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Anatomical Responses of Radical and Plumule Structures in Several Wheat Genotypes to Cadmium-induced Stress

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Abstract:

Wheat is the most important cereal crop on which more than half of the world's population depends. Therefore, they are at great risk of heavy metals e.g., Cd stress, and this compromises serious problems for humans when they enter the food chain. Therefore, this study aimed to investigate the effect of Cd on wheat genotypes to highlight their responses to this stress leading to select genotypes with less ability to accumulate Cd in their tissues. The study included 10 wheat genotypes suitable for arid and semi-arid areas and 4 concentrations of Cd (0, 75, 150, and 225 mg L^{-1}) laid as a factorial RCBD experiment with three replications. The important result of this experiment is that the superiority of G-4 in physiological traits, IRAQ, and G-9 were prominent in most anatomical traits. Genotypes G-3, G-9, and G-24 showed great ability of steady metabolism through the activation of defense mechanisms at high concentrations of Cd which led to the superiority of those genotypes at anatomical levels such as the number of xylem vessels. The anatomical traits were a clear indication of Cd toxicity in the genotypes but genotypes were varied in their responses to Cd stress. Therefore, further investigations are required to be able to select genotypes with low ability of Cd accumulation.

Keywords: Anatomy, Cadmium stress, Heavy metals, Wheat genotypes.

الاستجابات التشريحية لتركيب الجذير والرويشة في عدة تراكيب وراثية من الحنطة للإجهاد المستحث بالكادميوم

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الملخص:

الحنطة هي من اهم المحاصيل الحبوبية والتي يتغذى عليها أكثر من نصف سكان العالمز لذا، فهي أكثر محصول يقع تحت خطر التعرض الى اجهاد المعادن الثقيلة كالكادميوم وهذا بحد ذاته يشكل مشكلة خطيرة للإنسان اذا ما دخل هذا العنصر الى السلسلة الغذائية. لذلك اتت هذه الدراسة للتحري عن تأثير الكادميوم على تراكيب وراثية من الحنطة وتسليط الضوء على مدى وطريقة استجابتها لهذا النوع من الاجهاد وبالتالي ستمكن مربي النبات على اختيار التراكيب الوراثية التي تراكم الكادميوم في انسجتها بشكل اقل. تضمنت الدراسة 10 تراكيب وراثية من حنطة الخبز ملائمة للزراعة في الظروف الجافة وشبه الجافة و4 تراكيز من الكادميوم (0 و75 و150 و225 ملغم لتر$^{-1}$) في تجربة عاملية مطبقة بتصميم القطاعات العشوائية الكاملة بثلاث مكررات. تشير أهم النتائج في هذه التجربة الى تفوق التركيب الوراثي G-4 في الصفات الفسيولوجية والعراق وG-9 كانا متميزين في معظم الصفات التشريحية. التراكيب الوراثية G-3 و G-9 و G-24 كانت استجابتها مستقرة وثابته في العديد من العمليات الحياتية من خلال تحفيز الوسائل الدفاعية عند التراكيز العالية من الكادميوم والتي ادت بالنهاية الى تميز هذه التراكيب الوراثية في الصفات التشريحية كعدد الاوعية الخشبية في نسيج الخشب. الصفات التشريحية كانت مؤشرا واضحا لسمية عنصر الكادميوم في التراكيب الوراثية ولكن الاهم ان التراكيب الوراثية اختلفت في مدى استجابتها لهذا الاجهاد. لذلك، دراسات لاحقة تكون مهمة لتمكن مربي النبات من اختيار التراكيب الوراثية الاقل في تراكم الكادميوم في الانسجة.

الكلمات المفتاحية: التشريح، اجهاد الكادميوم، المعادن الثقيلة، التراكيب الوراثية للحنطة.

Introduction

Over half of the world's population is fed wheat as a source of vitamins, minerals, and carbohydrates (Al-Issawi et al., 2013). Since wheat is planted and harvested somewhere in the world every month of the year, it is therefore sown over quite large areas worldwide (more than 22% of cultivated areas globally) (Curtis & Halford, 2014). The popularity of wheat makes it vulnerable to all biotic and abiotic stresses, including pollutants.

Pollution has been a growing problem since the end of the 19th century and the beginning of the 20th century due to the industrial revolution (Engine, 2003; Jarrige et al., n.d.). This problem was classified as a global as a result of bad planning and the mismanagement of environmental resources (Masindi et al., 2018). Population growth, rapid industry development, various transportation systems, fertilizers and pesticides, and sewage contributed to the increase of pollution with heavy metals (Alengebawy et al., 2021; Srivastava et al., 2017). Heavy metals are dangerous due to their long life in the soil without chemical changes (Young, 2013), they can form complex compounds with most organic and inorganic compounds in plant cells and thus make them

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immobile (Shahid et al., 2017; Vandenbossche et al., 2015), therefore heavy metals could be considered from the hazardous chemicals in the soil (Tóth et al., 2016). Heavy metals affect not only plant growth but also accumulate in their edible parts (Alengebawy et al., 2021). Unlike some of the heavy metals (e.g., Mo, Cu, Cr, Co, V, Ni, Zn, Mn, Fe), there are other metals (e.g., Ag, Hg, As, U, Pb, Cd……etc.) that are not proven to have a role in plant life. On the contrary, their presence in the environment hinders plant growth. Thus, may enter the food chain, which in turn forms a great danger to human health because they are not soluble. Also, they can be accumulated in organism's cells, besides the life age of heavy metals is relatively long and this causes their accumulation in organisms' bodies (Jin et al., 2020; Tchounwou et al., 2012). There are no apparent symptoms of heavy metals once they enter the biological system; therefore, their impact is hidden and appears after many years due to their accumulation ability. Cadmium (Cd) is one of the widespread heavy metals in the environmental systems, and it comes third after mercury and lead (Wang et al., 2019). It has not been proven that Cd has a positive role in plant life and it is classified as one of the toxic substances according to the Agency of Toxic Substances and Disease Registry (Nordberg et al., 2018). It has been found that many cereal crops accumulate Cd in their grains such as wheat (MA et al., 2021), thus 90% of Cd in the human diet comes from wheat grains (Clemens et al., 2013). Plant functions start to decline when Cd concentrations get higher than 3 mg kg^{-1} TDM. Cd has a strong ability to bind with Sulphur causing protein damage. It also has a great ability to bind with oxygen and nitrogen compounds disturbing the oxidation balance in plant cells (MA et al., 2021). Cd competes with Ca^{+2} which is important to cell signaling through kinase enzymes; therefore, it disturbs cell signaling. As for Cd transporters, there is no evidence that Cd has specific transporter; however, some researchers proposed that Zn and Fe transporters can transport Cd with less efficiency. The Zn transporter ZNT1 had been found to transport Cd but with less efficiency (Song et al., 2017). Besides, Cd decrease from cell expansion through accumulating H_2O_2 in the cell wall which in turn makes it solid, and this explains the weakness in root growth in Cd-affected environments. Upon exposure to high concentrations of Cd, Auxins and Gibberellins, which are important in cell signaling might be damaged. On the other hand, Cd increases the content of Abscisic acid (ABA) and Ethylene (ET) in root cells and leads to plant vegetative growth reduction. Ethylene reduces cell expansions, while ABA helps plants adapt against water stress (Das et al., 1997; Kiran et al., 2022; Shahid et al., 2017).

Wheat genotypes are varied in their response to Cd stress therefore it requires better understanding of its behavior in plants. Few studies have been conducted on the effect of Cd on the anatomical parameters in wheat, therefore this study investigated the effects of Cd on the anatomical traits of root and plumule in promising wheat genotypes suitable for arid and semi-arid areas.

Materials and Methods:

A lab experiment was conducted in the plant physiology lab at the College of Agriculture- University of Anbar to evaluate 10 wheat genotypes (G-3, G-4, G-9, G-24, G-28, G-29, G-39, G-41, Al Diayr, and IRAQ) anatomically stressed by cadmium as stress (0, 75, 150, 225 mg Cd L^{-1}). The factorial experiment was conducted according to a Randomized Block Design with three replications of each treatment. 50 grains were laid in each petri dishes and then they were irrigated with required Cd concentration for each treatment.

Physiological parameters:

Standard lab germination (%) = Normal seedlings/total grains in each plate*100

Radical and Plumule Length (cm): the length was measured for 5 normal seedlings that were taken randomly.

Seed Vigor = Germination (%) * (Radical length + Plumule length) (International Rules for Seed Testing - International Seed Testing Association, n.d., 2013).

Anatomical traits

The study also included various anatomical characteristics of the radical and plumule, including the number of vessels in the 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 upper surface of the epidermis (adaxial) was prepared by putting the plumule upside down and the lower epidermis were gently scraped off. Some warm water drops were added to keep it fresh. Then, the prepared parts were transferred with forceps to warm water in order to clean it up from the residuals of other parts. Cross sections of radical were made manually by scraping them with a sharp sterilized blade while the root parts were held vertically by thumb and index fingers. Very thin slices were cut and then stained with 1% safranin for 30 min before moving them into ethanol (30, 70, and 90%) for 2 min in each concentration to completely remove the stain, and finally transferred to absolute ethanol (96%) for 2 min. Cross sections of radical (slices) were fixed on glass slides by placing them using Gel (local commercial) 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: 10X and 40X).

Results and Discussion:

Germination and seedling vigor: The genotypes in this study did not show any significant differences under the effect of either genotype or Cd stress (Table 1). However, genotype affected the seed vigor significantly. Genotype Al Diyar showed higher seed vigor (1933.96) in comparison with G-29 which recorded lower seed vigor of 1280.25 which did not differ from G-39, G-28, G-41, and G-3. Genetically, those genotypes were varied in many aspects as was proven by Abdul-Hassan & Al-Issawi (2021). Seedling vigor variation belongs to the significant variation in the length of radical and plumule (Table 2). Seedling vigor was prominent in control (2693.39) and started to decline with the increase of Cd to get to its lower value at 225 mg L^{-1} (692.91). The impact of Cd on this vigor might belong to the harmful role of Cd which disturb the enzyme function, cell division, and cell differentiation of either root or plumule leading to a reduction in plant growth and development (Zhao et al., 2023). The two-way interaction between G-4 and control recorded a mean of seedling vigor of 3148 for the trait, which did not significantly differ from the interaction between Al Diyar with either control or treatment of 75 mg L^{-1}, which gave a mean of seedling vigor 2926.36 and 2990, respectively. On the other hand, the lowest mean was recorded for the interaction between G-29 and the concentration of 225 mg L^{-1} (486) and also did not differ significantly from the interaction between G-3 and the concentrations of 150 and 225 mg L^{-1}. However, these results could be attributed to the variation in the genetic background of the genotypes under study which was reflected in their ability to absorb and accumulate cadmium in their parts (ÖZYİĞİT et al., 2021). The efficiency variation of those genotypes in their ability to chelate the cadmium as complexes and then move it to vacuoles of cells.

Table 1 Effect of Cd concentrations on grain germination (%) and Seedling vigor.

Genotypes	Percentage of germination (%) Cd conc. (mg L^{-1})				Mean	Vigor of seedlings Cd conc. (mg L^{-1})				Mean
	0	75	150	225		0	75	150	225	
G-3	100	100	100	100	**100**	2703	1734	645	593	**1418.75**
G-4	100	100	100	99.33	**99.83**	3148	1467	920	838	**1593.25**
G-9	100	100	100	100	**100**	2794	1117	1056	763.066	**1432.52**
G-24	100	100	100	100	**100**	2729	1208	1401	731	**1517.25**
G-28	100	100	100	100	**100**	2400	1160	1101	829	**1372.5**
G-29	100	100	100	100	**100**	2621	1178	836	486	**1280.25**
G-39	100	100	100	100	**100**	2012.5	1590	1007	597	**1301.63**
G-41	100	100	100	100	**100**	2814	1211.5	1058	559	**1410.63**
Al Diar	99.33	99.33	100	100	**99.67**	2926.36	2990.47	1106	713	**1933.96**
IRAQ	100	100	100	100	**100**	2786	1630	1341	819.999	**1644.25**
Mean	**99.93**	**99.93**	**100**	**99.93**	**G= N.S**	**2693.39**	**1528.6**	**1047.1**	**692.906**	**G=144.52**
LSD 0.05	**Cd, G*Cd= N.S**					**Cd= 91.4, G*Cd= 289.04**				

Length of radical and Plumule (cm): Statistical analysis in Table 2 showed that the genotype had a significant effect on the length of plumule only, while cadmium affected the length of both plumule and radical. The genotype G-4 recorded the highest length mean of plumule (10.05 cm), which in turn did not differ significantly from Al Diyar, IRAQ, G-24, G-39, G-9, and G-41, which recorded a mean of this trait ranging between 9.81 and 8.41 cm. While genotypes G-29, G-3, G-28, G-41, G-9, and G-39 had a length of plumule ranging between 7.17 and 8.74 cm. The variation of the genotypes among them in the trait of plumule length is considered one of the important indicators that indicate that there will be variation in the length of the plant in the stages of growth. However, the reason could be attributed to the structural differences in the genes responsible for the length of the plumule, which reflect the phenotype (AL-Obaidy, 2015; Hashem & Al-Issawi, 2023).

As for cadmium, the control was superior in giving the highest length of radical and plumule which reached 14.26 and 12.69 cm for the radical and plumule respectively, while the lowest mean was noticed at the treatment of Cd of 225 mg L^{-1} (1.9 and 5.04 cm for radical and plumule respectively). The reason for these results could be attributed to the vital role that cadmium plays in disrupting and deforming many enzymes inside plant cells, which are essential for plant growth processes, in addition to the changes that cadmium causes at the structural level of some proteins and the degradation of biological molecules (Feng et al., 2023; Wani et al., 2018), all of which negatively affects the length of the radical and plumule and many other traits.

Table 2 Effect of Cadmium concentration on Radical and Plumule Length (Cm)

Genotypes	Radicle length (cm) Cd Conc. (mg L^{-1})				Mean	Plumule length (cm) Cd Conc. (mg L^{-1})				Mean
	0	**75**	**150**	**225**		**0**	**75**	**150**	**225**	
G-3	15.34	7.95	1.61	1.67	**6.64**	11.69	9.39	4.84	4.26	**7.55**
G-4	15.79	3.92	1.93	1.88	**5.88**	15.69	10.75	7.27	6.5	**10.05**
G-9	16.05	2.09	2.79	2.55	**5.87**	11.89	9.08	7.77	5.12	**8.47**
G-24	15.53	2.51	4.39	1.67	**6.03**	11.76	9.57	9.62	5.64	**9.15**
G-28	13.44	3.16	3.3	2.01	**5.48**	10.56	8.44	7.71	6.28	**8.25**
G-29	15.04	3.65	2.26	1.58	**5.63**	11.17	8.13	6.1	3.28	**7.17**
G-39	7.88	4.7	2.81	1.74	**4.28**	12.25	11.2	7.26	4.23	**8.74**
G-41	15.07	3.21	3.17	1.34	**5.7**	13.07	8.91	7.41	4.25	**8.41**
Diar	14.2	18.57	3.51	2.28	**9.64**	15.26	11.56	7.55	4.85	**9.81**
IRAQ	14.25	5.39	5.01	2.23	**6.72**	13.61	10.91	8.4	5.97	**9.72**
Mean	**14.26**	**5.52**	**3.08**	**1.9**	**G= N.S**	**12.69**	**9.79**	**7.39**	**5.04**	**G= 2.39**
LSD0.05	**Cd= 3.81, A*B=N.S**					**Cd= 1.51, A*B=N.S**				

Chlorophyll Content: Figure 1 showed that there was a significant effect of Cd on the content of chlorophyll a and b. The low concentration of Cd led to a slight increase in the content of photosynthesis pigments, therefore the concentration 75 mg Cd L^{-1} gave content of Chl. a and b of 7.96 and 5.72 mg g^{-1} respectively. This result was consistent with what has been found by Ozyigit et al., (2021) as they explained that light stress of Cd stimulated the content of chlorophyll. This light stress might enhance some enzyme activities which led to the increase of photosynthesis pigments (Al-Ubaidy et al., 2021; Al-Yasary, 2022). On the other hand, moderate or high Cd stress led to a drastic reduction in chlorophyll content (Figure 1). A high concentration of Cd could exert oxidative stress in cell cytoplasm specifically in chloroplasts by which ROS will be accumulated and then damage photosynthesis (Zhao et al., 2023).

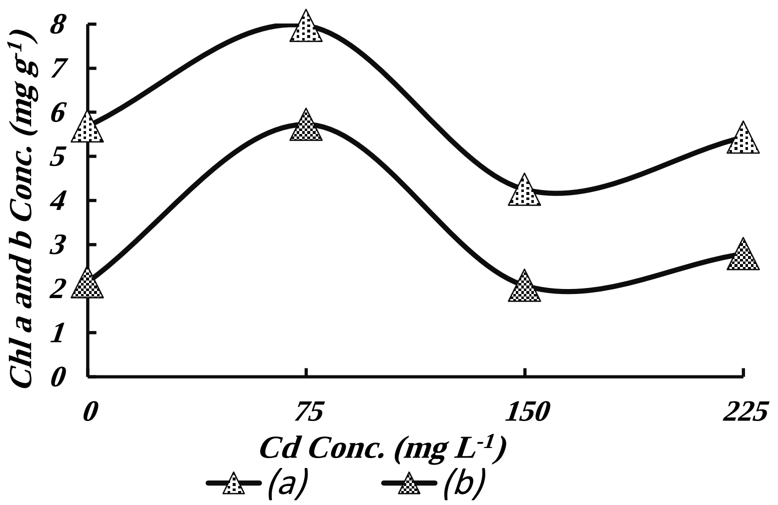


Figure 1. Effect of Cd concentrations on chlorophyll a and b content (mg g^{-1})

Anatomical traits:

Number of xylem vessels: figure 2-A and picture 1 indicated the clear effect of genotype, Cd, and their interaction on the number of vessels of xylem in the root of wheat seedlings. Al Diayr genotype showed the highest number (3.50) followed by IRAQ, G-24, and G-41 while G-29 recorded the lowest number of vessels number in the radical xylem. The reasons behind that might belong to the genetic background of those genotypes (Al-Yasary, 2022) as the variation in growth nature results in anatomical variation too. As for Cd, the treatment with 75 gm Cd L^{-1} led to get high number of xylem vessels (3.30) while the lowest number was observed in the control (2). It was noticeable from picture 1 that Cd affected the distribution of Parenchymal cells which made them unsystematic in the radical center which is considered the basis of xylem formation in comparison with the control. However, xylem tissue is the main transporter of Cd to other parts of plants (ÖZYİĞİT et al., 2021). Thus, the availability of Cd in the rhizosphere of plants leads to the increase of xylem vessels in the radical. On the other hand, the reduction in the diameter of xylem vessels because of the Cd might be the reason behind the increase in the number of vessels. This could be one of the adaptive mechanisms against Cd stress to compensate for the reduction of vessel diameter with the increase of their numbers.

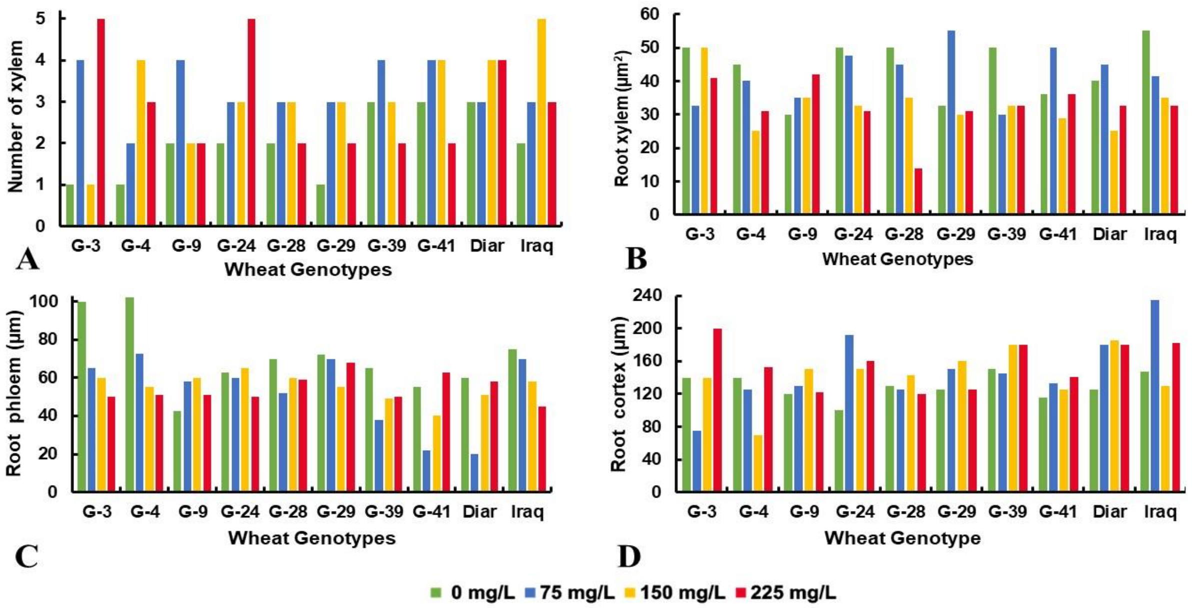
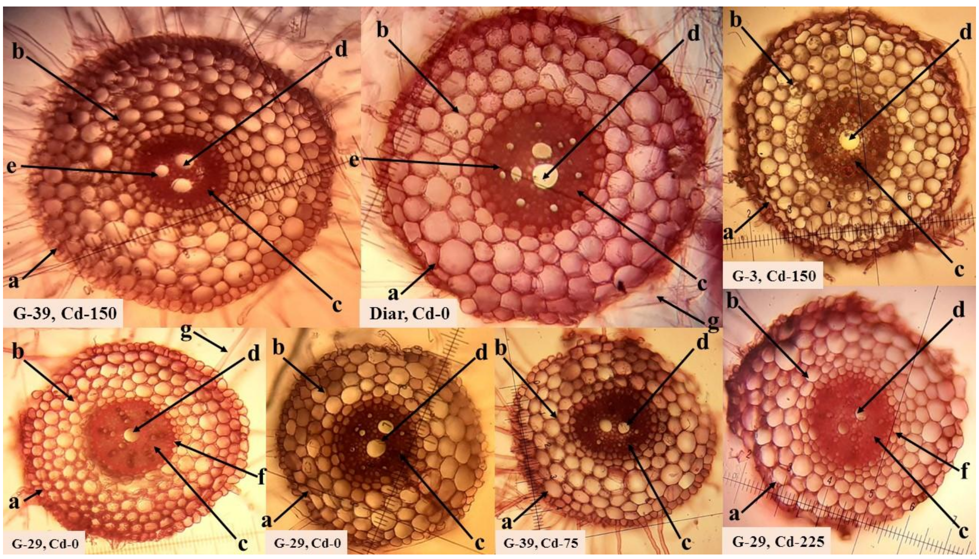


Figure 1 Effect of Cd concentrations on number of xylem vessels (A), Xylem dimensions μm^2 (B), Phloem thickness µm (C) and Cortex thickness µm (D).



Picture 1 Cross sections of radical of several wheat genotypes (10X). Epidermis (a), Cortex (b), Phloem (c), Xylem (d), Vascular cylinder (e), casparian strip (f), radical hairs (g)

Also, two-way interaction between the genotype and Cd affected the number of xylem vessels in the radical of wheat seedlings. The interactions between IRAQ*150 mg Cd L^{-1}, G-3*225 mg Cd L^{-1} and G-24 * 225 mg Cd L^{-1} recorded the lowest number of vessels in the xylem of the radical. The interaction between genotypes of G-3, G-4, and G-29 with the control and G-3*150 mg Cd L^{-1} recorded the lowest number of vessels of xylem in the seedling radical. This could be attributed to two factors, first the genetic variation of the genotypes under study reflected the ability of those genotypes to cope with Cd stress (Sabella et al., 2022). Secondly, the dose of Cd is the reason as exposing wheat genotypes leads to the increase of xylem vessels with the increase of Cd concentrations (ÖZYİĞİT et al., 2021). Thus G-3 has the big ability to accumulate Cd and could tolerate the high concentrations of Cd (150 and 225 mg Cd L^{-1}). Cd Concentrations stimulated the increase of xylem vessels while the reduction in their numbers was noticed at the control. This genotype (G-3) might have a defense system against the toxic effect of Cd which makes it prominent over the other genotypes under study. On the other hand, it was noticed that the IRAQ genotype was more stable genotype upon treatment with Cd, it was superior under the effect of 150 mg Cd L^{-1}.

Mean of Xylem Dimension (µm^2): Figure 2-B and Picture 1 showed a clear effect of genotype, Cd, and their interaction on the dimension of the xylem. Genotype G-3 gave the highest mean of dimension (43.38 µm^2) followed by IRAQ genotype (42 µm^2), while the genotypes G-4 and G-9 recorded the lowest mean of this trait (35.25 and 35.50 µm^2 respectively). The variation of the ancestors of those genotypes led to obtaining various dimensions of the xylem as well as many other anatomical traits (Ibrahim Mohammed et al., 2021). Cadmium had a negative impact on the dimension of the xylem in the radical of wheat genotypes. Therefore, the highest dimension was recorded at the control (43.85 µm^2) while the lowest mean was recorded at the treatment of 225 mg Cd L^{-1} (32.35 µm^2). This could be attributed to the negative impact of Cd on the photosynthesis and formation of carbohydrates and proteins (Paunov et al., 2018) eventually causes a reduction in biochemical processes responsible for cell division and differentiation which affects the dimension of the xylem. The two-way interaction IRAQ* control and G-29* 75 mg Cd L^{-1} gave the highest mean of xylem dimension (55 and 55 µm^2 respectively). Also, interaction G-3, G-39 and G-28 with control, G-41*75 mg Cd L^{-1} and G-3* 150 mg Cd L^{-1} all gave 50 µm^2. The interactions G-28*225 mg Cd L^{-1}, Al Diyar, and G-4 with 150 mg Cd L^{-1}. Generally, all wheat genotypes under study were affected by Cd and that might be attributed to the genetic background of those genotypes (Nielsen et al 2014) and this was reflected in the variation of their response to Cd stress. These findings were in consistency with what have been found by Al-Yasary (2022) who indicated that the genotypes differ in their response to Cd stress.

Thickness of Phloem (µm): Figure 1-C and picture 1 indicated a clear impact of genotype, Cd, and their interaction on the thickness of the phloem of wheat seedlings. The genotype G-4 recorded the highest mean thickness of phloem followed by G-3 which gave 70.18 and 68.75 µm for the aforementioned genotypes respectively. Genotype G-41 recorded the lowest mean of this trat (44.88 µm) followed by Al Diyar (47.25 µm). This could be attributed to the genetic variation among those genotypes which led to the variation in their radical efficiency in water absorption which eventually leads to different radical efficiency (Nakhforoosh et al., 2014). Cadmium also negatively affected the thickness of phloem in the radical of wheat seedlings. The control recorded the highest mean of thickness (70.42 µm), while the concentration of 75 mg Cd L^{-1} had the lowest mean of this trait (52.75 µm). This might be attributed to the toxic effect of Cd in the reduction of enzyme activities and hindering the nutrition to the root (Saleh et al., 2020). The interaction between G-4 and G-3 with the control recorded the highest mean of phloem thickness which gave 102.2 and 100 µm respectively. Contrary, the interaction Al Diayr*75 mg Cd L^{-1} and G-41*75 mg Cd L^{-1} gave the lowest mean of the trait (22 µm). From these results, Cd has a negative impact on this trait and that was because of Cd influx into phloem tissues causing ROS accumulation which leads to degradation of lipids and proteins (Genchi et al., 2020) finally affecting the phloem tissues negatively.

Thickness of Cortex (µm): Figure 1-D and picture 1 explained the significant effect of genotype and Cd as well as their interaction. Genotype IRAQ has the highest value for cortex thickness (173.75 µm) followed by Al Diyar and G-41 (121.88 and 128.38 µm respectively). These results proved that those genotypes differ in this trait and were consistent with the findings of Pandey et al., (2017) who indicated the variation of genotypes in terms of their field performances. The highest concentration of Cd (225 mg L^{-1}) increased the thickness of the cortex in the radical of wheat seedlings to reach its highest value of 156.35 µm, while the control recorded the lowest mean of cortex thickness (129.29 µm). It is clear from picture 1 that Cd of 225 mg L^{-1} increased the size of cells and this led to an increase in the cortex through the formation of more than one layer from the cortex. Those cells have oval or circle forms and also have thick walls as angular (Angular collenchymal). This could be attributed to the changes made by Cd in some metabolic pathways (Genchi et al., 2020), with potential accumulation of Cd in the Cortex as an adaptive mechanism towards Cd stress. The two-way interaction between the IRAQ genotype and 75 mg Cd L^{-1} gave the highest mean of this trait (235 µm) followed by the interaction of G-3 and 225 mg Cd L^{-1} with the value of 200 µm. On the other hand, the interaction between G-4 and 150 mg Cd L^{-1} recorded the lowest value of this trait (70 µm). This could be attributed to the genetic background of genotypes included in this study which led to different responses to Cd stress. In this context, Abedi & Mojiri (2020) pointed out this variation among wheat genotypes as they found that genotypes accumulate Cd differently eventually affecting their biochemical processes inside plants.

Length and width of Epidermis in radical (µm): Results shown in Figures 3-A, B, and picture 1 indicated significant effects of genotype and Cd along with their interaction on the length and width of the epidermis of the radical of wheat seedlings. Genotypes were greatly varied in these traits and that might belong to their ancestors. Genotypes G-24 and Al Diyar were prominent in those traits (34.5 and 34 µm respectively) and the lowest values were recorded for the genotypes G-28 and G-39. On the other hand, G-4 recorded the lowest width of the width of radical epidermis (17.75 µm). The metabolism of the plant also can be affected by the genotype and plants vary in how they transfer metabolites from root to shoot (AL-Obaidy, 2015). In this regard, Cd with a concentration of 150 mg L^{-1} enhanced both the length and width of the epidermis in radical of the wheat genotypes in comparison with control. This was consistent with what has been found by Khalil and Abdullah (2019) who indicated the thickness of the epidermis of radical as an adaptive strategy against Cd stress. Like other traits, the interaction between genotype and cadmium affected the length and width of epidermis cells in the radical of seedlings. It can be noticed that high concentration of Cd and this leads to the conclusion that wheat accumulates Cd in the tissue to mitigate its damaging effects. However, the level of accumulation and damage are varied among the genotypes under study. Most probably, the variation between genotypes makes some of them exclude Cd from their tissues while others accumulate it in their tissues (Al-Yasary, 2022).

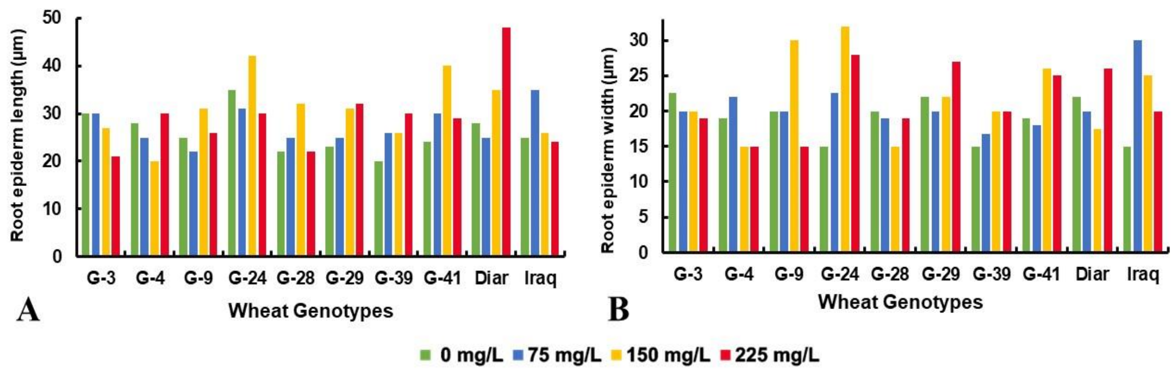


Figure 2 Effect of Cd concentrations on Length of root epidermis (A) and width of root epidermis (B).

Length and width of stomata Cells in plumule (µm): it is clear from Figure 3-A&B and Picture 2 that genotypes and Cd concentrations along with their interaction significantly affected the length and width of **stomata** in the plumule of 10 wheat genotypes. The genotype G-24 recorded the highest length of **stomata** (21.64 µm) followed by G-41 (20.51 µm) which also showed the highest width of the **stomata** (1.96 µm) in comparison with the IRAQ genotype which recorded the lowest width of **stomata** followed by Al Diyar genotype. These results could be attributed to the genetic behavior of those genotypes which leads to morphological variations (Hashem & Al-Issawi, 2023). Cadmium also affected these traits significantly, however, the highest mean of them was recorded at the control. Due to the increase of Cd concentration in the solution, either length or width were affected, therefore the lowest means of those traits were recorded at the concentration of 225 mg Cd L^{-1}. The harmful damage of Cd to the chloroplast led to a decrease in the metabolites to cells eventually affecting gas exchange and transpiration. Also, this could affect the absorption of water and nutrients. These results were consistent with have been found by Ozyigit et al (2021). Genotypes were also varied in their response to the Cd concentrations. The interaction G-9*75 mg Cd L^{-1} recorded the highest mean of guard cell width while G-41*225 mg Cd L^{-1} recorded the lowest mean of this trait. The genetic background of those genotypes could be the determinant factor for Cd absorption and accumulation in the different tissues of plants. The dose of Cd also played a negative role in plant responses to this stress (Sabella et al., 2022).

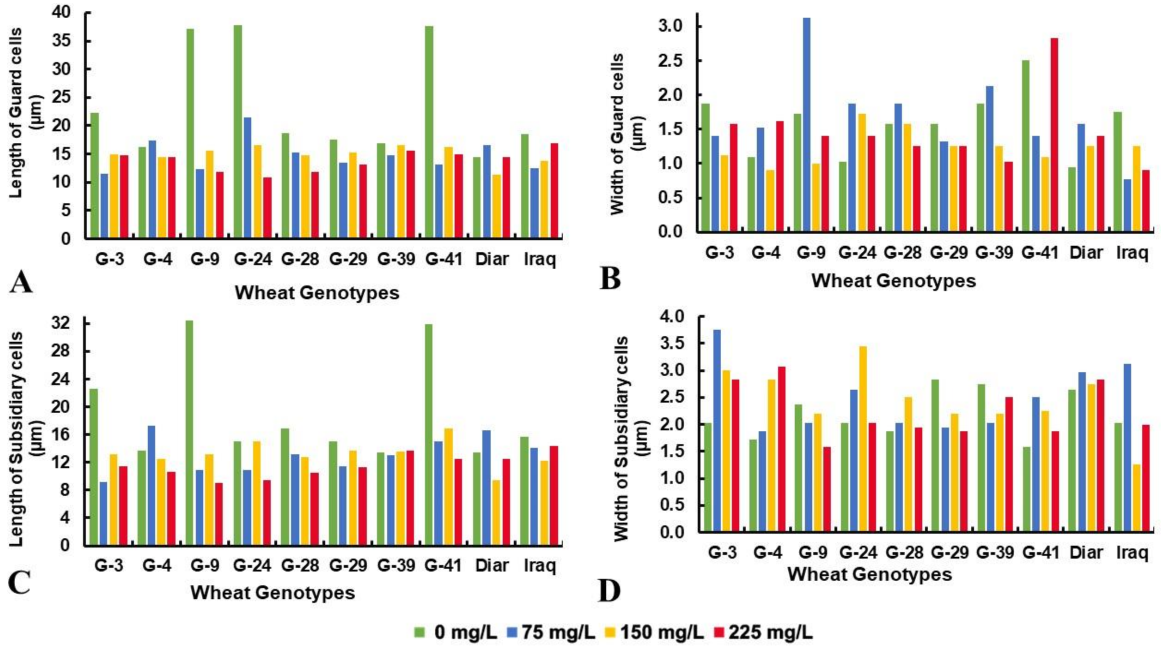
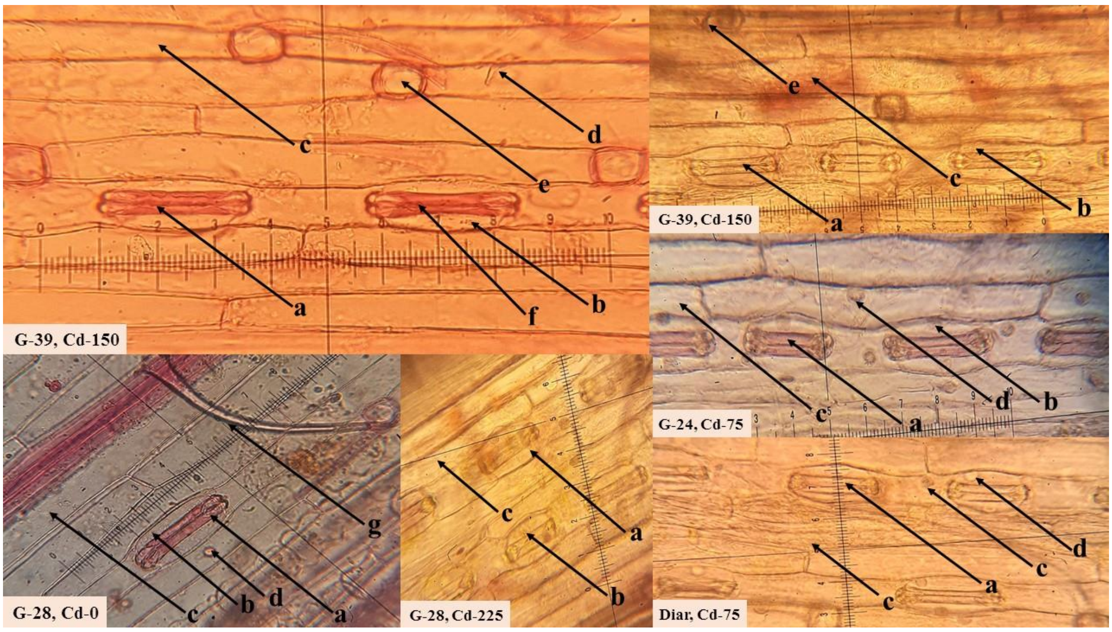


Figure 3. Effect of Cd concentrations on Length of Guard Cells (A), width of Guard Cells (B), Length of Auxillary Cells (C), and Width of Auxiliary cells (D).



Picture 2. Upper surface of Plumule of several wheat genotypes (40X). Guard Cells (a), auxiliary cells (b), Epidermis Cells (c), Nucleus (d), Origin of leaf hairs (e), stomatal aperture (f), Leaf hairs (g)

Length and width of auxiliary cells of plumule (µm): Figure 4-C&D and picture 2 indicated the length and width of auxiliary cells in wheat genotypes under the effect of Cd stress. Genotype, Cd, and their interaction significantly affected the shape of those cells. The genotype G-41 was prominent in the length of auxiliary cells (19.08 µm) in comparison with G-9 which gave the lowest mean of this trait. Wheat genotypes are varied in their response to Cd stress and this was proved by many researchers (Mahmoud Al-Amiry & Owaid Al-Ubaidi, 2016). Auxiliary cells in the plumule of wheat genotypes were affected by Cd. The highest values of either length or width of auxiliary cells were recorded at the control, then they started to decline as the concentration of Cd increased. Cadmium exerted many negative effects at the physiological level such as cell growth, protein synthesis, enzyme activity, transpiration, etc (Li et al., 2023), thus affecting the anatomical traits e.g., length and width of auxiliary cells of the epidermis. It was also clear that genotypes under control behaved in a better way in comparison with those treated with Cd. However, the degree was different as some were greatly affected in comparison with others. for instance, G-9 at control gave the highest mean length of auxiliary cells in comparison with the same genotype when treated with 225 mg Cd L^{-1}. The tolerance to the Cd stress can be varied according to the genotype (Sabella et al., 2022).

Length and width of Adaxial epidermis of plumule (µm): Those types of cells also were significantly affected by the study factors. Figure 5-A&B and picture 2 illustrate the measurements of adaxial cells in the epidermis of the plumule. Genotype IRAQ recorded the lowest value of these traits and the lowest value was recorded for G-3 and the rest of the genotypes under study were ranged between those two genotypes. The genetic variation among those genotypes led to variation in most anatomical traits (Abdul-Hassan & Al-Issawi, 2021). Cadmium effects were significant on the length and width of the adaxial epidermis in the plumule of wheat genotypes. The highest measurements were recorded at control and then declined as the concentration of Cd increased. Hormonal imbalance caused by Cd stress might be the reason for this reduction in adaxial epidermis cell dimensions (Khalil & Abdullah, 2019). The two-way interaction was consistent with the individual study factors, but the most interesting result is the variation among genotypes in their responses to the exerted stress (Zhang et al., 2020).

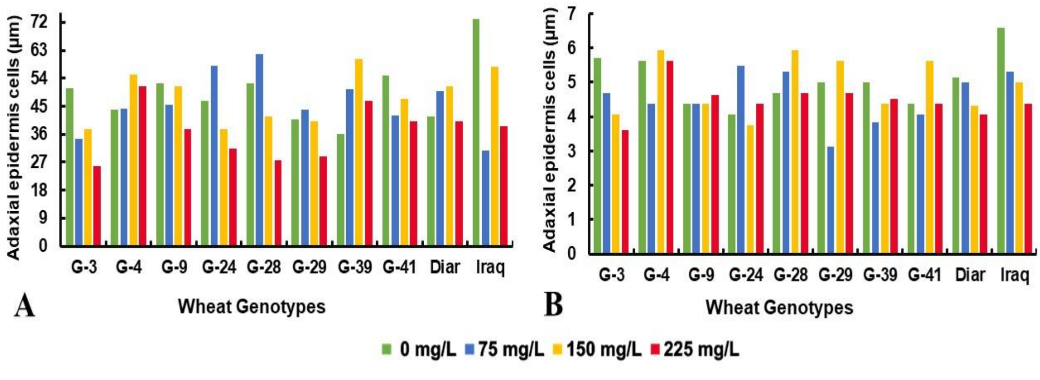


Figure 4. Effect of Cd concentrations on Length of Adaxial Epidermis cells (A), width of Adaxial Epidermis cells (B)

Conclusion:

It can be concluded from the results of the current study that Cd affected most of the physiological and anatomical traits included in this study for all wheat genotypes. This could be a clear indication that Cd played a role as a toxic factor, especially at high concentrations. Interestingly, Cd at low concentrations slightly enhanced some traits due to the adaptive response to this stress but traits declined with the increase of Cd up to 225 mg Cd L^{-1}. Also, the performances of genotypes included in this study and these greatly interesting results lead to conclusions that some genotypes tolerate Cd stress via accumulating it in the vacuole while others are sensitive to this stress. Therefore, a wheat breeder could select genotypes with low accumulating Cd in their tissues for human nutrition while they can exclude those that uptake Cd and accumulate it in their tissues which gives them a chance to transport it to the grains. Further investigations are required to clarify the behavior of Cd in cereal crops because of their importance to human nutrition and select genotypes with low Cd accumulation for this purpose.

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