DOI: 10.1007/s10535-015-0550-x BIOLOGIA PLANTARUM 59 (4): 701-707, 2015

# Differential gene expression in two contrasting wheat cultivars under cadmium stress

N. KUMARI, P. PARMAR, and V. SHARMA*

*Department of Bioscience and Biotechnology, Banasthali University, P.O. Banasthali Vidyapith, Rajasthan - 304022, India*

## Abstract

The present study investigated differences in cadmium resistance of two wheat (*Triticum aestivum* L.) cultivars. The cvs. RAJ 4161 (Cd resistant) and PBW 343 (Cd sensitive) were treated with 200 mg(Cd) $kg^{-1}$(soil) for 3, 5, 7, and 10 d. The effect of the Cd stress was estimated by measuring growth parameters, accumulation of cadmium, sulphur, and glutathione (GSH), and by expression of some defence genes [phytochelatin synthase (*PCS*), glutathione reductase (*GR*), and ascorbate peroxidase (*APX*)]. The Cd treatment resulted in a significant reduction in plant growth and in an increase in the accumulation of S and GSH. Further, the expressions of *PCS, GR*, and *APX* were also mostly enhanced. The *PCS* was upregulated significantly in roots of RAJ 4161 (0.6-fold) and downregulated (0.9-fold) in PBW 343 on day 3 of the Cd treatment. In RAJ 4161, the expressions of *APX* and *GR* recorded a maximum increase of 2.1- and 2.4-fold in roots and leaves, respectively, after 10 d of the stress. The results show that a different ability of RAJ 4161 and PBW 343 to modulate mRNA expression after the Cd treatment was related to their Cd tolerance.

*Additional keywords*: defence genes, glutathione content, real-time RT-PCR, sulphur, *Triticum aestivum*.

## Introduction

Heavy metal contamination has a large share in the environmental pollution (Chen *et al*. 2007). Cadmium is a non-essential heavy metal and is placed among the top twenty toxins (Morel 2008). Various natural and anthropogenic activities including **s**ewage discharges, phosphate fertilizers, industrial effluents, mining, Ni-Cd batteries, and volcanic eruptions contribute to its release and accumulation in arable lands (McLaughlin and Singh 1999, Yadav 2010). Cadmium exerts an adverse impact on plants, and its toxicity is related to inhibition of photosynthesis, activation or inhibition of many enzymes, disturbances in plant-water relations, and formation of free radicals. All these eventually lead to impaired growth and development (Skórzyńska-Polit *et al*. 2010, Valentovičová *et al*. 2010, Parmar *et al*. 2013). Plants in turn evoke a multitude of biochemical responses, *viz*., an increase in activity of antioxidative enzymes, binding of heavy metals to the cell wall or their accumulation in cell organelles *via* chelating agents, repair of damaged tissues or enzymes, and synthesis of osmolytes to maintain homeostasis (Pereira *et al*. 2002, Castiglione *et al*. 2007, Jozefczak *et al*. 2012).

Phytochelatins (PCs) are heavy metal binding peptides which act as crucial chelating agents sequestering heavy metals into vacuoles thereby rendering them non-toxic (Mishra *et al*. 2006, Yadav 2010). The PCs are synthesized from GSH and related thiols by glutathione γ-glutamylcysteinyl transferase (γ-ECS) commonly called phytochelatin synthase (PCS) (Tomaszewska *et al*. 1996) which is activated by a range of heavy metals including Cd, As, and Pb (Cobbett and Goldsbrough 2002, Clemens *et al*. 2009).

Besides PCs, the ascorbate-glutathione (AsA-GSH) cycle ameliorates oxidative stress caused by Cd exposure (Seth *et al*. 2012), and glutathione reductase (GR) helps in maintaining a GSH content to ensure proper

---

*Submitted* 20 October 2014, *last revision* 5 May 2015, *accepted* 15 June 2015.

*Abbreviations*: APX - ascorbate peroxidase; AsA-GSH - ascorbate-glutathione; Chl - chlorophyll; d.m. - dry mass; f.m. - fresh mass; GR - glutathione reductase; PC - phytochelatin; PCS - phytochelatin synthase; RT-qPCR - reverse transcription quantitative polymerase chain reaction.

*Acknowledgements*: The financial support provided by the Department of Science and Technology (DST), New Delhi, for the project 'Banasthali Centre for Education and Research in Basic Sciences' under the CURIE (Consolidation of University Research for Innovation and Excellence in Women Universities) program is gratefully acknowledged. Pooja Parmar acknowledges the financial support by the Department of Biotechnology (DBT), New Delhi, for providing a senior research fellowship.

* Corresponding author; fax: (+91) 1438 228365, e-mail: vinaysharma30@yahoo.co.uk

functioning of the AsA-GSH cycle (Szarka *et al*. 2012). Thus, AsA-GSH cycle related genes *GR* and *APX* are fundamental to the protection mechanism of plants against Cd stress.

The stress responses of plants vary within plant genera, species, and cultivars (Gaudet *et al*. 2011). Several biotechnological approaches have exploited targeting the overexpression of an *Arabidopsis PCS* (*AtPCS*) gene in tobacco (Pomponi *et al*. 2006) and *Brassica* (Gasic and Korban 2007) resulting in variable responses to Cd stress ranging from its hyperaccumulation to hypersensitivity. However, there is little information on naturally existing variations in wheat cultivars along with spatial differences in plant organs in the Cd defence mechanism. The present study was undertaken to record differences in plant growth, the content of chlorophyll (Chl), Cd, sulphate, and GSH, and expression of candidate genes (*APX*, *GR*, and *PCS*) between two contrasting cultivars: Cd tolerant RAJ 4161 and Cd sensitive PBW 343. They were obtained after screening 11 different wheat cultivars for Cd tolerance.

## Materials and methods

Seeds of two contrasting cultivars of wheat (*Triticum aestivum* L.) RAJ 4161 (Cd tolerant) and PBW 343 (Cd sensitive), which were selected after screening 11 cultivars on the basis of growth parameters and Chl content (Table 1 Suppl.), were procured from the Agricultural Research Station at the Banasthali University, Rajasthan. These cultivars are high yielding and suitable for cultivation in Rajasthan (Sharma 2013). The selected cultivars were released by the Central Variety Research Committee and developed as PBW 343 by the Punjab Agricultural University and as RAJ 4161 by the Swami Keshwanand Rajasthan Agricultural University. The seeds were surface sterilized in 0.1 % (m/v) $HgCl_2$ for 2 min and then washed several times with distilled water and germinated on filter paper placed in Petri plates supplied with 1/5 strength Hoagland's solution (Hoagland and Arnon 1950) in a growth chamber for 5 d. Then, 10 - 12 healthy seedlings were transferred to 15 cm diameter pots containing 200 g of sterilized *Soilrite* and grown under day/night temperatures of 20/18 °C, a relative humidity of 60/70 %, a 16-h photoperiod, and an irradiance of 160 ± 20 μmol $m^{-2}$ $s^{-1}$. The pots were divided into two groups: one group was used as control, and to the other $CdCl_2$ (200 mg $kg^{-1}$) was added.

Root and shoot lengths, and plant dry mass (d.m.) were measured at 3, 5, 7, and 10 d after the Cd treatment. For estimation of photosynthetic pigments, plant material (250 mg) was ground in chilled 80 % (v/v) acetone in the dark. After centrifugation at 10 000 *g* and 4 °C for 10 min, the absorbances of the supernatant were taken at 645, 663, and 700 nm (UV-VIS spectrophotometer, *Shimadzu*, Singapore). The Chl content was calculated using a formula given by Porra *et al*. (1989).

The content of Cd in roots and leaves was determined using an atomic absorption spectrophotometer (*Thermo scientific iCE 3000, Solaar House*, Cambridge, UK), following the method of Issac and Kerber (1971). Samples were washed thoroughly with distilled water and immersed in a 5 mM $CaCl_2$ solution for 5 min followed by washing with deionised water several times and then desorbed and blotted onto a filter paper. The samples were oven-dried at 80 °C for 3 d. The root and leaf samples (100 mg) were taken and ashed in a muffle furnace at 480 °C for 4 h. Then, 2 $cm^3$ of 5 M $HNO_3$ was added to the ashed samples and these were evaporated to dryness on a sand bath at 230 °C. Thereafter, the samples were placed in the furnace and heated to 400 °C for 15 min. Next, a 2 $cm^3$ of concentrated HCl was added and the solution was filtered through *Whatman No. 42* filter paper. Then, the volume of the acidified samples was made to 20 $cm^3$ using deionised water and absorbance was read. A standard curve of $CdCl_2$ (*Merck Biosciences*, Darmstadt, Germany) from 25 to 150 μg $cm^{-3}$ was used for calculation.

The content of S was determined by the method of Bhargava and Raghupati (1993). To 10 $cm^3$ of the plant digest, 1 $cm^3$ of 0.5 % (m/v) gum acacia and 1 $cm^3$ of 6 M HCl were added and mixed thoroughly followed by addition of $BaCl_2$ (0.5 g) and then allowed to stand for 1 min. The contents were then gently swirled and absorbance was taken at 420 nm. The glutathione content was estimated following the method of Anderson (1985). Fresh plant samples (0.3 g) were homogenized in 3 $cm^3$ of 5 % (m/v) sulfosalicylic acid at 4 °C. The homogenate was centrifuged at 10 000 *g* for 10 min. To 0.5 $cm^3$ of the supernatant, 0.5 $cm^3$ of a 0.1 M reaction buffer [0.5 mM $Na_2EDTA$ and 50 $mm^3$ of 3 mM 5´dithio-bis-(2-nitrobenzoic acid); pH 7.0] was added. After 5 min, absorbance was read at 412 nm.

Gene expressions in various Cd treated plant samples were detected based on a qRT-PCR using specific primers. For this, total RNA was extracted from roots and leaves (250 mg each) using a *TRIzol* reagent (*Life Technologies,* Carlsbad, CA, USA) according to the manufacturer's instructions. The total RNA was then diluted using nuclease free water and quantified by measuring absorbance at 260 nm using the formula: concentration [μg $cm^{-3}$] = $A_{260} \times 40 \times$ dilution factor. The purity of the RNA was checked by calculating an absorbance ratio at 260 and 280 nm ($A_{260}/A_{280} > 1.8$). The cDNA was prepared using a *M-MuLV* reverse transcriptase kit (*Merck Genei*, Bangalore, India). Total RNA (5 μg) was treated with *Dnase I* (*Merck Genei*, Bangalore, India) at 25 °C for 15 min and then at 75 °C for 5 min. Then, 3 μg of the total RNA was used for reverse transcription and a volume was made up to 9 $mm^3$ using nuclease free water. A random hexamer (2 $mm^3$)

was added and spun down at 1 000 rpm and incubated at 65 °C for 10 min followed by incubation at room temperature for 2 min. Different reagents were added in an indicated order as follows: 1 $mm^3$ of an RNase inhibitor, 1 $mm^3$ of 100 mM dithiotreitol, 4 $mm^3$ of a 5× assay buffer, 2 $mm^3$ of a 10 mM *dNTP* mix, 1 $mm^3$ of *M-MuLV* reverse transcriptase and 1 $mm^3$ of nuclease free water; mixed gently and spun slowly at 1000 rpm for 15 s. The tubes were incubated at 37 °C for 60 min. The reaction was terminated by heating at 70 °C for 15 min.

Primer pairs specific to *Triticum aestivum GR* (*TIGR* database accession number TC250996), *APX* (*NCBI* acc. No. AF093752.1), and *PCS* (*NCBI* acc. No. EF555121.1) cDNA (sense primer 5′-ACCTGTTGCTCTGATGGA GG-3' and antisense primer 5′-TCGGTATCAGCATCA ACCAC-3'; sense primer 5′-TTGGGCTGTTGGAGA TGTTA-3′ and antisense primer 5′-CTCTGGTTTGGT CGGTTCAT-3′; sense primer 5′CTACTGATAAGG CATTGTTGGAT-3′and antisense primer 5′-TGATCC GTGGTGTGAAGC-3′, respectively) were used. Primers for wheat *18s rRNA* (TIGR database accession number TC250996) gene cDNA (sense primer, 5′-GGCTAC CACATCCAAGGAA-3′ and antisense primer, 5′-CTA TTGGAGCTGGAATTACCG-3′) were used as internal control (Nicot *et al.* 2005). All the qRT-PCR experiments were conducted in three replicates and repeated twice. The expressions of *PCS*, *GR*, and *APX* were determined following the method of Rivera-Becerril *et al*. (2002) at various time intervals (3, 5, 7, and 10 d) after the Cd treatment. For the qRT-PCR, 2 $mm^3$ of 1:10 diluted cDNA was mixed with a *SYBR Green PCR* master mix (*Qiagen Gmbh*, Hilden, Germany), 5 pmol of a forward primer and 5 pmol of a reverse primer in a final volume of 20 $mm^3$. Template controls were analyzed for all genes. The PCR was performed using a *Rotor Gene Q Real-Time* system (*Qiagen Gmbh*, Hilden, Germany) with the following conditions: an initial activation step at 94 °C for 4 min, denaturation at 94 °C for 15 s, annealing at 58 °C for 15 s, and extension at 70 °C for 20 s. Melt curve analysis of the PCR product was carried out at 72 °C for 1 min and ramped from 75 to 95 °C with a rise by 1 °C every 5 s. The specificity of the reaction was confirmed by a melt curve and gel electrophoresis. Relative gene expressions were calculated in terms of fold changes using the ΔCt method.

The results are expressed as arithmetic means and standard deviations of six replicates. All experimental data were analyzed by *SPSS v. 16*. Two way interactions were performed between the cultivars and Cd concentrations using a generalized linear model. Significant differences of the measured parameters were analyzed using Tukey's post hoc multiple-comparison test.

## Results

After the Cd treatment (200 mg $kg^{-1}$), growth inhibition was significantly pronounced in roots and shoots of PBW 343 and progressively increased with time (Table 1). The inhibition in the roots and shoots was 11 and 13 % in RAJ 4161 whereas 38 and 41 % in PBW 343 after 10 d of the Cd treatment. In PBW 343, slight browning and branching roots were observed at longer durations of the stress treatment (7 d and 10 d). The content of Chl *a* decreased upon the Cd treatment in both the cultivars, however, merely 17 % in RAJ 4161 but

Table 1. Effects of Cd on plant growth parameters at 3, 5, 7, and 10 d after treatment with 200 mg(Cd) $kg^{-1}$(soil) in wheat cvs. RAJ 4161 and PBW 343. Means ± SD, $n = 6$. Values followed by different letters are significantly different ($P < 0.05$) according to Tukey's test.

| Parameter | Cultivar | Cd [mg $kg^{-1}$] | 3 d | 5 d | 7 d | 10 d |
|---|---|---|---|---|---|---|
| Root length [cm] | PBW 343 | 0 | 17.5 ± 0.90a | 18.9 ± 0.42ab | 20.0 ± 0.34ab | 20.3 ± 0.47c |
| | | 200 | 7.4 ± 0.11a | 11.5 ± 0.18b | 11.3 ± 0.38d | 12.7 ± 0.91c |
| | RAJ 4161 | 0 | 17.1 ± 0.57a | 19.7 ± 0.28b | 21.5 ± 1.02b | 22.6 ± 0.79b |
| | | 200 | 16.3 ± 0.07a | 17.6 ± 0.92b | 19.8 ± 0.54b | 20.1 ± 0.65c |
| Shoot length [cm] | PBW 343 | 0 | 11.8 ± 0.95a | 14.4 ± 0.22b | 18.2 ± 0.72c | 24.4 ± 0.39d |
| | | 200 | 7.2 ± 0.05a | 8.7 ± 0.56a | 12.7 ± 0.77b | 14.4 ± 0.54c |
| | RAJ 4161 | 0 | 14.2 ± 0.39a | 15.6 ± 0.90a | 25.1 ± 0.44b | 26.8 ± 0.25c |
| | | 200 | 13.6 ± 0.13a | 13.2 ± 0.05b | 20.4 ± 1.52c | 23.2 ± 0.74d |
| Root d.m. [g $plant^{-1}$] | PBW 343 | 0 | 0.005 ± 0.001a | 0.006 ± 0.001a | 0.006 ± 0.001a | 0.007 ± 0.001b |
| | | 200 | 0.004 ± 0.001a | 0.004 ± 0.001a | 0.006 ± 0.002b | 0.006 ± 0.001b |
| | RAJ 4161 | 0 | 0.007 ± 0.001a | 0.007 ± 0.001a | 0.007 ± 0.001a | 0.007 ± 0.001a |
| | | 200 | 0.005 ± 0.001a | 0.006 ± 0.001a | 0.006 ± 0.001a | 0.006 ± 0.001a |
| Shoot d.m. [g $plant^{-1}$] | PBW 343 | 0 | 0.013 ± 0.001a | 0.016 ± 0.002b | 0.016 ± 0.002b | 0.021 ± 0.003c |
| | | 200 | 0.006 ± 0.001a | 0.012 ± 0.003b | 0.014 ± 0.001c | 0.015 ± 0.001c |
| | RAJ 4161 | 0 | 0.014 ± 0.002a | 0.016 ± 0.003b | 0.018 ± 0.002c | 0.017 ± 0.002d |
| | | 200 | 0.015 ± 0.001a | 0.016 ± 0.002b | 0.016 ± 0.001b | 0.017 ± 0.002c |

Table 2. Effects of Cd on chlorophyll (Chl) *a*, Chl *b*, and Chl *a*/*b* ratio at 3, 5, 7, and 10 d after treatment with 200 mg(Cd) $kg^{-1}$(soil) in wheat cvs. RAJ 4161 and PBW 343. Means ± SD, $n = 6$. Values followed by different letters are significantly different at $P < 0.05$ according to Tukey's test.

| Parameter | Cultivar | Cd [mg $kg^{-1}$] | 3 d | 5 d | 7 d | 10 d |
|---|---|---|---|---|---|---|
| Chl *a* | PBW 343 | 0 | 1.5 ± 0.10a | 1.7 ± 0.12a | 1.8 ± 0.14ab | 1.8 ± 0.12c |
| [mg $g^{-1}$(f.m)] | | 200 | 1.1 ± 0.20a | 1.2 ± 0.16b | 1.2 ± 0.23b | 1.1 ± 0.18a |
| | RAJ 4161 | 0 | 1.8 ± 0.04a | 1.7 ± 0.03ab | 1.8 ± 0.16ab | 2.0 ± 0.23c |
| | | 200 | 1.7 ± 0.25a | 1.6 ± 0.18a | 1.7 ± 0.17a | 1.6 ± 0.08a |
| Chl *b* | PBW 343 | 0 | 0.3 ± 0.01a | 0.4 ± 0.03b | 0.4 ± 0.03b | 0.4 ± 0.00b |
| [mg $g^{-1}$(f.m)] | | 200 | 0.2 ± 0.04a | 0.3 ± 0.02a | 0.3 ± 0.01a | 0.3 ± 0.03a |
| | RAJ 4161 | 0 | 0.4 ± 0.08a | 0.3 ± 0.04a | 0.3 ± 0.05a | 0.4 ± 0.05b |
| | | 200 | 0.4 ± 0.01a | 0.3 ± 0.03a | 0.3 ± 0.04a | 0.3 ± 0.05a |
| Chl *a*/*b* | PBW 343 | 0 | 5.2 | 4.3 | 4.2 | 4.5 |
| | | 200 | 4.5 | 4.4 | 3.8 | 3.7 |
| | RAJ 4161 | 0 | 5.1 | 5.2 | 5.9 | 5.6 |
| | | 200 | 5.4 | 5.7 | 5.9 | 4.9 |

significantly (42 %) in PBW 343 after 10 d of the stress (Table 2). The ratio of Chl *a*/*b* decreased from 3 d to 10 d of the Cd treatment in both the cultivars: from 5.4 to 4.9 in RAJ 4161, and from 4.5 to 3.7 in PBW 343. This suggests a great damage to Chl *a*.

Uptake of Cd in wheat was cultivar dependent and a higher accumulation was recorded in PBW 343 than in RAJ 4161 (Table 3). The content of Cd in roots and leaves of PBW 343 was 201.3 and 35.6 μg $g^{-1}$(d.m.) as compared to 127.7 and 10.5 μg $g^{-1}$(d.m.) in RAJ 4161 after 10 d of the Cd stress.

The content of S also increased upon the Cd treatment in roots and leaves of both the cultivars. The increase was more pronounced in roots: cv. RAJ 4161 recorded an

Table 3. The content of GSH, cadmium, and sulphur in roots and leaves of wheat cvs. PBW 343 and RAJ 4161 at 3, 5, 7, and 10 d after treatment with 200 mg(Cd) $kg^{-1}$(soil). Means ± SD, $n = 6$. Values followed by different letters are significantly different at $P < 0.05$ according to Tukey's test.

| Parameter | Cultivar | Organ | Cd [mg $kg^{-1}$] | 3 d | 5 d | 7 d | 10 d |
|---|---|---|---|---|---|---|---|
| GSH | PBW 343 | root | 0 | 1.5 ± 0.12a | 2.2 ± 0.84b | 2.9 ± 0.02b | 2.1 ± 0.81c |
| [μg $g^{-1}$(f.m.)] | | | 200 | 1.9 ± 0.12a | 2.5 ± 0.08bc | 2.9 ± 0.71c | 2.4 ± 0.18ab |
| | | leaf | 0 | 3.0 ± 0.05a | 3.2 ± 0.08a | 3.2 ± 0.07a | 3.7 ± 0.08b |
| | | | 200 | 3.4 ± 0.05a | 3.7 ± 0.13b | 3.9 ± 0.05b | 4.1 ± 0.08b |
| | RAJ 4161 | root | 0 | 1.7 ± 0.07a | 1.9 ± 0.10ab | 2.0 ± 0.08ab | 2.1 ± 0.06c |
| | | | 200 | 2.5 ± 0.11a | 2.9 ± 0.21b | 3.2 ± 0.22b | 3.5 ± 0.01c |
| | | leaf | 0 | 3.5 ± 0.35a | 3.6 ± 0.87a | 3.7 ± 0.07a | 3.8 ± 0.18a |
| | | | 200 | 3.9 ± 0.08a | 4.2 ± 0.19ab | 4.4 ± 0.11bc | 4.6 ± 0.33c |
| Cadmium | PBW 343 | root | 0 | 0.3 ± 0.41a | 1.8 ± 0.82a | 1.9 ± 0.21a | 1.8 ± 0.67a |
| [μg $g^{-1}$(d.m.)] | | | 200 | 35.0 ± 9.09a | 100.8 ± 3.12b | 153.3 ± 2.07c | 201.0 ± 2.76d |
| | | leaf | 0 | 0.1 ± 0.03a | 0.3 ± 0.30a | 0.3 ± 0.25a | 0.7 ± 0.78a |
| | | | 200 | 5.9 ± 0.26a | 18.9 ± 0.85b | 26.6 ± 3.12c | 35.6 ± 4.59d |
| | RAJ 4161 | root | 0 | 0.1 ± 0.32a | 0.7 ± 0.53a | 0.8 ± 0.21a | 0.9 ± 0.31a |
| | | | 200 | 5.8 ± 4.13a | 63.3 ± 7.28b | 92.5 ± 8.66c | 127.9 ± 6.90c |
| | | leaf | 0 | 0.1 ± 0.20a | 0.2 ± 0.19a | 0.6 ± 0.22a | 0.6 ± 0.35a |
| | | | 200 | 0.8 ± 0.15a | 7.4 ± 0.8b | 9.7 ± 2.76b | 10.6 ± 2.13b |
| Sulphur | PBW 343 | root | 0 | 2601 ± 14.6a | 2649 ± 32.9a | 2777 ± 43.5b | 2865 ± 87.5b |
| [μg $g^{-1}$(d.m.)] | | | 200 | 2630 ± 6.9a | 2703 ± 21.7a | 2939 ± 26.2b | 3102 ± 54.9c |
| | | leaf | 0 | 1411 ± 14.6a | 1678 ± 33.9a | 1796 ± 13.3a | 1823 ± 67.8a |
| | | | 200 | 1420 ± 9.8a | 1833 ± 24.6b | 1920 ± 28.9b | 2008 ± 32.3b |
| | RAJ 4161 | root | 0 | 2617 ± 11.4a | 2681 ± 13.8a | 2802 ± 18.3a | 2996 ± 78.3b |
| | | | 200 | 2979 ± 23.2a | 3070 ± 10.6a | 3123 ± 22.8a | 3547 ± 36.4b |
| | | leaf | 0 | 1579 ± 21.4a | 1692 ± 35.2a | 1892 ± 17.9ab | 2102 ± 56.5b |
| | | | 200 | 1653 ± 9.6a | 1841 ± 20.2ab | 2027 ± 10.6b | 2397 ± 54.9c |

18 % increase as compared to 8 % in PBW 343 after 10 d of the stress (Table 3). The GSH content displayed marked differences in roots and increased by 26 and 40 % in PBW 343 and RAJ 4161, respectively, during the course of experiment. However in leaves, a rise in GSH content was similar in both the cultivars (Table 3).

The expressions of *GR, PCS*, and *APX* genes increased more in both roots and leaves of RAJ 4161 than of PBW 343. The expression of the *PCS* gene was induced even at the first sampling time (3 d after the Cd treatment) in RAJ 4161 whereas it was downregulated in PBW 343. Thereafter, a marked increase of 2.8- and 0.7-fold was observed for RAJ 4161 as compared to 0.7- and 0.3-fold in PBW 343 in roots and leaves, respectively, after 7 d of the stress (Table 4).

The expression of *GR* also displayed upregulation of 0.4-fold in RAJ 4161 and 0.1-fold in PBW 343 roots after 3 d of the stress (Table 5). Later, the expression increased to 3.1-fold in RAJ 4161 and 1.1-fold in PBW 343 roots after 10 d of the Cd treatment. In contrast, the expression pattern of *GR* in leaves was similar in both the cultivars, a 2.1-fold increase in RAJ 4161 and PBW 343 after 10 d of the treatment (Table 4).

The expression of *APX* in roots and leaves increased upon the Cd treatment. The *APX* expression in roots and leaves of RAJ 4161 was 2.5-fold; however in PBW 343, it was 0.6- and 1.3-fold after 10 d of the stress (Table 4).

Table 4. Expressions (log fold changes) of *PCS*, *GR* and *APX* genes in roots and leaves of wheat cvs. RAJ 4161 and PBW 343 at 3, 5, 7, and 10 d after treatment with 200 mg(Cd) $kg^{-1}$(soil). Means ± SD, $n = 6$. Values followed by different letters are significantly different at $P < 0.05$. Fold change = $2^{-\Delta(\Delta Ct)}$, where $\Delta Ct_{treated}$ = Ct(target) - Ct(normalizer), $\Delta Ct_{control}$ = Ct(target) - Ct(normalizer), $\Delta(\Delta Ct) = \Delta Ct(treated) - \Delta Ct(control)$. Log fold change = log $\Delta(\Delta Ct)$.

| Gene | Cultivar | Root | | | | Leaf | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 d | 5 d | 7 d | 10 d | 3 d | 5 d | 7 d | 10 d |
| *PCS* | PBW 343 | -0.9 ± 0.23a | 0.4 ± 0.11ab | 0.7 ± 0.26ab | 0.5 ± 0.15b | -0.80 ± 0.25a | 0.2 ± 0.09c | 0.3 ± 0.09b | -0.2 ± 0.05b |
| | RAJ 4161 | 0.6 ± 0.09a | 2.3 ± 0.32d | 2.8 ± 0.32b | 1.6 ± 0.23c | 0.04 ± 0.01a | 0.2 ± 0.08b | 0.5 ± 0.21c | 1.1 ± 0.17d |
| *GR* | PBW 343 | 0.1 ± 0.04a | 0.1 ± 0.02a | 0.4 ± 0.60a | 1.1 ± 0.12b | 0.03 ± 0.01a | 0.5 ± 0.26b | 1.6 ± 0.09c | 2.1 ± 0.05d |
| | RAJ 4161 | 0.4 ± 0.11a | 2.4 ± 0.16b | 2.5 ± 0.91b | 3.1 ± 0.14b | 0.10 ± 0.01a | 0.5 ± 0.23b | 1.0 ± 0.16c | 2.1 ± 0.24d |
| *APX* | PBW 343 | 0.1 ± 0.03a | 0.5 ± 0.09ab | 0.5 ± 0.01ab | 0.7 ± 0.07b | 0.02 ± 0.00a | 0.3 ± 0.03b | 0.6 ± 0.03c | 1.3 ± 0.11d |
| | RAJ 4161 | 0.5 ± 0.09a | 0.9 ± 0.12b | 1.5 ± 0.06c | 2.5 ± 0.13d | 0.40 ± 0.03a | 1.1 ± 0.06b | 2.1 ± 0.19c | 2.4 ± 0.29d |

## Discussion

Intraspecific differences in wheat provide a basis for unravelling the responses of Cd defence genes. Decreases in root and shoot lengths and plant dry mass were observed in the Cd treated plants as compared to the control plants. The Cd exposed plants of cv. PBW 343 were more severely affected than those of cv. RAJ 4161. The decrease in plant growth was proportional to the uptake of Cd. In the Cd treated plants, significant differences in Cd accumulation were observed in the two cultivars. It is concluded that the Cd accumulation had a distinct correlation with the reduction in the growth of *T. aestivum*. The results are in accordance with other studies with *Lepidium sativum, Brassica juncea*, and *Lycopersicon esculentum* (Gill *et al*. 2011, 2012, Gratão *et al*. 2012). Reduction in root growth appears to be due to Cd mediated cell damage, inhibition of mitosis, restricted cell wall synthesis and deposition of lignin in the cell wall (Pál *et al*. 2006). Chl destruction and inhibition of its biosynthesis have been known to be prime causes in leaf chlorosis in plants growing in Cd treated soils (Xue *et al*. 2013). In our study, 10 d of the Cd treatment caused the reduction of Chl *a* content in cv. PBW 343 whereas there was less decrease in Chl *a* content in tolerant cv. RAJ 4161.

In the current study, the increases in the content of S and GSH were observed in both the cultivars. A probable reason for this could be that the activity of an S assimilation pathway enzyme, ATP sulphurylase, increased after the Cd treatment (Herbette *et al*. 2006). The results of the current study are in agreement with the investigations of Bashir *et al*. (2013) who reported that Cd treated *Arabidopsis* plants show a higher content of S upon induction of ATP sulphurylase. The GSH content was higher in tolerant cv. RAJ 4161 in both roots and leaves as compared to cv. PBW 343. An increase in GSH has been observed in various plants such as *Brassica juncea* (Ferreira *et al.* 2002) and soybean (Mendoza-Cózatl *et al.* 2008). The results indicate that the regulation of GSH-related pathways was effective in imparting Cd tolerance to RAJ 4161.

The PCS aids in chelation of Cd by root tissue, and GR and APX help in ROS scavenging in leaves. The *PCS* gene expression was noticeably higher in cv. RAJ 4161 upon the Cd treatment as compared to cv. PBW 343. Some other reports have postulated that a higher expression of *PCS* is related to Cd tolerance (He *et al*. 2005, Li *et al*. 2009). The transcriptional up regulation pattern confirms previous findings about Cd immobilization in plant vacuoles (Cobbett 2000, Hirata *et al.*

2005). Despite a higher expression of *PCS* in the tolerant cultivar, the results do not show a higher accumulation of Cd in roots and its possible subsequent translocation to leaves in RAJ 4161. It can be inferred some excluder strategy in RAJ 4161. The expression of a transporter gene (*TaTM 20*) in wheat is related to a lesser accumulation of Cd (Kim *et al.* 2008). In addition, upregulation of some other transporter genes (pleiotropic drug resistance and multidrug and toxin extrusion) was observed, suggesting its involvement in the export of Cd from rice plants (Ogawa *et al.* 2009*)*.

Our data demonstrate that, as result of Cd treatment, the transcriptions of *GR* and *APX* were significantly enhanced in roots and leaves of wheat at all sampling times. Moreover, these genes were expressed more in the resistant cultivar. The expression patterns of both the genes suggest that they played a vital role in Cd defence. The high expression of *APX* even at 3 d of the Cd treatment suggests that its up regulation is among the most efficient defences (Gill and Tuteja 2010). Even in sensitive cv. PBW 343, the expressions of *APX* and *GR* in leaves were at par to the values obtained in RAJ 4161. Increased *GR* transcription has also been reported in response of *Camellia sinensis* to Cd stress (Mohanpuria *et al*. 2007). Luo *et al*. (2011) observed a higher expression of *APX* and *GR* in *Lolium perenne* upon Cd treatment.

In conclusion, the Cd tolerance exhibited by RAJ 4161 could be the result of increased expressions of the *PCS*, *GR* and *APX* genes and the tendency to accumulate lesser Cd in the plant. The plant growth parameters and gene expression patterns were found to be variable and stress duration dependent. The study also suggests that PCS and GR might play defence functions in roots whereas APX and GR in leaves.

## References


Anderson, M.E.: Determination of glutathione and glutathione disulfide in biological samples. - Methods Enzymol. **113**: 548-555, 1985.

Bashir, H., Ahmad, J., Bagheri, R., Nauman, M., Qureshi, M.I.: Limited sulfur resource forces *Arabidopsis thaliana* to shift towards non-sulfur tolerance under cadmium stress. - Environ. exp. Bot. **94**: 19-32, 2013.

Bhargava, B.S., Raghupathi, H.B.: Analysis of plant material for macro and micro nutrients. - In Tandon, H.L.S. (ed.): Methods of Analysis of Soils, Plant, Waters and Fertilizers. Pp. 49-82. FDCO, New Delhi 1993.

Castiglione, S., Franchin, C., Fossati, T., Lingua, G., Torrigiani, P., Biondi, S.: High zinc concentrations reduce rooting capacity and alter metallothioneins gene expression in white poplar (*Populus alba* L. cv. Villafranca). - Chemosphere **67**: 1117-1126, 2007.

Chen, F., Dong, J., Wang, F., Wu, F., Zhang, G., Li, G., Zheng, C., Jingxing, C., Wei, K.: Identification of barley genotypes with low grain Cd accumulation and its interaction with four microelements. - Chemosphere **67**: 2082-2088, 2007.

Clemens, S., Persoh, D.: Multi-tasking phytochelatin synthases. - Plant Sci. **177**: 266-271, 2009.

Cobbett, C., Goldsbrough, P.: Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. - Annu. Rev. Plant Biol. **53**: 159-182, 2002.

Cobbett, C.S.: Phytochelatins and their roles in heavy metal detoxification. - Plant Physiol. **123**: 825-832, 2000.

Ferreira, R.R., Fornazier, R.F., Vitória, A.P., Lea, P.J., Azevedo, R.A.: Changes in antioxidant enzyme activities in soybean under cadmium stress. - J. Plant Nutr. **25**: 327-342, 2002.

Gasic, K., Korban, S.S.: Transgenic Indian mustard (*Brassica juncea*) plants expressing an *Arabidopsis* phytochelatin synthase (AtPCS1) exhibit enhanced As and Cd tolerance. - Plant mol. Biol. **64**: 361-369, 2007.

Gaudet, M., Pietrini, F., Beritognolo, I., Iori, V., Zacchini, M., Massacci, A., Mugnozza, G.S., Sabatti, M.: Intraspecific variation of physiological and molecular response to cadmium stress in *Populus nigra* L. - Tree Physiol. **31**: 1309-1318, 2011.

Gill, S.S., Khan, N.A., Tuteja, N.: Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). - Plant Sci. **182**: 112-120, 2012.

Gill, S.S., Khan, N.A., Tuteja, N.: Differential cadmium stress tolerance in five indian mustard (*Brassica juncea* L.) cultivars. An evaluation of the role of antioxidant machinery. - Plant Signal. Behav. **6**: 293-300, 2011.

Gill, S.S, Tuteja, N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. - Plant Physiol. Biochem. **48**: 909-930, 2010.

Gratão, P.L., Monteiro, C.C., Carvalho, R.F., Tezotto, T., Piotto, F.A., Peres, L.E.P., Azevedo, R.A.: Biochemical dissection of diageotropica and never ripe tomato mutants to Cd-stressful conditions. - Plant Physiol. Biochem. **56**: 79-96, 2012.

He, Z., Li, J., Zhang, H., Ma. M.: Different effects of calcium and lanthanum on the expression of phytochelatin synthase gene and cadmium absorption in *Lactuca sativa*. - Plant Sci. **168**: 309-318, 2005.

Herbette, S., Taconnat, L., Hugouvieux, V., Piette, L., Magniette, M.L.M., Cuine, S., *et al*.: Genome-wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. - Biochimie **88**: 1751-1765, 2006.

Hirata, K., Tsuji, N., Miyamoto, K.: Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. - J. Biosci. Bioeng. **100**: 593-599, 2005.

Hoagland, D.R., Arnon, D.I.: The water culture method for growing plants without soil. - Calif. agr. exp. Station Circular **347**: 1-32, 1950.

Issac, R.A., Kerber, J.D.: Atomic absorption and flame photometry: techniques and uses in soil, plant and water analysis. - In: Walsh, L.M. (ed.): Instrumental Methods for Analysis of Soils and Plant Tissue. Pp. 17-37. Soil Science Society of America, Madison 1971.

Jozefczak, M., Remans, T., Vangronsveld, J., Cuypers, A.: Glutathione is a key player in metal-induced oxidative stress defences. - Int. J. mol. Sci. **13**: 3145-3175, 2012.

Kim, Y.Y., Kim, D.Y., Shim, D., Song, W.Y., Lee, J., Schroeder, J.I., Kim, S., Moran, N., Lee, Y.: Expression of

the novel wheat gene TM20 confers enhanced cadmium tolerance to bakers' yeast. - J. biol. Chem. **283**: 15893-15902, 2008.

Li, A.M., Yu, B.Y., Chen, F.H., Gan, H.Y., Yuan, J.G., Qiu, R., Huang, J.C., Yang, Z.Y., Z.F. Xu.: Characterization of the *Sesbania rostrata* phytochelatin synthase gene: Alternative splicing and function of four isoforms. - Int. J. mol. Sci. **10**: 3269-3282, 2009.

Luo, H., Li, H., Zhang, X., Fu, J.: Antioxidant responses and gene expression in perennial ryegrass (*Lolium perenne* L.) under Cd stress. - Ecotoxicology **20**: 770-778, 2011.

McLaughlin, M.J., Singh, B.R.: Cadmium in soil and plants: a global perspective. - In: McLaughlin, M.J., Singh, B.R. (ed.): Cadmium in Soils and Plants. Vol. 85. Pp. 1-9. Kluwer Academic Publishers, Dordrecht 1999.

Mendoza-Cózatl, D.G., Butko, E., Springer, F., Torpey, J.W., Komives, E.A., Kehr, J., Schroeder, J.I.: Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. - Plant J. **54**: 249-259, 2008.

Mishra, S., Srivastava, S., Tripathi, R.D., Govindarajan, R., Kuriakose, S.V., Prasad, M.N.V.: Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. - Plant Physiol. Biochem. **44**: 25-37, 2006.

Mohanpuria, P., Rana, N.K., Yadav, S.K.: Cadmium induced oxidative stress influence on glutathione metabolic genes of *Camellia sinensis* (L.) O. Kuntze. - Environ. Toxicol. **22**: 368-374, 2007.

Morel, F.M.M.: The co-evolution of phytoplankton and trace element cycles in the oceans. - Geobiology **6**: 318-324, 2008.

Nicot, N., Hausman, J.F., Hoffmann, L., Eves, D.: Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. - J. exp. Bot. **56**: 2907-2914, 2005.

Ogawa, I., Nakanishi, H., Mori, S., Nishizawa, N.K.: Time course analysis of gene regulation under cadmium stress in rice. - Plant Soil **325**: 97-108, 2009.

Pál, M., Horváth, E., Janda, T., Páldi, E., Szalai, G.: Physiological changes and defense mechanisms induced by cadmium stress in maize. - J. Plant Nutr. Soil Sci. **169**: 239-246, 2006.

Parmar, P., Kumari, N., Sharma, V.: Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. - Bot. Stud. **54**: 45-50, 2013.

Pereira, G.J.G., Molina, S.M.G., Lea, P. J., Azevedo, R.A.: Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. - Plant Soil **239**: 123-132, 2002.

Pomponi, M., Censi, V., Girolamo, V.D., Paolis, D.A., Di Toppi, L.S., Aromolo, R., Costantino, P., Cardarelli, M.: Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances $Cd^{2+}$ tolerance and accumulation but not translocation to the shoot. - Planta **223**: 180-190, 2006.

Porra, R.J., Thompson, W.A., Kriedemann, P.E.: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of concentration of chlorophyll standards by atomic absorption spectroscopy. - Biochim. biophys. Acta **975**: 384-394, 1989.

Rivera-Becerril, F., Calantzis, C., Turnau, K., Caussanel, J.P., Belismov, A.A., Gianinazzi, S., Strasser, J.R., Gianinazzi-Pearson, V.: Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. - J. exp. Bot. **53**: 1177-1185, 2002.

Seth, C.S., Remans, T., Keunen, E., Jozefczak, M., Gielen, H., Opdenakker, K., Weyens, N., Vangronsveld, J., Cupers, A.: Phytoextraction of toxic metals: a central role for glutathione. - Plant Cell Environ. **35**: 334-346, 2012.

Sharma, N.K.: Suitable varieties and agro-chemicals for yield optimization of wheat in arid Rajasthan. - J. Progr. Agr. **4**: 124-127, 2013

Skórzyńska-Polit, E., Drążkiewicz, M., Krupa, Z.: Lipid peroxidation and antioxidative response in *Arabidopsis thaliana* exposed to cadmium and copper. - Acta Physiol. Plant. **32**: 169-175, 2010.

Szarka, A., Tomasskovics, B., Bánhegyi, G.: The ascorbate-glutathione-α-tocopherol triad in abiotic stress response. - Int. J. mol. Sci. **13**: 4458-4483, 2012.

Tomaszewska, B., Tukendorf, A., Baralkiewicz, D.: The synthesis of phytochelatins in lupin roots treated with lead ions. - Sci. Legumes **3**: 206-217, 1996.

Valentovičová, K., Halušková, L., Huttová, J., Mistrík, I., Tamás, L.: Effect of cadmium on diaphorase activity and nitric oxide production in barley root tips. - J. Plant Physiol. **167**: 10-14, 2010.

Xue, X.C., Gao, H.Y., Zhang, L.T.: Effects of cadmium on growth, photosynthetic rate and chlorophyll content in leaves of soybean seedlings. - Biol. Plant. **57**: 587-590, 2013.

Yadav, S.K.: Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. - S. Afr. J. Bot. **76**: 167-179, 2010.