Modified Cryo-Preparation for Studying Salt Glands in the Turf Grass *Zoysia matrella*

Sheetal Rao, Michael W. Pendleton, Marla L. Binzel and E. Ann Ellis

Department of Horticulture and Microscopy and Imaging Center, Texas A&M University, College Station, TX

Zoysia, a common turf grass, is characterized by the presence of functional salt glands. These glands are specialized structures through which the plants excrete excess salt. Research on the mechanism of salt secretion in *Zoysia matrella* (Manila grass) prompted the development of a specimen preparation technique that would preserve the secreted salt and salt gland. Conventional aqueous preparative techniques wash away the secreted salt on the leaf surface. A specimen preparation technique was modified from a simple cryo-preparative technique for examining hydrogels in the transmission electron microscope [1].

Scanning electron microscopy (SEM) studies of functional salt glands were done on plants grown in the greenhouse and watered with either deionized water (control) or sodium chloride (300 mM NaCl). Salt treatment involved watering with 50 mM NaCl and increasing the salt in increments of 50 mM until reaching 300 mM. Then onwards, the plants were watered once weekly with 300 mM NaCl for six weeks before harvesting for microscopy studies.

The following procedure was conducted in a properly functioning fume hood with a minimum flow rate of 100 ft/min. Small leaf segments from the second youngest leaves were mounted on a small block of glass and placed in a petri dish containing 100 % acrolein in a plastic bottle cap. A beaker with hot water was placed on top of the covered petri dish to accelerate the reaction [2]. Leaf segments were exposed to acrolein vapors for one hour and then exposed to ruthenium vapors for 10 min. 0.02 g of ruthenium chloride was placed in a plastic bottle cap in the petri dish with the leaf segments and 1 ml of 10% sodium hypochlorite was added to the ruthenium chloride [3]. Following vapor fixation with acrolein and ruthenium vapors, the leaf segments were picked up one by one with locking forceps, and dipped in liquid nitrogen for 1 min. Leaf segments were then washed in methanol for 30 sec and then dipped in hexamethyl disilazane (HMDS) for 1 min. Leaf segments were allowed to air dry for 1-2 min and then mounted on stubs with double-sided carbon tape such that the adaxial (top) side of the leaf was facing upwards.

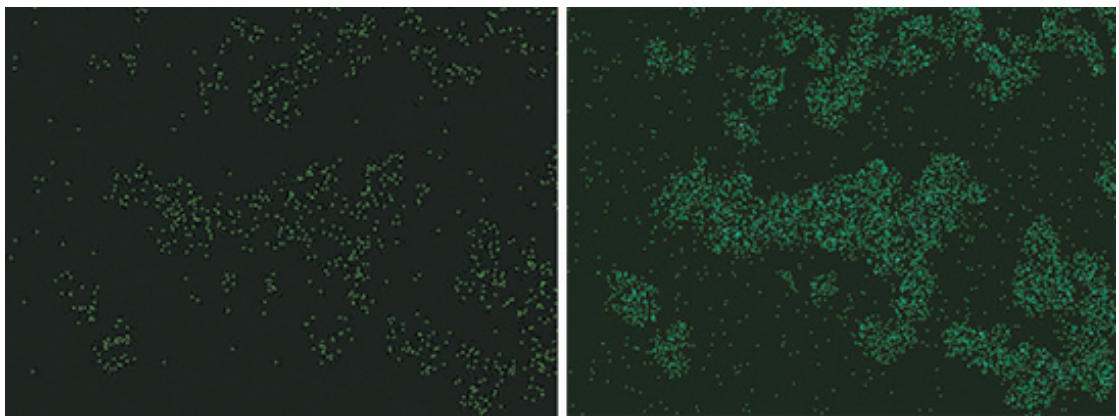


Figure 2a: An EDS map of the leaf area seen in Fig. 1 showing the presence of sodium ions where salt crystals were located.

Figure 2b: An EDS map of the leaf area seen in Fig. 1 showing the presence of chloride ions where salt crystals were located.

This method served as a cheap, yet very effective method for cryo-preparation of the leaves. The leaf segments on the stub were then coated with 30 nm of carbon and examined in a JEOL 6400 SEM at an accelerating voltage of 15 kV.

SEM micrographs showed salt glands located in rows parallel to stomata. In Diamond plants (the salt tolerant cultivar of *Zoysia matrella*) treated with 300 mM NaCl, salt crystals were deposited next to the salt glands in grooves of the leaf indicating active salt secretion. No salt secretion was seen on leaves of control plants or on the abaxial (bottom) side of salt secreting plants.

Excess salt is excreted onto the leaf surface through a salt gland pore (Fig. 1). Energy Dispersive Spectroscopy (EDS) was used for qualitative elemental analysis of the leaf surface. Figure 2 shows an EDS map of the leaf surface of salt treated plants and confirmed the presence of sodium chloride crystals.

Variable pressure SEMs could possibly be used in a study of this type after vapor fixation to stabilize the leaf structure. However, many research people have access to only conventional SEMs. This preparation technique is a simple, economical method for preserving sensitive materials for examination in a conventional SEM without a cryostage. ■

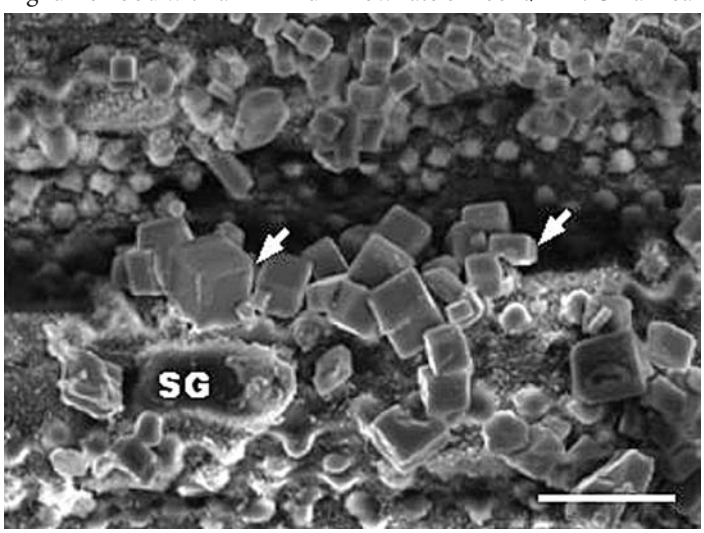


Figure 1: A scanning electron micrograph of the abaxial (top) side of a Diamond leaf showing a salt gland actively secreting salt onto the leaf surface. Arrows point to the cubical sodium chloride crystals. Salt gland, SG. The scale bar equals 25 μm.

References

[1] D. M. Hawkins, E. A. Ellis, D. Stevenson, A. Holzenburg and S. M. Reddy, *J. Mat. Sci.* 42(2007)9465.

[2] E. A. Ellis and M. W. Pendleton, *Microscopy Today* 15[3](2007)44.

[3] G. M. Brown and J. H. Butler, *Polymer* 38(1997)3937.

FEI COMPANY™
TOOLS FOR NANOTECH

Explore, discover, build

with unparalleled access to the nanoscale

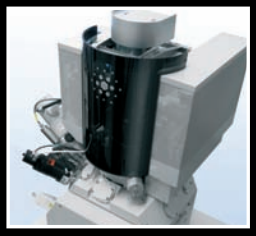
Today's advanced discovery and product development requires unimpeded 3D access to the nanoscale with the best resolution possible. FEI's Tools for Nanotech™ support the success of your most demanding projects by giving you the power you need to reach far into the nanoscale.

Titan™ 80-300 (S)TEM

The Titan™ breaks the Ångström barrier with atomicscale resolution. This dedicated platform for aberration corrected (scanning) electron microscopy delivers the highest resolution available on a commercially available (S)TEM system.

Tecnai™ G² Series

Any of the G² series offers a truly universal imaging and analysis solution that seamlessly embeds all peripherals and software in one powerful instrument. It offers total digital control of your advanced (S)TEM environment, and takes the proven Tecnai™ 'all-in-one' concept to a new level.



Helios™ NanoLab

The DualBeam™ for pushing the limits at the nanoscale, providing the best resolution and image quality down to the sub-nanometer level; producing the thinnest samples of the highest quality, enabling advanced 2D and 3D nanocharacterization, and delivering the most integrated solutions for nanoprototyping.

Nova™ NanoSEM

Experience the world's only true high resolution, low vacuum FEG-SEM for characterization of charging and/or contaminating nanomaterials or nanodevices.

Quanta™ 3D FEG

The Quanta™ 3D FEG is the most versatile high resolution, low vacuum FE-SEM/FIB for 2D and 3D material characterization and analysis. It can accommodate the widest range of samples of any SEM system and enables in situ study of the dynamic behavior of materials at different humidity levels (up to 100% RH) and temperatures (up to 1500 °C).

See more at fei.com