



**Full Length Article**

# Jasmonate Improved Cadmium Resistance in Maize Seedlings by Regulating Spermidine Synthesis

Jiakun Yan\*, Ningning Zhang and Furen Kang

*Shaanxi Key Laboratory of Ecological Restoration in Shanbei Mining Area, College of Life Science, Yulin University, Yulin, Shaanxi 719000, P.R. China*

\*For correspondence: [himingse@163.com](mailto:himingse@163.com); [zhangningning1986@163.com](mailto:zhangningning1986@163.com)

Received 23 September 2019; Accepted 24 February 2020; Published 31 May 2020

## Abstract

Cadmium (Cd) pollution threatens global food security and many plant growth regulators have been found to improve plant Cd resistance. Jasmonate, a low molecular-weight plant growth regulator, is involved in the processes by which plants respond to Cd stress. However, the underlying mechanisms involved in these processes are unclear. In the present study, we investigated the effects of methyl jasmonate (MeJA, a jasmonate active substance) on maize seedling growth under Cd stress conditions. We found that exogenous MeJA induced the accumulation of polyamines (PAs), especially spermidine (spd), and exogenous MeJA application partially mitigated the Cd-induced inhibition of whole-plant growth. Under Cd stress, the MeJA- and spd-treated plant leaves contained less Cd than the leaves of plants not treated with MeJA or spd. With the addition of the spd synthesis inhibitor dicyclohexylamine and MeJA under Cd stress conditions, the leaf Cd concentration was higher than that under the Cd treatment alone, and the JA-induced improvement in maize Cd resistance was eliminated. This suggests that spermidine plays an important role in JA-induced improved Cd resistance in maize seedlings by repressing Cd transport from roots to leaves. © 2020 Friends Science Publishers

**Keywords:** Cadmium; Jasmonate; Polyamines; Maize

## Introduction

Human activities, including mining, the utilization of wastewater in agriculture and other industrial/agricultural activities, induces the accumulation of heavy metals in plants, leading to a host of ultrastructural anomalies in plants (Jia *et al.* 2017; Rizwan *et al.* 2017; Scimeca *et al.* 2017). Among these heavy metals, cadmium (Cd) is one of the most aggressive and persistent; it is relatively mobile in soil and can be toxic to both plants and animals (Poschenrieder and Barceló 1999; Khan *et al.* 2016). According to the Chinese national survey of soil pollution, more than 7% of surveyed samples had excessive levels of cadmium (Ministry of Environmental Protection 2014). Therefore, Cd pollution is very serious. However, we seem to be powerless to reduce Cd inputs due to the pervasiveness of this metal, which even occurs in phosphorous fertilizers used in agriculture. To ensure food security while coping with the growing population, the exploration of ways to improve crop resistance to Cd stress is urgently needed.

Cadmium toxicity alters the uptake of water and mineral nutrients in plants and diminishes biomass, photosynthesis, grain yield, and quality in crops (Anh and Popova 2013; Moradi and Ehsanzadeh 2015). Cd toxicity also affects biochemical processes in plants, causing

oxidative stress by inducing reactive oxygen species (ROS) accumulation, which causes great damage to the antioxidant enzyme system (Mesnoua *et al.* 2016; Javed *et al.* 2017). Cd was also found to reduce leaf transpiration by affecting root water uptake (Rucińska-Sobkowiak 2016). In recent decades, researchers have performed fruitful work centered on reducing Cd uptake, translocation, and toxicity by applying soil amendments, biochar, manure, compost, silicon and other plant growth regulators, *e.g.*, jasmonic acid (Xu *et al.* 2014; Gondor *et al.* 2016; Xu *et al.* 2016; Abozeid *et al.* 2017).

Jasmonic acid, a plant growth regulator, has been found to play a vital role in the process of plant development and stress responses (Fragoso *et al.* 2014; Abouelsaad and Renault 2018). In the last decade, jasmonic acid (JA) has been mostly studied as an immune response factor in response to biotic stresses (herbivore attack and pathogen infection) (Farmer and Ryan 1992; Seo *et al.* 2001). More recently, studies have highlighted the important role of JA in improving plant resistance to abiotic stresses, including Cd stress (Li *et al.* 2003; Abouelsaad and Renault 2018; Ali *et al.* 2018). In general, treatment with exogenous methyl jasmonate (MeJA) significantly enhanced Cd stress tolerance in plant seedlings by decreasing the concentration of malondialdehyde (MDA)

and H<sub>2</sub>O<sub>2</sub> by increasing the transcription levels and activities of superoxide dismutase, peroxidase, catalase and ascorbate peroxidase. On the other hand, exogenous MeJA increased the contents of glutathione, Chl b and carotenoids to enhance the growth of Cd-stressed seedlings (Chen *et al.* 2014; Yan *et al.* 2015; Ali *et al.* 2018). Although the action of JA as a possible antioxidant in improving Cd stress is well understood, the underlying mechanisms involved in this process remain unclear.

Polyamines (PAs), another class of biomolecules that mainly consists of putrescine (put), spermidine (spd) and spermine (spm), have been found to be essential for normal plant growth, development and stress responses (Sanchez-Elordi *et al.* 2019). Under saline conditions, wheat seedlings treated with melatonin exhibited more free polyamines, especially spd, to mitigate salt stress and maintain plant growth (Ke *et al.* 2018). A primary study on JA indicated that exogenous MeJA induced polyamine accumulation to protect against powdery mildew in barley (Mitchell *et al.* 2002) and to delay peach fruit ripening (Ziosi *et al.* 2009). Taking all this into consideration, MeJA-induced polyamine accumulation functions in biotic stress responses and developmental regulation. However, whether MeJA induces polyamine accumulation and whether MeJA-induced polyamine accumulation plays a role in the plant abiotic stress response is unclear. Unraveling the underlying effects of MeJA-induced polyamine accumulation on the plant response to Cd stress could broaden our vision for improving crop Cd tolerance.

In the present study, we applied Cd and MeJA to maize (*Zea mays* L.) to evaluate the effect of MeJA on maize resistance to Cd stress. We also detected the accumulation of PAs with MeJA application under Cd stress. We added the spd synthetic inhibitor dicyclohexylamine (DCHA) to examine the role of PAs in MeJA-induced improved maize resistance to Cd stress.

## Materials and Methods

### Plant material and treatment

The widely cultivated maize variety Zhengdan 958, bred by the Henan Academy of Agronomy, was used in this study. Two experiments were conducted. Experiment 1 was performed to test whether MeJA affected PA content under Cd stress. First, seeds were disinfected with 3% NaClO and washed with double-distilled water. Then, six seeds were planted per pot (20 cm × 35 cm) with 9 kg soil, and the pots were placed in a rain-proof shed. The soil was a light silt loam (Heilutu series) collected from the top 0–20 cm of cropland. The soil organic carbon content, total nitrogen, available phosphorus, available potassium, pH and bulk density were 13.5 g kg<sup>-1</sup>, 0.62 g kg<sup>-1</sup>, 18.5 mg kg<sup>-1</sup>, 138.5 mg kg<sup>-1</sup>, 7.0 and 1.26 g cm<sup>-3</sup>, respectively. When seedlings grew to the three-leaf stage, thinning to three seedlings of the same size per pot was conducted. Then, the CK, Cd and Cd + MeJA treatments performed. The treatments were

conducted through irrigation with water (CK), 100 μM Cd<sup>2+</sup> (CdCl<sub>2</sub>) and 100 μM Cd<sup>2+</sup>+100 μM MeJA to maintain a soil water content of 75 ± 5% field water capacity. Each treatment had three replications. The MeJA and Cd concentrations were ascertained with reference to published studies (Chen *et al.* 2014; Yan *et al.* 2015; Ali *et al.* 2018). After treatment for two weeks, new fully expanded leaves (the sixth leaves) were collected to detect the PA content.

Experiment 2 was performed to detect the mechanism of JA in regulating Cd stress tolerance in maize. The soil and procedures used in experiment 1 were used to plant the maize seedlings and three seedlings were retained at the three-leaf stage. The experiment was comprised of six treatments: CK, Cd, Cd + MeJA (MeJA), Cd + spd (SPD), Cd + MeJA + spd (MeJA + SPD), and Cd + MeJA + DCHA (Cd + DCHA). Cd was applied in all treatments except CK. These treatments were performed through irrigating with water (CK), water containing 100 μM Cd<sup>2+</sup> (Cd), water containing 100 μM Cd<sup>2+</sup> + 100 μM MeJA (MeJA), water containing 100 μM Cd<sup>2+</sup> + 0.5 mM spd (SPD), water containing 100 μM Cd<sup>2+</sup> + 0.5 mM spd + 100 μM MeJA (MeJA + SPD) and water containing 100 μM Cd<sup>2+</sup> + 100 μM MeJA+1 mM DCHA (Cd + DCHA). Each treatment had 12 replications. After treatment for two weeks, the observations were recorded. All measurements (plant height, aboveground biomass, leaf gas exchange traits, chlorophyll contents, Hydrogen peroxide, MDA, antioxidant enzyme activities and tissue Cd concentration) except the polyamine content analyses were performed using the seedlings from experiment 2.

### Polyamine content analyses

This analysis was only performed in experiment 1. A new fully expanded leaf was used to test the PA content according to Ke's method (Ke *et al.* 2018). Briefly, a new fully expanded leaf (approximately 1 g) was sampled with liquid nitrogen, ground in a mortar to a fine powder and extracted in 5 mL of 5% (w/v) chilled perchloric acid. After 18 h of extraction at 25°C, the homogenate was centrifuged for 15 min at 15,000 g. The supernatant phase was used to detect the free polyamine content. For benzylation, 500 μL supernatant phase containing the free polyamine fraction was mixed with 1 mL 4 N NaOH and then 10 μL benzoyl chloride was immediately added. The benzyolated samples were separated on an Inertsil ODS-3 (5 μm, 4.6 × 250 mm, GL Science Inc., United States) under the following program: 0 ~ 15 min, 60% methanol; 15 ~ 35 min, 60 ~ 90%; 35 ~ 45 min, 90 ~ 60%; 45 ~ 60 min, 60% at a flow rate of 0.8 mL min<sup>-1</sup> at 35°C. Polyamine peaks were detected with a UV detector at 254 nm. The measurements included three biological replications.

### Plant height and aboveground biomass

In experiment 2, after two weeks of treatment, plant height was detected using a centimeter ruler. Dry biomass was

measured after two weeks of treatment using a previous method (Liu *et al.* 2014a). Briefly, the shoot was sampled and dried to a constant weight at 80°C. Measurements were taken for fifteen biological replications.

### Leaf gas exchange traits and chlorophyll contents

The gas exchange parameters of new fully expanded leaves were measured at 10:00 – 11:00 a.m. using a portable photosynthesis system with an IRGA analyzer (Li-6400; LICOR Inc., Lincoln, N.E., U.S.A.) after two weeks of the treatments in experiment 2. The leaf photosynthetic pigments were used to detect the chlorophyll contents with the method of Knudson *et al.* (1977). Measurements were taken for eight biological replications.

### Hydrogen peroxide, MDA and antioxidant enzyme activities

The hydrogen peroxide contents, and antioxidant enzyme activities were measured according to Liu *et al.* (2015). The superoxide dismutase activity was assayed by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The peroxidase activity was determined using the guaiacol oxidation method. The catalase activity was estimated by measuring the initial rate of the disappearance of H<sub>2</sub>O<sub>2</sub> at 240 nm. Hydrogen peroxide contents were measured as follows: Leaf samples (0.5 g fresh weight, FW) were homogenized in an ice bath with 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 12,000 g for 15 min, and 1 mL of supernatant was added to 1 mL of 10 mM potassium phosphate buffer (pH 7.0) and 0.8 mL of 1 M KI. The absorbance was measured at 390 nm. The H<sub>2</sub>O<sub>2</sub> content was calculated using a standard curve. MDA was detected using thiobarbituric acid (TBA) according to Li *et al.* (2017). For this measurement, a new fully expanded leaf was used. Measurements were taken for five biological replications.

### Determination of tissue Cd concentration

Leaves were dried at 80°C, ground artificially and used to detect the Cd concentration. Root samples were taken from the soil and then washed with double-distilled water to eliminate soil and cadmium from the root surfaces. The root was dried at 80°C, ground artificially and used to detect the Cd concentration. The Cd concentration was detected using an ICP-MS according to the instrument's specifications. Briefly, approximately 500 mg of dry, ground plant material was used for acid digestion. The digestion matrix contained 5 mL HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub>. Digestion was performed with a Multiwave 3000 (64 MG5 rotor, Anton Paar). After digestion, the microdigests were acidified with hydrofluoric acid to a final concentration of 0.01% to prevent the polymerization or precipitation of the ions. The Cd concentrations in the digests were measured with inductively

coupled plasma mass spectrometry (ICP-MS; IACP-MS-Qc, ThermoFisher Scientific, M.A., U.S.A.). Each measurement was performed for three biological replications.

### Statistical analysis

Statistical analyses were performed with SPSS version 18.0. One-way ANOVA was used to ascertain the effects of MeJA under the different treatments. Differences between the means were compared by the least significant difference (LSD) test at  $P < 0.05$ . The figures were produced using SigmaPlot version 12.01 (Systat Software, Inc.).

## Results

### Experiment 1

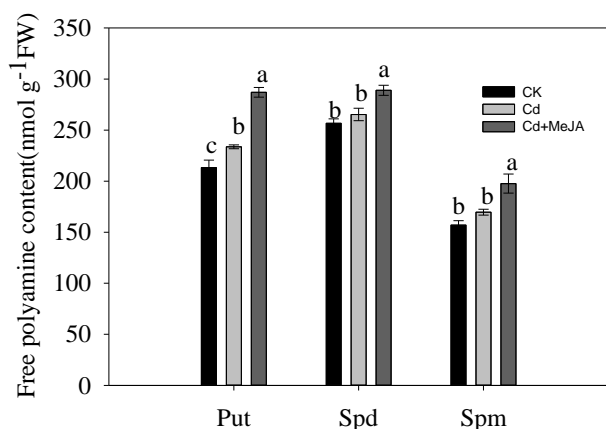
**Effect of exogenous MeJA on Pas:** After two weeks of treatment with Cd + MeJA and Cd, the maize leaves had significantly increased free put content ( $P < 0.05$ , Fig. 1). The exogenous Cd + MeJA group had the highest free put content, with increases of 34.5% and 22.8% compared with the CK and Cd groups, respectively. The free spd of the Cd + MeJA group was 12.9% and 9.1% higher than that of the CK and Cd groups, respectively. The free spm of the Cd + MeJA group was 25.5% and 16.6% higher than that of the CK and Cd groups, respectively. These results showed that exogenous MeJA could significantly increase endogenous PAs under Cd stress, which might be beneficial in improving maize Cd tolerance.

### Experiment 2

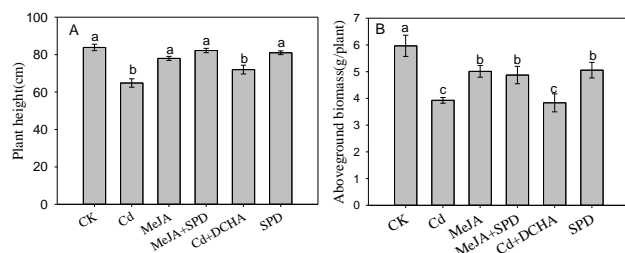
**Maize growth status:** After two weeks of treatment, the exogenous substances had obviously affected maize growth (Fig. 2). Cd stress significantly decreased the maize plant height and dry biomass ( $P < 0.05$ ) and the application of MeJA or spd mitigated the inhibition of maize growth under Cd stress (Fig. 2AB). However, the combined application of DCHA and MeJA did not improve maize seedling growth under Cd stress.

### Leaf gas exchange traits and chlorophyll contents

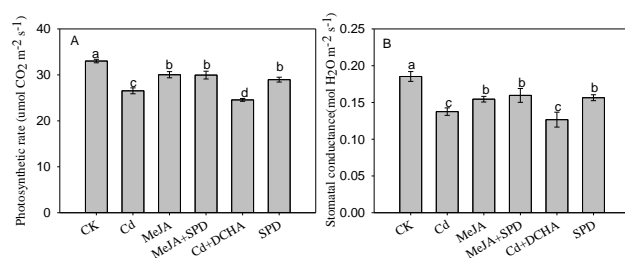
The trends in the net photosynthetic rate were similar to those of the biomass, and the CK group had the highest photosynthetic rate (Fig. 3A). The application of Cd decreased the photosynthetic rate compared with that in CK ( $P < 0.05$ ). MeJA+ SPD, SPD and MeJA application improved the photosynthetic rate, while the combined application of DCHA and MeJA under Cd stress did not improve the photosynthetic rate compared with that under only Cd application. In addition, exogenous MeJA and spd alleviated the Cd stress-induced inhibition of stomatal conductance, but the application of DCHA eliminated this



**Fig. 1:** Effects of exogenous MeJA on polyamine metabolism under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Cellular polyamines include putrescine (Put), spermidine (Spd) and spermine (Spm). Data represent the mean  $\pm$  SE of three biological replications; different lowercase letters above the bars indicate significant differences at  $P < 0.05$  according to the least significant difference method



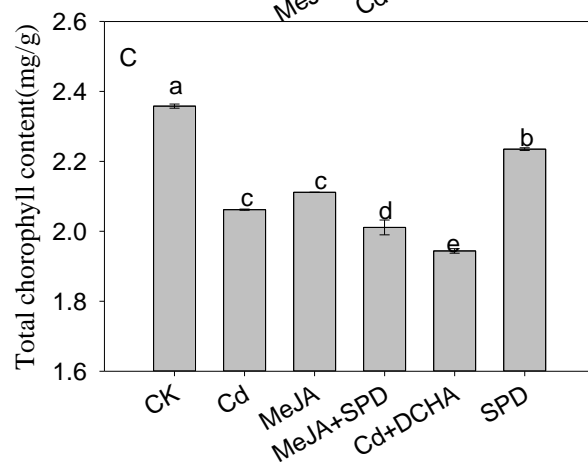
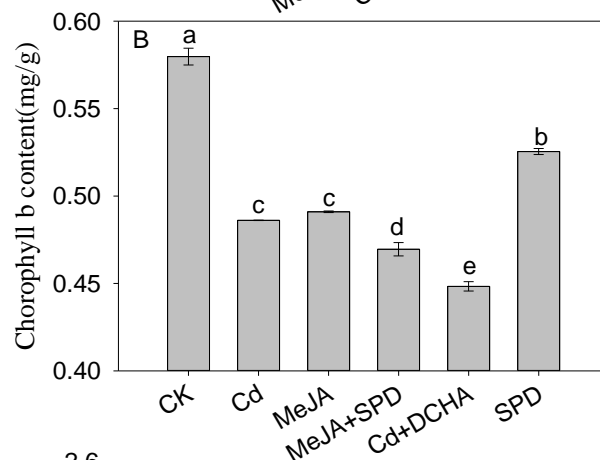
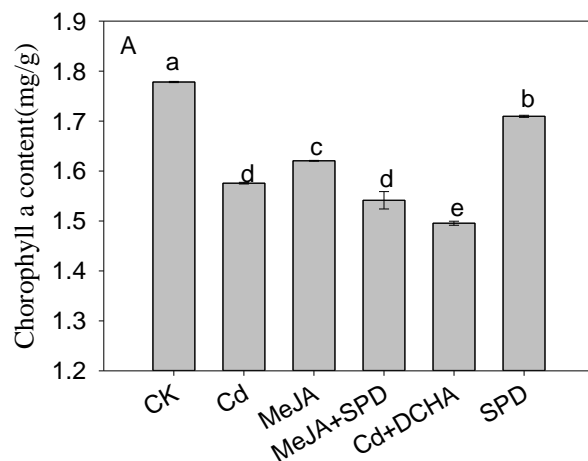
**Fig. 2:** Effects of MeJA on the growth of maize seedlings under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). (A) Plant height of maize seedlings. (B) Biomass of maize seedlings under Cd stress. Data represent the mean  $\pm$  SE of fifteen biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )



**Fig. 3:** Effects of MeJA on the photosynthetic rate (A) and stomatal conductance (B) of maize seedlings under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Data represent the mean  $\pm$  SE of eight biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )

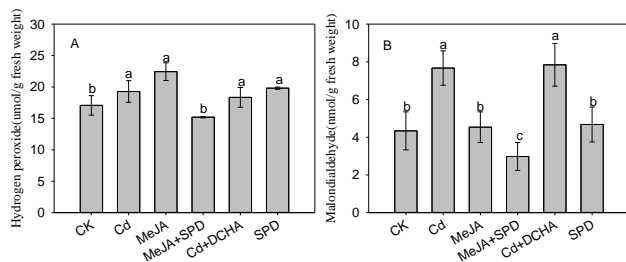
alleviating effect (Fig. 3B).

Compared with that in the CK group, Cd stress significantly decreased the chlorophyll content ( $P < 0.05$ , Fig. 4). When MeJA and spd were added under Cd stress, they significantly increased leaf chl a content ( $P < 0.05$ )



**Fig. 4:** Effect of MeJA on the chlorophyll contents of maize seedlings under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Data represent the mean  $\pm$  SE of eight biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )

compared with Cd application alone. When the combination of MeJA and spd was added, the effect was not obvious (Fig. 4A). The results for chl a, b and total chlorophyll are similar (Fig. 4B–C). Taking chl a and chl b together, exogenous MeJA and spd eliminated the decline in the total chlorophyll content induced by Cd stress.



**Fig. 5:** Effects of MeJA on the hydrogen peroxide and MDA content of maize seedlings grown under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Data represent the mean  $\pm$  SE of five biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )

### Hydrogen peroxide, MDA and antioxidant enzyme activities

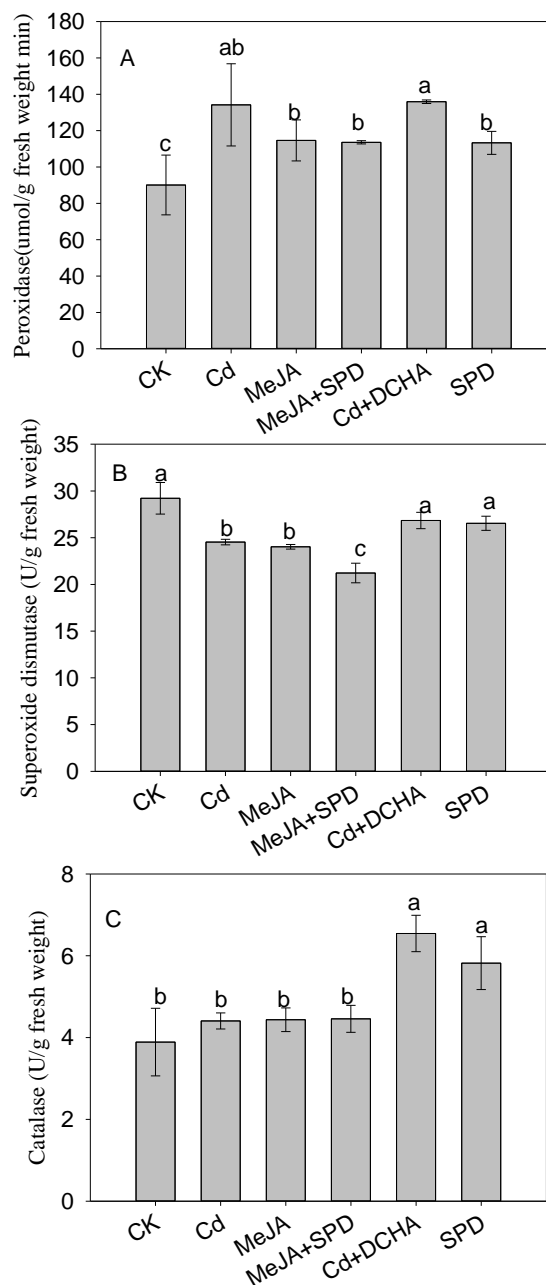
The results for the  $\text{H}_2\text{O}_2$  concentrations in leaves were very interesting. Compared to that in the CK group, treatment with Cd significantly increased the  $\text{H}_2\text{O}_2$  concentration (Fig. 5A). The application of MeJA, Cd + DCHA and SPD did not decrease the  $\text{H}_2\text{O}_2$  concentration compared with that under Cd stress. There were no significant differences among the treatments of Cd, MeJA, Cd + DCHA and SPD in terms of  $\text{H}_2\text{O}_2$  concentration ( $18.34\text{--}22.44 \mu\text{mol/g FW}$ ). Compared with the other treatments under Cd stress, the MeJA+ SPD group ( $15.19 \mu\text{mol/g FW}$ ) had a significantly decreased  $\text{H}_2\text{O}_2$  concentration ( $P < 0.05$ ) and was not significantly different from the CK group ( $17.07 \mu\text{mol/g FW}$ ).

As shown in Fig. 5B, Cd stress significantly increased the MDA content compared to that in the CK group ( $P < 0.05$ ). Exogenous MeJA, MeJA + SPD and SPD decreased this increment sharply. When DCHA and MeJA were added together, exogenous MeJA did not alleviate the Cd-induced increase in leaf MDA accumulation compared with that under the Cd treatment alone.

Unlike the MDA content, under Cd stress, the peroxidase activity was increased in these treatments. However, the Cd and Cd + DCHA combination exhibited higher peroxidase activity than the other treatments, and CK showed the lowest peroxidase activity (Fig. 6A). For superoxide dismutase activity, the SPD, Cd + DCHA combination and CK treatments exhibited the highest activity, while MeJA + SPD exhibited the lowest activity (Fig. 6B). For catalase, the Cd+DCHA combination had the maximum activity level, followed by the SPD, Cd, MeJA, MeJA + SPD and CK treatments (Fig. 6C).

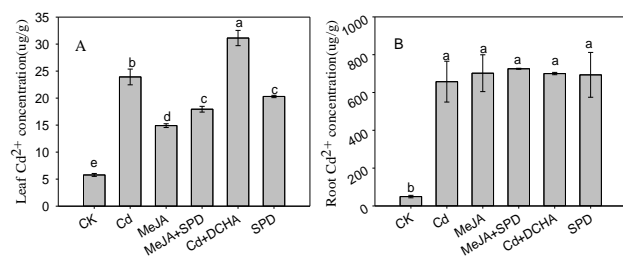
### Tissue Cd concentration

For leaf Cd concentration, under Cd stress, exogenous MeJA and Spd decreased leaf Cd accumulation, and the Cd concentration of these treatments was lower than those of the Cd and Cd + DCHA treatments (Fig. 7A).



**Fig. 6:** Effects of MeJA on the antioxidant enzyme activities of maize seedlings under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Data represent the mean  $\pm$  SE of five biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )

We also found that with the spd synthetic inhibitor DCHA, the leaves accumulated more Cd than in the only-Cd treatment. However, for the root Cd concentration, we found that exogenous MeJA and SPD did not change the root Cd uptake from the soil (Fig. 7B) because there was no significant difference in the root Cd concentration among the Cd treatments. As expected, the CK group had the lowest root Cd concentration.



**Fig. 7:** Effects of MeJA on the Cd concentration of maize seedlings grown under Cd stress ( $100 \mu\text{M Cd}^{2+}$ ). Data represent the mean  $\pm$  SE of three biological replications, and different lowercase letters above the bars indicate significant differences ( $P < 0.05$ )

## Discussion

Prior studies have documented the effectiveness of JA in improving plant resistance to abiotic stress (Seo *et al.* 2001; Chen *et al.* 2005; Han *et al.* 2018; Yan *et al.* 2019). In addition, numerous studies have confirmed that PAs play an important role in JA-induced resistance improvement in plants (Capitani *et al.* 2001; Mitchell *et al.* 2002; Chen *et al.* 2006). In the current study, under Cd stress, MeJA induced more PA accumulation than treatments without MeJA. In a further study, we found that exogenous DCHA could eliminate the improvements achieved with exogenous MeJA. These results indicate that PAs participate in JA-induced plant resistance to Cd.

Jasmonate has been reported to participate in many biotic and abiotic responses in plants (Chen *et al.* 2005; Harb *et al.* 2010). Here, we present evidence that Cd-induced growth inhibition in maize seedlings was alleviated by exogenous MeJA (Fig. 2). Numerous types of stress, such as salinity, cold, drought, and oxidative stress, severely repress the plant leaf photosynthetic rate (Yan *et al.* 2016). In research on improving resistance to environmental stress, an enhanced photosynthetic rate was considered an important index (Liu *et al.* 2015). Here, exogenous MeJA alleviated the Cd-induced inhibition of the leaf photosynthetic rate (Fig. 3), consistent with observations of previous studies (Yan *et al.* 2015; Ali *et al.* 2018).

In previous studies, almost all of the exogenous substances that enhanced plant Cd resistance were considered to improve antioxidant enzyme activities and decrease ROS contents (Singh and Shah 2014; Gondor *et al.* 2016). When rice plants were exposed to  $50 \mu\text{M Cd}^{2+}$  alone and/or with  $5 \mu\text{M MeJA}$ , MeJA increased the antioxidant enzyme activities to clear ROS (Singh and Shah 2014). However, in our study, we did not find any effective change in the antioxidant enzyme activities, including those of peroxidase, superoxide dismutase and catalase, with the application of MeJA (Fig. 6). In our study, we added  $100 \mu\text{M Cd}^{2+}$  to the soil by irrigation, while in other studies, seedlings were treated with Hoagland nutrient solution, and  $\text{Cd}^{2+}$  was added to the solution, which made the stress more intense. We speculate that the different treatment methods

and different species were the main source of this difference. In the present study, the highest  $\text{H}_2\text{O}_2$  content was  $22.44 \mu\text{mol/g FW}$ , which was lower than levels in a previous report (Souza *et al.* 2014). These results suggest that under this treatment, the accumulation of  $\text{H}_2\text{O}_2$  did not reach the threshold for causing damage, and there was no need for the initiation of antioxidant enzyme activities to clear it. In addition,  $\text{H}_2\text{O}_2$  plays an important role as a signal for regulating the stress response (Neill *et al.* 2002). Thus,  $\text{H}_2\text{O}_2$  may have acted as a signaling molecule in the current study, indicating that the oxidative damage was not serious. However, according to the MDA results, primary damage had begun, and exogenous MeJA and Spd reduced MDA production (Fig. 5B). These results showed that under Cd stress, exogenous MeJA could reduce membrane damage.

Based on the Cd concentration measurement, we found that exogenous MeJA reduced the transport of Cd from the root to the shoot but did not affect the root uptake of Cd (Fig. 7). In a previous study, MeJA was found to reduce leaf Cd in *Kandelia obovata* seedlings and it was suggested that the reduced uptake of Cd in the shoots of *K. obovata* might be a result of stomatal closure and decreased transpiration by exogenous MeJA (Chen *et al.* 2014). However, in this study, we found that exogenous MeJA increased stomatal conductance. Therefore, we speculate that the reduced leaf Cd accumulation was not the result of stomatal regulation. A previous study confirmed that silicon increased organic acid production to improve Cd resistance in sorghum seedlings (Liu *et al.* 2014b). Increased organic acid production by exogenous MeJA has been reported (Zhao *et al.* 2001). Here, it was possible that the MeJA treatments increased the organic acid content in the leaves of maize, thereby repressing the uptake of Cd in leaves. In addition, phenolic compounds also repress the uptake of Cd in shoots (Kováčik *et al.* 2011), which could be another reason for the MeJA-induced lower leaf Cd accumulation.

The role of PAs in processes involving cell division, such as the development of roots, reproductive organs, and embryos, fruit ripening and the control of aging processes is known to be significant (Ke *et al.* 2018). It has been reported that exogenous MeJA and spd alone could improve Cd tolerance (Ali *et al.* 2018; Tajti *et al.* 2018). Many studies have indicated that the involvement of the metabolic pathways of PAs could be involved in JA-induced plant resistance to Cd (Mitchell *et al.* 2002; Jia *et al.* 2015). MeJA was found to upregulate biosynthetic gene expression, oxidation and the conjugation of polyamines in tobacco (Capitani *et al.* 2001). Here, we found that under Cd stress, the MeJA-treated plants showed significantly increased PA production (Fig. 1). In addition, the exogenous application of the spd synthetic inhibitor DCHA with MeJA eliminated the MeJA-induced improvement in maize resistance to Cd stress (Fig. 2). These results confirmed our primary hypothesis that PAs play an important role in MeJA-induced improved resistance to Cd stress in maize.

## Conclusion

In the present study, we found that exogenous MeJA treatment partially mitigated the Cd-induced inhibition of whole-plant growth in maize. Under Cd stress, the MeJA- and spd-treated plant leaves contained a lower amount of Cd than the leaves of plants not treated with MeJA and spd. Through the addition of the spd synthesis inhibitor dicyclohexylamine under Cd stress conditions combined with MeJA, the leaf Cd concentration was higher than that under the Cd treatment alone, and the JA-induced improvement in maize Cd resistance was eliminated. Therefore, it could be concluded that spermidine plays an important role in JA-induced improved Cd resistance in maize seedlings by repressing Cd transport from roots to leaves.

## Acknowledgements

The study was sponsored by the PhD research startup fund of Yulin University (17GK19 and 17GK18), the Science and Technology Program of Yulin Science and Technology Bureau (2018-2-50) and the National Natural Science Foundation of China (31960223). The authors wish to express their sincere gratitude to the anonymous reviewers and editor for their important advice.

## Author contributions

Conceived and designed the experiments: JKY and FRK. Performed the experiments: JKY and NNZ. Analyzed the data and wrote the paper: JKY, NNZ and FRK.

## References

- Abouelsaad I, S Renault (2018). Enhanced oxidative stress in the jasmonic acid-deficient tomato mutant *def-1* exposed to NaCl stress. *J Plant Physiol* 226:136–144
- Abozeid A, Z Ying, Y Lin, J Liu, Z Zhang, Z Tang (2017). Ethylene improves root system development under cadmium stress by modulating superoxide anion concentration in *Arabidopsis thaliana*. *Front Plant Sci* 8; Article 253
- Ali E, N Hussain, IH Shamsi, Z Jabeen, MH Siddiqui, LX Jiang (2018). Role of jasmonic acid in improving tolerance of rapeseed (*Brassica napus* L.) to Cd toxicity. *J. Zhej Univ Sci B* 19:130–146
- Anh TT, LP Popova (2013). Functions and toxicity of cadmium in plants: recent advances and future prospects. *Turk J Bot* 37:1–13
- Capitani F, MM Altamura, P Torrigiani, S Scaramagli, S Biondi (2001). Methyl jasmonate upregulates biosynthetic gene expression, oxidation and conjugation of polyamines, and inhibits shoot formation in tobacco thin layers. *J Exp Bot* 52:231–242
- Chen H, AD Jones, GA Howe (2006). Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. *FEBS Lett* 580:2540–2546
- Chen H, CG Wilkerson, JA Kuchar, BS Phinney, GA Howe (2005). Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proc Natl Acad Sci* 102:19237–19242
- Chen J, Z Yan, X Li (2014). Effect of methyl jasmonate on cadmium uptake and antioxidative capacity in *Kandelia obovata* seedlings under cadmium stress. *Ecotoxicol Environ Saf* 104:349–356
- Farmer EE, CA Ryan (1992). Octadecanoid precursors of Jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *Plant Cell* 4:129–134
- Fragoso V, E Rothe, IT Baldwin, SG Kim (2014). Root jasmonic acid synthesis and perception regulate folivore-induced shoot metabolites and increase *Nicotiana attenuata* resistance. *New Phytol* 202:1335–1345
- Gondor OK, M Pal, E Darko, T Janda, G Szalai (2016). Salicylic acid and sodium salicylate alleviate cadmium toxicity to different extents in maize (*Zea mays* L.). *PLoS One* 11; e0160157
- Han X, Y Hu, G Zhang, Y Jiang, X Chen, D Yu (2018). Jasmonate negatively regulates stomatal development in *Arabidopsis* cotyledons. *Plant Physiol* 176:2871–2885
- Harb A, A Krishnan, MM Ambavaram, A Pereira (2010). Molecular and physiological analysis of drought stress in *Arabidopsis* reveals early responses leading to acclimation in plant growth. *Plant Physiol* 154:1254–1271
- Javed MT, MS Akram, K Tanwir, HJ Chaudhary, Q Ali, E Stoltz, S Lindberg (2017). Cadmium spiked soil modulates root organic acids exudation and ionic contents of two differentially Cd tolerant maize (*Zea mays* L.) cultivars. *Ecotoxicol Environ Saf* 141:216–225
- Jia L, L Yang, J Weimin, W Diaoe, W Xiaoxia, L Yuncheng (2015). The Effect of polyamine on growth of maize seedlings under cadmium stress and its associated mechanisms. *J Agro-Environ Sci* 34:1021–1027
- Jia Y, L Wang, Z Qu, C Wang, Z Yang (2017). Effects on heavy metal accumulation in freshwater fishes: species, tissues, and sizes. *Environ Sci Pollut Res* 24:9379–9386
- Ke Q, J Ye, B Wang, J Ren, L Yin, X Deng, S Wang (2018). Melatonin mitigates salt stress in wheat seedlings by modulating polyamine metabolism. *Front Plant Sci* 9; Article 914
- Khan AH, KMT Islam, KK Barman, KK Barua, M Abraham (2016). Outcome of surgical treatment in medically refractory epilepsy. *Bangl Med Res Counc Bull* 41:121–124
- Knudson LL, TW Tibbitts, GE Edwards (1977). Measurement of ozone injury by determination of leaf chlorophyll concentration. *Plant Physiol* 10:606–608
- Kováčik J, B Klejduš, F Štokr, J Hedbavny (2011). Nitrate deficiency reduces cadmium and nickel accumulation in chamomile plants. *J Agric Food Chem* 59:5139–5149
- Li CY, GH Liu, CC Xu, GI Lee, P Bauer, HQ Ling, MW Ganal, GA Howe (2003). The tomato suppressor of prosystemin-mediated responses2 gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. *Plant Cell* 15:1646–1661
- Li H, J Chang, H Chen, Z Wang, X Gu, C Wei, Y Zhang, J Ma, J Yang, X Zhang (2017). Exogenous melatonin confers salt stress tolerance to watermelon by improving photosynthesis and redox homeostasis. *Front Plant Sci* 8; Article 295
- Liu P, L Yin, S Wang, M Zhang, X Deng, S Zhang, K Tanaka (2015). Enhanced root hydraulic conductance by aquaporin regulation accounts for silicon alleviated salt-induced osmotic stress in *Sorghum bicolor* L. *Environ Exp Bot* 111:42–51
- Liu P, L Yin, X Deng, S Wang, K Tanaka, S Zhang (2014a). Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in *Sorghum bicolor* L. *J Exp Bot* 65:4747–4756
- Liu P, LN Yin, SW Wang, XP Deng (2014b). The effect and mechanism of silicon on sorghum seedlings growth under cadmium stress. *Res Soil Water Conserv* 21:229–234
- Mesnoui M, E Mateos-Naranjo, J Maria Barcia-Piedras, J Alberto Perez-Romero, B Lotmani, S Redondo-Gomez (2016). Physiological and biochemical mechanisms preventing Cd-toxicity in the hyperaccumulator *Atriplex halimus* L. *Plant Physiol Biochem* 106:30–38
- Ministry of Environmental Protection PRC (2014). *National Survey of Soil Pollution*, Beijing, China
- Mitchell A, D Walters, T Cowley (2002). Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. *J Exp Bot* 53:747–756

- Moradi L, P Ehsanzadeh (2015). Effects of Cd on photosynthesis and growth of safflower (*Carthamus tinctorius* L.) genotypes. *Photosynthetica* 53:506–518
- Neill S, R Desikan, A Clarke, R Hurst, J Hancock (2002). Hydrogen peroxide and nitric oxide as signaling molecules in plants. *J Exp Bot* 53:1237–1247
- Poschenrieder C, J Barceló (1999). *Water Relations in Heavy Metal Stressed Plants. Heavy Metal Stress in Plants: From Molecules to Ecosystems*, pp: 207–229. Springer, Berlin Heidelberg, Germany
- Rizwan M, S Ali, M Adrees, M Ibrahim, DCW Tsang, M Zia-Ur-Rehman, ZA Zahir, J Rinklebe, FMG Tack, YS Ok (2017). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere* 182:90–105
- Rucińska-Sobkowiak R (2016). Water relations in plants subjected to heavy metal stresses. *Acta Physiol Plantarum* 38:257–270
- Sanchez-Elordi E, LMD Los Rios, C Vicente, ME Legaz (2019). Polyamines levels increase in smut teliospores after contact with sugarcane glycoproteins as a plant defensive mechanism. *J Plant Res* 132:405–417
- Scimeca M, M Feola, L Romano, C Rao, E Gasbarra, E Bonanno, ML Brandi, U Tarantino (2017). Heavy metals accumulation affects bone microarchitecture in osteoporotic patients. *Environ Toxicol* 32:1333–1342
- Seo HS, JT Song, JJ Cheong, YH Lee, YW Lee, I Hwang, JS Lee, DC Yang (2001). Jasmonic Acid carboxyl methyltransferase: A key enzyme for Jasmonate-regulated plant responses. *Proc Natl Acad Sci USA* 98:4788–4793
- Singh I, K Shah (2014). Exogenous application of methyl jasmonate lowers the effect of cadmium-induced oxidative injury in rice seedlings. *Phytochemistry* 108:57–66
- Souza TCD, PC Magalhaes, EMD Castro, NP Carneiro, FA Padilha, CC Gomes (2014). ABA application to maize hybrids contrasting for drought tolerance: changes in water parameters and in antioxidant enzyme activity. *Plant Growth Regul* 73:205–217
- Tajti J, T Janda, I Majláth, G Szalai, M Pál (2018). Comparative study on the effects of putrescine and spermidine pre-treatment on cadmium stress in wheat. *Ecotoxicol Environ Saf* 148:546–554
- Xu D, Y Zhao, H Zhou, B Gao (2016). Effects of biochar amendment on relieving cadmium stress and reducing cadmium accumulation in pepper. *Environ Sci Pollut Res* 23:12323–12331
- Xu L, Y Dong, J Kong, S Liu (2014). Effects of root and foliar applications of exogenous NO on alleviating cadmium toxicity in lettuce seedlings. *Plant Growth Regul* 72:39–50
- Yan JK, NN Zhang, N Wang, YP Li, SQ Zhang, SW Wang (2016). Variations in adaptation strategies of wheat cultivar replacements under short-term osmotic stress. *Pak J Bot* 48:917–924
- Yan J, R Yan, Y Wang (2019). Impact of exogenous Methyl Jasmonate on water absorption of maize roots under salt stress. *Guangd Agric Sci* 16:1–6
- Yan Z, X Li, J Chen, NF Tam (2015). Combined toxicity of cadmium and copper in *Avicennia marina* seedlings and the regulation of exogenous jasmonic acid. *Ecotoxicol Environ Saf* 113:124–132
- Zhao J, K Fujita, J Yamada, K Sakai (2001). Improved  $\beta$ -thujaplicin production in *Cupressus lusitanica* suspension cultures by fungal elicitor and methyl jasmonate. *Appl Microbiol Biol* 55:301–305
- Ziosi V, AM Bregoli, F Fregola, G Costa, P Torrigiani (2009). Jasmonate-induced ripening delay is associated with up-regulation of polyamine levels in peach fruit. *J Plant Physiol* 166:938–946