ISSN: 2073-9524
eISSN: 2310-8746

Investigating the Diversity in Physiological, and Molecular Responses of Wheat (*Triticum aestivum* L.) Genotypes under Cadmium Stress

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Article history:

Received: 20 January 2023
Accepted: 17 March 2024
Published: 30 June 2024

Keywords: *Wheat genotypes, cadmium stress, anatomical traits, heavy metals, RWC.*

Abstract

Heavy metal toxicity is a real threat to the environment, crop productivity, and the health of humans and their animals, especially when the toxicity enters the food chain. This study investigates the effect of cadmium on the physiological and molecular levels of ten introduced wheat genotypes. Genotypes were stressed by cadmium (75 $mg.L^{-1}$) in comparison with unstressed treatment to highlight their response to the cadmium. The experiment was laid out as a factorial arrangement in RCBD, with three replicates. The genotypes included in this study varied in their response to cadmium stress during the laboratory tests. Notably, genotype G-41 was superior to the rest of the genotypes in terms of seed vigor (18.58), chlorophyll content (8.72 $mg.g^{-1}$), and carotenoid content (4.87 $mg.g^{-1}$), while genotype IRAQ had wider epidermis in the root (2.28 µm) and ordinary epidermis cells (3.10 µm). Cadmium boosted some physiological and anatomical traits (e.g., REC, Chlorophyll, carotenoids, length and width of root epidermis cells). However, cadmium concentration caused a deterioration in some anatomical traits, including cortex thickness and the length of the ordinary epidermis cells. Some wheat genotypes showed more resistance to cadmium stress than others, and G-3 was notably affected by cadmium treatment and this was associated with the high PCS1 expression of the enzyme that chelates Cd to the vacuole (39 folds). It can be concluded that cadmium reduced the physiological performances except for some genotypes that showed more tolerance. Those genotypes could therefore be investigated further to assess the accumulation of cadmium in their grains.

https://dx.doi.org/10.52951/dasj.24160106

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Introduction

Unlike some heavy metals (e.g., Mo (Molybdenum), Cu (Copper), Cr (Chrome), Co (Cobalt), V (Vanadium), Ni (Nickel), Zn (Zinc), Mn (Manganese), Fe (Iron)), there are other metals (e.g., Ag (Silver), Hg (Mercury), As (Arsenic), U (Uranium), Pb (Lead), Cd (Cadmium), etc.) that have not been proven to have a role in the development of normal plant life (Alloway, 2013; Naja and Volesky, 2017). On the contrary, their presence in the environment may hinder plant growth. They may enter the food chain, this will represent a real threat to human health because they are insoluble and can accumulate in the cells of the organism beyond the safe level. Considerably, the life span of heavy metals is relatively long and this causes their accumulation in the body of the organism (Sabella *et al.*, 2022; Zhao *et al*., 2023). Chemical weapons used in wars are a major source of heavy metals found in the environment, followed by the accumulated waste in cities, sludge places, dust, engine smoke, and wastes and gases from factories (Alloway, 2013). In addition, soil rock weathering is considered one of the sources of heavy metals, especially cadmium (Wen *et al*., 2020; Xia et al., 2020). The overuse of phosphate fertilizers can also be a source of

heavy metals. It should also be noted that sewerage discarded in rivers is one of the most dangerous sources of heavy metals (Kumar *et al.*, 2022; Wimalawansa, 2016).

There are no apparent symptoms of heavy metals once they enter the biological system; however, their impact appears after many years due to their accumulation (Mishra *et al*., 2019). Cadmium (Cd) is one of the most widespread heavy metals found in the environment, where it takes third place after mercury and lead (Balali-Mood *et al*., 2021; Rahman and Singh, 2019). It has not been proven that Cd has a positive role in plant life (Tran and Popova, 2013), but it has been shown that plant functions start to decline when Cd concentrations are higher than 3 mg kg^{-1} TDM (Dawar *et al*., 2023). The Cd has a strong ability to bind with sulphur, causing protein damage (Colovic *et al*., 2018). It also has a great ability to bind with oxygen and nitrogen compounds, thus disturbing the oxidation balance in plant cells (Demidchik, 2015; Kapoor *et al*., 2019). Cd competes with $Ca^{+2,}$ which is important to cell signaling through kinase enzymes, thus it has a disturbing effect on cell signaling (Thévenod and Lee, 2013b). As for Cd transporters, there is no evidence that Cd has a specific transporter; however, some researchers have proposed that Zn and Fe transporters can transport Cd, with less efficiency (Lin *et al*., 2016). The Zn transporter ZNT1 has been found to transport Cd but with less efficiency (Lin *et al*., 2016). Also, Cd decreases cell expansion through accumulating H_2O_2 in the cell wall, which in turn makes it solid This explains the weakness in root growth in Cd-affected environments (Demecsová and Tamás, 2019; Thévenod and Lee, 2013a). Upon exposure to high concentrations of Cd, Auxins and Gibberellins, which are important in cell signaling, may be damaged (Asgher *et al*., 2015; Singh *et al*., 2016). The Cd also increases the content of Abscisic acid (ABA) and Ethylene (ET) in root cells, leading to a reduction in plant vegetative growth. Ethylene reduces cell expansion, while ABA helps plants adapt to water stress (Fahad *et al.*, 2015; Ullah *et al*., 2018).

Genotoxic is serious damage that heavy metals can cause to organisms, as it may lead to unpredicted genetic variations during the replication and recombination of DNA (Dutta *et al*., 2018; Kocadal *et al*., 2020). Some researchers have indicated that DNA damage is due to the oxidation action by free radicals (ROS), which might partition DNA and cause it to lose some nitrogenous bases (Huang *et al*., 2019). There are many methods for removing or reducing cadmium effects by hindering Cd from reaching edible plant parts (Haider *et al*., 2021; Rizwan *et al*., 2017). This can be achieved by using plant breeding programs and molecular techniques (Nadeem *et al*., 2018; Alfalahi *et al.*, 2022). These give a clear image of how Cd is mobilized inside plants at physiological and genetic levels (Page and Feller, 2015). good plant nutrition reduces the toxicity of Cd and controls its accumulation in edible plant parts (Rizwan *et al.*, 2017). Phytochelatins are known to be produced by plants and are implicated in a number of their reactions to cadmium stress. In particular, Phytochelatin synthase 1 - PCS1 gene offered a valuable defense strategy against heavy metals such as Cd *(*Khan *et al.*, 2016). Its primary function is to bind to heavy metals and store them in cell vacuoles (Cong *et al.*, 2016).

Wheat is the most popular cereal used for human nutrition, especially in the Middle East (Iqbal *et al*., 2022). Wheat growing in polluted areas with heavy metals can be a source of Cd in the food chain and eventually in the human body (Mickovski Stefanović *et al*., 2023; Xing *et al*., 2023). The use of low-accumulated cadmium genotypes would be the most effective and eco-friendly method for excluding Cd from the food chain (Özyiğit *et al.*, 2021). In this context, the study investigated the effect of Cd on many genotypes of wheat to characterize the low Cd-accumulating genotypes suitable for human and animal nutrition, besides characterizing those that accumulate a higher amount of Cd which could be used as phytoremediation.

Materials and Methods

The experiment was conducted in the plant physiology laboratory at the College of

Agriculture, University of Anbar. It aimed to investigate the effect of Cd on germination, and the physiological and anatomical traits of 10 wheat genotypes recently introduced to Iraq with proven suitability to arid and semi-arid areas (Mansoor *et al.,* 2021). Factorial experiments in RCBD of two factors were applied. The first was wheat genotypes, namely G-3, G-4, G-9, G-24, G-28, G-29, G-39, G-41, Al Diar and IRAQ, which were chosen based on field performance in the last three seasons (Abdul-Hassan and Al-Issawi, 2021; Hashem and Al-Issawi, 2023; Mansoor *et al.,* 2021). The second factor was the two levels of Cd (0 and 75 mg L^{-1}). Cadmium Chloride (Cd Cl_2) (FW:183.317g mol^{-1}), (Thomas Baker, chemicals- India) was used as a source of Cd. Each treatment (combination) was repeated three times. After ten days of the experiment, standard lab germination (%), seed vigor, and Relative Electrical Conductivity (REC=EC1/EC2*100) (EC1: were measured after soaking seedlings in test tubes containing a fixed volume of distilled water for 24 h at lab temperature 25°± 2 C, EC2: was measured after exposing the test tubes to 100°C for 15 min in autoclave and then left, also for 24 h, at lab temp).

The experiment was conducted in the plant physiology laboratory at the College of Agriculture, University of Anbar. It aimed to investigate the effect of Cd on germination, physiological and anatomical traits of Eight genotypes recently introduced with two local varieties with proven suitability to arid and semi-arid areas. Factorial experiments in RCBD of two factors were applied. The first was wheat genotypes, namely G-3, G-4, G-9, G-24, G-28, G-G-29, G-39, and G-41, as well as two local Iraqi varieties (Al Diar and IRAQ), which were chosen based on their performance in the last three seasons (Abdul-Hassan and Al-Issawi, 2021; Hashem and Al-Issawi, 2023; Mansoor *et al.,* 2021). The second factor was the two levels of Cd (0 and 75 mg L^{-1}). Cadmium Chloride (Cd Cl_2) (FW:183.317g mol^{-1}), (Thomas Baker, chemicals- India) was used as a source of Cd. Each treatment (combination) was repeated three times. After ten days of the experiment, standard lab germination (%), seed vigor, and Relative Electrical Conductivity (REC=EC1/EC2*100) (EC1: were measured after soaking seedlings in test tubes containing a fixed volume of distilled water for 24 h at lab temperature 25± 2°C, EC2: was measured after exposing the test tubes to 100°C for 15 min in autoclave and then left, also for 24 h, at lab temp).

Physiological traits

Chlorophyll (mg g^{-1}) and carotenoid (µmole g^{-1}) concentrations were measured. Chlorophyll and carotenoid content were chemically measured in the plumules of wheat genotypes, in accordance with (Al-Saleh *et al*., 2019; Lichtenthaler, 1987; Pápista *et al*., 2002). The following equations were used:

$$\text{Chl.a} = (12.7\times A663) - (2.69\times A645) \times V/(1000\times W)$$

$$\text{Chl.b} = (22.9\times A645) - (4.68\times A663) \times V/(1000\times W)$$

$$\text{Total Chl.} = \text{chl.a} + \text{chl.b}$$

$$\text{Carotenoids} = (1.000 \times A470) - (1.8\ \text{Chl a} - 85.02\ \text{Chl b})/198$$

where ***V*** represents the volume of Ethanol used (99.99%), ***W*** represents the weight of plumules and ***A*** represents the absorbance at the specified wavelength of the spectrophotometer (APEL).

Anatomical traits

Some anatomical traits of radical and plumule were investigated, such as a number of root xylem vascular, cortex thickness(µM), length and width of epidermis cells (µM), length and width of ordinary epidermis cells in plumule (µM) (µM), length and width of ordinary epidermis cells in plumule (µM). Cross sections or radicals were made manually by scraping them with a sharp sterilized blade while the radical parts were held vertically by thumb and index fingers. Very thin slices were cut and then stained with safranin (1%) for 20-30 min before transferring them into ethanol (30, 70, and 90%) for 2 min in each concentration to remove the stain, finally transferred to absolute ethanol (96%) for 2 min.

A cross-section of radicals (slices) was fixed on glass slides by placing them using Gel (local commercial). The slide cover was placed and left for 3 hours to remove bubbles and afterward dried, and finally, a cross-section was obtained by the Microscope (Olympus: 100× and 400×) (Al-Ubaidy *et al*., 2021).

Total RNA Extraction and RT-qPCR:

The extraction of the total RNA from fresh seedlings samples was performed using GEN-ZOLTM TriRNA Pure Kit (CAT No: GZX050, Geneaid, ISO: 9001:2015 QMS) following the manufacturer's instruction by GEN-ZOl reagent. RT-PCR (Fluorescence Quantitative PCR Dector, Bioer: LineGene Plus (FQD-48A, Japan) was performed using GoTaq® 1-Step RT-qPCR System (CAT No: A6020, Technical Manual: TM255, Promega Corporation). The primers used in this experiment included primers for the phytochelatin synthase 1 (PCS1) gene (Forward: 3'CTGGCCATTTCTCACCGATC5' and Reverse: 3'GCGCCTTGATACAAGCATGA5') and for the housekeeping gene GAPDH (Forward: 3'TTGCCCTCAATGACCACTTTG5' and Reverse: 3'TTACTCCTTGGAGGCCATGTG5') which were designed in the aid of NCBI website.

Cadmium accumulation:

Cadmium concentration was determined using atomic absorption according to *Allen et al*., (1986), by digestion of the metal of the dried seedlings (1 g) in 100 ml beakers separately, to which 15 ml of the tri-acid mixture (70% high purity HNO_3, 65% $HClO_4$ and 70% H_2SO_4 in 5:1:1 ratio) was added. The mixture was then digested on a hot plate at 80^oC to get the transparent phase of the solution. The resulting solution was filtered and then diluted to 50 ml using deionized water and was analyzed for concentrations of Cd using an atomic absorption spectrophotometer. Data of the traits studied were analyzed according to ANOVA and significant means were distinguished by using MS Excel v. 2016.

Result and Discussion

It is well documented that wheat is grown in Cd- contaminated soils can easily accumulate it leading to many issues including hindering its growth, Reactive Oxygen Species (ROS) accumulation, nutrient imbalance, and most importantly entering the food chain and causing serious problems to human health (Zhou *et al.*, 2020). The high risk associated with Cd stress from its ability to readily dissolve and move within water, soil, and plant systems. Thus, strategies to control the level of Cd in the ecosystems are urgently needed. One of the environment-friendly strategies is to use low accumulating wheat genotypes. Therefore, a thorough examination is essential for characterizing wheat genotypes, prompting this study to investigate the response to Cd-induced stress in ten wheat genotypes, including two local cultivars. Understanding Cd's impact on the important stages of wheat life namely germination and seedling is very useful in determining the future of these crops *(Erenstein et al.*, 2022; Wang *et al.*, 2021).

Cd had no significant effect on the germination of wheat and also genotypes did not vary in the germination (%) ($P<0.05$) (Table 1). The response of wheat genotypes in Petri dishes is different from their response when sown in soils. However, (Ahmed *et al*., 2012) found that treating seeds of wheat genotypes with either 20, 50, or 80 mg L^{-1} had a negative impact on both germination and seed vigor. Unlike germination, seed vigor was drastically affected by cadmium treatment (Table 1). Cadmium application did not affect the germination and REC and this is in agreement with that of (Al-Ubaidy *et al*., 2021), who indicated that the lab performance of some genotypes was enhanced with the low concentration of Cd and promoted the metabolism inside seedlings, leading to relatively better performance. The findings of the present study revealed variations among genotypes in the majority of studied traits, particularly in terms of physiological and anatomical parameters. This variability may be attributed to differences in their genetic backgrounds, where the phenotypic and physiological performance mainly depends on

the genomic structure of any individual and the number of variations occurs either at the genetic or allelic level (Abdul-Hassan and Al-Issawi, 2021; Hashem and Al-Issawi, 2023; Mansoor *et al*., 2021). Cd did not have big effects on the investigated physiological traits which indicates a mild impact of this element on the physiological processes in plants (MA *et al*., 2021). Our finding indicated that Cd might have no effect on the metabolism combined with germination (Table 1) and these are consistent with what has been found by (Shao *et al*., 2011), as they indicated a deterioration in seed vigor when grain was treated with different concentrations of Cd. Additionally, many changes have been made in terms of root and plumule anatomy when Cd presented additionally in the irrigation solution.

Table 1. Germination (%), seed vigor, and relative electrical conductivity (REC) (%) of wheat genotypes under the effect of Cd stress (mg L^{-1})

Genotypes	Germination (%)		Mean	Seed Vigor		Mean	REC		Mean
	Cd0	**Cd75**		**Cd0**	**Cd75**		**Cd0**	**Cd75**	
G-3	100	100	100	20.9	12.02	16.46	41.21	40.79	41
G-4	100	100	100	18.759	13.96	16.36	45.42	46.35	45.89
G-9	100	100	100	17.85	10.42	14.14	56.95	37.15	47.05
G-24	100	100	100	18.42	11.99	15.21	46.67	42.06	44.37
G-28	100	100	100	17.75	11.72	14.74	40.2	38.96	39.58
G-29	100	100	100	20.57	11.54	16.06	45.79	41.33	43.56
G-39	100	100	100	16.67	13.32	15	40.35	43.97	42.16
G-41	100	100	100	22.179	14.99	18.58	41.59	44.45	43.02
Al Diar	99.33	99.33	99.33	20.02	13.76	16.89	38.62	31.75	35.19
IRAQ	100	100	100	21.25	14	17.63	44.68	28.04	36.36
Mean	99.933	99.933	G=NS	19.44	12.77	G=1.21	44.15	39.49	G=2.29
LSD (0.05)	Cd, G*Cd=NS			Cd=0.54, G*Cd=1.71			Cd=1.03, G*Cd=3.24		

*G: Genotypes and Cd: Cadmium (Cd0= control and Cd75= application of 75 mg L^{-1}).

Inhibition by cadmium to seed vigor was prominent at a concentration of 75 mg L^{-1}. Seed vigor declined from 19.44 in the control group to 12.77 when genotype seeds were exposed to 75 mg L^{-1}, indicating a notable reduction compared to the control ($P<0.05$). This reduction is owing to the negative effect of Cd on the plumule and eventually on seed vigor (Shao et al., 2011). Among different wheat genotypes, seed vigor was significantly ($P<0.05$) improved in G-41 and IRAQ genotypes, whereas it was reduced in genotypes such as G-9 (14.14), G-28 (14.74), G-39 (15), and G-24 (15.21). This variation may be due to the variation in the genetic background of the investigated genotypes. Certain genotypes, such as G-41, G-3, and G-29, exhibited a higher seed vigor in the absence of Cd stress. Conversely, genotype G-9 was in low seed vigor affected by 75 mg Cd L^{-1}, a characteristic that remained consistent with genotypes G-29, G-28, G-24, and G-3 at the same concentration (75 mg Cd L^{-1}). It also indicated a significant ($P<0.05$) of either genotype, cadmium stress, or their interaction on the relative electrical conductivity (%) (Table 1). It seems that cadmium enhanced this trait by reducing the REC from 44.15% to 39.49%. Local varieties namely IRAQ and Al Diar may leak fewer ions (36.36 and 35.19 respectively) in comparison with the rest of the genotypes included in this study. However, genotypes such as G-9 and G-4 showed high relative electrical conductivity, reaching 47.05 and 45.88% respectively. This proved that the genetic factor is the main reason for the variation and also indicates that there are genotypes that are tolerant and sensitive to Cd stress (Al-Ameri, 2011). On the other hand, there was a two-way interaction between genotype and Cd on the REC in wheat genotypes. Genotype G-9 recorded the highest REC in the control (56.95%) whereas the IRAQ genotype recorded a notable reduction in this trait (28.04%) in response to the higher level of

(75 mg Cd L^{-1}). followed by Al Diar (31.75%) confirming the protection role of Cd in relatively enhancing the integrity of plasma membrane when it was initially imposed on the plant.

Total chlorophyll content was significantly affected due to Cd treatments. Chlorophyll content was reduced from 7.16 mg g^{-1} in the control to 6.14 mg g^{-1} when 75 mg L^{-1} was applied (Table 2). On the other hand, the current results confirmed that the investigated genotypes were different in their chlorophyll content, however, G-41 was superior in this trait (8.72 mg g^{-1}) followed by Al Diar and G-24 (8.56 and 8.26 mg g^{-1}, respectively) compared to the lesser chlorophyll content recorded by G-4 (6.11 mg g^{-1}). Also, G-41 exhibited the highest level of chlorophyll in the control (10.02 mg g^{-1}) and was(10.02 mg g^{-1}) notably higher than the other interactions while G-3 gave the lowest level when treated with 75 mg g^{-1} which did not significantly differ from G-4 at the same concentration of cadmium, confirming the negative effect of this element on this pigment chlorophyll content. A decrease in chlorophyll content is always related to the availability of Cd in plants because of the damage in the ultrastructure of chloroplasts. In addition, Cd affects the photosynthesis components, such as the electron transport chain (Yotsova *et al*., 2020) and eventually causes pigment degradation and inhibition of CO_2 fixation. Previously, it was observed that the genotypes used in this experiment were varied in their chlorophyll content (Hashem and Al-Issawi, 2023).

Table 2. Total Chlorophyll (mg g^{-1}) and Carotenoids (µmole g^{-1}) content in wheat genotypes' seedlings under the effect of Cadmium stress (mg L^{-1})

Genotypes	Chlorophyll Content (mg g^{-1})		Mean	Carotenoids content (µmole g^{-1})		Mean
	Cd0	**Cd75**		**Cd0**	**Cd75**	
G-3	6.70	5.57	6.14	3.65	3.98	3.81
G-4	6.27	5.96	6.11	3.78	3.67	3.73
G-9	6.78	6.27	6.53	3.79	3.90	3.84
G-24	8.88	7.64	8.26	4.43	4.88	4.66
G-28	8.40	7.86	8.13	4.52	4.59	4.55
G-29	7.86	8.00	7.93	4.83	4.51	4.67
G-39	8.54	6.79	7.67	4.13	4.68	4.40
G-41	10.02	7.42	8.72	4.31	5.42	4.87
Al Diar	7.69	7.62	7.66	4.42	5.06	4.74
IRAQ	8.69	8.42	٨.٥٦	4.79	4.67	٤.٧٣
Mean	7.16	6.14	G=0.54	4.26	4.53	G=0.38
LSD (0.05)	Cd= 0.24 G*Cd=0.77			Cd=0.17 G*Cd=0.54		

Unlike chlorophyll, carotenoids slightly increased with the application of Cd. Carotenoid content was 4.26 µmole g^{-1} in the control and increased to 4.53 µmole g^{-1} when 75 mg L^{-1} was applied (Table 2). Cadmium might activate the antioxidant system in the plant as a response to Cd stress including carotenoids. Carotenoid content varied among wheat genotypes. Genotypes such as G-41, Al Diar, IRAQ, G-29, G-24 and G-28 were superior in carotenoid content reaching 4.87, 4.74, 4.73, 4.67, 4.66 and 4.55 µmole g^{-1} respectively. G-4 recorded the lowest content of carotenoids (3.73 µmole g^{-1}). The antioxidant content in some of the genotypes increased as a response to the abiotic stress, while other genotypes were relatively tolerant to stress and therefore slight response was detected, and produced less antioxidant. Genotypes varied in their responses to Cd application, and it was found that G-41 and Al Diar showed higher carotenoid content when treated with 75 mg L^{-1} in comparison with the rest of the genotypes. The Cd application significantly affected the radicals and plumule anatomy of wheat genotypes.

The results of this study also indicated significant changes in root cortex thickness, length and width of its epidermis (Table 3 and Figures 1 and 2). Genotype G-29 recorded the highest thickness of the cortex in the root (15.55

µM) followed by G-39, IRAQ, G-41, G-3, G-28, and Al Diar, compared with G-9, which recorded the lowest mean of thickness (12.38 µM). Cadmium (Cd) exerted discernible impacts on the anatomical features of wheat seedlings, irrespective of their genotypes. The application Cd at a rate of 75 mg L^{-1} resulted in significant alterations in the cortex thickness of the root (14.94 µM), compared to the control treatment which recorded only 13.81 µM. This observed change can be attributed to hormonal imbalances induced by the Cd treatment. The two-way interaction of G-39 and 75 mg Cd L^{-1} showed the highest mean of cortex thickness. On the other hand, G-9 at the control revealed the lowest mean of the root cortex thickness (11.50 µM). Heavy metal Cd caused considerable ultrastructural anatomical changes in either roots or plumules upon application (Sabella *et al*., 2022). These changes such as the width and length of either the root or plumule epidermis or the thickness of the cortex in the root were varied according to different genotypes included in this study. However, most of Cd changes to the anatomy of either root or plumule of those genotypes were negative and that could be due to the impact of Cd on hormone balance and metabolism (Zhao *et al.*, 2023). This in turn might lead to more thickness causing less elasticity in those tissues (Al-Ubaidy *et al*., 2021) and eventually growth abnormalities.

Table 3. The thickness of the root cortex (µM), length and width of root Epidermis (µM) of wheat genotypes under the effect of Cd stress (mg L^{-1})

Genotypes	Thickness of cortex (µM)		Mean	Length of Epidermis (µM)		Mean	Width of Epidermis (µM)		Mean
	Cd0	**Cd75**		**Cd0**	**Cd75**		**Cd0**	**Cd75**	
G-3	14.75	15.15	14.95	2.75	2.60	2.68	17.50	29.01	2.06
G-4	12.65	13.90	13.28	2.85	2.55	2.70	22.69	28.94	2.01
G-9	13.25	11.50	12.38	2.70	3.00	2.85	24.06	24.02	1.80
G-24	12.50	14.63	13.56	2.63	2.40	2.51	19.33	23.69	1.81
G-28	13.73	14.88	14.30	3.00	2.50	2.75	19.05	21.00	2.03
G-29	14.15	16.95	15.55	2.75	2.25	2.50	21.63	28.00	2.25
G-39	13.00	17.63	15.31	3.05	2.35	2.70	25.00	28.31	1.97
G-41	16.50	13.50	15.00	2.60	3.00	2.80	21.13	24.88	1.93
Al Diar	13.30	17.00	15.15	2.40	2.45	2.43	21.69	21.51	1.78
IRAQ	14.25	14.25	١٤.٢٥	2.75	2.45	٢.٦٠	22.93	23.75	٢.٢٨
Mean	13.81	14.94	*G=1.94*	2.75	2.56	*G=NS*	21.5	25.31	*G=0.13*
LSD (0.05)	Cd= 0.87, G*Cd=2.75			*Cd=0.13, G*Cd=0.42*			*Cd=0.13, G*Cd=NS*		

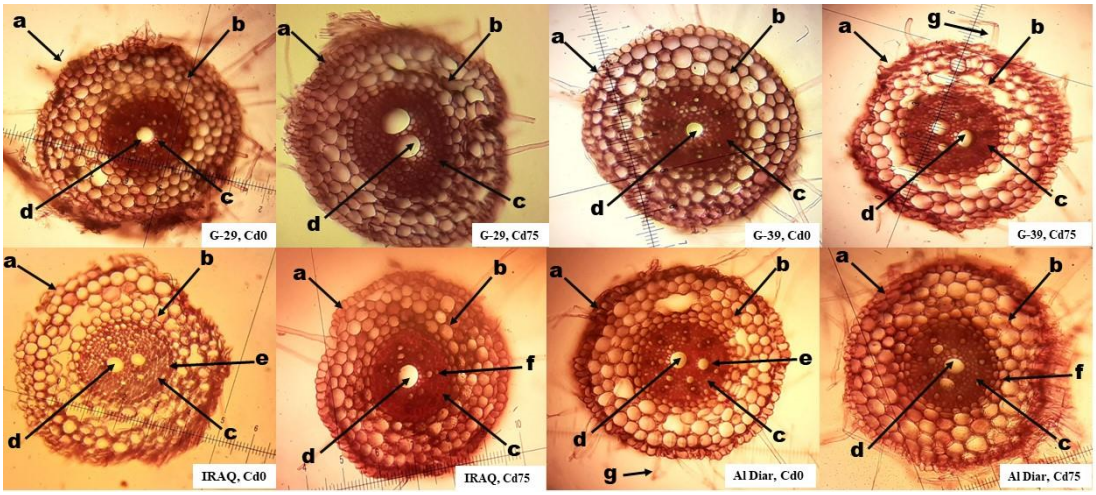

Figure 1. Cross sections of radicals (100X) of wheat genotypes (G-3, G-9, G-24, and G-41) under the effect of Cd stress (mg Cd L-1). (a: Epidermis, b: Cortex, c: Casparian strip, d: Phloem, e: xylem, f: vascular cylinder and g: root hair)

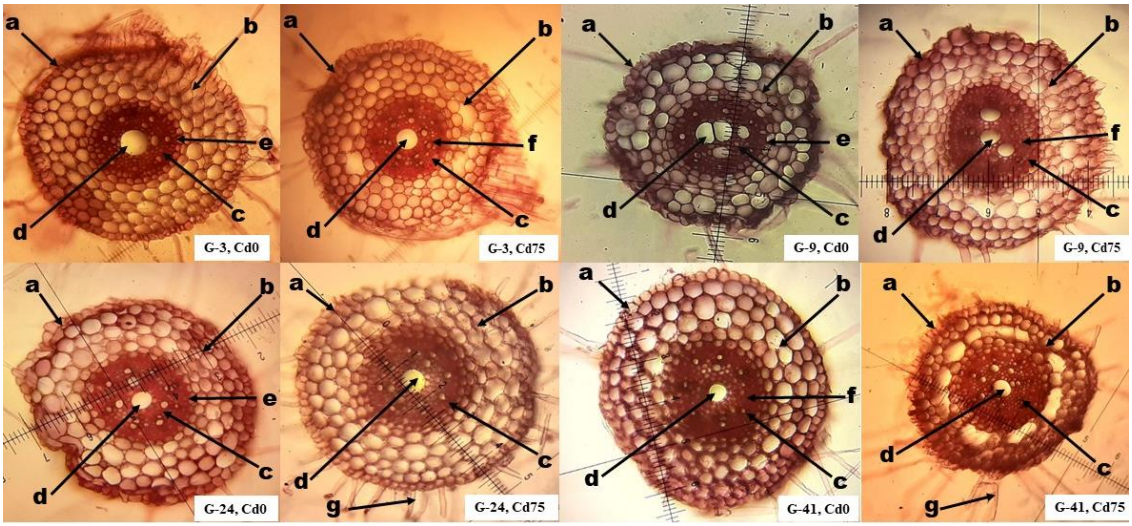

Figure 2. Cross sections of radicals (100X) of wheat genotypes (G-29, G-39, IRAQ, and Al Diar) under the effect of Cd stress (mg Cd L^{-1}). (a: Epidermis, b: Cortex, c: Casparian strip, d: Phloem and e: xylem, f: vascular cylinder and g: root hair)

Genotypic differences did not have the same effect on the epidermis length, as it on the width. For example, genotype IRAQ was superior in epidermis width (2.28 µM) followed by G-29, G-3, G-28, and G-4, meanwhile the Al Diar genotype had a negative effect on the trait by showing the lowest mean of epidermis width (1.78 µM) (Table 3 and Figure 1 and 2). This might be due to the genetic background, and this is consistent with the previous findings (Al-Amiry and Al-Ubaidi, 2016). Cadmium caused a significant reduction in the length of the root epidermis but increased its width. Cadmium induces the production of ROS, which in turn destroys the cell ultrastructure and also causes the degradation of DNA and protein (Özyiğit *et al.*, 2021). Genotype G-39, when treated with 75 mg Cd L^{-1}, recorded 3.05 µM, while G-29 at the control recorded the lowest mean of this trait (Table 3 and Figures 1 and 2). Also, leaf structure changed according to the application of Cd. Table 4 and Figures 3 and 4 indicated significant effects of genotype in the length and width of the upper ordinary epidermis cell structure (Table 4 and Figures 3 and 4). Genotype G-39 recorded prominent length and width of upper ordinary epidermis cells (26.66 µM and 2.78 µM respectively) on the other hand, genotypes such as G-28 (L 28.03 µM) and G-29 (W 2.05 µM) showed the lowest mean of those parameters. However, genotype IRAQ was superior in the width of the upper ordinary epidermis cells (3.10 µM). On the other hand, it has been found that Cd caused the reduction in the thickness of the epidermis due to the reduction in the dimensions of cells and not to the number of layers of cells (Özyiğit *et al.*, 2021). This effect is due to the destruction of the cell organelles, as well as biological compounds such as DNA and proteins, with an eventual reduction in the biomass and the cell structure (Sarwar *et al.*, 2015).

Table 4. Length and width of Upper Ordinary Epidermis (µM) of Plumules of wheat genotypes under the effect of Cd stress (mg L^{-1})

Genotypes	length and of upper ordinary epidermis cells (µM)		Mean	Width of upper ordinary epidermis cells (µM)		Mean
	Cd0	**Cd75**		**Cd0**	**Cd75**	
G-3	29.01	17.50	23.25	2.75	1.85	2.30
G-4	28.94	22.69	25.81	1.5	1.60	1.55
G-9	24.02	24.06	24.04	2.01	2.25	2.13
G-24	23.69	19.33	21.51	2.48	2.03	2.25
G-28	21.00	19.05	20.03	2.51	2.44	2.48
G-29	28.00	21.63	24.81	1.66	2.44	2.05
G-39	28.31	25.00	26.66	2.94	2.63	2.78
G-41	24.88	21.13	23.00	2.08	2.19	2.14
Al Diar	21.51	21.69	21.60	2.38	2.44	2.41
IRAQ	23.75	22.93	٢٣.٣٤	3.19	3.00	٣.١٠
Mean	25.31	21.5	*G=2.96*	2.35	2.29	*G=0.38*
LSD (0.05)	Cd= 1.32 G*Cd=4.18			*Cd=NS G*Cd=0.54*		

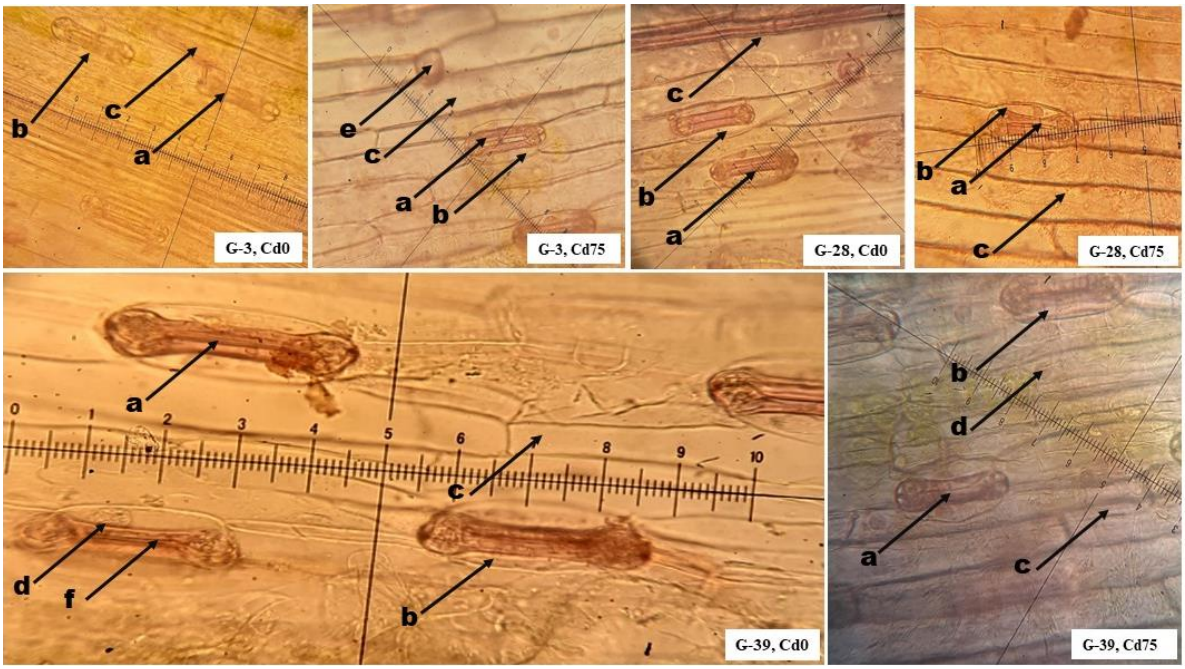

Figure 3. Cross sections of plumules (400X) of wheat genotypes (G-3, G-9, G-28, and G-39) under the effect of Cd stress (mg Cd L^{-1}). (a: Ordinary Epidermis Cells, b: Guard Cells, c: Auxiliary Cells, d: Stomata and e: Epidermis cell's nucleus)

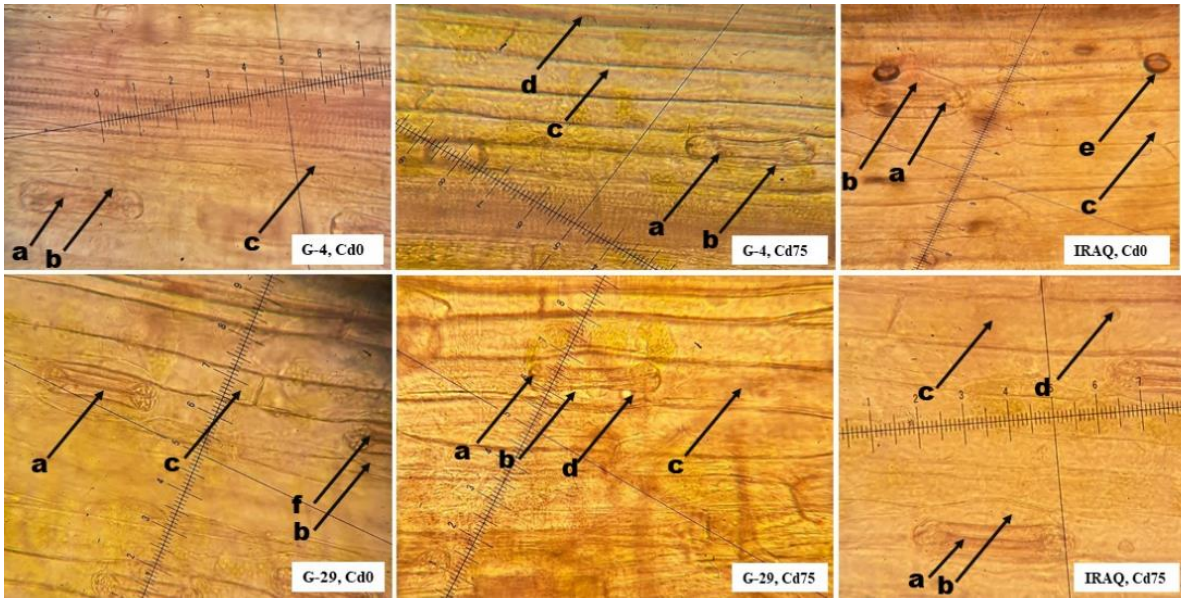

Figure 4. Cross sections of plumules (400X) of wheat genotypes (G-4, IRAQ, and G-29) under the effect of Cd stress (mg Cd L^{-1}). (a: Ordinary Epidermis Cells, b: Guard Cells, c: Auxiliary Cells, d: Stomata and e: Epidermis cell's nucleus)

The impact of cadmium on plumule ultrastructure was noteworthy, particularly regarding the length of upper epidermal cells in the ordinary epidermis. Under cadmium treatment, there was a discernible reduction in cell length, decreasing from 25.30 µM under control conditions to 21.50 µM when seedlings were exposed to 75 mg Cd L^{-1} (Table 4 and Figures 3 and 4). Cd did not have that clear effect in terms of the width of the upper ordinary epidermis cells. The two-way interaction indicated a significant effect on either the length or width of the upper ordinary cells of the plumules epidermis. In this context, in the control conditions, it was observed that G-3 exhibited elongated cells, measuring 29.01 µM. Similarly, G-29, G-39, and G-4 also demonstrated increased cell length under control conditions. However, the maximum level of 75 mg Cd L^{-1} at the same genotype (G-3) exhibited the shortest length among the epidermal cells. The widest cells found in genotype IRAQ at the control (3.19 µM) did not significantly vary from the same genotype when treated with cadmium, indicating that Cd had more effect on the length rather than the width of epidermis cells.

Data presented in Figure 5 indicate the basic concentration of Cd in the grain used in this experiment. It is however clear that genotypes included in this study were varied in their content of Cd which were planted in the same region (Anbar governorate-IRAQ). The basic concentration of Cd in the genotypes did not reach the safety threshold of Cd in wheat (0.2 mg kg^{-1}) according to the Codex Alimentarius Commission (Lu *et al*., 2021). On the other hand, Figure 6 shows that all the wheat genotypes at control had low Cd concentration in the root, while the concentration became higher after the treatment (75 mg L^{-1}). However, some genotypes behaved differently under Cd treatment: G-28, G-29, G-41 and IRAQ had lower Cd concentrations in the root in comparison with the rest of the genotypes in the study.

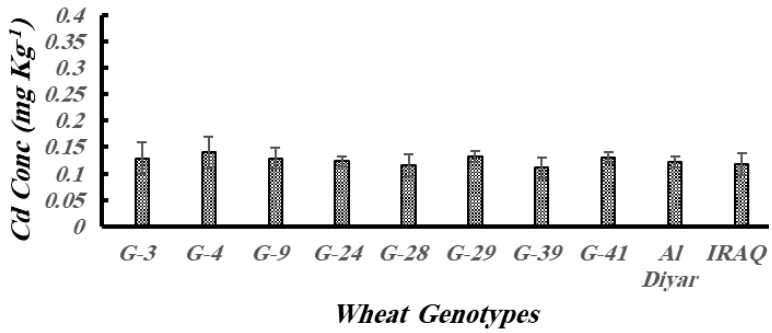

Figure 5. The Concentration of Cd in the grains of 10 wheat genotypes (Mean±SE)

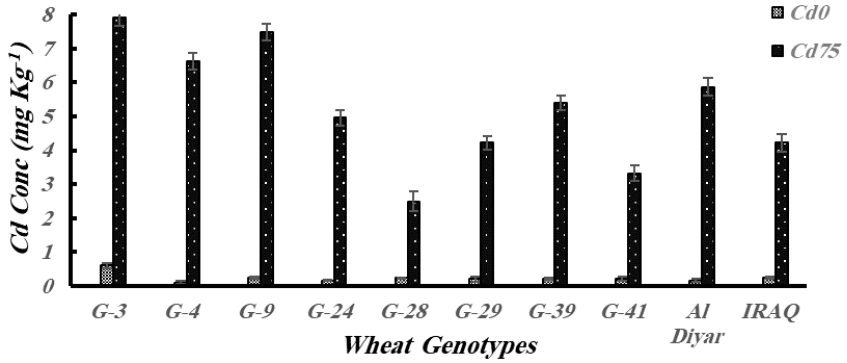

Figure 6. The concentration of Cd in the root of the 10 wheat genotypes under the effect of Cd (0 and 75 mg L^{-1}) (Mean±SE)

The responsiveness of PSC1 to heavy metals, notably cadmium, was demonstrated, with cadmium being identified as the most potent activator for this gene. PCS1 expression was detected in wheat genotypes included in this study relative to GAPDH. G-3 had relatively higher PSC1 expression in comparison with the rest of the genotypes (Table 5). The PCS1 gene demonstrates heightened activity in response to heavy metals, particularly cadmium (Feng *et al*., 2023; Zhang *et al*., 2022). PCS1 is typically associated with the Cd forming complexes and transferring them to the vacuole. This strategy is used by plants to reduce the effect of toxicity of heavy metals and keep plants growing normally. However, this does not eliminate Cd from the food chain. Genotype G-3 exhibited enhanced activity of the PCS1 gene, a correlation further substantiated by the pronounced concentration of cadmium (Cd) in that particular genotype. Therefore, a genotype demonstrating heightened activity of this gene could be a promising candidate serving for phytoremediation purposes, as opposed to utilizing it for human or animal nutrition due to the over-accumulation of cadmium in their tissues. Some other genotypes included in this study especially those accumulated Cd in their roots might be more safe candidates to be used for nutrition.

Table 5. Relative expression of PCS1 gene in wheat genotypes after treatment with 75 mg Cd L^{-1}

Genotypes	Relative Gene Expression of PCs1
G-3	39
G-4	$8.46*10^{-5}$
G-9	$7.43*10^{-12}$
G-24	$9.70*10^{-4}$
G-28	$3.67*10^{-13}$
G-29	$1.11*10^{-3}$

G-39	$5.32*10^{-6}$
G-41	$1.11*10^{-13}$
Al Diar	$6.99*10^{-13}$
IRAQ	$1.46*10^{-11}$

Conclusion

In conclusion, from an anatomical perspective, The IRAQ genotype was the most prominent, especially in the thickness of the root cells and the length and width of the epidermis cells. Genotypes such as IRAQ and G-28 and G-41 are expected to be used for human nutrition as they accumulate a low quantity of Cd in their tissues while G-3 is more suitable for phytoremediation of Cd-contaminated soils. Also, it can be concluded that genotypes G-28, G-29, G-41 and IRAQ were superior in most studied traits, while genotypes such as G-4 and G-9 performed negatively to the imposed stress. Genotype G-3 exhibited the high ability to express PCS1 which makes it a more important candidate to be served in phytoremediation. Further investigation is required to shed light on the chemical responses of wheat to the heavy metal stresses that have recently become widespread in the environment.

Conflict of interests

The authors of this manuscript have no conflict of interest to report. They all have seen and have agreed with the content of the paper and there is no financial interest to declare. Also, the authors certify that this submission is original work and it is not under review in other publications.

Acknowledgments

The authors are thankful for the support made by the College of Agriculture at the University Of Anbar for the use of its laboratories

References

Abdul-Hassan, A., and Al-Issawi, M. (2021). Introducing Several Wheat Genotypes and Testing Them for Planting Under Western Parts of Iraq Conditions. *Anbar Journal of Agricultural Sciences*, *19*(2), 315–328. https://doi.org/10.32649/ajas.2021.176184

Ahmed, I., Akhtar, M. J., Zahir, Z. A., and Amer Jamil. (2012). Effect of cadmium on seed germination and seedling growth of four wheat (Triticum aestivum L.) cultivars. *Pakistan Journal of Botany*, *44*(5), 1569–1574.

Al-Ameri, A. A. H. (2011). Effect of Iron Zinc Bicarbonate on Growth and Yield of Some Wheat Cultivars and Its Relationships with Some Antioxidant Enzymes. *PhD Thesis. College of Agriculture, University of Baghdad.* https://iqdr.iq/search?view=f839d2d092501533c7b6dd897d08d266

Al-Amiry, M. M., Al-Ubaidi, M. O. (2016). Evaluation of several genotypes of wheat and triticale under rain fed conditions in Sulaimaniyah province. *Undefined*.

Al-Saleh, L., Hyass, B., and Abbas, F. (2019). Effect of Osmotic Potential Induced by Polyethylene Glycol(PEG6000) on the Germination of Five Chickpea (Cicer arietinumL.) Varieties. *Syrian Journal of Agricultural Research*, *6*(3), 219–232.

Al-Ubaidy, H., Ismail, M., Abdulmajeed, A., and Al-Issawi, M. (2021). Using of Molybdenum To Improve Some of Anatomical Characteristics and Mitigation Induced Drought Damage By Peg-6000 in Mung Bean Seedlings (Vignaradiate L.). *Anbar Journal of Agricultural Sciences*, *19*(1), 1–19. https://doi.org/10.32649/ajas.2021.175729

Alfalahi, A. O., Hussein, Z. T., Khalofah, A., Sadder, M. T., Qasem, J. R., Al-Khayri, J. M., Jain, S. M., and Almehemdi, A. F. (2022). Epigenetic variation as a new plant breeding tool: A review. *Journal of King Saud University - Science*, *34*(8), 102302.

https://doi.org/10.1016/J.JKSUS.2022.102302

Allen, S.E., Grimshaw, H.M., Rowland, A. P. (1986). *Chemical analysis. Methods in plant ecology* (Eds). Blackwell Scientific Publication.

Alloway, B. J. (2013). *Sources of Heavy Metals and Metalloids in Soils*. 11–50. https://doi.org/10.1007/978-94-007-4470-7_2

Asgher, M., Khan, M. I. R., Anjum, N. A., and Khan, N. A. (2015). Minimising toxicity of cadmium in plants--role of plant growth regulators. *Protoplasma*, *252*(2), 399–413. https://doi.org/10.1007/S00709-014-0710-4

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., and Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology*, *12*, 643972. http://dx.doi.org/10.3389/fphar.2021.643972

Colovic, M. B., Vasic, V. M., Djuric, D. M., and Krstic, D. Z. (2018). Sulphur-containing Amino Acids: Protective Role Against Free Radicals and Heavy Metals. *Current Medicinal Chemistry*, *25*(3), 324–335. https://doi.org/10.2174/0929867324666170609075434

Cong, M., Zhao, J., Lü, J., Ren, Z., and Wu, H. (2016). Homologous cloning, characterization and expression of a new halophyte phytochelatin synthase gene in Suaeda salsa. *Chinese Journal of Oceanology and Limnology*, *34*(5), 1034–1043.http://dx.doi.org/10.1007/s00343-016-4382-0

Dawar, K., Mian, I. A., Khan, S., Zaman, A., Danish, S., Liu, K., Harrison, M. T., Saud, S., Hassan, S., Nawaz, T., Khan, I., Liu, H., Khan, M. A., Ullah, S., Ali, M. I., Jamal, A., Zhu, M., Khan, S. M., El-Kahtany, K., and Fahad, S. (2023). Alleviation of cadmium toxicity and fortification of zinc in wheat cultivars cultivated in Cd contaminated soil. *South African Journal of Botany*, *162*, 611–621. https://doi.org/10.1016/J.SAJB.2023.09.044

Demecsová, L., and Tamás, L. (2019). Reactive oxygen species, auxin and nitric oxide in metal-stressed roots: toxicity or defence. *Biometals : An International Journal on the Role of Metal Ions in Biology, Biochemistry, and Medicine*, *32*(5), 717–744. https://doi.org/10.1007/S10534-019-00214-3

Demidchik, V. (2015). Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. *Environmental and Experimental Botany*, *109*, 212–228. https://doi.org/10.1016/J.ENVEXPBOT.2014.06.021

Dutta, S., Mitra, M., Agarwal, P., Mahapatra, K., De, S., Sett, U., and Roy, S. (2018). Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. *Plant Signaling and Behavior*, *13*(8). https://doi.org/10.1080/15592324.2018.1460048

Erenstein, O., Jaleta, M., Abdul Mottaleb, K., Sonder, K., Donovan, J., and Braun, H. J. (2022). Global Trends in Wheat Production, Consumption and Trade. *Wheat Improvement: Food Security in a Changing Climate*, 47–66. https://doi.org/10.1007/978-3-030-90673-3_4

Fahad, S., Hussain, S., Bano, A., Saud, S., Hassan, S., Shan, D., Khan, F. A., Khan, F., Chen, Y., Wu, C., Tabassum, M. A., Chun, M. X., Afzal, M., Jan, A., Jan, M. T., and Huang, J. (2015). Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, *22*(7), 4907–4921. https://doi.org/10.1007/s11356-014-3754-2

Feng, D., Wang, R., Sun, X., Liu, L., Liu, P., Tang, J., Zhang, C., and Liu, H. (2023). Heavy metal stress in plants: Ways to alleviate with exogenous substances. *Science of The Total Environment*, *897*, 165397. https://doi.org/10.1016/J.SCITOTENV.2023.165397

Haider, F. U., Liqun, C., Coulter, J. A., Cheema, S. A., Wu, J., Zhang, R., Wenjun, M., and Farooq, M. (2021). Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety*, *211*, 111887. https://doi.org/10.1016/J.ECOENV.2020.111887

Hashem, L. F., and Al-Issawi, M. H. (2023). Chlorophyll Content Variation in Wheat Genotypes Planted in Semi-Arid Region. *IOP Conference Series: Earth and Environmental Science*, *1252*(1), 012025. https://doi.org/10.1088/1755-1315/1252/1/012025

Huang, H., Ullah, F., Zhou, D. X., Yi, M., and Zhao, Y. (2019). Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science*, *10*, 440478. https://doi.org/10.3389/fpls.2019.00800

Iqbal, M. J., Shams, N., Fatima, K., Iqbal, M. J., Shams, N., and Fatima, K. (2022). Nutritional Quality of Wheat. *Wheat*. https://doi.org/10.5772/INTECHOPEN.104659

Kapoor, D., Singh, S., Kumar, V., Romero, R., Prasad, R., and Singh, J. (2019). Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). *Plant Gene*, *19*, 100182. https://doi.org/10.1016/J.PLGENE.2019.100182

Khan, H. A., Ahmad, A., and Nawaz, S. (2016). Characterization and Analysis of PCS1 Gene from Wheat. *Journal of Bioresource Management*, *3*(4), 4, 24- 36. https://doi.org/https://doi.org/10.35691/JBM.6102.0063

Kocadal, K., Alkas, F. B., Battal, D., and Saygi, S. (2020). Cellular pathologies and genotoxic effects arising secondary to heavy metal exposure: A review. *Human and Experimental Toxicology*, *39*(1), 3–13. https://doi.org/10.1177/0960327119874439

Kumar, P., Goud, E. L., Devi, P., Dey, S. R., and Dwivedi, P. (2022). Heavy Metals: Transport in Plants and Their Physiological and Toxicological Effects. *Plant Metal and Metalloid Transporters*, 23–54. https://doi.org/10.1007/978-981-19-6103-8_2

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, *148*(C), 350–382. https://doi.org/10.1016/0076-6879(87)48036-1

Lin, Y. F., Hassan, Z., Talukdar, S., Schat, H., and Aarts, M. G. M. (2016). Expression of the ZNT1 Zinc Transporter from the Metal Hyperaccumulator Noccaea caerulescens Confers Enhanced Zinc and Cadmium Tolerance and Accumulation to Arabidopsis thaliana. *PLOS ONE*, *11*(3), e0149750. https://doi.org/10.1371/JOURNAL.PONE.0149750

Lu, M., Yu, S., Lian, J., Wang, Q., He, Z., Feng, Y., and Yang, X. (2021). Physiological and metabolomics responses of two wheat (Triticum aestivum L.) genotypes differing in grain cadmium accumulation. *Science of The Total Environment*, *769*, 145345. https://doi.org/10.1016/J.SCITOTENV.2021.145345

MA, J. F., SHEN, R. F., and SHAO, J. F. (2021). Transport of cadmium from soil to grain in cereal crops: A review. *Pedosphere*, *31*(1), 3–10. https://doi.org/10.1016/S1002-0160(20)60015-7

Mansoor, M. L., Al-Issawi, M. H., and Mhmood, J. N. (2021). Estimation of DREB Gene Expression in Wheat Genotypes (Triticum aestivum L.) Introduced to Anbar Governorate Under Water Stress. *IOP Conference Series: Earth and Environmental Science*, *761*(1), 012072. https://doi.org/10.1088/1755-1315/761/1/012072

Mickovski Stefanović, V., Roljević Nikolić, S., Matković Stojšin, M., Majstorović, H., Petreš, M., Cvikić, D., and Rački, G. (2023). Soil-to-Wheat Transfer of Heavy Metals Depending on the Distance from the Industrial Zone. *Agronomy*, *13*(4), 1016. https://doi.org/10.3390/agronomy13041016

Mishra, S., Bharagava, R. N., More, N., Yadav, A., Zainith, S., Mani, S., and Chowdhary, P. (2019). Heavy Metal Contamination: An Alarming Threat to Environment and Human Health. *Environmental Biotechnology: For Sustainable Future*, 103–125. https://doi.org/10.1007/978-981-10-7284-0_5

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., and Baloch, F. S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology and Biotechnological Equipment*, *32*(2), 261–285. https://doi.org/10.1080/13102818.2017.1400401

Naja, G. M., and Volesky, B. (2017). Toxicity and sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment. In *Handbook of Advanced Industrial and Hazardous Wastes Management* (pp. 855–903). CRC Press. https://doi.org/10.1201/9781315117423

Özyiğit, İ. İ., Baktibekova, D., Hocaoğlu-Özyiğit, A., K., G., Chekirov, K., and Yalçin, İ. E. (2021). The effects of cadmium on growth, some anatomical and physiological parameters of wheat (Triticum aestivum L.). *International Journal of Life Sciences and Biotechnology*, *4*(2), 235–253. https://doi.org/10.38001/ijlsb.833553

Page, V., and Feller, U. (2015). Heavy Metals in Crop Plants: Transport and Redistribution Processes on the Whole Plant Level. *Agronomy 2015, Vol. 5, Pages 447-463*, *5*(3), 447–463. https://doi.org/10.3390/AGRONOMY5030447

Pápista, É., Ács, É., and Böddi, B. (2002). Chlorophyll-a determination with ethanol - A critical test. *Hydrobiologia*, *485*(1), 191–198. https://doi.org/10.1023/A:1021329602685

Rahman, Z., and Singh, V. P. (2019). The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environmental Monitoring and Assessment*, *191*, (419), 1-21. https://doi.org/10.1007/S10661-019-7528-7

Rizwan, M., Ali, S., Adrees, M., Ibrahim, M., Tsang, D. C. W., Zia-ur-Rehman, M., Zahir, Z. A., Rinklebe, J., Tack, F. M. G., and Ok, Y. S. (2017). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere*, *182*, 90–105. https://doi.org/10.1016/J.CHEMOSPHERE.2017.05.013

Sabella, E., Aprile, A., Tenuzzo, B. A., Carata, E., Panzarini, E., Luvisi, A., De Bellis, L., and Vergine, M. (2022). Effects of Cadmium on Root Morpho-Physiology of Durum Wheat. *Frontiers in Plant Science*, *13*, 936020. https://doi.org/10.3389/fpls.2022.936020

Sarwar, N., Ishaq, W., Farid, G., Shaheen, M. R., Imran, M., Geng, M., and Hussain, S. (2015). Zinc–cadmium interactions: Impact on wheat physiology and mineral acquisition. *Ecotoxicology and Environmental Safety*, *122*, 528–536. https://doi.org/10.1016/J.ECOENV.2015.09.011

Shao, Y., Jiang, L., Zhang, D., Ma, L., and Li, C. (2011). Effects of arsenic, cadmium and lead on growth and respiratory enzymes activity in wheat seedlings. *African Journal of Agricultural Research*, *6*(19), 4505–4512.

Singh, S., Singh, A., Bashri, G., and Prasad, S. M. (2016). Impact of Cd stress on cellular functioning and its amelioration by phytohormones: An overview on regulatory network. *Plant Growth Regulation*, *80*(3), 253–263. https://doi.org/10.1007/s10725-016-0170-2

Thévenod, F., and Lee, W. K. (2013a). Toxicology of cadmium and its damage to mammalian organs. *Metal Ions in Life*

Sciences, *11*, 415–490. https://doi.org/10.1007/978-94-007-5179-8_14

Thévenod, F., and Lee, W. K. (2013b). Cadmium and cellular signaling cascades: Interactions between cell death and survival pathways. *Archives of Toxicology*, *87*(10), 1743–1786. https://doi.org/10.1007/s00204-013-1110-9

Tran, T. A., and Popova, L. P. (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turkish Journal of Botany*, *37*(1), 1–13. https://doi.org/10.3906/bot-1112-16

Ullah, A., Manghwar, H., Shaban, M., Khan, A. H., Akbar, A., Ali, U., Ali, E., and Fahad, S. (2018). Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environmental Science and Pollution Research*, *25*(33), 33103–33118. https://doi.org/10.1007/s11356-018-3364-5

Wang, M., Chen, Z., Song, W., Hong, D., Huang, L., and Li, Y. (2021). A review on Cadmium Exposure in the Population and Intervention Strategies Against Cadmium Toxicity. *Bulletin of Environmental Contamination and Toxicology*, *106*(1), 65–74. https://doi.org/10.1007/s00128-020-03088-1

Wen, Y., Li, W., Yang, Z., Zhang, Q., and Ji, J. (2020). Enrichment and source identification of Cd and other heavy metals in soils with high geochemical background in the karst region, Southwestern China. *Chemosphere*, *245*, 125620. https://doi.org/10.1016/J.CHEMOSPHERE.2019.125620

Wimalawansa, S. J. (2016). The role of ions, heavy metals, fluoride, and agrochemicals: critical evaluation of potential aetiological factors of chronic kidney disease of multifactorial origin (CKDmfo/CKDu) and recommendations for its eradication. *Environmental Geochemistry and Health*, *38*(3), 639–678. https://doi.org/10.1007/S10653-015-9768-Y

Xia, X., Ji, J., Yang, Z., Han, H., Huang, C., Li, Y., and Zhang, W. (2020). Cadmium risk in the soil-plant system caused by weathering of carbonate bedrock. *Chemosphere*, *254*, 126799. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126799

Xing, Q., Hasan, M. K., Li, Z., Yang, T., Jin, W., Qi, Z., Yang, P., Wang, G., Ahammed, G. J., and Zhou, J. (2023). Melatonin-induced plant adaptation to cadmium stress involves enhanced phytochelatin synthesis and nutrient homeostasis in Solanum lycopersicum L. *Journal of Hazardous Materials*, *456*, 131670. https://doi.org/10.1016/J.JHAZMAT.2023.131670

Yotsova, E., Dobrikova, A., Stefanov, M., Misheva, S., Bardáčová, M., Matušíková, I., Žideková, L., Blehová, A., and Apostolova, E. (2020). Effects of cadmium on two wheat cultivars depending on different nitrogen supply. *Plant Physiology and Biochemistry*, *155*, 789–799. https://doi.org/10.1016/J.PLAPHY.2020.06.042

Zhang, D., Zhou, H., Shao, L., Wang, H., Zhang, Y., Zhu, T., Ma, L., Ding, Q., and Ma, L. (2022). Root characteristics critical for cadmium tolerance and reduced accumulation in wheat (Triticum aestivum L.). *Journal of Environmental Management*, *305*, 114365. https://doi.org/10.1016/J.JENVMAN.2021.114365

Zhao, Y., Wang, J., Huang, W., Zhang, D., Wu, J., Li, B., Li, M., Liu, L., and Yan, M. (2023). Abscisic-Acid-Regulated Responses to Alleviate Cadmium Toxicity in Plants. *Plants 2023, Vol. 12, Page 1023*, *12*(5), 1023. https://doi.org/10.3390/PLANTS12051023

Zhou, J., Zhang, C., Du, B., Cui, H., Fan, X., Zhou, D., and Zhou, J. (2020). Effects of zinc application on cadmium (Cd) accumulation and plant growth through modulation of the antioxidant system and translocation of Cd in low- and high-Cd wheat cultivars. *Environmental Pollution*, *265*, 115045. https://doi.org/10.1016/J.ENVPOL.2020.115045