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ORIGINAL ARTICLE

Phytotoxicity of Chromium (VI) on Germination, Growth Attributes and Pigmentation in Cluster Bean

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An investigation was carried out to study the phytotoxic effect of chromium (VI) on seed germination, plant growth parameters and biosynthesis of pigments in cluster bean (*Cyamopsis tetragonoloba*). Four concentrations (1, 2, 4 and 8 µg ml^{-1}) of Cr (VI) as potassium dichromate were applied to cluster bean seeds in solution culture. The rate of seed germination was observed every hour. Growth parameters were measured at the early seedling stage. The concentrations of chlorophyll, carotenoid and anthocyanin in leaves were estimated. All the studied concentrations of Cr (VI) were found to be toxic to cluster bean. The speed of seed germination slowed down under Cr (VI) stress. The root-shoot length, root-shoot fresh weight and root-shoot dry weight decreased with increasing concentrations of Cr (VI). The most deleterious effect was observed at 8 µg ml^{-1}. Anthocyanin contents in cluster bean showed a significant ($P < 0.05$) correlation with applied Cr (VI) concentrations. Out of the various concentrations of Cr (VI) studied, 1 µg ml^{-1} and 2 µg ml^{-1} were moderately toxic while 4 µg ml^{-1} and 8 µg ml^{-1} were highly toxic to cluster bean.

Key words: Anthocyanin, chlorophyll, growth, seed germination, chromium toxicity

Cluster bean [C*yamopsis tetragonoloba* (L.) Taub.] is a multifaceted crop of the fabaceae family grown for food, fodder and industrial purposes (for the production of guar gum.). It is mainly grown in the north-western region of India and Pakistan and India produces about 80% of cluster bean across the world. In semi-arid regions of Rajasthan, it is largely used by agriculturists for crop rotation due to its nitrogen fixation ability. This legume lives in symbiosis with nitrogen-fixing bacteria and is also used to replenish the soil with essential fertilizer.

Chromium (Cr) is a transition element of group VI B of the Periodic Table and its average concentration in Earth's crust is 100 ppm (Estokova *et al.,* 2018). The occurrence of Cr in the environment is due to both natural and anthropogenic activities. Many industries like metallurgical, chemical industries, leather and tanning industries, electroplating, production of paint and pigment, wood preservation, pulp and paper production use Cr on a large scale (Kumar, 2020) and pollute the ecosystem by discharging in substantial amount. Leather industries contribute the maximum amount of Cr into the ecosystem and is 40% of the total industrial efflux (Barnhart, 1997).

Cr toxicity to living organisms depends upon its oxidation state. Cr (VI) is a mobile element and highly toxic whereas Cr (III) is less toxic to plants (Fendorf, 1995). Mostly chromium in soil is present in Cr (III) form its in agricultural soils varies and it can reach up to 350 mg kg^{-1} (Ertani *et al.,* 2017). Plants don't have a specific transport system for Cr and its uptake and transport depend upon the transporters of essential ions such as sulphate or iron (Shanker *et al.,* 2005). Cr is known to be very harmful to plants at high concentrations and can adversely affect seed germination and early seedling growth, inhibit the enzyme activity, reduce the protein content and cause necrosis and chlorosis (Kabata-Pendias, 2000). Cr toxicity is often associated with oxidative stress, caused by the formation of reactive oxygen species. The toxicity of Cr interferes with several metabolic processes in plants causing early seedling growth and reduced seed germination (Nath *et al.,* 2005). Cr can affect the germination process, growth and development of plants which ultimately results in decreased dry matter production and yield (Free *et al.,* 2010). However, to the best of our knowledge, no research has been conducted on the Cr-induced toxic effects on seed germination, pigment biosynthesis and anthocyanin mediated tolerance mechanism of cluster bean. Keeping this in view present study was carried out to assess phytotoxic effect of Cr (VI) on seed germination, plant growth parameters and pigmentation in cluster bean.

MATERIALS AND METHODS

The experiment was conducted in April, 2021 under the laboratory condition at Department of Botany, University of Rajasthan, Jaipur where the photoperiod was 8 hours per day. For the experimental purpose ten seeds of a certified variety NEELAM 51 PNB of C*yamopsis tetragonoloba* (L.) Taub. were placed in each Petri plate. Four concentrations of Cr (1, 2, 4 and 8 µg ml^{-1}) were used. The concentrations of Cr were prepared separately by taking the respective amount of potassium dichromate ($K_2Cr_2O_7$) (calculated on the basis of molecular weight). Except the concentration of Cr no other nutrient supplements were provided to the experimental plants. Control constituted only distilled water. Fix amount (10ml) of solution poured into each Petri plate to saturate the filter paper. Three replicates were used for each concentration. The rate of seed germination was observed every hour once the germination started. The seedlings were supplied with Cr concentrations through out the experiment. First trifoliate leaves were analyzed for pigment contents. Seedlings were harvested after seven days of treatment for root-shoot length and root-shoot fresh weight. Seedlings were dried in an oven at 80˚C for 48 hrs for dry weight. The relative yield of biomass was calculated by using the dry weight of control and Cr treated seedlings.

Coefficient of velocity of Germination (CVG): The coefficient of velocity of germination was computed by formula described by Al-ansari and Ksiksi (2016).

$$CVG = \frac{N_1+N_2\ldots\ldots+N_i*N_1T_1+\ldots\ldots\ldots\ldots NiTi}{100}$$

here N is the number of germinated seeds every hour; T = number of hours from seedling corresponding to N.

The tolerance index of seedlings was calculated by method given by Turner and Marshall (1972). Vigour index was calculated by formula described by Dhindwal *et al.,* (1991). The grade of growth inhibition was calculated after Aery (2010).

Quantification of pigments

The pigments were extracted from leaf samples in methanol. Total chlorophyll was determined according to Arnon (1949). The total carotenoid content was estimated using following formula:

Total carotenoid content (mg g^{-1}) =

$$\frac{1000\times A_{470}-2.86(15.65\times A_{666}-7.34\times A_{653})-129.2(27.05\times A_{653}-11.21\times A_{666})}{245}$$

The anthocyanin concentration was estimated after Murray and Hackett (1991).

Anthocyanin content (mg g^{-1})= $A_{530}-(0.24\times A_{653})$.

Statistical analysis

All the raw data obtained from three replicates were used to calculate standard error (SE), correlation coefficient (R), and regression equation (Y) using Origin 8.1.

RESULTS

The application of Cr (VI) adversely affected the seed germination, growth parameters and biomass production of cluster bean. A significant (*P = 0.01*) decrement in plant length, fresh and dry weight and relative yield was observed with the applied concentrations of Cr. Minimum root and shoot length was observed at the highest dose of Cr (8 µg ml^{-1}) and was 22.79% and 34.22% respectively, compared to the control (Fig. 1A).

Cr stress also reduces biomass production of cluster bean (Fig. 1B&C). Minimum fresh weight of root and shoot was observed at 8 µg ml^{-1} and was 45.30% and 32.58 % respectively, compared to the control (Fig.1B). The relative yield of cluster bean was decreased under Cr stress. The minimum relative yield was observed at highest applied concentrations of Cr (8 µg ml^{-1}) and was 33.9% lower, compared to the control (Fig. 1D).

In the present study, the coefficient of velocity of germination (CVG) was inhibited under all applied concentrations of Cr (VI). The minimum CVG was observed at the highest applied concentration of Cr (8 µg ml^{-1}) and was 57% lower, compared to control (Fig. 2).

In the present study, the TI of cluster bean was regularly decreased in all applied concentrations of Cr (1-8 µg ml^{-1}). Minimum TI was observed at the highest applied concentration (8 µg ml^{-1}) and was 43% lower compared to the control (Fig. 2). The VI of cluster bean was continuously decreased in increasing concentration of Cr (1-8 µg ml^{-1}). The minimum VI was observed at the highest applied concentration of Cr (8 µg ml^{-1}) and was 39.71% lower, compared to the control (Fig. 2). In the present investigation, the TI and VI of cluster bean decreased with increasing concentrations of Cr (VI) in solution culture (Fig. 2).

The grade of growth inhibition (GGI) was increased by increasing the concentration of Cr (VI). The minimum GGI was observed in the lowest applied concentration of Cr (VI) (1 µg ml^{-1}) and the maximum GGI was observed at highest applied concentration of Cr (8 µg ml^{-1}) (Fig. 2). This reveals the increasing toxicity of Cr (VI) in increasing concentration to cluster bean.

In the present study, a fluctuation the pigment content of cluster bean was observed under Cr (VI) stress. Interestingly, lower application of Cr positively influenced the synthesis of chlorophyll and carotenoid content in cluster bean. Maximum total chlorophyll was observed at Cr 1 µg ml^{-1} whereas maximum carotenoid content was observed at 2 µg ml^{-1} applied concentration of Cr. Beyond the above levels, total chlorophyll and carotenoid content were decreased. Minimum total chlorophyll was observed at the highest applied concentration (8 µg ml^{-1}) and were 9.6% lower, compared to the control (Fig. 3).

In the present investigation, the carotenoid contents of cluster bean decreased under Cr (VI) stress in plants. However, at lower applied concentrations of Cr (VI) (1-4 µg ml^{-1}) an increment in carotenoid contents is also observed.

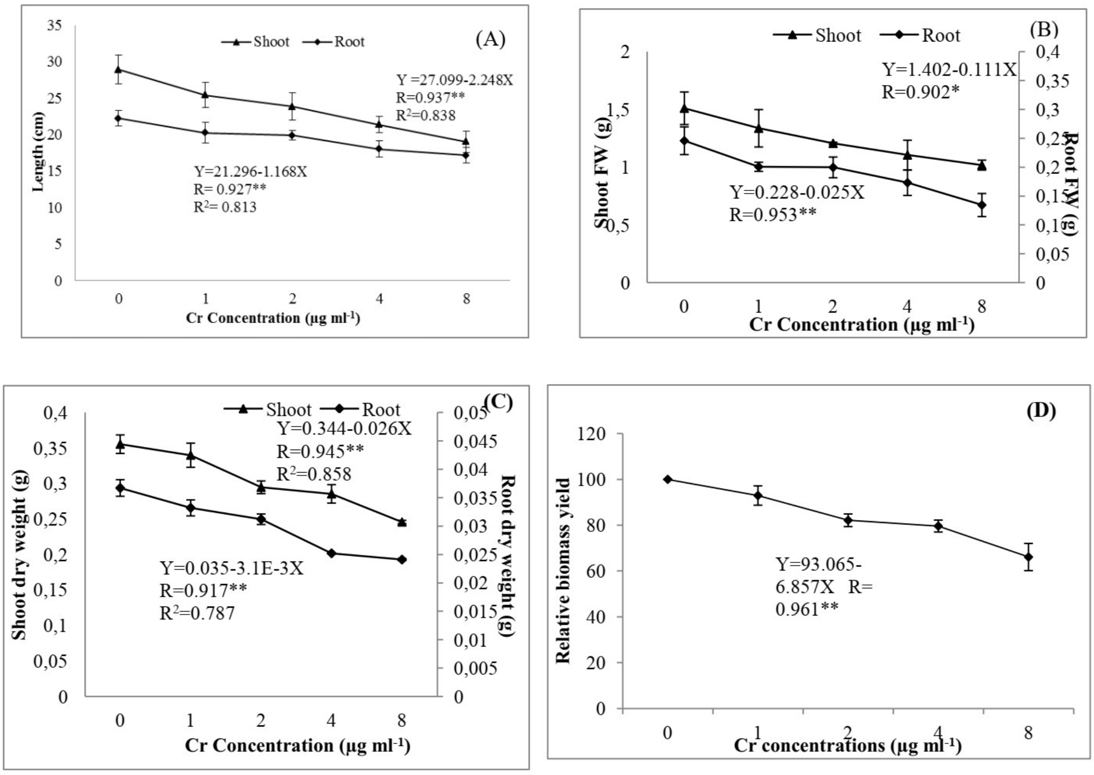

Figure 1. Effect of Cr(VI) on growth and biomass production of cluster bean. (A) Root-shoot length, (B) Root-shoot fresh weight, (C) Root-shoot dry weight, (D) relative yield (Error bars showing standard error of mean) *=Significant at 0.05% level, **=Significant at 0.01% level

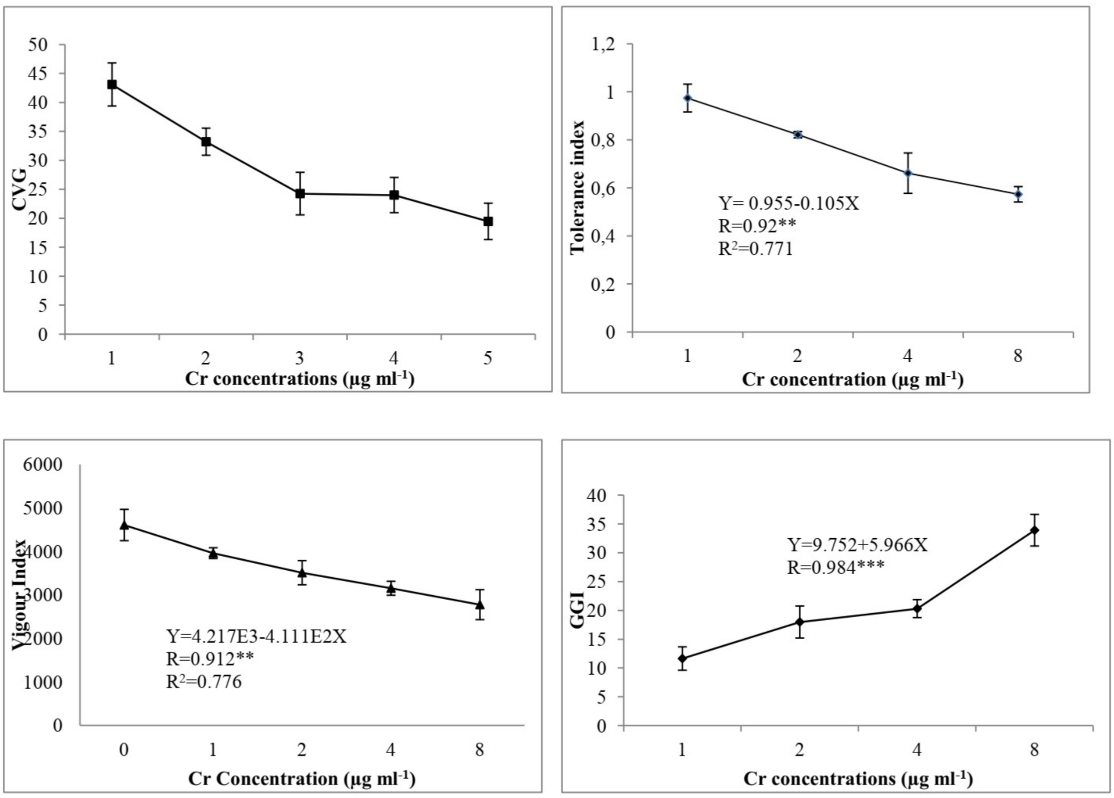

Figure 2. Effect of Cr(VI) on coefficient of velocity of germination, tolerance index, vigour index and grade of growth inhibition of cluster bean. (Error bars showing standard error of mean) *=Significant at 0.05% level, **=Significant at 0.01% level, ***=Significant at 0.001% level

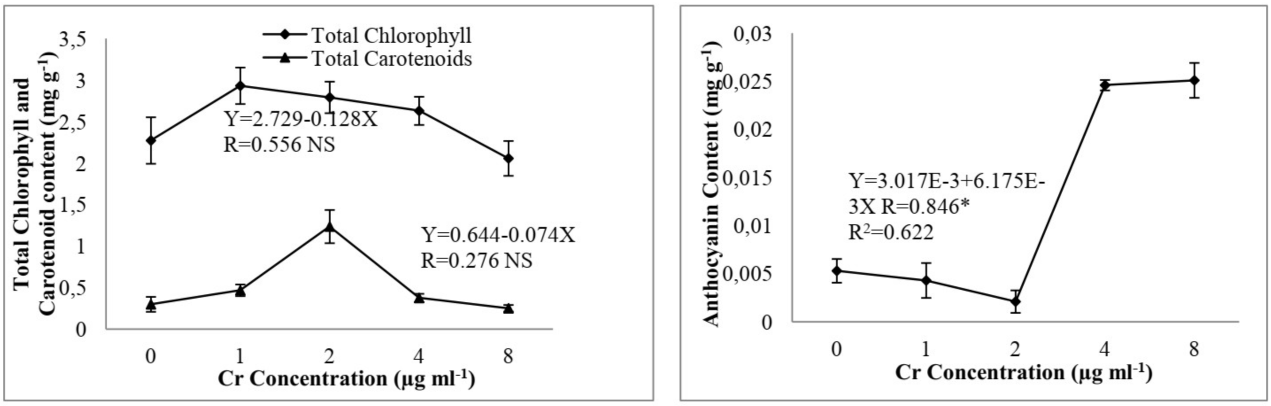

Figure 3. Effect of Cr(VI) on coefficient of velocity of germination, tolerance index, vigour index and grade of growth inhibition of cluster bean. (Error bars showing standard error of mean) *=Significant at 0.05% level, **=Significant at 0.01% level, ***=Significant at 0.001% level

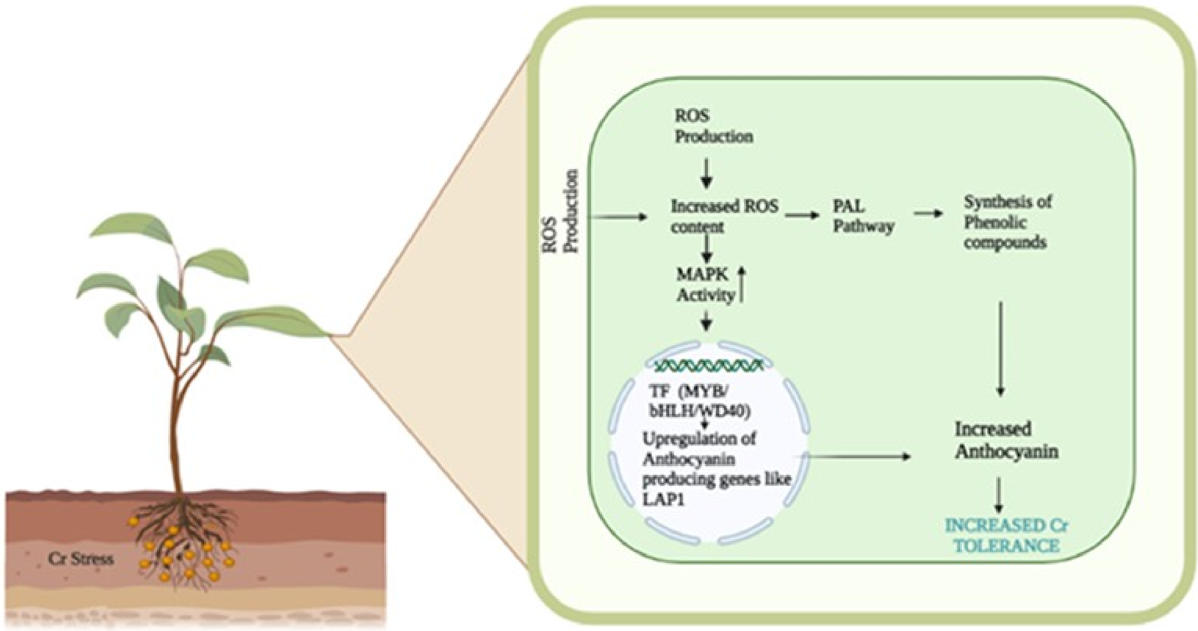

Figure 4. Diagrammatic representation of anthocyanin mediated chromium tolerance in cluster bean.

A decrement in anthocyanin content of cluster bean was observed in lower applied concentrations (1 – 2 µg ml^{-1}) of Cr. Minimum anthocyanin was observed at (1 µg ml^{-1}) and was 60.37% lower, compared to the control. Interestingly, in higher concentrations of Cr (4-8 µg ml^{-1}) huge biosynthesis of anthocyanin was observed. Maximum anthocyanin content was observed at the highest applied concentrations (8 µg ml^{-1}) of Cr and was 373.35% higher, compared to the control (Fig. 3).

DISCUSSION

Chromium toxicity adversely affected the growth and photosynthetic pigment synthesis in cluster bean. The application of Cr (VI) slowed down the seed germination of cluster bean in solution culture. The reduction in seed germination under Cr (VI) stress is reported by Akinci and Akinci (2010) in *Cucumis melon*, Rout *et al.* (1997) in mungbean, and Peralta *et al.* (2001) in alfalfa. The inhibitory effect of different metals such as Li (Surana and Aery, 2005), Ni (Jagetiya and Aery, 1994), W (Kumar and Aery, 2010) and Mo (Kumar and Aery, 2012) on seed germination has been reported.

In the present study, application of Cr (VI) to cluster bean was resulted in decreased plant length and biomass production (Fig. 1). Similarly, a reduction in root and shoot length of alfalfa under Cr stress has been reported (Barton *et al.,* 2000). The reduction in plant length might be due to higher deposition of Cr in cell walls which inhibits cell expansion and/or due to inhibition of cell division in the meristematic zone (Chidambaram *et al.,* 2009). The decrement in biomass production under Cr (VI) stress is reported in *Pisum sativum* (Bishnoi *et al.,* 1993), *Cucumis melon* (Akinci and Akinci, 2010) and *Helianthus annus* (Fozia *et al.,* 2008).

The tolerance of plants to metals is generally determined by the inhibitory effect of metal ions on root growth. An increment in vigour index (VI) and tolerance index (TI) at lower concentration and decrement at higher concentration in response to Co (Jayakumar *et al.,* 2008), W and Mo (Kumar and Aery, 2012) has been reported. In the present study, the TI and VI of cluster bean decreased with increasing concentrations of Cr (VI) (Fig. 2). Joshi *et al.* (2019) reported that increasing concentrations of Cr decreased the vigour index of *Vigna radiata, Trigonella foenum-graceum, Oryza sativa, Sorghum vulgare* and *Pennisetum glaucum*. Amin *et al.* (2013) observed that increasing concentrations of Cr decreased both vigour index and tolerance index of *Hibiscus esculentus.*

The reduction of chlorophyll under Cr stress is well reported by Sarkar and Jana (1986) in *Azolla pinnata*, Bassi *et al.* (1990) in *Lemna minor* and *Pistia stratiotes* and Sangwan *et al.* (2013) in cluster bean. The carotenoids in plants can be degraded under Cr stress (Rai *et al.,* 1992). In the present study, total chlorophyll and carotenoid content were decreased at higher application of Cr (4-8 µg ml^{-1}). The reduction in chlorophyll contents under Cr stress may be due to degraded d-aminolevulinic acid dehydratase that affects the utilization of d-aminolevulinic acid (Vajpayee *et al.,* 2000) or thylakoid disarrangement/swelling of the chloroplast (Bassi *et al.* 1990).

Increased biosynthesis of anthocyanin under adverse conditions is known as a marker of stress in plants. It is one of the responses of triggered tolerance mechanisms in plants.The results revealed that cluster bean overproduced anthocyanin under Cr (VI) toxicity. It is evident from the results that Cr (VI) is toxic to cluster bean and all the studied concentrations were found to be toxic (Fig. 3). An increase in carotenoid content was observed under Cr application in *Vallisneria spiralis* and other aquatic plants (Tripati and Smith, 2000; Vajpayee *et al.,* 2001). Under Cr (VI) stress, plants produce reactive oxygen species which triggers the activation of the tolerance mechanism via the transcription of the anthocyanin biosynthesis gene to neutralize the toxicity (Naing and Kim, 2021). This excessive accumulation of anthocyanin may help plants to tolerate Cr (VI) toxicity by activating the antioxidant activity (Ai *et al.*, 2018). There are two possible pathway's behind the tolerance mechanism of cluster bean under Cr toxicity. One is production of excess ROS in apoplast and cyctoplasm under such conditions that drive PAL pathway to synthesize phenolic compound which help plants to cope up against heavy metal stress condition and another possible pathway is activated MAP kinase in increased ROS which is responsible for translation of some anthocyanin producing gene like LAP 1 (Legume Anthocyanin Producing gene) and increased anthocyanin content help the plant to survive against heavy metal stress condition (Fig. 4). It is evident from the results that Cr (VI) is toxic to cluster bean and all the studied concentrations were found to be toxic.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest

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