

Exogenous Glycinebetaine Mediated Tolerance Mechanism Regulates the Contrasting Responses to Drought and Flooding Stresses in Leaf Physiochemical Attributes and Growth of Maize

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Abstract

Background

Flooding and drought are the two most devastating natural hazards limiting maize production. Exogenous glycinebetaine (GB), an osmotic adjustment agent, has been extensively used but limits focus on its role in mitigating the negative effects of different abiotic stress. The study aims to identify the different roles of GB in regulating the diverse defense mechanisms of maize against drought and flooding.

Methods

Hybrids of Yindieyu 9 and Heyu 397 grown in pots in a ventilated greenhouse were subjected to flooding (2–3 cm standing layer) and drought (40–45% field capacity) at the three-leaf stage for 8 d. The effects of different concentrations of foliar GB (0, 0.5, 1.0, 5.0, 10.0 mM) on the physiochemical attributes and growth of maize were tested.

Results

Both varieties were verified to have greater drought tolerance to combat oxidative stress associated with higher antioxidant activities, and proline content than flooding. While flooding decreased superoxide dismutase, peroxidase activities, and proline content, those all declined with stress duration, leading to a larger reactive oxygen species than drought. Heyu 397 was verified to be more sensitive to drought and flooding than Yindieyu 9. It was peroxidase under drought and ascorbate peroxidase under flooding that played crucial roles in tolerating water stress. Foliar GB further enhanced antioxidant ability which contributed the most effect to peroxidase to eliminate more hydrogen peroxide than superoxide anion under water stress, promoting growth especially for leaf. Furthermore, exogenous GB made a greater increment on Heyu 397 than Yindieyu 9 as well as flooding than drought.

Conclusion

Overall, a GB concentration of 5.0 mM with a non-toxic effect on well-watered maize was determined to be optimal for the effective mitigation of water stress damage to the physiochemical characteristics and growth of maize.

Introduction

China was recently ranked among the top 10 countries most severely impacted by frequent and alternating drought and flooding, leading to immense economic losses (EM-DAT2020-2022) [1]. As per EM-DAT2022, the notable drought and flooding events resulted in a total economic loss of US\$

12.6 billion in China in 2022. The simultaneous occurrence of drought and flooding has increased with global climate change, at the same time, severely limiting crop growth and production in Guangxi [2, 3]. Drought and flooding have immense inhibitory effects on plants leading to a series of physiochemical and growth alterations [4, 5]. The most typical response of plants to water stress (WS) is the excessive accumulation of reactive oxygen species (ROS), which causes oxidative damage to plants, inhibiting their growth. Undoubtedly, crop growth slows down under WS, which is reflected by the reduction in leaf area (LA), plant height, stem diameter, and yield [6, 7]. To avoid or tolerate WS, crops will generate a series of certain physiological responses through self-regulation to adapt to stress. For instance, the activities of antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX), and the content of osmotic adjustment substances, such as proline and soluble sugars, are enhanced to cope with oxidative stress damages [8, 9]. However, there are considerable inconsistencies in the literature regarding the mechanisms employed by different crops' responses to drought and flooding. Previous research have reported that flooding has no significant effect on LA but greatly increases stem dry matter, SOD, and POD activities [10–12], On the contrary, other studies have shown that SOD, POD, and APX activities and LA drastically decreased under flooding [4, 10, 13]. Recently, a few studies have highlighted the distinct stress response and defense mechanism of maize (*Zea mays* L.) seedlings based on their physiological and biochemical response to drought and flooding [5, 14, 15]. However, it will be still necessary to clarify the antioxidant defense stress mechanism of physiochemical attributes and growth of maize seedlings when maize suffers from drought and flooding stresses at the same time.

Despite being a water-loving crop, maize is sensitive to WS [6, 16]. The water sensitivity and tolerance of plants to WS vary with the growth stage and stress duration. For maize, the intensity of the adverse impacts from flooding generally improves with the lengthening of stress duration but declines when the crop reaches various growth stages [4, 17]. When a maize seedling is exposed to flooding, its most sensitive stage to water is generated at the three-leaf stage (V3); seedlings at the V3 accumulate more hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) than those at the three-leaf stage (V5) [12]. Concurrently, flooding causes considerable reductions in the grain yield, dry matter content, and LA of maize [4, 7, 18]. While short-term WS generally limits crop growth, long-term WS damages plants to the point of no recovery [19, 20]. Therefore, maize seedlings at the V3 should be used as a study object for understanding their defense and ROS scavenging mechanisms under WS.

To alleviate stress damage and improve stress tolerance in plants, exogenous application of growth regulators has been widely adopted [21–23]. Glycinebetaine (GB), as a quaternary amine compound, is an osmolyte, which mainly is distributed in chloroplasts and cytoplasm [24] The GB acts as an osmotic adjustment substance and helps in enhancing the tolerance in crops against abiotic stress by increasing the antioxidant ability and protecting the biological membranes from oxidative stress caused by excess ROS [25–27]. The GB has been widely applied in multiple plants such as maize [28], wheat [29], tomato [30], and cotton [25] under various abiotic stresses because it is easily extracted and highly stable existed in plant tissues [31]. Many studies have shown that GB accumulation is positively correlated with the stress tolerance of plants, but the relationship will be changed with variety, concentration, and application

method [23, 32]. Maize leaves have a high ability to absorb and accumulate exogenous GB, and can immediately transfer GB from leaf to root [33], indicating that foliar application of GB will be an optimum strategy. The most effective and efficient concentration of GB for stress tolerance varies with plant variety [34, 35]. The low dosage of GB when it is sprayed on leaves greatly promotes plant growth under non-stress conditions; however, the high dosage can have an inhibitory effect on plants [36]. Even at high concentrations, inhibition is present but has no harmful effects on plants [28, 33]). Yang and Lu [33] have reported that dry weight and plant height increase with a concentration of GB up to 10 mM, while are inhibited by GB > 10 mM. An optimum dosage of GB (5 mM) significantly increases the gas exchange parameter of cotton [23]. For maize, different concentrations of GB are optimum for the foliar spray in different studies, including 0.5 mM [37], 1.0 mM [38], and 10 mM [39], implying that concentrations vary with treatments. Therefore, the optimum dosage of foliar GB needs to be determined for maize response to drought and flooding stresses.

Further research is still needed to fully understand the numerous defense mechanisms and stress tolerance of plants to drought and flooding as well as the possible involvement of exogenous GB in the defense process. Therefore, our main objectives in this study were to 1) detect the different stress defense and ROS scavenging mechanisms of maize seedlings when separately subjected to drought and flooding; 2) determine the optimum concentration of GB for foliar spray on maize seedlings at V3; and 3) determine the different effects of GB on water-tolerant or -sensitive varieties of maize under drought and flooding. The results of this study will be crucial for promoting sustainable cultivation and management of maize.

Results

Glycinebetaine reduced the accumulation of reactive oxygen species under water stress

Without the influence of GB, prolonged WS and growth stage resulted in a marked increase in O_2^- content but a decrease in H_2O_2 content. (Fig. 1). After 8 d, the CK without GB had reduced H_2O_2 content by 25.52%, while increased O_2^- content by 47.99% compared with those after 4 d. Yindieyu 9 accumulated more H_2O_2 content and less O_2^- content under WS, but showed less increment ratio in H_2O_2 (65.00%) compared with Heyu 397 (140.83%) without GB. In addition, the H_2O_2 and O_2^- contents under flooding without GB were severally ~ 1.42 fold and ~ 1.15 fold as compared with those under drought after 8-d stress. The accumulation of ROS was significantly affected not only by WS but also by GB and the interaction between them ($P < 0.01$). The generation of ROS in all treatments was first attenuated and then rose with an increase in the concentration of GB. Although ROS in CK under high dosage of GB was increased, CK still exhibited lower levels of ROS compared with WS, in which the lowest H_2O_2 in CK from 4 d to 8 d was detected in GB1 to GB1 in Heyu 397 and GB1 to GB2 in Yindieyu 9, correspondingly for O_2^- was GB1 to GB1 and GB3 to GB2, indicating Heyu 397 under CK with low ROS required less GB than Yindieyu 9, and GB wasn't changed for total ROS with growth duration. Meanwhile, the ROS under WS was significantly reduced by GB ($P < 0.05$), and under the effect of GB, the ROS under flooding was still

higher than that under drought. The optimum dosage of GB for ROS after 8-d stress was detected in GB3. Meanwhile, more GB (~ 5.0 to 10.0 mM), especially for Heyu 397, was needed under the progressive WS to decrease O_2^- content. After 8 d, compared with GB0, GB promoted H_2O_2 content by 18.55%, -46.47%, and -31.57% under CK, flooding, and drought in variety Heyu 397, respectively, and correspondingly, by 11.34%, -11.72%, and -13.52% in variety Yindieyu 9. At the same time, there were increments in O_2^- content under CK, flooding, and drought in variety Heyu 397 by 0.74%, -12.21%, -5.15%, respectively, as well as in Yindieyu 9 by 11.32%, -12.05%, -1.84%, respectively. As a result, GB was more beneficial in attenuating the accumulation of ROS under flooding, especially for Heyu 397.

Gglycinebetaine was more beneficial in improving SOD activity under flooding stress

The interaction of either WS or variety with GB significantly affected SOD activity (Fig. 2, $P < 0.01$). Compared with that after 4 d, under CK, flooding and drought, the SOD activity without GB after 8 d in Heyu 397 was decreased by 13.24%, 2.24%, and 0.87%, respectively, and in Yindieyu 9 was increased by 1.16%, 27.21%, and -8.99%, respectively. When maize seedlings were exposed to WS, SOD activity exhibited a significant decrease under flooding, while an increase under drought, compared with CK. The GB played a significant role in improving SOD activity in all treatments ($P < 0.001$). After 8 d, under the effect of GB, drought still had a significantly higher SOD activity than flooding and CK ($P < 0.05$), while a lower SOD activity was under flooding compared with CK. The SOD activity first increased and then descended in response to the increase in concentration of GB. Where, the greatest increment in SOD activity was discovered in GB3 under flooding and drought in Heyu 397, GB3 (after 4 d), and GB2 (after 8 d) in Yindieyu 9, and GB2 in CK in both varieties. After 8 d, Yindieyu 9 exhibited a higher SOD activity than Heyu 397 under WS and CK, particularly under flooding, but the high SOD activity in Yindieyu 9 under drought was unaffected by GB. After 8 d, compared with GB0, under CK, flooding, and drought, SOD activity under the effect of GB increased in Heyu 397 by 11.24%, 14.21%, and 3.57%, respectively, and correspondingly, in Yindieyu 9 by 5.38%, 0.55%, and 4.51%, respectively. As a consequence, GB was more beneficial in improving the SOD activity of Heyu 397 under flooding.

Glycinebetaine enhanced the POD activity under water stress

From after 4 d to 8 d, in the absence of GB, the POD activities under CK, drought, and flooding declined in Heyu 397 by 31.97%, 31.27%, and 27.32%, respectively, and correspondingly, in Yindieyu 9 by 8.50%, 4.69%, 17.78%, respectively (Fig. 3). After 8 d, compared with CK in the absence of GB, WS greatly improved POD activity, especially drought stress that increased the POD activity by 22.62% in Heyu 397 and 25.30% in Yindieyu 9. After 8 d, Heyu 397 showed significantly higher POD activity under drought than under flooding and CK ($P < 0.05$), while Yindieyu 9 had no significant difference in the POD activity between drought and flooding ($P > 0.01$). Moreover, Yindieyu 9 had higher POD activity than Heyu 397 ($P < 0.05$). Foliar application of GB significantly increased POD activity no matter what the water conditions

applied ($P < 0.01$), where the rank for POD activity from high to low was drought, flooding, and CK in Heyu 397, and flooding, drought, and CK in Yindieyu 9 after 8 d. The POD activity was first significantly improved and then declined with the increase in the concentration of GB ($P < 0.01$), revealing that a high concentration of GB (GB4) inhibited the infinite increase of POD activity, but still improved the POD activity compared with CK. After 4 d, the highest POD activity was observed in GB3 in all treatments, except for GB2 in CK of Yindieyu 9. Nevertheless, after 8 d, the highest POD activity was observed in GB2 under CK and drought, in GB4 under flooding in Heyu 397, and correspondingly in GB1 under CK and drought, and GB3 under flooding in Yindieyu 9. After 8 d, compared with GB0, the POD activities under CK, drought, and flooding were increased by the foliar GB by 24.02%, 32.56%, 29.64% in Heyu 397, respectively, and by 14.62%, 27.58%, and 6.25% in Yindieyu 9, respectively. Results indicated that GB contributed more POD activity towards flooding as well as Heyu 397 than Yindieyu 9.

Glycinebetaine improved the APX activity under water stress

Prolonged WS and growth stage induced an obvious rise in APX activity (Fig. 4). Compared with after 4 d, APX activities after 8-d stress under CK, drought, and flooding were increased by 36.21%, 45.19%, and 57.74% in Heyu 397, respectively, and 63.20%, 56.97%, and 51.29% in Yindieyu 9, respectively. Yindieyu 9 accumulated higher APX activity under flooding relative to drought, in the absence of GB, after 8 d. In contrast to Yindieyu 9, Heyu 397 displayed lower APX activity under WS and CK and maintained higher APX activity under drought than under flooding. Nevertheless, there was no significant difference between drought and flooding treatments in APX for plants ($P > 0.05$). Water stress and GB significantly affected APX activity ($P < 0.01$), but the interaction between the two factors had no obvious effect on APX activity ($P > 0.05$). Throughout the whole period of treatment for Heyu 397, the highest APX activity was found in GB3 which was similar to that in GB2 under CK, and in GB3 under WS, those all were significantly higher than in other concentrations of GB ($P < 0.05$). Meanwhile, in Yindieyu 9, the highest APX activity was detected in GB1 under CK and in GB3 under WS, but there was no significant difference between treatments with GB after 8 d ($P > 0.05$). It was also revealed that Yindieyu 9 under the effect of GB still had significantly higher APX activity than Heyu 397 after 8 d ($P < 0.01$). Although a low dosage of GB was suitable for CK, high GB still improved the APX activity compared with the treatments without GB, and so did under WS. After 8 d, compared with GB0, GB was more beneficial to improving APX activity under flooding in Heyu 397 which about a 10.53% increment, and under drought in Yindieyu 9 by 13.33%.

Lower dosage of GB increased the proline content under water stress

Proline content steadily declined with the progression of WS and growth. Proline content of Yindieyu 9 showed a higher reduction from after 4 d to 8 d compared with Heyu 397. Furthermore, proline content without GB after 8-d stress significantly decreased under flooding and significantly increased under drought (~7-fold as compared with CK) (Table 1, $P < 0.01$). Yindieyu 9 accumulated less proline content than Heyu 397 in the absence of GB. Although GB significantly promoted the proline content under WS ($P < 0.01$), high concentrations of GB had adverse effects on maize seedlings under CK, because proline

content declined under CK after 8 d. With prolonged WS, maize's growth needed more and more GB to pronounce more proline. There was no significant difference in proline content induced by GB between flooding and CK ($P > 0.05$), which were all lower than drought. Proline content promoted by GB was significantly higher in Heyu 397 in comparison with Yindieyu 9. The proline content was enhanced most by GB2 under CK, GB1 under flooding, and GB3 under drought in Heyu 397, correspondingly by GB2, GB2, and GB1 in Yindieyu 9 after 4-d stress. After 8 d, the highest increment in proline content under WS at the effect of GB was detected in GB3, of which the average increment was 11.74% under flooding 136.95% under drought in Heyu 397, and 11.93% under flooding 99.68% under drought in Yindieyu 9. Consequently, GB contributed more proline content towards Heyu 397, especially for treatments under drought.

Table 1

The effect of exogenous glycinebetaine (GB) on the proline content ($\mu\text{g/g}$ FW) of maize seedlings under water stress.

Water stress/ GB (mM)	After 4 d of treatment		After 8 d of treatment		
	Heyu 397	Yindieyu 9	Heyu 397	Yindieyu 9	
CK	0	514.03 \pm 53.28d	516.97 \pm 32.46b	433.06 \pm 48.68a	351.74 \pm 59.51a
	0.5	773.46 \pm 13.20bc	484.00 \pm 29.75b	312.15 \pm 19.12bc	264.64 \pm 39.26b
	1.0	914.96 \pm 5.76a	658.77 \pm 22.34a	333.71 \pm 10.26b	257.60 \pm 31.10b
	5.0	731.70 \pm 20.56c	636.56 \pm 49.11a	286.30 \pm 2.85c	263.65 \pm 35.82b
	10.0	805.06 \pm 52.51b	496.00 \pm 15.93b	285.09 \pm 11.78c	218.50 \pm 22.50b
Flooding	0	450.08 \pm 51.47b	483.93 \pm 2.55c	315.87 \pm 44.52bc	271.27 \pm 1.07b
	0.5	765.04 \pm 90.03a	310.40 \pm 24.91d	289.94 \pm 14.43c	290.44 \pm 28.11b
	1.0	408.71 \pm 5.24b	868.75 \pm 49.61a	353.37 \pm 17.67b	285.66 \pm 2.24b
	5.0	359.11 \pm 23.79bc	567.70 \pm 44.73b	424.42 \pm 3.39a	333.84 \pm 8.23a
	10.0	296.97 \pm 51.38c	495.31 \pm 49.33c	344.03 \pm 46.18bc	295.52 \pm 15.18b
Drought	0	1545.92 \pm 175.38d	1472.65 \pm 13.69c	1128.34 \pm 42.66d	1109.00 \pm 28.02e
	0.5	1661.98 \pm 37.12cd	2875.10 \pm 65.66a	2477.36 \pm 225.42b	1963.93 \pm 35.93c
	1.0	1863.65 \pm 13.49c	1367.66 \pm 20.64d	2450.63 \pm 41.92b	2378.21 \pm 72.96b
	5.0	2708.21 \pm 108.50a	1729.74 \pm 74.63b	3904.41 \pm 33.75a	2694.72 \pm 58.82a
	10.0	2240.05 \pm 195.17b	1244.03 \pm 30.90e	1861.99 \pm 12.24c	1821.16 \pm 70.37d
Source of Variation	<i>P</i> > <i>F</i>				
Variety	< 0.0001		< 0.0001		
Water	< 0.0001		< 0.0001		
GB	< 0.0001		< 0.0001		
Variety \times Water	< 0.0001		< 0.0001		
Variety \times GB	< 0.0001		< 0.0001		

CK represents crops planted in the well-watered condition. FW is the fresh weight. Data are shown as the means \pm standard deviation ($n = 3$, biological replicates). Different letters in a water treatment indicate the least significant differences according to P value ≤ 0.05

Water stress/ GB (mM)	After 4 d of treatment		After 8 d of treatment	
	Heyu 397	Yindieyu 9	Heyu 397	Yindieyu 9
Water × GB	< 0.0001		< 0.0001	
Variety × Water × GB	< 0.0001		< 0.0001	

CK represents crops planted in the well-watered condition. FW is the fresh weight. Data are shown as the means ± standard deviation ($n = 3$, biological replicates). Different letters in a water treatment indicate the least significant differences according to P value ≤ 0.05

Glycinebetaine promoted the growth of maize seedlings under water stress

From after 4 d to 8 d, LA, and stem diameter in the absence of GB under flooding existed more increment than under drought, while plant height showed the exact opposite trend. Although there was no difference in the average increment between Yindieyu 9 and Heyu 7 under drought, a higher average increment occurred under flooding in Yindieyu 9 than in Heyu 397. Meanwhile, after 8 d, maize growth was significantly suppressed when subjected to drought in the absence of GB, resulting in smaller LA, thinner stem diameter, and shorter plant height compared with CK (Fig. 5, $P < 0.05$). By contrast, maize under flooding exhibited a thicker stem diameter, shorter plant height, and smaller LA than CK after 8 d. Although the difference in plant height between drought and flooding was not statistically significant, flooding resulted in higher plant height, stem diameter, and LA than drought in the absence of GB after 8 d ($P > 0.05$). Meanwhile, Yindieyu 9 maintained a significantly larger stem diameter and LA compared with Heyu 397 ($P < 0.05$). Foliar application of GB significantly mitigated the adverse effects of WS on maize growth, subsequently promoting crop growth ($P < 0.05$). With the progression of growth, GB played a more and more effect on LA and plant height, and less effect on stem diameter. However, the difference of morphological indicators affected by GB in WS and variety was similar to that without GB after 8 d. The morphological indicators were first increased and then reduced with an increase in GB concentration in all treatments. However, under the effect of GB, all morphological indicators were higher than those in GB0. After 8 d, to promote growth to survive from WS, Yindieyu 9 (GB2) required less GB than Heyu 397 (GB3) to improve stem diameter, while there was similar and optimum dosage of GB that was GB3 required between Yindieyu 9 and Heyu 397 to promote LA and plant height. Under CK, the optimum concentration for maize growth was GB3 after four times foliar application of GB, and other concentrations still maintained higher morphological indicators in comparison with GB0. The GB had the greatest effect on LA, followed by plant height, lowest on stem diameter. Where GB was more beneficial to increasing LA in Heyu 397 under flooding.

Glycinebetaine increased the biomass accumulation of maize seedlings under water stress

Water stress significantly slowed down the overall growth of maize seedlings, particularly in terms of leaf dry matter compared with CK (Fig. 6, $P < 0.05$). From 4 d to 8 d, under CK, drought, and flooding in the absence of GB, the total dry matter had grown by 78.18%, 19.81%, and 28.76% in Heyu 397, respectively, and 79.46%, 49.93%, 38.55% in Yindieyu 9, respectively, in which leaf dry matter was much more severely reduced by WS especially flooding. Additionally, stem dry matter under flooding in the absence of GB was reduced by 17.65% in Heyu 397 and 4.17% in Yindieyu 9 after 8 d, although not statistically significant between flooding and CK ($P > 0.05$). Drought also caused a significant reduction in leaf dry matter compared with CK ($P < 0.05$), which was also lower than flooding. Meanwhile, higher stem biomass was also observed under flooding rather than drought, with no significant difference ($P > 0.05$). Similarly, Heyu 397 showed significantly lower total biomass than Yindieyu 9 under flooding ($P < 0.05$), and no statistically significant compared with Yindieyu 9 under drought. The adverse effects of WS on the biomass accumulation of maize seedlings were significantly and steadily reduced with the increase of GB concentration ($P < 0.05$). The most significant increment in biomass accumulation was observed in GB3 after 4 d, while in GB3 in Heyu 397, and GB2 in Yindieyu 9 after 8 d. Although there was no adverse effect of GB on biomass accumulation of maize seedlings in all treatments, the higher dosage of GB had an inhibitory effect on biomass accumulation. Additionally, GB had contributed more biomass accumulation towards Heyu 397 than Yindieyu 9 especially in terms of leaf dry matter, which showed an increment of 21.95% under drought and 42.71% under flooding in Heyu 397. It also led to a similar dry matter between drought and flooding in Heyu 397, which was significantly lower than Yindieyu 9.

Glycinebetaine improved leaf relative water content under drought stress

Compared with CK, leaf relative water content (RWC) was not significantly altered under flooding ($P > 0.05$), but was significantly reduced during drought (Table 2, $P < 0.01$). Under drought without GB, the RWC of Heyu 397 with a decrement of 28.67% was significantly lower than that of Yindieyu 9 ($P < 0.05$). Foliar application of GB significantly increased RWC under drought by 22.90% in Heyu 397 and 13.23% in Yindieyu 9 ($P < 0.05$) but had no apparent impact on RWC under flooding and CK ($P > 0.05$). The most suitable concentration for plants grown under drought was GB3. Whereas, the higher dosage of GB had inhibitory but non-toxic effects on the RWC of maize seedlings.

Table 2

The effect of glycinebetaine (GB) on leaf relative water content (g/g, %) of maize seedlings after eight days of water stress.

Treatments		Heyu 397	Yindieyu 9
Concentration (mM)			
CK	0	95.39 ± 1.98a	97.12 ± 0.35ab
	0.5	97.48 ± 0.21a	96.59 ± 1.60ab
	1.0	96.80 ± 1.25a	98.35 ± 1.45a
	5.0	96.72 ± 1.59a	96.16 ± 0.79b
	10.0	96.71 ± 1.89a	95.65 ± 0.34b
Flooding	0	97.13 ± 0.67ab	95.84 ± 1.94a
	0.5	95.18 ± 1.80b	95.79 ± 0.59a
	1.0	97.44 ± 0.52a	96.35 ± 0.58a
	5.0	95.75 ± 0.41ab	97.23 ± 0.72a
	10.0	95.44 ± 1.43ab	97.19 ± 0.63a
Drought	0	68.04 ± 1.73c	71.50 ± 0.53c
	0.5	80.12 ± 1.64b	78.26 ± 2.66bc
	1.0	87.54 ± 0.69a	80.94 ± 0.57ab
	5.0	87.10 ± 4.45a	82.61 ± 2.78a
	10.0	79.76 ± 1.86b	82.04 ± 3.62ab
Source of Variation		<i>P</i> > <i>F</i>	
Variety		0.3565	
Water		< 0.0001	
GB		< 0.0001	
Variety × Water		0.0987	
Variety × GB		0.0188	
Water × GB		< 0.0001	
Variety × Water × GB		0.0005	
<p>CK represents crops planted in the well-watered condition. Data are shown as the mean ± standard deviation (<i>n</i> = 3, biological replicates). Different letters in a water treatment indicate the least significant differences according to <i>P</i> value ≤ 0.05</p>			

Analysis for correlation, stepwise regression, and tolerance

The correlation analysis revealed that the correlations among most of the indicators under WS were similar to those in CK (Fig. 7). However, SOD activity under flooding and proline content under drought showed the opposite correlation with some other indicators compared with CK, whereby either SOD activity under flooding or proline content under drought was positively correlated with morphological parameters and biomass. Additionally, POD activity significantly and positively correlated with SOD activity ($P < 0.05$). Furthermore, in all treatments, APX activity showed a significant positive correlation with morphological parameters and biomass ($P < 0.05$). Leaf area, plant height, stem diameter, and dry matter were significantly and positively correlated with each other ($P < 0.05$). It was APX activity under flooding, POD under drought, and plant height under both stresses that screened out by the stepwise regression analysis were the critical and representative indicators for leaf dry matter, which could determine a relatively high mean forecast accuracy of more than 80% (Table 3). Finally, D value, as an important indicator to evaluate water tolerance, was detected to be the highest in CK, followed by drought, and flooding (Table 4). The tolerance coefficient in a variety was more than 1.0 under drought, while lower than 1.0 under flooding. Yindieyu 9 had a higher tolerance to flooding and drought than Heyu 397, and its tolerance to flooding was ~ 3.17 fold as compared with Heyu 397 based on the D value.

Table 3

The stepwise regression analysis for eight indicators with leaf dry matter (LDM).

Water stress	Models	R^2	F	$P > F$
Flooding	$LDM_1 = -0.858 + 0.071X_{APX} + 0.014X_{PH}$	0.91	89.03	< 0.001
Drought	$LDM_2 = -0.504 - 0.051X_{POD} + 0.022X_{PH}$	0.80	32.35	< 0.001
POD, APX, and PH represent peroxidase, ascorbate peroxidase, and plant height.				

Table 4

Analysis for tolerance after 8 d based on comprehensive evaluation (D) value and tolerance coefficient.

Varieties	Water Stress	D value	Tolerance coefficient
Heyu 397	CK	0.53	-
	Flooding	0.10	0.82
	Drought	0.48	1.07
Yindieyu 9	CK	0.59	-
	Flooding	0.40	0.93
	Drought	0.50	1.12
CK represents crops planted in the well-watered condition.			

Discussion

Global climate change has resulted in an accelerating number of intense alternating drought and flooding incidents, which are extremely expensive in terms of crop production loss [40, 41]. It is also a major factor limiting maize production in the subtropical region of Guangxi, China. Few previous studies have compared the differences and similarities in responses of different maize genotypes under flooding and drought stresses. It is urgent to understand the adaptive mechanism and search for effective techniques to avoid or mitigate the damaging effect of both drought and flooding on maize growth in the area. When maize suffers from WS, its physiology and morphology occur a series of changes due to damage [7, 42], which the most typical damage for plants is oxidative damage [13]. Overproduction of ROS during water scarcity induces significant levels of oxidative stress in plants, damaging the cell membrane system, causing membrane lipid peroxidation, and either inhibiting plant growth or causing plant death [43, 44]. The study also found that a significant amount of ROS buildup occurred when plants were under WS; however, regardless of the WS or not, plant development duration increased O_2^- and declined H_2O_2 accumulation, which was similar to previous studies [12, 45]. Maize seedlings are more sensitive to V3 than V5 or V6, which has accumulated a higher ROS at V3 than V5 [12, 17]. By contrast, others have reported that ROS gradually improves with growth stage and stress duration [20, 46]. The distinct different responses of maize seedlings may result from a self-regulation compensation mechanism of different genotypes to enable plants to tolerate the damage from WS or the environment [47]. To cope with the stress to ensure survival, plants have developed an antioxidant defense system to scavenge ROS. From after 4 d to 8 d growth, maize seedlings reached a status of V5 stage, whose SOD and POD activities declined, while APX activity increased with the lengthening of WS or growth as were consistent with previous reports [48, 49]. A low SOD activity led the plants to convert less O_2^- to H_2O_2 . Meanwhile, although a low POD activity was detected, high APX has been identified as a key antioxidant enzyme to contribute most tolerance to WS [3, 43] as well as stress duration, which could still convert more H_2O_2 to oxygen. Eventually, more O_2^- , less H_2O_2 , accompanied by a higher increment of O_2^- than the reduction of H_2O_2 were accumulated with the progression of WS in the study same as the previous study [46]. Therefore, a decrease in proline which also acts as an amino acid in scavenging ROS [50, 51], accompanied by a reduction of SOD and POD activities in the lengthening of WS or growth, caused an excessive accumulation of ROS mainly focused on O_2^- that produced an even more destruction for plasmalemma than H_2O_2 [52].

Drought and flooding are two of the key environmental factors that cause varying degrees of oxidative damage to maize growth. Maize exposed to drought in the absence of GB before CK existed a more significant increment in the antioxidative enzyme of SOD, POD, and APX activities and proline content. By contrast, flooding just increased POD and APX activities which accelerated the conversion of H_2O_2 , while decreasing SOD activity and proline content compared with CK. Azahar et al. [13] also have reported that maize emerges a significant decrement in the activities of SOD and other antioxidant enzymes involved in the ascorbate-glutathione (AsA-GSH) cycle, causing a deregulation of the ROS scavenging system when maize suffered from flooding. Consequently, flooding deregulated ROS scavenging machinery

accompanied by a numerous accumulation of ROS associated with lower SOD and POD activities and proline content and ultimately reduced the tolerance of maize showing lower D value compared with drought. In addition, it was also further confirmed that it was APX under flooding and POD under drought screened out by a stepwise regression analysis that played the most key role in maize to combat the WS damages in the study same as proposed by prior studies [3, 43, 45]. Moreover, APX activity was significantly positively correlated with morphological parameters and biomass in all treatments, also revealing its importance in resisting WS. The aboveground plant parts exhibit growth inhibition under WS, and gradually undergo a series of morphological structure changes to adapt to WS [53]. In the study, it was found that four days of short flooding caused less damage to plant growth than drought, as indicated by the higher LA and stem diameter during flooding compared with those under drought. Particularly, the highest stem diameter occurred in flooding in the study, which has been confirmed to be one of the important elements that prevent plants from lodging to adoptive growth under flooding stress [17, 54]. Although flooding had higher LA than drought, leaf dry matter showed no significant difference between flooding and drought due to the higher RWC under flooding. Even though maize seedlings kept lower oxidation tolerance to flooding, short-term flooding of four days gave rise to a lighter effect on the leaf growth than drought. The flooding duration is probably short enough to avoid noticeable damage to root and electron transfer, short-term flooding can promote the photosynthetic rate of leaves and the uptake of nutrients by plant roots [55, 56]. Certainly, on the other hand, long-term flooding of eight days generated serious damage to growth to decline the increment of morphological characteristics and biomass compared with normal growth. The lowest RWC observed under drought in the study, which has been previously proved to restrict stomatal conductance to inhibit photosynthetic rate [53], and thus suppressed the growth in the study leading to a decrease in morphology characteristic and biomass. Overall, Maize growing under flooding mainly relies on POD and APX activities to scavenge ROS and maintain a strong stem to and adapt to stress, showing a weaker tolerance than under drought, while under drought mainly depends on antioxidative enzyme activities together with proline to eliminate ROS and resist stress for survival. However, maize adoptive growth depends not only on SOD, POD, APX activities, and proline content but also on other enzymes in the AsA-GSH cycle and osmotic adjustment substances [12, 13] as it is a comprehensive and complex regulatory mechanism for plants to combat WS.

However, the response of maize seedlings to drought and flooding had been slightly changed with genotypes. Yindieyu 9 before Heyu 397 had been identified to be more tolerant to both drought and flooding, but both varieties demonstrated a greater capacity to drought than flooding based on the D value (a comprehensive performance for all indicators) and tolerance coefficient. The tolerant variety demonstrated a greater antioxidant capacity, and higher, morphological characteristics (except plant height) and dry matter than the sensitive variety. Previous studies had reported that a tolerant cultivar to drought is also tolerant to waterlogging [14, 57], which was also verified in Yindieyu 9. With the lengthening of stage or stress, a distinct response for variety was revealed that Yindieyu 9 had a higher average increment of SOD, POD, and APX activities, morphological characteristics, and dry matter under WS, especially for flooding in comparison with Heyu 397. Heyu 397 possessed a higher antioxidant

activities and dry matter under drought relative to flooding, even though it was a sensitive genotype to drought and flooding. It may also be found that APX activity with the highest increment appears to be an important indicator of maize to withstand and survive stress duration. Therefore, water-tolerant variety not only had a high ability to suffer from serious stress but also long-term stress.

Glycinebetaine functions as an osmotic adjustment substance and plays an important role in protecting the structure and function of proteins, enzymes, and cell membranes, thus enabling the plants to defend themselves against WS-induced oxidative damage [11, 58]. Limit research has provided us limiting understanding of the differential role GB plays in the defense against drought and flooding stresses, even though GB has been applied in many plants under abiotic stress [22, 23, 39]. Therefore, the study was carried out to focus on the role of GB in various susceptible genotypes under either drought or flooding. The study revealed that whether under WS or not, foliar application of GB significantly increased SOD, POD, and APX activities, thereby enhancing the antioxidant capacity of maize in all treatments. Where, the GB contributed the most to the POD activity with the highest increment among the antioxidants for maize, which enhanced the crucial role in H_2O_2 scavenging system. The result of stepwise regression analysis also identified the result that POD activity under drought was screened out to be an efficient indicator for leaf dry matter. In addition, it is revealed that exogenous GB significantly increased proline content which mainly plays an important role in osmoregulation in plants under drought stress [27]. It is also verified by the correlation analysis that proline was positively correlated with morphological parameters and biomass under drought, while negatively correlated under CK and flooding conditions. The possible reason may be that the lower proline content under flooding and CK has less effect on the growth of maize, but GB can improve proline content and then reinforce the role of proline in flooding. Previous studies have also reported a similar increase induced by GB in antioxidant activity and osmolyte accumulation [11, 29]. The GB collaborated with the antioxidant activity and osmolyte accumulation in the leaves alleviating the oxidative damage caused by excess accumulation of ROS under WS. In the current study, the average contents of H_2O_2 and O_2^- were severely decreased by 25.82% and 7.81% under the function of foliar application of GB under WS. It is also indicated that exogenous GB was more beneficial to scavenging excess H_2O_2 under WS, which was associated with the essential role of POD activity induced by GB as suggested above. In addition, exogenous GB significantly improved leaf RWC under drought which was consistent with previous results [26, 36], but did not affect RWC under flooding as flooding induced no significant difference in RWC relative to the CK. High levels of antioxidant activity, osmolyte accumulation, and RWC (drought), and a low level of ROS accumulation under WS-induced foliar-GB promoted maize growth. Thereby, the LA, plant height, stem diameter, and dry matter for leaves and stems under WS or not had been improved under the effect of GB mediating with antioxidants and proline. It was also revealed that GB contributed the most effect on LA with the highest increment relative to other morphological characteristics, followed by plant height, resulting in the most effect on dry matter for leaf rather than stem. The plant height was also screened out as an efficient indicator for leaf dry matter under WS as other reports [3, 59].

The effect of exogenous GB on plants greatly varied according to crop cultivar, stress duration, stress type, and GB concentration, which was consistent with the previous findings [23, 26]. Despite previous studies that have reported the different roles of GB taking in tolerant cultivars and sensitive cultivars [26], the role is mainly researched based on the same stress. The reports regarding the different effects of foliar-applied GB on different tolerant cultivars to drought and flooding stresses have been edlimited reported so far. When the exogenous GB was sprayed to leaves after 8 d, maize still maintained higher oxidation tolerance to drought associated with higher SOD, POD, and APX activities, proline content, and ROS accumulation, accompanied by no statistical difference in morphological characteristics and dry matter compared with flooding, revealing that although GB couldn't change the trend of each index, could shorten the difference induced by drought and flooding. Nevertheless, the deleterious effect of flooding on maize rather than drought and CK was more mitigated by foliar GB. Heyu 397 was identified to be more sensitive to WS especially to flooding than Yindieyu 9, which was more enhanced by GB similar to the previous study [26]. Regardless of the variety, under WS and CK, antioxidant activities, proline (except CK), morphological characteristics, RWC, and biomass accumulation were all first increased and then reduced with the increase in the concentration of exogenous GB. Nevertheless, an excessive dosage of exogenous GB which reached 10 mM in the study exhibited inhibitory but non-toxic effects on maize seedlings. A high concentration of GB under WS or not still promoted the physiological and biochemical characteristics and growth of maize when compared with the treatment in CK without GB. Previous studies also demonstrated that excess dosage of GB has overwhelmed the beneficial effect as well as the non-toxic effect of GB on the growth characteristics [28, 33, 35]. Besides, less dosage of exogenous GB was generally needed for the water-tolerant variety Yindieyu 9 relative to the sensitive variety Heyu 397 under flooding accompanied with the lengthening of stress duration, which was closely associated with the strong flooding tolerance of Yindieyu 9. In contrast, the longer the stress duration, the more GB was required to meet Heyu 397 to survive from WS than Yindieyu 9, but just only small dosage of GB (GB2) could promote the growth under CK. The most effective and efficient dosage of GB was determined to be 5.0 mM under WS, which also greatly improved the growth of maize grown in CK.

Conclusion

Maize seedlings exhibited greater drought tolerance owing to higher antioxidant activities and proline content, and lower reactive oxygen species in comparison with flooding. It might be the decrease of superoxide dismutase activity and proline content that led to a larger accumulation of reactive oxygen species under flooding. The different responses to drought and flooding would be slightly changed by genotypes and stress duration. Specifically, peroxidase under drought and ascorbate peroxidase under flooding were significant variables for the accumulation of leaf dry matter. Foliar application of GB played an important role in improving tolerance, and promoting growth in all treatments, where, it contributed the most effect to POD and leaf growth to eliminate more hydrogen peroxide than superoxide anion. Furthermore, GB had a greater impact on flooding as well as the water-sensitive variety Heyu 397 than the water-tolerant variety Yindieyu 9 under drought, which could shorten the difference induced by drought and flooding. More and more GB was required in Heyu 397 to protect or survive from damage of

water stress, while less GB was needed for maize planted in well-watered conditions with growth stage. In general, irrespective of the maize variety, 5.0 mM emerged as the most effective and efficient dosage of GB under stress and also promoted maize growth in well-watered conditions. Overall, this study enhances the understanding of the contrasting defense mechanisms for alleviating oxidative damage in response to drought vs. flooding stresses and highlights the foliar application of GB as a valuable and effective strategy for alleviating water stress-induced damage to the physiochemical attributes and growth of maize.

Materials and Methods

Plant materials, and experimental design

The experiment was set up in a ventilated greenhouse at Guangxi University, an experimental station in Nanning, Guangxi, China (22°50'N, 108°17'E). The annual average of precipitation is 1599.0 mm, temperature is 20.5°C, and relative humidity is 79.1% in 30 years in the region, and the prevailing climate is humid subtropical monsoon climate.

Two maize (*Zea mays* L.) hybrids of Yindieyu 9 (tolerant to drought and flooding) and Heyu 397 (sensitive to drought and flooding) based on a previous study [3] designed as the main plot as planted in plastic pots (32.5 cm diameter, and 29.0 cm height) on 28th September 2020. The pots were arranged in a split-split plot design with three replications and placed in 60 cm row spacing and 32.5 cm center distance of pot. Ten seeds were planted per pot, and five seedlings per pot were thinned at the two-leaf stage. During the early growth period of the maize seedlings, the soil water content was maintained at the normal field capacity (FC) of 70–75%. The seedlings at V3 were imposed with progressive drought (40–45% FC), and flooding (2.0–3.0 cm standing layer) stresses for 8 d, additionally, normal FC was set as a control (CK), and those were designed as the split-plot. The soil moisture content for CK and drought stress was controlled based on daily measurements of pot weight, in which each pot weight was controlled between 9.59–9.72 kg (70–75%FC) for CK and 8.88–9.00 kg for drought stress (40–45% FC), the details showed in Fig. 8A. For flooding stress, the water level was maintained using an additional big pot without holes placed with each pot. Different concentrations of GB with 0, 0.5, 1.0, 5.0, 10.0 mM, designated hereafter as GB0, GB1, GB2, GB3, GB4, respectively, selected based on previous studies [23, 37, 38, 39] designed as a split-split plot, was sprayed using a 500 mL watering pot to each pot with 25.0 mL after 1, 3, 5 and 7 days of stress (Fig. 8B). After 4 d and 8 d of treatments, the indicators were measured, and all fully developed leaves per pot were sampled and stored at -80°C until further analysis for ROS, antioxidant enzyme activities and proline content (Fig. 8B).

Each plastic pot was filled with 8.0 kg of dry soil (soil water content of 3.79%) from arable topsoil mixed with 4.49 g urea (46.2% N), 1.38 g muriate of potash (60.0% K₂O), and 4.61 g calcium magnesium phosphate (18.0% P₂O₅). The arable topsoil was sandy clay loam (53.27% sand, 20.65% clay, and 26.28% silt, World Reference Base) with 31.21% FC (g/g, %), pH 6.83, soil bulk density of 1.24 g/cm³, soil organic

matter of 24.14 g/kg, and available nitrogen of 0.12 g/kg, available phosphorus of 22.10 mg/kg, and available potassium of 69.50 mg/kg.

Sampling and Measurements

Determination of reactive oxygen species

The extraction method was performed according to Wang et al. [3]. Briefly, 0.2 g fresh leaf was crushed at 4°C using a high-throughput cold grinding machine (Xinyi-48N, Ningbo Xinyi Ultrasonic Equipment Co., LTD, Zhejiang, China). Then, 2.0 mL pre-chilled phosphate buffer solution (PBS, 50 mM, pH 7.8) containing 1.0% polyvinyl pyrrolidone was added to the homogenate, and fully oscillated. Subsequently, the homogenate was centrifuged at 12,000 × g for 15 min at 4°C, and the supernatant was ready to measure the O₂⁻ content according to the slightly modified method of hydroxylamine oxidation [60]. The 0.5 mL of supernatant sequentially mixed with 0.5 mL of 50 mM PBS (pH 7.8) and 1.0 mL of 10 mM hydroxylamine hydrochloride were incubated at 25°C for 1 h. After that, 1.0 mL of 17 mM para-aminobenzoic acid and 1.0 mL of 7 mM α-naphthylamine were added in the order to the reaction mixture. After 20 min of incubation at 25°C, the absorbance of the mixture (AM) was measured at 540 nm using a luminometer (SpectraMax Plus384, Molecular Devices, CA, USA). The O₂⁻ content was calculated according to the formula (1):

$$O_2^- \text{ content } (\mu\text{g/g FW}) = (X \times V_s) / (FW \times V_r) \quad (1)$$

Where the *FW* is the fresh weight (g); *X* is the content of O₂⁻ according to standard curve (μg); *V_s* is the volume of sample extraction (mL), and *V_r* is the volume of supernatant participating in the reaction (mL).

The H₂O₂ content was determined based on the slightly modified method of Velikova et al. [61]. The 0.2 g ground fresh leaf homogenized in 2.0 mL trichloroacetic acid (0.1%, W/V) homogenate was centrifuged at 12,000 × g for 15 min at 4°C. After that, 0.5 mL supernatant was mixed with 0.5 mL of 10 mM PBS (pH 7.0) and 1 mL of 1.0 M potassium iodide. The AM was assayed at 390 nm using as described above luminometer and the content was calculated as follows:

$$H_2O_2 \text{ content } (\mu\text{M/g FW}) = (X \times V_s) / (FW \times V_r) \quad (2)$$

Where *X* is the content of H₂O₂ according to the standard curve (μM); others are the same as described in the O₂⁻ content.

Determination of antioxidant enzyme activities and proline content

The 0.1 mL supernatant extracted same as the method of O₂⁻ was mixed with 1.5 mL PBS (50 Mm, pH 7.8), 0.3 mL methionine (130 mM), 0.3 mL NBT (750 μM), 0.3 mL EDTA-Na₂ (100 μM), 0.3 mL riboflavin

(20 μM), 0.5 mL distilled water. The mixture was placed in an incubator at 30°C under 4000 Lux light intensity for 15 min [62]. In parallel, another mixture containing 0.1 mL distilled water instead of the supernatant was placed separately in the dark and light designed as a control. The AM was measured at 560 nm using a luminometer, as described above. The amount of enzyme required for inhibiting 50% reduction of NBT photochemical within 1 min per gram of FW was determined by the SOD activity (U/g FW/min), which is calculated as following formula (3):

$$\text{SOD activity (U/g FW/min)} = (OD_0 - OD_s) \times V_s / (OD_0 \times 0.5 \times V_r \times FW) \quad (3)$$

Where, OD_0 and OD_s are the absorbances of control under light and measurement for treatment, respectively; others are the same as described in the O_2^- content.

The POD activity was measured using the procedure of guaiacol reduction [63]. The 50 μL enzyme solution extracted same as the method of O_2^- mixed with 3 mL reaction mixture containing 50 mL PBS (0.2 mM, pH 6.0), 19 μL 30.0% H_2O_2 , and 28 μL guaiacol was measured quickly at 470 nm every 30 s for 2 min by a spectrophotometer (SP-1920, Shanghai Spectral Instrument Co., LTD, China). The AM at 470 nm was expressed as a U/g FW/min (formula 4).

$$\text{POD activity (U/g FW)} = (\Delta OD \times V_s) / (FW \times V_t \times 0.01 \times t) \quad (4)$$

Where ΔOD is the change of absorbance in 2 min (t); others are the same as described in the O_2^- content.

The APX activity was determined using the slightly modified procedure of ascorbic acid oxidation [63]. Approximately 2.6 mL PBS (50 mM, pH 7.0, containing 0.1 mM EDTA- Na_2), 0.15 mL ascorbic acid (5 mM), and 0.15 mL H_2O_2 (20 mM) were successively added into 0.1 mL supernatant (same as O_2^-). Then the absorbance was measured at 290 nm every 30 s for 2 min using a spectrophotometer (as described above). The reduction of absorbance at 290 nm was expressed as a U/g FW/min (formula 5).

$$\text{APX activity (U/g FW)} = (\Delta OD \times V_s) / (FW \times V_t \times 0.01 \times t) \quad (5)$$

Where ΔOD is the change of absorbance in 2 min (t); others are the same as described in the O_2^- content.

To extract proline, 0.2 g ground fresh leaf was homogenized in 2.0 mL sulfosalicylic acid and then boiled for 10 min [65], whereafter was centrifuged at $10,000 \times g$ for 10 min at 4°C. Then, 0.2 mL supernatant was added with 0.2 mL glacial acetic acid, 0.4 mL acid ninhydrin, and 0.2 mL sulfosalicylic acid and then boiled for 30 min. After cooling, the mixture was mixed with 0.4 mL toluene and then centrifuged at $900 \times g$ for 5 min at 4°C. The AM was measured at 520 nm using as described above luminometer.

$$\text{APX activity } (\mu\text{g/g FW}) = (X \times V_s) / (FW \times V_t) \quad (6)$$

Annotation is the same as described in the O_2^- content.

Assessment of morphological characteristics and crop biomass

Three pots containing nine plants with uniform growth were selected for measuring LA/plant and plant height by a straightedge, and stem diameter by a digital vernier caliper (DL3944, Ningbo Deli Tools Co., LTD, Zhejiang, China). Stem and leaf tissues were separately dried at 108°C for 30 min and then roasted at 75°C until reaching a constant weight, and the dry matter for stem and leaf was determined. The LA was determined as the following formula (2):

$$LA = 0.75 \times \sum_{i=1}^m \sum_{j=1}^n (L_{ij} \times W_{ij}) / m \quad (7)$$

Where 0.75 is the empirical correction coefficient for LA of maize; m is plant number per replication (three plants); n is the total number of leaves on the i th plant; L_{ij} and W_{ij} are the maximum leaf length and width of j th leaf in the i th plant, respectively.

Measurement of leaf relative water content

Top fully developed fresh leaves in three plants per replication were weighed using a 1/1000 balance recorded as FW. Then the leaves were immersed in water for 8 h. After soaking in the surface water, the leaves were weighed to determine their turgid weight (TW). Subsequently, the turgid leaves were placed in an oven at 108°C for 30 min before drying at 75°C to a constant weight to record the dry weight (DW). The RWC was calculated as the following formula (1) [66]:

$$RWC(\%) = (FW - DW) / (TW - DW) \times 100 \quad (8)$$

Tolerance analysis

The comprehensive evaluation (D) value and tolerance coefficient were adopted to predict the tolerance of varieties under WS. The SOD, POD, APX, LA, stem diameter, plant height, and dry matter were involved in the calculation of D value and tolerance coefficient as following formulas [67]:

$$u(X_{ij}) = (X_{ij} - X_{\min}) / (X_{\max} - X_{\min})$$

9

$$V_j = \sqrt{\sum_{i=1}^n (X_{ij} - \bar{X}_j)^2} / \bar{X}_j$$

10

$$W_j = V_j / \sum_{j=1}^n V_j$$

$$D = \sum_{j=1}^n [u(X_j) \times W_j] \quad (12)$$

$$\text{Tolerance coefficient} = \sum_{j=1}^n (\text{Stress}_j / \text{CK}_j) / n$$

Where the value of i th variety in the j th indicator was X_{ij} ; the membership function value, maximum value, minimum value, standard deviation coefficient, and average value for the j th indicator of two varieties were $\mu(X_{ij})$, X_{max} , X_{min} , V_j and \bar{X}_j , respectively, and the weight of the j th indicator in all indicators was W_j .

Statistical Analysis

The software of SAS 9.2 (SAS Institute, 2009) was used to carry out the one-way analysis of variance (ANOVA) for GB concentration, three-way ANOVA for variety, WS, and GB concentration to determine the interaction between each factor, and Pearson's correlation analysis among all parameters. Means with $n = 3$ biological replicates for all indicators were compared by the least significant difference (LSD) at $P \leq 0.05$. The stepwise regression analysis among SOD, POD, APX, proline, LA, plant height, stem diameter, and stem dry matter with leaf dry matter was performed by the software of SPSS Statistics v. 21 (IBM Inc., Armonk, N.Y., USA). Moreover, figures were generated using OriginPro 2021 (OriginLab Inc., Northampton, Massachusetts., USA).

Abbreviations

GB: Glycinebetaine

WS: Water stress

ROS: Reactive oxygen species

LA: Leaf area

SOD: Superoxide dismutase

POD: Peroxidase

APX: Ascorbate peroxidase

V3: Three-leaf stage

V5: Five-leaf stage

H₂O₂: Hydrogen peroxide

O₂⁻: Superoxide anion

RWC: Relative water content

CK: Control

FC: Field capacity

PBS: Phosphate buffer solution

AM: Absorbance of the mixture

FW: Fresh weight

D value: Comprehensive evaluation value

Declarations

Ethics approval and consent to participate

This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Consent for publication

The authors agreed to publish the manuscript in the journal.

Availability of data and materials

The data presented in this study are available in the graphs and table provided in the manuscript.

Competing interests

Author Li Bo Shi was employed by the company of MAP Division (Shandong) of Sinochem Agriculture Holdings, China. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

GYW, BWW, CQS and XBZ conceptualized the experiment and provided the funding acquisition and project administration. GYW and SA wrote the original manuscript, who were cooperated with YW helped in data curation and investigation. LBS, CQS, and XBZ supervised the study and provide resources. All authors have reviewed and agreed to the published version of the manuscript.

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Figures

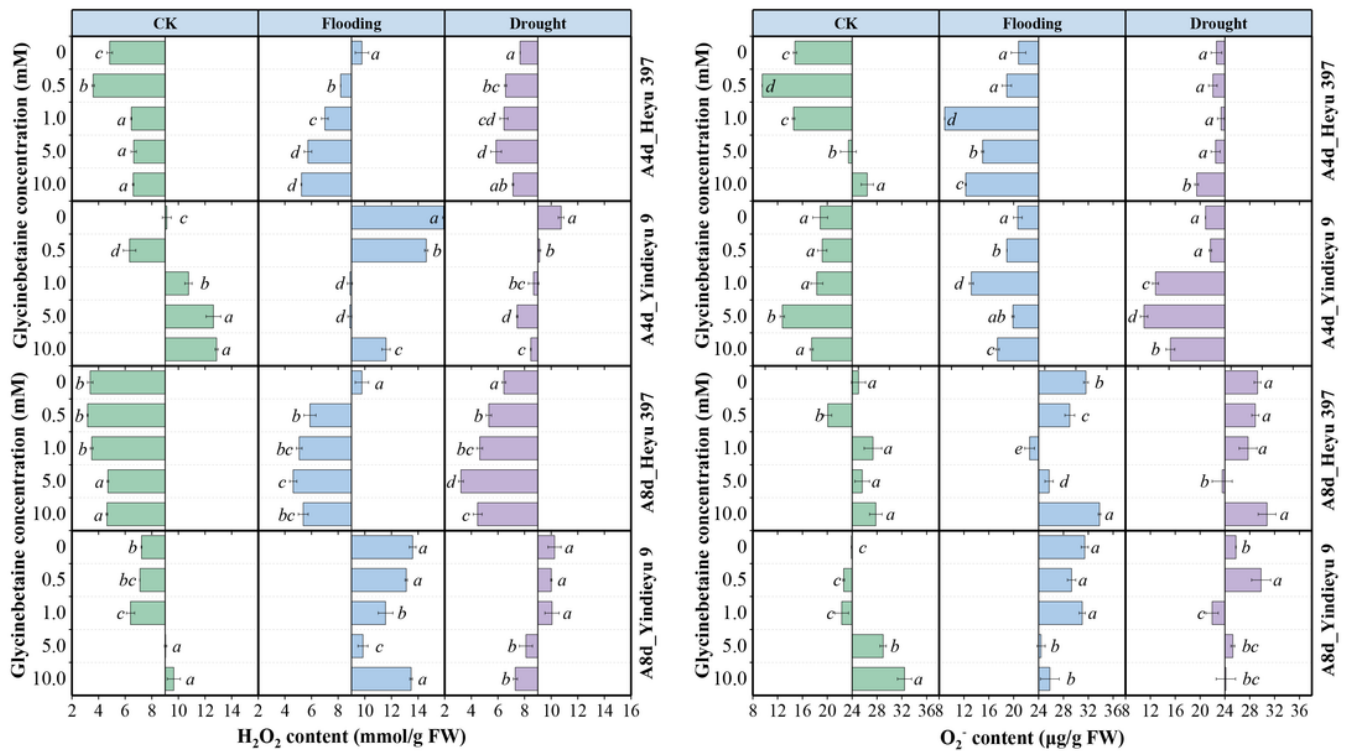


Fig. 1 The accumulations of hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) under the effect of glycinebetaine after 4 d (A4d) and 8 d (A8d) of water stress. CK represents crops planted in well-watered conditions. FW is the fresh weight. Vertical bars represent standard error (*n* = 3, biological replicates). Different letters in a water treatment indicate the least significant differences as *P* value ≤ 0.05.

Figure 1

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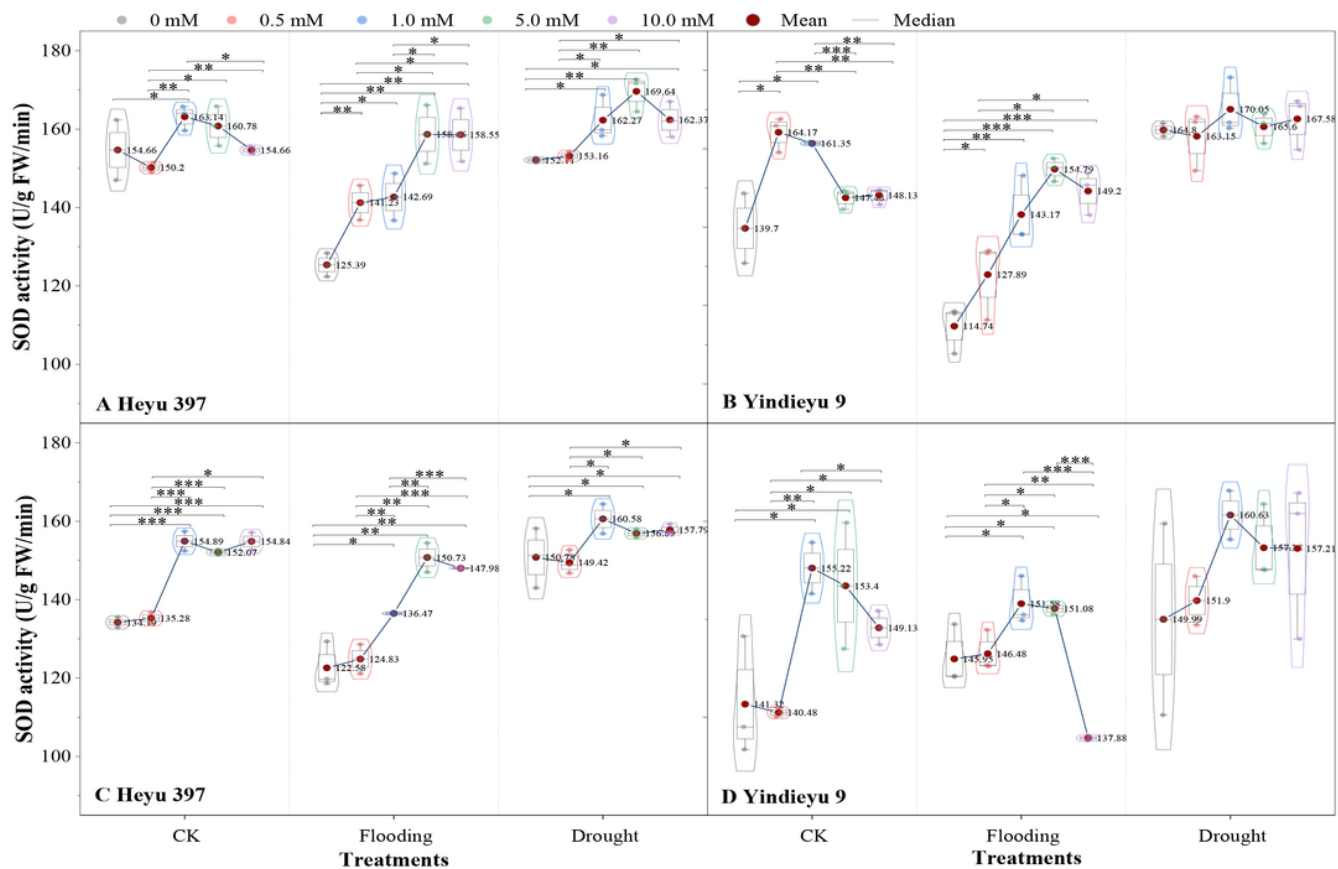


Fig. 2. The effects of glycinebetaine on superoxide dismutase (SOD) activity after 4 d (A, B) and 8 d (C, D) of water stress. Data are presented as three biological replicates with a vertical bar of standard error, and the numbers show means. CK represents crops planted in well-watered conditions, and 0, 0.5, 1.0, 5.0, and 10.0 mM are the different concentrations of glycinebetaine. FW is the fresh weight. *, **, *** mean significant P value ≤ 0.05 , ≤ 0.01 , ≤ 0.001 , respectively, if no symbol is presented between treatments, meaning P value > 0.05 .

Figure 2

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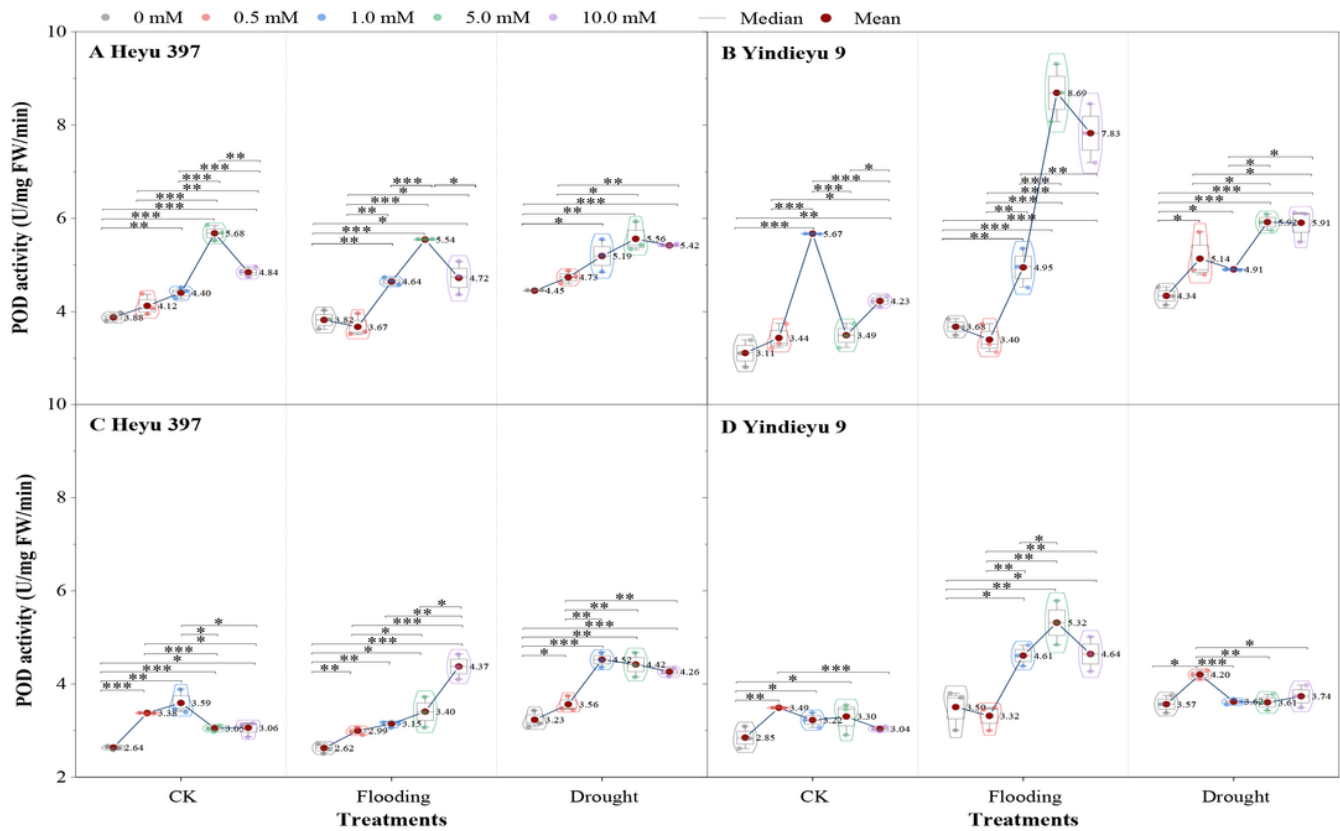


Fig. 3 The effects of glycinebetaine on peroxidase (POD) activity after four days (A, B) and eight days (C, D) of water stress. Data are presented as three biological replicates with a vertical bar of standard error, and the numbers show means. CK represents crops planted in the well-watered condition, and 0, 0.5, 1.0, 5.0, and 10.0 mM are the different concentrations of glycinebetaine. FW is the fresh weight. *, **, *** mean significant P value ≤ 0.05 , ≤ 0.01 , ≤ 0.001 , respectively, if no symbol is presented between treatments, meaning P value > 0.05 .

Figure 3

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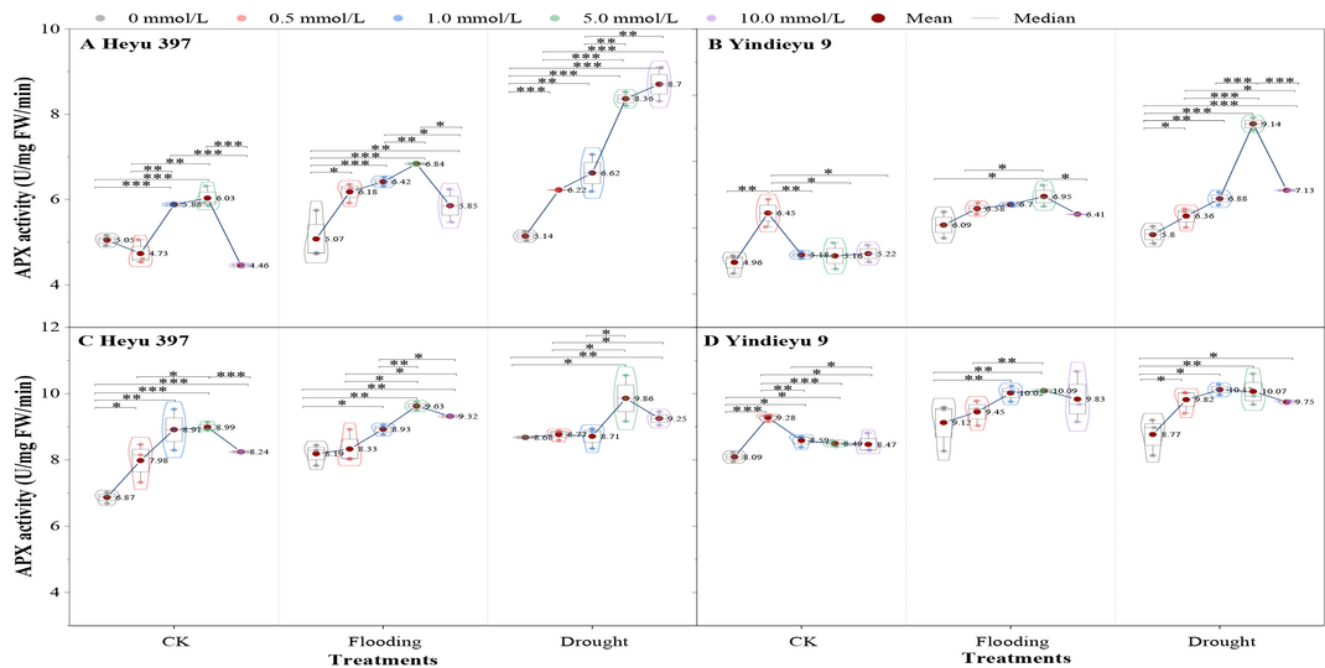


Fig. 4 The effects of glycinebetaine on ascorbate peroxidase (APX) activity after 4 d (A, B) and 8 d (C, D) of water stress. Data are presented as three biological replicates with the vertical bar of standard error, and the numbers show means. CK is crops planted in the well-watered condition, and 0, 0.5, 1.0, 5.0, and 10.0 mM are the different concentrations of glycinebetaine. FW is the fresh weight. *, **, *** mean significant P value ≤ 0.05 , ≤ 0.01 , ≤ 0.001 , respectively, if no symbol is presented between treatments, meaning P value > 0.05 .

Figure 4

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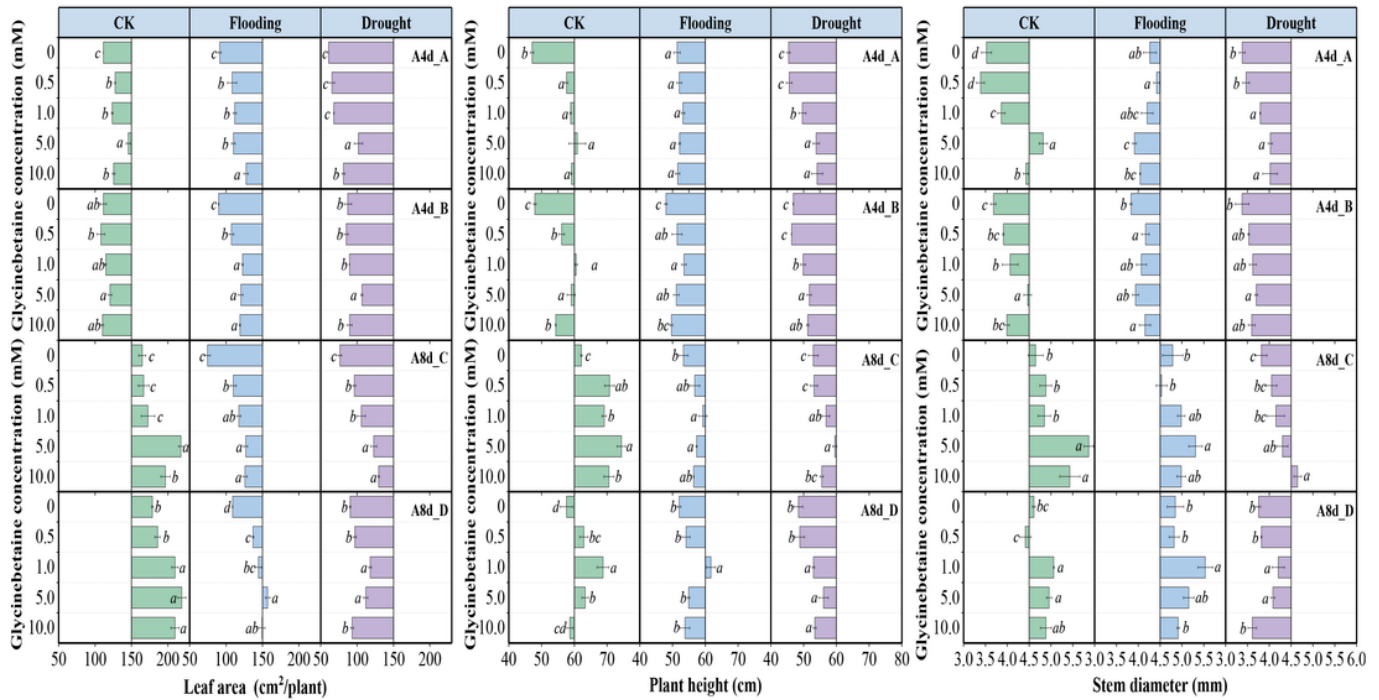


Fig. 5 The effect of glycinebetaine on maize morphology after 4 d (A4d) and 8 d (A8d) of water stress. CK represents crops planted in the well-watered condition. The letters A and C are the variety Heyu 397, and B and D represent the variety Yindieyu 9. Vertical bars represent standard error ($n = 3$, biological replicates). Different letters in a water treatment indicate the least significant differences as P value ≤ 0.05 .

Figure 5

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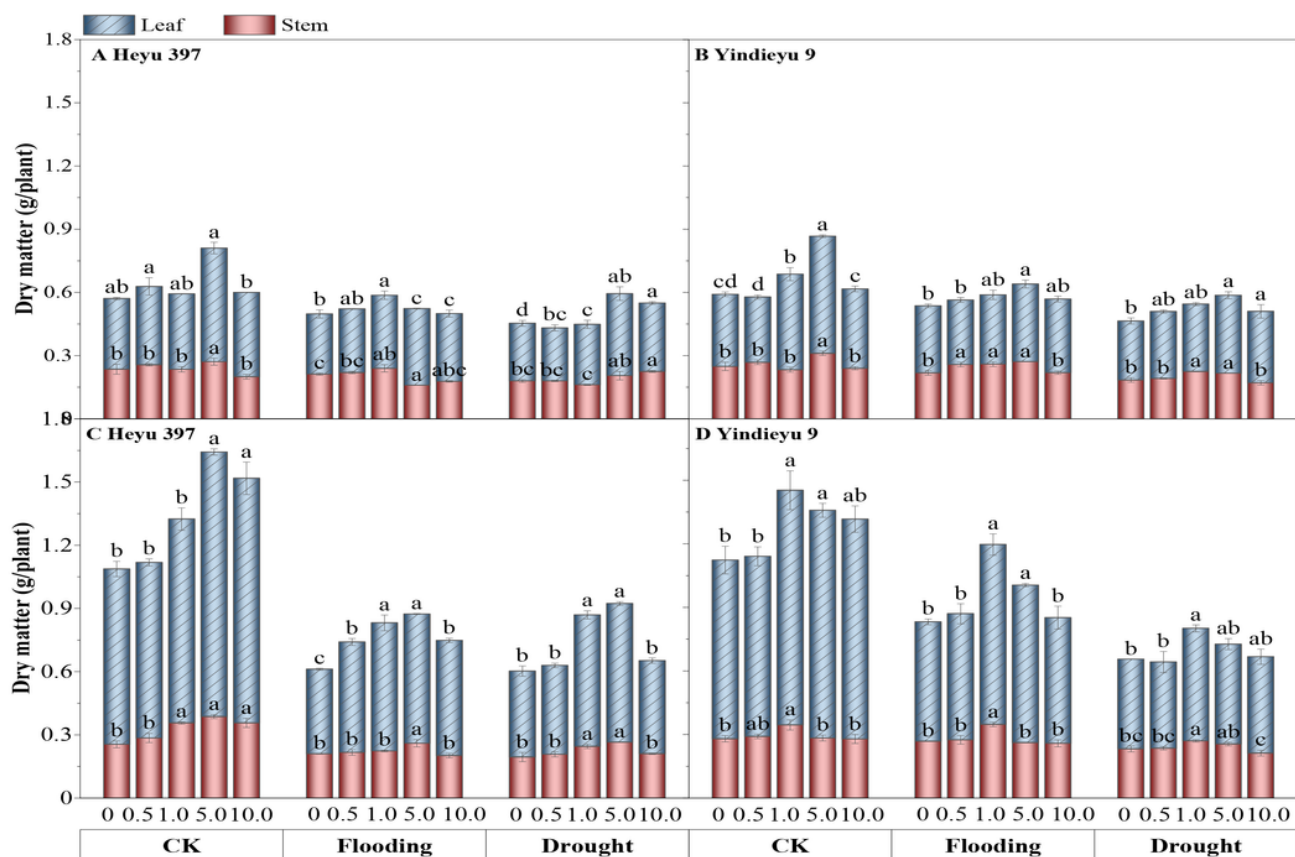


Fig. 6 The biomass accumulation under the effect of glycinebetaine after 4 d (A, B) and 8 d (C, D) of water stress. CK represents crops planted in the well-watered condition, and 0, 0.5, 1.0, 5.0, and 10.0 mM are the different concentrations of glycinebetaine. Vertical bars represent standard error ($n = 3$, biological replicates). Different letters in a water treatment indicate the least significant differences as P value ≤ 0.05 .

Figure 6

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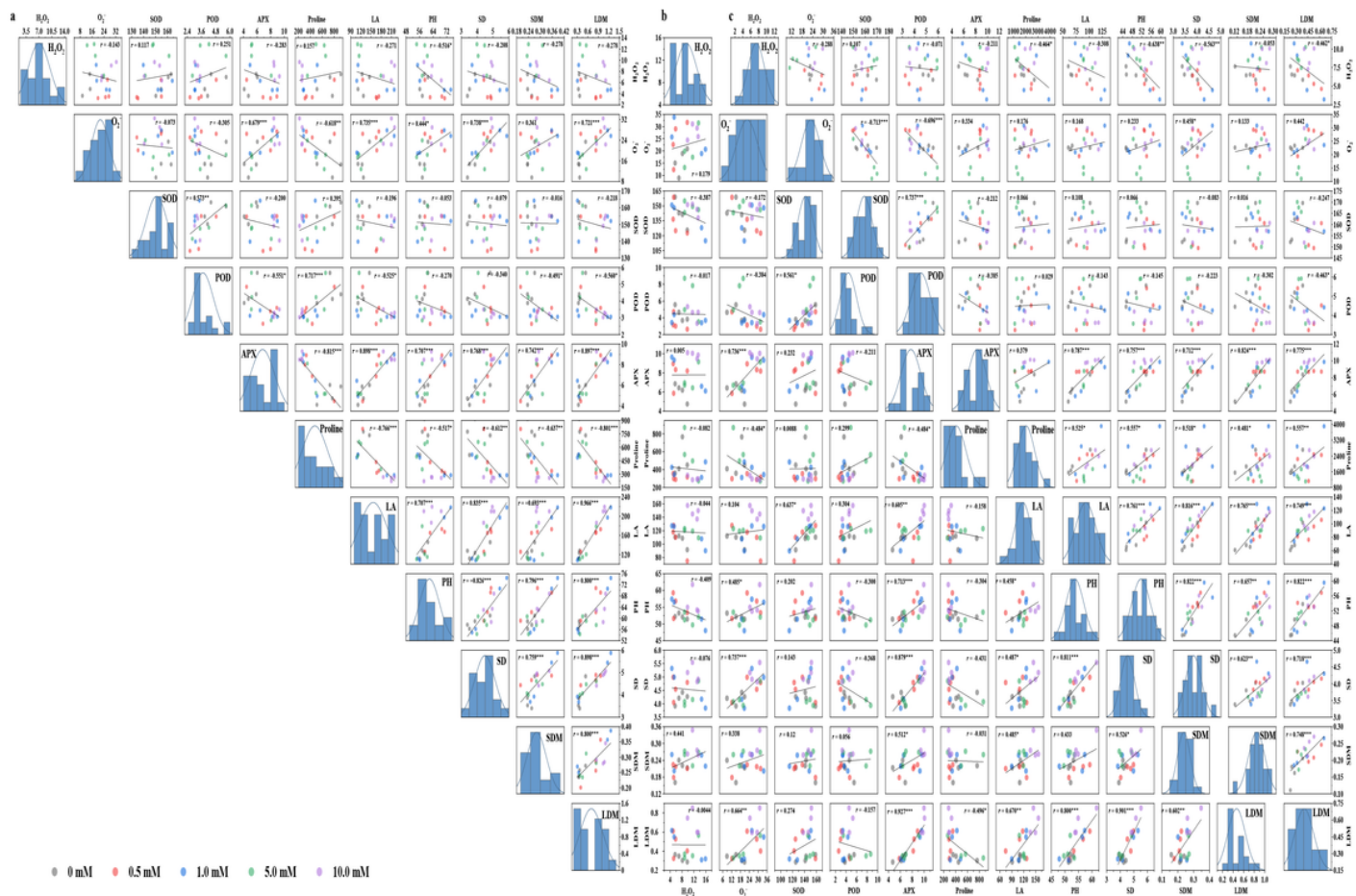


Figure 7. Correlation analyses of the inspected parameters of maize seedlings under CK (A), flooding (B) and drought (C) stresses in the influence of glycinebetaine. CK represents crops planted in the well-watered condition. Correlation coefficient r with the least significant difference according to P value ≤ 0.05 (*, **, *** mean significant P value ≤ 0.05 , ≤ 0.01 , ≤ 0.001) is shown. H₂O₂, O₂⁻, SOD, POD, APX, LA, PH, SD, SDM, and LDM represent hydrogen peroxide, superoxide anion, superoxide dismutase, peroxidase, ascorbate peroxidase, leaf area, plant height, stem diameter, stem dry matter, and leaf dry matter, respectively

Figure 7

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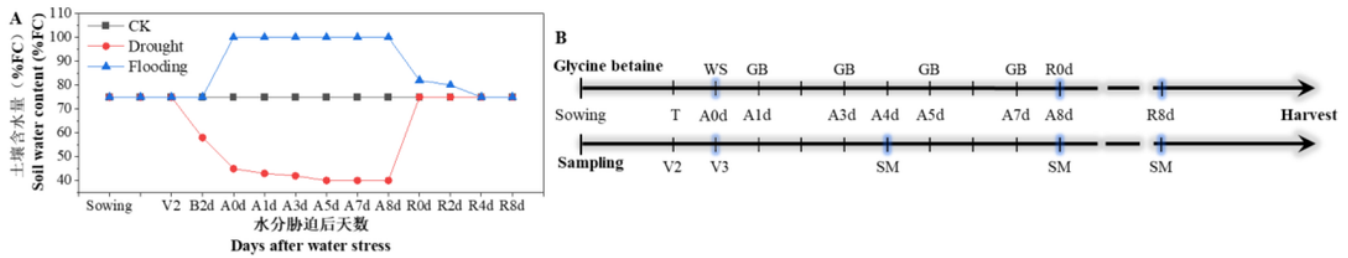


Fig. 8 The change of soil water content (A) and the diagram of the time (B) for foliar application of glycine betaine (GB) and sampling during experiment. FC represents maximum field capacity; CK represents crops planted in well-watered conditions; V2 and V3 are two-leaf stage and three-leaf stage for maize, respectively; B2d represent 2 d before WS treatment, and A0d, A1d, A3d, A4d, A5d, A7d, and A8d indicate 0, 1, 3, 4, 5, 7, 8 days after water stress, respectively, and R0d and R1d indicate 0 and 1 d after recovering growth, respectively; T, SM, and WS are abbreviations of thinning, sampling and measuring for indicators, and water stress, respectively.

Figure 8

See image above for figure legend

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