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Effects of exogenous glycinebetaine on cadmium-induced changes in photosynthetic performance, antioxidative metabolism and ATPase in cucumber seedlings

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Abstract: A hydroponic experiment was carried out to study the ameliorative effects of exogenous glycinebetaine (GB) upon cadmium (Cd) toxicity in cucumber seedlings. The results indicated that 50 μmol/L Cd stress decreased soil plant analysis development (SPAD) value, plant height, root length, seedling biomass, activities of ascorbate peroxidase (APX) and ATPase in leaves, stems and roots; however, increased peroxidase (POD) and superoxide dismutase (SOD) activities in all tissues, catalase (CAT) activities in stems/roots. Moreover, Cd stress also elevated leaf/root malondialdehyde (MDA), proline, phenols and flavonoid content in all cucumber tissues over the control. The supplementation of GB (Cd + GB) prominently alleviated Cd-induced growth inhibition and oxidative stress, increased SPAD value and stem ATPase, and improved photosynthetic performance compared with Cd treatment alone. Furthermore, external GB diminished leaf/root MDA accumulation and decreased leaf/root proline contents as well as phenols and flavonoid contents in all tissues. Meanwhile, exogenous GB counteracted Cd-induced alterations of certain antioxidant enzymes. For example, it brought all tissue POD and SOD activities and stem/root CAT activities down towards the control level and significantly increased APX activities, especially in leaves and stems. These data suggested the principal protective mechanism for the exogenous GB against Cd toxicity in cucumber seedlings is closely related to improved photosynthesis, diminished Cd-induced proline and MDA accumulation, enhanced ATPase as well as modulation of antioxidant enzymes.

Keywords: ascorbate peroxidase; malondialdehyde; proline; superoxide dismutase; toxic elements; *Cucumis sativus* L.

The rapid development of industry and agriculture, along with excessive human interference, makes environmental pollution more and more serious, and a large number of heavy metals enter the environment. Heavy metals are harmful to plants, plant-dependent animals, and ultimately human health. In water bodies and soil, cadmium (Cd) is one of the most toxic heavy metals, which is easily absorbed by plants, translocated to aerial parts, and then accumulated in the diet organs of crops, consequently resulting in negative impacts on crop productivity. Subsequently, Cd can be moved into the food chain, eventually posing health risks to humans.

The International Agency for Research on Cancer (IARC) has classified Cd as a human carcinogen on the basis of occupational studies (IARC 1993).

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In China, approximately 2.8 × 10^9 m^2 of farmland is contaminated by Cd (Liu et al. 2015, Xue et al. 2017). Cd concentration in vegetables was found to be 0.007–0.021 mg/kg (Song et al. 2017), close to the maximum Cd limit of 0.05 mg/kg in vegetables (GB2762-2017). Accordingly, vegetable consumption may be one of the primary Cd absorption pathways by humans, and more than 70% of Cd in humans comes from vegetables (Liu et al. 2011). Cucumber (*Cucumis sativus* L.) belongs to Cucurbitaceae, which is one of the most consumed vegetables in the world (Tatlioglu 1993). However, cucumber grown on the Cd contaminated soil is quickly acclimated to Cd uptake. Thus, trusted approaches to reduce Cd concentration in cucumber fruits are vitally and urgently needed. Chemical regulators application to mitigate the Cd-induced toxicity and minimise plant Cd accumulation in the medium contaminated farmlands might offer a cost-effective and practical strategy for safe cucumber production, food security, and human health.

Cd toxicity can cause plants to exhibit some typical symptoms, such as leaf margin curling and chlorosis, root shortening and thickness, plant growth and photosynthesis inhibition, membrane permeability changes, oxidative damage, and so on (Gallego et al. 2012, Rizwan et al. 2016). To cope with Cd toxicity, plants have developed some physiological and biochemical mechanisms. For instance, Cd-induced different response patterns in photosynthesis, internal metabolites, and ROS-scavenging enzymes, including antioxidative enzymes ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Wu et al. 2003, Wang et al. 2012). In addition, the accumulation of glycinebetaine (GB) is one of the self-defence mechanisms of abiotic stress-tolerant plants.

Glycinebetaine (GB) is a nontoxic, colourless, and odourless compound. It is easily accumulated by certain crops (Jokinen et al. 2022), which is also a secondary metabolite and an essential osmotic regulator. Exogenous GB plays a very crucial role for plants to enhance abiotic stress resistance, which can ameliorate plant growth under abiotic stress, such as chilling, drought, salt and Cd stress (Chen et al. 2000, He et al. 2019, Zhou et al. 2019), thereby enhancing subsequent growth and yield (Zhang and Yang 2019). However, levels of GB accumulation are bound up with the extent of increased tolerance by plants (Rhodes and Hanson 1993). According to the research of Huang et al. (2020), GB plays an important role in stabilising the structure and function of the photosystem II complex under abiotic stresses. It was also reported that GB could also effectively stabilise the quaternary structures of complex proteins and enzymes when salt concentrations or temperatures are extreme (Papageorgiou and Murata 1995). The regulation of exogenous GB on plant Cd stress has also been reported. For example, Islam et al. (2009) found that exogenous GB and proline confer Cd tolerance by increasing the ascorbate-glutathione cycle enzyme and certain antioxidase activities in cultured tobacco cells. Moreover, Zhang et al. (2021) indicated that GB improved rice plant growth under Al stress by regulating Al uptake and translocation. Simultaneously, Ali et al. (2015) reported that GB could alleviate wheat Cr toxicity by elevating the activities of antioxidase and suppressing heavy metal Cr uptake.

Although studies have shown GB played an essential role in improving plant tolerance resistance to heavy metals, the enhancing tolerance varies with the used GB concentration, which is diluted by the plant biomass, and the protective mechanisms until now remain poorly understood. Therefore, in order to verify the hypothesis of the beneficial effects of GB in the detoxification of Cd toxicity in cucumber, the present work was conducted to investigate the physiological effects of GB in alleviating the detrimental effects of Cd stress *via* a hydroponic experiment, including the changes in morphological attributes, photosynthesis parameters, certain antioxidant enzymes, MDA, proline, phenols and flavonoids content, and total ATPase activities of cucumber seedlings after seven days Cd exposure, and tried to explore the mechanisms of GB against Cd toxicity in cucumber.

MATERIAL AND METHODS

Plant material and experimental design. The hydroponic experiment was performed at Taiyuan University of Science and Technology, Taiyuan, China. Healthy cucumber seeds of genotype "Jinyan 4" were germinated in sterilised moist sand and grown for ten days in a greenhouse at 22 °C/25 °C (day/night) (Sun et al. 2016a). At the second leaf stage (10 days old), uniform healthy seedlings were transferred to 3 L plastic containers filled up with the basal nutrient solution (BSN) (Janicka-Russak et al. 2012), and the pH of the solution was adjusted to 5.6 ± 0.1 with HCl or NaOH as required. Seven averagely spaced holes were bored on the lid of the container, and there were two plants per hole, and the plants were fixed with the sponge.

Four treatments were imposed after seven days of transplanting: control (BNS); GB (BNS + 500 µmol/L GB); Cd (50 µmol/L $CdCl_2$), and Cd + GB (BNS + 50 µmol/L $CdCl_2$ + 500 µmol/L GB), and the solution was aerated continuously using gas pumps and replaced at three days interval. Each treatment was performed in three replicates. After seven days of exposure, the whole plants were collected for measurements of growth, physiological and biochemical analysis.

Photosynthesis and SPAD value analysis. At the end of treatments, the upper 2nd fully expanded cucumber leaves were used to measure the photosynthesis parameters and SPAD (soil plant analysis development) value. The net photosynthetic rate (P_n), transpiration rate (T_r), intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) were determined *via* a fully automatic portable photosynthesis system (LC Pro-SD, London, UK). SPAD value was measured by a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Tokyo, Japan).

Plant growth analysis. After seven days of exposure, all cucumber seedlings were uprooted for root length and plant height measurement. Then, the plants were separated into roots, leaves and stems, and all samples were dried at 80 °C until constant weight for biomass determination.

Enzyme activity assay. The fresh compound leaves, stems and roots of each plant were collected for enzyme determination after treatment, and all the samples were washed three times using distilled water. POD, SOD, CAT and APX activities were determined as described by Zhang et al. (2021). ATPase activities were carried out according to Greengard (1956).

Lipid peroxidation, proline, phenols and flavonoid content determination. The accumulation of lipid peroxidation was carried out by the TBA (thiobarbituric acid) reaction (Wu et al. 2003), which was quantitated by the amount of malondialdehyde (MDA). The level of proline was determined as described by Bates and Waldren (1973). Total phenols content was carried out based on the Folin-Ciocalteu reagent reduction as described by Singleton et al. (1999), while flavonoid content was determined as described by Jia et al. (1999). All the measurements were directly carried out with the fresh samples after seven days of treatments.

Statistic analysis. Data of each measurement was mean three times. Analysis of variance (ANOVA) was carried out with Data Processing System (DPS) statistical software package (Hangzhou, China), followed by Duncan's multiple range test to determine the significant difference at a level of $P \leq 0.05$.

RESULTS

Effect of exogenous GB on Cd-induced suppression in plant growth attributes. Cd stress (50 µmol/L) was found to reduce the SPAD value significantly, root length, plant height as well as dry weight (DW) in leaves, stems, and roots of cucumber seedlings compared with control (Figure 1). GB application significantly alleviated the detrimental effects of Cd stress on cucumber seedlings. However, such positive effects were more significant for root length, stem and root DW. Compared to Cd alone, GB (Cd + GB) increased the root length of cucumber seedlings by 35.8%, stem DW by 32.7%, and root DW by 27.5%. There was no apparent difference between the control and only GB treatment conditions regarding all growth parameters.

Effect of exogenous GB and Cd on photosynthesis. Photosynthesis-related parameters are shown in Table 1. After seven days of Cd treatment, the net photosynthetic rate, transpiration rate, and stomatal conductance of cucumber seedlings declined by 27.7, 32.1, and 31.5%, respectively, when compared with the controls. GB in combination with Cd improved P_n by 19.2%, T_r by 15.8%, and 10.2% in g_s over the corresponding Cd alone treatments. Nevertheless, Cd stress displayed a significant accumulation of intercellular CO_2 concentration (C_i) in comparison with control; exogenous GB (Cd + GB) addition decreased the accumulation of Cd-induced.

Effect of exogenous GB and Cd on certain antioxidant enzyme activities. As shown in Table 2, Cd or Cd + GB treatment resulted in a significant alteration in the antioxidative system in cucumber seedlings. Compared with control, the activities of POD and SOD were significantly higher in response to Cd stress in all tissues. In contrast, the control and GB treatment alone did not exhibit a significant difference (Table 2). Moreover, seedlings treated with Cd + GB had prominently lower POD and SOD activities at different levels as compared to Cd-treated alone. For instance, the POD/SOD activities under Cd + GB treatment were 16.2%/31.5% in leaves, 47.7%/14.4% in stems and 52.5%/25.2% in roots, respectively, lower than the seedlings with Cd-treated alone, and some of the POD and SOD activities in

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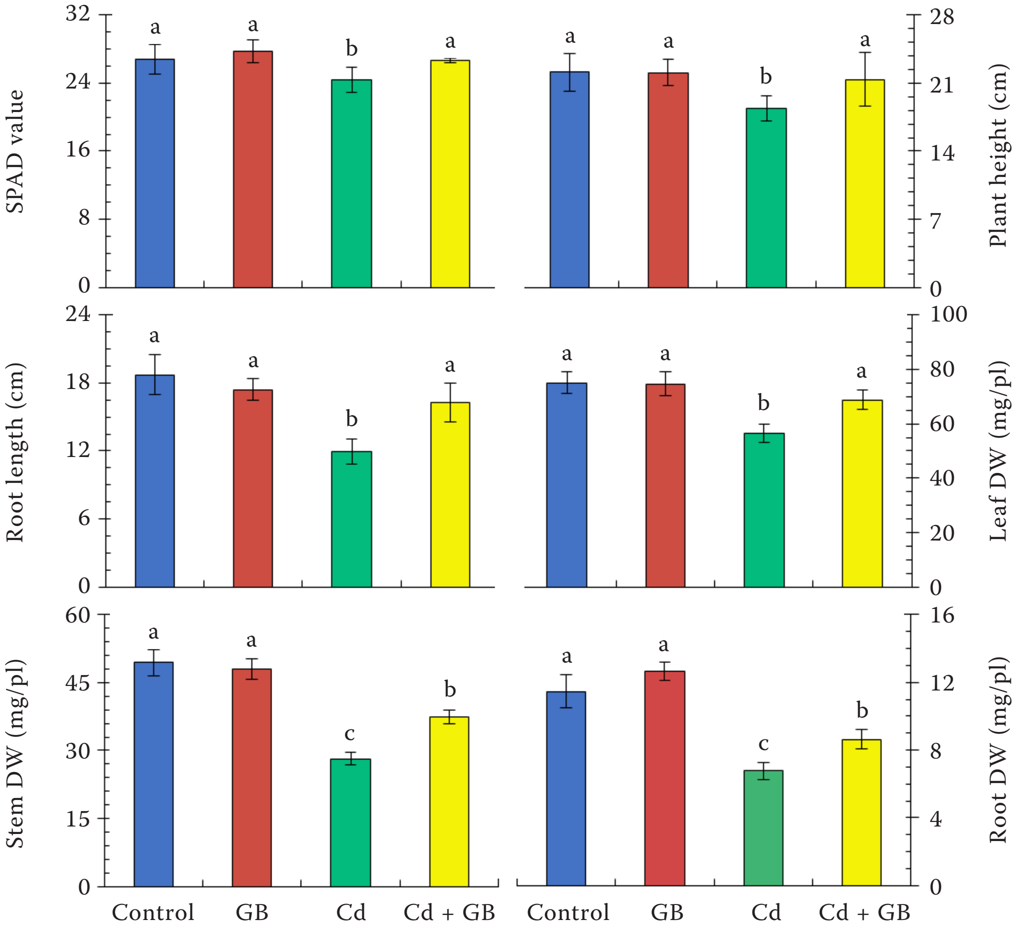

Figure 1. Effects of exogenous glycinebetaine (GB) on the soil plant analysis development (SPAD) value, plant height, root length and biomass of cucumber seedlings after 7 days cadmium (Cd) exposure. DW – dry weight. The error bars represent the standard deviation values ($n = 3$). The different letters indicate the significant differences ($P < 0.05$) among the 4 treatments within each sampling date

Cd + GB were even returned to the control level. On the other hand, in comparison with control, only stem CAT activity was significantly enhanced by 52.0% in response to Cd stress; leaves and roots CAT activities had no obvious difference after Cd stress. Exogenous application of GB (Cd + GB) significantly inhibited Cd-induced stem CAT activity enhancement. In contrast, compared with control, leaves, stems, and roots, APX activities decreased in response to Cd stress; moreover, seedlings treated

Table 1. Effect of exogenous glycinebetaine (GB) on photosynthesis parameters of cucumber seedlings after 7 days of cadmium (Cd) exposure

Treatment	P_n (μmol $CO_2/m^2/s$)	T_r (mmol $H_2O/m^2/s$)	g_s (mmol $H_2O/m^2/s$)	C_i (μmol CO_2/mol)
Control	20.2 ± 1.9^{a}	2.8 ± 0.3^{a}	121.5 ± 0.9^{a}	347 ± 4.3^{c}
GB	19.2 ± 1.7^{a}	2.5 ± 0.2^{a}	119.3 ± 0.5^{a}	339 ± 5.1^{c}
Cd	14.6 ± 0.5^{c}	1.9 ± 0.2^{b}	83.2 ± 2.1^{bc}	424 ± 6.3^{a}
Cd + GB	17.4 ± 1.3^{b}	2.2 ± 0.1^{ab}	91.7 ± 2.1^{b}	381 ± 2.9^{b}

P_n – net photosynthetic rate; T_r – transpiration rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration. Data are means ± standard deviation ($n = 3$). The different letters in each column indicate the significant differences ($P < 0.05$) among the 4 treatments

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Table 2. Effect of exogenous glycinebetaine (GB) on activities of certain antioxidant enzymes in leaves, stems and roots of cucumber seedlings after 7 days of cadmium (Cd) exposure

Tissue	Treatment	POD (mmol/g FW/min)	CAT (mmol/g FW/min)	SOD (U/g FW)	APX (mmol/g FW/min)
Leaf	control	2.594 ± 0.11^{b}	0.211 ± 0.092^{a}	5.851 ± 0.21^{b}	3.714 ± 0.16^{a}
	GB	2.251 ± 0.18^{b}	0.223 ± 0.183^{a}	5.192 ± 0.32^{b}	3.686 ± 0.12^{a}
	Cd	3.153 ± 0.21^{a}	0.195 ± 0.215^{a}	7.776 ± 0.31^{a}	2.655 ± 0.13^{b}
	Cd + GB	2.641 ± 0.27^{b}	0.231 ± 0.178^{a}	5.327 ± 0.38^{b}	3.541 ± 0.18^{a}
Stem	control	1.561 ± 0.15^{c}	0.025 ± 0.006^{b}	4.566 ± 0.27^{b}	3.205 ± 0.16^{a}
	GB	1.691 ± 0.12^{c}	0.019 ± 0.007^{b}	4.647 ± 0.39^{b}	3.485 ± 0.13^{a}
	Cd	4.329 ± 0.23^{a}	0.038 ± 0.011^{a}	5.938 ± 0.42^{a}	2.642 ± 0.10^{b}
	Cd + GB	2.264 ± 0.26^{b}	0.013 ± 0.004^{c}	5.085 ± 0.29^{b}	3.231 ± 0.14^{a}
Root	control	10.182 ± 0.58^{b}	0.024 ± 0.001^{a}	2.087 ± 0.32^{c}	4.067 ± 0.23^{a}
	GB	12.314 ± 0.55^{b}	0.028 ± 0.003^{a}	2.032 ± 0.27^{c}	4.143 ± 0.32^{a}
	Cd	22.541 ± 1.02^{a}	0.029 ± 0.004^{a}	3.219 ± 0.21^{a}	3.329 ± 0.24^{c}
	Cd + GB	10.716 ± 0.32^{b}	0.012 ± 0.002^{b}	2.407 ± 0.28^{b}	3.771 ± 0.32^{b}

POD – peroxidase; CAT – catalase; SOD – superoxide dismutase; APX – ascorbate peroxidase. Data are means ± standard deviation (n = 3). The different letters in each column indicate the significant differences ($P < 0.05$) among the 4 treatments. FW – fresh weight

with supplementation of GB (Cd + GB) had higher APX activities, increased by 33.4% in leaves, 22.3% and 13.3% in stems and roots over that seedlings with Cd-treated alone.

Effect of exogenous GB and Cd on MDA accumulation, proline, phenols and flavonoid contents. Exposure to 50 µmol/L Cd alone caused a significant increase in MDA content in leaves and roots of cucumber seedlings, whereas Cd + GB significantly decreased MDA content by 12.0% and 21.4% in leaves and roots, respectively, as compared to Cd treatment alone. Moreover, Cd decreased MDA content in stems; this decrease was increased with exogenous GB (Cd + GB treatment) by 46.2% (Figure 2A).

Data revealed that proline contents were significantly increased in all tissues of cucumber seedlings under Cd alone treatments (Figure 2B). Exogenous GB treatment (Cd + GB) significantly decreased proline levels in both leaves and roots by 11.4% and 19.3%, respectively, but remained unchanged in stems compared with Cd treatment alone.

Total phenols content (TPC) increased significantly in all tissues under Cd treatment relative to control, the application of exogenous GB (Cd + GB) decreased the levels of TPC by 28.9, 84.6, and 25.9% in leaves, stems, and roots, respectively, and the contents in all tissues almost recovered to the respective controls (Figure 2C). Similarly, compared to the control, exposure to Cd alone treatment caused an obvious increase in the flavonoid content in cucumber seedlings, a more significant increase was observed in stems and roots than leaves. However, exogenous GB addition displayed a notable decrease in flavonoid accumulation compared with 50 µmol/L Cd treatment alone; among the three tissues, the stems decreased more flavonoids as compared with leaves and roots (Figure 2D).

Effect of exogenous GB and Cd on total ATPase activity. Results showed that total ATPase activities in leaves, stems, and roots were significantly reduced under 50 µmol/L Cd stress in cucumber seedlings. Moreover, the difference among tissues in total ATPase was apparent; leaves recorded higher total ATPase than stems and roots, and stem total ATPase was the lowest. Exogenous GB (Cd + GB) significantly increased total ATPase only in stems and increased the total stem ATPase by 14.9% in comparison with Cd treatment alone (Figure 3).

DISCUSSION

Exogenous GB alleviates Cd-induced suppression in plant growth. Cd toxicity reduces plant growth and biomass, disturbs various physiological and biochemical processes of many crops, and ultimately leads to plant growth inhibition and even

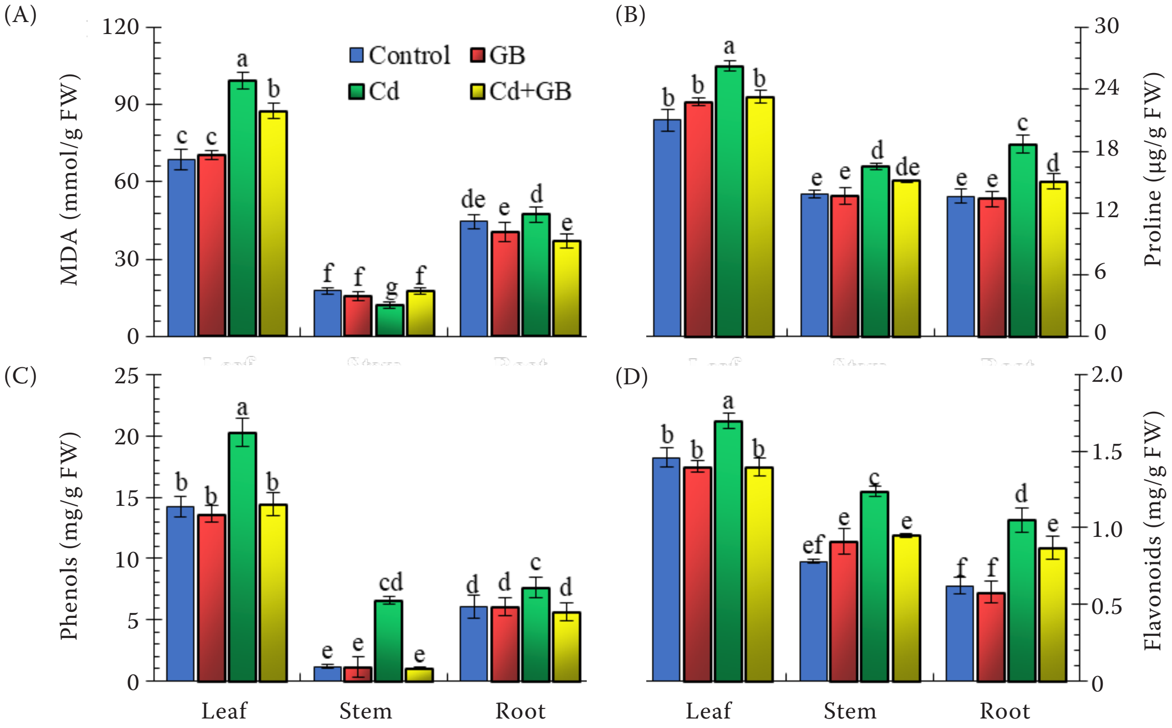

Figure 2. Effects of exogenous glycinebetaine (GB) on malondialdehyde (MDA), proline, phenols and flavonoids contents in leaves, stems and roots of cucumber seedlings after 7 days cadmium (Cd) exposure. The error bars represent the standard deviationvalues ($n = 3$). The different letters indicate the significant differences ($P < 0.05$) among the 4 treatments within each sampling date. FW – fresh weight

to death. Plants have to evolve all sorts of mechanisms to response to Cd stress, one of the impactful mechanisms is the application of chemical regulators (Fu et al. 2019, He et al. 2019). Glycinebetaine is the most common chemical regulator that occurs in the survival of a wide variety of plants under various abiotic stresses, including Cd stress (Demiral and Türkan 2006, Duman et al. 2011, Lou et al. 2015, He et al. 2019, Zhang et al. 2021).

The present study evaluated the effects of exogenous GB on Cd-induced changes in morphological attributes, photosynthesis, certain antioxidant enzyme activities, MDA, proline, phenols and flavonoids content, and total ATPase activity in cucumber seedlings. Our study indicated that 50 μmol/L Cd toxicity significantly reduced root and shoot growth and biomass of cucumber seedlings; GB supplementation (Cd + GB) enhanced the seedling growth attributes compared with those treated with Cd alone, especially the growth of cucumber roots (Figure 1), which states clearly that exogenous GB may play a positive role in cucumber safe production under Cd stress. On the other hand, the Cd-induced degradation of SPAD value was observably reverted after the application of GB, which is in accord with the previous study (He et al. 2019). It was indicated that exogenous GB might alleviate harmful effects of Cd-induced by increasing chlorophyll content.

Exogenous GB counteracts Cd-induced inhibition in photosynthesis. Photosynthetic and gas exchange parameters are considered powerful tools for evaluating the photosynthesis of plants (Kupper et al. 2007). In the present research, Cd stress decreased P_n and was accompanied by a notable reduction of T_r and g_s (Table 1). It may be suggested that 50 μmol/L Cd stress could inhibit P_n and induce the closing of stomata leading to a reduction in g_s, which is in line with studies on rice (Cai et al. 2011). Moreover, the addition of GB restored P_n to an appreciable level and also increased T_r and g_s. According to the change trends of these parameters under different treatments, P_n, T_r and g_s have a positive relation with plant growth indexes (Figure 1). Furthermore, C_i increased markedly after Cd stress; at the same time, Cd + GB suppressed Cd-induced increase; therefore, no stomatal limitation and decrease of SPAD value contributed to the inhibition of photosynthetic processes in Cd-stressed cucumber seedlings. This result

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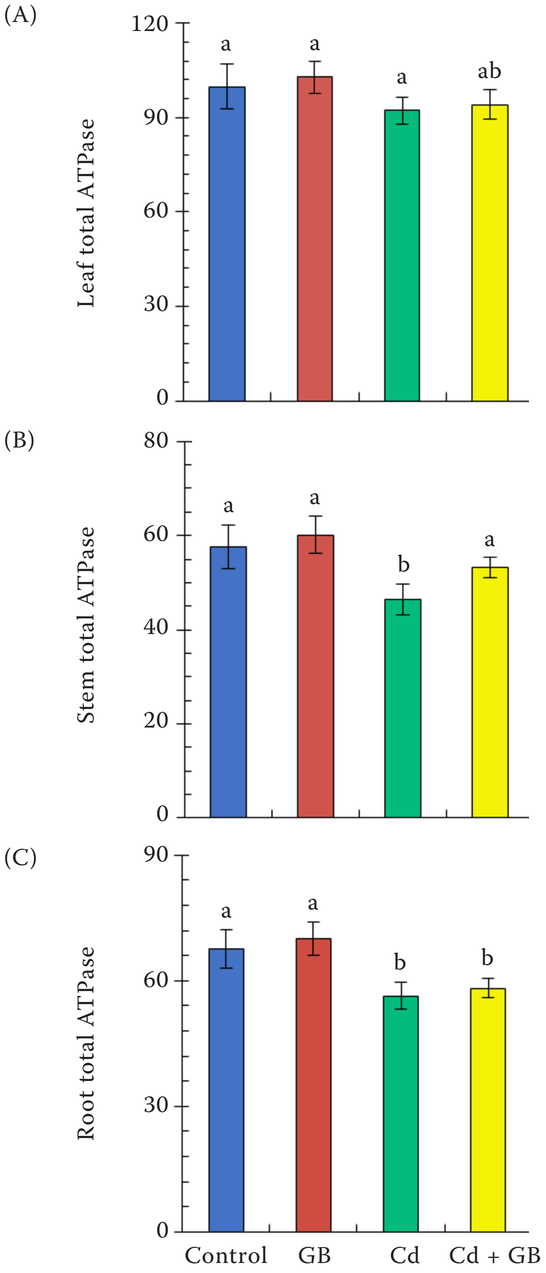

Figure 3. Effects of exogenous glycinebetaine (GB) on the total ATPase activities in leaves, stems and roots of cucumber seedlings after 7 days cadmium (Cd) exposure. Values are in µmol p_i/g FW (fresh weight)/h. The error bars represent the standard deviation values ($n = 3$). The different letters indicate the significant differences ($P < 0.05$) among the 4 treatments within each sampling date

was consistent with our previous results (Sun et al. 2016b). The present results showed that exogenous GB might positively affect photosynthesis and promote cucumber seedling growth under Cd toxicity.

Exogenous GB offsets Cd-induced alterations in the antioxidant system. Heavy metal stress induces oxidative stress and leads to the accumulation of ROS (Kim et al. 2017). Cd-induced oxidative stress is a common phenomenon observed in multiple studies. The inhibition in plant growth by Cd was linked with Cd-induced overproduction of ROS (Rasheed et al. 2014). In the present study, Cd stress-induced plant growth inhibition was consistent with MDA accumulation. The content of MDA is also considered to be an index of the degradation or deterioration of membranes (Hasanuzzaman et al. 2019). Present results indicated that Cd toxicity increased MDA contents both in leaves and roots (Figure 2A), in accordance with other metals which can cause oxidative damage (Yamamoto 2019). Dramatically, in the case of GB application under Cd stress, MDA contents were significantly depressed both in leaves and roots compared with Cd-stressed alone, which depicts the ability of GB to provide protection of membranes against Cd stress and thus provides protection against Cd toxicity.

Furthermore, to counteract oxidative stress, plants use antioxidant defense enzymes, including CAT, POD, SOD, and APX. Moreover, previous investigations showed that the application of GB could alleviate Cd-induced oxidative damage in wheat and tobacco (Rasheed et al. 2014, He et al. 2019). The current results observed that activities of POD, SOD and stem and root CAT were notably increased due to Cd stress; however, the pattern of the increase was distinctly counteracted with the addition of GB (Cd + GB). GB alleviated Cd stress in cucumber seedlings by enhancing the activities of APX in leaves, stems, and roots (Table 2). These results demonstrated that GB could harmonise the activities of antioxidant defense enzymes and protect cucumber tissues against oxidative damage caused by Cd stress.

Proline is one of the osmolytes, and high accumulation is a symptom of plant stress injury (Pei et al. 2010). In this study, proline content increased under Cd stress; GB addition (Cd + GB) decreased proline contents, particularly in cucumber roots (Figure 2B). Endogenous GB participated in the reduction of Cd-induced high proline levels in cucumber seedlings might be an indication of Cd stress relief and alleviation of Cd stress damage.

Phenolic compounds play a vital role in alleviating oxidative stress as they are involved in the detoxification of ROS (Wang et al. 2011). In accordance with this standpoint, cucumber seedlings, after Cd stress, displayed higher values of phenols and flavonoids and therefore appeared to have a greater capacity to eliminate ROS (Figure 2C, D). After the addition of GB (Cd + GB), an evident decrease was observed both in total phenolic and flavonoid contents compared with plants under Cd alone stress, which can be used as a stress indicator.

Effect of exogenous GB on Cd-induced alterations in the total ATPase. ATPase activity is a vital element for the survival of plants in response to multifarious environmental stresses such as Al stress (Dawood et al. 2012) and Cd + Cr stress (Cao et al. 2013). The previous report showed that Cd could inhibit DNA mismatch repair by suppressing ATPase activity in yeast (Banerjee and Flores-Rozas 2005). In this work, Cd stress induced prominent depression of total ATPase activity in all cucumber tissues; however, treatment with Cd + GB led to up-regulation of both leaves and stems total ATPase activities, especially in the stems (Figure 3). It suggested that GB – induced higher activities of total ATPase are beneficial to enhancing Cd tolerance in Cd exposed cucumber seedlings.

In conclusion, GB application had obvious beneficial effects on cucumber seedlings exposed to Cd stress. It effectively alleviated Cd-induced growth inhibition and toxicity. This alleviation was interrelated with a substantial reduction in MDA accumulation and improvement in photosynthetic performance. Furthermore, exogenous GB effectively decreased proline, phenols, and flavonoid contents and prominently improved stem total ATPase activity. Meanwhile, the addition of GB under Cd stress counteracted the Cd-induced response of antioxidant enzymes by suppressing a marked increase of POD and SOD in all tissues and stem/root CAT activities and *via* elevating Cd stress-depressed APX activities in cucumber seedlings. Therefore, GB might be used as a mitigator of Cd stress in cucumber seedlings, which would be useful in cucumber-safe production.

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