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Phytotoxicity of cadmium and its effect on two genotypes of *Brassica juncea* L.

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Abstract: By using two *Brassica juncea*, L. (Indian mustard) genotypes (Varuna and DHR-9504) a greenhouse experiment was carried out to study the effect of cadmium (Cd) on growth, yield and concentration of cadmium (Cd) in different plant parts at the Agricultural Research Station, Bilaspur Chhattisgarh, India during 2002-2003. Plants were grown under controlled climatic conditions and subjected to increasing Cd supply in the form of $CdCl2$ @ 0, 20, 40, 60, 80 and 100 mg Cd kg^{-1} soil. Cd phytotoxicity was shown by growth retardation of Varuna and DHR-9504. Varuna showed greater sensitivity to Cd toxicity than DHR-9504. Increasing Cd supply markedly reduced the seed, stem and root dry weight of both genotypes (Varuna and DHR-9504), and these decreases were more marked in Varuna. Due to increased level of Cd application, the Cd concentration increased significantly in various plant parts in both the *Brassica* genotypes tested. Increase in Cd concentration of about 5 times in seeds, 7 times in stems and 9 times in roots was noted with an application of 20 mg Cd kg^{-1} soil more than the control. Cd was accumulated in the roots in much higher amounts than in the stem and seeds, especially in the case of DHR-9504, indicating that there is limited transport of Cd from the root system to the above ground plant parts in DHR-9504 genotype due to the presence of strong Cd-binding proteins in the roots. Genotype DHR-9504 had significantly less uptake of Cd than genotype Varuna. More data are needed to ascertain the findings of this study.

Key words: *Brassica* genotypes, cadmium, Cd-binding protein, growth retardation, phytotoxicity.

سمية عنصر الكاديوم وتأثيرها على صنفين من نبات الخردل الهندي *Brassica juncea,* L

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كلية الدراسات العليا، بيلاسبور، جاتيسجار، الهند

ملخص: أجريت تجربة في البيوت الزجاجية لدراسة تأثير الكاديوم على نمو ومحصول صنفين من نبات الخردل الهندي (Varuna – DHR 9504) وكذلك على تركيز الكاديوم في الأجزاء المختلفة للنبات. وقد أجريت التجربة في محطة البحوث الزراعية في Bilaspur Chha Hisgarh في الهند خلال الموسم 2003/2002. النباتات كانت نامية تحت ظروف مناخية متحكم فيها مع إضافة الكاديوم (CdCl2) بتركيزات صفر، 20، 40، 60، 80، 100 ملجم / كجم تربة. لوحظ تأثير سمية الكاديوم على صنفي الخردل المستخدمين في التجربة حيث نقص معدل النمو. كان الصنف Varuna أكثر حساسية للكاديوم مقارنة بالصنف DHR 9504، وكلما زاد تركيز الكاديوم المضاف نقص بوضوح الوزن الجاف للبذور والساق والجذور لكلا الصنفين وهذا النقص كان أكثر وضوحاً في الصنف Varuna. ونظراً لزيادة تركيز الكاديوم المضاف فإن تركيز الكاديوم في أجزاء النبات زاد زيادة معنوية في كلا الصنفين، حيث زاد التركيز خمس مرات في البذور وسبع مرات في الساق وتسع مرات في الجذور عندما أضيف الكاديوم للتربة بتركيز 2 ملج / كجم من التربة مقارنة بنباتات المقارنة. ووجد أن الكاديوم تراكم في الجذور بمعدل كبير مقارنة بالساق والجذور وخاصة في صنف DHR 9504 مما يشير إلى أن هناك إنتقال محدود للكاديوم من الجذور إلى الأجزاء الخضرية، وذلك لوجود بروتينات رابطة للكاديوم بالجذور. كان امتصاص صنف DHR-9504 للكاديوم أقل معنويا مقارنة بالصنف Varuna. هناك معلومات أخرى مطلوبة لتفسير نتائج البحث.

كلمات مفتاحية: أصناف نبات الخردل الهندي، بروتين رابط للكاديوم، كاديوم، نقص نعدل النمو، سمية النبات

Introduction

Brassica juncea L. (Indian mustard) is an important oilseed crop of India and is quite sensitive to cadmium. This species originates from the hybridization of *Brassica nigra* and *Brassica campestris* which probably happened in South Western Asia and India where the natural distribution of the two species overlaps (Sauer, 1993). *Brassica juncea* L. has been identified as a high biomass-producing crop with the capacity to take up and accumulate heavy metals such as Cd, Cu, Ni, Zn and Pb (Kumar et al., 1995). *Brassica juncea* L. is able to accumulate more than 400 µg Cd g^{-1} in the shoots, a physiological trait which may be exploited for the bioremediation of contaminated soils and waters (Haag-Kerwer at al., 1999). Oilseed crops are generally grown in submarginal land under rainfed as well as irrigated conditions. Deshveer and Singh (2003) stated that the yield level of Indian mustard crop is of low magnitude, despite the use of N and P fertilizers. Foy et al. (1978) and Sheoran et al. (1991) found that indiscriminate usage of phosphatic fertilizers in agricultural fields leads to build up cadmium level in the soil which affects plant growth and economic yield.

Heavy metals constitute an important group of environmental pollutants, as these are non-biodegradable and are readily taken up by the plants. Among an array of heavy metal pollutants, Cd is of the major concern. Cadmium is one of the most toxic non-essential and mobile metallic elements found in soils, and it affects plant growth adversely. Nriagu and Pacgana (1988) reported that about 22000 tonnes of cadmium is globally discharged every year in to the soil. Cadmium contamination of the soils is regarded as a great danger for living organisms, because its concentration in top soils continuously increases and its surface flux from aerosols is greater than the losses by leaching and plant uptake (Kabata-Pendias and Pendias, 1989; Fergusson, 1991). The flux of Cd from the soil solution into the roots of plants occurs by passive diffusion or by active transfer (Cataldo et al., 1983; Clarkson and Lutge, 1989).

Cadium is easily taken up by plants and translocated to different plant parts. Patterson (1977) reported that plant species and even genotypes differ greatly in their ability to take up, transport and accumulate Cd within the plant. Many of the metal accumulating plants are members of the *Brassica* family. Generally, visible toxicity symptoms and impaired growth occur only at relatively high internal Cd concentrations (Adriano, 1986). Muramoto et al. (1990) reported that root and shoot weights of rice were reduced 32% and 21% by 100 mg Cd kg-1. Florijn and Van Beusichem (1993) found that internal distribution rather than uptake caused genotypic differences in shoot Cd concentration of maize inbred lines. The objective of the present study was to investigate the effect of Cd on growth, yield and distribution of Cd in different plant parts among two *Brassica juncea*, L. genotypes.

Materials and Methods

The experiment was carried out with two genotypes of *Brassica juncea*, L. (Varuna and DHR-9504) at the Agricultural Research Station, Bilaspur Chhattisgarh, India during 2002-2003. The plants were grown in a greenhouse under controlled environmental conditions (temperature 27°C, and humidity 50-60%).

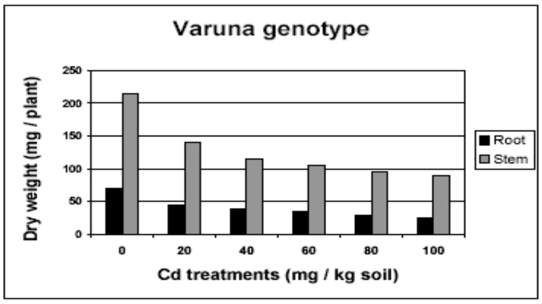
Physico-chemical properties of the soil were measured by the standard methods of soil chemical analysis (NIAST, 1988). The soil had 0.68% organic carbon, 240.3 kg ha^{-1} nitrogen, 194 kg ha^{-1} K_2O and Zn, B and

Mo 0.70, 0.31 and 0.04 mg kg^{-1} soil respectively, available sulphate-sulphur 9.1 mg kg^{-1} soil, available P 24.5 mg kg^{-1}soil and 0.40 mg Cd kg^{-1} soil with *p*H 7.85. 50 earthenware pots of 10 kg capacity having hole at their bottom were used for each replicate. The air-dry soil was sieved (< 2mm) and 8 kg soil was placed in each pot. N (urea) @ 100 kg ha^{-1}, P (single superphosphate, diammonium phosphate) @ 30 kg ha^{-1}, K (muriate of potash) @ 35 kg ha^{-1} and farmyard manure @ 25 tonnes ha^{-1} were applied as basal recommended fertilizers, along with Cd in the form of $CdCl_2$ @ 0, 20, 40, 60, 80 and 100 mg Cd kg^{-1} soil.

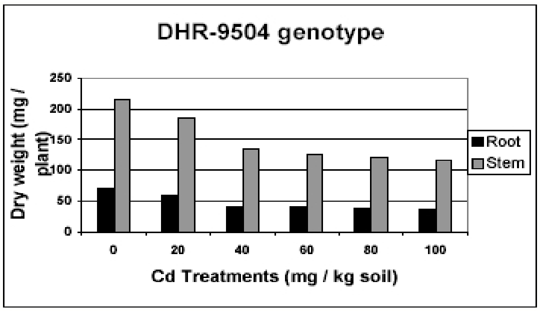
Each treatment was replicated three times, and the cd was mixed thoroughly with the soil and applied at the time of sowing. The water used for preparing the nutrient solution was deionized. Seeds of two genotypes of *Brassica juncea* L. (Varuna and DHR-9504) were sown in each pot on 12 November, 2002. After germination, the seedlings were thinned to four plants per pot and grown to maturity. Deionized water was used for irrigating the crop as and when required. At harvest on 17 March, 2003, the roots, stems and seeds were separated and dried at 70° C in order to determine dry weight and Cd concentration. Total content of Cd in plants was assayed by ICP after wet-acid (HNO3: H2SO4: HClO4=10:1:4) digestion (NIAST, 1988). Treatments were randomized throughout the greenhouse and the results given in the tables and figures are the means from three independent replications. The results were statistically analyzed by Duncan's test at a 5% probability level.

Results

Cd phytotoxicity was shown by growth retardation of Varuna and DHR-9504. The growth of Varuna and DHR-9504 was significantly reduced at the 0-20 mg kg-1 and 20-40 mg kg^{-1} of Cd treated soil, respectively (Figure 1A, 1B). An increasing supply of Cd resulted in significant decreases in the stem and root growth of both genotypes (Figure 1A, 1B). The decreases were more distinct in the Varuna genotype. For example, with a 20 mg kg^{-1} Cd supply, stem dry weight was reduced by around 15% in DHR-9504 and 35% in Varuna. Similar decreases were also noted for root dry weight (Figure 1A, 1B). Seed yield of *Brassica* genotypes (Varuna and DHR-9504) decreased to a greater extent than that of other plant parts, there was a significant reduction in yield of about 45% and 25% for seed at 100 mg Cd kg^{-1} soil compared with the control in genotypes Varuna and DHR-9504 respectively (Figure 2).



(A)



(B)

Figure 1A & 1B. Effect of increasing Cd supply on stem and root dry weight of Brassica juncea L. genotypes (Varuna and DHR-9504). The data represent means ± SD of three independent replications.

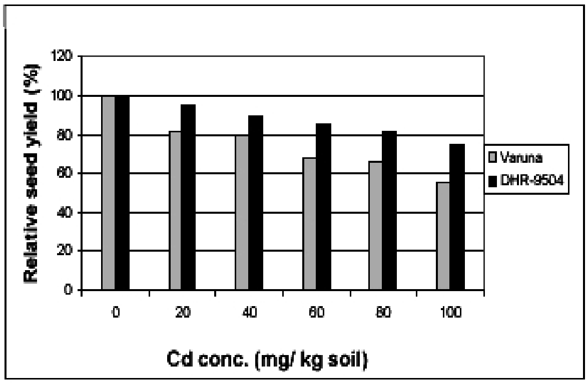


Figure 2. Relative seed yield of *Brassica juncea* L. genotypes (Varuna and DHR-9504) as influenced by Cd application.

Significant differences among both genotypes and Cd treatments were noted concerning Cd accumulation. As expected, increasing Cd supply markedly enhanced the Cd concentration of plants (Table 1). An increase in Cd concentration of about 5 times in seeds, 7 times in stems and 9 times in roots was noted with an application of 20 mg Cd kg^{-1}soil compared with the control (Table 1). Table 1 shows that Cd concentration in seeds of *Brassica juncea* plants varied from 3.2-68.5 µg g^{-1} (with a mean of 31.85 µg g^{-1}) and in the stem varied from 3.5 to 128 µg g^{-1} (with a mean of 51.65 µg g^{-1}) while that in the root varied from 7.2 to 316 µg g^{-1} (with a mean of 135.29 µg g^{-1}). Table 1 also shows that Cd was accumulated in the roots in much higher amounts than in the stems, especially in the case of the genotype DHR-9504. For example, with 40 mg kg^{-1} Cd supply, the Cd concentration in the roots was about 4 times higher than in the stems of genotype DHR-9504 and 2 times higher than in the stems of genotype Varuna.

Discussion

Genotypical variations in tolerance to Cd toxicity are well documented in the literature (Grant et al., 1998; Ozturk et al., 2003). Cd phytotoxicity was shown by growth retardation of Varuna and DHR-9504 (Figure 1). Marschner et al. (1988) also demonstrated Cd toxicity by growth retardation and leaf chlorosis of chinese cabbage in the early growing season. When compared to DHR-9504, Varuna showed higher sensitivity to Cd toxicity in this experiment (Figure 1A & 1B). Patel et al. (1980) also reported that Varuna showed greater sensitivity to Cd toxicity as compared to other genotypes of *Brassica* *juncea* L. this sensitivity might be due to higher Cd accumulation. Genotype Varuna had much higher Cd concentration in different plant parts indicating that it has better absorbing ability than DHR-9504 and has higher potential for removing Cd from moderately contaminated soil. Patel et al. (1980) and Sinha et al. (1981) also reported similar results. Singh and Nayyar (1994) reported that the relation between Cd in soil and Cd in plant is linear and the plant has an ability to exclude or accumulate large amounts of Cd at the higher Cd levels of soil. Simon (1998) also reported that Cd accumulated largely in roots and its concentration increased in shoots. High Cd contents in soils lead to a reduction in plant growth and dry matter yields. These findings are in close conformity with those of Haghiri (1973). The reduction in dry matter yield due to Cd application is in agreement with the findings of Lehoczky et al. (1996). Yield reductions in mustard plants have been attributed to the direct effect of higher Cd concentrations in plant tissue and not through an indirectly induced deficiency of other nutrients (Wilson, 1992). The results in Figure 1 show that the effect of Cd on stem and root growth is similar in both genotypes. In previous studies, Marschner et al. (1988) and Ozturk et al. (2003) also found that Cd supply reduced shoot and root dry matter production to a similar extent.

Table 1. Effect of increasing Cd supply on seed, stem and root Cd concentrations of two *Brassica juncea* L. (Indian mustard) genotypes (Varuna and DHR-9504). The data represent means ± SD of three independent replications.

Genotypes	Cd supply mg kg^{-1} soil	Cd concentration (µg g^{-1})		
		Seed	Stem	Root
Varuna	0	4.5±1	7.0±1	7.2±1
	20	22.0±1	49.1±1	65.2±1
	40	35.8±2	52.0 ±1	99.2±1
	60	44.5±3	72.3±3	114.1±3
	80	65.5±2	98.0 ±2	198.3±2
	100	68.5±4	128.0±3	298.4±2
DHR-9504	0	3.2±1	3.5±1	8.0±1
	20	16.3±2	22.2±2	72.3±4
	40	18.7±1	26.5±1	105.6±4
	60	23.3±2	41.2±2	125.4±4
	80	34.7±	2 53.7 ±3	215.1±5
	100	45.2± 4	67.5± 4	316.0±6

Genotype DHR-9504 had significantly less uptake of Cd than genotype Varuna (Table 2). Most of the Cd taken up in Cd treated plants remained in the roots. Seed Cd uptake was lowest as compared to other plant parts in both the genotypes (DHR-9504 and Varuna). These results show that Varuna plants accumulate more Cd than DHR-9504.

Table 2. Effect of increasing Cd supply on total Cd uptake in Varuna and DHR-9504 genotypes plants.

Cd conc. ($mg\ kg^{-1}$ soil)	Absorbed Cd concentration ($\mu g\ plant^{-1}$)		
	Varuna	DHR-9504	Mean
Control	12	11	11.5
20	190	138	164
40	289	189	239
60	307	237	272
80	487	358	422
100	689	499	594
Mean	329	238	

Genotype DHR-9504 had significantly less uptake of Cd than genotype Varuna (Table 2). Bhatia et al. (1990) also reported similar results. It seems that plant mechanisms affecting the root uptake and shoot transport of Cd can also affect the expression of Cd toxicity in plants. This result is in agreement with that reported by (Dunbar et al., 2003). Wilson (1992) and Hall (2002) also stated that differences in root uptake and shoot accumulation of Cd can be an important factor in explaining genotypical variations in tolerance to Cd toxicity. Therefore, the selection of plant genotypes with high ability to repress root uptake and shoot transport of Cd is a reasonable approach to alleviate adverse effects of Cd toxicity in crop plants. It is important to know the different Cd toxicity resistances among genotypes and the potential of Cd accumulation as well as their physiological responses to Cd toxicity. The results in Table (1) show that DHR-9504 had a higher Cd concentration in the roots indicating that there is a more limited transport of Cd from the root system to the above ground plant parts than Varuna. These findings are in close conformity with those of Bhatia et al. (1990) and Patel et al. (1980). DHR-9504 has a better genetic ability to retain Cd in the roots, possibly by binding and sequestering it in the vacuole, due to presence of strong Cd-binding proteins, which may contribute to higher Cd tolerance in this genotype. The present results are also in agreement with the findings of Marschner et al. (1988). Hall (2002) and Ozturk et al. (2003) also reported the importance of Cd-binding proteins in the development of Cd tolerance in plants.

Conclusion

In conclusion, the results presented show the existence of genotypical variations in the tolerance to Cd toxicity among *Brassica juncea* L. (Indian mustard) genotypes. The differential tolerance to Cd toxicity in *Brassica juncea* L. was related to Cd concentrations in the different plant parts and the retention of Cd in the roots.

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