} calcium nitrate $[\text{Ca}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}]$, 607 mg L^{-1} potassium

nitrate (KNO_3), 493 mg L^{-1} magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 20 mg L^{-1} ethylenediaminetetraacetic acid (EDTA)-iron (Fe), 2.86 mg L^{-1} boric acid (H_3BO_3), 1.81 mg L^{-1} manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 0.22 mg L^{-1} zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), and 0.08 mg L^{-1} copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). To maintain the concentration of nutrient solution throughout the experiment, the nutrient solutions was freshly prepared and renewed every three days. The pH of nutrient solution was closely monitored to maintain it between 6.0 and 6.5 with hydrochloric acid (HCl) or potassium hydroxide (KOH).

All containers used in the experiment were soaked in 5% (v/v) HCl solution for seven days, and then rinsed by deionized water four times at least. Deionized water was used for preparing nutrient solutions (Resistivity $>16 \text{ M}\Omega \cdot \text{cm}$ at 25°C) and all chemical reagents used were analytical grade to minimize possible Mo contamination.

Plants were grown in a controlled greenhouse with the following environmental conditions: a light/dark regime of 12/12 h, air temperatures of $25/18^\circ\text{C}$, photon flux density of $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and a relative humidity of 65%. Thirty five days after transferred, plants were taken from the treatments, washed several times with deionized water, oven dried at 70°C for 48h, separated as shoots and roots for Mo and P concentrations analysis, and their dry matter weights (DM) were recorded.

P and Mo Analysis

For the analysis of P concentration, oven-dried samples (about 100 mg) were digested in a sulfuric acid (H_2SO_4): perchloric acid (HClO_4) mixture (95:5). P concentrations in the digests were determined by the vanadomolybdo-phosphoric yellow colorimetric method using an autoanalyzer (FIAstar 5000, Foss, Hilleroed, Denmark) (Koenig and Johnson, 1942).

Some samples (about 300 mg) were dry-ashed at 550°C for 8h to determine the Mo concentration in plants, which was measured by the polarographic catalytic wave analysis method using a JP-4000 oscilloscope polarograph in a reaction solution (2 mL 2.5 mol L^{-1} sulfuric acid, 1 mL 0.5 mol L^{-1} benzohydroxyacetic acid, and 5 mL saturated sodium chlorate solution) (Wan et al., 1988). Nutrient element accumulations and translocation coefficients were calculated as $\text{DM} \times \text{nutrient concentrations}$ and shoot nutrient element concentrations/roots nutrient element concentrations.

Statistical Analysis

All data were statistically analyzed using ANOVA procedure in SAS 8.1 (SAS Institute, Cary, NC, USA). Main effects of factors and their interactions were assessed following the principle of F statistics, and the means of each treatment were multiply compared by the LSD-test ($P < 0.05$).

TABLE 1 Summary of the analysis of variance showing F- ratio for DM, P concentration and accumulation, and Mo concentration and accumulation in shoots and roots of *Brassica napus* seedlings in a double factorial experiment [3P × 4Mo]

Source of variance	DM		P concentration		P accumulation		Mo concentration		Mo accumulation	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
P	6.75**	NS	844**	294**	382**	141**	6.98**	313.46**	17.78**	129.74**
Mo	8.83**	9.43**	NS	NS	8.45**	8.76**	1432**	625.60**	857**	230.01**
P×Mo	NS	NS	NS	3.97*	3.17*	5.27**	2.71*	127.90**	7.29**	51.00**

NS = F ratio non-significant at $P > 0.05$; *significant at $P < 0.05$; **significant at $P < 0.01$.

RESULTS AND DISCUSSION

Interactive Effects of Mo and P on DM of Shoots and Roots of *Brassica napus* Seedlings

Dry matter of shoots were affected by both solution P and Mo concentrations ($P < 0.01$) (Table 1). Increasing solution P from 1 to 90 mg L⁻¹ stimulated ($P < 0.05$) the shoot DM by 23% and 33% at the levels of 0.01 and 0.1 mg L⁻¹ solution Mo, respectively (Figure 1a). Application of 0.01 mg L⁻¹ solution Mo also increased ($P < 0.05$) shoot DM at the levels of 30 and 90 mg L⁻¹ P. As solution Mo concentrations further increased, however, shoot DM was declined. Increasing solution Mo concentrations had no impact on shoot DM at the level of 1 mg L⁻¹ P. The results exhibited that 0.01 mg L⁻¹ of solution Mo was appropriate for promoting shoots growth of *Brassica napus* seedlings.

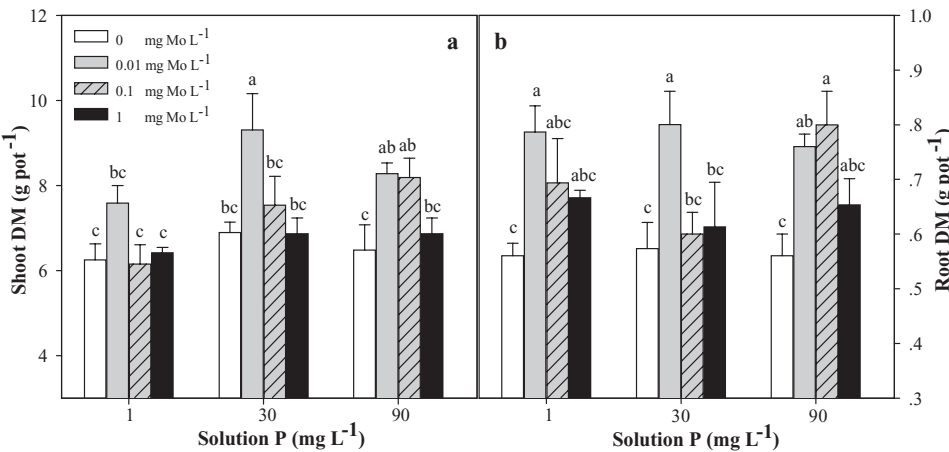


FIGURE 1 Effects of solution concentrations of Mo and P on DM of (a) shoots and (b) roots of *Brassica napus* seedlings. All data are means of three replicates. Vertical bars = ±SE. The different lowercase letters indicate significant differences by the LSD test ($P < 0.05$).

Dry matter of roots were only influenced by solution Mo concentrations ($P < 0.01$) (Table 1). Increasing solution Mo from 0 to 0.01 mg L⁻¹ remarkably enhanced ($P < 0.05$) roots growth at the levels of 1 and 30 mg L⁻¹ solution P, as did increasing solution Mo from 0 to 0.1 mg L⁻¹ at the level of 90 mg L⁻¹ solution P (Figure 1b). The results indicated that 0.01 mg L⁻¹ of solution Mo also had an obvious stimulating effect on root growth for *Brassica napus* seedlings, which were similarly reported by Basak et al. (1982) who suggested that the increase in the growth of rice may be due to the beneficial effect of Mo on the P metabolism of the plants.

Interactive Effects of Mo and P on the Uptake of P in Shoots and Roots of *Brassica napus* Seedlings

Shoot P concentrations were only significantly influenced by solution P concentrations and shoot P accumulations were affected by both solution P and Mo concentrations and interactions of P and Mo (Table 1). Solution Mo concentrations had no influences on shoot P concentrations at the three levels of solution P and shoot P accumulations at the level of 1 mg L⁻¹ P, but it stimulated ($P < 0.05$) shoot P accumulations at the levels of 30 and 90 mg L⁻¹ P (Figure 2a and 2b). These results showed that Mo could increase shoot P uptake of *Brassica napus* when sufficient P was supplied together and there was a positive interaction between Mo and P on the uptake of P.

Root P concentrations were dramatically influenced by solution P concentrations and interactions of P and Mo applications. Root P accumulations were impacted by both solution P and Mo concentrations and interactions of P and Mo (Table 1). Increasing solution Mo exhibited no significant influence on root P concentrations at the treatments of 1 and 30 mg L⁻¹ P, but it increased ($P < 0.05$) that at the treatment of 90 mg L⁻¹ P. The same trend of Mo effects on root P accumulations was also observed (Figure 2c and 2d). These results indicated that there was a synergistic effect between the two elements on P absorption by plants. A similar favorable effect of Mo on the concentration and uptake of P and the synergism between the two elements were also reported by Basak et al. (1982) and Singh and Kumar (1979). However, it has been reported that Mo had a decrease effect on the uptake of P by plants. For example, Mo significantly reduced P concentration of berseem, which might be attributed to the fact that both phosphates and molybdates were absorbed in anionic forms and might compete with each other for the absorption sites (Kumar and Singh, 1980). Molybdenum application resulted in the depression of the activities of acid phosphatase and the decline of the inorganic, organic and total P in mustard plants (Chatterjee et al., 1985). The effects of Mo on P uptake in plants and its possible mechanism are worth to further discussing.

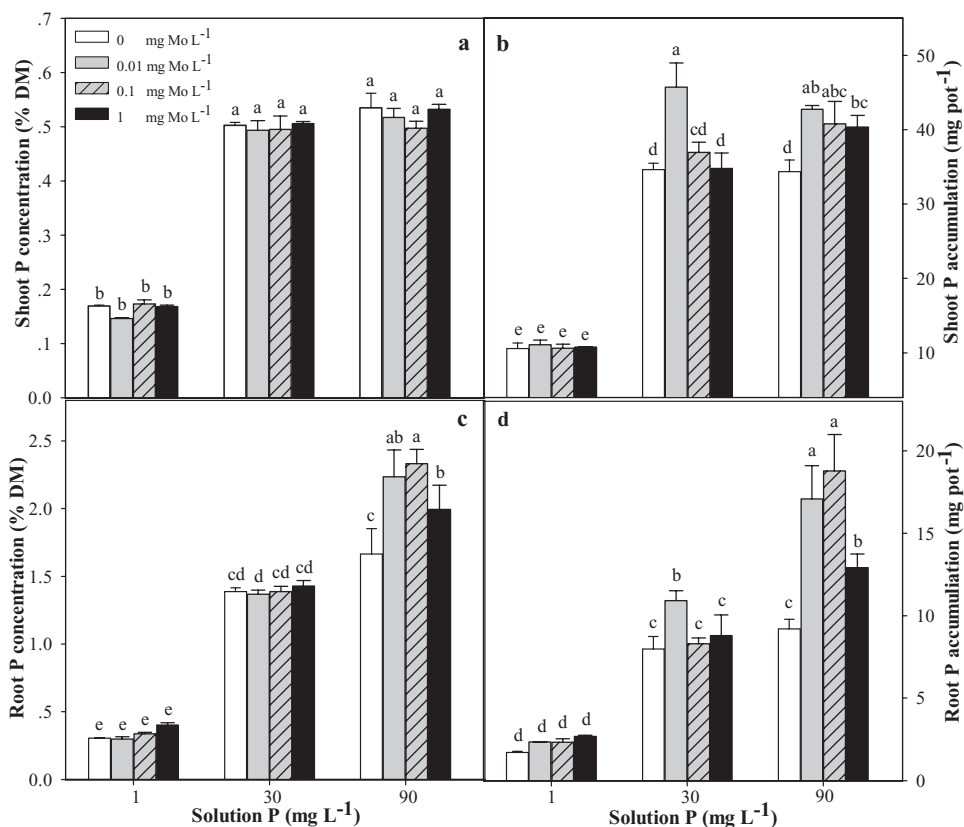


FIGURE 2 Effects of solution concentrations of Mo and P on P concentration and accumulation in (a and b) shoots and (c and d) roots of *Brassica napus* seedlings. All data are means of three replicates. Vertical bars = \pm SE. The different lowercase letters indicate significant differences by the LSD test (*P* < 0.05).

Interactive Effects of Solution Concentrations of Mo and P on the Uptake of Mo in Shoots and Roots of *Brassica napus* Seedlings

Molybdenum concentration and accumulation of shoots and roots were significantly influenced by solution Mo and P concentrations, and interactions of P and Mo (Table 1). As solution Mo concentration increasing, Mo concentrations and accumulations in shoots and roots were raised dramatically (*P* < 0.05) at the three levels of solution P, indicating that increasing solution Mo could enhance the uptake of Mo and improve the Mo nutrition conditions in *Brassica napus*. Shoot Mo concentrations and accumulations were also increased with the increase of solution P concentrations and it had more pronounced differences (*P* < 0.05) at the higher rates of solution Mo (0.1 and 1 mg L⁻¹) (Figures 3a and 3b). The results implied that the increase of solution P concentrations had a stimulating effect on shoot Mo concentration and uptake of *Brassica napus* and there was a positive

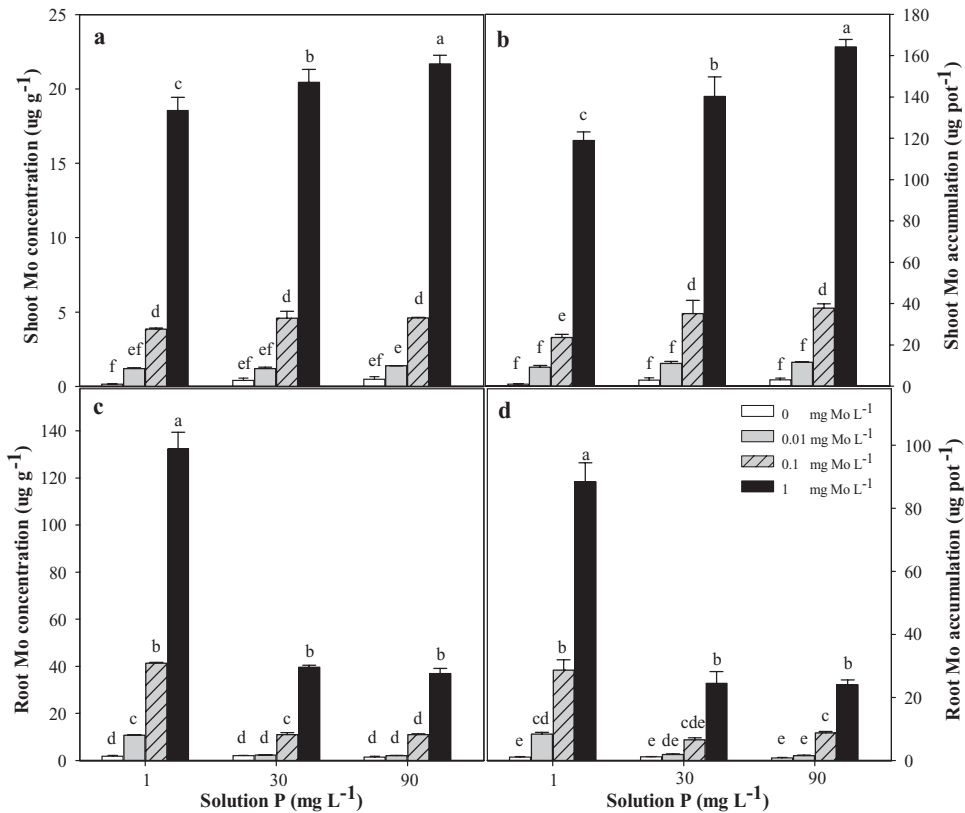


FIGURE 3 Effects of solution concentrations of Mo and P on Mo concentration and accumulation in (a and b) shoots and (c and d) roots of *Brassica napus* seedlings. All data are means of three replicates. Vertical bars = \pm SE. The different lowercase letters indicate significant differences by the LSD test ($P < 0.05$).

interaction between the two elements on Mo absorption by *Brassica napus* seedlings. The results were also reported by others (Stout et al., 1951; Barshad, 1951; Mandal et al., 1998). Some hypotheses have been put forward to explain the positive effects of P on Mo concentration and uptake in plants. For example, P application enhances Mo desorption from soil colloids (Stout et al., 1951; Xie and Mackenzie, 1991) and Mo absorption by plants due to the formation of a complex phosphomolybdate anion (Barshad, 1951). In addition, P application results in the formation of precipitation with iron, aluminum, and calcium components which could bind Mo to organic matter or particle surface, indirectly increasing Mo solubility in soils (Karimian and Cox, 1978). In our experiment, the behaviors of Mo and P in nutrient solution were very different from that in soil solution; however, the favorable influence of P on Mo uptake was also observed, and the possible causes were also worth exploring.

In contrast to shoot Mo concentrations and accumulations, increasing solution P concentrations remarkably depressed ($P < 0.05$) root Mo concentrations and accumulations, especially greater at the higher solution Mo rates (Figures 3c and 3d). A similar result was also observed by Heuwinkel et al. (1992), who inferred that P deficiency induced the increase of transport binding sites in the root cells plasma membrane and molybdate might be taken up by a phosphate transporter, and P deficiency induced increase of the root: shoot ratio and resulted in the increase of the number of these sites in the root cells plasma membrane (Heuwinkel et al., 1992). The stimulating effect of P deficiency on root:shoot ratio was also found in our experiment. In addition, two molybdate transport proteins MOT1 and AMA1 have been identified (Palmgren and Harper, 1999; Tomatsu et al., 2007) and molybdenum can also be absorbed through a sulfate transporter SHST1 in higher plants (Alhendawi et al., 2005; Fitzpatrick et al., 2008). Therefore, the relationship of P with molybdate and sulfate transporters also worth to be further studied.

Interactive Effects of Solution Concentrations of Mo and P on the Translocation of Mo and P from Shoots and Roots in *Brassica napus* Seedlings

Solution Mo concentrations had no influence on [P concentration]_{shoots/roots} at the level of 30 mg L⁻¹ P, but it obviously declined that at 1 and 90 mg L⁻¹ P (Figure 4a). The result implied that Mo addition had an inhibited effect on the translocation of P from roots to shoots at both lower and higher P rates. Therefore, more P was distributed to roots of *Brassica napus* seedlings due to Mo application, and P nutrition status for the

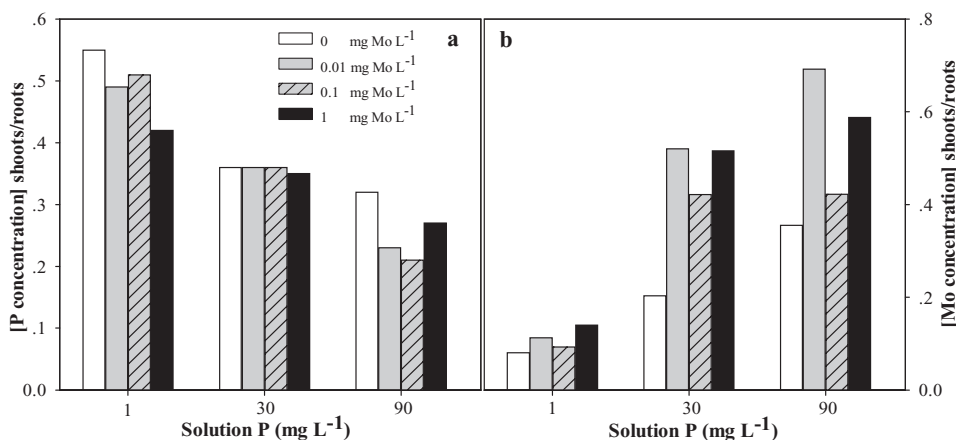


FIGURE 4 Effects of solution concentrations of Mo and P on translocation coefficients of a) P and b) Mo (shoots nutrient element concentrations/roots nutrient element concentrations).

growth of roots was also improved, which was in accordance with the result in this paper that roots growth was markedly enhanced by Mo application.

[Mo concentration]_{shoots/roots} was increased as solution P concentrations increasing, indicating that P addition resulted in stimulated translocation and distribution of Mo from roots to shoots (Figure 4b), which was also consistent with the result in this paper that P addition resulted in the increase of shoot Mo concentration and the decrease of root Mo concentration. The similar result has been reported by Basak et al. (1982) in rice, and a hypothesis that P addition increased the release of Mo from the root cells into the translocation system ensuring its effective utilization by plants, was also raised (Stout et al., 1951). Molybdenum was retained mainly in the roots of plants, particularly in the case of higher Mo supply (Kannan and Ramani, 1978). This result was very meaningful to further understand the effective utilization of Mo and the tolerance of higher Mo concentration for higher plants.

In conclusion, this study provided the nutrient absorption characteristics of Mo and P by *Brassica napus* seedlings when Mo and P nutrition was supplied together, in solution condition. The addition of Mo resulted in the increases of P concentration and uptake when P was applied together and more P nutrition was distributed to roots for its growth. P addition also increased shoot Mo concentration and uptake, but reduced root Mo concentration and uptake, thereby it enhanced the translocation of Mo from roots to shoots. And the effect of P was more remarkable when higher Mo concentration was supplied in nutrient solution. It will be of great importance to further understand the nutrient absorption and translocation characteristics of Mo and P by *Brassica napus* seedling and provide some instructions for the application of Mo and P fertilizer in rapeseed production.

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