Chromium (VI) Induced Physiological and Metabolic Responses in *Vigna Mungo L.* cv. BVN-3

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Research Article

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Abstract

The present study investigates the chromium (VI) induced phytoxicity and accumulation in the black gram (Vigna mungo L. cv. BVN-3) grown under refined sand pot culture. The phytotoxicity assessed with reference to growth behavior, water transport, metabolic alteration, yield, uptake and translocation of S, P, Fe and Cr under chromium (VI) stress. The black gram plants were treated with varied chromium (VI) at 0.00, 0.05, 0.10 and 0.25 mM concentration. After 5 d of Cr (VI) treatment, the foliar toxicity symptoms showed as loss of turgor and chlorosis of older leaves which also reflect in middle aged leaves later on at higher concentration of chromium (0.25 mM). At the later stage, chlorosis symptoms became critical and distorted to necrosis in patches with tapered lamina, thin tendrils and loosed coiling property. Cr (VI) induced toxicity observed on black gram as decreased growth and yield, impairment in photosynthesis activity, inhibition of metabolic and enzymatic activities and nutrient imbalances. Excess (0.25 mM) of Cr (VI) also caused a reduction in uptake and accumulation of iron in the leaves as compared to control (from 426.2 to 198.7 μg g^{-1} dw) with more uptake and accumulation of sulphur and phosphorus. Higher accumulation of Cr was recorded in the leaves (166.5 μg g^{-1} dw) followed by roots (123.4 μg g^{-1} dw) and stems (46.6 μg g^{-1} dw) at 0.25 mM after 29 d of treatment. Therefore, consumption of Cr containing black gram may have human health concern due to toxic Cr accumulation and nutrition imbalances.

Introduction

Despite multifarious industrial uses, chromium, the seventh most abundant metal on the earth's crust (Wakeel et al. 2020) is extensively established as a toxic metal. Extensive industrial uses of chromium encompass leather processing and finishing (Nriagu 1988), production of refractory steel, dyes and pigments, drilling mud’s, electroplating cleaning agents, catalyst and its ingredients, manufacture of chromic acid and expertise chemicals and many more. The critical level of accessible Cr (III) and Cr (VI) has been reported in the soil (Pratt 1966). Among the various forms of chromium with oxidation states (-2 to +6), the hexavalent chromate [Cr (VI)] and trivalent chromite [Cr (III)] are more predominant forms in the environment (Ashraf et al. 2017). Both the forms of chromium; Cr (III) and Cr (VI) differ in terms of mobility, bioavailability and toxicity.

About 2000-3200 tons of chromium discharged annually into the environment only from the tanning industry in India (Zayed and Terry 2003). Chromium content in leather tannery effluent generally ranges between 2000-5000 mg ml^{-1} in developing countries similar to India; the well-organized management of effluent discharge by the tanneries has become an alarming task. Hence, contamination of water resources through chromium containing wastewater is also posing serious health hazards to human beings and animals (Vajpayee et al. 2001). Chromium accumulates in the crops growing in contaminated sites and cause health hazards in the humans through Cr contaminated food (Broadway et al. 2010; Tiwari et al. 2009). As chromium is not a essential for plants growth and metabolism its uptake mechanisms in plants is not clear. The phytotoxicity of Cr depend on the oxidation states, bioavailability, uptake, translocation and bioaccumulation. Similarly, plants translocate and accumulate Cr with varying

capacity depend on the species of plants, oxidation state, and the background concentration (Dube et al. 2003; Tiwari et al. 2009).

Many investigators included Tiwari et al. (2013); Tiwari et al. (2009); Dube et al. (2003); Vajpayee et al. (2001) have been investigated that the effects of Cr in relation to phytotoxicity on various agricultural crop plants. The phytotoxicity of chromium resulted in appearance of retarded plant development, inhibition germination of seed, physiological changes, inhibit synthesis of photosynthetic pigment, essential nutrient uptake in term of translocation, yield production quality, antioxidant enzymes activities and induced oxidative stress (Poschenrieder et al. 1991; Panda et al. 2003, Tiwari et al. 2013). Beside, Cr can revolutionize the membrane ultrastructure of the chloroplast (Bassi et al. 1990). The transportation of Cr in plants does through the essential anions such as sulfate as a carrier in an active mechanisms (Cervantes et al. 2001). Chromium alters the uptake and accumulation mechanism of plasma membrane for essential nutrients such as nitrogen, phosphorus, potassium, iron, manganese, magnesium, molybdenum, zinc, copper, calcium and boron the plasma membrane in plant root cells limiting that the (Shanker et al. 2005).

The aim of present study to investigate the phytotoxicity symptoms, physiological and metabolic aspects of excess Cr concentration in the black gram (*Vigna mungo* L.) cv. BVN-3, a common growing crops worldwide with nutritional significance. The investigation focuses on the Cr (VI) induced phytotoxicity, yield production, antioxidant activities, nutrient uptake, translocation behavior and accumulation under chromium stress.

Materials And Methods

Experimental design

The plants of Black gram (*Vigna mungo* L.) cv. BVN-3 grown in sand pot culture (refined) in glasshouse conditions at neutral pH (6.8 to 7.0) and ambient temperature (Agarwala and Chatterjee 1996) with an amendment in the method of Hewitt (1966) feasible to the Indian climatic. A balance nutrient solution was made in distilled water to grow the Black gram with as follows: 4 mM KNO_3, 4 mM Ca $(NO_3)_2$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 100 mM Fe-EDTA, 10 mM $MnSO_4$, 30 mM H_3BO_3, 1 mM $CuSO_4$, 1 mM $ZnSO_4$, 0.2 mM Na_2 MoO_4, 0.1 mM $NiSO_4$, 0.1 mM $CoSO_4$, and 0.1 mM NaCl. For balance nutrients iron was supplied as Fe-EDTA (ferric ethylene diamine tetra acetic acid) as a chelate (Jacobson, 1951). Prepared nutrient solution was supplied to the experimental pots on regular basis for uniform plant growth and every pots were flushed weekly with distilled water for removal of absorbed nutrients and deleterious material from the root zone system. The pH of the nutrient solution was adjusted with buffers at 6.8±0.2 to supply to the plants during the experiment. Distilled water was used for watering the plants when needed throughout duration of the experiment. Plants of Black gram were treated with different concentration of Cr (0.00, 0.05, 0.10 and 0.25 mM) as potassium dichromate (AR grade salt) and supplemented with nutrient solution (after 40 d of growth) with control (without Cr). Plants were examined from time to time for toxicity and growth responses under Cr stress.

Analytical tools and adopted methods

On 47 d (7 d after Cr supply) relative water content (RWC) was estimated in middle leaves of Black gram as per the prescribed method by Barrs and Weatherley (1973). All measurements were made in the saturated condition of sand in the pots with nutrient solution in between 9 and 11 AM. The temperature and humidity recorded as 35-40^{o}C and 65-75% respectively, in the glasshouse during the experiment. At 12 d after chromium treatment the leaf area (cm^2) was measured by Delta-T leaf area measurement system to assess the growth behavior of the treated plant. At 48 d (8d after Cr treatment), chlorophylls content (a, b and total), hill reaction activity, concentration of sugars, starch, nitrogen, phenol and enzyme activity (catalase, peroxidase, acid phosphatase, and ribonuclease), soluble protein content were estimated in the crude leaves extract of Black gram (Table 1).

The treated plants of Black gram were harvested for metal analysis of in the plant tissue at 69 d growth (29 d after Cr treatment). Harvested plant samples first wiped with 0.01 N HCl followed by repeated washing with tap water and finally rinsed with distilled water and root, shoot and leaves were separated. Separated plant parts were chopped and oven dried at 70^{0}C for up to constant weight. Dried plants samples (100 mg) were digested with $HClO_4$:HNO_3 (1:4 v/v) and diluted with milli-Q water by using the method of Piper (1942). The concentration of iron and chromium in different plant parts were estimated by Inductively Coupled Plasma Spectrometer, Perkin Elmer Corporation (ICP Optima 3300 RL). All experiments were carried out in triplicate, to confirm the variability of data and validity of results, all data were analyzed statistically. Phosphorus was estimated calorimetrically and sulphur content by turbidimetrically (Table 1). Standard error of the mean is presented along with the mean values (Panse and Sukhatme 1954).

Quality control and quality assurance

The standard calibration reference material of Iron (BND 1101.02; provided by the National Physical Laboratory, New Delhi, India, Cr (Environmental Protection Agency quality control samples; E-Merck, Germany) were used for the calibration and quality assurance of the instrumental techniques. Analytical data quality of the metals were standardized through frequent analysis (n=6) of standard reference samples and the results were establish to be surrounded by ±2.01% of certified values. The mean resurgence was about 96 and 98 for Iron and Chromium respectively. The blanks were run in triplicate to check the accuracy of the method with every set of samples. The detection limits for Iron and Chromium were 0.3 and 0.5 ppb, respectively.

Results

Visual Phytotoxic symptoms of Cr

The present study, to assess the toxicity caused by excess levels of Cr supply, plants of black gram were grown in refined sand pot culture with excess amount of Cr at different concentration (0.05, 0.10 and 0.25 mM) supplied as potassium dichromate AR grade salt with a set of control pots. Plants were grown and

maintained up to the maturity, the toxic effects of differential levels of chromium (VI) stress have been observed in term of visible phytotoxic symptoms and growth behavior of black gram plants. At 5^{th} d of Cr supply the symptoms of excess Cr (0.50 mM) was observed on old leaves as chlorosis. After 7 d of Cr treatment, the toxicity reflected as wilting of leaves which later on hanged down from the petiole at higher concentration of Cr (0.10 and 0.25 mM Cr). On 10^{th} d of Cr supply, old leaves of treated plants turned golden yellow in colour. The number, size and shape of leaves reduced, chlorosis intensified and turned necrotic in next few days. Necrotic patches coalesced and large necrotic areas formed in the affected leaves. In successive few days, chlorotic leaves appeared wilted and dried followed by premature leaf fall. Similar symptoms observed in the middle and upper young leaf during the experiment. The development and growth of chlorosis in the leaves was comparatively delayed in plants grown at lower concentration of Cr after 14 d of the treatment.

Effects of Cr on biomass, grain yield, leaf area and relative water content in Black gram

The effect on biomass, grain yield, leaf area and relative water content of the Black gram plant grown under Cr treatment are depicted in Table 2. Dry biomass of Black gram decreased gradually with an increasing in Cr (VI) concentration in nutrient solution from 0.05 to 0.25 mM supply. The excess treatment of Cr (0.25 mM) at 69 d, resulted in reduced biomass which was 73.07% less as compared with control plant. In term of productivity, yield was produced only at 0.05 and 0.10 mM of Cr (VI), no pods were develop at other higher levels (0.25 mM). As compared to control plants, the grain weight reduced noticeably from 0.05 and 0.25 mM Cr, more decrease was evident at higher Cr concentration (0.25 mM). Seed size and shape were unusual, seed were deformed and shriveled in the Cr treated plants at higher concentration. There was a visible grain yield weight loss at 0.25 mM Cr (VI) treatment of black gram which was 76.95% with reduction observed in the current investigation.

At d 52 (12 d after Cr treatment), compare to the control plant, leaf area of Black gram plants decreased with increase in Cr exposure. The depression in leaf area was measured 56.16 % smaller as compared with control at the level of 0.25 mM of Cr treatment. The relative water content also decreased rapidly in Cr treated leaves as compared to the control plants with an increase in Cr Concentration. The decreased in RWC was observed 6.21%, 41.65 % and 59.37 % at 0.05, 0.1 and 0.25 mM of Cr concentration, respectively. Similarly, biomass reduction of Black gram recorded due to reflective loss of moisture and low RWC resulted in wilted leaves.

Cr alters changes in photosynthetic pigments, sugars, nitrogen, starch and phenols content in Black gram

Content of photosynthetic pigment, carotene, sugars (reducing and non-reducing), nitrogen (reducing and non-reducing), starch and phenols, in the Black gram grown under different Cr (VI) concentration are depicted in Table 3. The content of chlorophyll a, b and total decreased unpredictably and noticeably with increase in Cr (VI) supply in nutrient solution. The reduction in chlorophyll a, b and total in the leaves of Black gram observed more at 0.01 and 0.25 mM Cr exposure. The reduction in total chlorophyll content

recorded 60 % as compared to that of control plant leaves at 0.25 mM of Cr treatment. Decreased in chlorophyll content may be due to the inhibition of photosynthetic pigments with Cr (VI).

It was examined that the concentration of reducing and total sugars was notably high in the leaves of Black gram plants supplied by levels of Cr (VI) as comparison with control plants. Non-reducing sugars was found not to be affected significantly except with a small reduction. The reducing sugar was appreciably examined in higher order as compared with control plant leaves of Black gram. Total sugars, also showed a definite trend and its concentration increased slowly with an increase in Cr (VI) concentration in nutrient solution, however, non-reducing sugars observed in reducing trends as compared with control level. The content of starch decreased due to different levels of Cr (VI) supply in Black gram leaves, maximum reduction (67.43%) was most observed at 0.25 mM Cr (VI) exposure. In our observation, concentration of phenols increased with increase in the Cr (VI) supply in leaves of Black gram as compared to control plant, maximum increased (57.14%) was recorded at 0.25 mM of Cr (VI) treatment level.

Effect of Cr on protein content and activities of catalase, peroxidase, ribonuclease, hill activity, acid phosphatase activity in black gram

The concentration obtained at control levels, protein N was decreased with an increase in Cr (VI) stress, however, non- protein N was recorded in increased levels. Total N content also decreased with increase in Cr exposure along with nutrient solution. *In the present investigation results of diverse biochemical parameters in the leaves of Black gram exposure with excess levels of Cr (VI) grown under sand culture techniques are presented in Fig.1.* The experimental results showed protein concentration was affected by the excess Cr (VI). The percentage of protein progressively decreased with an increase in Cr (VI) supply in nutrient solution. The reduction in its concentration was pronounced at 0.10 (46.39%) and 0.25 (79.38%) mM Cr (VI) was measured respectively. At d 49 (9 days after Cr treatment) the activity of catalase in leaves of black gram decreased with increased in Cr (VI) exposure was estimated. The activity of peroxidase at d 9 after Cr (VI) supply was increased in leaves gradually of Black gram. The activity of ribonuclease was also increased gradually from 0.05 to 0.25 mM Cr exposure in black gram leaves. The present measurement showed that the activity of acid phosphatase in all levels of treatment increased with an increased in Cr supply in nutrient solution from 0.05 to 0.50 mM. In the present investigation the activity of acid phosphatase was enhanced up to 90.34% at 0.25 mm exposure of Cr (VI). Hill reaction activity in leaves of Black gram plant was decreased up to 51.94% at 0.25 mM treatment level by increasing the concentrations of Cr (VI) in nutrient solution.

Effects of Cr (VI) on phosphorus and sulphur

In Black gram plant the accumulation and translocation of phosphorus and sulphur from roots to different parts of shoots was also affected by induced levels of Cr (VI). In the present observation, excess supply of Cr in black gram plants resulted increased in the concentration of phosphorus and sulphur in various plant parts (leaves, stem, roots, seed and husk) (Figure 2). However the diagnostic outcome showed that the all these nutrients were accumulated significantly in roots parts of black gram.

Exposure of Cr (VI) on iron and chromium accumulation

Excess Cr (VI) supply resulted in decreased the concentration of iron in seed, husk, leaves and stem and increased in root parts of black gram plant (Figure 3). Uptake, translocation and accumulation of Cr in different parts of Black gram plant has been found to vary in root, shoot and leaves at different levels of excess Cr (VI) treatment. The prominent accumulation of Cr (VI) was measured in roots followed by leaves and shoot at all the exposure levels. It addition it was also reported that the uptake and translocation of Cr vary in different plant parts and levels of supply along with nutrient solution.

Discussion

Visual phytotoxic symptoms under Cr (VI) stress

Black gram plants were grown up to maturity along with essential nutrient to observe grain yield production under chromium stress in relation with control level. After 5 d of Cr supply (at d 45) the visible physiological symptoms of excess Cr (0.25 mM) were observed on old leaves as chlorosis in the aerial part of leaves. Subsequently 7 days of Cr supply, the visual symptoms appeared as wilting of affected leaves which afterward hanged losing from the petiole at t 0.1 and 0.25 mM of Cr. On 10th day of metal supply old leaves of these plants turned golden yellow in colour. In same duration the size and number of leaves reduced, chlorosis intensified and turned necrotic in next few days. Necrotic patches coalesced, produced large necrotic areas, leaves appeared permanently wilted and dry followed by early leaf fall was observed. These visible phytotoxic symptoms of excess Cr in black gram plants species are new to literature and some extent works be comparable to that previously observed by Tiwari et al. (2008) in citrullus plants. Investigation of Chatterjee and Chatterjee (2000) described on cauliflower plants and concluded that the growth depression is a general characteristic of excess Cr.

Effect of induced chromium exposure on biomass, grain, leaf area and RWC

In Black gram plants, reduction in plant biomass, grain yield production, leaf area and RWC (Table 2) was observed with increasing in the concentration of chromium in essential nutrient supplied during the present experiment. The decline in black gram plant biomass may be generally due to the reduced shoot, root development. Subsequently very low amounts of essential nutrients and water transfer to the shoot parts via plant roots. Pandey et al. (2009) and Tiwari et al (2009) observed that the leaf area is usually decreased in response to increase of Cr content. Several experimental theories available in relation to plants exposed to Cr, develop metabolic impairment and results in alteration of several physiological processes including reduction in germination rate, growth, chlorosis, stunting, and finally plant death (Rai et al. 2004; Shanker et al. 2005). Some of other investigation showed and support our present examination that the physiological and biochemical processes were cruelly affect by excess Cr and as outcome, the yield and productivity of the crops were equally affected (Dube et al. 2003; Tiwari et al. 2009). In black gram, decreased in RWC at 0.05, 0.1 and 0.25 mM Cr were 18.5%, 37.5% and 56.2% was observed respectively. Reduction in the fresh biomass in black gram plants owing to higher loss of moisture as was evident from the low RWC in leaves which outcome in their wilted manifestation. Tiwari

et al. (2013); Dube et al. (2003) reported that the excess supply of Cr (VI) exhibited poorer water potential and relative water content in plant leaves.

Content of chlorophyll, sugars, starch, nitrogen and phenols under dissimilar levels of Cr (VI)

The concentration of chlorophyll, sugars, starch, nitrogen and phenols in black gram under excess chromium stress are depicted in Table 3. It was observed that the increased concentration of reducing sugars, total sugars, non protein nitrogen and phenol content however the concentration of chlorophyll, protein nitrogen, total nitrogen and starch in the black gram leaves decreased *significantly in black gram plants* with the increase in Cr (VI) supply. The results of the present study have shown that the depression in chlorophyll content at 0.25 mM of Cr stress was about 60% as compared to that of control leaves. Chlorophyll is established in the chloroplasts of green plants. It is consists of a central magnesium atom bounded by a nitrogen hold formation connected with an extended ring of carbon hydrogen side chain, known as a phytol chain. Excess Cr concentration leads to a significant reduction in the leaf area and leaf biomass, which is accompanied by decline photosynthesis and induction of chlorosis and necrosis of leaves (Gill et al. 2015; Tiwari et al. 2009). Due to excess supply of Cr, many critical processes take place in plant leaves was observed. Those contain inhibition of chlorophyll synthesis, chloroplast ultra structure disruption, inhibition of photosynthetic electron transport, and release of magnesium ions from the molecule of chlorophyll (Rai et al. 2004; Panda and Chaudhary 2005).

It was examined that the concentration of reducing and total sugars was notably high in the leaves of black gram plants supplied by levels of Cr (VI) as comparison with control plants. Non-reducing sugars was found not to be affected significantly except with a small reduction. Alteration of non-reducing sugars concentration in present investigation might be due to Cr induced changes in carbohydrate metabolism and another explanation would be that metal reduced vein loading hence inhibiting photo-assimilate export with a resultant carbohydrate accumulation (Rauser and Samarukoon, 1980). The effects of Cr (VI) on the concentration of non-reducing sugars was not significant, as has been investigated by some other earlier investigator in barley by Agarwala et al. (1977) and in pea plant by Tiwari et al. (2009).

In Black gram plant leaves after 29 day exposed with excess levels of Cr (VI) showed reduction in starch formation. The observations of Tiwari et al. (2008) suggested that the Cr accumulation reduced the biosynthesis of starch in citrullus plant. The concentration of protein nitrogen total N decreased and increase in non-protein N in black gram leaves was found with differential Cr (VI) exposure compared to that of control. Earlier findings of Sharma et al. (1995) investigated that the Cr affects nitrogen uptake and absorption which is evident from the decreased in the content in protein N. At present observation the excess Cr (VI) treatment in nutrient solution increase in the concentration of phenols in black gram leaves. The activity and antioxidant defense property of phenolic compounds is ascribed to the ability of chelate metal cations, donating hydrogen atoms, scavenging free radicals and or electrons. Similar observation by Tewari et al. (2002) they viewed that the enhancement of phenols might be attributed to

rapid diffusivity of H_2O_2 produced in the cytosol or owing to uptake of higher phenols and low protein production in such situation.

Chromium induced changes in catalase, peroxidase and ribonuclease, hill activity, acid phosphatase activity and protein content

The activity of catalase, peroxidase and ribonuclease, hill activity, acid phosphatase with protein content of black gram under Cr stress are presented in Figure 1. At 49 days (9 days after Cr exposure) the activity of catalase in fresh leaves of black gram was increased due to varying levels of Cr (VI) exposure compared to that of control plant. The activity of catalase in black plants has been examined in a concentration dependant way and the maximum concentration was observed at 0.25 Mm exposure of Cr (VI). Likewise, Rai et al. (2004) observed and reported the increased movement of catalase in *Oscimum tenuiflorum* exposed to Cr. The essential activity of peroxidase in black gram plant fresh leaves increased under diverse levels of Cr (VI) in nutrient solution. Shahid et al., (2016a) documented that the peroxidase is a large family of enzymes that usually catalyzes the deduction of H_2O_2 into H_2O. Within plant cells, ascorbate acts as a reductant for the decrease of H_2O_2.

In Black gram, the activity of ribonuclease in fresh leaves at excess treatment of Cr (VI) was with increase in Cr concentration along with essential nutrient solution. Cr (VI) toxicity stimulates the movement of ribonuclease (37.94%) in plant leaves as compare to control at 0.25 mM exposure. Cr treatment up to excess of 0.10 mM caused a slight increase in ribonuclease activity which contradicted past findings by Dua and Sawhney (1991) and Tiwari et al. (2013) in different plant species. Increase in the activity under induced levels of Cr treatment contradicts with the prior outcome in grapevines plants (Strakhov and Chazova 1981). However, that of such phenomena like the enhanced ribonuclease activity seems similar to those degradative pathways occurring in senescent tissue. Protein concentration in black gram plant leaves was also decreased with increase in the Cr (VI) levels. Our measurements supported with previous findings by Chatterjee and Chatterjee (2000) observed that the restricted biomass of cauliflower in the presence of Co, Cu and Cr might be the result of lower protein formation in such conditions.

The activity of Hill reaction in the leaves of black gram plant reduced extensively due to excess treatment of Cr (VI) at 9 days after supply. Our findings correlate with Krupa and Baszynski (1995) observation that Cr can reduce the hill reaction, affecting equally dark and light reactions. Increased acid phosphatase activity was measured in black gram plant leaves under excess stress of Cr treatment. Maximum movement was observed at 0.25 mM of Cr (VI) exposure. In our present investigation of black gram contradict with the prior observation in grapevines by Strakhov and Chazova (1981).

Effects of Cr (VI) on phosphorus and sulphur accumulation and translocation

In black gram the concentration of phosphorus and sulphur in different plant parts significantly increased (Figure 2) due to excess diverse Cr (VI) exposure as compared to that of control plant. However it is observed that the all these nutrients elements were accumulated significantly in black gram plant roots. In present investigation the translocation and accumulation of phosphorus and sulphur from roots to

upper parts of plants is moreover affected by excess levels of Cr (VI) in essential nutrient solution. Earlier study carried out by Chatterjee and Chatterjee (2000) concluded that the excess supply of Cr could affect the translocation of Mn, Zn, P, S and Cu from roots to tops in cauliflower plants. Cr (VI) affects the translocation and accumulation of S and P in citrillus plant under varying levels of exposures (Dube et al., 2003). Some other findings supports our present investigation in black gram plants that the accumulation of phosphorus may be due to the direct intrusion of Cr with the metabolism of phosphorus in plants as recommended by (Spence and Millar 1963).

Exposure of Cr (VI) on iron and chromium accumulation and translocation

After 29 days of excess Cr (VI) supply along with nutrient solution, maximum accumulation of Fe was found in roots at 0.25 mM exposure. Excess Cr (VI) resulted in a decreased in the concentration of iron in seed, husk, leaves and stem and increased in root parts (Figure 3b). In context to that the previous findings by Dube et al. (2003) and Tiwari el al., (2013) founds that Cr affects the availability, uptake and translocation of Fe by plants. Under Fe-deficient situation, dicotyledonous plants enhanced root Fe (III) reductase activity, therefore increasing the ability to decrease Fe (III) to Fe (II), the form in which roots absorb Fe (Alcantara et al. 1994). The accumulation and translocation of chromium (Figure 3a) in diverse plant parts of black gram was observed to be changeable in response to excess Cr (VI) exposure. Lahouti and Peterson (1979) was suggested that the uptake and translocation of Cr differ in varied plant parts and also depend upon the genus and species diversity. It is observed that the higher accumulation of Cr was observed in black gram plant roots at all levels of Cr exposures. Tauchnitze and Schnabel (1983) was reported that the Cr was least mobilized in roots.

Conclusion

It is concluded in the present experimental observation in term of visual phytotoxic symptoms, inhibition of plant growth, changes in various metabolic activities, decrease in biomass production and yield quality was directly affected by variable chromium (VI) stress in black gram. Higher levels of chromium stress was absolutely concerned with plant metabolism through competition for uptake, translocation, inactivation of several enzymatic activity, and displacement of certain essential nutrient in the functional sites. Naturally, the uptake of chromium from the polluted agriculture field-grown crop plants might cause hazardous effects and injurious disorder in human beings as well as in ruminants in term of levels of contamination though food chain. The physiology and biochemistry of Cr (VI) toxicity have been less studied in intact plants system. Findings and data from the present investigation may assist on the way to improve understanding in screening and selecting low risk crops to grow in chromium contaminated sites and minimize food chain for health and environmental safety.

Declarations

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Consent for publication: Not applicable

Availability of data and materials: Not applicable

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Authors' contributions

Chromium analyzed and interpreted the metal accumulation data regarding the phyto-toxicity and metabolic changes by the author Kamlesh Kumar Tiwari.Experimental designing and uptake and accumulation analysis of nutrients in the plants Vigna mungo major contribution in writing the manuscript by Naveen Kumar Singh. All authors read and approved the final manuscript.

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Tables

Table 1. Analytical parameters and adapted reference methods

Sr. No.	Parameter	Reference of Method
1	Phosphorus	Wallace (1951)
2	Sulphur	Chesnin and Yien (1951)
3	Relative water content	Barrs and Weatherly (1973)
4	Chlorophyll contents	Arnon (1949)
5	Hill activity	Brewer and Jogendorf (1965)
6	Protein	Bradford (1976)
7	Starch	Montgomery (1957)
8	Nitrogen	Swain and Hillis (1959)
9	Sugars	Nelson (1944)
12	Catalase	Bisht et al. (1989)
13	Peroxidase	Luck (1963)
14	Ribonuclease	Tuve and Anfinsen (1960)

Table 2. Phytotoxic effect of variable chromium exposure on biomass, grain yield, leaf area and relative water content of black gram plants. Values are means ± SE (n=5).

Days of growth	Days after metal supply		mM chromium				LSD (*P*=0.05)
			Control	0.05	0.10	0.25	
69	29	Biomass:g plant^{-1}	15.60 ±1.20	10.90 ±0.80	6.70 ±0.30	4.20 ±0.10	0.78
69	29	Grains : g plant^{-1}	4.86 ±0.06	2.15 ±0.06	1.12 ±0.01	-	0.23
52	12	Leaf area : cm^2	92.12 ±1.42	75.10 ±1.42	57.59 ±0.66	40.38 ±0.70	2.56
47	7	RWC: %	96.50 ±3.82	90.50 ±2.30	56.30 ±1.29	39.20 ±0.81	3.51

Table 3. Variable chromium exposure on concentration of chlorophyll, hill activity, carbohydrate fraction, starch, nitrogen and phenols in leaves of black gram (at d 48; 8 d after metal exposure). Values are mean ±SE (n=5).

Parameters evaluated	mM chromium				LSD (P=0.05)
	Control	0.05	0.10	0.25	
Chlorophyll: mg g^{-1} fresh wt					
a	0.981 ±0.02	0.597 ±0.01	0.398 ±0.01	0.309 ±0.00	0.05
b	0.405 ±0.04	0.303 ±0.01	0.263 ±0.02	0.169 ±0.03	0.02
Total	1.386 ±0.04	0.900 ±0.02	0.661 ±0.02	0.478 ±0.03	0.08
Sugars: % fresh weight					
Reducing	0.22 ±0.02	0.37 ±0.01	0.42 ±0.02	0.47 ±0.01	0.05
Non reducing	0.09 ±0.02	0.08 ±0.01	0.07 ±0.00	0.02 ±0.00	0.00
Total sugars	0.31± 0.01	0.45 ±0.02	0.49 ±0.01	0.49 ±0.03	0.02
Nitrogen: % fresh weight					
protein nitrogen	1.02 ±0.02	0.90± 0.01	0.70± 0.01	0.60± 0.01	0.06
non protein nitrogen	0.276± 0.01	0.264 ±0.03	0.421 ±0.04	0.484 ±0.04	0.03
total nitrogen	1.296 ±0.11	1.164 ±0.05	1.121 ±0.06	1.084 ±0.03	0.06
Starch: % fresh weight	1.210 ±0.09	1.031 ±0.07	0.613 ±0.08	0.394 ±0.00	0.04
Phenols: % fresh weight	0.003± 0.00	0.004± 0.00	0.005± 0.001	0.007± 0.001	0.001

Figures

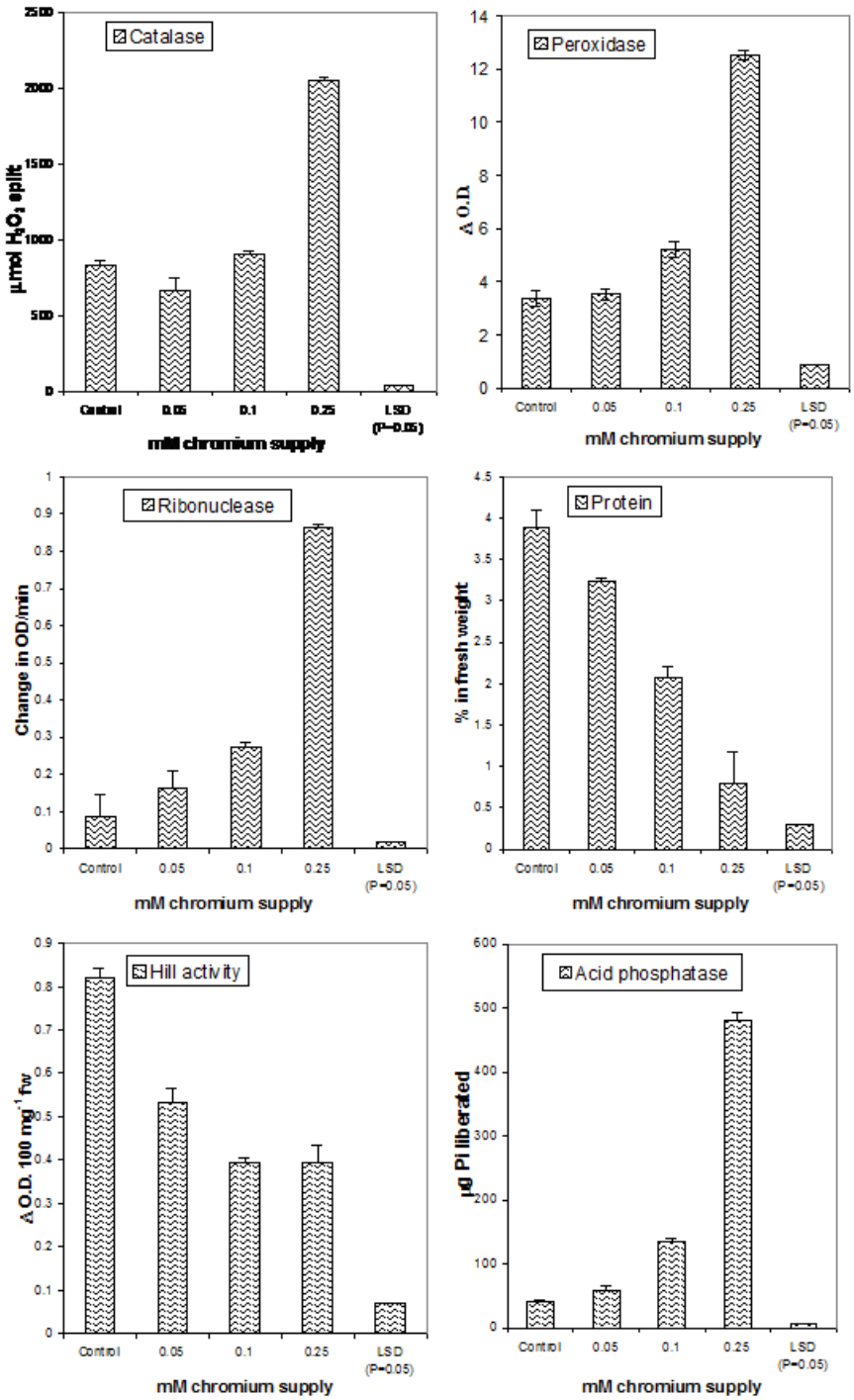

Figure 1

Variable chromium treatment and activities of some enzymes, hill reaction activities and protein concentration in leaves of Blackgram 49 d (9 d after metal exposure). (Values are means ±SE (n=5)

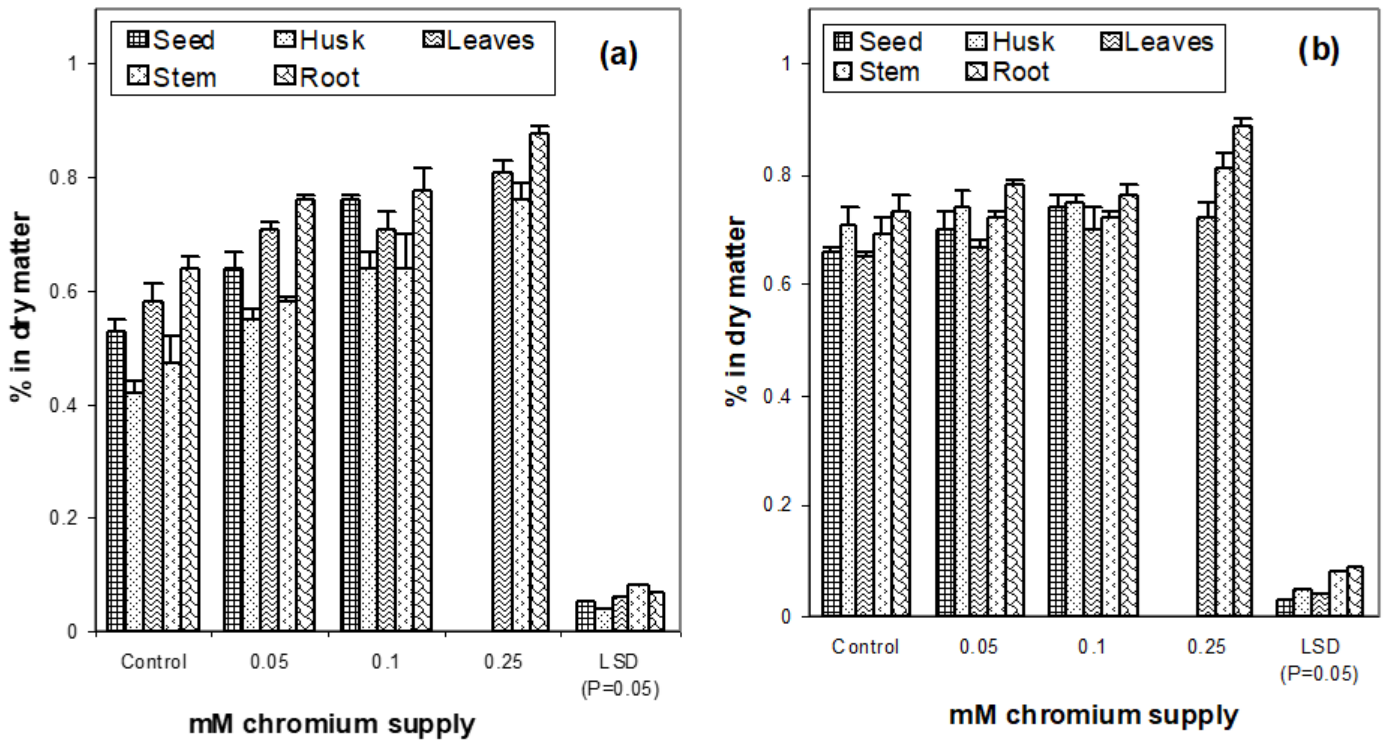

Figure 2

Variable chromium treatment and S (a) and P (b) uptake and accumulation in different part of Blackgram 69 d (29 d after Cr exposure). (Vertical bars represent values± SE (n=5).

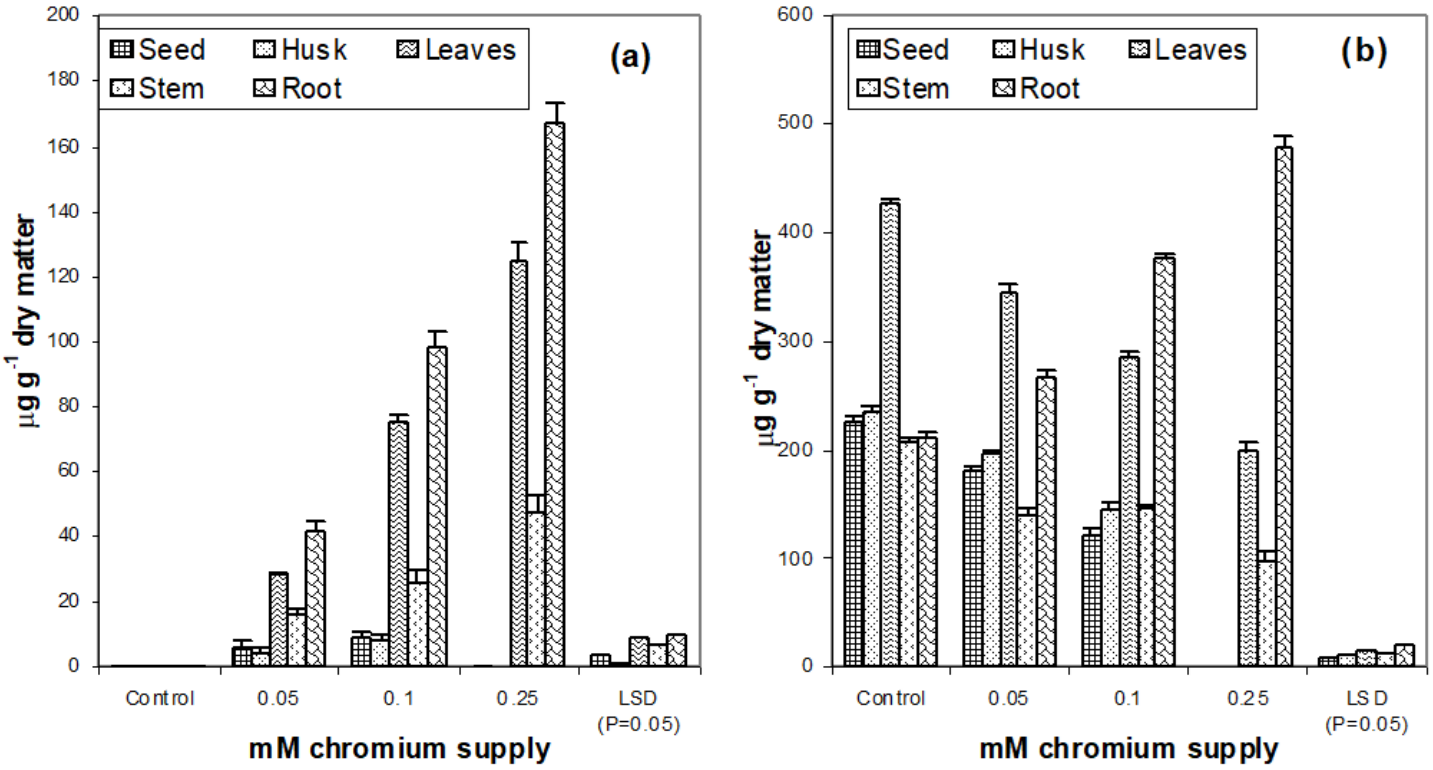

Figure 3

Variable chromium treatment and Cr (a) and Fe (b) uptake and accumulation in different part of Blackgram 69 d (29 d after Cr exposure). (Vertical bars represent values± SE (n=5).