Studies on postinvasive resistance of *Arabidopsis thaliana* against multiple fungal pathogens

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Summary

Chapter I. CYP71A12-dependent biosynthesis of indole-3-carboxylic acids is involved in the postinvasive resistance against *Colletotrichum tropicale*

Arabidopsis thaliana mounts nonhost resistance response against nonadapted hemibiotrophic fungus *Colletotrichum tropicale* (*Ctro*), which requires PEN2-dependent synthesis of antifungal metabolites derived from tryptophan (Trp). When *C. tropicale* invades *Arabidopsis* defective in this entry control, postinvasive resistance is newly activated as a second layered defense, which blocks further expansion of invasive hyphae. CYP79B2 and CYP79B3 are key enzyme for the biosynthesis of Trp-derived secondary metabolites. *cyp79B2 cyp79B3* mutant is fully susceptible to *Ctro*, suggesting the importance of Trp-pathway derived secondary metabolites for the immunity. This mutant is also defective in both pre- and postinvasive resistance against *Ctro*, indicating the importance of Trp-pathway derived secondary metabolites. Analysis of series of *Arabidopsis* mutants defective in Trp-metabolism pathway revealed that indole-3-carboxylic acids derivatives (ICAs) as well as camalexin are indispensable for the postinvasive resistance, whereas these secondary metabolites were dispensable for the preinvasive resistance to *Ctro*. Metabolites profiling of *Arabidopsis* mutants treated with *Ctro* showed that CYP71A12 has an important contribution to the accumulation of ICAs whereas CYP71A13 is critical for camalexin accumulation upon pathogen infection. These findings suggest distinct roles of these two homologous P450 monooxygenases in the immune responses. The postinvasive resistance to *Ctro* was associated with plant cell death similar to HR cell death. To assess whether plant cell death observed in postinvasive resistance to *Ctro* is mediated by NLRs, I utilized mutations in *RAR1* and *SGT1*. Analysis of fungal hyphal expansion showed that *RAR1* and *SGT1* have no clear contribution to postinvasive resistance and HR-like cell death in response to the invasion by *Ctro*.

Chapter II. *bak1–5* mutation uncouples tryptophan-dependent and independent postinvasive immune pathways triggered in *Arabidopsis thaliana* by multiple fungal pathogens

I found that both CYP71A12 and CYP71A13 are critical for *Arabidopsis*' postinvasive resistance toward both the necrotrophic *Alternaria brassicicola* and the adapted hemibiotrophic *C. higginsianum* in addition to *Ctro*. Metabolite analyses suggest that the production of ICAs and camalexin is induced upon pathogen invasion, while phenotypic comparison of *cyp79B2 cyp79B3* and *pen2 cyp71A12 cyp71A13* plants indicates that the contribution of ICAs to postinvasive resistance is dose-dependent. I also found that the *bak1–5* mutation significantly reduced postinvasive resistance against *C. tropicale* and *A. brassicicola*, indicating that a pattern recognition receptor complex commonly contributes to this second defense-layer against pathogens with distinct infection strategies. Unexpectedly, I revealed that the *bak1–5* mutation had no detectable effects on Trp-metabolite accumulation triggered by pathogen invasion. Further comparative gene expression analyses suggested that pathogen invasion in *Arabidopsis* activates (i) *bak1–5* insensitive Trp-metabolism that leads to antimicrobial small molecules and (ii) a *bak1–5* sensitive immune pathway that activates the expression of antimicrobial protein genes such as *AED1*, *BGL2*/*PR2*, *GLIP1* and *RLP23*.

Chapter III. EDR1- and ORA59-dependent expression of plant defensin contributes to the postinvasive resistance against fungal pathogen

Arabidopsis exhibits durable resistance that is composed by two layers of defense: preinvasive resistance and postinvasive resistance. *PEN2* and *EDR1* is involved in the preinvasive resistance against *Ctro*. In preinvasive resistance to *Ctro*, both PEN2-dependent synthesis of Trp-related antifungal metabolites and EDR1-regulated expression of plant defensins (PDFs) are necessary. However, it remains to be elucidated (i) whether EDR1 also regulates the expression of PDFs during postinvasive defense and (ii) whether the expression of PDFs is involved in postinvasive defense against fungal pathogens. I found that the invasion by *A. brassicicola* resulted in the transient expression of *PDFs* where it resulted in the sustained expression of Trp-metabolism pathway genes. I also revealed that *EDR1* is required for the induced expression of the *PDF* genes upon the pathogen invasion. ORA59 is a transcription factor that regulates the expression of *PDFs*. Here, I also found that *ORA59* is critical for the induced expression of the *PDF* genes upon the pathogen invasion. The pathogen-induced expression of *PDFs* is not canceled by the *bak1-5* mutation. Importantly, the inoculation assay of *A. brassicicola* indicated that *EDR1* and *ORA59* are involved in the postinvasive resistance against *A. brassicicola*, suggesting the involvement of PDFs in the *Arabidopsis* postinvasive resistance in addition to the Trp-related secondary metabolites.