Analysis of Biochemical Stress Signaling in Plants Using Fluorescent Nanosensors

By

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Climate change and population growth are straining global agriculture and supply chains, accelerating the risks of famine and under-nutrition. One promising technological approach is to improve yields by tapping into the real time information collected and accumulated by plants and crops about their stress and growth environment. Nanosensors are capable of rapidly measuring this internal signaling and facilitating its communication to users in time to prevent yield loss. Understanding how this new technology can inform the broader internal, highly informative signaling network remains an ongoing challenge addressed in this thesis work.

Plants, as sessile organisms, must sense and rapidly respond to constantly changing environmental conditions. They encounter a broad range of abiotic and biotic stresses, some of which are acute and localized, such as insect feeding or mechanical wounding, and others that develop more broadly across the plant, such as drought or temperature stress. In each case, plants must not only perceive the initial stress but also transmit that information systemically to coordinate appropriate responses. Unlike animals, plants do not rely on mobile immune cells to propagate information, and instead depend on the transport of chemical, electrical, and mechanical signals between tissues. Reactive oxygen species (ROS), especially H_2O_2, are among the earliest signals, propagating systemically within minutes to hours upon stress perception. Notably, H_2O_2 exhibits stress-dependent spatial and temporal dynamics, suggesting that it may contribute to encoding stress-specific information before slower phenotypic changes emerge. Directly resolving these early dynamics could therefore provide a more immediate and mechanistic basis for agricultural intervention but remains challenging *in vivo* because conventional sensing approaches are often limited by poor chemical specificity or the requirement for genetic transformation, which restricts use across species. To address this gap, we previously demonstrated the application of ss$(GT)_{15}$-DNA wrapped single-walled carbon nanotube (G-SWNT) fluorescent sensors for selective, reversible measurement of H_2O_2 in leaf lamina. Building on this work, this thesis develops analytical frameworks for interpreting nanosensor-based H_2O_2 measurements and establishes the application of G-SWNT in previously inaccessible vascular tissue that plays a central role in systemic stress signaling.

During normal plant growth, ROS are continuously produced as metabolic byproducts and their concentrations are tightly regulated by scavengers to prevent toxic accumulation. In acute stress responses, NADPH oxidases drive autocatalytic H_2O_2 production, generating a self-sustaining propagating wave known as the H_2O_2 wave. G-SWNT measurements in leaf lamina reveal a characteristic temporal waveform in response to mechanical wounding that describe the production and degradation dynamics. We develop a reaction-diffusion model, showing that nonlinear, autocatalytic production and Fickian diffusion of H_2O_2 followed by first order decay well describes the concentration profile. The solution to this model is a soliton, or traveling wave, that maintains its shape as it propagates through space and time with constant speed. We develop a closed form approximate solution based on a single term logistic function ansatz and fit to measured waveforms across several plant species. Despite a conserved underlying signaling mechanism, waveform characteristics such as amplitude, full width at half maximum (FWHM), lag time, and asymmetry varied across species and were captured by a dimensionless H_2O_2 degradation parameter. This

model lays the foundation for describing stress-specific H_2O_2 signaling and its integration with concurrent and downstream signaling to drive adaptive plant responses. Building on this model, we develop a modeling framework for integrating pre-stress baseline physiology, describing stress signal initiation from a non-zero basal H_2O_2 level. Our analysis shows how stress signaling waveform shape and intensity can be mathematically decoupled from basal level, suggesting that the basal concentration may operate as a distinct, orthogonal signaling channel, separate from the acute waveform following a discrete stress event.

G-SWNT application to leaf lamina involves pressure infiltration via needleless syringe, relying on transport through stomata. However, this limits investigation of systemic signaling, which relies on long distance transport through the vasculature. We develop vascular sensor delivery via direct injection with sensor-coated silk microneedle patches or 30 gauge needles. This delivery approach is compatible with leaf imaging, facilitating characterization of SWNT fluorescence equilibration to the *in vivo* vascular environment. We observe that SWNTs quench upon injection and exhibit a fluorescence turn-on response during H_2O_2 wound signaling, which is opposite of the behavior observed in the lamina. We link the inverted sensor response to the distinct proteomic composition of the vasculature, which notably contains elevated levels of peroxidases that can adsorb to G-SWNT with high affinity and quench fluorescence. Spatial analysis of H_2O_2 wavefront propagation revealed velocities approximately one order of magnitude faster than those observed in the lamina. Neither velocity nor amplitude varied substantially with sensor location, wound location, or the number of consecutive wounds, highlighting the robustness of the underlying signaling network. We modeled the temporal waveform based on our previous nonlinear autocatalytic reaction-diffusion model, accounting for advection in the vasculature via a modified wave coordinate. Our analysis revealed similar reaction-diffusion velocities between lamina and vasculature, with advection accounting for the difference in apparent propagation speed observed in the vasculature.

Overall, this thesis advances the quantitative interpretation of nanosensor-enabled spatiotemporal mapping of plant signaling. Changes in nanosensor performance and behavior upon protein adsorption in biological fluids are quantified, informing accurate interpretation of *in vivo* measurements. In parallel, experimental methods are developed to establish sensor delivery into vasculature. Together, these advances enable investigation of stress-specific signaling dynamics and signal propagation across plant tissues, with potential applications in precision agriculture diagnostics.

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