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Physiological, biochemical, and transcriptomic responses to waterlogging stress in *Panicum bisulcatum*, an emerging weed in rice fields

Jinqiu Sun¹, Xiaoyue Yu¹, Yongjie Yang¹, Hongxing Xu², Mengjie Liu¹, Zhongxian Lü^{2*} and Wei Tang^{1*}

Abstract

Background *Panicum bisulcatum*, an annual grass weed of the *Panicum* genus, is typically found in arid land crop fields, has been observed invaded and become a predominant troublesome weed in rice fields of China in recent years. This study aimed to explore the effect of waterlogging on the control of *P. bisulcatum* and to elucidate its physiological, biochemical, and molecular response mechanisms under waterlogging stress.

Results The results revealed that different water depths showed varied impact on the growth of *P. bisulcatum* seedlings. A 2 cm water depth had no significant effect on growth, while a 4 cm water depth completely inhibited plant height and aboveground biomass accumulation. Under waterlogging stress, the activities of key anaerobic respiration enzymes (alcohol dehydrogenase [ADH], pyruvate decarboxylase [PDC], and lactate dehydrogenase [LDH]), malondialdehyde (MDA) content, and antioxidant enzyme activities (superoxide dismutase [SOD], peroxidase [POD], and catalase [CAT]) were significantly increased. Comparative transcriptomic analysis at two waterlogging time points identified 11,986 and 1295 differentially expressed genes (DEGs) at 1 day and 7 days, respectively. These DEGs were significantly enriched in signal transduction, carbohydrate metabolism, and translation pathways. Notably, 18 genes associated with anaerobic respiration, ethylene biosynthesis, and gibberellin synthesis were upregulated after one day of waterlogging stress. Following the identification of *GAPDH* as the most stable reference gene for normalization, quantitative real-time PCR analysis revealed significant upregulation of key pathway-related genes in *P. bisulcatum* under waterlogging stress, including *LDH*, *GA20ox*, *GA2ox-1*, *SAM-synthase*, *ACO-1*, and *ACO-5*. These findings are consistent with the enhanced waterlogging tolerance observed following exogenous gibberellin treatment.

Conclusion This study provides valuable information for unrevealing the adaptive mechanisms of *P. bisulcatum* under waterlogging conditions and helps developing strategies for the management of *P. bisulcatum* in rice fields.

Keywords Waterlogging, Transcriptome, Anaerobic respiration, *Panicum bisulcatum*

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Introduction

In recent years, the escalating frequency of extreme climatic events, driven by global warming, has led to a surge in flooding disasters worldwide [1]. Climate models predict that the intensity and frequency of flooding will further increase in certain regions over the coming decades [2]. Consequently, waterlogging has emerged as one of the most devastating abiotic stresses, affecting approximately 17 million square kilometers of terrestrial ecosystems and posing a significant constraint on plant growth and productivity [3]. Waterlogging disrupts gas exchange between plants and the atmosphere, causing oxygen levels in the rhizosphere to plummet to as low as 1/10,000th of normal conditions [4]. Under such hypoxic stress, root oxidative phosphorylation is severely inhibited, leading to a drastic reduction in ATP synthesis [5]. Concurrently, the accumulation of reactive oxygen species (ROS) exacerbates cellular damage [6], resulting in a cascade of growth impairments, including diminished primary root vigor, leaf chlorosis, and disrupted developmental processes [7].

To mitigate the adverse effects of waterlogging, plants have evolved a suite of phenotypic, physiological, and biochemical adaptations. Under waterlogging stress, aerobic respiration is suppressed, and plants rapidly shift to anaerobic respiration pathways, such as ethanol fermentation, to sustain energy production [8]. Key enzymes involved in this metabolic shift include alcohol dehydrogenase (ADH), pyruvate decarboxylase (PDC), and lactate dehydrogenase (LDH). Notably, the activities and gene expression levels of these enzymes are significantly upregulated in response to flooding stress in various plant species [9–11].

Plants adapt to waterlogging and associated environmental stressors through dynamic modulation of gene expression, which is precisely controlled through a multi-layered regulatory framework spanning epigenetic modifications [12], translational control [13], and transcriptional regulation mechanisms [14]. Current investigations into waterlogging tolerance-related genes predominantly concentrate on three key categories: anaerobic metabolic genes, ERF and WRKY family transcription factors, as well as hemoglobin-regulatory genes [15].

In addition to metabolic adjustments, plants exhibit morphological adaptations to cope with waterlogging. For instance, under prolonged but shallow waterlogging, many species undergo internode elongation to facilitate oxygen uptake, a strategy termed low oxygen escape syndrome [16]. A classic example is *Rumex palustris*, which reorients its leaves from horizontal to vertical and rapidly elongates its petioles upon flooding [17, 18]. These morphological changes are primarily regulated by phytohormones, particularly ethylene and gibberellins, which

orchestrate escape strategies to promote internode elongation [19]. In deep water rice, flooding triggers rapid internode elongation over several months, ensuring that leaf tips remain above the water surface. This response is accompanied by elevated levels of ethylene and gibberellins [20, 21]. Kuroha et al. identified *OsGA20ox2* (also known as SD1), a gene encoding gibberellin biosynthesis enzymes, as a critical regulator of internode elongation in deepwater rice. This gene, along with the ethylene response factor *OsEILa*, modulates gibberellin levels to drive internode elongation under flooding conditions [22].

Waterlogging has been increasingly utilized as a weed management strategy in rice paddies, particularly with the development of rice varieties exhibiting enhanced submergence tolerance [23–26]. However, the emergence of *Panicum bisulcatum* Thunb., an annual grass weed, as a predominant species in rice fields in northern Zhejiang Province, has posed new challenges. At densities of 125 plants/m², *P. bisulcatum* can inhibit rice plant height by 4.17% and reduce yield by up to 61.42% [27]. In our previous study, *P. bisulcatum* seeds were able to germinate under waterlogging stress from 0.5 to 6.0 cm water layer with germination rate higher than 88% [28]. However, the submergence tolerance of *P. bisulcatum* seedling remains unclear.

Advances in RNA sequencing (RNA-Seq) have enabled high-resolution transcriptomic profiling to dissect plant stress adaptation mechanisms, yet its application in weed biology remains underexplored. While RNA-Seq has been widely adopted to study herbicide resistance and genome evolution in weeds [29–31], their molecular adaptation to waterlogging remains poorly characterized. In this study, we systematically evaluated *P. bisulcatum*'s response to waterlogging stress by integrating physiological metrics (aboveground biomass, anaerobic respiration enzymes, malondialdehyde (MDA) levels, and antioxidant activities) with transcriptomic profiling. We identified stable reference genes and delineated dynamic transcriptional changes in key pathways, including anaerobic respiration and escape strategies mediated by ethylene and gibberellin signaling. The objectives of this study were twofold: (1) to evaluate the response of *P. bisulcatum*, an emerging weed, to varying water depths, and (2) to uncover the physiological and molecular mechanisms underlying its adaptation to waterlogging stress. These findings provide valuable genetic insights for the development of targeted weed control strategies in rice fields.

Materials and methods

Plant material and waterlogging treatment

P. bisulcatum seeds were collected from a direct-seeded rice field in Nanhu District, Jiaxing City, Zhejiang Province, China (30.67°N, 121.13°E) in 2021. This material was

also used in our previous research [28]. For germination, seeds were surface-sterilized and placed in sterile Petri dishes (9 cm diameter) containing two layers of filter paper moistened with 4 mL of distilled water. The Petri dishes were incubated in a growth chamber (GXZ-300 C, Southeast Instrument Factory, Ningbo, Zhejiang, China) under controlled conditions: 30/20°C day/night temperature regime with a 12-h photoperiod and 65% relative humidity.

Following germination, fifty uniform seedlings were carefully transplanted into plastic pots (13.5 cm diameter × 11.0 cm height) containing paddy soil collected from the Fuyang experimental base of the China Rice Research Institute (30.04°N, 119.55°E). Each pot was filled to approximately 50% capacity, resulting in a soil depth of 5 cm. The transplanted seedlings were maintained in a climate-controlled chamber under the following conditions: 25/20°C day/night temperature, 12-h photoperiod, and 65% relative humidity.

The experimental design included three water regime treatments: (1) control (0 cm water depth, non-flooded conditions) with soil maintained at field capacity; (2) moderate waterlogging (2 cm standing water); and (3) severe waterlogging (4 cm standing water). Waterlogging treatments were initiated at the two-leaf stage (emergence of the second true leaf) by slowly adding deionized water along the inner wall of the pots to achieve the desired water depths, which were maintained throughout the experimental period. The control treatment was maintained by daily watering to prevent surface water accumulation while ensuring adequate soil moisture. The control treatment was maintained by daily watering to prevent surface water accumulation while ensuring adequate soil moisture.

This experimental setup was designed to simulate natural waterlogging conditions in rice fields and to investigate the physiological and biochemical responses of *P. bisulcatum* seedlings to differential water stress conditions. The experimental design followed a completely randomized block design with appropriate replication to ensure statistical validity.

Sample collection and measurement of physiological indicators

For each experimental group, seedlings were randomly sampled prior to the initiation of waterlogging treatments. The collected samples were thoroughly rinsed under running tap water to remove surface debris, followed by three successive washes with distilled water to eliminate any residual impurities. Excess water was carefully removed by blotting the seedlings with sterile filter paper.

This sampling protocol was consistently repeated daily at a fixed time following the implementation of the

flooded treatments. At each sampling point, plant height was measured from the base of the stem to the apex using a digital caliper, and fresh weight was determined using a precision analytical balance (accuracy ± 0.0001 g). All measurements were performed in triplicate to ensure data accuracy and reproducibility. The standardized sampling procedure was maintained throughout the experimental period to minimize variability and ensure reliable comparison across treatment groups.

Determination of anaerobic respiration enzymes activities, malondialdehyde (MDA) levels, and antioxidant enzyme activities

Under waterlogging stress, plants undergo anaerobic respiration, during which ADH, PDC, and LDH serve as key enzymes in this metabolic pathway. Plant samples (2 g) were collected at 0, 12 h, 1, 3, 5, 7, and 14 days post-treatment to measure the activities of these three enzymes in both the control group (0 cm water depth) and the waterlogged group (2 cm water depth). Additionally, MDA, SOD, CAT, and POD were selected as physiological indicators of waterlogging stress. Samples (2 g, weighed prior to grinding) were collected at 1 and 7 days post-treatment to determine the activities of these enzymes and MDA levels in both the control and waterlogged groups.

Leaf tissues were rinsed with phosphate-buffered saline (PBS), flash-frozen in liquid nitrogen, and stored at −80°C. For enzyme assays, 0.1 g of tissue was homogenized in PBS (pH 7.4), centrifuged (3,000 rpm, 20 min, 4°C), and supernatants were analyzed for enzyme activities (ADH, PDC, LDH, SOD, CAT, and POD) and MDA levels using ELISA kits (Jiangsu Meimian Industrial Co., Ltd.), following protocols standardized in prior studies on plant stress physiology [32].

Transcriptomic analysis

Plant samples (2 g) were collected at 1 and 7 days after treatment (DAT) (designated as WA/WB groups) and corresponding controls (CA/CB groups), flash-frozen in liquid nitrogen, and stored at −80°C. Total RNA was extracted from leaves and stems using the RNA simple Total RNA Kit (QIAGEN), with quality verified by 1% agarose gel electrophoresis, NanoPhotometer® spectrophotometry (IMPLEN), and Agilent 2100 Bioanalyzer. Sequencing libraries were constructed with the NEBNext® Ultra™ RNA Library Prep Kit and paired-end sequenced on the Illumina NovaSeq 6000 platform (Novogene Co., Ltd).

Raw reads were processed to remove adapters, low-quality sequences (Qphred ≤ 20 in > 50% bases), and ambiguous nucleotides. Clean reads were de novo assembled using Trinity [33], followed by functional annotation against NR, NT, KOG, Swiss-Prot (UniProt), and Pfam databases with DIAMOND (v0.8.22) and HMMER3.0.

Gene Ontology (GO) and KEGG pathway annotations were generated using BLAST2GO (v2.5.0).

Gene expression was quantified as FPKM [34]. Differential expression analysis ($|\log_2FC| > 1$, $\text{padj} < 0.05$) between waterlogged and control groups at 1/7 DAT was performed using DEGseq [35]. Enriched GO terms and KEGG pathways ($\text{padj} < 0.05$) were identified through hypergeometric testing.

Statistical analysis

Data are presented as mean $\pm$ standard error (SE) of three biological replicates ($n=3$). Differences in plant responses to varying waterlogging depths (0 cm, 2 cm, 4 cm) and anaerobic respiration enzymes were analyzed using t-tests with significance levels set at $*p < 0.05$ and $**p < 0.01$. For MDA content, antioxidant enzyme activities, and exogenous hormone treatments (GA, PBZ), one-way ANOVA followed by LSD test ($p < 0.05$) was performed. Significant differences among treatments are denoted by lowercase letters (shared letters indicate no significant difference). All analyses were conducted using SPSS22.0.

Validation of exogenous hormones and RT-qPCR validation

After subjecting *P. bisulcatum* seedlings to waterlogging stress with a 2 cm water layer, treatments involving 1000 μM gibberellic acid (GA) and 6000 μM paclobutrazol (PA) were applied. Seedlings were maintained under these conditions for 14 days, after which plant height and shoot fresh weight were measured.

To validate the reliability of the transcriptomic data, key differentially expressed genes (DEGs) identified through RNA-Seq analysis as responsive to waterlogging stress were selected for further validation using quantitative real-time PCR (RT-qPCR). Gene-specific primers were designed based on the coding sequences (CDS) of the target genes using Primer3.0 (<http://primer3.ut.ee/>; see Supplemental Table S1 for primer details). The most stably expressed reference gene identified in Supplementary material was used as an internal control for normalization. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, and statistical analysis was performed to confirm the consistency between RNA-Seq and RT-qPCR results. This approach ensured the accuracy and reliability of the transcriptional profiling data.

Results

Effect of waterlogging stress on the growth of *P. bisulcatum*

Here, the effects of 0 cm, 2 cm and 4 cm water depth on plant height and aboveground fresh weight of *P. bisulcatum* seedlings were examined. The results indicated that waterlogging treatment (4 cm) inhibited plant growth, and the leaves gradually yellowed (Figure S1). Compared to the control treatment (0 cm), the plant height

and fresh weight of the plants decreased by 35.5% and 50.6% at 7 DAT, and the plant height and fresh weight declined by 80.9% and 86.2% at 28 DAT. Plant height was consistently lower than the control treatment (0 cm) after the waterlogging treatment (2 cm). However, the above ground fresh weight started to be higher than the control treatment after 21 DAT (Fig. 1a, b). Based on these data, *P. bisulcatum* exhibits differential adaptation to waterlogging stress: low-level flooding (2 cm) stimulates biomass growth, whereas deeper waterlogging (4 cm) severely inhibits plant development.

ADH, PDC and LDH activities assay

The increases in ADH, PDC and LDH activities were more pronounced under waterlogging condition compared to control treatment (Fig. 2). By 5 DAT, the ADH activity in waterlogging was 0.33 U/g, higher than in control (0.26 U/g), meanwhile the LDH activity of waterlogging (0.06U/g) peaked which was 1.2 times that of control (0.05 U/g). The LDH activity of the control group peaked on the third day and the activity did not differ significantly from that of the waterlogging group, with enzyme activities of 0.050 and 0.052 U/g, respectively. After waterlogging treatment, PDC activity of *P. bisulcatum* reached a peak of 1.32-fold their control level by day 3. Enzyme activities peaked at 3 DAT, followed by a gradual decline. By 7 DAT, PDC activity returned to baseline levels, while ADH and LDH remained elevated, suggesting a transient dominance of ethanol fermentation over lactate metabolism during prolonged hypoxia.

The contents of malondialdehyde (MDA) and antioxidant enzymes activities assay

In this study, the contents of malondialdehyde (MDA) and three antioxidant enzymes activity were determined. As shown in Fig. 3, there was no significant change in MDA content between the treated and control groups after 7 days of waterlogging compared with 1 day of waterlogging. However, the content of MDA in the waterlogging group increased by 16.1% and 12.1% after 1 and 7 days of treatment, respectively compared to control. Further, antioxidant enzyme activities were induced after waterlogging. The activity of SOD, POD and CAT at 1DAT and 7DAT were higher than that of correspond control group. The activities of SOD and POD in the treatment and control groups decreased significantly with the increasing waterlogging time. the SOD and POD activity after waterlogging decreased to 1619.5 U/g and 2.0 U/g at 7 DAT, significantly lower than that observed at 1 DAT (2367.5 U/g and 2.4 U/g). The opposite pattern was observed for CAT activity and there was a continuous increase during the waterlogging time.

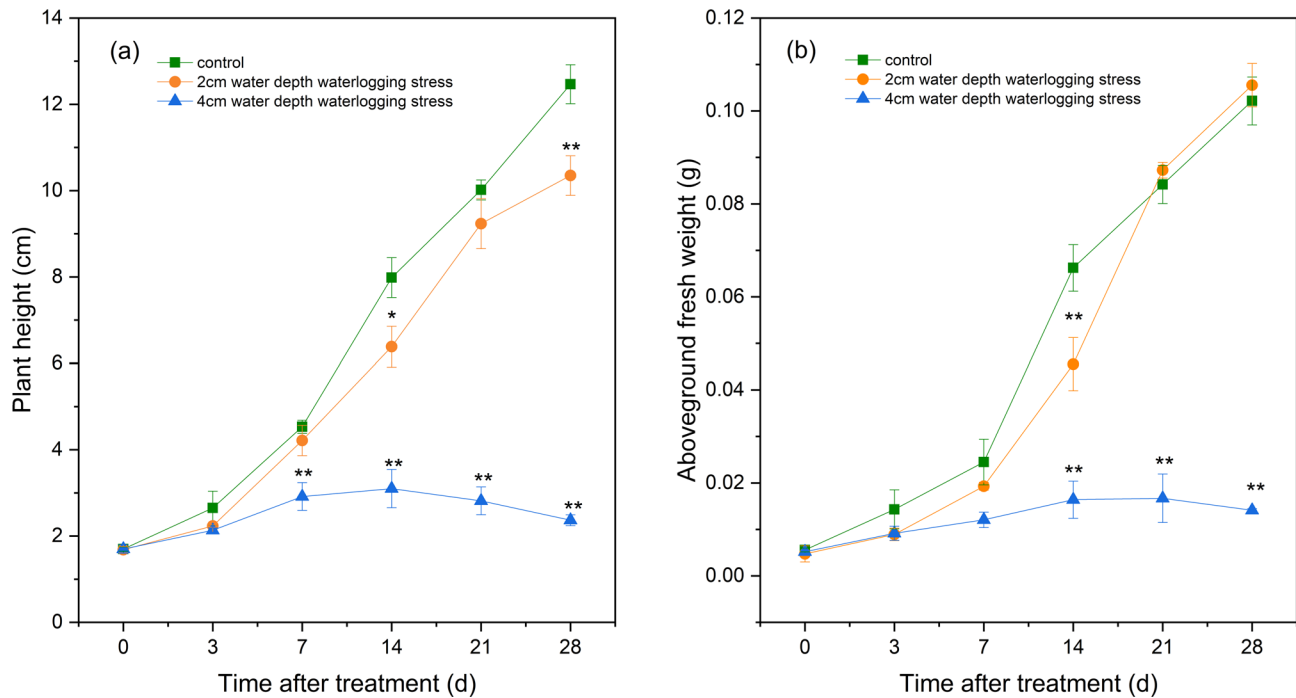


Fig. 1 Effects of waterlogging stress with different water depths on plant height (a) and aboveground fresh weight (b). Data points represent mean values $\pm$ standard error (SE) of three biological replicates. Significant differences versus the control group were determined by t-tests (* $P < 0.05$; ** $P < 0.01$)

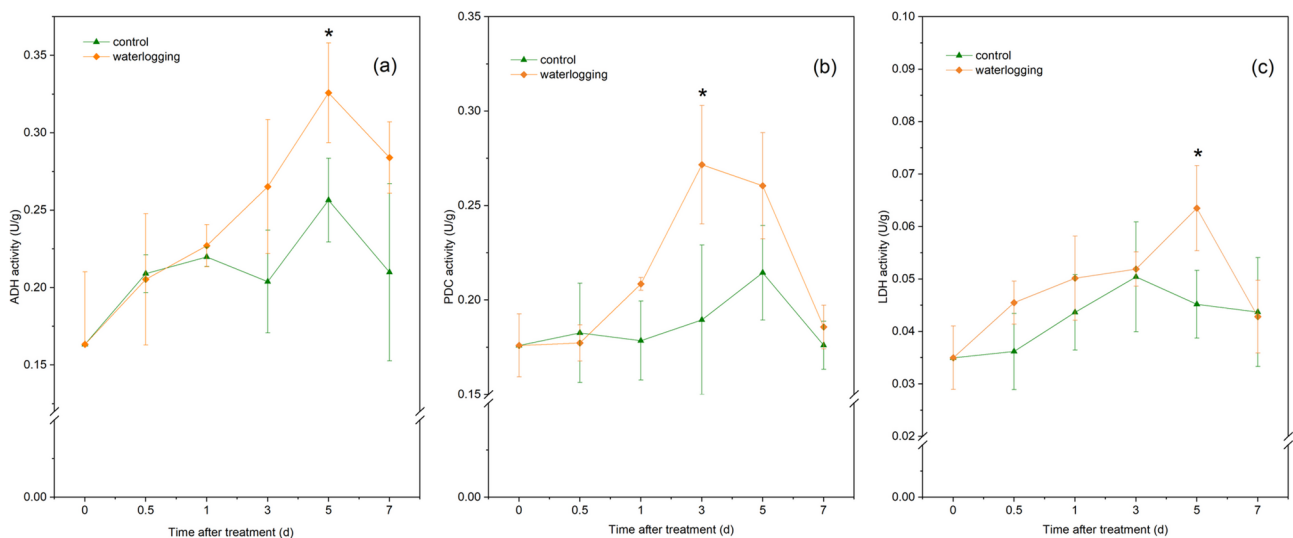


Fig. 2 The effect of waterlogging on anaerobic respiratory enzymes including (a) ADH, (b) PDC and (c) LDH. Values represent mean $\pm$ standard error based on three replicates, Significant differences versus the control group were determined by t-tests (* $P < 0.05$)

Quality control, de Novo assembly and functional annotation of transcriptome

RNA sequencing generated 258,980,330 raw reads, with 251,682,620 high-quality reads retained following rigorous quality control. The filtered sequences exhibited size ranges between 5.9 and 6.9 Gb, with quality scores exceeding Q20 > 97% and Q30 > 90%, indicating superior sequencing accuracy. GC content ranged from 55.28 to 58.61%, consistent with typical monocot transcriptome

profiles (Table 1). A comprehensive transcriptome assembly yielded 191,271 transcripts that were clustered into 65,485 non-redundant unigenes. Length distribution analysis revealed substantial contig continuity (Fig. 4a). Functional annotation against the NCBI non-redundant (NR) database identified 36,825 protein-coding genes with significant homology matches. Phylogenetic analysis of top BLAST hits demonstrated closest sequence similarity to established Poaceae species: *Setaria italica*

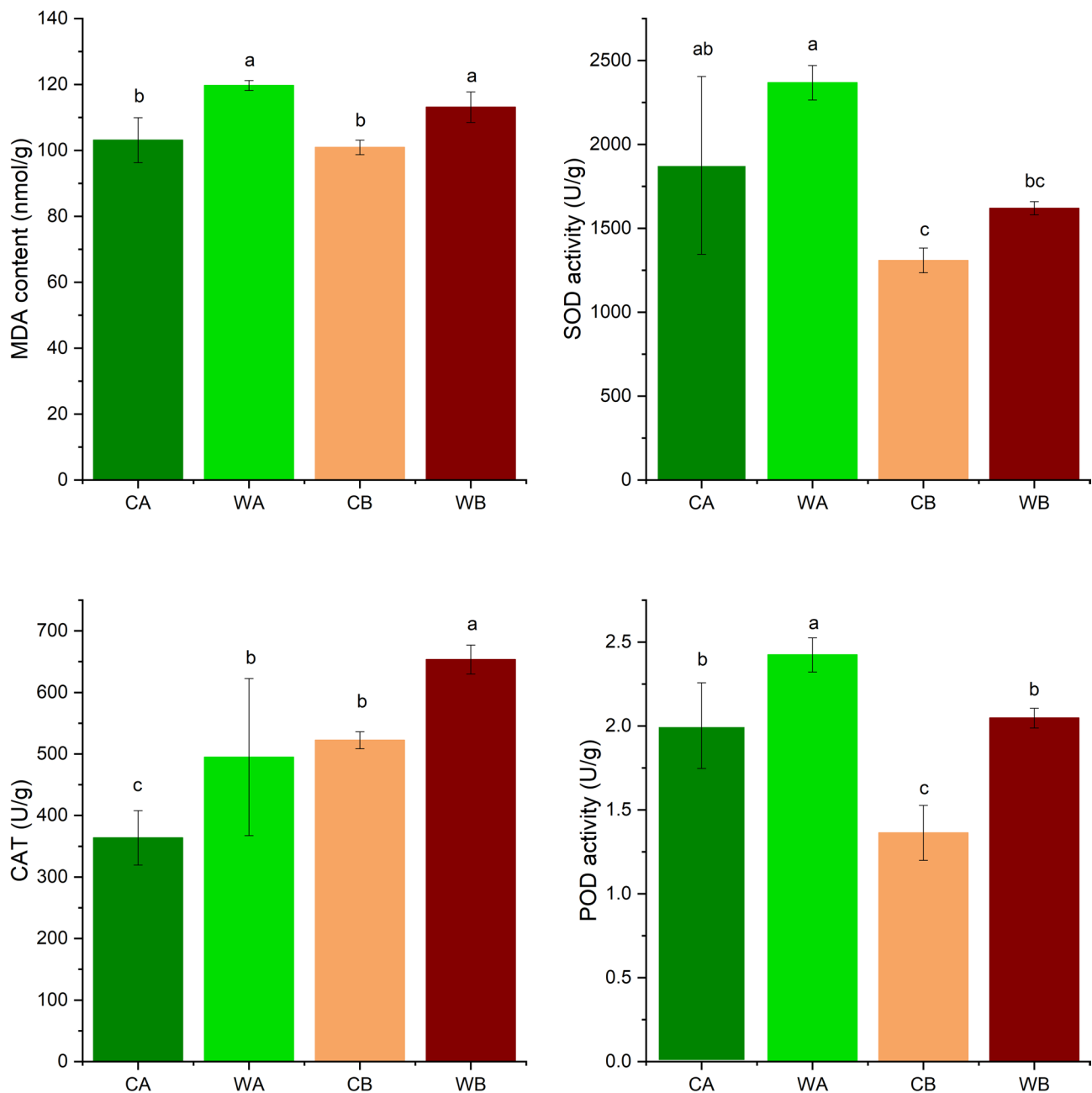


Fig. 3 The contents of malondialdehyde (MDA) and activities of catalase (CAT), Peroxidase (POD) and superoxide dismutase (SOD) in seedling treated for 1 day and 7 day. Significant differences from LSD test at $p < 0.05$. Data are mean $\pm$ SD ($n = 3$). Different letters mark significant differences between groups (one-way ANOVA with LSD test, $p < 0.05$) WA and WB represent the waterlogging stress for 1 d and waterlogging stress for 7 d, respectively, and CA and CB correspond to the control treatments without waterlogging stress for WA and WB, respectively

(24.2%), *Panicum miliaceum* (14.3%), *Panicum hallii* (13.3%), *Dichanthelium oligosanthes* (11.8%), and *Panicum hallii* var. *hallii* (9.5%) (Fig. 4b). This evolutionary conservation pattern aligns with current understanding of panicoid grass phylogenetics.

Identification of differentially expressed genes (DEGs)

To investigate the transcriptional responses of *P. bisulcatum* to waterlogging stress, a comprehensive

transcriptomic analysis was conducted across different time points of waterlogging treatment. The analysis revealed significant changes in gene expression profiles, providing insights into the molecular mechanisms underlying plant adaptation to waterlogging. A total of 11,986 DEGs were identified when comparing the transcriptomes of waterlogged plants at day 1 (WA) and control plants (CA), with 6,077 genes showing upregulation and 5,909 genes exhibiting

Table 1 Sequencing data quality statistics

Sample	Raw reads	Clean reads	Raw reads Base (G)	Clean reads base(G)	Clean reads Q20(%)	Clean reads Q30(%)	GC content(%)
CA1	21,730,741	21,187,543	6.5	6.4	97.37	92.83	57.34
CA2	20,574,826	19,994,569	6.2	6	97.5	93.23	57.09
CA3	20,164,042	19,539,084	6	5.9	97.44	93.03	56.9
WA1	21,773,341	21,365,477	6.5	6.4	97.42	93	57.79
WA2	20,675,104	20,188,745	6.2	6.1	97.56	93.37	58.04
WA3	23,815,460	23,151,016	7.1	6.9	97.42	93.02	56.53
CB1	22,539,105	21,855,103	6.8	6.6	97.48	93.1	56.95
CB2	21,504,221	20,802,965	6.5	6.2	97.42	93.02	55.66
CB3	20,461,012	19,982,458	6.1	6	97.47	93.08	56.62
WB1	20,514,981	19,992,074	6.2	6	97.56	93.33	56.77
WB2	22,526,770	21,647,918	6.8	6.5	97.44	93	58.61
WB3	22,700,727	21,975,668	6.8	6.6	97.55	93.33	55.28

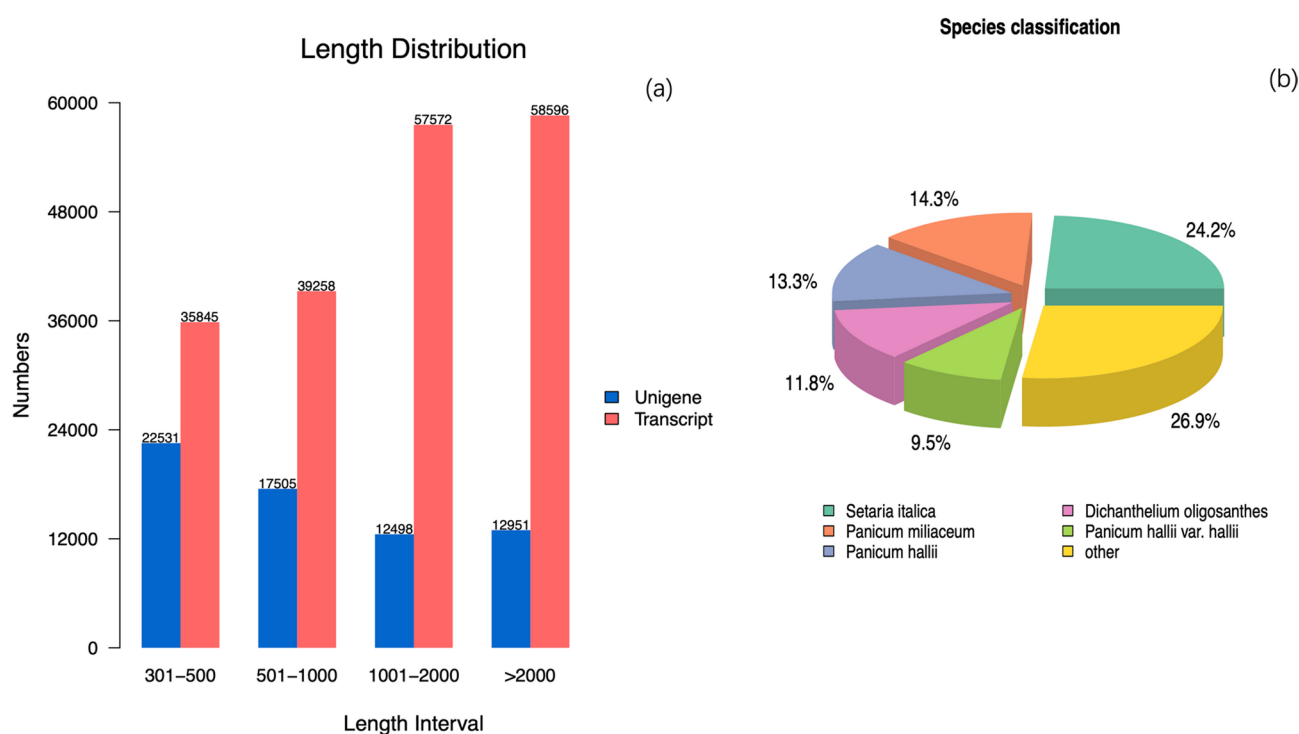


Fig. 4 Length distribution and species classification of transcriptome. **a** The unigenes and transcript length distributions; **b** Species classification according to the NR (NCBI Nonredundant Protein Sequences) database annotation. Those numbers near the pie charts refer to the percentage of unigenes in each category

downregulation (Fig. 5a). This indicates a substantial reprogramming of the transcriptional landscape in response to early-stage waterlogging stress. Notably, the number of DEGs decreased significantly at day 7 of waterlogging (WB vs. CB), with 1295 DEGs detected, including 654 upregulated and 641 down-regulated genes (Fig. 5b). This reduction suggests that the plant may have initiated adaptive mechanisms to mitigate the effects of prolonged waterlogging. To further explore the dynamics of gene expression changes, a comparative analysis was performed using a Venn

diagram approach. The results revealed that 35,739 genes were commonly expressed under both WA and CA conditions, indicating a core set of genes that remain consistently expressed regardless of waterlogging stress. However, 13,706 and 8,268 genes were uniquely differentially expressed in WA and CA, respectively (Fig. 5c). Similarly, in the comparison between CB and WB, 9,318 unique DEGs were identified in the CB group, and 7,105 unique DEGs were specific to the WB group. Importantly, 39,013 DEGs were shared between CB and WB groups (Fig. 5d).

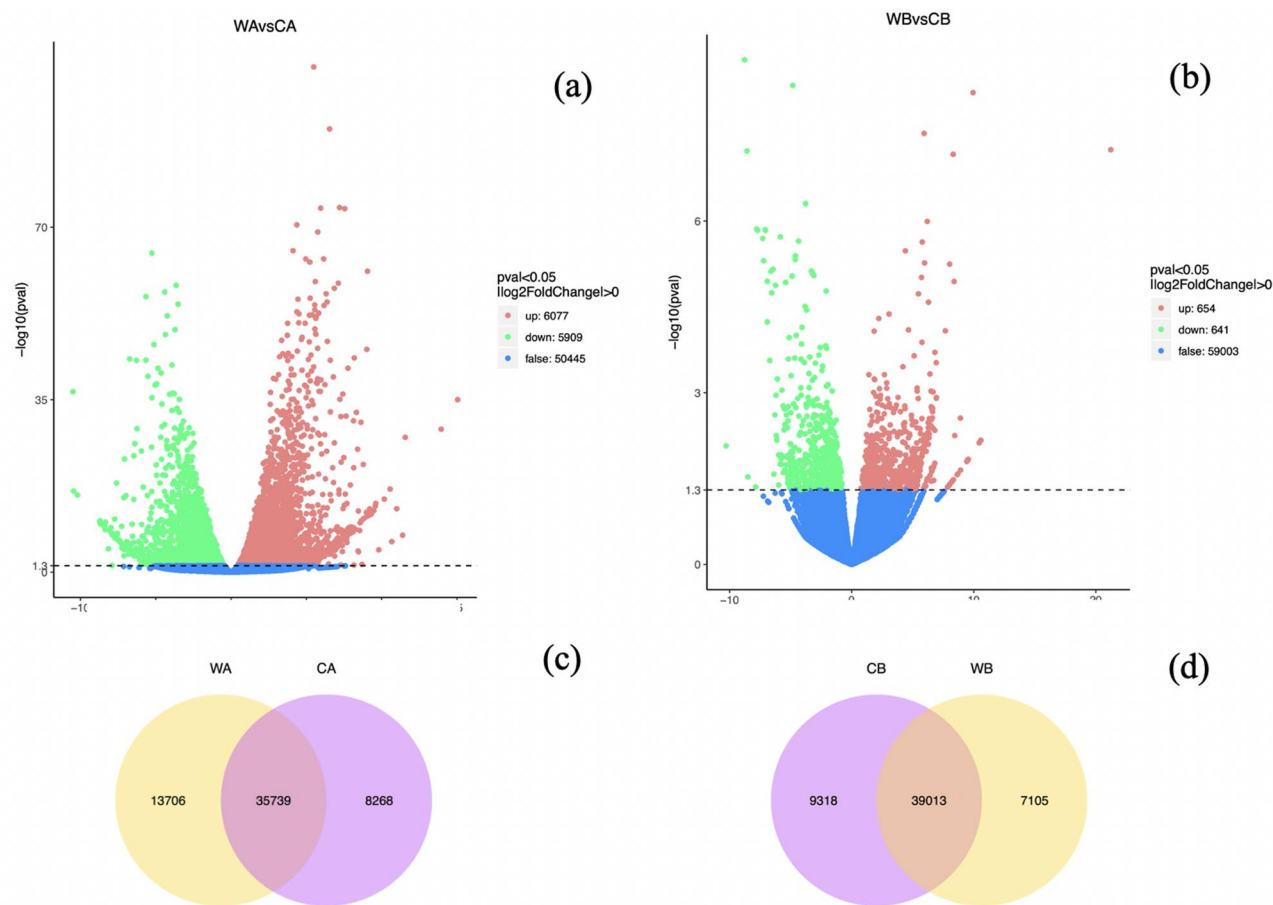


Fig. 5 The analysis of statistics of differentially expressed genes in the two comparison groups (WA vs. CA and WB vs. CB). The volcano plot of DEGs in WA vs. CA (a) and WB vs. CB (b). Significantly down-regulated and up-regulated DEGs were indicated by green and red dots, respectively ($\text{padj} < 0.05$). Venn diagram showing genes expressed under waterlogged conditions in the two comparison groups (c, d)

KEGG (Kyoto encyclopedia of genes and genomes) enrichment of DEGs

The annotation results of 13415DEGs between waterlogging and control groups were categorized according to the types of pathways in KEGG which were classified into five major categories of specific KEGG pathways, including cellular processes (1415DEGs, 10.55%), environmental information processing (1387DEGs, 10.34%), genetic information processing (2415DEGs, 18.00%), metabolic mechanisms (5952DEGs, 44.37%), and tissue systems (2246DEGs, 16.74%). Among them, 1290, 1048 and 1040 DEGs are annotated to the Signal transduction, Carbohydrate metabolism and Translation pathways, respectively (Fig. 6a). As detailed in Fig. 6b, c, the top 20 pathways enriched for DEGs in WA vs. CA and WB vs. CB comparisons, of which 5 pathways including Stilbenoid, diarylheptanoid and gingerol biosynthesis; Metabolic pathways; Flavonoid biosynthesis; Carbon fixation in photosynthetic organisms and Biosynthesis of secondary metabolites, are the same in two comparisons.

Among them, the DEGs in both comparison groups were significantly enriched in metabolic pathways.

DEGs related to anaerobic respiration, Gibberellin synthesis and ethylene synthesis

Based on the biological functions of DEGs and the processes associated with waterlogging stress, we selected pathways related to waterlogging to analysis the different regulatory networks in the two comparison groups. Anaerobic respiration, gibberellin synthesis and ethylene synthesis pathways played important roles in plant response to waterlogging stress. Therefore, we analyzed six genes in each pathway for expression changes in two comparison groups. Cluster-21538.18870 encoded ADH, which is a key enzyme in the respiratory process significantly downregulated in the WA vs. CA comparison group and significantly up-regulated in the WB vs. CB. Cluster-21538.31581 encoded KS is involved in gibberellin synthesis, and this gene was significantly upregulated in both comparison groups.

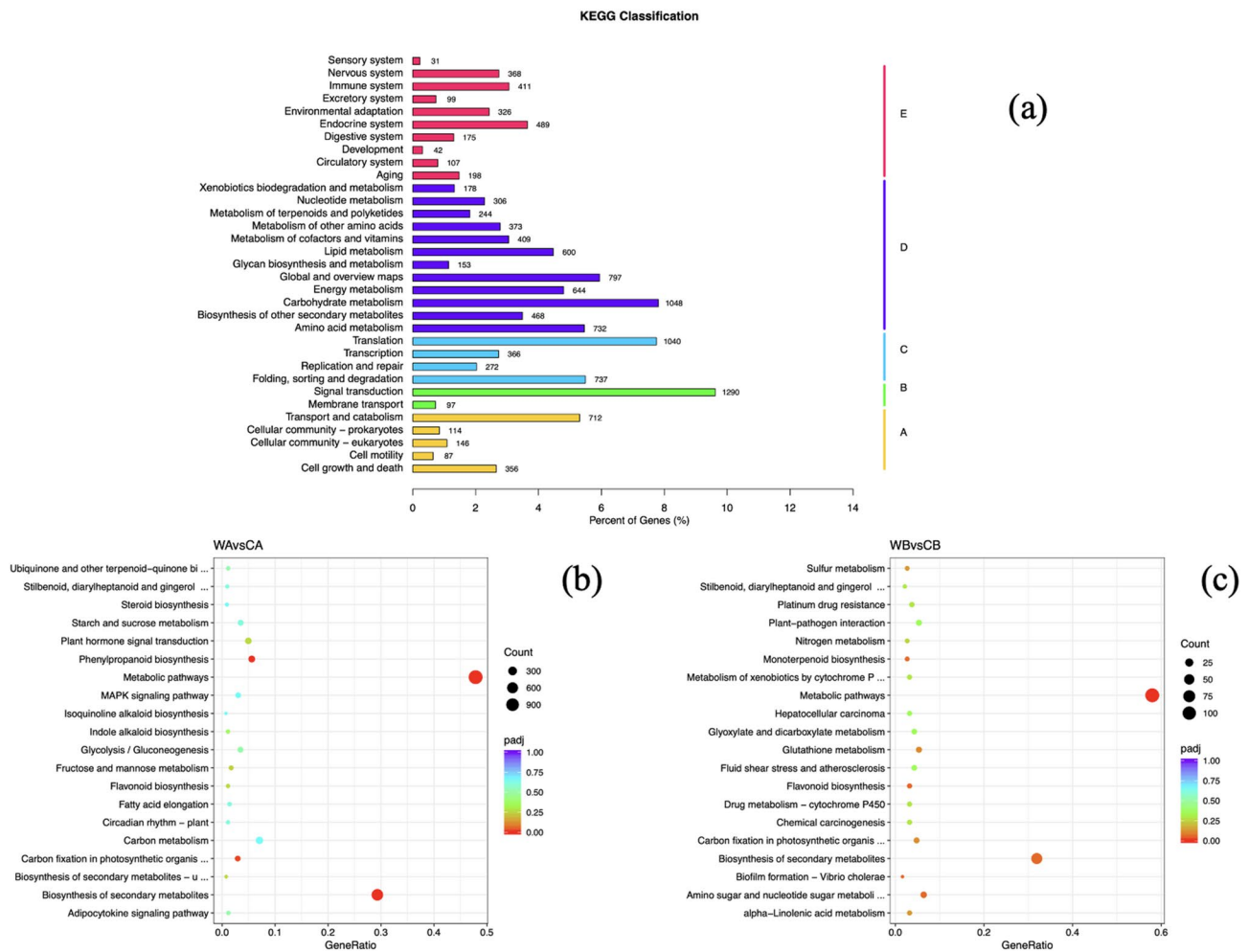


Fig. 6 KEGG enrichment analysis of the DEGs. **a** Classification map of differentially expressed gene KEGG A: Cellular processes B: Environmental information processing C: Genetic information processing D: Metabolic mechanisms E: Organizational systems. **b** Top 20 enriched pathways of DEGs in WA vs. CA. **c** Top 20 enriched pathways of DEGs in WB vs. CB

The expression of Cluster-21538.16638, encoding ACO, was significantly up-regulated at both stages of waterlogging. However, Cluster-21538.15371, also encoding ACO, was significantly upregulated in WA vs. CA and downregulated in WB vs. CB. Except for the above four genes, the expression of the remaining genes is significantly up-regulated in WA vs. CA and down-regulated in WB vs. CB, but the decrease was not significant (Table 2).

Validation of DEGs from RNA-seq by RT-qPCR

To validate the accuracy of gene expression patterns analyzed by RNA-seq, six related genes were selected for RT-qPCR analysis. As shown in Fig. 7A, among which the expression levels of five genes (*LDH*, *GA20ox*, *GA2ox-1*, *SAM-synthase*, *ACO-5*) were induced at 1 DAT (CA vs. WA), and these gene were reduced at 7 DAT (CB vs. WA). The *ACO-1* gene was induced after waterlogging. These results were

consistent with the RNA-seq results with a similar expression of these DEGs, indicating that the transcriptome data were reliable for subsequent analysis.

Hormones regulate the waterlogging tolerance of the *P. bisulcatum*

According to the results of RNA-seq, Gibberellin (GA) synthesis pathways mediate waterlogging tolerance in *P. bisulcatum*. Seedlings completely submerged under water were treated with exogenous GA and their synthesis inhibitors, paclobutrazol (PBL), to observe the aboveground growth (Fig. 7B). Compared to waterlogging condition (WL), exogenous GA application significantly promoted plant height from 4.1 cm to 7.6 cm and fresh weight from 0.03 g to 0.06 g, suggesting that exogenous GA promotes elongation of seedling stem internodes. After the application of PBL, the plant height of seedlings decreased to 0.17 cm and the fresh weight decreased to 0.02 g, indicating that PBL inhibited GA

Table 2 DEGs significantly expressed in WA vs. CA and WB vs. CB comparison settings

Pathways	Gene ID	Log ₂ FC (WA vs. CA)	Log ₂ FC (WB vs. CB)	Encoded Protein	Description
Anaerobic respiration	Cluster-21538.29506	4.89	−0.41	ADH	Alcohol dehydrogenase
	Cluster-21538.9197	3.88	−0.53		
	Cluster-21538.18870	−3.46	1.38		
	Cluster-21538.7142	2.81	0.65	PDC	Pyruvate decarboxylase
	Cluster-21538.21325	2.89	0.00		
	Cluster-21538.19349	1.04	−0.12	LDH	Lactate dehydrogenase
Gibberellin synthesis	Cluster-21538.31581	7.98	2.65	KS	Ent-kaurene synthase
	Cluster-21538.9011	2.78	−0.09		
	Cluster-21538.7110	3.15	0.85	GA13ox	Gibberellin13 oxidase
	Cluster-21538.16609	1.58	−0.98	GA20ox	Gibberellin20 oxidase
	Cluster-21538.1263	3.32	−0.88	GA2ox	Gibberellin2 oxidase
	Cluster-21538.8196	3.18	−1.06		
Ethylene synthesis	Cluster-21538.24304	0.95	−0.21	SAM-synthase	S-adenosyl-L-methionine-synthase
	Cluster-21538.16638	1.12	1.28	ACO	1-aminocyclopropane-1-carboxylic acid oxidase
	Cluster-21538.15371	1.84	−2.37		
	Cluster-21538.8626	6.30	−0.93		
	Cluster-21538.25375	3.85	−0.45		
	Cluster-21538.19486	1.48	−0.26		

synthesis and seedlings were unable to adapt to the waterlogged conditions.

Discussion

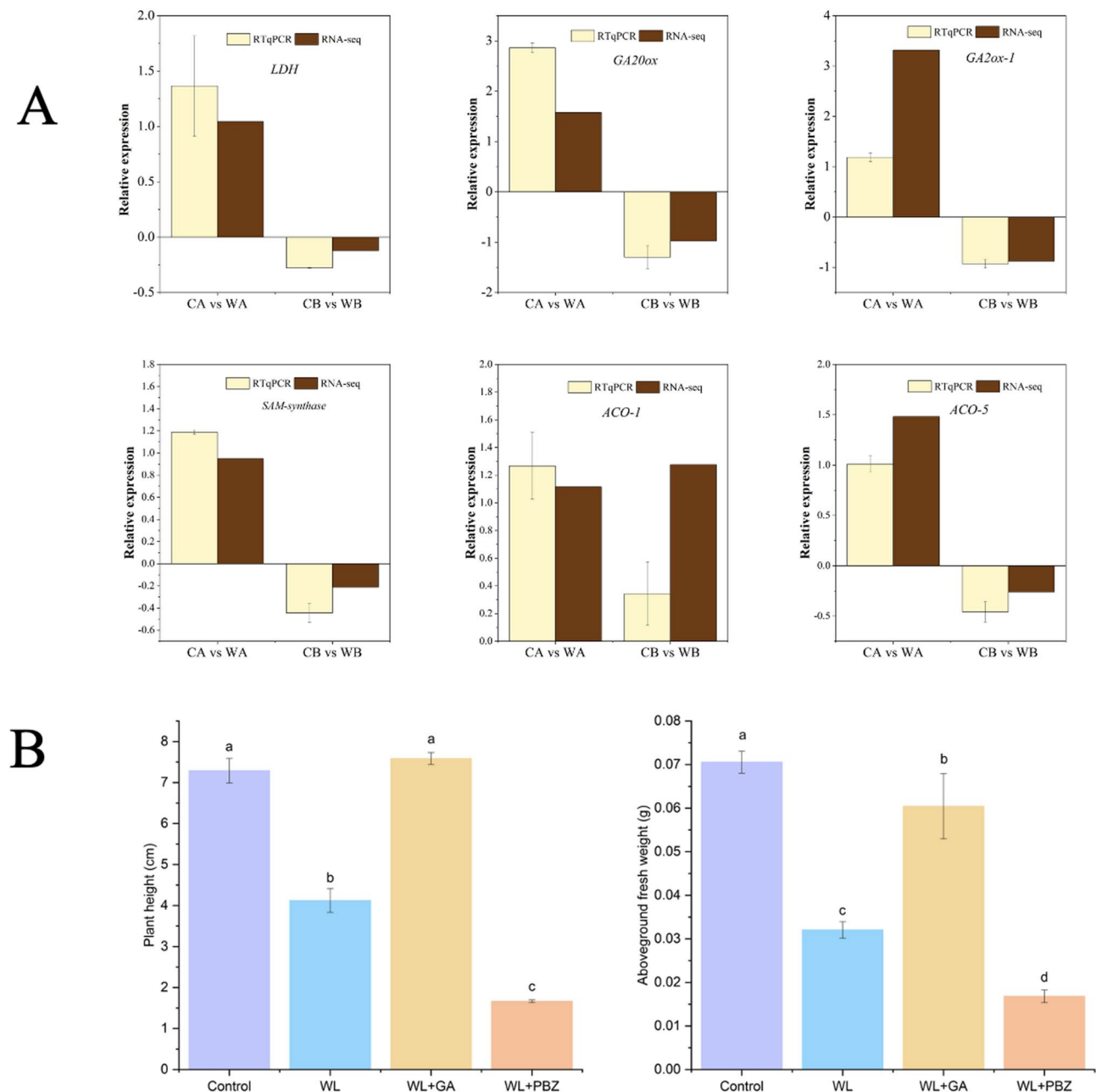
Morphological responses to waterlogging stress in *P. bisulcatum*

The increasing ability of some weeds to tolerate waterlogging has caused concern among rice farmers, as waterlogging is an important method of weed control. In the present study, we observed that depth (2 cm) of flooding had little effect on the growth of *P. bisulcatum* seedling due to breakthrough of the water surface, indicating that prolonged submergence could be avoided by an escape strategy. However, depth of waterlogging (4 cm) inhibited the growth of *P. bisulcatum* seedling with plant height and weight of 19.0% and 13.8% of the control treatment (0 cm), respectively, after 28 days of waterlogging. This result is similar to previous reports that waterlogging depth is usually negatively correlated with weed growth, and barnyardgrass (*Echinochloa crus-galli*), which is also a grass weed, had an average reduction of 26.82% in plant height and 74.95% in aboveground dry biomass under the 7.5 cm flooding depth treatment compared to saturated soil treatments [36]. Response strategies of plants to waterlogging depend on water depth, which determines the distance of plant shoots from the water surface [37]. The growth inhibition of *P. bisulcatum* by water depth (4 cm) may be due to the lower availability of oxygen and light energy reaching the submerged plant tissues [38, 39] and the reduction in photosynthetic rate [40]. The differential responses to water depth (2 cm vs. 4 cm) reveal a critical threshold for effective weed suppression. While shallow flooding (2 cm) allows *P. bisulcatum* to partially

recover through LOES mechanisms, deeper flooding (4 cm) overwhelms these adaptations, causing irreversible growth inhibition. This suggests that maintaining water levels above 4 cm in rice paddies could synergize with flood-tolerant rice varieties to suppress weeds without crop yield penalties.

Effect of waterlogging on anaerobic respiratory enzymes

Waterlogging disrupts physiological and metabolic homeostasis in plants, triggering adaptive responses such as glycolytic fermentation—a critical mechanism for energy maintenance under hypoxia [41]. In hypoxic environments, LDH catalyzes the conversion of pyruvate to lactate, transiently acidifying the cytoplasm [42]. Concurrently, PDC initiates the alcoholic fermentation pathway by converting pyruvate to acetaldehyde, which is subsequently reduced to ethanol via ADH. This dual fermentation system mitigates pyruvate accumulation, facilitates NAD⁺ regeneration, and sustains substrate-level ATP production under oxygen deprivation [43]. In *P. bisulcatum*, we observed a rapid induction of fermentation enzyme activities following waterlogging. LDH activity increased within 12 h of stress exposure, preceding the upregulation of ADH and PDC activities, which were higher than those in control group at 1 day post-treatment. These findings align with prior reports in flooded plants, where ADH/PDC-mediated ethanol fermentation (catalyzed by ADH and PDC) [44–46] and LDH-driven lactate production [47–49] are conserved responses to oxygen limitation. The observed enzymatic dynamics align with conserved biomarkers of anaerobic metabolism. ADH activity strongly correlates with ethanol accumulation during hypoxia [50], while LDH-driven



lactate production stabilizes cytoplasmic pH under prolonged submergence [47]. Although ethanol and lactate levels were not quantified here, the temporal patterns of ADH and LDH activities in *P. bisulcatum* mirror metabolite dynamics reported in flood-tolerant species [51], suggesting conserved metabolic trade-offs. The concurrent activation of both pathways in *P. bisulcatum* underscores

their complementary roles in metabolic acclimation: while lactic acid fermentation provides an immediate response to acute hypoxia, alcoholic fermentation may dominate during prolonged stress to minimize cytoplasmic acidosis. This dual strategy highlights the evolutionary optimization of energy conservation mechanisms in waterlogging-tolerant species.

Effect of waterlogging on the contents of MDA and antioxidant enzymes

Lipid peroxidation generates MDA, and the level of MDA reflects the degree of oxidative damage caused by reactive oxygen species (ROS) in the tissue [52]. The results of the current study revealed a marked elevation in MDA content in *P. bisulcatum* compared to the control group, suggesting substantial ROS accumulation, enhanced lipid peroxidation, and increased cell membrane permeability. Compared to 1 day of waterlogging, plants treated with 7 days of waterlogging demonstrated a reduced lipid peroxidation effect, as evidenced by their significantly lower MDA content. This observed reduction may be attributed to the partial emergence of plants from the water layer by the seventh day, allowing some leaves to be exposed to the air. This exposure likely increased oxygen availability, thereby supporting normal respiratory and metabolic processes. The improved oxygen supply likely contributed to the stabilization of cellular membrane structure and function, ultimately reducing the extent of membrane lipid peroxidation. After waterlogging treatment, the activities of antioxidant enzymes (SOD, POD, and CAT) in *P. bisulcatum* seedlings exhibited an increasing trend. The activities of these antioxidant enzymes in the waterlogged group were significantly higher compared to the control group at 1 DAT and 7DAT. The increase in enzyme activities is closely associated with the accumulation of reactive oxygen species (ROS) under stress conditions. Similar phenomena have been reported in rice [53], wheat [54] and onion [55], where the activities of SOD, CAT, POD, and APX were significantly elevated after waterlogging treatment. Additionally, studies have shown that the activity of catalase (CAT) in ramie continuously increases under waterlogging condition [56, 57]. The enhanced activities of these antioxidant enzymes contribute to the reduction of ROS-induced cellular damage [58]. The rapid induction of antioxidant enzymes and anaerobic respiration genes in *P. bisulcatum* under waterlogging underscores a dual survival strategy: (1) mitigating oxidative damage through ROS scavenging, and (2) sustaining energy production via fermentation pathways under hypoxia. The transient surge in MDA levels at 1 DAT (Fig. 3) reflects acute membrane peroxidation, which is subsequently alleviated by elevated antioxidant activity, mirroring adaptive responses in flood-tolerant crops like rice [53].

DEGs involved in anaerobic respiration, Gibberellin synthesis and ethylene synthesis under waterlogging condition

Waterlogging induces significant transcriptional changes in plants, particularly in genes associated with anaerobic respiration. In this study, we observed that *P. bisulcatum* upregulated key response genes, including PDC, ADH,

and LDH (except ADH3), at 1 DAT (Fig. 8). These findings indicate that *P. bisulcatum* enhances anaerobic respiration to metabolize amino acids and carbohydrates, thereby accumulating energy reserves to cope with hypoxic conditions. Such transcriptional adjustments under waterlogging stress have been previously documented in cucumber [59], cotton [60], and soybean [61]. However, by 7 DAT, gene expression returned to baseline levels, likely due to the plants breaking through the water surface and resuming aerobic respiration.

Gibberellins (GAs) play a pivotal role in regulating plant growth and stress responses, particularly under waterlogging conditions [62]. Our study revealed that six GA biosynthesis-related genes were upregulated in response to waterlogging, underscoring the close association between GA signaling and low-oxygen stress adaptation. This aligns with previous findings in soybean, where GA content increased significantly in waterlogging-tolerant genotypes [63]. Exogenous gibberellin (GA) application has been shown to enhance plant resilience under waterlogging by modulating reactive oxygen species (ROS) levels and improving yield. This aligns with findings in *Glycine max* (soybean), where GA application significantly ameliorated the adverse effects of short-term flooding by promoting stem elongation and reducing oxidative stress [64, 65]. Specifically, GA enhances hypoxia escape through internode elongation, a strategy observed in flood-tolerant species such as lotus (*Nelumbo nucifera*). In lotus, submergence-induced petiole elongation is regulated by synergistic interactions between ethylene, GA, and abscisic acid (ABA), where GA counteracts ABA-mediated growth suppression and synergizes with ethylene to activate cell wall loosening enzymes [66]. Notably, GA-responsive genes such as ACE1 and DEC1 regulate internode elongation in rice, with ACE1 promoting growth and DEC1 acting as a negative regulator [67]. In this study, six genes involved in gibberellin synthesis were up-regulated after waterlogging. This evidence demonstrated that GA is closely associated with LOES pathways (Fig. 8).

Hattori et al. (2009) obtained two ethylene-inducible genes, SNORKEL1 (SK1) and SNORKEL2 (SK2), by mapping QTL for internode elongation on chromosome 12. SK1 and SK2 are two transcription factors encoding ethylene-responsive factors that regulate internode elongation through ethylene-induced GA synthesis [68]. OsEILa, another ethylene-responsive gene in deepwater rice, can activate SD1 to induce a rise in GA4 content, thereby inducing internode elongation [22]. In this study, both SAM-synthesis and ACO, key enzyme genes involved in the ethylene synthesis pathway (Fig. 8), were up-regulated after waterlogging, suggesting that ethylene plays an important role in the adaptation of myrmecophyte to waterlogging stress.

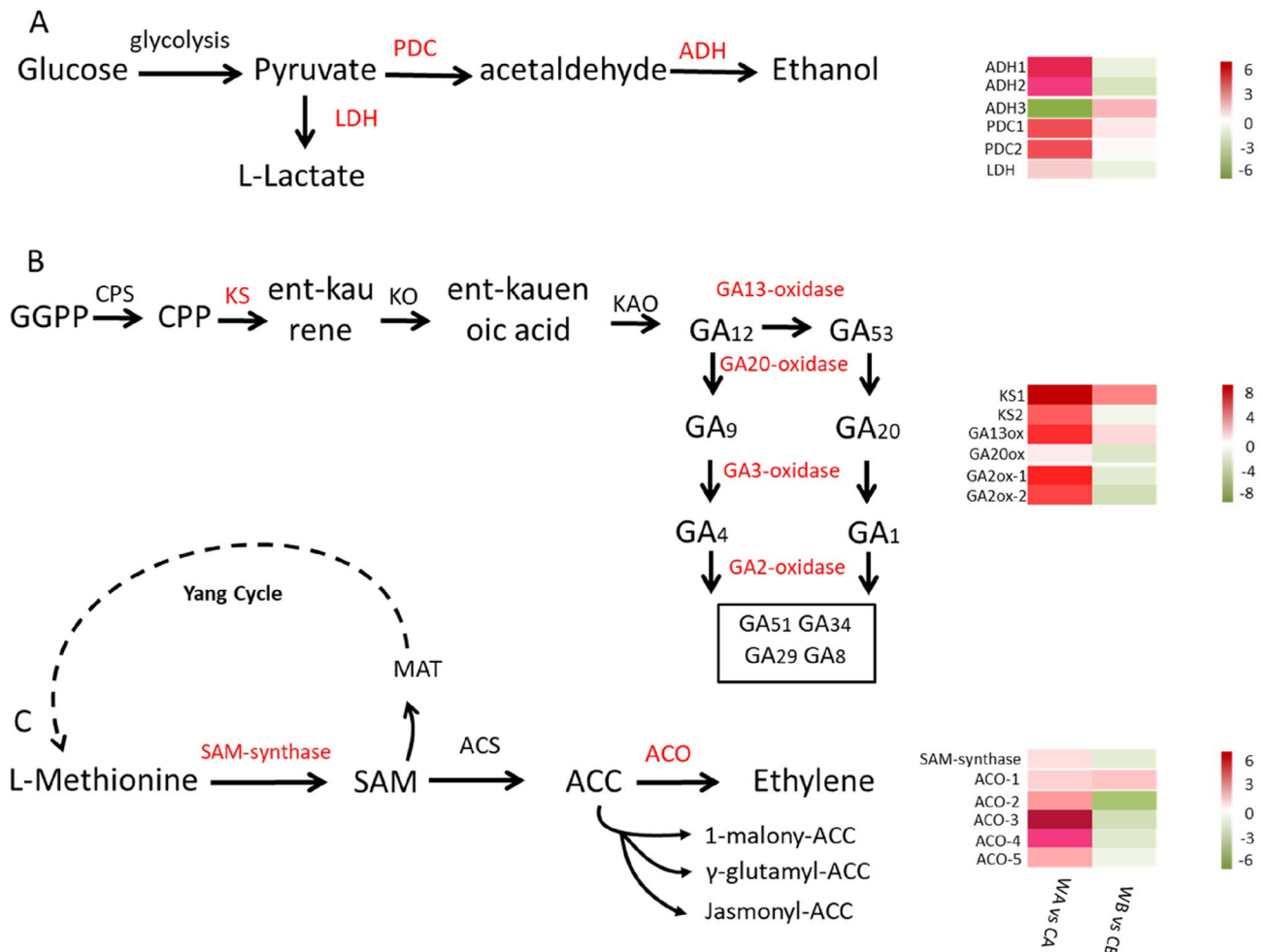


Fig. 8 Visualization of the key DEGs involved in anaerobic respiration (A), Gibberellin synthesis (B) and ethylene synthesis (C) pathway. The scale bar represents fold change of up- (red) and down- (green) regulation. The color of boxes represents Log₂ (fold change) values based on the transcriptome results

Targeting key nodes in ethylene/gibberellin crosstalk may further disrupt weed-specific stress responses, offering a dual approach—environmental control and molecular intervention—to enhance rice productivity in flood-prone regions.

Conclusions

This study systematically explored the impact of varying water depths on the growth dynamics of *P. bisulcatum*, with a particular focus on its physiological adaptations to waterlogging stress under a 2 cm water depth. Furthermore, leveraging high-throughput transcriptome sequencing, we deciphered the molecular mechanisms underpinning response of *P. bisulcatum* to waterlogging stress, providing a comprehensive understanding of its adaptive strategies at the genetic level. *P. bisulcatum* exhibits adaptive capacity to waterlogging stress at a 2 cm water depth (Figure S2). During the early stages of stress, malondialdehyde (MDA) levels in seedlings increase, but the damage induced by stress can

be mitigated through the upregulation of antioxidant enzyme activities. Transcriptomic analysis reveals that *P. bisulcatum* likely responds to waterlogging stress by upregulating genes associated with anaerobic respiration, as well as ethylene and gibberellin biosynthesis. Collectively, the physiological and transcriptomic data presented in this study provide novel insights into the mechanisms underlying the response *P. bisulcatum* to waterlogging stress and offer a scientific foundation for leveraging waterlogging as a strategy to control *P. bisulcatum* in rice paddies. By unraveling the waterlogging-tolerance mechanisms of *P. bisulcatum*, this study highlights pathways to enhance rice tolerance under waterlogging. Insights into conserved stress-responsive genes and hormonal regulation can guide breeding strategies for developing flood-resistant rice varieties. Coupling such genetic advancements with improved agricultural practices could mitigate yield losses in flood-prone regions, bolstering global food security amid climate challenges.

Abbreviations

ADH	Alcohol dehydrogenase
PDC	Pyruvate decarboxylase
LDH	Lactate dehydrogenase
MDA	Malondialdehyde
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
TUB	Beta-tubulin
EIF4A	Eukaryotic initiation factor 4 A
DEGs	Differentially expressed genes
CDS	Coding sequences
RT-qPCR	Quantitative real-time PCR
GA	Gibberellic acid
PA	Paclobutrazol

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-06874-3>.

Supplementary Material 1

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Authors' contributions

J.Q.S: conceptualization, Investigation and manuscript writing and editing. X.Y.Y, Y.Y.J, H.X.X and M.J.L: Formal analysis and Investigation. Z.X.L and W.T: Funding acquisition, conceptualization, Investigation, draft designing and revising, supervision. All authors have reviewed and agreed to the final version of the manuscript.

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Data availability

Data will be made available on request and the raw sequencing data from this study have been deposited at the NCBI under BioProject ID PRJNA1244082.

Declarations

Ethics approval and consent to participate

Our study was conducted in full compliance with local regulations. This article did not involve any studies with human, animals, or endangered species. The collection of plant materials and the experimental procedures conducted in this study adhered to institutional, national and international guidelines and legal requirements.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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