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Physiological and ultra-structural changes in *Brassica napus* seedlings induced by cadmium stress

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Abstract

The effects of cadmium on physiological and ultrastructural characteristics were evaluated in 6-d-old seedlings of two *Brassica napus* L. cultivars Zheda 619 and ZS 758. Results show that Cd at lower concentration (100 µM) stimulated the seedling growth but at higher concentration (500 µM) inhibited the growth of both cultivars, decreased content of photosynthetic pigments, activities of antioxidant enzymes, and increased the content of malondialdehyde and reactive oxygen species. Cd content in different parts of seedlings was higher in ZS 758 than in Zheda 619. Electron micrographs illustrated that 500 µM Cd severely damaged the leaf and root tip cells of both cultivars. Under Cd stress, the size and number of starch grains, plastoglobuli, and lipid bodies in the chloroplasts increased. In the root tip cells, enlarged vacuoles, diffused cell walls, and undeveloped mitochondria were detected.

Additional key words: antioxidant enzymes, chlorophyll, chloroplasts, mitochondria, oilseed rape, reactive oxygen species.

Introduction

Cadmium (Cd) is released from a variety of sources, such as metallurgic industries, cement factories, waste materials, and phosphate fertilizers (Sanita di Toppi and Gabbrielli 1999). Cd is considered as most toxic heavy metal due to its high solubility in water (Pinto *et al.* 2004). It is not essential for the plants but it can be easily taken up by plant roots (Vitoria *et al.* 2001). Above a threshold level, it causes reduction in the yield of crops and also downgrades their quality (Hassan *et al.* 2005a). In soil, Cd competes with micronutrients because of the similarities in physiochemical properties (Papoyan *et al.* 2007). Cd can cause damage to plant tissue membranes, production of reactive oxygen species, and inhibition of photosynthesis (Hassan *et al.* 2005b, Ali *et al.* 2013a). Cd can inhibit seed germination, root elongation, cause chlorosis, and decreased activities of some enzymes (Fodor *et al.* 1995, Ouariti *et al.* 1997, Tian *et al*. 2012, Ali *et al.* 2013b). In different crops, exposure to Cd reduces plant size, the number of lateral roots (Sandalio *et al.* 2001, Rodriguez-Serrano *et al.* 2009), chlorophyll content, and photosynthetic rate, whereas elevates malondialdehyde (MDA) content, and superoxide dismutase (SOD) and peroxidase (POD) activities (Shamsi *et al.* 2008, Tao *et al.* 2012). It has been shown that higher Cd concentrations decrease growth of *Hordeum vulgare* and *Thlaspi arvense* due to a reduction of antioxidant enzyme activities in root tips and leaves, respectively (Bočová *et al*. 2012, Martin *et al*. 2012). Cd brings about the oxidative stress (Schutzendubel *et al.* 2002) leading to cell aging (Quirino *et al.* 2001, Ali *et al*. 2013a).

The Cd accumulation in maize (*Zea mays* L.) causes disorganization of grana and an increase in the number and size of plastoglobuli in chloroplasts, increases cell and vacuole sizes, and induces vesiculation (Rascio *et al.* 1993). The waxy form of granal and agranal thylakoids and dilatation of thylakoid membranes in chloroplast under Cd stress were observed in wheat by Ouzounidou *et al.* (1997). Further, Cd can increase number of nucleoli and vacuoles, cause diminution of mitochondrial cristae, disorganization of chloroplasts, disruption of nuclear

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; Car - carotenoids; Chl - chlorophyll; GR - glutathione reductase; MDA - malondialdehyde; POD - peroxidase; SOD - superoxide dismutase; TSP - total soluble proteins.
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envelope, and rigorous plasmolysis (Victoria *et al.* 2003, Daud *et al.* 2009a). Very few studies are available which combine structural and ultra-structural modifications with physiological dysfunctions (Barcelo *et al.* 1988).

Cd is increasing to toxic levels in productive lands, so it is need to discover plant species which are capable of degrading and dissipating Cd from contaminated soils. Generally, *Brassica* species are tolerant to heavy metals due to their fast growth, high biomass production, and ability to uptake greater amount of heavy metals (Banuelos and Meek 1990, Momoh and Zhou 2001, Meng *et al.* 2009). Oilseed rape is cultivated all around the world for the production of edible oil. Therefore, two leading cultivars of this species were examined under different Cd concentrations.

Materials and methods

Seeds of two cultivars (Zheda 619 and ZS 758) of oilseed rape (*Brassica napus* L.) were obtained from the College of Agriculture and Biotechnology, Zhejiang University. They were treated with 70 % (v/v) ethanol for 3 min, transferred into 0.1 % (m/v) $HgCl_2$ for 8 min, and then rinsed with deionized water. The $CdCl_2$ solutions at different concentrations (0, 100, 200, 300, 500 μM Cd) were applied to 50 seeds in Petri dishes and treatments were replicated three times. The seeds germinated in a growth chamber under day/night temperatures of 25/20 °C, a 16-h photoperiod, irradiance of 300 μmol m^{-2} s^{-1}, and relative humidity of 60 - 70 %. Six days later, the seedlings were harvested and samples were collected for enzyme analyses and microscopic studies.

Germination test and measurements of hypocotyl and radicle lengths were carried out according to Daud *et al.* (2009a). Chlorophyll content was assessed according to the method of Porra *et al.* (1989) and Pei *et al.* (2010). Lipid peroxidation in the cotyledons, measured in terms of malondialdehyde (MDA) content, was analyzed according to Zhou and Leul (1999). Antioxidant enzymes were examined according to Zhang (1992) with some modifications. The fresh cotyledons (0.3 g) were homogenized in 7 cm^3 of 50 mM potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at 10 000 *g* and 4 °C for 20 min and the supernatant was utilized for enzyme assays (Leul and Zhou 1999). Ascorbate peroxidase (APX, EC1.11.1.11) activity was measured in 3 cm^3 of a mixture of 100 mM phosphate buffer (pH 7.0), 0.1 mM Na_2-EDTA, 0.3 mM ascorbic acid, 0.06 mM H_2O_2, and 0.1 cm^3 of the enzyme extract. The change in absorbance was recorded at 290 nm for 30 s after addition of H_2O_2 (Nakano and Asada 1981). Peroxidase (POD, EC1.11.1.7) activity was determined according to Zhou and Leul (1999) with some modifications. The mixture contained 50 mM potassium phosphate buffer (pH 7.0), 1 % (m/v) guaiacol, 0.4 % (v/v) H_2O_2, and 0.1 cm^3 of the enzyme extract. Changes in absorbance was measured at 470 nm. Catalase (CAT, EC 1.11.1.6) activity was analyzed according to Aebi (1984) in 3 cm^3 of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 2 mM Na_2-EDTA, 10 mM H_2O_2, and 0.1 cm^3 of the enzyme extract. Absorbance at 240 nm was measured for 1 min (coefficient of absorbance 39.4 mM^{-1} cm^{-1}). Total superoxide dismutase (SOD, EC 1.15.1.1) activity was assessed using the method of Zhang *et al.* (2008) following the inhibition of photochemical reduction due to nitroblue tetrazolium (NBT). One unit of SOD activity was defined as the quantity of enzyme required to cause 50 % inhibition of the NBT reduction measured at 560 nm. 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 for 1 min (coefficient of absorbance 6.2 mM^{-1} cm^{-1}). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 2 mM Na_2-EDTA, 0.15 mM NADPH, 0.5 mM oxidised glutathione (GSSG), and 0.1 cm^3 of the enzyme extract in a 1 cm^3 volume. NADPH was used to initiate the reaction. Total soluble protein content was determined according to Bradford (1976) using bovine serum albumin as standard.

Superoxide radical ($O_2^{\cdot-}$) was analyzed according to Jiang and Zhang (2001) method with some modification. The supernatant (1 cm^3) was mixed with 0.9 cm^3 of 65 mM potassium phosphate buffer (pH 7.8) and 0.1 cm^3 of 10 mM hydroxylamine hydrochloride, and then incubated at 25 °C for 20 min. The incubation solution (1 cm^3) was then mixed with 1 cm^3 of 17 mM sulphanilamide and 1 cm^3 of 7 mM α-naphthylamine and incubated for further 20 min at 25 °C. After incubation, n-butanol of the same volume was added and then centrifuged at 1 500 g for 5 min. The absorbance in the supernatant was read at 530 nm. A standard curve was used to calculate the generation rate of $O_2^{\cdot-}$. Hydrogen peroxide was examined according to Gong *et al.* (2008). The 0.5 cm^3 supernatant was mixed with 0.5 cm^3 of 10 mM potassium phosphate buffer (pH 7.0) and 1 cm^3 of 1 M KI was added. The absorbance was read at 390 nm and the H_2O_2 content was calculated by using a standard curve.

For determination of Cd content, roots and shoots were dried at 70 °C for 48 h. The samples were ground to fine powder and wet digested in a 5 cm^3 mixture of HNO_3:$HClO_4$ (2:1, v/v). After heating the mixture at 80 °C in water bath for about 2 h, Cd content was measured using atomic absorption spectrometry (*PE-100*, *Perkin Elmer*, USA).

For microscopy, leaf fragments without veins (about 1 mm^2) and root tips (about 2 - 3 mm) were fixed in 4 % (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer (PBS, pH 7.4) overnight and then washed three times with PBS. The samples were post fixed in 1 % (m/v) OsO_4 for 1 h and washed again three times with PBS. After that, the samples were dehydrated in a graded series

of ethanol (50, 60, 70, 80, 90, 95, and 100 %, v/v) for 15 - 20 min each and then in absolute acetone for 20 min. After dehydration, the samples were embedded in Spurr's resin overnight. After heating the specimens at 70 °C for 9 h, the ultra thin sections (80 nm) were cut and mounted on copper grids for observation in the transmission electron microscope (*TEM 1230EX*, *JEOL*, Japan) at 60.0 kV.

The data were analyzed using a statistical package *SPSS v. 16.0* (*SPSS*, Chicago, IL, USA). A two-way *ANOVA* was carried out, followed by the Duncan's multiple range test.

Results

Maximum seed germination and hypocotyl length after 24 h was noted under 100 µM Cd in both cultivars but the higher Cd concentrations (300 and 500 µM) inhibited seed germination and hypocotyl growth (Table 1). After 6 d, maximum growth was in the control plants (no Cd added) and ZS 758 exhibited a shorter hypocotyl and radicle as compared to Zheda 619. Also fresh and dry masses of the roots and leaves were significantly decreased with increasing Cd concentration in both cultivars but more in ZS 758 than in Zheda 619 (Table 2). The chlorophyll and carotenoid content decreased with increasing Cd concentration in both cultivars but this decline was lower in ZS 758 than in Zheda 619 (Table 3).

As concerns antioxidative enzymes, the maximum APX activity was in the controls of both cultivars and with increasing Cd concentration, APX activity decreased by 44.40 % in Zheda 619 and 62.95 % in ZS 758. Maximum CAT, SOD, and POD activities in Zheda 619 were in the control plants but in ZS 758, maximum CAT, SOD, and POD activities were at 100 µM Cd but then these activities decreased with increasing Cd concentration. The GR activity displayed a linear decrease with increasing Cd concentration in ZS 758. In Zheda 619, GR activity increased a little bit at lower Cd concentrations

Table 1. Seed germination and radical and hypocotyl lengths in two cultivars of *Brassica napus* grown under different Cd concentrations for 6 d. Means ± SD, $n = 3$. Means with different letters in individual columns are statistically different at $P < 0.05$.

Cultivar	Cd conc. [µM]	Germination rate [%]	Radical and hypocotyl lengths [cm plant^{-1}] after 24 h radicle	hypocotyl	after 6 d radicle	hypocotyl
Zheda 619	0	85.73 ± 1.64 bc	0.26 ± 0.01 de	0.12 ± 0.03 abc	5.90 ± 0.62 a	6.60 ± 0.26 a
	100	94.51 ± 2.17 a	0.35 ± 0.02 a	0.14 ± 0.01 a	5.13 ± 0.15 b	6.00 ± 0.26 bc
	200	92.26 ± 2.62 a	0.33 ± 0.02 ab	0.15 ± 0.03 a	5.10 ± 0.26 bc	5.90 ± 0.10 cd
	300	82.52 ± 3.30 cd	0.25 ± 0.02 de	0.10 ± 0.02 abc	4.96 ± 0.11 bc	5.47 ± 0.31 de
	500	75.81 ± 0.52 e	0.18 ± 0.01 f	0.09 ± 0.02 bcd	4.76 ±0.05 bcd	5.10 ± 0.26 e
ZS 758	0	83.63 ± 3.47 cd	0.25 ±0.04 de	0.11 ± 0.02 abc	5.76 ± 0.11 a	6.40 ± 0.36 ab
	100	90.67 ± 2.49 ab	0.30 ± 0.01 bc	0.13 ± 0.02 ab	4.93 ± 0.20 bc	5.67 ± 0.25 cd
	200	89.55 ± 2.67 ab	0.29 ± 0.01 cd	0.13 ± 0.01 a	4.83 ± 0.15 bcd	5.47 ± 0.20 de
	300	79.43 ± 0.61 de	0.23 ± 0.00 e	0.08 ± 0.01 cd	4.63 ± 0.11 cd	5.13 ± 0.15 e
	500	67.57 ± 0.32 f	0.14 ± 0.00 g	0.05 ± 0.00 d	4.40 ± 0.10 d	4.57 ± 0.05 f

Table 2. Fresh and dry biomasses in two cultivars of *Brassica napus* grown under different Cd concentrations for 6 d. Means ± SD, $n = 3$. Means with different letters in individual columns are statistically different at $P < 0.05$.

Cultivar	Cd conc. [µM]	Root biomass [g plant^{-1}] fresh mass	dry mass	Leaf biomass [g plant^{-1}] fresh mass	dry mass
Zheda 619	0	0.012 ± 0.0012 a	0.0042 ± 0.0001 a	0.079 ± 0.001 a	0.009 ± 0.0004 a
	100	0.010 ± 0.0002 c	0.0038 ± 0.0002 b	0.076 ± 0.000 b	0.008 ± 0.0003 b
	200	0.010 ± 0.0001 c	0.0035 ± 0.000 c	0.071 ± 0.001 c	0.008 ± 0.0003 bc
	300	0.008 ± 0.0002 ef	0.0030 ± 0.0001 d	0.067 ± 0.001 d	0.007 ± 0.0002 d
	500	0.007 ± 0.0001 g	0.0019 ± 0.0001 fg	0.065 ± 0.000 e	0.006 ± 0.0001 e
ZS 758	0	0.011 ± 0.0004 b	0.0039 ± 0.0002 ab	0.079 ± 0.000 a	0.009 ± 0.0002 a
	100	0.010 ± 0.0000 cd	0.0033 ± 0.0003 c	0.075 ± 0.001 b	0.007 ± 0.0002 c
	200	0.009 ± 0.0003 de	0.0027 ± 0.0002 e	0.068 ± 0.001 d	0.006 ± 0.0003 e
	300	0.008 ± 0.0001 f	0.0019 ± 0.0003 f	0.065 ± 0.000 e	0.006 ± 0.0002 e
	500	0.006 ± 0.0000 h	0.0016 ± 0.0000 g	0.062 ± 0.001 f	0.004 ± 0.0002 f

Table 3. Effects of different Cd concentrations on content of chlorophylls, carotenoids, and soluble proteins (TSP) [mg g^{-1}(f.m.)] in cotyledons of two *Brassica napus* cultivars. Means ± SD, *n* = 3. Means with different letters in individual columns are statistically different at $P < 0.05$.

Cultivar	Cd [μM]	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids	TSP
Zheda 619	0	6.27 ± 1.05 a	2.33 ± 0.37 a	8.60 ± 0.37 a	0.51 ± 0.01 a	14.04 ± 0.53 a
	100	5.75 ± 2.12 b	1.56 ± 0.03 b	7.31 ± 0.18 b	0.47 ± 0.02 b	12.42 ± 0.19 c
	200	4.92 ± 0.17 de	1.45 ± 0.16 bc	6.37 ± 0.31 c	0.38 ± 0.01 d	10.03 ± 0.18 de
	300	3.90 ± 0.30 f	1.34 ± 0.09 bcd	5.24 ± 0.25 ef	0.32 ± 0.01 e	9.52 ± 0.43 ef
	500	3.78 ± 0.15 f	1.13 ± 0.09 d	4.91 ± 0.14 f	0.25 ± 0.00 g	9.32 ± 0.59 f
ZS 758	0	5.28 ± 1.15 c	1.29 ± 0.08 bcd	6.57 ± 0.15 c	0.46 ± 0.01 bc	13.34 ± 0.21 b
	100	5.11 ± 0.35 cd	1.19 ± 0.16 cd	6.31 ± 0.11 c	0.44 ± 0.00 c	12.37 ± 0.33 c
	200	4.79 ± 1.82 de	1.09 ± 0.16 d	5.88 ± 0.02 d	0.38 ± 0.00 d	10.24 ± 0.25 d
	300	4.67 ± 0.51 e	0.76 ± 0.10 e	5.43 ± 0.10 e	0.29 ± 0.01 f	8.86 ± 0.20 f
	500	3.37 ± 0.58 g	0.65 ± 0.00 e	4.08 ± 0.05 g	0.16 ± 0.00 h	8.94 ± 0.54 f

Table 4. Effects of different Cd concentrations on activities of ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), glutathione reductase (GR) [μmol mg^{-1}(protein) min^{-1}], and superoxide dismutase (SOD) [U mg^{-1}(protein)] in cotyledons of two *Brassica napus* cultivars. Means ± SD, *n* = 3. Means with different letters in individual columns are statistically different at $P < 0.05$.

Cultivar	Cd [μM]	APX	CAT	POD	GR	SOD
Zheda 619	0	1.56 ± 0.15 b	0.59 ± 0.02 a	1.41 ± 0.27 a	21.34 ± 3.45 f	171.00 ± 4.58 a
	100	1.40 ± 0.01 bc	0.43 ± 0.01 c	0.82 ± 0.02 b	27.18 ± 2.39 bcd	148.66 ± 6.65 b
	200	1.26 ± 0.03 c	0.40 ± 0.02 c	0.76 ± 0.02 bc	28.99 ± 2.61 bc	113.66 ± 6.42 c
	300	0.99 ± 0.01 d	0.28 ± 0.02 e	0.78 ± 0.04 bc	23.16 ± 2.29 ef	90.00 ± 6.24 d
	500	0.86 ± 0.01 d	0.25 ± 0.01 e	0.64 ± 0.04 cde	20.68 ± 1.23 f	77.64 ± 4.50 d
ZS 758	0	1.76 ± 0.24 a	0.25 ± 0.01 e	0.50 ± 0.01 cde	34.57 ± 1.70 a	106.66 ± 5.50 c
	100	1.23 ± 0.10 c	0.55 ± 0.01 ab	0.64 ± 0.03 bcd	30.70 ± 1.72 b	140.66 ± 11.56 b
	200	0.96 ± 0.01 d	0.53 ± 0.03 b	0.68 ± 0.02 bcd	25.51 ± 1.77 cde	116.00 ± 5.56 c
	300	0.85 ± 0.06 d	0.36 ± 0.02 d	0.52 ± 0.01 de	23.79 ± 2.01 def	88.16 ± 10.06 d
	500	0.65 ± 0.02 e	0.20 ± 0.01 f	0.29 ± 0.02 f	19.99 ± 1.79 f	56.03 ± 11.57 e

Table 5. Effects of different Cd concentrations on malondialdehyde (MDA) content [nmol mg^{-1}(protein], hydrogen peroxide content [μmol g^{-1}(d.m.)], superoxide radical production [nmol g^{-1}(d.m.) min^{-1}] in cotyledons, and Cd content in roots and shoots [mg kg^{-1}(d.m.)] of two *Brassica napus* cultivars. Means ± SD, *n* = 3. Means with different letters in individual columns are statistically different at $P < 0.05$.

Cultivar	Cd [μM]	MDA	H_2O_2	$O_2^{\cdot-}$	Root Cd	Shoot Cd
Zheda 619	0	94.5 ± 9.8 de	6.94 ± 1.00 de	23.10 ± 5.36 d	0.3 ± 0.0 i	0.11 ± 0.00 h
	100	87.4 ± 4.7 ef	5.13 ± 0.36 f	31.63 ± 5.23 c	114.1 ± 13.0 h	88.70 ± 3.23 g
	200	101.0 ± 5.5 de	6.55 ± 0.91 e	33.45 ± 5.75 c	221.2 ± 7.9 f	117.53 ± 5.36 e
	300	118.3 ± 3.5 c	7.07 ± 0.38 de	38.03 ± 5.60 c	253.9 ± 11.0 d	129.33 ± 9.34 d
	500	144.3 ± 11.3 b	7.83 ± 0.16 cde	49.26 ± 2.74 ab	392.9 ± 6.3 b	199.46 ± 6.71 b
ZS 758	0	63.1 ± 7.5 g	7.19 ± 0.16 de	19.43 ± 2.70 d	0.3 ± 0.0 i	0.12 ± 0.01 h
	100	77.4 ± 8.4 f	7.96 ± 0.75 cd	21.80 ± 1.95 d	148.4 ± 7.1 g	100.50 ± 3.17 f
	200	104.7 ± 6.2 d	8.81 ± 0.31 bc	35.23 ± 2.15 c	237.7 ± 2.1 e	132.46 ± 13.01 d
	300	152.2 ± 11.3 b	9.76 ± 0.55 b	46.96 ± 1.26 b	280.4 ± 19.0 c	145.53 ± 4.30 c
	500	191.3 ± 7.4 a	14.01 ± 0.65 a	56.00 ± 7.01 a	413.5 ± 7.3 a	218.93 ± 7.27 a

(100 and 200 μM) and then decreased. In the Cd-treated plants, there was a decline in protein content in both cultivars and maximum reduction was at 500 μM Cd (33.6 % in Zheda 619 and 33.0 % in ZS 758; Table 4).

The MDA content increased with increasing Cd concentration more in ZS 758 than in Zheda 619. In ZS 758, the production of H_2O_2 increased with increasing Cd concentration, however, in Zheda 619, H_2O_2 content increased only at 300 and 500 μM Cd. The production of $O_2^{\cdot-}$ enhanced due to addition of Cd and reached

maximum at 500 μM Cd in both cultivars. Cd content in the shoots and roots increased with increasing Cd concentration in both cultivars (Table 5). Cd content was higher in ZS 758 than in Zheda 619 and accumulation of Cd was higher in the roots than in the shoots of both cultivars.

TEM micrographs of the control leaf mesophyll cells of Zheda 619 and ZS 758 show clean and thin cell walls. The cytoplasm contained flattened cell membranes and

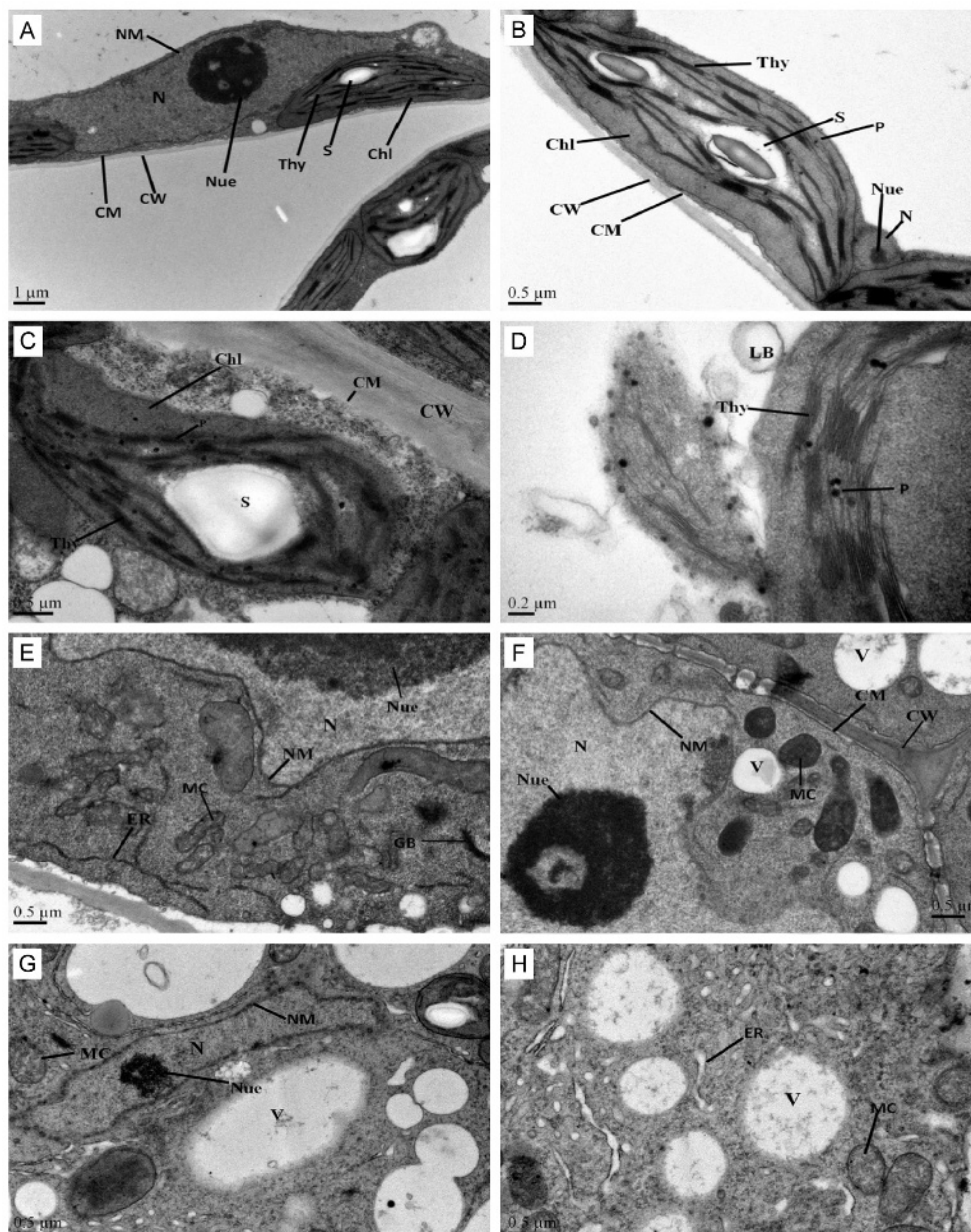

Fig. 1. Electron micrographs of leaf mesophyll and root tip cells of 6-d-old seedlings of two cultivars of *Brassica napus* (Zheda 619 and ZS 758) grown without (control) and with 500 μM Cd. *A*,*B* - leaf mesophyll cells of Zheda 619 and ZS 758 controls, respectively, show well-developed nucleus (N) with nucleoli (Nue), cell membrane (CM), cell wall (CW), well-developed chloroplast (Chl), and numerous starch grains (S). *C* - leaf mesophyll cells of Zheda 619 at 500 μM Cd show a damaged chloroplast with a large starch grain (S). *D* - leaf mesophyll cell of ZS 758 show a disruptured chloroplast with totally damaged thylakoid membranes. Accumulation of Cd is present in the form of electron dense granules inside vacuoles and attached to the cell walls. *E*,*F* - control root tip cells of Zheda 619 and ZS 758, respectively, show a well-developed nucleus (N) with nucleoli (Nue), a distinct nuclear membrane (NM), a number of vacuoles (V), mitochondria (MC), endoplasmic reticulum (ER), cell membrane (CM), and cell wall (CW). *G* - root tip cells of Zheda 619 at 500 μmol Cd show an elongated nucleus (N), a number of large vacuoles (V), and undeveloped mitochondria (MC). *H* - root tip cells of ZS 758 at 500 μmol Cd show a number of large vacuoles (V), undeveloped mitochondria (MC), pieces of endoplasmic reticulum (ER) and nucleus disappeared. Accumulation of Cd in the form of electron dense granules is present inside vacuoles.

nearly well-developed nucleus. Moreover, properly developed chloroplasts having closely-arranged and well-aligned granum thylakoids along with number of starch grains and plastoglobuli were observed (Fig. 1*A*,*B*). However, at 500 µM Cd, chloroplasts were elongated and starch grains larger. In ZS 758, the outer membrane of chloroplasts was disrupted and the thylakoid membranes were broken at different places. The Cd deposition in the form of electron dense granules in the leaf mesophyll cells could be seen in both cultivars but more prominently in the ZS 758 (Fig. 1*C*,*D*).

TEM micrographs of the control root tip cells show dense cytoplasm, flattened cell membranes, and centrally located well-shaped nucleus. There were small vacuoles, round-shaped mitochondria, and number of plastids (Fig. 1*E*,*F*). The root tip cells at 500 µM Cd show destroyed cellular structures and the root tip cells of ZS 758 were more damaged. Increased vacuoles, smaller nucleus, and ruptured nuclear envelope were some of the apparent variations. Moreover, Cd accumulation was observed (the appearance of electron dense granules in vacuoles and cell walls) in both cultivars (Fig. 1*G*,*H*).

Discussion

Plants can be distinguished into metal tolerant and metal sensitive on the basis of their growth in metal contaminated soils (Macnair 1993). Germination assay is considered as a basic way to determine toxic effect of heavy metals (An 2006, Labra 2006). We found that low Cd concentration (100 µM) stimulated the oil-seed rape germination and the seedling growth within 24 h of Cd stress. After 6 d, the lengths of the hypocotyl and radical were negatively affected by the higher Cd concentrations (200 - 500 µM) as compared to the respective controls. Our results confirm the findings of Chugh and Sawhney (1996), who observed similar effect of Cd on seed germination of different crops. The sensitivity of seedling growth has been found previously in cotton (Daud *et al.* (2009a) and barley (Wu and Zhang 2002). Moreover, our investigation also shows that the biomass of the seedlings was significantly decreased due to elevated Cd concentrations. These results coincide with the findings of Daud *et al.* (2009a,b) and Bachir *et al.* (2004) in cotton. Inhibition of plant growth might be due to inhibitions of photosynthesis, both photosystems, and the enzymes of carbon reduction cycles (Greger and Ogren 1991, Somashekaraiah *et al*. 1992, Skorzynska and Baszynski 1997). In our study, chlorophyll content decreased with increasing Cd concentration which might be due to disturbance in the supply of Fe^{2+}, Zn^{2+}, and Mg^{2+} (Kupper *et al.* 2000) or due to inhibition of protochlorophyllide reduction and inhibition of aminolevulinic acid synthesis under Cd stress (Stobart *et al*. 1985).

In the present study, most antioxidant enzyme activities decreased under Cd stress, although some of them remained high at 100 or 200 µM Cd. We found some contrasting reports in literature about Cd effects on activities of antioxidant enzymes, *e.g*., Gallego (1996) observed decreased activities of SOD, CAT, APX, and GR in sunflower leaves but Shamsi *et al.* (2008) observed that Cd increased activities of SOD and POD in soybean plants. Najeeb *et al.* (2011) also found reduced activities of CAT, APX, and POD in *Juncus effusus* when Cd was applied at high concentrations. Reduction in antioxidant enzyme activities might be due to inhibition of enzyme syntheses and due to change in the assemblage of enzyme subunits (MacRae and Ferguson 1985, Garnier *et al*. 2006).

Total soluble protein content was reduced by Cd in both cultivars which was in line with Singh and Sinha (2005) who observed decreased soluble protein content in *Brassica juncea* under heavy metal stress. We observed that addition of Cd produced oxidative stress and significantly increased the MDA content in both cultivars. The production H_2O_2 and $O_2^{\cdot-}$ can be a result of various mechanisms involving electron transfer (Dietz *et al*. 1999).

As we expected, Cd content in different parts of a plant was concentration dependent. Cd content in the roots was higher than in the shoots of both cultivars due to the longer radicles than the hypocotyls and lower translocation (Wong *et al.* 1984, Chugh *et al.* 1992). It is important to mention that high retention of heavy metals in roots is desirable in a crop like *Brassica* of which mainly seeds are used.

Electron microscopy helps to evaluate the damage of cell structures induced by Cd (Caasilit *et al.* 1997). In the present study, the mesophyll cells were totally damaged under 500 µM Cd and ZS 758 was more affected than Zheda 619. Nucleus which has a role to control the action of cell by selective expression of genes (Jiang *et al.* 2007) became irregular and finally disappeared. Chloroplasts were deformed, thylakoid membranes were disorganized, and outer membranes of chloroplasts were ruptured. It has been stated that chloroplasts are highly susceptible to presence of metal ions in their micro-environment (Zhang *et al.* 2003). In *Juncus effusus*, Cd also increases the number of plastogobuli and disrupted thylakoid membranes (Najeeb *et al*. 2011). Our results confirm the findings of Jin *et al*. (2008), who have concluded that Cd damages the cell ultrastructure due to oxidative stress.

In conclusion, the two oilseed rape cultivars used in the present study had different capability to face the Cd toxicity. Under the light of morphological, physiological, and ultrastructural results, we conclude that Zheda 619 was more tolerant to Cd-stress than ZS 758. Moreover, our study brings results useful for the phytoremediation and related areas. To determine toxic effects of Cd on these *Brassica* cultivars in the field, further investigation is needed.

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