Transcriptional regulation of effector-triggered immunity (ETI) in plants: from tissue to cells

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Summary

Plants possess a sophisticated innate immune system composed of two major defense layers: pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Rather acting as independent pathways, PTI and ETI are highly interconnected and mutually reinforcing, together generating robust and durable immune responses. Over the past decades, extensive research has elucidated key components of plant immune architecture and signalling. However, the complexity and interconnectedness of immune signalling mean that key gaps remain in our understanding of the organisation and execution of ETI The research described in this thesis employs genetics and transcriptomics to define how ETI signalling is partitioned across distinct pathways and leaf cell types in *Arabidopsis thaliana* (Arabidopsis).

Chapter 1 provides an overview of the organizational principles underlying plant immunity, from pathogen perception at the cell surface and intracellular immune receptors to downstream signalling cascades and transcriptional reprogramming. Particular emphasis is placed on the regulatory layers that shape immune output, including calcium signaling and the CALMODULIN-BINDING PROTEIN 60 (CBP60) family of transcription factors, CBP60g and SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1)**,** which are central regulators of salicylic acid (SA)-dependent immunity. Although recent studies have demonstrated that PTI and ETI synergize to amplify immune responses, this interdependence has made it difficult to disentangle ETI-specific signalling. Because natural pathogen infection invariably activates PTI prior to ETI, dissecting ETI in isolation has remained a major experimental challenge. To overcome this limitation, this thesis employs a synthetic, estradiol-inducible ETI (SETI)

system, enabling precise activation and analysis of ETI signaling in absence of confounding PTI inputs.

Chapter 2 uses this inducible ETI system to dissect the functional contributions of two partially redundant signaling branches in Arabidopsis TIR-NLR–mediated immunity. We demonstrate that the two ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) associated modules, EDS1-PHYTOALEXIN DEFICIENT 4 (PAD4)-ACTIVATED DISEASE RESISTANCE 1 (ADR1) and EDS1-SENESCENCE-ASSOCIATED GENE 101 (SAG101)-N REQUIREMENT GENE 1 (NRG1), play distinct roles during ETI execution. The PAD4-ADR1 is involved in disease resistance while NRG1-SAG101 branch is primarily responsible for triggering the hypersensitive response (HR), a localized form of programmed cell death. These results establish that disease resistance and HR represent separable outputs of ETI, rather than obligatorily coupled consequences of the same signalling process.

To uncover the transcriptional basis of this functional separation, we performed global transcriptome profiling in SETI_*pad4*, SETI_*sag101*, and SETI_*pad4 sag101* backgrounds following PTI, ETI, and combined PTI+ETI activation. These analyses revealed that PAD4 is the dominant driver of immune-induced transcriptional reprogramming, whereas the contribution of SAG101 becomes evident mainly in the SETI_*pad4 sag101* double mutant, highlighting a context-dependent redundancy between the two signalling branches. Furthermore, we identified node-specific gene regulatory signatures, with distinct sets of defense-related genes preferentially controlled by the PAD4–ADR1 or SAG101–NRG1 modules. Importantly, this chapter also demonstrates that immune priming can be retained even if immune signaling is partially compromised, indicating that plants can maintain immune memory without fully engaging

all ETI branches. This finding has practical implications for crop improvement, as it suggests that durable disease resistance may be achieved while minimizing the fitness costs associated with excessive immune activation.

Chapter 3 extends the concept of ETI modularity from signalling pathways to cellular and spatial organization. Using single-cell RNA sequencing (scRNA-seq), we resolved immune ETI-induced transcriptional responses at the level of individual leaf cell types. Although ETI activation is initiated broadly across the tissue, its transcriptional execution varies substantially between cell identities. A conserved core defense program is activated in most cell types, representing a shared ETI backbone, while additional immune modules are selectively deployed in specific cell types, reflecting differences in developmental state, chromatin accessibility, and transcription factor availability.

Comparison of single-cell ETI-responsive genes with bulk RNA-seq data confirms that these transcriptional programs represent authentic ETI outputs rather than artifacts of cell isolation. We further classify ETI-responsive genes into “generalists”, expressed across multiple cell types, and “specialists”, restricted to one or a few cell types. This distinction reveals a hierarchical immune organization in which broadly distributed defenses coexist with highly localized, cell-type-specific immune functions.

A key finding of this chapter is that transcription factors CBP60g and SARD1 are preferentially induced in epidermal pavement cells, the plant’s first physical barrier against pathogen invasion. Functional analyses show that loss of these regulators compromises the epidermis ability to restrict pathogen entry, even when inner tissues retain immune competence. These results demonstrate that effective ETI requires not only robust

signaling but also precise spatial deployment of immune regulators across tissue layers.

Chapter 4 focuses on CBP60g and SARD1 as central transcriptional regulators that enable plants to balance immune activation with cellular viability during ETI. Through genetic analysis, inducible ETI activation, and transcriptome profiling, we show that these transcription factors function not merely as positive regulators of SA-mediated immunity, but as master coordinators that integrate immune-promoting and immune-restraining programs.

The *cbp60g sard1* double mutant provides a striking genetic uncoupling of ETI outputs: ETI induction triggers exaggerated HR, yet pathogen resistance is markedly reduced. This demonstrates that HR is neither sufficient nor required for effective pathogen restriction, challenging the long-standing assumption that stronger cell death necessarily correlates with stronger immunity. Notably, the absence of major developmental defects in this mutant allows immune-specific phenotypes to be interpreted without confounding pleiotropic effects. Transcriptome analyses reveal that CBP60g and SARD1 simultaneously activate defense genes while maintaining expression of negative immune regulators, including Nudix hydrolases, thereby preventing uncontrolled or self-destructive immune activation. Their regulatory role can thus be understood as establishing a dynamic equilibrium that permits strong pathogen control while safeguarding cellular integrity.

In the concluding **Chapter 5**, these findings are integrated into a broader conceptual framework of plant immunity, and future research directions are discussed. Collectively, this thesis demonstrates that ETI is not a uniform or binary response, but a modular, tunable, and spatially coordinated immune system. By elucidating how core immune signaling

nodes interface with central transcriptional regulators across distinct cellular contexts, this work provides a foundation for developing strategies to engineer disease-resistant crops that maintain growth and yield, an essential goal for sustainable agriculture and plant biotechnology.