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Responses of *Phaseolus vulgaris* to chromium and cobalt treatments

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Abstract

The effects of Cr and Co supplied either individually or mixed together in the nutrient solution on seed germination, enzyme activities, photosynthesis, metabolic products, and yield were investigated. Cr and Co reduced germination percentage only at the highest concentration used but markedly decreased radicle growth which might be attributed to depressive effect of Cr and Co on the activity of amylases and subsequent transport of sugars to the embryo axes. Protease activity, on the other hand, increased with the metal treatment. The highest concentration (10^{-2} M) tested of both metals was harmful on plant growth, while the low and moderate concentrations (10^{-6} and 10^{-4} M) enhanced the contents of chlorophylls and sugars, and activity of Hill reaction. Fresh mass of the produced pods increased at low and moderate concentrations of Cr and at Cr+Co treatment, but decreased in plants treated with Co.

Additional key words: carotenoids, chlorophyll, enzyme activity, heavy metals, Hill reaction.

Introduction

Recently, the world has become disturbed by the problem of pollution of the environment by trace elements. Deviation in trace element availability beyond the normal physiological range may affect many physiological processes, growth and yield of plants. Some heavy metals, *e.g.* copper, zinc and nickel are essential for growth at very low concentrations but toxic at slightly ones (Gadd and Griffiths 1978, Reed and Gadd 1989). Heavy metals may affect chlorophyll content, enzyme activities, and cause degeneration of chloroplasts and mitochondria (Reddy and Prasad 1990). Chromium decreased germination percentage in rice (Samantaray and Das 1991) but not in pea (Bishnoi *et al.* 1993), and decreased radicle and plumule growth of tomato, pea, *Salvia* and *Allium* (Atta Aly *et al.* 1991, Corradi *et al.* 1993, Liu *et al.* 1993, Nyarai *et al.* 1997). High concentrations of Co^{2+} had a toxic effect on cell division and induced chromosomal aberrations in root tips of *Allium cepa* (Liu *et al.* 1994).

The present investigation was conducted to evaluate the response of bean to chromium and cobalt treatment. Their effect on the activity of amylases and proteases was investigated in the germinating seeds; while on photosynthesis, chlorophyll, sugars and protein content at the seedling stage and at the flowering stage.

Materials and methods

The seeds of bean (*Phaseolus vulgaris* L., cv. Giza-3) were sown in pots (20 cm diameter) in pure sand irrigated with full Hoagland's nutrient solution. The pots were divided into ten groups, each group consisted of four replicates. In a preliminary experiment was found that 0.1 M of Cr and Co completely inhibited seed germination of been. Therefore, chromium was added in the nutrient solution to the first three groups at concentrations 10^{-6}, 10^{-4} and 10^{-2} M as $CrCl_3 \cdot 6\ H_2O$. Cobalt was added to the second three groups as 10^{-6}, 10^{-4} and 10^{-2} M of $CoCl_2 \cdot 6\ H_2O$. A mixture of both metals was added to the third three groups at the concentrations mentioned above. The last group served as control plants. The plant samples were taken for analysis at the seedling stage (21 d after sowing) and at the flowering stage (45-d-old). Percentage of seed germination and enzyme activities were assayed in seeds grown at 0, 10^{-6}, 10^{-4}, 10^{-2} and 5×10^{-2} M of Cr and/or Co for 4 d. The cell free extracts of the germinating seeds were prepared at 0 - 4 °C by macerating the seeds with a chilled pestle and

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mortar. The tissue homogenate was centrifuged at 10 000 *g* for 20 min and the supernatant obtained was used directly for determining enzyme activity. For assaying of α- and β-amylases activity 3,5-dinitrosalicylic acid reagent was used as described by Rick and Stegbauer (1974). Protease activity was measured according to Gallop *et al.* (1957). Contents of chlorophylls and carotenoids content were estimated spectrophotometrically (*Spectronic 20 D*) of plant leaves extracted in 85 % acetone according to Metzner *et al.* (1965). Fresh leaves were extracted in 70 % ethanol and completed to a known volume with distilled water and used for estimation of sugars and proteins. The saccharides and polysacharides were determined using anthrone reagent (Umbreit *et al.* 1959). The total protein content of leaves was estimated following the procedure described by Lowry *et al.* (1951). For extraction of DNA, the method of Marmur (1961) and Mohamed and Capesius (1980) was followed using Tris-EDTA buffer (pH 8), and the DNA content was determined spectrophotometrically at 595 nm using diphenylamine reagent as described by Stahl (1937). For isolation of chloroplasts, according to the method of Aronoff (1946) and Osman *et al.* (1982), fresh leaves were blended in cold buffered combined solution of 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM $MgCl_2$, 4 mM sodium ascorbate and 0.1 % bovine serum albumin. The mash was stained through cheese-cloth and filtered immediately through a small plug in glass funnel. The suspension was centrifuged at 4 °C (1 min at 800 *g*). The pellet was resuspended in the isolation buffer and centrifuged for 5 min at 300 *g* and the supernatant was then centrifuged for 10 min at 1000 *g*. Chloroplasts (residue) were resuspended in the buffer solution, The chlorophyll content of the obtained chloroplasts was determined by the method described by Mackinney (1941). Hill reaction of the isolated chloroplasts was measured by using potassium ferricyanide as electron acceptor. Reduction of ferricyanide was followed spectrophotometrically at 420 nm at room temperature.

Statistical analysis was carried out according to Snedecor and Cochran (1980) using analysis of variance and the significance was determined using LSD values at $P = 0.05$ and 0.01.

Results and discussion

Treatment of bean with both trace elements either individually or as mixture did not inhibit the radicle protrusion of bean seeds up to concentration of 10^{-1} M. Germination percentage of seeds was reduced by 34, 67, and 50 % at 5×10^{-2} M of Cr, Co and Cr+Co, respectively. The toxicity of Co was more obvious than that of Cr. The presence of Cr with Co in the mixture reduced the toxicity of high concentration of Co (Table 1). The radicle length was markedly reduced at 10^{-2} and 5×10^{-2} M of Cr and/or Co. In this respect, Corradi *et al.* (1993) reported that seed germination may not be affected by Cr treatment, but when the emergent radicle came into contact with the Cr solution, its growth was inhibited. Similar results have been obtained by Atta Aly *et al.* (1991), Liu *et al.* (1993), and Nyarai Horvath *et al.* (1997).

Table 1. Effect of Cr and Co on germination [%], DNA content [μg g^{-1}(f.m.)], radicle length [cm], activities of amylases and protease [U g^{-1}(f.m.) s^{-1}] in germinating bean seeds, and on pods fresh mass [g].

Treatment	[M]	Germination	DNA	Radicle length	α-amylase	β-amylase	Protease	Pods fresh mass
Control	0	100	5.12	4.53	0.182	0.181	0.009	10.05
Cr	10^{-6}	100	4.30	4.38	0.213	0.160	0.011	12.22
	10^{-4}	100	5.26	4.67	0.155	0.119	0.014	11.49
	10^{-2}	100	5.98	3.43	0.120	0.106	0.014	0
	5×10^{-2}	66	8.78	0.76	0.112	0.099	0.015	0
Co	10^{-6}	100	4.10	5.62	0.236	0.184	0.009	8.40
	10^{-4}	100	5.21	4.58	0.119	0.085	0.019	8.58
	10^{-2}	100	5.82	3.24	0.103	0.074	0.012	0
	5×10^{-2}	33	7.83	0.64	0.084	0.068	0.016	0
Cr + Co	10^{-6}	100	4.26	5.35	0.250	0.164	0.010	11.69
	10^{-4}	100	4.96	4.52	0.105	0.072	0.010	12.47
	10^{-2}	100	6.39	3.50	0.091	0.059	0.011	0
	5×10^{-2}	50	8.17	0.70	0.078	0.060	0.140	0
$LSD_{0.05}$			1.01	0.21	0.017	0.008	0.002	1.42
$LSD_{0.01}$			1.45	0.30	0.024	0.012	0.003	2.06

DNA content of bean germinating seeds increased progressively with increasing the concentration of Cr and/or Co in the external medium (Table 1). Therefore, the DNA content showed a reversed trend with that of the radicle growth. It has been suggested that high concentrations of Cr and Co inhibit cell division and elongation of bean roots. Bishnoi *et al.* (1993) reported a similar results on pea. However, Villalobos Pietrini *et al.* (1993) and Liu *et al.* (1994) recorded a toxic effect of high concentrations of Cr and Co on cell division and induction of chromosomal aberrations.

Hydrolysis of stored reserve materials such as starch and proteins occurs during seed germination for providing precursors such as sugars and amino acids for the growth of embryo axes as well as substrates for various metabolic processes. Activity of α- and β-amylases of the germinating seeds decreased with the progressive increase in the metal concentrations (Table 1). However, the activity of α-amylase seems to be stimulated by very low concentration (10^{-6} M) of Cr and/or Co. It has been suggested that the reduced germination percentage and radicle growth of seeds grown under severe stress of both metals may be attributed to the depressive effect of these metals on amylase activity and consequently, the sugar supply to the embryo axes. On the other hand, protease activity seemed to be stimulated by the metal treatment and the mobilization of cotyledonary reserve proteins increased during early germination of bean seeds (Table 1). The obtained results are in agreement with that of Dua and Sawhney (1991), who found a depressed activity of amylases, whereas the protease activity was enhanced during the initial phases, followed by insignificant effects at later stages of pea seed germination in the presence of Cr. Sheudzhen *et al.* (1994) recorded a similar results on rice seeds in response to Co treatment.

Seedling growth was inhibited by 10^{-2} M of Co after 10 d. Wilting occurred in plants treated with 10^{-2} M of Cr+Co after 30 d (data not shown). The same concentration of Cr suppressed plant growth after 45 d. Moderate and low concentrations (10^{-4} and 10^{-6} M) of Cr and Cr+Co were beneficial and resulted in increasing the fresh mass of the produced pods. On the other hand, the same concentrations of Co resulted in a considerable reduction in the fresh mass of pods (Table 1). Similar effects of high concentrations of Cr have been reported by Hara *et al.* (1976), and Sharma and Sharma (1993). Naguib *et al.* (1986a) also observed a beneficial effect of

Table 2. Effect of Cr and Co on chloroplast pigments [mg g^{-1}(d.m.)], Hill reaction activity [μmol(ferricyanide) g^{-1}(chlorophyll) s^{-1}], and saccharides and protein contents [mg g^{-1}(d.m.)] of bean leaves.

Age [d]	Treatment	[M]	Chl *a*	Chl *b*	Carotenoids	Hill reaction activity	Soluble saccharides	Polysaccharides	Protein
21	Control	0	12.79	8.15	2.52	10.28	123.8	151.6	66.6
	Cr	10^{-6}	13.64	8.45	2.68	12.78	144.7	148.8	63.8
		10^{-4}	13.72	8.77	2.69	13.06	125.5	149.8	65.1
		10^{-2}	12.36	8.05	2.14	5.83	134.4	136.0	62.2
	Co	10^{-6}	15.21	9.55	3.28	14.72	167.8	160.2	62.3
		10^{-4}	14.91	9.09	3.27	16.11	147.3	157.4	69.8
		10^{-2}	0	0	0	0	0	0	0
	Cr + Co	10^{-6}	13.52	8.63	2.73	12.78	153.1	145.5	64.5
		10^{-4}	14.65	9.09	2.87	13.61	133.1	147.5	64.3
		10^{-2}	8.61	4.79	0.86	5.56	88.0	141.6	70.7
45	Control	0	13.71	7.75	6.28	11.67	95.4	194.4	40.7
	Cr	10^{-6}	14.31	9.70	6.50	15.00	119.9	194.4	39.4
		10^{-4}	17.96	10.96	7.51	15.56	102.6	151.2	36.3
		10^{-2}	9.93	5.96	3.43	9.17	117.6	230.6	25.5
	Co	10^{-6}	14.23	8.69	7.02	14.17	129.4	192.8	37.2
		10^{-4}	13.02	7.37	6.08	14.72	112.5	185.3	42.0
		10^{-2}	0	0	0	0	0	0	0
	Cr + Co	10^{-6}	16.76	9.62	7.69	15.28	145.2	170.0	43.1
		10^{-4}	16.05	9.45	7.10	16.39	113.6	177.5	45.3
		10^{-2}	0	0	0	0	0	0	0
$LSD_{0.05}$			2.13	0.39	0.93	2.47	20.5	11.2	14.1
$LSD_{0.01}$			3.06	0.56	1.33	3.53	29.4	16.1	20.3

low Cr doses on fruit set and seed formation of *Datura stramonium*. In contrast to the present data, low concentrations of Co increased yield of cabbage (Kosinova 1975). This indicates a variable response of different plant species to the metal treatments.

Low and moderate concentrations (10^{-6} and 10^{-4} M) of Cr and/or Co increased pigment content in bean leaves (Table 2), but the higher concentration (10^{-2} M) reduced chlorophyll *a*, chlorophyll *b*, and carotenoids content, particularly at the mature stage (45-d-old). The positive effect of low and moderate concentrations of Cr and/or Co on chlorophyll synthesis may be attributed to the increased transport of Mg as a result of these treatments (Barcelo *et al.* 1985, Zeid and Kadi 1998). Chromium-induced chlorosis (at high concentrations) may be due to inhibition of Fe (Zeid and Kadi 1998) and Zn transport (Barcelo *et al.* 1986).

Treatment with 10^{-6} and 10^{-4} M of both metals either added individually or mixed together positively affected the Hill reaction of isolated chloroplasts but the higher concentration (10^{-2} M) suppressed it (Table 2). Krupa and Baszynski (1995) reported that Co and Cr affected both light and dark reactions of photosynthesis in cereal and legume crops. Mohanty *et al.* (1989) implied that Co ions inhibited either the reaction centre or the components of PS II acceptor site.

Metal treatment (10^{-6} and 10^{-4} M) increased soluble saccharides and polysaccharides content. However, the saccharide content of bean leaves was significantly reduced with 10^{-2} M of Cr and/or Co. At the mature stage (45-d-old) and during the wilting of the plants treated with 10^{-2} M of Cr, an accumulation of saccharides was observed, whilst the protein content was markedly reduced. This may be attributed to the reduction in N content under sever stress of heavy metals (Barcelo *et al.* 1985). However, the low concentrations of the metals did not significantly affect the total protein content of bean leaves (Table 2). These results are in general agreement with that of Naguib *et al.* (1986b) and Milenysheva (1986).

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