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Original Article

Physiological Adaptation Strategies and Comprehensive Waterlogging Tolerance Evaluation of Alpine Swamp Wetland Plants under Waterlogging Stress

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

[1]College of Animal Husbandry and Veterinary Medicine, Qinghai University, Xining 810016, Qinghai, China

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

[*]Corresponding Author: Li Changhui

Abstract:
To investigate the effects of waterlogging stress on the morphological structure, physiological characteristics, and adaptation mechanisms of alpine wetland plants, five representative alpine swamp wetland plant species with similar specifications and healthy growth were selected as test materials. Pot-based waterlogging experiments were conducted, including a control group (CK) and a waterlogging stress treatment group (MW), with treatment durations of 20, 40, and 60 days. The growth status and physiological-biochemical indicators of the five plant species were recorded and measured. A comprehensive evaluation was performed using waterlogging tolerance coefficients, significance analysis, correlation analysis, principal component analysis (PCA), and subordinate function methods. The results showed that: with prolonged waterlogging stress, the contents of chlorophyll and carotenoid first increased and then decreased, while the malondialdehyde content first decreased and then increased; the activities of antioxidant enzymes and the contents of osmotic adjustment substances exhibited varied trends over time; correlation analysis revealed D-values of 0.739, 0.616, 0.660, 0.200, and 0.534 for the five species; and the ranking of waterlogging adaptability was as follows: Hippuris vulgaris > Halerpestes tricuspis > Poa psilolepis > Blysmus sinocompressus > Pedicularis chinensis.

Keywords: Alpine wetland plants; Waterlogging stress; Physiological traits; Adaptability assessment

1. Introduction

Wetlands, as transitional ecosystems between aquatic and terrestrial systems, are widely distributed globally [1]. Global ecological statistics indicate that although wetlands cover only 6% of the Earth's surface, they rank among the most productive ecosystems worldwide, providing unique habitats for biodiversity and shelter for over 40% of global species [2]. China possesses the world's fourth-largest wetland area, including the unique Qinghai-Tibet Plateau wetlands spanning $>1\times10^5$ km^2 (≈30% of China’s total wetland area) [3,4]. These plateau wetlands feature distinctive landscape resources and exceptional ecological functions, yet their water conservation capacity and ecological barrier role face threats from intensifying human activities [5]. In recent years, the Qinghai-Tibet Plateau has exhibited a pronounced "warming and humidification" trend: rising temperatures have caused the retreat of permafrost distribution, increased soil moisture, and enhanced water recharge to wetlands; inland marsh areas in western endorheic basins have expanded; and increased frequency and intensity of rainy-season precipitation have enlarged waterlogged areas in alpine swamp wetlands, exposing vegetation to widespread waterlogging stress [6,7].

Waterlogging stress refers to an environmental constraint triggered by excessive soil water saturation, which reduces soil oxygen partial pressure and thereby affects plant growth and metabolic processes [8]. Under waterlogging stress: mineral element transport mechanisms in plant roots are disrupted, causing nutrient deficiency and decreased chlorophyll content. Plants activate physiological response mechanisms including photosynthetic system adjustments, contingent changes in osmotic adjustment substances, and antioxidant enzyme system activation [9]. However, plant responses exhibit significant interspecific and intraspecific variations: under short-term waterlogging, physiological indicators show divergent response patterns across species under identical stress intensity, while individual species display varied adaptability to different stress levels [10]. Long-term waterlogging exerts species-specific growth inhibition, though it may promote growth in certain plants; for example,Medicago sativa exhibits initial growth acceleration followed by decline under short-term flooding [11]. Current research on waterlogging adaptation in alpine swamp wetland plants remains limited: existing studies confirm differences in leaf functional traits and chlorophyll fluorescence parameters along water level gradients, but systematic investigations into physiological-biochemical adaptations are lacking. Thus, studying the physiological-biochemical responses of representative alpine swamp wetland plants under waterlogging stress holds significant scientific value for revealing their unique adaptation strategies.

1.Materials and Methods

1.1 Study Area Overview

The research was conducted in the Sanjiangyuan region (33°30′–35°40′N, 97°20′–101°50′E), a core area of China's Qinghai-Tibet Plateau that encompasses the headwaters of the Yangtze, Yellow, and Lancang Rivers. Situated at an elevation of 4,000–4,800 m, the region has a harsh plateau continental climate, with a mean annual temperature of −0.8°C, annual precipitation of 290–590 mm (80% of which falls between May and September), a frost-free period of fewer than 30 days, and permafrost covering over 70% of the area. Dominated by alpine swamp meadows and peatlands, its typical vegetation includes Kobresia tibetica, Blysmus sinocompressus, and Hippuris vulgaris. This establishes the area as a globally critical biodiversity hotspot and a key water conservation zone for East Asia.1.2 Experimental Materials

This study employed five dominant alpine hygrophytic species—Halerpestes tricuspis, Hippuris vulgaris L., Trichophorum distigmaticum, Pedicularis chinensis, and Poa psilolepis Keng—collected via intact soil core transplantation (30 cm in diameter × 40 cm in depth) from Zeku Zequ National Wetland Park (Qinghai Province) in June 2024. The plants were acclimatized under controlled conditions at Qinghai University for 7 days with routine irrigation, after which waterlogging treatments were initiated: treatment groups (MW) were submerged to 3 cm above the soil surface, while control groups (CK) received standard irrigation without submergence. The experiment comprised 3 replicates per treatment, with 10 plants per replicate, and physiological measurements were conducted after 20, 40, and 60 days of stress exposure.

1.3 Experimental Methods

Following 20, 40, and 60 days of waterlogging treatment, physiological and morphological parameters (plant height, biomass, root-shoot ratio) were measured for both control (CK) and waterlogged (MW) groups of the five alpine hygrophytic species. For photosynthetic pigment analysis, 0.1 g of fresh leaf tissue was fragmented and immersed in 10 mL of a 1:1 (v/v) ethanol-acetone mixture (95%) in light-proof containers for 24 hours until complete decolorization. Absorbance was measured at 665 nm, 649 nm, and 470 nm, and the contents of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl), and carotenoids were calculated using standard formulas (three biological replicates per group).

For biochemical assays, flash-frozen samples (liquid nitrogen) were analyzed with Nanjing Jiancheng Bioengineering Institute kits:

Parameter	Kit Code	Method

Malondialdehyde (MDA)	A003-1-2	Thiobarbituric acid (TBA) assay
Free proline (Pro)	A107-1-1	Ninhydrin colorimetry
Soluble sugar (SS)	A145-1-1	Anthrone colorimetry
Soluble protein (SP)	A045-2-1	Coomassie brilliant blue
Catalase (CAT) activity	A007-1-1	Ultraviolet spectrophotometry
Peroxidase (POD) activity	A084-3-1	Guaiacol method
Superoxide dismutase (SOD) activity	A001-1-1	Nitroblue tetrazolium (NBT) photoreduction

1.4 Data Analysis

Statistical analyses were performed using SPSS 29.0 and Microsoft Excel® 2021 (version 2108), including: Significance analysis (Independent samples t-test at $P<0.05$);Correlation analysis (Pearson/Spearman based on data distribution);Principal Component Analysis (PCA) ; Computational statistics for waterlogging tolerance coefficients;Data visualization was implemented with Origin® 2024 (OriginLab Corporation). All values are presented as mean±standard error (SE) with different lowercase letters indicating statistically significant differences ($P < 0.05$).

1.4.1 Waterlogging Tolerance Coefficient

The waterlogging tolerance coefficient (WTC) was calculated for 20 physiological parameters using the formula:WTC = (MW group value / CK group value) × 100% (1) Following the methodology of LU et al[12]. Pairwise correlation analysis of WTC values among the five alpine hygrophytic species was performed to determine inter-indicator correlation coefficients.

1.4.2 Principal Component Analysis

Principal Component Analysis (PCA) was performed on the waterlogging tolerance coefficients (WTC) of all 20 individual indicators. This analysis yielded the eigenvalues,contribution rates, and factor scores for the principal components (PCs).The comprehensive evaluation indices (i.e., major PCs) were selected based on cumulative contribution rates ≥ 85%.

1.4.3 Comprehensive Evaluation via Subordinate Function

Given that plant waterlogging tolerance is a polygenic trait involving multi-factor interactions, single-indicator assessments cannot accurately evaluate the waterlogging resistance of the five alpine hygrophytic species. Thus, we employed the subordinate function method for comprehensive evaluation of growth and physiological-biochemical parameters. Higher mean subordinate function values indicate stronger waterlogging stress resistance.

The calculations were performed as follows:

Subordinate function value for each comprehensive indicator:

$$\mu（Xj） = (Xj - Xmin)/(Xmax - Xmin) \quad (2)$$

Where XjXj is the measured value of indicator jj, and Xmin/Xmin/Xmax/Xmaxare the minimum/maximum values among all test materials.

Weight coefficient of comprehensive indicators:

$$Wj = Pj/\sum Pj \ \ (j=1,2,......,n) \quad (3)$$

Where Pjis the contribution rate of the jj-th principal component.

Comprehensive evaluation value （D）

$$D = \sum[\mu(Wj) \times Wj] \quad (4)$$

Here, Drepresents the integrated adaptability assessment to waterlogging stress.

2 Results and Analysis

2.1 Effects of Waterlogging Stress on Phenotypic Morphology of Alpine Hygrophytic Plants

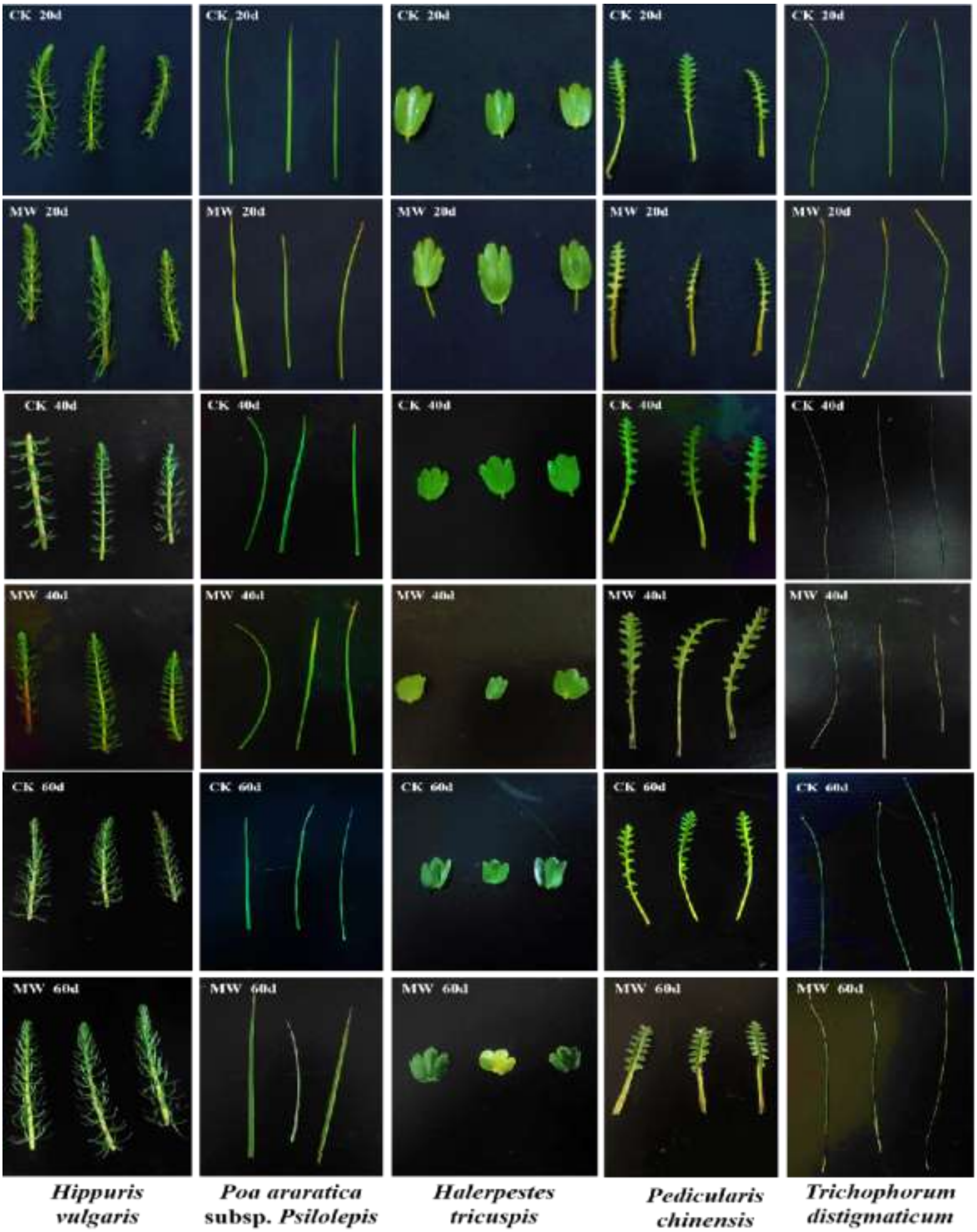


Figure 1 Leaf morphological changes of 5 species of alpine wetland plants under flooding stress

Under prolonged waterlogging stress, the five alpine hygrophytic species exhibited distinct leaf morphological adaptations. After 60 days of treatment (Figure 1):Hippuris vulgaris maintained stable leaf pigmentation but developed significantly elongated lanceolate leaves compared to controls;Halerpestes tricuspis, Pedicularis chinensis, and Poa psilolepis displayed pronounced chlorophyll degradation accompanied by progressive yellowing and reduced surface luster;while Trichophorum distigmaticum manifested systemic chlorosis with marked chlorophyll deficiency in both apical meristems and leaf tissues.

These differential responses reflect species-specific adaptation strategies, where chlorophyll reduction and yellowing represent hallmark physiological characteristics of anaerobic stress acclimation.

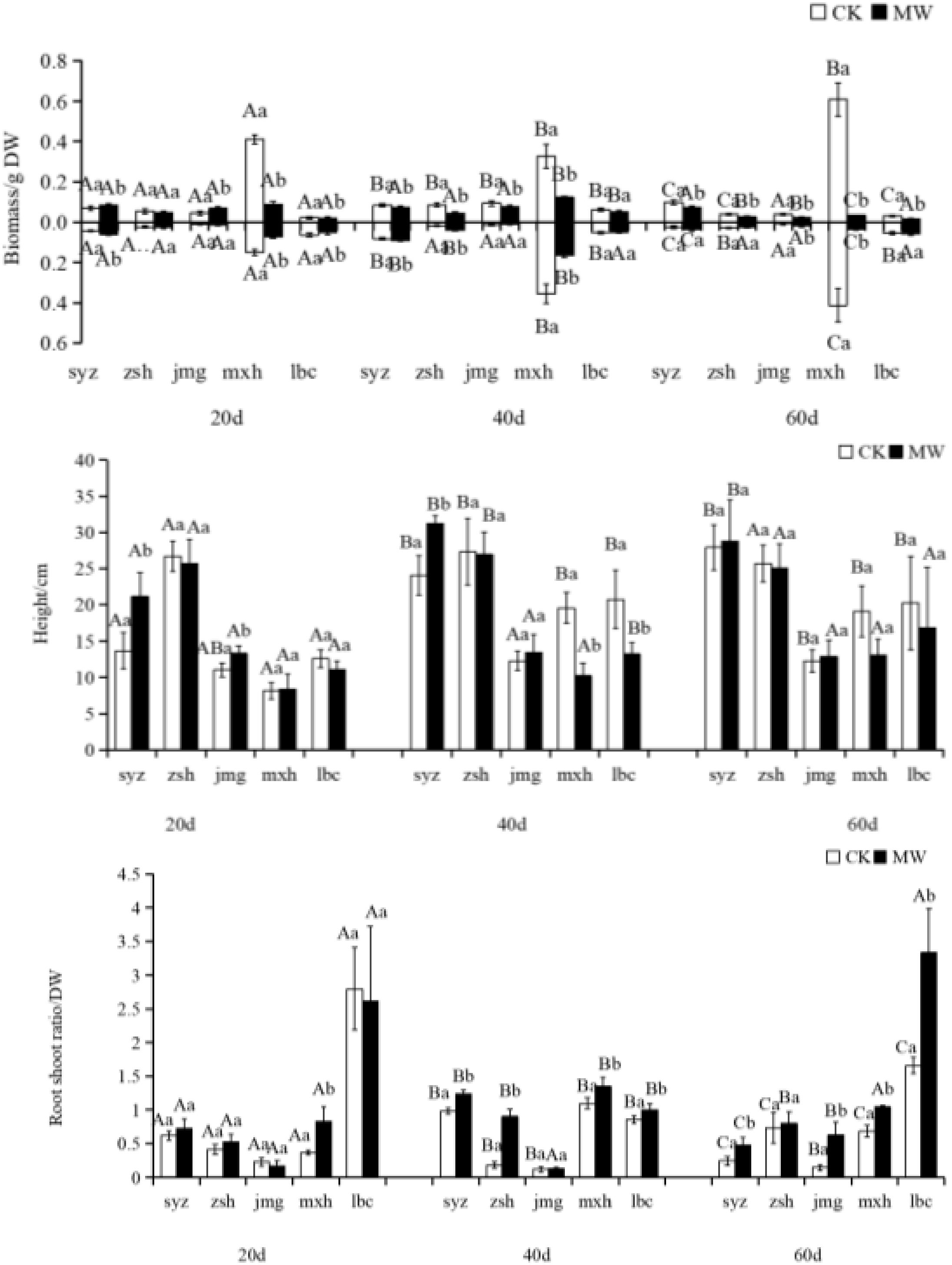


Fig.2 Changes of plant height and biomass in 5 alpine marsh wetland plants under waterlogging Environment

Note:Different lowercase letters above bars indicate significant differences ($P<0.05$) among treatments for the same species at identical time points, while different uppercase letters denote significant differences ($P<0.05$) across time points under the same treatment for identical species.Species abbreviations:syz: Hippuris vulgaris;zsh: Poa psilolepis ;jmg: Halerpestes tricuspis ;mxh: Pedicularis chinensis ;lbc: Trichophorum distigmaticum

As shown in Figure 2, analysis of growth parameters in five alpine swamp wetland plants under 20-, 40-, and 60-day waterlogging treatments revealed significant interspecific variations:At 20 days:Hippuris vulgaris (syz) and Halerpestes tricuspis (jmg) exhibited positive growth responses (biomass increased by 18.7% and 12.5%, respectively),Pedicularis chinensis (mxh) and Trichophorum distigmaticum (lbc) showed biomass reduction (15.3% and 9.8%),Poa psilolepis (zsh) displayed no significant change.At 40 days: Species-specific divergent responses emerged (Table 2),At 60 days:Most species exhibited reduced biomass and elevated root-shoot ratios P. chinensis and T. distigmaticum developed root necrosis.

Compared to controls, all five species demonstrated convergent biomass reduction trends under prolonged stress, though height progression and phenological phases varied interspecifically. The universal increase in root-shoot ratios indicates strategic biomass reallocation from shoots to roots as a waterlogging adaptation mechanism, with P. chinensis and T. distigmaticum exhibiting the weakest tolerance.

2.2 Effects of Flooding Environment on Photosynthetic Pigment Content in Plants of Alpine Swamp Wetland

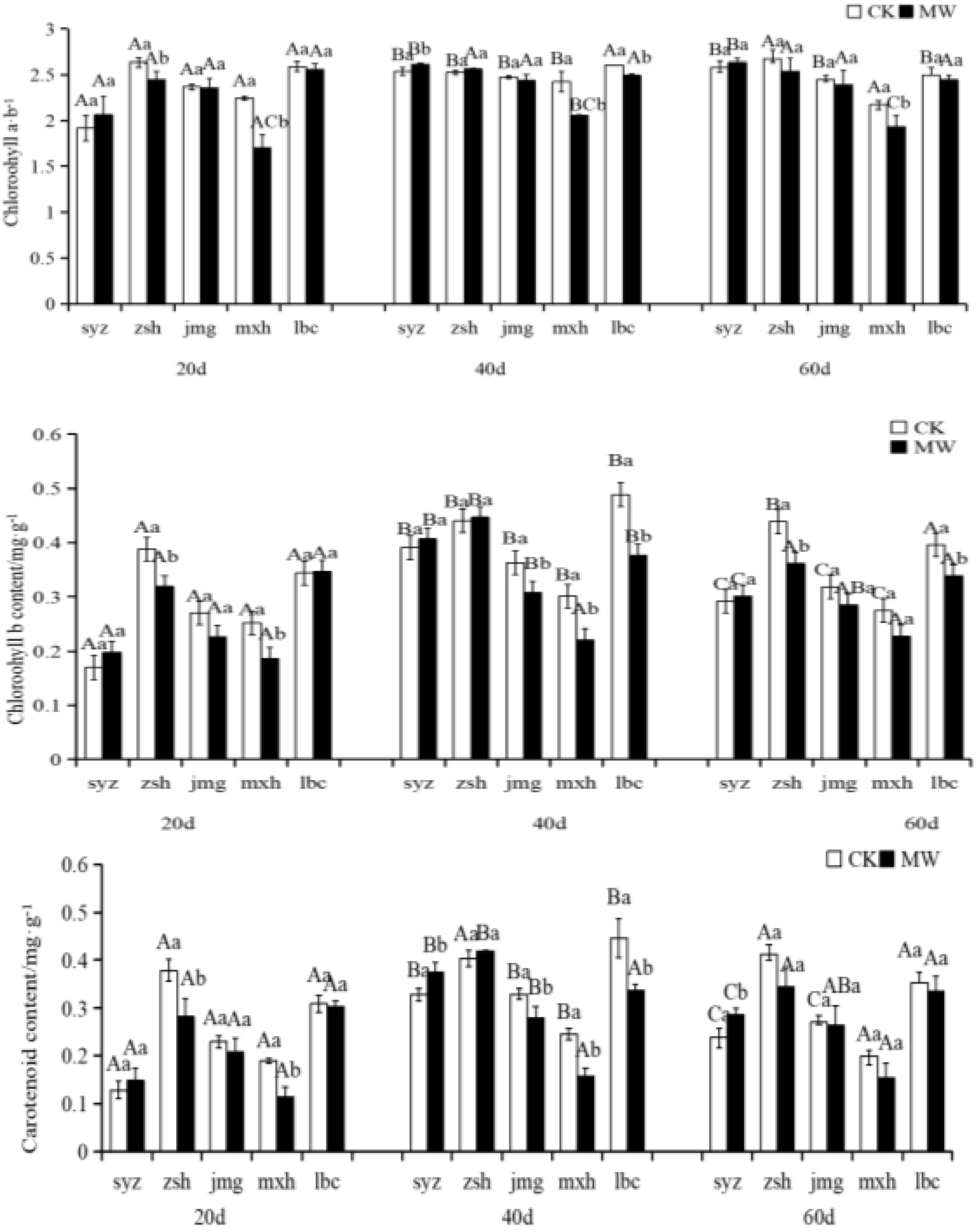


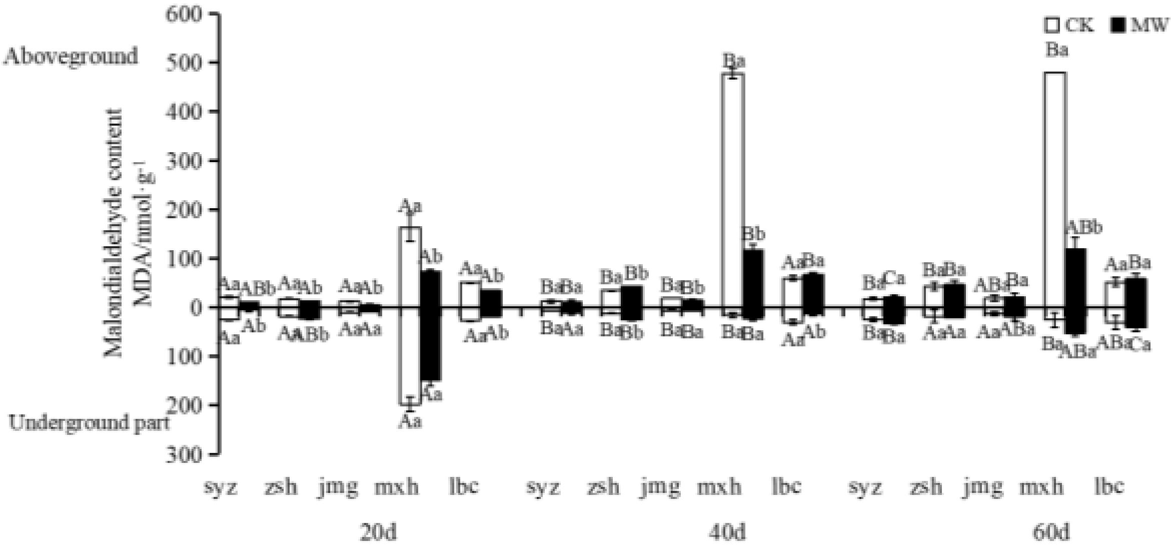
Fig.3 Photosynthetic pigment content of 5 alpine marsh wetland plants under waterlogging

environment

Note: Different lowercase letters above bars indicate significant differences ($P < 0.05$) between the same species under different treatments at the same time point, while different uppercase letters indicate significant differences ($P < 0.05$) between the same species under the same treatment at different time points. syz: Hippuris vulgaris; zsh: Poa psilolepis; jmg: Halerpestes tricuspis; mxh: Pedicularis chinensis; lbc: Blysmus sinocompressus.

Figure 3 demonstrates interspecific variation in photosynthetic pigment responses to flooding duration among five alpine swamp wetland plants. Under 20-day flooding, Hippuris vulgaris, Halerpestes tricuspis, and Blysmus sinocompressus exhibited no significant changes in photosynthetic pigments, while Pedicularis chinensis showed significant reductions in chlorophyll content, carotenoid content, and chlorophyll a/b ratio, and Poa psilolepis displayed significantly decreased pigment content only. At 40-day flooding, H. vulgaris manifested increased chlorophyll content (non-significant trend) with significant rises in carotenoids and chlorophyll a/b, whereas P. psilolepis showed non-significant upward trends across all indicators; conversely, H. tricuspis experienced significant declines in chlorophyll and carotenoids, and both P. chinensis and B. sinocompressus demonstrated significant decreases in all three parameters. After 60-day flooding, H. vulgaris displayed significantly elevated carotenoids, while P. psilolepis showed significant chlorophyll and carotenoid reductions; H. tricuspis exhibited declining chlorophyll trends, P. chinensis had significantly reduced chlorophyll a/b, and B. sinocompressus showed significant chlorophyll decreases. Collectively, chlorophyll a/b ratios remained relatively stable though declining with prolonged flooding in all species except H. vulgaris; chlorophyll and carotenoid contents followed parallel initial-increase-then-decrease patterns, with only H. vulgaris maintaining consistently higher levels than controls, while the other four species ultimately registered significantly lower photosynthetic pigment content versus control groups.

2.3 Effect of Flooding Environment on Membrane Lipid Peroxidation (MDA) and Antioxidant Enzyme Activities in Alpine Swamp Wetland Plants



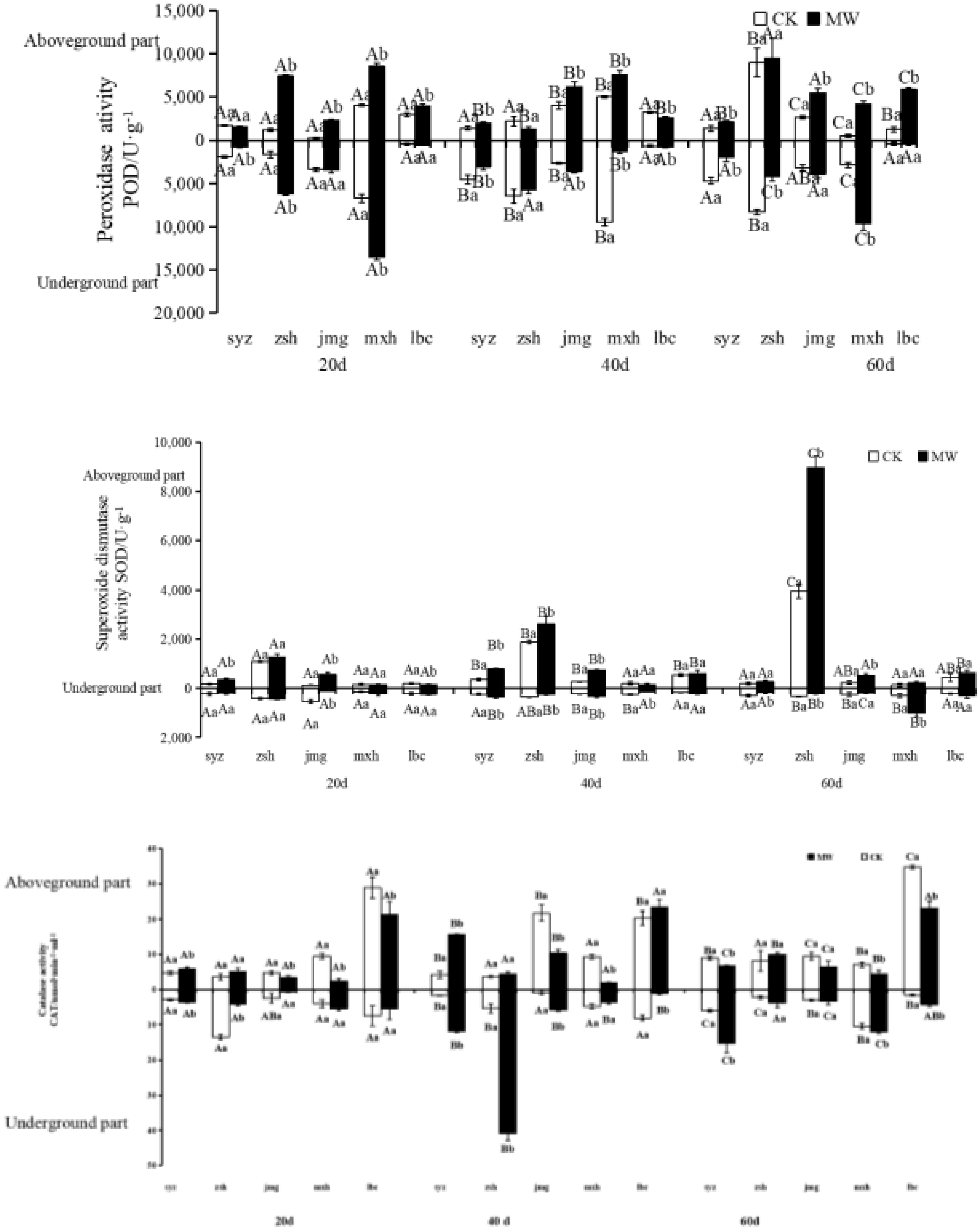


Fig.4 Changes of MDA content and antioxidant enzyme activity in 5 alpine marsh wetland plants under different waterlogging treatment time

Figure 4 reveals significant interspecific differences and temporal dynamics in malondialdehyde (MDA) content and antioxidant enzyme activities (POD, CAT, SOD) among five alpine swamp wetland plant species under varying flooding durations. MDA content generally exhibited an initial decrease followed by an increase, with species-specific patterns: Hippuris vulgaris showed "low-stable-increasing",Poa psilolepis manifested “decreasing-increasing-stable” in aboveground tissues and “increasing-increasing-stable” in belowground tissues, while Halerpestes tricuspis and Pedicularis chinensis displayed early increases in aboveground MDA with divergent late-stage trends, and Blysmus sinocompressus followed a "decreasing-decreasing-increasing" trajectory. Antioxidant enzyme activities demonstrated diverse patterns across species and tissue types (aboveground/ belowground) during different treatment phases: POD activity initially increased in aboveground tissues of some species, diverged at mid-term, and commonly elevated in aboveground tissues later; CAT activity showed significant mid-term variations, with late-stage increases throughout P. psilolepis but declines in aboveground tissues and rises in belowground tissues of other species; SOD activity exhibited inconsistent trends across stages. These results collectively illustrate the complexity and interspecific variations in plant adaptive responses to flooding stress.

2.4 Effect of Flooding Environment on Osmotic Adjustment Substances in Alpine Swamp Wetland Plants

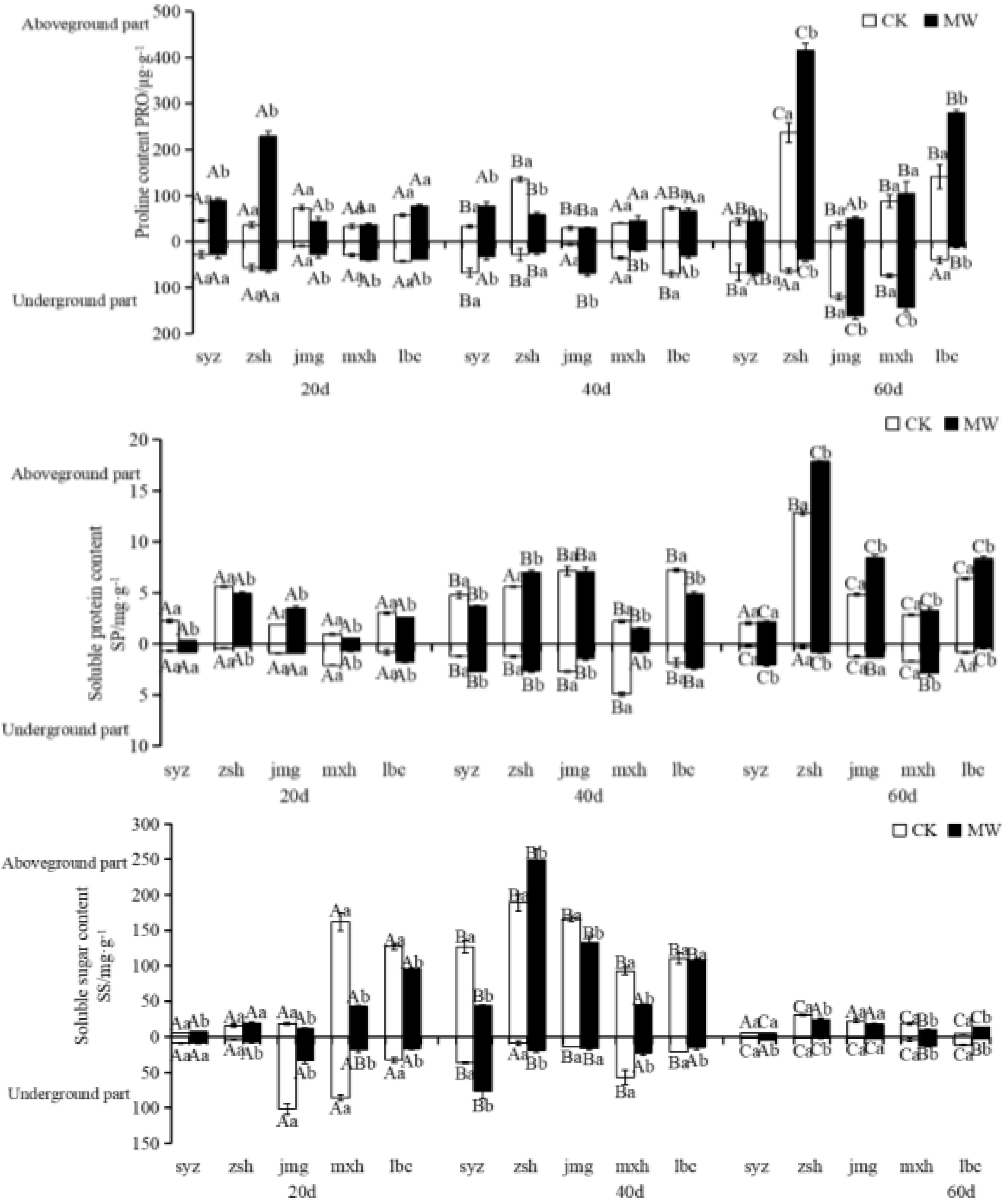


Fig.5 Changes of osmoregulatory substances contents of 5 alpine marsh wetland plants under different waterlogging treatment times

Figure 5 illustrates significant spatiotemporal dynamics and interspecific variations in osmotic adjustment substances (sucrose [SS], soluble protein [SP], and proline [Pro]) within aboveground and belowground tissues of five alpine swamp wetland plant species under different flooding durations. During short-term flooding (20 days), SS content significantly increased in Hippuris vulgaris aboveground tissues and Poa psilolepis belowground tissues but decreased in other species; SP content declined in aboveground tissues of all species except Halerpestes tricuspis, while Pro content rose significantly in H. vulgaris and P. psilolepis aboveground tissues with species-specific tissue variations. At mid-term flooding (40 days), SS content exhibited opposite aboveground-belowground trends in some species, SP displayed diverse patterns (increase/decrease/stability), and Pro trends diverged across species. Under long-term flooding (60 days), SS content was consistently lower than controls in all species, most species showed significantly elevated SP in both tissues (except Blysmus sinocompressus belowground), and all species except H. vulgaris (aboveground Pro decrease) accumulated Pro to varying degrees. Collectively, plants dynamically adapt to flooding durations through spatiotemporally specific accumulation and allocation of osmolytes, reflecting the complexity and diversity of osmotic adjustment mechanisms.

2.5 The changes in the relative expression levels of waterflood-tolerant genes in five alpine hygrophytic plants under different waterlogging durations

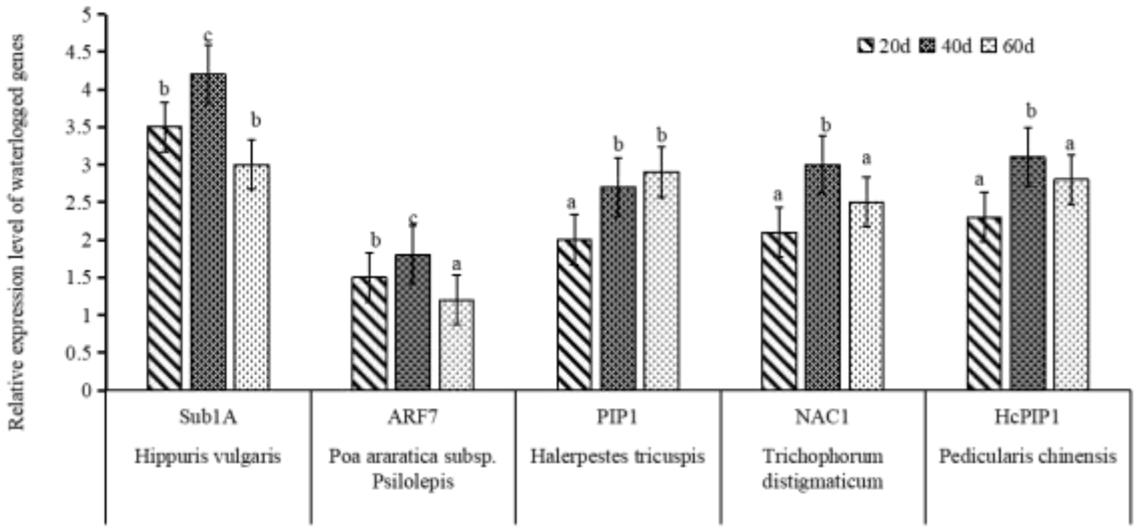


Fig.6 The changes in the relative expression contents of waterflood-tolerant genes in five species of alpine marsh wetland plants under different waterlogging treatment times

The results in Figure 6 showed that: For the Sub1A gene in *Hippuris vulgaris*, there was a certain level of expression at 20 days. The expression level increased significantly and reached a peak at 40 days. Although it decreased at 60 days, it still maintained a relatively high level, showing a dynamic trend of first increasing and then decreasing. For the ARF7 gene in *Poa glauca*, there was a basal expression at 20 days, the expression was up-regulated at 40 days, and then significantly decreased at 60 days, showing a "rise-fall" pattern. For the PIP1 gene in *Halerpestes tricuspis*, the expression was relatively low at 20 days, increased significantly at 40 days, and maintained a high expression level similar to that at 40 days at 60 days, reflecting the continuous induction effect of medium-term waterlogging. For the NAC1 gene in *Scirpus distigmaticus*, the expression level was moderate at 20 days, significantly up-regulated at 40 days, and decreased at 60 days, showing a trend of "first increasing and then decreasing". For the HcPIP1 gene in *Pedicularis chinensis*, there was a certain level of expression at 20 days, reached a relative peak at 40 days, and declined at 60 days. The letters a, b, and c in the figure are markers for significant differences ($P < 0.05$), which are used to determine the statistical significance of differences in expression between groups. Different letters indicate significant differences, while the same letters indicate no significant differences.

2.6 Changes in the relative expression contents of antioxidant genes in five alpine marsh wetland plants under different waterlogging treatment times

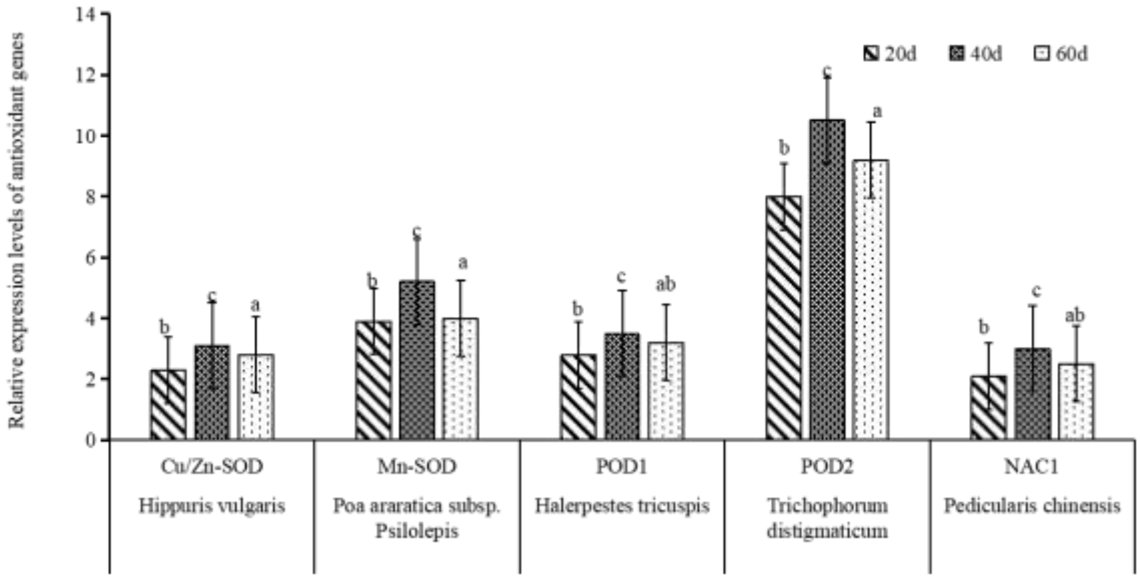


Fig.7 Figure 7 shows the changes in the relative expression contents of antioxidant genes in five alpine marsh wetland plants under different waterlogging treatment times

The results in Figure 7 showed that: For the Cu/Zn - SOD gene in Hippuris vulgaris, the expression level showed a trend of first increasing and then decreasing with the duration of waterlogging, reaching a relative peak at 40 days, which reflected the induction effect of short-term waterlogging (40 days) on this gene. For the Mn - SOD gene in Poa glauca, there was a basal expression at 20 days, it was significantly up-regulated at 40 days, and although it declined at 60 days, it still maintained a relatively high level, reflecting the temporal dynamics of the gene in response to waterlogging. For the POD1 gene in Halerpestes tricuspis, the expression level at 40 days was significantly higher than that at 20 days and 60 days, showing an expression pattern of "high in the middle and low at both ends". For the POD2 gene in Scirpus distigmaticus, the expression increased significantly from 20 days to 40 days, and slightly decreased from 40 days to 60 days but remained highly expressed overall, indicating that this gene was activated during moderate waterlogging (40 days) and involved in the oxidative stress response. For the NAC1 gene in Pedicularis chinensis, the expression level at 40 days was higher than that at 20 days and 60 days, showing a "peak - type" change, reflecting the specific regulation of the transcription factor gene in response to waterlogging. In the figure, * ($P < 0.05$) and **($P < 0.01$) mark the significance of differences, verifying the statistical significance of differences in expression between groups. In conclusion, different antioxidant genes showed unique expression sequences due to species and functional differentiation of genes in the response of plants to waterlogging stress, and they coordinately regulated the redox homeostasis of plants, supporting the adaptability to waterlogging habitats.

2.7 Evaluation of Alpine Swamp Wetland Plants' Adaptability to Seasonal Flooding Environments

Table 1 Waterlogging tolerance coefficient of 5 alpine marsh wetland plants after 20 days of waterlogging treatment

Index	syz	zsh	jmg	mxh	lbc
Height	1.549	0.964	1.204	1.027	0.878
Biomass	1.274	0.943	1.418	0.283	0.794
Root shoot ratio	1.165	1.261	0.754	2.300	0.936

Chloroohyll a/b	1.074	0.929	0.995	0.757	0.990
Chloroohyll	1.179	0.780	0.838	0.620	1.001
Carotenoid	1.156	0.746	0.904	0.603	0.981
MDA1	0.500	0.710	0.450	0.445	0.676
MDA2	0.177	1.290	0.906	0.756	0.660
POD1	0.883	6.241	8.767	2.118	1.337
POD2	0.382	3.727	1.026	2.012	1.281
SOD1	1.953	1.167	4.217	0.994	0.742
SOD2	0.839	1.041	0.195	1.630	1.072
CAT1	1.251	1.441	0.702	0.248	0.739
CAT2	1.286	0.312	0.256	1.373	0.735
SS1	1.277	1.232	0.660	0.264	0.751
SS2	0.964	1.780	0.332	0.214	0.503
SP1	0.137	0.883	1.860	0.608	0.860
SP2	1.182	0.561	0.951	0.348	2.161
Pro1	1.970	6.358	0.608	1.129	1.337
Pro2	1.004	1.089	3.028	1.352	0.890

Table 2 Waterlogging tolerance coefficient of 5 alpine marsh wetland plants after 40 days of waterlogging treatment

Index	syz	zsh	jmg	mxh	lbc
Height	1.3	0.986	1.093	0.524	0.638
Biomass	0.982	0.8	0.829	0.42	0.919
Root shoot ratio	1.257	4.918	1.107	1.231	1.174
Chloroohyll a/b	1.072	0.972	0.835	0.623	0.737
Chloroohyll	1.041	0.987	0.848	0.734	0.771
Carotenoid	1.03	1.014	0.985	0.849	0.955
MDA1	1.064	0.987	0.839	0.656	0.746
MDA2	1.143	0.977	0.848	0.641	0.756
POD1	0.863	1.244	0.798	0.244	1.113
POD2	1.493	2.124	1.194	1.512	0.441
SOD1	1.429	0.603	1.544	1.5	0.794
SOD2	0.667	0.883	1.345	0.134	1.093
CAT1	2.207	1.393	2.73	0.716	1.131
CAT2	1.556	0.706	1.393	0.539	1.175
SS1	2.833	1.153	0.522	0.34	1.14
SS2	3.758	5.797	2.705	0.821	0.309
SP1	0.344	1.321	0.796	0.482	0.974
SP2	2.099	2.249	1.122	0.394	0.703
Pro1	0.773	1.249	0.992	0.688	0.678
Pro2	2.16	2.074	0.539	0.155	1.233

Table 3 Waterlogging tolerance coefficient of 5 alpine marsh wetland plants after 60 days of waterlogging treatment

Index	syz	zsh	jmg	mxh	lbc
Height	1.032	0.974	1.028	0.486	0.831

Biomass	0.868	0.764	0.885	0.068	0.885
Root shoot ratio	1.975	1.096	4.167	1.518	2.011
Chloroohyll a/b	1.022	0.952	0.977	0.890	0.981
Chloroohyll	1.047	0.797	0.884	0.765	0.845
Carotenoid	1.197	0.835	0.978	0.774	0.952
MDA1	1.164	1.078	1.097	0.249	1.129
MDA2	1.271	1.083	1.459	2.033	1.329
POD1	1.543	1.000	3.567	10.442	3.305
POD2	0.429	0.514	1.244	3.381	0.998
SOD1	1.318	2.266	2.198	1.640	1.420
SOD2	0.578	0.702	0.840	3.400	1.456
CAT1	0.766	1.224	0.679	0.613	0.664
CAT2	2.557	1.729	1.108	1.150	2.717
SS1	0.935	0.798	0.809	0.540	2.970
SS2	3.241	5.247	0.767	3.222	0.182
SP1	1.077	1.393	1.732	1.169	1.301
SP2	7.320	2.634	1.060	1.666	0.465
Pro1	1.006	1.745	1.433	1.185	1.977
Pro2	1.017	0.616	1.336	1.922	0.289

Note: The number 1 after the indicator indicates the shoot, and the number 2 indicates the root.

Based on principal component analysis (PCA), dimensionality reduction was performed on 20 physiological indicators from five alpine wetland plant species subjected to 20-, 40-, and 60-day flooding stress. Four orthogonal principal components (PC1–PC4) were extracted, collectively explaining 100% of the cumulative variance and fully capturing the stress-response information. Temporal analysis revealed that: under **short-term stress (20 days)**, PC1 was governed by chlorophyll a/b ratio and biomass, while PC2–PC4 were driven by antioxidant enzymes–membrane lipid peroxidation products (e.g., MDA) and osmotic adjustment substances; during **mid-term stress (40 days)**, PC1's core factors shifted to chlorophyll a/bratio and aboveground MDA, with PC2–PC4 linked to root-shoot ratio (R/S) and belowground antioxidant enzyme activities; under **long-term stress (60 days)**, PC1 loadings concentrated on aboveground MDA and antioxidant enzymes, while PC2–PC4 responded to aboveground antioxidant enzymes, belowground osmolytes, and R/S, respectively. This analysis systematically elucidates differentiated driving mechanisms across photosynthetic efficiency, oxidative damage, osmotic balance, and resource allocation dimensions, establishing a multidimensional statistical framework for quantitative evaluation of flooding adaptation strategies in alpine wetland plants.

Table 4 Coefficient, eigenvalue and contribution rate of comprehensive evaluation index of waterlogged environment adaptability of alpine marsh wetland plants after 20d waterlogged treatment

	Principle component			
Index	PC1	PC2	PC3	PC4
Plant height	0.597	-0.409	-0.19	0.664
Biomass	0.952	0.247	-0.059	0.168
Root shoot ratio	-0.885	-0.284	-0.062	0.363
Chloroohyll a/b	0.972	-0.133	0.174	-0.09
Chloroohyll	0.81	-0.533	0.222	-0.099
Carotenoid	0.887	-0.445	0.072	-0.102
MDA1	0.023	0.208	0.844	-0.493

MDA2	-0.357	0.899	0.234	-0.097
POD1	0.205	0.943	-0.218	0.145
POD2	-0.545	0.598	0.584	0.069
SOD1	0.585	0.442	-0.626	0.265
SOD2	-0.85	-0.433	0.299	-0.015
CAT1	0.554	0.15	0.776	0.263
CAT2	-0.35	-0.886	-0.125	0.277
SS1	0.634	-0.012	0.73	0.256
SS2	0.174	0.284	0.898	0.288
SP1	0.185	0.806	-0.49	-0.275
SP2	0.484	-0.36	0.069	-0.795
Pro1	-0.093	0.409	0.876	0.238
Pro2	0.276	0.594	-0.744	0.131
Eigenvalue	7.18	5.48	5.288	2.052
Contribution rate（%）	35.9	27.398	26.441	10.261
Cumulative contribution rate(%)	35.9	63.297	89.739	100

Table 5 Coefficient, eigenvalue and contribution rate of comprehensive evaluation index of waterlogged environment adaptability of alpine marsh wetland plants after 40d waterlogged treatment

	Principle component			
Index	PC1	PC2	PC3	PC4
Plant height	0.597	-0.409	-0.19	0.664
Biomass	0.952	0.247	-0.059	0.168
Root shoot ratio	-0.885	-0.284	-0.062	0.363
Chloroohyll a/b	0.972	-0.133	0.174	-0.09
Chloroohyll	0.81	-0.533	0.222	-0.099
Carotenoid	0.887	-0.445	0.072	-0.102
MDA1	0.023	0.208	0.844	-0.493
MDA2	-0.357	0.899	0.234	-0.097
POD1	0.205	0.943	-0.218	0.145
POD2	-0.545	0.598	0.584	0.069
SOD1	0.585	0.442	-0.626	0.265
SOD2	-0.85	-0.433	0.299	-0.015
CAT1	0.554	0.15	0.776	0.263
CAT2	-0.35	-0.886	-0.125	0.277
SS1	0.634	-0.012	0.73	0.256
SS2	0.174	0.284	0.898	0.288
SP1	0.185	0.806	-0.49	-0.275
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Pro1	-0.093	0.409	0.876	0.238
Pro2	0.276	0.594	-0.744	0.131
Eigenvalue	7.18	5.48	5.288	2.052
Contribution rate(%)	35.9	27.398	26.441	10.261
Cumulative contribution rate(%)	35.9	63.297	89.739	100

Table6 Coefficient, eigenvalue and contribution rate of comprehensive evaluation index of waterlogged environment adaptability of alpine marsh wetland plants after 60d waterlogged treatment

	Principle component			
Index	PC1	PC2	PC3	PC4
Plant height	0.922	-0.121	-0.186	0.316
Biomass	0.963	-0.171	0.164	0.131
Root shoot ratio	0.181	-0.19	0.399	0.879
Chloroohyll a/b	0.932	0.29	0.142	0.167
Chloroohyll	0.671	0.669	0.002	0.319
Carotenoid	0.729	0.608	0.118	0.289
MDA1	0.984	-0.15	0.076	0.065
MDA2	-0.925	0.238	0.219	0.199
POD1	-0.966	0.149	0.205	0.058
POD2	-0.983	0.082	0.148	0.072
SOD1	-0.024	-0.816	-0.467	0.341
SOD2	-0.954	0.132	0.203	-0.179
CAT1	0.372	-0.418	-0.75	-0.354
CAT2	0.651	0.399	0.318	-0.562
SS1	0.357	-0.118	0.786	-0.491
SS2	-0.106	0.074	-0.942	-0.309
SP1	0.145	-0.78	0.114	0.598
SP2	0.405	0.77	-0.492	0.036
Pro1	0.252	-0.753	0.366	-0.485
Pro2	-0.767	0.284	-0.207	0.538
Eigenvalue	9.829	3.974	3.231	2.965
Contribution rate（%）	49.146	19.871	16.156	14.827
Cumulative contribution rate(%)	49.146	69.017	85.173	100

Table 7 Comprehensive index values, Wj, μ(Xj), D values of five alpine marsh wetland plants at 20 days of waterlogging treatment and the ranking of waterlogging environmental adaptability

Species	D1	D2	D3	D4	μ(X1)	μ(X2)	μ(X3)	μ(X4)	D-Value	Ranking
syz	2.55	-2.797	0.612	1.277	1	0	0.573	1	0.613	3
zsh	-0.66	2.446	3.188	0.508	0.523	1	1	0.793	0.808	1
jmg	2.053	2.433	-2.844	0.069	0.926	0.997	0	0.675	0.675	2
mxh	-4.184	-0.961	-1.493	0.592	0	0.35	0.224	0.816	0.239	5
lbc	0.241	-1.12	0.537	-2.445	0.657	0.32	0.561	0	0.472	4
Wj					0.359	0.274	0.264	0.102		

Table 8 Comprehensive index values, Wj, μ(Xj), D values of five alpine marsh wetland plants at 40 days of waterlogging treatment and the ranking of waterlogging environmental adaptability

Species	D1	D2	D3	D4	μ(X1)	μ(X2)	μ(X3)	μ(X4)	D-Value	Ranking
syz	3.268	2.278	-1.31	-0.851	1	1	0.11	0.115	0.798	1
zsh	2.985	-3.2	-0.155	0.082	0.965	0	0.385	0.394	0.627	3
jmg	0.054	1.174	0.808	2.113	0.6	0.798	0.614	1	0.678	2
mxh	-4.768	-0.513	-1.771	-0.108	0	0.491	0	0.337	0.133	5
lbc	-1.538	0.261	2.428	-1.236	0.402	0.632	1	0	0.503	4

Wj					0.559	0.214	0.143	0.084		

Table 9 Comprehensive index values, Wj, μ(Xj), D values of five alpine marsh wetland plants at 60 days of waterlogging treatment and the ranking of waterlogging environmental adaptability

Species	D1	D2	D3	D4	μ(X1)	μ(X2)	μ(X3)	μ(X4)	D-Value	Ranking
syz	2.594	3.045	-0.708	0.286	1	1	0.324	0.433	0.807	1
zsh	1.097	-1.925	-2.3	-1.23	0.814	0	0	0.086	0.413	4
jmg	0.514	-1.413	0.555	2.764	0.741	0.103	0.581	1	0.627	2
mxh	-5.441	0.813	-0.158	-0.214	0	0.551	0.436	0.319	0.227	5
lbc	1.236	-0.52	2.61	-1.606	0.831	0.283	1	0	0.626	3
Wj					0.491	0.199	0.162	0.148		

Membership function values μ(X) calculated via Formula ② based on PCA scores reveal distinct interspecific adaptation patterns across principal components. At 20-day flooding:In PC1 & PC4 dimensions, Hippuris vulgaris achieved the highest μ(X1) and μ(X4), indicating optimal adaptability in these composite metrics.In PC2 & PC3 dimensions, Poa psilolepis attained peak μ(X2) and μ(X3), signifying superior adaptation within these parameters.Minimal adaptability manifested as: lowest μ(X1) in Pedicularis chinensis (PC1), lowest μ(X2) in H. vulgaris (PC2), lowest μ(X3) in Halerpestes tricuspis (PC3), and lowest μ(X4) in Blysmus sinocompressus (PC4).Comprehensive evaluation integrating principal component contributions via Formulas ③-④ yielded weight-adjusted values (Wj) and composite indices (D), quantitatively ranking relative adaptability: P. psilolepis showed the highest D (optimal adaptability), while P. chinensis exhibited the lowest D (poorest adaptability) (Table 7).

At 40-day flooding:H. vulgaris dominated PC1 & PC2 with maximal μ(X1) and μ(X2), reflecting peak adaptability.B. sinocompressus achieved highest μ(X3) (PC3), and H. tricuspis peaked in μ(X4) (PC4), indicating dimension-specific advantages.Minimal values occurred in: P. chinensis (lowest μ(X1) & μ(X3) for PC1/PC3), P. psilolepis (lowest μ(X2) for PC2), and B. sinocompressus (lowest μ(X4) for PC4).Composite indices weighted by contribution rates (Wj) confirmed H. vulgaris with the highest D (optimal) and P. chinensis with the lowest D (poorest) (Table 8).

60-day flooding results mirrored 40-day patterns:H. vulgaris maintained highest μ(X1) & μ(X2) (PC1/PC2), sustaining optimal adaptability.B. sinocompressus and H. tricuspis retained peak μ(X3) (PC3) and μ(X4) (PC4), respectively.Consistently poorest performers: P. chinensis (lowest μ(X1) & μ(X3)), P. psilolepis (lowest μ(X2)), and B. sinocompressus (lowest μ(X4)).Final composite indices again positioned H. vulgaris with maximal D (optimal) and P. chinensis with minimal D (poorest) (Table 9).

Conclusions

Membership function values (μ(X)) derived from principal component analysis (PCA) and weighted composite evaluation indices (D) based on principal component contribution rates demonstrate that: Under **20-day flooding stress**, *Hippuris vulgaris* exhibited optimal adaptability in PC1 and PC4 dimensions (highest μ(X₁) and μ(X₄)), while *Poa psilolepis* dominated PC2 and PC3 (highest μ(X₂) and μ(X₃)), with the latter showing the highest overall adaptability (peak D-value).During **40- and 60-day flooding**, *H. vulgaris* maintained superior adaptability in PC1 and PC2 (highest μ(X₁) and μ(X₂), *Blysmus sinocompressus* excelled in PC3 (highest μ(X₃)), and *Halerpestes tricuspis* led in PC4 (highest μ(X₄). *H. vulgaris* consistently achieved significantly superior comprehensive adaptability (highest D-value).*Pedicularis chinensis* displayed the **lowest adaptability** at all timepoints (persistently minimal D-value), with consistently lowest μ(X) values across multiple principal components (PC1, PC3), highlighting its consistently weakest flooding tolerance.**Comprehensive adaptability ranking** integrating all metrics and D-values:*Hippuris vulgaris*>*Halerpestes tricuspis*>*Poa psilolepis*>*Blysmus sinocompressus* > *Pedicularis chinensis*.

Discussion

This study reveals differential flooding adaptability among five alpine swamp wetland plants through multidimensional physiological and biochemical evaluations.Hippuris vulgaris exhibits the strongest comprehensive flooding tolerance (highest composite index D),attributable to morphological optimization and efficient antioxidant regulation. Its elongated needle-like leaves minimize hydraulic resistance—analogous to the aerenchyma strategy in Phragmites australis [13]. Stable chlorophyll a/b ratio after 60-day flooding indicates intact Photosystem II (PSII) core complexes[14,15],contrasting with Pedicularis chinensis where severe chlorosis and declining chlorophyll a/b reflect PSII damage, aligning with photoinhibition observed in flooded Astragalus sinicus [16].

Antioxidant synergy underpins flooding resilience. H. vulgaris shows significant SOD and CAT upregulation during prolonged flooding[17],effectively scavenging ROS[18]and limiting MDA increase (only 16.4% rise from 20d to 60d). This enzymatic barrier mirrors mechanisms in flood-tolerant Oryza sativacultivars[19,20].Conversely, erratic SOD activity in Blysmus sinocompressus(20.6% decrease at 40d; 242% surge at 60d) exacerbates membrane peroxidation (MDA 132.9% of control at 60d), indicating antioxidant dysregulation consistent with flooding sensitivity reported for Kobresia littledalei[21,22].

Osmolyte accumulation displays tissue specificity.Belowground proline in Poa psilolepis peaks at 2.07-fold control levels under 40d flooding, maintaining osmotic potential akin to Triticum aestivumroot hypoxia adaptation[23–25].Meanwhile, aboveground soluble sugars (SS) in H. vulgaris increase 2.83-fold at 40d,providing energy substrates and osmotic buffering—paralleling carbon partitioning in aquatic Typha angustifolia[26–28].

Biomass allocation strategies diverge: Halerpestes tricuspis elevates root-shoot ratio (R/S) to 4.17×control after 60d flooding,enhancing root anchorage/absorption—a“conservative strategy”contrasting with Medicago sativa's energy-saving R/S reduction under short-term flooding[29].While Carex moorcroftii exhibits limited leaf trait plasticity along moisture gradients [30,31],stable chlorophyll a/b in flooded P. psilolepis suggests photosynthetic reorganization in alpine graminoids.

Restoration implications:Superior tolerance (D>0.6)in H. vulgaris and H. tricuspis designates them as prime species for Three-River-Source wetland rehabilitation. Their adaptation mechanisms are critical for maintaining carbon sink functions under climate warming-wetting trends[32].Conversely, the sensitivity of P.chinensis (D<0.25) necessitates water-level control to prevent community degradation.

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