Current Science
CS 5 (5), 4715-4729 (2025)
Received 10 July 2025 | **Revised 13 August** 2025 | **Accepted 11 September** 2025 | **Online Available 08 October** 2025
https://doi.org0/10.52845/CS/2025-5-5-30

Current Science
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OPEN ACCESS
ISSN(O)2795-8639

Research Article

Growth Physiology and Transcriptome Analysis of Alpine Wet Plants in Response to Flooding Stress

Xiaoyan Su[1], Yu Zhan[2], Changhui Li[1,2,3]

[1] Qinghai Academy of Animal Husbandry and Veterinary Sciences, Qinghai University;

[2] College of Agriculture and Animal Husbandry, Qinghai University ;

[3] Key Laboratory of Alpine Grassland Ecology, Ministry of Education, Sanjiangyuan Region, Xining 810016, Qinghai, China

***Corresponding Author: Changhui Li**

Abstract:
This study focuses on five dominant alpine plants, namely *Halerpestes tricuspis*, *Hippuris vulgaris L*, *Trichophorum distigmaticum*, and *Poa psiolepis*, to explore the adaptive mechanisms of alpine hygrophytes to waterlogging stress. These plants were selected as the research subjects. After 60 days of waterlogging stress treatment, their adaptability was evaluated through morphological observation, determination of physiological and biochemical indicators, and transcriptome sequencing analysis. The results showed that: (1) Waterlogging stress caused significant leaf yellowing in all species except Sarcosum (note: this species was not mentioned in the earlier list and needs clarification), and the root-crown ratio of Pedicularis chinensis (note: this species was not mentioned in the earlier list and needs clarification) increased significantly (P<0.05); (2) The chlorophyll a/b content decreased significantly, while the carotenoid content increased; the MDA content increased by 1–3 folds; the activities of SOD and POD increased, but the activity of CAT decreased; (3) Interspecific differences were observed in osmotic regulatory substances while increasing in Ranunculus triphyllus (note: this species was not mentioned in the earlier list and needs clarification)); (4) Transcriptome analysis revealed significant enrichment of the glycolysis, glutathione metabolism, and ethylene signalling pathways (with key genes such as ADH, GST, and ERF1). These results indicate that alpine wetland plants exhibit strong adaptability to waterlogging stress by coordinately regulating the stability of photosynthetic structures , antioxidant systems, and osmotic balance. This study provides a basis for species screening in the restoration of alpine wetland vegetation.

Key words: **Alpine wetland plants**, **Flooding stress**, **Growth physiology response**, **Transcriptome analysis**

1. Introduction

Alpine wetlands are a vital component of the Tibetan Plateau's ecosystem, playing a key role in maintaining ecological balance and biodiversity. Their unique plant communities are essential for regional climate regulation and water conservation[1]. However, climate change and human activities have led to frequent flooding events in alpine wetlands, which seriously threaten the survival of wetland plants and the functioning of ecosystems[2]. Understanding how alpine wetland plants adapt to flooding stress could provide a theoretical basis for protecting and restoring alpine wetland ecosystems. It could also offer technical support for selecting and breeding flood-tolerant forage grass varieties, as well as for restoring degraded grasslands, which holds great ecological significance and practical applications[3,4]. Flooding stress is characterised by excessive soil moisture and reduced soil oxygen content, both of which affect plant growth and metabolism[5].Under flooding stress, mineral transport in plant root systems is impeded, resulting in nutrient deficiencies and reduced chlorophyll content[6,7]. However, plants exhibit a series of physiological responses to adapt to flooded environments[8], such as altered photosynthetic capacity, significant changes in the content of osmoregulatory substances, and activation of antioxidant enzyme systems. Nevertheless, different plant species respond differently to the same flooding conditions[9,10].Under short-term flooding stress, different species show distinct responses to the same degree of flooding, and the same species responds differently to varying degrees of flooding[11]. The extent to which long-term flooding inhibits plant growth varies among grass species, and to a certain extent, it can even promote the growth of certain plants[12]. For example, Flocco et al[13]. studied different alfalfa varieties and found that their growth rates first increased and then decreased under short-term flooding.Currently, research on the adaptation of alpine swamp wetland plants to flooded environments is scarce. Only one study has reported differences in leaf functional traits and chlorophyll fluorescence parameters among different alpine wetland plants under varying water gradients[14]. Additionally, José et al[15,16].investigated changes in morphological characteristics and biomass allocation of Qinghai-Tibetan tussocks along water gradients in alpine wetlands. Since there have been no studies on the physiological and biochemical adaptations of plants in alpine swamp wetlands, it is necessary to conduct such research on representative wetland plant species under flooded conditions to further our understanding of their response strategies.Currently, most studies on plant responses to flooding stress focus on crops such as maize[17], trees such as poplar [18], and herbaceous plants [19], among others. However, there remains a lack of research targeting alpine wetland plants. Revealing the morphological adaptive characteristics and physiological response mechanisms of alpine wetland plants under flooding stress is crucial for clarifying the effects of flooding stress on these plants and establishing a flooding tolerance evaluation system. This would provide a theoretical basis for the ecological protection and restoration of alpine wetlands.

1. Materials and Methods

1.1 Overview of the study area

This experiment was conducted in Zeku Zequ National Wetland Park, Huangnan Prefecture, Qinghai Province, China (34°55′–35°15′N, 101°25′–101°45′E). The ecosystem of this wetland park is a typical example of an alpine wetland ecosystem on the Tibetan Plateau. It is situated in the Zequ River Basin, which is one of the sources of the Zequ River and an important upper reach of the Yellow River. The area has a typical plateau continental climate, characterized by low temperatures, oxygen deficiency, and strong solar radiation. It encompasses various wetland types, including permanent rivers, herbaceous swamps, and swampy meadows, with rich biodiversity. The average elevation of the area is approximately 3,600 m. Located at the eastern edge of the alpine wetland distribution area on the Tibetan Plateau, the park serves as an important ecological corridor connecting the Sanjiangyuan Nature Reserve and the Ruoergai Wetland. This corridor plays a vital role in maintaining regional ecological balance, protecting biodiversity,and regulating the climate[20].

1.2 Experimental materials

In this study, five alpine wetland plants were selected as experimental materials, namely *Halerpestes tricuspis*, *Hippuris vulgaris L*, *Trichophorum distigmaticum*, *Pedicularis chinensis*, and *Poa psiolepis Keng*. Additionally, another five alpine wetland plants were used as test materials.

1.2.1 Experimental methods

In June 2024, specimens were collected from Zeku Zequ National Wetland Park in Zeku, Qinghai Province. These were then manually transplanted and indoor experiments were conducted. Acclimatisation, cultivation and observation were also conducted in the Agricultural and Animal Husbandry Experimental Building at Qinghai University. After 60 days of flooding stress, the relevant growth and physiological indexes were measured. Normal water management measures were carried out during this period. One week later, the flooding treatment was implemented: the plants in the treatment group (MW) were submerged 3 cm above the soil. Three replicates were set up for each treatment group in the experiment. Ten plants were used for each replicate measurement. The control group (CK) was cultivated under the same conditions as the flooded group, with the same water management. Three plants were randomly selected as biological replicates and the treatment was carried out for 60 days. After 60 days, the healthy, mature plants were collected separately. After 60 days of treatment, 10 g of healthy, mature aboveground tissue was collected from each plant for measurement of the physiological index. A total of 15 samples were sent to Shanghai Meiji Biotechnology Co. for transcriptome sequencing and analysis.

1.2.2 Determination of growth indexes

Measure the plant height and ground diameter respectively before and at the end of the treatment. The plant height was measured with a steel tape measure, accurate to 0.1 cm, and the ground diameter was measured with a vernier caliper, accurate to 0.01 cm.

1.2.3 Determination of photosynthetic pigment content

0.1 g of freshly cut leaves were placed in a 10 ml centrifuge tube and 10 ml of a 1:1 mixture of 95% ethanol and acetone was added. The leaves were then treated with light for 24 hours until they became colourless and the absorbance was measured at wavelengths of 665 nm, 470 nm and 649 nm, respectively. The chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl) and carotenoid content were then calculated using the formula for chlorophyll content.

1.2.4 Determination of antioxidant enzyme activity indexes

The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in leaves were determined using NBT photochemical reduction, guaiacol and hydrogen peroxide deoxygenation, and UV spectrophotometry, respectively.

1.2.5 Determination of osmoregulatory substances

The concentrations of soluble sugars (SS), soluble proteins (SP) and proline (Pro) were determined using the anthrone method, protein quantification kit and ninhydrin colourimetric method, respectively.

1.2.6 Measurement of membrane lipid peroxidation indices

The malondialdehyde (MDA) content was measured using the thiobarbituric acid method.

1.2.7 Quality control and comparative analysis of transcriptome sequencing data

To obtain high-quality transcriptome data, Fastp was used to perform quality control on the raw data and filter out the low-quality data. Sequences containing junctions and poly-N were removed, as were low-quality sequences and those with an N ratio greater than 10%. The Q20 and Q30 values, as well as the GC content, were then calculated for the clean reads.

1.2.8 Real-time fluorescence quantitative PCR validation

To test the accuracy of the transcription data, total RNA was extracted from leaves and reverse transcribed into cDNA using a MonScript™ RTIII All-in-One First Strand cDNA Synthesis Kit. The consistency of the transcriptome data with the qRT-PCR results was then tested using fluorescence quantitative PCR. Consistency. Primer Premier 5.0 software was used to design primers for the fluorescence quantitative PCR assay (Table 1). *β-actin* was used as the internal reference gene and relative expression levels were calculated using the $2^{\wedge(-\Delta\Delta)Ct}$ method and plotted using GraphPad Prism 8.

Table 1 Primers used for fluorescence quantitative PCR detection

Genes	Forward Primer (5'→3')	Reverse Primer (3'→5')
β-actin	GTTGGGATGGGGCAGAAAGA	TCTCCACGGCAGAACTGTTC
HcSOD2	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
HsERF1	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
HsADH1	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
PmLEA3	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
ScNAC1	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
ScAPX2	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG

PaPOD1	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG
PaARF7	TTCCCTAAACCCGAAACCGG	AATAGAGCATCGTCGAGGCG

1.3 Data processing

The SPSS 29.0 and Excel 20.0 software packages were used to analyse the significance of the experimental data. Graphs were created using Origin 2024 graphing software, and statistical significance was analysed using an independent samples t-test with a significance level of $P < 0.05$. The transcriptome was analysed using the BMKCloud online platform (www.biocloud.net). The results of the transcriptome sequencing were analysed using the BMKCloud online platform.

2. Results and analysis

2.1 The effects of flooding stress on the phenotypic morphology of alpine wetland plants

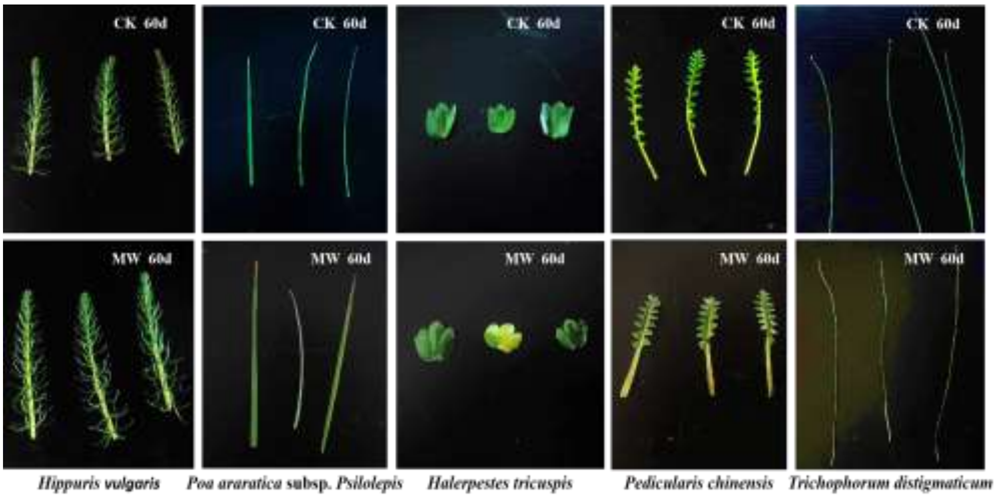
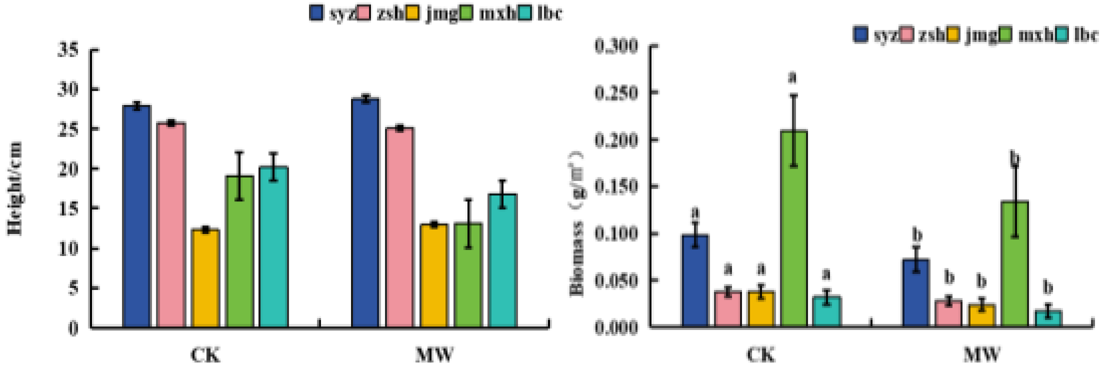


Figure 1 Leaf morphological changes of 5 species of alpine wetland plants under flooding stress

Significant adaptive changes in leaf blade morphology and structure occurred in five species of alpine hygrophilous plants under flooding stress conditions. As shown in Figure. 1, after 60 days of flooding treatment, the different species exhibited distinct morphological responses. The leaf colour of cedar leaf alga did not change significantly, but the leaf length increased compared with the control group and the acicular leaf shape became more pronounced. The leaves of Triticum, Alkali buttercup, Artemisia sinensis and Light-lemma Early-morning glory showed obvious chlorophyll degradation and were noticeably yellowed, accompanied by a significant reduction in the glossy surface of the leaf blade. The Two-stigma rushes exhibited typical yellowing symptoms and had obvious chlorophyll deficiency in their apical meristematic and leaf blade tissues. These morphological changes reflect the different species' adaptation strategies to flooding stress, with reduced chlorophyll content and leaf yellowing being common physiological responses of plants to an anaerobic environment.



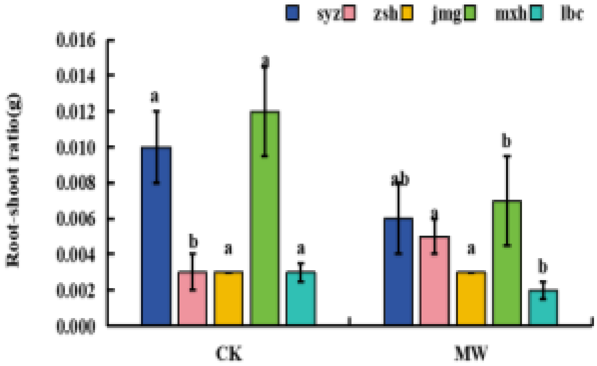


Figure 2 Changes in plant height and biomass of five alpine hygrophytes under waterlogging stress
Note: jmg: Halerpestes tricuspis; syz:Hippuris vulgaris L.; mxh:Pedicularis chinensis Maxim.; lbc:Trichophorum distigmaticum;zsh：Poa psiolepis Keng

As shown in Figure. 2, after 60 days of flooding stress treatment (MW),the plant height and biomass indexes of five wet plants, including S. cedarica, did not reach statistically significant levels (P>0.05) compared with the control (CK). However, the root-crown ratios of Artemisia sinensis exhibited significant differences (P<0.05) under the same treatment conditions.This phenomenon may be attributed to plants' adaptive mechanisms in response to hypoxic environments. Artemisia chinensis enhanced oxygen acquisition, nutrient absorption, and water uptake by increasing root biomass allocation. This maintains respiratory metabolism and root physiological functioning in aquatic environments. This suggests that A. chinensis can effectively adapt to flooding stress through significant changes in the root-crown ratio, ensuring its survival and growth in a long-term anoxic environment.

2.2 Changes in the pigments of waterlogged alpine plants

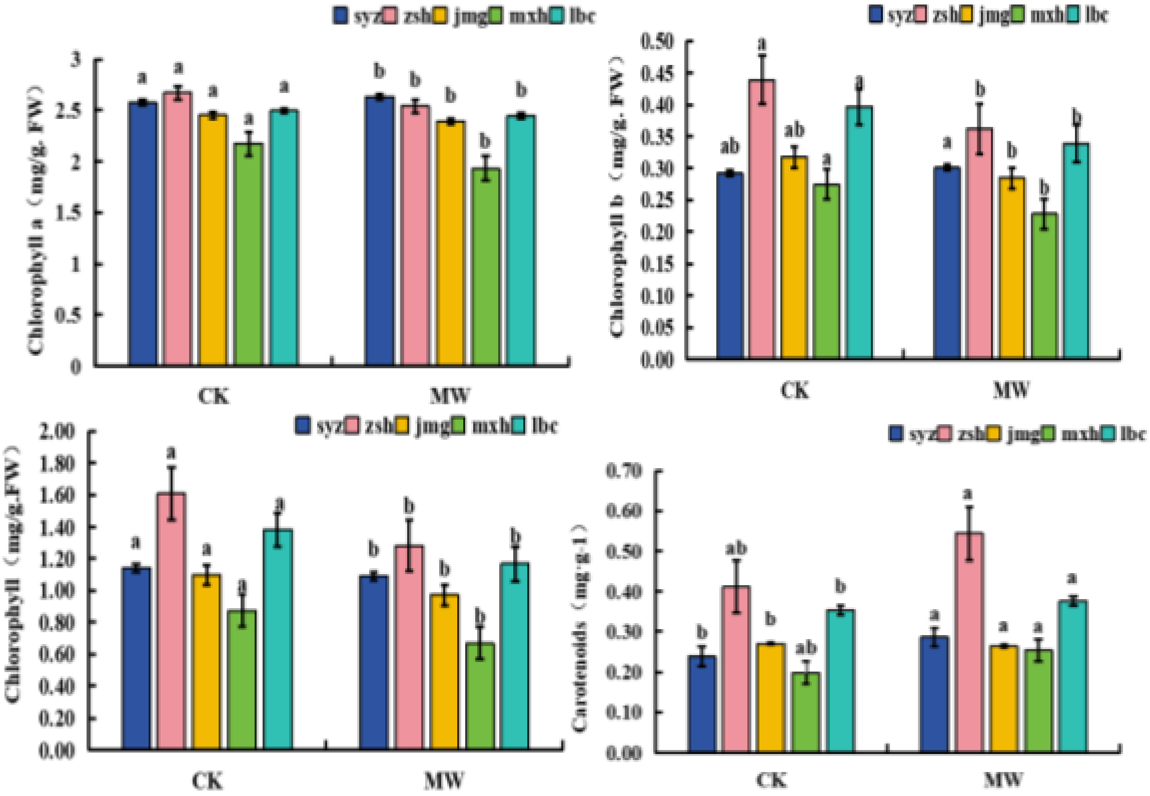


Figure 3 Changes in photosynthetic pigment content of five alpine wetland plants under flooding stress

As shown in Figure 3, the chlorophyll a, chlorophyll b and total chlorophyll content of five alpine wetland plants, including S. cedarica, gradually decreased after 60 days of flooding stress treatment (MW), showing significant differences (P<0.05). Flooding stress limits the photosynthetic efficiency of plants, resulting in impairment of photosystem II (PSII) function. The balance between the synthesis and degradation of chlorophyll, a key pigment of photosynthesis, was disrupted, causing a decrease in content. However, after 60 days of flooding stress, the carotenoid content of the plants increased significantly (P<0.05) compared with the control group. This may be because flooding stress blocks photosynthesis and causes excess light energy absorption. Carotenoids can dissipate this excess light energy through non-photochemical quenching, which prevents damage to PSII and enhances the adaptive ability of wet plants to flooding stress.

2.3 Changes in malondialdehyde content. Changes in antioxidant enzyme activities. In wet plants under flooding stress.

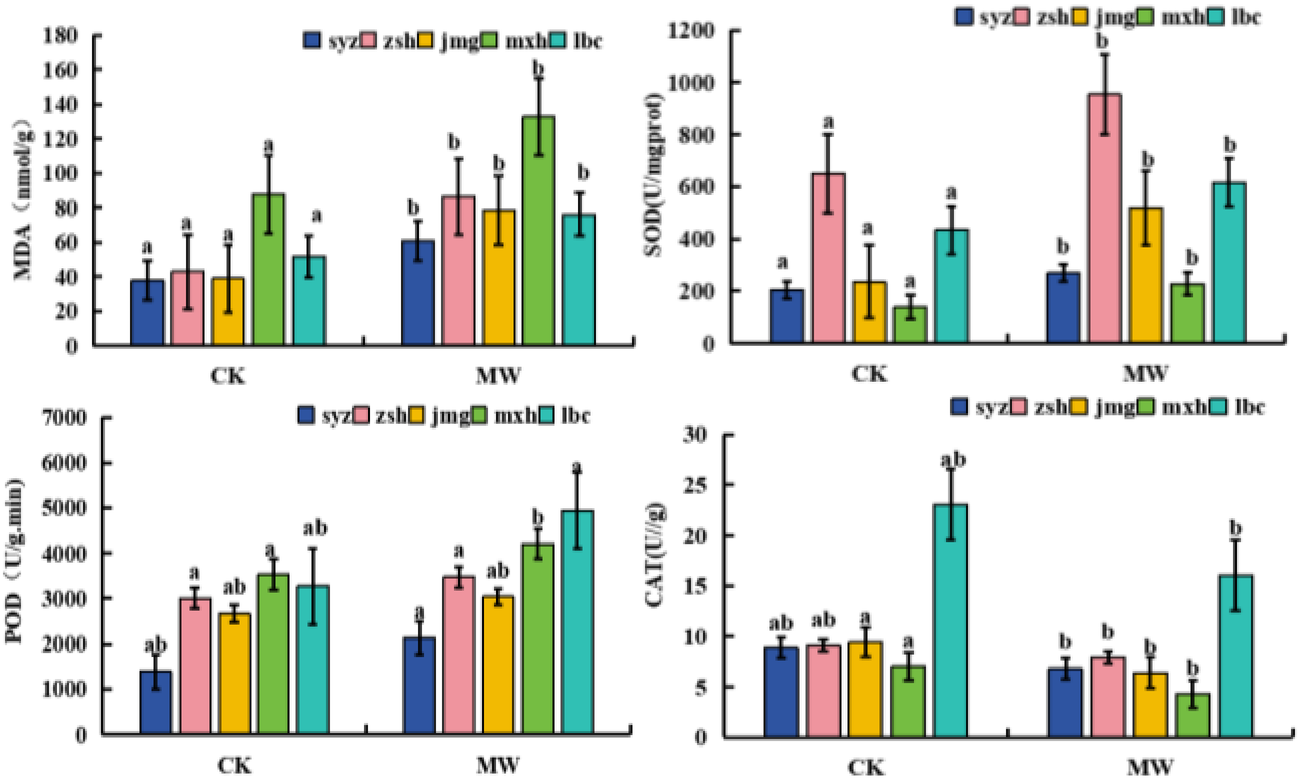


Figure 4 Changes of malondialdehyde content and antioxidant enzyme activity in 5 alpine wetland plants under waterlogging stress

As shown in Figure.4, the MDA, POD and SOD contents of five alpine wetland plants, including S. cinerea, gradually increased after 60 days of flooding stress treatment (MW) (P<0.05), while the CAT content decreased (P<0.05). This indicates that lipid peroxidation damaged the cell membrane due to the accumulation of reactive oxygen species (ROS).The increase in POD and SOD content reflects the plants' ability to scavenge ROS by enhancing antioxidant enzyme activity, thereby reducing oxidative stress. In contrast, the decrease in CAT content may be due to its activity or synthesis being inhibited by hypoxia, resulting in diminished hydrogen peroxide scavenging ability. Together, these changes reflect the physiological adaptation mechanisms of plants in response to hypoxia and oxidative stress in flooded environments.

2.4 Changes in osmoregulatory substances. This happens in wet plants. It happens under flooding stress.

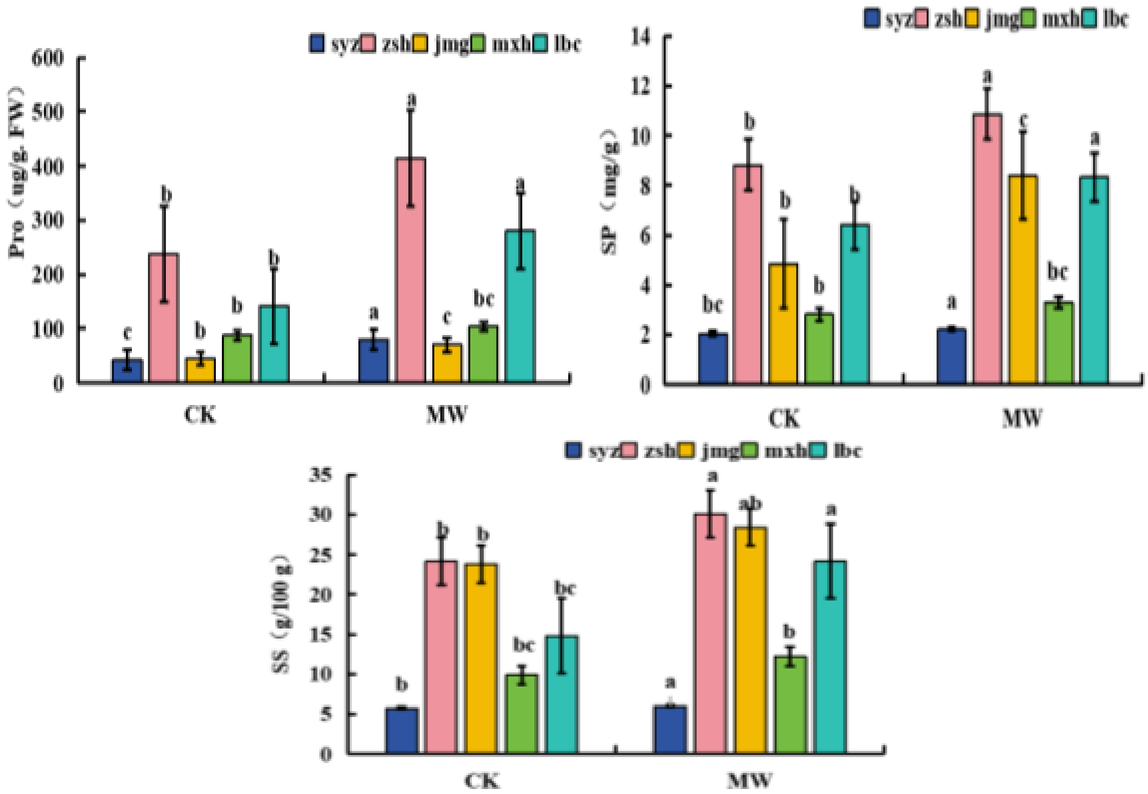


Figure 5 Changes of osmoregulatory substances contents in 5 high-cold-dampness plants under waterlogging stress

As can be seen in Figure 5, the proline (Pro) content of S. cedarica decreased significantly ($P < 0.05$), while the Pro content of Trichoderma lucidum increased significantly ($P<0.05$) in the flooded treatment group (MW) compared to the control group (CK). Plants reduce the intracellular osmotic potential by increasing Pro synthesis to maintain normal cellular metabolism. cedarica increases antioxidant enzyme activity and the root system through above-ground growth and development to cope with a flooded environment, without requiring a significant increase in proline content.

3. Quality control and comparative analysis of transcriptome sequencing data

To deeply analyse the changes in the gene expression levels of dominant plants in alpine wetlands in response to simultaneous long-term flooding stress, the raw transcriptome sequencing data from 15 samples were filtered and quality-controlled. The average percentage of Q20 and Q30 in the high-quality sequences was 97.38 % and 95.17 %, respectively, with an average GC content of 47.06% (Table 2). This indicates that the transcriptome sequencing data were well controlled and can be used for subsequent bioinformatics analyses. This indicates that the data had good quality control and could be used for subsequent bioinformatics analysis. Further comparison of the clean data with the Quercus serrata reference genome showed that the alignment rate of the 15 samples was greater than 85% (Table 2), indicating that gene expression levels could be accurately predicted.

Table 2 Transcriptome sequencing data quality control and comparison analysis with reference genome

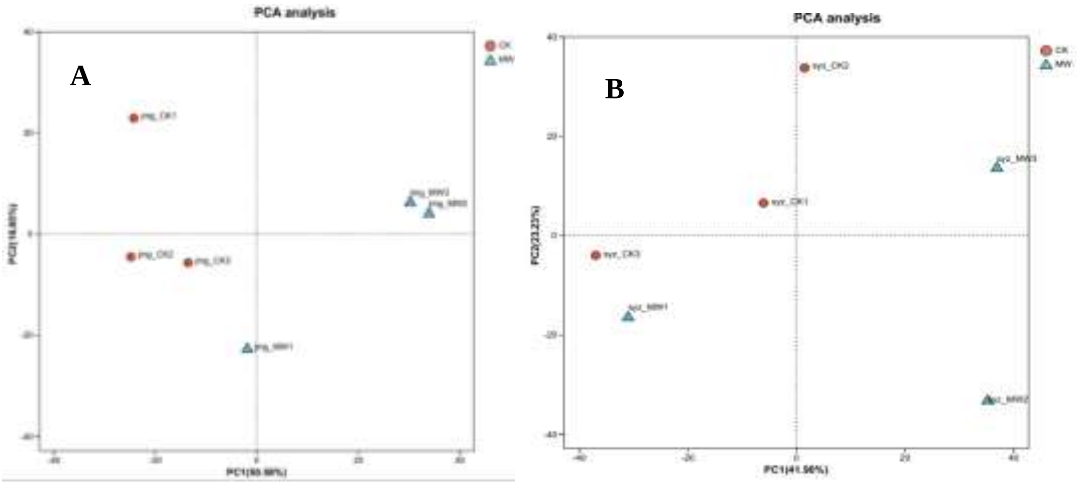
Sample No	Clean reads	Mapped reads	Mapped ratio（%）	Multimapped reads	uniqmapped reads
syz_MW1	24952653	20715427	83.02%	14 653 875（80.12%）	3 543 874（18.63%）
syz_MW2	22876096	18863826	82.46%	15 865 437（84.21%）	3 162 763（18.98%）
syz_MW3	21992374	17843446	81.13%	17 279 743（78.63%）	3 585 327（18.54%）

mxh_MW1	21790511	16747752	76.86%	16 742 754（80.42%）	2 674 284（17.13%）
mxh_MW2	21804633	17067238	78.27%	17 642 762（79.67%）	2 621 327（16.87%）
mxh_MW3	23668405	18323624	77.42%	17 632 879（80.34%）	2 587 959（16.65%）
jmg_MW1	48028902	48531780	84.32%	15 352 862（81.02%）	3 356 687（17.83%）
jmg_MW2	40700306	41093996	83.78%	16 735 457（84.21%）	3 162 763（18.96%）
jmg_MW3	41629742	42067378	85.30%	16 546 942（81.03%）	3 576 467（18.34%）
zsh_MW1	44122746	44621608	82.72%	15 562 158（82.45%）	2 568 268（16.85%）
zsh_MW2	43636276	44090920	83.23%	15 648 378（83.66%）	2 753 237（17.86%）
zsh_MW3	43333754	43767134	83.24%	16 335 849（82.14%）	2 487 967（16.57%）
szt_MW1	44122746	546253218	84.43%	15 687 975（82.12%）	3 543 874（18.95%）
szt_MW2	43636276	481182066	82.36%	14 746 854（83.21%）	3 174 763（18.38%）
szt_MW3	43333754	434197006	83.22%	16 634 825（81.53%）	3 598 427（18.67%）

Table 3 Sample sequencing data evaluation

Samp**le No**	Base number	GC Content(%)	Clean reads Q20(%)	Clean reads Q30(%)
syz_MW1	7612077308	47.04	95.47	92.93
syz_MW2	6980948346	47.27	95.3	92.67
syz_MW3	6714456298	47.23	95.34	92.68
mxh_MW1	6649660990	46.92	95.59	93.03
mxh_MW2	6665176240	47.01	95.21	92.46
mxh_MW3	7277599926	47.03	95.12	91.98
jmg_MW1	7328298780	52.34	95.92	93.57
jmg_MW2	6205193396	51.96	95.6	93.12
jmg_MW3	6352174078	52.18	95.48	92.95
zsh_MW1	6737862808	52.49	95.76	93.27
zsh_MW2	6657728920	52.64	95.63	93.15
zsh_MW3	6608837234	52.14	95.73	93.28
szt_MW1	6205193396	52.34	95.6	93.57
szt_MW2	6352174078	51.96	95.48	93.12
szt_MW3	7328298780	52.18	95.76	92.95

3.1 Analysis of DEGs of alpine wet plants under flooding stress treatment



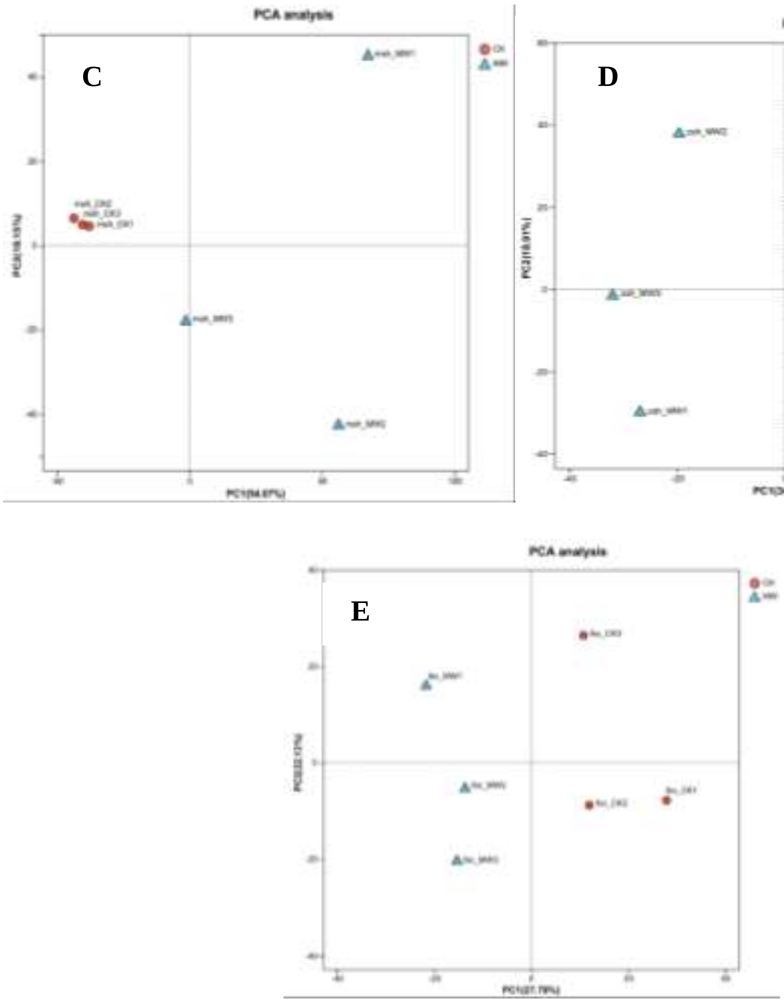
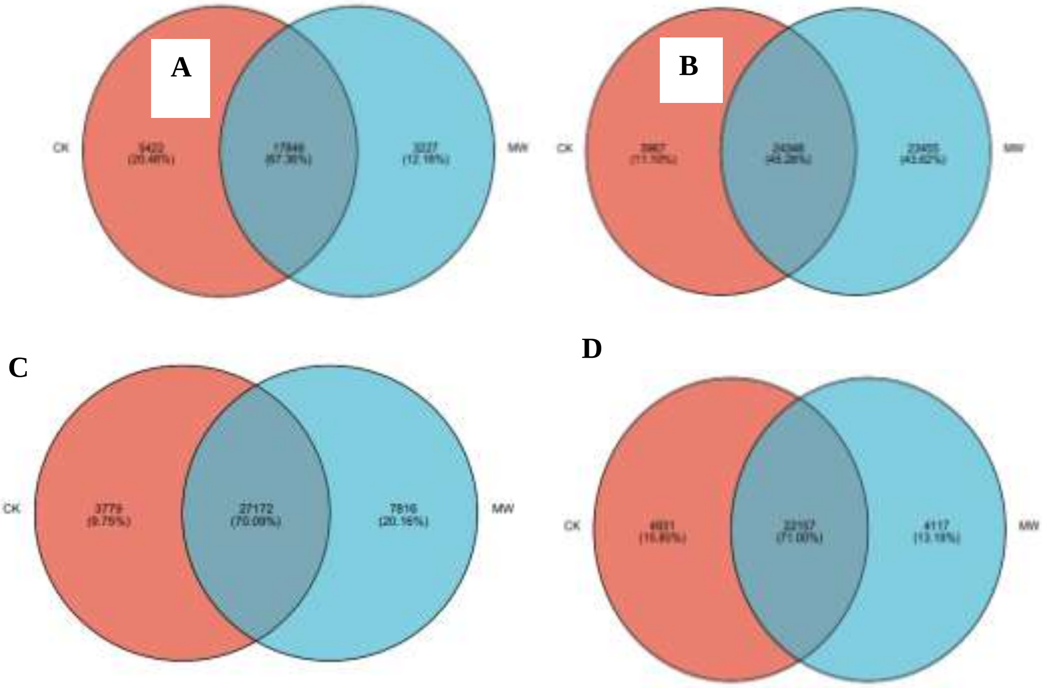


Figure 6 Analysis of differentially expressed genes (DEGs) in five alpine wetland plants under waterlogging stress for 60 days (A) Principal component analysis (PCA) of Ranunculus ternatus; (B) PCA of Hippuris vulgaris; (C) PCA of Pedicularis kansuensis; (D) PCA of Elymus nutans; (E) PCA of Carex biglumis.



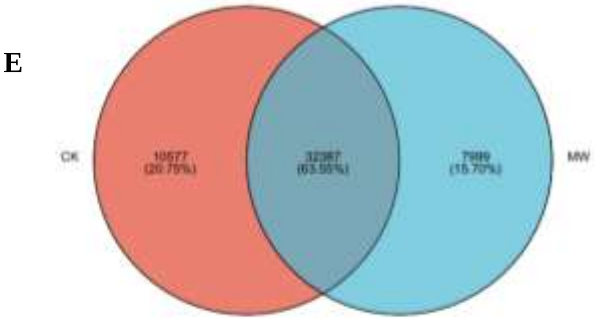


Figure 7 Venn diagrams of up-regulated and down-regulated differentially expressed genes in different alpine wetland plants under waterlogging stress for 60 days (A) Venn diagram of up-regulated and down-regulated differentially expressed genes in Ranunculus tripartitus; (B) Venn diagram of up-regulated and down-regulated differentially expressed genes in Pedicularis kansuensis; (C) Venn diagram of up-regulated and down-regulated differentially expressed genes in Chara constricta; (D) Venn diagram of up-regulated and down-regulated differentially expressed genes in Carex biseriata; (E) Venn diagram of up-regulated and down-regulated differentially expressed genes in Elymus nutans

Table 4 KEGG enrichment pathway of DEGs in Q. robur under different water treatments

ko ID	KEGG enrichment pathway	Unigene annotated to the pathway	*p-value*	Core gene function	KEGG functional classification
ko00010	Glycolysis/glycolysis	42	1.2E-08	ADH, PDC, SUS (Enhanced anaerobic respiration)	energy metabolic reconfiguration
ko00196	Photosynthetic antenna protein	35	7.8E-10	LHCB1-6 (inhibition of photosynthesis)	
ko00040	Pentose phosphate pathway	28	3.5E-05	G6PD, PGL (Supply of NADPH)	
ko00480	Glutathione metabolism	26	5.6E-05	GST, GPX, GR (ROS Clearance)	Antioxidant defense system
ko00940	Phenylpropane Biosynthesis	57	2.1E-12	PAL, 4CL (Lignin deposition enhances ventilatory organization)	
ko04075	Plant Hormone Signaling	63	4.30E-15	ERF1/2, EIN3（Vinyl Pathway） ARF, AUX/IAA（Growth hormone pathway）	Hormone signaling regulation

3.2 qRT-PCR validation

Ten differentially expressed genes (DEGs) were randomly selected from five wetland plants for qRT-PCR validation. The qRT-PCR and RNA-Seq data were highly concordant (Pearson r=0.92，$p<0.001$),proving that the transcriptome results were reliable (Figure.8).

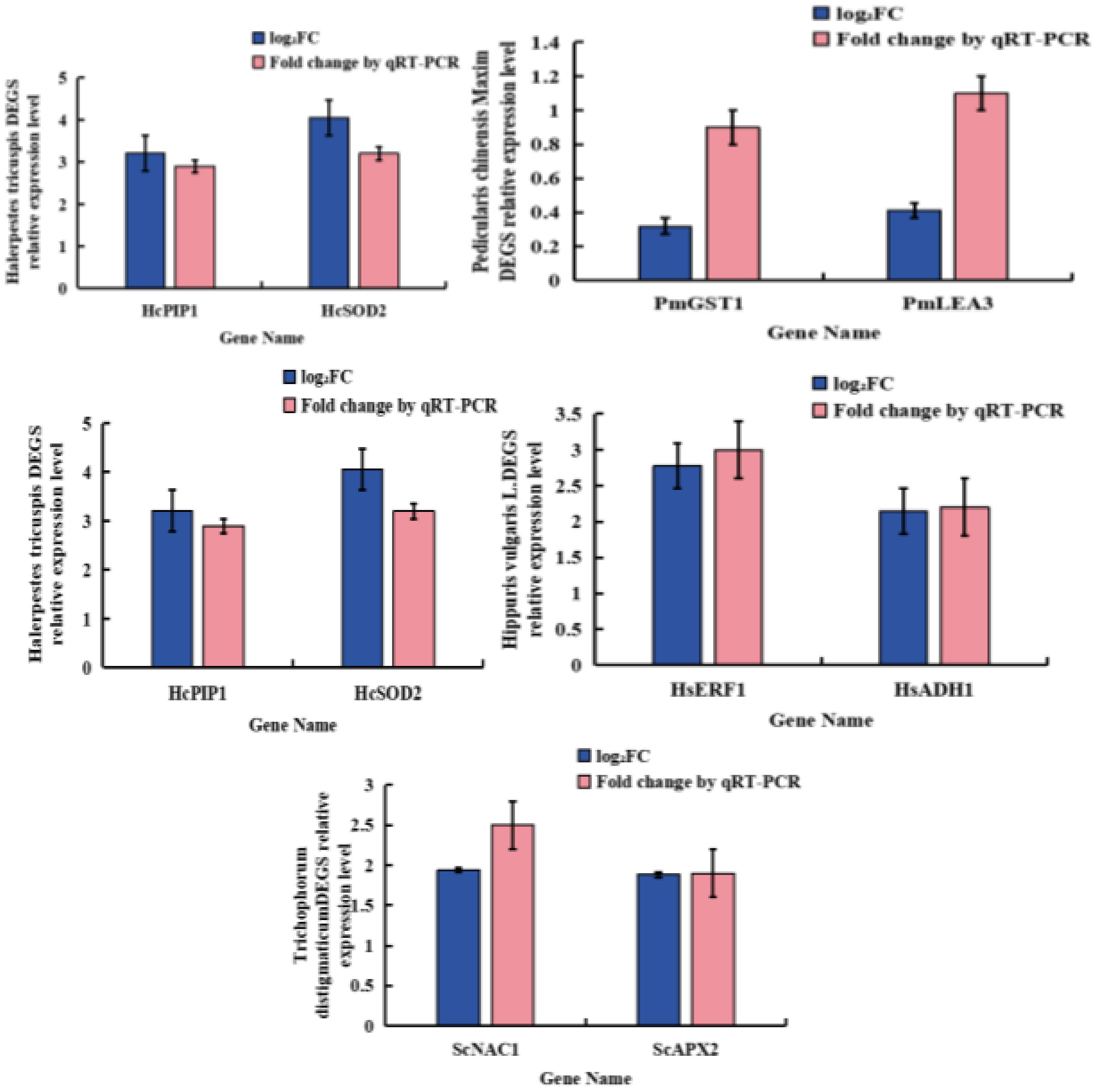


Figure 8 qRT-PCR validation of transcriptome analysis

3 Discussion

3.1 Changes in morphological structure of plants under flooding stress

Under flooding conditions, many plants adapt to the environment by adjusting the morphology and structure of their leaves. Changes in leaf structure and morphology are important indicators of a plant's response to flooding[21]. For instance, flooding stress has been shown to reduce soil oxygen levels and decrease hormone levels, thereby inhibiting plant growth and causing leaf yellowing in studies on the Lianxiang tree[22](Cercidiphyllum japonicum) and the maple poplar (Pterocarya stenoptera)[23]. Trillium (Bougainvillea spp.) showed bract shriveling, yellowing, yellow spotting, leaf drooping and dropping, and even bract rotting with an unpleasant odour, as well as extensive flower and leaf drop as the duration of flooding stress increased. The degree and duration of flooding can significantly affect plant growth and development; the more severe and prolonged the flooding, the greater the inhibitory effect on growth. Mommer et al.[24] found that the specific leaf area of the leaves of the aquatic plant Polygonum acidum (Rumex palustris) doubled under complete flooding. The thickness of the leaf blade was reduced by 20%, and the chloroplasts moved towards the epidermal layer of the leaf blade.Flesch **et al.**[25] found that the size and inclination angle of the leaf blades of hollow lotus grass increased significantly under flooding. Grabowski et al. [26] found that the stomata of crown grass (Echinodorus amazonicus) leaf blades became smaller, and that the stomatal density in the upper epidermis increased. They also found that the leaf blades of descending sandalwood (Dalbergia odorifera) gradually wilted and fell off in prolonged flooding, eventually leading to plant death. Leaves gradually wither and fall off in long-term flooding, eventually leading to plant death. Under flooding stress conditions, leaves often exhibit symptoms such as curling, yellowing, wilting, abscission, and rotting. Studies by Yadav et al.[27] on Nandia lutarioriparia (Triarrheca lutarioriparia) and Huang et al[28] on Begonia hupehensis (Malus hupehensis) showed that excessive water content in the soil inhibits normal leaf growth, resulting in dry leaf margins and tips, leaf yellowing, and even leaf shedding and rotting. However, not all plant leaf morphology and structural changes are consistent; they vary according to plant species. In this study, five species of wet plant, such as cedar leaf algae, developed a certain degree of tolerance to the flooded environment because they had been living in it for a long time [29]. The cedar leaf algae did not undergo a very obvious change in structure or morphology after 60 days of flooding stress. The leaf blades were acicular, most likely to reduce transpiration and adapt to the flooded environment. Compared with the control group, the leaves of light lemna, morning glory, Trichoderma buttercup, Chinese artemisia, double stigma rush and cassis appeared yellow due to chlorophyll degradation.

3.2 Changes in the photosynthetic parameters of wetland plants under flooding stress

Photosynthetic parameters are important indicators of plant growth and resistance. Under flooding stress, wetland plants maintain the relative stability of their photosynthetic apparatus by integrating mechanisms such as stomatal regulation, photoprotection, and morphological adjustment These adaptive characteristics provide the physiological basis for their dominant position in wetland ecosystems. Under short-term or mild flooding stress, most plants will increase their transpiration rate and lose excessive water by increasing stomatal conductance. However, the increased stomatal conductance also provides more CO_2 to promote photosynthesis and maintain normal growth[30]. Blue-fruited Lonicera (Lonicera caerulea L.) showed a decreasing trend in chlorophyll a and b content with increasing flooding time. Yan Shangbo's study of Potentilla chinensis (Potentilla chinensis Ser.) also found that the chlorophyll content of this species remained consistent under excessive flooding or drought. This is consistent with the results observed for the five wet plants in this experiment after 60 days of flooding. The reason for this may be that flooding stress reduces the activity of photosynthetic pigment synthase in wet plants, resulting in a lower rate of chlorophyll synthesis than decomposition.

3.3 Changes in the activities of antioxidant enzymes and osmoregulatory substances in plants growing in wet conditions when subjected to waterlogging stress.

Significant changes in the physiological indices of wet plants occurred under flooding stress conditions, including a decrease in photosynthetic rate, chlorophyll content and aerobic respiration, and an increase in anaerobic respiration. Meanwhile, the activities of antioxidant enzymes (e.g. SOD, CAT and POD) increased in order to scavenge excess reactive oxygen species (ROS). However, an increase in malondialdehyde (MDA) content indicated an increase in membrane lipid peroxidation[31-33]. Additionally, osmoregulatory substances (e.g.proline and soluble sugars) accumulate to maintain cellular osmotic balance and changes in hormone levels regulate stomatal closure and acclimatisation growth. However, disturbances in nitrogen metabolism and mineral imbalances affect nutrient uptake and metabolism[34].In terms of energy metabolism, an insufficient supply of ATP prompts plants to turn to anaerobic metabolic pathways to maintain the energy supply.Together, these physiological changes reflect the adaptive strategies of plants in response to flooding stress, aimed at mitigating injury and maintaining essential physiological functions. The response mechanisms may vary among species, depending on their genetic characteristics and the environmental conditions they encounter. A study of blue-fruited Lonicera japonica seedlings found that low levels of water stress did not significantly affect seedling height, ground stem increment or biomass accumulation. However, each index was significantly reduced in the heavy drought and heavy flooding treatment groups. Overall, there were no significant changes in chlorophyll and carotenoid content or chlorophyll fluorescence parameters (Fv/Fm, ΦPSII, qP and qN) in the control group. However, in the other groups, chlorophyll content and chlorophyll fluorescence parameters decreased with increasing stress time and degree, while carotenoid content initially increased and then decreased. Water stress significantly increased the contents of malondialdehyde, soluble sugars, and proline, as well as increasing SOD and CAT activities. POD activity showed a tendency to first increase and then decrease at low degrees of water stress. These results are consistent with those of the present study, in which the contents of MDA, SS and SP increased gradually and the activities of the enzymes SOD and CAT increased after flooding for 60 days compared with the control group in five species of wet plant. The changes in these physiological indices suggest that wet plants respond to water stress by accumulating osmoregulatory substances and enhancing antioxidant enzyme systems, thereby reducing oxidative damage and maintaining cellular homeostasis. However, the trend of POD activity may be related to its different functional regulation under low and high stress.

4. Conclusions

Under flooding stress conditions, alpine wetland plants effectively scavenge the excessive accumulation of reactive oxygen species (ROS) by activating their endogenous antioxidant enzyme systems. This reduces damage to cell membranes caused by membrane lipid peroxidation and enhances their adaptive capacity to flooding stress. The study showed that the adaptability of the five alpine wetland plants to flooding stress differed significantly, in the following order: Fagus sylvatica > Trichocarpus buttercup > Lemna lucida > Ruscus bicolor > Artemisia chinensis. Furthermore, physiological indicators such as plant height, chlorophyll a and b, carotenoids, soluble sugars and proteins could be used as an important reference for screening the adaptation of alpine wetland plants to flooding stress. Comprehensive analysis of these indicators provides a scientific basis for revealing the response mechanisms of alpine wetland plants to flooding stress and evaluating their adaptability.

ACKNOWLEDGEMENTS

We sincerely thank all the fellow students who contributed to and participated in this study. We are deeply grateful to Professor Changhui Li for his invaluable guidance. This work was supported by the research project "Physiological and Molecular Mechanisms of Alpine Hygrophytic Plant Adaptation to Waterlogging Environments" (Grant No. 2025GJLCGCZX003).

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