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Original Research Paper

Effect of molybdenum on growth and nitrogen metabolism of *Brassica parachinensis* L. and *Brassica integrifolia* L. under drought stress

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ABSTRACT

Molybdenum (Mo) is an essential trace element that plays a critical role in various physiological processes of plants. Drought stress poses a significant threat to plant growth, making it imperative to study the effects of Mo in mitigating its impact on *Brassica parachinensis* L. and *Brassica integrifolia* L. This study aims to investigate the influence of molybdenum on the growth and nitrogen metabolism of Brassica species under drought-stress conditions. The study delves into the physiological and biochemical responses of these plants to Mo supplementation to comprehend the mechanisms by which Mo enhances drought tolerance and nitrogen assimilation. The results revealed that Mo supplementation (150 g ha^{-1}) significantly improves the growth and nitrogen metabolism of Brassica species under drought-stress conditions. In particular, the application of Mo under drought stress leads to a notable increase in yield, as indicated by the improvement in productivity from 3.41 to 4.25 (kg m^{-2}) and 3.89 to 4.97 (kg m^{-2}) in *Brassica parachinensis* and *Brassica integrifolia*, respectively. Furthermore, Mo supplementation enhances chlorophyll levels, thereby promoting efficient photosynthesis. Additionally, it positively affects the accumulation of soluble sugars, starch, and proteins, indicating improved nutrient assimilation and utilization in the plants. These findings suggest that Mo supplementation plays a crucial role in enhancing drought tolerance and nitrogen assimilation in Brassica species. The study highlights the potential of Mo as a valuable tool for improving crop productivity and resilience under drought-stress conditions.

Keywords: Brassica, drought stress, molybdenum, nitrogen metabolism

INTRODUCTION

Molybdenum (Mo) is a trace element that has gained significant attention in plant research due to its essential role in various physiological processes. Mo is a critical component of enzymes involved in nitrogen assimilation, including nitrate reductase, nitrite reductase, and nitrogenase (Kaur et al., 2023). These enzymes convert nitrate into ammonia, which is then used to synthesize amino acids, nucleotides, and other nitrogen-containing compounds (Imran et al., 2019). Thus, Mo is pivotal in ensuring efficient nitrogen utilization and maintaining proper nitrogen balance within plant cells. Furthermore, Mo regulates various enzymatic reactions and redox processes within plant cells (Alamri et al., 2022). It acts as a cofactor for enzymes such as sulfite oxidase, xanthine oxidase, and aldehyde oxidase, which are involved in detoxifying harmful compounds and metabolizing various organic molecules. Mo also participates in the biosynthesis of phytohormones, such as abscisic acid (ABA), which plays a crucial role in plant responses to abiotic stresses, including drought stress (Rana et al., 2020).

Drought stress poses a significant threat to plant growth and agricultural productivity worldwide. In this context, studying Mo's role in mitigating drought stress's detrimental effects on plant growth and metabolism becomes particularly relevant. *Brassica parachinensis* L. and *Brassica integrifolia* L. are two economically important *Brassica* species known for their nutritional value and adaptability to diverse environmental conditions. However, their growth and nitrogen metabolism are severely affected by drought stress. Therefore, exploring the potential role of Mo in mitigating the adverse effects of drought stress on these Brassica species is of great significance.

This study aims to investigate the influence of molybdenum on the growth and nitrogen metabolism of *Brassica parachinensis* L. and *Brassica integrifolia* L. under drought-stress conditions. By examining these plants' physiological and biochemical responses to Mo supplementation, we seek to gain insights into the underlying mechanisms by which Mo enhances drought tolerance and improves nitrogen assimilation.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of the two Brassica species were sown in the soil beds. These soil samples were characterized by specific chemical properties and nutrient compositions: organic matter (24.91 g kg^{-1}), total nitrogen (0.165%), phosphorous (0.062%), potassium (0.93%), zinc (733 mg kg^{-1}), boric (98 mg kg^{-1}). The plants were subjected to two moisture treatments: drought stress (DS) at 65% field capacity and the control at 85% field capacity. After 15 days of seed sowing, the plants were treated with varying concentrations of Na_2MoO_4: 0, 50, 100, 150, and 200 g ha^{-1}. It should be noted that the Mo treatments were applied exclusively within the context of drought stress conditions. After 40 days of growth, various agronomic parameters such as plant height, leaf number, and fresh weight were measured to evaluate plant growth performance. Additionally, physiological and biochemical parameters were assessed.

Chlorophyll and carotenoid content measurement

The content of chlorophyll a, chlorophyll b, and carotenoids in the leaves was measured following the method described by Lichtenthaler & Buschmann (2001). Leaf samples were ground with ethanol, and the extract was centrifuged. A spectrophotometer measured absorption at 664 nm, 648 nm, and 470 nm.

Respiration and photosynthesis rate measurement

Gas exchange capacity, including respiration and photosynthesis rates, was assessed using a CO_2 sensor (EA80, Extech, USA), following the methodology outlined by Thang et al. (2022).

Soluble sugar, starch, and protein content measurement

For soluble sugar quantification, leaf samples were ground with ethanol, and the supernatant was assayed with phenol and sulfuric acid (Dubois et al., 1951). The residue was hydrolyzed for starch quantification, and the supernatant was assayed with the DNS reagent (Masuko et al., 2005). Absorbance readings were taken at 490 nm (soluble sugar) and 540 nm (starch) and converted to soluble sugar and starch content using standard curves. The leaf sample was ground in a phosphate buffer (pH 7.5). After centrifugation, the protein extract was collected. A portion of the protein extract was mixed with the Bradford reagent. The optical density was measured at 595 nm, and the protein content was calculated using a standard albumin curve (He, 2011).

Nitrate, nitrite, ammonium content measurement

The nitrate in the samples was extracted using microwave energy and converted to nitrophenol-disulfonic in an alkaline environment, resulting in a yellow-colored product measured at 410 nm (Middleton, 1958). The nitrite in leaves was extracted using ethanol, and assayed with sulfanilic acid solution to complete the diazotization reaction. Ethyl acetoacetate and sodium hydroxide solutions were added, and the absorbance of the colored azo dye was measured at 356 nm (Sreekumar et al., 2003). In another procedure, the content of ammonium from leaf was extracted with formic acid, and the resulting supernatant was mixed with phenol, sodium nitroprusside, and an oxidizing solution (a mixture of sodium citrate and sodium hypochlorite). The color development occurred at room temperature, and absorbance was measured at 640 nm (Sasongko, 2018).

Statistical analysis

The experiment was replicated three times following a randomized block design, and the obtained data were subjected to analysis of variance (ANOVA). To determine significant differences between means with a 5% probability level, Duncan's Multiple Range Test was performed using SPSS 20.0 software. The results are presented as means accompanied by their corresponding standard deviations.

RESULTS AND DISCUSSION

Effect of molybdenum on plant growth under drought stress

In the context of *Brassica parachinensis* L., adverse drought conditions have been shown to result in a significant reduction in plant height, leaf count, fresh weight, and yield. However, the application of molybdenum at varying concentrations has demonstrated significant ameliorative effects on the growth of these plants. Specifically, Mo application at a concentration of 150 g ha^{-1} has shown growth

Table 1 : Effect of molybdenum on shoot height and leaf number of *Brassica parachinensis* L. and *Brassica integrifolia* L. under drought stress at harvest time

Parameter	Mo treatments (A)	Varieties (B) *Brassica parachinensis*	*Brassica integrifolia*	Mean (A)
Shoot height (cm)	Control	17.93	20.53	19.23[a]
	Drought stress (DS)	13.00	15.33	14.17[c]
	DS + Mo 50	15.23	16.87	16.05[b]
	DS + Mo 100	16.87	18.50	17.68[ab]
	DS + Mo 150	17.87	20.10	18.98[a]
	DS + Mo 200	15.93	19.23	17.58[ab]
	Mean (B)	16.13[b]	18.42[a]	-
Significance of fixed factors		F_A = 98.40**	F_B = 212.7**	F_{AB} = 2.69 [ns]
Leaf number	Control	9.67	8.10	8.88[a]
	Drought stress (DS)	7.20	5.23	6.22[b]
	DS + Mo 50	7.47	5.77	6.62[b]
	DS + Mo 100	8.60	6.93	7.77[a]
	DS + Mo 150	9.17	8.17	8.67[a]
	DS + Mo 200	8.10	7.83	7.97[a]
	Mean (B)	8.37[a]	7.01[b]	-
Significance of fixed factors		F_A = 73.65**	F_B = 30.65**	F_{AB} = 2.59 [ns]

Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

Table 2 : Effect of molybdenum on fresh weight and yield of *Brassica parachinensis* L. and *Brassica integrifolia* L. under drought stress at harvest time

Parameter Mean (A)	(A)	Mo treatments *Brassica parachinensis*	*Brassica integrifolia*	Varieties (B)
Fresh weight (g)	Control	54.00	62.43	58.22 [a]
	Drought stress (DS)	42.67	48.67	45.67 [d]
	DS + Mo 50	46.23	54.67	50.45 [c]
	DS + Mo 100	51.40	56.73	54.07 [b]
	DS + Mo 150	53.07	62.13	57.60 [a]
	DS + Mo 200	52.10	60.67	56.38 [a]
	Mean (B)	49.91 [b]	57.55 [a]	-
Significance of fixed factors		F_A = 48.41**	F_B = 179.50 **	F_{AB} = 1.25 [ns]
Yield(kg m^{-2})	Control	4.32	4.99	4.66 [a]
	Drought stress (DS)	3.41	3.89	3.65 [d]
	DS + Mo 50	3.70	4.37	4.04 [c]
	DS + Mo 100	4.11	4.54	4.33 [b]
	DS + Mo 150	4.25	4.97	4.61 [a]
	DS + Mo 200	4.17	4.85	4.51 [a]
	Mean (B)	3.99 [b]	4.60 [a]	-
Significance of fixed factors		F_A = 48.41**	F_B = 179.50**	F_{AB} = 1.25 [ns]

Values with different letters in a column are significantly different according to Duncan's test (p=0.05) enhancements that are comparable to the non-stressed control group. Correspondingly, the application of Mo at concentrations of 150 and 200 g ha^{-1} has been found to increase the yield of *Brassica integrifolia* L. plants experiencing drought stress, with the yield increment being equivalent to that observed in the control group (Fig.1). Moreover, the statistical analysis results indicate that there is no interaction between the planting variety factor and the Mo treatment factor on the growth parameters (Table 1 & 2).

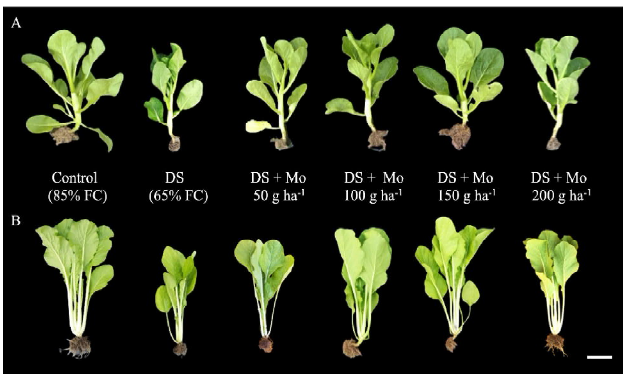

Fig. 1 : Effect of molybdenum on the growth of *Brassica parachinensis* L. (A) and *Brassica integrifolia* L. (B) under drought stress (DS). Scale bar = 5 cm

Changes in chlorophyll and carotenoid content

Under drought stress, both *Brassica* species experience a significant decrease in chlorophyll a and b levels, while carotenoid content increases in comparison to the control group. However, administering Mo results in an increase in both chlorophyll a and b levels, while carotenoid levels decrease. Notably, when Mo is applied at concentrations of 150 g ha^{-1} or higher, chlorophyll a and b levels are similar to those of the control group (Fig. 2).

Changes in respiration and photosynthesis rate

During periods of drought, the rate of photosynthesis in Brassica species experiences a significant decrease, dropping by approximately 40% compared to the control group. However, Mo application at concentrations ranging from 50 to 200 g ha^{-1} gradually improves the intensity of photosynthesis. The best results are seen with a Mo treatment concentration of 150 g ha^{-1}, which is similar to that of the control group. Conversely, drought stress causes the respiratory rate to intensify, increasing by around 20% when compared to the control. On the other hand, Mo application reduces the respiratory intensity of the leaves in both Brassica species (Fig. 2).

Changes in soluble sugar, starch, and protein content

In *Brassica* species, exposure to drought stress has been found to result in a reduction in starch, total sugars, and protein content in leaves. However, the application of Mo has been shown to positively influence the content of these three compounds in

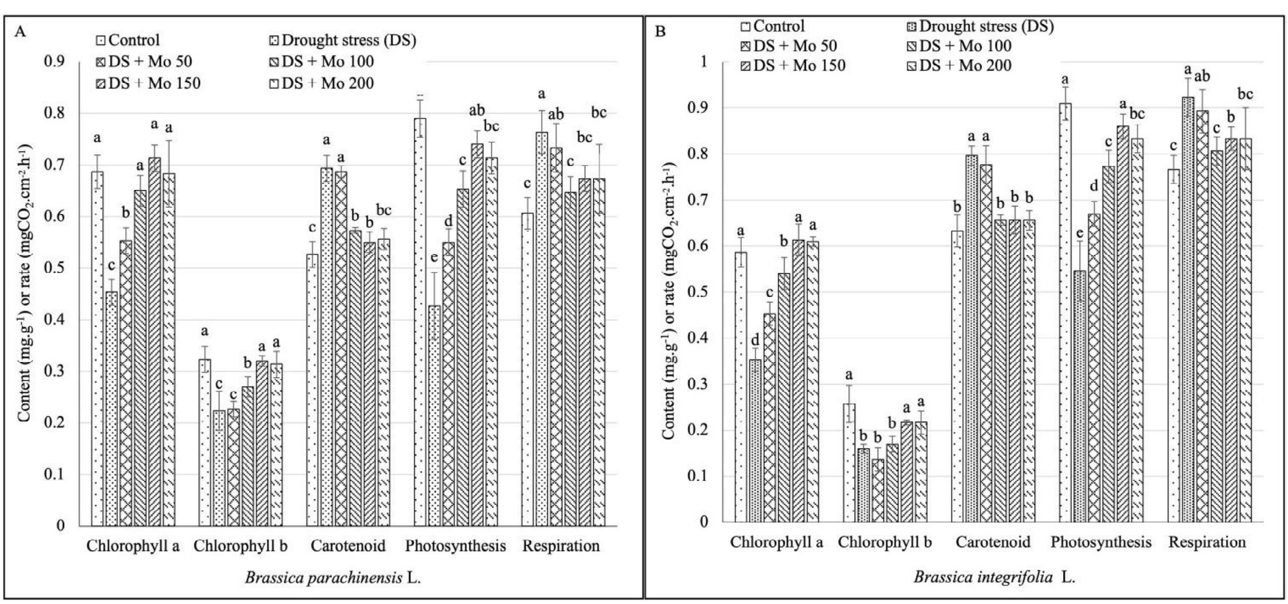

Fig. 2 : Effect of molybdenum on changes in chlorophyll, carotenoid content, respiration and photosynthesis rate of *Brassica parachinensis* L. (A) and *Brassica integrifolia* L. (B) under drought stress (DS). Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

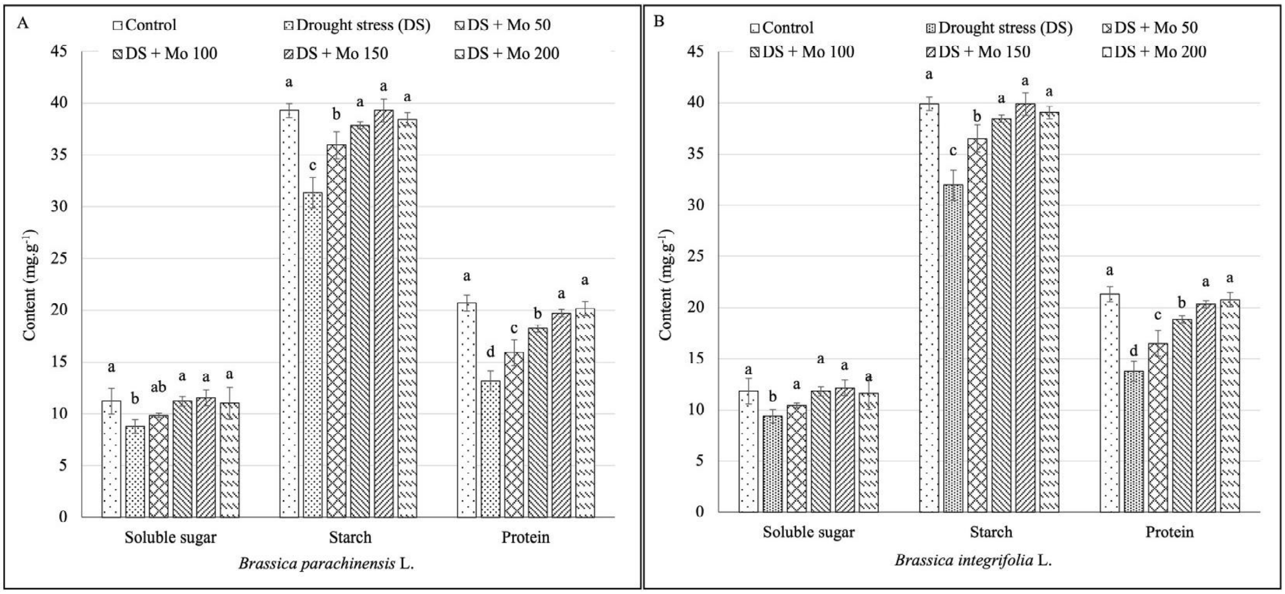

Fig. 3 : Effect of molybdenum on changes in soluble sugar, starch, and protein content of *Brassica parachinensis* L. (A) and *Brassica integrifolia* L. (B) under drought stress (DS). Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

leaves. Specifically, the optimal concentration of Mo was observed to be at 150 g ha^{-1}, beyond which increasing the Mo concentration to 200 g ha^{-1} did not result in any significant changes in these values (Fig. 3).

Changes in nitrate, nitrite, ammonium content

Both Brassica species have significantly lower nitrite content in their leaves when compared to nitrate and ammonium. Under drought conditions, the levels of these three compounds decrease. On treatment with Mo, only the nitrate content increases. However, it remains lower than that in the control group. Meanwhile, the nitrite and ammonium levels continue to decrease with increasing Mo treatment concentration (Fig. 4).

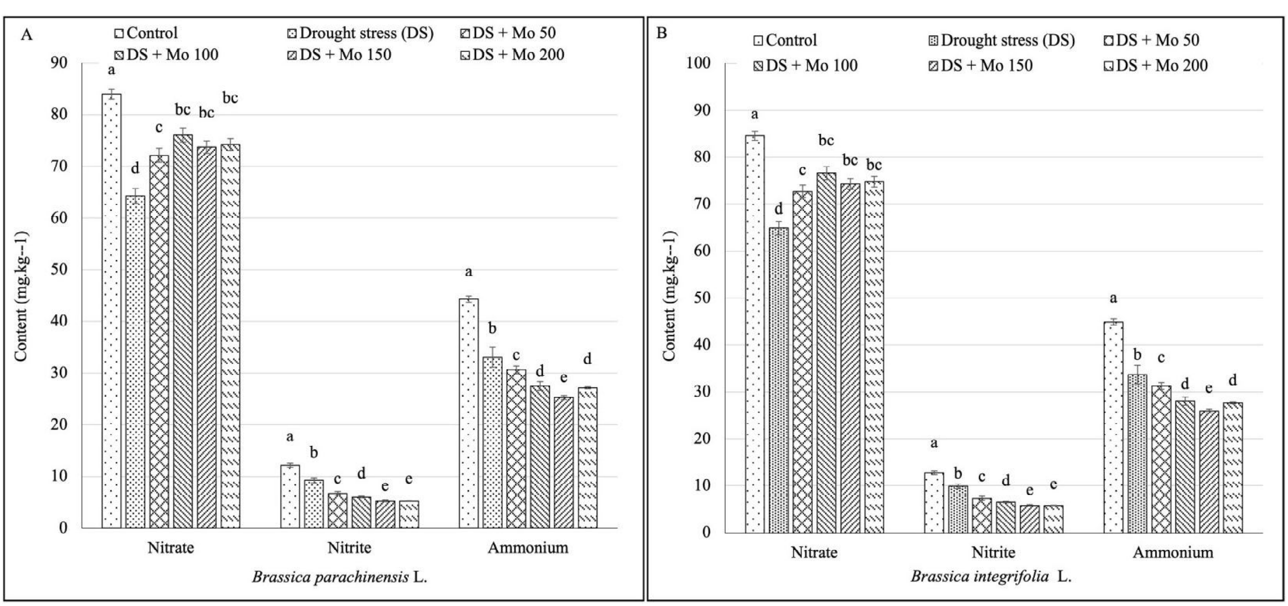

Fig. 4 : Effect of molybdenum on changes in nitrate, nitrite, ammonium content of *Brassica parachinensis* L. (A) and *Brassica integrifolia* L. (B) under drought stress (DS). Values with different letters in a column are significantly different according to Duncan's test (p=0.05)

Under conditions of drought stress, plants undergo a series of physiological changes that include alterations in pigment composition. In the case of *Brassica* species, exposure to drought stress leads to a notable decrease in the levels of chlorophyll a and b, while, the content of carotenoid increases as compared to the control group. Research has shown that the administration of Mo to drought-stressed plants has a positive influence on chlorophyll levels (Fig. 2). The addition of Mo increases the levels of chlorophyll a and b, bringing them closer to the levels observed in the control group. Mo is hypothesized to play an essential role in enzymatic reactions within the chlorophyll biosynthesis pathway, contributing to its positive impact. As a cofactor for enzymes involved in chlorophyll synthesis, Mo enhances the production and stability of chlorophyll molecules (Chen et al., 2023). Interestingly, Mo application also leads to a decrease in carotenoid levels in drought-stressed plants. Mo supplementation may alter the balance between chlorophylls and carotenoids, favoring chlorophyll accumulation while reducing carotenoid synthesis (Rudi et al., 2023). By enhancing chlorophyll synthesis and maintenance, Mo ensures an optimal photosynthetic pigment content, improving photosynthetic efficiency. This leads to increased synthesis of soluble sugar, which is the building block for starch and protein (Fig. 3). Additionally, Mo application has been found to reduce the respiratory intensity of leaves in Brassica species under drought stress (Fig. 2). This observation suggests that Mo supplementation helps optimize energy utilization and reduces excessive energy consumption through respiration. By regulating respiratory processes, Mo contributes to better resource allocation and improved plant adaptation to drought stress.

Nitrite, nitrate, and ammonium are different nitrogen compounds that play an essential role in plant nutrition. This research has shown that under drought conditions, the levels of these nitrogen compounds, including nitrite, nitrate, and ammonium, decrease in the leaves (Fig. 4). Drought stress can affect various physiological processes, including nitrogen uptake, assimilation, and metabolism, reducing nitrogen availability and altering nitrogen compound concentrations. However, Mo treatment has been found to increase the nitrate content in the leaves. Mo treatment can improve the plant’s ability to take up nitrate as it interacts with cellular structures and transport processes within the plant (Zayed et al., 2023). This leads to higher efficiency in nitrate uptake from the environment, increasing plant nitrate availability. Furthermore, the levels of nitrite and ammonium continue to decrease with increasing Mo treatment concentrations (Fig. 4). Mo acts as a cofactor for nitrate reductase, influencing the activity of enzymes involved in nitrite and ammonium metabolism (Mendel, 2022). Stronger enzyme activity may result in faster conversion of nitrite and ammonium to other nitrogen compounds, leading to lower levels of nitrite and ammonium in the leaves.

CONCLUSION

The application of molybdenum has shown significant ameliorative effects on the growth and physiology of Brassica species under drought stress. Mo enhances nutrient metabolism by stimulating nitrogen assimilation, starch synthesis, and protein production. It promotes chlorophyll synthesis, leading to improved photosynthesis and growth. Furthermore, Mo regulates respiration, conserving energy and enhancing plant resilience to drought conditions. Further research is warranted to explore optimal Mo concentrations and application methods to maximize its benefits in drought-stressed *Brassica* species.

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