

γ -aminobutyric acid enhances the antioxidant defense system and photosynthetic performance of wheat seedlings under cadmium stress

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Abstract: In this paper, I elucidated the influence of γ -aminobutyric acid (GABA) on wheat cadmium (Cd) tolerance. Research results manifested that Cd stress increased superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and glutathione peroxidase activities, and ascorbic acid (AsA) and glutathione (GSH) contents. However, Cd stress decreased AsA/dehydroascorbic acid (DHA) and GSH/oxidised glutathione (GSSG) ratios, and inhibited photosynthetic performance and plant growth. Compared to Cd alone, GABA plus Cd improved wheat Cd tolerance by increasing the activities of above antioxidant enzymes, AsA and GSH contents, and AsA/DHA and GSH/GSSG ratios. Meanwhile, compared with Cd alone, GABA plus Cd also enhanced the photosynthetic performance by improving chlorophyll (*Chl*) and carotenoid (*Car*) contents and *Car/Chl* ratio, photosynthetic rate, transpiration rate, stomatal conductance, intercellular carbon dioxide concentration, and *Chl* fluorescence parameters maximum photochemical efficiency of PSII, photochemical quenching, nonphotochemical quenching and quantum efficiency of PSII photochemistry, which further promoted plant height and biomass. Compared to control, GABA alone also improved above indicators. Current results suggested that GABA can be applied as an anti-cadmium agent in wheat production practice.

Keywords: antioxidant compound; cadmium toxicity; plant growth regulator; *Triticum aestivum* L.; stress tolerance

Cadmium (Cd) stress can induce serious injuries to plants, which further impedes plant growth and reduces production (Liu et al. 2023b, Chi et al. 2024). For the main reason, Cd stress induces the imbalance of active oxygen metabolism, which further results in the peroxide damage to plants (Liu et al. 2023b, Chi et al. 2024). To counteracting the peroxide damage, plants employ two types of antioxidant defense systems in their bodies. One is the antioxidant enzyme protection system, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) (Li et al. 2022, Song et al. 2024). The other is the non-enzymatic antioxidant defense system, such as ascorbic acid (AsA) and glutathione (GSH) (da Silva et al. 2021). Previous researches demonstrated that many exogenous substances

alleviated the peroxide damage in Cd-stressed plants *via* regulating above two antioxidant defense systems, including plant growth regulators and plant mineral nutrients (Liu et al. 2023b, Song et al. 2024). Song et al. (2024) demonstrated that melatonin reinforced Cd tolerance of tomato seedlings by improving SOD, CAT and APX activities. Li et al. (2022) displayed that brassinosteroids enhanced grape Cd tolerance by strengthening SOD, POD and APX activities and upgrading AsA and GSH contents. Liu et al. (2023b) proved that silicon enhanced Cd tolerance of tomato seedlings by raising SOD, POD, CAT and APX activities and boosting AsA and GSH contents. The findings of above researches indicated that we can apply corresponding exogenous substances to improve plant Cd tolerance.

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As reported, γ -aminobutyric acid (GABA) could regulate plant tolerance to many stresses, including salt, cold, heat, chilling and heavy metal stresses, etc. (Qi et al. 2021, Aljuaid and Ashour 2022, Liu et al. 2023a, Waris et al. 2023, Cao et al. 2024). For Cd stress, it has been reported that GABA could enhance Cd tolerance of plants, such as lettuce (*Lactuca sativa* L.) and maize (*Zea mays* L.) (Seifikalhor et al. 2020, Waris et al. 2023). In these studies, previous researchers all found that GABA could enhance Cd tolerance by reinforcing the antioxidant defense system, including SOD and POD, etc. For wheat crops, GABA could enhance their tolerance to ozone, salt and heat stresses (Wang et al. 2022, Kumari et al. 2024, Wang et al. 2024). Whereas, it is still unclear that whether GABA could enhance wheat Cd tolerance. Hence, it is important to study the influence of GABA on the antioxidant defence system of wheat crops exposed to Cd. Meanwhile, plant Cd tolerance can be reflected by the growth. While, plant growth has close relationship with the photosynthetic performance. Therefore, it is also important to uncover the influence of GABA on the photosynthetic performance of Cd-stressed wheat crops.

In current research, I hypothesised that GABA could mitigate wheat Cd toxicity by enhancing the activity of the antioxidant defense system, which further improved the photosynthetic performance and plant growth. To validate this hypothesis, I explored the influence of GABA on the activities of antioxidant enzymes, AsA and GSH contents and their redox status, the contents of oxidative stress markers, the contents of photosynthetic pigments, gas exchange parameters (GEP), *Chl* fluorescence parameters (CFP) and growth indexes. By this research, I also wanted to provide new knowledge for the physiological mechanism of GABA in enhancing wheat Cd tolerance, which can provide the theoretical reference basis for the potential application of GABA in wheat production, especially for those cultivated in Cd-contaminated soil.

MATERIAL AND METHODS

Plant material and treatments. Wheat seeds of the cv. Bainong 207 were germinated on distilled water-moistened filter paper and wheat seedlings were cultivated in the artificial climate chamber under below conditions. Day/night temperature was 25 °C/15 °C, the photosynthetic active radiation was 500 $\mu\text{mol}/\text{m}^2/\text{s}$, and the photoperiod was 12 h. When first leaves of

seedlings were fully unfolded, roots were immersed in half-strength Hoagland's nutrient solution and kept them in dark. The nutrient solution was changed every 2 days. When second leaves were fully unfolded, wheat seedlings were used for next experiment.

Cd stress was applied to wheat seedlings by selecting the concentration of CdCl_2 (50 mg/L CdCl_2) from 25, 50 and 100 mg/L CdCl_2 . After 4 days of CdCl_2 treatment, the treatment of 100 mg/L CdCl_2 led to significant yellowing and wilting phenomenon of wheat leaves. While wheat seedlings treated by 25 and 50 mg/L CdCl_2 showed no significant yellowing and wilting phenomenon. Thus, 50 mg/L CdCl_2 was used as the suitable concentration to apply Cd stress on wheat seedlings. Roots of seedlings were immersed 100 mL 50 mg/L CdCl_2 in beakers for 8 days. To create a dark environment for roots, the aluminium foil was used to wrap beakers. To explore the effects of different GABA concentrations, three groups of wheat seedlings were respectively firstly treated by 50, 100 and 200 $\mu\text{mol}/\text{L}$ GABA for 10 h and then treated by CdCl_2 or the nutrient solution. Control seedlings were only treated by the nutrient solution. After 4 days of treatment, top fully unfolded leaves were sampled and rapidly frozen by liquid N_2 . Samples were kept in -80 °C until assay. After 8 days of treatment, plant height and biomass were measured.

Assays of antioxidant enzymes. All antioxidant enzymes were analysed by using UV-5200 spectrophotometer (Shanghai Metash Instruments Co., Ltd, Shanghai, China). SOD activity was analysed according to Giannopolitis and Ries (1977) by monitoring the absorbance value at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to cause a 50% inhibition of NBT reduction. POD, CAT, APX and glutathione peroxidase (GPX) activities were analysed according to Nickel and Cunningham (1969), Aebi (1984), Nakano and Asada (1981) and Shan and Zhao (2015), respectively. One unit of POD, CAT, APX and GPX activities was all defined as the reduction in the absorbance value by 0.01 per min. Their specific activities were all expressed as U/g fresh weight (FW).

Assays of AsA and GSH contents and their redox status. AsA and DHA were analysed according to Hodges et al. (1996). The redox status of AsA was expressed as the ratio of AsA content to DHA content. GSH and GSSG were analysed according to Griffith (1980). The redox status of GSH was expressed as the ratio of GSH content to DGSSG content.

Measurement of malondialdehyde and hydrogen peroxide. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were analysed based on spectrophotometric method by using UV-5200 spectrophotometer (Shanghai Metash Instruments Co., Ltd, Shanghai, China). MDA content was analysed based on thiobarbituric acid method reported by Heath and Packer (1968) by recording the absorbance at 532 nm. H_2O_2 content was analysed based on titanium sulfate colourimetric method reported by Brennan and Frenkel (1977) by recording the absorbance at 415 nm.

Measurement of chlorophyll and carotenoids and Car/*Chl* ratio. Chlorophyll (*Chl*) and carotenoids (Car) contents were analysed based on spectrophotometric method according to Song et al. (2016) by using UV-5200 spectrophotometer (Shanghai Metash Instruments Co., Ltd, Shanghai, China). *Chl* and Car were extracted using 95% ethanol, and the absorbances at 665, 649, and 470 nm were recorded. The ratio of Car/*Chl* was expressed as the quotient of Car content and *Chl* content.

Measurement of gas exchange parameters. Gas exchange parameters (GEP), photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (g_s) and internal CO_2 concentration (c_i) were measured by using LICOR-6400 photosynthetic instrument (Lincoln, USA). In leaf chamber, the temperature remained around 26 °C, the actinic light intensity and the CO_2 concentration was set to 1 200 $\mu\text{mol}/\text{m}^2/\text{s}$ and 400 $\mu\text{mol}/\text{mol}$. After the leaf was placed in the chamber for around 2 min until the readings became stable, P_n , T_r , g_s and c_i were recorded. These parameters were measured from 9:30 to 11:00 in the morning.

Measurement of CFP. Maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_p), nonphotochemical quenching (NPQ) and quantum efficiency of PSII photochemistry ($Y_{(II)}$) were measured by PAM-2500 chlorophyll fluorometer (Walz, Effeltrich, Germany). The leaves were covered by leaf clips for 30 min before light adaptation. The minimal fluorescence (F_0) and the maximum fluorescence (F_m) under dark adaptation were measured by using weak actinic light (0.12 $\mu\text{mol}/\text{m}^2/\text{s}$, 620 nm) and a saturated pulse of actinic white light (3 500 $\mu\text{mol}/\text{m}^2/\text{s}$, 650 nm, pulse time 0.8 s), respectively. The steady-state fluorescence (F_s) of the light-adapted leaves was measured when the sample achieved stable status. The maximum fluorescence (F_m') was measured under saturation pulse light. According to Zai et al.

(2021), F_v/F_m and $Y_{(II)}$ were calculated as $(F_m - F_0)/F_m$ and $(F_m' - F_s)/F_m'$, respectively. q_p was calculated as $(F_m' - F_s)/(F_m' - F_0)$. NPQ was calculated as $(F_m/F_m') - 1$. These parameters were measured from 7:00 to 13:00.

Plant height and biomass were assayed. Plant height was measured by a ruler. Fresh weights of all seedlings were recorded, and they were oven-dried for 72 h at 80 °C. Dry weights were then recorded.

Statistical analysis. The data in Tables 1–4 and Figures 1–3 were expressed as the mean of five replications. Means were compared by one-way analysis of variance and Duncan's multiple range test at the 5% level of significance using SPSS software (version 25.0, Chicago, USA).

RESULTS

Influence of GABA on antioxidant enzymes. Compared to control, Cd significantly increased SOD, POD, CAT, APX and GPX activities (Table 1). Compared to Cd alone, GABA plus Cd significantly improved the activities of these antioxidant enzymes. Among three concentrations, 100 $\mu\text{mol}/\text{L}$ GABA positively influenced these antioxidant enzymes. When compared to Cd alone, 100 $\mu\text{mol}/\text{L}$ GABA plus Cd improved SOD, POD, CAT, APX and GPX activities by 35.8, 41.6, 52.9, 75.7 and 41.7%, respectively. Compared with the control, GABA alone had the same positive influence on antioxidant enzymes. Above results indicated that GABA strengthened the activities of antioxidant enzymes in Cd-stressed wheat seedlings, especially for 100 $\mu\text{mol}/\text{L}$ GABA.

Influence of GABA on AsA and GSH contents and their redox status. Compared to control, Cd significantly increased AsA and GSH contents, but reduced AsA/DHA and GSH/GSSG ratios (Table 2). Compared to Cd, GABA plus Cd significantly improved AsA and GSH contents and their redox status. Among three concentrations, 100 $\mu\text{mol}/\text{L}$ GABA positively influenced these indicators. When compared to Cd, 100 $\mu\text{mol}/\text{L}$ GABA plus Cd improved AsA content, GSH content, AsA/DHA and GSH/GSSG by 59.2, 71.3, 36.2 and 43.4%, respectively. Compared to control, GABA alone also improved these indicators. Above results indicated that GABA improved antioxidants AsA and GSH contents and their redox status under Cd treatment, especially for 100 $\mu\text{mol}/\text{L}$ GABA.

Influence of GABA on MDA and H_2O_2 . Cd significantly promoted MDA and H_2O_2 production,

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Table 1. Influence of γ -aminobutyric acid (GABA) on superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX) activities

Treatment	SOD	POD	CAT	APX	GPX
	(U/g FW)				
Control	20.0 $\pm$ 0.97 ^d	15.4 $\pm$ 0.88 ^e	1.58 $\pm$ 0.08 ^f	1.40 $\pm$ 0.06 ^e	2.72 $\pm$ 0.13 ^e
50 μ mol/L GABA	23.0 $\pm$ 1.10 ^c	17.5 $\pm$ 0.80 ^d	1.90 $\pm$ 0.09 ^e	1.65 $\pm$ 0.07 ^d	3.06 $\pm$ 0.15 ^d
100 μ mol/L GABA	26.8 $\pm$ 1.24 ^{bc}	21.3 $\pm$ 0.95 ^c	2.36 $\pm$ 0.12 ^{cd}	1.98 $\pm$ 0.10 ^c	3.53 $\pm$ 0.18 ^c
200 μ mol/L GABA	24.0 $\pm$ 1.17 ^c	18.8 $\pm$ 0.88 ^d	2.17 $\pm$ 0.12 ^d	1.80 $\pm$ 0.08 ^{cd}	3.27 $\pm$ 0.15 ^{cd}
Cd	24.6 $\pm$ 1.16 ^c	20.9 $\pm$ 1.10 ^c	2.57 $\pm$ 0.14 ^c	1.85 $\pm$ 0.10 ^c	3.60 $\pm$ 0.19 ^c
50 μ mol/L GABA + Cd	28.5 $\pm$ 1.35 ^b	25.0 $\pm$ 1.24 ^b	3.10 $\pm$ 0.15 ^b	2.50 $\pm$ 0.13 ^b	4.35 $\pm$ 0.20 ^b
100 μ mol/L GABA + Cd	33.4 $\pm$ 1.63 ^a	29.6 $\pm$ 1.67 ^a	3.93 $\pm$ 0.22 ^a	3.25 $\pm$ 0.16 ^a	5.10 $\pm$ 0.24 ^a
200 μ mol/L GABA + Cd	29.8 $\pm$ 1.48 ^b	26.3 $\pm$ 1.25 ^b	3.36 $\pm$ 0.18 ^b	2.74 $\pm$ 0.12 ^b	4.66 $\pm$ 0.25 ^b

Wheat seedlings were treated by GABA for 10 h, and then exposed to cadmium (Cd) or the half-strength Hoagland's solution for 4 days. FW – fresh weight

compared to control (Figure 1). Compared with Cd alone, GABA plus Cd significantly reduced MDA and H₂O₂ contents. Among three concentrations, 100 μ mol/L GABA had more positive influence on these indicators under Cd stress. When compared to Cd alone, 100 μ mol/L GABA plus Cd reduced MDA and H₂O₂ contents by 44.4% and 45.4%, respectively. Compared to control, GABA alone also reduced these indicators. Above results indicated that GABA enhanced wheat Cd tolerance, especially for 100 μ mol/L GABA.

Influence of GABA on *Chl* and *Car* contents and *Car/Chl* ratio. Compared to control, Cd significantly reduced *Chl* and *Car* contents and *Car/Chl* ratio (Figure 2). Compared to Cd alone, GABA plus Cd significantly improved these indicators. Among three concentrations, 100 μ mol/L GABA positively influenced these indicators. When compared to Cd alone,

100 μ mol/L GABA plus Cd increased *Chl* and *Car* contents and *Car/Chl* ratio by 40.6, 95.5 and 35.3%, respectively. Compared to control, GABA alone also improved these indicators. These results indicated that GABA improved photosynthetic pigments under Cd stress, especially for 100 μ mol/L GABA.

Influence of GABA on GEP. Compared to control, Cd significantly decreased P_n , g_s and c_i (Table 3). Compared with Cd alone, GABA plus Cd significantly improved these GEP. Among three concentrations, 100 μ mol/L GABA positively influenced these GEP under Cd stress. When compared to Cd alone, 100 μ mol/L GABA plus Cd increased P_n , g_s and c_i by 62.8, 35.7 and 38.3%, respectively. Compared to control, GABA alone also improved these indicators. These results indicated that GABA promoted the function of the photosynthetic system under Cd stress, especially for 100 μ mol/L GABA.

Table 2. Influence of γ -aminobutyric acid (GABA) on ascorbic acid (AsA) and glutathione (GSH) contents and their redox status in cadmium (Cd)-stressed seedlings

Treatment	AsA (μ mol/g FW)	GSH (μ mol/g FW)	AsA/DHA	GSH/GSSG
Control	2.20 $\pm$ 0.10 ^e	0.80 $\pm$ 0.04 ^f	23.7 $\pm$ 1.09 ^b	24.3 $\pm$ 1.20 ^b
50 μ mol/L GABA	2.47 $\pm$ 0.12 ^d	0.96 $\pm$ 0.04 ^e	24.9 $\pm$ 1.10 ^b	25.0 $\pm$ 1.14 ^b
100 μ mol/L GABA	2.99 $\pm$ 0.14 ^c	1.28 $\pm$ 0.06 ^c	27.6 $\pm$ 1.30 ^a	27.5 $\pm$ 1.30 ^a
200 μ mol/L GABA	2.70 $\pm$ 0.12 ^d	1.10 $\pm$ 0.05 ^d	25.8 $\pm$ 1.24 ^{ab}	26.2 $\pm$ 1.23 ^{ab}
Cd	2.94 $\pm$ 0.14 ^c	1.15 $\pm$ 0.06 ^d	14.9 $\pm$ 0.80 ^e	14.5 $\pm$ 0.63 ^e
50 μ mol/L GABA + Cd	3.42 $\pm$ 0.16 ^b	1.44 $\pm$ 0.08 ^b	17.3 $\pm$ 0.74 ^d	17.7 $\pm$ 0.80 ^d
100 μ mol/L GABA + Cd	4.68 $\pm$ 0.22 ^a	1.97 $\pm$ 0.09 ^a	20.3 $\pm$ 1.00 ^c	20.8 $\pm$ 0.96 ^c
200 μ mol/L GABA + Cd	3.70 $\pm$ 0.18 ^b	1.66 $\pm$ 0.07 ^b	18.2 $\pm$ 0.73 ^d	18.8 $\pm$ 0.84 ^d

Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 4 days. DHA – dehydroascorbic acid; GSSG – oxidised glutathione; FW – fresh weight

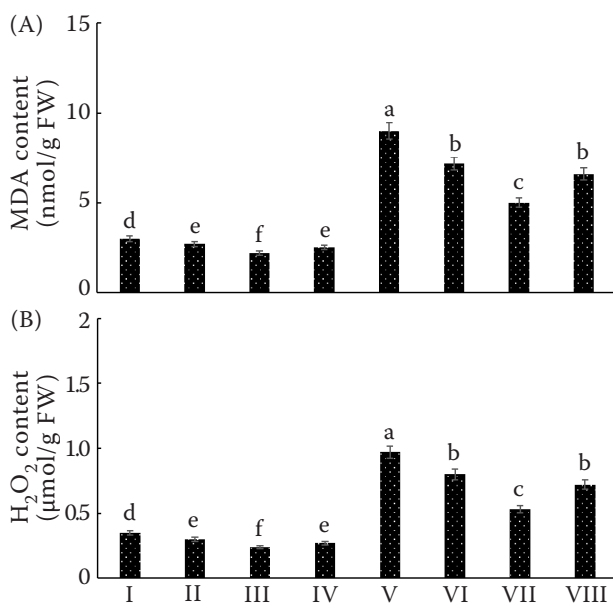


Figure 1. Influence of γ -aminobutyric acid (GABA) on malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) contents in cadmium (Cd)-stressed seedlings. Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 4 days. I – control; II – 50 μ mol/L GABA; III – 100 μ mol/L GABA; IV – 200 μ mol/L GABA; V – Cd; VI – 50 μ mol/L GABA + Cd; VII – 100 μ mol/L GABA + Cd; VIII – 200 μ mol/L GABA + Cd; FW – fresh weight

Influence of GABA on CFP. Compared to control, Cd significantly increased NPQ and reduced F_v/F_m , qP and $Y_{(II)}$ (Table 4). Compared with Cd alone, GABA plus Cd significantly decreased NPQ and improved other CFP. Among three concentrations, 100 μ mol/L GABA had more influence on these CFP under Cd stress. When compared to Cd alone, 100 μ mol/L GABA plus Cd decreased NPQ by 137% and respectively increased F_v/F_m , qP and $Y_{(II)}$ by 22.4, 41.9 and 25.0%, respectively. Compared to control, GABA alone also decreased NPQ and improved other CFP. These results indicated that GABA improved the function of photosynthetic system under Cd stress, especially for 100 μ mol/L GABA.

Influence of GABA on plant height and biomass. Compared to control, Cd significantly declined plant height and biomass (Figure 3). Compared to Cd alone, GABA plus Cd significantly improved these growth indicators. Among three concentrations, 100 μ mol/L GABA positively influenced these growth indicators under Cd stress. When compared to Cd alone, 100 μ mol/L GABA plus Cd increased plant height and biomass by 32.0% and 39.1%, respectively. Compared

to control, GABA alone also improved these indicators. Above results indicated that GABA promoted wheat growth under Cd stress, especially for 100 μ mol/L GABA.

DISCUSSION

Antioxidant enzyme activity is an important indicator for evaluating plant antioxidant capacity, especially under stresses. More and more researches demonstrated that Cd-stressed plants could strengthen the activities of antioxidant enzymes (Alghamdi et al. 2023, Jia et al. 2023). In this research, I also revealed that Cd enhanced antioxidant enzymes SOD, POD, CAT and APX in wheat seedlings. Besides,

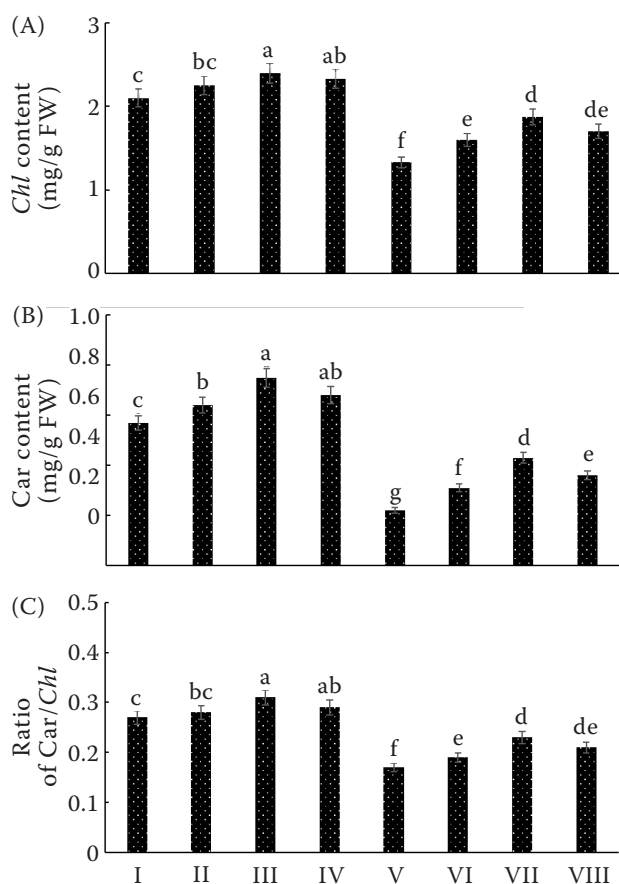


Figure 2. Effects of γ -aminobutyric acid (GABA) on chlorophyll (Chl) and carotenoids (Car) contents and the ratio of Car/Chl of cadmium (Cd)-stressed seedlings. Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 4 days. I – control; II – 50 μ mol/L GABA; III – 100 μ mol/L GABA; IV – 200 μ mol/L GABA; V – Cd; VI – 50 μ mol/L GABA + Cd; VII – 100 μ mol/L GABA + Cd; VIII – 200 μ mol/L GABA + Cd; FW – fresh weight

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Table 3. Influence of γ -aminobutyric acid (GABA) on gas exchange parameters of cadmium (Cd)-stressed seedlings

Treatment	P_n ($\mu\text{mol}/\text{m}^2/\text{s}$)	T_r ($\text{mmol}/\text{m}^2/\text{s}$)	g_s ($\text{mol}/\text{m}^2/\text{s}$)	c_i ($\mu\text{mol}/\text{mol}$)
Control	23.5 ± 1.00^b	3.30 ± 0.13^b	0.21 ± 0.01^b	180.0 ± 8.50^b
50 $\mu\text{mol}/\text{L}$ GABA + Cd	24.5 ± 1.13^b	3.42 ± 0.14^{ab}	0.23 ± 0.01^{ab}	188.3 ± 9.11^{ab}
100 $\mu\text{mol}/\text{L}$ GABA + Cd	26.9 ± 1.20^a	3.60 ± 0.17^a	0.25 ± 0.01^a	198.5 ± 8.18^a
200 $\mu\text{mol}/\text{L}$ GABA + Cd	25.5 ± 1.30^{ab}	3.51 ± 0.15^a	0.24 ± 0.01^a	191.0 ± 7.67^a
Cd	11.3 ± 0.68^e	2.10 ± 0.10^f	0.14 ± 0.00^e	115.7 ± 5.20^e
50 $\mu\text{mol}/\text{L}$ GABA + Cd	13.0 ± 0.67^d	2.43 ± 0.10^e	0.16 ± 0.00^d	132.4 ± 5.33^d
100 $\mu\text{mol}/\text{L}$ GABA + Cd	18.4 ± 0.80^c	3.01 ± 0.11^c	0.19 ± 0.01^c	160.0 ± 7.10^c
200 $\mu\text{mol}/\text{L}$ GABA + Cd	15.2 ± 0.63^d	2.66 ± 0.11^d	0.18 ± 0.01^c	147.6 ± 6.44^c

Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 4 days. P_n – photosynthetic rate; T_r – transpiration rate; g_s – stomatal conductance; c_i – internal CO_2 concentration

I also found that Cd enhanced GPX activity in wheat crops. For wheat crops, GABA could enhance antioxidant enzymes under ozone, salt and heat stresses (Wang et al. 2022, Kumari et al. 2024, Wang et al. 2024). Whereas, there is no report for the influence of GABA on antioxidant enzymes in Cd-stressed wheat crops. For this study, I found that GABA could enhance wheat Cd tolerance by improving the activities of SOD, POD, CAT, APX and GPX. Current results indicated that GABA could enhance wheat Cd tolerance by improving the activity of the antioxidant defense system through above antioxidant enzymes.

Antioxidant content and redox status are important indicators for evaluating plant antioxidant capacity, especially under stress. More and more researches demonstrated that Cd-stressed plants could improve the contents of antioxidants (Ali et al. 2022, Rehman et al. 2022). In this research, I also demonstrated that Cd stress improved antioxidants AsA and GSH

contents in wheat crops, which agreed with Ali et al. (2022). Besides, Ali et al. (2022) uncovered that Cd also improved AsA and GSH redox status of wheat, indicated by AsA/DHA and GSH/GSSG. Whereas, I showed that Cd reduced AsA/DHA and GSH/GSSG ratios. This discrepancy may be due to the difference in the Cd treatment concentration and/or wheat cultivar. Besides, I also found that GABA could enhance wheat Cd tolerance by improving AsA and GSH contents and their redox status. Current results suggested that GABA not only enhanced AsA and GSH levels, but also maintained their redox status in wheat plants. It has been reported that the contents of AsA and GSH and their redox status can be modulated by key enzymes in charge of the recycling and biosynthetic pathways of AsA and GSH. The recycling pathway is called ascorbate-glutathione (AsA-GSH) cycle, in which APX, GR, DHAR and MDHAR are important key enzymes for this cy-

Table 4. Influence of γ -aminobutyric acid (GABA) on chlorophyll (*Chl*) fluorescence parameters of cadmium (Cd)-stressed seedlings

Treatment	F_v/F_m	qP	NPQ	$Y_{(II)}$
Control	0.80 ± 0.02^b	0.50 ± 0.02^b	0.98 ± 0.04^c	0.51 ± 0.02^b
50 $\mu\text{mol}/\text{L}$ GABA	0.83 ± 0.02^{ab}	0.53 ± 0.02^{ab}	0.91 ± 0.04^{cd}	0.55 ± 0.02^{ab}
100 $\mu\text{mol}/\text{L}$ GABA	0.86 ± 0.03^a	0.57 ± 0.02^a	0.80 ± 0.03^e	0.60 ± 0.03^a
200 $\mu\text{mol}/\text{L}$ GABA	0.85 ± 0.02^a	0.55 ± 0.02^a	0.85 ± 0.03^{de}	0.58 ± 0.03^a
Cd	0.58 ± 0.01^e	0.31 ± 0.01^e	1.90 ± 0.11^a	0.36 ± 0.01^e
50 $\mu\text{mol}/\text{L}$ GABA + Cd	0.63 ± 0.01^d	0.38 ± 0.01^d	1.75 ± 0.10^{ab}	0.40 ± 0.01^d
100 $\mu\text{mol}/\text{L}$ GABA + Cd	0.71 ± 0.02^c	0.44 ± 0.02^c	1.64 ± 0.06^b	0.45 ± 0.02^c
200 $\mu\text{mol}/\text{L}$ GABA + Cd	0.67 ± 0.02^c	0.41 ± 0.02^{cd}	1.72 ± 0.08^{ab}	0.43 ± 0.02^{cd}

Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 4 day. F_v/F_m – maximum photochemical efficiency of PSII; qP – photochemical quenching; NPQ – nonphotochemical quenching; $Y_{(II)}$ – quantum efficiency of PSII photochemistry

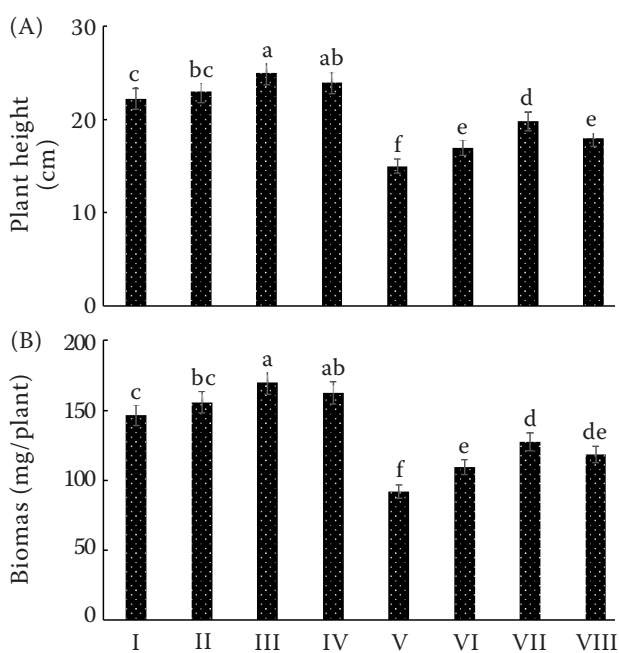


Figure 3. Influence of γ -aminobutyric acid (GABA) on the growth of cadmium (Cd)-stressed seedlings. Wheat seedlings were treated by GABA for 10 h, and then exposed to Cd stress or the half-strength Hoagland's solution for 8 days. I – control; II – 50 μ mol/L GABA; III – 100 μ mol/L GABA; IV – 200 μ mol/L GABA; V – Cd; VI – 50 μ mol/L GABA + Cd; VII – 100 μ mol/L GABA + Cd; VIII – 200 μ mol/L GABA + Cd

cle (Shan and Liang 2010). In plants, GalLDH and γ -ECS are important key enzymes in charge of AsA and GSH biosynthesis, respectively (Shan and Liang 2010). Currently, I only investigated the influence of GABA on APX activity in Cd-stressed wheat crops, which indicated that GABA could regulate AsA and GSH contents and their redox status through AsA-GSH cycle. However, this study did not explore the influence of GABA on other enzymes in AsA-GSH cycle, AsA biosynthetic key enzyme GalLDH and GSH biosynthetic key γ -ECS. Thus, it is interesting to explore further the influence of GABA on the above enzymes under Cd stress, which will provide more information on the regulatory mechanism of GABA in enhancing wheat Cd tolerance.

Previous research demonstrated that the peroxide damage inhibited the photosynthetic performance by degrading photosynthetic pigments and destroying photosynthetic apparatus, which further decreased the growth (Rady et al. 2023, Zhang et al. 2023). Therefore, photosynthetic performance has close relationship with plant antioxidant capacity under stresses. As the above results for the influ-

ence of GABA on wheat antioxidant capacity under Cd stress, the current study indicated that GABA could improve wheat photosynthetic performance by enhancing the antioxidant capacity. Besides, Car is also an important type of antioxidants in removing ROS. In this study, I found that GABA improved *Chl* and Car contents, as well as the ratio of Car/*Chl* in Cd-stressed wheat plants. This result suggested that GABA could inhibit the reduction in the contents of photosynthetic pigments and protect the photosynthetic apparatus against peroxide damage. Meanwhile, GABA improved P_n , F_v/F_m , qP , NPQ and $Y_{(II)}$ in Cd-stressed wheat plants. Thus, current study clearly proved that GABA could improve photosynthetic performance by enhancing their antioxidant capacity, which further promoted wheat growth under Cd stress.

Water metabolism is also an important physiological process related with plant growth. Cd stress could reduce the water metabolism capacity by decreasing T_r and g_s of plants, such as soybean and wheat (EI-Esawi et al. 2020, Iqbal et al. 2023). Currently, I found the same influence of Cd stress on T_r and g_s . Besides, I also showed that Cd stress led to the reduction in c_i , which indicated that Cd prevented the entrance of CO_2 into leaves by reducing the opening of stomata. In this way, Cd inhibited P_n , which further decreased wheat growth. Meanwhile, I found that GABA could improve the values of T_r , g_s and c_i in Cd-stressed wheat plants, which suggested that GABA enhanced the water metabolism capacity and promoted the entrance of CO_2 into leaves by improving the opening of stomata under Cd stress. In this way, GABA improved P_n , which further promoted wheat growth under Cd stress. It has been reported that the limitation of photosynthesis may be due to stomatal effects and non-stomatal effects (Maroco et al. 2002, Chaves and Oliveira 2004, Foyer and Noctor 2009). Zivcak et al. (2013) showed that the dominant limitation of photosynthesis of wheat under drought stress was through stomatal effects. The current results demonstrated that the difference in the limitation of photosynthesis between different treatments were also mainly caused by stomatal effects. Therefore, my results manifested that GABA promoted photosynthesis and wheat growth by enhancing the water metabolism capacity through the regulation of the stomatal aperture under Cd stress. In this study, different doses of GABA all enhanced wheat Cd tolerance by improving the activity of antioxidant defense system and

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photosynthetic performance. However, different doses of GABA had different influence on wheat Cd tolerance. Among three tested doses of GABA, the influence of 100 $\mu\text{mol/L}$ GABA on wheat Cd tolerance was better than 50 and 200 $\mu\text{mol/L}$ GABA. This phenomenon indicated that GABA had a dose-response effect in enhancing wheat Cd tolerance. Thus, it is necessary to firstly select the appropriate concentration of GABA before its application in the production. Meanwhile, this study also showed that GABA positively influenced the antioxidant defence system, photosynthetic performance and wheat growth under normal conditions. Therefore, 100 $\mu\text{mol/L}$ GABA has also potential application in the production of tested wheat cv. Bainong 207 under normal condition.

Under Cd stress, it has been documented that GABA also positively influenced the antioxidant capacity of *Monoraphidium* sp. QLY-1, lettuce (*Lactuca sativa*) and maize (*Zea mays*) by regulating corresponding antioxidant enzymes and antioxidants (Seifikalhor et al. 2020, Zhao et al. 2020, Waris et al. 2023). Besides, previous studies showed that GABA improved the photosynthetic performance by increasing *Chl* content in the leaves of *Solanum nigrum* var. *humile* and F_v/F_m of maize under Cd stress (Seifikalhor et al. 2020, Li et al. 2024). The present research also proved that GABA could enhance the antioxidant capacity and the photosynthetic performance of wheat crops under Cd stress, which agreed with these previous studies. Thus, this study and previous studies all indicated that GABA could enhance the Cd tolerance of plants by regulating their antioxidant capacity and the photosynthetic performance, which provided the basis for its application in crop production.

It has been reported that the gas signal molecule nitric oxide (NO) acted as a downstream signal of GABA to activate the antioxidant defence system, enhancing muskmelon salinity-alkalinity tolerance and promoting growth (Xu et al. 2021). Tang et al. (2020) reported that NO also acted as a downstream signal of GABA to enhance the water stress tolerance of creeping bentgrass by improving the antioxidant defense system. However, it is still unclear whether NO is involved in the regulation of GABA in enhancing the antioxidant defence system of Cd-stressed wheat crops. Therefore, it is worth exploring the role of gas signal molecule NO in the regulation of GABA in enhancing the antioxidant defence system in Cd-stressed wheat crops. This part of work can further provide more information for the modula-

tory mechanism of GABA in enhancing wheat Cd tolerance.

In conclusion, my findings implied that GABA enhanced wheat's antioxidant capacity and water metabolism capacity, which further promoted wheat photosynthetic performance and growth. Current results showed new information for the modulatory mechanism of GABA in enhancing wheat Cd tolerance and provided theory basis for its application in wheat production.

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