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PHYTOMELATONIN MITIGATES CADMIUM STRESS FOR BREAD WHEAT TRITICUM AESTIVUM L.

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Abstract

To elucidate the role of melatonin in mitigating the cadmium stress effect on wheat in terms of physiological and anatomical responses (e.g., root and leaf), this study was conducted in the laboratories of the College of Agriculture, University of Anbar. The aim was to investigate the important role of melatonin in alleviating Cd stress in wheat. The study included three factors: two wheat genotypes—introduced genotype G-31 (newly introduced to the country through the Iraqi Ministry of Science and Technology) and local cultivar IRAQ; four Cd concentrations (0, 75, 150, and 225 mg Cd L^{-1} as $CdCl_2$); and three concentrations of melatonin (0, 100, and 200 mmole as pure melatonin). The study highlighted melatonin's role in terms of physiological and anatomical responses (e.g., root and leaf). The main results of this study confirmed the negative impact of cadmium on wheat growth, primarily affecting roots more than other plant parts. The epidermis, cortex, and vascular tissues in the xylem and phloem were negatively affected by the application of Cd. Melatonin played a vital role in boosting some physiological traits such as root growth and relative electrical conductivity. The most prominent role of melatonin was observed at the cellular level, as confirmed by the anatomical study. Melatonin helped plants cope with stress through modifications in cell organization and the thickening of either epidermal or cortical cells. Regarding the leaf, melatonin's important role was recorded in the stomatal system. More investigations are required to highlight melatonin's vital role in terms of gene expression and enzymatic and non-enzymatic antioxidants in wheat.

Keywords: Melatonin, Cadmium, Wheat genotypes, Anatomical traits, REC.

استخدام الميلاتونين لتخفيف ضرر اجهاد الكادميوم على حنطة الخبز

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الخلاصة

لتسليط الضوء على دور الميلاتونين في التخفيف من تأثير اجهاد الكادميوم في الحنطة من حيث الاستجابات الفسيولوجية والتشريحية (مثل الجذر والأوراق). أجريت هذه الدراسة في مختبرات كلية الزراعة– جامعة الأنبار لمعرفة دور الميلاتونين المهم في التخفيف من إجهاد الكادميوم في الحنطة. وتضمنت الدراسة ثلاثة عوامل وهي؛ نوعان وراثيان من الحنطة (التركيب الوراثي المُدخل G-31 والصنف المحلي IRAQ)، وأربعة تراكيز من الكادميوم 0، 75، 150، و225 ملغم لتر$^{-1}$ من الكادميوم بشكل كلوريد الكادميوم $CdCl_2$ وثلاثة تراكيز من الميلاتونين النقي 0، 100، 200 ملي مول. وسلط الضوء في هذه الدراسة على دور الميلاتونين من حيث الاستجابات الفسيولوجية والتشريحية (مثل الجذر والأوراق). أكدت النتائج الرئيسية لهذه الدراسة التأثير السلبي للكادميوم على نمو الحنطة. يلعب الميلاتونين دورًا حيويًا في تعزيز بعض الصفات الفسيولوجية مثل تطور الجذر والايصالية الكهربائية النسبية. وسجل الدور الأبرز للميلاتونين على مستوى الخلايا وهو ما أكدته نتائج الدراسة التشريحية. بشكل رئيسي، الكادميوم له تأثيرات أكبر على الجذور مقارنة بأجزاء النبات الأخرى. تأثرت البشرة والقشرة والاسطوانة الوعائية في نسيج الخشب واللحاء سلبًا باستخدام الكادميوم، والأهم من ذلك أن الميلاتونين ساعد النباتات على التغلب على هذا الاجهاد من خلال بعض التعديلات على تنظيم الخلايا وزيادة سماكة خلايا البشرة والقشرة. أما بالنسبة للورقة فقد تم تسجيل الدور الهام للميلاتونين في نظام الثغور. هناك حاجة إلى مزيد من التحقيقات لتسليط الضوء على الدور الحيوي للميلاتونين من حيث التعبير الجيني ومضادات الأكسدة الأنزيمية وغير الأنزيمية في الحنطة.

كلمات مفتاحية: الميلاتونين، الكادميوم، التراكيب الوراثية للحنطة، الصفات التشريحية، الايصالية الكهربائية.

Introduction

Cadmium is one of the most risky heavy metals to the environment and has a harmful impact on plant growth, development, and productivity. Its availability in the environment is at relatively low levels, but with the increase of human activities, its level got higher which can affect human lives especially when it occurs in the food chain (1). Humans can be at high risk specifically kidney, lung, bones, etc., because cadmium was classified as a carcinogenic material (24). Cadmium enters plants through either roots or shoots with the aid of transporters such as HAM, MTPs, NRAMP, ZIP, and ZRT-IRT. Cadmium's negative impact normally comes from the formation of Reactive Oxygen Species (ROS) that occur in the metabolic pathways. Besides, it can damage the photosynthesis systems, root growth, and absorption of water and nutrients (35). On the other hand, the genetic background of plants might play a vital role in the mitigation of Cadmium effects in tissues (34). The strategy of avoiding exposure to Cd through low-Cd accumulating varieties is one of the important strategies, specifically in wheat as it is the most consumed crop in the world (31). Hence wheat is the daily food for more than 50% of the world's population, its importance increased especially after the Ukraine- Russia war, the most producing countries of this crop (15). It is expected that the demand for this crop will increase by 70% by 2050 in parallel with the increase of the world population which is also expected to get to 10 billion. Therefore, the need to increase the productivity of this crop should be increased to meet the food requirement, in addition, the cultivated area of this crop should also be increased over the current percentage (20% of cultivated land globally) (29). In Iraq, the cultivation of wheat is facing big challenges due to the biotic and abiotic stresses which cause huge quantitative and qualitative losses of wheat grains. As a result of exposing plants to external stresses, they produce many of ROS which eventually hinder plant growth and development. Therefore, as a response plants rise their internal content of antioxidants to naturalize the harmful effects of ROS (4). The application of antioxidants exogenously also can increase the plants ability to cope with the oxidative stress exerted to plant by environmental stresses (2). Melatonin is one of those antioxidants that can be produced endogenously and also can be added exogenously. Melatonin (N-acetyl-5-methoxytryptamine) was discovered as a plant hormone during 1995 which was synthesized in plants from tryptophan amino acid (26), and reduces the harmful effect of oxidative stress combined with environmental stresses (32). (11) indicated that melatonin has a vital role in plant mitigation of the harmful effect of cadmium through increasing the activity of antioxidants and eventually plant growth. Many studies proved the important role of this hormone in plants as a response to Cd stress through protecting photosynthesis and modulating the expression of genes responsible for heavy metals transportation. Melatonin regulates many signal pathways such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) which is important in increasing the tolerance of plants against Cd toxicity as well as changing endogenous chelating compounds and eventually decreasing the accumulation of Cd in plants (11 and 13). The role of melatonin in the mitigation of environmental stresses in plants have recently gained more attention. Therefore, the role of this hormone against Cd stress will be the focus of this study in wheat and determine the best concentration that could alleviate the immune systems of wheat against the toxicity of Cd.

Materials and Methods

A laboratory experiment has been conducted in the College of Agriculture – University of Anbar to study the important role of melatonin 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^{-1} as $CdCl_2$ (MW: 183.32 gm $mole^{-1}$) (Thomas Baker) and three concentrations of melatonin 0, 100 and 200 mmoles as pure melatonin (MW: 232.278 from Pure Bulk Company). All these factors were laid out as factorial arrangements in CRD with three replications. Grains of the two wheat genotypes were soaked with melatonin solutions for 8 hours and then 100 grains from each cultivar were distributed in petri dishes for each treatment and treated with the required concentration of Cd. Parameters were investigated: germination (%), radicle and plumule length (cm), relative water content RWC (%) and relative electrical conductivity REC (%).

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

Where:
TW: turgid weight
FW: fresh weight
DW: dry weight (Al-Sheibany & El-Karhi, 2017)

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

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 (10).

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 glass slides. The cover of the slide was placed and left for 3 hours to remove bubbles 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 the means were calculated according to the LSD test at 5% probability level.

Results and Discussion

The risk of heavy metals especially cadmium dramatically increased due to industrial and agricultural activities. This threat leads to a negative impact on living organisms (28). On the other hand, Cd stress affected wheat seedlings' growth as indicated in Figure 1. Germination has not been affected at low concentrations of Cd but it declined when concentration got higher ($P<0.05$, $n=3$, $LSD= 0.97$) and this supports the fact that Cd boosts germination of wheat at low concentrations (17). Cd application negatively impacted the growth of radicle and plumule of wheat seedlings at all studied concentrations ($P<0.05$, n=3, $LSD_{(radicle)}$= 0.395, $LSD_{(plumule)}$= 0.594). A reduction in the growth of wheat seedlings was reported by many researchers (23 and 33). However, relative water content decreased upon the application of Cd (P<0.05, n=3, LSD=3.750). Electrolyte leakage (%) slightly increased at the low concentrations of Cd while it got higher at 150 and 225 mg Cd L^{-1} ($P<0.05$, n=3, LSD=2.602). These findings were consistent with what has been found by (28). Melatonin is the bio stimulator in plants as it enhances the tolerance against abiotic stresses including heavy metals stress.

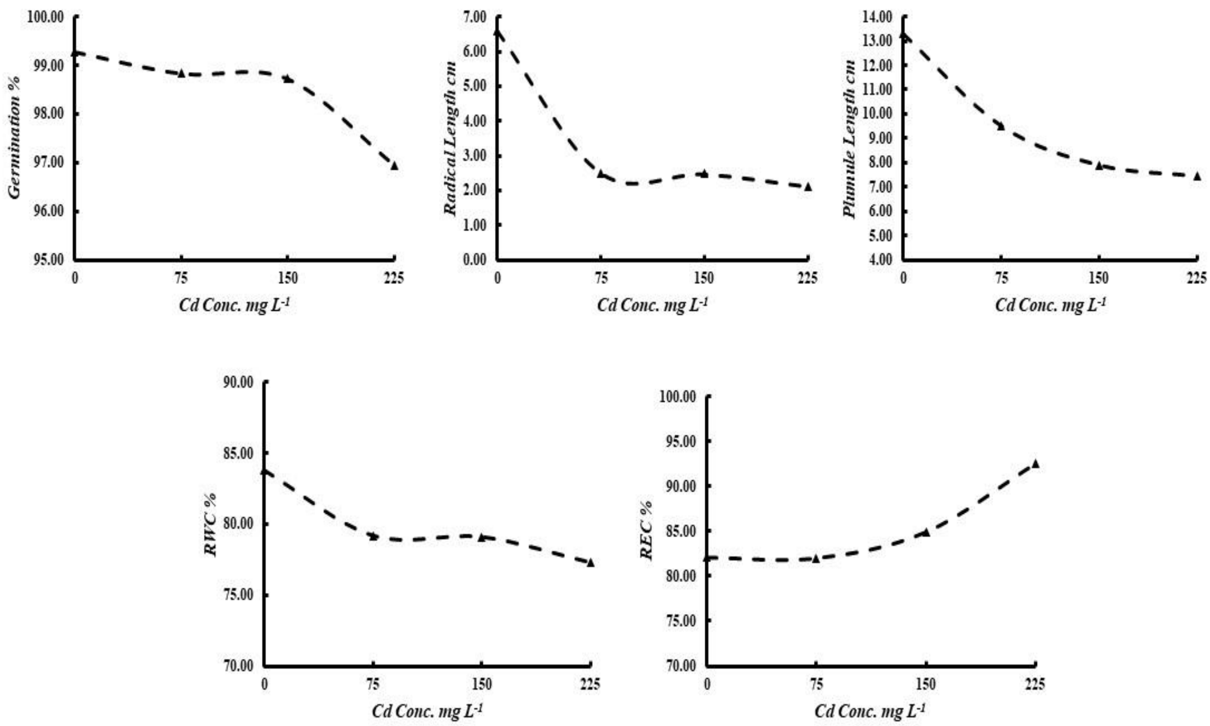

Figure 1: Effect of Cd on germination (%), Length of radicle and plumule (cm), Relative water content (%), and Relative electrical conductivity (%) in wheat seedlings.

The exogenous application of melatonin boosts the radicle length of wheat at a concentration of 100 mmol (3.73 cm). The increase percentage was 10.36 % in comparison with the control. On the other hand, the application of melatonin significantly decreased the REC at the two concentrations 100 and 200 mmole with a decrease rate of 3.17 and 2.46 % sequentially in comparison with the control (Table 1). Unlike what has been found by (30), the results of this study proved that plasma membranes in seedlings were not integrated. While there was no clear effect of melatonin on either germination, plumule length of RWC%. Many studies have proved the vital role of melatonin at certain concentrations in enhancing the growth of the root and increasing its surface area which eventually might affect the absorption of water and nutrients (25). Besides, this hormone has a role in secondary growth and development of roots which increases the ratio of Root/Shoot (7 and 8). Collectively, by enhancing root growth and development, melatonin might also enhance the absorption of water and nutrients and then photosynthesis reactions.

Table 1: Effect of melatonin on some physiological growth traits of wheat.

MET mmole	Germination (%)	Radicle Length (cm)	Plumule Length (cm)	RWC (%)	REC (%)
0	98.38	3.38	9.45	80.52	87.00
100	98.25	3.73	9.44	80.89	84.24
200	98.71	3.15	9.73	78.12	84.86
LSD (0.05)	**NS**	**0.34**	**NS**	**NS**	**3.008**

The two genotypes under investigation varied in their physiological responses to melatonin application (Table 2). The IRAQ cultivar was superior in radicle length by an increased rate of 20.41 % over the other genotype (G-31) at the concentration of 100 mmole of melatonin. The genotype G-31 was superior by giving the lowest mean of REC% with a reduction rate of 13.73% when treated with 100 mmole of melatonin in comparison with control. Exogenous application of melatonin specifically the concentration of 100 moles enhanced some of the physiological traits of wheat genotypes. These results were consistent with the findings of previous studies (9). Some of the variation in the response might belong to the background genetics of the used genotypes as slight superiority of IRAQ genotypes over the other genotype (G-31) while in other traits the superiority of G-31 was detected (e.g., REC%). The superiority of both genotypes was noticed when they were treated with 100 mmole of melatonin. This might be beneficial in choosing a superior genotype along with the optimum concentration of the exogenous melatonin for best stress tolerance and considerable productivity.

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

Genotypes	MET mmole	Germination (%)	Radicle Length (cm)	Plumule Length (cm)	RWC (%)	REC (%)
G-31	0	99.00	3.17	9.76	79.81	92.47
	100	98.33	3.38	9.78	81.88	79.77
	200	99.50	2.79	9.98	75.33	89.88
IRAQ	0	97.75	3.60	9.15	81.22	81.53
	100	98.17	4.07	9.10	79.89	88.71
	200	97.92	3.52	9.48	80.90	79.83
LSD (0.05)		**NS**	**0.40**	**NS**	**NS**	**3.19**

To date, there is no biological role of Cd in plants. On the contrary, Cd when occurs in the food chain causes harmful effects on human health. On the other hand, some researchers speculated a possible role of it in plants by competing with some other nutrients e.g., Zn (6). However, the reports are certain about its significant toxic effects even at a low concentration in the plant tissues (18). Wheat seedlings' growth was significantly reduced by the addition of Cd to the medium. On the other hand, melatonin by interacting with other plant hormones regulates plant responses against environmental stresses (16). Some reports indicated that melatonin treatment increased endogenous levels of other hormones such as CK, ABA, and GA (14 and 16). These findings suggested the role of melatonin in the homeostasis of hormones which eventually contribute to the tolerance to environmental stresses. Results of the current investigation proved the damaging effect of Cd on wheat seedlings and melatonin mitigates this effect. Melatonin has a noticeable effect on some physiological traits (Table 3). The current investigation indicated an increased rate of 5.31% of radicle length when the plant was treated with 100 mmoles of melatonin under the effect of 225 mg Cd L^{-1} in comparison with the control. This result was consistent with most of the previous findings which explained the vital role of melatonin in the wheat seedlings' growth inhibition due to Cd treatment, besides its role in increasing the endogenous melatonin (11). The clear effect of melatonin was at the low concentration 100 mmoles in comparison with the high concentration 200 mmoles with the increase rate of 5.31 and 1.45% over the control respectively. These results also agreed with the findings of (27) who proved that exogenous melatonin alleviates the toxicity of Cd. Melatonin also reduced the REC% as indicated in table 3. Melatonin at a concentration of 100 mmoles decreased the REC% at the decrease rate of 6.15% under the effect of Cd (225 mg Cd L^{-1}). The Cd application led to the production of ROS which has a deterioration effect on the plasma membranes through the interaction with lipids and proteins (35). However, melatonin has the effective ability to protect the plasma membranes, especially during environmental stresses (27). The harmful effect of Cd on the permeability of membranes might be due to the accumulation of Cd in the root (4 and 20).

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

Cadmium mg L-1	MET mmole	Germination (%)	Radicle Length (cm)	Plumule Length (cm)	RWC (%)	REC (%)
0	**0**	99.33	6.63	13.47	85.77	73.04
	100	99.50	7.60	13.07	81.62	88.61
	200	99.00	5.52	13.43	84.02	84.53
75	**0**	98.33	2.45	9.65	80.72	95.02
	100	98.67	2.62	9.45	80.71	71.29
	200	99.50	2.45	9.45	76.13	79.63
150	**0**	98.17	2.38	7.62	79.90	85.47
	100	98.83	2.50	7.87	81.42	88.42
	200	99.17	2.55	8.18	75.95	80.81
225	**0**	97.67	2.07	7.08	75.69	94.46
	100	96.00	2.18	7.38	79.80	88.65
	200	97.17	2.10	7.85	76.36	94.46
LSD (0.05)		**NS**	**0.68**	**NS**	**NS**	**4.51**

The previous studies showed that Cd is accumulating in the root of wheat more than in other parts (19) and this was confirmed by the anatomical traits in the current study. However, Cd increased the thickness of the cell wall in the root, and vacuole volume and damaged the plasma membrane in comparison with the control (Figure 2). The cross-section of 10-day-old roots of wheat explained that the epidermis cells were abnormal upon treating them with Cd, hence the epidermis became disorderly thicker than those untreated with Cd. Also, cortex cells which potentially responsible for reducing Cd toxicity by eliminating it to the vacuole and eventually stopping transporting it to the vegetative and reproductive parts and this was consistent with what has been found by (21). A slight increase in cortex cell thickness was noticed in seedlings treated with Cd due to the increase in thickness of parenchyma cells, while the number of xylem vessels increased under the effect of Cd compared to the control (Figure 2).

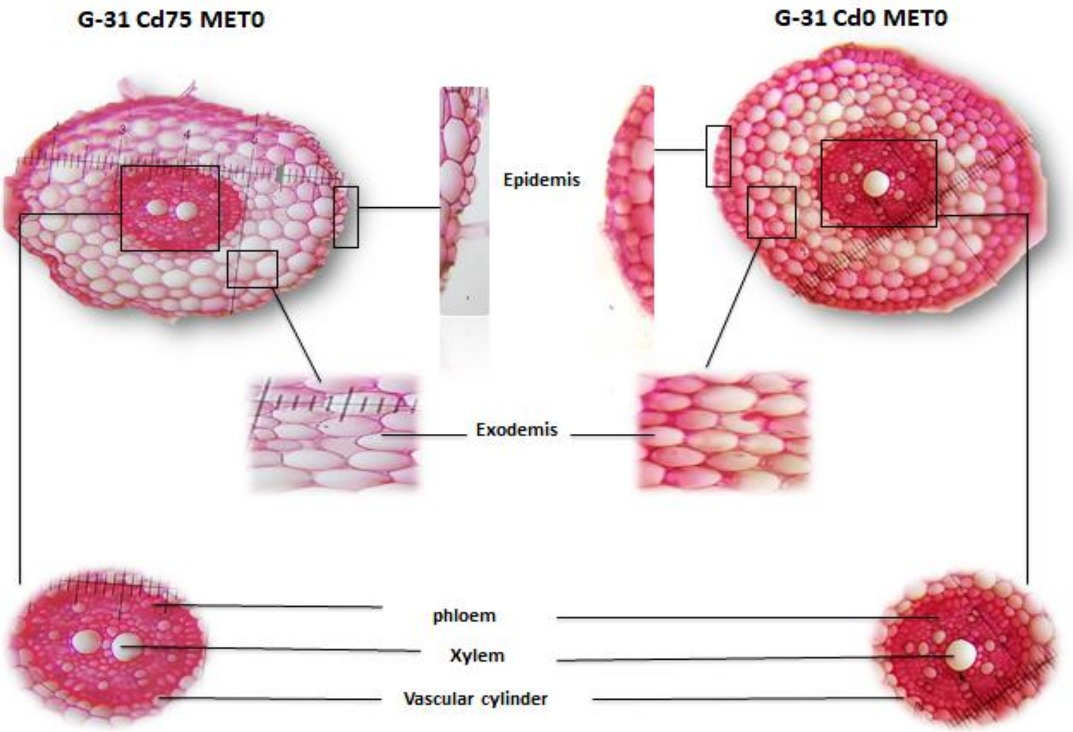

Figure 2: Cross-section of wheat genotype (G-31) radicle treated without application of MET under the effect of Cd (X10).

It was noticed that the thickness of the epidermis was increased (Figure 3). This expansion might be related to the promotion of producing new phloem and xylem tissues to potentially cope with the accumulation of Cd. It was clear that Cd stress caused a Suberization phenomenon in the xylem vessels and this is considered a defense mechanism that plants use against the high concentration of Cd and limits the uptake of it and eventually reduces its harmful effects on tissues. This result is in agreement with what has been found by (21), (7) who believed that suberization promotes roots to increase the xylem vessels as a response to Cd stress. Based on the cross-section of wheat radicles, no clear differences were noticed in terms of root diameter in Cd-treated and untreated seedlings. However, root instruction was changed upon treatment with Cd which caused relative changes in the thickness of cell walls of the epidermis and cortex of the xylem. This result was in agreement with the findings of (21) who confirmed that the root cell's vital role in the mitigation of harmful effects of Cd due to the increase in water and nutrient uptake which hinders transporting Cd to aerial parts of the plant.

In the current investigation, melatonin was found to have a positive role at the cellular level (Figure 3). Throughout the anatomical study, it has been found that melatonin organizes the epidermis cells that were affected by Cd stress. Also, it has been noticed that cortex tissues were thicker upon treating it with Cd and the number of cells was also higher in comparison with the control. This could be due to the physiological mechanism that plants use against exposed stress with the availability of melatonin on the cell level. This effect was obvious in genotype G-31 when treated with 75 mg Cd L^{-1} especially at 100 MET mmole compared to IRAQ cultivar (Figure 3). It was clear that melatonin effects on the xylem led to mitigation of Cd toxicity through depositing it into vacuoles which in turn limiting the translocation of Cd to the other plant parts. In this case, cell walls and vacuoles of the epidermis accommodate most of Cd uptake through chelating it by NA (Nicotianamine) and this also explains the thickness of the cells in roots of wheat. These findings were mostly when wheat was treated with 100 MET mmole when it increased the thickness of cell walls as well as the number of xylem vessels was less in comparison with the control group (Figure 3) and this is in agreement with the findings of (21). It has also been Noticed that the vascular cylinder size is relatively increased when wheat plants are treated with 75 mg Cd L^{-1} and 200 MET mmole.

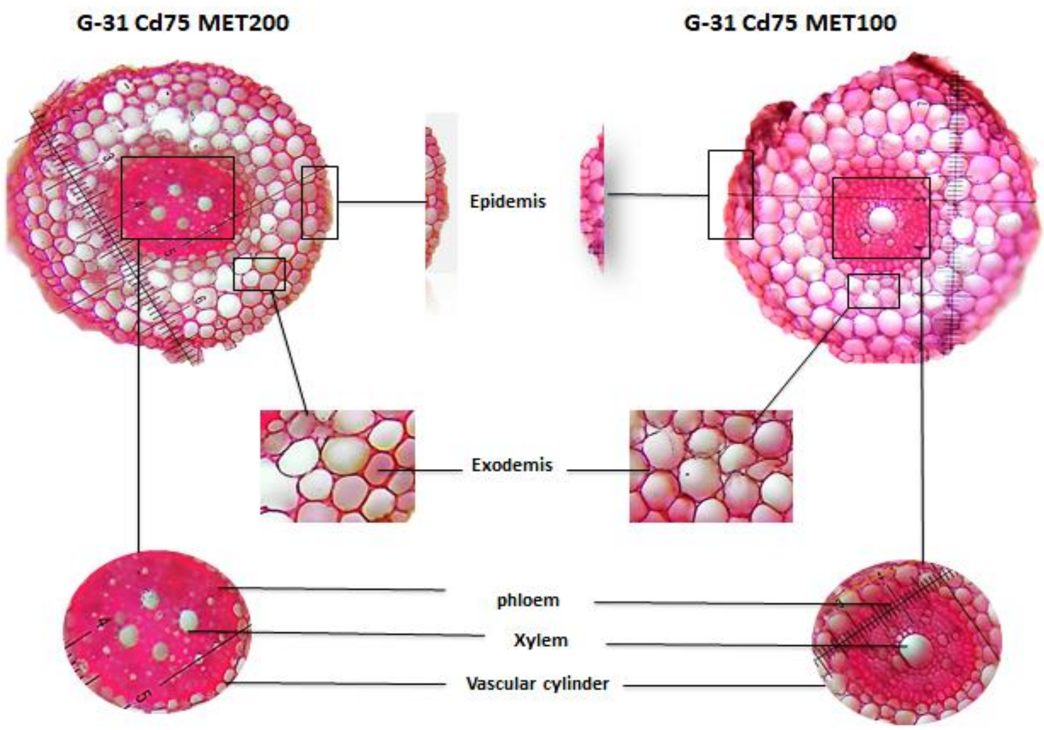

Figure 3: Cross-section of wheat genotype (G-31) radicle treated with the application of MET (100 and 200 mmoles) under the effect of Cd (X10).

As per leaf anatomy presented in Figure 4, it is clear that Cd relatively has less effect in comparison with its effect on roots which might be due to the genetic background of the used genotypes in the current investigation. However, the clear effect of Cd was on the extension of the ordinary epidermis cells and increased the motor cells in comparison with the control group (Figure 4). On the other hand, the effect of Cd was clear on the stomata numbers and shape. The guard and subsidiary cell dimensions were also affected by Cd compared to the untreated group (Figure 4). In the current investigation, it has been noticed that melatonin reduced the accumulation of Cd in leaf tissues. The epidermis cells of wheat leaf were affected by Cd application while melatonin treatment enhanced from the dimension of epidermis cells and this result is consistent with the findings of (22) who confirmed that melatonin has a vital role on leaf cells. Also, it was noticed through the anatomy investigation on the surface section of the wheat leaf that Cd expanded the ordinary epidermis cells (length) by which the stomata system was affected. This was noticed with the increase in Cd concentration (Figure 4).

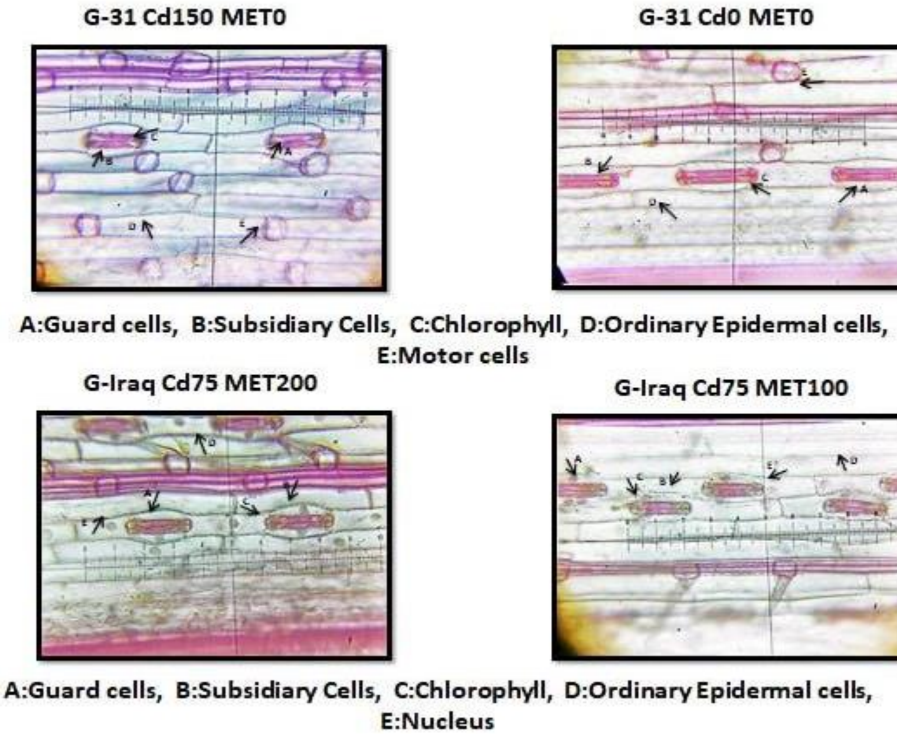

Figure 4: Longitudinal section of wheat plumules (G-31 and IRAQ genotypes) with the application of MET under the effect of Cd.

Generally, melatonin reduces the Cd toxicity on leaf cell levels through the reduction of the cell dimensions gradually. However, the two-way interaction of the application with 75 mg Cd L^{-1} and 200 MET mmole contributed to the reduction of guard, Subsidiary, and motor cell dimensions in comparison with plants treated with Cd only. (12) highlighted the protective role of melatonin in the plasma membranes by scavenging the ROS that occurs because of the abiotic stresses including Cd stress.

Conclusions

Based on the findings of the current study, it can be concluded that the relative increase in Cd accumulation in wheat seedlings led to the inhibition of most metabolism processes in plants such as photosynthesis which in turn could be reflected in the productivity. This study proved the vital role of melatonin in mitigating the toxic effect of the heavy metal (Cd) in wheat seedlings especially when was used at the concentration of 100 mmole as it was superior over the other levels 0 and 200 mmole in most studied traits. As per the anatomy study, the results confirmed that Cd has a negative impact on wheat growth for both genotypes under investigation. This negative impact was obvious on root in comparison with leaf. More importantly, melatonin has a very positive role in terms of cellular levels. It limits the uptake and translocation of Cd. Also, melatonin positively affected the organization of cells that were affected by Cd application. Melatonin increases the thickness of the cortex and increases its cell number as a physiological modification against the Cd stress. Finally, based on the aforementioned findings it can be recommended to study the effect of melatonin on the gene levels as it can interfere with the gene expression of stress genes. Besides, the effect of melatonin on the activity of antioxidants can be studied to highlight the mechanism by which melatonin mitigates the negative impact of abiotic stresses in wheat.

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Author1; methodology, writing—original draft preparation, Author 1 and Author 2 writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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