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# Molybdenum mitigates cadmium stress for bread wheat (*Triticum aestivum* L.) seedlings

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Summary: Pollution with heavy metals has become a serious problem that threatens all living organisms. Cadmium (Cd) pollution has become focus of the environmental studies and can negatively affect human health and plant growth. Molybdenum has been highlighted as a stressresistant micronutrient that supports the defense system against environmental stresses. In this context, this study highlighted the role of molybdenum in the mitigation of the toxic effect of cadmium in wheat seedlings. The study included four concentrations of cadmium (0, 75, 150, and 225 mg L-1), three concentrations of molybdenum (0, 15, and 30 mg L-1), and two wheat genotypes (G-31 and IRAQ). These factors were laid as a factorial experiment according to a completely randomized design (CRD) with three replications. The results of this study indicated the negative impact of cadmium on wheat seedlings' growth in terms of physiological and anatomical traits. It also highlighted the positive role of molybdenum in the mitigation of the toxic effect of cadmium. Molybdenum at a concentration of 15 mg L-1 enhanced the structure of the root and leaf of cadmiumtreated wheat. The two wheat genotypes did not vary in terms of anatomy when they were treated with molybdenum. Further studies are required on a wide range of wheat genotypes to investigate their ability to withstand heavy metal stress. Therefore, heavy metal-tolerant genotypes could be utilized in heavy metal-contaminated soils.

Keywords: anatomy, cadmium, genotypes, molybdenum, heavy metals, wheat

### Introduction

Cadmium poses significant risks to all organisms including humans especially when it occurs in the food chain. By consuming cadmium-contaminated food or air, smoking, or even touching it through the skin, cadmium can be available in living organisms. Cadmium exposure is associated with severe health implications, including the development of malignant tumors in vital organs such as the breast, lung, prostate, and kidney, as well as potentially contributing to conditions like osteoporosis due to its extended half-life of 25-30 years in human organs (Khan et al., 2022). Cadmium stands out

as one of the most hazardous heavy metal pollutants, with its excessive accumulation in plants posing a direct threat to human and animal health (Peana et al., 2023). Therefore, it becomes imperative to explore strategies to mitigate its impact on living organisms.

In plants, it causes physical, chemical, morphological, and structural changes. These changes culminate in producing free radicals (e.g., Reactive Oxygen Species - ROS), which in turn facilitate the DNA damage and breakdown of the enzymatic activity of antioxidants within the plant cells. Regarding cellular processes, cadmium increases programmed cell death mechanisms. Furthermore, it disrupts nutrient balance and induces oxidative stress, thereby compromising the efficiency of photosynthesis, if this stress continues, it ultimately leads to the death of the plant (Zulfiqar et al., 2022).

Wheat is one of the most important cereals in the world due to its significant nutritional value as a rich source of carbohydrates and calories. Consequently, it plays a vital role in ensuring food security worldwide. However, abiotic stresses pose massive challenges to agricultural productivity, thereby impeding efforts to meet escalating global food demands. Extensive research has demonstrated susceptibility of wheat to heavy metal toxicity, particularly cadmium, which detrimentally impacts various stages starting from germination to yield (Rizwan et al., 2016). Balancing nutrients can enhance the plants tolerance to abiotic stress including cadmium stress by the activation of the enzymatic and non-enzymatic antioxidant defense systems to mitigate the damages caused by the ROS. Many studies proved the vital role of molybdenum in increasing plant tolerance to various abiotic stress (Al-Issawi et al., 2013; Al-Issawi et al., 2016; Wu et al., 2020; Imran et al., 2021). This micronutrient has a very important role in activating the enzymatic and non-enzymatic systems of defense. Investigations proved that Mo increases the activities of key enzymes related to abiotic stress tolerance (Mohammed et al., 2019; Hayyawi et al., 2020). Besides, it helps in producing abscisic acid (ABA) during stress which in turn contributes to tolerance (Wu et al., 2018).

Furthermore, molybdenum is an essential element for plant growth and in many physiological and biochemical processes. While plants require it in small amounts, its regulatory role across various plant functions is indispensable. Notably, molybdenum serves as a key element for resisting abiotic stresses (such as drought, salinity, and heavy metals toxicity). Insufficiency of molybdenum adversely impacts crop growth and quality, particularly enhancing resistance against oxidative stress associated with environmental stresses, especially heavy metals (Hadi et al., 2016).

The interaction between cadmium and molybdenum toxicity in wheat is complex and not fully understood. However, several studies have shown that molybdenum can reduce the toxic effects of cadmium in wheat (Imran et al. 2021; Imran et al. 2020). Plants grown in cadmium-contaminated soils were affected in terms of growth and photosynthesis, in addition to increased oxidative stress and damage to cell membranes (Rehman et al., 2020). However, it is believed that Mo application to the soil might reduce the toxic effects of cadmium and improve plant growth and photosynthesis (Han et al., 2020). Numerous studies have provided evidence that molybdenum could alleviate the harmful effects of cadmium on crops cultivated in cadmium-contaminated soils. Consequently, the incorporation of molybdenum into agricultural practices holds the potential to contribute to the objectives of sustainable agricultural development and enhancing food quality in the United States by 2030 (Imran et al., 2021). Besides, previous research has demonstrated that molybdenum supplementation decreases the absorption and translocation of cadmium within plants, thereby reducing its accumulation in vegetative parts growing above the soil surface (Han et al., 2020).

Based on the aforementioned information, it can be concluded that molybdenum possesses the potential to mitigate the adverse impacts of cadmium on wheat by employing various mechanisms. These mechanisms encompass the reduction of cadmium uptake, mitigation of oxidative stress, and boosting of plant growth and photosynthetic activity. Nevertheless, it is essential to recognize that the

effects of cadmium and molybdenum on wheat toxicity can be contingent upon the concentrations of these elements and the duration of exposure (Imran et al. 2021; Imran et al. 2020).

Despite the ongoing inquiries, there remains a notable scarcity of studies investigating the role of molybdenum in mitigating cadmium-induced damage to wheat, particularly in reducing oxidative stress and enhancing antioxidant defenses. Furthermore, the necessity for additional research and exploration into the effects of molybdenum in this field is underscored. This study aimed to determine whether molybdenum has a fundamental role in confronting the threat of heavy metals for the management and sustainability of strategic crops, diagnosing this through physiological and anatomical investigations.

#### Materials and Methods

A laboratory experiment has been conducted in the College of Agriculture – University of Anbar to investigate the role of molybdenum in alleviating Cd stress in wheat. The experiment included three factors namely; two wheat genotypes (introduced genotype G-31 and local cultivar IRAQ), four Cd concentrations (0, 75, 150, and 225 mg Cd L<sup>-1</sup>) as CdCl<sub>2</sub> (MW: 183.32 gm mole<sup>-1</sup>) (Thomas Baker) and three concentrations of molybdenum (0, 15 and 30 mg l<sup>-1</sup>) as molybdenum (MW: 1235.86 from STANFORD Company). All these factors were laid as factorial arrangements in completely randomized design (CRD) with three replications. Grains of the two wheat genotypes were soaked with molybdenum solutions for 8 hours (Al-Issawi et al., 2016) and then 100 grains from each cultivar were distributed in petri dishes for each treatment and were treated with the required concentration of cadmium. Parameters were investigated:

germination (%) which was measured according to the following equation:

Germination (%) =  $\frac{Number\ of\ germinated\ grains}{total\ number\ of\ grain\ per\ petri\ dish} \times 100$ 

**Radicle and plumule length (cm):** the length was measured for 10 seedlings and then we calculated the mean.

Relative water content RWC (%): RWC was obtained according to the following equation:

$$RWC\% = \frac{TW - FW}{DW - FW}$$

Where:

TW: turgid weight FW: fresh weight

DW: dry weight (Al-Sheibany and El-Karhi 2017).

Relative electrical conductivity REC (%): REC was measured according to the following equation:

$$REC\% = \frac{EC1}{EC2} \times 100$$

Where:

EC1: electrical conductivity after soaking seedlings in test tubes containing distilled water overnight at room temperature

EC2: electrical conductivity after exposing test tubes from EC1 to autoclave at 115 °C for 15 min (Al-Ubaidy et al. 2021).

#### Anatomical traits

Characteristics of the radicle and plumule anatomy, including the number of vascular root xylem, the thickness of the cortex (µM), the length and width of the epidermis cells (µM), and the length and width of the ordinary epidermis cells in the plumule (µM). The radicle portions were held vertically by the thumb and index fingers, and cross sections or radicles were manually created by scraping them with a sharp, sterile blade. Very thin slices were cut, and after staining them for 20–30 minutes with safranin (1%), they were then removed from the stain by moving them to ethanol (30, 70, and 90%) for 2 minutes at a time in each concentration, and lastly to absolute ethanol (96%) for 2 minutes. Using Gel (local commercial), a cross-section of the radicle (slices) was adhered to on glass slides. The cover of the slide was placed and left for 3 hours to remove bubbles and afterward dried, and finally, a cross-section was obtained by the Microscope (Olympus: 100X and 400X). Collected data were then subjected to ANOVA analysis according to the used experimental design and the significant differences between mean were calculated according to the LSD test at 5% probability level.

#### Results and Discussion

Soaking wheat grains with molybdenum led to the stimulation of wheat seedling growth, as evidenced by significant improvements in various morphological and physiological traits (Table 1). Notably, molybdenum exhibited a clear impact on certain traits, namely germination percentage, plumule length, and relative water content, with observed increases of 4.5%, 1.08%, and 6.63%, respectively, compared to the control group (without molybdenum treatment). It is noteworthy that the initial concentration of molybdenum (15 mg L<sup>-1</sup>) had a noticeable effect, particularly on the relative water content (%), which holds significant cellular physiological importance potentially conducive to enhancing wheat crop growth and productivity. However, molybdenum did not demonstrate a pronounced effect on other traits under investigation (Table 1).

Soaking wheat grains with molybdenum contributed to increasing the percentage of soluble sugars, which promotes increased germination rates and the development of healthy seedlings, reflecting the beneficial role of molybdenum in facilitating favorable field establishment (Oliveira et al., 2022). The current study findings align with prior research, attributing them to molybdenum's ability to enhance chlorophyll effectiveness in leaves (Oliveira et al., 2022; Imran et al., 2019; Hayyawi et al., 2020). This enhancement subsequently promotes crop growth and increased photosynthetic efficiency, thereby increasing wheat crop growth and productivity (Liu et al., 2019). Meanwhile, molybdenum is a cofactor for the nitrate reductase enzyme, which plays a pivotal role in nitrogen uptake by plants, and increases the efficiency of photosynthesis and nutrient absorption. Consequently, molybdenum has the potential to improve the absorption and accumulation of macronutrients, thus significantly enhancing nitrogen use efficiency in wheat. This is consistent with previous studies (Moussa et al., 2022; Watanabe et al., 2022; Bazzo et al., 2018). Contrary, the findings of this study differ from some prior research, such as the study by Alkhamisi et al. (2017), who reported that molybdenum had an insignificant effect on germination despite a slight impact, while it agreed with a noticeable effect of molybdenum on the length of the plumule. Moreover, molybdenum was observed to enhance membrane stability, optimizing leaf water status and potentially aiding plants in mitigating damage from environmental stresses. This outcome aligns with previous studies indicating that molybdenum supplementation promotes abscisic acid (ABA) production, which plays a vital role in regulating wheat leaf water balance, thereby enhancing the water retention efficiency of plant organs and tissues (Sun et al. 2009; Wu et al. 2018).

Also, molybdenum plays a positive role in preserving the structure and integrity of membranes in wheat leaves, consequently enhancing growth and productivity (Wu et al., 2020). These findings

align with Al-Ubaidy et al. (2021) results which demonstrated molybdenum's influence on the electrical conductivity of mung seedlings during drought conditions in the laboratory. Furthermore, the elevated concentration of molybdenum exceeding 15 mg L<sup>-1</sup> in terms of relative water content may be attributed to its role in increasing the production and formation of ascorbic acid (Khazaei & Estaji, 2020).

Table 1. Effect of Molybdenum on some physiological traits of wheat

| Mo<br>Mg L <sup>-1</sup> | Germination (%) | Radicle<br>Length<br>(cm) | Plumule<br>Length<br>(cm) | RWC<br>(%) | REC<br>(%) |
|--------------------------|-----------------|---------------------------|---------------------------|------------|------------|
| 0                        | 88.73           | 3.15                      | 8.15                      | 74.05      | 77.21      |
| 15                       | 91.47           | 3.14                      | 8.53                      | 80.68      | 77.56      |
| 30                       | 93.23           | 3.30                      | 9.23                      | 76.20      | 75.86      |
| LSD (0.05)               | 1.86            | NS                        | 0.33                      | 3.72       | NS         |

<sup>\*</sup>RWC: Relative water content, REC: Relative Electrical Conductivity, NS: Non-significant

A significant interaction was found between molybdenum and the genotypes used in the current investigation (Table 2). The highest germination mean was recorded for genotype G-31 when treated with 30 mg L<sup>-1</sup>, with an increase rate of 19.8% compared to the Mo-untreated local variety IRAQ. Molybdenum did not have a significant impact on certain traits in this study, including root length, shoot length, and relative water content. In contrast, molybdenum and genotypes had a significant effect on the electrical conductivity mean of wheat seedlings (Table 2), as molybdenum (15 mg. L<sup>-1</sup>) significantly reduced the mean characteristic of the local variety IRAQ by 8.42% compared to the Mo- untreated genotype G-31.

The Molybdenum affected the germination (%), these results were consistent with the findings of Kumar & Aery (2012), who confirmed its effectiveness in germinating wheat grains at low concentrations. Conversely, a negative effect was observed when molybdenum was applied at higher concentrations. Treatment of both genotypes with molybdenum resulted in a noticeable increase in germination (%), with the effect being more pronounced in the G-31 genotype, exhibiting an 11.96% increase rate compared to the local variety IRAQ (Table 2). Identifying wheat genotypes that are physiologically significant in terms of growth traits and further yields, holds the potential for significantly enhancing yields and adaptation to unfavorable environmental conditions. The local variety (IRAQ) exhibited significant value of electrical conductivity compared to the genotype G-31, likely due to genetic background differences between the genotypes under investigation. Molybdenum significantly promoted physiological growth in both genotypes (Table 2). Treatment of wheat seedlings with molybdenum (15 mg L<sup>-1</sup>) resulted in a reduction in average electrical conductivity, particularly evident in the IRAQ cultivar. This indicates its role in alleviating or limiting cell membrane deterioration as an adaptive mechanism by molybdenum to stabilize or maintain cell membranes against any possible stress. This interpretation agreed with previous studies on cereals conducted by Wu et al. (2020) and Imran et. al. (2020).

Table 2. Effect of Molybdenum on some physiological traits of two wheat genotypes

| Genotypes | Mo<br>Mg L <sup>-1</sup> | Germination (%) | Radicle<br>Length<br>(cm) | Plumule<br>Length<br>(cm) | RWC<br>(%) | REC<br>(%) |
|-----------|--------------------------|-----------------|---------------------------|---------------------------|------------|------------|
| G-31      | 0                        | 98.08           | 3.14                      | 8.36                      | 73.19      | 81.42      |
|           | 15                       | 97.83           | 3.15                      | 8.72                      | 77.45      | 81.17      |
|           | 30                       | 99.25           | 3.22                      | 9.74                      | 73.58      | 77.36      |
| IRAQ      | 0                        | 79.45           | 3.14                      | 7.95                      | 74.92      | 73.95      |
|           | 15                       | 85.16           | 3.12                      | 8.34                      | 83.91      | 73.00      |
|           | 30                       | 87.29           | 3.38                      | 8.76                      | 78.81      | 74.36      |
| LSD (0    | ).05)                    | 4.16            | NS                        | NS                        | NS         | 8.19       |

<sup>\*</sup>RWC: Relative water content, REC: Relative Electrical Conductivity, NS: Non-significant

Table 3 shows that there was no significant impact of Cd on all physiological traits. On the other hand, the protective role of molybdenum proved to be statistically significant in mitigating cadmium stress, making the plant relatively more tolerant to high-stress conditions. Treatment with molybdenum resulted in an increase in plumule length by 1.03%, 2.25%, and 0.95% under the influence of cadmium stress concentrations of 75, 150, and 225 mg L<sup>-1</sup>, respectively. Remarkably, the effect of a low concentration of molybdenum (15 mg L<sup>-1</sup>) was particularly pronounced at a high cadmium concentration (225 mg L<sup>-1</sup>) compared to higher molybdenum concentrations and the control treatment, achieving a relative increase in the trait mean of 0.69 and 0.95% respectively.

Previous studies reported that cadmium significantly reduced photosynthetic activity and chlorophyll content in wheat (Sarwar et al., 2015; Li et al., 2023; Paunov et al., 2018). It is certain that molybdenum treatment significantly enhanced the photosynthesis process and chlorophyll content, and this may result from its role in improving the activity and expression of nitrogen assimilation enzymes, thus increasing nitrogen accumulation further in the leaves (Hashem & Al-Issawi, 2023). These results are consistent with previous studies that reported that the efficiency of chlorophyll content, photosynthesis, and chloroplasts have a direct relationship with the efficiency of nitrogen content accumulation under the influence of cadmium stress (Imran et al., 2021; Al-Obaidi et al., 2021). Molybdenum did not have a significant noticeable effect on the rest of the studied traits, although molybdenum slightly increased the averages of morphological and physiological traits for this study they did not reach the significant levels, slightly, except the length of the plumule, which molybdenum significantly affected, and this agreed with the results of Han et al. (2020) and Al-Obaidi et al. (2021) who confirmed the positive role of Mo on growth under the effect abiotic stresses such heavy metal and drought. This effect might be attributed to the role of molybdenum in stimulating physiological processes, promoting vegetative growth, and depositing cadmium in the soil (Schat et al., 1997), or might belong to the improvement the traits through its effect on the processes of carbon synthesis and hydrogen metabolism, and nitrogen has a major role in promoting vegetative growth, especially the length of the plumule (Crusciol et al., 2019). It is also noted that cadmium at a low concentration (75 mg L<sup>-1</sup>) improved some growth traits, especially the germination rate, and these results are consistent with the findings of Ismael et al. (2018), who found that the low concentration of cadmium slightly enhanced the growth traits, and this might be attributed to increased enzymes activities at low concentrations of cadmium, while its effect at high concentrations was harmful to all the traits under investigation.

Table 3. Effect of Cadmium and Molybdenum interaction on some physiological traits of wheat

| Cadmium<br>mg L <sup>-1</sup> | Mo<br>Mg L <sup>-1</sup> | Germination (%) | Radicle<br>Length<br>(cm) | Plumule<br>Length<br>(cm) | RWC<br>(%) | REC<br>(%) |
|-------------------------------|--------------------------|-----------------|---------------------------|---------------------------|------------|------------|
| 0                             | 0                        | 94.17           | 7.34                      | 11.60                     | 77.85      | 95.94      |
|                               | 15                       | 95.50           | 6.97                      | 10.65                     | 85.00      | 84.48      |
|                               | 30                       | 96.00           | 7.13                      | 12.44                     | 78.14      | 86.47      |
| <b>7</b> 5                    | 0                        | 96.83           | 2.00                      | 7.49                      | 70.09      | 74.68      |
|                               | 15                       | 97.00           | 2.00                      | 8.24                      | 83.49      | 83.04      |
|                               | 30                       | 96.34           | 2.04                      | 8.52                      | 75.58      | 72.60      |
| 150                           | 0                        | 85.50           | 1.58                      | 7.39                      | 73.91      | 69.61      |
|                               | 15                       | 87.17           | 1.69                      | 8.14                      | 77.25      | 71.83      |
|                               | 30                       | 92.32           | 1.94                      | 9.64                      | 75.02      | 76.76      |
| 225                           | 0                        | 78.59           | 1.68                      | 6.17                      | 75.69      | 68.63      |
|                               | 15                       | 86.33           | 1.90                      | 7.12                      | 77.02      | 70.91      |
|                               | 30                       | 88.42           | 2.10                      | 6.43                      | 76.08      | 67.32      |
| LSD (                         | 0.05)                    | NS              | NS                        | 0.66                      | NS         | NS         |

In the anatomical study, it was observed that all cadmium concentrations included in this study affected the cellular structure of the root and its overall morphology. Sabella et al. (2022) reported similar results in terms of the anatomy of wheat seedlings. Previous studies have also confirmed that cadmium accumulation in the root of wheat exceeds that in other plant organs (Özviğit et al., 2021). Furthermore, the thickness of the cortex tissue noticeably increased concomitantly with the increase in the concentration of added cadmium. This phenomenon may be attributed to an increase in the average diameter of the vascular cylinder of the root (Figure 1), prompting the plant to generate new xylem and phloem cells as a response to reduce cadmium toxicity. The increase in cortex thickness observed with higher cadmium concentrations might be attributed to the enlargement of parenchymal cells within the cortex, as some types of cortex cells function to eliminate cadmium in vacuoles as much as possible, thereby restraining its translocation to vegetative parts. This was noticed by the expansion of cell walls in the epidermal tissue of cadmium-treated roots compared to the control treatment (Figure 1). Similar findings were reported in a study on wheat roots by Sabella et al. (2022). The root of wheat retains large amounts of cadmium and reduces the amounts transferred to the aerial parts (Sabella et al., 2022 & Payandeh et al., 2018). This leads to the conclusion that cadmium effects are greater on the root compared to other parts of wheat (Payandeh et al., 2018). The response of genotype G-31 to the cadmium treatment in the root was similar to that of the local variety IRAQ. As an important mechanism, the roots of these genotypes - adopted in this study - relatively retained cadmium in the epidermis or cortex as much as possible to reduce its accumulation in the vegetative parts above the soil surface. This result agreed with a study that showed that when the roots of some wheat genotypes are exposed to cadmium, the root parts, such as the epidermis and cortex, eliminate or decrease the effect of cadmium toxicity to the vascular cylinder and then to other parts of the plant (Parrotta et al., 2015). This is what was observed in the roots of the genotypes under investigation and when were exposed to a high concentration of cadmium (Figure 1). The ability of roots to retain an

amount of cadmium in their tissue is a very effective mechanism to eliminate or reduce the damage and toxicity of heavy metals and limit their negative impacts on the other parts of the plant. It is believed that cadmium affects the cell wall of the root by increasing the proportion of lignin that makes up the cell wall and thus losing or reducing the intercellular spaces (Janicka-Russak et al., 2012).

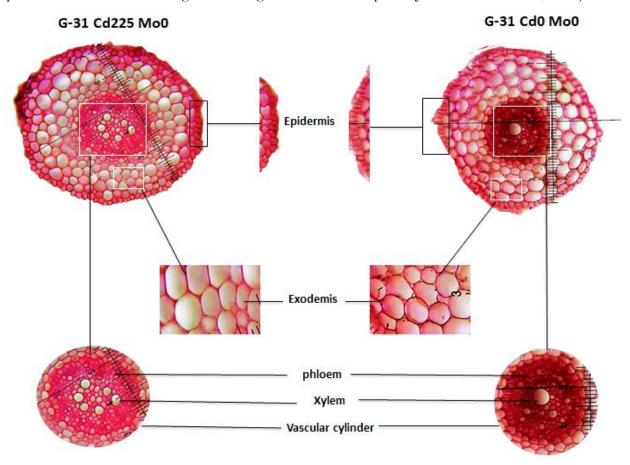


Figure 1. Cross-section of wheat genotype (G-31) radicle treated with cadmium (0 and 225 mg L<sup>-1</sup>) (X10)

It is believed that the stress induced by cadmium triggers the formation of a phenomenon known as suberin in the xylem vessels (Sabella et al., 2022). This phenomenon may stimulate the root to generate additional xylem vessels in response as a response to cadmium exposure. Sabella et al. (2022) reported similar results. Suberin is a waxy substance present in the cell walls of xylem tissues, which may be induced by abiotic stresses. It serves as a defensive mechanism within the plant to combat stressors. As the plant cell surrounds its four walls or on two sides only, and by deposition of the waxy substance, the plant cell limits the uptake of cadmium, thereby reducing its detrimental effects on the cell, tissue, and ultimately the whole organs, as outlined by Han et al. (2020).

The alteration observed in the root cells and tissues of genotype G-31 when exposed to a molybdenum concentration of 15 mg L<sup>-1</sup> under the influence of cadmium gave a positive consequence (Figure 2). This contributed to the overall restructuring of the root, starting from the epidermis and extending to the xylem tissue. These findings affirm the beneficial role of molybdenum at the cellular level of the root, as it facilitated the reorganization of epidermal cell morphology induced by cadmium

exposure. Furthermore, at the cortex tissue level, molybdenum promoted an increase in thickness and cell count. This is attributed to the plant's physiological mechanism triggered by molybdenum as a defensive response to cadmium toxicity. This effect was particularly noticeable at the initial concentration of molybdenum (15 mg L<sup>-1</sup>).

Anatomically, no distinction was noted between genotype G-31 and the local variety IRAQ included in this study. Additionally, molybdenum has improved the integrity of cell walls in the root, these results align with the findings of Huang et al. (2021), who indicated a reduction in the accumulation of free radicals (ROS). Consequently, the anticipated damage to the root structure and architecture caused by cadmium is mitigated, thereby enhancing root efficiency in absorbing water and nutrients.

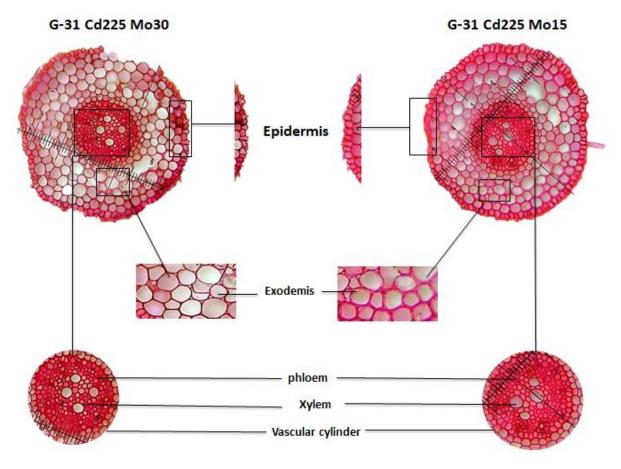
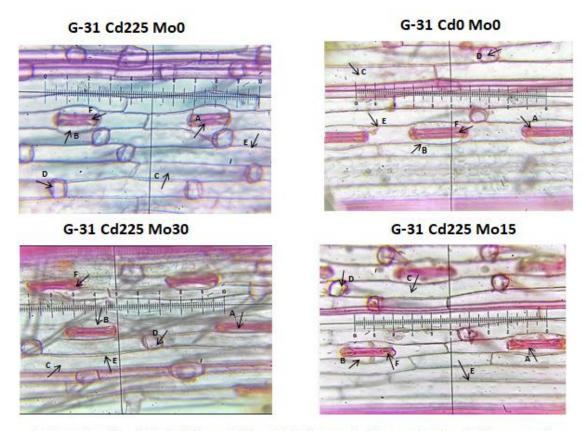


Figure 2. Cross-section of wheat genotype (G-31) radicle treated with the application of Mo (30 and 15 mg L<sup>-1</sup>) under the effect of Cd (225 mg L<sup>-1</sup>) (X10)

As per leaf anatomy, the current investigation showed that cadmium harmed normal epidermal cells, through their increased expansion or division, resulting in a reduction in the malfunction of the stomatal system and an increase in the number of epidermal and motor cells. These effects increased as the cadmium concentrations increased in comparison with the control group (Figure 3). These results align with what has been found by Piršelová et al. (2021), who noted a decrease in stomatal length and width, besides an increase in the number of epidermal cells, particularly at the high concentration of cadmium. Whereas treating the plant with molybdenum, reduced the toxicity of

cadmium at the level of leaf cells in general by gradually reducing the dimensions of the cells, as it reduced the rate of dimensions of both guard and auxiliary cells and ordinary epidermal cells as well as motor cells compared to the control group.

In contradiction, there was no obvious difference in the response of the genotypes under study at the level of leaf cells under stress conditions. Furthermore, this anatomical investigation revealed a relative increase in the thickness of cell walls in both the roots and leaves of wheat. This increase was positively proportional to the concentration of cadmium, possibly indicating a higher accumulation of suberin in cell walls as a physiological response to cadmium stress in wheat. These findings align with what has been reported by Wu et al. (2018).



A:Guard cells, B:Subsidiary Cells, C:Ordinary Epidermal cells, D:Motor cells, E:Cell wall, F:Chlorophyll

Figure 3. Longitudinal section of wheat plumules (G-31 genotypes) with the application of Mo under the effect of Cd

Cadmium hinders the process of photosynthesis, inducing alterations in physiological, chemical, and cellular characteristics (Song et al., 2019), by impeding Calvin cycle enzymes. Additionally, it interferes with carbohydrate synthesis and impacts the permeability of the plasma membrane by inhibiting ATPase activity (Greco et al., 2012). Consequently, this leads to distortions in cell membranes and the degradation of cellular and organelle biomolecules due to the excessive production of free radicals triggered by cadmium (Ullah et al., 2021).

Treating plants with molybdenum significantly increased the average length and width of leaf epidermis cells (Figure 3), and this is consistent with the findings of Rana et al. (2020), who

demonstrated the positive role of molybdenum at the level of leaf epidermis cells compared to Mountreated plants under the influence of cadmium. Molybdenum reduced the accumulation of cadmium in the leaf tissues of wheat. This may be due to the effectiveness of molybdenum at the root level and thus reduced its accumulation in the vegetative parts. Upon exposure to cadmium, wheat leaves showed alterations in stomatal patterns, with cadmium reducing both the length and width of stomata (Figure 3). Molybdenum effectively maintained the stomatal structure under the influence of cadmium stress. These findings are consistent with the results reported by Rana et al. (2020), who demonstrated the beneficial role of molybdenum in increasing both the length and width of stomata in leaves that are affected by cadmium through improved nutrient availability and enhanced plant growth. Additionally, the treatment of molybdenum inhibited cell deterioration, thereby improving the osmosis status under unfavorable conditions.

These results agreed with previous studies of Ismael et al. (2018), who confirmed that foliar application of molybdenum effectively reduces the absorption and accumulation of cadmium, despite its increased accumulation in the root. Furthermore, optimal concentrations of molybdenum can significantly mitigate damage caused by heavy metals by decreasing their absorption and accumulation in plant tissues.

#### Conclusion

The findings of the current investigations gave a clear and concise idea regarding the effect of cadmium on the physiological and anatomical traits of wheat seedlings. Besides, it investigated the role of molybdenum in mitigating the toxic effect of cadmium on wheat in terms of study traits. Thus, the negative impact of cadmium was highlighted in all studied traits. While Mo alleviates the toxicity of this heavy metal especially at the concentration of 15 mg Mo L<sup>-1</sup> by boosting the growth of wheat seedlings in both treated and untreated seedlings. The overall performance of the wheat seedlings was enhanced in the presence of Mo. In addition, Mo has a distinct role in terms of anatomical traits of either root or leaf. Thus, the epidermis, cortex, and vascular cylinder were enhanced in the root upon the application of Mo while the stomatal system was also enhanced in the leaves in comparison with seedlings not treated with Mo. The variation between the two genotypes was not clear in most studied traits especially at the level of anatomy due to the treatment of Mo. This might belong to the role of Mo in hindering the uptake of Cd or might belong to the role of Mo in enhancing the activities of enzymatic and nonenzymatic antioxidants in plants. Mo could also be involved in the alteration of stress gene expression. In this context, further studies are needed on a wide range of genotypes to investigate their ability to withstand heavy metal stress. Therefore, heavy metal-tolerant genotypes could be exploited in heavy metal-contaminated soils.

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## Molibden ublažava stres izazvan kadmijumom za klijance hlebne pšenice (Triticum aestivum L.)

## Marwan Magid Khalid · Mohammed Hamdan Al-Issawi

Sažetak: Zagađenje teškim metalima je postalo ozbiljan problem koji ugrožava sve žive organizme. Zagađenje kadmijumom (Cd) je postalo fokus istraživanja životne sredine i može negativno uticati na zdravlje ljudi i rast biljaka. Molibden se izdvaja kao mikroelemenat otporan na stres koji podržava odbrambeni sistem protiv stresa koji potiče od životne sredine. S tim u vezi, ovo istraživanje ističe ulogu molibdena u ublažavanju toksičnog uticaja kadmijuma na klijance pšenice. Istraživanje je uključilo četiri koncentracije kadmijuma (0, 75, 150 i 225 mg L<sup>-1</sup>), tri koncentracije molibdena (0, 15 i 30 mg L<sup>-1</sup>) i dva genotipa pšenice (G-31 i IRAQ). Ovi faktori su postavljeni kao faktorski eksperiment prema potpuno nasumičnom rasporedu (CRD) sa tri ponavljanja. Rezultati ovog istraživanja ukazali su na negativan uticaj kadmijuma na rast klijanaca pšenice u pogledu fizioloških i anatomskih osobina. Takođe su istakli pozitivnu ulogu molibdena u ublažavanju toksičnog uticaja kadmijuma. Molibden u koncentraciji od 15 mg L<sup>-1</sup> je poboljšao strukturu korena i lista pšenice tretirane kadmijumom. Dva genotipa pšenice nisu se razlikovala u pogledu anatomije kada su tretirani molibdenom. Potrebna su dalja istraživanja na velikom broju genotipova pšenice da bi se ispitala njihova sposobnost da podnesu stres izazvan teškim metalima. Stoga bi se genotipovi tolerantni na teške metale mogli koristiti u zemljištima koja su zagađena teškim metalima.

Ključne reči: anatomija, genotipovi, kadmijum, molibden, pšenica, teški metali