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Mechanism of Water Lily (Nymphaeaceae) to Salinity, Cold, and Heavy Metal Stresses

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Abstract

Water lilies, significant in ecology and economy, play a vital role in aquatic environments by shading water surfaces and oxygenating water through their root systems. However, they face growing challenges from environmental stressors like salinity, cold, and heavy metal contamination, which impact their germination, growth, and physiological processes. This review investigates water lily's mechanisms to salinity, cold, and heavy metal stress. The paper explores how water lilies handle stresses such as salinity, cold, and heavy metals. Discussions include survival and growth strategies under salt stress, management of essential ions, and gene regulation. The document also addresses adaptations to cold temperatures, focusing on structural changes, prevention of cell damage, and gene response. Furthermore, the review examines the plant's unique ability to absorb heavy metals, highlighting specialized structures and detoxification processes. The conclusion points out the need for more research into the genetic and molecular aspects of water lilies to improve their cultivation and use in environmental cleanup.

Keywords: Water lily, salinity tolerance, cold stress tolerance, heavy metal uptake, phytoremediation

Introduction

The Nymphaeales, with three families (Hydatellaceae, Cabombaceae, Nymphaeaceae) and 85 species [2], known as water lily, are important in economy, ecology, and evolutionary biology [23] . Their broad leaves shade the water, reducing algae growth, and their root systems help to filter and oxygenate the water, fostering a healthy aquatic environment [3].

Water lilies face increasing threats from various environmental stressors, notably salinity, cold, and heavy metal contamination. Salinity stress can inhibit germination and growth, altering plant physiology [(7)]. Cold stress, particularly significant for tropical varieties, poses challenges to survival in non-native colder climates, affecting physiological functions like membrane integrity and enzymatic activity [(21)] . Heavy metals can adversely affect water lilies by interfering with their physiological processes, such as nutrient uptake and growth, leading to reduced vitality and impaired development [(8)]. Today, the effects of salinity, cold temperatures, and heavy metals are becoming more severe due to the impact of climate change, and climate change is becoming increasingly serious [(5)].

This review compiles research on how water lily responds morphologically, biochemically, physiologically, and molecularly to salinity, cold, and heavy metals. It will identify research gaps and opportunities, aiding breeders in developing water lilies for challenging environments like extreme temperatures and heavy metal pollution, and in using these plants for environmental clean-up.

Salinity Tolerance Mechanisms

Germination and early seedling growth of four native Australian *Nymphaea* species are markedly affected by salinity [(7)]. This reduction in germination and growth is an adaptive mechanism in plants facing salinity stress, aimed at conserving resources and managing ion toxicity, thereby enhancing their long-term survival and resilience in saline conditions. Under one month of salinity stress, water lilies adapt by quickly regenerating floating leaves and reducing leaf number and size [(17)]. These changes support sustained photosynthesis in saline conditions and reduce water loss and salt uptake, collectively enhancing the plant's resilience and survival in such environments. [(9)] reported that plant mortality occurred at a salinity of 15 ppt, and there was a noticeable decline in shoot growth at 4 ppt salinity following six weeks of exposure. Salt also reduced root growth [(9)] , inhibited root biomass, and altered root architecture (root length, surface area, volume, and tip number) [(4)]. These changes reduce the intake of harmful salts and enhance the plant's overall ability to survive in saline environments. Roots of water lily displayed a high concentration of Na^+ in 150 mM salinity treatment, which reached 43.71 g/kg DW, nearly twice that of control, which demonstrates the ability to hyper-accumulate sodium. This accumulation is a strategy for coping with high-salinity environments. By storing excess sodium in the roots, water lilies can mitigate the harmful effects of saline conditions, contributing to their overall salt tolerance. This process helps in maintaining cellular and physiological balance in a high-salt environment.

Water lilies demonstrate physiological adaptations to cope with salinity stress. Photosynthesis rate and chlorophyll fluorescence in water lily decrease after salt exposure, but recovery is possible under mild stress (100 mM or less) within 18 days, suggesting tolerance to salt stress [(17)].

Research on water lilies under salt stress has revealed several molecular responses. [(17)] found that exposure to salt stress led to the activation of 120 genes and the suppression of 1214 genes, predominantly impacting oxidoreductase activity, structural molecule activity, and transmembrane transporter activity. Notably, there was a widespread downregulation of ion transporter genes, suggesting a strategy to regulate ion channels and transporters, thus preventing excessive ion accumulation and maintaining ion balance. Specifically, genes associated with nitrate transport varied in their activity levels, whereas those for ammonium transport consistently decreased, indicating a potential adjustment in nitrogen metabolism. Similarly, [17] noted a preference for nitrate absorption over ammonium in response to salt stress, with increased activity in nitrate transport genes and decreased activity in ammonium transport genes in both leaves and petioles. Additionally, [4] observed a decrease in sodium transporter genes alongside mixed regulation in potassium transporter genes, implying an adaptive strategy to limit sodium uptake while regulating potassium levels. Complementing these findings, [17] reported a decline in genes responsible for auxin signal transduction. Lastly, ([13] highlighted the significant role of *NcSODs* in salt stress response in *Nymphaea colorata*, with NcCSD showing high expression at 6 hours and *NcMnSD1*, *NcFSD1*, *NcFSD2*, and *NcFSD5* being strongly induced under such conditions, suggesting their crucial involvement in salt stress adaptation.

Despite advancements in understanding the mechanisms of water lily's response to salinity, a gap exists in genetically enhancing their tolerance. Future efforts should integrate gene-editing to target key adaptive genes and implement breeding programs, aiming to cultivate water lilies capable of thriving in saline conditions, thus expanding their ecological range.

Cold Tolerance Mechanisms

Water lilies display curled leaf edges and chlorosis (leaf yellowing) to cold stress. These morphological adaptations help the plants reduce their exposure to cold and conserve vital resources [18] . Furthermore, other morphological traits are used in assessing their cold tolerance, such as the bifacial nature of leaves, the arrangement of stomata, variations in epidermal thickness, overall leaf thickness, the density of the leaf structure, the frequency of stomata, and the ratio of palisade to spongy tissues, [22].

They also resist cold stress through sophisticated biochemical strategies. They reduce cellular damage by decreasing malondialdehyde levels and increasing betaine [21] or reduce photosynthetic pigments, changes in soluble sugars, proline [10], and enzyme activities like SOD and CAT, helping in osmoprotection and minimizing oxidative stress [18]. These mechanisms illustrate the water lily' adaptive response to maintain vital functions in cold conditions.

Water lilies exhibit distinct physiological mechanisms to tolerate cold stress. [21] observed that transgenic water lilies have lower conductivity, suggesting reduced cellular damage, and increased activity in antioxidative enzymes like superoxide dismutase, catalase, and peroxidase, indicating a robust defense against oxidative stress caused by cold exposure. [18] found changes in membrane permeability due to varying degrees of membrane peroxidation, affecting cell health and function in cold conditions. Additionally, [10] noted an increase in catalase activity in response to cold temperatures, a crucial adaptation for mitigating oxidative stress by decomposing hydrogen peroxide, a harmful byproduct intensified under stress.

Cold stress tolerance in tropical water lilies at the molecular level is also analyzed. [21] enhanced this tolerance by genetically modifying water lily with a gene for choline oxidase (CodA). Cold stress alters the expression of genes related to hormone signaling and metabolism, notably highlighting the role of the *NlZAT12* gene [18]. Overexpressing *NlZAT12* in *Arabidopsis thaliana* increased cold tolerance by altering cold-responsive protein genes and reducing oxidative stress indicators, offering insights into the genetic pathways that equip these plants to survive in cold conditions [18].

To advance the understanding of cold tolerance in tropical water lily, research should focus on exploring a wider range of species, as current studies are limited in scope. It's also crucial to delve deeper into the functions of specific genes involved in cold tolerance, particularly their interactions and impacts on plant physiology. Furthermore, employing advanced genetic modification techniques, like CRISPR/Cas9, could aid in developing varieties with enhanced cold resistance. Additionally, field studies are essential to observe the plants' natural adaptive mechanisms to cold stress, offering practical insights beyond laboratory findings. This targeted research approach could lead to more effective cultivation and conservation strategies for these ecologically and aesthetically valuable plants.

Heavy Metal Uptake Mechanism

Water lilies, as specialized aquatic plants, exhibit a great ability for heavy metal uptake [19; 1; 6; 11; 20]. Distinct from terrestrial plants, their constant contact with the aquatic environment allows them to absorb heavy metals through both their roots and submerged leaves and stems, a dual absorption mode that enhances their capacity for metal uptake from water [14]. They possess specialized anatomical structures, notably epidermal glands and glandular trichomes located on the abaxial side of the leaf laminae, petioles, and rhizomes, facilitating effective heavy metal accumulation [16].

Once heavy metals enter the plant tissues, water lilies activate biochemical pathways for mitigation. They produce polyphenols and other chelating agents to bind and neutralize these metals, aiding in their safe cellular storage [15]. Additionally, enzymes like peroxidases and polyphenol oxidases play a key role in further detoxifying and transforming these metals into less harmful forms [16]. To protect critical cellular functions, water lilies likely utilize compartmentalization and regulation mechanisms, managing heavy metal concentrations within the plant. The increase in astrosclereids, a supportive parenchyma tissue, especially under chromium stress, suggests an adaptive response for heavy metal sequestration, thereby reducing their toxic impacts [12].

The molecular response of water lilies to heavy metal exposure involves distinct reactions from *NcSOD* genes [13]. When subjected to copper sulfate, the expressions of *NcSOD* genes fluctuated at various intervals. In the case of cadmium chloride exposure, all *NcSOD* genes showed an upsurge in activity at the 2-hour mark. Particularly, *NcCSD3* maintained high expression levels consistently over time. However, a subset of the nine *NcSOD* genes demonstrated an initial increase followed by a decrease in expression, a pattern observed under both heavy metal treatments.

To enhance the understanding of water lilies' response to heavy metal exposure and their potential in phytoremediation, two key research gaps need to be addressed. Firstly, there's a notable gap in our understanding of the long-term effects of heavy metal exposure on water lilies. Current research primarily focuses on immediate or short-term responses, leaving the long-term impacts of fluctuating environmental conditions largely unexplored. Future research should, therefore, focus on conducting longitudinal studies. These studies would monitor the effects of continuous exposure to heavy metals over extended periods, examining changes in growth, reproduction, and overall survival in contaminated environments.

Secondly, while some molecular responses to heavy metals in water lilies are known, the detailed mechanisms of how these plants tolerate and accumulate heavy metals at cellular and systemic levels are not fully understood. Future research should delve into the cellular and physiological processes underlying water lilies' ability to tolerate and accumulate heavy metals. Investigating the roles of specific genes, proteins, and cellular structures involved in the detoxification and sequestration processes would provide deeper insight into the plant's adaptive mechanisms. This research could significantly advance the knowledge of water lilies' potential in heavy metal phytoremediation, especially regarding their long-term application and understanding of the underlying mechanisms of tolerance and accumulation.

Conclusion

In summary, this review underscores the crucial ecological and economic roles of water lilies and the challenges they face from environmental stressors such as salinity, cold, and heavy metal contamination. These factors significantly affect their growth, germination, and physiological processes. The adaptations of water lilies to these stressors are explored, including responses to salinity in terms of ion homeostasis and gene regulation, morphological and cellular adaptations for cold tolerance, and unique capabilities in heavy metal absorption. The review highlights the need for further research to enhance genetic and molecular understanding of these adaptations. Such research is vital for improving water lily cultivation and their application in environmental remediation, contributing significantly to environmental sustainability.

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