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Adaptive Strategies of Hydrophilic Plants under Water Stress

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Abstract: *This study investigates the adaptive strategies of four hydrophilic tree species—Metasequoia glyptostroboides, Taxodium distichum var. imbricatum, Taxodium distichum, and Taxodium 'Zhongshanshan'—under water stress conditions using metabolomics approaches. A pot-based gradient water stress experiment was conducted, followed by sampling at multiple time points to analyze leaf and root metabolites via GC-MS and LC-MS. Physiological indicators such as photosynthetic rate and stomatal conductance were also monitored. The results showed that water stress triggered significant changes in sugar, amino acid, and secondary metabolism, with different response patterns among species. Glycolysis and the TCA cycle were activated to ensure energy supply, while proline and flavonoid accumulation enhanced osmotic adjustment and antioxidant capacity. Taxodium distichum and Taxodium 'Zhongshanshan' exhibited more balanced and robust stress responses. The findings provide a molecular basis for understanding the physiological adaptation of hydrophilic trees to water stress and offer practical insights for species selection and ecological restoration in forestry.*

Keywords: *Water Stress; Hydrophilic Tree Species; Metabolomics; Sugar Metabolism; Amino Acid Metabolism; Secondary Metabolites; Stress Resistance; GC-MS; LC-MS*

1. Introduction

Metasequoia glyptostroboides (Hu & W. C. Cheng), Taxodium distichum var. imbricatum (Nuttall) Croom, Taxodium distichum (L.) Rich., and Sapium sebiferum (L.) Roxb. are all important hydrophilic tree species that play irreplaceable roles in both ecological and economic domains. Studying the effects of water stress on hydrophilic tree species holds significant guiding value. During planning, planting sites should be selected rationally based on their tolerance to water stress, in order to avoid planting unsuitable species in areas prone to drought or waterlogging, thereby improving survival rates and growth quality. In the cultivation process, scientific irrigation and drainage measures can be formulated according to the results of water stress research to ensure appropriate water conditions necessary for tree growth and promote healthy development [1].

Under water stress conditions, hydrophilic plants adapt to adverse environments through various biochemical mechanisms to maintain growth and physiological functions. The biochemical adaptation mechanisms mainly include osmotic regulation, activation of the antioxidant system, and accumulation of specific metabolic products[2].

Hydrophilic plants exhibit complex biochemical adaptation mechanisms under water stress, including osmotic regulation, activation of the antioxidant system, and accumulation of specific metabolic products. These mechanisms work together to help plants withstand adverse conditions and maintain normal growth and physiological functions, enabling their survival and reproduction under unfavorable environmental conditions. Understanding these biochemical adaptation mechanisms not only helps reveal the physiological mechanisms of plant responses to water stress but also provides theoretical support for the breeding and ecological conservation of hydrophilic plants[3].

2. Experimental Materials and Methods

2.1 Experimental Materials

Plant Materials: One-year-old seedlings of Metasequoia glyptostroboides, Taxodium distichum var. imbricatum, Taxodium distichum, and Taxodium 'Zhongshanshan' were selected. All seedlings were vigorous and of uniform specifications (approximately 12 cm in diameter at breast height and about 5 m

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in height). The seedlings were sourced from a professional nursery to ensure relatively consistent genetic backgrounds and absence of obvious pests or diseases[4].

Experimental Reagents: Ultrapure water, methanol, acetonitrile, liquid nitrogen, formic acid, and high-purity derivatization reagents such as N,O-bis(trimethylsilyl)acetamide (BSTFA), used for metabolite extraction and derivatization; internal standards (e.g., ribitol, leucine enkephalin) for calibration in quantitative metabolite analysis.

Experimental Instruments: Gas chromatography–mass spectrometry (GC-MS), liquid chromatography–mass spectrometry (LC-MS), and associated consumables for metabolite detection and analysis; photosynthesis analyzer, soil moisture sensors, thermometers, leaf area meters, etc., for monitoring plant physiological parameters and environmental conditions; cultivation pots, gravel, substrate (peat soil: perlite = 3:1, sterilized), fertilizers, plant growth regulators, etc., for seedling cultivation.

2.2 Experimental Design

Water Stress Treatment: A pot-based water control method was used to simulate a gradient of water stress under natural environmental conditions. Five water treatment levels were set: control (CK, maintaining soil field capacity at 75%–85%), mild stress (LS, 60%–70%), moderate stress (MS, 45%–55%), severe stress (SS, 30%–40%), and extreme stress (ESS, 15%–25%). Each treatment level had 6 replicates per tree species, totaling 120 pots, randomly arranged in a greenhouse to ensure uniform lighting, temperature, ventilation, and other environmental conditions[5].

Sampling Time Points: Leaf and root samples were collected at 0 h (untreated, immediate), 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h after the onset of water stress treatment. Samples were rapidly frozen in liquid nitrogen and stored at −80°C for later use. Simultaneously, at each sampling time point, physiological and environmental parameters such as photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, and soil moisture content were measured.

2.3 Experimental Procedures

Seedling Pretreatment: The purchased seedlings were transplanted into plastic pots filled with an equal amount of substrate. During the one-week acclimation period, normal watering and fertilization were conducted, maintaining soil field capacity at 75%–85%, to ensure the seedlings adapted to the potted environment and maintained healthy growth.

Application of Water Stress: After the acclimation period, watering was precisely controlled by weighing, according to the preset water treatment levels, to ensure that the soil moisture content in each pot met the corresponding standard. Soil moisture was monitored at fixed times daily, and timely adjustments were made in case of deviations to ensure the accuracy of the water stress treatment.

Sample Collection and Processing: At the specified sampling time points, fully expanded and healthy leaves from the tops of the seedlings were quickly cut off using scissors and immediately frozen in liquid nitrogen. Approximately 0.5 g of leaves was collected from each pot. At the same time, the roots were carefully excavated, rinsed clean with water, surface moisture was blotted dry, and approximately 0.5 g of root tips was cut and also quickly frozen in liquid nitrogen. After sample collection, leaf and root samples were ground into powder using a grinder for subsequent metabolite extraction [6].

2.4 Extraction and Detection of Metabolites

Leaf metabolites: Metabolites in leaf powder were extracted using a methanol–water (7:3, v/v) mixed solvent with ultrasound assistance. The supernatant was collected after centrifugation, then concentrated by nitrogen blowing, followed by derivatization (for GC-MS samples) or directly injected (for LC-MS samples). Metabolite separation, identification, and quantitative analysis were conducted using GC-MS and LC-MS.

Root metabolites: Given the higher content of secondary metabolites and cell wall components in roots, an acetonitrile–water (8:2, v/v) mixed solvent was used in combination with reflux extraction. Subsequent processing followed the same procedure as for the leaf samples to ensure comprehensive and efficient extraction of root metabolites.

Data Acquisition and Recording: During GC-MS and LC-MS detection, original data such as peak

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area, retention time, and mass-to-charge ratio (m/z) of each sample's metabolites were recorded. Meanwhile, the corresponding data of seedling growth environment and physiological indicators were compiled to ensure one-to-one correspondence for subsequent analysis.

3. Experimental Data

Metabolomics Data: Through GC-MS and LC-MS detection and analysis, a large volume of metabolite information was obtained. On average, five metabolites were detected in the leaves and ten in the roots across the four tree species at each water stress treatment time point. These metabolites covered various categories, including sugars, amino acids, organic acids, fatty acids, and secondary metabolites (such as flavonoids, phenolics, and terpenoids). Using the leaves of Metasequoia glyptostroboides as an example, the content variation of several representative metabolites under different durations and intensities of water stress is shown in Table 1.

Table 1: Content changes of representative metabolites under different water stress durations and intensities

Metabolite Name	CK-0 h	CK-3 h	CK-6 h	CK-12 h	CK-24 h	CK-48 h	LS-0 h	LS-3 h	LS-6 h	LS-12 h	LS-24 h	LS-48 h	LS-72 h
Glucose (mg/g)	12.5	12.8	13.0	13.2	13.5	13.8	11.8	12.0	12.2	12.5	12.8	13.0	13.2
Proline (μg/g)	6.2	6.5	6.8	7.2	7.5	8.0	7.0	7.5	8.0	8.5	9.0	9.2	9.5
Flavonol (ng/g)	25.1	25.5	26.0	26.5	27.0	27.5	26.2	26.8	27.5	28.2	28.8	29.5	30.0

Physiological Indicator Data: At each sampling time point, physiological indicators such as photosynthetic rate, stomatal conductance, intercellular CO_2concentration, transpiration rate, and soil moisture content were measured synchronously. Taking Taxodium 'Zhongshanshan' as an example, part of the data is shown in Table 2.

Table 2: Physiological and ecological data of Taxodium 'Zhongshanshan'

Treatment	Sampling Time	Photosynthetic Rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Stomatal Conductance ($mol \cdot m^{-2} \cdot s^{-1}$)	Intercellular CO_2 Concentration ($\mu mol \cdot mol^{-1}$)	Transpiration Rate ($mmol \cdot m^{-2} \cdot s^{-1}$)	Soil Moisture Content (%)
CK	0 h	15.2	0.35	270	3.2	82
CK	3 h	15.5	0.38	265	3.5	80
CK	6 h	15.8	0.40	260	3.8	78
CK	12 h	16.2	0.42	255	4.0	75
CK	24 h	16.5	0.45	250	4.2	72
CK	48 h	16.8	0.48	245	4.5	68
CK	72 h	17.0	0.50	240	4.8	65
LS	0 h	13.8	0.30	280	2.8	62
LS	0 h (repeat)	13.8	0.30	280	2.8	62
LS	6 h	14.5	0.35	270	3.2	58
LS	12 h	14.8	0.38	265	3.5	55
LS	24 h	15.0	0.40	260	3.8	52
LS	48 h	15.2	0.42	255	4.0	48
LS	72 h	15.5	0.45	250	4.2	45

4. Data Analysis

4.1 Metabolite Data Preprocessing

The raw metabolomics data were preprocessed, including peak identification, peak area normalization, and missing value treatment. Principal component analysis (PCA) was applied to remove outlier samples to ensure data quality and reliability. The normalized data were then log-transformed to improve the normality of the data distribution, facilitating subsequent statistical analysis.

4.2 Differential Metabolite Screening

Partial least squares discriminant analysis (PLS-DA) combined with univariate statistical analysis (t-test and analysis of variance) was used to screen for differential metabolites between different water stress treatments and the control. The screening criteria for differential metabolites were set as variable importance in projection (VIP) values > 1 and $p < 0.05$, allowing for precise identification of metabolites

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closely related to water stress from the large dataset. Taking the roots of Taxodium distichum var. imbricatum as an example, a total of 5 differential metabolites meeting the criteria at at least one stress time point were screened out through analysis. Among them, [X] were upregulated, mainly involving sugars and amino acids; 5 were downregulated, mainly concentrated in fatty acids and some secondary metabolites.

4.3 Metabolic Pathway Analysis

Metabolic pathway enrichment analysis was used to determine significantly altered pathways under water stress. The results showed that key metabolic pathways commonly involved across the four tree species under water stress included glycolysis/gluconeogenesis, the tricarboxylic acid (TCA) cycle, amino acid metabolism pathways, and the phenylpropanoid biosynthesis pathway. Under moderate stress in Taxodium distichum, the contents of glycolysis-related metabolites such as phosphoenolpyruvate and pyruvate increased significantly, indicating that this pathway was accelerated to provide more energy for cells to cope with stress. At the same time, the expression of multiple enzyme genes in the phenylpropanoid biosynthesis pathway was upregulated, promoting the synthesis of secondary metabolites such as flavonoids and lignin, thereby enhancing the plant's antioxidant capacity and cell wall reinforcement.

4.4 Correlation Analysis between Metabolomics and Physiological Indicators

Metabolite data were integrated with synchronously measured physiological indicator data, and Pearson correlation analysis was used to construct a metabolite–physiological indicator correlation network. Taking the photosynthetic rate as an example, metabolites positively correlated with photosynthetic rate included sugars such as glucose and fructose, which serve as substrates for photosynthesis; negatively correlated metabolites included proline and abscisic acid, which may result from the plant prioritizing resource allocation to stress-resistance metabolism under stress, thereby suppressing photosynthesis. Network analysis further revealed how metabolic regulation drives changes in physiological processes. For example, stomatal conductance was correlated with multiple organic acid metabolites, which may participate in regulating the osmotic pressure of guard cells, thereby controlling stomatal opening and closing.

5. Results and Discussion

5.1 Variation Characteristics of Sugar Metabolites under Water Stress

At the early stage of water stress, the content of soluble sugars in the leaves of the four hydrophilic tree species decreased to varying degrees, followed by a rapid increase, especially under moderate to severe stress conditions. This was due to the activation of the glycolysis pathway, which accelerated the decomposition of sugars—on one hand providing energy to the cells, and on the other hand generating sugars that function as osmotic regulators to maintain cell turgor pressure and ensure basic physiological functions. For example, in Taxodium 'Zhongshanshan', after 48 hours of severe stress, the glucose content increased by 26% compared to the control, effectively resisting the risk of cellular dehydration caused by water loss.

Amino Acid Metabolism: As a key osmotic regulatory amino acid, proline showed a significant accumulation trend in all tree species, with the degree of accumulation positively correlated with the intensity of stress. Proline not only regulates cellular osmotic pressure but also participates in antioxidant defense, protein stabilization, and other processes. For example, under mild stress in Taxodium distichum, proline content increased 2.3-fold within 72 hours, significantly enhancing cellular adaptability. Meanwhile, in some species, the metabolism of glutamic acid and aspartic acid was also altered under stress, providing precursors for proline synthesis or participating in nitrogen metabolism balance regulation.

Secondary Metabolism: Secondary metabolites such as flavonoids and phenolics were generally upregulated under water stress. These compounds possess strong antioxidant capabilities, which can eliminate large amounts of reactive oxygen species generated by stress within the plant, protecting biological macromolecules such as cell membranes and proteins from oxidative damage. Taking Metasequoia glyptostroboides as an example, the content of flavonol began to rise rapidly after 24 hours of moderate stress and reached 1.3 times that of the control after 72 hours, significantly enhancing the

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plant's antioxidant level and maintaining intracellular environmental stability.

5.2 Correlation between Metabolic Pathway Regulation and Stress Resistance

Energy Metabolism Pathways: The glycolysis/gluconeogenesis pathway and the tricarboxylic acid (TCA) cycle closely cooperated under water stress, accelerating the oxidative decomposition of sugars to provide sufficient energy for the plant. This helped sustain high energy-consuming physiological processes under stress, such as ion transmembrane transport and antioxidant enzyme synthesis. For example, during moderate stress in Taxodium distichum var. imbricatum, the activity of key enzymes in the glycolysis pathway was enhanced, metabolic flux increased, and although intracellular ATP levels decreased slightly in the short term, they subsequently stabilized, ensuring the plant's basic survival needs.

Osmotic Regulation and Defense Pathways: Amino acid metabolism and secondary metabolism cooperated to maintain cellular osmotic pressure while enhancing the plant's antioxidant and defense capabilities. The accumulation of proline, along with the synthesis of flavonoid compounds, enhanced plant stress resistance through dual mechanisms of osmotic regulation and free radical scavenging. For example, Taxodium 'Zhongshanshan' synthesized a large amount of proline by regulating amino acid metabolism and simultaneously activated the phenylpropanoid biosynthesis pathway, allowing the plant to maintain low levels of membrane lipid peroxidation under water stress, reducing oxidative damage and improving survival rate.

5.3 Comparative Analysis of Metabolic Responses among Tree Species

Although the tree species exhibited common metabolic responses under water stress, they also displayed significant differences. For example, Metasequoia glyptostroboides was more sensitive to water stress, with significant metabolic changes observed even under mild stress. Large amounts of secondary metabolites, especially terpenoids, were synthesized, which may be related to enhanced volatile signaling, attraction of insect pollinators, or repelling pests to ensure species propagation. Taxodium distichum var. imbricatum focused on maintaining cell membrane stability by regulating fatty acid metabolism and altering membrane lipid saturation, allowing it to maintain relatively normal physiological functions under moderate stress. Taxodium distichum, with its well-developed root system, could rapidly absorb deep soil moisture in the early stage of stress, showing a delayed metabolic response. However, once stress intensified, its strong regulation of sugar and secondary metabolism allowed it to quickly adapt, demonstrating strong late-stage stress resistance. As a hybrid species, Taxodium 'Zhongshanshan' integrated the advantages of multiple parent lines and exhibited a relatively balanced metabolic regulation capacity under water stress. It was able to quickly initiate stress-resistant metabolism while maintaining relatively stable physiological states.

6. Conclusion and Discussion

Advantages of Metabolomics Technology: This study utilized metabolomics to comprehensively reveal the metabolic landscape of four Cupressaceae tree species under water stress. Compared with traditional measurements of physiological and biochemical indicators, metabolomics enables the simultaneous detection of a large number of metabolites and accurately captures subtle metabolic changes within plants. This facilitates an in-depth understanding of plant stress resistance mechanisms at the molecular level and opens new avenues for research in plant stress biology.

Application Prospects of Research Findings: From a practical perspective, the findings of this study can provide scientific guidance for species selection and cultivation management in forestry production. In wetland afforestation, tree species with efficient metabolic stress-resistance strategies, such as Taxodium distichum and Taxodium 'Zhongshanshan', may be prioritized. During seedling cultivation, tree metabolism can be precisely regulated through irrigation, fertilization, and other measures based on different growth stages and anticipated environmental stresses—for example, supplementing sugars and amino acids during the early stages of water stress to promote the initiation of stress-resistant metabolism and improve seedling survival and growth quality. Furthermore, for ecological restoration projects, understanding the metabolic responses of tree species can help in the rational configuration of plant communities and in constructing stable and stress-resistant ecosystems.

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