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CADMIUM PARTITIONING AND SEED QUALITY IN TWO VARIETIES OF PEA AND THEIR HYBRID AS INFLUENCED BY RHIZOSPHERIC CADMIUM

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SUMMARY

Cadmium partitioning within pea plants and its effect on biomass production and seed quality were studied in a pot experiment using river sand as rooting medium. The two varieties (Arkel and HPF-4) and their hybrid (Arkel x HFP-4) differed in biomass production. Low dose of Cd^{2+} (2.5 mM) increased biomass of roots and above ground parts, while higher doses decreased shoot biomass in both cvs, root biomass only in HFP-4. Cadmium decreased both root and above ground biomass in the hybrid. Cadmium content in root and seed was maximum in HFP-4 and least in the hybrid and Arketl, respectively. Pod wall of the hybrid retained maximum amount of cadimum. Cadmium decreased starch content of seeds and such a reduction was evident even at 2.5 mM Cd^{2+} in HFP-4. It, however, did not affect total soluble proteins significantly except in the bybrid seeds. Cd^{2+} increased seed phytate and increase was significantly more in Arkel than HFP-4 and the hybrid.

***Key words*: Cadmium-partitioning, phytate, protein, seed quality, starch, pea.**

INTRODUCTION

Disposal of sewage sludge mixed with industial effluents in an arable land coupled with the application of agricultural chemicals like pesticides, herbicides and fertilizers contaminate plant rhizosphere with a variety of heavy metals (HM). The problem of HM toxicity is further aggravated due to the persistence of these metals in the environment. Among an array of HMs, special attention has been drawn to Cadmium (Cd^{+2}) due to its high toxicity. In most plants, exposure to elevated levels of Cd^{2+} results in growth inhibition, which is coupled with chlorosis and necrotic lesions. Severity of Cd^{2+} toxicity depends upon its uptkae and partitioning within the plant. Florijn and Van Beausichem (1993) reported varietal differences in Cd^{2+} uptake pattern in maize. In pea, some varieties are used as green vegetable, while others as pulses. Varieties used as green vegetable (Arkel, PH-1 etc.) are leafy, tall, early flowering and sensitive to water-logging and powdery mildew, while those used as pulse (HFP-4, HFP-8712 etc.) are completely tendrillar except the stipules, dwarf, late flowering and resistant to above said stresses. Cadmium content of seeds of these plants may vary widely. Moreover, Cd^{2+} may deteriorate the quality of seeds due to altered plant as well as seed metabolism. Therefore, it was of interest to know if and how rhizospheric Cd^{2+} supply affects its partitioning within the pea plant and in turn the quality of seeds.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L.) varieties Arkel, HFP-4 and hybrid (Arkel x HFP-4) were first surface sterilized with 20% sodium hypochlorite. Surface sterilized

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seeds were washed thoroughly with distilled water (5-6 times) inoculated in a broth of *Rhizobium leguminosarum* strain bv. viciae for half an hour and sown in cement pots. Each pot was lined with a polythene bag with a central drainage hole and filled with six kg of river sand. Three plants of uniform size were maintained in each pot. Plants were supplied with N-free nutrient solution (Wilson and Reisenauer 1963) at an interval of 10 days throughout the course of crop growth except a starter dose of NO_3^- N (45 mg/pot).

Plants were exposed to a range of cadmium ($CdCl_2.H_2O$) concentrations *i.e.* 0, 2.5, 5.0, 7.5 and 10.0 mM, approx. 15 days prior to anticipated date of initiation of flowering. One hundred and fifty mg fresh weight of physiologically mature seeds were homogenized in 80% ethanol (v/v) using acid washed sand as an abrasive. The homogenate was refluxed for 15 minutes on a water bath at 60ºC and centrifuged. The residue was refluxed thrice with 80% ethanol. The supernatants were pooled together and used for estimation of soluble sugars (Yemm and Willis 1954). The left over pellet was further hydrolysed with 4 ml of chilled 0.2 N $HClO_4$ for 24 h at 4ºC and hydrolysate was used for starch estimation (McCreddy *et al.* 1958). The pellet left after starch hydrolysis was further hydrolysed with 1N NaOH and used for estimation of total soluble proteins (Lowry *et. al.* 1951). Proline was extracted with 3% sulphosalicylic acid (Bates *et. al.* 1973) and content was estimated by using acid ninhydrin and toluene. Phytate was extracted and determined according to the procedure of Davies and Reid (1979). At maturity, biomass of roots and aboveground parts were recorded. For determination of cadmium content, 500 mg of dried samples of root, shoot, pod wall and seeds, were digested in 10 ml of diacid mixture (perchloric acid and sulphuric acid; 1 : 4). Resulting digested solution was cooled to room termperature and its volume was made to 25 ml with distilled water and cadmium content was determined by Atomic Absorption Spectrophotometer (Perkin-Elmer Analyst 100).

RESULTS AND DISCUSSION

Under normal condition, accumulation of dry matter in the roots as well as above ground parts was higher in HFP-4 followed by the hybrid and least in Arkel. Above ground biomass of HFP-4 and the hybrid was nearly identical, while root biomass of HFP-4 was nearly three times that of Arkel. Cadmium treatment did not affect dry matter accumulation in the roots and above ground parts of Arkel except 7.5 mM Cd^{2+} which was inhibitory to accumulation of dry matter in above ground parts. On the other hand, 2.5 mM Cd^{2+} enhanced the dry matter accumulation in roots as well as above ground parts of HFP-4 (Table 1). Similar stimulatory effect of low dose of Cd^{2+} on dry matter accumulation has been reported by Chugh (1991). Root biomass of the hybrid was suppressed by even lower dose of Cd^{2+} (2.5 mM), which did not

Table 1. Effect of Cd^{2+} levels on root and above ground biomass production (g) in two cvs. of pea and their hybrid.

Cd^{2+} treatment (mM)	Root biomass				Above ground biomass			
	Arkel	HFP-4	Hybrid	Mean	Arkel	HFP-4	Hybrid	Mean
C	0.11±0.01	0.30±0.04	0.18±0.01	0.20	3.26±0.04	5.78±0.06	5.21±0.01	4.75
2.5	0.11±0.02	0.40±0.01	0.10±0.00	0.20	2.97±0.58	6.83±0.20	4.17±0.44	4.66
5.0	0.11±0.00	0.33±0.06	0.09±0.00	0.18	2.87±0.27	5.63±0.16	3.63±0.24	4.04
7.5	0.09±0.03	0.25±0.04	0.09±0.01	0.14	2.57±0.64	5.63±0.03	3.07±0.30	3.76
10.0	*	0.14±0.04	*		*	5.63±0.03	*	
Mean	0.10	0.28	0.11		2.92	5.90	4.02	

*Plants did not survive.

CD (P=0.05)		Root biomass	Above ground biomass
Cultivatar	=	0.03	0.35
Treatment	=	0.03	0.40
Cultivar x Treatment	=	0.06	0.70

change with the further increase in the level of Cd^{2+}, while aboveground biomass decreased gradually with the increase in the level of Cd^{2+}. Ouariti *et. al.* (1997) reported a sharp decline in the accumulation of dry weight of root and shoot in bean even at lowest concentration used.

Cadmium treatment lead to accumulation of Cd^{2+} in root, being higher in HFP-4 and least in the hybrid. There was considerable increase in root Cd^{2+} in Arkel even by 2.5 mM Cd^{2+}, which remained nearly unchanged with further increase in rhizospheric Cd^{2+}, while in HFP-4 and the hybrid there was gradual increase in Cd^{2+} content (Table 2). Accumulation of Cd^{2+} in the shoot depended upon concentration of rhizospheric cadmium and it was relatively more in Arkel than HFP-4. At 2.5 mM Cd^{2+}, shoot Cd^{2+} in the two parent cvs. was nearly identical but was much less in the hybrid (Table 2). Cadmium treatment lead to an increase in cadmium in the pod wall and accumulation in the hybrid was more than the two parents. There was a gradual dose dependent increase in Cd^{2+} in Arkel and the hybrid. While in HFP-4 such an increase in cadmium was evident only up to 2.5 mM rhizospheric Cd^{2+}, which then remained unaltered with the further increase in cadmium (Table 2). Accumulation of cadmium was significantly higher in HFP-4 than Arkel and the bybrid. Divya (1999) also reported that HFP-4 seedlings accumulated more cadmium than the Arkel and accumulation was more in the radicle than the plumule. Cultivar HFP-4, seems to be Cd^{2+} shoot excluders. This behaviour is one of the several strategies of Cd^{2+} tolerance adopted by plants (Fett *et al.* 1994). Cd^{2+} retention in roots might be due to cross linking of Cd^{2+} to carboxyl groups of the cell wall (Barcelo and Pschenreider 1990) and/or to an interaction with thiol residues of soluble proteins (Lieta *et al.* 1991) or, binding of Cd^{2+} with phytochelatins (Prasad 1995, Jemal *et al.* 1998). John (1973), however, detected very low levels of Cd^{2+} in pea seeds of the plants raised with 40 and 200 µg Cd^{2+} g^{-1} soil. These differences in seed cadmium might be due to differences in the genotype handled by different workers.

Starch, which constitutes the major polysaccharide reseve in pea seeds, was found to account for about 40% of dry weight Starch content of seeds was affected differently by cadmium. Low concentration of Cd^{2+} (up to 5.0 mM) did not affect starch content in seeds of Arkel and the hybrid, while higher concentraion decreased it. On the other hand, even low dose of Cd^{2+} (2.5 mM) caused a significant decrease in starch content in HFP-4 (Table 3). Soluble sugar content in the hybrid was higher than the two parents. Low dose of Cd^{2+} (2.5 mM) did not affect soluble sugar content in the two parents but induced a significant increase at 5.0 mM level, which remained nearly unchnaged with further increase in dose of Cd^{2+} treatment except at 10 mM cadmium in HFP-4 (Table 3).

Protien content was found to be higher in Arkel and the hybrid than HFP-4 (Table 4). Cadmium treatment in general did not affect the seed protein appreciably in the two parent cvs. and their hybrid except in HFP-4, where a decrease in protein content by higher doses (7.5 mM) was evident. Singh *et al.* (1988) reported that soluble protein content of pea leaves decreased at higher concentrations of applied cadmium, which was further confirmed in *Phaseolus vulgaris* (Poschenrieder *et al.* 1983) and pigeon pea (Sheoran *et al.* 1990). Seeds of HFP-4 and the hybrid had higher proline content than Arkel. Cadmium treatment up to 5.0 mM did not affect the proline content in Arkel, while higher treatments increased it. There was an increase in seed proline content in HFP-4 and the hybrid even at lower doses (2.5 mM) of Cd^{2+}, which increased with the further rise in cadmium (Table 4). Phytin a calcium and magnesium salt of inositol hexaphosphate is present in abundance in cereal and legume seeds and may contribute upto 80 per cent of the total phosphorus (Mayer and Poljakoff-Mayber 1989). Present investigation revealed that physiologically mature seeds of HFP-4, plants have high phytate content, while it was low and nearly identical in the Arkel and the hybrid.Cadmium treatment up to 5.0 mM increased seed phytate in Arkel, which did not change with the further increase in Cd^{2+}. No change in seed phytate content was evident in HFP-4 up to Cd^{2+} treatment of 5.0 mM, which increased at 7.5 mM level. In case of hybrid, 2.5 mM cadmium increased seed phytate content, while higher doses did not affect it significantly (Table 5). It is thus evident that cadmium treatment increased the phytate content in seeds of two cvs. and their hybrid, increase being more in HFP-4 and least in hybrid. Variation in phytic acid content, however, are reported to have no bearing on seed germination and seedling growth in

Table 2. Effect of Cd^{2+} levels on Cd^{2+} distribution in root, shoot, pod wall and seeds (µg g^{-1}) in two cvs. of pea and their hybrid.

Cd^{2+} treatment (mM)	**Root**			**Shoot**			**Pod wall**			**Seed**		
	Arkel	**HFP-4**	**Hybrid**	**Arkel**	**HFP-4**	**Hybrid**	**Arkel**	**HFP-4**	**Hybrid**	**Arkel**	**HFP-4**	**Hybrid**
C	1.25 ±0.35	1.75 ±0.35	1.25 ±0.35	1.00 ±0.00	1.50 ±0.71	3.75 ±1.06	0.75 ±0.35	1.50 ±0.00	2.25 ±1.06	1.75 ±0.35	1.75 ±0.35	3.25 ±1.77
2.5	33.0 ±0.71	30.50 ±1.41	3.75 ±1.06	25.00 ±7.78	23.75 ±0.35	16.25 ±4.60	17.50 ±0.71	28.25 ±10.25	33.50 ±1.41	22.25 ±3.89	31.00 ±10.61	28.75 ±1.77
5.0	35.00 ±0.71	66.50 ±9.19	7.75 ±1.77	27.00 ±12.73	25.00 ±0.71	31.25 ±6.01	27.75 ±2.47	34.75 ±0.35	39.25 ±5.30	27.75 ±1.77	66.00 ±3.54	35.50 ±6.36
7.5	38.50 ±1.41	72.75 ±0.35	12.75 ±0.35	39.25 ±0.35	25.00 ±3.53	33.50 ±6.36	38.75 ±6.01	32,25 ±6.72	45.25 ±1.06	39.75 ±0.35	89.00 ±1.41	44.85 ±6.58
10.0	*	80.00 ±4.41	*	*	29.75 ±0.35	*	*	33.25 ±1.77	*	*	91.00 ±1.41	*
Mean	26.94	50.30	6.38	23.06	21.00	21.19	21.19	26.00	30.06	22.88	55.75	28.09

*Plants did not survive

CD (P=0.05)

Cultivar	=	3.05	5.76	4.72	4.83
Treatment	=	3.53	6.65	5.45	5.57
Cultivar × Treatment (C×T)	=	6.11	11.52	9-45	9.66

Table 3. Effect of Cd^{2+} levels on starch and soluble sugar (in parentheses) contents (mg g^{-1}) in physiologically mature seeds of two cvs of pea and their hybrid.

Cd^{2+} treatment (mM)	**Arkel**	**HFP-4**	**Hybrid**	**Mean**
C	336±8.91 (39±0.35)	381±14.42 (34±3.04)	365±18.38 (53±1.44)	360.67 (42.00)
2.5	397±19.37 (36±3.08)	299±24.61 (48±0.00)	374±71.28 (68±8.60)	356.67 (50.67)
5.0	360±15.56 (64±8.43)	276±19.02 (101±9.98)	333±7.64 (32±7.48)	323.00 (65.67)
7.5	229±12.73 (70±5.72)	286±23.83 (94±20.48)	229±33.30 (40±2.49)	248.00 (68.00)
10.0	*	311±8.63 (147±32.31)	*	
Mean	330.50 (52.25)	310.60 (84.77)	325.00 (48.25)	

*Plants did not survive.

CD at (P=0.05)

		For soluble sugars	For starch
Cultivatar	=	7.87	30.14
Treatment	=	10.14	34.81
Cultivar x Treatment	=	17.57	60.32

Table 4. Effect of Cd^{2+} levels on protein (mg g^{-1} dry wt.) and proline content (µg g^{-1}) (in parentheses) of physiologically mature seeds in two cvs of pea and their hybrid.

Cd^{2+} treatment (mM)	Arkel	HFP-4	Hybrid	Mean
C	256±1.34 (66±12.33)	219±4.95 (119±0.00)	262±14.64 (154±27.72)	245.67 (114.00)
2.5	261±12.30 (80±22.63)	224±6.08 (177±1.41)	268±4.38 (250±35.43)	251.00 (169.00)
5.0	280±3.75 (64±18.03)	227±7.14 (301±60.81)	272±13.08 (217±18.10)	259.67 (194.00)
7.5	239±18.24 (314±110.87)	186.6.08 (412±0.00)	249±17.39 (289±12.23)	224.67 (338.33)
10.0	*	173±1.84 (627±55.44)	*	
Mean	259.00 (131.00)	205.80 (327.20)	262.75 (228.25)	

*Plants did not survive.

CD (P=0.05)

		For proline	For proteins
Cultivatar	=	50.68	11.58
Treatment	=	43.89	13.38
Cultivar x Treatment	=	87.83	23.78

Table 5. Effect of Cd^{2+} levels on phytate content (mg g^{-1}) of mature seeds in two cvs. of pea and their hybrid.

Cd^{2+} treatment (mM)	Arkel	HFP-4	Hybrid	Mean
0	4.10±1.27	10.60±3.68	5.00±0.00	6.57
2.5	8.65±1.20	10.20±3.11	8.55±0.78	9.13
5.0	10.10±1.98	13.20±3.96	4.50±0.71	9.27
7.5	7.90±2.26	17.45±1.34	3.20±1.13	9.52
10.0	*	7.65±3.04	*	
Mean	7.69	11.82	5.31	

*Plants did not survive.

CD (P=0.05)

Cultivatar	=	2.34
Treatment	=	2.70
Cultivar x Treatment	=	4.67

soybean (Raboy *et al.* 1985). Thus, present study indicate that dry matter accumulation is significantly reduced by 7.5 mM and 10 mM cadmium in hybrid and HFP-4, respectively. Cadmium treatment failed to have any impact on the root biomass in Arkel. On the other hand, cadmium did not affect above ground biomass in HFP-4 but decreased in Arkel and the hybrid even at 2.5 mM level. Greater reduction in root biomass in HFP-4 may be explained on the basis of retention of most of the cadmium absorbed in roots, while a major proportion of it is translocated to above ground parts in Arkel and the hybrid. A major proportion of cadmium transported to shoots in HFP-4 seems to be preferentially stored in the seeds. Accumulation of cadmium has a bearing on the accumulation of seeds reserves. Consequently, seeds of HFP-4 have lower starch and protein contents than Arkel and the hybrid. This mean that deterioration in the quality of seeds of HFP-4 is more than Arkel and the hybrid. Furthernore, cadmium increased seed phytate was significantly more in Arkel than HFP-4.

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REFERENCES

Anita Rani (1991). Effect of Cr(IV) on carbon metabolism in germinating pea seeds. Ph.D. Thesis, CCS HAU, Hisar, India.

Barelo, J. and Poschenrieder, C. (1990). Plant water relations as affected by heavy metal stress. A Review. *J. Plant Nutr.* **13:** 37-40.

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil* **39:** 205-207.

Chugh, L.K. (1991). Effect of cadmium on carbon and nitrogen metabolism of pea (*Pisum sativum*). Ph. D. Thesis, CCS HAU, Hisar, India.

Davies, N.T. and Reid, H. (1979). An evaluation of the phytate, zinc, copper, iron and manganese contents of, and zinc availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. *British J. Nutr.* **41:** 579-589.

Divya (1999). Screening of pea cultivars for cadmium toxicity and mechanism of cadmium-calcium interaction. Ph.D. Thesis, CCS HAU, Hisar, India.

Fett, J.P., Cambria, J., Oliva, M.A. and Jordao, C.P. (1994). Absorption and distribution of cadmium in water hyacinth plants. *J. Plant Nutr.* **17:** 1219-1230.

Florijn, P.J. and Van Beusichem, M.L. (1993). Uptake and distribution of cadmium in maize inbred lines. *Plant Soil* **150:** 25-32.

Jemal, F., Didierjean, L., Ghrir, R., Ghorbal, M.H. and Burkard, G. (1998). Characterization of cadmium binding peptides from pepper (*Capsicum annuum*). *Plant Sci.* **137:** 143-154.

John, M.K. (1973). Cadmium uptake by eight food crops as influenced by various soil levels of cadmium. *Environ. Pollut.* **4:** 7-15.

Leita, L., Contin, M. and Maggioni, A. (1991). Distribution of cadmium and induced Cd-binding proteins in roots, stem and leaves of *Phaseolus vulgaris. Plant Sci.* **77:** 139-147.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with folin-phenol reagent. *J. Biol. Chem.* **193:** 265-275.

Mayer, A.M. and Poljakoff-Mayber, A**.** (1989). The germination of seeds. 4th edition. Pergamon Press, Oxford.

McCreddy, R.M., Guggolz, J., Silviera, V. and Owens, H.S. (1958). Determination of starch and amylase in vegetables. *Anal. Chem.* **22:** 1156.

Ouariti, O., Gouia, H. and Ghorbal, M.H. (1997). Responses of bean and tomato plants to cadmium : Growth, mineral nutrition and nitrate reduction. *Plant Physiol. Biochem.* **35:** 347-354.

Poschenrieder, C., Cabot, C. and Barcelo, J. (1983). Influence of high concentration of cadmium on the growth, development and photosynthetic pigments of *Phaseolus vulgaris*. *Anales de Edafologia y Agrobiologia* **42:** 315-327.

Prasad, M.N.V. (1995). Cadmium toxicity and tolerance in vascular plants. Environ. Expt. Bot. **35:** 525-545.

Raboy, V., Hudson, S.J. and Dickson, D.B. (1985). Reduced phytic acid content does not have any adverse effect on germination of soybean seeds. *Plant Physiol.* **79:** 323-325.

Sheoran, I.S., Singal, H.R. and Singh, R. (1990). Effect of cadmium and nickel on photosynthesis and the enzymes of photosynthetic carbon reduction cycle in pigeonpea. *Phytosynth. Res.* **23:** 343-351.

Singh, D.N., Srivastava, H.S. and Singh, R.P. (1988). Nitrate assimilation in pea leaves in the presence of cadmium. *Water, Air, Soil Pollut.* **42:** 1-5.

Thompson, L.U. (1989). In : Kinsella, J.E., Soucie, W.G. (eds.), Food Proteins. pp. 410-431. American Oil Chemical Society, Champaign, IL.

Wilson, D.O. and Reisenauer, H.M. (1963). Cobalt requirement of symbiotically grown alfalfa. *Plant Soil* **18:** 364-373.

Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.* **57:** 508-514.