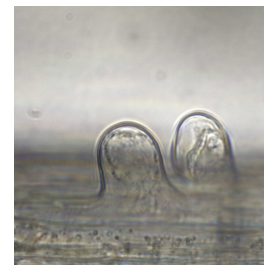


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Inducing Plasmolysis in Root Hair Cell Membranes: A Sensitive Assay for Studying Cell Membrane Physiology

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We use this protocol and it's working

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Abstract

Plasmolysis, a fundamental phenomenon in plant physiology, occurs when plant cells are exposed to a hypertonic extracellular medium, resulting in the loss of water and shrinkage of the cell contents. This process highlights the semipermeability of plant cell membranes, leading to the separation of the plasma membrane from the cell wall. The unique characteristics of plasmolysis make it a valuable tool for studying various aspects of cell membrane physiology.

The protocol outlined in this article focuses on inducing plasmolysis, specifically in root hairy cell membranes. Root hairs, being highly specialized structures involved in nutrient uptake and interaction with the surrounding soil environment, provide an ideal model system for studying membrane physiology. Plasmolysis offers researchers a controlled environment to observe and analyze various molecular and phenotypic characteristics of the plasma membrane. By inducing plasmolysis, researchers can investigate changes in membrane integrity, permeability, and functionality under different experimental conditions. Additionally, plasmolysis can facilitate the study of cellular responses to osmotic stress, providing insights into plant adaptation mechanisms.

This protocol provides a step-by-step guide to efficiently obtaining protoplasts from plant root cells undergoing plasmolysis. Protoplasts, or plant cells with their cell walls removed, offer a simplified system for studying membrane properties and dynamics. The simplicity and cost-effectiveness of the method make it accessible to a wide range of researchers interested in membrane physiology studies.

The protocol presented here offers a valuable tool for researchers interested in investigating the intricate mechanisms underlying plant cell membrane physiology. By inducing plasmolysis in root hairy cell membranes and obtaining protoplasts, researchers can delve deeper into understanding the fundamental processes governing plant cell biology and adaptation to environmental cues.

Image Attribution

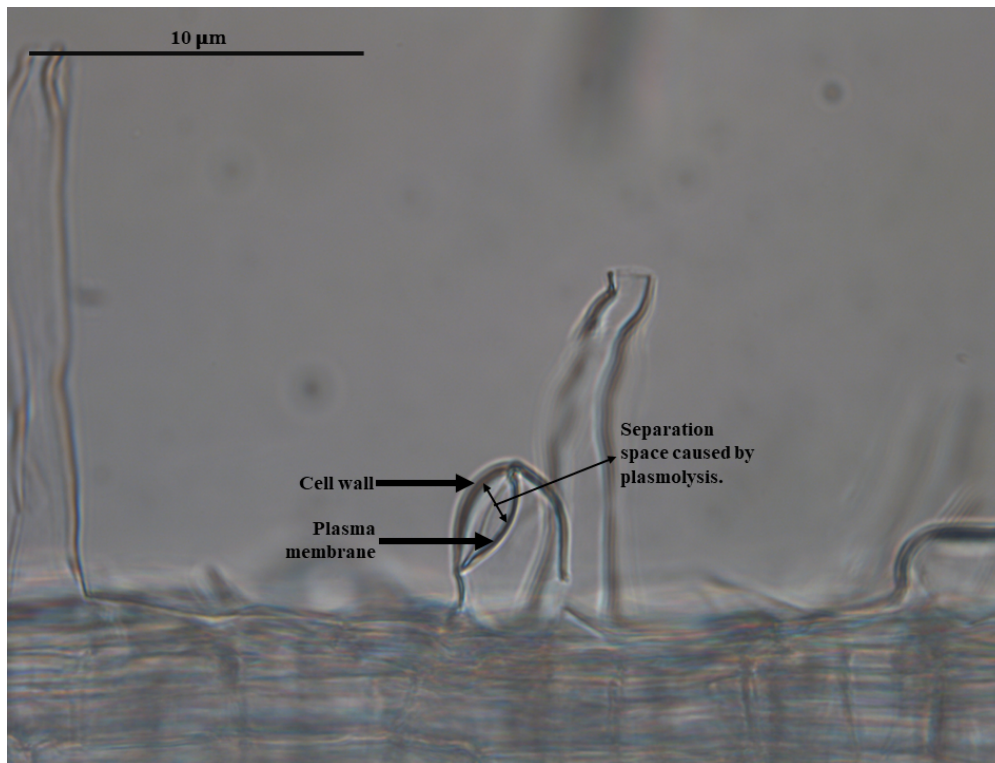


Figure 4. The separation between the plasma membrane and the cell wall of root hairs of a bean plant is observed.

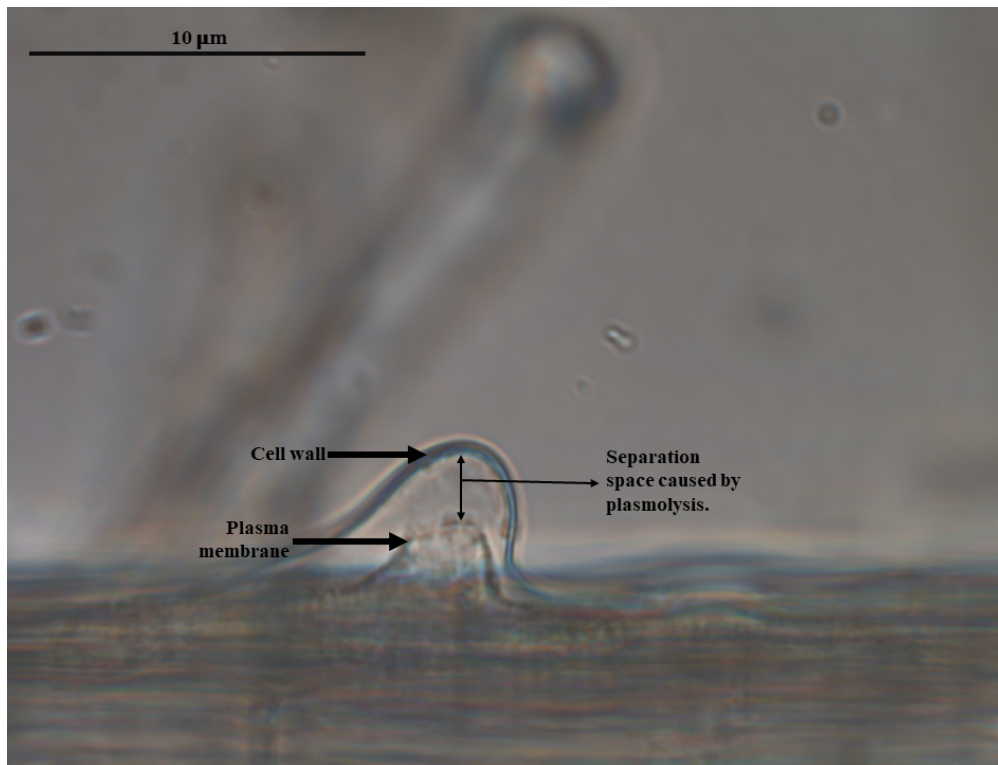


Figure 5. The separation between the plasma membrane and the cell wall of root hairs of a bean plant is observed.

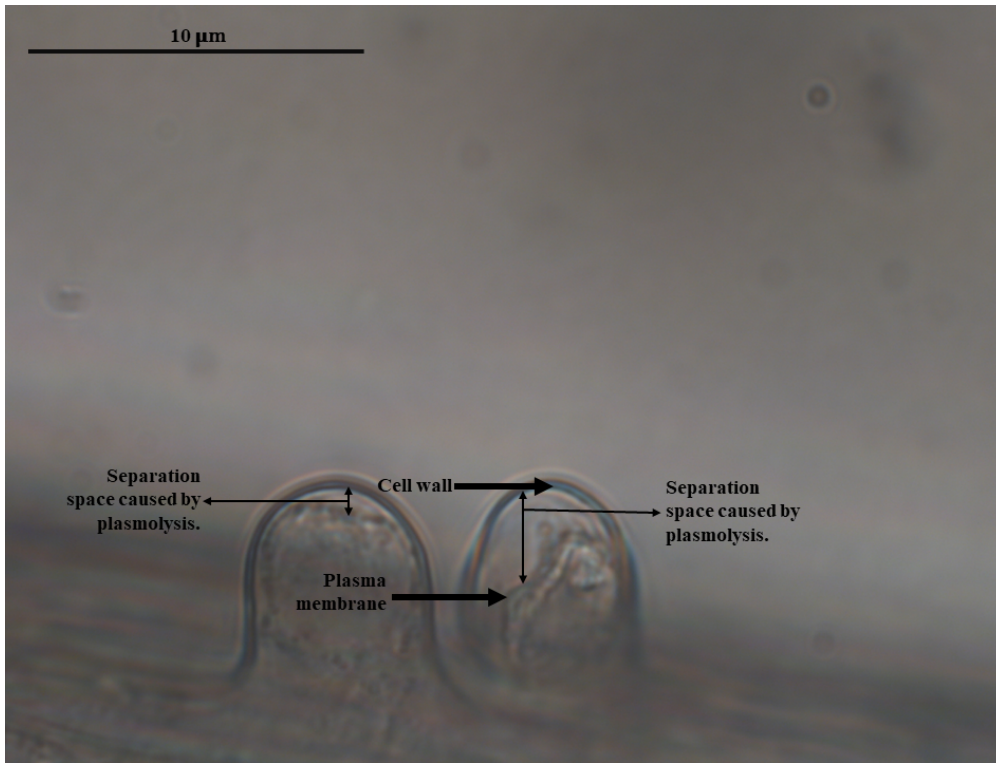


Figure 6. The separation between the plasma membrane and the cell wall of root hairs of a bean plant is observed.

Guidelines

It is imperative to wear gloves consistently throughout the protocol to prevent sample contamination and ensure optimal observation and manipulation of the protoplasts under the microscope.

Materials

- 1.- NaCl 150 mM
- 2.- Glyrecol 2 % (9.8 ml of water + 0.2 ml of glycerol)
- 3.- Sterile distilled and deionized water
- 4.- Micropipettes
- 5.- Sterile petri dishes
- 6.- Flask
- 7.- Slide and coverslips
- 8.- Fine forceps
- 9.- Gloves
- 10.- Surgical scissors or a scalpel
- 11.- Microscope

Safety warnings

 Do not squeeze or press the root tissues. Handle the roots gently.

Before start

Verify that the roots have reached the appropriate stage for inducing plasmolysis.

Preparation of solutions

- 1 Prepare a **100 ml NaCl** solution at a concentration of **150 mM**, following the instructions outlined in the materials section of this protocol.
- 2 Prepare a **2% glycerol** solution, sterilize it using autoclaving, and store it appropriately.



Plant growth

- 3 The bean plants were cultivated under controlled in vitro conditions for a duration of 12 days, as depicted in **Figure 1**. During this period, they were regularly irrigated with a nutrient solution every 2 days to ensure optimal growth and development.



IN VITRO PLANT GROWTH

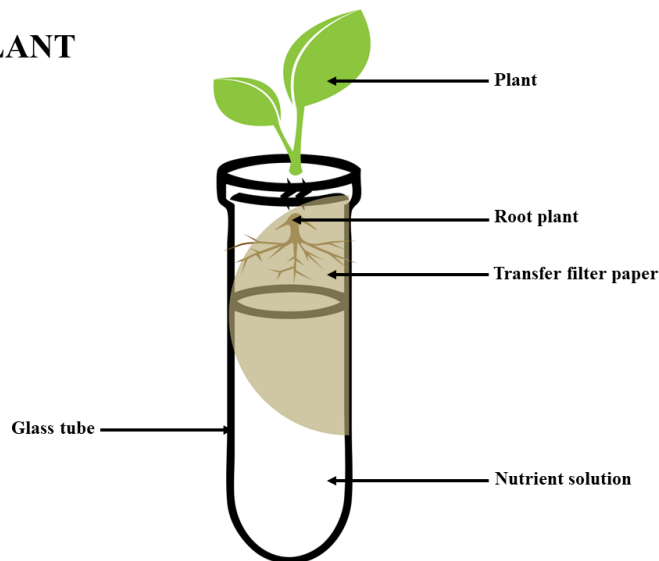


Figure 1: illustrates the in vitro plant growth method designed to minimize potential mechanical damage to the roots during their developmental stages. Utilizing capillary action, a filter paper acts as a conduit for transferring the nutrient solution, ensuring gentle and efficient delivery to the roots.

Induction of plasmolysis

- 4 Harvest plant tissue from the roots using surgical scissors or a scalpel, ensuring careful handling. Submerge the harvested tissue in sterile water within sterile petri dishes.

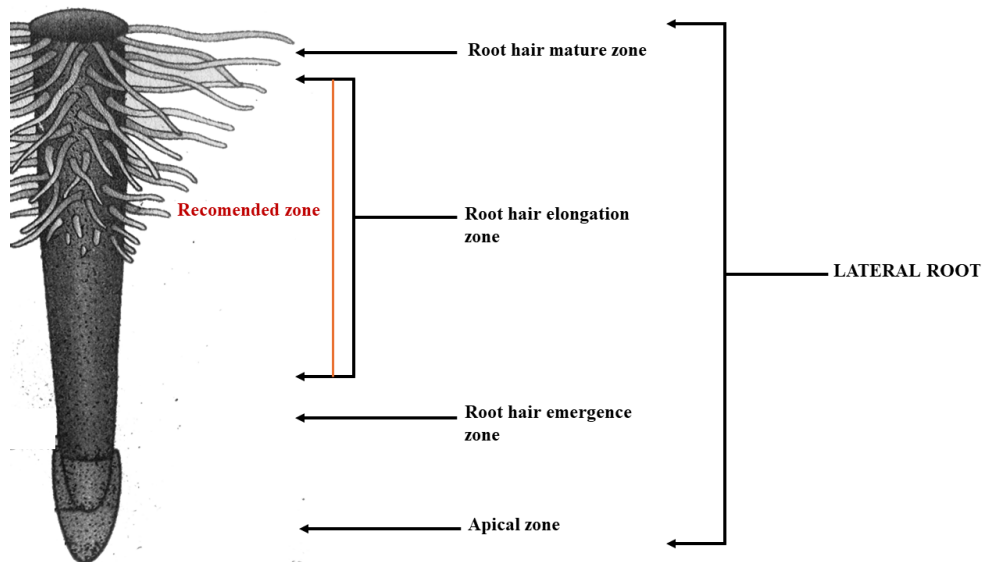


Figure 2: Diagram showing different root hair zones on a lateral root. The marked area in red designates the recommended region for tissue extraction.

- 4.1 **NOTE:** For optimal results, it is advised to target the root hair elongation zone of the lateral root, In simpler terms, specifically focus on the midpoint of the lateral roots, as depicted in **Figure 2** for clarity.
- 5 Replace sterile water with **10 ml** of a **150 mM NaCl** solution, ensuring complete coverage of the plant root tissue.
- 6 Incubate the sample at **4°C** for **12 min**.
- 7 Transfer the tissue to a fresh petri dish containing sterile water at **4°C** and gently rinse it for **1 minute**. Repeat this washing process **three** times to remove any excess NaCl.
- 8 After rinsing, promptly immerse the root tissues into a 2% glycerol solution and store at **4°C**.

12m



Microscopic observation of root hair cells

- 9 Arrange the roots without overlapping on a microscope slide, ensuring they are immersed in the **2% glycerol** solution. Gently position a coverslip over the glycerol-treated tissue.

- 10 The samples are examined using an optical light microscope at magnifications of **20X**, **40X**, and **60X** to confirm the effectiveness of the plasmolysis process (**Figure 3**).



Figure 3: illustrates a root hair visualized under optical microscopy at a magnification of 40X, exhibiting plasmolysis.

Protocol references

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