J. Int. Environmental Application & Science, Vol. 13(1): 27-32 (2018)

Research Paper

Effects of Chromium on Anatomical Characteristics of Bread Wheat (*Triticum aestivum* L. cv. "*Ekiz*")

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Received January 03, 2018; Accepted March 22, 2018

Abstract: Heavy metal toxicity is one of the important abiotic stresses. When a few metals such as copper, manganase, cobalt, zinc and chromium are present at excessive levels, they have potential to become toxic to plants. In the study, the important anatomical changes caused by chromium (Cr) in *Triticum aestivum* cv. "*Ekiz*" were investigated. For this aim, plants were treated with 0.3 and 0.4 mmol Cr for 21 days. Microscopic studies showed that Cr accumulated mainly in the roots of the metal-treated plants. The stems of Cr-treated plants showed an increase in diameter of parenchyma cells and a decrease in thickness of sclerenchyma. In the leaves of plants exposed to Cr treatment, the thickness of phloem, xylem and mesophyll tissue was decreased compared with control plants. The anatomical changes in the root, stem and leaves of Cr- treated plants indicated that Cr has an important impact on *Triticum aestivum* cv. "*Ekiz*".

Keywords*: Anatomy, bread wheat, chromium*

Introduction

Heavy metals are among the most dangerous substances that exert negative influences on environment because of their high level of harmful to live organism (Sethy, 2013). Heavy metal toxicity results in chlorosis, weak plant growth, decrease in germination and reduction in reproductive capability (Al-Saadi *et al*., 2013). Chromium (Cr) is serious metal contaminant in all all phases of the environment including air, water and soil (Shanker *et al*., 2005). The stable forms of Cr are the trivalent Cr (III) and hexavalent Cr (IV). Cr (IV) is considered the most toxic form Cr which usually occurs associated with oxygen (Shahandeh & Hossner, 2000; Mei et al., 2002). Cr (III) is less toxic and is mainly found bound to organic matter in soil and aquatic environments (Becquer *et al*., 2003). Toxic effects of Cr observed at multiple alterations in the germination as well as in the growth of roots, stems and leaves which may affect total dry matter production and yield. Sharma and Sharma (1993) found that Cr inhibited leaf number per plant in wheat. Cr also causes deleterious effect on plant physiological processes such as photosynthesis, water relations, mineral nutrition and enzmatic activities (Shanker *et al*., 2005).

It was reported that Cr accumulation in plants is always higher in roots than shoots (Shahandeh & Hossner, 2000; Han *et al*., 2004). The higher concentration of heavy metals in roots was mainly the result of accumulation of Cr in the vacuoles of the root cells (Zayed *et al*., 1998). In the presence of high accumulation of heavy metals in roots, plants showed some anatomical changes in diameter of root, xylem and phloem, central vein, size of pericycle, epiderma and parenchyma cells (Stohs et al., 2000). Barcelo *et al*. (1988) reported that some invisible anatomical changes such as thickenings in vascular bundles and low cellular differentiation of bush bean stems due to heavy metal stress. Zhao et al. (2000) also determined that the bioaccumulation of metal has resulted in decrease in the size of mesophyll cells in *Arabidopsis halleri* (L.) O'Kane & Al-Shehbaz. Similar findings were reported by Mangabeira et al. (2001) for Cr (IV) in tomato plants (root, stem and leaf) and argued that Cr (IV) induced changes in the ultrastructure of these organs. It was found that copper, magnesium and sulphate have an inhibitory effect on stomata of wheat (Velichkova *et al*., 2011).

Among cereal plants, wheat and barley have shown greater adaptability to contamined soils and main interest concerning biomass crops is focused on cereals (Wang et al., 2009). Wheat, which is one important cereal crop, usually cultivated by farmers in this area would accumulate relatively high amounts of heavy metals in roots, stems and leaves. Although a number of studies have been

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documented on the toxic effects of chromium, there are little reports on the concerned anatomical alterations especially in cultivated plants. Therefore, the main objective of our study was to determine the anatomical effects of chromium on widely cultivated plants in Turkey, *Triticum aestivum* L. cv. "*Ekiz*".

Materials and Methods

Plant samples (known as Ekiz bread wheat) were collected from the agricultural fields in Suluova, Amasya. The plants were transplanted into plastic pots and were treated with 0.3 mmol and 0.4 mmol of Cr for 21 days, starting one week after transplanting. Pure distilled water was used as control (untreated with Cr) for the study. For anatomical studies, root, stem and leaf samples taken from about same position were cut into 10-15 cm. pieces. Free hand cut sections were treated in 0.2 % Chrome Azurol S solution (CAS) for 24 hours at room temperature to localize chrome (Suzuki et al., 1978). Their photographs were taken with a Nicon Coolpix 5200 digital camera. Image-J program was used for anatomical measurements. All data were subjected to analysis of variance (ANOVA) using the SPSS package program (version 10.00).

Results and Discusssions

The results obtained from present study indicated that the accumulation of Cr in the root of *Triticum aestivum* L. cv. "*Ekiz*" was much higher than its stem and leaf (Table 1-3). In aggrement with our results, Mangabeira *et al*. (2001) and Sridhar *et al*. (2011) showed that the Cr was mainly accumulated in the roots of the metal treated plants. Mac Farlane and Burchlett (2000) was also reported more higher accumulation in root cell walls due to heavy metal stress. It was suggested that generally in plants chromium translocation from root to shoot is very slow (Shanker et al., 2005). An important reason for enhanced accumulation of chromium in the root may be due the presence of organic acids in the root which from complexes with chromium there by making them available for the uptake by root (Sridhar *et al*., 2011).

Table1. Effects of Cr on root anatomical characters of *T. aestivum*. All the values are mean of three replicates ± S.D.

Characters	Control	0.3mM Cr	0.4 mM Cr
Thickness of cortex	207.04±23.793**a**	264.74 ±29.788**b**	294.02 ±30.615**c**
Stele	464.67±30.879**c**	440.47 ±18.913**b**	398.08± 31.881**a**
The width of epidermis cells	24.83 ±5.842**a**	28.24± 4.824**b**	33.74 ±5.539**c**
The lenght of epidermis cells	30.52± 5.083**c**	25.68 ±6.360**b**	20.81± 4.637**a**
Thickness of exodermis	51.96 ± 10.378**a**	66.96± 11.236**b**	90.66 ±20.024**c**
The width of exodermis cells	26.27 ±5.271**b**	24.57 ±4.499**ab**	21.75 ±5.969**a**
The lenght of exodermis cells	27.01 ±3.956**b**	25.53± 5.289**b**	21.78± 3.540**a**
The diameter of cortex cells	37.91± 9.416**a**	37.62± 7.543**a**	42.08± 7.494**a**
The width of endodermis cells	23.26 ±4.339**b**	21.75± 3.606**b**	17.87± 3.200**a**
The lenght of endodermis cells	22.18± 3.009**a**	23.26± 4.339**a**	27.44± 5.807**b**
The diameter of vessel elements	57.29± 5.548**c**	47.92 ±7.320**b**	38.84 ±6.278**a**

Table 2. Effects of Cr on stem anatomical characters of *T. aestivum*. All the values are mean of three replicates ± S.D.

Characters	Control	0.3 mM Cr	0.4 mM Cr
The width of epidermis cells	12.36 ±2.172**b**	10.08± 1.730**a**	9.90± 1.385**a**
The lenght of epidermis cells	14.03± 1.983**a**	15.03 ±2.609**a**	19.26± 2.092**b**
Thickness of sclerenchyma	54.02± 9.860**b**	52.32 ±9.860**b**	36.17± 7.068**a**
The diameter of parenchyma cells	28.83 ±9.359**a**	46.41± 9.359**b**	66.82 ±17.212**c**
Thickness of phloem	30.70 ±4.623**a**	34.06 ±4.966**a**	48.11± 10.715**b**
Thickness of xylem	59.82± 8.71**a**	65.98 ±17.715**ab**	75.76 ±19.587**b**
The diameter of vessel elements	35.32 ±5.828 **a**	28.40 ±3.381**b**	22.87± 2.585**c**

For Cr-treated plants, there were some spesific anatomical changes on root anatomy compared to the control group (Figs. 1A-D). In the cross sections taken from the Cr-treated roots was seen Cr depositions along the exodermis cells (Fig.1B, D). Under higher concentration of Cr, a significantly

increase determined in thickness of exodermis (Table 1). These changes may attributed to the oxidative properties of Cr in the roots. Some authors suggest that in plant the capacity to bind heavy metal in the cell wall has a protective action against the deleterious effect of heavy metals (Vazquez, 1992; Wojcik, 2005). The other remarkable structural change observe for roots of Cr-treated plants was the breakdown of epidermal cells (Fig.1C). Previous studies carried out on the toxic effects of Cr in different plants showed reduction in root size, damaged epidermal cells and collapsed trichomes and root hairs (Maleci et al., 2001; Mangabeira et al., 2001). Our results in aggrement with these data reported by previous researchers. The present results indicated that Cr lead to a significant increase in thickness of cortex (Table 1). The highest increase was seen in 0.4 mM Cr treatment. Previous studies reported that reduction in root growth may be due to a decrease in cell division or disorder in the activity and contents of phytohormones in the roots exposed to heavy metals (Sharma and Dietz, 2006; Soudeh and Zarinkamar, 2012). Aery and Sarker (2012) reported that the inhibition of root elongation under the influence of heavy metals may include abnormal mitosis and cell elongation.

Table 3. Effects of Cr on leaf anatomical characters of *T. aestivum*. All the values are mean of three replicates ± S.D.

Characters	Control	0.3 mM Cr	0.4 mM Cr
The width of adaxial epidermis	16.36± 3.575**a**	18.37 ±3.864**a**	21.86± 6.958**b**
The lenght of adaxial epidermis	21.04 ±3.215**a**	23.08± 3.547**a**	29.98 ±4.137**b**
The width of abaxial epidermis	17.10 ±2.233**a**	18.71 ±3.078**a**	22.97± 3.788**b**
The lenght of abaxial epidermis	21.17 ±2.961 **a**	24.48 ±3.461**b**	26.26± 4.108**b**
The thickness of mesophyll	208.17 ±8.461**b**	196.04± 9.957**b**	154.29 ±9.37**a**
The thickness of phloem	43.83± 9.039**b**	41.29 ±8.473**b**	31.47 ±5.398**a**
The thickness of xylem	67.95 ±8.823**c**	51.86 ±9.301**b**	36.81± 5.859**a**
The diameter of vessel elements	34.92± 7.856**c**	26.89 ±5.592**b**	19.50± 3.668**a**
The width of bulliform cells	24.80± 5.322**a**	29.27 ±8.174**a**	46.97± 8.465**b**
The lenght of bulliform cells	37.88± 6.615**a**	39.99± 8.363**a**	41.22± 3.255**a**
The width of sclerenchyma cells	14.14 ±2.554**a**	16.70± 4.538**b**	17.04± 3.254**b**
The lenght of sclerenchyma cells	21.07 ±3.043**a**	23.15 ±4.284**a**	22.22 ±3.740**a**

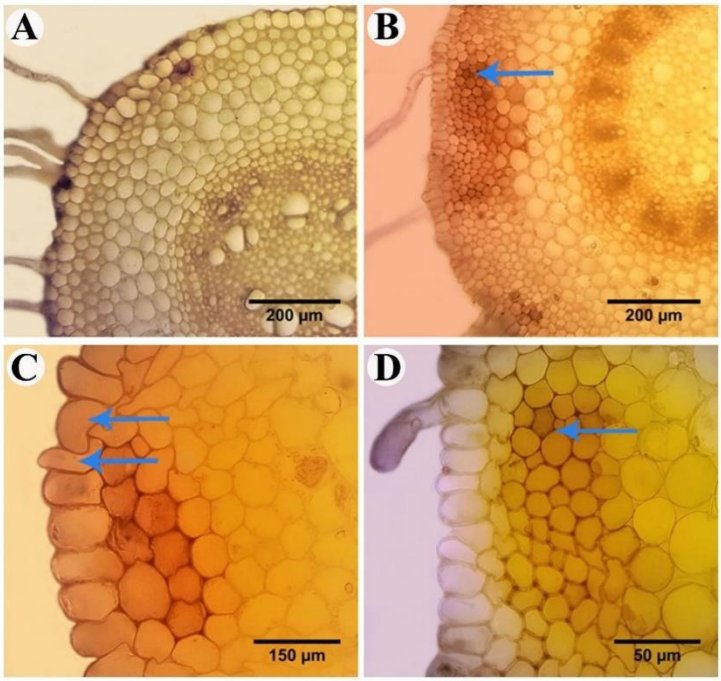

Figure 1. The light micrographs showing the transverse section of root of *T. aestivum*. A-The transverse section of root of control, B-The transverse section of root of plants treated with 0.3 mM Cr, C- The light micrograph showing the breakdown of epidermal cells of 0.4 mM Cr-treated roots, D- The root of plants treated with 0.4 mM Cr. ⟶ indicates the densely stained deposits of Cr in the root (B, D) and the breakdown of epidermal cells (C).

The plants treated with Cr was showed some specific changes on stem anatomy (Figs. 2A-B). In the present study, it was observed that Cr in stem cells especially accumulates along with vascular bundles (Fig. 2B). These depositions can be suggested as being possible mechanism of detoxification and adaptation in stem (Sridhar et al., 2011). Similar observations were supported by findings of Ambo-Rappe et al. (2011). The anatomical changes in the stem treated with Cr resulted with an increase in the diameter of parenchyma cells compared with the control group (Table 2). The changes in shape and size of cortical parenchyma cells suggest heavy metals may disrupt the hormonal balance in the stem (Gomes, 2011). Al-Saadi et al. (2013) reported that some *Potamogeton* L. species exposed to metal treatments of Ag and Cu had widened intercellular spaces in the cortical parenchyma. The accumulation of heavy metal in intercellular spaces of these cells could be a plant strategy to tolerate harmful levels of heavy metal of accumulator species (Al-Saadi et al., 2013). However, the thickness of sclerenchyma and the diameter of vessel elements in stem exposed to Cr treatment presented thinner than the control (Table 2). The decrease in the diameter of vessel elements in the stem may be a result of response to heavy metals (Al-Saadi et al., 2013). The reduction in the number of vessel elements of the xylem has also been reported by previous studies (Barnabas, 1996; Sridhar et al., 2011).

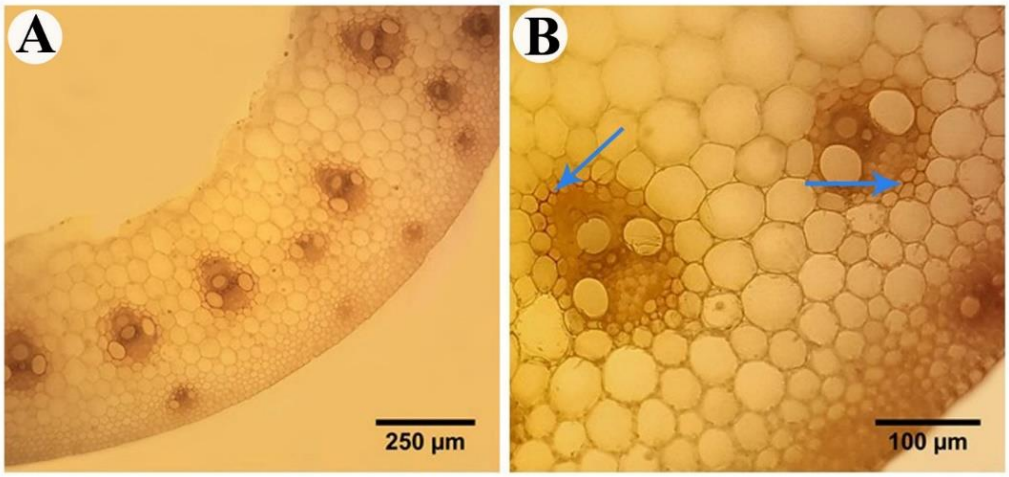

Figure 2. The transverse sections of stem of *T. aestivum.* A-The transverse section of stem of control, B-The transverse section of stem of plants treated with 0.4 mM Cr. ⟶ indicates the densely stained depositions of Cr along with vascular bundles in the stem.

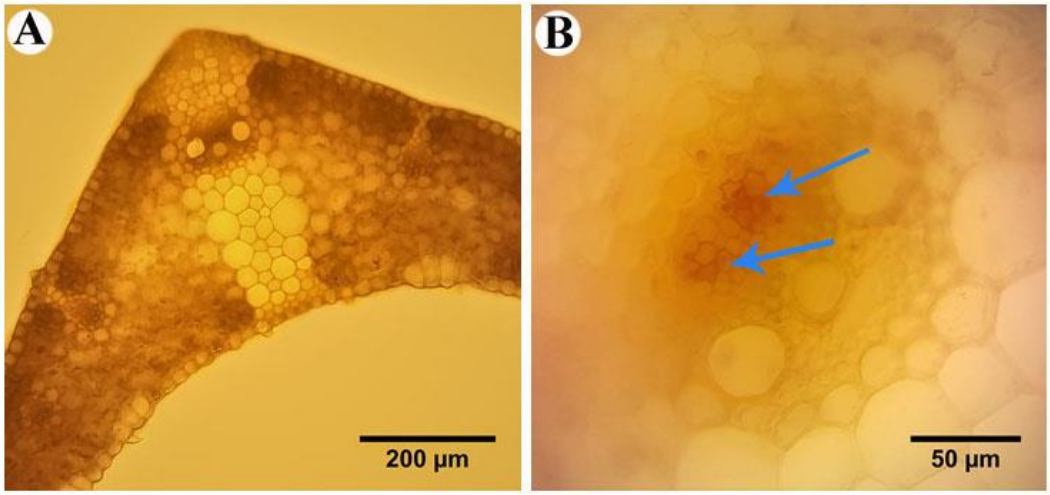

Figure 3. The transverse sections of leaf of *T. aestivum.* A-The transverse section of leaf of control, B-The transverse section of leaf of plants treated with 0.4 mM Cr. ⟶ indicates intensely coloured depositions of Cr in vascular bundles of leaves.

Light micrographs of Cr-treated leaves showed intensely coloured areas in vascular bundle of midrib compared to control leaves (Figs. 3A-B). Similar results were reported by Sridhar et al. (2011) and Al-Saadi et al. (2013) where the heavy metal accumulation predominantly found in the vascular bundles of stem. It was also reported that heavy metals caused to detoration in shape of vascular bundles in stem (Al-Saadi et al., 2013). Exposure to high Cr concentrations was lead to a reduction of mesophyll thickness. The mesophyll thickness of control 208.17±8.461 µm, while it was decreased to 154.29±9.371 µm in 0.3 mmol Cr concentration (Table 3). Sridhar et al. (2011) determined that Cr accumulation resulted a decreasing in leaf thickness and shrinkage of epidermal, spongy and palisade parenchyma cells in brake fern (*Pteris vittata* L.). Al-Saadi et al. (2013) reported that exposure to heavy metals caused a reduction in the blade thickness in some *Potamogeton* plants. These decrease in leaf thickness was mainly the result of Cr-induced water stress or Cr accumulation in leaves or the combination of both (Sridhar et al., 2011). In addition to, the thickness of phloem end xylem and the diameter of vessel elements were decreased compared with the control group (Table 3). A decrease in vascular bundle, metaxylem and phloem areas has also reported in *Sorghum bicolor* (L.) Moench. and *Brachiaria decumbens* Staph. due to Cd stress. Earlier it was shown that xylem and phloem deformation presented in stem sections treated with heavy metals of *Bruguiera sexangula* (Lour.) Poir (Gupta and Chakrabarti, 2013). The decreased xylem and phloem thickness could be related to low translocation effeciency of Cr and preventing their translocation to photosynthetic tissues (Gomes et al., 2011).

Conclusion

The anatomical changes in the root, stems and leaves of Cr-treated plants indicated that Cr has a significant impact on the anatomy of *T. aestivum* cv. Ekiz. The remarkable reduction in cell sizes of root, stem and leaf may be due to growth under heavy metal stressed. As a consequence, plant tissues exposed to Cr showed some spesific anatomical changes to survive in polluted environment. Results obtained from this study will help us for better understanding of effects of heavy metals to different vegetative organs.

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