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CHROMIUM PHYTOACCUMULATION BY *ALTERNANTHERA SESSILIS IN VITRO* – A FIRST REPORT

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ABSTRACT

The present study established a protocol for assessing *in vitro* chromium absorption potential of *Alternanthera sessilis*. The study also investigated the effect of chromium on morphology of the micropropagated plants. Murashige and Skoog (1962) medium fortified with 0.5 mg/l of 6-benzylaminopurine (BAP) and 0.2 mg/l Indole-3 acetic acid (IAA) was selected as '*in vitro* plantlet induction medium (IVPI)' for *A. sessilis*. Optimum chromium uptake (OCU) concentration was determined as 8 mg/l $K_2Cr_2O_7$ on which moderate effects in length of shoot (4.5 cm) and root (1.2 cm) and survival rate (58.6%) was recorded. The chromium accumulation of *A. sessilis* at OCU concentration is comparatively similar in both *in vitro* condition (1011 µg/g in root; 201 µg/g in shoot) and Hydroponics (996 µg/g in root; 195 µg/g in shoot). The *in vitro* method was found to be a valuable tool in identifying prospective phytoremediation candidates to reduce environmental toxic metals.

KEYWORDS:Phytoremediation, direct regeneration, chromium, environmental pollutants, detoxification

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INTRODUCTION

Toxic metals in soil and groundwater, with increasing industrial and municipal wastewater, are major concern for environment and human health issues. Elemental pollutants like arsenic, cadmium, caesium, chromium, lead, mercury, strontium, technetium, tritium and uranium are toxic heavy metals and radionucleotides [1,2,3]. Chromium is abundant in the effluents of tanning industries. Chromium with oxidation state VI is considered to be more toxic. There are many methods to detoxify the heavy metals in the environment. Unfortunately, most of them are extremely costly. Bioremediation has been the recent technology for separating toxic metals from affected areas. Many bacteria, fungi and plants are being tested for their ability to detoxify metalliferous substrates. Among which, phytoremediation is cost-effective and guarantees commercial development [2]. Uptake of metals by terrestrial plants has been studied by several workers including [4,5,6]. It is meant that a plant with more than 0.1% of Ni, Co, Cu, Cr or Pb or 1% of Zn in its leaves on a dry weight basis is called a hyperaccumulator regardless of the concentration of the metal in soil [7]. About 400 plants are known to be hyperaccumulators of metals [8]. Many plants possess the ability to accumulate heavy metals like Cd, Cr, Pb, Co, Ag, Se and Hg having no known biological role [9,10]. Use of aquatic or semi aquatic vascular plants for sequestration of heavy metals from aqueous environment has become relatively common recently. This is because of the ability of plants' roots to absorb, concentrate and precipitate the pollutants from effluents [11]. *Alternanthera sessilis*, a member of Amaranthaceae family, known as sessile joyweed occurs in both wetlands and uplands and can grow on a variety of soil types.The efficiency of phytoextraction depends, besides the substrate type, on several characteristics of the plant, such as the ability to accumulate and translocate metals to the aerial parts, a fast growth and a deep and extended root system [12]. An *in vitro* screening reduces not only the growth period and the treatment time length of the plants but also the space required for the experiments. Moreover, environmental factor variability is also reduced. Hence, the present study attempts to assess chromium absorption of *A. sessilis* under *in vitro* condition for the first time.

MATERIALS AND METHODS

i. Collection of Plants

Plants of *Alternanthera sessilis* were collected near Pallikaranai Lake, Chennai. The collected plant specimens were identified using regional floras [13] in Centre for Floristic Research, Department of Botany, Madras Christian College, Chennai, India.

ii.Surface sterilization and in vitro plant regeneration

Young shoots were washed with running tap water and surface sterilized using 0.1% mercuric chloride for 8 min. After rinsing 3-4 times with sterile distilled water, shoot tip, stem nodes and internodes were cut into smaller segments (0.5 to 1.0 cm) and used as explants. The explants were placed vertically on solid basal Murashige and Skoog [14] medium with 3% sucrose, 0.75% (w/v) agar and combination of 0.5 mg/l BAP and 0.2 mg/l IAA for simultaneous *in vitro* plantlet regeneration [15] . This medium combination was named as '*in vitro* plantlet induction medium (IVPI)'. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. The cultures were incubated at 25 ± 2°C under 16/8 hrs light/dark photoperiod.

iii. In vitro chromium treatment

Fresh nodal explants were inoculated on IVPI medium containing different concentrations (2.0-10 mg/l) of $K_2Cr_2O_7$ for 25 days. Each experiment was performed with 30 replicates and repeated three times.

iv. Chromium absorption in Hydroponics

Uniform size of plants *A. sessilis* (10–15 nodes long) was detached from a mother plant and kept for acclimatization in 10% Hoagland's solution [16] for 25 days. The proposed optimum chromium uptake (OCU) concentration of *in vitro* condition was selected as the common concentrations of Cr and the solution were prepared in 10% Hoagland's solution [17].

v. Estimation of Chromium

The regenerated plants were harvested from the medium, washed five times with double distilled water, oven-dried at 80°C and digested in concentrated HNO_3 (70%) using Microwave Digestion System. Chromium was estimated by Atomic Absorption Spectrophotometer.

vi. Statistical Analysis

Analysis of variance in completely randomized block design involving four Cr concentrations and three durations was performed to confirm the validity of data [18].

Direct regeneration of *Alternanthera sessilis*

RESULTS

i. Direct regeneration of A. sessilis

IVPI medium showed very good response with respect to shoot length (5.7 cm) and root regeneration (2 cm), concurrent with 92.4% survival rate from nodal explants (Figure 1).

Figure 1
In vitro plantlet induction (IVPI) medium

Figure 2
IVPI + with optimum chromium uptake concentration (8 mg/l $K_2Cr_2O_7$)

Figure 3
IVPI + 10 mg/l $K_2Cr_2O_7$

ii. Effect of different concentration of K2Cr2O7 on in vitro plant regeneration

A. sessilis showed significant change in the growth rate with different concentrations of $K_2Cr_2O_7$ (Table 1). Even though maximum tolerance was observed at 10 mg/l $K_2Cr_2O_7$, (Figure 2) very lower survival rate (18.2 %) was recorded (Table 1); also many explants died at developmental stages. The medium amended with 8 mg/l $K_2Cr_2O_7$ showed moderate effects in length of shoot (4.5 cm) and root (1.2 cm) and survival rate (58.6 %). In concentrations of 2 mg/l and 4 mg/l $K_2Cr_2O_7$, the growth data were slightly similar to the control. Based on this data interpretation and statistical analysis, 8 mg/l $K_2Cr_2O_7$ was considered as optimum chromium uptake concentration and the plants regenerated in this concentration were taken for further analysis (Table 1; Figure 2).

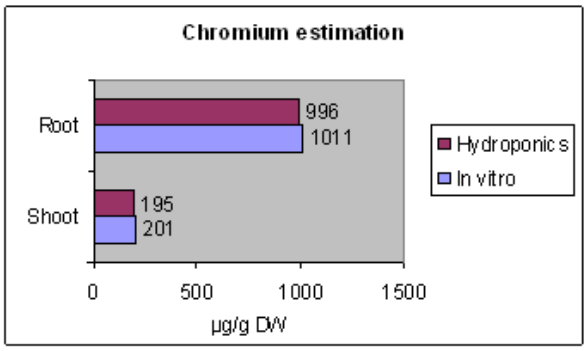
Table 1
Effect of $K_2Cr_2O_7$ on in vitro regeneration of A. sessilis.

$K_2Cr_2O_7$ mg/l	Survival rate %	Length of shoot cm mean ± SE	Length of root cm mean ± SE
2.0	82.6	5.5 ± 0.14	1.8 ± 0.12
4.0	76.2	5.1 ± 0.17	1.6 ± 0.13
6.0	60.8	4.8 ± 0.19	1.5 ± 0.17
8.0	58.6	4.5 ± 0.26	1.2 ± 0.19
10.0	18.2	1.8 ± 0.57	0.6 ± 0.52

iii. Estimation of chromium

The results of Cr accumulation in the roots and shoots of *A. sessilis* after 25 days of inoculation are shown in Graph 1. The accumulation of Cr in roots was higher than that of shoots in both *in vitro* and hydroponics conditions at 8 mg/l on 25th day of exposure (Graph 1).

Graph 1
Chromium adsorption of the regenerated plants



One way ANOVA was performed between *in vitro* and hydroponics data of accumulation of chromium. The P value all the four treatments showed less than 0.01 shows the values are significant.

DISCUSSION

In the present investigation, nodal explants of *A. sessilis* responded well on MS medium supplemented with 0.5 mg/l BAP. Similar results in shoot initiation from callus tissue were observed with higher level of BAP at 1.0 mg/l [19]. The study showed that combination of auxin and cytokinin concentrations stimulates simultaneous regeneration of shoots and roots. This correlation in results can be attributed to many factors. However, Murashige and Skoog (1962) stated that the combination of IAA and different cytokinin levels allowed vigorous organ development. Uptake of metals by terrestrial plants has been studied by several workers by several workers like [4,6] . Various categories of aquatic plants useful in phytoremediation have been identified by Outridge and Noller [20]. The results of the present study suggested that the rooted plants of *A. sessilis* have shown more accumulation in Cr containing medium. Similar results have also been reported in rooted submerged plants of *Vallisneria spiralis* treated with Cu and Cd [21] showing higher accumulation of metals than the emergent macrophyte, *Bacopa monnieri* [22].Hydroponics has also been effectively used for the evaluation of heavy metal accumulation *A. sessilis* [17]. Our study is the first to demonstrate the heavy metal accumulation potential of *A. sessilis* under *in vitro* condition. The limitations of *in vitro* analysis include lack of testing the consistency of accumulation potential in offspring as environmental factors play a major role in influencing plant performance and lack of field trials to check the remediation potential at polluted areas. Hence, the results obtained in *in vitro* condition were compared with hydroponics. Similarly, Watson and Pulford [23,24] pointed out that results obtained in hydroponics and in field experiments have a good agreement.Multidisciplinary research can increase our knowledge and promote practical application of phytoremediation. Data demonstrated that *in vitro* screening represents a valuable way of assessing the ability to take up, tolerate and survive metal stress. Hence, Plant tissue culture could be an effective tool for analyzing phytoremediation mechanism. Our results are also emphasis that *A. sessilis* to be a promising phyto-remediating candidate for exploiting in metal-polluted habitats.

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