https://doi.org/10.17221/154/2024-PSE

Poly-glutamic acid reinforces wheat cadmium tolerance by modulating ascorbic acid and glutathione metabolism

Limin Wu[1], *Qiumei Zhang*[2], *Ninghai Lu*[1]*

[1]*Henan Institute of Science and Technology, Xinxiang, P.R. China*
[2]*Henan Fengyuan Seed Company Limited, Xinxiang, P.R. China*
**Corresponding author: luninghai2019@aliyun.com*

Citation: Wu L.M., Zhang Q.M., Lu N.H. (2024): Poly-glutamic acid reinforces wheat cadmium tolerance by modulating ascorbic acid and glutathione metabolism. Plant Soil Environ., 70: 483–491.

Abstract: We investigated the influence of poly-glutamic acid (PGA) on ascorbic acid (AsA) and glutathione (GSH) metabolism in leaves of wheat seedlings under cadmium (Cd) stress. The results showed that Cd stress enhanced ascorbate peroxidase, dehydroascorbate reductase, monodehydroascorbate reductase, glutathione reductase, gamma-glutamylcysteine synthetase and L-galactono-1,4-lactone dehydrogenase activities, and increased AsA and GSH contents. Whereas Cd reduced AsA/dehydroascorbic acid (DHA) and GSH/oxidised glutathione (GSSG) ratios and increased malondialdehyde (MDA) content and electrolyte leakage (EL). Meanwhile, Cd stress improved Cd accumulation and nonphotochemical quenching (q_N) and decreased soil and plant analyser development (SPAD) value, net photosynthetic rate (P_n), maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_P), quantum efficiency of PSII photochemistry (Φ_{PSII}), wheat height and dry biomass. Compared to Cd alone, PGA plus Cd stress reinforced AsA and GSH metabolism *via* the above enzymes and increased AsA and GSH contents and their redox status. PGA plus Cd stress also decreased MDA content and EL. Besides, PGA plus Cd stress decreased Cd accumulation and increased SPAD value, P_n, q_N, F_v/F_m, q_P, Φ_{PSII}, wheat height and dry biomass. Moreover, PGA alone showed positive effects on the indicators mentioned above. Our results clearly indicated that PGA enhanced wheat Cd tolerance by preventing Cd uptake and enhancing AsA and GSH metabolism. Therefore, PGA can be applied to enhance wheat Cd tolerance in production.

Keywords: cadmium toxicity; biostimulant; antioxidant capacity; redox state; photosynthetic traits

The rapid development of cities and modern industry has brought many environmental problems, among which heavy metal pollution has become a widely concerned environmental issue. Excess heavy metals pollute water and soil and further induce plant stress, thereby inhibiting growth and production, especially for food crops. It has been documented that many heavy metals could induce stress to wheat crops, such as cadmium (Cd) (Hussain et al. 2022), mercury (Hg) (Ibrahim et al. 2022), chromium (Cr) (Rafique et al. 2022), copper (Cu) (Alshegaihi et al. 2023) and lead (Pb) (Perveen et al. 2022). Among these heavy metals, Cd usually causes excessive production and massive accumulation of reactive oxygen species (ROS), thereby inducing plant peroxide damage. In this way, Cd breaks plant redox homeostasis and inhibits growth. Fortunately, plants can adapt the antioxidant system to keep ROS at a low level. Thus, plants can maintain redox homeostasis by employing the antioxidant system to relieve the toxic influence of Cd. As reported, the antioxidant defence system contained enzymatic and non-enzymatic antioxidants (Mir et al. 2022, Gao et al. 2024). Ascorbic acid (AsA) and glutathione (GSH) are two non-enzymatic antioxidants and display an important part in keeping the redox homeostasis, thereby protecting plants against the peroxide damage caused by various environmental stresses (Zhu et al. 2021, Zhao et al. 2023). Plants may modulate AsA and GSH contents by controlling their recycling and biosynthetic metabolism (Zhu et al. 2021, Zhao et al. 2023). In plants, AsA biosynthesis is mainly

https://doi.org/10.17221/154/2024-PSE

through the L-galactose pathway. In this pathway, L-galactono-1,4-lactone dehydrogenase (GalLDH) is the key enzyme (Zhu et al. 2021, Zhao et al. 2023). Gamma-glutamylcysteine synthetase (γ-ECS) is the key enzyme of GSH biosynthesis (Zhu et al. 2021, Zhao et al. 2023). The ascorbate-glutathione (AsA-GSH) cycle is responsible for the metabolism of AsA and GSH recycling. The operation of this cycle is controlled by four enzymes, namely ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and glutathione reductase (GR). Many exogenous substances have been proven to control AsA and GSH contents through recycling and biosynthetic metabolism under stress (Khan 2023, Zhao et al. 2023). For Cd stress, Kaushik et al. (2024) clarified that methyl jasmonate (Me-JA) could improve AsA and GSH contents by promoting the AsA-GSH cycle in Cd-stressed pigeon peas. Zhu et al. (2021) showed that rare earth element praseodymium (Pr) mitigated maize Cd toxicity by improving AsA and GSH contents through their recycling and biosynthetic pathways. The research mentioned above manifested that we may use corresponding exogenous substances to improve AsA and GSH contents through their regeneration and biosynthesis, thereby reinforcing plant Cd tolerance.

Poly-glutamic acid (PGA), also known as natto gum, is usually obtained through microbial fermentation. It has many advantages, such as water solubility, biodegradability, non-toxicity and environmental friendliness. As reported, PGA played important roles in chelating heavy metal elements, improving soil physical and chemical properties, fertiliser utilisation efficiency and stress tolerance, and promoting crop growth, yield and quality (Wang et al. 2023). PGA also improved plant tolerance under various stresses, such as salt, cold and drought stresses (Xu et al. 2017, Skalski et al. 2024). However, whether or not PGA enhances plant Cd tolerance by controlling AsA and GSH metabolism is unknown. Therefore, it is interesting to carry out this part of the work. Besides, the photosynthetic performance and growth can directly reflect the response of plants to stresses. Accordingly, it is interesting to look into PGA action in modulating plant photosynthetic performance and growth when exposed to Cd. From the above two aspects, we may add new useful and deep knowledge for PGA action in modulating plant Cd tolerance.

In this study, we explored the influence of PGA on enzymes in AsA and GSH metabolism, AsA and GSH contents and their redox status, malondialdehyde (MDA) content, electrolyte leakage (EL), photosynthetic performance indicators, Cd accumulation and growth indicators of wheat plants exposed to Cd stress. We simultaneously aimed to elucidate a more theoretical foundation for PGA application in the agricultural management of wheat crops exposed to Cd-contaminated soil.

MATERIAL AND METHODS

Plant material and treatments. Wheat seeds of cv. Yuanfeng16 were germinated and cultivated in a plant growth chamber with controllable temperature, humidity and lighting. In the plant growth chamber, the temperature, relative humidity, light intensity, and photoperiod were respectively set as 25/15 °C (day/night), 60%, 500 μmol/m^2/s and 12-h. After wheat plants fully unfolded their second leaves, they were cultivated by using hydroponic methods. Seedlings were transferred into 1 000 mL half-strength Hoagland's nutrient solution by immersing their roots in the nutrient solution, and the roots were kept in the dark. The nutrient solution was changed once a day. After the 3rd leaves had fully unfolded, wheat plants were used to explore the influence of Cd and PGA on AsA and GSH metabolism, photosynthetic performance, and growth.

Appropriate Cd concentration was chosen from four concentrations (25, 50, 75 and 100 mg/L $CdCl_2$). After 10 days of Cd treatment, plants treated with 25 mg/L $CdCl_2$ presented no obvious wilting symptoms, and those exposed to 50 mg/L $CdCl_2$ presented only slight wilting symptoms. However, the plants exposed to 75 and 100 mg/L $CdCl_2$ presented obvious wilting symptoms. Thus, we chose 50 mg/L $CdCl_2$ as the appropriate treatment concentration. All plants were transferred into beakers containing 500 mL 50 mg/L $CdCl_2$ by immersing their roots in the solution, and their roots were kept in the dark. The period of Cd treatment was 10 days. To study the effects of PGA, plants were exposed to 0.1, 0.3 and 0.6% PGA for 12 h and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 10 days. Control plants were exposed to the nutrient solution alone. After 5 days of Cd treatment, the 3rd leaves were sampled and kept in a –80 °C refrigerator. The above samples were then used to analyse physiological and biochemical indicators. After 10 days of Cd treatment, growth indicators and Cd accumulation were analysed.

Analysis of APX, GR, DHAR and MDHAR. The crude enzyme solution was extracted using Shan and

https://doi.org/10.17221/154/2024-PSE

Liang's method (2010). The activities of APX, GR, MDHAR and DHAR were respectively measured following the method of Nakano and Asada (1981), Grace and Logan (1996), Miyake and Asada (1992) and Dalton et al. (1986). For APX, the wavelength used to measure the absorbance was 290 nm. For GR, the wavelength used to measure the absorbance was 340 nm. For MDHAR, the wavelength used to measure the absorbance was 340 nm. For DHAR, the wavelength was used to measure the absorbance. The specific activities of these enzymes were all expressed as U/g fresh weight (FW).

Analysis of GalLDH and γ-ECS. For GalLDH analysis, samples were homogenised in potassium phosphate buffer (pH 7.4) and then centrifuged at 300 g for 10 min at 2 °C. The supernatant was centrifuged at 10 000 *g* for 20 min at 2 °C. The sediment was suspended in the above buffer and then used to measure GalLDH activity following the method of Shan and Liang (2010). The absorbance at 550 nm was recorded. For γ-ECS analysis, samples were homogenised in HCl and then centrifuged at 20 000 *g* for 10 min at 2 °C. The supernatant was then used to measure γ-ECS activity following the method of Shan and Liang (2010). The specific activities of the above enzymes were expressed as U/g FW.

Analysis of AsA and GSH contents and their redox status. AsA and DHA were detected following the method of Hodges et al. (1996). AsA redox status was expressed as the ratio of AsA/DHA. GSSG and GSH were detected using Griffith's method (1980). GSH redox status was expressed as the ratio of GSH/GSSG.

Analysis of MDA and EL. MDA content and EL were respectively measured following the Hodges et al. (1999) and Zhao et al. (2004) method. For MDA analysis, samples were homogenised with phosphate buffer solution (pH 7.8) and then centrifugated at 12 000 × *g* for 10 min at 4 °C. The supernatant was used to determine MDA content by recording the absorbances at 532, 600, and 450 nm. For EL analysis, 20 discs of fresh leaves were submerged in 20 mL of deionised water at 25 °C for 3 h. Then, the electrical conductivity was measured and recorded as L_0. After boiling, the electrical conductivity was measured and recorded as L_1. EL was calculated as the percentage of L_0 to L_1.

Analysis of photosynthetic performance. The SPAD value was measured through SPAD-502 plus chlorophyll meter (Konica Minolta, Tokyo, Japan). P_n was measured through a photosynthesis system (LI-COR 6400, Lincoln, USA). F_v/F_m, q_P, q_N and Φ_{PSII} were measured and recorded using a Yaxin-1161G fluorometer (Yaxin, China). P_n, F_v/F_m, q_P, q_N and Φ_{PSII} were all determined from 10:00 to 11:30 A.M.

Analysis of wheat height and biomass. After 10 days of Cd treatment, the height was measured using a plastic meter ruler with a measuring range of 100 cm. The drying method was used to measure wheat biomass. After 10 days of Cd treatment, each seedling was harvested, weighed, and placed in an 80 °C oven for 72 h. Finally, the dry biomass was weighed and recorded.

Measurement of Cd accumulation. Cd accumulation in both leaves and roots was measured according to Xu et al. (2011). Firstly, 10 mmol/L EDTA was used to wash all samples to remove metals from their surface. Then, the above samples were placed in a 70 °C drying oven to be dried until they reached a constant weight. Dry samples were then digested with 3:1 $HNO_3/HClO_4$ at 95 °C. The digested residue was dissolved in 0.7 mL 1 mol/L HCl and then diluted by distilled water to 10 mL. Then, Cd content was analysed through the flame atomic absorbance spectrometry (Hitachi 180-80, Tokyo, Japan).

Statistical analysis. The results are presented as the mean of three replications. Excel software (Washington, USA) was used to organise data and draw tables and figures. SPSS software (Chicago, USA) was used to compare means by one-way analysis of variance and Duncan's test at the 5% significance level.

RESULTS

Influence of PGA on enzymes responsible for AsA and GSH metabolism. Cd and PGA alone all significantly improved APX, GR, DHAR, MDHAR, GalLDH and γ-ECS activities than the control (Table 1). Comparatively, PGA plus Cd significantly increased these enzyme activities more than Cd treatment alone. In comparison with Cd treatment, 0.1% PGA plus Cd improved APX, GR, DHAR, MDHAR, GalLDH and γ-ECS activities by 29.4, 20.6, 22.0, 26.1, 25.3 and 20.9%, respectively. The application of 0.3% PGA plus Cd stress increased APX, GR, DHAR, MDHAR, GalLDH and γ-ECS activities by 63.5, 48.1, 65.4, 50.0, 56.6 and 54.5%, respectively. The application of 0.6% PGA plus Cd increased APX, GR, DHAR, MDHAR, GalLDH and γ-ECS activities by 72.6, 53.4, 76.1, 61.1, 66.3 and 65.5%, respectively. However, there is no significant difference between 0.3% and 0.6% PGA in strengthening the above enzymes. The research

https://doi.org/10.17221/154/2024-PSE

Table 1. Influence of poly-glutamic acid (PGA) on enzymes responsible for ascorbic acid (AsA) and glutathione (GSH) metabolism

Treatment	APX	GR	DHAR	MDHAR	GalLDH	γ-ECS
	(U/g FW)					
Control	1.64 ± 0.08^{d}	1.33 ± 0.06^{e}	1.00 ± 0.05^{e}	1.26 ± 0.06^{e}	1.57 ± 0.12^{c}	2.10 ± 0.11^{c}
0.1% PGA	1.90 ± 0.08^{c}	1.67 ± 0.08^{d}	1.34 ± 0.07^{d}	1.50 ± 0.07^{d}	1.87 ± 0.09^{b}	2.40 ± 0.12^{b}
0.3% PGA	2.48 ± 0.12^{b}	2.24 ± 0.11^{b}	1.88 ± 0.09^{b}	1.85 ± 0.09^{c}	2.30 ± 0.12^{a}	2.85 ± 0.14^{a}
0.6% PGA	2.60 ± 0.10^{b}	2.36 ± 0.09^{b}	1.97 ± 0.08^{b}	2.02 ± 0.10^{c}	2.45 ± 0.10^{a}	3.00 ± 0.12^{a}
Cadmuim (Cd)	1.97 ± 0.09^{c}	1.89 ± 0.09^{c}	1.59 ± 0.06^{c}	1.80 ± 0.08^{c}	0.83 ± 0.04^{f}	1.10 ± 0.06^{f}
0.1% PGA + Cd	2.55 ± 0.12^{b}	2.28 ± 0.10^{b}	1.94 ± 0.08^{b}	2.27 ± 0.10^{b}	1.04 ± 0.05^{e}	1.33 ± 0.06^{e}
0.3% PGA + Cd	3.22 ± 0.15^{a}	2.80 ± 0.14^{a}	2.63 ± 0.13^{a}	2.70 ± 0.14^{a}	1.30 ± 0.06^{d}	1.70 ± 0.08^{d}
0.6% PGA + Cd	3.40 ± 0.14^{a}	2.90 ± 0.12^{a}	2.80 ± 0.11^{a}	2.90 ± 0.12^{a}	1.38 ± 0.06^{d}	1.82 ± 0.07^{d}

Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 5 days. APX – ascorbate peroxidase; GR – glutathione reductase; DHAR – dehydroascorbate reductases; MDHAR – monodehydroascorbate reductase; GalLDH – L-galactono-1,4-lactone dehydrogenase; γ-ECS – gamma-glutamylcysteine synthetase; FW – fresh weight

above demonstrated that PGA enhanced AsA and GSH metabolism by strengthening the above enzymes responsible for their regeneration and biosynthesis under Cd stress.

Influence of PGA on AsA and GSH contents and their redox status. Cd alone significantly improved AsA and GSH contents but decreased the value of their redox status (Table 2). While, PGA alone significantly improved these indicators. Comparatively, PGA plus Cd also significantly improved these indicators compared to Cd alone. Compared to Cd treatment, 0.1% PGA plus Cd improved AsA and GSH contents, AsA/DHA and GSH/GSSG by 23.1, 22.7, 7.1 and 8.0%, respectively. The application of 0.3% PGA plus Cd stress improved AsA and GSH contents, AsA/DHA and GSH/GSSG by 54.5, 66.7, 20.2 and 19.3%, respectively. The application of 0.6% PGA plus Cd stress, respectively, improved AsA and GSH contents, AsA/DHA and GSH/GSSG by 58.6, 79.3, 22.6 and 20.5%. However, there is no significant difference between 0.3% and 0.6% PGA in increasing these indicators. These results demonstrated that PGA improved AsA and GSH contents and kept the redox homeostasis of Cd-stressed seedlings.

Table 2. Influence of poly-glutamic acid (PGA) on ascorbic acid (AsA) and glutathione (GSH) contents and their redox status

Treatment	AsA	GSH	AsA/DHA	GSH/GSSG
	(μmol/g FW)			
Control	2.10 ± 0.10^{e}	1.03 ± 0.05^{e}	23.0 ± 1.10^{b}	24.1 ± 1.22^{b}
0.1% PGA	2.41 ± 0.12^{d}	1.35 ± 0.07^{d}	24.2 ± 1.14^{ab}	25.0 ± 1.20^{ab}
0.3% PGA	2.86 ± 0.13^{c}	1.80 ± 0.09^{b}	26.0 ± 1.33^{a}	27.0 ± 1.30^{a}
0.6% PGA	3.00 ± 0.11^{c}	1.92 ± 0.08^{b}	26.5 ± 1.25^{a}	27.3 ± 1.33^{a}
Cadmium (Cd)	2.90 ± 0.15^{c}	1.50 ± 0.07^{c}	16.8 ± 0.82^{d}	17.6 ± 0.80^{d}
0.1% PGA + Cd	3.57 ± 0.17^{b}	1.84 ± 0.08^{b}	18.0 ± 0.90^{d}	19.0 ± 0.90^{d}
0.3% PGA + Cd	4.48 ± 0.22^{a}	2.50 ± 0.13^{a}	20.2 ± 1.00^{c}	21.0 ± 1.00^{c}
0.6% PGA + Cd	4.60 ± 0.20^{a}	2.69 ± 0.10^{a}	20.6 ± 0.88^{c}	21.2 ± 0.95^{c}

Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 5 days. DHA – dehydroascorbic acid; GSSG – oxidised glutathione; FW – fresh weight

https://doi.org/10.17221/154/2024-PSE

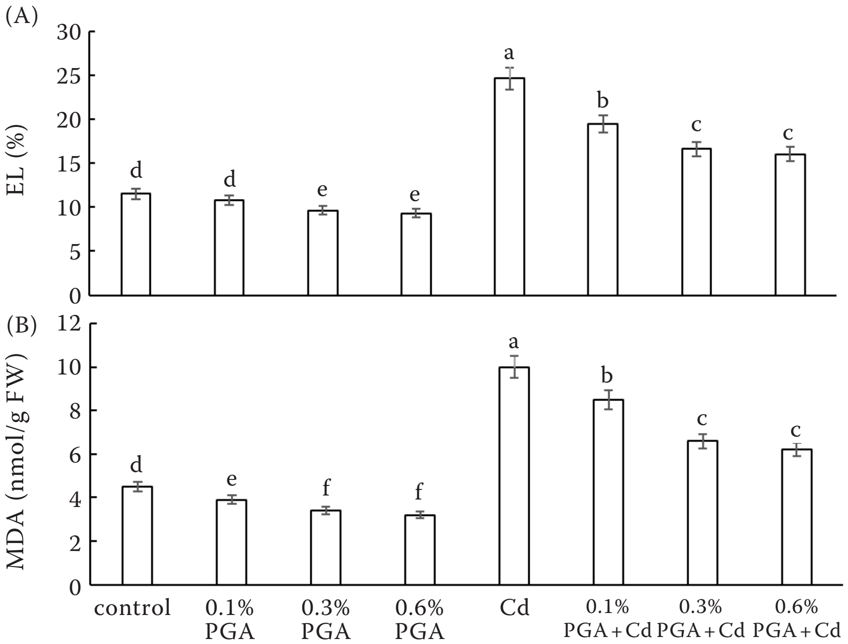

Figure 1. Influence of poly-glutamic acid (PGA) on malondialdehyde (MDA) and electrolyte leakage (EL) levels. Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 5 days. FW – fresh weight

Influence of PGA on MDA and EL. Compared to control, Cd alone significantly improved MDA and EL levels (Figure 1). While, PGA alone significantly decreased these indicators. Comparatively, PGA plus Cd significantly reduced MDA and EL levels than Cd alone. Compared to Cd treatment, 0.1% PGA plus Cd, respectively, decreased MDA and EL levels by 21.1% and 15.0%. The application of 0.3% PGA plus Cd, respectively, decreased MDA and EL levels by 32.5% and 34.0%. The application of 0.6% PGA plus Cd decreased MDA and EL levels by 35.0% and 38.0%, respectively. However, there is no significant difference between 0.3% and 0.6% PGA in decreasing the levels of MDA and EL. The above findings demonstrated that PGA played an important role in enhancing the tolerance of wheat plants to Cd toxicity.

Influence of PGA on the photosynthetic performance. Compared to the control, Cd alone remarkably increased q_N and reduced SPAD value, P_n, F_v/F_m, q_P and Φ_{PSII} (Table 3). While, PGA alone remarkably increased these indicators. Comparatively, PGA plus Cd also significantly increased these indicators more than Cd alone. Compared to Cd treatment, 0.1% PGA plus Cd increased SPAD value, P_n, F_v/F_m, q_P, q_N and Φ_{PSII}

Table 3. Influence of poly-glutamic acid (PGA) on wheat photosynthetic performance

Treatment	SPAD value	P_n (μmol/m²/s)	F_v/F_m	q_P	q_N	Φ_{PSII}
Control	31.5 ± 1.24^b	15.8 ± 0.70^b	0.78 ± 0.03^b	0.50 ± 0.02^b	0.24 ± 0.01^e	0.40 ± 0.02^b
0.1% PGA	33.0 ± 1.30^b	17.0 ± 0.81^b	0.80 ± 0.03^{ab}	0.53 ± 0.02^b	0.26 ± 0.01^d	0.42 ± 0.02^b
0.3% PGA	35.8 ± 1.43^a	18.8 ± 0.90^a	0.84 ± 0.03^a	0.57 ± 0.02^a	0.29 ± 0.01^c	0.47 ± 0.02^a
0.6% PGA	36.3 ± 1.35^a	19.3 ± 0.87^a	0.86 ± 0.03^a	0.59 ± 0.02^a	0.31 ± 0.01^b	0.49 ± 0.02^a
Cadmium (Cd)	22.4 ± 1.08^d	10.0 ± 0.55^e	0.55 ± 0.02^e	0.36 ± 0.01^e	0.31 ± 0.01^b	0.29 ± 0.01^e
0.1% PGA + Cd	24.3 ± 1.13^d	11.6 ± 0.52^d	0.60 ± 0.02^d	0.40 ± 0.02^d	0.32 ± 0.01^b	0.32 ± 0.01^d
0.3% PGA + Cd	26.9 ± 1.24^c	13.2 ± 0.60^c	0.67 ± 0.03^c	0.45 ± 0.02^c	0.36 ± 0.02^a	0.35 ± 0.01^c
0.6% PGA + Cd	27.7 ± 1.15^c	13.8 ± 0.60^c	0.68 ± 0.02^c	0.46 ± 0.02^c	0.37 ± 0.02^a	0.36 ± 0.01^c

Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and then followed by 50 mg/L $CdCl_2$ or the nutrient solution for 5 days. SPAD – soil and plant analyser development; P_n – photosynthetic rate; F_v/F_m – maximum photochemical efficiency of photosystem II (PSII); q_P – photochemical quenching; q_N – nonphotochemical quenching; Φ_{PSII} – quantum efficiency of PSII photochemistry

https://doi.org/10.17221/154/2024-PSE

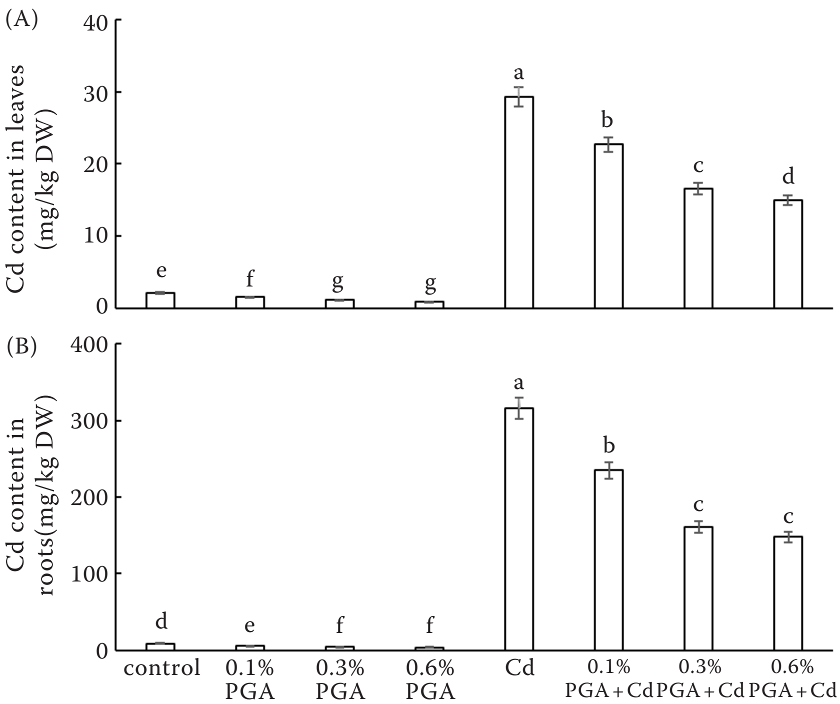

Figure 2. Influence of poly-glutamic acid (PGA) on cadmium (Cd) accumulation.Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 10 days. DW – dry weight

by 8.5, 16.0, 9.1, 11.1, 3.2 and 10.3%, respectively. The application of 0.3% PGA plus Cd stress increased SPAD value, P_n, F_v/F_m, q_P, q_N and Φ_{PSII} by 20.1, 32.0, 21.8, 25.0, 16.1 and 20.7%, respectively. The application of 0.6% PGA plus Cd stress respectively increased SPAD value, P_n, F_v/F_m, q_P, q_N and Φ_{PSII} by 23.7, 38.0, 23.6, 27.8, 19.4 and 24.1%. However, there is no significant difference between 0.3% and 0.6% PGA in increasing the abovementioned indicators. The above findings demonstrated that PGA also improved wheat photosynthetic performance when exposed to Cd.

Influence of PGA on Cd accumulation. Cd alone significantly promoted Cd accumulation in leaves and roots compared to the control (Figure 2). Comparatively, PGA plus Cd significantly reduced Cd accumulation more than Cd alone. Compared to Cd treatment, the application of 0.1% PGA plus Cd stress reduced Cd accumulation in leaves and roots by 22.5% and 25.6%, respectively. The application of 0.3% PGA plus Cd stress, respectively, decreased Cd accumulation in leaves and roots by 43.3% and 49.1%. The application of 0.6% PGA plus Cd stress, respectively, decreased Cd accumulation in leaves and roots by 48.8% and 53.2%. However, there is no significant difference between 0.3% and 0.6% PGA in decreasing Cd accumulation in roots. The abovementioned research demonstrated that PGA prevented wheat Cd uptake under Cd stress.

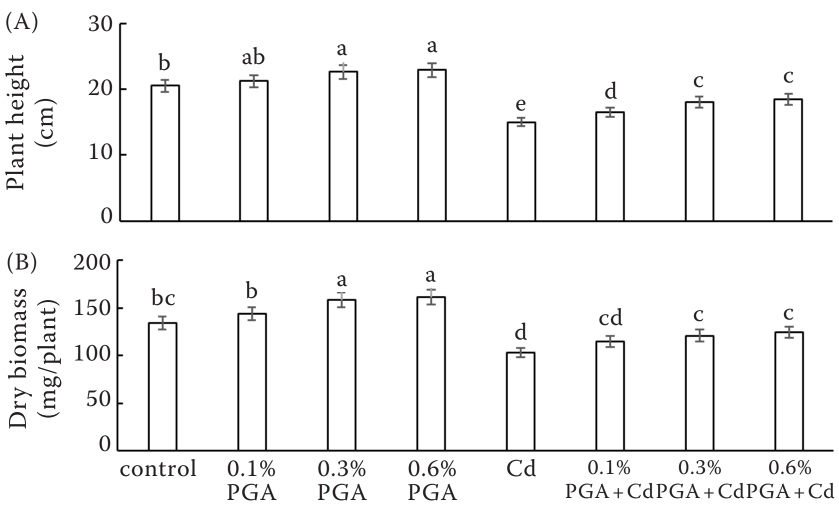

Figure 3. Influence of poly-glutamic acid (PGA) on wheat height and biomass. Seedlings were treated as below: control – half-strength Hoagland's solution; 0.1%PGA; 0.3%PGA; 0.6%PGA; Cd, 50 mg/L $CdCl_2$; 0.1%PGA + Cd, 0.1% PGA + $CdCl_2$; 0.3% PGA + Cd, 0.3% PGA + $CdCl_2$; 0.6% PGA + Cd, 0.6% PGA + $CdCl_2$. The seedlings were exposed to PGA for 12 h, and followed by 50 mg/L $CdCl_2$ or the nutrient solution for 10 days

https://doi.org/10.17221/154/2024-PSE

Influence of PGA on plant height and biomass. Compared to the control, Cd alone remarkably reduced the height and biomass (Figure 3). While, PGA treatment alone remarkably increased these indicators. Comparatively, PGA plus Cd significantly improved these growth parameters more than Cd alone. Compared with Cd treatment, 0.1% PGA plus Cd stress improved the height and biomass by 10.0% and 11.2%, respectively. The application of 0.3% PGA plus Cd, respectively, improved the height and biomass by 20.0% and 17.5%. The application of 0.6% PGA plus Cd, respectively, improved the height and biomass by 22.6.0% and 21.0%. However, there is no significant difference between 0.3% and 0.6% PGA in improving wheat height and biomass. These findings once more demonstrated that PGA played an important role in enhancing wheat Cd tolerance.

DISCUSSION

Cd stress-induced membrane lipid peroxidation in plants, such as cabbage, rapeseed, wheat and maize (Wang et al. 2021, Hussain et al. 2022, Jia et al. 2023, Nie et al. 2023). The level of the membrane lipid peroxidation can be evaluated by MDA and EL levels. The present study displayed that MDA and EL levels of wheat plants were increased after Cd exposure. As reported, exogenous substances could protect wheat plants against Cd stress, such as zeatin, silymarin, isosteviol, boron and biochar (Zhang and Gao 2021, Ali et al. 2022, Hussain et al. 2022). However, it is unclear whether or not PGA affects AsA and GSH metabolism after Cd exposure. The current research demonstrated that PGA strengthened AsA and GSH metabolism through enzymes responsible for their regeneration and biosynthesis, thereby reducing MDA and EL levels of wheat plants after Cd exposure. These findings are an important novel in this study.

As an important antioxidant, AsA plays a vital role in controlling peroxide damage in plants under stress (Wu et al. 2024). AsA content is usually regulated by GalLDH, DHAR, MDHAR and APX. Shan et al. (2024) clarified that PGA strengthened APX activity and increased AsA content in the leaves of *Salvia miltiorrhiza*. The current study uncovered that PGA also strengthened APX activity and increased AsA content, which was consistent with Shan et al. (2024). Additionally, the present study manifested that PGA could enhance DHAR, MDHAR and GalLDH activities of Cd-stressed plants. At the same time, we found that PGA plus Cd also increased the AsA/DHA ratio. Therefore, current research demonstrated that PGA regulated AsA regeneration *via* APX, MDHAR and DHAR and modulated AsA biosynthesis *via* GalLDH in Cd-stressed wheat seedlings. This way, PGA enhanced AsA metabolism and the ability to scanvage ROS hydrogen peroxide, thereby increasing AsA/DHA ratio and keeping the redox homeostasis.

GSH is also an important antioxidant in controlling the peroxide damage at low level under stresses (Rai et al. 2023). GSH content is usually regulated by γ-ECS and GR. Shan et al. (2024) uncovered that PGA strengthened GR activity and improved GSH content in *Salvia miltiorrhiza*. Our research showed that PGA also strengthened GR activity and improved the GSH content of wheat plants after Cd exposure. Additionally, current research showed that PGA strengthened the γ-ECS activity of wheat plants after Cd exposure. Meanwhile, we displayed that PGA plus Cd increased the GSH/GSSG ratio. Hence, our research demonstrated that PGA regulated GSH regeneration *via* GR and modulated GSH biosynthesis *via* γ-ECS of wheat plants after Cd exposure. In this way, PGA increased GSH content by strengthening GSH metabolism, which in turn improved GSH/GSSG ratio and kept the redox homeostasis.

The damage induced by Cd is closely related to Cd excessive accumulation in the plant body. Therefore, reducing Cd accumulation in plants is an effective measure to enhance Cd tolerance. It has been documented that Cd can be chelated by GSH (Wang et al. 2021). In addition, phytochelatin (PC) also could chelate Cd. GSH can also act as a substrate of PC synthesis (Clemens and Ma 2016). Thus, GSH content had a close relationship with the PC level. Currently, PGA has improved GSH content, thereby enhancing the ability to chelate Cd. In addition, our results manifested that PGA reduced wheat Cd accumulation. Therefore, the present study implied that PGA can also enhance wheat Cd tolerance by enhancing the ability to chelate Cd and reduce Cd uptake. Therefore, PGA application will be an effective measure to enhance wheat Cd tolerance.

Photosynthetic performance and plant growth are the intuitive manifestations of plants under stresses at the physiological and morphological levels. Previous research demonstrated that Cd seriously inhibited the photosynthetic performance and growth of mustard, alfalfa, *Mentha piperita* "chocolate" and *Mentha spicata* (Jiang et al. 2022, Liu et al. 2022, Mir et al. 2022). Our present findings displayed that

https://doi.org/10.17221/154/2024-PSE

Cd inhibited wheat photosynthetic performance and growth, which agreed with previous research about other plants. Additionally, the present study uncovered that PGA increased SPAD value, P_n, F_v/F_m, q_P, q_N and Φ_{PSII}, which further improved wheat photosynthetic performance and growth. The above results once more indicated that PGA could enhance wheat Cd tolerance.

The current study also found that different PGA concentrations had various effects on AsA and GSH metabolism, Cd accumulation, photosynthetic performance and plant growth. Among different concentrations, 0.3% and 0.6% PGA showed more effectiveness than 0.1% PGA in reinforcing wheat Cd tolerance. However, the difference between 0.3% and 0.6% PGA is insignificant. This phenomenon dropped a hint that PGA displayed the dose effect in regulating wheat Cd tolerance. Therefore, a suitable PGA concentration should be selected before its application. For this study, the results clearly showed that 0.3% PGA may be used to improve the tolerance of wheat cv. Yuanfeng16 to Cd toxicity.

Our research manifested that PGA regulated AsA and GSH metabolism by activating the enzymes responsible for their regeneration and biosynthesis in Cd-stressed wheat seedlings. In this way, PGA increased AsA and GSH contents and the redox status of wheat plants when exposed to Cd, thereby enhancing wheat's antioxidant ability, photosynthetic performance, and growth. The current study's findings provide useful knowledge for PGA action in modulating the antioxidant metabolism of wheat plants when exposed to Cd. Therefore, we can apply PGA to field production to improve wheat Cd tolerance, especially for 0.3% PGA.

REFERENCES

Ali E.F., Aljarani A.M., Mohammed F.A., Desoky E.-S.M., Mohamed I.A.A., El-Sharnouby M., Tammam S.A., Hassan F.A.S., Rady M.M., Shaaban A. (2022): Exploring the potential enhancing effects of trans-zeatin and silymarin on the productivity and antioxidant defence capacity of cadmium-stressed wheat. Biology, 11: 1173.

Alshegaihi R.M., Saleem M.H., Saleem A., Ali B., Aziz H., Fahad S., Alataway A., Dewidar A.Z., Elansary H.O. (2023): Silicon and titanium dioxide mitigate copper stress in wheat (*Triticum aestivum* L.) through regulating antioxidant defence mechanisms. Journal of Plant Growth Regulation, 342: 123013

Clemens S., Ma J.F. (2016): Toxic heavy metal and metalloid accumulation in crop plants and foods. Annual Review of Plant Biology, 29: 489–512.

Dalton D.A., Russell S.A., Hanus F.J., Pascoe G.A., Evans H.J. (1986): Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. Proceedings of the National Academy of Sciences, 83: 3811–3815.

Gao Y., Dong X., Wang R., Hao F., Zhang H., Zhang Y., Lin G. (2024): Exogenous calcium alleviates oxidative stress caused by salt stress in peanut seedling roots by regulating the antioxidant enzyme system and flavonoid biosynthesis. Antioxidants, 13: 233.

Grace S.C., Logan B.A. (1996): Acclimation of foliar antioxidant systems to growth irradiance in three broad-leaved evergreen species. Plant Physiology, 112: 1631–1640.

Griffith O.W. (1980): Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical Biochemistry, 106: 207–212.

Hodges D.M., Andrews C.J., Johnson D.A., Hamilton R.I. (1996): Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. Plant Physiology, 98: 685–692.

Hodges M.D., DeLong J.M., Forney C.F., Prange R.K. (1999): Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta, 207: 604–611.

Hussain S., Irfan M., Sattar A., Hussain S., Ullah S., Abbas T., Ur-Rehman H., Nawaz F., Al-Hashimi A., Elshikh M.S. (2022): Alleviation of cadmium stress in wheat through the combined application of boron and biochar *via* regulating morpho-physiological and antioxidant defense mechanisms. Agronomy, 12: 434.

Ibrahim M., Nawaz S., Iqbal K., Rehman S., Ullah R., Nawaz G., Almeer R., Sayed A.A., Peluso I. (2022): Plant-derived smoke solution alleviates cellular oxidative stress caused by arsenic and mercury by modulating the cellular antioxidative defense system in wheat. Plants, 11: 1379.

Jia K., Zhan Z., Wang B., Wang W., Wei W., Li D., Huang W., Xu Z. (2023): Exogenous selenium enhances cadmium stress tolerance by improving physiological characteristics of cabbage (*Brassica oleracea* L. var. *capitata*) seedlings. Horticulturae, 9: 1016.

Jiang W., Xu L., Liu Y., Su W., Yan J., Xu D. (2022): Effect of biochar on the growth, photosynthesis, antioxidant system and cadmium content of *Mentha piperita* 'Chocolate' and *Mentha spicata* in cadmium-contaminated soil. Agronomy, 12: 2737.

Kaushik S., Ranjan A., Singh A.K., Sirhindi G. (2024): Methyl jasmonate reduces cadmium toxicity by enhancing phenol and flavonoid metabolism and activating the antioxidant defense system in pigeon pea (*Cajanus cajan*). Chemosphere, 346: 140681.

Khan M.N. (2023): Melatonin regulates mitochondrial enzymes and ascorbate-glutathione system during plant responses to drought stress through involving endogenous calcium. South African Journal of Botany, 162: 622–632.

Liu B., An C., Jiao S., Jia F., Liu R., Wu Q., Dong Z. (2022): Impacts of the inoculation of *Piriformospora indica* on photosynthesis, osmoregulatory substances, and antioxidant enzymes of alfalfa seedlings under cadmium stress. Agriculture, 12: 1928.

https://doi.org/10.17221/154/2024-PSE

Mir I.R., Rather B.A., Masood A., Khan N.A. (2022): Nitric oxide- and sulfur-mediated reversal of cadmium-inhibited photosynthetic performance involves hydrogen sulfide and regulation of nitrogen, sulfur, and antioxidant metabolism in mustard. Stresses, 2: 550–577.

Miyake C., Asada K. (1992): Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. Plant and Cell Physiology, 33: 541–553.

Nakano Y., Asada K. (1981): Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant and Cell Physiology, 22: 867–880.

Nie L., Zhou B., Hong B., Wang X., Chang T., Guan C., Guan M. (2023): Application of selenium can alleviate the stress of cadmium on rapeseed at different growth stages in soil. Agronomy, 13: 2228.

Perveen S., Parveen A., Saeed M. (2022): Interactive effect of glycine, alanine, and calcium nitrate $Ca(NO_3)_2$ on wheat (*Triticum aestivum* L.) under lead (Pb) stress. Environmental Science and Pollution Research, 29: 37954–37968.

Rafique M.I., Ahmad M., Al-Wabel M.I., Ahmad J., Al-Farraj A.S. (2022): Mitigating the toxic effects of chromium on wheat (*Triticum aestivum* L.) seed germination and seedling growth by using biochar and polymer-modified biochar in contaminated soil. Sustainability, 14: 16093.

Rai G.K., Kumar P., Choudhary S.M., Singh H., Adab K., Kosser R., Magotra I., Kumar R.R., Singh M., Sharma R. (2023): Antioxidant potential of glutathione and crosstalk with phytohormones in enhancing abiotic stress tolerance in crop plants. Plants, 12: 1133.

Shan C., Liang Z. (2010): Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. Plant Science, 178: 130–139.

Shan C., Zhang X., Luo Y., Yang D. (2024): Poly-glutamic acid promotes the growth and the accumulation of main medicinal components in *Salvia miltiorrhiza*. Agronomy, 14: 252.

Skalski T., Zając E., Jędrszczyk E. (2024): Effects of γ-polyglutamic acid on grassland sandy soil properties and plant functional traits exposed to drought stress. Scientific Reports, 14: 3769.

Wang R., Lin K., Chen H., Qi Z., Liu B., Cao F., Chen H., Wu F. (2021): Metabolome analysis revealed the mechanism of exogenous glutathione to alleviate cadmium stress in maize (*Zea mays* L.) seedlings. Plants, 10: 105.

Wang Y., Duan W., Lv C. (2023): Citric acid and poly-glutamic acid promote the phytoextraction of cadmium and lead in *Solanum nigrum* L. grown in compound Cd-Pb contaminated soils. Bulletin of Environmental Contamination and Toxicology, 110: 37.

Wu P., Li B., Liu Y., Bian Z., Xiong J., Wang Y., Zhu B. (2024): Multiple physiological and biochemical functions of ascorbic acid in plant growth, development, and abiotic stress response. International Journal of Molecular Sciences, 25: 1832.

Xu X., Shi G., Ding C. (2011): Regulation of exogenous spermidine on the reactive oxygen species level and polyamine metabolism in *Alternanthera philoxeroides* (Mart.) Griseb under copper stress. Plant Growth Regulation, 63: 251–258.

Xu Z., Lei P., Pang X., Li H., Feng X., Xu H. (2017): Exogenous application of poly-γ-glutamic acid enhances stress defense in *Brassica napus* L. seedlings by inducing cross-talks between Ca^{2+}, H_2O_2, brassinolide, and jasmonic acid in leaves. Plant Physiology and Biochemistry, 118: 460–470.

Zhang L., Gao B. (2021): Effect of isosteviol on wheat seed germination and seedling growth under cadmium stress. Plants, 10: 1779.

Zhao L.Q., Zhang F., Guo J.K., Yang Y.L., Li B.B., Zhang L.X. (2004): Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. Plant Physiology, 134: 849–857.

Zhao X., Zhang Y., Zhang X., Shan C. (2023): Putrescine improves salt tolerance of wheat seedlings by regulating ascorbate and glutathione metabolism, photosynthetic performance, and ion homeostasis. Plant, Soil and Environment, 69: 512–521.

Zhu Q., Li Y., Gao S., Shan C. (2021): Praseodymium enhanced the tolerance of maize seedlings subjected to cadmium stress by up-regulating the enzymes in the regeneration and biosynthetic pathways of ascorbate and glutathione. Plant, Soil and Environment, 67: 633–642.

Received: April 8, 2024
Accepted: June 4, 2024
Published online: July 4, 2024