BIOLOGIA PLANTARUM 35 (4): 583-589, 1993

Effect of cadmium and nickel on mobilisation of food reserves and activities of hydrolytic enzymes in germinating pigeon pea seeds

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Abstract

Inclusion of cadmium as cadmium chloride and nickel as nickel chloride in media differentially induced dry matter mobilisation from cotyledons of germinating pigeon pea seeds. Cadmium depressed the activities of total amylase, protease, acid phosphatase and peroxidase in germinating seeds. The activities of these enzymes were stimulated at the lower concentrations of nickel and suppressed at the higher ones. This dual response of hydrolytic enzymes to cadmium and nickel is postulated to account for the promotory and inhibitory effects of these heavy metals on dry matter mobilisation.

Introduction

Unabated, indiscriminated, and uncontrolled discharge of hazardous chemicals into the environment has become a matter of major concern. Release of phytotoxic gases into the atmosphere and the contamination of arable lands with heavy metals are of immediate relevance to agriculture. Heavy metals such as Cd^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+} are non-degradable, quite easily taken up by plants, and hence readily enter the food chain (Lepp 1981). Heavy metals are toxic to plants and depress growth and yield of crops (*e.g.* Mishra and Kar 1974, Foy *et al.* 1978, Lepp 1981, Sheoran *et al.* 1990). Cd^{2+} being one of the more toxic elements, interfers with seed germination and seedling growth (Mrozek 1980). However, investigations on the effect of nickel on seed germination, growth and crop yield have given conflicting results. Seed germination represents an important and initial phase in the life cycle of plants. One of the early crucial events during seed germination entails mobilization of seed reserves because it supplies substrates for functioning of different metabolic processes including respiration and various anabolic pathways, which are essential for growth of embryonic axis (Bewley and Black 1978, Mayer and Poljakoff-Mayber 1982). The concomitant rise in metabolic activity is partly due to the activation by

Received 18 September 1992, *accepted* 3 February 1993.

hydration of enzymes already present in the dry seeds (Opik and Simon 1963, Dua and Sawhney 1991). In view of this, the present investigations were conducted to verify whether cadmium and nickel inhibit germination of pigeon pea seeds by diminishing mobilization of reserve material due to their deleterious effects on the activities of hydrolytic enzymes.

Materials and methods

Germination of seeds: Seeds of pigeon pea (*Cajanus cajan* L. cv. UPAS-120) were selected for uniformity and surface-sterilized with 0.2 % mercuric chloride solution for 5 min, thoroughly washed for one hour under running tap water and than placed on two layers of moistened Whatman No.1 filter paper disks in Petri plates (∅ 15 cm). Ten cm^3 of either distilled water (control) or solutions of $CdCl_2$ or $NiCl_2$ containing specified concentrations of Cd^{2+} and Ni^{2+} were poured into each Petri plate. Streptomycin sulphate (25 $\mu g\ cm^{-3}$) was included in all solutions to suppress microbial growth. The seeds were kept for germination at 25 ± 2 °C in the dark in an incubator. Samples were taken at 2, 4 and 6 d from soaking and the growth measurements were recorded. Experiments were done in duplicate and were repeated once.

Preparation of cell free extract: On the indicated days, germinating seeds were removed, thoroughly washed under running tap water and rinsed twice with glass distilled water. Cotyledons and embryo axis were separated. Cell free extracts was prepared at 0 - 4 °C by macerating the tissue in a chilled pestle and mortar. The tissue homogenate was centrifuged at 10 000 *g* for 20 min and the supernatant obtained was used for determining enzyme activity.

Enzyme assays: The extraction media and assay procedure for various enzymes were those as described by Beevers (1968) for protease, by Johnson *et al.* (1973) for acid phosphatase and by Seevers *et al.* (1971) for peroxidase. Total amylase activity was determined according to the method of Swain and Dekker (1966) except that the extraction buffer contained 1 mM $CaCl_2$ and 3 mM of 2-mercaptoethanol. Under the experimental conditions employed, the rate of reaction was strictly proportional to the amount of enzyme. For each treatment three replicates were taken and the enzyme activity in each of the extract was assayed in duplicate.

The data were statistically analysed for the mean ± SEM and C.D. at 5 % of six different determinations. Experiments were done in duplicate and were repeated once.

Results and discussion

The mobilisation of stored reserve material is a crucial event during seed germination for providing precursors for the growth of embryonic axis as well as substrate for various metabolic processes (Bewley and Black 1978, Mayer and Poljakoff-Mayber

1982). The dry mass of embryo axis increases with seedling age and is associated with decline in cotyledonary dry mass (Table 1). The extent of this inverse relationship representing mobilization from the cotyledons is influenced by Cd^{2+} and Ni^{2+}. The mobilization is significantly stimulated by Ni^{2+}, whereas with Cd^{2+} it is markedly suppressed. Although the extent of mobilization is affected by Cd^{2+} and Ni^{2+}, the pattern of mobilization remains unaltered with both the heavy metals. Our observations can be compared with those of Chugh (1992) who demonstrated that the magnitude of inhibition in pea was enhanced with increasing concentration of Cd^{2+}. During germination, starch which is the principal reserve carbohydrate is degraded to provide sugar either *via* amylolytic or phosphorylytic pathways (Swain and Dekker 1966).

Table 1. Effect of cadmium and nickel on dry mass of embryonic axis [mg per plant] and cotyledons [mg per 2 cotyledons] in pigeon pea seedlings germinated for 2, 4 or 6 d.

Treatment [mM]	Embryonic axis 2 d	4 d	6 d	Cotyledon 2 d	4 d	6 d
Control	14 ± 1.1	19 ± 1.2	65 ± 5.1	678 ± 16.1	617 ± 12.1	553 ± 19.2
Cd^{2+} 0.5	11 ± 0.8	13 ± 0.8	14 ± 1.0	679 ± 9.2	629 ± 10.3	578 ± 7.2
1.0	7 ± 0.2	12 ± 1.1	12 ± 0.7	687 ± 6.2	631 ± 9.7	592 ± 4.7
2.0	4 ± 0.4	6 ± 0.3	7 ± 0.2	709 ± 5.7	659 ± 8.4	610 ± 8.7
Ni^{2+} 0.5	15 ± 0.2	23 ± 0.3	67 ± 0.8	663 ± 9.3	602 ± 11.2	548 ± 8.9
1.0	16 ± 0.1	21 ± 0.5	62 ± 1.3	667 ± 6.7	614 ± 10.9	559 ± 7.7
2.0	16 ± 0.2	20 ± 0.2	59 ± 1.5	679 ± 8.2	623 ± 6.5	583 ± 9.2
C.D. at 5 %	0.9	1.2	2.6	8.4	10.7	13.9

A substantial activity of total amylase was detected in 2-d old germinating seeds in water, which increases linearly in embryonic axis and cotyledons (Table 2). A similar quantitative changes in enzyme profile occurred in the presence of 0.5 mM Cd^{2+} up to 4 d of germination in embryonic axis as well as in cotyledons, whereas its activity began to decline after 2 d with 1.0 and 2.0 mM Cd^{2+}. In the presence of Cd^{2+}, the enzyme activity was consistently and significantly lower than that of control. On the 6th day, when control embryonic axis and cotyledons exhibited maximum amylase activity, 0.5, 1.0 and 2.0 mM Cd^{2+} diminished activity level by 10, 27 and 38 % in embryonic axis and 31, 48 and 66 % in cotyledons, respectively. Total amylase activity was increased in the presence of 0.5, 1.0 and 2.0 mM Ni^{2+} (Table 2). Thus in the presence of 2.0 mM Ni^{2+} the total amylotic activity was depressed by 10 and 19 % in embryonic axis and by 16 and 24 % in cotyledons on the 4th and 6th day of germination, respectively.

During germination, mobilization of cotyledonary reserve proteins is mediated by proteases (Beevers 1968, Yomo and Varner 1973). In control, protease activity increased rapidly between 2 and 6 d (Table 3). Increase in caseolytic activity during initial stages of germination has been reported by Beevers (1968), Yomo and Varner

Table 2. Total amylase activity [μmol(maltose liberated) seed^{-1} s^{-1}] and in [% of control - numbers in parentheses] in the embryonic axis and cotyledons of the pigeon pea seeds germinated 2, 4 or 6 d in the presence of cadmium or nickel.

Treatment [mM]	Embryonic axis			Cotyledons		
	2 d	4 d	6 d	2 d	4 d	6 d
Control	1.17 ± 0.08 (100)	2.11 ± 0.22 (100)	5.67 ± 0.44 (100)	0.67 ± 0.08 (100)	1.14 ± 0.06 (100)	3.81 ± 0.31 (100)
Cd^{2+} 0.5	1.14 ± 0.03 (97)	2.11 ± 0.17 (100)	5.12 ± 0.22 (90)	0.78 ± 0.06 (116)	1.06 ± 0.03 (93)	2.61 ± 0.22 (69)
1.0	1.08 ± 0.11 (92)	1.72 ± 0.08 (82)	4.14 ± 0.36 (73)	0.58 ± 0.03 (87)	0.81 ± 0.08 (71)	1.97 ± 0.17 (52)
2.0	1.00 ± 0.06 (86)	1.50 ± 0.13 (71)	3.50 ± 0.28 (62)	0.53 ± 0.06 (79)	0.61 ± 0.03 (54)	1.25 ± 0.11 (33)
Ni^{2+} 0.5	1.61 ± 0.08 (137)	2.61 ± 0.19 (124)	6.42 ± 0.33 (113)	0.83 ± 0.03 (124)	1.31 ± 0.08 (114)	4.00 ± 0.31 (105)
1.0	1.42 ± 0.03 (121)	2.22 ± 0.08 (105)	5.56 ± 0.58 (98)	0.78 ± 0.06 (116)	1.08 ± 0.11 (96)	3.39 ± 0.20 (89)
2.0	1.39 ± 0.06 (118)	1.89 ± 0.14 (90)	4.59 ± 0.47 (81)	0.70 ± 0.06 (104)	0.95 ± 0.06 (84)	2.89 ± 0.25 (76)
C.D. at 5 %	0.08	0.21	0.57	0.17	0.11	0.42

(1973), Dua and Sawhney (1991). The influence of heavy metals on protease followed a pattern similar to that of total amylase, though the effect on protease was greater than that on total amylase during initial stage. Thus, on the 2nd day of germination, the level of protease activity in 0.5, 1.0 and 2.0 mM Cd^{2+} was 95, 87 and 80 % in embryonic axis of that in control, respectively. Reverse was found with nickel. On the 2nd day of germination, the level of protease activity in 0.5, 1.0 and 2.0 Ni^{2+} was increased by 53, 42 and 20 % in embryonic axis and 36, 21 and 12 % in cotyledons, respectively (Table 3).

Acid phosphatase is an important enzyme of phosphate metabolism and is considered to play an important role during seed germination (Bewley and Black 1978); as shown in Table 4, its activity was significantly depressed by cadmium. On the 6th day, 0.5, 1.0 and 2.0 mM Cd^{2+} caused 25, 33 and 43 % decrease in enzyme activity in embryonic axis and 28, 44 and 54 % decrease in enzyme activity in cotyledons, respectively. The influence of Ni^{2+} on acid phosphatase followed a pattern similar to that on total amylase.

A substantial activity of peroxidase was detected in seeds germinated in water on the 2nd day of germination which increases linearly in embryonic axis as well as cotyledons upto 4 d of germination and then begins to decline (Table 5). A quantitatively similar profile in change the enzyme level occurred in the 0.5, 1.0 and 2.0 mM Ni^{2+} in embryonic axis as well as in cotyledons, except 2.0 mM Ni^{2+} had no effect on the peroxidase activity in cotyledons (Table 5).

Table 3. Protease activity [μmol(amino acids released) $seed^{-1}$ s^{-1}], [% of control - numbers in parentheses] in the embryonic axis and cotyledons of the pigeon pea seeds germinated for 2, 4 or 6 d in the presence of cadmium and nickel.

Treatment [mM]	Embryonic axis 2 d	4 d	6 d	Cotyledons 2 d	4 d	6 d
Control	2.1+ ± 0.14 (100)	5.06 ± 0.47 (100)	10.65 ± 0.73 (100)	1.20 ± 0.06 (100)	2.97 ± 0.19 (100)	7.42 ± 0.53 (100)
Cd^{2+} 0.5	2.00 ± 0.22 (95)	4.61 ± 0.34 (91)	9.15 ± 0.61 (86)	1.11 ± 0.06 (92)	2.56 ± 0.19 (86)	6.00 ± 0.33 (81)
1.0	1.83 ± 0.11 (87)	4.09 ± 0.42 (81)	7.87 ± 0.36 (74)	1.00 ± 0.03 (84)	2.22 ± 0.14 (75)	5.25 ± 0.28 (71)
2.0	1.70 ± 0.08 (80)	3.84 ± 0.31 (76)	7.23 ± 0.47 (68)	0.92 ± 0.06 (76)	2.53 ± 0.17 (85)	5.77 ± 0.36 (70)
Ni^{2+} 0.5	3.22 ± 0.22 (153)	6.12 ± 0.36 (121)	10.95 ± 0.67 (103)	1.61 ± 0.08 (136)	3.70 ± 0.25 (124)	8.40 ± 0.36 (113)
1.0	3.00 ± 0.14 (142)	5.75 ± 0.50 (114)	10.12 ± 0.86 (95)	1.45 ± 0.03 (121)	3.11 ± 0.11 (105)	7.28 ± 0.61 (98)
2.0	2.53 ± 0.11 (120)	4.95 ± 0.39 (98)	9.56 ± 0.81 (90)	1.33 ± 0.08 (112)	2.72 ± 0.17 (92)	5.64 ± 0.50 (76)
C.D. at 5 %	0.09	0.24	0.21	0.06	0.31	0.72

Table 4. Acid phosphatase activity [nmol(P-nitrophenol produced) $seed^{-1}$ s^{-1}], [% of control - numbers in parentheses] in the embryonic axis and cotyledons of the pigeon pea seeds germinated for 2, 4 or 6 d in the presence of cadmium and nickel.

Treatment [mM]	Embryonic axis 2 d	4 d	6 d	Cotyledons 2 d	4 d	6 d
Control	11.70 ± 0.75 (100)	20.93± 1.20 (100)	36.06 ± 2.09 (100)	8.37 ± 0.61 (100)	17.57 ± 1.45 (100)	30.08 ± 2.53 (100)
Cd^{2+} 0.5	10.17 ± 0.47 (87)	16.54 ± 0.89 (79)	27.05 ± 1.83 (75)	6.92 ± 0.33 (83)	13.34 ± 1.86 (76)	21.66 ± 1.31 (72)
1.0	8:90 ± 0.67 (76)	14.87 ± 1.28 (71)	24.16 ± 1.64 (67)	5.95 ± 0.53 (71)	11.06 ± 0.61 (63)	16.85 ± 1.45 (56)
2.0	7.48 ± 0.33 (64)	12.56 ± 0.81 (60)	20.54 ± 1.97 (57)	4.42 ± 0.47 (53)	8.42 ± 0.36 (48)	13.82 ± 0.95 (46)
Ni^{2+} 0.5	14.73 ± 1.20 (126)	24.91 ± 2.00 (119)	37.14 ± 2.56 (103)	9.87 ± 0.78 (118)	19.32 ± 1.45 (110)	31.28 ± 2.72 (104)
1.0	13.46 ± 0.78 (115)	25.13 ± 1.78 (120)	34.25 ± 2.42 (95)	9.04 ± 0.86 (108)	16.68 ± 1.75 (95)	25.88 ± 2.42 (86)
2.0	11.45 ± 0.58 (98)	19.68 ± 1.20 (94)	31.36 ± 1.03 (87)	7.70 ± 0.53 (92)	14.60 ± 1.58 (83)	21.66 ± 1.50 (72)
C.D. at 5 %	0.65	1.83	2.94	0.80	1.12	3.02

Table 5. Peroxidase activity [nmol(H_2O_2 consumed) $seed^{-1}$ s^{-1}], [% of control - numbers in parentheses] in the embryonic axis and cotyledons of the pigeon pea seeds germinated 2, 4 or 6 d in the presence of cadmium and nickel.

Treatment [mM]	Embryonic axis			Cotyledons		
	2 d	4 d	6 d	2 d	4 d	6 d
Control	45.31 ± 3.95 (100)	85.62± 6.03 (100)	66.44 ± 4.89 (100)	37.81 ± 2.56 (100)	68.67 ± 4.81 (100)	60.06 ± 3.25 (100)
Cd^{2+} 0.5	43.09 ± 2.70 (95)	69.22 ± 2.97 (81)	45.87 ± 3.11 (69)	36.97 ± 2.97 (98)	57.55 ±11.45 (84)	43.92 ± 2.14 (73)
1.0	35.86 ± 3.14 (79)	53.10 ± 4.87 (62)	30.58 ± 2.70 (46)	32.53 ± 2.00 (86)	52.82 ± 2.31 (77)	39.75 ± 1.61 (66)
2.0	30.02 ± 2.00 (66)	41.14 ± 3.95 (48)	21.13 ± 1.72 (32)	28.36 ± 1.42 (75)	43.92 ± 2.11 (64)	29.45 ± 1.86 (49)
Ni^{2+} 0.5	61.99 ± 3.14 (137)	131.77 ± 5.89 (154)	85.07 ± 4.78 (128)	47.51 ± 3.36 (126)	83.68 ± 5.09 (122)	66.75 ± 2.56 (111)
1.0	57.55 ± 2.59 (127)	115.65 ± 4.36 (135)	77.01 ± 4.11 (116)	45.04 ± 5.34 (119)	77.00 ± 3.25 (112)	65.33 ± 1.89 (109)
2.0	53.38 ± 1.86 (118)	95.91 ± 3.81 (112)	71.17 ± 3.25 (107)	38.64 ± 2.72 (102)	71.45 ± 4.36 (104)	58.94 ± 2.92 (98)
C.D. at 5 %	2.19	7.41	5.43	2.03	4.25	3.32

In contrast, peroxidase activity was increasingly depressed with higher concentrations of Cd^{2+}. Thus, on the 6th d of germination, the level of this activity in 0.5, 1.0 and 2.0 mM Cd^{2+} was 69, 46 and 32 % as that in control in embryonic axis and 73, 66 and 49 % in cotyledons, respectively. Results also indicate that during germination, the extent of inhibition increased with time. Thus, in the presence of 2.0 mM Cd^{2+}, the peroxidase activity was depressed by 34, 52 and 68 % in embryonic axis and by 25, 36 and 51 % in cotyledons on 2, 4 and 6 d of germination.

Thus, in conclusion, nickel at lower concentration might be enhancing the availability of substances by stimulating the activity of hydrolytic enzymes and thus causing the enhancement of dry matter mobilization. Similarly, a restricted availability of potentially mobile matter, through the suppression of activity of hydrolytic enzymes by cadmium, could be responsible for suppression of dry matter mobilization from cotyledons. However, precise explanation of the mechanism of action requires further work.

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