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ORIGINAL ARTICLE

Glycinebetaine: a versatile protectant to improve rice performance against aluminium stress by regulating aluminium uptake and translocation

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Abstract

Key message **Glycinebetaine alleviates the detrimental effects of aluminium stress by regulating aluminium uptake and translocation, maintaining PSII activity, and activating the oxidative defence, thereby maintaining the growth and development of rice.**

Abstract Aluminium (Al) toxicity is one of the primary growth-limiting factors that limits plant growth and crop productivity in acidic soils. Rice (*Oryza sativa* L.) plants are susceptible to Al stress and do not naturally accumulate glycinebetaine (GB), one of the most effective protectants. Therefore, the objective of this study was to investigate whether exogenous GB can ameliorate the detrimental effects of Al stress on rice plants. Our results showed that the growth, development and biomass of rice were clearly inhibited under Al stress. However, exogenous GB application increased rice shoot growth and photosynthetic pigments contents, maintained photosystem II (PSII) activity, and activated the antioxidant defence system under Al stress. More importantly, GB may mediate the expression of Al uptake- and translocation-related genes, including *OsALS1*, *OsNrat1*, *OsSTAR1* and *OsSTAR2*, and the galacturonic acid contents in rice roots under Al stress. Therefore, our findings highlight exogenous GB application is a valid approach to effectively combat Al toxicity by regulating physiological and biochemical processes in crops.

Keywords Glycinebetaine · Aluminium stress · Aluminium uptake and translocation · Oxidative defence · Rice

Introduction

As sessile organisms, plants are frequently and repeatedly suffer from various abiotic stresses during their lifetime, such as extreme temperature, drought, salinity, high light intensity, UV radiation, flooding and submergence, nutrient deficiency, metal ion toxicity (Zhu 2016; Kumar et al. 2017; Gong et al. 2020). Aluminium (Al) stress is a global agricultural problem in acidic soils that limits crop productivity, as approximately 50% of the potentially arable soil is acidic worldwide (Kochian et al. 2004, 2015; Zhang et al. 2018). Al is the most abundant metal element in the Earth's crust and exists in the form of rather insoluble aluminosilicates or oxides in the soil (Kochian et al. 2004; Zhang et al. 2018; Lou et al. 2020). However, when the soil pH drops below 5.5, Al becomes increasingly soluble and dissolves in the soil (Kochian et al. 2015). In particular, Al^{3+}, the most toxic form among the various ionic forms, can cause phytotoxicity, including severe inhibition

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of plant root growth and development, thereby resulting in reduced efficiency to take up water and nutrients (Yang et al. 2017; Yamamoto 2019).

Currently, osmoprotection is one of the most important mechanisms that protects plants from various abiotic stresses (Chen and Murata 2002). It utilises a variety of low molecular weight compounds called compatible solutes to alleviate the negative effects of abiotic stresses (Chen and Murata 2008, 2011; Zulfiqar et al. 2020). Glycinebetaine (GB) is one of the most-effective compatible solutes that can increase plant resistance to various environmental stresses (Li et al. 2011; Wei et al. 2017; Huang et al. 2020). It has been reported that GB has beneficial physiological effects on plants suffering from ionic toxicity. In mung bean (*Vigna radiata* L.) seedlings, GB application can enhance cadmium (Cd) tolerance by reducing the levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) and increasing the activities of the enzymes in the systems that detoxify reactive oxygen species (ROS) and methylglyoxal (MG) (Hossain et al. 2010). Likewise, under Cd stress, the exogenous application of GB could increase endogenous proline accumulation and the activities of antioxidant enzymes and photosystems, as well as decrease the levels of ROS and MDA in the aquatic plant *Lemna gibba* L. (Duman et al. 2011). Furthermore, in perennial ryegrass, the use of exogenous GB relieved the negative effects of Cd stress by elevating the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and the related stress-responsive gene expression (Lou et al. 2015). In addition, the concentrations of oxalic acid and tartaric acid increased, leading to the chelation of more heavy metal ions and resulting in less Cd^{2+} translocation from the roots to shoots (Lou et al. 2015). In addition to Cd stress, the exogenous application of GB could also alleviate lead (Pb) toxicity by increasing the activity of antioxidant enzymes and photosynthesis in cotton (Bharwana et al. 2014), and mitigate the toxic effects of chromium (Cr) by improving the protein and chlorophyll contents of wheat plants (Ali et al. 2015). Moreover, GB can increase photosynthesis and antioxidant enzyme activity in cauliflower, which is of great significance for food safety (Ahmad et al. 2020).

Although GB has been reported to play an important role in enhancing metal toxicity tolerance in plants, the protective mechanisms of GB against Al stress remain poorly understood. To the best of our knowledge, this is the first report on the beneficial physiological effects of GB against Al stress in higher plants. In the present study, we used the exogenous application of 5 mM GB to rice (*Oryza sativa* L. spp. japonica) seedlings to investigate whether GB would alleviate the detrimental effects of Al stress (500 µM). In addition, we explored the mechanism of Al tolerance in rice.

Materials and methods

Plant growth and stress treatment

Rice seeds were soaked in the deionized water for 2 days. Afterwards, the sprouted seeds were hydroponically sown in plastic pots and incubated in a cultivation chamber. All plants were facilitated growing under normal conditions at 25/22 °C (day/night temperature), 350 µmol photon m^{-1} s^{-2} photosynthetic photon flux density (PPFD), 65–70% relative humidity and a photoperiod of 16/8 h (light/dark). During the growing period, the seedlings were nourished with nutrient solution at pH 4.5. The culture solution (0.04 mmol L^{-1} Na EDTA-Fe_3H_2O, 0.05 mmol L^{-1} K_2SO_4, 0.09 mmol L^{-1} KH_2PO_4, 0.09 mmol L^{-1} KNO_3, 0.27 mmol L^{-1} $MgSO_4{\cdot}7H_2O$, 0.8 mmol L^{-1} Na_2SiO_3, 0.18 mmol L^{-1} Ca $(NO_3)_2{\cdot}4H_2O$, 1.18 mmol L^{-1} $(NH_4)_2SO_4$) was replaced every 3 days. On the fourth day, the seedlings were treated with $AlCl_3$ (500 µM) and GB (5 mM) individually and in combination. In preliminary experiments, the concentrations for $AlCl_3$ treatment were 100 µM, 500 µM and 1000 µM, and the concentrations for GB treatment were 3 mM, 5 mM and 8 mM. Finally, according to rice phenotype, we determined that the concentrations of $AlCl_3$ and GB should be 500 µM and 5 mM, respectively. The experimental groups were as follows. CK: control check, GB: 5 mM GB treatment, Al: 500 µM $AlCl_3$ treatment, and Al + GB: 500 µM $AlCl_3$ + 5 mM GB treatment.

Plant growth and biomass measurements

After 2 weeks, the lengths of the shoots and roots of 10 randomly selected rice seedlings from each treatment group were measured. The fresh weights of the shoots and roots were recorded after cutting the junction between the shoots and roots.

Analysis of chlorophyll fluorescence and the rapid light-response curves of ΦPSII

According to Pfündel et al. (2008), the modulated chlorophyll fluorescence and rapid light curves of ΦPSII were measured with a Dual PAM-100 (Walz, Germany) in this study. The measurement protocol for rapid light curves of ΦPSII was as follows. During the 60 s illumination periods, the measurement sequence was 23, 37, 53, 107, 179, 232, 378, 576, 875 and 1324 µmol photons m^{-2} s^{-1}. The actual efficiency of PSII was calculated as $\Phi PSII = (Fm' - Fs)/Fm'$; the electron transport rate of PSII was calculated as $ETR(II) = 0.84 \times 0.5 \times \Phi PSII \times PPFD$; the non-photochemical quenching was calculated as $NPQ = (Fm - Fm')/Fm'$; and the photochemical quenching coefficient was calculated as qP = (Fm' − Fs)/(Fm' − F_0'). Here, Fm is the maximal fluorescence, Fs is the stationary fluorescence, Fm' is the maximal chlorophyll fluorescence in light-adapted leaves and F_0' is the minimal fluorescence in the illuminated samples.

Determination of $O_2^{\cdot -}$ productivity rate and MDA, photosynthetic pigment, protein, and proline contents

The $O_2^{\cdot -}$ production rate was quantified using the method of Rauckman et al. (1979). MDA content was determined according to the method of Cavalcanti et al. (2004). Photosynthetic pigment content was estimated according to Wellburn (1994). The proline content was estimated according to Bates and Waldren (1973). The protein content in each sample was determined according to Bradford (1976).

Enzyme activity assay

Rice roots and shoots (0.5 g) were ground into homogenates with buffer. The homogenates were centrifuged at 10,000*g* for 20 min, and the obtained supernatant was used to determine enzyme activity. The temperature was maintained at 0–4 °C.

SOD activity was measured using the photochemical NBT method of Dhindsa and Matowe (1981). First, 0.1 mL of enzyme solution and 2.9 mL of reaction mixture were mixed well for 20 min of reaction under light conditions. Then, the absorbance of the solution was measured at 560 nm with a spectrophotometer.

APX activity was determined as stated by Nakano and Asada (1981). First, 0.1 mL of enzyme solution and 2.9 mL of reaction mixture were mixed well for 40 s of reaction. Then, the absorbance of the solution was measured at 290 nm with a spectrophotometer.

CAT activity was assayed following the method of Patra et al. (1978). First, 0.1 mL of enzyme solution and 2.9 mL of reaction mixture were mixed for 20 s of reaction. Then, the absorbance of the solution was measured at 240 nm with a spectrophotometer.

POD activity was assayed following the method of Upadhyaya et al. (1985). First, 0.02 mL of enzyme solution and 3 mL of reaction mixture were mixed well for 30 s of reaction. Then, the absorbance of the solution was measured at 470 nm with a spectrophotometer.

Cell wall extraction and measurement of galacturonic acid content

The cell walls of the rice roots were extracted according to Zhong and Lauchli (1993). The pectin component was extracted twice in a boiling water bath with 0.5% ammonium oxalate buffer containing 0.1% $NaBH_4$ for 1 h each time, and then the supernatants were combined. The content of galacturonic acid in pectin was measured according to Blumenkrantz and Asboe-Hansen (1973). To 150 μL of supernatant was added 1 mL of H_2SO_4–$Na_2B_4O_7 \cdot 10H_2O$ (0.125 M) followed by boiling in a water bath for 5 min. After cooling, 20 μL of 0.15% m-hydroxybiphenyl (dissolved in 0.5% NaOH) was added. Using galacturonic acid as the standard, the pectin content was determined by spectrophotometry at 520 nm. Galacturonic acid was used as a calibration standard; thus, the pectin content in rice roots is expressed as galacturonic acid equivalents.

Measurement of Al and Ca contents

The Al and Ca contents were measured according to Zasoski and Burau (1977). After treatment for 2 weeks, the roots and shoots of rice seedlings were dried for 72 h at 75 °C, milled into a power and weighed, and then the dried sample and extracted cell wall were digested with acid (HNO_3:$HClO_4$, 5:1, v/v) at a constant volume of 50 ml. Then, the contents of Al and Ca were measured by inductively coupled plasma mass spectrometry (ICP-MS).

Gene expression analysis

Total RNA was extracted from rice roots using a RNAprep Pure Plant Kit (TIANGEN, Beijing, China). Two micrograms of total RNA was used to synthesise cDNA using a Transcriptional First Stand cDNA Synthesis Kit (TRANS, Beijing, China). The gene expression levels were determined by quantitative real-time polymerase chain reaction (qRT-PCR) using a SYBR Premix Ex Taq™ kit (Vazyme, Nanjing, China). The relative gene expression was calculated by the Formula $2^{-\Delta\Delta \mathrm{Cp\ (Crossing\ point)}}$. The experiment was performed with three biological and technical replicates. The primers for each gene used in this study are listed in Table S1.

Statistical analysis

The data were preprocessed using Microsoft Excel 2010 (Microsoft, Redmond, WA), and the graphics were generated using GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, USA). Significant differences among the data were determined using one-way analysis of variance (ANOVA) with SPSS (ver. 22.0) followed by LSD (Duncan's test, $P < 0.05$). Mean values ± standard deviation (SD) of at least three replicates of all data are presented.

Results

Exogenous GB mitigates the Al-induced growth inhibition of rice shoots

To test the beneficial physiological effect of GB on the growth of rice seedlings under Al stress, we examined the plant phenotype and measured the lengths and fresh weights of the shoots and roots (Fig. 1). Exposure of rice seedlings to Al stress (500 µM) led to a significant decrease in the lengths and fresh weights of the shoots and roots (Fig. 1), but the use of exogenous GB (5 µM) markedly mitigated the inhibitory effects of Al stress on the growth of rice seedlings, especially rice shoot growth (Fig. 1B, C). Interestingly, compared to the control, application of GB alone to the control culture solution caused an evident increase in shoot growth, whereas, GB-treated plants had shorter roots under Al stress conditions (Fig. 1E). However, there was no notable difference between the root fresh weights of Al-treated and Al + GB-treated plants (Fig. 1D). These results demonstrated that exogenous GB alleviated Al-induced growth inhibition, especially the growth of rice shoots.

Moreover, exposure of the rice seedlings to Al stress conditions for 2 weeks markedly decreased chlorophyll *a* and carotenoid contents by 25 and 14%, respectively, compared with control (Fig. S1A, D), while there was no substantial difference between the chlorophyll *b* and total chlorophyll contents among the control and Al-treated plants (Fig. S1B, C). However, GB application significantly mitigated the Al stress-induced decrease in chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids, and these values returned close to those of the control or were even higher (Fig. S1), suggesting that GB application could mitigate the reduction in chlorophyll and carotenoid contents under Al stress.

Exogenous GB alleviates PSII photoinhibition induced by Al stress

To investigate the effects of GB on PSII photoinhibition under Al stress, the actual efficiency of PSII (ΦPSII), photosynthetic electron flow through PSII [ETR(II)], photochemical quenching (qP) and non-photochemical quenching (NPQ) were determined. Under non-Al conditions, there were no significant differences in these parameters between the control and GB-treated plants (Fig. 2). However, under Al stress, the ΦPSII, ETR(II) and qP values decreased in rice leaves, but exogenous application of GB mitigated the detrimental effects of Al stress; the ΦPSII, ETR(II) and qP values after GB treatment were 26, 25 and 31% higher, respectively, than those after Al treatment alone (Fig. 2A, B, D). Furthermore, NPQ plays an important role in PSII light

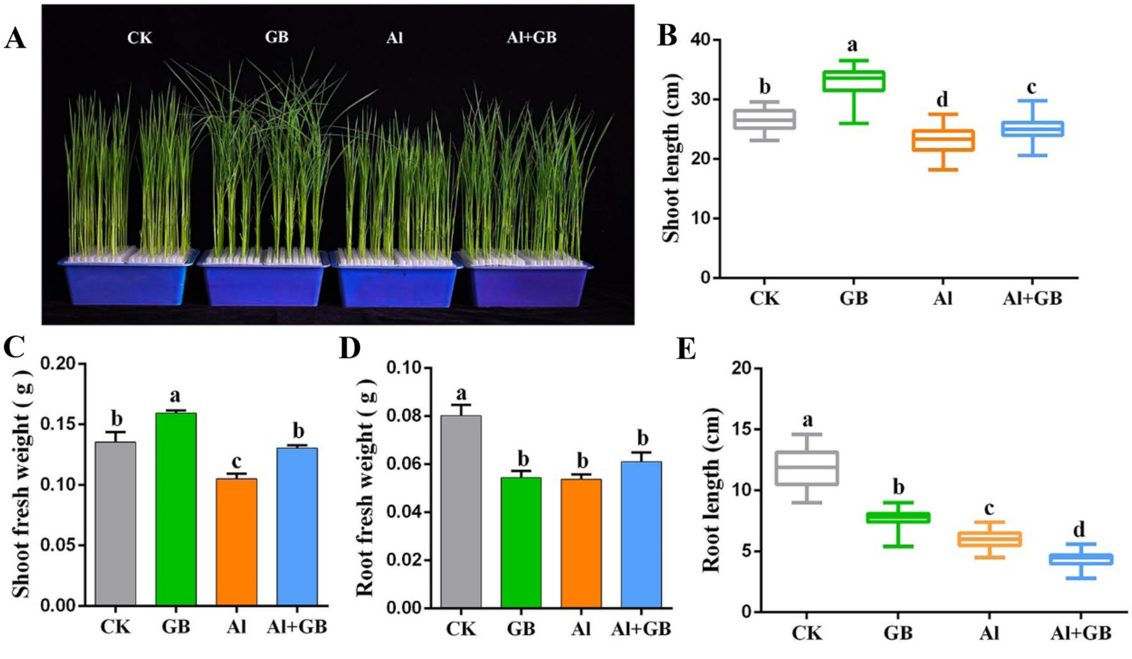

Fig. 1 Effects of glycinebetaine on the growth phenotype and biomass of rice under aluminium stress. **a** Growth phenotype of rice. **b** Height of rice shoots. **c** Fresh weights of rice shoots. **d** Fresh weights of rice roots. **e** Rice root lengths. CK: control check. GB: 5 mM GB treatment. Al: 500 µM $AlCl_3$ treatment. Al + GB: 500 µM $AlCl_3$ + 5 mM GB treatment. Different letters indicate significant differences between different treatments at $P < 0.05$

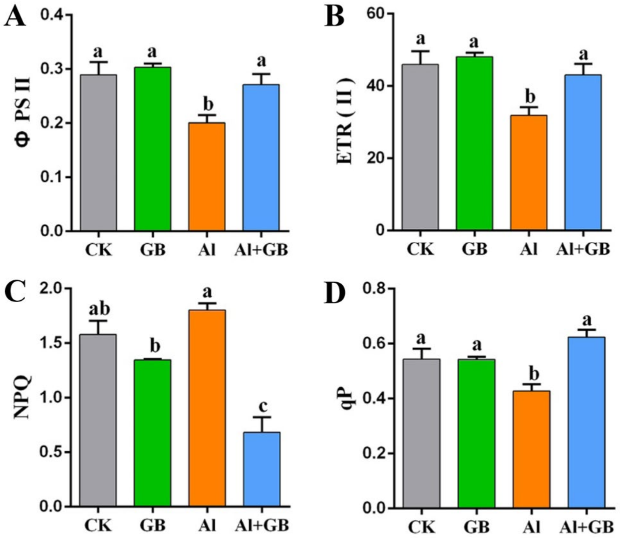

Fig. 2 Effects of glycinebetaine on PSII activity in rice leaves under aluminium stress. **a** The effective quantum yield of PSII, ΦPSII. **b** Photosynthetic electron flow through PSII, ETR(II). **c** Non-photochemical quenching, NPQ. **d** Photochemical quenching, qP. CK: control check. GB: 5 mM GB treatment. Al: 500 μM $AlCl_3$ treatment. Al + GB: 500 μM $AlCl_3$ + 5 mM GB treatment. Different letters indicate significant differences between different treatments at $P < 0.05$

protection and is a symbol of excess excitation energy (Joliot and Johnson 2011; Sasi et al. 2018). As shown in Fig. 2C, Al stress leads to an increase in excess excitation energy in the reaction centre, and the exogenous application of GB can reduce this increase. Moreover, the energy distribution in PSII differed between the control and Al stress treatments. From the result of the light response change in ΦPSII, in comparison to control plants, the ΦPSII of Al stressed plants was much lower, but under Al stress, GB treatment led to a higher ΦPSII than that in Al-treated plants (Fig. S2). These results demonstrated that the degree of photoinhibition in the GB-treated plants was slight under Al stress, and GB application could alleviate Al stress-induced photoinhibition.

Effects of GB on the contents of $O_2\cdot^-$, MDA, proline and soluble protein in rice under Al stress

Under metal toxicity stress, plants will produce a large amount of ROS, such as superoxide anion radicals ($O_2\cdot^-$), which act as an important indicator that reflects the degree of damage to the plants. As shown in Fig. 3A, there was no clear difference in the $O_2\cdot^-$ production rate among all rice plants in absence of Al stress. However, in comparison with the control, Al stress induced 43% and 49% more $O_2\cdot^-$ production in the shoots and roots, respectively (Fig. 3A). Moreover, while under Al stress, GB application

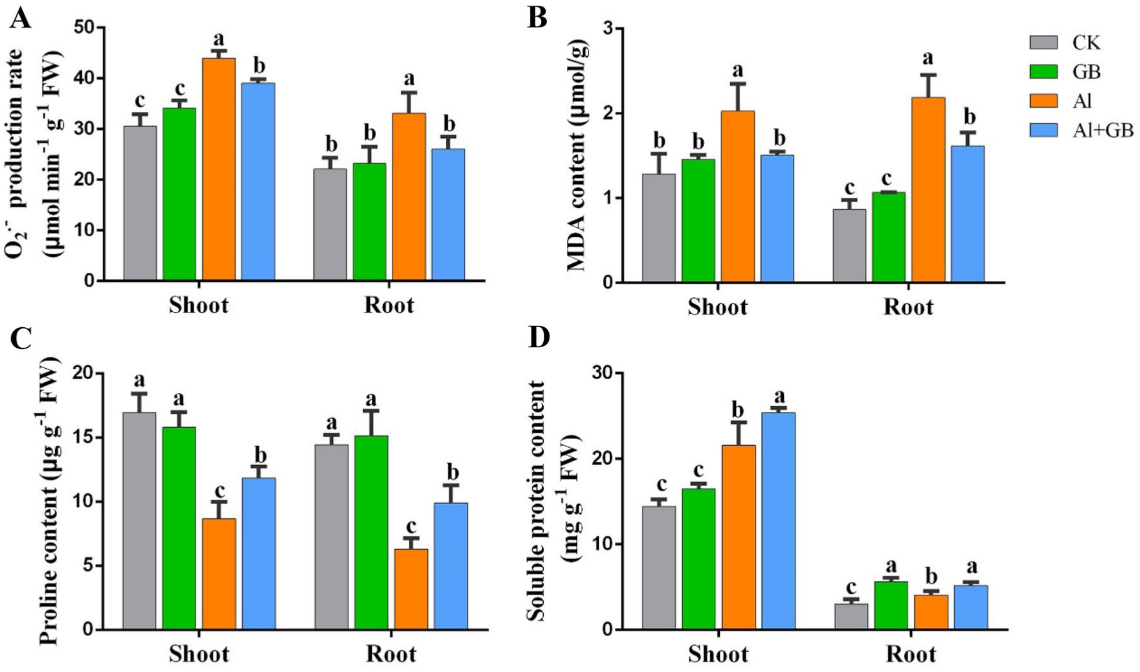

Fig. 3 Effects of glycinebetaine on the contents of $O_2\cdot^-$, MDA, proline and soluble protein in rice under aluminium stress. **a** The rate of $O_2\cdot^-$ production in the shoots and roots of rice. **b** MDA contents in the shoots and roots of rice. **c** Proline contents in the shoots and roots of rice. **d** Soluble protein contents in the shoots and roots of rice. CK: control check. GB: 5 mM GB treatment. Al: 500 μM $AlCl_3$ treatment. Al + GB: 500 μM $AlCl_3$ + 5 mM GB treatment. FW: fresh weight. Different letters indicate significant differences between different treatments at $P < 0.05$

significantly decreased the production rate of $O_2^{\cdot -}$ by 11% and 21% in the shoots and roots, respectively, compared with Al-treated plants (Fig. 3A). Likewise, Al stress also resulted in a notable increase in the contents of MDA in rice seedlings, but exogenous GB treatment significantly decreased the MDA levels compared with Al-treated plants (Fig. 3B).

Furthermore, we examined the contents of intracellular proline and soluble protein in the shoots and roots (Fig. 3C, D). Under normal conditions, there was no significant difference in the proline contents of rice seedlings (Fig. 3C). However, in comparison with the control, Al stress markedly reduced the proline contents by 48 and 56% in the shoots and roots, respectively (Fig. 3C), but GB treatment greatly relieved the Al-induced reduction in proline contents, and these values increased by 26 and 36% in the shoots and roots, respectively, compared with Al-treated plants (Fig. 3C). Interestingly, compared with the control, exogenous application of GB led to an increase in the contents of soluble protein in rice roots in the absence of Al stress, but there was no evident difference in the shoots (Fig. 3D). Moreover, although the contents of soluble protein were clearly induced by Al stress treatment in rice seedlings, exogenous application of GB maintained higher soluble protein contents than that in Al-treated rice plants (Fig. 3D).

These results demonstrated that GB application could mitigate oxidative damage, reduce the degree of lipid peroxidation, and increase the contents of proline and soluble protein under Al stress.

Exogenous GB enhances antioxidant enzyme activities in rice under Al stress

We also measured the activities of antioxidant enzymes involved in scavenging ROS, including SOD, CAT, ascorbate peroxidase (APX) and POD to explore the antioxidant defence mechanisms of GB under Al stress. As shown in Fig. 4, Al stress resulted in multiple changes to the antioxidative system in rice seedlings. Exposure of the seedlings to Al stress conditions showed that the SOD, POD and CAT activities increased by 8, 16 and 18% in the shoots, respectively, in comparison with control seedlings (Fig. 4A, B, C). Notably, Al + GB treatment markedly increased the activities of SOD, POD and CAT by 26, 26 and 9% in shoots, respectively, compared with Al-treated plants (Fig. 4A, B, C). However, APX activity in shoots did not vary significantly (Fig. 4D).

Likewise, Al stress treatment led to a clear increase in the activities of SOD, CAT, APX and POD in the roots (16, 48, 25, and 27%) in comparison with control seedlings (Fig. 4). Moreover, Al + GB-treated roots showed 30 and 61% higher POD and CAT activities, respectively, than the seedlings treated with Al alone (Fig. 4B, C). This revealed

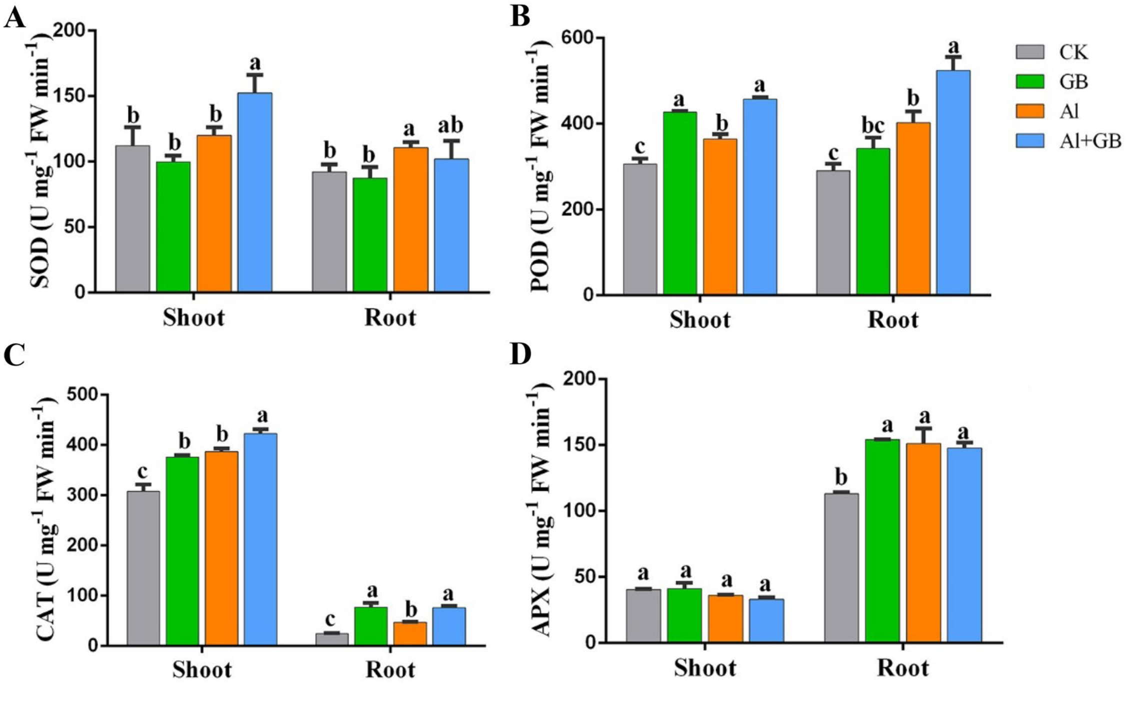

Fig. 4 Effects of glycinebetaine on the activity of antioxidant enzymes in the shoots and roots of rice under aluminium stress. **a** Superoxide dismutase, SOD. **b** Catalase, CAT. **c** Ascorbic peroxide, APX. **d** Peroxidase, POD. CK: control check. GB: 5 mM GB treatment. Al: 500 μM $AlCl_3$ treatment. Al + GB: 500 μM $AlCl_3$ + 5 mM GB treatment. FW: fresh weight. Different letters indicate significant differences between different treatments at $P < 0.05$

that exogenous application of GB could enhance the antioxidant enzyme activities in rice seedlings under Al stress, which contributes to scavenging more of the ROS induced by Al stress.

Exogenous GB affects Al accumulation and calcium contents in rice plants under Al stress

To further understand the mechanism responsible for Al tolerance after exogenous GB application, the contents of Al in rice seedlings were determined under Al stress. Al stress caused an evident increase in Al concentration in the shoots and roots of rice seedlings, especially the roots, which was significantly higher than that in the shoots (Fig. 5A, B). However, exogenously applied GB during Al stress reduced the Al contents by 48% in the shoots compared with Al-treated plants (Fig. 5A). Interestingly, under Al stress, GB-treated roots showed a threefold higher Al content than the seedlings treated with Al alone (Fig. 5B). Moreover, after the application of GB, Al accumulation increased significantly in the cell walls of rice roots, giving a value that was 4.3-fold higher than that in Al-treated rice roots (Fig. 5C). Furthermore, the ratio of the Al content in the cell wall to that in the whole cell in Al + GB-treated roots was notably higher than that of the other treatments (Fig. 5D). These results demonstrated that GB application could alleviate the toxic effects of Al stress by increasing the accumulation of absorbed Al^{3+} in the root cell walls and preventing Al^{3+} translocation from the roots to shoots in rice plants.

In addition, we examined the contents of calcium (Ca) in rice shoots and roots under Al stress. As shown in Fig. S3, exposure to Al stress caused an obvious reduction in Ca content by 30 and 34% in the shoots and roots, respectively, in comparison to control plants. However, under Al stress, exogenous GB treatment notably increased the Ca concentration by 25 and 28% in the shoots and roots, respectively, compared with Al-treated rice seedlings (Fig. S3A, B). This result suggested that the variable uptake and distribution of Ca^{2+} may also be involved in plant tolerance to Al stress.

Exogenous GB mediates the expression of Al uptake- and translocation-related genes and galacturonic acid content in rice roots under Al stress

To further explore the physiological mechanisms of GB in rice tolerance to Al stress, we analysed the expression of certain Al uptake- and translocation-related genes, including *OsALS1* (*aluminium sensitive 1*), *OsNrat1* (*rice Nramp aluminium transporter 1*), *OsSTAR1* (*sensitive to Al rhizotoxicity 1*) and *OsSTAR2* (*sensitive to Al rhizotoxicity 2*). As illustrated in Fig. 6, there was no obvious difference in the expression of these genes in all rice roots between the control plants and those treated with GB alone (Fig. 6).

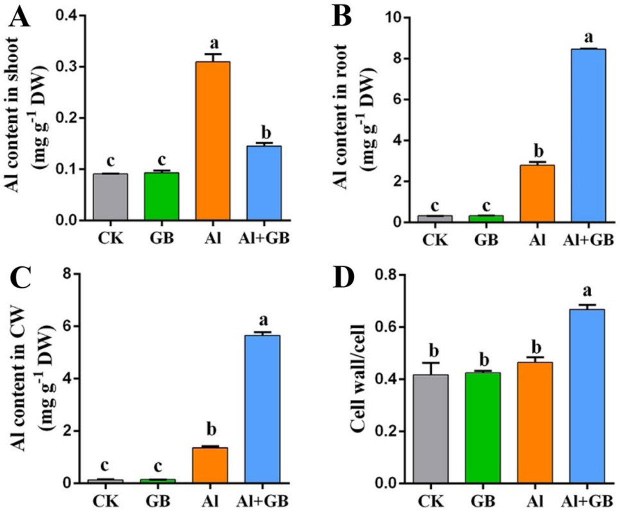

Fig. 5 Effects of glycinebetaine on aluminium contents in different parts of rice plants under aluminium stress. **a** Aluminium contents in the shoots. **b** Aluminium contents in the roots. **c** Aluminium contents in the root cell walls. **d** The ratio of aluminium content in the cell wall to that in the roots. *CK* control check. GB: 5 mM GB treatment. Al: 500 μM $AlCl_3$ treatment. Al + GB: 500 μM $AlCl_3$ + 5 mM GB treatment. DW: Dry weight. Different letters indicate significant differences between different treatments at $P < 0.05$

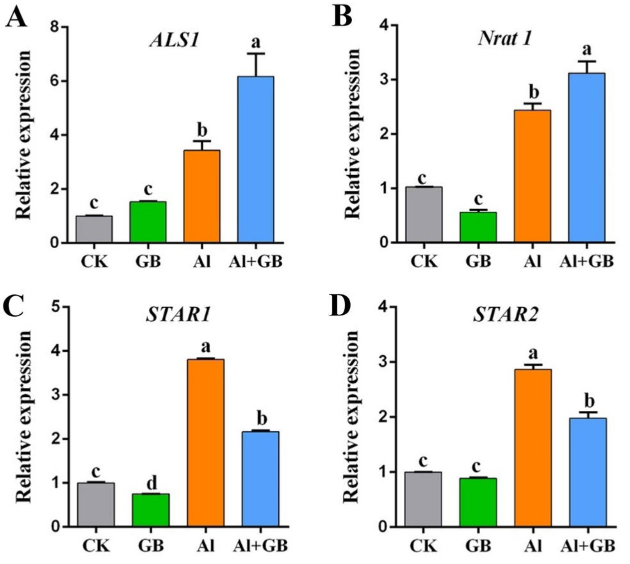

Fig. 6 Effects of glycinebetaine on the expression of aluminium uptake- and translocation-related genes under aluminium stress. **a** *Aluminium sensitive 1*, *OsALS1*. **b** *Nramp aluminium transporter 1*, *OsNrat1*. **c** *Sensitive to Al rhizotoxicity 1*, *OsSTAR1*. **d** *Sensitive to Al rhizotoxicity 2*, *OsSTAR2*. CK: control check. GB: 5 mM GB treatment. Al: 500 μM $AlCl_3$ treatment. Al + GB: 500 μM $AlCl_3$ + 5 mM GB treatment. Different letters indicate significant differences between different treatments at $P < 0.05$

However, exposure to Al stress caused an obvious increase in the expression of these genes by 3.8-, 2.6-, 3.8- and 2.9-fold, respectively, compared with control plants. Interestingly, GB-treated Al-stressed roots showed 79% and 27% higher expression levels of *OsALS1* and *OsNrat1* but 43% and 35% lower expression levels of *OsSTAR1* and *OsSTAR2*, respectively, than the seedlings treated with Al alone (Fig. 6). These data indicate that GB may modulate the expression of certain genes related to Al uptake and translocation.

In addition, the pectin in the cell walls of dicots is mainly composed of galacturonic acid, while the galacturonic acid in monocots mainly exists in pectin and hemicellulose. To a certain extent, the content of galacturonic acid shows the ability of plants to absorb Al^{3+}. Herein, we found that there were no notable differences in galacturonic acid contents between the control and Al-treated plants, but Al + GB-treated roots showed a 43% higher galacturonic acid content than Al-treated rice plants (Fig. S4), which is perhaps a crucial reason for the higher Al accumulation in the cell walls of GB-treated rice roots under Al stress.

Discussion

It was previously reported that GB could enhance the tolerance of plants subjected to metal toxicity, such as Cd (Hossain et al. 2010; Duman et al. 2011; Lou et al. 2015), Pb (Bharwana et al. 2014), and Cr (Ali et al. 2015; Ahmad et al. 2020). However, to date, there have been no research reports on the effects of GB application on Al stress.

From our results, the growth, development and biomass of rice seedlings decreased significantly under Al stress (Fig. 1), which was in accordance with numerous studies showing that Al inhibits the growth and development of plants (Kochian et al. 2015; Zhang et al. 2018). Exogenous GB alleviated Al-induced growth inhibition, especially the growth of rice shoots (Fig. 1B, C). Interestingly, GB-treated plants had shorter roots under Al stress conditions (Fig. 1E), but there was no evident difference between the root fresh weights of the Al-treated and Al + GB-treated plants (Fig. 1D), which may be associated with the higher content of Al in rice roots (Fig. 5B). With the application of GB, Al accumulation significantly increased in the cell walls of the rice roots, showing a 4.3-fold increase compared with that in rice roots under Al stress (Fig. 5C). Exogenously applied GB to Al-stressed plants reduced the Al concentration by 48% in the shoots compared with Al stress alone (Fig. 5A), indicating that GB may mitigate the toxic effects of Al stress by absorbing and accumulating more Al^{3+} in the cell walls of the roots and preventing Al translocation from the roots to shoots in rice plants.

Previous studies have suggested that GB application might alleviate the deleterious effects of drought and salinity stress by increasing chlorophyll content (Zhao et al. 2007; Hu et al. 2012). In the present study, the Al-induced degradation of chlorophyll and carotenoids in rice seedlings was markedly reverted after GB treatment, and their contents were even greater than those of the control (Fig. S1), suggesting that GB application could positively affect photosynthetic pigments to increase photosynthesis and promote plant growth under Al stress. Furthermore, many studies have demonstrated that exogenous GB application could improve the photochemical properties and stability of PSII reaction centres (Yang and Lu 2005; Zhang and Yang 2019). From our results, we observed that Al stress resulted in an evident decrease in ΦPSII, ETR(II) and qP (Figs. 2, S2). More importantly, GB application alleviated the PSII photoinhibition induced by Al stress, and the values of ΦPSII, ETR(II) and qP were 26, 25, and 31% higher, respectively, than Al treatment alone (Figs. 2, S2). These results suggested that exogenous GB application could increase the excitation energy for the electron transport process during Al stress by holding more PSII reaction centres in an open state. Moreover, over excitation of PSII can lead to the formation of ROS, photodamage, and photoinhibition (Derks et al. 2015; Derks and Bruce 2018). In the pigment beds of light-harvesting complexes, NPQ is closely correlated with the excess energy that can be harmlessly dissipated in the form of heat (Yang and Lu 2005; Joliot and Johnson 2011; Sasi et al. 2018). Notably, a clear increase in NPQ was observed in rice leaves under Al stress, but GB application led to a smaller increase in NPQ in Al-stressed rice leaves (Fig. 2).

$O_2{\cdot}^-$, a lethal ROS, can result in the degradation of lipids and proteins in biomembranes; in addition, MDA content is usually regarded as an index of the damage or degradation of membranes (Li et al. 2014b; Hasanuzzaman et al. 2019). Our results showed that Al stress enhanced $O_2{\cdot}^-$ and MDA contents (Fig. 3A, B) (Yamamoto 2019), which was similar to other metals that can cause oxidative damage (Duman et al. 2011; Bharwana et al. 2014). However, exogenous GB application could moderate the destructive effects of Al stress in rice seedlings by decreasing the $O_2{\cdot}^-$ and MDA contents compared with Al-stressed plants (Fig. 3A, B). Furthermore, GB alleviated Al stress in rice by enhancing the activities of antioxidant enzymes, including SOD, POD, CAT and APX (Fig. 4), suggesting that the use of exogenous GB could significantly enhance the defence capacity to counteract Al-induced oxidative damage in rice plants.

Based on previous studies, GB, either synthesised in vivo in transgenic plants or applied exogenously, can induce and activate specific genes, especially those related to stress tolerance (Einset et al. 2007; Li et al. 2019; Zhang et al. 2019). The genes *OsALS1* and *OsNrat1* have been identified as important during Al^{3+} uptake and translocation; OsNrat1 is the plasma membrane–localised Nramp Al^{3+} uptake transporter, and OsALS1 is a vacuolar ABC

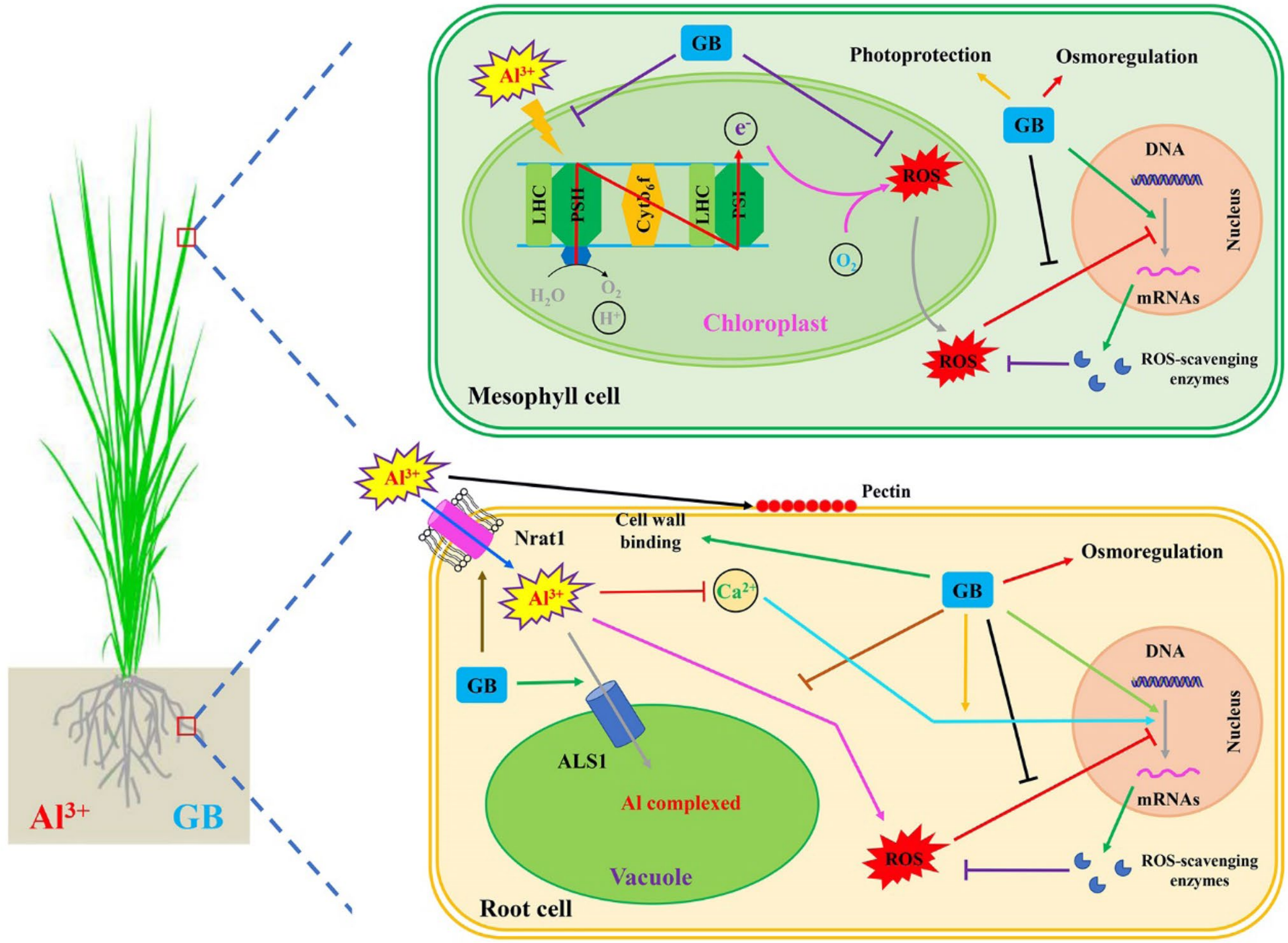

Fig. 7 A possible model for the mechanism of action of exogenous glycinebetaine treatment on aluminium tolerance in rice. Exogenous application of GB effectively alleviated the detrimental effects of Al stress on rice. In rice roots, GB enhanced the Al-binding capacity of the cell wall via the increased galacturonic acid content and the decreased expression levels of *OsSTAR1* and *OsSTAR2*. On the other hand, GB may sequester Al^{3+} in the root cell vacuoles by regulating the expression of *OsALS1* and *OsNrat1*, thereby mitigating Al toxicity in rice. In rice shoots, GB application increased the contents of chlorophyll and carotenoids, alleviated photosystem photoinhibition induced by Al stress and maintained PSII activity, which contributed to maintaining a higher photosynthetic rate under Al stress. Moreover, GB enhanced the elevated antioxidant enzyme activity and increase the proline and soluble protein contents to scavenge more $O_2\cdot^-$ and MDA, thereby improving the growth and development of rice plants

transporter that sequesters Al^{3+} in root cell vacuoles (Xia et al. 2010; Huang et al. 2012). In fact, the expression of *OsALS1* and *OsNrat1* was significantly induced after GB treatment under Al stress (Fig. 6A, B), indicating that GB may modulate Al^{3+} uptake and translocation. Moreover, an ATP-binding cassette (ABC) transporter complex in rice is encoded by *OsSTAR1* and *OsSTAR2* in response to Al tolerance, which seems to regulate UDP-glucose efflux into the cell wall, thereby leading to alterations in the cell wall components and a decrease in Al-binding ability (Huang et al. 2009; Kochian et al. 2015). From our results, the lower expression levels of *OsSTAR1* and *OsSTAR2* were consistent with the higher Al contents in the cell walls of rice roots after GB treatment under Al stress (Fig. 6C, D). In addition, the content of galacturonic acid shows the ability of plants to absorb Al^{3+} to a certain extent (Geng et al. 2017), and Al + GB-treated roots showed 43% higher galacturonic acid contents than rice plants treated with Al alone (Fig. S4). This is another important reason for the higher Al contents in the cell walls of GB-treated rice roots and the improved shoot development under Al stress. In addition, some studies have reported that Al stress causes changes in cytosolic Ca^{2+} activity (Jones et al. 1998a; b; Rengel and Zhang 2003), and Ca^{2+} may play a pivotal role in the mechanism of Al resistance (Konarska 2010; Sade et al. 2016). Interestingly, GB has a certain impact on Ca^{2+} absorption under abiotic stresses (Gobinathan et al. 2009; Li et al. 2014a). Herein, we found that GB application also caused obvious changes in the contents of Ca under Al stress (Fig. S3), suggesting that GB could enhance plant tolerance against Al stress by regulating the uptake and distribution of Ca^{2+}.

Finally, a putative model is proposed in Fig. 7. The exogenous application of GB effectively alleviated the detrimental effects of Al stress on rice. In rice roots, GB may enhance the Al-binding capacity of the cell wall via the increased contents of galacturonic acid and the lower expression levels of *OsSTAR1* and *OsSTAR2*; on the other hand, GB may sequester Al^{3+} in the root cell vacuoles by regulating the expression of *OsALS1* and *OsNrat1*, thereby mitigating Al toxicity in rice. In rice shoots, GB application increased the contents of chlorophyll and carotenoids, alleviated photosystem photoinhibition induced by Al stress and maintained PSII activity, which contributed to maintaining a higher photosynthetic rate under Al stress. Moreover, GB also enhanced the activity of antioxidant enzymes to scavenge more $O_2\cdot^-$, decrease MDA levels, increase the contents of proline and soluble protein, and regulate the uptake and distribution of Ca^{2+} under Al stress conditions, thereby improving the growth and development of rice plants. Therefore, exogenous GB application is a viable approach to effectively combat Al toxicity in crops.

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Author contribution statement XY and MB conceived and supervised the project, and XY, YL and TZ designed the experiments. TZ, WZ and DL performed most of the experiments. FZ, XC, CL and SY performed some of the experiments. TZ, WZ and XY analysed data and wrote the manuscript. MB and YL gave positive suggestion about this article. All authors read and approved the manuscript.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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