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Chromium toxicity and ultrastructural deformation of *Cicer arietinum* with special reference of root elongation and coleoptile growth

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ABSTRACT

Chromium (Cr) is a potent heavy metal which pollutes both soil and aquatic body. Therefore, it should be considered as a hazardous element. Cr can exists in various from in the environment, among them most stable from of chromium is Cr(VI). The present study demonstrate the adverse impacts of hexavalent chromium on *Cicer arietinum*, development and growth morphology, including elongation of coleoptyl, number of metabolic responses related to growth and ultra structural deformation of root and shoot. Exogenous application of Cr(VI) from 20 ppm to 100 ppm progressively inhibited seed germination and coleoptyl growth and dramatically damaged root aperture (xylem and phloem) even at the lowest dose. Results also revealed that the maximum reduction of germination was recorded at 80 mg/L Cr(VI) solution during 72 h of incubation. However, at 80 mg/L of Cr(VI) solution, coleoptyle growth reduces to 30% with respect to control. The chlorophyll ('a' and 'b' and total chlorophyll) level significantly declined by chromium treatment. On the other hand, SEM picture clearly indicate the distinct structural change of xylem and phloem with increasing the concentration of hexavalent chromium from 20 ppm to 100 ppm.

Introduction

The pollution load due to heavy metals is originated from industrial development, huge traffic, mining activity, etc. leads to the negative impact on plants community [1–3]. Chromium is considered one of the such heavy metal which pollute both soil and water and it mainly discharges from leather tanning, textile, electroplating and metal extraction factory [4]. Among the several oxidation state only two states of chromium (trivalent and hexavalent) are very stable [5]. Hexavalent chromium is considered the most toxic form of chromium, which normally combined with oxygen and form CrO_4^{2-} and Cr_2O^{2-} oxoanions. Trivalent chromium is less mobile, less toxic and strongly associated with soil organic matter. Scientific literature highlighted that trivalent chromium can be transformed to hexavalent chromium inside the plant cell organs and or in leaves. The accumulation of chromium in roots is 100 times more than other parts (shoot and leaf) of the plant [6,7]. Shallari et al. [8], collecting plants growing in serpentine soils, found that *Herniaria hirusta* was a Cr high-accumulator. Uptake and translocation of Cr from soil to plant by various crops are well documented [9–11]. Independent uptake mechanisms for Cr(VI) and Cr(III) have been reported in barley [5].

Anthropogenic activities are the main causes of chromium pollution in both soil and underground water over the last couple of years. According to Dotaniya et al. [2], the most adverse impact of chromium on overall growth pattern including germination, growth of root, shoot and leaves which directly or indirectly influence on whole plant biomass and yield. Moreover, it also causes tremendous negative impacts on harvesting of sunlight by green leaves, water balance and water uptake. Sanker et al. [5] reported that the toxicity of Cr is due to its mobilization and subsequent uptake in different plant parts.

Cicer arietinum is a legume of the family Fabaceae, subfamily Faboideae. It is a winter season crop but severe cold and frost are injurious to it. It is best suited to areas having moderate rainfall of 60–90 cm per annum. Currently, *Cicer arietinum* is grown in over fifty countries across the Indian subcontinent, North Africa, the middle East, Southern Europe, the Americas and Australia. Globally, *Cicer arietinum* is the third most important pulse crop in production, next to dry bean and field peas. India is the largest *Cicer arietinum* producing countries including Pakistan, Turkey, Australia, Mayanmar, Iran, Ethiopia, Mexico, Canada and the USA, with an average production of 6.38 million metric tons during 2006-9,

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accounting for 66% of global *Cicer arietinum* production. In order to supplement the exponential growth of population, more production of food grain is extremely inevitable. There is tremendous scarcity of potable water, as a matter of fact farmers are force to use industrial waste water for their crop production [12]. As most of the industrial waste water content substantial amount of heavy metals including chromium, the growth as well as production of yield significantly reduced and the quality of soil health hamper.

Keeping in view the increasing use of effluent water for irrigation and resultant excessive accumulation of chromium in different soil profiles, it was tremendous importance to evaluate their toxic effects on germination, root elongation, coleoptiles growth, and ultra structural deformation of gram seedlings.

Materials and methods

Experimental details

Present experiment executed at Environmental Chemistry Laboratory, Environmental Science Department, Burdwan University, India. In this experiment, five treatments (0, 20, 40, 80 and 100 mg/L) of chromium was applied on *Cicer aretinum*. Initially, the surface of seeds were sterilized with 0.1% mercuric chloride solution for 30 s and thoroughly washed with double distilled water. The sterilized seeds were used for germination experiment in a petridish (Diameter 10 cm) with above mentioned chromium concentration along with a control. After 24 h of incubation, germination was calculated in percentage. The better germination was considered if the radical length is 1 mm. The calculation for root and coleoptiles growth were measured at 48, 72, 96 and 120 h of incubation. Ultra structural deformations were recorded by using scanning electron micrograph (HITACHI-S-530) operating at an accelerating voltage of 20 KV.

Chlorophyll (a, b and total) analysis

For the estimation of pigment level in leaves, 0.1 g of fresh young leaves were collected from each treatment during the end of the experiment. The leaves were chopped into small pieces so that pigment can easily extracted during treatment with 80% acetone [13]. The extracted pigment concentration can be measured spectrophotometrically at different wave length (645, 652 and 663 nm). The concentration of chlorophyll can be express as $mg.g^{-1}$.f.w. For entire calculation following equation can be used (1–3):

$$chl'a'\left(mg.g^{-1}.f.w\right) = [12.7xD_{663} - 2.69xD_{645}]x\frac{v}{1000xw} \quad (1)$$

$$chl'b'\left(mg.g^{-1}.f.w\right) = [22.9xD_{645} - 2.69xD_{663}]x\frac{v}{1000xw} \quad (2)$$

$$TotalChl\left(mg.g^{-1}.f.w\right) = D_{652}x1000x\frac{v}{1000xw} \quad (3)$$

Here, D = absorbance, v = final volume of 80% acetone; w = mass of sample; fw = fresh weight of the sample.

Germination percentage

Seed germination was recorded daily up to 3 days after the initial day of the experiment. Seeds were considered as germinated the radical reached a length of 1 mm [14] and the germination percentage was calculated as per the following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}x100 \quad (4)$$

Coleoptile length, Root and shoot length (early seedling growth) Length of root and shoot and coleoptiles was measured with the help of a scale and reading was taken from both treated and controlled plants.

Biomass production

Fresh weight (FW, biomass) and dry weight (DW) of all experimental sets were recorded before starting the experiment and after 5 days of incubation. To measure dry weight, plants were dried at 80 °C [15] up to constant weight (usually 24 h).

Scanning Electron Microscopic (SEM) study

Root, shoot, nodule and leaf specimens were prepared for SEM study using the protocol adapted from standard procedures [16]. The fresh root and shoot samples (5 mm square from similar middle portion) from the control and Cr treatments were dissected and immediately fixed in a solution of 2% gluteraldehyde prepared in a 0.1 M sodium phosphate buffer (pH 7.0) for 12 h at room temperature. 25 mM sodium phosphate buffer (pH 6.8) was used for washing the plant specimens and temperature was kept at 4 °C for overnight and then dehydrated in absolute ethanol using 10 min series samples of 25%, 50%, 75%, 95% and 100% ethanol and then stored at −20 °C until examination. The specimens were rinsed, post fixed in 2% osmium tetraoxide, critical point dried and sputter coated with gold/palladium before being mounted on aluminum stubs. The specimens were viewed and photographed using a 20 KV scanning electron microscope (HITACHI-S-530).

Data analysis

The experiment was conducted by using randomized design (CRD) matrix with three replicates. All Statistical calculation was performed with MINITAB version 13 software. The experimental results were statistically assessed through analysis of variance (ANOVA). The significance of the experimental data were evaluated at the significance level at $p < 0.05$.

Results and discussion

Morphology of germinated seed

From Fig. 1 it is clear that gradual variation of both radical and coleoptiles length during 24 h of incubation. Moreover, colour of seed coat gradually changes from brown to chocolate. This is perhaps due to oxidation of phenolic compounds [17]. On the other

Fig. 1. Root elongation and coleoptyel growth under different concentration of Cr: **1**:control, **2**: 20 mg/L, **3**: 40 mg/L, **4**: 80, **5**: 100 mg/L.

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