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Effect of Hydroxybenzoic Acid Foliar Spray on Selected Wheat Varieties under Induced Heavy Metal Stress

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Abstract

This study was aimed to evaluate the effect of chromium heavy metal on the physicochemical and agronomic characters of wheat (*Triticum aestivum* L.) under hydroxybenzoic acid (HBA) foliar spray for the selected wheat varieties (Siran and Galaxy 2013). Different concentrations of chromium along with hydroxybenzoic acid (HBA) (2ppm, 2ppm+HBA, 4ppm, and 4ppm+HBA) were applied to the cultivars. Each treatment was replicated thrice in a randomized block design. The results of the present study revealed that different concentrations of chromium affected the agronomic parameters but hydroxybenzoic acid at a low concentration of Cr^{+3} enhanced the growth of the wheat (*Triticum aestivum* L.). Significant inhibition of seed germination was recorded when seeds were exposed to a higher concentration of Cr^{+3} (4ppm) as compared to T5 (untreated control). The agronomic characters were found highest in T2 (2ppm+HBA) of both varieties, a significant reduction in agronomic characters was observed in T1 (2ppm) and T3 (4ppm) because both treatments were without hydroxybenzoic acid (HBA) foliar spray. On the basis of agronomic characters, Galaxy 2013 was found a bit tolerant as compare to Siran.

Keywords: Wheat cultivars; Heavy metal toxicity; Chromium; HBA; Growth parameters

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Introduction

Triticum aestivum L. (common bread Wheat) belongs to family Poaceae, the inflorescence is in form of a distichous spike. Different varieties of the plant are cultivated from the plains to over 4000 m elevations from the sea; the grains of wheat (*Triticum aestivum* L.) are an essential ingredient of human diet worldwide. Wheat (*Triticum aestivum* L.) is the tack food of the people in Pakistan, besides that its straw is incorporated part of a daily share for the livestock [1]. Being a major staple crop, Pakistan's population derives 40 to 45% of nutrition from wheat (*Triticum aestivum* L.). Wheat (*Triticum aestivum* L.) has been the most important crop of Pakistan since the 1960s and considered the major contributor to GDP.

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term heavy metals refer to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. Heavy metals in a general collective term applied to the group of metals and metalloids with an atomic density greater than 4 g/cm^3, or 5 times or more, greater than water [2]. Common sources of heavy metal pollution include discharge from industries such as electroplating, plastics manufacturing, fertilizer producing plants and wastes left after mining and metallurgical processes [3].

Chromium (Cr^{+3}) was first discovered in the Siberian red lead ore (crocoite) in 1798 by the French chemist Prof Louis Nicolas Vauquelin. Chromium is the seventh most abundant element on earth [4]. Chromium occurs naturally by the burning of oil and coal, petroleum from a ferrous chromate refractory material, pigment oxidants, catalyst, chromium steel, fertilizers, oil well drilling, and metal plating tanneries. Chromium (Cr^{+3}) compounds are highly toxic to plants and are detrimental to their growth and development. Chromium toxicity greatly affects the biological processes in various plants such as maize, wheat (*Triticum aestivum* L.), barley, cauliflower, Citrullus and vegetables by reducing growth parameters and chlorophyll content in them [5-8].

The present study was aimed to determine the effect of Chromium on the physicochemical characteristics of the soil, agronomic characters of cultivars and role of hydroxybenzoic acid (HBA) foliar spray on the agronomic characters of cultivars under induced heavy metal stress condition.

Material and Methods

Plant material and growing conditions

The experiment was conducted in the Department of Botany, Bacha Khan University, Charsadda, during the wheat (*Triticum aestivum* L.) growing seasons. Seeds of two varieties of wheat (*Triticum aestivum* L.) were selected, including Siran and Galaxy 2013.

The seeds were sterilized in 5% Clorox for 2 minutes then washed with 50% ethanol for 3 minutes. After that, the seeds were rinsed with distilled water and sown in plastic pots containing soil and sand in a ratio of 3:1. Pots were protected from rain. 3 replicates were taken for each treatment. The plants in pots were watered as required.

Sampling

Sampling was done 5, 10 and 15 days after the start of heavy metal treatment (Cr^{+3}). The samples were stored after being weighted until further analysis. For further analysis, the samples were dried and powdered and stored in moisture free plastic bags.

Assessment and measurement of agronomic characters

Root length, shoot length, a number of leaves, leaf length and leaf width were calculated for all the replicates. Root, shoot, leaf, and soil fresh weight in grams (g) were precise for all the replicates by the help of electric balance.

Physicochemical analysis of rhizospheric soil

Soil moisture content: Soil (10) gram was taken from uniform depth i.e. 6 inches from the surfaces of pots. Dry weight was determined after drying the soil in an oven for 72 hrs at 70˚C till constant weight.

The % age moisture content of the soil is calculated by the following formula.

$$\%\text{ age moisture content} = \frac{\text{Fresh weight of soil}(g) - \text{Dry weight of soil}(g) \text{x} 100}{\text{Fresh weight of soil}}$$

Field capacity of rhizospheric soil: The field capacity of rhizospheric soil was determined following the method:

$$\%\text{ Field Capacity} = \frac{\text{Weight of wet soil}(g) - \text{Weight of dry soil}(g) \times 100}{\text{Weight of dry soil}(g)}$$

Heavy metal analysis of rhizospheric soil and plant powder: The rhizospheric soil and Plant powder were analyzed for chromium (Cr+3), samples were digested with a mixture of 5: 1: 0.1 (HNO3: H_2SO_4: $HClO_4$) to measure the concentration of Ni by AAS [9]. Method for preparation of reagents, stock solutions, working solution and standards solutions is given in the appendix.

Analysis of heavy metals in rhizospheric soil: 0.25 gm of rhizospheric soil extract and 10-20 ml of distilled water was taken in a test tube and analyzed for Cr^{+3} on atomic absorption Spectrophotometer.

Elemental analysis in selected plants: For the determination of Cr^{+3} elements, stock solutions were made. 100 ppm stock solution of the Cr^{+3} was prepared by dissolving the required amount of salts in distilled water. The availability of Cr^{+3} elements in the selected varieties of plants from Charsadda district was determined by Perchloric-acid digestion method [10].

Oven dried sample (0.25 g) was taken in 100 ml beaker and added 5 ml of mixed acid solution i.e. Nitric acid, Sulfuric acid, Perchloric acid (5:1:0.1) in it and boiled it in fume hood on hot plate till the digestion has been completed which was indicated by white fumes coming out from the flasks. Thereafter, few drops of distilled water were added and allowed to cool. Then these digested samples were transferred in 100 ml volumetric flasks and the volume was made up to 100 ml by adding distilled water in them. Then filter the extract with Whitman No 42 filter paper and filtrate were collected in labeled plastic bottles. The Concentration of these elements in the entire sample was determined by Shimadzu AA-670 Atomic Absorption Spectrophotometer.

Results and Discussion

Effect of HBA on seed germination of wheat under heavy metal stress

Wheat (*Triticum aestivum* L.) is a most essential cereal for the world population. Wheat (*Triticum aestivum* L.) at seedling germination and seedling stages are sensitive to environmental influences [11]. Chromium toxicity greatly affects the biological processes in various plants such as maize, wheat (*Triticum aestivum* L.), barley, cauliflower, Citrullus and in vegetables. Cr (VI) decreases the seedling growth, root length; shoot length, number of leaves [7]. The consequences of the present study showed significant changes in all agronomic and physicochemical measures in both the selected varieties of wheat (*Triticum aestivum* L.). All treatments showed momentous changes in agronomic characters under both control and heavy metal induce stress condition. Since seed germination is the first physiological process affected by Cr^{+3}, the ability of a seed to germinate in a medium containing Cr^{+3} would be indicative of its level of tolerance to this metal [12]. In our present work, the % germination was recorded 62% in Siran and 73% in Galaxy 2013 when plants were exposed to the higher concentration (4ppm) of Cr^{+3}. Control treatment (T5) showed the highest % germination of both verities (**Figure 1**). In previous studies % germination of wheat (*Triticum aestivum* L.) was adversely affected when a higher concentration of Cr^{+3} was applied [13,14]. Seed germination of the weed *Echinochloa colona* was reduced to 25% [13], 48% in the bush bean *Phaseolus vulgaris* [14] and 23% in *Medicago sativa* cv. Malone [12] due to the Cr^{+3} exposures. Cr inhibits the transport of sugar to the embryo and reduces seed germination [15]. Chromium elevates the Protease activity which could also contribute to the reduction in germination of chromium treated seeds [15].

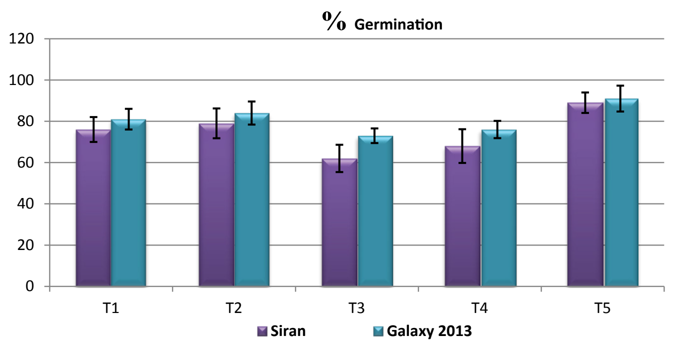

Figure 1 Effect of chromium on the % germination of wheat varieties.

Effect of HBA on the shoot of wheat varieties under heavy metal stress

In the present research work, it was clearly observed that a higher concentration of Cr decreased plant height. Shoot length, shoot fresh weight and shoot dry weight of all sample collected after 5, 10 and 15 days of treatment was decreased in treatment T1 (2ppm) and T3 (4ppm) as compared to treatment T5 (untreated control), T2 (2ppm+HBA) and T4 (4ppm+HBA) of both the varieties. Among all the samples, treatment T2 (2ppm+HBA) of galaxy 2013 showed the highest shoot length, 31.5 ± 12.587 cm (**Table 1**), indicating that hydroxybenzoic acid (HBA) can help in the expansion of shoot length and tolerance against Cr^{+3} toxicity. While among all the samples, treatment T3 (4ppm) of siran showed the least height of plant (11.8 ± 0.141 cm), indicating that a higher concentration of heavy metal cause reduction in plant height. The reduction in plant height might be due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this, Cr^{+3} transports to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction in plant height. Increase in Cr uptake reduces plant growth and development because Chromium starts interacting with the micronutrient and limits their availability to the plant [16]. Chromium affects plant growth adversely which depends upon its concentration and plant species [16,17]. Reduction in plant height due to Cr(VI) is reported in numerous plants, 11%, 22% and 41% reduction in height of oats [15], in *Curcumas sativus, Lactuca sativa* and *Panicum miliaceum* [16], *Sinapsis alba* [17], in Lucerne [18] and wheat (*Triticum aestivum* L.) cv [6,7,13,14]. Not only shoot length, but the moisture content in shoots was also reduced when treated with higher concentration of Cr. As compare to Siran, Galaxy 2013 accumulated bit more moisture content and had a fresher and dry weight of shoot. Least shoot dry and fresh weight after 15 days of treatment was recorded in Treatment T1 and T4 of Siran (**Table 1**).

1= (2ppm Cr^{+3} solution), T2= (2ppm Cr^{+3} solution+HBA), T3= (4ppm Cr^{+3} solution), T4= (4ppm Cr^{+3} solution +HBA), T5= (untreated control), 5D = (After 5 days), 10D= (After 10 days), 15D= (After 15 days).

Effect of HBA on Root of wheat varieties under heavy metal stress

The present investigation revealed that the root length, root fresh weight, and root dry weight was also adversely affected by the higher concentration of Cr^{+3} (4ppm) in all collected samples. The root length was recorded maximum in treatment T5 (untreated control) along with treatment T2 (2ppm+HBA) and T4 (4ppm+HBA), while a clear decline in root length of treatment T1 (2ppm) and T3 (4ppm) of both varieties. Treatment T2 (2ppm+HBA) of galaxy 2013 showed the maximum root length in all samples, 10 ± 3.748 cm (**Table 2**) indicating that hydroxybenzoic acid (HBA) can help in the expansion of root length and ameliorate the tolerance against Cr^{+3} toxicity. Whereas treatment T3 (4ppm) of siran had the minimum root length in all samples. The reduction in root growth could be due to the direct contact of seedlings roots with Cr^{+3} in the medium causing a collapse and subsequent inability of the roots to absorb water from the medium and inhibition of root cell division [19]. Chromium toxicity causes inhibition of root cell division and elongation or extension of the cell cycle in the roots. Also, heavy metals have been reported to impair the growth of new roots and seedling establishment [20]. In previous studies it has been reported that root length and dry weight of the important arid tree *Caesalpinia pulcherrima* was inhibited by 100ppm Cr^{+3} [21], total root weight and root length of wheat (*Triticum aestivum* L.) was also affected by 20 mg Cr(VI) kg^{-1} [5]. In another study, no or retarded root formation was observed in the Wheat plant when exposed to higher concentration of Cr [13]. Accumulation of moisture content was also inhibited by the Cr stress in both varieties. Along with reduced root length reduced fresh and dry weight was also observed in the same treatments (**Table 2**).

T1= (2ppm Cr^{+3} solution), T2= (2ppm Cr^{+3} solution+HBA), T3= (4ppm Cr^{+3} solution), T4= (4ppm Cr^{+3}solution +HBA), T5= (untreated control), 5D = (After 5 days), 10D= (After 10 days), 15D= (After 15 days).

Effect of HBA on leaf of wheat varieties under heavy metal stress

The leaf growth traits might serve as suitable bioindicators of heavy metal pollution and in the selection of resistant species. Leaf growth, area development, and total leaf number decisively determine the yield of crops. The number of leaves per plant was reduced by 50% in wheat (*Triticum aestivum* L.) when 0.5 mM Cr^{+3} was added in nutrient solution [6], our present work also highlighted that the samples collected after 5, 10 and 15 days had reduced almost 50% of leaves over control. Treatment T5 (untreated control) along with treatment T2 (2ppm+HBA) and T4 (4ppm+HBA) showed the maximum number of leaves in all samples ranges from 6 ± 0.707 to 7 ± 0 (**Table 3**), while treatment T1 (2ppm), and T3 (4ppm) showed minimum number of leaves in all samples, ranges from 3 ± 0 to 5 ± 0.707. This indicates that chromium cause reduction in a number of leaves of the plant but hydroxybenzoic acid (HBA) can help a plant to tolerate against heavy metal (Cr^{+3}) toxicity and even enhance the growth of a plant. Along with number of leaves in plant, Cr^{+3} also affect the other aspects of leaves of different plants such as Cr^{+3} effect leaf

Table 1: Effect of hydroxyl benzoic acid foliar spray on shoot length, shoot fresh weight and dry weight of wheat (*Triticum aestivum*) under inoculation of Chromium.

Treatments	Siran									Galaxy 2013								
	Shoot length			Shoot fresh weight			Shoot dry weight			Shoot length			Shoot fresh weight			Shoot dry weight		
	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D
T1	12.6 ± 4.525	14.3 ± 1.626	16.4 ± 0.354	0.115 ± 0.028	0.155 ± 0.016	0.141 ± 0.007	0.028 ± 0.005	0.030 ± 0.001	0.038 ± 0.003	12.7 ± 4.808	20.1 ± 2.828	25.5 ± 9.687	0.165 ± 0.020	0.296 ± 0.089	0.318 ± 0.134	0.038 ± 0.015	0.033 ± 0.008	0.068 ± 0.013
T2	18.4 ± 7.142	20.5 ± 5.940	22.7 ± 7.707	0.299 ± 0.183	0.261 ± 0.113	0.258 ± 0.141	0.043 ± 0.012	0.042 ± 0.006	0.050 ± 0.016	17.8 ± 1.061	22.1 ± 3.253	31.5 ± 12.58	0.221 ± 0.047	0.400 ± 0.097	0.558 ± 0.424	0.042 ± 0.016	0.043 ± 0.004	0.072 ± 0.023
T3	11.8 ± 0.141	14.7 ± 0.354	18.0 ± 2.546	0.146 ± 0.064	0.158 ± 0.028	0.211 ± 0.027	0.029 ± 0.011	0.025 ± 0.001	0.041 ± 0.011	15.6 ± 2.475	19.9 ± 3.960	26.5 ± 7.354	0.130 ± 0.012	0.267 ± 0.007	0.267 ± 0.086	0.033 ± 0.005	0.037 ± 0.001	0.053 ± 0.012
T4	12.8 ± 3.111	13.4 ± 0.071	16.9 ± 1.768	0.121 ± 0.025	0.188 ± 0.035	0.199 ± 0.010	0.026 ± 0.001	0.034 ± 0.006	0.039 ± 0.003	15.7 ± 0.636	18.0 ± 1.202	26.0 ± 4.808	0.148 ± 0.055	0.339 ± 0.071	0.443 ± 0.267	0.026 ± 0.001	0.040 ± 0.008	0.073 ± 0.030
T5	13.9 ± 0.141	18.5 ± 7.000	23.1 ± 8.273	0.143 ± 0.012	0.223 ± 0.076	0.218 ± 0.044	0.041 ± 0.004	0.032 ± 0.010	0.042 ± 0.013	15.8 ± 0.919	22.6 ± 0.919	27.4 ± 2.758	0.201 ± 0.035	0.441 ± 0.111	0.511 ± 0.075	0.038 ± 0.001	0.046 ± 0.005	0.085 ± 0.005

Table 2: Effect of hydroxyl benzoic acid foliar spray on root length, root fresh weight and dry weight of root of wheat (*Triticum aestivum*) under inoculation of Chromium.

Treatments	Siran									Galaxy 2013								
	Root length			Root Fresh weight			Root dry weight			Root length			Root Fresh weight			Root dry weight		
	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D
T1	5.9 ± 1.909	4.0 ± 1.556	4.3 ± 0.071	0.034 ± 0.006	0.027 ± 0.002	0.022 ± 0.010	0.014 ± 0.004	0.009 ± 0.001	0.012 ± 0.001	6.5 ± 3.182	4.4 ± 1.131	6.6 ± 0.141	0.060 ± 0.020	0.050 ± 0.017	0.034 ± 0.008	0.026 ± 0.020	0.010 ± 0.001	0.017 ± 0.001
T2	6.8 ± 3.784	4.9 ± 2.475	5.1 ± 1.061	0.055 ± 0.019	0.057 ± 0.034	0.035 ± 0.003	0.019 ± 0.001	0.016 ± 0.001	0.016 ± 0.001	10 ± 3.748	5.0 ± 3.182	6.9 ± 0.071	0.085 ± 0.002	0.060 ± 0.018	0.053 ± 0.033	0.036 ± 0.002	0.013 ± 0	0.022 ± 0.004
T3	4.3 ± 1.980	3.1 ± 0.495	4.6 ± 1.061	0.029 ± 0.014	0.036 ± 0.004	0.025 ± 0.006	0.012 ± 0.001	0.011 ± 0	0.013 ± 0.004	4.5 ± 2.121	4.1 ± 1.556	6.0 ± 1.556	0.032 ± 0.006	0.040 ± 0.004	0.028 ± 0.013	0.010 ± 0.001	0.009 ± 0.001	0.014 ± 0.001
T4	5.3 ± 1.273	3.3 ± 0.283	4.6 ± 0.707	0.035 ± 0.011	0.031 ± 0.009	0.028 ± 0.004	0.014 ± 0.004	0.010 ± 0.001	0.015 ± 0.001	5.8 ± 0.141	4.6 ± 1.202	6.3 ± 0.495	0.065 ± 0.039	0.050 ± 0.010	0.033 ± 0.003	0.027 ± 0.011	0.011 ± 0	0.018 ± 0.002
T5	6.0 ± 1.980	6.3 ± 3.536	5.5 ± 0.141	0.047 ± 0.044	0.059 ± 0.042	0.037 ± 0.001	0.025 ± 0.011	0.012 ± 0.003	0.017 ± 0.001	6.7 ± 2.828	5.4 ± 1.061	6.7 ± 0.566	0.075 ± 0.055	0.056 ± 0.046	0.036 ± 0.007	0.031 ± 0.022	0.012 ± 0.002	0.019 ± 0.001

Table 3: Effect of hydroxyl benzoic acid foliar spray on leaf fresh weight, dry weight of leaf and number of leaves of wheat (*Triticum aestivum*) under inoculation of Chromium.

Treatments	Siran									Galaxy 2013								
	Number of leaves			Leaf fresh weight			Leaf dry weight			Number of leaves			Leaf fresh weight			Leaf dry weight		
	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D
T1	4 ± 0.707	6 ± 0.707	4 ± 0.707	0.019 ± 0.004	0.021 ± 0.001	0.020 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.006 ± 0.002	5 ± 0.707	6 ± 0	6 ± 0.707	0.030 ± 0.008	0.058 ± 0.005	0.037 ± 0.004	0.006 ± 0.001	0.006 ± 0.002	0.009 ± 0.002
T2	5 ± 0.707	6 ± 0	7 ± 1.414	0.053 ± 0.037	0.029 ± 0.014	0.033 ± 0.017	0.006 ± 0.001	0.006 ± 0.002	0.007 ± 0.001	5 ± 1.414	6 ± 0.707	6 ± 0.707	0.041 ± 0.011	0.065 ± 0.002	0.050 ± 0.030	0.007 ± 0.001	0.008 ± 0	0.012 ± 0.004
T3	5 ± 0.707	5 ± 0	5 ± 1.414	0.027 ± 0.001	0.020 ± 0.006	0.023 ± 0.006	0.003 ± 0.001	0.003 ± 0	0.005 ± 0.001	3 ± 0	5 ± 0.707	5 ± 0.707	0.017 ± 0.004	0.039 ± 0.001	0.031 ± 0.012	0.003 ± 0.001	0.005 ± 0.001	0.007 ± 0.001
T4	5 ± 0.707	6 ± 0	6 ± 0.707	0.023 ± 0.003	0.027 ± 0.003	0.017 ± 0.008	0.004 ± 0	0.004 ± 0.001	0.004 ± 0	4 ± 0	6 ± 0	6 ± 0.707	0.024 ± 0.007	0.056 ± 0.017	0.045 ± 0.004	0.004 ± 0.001	0.006 ± 0	0.010 ± 0.004
T5	6 ± 0.707	7 ± 0.707	5 ± 0.707	0.027 ± 0.002	0.040 ± 0.016	0.028 ± 0.002	0.005 ± 0.001	0.005 ± 0.002	0.006 ± 0.001	6 ± 0	7 ± 0	7 ± 0.707	0.039 ± 0.015	0.067 ± 0.001	0.054 ± 0.025	0.006 ± 0.001	0.009 ± 0.001	0.013 ± 0.001

area and biomass of *Albizia lebbek* [22], decrease in leaf area of primary and trifoliate leaves of bush bean plant [23], dry leaf yield of bush bean plants was also decreased [24], decrease in leaf area and leaf dry weight in *Oryza sativa, Acacia holosericea*, *Leucaena leucocephala* [25], and *P. vulgaris* [26], reduced leaf size of spinach [27], affected young leaves in tomato plants [28], this sort of reduction was also observed in treatment T1 (2ppm) and T3 (4ppm) of our collected samples of leaves as well (**Figure 2**). Leave the area in T1 (2ppm), T3 (4ppm) in all samples ranges from 2.76 ± 0.208 to 5.12 ± 0.312 cm^2. As compare to other touched treatments, treatment T2 (2ppm+HBA) of galaxy 2013 showed the maximum leaf area, 6.95±0.326 cm^2, followed by treatment T4 (6.55 ± 0.291 cm^2).

T1= (2ppm Cr^{+3} solution), T2= (2ppm Cr^{+3} solution+HBA), T3= (4ppm Cr^{+3} solution), T4= (4ppm Cr^{+3} solution +HBA), T5= (untreated control), 5D = (After 5 days), 10D= (After 10 days), 15D= (After 15 days).

Effect of HBA on the accumulation of Cr in the wheat plant

Results of AAS (atomic absorption spectroscopy) indicated that the level of heavy metal in samples was kept on increasing by the increase in the concentration of Cr exogenous supply. The lowest level of heavy metal was detected in controls however a significant level of Cr was detected in all samples of rhizospheric soil and whole plant of both varieties (**Table 4**). In the present study, it was also examined that HBA foliar spray significantly inhibited the accumulation of Chromium in the whole plant as compared to unsprayed plants. The lower amount of Cr was detected in plants of treatment T2 (2ppm Cr^{+3} +HBA) and T4 (4ppm Cr^{+3} +HBA) as compared to plants without HBA foliar spray (T1 and T3). It was reported previously that GB (glycine betaine) and FA (Fulvic acid) significantly inhibited the accumulation of Chromium in different parts of the wheat plant [29,30]. However, accumulation of Cr is reported in many plant species such as rice [31], *Vicia faba* [32], barley [33-45] and *Brassica napus* [18]. In a comparison of wheat varieties, Galaxy 2013 variety of wheat accumulated more Cr in it as compared to Siran. However enhanced growth parameters were observed in Galaxy 2013, resistance to heavy metal stress could be the reason of higher biomass formation and growth.

T1= (2ppm Cr^{+3} solution), T2= (2ppm Cr^{+3} solution+HBA), T3= (4ppm Cr^{+3} solution), T4= (4ppm Cr^{+3} solution+HBA), T5= (untreated control), 5D = (After 5 days), 10D= (After 10 days), 15D= (After 15 days).

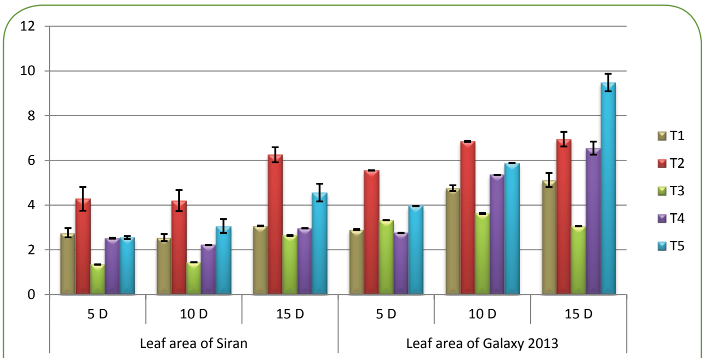

Figure 2 Effect of chromium on Leaf area of both wheat varieties.

Effect of HBA on % moisture content, vigor index and % field capacity under heavy metal stress conditions

Plant Growth Regulators (PGRs) are vastly used for modifying the growth and development of many agriculture crops. Phytohormones are the chemicals generally related to the enhancement of plant growth in minute quantity [46], hydroxybenzoic acid (HBA) was used as foliar spray and it played a vital role in amelioration of tolerance and enhancement of agronomic characters of plant against induced heavy metal (Cr^{+3}) stress such as in treatment T2 (2ppm+HBA) and T4 (4ppm+HBA). The results in all the tables indicate that the treatment T2 (2ppm+HBA) in both varieties showed the maximum values for the majority of agronomic characters among all the treatments. Treatment T2 (2ppm+HBA) showed the maximum vigor index in both varieties (**Table 5**) and was the best treatment of our research work. Maximum Vigor index was recorded in treatment T2 of Galaxy 2013 (2546.2 ± 992.778), followed by treatment T4 of the same variety. Whereas, treatment T3 (4ppm) of both varieties was the worst treatment with the lowest vigor index in all samples [47-58].

T1= (2ppm Cr^{+3} solution), T2= (2ppm Cr^{+3} solution+HBA), T3= (4ppm Cr^{+3} solution), T4= (4ppm Cr^{+3}solution +HBA), T5= (untreated control), 5D = (After 5 days), 10D= (After 10 days), 15D= (After 15 days).

Conclusion

It was concluded that varieties, Siran and Galaxy 2013 showed different level of heavy metal tolerances, all of the agronomic attributes were found to be maximum in treatment T2(2ppm+HBA) and T5(untreated control) in both varieties while treatment T1(2ppm) and T3(4ppm) were found to be adversely affected by the heavy metal. It was also concluded that exogenous supply of hydroxybenzoic acid (HBA) foliar spray played a vital role to ameliorate the stress and enhance the growth and development of cultivars against induced Cr^{+3} toxicity. Among the two cultivars, Galaxy 2013 showed better germination, seedling growth, high vigor index and high tolerance index at tested Cr concentrations as compared to cv. Siran indicating a high potential of Galaxy 2013 to grow in soils with high Cr concentration as compared to Siran. Further studies are suggested in this regard to evaluating the best method of application of the bioregulators to achieve the optimum effect and possible combined treatments of bioregulators to improve plant productivity and also find out the differences among the different cultivars at the molecular level.

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Conflict of Interest

The authors declare no conflict of interest.

Table 4: Effect of hydroxyl benzoic acid foliar spray on the accumulation of Cr^{+3} in rhizospheric soils and wheat plant.

Treatments	Siran						Galaxy 2013					
	Rhizospheric soil			Whole plant			Rhizospheric soil			Whole plant		
	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D
T1	0.922 ± 0.1283	1.368 ± 0.052	1.576 ± 0.017	1.183 ± 0.047	1.419 ± 0.032	1.564 ± 0.087	1.216 ± 0.028	1.495 ± 0.013	1.628 ± 0.131	1.495 ± 0.065	1.603 ± 0.074	1.732 ± 0.126
T2	1.061 ± 0.0612	1.394 ± 0.058	1.465 ± 0.039	0.964 ± 0.110	1.233 ± 0.126	1.416 ± 0.123	1.293 ± 0.032	1.543 ± 0.038	1.702 ± 0.031	1.294 ± 0.083	1.381 ± 0.009	1.448 ± 0.085
T3	1.061 ± 0.0439	1.481 ± 0.026	1.502 ± 0.028	1.264 ± 0.076	1.609 ± 0.094	1.776 ± 0.095	1.272 ± 0.015	1.649 ± 0.046	1.764 ± 0.093	1.513 ± 0.030	1.629 ± 0.090	1.710 ± 0.041
T4	1.097 ± 0.0225	1.467 ± 0.076	1.555 ± 0.077	1.106 ± 0.053	1.371 ± 0.029	1.451 ± 0.108	1.350 ± 0.097	1.568 ± 0.115	1.724 ± 0.041	1.387 ± 0.080	1.447 ± 0.153	1.566 ± 0.072
T5	0.176 ± 0.082	0.312 ± 0.037	0.399 ± 0.041	0.418 ± 0.068	0.584 ± 0.059	0.626 ± 0.110	0.432 ± 0.072	0.521 ± 0.053	0.589 ± 0.021	0.500 ± 0.013	0.563 ± 0.072	0.633 ± 0.076

Table 5: Effect of hydroxyl benzoic acid foliar spray on soil moisture, field capacity and %germination of wheat (*Triticum aestivum*) under inoculation of Chromium.

Treatments	Siran									Galaxy 2013								
	% Moisture content			Vigor Index			% Field capacity			% Moisture content			Vigor Index			% Field capacity		
	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D	5D	10D	15D
T1	12.27 ± 1.91	10.66 ± 1.65	7.650 ± 0.04	961.0 ± 625.1	699.2 ± 344.0	834 ± 31.90	13.986 ± 2.49	11.93 ± 2.07	8.284 ± 0.05	10.89 ± 0.35	10.40 ± 7.65	9.680 ± 6.08	1152.2 ± 337.7	1080.5 ± 422.2	1943.5 ± 312.0	12.221 ± 0.44	11.607 ± 9.575	7.273 ± 2.27
T2	12.83 ± 2.34	14.08 ± 6.43	10.20 ± 5.50	1646.9 ± 1403.720	1281.3 ± 954.509	1270.9 ± 396.263	14.982 ± 9.021	16.387 ± 8.741	11.359 ± 6.835	18.35 ± 9.758	14.86 ± 12.756	11.43 ± 8.846	1364.9 ± 263.553	1374.8 ± 1037.071	2546.2 ± 992.778	22.474 ± 14.742	17.454 ± 17.797	12.905 ± 11.33
T3	7.800 ± 2.390	7.570 ± 0.997	10.07 ± 0.686	697.4 ± 464.654	496.5 ± 42.681	937.6 ± 240.558	8.460 ± 2.812	8.190 ± 1.167	11.198 ± 0.848	8.160 ± 0.495	5.980 ± 0.509	6.240 ± 0.035	838.8 ± 351.291	1016.0 ± 566.308	1937.5 ± 877.718	8.885 ± 0.587	6.360 ± 0.576	6.655 ± 0.040
T4	13.03 ± 6.802	8.180 ± 1.909	11.10 ± 1.082	749.4 ± 171.233	537.2 ± 99.957	999 ± 368.092	13.417 ± 3.012	8.909 ± 2.265	11.235 ± 1.339	9.990 ± 0.580	7.860 ± 2.234	6.780 ± 1.980	1088.7 ± 17.734	1073.9 ± 299.870	2125.7 ± 436.483	16.347 ± 9.450	8.530 ± 2.633	10.951 ± 1.306
T5	13.74 ± 8.683	19.76 ± 4.349	11.62 ± 6.703	977.4 ± 328.380	1543.3 ± 1311.994	1443.4 ± 793.459	15.929 ± 11.729	24.626 ± 6.765	13.148 ± 8.607	14.05 ± 6.958	21.06 ± 11.816	9.870 ± 1.061	1353.6 ± 595.667	1224.6 ± 275.093	2155.6 ± 771.142	11.099 ± 3.187	26.678 ± 19.179	10.717 ± 7.481

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