B A
S E

Biotechnol. Agron. Soc. Environ. 2011 **15**(3), 401-414

Growth, nitrate reductase activity and antioxidant system in cadmium stressed tomato (*Lycopersicon esculentum* Mill.) cultivars

Shamsul Hayat, Syed Aiman Hasan, Aqil Ahmad

Aligarh Muslim University. Plant Physiology Section. Department of Botany. IND-Aligarh 202 002 (India).
E-mail: shayat@lycos.com

Received on March 6, 2010; accepted on December 7, 2010.

Ten cultivars of tomato were subjected to different cadmium (Cd^{2+}) concentrations, to find out their degree of tolerance towards these metal ions during the tomato ontogeny. Seeds of tomato cultivars (*i.e.* 'K-25', 'K-21', 'NTS-9', 'Kaveri', 'NBR-Uday', 'Swarnodya', 'Sarvodya', 'NBR-Uttam', 'Malti' and 'S-22') were soaked in 0, 50, 100 or 150 µM of Cd^{2+} for 0, 4, 8 or 12 h. Despite substantial varietal differences, increases in Cd^{2+} concentration and the soaking duration caused a linear decrease in growth and a reduced activity of catalase and peroxidase for all varieties. Variety 'K-25' was found to be the most resistant cultivar as it possessed maximum activity of antioxidative enzymes reflecting one of the possible reasons to overcome stress conditions. However, the seeds of 'S-22' could not germinate in the presence of even the lowest Cd^{2+} concentration.
Keywords. *Lycopersicon esculentum*, abiotic stress, heavy metals, tolerance, varieties, plant defense reactions.

Croissance, activité de la nitrate réductase et du système antioxydant chez les cultivars de tomate (*Lycopersicon esculentum* Mill.) soumis à un stress au cadmium. Dix cultivars de tomate ont été soumis à différentes concentrations en cadmium (Cd^{2+}) afin de déterminer leur degré de tolérance vis-à-vis de ces ions métalliques durant l'ontogénèse de la tomate. Des semences de cultivars de tomate ('K-25', 'K-21', 'NTS-9', 'Kaveri', 'NBR-Uday', 'Swarnoday', 'Sarvodya', 'NBR-Uttam', 'Malti' et 'S-22') ont été trempées dans 0, 50, 100 et 150 µM de Cd^{2+} pendant 0, 4, 8 et 12 h. Malgré des différences variétales évidentes, des augmentations de la concentration en Cd^{2+} et de la durée de trempage provoquent une décroissance linéaire de la croissance et de l'activité catalase et peroxydase chez toutes les variétés. La variété 'K-25' s'est montrée la plus résistante et présente aussi la plus grande activité des enzymes antioxydants, ce qui représente une des raisons possibles de sa résistance aux conditions de stress. À l'inverse, les semences de 'S-22' ne germent pas du tout en présence de Cd^{2+}, même à faible concentration.
Mots-clés. *Lycopersicon esculentum*, stress abiotique, métal lourd, tolérance, variété, mécanisme de défense.

1. INTRODUCTION

Plants are good bioindicators as they play a significant role in food chain transfer and in defining environmental health (Gianazza et al., 2007). They are easy to grow and adaptable to environmental stress and also reflect toxicant damage in other organisms, such as animals (Minissi et al., 1997). Heavy metals in soil and plants have received increasing attention in recent years because of the harmful effects on dietary intake (Usha et al., 2002). Cadmium is one of the most toxic heavy metal pollutant in the environment, soil and water, and the sources of its contamination are fossil fuel, mining, waste water, household waste, municipal and industrial waste, use of metal containing pesticides and fertilizers in agricultural soil (Radotic et al., 2000; Akinola et al., 2006). Cadmium (Cd^{2+}) is a non-essential and highly toxic metal ion that at higher concentrations inhibits growth and cell division (Liu et al., 2003). Moreover, species and cultivars display marked difference for cadmium tolerance (Wu et al., 2004; He et al., 2006; Hasan et al., 2009). Cd^{2+} causes severe morphological, physiological and biochemical effects on plants such as stunted growth (Ali et al., 2007; Hasan et al., 2008), leaf rolling and chlorosis (Ghani et al., 2007), interferes with uptake, transport and use of several elements (Cu, Zn, Ni, Pb and Cr) and that of water by plants (Das et al., 1997). At the cellular level Cd^{2+} interacts with biomolecules such as protein and nucleic acid, as it affects enzyme activities and causes alteration in the membrane permeability (Sanita di Toppi et al., 1999). Studies suggested that Cd^{2+} reduces ATPase

activity of plasmalemma fraction (Astolfi et al., 2005), changes lipid composition by enhancing reactive oxygen species (ROS) production (Gomes-Junior et al., 2006). Moreover Cd^{2+} is an effective inhibitor of photosynthesis (Vessiliev et al., 1998; Hasan et al., 2009). A linear relationship between photosynthesis and inhibition of transpiration was observed, that suggests Cd^{2+} inhibited stomatal opening (Barcelo et al., 1986) and it also decreases the activity of several other enzymes (Hayat et al., 2007; Hasan et al., 2009).

The present study was conducted with the aim to find out the degree of tolerance among ten cultivars of tomato by soaking (*i.e.* shotgun approach) their seeds in varied cadmium chloride concentrations for different durations, and to find out the most sensitive variety and resistant one by assessing the plant through its growth and antioxidative system.

2. MATERIALS AND METHODS

2.1. Biological materials

The seeds of *Lycopersicon esculentum* L. cv. 'K-25', 'K-21', 'NTS-9', 'Kaveri', 'NBR-Uday', 'Swarnodya', 'Sarvodya', 'NBR-Uttam', 'Malti' and 'S-22' were purchased from National Seed Corporation Ltd., New Delhi, India. The healthy seeds were surface sterilized with 5% hypochlorite and were soaked in 0, 50, 100 or 150 µM of Cd^{2+} for 0, 4, 8 or 12 h. These seeds were sown in earthen pots (6 inches diameter) filled with sandy loam soil and farmyard manure mixed in a ratio of 6:1 to create a nursery. At 15 day stage these seedlings were uprooted and transplanted to the pots, under similar conditions as that of nursery. The plants were grown in a net house under natural environmental conditions. The average temperature, humidity, and day/night photoperiods were 26 ± 2°C, 65 ± 5% and 14/10 h, respectively. Plants were watered at regular intervals. The plants were removed at 30 day stage along with the soil and dipped in a bucket, filled with water, to remove the adhering soil particles, ensuring the safety of roots. The plants were blotted and the lengths of roots and shoots were measured, followed by their subsequent weighing to record their fresh mass.

2.2. Estimation of nitrate reductase (NR) activity

The activity of NR was measured following the method adopted by Jaworski (1971). The fresh leaf samples were cut into small pieces and transferred to plastic vials, containing phosphate buffer (pH 7.5) followed by the addition of potassium nitrate and isopropanol solutions. The reaction mixture was incubated at 30°C, for 2 h followed with the addition of *N*-1 naphthylethylenediamine dihydrochloride and sulphanilamide. The absorbance of the color was read at 540 nm and was compared with that of the calibration curve. The activity of NR (nmol $NO_2 \cdot g^{-1} \cdot h^{-1}$) was computed on fresh mass basis.

2.3. Assay of antioxidative enzymes

Leaf tissue (0.5 g) was homogenized in 5 ml of 50 M phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrolidone. The homogenate was centrifuged at 15,000 rpm for 10 min and the supernatant was used as the source of enzyme. The extraction was carried out at 4°C. Peroxidase and catalase activities were assayed following the procedure described by Chance et al. (1955). Catalase activity was estimated by titrating the reaction mixture, consisting of phosphate buffer (pH 6.8), 0.1M H_2O_2 enzyme extract and 2% H_2SO_4 against potassium permanganate. The reaction mixture for peroxidase consisted of pyrogallol phosphate buffer (pH 6.8), 1% H_2O_2 and enzyme extract. Change in absorbance, due to catalytic conversion of pyrogallol to perpurogallin, was noted at an interval of 20 s for 2 h at 420 nm. A control set was prepared by using distilled water instead of enzyme extract. The activity of superoxide dismutase was assayed using the method of Beauchamp et al. (1971). The reaction mixture contained 50 mmol phosphate buffer (pH 7.8), 13 mmol methionine, 74 µmol NBT, 2 µmol riboflavin, 0.1 mmol EDTA and 0-50 µl enzyme extract, and was placed under 15W fluorescent lamp. The reaction was started by switching on the light and was allowed to run for 10 min. The reaction was stopped by switching off the light. Fifty per cent inhibition by light was considered as one enzyme unit.

2.4. Statistical analysis

The experiment was conducted according to simple randomized block design. A total of ten replicates for each treatment were taken. Treatment means were compared by analysis of variance using SPSS (SPSS, Chicago, IL, USA). Least Significance Difference (LSD) was calculated at the 5% level of probability.

3. RESULTS

3.1. Root length

The observations shown in **table 1** clearly indicate that the Cd^{2+} treatment resulted in a significant decrease in the length of roots of the resulting plants. However, the response to the Cd^{2+} stress showed significant variation when the varieties were taken into consideration. The highest concentration of Cd^{2+} (150 µM) supplied for any of the duration (4, 8 or 12 h) of seed soaking

Table 1. Effect on root length (cm) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Longueurs des racines (cm) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) mesurées à 30 jours après semis à la suite d'un traitement des semences par trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	**Duration of seed soaking**																
	Unsoaked control	**4 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**8 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**12 h $CdCl_2$ concentration (µM)**				
		0	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**
K-25	6.60	6.62	6.18	5.75	5.20	6.07	6.60	6.63	5.76	5.07	4.46	5.64	6.60	6.60	5.17	4.29	3.79	5.29
K-21	6.45	6.45	5.54	5.15	4.60	5.63	6.45	6.47	5.27	4.57	4.03	5.35	6.45	6.50	4.70	4.00	3.50	5.03
NTS-9	6.48	6.50	5.20	4.86	4.15	5.43	6.48	6.53	4.78	4.45	3.46	5.14	6.48	6.55	4.61	3.62	3.00	4.85
Kaveri	6.04	6.01	4.83	4.81	3.63	5.06	6.04	6.03	4.71	4.00	2.95	2.37	6.04	6.04	5.17	4.29	-	4.30
NBR-UDAY	6.40	6.43	5.19	4.22	3.12	5.07	6.40	6.46	4.60	3.50	2.45	4.68	6.40	6.49	4.49	3.50	-	4.17
SWARNODYA	5.53	5.56	5.05	3.72	3.00	4.57	5.53	5.58	4.00	3.10	2.00	4.04	5.53	5.60	4.81	4.13	-	4.01
SARVODYA	5.32	5.35	4.80	3.66	2.89	4.40	5.32	5.39	3.50	3.07	2.00	3.85	5.32	5.42	3.62	2.74	-	3.42
NBR-UTTAM	6.16	6.18	4.50	3.08	2.35	4.45	6.16	6.20	3.31	3.00	-	3.73	6.16	6.22	3.12	-	-	3.10
MALTI	5.60	5.61	4.45	2.56	2.00	4.04	5.60	5.64	3.00	2.45	-	3.33	5.60	5.65	4.32	-	-	3.11
S-22	4.29	4.30	-	-	-	1.71	4.29	4.32	-	-	-	1.72	4.29	4.33	-	-	-	1.72
Mean	5.88	5.90	4.57	3.78	3.09		5.88	5.92	3.89	3.32	2.10		5.88	5.94	4.00	2.65	1.02	
LSD at 5%	Varieties (V) = 0.15 Cadmium concentration (C) = 0.40 V × C = 0.66						Varieties (V) = 0.18 Cadmium concentration (C) = 0.42 V × C = 0.73						Varieties (V) = 0.15 Cadmium concentration (C) = 0.49 V × C = 0.77					

- = not survived — *n'a pas survécu.*

caused the maximum reduction in the root length. The varieties which were severely affected by the Cd^{2+} were 'Sarvodya', 'NBR-Uttam' and 'Malti'. These varieties also experienced a significant damage at the lowest concentration (50 µM) supplied for minimum duration (4 h). Here the root length was 10.2%, 27.1% and 20.6% lower than the respective controls. The varieties K-25, K-21 and NTS-9 showed the maximum resistance to Cd^{2+}, however the higher concentration (150 µM) of Cd^{2+} fed for 12 h reduced the root length by 42.5%, 46.1% and 54.1% compared to their controls. The pattern of resistance exhibited by three varieties were 'K-25' > 'K-21' > 'NTS-9'. The varieties 'Kaveri', 'NBR-Uday' and 'Swarnodya' showed maximum inhibition of root elongation at 100 µM Cd^{2+} concentration, soaked for 12 h. However, treating the seeds for shorter time duration (4 or 8 h) caused a lesser toxicity than 12 h soaking duration.

3.2. Root fresh mass

The shotgun approach of Cd^{2+} (50, 100 or 150 µM) significantly reduced the root fresh mass and all the varieties differed significantly in their response to the metal concentrations (**Table 2**). The treatment combination of the highest concentration (150 µM) of Cd^{2+} and longest soaking duration (12 h) was most toxic, which was tolerated only by the varieties 'K-25', 'K-21' and 'NTS-9'. In response to this treatment combination these varieties exhibited 32.7%, 28.1% and 36.6% decrease, compared to their respective control. The combined effect of 50 µM of Cd^{2+} and 4 h soaking duration was the least damaging. The varieties 'Sarvodya', 'NBR-Uttam' and 'Malti' were so sensitive that even at the lowest level of Cd^{2+} (50 µM) and minimum soaking duration (4 h) showed 14.2%, 16.8% and 13.6% decrease, over their respective controls. The variety 'S-22' was the most sensitive that could not survive in the presence of any of the cadmium concentration.

3.3. Shoot length

The data in **table 3** indicate that the pre-sowing seed soaking treatment (shotgun approach) caused a significant decrease in the shoot length of the resulting plants. The decrease was proportionate to the concentration of the metal as well as to the duration of soaking. The treatment for the longest duration (12 h) caused the maximum damage. The variety 'S-22' could not survive in the presence of any of the Cd^{2+} concentration, whereas the varieties 'NBR-Uttam' and 'Malti' could not survive to the pre-sowing soaking with Cd^{2+} 100 and/or 150 µM for 12 or 8 h, respectively. The variety 'K-25' was the most resistant that exhibited the minimum damage in response to the stress. This variety showed 8.2%, 12.6%, 21.4%; 11.4%, 15.5%, 25.4% and 17.2%, 26.0% and 41.3% reduction in response to 50, 100 or 150 µM of Cd^{2+} supplemented for 4, 8 or 12 h, respectively. The variety 'K-21' showed a response comparable to that of the most resistant one ('K-25'). The varieties 'Kaveri', 'NBR-Uday' and 'Swarnodya' were moderately affected by the cadmium. However, they could not resist the pre-sowing soaking in 150 µM of Cd^{2+} for 12 h. The varieties 'Sarvodya', 'NBR-Uttam' and 'Malti' experienced a severe damage and among these three varieties the order of susceptibility/sensitivity was 'Sarvodya' > 'NBR-Uttam' > 'Malti'.

3.4. Shoot fresh mass

The plants raised from the seeds soaked in different concentrations of Cd^{2+} for 12 h duration exhibited maximum inhibition in shoot fresh mass production (**Table 4**). The varieties 'K-25', 'K-21' and 'NTS-9' showed least inhibition to 12 h soaking in 150 µM of Cd^{2+} where the values were 51.8%, 59.6% and 55.2% below their respective controls. However the other varieties could not survive in higher concentration of the metal. The varieties 'Kaveri', 'NBR-Uday', 'Swarnodya' and 'Sarvodya' were moderately affected by Cd^{2+} and showed 31.6%, 31.1%, 45.6% and 44.1%; 49.5%, 50.0%, 52.7% and 63.9%, decrease compared to their respective controls when Cd^{2+} (150 µM) was given for 4 or 8 h respectively.

3.5. Nitrate reductase (NR) activity

Table 5 shows that the activity of NR significantly decreased with the increasing concentration of Cd^{2+} as well as duration of soaking. The lowest concentration of Cd^{2+} (50 µM) was least toxic for all the varieties. The other two concentrations (100 and 150 µM) showed a higher magnitude of toxicity, which shifted from variety to variety. Among the varieties 'K-25', 'K-21' and 'NTS-9' were comparatively resistant in terms of the NR activity. The varieties 'Kaveri', 'NBR-Uday' and 'Swarnodya' were neither too resistant nor too sensitive. In these three varieties seed soaking in 100 µM of Cd^{2+} for 4, 8 or 12 h inhibited the activity of NR by 16.9%, 20.2%, 26.1; and 17.5%, 24.3%, 27.7%; and 14.4%, 23.3%, 26.7% compared to their respective control. The varieties 'Sarvodya', 'NBR-Uttam' and 'Malti' were highly sensitive to the cadmium ion.

3.6. Antioxidative enzyme activities

The activities of catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) were positively affected by the Cd^{2+} as well as its duration of soaking in all the varieties (**Tables 6**, **7**, **8**) and this increase was directly proportional to the both treatment components

Table 2. Effect on root fresh mass (g) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Poids frais des racines (g) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) mesurés à 30 jours après semis à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	**Duration of seed soaking**																
	Unsoaked control	**4 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**8 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**12 h $CdCl_2$ concentration (µM)**				
		0	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**
K-25	0.91	0.91	0.86	0.81	0.75	0.85	0.91	0.92	0.81	0.76	0.70	0.82	0.91	0.92	0.78	0.70	0.62	0.77
K-21	0.73	0.74	0.69	0.67	0.64	0.69	0.73	0.74	0.69	0.64	0.59	0.68	0.73	0.75	0.63	0.59	0.54	0.65
NTS-9	0.71	0.71	0.66	0.63	0.58	0.66	0.71	0.72	0.66	0.61	0.53	0.64	0.71	0.72	0.61	0.54	0.46	0.61
Kaveri	0.66	0.67	0.62	0.56	0.52	0.60	0.66	0.67	0.61	0.56	0.52	0.60	0.66	0.67	0.56	0.47	-	0.47
NBR-UDAY	0.64	0.64	0.59	0.54	0.49	0.58	0.64	0.65	0.59	0.45	0.44	0.55	0.64	0.65	0.56	0.38	-	0.44
SWARNODYA	0.60	0.61	0.54	0.48	0.43	0.53	0.60	0.61	0.44	0.40	0.38	0.48	0.60	0.61	0.47	0.35	-	0.40
SARVODYA	0.55	0.56	0.48	0.42	0.37	0.48	0.55	0.56	0.42	0.35	0.30	0.43	0.55	0.56	0.37	0.30	-	0.36
NBR-UTTAM	0.50	0.50	0.41	0.37	0.32	0.42	0.50	0.50	0.37	0.30	-	0.33	0.50	0.51	0.32	-	-	0.26
MALTI	0.43	0.44	0.38	0.32	0.25	0.36	0.43	0.45	0.32	0.27	-	0.29	0.43	0.44	0.32	-	-	0.24
S-22	0.46	0.46	-	-	-	0.18	0.46	0.47	-	-	-	0.18	0.46	0.47	-	-	-	0.18
Mean	0.62	0.62	0.52	0.48	0.43		0.62	0.63	0.49	0.43	0.34		0.62	0.63	0.46	0.33	0.16	
LSD at 5%	Varieties (V) = 0.024 Cadmium concentration (C) = 0.040 V × C = 0.076						Varieties (V) = 0.020 Cadmium concentration (C) = 0.049 V × C = 0.083						Varieties (V) = 0.024 Cadmium concentration (C) = 0.050 V × C = 0.090					

- = not survived — *n'a pas survécu.*

Table 3. Effect on shoot length (cm) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Longueurs des tiges (cm) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) mesurées à 30 jours après semis à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	Duration of seed soaking																
	Unsoaked control	4 h $CdCl_2$ concentration (µM)					Unsoaked control	8 h $CdCl_2$ concentration (µM)					Unsoaked control	12 h $CdCl_2$ concentration (µM)				
		0	50	100	150	Mean		0	50	100	150	Mean		0	50	100	150	Mean
K-25	7.60	7.60	6.97	6.64	5.97	6.95	7.60	7.71	6.83	6.51	5.75	6.88	7.60	7.84	6.49	5.80	4.60	6.46
K-21	7.78	7.81	6.85	6.30	5.83	6.91	7.78	7.87	6.79	6.10	5.60	6.82	7.78	7.91	6.18	5.47	4.32	6.33
NTS-9	6.90	6.90	6.60	6.18	5.67	6.45	6.90	6.99	6.43	6.06	5.00	6.27	6.90	7.12	6.03	5.05	4.19	5.85
Kaveri	6.86	6.89	6.49	6.10	5.43	6.35	6.86	6.90	5.81	5.64	4.80	6.00	6.86	7.08	5.49	4.66	-	4.81
NBR-UDAY	6.50	6.52	6.50	5.99	5.32	6.16	6.50	6.45	5.80	4.81	4.07	5.52	6.50	6.66	5.07	4.36	-	4.51
SWARNODYA	6.37	6.41	5.41	5.18	4.93	5.66	6.37	6.57	5.36	4.60	3.90	5.36	6.37	6.63	4.30	4.00	-	4.26
SARVODYA	6.29	6.31	5.07	4.63	3.93	5.24	6.29	6.44	4.91	4.43	2.83	4.98	6.29	6.47	4.20	3.87	-	4.16
NBR-UTTAM	6.18	6.19	5.29	4.01	3.98	5.13	6.18	6.23	5.10	4.51	-	4.40	6.18	6.30	4.69	-	-	3.43
MALTI	5.87	5.90	5.04	3.87	3.25	4.78	5.87	5.97	4.81	3.16	-	3.96	5.87	6.03	3.21	-	-	3.02
S-22	5.64	5.57	-	-	-	2.24	5.64	5.69	-	-	-	2.26	5.64	5.73	-	-	-	2.27
Mean	6.59	6.61	5.42	4.89	4.43		6.59	6.68	5.18	4.58	3.19		6.59	6.77	4.56	3.32	1.31	
LSD at 5%	Varieties (V) = 0.08 Cadmium concentration (C) = 0.06 V × C = 0.17						Varieties (V) = 0.25 Cadmium concentration (C) = 0.20 V × C = 0.51						Varieties (V) = 0.12 Cadmium concentration (C) = 0.67 V × C = 0.97					

- = not survived — *n'a pas survécu.*

Table 4. Effect on shoot fresh mass (g) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 μM) for 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Effet observé à 30 jours après le semis sur le poids frais des tiges (g) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 μM pendant 4, 8 et 12 h.*

Varieties	**Duration of seed soaking**																
	Unsoaked control	**4 h $CdCl_2$ concentration (μM)**					**Unsoaked control**	**8 h $CdCl_2$ concentration (μM)**					**Unsoaked control**	**12 h $CdCl_2$ concentration (μM)**				
		0	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**
K-25	3.02	3.05	2.96	2.29	2.04	2.67	3.02	3.02	2.82	1.91	1.83	2.52	3.02	2.97	2.39	1.90	1.43	2.34
K-21	2.70	2.72	2.66	2.24	1.80	2.42	2.70	2.78	2.26	1.87	1.55	2.22	2.70	2.80	1.80	1.46	1.13	1.97
NTS-9	2.41	2.42	2.40	2.18	1.79	2.24	2.41	2.44	2.00	1.79	1.50	2.02	2.41	2.46	1.60	1.32	1.10	1.77
Kaveri	2.32	2.34	2.29	2.10	1.60	2.13	2.32	2.36	1.90	1.58	1.19	1.87	2.32	2.38	1.47	1.16	-	1.46
NBR-UDAY	2.20	2.25	2.16	1.61	1.55	1.95	2.20	2.28	1.91	1.45	1.14	1.79	2.20	2.30	1.30	1.08	-	1.37
SWARNODYA	2.15	2.17	1.60	1.55	1.18	1.73	2.15	2.20	1.51	1.27	1.04	1.63	2.15	2.24	1.18	1.05	-	1.32
SARVODYA	2.03	2.06	1.86	1.50	1.15	1.72	2.03	2.08	1.40	1.00	0.75	1.45	2.03	2.10	1.10	0.52	-	1.15
NBR-UTTAM	1.91	1.93	1.54	1.47	1.01	1.57	1.91	1.96	1.10	1.00	-	1.19	1.91	1.99	0.96	-	-	0.972
MALTI	1.84	1.88	1.37	1.05	0.96	1.42	1.84	1.90	1.04	0.91	-	1.13	1.84	1.92	0.66	-	-	0.884
S-22	1.79	1.80	-	-	-	0.718	1.79	1.81	-	-	-	0.720	1.79	1.83	-	-	-	0.724
Mean	2.23	2.26	1.88	1.59	1.30		2.23	2.28	1.58	1.27	.900		2.23	2.29	1.24	.849	.366	
LSD at 5%	Varieties (V) = 0.07 Cadmium concentration (C) = 0.18 V × C = 0.30						Varieties (V) = 0.10 Cadmium concentration (C) = 0.20 V × C = 0.36						Varieties (V) = 0.07 Cadmium concentration (C) = 0.30 V × C = 0.44					

- = not survived — *n'a pas survécu.*

Table 5. Effect on leaf nitrate reductase (NR) activity (nM $NO_2 \cdot g^{-1} \cdot h^{-1}$) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) for 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Activités de la nitrate réductase (nM $NO_2 \cdot g^{-1} \cdot h^{-1}$) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) mesurées à 30 jours après semis à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	Duration of seed soaking																	
	Unsoaked control	4 h $CdCl_2$ concentration (µM)					Unsoaked control	8 h $CdCl_2$ concentration (µM)					Unsoaked control	12 h $CdCl_2$ concentration (µM)				
		0	50	100	150	Mean		0	50	100	150	Mean		0	50	100	150	Mean
K-25	312	315	307	290	250	294.8	312	318	296	266	241	286.6	312	311	275	249	214	272.2
K-21	300	303	285	263	238	277.8	300	305	266	244	225	268.0	300	308	251	230	210	259.8
NTS-9	290	291	271	254	230	267.2	290	293	250	232	222	257.4	290	294	240	227	202	250.6
Kaveri	280	283	260	235	221	255.8	280	286	245	228	212	250.2	280	287	230	212	-	201.8
NBR-UDAY	271	273	248	225	206	244.6	271	275	226	208	189	233.8	271	277	209	200	-	191.4
SWARNODYA	255	256	235	219	197	232.4	255	257	211	197	180	220.0	255	258	201	189	-	1806
SARVODYA	247	248	215	192	180	216.4	247	249	194	178	160	205.6	247	251	175	157	-	166.0
NBR-UTTAM	244	246	204	184	160	207.6	244	248	187	170	-	169.8	244	249	160	-	-	130.6
MALTI	540	242	216	194	166	211.6	240	242	168	153	-	160.6	240	244	151	-	-	128.0
S-22	231	233	-	-	-	92.8	231	234	-	-	-	93.0	231	237	-	-	-	93.6
Mean	267	269	224	205	184		267	270	204	187	192		267	272	189	146	62.6	
LSD at 5%	Varieties (V) = 8.00 Cadmium concentration (C) = 18.00 V × C = 31.72						Varieties (V) = 10.00 Cadmium concentration (C) = 16.00 V × C = 31.98						Varieties (V) = 10.00 Cadmium concentration (C) = 17.00 V × C = 32.67					

- = not survived — *n'a pas survécu.*

Table 6. Effect on leaf catalase activity (µmol H_2O_2 decomposed in g) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Effet observé à 30 jours après le semis sur l'activité catalase (µmol H_2O_2 décomposé en g) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h*

Varieties	Duration of seed soaking																	
	Unsoaked control	4 h $CdCl_2$ concentration (µM)					Unsoaked control	8 h $CdCl_2$ concentration (µM)					Unsoaked control	12 h $CdCl_2$ concentration (µM)				
		0	50	100	150	Mean		0	50	100	150	Mean		0	50	100	150	Mean
K-25	415	418	433	455	477	439.6	415	421	454	480	491	452.2	415	425	476	503	521	468.0
K-21	405	407	415	434	436	419.4	405	410	433	455	460	432.6	405	412	454	465	482	443.6
NTS-9	397	400	420	422	461	420.0	397	404	430	440	480	430.2	395	407	435	450	500	437.4
Kaveri	350	354	368	385	398	371.0	350	360	388	412	430	388.0	350	365	392	430	-	307.4
NBR-UDAY	336	339	340	366	390	354.2	336	341	365	392	410	368.8	336	344	390	411	-	296.2
SWARNODYA	320	325	350	360	385	348.0	320	330	360	388	400	359.6	320	333	380	410	-	288.6
SARVODYA	295	298	319	340	362	322.8	295	300	335	360	381	334.2	295	305	340	379	-	263.8
NBR-UTTAM	270	273	287	307	320	291.4	270	275	310	339	-	238.8	270	280	331	-	-	176.2
MALTI	264	267	288	300	315	286.8	264	270	307	320	-	232.2	264	273	310	-	-	169.4
S-22	251	253	-	-	-	100.8	251	255	-	-	-	101.2	251	256	-	-	-	101.4
Mean	330	333	322	336	354		330	336	338	358	305		330	340	350	304	150	
LSD at 5%	Varieties (V) = 11.0 Cadmium concentration (C) = 10.0 V × C = 25.6						Varieties (V) = 14.0 Cadmium concentration (C) = 17.0 V × C = 38.1						Varieties (V) = 8.00 Cadmium concentration (C) = 25.0 V × C = 39.9					

- = not survived — *n'a pas survécu*.

Table 7. Effect on leaf peroxidase (g^{-1} F.M.) of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Activités de la peroxydase racinaire (en µmol d'H_2O_2 décomposé ou en unités g^{-1} M.F.) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) mesurées à 30 jours après semis à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	Duration of seed soaking																	
	Unsoaked control	4 h $CdCl_2$ concentration (µM)					Unsoaked control	8 h $CdCl_2$ concentration (µM)					Unsoaked control	12 h $CdCl_2$ concentration (µM)				
		0	50	100	150	Mean		0	50	100	150	Mean		0	50	100	150	Mean
K-25	9.50	9.56	11.30	15.9	18.9	13.0	9.50	10.7	12.6	16.8	20.6	14.0	9.0	10.00	14.7	19.5	22.3	15.22
K-21	9.10	9.10	11.20	15.6	16.3	12.2	9.10	9.46	12.9	16.9	18.7	13.4	9.10	9.00	13.0	17.0	20.6	13.86
NTS-9	7.38	7.39	9.75	11.0	13.5	9.80	7.38	7.42	10.0	12.7	15.4	10.5	7.38	7.50	10.6	14.0	17.2	11.36
Kaveri	6.90	6.91	7.80	9.11	11.7	8.50	6.90	6.95	9.00	10.0	13.0	9.17	6.90	7.00	10.1	12.1	-	7.24
NBR-UDAY	6.23	6.25	7.54	7.64	8.59	7.25	6.28	6.29	7.84	8.88	10.3	7.92	6.28	6.32	9.89	10.0	-	6.49
SWARNODYA	5.76	5.80	6.00	6.09	6.50	5.97	5.76	5.84	6.20	6.80	7.25	6.37	5.76	5.90	8.90	9.00	-	5.91
SARVODYA	5.48	5.50	6.07	6.62	6.97	6.12	5.48	5.55	6.15	6.91	7.00	6.21	5.48	5.59	8.60	9.11	-	5.75
NBR-UTTAM	4.90	4.91	5.10	6.30	6.35	5.51	4.90	4.96	5.70	6.08	-	4.62	4.90	4.97	6.10	-	-	3.19
MALTI	4.75	4.77	5.00	5.80	5.92	5.24	4.75	4.79	5.00	5.75	-	4.05	4.75	4.80	5.28	-	-	2.96
S-22	4.60	4.62	-	-	-	1.84	4.60	4.66	-	-	-	1.85	4.60	4.67	-	-	-	1.85
Mean	4046	6.48	6.97	8.41	9.46		6.46	6.66	7.54	9.08	9.23		6.46	6.62	8.74	9.07	6.03	
LSD at 5%	Varieties (V) = 0.30 Cadmium concentration (C) = 0.80 V × C = 1.33						Varieties (V) = 0.40 Cadmium concentration (C) = 0.60 V × C = 1.21						Varieties (V) = 0.50 Cadmium concentration (C) = 1.00 V × C = 1.83					

- = not survived — *n'a pas survécu.*

Table 8. Effect on leaf superoxide dismutase activity [units g^{-1} (F.M.)] of a pre-sowing seed soaking treatment with cadmium chloride ($CdCl_2$) (0, 50, 100 or 150 µM) during 4, 8 or 12 h for ten different varieties of tomato (*Lycopersicon esculentum* Mill.) at 30 days after sowing — *Effet observé à 30 jours après le semis sur l'activité superoxide dismutase (unité g^{-1} F.M.) de dix variétés de tomate (*Lycopersicon esculentum *Mill.) à la suite d'un traitement en pré-semis des semences par leur trempage dans du chlorure de cadmium ($CdCl_2$) aux concentrations de 0, 50, 100, 150 µM pendant 4, 8 et 12 h.*

Varieties	**Duration of seed soaking**																	
	Unsoaked control	**4 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**8 h $CdCl_2$ concentration (µM)**					**Unsoaked control**	**12 h $CdCl_2$ concentration (µM)**				
		0	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**		**0**	**50**	**100**	**150**	**Mean**
K-25	125	127	140	149	160	144	125	129	143	155	150	144	125	130	162	172	157	155
K-21	122	123	134	142	151	137	122	125	138	150	168	145	122	126	155	166	178	156
NTS-9	119	120	129	137	145	132	119	122	134	145	162	140	119	123	149	160	171	150
Kaveri	114	116	124	131	139	127	114	119	131	141	157	137	114	120	145	155	-	140
NBR-UDAY	110	111	117	124	131	120	110	114	125	134	152	131	110	115	139	148	-	134
SWARNODYA	106	107	112	118	125	115	106	110	121	130	143	126	106	112	133	142	-	129
SARVODYA	108	110	115	123	132	120	108	113	123	133	138	126	108	115	126	137	-	126
NBR-UTTAM	101	103	107	113	120	110	101	105	114	122	-	113	101	108	120	-	-	114
MALTI	100	101	105	111	116	108	100	105	113	120	-	112	100	105	114	-	-	109
S-22	101	100	-	-	-	100	101	102	-	-	-	102	101	103	-	-	-	103
Mean	111	120	127	135			111	114	126	136	152	111		115	138	154	168	
LSD at 5%	Varieties (V) = 0.30 Cadmium concentration (C) = 0.80 V × C = 1.33						Varieties (V) = 0.40 Cadmium concentration (C) = 0.60 V × C = 1.21						Varieties (V) = 0.50 Cadmium concentration (C) = 1.00 V × C = 1.83					

- = not survived — *n'a pas survécu.*

(concentration and duration of soaking). The activity of the enzymes was maximum in variety 'K-25', at all the Cd^{2+} levels. The plants of 'K-25' raised from the seeds exposed to 50, 100 or 150 µm of Cd^{2+} for 12 h possessed the activity of the enzymes which was 12.0%, 18.3% and 22.5% higher for catalase and 47.5%, 95.0% and 123.9% higher for peroxidase over the control, respectively. The varieties 'Sarvodya', 'NBR-Uttam' and 'Malti' possessed least value among all the varieties. The treatment combination of Cd^{2+} (100 µM) with 8 h soaking duration elevated the level of the enzymes, which were 20%, 23.2% and 18.5% higher for catalase, 24.5%, 22.5% and 20.0% higher for peroxidase and 24.6%, 32.3% and 20.7% higher for SOD in the varieties 'K-25', 'NBR-Uttam' and 'Malti', respectively. This increase was maximum for these three varieties among all the Cd^{2+} doses and the soaking durations. The varieties 'Kaveri', 'NBR-Uday' and 'Swarnodya' showed an intermediate response, which possessed the maximum activities of the enzymes at 100 µM Cd^{2+} supplied for 12 h treatment. The trend followed by different treatment durations was 12 h > 8 h > 4 h.

4. DISCUSSION

Plants have a well equipped natural antioxidative defense system to maintain the redox equilibrium. In non-stress conditions, ROS and other oxidants are balanced by the antioxidative defense system, which is composed of enzymes (CAT, POX and SOD) and metabolites (tocopherol, ascorbate and proline). However under stress this redox equilibrium is disturbed and the increased ROS accumulation causes a specific oxidative stress response (Cuypers et al., 2001). In the present study, the increase in the activity of SOD and CAT is a consequence of dis-equilibrium, provoked by the increased availability of the Cd^{2+}. Previous studies have also shown that Cd^{2+} is able to induce oxidative stress, which provokes an increase in metabolite content as well as the activation of several antioxidative enzymes (Smeets et al., 2007; Hasan et al., 2008). This increase in SOD and CAT activities with the increasing concentration of cadmium (**Tables 6**, **7**, **8**) indicated that these cultivars had the capacity to adapt to different Cd^{2+} concentrations by developing an antioxidative defence system. However the varieties differed widely in their ability to tolerate Cd^{2+} stress. The variety 'S-22' could not tolerate the presence of even the lowest concentration of Cd^{2+}. 'Sarvodya', 'NBR-Uttam' and 'Malti' are also severely affected by cadmium, however 'Kaveri', 'NBR-Uday' and 'Swarnodya' experienced moderate damage. Moreover, varieties 'K-25', 'K-21' and 'NTS-9' showed maximum resistance to cadmium concentration as they possessed highest level of the antioxidative enzymes.

The activity of NR enzyme decreased with the increasing concentration of cadmium where Cd^{2+} is known to restrict the uptake of nitrate by the roots by damaging the normal function of plasma-membrane bound proton pump (Obata et al., 1996) and the fluidity of membrane (Meharg, 1993). Therefore, the restricted supply of the NR inducer and the substrate hamper the activity of NR. Moreover, toxicity generated by Cd^{2+} impaired root and shoot growth in all the tomato cultivars. However, it should be noted that although both root and shoot growth were affected by Cd^{2+} treatment but the decrease in the root length and its fresh mass was stronger than in shoot. The primary reason for high root sensitivity to Cd^{2+} might be related to the fact that root is the first organ exposed to Cd^{2+} and hence accumulates metal at much higher concentrations than the shoot (Tiryakioglie et al., 2006). Secondly Cd^{2+} is known to cause physiological drought by altering plant water balance, nutrient uptake and permeability of plasma membrane (Barcelo et al., 1990; Hernandez et al., 1996) which in turn affect cell enlargement and resulted in stunted growth. The extent of changes in growth attributes and enzymatic activity revealed the existence of great varietal differences throughout the tomato ontogeny for Cd^{2+} tolerance.

Acknowledgements

This work was funded by University Grants Commission [Project No 32-403/ 2006 (SR)], New Delhi, India.

Bibliography

Akinola M.O. & Ekiyoho T.A., 2006. Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Rabillia at Odonla area of Ikirodu, Lagos state, Nigeria. *J. Environ. Biol.*, **27**, 597-599.

Ali B., Hayat S. & Ahmad A., 2007. 28-Homobrassinolide ameliorates the saline stress in chickpea (*Cicer arietinum* L.). *Environ. Exp. Bot.*, **59**, 217-233.

Astolfi S., Zuch S. & Passera C., 2005. Effect of cadmium on H^+-ATPase activity of plasma membrane vesicles isolated from roots of different S supplied maize (*Zea mays* L.) plants. *Plant Sci.*, **169**, 361-368.

Barcelo J., Poschenrieder C., Andreu I. & Gunse B., 1986. Cadmium induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. 'Contender'). I. Effect on water potential, relative water content and cell wall elasticity. *J. Plant Physiol.*, **125**, 17-25.

Barcelo J. & Poschenrieder C., 1990. Plant water relations are affected by heavy metal stress: a review. *Plant Nutr.*, **13**, 1-37.

Beauchamp C.O. & Fridovich I., 1971. Superoxide dismutase: improved assays and assay applicable to acrylamide gels. *Anal. Biochem.*, **44**, 276-287.

Chance B. & Maehly A.C., 1955. Assay of catalase and peroxidase. *Methods Enzymol.*, **2**, 764-775.

Cuypers A., Vangronsveld J. & Clijsters H., 2001. The redox status of plant cells (asa and gsh) is sensitive to zinc imposed oxidative stress in roots and primary leaves of *Phaseolus vulgaris*. *Plant Physiol. Biochem.*, **39**, 657-669.

Das P., Samantaray S. & Rout G.R., 1997. Studies on cadmium toxicity in plants. A review. *Environ. Pollut.*, **98**, 29-36.

Ghani A. & Wahid A., 2007. Varietal difference for cadmium induced seedling mortality and foliar toxicity symptoms in mungbean (*Vigna radiate*). *Int. J. Agric. Biol.*, **9**, 555-558.

Gianazza E. et al., 2007. Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. *Environ. Exp. Bot.*, **59**, 179-187.

Gomes-Junior R.A. et al., 2006. Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. *Chemosphere*, **65**, 1330-1337.

Hasan S.A., Hayat S., Ali B. & Ahmad A., 2008. 28-Homobrassinolide protect chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidant. *Environ. Pollut.*, **151**, 60-66.

Hasan S.A., Hayat S. & Ahmad A., 2009. Screening of tomato (*Lycopersicon esculentum*) cultivars against cadmium through shotgun approach. *J. Plant Interact.*, **4**, 187-201.

Hayat S., Ali B., Hasan S.A. & Ahmad A., 2007. Brassinosteroids enhanced the level of antioxidant under cadmium stress in *Brassica juncea*. *Environ. Exp. Bot.*, **60**, 33-41.

He J. et al., 2006. Genotypic variation in grain cadmium concentration of lowland rice. *J. Plant Nutr. Soil Sci.*, **169**, 711-716.

Hernandez L.E., Carpena-Ruiz R. & Garate A., 1996. Alteration mineral nutrition of pea seedlings exposed to cadmium. *J. Plant Nutr.*, **19**, 1581-1598.

Jaworski E.G., 1971. Nitrate reductase assay in intact plant tissue. *Biochem. Biophys. Res. Commun.*, **43**, 1274-1279.

Liu D.H., Jiang W.S. & Gao X.Z., 2003. Effects of cadmium on root growth, cell division and nuclei in root tip cells of garlic. *Biol. Plant.*, **47**, 78-83.

Meharg A.A., 1993. Integrated tolerance mechanisms constitutive and adaptive plant response to elevated metal concentrations in the environment. *Plant Cell Environ.*, **17**, 989-993.

Minissi S. & Lombi E., 1997. Heavy metal content and mutagenic activity, evaluated by *Vicia faba* micronucleus test, of Tiber river sediments. *Mutat. Res.*, **393**, 17-21.

Obata H., Inone N. & Umebayshi M., 1996. Effect of cadmium on plasma membrane ATPase from plant root differing in tolerance to cadmium. *Soil Sci. Plant Nutr.*, **42**, 361-366.

Radotic K., Ducic T. & Mutavdzic D., 2000. Changes in peroxidase activity and iso-enzymes in spruce needles after exposure to different concentrations of cadmium. *Environ. Exp. Bot.*, **44**, 105-113.

Sanita di Toppi L. & Gabbrielli R., 1999. Response to cadmium in higher plants. *Environ. Exp. Bot.*, **41**, 105-130.

Smeets K. et al., 2007. Cadmium induced transcriptional and enzymatic alterations related to oxidative stress. *Environ. Exp. Bot.*, **63**, 1-8.

Tiryakioglie M. et al., 2006. Antioxidant defence system and cadmium uptake in barley genotype differing in cadmium tolerance. *J. Trace Elem. Med. Biol.*, **20**, 181-190.

Usha M., Venugopoj V.K. & Saraswathi P., 2002. Cadmium content of plants as affected by soil application of cadmium and farmyard manure. *J. Trop. Agric.*, **40**, 70-80.

Vessiliev A., Berora M. & Zlateve Z., 1998. Influence of Cd^{2+} on growth, chlorophyll content and water relations in young barley plant. *Biol. Plant.*, **41**, 601-606.

Wu F., Wu H., Zhang G. & Bachir D.M.L., 2004. Differences in growth and yield in response to cadmium toxicity in cotton genotypes. *J. Plant Nutr. Soil Sci.*, **167**, 85-90.

(29 ref.)