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Nuclear Ultrastructure in Mesophyll Cells of Salt-Tolerant Artemisia Marschalliana Leaves

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Abstract: The nucleus, as the control center of the eukaryotic cell, plays a pivotal role in orchestrating cellular responses to environmental stresses, including salinity. Salt-tolerant plants, such as Artemisia marschalliana, possess unique adaptive mechanisms to thrive in high-salinity environments. This study investigates the distinct structural features of nuclei within the leaf mesophyll cells of Artemisia marschalliana, aiming to elucidate potential ultrastructural adaptations associated with its salt tolerance. Using transmission electron microscopy, we analyzed the chromatin organization, nucleolar morphology, and nuclear envelope integrity. Our findings reveal specific nuclear characteristics, including a well-defined nucleolus with distinct fibrillar and granular components and a relatively dispersed chromatin pattern, suggesting active transcriptional and metabolic processes. These ultrastructural observations provide insights into the cellular strategies employed by Artemisia marschalliana to maintain nuclear homeostasis and cellular function under saline conditions, contributing to a deeper understanding of plant salt tolerance mechanisms.

Keywords: Artemisia marschalliana, Salt Tolerance, Nucleus, Ultrastructure, Mesophyll Cells, Transmission Electron Microscopy, Chromatin, Nucleolus

Introduction: Salinity is a major abiotic stress factor that severely limits plant growth and agricultural productivity worldwide. High concentrations of soluble salts in the

soil adversely affect plants by imposing osmotic stress, ion toxicity, and oxidative stress, leading to a cascade of physiological and biochemical disturbances [1]. Plants have evolved diverse strategies to cope with saline environments, ranging from salt exclusion and compartmentalization to osmotic adjustment and antioxidant defense [1, 3]. Halophytes, such as *Artemisia marschalliana*, are naturally adapted plants capable of completing their life cycle in high-salt conditions, making them valuable models for studying salt tolerance mechanisms.

The nucleus, housing the plant's genetic material, is central to orchestrating cellular responses to environmental cues, including stress [4]. Gene expression regulation, DNA repair, and ribosomal biogenesis—all critical processes for cellular survival and adaptation—are tightly controlled within the nuclear compartment. Previous research has shown that salt stress can induce significant changes in cellular ultrastructure, including alterations in root cells [1] and mesophyll cells [3], and can even lead to nuclear and DNA degradation in sensitive plant species [2]. These changes often reflect the cell's struggle to maintain homeostasis under adverse conditions. Furthermore, metabolism of proteins and nucleic acids undergoes formative changes under salinization conditions [6], underscoring the dynamic nature of nuclear activity during stress.

Despite the general understanding of salt stress impacts on plant cells, specific ultrastructural adaptations of the nucleus in highly salt-tolerant species like *Artemisia marschalliana* remain underexplored. Understanding the fine structural organization of the nucleus in such resilient plants can provide crucial insights into how they maintain genetic integrity, regulate gene expression, and sustain metabolic activity under conditions that would be detrimental to glycophytes (salt-sensitive plants). This study aims to characterize the structural features of nuclei within the leaf mesophyll cells of *Artemisia marschalliana*, thereby contributing to a more comprehensive understanding of the cellular basis of salt tolerance.

2. METHODOLOGY

To investigate the ultrastructural features of nuclei in leaf mesophyll cells of salt-tolerant *Artemisia marschalliana*, standard protocols for transmission electron microscopy (TEM) sample preparation and observation were followed.

2.1. Plant Material:

Leaves were collected from mature, healthy specimens of Artemisia marschalliana grown under natural, salt-tolerant conditions. The plant material was immediately processed to preserve cellular integrity.

2.2. Tissue Preparation for Electron Microscopy:

Small leaf segments (approximately 1-2 mm^2) were excised from the mesophyll tissue and subjected to a meticulous fixation process.

- Primary Fixation: Samples were immersed in a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 4°C. This step cross-links proteins, preserving cellular architecture.
- Washing: Fixed samples were rinsed thoroughly three times, for 15 minutes each, with 0.1 M phosphate buffer to remove excess glutaraldehyde.
- Secondary Fixation: Samples were then post-fixed in 1% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer (pH 7.2) for 2 hours at 4°C. Osmium tetroxide stains lipids and further stabilizes cellular components, enhancing contrast for electron microscopy.
- Dehydration: A graded series of ethanol solutions (50%, 70%, 90%, 100% absolute ethanol) was used to dehydrate the samples. Each step involved 15-minute immersions, with three changes in absolute ethanol to ensure complete water removal.
- Infiltration and Embedding: Dehydrated samples were infiltrated with propylene oxide for 30 minutes, followed by a gradual infiltration with Spurr's resin. Samples were placed in a mixture of propylene oxide and resin (1:1) for 1 hour, then in pure resin for 2 hours. Finally, samples were embedded in fresh Spurr's resin in flat molds and polymerized at 70°C for 48 hours.

2.3. Sectioning and Staining:

Ultrathin sections (approximately 70-90 nm thickness) were cut from the embedded blocks using an LKB Ultramicrotome with a diamond knife. These sections were then mounted on copper grids (200 mesh). For

enhanced contrast, the sections were stained sequentially:

- Uranyl Acetate Staining: Sections were stained with 2% uranyl acetate solution for 15 minutes at room temperature.
- Lead Citrate Staining: Following rinsing, sections were stained with lead citrate solution for 5 minutes at room temperature.

2.4. Microscopy and Analysis:

Stained ultrathin sections were examined using a JEM-100CX Transmission Electron Microscope operating at 80 kV. Images were captured at various magnifications to observe the overall morphology of mesophyll cells and, specifically, the detailed ultrastructure of their nuclei. Qualitative analysis focused on describing the organization of chromatin (euchromatin and heterochromatin distribution), the morphology and internal components of the nucleolus (fibrillar and granular regions), and the integrity and pore distribution of the nuclear envelope. Observations were compared against general knowledge of plant cell nuclear ultrastructure to identify any distinctive features potentially related to salt tolerance.

3. Results

Transmission electron microscopic examination of leaf mesophyll cells from salt-tolerant *Artemisia marschalliana* revealed distinct structural features within their nuclei, indicative of active metabolic states and potential adaptations to saline conditions.

3.1. General Nuclear Morphology:

The nuclei in the mesophyll cells were typically spherical to ovoid in shape, prominently located within the cytoplasm, and often surrounded by chloroplasts and vacuoles. The nuclear envelope appeared intact and well-defined, consisting of inner and outer membranes with clearly discernible nuclear pores, suggesting active transport between the nucleoplasm and cytoplasm.

3.2. Chromatin Organization:

A notable feature was the organization of chromatin. While both euchromatin (electron-lucent, dispersed chromatin) and heterochromatin (electron-dense, condensed chromatin) were present, euchromatin appeared relatively abundant and widely dispersed throughout the nucleoplasm. Heterochromatin was primarily observed as small, condensed clumps associated with the inner nuclear membrane and scattered within the nucleoplasm. This relatively decondensed chromatin state suggests a high level of transcriptional activity, which is crucial for gene expression and cellular responses.

3.3. Nucleolar Ultrastructure:

The nucleolus was a prominent and well-developed organelle within the nucleus, typically appearing as a single, large, and often irregularly shaped body. Its internal organization was clearly differentiated into two main components:

- Fibrillar Centers (FCs): These appeared as pale, electron-lucent regions, often surrounded by the dense fibrillar component.
- Dense Fibrillar Component (DFC): This was observed as a highly electron-dense network surrounding the fibrillar centers.
- Granular Component (GC): This appeared as numerous electron-dense granules, typically located at the periphery of the nucleolus.

The distinct and well-organized appearance of the nucleolus, particularly the prominent granular component, indicates active ribosomal RNA (rRNA) synthesis and ribosome biogenesis. This suggests a high capacity for protein synthesis, which is essential for cellular maintenance and stress response mechanisms.

3.4. Absence of Stress-Induced Degradation:

Crucially, there was no widespread evidence of nuclear or DNA degradation, such as chromatin condensation into large, irregular masses or fragmentation of the nuclear envelope, which are often observed in salt-sensitive plants subjected to severe salt stress [2]. The integrity of the nuclear envelope and the organized chromatin structure suggest that Artemisia marschalliana is able to maintain nuclear homeostasis under its salt-tolerant conditions.

These ultrastructural findings collectively point towards a highly active and well-preserved nuclear machinery in the mesophyll cells of *Artemisia marschalliana*, likely contributing to its remarkable ability to tolerate high salinity.

4. DISCUSSION

The ultrastructural features of nuclei in leaf mesophyll cells of *Artemisia marschalliana* provide compelling insights into the cellular adaptations that underpin its salt tolerance. The observed nuclear characteristics, particularly the dispersed chromatin and the well-developed nucleolus, suggest an active and robust transcriptional and translational machinery, which is crucial for coping with environmental stress.

The prevalence of euchromatin, indicative of actively transcribed genes, implies that *Artemisia marschalliana* maintains a high level of gene expression even under saline conditions. This contrasts with observations in salt-sensitive plants, where severe salt stress can lead to chromatin condensation and reduced transcriptional activity, signaling cellular damage or programmed cell death [2]. The ability to sustain active gene expression is paramount for synthesizing stress-response proteins, enzymes involved in ion homeostasis, and osmolytes necessary for osmotic adjustment, all of which are vital for salt tolerance [6].

The prominent and well-organized nucleolus, with its distinct fibrillar and granular components, further supports the notion of high metabolic activity. The nucleolus is the primary site of ribosomal RNA (rRNA) synthesis and ribosome assembly, processes fundamental for protein synthesis. A well-developed nucleolus suggests an efficient capacity for producing the ribosomes required for the extensive protein synthesis needed to manage salt stress, including the production of transporters, detoxification enzymes, and structural proteins [6]. This aligns with studies indicating the importance of protein and nucleic acid metabolism in plants under salinization [6].

The intact nuclear envelope and the absence of widespread nuclear degradation or DNA fragmentation are critical findings. In contrast, salt stress can induce nuclear and DNA degradation in meristematic cells of sensitive plants like barley [2]. The preservation of nuclear integrity in *Artemisia marschalliana* highlights its effective cellular defense mechanisms against salt-induced damage, allowing the cell to maintain its genetic stability and functional capacity. This resilience at the nuclear level likely contributes significantly to the overall salt tolerance of the plant.

These ultrastructural observations complement physiological and biochemical studies on halophytes. The ability to maintain nuclear homeostasis and active gene expression under saline conditions is a fundamental adaptive trait. While this study provides qualitative insights into nuclear morphology, future research could benefit from quantitative analyses of chromatin density, nucleolar volume, and nuclear pore distribution under varying salt concentrations to establish a more precise correlation with salt tolerance levels. Furthermore, integrating these ultrastructural findings with molecular studies (e.g., gene expression profiling, proteomics) could provide a more comprehensive understanding of the specific genes and proteins regulated by this active nuclear machinery in response to salinity.

5. CONCLUSION

This study provides a detailed ultrastructural analysis of nuclei in leaf mesophyll cells of salt-tolerant *Artemisia marschalliana*. Our findings demonstrate that these nuclei exhibit characteristics indicative of high metabolic activity and robust cellular maintenance under saline conditions, including a dispersed chromatin pattern and a well-developed, organized nucleolus. Crucially, the absence of widespread nuclear degradation suggests effective mechanisms for preserving nuclear integrity in this halophytic species. These structural adaptations at the nuclear level are likely fundamental to *Artemisia marschalliana*'s remarkable ability to tolerate high salinity. The insights gained from this study contribute to our understanding of the cellular and subcellular basis of plant salt tolerance, providing valuable information for future research aimed at enhancing crop resilience in saline environments.

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