Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

Accumulation of chromium and its effects on physiological and biochemical parameters of *Alternanthera philoxeroides* seedlings

*Sindhujaa Vajravel**, *Poornima Saravanan*

Dept. of Plant Morphology and Algology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625021, India

ARTICLE INFO

Article history:
Received 24 April 2013
Accepted 22 July 2013
Available online 22 August 2013

Keywords:
Antioxidative enzymes
Alternanthera philoxeroides
Chromium

ABSTRACT

Aim: To assess the effects of different concentrations of chromium (25; 50; 100; 150 mg/l) in the plant, *Alternanthera philoxeroides*.

Method: Different concentrations of chromium (25; 50; 100; 150 mg/l) were applied for 12 days and assessed by measuring changes in the growth; photosynthetic pigments activities; and antioxidative enzymes: catalase (CAT); peroxidase (POD); ascorbate peroxidase (APX) and total soluble protein changes. Metabolic responses to chromium (Cr) exposure and metal uptake were also experimentated.

Results: It was found that chromium was accumulated in shoots and roots of *A. philoxeroides*. The shoots accumulated 111.27 mg Cr/g of their dry weight at 150 mg/l Cr concentration, while the roots accumulated 751.71 mg Cr/g. The photosynthetic pigment contents increased with the higher concentration of Cr. Both in shoots and roots Cr could induce rise of the activity of CAT; POD and APX. The total soluble protein contents also increased with the increased concentration of Cr.

Conclusion: The results from the present experiments suggest that high concentrations of Cr cause oxidative damage as evidenced by increased antioxidative enzymes, photosynthetic pigments and changes in total soluble protein content. Induction of antioxidative enzymes could the reason for tolerating higher levels of metals by *A. philoxeroides* plants.

1. Introduction

Chromium is one of the toxic metals of wide spread use. The International Agency for Research on Cancer (IARC) has reported that Cr (VI) is carcinogenic to humans and in addition it can cause liver damage; pulmonary congestion and causes skin irritation resulting in ulcer formation. It is mostly used in many industries such as wood preservation, leather tanning, electroplating and steel productions.[1,2] Phytoremediation is a promising cleanup technology for contami

* *Corresponding author*. Tel.: +91 9952350916 (mobile).
E-mail address: Sindhujaapsr@gmail.com (S. Vajravel).

0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.07.028

nated soils, groundwater and waste water that is both low-tech and low-cost. *Alternanthera philoxeroides* is one of the aquatic macrophytes which are commonly known as alligator weed. It coexists abundantly in natural habitat all over the world. Therefore it can be used as a convenient plant material for heavy metal toxicity investigations.[3] In many reports chromium has been demonstrated to induce the formation of reactive oxygen species (ROS) and free radicals (FR) in plants such as hydrogen peroxide (H_2O_2) hydroxyl radicals (•OH) and superoxide radicals (O_2^-•); either by direct electron transfer involving metal cations or as a consequence of metal mediated inhibition of metabolic reactions.[4] Free radicals can cause oxidative damage to the biomolecules such as lipids, proteins and nucleic acids.[5] To avoid this kind of cellular damage, plants posses a complex system of antioxidative enzymes like catalase, peroxidase and ascorbate peroxidase. Those play a major to tolerate the plants by scavenging ROS produced under heavy metal stress.[6]

The present study was undertaken to examine Accumulation of Chromium and its Effects on Physiological and Biochemical Parameters of *Alternanthera philoxeroides* Seedlings under hydroponic systems.

2. Research methods

2.1. Plant collection and chromium treatment

Alternanthera philoxeroides were collected and then washed several times in running tap water to wash out the soil particles from plants. Approximately same height and weights of plants were carefully selected and transferred into plastic container filled with full strength Hoagland Nutrient Solution for hydroponic settings.[7] The hydroponic system was set up in the Green House. After 12 days both the root and shoot lengths of hydroponically growing plants were determined and treated with Cr (potassium dichromate) in different concentrations 0; 25; 50; 100; 150 mg/l; while medium without these heavy metals served as control. The physiological and biochemical parameters were investigated after 12 days of Cr treatment.

2.2. Physiological parameters

2.2.1. Growth parameters

Both shoot and root lengths were measured before and after treatment of Cr in *A. philoxeroides* seedlings. The biomass was estimated by the measurement of shoot and root dry weight. Index of tolerance (IT) for the root and shoot was calculated. Water content of leaves was calculated, using the values obtained from fresh and dry weights of Cr treated plants, according to (FW-DW)*100/FW.[8]

2.2.2. Photosynthetic pigment assay

A. philoxeroides leaf tissues samples (100 mg) were extracted in ice – cold pestle and mortar with 2 ml of 80% acetone (v/v) as described by Arnon.[9] Leaf extracts were centrifuged at 5000 rpm for 10 min and upper layer was collected for chlorophyll a/b and carotenoid estimation. The absorbance was measured at 470; 645; 663 nm in the UV–Visible spectrophotometer. The cholorophyll pigments and carotenoids were estimated according to the standard calculations.

$$\text{Chl a} = [(13.95A_{665} - 6.88A_{649}) \times 10]/100;$$
$$\text{Chl b} = [(24.96A_{649} - 7.32A_{665} \times 10)/100];$$
$$\text{Car} = [(1000A_{470} - 2.05\text{Ca} - 114.8\text{Cb})/245] \times 10/100$$

2.2.3. Chromium accumulation analysis by ICP-AES

The Cr heavy metal accumulation was analysed by ICP-AES.[10]

2.3. Biochemical assays

2.3.1. Ascorbate peroxidase assay (APX)

APX activity was determined according to the method mentioned by Nakano and Asada.[11] The reaction mixture used for this assay contained 50 mM phosphate buffer (pH 7.8); 0.5 Mm ascorbic acid 0.1 mM EDTA; 65 Mm H_2O_2; enzyme extract and distilled water. The oxidation of ascorbic acid was at 290 nm absorbance for 30 s using UV–visible spectrophotometer (Double Beam Spectrophotometer 2203).

2.3.2. Assay of catalase (CAT)

The CAT activity was performed by Aebi method.[12] The reaction mixture used for this assay; 50 mM phosphate buffer (pH 7.8); 75 mM H_2O_2, enzyme extract and distilled water. The reaction was started by adding H_2O_2 and CAT activity was at 240 nm absorbance.

2.3.3. Assay of peroxidase (POD)

POX activity was measured using Castillo et al, method.[13] The 3 ml of reaction mixture contained; 50 mM phosphate buffer (pH 6.1); Guaiacol (16 mM); H_2O_2 (2 mM); enzyme and distilled water. POX activity was measured at 470 nm absorbance.

2.3.4. Determination of protein content

Total soluble protein supernatant was determined according to Bradford method[14] using Bovine Serum Albumin (BSA) as standard and was expressed in mg/g fresh weight.

3. Analysis of results

3.1. Cr toxicity on A. philoxeroides plant growth

A. philoxeroides seedlings were exposed to different concentrations (25; 50; 100; 150 mg/l) of Cr for 12 days. Both the shoot and root growth were affected in all the concentrations used in the experiments. Table 1 depicted the effect of

Table 1 – Effect of Cr on shoots and root length of *A. philoxeroides* after 12 days treatment.

Cr concentration (mg/L)	Shoot length (cm)	Root length (cm)	IT values (%)		RWC (%)
			Root	Shoot	
Control	25.7 ± 0.72	16.8 ± 0.82	0.00	0.00	66.0
25	23.5 ± 0.29	12.3 ± 0.14	88.3	92.7	65.8
50	22.2 ± 0.47	10.8 ± 0.83	85.7	75.7	65.8
100	20.3 ± 0.27	11.6 ± 0.81	78.6	77.0	64.5
150	20.4 ± 0.54	10.2 ± 0.20	80.8	64.0	64.5