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# Phytotoxic effect of chromium on the germination, seedling growth of some wheat (*Triticum aestivum* L.) cultivars under laboratory condition.

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A laboratory experiment was conducted to determine the phytotoxic effect of chromium on seed germination and seedling growth of some wheat cultivars (*Triticum aestivum* L.). Seeds of five wheat cultivars (HD2956, HD2932, DBW14, KO512, WH775) were treated under 25, 50, 75, 100 and 125 ppm of Cr(VI) concentration solutions individually. Each treatment were replicated thrice in a randomized block design. Observations were made on germination percentage, root and shoot length, fresh weight and dry weight of seedling, % phytotoxicity of root and shoot , sugar , protein and chlorophyll content in the leaves of both treated and control plants . Gradual increase in Cr (VI) concentration under various treatment significantly lead to inhibition of seed germination and other growth parameters. Percentage phytotoxicity showed an increasing trend with gradual increase in Cr VI concentration for all the wheat cultivars. Maximum inhibition of root growth were recorded. Attempts are being made in different laboratories to construct novel plants using genetic manipulation technologies that may have a greater tolerance o the presence of toxic metals. The result of the present study may help in understanding the mechanisms involved and their possible use in phytoremediation.

**Key words:** chromium, wheat cultivars, germination, phytotoxicity

## Introduction

Heavy metal phytotoxicity is considered to be main factor limiting plant growth, and thus crop cultivation in acid soils (Foy, 1988). Chromium is considered as a strong toxic element. Chromium ions are tightly bound to humus and clay particles and are more or less insoluble in the soil. Its availability in plants is therefore generally low but mobility and availability are relatively decreased with the increasing pH. Since seed germination is the first physiological process affected by chromium (Cr), the ability of seed to

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germinate in a medium containing Cr would be indicative of its level of tolerance to his metal (Peralta *et al.*, 2001) . When the concentration of the chromium in the oil reaches a threshold level, the ability of the plant to hold the Cr breaks down and thus the metal exerts their toxic effect in any system of cell metabolism and will kill the seed if it is present in large amount at that condition, sensitive species serve as an indicator and tolerant sp, which collect large amount of metals in their cell wall without damaging detected as accumulators (Bradshaw *et al.*, 1965). Cr (VI) is considered the most toxic form of Cr, which usually occurs associated with oxygen as chromate ($CrO_4^{2-}$) or dichromate ($Cr_2O^{-7}$) oxy anions that have a long residence time and high solubility in the water (Klieman and Cogliatts, 1998). Cr interferes with several metabolic process causing toxicity to the plants as exhibited by reduced root growth and phytomass chlorosis, photosynthetic impairing, stunting and finally plant death. (Huffman and Allaway, 1973; Kocik and Havsky,1994; Gardea – Torresday *et al*, 2004; Mengel and Kirkby, 1987) mentioned that 0.5 ppm of chromium as chromium sulphate stimulate growth in hydrophonic experiment with maize .They found that the growth was inhibited at 5ppm and strongly inhibited at 50 ppm . El-Bassam (1978) reported that low $Cr^{+3}$ concentration promote plant growth and also stimulate chlorophyll synthesis and photosynthetic activity. Hexavalent anionic form of chromium is several times as toxic as the trivalent cation $Cr^{+3}$. A number of studies have been conducted on relative root growth inhibition to define categories of sensitivity and to identify more tolerant cultivar of wheat (Basu *et al.*,1994; Delhaize *et al.*, 1993a,b).Wheat (*T.aestivum* L) is the staple food for a large part of the world population including India.. With increasing population the over demanding production of wheat can be increased either by bringing more area under wheat cultivation or by introducing high yielding wheat varieties which are resistant against biotic and abiotic stresses. The present study was carried out to determine the effect of chromium (Cr VI) on seed germination and seedling growth of five wheat varieties.

## Materials and methods

The seeds of five wheat cultivars HD2956, HD2932, DBW14, KO512, WH775 were collected from Field Crop Research Station, Department of Agriculture, Government of West Bengal. The collected seeds were kept at room temperature in air tight packets.

The source of Cr (VI) was $K_2Cr_2O_7$. The treatment concentration viz, $T_1$-25 ppm, $T_2$-50 ppm, $T_3$-75 ppm, $T_4$-100 ppm,$T_5$-125 ppm were prepared from stock solution (1000ppm)along with a control ($T_6$) .

The seeds were surface sterilized with 0.1% $HgCl_2$ for 30seconds and then washed with fresh water, followed by distilled water. 180 healthy and uniform sized seeds were selected and sown at equal distance in a petridish lined with filter paper. 10ml of test solution was added to each petridishes and kept inside the germination cage. The petridishes were kept moist by regularly adding 5ml of test solution.

The rate of seed germination was recorded for every 24 hours upto five days (ISTA, 1976). The petridishes were covered with a net and kept in a growth room under optimum temperature and light condition. The length of shoot and root were recorded by using a centimeter scale, % Phytotoxicity for shoot and root of 7day old seedlings were calculated by the following formula (Chou and Lin, 1976)

$$\% \text{ Phytoxicity} = \frac{\text{Shoot or root length of control - Shoot or root length of treatment X100}}{\text{Shoot or root length of control}}$$

For fresh weight 7day old seedlings were collected and soaked in blotting paper for removal of excess water and the final weight was recorded. The dry mass of shoot and root was recorded from 7day old seedlings after keeping them in an oven at 80$^{o}$C for 72 hrs.

For biochemical estimation of Total chlorophyll (Arnon, 1949), protein (Lowry *et al.*, 1951), sugar (Mc Cready, 1950) were mediated from the leaves of the plants under various treatments of Cr (VI) solutions using standard methods.

Observed data were analysed statistically by one-way ANOVA and for interpretation of the experimental results Standard methods (Cochran and Cox, 1959; Panse and Sukhatme, 1967) were consulted. For interpreting the effect of different treatments under different cases, treatment means were compared through Duncan's Multiple Range Test (DMRT).

## Results

Seeds of five varieties of wheat germinated well under all treatments (Table 1-5). All the seeds germinated next day. Seed germination of all the varieties was reduced at different concentration as compared to control (Table 1-5). Control showed 100% germination whereas the germination % ranged from 65-80% for HD-2956, 60-95% for HD-2932, 75-90% for DBW-14, 65-100% for KO512, 65-95% for WH 775.

Results showed that four wheat varieties (HD2956, HD2932, KO512, WH775) showed significantly ($p<0.05$) affected root, shoot length in comparison to control in all the five varieties of wheat (Table 1-5). Again the

deleterious effect was more pronounced in case of roots in all the varieties of root. At 75, 100, 125 ppm of treatments the root growth of variety HD-2956, HD-2932 were completely inhibited (Table 1 and 2) whereas Cr (VI) treatments of 100 ppm and 125 ppm showed complete inhibition of root growth in varieties DBW14, KO512 and WH775 (Table 3, 4 and 5) .

The % phytotoxicity of shoot of five wheat (*Triticum aestivum*) cultivars under Cr (VI) treatment showed an increasing trend with increasing Cr (VI) concentration in case of all wheat cultivars. The highest % phytotoxicity value of shoot was found at 25 ppm Cr (VI) concentration. In case of % phytotoxicity of root similar observation was found but highest % phytotoxicity was found at 100 ppm Cr (VI) concentration in all the varieties (Table1-5). The different graded dose of Cr (VI) concentration significantly ($P>0.05$) contributed towards % phytotoxicity of root and shoot.

Among the different biochemical parameters sugar content showed an increasing trend with increasing Cr (VI) concentration in all varieties of wheat. The maximum sugar concentration was observed in 75 ppm, and minimum was observed in control condition. The Cr (VI) concentration significantly ($P<0.05$) contributed towards the increase in the sugar concentration. Protein content showed a decreasing trend with increasing concentration of Cr (VI) concentration. The lowest protein concentration was observed in 25 ppm in case of all wheat varieties. Here also there is significant contribution of Cr (VI) concentration towards increase in the protein content in the leaves under different treatments and control conditions. The total chlorophyll content was found highest in case of control conditions and the total chlorophyll content decreased with increasing concentration of Cr (VI) solutions in all the varieties significantly (Table1-5).

## Discussion

All the five wheat varieties HD2956, HD2932, DBW14, KO512, WH775 showed similar reduced response in germination with subsequent increase in Cr concentration Cr (VI) concentration of 100 ppm, 125 ppm significantly inhibited the germination of seeds of all the wheat cultivars. The reduced germination of seeds under Cr stress would be due to the depressive effect of Cr on the subsequent transport of sugars to the embryo axis (Zeid, 2001). Protease activity increases simultaneously with the chromium treatment which could also contribute to the reduction in germination of chromium treated seeds. (Zeid, 2001).

The growth of all the five wheat cultivars showed inhibitory effect with increasing concentration of Cr (VI) solutions. Chromium was found to be more toxic affecting root, shoot length .The reduction in the plant height might be

mainly due to the reduced root growth and consequent lesser nutrient and water transport to the above parts of the plant. In addition to this, chromium transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction of plant height (Shankar *et al.*, 2005). Root was found to be more affected than shoot. This is due to the fact that heavy metals (Cr-VI) accumulated on root due to binding of metals (Cr-VI) on the cell wall of root and retard cell division and cell elongation (Woolhouse, 1983). General decreased root growth due to chromium toxicity could be due to inhibition of root cell division/root elongation or to the extension of cell cycle in the roots. The reason of the high accumulation in roots of the plants could be because chromium is immobilized in the vacuoles of the root cells, thus rendering it less toxic , which may be a natural toxicity response o plant.(Shankar *et al.*, 2004) Also heavy metals have been reported to impair the growth of new roots and seedling establishment. (Rellén-Álvarez *et al.*, 2006)

Percentage phytotoxicity of root and shoot showed increasing trend with increasing Cr (VI) concentration in case of all wheat cultivars which may be due to the Cr (VI) stress upon the plants of five wheat varieties.

Total chlorophyll content decreased with increasing concentration of Cr (VI). Fom the experimental results of the present investigation the increased total chlorophyll content at the lower level of Cr (VI) was obviously due to better growth. The formation of chlorophyll pigment depends on the adequate supply of iron as it is the main component of the protoporphyrin, a precursor of chlorophyll synthesis. An excessive supply of chromium seems to prevent the incorporation of iron into the protoporphyin molecule, resulting in the reduction of chlorophyll pigment. Our findings corroborates with earlier findings of Bera *et al*. (1999). Again the protein content in the leaves of wheat plants decreased which may be due to decrease in the nitrogen content and as nitrogen is the precursor for the synthesis of amino acids which are the building blocks of protein in case of rice plants. (Nag *et al.,*1981). Again the increase in sugar content in the leaves under different treatment of Cr (VI) salt solutions might be to overcome the Cr stress on plants by increasing carbohydrate synthesis.. Mechanism of resistance in plants continues to remain to be poorly understood. Such resistance arises either by the plant ability to exclude heavy metals in roots or its ability to detoxify heavy metals with in the plants (Hall, 2000; Kochian, 1995; Rellen-alvarez *et al.*, 2006). Detoxification also depends upon organic compounds present in soil and inside plants. Wheat plants are very sensitive to this heavy metals as they have very little external ability to detoxify the toxic metals .wheat seeds were germinated in the petriplates which were devoid of other minerals. The metals applied did not get detoxified and were absorbed by plants which resulted in the inhibition of root, shoot length.

**Table 1.** Effect of Chromium on germination and growth of HD 2956 wheat cultivar.

| Treatment | Conc (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | %Phytot oxicity of shoot | %Phytot oxicity of root | Sugar (mg/g) | Protein (mg/g) | Total Chlorophyll (mg/g fw) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | $100^{a}$ | $10.5^{c}$ | $9.74^{a}$ | $0^{f}$ | $0^{d}$ | $0.0007^{d}$ | $0.0086^{a}$ | $0.196^{a}$ |
| Cr(VI) | 25 | $100^{a}$ | $8.36^{b}$ | $2.29^{b}$ | $20.38^{e}$ | $75.81^{c}$ | $0.0032^{b}$ | $0.0079^{ab}$ | $0.1811^{b}$ |
| | 50 | $100^{a}$ | $6.53^{c}$ | $0.87^{c}$ | $37.8^{d}$ | $90.81^{b}$ | $0.0044^{d}$ | $0.0071^{bc}$ | $0.1666^{c}$ |
| | 75 | $90^{b}$ | $5.15^{d}$ | $0^{d}$ | $50.95^{c}$ | $100^{a}$ | $0.0064^{a}$ | $0.0064^{d}$ | $0.1231^{d}$ |
| | 100 | $80^{c}$ | $3.98^{e}$ | $0^{d}$ | $62.09^{b}$ | $100^{c}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |
| | 125 | $65^{d}$ | $2.86^{f}$ | $0^{d}$ | $72.76^{a}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT).

**Table 2.** Effect of Chromium on germination and growth of HD 2932 wheat cultivar.

| Treatment | Conc (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | %Phytot oxicity of shoot | %Phyto toxicity of root | Sugar (mg/g) | Protein (mg/g) | Total Chlorophyll (mg/g fw) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | $100^{a}$ | $12.65^{a}$ | $13.31^{a}$ | $0^{d}$ | $0^{d}$ | $0.0057^{d}$ | $0.0083^{a}$ | $0.417^{a}$ |
| Cr(VI) | 25 | $95^{b}$ | $7.94^{b}$ | $1.53^{b}$ | $37.23^{c}$ | $88.5^{c}$ | $0.0117^{c}$ | $0.0077^{b}$ | $0.371^{b}$ |
| | 50 | $90^{c}$ | 6.48c | $0.88^{c}$ | $48.77^{b}$ | $93.38^{b}$ | $0.0140^{b}$ | $0.0071^{c}$ | $0.255^{c}$ |
| | 75 | $85^{d}$ | $5.33^{d}$ | $0^{d}$ | $57.86^{a}$ | $100^{a}$ | $0.0156^{a}$ | $0.0061^{d}$ | $0.122^{d}$ |
| | 100 | $65^{e}$ | $3.71^{e}$ | $0^{d}$ | $70.67^{ab}$ | $100^{ab}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |
| | 125 | $60^{f}$ | $2.93^{f}$ | $0^{d}$ | $76.83^{ab}$ | $100^{ab}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT).

**Table 3**. Effect of Chromium on germination and growth of DBW14 wheat cultivar.

| Treatment | Conc (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | %Phytot oxicity of shoot | %Phytot oxicity of root | Sugar (mg/g) | Protein (mg/g) | Total Chlorophyll (mg/g fw) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | $95^{a}$ | $13.02^{a}$ | $14.88^{a}$ | $0^{d}$ | $0^{e}$ | $0.0056^{d}$ | $0.0105^{a}$ | $0.5536^{a}$ |
| Cr(VI) | 25 | $90^{b}$ | $9.19^{b}$ | $2.45^{b}$ | $29.41^{e}$ | $83.35^{d}$ | $0.0080^{c}$ | $0.099^{b}$ | $0.3594^{b}$ |
| | 50 | $90^{bc}$ | $6.73^{c}$ | $1.00^{c}$ | $48.31^{d}$ | $93.27^{bc}$ | $0.0099^{b}$ | $0.0091^{c}$ | $0.1768^{c}$ |
| | 75 | $90^{bcd}$ | $5.75^{d}$ | $0.61^{d}$ | $55.83^{c}$ | $95.5^{b}$ | $0.0109^{a}$ | $0.0083^{d}$ | $0.0695^{d}$ |
| | 100 | $75^{de}$ | $3.79^{e}$ | $0^{d}$ | $70.89^{b}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |
| | 125 | $75^{de}$ | $2.3^{f}$ | $0^{d}$ | $82.33^{a}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT).

**Table 4**. Effect of Chromium on germination and growth of KO512 wheat cultivar.

| Treatment | Conc (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | %Phyto toxicity of shoot | %Phytotoxicity of root | Sugar (mg/g) | Protein (mg/g) | Total Chlorophyll (mg/g fw) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | $100^{a}$ | $12.54^{a}$ | $17.38^{a}$ | $0^{f}$ | $0^{e}$ | $0.018^{d}$ | $0.018^{a}$ | $0.915^{a}$ |
| Cr(VI) | 25 | $100^{b}$ | $7.28^{b}$ | $2.05^{b}$ | $41.94^{e}$ | $88.2^{d}$ | $0.025^{c}$ | $0.015^{b}$ | $0.614^{b}$ |
| | 50 | $95^{c}$ | $6.36^{c}$ | $0.845^{c}$ | $49.28^{d}$ | $95.13^{c}$ | $0.035^{b}$ | $0.012^{c}$ | $0.455^{c}$ |
| | 75 | $90^{d}$ | $5.97^{d}$ | $0.48^{d}$ | $52.39^{c}$ | $97.23^{b}$ | $0.046^{a}$ | $0.010^{d}$ | $0.376^{d}$ |
| | 100 | $80^{e}$ | $4.97^{e}$ | $0^{e}$ | $60.36^{b}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |
| | 125 | $65^{f}$ | $2.53^{f}$ | $0^{e}$ | $79.82^{a}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT).

**Table 5**. Effect of Chromium on germination and growth of WH775 wheat cultivar.

| Treatment | Conc (ppm) | Germination (%) | Shoot length (cm) | Root length (cm) | %Phytotoxicity of shoot | %Phytotoxicity of root | Sugar (mg/g) | Protein (mg/g) | Total Chlorophyll (mg/g fw) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | $100^{a}$ | $12.94^{a}$ | $17.23^{a}$ | $0^{e}$ | $0^{f}$ | $0.008^{d}$ | $0.017^{a}$ | $0.881^{a}$ |
| Cr(VI) | 25 | $95^{b}$ | $7.79^{b}$ | $2.29^{b}$ | $39.79^{d}$ | $86.7^{e}$ | $0.035^{c}$ | $0.015^{b}$ | $0.597^{b}$ |
| | 50 | $95^{bc}$ | $6.37^{c}$ | $0.876^{c}$ | $50.77^{c}$ | $94.91^{d}$ | $0.047^{b}$ | $0.013^{c}$ | $0.414^{c}$ |
| | 75 | $95^{bcd}$ | $6.0^{d}$ | $0.47^{d}$ | $53.63^{b}$ | $97.27^{c}$ | $0.068^{a}$ | $0.011^{d}$ | $0.330^{d}$ |
| | 100 | $80^{e}$ | $4.39^{e}$ | $0^{e}$ | $60.07^{e}$ | $100^{a}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |
| | 125 | $65^{f}$ | $2.016^{f}$ | $0^{e}$ | $83.3^{a}$ | $100^{ab}$ | $0^{e}$ | $0^{e}$ | $0^{e}$ |

Means followed by the same letter (S) within treatment are not significantly different at 5% using Duncan's multiple range test (DMRT).

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