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Full Length Article

Application of Fulvic Acid Modulates Photosynthetic Pigments and Malondialdehyde Content in Bread Wheat (*Triticum aestivum* L. cv. Ekiz) to Increase Resistance to Chromium Stress

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Abstract

This study investigated the effects of fulvic acid (FA) on photosynthetic pigments, carotenoids and malondialdehyde content in bread wheat (cv. Ekiz) under chromium (Cr) stress. Wheat seedlings were divided into two groups except control including 0.10, 0.20, 0.30 and 0.50 m*M* Cr solution applied and second group included Cr solution and 1.5 mg/L FA at the same concentration. The FA was sprayed to wheat plants. The highest Cr-accumulation was found in the roots compared to other parts of the plant. Chlorophyll *a*, *b*, total chlorophyll and carotenoid contents decreased and this reduction was higher in only Cr treated plants than with FA+Cr. In contrast, the chlorophyll *a/b* ratio and malondialdehyde (MDA) content increased depending on the Cr stress. With FA+Cr application, the increase in only Cr-treated plants was higher. The FA application showed positive effects on chlorophyll pigments and carotenoids in preventing Cr stress in wheat seedlings and to reduce the detrimental effects of Cr. © 2020 Friends Science Publishers

Keywords: Carotenoid; Chlorophyll; Heavy metal; Malondialdehyde; Photosynthetic pigment

Introduction

In recent years, rapid industrialization, anthropogenic activities, modern agricultural practices and urbanization has led to an increase in the threshold levels of various heavy metals in soil and aquatic environments. Thus, negative effects are observed on living forms (Sohail *et al.*, 2016; Shahid *et al.*, 2017). Chromium (Cr) is one of the most used heavy metals in industrial activities and the main areas of Cr application are industrial leather processing and finishing, refractory steel production, drilling muds, electroplating cleaning agents, catalytic manufacture and production of chromic acid and specialty chemicals. As a result of industrial activities, Cr compounds released into the environment through solid, liquid and gas wastes showed significant negative biological effects (Shankar *et al.*, 2005). Under Cr and physico-chemical stress, plant development reduced and different structural changes occur according to plant species. These negative conditions affect the nutrient uptake of plants, the roots are damaged and the plants eventually die (Ali *et al.*, 2011a; 2011b; Gill *et al.*, 2015; Ertani *et al.*, 2017).

Chlorophylls are the most important pigment subgroup containing tetrapyrrole and found in chloroplasts of higher plants and of most algae. Chlorophyll *a* and *b* are found in higher plants, ferns, mosses and green algae. Other types of chlorophyll are present in algae and bacteria. Chlorophylls carry out the most important task in the process of photosynthesis (Delgado-Vargas *et al.*, 2000).

Likely, carotenoids are the most widespread within pigments produced by photosynthetic and non-photosynthetic organisms such as higher plants, algae, fungi, bacteria and other animals (Delgado-Vargas *et al.*, 2000). Krinsky (1994) reported that carotenoids have a functional role in photosynthetic organisms such as plants. Because these compounds can transfer energy in photosynthesis and photoprotection. It was also determined that this function can protect cells and tissues from cellular damage in plants and microorganisms (Rock, 1997).

Lipid peroxidation and malondialdehyde (MDA) causes membrane damage formed by the decomposition of polyunsaturated fatty acids of biomembranes. Heavy metal toxicity is one of the main causes of MDA formation (Weber *et al.*, 2004; Sajedi *et al.*, 2011; Osuala, 2012; King *et al.*, 2012). Reactive oxygen species (ROS) are important signal molecules produced as a result of biotic and abiotic stresses. When produced high concentration, it causes disruption of macromolecules such as lipids, proteins and nucleic acids with important functions in the cell. The MDA content increases due to lipid peroxidation during oxidative stress (Bailly *et al.*, 1996; Kranner *et al.*, 2010).

Organic acids are formed by decomposition of plants in soil (Morales *et al.*, 2012) which ingenerate from fulvic acid (FA) and humic acid (HA). These organic acids are called as humic substances and constitutes 60 to 70% of total organic matter. FA has a lower molecular weight than HA, however, former has more oxygen and carbon-poor functional groups (Schnitzer and Khan, 1972; Weng *et al.*, 2006). It is known that FA increases nutrient uptake from soil and resistance to drought in plants. It has shown significant effects in reducing fertilizer usage and stabilizing soil pH (Aiken *et al.*, 1985).With the application of FA to the soil by spraying, seed germination is increased. The application of FA to the leaves increased the seedling growth and the root weight of the wheat plants (Katkat *et al.*, 2009).

Bread wheat (*Triticum aestivum* L.) is the best adapted species in the world supplying the energy and protein needs of a significant part of the world's population (Ulukan, 2008). Approximately 95% of wheat is produced as bread wheat and grown widely in 67% of crop areas in temperate Mediterranean and subtropical regions all over the world. It is basic foodstuff for 40% of the world's population (Peng *et al.*, 2011). About 50% of the cultivated area is under cereals cultivation and 70% of this is wheat in Turkey. Wheat varieties with different properties are grown widely in Turkey (Güleç *et al.*, 2010). It is also known that wheat plants are more sensitive to Cr stress than other crops (Dey *et al.*, 2009; Diwan *et al.*, 2012). With the increase in the world population, wheat requirement is also increasing significantly. To meet this demand, wheat varieties with best tolerance to toxic metals, biotic and abiotic environmental stresses should be cultivated (Ali *et al.*, 2015a).

Therefore, the present study was conducted with objective to identify the effects of FA in root, stem and leaf of *Triticum aestivum* (cv. Ekiz) seedlings against Cr stress application. In addition, effects of FA on photosynthetic pigments and MDA content against stress caused by Cr metal were also determined.

Materials and Methods

Plant Materials, Experimental Design and Treatments

In this study, *Triticum aestivum* L. (cv. Ekiz) grown as bread wheat in the province of Amasya was selected as plant material. After germination, wheat seedlings were transferred to plastic pots containing agricultural soil and sand (1:1). The wheat seedlings were grown in a growth chamber with a photoperiod of 16 h light/8 h dark with light intensity of 200 μmol m^{-2} s^{-1}. Apart from the control, wheat seedlings were divided into two groups each applied with 0.10, 0.20, 0.30 and 0.50 m*M* Cr. Only 1.5 mg/L FA was sprayed to seedlings. Hoagland’s nutrient solution was applied to all plants (Ali *et al.*, 2015a).

Determination of Chromium Contents

Harvested wheat plants were washed with distilled water for total Cr accumulation. Wheat plants were dried in an oven at 105°C for 24 h, then the root, stem and leaves were separated. These samples were grinded and kept for Cr analysis. 500 mg (root, stem and leaf) of dried samples were weighed and transferred to pyrex tubes. Dried plant tissues were digested for 65% HNO_3 (7.5 mL) and 36% HCl (2.5 mL) at 25°C for 12 h. Then the samples were heated at 105°C in the incubator for 2 h. Atomic absorption spectroscopy with a Thermo scientific ice 3000 series was used for determination of Cr contents. (Novoa-Munoz *et al.*, 2008; Lamhamdi *et al.*, 2013).

Measurement of Photosynthetic Pigments

After four weeks of application to wheat seedlings, chlorophyll and carotenoid contents were determined. The uppermost leaves of wheat seedlings were used for pigment contents. 200 mg leaf pieces were homogenized in 96% acetone. The homogenate was measured spectrophotometrically after filtration the Chlorophyll *a*, *b* and carotenoids contents were measured using a UV visible spectrophotometer at 645, 652 and 470 nm wavelengths. The following equations are used for calculations (Lichtenthaler and Wellburn, 1983). A solution of 96% acetone was used as a blank.

Chlorophyll *a* $=(11.75XA_{662}-2.35XA_{645})X20/$ mg fresh leaf weight

Chlorophyll *b* $=(18.61XA_{645}-3.96XA_{662})X20/$mg fresh leaf weight

Total chlorophyll$=A_{652}X27.8X20/$mg fresh leaf weight

Total carotenoid$=(1000XA_{470}-2.27XKl$ $a-81.4XKl$ $b/227)X20/$ mg fresh leaf weight.

Malondialdehyde (MDA) Content Determination

The lipid peroxidation was measured by a procedure based on the method of Heath and Packer (1968). 500 mg of fresh leaf pieces were homogenized in 1.5 mL of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 g for 15 min. 2 mL of the supernatant was then added to 4 mL of 0.5% (w/v) 2-thiobarbituric acid (TBA) in 20% (w/v) TCA). The mixture was heated at 90°C for 30 min, then quickly cooled in an ice-bath and centrifuged at 15000 g for 15 min. Absorbance of the aqueous phase at 450, 532 and 600 nm were measured, respectively. The concentration of MDA was calculated using 155 mM^{-1} cm^{-1} as the coefficient of absorbance.

Concentration (μmol L^{-1}) $= 6.45\times(A_{532}-A_{600})-0.56\times A_{450}$

Statistical Analysis

All data were subjected to one-way ANOVA and analysis of variance was done by using the statistical package programme SPSS version 10.0. Unless, differences were considered statistically significant when $p < 0.05$ and checked with Tukey's multiple comparison test. Data presented are the means of three replicates.

Results

Cr Accumulation in Root, Stem and Leaf

Cr were not detected in control plants and 0.10 m*M* concentration in the root. Cr accumulation increased with increase in Cr concentration in root and its accumulation was higher only in Cr treated plants compared to the plants treated with FA (Fig. 1). In the stem, Cr was not detected in the control and 0.10 m*M* concentration plants. With the increase in Cr concentration, the accumulation of Cr in the root increased at 0.20, 0.30 and 0.50 m*M* concentrations and higher accumulation was observed in Cr treated plants compared to the FA treated plants (Fig. 2). In the leaf, no Cr was found in control and at 0.10, 0.20 m*M* Cr concentrations. In Cr treated plants, its accumulation was higher as compared to plants applied with FA (Fig. 3). According to results, most of Cr was accumulated in the root and stem and leaf similar concentration was found.

Changes in Pigment and Carotenoid Content

Due to the increase in Cr concentration, chlorophyll *a* content decreased in both FA and Cr treated plants. Chlorophyll *a* content decreased in plants containing 0.30 and 0.50 m*M* Cr. The chlorophyll *a* contents was lower in the Cr treated plants compared with FA treatments (Fig. 4). Chlorophyll *b* content also decreased in all plants compared to control due to increased Cr concentrations and were lower at the 0.30 and 0.50 m*M* Cr concentrations. The reduction in chlorophyll *b* content was more reduced in Cr treated plants compared to the plants treated with FA (Fig. 5). Chlorophyll *a/b* ratio increased in all plants compared to the control with low ratio. The highest increase in chlorophyll *a/b* ratio was observed at 0.50 m*M* Cr concentration. The increase in only Cr treated plants was higher than FA treated plants (Fig. 6). Total chlorophyll content decreased in all plants compared to the control plants due to the increase in Cr treated plants. Total chlorophyll content at the 0.30 and 0.50 m*M* Cr concentrations was more less than the other plants. In the only Cr treated plants, the total chlorophyll content was more less in all plants compared to the FA treated plants (Fig. 7).

Total carotenoid content characteristics behaved similar to total chlorophyll content. However, total carotenoid content decreased in plants treated with 0.30 and 0.50 m*M* Cr. In addition, the total carotenoid content decreased only Cr treated plants compared to the plants treated with FA (Fig. 8).

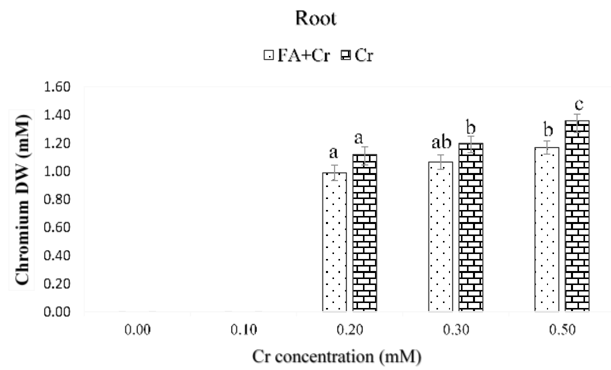

Fig. 1: Cr concentrations in root of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at p<0.05

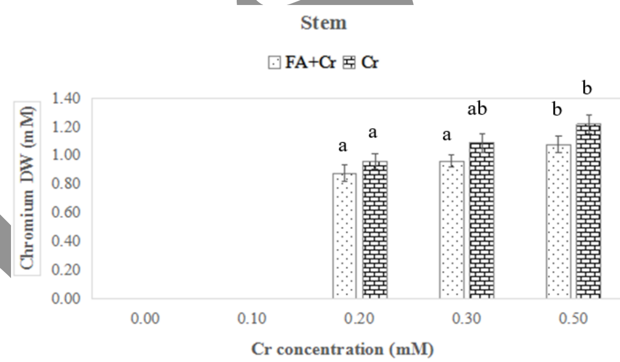

Fig. 2: Cr concentrations in stem of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at p<0.05

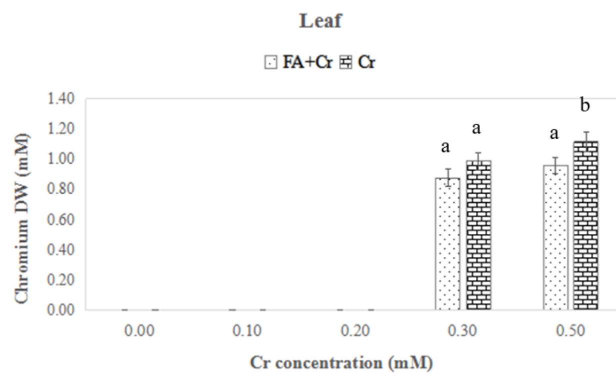

Fig. 3: Cr concentrations in leaf of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at p<0.05

According to results of the present study, the MDA contents showed response similar to the chlorophyll *a/b* ratio while MDA content increased with increasing Cr concentrations. The MDA content was higher in only Cr treated plants compared to the plants applied with FA (Fig. 9).

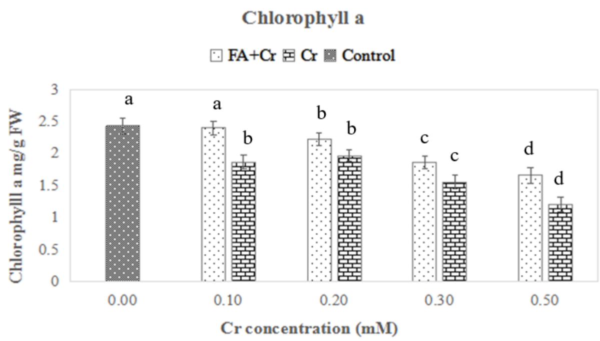

Fig. 4: Chlorophyll a contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

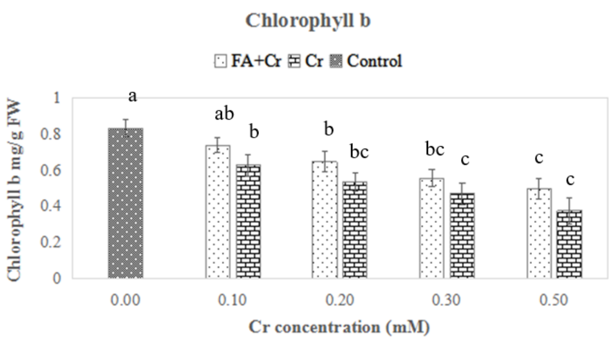

Fig. 5: Chlorophyll b contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

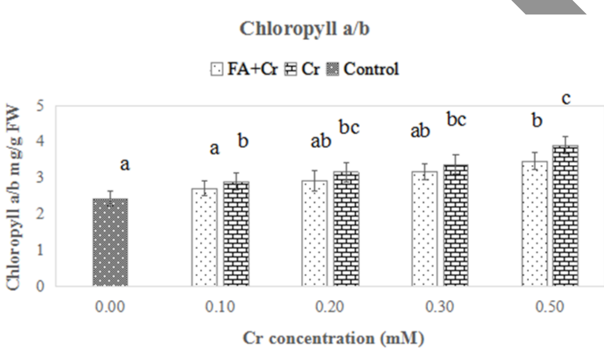

Fig. 6: Chlorophyll a/b contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

Discussion

In this study, Cr was not detected in the roots of the control and 0.10 m*M* concentration. The accumulation of Cr, in the roots wheat plants was higher than the other parts of plants and was higher in only Cr treated plants compared to FA application (Fig. 1). Some researchers have reported that Cr and Cd more accumulated in the roots of wheat plants

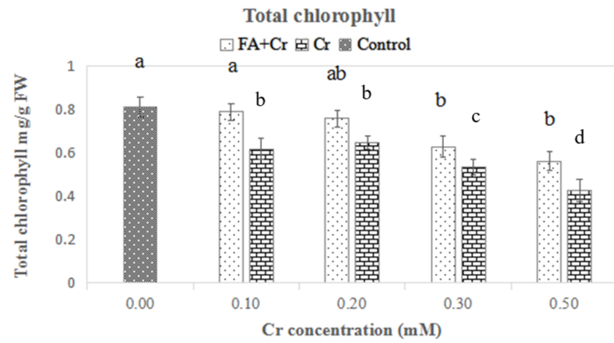

Fig. 7: Total chlorophyll contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

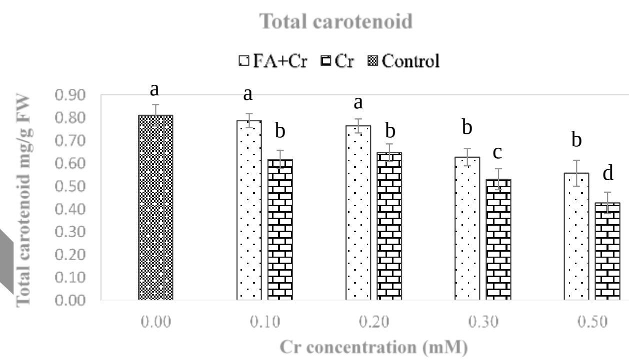

Fig. 8: Total carotenoid contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

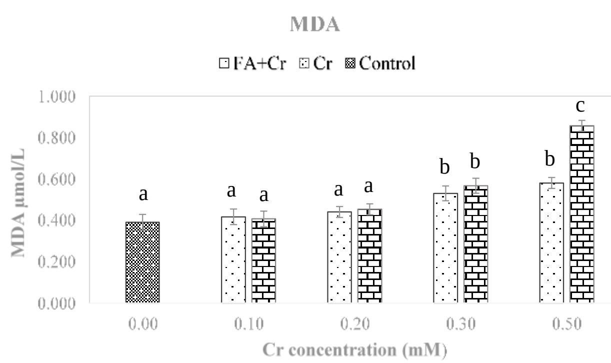

Fig. 9: MDA contents of wheat plants exposed to various Cr concentrations with and without applied FA. Bars represent SD of three replicates. Different letters on the bars indicate significant differences among the treatments at $p<0.05$

(Subrahmanyam, 2008; Ali *et al.*, 2015a; Ali *et al.*, 2015b; Akcin *et al.*, 2018; Hussain *et al.*, 2018). Also depending on the application of Cu, Cd and Cr to wheat plants at increasing concentrations, these metals have accumulated more in the roots of the plants (Rizvi and Khan, 2017).

Similarly, Liu *et al.* (2009) determined that the highest accumulation of Cd, Cr, Pb, As and Hg was found in roots, compared to other parts of wheat plants. Furthermore, Cr accumulates along the exodermis cells in the root of the wheat plants increased cortex thickness and led to deterioration of epidermal cells in roots (Akcin *et al.,* 2018). With the increase of Cr sequestration in the roots, Cr precipitates in the form insoluble salts (Ali *et al.,* 2015b) and Shahid *et al.* (2013) reported this occurrence by immobilizing Cr by other molecules such as cellulose, hemicelluloses, pectins and sugars.

In present study, the pigment contents obtained in FA+Cr and only Cr treated were compared with the control. Total carotenoid, total chlorophyll, chlorophyll *a* and *b* contents decreased compared to the control treatments with Cr. This decrease was higher in FA+Cr treated plants than in only Cr treatments. Heavy metals such as mercury (Hg), copper (Cu) and cadmium (Cd) applied to the bean plant decreased the chlorophyll *a* concentration compared to the control (Zengin and Munzuroğlu, 2005). Depending on the Cr application, the chlorophyll *a* and *b* values of wheat and spinach plants were lower than the control (Sharma *et al.,* 1995). In a similar study, Lamhamdi *et al.* (2013) reported that chlorophyll *a*, *b* and total chlorophyll values were lower in wheat and spinach plants after Pb application. In addition, the total chlorophyll content of wheat plants decreased with increasing Cr application (Ali *et al.*, 2015a). The decrease in photosynthetic pigment contents with Cr stress is thought to be due to the increase in chlorophyllase activity leading to the deterioration of chlorophyll (Hegedus *et al.,* 2001; Gill *et al.,* 2015). As a result of heavy metals, ROS may cause a decrease in chlorophyll levels (Ehsan *et al.,* 2014). One of the most sensitive indicators of the toxicity of metals in plants is thought to be the change in total chlorophyll content (Sinha *et al.,* 2005). The carotenoid contents in rice seedlings decreased in Cd, Pb and Cd+Pb applications compared to controls (Srivastava *et al.,* 2014). It has been determined that Cd, Cu and Zn metals in wheat plants are decreased in dose-dependent experiments compared to control (Ciobanu *et al.,* 2017). Moreover, due to metal stress in wheat and other crops, carotenoid contents decreased (Ali *et al.,* 2013; Yadav and Singh, 2013). Carotenoids are antioxidant molecules, preventing the formation of ROS and lipid peroxidation (Panda and Coundhury, 2005).

In present study findings, although the content of pigments decreased with Cr stress, chlorophyll *a/b* ratio and MDA content increased compared to the control. However, this increase was more less in the FA+Cr treated plants compared to the only Cr treated plants (Fig. 6 and 9). Chlorophyll *a/b* ratio increases in plants where chlorophyll *b* decreased more than chlorophyll *a*. After application of Cr stress to *Salvinia*, cauliflower and wheat, chlorophyll *b* significantly more decreased than chlorophyll *a* (Chatterjee and Chatterjee, 2000; Nichols *et al.,* 2000).This reduction in the level of chlorophyll *b* is associated with the deterioration of proteins around the antenna complex (Shankar, 2003). The application of Cd to the wheat plants (cv. Bolal 2973) caused a significant increase in the chlorophyll *a/b* ratio. However, chlorophyll *a/b* ratio decreased at high Cd concentrations (Zengin and Munzuroğlu, 2005). Öncel *et al.* (2000) found a reduction in chlorophyll *a/b* ratio at high temperature in wheat (cv. Gerek 79). The MDA content was increased with increasing levels of Cr applied to wheat seedlings compared to the control. The increase in MDA content at 0.30 and 0.50 m*M* concentrations was more prominent. However, in the plants treated with FA+Cr, the MDA content was less than only Cr treated plants (Fig. 9). MDA content was significantly increased depending on the amount of Cr and the duration of application in wheat plants (Subrahmanyam, 2008). Mutlu *et al.* (2018) stated that MDA content increased after application of Cd stress to wheats (cv. Sönmez 2001 and cv. Quality). Cr metal caused an increase in MDA content in Albares wheat and Pedrezuela barley compared to control (González *et al.,* 2017).

The present study showed that chlorophyll pigment and carotenoid contents were increased with the application of FA in wheat plants under Cr stress (Ali *et al.* 2015a).These results agree with the findings of Shahid *et al.* (2012) that a reduction in Pb accumulation with application of FA in *V. faba* plants. Addition of humic substances to nutrient solution of gerbera plant was reported to improve Zn and Fe uptake by scaps and leaves. In addition, a reduction in Zn and Fe content scaps and leaves at the rate of 1000 mg/L. Due to the adsorption of free Cr ions to FA in living cells, metal concentration decreases and chlorophyll content increases (Nikbakht *et al.*, 2008). The content of FA in leaves may cause an increase in pigment concentration due to the decrease of ROS production. (Shahid *et al.,* 2012). In present study, this effect might be due to the reduced Cr concentration in leaves of FA applied plants. It was observed that application of FA significantly reduced the MDA contents in wheat plants under different levels of Cr. This reduction in MDA content by application of FA might be due to improved free radical scavenging (Anjum *et al.,* 2011) and reduced ROS production (Ali *et al.,* 2015a). The another possible explanation might be a reduction in membrane damage due to the adsorption of free radicals with FA (Ali *et al.,* 2018).

Conclusion

The Cr accumulation in the root, stem and leaf was measured depending on the Cr stress applied at increasing concentrations to the wheat plants (cv. Ekiz). The highest Cr accumulation in the wheat plant was determined in the root than other parts. The amount of Cr accumulated in the FA+Cr treated plants was more less than the only Cr treated plants. This result shows the positive importance of FA in preventing Cr accumulation in wheat plants. Cr produces ROS in plants. ROS causes a decrease in chlorophyll pigments and carotenoids. In contrast, the amount of MDA increases as a result of lipid peroxidation. FA, which is a macromolecule, is composed of different groups and shows solubility in water. The chlorophyll pigment contents may have increased due to the absorption of free Cr ions by FA. In addition, the decrease in ROS production caused by FA application may be another factor increasing the pigment concentration. Therefore, FA has positive effects on chlorophyll *a, b*, total chlorophyll and carotenoids in wheat plants. In addition, FA was reduced the MDA content in wheat plants. FA helps to increase the activity of antioxidant enzymes by preventing the accumulation of metal in the plants. Thus, it supports the growth and development of the plants against to metal stress. In order to maintain the existence of FA in the soil, it is necessary to contend with erosion. Burning stubble in agricultural areas causes the destruction of valuable organic acids such as FA. So, it should formulate appropriate agricultural policies to enhance the importance of FA in country.

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