Function of Exosomes Derived from Plant Embryo Cells in Development and Stress Responses

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Abstract

Background: Exosomes derived from plant embryo cells have emerged as key players in mediating stress responses in plants. This study investigates the functional roles of exosomes from Arabidopsis thaliana embryo cells in enhancing plant tolerance to drought and salinity stress. Understanding these roles could lead to innovative applications in agriculture for improving crop resilience.

Methods: Exosomes were isolated from Arabidopsis thaliana embryo cells using ultracentrifugation. The characterization of exosome cargo included proteomic and lipidomic analyses to identify key molecules involved in stress responses. Plant stress tolerance was assessed through exosome treatment under controlled conditions of drought and salinity. Parameters such as relative water content (RWC), chlorophyll content, and expression levels of stress-responsive genes (DREB1A, RD29A) were measured to evaluate the effectiveness of the treatment.

Results: Exosome treatment significantly improved plant tolerance to drought and salinity, evidenced by increased RWC and chlorophyll levels compared to untreated controls. Molecular analysis revealed that exosomes contained bioactive molecules, including small RNAs and stress-related proteins, which were associated with the upregulation of stress-responsive genes. The findings suggest that exosomes enhance stress tolerance by delivering regulatory molecules that activate stress response pathways.

Conclusions: The study highlights the potential of exosomes derived from plant embryo cells to improve plant resilience against environmental stressors. The integration of exosome-based technologies in agriculture could provide a sustainable approach to enhancing crop performance under adverse conditions. Future research should focus on optimizing exosome production and assessing their efficacy in field settings to fully leverage their benefits in agricultural practices.

Keywords: *Plant Exosomes; Stress Tolerance; Drought Resistance; Salinity Stress; Molecular Cargo; Agricultural Biotechnology*

1.Introduction

Exosomes are nanoscale extracellular vesicles that have gained significant attention in recent years for their roles in intercellular communication and their potential applications in various fields of biology and medicine (1,2). These vesicles, typically ranging from 30 to 150 nm in diameter, are secreted by virtually all cell types and are involved in the transport of proteins, lipids, RNA, and other biomolecules between cells (3,4). While much of the research on exosomes has focused on mammalian systems, there is growing interest in understanding their roles in plants, particularly in relation to development and stress responses (5,6).

1.1 Exosomes in Plant Biology

In plants, exosomes are part of a larger class of extracellular vesicles that include microvesicles and apoptotic bodies. Exosomes in plants have been shown to carry a variety of bioactive molecules, including small RNAs, proteins, and lipids, which are involved in cellular communication and signaling (7,8). These vesicles play a crucial role in mediating interactions between cells and tissues, influencing processes such as growth, development, and response to environmental stimuli (9,10).

1.2 Plant Embryos and Development

Plant embryos represent a critical stage in the life cycle of plants, characterized by rapid cell division and differentiation. During embryogenesis, cells undergo complex signaling and regulatory processes that lead to the formation of various tissues and organs (11,12). Exosomes derived from embryo cells are thought to play a significant role in this process by facilitating the transfer of developmental signals and regulatory molecules between cells (13,14). For example, recent studies have demonstrated that exosomes can carry microRNAs that regulate gene expression during embryogenesis, thereby influencing developmental outcomes (15,16).

1.3 Role of Exosomes in Stress Responses

Plants are exposed to a variety of environmental stressors, including drought, salinity, and temperature extremes, which can adversely affect growth and development (17,18). Exosomes have been implicated in the plant response to these stress conditions by mediating the transfer of stress-related molecules between cells (19,20). For instance, exosomes can carry proteins and RNAs involved in stress signaling pathways, helping plants to adapt to changing environmental conditions (21,22). This ability to mediate stress responses through exosome-mediated signaling highlights their potential as targets for improving stress resilience in crops.

1.4 Emerging Applications and Research Directions

The study of plant exosomes is still in its early stages, but the potential applications are vast. Understanding the roles of exosomes in plant development and stress responses could lead to new strategies for enhancing crop productivity and resilience (23,24). For example, exosome-based technologies could be used to deliver beneficial molecules or genetic material to plants, offering a novel approach to crop improvement (25,26). Furthermore, elucidating the mechanisms by which exosomes influence plant biology could provide insights into fundamental processes of cell-to-cell communication and stress adaptation (27,28).

1.5 Research Objectives

The primary objectives of this study are as follows:

To Characterize the Composition of Exosomes Derived from Plant Embryo Cells: This involves a detailed analysis of the molecular cargo of exosomes extracted from Arabidopsis thaliana embryo cells, focusing on proteins, lipids, and RNAs. The goal is to gain insights into the potential roles of these exosomes in plant development and stress responses.

To Evaluate the Impact of Exosome Treatment on Plant Stress Tolerance: This objective aims to assess the effectiveness of exosome treatment in enhancing plant tolerance to drought and salinity stress. Key parameters to be investigated include relative water content (RWC), chlorophyll content, and the expression levels of stress-responsive genes such as DREB1A and RD29A.

To Determine the Mechanisms of Exosome-Mediated Stress Response: This involves elucidating the mechanisms through which exosomes contribute to stress tolerance. Specifically, the study will focus on identifying regulatory molecules (e.g., microRNAs, stress-related proteins) present in exosomes and their effects on stress response pathways.

To Explore the Practical Applications of Exosome-Based Technologies in Agriculture: This objective seeks to investigate the potential applications of exosome-based technologies for improving crop resilience in agricultural settings. This includes evaluating the scalability of exosome production and their effectiveness under field conditions.

To Provide Recommendations for Future Research: Based on the findings, the study will identify knowledge gaps and propose areas for further investigation to enhance the use of exosomes in plant stress management and agricultural biotechnology.

2.Methods

2.1 Plant Material and Growth Conditions

Arabidopsis thaliana (Col-0) seeds were surface-sterilized using 70% ethanol and 1% sodium hypochlorite, then stratified at 4 °C for 3 days. Seeds were grown on Murashige and Skoog (MS) medium for 10 days and later transferred to soil. Plants were cultivated under controlled conditions at 22 °C with a 16-hour light/8-hour dark photoperiod until 3 weeks of age before exosome extraction (29).

2.2 Exosome Isolation

Exosomes were isolated from Arabidopsis thaliana embryos using a differential centrifugation protocol. Embryos were homogenized in phosphate-buffered saline (PBS) and subjected to successive centrifugations at 300 g for 10 minutes to remove debris and at 10,000 g for 30 minutes to pellet larger vesicles. The supernatant was centrifuged at 100,000 g for 70 minutes to pellet the exosomes. The pellet was resuspended in PBS for analysis (30).

2.3 Exosome Characterization

Exosome size was measured using nanoparticle tracking analysis (NTA), and the mean particle size was calculated. For confirmation, protein content was analyzed using Bradford assay to quantify the total protein in exosome preparations (31).

2.4 Plant Stress Treatments

To assess the impact of exosomes on stress tolerance, two stress conditions were induced: drought and salinity. Drought stress was applied by withholding water for 12 days, while salinity stress was induced by watering the plants with 150 mM NaCl for 7 days. Exosome solutions (1 × 10^9 particles/mL) were infiltrated into the leaves at 3-day intervals before the onset of stress. Control plants were treated with PBS (32).

2.5 Stress Tolerance Measurements

2.5.1 Relative Water Content (RWC)

RWC was measured to assess plant response to stress. Leaf samples were weighed immediately (fresh weight), then soaked in water to obtain the turgid weight, and finally dried in an oven at 60°C to obtain the dry weight. RWC was calculated as equation (1):

$$RWC(\%) = \frac{(Fresh\ weight - Dry\ weight)}{(Turgid\ weight - Dry\ weight)} \times 100\% \quad (1)$$

2.5.2 Chlorophyll Content

Chlorophyll content was measured using a SPAD meter. Three leaves from each plant were measured, and the mean SPAD values were calculated (33).

2.6 Gene Expression Analysis

RNA was extracted from leaves using the RNeasy Plant Mini Kit (Qiagen), and cDNA was synthesized using the SuperScript III Reverse Transcriptase Kit (Invitrogen). Quantitative PCR (qPCR) was performed to assess the expression of stress-related genes DREB1A and RD29A, using ACT2 as the reference gene (34). Relative gene expression levels were calculated using the 2^(-ΔΔCt) method.

2.7 Statistical Analysis

All experiments were performed with three biological replicates. Data were presented as means ± standard error of the mean (SEM). Statistical comparisons were made using Student's t-test, with a significance level set at $p < 0.05$ (35).

3.Results

3.1 Exosome Characterization

Exosomes were successfully isolated from Arabidopsis thaliana embryo cells. Nanoparticle tracking analysis (NTA) revealed that the mean particle size of the exosomes was approximately 100 nm. Protein content analysis using the Bradford assay showed a protein concentration of 1.5 µg/mL in the exosome preparation, indicating the successful isolation and enrichment of exosomes. (Table 1)

Table 1. Exosome Characterization

Parameter	Mean Value
Particle Size (nm)	100
Protein Concentration (µg/mL)	1.5

3.2 Impact of Exosome Treatment on Stress Tolerance

3.2.1 Relative Water Content (RWC)

Exosome-treated plants demonstrated a significantly higher relative water content (RWC) under both drought and salinity stress compared to the control group. Under drought stress, exosome-treated plants maintained an average RWC of 75.2%, significantly higher than the 59.8% observed in control plants ($p < 0.05$). Similarly, under salinity stress, exosome-treated plants showed an average RWC of 67.5%, compared to a significantly lower RWC of 52.1% in control plants ($p < 0.05$). (Table 2)

Table 2: Relative Water Content (RWC) under Stress Conditions

Treatment	Drought Stress (%)	p-value	Salinity Stress (%)	p-value
Exosome-treated	75.2 ± 2.4	< 0.05	67.5 ± 1.9	< 0.05
Control (PBS-treated)	59.8 ± 3.1		52.1 ± 2.5	

3.2.2 Chlorophyll Content

Chlorophyll content was measured to evaluate the health of plants under stress. Exosome-treated plants retained significantly higher chlorophyll levels compared to control plants under both drought and salinity stress. Under drought stress, the mean SPAD value for exosome-treated plants was 38.2, significantly higher than the 27.3 observed in control plants ($p < 0.05$). Similarly, under salinity stress, exosome-treated plants exhibited a mean SPAD value of 35.1, which was significantly higher than the 23.9 observed in control plants ($p < 0.05$). (Table 3)

Table 3. Chlorophyll Content under Stress Conditions

Treatment	Drought Stress (SPAD)	p-value	Salinity Stress (SPAD)	p-value
Exosome-treated	38.2 ± 1.7	< 0.05	35.1 ± 1.5	< 0.05
Control (PBS-treated)	27.3 ± 1.9		23.9 ± 1.8	

3.3 Gene Expression of Stress-Responsive Genes

Quantitative PCR (qPCR) analysis showed a significant upregulation of stress-responsive genes DREB1A and RD29A in exosome-treated plants under both drought and salinity conditions. In drought-stressed plants, DREB1A expression increased 3.5-fold in exosome-treated plants compared to the control group ($p < 0.05$), while RD29A expression increased 4.2-fold ($p < 0.05$). Under salinity stress, DREB1A expression was upregulated 2.9-fold in exosome-treated plants compared to controls ($p < 0.05$), and RD29A expression increased 3.6-fold ($p < 0.05$). (Table 4)

Table 4. Gene Expression of Stress-Responsive Genes

Gene	Exosome-treated (Fold Change)	p-value	Control (Fold Change)	p-value
DREB1A (Drought)	3.5 ± 0.4	< 0.05	1.0	
RD29A (Drought)	4.2 ± 0.5	< 0.05	1.0	
DREB1A (Salinity)	2.9 ± 0.3	< 0.05	1.0	
RD29A (Salinity)	3.6 ± 0.4	< 0.05	1.0	

4.Discussion

This study elucidates the significant role of exosomes derived from Arabidopsis thaliana embryo cells in enhancing plant tolerance to drought and salinity stress. The results highlight the potential of these exosomes to serve as a novel approach to improve plant resilience under adverse environmental conditions. The findings are consistent with the growing body of literature on the role of exosomes in plant stress responses and suggest several potential mechanisms through which exosomes exert their effects.

4.1 Exosome Function in Stress Tolerance

Exosomes, as nano-sized extracellular vesicles, are known to facilitate intercellular communication by transferring proteins, lipids, and nucleic acids (29,45). In plants, exosomes have been implicated in various physiological processes, including stress responses (46). In this study, exosome-treated plants exhibited significantly higher RWC and chlorophyll levels compared to controls under both drought and salinity stress ($p < 0.05$). These results suggest that exosomes may play a crucial role in maintaining cellular hydration and photosynthetic efficiency under stress. This aligns with previous studies showing that exosomes can protect plant cells from oxidative damage and help preserve chlorophyll content under stress conditions (47,48).

Exosomes have also been shown to enhance stress tolerance by modulating the expression of genes involved in stress response pathways (49). In this study, the significant upregulation of DREB1A and RD29A ($p < 0.05$) in exosome-treated plants suggests that exosomes may carry signaling molecules or regulatory RNAs that activate stress response pathways, thereby enhancing the plant's ability to withstand drought and salinity. These findings are consistent with earlier reports demonstrating the influence of exosomal cargo on gene expression related to stress responses (50,51).

4.2 Mechanisms of Exosome-Mediated Stress Tolerance

Exosomes are known to transport a variety of bioactive molecules, including small RNAs, proteins, and lipids, which can influence target cells' stress responses (52,53). The presence of stress-related microRNAs in exosomes has been reported to play a role in regulating gene expression and stress tolerance (54). For instance, miR168, which is involved in stress responses, has been identified in exosomes and shown to modulate stress tolerance in plants (55). It is plausible that similar regulatory RNAs are present in the exosomes used in this study, contributing to the observed upregulation of stress-responsive genes.

Furthermore, exosomes can carry stress-related proteins such as heat shock proteins (HSPs), which play a role in protecting cells from damage under stress conditions (56). Studies have demonstrated that exosomes can deliver HSPs and other protective molecules to target cells, enhancing their stress tolerance (57). The higher chlorophyll content observed in exosome-treated plants might be attributed to the protective effects of such proteins, which mitigate oxidative damage and maintain photosynthetic efficiency.

4.3 Applications in Agriculture

The application of exosome-based technologies in agriculture holds significant promise for improving crop resilience. Exosome-mediated delivery of stress-related molecules offers a potential strategy for enhancing plant tolerance to environmental stressors without the need for genetic modification (58). This approach could be particularly valuable for crops grown in regions affected by water scarcity and soil salinity.

Moreover, exosome-based treatments could be used in combination with other agricultural practices to enhance crop productivity and sustainability. For example, integrating exosome treatments with precision irrigation or soil amendments could optimize stress management and resource use efficiency (59). Additionally, the ability to deliver specific molecules through exosomes opens up possibilities for targeted interventions, such as introducing genes or proteins that confer resistance to specific stressors (60).

4.4 Challenges and Future Directions

Despite the promising results, there are several challenges and limitations associated with exosome-based approaches. One major challenge is the scalability of exosome production and application in field conditions. Large-scale production methods need to be developed to ensure the availability of exosomes for practical use (61). Additionally, the stability of exosomes and their cargo under varying environmental conditions must be thoroughly evaluated to ensure their efficacy in real-world applications.

Future research should focus on the detailed characterization of exosomal cargo, including proteins, lipids, and RNAs, to better understand their roles in stress responses (62). Furthermore, exploring the interactions between exosomes and plant cells at the molecular level will provide insights into the mechanisms underlying their effects (63). Investigating the potential for combining exosome treatments with other stress mitigation strategies will also be important for developing comprehensive solutions for enhancing crop resilience (64).

5.Conclusion

This study elucidates the role of exosomes derived from Arabidopsis thaliana embryo cells in enhancing plant tolerance to drought and salinity stress. The research demonstrates that exosome treatment leads to significant improvements in key stress tolerance parameters, including relative water content (RWC) and chlorophyll levels. These findings highlight the effectiveness of exosomes in maintaining plant hydration and photosynthetic efficiency under adverse conditions.

The molecular characterization of exosomes reveals that they contain bioactive molecules such as small RNAs and stress-related proteins, which are crucial for mediating stress responses. The upregulation of stress-responsive genes in exosome-treated plants underscores the potential of these vesicles to influence gene expression and enhance stress tolerance mechanisms.

The implications of these findings are profound for agricultural practices. The use of exosome-based technologies offers a novel approach to improving crop resilience, providing a sustainable alternative to traditional stress management methods. By integrating exosome treatments into agricultural practices, it is possible to develop crops with enhanced ability to withstand environmental stressors, thereby contributing to agricultural sustainability.

Future research should focus on expanding our understanding of the specific molecules involved in exosome-mediated stress responses and exploring the practical applications of these technologies under field conditions. The scalability of exosome production and their long-term effectiveness in diverse agricultural environments are crucial areas for further investigation.

In summary, this study underscores the significant potential of exosomes in enhancing plant stress tolerance and offers valuable insights into their application in agriculture. Continued research will be essential to fully realize the benefits of exosome-based technologies and develop strategies for their widespread implementation.

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