“Beating Back the Bugs! Innovative Approaches to Sustainable Agriculture”

Hyrum Gillespie[1], Elenor Castillo[1], and J. Mitch Elmore[2]

[1]Department of Plant Sciences and [2]Department of Plant Pathology

University of California at Davis

CREATE IGERT — COLLABORATIVE RESEARCH & EDUCATION IN AGRICULTURAL TECHNOLOGIES & ENGINEERING

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Plants are constantly attacked by microbes looking for a meal. In fact, crop losses to pathogens can be a major factor to field productivity and have a big influence on the prices we pay at the grocery store. Many people do not realize that plants are not helpless in their fight against pathogenic organisms; plants can recognize and actively respond to pathogen threats. Plants use their immune system to sense attacking pests and mount a defense response. In fact, the plant immune system ensures that most plants are resistant to most pathogens. In order to understand plant defense responses, we are using large-scale proteomic profiling of plant tissue after activation of immune receptors. In addition, we are investigating ways to disrupt bacterial communication signals (quorum sensing) and modulate the behavior of insects that vector important plant pathogenic bacteria. By studying different aspects of plant-pathogen interactions we hope to gain a comprehensive understanding of pathogen virulence strategies and plant immune responses. Ultimately, these approaches should contribute to the development of novel methods of plant disease control in agriculture.

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Understanding Plant Immune Responses

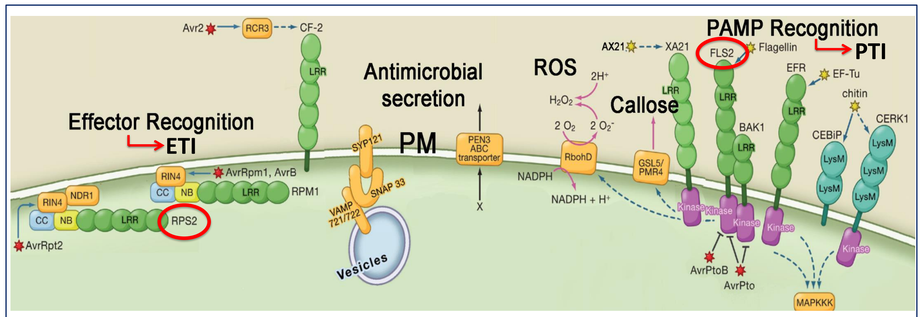


Figure 1. Plasma membrane (PM) proteins regulate many plant responses to microbial infection

Plant immune receptors can recognize pathogen-associated molecular patterns (PAMPs) or pathogen effector proteins and initiate intracellular immune signaling. Activation of these signaling cascades results in cellular reprogramming to mount an effective defense response including secretion of antimicrobial compounds, production of reactive oxygen species (ROS), and callose deposition in the plant cell wall. Immune receptors studied in this work are circled in red. *ETI, effector-triggered immunity; PTI, pattern-triggered immunity.*

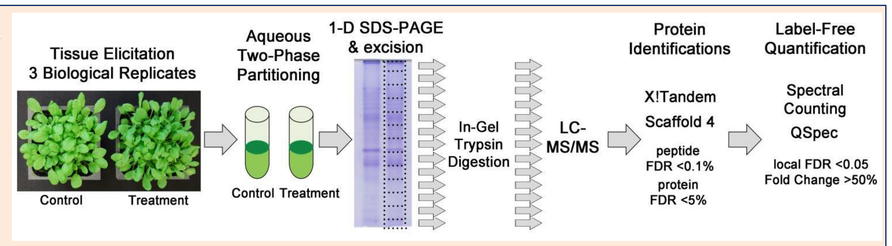


Figure 2. A proteomics workflow allows identification of proteins involved in immune responses at the plant PM

A. To activate RPS2-ETI, dexamethasone(Dex)-inducible GVG-AvrRpt2 transgenic Arabidopsis lines were sprayed with 30μM Dex and leaves were harvested after 6 hours. To activate FLS2-PTI, Col-0 WT plants were sprayed with 10 μM flg22 peptide. LC-MS/MS, Liquid chromatography-tandem mass spectrometry.

B. Immunoblot analysis of PM fractions demonstrates enrichment of PM proteins and depletion of other subcellular compartments after two-phase partitioning. 10 μg total protein loaded per lane.

Condition/ Time	Total # SpC IDs	Total # Protein IDs	# Quantifiable	% Quantifiable	# DE (%)	# UP (%)	# DOWN (%)
FLS2-PTI 10min	2136198	4857	4287	88.3%	80 (1.9%)	37 (0.9%)	43 (1.0%)
FLS2-PTI 3hr	1496265	4726	4096	86.7%	142 (3.5%)	99 (2.4%)	43 (1.0%)
FLS2-PTI 12hr	2566199	4540	4102	90.4%	266 (6.5%)	155 (3.8%)	111 (2.7%)
RPS2-ETI 6hr	416329	3909	2858	72.7%	416 (14.6%)	252 (8.8%)	164 (5.7%)

Table 1. Dynamic changes in protein composition occur at the plant PM during immune responses

(Abbreviations: FLS2-PTI, PAMP-triggered immunity; RPS2-ETI, Effector-triggered immunity; SpC, Spectral count; DE, differentially expressed)

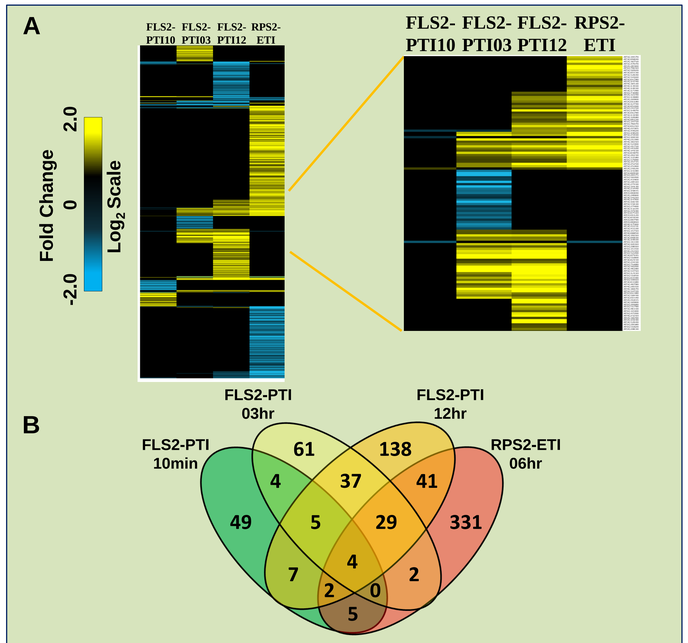


Figure 3. Overlapping patterns of PM protein regulation have been observed after activation of different types of plant immune receptors

A. Hierarchical clustering of differentially regulated proteins based on fold change.

B. Venn Diagram showing overlap in differentially regulated proteins.

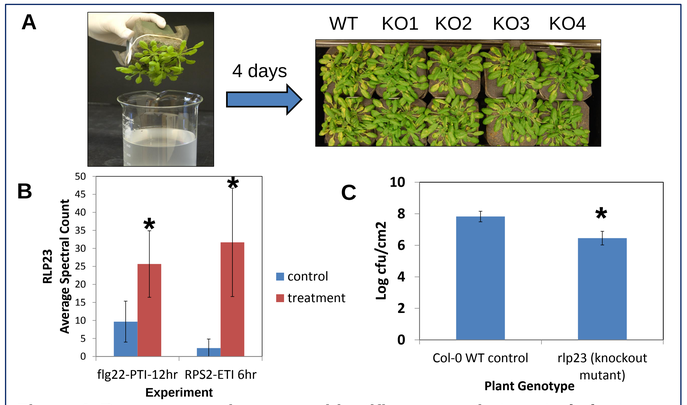


Figure 4. Reverse-genetics screen identifies genes that control plant immune responses

A. High-throughput disease phenotyping of *Arabidopsis* knock-out lines. Plants are dip-inoculated with *Pseudomonas syringae* pv. *tomato* strain DC3000.

B. Receptor-like Protein 23 increase in abundance at the PM during PTI & ETI

C. rlp23 knockout mutant exhibits enhanced disease resistance to bacterial pathogens. *cfu, colony forming units*.

Novel Strategies for Disease Control

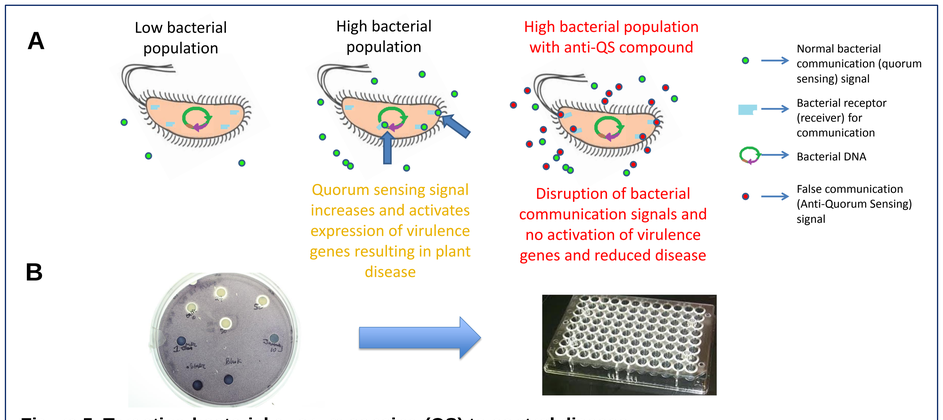


Figure 5. Targeting bacterial quorum sensing (QS) to control disease

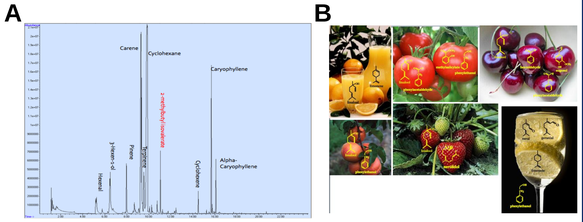
A. Concept for disruption of bacterial QS.

B. High-throughput screen for anti-QS compounds. Bacterial biomonitor strain (left) shows phenotypic color change upon inhibition of quorum sensing. Thousands of compounds can be incubated with biomonitor strain and screened (right) for potential anti-QS properties.

Figure 6. Investigating plant volatiles that modulate insect behavior.

A. Identification of volatile compounds that can attract or repel specific insects.

B. The goal is to determine which compounds repel insects that vector pathogenic organisms.



Conclusions and Future Directions

- **Understanding the genes and proteins that regulate plant immunity will allow us to breed or engineer plants to be more resistant to pathogens**
  - **A quantitative proteomics strategy identifies proteins that regulate plant immunity to bacterial pathogens.**
- **Novel strategies for disease control can help mitigate crop disease**
  - **Identification of anti-quorum sensing compounds should result in the development of new ways to control important bacterial plant pathogens.**
  - **Sulfur volatiles may be useful in deterring several insect vectors of citrus pathogens.**

References

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