INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–487/2016/18–3–557–564
DOI: 10.17957/IJAB/15.0124
http://www.fspublishers.org

Full Length Article

Alleviation of Cadmium Toxicity by 5-Aminolevulinic Acid is related to Improved Nutrients Uptake and Lowered Oxidative Stress in *Brassica napus*

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Abstract

The oilseed rape production (*Brassica napus* L.) has been shown to decrease under heavy-metal stress. This study tested the hypothesis that 5-aminolevulinic acid (ALA) has ameliorating role under cadmium (Cd)-toxicity in *B. napus*. In the present study, three levels (0, 100 and 500 µM) of Cd and three levels (0, 12.5 and 25 mg/L) of foliar ALA were applied hydroponically in greenhouse. Results demonstrated that ALA significantly enhanced the leaf chlorophyll contents under Cd stress as compared to control. Exogenously applied ALA enhanced the concentration of macro- and micronutrients in the leaves and roots of *B. napus* under Cd stress. Further, foliar application of ALA significantly decreased malondialdehyde and reactive oxygen species in the leaves of *B. napus* under Cd stress. Moreover, the present study revealed that leaves treated with ALA at different concentrations increased the concentration of glutathione reduced (GSH), glutathione oxidized (GSSG), total glutathione and soluble protein contents as well as decreased the GSH/GSSG ratio under Cd-toxicity. The present study stated that ALA improved the concentration of nutrients and decreased oxidative stress due to its ameliorative capability under Cd stress conditions in *B. napus*. © 2016 Friends Science Publishers

Keywords: Cadmium; Non-antioxidant enzyme activities; Elements uptake; Oilseed rape; Reactive oxygen species

Introduction

In atmosphere, various anthropogenic activities such as mining, industrialization, and phosphate fertilizers are directly or indirectly responsible for the environmental pollution (Zhou and Qiu, 2005). Metal toxicity has received great attention worldwide because large amounts of these metals are released into the environment every year. Heavy metal toxicity, especially by cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) constitute serious threats to human health (Wenneberg, 1994). A part of agricultural soils in the world is contaminated with Cd as slightly to moderately due to the usage of phosphate fertilizers, sewage water as well as sedimentation (Thawornchaisit and Polprasert, 2009). Cd is most toxic element to plants and at high concentration (5 mM), can reduce chlorophyll contents and photosynthesis in *B. napus* (Baryla *et al.,* 2001; Zhou and Huang, 2001). Cd can disturb the uptake and distribution of macro- and micronutrients in the plants (Ramos *et al.,* 2002). Previously, Rivetta *et al.* (1997) reported the Cd competition with elements i.e. Fe, Zn, Mn, Cu, Ca, Mg and P in radish plants. Aerobic metabolism faces a constant risk of being disturbed by ROS like free radicals O_2^- and $^{\cdot}OH$, and H_2O_2 (Kanazawa *et al.,* 2000). Cd-toxicity causes the oxidative stress through accumulation of ROS in plant, which ultimately lead to excessive lipid peroxidation (Shah *et al.,* 2001).

Application of plant growth regulators (PGRs) is a promising approach to increase the plant tolerance against abiotic stresses (Ai *et al.,* 2008; Ali *et al.,* 2013a). PGRs can improve the productivity of plants by regulating the physiological characters. In fact, many PGRs have been found to enhance the stress tolerance in plants (El-Tayeb, 2005; Zhang *et al.,* 2008). ALA is an important PGR and it is a precursor for biosynthesis of chlorophyll (Von Wettstein *et al.,* 1995). ALA exerts positive effects on the plants and increases the chlorophyll content and antioxidants under saline conditions (Watanabe *et al.,* 2000; Naeem *et al.,* 2012). Recently, it was found that ALA improved the concentration of different nutrients in *B. napus* under salinity stress conditions (Naeem *et al.,* 2010). Similarly, an increase in antioxidant enzymes activities was reported by ALA under salinity in Indian mustard and sugar beet (Bor *et al.,* 2003; Youssef and Awad, 2008). ALA and ascorbic acid have been shown to ameliorate the effect of water stress in *B. napus* and *Zea mays*, respectively (Liu *et*

To cite this paper: Xu, L., B. Ali, R.A. Gill, L. Li and W. Zhou, 2016. Alleviation of cadmium toxicity by 5-aminolevulinic acid is related to improved nutrients uptake and lowered oxidative stress in *Brassica napus. Int. J. Agric. Biol*., 18: 557–564

al., 2011; Dolatabadian *et al.,* 2009).

Nowadays, rapeseed *Brassica* crops are used in food and feed applications as well as in biofuel production. Phytoremediation with *B. napus* has greater potential to become a profitable enterprise (Grispen *et al.,* 2006). Rapeseed is the oldest cultivated oil-producing plant. A rapid increase in *B. napus* production has been observed during the last two decades all over the world (FAOSTAT, 2005). Therefore, it is necessary to resolve the Cd-toxicity problems in *B. napus* fields on urgent basis. However, in the previous study, we found that ALA improved the root growth in *B. napus* by improving biochemical and ultrastructural changes under Cd stress (Ali *et al.,* 2013b). Keeping in view the alleviating ability of ALA, this study was planned to clarify the hypothesis that ALA has the ameliorating role to improve Cd-induced growth and nutrients uptake, and decrease the oxidative stress in *B. napus.*

Materials and Methods

Plant Material and Treatments

Seeds of oilseed rape (*Brassica napus* L. cv. ZS 758) were obtained from the College of Agriculture and Biotechnology, Zhejiang University. Seeds were washed with distilled water and then air-dried. Seeds were sown in plastic pots (170 mm × 220 mm) filled with peat moss. After thirty days of sowing, morphologically uniform seedlings were selected and plugged into plate holes on plastic pots (five plants per pot) containing a half strength nutrient solution (Arnon and Hoagland, 1940), aerated continuously with air pump in the greenhouse. The pH of solution was maintained at 6.0 and light intensity was in the range of 250–350 µmol m^{-2} s^{-1}. Temperature and relative humidity were adjusted at 16–20°C and 55–60% respectively.

After acclimatization period, desired Cd concentrations (0, 100 and 500 µM Cd) were adjusted and plants were simultaneously sprayed with an aqueous solution of ALA (Cosmo Oil, Japan) at three concentrations (0, 12.5 and 25 mg/L ALA). The treatment concentrations were based on pre-experimental studies, in which Cd at 100 µM concentration showed a little damage on plant growth and 500 µM Cd imposed a significant damage to plant growth, while those higher than 500 µM were too toxic for plant growth (Ali *et al.,* 2014). After every 5 days, a subsequent foliar spray of ALA was applied. The control plants were sprayed with distilled water. After fifteen days, all physiological data were measured and samples were collected for nutrient uptake and biochemical analysis.

Morphological and Elemental Measurements

Plant biomass (% of control) against each treatment was calculated according to Ali *et al.* (2011) by using this formula: (% of control) = (Mean value in treatment - Mean value in control/Mean value in control × 100). A chlorophyll meter (Minolta Co. Ltd., Japan) was used to take SPAD values of the fully expanded functional leaves (the 4th from the apex), which provided a rapid, accurate, and non-destructive estimate of leaf chlorophyll content. For this purpose, a total of 20 readings per treatment were taken from randomly selected plants to determine chlorophyll content according to Wu *et al.* (1998).

After 15 days of treatments, plants were differentiated to leaves and roots, washed with tap water and distilled water thrice, respectively and then blotted to remove the excessive water. Thereafter, plants were separated into roots and leaves (topmost fully expanded leaf) and then dried at 80°C in an oven for 48 h, and ground into powder. Plant samples of roots and leaves (0.2 g) were digested with a mix of 5 mL of HNO_3 (65%) + 1 mL of $HClO_4$ (72%). After that the resultant solutions were diluted to 25 mL using 2% HNO_3 and filtered. The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+} and Zn^{2+} in the filtrate were determined using inductively coupled plasma-mass spectrometry (ICP-MS, Agilent, 7500a) following a standard procedure.

The nitrogen content was determined according to the method of Lindner (1944). Plant dried samples (leaves and roots) were digested with 3 mL of H_2SO_4-H_2O_2 (30%) to obtain a clear solution. To the digested material, 2.5 N NaOH, 1% sodium silicate and Nessler's reagent were added. The absorbance of solution was read at 525 nm, using the spectrophotometer. Phosphorus content was estimated according to the method of Murphey and Riley (1962) by using molybdenum blue method and absorbance was read at 660 nm in a spectrophotometer. Sulphur content was measured by Butters and Chenery (1959) method using barium sulfate and absorbance was taken at 440 nm with spectrophotometer.

Malondialdehyde and Reactive Oxygen Species

Lipid peroxidation was estimated in terms of malondialdehyde (MDA) contents and was determined as 2-thiobarbituric acid (TBA) reactive substances following the method of Zhou and Leul (1998). H_2O_2 contents were determined according to the method of Velikova *et al.* (2000) with some modifications. To 0.5 mL of the supernatant, 0.5 mL of phosphate buffer (pH 7.0) and 1.0 mL of potassium iodide (1 mM) were added. The absorbance of the mixture was taken at 390 nm. H_2O_2 content was determined using an extinction coefficient of 0.28 µM cm^{-1} and expressed as nmol g^{-1} FW. The O_2^- contents were determined according to Jiang and Zhang (2001) method. For estimation of ^-OH contents in leaves, 50 mg of leaves were incubated in 1 mL of 10 mM Na-phosphate buffer (pH 7.4) consisting 15 mM 2-deoxy-D-ribose at 37°C for 2 h (Halliwell *et al.,* 1987). Following incubation, an aliquot of 0.7 mL from the above mixture

were added to reaction mixture containing 3 mL of 0.5% (w/v) TBA and 1 mL glacial acetic acid, heated at 100°C in a water bath for 30 min and cooled down to 41°C for 10 min before measurement. The absorbance was measured at 532 nm and concentration was calculated using an extinction coefficient (155 mM cm^{-1}) and expressed in mmol g^{-1} FW.

Total Soluble Protein and Non-enzymatic Antioxidants Analysis

TSP contents were determined according to the method of Bradford (1976). GSH and GSSG contents were measured by following the method of Law *et al.* (1983) with some modifications. Leaves (0.3 g) were homogenized with 5 mL 10% (w/v) TCA and homogenate was centrifuged at 15,000 g for 15 min. The supernatant was used for the determination of GSH and GSSG contents. To assay total glutathione, 150 µL supernatant was added to 100 µL 6 mM DTNB, 50 µL glutathione reductase (10 units mL^{-1}), and 700 µL 0.3 mM NADPH. The total glutathione content was calculated from the standard curve. All the reagents were prepared in 125 mM NaH_2PO_4 buffer, containing 6.3 mM EDTA, at pH 7.5. To measure GSSG content, 120 µL of supernatant was added to 10 µL 2-vinylpyridine followed by 20 µL of 50% (v/v) triethanolamine. The solution was vortex-mixed for 30 s and incubated at 25°C for 25 min. Calibration curve was developed using GSSG samples treated exactly as above and GSH content was determined by subtracting GSSG content from the total glutathione content. Ascorbic acid (AsA) contents were measured according to Singh *et al.* (2006).

Statistical Analysis

The experiment was carried out through a randomized design. The analysis of variance was computed for statistically significant differences determined based on the appropriate two-way variance analysis (ANOVA). The results were the mean ± SD of at least three independent replicates and were analyzed using data processing system SPSS version 16.0 (SPSS, Chicago, IL, USA), followed by the Duncan's Multiple Range Test (DMRT).

Results

ALA Regulates the Plant Growth under Cd Stress

The data regarding plant biomass (% of control) and chlorophyll contents (SPAD value) are shown in Fig. 1. Results showed that Cd alone inhibited the plant growth significantly, which led to the significant decrease in plant biomass. However, exogenously applied ALA improved the biomass of *B. napus* plants under Cd stress. The maximum plant biomass (fresh and dry weight) was observed with ALA application alone at 25 mg/L dosage. The contents of chlorophyll were decreased by 30% and 40% under 100 and 500 µM Cd stress alone, respectively. However, application of ALA at higher concentration (25 mg/L) increased chlorophyll contents (26% and 21%) under both Cd levels (100 and 500 µM) respectively, compared to their respective controls.

ALA Improves the Nutrient Concentration in Leaves and Roots

Results showed that concentration of Na^+ increased as concentration of Cd increased alone in the solution and this uptake was more obvious in leaves than roots (Table 1). The maximum Na^+ contents were found at 500 µM Cd alone; however, application of ALA decreased the Na^+ concentration in different plant parts under Cd-toxicity. Higher dosage of ALA (25 mg/L) reduced the concentration of Na^+ in leaf (by 25% and 35%) and in root (by 14% and 28%) under 100 and 500 µM Cd stress respectively, than that of untreated plants. Exogenous ALA alone at 25 mg/L increased concentration of K^+ in leaf and root and increased the K^+ concentration in leaf (by 29% and 62%) under 100 and 500 µM Cd stress respectively, than that of untreated plants. However, K^+ concentration in root did not increase significantly under different concentrations of ALA and Cd. Moreover, this study revealed that ALA significantly decreased the Na^+/K^+ ratio in leaves and roots. Lower concentration of Cd (100 µM) alone showed no effect on the concentrations of Ca^{2+} and Mg^{2+} in leaves and roots, but higher concentration of Cd (500 µM) significantly reduced the concentrations of both elements (Table 1). ALA at 25 mg/L enhanced the contents of Ca^{2+} by 45% and 24% in leaf and root respectively; Mg^{2+} by 80% and 90% in leaf and root respectively, under 500 µM Cd stress level (Table 1).

The magnitude of macro- (N, P and S) and micro-nutrients (Mn, Zn, Fe and Cu) in leaves and roots under different levels of ALA and Cd are presented in Table 2. Higher level of Cd (500 µM) alone significantly reduced the concentration of macro- and micro-nutrients except Fe. This decline was 29% in N, 48% in P, 37% in S, 41% in Mn, 18% in Zn and 44% in Cu as compared to control. However, when ALA at 25 mg/L was foliar applied to plants under 500 µM Cd, the concentrations of all the nutrients was enhanced. This increase was 35% in N, 65% in P, 12% in S, 22% in Mn, 67% in Zn, 25% in Fe and 29% in Cu as than that of their respective controls. Cd alone at different levels gradually decreased the concentrations of macro- and micro-nutrients in roots of *B. napus* plants except Fe which was increased. Moreover, ALA under different Cd levels did not increase the concentrations of macro-nutrients. Whereas, ALA at 25 mg/L increased the concentration of Mn by 28% and 43%, Zn by 26% and 20%, Fe by 62% and 32%, Cu by 41% and 71% in roots under 100 and 500 µM Cd stress respectively, as compared to their respective controls.

Table 1: Effects of different treatments of 5-aminolevulinic acid (ALA) (mg/L) and cadmium (Cd) (μM) on the concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} and Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios in leaves and roots of *B. napus*

ALA conc.	Cd conc.	Leaf (mg g^{-1} DW)						Root (mg g^{-1} DW)					
		Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+ ratio	Ca^{2+}/Mg^{2+} ratio	Na^+	K^+	Ca^{2+}	Mg^{2+}	Na^+/K^+ ratio	Ca^{2+}/Mg^{2+} ratio
0	0	1.03 g	55.07 b	29.63 ab	4.95cd	0.02 e	5.97 ab	1.05 f	38.63 b	6.66 cd	2.07 bc	0.03 de	3.21 b
	100	1.79 b	39.89 e	29.16 ab	4.56 d	0.04 c	6.37 ab	1.48 c	35.44 bc	6.99 cd	2.03 bc	0.04 b	3.45 b
	500	2.34 a	26.12 g	17.61 d	2.65 f	0.09 a	6.70 a	2.04 a	30.41 c	4.74 f	0.96 e	0.07 a	4.95 a
12.5	0	1.13 f	53.95 b	28.97 ab	4.76cd	0.02 e	6.07 ab	1.21 de	40.69 b	6.56 d	1.98 bc	0.02 de	3.34 b
	100	1.62 c	47.02cd	31.29 ab	5.10 c	0.03 d	6.10 ab	1.34 cd	35.79 bc	7.66 b	2.33 b	0.04 bc	3.30 b
	500	1.83 b	32.99 f	19.45 cd	3.53 e	0.06 b	5.54 ab	1.76 b	29.57 c	5.20 f	1.53 d	0.06 a	3.54 b
25	0	1.27 e	61.70 a	31.86 ab	6.35 a	0.02 e	5.01 ab	1.07 ef	47.66 a	7.13 c	2.20 bc	0.02 e	3.25 b
	100	1.35 e	51.46bc	34.82 a	5.88 b	0.03 e	5.96 b	1.27 d	40.22 b	8.80 a	2.75 a	0.03 cd	3.21 b
	500	1.52 d	42.28de	25.45 bc	4.77cd	0.04 d	5.33 ab	1.46 c	35.60 bc	5.88 e	1.83 cd	0.04 b	3.21 b

Each value represents the mean of three replicates of each treatment. Within each column, means followed by same lowercase letters are not significantly different by the LSD test at $P \leq 0.05$

Table 2: Effects of different treatments of 5-aminolevulinic acid (ALA) (mg/L) and cadmium (Cd) (μM) on the concentrations of macronutrients (mg g^{-1} DW) and micronutrients (μg g^{-1} DW) contents in leaves and roots of *B. napus*

ALA conc.	Cd conc.	Leaf							Root						
		N^a	P^a	S^a	Mn^b	Zn^b	Fe^b	Cu^b	N^a	P^a	S^a	Mn^b	Zn^b	Fe^b	Cu^b
0	0	48.90 c	12.40 ab	4.46 a	0.06 cd	0.11 de	0.32 f	4.64 c	29.49 ab	4.25 b	3.38 a	0.36 c	0.24 bc	0.57 f	26.53 ab
	100	45.42 cd	9.38 bcd	3.51 ab	0.06 d	0.15 c	0.37 cd	3.65 e	21.46 cde	3.44 bc	2.65 ab	0.25 f	0.23 c	0.60 f	17.42 d
	500	34.58 e	6.39 d	2.82 b	0.04 f	0.09 f	0.32 f	2.62 h	16.66 e	2.67 c	2.23 b	0.16 h	0.15 f	0.99 c	11.55 e
12.5	0	52.63 bc	13.64 ab	4.45 a	0.06 d	0.10 ef	0.35 e	4.81 b	27.63 abc	4.43 b	3.33 a	0.37 b	0.20 d	0.87 d	24.67abc
	100	47.59 c	11.41abc	3.62 ab	0.06 c	0.17 b	0.39 b	4.18 d	23.72bcde	3.84 bc	2.69 ab	0.28 e	0.26 b	0.71 e	21.44bcd
	500	38.68 de	8.05 cd	2.96 b	0.04 f	0.12 d	0.35 de	3.11 g	19.57 de	3.28 bc	2.16 b	0.18 g	0.17 e	1.16 b	15.90 de
25	0	62.66 a	15.56 a	4.48 a	0.08 a	0.14 c	0.38 c	5.55 a	30.94 a	6.73 a	3.27 a	0.41 a	0.24 bc	0.87 d	27.77 a
	100	57.68 ab	13.48 ab	3.74 ab	0.07 b	0.21 a	0.42 a	4.58 c	26.64abcd	4.35 b	2.75 ab	0.32 d	0.29 a	0.97 c	24.63abc
	500	46.75 c	10.57bcd	3.17 b	0.04 e	0.15 c	0.40 b	3.37 f	21.72 cde	3.54 bc	2.30 b	0.23 f	0.18 de	1.31 a	19.72 cd

Each value represents the mean of three replicates of each treatment. Within each column, means followed by same lowercase letters are not significantly different by the LSD test at $P \leq 0.05$

[a] mg g^{-1} DW; [b] μg g^{-1} DW

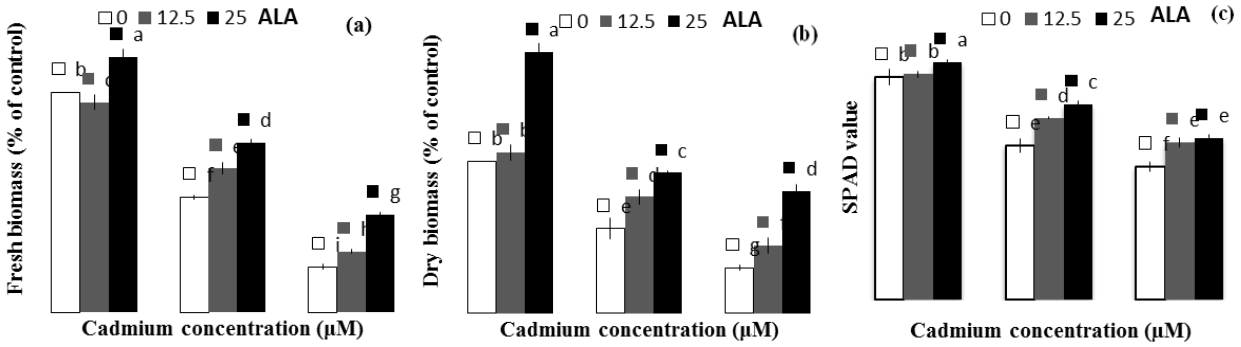

Fig. 1: Effects of different treatments of 5-aminolevulinic acid (ALA) (mg/L) and cadmium (Cd) (μM) on (a) fresh biomass (% of control), (b) dry biomass (% of control), and (c) SPAD value in the leaves of *B. napus* cv. ZS 758. Values are mean ± S.D. (n = 3). Means followed by the same letter did not significantly differ at $P \leq 0.05$ according to Duncan's multiple range test

ALA Reduces Cd-induced MDA and Various ROS

The contents of MDA, H_2O_2, O_2^- and ^-OH in the leaves of *B. napus* under different concentrations of Cd and ALA have been shown in Fig. 2. Figure illustrated that higher concentration of Cd (500 μM) alone significantly increased the MDA contents (78%) than that of control. Exogenous ALA at 25 mg/L decreased the MDA contents by (28% and 34%) under 100 and 500 μM Cd stress, respectively. Moreover, the higher level of Cd (500 μM) alone elevated the concentration of ROS contents in the leaves. Lower level of ALA (12.5 mg/L) showed no significant effect on H_2O_2 contents; however, it greatly decreased the level of O_2^- and ^-OH under Cd stress. Higher level of ALA (25 mg/L) significantly reduced the H_2O_2 content by 18 and 22%, O_2^- content by 36% and 32% and ^-OH content by 77 and 55% under 100 and 500 μM Cd stress, respectively as compared to their respective controls.

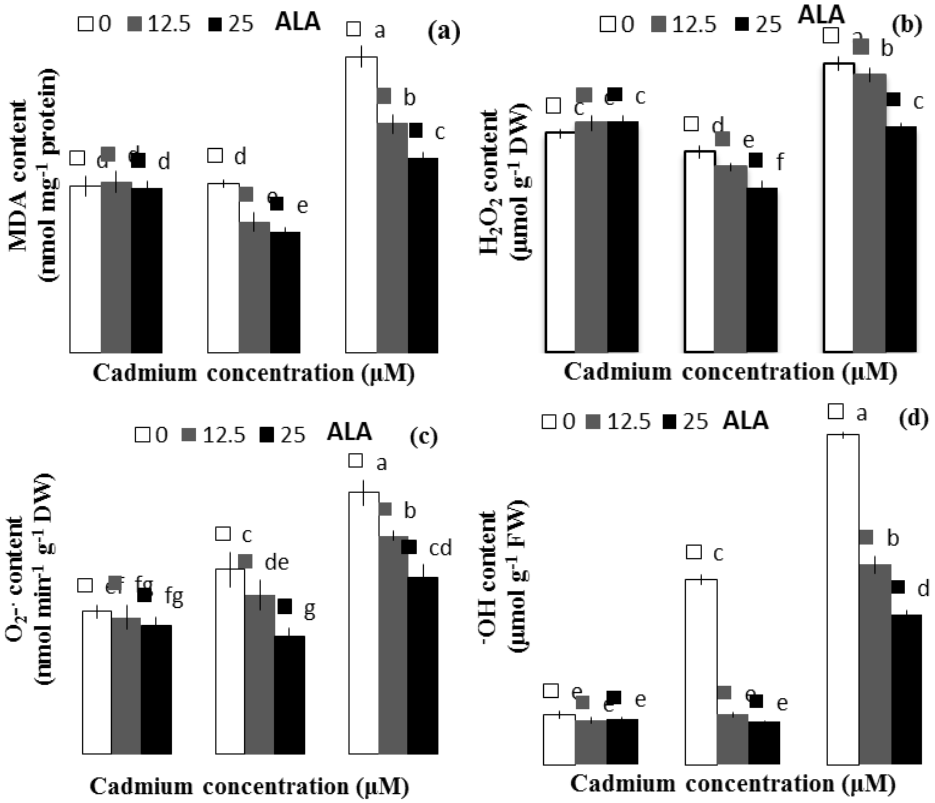

Fig. 2: Effects of different treatments of 5-aminolevulinic acid (ALA) (mg/L) and cadmium (Cd) (µM) on (a) malondialdehyde (MDA) content, (b) hydrogen peroxide (H_2O_2) content, (c) superoxide radical (O_2^{-}) content, and (d) hydroxyl ion (^{-}OH) content in the leaves of *B. napus* cv. ZS 758. Values are mean ± S.D. (n = 3). Means followed by the same letter did not significantly differ at $P \leq 0.05$ according to Duncan's multiple range test

ALA Alleviates Non-enzymatic Antioxidants and Soluble Protein Contents

Results showed that different levels of Cd (100 and 500 µM) alone did not show any significant changes in GSH (Fig. 3a) and GSSG (Fig. 3b) contents in *B. napus* leaves. However, ALA alone increased GSH and GSSG contents significantly as compared to control. This increase was (31% and 77%) in GSH contents and (by 81% and 53%) in GSSG contents under 12.5 and 25 mg/L ALA, respectively as compared to control. The GSH+GSSG contents were also increased significantly with different concentrations of ALA alone (Fig. 3c), and maximum total glutathione contents were observed at 25 mg/L ALA alone. This increase in GSH+GSSG contents was 76% more as compared to control. However, Cd alone with different concentrations showed no significant change in total glutathione contents. However, when exogenous ALA was applied to Cd-stressed plants, GSH+GSSG contents were increased significantly. The present study showed that GSH/GSSG ratio exhibited no change under various concentrations of Cd (Fig. 3d). The higher level of ALA alone enhanced the ratio of GSH/GSSG and showed maximum GSH/GSSG ratio (1225.39) in the leaves of *B. napus*.

Results described that lower level of Cd (100 µM) alone decreased the AsA contents (Fig. 3e) significantly, but higher level of Cd (500 µM) increased AsA contents in the leaves. This increase was 12% more as compared to untreated plants. ALA alone also improved the AsA contents by 61% and 91%, under 12.5 and 25 mg/L ALA treatment respectively. The Cd-treated plants sprayed with ALA showed significant increase in AsA contents. The AsA contents were increased by 96% and 115% with application of ALA at 25 mg/L under 100 and 500 µM Cd stress, respectively. TSP contents showed no significant change under ALA alone or Cd alone conditions (Fig. 3f). However, TSP contents were increased under the combine treatment of ALA and Cd at different concentrations. Maximum TSP contents were observed with the application of 25 mg/L ALA under the higher level of Cd (500 µM), and this increase was 39% more as compared to 500 µM Cd treatment alone (Fig. 3f).

Discussion

Metal toxicity is a complex phenomenon which shows negative effects on physiology of plants through element uptake and oxidative stress. In our previous study, we

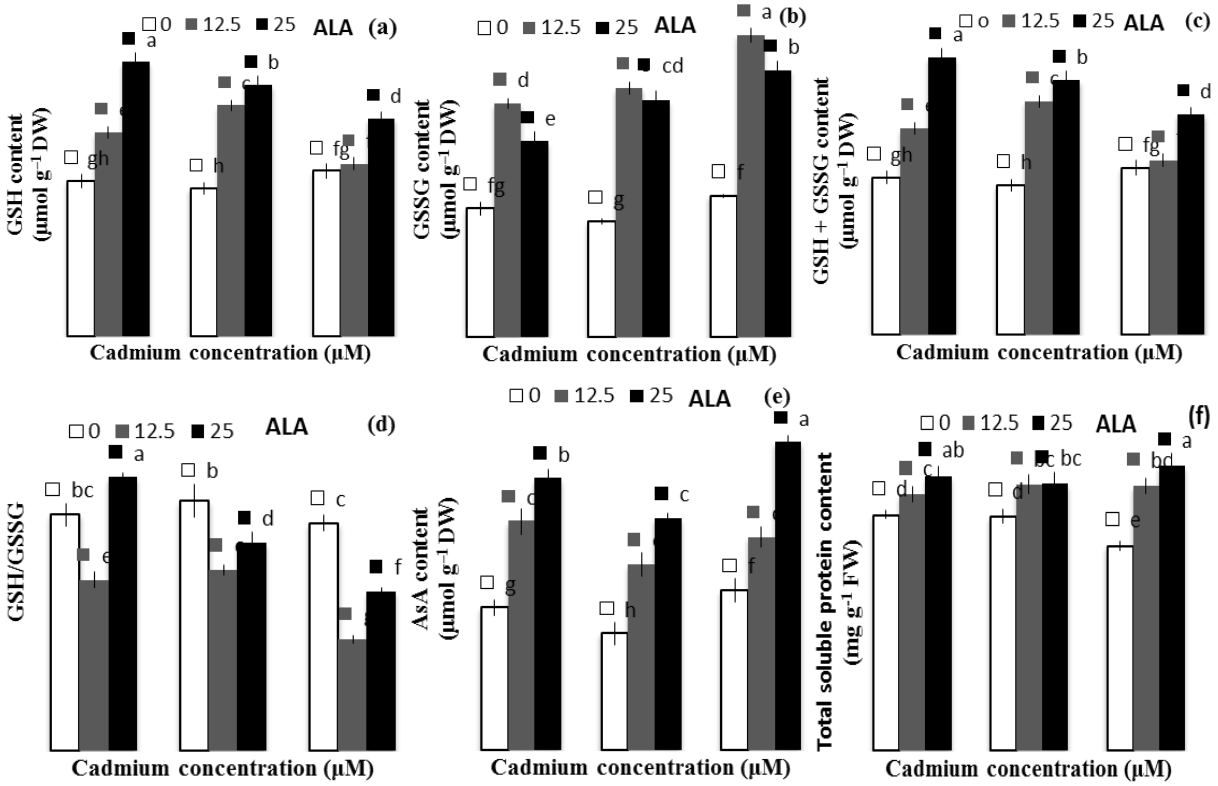

Fig. 3: Effects of different treatments of 5-aminolevulinic acid (ALA) (mg/L) and cadmium (Cd) (µM) on (a) reduced glutathione (GSH), (b) oxidized glutathione (GSSG), (c) total glutathione (GSH + GSSG) contents, (d) reduced/oxidized glutathione (GSH/GSSG) ratio, (e) ascorbic acid (AsA) and (f) total soluble protein (TSP) contents in the leaves of *B. napus* cv. ZS 758. Values are mean ± S.D. (n = 3). Means followed by the same letter did not significantly differ at $P \leq 0.05$ according to Duncan's multiple range test

evaluated that exogenous application of ALA under Cd-toxicity promoted the plant growth by improving root morphology, antioxidant enzyme activities and ultrastructural changes in *B. napus* (Ali *et al.*, 2013b). To understand how ALA ameliorates the Cd-induced plant growth by regulating elements uptake and oxidative stress in the leaves of *B. napus*, the current study was conducted. In the present study, plant growth parameter like plant biomass (% of control) significantly increased, when ALA was applied to Cd-stressed plants (Fig. 1). This is possibly because of the potential role of ALA to improve tolerance by decreasing lipid peroxidation through activation of antioxidant systems (Nishihara *et al.*, 2003; Youssef and Awad, 2008). It has been shown that ALA improves the plant growth by decreasing the effect of herbicide and salinity stress in oilseed rape and cotton respectively (Watanabe *et al.* 2000; Zhang *et al.* 2008). It was found that chlorophyll contents were reduced under the Cd stress alone, which might be due to destruction of photosynthetic apparatus and protein complex (Vassilev *et al.*, 1995; Ali *et al.*, 2013a), and inhibition of photosynthetic electron transport chain (Mohanty *et al.*, 1989). It was also observed that ALA alone at 25 mg/L significantly improved the chlorophyll pigments in the leaves of *B. napus* under Cd-toxicity (Fig. 1). ALA improves chlorophyll biosynthesis because it is a key precursor of biomolecules (Hotta *et al.*, 1997a, b; Naeem *et al.*, 2010).

Cd at different levels decreases the concentration of elements in plants (Larbi *et al.*, 2002; Ramos *et al.*, 2002). In the present investigation, effects of ALA on Na^+/K^+ and Ca^{2+}/Mg^{2+} ratios under Cd stress were measured. Recently, it is stated that enhanced ratios of Na^+/K^+ and Ca^{2+}/Mg^{2+} can affect the plant growth (Naeem *et al.*, 2012). Results showed that these ratios were increased as Cd level increased in the solution; however, at the same time, application of ALA minimized these ratios under Cd-toxicity (Table 1). Previously, Watanabe *et al.* (2000) also concluded that ALA at different concentrations notably reduced the Na^+/K^+ ratio in roots and leaves of cotton seedlings under salinity stress. Moreover, the reduction in K^+ uptake in leaves and roots under Cd stress might be due to antagonistic effect of Cd (Murphy *et al.*, 1999). This study also revealed that nutrient concentrations in roots and leaves were reduced under Cd-toxicity. Our observations about reduction of nutrients under Cd stress are in line with Ouariti *et al.* (1997) and Lagriffoul *et al.* (1998), who stated that toxicity of heavy metals, can reduce the uptake of nutrients. Moreover, in the previous study we found that Cd

stress alone increased the Cd concentration in shoots and roots of *B. napus* plants, thus, might be Cd reduced the nutrients concentrations due to antagonistic effect in the present study (Ali *et al.,* 2013a). It was found that exogenously applied ALA mitigated the Cd stress and enhanced the capacity of plants to store essential nutrients (Table 2). It may be due to the fact that ALA has a promotive role in regulating a number of metabolic processes, thereby, improving nutrient uptake under abiotic stress (Zhang *et al.,* 2008). Previously, it was found that ALA increased the uptake of different elements in the leaves of *B. napus* under salinity stress (Naeem *et al.,* 2010, 2012). Previously, Ahmad *et al.* (2011) applied the salicylic acid under Cd stress and noted that salicylic acid can improve the nutrient uptake in mustard plants.

Cd stress alone increased the production of MDA in the leaves of *B. napus*, however exogenously applied ALA significantly decreased the MDA contents under Cd stress (Fig. 2). Previously, Naeem *et al.* (2011) also found that exogenous ALA decreased the MDA production in the leaves of *B. napus* under salinity stress. Similar to these findings, in previous study, we found that ALA suppressed the MDA contents under Cd stress in *B. napus* roots (Ali *et al.,* 2013b). Moreover, ROS contents were increased under the Cd-toxicity alone conditions (Fig. 2). ROS production in plants at sub-cellular level under Cd stress was also demonstrated previously (Rodriguez-Serrano *et al.*, 2006; Ortega-Villasante *et al.*, 2007). Cd enhanced the level of ROS, whereas, exogenous ALA helped the plants to remove ROS by activating the activities of antioxidant enzymes as found in spinach (Nishihara *et al.*, 2003).

GSH and some emerging antioxidants like carbon monoxide and proline are considered as non-enzymatic antioxidant systems (Zhang and Chen, 2011) and play a crucial role in response to heavy metal stress. The present results showed that GSH contents were increased with the higher concentration of ALA (25 mg/L) alone (Fig. 3a). Moreover, findings of Nishihara *et al.* (2003) are agreement of our results who stated that ALA increases the GSH/GSSG ratio in spinach leaves under NaCl stress. In the present research, contents of AsA were enhanced under the higher Cd concentration (500 μM). Previously, Zengin and Munzuroglu (2005) also found similar results in *Phaseolus vulgaris* under metal stress. However, ALA showed a synergetic effect and enhanced the AsA contents in the leaves of *B. napus* under Cd stress (Fig. 3). Similar to these findings, recently, we also found that exogenously applied ALA ameliorated the Cd stress and improved the AsA contents in the roots of *B. napus* (Ali *et al.,* 2013b). As conclusion, it is found that Cd-toxicity can reduce the concentration of nutrients in the leaves and roots of *B. napus*. Moreover, it was also observed that ROS contents were enhanced in the leaves of *B. napus* under Cd-stressed alone conditions. Meanwhile, exogenously applied ALA reduced the ROS production by promoting non-antioxidant activities in the leaves of *B. napus* under Cd stress. Thus, it can be concluded that ALA has ameliorative role on *B. napus* under Cd-toxicity.

Acknowledgements

Authors would like to appreciate the Jiangsu Collaborative Innovation Center for Modern Crop Production, Zhejiang Provincial Top Key Discipline of Biology and its Open Foundation (2014C03, 2015D24), the Science and Technology Department of Zhejiang Province (2012C12902-1, 2011R50026-25), Zhejiang Provincial Education Department (Y201533983), and the Science Foundation of Zhejiang Sci-Tech University (14042216-Y) to provide the financial support for this study.

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(Received 06 May 2015; Accepted 26 October 2015)