Research Paper Botany

Phytotoxic Effect of Hexavalent Chromium on Total Protein, Fatty Acid and Dietary Fibre Content of Amaranthus Dubius Mart.ex Thell

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ABSTRACT

Hexavalent chromium (CrVI) is a serious environmental pollutant and contamination of soil and water by this metal is a matter of serious discussion. Toxic effects of Cr on plant growth and development which affect the total dry matter production and yield is due to its deleterious effects on plant physiology and metabolism. The present study was aimed at assessing the phytotoxic effect of hexavalent chromium on Amaranthus dubius Mart. Ex Thell raised over standard potting mixture which is amended with different concentration levels of hexavalent chromium. Potassium dichromate salt (K2Cr2O7) was used as the source of chromium. The parameters considered and analyzed for the study includes fatty acid, total protein and total dietary fibre content. The investigation reveals fatty acid and total dietary fibre content of A. dubius is having a negative correlation with concentration of metal chromium in the potting mixture. However, the overall protein content exhibited a positive correlation during lower level concentration treatments whereas negative correlation was observed at higher levels

KEYWORDS Amaranthus dubius Mart. Ex Thell, hexavalent chromium stress, fatty acid, total protein and total dietary fibre

INTRODUCTION

Chromium is a toxic and nonessential element to plants and hence they do not possess specific mechanisms for its uptake. However, plants uptake this heavy metal through carriers used for the uptake of essential metals for plant metabolism. Soil and ground water contamination due to heavy metal chromium resulting from various anthropogenic activities has become a serious source of concern to plant and animal scientists over the past decade. The impact of Cr toxicity on the physiology and metabolism of plants depends on the metal speciation, which determines its uptake, translocation, accumulation and resultant toxicity in the plant system. Hexavalent chromium (Cr^{VI}) is considered the most toxic form of Cr as it is a strong oxidant with a high redox potential in the range of 1.33–1.38 eV accounting for a rapid and high generation of ROS and its resultant toxicity (Shanker et al., 2004a, b). Hexavalent chromium compounds are used in industries for metal plating, leathering, tanning, wood preservation etc. and these anthropogenic activities have led to the widespread contamination and have increased its bioavailability and biomobility (Arun et al., 2005). However, in contrast to other toxic trace metals like mercury, lead, cadmium etc, Cr has not received much attention from plant scientists. The aim of the present study was to investigate the effects of hexavalent chromium on the content of fatty acid, total protein and total dietary fibre in ***Amaranthus dubius*** Mart. Ex Thell.

MATERIALS AND METHODS

Healthy seeds of ***Amaranthus dubius*** collected from Kerala Agricultural University, Thrissur is subjected to germination and healthy germinated seeds which emerge out of the growing media are transferred carefully into plastic pots containing standard potting mixture prepared by using farmyard manure, red soil and sand in the ratio 1:1:1. One week after transplantation, healthy uniform sized and aged seedlings are selected in triplicate blocks of 30 each for each treatment. Treatments include eight different specific concentration levels of heavy metal chromium which are selected based on a preliminary germination study. Different concentrations include 1mg Cr/kg potting mixture, 5mg Cr/kg potting mixture, 10mg Cr/kg potting mixture, 20mgCr/kg potting mixture, 30mg Cr/kg potting mixture, 50mg Cr/kg potting mixture, 70mg Cr/kg potting mixture and 0.00mg Cr/kg potting mixture (control). Different concentrations of metal chromium are prepared from potassium dichromate salt ($K_2Cr_2O_7$) of an analytical reagent grade. The required concentrations of chromium are applied to the potting mixture as aqueous solution in such a way that every 200ml contains required quantities of chromium. 200ml of distilled water alone is used as control (0.0mg Cr/200ml). Plant materials for analysis were collected from tender shoot parts of all treated plants on twentieth day of metal treatment. Different parameters such as fatty acid content, total protein content and total dietary fibre content were selected and analyzed for all the Cr treated as well as the control plants of ***A. dubius,*** to determine the impact of different levels of Cr^{VI} stress.

Estimation of Fatty Acids Content

Fatty acid content was estimated by the method of Cox and Pearson (1962). Dissolve 1 to 10g of sample in 50ml of the neutral solvent in a 250 ml conical flask. Add a few drops of 1% phenolphthalein. Titrate the content against 0.1N potassium hydroxide. Shake constantly until a pink color, which persists for fifteen seconds, is obtained.

Calculation

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titer value x Normality of KOH x 56.1}}{\text{Weight of the sample (g)}}$$

Fatty acid is calculated as oleic acid using the equation 1ml N/10KOH =0.028g oleic acid.

Estimation of Total Protein Content

Protein was estimated by the method of Lowry et al. (1951). 100 mg plant material was homogenized with 3 ml of 10% trichloroacetic acid. The homogenate was centrifuged at 10,000 rpm and supernatant was discarded. The pellets obtained after centrifugation were treated with 3 ml 1 N NaOH, heated on water bath for 7 minutes and cooled down to room temperature. Again, the solution was centrifuged for five to ten minutes at 5000 rpm. 5 ml reagent containing 100 parts of 2% solution of sodium carbonate and one part of 2% solution of sodium potassium tartrate was added to

0.5 ml of supernatant thus obtained after centrifugation and allowed to stand for ten to fifteen minutes. Then 5 ml Folin and Ciocalteu's Phenol reagent (diluted with distilled water in ratio of 1:1) was added and allowed to stand for half an hour for development of colour, and then finally absorbance was measured at 700 nm.

Estimation of Total Dietary Fibre Content

Total dietary fibre (TDF) content was estimated by the method followed by Prosky et al. (1988). With each assay, run blanks along with samples to measure any contribution from reagents to residue. Weigh out duplicate 1.0g samples accurately into 400 ml tall-form beakers. Add 40 ml MES-TRIS blend buffer solution (pH 8.2) to each beaker. Stir on magnetic stirrer until sample is completely dispersed in solution. Incubation with heat-stable α-amylase: Add 50 µl heat-stable α-amylase solution, while stirring at low speed. Cover each beaker with aluminium foil and incubate for 30 min with continuous agitation in a shaking water bath at 98-100°C. Remove all sample beakers from shaking water bath and cool to 60°C. Scrape any ring around beaker and gels in bottom of beaker with spatula and rinse side wall of beaker and spatula with 10 ml distilled water by using pipette. Adjust temperature of water bath to 60°C by draining some of hot water from water bath and adding cold water. Add 100 µl protease solution to each sample. Cover with aluminium foil and incubate for 30 min with continuous agitation in a shaking water bath at 60±1°C. Remove sample beakers from shaking water bath. Dispense 5 ml of 0.56 1N HCl solution into sample while stirring. Check pH and adjust to 4.1-4.8 by adding either 5% NaOH solution or 5% HCl solution. Add 200 µl amyloglucosidase solution while stirring on magnetic stirrer. Cover with aluminium foil and incubate for 30 min with continuous agitation in a shaking water bath at 60±1°C. To each sample, add 225 ml 95% EtOH pre-heated to 60°C. Ratio of EtOH volume to sample volume should be 4:1. Cover all samples with aluminium foil and incubate at room temperature for 60 min to allow precipitate to form. Tare crucible containing Celite to nearest 0.1 mg. Wet and redistribute the bed of Celite in the crucible, using 15 ml of 78% EtOH from wash bottle. Apply suction to crucible to draw Celite onto fritted glass as an even mat. Quantitatively transfer all the precipitate and suspension from each beaker by using a wash bottle with 78% EtOH and a rubber spatula, to its respective crucible. Use vacuum and wash the residue successively with two 15 ml portions of (a) 78% EtOH, (b) 95% EtOH and (c) Acetone. Dry the crucible containing residue overnight in an air oven at 103°C. Cool crucible in desiccators for approximately 1 h. Weigh crucible containing dietary fibre residue and Celite to nearest 0.1 mg. To obtain residue weight, subtract tare weight, i.e. weight of dried crucible and Celite. Protein and ash determination: One residue sample and the corresponding blank are analyzed for protein using Kjeldahl method. Use 6.25 factors for all cases to calculate gram of protein. For ash analysis, incinerate the second residue sample and corresponding blank for 5 hours at 525°C. Cool in desiccators and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash content.

Calculations:

$$\text{Dietary Fibre (\%)} = \frac{\frac{R1+R2}{2} - p - A - B}{\frac{m1+m2}{2}} \times 100$$

Where:

R1 = residue weight 1 from m1; R2 = residue weight 2 from m2; m1 = sample weight 1; m2 = sample weight 2; p = protein weight from R_1; A = ash weight from R_2 and B = blank

$$B = \frac{BR_1 + BR_2}{2} - BP - BA$$

Where:

BR = blank residue; BP = blank protein from BR1 and BA = blank ash from BR2.

RESULT AND DISCUSSION

Effect of different concentrations of hexavalent chromium (Cr^{VI}) on the content of fatty acid, total protein and total dietary fibre in ***Amaranthus dubius*** Mart. Ex Thell is shown in table 1. Fatty acid content showed a decreasing trend with progressive increase in Cr concentration. Reduction in fatty acid content was more prominent from a concentration of 20 mg Cr treatment per kg potting mixture onwards. Maximum reduction was recorded at the highest concentration of 70mg Cr/ kg potting mixture while the least reduction was recorded at 5mg Cr/ kg potting mixture and was 34.2% and 2.1% respectively over control; however the treatment of 1mg Cr did not contribute to any change in concentration of fatty acid content (Figure 1). The decrease in fatty acid content in ***A.dubius*** observed in the present study when subjected to increasing concentration of heavy metal Cr may be attributed to the decomposition of polyunsaturated fatty acids under oxidative stress. This may be considered as one of the potential parameter due to heavy metals toxicity. The present inferences are in similar lines with the observations of Le Guédard et al. (2008) in ***Lactuca sativa*** and Fikriye (2013) in ***phaseolus vulgaris*** seedlings which are exposed to different concentration of heavy metals including chromium, showed a decrease in fatty acid composition linearly with increased heavy metal concentration.

TABLE 1
Effects of different concentrations of hexavalent chromium (Cr^{VI}) on the content of fatty acid, total protein and total dietary fibre in *Amaranthus dubius*

Treatment Cr^{VI} (mg/kg soil)	Fatty acid content calculated as oleic acid (g)	Total Protein content (mg/g)	Total dietary fibre content (%)
1mg	0.187±0.012	18.07±0.12	43±0.82
5mg	0.183±0.012	18.2***±0.14***	42.67±0.47
10mg	0.180±0.014	17.8±0.16	41.67±0.94
20mg	0.160±0.009	17.13±0.48	34±3.27
30mg	0.140±0.016	11.4±1.39	28.33±3.40
50gm	0.130±0.163	6.56±0.94	26.33±2.62
70mg	0.123±0.012	5.9±0.50	26± 1.63
Control	0.187±0.005	18.03***±0.12***	43.33±0.47

The data presented in the table 1 indicates an increasing trend in total protein content with lower level concentrations and decreasing trend with higher level concentrations of hexavalent chromium (Cr^{VI}) in ***A. dubius***. Slight increase in the overall protein content recorded in 1mg Cr/ kg potting mixture and 5mg Cr/kg potting mixture was 0.22% and 0.94% respectively over control. Stress due to lower level concentrations of heavy metal has been reported to induce a variety of proteins including various enzymes and proline, resulting in an overall increase in protein content (Shah and Dubey. 1997). However the study reveals further increase in the concentration of metal Cr found to cause reduction in total protein content and maximum reduction was recorded at treatment of 70mg Cr/kg potting mixture and was 67.28% reduction over control (Figure 2). Decrease in total protein content in ***A.dubius*** observed at higher level concentrations may be because of enhanced protein degradation process under increased protease or other catabolic enzyme activities together with induced lipid peroxidation and fragmentation of proteins due to increased Cr toxicity. Decrease in the synthesis of proteins due to disturbance in nitrogen metabolism under Cr toxicity may also be the reason contributed (Auda and Ali, 2010).

Figure 3 and table 1 summarize the results for the effects of different concentrations of hexavalent chromium (Cr^{VI}) on total dietary fibre content of ***A.dubius.*** Data reveals a negative correlation exist between total dietary fibre content and concentration of heavy metal Cr. The present investigation observed

the maximum TDF content was obtained in lowest concentration of 1mg Cr/kg potting mixture (43±0.82%) which was 0.76% decrease over control and further decreases as the concentration of Cr increases. The decrease was more prominent from treatment of 20mg Cr/kg potting mixture onwards and the lowest TDF content was recorded in plants treated with highest concentration of 70mg Cr/kg potting mixture and it was about 40% decrease over control. The present study results are in conformity with earlier findings of Gill and Saggoo (2010) in turnip plants and Emmanuel et al. (2013) in eight different edible vegetable plants, when subjected to chromium stress. Decrease in plant growth due to low utilization of nitrogen together with reduction in photosynthesis and degradation of important cellular components under oxidative stress resulting from Cr toxicity might be the reason for reduction in TDF content.

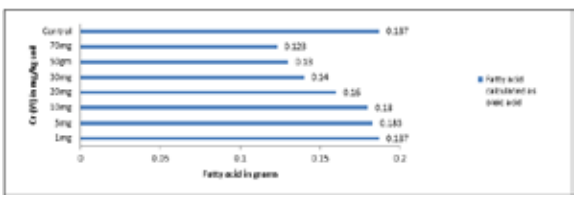

Figure1: Changes in fatty acid content of *Amaranthus dubius* under different concentrations of Cr^{VI}. Data are mean of 3 replicates

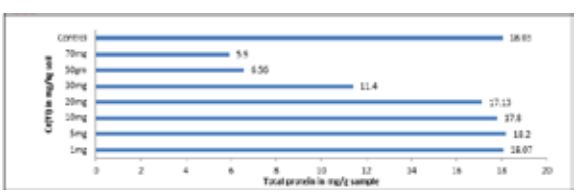

Figure 2: Changes in total protein content of *Amaranthus dubius* under different concentrations of Cr^{VI}. Data are mean of 3 replicates

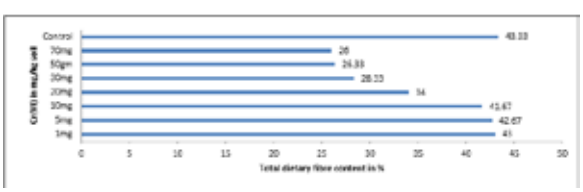

Figure 3: Changes in total dietary fibre content of *Amaranthus dubius* under different concentrations of Cr^{VI}. Data are mean of 3 replicates

Figure 4: Difference in growth performance of *Amaranthus dubius* at 20th day after treatment with different concentrations of hexavalent chromium (Cr^{VI})

CONCLUSION

In the present study, exposure to hexavalent chromium (Cr^{VI}) affected different parameters of ***Amaranthus dubius*** Mart. Ex Thell: fatty acid content, overall protein content and total dietary fibre content. Exposure of ***A. dubius*** seedlings to Cr^{VI} decreased all the parameters selected for the study with an exception during the low level concentration treatment with respect to overall protein content which might be due to the induction of certain proteins including enzymes and proline by the plant as an attempt to resist the oxidative stress due to chromium toxicity. The decreasing trend was found to increase as the concentration of the metal chromium is increasing in the growing media and hence the investigation clearly reveals, Cr^{VI} is toxic to ***A. dubius.*** The degree of toxicity is concentration dependant and as the concentration of metal chromium is increasing, the quality as well as the yield of the plant is severely affected.

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