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RESEARCH ARTICLE

Effect of Chromium and Copper on *invitro* seedlings of *Arachis hypogaea* L. and *Brassica juncea*

***Febina Bernice Sharon S., Priscilla Sweetlin G., Sasikala S., Anbumani V., Subathra R.**

Department of Botany and Microbiology, Lady Doak College, Madurai-625002, Tamil Nadu, India

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ABSTRACT

Due to wide industrial use, chromium and copper are considered as serious environmental pollutants. Hexavalent chromium is one of the heavy metal and it was found to cause variety of clinical problems like asthma, pneumonitis, bronchogenic carcinoma, skin allergies and so on. Cu toxicity also causes problems like abdominal pain, nausea, vomiting, headache, diarrhoea, respiratory difficulties, anaemia, gastrointestinal bleeding, kidney failure and death. Plants are becoming more efficient producers of food, fiber and medicines. Apart from these conventional uses, biotechnology unlocks the doors to unique uses of plants that are gaining greater acceptance and attention from the people and the scientific community. These are called ''value-added'' uses include phytoremediation and hence the objective of the present study is to explore the full potential of plant tissue culture techniques to study the metal tolerance in whole plant in culture. The model plant system used in this study is a cultivated variety of mustard, groundnut and the metal that had been used is Chromium and Copper in six different concentrations.

INTRODUCTION

Copper toxicity is one of the problems of both agronomic and environmental importance. Sources of copper contamination include mining and smelting from urban, industrial and agricultural wastes and the use of agrochemicals (Anna Sheldon and Neal Menzies, 2004). The wide use of chromium, mostly in the trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) forms were used in steel, alloys, cast iron, chrome plating, dyes, pigments, textile, leather, tanning and wood preserving make the industries the possible sources of chromium pollution in the environment (Dixit *et al*., 2002). There are various plants which can store high level of the toxic metals in their tissues. These plants will be helpful to eliminate the pollutants from soil and also in water (Singh *et al*., 1997, 2001, 2007; Asha Sharma *et al*., 2010) to reduce the contamination; this technique is commonly known as phytoremediation. Indian mustard (*Brassica juncea*) was found to remove heavy metals from soil (Zhu *et al*., 1999; Singh *et al*., 2001, 2007; Qadir *et al*., 2004; Gupta and Sinha, 2006; Asha Sharma *et al*., 2010). *Brassica juncea* found to store more Cr from polluted soils than other plant species (Shahandeh and Hossner, 2000 and Fengxiang Han *et al*., 2003). Cr^{6+} is the most biologically toxic oxidation state of Cr. In this present study, the ability of *Brassica juncea and Arachis hypogaea* to grow in different concentrations of Chromium and Copper were studied.

MATERIALS AND METHODS

Media and Media components

In order to grow the plants *invitro*, researchers have formulated media that provide nutrients that are usually available in soil. One of the most commonly used basal medium is MS medium (John *et al*., 1996). This media was formulated by Murashige and Skoog in 1962.

Corresponding author: febina.623@gmail.com

Source of explants

The seeds used for the experiment and as a source of explants for experiments on embryo axes were procured from the local market.

Surface sterilization of explants

For initiation of culture from non sterile plant materials like seed and embryo axes, the surface of the plant materials were sterilized to eliminate the adhering microbes. The plant materials were treated with chemicals like savlon, mercuric chloride and hypochlorite, before culturing in tissue culture medium.

Effect of various concentrations of $K_2Cr_2O_7$ (CrVI) and $CuSO_4$ on peanut seed germination

Seeds were surface sterilized and the seed coats were removed aseptically. These were cultured in MS half strength medium with various concentrations of $K_2Cr_2O_7$ (25µM, 50µM, 75µM, 100µM and 125µM) and $CuSO_4$ (25µM, 50µM, 75µM, 100µM and 125µM). MS half strength medium without Cr and Cu was used as control. After 14 and 21 days of incubation, number of seeds germinated, length of the root of the seedling, length of the shoot of the seedlings and number of leaflets were noted. The frequency of germination average length of roots, shoots and number of leaflets was also scored.

Effect of various concentrations of $K_2Cr_2O_7$ (CrVI) and $CuSO_4$ on embryo axes derived plantlet

Embryo axes were surface sterilized and cultured in MS half strength medium with various concentrations of $K_2Cr_2O_7$ (25µM, 50µM, 75µM, 100µM and 125µM) and $CuSO_4$ (25µM, 50µM, 75µM, 100µM and 125µM). MS half strength medium without Cr and Cu was used as control. After 14 and 21 days of incubation, number of embryo axes germinated, length of the root of the seedling, length of the shoot of the seedlings and number of leaflets were noted. The

frequency of germination average length of roots, shoots and number of leaflets was also scored.

Effect of various concentrations of $K_2Cr_2O_7$ (CrVI) and $CuSO_4$ on mustard seed germination

Seeds were surface sterilized and were cultured in MS half strength medium with various concentrations of $K_2Cr_2O_7$ (25µM, 50µM, 75µM, 100µM and 125µM) and $CuSO_4$ (25µM, 50µM, 75µM, 100µM and 125µM). MS half strength medium without Cr and Cu was used as control. After 14 and 21 days of incubation, number of seeds germinated, length of the root of the seedling, length of the shoot of the seedlings and number of leaflets were noted. The frequency of germination average length of roots, shoots and number of leaflets was also scored.

RESULTS AND DISCUSSION

The technique of plant tissue culture offers the opportunity to test the effect of altered conditions on growth and differentiation of plants and isolated explants by manipulating the medium composition. In the present study this technique is applied, to study the effect of Cr and Cu on developing peanut seedling rose from seeds and isolated embryo axes and mustard seedling rose to seeds.

Effect of various concentrations of $K_2Cr_2O_7$ on Peanut seed Germination

The frequency of germination in seeds ranged between 82% and it was decreased to 73% on twenty first day. In medium without metal, 50µM and 100µM chromium containing medium, the germination frequency was 100%. The growth was good in 25µM, 50µM and 75µM concentrations. The seedling appears healthy and green with opened leaves in all the concentrations of chromium tested. The mean heights of the seedling shoots in chromium ranged between 13 cm to 14.4 cm. In medium devoid of chromium the height was 9 cm (Table 1).

Effect of various concentrations of $CuSO_4$ on Peanut seed Germination

The frequency of germination ranged between 78% and increased to 85% on twenty first day. The effect was good when compared with chromium. At 75µM and 100µM the growth was very good. Even in few days we got flowers from the plant. The germination frequency is 100% in 0µM, 25µM and 50µM concentrations. When the concentration of copper increased, it retards the growth of the plant (Table 2).

Figure 1. Effect of various concentrations of $CuSO_4$ on Peanut seedling germination

Table 1. Effect of various concentrations of $K_2Cr_2O_7$ on Peanut seed Germination

Concentration of Cr(VI)	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	5.5±0.71	6.0±0.5	6±1.41	9±0	16±0	18±0
25 µM	2.0±3.58	6.0±0.5	10±5.2	13.0±3.3	16±8.7	24±0
50 µM	4.0±2.49	6.4±1.5	10±0.55	13.4±0.55	16±0	20±0.3
75 µM	4.0±2.34	7.4±2.34	10.2±4.2	13.7±4.5	16±7.16	16±7.16
100 µM	4.2±1.34	7.4±1	10.5±1.87	14.2±1.87	24±2.19	24±2.19
125 µM	4.5±1.50	7.5±0.5	10.7±1.50	14.4±0.5	24±0	24±0

Table 2. Effect of various concentrations of $CuSO_4$ on Peanut seed Germination

Concentration of Cu	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	3.2±0.7	7±0.5	6±1.41	9±0	16±0	16±0
25 µM	3.3±0.74	10±1.9	4.5±0.95	12.5±0	12±3.35	24±6.26
50 µM	4.0±1.28	8.5±5.6	2.8±1.75	13.1±4.7	12±6.57	24±4.98
75 µM	5.8±3.35	8.5±4.3	3.8±1.83	14.5±6.5	12±5.37	18±10.62
100 µM	5.8±2.68	5.9±2.5	3.7±2.57	14.5±4.5	12±6.57	12±8.79
125 µM	1.5±0.82	3.6±6.3	2±1.24	9±4.9	8±4.38	12±7.48

Effect of various concentrations of $K_2Cr_2O_7$ on Embryo axes derived Plantlet

Above experiment was repeated with isolated embryo axes without cotyledons. The frequency of the seed germination in control was 75% where as 51.2% of embryo axes differentiated to develop into plantlets. However in contrast to seedling, the vigor of the embryo axes derived plants was reduced and organs were less differentiated. The frequency of response shoot length and number of leaves were more in the seedling developed in media with or without chromium compared to the embryo axes derived plants. The frequency of response, shoot length, root length, number of leaves were higher compared to the control. In increased concentrations (200µM, 300µM) there was gradual decrease in all these responses. Thus it is apparent that low concentration of Cr support plant development and differentiated where as it is inhibitory at higher concentration (Table 3).

Effect of various concentrations of $CuSO_4$ on Embryo axes derived plantlet

The frequency of seed germination in control was 75% but it was reduced to 69% on twenty first day where as 50% of embryo axes differentiated to develop into plantlets. Comparing the data generated from the experiments on seedlings and embryo axes derived plantlets, it is assumed that possibly cotyledon plays a protective role in high concentration of Cr. There was no significant inhibition of growth in the seedlings were as the growth of embryo axes derived plants was significantly retarded in the presence of higher concentration of Cr (Table 4).

Effect of various concentrations of $K_2Cr_2O_7$ on Mustard plant seedling

The frequency of germination in seeds ranged between 81% and frequency of germination is 88% in 25µM, 50µM and 100µM concen tration.

Table 3. Effect of various concentrations of $K_2Cr_2O_7$ on Embryo axes derived Plantlet

Concentration of Cr(VI)	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	0.3±0.23	0.6±0.2	0.75±0.46	1.13±0.4	8±0.91	12±1.38
25 µM	1.5±0.7	2.5±0.70	0.62±0.40	1.0±0.86	6±0.84	16±0.84
50 µM	1.55±1.03	2.6±0.97	0.70±0.74	1.53±0.29	7±0.84	10±0.84
75 µM	1.6±0.07	2.7±0.07	1.20±0.40	2.0±1.11	8±2.26	14±2.26
100 µM	1.9±1.10	2.7±0.76	1.20±0.9	2.80±0.65	8±0.84	14±0.84
125 µM	3.3±2.5	2.9±0.71	1.10±0.43	3.30±0.50	8±1.15	16±1.63

Table 4. Effect of various concentrations of $CuSO_4$ on Embryo axes derived plantlet

Concentration of Cu	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	0.30±0.23	0.60±0.20	0.75±0.46	1.13±0.40	2±0.91	2±1.38
25 µM	2.80±1.80	2.80±0.88	1.6±0.99	2.60±0.54	3±1.50	10±0.89
50 µM	2.80±1.30	2.80±0.30	1.70±0.80	3.1±0.65	4±0.84	12±0.55
75 µM	2.83±0.40	3.40±0.15	2.20±0.50	3.40±0.52	4±1.14	16±1.60
100 µM	3.0±1.50	3.60±0.49	2.40±0.60	3.40±0.63	6±0.84	12±0.27
125 µM	3.50±1.10	4.25±0.82	2.65±0.80	3.80±0.37	6±0.55	9±1.30

Figure 2. Effect on various concentrations of $CuSO_4$ on mustard seedlings

Table 5. Effect of various concentrations of $K_2Cr_2O_7$ on Mustard plant seedling

Concentration of Cr(VI)	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	1.80±0.47	1.80±0.57	1.67±0.57	2.96±0.78	12.3±1.30	2.30±1.30
25 µM	1.6±4.12	1.80±0.65	1.47±0.30	2.56±0.27	2.30±1.80	2.50±0.63
50 µM	2±0.52	2.80±1.80	1.53±0.13	2.60±1.17	2.50±0.90	3.30±1.26
75 µM	1.9±0.84	2.30±0.78	1.6±0.95	2.60±0.62	1.30±1.30	3.10±0.43
100 µM	1.81±0.22	2.1±0.41	1.56±0.91	2.68±0.55	2.20±1.05	2.0±0.65
125 µM	1.81±0.35	3.3±1.23	1.96±0.68	3.20±0.98	1.40±0.90	4.30±1.19

Table 6. Effect of various concentrations of $CuSO_4$ on Mustard plant seedling

Concentration of Cu	Root length (cm) Mean±SD		Shoot length (cm) Mean±SD		No of Leaves Mean±SD	
	14th day	21th day	14th day	21th day	14th day	21th day
Control	1.80±0.40	1.96±0.78	1.67±0.57	1.80±0.57	2.30±1.30	2.30±1.30
25 µM	2.60±4.12	3.13±1.15	1.47±0.30	1.50±0.21	2.25±1.80	2.25±1.80
50 µM	2.0±0.52	2.88±0.70	1.63±0.13	1.90±0.21	2.50±0.90	2.50±0.90
75 µM	1.80±0.84	2.44±1.36	1.40±0.90	2.0±0.99	1.30±1.30	1.30±1.30
100 µM	1.80±0.35	2.29±0.71	1.56±0.90	1.90±0.86	2.10±1.05	2.10±1.05
125 µM	1.80±0.35	2.23±0.89	1.96±0.68	2.10±0.67	1.35±0.90	1.33±0.90

The growth was good in 125µM concentration but in the rest of the concentration, the growth was not so good when compared to the control. Cr can be accumulated in the plant roots especially in the vacuoles. But here the root growth is well when compared to the shoot growth (Table 5).

Effect of various concentrations of $CuSO_4$ on Mustard plant seedling

The frequency of germination in seeds ranged between 79% and increased to 82% on twenty first day. At 125µM concentration the growth of shoot was good (Table 6). Cu have a tendency to store in the root tissue and few were translocated to the shoots (Marschner, 1995).

Conclusion

The experiment described in this study was designed to test the metal tolerance of these plants *Arachis hypogaea* L and *Brassica juncea* on effect of Cr and Cu on whole plant. For studies of this nature, it is essential to use plant tissue culture system. *Brassica juncea* is chosen as a model system for this study has certain advantages. First being herbaceous species and an annual plant of short duration, the growth of tissue in culture is fast. This is same for *Arachis hypogaea* L. also. The data is preliminary in nature. More extensive studies need to be conducted to confirm the results. However from over all assessment of the preliminary data it appears that low concentration of Cr VI supports the growth of peanut tissue in culture where as mustard growth is inhibited at Cu concentration that has been already reported that lower concentrations of Cr stimulate the growth of the plant.

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