

## Deteriorative Effects of Cadmium Stress on Antioxidant System and Cellular Structure in Germinating Seeds of *Brassica napus* L.

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### ABSTRACT

Cadmium (Cd), known as a non-essential heavy metal, can cause oxidative stress in plants. In this study, an attempt was carried out to find out whether Cd-induced oxidative and microscopic changes could be observed in the early stage of seedling growth. Seeds of *Brassica napus* (cvs. Zheda 619 and ZS 758) were germinated *in vitro* at 0, 100, 200, 300, and 500  $\mu\text{M}$  Cd concentrations in dark for 48 hours. Results showed that the higher concentration of Cd (500  $\mu\text{M}$ ) significantly reduced plant growth in both cultivars. However, Cd concentration in both cultivars increased linearly with the Cd concentration, but was more pronounced in ZS 758 than the other cultivar. Moreover, Cd caused oxidative stress in germinated seeds by increasing reactive oxygen species, however, no relationship was found between antioxidative defense capacity including catalase, peroxidase, superoxide dismutase and glutathione reductase activities and Cd tolerance in the two cultivars. Cd toxicity showed significant ultrastructural changes in germinated seed cells, but cell structure in Zheda 619 did not show any difference, while cell structure in ZS 758 was totally damaged under higher concentration of Cd (500  $\mu\text{M}$ ). Based on the present findings, it can be concluded that cultivar Zheda 619 showed comparatively higher tolerance and integrity of cell organelles under Cd stress.

**Keywords:** Defense system, Malondialdehyde, Microscopic analysis, Oilseed rape, Phytoremediation.

### INTRODUCTION

Heavy metal intoxications, especially toxicity caused by lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) constitute serious threat to human health (Wenneberg, 1994). However, anthropogenic inputs associated with agricultural practices, mineral exploration, industrial processes and solid waste management play a significant role in contamination of terrestrial ecosystem through heavy metals (Alumaa *et al.*, 2002; Nazar *et al.*, 2012). Cd is one of

those heavy metals which can cause serious toxicity in human body and has a half-life of 10 to 30 years. Therefore, it has the ability to stay for a long time in the environment and cause toxicity (Jarup *et al.*, 1998; Prabu *et al.*, 2011). Cd is also toxic for plant; when entering into the plant body, it reduces the photosynthetic activity, chlorophyll content, plant growth, and induces oxidative stress (Zhou *et al.*, 2003; Masood *et al.*, 2012; Latef, 2013).

Reactive oxygen species (ROS) including the superoxide radical ( $\text{O}_2^-$ ) and hydrogen

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peroxide ( $H_2O_2$ ), which are inevitably generated via a number of metabolic pathways (Kanazawa *et al.*, 2000), are constant risk for organisms with aerobic metabolism. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids of membrane, is considered as one of the reliable indicators of oxidative stress (Demiral and Turkan, 2005). Moreover, plant cells are equipped with enzymatic mechanisms to eliminate or reduce the oxidative damage. These protective enzymes include SOD, CAT, APX, GPX and GR (Larson, 1988; Singh and Agrawal, 2013). SOD works as the first line of defense, converting superoxide radical to hydrogen peroxide ( $H_2O_2$ ), which is then reduced to water and oxygen either by APX in ascorbate-glutathione cycle or by GPX and CAT in cytoplasm and other cellular compartments. GR is another important enzymatic constituent of this orchestrated antioxidative defense system that helps in maintaining a high GSH/GSSG ratio (Asada, 1994).

In plants, anatomical and structural changes are known to be some of the worst effects of Cd (Vitoria, 2004). Previously, Shah and Dubey (1995) observed changes such as the occurrence of low mitotic index and pycnosis, cell division and cell proliferation, chromosomal aberrations, alteration in the synthesis of RNA, and slowing down of ribo-nuclease activity in various crops. The use of metal-accumulating plants to remove toxic metals, including Cd, from soil and aqueous streams has been proposed as a possible solution to this problem (Salt and Rauser, 1995). *Brassica napus* is the oldest cultivated oil-producing crop and a rapid increase in its production has been observed recently in all over the world (FAOSTAT, 2011; Samizadeh *et al.*, 2007). So far, most of the experiments have studied adult *B. napus* plants under heavy metal stress (Wang *et al.*, 2004), and previous experiments have revealed that Cd can reduce the germination rate (Munzuroglu and Geckil, 2002), or inhibit the germination and growth of plants

(Li *et al.*, 2005). On the basis of this background information and importance of *B. napus*, it would be useful to know what biochemical and ultrastructural changes occur in germinating seeds of *B. napus* under the Cd-stressed conditions and, for this purpose, the present study was planned.

## MATERIALS AND METHODS

### Plant Material and Growth Condition

Seeds of two leading cultivars of oilseed rape (*Brassica napus* L. cvs. Zheda 619 and ZS 758) were obtained from the College of Agriculture and Biotechnology, Zhejiang University. Mature seeds of two cultivars were treated with 70% ethanol for 3 minutes and then transferred into 0.1%  $HgCl_2$  for 10 minutes. After that, seeds were washed with deionized water and finally with distilled water. To study the stress of cadmium (Cd) on these cultivars, solutions of different Cd concentrations (0, 100, 200, 300, and 500  $\mu M$ ) were prepared. All these solution were transferred in Petri dishes and the treatments were replicated three times. The treatment concentrations were based on pre-experimental studies, in which several lower and higher levels of the metal were used, i.e., 100, 200, 300, 400, 500 and 1000  $\mu M$  of Cd. Cadmium at 100  $\mu M$  concentration showed little damage on seed growth while 500  $\mu M$  Cd imposed a significant damage; concentrations higher than 500  $\mu M$  were too toxic for seed growth. In every Petri dish, 70-80 seeds were placed and seeds were cultured in a growth chamber under the control environmental conditions (temperatures of 25/20°C (day/night), 16-hour photoperiod (light intensity of 300  $\mu mol m^{-2} s^{-1}$ ) and relative humidity of 60-70%). After 48 hours, germinated seeds were harvested and data regarding germination and fresh weight were measured. Samples were collected for determining biochemical and ultrastructural changes by following proper procedures.

### Plant Growth Parameters

For germination test, another set of experiment was designed. Five seeds per plate were randomly selected from each replication of all treatments and 2 mm radical length was considered as germinated seed. For fresh biomass, a total of 10 seeds per replication were randomly selected and their average values were counted.

### Biochemical Analysis

For enzyme assays, 0.3 g of fresh germinated seeds was homogenized by using 7 ml of 50 mM potassium phosphate buffer solution (pH 7.8). Homogenate was centrifuged at 10,000×g for 20 minutes at 4°C and the supernatant was used for the determination of the enzyme activities. Total soluble protein content was determined by following the method of Bradford (1976), using bovine serum as standard. Ascorbate peroxidase (APX, EC1.11.1.11) activity was measured in a 3 ml reactant mixture having 100 mM phosphate (pH 7.0), 0.1 mM EDTA-Na<sub>2</sub>, 0.3 mM ascorbic acid, 0.06 mM H<sub>2</sub>O<sub>2</sub> and 100 µl enzyme extract. The change in absorption was recorded at 290 nm for 30 seconds after addition of H<sub>2</sub>O<sub>2</sub> (Nakano and Asada, 1981). Peroxidase (POD, EC1.11.1.7) activity was analyzed by following Zhou and Leul (1999) method with some modifications. The guaiacol was used as substrate. The reactant mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1% guaiacol, 0.4% H<sub>2</sub>O<sub>2</sub> and 100 µl enzyme extract. Variation in absorbance due to guaiacol oxidation was measured at 470 nm.

Catalase (CAT, EC 1.11.1.6) activity was measured with the consumption of H<sub>2</sub>O<sub>2</sub> (extinction coefficient 39.4 mM<sup>-1</sup> cm<sup>-1</sup>) for 1 minute at 240 nm in 3 ml of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), to initiate reaction 100 µl enzyme extract was used, 2 mM EDTA-Na<sub>2</sub>, 10 mM H<sub>2</sub>O<sub>2</sub> (Aebi, 1984). Glutathione reductase (GR, EC 1.6.4.2)

activity was determined according to Jiang and Zhang (2002) by the oxidation of NADPH at 340 nm (extinction coefficient 6.2 mM<sup>-1</sup> cm<sup>-1</sup>) for 1 min. The reaction mixture was comprised of 50 mM potassium phosphate buffer (pH 7.0), 2 mM EDTA-Na<sub>2</sub>, 0.15 mM NADPH, 0.5 mM GSSG and 100 µL enzyme extract in a 1 ml volume. NADPH was used to start the reaction. Zhang *et al.* (2008) method was used to measure total superoxide dismutase (SOD, EC 1.15.1.1) activity, following the inhibition of photochemical reduction due to nitro blue tetrazolium (NBT). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA and 100 µL of enzyme extract in a 3-ml volume. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction measured at 560 nm. Glutathione reduced (GSH) content was evaluated using the method of Sedlak and Lindsay (1968). In total, 0.5 ml of plant extract and 2 ml of 5% (w/v) TCA were mixed and centrifuged for 10 minutes at 10,000×g. Then, the supernatant was used for the measurement by adding to 0.4 M Tris buffer (pH 8.9) and 5,5'-dithiobis (2 nitro benzoic acid) (DTNB) and absorbance was detected spectrophotometrically at 412 nm.

Lipid peroxidation malondialdehyde (MDA) in the germinated seeds was determined as 2-thiobarbituric acid (TBA) reactive substances using the method of Zhou and Leul (1998). Superoxide radical (O<sub>2</sub><sup>-</sup>) was determined according to Jiang and Zhang (2001) method with some modifications. The fresh samples (0.6 g) were homogenized in 3 ml of 65 mM potassium phosphate buffer (pH 7.8) and then the homogenate was centrifuged at 5,000×g for 10 minutes at 4°C. After that, the supernatant (1 ml) was mixed with 0.9 ml of 65 mM potassium phosphate buffer (pH 7.8) and 0.1 ml of 10 mM hydroxylamine hydrochloride, and then incubated at 25°C for 24 hours. After incubation, 1 ml of 17 mM sulphanilamide



and 1 ml of 7 mM a-naphthylamine was mixed in 1 ml solution for further 20 minutes at 25°C. Later, n-butanol was added to the same volume and centrifuged at 1,500×g for 5 minutes. The absorbance in the supernatant was read at 530 nm. A standard curve was used to calculate the generation rate of O<sub>2</sub><sup>-</sup>. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content was determined according to Gong *et al.* (2008). Samples (0.3 g) were homogenized with 4 ml 0.1% (w/v) TCA in ice bath. The homogenate was centrifuged at 12,000×g at 4°C for 15 minutes and mixed with 0.5 ml of the supernatant with 0.5 ml 10 mM potassium phosphate buffer (pH 7.0) and 1 ml KI was also added. Then, absorbance was read at 390 nm. Finally, H<sub>2</sub>O<sub>2</sub> content was calculated by using standard curve.

#### Determination of Cadmium Contents

For determination of Cd concentration in germinated seeds, samples were washed with distilled water and then with 20 mM Na<sub>2</sub>-EDTA for 15 minutes. After three times washing with distilled water, samples were washed with deionized water. When the washed samples were dried at 65°C for 24 hours, they were ashed in Muffle furnace at 550°C for 20 hours and the ash was incubated with 31% HNO<sub>3</sub> and 17.5% H<sub>2</sub>O<sub>2</sub> at 70°C for 2 hours, and dissolved in distilled water. Cd concentration in the digest was determined using an atomic absorption spectrophotometer (PE-100, PerkinElmer).

#### Transmission Electron Microscopy

For microscopic analysis, germinated seeds were cut into cross sections and fixed overnight in 4% glutaraldehyde (v/v) in 0.1M sodium phosphate buffer (PBS, pH 7.4). Then, all the samples were washed three times with PBS. The samples were post fixed for 1 hour in 1% OsO<sub>4</sub> (osmium (VIII) oxide), and again washed three times

in 0.1M PBS (pH 7.4) after every 15 minutes interval. Later, the samples were dehydrated with a graded series of ethanol (50, 60, 70, 80, 90, 95, and 100 %) for every 15-20 minutes interval and, finally, by absolute acetone for 20 minutes. After this, the samples were filtered and embedded in Spurr's resin overnight. Following the heating of the specimens at 70°C for 9 hours, the ultra thin sections (80 nm) were prepared and mounted on copper grids for viewing in the transmission electron microscope (JEOL TEM1230EX) at an accelerating voltage of 60.0 kV.

#### Statistical Analysis

The data were analyzed using a statistical package, SPSS version 16.0 (SPSS, Chicago, IL). Two-way variance analysis (ANOVA) was carried out, followed by the Duncan's multiple range test.

## RESULTS

The contents of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide radical (O<sub>2</sub><sup>-</sup>), malondialdehyde (MDA) and Cd contents in germinated seeds are shown in Table 1. The maximum H<sub>2</sub>O<sub>2</sub> contents were observed under the lower level of Cd (100 μM) in Zheda 619 but in case of ZS 758, maximum H<sub>2</sub>O<sub>2</sub> contents were found under the higher level of Cd (500 μM). O<sub>2</sub><sup>-</sup> contents increased linearly as Cd concentration increased in both cultivars, however, more concentration was found in ZS 758 than Zheda 619. This increase was 44.96% in Zheda 619 and 84.91% in ZS 758 under 500 μM Cd as compared to their respective controls. Results showed that MDA contents increased in ZS 758 as Cd concentration increased, but, in case of Zheda 619, higher levels of Cd produced lower amount of MDA. Cd contents in germinated seeds were significantly higher in ZS 758 than Zheda 619 under different concentrations of Cd.

**Table 1.** Effects of different cadmium (Cd) concentrations on reactive oxygen species and Cd contents in germinated seeds of two cultivars of *Brassica napus* L. grown for 48 hours.<sup>a</sup>

Variety	Cd conc. (μM)	H <sub>2</sub> O <sub>2</sub> (μmol g <sup>-1</sup> DW)	O <sub>2</sub> <sup>-</sup> (nmol min <sup>-1</sup> g <sup>-1</sup> DW)	MDA (nmol mg <sup>-1</sup> protein)	Cd content (mg kg <sup>-1</sup> DW)
Zheda 619	0	183.34 ± 6.33 bc	67.16 ± 3.84 e	194.83 ± 7.02 f	0.02 ± 0.00 f
	100	210.92 ± 8.60 a	72.97 ± 4.75 e	220.13 ± 7.85 e	146.13 ± 4.37 e
	200	185.23 ± 3.48 bc	87.73 ± 5.20 d	222.70 ± 9.07 e	154.53 ± 8.57 e
	300	145.77 ± 7.30 d	91.36 ± 6.15 cd	220.47 ± 10.41 e	205.77 ± 12.37 c
	500	121.90 ± 8.54 e	97.36 ± 4.32 cd	202.80 ± 12.70 f	265.59 ± 7.61 b
ZS 758	0	173.13 ± 6.10 c	75.06 ± 5.81 e	243.93 ± 7.55 d	0.01 ± 0.00 f
	100	144.13 ± 7.10 d	96.06 ± 3.27 cd	252.70 ± 7.45 cd	175.04 ± 7.68 d
	200	189.40 ± 6.95 b	102.77 ± 6.37 c	265.40 ± 6.51 c	196.76 ± 7.79 c
	300	192.03 ± 7.19 b	120.97 ± 7.17 b	291.87 ± 7.26 b	205.54 ± 15.04 c
	500	212.47 ± 12.93 a	138.80 ± 11.21 a	324.10 ± 2.65 a	295.63 ± 12.58 a

<sup>a</sup> Values are the means of three replications (n=3) ±SD and variants possessing the same letter are not statistically significant at  $P < 0.05$ .

The effect of different Cd concentrations on the germination, fresh weight, activities of catalase (CAT) and peroxidase (POD) in germinated seeds of the two *B. napus* cultivars is shown in Table 2. In both cultivars, low levels of Cd (100, 200 μM) did not show any significant effect, while higher concentration of Cd (500 μM) significantly reduced the germination percentage and this decrease was 18.76% in Zheda 619 and 22.26% in ZS 758 under 500 μM Cd as compared to their respective controls. The results proved that lower dose of Cd (100 μM) did not show any effect on fresh weight of germinated seeds, but higher concentration of Cd (500 μM) significantly reduced the fresh weight of the germinated seeds. This decrease was 21.69% in Zheda 619 and 25.51% in ZS 758 under 500 μM Cd as compared to their respective controls. CAT activity showed obvious changes under different Cd concentrations, and minimum values were obtained under higher level of Cd (500 μM) in both cultivars. The activity of POD showed decline under lower levels of Cd in Zheda 619 but under higher levels of Cd, it increased the POD activity. In case of ZS 758, lower levels of Cd increased the POD activity but higher levels of Cd significantly decreased the activity of POD.

Figure 1 illustrates the changes in the activities of ascorbate peroxidase (APX), superoxide dismutase (SOD), total soluble protein (TSP), glutathione reductase (GR)

and glutathione reduced (GSH) contents in the germinated seeds of the two cultivars of *B. napus* under Cd stress. Results depicted that higher level of Cd (500 μM) significantly reduced the APX activity in both cultivars and this decrease was 29.55% in Zheda 619 and 61.27% in ZS 758 (Figure 1-a). Higher level of Cd (500 μM) increased the SOD activity in Zheda 619 and significantly decreased the SOD activity in ZS 758 (Figure 1-b). There was no significant change in TSP contents in Zheda 619 under Cd stress. Moreover, ZS 758 showed a significant decline in TSP contents under 500 μM Cd (Figure 1-c). The activity of GR was significantly enhanced under Cd stress in Zheda 619 (Figure 1-d). However, GR activity decreased linearly as Cd concentration increased in ZS 758. Lower level of Cd (100 μM) showed a synergetic effect on contents of GSH and produced maximum GSH contents in both cultivars and GSH contents decreased as Cd concentration increased in both cultivars (Figure 1-e).

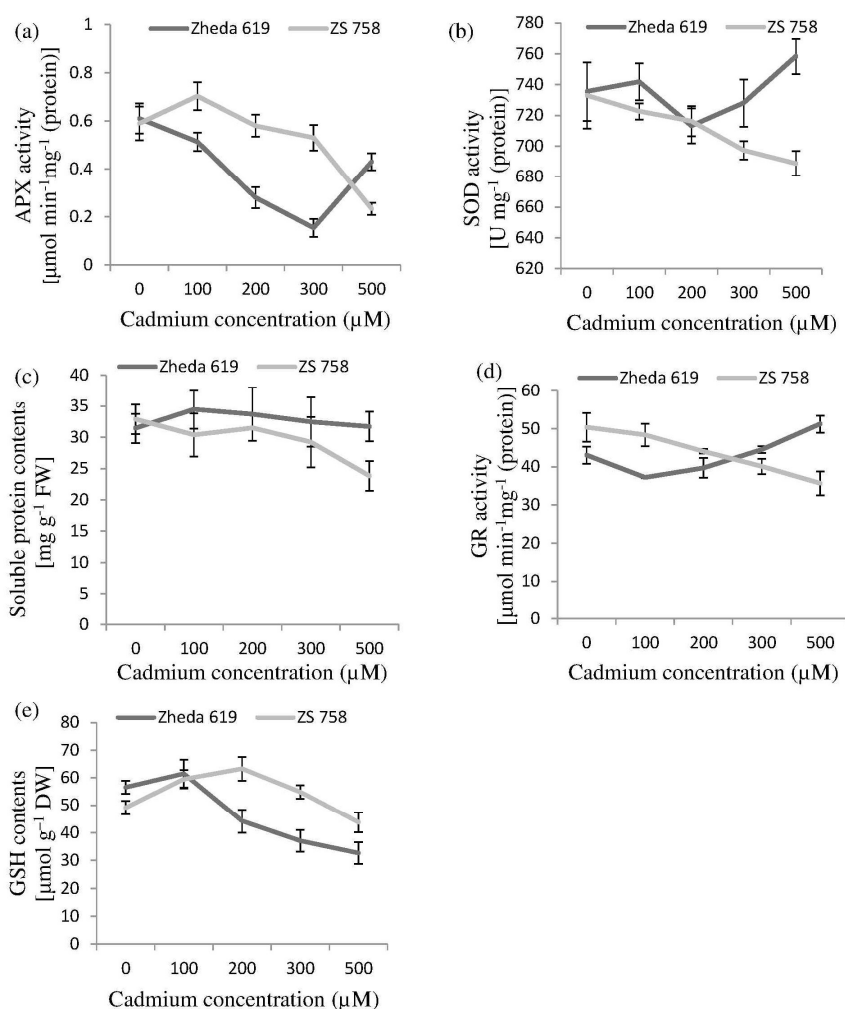
Ultrastructural changes in the germinated seeds of two cultivars of *B. napus* under control and 500 μM Cd level at low and high magnification, respectively, are shown in Figure 2 (A-H). The microscopic study showed that overall shape of the cell was normal and cell wall was clear at control in both cultivars. There was a big nucleus with well-developed nucleolus and nuclear



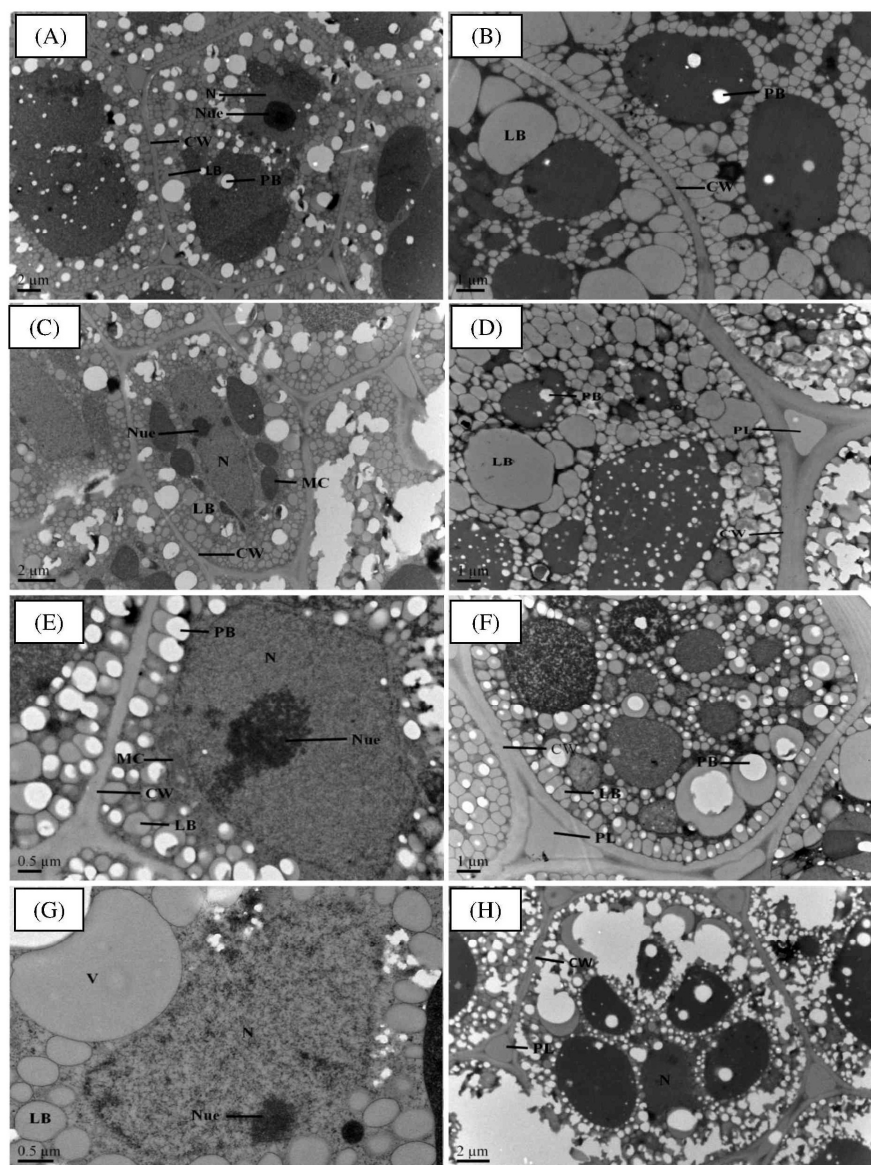
**Table 2.** Effects of different cadmium (Cd) concentrations ( $\mu\text{M}$ ) on seed germination (%), fresh weight per 10 seeds (g), and activities of catalase (CAT) and peroxidase (POD) ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$  protein) in germinated seeds of two cultivars of *Brassica napus* L. grown for 48 hours.<sup>a</sup>

Variety	Cd conc.	Germination	Fresh weight	CAT activity	POD activity
Zheda 619	0	91.49 $\pm$ 6.71 a	0.922 $\pm$ 0.025 ab	0.14 $\pm$ 0.03 bcd	0.040 $\pm$ 0.009 abc
	100	92.90 $\pm$ 4.54 a	0.963 $\pm$ .023 a	0.21 $\pm$ 0.05 ab	0.031 $\pm$ 0.003 cde
	200	84.73 $\pm$ 6.76 ab	0.854 $\pm$ .024 cd	0.17 $\pm$ 0.07 abc	0.020 $\pm$ 0.003 ef
	300	82.52 $\pm$ 7.27 ab	0.816 $\pm$ .023 d	0.23 $\pm$ 0.01 a	0.025 $\pm$ 0.001 def
	500	74.32 $\pm$ 8.95 bc	0.722 $\pm$ .052 ef	0.19 $\pm$ 0.06 ab	0.049 $\pm$ 0.008 a
ZS 758	0	90.29 $\pm$ 3.70 a	0.915 $\pm$ .024 ab	0.11 $\pm$ 0.02 cde	0.028 $\pm$ 0.004 cdef
	100	85.76 $\pm$ 5.71 ab	0.879 $\pm$ .043 bc	0.10 $\pm$ 0.03 cde	0.035 $\pm$ 0.004 bcd
	200	83.80 $\pm$ 5.61 ab	0.847 $\pm$ 0.021 cd	0.11 $\pm$ 0.02 cde	0.047 $\pm$ 0.003 ab
	300	77.76 $\pm$ 6.67 bc	0.753 $\pm$ 0.037 e	0.09 $\pm$ 0.03 de	0.032 $\pm$ 0.002 cde
	500	70.19 $\pm$ 4.84 c	0.681 $\pm$ .009 f	0.06 $\pm$ 0.02 e	0.016 $\pm$ 0.001 f

<sup>a</sup> Values are the means of three replications (n=3)  $\pm$ SD and variants possessing the same letter are not statistically significant at  $P < 0.05$ .



**Figure 1.** Effects of different cadmium (Cd) concentrations ( $\mu\text{M}$ ) on: (a) Ascorbate peroxidase (APX); (b) Superoxide dismutase (SOD) activities; (c) Total soluble protein contents (TSP); (d) Glutathione reductase (GR), and (e) Glutathione reduced (GSH) contents in germinated seeds of two cultivars of *Brassica napus* L. grown for 48 hours. Values are mean $\pm$ SD (n= 3) and bars indicate SD.



**Figure 2.** Electron micrographs of cross sections of germinated seeds of two cultivars of *Brassica napus* L. (Zheda 619 and ZS 758) grown for 48 hours at control (without Cd treatment) and 500  $\mu\text{M}$  Cd level. (A–B) TEM micrographs of cross section of germinated seeds of Zheda 619 respectively at low and high magnifications under control show a well-developed nucleus (N) with nucleoli (Nuc) and a distinct nuclear membrane (NM), cell wall (CW) as well as a number of lipid bodies (LB) and protein bodies (PB); (C–D) TEM micrographs of cross section of germinated seeds of ZS 758 respectively at low and high magnifications under control show a well-developed elongated nucleus (N) with nucleoli (Nuc) and a distinct nuclear membrane (NM), mitochondria (MC), and cell wall (CW) as well as a number of lipid bodies (LB) and protein bodies (PB); (E–F) TEM micrographs of cross section of germinated seeds of Zheda 619 respectively at low and high magnifications under 500  $\mu\text{M}$  Cd level show a nucleus (N) with nucleoli (Nuc), well developed cell wall (CW) as well as a number of lipid bodies (LB) and protein bodies (PB). Moreover, mitochondrion is also present in the cell, and (G–H) TEM micrographs of cross section of germinated seeds of ZS 758 respectively at low and high magnifications under 500  $\mu\text{M}$  Cd level show un-matured nucleus (N) with nucleoli (Nuc), and totally ruptured cell wall (CW) as well as mitochondria (M) was also not present.



membrane in both cultivars. Mitochondrion was also found and a number of lipid and protein bodies could be observed in the cells of both cultivars under control conditions (Figure 2, A-D). The ultrastructures at 500 $\mu$ M Cd level showed that major toxicity target sites of Cd were nucleus and cell wall (Figure 2, E-H). TEM structures depicted that ZS 758 was more affected than Zheda 619 under the Cd stress. The nucleus was present with well-developed nucleolus in Zheda 619 and nuclear membrane was also found (Figure 2, E-F). The cell wall was not damaged under Cd stress in Zheda 619. Moreover, mitochondria could be found in the micrograph. In the case of ZS 758, cell wall was totally damaged under the high concentration of Cd (500  $\mu$ M). The nucleus was not clear and nuclear membrane was also totally damaged (Figure 2, G-H).

## DISCUSSION

In the present research, toxicity effects of Cd were examined on plant growth attributes, biochemical changes, and ultrastructural changes in the germinated seeds of two cultivars of *B. napus*. It is a fact that seed coat is the first barrier to Cd uptake and embryo protection, therefore, it is very important to study the biochemical and ultrastructural changes taken place in germinating seeds under Cd stress conditions. Results revealed that lower concentrations of Cd did not have any significant impact on the seed germination and fresh weight of germinated seeds. However, with an increase in Cd concentrations, a significant decrease in germination and fresh weight was observed in both cultivars (Table 2). Previously, different studies showed that, similar to Cd, other heavy metals like Cu and Pb had stimulating effect on seed germination (Dorn and Salanitro, 2000). However, the sensitivity of early growth seedlings has been found previously in cotton by Daud *et al.* (2009). Cd contents in germinated seeds increased as Cd concentration increased in

both cultivars (Table 1). In the past, Xiong and Wang (2005) also confirmed that uptake of heavy metal by plants is correlated with the increasing metal concentration.

It was found that MDA content was lower in cultivar Zheda 619 compared to ZS 758 at all Cd concentrations (Table 1). This could be explained in terms of higher degree of protection against oxidative damage in Zheda 619 by fast removal of H<sub>2</sub>O<sub>2</sub> or by other scavenging systems (Mobin and Khan, 2007). This decrease in MDA contents in treated seeds may be due to seed coat that can be a barrier to prevent the entry of Cd into the germinating seeds (Seregin and Ivanov, 2001), protecting the embryo. Moreover, in the present study, ROS (H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup>) production was lower in Zheda 619 than ZS 758 even in the presence of higher levels of Cd (Table 1), which implies that the generation of H<sub>2</sub>O<sub>2</sub> was quenched by the efficient antioxidative mechanism of Zheda 619 (Mobin and Khan, 2007).

Therefore, a delicate antioxidant system is indispensably required to supervise the cytotoxic effects of ROS. According to the results, activities of CAT, POD, APX, SOD and GR were found to increase in Zheda 619 under higher Cd treatment showing efficiency of the antioxidative enzyme mechanism that led to the protection of the photosynthetic machinery to some extent (Gill *et al.*, 2012) and lesser reduction in growth (germination and fresh weight) in the present study. The reduction in the activities of antioxidant enzymes in ZS 758 may be due to direct targets of heavy metal and that binding to SH groups in proteins leads to a defect of activity and/or structure (Seregin and Ivanov, 2001). The soluble protein contents showed no change under the different concentrations of Cd in both cultivars, except that higher concentration of Cd (500  $\mu$ M) decreased the protein contents in ZS 758 (Figure 1-c), which could be probably due to the induction of the synthesis of stress proteins (Seth *et al.*, 2008). In the present study, GSH contents decreased as Cd concentration increased in both studied cultivars, and the minimum



GSH contents were found under 500  $\mu\text{M}$  Cd stress (Figure 1-e). Previously, Gallego *et al.* (1999) also observed a decrease in GSH content in sunflower cotyledons at 200 mM Cd as compared to the control seeds.

The effect of heavy metals on cellular organization is an important factor in understanding the physiological alterations induced by heavy metals due to complementarity of structure and function. Ultrastructural observations of germinated seed cells showed that these cells possessed numerous organelles which seemed to be without any obvious damage under control conditions in both the cultivars (Figure 2, A-D). Cd exposure resulted in ultrastructural changes in seed cells of both cultivars but damage was more pronounced in ZS 758 than Zheda 619 (Figure 2, E-H). Other similar studies have reported that Cd induces premature senescence in leaves of several plant species including wheat (Ouzounidou *et al.*, 1997), pea (McCarthy *et al.*, 2001) and *Elodea canadensis* (Vecchia *et al.*, 2005). Moreover, Ciamporova and Mistrik (1993) also noticed more lipid bodies in cytoplasm of maize cell under water and Cu stress conditions.

### CONCLUSIONS

The findings of the present study showed that sub-cellular and antioxidative changes could occur early in plant development, during germination. This was observed in oilseed *Brassica* plants, even though they are relatively tolerant to heavy metals due to fast growth and higher biomass production, and are used in phytoremediation (Momoh and Zhou, 2001). However, this study suggests that cultivar Zheda 619 has a greater ability to adapt to Cd toxicity. Selection of tolerant varieties towards better production under the heavy metal stress enhances the yield of oilseed rape. Moreover, our present study intends to investigate the toxic effects of Cd by using lab-based approach, and these findings would be of great interest to the scientists

working on the phytoremediation and related area.

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## اثرات تخریبی کادمیوم روی سامانه آنتی اکسیدان و ساختمان سلولی در بذر جوانه *Brassica napus* L. زده

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### چکیده

کادمیوم که به عنوان یک عنصر سنگین غیر ضروری شناخته می شود، می تواند موجب تنش (استرس) اکسیدی در گیاه شود. در پژوهش حاضر، تلاش شد تا تعیین شود که آیا تغییرات اکسایشی یا میکروسکوپی ناشی از کادمیوم در مراحل اولیه رشد قابل مشاهده است یا خیر. به این منظور، بذرهای *Brassica napus* (کالتیوارهای Zheda 619 و ZS 758) برای جوانه زنی درون شیشه در غلظت های ۲۰۰، ۱۰۰، ۳۰۰، ۵۰۰ میکرومول کادمیوم به مدت ۴۸ ساعت در تاریکی نگهداری شدند. نتایج نشان داد که کادمیوم در غلظت بالا (۵۰۰ میکرومول) به طور معنی داری رشد هر دو کالتیوار را کم کرد و غلظت کادمیوم در هر دو کالتیوار به طور خطی با افزایش غلظت کادمیوم در محیط زیاد شد هر چند که این اثر در کالتیوار ZS 758 بیشتر از کالتیوار دیگر بود. همچنین، کادمیوم با زیاد کردن ترکیبات اکسیژنی فعال منجر به تنش اکسیدی در بذرهای جوانه زده شد. با این وجود، هیچ رابطه ای بین ظرفیت دفاعی آنتی اکسیدانی (شامل کاتالیز، پراکسیداز، superoxide dismutase و فعالیت رد تاکتاز گلوتایون) و تحمل به کادمیوم در این دو کالتیوار به دست نیامد. مسمومیت کادمیومی تغییرات فرا ساختمانی معنی داری در سلول های بذر ها پدید آورد ولی ساختمان سلولی در کالتیوار Zheda 619 تغییری نشان نداد در حالی که ساختمان سلولی در ZS 758 در غلظت بالای کادمیوم (۵۰۰ میکرومول) به طور کامل تخریب شد. بر پایه این یافته ها می توان نتیجه گرفت که کالتیوار Zheda 619 به طور نسبی مقاومت بیشتری به کادمیوم دارد و اجزای سلول آن در شرایط تنش کادمیوم از دوام و استحکام بیشتری برخوردارند.