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Research Article

Molybdenum Induces Growth, Yield, and Defence System Mechanisms of the Mung Bean (*Vigna radiata* L.) under Water Stress Conditions

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Water stress has a negative impact on the yield and growth of crops worldwide and consequently has a global impact on food security. Many biochemical changes occur in plants as a response to water stress, such as activation of antioxidant systems. Molybdenum (Mo) plays an important part in activating the expression of many enzymes, such as CAT, POD, and SOD, as well as increasing the proline content. Mo therefore supports the defence system in plants and plays an important role in the defence system of mung bean plants growing under water stress conditions. Four concentrations of Mo (0, 15, 30, and 45 mg·L^{-1}) were applied to plants, using two approaches: (a) seed soaking and (b) foliar application. Mung bean plants were subjected to three irrigation intervals (4 days control, 8 days-moderate water stress, and 12 days severe water stress). Irrigation intervals caused a reduction in the growth and production of mung beans, especially when the plants were irrigated every 12 days. It also led to the accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in mung bean leaves, and these are considered to be indicators of lipid peroxidation and Reactive Oxygen Species (ROS) accumulation, respectively. On the other hand, applying Mo enhanced some growth and yield traits and also enhanced the defence system by upregulating antioxidant expressions, such as proline, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). The MDA content did not change under the effect of Mo treatments. However, H_2O_2 content slightly increased with an increase of Mo concentration of up to 30 mg·L^{-1} followed by a significant decrease when Mo concentration was increased to 45 mg·L^{-1}. It can be concluded that Mo is a robust tool for the activation of the defence system in mung beans.

1. Introduction

Water stress is one of the most significant agricultural problems worldwide, due to its effects on the productivity of crops [1]. Climate change is expected to increase water stress by about 20% in the current century. The threat is due to water scarcity and also to stress caused by extreme temperatures and salinity. The world population is expected to grow by 50% in the coming years, thus increasing the demand for food [2]. A great deal of research has focused on the influence of drought stress on crop development and productivity. It has been demonstrated that plants vary in their response to water deficit, depending on the severity of the stress and the developmental stage at which stress to the plant takes place [3].

Legume crops, such as mung beans (vigna radiata L), come second only to cereal crops in terms of importance. About one-third of human dietary protein is derived from

grain legumes [4]. Mung beans can be used in various forms and are used as a whole snack and as bean sprouts or bean noodles. They have important biological functions, such as detoxification, the reduction of cholesterol, and antitumour and anti-inflammatory activities [5], and contain high levels of vitamins, minerals, proteins, and essential amino acids [6]. They constitute a significant part of the human diet and are also used as animal feed, since the seeds contain high levels of protein (240 g·kg^{-1}) and carbohydrates (630 g·kg^{-1}). They are more easily digestible than other legumes, and they cause less flatulence and are better tolerated by children [7, 8].

The physiological mechanism of crop responses to water deficit stress in dry conditions is characterised by reduction of the transpiration process through closure of the stomata. This, in turn, affects the movement of CO_2 into the plant. Drought stress is also associated with a decrease of the leaf area to maintain the high water potential of tissue and to protect the metabolic process functions from the damaging effects of stress [9, 10]. Persistent exposure of mung beans to water stress modifies the plant's physiological, biochemical, and molecular responses, thereby affecting a series of processes, including growth, yield, and quality. Lack of moisture affects biochemical and physiological processes, especially the photosynthetic system and enzyme activity, and eventually leads to the production of Reactive Oxygen Species (ROS), such as H2O2. This, in turn, causes oxidative stress and consequently cell death through interaction with important cellular components, such as lipids, proteins, DNA, and RNA [11]. Lipid peroxidation is one of the harmful reactions that occur in plants when they are exposed to abiotic stresses. Malondialdehyde-MDA is the final product of lipid peroxidation and is an indicator of ROS production in the plant as a response to abiotic stress [12].

Plants have unique mechanisms to cope with various abiotic stresses. They are able to raise the level of their enzymatic antioxidants (e.g., CAT, POD, and SOD) and nonenzymatic antioxidant (e.g., proline) systems to balance the negative effects of ROS production [13]. It has been reported that micronutrients (e.g., molybdenum) support the defence system through activating the expression of many enzymes involved in various metabolic processes in the plant [14–17]. Molybdenum (Mo) is an essential micronutrient that exists in a wide range of metalloenzymes in plants, fungi, algae, and animals, where it is a part of the active sites of these enzymes [18].

Molybdenum (as molybdate) is a very important element in the healthy development of crops and plays an essential part in several physiological processes of plants [19]. Molybdate is the main form of molybdenum available to plants. Although molybdenum plays an important role in different redox reactions, it needs to be at very low concentrations [20] and the amount of this element that is required is one of the lowest among the micronutrients essential for plant growth [21]. Molybdenum is a crucial element of more than 40 enzymes, four of which have been found in plants [22]. These are nitrate reductase (NR), which is important for nitrogen fixation and assimilation; xanthine dehydrogenase/oxidase (XDH), which is involved in purine catabolism; aldehyde oxidase (AO), which plays an important role in the synthesis of indole-3 acetic acid (IAA) and abscisic acid (ABA); and sulphite oxidase (SO), which is important for sulphur metabolism [17]. Molybdenum is biologically inactive and cannot function as a facilitator in the biological system unless it is united with specific cofactors [12]. In many studies of the upregulation of cold tolerance, Mo has been found to enhance the expression of CBF genes [15, 17, 19]. The role of Mo in drought tolerance, however, has not been comprehensively explored in the previous literature. Since there is cross talk between most abiotic stresses, such as drought and low temperature, there is a significant possibility that Mo can also play a vital role in drought tolerance.

This study aims to investigate the effect of Mo on the growth, yield, and development of mung beans grown under water stress conditions. It aims, moreover, to investigate the physiological mechanism by which Mo improves the drought tolerance of plants and includes an investigation of the impact of this element on several antioxidant activities in mung beans.

2. Materials and Methods

A field experiment was carried out in the autumn of 2018 in western Iraq (decimal latitude and longitude coordinates: 33.4366°N, 43.2683°E). Mung bean seeds were sown in a well-prepared field, following established agricultural practice. The field was divided into main and subplots. According to split-plot-RCBD, in order to attain space between main plots sufficient to prevent interaction between irrigation treatments, the same water volume was used per treatment at each irrigation.

Three concentrations of Mo in the form of molybdate [$(NH_4)6Mo_7O_{24}.4H_2O$] (15, 30, 45 mg·L^{-1}) (0 mg·L^{-1} Mo was considered as control) were applied to the seed of mung beans (seed primer), and seeds were soaked in Mo solution for 4 hours at room temperature before sowing. Growing plants were, then, treated with foliar application with the same concentration of Mo for each treatment in order to maintain the provision of Mo until the end of the experiment. Foliar application was conducted one month after sowing. Three flood irrigation intervals were applied every 4, 8, and 12 days. The irrigation was scheduled after the full germination, and uniformity of seedlings were achieved. The studied traits were measured on a random sample of (N:10) plants and for three biological replicates. Three types of parameters were collected as follows.

2.1. Growth Traits. These included plant height (cm), number of branches per plant, chlorophyll content (SPAD) using the SPAD meter, leaf area (cm^2 plant^{-1}), and plant dry weight (g plant^{-1}).

2.2. Yield Traits. These included the number of pods per plant (pod plant^{-1}), pod length (cm), number of seeds per pod (seed·pod^{-1}), weight of 100 seeds (g), plant yield (g·plant^{-1}), total yield (ton·ha^{-1}), biochemical traits

(malondialdehyde content (MDA) (μmol g^{-1}·FW), hydrogen peroxide (H_2O_2) (μmol·g^{-1}·FW), proline content (mg·g^{-1}·FW), catalase enzyme activity (Unit mL^{-1}), and peroxidase enzyme activity (mL^{-1}) superoxide dismutase enzyme activity (mL^{-1}).

2.3. Biochemical Trait Assays

2.3.1. Lipid Peroxidation (Malondialdehyde (MDA) Content (μmol G^{-1}·FW)). The content of MDA was estimated following procedures described by [23]. One gram (1 g) of fresh leaf was ground, and then, 3 mL of trichloroacetic acid (TCA) were added. Samples were separated by centrifugation (1000 rpm) for 30 min, and then, 0.5 mL from the supernatant was added to 3 mL of thiobarbituric acid (0.5%). Samples were put in a water bath for 50 min and, then, left to cool. Next, samples were centrifuged (100 rpm) for 10 min. The supernatant was taken in order to estimate MDA content (μmol g^{-1} FW), using a spectrophotometer at 450, 532, and 600 nm. The following equation was used:

$$\text{MDA}\left(\mu\text{mol}\cdot\text{g}^{-1}\right) = \frac{\text{absorbance of sample}}{E0 * \text{light path} * \text{dilution factor}}. \quad (1)$$

E0 : Extinction Coefficient (1.56)

2.3.2. Estimation of H_2O_2 (μmol G^{-1} FW). The content of H_2O_2 was estimated in accordance with the method in [24]. A solution of TCA 0.01% was used along with phosphate buffer (0.01 mole, pH = 7). A solution of KI (1 M) was prepared, and H_2O_2 (0.01 M) was used. The leaf sample was ground in TCA solution and, then, centrifuged (12000 rpm) for 15 min. 0.5 mL supernatant was added to 1 mL phosphate buffer and 1 mL KI solution. A blank sample was prepared in the same way, except that the phosphate buffer was added instead of the plant sample. The content of H_2O_2 was measured, using a spectrophotometer at a wave length of 390 nm.

2.3.3. Nonenzymatic Antioxidant: Proline Content (mg g^{-1} FW). A modification of the method in [25] was used in order to estimate proline content, based on ninhydrin. A fresh leaf sample of 100 mg was used for the extraction and calculation of proline content (μg g^{-1} FW) in 2 mL of aqueous sulphosalicylic acid (3%). The supernatant was filtered, and 2 mL of glacial acetic acid was added and, then, incubated in a water bath. After samples were cooled, 4 mL of toluene was added, and then, the mixture was shaken for 20 min and incubated at room temperature in order to separate the toluene layer with proline in it. 1 mL from the upper liquid was taken and then subjected to 520 nm, using a spectrophotometer. Finally, the following equation was used:

$$\text{Proline content}\left(\text{mg}\cdot\text{g}^{-1}\cdot\text{FW}\right) = \frac{\text{absorbance} * 20}{\text{weight of sample} * 1.47}. \quad (2)$$

2.4. Enzymatic Antioxidant Assays

2.4.1. Catalase (CAT) Assay (EC 1.11.1.6). Catalase enzyme activity was estimated by the modified method in [26], which depended on the change in the absorbency of light at 240 nm. A buffer solution of phosphate solution (50 m mole, pH = 7) and hydrogen peroxide solution (30 m mole) was used. 0.34 mL from 30% H2O2 and the volume was raised to 100 mL with the phosphate buffer solution. Samples were ground in 10 mL of cold phosphate buffer (0.1 molar, pH = 7.8) and, then, filtered and centrifuged in a cooling centrifuge (4°C) at 4000 rpm for 30 min [27]. From the supernatant of samples, 0.1 mL was mixed with 1.9 mL from the buffer solution and, then, 1 mL from H2O2. The tubes were shaken thoroughly for reaction to occur, and then, samples were subjected to 240 nm using a spectrometer (UV-Spectrophotometer- Sp 300 nm Optic). Changes were read each 30 seconds for 3 min. The following equation was used in order to estimate the activity of CAT:

$$\text{CAT activity}\left(\text{U}\cdot\text{mL}^{-1}\right) = \left(\frac{\Delta\text{Absorbency}/\Delta\text{Time}}{0.1 * 0.01}\right). \quad (3)$$

2.4.2. Peroxidase (POD) Assay (EC: 1.11.1.7). The samples were prepared, following the same procedure as was used with the CAT samples, and the activity of POD was measured in accordance with [28]. A guaicol solution was prepared by mixing 1.36 mL of guaicol with 250 mL dH_2O. A solution of H_2O_2 (0.1%) was prepared by taking 0.4 mL from 30% H_2O_2. The volume was, then, brought up to 120 mL using dH_2O. Then, 1 mL from the first solution (guaicol) with 1 mL from the second solution (0.1% H_2O_2) and 2 mL from the mixture was added to each sample, and the activity of POD was measured following changes in the absorbency, using a spectrophotometer each 3 sec for 3 min at 420 nm. Finally, the following equation was used:

$$\text{POD activity}\left(\text{Unit}\cdot\text{mL}^{-1}\right) = \left(\frac{\Delta\text{Absorbency}/\Delta\text{Time}}{0.1 * 0.01}\right). \quad (4)$$

2.5. Superoxide Dismutase (SOD) Assay (EC: 1.15.1.1). The activity of SOD was estimated according to its ability to inhibit nitro blue tetrazolium (NBT), as described in [29]. The following solutions were used: solution A (phosphate buffer 28.4 mmole, 18.35 mL), solution B (L-methionine 14 mmole, 1.5 mL), solution C (Triton X-100, 1%, 0.75 mL), and solution *D* (NBT 14.4 mg + 10 μL dH2O in 1 mL). The total volume was 21.6 mL in addition to the solution F (riboflavin 47.4 μmole, by solving 0.0018 g in dH2O, and the volume brought up to 100 mL). Plant samples were prepared as previously described (see the procedure of CAT), and 40 μL was added to 1.5 μL from the reaction mixture. Then, 40 μL from solution F were also added. The absorbency was read at 560 nm. The activity was estimated as follows:

$$\%\ \text{SOD inhibition} = \frac{(A_1B - A_2B) - (A_1S - A_2S)}{(A_1B - A_2B)}, \quad (5)$$

where A_1B: absorbency of blank before subjection to light. A_2B: absorbency of blank after subjection to light. A_1S: absorbency of the sample before subjection to light. A_2S: absorbency of the sample after subjection to light.

The unit of SOD is the unit that inhibits 50% of NBT, and therefore, the activity of SOD was calculated by the following equation:

$$\text{POD activity}\left(\text{Unit}\cdot\text{mL}^{-1}\right) = \left(\frac{(\%\text{sample inhibition/max inhibition})}{(\text{dilution factor/sample vol})}\right). \quad (6)$$

(Dilution factor = 2000 μL, Sample vol. = 40 μL).

2.6. Statistical Analysis. Data were subjected to ANOVA analysis according to the split-plot arrangement in RCBD design, using MS : Excel 2010. The significant differences between means were obtained by using the Least Significant Difference test (LSD) at a probability of 0.05 ($p > 0.05$).

3. Results

3.1. Growth Parameters. Drought application had a significant negative impact on growth parameters, including plant height, number of branches, chlorophyll content, leaf area, and plant dry weight but not on Chlorophyll SPAD, where the effect was not significant (Figure 1 and Table 1). The highest mean of the growth traits was observed when plants were irrigated every 4 days, and growth significantly declined when the interval of irrigation was extended to 12 days.

Mo application significantly enhanced leaf area, plant dry weight, and chlorophyll SPAD. However, there was no significant impact of Mo on plant height and number of branches (Figure 1 and Table 1).

A significant interaction between Mo treatments and irrigation treatments was observed in terms of the effect on all growth parameters. While 45 mg·L^{-1} with 4 days irrigation intervals provided the best plant height, dry weight, leaf area, and chlorophyll content, 30 m·L^{-1} at 4 days irrigation intervals gave the largest number of branches (Figure 1 and Table 1).

It was observed that, under drought conditions (12 days irrigation interval), 15 mg·L^{-1} significantly increased most of the investigated growth parameters, such as plant height, dry weight, and leaf area.

3.2. Yield Components. Drought (longer irrigation intervals) had a significant impact on pod lengths, number of seeds per pod, weight of 100 seeds, plant yield, and total yield. However, no significant impact of drought on the number of pods of mug beans was observed (Figure 2 and Table 2).

While Mo significantly improved number of pods, pod lengths, and the number of seeds per pod, no significant impact of Mo was observed on the weight of 100 seeds, plant yield, and total yield (Figure 2 and Table 2).

A significant interaction between Mo treatments and irrigation intervals was observed in terms of the effect on the number of pods, length of pods, number of seeds per pod, weight of 100 seeds, plant yield, and total yield (Figure 2 and Table 2). The highest number of pods per plant and greatest length of pods were achieved at 4 day irrigation intervals when plants were treated with 30 mg·L^{-1} (48.10 pod plant-1 and 7.15 cm, respectively), while the number significantly diminished at 12-day irrigation intervals when they were not treated with Mo (26.68 pod plant-1 and 7.15 cm for the aforementioned traits, respectively). The response of seed weight was slightly different, as the highest mean was obtained when plants were irrigated every 8 days and treated with 45 mg Mo·L^{-1} (4.5 g). Plants gave the lowest trait values when they were irrigated every 12 days without Mo application (3.07 g). Yield per plant and total yield increased to 15 mg Mo·L^{-1} when plants were irrigated every 4 days (16.58 g plant^{-1} and 2.07 ton ha^{-1}, respectively), while they showed the lowest values of the aforementioned traits. 15 mg·L^{-1} significantly improved all yield components at 12 days irrigation intervals, and this, in turn, highlights the importance of this element in improving the drought tolerance of mung beans (Figure 2 and Table 2).

At high drought level (12 days irrigation intervals), the use of 15 mg L^{-1} signficantly improved most of the yield components such as length of pods, number of seeds per pod, yield per plant, and total yield per plant.

There was a significant interaction between Mo concentration and irrigation intervals in terms of the effect on the yield components of mung beans.

3.3. Biochemical Traits. Many changes in terms of biochemical processes occurred inside plants, as they are sessile in their place according to changes in the environment. All the biochemical traits significantly increased under the effect of drought treatment (Table 3).

It was found that Mo supported the defence system in mung beans. Mo did not affect MDA content (lipid peroxidation). However, it significantly increased the proline content and the activity of antioxidant enzymes (CAT, POD, and SOD) (Table 4).

Hydrogen peroxide (H_2O_2) also slightly increased with the application of Mo, but then significantly decreased when plants were treated with a relatively high concentration of Mo (45 mg·L^{-1}). The nonenzymatic antioxidant (proline) significantly increased with the treatment of Mo from

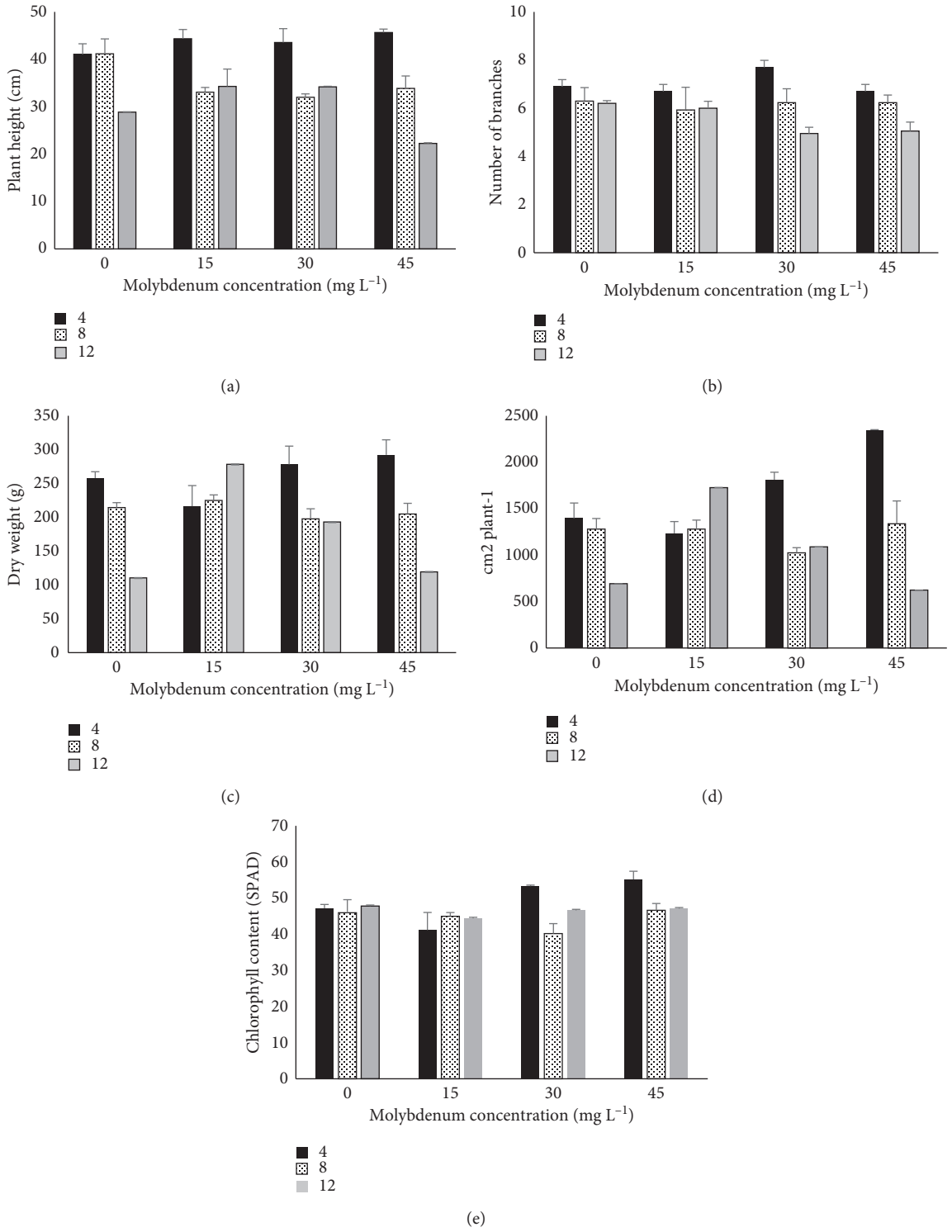

Figure 1: The effect of molybdenum treatment on growth parameters. (a) Plant height (cm) $p > 0.0006$, (b) number of branches $p > 0.0006$, (c) dry weight (g) $p > 0.0003$, (d) leaf area (cm^2 $plant^{-1}$) $p > 0.00003$, and (e) chlorophyll content (SPAD), $p > 0.004$ of mung bean.

456.08 $mg \cdot g^{-1}$ FW at the control treatment to 499.33 $mg \cdot g^{-1} \cdot$FW at the highest concentration of Mo (45 $mg \cdot L^{-1}$). The baseline activities of the antioxidant enzymes increased significantly with the increase of Mo concentration (Table 4).

There was significant interaction between Mo treatments and irrigation intervals for the proline content, antioxidant activities, MDA, and H_2O_2 (Table 5). Lipid peroxidation did not change with Mo application when plants were irrigated at 4-day intervals. However, the highest lipid peroxidation

Table 1: Least significant differences (LSD) of the impact of molybdenum (Mo), irrigation intervals (I), and the interaction between Mo and I treatments on the growth traits.

Trait	Mo	Irrigation intervals	Interaction between Mo and irrigation intervals
Plant height	NS	5.41	5.45
No. of branches	NS	1.08	0.59
Dry weight	11.83	45.18	20.5
Leaf area	206.25	67.07	357.23
Chlorophyll SPAD	3.12	NS	5.41

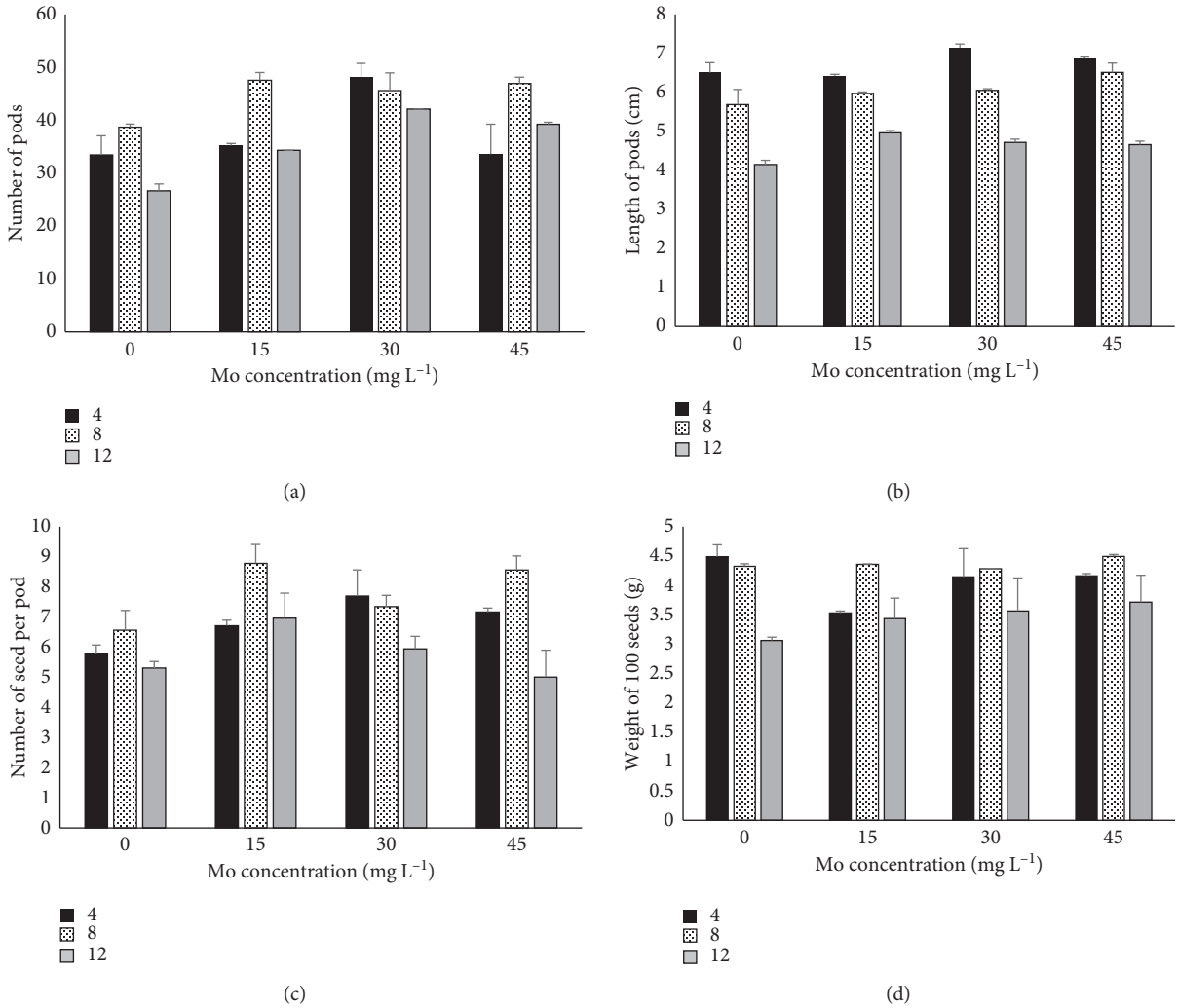

Figure 2: Continued.

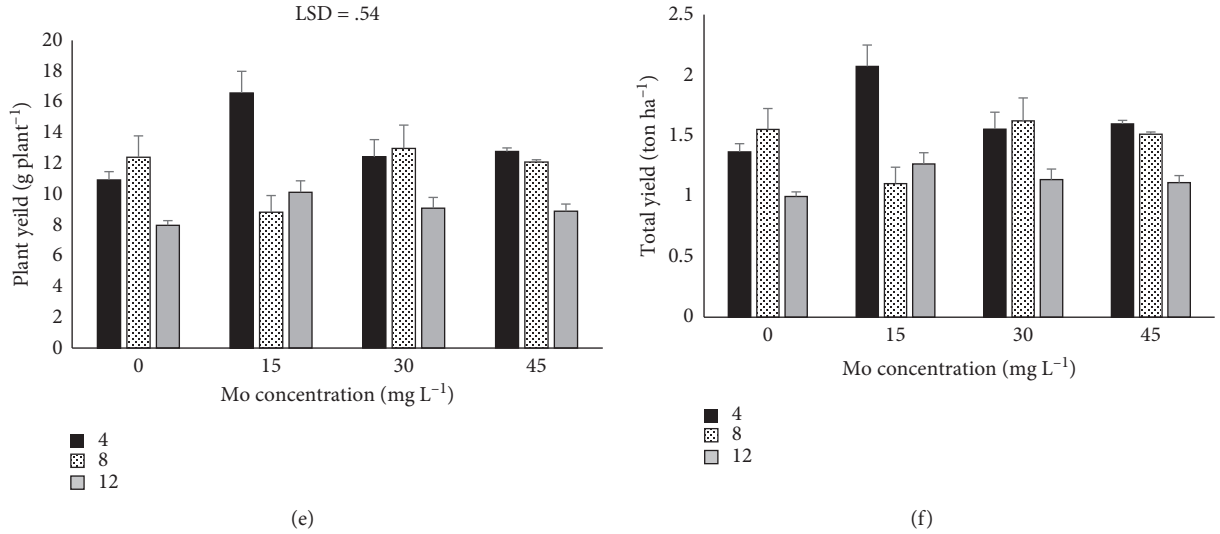

Figure 2: The effect of molybdenum treatment on the yield components: (a) number of pods, $p = 0.002$,(b) length of pods (cm), $p = 0.02$, (c) number of seeds per pod, $p = 0.01$, (d) weight of 100 seeds (g) $p = 0.04$, (e) plant yield (g plant^{-1}), $p \geq 0.001$ of mung bean, and (f) total yield (ton ha^{-1}) $p = 0.04$.

Table 2: Least significant differences (LSD) of the impact of molybdenum (Mo), irrigation (I), and the interaction between Mo and I treatments on the yield traits; number of pods, pod length, number of seeds/pod, weight of 100 seeds, plant yield, and total yield of mung beans.

Trait	Mo	Irrigation intervals	Interaction between Mo and irrigation intervals
No. of pods	3.14	NS	5.43
Pod length	0.26	0.26	0.45
No. of seeds pod^{-1}	0.89	1.47	NS
Weight of 100 seeds	NS	0.7	0.55
Plant yield	NS	2.44	2.54
Total yield	NS	0.31	0.32

Table 3: Impact of irrigation intervals on some biochemical traits of mung bean crops growing under water stress conditions (Mean ± SE).

Irrigation intervals (days)	MDA (μmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	Proline (mg g^{-1} FW)	CAT (mL^{-1})	POD (mL^{-1})	SOD (mL^{-1})
4	22.27 ± 0.46	1.81 ± 0.06	451.75 ± 7.36	20.89 ± 0.17	31.22 ± 0.45	94.56 ± 0.67
8	25.46 ± 0.34	3.07 ± 0.13	492.00 ± 3.61	26.54 ± 0.25	34.62 ± 0.32	123.22 ± 0.46
12	27.84 ± 0.24	4.45 ± 0.19	483.38 ± 4.11	33.18 ± 0.37	36.56 ± 0.89	136.41 ± 0.48
LSD (0.05)	**0.96**	**0.09**	**14.80**	**0.56**	**0.91**	**0.79**

Table 4: Impact of Mo application on some biochemical traits of the mung bean crop growing under water stress conditions (Means ± SE).

Mo (mg L^{-1})	MDA (μmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW	Proline (mg g^{-1} FW)	CAT (mL^{-1})	POD (mL^{-1})	SOD (mL^{-1})
0	25.17 ± 0.45	2.79 ± 0.15	456.08 ± 3.56	24.41 ± 0.20	32.41 ± 0.71	113.61 ± 0.31
15	24.66 ± 0.33	3.65 ± 0.16	473.25 ± 3.32	26.69 ± 0.14	33.76 ± 0.52	116.92 ± 0.35
30	25.57 ± 0.45	3.25 ± 0.15	474.17 ± 3.56	27.42 ± 0.20	34.91 ± 0.71	118.93 ± 0.31
45	25.36 ± 0.35	2.74 ± 0.14	499.33 ± 3.08	28.95 ± 0.25	35.45 ± 0.34	122.78 ± 0.40
LSD (0.05)	**NS**	**0.27**	**10.35**	**0.57**	**1.12**	**1.29**

events occurred in plants irrigated every 12 days when they were treated with 30 mg·L^{-1} Mo (28.39 μmol·g^{-1}·FW), compared with the lowest MDA content, which was observed in plants irrigated every 4 days (21.02 μmol·g^{-1} FW).

H_2O_2 content increased under the effect of relatively low Mo concentration (15 and 30 mg L^{-1}). However, H_2O_2 content decreased when MO was used at a relatively high concentration (Table 5).

Table 5: The effect of interaction between Mo concentration and irrigation intervals on some biochemical traits of the mung bean crop (Means ± SE).

Irrigation intervals (days)	Mo (mg L^{-1})	MDA (μmol g^{-1} FW)	H_2O_2 (μmol g^{-1} FW)	Proline (mg g^{-1} FW)	CAT (mL^{-1})	POD (mL^{-1})	SOD (mL^{-1})
4	**0**	21.02 ± 0.24	2.135 ± 0.04	433.75 ± 15.19	19.15 ± 0.33	26.91 ± 0.32	90.94 ± 1.43
	15	22.42 ± 0.39	1.720 ± 0.05	462.75 ± 7.07	20.00 ± 0.08	31.17 ± 0.40	90.71 ± 0.60
	30	22.86 ± 0.85	2.005 ± 0.07	418.00 ± 2.89	21.48 ± 0.05	32.42 ± 0.65	94.99 ± 0.25
	45	22.79 ± 0.36	1.365 ± 0.09	492.50 ± 4.33	22.93 ± 0.20	34.38 ± 0.42	101.59 ± 0.40
8	**0**	26.12 ± 0.35	2.395 ± 0.07	475.00 ± 6.35	25.10 ± 0.22	35.12 ± 0.23	117.26 ± 1.43
	15	24.68 ± 0.17	4.079 ± 0.17	484.00 ± 0.58	26.77 ± 0.04	33.97 ± 0.40	124.09 ± 0.26
	30	25.47 ± 0.18	3.195 ± 0.12	504.00 ± 4.04	26.39 ± 0.43	34.31 ± 0.46	125.05 ± 0.05
	45	25.56 ± 0.64	2.590 ± 0.14	505.00 ± 3.46	27.89 ± 0.32	35.07 ± 0.18	126.47 ± 0.17
12	**0**	28.38 ± 0.17	3.835 ± 0.04	459.50 ± 8.95	28.99 ± 0.82	35.21 ± 1.39	132.64 ± 0.48
	15	26.87 ± 0.42	5.149 ± 0.27	473.00 ± 2.31	33.29 ± 0.31	36.14 ± 0.76	135.95 ± 0.19
	30	28.39 ± 0.32	4.560 ± 0.25	500.50 ± 3.75	34.40 ± 0.12	37.99 ± 1.02	136.76 ± 0.62
	45	27.73 ± 0.05	4.260 ± 0.18	500.50 ± 1.44	36.04 ± 0.22	36.90 ± 0.41	140.29 ± 0.62
LSD (0.05)	**1.17**	**0.47**	**17.2**	**0.98**	**1.94**	**2.23**	

Proline content significantly increased with the increase of Mo concentration and irrigation intervals. Proline content was 433.75 mg·g^{-1} FW at the control treatment and increased to 500.50 mg·g^{-1} when plants were subjected to 12-day intervals and treated with 45 mg Mo L^{-1}.

The antioxidant enzymes (CAT, POD and SOD) followed almost the same pattern as in the proline response. They increased from 19.15, 26.91, and 90.94 mL^{-1} at control to 36.04, 36.90, and 140.29 mL^{-1} in plants irrigated every 12 days and treated with a relatively high concentration of Mo (45 mg·L^{-1}).

4. Discussion

Water stress is always accompanied by oxidative damage. Water stress exposes plants to excessive ROS production, and the balance between ROS production and their scavenging is an indication of the plants' tolerance to water stress. The permanence of ROS, such as HO.-, O_2.-, 1O2, and H_2O_2, rapidly affects macromolecules in plant cells, i.e., lipid, protein, DNA, and RNA, which leads to cell damage, and therefore, plants need to augment their defence systems in order to protect their cell components from the accumulation of ROS compounds. Oxidative stress could be a result of water stress. It was clear that lipid peroxidation and H2O2 content significantly increased in mung bean leaves (Tables 1–3) under the effect of water stress: it increased 4-fold in comparison to the control (Table 1). This effect accords with the findings of Nahar et al. [11] who reported that drought treatments doubled the content of H_2O_2.

It was noticed that Mo application slightly increased H_2O_2 content, which then significantly declined when the concentration of Mo was raised to 45 mg L^{-1}. This increase was consistent with the increase in enzymatic and nonenzymatic defence systems [30]. These results were consistent with the findings of other researchers [14–16, 31].

It is commonly reported that water stress significantly decreases the growth and production of plants [32, 33]. The results of of the current study indicate that the increase of irrigation intervals leads to a significant reduction in mung bean growth and production, as well as a significant increase in the production of ROS which, in turn, leads to the major effect on important cellular compartments, such as chloroplast, mitochondria, and peroxisomes [34]. It has been shown that Mo is more effective when used for seed soaking or as a foliar application than when it is added to soil. This is because of its sensitivity to soil pH [20]. In the present study, therefore, Mo was applied using the two methods that had proved to be superior to its addition to soil [16]. In the current study, results showed that micronutrient Mo application enhanced chlorophyll content. In agreement with the current results, it has been demonstrated that the net photosynthetic rate (Pn) was affected and chlorophyll biosynthesis was repressed in Mo-deficient winter wheat [35], reported in [17]. The biosynthesis of chlorophyll can be described as follows: glutamate (Glu), aminolaevulinic acid (ALA), porphobilinogen (PBG), uroporphyrinogen 111 (Uro I11), protoporphyrin IX (Proto IX), Mg-protoporphyrin JX (Mg-Proto IX), protochlorophyll (Pchl), chlorophyll a (Chl a), and chlorophyll b (Chl b) [36]. It was demonstrated that Mo deficiency blocked the conversion of ALA to uro111, causing a decrease in chlorophyll biosynthesis [37]. Mo also enhanced the leaf area and, eventually, the plant dry matter of mung beans grown in field conditions. Additionally, Mo application in the form of seed soaking and foliar application enhanced some of the yield components, such as the number of pods per plant, the length of pods, and the number of seeds per pod. The significant role of Mo in enhancing some growth and yield traits might be due to its vital role in upregulation pathways related to growth and production through the activation of many enzymes involved in metabolic processes, such as nitrogen fixation and assimilation [38]. Moreover, Mo application markedly enhanced the defence system in mung bean plants. Nonenzymatic antioxidants represented as free proline in this study accumulated significantly in plant leaves under water stress conditions. These results were consistent with the findings of other researchers [12, 14, 19]. Proline has been reported to act as an environmental stress indicator [39–43]. It is also reported to have increased in different

plant species in response to various kinds of abiotic stress [44, 45]. Mo application significantly increased the concentration of free proline in mung bean leaves under water stress conditions, providing evidence that Mo plays an important part in the stress tolerance of plants, possibly due to its role in nitrogen assimilation [46]. Enzymatic antioxidant defence systems were upregulated, either by increasing irrigation intervals or by providing the plant with Mo. It can be concluded that the application of Mo is a very robust tool in upregulating the defence system of mung bean plants when the latter are exposed to abiotic stress, especially when it is applied as a seed primer or by foliar application to the shoots. Mo is a very important micronutrient and has been shown to participate in many cell signaling pathways, such as ABA production in the plant through the activation of AO and in N fixation and assimilation by activation of NR or nitrogenase. In addition, it supports the defence system in plants, e.g., by inducing enzymatic and nonenzymatic antioxidants.

5. Conclusions

This study has described the negative impact of drought stress on the growth, yield, and physiological parameters of the mung bean. Molybdenum has a positive impact on the drought tolerance of the mung bean and enhances its growth yield and its physiological response to water deficit. The use of 15 and 30 $mg \cdot L^{-1}$ of Mo significantly enhanced yield parameters. The use of relatively high concentrations of Mo had a negative impact on some of the growth and yield parameters. This study is a significant contribution to the understanding of the mechanism of drought tolerance and response to drought. It also demonstrates the positive impact of molybdenum treatment, which in turn could have wider practical applications.

Data Availability

The [DATA TYPE] data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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