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Research Article

Jasmonic acid priming and foliar application of spermidine up-regulates the tolerance mechanisms to alleviate the damaging effects of cadmium stress on growth and photosynthesis in wheat

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Abstract

The study examined the effects of jasmonic acid (100 nmol, JA) priming and foliar application of spermidine (1 mM, Spd), both individually and combined, on mitigating cadmium (100 µM, Cd) stress-induced oxidative damage in wheat. Cadmium stress reduced plant height and dry mass, but JA priming and/or Spd treatment increased resistance. Cd stress significantly decreased carotenoids, total chlorophylls, glutamate 1-semialdehyde (GSA), and δ-aminolevulinic acid (ALA), but JA and Spd treatments counteracted these reductions. Photosynthetic parameters improved under JA and Spd treatments, with combined treatment showing greater alleviation. Cd exposure increased lipid peroxidation, hydrogen peroxide, electrolyte leakage, and superoxide, but these oxidative stress indicators were significantly reduced after JA and Spd treatment. Antioxidant enzyme activity was upregulated by JA priming and Spd application, both under unstressed and Cd-stressed conditions. JA and/or Spd treatments also increased ascorbic acid, lowered glutathione concentration, and upregulated glyoxylase activity, reducing methylglyoxal accumulation. Additionally, secondary compounds (phenols and flavonoids) and osmolytes (proline and glycine betaine) levels improved. Proline oxidase activity decreased, indicating controlled proline buildup, while γ-glutamyl kinase activity increased. JA and/or Spd treatments significantly reduced Cd accumulation in seedlings. The study concluded

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that JA and Spd treatments enhance the plant's defensive mechanisms against oxidative stress by boosting antioxidant enzymes and secondary metabolism.

Keywords: antioxidants; cadmium; glyoxylase; jasmonic acid priming; oxidative stress; spermidine; wheat

Introduction

Cadmium (Cd) is considered toxic metals because it has harmful effects on human health and the environment. When released into the environment, it can accumulate in soil, water bodies, and plants. Human exposure to cadmium can occur through the consumption of contaminated food, particularly crops grown in cadmium-contaminated soil, as well as through inhalation of cadmium-containing dust and fumes (Qadir *et al.,* 2014). At lower concentrations, Cd has been reported to impart toxic effects in plants and animals. Being highly soluble in water, Cd has high fluidity and toxicity, therefore, is easily absorbed by plants (Delangiz *et al.,* 2020; Zhao *et al.,* 2021). Once taken up, it alters the structure and function of cellular organelles and other macromolecules (Jia *et al.,* 2015; Haider *et al.,* 2021). Cadmium inhibits germination, root growth, water uptake, photosynthesis, respiration, enzyme functioning, etc., resulting in substantial failure in plant development (Alyemeni *et al.,* 2018; Zhao *et al.,* 2021). Additionally, according to Ahanger *et al.* (2020), Cd may interfere with the osmolyte accumulation, glyoxylase cycle, and antioxidant system, which are all part of the body's tolerance mechanisms. According to research by Jan *et al.* (2018), plants exposed to Cd produce more reactive oxygen species and other harmful compounds, such as methylglyoxal, which can impact photosynthesis, enzyme activity, mineral uptake, and assimilation. The mechanisms of tolerance, such as the antioxidant system and osmolyte, respond to stressors like Cd and secondary compound production, exclusion, compartmentation, and chelation of toxic metal ions, are triggered to lessen the damage on plant functioning (Delangiz *et al.,* 2020; Haider *et al.,* 2021).

Jasmonic acid (JA) is any important phytohormone belonging to cyclopentanone compounds having linolenic acid as precursor molecules. It has been reported that JA is actively involved in several developmental events, involving the germination, development, and photosynthesis of roots and shoots, respiration, protein synthesis, signalling, and tolerance to stresses (Ali *et al.,* 2018; Manzoor *et al.,* 2022). Several research reports have shown that exogenous treatments of lower concentrations of JA benefit plants significantly and result in improved germination, growth, biomass production, photosynthesis, enzyme activity etc (Manzoor *et al.,* 2022; Abbas *et al.,* 2022). It has been reported that JA shows positive interaction with gibberellic acid (Ahmad *et al.,* 2021) and potassium (Abbas *et al.,* 2022), leading to tolerance against Cd stress. Treatment of JA increases osmolyte accumulation and gene expression, upregulates antioxidant system activity, and significantly protects photosynthesis, enzyme activity, and general plant function (Ali *et al.,* 2018). Spermidine (Spd) is a polyamine that is vital in plants. Polyamines are bioactive molecules with aliphatic nitrogen groups that have a low molecular weight (Singh *et al.,* 2018). They have been reported to show a crucial role in regulating different physiological and developmental events like photosynthesis, chlorophyll synthesis, enzyme activity, antioxidant functioning, gene expression, and tolerance to stresses (Huang *et al.,* 2021; Qin *et al.,* 2022; He *et al.,* 2022). It has been reported that polyamines show crosstalk with other molecules including phytohormones and mineral nutrients to better benefit the plants and counter the adverse environment by activating tolerance mechanisms (Nahar *et al.,* 2016; Qin *et al.,* 2022). Plants exhibiting further build-up of polyamines have better stress withstanding potential and also influence the activation or upregulation of specific genes in response to stress conditions (Kasukabe *et al.,* 2004).

Wheat is a prominent cereal crop due to its global production and consumption. Heavy metal pollution in soils has a negative impact on wheat productivity and growth. Rising levels of heavy metal accumulation in the soil have been linked to a major global decrease in wheat output (Rizvi *et al.*, 2020). Given this context, the current work postulated that JA priming and the exogenous use of spermidine could mitigate the detrimental effects of Cd on wheat metabolism and growth.

Materials and Methods

Design and treatments

Healthy seeds of wheat (*Triticum aestivum* L.) seeds were chosen, following a five-minute disinfection with $HgCl_2$ (0.001%), ethanol was used as a solvent to prepare the $HgCl_2$ solution. The seeds were thereafter completely cleaned with deionized water and patted dry. Seeds that had been blot dried were soaked for twelve hours in either distilled water or 100 nM JA (methyl jasmonate, or Me-J; Sigma-Aldrich). Reconstituted soil that contained sand, peat, and compost at the rate of 4:1:1 proportion and put in pots for seedling emergence. Each pot contained ten soaked seeds, and 300 mL of nutrient solution was added to each pot. Pots were split into two groups one week after germination. One group received normal nutrient, while the other group was given a nutrient solution containing 100 µM Cd ($CdSO_4 \cdot 8H_2O$). the normal nutrient solution contains NKP. Foliar application of 1 mM spermidine (Spermidine trihydrochloride, Sigma-Aldrich) was started ten days after germination and was given twice a week, along with surfactant. Treatment of Cd and Spd was given for three weeks. Overall treatments in the experiment included: control (normal nutrient solution, without Spd application and JA priming), JA priming, foliar Spd, JA + Spd, Cd, Cd + JA, Cd + Spd and Cd + JA + Spd. Pots were kept randomized complete block (RCB) design, with five replicates. Four-week-old seedlings that had been treated and those that hadn't were uprooted and examined for the various parameters listed below.

Glutamate 1-semialdehyde (GSA), ALA (amino levulinic acid)

A method developed by Kannangara and Schouboe (1985) was used to estimate the GSA's content. In a nutshell, 200 mg of fresh leaf tissue was obtained from two independent sets of leaves from each treatment. While one leaf set was extracted after 4 hours of incubation in 500 µM gabaculine under light, the other was homogenized in 0.1 N HCl. The liquid part above the pellet that was obtained after centrifuging the homogenate at 15,000 g for 10 minutes was gathered and added to the subsequent stage of the experiment. After that, HCl and 3-methyl-2-benzothiazolinonehydrazone (MBTH) were added at the rate of 20% kg HCL/kg and 1.5 mg/ml MBTH respectively, and the mixture was heated for two minutes at a boil in a water bath. The samples were cooled before adding $FeCl_3$ (10 g), and the absorbance at 620 nm was recorded.

The content of -ALA was estimated using two sets of 200 mg fresh leaf samples. In one group, levulinic acid (60 mM) was applied for four hours under light, and the other set was immediately extracted in 1 M sodium acetate buffer (pH 4.6). Following a quick centrifugation of the homogenate the supernatant was boiled in boiling water with acetyl-acetone. Then, after properly mixing, the mixture was added to the cold samples together with Ehrlich's reagent, glacial acetic acid, and 70% perchloric acid. The combination was then allowed to sit for 10 minutes. Harel and Klein (1972) measured absorbance at 555 nm. The treatments were applied for the entire duration of the experiment.

Total levels of carotenoids, chlorophyll, and photosynthetic parameters

Macerating fresh leaf tissue in acetone allowed for the extraction of total chlorophylls and carotenoids. Following a 20-minute centrifugation of the supernatant's optical density at three distinct wavelengths (480 nm, 645 nm, and 663 nm) after the extract was centrifuged at 3000 g (Arnon, 1949). Using the transportable Li-6400 photosynthetic apparatus (LI-COR Inc., USA), net photosynthesis and intercellular CO_2 were

determined. A modulated chlorophyll fluorometer (PAM 2500; Walz, Germany) was used to assess PSII activity (Fv/Fm) in leaves that had been dark-adapted for 30 minutes.

Evaluation of free proline, glycine betaine, and RWC

Estimations of free proline and glycine betaine were carried out as a result of Bates *et al*. (1973); Grieve and Grattan (1983). Relative water content, or RWC, was taken into consideration, and measurement was done using the following, as per the guidelines of Smart and Bingham (1974) formula stated below.

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

where; FW = fresh weight, DW = dry weight and TW = turgid weight

Estimation of Superoxide, hydrogen peroxide, and lipid peroxidation

The concentration of hydrogen peroxide was measured using the Velikova *et al*. (2000) method. Following the extraction of fresh tissue in 0.1% trichloroacetic acid (TCA), the supernatant was mixed with potassium phosphate buffer (pH 7.0), potassium iodide, and the absorbance at 390 nm was measured. The method described by Yang *et al*. (2011) was used to determine the superoxide content. Sulfanilamide and naphthylamine were added after the supernatant underwent a reaction with 10 mM hydroxylamine hydrochloride. The optical density was measured at 530 nm. The amount of malonaldehyde (MDA) formation content was determined using Heath and Packer's (1968) methodology to assess the extent of lipid peroxidation. After removing fresh tissue with 1% TCA, the sample was centrifuged at 10,000 g. To be clear, the supernatant was centrifuged once more at 5000 g following a 30-minute exposure to 0.5% thiobarbituric acid at 95 °C. Measurements of optical density were taken at 532 and 600 nm.

Tests for proline oxidase and glutamyl kinase

The activities of glutamyl kinase (EC 2.7.2.11) and proline oxidase (EC 1.4.3.1) were measured by controlling 500 mg of fresh tissue in 100 mM Tris buffer (pH 7.5) with 1 mM (DTT). A 30-minute, 30,000-g centrifugation was performed on the homogenate. The methods employed by Hayzer and Leisinger (1980) and Huang and Cavalieri (1979) for measuring -GK and PROX activities, respectively, were utilized. The hydroxamate-HCl assay combination for -GK, which included the enzyme to initiate the reaction, 50 mM Tris buffer (pH 7.0), 50 mM L-glutamate, 20 mM MgCl2, and 10 mM ATP, probably reflects the make-up of an assay mixture or reaction employed in a particular experimental process. The reaction was stopped using a stop mixture made up of $FeCl_3$ and TCA. 535 nm was used to measure optical density. Proline, $MgCl_2$, KCN, NADP, phenazine methanosulphate, 2, 6-Dichlorophenol indophenol (DCPIP), and 50 mM Tris buffer (pH 8.5) were the ingredients in the PROX assay mixture. It was measured for three minutes at 600 nm.

Nitrate reductase activity

According to Jaworski (1971), the movement of nitrate reductase (E.C. 1.6.6.1) was assessed. Fresh 500 mg of leaf was incubated for two hours in phosphate buffer (pH 7.5) with 20 mM potassium nitrate and 5% isopropanol. Then, in a different test tube, each sample was given a dose of sulphanilamide (1%), followed by a dose of naphthylethylene diamine hydrochloride (0.02%). The optical density at 540 nm was measured after twenty minutes.

Screening for antioxidant enzymes

In order to isolate the antioxidant enzymes, 500 mg of fresh leaf material was homogenized in 100 mM of phosphate buffer (pH 7.8) that contained 0.1 mM PMSF, 1% polyvinyl pyrrolidine, and EDTA. The homogenate was subjected to a 15-minute 12,000 g centrifugation, after which the supernatant was extracted and utilized to determine the enzyme activity. According to Bayer and Fridovich (1987), superoxide dismutase

(SOD, EC 1.15.1.1) activity was measured by measuring the degree to which the enzyme impeded the photochemical reduction of nitroblue tetrazolium chloride. This procedure was carried out at a wavelength of 560 nm. The optical density change at 240 nm was measured for two minutes using Aebi's (1984) method to determine the activity of catalase (EC 1.11.1.6). The activity of ascorbate peroxidase (APX, EC 1.11.1.11) was assessed using the methodology of Nakano and Asada (1981), and the elimination of H_2O_2 was observed at 290 nm. The glutathione reductase activity (GR; EC 1.6.4.2) was determined by Foyer and Halliwal (1976). The optical density was recorded for two minutes at 340 nm. In order to assess the activity of dehydroascorbate reductase (DHAR, EC 1.8.5.1), absorbance change was measured at 265 nm for two minutes using the Nakano and Asada (1981) method. Hossain *et al*., (1984) state that absorbance measurements taken at 340 nm over a two-minute period were used to determine the monodehydroascorbate reductase activity (MDHAR, EC 1.6.5.4). Utilizing the procedure outlined in Hasanuzzaman and Fujita (2013), variations in optical density were measured for two minutes at 340 nm to ascertain glutathione S-transferase (GST, EC: 2.5.1.18) activity. Protein was evaluated by Lowry et al. (1951), and EU mg-1 protein is the antioxidant enzyme activity.

Methylglyoxal and glyoxalase I activity

Following the guidelines of Hasanuzzaman *et al*. (2011), when glyoxylase I (EC: 4.4.1.5) activity was measured, the optical density rose up at 240 nm. After 500 mg of fresh tissue were extracted, the homogenate was briefly centrifuged at 11,500 g for 15 minutes. A combination of 35 mM methylglyoxal, 16 mM $MgSO_4$, 100 mM GSH, and 0.1 M phosphate buffer make up the experiment. The quantity of methylglyoxal was measured using the procedure described by Wild *et al*. (2012). To put it briefly, the leaf tissue was extracted using 5% perchloric acid, and the supernatant was subsequently neutralized with a saturated potassium carbonate solution and blackened with charcoal. Following treatment of the supernatant with sodium dihydrogen phosphate and N-acetyl-L-cysteine, 288 nM of N-acetyl-S-(1-hydroxy-2- oxoprop-1-yl) cysteine were produced.

Quantification of reduced glutathione and ascorbic acid

Ascorbic acid (AsA) was calculated using a method given by Mukherjee and Choudhuri (1983), whilst reduced glutathione (GSH) was calculated using a method described by Ellman (1959). Calculation was done using the AsA and GSH standard curves.

Total phenols

The method of Singleton and Rossi (1965) was used to estimate the total phenol content. The phenols in the supernatant from the methanol-based extraction of dry plant tissue were identified using the Folin-Ciocalteu reagent. Catechol was employed as the standard optical density measurement at 765 nm.

Estimation of Cd

Dry plant tissue was digested in sulphuric and nitric acid (1:5) at 60 °C, and Cd Cadmium was measured using an atomic absorption spectrophotometer Model: SP-MUV1000. Samples were read on an atomic absorption spectrophotometer Model: SP-MUV1000 following digestion.

Data analysis

The least significant difference (LSD) was set at p 0.05 when Duncan's Multiple Range Test was conducted using ANOVA, and the data were the mean (SE) of three replicates.

Results

Illustration of Cd, JA, and Spd affect plant height as well as the fresh and dry weight of the shoot and root was show in Figure 1. In comparison to the control, the Cd treatment lowered shoot length by 52.26%, fresh weight by 30.95%, dry weight by 39.49%, fresh root weight by 49.31%, and dry root weight by 54.58%. The Cd-induced reduction on these parameters was well mitigated by both JA and Spd, whether used separately and in combination. Only 8.86%, 15.20%, 16.93%, 30.59%, and 29.09% of the height and dry weight of the plants treated with Cd + JA + Spd decreased in comparison to the control (Figure 1 A, B, C, D, E).

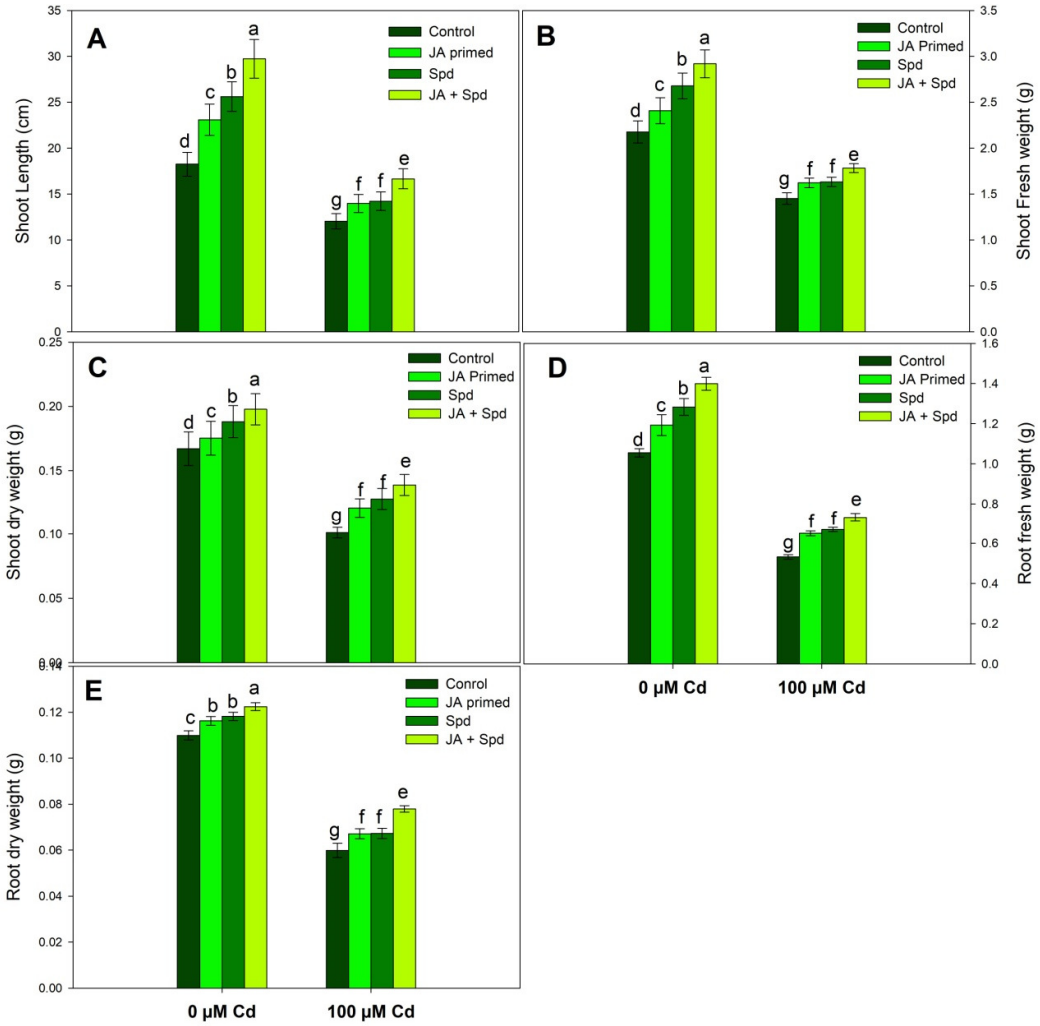

Figure 1. Effect of cadmium stress on (A) shoot length, (B) shoot fresh weight, (C) shoot dry weight, (D) root fresh weight and (E) root dry weight in *Triticum aestivum* L. with and without jasmonic acid priming and foliar spermidine treatments

Data is mean (±SE) of three replicates and different letters on bars show significant difference at $P<0.05$.

The foliar application of Spd and the treatment of JA priming resulted in a reduction of the cadmium level in the roots and leaves. The Cd concentration in root and shoot decreased by 16.30% and 19.63% in Cd + JA, by 30.79% and 36.25% in Cd + Spd, and by 41.78% and 54.04% in Cd + JA + Spd in comparison to Cd-stressed plants (Figure 2A and B).

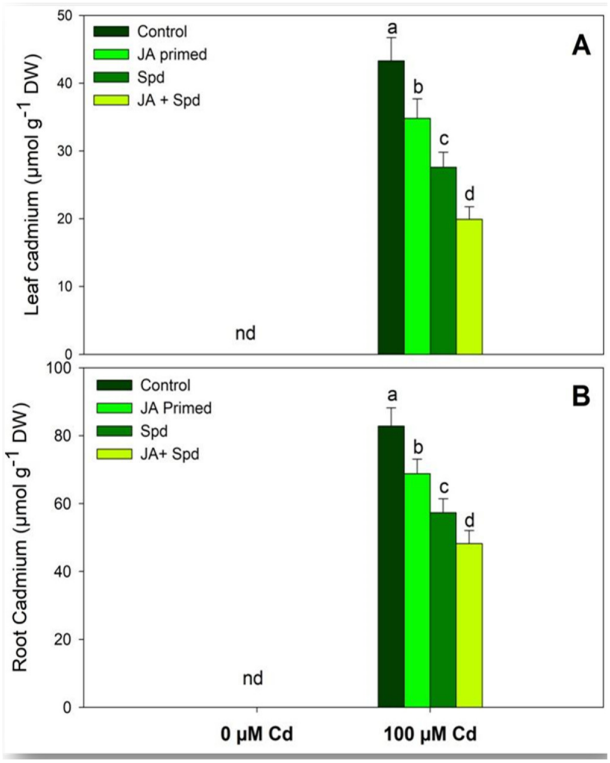

Figure 2. Effect of jasmonic acid priming and foliar spermidine treatments on cadmium content in cadmium stressed *Triticum aestivum* L

Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

Treatment with JA and Spd over control resulted in a considerable increase in content of δ-ALA, GSA, total chlorophyll, and carotenoids, and it also mitigated the loss induced by Cd stress. In plants treated with both JA and Spd, the maximum increases in δ-ALA, GSA, total chlorophyll, and carotenoids were 35.33%, 53.09%, 74.85%, and 32.58%, respectively, compared to the control. A decrease of 32.19% in δ-ALA, 52.00% in GSA, 50.35% in total chlorophyll, and 41.43% in carotenoids was observed under cadmium stress compared to control. Treatment for Spd and JA, however, greatly reduced the decrease. When treated with both JA and Spd, plants stressed by cadmium showed the greatest alleviation; the percentage drop in δ-ALA, GSA, total chlorophyll, and carotenoids compared to control was only 10.15%, 13.21%, 11.63%, and 11.92% (Figure 3A-B and 4A-B.

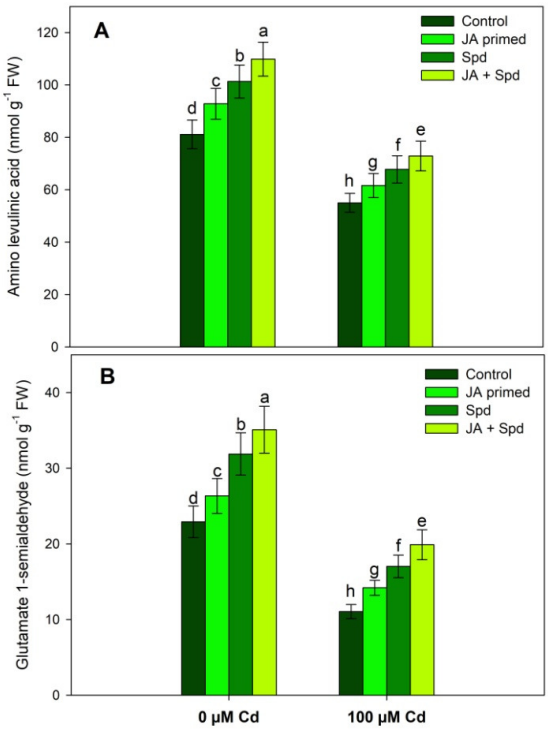

Figure 3. Effect of cadmium stress on (A) amino levulinic acid and (B) glutamate 1-semialdehyde in *Triticum aestivum* L. with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

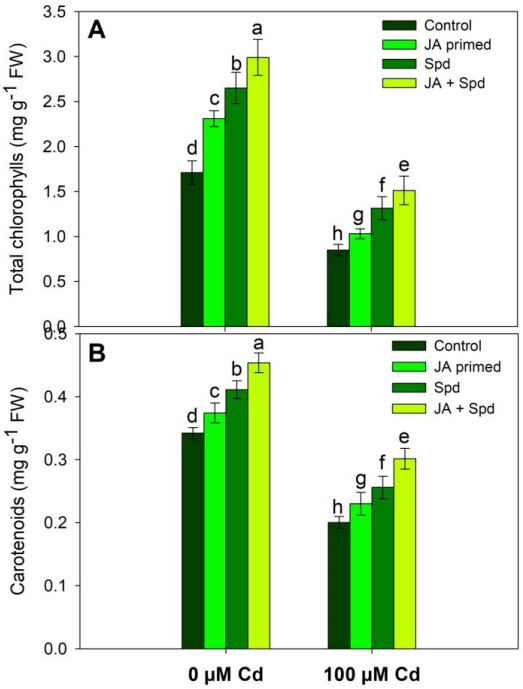

Figure 4. Effect of cadmium stress on (A) total chlorophylls and (B) carotenoids in *Triticum aestivum* L. with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

By 40.01% and 35.26%, respectively, compared to the control, cadmium decreased photosynthesis and intercellular CO_2. Photosynthesis and intercellular CO_2 declined, but were mitigated to a maximum extent in plants treated with both JA and Spd compared to their Cd-stressed counterparts (Figure 5A and B). This was achieved through priming with JA and foliar application of Spd.

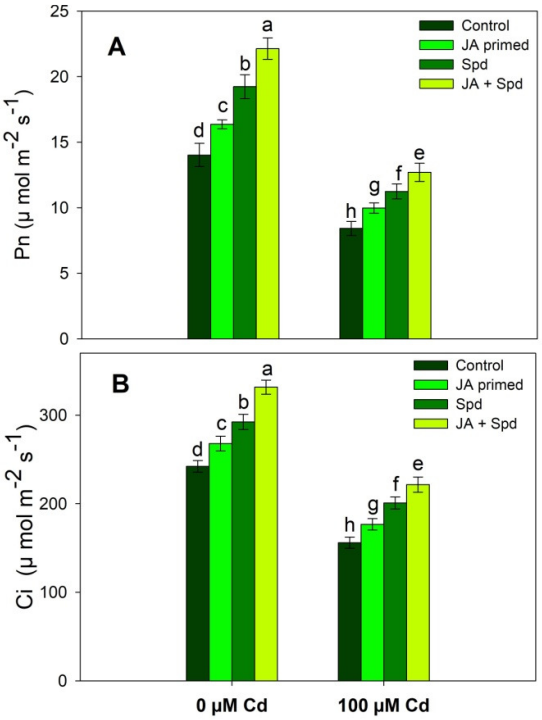

Figure 5. Effect of cadmium stress on (A) photosynthesis and (B) intercellular carbon dioxide in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

When JA and Spd were administered to wheat, the level of proline and glycine betaine rose above control. This increase was further exacerbated when the plants were exposed to Cd stress. Proline and glycine betaine improved by 118.28% and 51.79%, respectively, in comparison to the control when both JA and Spd were treated in an unstressed environment. Proline and glycine betaine grew by 204.33% and 119.90% over control as a result of Cd stress; however, therapy for JA and Spd resulted in an additional rise. Proline and glycine betaine levels were found to be 330.58% and 186.01% higher in Cd + JA + Spd treated wheat plants compared to control (Figure 6 A, B, C). While JA and Spd treatments significantly attenuated the decline, cadmium stress lowered RWC by 38.03%. Under Cd stress, proline oxidase and γ-glutamyl kinase activities rose (71.25% and 27.50%, respectively). Treatment of JA and Spd resulted in a decrease in proline oxidase activity and an increase in γ-glutamyl kinase activity. The wheat plants treated with Cd + JA + Spd showed a maximum rise of 110.17% in γ-glutamyl kinase activity and a maximum decrease of 44.86% in proline oxidase activity, respectively, compared to the control group (Figure 7A and B)

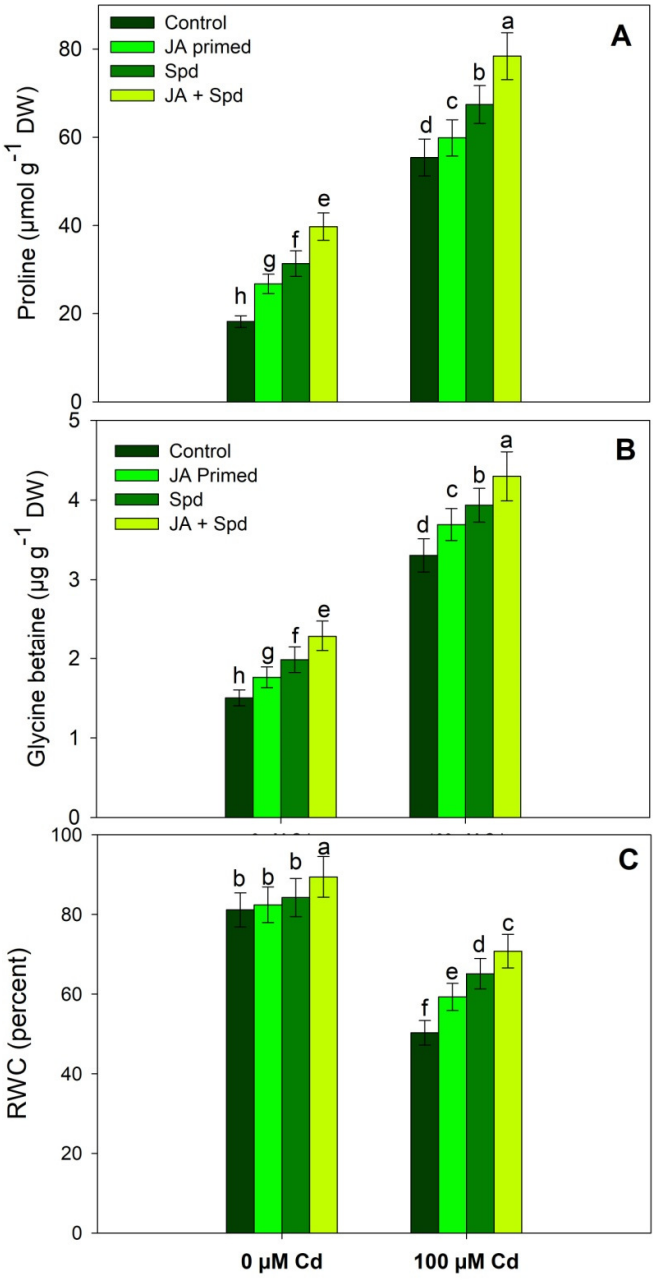

Figure 6. Effect of cadmium stress on (A) proline, (B) glycine betaine and (C) relative water content in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments

Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

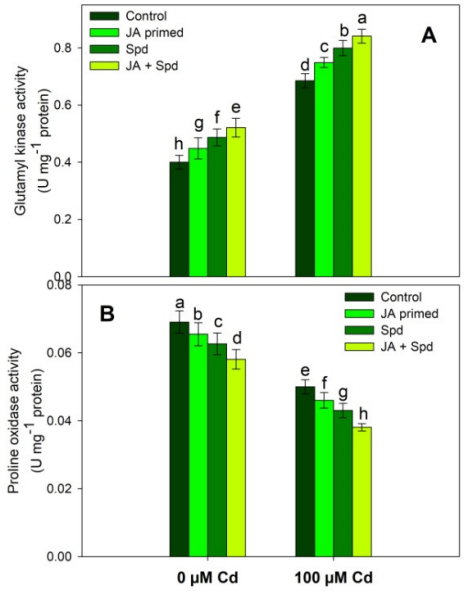

Figure 7. Effect of cadmium stress on activity of (A) γ-glutamyl kinase and (B) proline oxidase in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

Lipid peroxidation and the amount of H_2O_2 and O_2 were decreased in both unstressed and stressed circumstances when JA and Spd were treated separately and in combination. Both JA and Spd-treated plants showed a 62.11%, 50.91%, and 53.11% reduction in H_2O_2, O_2, and lipid peroxidation, respectively, compared to the control. When Cd stress was treated, there was a rise in H_2O_2, O_2, and lipid peroxidation of 143.06%, 113.22%, and 103.06%, respectively. Figure 8A-B-C shows that the combined application of JA and Spd to Cd-stressed plants resulted in a 56.94%, 40.88%, and 43.62% reduction in H_2O_2, O_2, and lipid peroxidation; however, no additional treatment was necessary. The treatment of Cd above control resulted in a considerable increase in the activity of antioxidant enzymes tested.

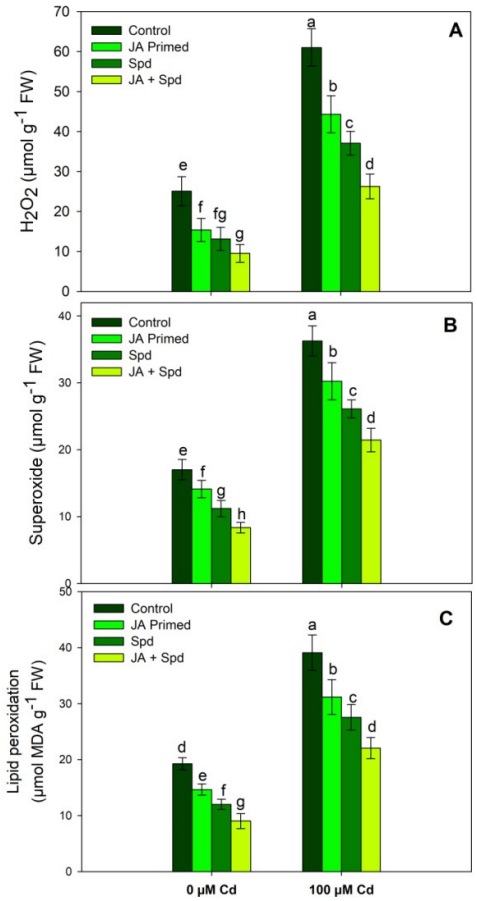

Figure 8. Effect of cadmium stress on (A) hydrogen peroxide, (B) superoxide and (C) lipid peroxidation in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

Due to cadmium stress, the percentage increase in SOD, CAT, GST, APX, DHAR, MDHAR, and GR activity over the control was 230.13%, 89.38%, 139.14%, 94.55%, 175.35%, 88.08%, and 81.86%, respectively. The antioxidant enzymes' activity was greatly enhanced by both the solo and combined treatments of JA and Spd under growth conditions that were not stressed. In plants treated with Cd + JA + Spd over control, the activities revealed an increase of 5.55-fold for SOD, 2.77-fold for CAT, 3.96-fold for GST, 2.52-fold for APX, 4.24-fold for DHAR, 2.48-fold for MDHAR, and 2.98-fold for GR. (Table 1). In contrast, treatment of AsA and GSH under normal and Cd treatment enhanced both AsA and GSH. Cadmium stress lowered AsA by 31.33% and increased GSH by 46.41% over control. In unstressed plants, the increases in AsA and GSH were 39.83% and 30.33%, respectively, in plants treated with both JA and Spd treatments. AsA declined less when JA and Spd were given together to Cd-stressed plants, and the GSH content rose even more (Table 1).

Table 1. Effect of cadmium (100 µM) stress on activity of superoxide dismutase, catalase, glutathione S-transferase, ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase, and ascorbic acid and reduced glutathione content in wheat (*Triticum aestivum* L) with and without jasmonic acid (100 nmol) priming and foliar application of spermidine (1 mM)

	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GST (U mg^{-1} protein)	APX (U mg^{-1} protein)	DHAR (U mg^{-1} protein)	MDHAR (U mg^{-1} protein)	GR (U mg^{-1} protein)	AsA (nmol g^{-1} FW)	GSH (nmol g^{-1} FW)
Control	3.02 ±0.21h	40.10 ±3.1h	11.52 ±.88g	0.312 ±0.020g	20.1 ±1.5h	31.1 ±3.1h	0.1003 ±0.010g	157.4 ±8.3h	201.1 ±9.2g
JA	4.10 ±0.29g	50.35 ±3.7g	15.04 ±.92f	0.382 ±0.025f	26.3 ±1.7g	37.6 ±3.5g	0.1192 ±0.011f	175.6 ±10.1g	232.2 ±9.7f
Spd	4.98 ±0.32f	61.28 ±4.0f	17.45 ±1.4ef	0.428 ±0.031ef	36.2 ±2.1f	45.0 ±3.6f	0.1309 ±0.014ef	193.9 ±12.2f	241.9 ±10.1ef
JA + Spd	6.36 ±0.38e	68.03 ±4.2e	20.31 ±1.6e	0.451 ±0.036e	43.5 ±3.3e	53.1 ±3.7e	0.1389 ±0.010e	217.3 ±12.9e	257.1 ±10.6e
Cd	9.97 ±0.56d	79.73 ±5.1d	27.55 ±2.1d	0.567 ±0.042d	57.1 ±3.8d	63.0 ±4.5d	0.1806 ±0.017d	102.7 ±6.7d	284.0 ±9.9d
Cd + JA	12.01 ±0.88c	87.66 ±5.6c	33.88 ±2.5c	0.659 ±0.041c	66.5 ±4.1c	72.1 ±4.3c	0.2351 ±0.017c	116.2 ±6.9c	308.3 ±11.2c
Cd + Spd	13.62 ±0.98b	103.66 ±6.2b	37.50 ±3.0b	0.723 ±0.045b	74.8 ±4.9b	80.7 ±4.6b	0.2799 ±0.021b	130.2 ±7.1b	334.3 ±12.3b
Cd + JA + Spd	16.78 ±1.2a	116.93 ±6.6a	45.72 ±3.3a	0.798 ±0.043a	89.6 ±5.4a	89.8 ±4.8a	0.3395 ±0.026a	143.3 ±8.0a	359.2 ±13.2a

Data is mean of three replicates and different letters show significant difference at $P<0.05$.

Under Cd stress, phenols increased by 82.92% and nitrate reductase activity reduced by 43.77%, respectively. When JA and Spd were applied together, unstressed plants showed a 39.02% increase in phenols and a 33.47% increase in nitrate reductase activity. Comparing plants under Cd stress with those treated with JA and Spd together resulted in an additional 14.0 percent rise in phenols. By treating JA and Spd together, the activity of nitrate reductase decreased by 42.74% compared to counterparts under Cd stress (Figure 9A and B). Methylglyoxal (103.00%) and glyoxylase I activity (90.97%) were higher under cadmium stress than under control. Treatment with either JA or Spd alone or in combination reduced the amount of methylglyoxal by 18.87% in JA primed seedlings, 33.15% in Spd treated seedlings, and 47.29% in JA + Spd treated seedlings compared to control. Methylglyoxal was reduced by 37.00% in Cd-stressed plants when JA and Spd were treated together. Over the control, glyoxylase I activity rose by 11.39% as a result of JA, 24.38% as a result of Spd, and 34.12% as a result of JA + Spd. Plants treated with both JA and Spd over control showed the largest increase in glyoxylase I activity, rising by 153.39%, after treatment with JA and Spd for Cd-stressed plants (Figure 10A and B).

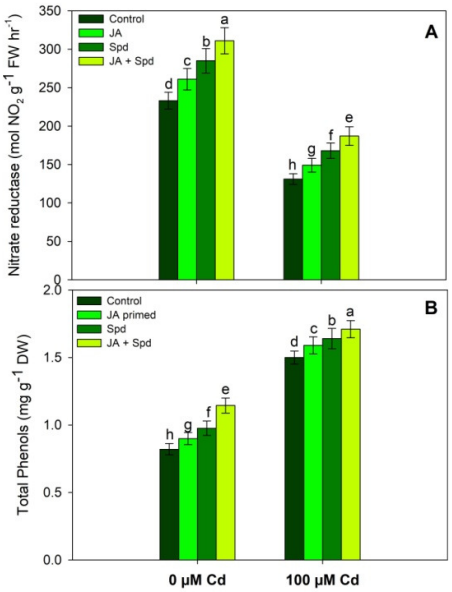

Figure 9. Effect of cadmium stress on activity of (A) nitrate reductase and the (B) total phenols in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

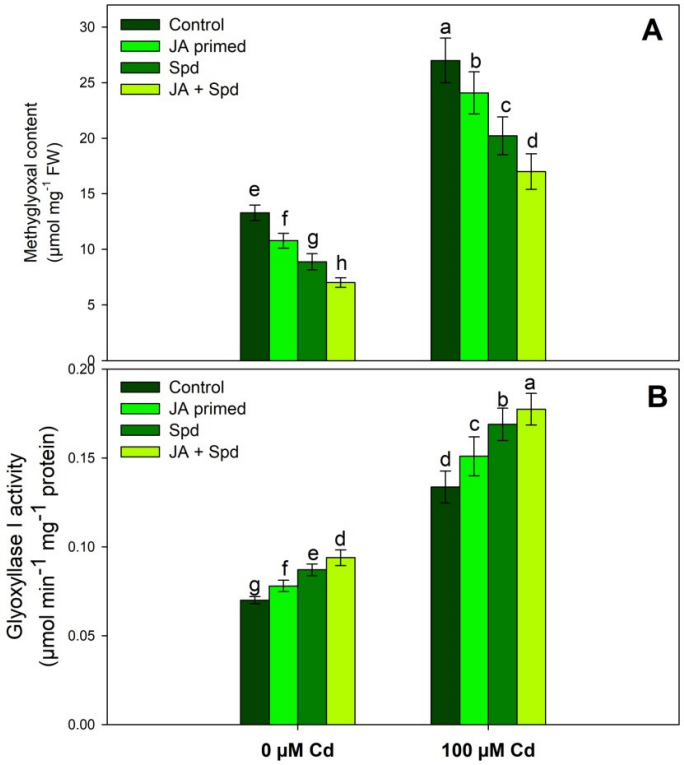

Figure 10. Effect of cadmium stress on (A) methylglyoxal content and the (B) activity of glyoxylase I in *Triticum aestivum* L with and without jasmonic acid priming and foliar spermidine treatments
Data is mean (SE) of three replicates and different letters on bars show significant difference at P<0.05.

Discussion

There has been increasing evidence that heavy metal pollution is increasing due to significant human interferences through various means. In the current study, the influence of JA priming and foliar Spd application in alleviating the Cd stress mediated deleterious impact on wheat growth. Treatments of JA and Spd caused in an important increase in growth measured as plant height and plant dry weight and also alleviated the Cd induced reduction in these parameters. Our results of increased plant height and dry weight due to JA priming corroborate with the findings of Bali *et al.* (2020) and Kaushik *et al.* (2022) in tomato and *Cajanus cajan,* respectively. Similarly, foliar treatment of Spd resulted in increased growth of *Potamogeton malaianus* (Yang *et al.,* 2011). Several plant species have observed growth decline due to Cd stress (Hassan *et al.,* 2020; Zhao *et al.,* 2021; Liu *et al.,* 2018*)*. It has been observed that Cd triggers mitotic irregularities like c-mitoses, breaks, stickiness lagging and vagrant chromosomes resulting in significant alterations in cellular division and chromosome morphology (Zhang and Yang, 1994*)*. Heavy metals restrict cellular proliferation resulting in declined growth (Jaiswal *et al.,* 2021). The effect of combined treatments of JA and Spd on Cd tolerance has not been reported. Alleviation of Cd mediated decline in growth by JA (Ahmad *et al.,* 2021; Abbas *et al.,* 2022) and Spd (Ahanger *et al.,* 2020; Gu *et al.,* 2022) treatments have been reported earlier also. Increased growth in JA and Spd treated plants may be due to increased enzyme functioning and water uptake concomitant with reduced Cd accumulation. Further growth enhancement in JA and Spd grown seedlings can be achieved through improved chlorophyll synthesis, photosynthesis and reduced ROS accumulation. Treatment of either Cd or Spd or both caused in a deterioration in the uptake of Cd. The uptake and transport of Cd occur through membrane transporters like ZIP and NRAMP in competition with the essential nutrients (Haider *et al.,* 2021). Reducing the uptake and increase of lethal metal ions is one of the efficient mechanisms for tolerating the toxics effect of metal ions (Alyemeni *et al.,* 2018; Ahmad *et al.,* 2021).

Seedlings stresses with Cd revealed a substantial decrease in the components of the chlorophyll biosynthesis pathway, reflecting in declined chlorophyll content. However, treatments of JA and Spd alone and combinedly increased the synthesis of chlorophyll and the intermediates compounds of the chlorophyll biosynthesis pathway, which were also obvious as increased photosynthesis. Results showing the influence of Cd on the synthesis of δ-ALA and GSA are scanty. However, it has been demonstrated the stresses significantly decline the synthesis of intermediate compounds of the chlorophyll biosynthesis pathway, including δ-ALA and GSA (Zhao *et al.,* 2020; Qin *et al.,* 2022). Reduced synthesis of δ-ALA, GSA and chlorophylls due to stresses have been attributed to decline activities of enzymes mediating the interconversion of these intermediate compounds (Zhao *et al*., 2020; Qin *et al.,* 2020, 2022). It was detected that plants treated with JA priming and foliar Spd supplication exhibited much increase in δ-ALA, GSA, chlorophylls and carotenoids under unstressed conditions, and exhibited maximal alleviation of Cd-induced decline. Similar to our results increase in the content of chlorophylls and carotenoids due to JA (Ahmad *et al.,* 2021; Abbas *et al.,* 2022) and Spd (Ahanger *et al*., 2020) treatments under Cd stress has been reported. Cadmium induced reduction in chlorophyll synthesis significantly contributes to photosynthetic decline. In addition, there have been considerable evidence that Cd inhibits stable chlorophyll protein binding, damages light harvesting complexes and photosystems and reduces the transcription of key genes including psbA, psaB and rbcL photosynthesis thereby slowing down the significant yield and photosynthesis (Shukla *et al.,* 2008; Qian *et al.*, 2009; Hendrik *et al.,* 2010; Goussi *et al.,* 2018). It has been shown that JA (Manzoor *et al.,* 2022) and Spd (Ahanger *et al.,* 2020) treatment enhances and alleviates the Cd induced alterations in chlorophylls, carotenoids and PSII functioning by maintaining the redox homeostasis and antioxidant functioning. Cadmium affects the photosynthetic functioning by adversely affecting the structure of chloroplast, thylakoids and membranes (Parmar *et al.,* 2013), however exogenously applied protectants, including JA (Per *et al.,* 2016) and Spd (Li *et al.,* 2015) stabilizes the structure and function of cellular organelles.

Treatment of JA and Spd reduced the content of ROS, including superoxide and hydrogen peroxide reducing lipid peroxidation considerably. Several reports discussing increased oxidative damage due to Cd stress through increased ROS accumulation in several plant species are available (Hasanuzzaman *et al.,* 2017; Alyemeni *et al.,* 2018; Hassan *et al.,* 2020; Kaleem *et al.*, 2022). Greater generation of ROS imparts damaging effects on cellular structures and pathways, leading to damage to normal plant functioning. The ideal application of ROS can be beneficial in stress signalling and elicitation of tolerance mechanisms (Apel and Hirt 2004). Muhammad *et al.,* (2023) have recently reported increased oxidative damage in terms of lipid peroxidation in Cd stress *Dendranthema morifolium* cultivars affecting photosynthesis and growth significantly. Like our results, the decline in ROS and lipid peroxidation due to the treatment of JA (Ahmad *et al.,* 2021; Manzoor *et al.,* 2022) and Spd (Yang *et al.,* 2011; Ahanger *et al*., 2020) under Cd stress has been reported earlier; however, the combined effect has not been evaluated.

Because antioxidant enzyme activity is significantly increased in JA and Spd-treated seedlings, excess ROS is quickly eliminated, reducing oxidative damage. Enzymes of the antioxidant system eliminate ROS in different cellular compartments, thereby protecting the structure and function of major cellular organelles (Hasanuzzaman *et al*., 2020). Others have also reported that Cd stress causes an increase in the activity of antioxidant enzymes (Muradoglu *et al.,* 2015; Alyemeni *et al*., 2018; *Kang et al.,* 2021; Ahmad *et al.,* 2021). Among the antioxidant enzymes, SOD is specific for neutralising superoxide, while H_2O_2 is neutralised by CAT and ascorbate-glutathione cycle (Ahanger *et al.,* 2017). Plants displaying increased functioning of the antioxidant system exhibit a significant decline in ROS and lipid peroxidation, thereby showing greater protection to metabolism (Kang *et al.,* 2021). Priming with JA and foliar application of Spd resulted in an increase in the activities of antioxidant enzymes assayed, thereby contributing to reduced ROS accumulation and membrane lipid peroxidation. In support of the results, earlier up-regulation of antioxidant enzymes due to JA (Ali *et al.,* 2018; Mir *et al.,* 2018; Kamran *et al.,* 2021) and Spd (Nahar *et al.,* 2016; Jiang *et al.,* 2021) has been described in numerous plant species. Glutathione S-transferase (GST) quenches ROS using glutathione, thereby protecting cells (Kumar and Trivedi 2018). Increased GST activity due to JA (Ahmad *et al.,* 2021) and Spd *(Li et al. 2020)* has also been reported in other plants. However, reports showing the combined influence of JA priming and foliar Spd application in alleviating the Cd stress induced damaging effects are not available. Further, JA and Spd enhanced ascorbic acid content and reduced glutathione significantly, and the maximum growth was detected in seedlings preserved with both JA and Spd. Cadmium stress resulted in reduced ascorbic acid content while increased GSH. Earlier, the alleviation of Cd mediated decline in AsA and increase in GSH due to JA treatment has been reported by Ahmad *et al.,* (2021). Similarly, in justification with our results, Kamran *et al.* (2021) have also confirmed a similar effect of JA on AsA and GSH in chromium stressed *Brassica parachinensis*. Both AsA and GSH are important redox components, and ROS scavengers and maintaining their increased concentrations due to JA and Spd treatments can be beneficial to tolerate stressful conditions (Ahanger *et al.,* 2018). Plants exhibiting increased AsA and GSH content exhibit improved photosynthesis, enzyme activity and cellular functioning (Akram *et al.,* 2017; Hasanuzzaman *et al.,* 2017).

Cadmium stress resulted in increased accumulation of phenols, and treatment of JA and Spd induced further increase. Earlier, Kisa *et al.,* (2016) and Janczak-Pieniazek *et al.,* (2023) have also reported an increase in the content of phenolics due to Cd stress. Phenols are important secondary compounds involved in several plant functions, including growth, metal chelation, ROS scavenging, membrane stabilization and stress signalling (Ghori *et al.,* 2019; Anjitha *et al.,* 2021). In confirmation with our results, Abbas *et al.* (2022) have also observed an increase in phenols due to JA treatment in Cd stressed pea. Increased phenol accumulation due to Spd application has been described under Cd stress (Ahanger *et al.,* 2020). Increased secondary compound accumulation results due to up-regulation of the activity of enzymes mediating synthesis of protection (Ahanger *et al.,* 2020; Janczak-Pieniazek *et al.,* 2023).

The activity of nitrate reductase was increased significantly due to JA and Spd treatments. Cadmium mediated decline in nitrate reductase activity were mitigated by the treatments if JA and Spd had the highest alleviation observed in seedlings treated with both JA and Spd. Nitrate reductase is a rate limiting enzyme in Nitrogen breakdown and a decline in its activity severely influences the nitrogen assimilation and incorporation of nitrogen into amino acids (Chaffei *et al.,* 2004; Singh *et al.,* 2019). Improved activity of nitrate reductase due to Spd in pea (Kapoor *et al*., 2021). Spermidine has a beneficial impact on the nitrogen metabolism of tomatoes under stressed conditions (Zhang *et al*., 2013). Similarly, treatment of JA increased nitrate reductase activity in Cd stressed peas been reported by Abbas *et al.* (2022). Treatment of JA and Spd resulted in optimising the activity of nitrate reductase and preventing the Cd induced decline. Optimising the nitrogen metabolism can increase growth and yield under stress (Dong *et al.*, 2022).

Moreover, the activity of glyoxylase I enzyme was significantly up-regulated in JA and Spd treated plants. Glyoxylase system eliminates the excessively generated methylglyoxal thereby preventing the cells from its damaging effects (Kamran *et al.,* 2021; Qin *et al.,* 2022). Similar reports about increased methylglyoxal content and glyoxylase I activity under Cd stress are available (Hossain *et al.,* 2010; Nahar *et al.,* 2016; Ahmad *et al.,* 2021). In confirmation with our results of increased glyoxylase I activity and reduced accumulation of methylglyoxal due to JA treatment are the results of Ahmad *et al.* (2021) under Cd stress, Kamran *et al.* (2021) under chromium stress and Siddiqui *et al.* (2022) under arsenic stress. In addition, up-regulation of glyoxylase activity due to Spd treatment under different stresses, including Cd (Nahar *et al*., 2016), arsenic (Kapoor *et al*., 2021). Mitigation of the damaging effects of methylglyoxal under heavy metal due to Spd induced up-regulation of glyoxylase system.

Treatment of JA and Spd triggered the gathering of osmoprotectants, including proline and glycine betaine. A significant enhancement in the proline and glycine betaine content in Cd stressed plants has been stated earlier (Hossain *et al.,* 2010; Ahanger *et al.,* 2020; Ahmad *et al.*, 2021). Improved accumulation of osmoprotectants benefits plants by alleviating the adverse effects of stresses on key metabolic pathways, including photosynthesis by scavenging ROS, maintaining tissue water content and protecting enzyme activity (Ahmad *et al*., 2018; Alyemeni *et al*., 2018). Increased accumulation of proline due to JA and Spd was observed to be regulated by modulation in the activity of enzymes involved in biosynthesis and catabolism. It has been stated that increased γ-glutamyl kinase activity and decreased proline oxidase activity significantly reflects in proline accumulation (Elkelish *et al*., 2019; Khan *et al*., 2020).

Conclusions

In conclusion, the current study investigated the effects of jasmonic acid (JA) priming and foliar spermidine (Spd) application, both individually and in combination, on the mitigation of cadmium (Cd) stress-induced oxidative damage in wheat. The findings exhibited that Cd stress has a negative impact on plant growth as well as various physiological and biochemical parameters. However, the use of JA and Spd, both separately and in combination, demonstrated promising results in minimizing these unfavorable effects. Furthermore, the use of JA and Spd reduced oxidative stress markers while increasing antioxidant enzymes and secondary metabolism. These alterations showed an improvement in the plant's oxidative stress defense mechanisms. These findings contribute to our understanding of plant stress responses and offer valuable insights into the development of strategies for improving the tolerance of crops to heavy metal stress. More research and exploration of the mechanisms of action of these priming agents is required to understand their potential in sustainable agriculture and environmental restoration fully.

Authors' Contributions

Data curation: KA; Formal Analysis: GSHA; Funding Acquisition: KA; Investigation: MHS and SMSA; Methodology and data curation: IMA & KA; Project Administration: MHS, NB and IK; Resources: AAA, KA and SMSA; Supervision and review: MIK; Writing original draft, KA, MIK and MHS. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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