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Full Length Research Paper

Comparative evaluation of copper, cobalt, cadmium and iron scavenging efficiency by *in-vivo* and *in-vitro* grown *Momordica charantia* using atomic absorption spectroscopy

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Phytoextraction, the use of hyperaccumulator plant species to scavenge toxic heavy metals from contaminated soils are considered as an emerging technique for cost effective and environment friendly detoxification. The present study was conducted to evaluate and compare the scavenging efficiency of *in-vivo* and *in-vitro* grown *Momordica charantia* for the uptake of copper, cobalt, cadmium and iron using atomic absorption spectroscopy (AAS). *In-vitro* plants were cultured on a mixture of 2,4-D (2.5 mg/L) and NAA (2.0 mg/L) in MS basal medium. Bioassays of *in-vitro* and field grown (*In-vivo*) *M. charantia* plants were subjected to atomic absorption spectrophotometer for the analysis of scavenging efficiency of the aforementioned four elements, after a 30 days of growth in soils, contaminated with each of these, separately. The *in-vitro M. charantia* absorbed much lesser amounts, that is, 0.79, 1.05, 0.45 and 1.61 ppm as compared to field grown plants which showed higher ranges of uptake, that is, 2.09, 10.39, 4.77 and 8.29 ppm for Cu, Co, Cd and Fe, respectively. The metal uptake ratios were proportional to their concentrations in the contaminated soils. Maximum uptake for all the four heavy metals was observed by the roots in all plants, due to localization of their ions in the apoplasm. Nodes also showed high heavy metal accumulation in the aerial region of the plant exhibiting their role during xylem-phloem transportation. Higher concentration of heavy metals in the soil showed a negative effect on their growth.

Key words: Phytoremediation, *in-vitro* growth, micropropagation, atomic absorption, spectroscopy, hyperaccumulates.

INTRODUCTION

Phytoremediation is the most emerging field of environmental biotechnology. Most of the soil contaminants can be removed by many other physical methods but the heavy metal pollution of vast cultivated land areas are a serious threat to the agricultural biology. The plant roots have natural ability to absorb the heavy metals of the soil, behaving as natural phytoremediates.

Momordica charantia is a member of family Cucurbitaceae having astringent, antihaemorrhoidal, hypoglycemic, stomachic, emmenaagogue, galactogogue, hepaticstimulant, anthelmentic, anti-timorous and antimicrobial characteristics (Brain, 2008). The phytochemcals of *M. charantia* include glycosides, peptides, Sterols, insulin, zeatin and ribosides etc.

Extensive work is reported for *M. charantia* with respect to its tissue cultural aspects. Thiruvengadam et al. (2004) optimized a system for the somatic embryogenic suspension cultures of bitter melon. Friable calluses could be induced in 30 days-old leaves on semi-solids MS supplemented with 1.0 mg/L 2,4-D. Wang et al. (2004) observed flower formation from shoot tips of bitter melon cultured on MS medium supplemented with 90 mM

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sucrose, 0.05 mM iron and 4 mM N^6-benzyladenine (BA). Huda and Sikdar (2006) observed the growth of meristem of bitter gourd on semi solid MS medium supplemented with 0.05 mg/L Kn, IBA, NAA and IAA singly or in combination for shoot elongation and root initiation. Malik et al. (2007) analyzed the effects of plant growth regulators on callogenesis and direct and indirect organogenesis of *M. charantia*.

Detection of harmful elements in *M. charantia* and other plant species is also being reported on wider scale by many workers. Sahito et al. (2002) reported the presence of fifteen essential, trace and toxic elements, that is, Zn, Cr, K, Mg, Ca, Na, Cu, Fe, Pb, Al, Ba, Mn, Co, Ni and Cd in *M. charantia and Syzigium jambolana*. Ansari et al. (2004) studied the concentrations of essential trace metals, that is, Zn, Mn, Cu and Fe in thirty five different spices and plants having folk medicinal uses. Metals uptake using plants is an *in-situ* solution of the soil pollution, which is a low cost, environmental friendly and is operated by solar energy (McCutcheon and Schnoor, 2003).

Some plants species act as hyper accumulators of the metals, depending upon their scavenging capacity and ability to deposit these metals in the different cellular compartments. These metals pass through the root cell membrane to the symplast, then metals could be passed to the vacuoles, (where their degradation occurs by enzymes) by membrane metal transporters, and could be deposited there with the help of special metal-binding proteins called metallothioneins. Heavy metals are supposed to replace other essential metals in pigments of the cellular structure, destroying their natural balance (Manios et al., 2003). These metals may be a cause of oxidative stress too, especially transition metals, for example, $Fe^{2+/3+}$ and $Cu^{+/2+}$ (Rivetta et al*.,* 1997). Plant tissue culture provides a selected environment for the evaluation of many limiting factors. It is in extensive use nowadays, to obtain variants with variable tolerance to different biotic stresses (Ben-Hayyim, 1987; Santos-Díaz et al., 1994).

This technique is also useful for cultured plant organs to know the metal accumulation properties of each separate plant part, for example, the removal of Sr^{2+} using shoots of *Solanum laciniatum* (Kartosentono et al., 2001), and Cd hyper-accumulation by roots of *Thlaspi caerulescens* (Nedelkoska and Doran, 2000). Atomic absorption spectroscopy (AAS) is an alternative, simple and rapid technique for quantitative isolation of the group of eight elements (Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn) from biological material. Therefore the main objective of the present study is to evaluate and compare the Cobalt and Cadmium uptake by *in vivo* and *in vitro* grown *M. charantia* using AAS.

MATERIALS AND METHODS

The work was divided into two steps:

The *in vivo* and *in-vitro* growth of *M. charantia*

For *in-vivo* growth the certified seeds of *M. charantia* were sown in the back lawn of LCW University and plants were grown for 60 days. For the *in-vitro* growth, the explants were taken from the wild *M. charantia*, cultured and then subcultured in the PGRs optimium media for 60 days. For the *in-vitro* growth, following protocols were followed.

Basal medium and phyto growth regulators (PGRs)

MS (Mrashaige and Skoog, 1962) basal medium was used. Following PGRs were used separately and in combinations in MS basal medium.

1. BAP
2. NAA
3. 2,4-D
4. BAP+NAA
5. 2,4-D+NAA
6. BAP+ 2,4-D

Physical factors

Sucrose was added to medium at 3% concentration (30 g/L). The optimum temperature required for culture environment was maintained at 25±2℃. The cultures were incubated at 16 h photoperiod (under cool light fluorescent tubes with light intensity of 2000 to 3000 lux. The pH of the medium was adjusted between 5.6 to 5.7.

Plan of experiment and data recording

Three sets of each experiment were maintained with three replica of each experiment. The cultured explants were observed after inoculation and the contamination percentage, percentage of callus formation and number of frequency of micro-propagated plants per explants after given culture period was worked out. Mean deviation was calculated after Steel and Torrie (1980).

Estimation of copper, cobalt, cadmium and iron uptake by *in-vivo* and *in-vitro M. charantia* using AAS

The second step of the study was to estimate the, copper, cobalt, cadmium and iron uptake by *M. charantia* using AAS. All chemicals and reagents used in the study were of analytical grade and were used without further purification. Solutions were prepared in double distilled water.

Preparation of biomass

Elements in plants parts cannot be detected directly by AAS, so solutions for plants were prepared by wet digestion method and then samples were analyzed to determine the concentration of metal ions. After collecting leaves of plant, they were washed with double distilled water to remove dust from plant. These leaves were then dried in an oven. The dried plants were then digested. The same procedure was done for *in-vitro* grown plants except that regenerated plants were not sterilized.

Method for digestion

The dried plant leaves were weighed separately and 5.0 g was

Table 1. Effect of different PGRs on micropropagation of different explants of *Momordica charantia* on MS medium.

PGRs used	Explants used	Micropropagation (% Mean)
2,4-D (2.5 mg/L)	Node	100
2.5 (mg/L) 2,4-D (2.5 mg/L)	Cotyledon	70±1.79
2, 4-D (2.5 mg/L) and NAA (2.0 mg/L)	Leaf	100±0.21
2,4-D (2.5 mg/L)	Node	70±0.91
2,4-D (2.00 mg/L)	Node	80±2.60

Table 2. Concentration of copper, cobolt, cadmium and iron in *in-vivo* (field) grown *Momordica charantia* determined by AAS.

S/N	Elements	Biomass used	Mean conc. of element (ppm)
1	Cu	*In-vivo* grown plants	2.09
2	Co	*In-vivo* grown plants	10.39
3	Cd	*In-vivo* grown plants	4.77
4	Fe	*In-vivo* grown plants	8.29

taken in a round bottom flask. The dried material was ashed in crucible muffle furnace at 500℃ for 1 h. The residue was then wet digested by HCl/HNo_3 5 ml (1:3) and heated till dryness. After drying 5 ml of HNO_3 was added in the same beaker and heated for 5 to 10 min. The volume was adjusted up to 50 ml with double distilled water and then was filtered. The sample solutions were ready to be aspirated in AAS. These sample solutions of *in-vivo* and *in-vitro* grown leaves were kept at 4℃ with UV protection in amber bottles.

Calibration range

These samples were subjected to Z-5000 series polarized Zeeman Atomic Absorption Spectrophotometer. The calibration curve was obtained by running the standards of 0, 2, 4 and 8 ppm. A straight line was obtained between concentration and absorbance. All the points of the standard were tried to lie in the straight line, because the accuracy of the sample results is depends on exact absorbance of these standards.

Statistical analysis

All the data were statistically analyzed following Steel and Torrie (1980).

RESULTS AND DISCUSSION

For *in-vitro* growth, leaf, cotyledons, internodes and root explants of *M. charantia* were cultured on MS basal medium. Leaf explants formed actively growing callus on MS medium with a mixture of 2, 4-D (2.5 mg/L) and NAA (2.0 mg/L) and whole explants converted into calli which later on regenerated multiple shoots (Table 1). Regeneration potential shown by *M. charantia* leaf explants during the present study resembles to that reported by Yang et al. (2004) who induced adventitious buds and multiple shoots in green calli of *M. charantia* leaf explants on MS medium supplemented with kinetin, BAP and Zeatin.

The rest of the explants, cotyledons and internodal explants of *M. charantia* form calli on 2,4-D. These were moderately growing green calli. Somatic regeneration was not observed in either case although the callus texture was compact and nodular only leaf callus formed on 2,4-D produced multiple shoots as mentioned earlier.

Regeneration from leaf and internodes is quoted during *in-vitro* clonal propagation of *M. charantia* by Malik et al. (2007) on BAP and NAA; they also reported regeneration in MS culture with TDZ. During direct regeneration from *M. charantia* leaf explants were induced on NAA and BAP (0.4 to 1.0 mg/L) in MS media.

Present findings show somatic embryogenesis in liquid MS (Murashige and Skoog, 1962) media with different concentration of 2,4-D (1.0 to 3.0 mg/L). Somatic embryos with high growth rate, globular in shape were observed in 2.5mg/L 2, 4-D. Thiruvengadam et al. (2006) also observed the similar results for *M. charantia* using 2,4-D.

The second step of this research work was to determine concentration of four mineral elements, that is, copper, cobolt, cadmium and iron in *in-vivo* and *in-vitro* grown plant tissues (Tables 2 and 3). The *in-vitro M. charantia* absorbed much lesser amounts of all these metals, that is, 0.79, 1.05, 0.45 and1.61 ppm as compared to *in-vivo* plants which showed higher ranges of uptake, that is, 2.09, 10.39, 4.77 and 8.29 ppm for Cu, Co, Cd and Fe, respectively, which was proportional to the metal concentration in the contaminated soil. The concentration of copper and iron was determined by Yuwai et al. (1991) in *in-vivo* grown plant tissues of

Table 3. Concentration of Copper, Cobolt, Cadmium and Iron (Fe) in the *in-vitro* grown plants of *Momordica charantia* determined by AAS.

S/N	Elements	Biomass used	Mean conc. of element (ppm)
1	Cu	*In-vitro* grown plants	0.79
2	Co	*In-vitro* grown plants	1.05
3	Cd	*In-vitro* grown plants	0.45
4	Fe	*In-vitro* grown plants	1.61

of *M. charantia*. According to them copper was 3.54 ppm and iron was 5.97 ppm. One of the major factors influencing trace mineral uptake in plants is the composition of the soil. Fatima et al. (2004) also studied metal contents of *in-vivo* grown *M. charantia* and found that no metal was above the toxic limit. Sahito et al. (2002) also studied the trace and essential elements of *M. charantia* plants using atomic absorption spectroscopy technique. The levels of essential elements were found high as compared to the levels of toxic elements.

Lin et al. (2007) worked on effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum* as a newly found cadmium hyper accumulator. They reported that hyper accumulators are ideal plant species used for phytoremediation of soils contaminated by heavy metals. A full understanding of metal tolerance mechanisms of hyperaccumulators will facilitate enhancing their phyto-remediation efficiency. However, how Cd affects nitrogen metabolism is still unknown.

During present study the concentrations of copper, cadmium, cadmium and iron in *in-vivo* plant tissues and *in-vitro* grown plant tissues is predicted in Tables 2 and 3. Results show that all the above mentioned four elements were in high concentration in field grown plant tissues as compared to *in-vitro* grown plant tissues which indicate that chemical composition of soil is different from cultural medium. These results demonstrate that the composition of the media and soil plays an important role in mineral uptake of plants.

There has been phytoremediation work done on *Amaranthus hybridus* (Jonnalagadda and Nenzou, 1997) which was used to determine the effect of coal mine contamination on the uptake and distribution of lead, cadmium, mercury, nickel, manganese and iron. *Amaranthus tricolor* and *Amaranthus retroflexus* (Bigaliev et al., 2003) were used for the uptake of cadmium, mercury, zinc and copper; and *Amaranthus spinosus* (Prasad and de Oliveira, 2003) was used for the accumulation of cadmium, zinc and iron.

This study also leads to the conclusion that *in-vitro* grown plants of *M. charantia* can behave as natural scavengers if planted to the chemically polluted soils on large scale in future. The present piece of work fully supports our idea that purified *in-vitro* grown hyper accumulators, like *M. charantia* can be used as the natural phytoremediates and heavy metal scavengers of the toxic elements for the treatment of contaminated and polluted agricultural lands on commercial scale.

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