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RESEARCH ARTICLE

INFLUENCE OF CHROMIUM ON SEEDLING GROWTH, BIOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF BLACKGRAM

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ABSTRACT

Vignamungo is an important pulse crop of South India which belongs to the family Fabaceae. *Vignamungo* also commonly known asblackgram is cultivated in wider areas of Tamil Nadu. The present investigation has been carried out to assess the influence of chromium on seedling growth, biochemical constituents and antioxidant properties of *Vignamungo*. Blackgram seeds were germinated under various concentrations of chromium (5, 10, 25, 50, 75 and 100 mgl^{-1}). One set was irrigated with distilled water served as control (0 mgl^{-1}). Seed germination rate, seedling growth parameters were recorded on the 9th day after sowing of seeds. The biochemical constituents and antioxidant enzymes were analyzed on the same day. All the growth parameters, biochemical constituents and antioxidant enzyme activities of blackgram decreased in proportion to the concentration of metal applied to the seedlings.

INTRODUCTION

which results in deleterious effects on plants and other organisms of ecosystem. The problem of heavy metal contamination is getting serious in developing countries. Heavy metals can be emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources specifically mining operations (Nriagu, 1989). Heavy metal pollution is considerably important and relevant to the present scenario due to the increasing levels of pollution and its impact on human health through food chain. Heavy metal stress causes multiple direct and indirect effects on all physiological processes of plants. The biochemical impact of metalions on the cell is as diverse as this chemical nature. Distinct concentrations of metal induce different biochemical responses in plants. In sensitive plants, high concentrations of heavy metals inhibit enzymes involved in photosynthetic reaction (Singh *et al.,* 1997). Excessive amount of toxic element usually caused reduction in plant growth (Prodgers and Inskep, 1981). Studies on higher concentration of chromium affected germination and early growth of *Allium cepa* was reported by Mathur*et al.,* (1987). The present study examined the effect of chromium on seed germination, seedling growth, biochemical constituents and antioxidant enzymes activities of blackgram.

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MATERIALS AND METHODS

*Vignamungo*seeds were obtained from Regional Agricultural Research Station, Aaduthurai. Healthy seeds were selected and surface sterilized with 3% v/v formaldehyde solution for 5 minutes to avoid fungal contamination and washed thoroughly thrice with distilled water for 15 minutes. Seeds were arranged equispacially in petriplates lined with filter paper. Different concentrations of chromium (0, 5, 10, 25, 50, 75 and 100 mgl^{-1}) were prepared and used for this experiment. Seeds were irrigated with respective concentration of metal. Control plants (0 mgl^{-1}) were supplied with distilled water. The seed germination rate and seedling growth parameters were recorded on the 9th day after sowing. The biochemical constituents like chlorophyll (Arnon, 1949), carotenoids (Kirk and Allen, 1965), total sugars (Nelson, 1944), free amino acid (Moore and Stein, 1948), protein (Lavory *et al.,* 1951), proline (Bates *et al.,* 1973), total phenols (Bloin, 1958), antioxidant activity enzymes like catalase (Pesisseroth and Poune, 1970), peroxidase (Dawson, 1988) and nitrate reductase (Klepper, 1971) were analyzed and recorded on the same day.

RESULTS AND DISCUSSION

The seed germination percentage was reduced under chromium treatments. The control plants showed the maximum percentage of seed germination. It decreased in accordance with chromium applied. Maury *et al.* (1986) was

Table 1. Effect of chromium on germination rate and seedling growth parameters of blackgram (*Vignamungo*(L.)Hepper.)cultivar

Concentration (mgl^{-1})	Seed Germination Rate (%)	Root Length (cm $plant^{-1}$)	Shoot Length (cm $plant^{-1}$)	Number of Lateral Roots ($plant^{-1}$)
0	100	8.51	16.32	8.20
5	95	7.92	15.91	7.80
	(-5.00)	(-6.93)	(-2.51)	(-4.88)
10	92	7.24	15.10	7.00
	(-8.00)	(-14.92)	(-7.47)	(-14.63)
25	85	6.78	14.30	6.60
	(-15.00)	(-20.32)	(-12.37)	(-19.51)
50	72	6.12	13.51	6.00
	(-28.00)	(-28.08)	(-17.22)	(-26.82)
75	65	5.50	12.24	5.20
	(-35.00)	(-35.37)	(-25.00)	(-36.58)
100	57	5.03	11.02	4.40
	(-43.00)	(-40.89)	(-32.47)	(-46.34)

F – Test value for the variance between the cultivars 38.76** F- Test value for the variance between the concentrations 42.46**
Percentage of reduction over control values are given in parentheses(-). ** - Significant at 1 percent level.

Table 2. Impact of chromium on Photosynthetic pigments (mg g^{-1}fr. wt.) of blackgram (*Vignamungo*(L.)Hepper.)cultivar

Concentration (mgl^{-1})	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoid
0	0.435	0.245	0.680	0.340
5	0.402	0.231	0.633	0.315
	(-7.59)	(-5.71)	(-6.91)	(-7.35)
10	0.356	0.202	0.558	0.290
	(-18.16)	(-17.55)	(-17.94)	(-14.70)
25	0286	0.187	0.473	0.275
	(-34.25)	(-23.67)	(-30.44)	(-19.12)
50	0.252	0.162	0.414	0.250
	(-42.06)	(-33.88)	(-39.12)	(-26.47)
75	0.220	0.130	0.350	0.232
	(-49.42)	(-46.94)	(-48.53)	(-31.76)
100	0.198	0.115	0.313	0.212
	(-54.49)	(-53.06)	(-53.97)	(-37.65)

F – Test value for the variance between the cultivars 74.25** F- Test value for the variance between the concentrations 36.12**
Percentage of reduction over control values are given in parentheses(-). ** - Significant at 1 percent level

Table 3. Influence of chromium on Total sugars and Protein content (mg g^{-1}fr. wt.) of blackgram (*Vignamungo*(L.)Hepper.)cultivar

Concentration (mgl^{-1})	Total sugars			Protein		
	Root	Stem	Leaf	Root	Stem	Leaf
0	2.67	4.02	5.67	10.72	12.36	15.80
5	2.40	3.85	5.20	9.80	11.80	15.02
	(-10.11)	(-4.23)	(-8.29)	(-8.58)	(-4.53)	(-4.94)
10	2.12	3.62	4.78	9.10	10.98	14.30
	(-20.60)	(-9.95)	(-15.70)	(-15.1)	(-11.16)	(-9.49)
25	1.88	3.43	4.23	8.56	10.12	13.78
	(-29.59)	(-14.68)	(-25.40)	(-20.15)	(-18.12)	(-12.78)
50	1.75	3.05	3.95	8.04	9.79	12.58
	(-34.45)	(-24.13)	(-30.33)	(-25.00)	(-20.79)	(-20.38)
75	1.42	2.86	3.40	7.48	9.10	11.85
	(-46.82)	(-28.85)	(-40.03)	(-30.22)	(-26.37)	(-25.00)
100	1.14	2.24	3.02	6.85	8.24	10.48
	(-57.30)	(-44.28)	(-46.74)	(-36.10)	(-33.36)	(-33.67)

F – Test value for the variance between the cultivars 41.52** F- Test value for the variance between the concentrations 22.31**
Percentage of reduction over control values are given in parentheses(-). ** - Significant at 1 percent level

Table 4. Effect of chromium on Free Aminoacids and Proline content (mg g^{-1}fr. wt.) of blackgram (*Vignamungo*(L.)Hepper.)cultivar

Concentration (mgl^{-1})	Free Aminoacids			Proline		
	Root	Stem	Leaf	Root	Stem	Leaf
0	6.50	7.59	8.36	3.26	2.94	2.12
5	6.11	7.02	7.94	3.95	3.15	2.56
	(-6.00)	(-7.51)	(-5.02)	(+21.16)	(+7.14)	(+20.75)
10	5.78	6.80	7.20	4.24	3.50	2.89
	(-11.08)	(-10.41)	(-13.87)	(+30.06)	(+19.05)	(+36.32)
25	5.12	6.02	6.49	4.90	3.94	3.28
	(-21.23)	(-20.68)	(-25.24)	(+50.31)	(+34.01)	(+54.72)
50	4.70	5.82	5.95	5.65	4.56	4.18
	(-27.69)	(-23.32)	(-28.83)	(+73.31)	(+55.10)	(+97.17)
75	4.13	4.95	5.78	6.28	5.12	4.79
	(-36.46)	(-34.78)	(-30.86)	(+92.64)	(+74.15)	(+125.94)
100	3.65	4.26	5.14	7.24	5.85	5.12
	(-43.85)	(-43.87)	(-38.52)	(+122.08)	(+98.98)	(+141.51)

F – Test value for the variance between the cultivars 86.43** F- Test value for the variance between the concentrations 52.35**
Percentage of reduction over control values are given in parentheses(-). ** - Significant at 1 percent level

observed similar inhibition of germination in *Indigofera, Dactloctenium* and Desmodim. The delay in germination may also due to inhibition by chromium ions (Dua and Sawhney, 1991). Table-1.shows clearly that plants exhibited a marked decrease in root and shoot length due to deleterious effect of chromium. Root length was found to be more affected than shoot length. The number of lateral roots were reduced with increase in chromium concentration. The lateral roots were very short and brown in colour in chromium treated seedlings. The reduction in seedling length was mainly due to the reduced root growth and consequent lesser nutrients and water transport to the above parts of the plant. In addition to this chromium transport to the aerial part of the plant have a direct impact on the shoot metabolism contributing to the reduction in seedling length (Unnikannan *et al.,* 2014). The levels of chlorophyll a, chlorophyll b and carotenoid in control plants were found to be high when compare to chromium treated ones (Table-2). There was a progressive decrease in the photosynthetic pigments with increase in chromium concentration. The decrease in chlorophyll and carotenoid content is due to determinental effect of increased metal content (Larsen *et al.,* 1998). The decreased chlorophyll and carotenoid content would ultimately decrease the rate of photosynthesis (Zhang *et al.,* 2003; Mobin and Khan, 2007). The reduction in photosynthetic pigments may also be due to disruption of chloroplast phosphorylation observed in various plants as suggested by Data *et al.* (2011). The results related to the effect of chromium on total sugars shows the same pattern of response as in the case of photosynthetic pigments (Table-3). Such changes in sugar content have been reported by Mahadeswaraswamy and Theresa (1992).

Study with the estimation of free amino acids and protein showed a declining trend with progressive increase in chromium concentration. Similar trend was also recorded by Jana *et al.* (1987). The degradation of amino acids and protein content might be due to the increased protease or other catabolic enzymes by chromium activity stress (Vajpayee *et al.,* 2001). The level of proline content in root and shoot showed an increasing trend with increase in metal concentration (Table-4). Proline accumulation is a symptoms of injury which does not confer tolerance against metal and other stresses (Lutts *et al.,* 1996). According to Chen *et al.* (2003), the indication to self protection by plants growing under stress conditions is their free proline accumulating capacity. In plants subject to metals stress condition, free proline content rapidly increases as a means of protection against stress. Antioxidants are important species which possess the ability of protecting organisms from damage caused by free radical induced oxidative stress (Canada novic – Brunet *et al.,* 2005). An antioxidant is a substance capable of slowing or preventing oxidation of other molecules. The antioxidant enzymes catalase and peroxidase increased significantly in the plant samples (Table-5. Functionally catalases are related to peroxidases, both promote hydrogen peroxidase oxidation by mechanisms that involve ferryl intermediates (Desisseroth and Dounce, 1970; Dawson, 1988). Catalases are believed to be involved in the protective destruction of hydrogen peroxidase that is generating in respiring cells (Shaffer *et al.,* 1987). Nitrate reductase (NR) is a key enzyme in the conversion of nitrate to nivrite and its sustained activity is crucial for nitrogen assimilation (Gouia *et al.,* 2005). Reduction in NR activity with increase in metal concentration reduced the nitrogen function and ammonia assimilation in nodules have been reported in legumes with applied cadmium (Balestrasse *et al.,* 2004).

From the present investigation it can be concluded that the phytotoxicity effect of chromium have severely affected the germination, seedling growth, biochemical changes and antioxidant properties of *Vignamungo.*

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