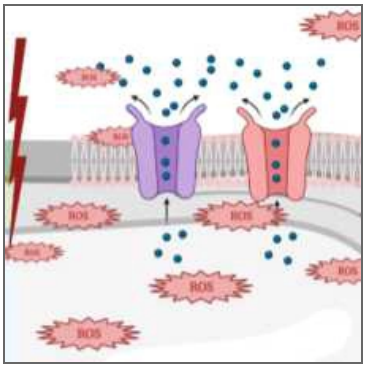

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Protocol status: Working
We use this protocol and it's working

Electrolyte Leakage Assay to Analyze Membrane Integrity in Leaves

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DISCLAIMER

ABSTRACT

The production of reactive oxygen species occurs naturally in certain cellular organelles as metabolic byproducts. However, under stress conditions, their accumulation increases, resulting in cell death and the leakage of intracellular electrolytes. Consequently, quantifying electrolyte leakage has been widely accepted as an indicator of membrane integrity. This protocol delineates specific steps for measuring electrolyte leakage from plant tissues, providing insights into cellular damage caused by various stressors, including biotic, abiotic, and other factors.

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Keywords: ion leakage, cell death

MATERIALS

1. Cork borer for cutting leaf disks
2. Sterile paper towels
3. Sterile deionized water
4. Alcohol
5. Lighter
6. 15 ml centrifuge tubes
7. 50 ml test tubes with caps
8. Fine forceps
9. Gloves

BEFORE START INSTRUCTIONS

During the process of sample selection, it is imperative to choose a region on the leaves that avoids the major vascular bundles and noticeable signs of mechanical damage, as these factors may compromise the quality of the assay. Moreover, careful consideration of the timing of tissue collection is advised, ensuring alignment with the specific stage of plant growth relevant to your research objectives. Selecting leaves in similar developmental stages is crucial not only to ensure a consistent amount of tissue for each leaf disk but also to elicit similar responses to stresses. Additionally, if multiple repetitions are planned, it is essential to maintain uniformity in sample collection conditions across each repetition. Consistency in sample collection procedures helps preserve the integrity and reproducibility of the experimental results.

Preparation of Samples

30m

1 Using a cork borer, cut leaf disks of the desired diameter (at least 5 mm) from one plant, ensuring at least one disk per leaf. Perform this process on sterilized paper towels. With the assistance of fine forceps and gloves, carefully detach the disks from the vascular bundles.

Note

Clean the cork borer by heating it with alcohol and a lighter before cutting each new disk. Ensure that the cork borer is cold and sharp enough to minimize mechanical damage and prevent the release of additional ions beyond those caused by the treatment being applied.

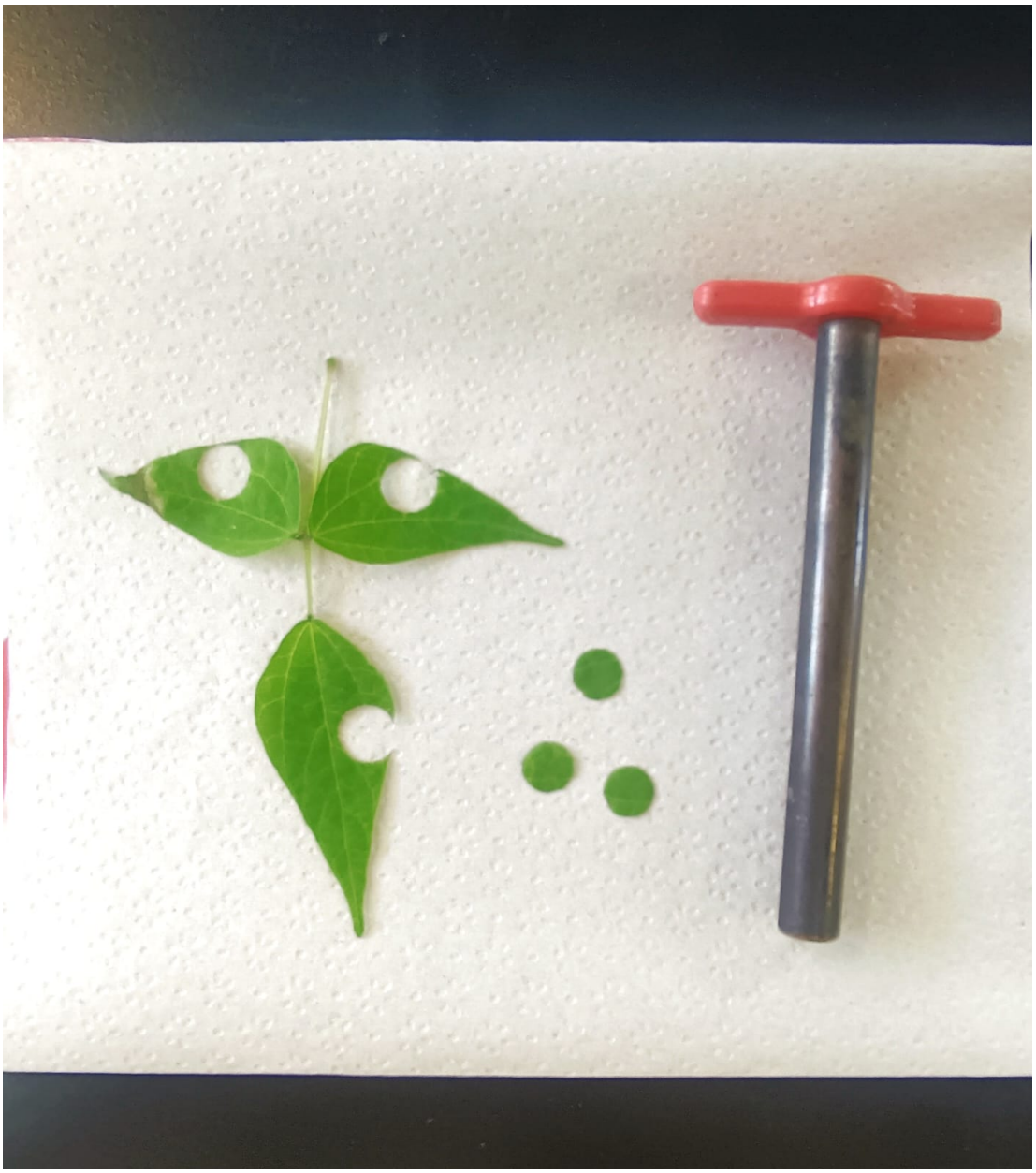

Figure 1. Leaf disk obtained from a trifoliate leaf using a cork borer.

2 Immediately after cutting each leaf tissue, submerge three disks, positioned with the adaxial surface facing downward, from a single plant into 10 mL of sterilized deionized water within individual tubes. Each tube should correspond to a different plant under examination.

3 Let the samples sit for 00:30:00 to allow the removal of electrolytes adhered to the leaf surface and those released during the cutting process. Subsequently, replace the deionized water with fresh water after rinsing. 30m

Measurement Procedure 5h

4 Keep the samples immersed in 50 mL of sterilized deionized water for an additional 05:00:00 following rinsing. 5h

5 Utilize a conductivity meter (SevenCompact, Mettler-Toledo) to measure conductivity.

Insert the electrode into the sample tubes for measurement, ensuring that the disks do not make contact with the electrode during the process to avoid interference.

Note

Before each measurement, rinse the electrode with sterilized deionized water to prevent contamination between samples.

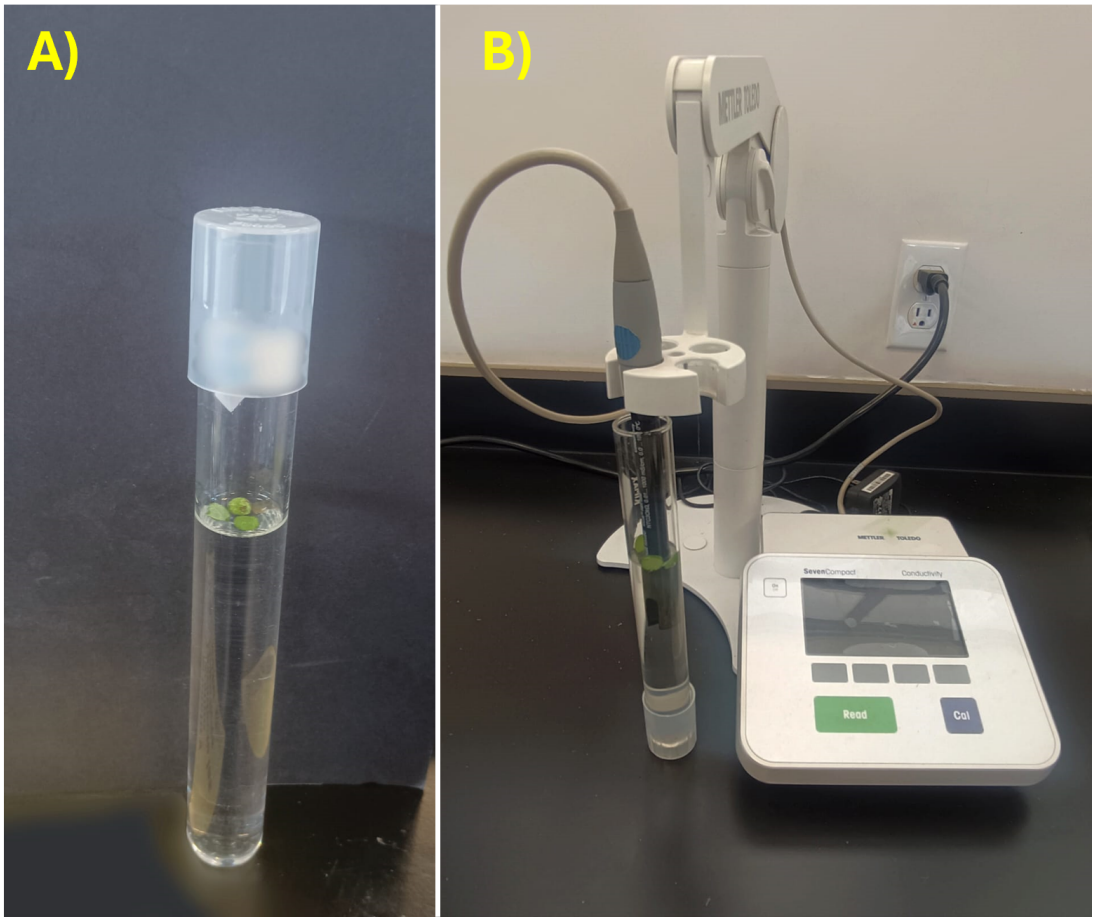

Figure 2. A) Leaf disks resting in sterilized deionized water after washing. B) Measurement of conductivity in the water of the sample at rest.

5.1 *Optional:* If conducting an experiment over time (hours), return the sampled water to the tube to maintain a constant volume of water throughout the time course.

Data Analysis

6 For the analysis of data, utilize the conductivity values directly obtained from the conductivity meter. It is advisable to incorporate untreated leaf disks in the experiment as a negative control.

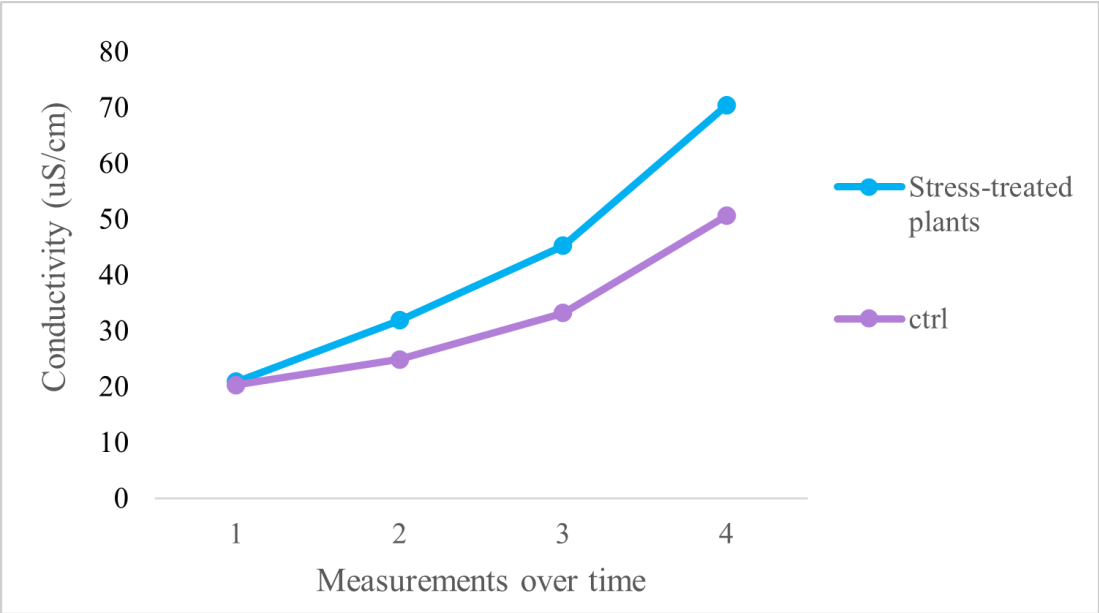

Figure 3. Representative data of electrolyte leakage from leaf disks.

Applications

7

1. Assessment of Plant Stress Response: This protocol can be used to evaluate the response of plants to various stressors, such as drought, salinity, or pathogen infection, by measuring electrolyte leakage as an indicator of cell membrane integrity.

2. Analysis of Rhizosymbiont Effects on Plants: This protocol can be employed to investigate the impact of rhizosymbionts, such as mycorrhizal fungi or nitrogen-fixing bacteria, on plant response to stress. By measuring electrolyte leakage, researchers can assess how rhizosymbionts influence plant tolerance to environmental stresses.

3. Screening Plant Genotypes: Researchers can utilize this protocol to screen different plant genotypes for their tolerance to environmental stresses. By comparing electrolyte leakage levels among genotypes, scientists can identify those with improved stress tolerance.

4. Testing Efficacy of Stress Mitigation Strategies: This protocol can also be employed to assess the effectiveness of different stress mitigation strategies, such as the application of growth regulators or soil amendments, in reducing cellular damage under stress conditions.