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# Gamma-aminobutyric acid improves cold tolerance of wheat seedlings

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**Abstract:** To provide a new agent to enhance wheat cold tolerance, we investigated the impacts of gamma-aminobutyric acid (GABA) on wheat antioxidant and photosynthetic capacity and growth parameters under cold stress (CS). CS significantly improved superoxide dismutase, catalase, peroxidase, ascorbate peroxidase and gluathione reductase in wheat leaves. Meanwhile, CS also increased the contents of reduced ascorbate (AsA) and reduced glutathione (GSH). However, CS significantly increased electrolyte leakage (EL) and malondialdehyde (MDA) levels. Compared with CS, GABA + CS improved the activities of the above antioxidant enzymes and the contents of antioxidants. In this way, GABA improved wheat antioxidant capacity and decreased MDA content and EL under CS. Whereas CS significantly increased non-photochemical quenching coefficient (qN) and reduced soil and plant analyser development (SPAD) value, net photosynthetic rate ($P_n$), maximum photochemical efficiency of PSII ($F_v/F_m$), effective quantum yield of PS II (Y(II)), photochemical quenching coefficient (qP), plant height and biomass. Compared to CS, GABA + CS significantly promoted the photosynthetic capacity by reducing qN and increasing SPAD value, $P_n$, $F_v/F_m$, Y(II) and qP. In this way, GABA improved plant growth under CS. Our results indicated that GABA can be used as a new agent to improve wheat cold tolerance.

**Keywords:** cold damage; chlorophyll fluorescence parameters; osmoregulation; photosynthetic pigments; reactive oxygen species

Wheat is an important grain crop in the world. In China, winter wheat plays a major role in wheat production, with its sowing area accounting for 93.68% of the total wheat area (Zhao et al. 2018). However, winter wheat often suffers from different stresses during its growth and development, such as salt, drought, and cadmium stress (Coppa et al. 2024, Katerova et al. 2024, Tobiasz-Salach et al. 2024). Among different abiotic stresses, cold stress (CS) usually inhibits the growth and yield of winter wheat during the overwintering period and spring. As reported, CS had an important influence on wheat antioxidant and photosynthetic capacity, which further inhibited the growth and yield of wheat plants (Malko et al. 2023, Fang et al. 2024). Meanwhile, previous studies also showed that exogenous chemicals improved plant cold tolerance, such as hydrogen sulfide ($H_2S$), salicylic acid (SA), melatonin (MT) and brassinolide (BR) (Sun et al. 2020, Kołodziejczyk et al. 2021, Hmmam et al. 2022, Wu et al. 2024). Therefore, we can improve wheat cold tolerance by using exogenous chemicals.

Gamma-aminobutyric acid (GABA) is an environmentally friendly chemical substance. It has been documented that GABA plays an important role in regulating plant growth and development (Kumari et al. 2024, Wang et al. 2025). Meanwhile, more and more research showed that GABA also plays important roles in enhancing plant tolerance under many stresses, including salt stress, CS, heat stress, water deficit stress, low light stress, osmotic stress, chromium stress, etc. (Vijayakumari and Puthur 2016,

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Jia et al. 2017, Li et al. 2017, Mahmud et al. 2017, Rezaei-Chiyaneh et al. 2018, Aljuaid and Ashour 2022, Liu et al. 2024). Besides, GABA could enhance the tolerance of rice, tea and cucumber to CS (Jia et al. 2017, Zhu et al. 2019, Qin et al. 2024). Previous studies manifested that GABA enhanced cold tolerance by increasing the activities of antioxidant enzymes, such as superoxide dismutase (SOD), Catalase (CAT), ascorbate peroxidase (APX) and peroxidases (POD) (Malekzadeh et al. 2017, Rabiei et al. 2019). Meanwhile, crop cold tolerance can be directly reflected by the growth, closely related to the photosynthetic performance. Jia et al. (2017) discovered that GABA enhanced rice photosynthetic capacity by increasing the maximum photochemical efficiency of PSII ($F_v/F_m$) and PSII efficiency under CS, improving growth. Previous research also showed that GABA enhanced wheat hypoxia tolerance, ozone tolerance, arsenic tolerance, salt tolerance and heat tolerance (Wang et al. 2018, Badr et al. 2024, Kolupaev et al. 2024, Kumari et al. 2024, Wang et al. 2024, Kumari et al. 2025). However, there is still no study on GABA's influence on wheat seedlings' cold tolerance. Therefore, it is necessary to explore whether GABA regulates wheat's antioxidant and photosynthetic capacity under CS.

In the current study, we supposed that GABA enhanced wheat cold tolerance by improving the antioxidant and photosynthetic capacity. To verify this supposition, we explored the effects of GABA on malondialdehyde (MDA) content, electrolyte leakage (EL), the activities of antioxidant enzymes (SOD, POD, CAT, APX and glutathione reductase (GR)), the contents of reduced ascorbate (AsA) and reduced glutathione (GSH), soil and plant analyser development (SPAD) value, net photosynthetic rate ($P_n$), chlorophyll fluorescence parameters, and growth parameters under CS. Through this study, we aimed to add information to elucidate the protective role of GABA in helping wheat fight against CS and offer the theoretical basis for GABA usage in the cultivation and production of wheat under lower temperatures.

## MATERIAL AND METHODS

**Plant material and treatments.** Wheat cv. Zhengmai9023 with weak cold tolerance was used as the material for this study (You et al. 2015). Wheat seeds with whole kernels and similar sizes were selected to cultivate wheat seedlings. Firstly, seeds were soaked in distilled water for 4 h and then sterilised with 5% sodium hypochlorite (NaClO) for 10 min. Secondly, seeds were rinsed with distilled water to remove NaClO from the seed surface completely and then soaked in distilled water in a 25 °C incubator for 20 h. Thirdly, seeds were placed on moist filter paper in Petri dishes. Then, all dishes were placed in an incubator to culture seedlings under the following conditions: 25 °C/15 °C day/night temperature, 400 $\mu mol/m^2/s$ photosynthetic active radiation and a 12-h photoperiod. The seedlings were transferred into the half-strength Hoagland's nutrient solution by submerging their roots in the nutrient solution when they fully expanded the first leaves. To create an environment for the growth of the root system, the oxygen pump was used to supply oxygen to the nutrient solution, and the roots were kept in the dark. The nutrient solution was renewed every three days. To uncover the influence of CS, wheat seedlings (BBCH 13) were selected and placed in beakers containing 200 mL nutrient solution and then placed in a climatic chamber at 4 °C, keeping other conditions unchanged. After 4 days of CS treatment, the temperature was returned to 25 °C/15 °C (day/night) for 10 days.

To select suitable GABA concentration, four groups of seedlings were treated with 1.0, 2.0, 4.0 and 6.0 mmol/L GABA for 12 h by submerging their roots in these GABA solutions and then treated with CS. Control seedlings (BBCH 13) were only treated with the nutrient solution alone. There were four replications for each treatment. For each replication, there were 20 seedlings. The significant physiological response of wheat seedlings to CS could first be reflected by photosynthetic and antioxidant indicators after several days. However, significant differences in wheat growth under normal temperatures and CS needed more time to be detected. Therefore, the growth parameters were measured only at 14 days. After 4 days of CS treatment, the third leaves were used to measure the indicators related to the photosynthetic capacity, including SPAD value, $P_n$, maximum photochemical efficiency of PSII ($F_v/F_m$), non-photochemical quenching coefficient (NPQ), photochemical quenching coefficient (qP) and actual photochemical efficiency of PSII (Y(II)). Then, these leaves were sampled and kept in an ultra-low temperature freezer at –80 °C until the analysis for MDA content, EL, antioxidant enzymes, AsA, and GSH contents was done. After 14 days of CS treatment, the growth indicators were measured.

**Assay of antioxidant enzymes.** As reported by Shan and Zhao (2015), SOD, POD and CAT activi-

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ties were measured through the spectrophotometric method. Leaf samples were homogenised with 5 mL 50 mmol/L $KH_2PO_4$ buffer (pH 7.0) and centrifugated at 10 000 × *g* for 10 min at 4 °C. Then the supernatant was used to measure the absorbance value at 560 nm for SOD, 470 nm for POD and 240 nm for CAT through UV759 visible-ultraviolet spectrophotometer (Shanghai Jingke Scientific Instrument Co. Ltd., Shanghai, China). According to Shan and Liang (2010), APX and GR activities were measured through the spectrophotometric method. Leaf samples were homogenised with 5 mL 50 mmol/L $KH_2PO_4$ buffer (pH 7.5) and then centrifuged at 13 000 *g* for 15 min at 2 °C. Then, the supernatant was used to measure the absorbance value at 290 nm for APX and 340 nm for GR through the UV759 visible-ultraviolet spectrophotometer. The activities of the above antioxidant enzymes were expressed as U/g fresh weight (FW).

**Assay of reduced ascorbate and reduced glutathione.** Reduced ascorbate was measured, as reported by Hodges et al. (1996). Leaf samples were homogenised with 6% trichloroacetic acid (TCA) and centrifuged. The supernatant was added to the reaction mixture and incubated at 42 °C for 60 min. Then, the absorbance value at 525 nm was recorded through a UV759 visible-ultraviolet spectrophotometer. Reduced glutathione was measured, as reported by Griffith (1980), by using GR and 2-vinylpyridine, and then the absorbance value at 412 nm was recorded through a UV759 visible-ultraviolet spectrophotometer.

**Assay of MDA and EL.** Hodges et al. (1999) reported that MDA content was measured using the thiobarbituric acid (TBA) method. To determine MDA content, leaf samples were homogenised with $KH_2PO_4$ buffer (pH 7.8), and then centrifugated at 12 000 × *g* for 10 min at 4 °C. Then the supernatant was used to determine MDA content by recording the absorbances at 532, 600, and 450 nm through UV759 visible-ultraviolet spectrophotometer. EL were measured according to Zhao et al. (2004). To determine EL, 20 discs of fresh leaves were submerged in 20 mL of deionised water at 25 °C for 3 h. Then, the electrical conductivity (EC) was measured through the DDS-307 conductivity meter (Shanghai INESA Scientific Instrument Co. Ltd., Shanghai, China) and recorded as $EC_0$. After boiling, EC was measured through a DDS-307 conductivity meter and recorded as $EC_1$. EL was calculated as the percentage of $EC_0$ to $EC_1$.

**Assay of SPAD and $P_n$.** SPAD value was measured through the SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan). $P_n$ was measured through the Licor-6400 photosynthesis system (Lincoln, USA) from 9:00–12:00. The conditions in the leaf chamber were set as light intensity of 1 000 μmol(photon)/$m^2$/s, $CO_2$ concentration of 400 ppm, and leaf temperature of 25.0 °C. The actinic light was provided by an LED light source. The top fully expanded leaves were first equilibrated, and then a steady-state value of $P_n$ was recorded.

**Assay of chlorophyll fluorescence parameters.** Maximum photochemical efficiency of PSII, non-photochemical quenching coefficient, photochemical quenching coefficient and actual photochemical efficiency of PSII (Y(II)) were measured through PAM-2500 chlorophyll fluorometer (Walz, Effeltrich, Germany). These indicators were all measured from 9:00–12:00. For dark adaptation; the leaves were covered by leaf clips for 30 min. The minimal and maximum fluorescences ($F_0$ and $F_m$) under dark adaptation were respectively measured under weak modulating radiation (0.5 μmol/$m^2$/s) and a saturating pulse of radiation (2 400 μmol/$m^2$/s, pulse time 0.8 s). The light-adapted leaves' steady-state fluorescence ($F_s$) was measured when the sample achieved stable status under the actinic light intensity of 600 μmol/$m^2$/s. The maximum fluorescence ($F_{m'}$) was measured under the saturation pulse light. $F_v/F_m$, Y(II), qP, and NPQ were calculated using the below equations:

$$F_v/F_m = (F_m - F_0)/F_m$$

$$Y(II) = (F_{m'} - F_s)/F_{m'}$$

$$qP = (F_{m'} - F_s)/(F_{m'} - F_0)$$

$$NPQ = (F_m/F_{m'}) - 1.$$

**Assay of plant growth parameters.** After 14 days of CS treatment, the vertical height of the shoot was measured through the ruler and recorded as plant height. Every whole seedling was dried in the oven (Dongguan Cree Instrument Technology Co. Ltd., Dongguan, China) at 80 °C until the constant mass was recorded as wheat biomass per plant.

**Statistical analysis.** Data were expressed as the mean value of four replications ± standard deviation (SD). Values were compared by one-way analysis of variance (ANOVA) through SPSS software 22.0 (IBM, Chicago, USA). Duncan's multiple range test (DMRT) was employed to analyse the differences among treatments at 0.05. Pearson correlation analysis was performed using SPSS software 22.0 (Chicago, USA).

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## RESULTS AND DISCUSSION

**The selection of GABA treatment concentration.** CS markedly promoted the accumulation of MDA in wheat leaves and reduced wheat height and biomass against the control (Table 1). Compared to CS, all GABA treatments markedly decreased MDA accumulation in leaves and improved plant height and biomass under CS. Among four concentrations, 4.0 mmol/L GABA markedly enhanced wheat cold tolerance by decreasing MDA content by 28.7% and increasing plant height and biomass by 21.0% and 18.7%. At one time, all concentrations of GABA alone also reduced MDA content in leaves and improved wheat height and biomass against the control, especially for 4.0 mmol/L GABA. Therefore, 4.0 mmol/L GABA was selected to uncover the influence of GABA on wheat cold tolerance.

**Effects of GABA on antioxidant enzymes under CS.** Figure 1 showed that CS markedly increased SOD, POD, CAT, APX and GR activities by 91.3, 120.9, 177.8, 124.0 and 172.7%, compared with control. GABA + CS markedly increased the activities of these enzymes against CS alone. When compared to CS, GABA + CS respectively increased SOD, POD, CAT, APX and GR activities by 44.8, 44.0, 64.0, 37.5 and 66.7%. GABA alone also enhanced the activities of these enzymes against the control. When compared to control, GABA alone increased SOD, POD, CAT, APX and GR activities by 46.4, 71.8, 94.4, 60.0 and 81.8%, respectively. It has been reported that plant antioxidant capacity was closely related to the activities of these antioxidant enzymes. A current study discovered that GABA enhanced SOD, POD, CAT, APX and GR activities of wheat crops under CS, which suggested that GABA enhanced wheat antioxidant capacity through the antioxidative enzyme system under CS.

As reported, GABA enhanced the activities of antioxidant enzymes under salt stress, water deficit and cadmium stress (Akbarzadeh et al. 2023, Dong et al. 2024, Hao et al. 2024). At one time, GABA improved the cold tolerance of several fruits during low-temperature storage, such as aonla fruit, zucchini fruit and cherry fruit (Palma et al. 2019, Rabiei et al. 2019, Ali et al. 2022). Rabiei et al. (2019) showed that GABA could enhance SOD, POD, APX and GR activities in cherry fruit under CS. Saleem and Hasan (2022) showed that GABA could enhance SOD, POD, CAT and APX activities in aonla fruit under CS. Besides, GABA enhanced the tolerance of rice to CS by restoring the membrane integrity (Jia et al. 2017). Zhu et al. (2019) reported that GABA could enhance the tolerance of tea to CS by enhanc-

Table 1. Impacts of different gamma-aminobutyric acid (GABA) concentrations on malondialdehyde (MDA) content, plant height and biomass

| Treatment | MDA (nmol/g FW) | Plant height (cm) | Plant biomass (mg/plant) |
|---|---|---|---|
| Control | 5.10 ± 0.22$^{d}$ | 21.5 ± 0.71$^{b}$ | 188.5 ± 6.59$^{c}$ |
| CS | 9.85 ± 0.49$^{a}$ | 16.2 ± 0.50$^{e}$ | 143.2 ± 3.58$^{e}$ |
| 1.0 GABA | 4.88 ± 0.19$^{de}$ | 22.5 ± 0.73$^{b}$ | 196.7 ± 6.68$^{bc}$ |
| 2.0 GABA | 4.60 ± 0.23$^{e}$ | 23.7 ± 0.70$^{ab}$ | 205.6 ± 6.19$^{b}$ |
| 4.0 GABA | 4.08 ± 0.15$^{f}$ | 24.9 ± 0.88$^{a}$ | 219.9 ± 7.44$^{a}$ |
| 6.0 GABA | 4.44 ± 0.22e$^{f}$ | 24.3 ± 0.71$^{a}$ | 213.5 ± 7.47$^{ab}$ |
| 1.0 GABA + CS | 9.15 ± 0.40$^{a}$ | 17.0 ± 0.54$^{de}$ | 150.1 ± 5.10$^{e}$ |
| 2.0 GABA + CS | 8.27 ± 0.41$^{b}$ | 18.1 ± 0.59$^{cd}$ | 159.8 ± 5.29$^{de}$ |
| 4.0 GABA + CS | 7.02 ± 0.28$^{c}$ | 19.2 ± 0.65$^{c}$ | 170.0 ± 6.60$^{d}$ |
| 6.0 GABA + CS | 7.88 ± 0.33$^{b}$ | 18.9 ± 0.70$^{c}$ | 165.4 ± 6.73$^{d}$ |

Wheat seedlings were treated as: control – no GABA treated and no cold stress; CS – cold stress; 1.0 GABA – 1.0 mmol/L GABA treated with no cold stress; 2.0 GABA – 2.0 mmol/L GABA treated with no cold stress; 4.0 GABA – 4.0 mmol/L GABA treated with no cold stress; 6.0 GABA – 6.0 mmol/L GABA treated and no cold stress; CS + 1.0 GABA – 1.0 mmol/L GABA treated with cold stress; CS + 2.0 GABA – 2.0 mmol/L GABA treated with cold stress; CS + 4.0 GABA – 4.0 mmol/L GABA treated with cold stress; CS + 6.0 GABA – 6.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then to CS or normal temperature for 4 days. After CS treatment, the temperature was returned to normal temperature. MDA content was measured after 4 days of CS treatment. Plant height and biomass were measured after 14 days of CS treatment; FW – fresh weight

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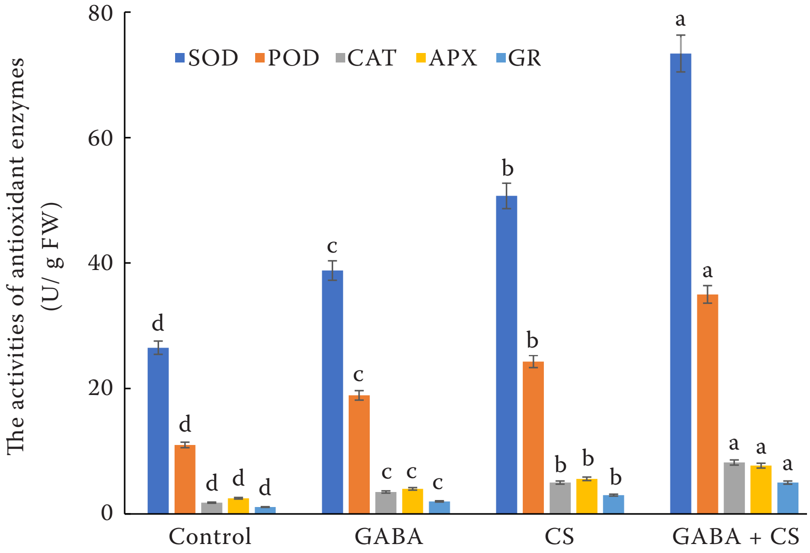

Figure 1. Impacts of gamma-aminobutyric acid (GABA) on antioxidant enzymes activities under cold stress (CS). Wheat seedlings were treated as: control – no GABA treated and no cold stress; GABA – 4.0 mmol/L GABA treated with no cold stress; CS + GABA – 4.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then to CS or normal temperature for 4 days. After 4 days of CS treatment, the temperature was returned to normal temperature. The activities of antioxidant enzymes were measured after 4 days of CS treatment; SOD – superoxide dismutase; POD – peroxidases; CAT – catalase; APX – ascorbate peroxidase; GR – glutathione reductase; FW – fresh weight

ing SOD activity and decreasing MDA content. Qin et al. (2024) showed that GABA enhanced the tolerance of cucumber to CS by improving SOD, POD, CAT and APX activities. For wheat crops, previous reports showed that the accumulation of endogenous GABA could protect themselves against stresses, such as ozone stress, hypoxia stress, salt and osmotic stress (Al-Quraan et al. 2013, Wang et al. 2018, 2024). Meanwhile, previous research also proved that exogenous GABA protected wheat crops against heat and salt stress (Kolupaev et al. 2024, Kumari et al. 2024). However, there is still no report on the role of GABA in regulating wheat cold tolerance. The present study showed that exogenous GABA enhanced wheat cold tolerance by increasing SOD, POD, CAT and APX activities, which was consistent with previous studies on tea and cucumber (Zhu et al. 2019, Qin et al. 2024). Besides, exogenous GABA enhanced wheat cold tolerance by increasing GR activity. Kolupaev et al. (2024) discovered that GABA enhanced wheat heat tolerance by enhancing SOD, POD and CAT activities, which was consistent with our current study. Kumari et al. (2024) discovered that GABA enhanced wheat salt tolerance by activating the ascorbate-glutathione pathway through APX, GR, DHAR and MDHAR. This study also showed that GABA could enhance APX and GR activities under CS, indicating that exogenous GABA application enhances wheat cold tolerance by activating the ascorbate-glutathione pathway. Therefore, the above results of previous studies and our current study indicated GABA could enhance plant tolerance to abiotic stress by enhancing the antioxidant enzyme system, including SOD, POD, CAT and ascorbate-glutathione pathway. However, we only explored the effects of GABA on APX and GR activities of wheat crops under CS but did not explore the influence of GABA on dehydro- ascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) activities under CS. Thus, it will be interesting to carry out this work to provide more information to elucidate the physiological mechanism of GABA in enhancing wheat cold tolerance. Besides, it has been documented that GABA enhanced wheat salt tolerance by improving GPX and GST activities. However, there is still no report on the effects of GABA on these antioxidant enzymes of wheat crops under CS. Therefore, it is also interesting to investigate the effects of GABA on the activities of GPX and GST of wheat crops under CS, which can offer more information on the physiological mechanism of GABA in improving wheat cold tolerance.

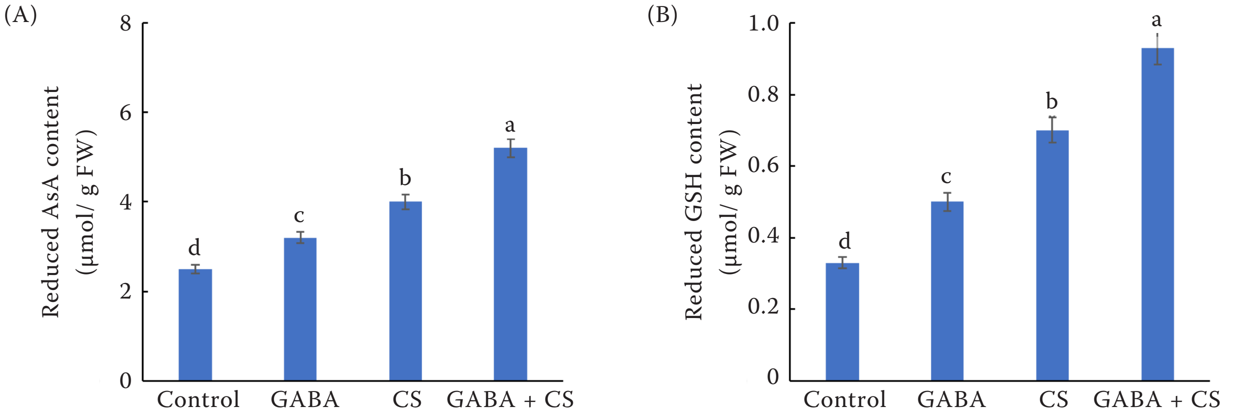

Figure 2. Impacts of gamma-aminobutyric acid (GABA) on (A) reduced ascorbate (AsA) content and (B) reduced glutathione (GSH) content in wheat leaves under cold stress (CS). Wheat seedlings were treated as: control – no GABA treated and no cold stress; GABA – 4.0 mmol/L GABA treated with no cold stress; CS + GABA – 4.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then subjected to CS or normal temperature for 4 days. After 4 days of CS treatment, the temperature was returned to normal temperature. The contents of these two antioxidants were measured after 4 days of CS treatment; FW – fresh weight

**Effects of GABA on reduced ascorbate and reduced glutathione contents under CS.** When compared to control, CS enhanced the accumulation of ascorbate (AsA) and glutathione (GSH) by 60.0% and 112.1%, respectively (Figure 2). Compared to CS, GABA further increased the accumulation of these two antioxidants under CS. Compared with CS, GABA + CS improved AsA and GSH contents by 30.0% and 32.9%, respectively. GABA alone also increased AsA and GSH contents by 28.0% and 51.5% against the control. These results indicated that GABA enhanced wheat antioxidant capacity by increasing AsA and GSH accumulation in wheat leaves under CS.

As reported, exogenous GABA increased non-enzymatic antioxidants AsA and GSH contents in aonla fruit under CS (Ali et al. 2022). For wheat crops, Kumari et al. (2024) discovered that GABA enhanced wheat salt tolerance by increasing AsA and GSH contents. In this study, we discovered that exogenous application of GABA could also increase AsA and GSH contents in wheat leaves under CS. Previous and current results indicated that GABA enhanced wheat antioxidant capacity under both salt and cold stresses by increasing non-enzymatic antioxidants AsA and GSH accumulation in wheat crops. As reported, AsA and GSH contents were controlled by the enzymes in the ascorbate-glutathione pathway and their biosynthetic pathway, mainly including APX, GR, DHAR, MDHAR, L-galactono-1,4-lactone dehydrogenase (GalLDH) and γ-glutamylcysteine synthetase (γ-ECS). Kumari et al. (2024) discovered that GABA increased the contents of AsA and GSH of wheat crops under salt stress by enhancing APX, GR, DHAR and MDHAR activities. For the current study, we only found that GABA improved the APX and GR activities of wheat crops under CS. However, it is still unknown whether GABA can regulate AsA and GSH contents of wheat seedlings under CS by improving DHAR, MDHAR, GalLDH and γ-ECS. Consequently, it will be interesting to explore the impact of GABA on other enzymes responsible for AsA and GSH recycling and biosynthesis. Through this further study, we can add more information to elucidate the physiological mechanism of GABA in improving wheat antioxidant capacity under CS.

**Effects of GABA on MDA and EL under CS.** Compared with the control, CS significantly increased the levels of MDA and EL by 80.0% and 107.5%, respectively (Figure 3). Compared to CS, GABA + CS significantly decreased MDA and EL levels by 26.7% and 33.6%, respectively. CS alone also reduced MDA and EL levels by 20.0% and 23.6% against the control, respectively. The above results indicated that GABA could alleviate CS-induced peroxide damage in wheat crops. As reported, GABA enhanced the cold tolerance of aonla fruit, zucchini fruit and cherry fruit by lowering the levels of MDA and EL (Palma et al. 2019, Rabiei et al. 2019, Saleem and Hasan 2022). For crops, previous studies showed that GABA enhanced the cold tolerance of rice, tea and cucumber by decreasing MDA content and EL (Jia et al. 2017, Zhu et al. 2019, Qin et al. 2024).

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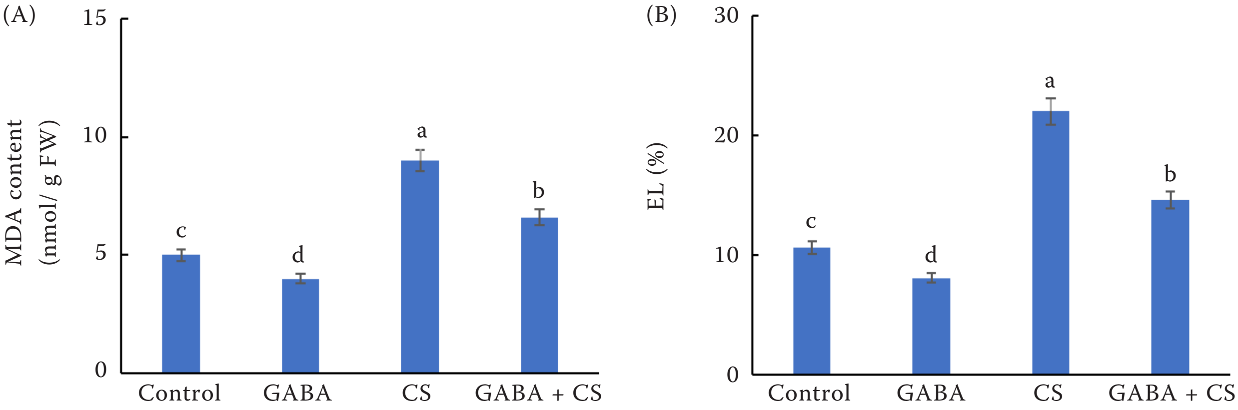

Figure 3. Impacts of gamma-aminobutyric acid (GABA) on the levels of (A) malondialdehyde (MDA) and (B) electrolyte leakage (EL) under cold stress (CS). Wheat seedlings were treated as: control – no GABA treated and no cold stress; GABA – 4.0 mmol/L GABA treated with no cold stress; CS + GABA – 4.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then subjected to CS or normal temperature for 4 days. After 4 days of CS treatment, the temperature was returned to normal temperature. MDA content and EL were measured after 4 days of CS treatment; FW – fresh weight

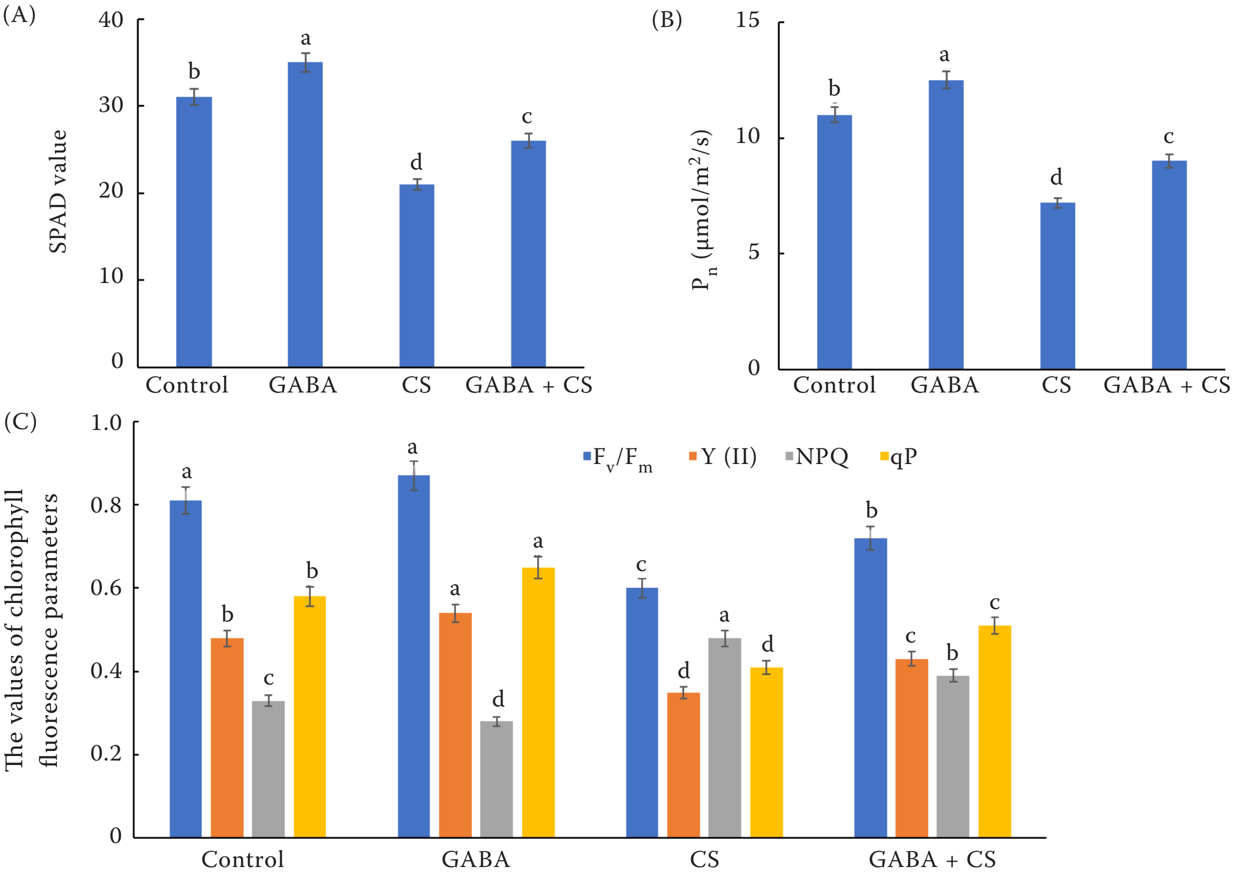

Figure 4. Impacts of gamma-aminobutyric acid (GABA) on (A) soil and plant analyser development (SPAD) value; (B) net photosynthetic rate ($P_n$) and (C) chlorophyll fluorescence parameters under cold stress (CS). Wheat seedlings were treated as: control – no GABA treated and no cold stress; GABA – 4.0 mmol/L GABA treated with no cold stress; CS + GABA, 4.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then subjected to CS or normal temperature for 4 days. After 4 days of CS treatment, the temperature was returned to normal temperature. $F_v/F_m$ – maximum photochemical efficiency of PSII; Y(II) – actual photochemical efficiency of PSII; NPQ – non-photochemical quenching coefficient; qP – photochemical quenching coefficient

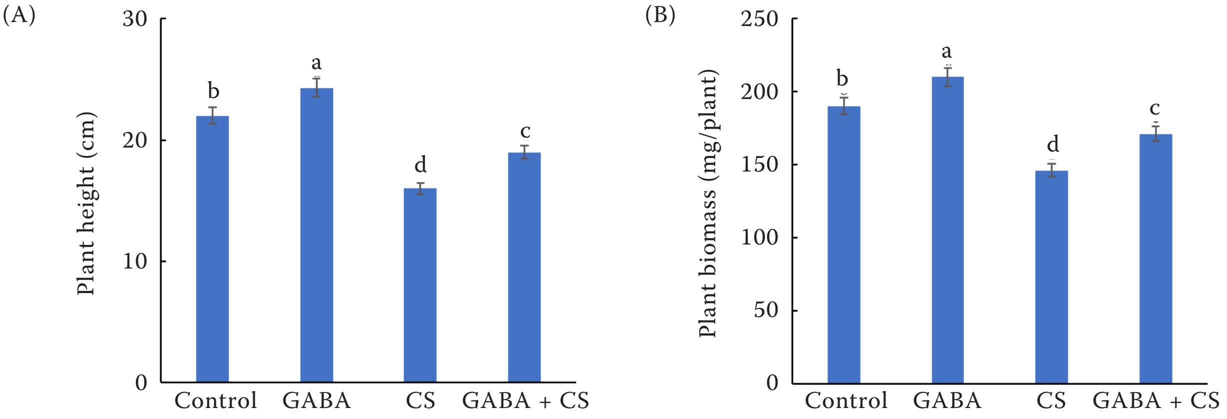

Figure 5. Impacts of gamma-aminobutyric acid (GABA) on (A) plant height and (B) plant biomass. Wheat seedlings were treated as: control – no GABA treated and no cold stress (CS); GABA – 4.0 mmol/L GABA treated with no cold stress; CS + GABA – 4.0 mmol/L GABA treated with cold stress. Wheat seedlings were subjected to GABA for 12 h and then subjected to CS or normal temperature for 4 days. After 4 days of CS treatment, the temperature was returned to normal temperature

We discovered that GABA also lowered the levels of MDA and EL of wheat leaves, which hinted that GABA also enhanced wheat cold tolerance by alleviating CS-induced peroxide damage. Therefore, current and previous studies proved that GABA was important in mitigating crop stress-induced injury.

**Effects of GABA on photosynthetic parameters under CS.** Compared to control, CS significantly increased NPQ by 45.4% and respectively decreased SPAD value, $P_n$, $F_v/F_m$, Y(II) and qP by 32.3, 34.5, 26.3, 27.1 and 29.3% (Figure 4). Compared to CS, GABA significantly decreased NPQ by 18.8% and increased SPAD value, $P_n$, $F_v/F_m$, Y(II) and qP by 23.8, 25.0, 20.0, 22.9 and 24.4%. Compared with control, GABA alone decreased NPQ and increased SPAD value, $P_n$, $F_v/F_m$, Y(II) and qP. These results showed that GABA enhanced wheat seedlings' photosynthetic function under CS.

Malko et al. (2023) reported that CS decreased wheat photosynthetic capacity by reducing $P_n$, Y(II) and qP. CS decreased wheat photosynthetic capacity by reducing $P_n$, Y(II) and qP, which agreed with Malko et al. (2023). Moreover, we showed that CS decreased wheat photosynthetic capacity by increasing NPQ and reducing SPAD value and $F_v/F_m$. SPAD value stands for the level of photosynthetic pigment *Chl*, which plays an important role in light absorption and utilisation. $F_v/F_m$ stands for the maximum photochemical efficiency of PSII. NPQ represents the proportion of light energy absorbed by photosystem II (PSII) dissipated in heat. Thus, this study indicated that CS decreased wheat light use efficiency (LUE). It has been reported that GABA improved the $F_v/F_m$ of cucumber seedlings under CS (Qin et al. 2024). Zhu et al. (2019) found that GABA improved the SPAD value of tea plants under CS. Besides, GABA enhanced rice photosynthetic capacity by increasing $F_v/F_m$ and PSII efficiency under CS (Jia et al. 2017). In this study, we found that GABA increased SPAD value and $F_v/F_m$ of wheat crops under CS, which agreed with previous results on other crops, including cucumber, tea and rice (Jia et al. 2017, Zhu et al. 2019, Qin et al. 2024). This study also uncovered that GABA enhanced wheat photosynthetic capacity under CS by decreasing NPQ and increasing $P_n$, Y(II) and qP. These results hinted that GABA could improve wheat LUE under CS, which enhanced the photosynthetic capacity. Under heat and cadmium stresses, it has also been reported that GABA could enhance plant photosynthetic capacity by increasing $P_n$ and *Chl* content (Zeng et al. 2021, Hao et al. 2024), which agreed with the results of our study on wheat crops. Our previous study indicated that GABA could enhance plant photosynthetic capacity under various stresses. Therefore, we can use GABA to promote crop photosynthetic capacity.

**Effects of GABA on plant growth parameters under CS.** CS significantly decreased plant height and biomass by 27.3% and 23.2%, respectively, compared to the control (Figure 5). When compared to CS, GABA + CS significantly increased these growth parameters. Compared with CS, GABA + CS increased plant height to 19.0 cm and biomass to 171.0 mg/plant. GABA alone also increased these two growth param-

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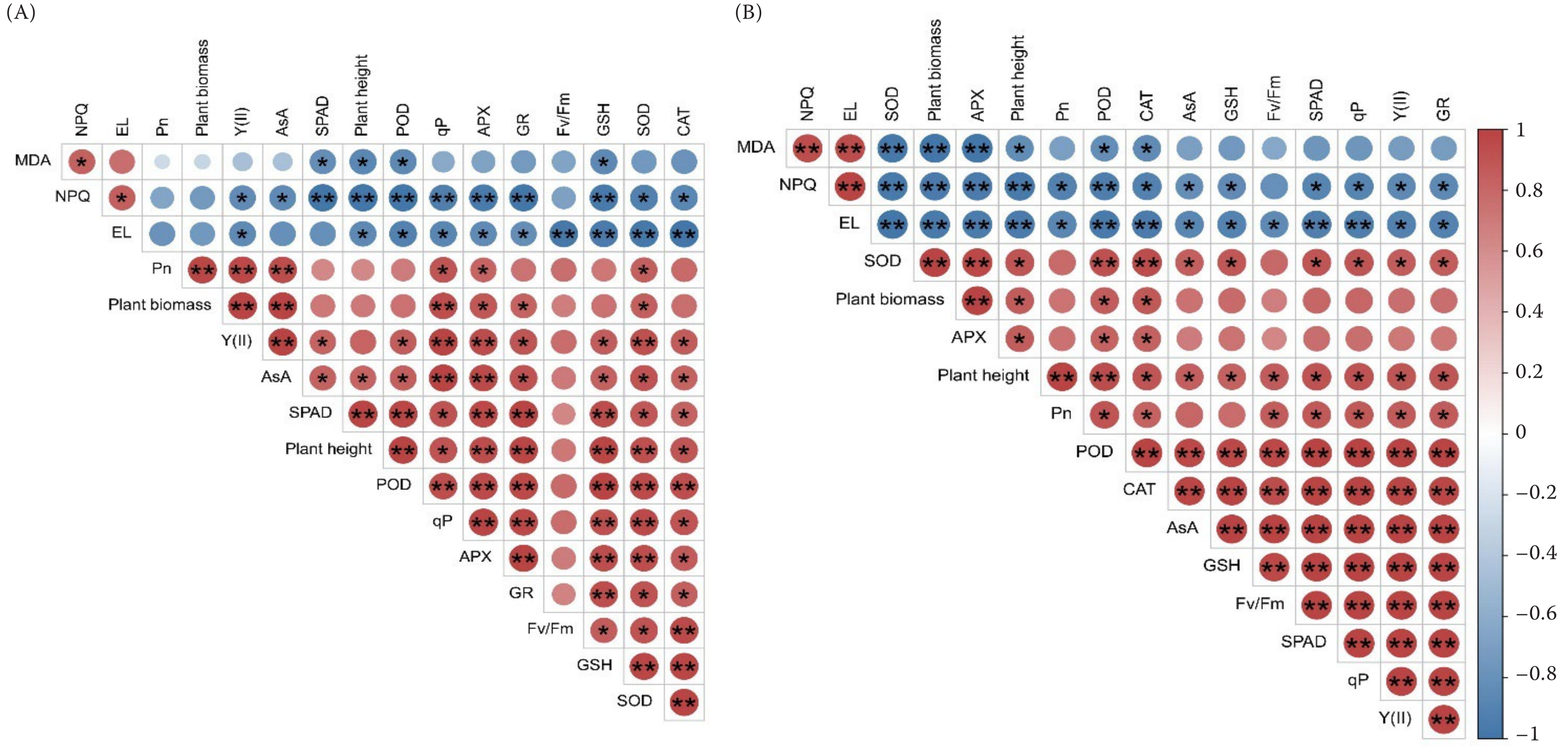

Figure 6. Pearson correlation analysis among different parameters under normal temperature (A) and cold stress (B). One asterisk (*) stands for the significant correlation at 0.05 level, and two asterisk (**) stands for the significant correlation at 0.01 level. AsA – reduced ascorbate; APX – ascorbate peroxidase; CAT – catalase; EL – electrolyte leakage; $F_v/F_m$ – maximum photochemical efficiency of PSII; GR – glutathione reductase; GSH– reduced glutathione; MDA – malondialdehyde; NPQ – non-photochemical quenching coefficient; qP – photochemical quenching coefficient; $P_n$ – net photosynthetic rate; POD –peroxidase; SOD – superoxide dismutase; SPAD – soil and plant analyzer development; Y(II) – effective quantum yield of PS II

eters against the control. These results directly showed that GABA improved wheat CS tolerance.

Plant growth parameters can directly reflect plant cold tolerance. Zhao et al. (2024) displayed that CS significantly inhibited flax height and biomass. Li et al. (2024) discovered that CS inhibited annual bluegrass growth. Previous studies proved that CS also decreased wheat growth by decreasing plant biomass (Osman et al. 2013). As reported, GABA could improve plant growth under salt-alkaline stress and cadmium stress (Hao et al. 2024, Yang et al. 2024). However, there is still no report on the influence of GABA on wheat growth under CS. We discovered that GABA increased the height and biomass of wheat seedlings under CS, which directly showed that GABA enhanced wheat cold tolerance.

**Pearson correlation analysis among different parameters under normal temperature and CS.** Under normal temperatures, Pearson correlation analysis showed that plant height and biomass had positive relationships with SPAD, $P_n$, $F_v/F_m$, Y (II), qP, SOD, POD, CAT, APX, GR, AsA and GSH but had negative relationships with NPQ, MDA and EL (Figure 6A). Under CS, Pearson correlation analysis also showed that plant height and biomass had positive relationships with SPAD, $P_n$, $F_v/F_m$, Y (II), qP, SOD, POD, CAT, APX, GR, AsA and GSH but had negative relationships with NPQ, MDA and EL (Figure 6B). These results indicated that plant growth was closely related to wheat photosynthetic performance and antioxidant capacity under normal temperature and CS. As shown in Figures 1–4, GABA could increase SPAD, $P_n$, $F_v/F_m$, Y (II), qP, SOD, POD, CAT, APX, GR, AsA and GSH, and decreased NPQ, MDA and EL. Therefore, GABA could enhance wheat photosynthetic performance and antioxidant capacity, improving wheat growth under normal temperatures and CS. Our research showed new information to elucidate the physiological mechanism of GABA in enhancing wheat cold tolerance. It also provided the foundation for its application in future production practices to alleviate the negative influence of low temperatures on wheat seedlings. This study only investigated the influence of GABA on photosynthetic performance and antioxidant capacity of wheat seedlings under CS at the physiological level but did not investigate the deeper mechanism of GABA in enhancing wheat tolerance under CS at the molecular level. Therefore, exploring the deeper mechanisms of GABA in enhancing wheat tolerance under CS through multi-omics, such as transcriptomics and metabolomics, will be very interesting. In this way, we can elucidate the deeper mechanism of GABA in enhancing wheat tolerance under CS.

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