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Research Paper

Protective effects of humic acid against chromium stress in wheat (Triticum aestivum L. cv. Delabrad-2)

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Abstract: Wheat (*T. aestivum*) is the world's most widely cereal crop and is a staple food for a over 50 % world's population. Soils contaminated with heavy metals may cause deleterious effects on human health. However, humic substances (humic acid and fulvic acid) might benefit plant growth by improving nutrient uptake and the activation of biomass production. Hence, the objective of the current study was to investigate the effects of humic acid (HA) on photosynthetic pigment and malondialdehyde content (MDA) against chromium stress in *Triticum aestivum* L. cv. Delabrad-2. For this purpose, four Cr treatments (0.10, 0.20, 0.30, 0.50 mM) were applied to wheat seedlings and the liquid humic acid sprayed on the leaves at 1.5 mg L^{-1} dose alone or in combination with chromium stress for 21 days. According to our results, the higher concentration of Cr was found in the leaves in comparison with roots and stems of wheat plants. The treatment with 0.6 mM Cr concentration was the most effective for wheat. Total carotenoid, total chlorophyll, chlorophyll a and b contents decreased in groups only chromium compared to HA+Cr groups depending on the increased chromium dose. However, the application of HA increased the chlorophyll a/b ratio and MDA content in plants as compared with Cr treatment alone. We conclude that HA application eliminated the toxicity of Cr stress by modulating the photosynthetic activities in wheat.

Keywords: *Wheat, Heavy metal stress, Humic acid, photosynthetic pigment,*

Introduction

In recent years, rapid grown in the agricultural and industrial sectors has also led to an increase in the levels of various heavy metals in soil and aquatic environment (Sohail et al., 2016). Soils polluted with heavy metals have threatened living organisms including plants and animals (Ali et al., 2015; Adrees et al., 2015). Chromium (Cr) is one of the 18 core hazardous air pollutants and causes serious environmental contamination in soil and groundwater (Shankar et al., 2005). In plants, Cr is found in the forms of trivalent and hexavalent and Cr (III) is relatively stable and less toxic than Cr (IV) (Chattopadyay et al., 2010; Oliveira, 2012). This toxicity of Cr(IV) includes reduced plant growth and development, the inhibition of photosynthesis and enzymatic activities, chlorosis and ultimately plant death (Gill et al., 2015; Bukhari et al., 2016).

Bioaccumulation and toxicity of Cr has been reported in various crops (Mishra et al., 1997; Singh, 2001; Shanker, 2003). It is known that Cr is toxic to most higher plants at about 0.5 to 5.0 mg mL^{-1} in nutrient solution and 5 to 100 mg g^{-1} in soil (Davies et al., 2002; Oliveira, 2012). Chromium eventually accumulates in crops from contaminated soils and is mainly retained in the root tissues(Ahmed et al., 2016; Jaison and Muthukumar, 2016). Studies also reported that Cr stress affects photosynthesis in terms of carbon assimilation, electron transport and photophosphorylation in plants (Barbosa et al., 2007; Rodriguez et al., 2012). Decrease in photosynthetic pigments by chromium can be ascribed to the inhibition of the electron transport processes and to the disorder of ultrastructure of chloroplasts (Pandey and Sharma, 2003; Shanker et al., 2005). Moreover, it was found that Cr toxicity caused the ultrastructural changes in the form of lamellar system (Ali et al., 2013). The reduction in the content of photosynthetic pigments due to Cr toxicity has been reported in many plants (Sharma and Sharma, 1996; Nichols et al, 2000; Shanker, 2003).

Exposure of plants to high levels of Cr also leads to enhanced production of reactive oxygen species (ROS) (Islam et al, 2014; Gill et al., 2015). Lipid peroxidation is the most deleterious influence caused

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by Cr and heavy metals induced ROS (Mithofer et al., 2004). Malondialdehyde (MDA) is one of the cytotoxic products of lipid peroxidation and an indicator of free radical production and tissue damage (Ohkawa et al., 1979). It was also reported that over production of ROS in plants under stress conditions can damage in selective permeability of biological membrane structure. Absorption of Cr is facilitated by a carrier membrane, the cell membrane stability are very important (Maiti et al., 2012).

Humic substances (fulvic acid and humic acid) are the main components of soil organic matter (Chen and Aviad, 1990). Humic acids (HA) are characterized as having high molecular weight and a heterogenous natural resource (Larcher, 2003). It has been reported that humic substances in the soil caused to increase the uptake of mineral elements and the weights of crop plants (Kauser et al., 1985; Chen et al., 2004). Eyheraguibel et al. (2008) showed that humic acid application increased the length and dry weight of maize plant roots. Furthermore, it was reported that humic acid leads to increased biological yield through increasing nitrogen content of the plant (Ayas and Gülser, 2005). According to Tufail et al. (2014), humic acid stimulated the growth of root and shoot of wheat plants. Studies on the effects of humic substances on plant growth showed that the promoting effects of humic acid was associated with increasing cell membrane permeability, oxygen and phosphate uptake, respiration and photosynthesis and root cell elongation (Tan, 2003; Türkmen et al., 2004). Recently, it has been reported that humic substances is used for effectively removal of heavy metals from aqueous media. (Tang et al., 2014). However, little information is available on the effects of humic acid against Cr stress. The aim of this study was to determine the effects of humic acid in terms of photosynthetic pigments and malondialdehyde content against Cr stress on wheat plants.

Materials and Methods

Plant Material and Experimental Design

The wheat seedings (*Triticum aestivum* cv. Delabrad-2), which grown as bread wheat in Amasya (Suluova), were used in this study. Germinated wheat seedlings were transferred to plastic pots each containing sand and soil and were grown under controlled conditions (light/dark regime of 16/8 h at 25 C^0, relative humidity of 70 %). The seedlings were grown for four weeks and 2.0 mg/L humic acid were treated alone or in combination with various concentrations chromium stress (0.20, 0.40 and 0.60mM).

Determination of Pigment and Malondialdehyde Content (MDA)

Carotenoid and chlorophyll contents were extracted from the uppermost leaves of wheat plants. Concentrations of chlorophyll a, chlorophyll b and carotenoids were calculated using the method of Lichtenthaler and Welburn (1983). MDA content was determined spectrophotometrically as described by Heath and Packer (1968). The absorbance of supernatant was recorded at 532, 600 and 450 nm. The MDA content was calculated by using its molar extinction coefficient of 155 mM^{-1} cm^{-1}.

Determination of Cr Contents

After washing, the samples were divided into root, stem and leaves. Then all samples are washed with 2% HCl and with tap and distilled water. The samples were dried in an oven at 105 ° C. This process was continued until a constant weight was reached. Plant samples (1/2 g dry weight) were transferred to pyrex tubes. Heavy metals were digested for (7.5 mL) 65% HNO_3 and (2.5 mL) 36% HCl at 25 °C for 12 hours. Then, the samples were heated at 105 °C in the incubator for 2 hours. Chromium contents in samples were determined by using atomic absorption spectrometry (Thermo scientific ice 3000 series) using the method by Lamhamdi et al. (2013).

Statistical Analysis

All values in results are mean of at least three replicates±standard deviation (SD). The data were analysed using SPSS version 12.0. Tukey's post-test (at a significance level of $p < 0.05$) was used to compare the treatment groups.

Results and Discussion

Effect of HA on Cr Uptake by Wheat Plants

In this study, the amount of Cr in the root, stem and leaf parts increased significantly with increasing Cr levels (Figures 1-3). The higher concentration of Cr was found in the leaves in comparison with roots and stems of wheat plants. The treatment with 0.6 mM Cr concentration was the most effective for wheat

(Figure 3). However, it was found that the Cr toxicity is more predominant in root compared to leaf and shoot in many plant species such as wheat in the previous study (Ali et al., 2013; Dotaniya et al., 2014; Gill et al., 2015). Ali et al. (2018) also reported that the accumulation of Cr in roots was significantly higher than both stem and leaves in wheat plants. In this study, higher Cr amounts in leaves might be due to fast translocation and more accumulation of Cr in leaves compared to root and stem.

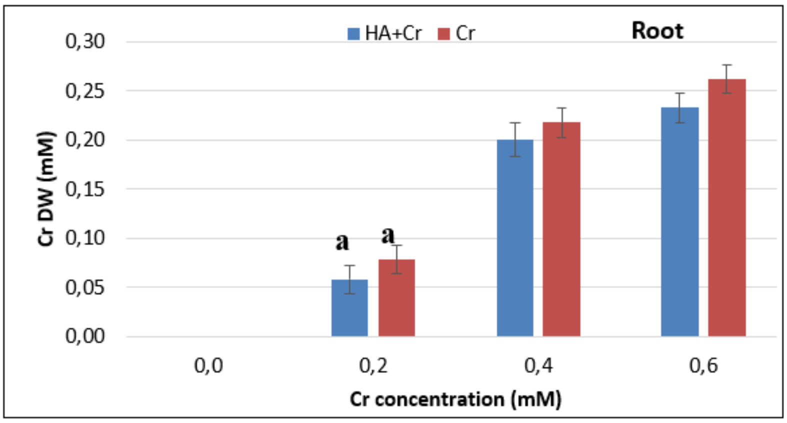

Figure 1. Effects of HA on Cr accumulation in root of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

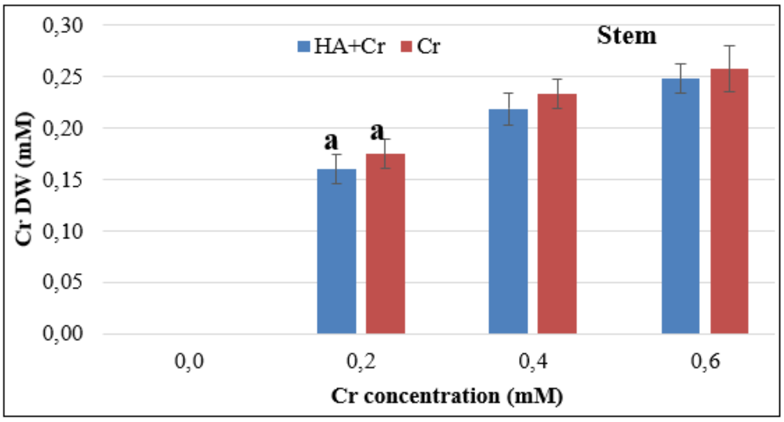

Figure 2. Effects of HA on Cr accumulation in stem of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

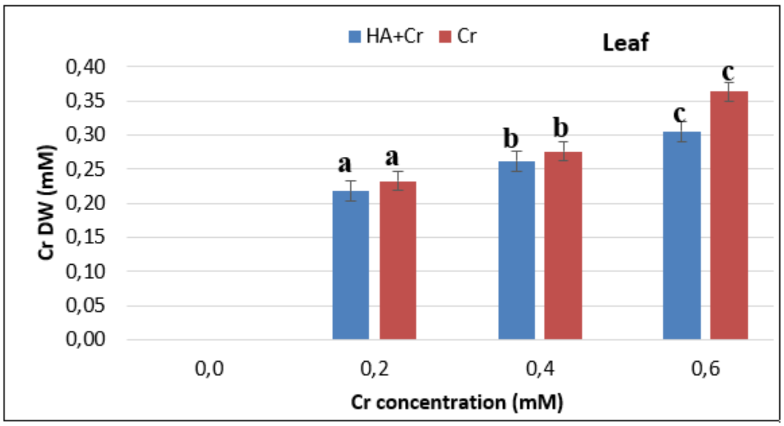

Figure 3. Effects of HA on Cr accumulation in leaves of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

Application of HA markedly ($p<0.05$) decreased Cr toxicity compared to Cr-treated groups only. (Figures 1-3). The role of humic acid in reducing heavy metal stress in soil has been reported earlier by some researchers (Harter and Naidu, 2001; Billingham, 2015). Chen and Aviad (1990) reported that humic substances have more profound effects in growth, plant height and dry weight. Similarly, root and shoot weight were increased in response to foliar application of humic acid to tomato plants (Yildirim, 2007).A remarkable reduction in Cr uptake was reported in the treatment with fulvic acid (FA) in *Triticum aestivum* L (Ali et al., 2015).Decreased Cr uptake with FA might be caused by competition between other essential nutrients and Cr in the soil (Matysiak *et al*., 2011; Ali *et al.,* 2013).

Effect of HA on Photosynthetic pigments and MDA

Total Chlorophyll, total carotenoid, chlorophyll a and chlorophyll b, concentrations were decreased with the application of Cr alone. This reduction in photosynthetic pigments was more higher especially under highest Cr stress (Figures 4- 8). Decreased chlorophyll content associated with Cr stress in various plant was also previously reported by some researchers (Sharma and Sharma, 1993; Nichols et al., 2000; Zengin and Munzuroğlu, 2006). The reduction of photosynthetic pigments induced by Cr was attributed to disorganizations in the chloroplast membranes and to the inhibition of gas exchange parameters and electron transport (Vazquez et al., 1987; Ali et al., 2011; Gill et al., 2015). Ehsan et al. (2013) was also reported that ROS generation under metal stress caused reduction in chlorophyll pigments. In earlier studies, chromium also caused a reduction in the chlorophyll concentration of wheat plants (Sharma et al., 1995; Subrahmanyam, 2008). In our study, increase in chlorophyll a/b ratio indicates that chlorophyll b is more sensitive to Cr toxicity than chlorophyll a in *Triticum aestivum* cv. Delabrad-2 (Figure 6). These findings are in agreement with the findings of Zengin and Munzuroğlu (2005) and Subrahmanyam (2008).

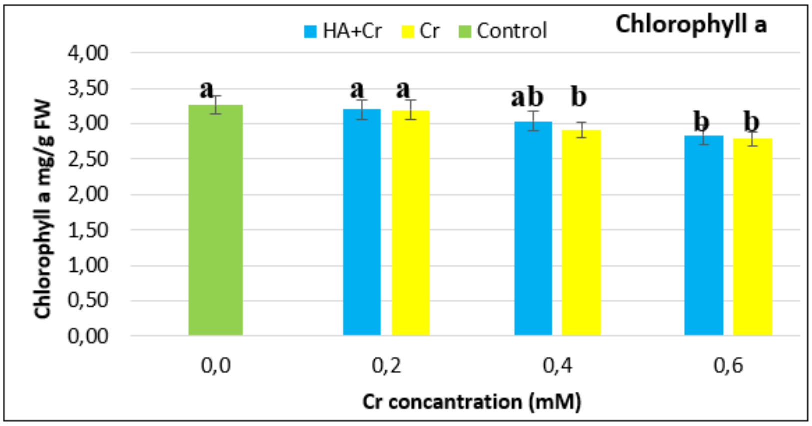

Figure 4. Effects of HA on the content of chlorophyll a of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

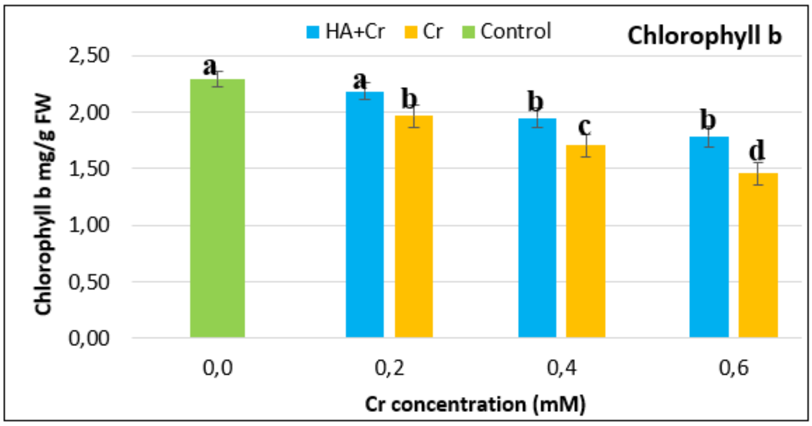

Figure 5. Effects of HA on the content of chlorophyll b of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

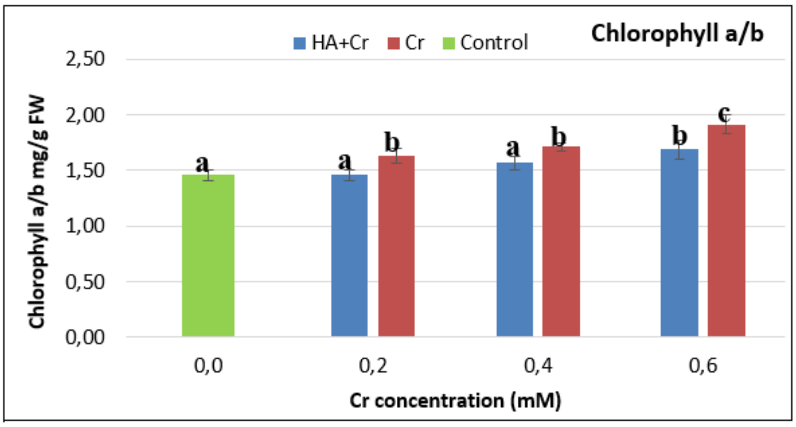

Figure 6. Effects of HA on the content of Chlorophyll a/b of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

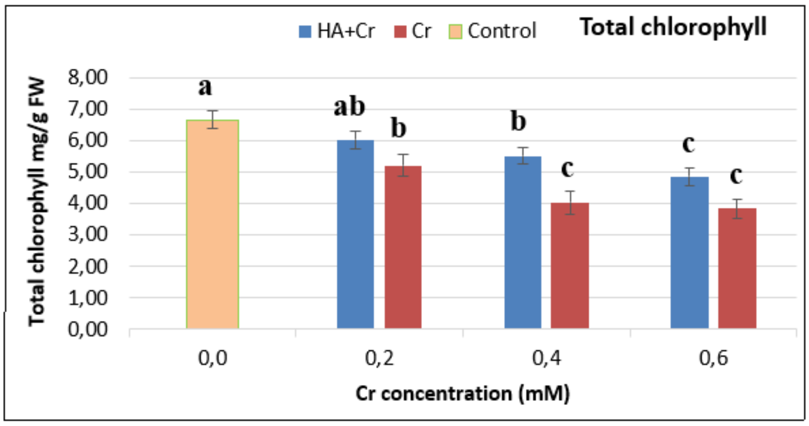

Figure 7. Effects of HA on the content of total chlorophyll of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

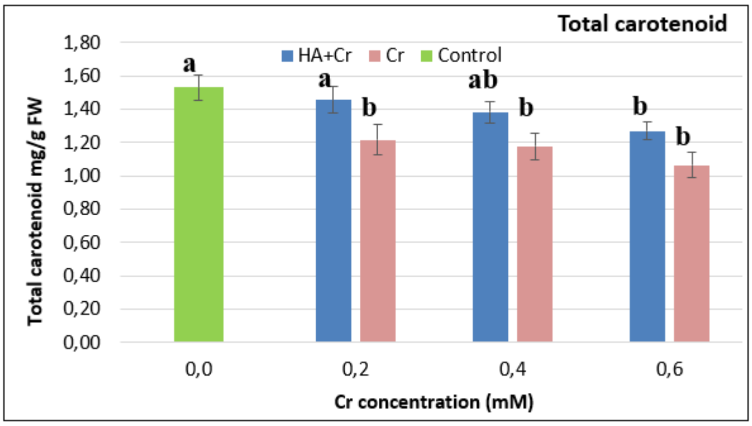

Figure 8. Effects of HA on the content of total carotenoid of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

Zhang et al. (2013) and Lotfi et al. (2015) were also reported that the HA application caused to increase chlorophyll content and photosynthesis rate in heavy metals treated plants. It was also determined that humic acid maintained water content and water uptake by cadmium stress in wheat leaves (Konakçı et al., 2018). In a similar study, humic substances caused a positive role on chlorophyll contents in wheat under Cr stress (Ali et al., 2015). It could be connected with the decrease in chlorophyll degradation and chloroplast damage (Shahid et al., 2012). In addition, absorption of free Cr ions by HA may cause an increase in chlorophyll content (Ali et al., 2015). As shown in Figures 4- 8, a significant decrease in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid pigments was observed at 0.4

and 0.6 mM Cr plus HA as compared to the control. These findings are consistent with the results of Gill et al. (2015) and Ali et al. (2015). Previous studies showed that carotenoid content decreased in wheat and other crops exposed to heavy metal stress. (Ali et al., 2013; Yadav and Singh, 2013). In our study, it was determined a reduction of carotenoid content after Cr stress application. This reduction of carotenoid may be result of a production of ROS (Ghnaya et al., 2009).

MDA is commonly an indicator of lipid peroxidation and oxidative damage to a membrane under heavy metal stress (Chaoui et al., 1997; Dhir et al., 2004). Our study showed that MDA content were significantly enhanced compared to control in wheat plants after exposure to different Cr concentrations (Figure 9). Moreover, the MDA content also increased with increasing doses of Cr. Similar results have already been reported in many earlier studies (Singh et al., 2013; Liu et al., 2014; Gonzales et al., 2017). It was also determined that MDA amounts were increased in wheat and rice under lead stress (Aziz et al., 2015). Furthermore, a similar increase in malondialdehyde content in the maize plants under heavy metal stress was reported (Rizvi and Khan, 2018). In the present study, application of HA reduced the MDA content under Cr stress in wheat seedlings. In addition, the MDA content was reduced with application of HA in wheat plants under Cr stress as compared with Cr alone. This effect might be due to reduction in membrane damage by adsorption of free radicals by humic substances (Ali *et al*., 2018).

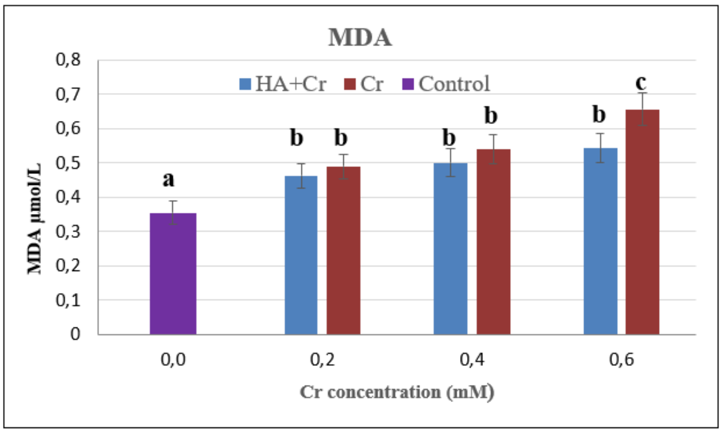

Figure 9. Effects of HA on the content of MDA of wheat plants under Cr stress. Values are means of three replicates. Different letters indicate significant difference at $p<0.05$ (Tukey's multiple range test).

Conclusion

This study shows the significant effect of HA on the amount of Cr taken by wheat seedlings. Photosynthetic pigments decreased under Cr stress. However, MDA content were significantly enhanced compared to control in wheat plants after exposure to different Cr concentrations. The HA can reduce the adverse effects of Cr by restricting its uptake and transport by wheat plants. Chromium concentration was larger in leaves and shoots of wheat plants with Cr treatments. From these observations, it was concluded that HA application can improve the Cr stress in the important crops such as wheat. In addition, further studies on the different metal types and tolerance mechanisms of HA should be performed.

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