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Genotypic variation in *Brassica juncea* (L.) Czern. cultivars in growth, nitrate assimilation, antioxidant responses and phytoremediation potential during cadmium stress

Asha Sharma[1], Manish Sainger[1,2], Sanjay Dwivedi[3], Sudhakar Srivastava[3,4], R.D. Tripathi[3] and Rana P. Singh*[2]

[1]Department of Biosciences, M.D. University, Rohtak - 124 001, India
[2]Department of Environmental Science, B.B.A. University, Rae Bareliy Road, Lucknow - 226 025, India
[3]Ecotoxicology and Bioremediation Group, National Botanical Research Institute, Lucknow - 226 001, India
[4]Functional Plant Biology Section, Nuclear Agriculture and Biotechnology Division,
FIPLY, Bhabha Atomic Research Centre, Mumbai - 400 085, India

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Abstract: *Four cultivars of Brassica juncea i.e. TM-4, TM-2, RH-30 and T-59 were screened for seed germination and seedling growth up to 15 days in the presence of 0.5-1.5 mM cadmium (Cd). The exposure to Cd reduced seed germination and seedling growth (root and shoot length and dry weight) in all four cultivars; the effect being more severe in TM-2 and RH-30 than in TM-4 and T-59 and at 3 d than at 7 d and 15 d. The cultivars TM-4 and T-59, with higher tolerance to Cd toxicity, were selected for further analysis including the estimation of nitrate reductase (NR) and peroxidase (POD) activities, total organic nitrogen (TON), total soluble proteins, proline levels and Cd accumulation. The NR activity and total soluble proteins decreased upon Cd exposure in a concentration dependent manner, whereas TON increased significantly in 3 d seedlings upon Cd exposure. The activity of POD and proline level increased significantly as compared to the respective controls. The level of Cd accumulation was higher in T-59 than in TM-4. Therefore, T-59 was found to be the most tolerant cultivar to Cd than other three cultivars possibly due to a better capacity to transport Cd in their vacuolar sink. The variety T-59, thus, appears to be suitable for Cd phytoremediation.*

Key words: *Brassica juncea, Cadmium, Growth, Phytoremediation*
PDF of full length paper is available online

Introduction

Cadmium (Cd) is one of the most toxic heavy metals, which is released into the environment by anthropogenic pathways *e.g.* mining and smelting, dispersal of sewage sludge, power stations and Cd-rich phosphate fertilizers (Chaney, 1998). Its long persistence in the environment and accumulation by food-chain organisms make it a potential environmental hazard (Dudka and Miller, 1999; Aina *et al*., 2007; Siddhu *et al.,* 2008; Hasan *et al*., 2009). The toxic effects of Cd on biological systems have been reported extensively (di Toppi and Gabbrielli, 1999; Kirkham, 2006). Cadmium is reported to cause chlorosis with stunted growth, ultrastructural and physiological damages (Vecchia *et al.,* 2005; Prasad, 1995; Dinakar *et al.,* 2009; Chaudhary and Sharma, 2009), inhibit the biosynthesis of chlorophyll (Mobin and Khan, 2007), alter water balance (Barcelo and Poshenrieder, 1990), decrease activity of various enzymes (Chaudhary and Singh, 2000; Gouia *et al*., 2003; Dong *et al*., 2006; Mishra *et al*., 2006) and interfere with general and membrane physiology (di Toppi and Gabbrielli 1999; Nouairi *et al*., 2006; Chaudhary and Singh, 2000; Liu *et al*., 2003; Lin *et al*., 2007).

There are many plants, both aquatic and terrestrial, which can accumulate high level of the toxic metals in their tissues. Such plants can be helpful to remove the pollutants from soil and water (Singh *et al*., 1997, 2001, 2007) to reduce their contamination; the technique is popularly known as phytoremediation. In recent times, Indian mustard *viz.*, *Brassica juncea* (L.) Czern has been one of the most studied terrestrial plants for its potentiality to extract heavy metals from soil, fly-ash and sediments (Zhu *et al*., 1999; Singh *et al*., 2001, 2007; Qadir *et al*., 2004; Gupta and Sinha, 2006). This plant has been demonstrated to be a hyperaccumulator of Cd, is fast growing and possesses higher aerial biomass than many other potential metal accumulators and hence, can be used as a phytoremediator (Singh *et al*., 2007; Nouairi *et al*., 2006). To achieve this goal, screening high yielding cultivars of Indian mustard suitable for the various agroclimatic zones and having tolerance to heavy metals including Cd, is needed to develop phytoremediation technology for northern Indian soil contaminated with Cd. The genotypic variation in phytoremediation potential of *B. juncea* cultivars (Qadir *et al*., 2004; Mobin and Khan, 2007) has been reported. In addition, the use of *Brassica* plants in the phytoremediation of Cd and other metals has also been demonstrated at field level or in simulated pot experiments (Ahmed *et al.,* 2001; Su and Wong, 2004; Ishikawa *et al.,* 2006). Thus, it seems worthwhile to screen appropriate plant cultivars for tolerance and accumulation of Cd, so that the best cultivar be used for phytoremediation of Cd-polluted sites without affecting growth and yield of the crop.

* **Corresponding author:** ranapsingh1@hotmail.com

In this context, the present study was planned to study the responses of four cultivars of *B. juncea i.e.* TM-2, TM-4, T-59 and RH-30 under Cd stress. Stress exerted by Cd to plant growth was measured in terms of effects on the seed germination, shoot and root length and biomass (dry weight). Two cultivars *viz.*, TM-4 and T-59, which were found to have greater tolerance to Cd, were further analyzed for total organic nitrogen, protein and proline contents, nitrate reductase and peroxidase activities, and Cd accumulation in Cd-exposed seedlings after 3, 7 and 15 d.

Materials and Methods

Seeds of *B. juncea* L. cv TM-4 and TM-2 were obtained from, Plant-Biotech Division, Bhabha Atomic Research Centre, Trombay, Mumbai, and that of cultivars T-59 and RH-30 were purchased from the local dealer. Seeds were sterilized in 70% ethanol for 2-3 min and washed under running tap water, followed by distilled water till the alcohol is removed. Thirty sterilized seeds were put in each petriplate on moist filter paper and watered with 50% Hoagland nutrient medium (Hoagland and Arnon, 1950) with/without Cd (0, 0.5, 1.0 and 1.5 mM; prepared using the salt cadmium acetate; E-Merck) for germination. The number of germinated seeds was counted manually after 7 d. After germination, seedlings were transferred to small beakers and were exposed to different concentrations of Cd maintained in 50% Hoagland nutrient medium for a period of 3, 7 or 15 d under controlled laboratory conditions (15 hr photoperiod using a light intensity of 115 mmol m^{-2} s^{-1}, day/night temperature of 25±2°C and relative humidity of 55-75%). At each harvesting period, plants were washed thoroughly with double distilled water and were used for the analysis of various parameters.

For measurement of dry weight, seedlings were dried in an oven for 2d to constant weight and then weighed using an electronic balance. The root and shoot lengths of the seedlings were measured using a metric scale. The activity of nitrate reductase (NR; EC 1.6.6.1) in leaves and roots was assayed by *in vivo* method given by Srivastava (1975). For estimation of organic nitrogen (TON), the modified micro Kjeldahl method was used (Lang, 1958). The protein content was estimated by the method of Bradford (1976). For estimation of peroxidase (POD; EC 1.11.1.7) activity in the plant parts, the method given by Putter (1974) was used and the proline content was estimated following the method by Bates *et al.* (1973).

For Cd estimation, oven-dried seedlings were digested in $HClO_4$:HNO_3 (1:3 v/v) at 100°C and then diluted with demineralized water. Cadmium concentration was determined on a Flame Atomic Absorption Spectrophotometer (GBC Avanta O, Australia). The standard reference material of Cd (E-Merck, Germany) was used for the calibration and quality assurance. Analytical data quality of Cd was ensured through repeated analysis (n = 6) of EPA quality control samples (Lot TMA 989) and the results were found to be within ±2.79% of certified values. Recovery of Cd from the plant tissues was found to be more than 98.5%. The blanks were run in triplicate to check the precision of the method with each set of samples. The detection limit of Cd was 0.013 ppm.

The experiments were carried out in a randomized block design. Two-way analysis of variance (ANOVA) was done on all the data to confirm the variability of data and validity of results, and Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments (Gomez and Gomez, 1984).

Results and Discussion

Differential effects of cadmium exposure on the germination percentage and growth of four cultivars of *Brassica juncea*: A significant inhibition of seed germination was observed in all varieties in presence of Cd after 7 d (Table 1). A significant decline in germination percentage was noticed at 0.5 mM Cd in RH-30 and TM-2, at 1.0 mM Cd in TM-4 while only at 1.5 mM Cd in T-59. The metal caused a delay in germination. An inhibition of seed germination upon exposure to Cd has also been reported in other plants also including *Vicia faba* and *Sesamum indicum* (Bharti and Singh, 1994; Singh *et al*., 1988,1991; Bharti *et al*., 1996; Liu *et al*., 2003).

Cadmium exposure caused significant decrease in the seedlings length as a function of the level and duration of exposure (Table 2). After 3 d seed sowing, the seedling length was inhibited by about 75, 67, 66 and 55% in RH-30, TM-2, TM-4 and T-59, respectively at 1.5 mM Cd. After 7 and 15 d, decrease in total seedling length was higher in RH-30 and TM-2 (up to 79-82%) than in TM-4 and T-59 (up to 50-57%). In all four varieties, decrease in root length was more severe than that in the shoot length. Dry weights of all four varieties were also significantly reduced upon exposure to Cd for 3, 7 and 15 d after sowing (Table 3). In 3 d seedlings, reduction in dry weights was about 80% in RH-30 and TM-2 while it was about 50% in TM-4 and T-59. With an increase in duration, reduction in dry weights decreased in all varieties. After 15 d, reduction in dry weights in RH-30 and TM-2 was about 55-60% while in TM-4 and T-59 it decreased to about 41%. Similar to seedling length, the lowest reduction in dry weigh was noticed in T-59, while the maximum reduction was observed in TM-2.

The most common effect of Cd toxicity in plants has been shown to be stunted growth, leaf chlorosis and alteration in the

Table - 1: Percentage of seed germination of *B. juncea* varieties as calculated for 7d old seedlings in presence of cadmium

Concentration	% of seed germinated			
(mM)	T-59	TM-4	RH-30	TM-2
00	79.33^{a}±2.82	70.12^{a}±3.93	82.01^{a}±3.2	86.21^{a}±4.23
0.5	77.66^{a}±3.38	66.66^{ab}±4.23	77.02^{b}±2.37	72.66^{b}±3.63
1.0	76.77^{ab}±4.27	64.66^{bc}±4.56	64.66^{b}±4.56	71.01^{b}±4.96
1.5	74.01^{b}±4.29	61.33^{c}±2.67	61.33^{c}±2.67	70.66^{b}±3.38

Data represent the mean ±S.E. (n = 9), ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c and d) indicate significantly different values (DMRT, $p \leq 0.05$)

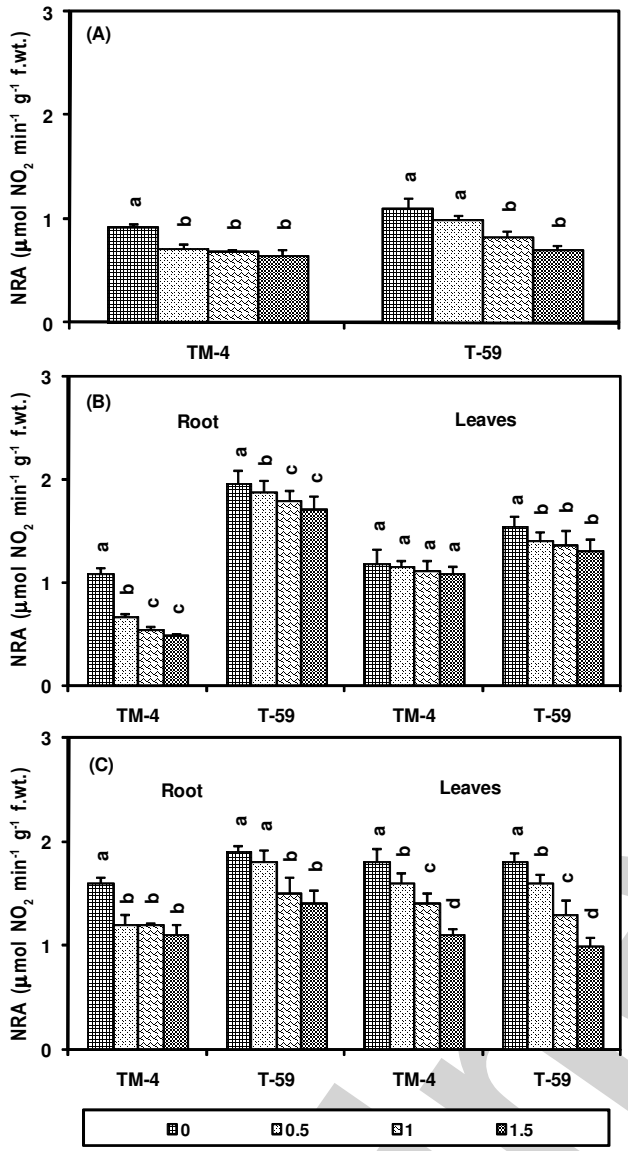

Fig. 1: Nitrate reductase activity (NRA) of the various plant parts of *Brassica juncea* seedlings at 3d (A), 7d (B) and 15d (C) upon exposure to different cadmium concentrations. Values are mean ± SD (n=3). ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c, and d) indicate significantly different values (DMRT, $p \leq 0.05$)

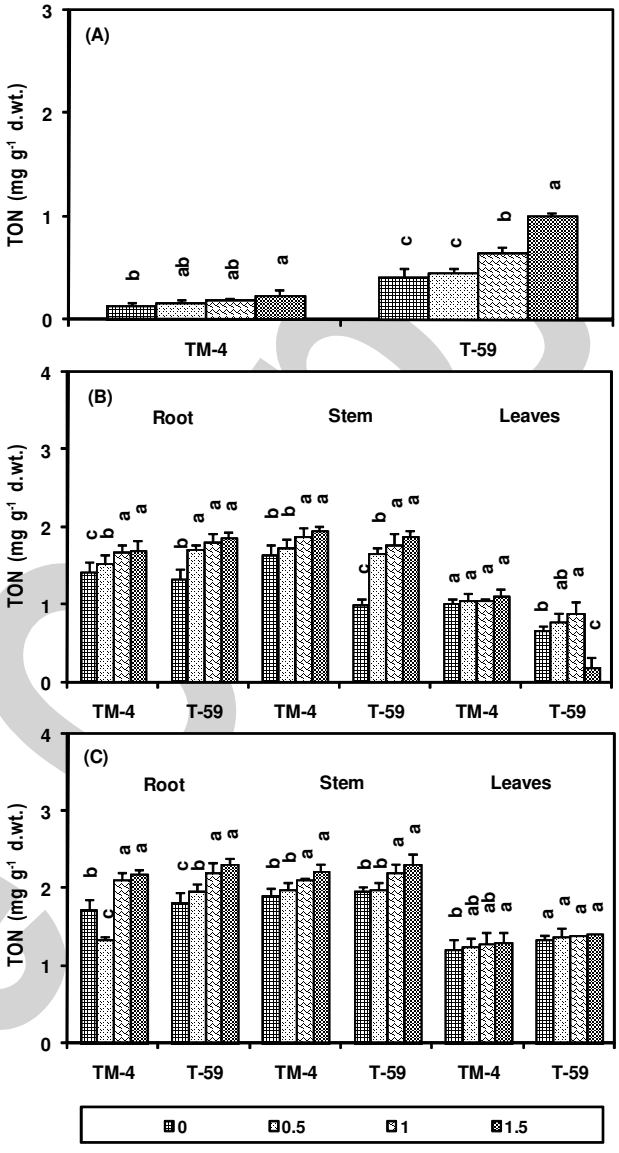

Fig. 2: Total organic nitrogen (TON) content of the various plant parts of *Brassica juncea* seedlings at 3d (A), 7d (B) and 15d (C) upon exposure to different cadmium concentrations. Values are mean ± SD (n=3). ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c, and d) indicate significantly different values (DMRT, $p \leq 0.05$)

activity of many key enzymes of various metabolic pathways (di Toppi and Gabbrielli, 1999). The present results of the length and dry weights of the seedlings indicate that varietal differences in response to Cd toxicity existed in four varieties and the initial responses were not only linked not only with the genotype but also with the metal concentration (Singh *et al*., 1988, 1991). These results are in conformity with the study of Qadir *et al.* (2004) who also demonstrated a significant decline in seedling height and biomass as a function of Cd concentration. Seedling growth of other plant species has also been reported to be inhibited variously in the presence of heavy metals including Cd (Bharti and Singh, 1994; Bharti *et al*., 1996; Liu *et al*., 2003). The observed growth reduction may be attributed to reduced photosynthetic efficiency of the plants upon Cd exposure (Hayat *et al*., 2007; Mobin and Khan, 2007; Singh *et al*., 2007). However, with an increase in duration some recovery over growth inhibition was noticed in all four varieties, which might be linked to an induction of various defense strategies as an adaptive response including probably the synthesis of metal binding ligands, such as phytochelatins (Salt *et al*., 1995) and metallothioneins (Schafer *et al*., 1997) and other stress responsive proteins likely the antioxidant enzymes (Hayat *et al*., 2007). An up-regulation of a number of genes in response to Cd exposure has been observed in *B. juncea* (Minglin *et al*., 2005) including those of catalase, an important antioxidant enzyme of the cell (Mishra *et al.,* 2006) and ATP sulfurylase and APS reductase, the important enzymes of cysteine biosynthesis. In general, TM-4 and T-59 were

Table - 2: Seedling length (root + shoot) in 3, 7 and 15d old *B. juncea* in the presence of cadmium

Concentration (mM)	*B. juncea* varieties											
	3 d old seedlings (cm ± S.E.)				7 d old seedlings (cm ± S.E.)				15 d old seedlings (cm ± S.E.)			
	T-59	TM-4	RH-30	TM-2	T-59	TM-4	RH-30	TM-2	T-59	TM-4	RH-30	TM-2
0.0	2.7^{a} ±0.11 (100)	2.9^{a}±0.15 (100)	2.8^{a}±0.11 (100)	2.7^{a}±0.16 (100)	11.9^{a}±0.91 (6.4+5.5)	11.7^{a}±0.83 (6.1+5.6)	9.9^{a}±0.44 (4.7+5.2)	11.1^{a}±1.12 (5.7+5.4)	14.9^{a}±0.91 (7.2+7.7)	14.3^{a}±0.83 (6.9+7.4)	12.9^{a}±0.86 (6.4+6.5)	13.1^{a}±0.72 (6.1+7.0)
0.5	2.1^{b} ±0.05	1.8^{b}±0.11	1.2^{b}±0.06	1.4^{b}±0.13	10.5^{b}±0.94 (5.4+5.1)	9.8^{b}±0.57 (5.0+4.8)	4.3^{b}±0.51 (1.9+2.4)	4.8^{b}±0.46 (2.5+2.3)	12.3^{b}±0.57 (6.1+6.2)	11.3^{b}±0.81 (5.4+5.9)	4.9^{b}±0.52 (2.0+2.9)	6.4^{b}±0.46 (2.7+3.7)
1.0	1.7^{c} ±0.08	1.6^{b}±0.09	1.1^{b}±0.07	1.3^{b}±0.09	8.1^{c}±0.55 (4.2+3.9)	7.5^{c}±0.49 (3.8+3.7)	3.2^{c}±0.33 (1.5+1.7)	3.2^{b}±0.26 (1.5+1.7)	9.6^{c}±0.33 (4.6+5.0)	9.1^{c}±0.46 (4.3+4.8)	3.6^{b}±0.26 (1.5+2.1)	4.1^{c}±0.29 (1.6+2.5)
1.5	1.2^{d} ±0.06	1.0^{c}±0.05	0.7^{c}±0.01	0.9^{c}±0.03	5.9^{d}±0.44 (2.9+3.0)	5.2^{d}±0.27 (2.7+2.5)	1.8^{d}±0.33 (0.9+0.9)	2.3^{c}±0.25 (1.0+1.3)	7.3^{d}±0.49 (3.1+4.2)	6.1^{d}±0.31 (3.0+3.1)	2.3^{c}±0.22 (1.0+1.3)	2.5^{d}±0.17 (1.0+1.5)

Data ±S.E. (n = 15), ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c, and d) indicate significantly different values (DMRT, $p \leq 0.05$)

Table - 3: Dry weight 3, 7 and 15 d old *Brassica juncea* seedling the presence of cadmium

Concentration (mM)	*B. juncea* varieties											
	3 d old seedlings (mg fw ± S.E.)				7 d old seedlings (mg fw ± S.E.)				15 d old seedlings (mg fw ± S.E.)			
	T-59	TM-4	RH-30	TM-2	T-59	TM-4	RH-30	TM-2	T-59	TM-4	RH-30	TM-2
0.0	0.51^{a}±0.03 (100)	0.54^{a}±0.01 (100)	0.38^{a}±0.05 (100)	0.44^{a}±0.03 (100)	2.25^{a}±0.59 (100)	1.83^{a}±0.19 (100)	1.41^{a}±0.06 (100)	1.62^{a}±0.11 (100)	2.56^{a}±0.34 (100)	2.04^{a}±0.39 (100)	1.61^{a}±0.51 (100)	1.89^{a}±0.31 (100)
0.5	0.42^{b}±0.04	0.41^{b}±0.02	0.24^{b}±0.02	0.31^{b}±0.01	1.89^{ab}±0.26	1.46^{b}±0.16	1.09^{b}±0.06	1.13^{b}±0.08	2.01^{b}±0.31	1.79^{a}±0.21	1.29^{ab}±0.16	1.47^{b}±0.26
1.0	0.35^{b}±0.04	0.34^{c}±0.01	0.16^{b}±0.01	0.21^{c}±0.01	1.21^{b}±0.33	1.23^{b}±0.21	0.86^{c}±0.07	0.91^{c}±0.06	1.86^{bc}±0.27	1.61^{ab}±0.17	1.07^{ab}±0.19	1.09^{bc}±0.43
1.5	0.25^{c}±0.01	0.26^{d}±0.03	0.07^{c}±0.04	0.09^{d}±0.04	1.11^{b}±0.18	0.92^{b}±0.05	0.39^{d}±0.13	0.43^{d}±0.08	1.51^{c}±0.16	1.19^{b}±0.12	0.71^{b}±0.21	0.74^{c}±0.11

Data ±S.E. (n = 15), ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c, and d) indicate significantly different values (DMRT, $p \leq 0.05$)

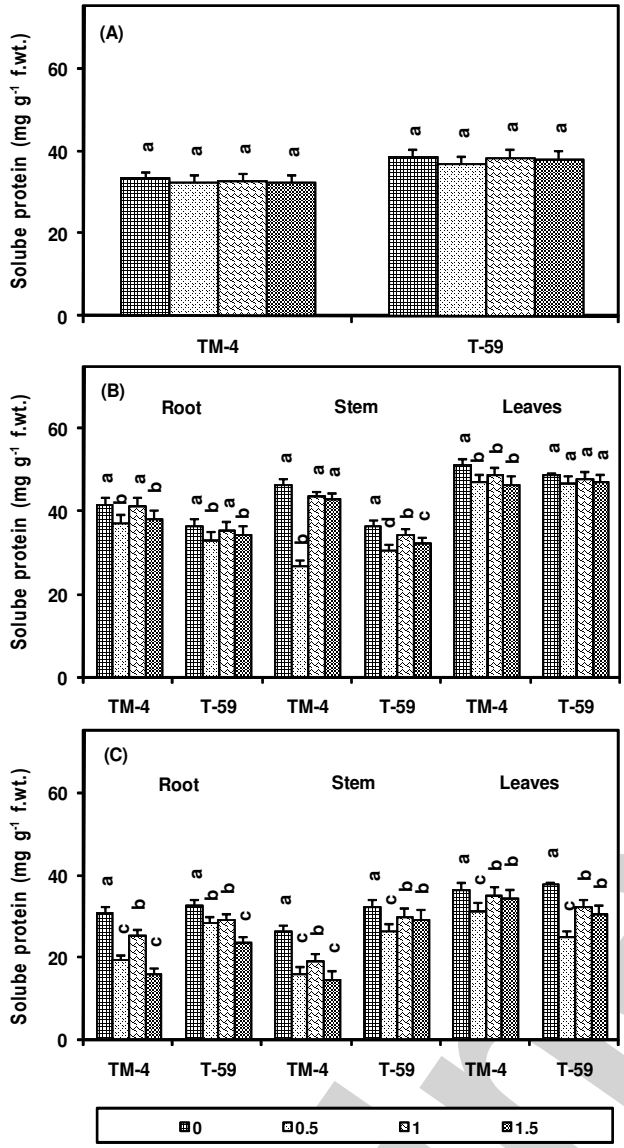

Fig. 3: Total soluble protein content of the various plant parts of *Brassica juncea* seedlings at 3d (A), 7d (B) and 15d (C) upon exposure to different cadmium concentrations. Values are mean ± SD (n=3). ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c and d) indicate significantly different values (DMRT, $p \leq 0.05$)

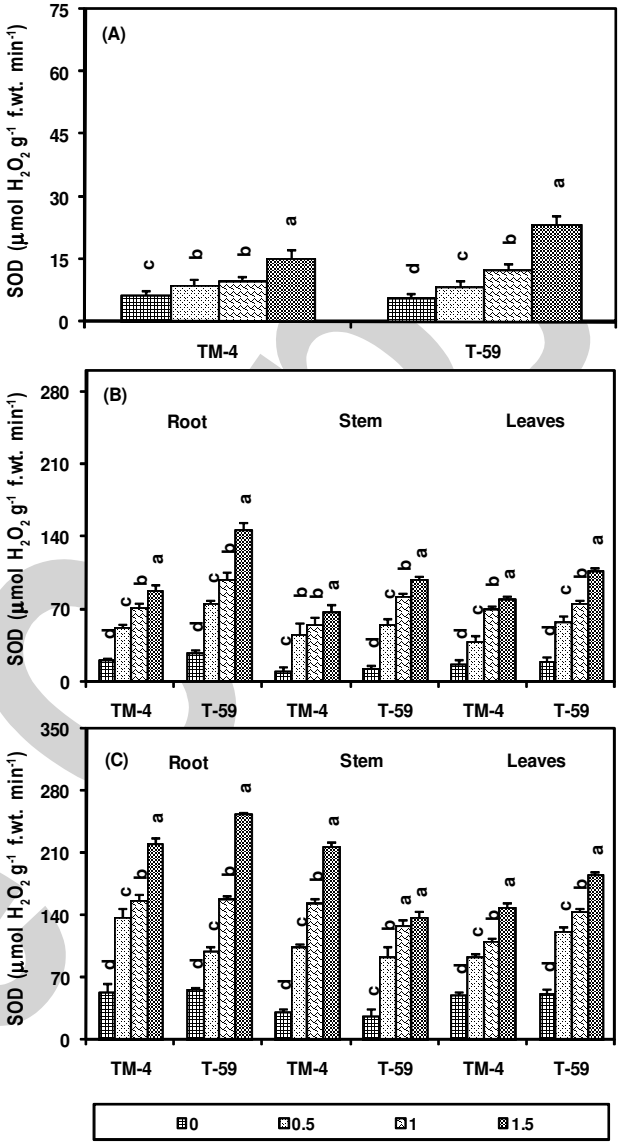

Fig. 4: Specific peroxidase (SPOD) activity of the various plant parts of *Brassica juncea* seedlings at 3d(A), 7d(B) and 15d(C) upon exposure to different cadmium concentrations. Values are mean ± SD (n=3). ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c and d) indicate significantly different values (DMRT, $p \leq 0.05$)

Table - 4: Level of Cadmium in *Brassica juncea* L cv. TM-4 and T-59 whole seedling in lab conditions when supplied with different concentrates

Concentration (mM)	15 d old seedlings (µg Cd g^{-1} dw)	
	T-59	TM-4
0.0	7.4^{d}±2.3	8.2^{d}±1.1
0.5	464^{c}±31	414^{b}±30
1.0	684^{b}±26	627^{c}±51
1.5	1292^{a}±79	1227^{a}±92

Data represent the mean ±S.E. (n = 9), ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c and d) indicate significantly different values (DMRT, $p \leq 0.05$)

more tolerant/resistant to Cd toxicity than RH-30 and TM-2. Therefore, further analyses for Cd accumulation and biochemical responses were performed using these two varieties.

Effect of cadmium exposure on nitrate reductase (NR) activity, total organic nitrogen and soluble protein content of *Brassica juncea*: The NR activity was decreased upon Cd exposure at all durations in roots and leaves of both varieties except in leaves of TM-4 after 7 d (Fig. 1). In 3 d seedlings, NR activity was reduced more drastically in T-59 (36%) than in TM-4 (30%) at 1.5 mM Cd. After 7 and 15 d, decline in NR activity in roots of TM-4 (31-55%) was higher than that in roots of T-59 (12-26%); while

in leaves, the decline was higher in T-59 (15-44%) than in TM-4 (8-39%) (Fig. 1). However, the level of total organic nitrogen generally, with a few exceptions, increased significantly in all plant parts *viz.*, roots, stem and leaves of both varieties at all durations as a function of Cd concentration (Fig. 2). The increase in total organic nitrogen was highly significant after 3 d (64% in TM-4 and 146% in T-59 at 1.5 mM Cd) that decreased with increase in duration (7-26% in TM-4 and 7-90% in T-59 at 1.5 mM Cd in various plant parts after 7 and 15 d). Further, the increase in total organic nitrogen was higher in T-59 than in TM-4 at all durations and in all plant parts but leaves. Total soluble proteins in general showed significant/non-significant decrease at all durations and concentrations in comparison to control (Fig. 3). After 3 d, the decline was not significant at any concentration in both TM-4 and T-59. After 7 and 15 d, proteins showed a significant decline at 0.5 mM Cd in all the plant parts in both varieties while at 1.0 and 1.5 mM Cd, protein levels were again recovered to some extent and even approached control levels in some cases. The maximum decline in protein levels after 3 and 7 d was observed at 0.5 mM Cd. After 15 d, the maximum decline was noticed at 1.5 mM Cd in roots (47% in TM-4 and 27% in T-59) and stems (43% in TM-4 and 10% in T-59), however in leaves the maximum decline in protein levels of TM-4 (6%) was observed at 1.5 mM Cd while in T-59 (34%) at 0.5 mM Cd.

Nitrate reductase is considered as a key enzyme and a rate limiting step in the nitrogen metabolism of the plants (Bharti and Singh, 1994; Bharti *et al*., 1996; Singh *et al*., 1997). The enzyme catalyzes reduction of nitrate to nitrite and its activity is often correlated with the nitrogen status of the plant. Variable effects of heavy metals especially Pb and Cd on NR activity have been reported (Singh *et al*., 1997; Hayat *et al.,* 2007). The reason for the inhibition of NR activity in response to Cd exposure might be multi-facial. It might be due to (i) reduced supply of NADH, which may either be oxidized due to the presence of metal at the site or its generation may be inhibited due to the modification of structure and function of cellular components, (ii) reduced uptake of nitrate or water stress created in the plants due to Cd toxicity (Singh *et al*., 1997) and (iii) a direct effect of the Cd due to its strong affinity with the functional -SH group of the enzyme (Choudhary and Singh, 2000). Total soluble proteins of the plants were inhibited upon Cd exposure whereas total organic nitrogen was generally increased as a function of the stress. The cadmium applied to the seedlings may increase the translocation of reserve N from the cotyledons to the growing roots and shoots in the early growth phase as it has been correlated with a decreased organic N content in the cotyledons in *Sesamum indicum* under a Pb^{+2} environment (Kumar *et al*., 1993). It seems that reduced activity of NR and use of significant amount of nitrogen for the synthesis of PCs, MTs and other stress responsive proteins probably resulted in a decrease in the levels of total soluble proteins but the Cd inducible proteins. We observed an induction of some stress proteins upon Cd exposure as revealed by SDS-PAGE protein profiling of the two varieties, which indicates towards an induction of stress responsive antioxidant enzymes or *de novo* synthesis of stress proteins (Blinda *et al*., 1997). In mung bean Choudhary and Singh, (2000) has reported synthesis of both low and high molecular weight proteins in the presence of Cd and Pb but no specific function for these induced proteins could be assigned.

Effect of cadmium exposure on the peroxidase activity and proline content in *Brassica juncea*: Specific peroxidase activity increased to highly significant levels upon Cd exposure at all Cd concentrations and durations in various plant parts of both varieties (Fig. 4). The increase in peroxidase activity was higher in T-59 than in TM-4 in all plant parts and under various conditions except in stem of TM-4 after 15 d. The maximum increases in peroxidase activity in root, stem and leaves after 15 d were 310, 586 and 195%, respectively in TM-4 and 353, 433 and 257%, respectively in T-59. Similar to peroxidase, the proline content in root, stem and leaves of the two varieties was also increased significantly by many-fold at all durations and concentrations (Fig. 5). By contrast to peroxidase, the increase in proline levels was always higher in TM-4 than in T-59. The maximum increases in proline content in root, stem and leaves after 15 d were 650, 375 and 200%, respectively in TM-4 and 250, 186 and 144%, respectively in T-59.

Significant increase observed in the levels of peroxidase and proline appears to be an adaptive response of the plants to combat Cd toxicity. Peroxidases are located in cytosol, cell wall, vacuole and extracellular spaces. This is considered as a stress marker enzyme having broad specificity for phenolic substrates and higher affinity for H_2O_2 than catalase (Reddy *et al*., 2005). Proline has been suggested to play an important role in osmoregulation, protection of enzymes, stabilization of the machinery of protein synthesis, regulation of cytosolic acidity, as well as in non-enzymatic free radical detoxification (Alia and Saradhi, 1991; Alia *et al.,* 1995). Significant increase observed in the peroxidase activity and proline levels appears to be correlated to Cd stress suggesting it to be an intrinsic defense tool (Mishra *et al*., 2006; 2008). Significant increase in peroxidase activity and/or proline levels in *Brassica junea* plants upon exposure to Cd has been reported previously (Dhir *et al*., 2004; Hayat *et al*., 2007). An increase in peroxidase activity upon Cd exposure has also been observed in other terrestrial plants, such as *Lycopersicon esculentum* and *Triticum aestivum* (Dong *et al.,* 2006; Lin *et al*., 2007).

Cadmium accumulation: The level of Cd accumulated by the plants was analyzed after 15 d of seedling growth at whole plant level. Plants showed a progressive increase in Cd accumulation with an increase in Cd concentration (Table 4). The maximum level of Cd was observed at 1.5 mM Cd that was higher in T-59 (1292 mg g^{-1} dw) than in TM-4 (1227 mg g^{-1} dw). Differential Cd accumulation of Cd by various cultivars of *Brassica juncea* (Qadir *et al*., 2004) or different species of *Brassica viz*., *B. juncea* and *B. napus* has been reported previously (Nouairi *et al*., 2006). The level of Cd accumulation observed in this study was found to be higher than that reported previously (Qadir *et al.,* 2004). In *B. juncea*, it has been demonstrated that Cd accumulates preferentially

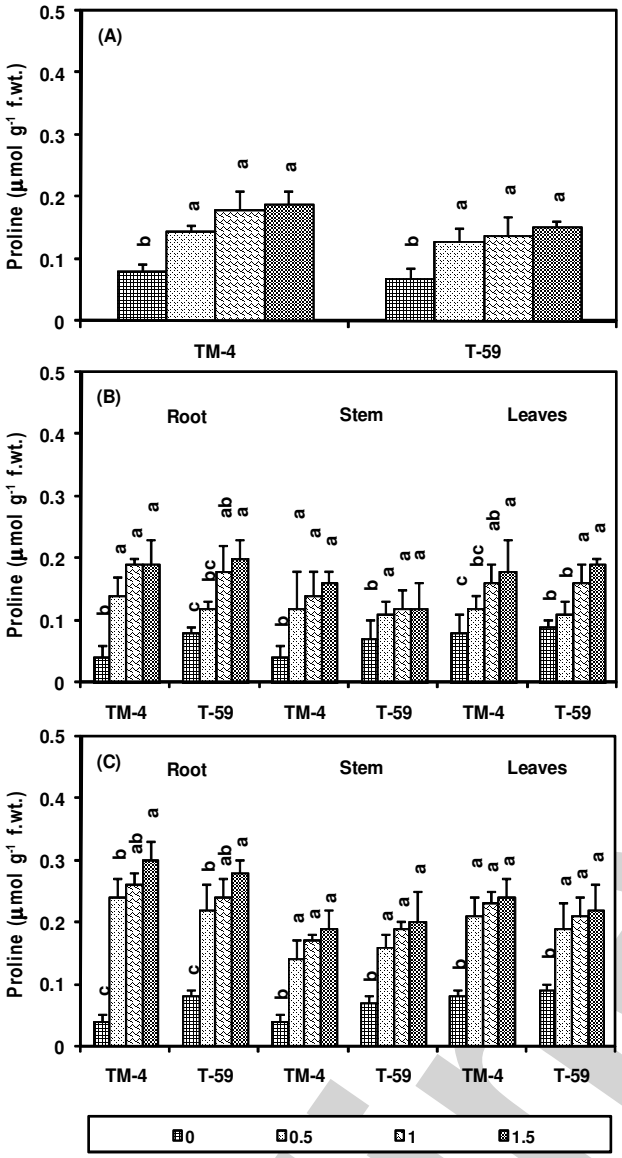

Fig. 5: Proline level of the various plant parts of *Brassica juncea* seedlings at 3d (A), 7d (B) and 15d (C) upon exposure to different cadmium concentrations. Values are mean ± SD (n=3). ANOVA significant at $p \leq 0.01$. For a particular variety, different letters (a,b,c, and d) indicate significantly different values (DMRT, $p \leq 0.05$)

in the trichomes of the youngest leaf surface (Salt *et al*., 1995). The storage of Cd in trichomes may represent a detoxification mechanism, since trichomes represent an external tissue to the leaf. The high Cd accumulation potential of *B. juncea* might be attributed to its potential to chelate it through metal binding ligands, phytochelatins (Salt *et al*., 1995; Mishra *et al*., 2006) and low molecular weight proteins, MTs (Schäfer *et al*., 1997) in addition to its capacity to tolerate Cd induced oxidative stress through induced antioxidant systems (Qadir *et al*., 2004; Hayat *et al*., 2007).

Of the four varieties screened for tolerance to Cd toxicity, RH-30 and TM-2 were found more sensitive than TM-4 and T-59 as evaluated in terms of seedling growth. Variety T-59 was found to possess the greatest tolerance among the four tested cultivars. In addition, T-59 showed higher accumulation of Cd than that observed in TM-4. Hence, the *B. juncea* cultivar T-59 appears to possess a greater tolerance to Cd toxicity and thus seems a suitable candidate for phytoremediation of Cd. There is a need of further studies at field level to utilize the potential of suitable varieties for the purpose of phytoremediation in coming future.

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