A Nanotool for Phase Equilibrium and Water Potential Measurements in Living and Synthetic Systems

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Abstract:

The multiphases water transport in unsaturated porous media has been investigated for a long period yet remains unknown fundamental questions for exploration. In this report, our group developed a nanotool, AquaDust, to measure the local water potential in both living and synthetic unsaturated porous media. In leaves, the disequilibrium in water potential between symplast and apoplast points out a large hydraulic resistance at the plasma membrane of mesophyll cells. In synthetic porous media, a water potential distribution of the wetting front in an imbibition process can enhance the understanding of the capillary properties and the dynamic of the process.

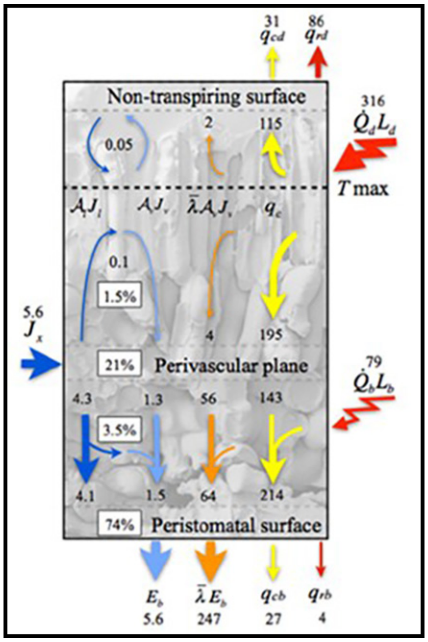

Figure 1: Model prediction of heat and mass flux within a transpiring leaf [1].

Summary of Research:

The mesophyll in leaves operates as an unsaturated porous medium, with vapor-filled spaces interspersed within a matrix that is wetted by the condensed liquid phase. The undersaturated state introduced a local coupling between phase equilibrium and transport processes in the two phases, and resulted in the strong global coupling of heat and mass transfer. The physic and mathematical model developed by Rockwell et al. shows the competition between these two phases in mesophyll through modeling in Figure 1 [1]. This model strengthened the understanding of the fundamental questions in transport processes, and open a new route for the experimental exploration in both living and synthetic systems.

In a previous study by our group, a new hydrogel nanoreporter, AquaDust, was developed to report local water potential (Ψ) for *in planta* measurements [2]. Figure 2a shows the mechanism that the gel matrix responds to water potential by shrinking and swelling under dry and wet environments, respectively. The volumetric changes of the matrix will lead to the distance changes between donor and acceptor dyes. Accordingly, the emission spectrum via Förster Resonance Energy Transfer (FRET) between donor and acceptor dyes provides quantitative measurement of water potential in Figure 2b.

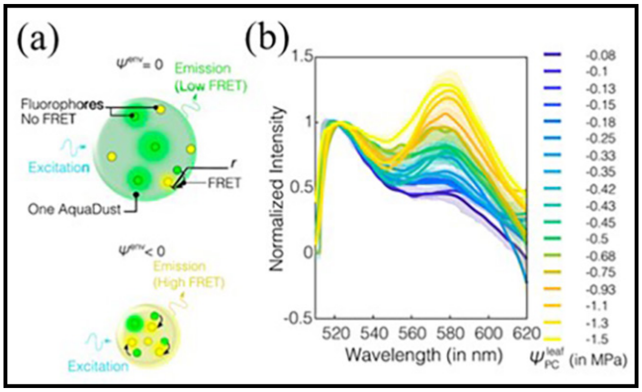

Figure 2: (a) Mechanism of AquaDust responses under wet and dry environments. (b) Spectra of AquaDust in maize leaves at different water potentials. (Jain, et al., 2021.)

The *in planta* measurement of AquaDust provided local water potential in mesophyll. The gradient in water potential of through-thickness section of a maize leaf are depicted in Figure 3a. The inset highlights the disequilibrium in water potential between the symplast

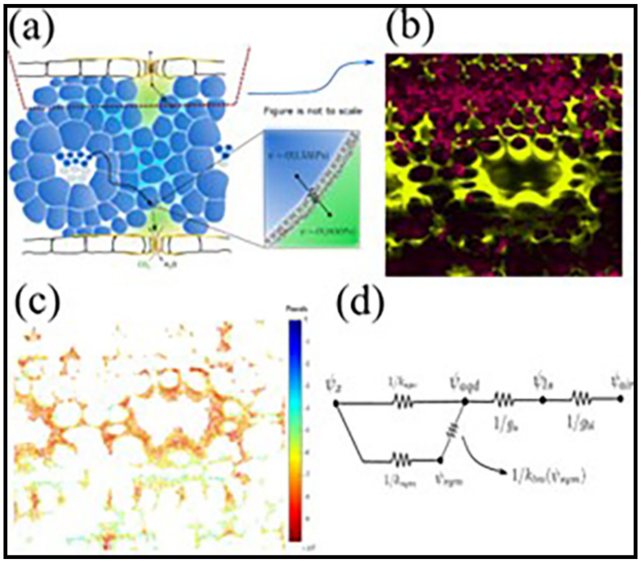

Figure 3: (a) Through-thickness section of a maize leaf depicting the gradient in water potential from xylem to stomates. (b) Confocal microscope image of a horizontal section in a maize leaf. (c) Spatial distribution water potential for the location shown in (b). (d) A model of hydraulic resistances in a maize leaf.

and apoplast. In Figure 3b, the confocal image shows turgid mesophyll cells, with chloroplasts (pink) pushing against cell boundaries (in turgid state), having their cell walls coated with AquaDust (yellow). In Figure 3c, the AquaDust response shows that water potentials in the vapor phase as low as -9 MPa are observed adjacent to turgid cells.

To explain this disequilibrium, we invoke a large hydraulic resistance at the plasma membrane of mesophyll cells, and a model of hydraulic resistances in a maize leaf is shown in Figure 3d. The xylem (Ψ_x) and AquaDust/mesophyll-apoplast (Ψ_{AQD}) nodes are connected by an apoplasmic resistance ($1/k_{apo}$) in parallel with a transmembrane resistance ($1/k_{tm}(\Psi_{sym})$), with the latter assumed to be a variable function of the symplastic water potential; this 'outside-xylem' part of the network is in series with the stomatal resistance ($1/g_s$) and boundary-layer resistance ($1/g_{bl}$).

In the synthetic system, we developed an experimental method to measure water potential distribution in synthetic porous media. First, the relationship between relative FRET efficiency and water potential in Figure 4a was built through measuring relative FRET efficiency under control relative humidity. The relative humidities were converted into water potentials through Kelvin equation [3] and the results were anastomotic with the theory presented in Jain, et al. [2].

Then, an imbibition process was performed in an AquaDust-filled cellulose acetate filter paper to investigate water potential distribution in capillary-driven process. The results before and after imbibition are shown in Figure 4b-c. Within the imbibition area, lower relative FRET efficiency and transition area of wetting front are observed. The detailed water potential distribution in synthetic porous media can enable investigations on capillary properties and dynamic processes.

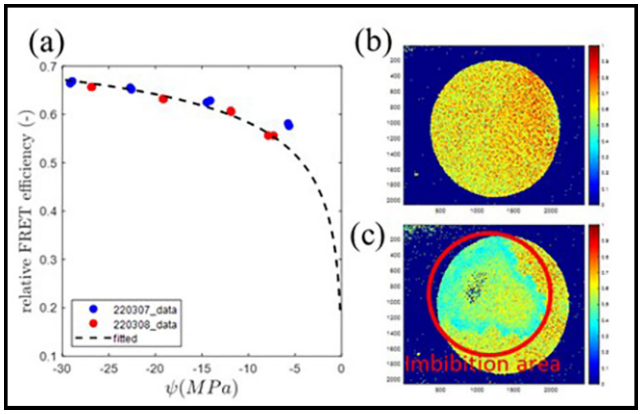

Figure 4: (a) The relative FRET efficiency to water potential data. Dashed line is the theoretical prediction as obtained from the Flory–Rehner theory and dipole-plane FRET model presented in Jain, et al., 2021. (b-c) The relative FRET efficiency (b) before and (c) after imbibition.

Conclusions and Next Steps:

AquaDust can provide an outstanding and reliable tool to measure water potential in both living and synthetic systems. For living system, water potential measured by AquaDust shows the disequilibrium in water potential between the symplast and apoplast, indicating a large hydraulic resistance at the plasma membrane of mesophyll cells. In synthetic system, the transition area of water potential in an imbibition process can be clearly recorded by AquaDust.

The next steps of *in planta* experiments presented in Figure 3 should include understanding the molecular mechanism regulating outside-xylem hydraulic conductance (K_{ox}) using tools from genetics such as aquaporin mutants, transcriptomic analysis and treatments using abscisic acid (ABA) and blue light responses [4]. The next steps of synthetic system will be to investigate the coupled heat and mass transport in the thermally loaded unsaturated porous medium by measuring the spatiotemporal water potential.

References:

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[3] Skinner, L. and J. Sambles, The Kelvin equation—a review. Journal of Aerosol Science, 1972. 3(3): p. 199-210.

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