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Role of jasmonic acid in improving tolerance of rapeseed (*Brassica napus* L.) to Cd toxicity*

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Abstract: The well-known detrimental effects of cadmium (Cd) on plants are chloroplast destruction, photosynthetic pigment inhibition, imbalance of essential plant nutrients, and membrane damage. Jasmonic acid (JA) is an alleviator against different stresses such as salinity and drought. However, the functional attributes of JA in plants such as the interactive effects of JA application and Cd on rapeseed in response to heavy metal stress remain unclear. JA at 50 μmol/L was observed in literature to have senescence effects in plants. In the present study, 25 μmol/L JA is observed to be a "stress ameliorating molecule" by improving the tolerance of rapeseed plants to Cd toxicity. JA reduces the Cd uptake in the leaves, thereby reducing membrane damage and malondialdehyde content and increasing the essential nutrient uptake. Furthermore, JA shields the chloroplast against the damaging effects of Cd, thereby increasing gas exchange and photosynthetic pigments. Moreover, JA modulates the antioxidant enzyme activity to strengthen the internal defense system. Our results demonstrate the function of JA in alleviating Cd toxicity and its underlying mechanism. Moreover, JA attenuates the damage of Cd to plants. This study enriches our knowledge regarding the use of and protection provided by JA in Cd stress.

Key words: Rapeseed; Cadmium; Jasmonic acid; Antioxidant enzyme; Malondialdehyde; Ultrastructure
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1 Introduction

The last few decades have been significant in terms of the studies conducted on the metal pollutant accumulation in soil. Metal pollutants reduce crop productivity and quality and are extremely dangerous to the food chain. Moreover, they disturb the ecosystem balance and threaten human health (Chen et al., 1999). Anthropogenic activities, including chemical fertilizer application, waste water irrigation, and animal waste, are dominant sources of heavy metal (e.g. cadmium (Cd), plumbum (Pb), zinc (Zn), and copper (Cu)) deposition into the soil (Wu and Zhang, 2010; Jiao et al., 2012).

Cd, which is often applied with phosphorous fertilizers, is among the most hazardous metal pollutants to plants and humans (Pinto et al., 2004). Various strategies, such as split nitrogen application in form of ammonium or urea fertilizer, are being adopted for vegetable cultivation in Cd-contaminated soil (Fan et al., 2017), but still Cd has the highest pollution index in China (Niu et al., 2013) and is known for its carcinogenic, mutagenic, and teratogenic effects on human health. Despite being a non-essential element for plant metabolism, Cd uptake by the roots and transportation to the shoot can easily be accomplished in plants.

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Cd is mobile in nature and it can bring physiological, biochemical, and genetic changes in plants. The well-recognized detrimental effects of Cd on plants are: (1) chloroplast structure destruction that leads to the inhibition of photosynthetic pigments and photosynthesis that ultimately decreases growth and yield (Shamsi et al., 2010); (2) irregular homeostasis of essential plant nutrients (e.g. calcium (Ca), magnesium (Mg), iron (Fe), and Zn), thereby leading to nutrient deficiency and eventual plant death (López-Millán et al., 2009); and (3) membrane degradation due to the production of reactive oxygen species (ROS) (Shamsi et al., 2008).

Flowering plants usually have defense responses that protect them from a continuously changing environment. Initially, the cell wall could resist the entry of Cd or other harmful metals into the cells. However, after Cd has crossed the cell wall, plant cells launch other detoxifying mechanisms such as phytochelation and stress protein upregulation against Cd. In addition, plant cells contain superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase, a group of antioxidant enzymes that actively participate in stress conditions (Larson, 1988). SOD produces hydrogen peroxide (H_2O_2) from ROS generated during oxidative stress. H_2O_2 is reduced to H_2O and O_2 by CAT and GPX (in the cytoplasm and other cellular compartments) or APX (in the ascorbate-glutathione cycle). This enzymatic defense system is augmented by stress signaling molecules that further improve defense system regulation in response to Cd stress. These signaling molecules, including jasmonic acid (JA), nitric oxide, salicylic acid, and ethylene, can directly or indirectly attenuate Cd phytotoxicity. In China, especially in the Zhejiang Province, rapeseed and rice are grown in rotation. Irrigation with contaminated water greatly pollutes the fields with heavy metals, especially Cd, that affect crops and pose serious threats to human health (Meng et al., 2009). Cd is one of the most toxic elements worldwide and has the highest pollution index in China (Niu et al., 2013).

JA is a natural plant hormone involved in many biological processes (Creelman and Mullet, 1997). The intensive research in stress physiology revealed its role as a signaling or stress-modulating molecule against various environmental stresses. Currently, JA is receiving much attention due to its multifunctional defense properties in plants against several abiotic stresses, including salinity (Tsonev et al., 1998), drought (Creelman and Mullet, 1997), and herbicides (Kaya and Doganlar, 2016). However, studies of its role in plants under heavy metal stress are few (Chen et al., 2014), especially regarding the interactive effects of Cd and JA application on rapeseed oil. Thus, the aim of present study was to know: (1) the effects of exogenous JA application in rapeseed genotypes under Cd stress; (2) whether JA enhances gas exchange by protecting the chloroplast against oxidative stress and thereby keeping the ionic balance by reducing Cd uptake to the plant shoots; and (3) whether JA application modulates the antioxidant enzymes that can reduce and/or negate the detrimental effects of Cd on rapeseed oil.

2 Materials and methods

2.1 Soil properties

The soil collected from the experimental field of Huajiachi Campus, Zhejiang University, Hangzhou, China, was air-dried at room temperature with a water content of around 8%–10% with regular mixing. Approximately 8 kg of ground and sieved soil was loaded into a plastic pot (10 L, 20 cm height) to the brim. The physicochemical properties were the same as in our previous experiment (Ali et al., 2015).

2.2 Plant materials and experimental treatments

The pot experiment was done in the experimental wire house in Zhejiang University under natural light conditions. This study was conducted on three rapeseed genotypes (Zheshuang-72 (ZS72), Zhejiang-619 (ZJ619), and Zheshuang-758 (ZS758)) that were characterized and certified in different decades (Guan et al., 2012; Hussain et al., 2013, 2014).

Seeds were initially grown in trays containing a growing medium of 1/2 compost, 1/4 vermiculite, and 1/4 sand (v/v/v). Seedlings were kept at low temperature in the growth chamber for a month to provide vernalization treatment. Morphologically homogenous seedlings were selected and transplanted into pots for further investigation. The experiment used a completely randomized design with three replicates. Five seedlings per pot per treatment per replication were grown initially and only two healthy seedlings

per pot were kept until the end of the experiment. After two weeks of acclimatization, treatments were applied as follows: (1) control (untreated), (2) 75 mg/kg Cd, (3) 150 mg/kg Cd, (4) 300 mg/kg Cd, (5) 25 μmol/L JA, (6) 75 mg/kg Cd+25 μmol/L JA foliar spray, (7) 150 mg/kg Cd+25 μmol/L JA foliar spray, and (8) 300 mg/kg Cd+25 μmol/L JA foliar spray. $CdCl_2$ was used as a source of Cd and applied only once to the soil. Exogenous applications of JA (25 μmol/L) and distilled water containing acetone (control) were made four times with an interval of 3 d per application using a foliar spray. For the foliar spray, JA solution was prepared by dissolving JA in 100 μl of absolute ethanol and a concentration of 25 μmol/L was achieved with the appropriate volume of distilled water. The control solution contained acetone dissolved in water (Thaler et al., 1999). Data on physiological parameters were collected after a week of the last treatment. Leaf samples were taken per treatment and immediately stored at −80 °C for further analysis.

2.3 Measurement of leaf gas exchange

Net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i), and transpiration rate (T_r) were measured in the leaves of rapeseed genotypes using an infrared gas analyzer and portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA) on a clear sunny morning, with an air temperature of 25 to 30 °C, CO_2 concentration of 400 μmol/mol, relative air humidity of 80%–90%, and photosynthetic photon flux density (PPFD) of 1000 μmol/(m^2·s).

2.4 Analysis of photosynthetic pigments in leaves

Chlorophyll (chl *a* and chl *b*) content and carotenoid content were analyzed following the method of Wang et al. (2009). Briefly, 0.1 g of fresh leaf sample was sliced with scissors to provide maximum contact with the extract (acetone, ethanol, and distilled water; 4.5:4.5:1, v/v/v). The samples were placed in a glass tube containing the extract and stored at 4 °C for 48 h. After that, the solution absorbance was recorded at 645 and 663 nm spectrophotometrically.

2.5 Analyses of malondialdehyde content and antioxidant enzyme activity

The leaf sample (0.5 g) was ground and homogenized in 8 ml ice-cold 50 mmol/L phosphate buffer solution (PBS, pH 7.8) containing $Na_2HPO_4·12H_2O$ (16.385 g/L)+$NaH_2PO_4·2H_2O$ (0.663 g/L). The homogenized solution was centrifuged at 4 °C for 15 min at 12000 r/min. The supernatant of each sample was collected and stored at 4 °C before being used to determine the malondialdehyde (MDA) content and antioxidant enzyme activity.

MDA was analyzed according to the method described by Hodges et al. (1999). In brief, a solution containing 5% (v/v) trichloracetic acid with 2.5 g of thiobarbituric acid and enzyme extract was heated in a hot water bath at 95 °C for 15 min and cooled immediately on ice. To collect the supernatant, the samples were centrifuged at 4800 r/min for 10 min and measured spectrophotometrically at 532 nm (E_{532}= 0.155 L/(mol·cm)). Non-specific turbidity was corrected by subtracting the absorbance index obtained at 600 nm.

SOD (EC 1.15.1.1) analysis was done according to Giannopolitis and Ries (1977) as follows: one unit of SOD activity is the amount of enzyme required to inhibit 50% of *p*-nitro blue tetrazolium chloride photoreduction at 560 nm. POD (EC 1.11.1.7) activity and CAT (EC 1.11.1.6) activity were measured according to the methods of Cakmak and Marschner (1992). POD activity was determined using the increasing absorbance value of a solution, containing 50 mmol/L PBS (pH 7.8), 1.5 mmol/L guaiacol, 300 mmol/L H_2O_2, and enzyme extract, at 470 nm from guaiacol oxidation (E_{470}=26.6 L/(mol·cm)). CAT activity was measured by using the reduction in the absorbance index of a reaction mixture (50 mmol/L PBS (pH 7.8), 300 mmol/L H_2O_2, and the enzyme extract) caused by the decomposition of H_2O_2 (E_{240}=39.4 L/(mol·cm)) at 240 nm. APX (EC 1.11.1.11) activity was measured by analyzing a reaction solution containing 50 mmol/L PBS (pH 7.8), 7.5 mmol/L ascorbate, 300 mmol/L H_2O_2, and enzyme extract, following the method of Nakano and Asada (1981). This measurement is technically based on the principle of monitoring ascorbic acid oxidation by H_2O_2 at 290 nm (E_{290}=2.8 L/(mol·cm)).

2.6 Analysis of nutrient elements

Oven-dried (65 to 70 °C for 72 h) leaf samples were ground, weighed, and placed in a muffle furnace to be incinerated for 12 h at 500 °C. The ash was digested with 5 ml of 30% HNO_3, and diluted using

deionized H_2O. A flame atomic absorption spectrometer (Shimadzu, AA-6300, Kyoto, Japan) was used to analyze the concentrations of Cd and other mineral elements including Ca, Mg, Fe, and Zn.

2.7 Subcellular analysis using transmission electron microscopy

For subcellular analysis, transmission electron microscopy (TEM) studies were conducted on the fresh leaf sections (about 1 mm^2) from the middle of the topmost leaf in all three rapeseed genotypes. The assay was done as follows: (1) fixation of the leaf sample in 4% (v/v) glutaraldehyde in 0.2 mol/L PBS (pH 7.2) overnight; (2) postfixation in 1% (0.01 g/ml) osmium tetroxide (OsO_4) for 1 h; (3) washing in 0.2 mol/L PBS (pH 7.2) for 1–2 h; (4) dehydration in a graded ethanol series (50%, 60%, 70%, 80%, 90%, 95%, and 100%) followed by acetone (100%); (5) filtration of the samples and embedding in Spurr's resin; and finally, (6) preparation of ultra-thin sections (80 nm) and mounting of the samples on copper grids for visualization under TEM (JEOL TEM-1230 EX) at an accelerating voltage of 60.0 kV.

2.8 Statistical analysis

MSTAT-C software for DOS (MSTATC version 2.10, 1989) was used for statistical analyses. The data were mainly presented as mean±standard error (SE) and analyzed using the one-way analysis of variance (ANOVA) technique. The significance levels considered were $P\leq0.01$ or $P\leq0.05$. Summaries of the analyses are given in Tables 1 and 2. To conduct multiple comparisons of the significant means, the least significant difference (LSD) test was applied (Steel and Torrie, 1980). OriginPro v7.5 (OriginLab, Northampton, MA) was used to design the graphs and allot the SE bars.

3 Results

3.1 Leaf gas exchange under Cd and JA application

To assess the sole and interactive effects of Cd, JA, and genotypes on photosynthesis, the leaf gas exchange parameters were measured (Figs. 1 and 2). Increased Cd levels resulted in decreased gas exchange in leaves. For instance 42.56%, 35.99%, 11.87%, and 43.43% decreases are shown by P_n, G_s, C_i, and T_r, respectively, at 300 mg/kg Cd (Figs. 1a–1d). Significant differences were observed in all the genotypes for gas exchange except for C_i (Figs. 1i–1l). In contrast to Cd, a sole application of JA positively regulated all gas exchange parameters. Increases of 38.39% (P_n), 40.60% (G_s), 5.75% (C_i), and 45.36% (T_r) took place after JA application compared with those in the untreated plants (Figs. 1e–1h).

Interactive effects of genotypes with Cd and JA application were significantly different among genotypes regarding gas exchange parameters in different Cd levels (Figs. 2a–2d) and JA application (Figs. 2e–2h). The largest decrease was shown by rapeseed genotypes at the highest Cd level (300 mg/kg). For instance, at 300 mg/kg Cd, ZS72, ZJ619, and ZS758 decreased by 42.88%, 43.94%, and 40.57% in P_n, respectively (Fig. 2a); 35.09%, 37.42%, and 35.53% in G_s, respectively (Fig. 2b); 14.00%, 10.45%, and 11.11% in C_i, respectively (Fig. 2c); and 46.28%, 37.82%, and 46.55% in T_r, respectively (Fig. 2d). Moreover, JA application increased the levels of all the gas exchange parameters. For ZS72, after JA application, compared with the control, P_n, G_s, C_i, and T_r increased by 42.00%, 45.94%, 5.45%, and 46.85%, respectively. Similarly, JA improved gas exchange in terms of JA and Cd interaction (Figs. 2i–2l). Compared with their respective controls, JA application on leaves under 300 mg/kg Cd improved P_n, G_s, C_i, and T_r by 75.21%, 75.83%, 10.97%, and 89.37%, respectively (Figs. 2i–2l).

3.2 Role of JA on leaf pigment composition under Cd stress

Sole effects of Cd, JA, and genotypes on photosynthetic pigment composition are shown in Fig. 3. Cd negatively regulated the photosynthetic pigments in all concentrations, but an obvious decrease was observed with 300 mg/kg Cd. The average decreases in chl *a* (30.04% and 40.90%), chl *b* (26.23% and 35.91%), chl *a*+chl *b* (28.43% and 38.78%), and carotenoids (26.27% and 39.24%) were recorded at 75 and 300 mg/kg Cd (Figs. 3a–3d); however, JA application relatively increased chl *a* by 37.35%, chl *b* by 25.06%, chl *a*+chl *b* by 31.86%, and carotenoids by 23.50% compared with their respective controls (Figs. 3e–3h). Genotypic differences were found in rapeseed genotypes based on photosynthetic pigment composition (Figs. 3i–3l). Except for chl *b* (Fig. 3j), all genotypes were significantly different in their photosynthetic pigments.

Table 1 Analysis of variance for gas exchange, pigment composition, and MDA content

Treatment	DF	P_n	G_s	C_i	T_r	Chl *a*	Chl *b*	Chl *a*+*b*	Car	MDA
G	2	**	**	ns	**	**	**	**	**	**
Cd	3	**	**	**	**	**	**	**	**	**
JA	1	**	**	**	**	**	**	**	**	**
G×Cd	6	ns	ns	ns	ns	ns	ns	ns	ns	ns
G×JA	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cd×JA	3	**	**	**	**	**	**	**	**	**
G×Cd×JA	6	ns	ns	ns	ns	ns	ns	ns	ns	ns

G: genotypes; Cd: cadmium; JA: jasmonic acid; DF: degree of freedom; P_n: photosynthetic rate; G_s: stomatal conductance; C_i: intercellular CO_2 concentration; T_r: transpiration rate; Chl *a*: chlorophyll *a*; Chl *b*: chlorophyll *b*; Chl *a*+*b*: chlorophyll *a*+chlorophyll *b*; Car: carotenoid; MDA: malondialdehyde; ns: not significant; **: significant at $P \leq 0.01$

Table 2 Analysis of variance of antioxidant enzyme activity, Cd accumulation, and essential elements

Treatment	DF	CAT	APX	POD	SOD	Cd	Ca	Mg	Zn	Fe
G	2	**	**	ns	**	**	**	**	ns	**
Cd	3	ns	**	**	**	**	**	**	**	**
JA	1	**	**	**	**	**	**	**	**	**
G×Cd	6	ns	ns	ns	**	**	ns	ns	ns	**
G×JA	2	**	ns	ns	ns	*	ns	**	ns	**
Cd×JA	3	**	*	**	**	**	**	ns	ns	**
G×Cd×JA	6	ns	ns	ns	ns	ns	ns	ns	ns	**

G: genotypes; Cd: cadmium; JA: jasmonic acid; DF: degree of freedom; CAT: catalase; APX: ascorbate peroxidase; POD: peroxidase; SOD: superoxide dismutase; Cd: cadmium; Ca: calcium; Mg: magnesium; Zn: zinc; Fe: iron; ns: not significant; *: significant at $P \leq 0.05$; **: significant at $P \leq 0.01$

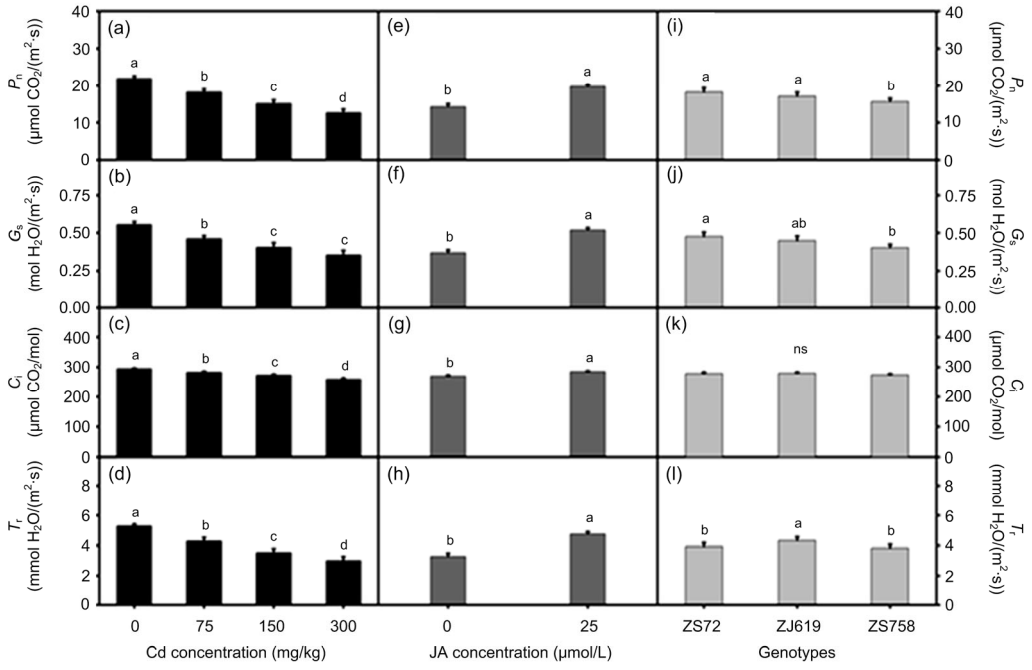

Fig. 1 Sole effects of genotypes, cadmium, and jasmonic acid on leaf gas exchange in rapeseed

(a–d) Cadmium; (e–h) Jasmonic acid; (i–l) Genotypes. (a, e, i) P_n: photosynthetic rate; (b, f, j) G_s: stomatal conductance; (c, g, k) C_i: intercellular CO_2 concentration; (d, h, l) T_r: transpiration rate. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter; ns: not significant. Data represent the mean±SE of three measurements

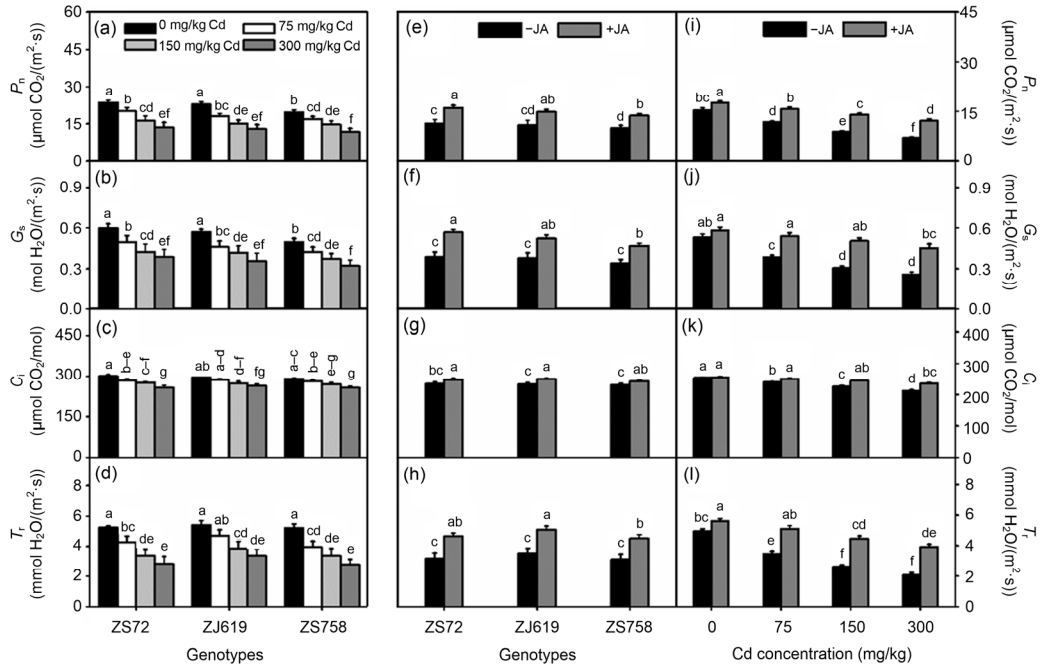

Fig. 2 Interactive effects of genotypes, cadmium, and jasmonic acid on leaf gas exchange in rapeseed
(a–d) Genotype×cadmium; (e–h) Genotype×jasmonic acid; (i–l) jasmonic acid×cadmium. (a, e, i) P_n: photosynthetic rate; (b, f, j) G_s: stomatal conductance; (c, g, k) C_i: intercellular CO_2 concentration; (d, h, l) T_r: transpiration rate. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference (P≤0.01) among the treatments for each parameter. Data represent the mean±SE of three measurements

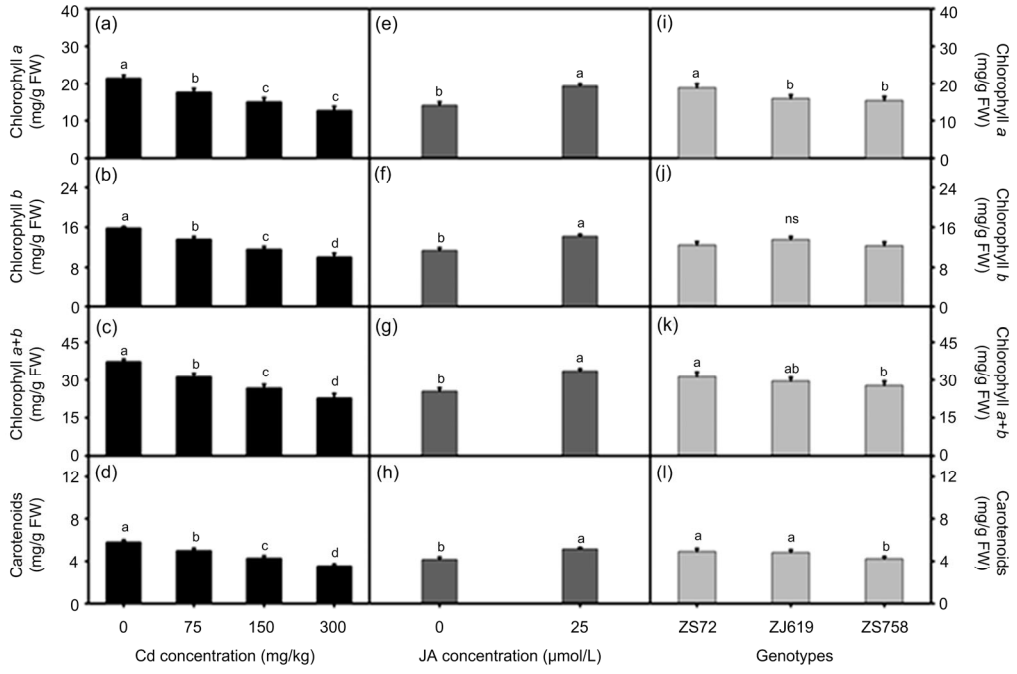

Fig. 3 Sole effects of genotypes, cadmium, and jasmonic acid on leaf photosynthetic pigments in rapeseed
(a–d) Cadmium; (e–h) Jasmonic acid; (i–l) Genotypes. (a, e, i) Chlorophyll *a*; (b, f, j) Chlorophyll *b*; (c, g, k) Chlorophyll *a*+*b*; (d, h, l) Carotenoids. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference (P≤0.01) among the treatments for each parameter; ns: not significant. Data represent the mean±SE of three measurements

Interaction among genotypes, Cd, and JA is shown in Fig. 4. As Cd concentration increased, a significant decrease was noticed in all genotype pigments (Figs. 4a–4d). However, JA application in genotypes and JA interaction showed a positive and significant effect. The average increases of 46.95%, 25.13%, 37.74%, and 18.80% in ZS72, 30.13%, 21.94%, 26.27%, and 25.40% in ZJ619, and 33.99%, 28.54%, 31.47%, and 27.00% in ZS758 were recorded for chl *a*, chl *b*, chl *a*+*b*, and carotenoids, respectively, after JA application compared with their respective controls (Figs. 4e–4h). Moreover, JA rectified the deleterious effects of Cd on pigments in co-applications of Cd and JA. For instance, under 300 mg/kg Cd application, average increases of 75.40% (chl *a*), 54.51% (chl *b*), 65.67% (chl *a*+*b*), and 51.06% (carotenoids) were noticed after foliar JA application (Figs. 4i–4l).

3.3 Decrease in Cd accumulation and MDA content by JA treatment under Cd stress

Fig. 5 shows the sole effects of Cd concentration, genotype, and JA levels on the Cd accumulation and MDA content in rapeseed leaves. As Cd concentration increased, Cd accumulation and MDA content in leaves were increased. For instance, average increases of 100.67, 144.73, and 167.59 mg/g DW in Cd accumulation and 24.93%, 92.46%, and 162.80% in MDA content were observed at 75, 150, and 300 mg/kg Cd, respectively (Figs. 5a and 5b). In contrast to Cd treatment, JA application decreased both Cd accumulation and MDA content in rapeseed leaves at an average of 29.12% (Cd) and 20.92% (MDA) compared with their respective controls (Figs. 5c and 5d). Moreover, genotypes showed significant differences in Cd accumulation and MDA content (Figs. 5e and 5f).

Interactions among Cd concentration, JA levels, and genotypes are shown in Fig. 6. As Cd concentration increased, a substantial increase was observed in the leaf Cd accumulation and MDA content in three rapeseed genotypes. At 300 mg/kg Cd treatment, average increases of 197.78, 162.63, and 142.38 mg/g DW in Cd accumulation were observed in ZS72, ZJ619, and ZS758, respectively (Fig. 6a), whereas 159.05%, 198.98%, and 136.77% increases were recorded for MDA content in ZS72, ZJ619, and ZS758, respectively, compared with the control (Fig. 6b). JA showed its effects by decreasing the Cd accumulation and MDA content in all genotypes. Average decreases of 33.79%, 30.01%, and 21.32% in Cd accumulation, and 19.66%, 23.38%, and 19.77% in MDA content were noticed in ZS72, ZJ619, and ZS758, respectively, after JA application (Figs. 6c and 6d). Interaction between Cd and JA application showed the alleviatory effects of JA over Cd treatments. JA application reduced Cd accumulation by 31.70%, 36.18%, 22.79%, and 29.48% and MDA by 8.18%, 20.06%, 21.83%, and 25.09% at 0, 75, 150, and 300 mg/kg Cd treatments, respectively (Figs. 6e and 6f).

3.4 Effects of JA application on antioxidant enzyme activity under Cd stress

Antioxidant enzymes mainly protect plants from stress. To keep the ionic balance in stressful environments, these enzymes exhibit different increasing and decreasing patterns. Sole and interactive effects of Cd, JA, and genotypes are shown in Figs. 7 and 8. Cd application significantly enhanced antioxidant enzyme activity in a dose-dependent manner, except for CAT (Figs. 7a–7d). JA application not only increased CAT activity but also decreased SOD, POD, and APX activity (Figs. 7e–7h). Moreover, different responses of genotypes to JA application were observed in all antioxidant enzyme activity, except in POD (Figs. 7i–7l). The interaction between genotypes and Cd showed that CAT activity remained non-significant in genotypes ZS72, ZJ619, and ZS758, compared with the control. Other enzymes (e.g. SOD, POD, and APX) showed an increasing trend in response to increased Cd concentration. Average increases of 44.61%, 53.54%, and 53.25% in SOD, 42.85%, 52.14%, and 79.08% in POD, and 47.65%, 20.47%, and 37.07% in APX activity were noted in ZS72, ZJ619, and ZS758, respectively, at 300 mg/kg Cd (Figs. 8a–8d). In genotype and JA interaction, JA treatment decreased all enzyme activity except for CAT, whose activity is increased after JA application in rapeseed genotypes (Figs. 8e–8h). JA decreased SOD, POD, and APX activity when co-applied with Cd and increased CAT activity in all applied Cd levels except 150 mg/kg. Average decreases of 10.74% (SOD), 28.19% (POD), and 18.97% (APX) were noted at 300 mg/kg after JA application (Figs. 8i–8l).

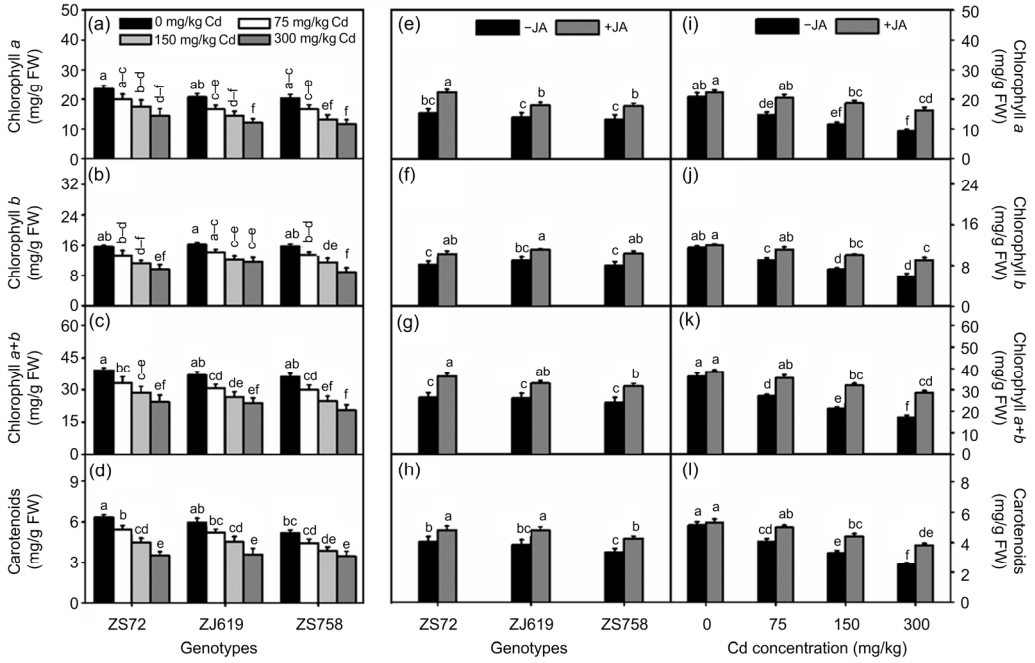

Fig. 4 Interactive effects of genotypes, cadmium, and jasmonic acid on leaf photosynthetic pigments in rapeseed
(a–d) Genotype×cadmium; (e–h) Genotype×jasmonic acid; (i–l) Jasmonic acid×cadmium. (a, e, i) Chlorophyll *a*; (b, f, j) Chlorophyll *b*; (c, g, k) Chlorophyll *a*+*b*; (d, h, l) Carotenoids. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter. Data represent the mean±SE of three measurements

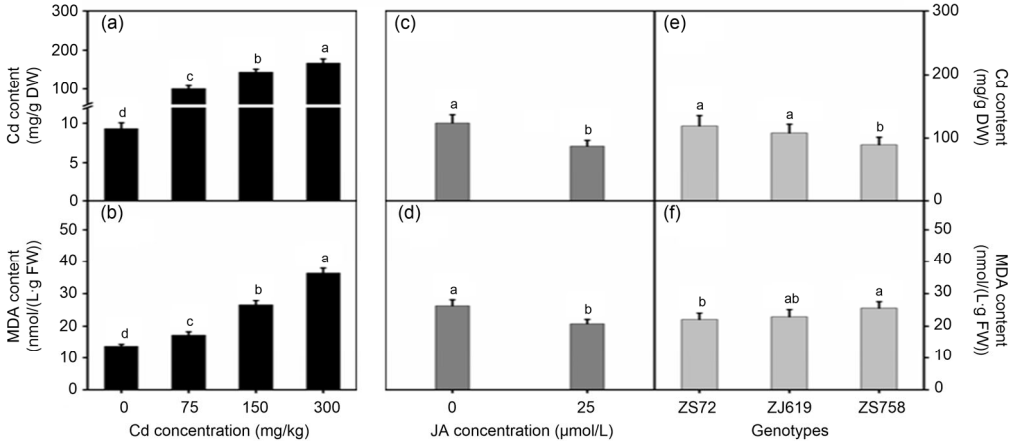

Fig. 5 Sole effects of genotypes, cadmium, and jasmonic acid on Cd accumulation and MDA content in rapeseed
(a, b) Cadmium; (c, d) Jasmonic acid; (e, f) Genotypes. (a, c, e) Cd: cadmium content; (b, d, f) MDA: malondialdehyde content. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter. Data represent the mean±SE of three measurements

3.5 Mineral elements under JA and Cd treatments

The sole effects of Cd, genotypes, and JA on leaf nutrient elements, except Zn, in rapeseed were found to be significant (Table 3). A significant and negative effect on elements was observed after Cd application. Cd treatment decreased all the elements in a dose-dependent manner and the highest decrease was found with 300 mg/kg Cd. In contrast with Cd, JA application significantly increased nutrient elements in rapeseed leaves (Table 3).

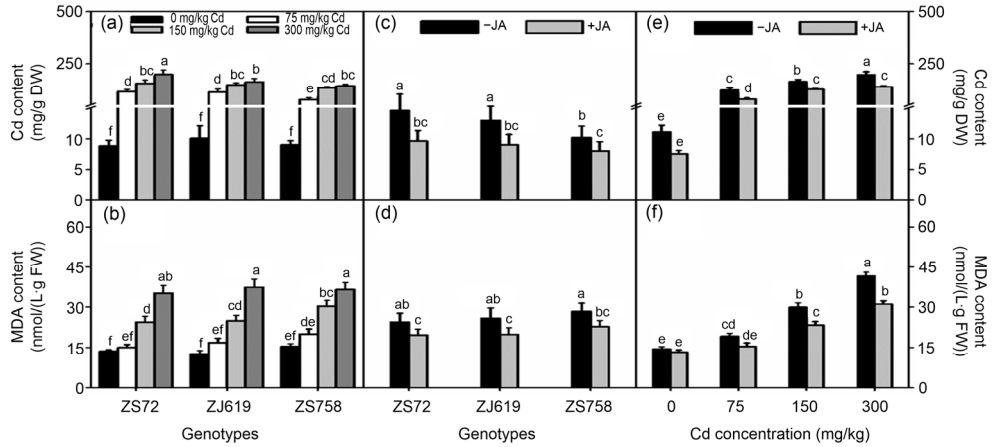

Fig. 6 Interactive effects of genotypes, cadmium, and jasmonic acid on Cd accumulation and MDA content in rapeseed
(a, b) Genotype×cadmium; (c, d) Genotype×jasmonic acid; (e, f) Jasmonic acid×cadmium. (a, c, e) Cd: cadmium content; (b, d, f) MDA: malondialdehyde content. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter. Data represent the mean±SE of three measurements

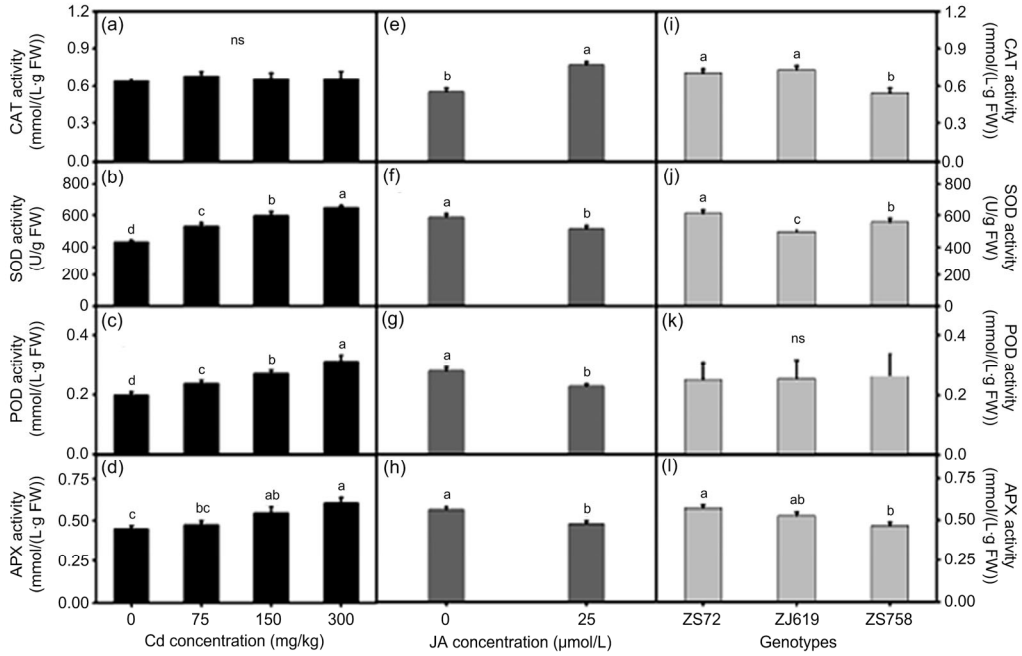

Fig. 7 Sole effects of genotypes, cadmium, and jasmonic acid on leaf antioxidant enzyme activity in rapeseed
(a–d) Cadmium; (e–h) Jasmonic acid; (i–l) Genotypes. (a, e, i) CAT: catalase; (b, f, j) SOD: superoxide dismutase; (c, g, k) POD: peroxidase; (d, h, l) APX: ascorbate peroxidase. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter; ns: not significant. Data represent the mean±SE of three measurements

The interactive effects of genotypes and Cd levels negatively regulated the mineral elements in leaves. Cd application decreased all the nutrient elements in a dose-dependent manner in all genotypes. The average decreases of 41.24%, 42.58%, 53.13%, and 56.66% in ZS72, 34.78%, 48.19%, 61.09%, and 52.72% in ZJ619, and 48.01%, 37.78%, 52.55%, and 54.55% in ZS758 were noted for Ca, Mg, Fe, and Zn, respectively, at 300 mg/kg Cd (Table 4).

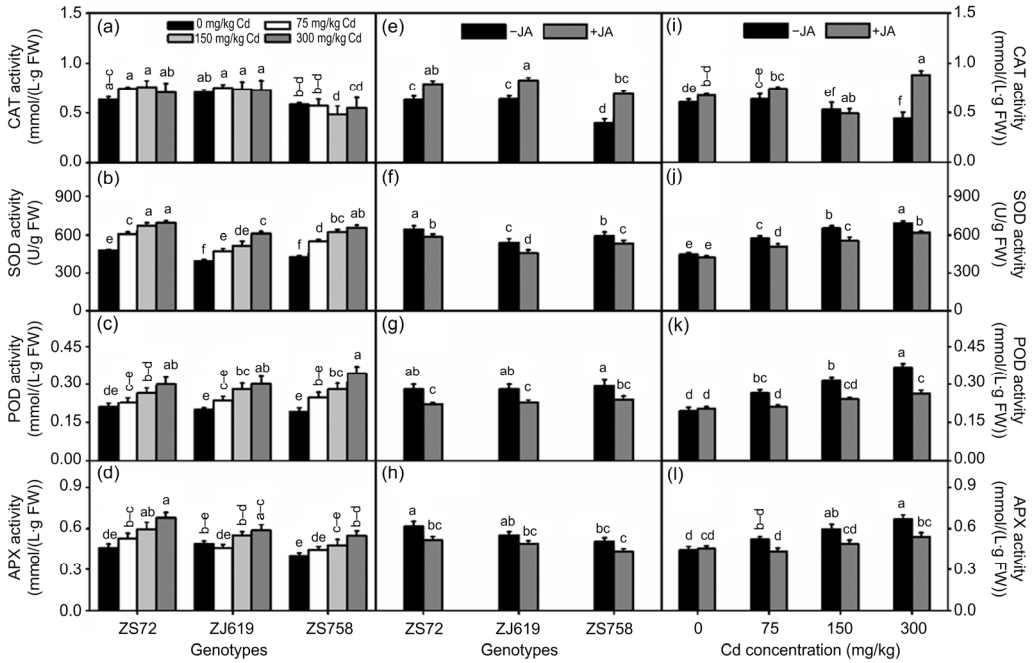

Fig. 8 Interactive effects of genotypes, cadmium, and jasmonic acid on leaf antioxidant enzyme activity in rapeseed
(a–d) Genotype×cadmium; (e–h) Genotype×jasmonic acid; (i–l) Jasmonic acid×cadmium. (a, e, i) CAT: catalase; (b, f, j) SOD: superoxide dismutase; (c, g, k) POD: peroxidase; (d, h, l) APX: ascorbate peroxidase. Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Differently lettering indicates statistical difference ($P \leq 0.01$) among the treatments for each parameter. Data represent the mean±SE of three measurements

Table 3 Sole effects of genotypes, cadmium, and jasmonic acid application on leaf nutrient elements in *Brassica napus*

Factor	Ca content (mg/g DW)	Mg content (mg/g DW)	Fe content (mg/g DW)	Zn content (mg/g DW)
Genotype				
ZS72	45.10±2.48^{a}	8.72±0.82^{a}	0.25±0.01^{a}	0.21±0.01^{a}
ZJ619	43.73±2.29^{a}	6.28±0.46^{b}	0.24±0.02^{a}	0.22±0.01^{a}
ZS758	38.12±2.61^{b}	4.90±0.26^{b}	0.18±0.01^{b}	0.20±0.01^{a}
Level of significance	**	**	**	ns
Cd concentration (mg/kg)				
0	53.32±1.53^{a}	8.73±0.73^{a}	0.32±0.02^{a}	0.30±0.01^{a}
75	45.66±2.17^{b}	7.16±0.69^{b}	0.25±0.02^{b}	0.23±0.01^{b}
150	39.03±2.70^{c}	5.79±0.64^{bc}	0.19±0.02^{c}	0.17±0.01^{c}
300	31.26±2.22^{d}	4.96±0.61^{c}	0.14±0.02^{d}	0.13±0.01^{c}
Level of significance	**	**	**	**
JA concentration (μmol/L)				
0	36.09±2.02^{b}	5.17±0.35^{b}	0.17±0.02^{b}	0.17±0.01^{b}
25	48.54±1.47^{a}	8.15±0.55^{a}	0.27±0.01^{a}	0.24±0.01^{a}
Level of significance	**	**	**	**

Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc; Cd: cadmium; JA: jasmonic acid; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Data represent the mean±SE of three measurements. Differently lettering indicates statistical difference ($P \leq 0.01$) in the same column. ** Significant at $P \leq 0.01$; ns: not significant

However, applying JA with Cd mitigated the negative effects of Cd by increasing the nutrient element absorption. After JA application, Ca increased by 35.66%, 56.35%, and 62.02%, and Mg increased by 57.55%, 84.52%, 106.81%, in leaves under 75, 150, and 300 mg/kg Cd, respectively. In addition, Fe and Zn increased by 58.89% and 40.02% at 75 mg/kg Cd, 83.55% and 60.93% at 150 mg/kg Cd, and 139.57% and 89.43% at 300 mg/kg Cd treatment, respectively (Table 5). Moreover, JA increased the mineral elements in rapeseed genotypes. In ZS72, ZJ619, and ZS758, the average increases after JA application were respectively

35.53%, 28.47%, and 40.47% in Ca; 89.70%, 44.07%, and 29.26% in Mg; 52.73%, 59.57%, and 63.15% in Fe; and 36.55%, 34.53%, and 45.43% in Zn (Table 6).

3.6 Subcellular study of rapeseed leaves under JA and Cd treatments

The physiological and biochemical changes observed in the leaves of rapeseed genotypes after Cd and JA treatments are observed at a subcellular level (Fig. 9). In untreated (control) plants, cells were metabolically active in terms of normal chloroplasts with starch granules in the stroma, intact cell walls, and membrane systems, active mitochondria, normal peroxisomes, and typical nuclei with nucleoli. Granal and stromal lamella maintained their architecture (Figs. 9a–9c). However, in Cd-treated leaves, the lamella were fragmented and disorganized, thylakoid membranes were disorganized with disrupted granal and stromal lamella, starch grains were present, and the nuclei had disintegrated nucleoli (Figs. 9d–9f). JA treatment alleviated the damage caused by Cd on the chloroplast. Exogenous JA application on Cd-treated plants retained the normal structures of the chloroplast and thylakoid membrane (Figs. 9g–9i).

Table 4 Interactive effects of genotypes and cadmium application on leaf nutrient elements in *Brassica napus*

Genotype	Cd (mg/kg)	Ca (mg/g DW)	Mg (mg/g DW)	Fe (mg/g DW)	Zn (mg/g DW)
ZS72	0	57.00±2.00^{a}	11.60±1.40^{a}	0.34±0.02^{ab}	0.30±0.02^{a}
	75	47.54±3.30^{bc}	9.11±1.70^{ab}	0.28±0.03^{bc}	0.23±0.02^{abc}
		(−16.62%)	(−21.93%)	(−18.84%)	(−23.33%)
	150	42.34±4.90^{cd}	7.41±1.50^{bcd}	0.22±0.02^{cde}	0.18±0.02$^{b–e}$
		(−25.74%)	(−36.50%)	(−33.82%)	(−40.00%)
	300	33.50±3.30^{de}	6.70±1.40$^{b–e}$	0.16±0.02^{efg}	0.13±0.02^{de}
		(−41.24%)	(−42.58%)	(−53.13%)	(−56.66%)
ZJ619	0	52.90±2.70^{ab}	8.32±0.50^{bc}	0.36±0.02^{a}	0.30±0.02^{a}
	75	46.73±3.70^{bc}	6.92±0.60$^{b–e}$	0.27±0.03^{bc}	0.25±0.02^{ab}
		(−11.66%)	(−16.82%)	(−24.87%)	(−17.41%)
	150	41.07±4.50^{cd}	5.59±0.80$^{d–f}$	0.18±0.03^{efg}	0.19±0.02$^{b–e}$
		(−22.36%)	(−32.81%)	(−49.76%)	(−38.05%)
	300	34.20±4.00^{de}	4.31±0.80^{ef}	0.14±0.03^{fg}	0.14±0.02^{cde}
		(−34.78%)	(−48.19%)	(−61.09%)	(−52.72%)
ZS758	0	50.00±2.80^{abc}	6.22±0.30$^{c–f}$	0.26±0.01^{cd}	0.29±0.02^{a}
	75	42.72±4.00^{cd}	5.45±0.30$^{d–f}$	0.19±0.03^{def}	0.21±0.02^{bcd}
		(−14.64%)	(−12.37%)	(−24.68%)	(−28.40%)
	150	33.68±4.50^{de}	4.37±0.40^{ef}	0.16±0.02^{efg}	0.16±0.02^{cde}
		(−32.70%)	(−23.95%)	(−37.97%)	(−43.74%)
	300	26.00±3.70^{e}	3.87±0.50^{f}	0.12±0.02^{g}	0.13±0.02^{e}
		(−48.01%)	(−37.78%)	(−52.55%)	(−54.55%)
Level of significance		**	**	**	**

Cd: cadmium; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc; ZS72: Zheshuang-72; ZJ619: Zhejiamg-619; ZS758: Zheshuang-758. Data represent the mean±SE of three measurements (percent changes compared to 0 mg/kg Cd in the same genotype). Differently lettering indicates statistical difference ($P\leq0.01$) in the same column. ** Significant at $P\leq0.01$

Table 5 Interactive effects of jasmonic acid and cadmium application on leaf nutrient elements in *Brassica napus*

Cd (mg/kg)	JA (μmol/L)	Ca (mg/g DW)	Mg (mg/g DW)	Fe (mg/g DW)	Zn (mg/g DW)
0	0	51.32±2.20^{ab}	7.81±0.70^{ab}	0.29±0.01^{bc}	0.28±0.02^{a}
	25	55.32±2.00^{a}	9.66±1.20^{a}	0.35±0.02^{a}	0.31±0.02^{a}
		(7.80%)	(23.68%)	(21.99%)	(9.27%)
75	0	38.75±2.20^{c}	5.56±0.20^{cd}	0.19±0.02^{d}	0.19±0.01^{cd}
	25	52.57±1.70^{ab}	8.76±1.10^{ab}	0.30±0.02^{ab}	0.27±0.05^{ab}
		(35.66%)	(57.55%)	(58.89%)	(40.02%)
150	0	30.45±2.10^{d}	4.07±0.20^{de}	0.13±0.01^{e}	0.13±0.01^{de}
	25	47.61±2.00^{b}	7.51±0.90^{abc}	0.24±0.01^{cd}	0.22±0.01^{bc}
		(56.35%)	(84.52%)	(83.55%)	(60.93%)
300	0	23.86±2.00^{d}	3.23±0.20^{e}	0.08±0.01^{e}	0.09±0.01^{e}
	25	38.66±1.70^{c}	6.68±0.80^{bc}	0.20±0.01^{d}	0.18±0.01^{cd}
		(62.02%)	(106.81%)	(139.57%)	(89.43%)
Level of significance		*	**	**	**

Cd: cadmium; JA: jasmonic acid; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc. Data represent the mean±SE of three measurements (percent changes compared to 0 μmol/L JA at the same Cd concentration). Differently lettering indicates statistical difference ($P\leq0.01$) in the same column. * Significant at $P\leq0.05$; ** Significant at $P\leq0.01$

Table 6 Interactive effects of genotypes and jasmonic acid application on leaf nutrient elements in *Brassica napus*

Genotype	JA (μmol/L)	Ca (mg/g DW)	Mg (mg/g DW)	Fe (mg/g DW)	Zn (mg/g DW)
ZS72	0	38.30±3.32^{cd}	6.00±0.70^{bc}	0.20±0.02^{b}	0.18±0.02^{c}
	25	51.90±2.61^{a}	11.40±0.90^{a}	0.30±0.02^{a}	0.24±0.01^{a}
		(35.53%)	(89.70%)	(52.73%)	(36.55%)
ZJ619	0	38.20±3.63^{cd}	5.10±0.60^{c}	0.18±0.03^{bc}	0.19±0.02^{bc}
	25	49.10±2.11^{ab}	7.40±0.40^{b}	0.20±0.02^{a}	0.25±0.02^{a}
		(28.47%)	(44.07%)	(59.57%)	(34.53%)
ZS758	0	31.70±3.73^{d}	4.30±0.40^{c}	0.14±0.02^{c}	0.16±0.02^{c}
	25	44.50±2.66^{bc}	5.60±0.20^{bc}	0.20±0.01^{b}	0.23±0.02^{ab}
		(40.47%)	(29.26%)	(63.15%)	(45.43%)
Level of significance		**	**	**	**

JA: jasmonic acid; Ca: calcium; Mg: magnesium; Fe: iron; Zn: zinc; ZS72: Zheshuang-72; ZJ619: Zhejiang-619; ZS758: Zheshuang-758. Data represent the mean±SE of three measurements (percent changes compared to 0 μmol/L JA in the same genotype). Differently lettering indicates statistical difference (P≤0.01) in the same column. ** Significant at P≤0.01

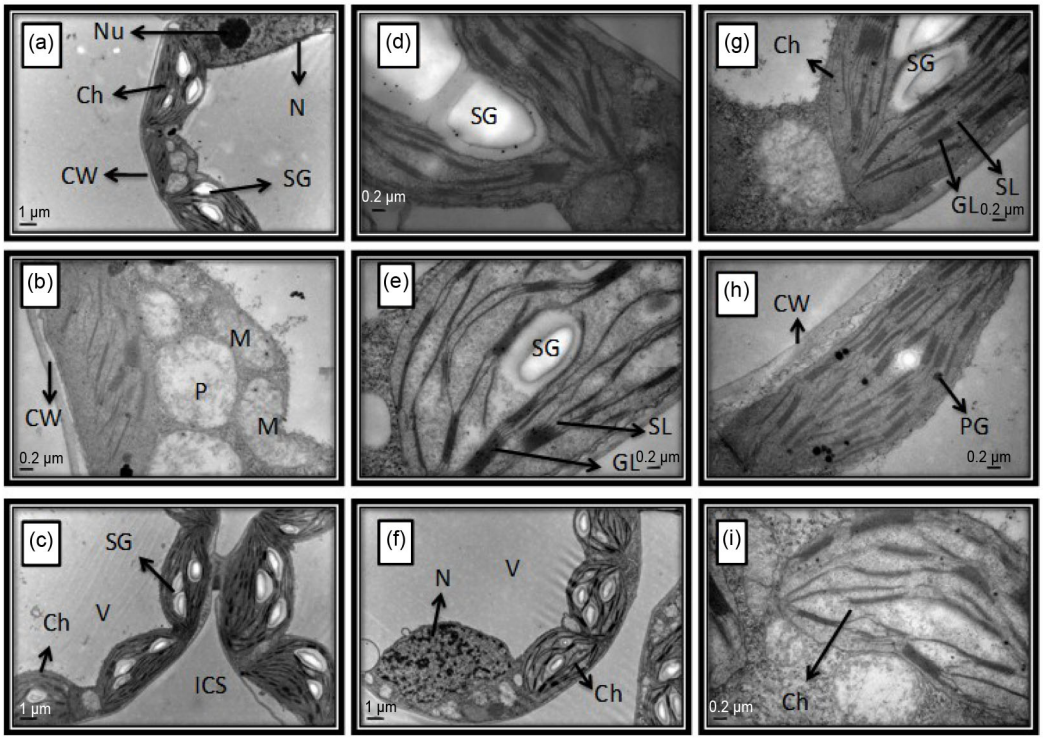

Fig. 9 Transmission electron micrograph of the leaf mesophyll cells

(a, d, g) Zheshuang-72; (b, e, h) Zhejiang-619; (c, f, i) Zheshuang-758. (a, b, c) Control; (d, e, f) 150 mg/kg Cd; (g, h, i) 150 mg/kg Cd+25 μmol/L JA. Labels: Ch, chloroplast; SG, starch grain; PG, plastoglobule; M, mitochondrion; GL, granal lamella; SL, stromal lamella; N, nucleus; Nu, nucleolus; P, peroxysome; V, vacoule; ICS, intercellular spaces; CW, cell wall

4 Discussion

Cd stress evokes a series of complex responses in higher plants in terms of disturbing the regular physiological and morphological processes. Thus, the mechanism(s) involved in such responses can be regulated as a cause and/or an effect of metabolic changes regarding Cd stress management. The current experiment was conducted to unravel the underlying mechanisms and effects of JA in enhancing the tolerance of rapeseed genotypes to Cd toxicity.

Significant reduction of gas exchange in plants was observed under Cd stress; however, exogenous JA application repaired the damage caused by Cd

(Figs. 1 and 2). This reduction can be attributed to possible reduction in G_s due to the toxic effects of Cd on guard cells, which is a major consequence of Cd stress in plants (Satler and Thimann, 1981). As a result, limited diffusion of CO_2 to the site of carboxylation would take place and this can reduce P_n, C_i, and T_r (Perfus-Barbeoch et al., 2002). JA induced improvement in G_s when applied alone (Fig. 1f) and/or in a genotypic-dependent manner in response to Cd treatment (Figs. 2f and 2j). These results are contrary to the findings that jasmonates cause stomatal closure (Satler and Thimann, 1981). However, numerous studies reported that the response of G_s to jasmonates depends on the concentration and time of exposure (Metodiev et al., 1996). In general, 50 μmol/L JA induces senescence-related responses (Creelman and Mullet, 1995); however, we used a concentration of 25 μmol/L JA in this study. JA-induced improvement in gas exchange can be attributed to the reduced Cd accumulation in upper parts of plant (Fig. 6e) that can protect against stomatal closure and pigment degradation. Hence, this improvement increases the photosynthetic rate (Figs. 2e and 2i) and related parameters.

The disruption of photosynthetic pigments can explain the degree of damage to the photosynthetic system from environmental stressors (Maxwell and Johnson, 2000). Drastic reductions of chlorophyll and carotenoids were observed in response to Cd stress application, whereas JA application increased photosynthetic pigments (Figs. 3 and 4), showing the negative effects of Cd in pigment regulation and the positive role of JA in protecting photosynthetic pigments and apparatuses. Little or no accumulation of photosynthetic pigments under heavy metal stress might be a consequence of chloroplast membrane peroxidation through enhanced rates of H_2O_2 production (Piotrowska-Niczyporuk et al., 2012). JA usually protects chlorophyll under toxic metal stress (Chen et al., 2014), whether applied alone (Figs. 3e–3h) or in combination with metals (Figs. 4i–4l), as seen in this study. These results suggested that JA elicits protective effects during photosynthesis under Cd stress. Other studies demonstrated similar findings in JA- and Pb-treated *Wolffia arrhiza* (Piotrowska et al., 2009) and soybean plants (Keramat et al., 2009). JA-induced protection of photosynthetic pigments can be attributed to the production of multiple secondary metabolite classes, alkaloids and phenolics, and anthocyanins in various plant species (Memelink et al., 2001). Moreover, anthocynin accumulation in epidermal cells and increased carotenoid content under JA and metal stress can protect the chloroplast (Czerpak et al., 2006).

Cd accumulation in the aboveground plant parts increased with Cd application in a dose-dependent manner. However, JA application significantly reduced Cd accumulation in rapeseed genotypes (Figs. 5a and 6e). These results agree with the findings of Piotrowska et al. (2009) which state that JA application significantly inhibits Pb accumulation in *W. arrhiza*. In addition, other stress hormones, such as salicylic acid (SA), abscisic acid (ABA), and brassinolide (2,4-epibrassinolide), can reduce the uptake of toxic metals (Cd or nickel (Ni)) in hydroponic and soil-grown plants (Kanwar et al., 2012; Ali et al., 2015). Reduction in heavy metal uptake in response to exogenous application of stress hormones can be attributed to the reduced T_r and symplastic loading of Cd into the xylem (Lux et al., 2011). However, a relatively higher T_r and reduced Cd translocation into the leaves in JA-treated plants were observed and correspond with our previous findings (Ali et al., 2015), where low Cd accumulation and high T_r were observed in rapeseed after SA application. Besides transpiration, metal uptake in the plant can also be influenced by other factors, such as accumulation/exudation of organic compounds such as phenolic compounds. Kováčik et al. (2011) reported that accumulation of phenolic compounds in the aboveground parts of plants significantly repressed Ni and Cd uptakes in shoots. Moreover, significantly increased phenolic compound accumulation in plants in response to exogenous JA application was reported (Kim et al., 2007). JA treatments might increase the phenolic compounds in rapeseed leaves, thereby repressing Cd uptake in leaves.

Cd stress indirectly induces the overproduction of various ROS, such as H_2O_2, O_2^-, and OH^-, to cause membrane lipid peroxidation and MDA accumulation. MDA is a product of lipid peroxidation and indicates oxidative membrane damage. Significant increase in MDA content indicates oxidative impairment in the leaves of rapeseed genotypes under Cd stress in this study (Figs. 5b and 6b). A similar trend was observed in the pea (Popova et al., 2009) and the results can link Cd toxicity to the production of free radicals,

which can hinder membrane stability and increase membrane permeability to the outside environment. However, JA protects the cell membrane lipid by alleviating lipid peroxidation during Cd stress. The benefit of exogenous JA application to plants was observed in terms of reduced oxidative stress, evidenced by the decreased MDA level.

Plant cells are equipped with enzymatic machinery that helps eliminate or reduce oxidative damage. Plant cells have defensive enzymes, including SOD, POD, APX, and CAT, against oxidative damage (Larson, 1988). Among these enzymes, SOD defends by catching superoxide radicals and converts them to H_2O_2, which is then moved to either APX (in the ascorbate–glutathione cycle) or CAT (in cytoplasm and other cellular compartments) and split into water and oxygen. Previously, Cd was reported to alter the antioxidative system of plants (Ahmad et al., 2011). Significant increases in the enzymatic activity of SOD, POD, and APX under Cd stress were observed, except that of CAT, which remained non-significant in all Cd levels (Figs. 7a–7d). A similar trend was reported in previous studies on different crops, such as pea (Popova et al., 2009) and *Brassica juncea* (Mobin and Khan, 2007), where the enzymatic activity increased as Cd concentration increased. However, CAT activity is balanced by the increase in APX activity (Fig. 7d). In contrast, a sole application of JA decreases the activity of all antioxidant enzymes, except CAT (Sorial et al., 2010). Moreover, JA also decreases SOD, POD, and APX activity when combined with Cd but increases CAT activity in all Cd levels except 150 mg/kg Cd (Figs. 8i–8l). Decreased SOD, POD, and APX activity can be attributed to the decreased Cd uptake (Fig. 6e), reduction in lipid peroxidation and ROS production by JA, and this can be confirmed from the decline in MDA content (Fig. 6f). CAT is a heme-containing enzyme; hence, increased Fe content after JA application (Table 2) can cause the increased CAT activity (Garg and Manchanda, 2009).

Cd stress can disrupt the homeostasis of macro- and micro-nutrients and related processes in plants (Ramos et al., 2002). For instance, reduced Ca, Mg, Fe, and Zn concentrations were reported in plants under Cd stress (Azevedo et al., 2005). Cd can enter the cells through an uptake system similar to one used by cations, such as Fe, Ca, and Zn. Cd could reduce the uptake and accumulation of these cations by binding with the related transporters (Clemens, 2006). However, JA treatment improves ion homeostasis either solely or in combination with Cd (Tables 2 and 4) by maintaining normal T_r and stomatal openings. The JA-improved ion homeostasis can be attributed to the inhibition of Cd uptake in aboveground plant parts (Chen et al., 2014), thereby providing a clear uptake channel for the ions.

Ultra structural studies under environmental stresses are important for studying the probable mechanisms of plant defenses at the subcellular level. Previous studies on Cd effects at the subcellular level in plants revealed the chloroplast to be the most sensitive organelle to Cd toxicity (Ali et al., 2015). However, studies on the combined effects of Cd and JA in rapeseed are scarce. Severe chloroplast damage was noticed in plants under Cd stress compared with their respective controls. The damage can be explained in terms of enlarged but disrupted chloroplasts with entirely deformed thylakoid membranes and disintegrated nucleoli (Vijaranakul et al., 2001; Borges et al., 2004). Despite reduced chlorophyll and low photosynthetic activity in Cd-treated leaves, the presence of starch granules was surprising in this study (Fig. 9d). Disrupted chloroplasts with a low amount of chlorophyll are unlikely to have an energy surplus. This suggests the inability of chloroplasts to metabolize stored starch deposits (Uzunova and Popova, 2000). Nutrient deficiencies may be responsible for starch grain accumulation in Cd-treated leaves (Sandalio et al., 2001).

The recovery of chloroplast through JA application after Cd stress (Figs. 9g–9i) can be attributed to the reduced Cd accumulation and antioxidant enzyme modulation against ROS, and this could be verified by both the increase in photosynthetic pigments and gas exchange parameters in this study. Moreover, JA increases the uptake and accumulation of essential nutrient elements required for chlorophyll and other metabolic activities.

5 Conclusions

JA is helpful to plants in attenuating damage by Cd. The relief provided by JA to plants under Cd stress might be the result of the inhibition of Cd

accumulation in leaves, thereby decreasing the damage intensity to the membrane system by ROS produced during oxidative stresses. Moreover, JA increases the uptake of essential nutrients that are required for normal growth, and some of these nutrients act as cofactors for key enzymes involved in photosynthesis; therefore JA enhances photosynthesis-related activity. Similarly, JA modulates the activity of stress enzymes to ensure maximum protection to plants. Furthermore, normal chloroplast, and increased gas exchange and photosynthetic pigments suggested that JA protects chloroplasts against ROS produced from Cd stress. This study may clarify the possible mechanisms involved in JA-induced tolerance in rapeseed against Cd toxicity. However, molecular studies should be conducted to further understand the underlying mechanisms of JA-induced tolerance in plants under Cd stress.

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Compliance with ethics guidelines

Essa ALI, Nazim HUSSAIN, Imran Haider SHAMSI, Zahra JABEEN, Muzammil Hussain SIDDIQUI, and Li-xi JIANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：茉莉酸对油菜（*Brassica napus* L.）受镉毒害的缓解作用

目　的：本研究目的在于了解：（1）喷施外源茉莉酸对受到镉胁迫油菜的作用；（2）是否茉莉酸能够通过增强气体交换，从而保护受到氧化胁迫的地上部分组织的叶绿体，进而通过减少镉的吸收来维持离子平衡；（3）是否通过喷施茉莉酸来对具有减缓镉毒害效应的抗氧化酶的活性进行调节。

创新点：茉莉酸能够调节响应胁迫的抗氧化酶的活性，从而通过保护叶绿体免受活性氧（ROS）伤害而提高光合产物的能力，最大限度地缓解油菜植株受到的镉毒害。

方　法：（1）叶片气体交换；（2）叶片光合色素分析；（3）丙二醛与抗氧化酶活性分析；（4）营养成分分析；（5）透射电镜亚细胞水平观察。

结　论：茉莉酸对于植物受镉毒害的缓解作用的机理在于减少叶片中镉的积累，从而减轻氧化胁迫过程中产生的 ROS 对于膜系统的损害程度。

关键词：油菜；镉；茉莉酸；抗氧化酶；丙二醛；超微结构