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ASSESSMENT OF GENETIC STABILITY AND PHOTORECEPTOR GENE EXPRESSION (*CmPHYA* AND *CmPHYB*) OF *Chrysanthemum indicium* PLANTLETS CULTURE ON MEDIUM CONTAINING MOLYBDENUM TRIOXIDE NANOPARTICLES

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ABSTRACT

This study investigated the effects of molybdenum trioxide nanoparticles (MoO_3NPs) on the rooting, genetic stability, photosynthesis-related gene expression, and nursery-stage growth of *Chrysanthemum indicum*. The rooting stage analysis revealed that the 6.4 µg/L MoO_3NPs treatment significantly enhanced rooting rate, plantlet height, leaf number, root number, root length, SPAD value, and fresh weight compared to control treatments. ITS sequence analysis confirmed that MoO_3NPs did not induce mutations or genetic instability, with the ITS sequences of treated plantlets showing 100% similarity with those in the NCBI database and control samples. MoO_3NPs supplementation also upregulated the expression of *CmPHYA* and *CmPHYB*, critical genes encoding photoreceptors involved in red and far-red light perception, enhancing photosynthetic efficiency. At the nursery stage, plantlets derived from MoO_3NPs-supplemented media demonstrated superior growth parameters, including plant height, leaf and root numbers, fresh and dry weight, and SPAD value. These findings underscore the potential of MoO_3NPs to improve plantlet

development, photosynthetic performance, and nutrient absorption, thereby highlighting their role in sustainable plant production systems.

Keywords: *Chrysanthemum indicum*, *in vitro* culture, gene expression, genetic stability, molybdenum trioxide nanoparticles.

INTRODUCTION

In recent years, metal nanoparticles have garnered significant attention due to their potential applications in various fields, including plant science (Tymoszuk & Miler, 2019). A diverse range of metal nanoparticles, such as silver (Ag), copper (Cu), iron (Fe), cobalt (Co), and selenium (Se) nanoparticles, have been extensively investigated for their effects on plant micropropagation. Studies indicate that the impact of these nanoparticles on plant growth and development can be both beneficial and detrimental, with outcomes highly dependent on factors such as nanoparticle composition, structure, concentration, and the specific plant species under study (Kim *et al*., 2017; Siddiqui *et al*., 2015). In plant cell technology, there have been reports highlighting the positive effects of metal nanoparticles, including increased germination rates (Gopinath *et al*., 2014) and enhanced growth rates (Tymoszuk & Miler, 2019). For instance, molybdenum trioxide nanoparticles (MoO_3NPs) have been shown to improve growth and optimize nitrate utilization in rice (Zhang *et al*., 2012). Furthermore, MoO_3NPs are readily absorbed, accumulated, transported, and metabolized by plants, thereby facilitating their beneficial effects (Mendel & Leimkühler, 2015). Recent research by Nguyen *et al*. (2024) demonstrated that MoO_3NPs positively influenced shoot regeneration from leaf explants and accelerated shoot multiplication from chrysanthemum stem node explants under *in vitro* conditions. Additionally, residual MoO_3NPs in shoot explants promoted rooting and improved certain physiological and biochemical traits of chrysanthemum plantlets (Nguyen *et al*., 2024).

Despite the increasing use of nanotechnology in agriculture, limited research has been conducted on the genotoxic effects of nanoparticles in plants, with most studies focusing on mammals and bacteria (Mehrian & De Lima, 2016). Nanoparticles can exert genotoxic effects either directly or indirectly. In the direct mechanism, nanoparticles penetrate the cell and nuclear membranes and interact with DNA via mechanical or chemical bonds. Indirect genotoxicity results from interactions with nuclear proteins involved in replication, transcription, translation, and oxidative stress (Mehrian & De Lima, 2016). Chromosomal abnormalities, such as chromatin bridges, adhesions, metaphase disturbances, and chromosome breakages, have been reported in the root tips of *Vicia faba* treated with silver nanoparticles (Patllola *et al*., 2012), and in the root tip meristem of *Allium cepa* exposed to zinc oxide nanoparticles (Kumari *et al*., 2011), among others (Ghosh *et al*., 2019). These findings suggest that nanoparticles can influence plant growth, metabolism, and phenotype. However, the effects of nanoparticles on plant development are intricately linked to their chemical composition, size, shape, surface area, surface coatings, concentration, synthesis

method (chemical or biological), as well as the genotype, age, developmental stage, and chemical environment of plant cells (Barbasz *et al*., 2018).

The utility of molecular biology techniques for assessing genetic stability has been highlighted in previous studies. Methods such as Sequence-Related Amplified Polymorphism (SRAP), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), and Restriction Fragment Length Polymorphism (RFLP) have been successfully employed (Bekheet *et al*., 2015; Narula *et al*., 2007). Recently, DNA barcoding has emerged as a promising tool for genetic stability studies. This molecular technique relies on short conserved DNA sequences to identify plant species, overcoming the limitations posed by morphological and physiological variations (Wang *et al*., 2015). DNA barcoding is particularly useful in plant tissue culture to ensure genetic uniformity of *in vitro* regenerated plants (Adeniran *et al*., 2018). For example, the trnH-psbA and rbcL regions were used to assess the genetic stability in *Dioscorea bulbifera* L. and *Dioscorea hirtiflora* Benth. (Adeniran *et al*., 2018), whereas the rbcL region was used for genetic analysis in *Artemisia vulgaris* L. tissue culture (Jogam *et al*., 2020). The Internal Transcribed Spacer (ITS) region has also proven to be an effective DNA barcode, particularly for species discrimination and sequence recovery across a wide range of plant groups, particularly herbaceous species (Chen *et al*., 2010; Yao *et al*., 2010).

In the present study, the ITS region was used to evaluate the genetic stability of *Chrysanthemum indicum* cultured on medium supplement MoO_3NPs. In addition, the expression of photosensitive genes in chrysanthemum plants grown *in vitro* was examined. The growth performance of chrysanthemum plantlets derived from MoO_3NPs-containing medium was also recorded under nursery conditions, contributing to a broader understanding of the effects of MoO_3NPs on plant development and genetic integrity.

MATERIALS AND METHODS

Plant material

One-month-old *in vitro* chrysanthemum (*Chrysanthemum indicum*) shoots were cultured on Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) (Sigma-Aldrich, USA) supplemented with 8 g/L agar (Viet Xo, Viet Nam), 30 g/L sucrose and without plant growth regulators (PGRs) and were used as initial material.

Nano molybdenum trioxide solution

MoO_3NPs solution (nanoparticles with an average size from 20 to 60 nm) was provided by the Institute of Environmental Technology (VAST). The initial concentration of MoO_3NPs solution was 500 mg/L.

Effects of MoO_3NPs on rooting stage

One-month-old *in vitro* chrysanthemum shoots were cultured on MS medium, replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs at different concentrations (1.6, 3.2, 6.4, 9.6, and 12.8 µL/L corresponding to 1/4, 1/2, 1, 3/2, and 2 times the $Na_2MoO_4.2H_2O$

concentration present in the standard MS medium). The negative control [C(-)] was MS medium without $Na_2MoO_4.2H_2O$ salt and the positive control (MS) was standard MS medium. Rooting rate (%), plantlet height (cm), number of leaves, number of roots, root length (cm), SPAD (SPAD-502, Minolta Co., Ltd., Japan), and fresh weight (mg) were measured after 15 days of culture.

Total DNA extraction, PCR reaction and sequence analysis

The young leaves of the three studied samples were isolated using the modified CTAB 2X method (Aboul-Maaty & Oraby, 2019). The ITS region (White, 1990) was amplified on a Mastercycler PCR machine (Eppendorf, Germany) with the following reaction components: 12.5 µL master mix (Phu Sa, Vietnam); 1.25 µL (10 µM concentration) for each forward and reverse primer (ITS1: 5' TCCGTAGGTGAACCTGCGG 3'/ITS4 :5' TCCTCCGCTTATTGATATGC 3'); 9.0 µL of deionized water and 1.0 µL of template DNA. The PCR reaction was performed with the following thermal cycle: 5 minutes at 95^{o}C; 35 cycles including (1 minute at 94^{o}C, 1 minute at 55^{o}C, and 1 minute 30 s at 72^{o}C); and extension at 72^{o}C°C for 10 min. The PCR products were electrophoresed on a 1.0% agarose gel and then sequenced at APOLLO Company Limited, Thu Duc City, Ho Chi Minh City, Vietnam) using the ABI 3500 machine: DNA analysis system 3500 Series Genetic Analyzer (Applied Biosystems™ 3500 XL Genetic Analyzer). The ITS sequences were aligned using the FinchTV and SeaView software. The Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI) database was used to search for homologous gene regions. Finally, the ITS sequences of the three studied samples were aligned with the ITS sequences of *Chrysanthemum* species using the BioEdit software. The ClustalX2 and Bioedit software were used to to align studied sequences with those of four taxa of C. *indicum* from NCBI database with the accession number of KC694453, KC694311, KC694223, and KC694224 (Li *et al*., 2014).

Gene expression assessment

In this study, RNA was extracted from cultures grown on three distinct media using a modified Trizol-based protocol (Mai *et al*., 2016). Residual DNA contamination was eliminated by treatment with DNase I (Thermo Scientific). The purified RNA served as the template for complementary DNA (cDNA) synthesis, which was performed using the RevertAid H Minus Reverse Transcriptase Kit (Thermo Scientific). The resulting cDNA was subsequently used for quantitative real-time polymerase chain reaction (qRT-PCR) to assess the expression levels of two photoreceptor-associated genes, *CmPHYA* and *CmPHYB*.

qRT-PCR was conducted using SYBR Green qPCR Master Mix (Thermo Scientific), according to the manufacturer's protocol. Specific primer sets were designed and used for the amplification of *CmPHYA* (Forward primer: 5'-AAAGGGTGGATAGCGAGGGT-3', Reverse primer: 5'-CCAAATACCCGTCTACGATGC-3') and *CmPHYB* (Forward primer: 5'-GGAGAAACTCACAGGGTGGG-3',

Reverse primer: 5'-ACAAAAAGGCACCCGTAACCT-3').

The reaction mixture for qRT-PCR was prepared as follows: 1 µL of cDNA (25 ng/µL), 7 µL of nuclease-free water, 7.5 µL of SYBR Green Master Mix, 0.5 µL of forward primer (50 ng/µL), and 0.5 µL of reverse primer (50 ng/µL), resulting in a total reaction volume of 16.5 µL. Amplification was performed under the following thermal cycling conditions: an initial denaturation step at 94°C for 5 min, 35 cycles of denaturation at 94°C for 50 s, annealing at a primer-specific temperature (50 - 60°C depending on the melting temperature [Tm] of the primers) for 50 s, and extension at 72°C for 90 s, followed by a final extension step at 72°C for 5 min. This experimental approach ensured robust quantification of gene expression, enabling the precise evaluation of photoreceptor-related genes under varying culture conditions.

The effect of MoO_3NPs on the growth of chrysanthemum plantlets in the greenhouse

Plantlets derived from the DC, MS, and 1MoO_3NP treatments were transferred to a greenhouse and grown on TS1 clean soil substrate (Vietnam Flower Seeds Co., Ltd., Ho Chi Minh City). Each experiment involved 30 plantlets. Parameters such as plant height (cm), number of leaves per plant, number of roots per plant, fresh weight (g), dry weight (g), and SPAD were measured after 30 days.

Statistical analysis

All experiments in this study were repeated three times with 30 explants/treatments. All data were processed using Microsoft Excel 2016 software and SPSS 20.0, and statistical analysis was performed using Duncan's test method with $P < 0.05$ (Duncan, 1955).

RESULTS AND DISCISSION

Effect of MoO_3NPs on rooting stage of chrysanthemum plantlets

The effects of MoO_3NPs on the rooting stage of chrysanthemum plantlets after 15 days of culture were recorded (Table 1 and Figure 1). Significant differences were observed across treatments in parameters such as rooting rate, plantlet height, number of leaves, number of roots, root length, SPAD value, and fresh weight. The 6.4 µg/L MoO_3NPs treatment consistently outperformed others, achieving the highest plantlet height (18.67 cm), number of leaves (12.73), number of roots (14.67), root length (59.60 cm), SPAD value (59.60), and fresh weight (0.47 g), indicating it as the optimal concentration for promoting rooting and growth.

The negative control (without MoO_3NPs and without $Na_2MoO_4 \cdot 2H_2O$) exhibited the lowest performance, while the positive control (MS medium with $Na_2MoO_4 \cdot 2H_2O$) showed moderate improvements but was outperformed by MoO_3NPs treatments. Concentrations of 3.2 µg/L and 9.6 µg/L MoO_3NPs also enhanced growth, but were less effective than 6.4 µg/L. At 12.8 µg/L, a decline in growth was observed, suggesting toxicity at higher concentrations.

Nguyen *et al*. (2024) demonstrated that the rooting capacity of chrysanthemum (*Chrysanthemum morifolium*) plantlets cultured on media containing MoO_3NPs

was significantly superior to that observed in plantlets grown on conventional MS culture medium. These results highlight the importance of MoO_3NPs in improving the rooting and growth of chrysanthemum plantlets, with the 6.4 µg/L treatment being the most effective.

Table 1. The rooting stage of chrysanthemum plantlet culture on medium supplemented with MoO_3NPs after 15 days of culture

Treatment (µL/L)	Rooting rate (%)	Plantlet height (cm)	No. of leaf	No. of root	Root length (cm)	SPAD	Fresh weight (g)
C(-)	100a*	10.50f	7.33d	4.77d	7.33d	31.90d	0.25c
MS		12.93d	14.67b	6.53c	12.00b	43.83b	0.38b
1.6		11.70e	11.00c	5.37d	7.67d	31.13d	0.28c
3.2		14.03c	15.00b	7.40b	9.67c	41.60b	0.35b
6.4		18.83a	18.67a	12.73a	14.67a	59.60a	0.47a
9.6		14.80b	13.67b	7.97b	10.67c	35.17c	0.38bb
12.8		12.57d	11.67c	6.43c	7.33d	36.50c	0.18d

C(-): MS medium without $Na_2MoO_4.2H_2O$

MS: MS medium with $Na_2MoO_4.2H_2O$

*Mean ± SD (Std. Deviation) and values with different letters are significantly different among treatments

Figure 1. Effect of MoO_3NPs on rooting stage of chrysanthemum plantlets after 15 days of culture [C(-), MS, 37.25, 74.5, 149, 223.5 and 298 µL/L; left to right]. Bar = 2 cm.

Evaluation of genetic stability of chrysanthemum plantlets cultured on medium containing MoO3NPs

The final length of the ITS sequences of all studied samples was 540 bp, and these sequences were deposited in the GenBank database with the accession numbers PP982908, PP982909, and PP982910, respectively. Figure 2 shows that the ITS sequences of the three specimens from this study and four taxa of *C. indicum* from the GenBank database (KC694453, KC694311, KC694223, and KC694224) had 100% similarity, whereas their ITS regions differed from those of other

Chrysanthemum species (Li *et al*., 2014). Additionally, the studied samples have the morphological characteristics of *C. indicum*: upright plants, grooved and short-haired stems, pointed, oval-shaped, lobed leaves with many teeth, green leaves on both sides, yellow flower heads, short stalks, etc. (Sha *et al*., 2020). Therefore, careful examination of its morphological features and ITS region indicated that the specimens studied in this study were *C. indicum*.

The results showed that *C. indicum* cultured in the medium replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs ($1MoO_3$NPs) showed no change in the ITS region compared to that of the positive control sample (MS sample). Notably, the removal of $Na_2MoO_4.2H_2O$ from the culture medium [C(-)] of *C. indicum* did not differ in the ITS region. In addition, the ITS regions of these samples had 100% similarity with those of *C. indicum* published in the NCBI database (Figure 2) (Li *et al*., 2014).

According to Gao *et al*. (2023), nanoparticles that penetrate plant cells and leaves can transport DNA and chemicals into plant cells, thereby affecting the level of genetic stability. Recent studies have shown that heavy metals such as Cd, Pb, Co, Ni, and Zn cause changes in DNA methylation (Gao *et al*., 2023). However, studies on the effects of nanoparticles on the induction of specific gene mutations in plants and changes in DNA methylation have been limited. Determining the effect of nanomaterials on the genetic stability of crops is important; in particular, the use of nanomaterials in fertilizers as well as applications in plant cell technology has become increasingly popular. The current study is the first to investigate the effects of MoO_3NPs on plant stability under *in vitro* culture conditions. Adding MoO_3NPs at appropriate concentrations to the culture medium not only helps increase the growth and development of plantlets (Nguyen *et al*., 2024) but also does not change the DNA sequence in chrysanthemum plantlets.

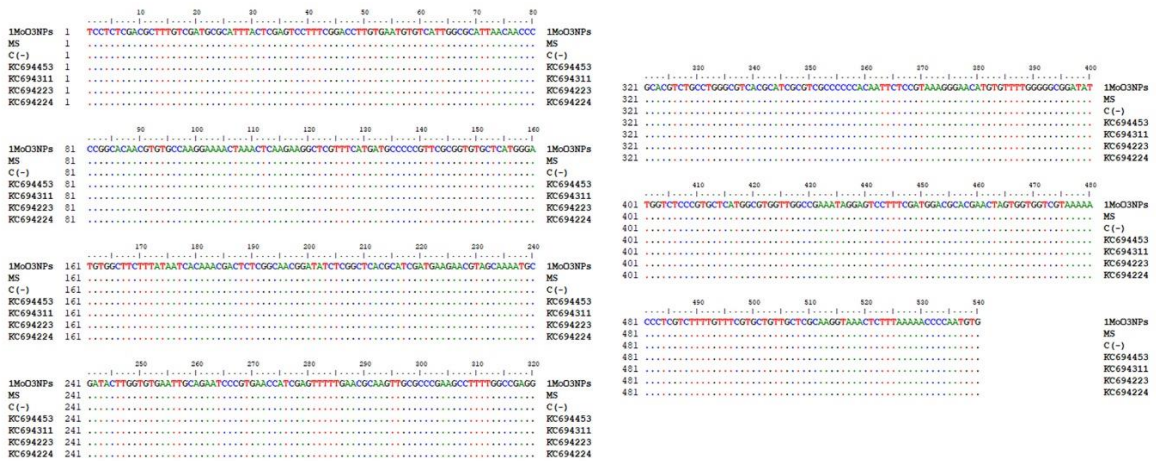

Figure 2. Pairwise sequence alignment of ITS regions between three studied samples and other Chrysanthemum plants on the NCBI database. Note: dots (.) show the positions of similarity between sequences. $1MoO_3$NPs: MS medium replacing $Na_2MoO_4.2H_2O$ by MoO_3NPs at an equivalent concentration. C(-): MS medium without $Na_2MoO_4.2H_2O$. MS: MS medium with $Na_2MoO_4.2H_2O$.

Effect of molybdenum trioxide nanoparticles on photosensitive gene expression

The gene expression levels of *CmPHYA* and *CmPHYB* are shown in Figure 3. Both genes exhibited the highest expression levels in chrysanthemum plantlets derived from cultures supplemented with MoO_3NPs followed by explants from the MS medium treatment. The lowest expression levels were observed in the C(-) treatment. These results indicate that substituting $Na_2MoO_4 \cdot 2H_2O$ with MoO_3NPs is the most effective approach for stimulating the expression of *CmPHYA* and *CmPHYB*. Conversely, the absence of Mo^{3+} in the culture medium has a detrimental effect on the expression of these genes, ultimately impairing the photosynthetic capacity of chrysanthemum plantlets.

Light serves as the primary energy source for photosynthesis and is a critical environmental factor that influences plant growth and development (Li *et al*., 2011). Variations in light intensity, duration, and quality significantly affect plant morphology and physiological responses (Roso *et al*., 2020). Previous research has demonstrated that light quality influences leaf photosynthetic pigments (Wang *et al*., 2022), flowering time, and stress tolerance, primarily through the regulation of gene expression (Shang *et al*., 2023). *CmPHYA* and *CmPHYB* encode red light photoreceptors (Casal *et al*., 2014; Legris *et al*., 2016), which play pivotal roles in light perception and response, thereby influencing numerous growth and developmental processes in plants. *CmPHYA* encodes the phytochrome A protein, which primarily detects light in the far-red spectrum (Casal *et al*., 2014). This gene is involved in critical processes such as seed germination, stem elongation, and flower development. Moreover, *CmPHYA* enables plants to respond to shade conditions by sensing the red to far-red light ratio, which is altered in dense canopies where neighboring plants absorb red light and reflect far-red light (Legris *et al*., 2016).

CmPHYB encodes the phytochrome B protein, which is primarily sensitive to red light and plays a dominant role in regulating light responses under natural sunlight conditions. *CmPHYB* influences key developmental processes, including seedling growth, stem elongation, and flowering time, in response to diurnal light and dark cycles (Legris *et al*., 2016). In the present study, the enhanced expression of *CmPHYA* and *CmPHYB* under *in vitro* conditions enabled seedlings to better absorb red light, thereby improving their photosynthetic capacity and growth potential when transitioned to nursery conditions.

The application of MoO_3NPs significantly enhanced photosynthesis and plant performance owing to their role in boosting photosynthetic efficiency and mitigating oxidative stress. Zheng *et al*. (2020) reported that MoO_3NPs improve the conversion of light energy into chemical energy during photosynthesis, thereby increasing photosynthetic efficiency. Additionally, these nanoparticles minimize oxidative stress-induced damage and enhance crop yield and plant tolerance to adverse environmental conditions. Overall, these findings underscore the potential of MoO_3NPs in improving photosynthetic performance and promoting robust plant growth by upregulating photoreceptor-

related genes and mitigating abiotic stress factors.

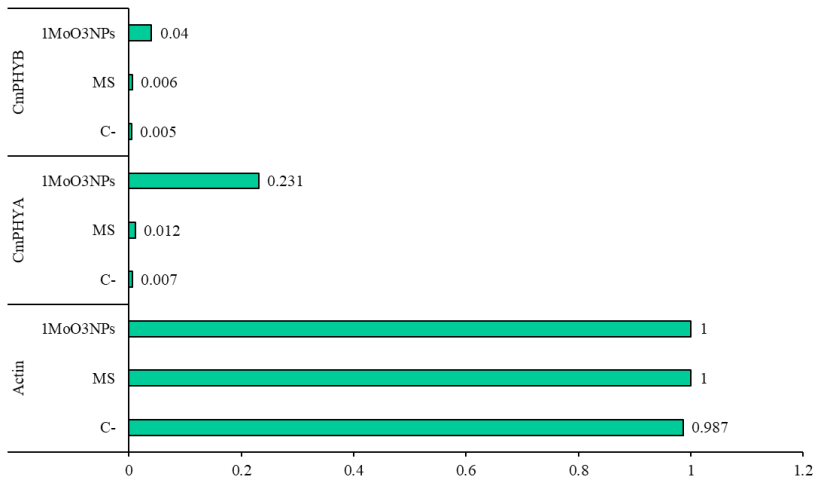

Figure 3. Expression of genes related to photosynthesis in chrysanthemum plantlets. 1MoO_3NPs: MS medium replacing $Na_2MoO_4.2H_2O$ by MoO_3NPs at an equivalent concentration. C(-): MS medium without $Na_2MoO_4.2H_2O$. MS: MS medium with $Na_2MoO_4.2H_2O$.

Effect of nano molybdenum trioxide on the growth of chrysanthemum plants at nursery stage

The replacement of $Na_2MoO_4.2H_2O$ with MoO_3NPs in the culture medium had a positive effect on all growth parameters of seedlings after four weeks under nursery conditions, including plant height (12.133 cm), number of leaves (8.333 leaves/ plant), number of roots (14.333 roots/plant), fresh weight (2.431 g), dry weight (0.439 g), and SPAD (46.433). Meanwhile, the lack of Mo in the culture medium caused poor growth of chrysanthemum plants under nursery conditions, yellow leaves, short plants, and little formation of new leaves (Table 2 and Figure 4). This suggests that MoO_3NPs can improve the nutrient absorption efficiency of plants, thereby promoting comprehensive development of the root system, leaves, and biological mass of chrysanthemum plants. In addition, MoO_3NPs have a positive effect on enhancing the expression levels of the two genes *CmPHYA* and *CmPHYB* (Figure 4), thereby helping plants increase their ability to absorb red light and helping seedlings grow better in nursery conditions.

Table 2. The growth of chrysanthemum plantlets derived from the medium replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs after 30 days in the greenhouse

Treatment	Plant height (cm)	No. of leaf	No. of root	Fresh weight (g)	Dry weight (g)	SPAD
C(-)	9,533c*	6,333b	6,000c	1,253b	0,144c	34,233b
MS	10,867b	7,333ab	10,000b	1,960a	0,257b	36,633b
1MoO_3NPs	12,133a	8,333a	14,333a	2,431a	0,439a	46,433a

C(-): MS medium without $Na_2MoO_4.2H_2O$

MS: MS medium with $Na_2MoO_4.2H_2O$

*Mean ± SD (Std. Deviation) and values with different letters are significantly different among treatments

Molybdenum is a vital trace element found in the soil and is indispensable for plant growth and development. Mo facilitates critical redox reactions across various metabolic pathways and acts as a cofactor for a range of plant enzymes. Among more than 50 enzymes that require Mo, key examples include those involved in nitrogen metabolism, such as nitrogenase (the enzyme responsible for biological nitrogen fixation), nitrate reductase, and enzymes essential for legume nodule functionality. Furthermore, Mo plays a pivotal role in ureide synthesis and purine catabolism by contributing to xanthine dehydrogenase/oxidase activity, highlighting its broad metabolic importance (Zhang *et al*., 2012).

In addition to its role in metabolic processes, Mo also contributes to enhanced plant growth and abiotic stress tolerance. This is achieved by stimulating antioxidant enzyme activities, promoting abscisic acid (ABA) production, and facilitating the assimilation of nitrogen and iron. These biochemical processes collectively bolster a plant's oxidative stress resistance and optimize its physiological performance (Wu *et al*., 2018; Arnon & Stout, 1939).

The foundational understanding of the role of Mo in plant development was first established by Arnon & Stout (1939), who investigated Mo-deficient hydroponic nutrient solutions in tomato plants (*Solanum lycopersicum*). Plants subjected to Mo deficiency exhibited distinctive symptoms, including leaf mottling and morphological anomalies such as inward curling of leaf blades, a condition commonly referred to as the "whiptail" phenotype (Arnon & Stout, 1939).

Interestingly, similar Mo-deficient phenotypes have not been observed in *Chrysanthemum morifolium*, suggesting potential species-specific differences in Mo sensitivity. These differences may arise from variations in nutrient requirements or adaptive mechanisms that regulate Mo utilization. These findings underscore the nuanced and species-dependent nature of micronutrient requirements in plants.

The indispensable role of Mo in processes such as nitrogen metabolism, antioxidant defense, and other fundamental biochemical pathways highlights its critical importance in plant growth, stress resilience, and overall agricultural productivity. Given its profound impact, Mo nutrition remains a key focus in agronomic research, with significant implications for optimizing crop performance under diverse environmental conditions.

Figure 4. The growth of chrysanthemum plantlets after 4 weeks in the greenhouse. Bar = 5 cm. 1MoO_3NPs: MS medium replacing $Na_2MoO_4.2H_2O$ by MoO_3NPs at an equivalent concentration. C(-): MS medium without $Na_2MoO_4.2H_2O$. MS: MS medium with $Na_2MoO_4.2H_2O$.

CONCLUSION

This study demonstrates that MoO_3NPs play a vital role in promoting the rooting and growth of *C. indicum* through their ability to enhance photosynthetic efficiency and nutrient absorption without compromising genetic stability. The 6.4 µg/L concentration of MoO_3NPs was identified as optimal for maximizing rooting and growth parameters, significantly outperforming both negative and positive controls. ITS sequence analysis confirmed that MoO_3NPs supplementation did not induce DNA mutations, ensuring genetic stability during *in vitro* culture. Additionally, MoO_3NPs upregulated the expression of photosynthesis-related genes (*CmPHYA* and *CmPHYB*), enhancing the capacity of plantlets to absorb red light and improving their adaptability to nursery conditions.

At the nursery stage, plantlets derived from MoO_3NPs-supplemented media exhibited improved growth metrics, including plant height, root and leaf numbers, SPAD value, and biomass. These findings highlight the potential of MoO_3NPs as a sustainable tool to improve plantlet quality and productivity in chrysanthemum cultivation systems. Future studies should explore the long-term effects of MoO_3NPs on field-grown chrysanthemums and their broader application in horticultural and agricultural practices.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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