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SEXUAL REPRODUCTION AND CADMIUM PARTITIONING IN TWO MUNGBEAN GENOTYPES RAISED IN SOILS CONTAMINATED WITH CADMIUM

SURESH KUMAR AND H. R. DHINGRA*

Reproductive Biology Laboratory
Department of Botany and Plant Physiology
CCS Haryana Agricultural University, Hisar-125004

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SUMMARY

Plants of two mungbean genotypes MH 85-111 and MH 98-6 were exposed to different levels of cadmium 28 days after sowing. Plants exposed to 3.0 and 4.0 mM Cd^{+2} did not survive and died before entering into reproductive phase. Cadmium induced reduction in the number of flowers and *in vitro* pollen germination but did not affect pollen viability. However, it stimulated tube growth. Cadmium although did not affect pistil length, it decreased number of ovules/ pistil. Ovules were morphologically normal and receptive. *In vivo* stylar studies revealed all the ovules were not penetrated by pollen tube and number of unpenetrated proximal ovules was increased by Cd^{+2} and cv. MH 85-111 was affected more adversely than MH 98-6. Cadmium inhibited number of pods, seeds, seed weight / plant and 100 seed weight, inhibition being more in MH 85-111 than MH 98-6. Cadmium treatment did not affect starch content but increased protein content in physiologically mature seeds. Accumulation of Cd^{+2} was maximum in the roots and least in the seeds. Cadmium accumulation, in general was higher in MH 85-111 than MH 98-6 and stem of MH 85-111 accumulated four times Cd^{+2} than MH 98-6. Seed cadmium however, was comparable in both the genotypes.

Key words : **Cadmium, mungbean, pollen, sexual reproduction**

INTRODUCTION

Use of municipal based composts as fertilizer and irrigation of crop fields with sewer water is being increasingly adopted by vegetable growers and marginal farmers. Though this strategy is intended to increase the fertility of soil and to circumvent scarcity of irrigation water, it inadvertently leads to addition of high quantity of heavy metals (HMs) to the agro-ecosystem, which results into the deterioration of soil quality, diminution of crop yield, concomitant with deteriorated seed quality. Among different HMs, Cd is of major concern. Nriagu and Pacyna (1988) reported that about 22 thousand metric tonnes of cadmium is globally discharged annually into the soil. Application of phosphatic fertilizers to the agricultural fields worsens the situation further. Cadmium affects plant growth, photosynthesis and metabolic processes adversely, which lead to diminished economic yield. Though reproductive characters have a direct relevance to seed production, their contribution in decreased crop yield in soils contaminated with cadmium have not received the desired attention. Therefore, it is relevant to evaluate its effect on reproductive characters.

Mungbean seeds are highly nutritious, easily digestible and non-flatulent, which make it suitable for consumption

* Corresponding authors, E-mail: hrdhingra@hau.ernet.in

by infants, old and sick persons. It provides a very good protein supplement to cereal-based diets in Indian population. Pulse breeders of CCS Haryana Agricultural University, Hisar, have developed a number of genotypes of mungbean but, so far, practically no information is available on their performance in soils polluted with cadmium. Present study was, therefore, undertaken to evaluate the effect of cadmium on competence of different reproductive components and cadmium partitioning within the plant.

MATERIALS AND METHODS

Plants from uniform sized seeds of two genotypes (MH85-111 and MH 98-6) of mungbean were raised in the screen house. Surface sterilized seeds were inoculated in a broth of *Rhizobium leguminosarum* strain S-24 and sown in cement pots lined with polythene bags. Each bag was having a central drainage hole and filled with 8 kg of dune sand. Eight seeds were sown in each pot. Seedlings 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). Thinning was carried out to leave four plants of uniform size in each pot. Plants were exposed to a range of cadmium concentrations (Control, 0.5, 1.0, 2.0, 3.0 and 4.0 mM) by applying cadmium solutions @ 650 ml per pot 28 days after sowing. Intermittent canal water irrigation was given as and when required.

Number of flowers produced / plant was recorded on alternate days until the flowering was complete. Floral buds were collected a day before anthesis and pollen from these flower buds were mixed thoroughly on a glazed paper. Quality of pollen was assessed in terms of their viability by TTC test (Hauser and Morrison 1964) and *in vitro* germination. Pollen were germinated on semisolid medium (sucrose 35%, boric acid and calcium nitrate 100 ppm each and agar 0.8%) contained in petri dishes supplementd with 0, 25, 50, 75, 100, 150, 200 and 250 µM Cd^{+2}. Petriplates were incubated at 30 ± 2 ºC for 3 h in dark in a BOD incubator. Three petriplates per treatment were used. After incubation, the pollen activity was terminated by flooding the surfaces of the media with killing and fixing solution (Sass 1951). Pollen producing a tube length of a size greater than its diameter was designated as germinated. Ten readings for pollen germination and 30 for tube length from different microscopic fields of each petriplates were made. Receptivity of the ovules was adjudged on the basis of callose deposition as evinced by aniline blue test. *In vivo* pollen tube growth was studied by collecting pistil samples 48-72 h after anther dehiscence and processed by aniline blue test (Dumas and Knox 1983).

To determine biochemical composition, 100 mg of physiologically mature seeds were homogenized in 80% ethanol (v/v) using acid washed sand as an abrasive. The homogenate was refluxed thrice for 15 min on a water bath at 60ºC and centrifuged. The left over pellet was further hydrolyzed with 4 ml of chilled 0.2 N $HClO_4$ and allowed to stand for 24 h at 4ºC.The hydrolysate was used for starch estimation (McCreddy *et al.* 1958), while the leftover pellet was suspended in 1N NaOH at room temperature for 24 h, centrifuged and the supernatant was used for estimation of proteins according to the method of Lowry *et al.* (1951).

For determination of cadmium content, 500 mg of dried plant material was digested in 20 ml of diacid mixture (nitric acid and perchloric acid, 4:1), cooled to room temperature and volume was made to 25 ml. Cadmium content was determined by atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

Pollen viability was more than 94% in both the mungbean genotypes. Cadmium treatment did not bring about any significant change in the pollen viability (Table 1). These results are in conformity with the findings of Nirmal *et al.* (1996) in pea with regards to chromium. Manisha and Dhingra (2003) reported that Cd^{+2} did not affect pollen viability significantly in pea cultivars except HFP-4, where viability decreased at and beyond 7.5 mM Cd^{+2}. *In vitro* germination studies (Table 1) showed that pollen germination of untreated control plants was more than 85% in both the genotypes but tube length was more in MH 85-111 than MH 98-6. Pollen from plants grown under 0.5 mM Cd^{2+} did not exhibit any change in germination and tube growth, while at higher levels it reduced germination but stimulated tube growth significantly in MH 98-6 (Table 1). Cadmium mediated

Table 1. Effect of cadmium on sexual competence and yield components in mungbean genotypes.

Characters	Genotypes/ Cd Treatment (mM)								CD at 5% LS
	MH 85-111				MH 98-6				
	Control	0.5	1.0	2.0	Control	0.5	1.0	2.0	
Pollen viability(%)	96.20	92.55	92.79	91.73	94.33	90.71	93.82	91.96	C=NS T=NS CxT=NS
Pollen germination(%)	89.19	83.37	80.36	78.04	82.23	80.23	72.66	70.57	C=NS T=8.92 CxT=NS
Tube length(µm)	663.6	676.6	705.0	731.4	464.4	512.4	643.6	671.6	C=39.59 T=55.98 CxT=79.18
Style length (cm)	1.58	1.64	1.64	1.64	1.50	1.57	1.57	1.65	C=0.05 T=0.07 CxT=NS
Number of ovules/pistil	11.52	10.53	10.47	10.33	11.61	10.74	10.61	10.41	C=NS T=0.36 CxT=NS
Number of flowers/plant	17.62	14.73	12.58	9.87	16.80	15.37	13.87	9.50	C=NS T=2.92 CxT=NS
Number of pods/plant	12.93	9.75	8.25	6.37	12.37	10.97	9.75	6.56	C=NS T=1.63 CxT=NS
Pod setting (%)	73.38	66.19	65.58	64.54	74.97	70.72	70.29	69.05	
Number of seeds/pod	9.19	8.22	7.57	6.15	9.31	9.06	7.61	7.31	C=NS T=0.74 C×T=NS
Number of seeds/ plant	118.6	86.1	64.1	40.9	115.0	98.0	74.6	49.0	C=NS T=15.09 C×T=NS
Seed weight /plant (g)	2.69	2.07	1.53	1.11	2.87	2.15	1.65	1.35	C=NS T=0.46 CxT=NS
Test weight of 100 seeds (g)	3.02	2.94	2.56	2.54	3.12	2.98	2.90	2.86	C=0.09 T=0.13 CxT=NS

C= Genotype T= Treatment

stimulation in tube growth and stimulation of pollen germination by lower Cd^{2+} level has been reported in *Catharanthus roseus* (Salgare and Pathak 2001)

Pistils of MH 85-111 were longer than MH 98-6. Cadmium treatment, in general, did not affect pistil length in MH 85-111 but increased it in MH 98-6. Ovary length was not affected by Cd^{2+}. However, style length was increased by Cd^{2+} in MH 98-6 plants only. Overall interaction between genotypes and Cd^{2+} for the length of pistil and its component was statistically non-significant. The number of ovules in the pistils of both the genotypes did not differ significantly. Cadmium treatment decreased the number of ovules (Table 1). Similar reduction in the number of ovules by water stress has been reported in *Trifolium repens* (Turner 1993). These results support the findings of Manisha and Dhingra (2003) who found that higher doses of Cd^{+2} reduced pistil length and number of ovules /pistil in pea. Similarly, Surekha (2002) also observed a reduction in the number of ovules and pistil length in pea genotypes by chromium.

Pollen grains germinated lavishly on the surface of stigma of both mungbean genotypes raised under sand culture conditions with and without applied Cd^{2+}. These pollen tubes penetrated stigma, passed through the transmitting tissue of the style and entered the ovarian cavity. All the ovules were not penetrated by the pollen tube and the number of unpenetrated proximal ovules was increased by the rhizospheric Cd^{2+} in both the genotypes, MH 85-111 being affected more adversely than MH 98-6.

Total number of flowers/plant was comparable in both the genotypes. Cadmium decreased the number of flowers. Even the lowest dose of Cd^{2+} (0.5mM) caused a significant reduction in the number of flowers in MH 85-111 (Table 1), while no such effect was evident in MH 98-6. Similar inhibitory effects of cadmium on flower production has been reported in bean (Jain 1978) and tomato and egg plant (Khan and Khan 1983). Cadmium and chromium mediated impairment of flower production was found to vary with the genotype in pea (Nirmal *et al*. 1996, Dhingra 2002 and Manisha and Dhingra 2003). Cadmium also decreased the size of floral buds (Kumar *et al*. 2000).

Pod setting in untreated control plant was around 75% in both the mungbean genotypes, which decreased with Cd^{2+} treatment. Per cent reduction in pod set was more in MH 85-111 than MH 98-6. This suggests that Cd^{+2} interferes with the process of pod setting. Similar reduction in the number of pods to the extent of 60% by 10 mM Cd^{+2} has been reported in pea (Chugh 1991). This was coupled with reduction in pod size (Setia *et al*. 1989). Such a reduction in pod setting may be ascribed to formation of non-functional flowers possibly at the fag end of flowering, which abscised without being converted into pods or due to lack of fertilization of sufficient number of ovules in the pistil to support pod growth (Dhingra 2002)

Number of seeds/pod, number of seeds/plant and total seed weight/plant were identical in MH 98-6 and MH 85-111. Cd^{2+} treatment decreased all these parameters significantly. Overall comparison did not reveal any significant difference in tolerance status of two genotypes. The test weight of 100 healthy seeds was comparable in both the tested genotypes. It decreased with the increasing levels of Cd^{2+} treatment and the decrease was more in MH 85-111 than MH 98-6. The interactive effect of genotype and cadmium was statistically non-significant. Deleterious effect of cadmium on seed yield has also been reported in soybean (Aery and Sarkar 1991) and pea (Manisha and Dhingra 2003). Results obtained by Poschenreider *et al*. (1983) indicated that that reduction in seed size is mainly responsible for diminished yield in dwarf beans. Reduction in seed yield in pea has been found to be associated with decline in number of flowers, per cent pod set, number of seeds and seed size (Manisha and Dhingra 2003).

Starch, which constitutes the major polysaccharide seed reserve in mungbean, was identical in both genotypes. It was not affected significantly by cadmium except at 2.0 mM Cd^{2+} in MH 98-6. Protein content in physiologically mature seeds was also comparable in both the genotypes. Lower treatment of Cd^{2+}(0.5 mM) did not affect protein content, while higher treatments increased the protein content in both the genotypes (Table 2). Similar increase in protein content have also been reported in linseed (Chakravarty and Srivastava 1997). Plants are known to produce small cysteine containing peptides – phytochelatins in response to HMs (Prasad

Table 2. Effect of cadmium on starch and protein content (mg/g^{-1}) of physiologically mature mungbean seeds.

Cd Treatment (mM)	Starch		Protein	
	MH 85-111	MH 98-6	MH 85-111	MH 98-6
Control	400.8	341.1	233.9	247.9
0.5	418.7	338.8	271.3	249.1
1.0	340.2	433.8	277.4	291.8
2.0	344.4	457.3	358.8	326.8
Mean	376.0	392.7	285.3	278.7
CD at 5% LS	C(Genotype)=NS T(Treatment)=NS CxT =91.8		C=NS T=29.61 CxT=NS	

1995, Jemal *et al.* 1998) and these bind to HMs to detoxify them. Such low molecular weight proteins are anticipated to be synthesized in seeds as evident from their high protein content at elevated endogenous Cd^{+2}.

Among different plant components, accumulation of Cd^{2+} was maximum in the roots followed by stem, leaf, pod wall and seeds and this accumulation, in general, was significantly more in MH 85-111 than MH 98-6 (Table 3). Cadmium content in stem, leaf and pod wall of MH 85-111 was nearly 4.0, 1.5 and 2.0 times higher, respectively, than MH 98-6 at 2.0mM applied Cd^{2+}. Interestingly seed Cd^{2+} was identical in both the genotypes. Chugh (1991) recorded maximum concentration of Cd^{+2} in roots of Bonneville cv. of pea followed by leaves, stem, pod wall and least in seeds. Divya (1999) also reported higher concentrations of cadmium in the radicle of pea genotypes. Cd^{+2} retention

Table 3. Effect of rhizospheric cadmium on its partitioning in roots, stem, leaf, pod wall and seeds (µg g^{-1} dry wt.) in mungbean genotypes. Values in parentheses indicate per cent distribution in each component

Plant part	Genotypes / Cd treatment (mM)								Cd at
	MH 85-111				MH 98-6				5% LS
	Control	0.5	1.0	2.0	Control	0.5	1.0	2.0	
Root	2.0	45.00	99.40	130.90	2.05	27.50	74.70	89.60	C=5.58
	(24.33)	(36.73)	(51.80)	(41.79)	(23.86)	(34.37)	(56.51)	(52.83)	T=7.89
									CxT=11.10
Stem	1.70	27.50	40.00	100.00	1.90	12.50	12.50	25.00	C=7.39
	(20.68)	(22.45)	(26.84)	(31.93)	(22.12)	(15.62)	(9.45)	(14.74)	T=10.45
									CxT=10.45
Leaf	1.82	20.00	22.50	47.50	1.84	15.00	20.00	30.00	C=2.04
	(22.14)	(16.33)	(11.72)	(15.17)	(21.42)	(18.75)	(15.13)	(17.69)	T=2.88
									CxT= 4.08
Pod wall	1.45	17.50	17.50	20.00	1.55	10.00	10.00	10.00	C=2.04
	(17.64)	(14.29)	(9.14)	(6.39)	(18.04)	(12.50)	(7.56)	(5.90)	T=2.88
									CxT=4.08
Seed	1.25	12.50	12.50	15.00	1.25	15.00	15.00	15.00	C=NS
	(15.21)	(10.20)	(6.51)	(4.80)	(14.55)	(18.75)	(11.25)	(8.84)	T=2.88
									CxT=NS
Total	8.22	122.50	191.90	313.20	8.59	80.00	132.20	169.60	

in roots has been ascribed to cross linking of Cd^{+2} to carboxyl group of the cell wall (Barcelo and Poschenreider 1990) and/or to an interaction with thiol residues of soluble proteins (Leita *et al*. 1991) or binding of Cd^{+2} with phytochelatins (Prasad 1995, Jemal *et al*. 1998).

Perusal of various yield determinants has revealed that Cd^{+2} affects flower production considerably. Microsporogenesis does not seem to be affected by Cd^{+2} as pollen grains looked normal and had high viability. It may be acting through its effect on pollen production (Manisha and Dhingra 2003) and decreased germination coupled with stimulation of tube growth particularly in MH98-6. But this reduction in male fecundity does not seem to contribute significantly towards seed production since a large number of pollen grains are still available to sire seeds in mungbean. Reduction in the number of ovules is another strong determinant of seed yield, as has been observed in pea also (Manisha and Dhingra 2003). Moreover seed setting is not hundred per cent since some of the basal ovules are not penetrated by pollen tubes as evident from *in vivo* tube growth studies or those fertilized abort due to sibling rivalry for limited availability of photoassimilates. This contention is supported by the co-existence of undersized seeds with healthy seeds. Cadmium not only reduces seed production but affects their growth (weight) adversely. Abortion of genetically competent zygotes seems to be an adaptive measure to adjust the number of seeds to the bearing capacity of maternal parent. Cadmium although did not affect starch content of physiologically mature seeds but increased protein content, the increase being more in MH85-111 than MH98-6.

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