

**Exploring the molecular basis of nonhost
resistance in evolutionarily divergent plants**

by

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ABSTRACT

Over more than 500 million years of coevolution, plants have continuously updated their immune systems to resist surrounding pathogens. Most potential pathogens fail to infect a given plant species, and this phenomenon is collectively termed nonhost resistance (NHR). NHR is supported by a diverse repertoire of biochemical, cellular, and molecular mechanisms that restrict non-adapted pathogens. Although these mechanisms have been extensively studied in angiosperms, their origins and evolutionary trajectories across land plants remain largely unexplored. In this thesis, I show that key components of the immune chaperone complex underlying NHR, RAR1 (REQUIRED FOR MLA12 RESISTANCE 1) and SGT1 (SUPPRESSOR OF G2 ALLELE OF SKP1), as well as their interaction, are conserved across streptophytes, with a notable exception in ferns. In leptosporangiate ferns, the RAR1–SGT1 binding interface has diverged while maintaining the protein–protein interaction. This molecular coevolution was facilitated by promiscuous SGT1 intermediates. My findings illustrate how conserved protein–protein interactions can be rewired without disrupting their essential functions. I further investigated chaperone-mediated NHR in the liverwort *Marchantia polymorpha* by testing *rar1* mutants and wild-type plants with diverse strains of the oomycete phytopathogen *Phytophthora*. I identified candidate pathogens whose resistance depends on a functional immune chaperone complex, establishing *M. polymorpha* as a valuable system to discover resistance genes and elucidate the molecular basis of pathogenesis in understudied *Phytophthora* species. Finally, I examined transcriptomic responses of plants and pathogens across diverse interactions. These analyses revealed that both hosts and pathogens deploy core transcriptomic programs during infection, rather than relying on host- or pathogen-specific strategies. In conclusion, my work on NHR and host range using diverse non-seed plants highlights deeply conserved mechanisms underlying plant–pathogen interactions, while uncovering how immune systems diversify through the rewiring of conserved molecular components across land plants.

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GLOSSARY

ABA	Absciscic acid
ABC	ATP-binding cassette
ADR1	Activated Disease Resistance 1
AMS	Arbuscular mycorrhizal symbiosis
AOS	Allene oxide synthase
AP2	APETALA2
ASR	Ancestral state reconstruction
avr gene	Avirulence gene
BAHD	BEAT, AHCT, HCBT, and DAT
BAK1	BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1
BF	Bright field
BIR1	BAK1-INTERACTING RECEPTOR-LIKE KINASES 1
C1HDZ	Class I homeodomain leucine-zipper
CARD	Caspase activation and recruitment domain
CC	Coiled-coil
CERK1	CHITIN ELICITOR RECEPTOR KINASE 1
CHORD	Cysteine- and histidine- rich domain
CHP	CHORD-containing protein
CNL	CC-NLR
COI1	CORONATINE-INSENSITIVE PROTEIN 1
CRISPR	Clustered regularly interspaced short palindromic repeats
CrRLK1L	<i>Catharanthus roseus</i> RLK1-like
CS	CHORD-containing protein and SGT1
CWA	Cell wall apposition
DAMP	Damage-associated molecular pattern
DEG	Differentially expressed gene
DEG	Differential expressing genes
DM2	DANGEROUS MIX2
dpi	days post infection
EDS1	ENHANCED DISEASE SUSCEPTIBILITY 1
EFR	EF-TU RECEPTOR
eIF4E	eukaryotic translation initiation factor 4E
EPIC1	Extracellular Protease Inhibitor with Cystatin-like domain 1
ERC	early root colonization
ERF	ETHYLENE RESPONSIVE FACTOR
ETI	Effector-triggered immunity
EvoMPMI	Evolutionary molecular plant-microbe interaction
FDR	false discovery rate
FER	FERONIA
FLS2	FLAGELLIN-SENSING 2
GAL4DBD	GAL4 DNA-binding domain
GLV	Green leaf volatile

GWAS	genome-wide association study
HIV	Human immunodeficiency virus
Hop	Hsp70-Hsp90 organising protein
HPL	Hydroperoxide lyase
HSF	Heat shock factor
HSP	HEAT SHOCK PROTEIN
JA	Jasmonic acid
JAZ	JASMONATE ZIM-DOMAIN
LFC	log2 fold change
LFY	LEAFY
LRR	Leucine rich repeat
LYK1	LysM-receptor-like kinase 1
LYR	LYK-related
LysM	Lysin motif
MAPK	mitogen-activated protein kinase
MLA	Mildew Locus A
MPMI	Molecular plant-microbe interaction
NAD	Nicotinamide adenine dinucleotide
NADase	NAD ⁺ glycohydrolase
NB-ARC	Nucleotide-binding adaptor shared by Apaf-1, Resistance proteins, and CED-4
NHR	Nonhost resistance
NLR	Nucleotide binding and leucine rich repeat
NLRC4	NOD-like receptor family CARD-containing 4 protein
NOD	Nucleotide-binding oligomerization domain
NOP1	NOPPERABO 1
NRC3	NLR-required for cell death 3
NRG1	N requirement gene 1
oah	oxaloacetate acetylhydrolase
OPDA	12-oxo-phytodienoic acid
PAD	PHYTOALEXIN-DEFICIENT
PAL	phenylalanine ammonia-lyase
PAMP	Pathogen-associated molecular pattern
PBL	AVRPPHB SUSCEPTIBLE 1 (PBS1)-like
PCA	principal component analysis
PCD	Programmed cell death
PDR8	PLEIOTROPIC DRUG RESISTANCE 8
PEN	PENETRATION
Pin I	potato inhibitor type I
PIP113	plant immune protein 113
PPI	Protein-protein interaction
PR	Pathogenesis-related
PRR	Pattern recognition receptor
R gene	Resistance gene
RALF	RAPID ALKALINIZATION FACTOR
RAR1	REQUIRED FOR MLA12 RESISTANCE 1

RBOH	RESPIRATORY BURST OXIDASE HOMOLOG
RFP	red fluorescence protein
RIN4	RPM1-INTERACTING PROTEIN 4
RLK	receptor-like kinase
ROQ1	Recognition of XopQ 1
ROS	Reactive oxygen species
RPM1	RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1
RPP1	RECOGNITION OF PERONOSPORA PARASITICA 1
RPS2	RESISTANCE TO PSEUDOMONAS SYRINGAE PROTEIN 2
RPW8	RESISTANCE TO POWDERY MILDEW 8
Rxo1	Reaction to X. o. pv. oryzae
SA	Salicylic acid
SAD	SAPONIN-DEFICIENT
SAP	Single amino acid polymorphism
SEP3	SEPALLATA3
SERK	SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE
serpin	serine proteinase inhibitor
SGS	SGT1-specific
SGT1	SUPPRESSOR OF G2 ALLELE OF SKP1
SHIV	Simian/human immunodeficiency virus
Six	secreted in xylem
SSVP1	small secreted virulence-related protein 1
STAND	Signal transduction ATPases with numerous domains
Sti1	Stress-induced protein 1
SYN121	SYNTAXIN OF PLANTS 121
T3SS	Type III secretion system
TIR	Toll-interleukin 1 receptor
TNL	TIR-NLR
TPR	Tetratricopeptide repeat
VOC	Volatile organic compound
Y2H	Yeast two-hybrids
ZAR1	HOPZ-ACTIVATED RESISTANCE 1

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CHAPTER 1. Introduction

1.1 Plant immune system

Defense mechanisms against pathogens are genetically encoded in plants. Throughout their life, plants interact with a variety of microorganisms, including both beneficial symbionts and pathogenic species that can negatively impact their growth and survival. Historically, plant pathogens have garnered the attention of crop scientists due to their substantial impact on food security (Berg, 1926).

Plants have evolved a two-layered immune system governed by two major classes of receptors: cell surface pattern recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat (NLR) proteins (Jones and Dangl, 2006). PRRs perceive conserved pathogen-associated molecular patterns (PAMPs), such as peptides derived from bacterial flagellin and fungal chitin, as well as damage-associated molecular patterns (DAMPs), which are endogenous molecules released by cell damage, such as fragments of cytosolic proteins (Chinchilla et al., 2006; Flury et al., 2013; Cuoto and Zipfel, 2016). When these molecules are detected by PRRs, the recognition triggers PAMP-triggered immunity (PTI), accompanying a suite of cellular and molecular responses to sequester pathogen growth (Couto and Zipfel, 2016).

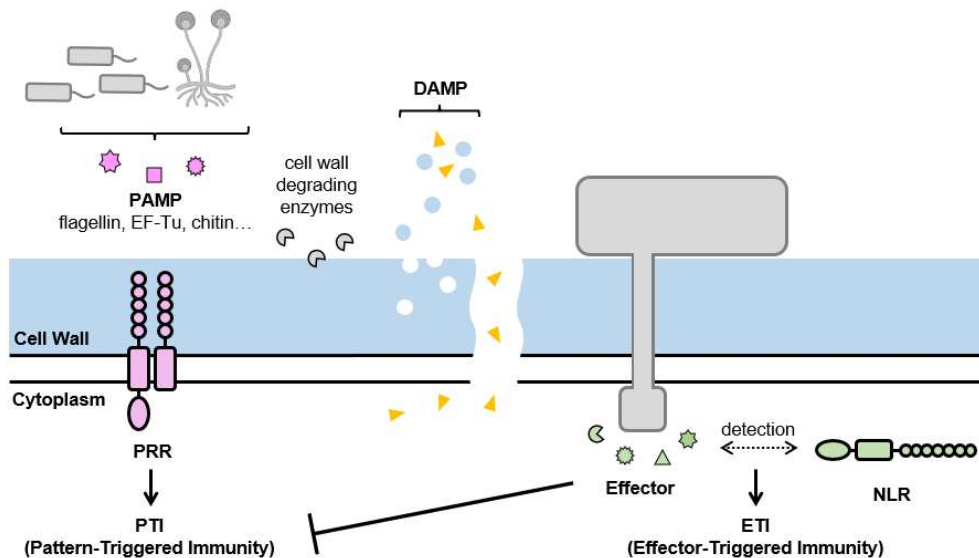


Figure 1.1 Overview of plant immunity. The genetic components of plant immune system include two major classes of receptors: pattern recognition receptors (PRRs) and nucleotide-binding leucine-rich repeat (NLR) proteins. PRRs recognize the presence of pathogens by detecting pathogen-associated molecular patterns (PAMPs), as well as damage-associated molecular patterns (DAMPs), and activate pattern-triggered immunity (PTI). Pathogens employ effectors to dampen PTI, while NLR proteins monitor effector activity in cytoplasm and activate effector-triggered immunity (ETI).

To overcome PTI, pathogens secrete small virulence proteins known as effectors. The primary function of effectors is to suppress PTI and hence promote infection. Plants employ another class of receptors, NLR proteins, to detect effector activity. NLR proteins are cytosolic sensors that can detect pathogen activity either directly or indirectly. The recognition can occur through direct interactions between two proteins, or by sensing effector-mediated modifications of host proteins. Activation of NLR proteins results in effector-triggered immunity (ETI), a robust defense response often associated with rapid and localized programmed cell death (PCD) at the infection site (Figure 1.1) (Cui et al., 2015; Kourcelis and van der Hoorn, 2018).

The gene-for-gene paradigm established the molecular basis of plant disease resistance. Extensive genetic studies on flax and its pathogen *Melampsora lini* led to the gene-for-gene hypothesis, which explains the molecular basis of disease resistance and susceptibility (Flor, 1971). The gene-for-gene hypothesis proposes two categories of genes in plant–pathogen interactions: resistance (R) genes in the plant, which confer resistance to specific pathogens, and avirulence (Avr) genes in the pathogen, which correspond to the R genes of plants (Keen, 1990; van der Biezen and Jones, 1998) (Figure 1.2). A classic example of the gene-for-gene relationship is *RPS2* (*RESISTANCE TO PSEUDOMONAS SYRINGAE PROTEIN 2*) in *Arabidopsis thaliana* and *avrRpt2* in the bacterial pathogen *Pseudomonas syringae*. *avrRpt2* encodes a protease effector, AvrRpt2, that cleaves plant proteins, such as RIN4 (RPM1-INTERACTING PROTEIN 4), which is involved in immune signaling, thereby promoting infection (Mackey et al., 2002). *A. thaliana* ecotypes with a functional *RPS2* allele exhibit resistance to *P. syringae* strains expressing *avrRpt2*, as RPS2 detects the cleavage products of AvrRpt2 and triggers immune responses. Conversely, *A. thaliana rps2* mutants, or ecotypes lacking *RPS2*, are susceptible to *P. syringae* strains carrying *avrRpt2* (Innes et al., 1993; Kunkel et al., 1993).

1.1.1 Effectors

The primary monitoring target of plant NLR proteins is the activities of small proteins secreted by pathogens, known as effectors, which are encoded by Avr genes. (Bent, 1996; Jones, 1996; Michelmore et al., 2013). Effectors are mainly known to dampen prior immune responses, commonly referred to as PTI (Hogenhout et al., 2009; Bozkurt et al., 2012; Toruño et al., 2016). Three effectors of *P. syringae*—AvrRpt2, AvrRpm1, and AvrB—target the same host protein, RIN4, which is a negative regulator of basal immune response governed by PRRs, but in distinctive manners. AvrRpt2 cleaves RIN4 to suppress the immune responses, whereas AvrRpm1 and AvrB, which are unrelated, biochemically modify RIN4 to disturb its regulatory function (Mackey et al., 2002; Chung et al., 2011; Peng et al., 2024). Similarly, effectors from different pathogens converge on the same step in plant immunity. Two bacterial effectors, AvrPto and AvrPtoB, and an effector from the oomycete

pathogen *Phytophthora infestans*, Avr3a, both interfere with signaling initiated by the activated PRR protein, FLAGELLIN-SENSING 2 (FLS2) (Göhre et al., 2008; Xiang et al., 2008; Chaparro-Garcia et al., 2015). This convergence of effectors on critical immune nodes highlights the importance of effectors in the evolutionary arms race with plants.

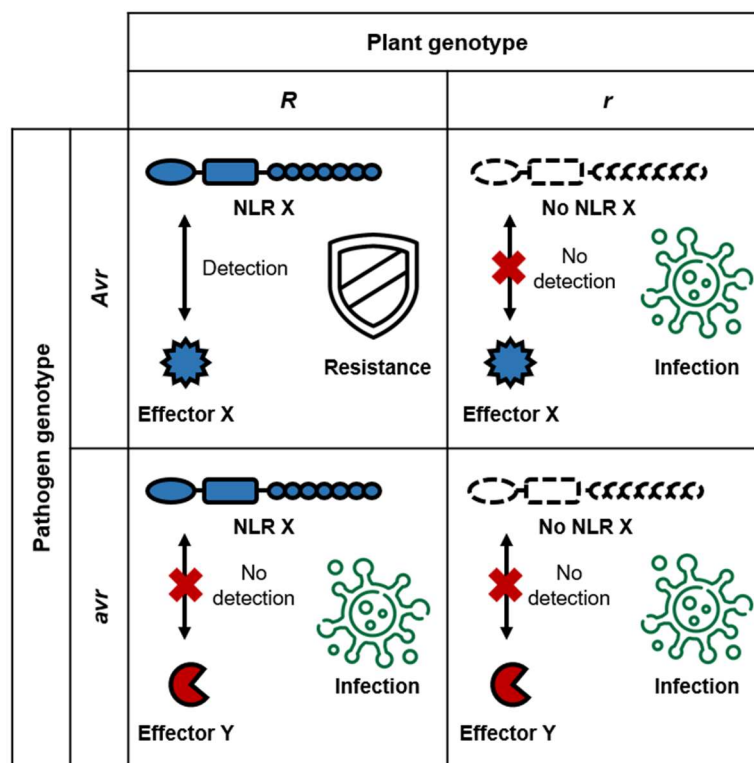


Figure 1.2 Gene-for-gene hypothesis. Disease resistance can exist when the protein encoded by a plant *R* gene recognizes the corresponding *Avr* protein from the pathogen. In the absence of this matching interaction, the pathogen can successfully infect the host.

Diverse organisms across distant taxonomic clades—including bacteria, viruses, fungi, oomycetes, nematodes and insect herbivores—employ effectors to aid their propagation on plants despite their distinct ecologies (Hogenhout et al., 2009; Bozkurt et al., 2012; Toruño et al., 2016). The mechanisms of effector delivery vary among pathogen species. In Gram-negative bacteria, mutations in the *Hrp* (*hypersensitive reaction and pathogenicity*) genes, resulting in so-called *Hrp*[−] mutants, abolish pathogenicity. *Hrp* genes are typically clustered in the genome, and many encode components of the type III secretion system (T3SS), which delivers effectors directly into plant cells (Lindgren, 1997; Alfano and Collmer, 1997). Consequently, many effectors cannot promote infection in *Hrp*[−] bacterial backgrounds (Alfano and Collmer, 2004). Filamentous pathogens, such as fungi and oomycetes, often form specialized structures called haustoria, where effectors are translocated into host cells. Although the exact delivery mechanisms are less well understood compared to T3SS, several

conserved motifs involved in host-cell translocation have been identified among effectors (Bozkurt et al., 2012; Giraldo et al., 2013; Bozkurt and Kamoun, 2020). Aphids, which are among the best-studied insect herbivores, deliver effectors through their stylet mouthparts. Heterologous expressions of aphid effectors can trigger plant immune responses, and plants possessing the corresponding R genes are able to recognize them and trigger defense responses against these aphids (Hogenhout and Bos, 2011; Rodriguez and Bos, 2013).

1.1.2 NLR proteins

Most plant R genes are members of the NLR gene family (Bent, 1996; Jones, 1996; Michelmore et al., 2013). Plant NLR proteins have a conserved tripartite modular structure, which is also a conserved characteristic of NLR proteins in animals. Both plant and animal NLR proteins are in the STAND (signal transduction ATPases with numerous domains) superfamily, which function as molecular switches controlled by nucleotide binding and hydrolysis. Plant NLR proteins typically consist of three domains: an N-terminal domain, a central NB-ARC domain (nucleotide-binding adaptor shared by Apaf-1, Resistance proteins, and CED-4), and a C-terminal LRR (leucine-rich repeat) domain (Ye and Ting, 2008; Qi and Innes, 2013; Saur et al., 2021). There are three major types and two less common types of N-terminal domains in plants. Toll-interleukin 1 receptor (TIR) domain, coiled-coil (CC) domain containing a four-helix bundle, and RPW8 (RESISTANCE TO POWDERY MILDEW 8)-like domain are commonly found in flowering plants (Cui et al., 2015; Monteiro and Nishimura, 2018), whereas α/β -hydrolase domain and protein kinase domain are restricted to specific clades (Chia et al., 2024).

Each domain of plant NLR protein contributes to plant immunity by distinct biochemical functions. The NB-ARC domain is the actual molecular switch, where conformational changes by ADP/ATP-bound state result in different outputs: the active state (ATP-bound state) and the resting state (ADP-bound state). Upon activation, the resulting conformation change promotes oligomerization, which facilitates downstream signaling (Cui et al., 2015). The LRR domain has dual roles during NLR activation. In the resting state, the LRR domain folds back onto the NB-ARC domain and provides autoinhibition by acting as a molecular lid. Upon activation, the LRR domain functions as a ligand-binding site and detects the pathogen activity either through direct recognition of pathogen proteins or indirect sensing of pathogen-induced modifications on the host targets (Qi and Innes, 2013; Saur et al., 2021). A parallel is observed in animal NLR proteins, such as NOD (nucleotide-binding oligomerization domain)-like receptors and NLRC4 (NOD-like receptor family CARD (caspase activation and recruitment domain)-containing protein 4). The LRR domain of NLRC4 keeps the protein in the resting state before the inflammasome assembly. In NOD1 and NOD2, the LRR domains directly recognize bacterial peptidoglycan fragments, which indicate the presence of

bacterial peptidoglycan, a hallmark of bacterial pathogens (Caruso et al., 2014; Moghaddas et al., 2018; Yang et al., 2019). Lastly, the N-terminal domains are ‘signaling’ or ‘executioner’ domains that initiate the downstream signaling after the assembly of the activated NLR proteins (Qi and Innes, 2013; Saur et al., 2021)

1.1.3 NLR diversity in land plants

Land plants possess a wide variety of NLRs. Bioinformatic analyses of plant and algal genomes have shown that these three major N-terminal domains are broadly conserved in land plants, although TIR-NLRs (TNLs) are absent in monocots and in specific clades of dicots (Tian et al., 2004; Tarr and Alexander, 2009; Yue et al., 2012; Gao et al., 2018; Chia et al., 2024). Notably, specific land plant lineages harbor NLRs with atypical N-terminal domains. NLR proteins with an α/β -hydrolase domain are found in bryophyte genomes, especially in liverworts and mosses. Mosses also have another class of atypical NLRs with protein kinase domains at the N-terminus, a class otherwise restricted to only monocots among vascular plants (Xue et al., 2012; Chia et al., 2024). Although the functional conservation of full-length NLR proteins from non-seed plants remains untested, recent studies have shown that the TIR and CC domains from non-seed plants and streptophyte algae can trigger immunity when expressed in angiosperms. Interestingly, only two land plant species are known to possess all types of NLRs: the moss *Sphagnum fallax* and *Ceratodon purpureus* (Chia et al., 2024). Despite our current understanding of NLR genetic diversity in flowering plants, many aspects of NLR functional diversity in underrepresented plant lineages, such as non-seed plants, remain to be investigated (Figure 1.3). Interestingly, NLRs are present in the animal kingdom as components of the innate immune system, although they belong to a different subtype of NLRs, highlighting the deep evolutionary roots of *NLRs* as immunity genes (Jones et al., 2016).

1.1.4 Effector-triggered immunity (ETI)

Monitoring effector activity by NLR proteins plays a crucial role in plant immunity. Plant genomes encode a larger number of NLR genes compared to other gene families (Cui et al., 2015; Li et al., 2015). Immune responses triggered by NLR proteins, commonly referred to as effector-triggered immunity (ETI), involve rapid responses in plant cells, including locally restricted PCD, a burst of reactive oxygen species (ROS), calcium influx, transcriptional reprogramming, induction of PR (Pathogenesis-related) genes, and defense hormone accumulation (Cui et al., 2015). Among these responses, rapid and local PCD, referred to as the hypersensitive response (HR), limits further infection by quenching accessible nutrients and other resources from pathogens (Dangl et al., 1996; Jacob et al., 2013; Cui et al., 2015). Additionally, HR is frequently used as a functional readout for

experimental studies as it produces visible patches of dead tissue and causes leaf deformation in many model angiosperms. For example, HR induced by two well-studied NLR proteins from *A. thaliana*, RPM1 (RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1) and RPS2, results in leaf curling due to rapid tissue collapse, reflecting successful recognition of the effectors by RPM1 and RPS2 (Axtell et al., 2003; Kim et al., 2005; Redditt et al., 2019).

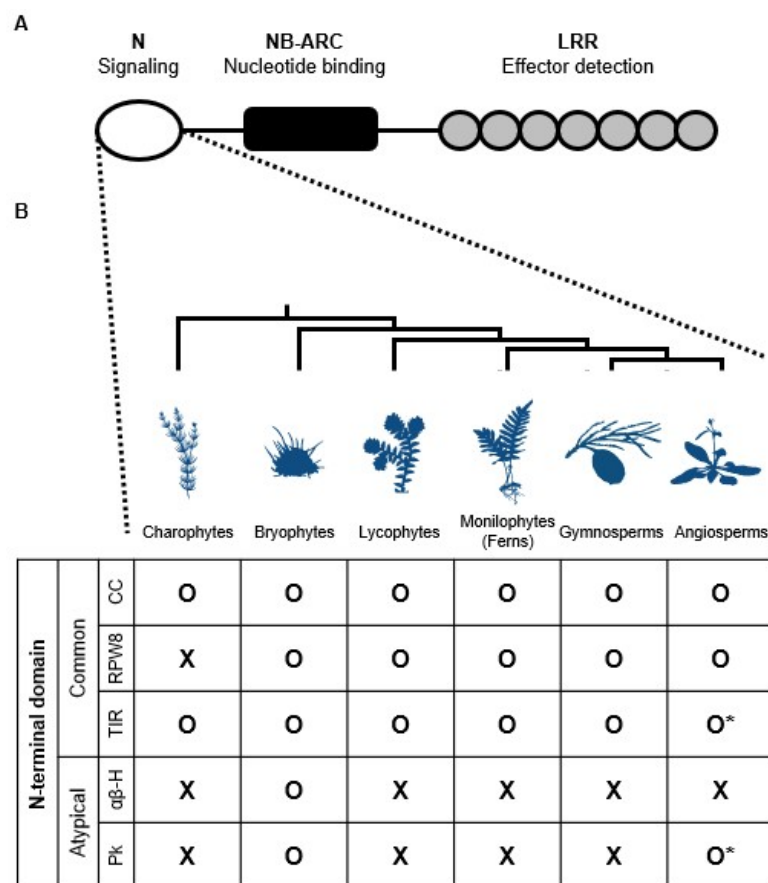


Figure 1.3 NLR diversity across land plants. **(A)** Canonical domain structure of NLR proteins. **(B)** A simplified phylogeny of streptophytes, including land plants and charophytes (algae). Presence and absence of five types of N-terminal domains across clades are illustrated. *These domains are restricted to specific lineages of angiosperms: Protein kinase domains are found in monocots, whereas TIR domains are specific to dicots. Adapted from Chia and Carella, (2023). (Abbreviations: $\alpha\beta$ -H, α/β -hydrolase domain; Pk, protein kinase domain)

NLRs can also cooperate with other non-NLR plant proteins to trigger ETI. For example, bacterial effectors AvrPto and AvrPtoB are primarily detected by the host kinase Pto in tomato. Upon recognition of the effectors, Pto forms a complex with the NLR protein, Prf, and the resulting Pto/Prf complex induces HR (Xiang et al., 2008; Ntoukakis et al., 2014).

Recent structural studies have elucidated the biochemical mechanisms underlying the ETI. ZAR1 (HOPZ-ACTIVATED RESISTANCE 1), a CC-NLR (CNL) protein of *A. thaliana* that senses an uridylated host protein, assembles into a pentameric resistosome with the modified host protein and additional host factors. At the N terminus, α -helices containing the conserved MADA motif, named after the consensus amino acid sequence, form a funnel-like structure that inserts into the plasma membrane and functions as Ca^{2+} permeable channel. The resulting Ca^{2+} influx acts as a secondary messenger to trigger downstream ETI responses including HR at the infection site (Adachi et al., 2019; Wang et al., 2019; Bi et al., 2021). Intriguingly, a different CNL protein from monocots, Sr35 found in einkorn wheat, shares a similar assembly and mode of action, implying evolutionary conservation of CC-NLR resistosomes in flowering plants (Förderer et al., 2022).

In addition to CC-NLR proteins, two TNL proteins, RPP1 (RECOGNITION OF PERONOSPORA PARASITICA 1) and ROQ1 (Recognition of XopQ 1), also assemble into higher-order complexes in the presence of their respective effectors (Ma et al., 2020; Martin et al., 2020). The associated TIR domains in the complex form NADase (NAD⁺ glycohydrolase) active sites, and convert NAD⁺ (nicotinamide adenine dinucleotide), either alone or together with ATP, into small signaling molecules that activate downstream immune signaling pathways (Jia et al., 2022; Huang et al., 2022). This enzymatic activity is required to induce PCD in infected tissues (Horsefield et al., 2019; Wan et al., 2019). The resulting nucleotide-derived small molecules are recognized and are amplified by the EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1) complex and helper NLRs such as ADR1 (Activated Disease Resistance 1) and NRG1 (N requirement gene 1) (Jia et al., 2022; Huang et al., 2022). Strikingly, bacterial TIR domains also produce small nucleotide-derived molecules, although the resulting signaling molecules are structurally distinct from those produced by plant TNLs. These molecules activate the downstream signaling pathways that confer anti-phage resistance to bacteria (Essuman et al., 2022). This highlights a conserved biochemical principle of TIR enzymatic activity across kingdoms.

1.2 Molecular and cellular mechanisms of nonhost resistance

1.2.1 What is nonhost resistance?

Plants are naturally resistant to most potential pathogens around them, a phenomenon termed nonhost resistance (NHR). NHR is considered highly durable and of great interest for crop protection. Despite its significance and applicability, the molecular and cellular mechanisms underlying NHR remain incompletely elucidated and fragmented (Heath, 2000; Lee et al., 2017; Panstruga and Moscou, 2020). A major challenge in NHR research is the conditional nature of infection: plant-pathogen interactions are influenced by diverse endogenous and environmental factors, such as plant age, tissue type, time of day, humidity and temperature (Ayliffe and Sørensen, 2019; Panstruga and Moscou, 2020). Such

variability can result in “marginal” or “intermediate host” resistance, as illustrated in previous studies using the barley-*Puccinia* pathosystem (Atienza et al., 2004; Niks and Marcel, 2009). Senescence also plays a role in NHR, as mature and immature plants often displaying different degrees of resistance (Atienza et al., 2004; Shibata et al., 2011). The interaction between *A. thaliana* and its non-adapted pathogen *Magnaporthe oryzae*, which is a rice pathogen, further demonstrates the conditionality of NHR: penetration is more efficient at dawn than at dusk (Yamaguchi et al., 2017).

1.2.2 Pre-invasive NHR

NHR can be categorized into two subtypes based on the stage where pathogenesis is halted by plants. In pre-invasive NHR, pathogens fail to enter the nonhost plant due to unsuitable physical and chemical microenvironments (Lee et al., 2017; Fonseca and Mysore, 2018). The leaf epidermis is the frontline of plant-pathogen interactions. For fungal pathogens, the cuticular wax chemistry often has a more significant impact on successful infection than its physical properties (Arya et al., 2021). For example, the barley powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* develops fewer penetrating structures, called appressoria, on cabbage, which is a nonhost. A key difference between barley and cabbage leaves is the fatty acid and fatty aldehyde profile of the epidermal wax. Interestingly, C26 fatty aldehydes, which is abundant in barley leaves but absent in cabbage, promote appressorium formation and germ tube development *in vitro*, implicating that cuticular wax composition is a more important determinant of compatibility than surface structure (Tsuba et al., 2002). The importance of cuticular wax composition is further supported by genetic studies: mutations in genes involved in cuticular wax biosynthesis alter spore germination or differentiation on leaves (Uppalapati et al., 2012; Wang et al., 2019; Kong et al., 2020).

1.2.2.1 Constitutive chemical defenses

Plants also constitutively produce antimicrobial secondary metabolites, forming an additional layer on NHR. Saponins, triterpene glycosides found in many flowering plants, have antifungal activity, then shape plant-pathogen compatibility (Osbourn, 1996; Mysore and Ryu., 2004). A fungal pathogen *Gaeumannomyces graminis* var. *tritici* infects roots of wheat and barley, but not oat. Oat roots contain the oat-specific saponin named avenacin that prohibits the fungal pathogen. Both *sad* (*saponin-deficient*) mutants of diploid oat, *Avena strigosa*, and other diploid oat species naturally lacking avenacin, like *A. longiglumis*, are susceptible to the same pathogen, highlighting the role of avenacin in NHR (Osbourn et al., 1994; Papadopoulou et al., 1999).

Two-component chemical defense systems in angiosperms, composed of toxin precursors and activating enzymes, highlight the contribution of specialized secondary metabolism to NHR. One

well-characterized system consisting of cyanogenic glucosides and β -glucosidases is present in more than 3,000 different species and has been acquired through multiple independent evolutionary events (Sánchez-Pérez and Neilson, 2024). In these plants, the cyanogenic glucoside substrates are physically sequestered in vacuoles from β -glucosidases. Upon tissue damage by microbial pathogens or insect herbivores, the substrates and enzymes colocalize, and the resulting hydrolysis produces hydrogen cyanide. This induced cyanogenesis provides an acute toxic barrier that deters further pathogen proliferation and feeding (Zagrobelny et al., 2004; Møller, 2010).

Principle of the two-component chemical defense can be found in different organisms. In Brassicales, which do not possess the cyanogenesis mechanism, another two-component system exists. The substrates, glucosinolates, are stored in vacuoles of most cells, whereas the enzymes, myrosinases, are localized to specialized idioblasts called myrosin cells. Tissue damage allows myrosinases to convert glucosinolates into isothiocyanates, the lineage-specific defensive compounds (Shirakawa and Hara-Nishimura, 2018). Beyond angiosperms, cyanogenic glucosides are also found in distantly related species, including certain ferns, and even in some arthropods, implying that chemical defense by secondary metabolites is a broadly conserved and fundamental defense strategy across diverse organisms (Bak et al., 2006; Jensen et al., 2011; Thodberg et al., 2020).

1.2.2.2 Cell wall appositions (CWAs)

When filamentous pathogens penetrate epidermal cells, they trigger the formation of localized subcellular structures known as cell wall appositions (CWAs) or papillae. CWAs are locally reinforced regions characterized by the site-directed deposition of callose and the secretion of antimicrobial materials (Hardham et al., 2007). This cellular response is facilitated by rapid reorganization of the cytoskeleton. When reorganization of actin filaments and microtubules are chemically inhibited, it results in increased penetration rates by non-adapted pathogens (Kobayashi et al., 2003).

Pre-invasive NHR against penetration of filamentous pathogens requires a combination of different cellular processes. In *A. thaliana*, mutations in *PEN* (*PENETRATION*) genes enable penetration by its non-adapted pathogen, barley powdery mildew fungus *B. graminis* f. sp. *hordei*. *PEN1* (*PENETRATION 1*), which is also known as *SYPI21* (*SYNTAXIN OF PLANTS 121*), encodes a syntaxin, a protein essential for cellular membrane fusion and exocytosis (Assaad et al., 2004). *PEN2* encodes a glycosyl hydrolase localized to peroxisomes and mitochondria, which catalyzes indole glucosinolates into their active form as defensive chemical (Lipka et al., 2005; Fuchs et al., 2015). *PEN3*, which referred to also as *PDR8* (*PLEIOTROPIC DRUG RESISTANCE 8*), is an ATP-binding cassette (ABC) transporter whose substrates include antimicrobial compounds such as camalexin (Stein et al., 2006; He et al., 2019). The distinct biochemical functions of the *PEN* proteins suggest

that CWA formation is a combination of diverse cellular processes, including cell wall rearrangement, metabolite transport, and exocytosis.

1.2.3 Post-invasive NHR

Pathogens must overcome post-invasive NHR mechanisms to complete their life cycle and propagate within plant tissues. To restrict pathogen growth, plants employ diverse strategies, including the formation of specialized subcellular structures and the induced biosynthesis of antimicrobial compounds. These antimicrobial chemicals can present as lineage-specific. For example, camalexin, one of the most well-studied examples, is an indole alkaloid produced by members of the Brassicaceae family (Piasecka et al., 2015). Forward genetic screens identified five *pad* (*phytoalexin-deficient*) mutants of *A. thaliana* that are unable to produce camalexin (Glazebrook and Ausubel, 1994; Glazebrook et al., 1996, 1997). Mutations in *PAD3*, which encodes a cytochrome P450 monooxygenase responsible for catalyzing the final two steps of camalexin biosynthesis (Zhou et al., 1999; Schuhegger et al., 2006; Bottcher et al., 2009). Notably, resistance against several non-adapted pathogens, including the fungal pathogen *Alternaria brassicicola* and camalexin-sensitive isolates of the fungal pathogen *Botrytis cinerea*, is compromised in *pad3* mutants (Thomma et al., 1999; Kliebenstein et al., 2005).

1.2.3.1 NLRs in NHR

ETI contributes to NHR, as evidenced by previous studies have shown that ectopic expression of effectors induces HR in nonhost plants. Several NLR genes have been identified as responsible for NHR (Lee et al., 2017; Oh and Choi, 2022). Importantly, NLR-mediated NHR is not always conferred by a single NLR gene. For example, extensive genetic study in pepper revealed that pepper's NHR to the potato pathogen *P. infestans* is layered by multiple loci, indicating several R genes act in together to provide the resistance (Lee et al., 2014).

NLR proteins can contribute to NHR when a plant is phylogenetically close to the host of a specific pathogen (Panstruga and Moscou, 2020). Several cases demonstrate that NLR-mediated NHR can be controlled by a single NLR protein, underscoring the potential for resistance transferability between closely related species. One example is the interaction between maize and the bacterial pathogen, *Xanthomonas oryzae* pv. *oryzicola*, which causes bacterial leaf streak on rice. Rxo1 (Reaction to X. o. pv. *oryzicola*), a maize NLR protein, recognizes the respective effector, AvrRxo1, and activated ETI. Transgenic rice lines expressing *Rxo1* acquire resistance against *X. oryzae* pv. *oryzicola*. Interestingly, *Rxo1* also functions as the R gene against the maize pathogen, *Burkholderia andropogonis* (Zhao et al., 2004a; 2004b; 2005).

Albugo candida is the causal agent of white rust, a major disease of many cruciferous crops, including oilseed rapese and mustards. Interestingly, several accessions of *A. thaliana*, which is the model angiosperm species in the Brassicaceae family as these cruciferous crops, exhibit partial to nearly complete NHR against *A. candida* race isolated from these crops. A key NHR gene identified from *A. thaliana* is *WRR4* (*WHITE RUST RESISTANCE 4*), which encodes a TNL protein (Borhan et al., 2008). Notably, transgenic oilseed rape expressing *WRR4* acquires the resistance to an *A. candida* race, demonstrating another example of NHR governed by a single *NLR* gene (Borhan et al., 2010).

1.3 Immune chaperone complex in NHR

The stabilization and maturation of NLR proteins is maintained by an immune chaperone complex composed of a chaperone protein, HSP90 (HEAT SHOCK PROTEIN 90), and two co-chaperone proteins, RAR1 (REQUIRED FOR MLA12 RESISTANCE 1) and SGT1 (SUPPRESSOR OF G2 ALLELE OF SKP1) (Kadota et al., 2010). By forming this complex, RAR1 and SGT1 direct part of the chaperone capacity of HSP90 to the NLR-mediated immunity (Figure 1.4). Consistently, *rar1* and *sgt1* mutants show enhanced disease susceptibility in multiple pathosystems, including *A. thaliana* downy mildew (Austin et al., 2002; Muskett et al., 2002; Tör et al., 2002), powdery mildew in barley (Shirasu et al., 1999; Azevedo et al., 2002) and tobacco mosaic virus resistance in *Nicotiana* species (Liu et al., 2002a; Liu et al., 2002b).

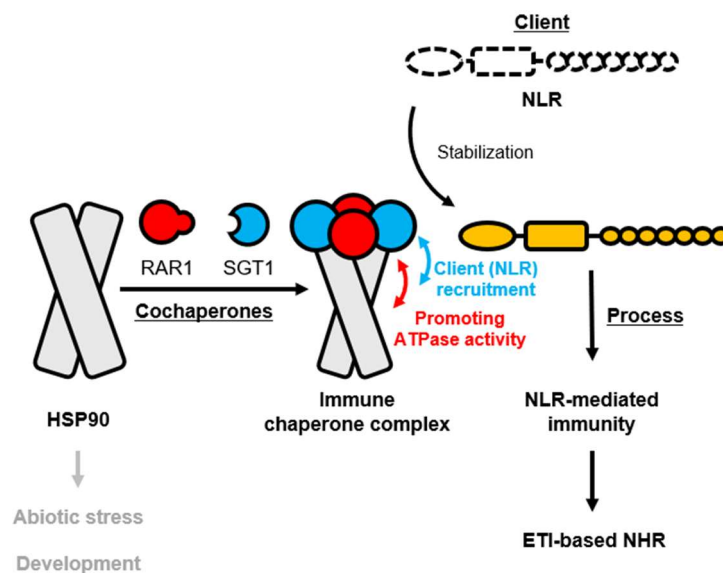


Figure 1.4 Role of RAR1 and SGT1 in NHR. RAR1 (REQUIRED FOR MLA12 RESISTANCE 1) and SGT1 (SUPPRESSOR OF G2 ALLELE OF SKP1) direct chaperone activity to NHR by forming an immune chaperone complex. The immune chaperone complex maintains the steady-state levels of NLR proteins, providing basis of ETI-based NHR

In many cases, defects in the function of the immune chaperone complex correlate with decrease of NLR protein accumulation and corresponding disease phenotypes (Table 1.1; Table 1.2). For example, *rar1* and *hsp90* mutants exhibit a dramatic decrease of cellular RPM1 protein levels, accompanied by enhanced pathogen growth (Tornero et al., 2002; Hubert et al., 2003) (Figure 1.5). However, not all NLR proteins are equally dependent on the immune chaperone complex. For instance, barley (*H. vulgare*) has two NLR genes, *MLA1* (*Mildew Locus A 1*) and *MLA6*, to detect the barley powdery mildew isolates carrying *avrMla1* and *avrMla6*, respectively. *MLA6* requires *RAR1* and *SGT1* to confer disease resistance, whereas the disease resistance provided by *MLA1* remains functional when *RAR1*, *SGT1*, or even both genes are silenced (Azevedo et al., 2002). In barley *rar1* knock-out backgrounds, the steady state level of both *MLA* isoforms is decreased, but again *MLA1*-mediated resistance is maintained, unlike *MLA6*-mediated resistance. Similarly, silencing of *RAR1* reduces the concentration of Rx, the NLR protein conferring resistance to potato virus X (PVX), but the resistance is retained (Bieri et al., 2004). In summary, this dependency on the chaperone complex varies by protein, although the dependency of many NLR proteins has been addressed. These observations indicate that the functional threshold of NLR proteins differs by protein and context.

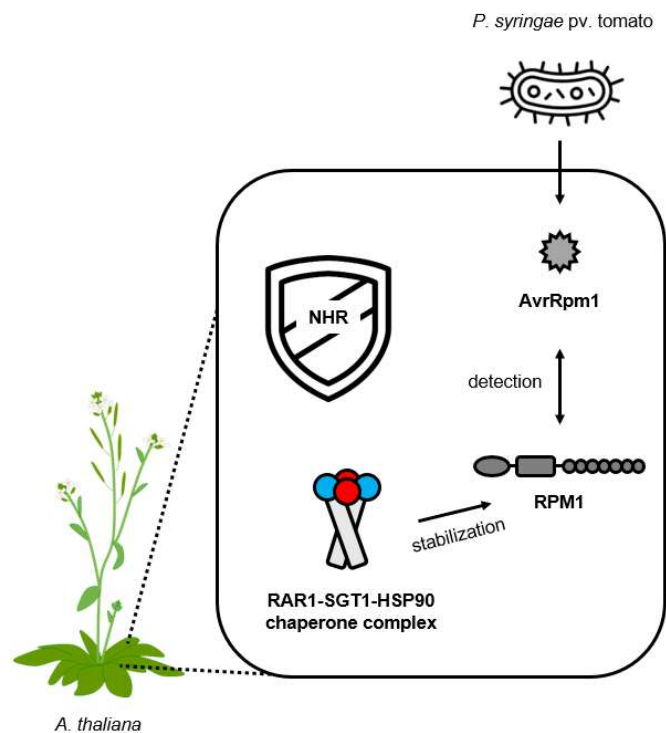


Figure 1.5 Contribution of RAR1 and SGT1 to NHR in *A. thaliana*. The RAR1–SGT1–HSP90 complex maintains cytosolic levels of NLR protein RPM1, which recognizes AvrRpm1 from *P. syringae* pv. tomato. When this chaperone complex is disrupted, plants lose NHR against *P. syringae* strains carrying AvrRpm1 (Tornero et al., 2002; Hubert et al., 2003).

1.3.1 RAR1

RAR1 was first described in barley *rar1* mutants which lost resistance against barley powdery mildew *B. graminis* f. sp. *hordei* (formerly known as *Erysiphe graminis* f. sp. *hordei*) isolate A6 (Freialdenhoven et al., 1994). RAR1 contains two tandem CHORD (cysteine- and histidine- rich domain) domains, CHORD I and CHORD II, located at the N- and C-terminus, respectively. CHORD-containing proteins are also found in non-plant species including fungi and animals. However, plant RAR1 homologs uniquely contain a short and conserved stretch with three cysteines and a histidine, designated as CCCH motif, between CHORD I and CHORD II (Shirasu et al., 1999; Shirasu, 2009). In plants, RAR1 is typically a single-copy gene. *rar1* mutants show no developmental phenotypes and their phenotypes are restricted to breakdown of NLR-mediated immunity and NHR to non-adapted pathogens (Shirasu et al., 1999; Muskett et al., 2002; Tornero et al., 2002).

There are no true RAR1 homologs outside plants, but CHORD-containing proteins with the same tandem domain structure (Shirasu, 2009). CHORD-containing proteins are involved in viability at the diploid state of diverse eukaryotic lineages such as fungi (Sadanandom et al., 2004), and nematodes (Shirasu et al., 1999). Mice possess two genes encoding CHORD-containing proteins, *Chordc1* (also known as *Morgana* or *Chp-1*) and *Melusin*. Homozygous *Chordc1* *-/-* mice exhibit embryo lethality (Groza et al., 2023) while *Melusin* is specifically involved in cardiac hypertrophy, which is an adaptive response that helps the heart to retain its function under pressure overload caused by external stimuli (Brancaccio et al., 2002). Interestingly, many non-plant CHORD-containing proteins, but not all, have a C-terminal extension.

1.3.2 SGT1

SGT1 was first discovered as an interaction partner of RAR1 in two model plant species, *A. thaliana* and *Nicotiana benthamiana* (Azevedo et al., 2002; Liu et al., 2002b). Independently, SGT1 was originally identified in the bread yeast *Saccharomyces cerevisiae* as a component of the SCF (SKP1–Cullin–F-box) ubiquitin ligase complex required for the yeast kinetochore assembly pathway (Kitagawa et al., 1999). *A. thaliana* encodes two *SGT1* paralogs, *AtSGT1a* and *AtSGT1b*, which have overlapping but also partially distinct functions in immunity and development. *Atsgt1a Atsgt1b* double mutants show embryo lethality phenotype, consistent with essentiality of *SGT1* in yeasts (Austin et al., 2002; Azevedo et al., 2006). Remarkably, *A. thaliana* *SGT1* homologs can rescue yeast *sgt1* mutants, highlighting the deep evolutionary conservation of *SGT1* in eukaryotes (Azevedo et al., 2002).

SGT1 proteins contain three conserved domains: an N-terminal tetratricopeptide repeat (TPR) domain, a central CS (CHORD-containing protein and SGT1) domain, and a C-terminal SGS (SGT1-

specific) domain (Azevedo et al., 2002; Peart et al., 2002). The CS domain interacts with both HSP90 and RAR1, using distinct binding interfaces (Zhang et al., 2010). The SGS domain mediates recruitment of NLR proteins to the immune chaperone complex through clients' LRR domains. Interestingly, domain–domain interactions are conserved between plant and animal immune systems, where SGT1 also acts as a co-factor for the immune sensor NOD1 (Bieri et al., 2004; da Silva Correia et al., 2007). The TPR domain remains comparatively less characterized in plant SGT1 homologs. In yeast and human SGT1 proteins, the TPR domains interact with components of SCF E3 ubiquitin ligase complexes (Bansal et al., 2004; Catlett and Kaplan, 2006; Willhoft et al., 2017). In plants, AtSGT1b modulates auxin response through interaction with the SCR^{TIR1} E3 ubiquitin ligase complex (Gray et al., 2003). The TPR domain may be involved in biological functions beyond plant immunity through the ubiquitin ligase pathways.

Table 1.1 List of RAR1-dependent NLR proteins.

Organism	NLR	Tested system	Pathogen	Effector	References
<i>Arabidopsis thaliana</i>	RPM1	<i>Arabidopsis thaliana</i>	<i>P. syringae</i> pv. tomato DC3000	AvrRpm1	Tomero et al., 2002
<i>Glycine max</i>	Rsv1*	<i>Glycine max</i>	Soybean mosaic virus		Fu et al., 2009
	Rpg1-b	<i>Glycine max</i>	<i>P. syringae</i> pv. glycinea	AvrB	Fu et al., 2009
<i>Hordeum vulgare</i>	MLA6	<i>Hordeum vulgare</i>	<i>B. graminis</i> f. sp. <i>hordei</i>	Bgh-A6 (AvrMla6)	Bieri et al., 2004
<i>Nicotiana tabacum</i>	N	<i>Nicotiana benthamiana</i> , transgenic (NN)	Tobacco mosaic virus		Liu et al., 2002a
<i>Solanum lycopersicum</i>	Pto**	<i>Solanum lycopersicum</i>	<i>P. syringae</i> pv. tomato T1	AvrPto	Ekengren et al., 2003

* possibly a locus with multiple NLR genes (Ross et al., 2021)

** indispensable for NLR protein Prf to detect the effectors

Table 1.2 List of SGT1-dependent NLR proteins.

Organism	NLR	Tested system	Pathogen	Effector	References
<i>Arabidopsis thaliana</i>	RPW8.1, RPW8.2	<i>Nicotiana benthamiana</i>		*	Peart et al., 2002
<i>Glycine max</i>	Rsv1**	<i>Glycine max</i>	Soybean mosaic virus		Fu et al., 2009
	Rpg1-b	<i>Glycine max</i>	<i>P. syringae</i> pv. glycinea	AvrB	Fu et al., 2009
<i>Hordeum vulgare</i>	MLA6	<i>Hordeum vulgare</i>	<i>B. graminis</i> f. sp. <i>hordei</i>	Bgh-A6 (AvrMla6)	Azevedo et al., 2002
<i>Nicotiana glutinosa</i>	N	<i>Nicotiana benthamiana</i>	Tobacco mosaic virus		Peart et al., 2002
<i>Solanum lycopersicum</i>	Pto***	<i>Nicotiana benthamiana</i>		AvrPto	Peart et al., 2002
		<i>Nicotiana benthamiana</i>	<i>P. syringae</i> pv. tabaci	AvrPto	Peart et al., 2002
	Prf	<i>Nicotiana benthamiana</i>		****	Kud et al., 2013
		<i>Nicotiana benthamiana</i>	<i>P. syringae</i> pv. tomato DC3000	AvrPto, AvrPtoB	Kud et al., 2013
		<i>Nicotiana benthamiana</i>		****	Van Ooijen et al., 2010
<i>Solanum hirsutum</i>	I-2	<i>Nicotiana benthamiana</i>		****	Van Ooijen et al., 2010
	Mi-1	<i>Nicotiana benthamiana</i>		****	Van Ooijen et al., 2010
	Sw-5b	<i>Nicotiana benthamiana</i>	Tomato spotted wilt virus		Chen et al., 2021
	Cf-4	<i>Nicotiana benthamiana</i>		Avr4	Peart et al., 2002
	Cf-9	<i>Nicotiana benthamiana</i>		Avr9	Peart et al., 2002
<i>Solanum tuberosum</i>	Rx	<i>Nicotiana benthamiana</i>		PVX-CP	Peart et al., 2002
		<i>Nicotiana benthamiana</i>	PVX		Peart et al., 2002
		<i>Nicotiana benthamiana</i>	PVX		Azevedo et al., 2006

* simultaneous overexpression causes HR

** possibly a locus with multiple NLR genes (Ross et al., 2021)

*** indispensable for NLR protein Prf to detect the effectors

**** autoactive mutations were used to examine function of these NLR proteins: Prf^{D1416V}, I-2^{D495V}, and Mi-1^{D841V}

1.3.3 RAR1–SGT1 interaction

The protein-protein interaction (PPI) between RAR1 and SGT1 is considered highly robust in plants, supported by multiple independent studies across species and experimental methods (Table 1.3). The first evidence came from yeast two-hybrid (Y2H) assay, which identified a direct interaction between *A. thaliana* RAR1 and SGT1. Subsequent Y2H studies confirmed this interaction in many traditional plant model systems, such as rice, soybean, and wheat (Wang et al., 2008; Fu et al., 2009; Cantu et al., 2013; Tai, 2015). Moreover, conservation of the RAR1–SGT1 interaction was also demonstrated in tetraploid peanut, banana, and rubber tree, species that only recently gained genomic resources (Yuan et al., 2021; Wang et al., 2024; Liu et al., 2026). Importantly, Y2H assays have also shown that RAR1 and SGT1 homologs from different angiosperms can interact, highlighting conservation of the binding interface across flowering plants (Azevedo et al., 2002; Cantu et al., 2013). In several cases, the RAR1–SGT1 interaction has been further validated by alternative PPI assays such as co-immunoprecipitation (Azevedo et al., 2002) and bimolecular fluorescence complementation (Wang et al., 2024; Liu et al., 2026). Outside flowering plants, the model moss species *Physcomitrium patens* contains a RAR1 homolog with a conserved domain structure similar to that of RAR1 homologs (Shirasu, 2009). However, the RAR1–SGT1 interaction in non-flowering land plants, including *P. patens*, has not yet been tested.

Table 1.3 List of tested RAR1–SGT1 interactions across angiosperms.

Species	RAR1	SGT1	Methods	References
<i>A. thaliana</i>	AtRAR1	AtSGT1a AtSGT1b	Y2H	Azevedo et al., 2002
<i>N. benthamiana</i>	NbRAR1	NbSGT1	Y2H	Liu et al., 2002b
Rice (<i>Oryza sativa</i>)	OsRAR1	OsSGT1	Y2H	Wang et al., 2008
Soybean (<i>G. max</i>)	GmRAR1	GmSGT1-1 GmSGT1-2	Y2H	Fu et al., 2009
Wheat (<i>Triticum aestivum</i>)	TaRAR1	TaSGT1	Y2H	Cantu et al., 2013; Tai et al., 2015
Tetraploid peanut (<i>Arachis hypogaea</i>)	AhRAR1-A AhRAR1-B	AhSGT1-A2 AhSGT1-A3 AhSGT1-B1 AhSGT1-B2 AhSGT1-B3	Y2H	Yuan et al., 2021
Banana (<i>Musa acuminata</i>)	MaRAR1	MaSGT1a	Y2H, BiFC	Wang et al., 2024
Rubber tree (<i>Hevea brasiliensis</i>)	HbRAR1-1 HbRAR1-2	HbSGT1b	Y2H, BiFC	Liu et al., 2026
Barley (<i>H. vulgare</i>)	HvRAR1	HvSGT1	co-IP	Azevedo et al., 2002
Cross-species interaction	HvRAR1	AtSGT1a AtSGT1b	Y2H	Azevedo et al., 2002
	OsRAR1	TaSGT1	Y2H	Cantu et al., 2013
	TaRAR1	OsSGT1	Y2H	Cantu et al., 2013

The CHORD II domain of RAR1 and the CS domain of SGT1 were identified as the responsible domains for RAR1–SGT1 interactions using *A. thaliana* homologs and Y2H (Azevedo et al., 2002). The RAR1^{CHORD II}–SGT1^{CS} binding interface was further resolved by crystal structure of a HvHsp90^N–AtSGT1a^{CS}–AtRAR1^{CHORD II} ternary complex (Figure 1.6). Critical residues in both domains are conserved across plants, and mutations in these sites abolish the RAR1–SGT1 interaction and the chaperone-mediated NHR (Zhang et al., 2010). These include a conserved isoleucine in RAR1 and conserved isoleucine residue in SGT1 (discussed in detail in Chapter 3). Due to the reproducibility and robustness of the RAR1–SGT1 interaction, it is widely used as a benchmark for evaluating novel *in planta* PPI assays (Chen et al., 2008; Chen et al., 2023).

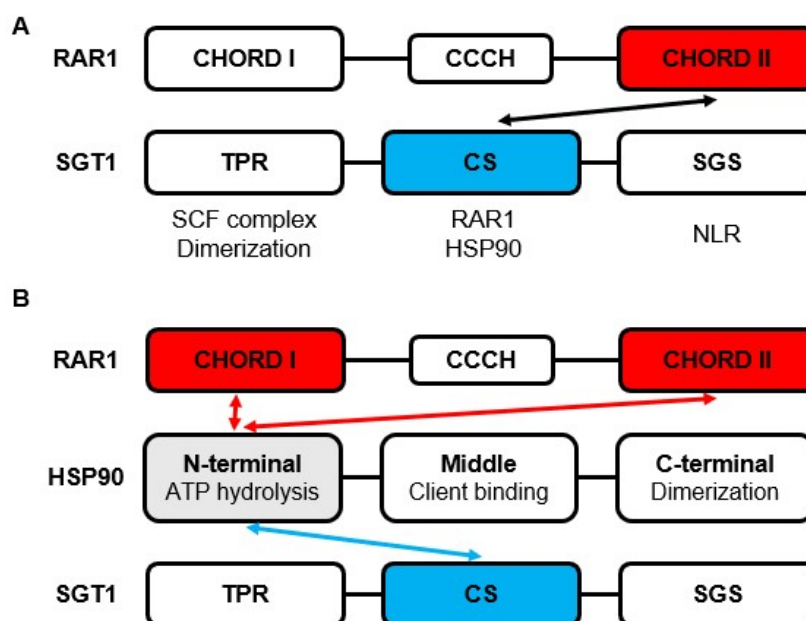


Figure 1.6 Interaction dynamics in the RAR1–SGT1–HSP90 complex. **(A)** RAR1 and SGT1 interacts through the CHORD II domain and CS domain **(B)** The CHORD domains of RAR1 and the CS domain of SGT1 interacts with the N-terminal domain of HSP90. Adapted from Kadota and Shirasu (2010).

1.3.4 HSP90-mediated immunity without RAR1 and SGT1

Several lines of evidence indicate that HSP90 can contribute to plant immunity independently of the canonical immune chaperone complex assembly. In rice, the chitin-recognizing PRR OsCERK1 (CHITIN ELICITOR RECEPTOR KINASE 1) is stabilized by HSP90 together with a different co-chaperone, the plant homolog of Hop (Hsp70-Hsp90 organising protein), also known as Sti1 (stress-induced protein 1). HSP90 and Hop/Sti1a interact with OsCERK1 at the endoplasmic reticulum, mediating its proper trafficking to the plasma membrane. When *Hop/Sti1a* is silenced, chitin-triggered immunity and resistance to the rice blast fungus are impaired (Chen et al., 2010).

HSP90 also contributes to plant immunity by regulating signaling pathways of two defense hormones, jasmonic acid (JA) and salicylic acid (SA), independently of RAR1 or SGT1. The F-box protein COI1 (CORONATINE-INSENSITIVE PROTEIN 1), which is the JA receptor, is stabilized by an HSP90–HSP70–AtSGT1b chaperone complex in *A. thaliana*. (Zhang et al., 2015). Although epistasis between *COI1*, *HSP90*, *HSP70*, and *AtSGT1b* has not been tested, mutations in *COI1* are known to impact disease resistance. *Atcoil-16* mutants, which possess a partially functional allele, are compromised in immune responses triggered by the DAMP, AtPep1 (Holmes et al., 2018). Furthermore, silencing *COI1* impairs inducible defenses against the necrotrophic fungal pathogen *A. brassicicola* in the ornamental plant *Gladiolus hybridus* (Seng et al., 2020). Considering that the JA signaling is primarily involved in defense against necrotrophic pathogens, these findings suggest that HSP90 may contribute to necrotroph resistance through the JA signaling pathway components.

SA-mediated disease resistance in cassava (*Manihot esculenta*) is also controlled by a specific HSP90 isoform and its co-chaperone. Infection with cassava bacterial blight (CBB) upregulates the heat shock factor MeHsf8, which induces expression of MeHSP90.9. MeHSP90.9, together with the co-chaperone MeSRS1 (SHI-related sequence 1), promotes SA biosynthetic pathway and immune responses, including induction of MePR1 and MePR2. The role of MeHsf8–MeHSP90.9 module and MeHSP90.9–MeSRS1 chaperone complex was further examined by silencing of MeHsf8, MeHSP90.9, MeSRS1. Silencing of these genes resulted in increased susceptibility to the CBB agent *Xanthomonas axonopodis* pv. manihotis (Wei et al., 2021). This indicates that HSP90 can modulate disease resistance through the SA signaling pathway outside the canonical immune chaperone complex.

1.4 Evolutionary molecular plant-microbe interactions (EvoMPMI)

Plant–microbe interactions provide a valuable system to study the dynamics of coevolution. When pathogens successfully infect a plant species, they reduce the host’s fitness and survival. Simultaneously, the plant immune system places pathogens under strong selective pressure (Han, 2019). This reciprocal selective pressure during plant–pathogen coevolution is especially well-illustrated in the interactions between pathogen effectors and host proteins (Jones and Dangl, 2006; Upson et al., 2018). Two closely related oomycete pathogens in *Phytophthora* genus, *P. infestans*, the potato late blight pathogen, and *P. mirabilis*, are adapted to different host species. *P. infestans* secretes a protease inhibitor named EPIC1 (Extracellular Protease Inhibitor with Cystatin-like domain 1) to neutralize host proteases involved in defense responses (Tian et al., 2007; Song et al., 2009). The nucleotide sequence of PmepiC1, the homolog from *P. mirabilis*, exhibits signatures of positive selection, which indicates molecular adaptation associated with host jump. Notably, when a single amino acid polymorphism from PmepiC1 is introduced into EPIC1, the modified EPIC1 shows

enhanced inhibitory activity against the protease homolog from *Mirabilis jalapa*, the host species of *P. mirabilis*, while displaying reduced inhibition on its original targets (Dong et al., 2014).

1.4.1 Fossil evidence for ancient plant–microbe interactions

Plant fossils offer a unique window into the earliest documented plant–microbe interactions. The Rhynie chert, which preserves ancient vascular plants approximately 407 million years ago, captures two types of interactions between plants and fungal endophytes that resemble those observed in modern angiosperms (Strullu-Derrien, 2018). Strikingly, fungal endophytes were found forming complex dendritic structures reminiscent of arbuscules built by mycorrhizal fungi during symbiosis in extinct early Devonian vascular plants, such as *Horneophyton lignieri* and *Aglaophyton major* (Remy et al., 1994; Taylor et al., 1995; Strullu-Derrien et al., 2014). Another type of interaction, parasitic interactions, is also documented in the same deposit. The fossils show anatomical features of successful infection, including invasive hyphae, asexual propagation structures (conidiophore), and reproductive structures (zoosporangia). Putative infection sites occasionally exhibit signs of tissue degradation, suggesting the possibility of PCD as a defense mechanism in ancient land plants (Kring et al., 2007; Strullu-Derrien et al., 2023). Collectively, these snapshots in the fossils underscore the deep evolutionary history of plant–microbe interactions during land plant evolution.

The ‘mycorrhizal landing hypothesis’ proposes that symbiotic microbes played a pivotal role in aiding the ‘landing’ of the earliest land plants (Rensing, 2018). Comparative analyses of green algal genomes and transcriptomes support this hypothesis, revealing that key components of symbiotic signaling are conserved in streptophyte algae (Delaux et al., 2015). The liverwort *Marchantia paleacea* is compatible with arbuscular mycorrhizal symbiosis (AMS) unlike its close relative, the model liverwort *Marchantia polymorpha*. Experimental studies demonstrated that the AP2 (APETALA2) transcription factor MpaWRINKLED regulates lipid transport between the host plant and its associated mycorrhizal fungi, in a manner similar to angiosperms (Rich et al., 2021). In angiosperms, signaling pathways for AMS, from cell surface receptors to nucleic calcium-dependent kinases, are well characterized. Recent studies reveal that *M. paleacea* employs the conserved pathways to establish AMS (Teyssier et al., 2025; Tan et al., 2025; Vernié et al., 2025).

1.4.2 Non-seed plants as EvoMPMI model systems

Non-seed plant models enable EvoMPMI to study the plant–microbe interactions in a macroevolutionary perspective (Figure 1.7). Widely used model species in plant evolutionary developmental biology, such as the liverwort *M. polymorpha*, the moss *P. patens* and the fern *Ceratopteris richardii*, are compatible hosts of pathogens originally isolated from angiosperms

(Ponce de Leon and Montesano, 2017; Carella et al., 2018; Gimenez-Ibanez et al., 2018; Grenz et al., 2025). In contrast, relatively few pathogens have been isolated from infected non-seed plants, often by market-driven needs, such as the ornamental fern *Asplenium nidus* (Chase et al., 1984) and moss panels used in urban greening architecture (Akita et al., 2011; Marttinen et al., 2020). Natural fungal and bacterial pathogens have been also isolated from *M. polymorpha* and shown to reinfect this species in laboratory conditions (Matsui et al., 2020; Robinson et al., 2025). Strikingly, the host range of these isolates is not limited to the original non-seed plants but can extend to distantly related species. For example, *Pseudomonas asplenii* isolated from the fern *A. nidus* and *P. viridiflava* MPG32 isolated from the liverwort *M. polymorpha* also infect angiosperm species, gladiolus and *N. benthamiana*, respectively (Chase et al., 1984; Robinson et al., 2025). Furthermore, *P. viridiflava* MPG32 loses its ability to infect both *M. polymorpha* and *N. benthamiana* when two conserved effectors, AvrE and HopM1, are deleted, highlighting the shared infection strategy for distantly related hosts (Robinson et al., 2025). This parallel is further supported in the lab-based pathosystem between *M. polymorpha* and *P. syringae*, two effectors, AvrPto and AvrPtoD, are required to colonize the host efficiently (Gimenez-Ibanez et al., 2019). Although much of our current understanding derives from *M. polymorpha* and *P. patens*, additional non-seed lineages are increasingly being incorporated into EvoMPMI research (Ponce de León, 2011; Poveda, 2020; Jeong et al., 2023).

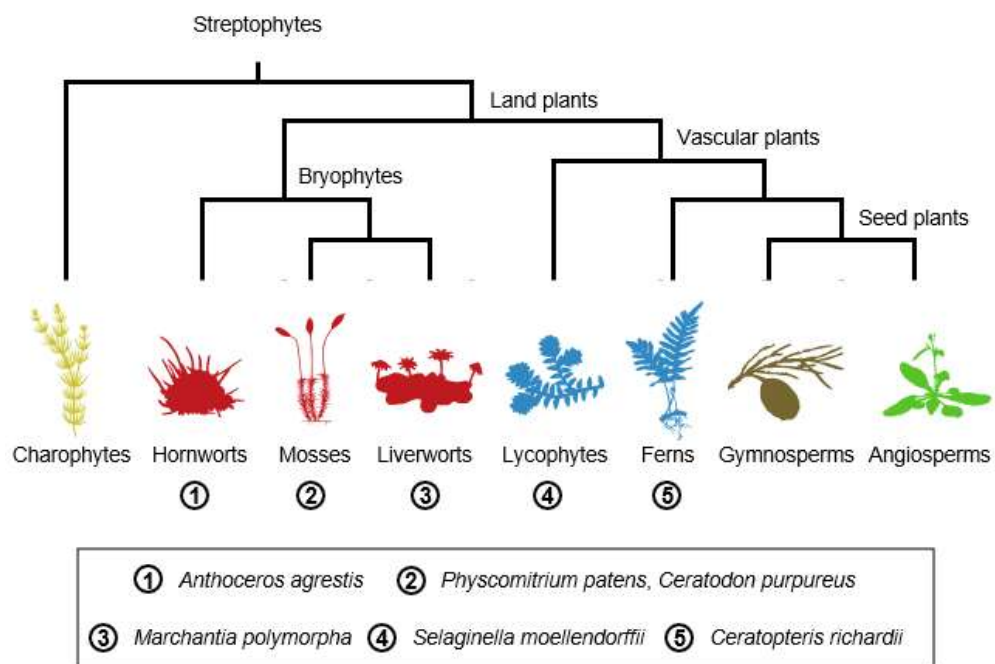


Figure 1.7 Emerging non-seed plant models in EvoMPMI. Simplified phylogenetic relationships between major clades in land plants and charophytes (a sister lineage of land plants) are illustrated. From 1 to 5, the representative model species in each clade is explained.

1.4.2.1 *Marchantia polymorpha*

M. polymorpha is one of the most studied non-seed plants in terms of plant–pathogen interactions (Bowman et al., 2022; Jeong et al., 2023). It is a compatible host for diverse viral and microbial pathogens (bacteria, oomycetes, and fungi) as well as arthropod and mollusc herbivores (Gimenez-Ibáñez et al., 2018; Carella et al., 2018; Kanazawa et al., 2020; Romani et al., 2020; Redkar et al., 2022; Ros-Moner et al., 2024; Schweizer et al., 2025).

Pathogenic interactions between *M. polymorpha* and two broad-host range pathogens, the oomycete *Phytophthora palmivora* and the fungus *Fusarium oxysporum*, illustrate how such pathogens exploit to lineage-specific features of their hosts. *P. palmivora* enters the host through the open pores of liverworts, called air chambers, and subsequently colonizes the underlying photosynthetic layer (Carella et al., 2018). Notably, *nop1* (*nopperabo 1*) mutants, which lack air chambers, are more resistant to *P. palmivora* infection, demonstrating that this liverwort-specific structure provides a critical entry route for infection. In contrast, *M. polymorpha* lacks vascular tissues, which is the major infection route of *F. oxysporum* in tomato. The fungus is still able to infect the liverwort tissues, indicating that some pathogens can infect unfamiliar hosts through alternative colonization strategies (Redkar et al., 2022).

The low-redundancy genome of *M. polymorpha* provides a powerful platform for genomic and transcriptomic approaches, allowing immune responses to be dissected without the complications of gene redundancy (Bowman et al., 2017). During infection of *M. polymorpha*, well-characterized molecular signatures of plant immune responses can be observed, including defensive metabolites and antagonism between defense hormones (Gimenez-Ibanez et al., 2018; Carella et al., 2019). Recent reverse genetics approaches further demonstrate that *M. polymorpha* harbors conserved but simpler PRR signaling pathways compared to flowering plants (Chu et al., 2023; Yotsui et al., 2023; Yan et al., 2025). In addition, it contains far fewer NLR genes than most other land plants, whereas other bryophytes such as the moss *P. patens* encode hundreds of NLRs in numbers comparable to angiosperms (Chia et al., 2024). Together, these features make *M. polymorpha* a powerful model for uncovering the evolutionary foundations of plant immune signaling.

1.4.2.2 *Physcomitrium patens*

P. patens (formerly *Physcomitrella patens*) is a compatible host for a variety of microbial pathogens, including the oomycete *Pythium irregulare*, the fungal pathogens *B. cinerea* and *Fusarium solani*, and the bacterial soft-rot pathogen *Pectobacterium carotovorum* (formerly *Erwinia carotovora*) (Ponce de León et al., 2007; Oliver et al., 2009; Reboledo et al., 2015; Mamaeva et al., 2023). *P. patens* has single-cell layer leaflets and its protonemal filaments are composed of single-cell threads.

These anatomical features provide a unique window to observe cellular immune responses in non-seed plants, including HR-like cell death, cell wall fortification, and ROS bursts (Overdijk et al., 2016; Ponce de Leon and Montesano, 2017).

The cellular and molecular immune responses of *P. patens* also show similarities to those found in angiosperms. For example, the broad host oomycete *P. palmivora* rarely infects *P. patens* protonemata, as most infection attempts are blocked by papilla-like structures, paralleling papillae (CWAs) in angiosperms (Overdijk et al., 2016). In addition, *P. patens* upregulates *PR10* homologs in response to diverse pathogen-related signals, including infection by *B. cinerea*, elicitors derived from *P. carotovorum*, and SA treatments. Notably, ectopic expression of *PpPR10* in *A. thaliana* enhances disease resistance against *P. irregulare*, which can infect both species, demonstrating functional conservation of *PR* genes between distantly related species (Castro et al., 2016).

Beyond defense mechanisms, studies in *P. patens* also have addressed conserved susceptibility factors. *P. infestans*, the oomycete potato pathogen, can colonize *P. patens* (Overdijk et al., 2016). Its effector AVR1, which targets Sec5 to disturb the exocyst trafficking complex in potato, also binds to two Sec5 homologs in *P. patens*, *PpSec5b* and *PpSec5d*. Ectopic expression of AVR1 and silencing of *PpSec5* both result in similar developmental defects in protonema. These results suggest that *P. infestans* may exploit AVR1 during moss infection as it does in potato (Overdijk et al., 2021). Another conserved susceptibility factor in *P. patens* is RALF (RAPID ALKALINIZATION FACTOR) peptides. In *A. thaliana*, RALFs bind to the receptor-like kinase FERONIA (FER) and suppress PRR-mediated immunity, thus acting as negative regulators of defense (Stegmann et al., 2017). Interestingly, pathogens have co-opted these susceptibility factors: *F. oxysporum* secretes RALF-like peptides to hijack RALF–FER signaling and promote infection (Masachis et al., 2016). Mutations in *PpRALF2* and *PpRALF3* result in enhanced resistance of *P. patens* to the bacterial pathogen *P. carotovorum* and the fungal pathogen *F. solani*, implying that these peptides also act as negative regulators of immunity in mosses (Mamaeva et al., 2023). *P. patens* encodes multiple CrRLK1L (*Catharanthus roseus* RLK1-like) receptor-like kinases, the clade where *FER* belongs, which complicates cross-lineage functional analyses (Galindo-Trigo et al., 2016). Together, these findings suggest a conservation of the RALF–CrRLK1L signaling pathway in mosses, as well as its exploitation by fungal pathogens during infection.

1.4.2.3 Lycophytes and monilophytes

Although several lycophytes and monilophytes (ferns) have also contributed to our understanding of EvoMPMI, these lineages remain underexplored compared to bryophyte models, possibly due to the limited availability of genetic transformation tools. One accessible approach to investigate immunity in these lineages is through metabolomics. Green leaf volatiles (GLVs) are six-carbon volatile

organic compounds (VOCs), including aldehydes, alcohols, and their esters, released after tissue damage. These compounds are produced by hydroperoxide lyases (HPLs) and allene oxide synthases (AOSs) which are members of the cytochrome CYP74 family. Comparative metabolomics studies show that a rapid GLV burst by wounding occurs across vascular plants including lycophytes and ferns (Imbiscuso et al., 2009; Tanaka et al., 2021). In the model lycophyte *Selaginella moellendorffii*, HPLs and AOSs catalyze the same reactions as their counterparts in angiosperms (Tanaka et al., 2021). By contrast, bryophytes lack the GLV burst but instead emit eight- and nine-carbon VOCs. For example, a HPL homolog in the moss *P. patens* (PpHPL) is involved in production of C9 VOCs (Stumpe et al., 2006). Interestingly, *P. patens* retains both HPL and AOS genes, whereas the liverwort *M. polymorpha* and the streptophyte alga *Klebsormidium nitens* encode only AOS homologs (Tanaka et al., 2021), suggesting lineage-specific divergence or loss of CYP74 enzyme repertoires.

1.4.3 Conservation of PRR- and NLR-mediated immunity

Plant immunity is governed by two major groups of receptors: PRRs, which detect PAMPs at the cell surface, and NLRs, which monitor pathogen activities in the cytoplasm. Activation of these receptors triggers rapid immune responses including the accumulation of ROS and HR. In *M. polymorpha*, lysin motif (LysM)-domain-containing receptors, MpLYK1 (LysM-receptor-like kinase 1) and MpLYR (LYK-related) detect microbial PAMPs, chitin and peptidoglycan, analogous to how *A. thaliana* FLS2 recognizes peptide fragments from bacterial flagella (Yotsui et al., 2023). The homolog of another well studied PRR, MpSERK (SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE) functions in immunity together with MpBIR1 (BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1)-INTERACTING RECEPTOR-LIKE KINASES 1), as their counterparts in angiosperms (Yan et al., 2025). Components mediating the ROS burst are also conserved in *M. polymorpha*. Functional homologs of RBOH (RESPIRATORY BURST OXIDASE HOMOLOG) and PBL (PBS1 (AVRPPHB SUSCEPTIBLE 1)-like), MprBOH1 and MpPBLa, are required for the chitin-induced ROS burst (Chu et al., 2023). In *P. patens*, a homolog of an angiosperm LysM PRR, CERK1 mediates immune responses against chitin (Bressendorff et al., 2016). Additionally, class III peroxidase PpPrx34 is required for chitosan-responsive ROS production (Lehtonen et al., 2009; Lehtonen et al., 2012).

Despite fewer studies on intracellular immunity in non-seed plants, key NLR functions are clearly conserved across land plant lineages. Recent work shows that CC and TIR domains are functionally conserved from streptophyte algae to angiosperms. Notably, non-seed plant CC domains possess a conserved motif in the first α -helix, referred to as MAPEL, analogous to the MADA motif found in angiosperm CNL proteins. Deletion of the MAEPL motif abolished HR in *N. benthamiana* by MAEPL-containing CC domains. Consistently, inducible expression of a MAPEL-containing CC

domain in *M. polymorpha* triggered HR-like responses and promoted focal accumulation of this protein at the plasma membrane, whereas deletion of the MAEPL motif eliminated these responses. These findings suggest that MAPEL is required for CNL function in non-seed plants, analogous to the role of the MADA motif in angiosperm CNL proteins. Strikingly, atypical N-terminal domains, α/β -hydrolase and protein kinase domain, could also trigger HR in *N. benthamiana*, which lacks NLR proteins with these domains. This cross-lineage HR induction by atypical domains implies overlap in downstream immune signaling between canonical (CC, TIR, and RPW8) and atypical (α/β -hydrolase and protein kinase) N-terminal domains (Chia et al., 2024). Together, these findings indicate that NLR-mediated immunity is conserved in non-seed plants, alongside PRR-mediated immunity.

1.4.4 SA–JA antagonism in non-seed plants

Antagonism between the two major defense hormones, SA and JA, fine-tunes immune responses against multiple pathogens. SA primarily controls biotrophic and hemibiotrophic pathogens, whereas JA signaling is more specialized for defense against necrotrophic pathogens. SA has been detected in field-collected bryophytes (Drábková et al., 2015), and SA treatment enhances disease resistance of *P. patens* as observed in angiosperms (Ponce De León et al., 2012). Intriguingly, although SA functions similarly in disease resistance across distantly related species, downstream transcriptional reprogramming induced by SA appears to be only partially conserved in bryophytes (Peng et al., 2017; Jeon et al., 2024). In contrast, bryophytes are insensitive to the active form of JA in angiosperms, JA-Ile, but they respond to precursors such as 12-oxo-phytodienoic acid (OPDA), which can inhibit their growth (Ponce De León et al., 2012; Monte et al., 2018). In *M. polymorpha*, the JA receptor MpCOI1 binds to a specific precursor, dn-OPDA, than other chemical analogs appearing during JA biosynthesis, together with the co-receptor MpJAZ (JASMONATE ZIM-DOMAIN) (Monte et al., 2018; 2022). In parallel of SA–JA antagonism in angiosperms, OPDA applications promotes resistance against a necrotrophic pathogen but weakens resistance against the hemibiotrophic pathogens in previous studies (Gimenez-Ibanez et al., 2019; Matsui et al., 2020). These observations suggest that fine-tuning of disease resistance through hormone antagonism is deeply conserved in land plants, although lineage-specific divergences are found in the mechanistic details.

1.4.5 Transcriptional regulators in non-seed plant defense

Defense-related transcription factors in bryophytes are clear examples of lineage-specific divergence of plant immune systems. In *P. patens*, a moss-specific AP2/ERF (APETALA2/ETHYLENE

RESPONSIVE FACTOR) transcription factor, PpERF24, acts as a master regulator of defense responses, including PR gene induction, ROS production, JA signaling, and phenylpropanoid biosynthesis (Reboledo et al., 2022). In *M. polymorpha*, a R2R3-MYB transcription factor MpMYB14 is induced by various environmental stresses (Kubo et al., 2018; Carella et al., 2019). Activation of MpMYB14 leads to accumulation of phenylpropanoid-related molecules including riccionidin A, which is a liverwort-specific anthocyanin-like compound (Kubo et al., 2018). Infection by the oomycete pathogen *P. palmivora* also upregulates MpMYB14, and the resulting riccionidin A embedded in cell walls confers penetration resistance (Carella et al., 2019), possibly by modifying cell wall properties (Jibran et al., 2024). Phenylpropanoid and flavonoid biosynthetic pathways are also upregulated by *P. palmivora* infection in the angiosperm *N. benthamiana* (Carella et al., 2019), highlighting secondary metabolites as a conserved defense strategy across land plants.

In addition to transcriptional regulation of phenylpropanoid pathways, liverworts possess a unique cellular structure known as the oil body, which stores terpenoids and other specialized metabolites. Recent studies demonstrated that oil body formation in *M. polymorpha* is regulated by specific transcription factors, MpERF13 and MpC1HDZ (class I homeodomain leucine-zipper), and that disruption of oil body biogenesis increases susceptibility to herbivory (Kanazawa et al., 2020; Romani et al., 2020). These findings highlight oil bodies as lineage-specific innovations that contribute to herbivore defense.

1.5 Main objectives of this thesis

1.5.1 Hypothesis 1: Nonhost resistance mediated by the RAR1–SGT1–HSP90 chaperone complex is conserved in land plants.

RAR1 and SGT1 are broadly conserved in eukaryotes and their roles in plant immunity are well characterized in angiosperms (Shirasu, 2009). Given the robustness of the RAR1–SGT1 interaction in angiosperms, I hypothesize that the RAR1–SGT1 interaction, and consequently the RAR1–SGT1–HSP90 immune chaperone complex and its function, are conserved across land plants.

To address this hypothesis, I investigated chaperone-mediated nonhost resistance from both the plant and pathogen perspectives.

In Chapter 3 (plants), I examined whether *RAR1*, *SGT1*, and RAR1–SGT1 interaction are conserved in land plants. Phylogenetic analyses of land plant *RAR1* and *SGT1* were combined with protein-protein interaction assays with a set of representative lineages. I found that *RAR1*, *SGT1*, and their interaction are conserved, while their binding interface has diverged in leptosporangiate ferns through promiscuous SGT1 intermediates.

In Chapter 4 (pathogens), I interrogated the presence of RAR1-mediated resistance in non-seed plants with a set of *Phytophthora* pathogen strains and *M. polymorpha*. I identified several *Phytophthora* species that showed enhanced infection in *rar1* mutants compared to wildtype. Interestingly, the candidates included understudied *Phytophthora* species. These findings position *M. polymorpha* as a tractable system both for discovering R genes against them and for dissecting their pathogenesis mechanisms.

1.5.2 Hypothesis 2: There are conserved transcriptomic signatures of broad host range pathogens

The broad host range pathogen *P. palmivora* infects the model angiosperm *N. benthamiana* and the model bryophyte species from three clades: liverworts, mosses, and hornworts.

In Chapter 5, I analyzed pathogen transcriptomes during infection of distantly related hosts. I found that conserved transcriptional shifts constitute a core infection program, rather than host-tailored programs. A similar pattern was detected when *M. polymorpha* was infected by diverse *Phytophthora* pathogens, suggesting both plants and pathogens employ ‘master key’ solutions during interactions.

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CHAPTER 2. Materials and Methods

2.1 Plant and pathogen materials

2.1.1 Bryophyte growth conditions

Marchantia polymorpha Tak1 strain (WT) and *Mprarl-13* mutants were propagated via gemmae and grown on half-strength Gamborg's B5 medium (pH 6.7) supplemented with B5 vitamins. *Ceratodon purpureus* GG1 strain was propagated by transferring protonemal tissue to fresh BCDAT medium (Cove et al., 2009). *Anthoceros agrestis* Oxford strain was subcultured by cut thallus pieces and grown on modified KNOP medium (Szövényi et al., 2015). All plants were grown under long day conditions (16 h light/8 h dark) at 22 °C.

2.1.2 *Nicotiana benthamiana* growth conditions

N. benthamiana plants were cultivated in soil under controlled conditions at 22 °C with a long-day photoperiod (16 h light; 160–200 µE) provided by fluorescent lamps (Sylvania F58W/GRO).

2.1.3 *Ceratopteris richardii* growth conditions

C. richardii Hn-n strain gametophytes were cultured on C-fern nutrient medium solidified with 1% agar (pH 6.0) under long-day conditions (16 h light/8 h dark) at 28 °C (Plackett et al., 2015).

2.1.4 Oomycete growth conditions

Phytophthora strains used in this study are listed in Table S1. Each *Phytophthora* strain was maintained by transferring an agar plug onto the appropriate medium and incubated at the temperature specified in Table S1.

2.2 Phylogenetic analyses of extant RAR1 and SGT1

2.2.1 Transcriptome and genome sequence mining

Sequence information of most species used in this study was obtained from SymDB (<https://www.polebio.lrsv.ups-tlse.fr/symdb/web/>). The transcriptome or genome of each species was searched using the hidden Markov model-based software HMMER v3.4 (<http://hmmer.org/>). RAR1 and SGT1 homologs of species not included in SymDB were identified using BLASTp against online databases or local BLASTp searches using NCBI BLAST+ (Camacho et al., 2009) version

2.13.0 with e-value threshold $< 10^{-50}$ (Table S2). Positive hits from the search were examined by reciprocal BLAST using two thoroughly annotated plant genomes, *Arabidopsis thaliana* (flowering plants, TAIR10 at Phytozome (<https://phytozome-next.jgi.doe.gov/>)) and *Marchantia polymorpha* (bryophytes, <https://marchantia.info/>). Following five sequences were used as the queries: MpRAR1 (Mp4g22990.1), AtRAR1 (AT5G51700.1), MpSGT1 (Mp5g04260.1), AtSGT1a (AT4G23570.1), and AtSGT1b (AT4G11260.1). The integrity of RAR1 and SGT1 sequences was examined based on the presence of key domains described by previous studies (Shirasu et al., 1999; Azevedo et al., 2002; Zhang et al., 2010) after multiple sequence alignment by MUSCLE algorithm (Edgar, 2004) version 3.8.1551 using the default settings of SnapGene (<https://www.snapgene.com/>).

2.2.2 Identification of CrRAR1 and CrSGT1 from *C. richardii* cDNA

Total RNA was isolated from flash-frozen *C. richardii* gametophyte tissue using the PureLink Plant RNA Reagent (Invitrogen, 12322012). Genomic DNA was removed by using the TURBO DNA-free kit (Invitrogen, AM1907). First-strand cDNA was synthesized from 2 µg of RNA with SuperScript II Reverse Transcriptase (Invitrogen, 18064014). All procedures from RNA extraction to cDNA synthesis were carried out according to the manufacturers' protocols.

To identify CrSGT1 paralogs, CrSGT1 (from Geng et al., 2021), CrSGT1N (Ceric.25g062300.1), and CrSGT1C (Ceric.1Z100300.1) were amplified along with CrRAR1 (Ceric.17G016600.1) and a fragment of CrACT1 (Ceric.08g028400.1). Amplicons corresponding to CrRAR1 and CrSGT1 were purified with the QIAquick Gel Extraction Kit (QIAGEN, 28704) and subsequently cloned into pDONR221 using Gateway BP Clonase II Enzyme Mix (Invitrogen, 11789020). Five independent entry clones containing either CrRAR1 or CrSGT1 were verified by Sanger sequencing (Genewiz, <https://www.genewiz.com/>) to exclude potential PCR-induced mutations. Primer sequences used for cloning are listed in Table S3.

Validated CrRAR1 and CrSGT1 sequences were used in subsequent phylogenetic analyses and experimental assays. Additionally, CrSGT1 was domesticated to remove BsaI sites and optimized for the codon usage of *A. thaliana*, prior to cloning into a Golden Gate-compatible split GAL4 Ruby system.

2.2.3 Sequence alignment and phylogenetic tree construction

The sets of confirmed RAR1 and SGT1 sequences were aligned using MAFFT v7.505 using the options "--maxiterate 1000 --localpair" (Kato and Standley, 2013). Maximum likelihood trees were constructed based on the alignments using IQ-TREE multicore version 2.3.2 (Nguyen et al., 2014).

The best-fit model found by ModelFinder was “JTTDCMut+I+G4” for RAR1 and “Q.plant+I+G4” for SGT1, according to the Bayesian Information Criterion (BIC; Kalyaanamoorthy et al., 2017). Each full maximum likelihood tree was constructed using the selected best-fit model with 1,000 ultrafast bootstrap replicates (Hoang et al., 2018). The resulting trees were visualized using tvBOT (<https://www.chiplot.online/tvbot.html>) (Xie et al., 2023). Species included in the phylogenetic analyses are listed in Table S2.

2.3 Protein–protein interaction assays

2.3.1 Yeast two-hybrid assay

Coding sequences of *RAR1* and *SGT1* homologs from *Chara braunii* and *Azolla filliculoides*, Cb*RAR1* (CBR_g32001), Cb*SGT1* (CBR_g30800), Af*RAR1* (Azfi_s0014.g013658), and Af*SGT1* (Azfi_s0197.g057330), were synthesized based on their genome annotations. Coding sequences of *RAR1* and *SGT1* homologs of *Hymenophyllum dentatum* and *Osmunda* sp., Hd*RAR1*, Hd*SGT1*, Osp*RAR1*, and Osp*SGT1*, were identified in their transcriptome data (Table S2), codon-optimized for yeast and synthesized. Gene synthesis was performed by TWIST Bioscience (<https://www.twistbioscience.com/>) and prepared as Gateway entry clones. Two *A. thaliana* *SGT1* prey plasmids, pDEST-AD-AtSGT1a (PDEST-AD008B04) and pDEST-AD-AtSGT1b (PDEST-AD013B12) were obtained from the Arabidopsis Biological Resource Center (ABRC; <https://abrc.osu.edu/>).

At*RAR1* and AtSGT1b variants carrying single amino acid polymorphisms were synthesized following the same procedures described above. BsaI domestication of AtSGT1b and point mutations were designed according to the codon usage of *A. thaliana*. AtSGT1b variants carrying multiple single amino acid polymorphisms (AtSGT1b^{EI+IS}, AtSGT1b^{EI+SK}, and AtSGT1b^{EI+IS+SK}) and Cr*RAR1* variant carrying single amino acid polymorphism (Cr^{FI}) were generated by a Golden Gate compatible system. The resulting variants, AtSGT1b^{EI+IS}, AtSGT1b^{EI+SK}, and AtSGT1b^{EI+IS+SK}, and Cr^{FI}, were subcloned into pDONR221 and verified by Sanger sequencing for subsequent yeast-two hybrid assays. Sequences of primers used for the cloning are provided in Table S3.

Expression clones in Gateway-compatible pDEST32 (GAL4 DNA Binding Domain, DBD) and pDEST22 (GAL4 DNA Activation Domain, AD) backbones were generated using the Gateway recombination system (Invitrogen). The bread yeast (*Saccharomyces cerevisiae*) strain MaV203 was used for yeast-two hybrid assays. Synthetic complete (SC) medium was prepared from yeast nitrogen base without amino acids (Formedium, CYN0405), agar (Formedium, AGA03), glucose (Fisher, G/0500/53), and SC amino acid drop-out mix (-Leu -Trp, Formedium DSCCK172; -Leu -Trp -His, Formedium DSCCK242). For stringent His auxotrophic selection, 15 mM of 3-amino-1,2,4-triazole

(Sigma-Aldrich, A8056) was supplemented to SC -Leu -Trp -His plates. For URA3 counter-selection, SC -Leu -Trp medium was supplemented with 1 mg/ml 5-Fluoroorotic Acid (Fisher, R0811) with 1 µg/ml final concentration. Growth of yeasts on selective and counter-selective plates were assessed at 3 days after inoculation. The strength of protein-protein interaction (PPI) was estimated based on manufacturer's protocol (Table 2.1).

Table 2.1 Estimation of PPI strength in *S. cerevisiae* MaV203 system.

Colony growth		Strength of PPI
His selection media (SC -LWH + 3-AT)	Ura counter selection media (SC -LW + 5-FOA)	
Yes	No	Strong interaction
Yes	Yes	Weak interaction
No	Yes	No interaction

2.3.2 Split GAL4 Ruby assay

Split GAL4 Ruby assay was used to quantify RAR1-SGT1 interaction *in planta*. *RAR1* and *SGT1* wild type coding sequences and variants were amplified by PCR using primers containing BsaI cloning sites to be cloned into level 2 Golden Gate-compatible acceptors for split GAL4 Ruby assay, pICSL22026 (TSL SynBio) and pICSL22027 (TSL SynBio; containing the Ruby expression cassette). Purified SGT1 fragments or the GUS control (Addgene #50327), were assembled with one of two promoters, pICH85281 (Promoter mas, (*Agrobacterium tumefaciens*) + 5' UTR, Ω (Tobacco Mosaic Virus), Addgene #50272) or pICH87633 (Promoter Nos, (*A. tumefaciens*) + 5' UTR, Ω (Tobacco Mosaic Virus), Addgene #50271), pICSL50065 (GAL4DBD; TSL SynBio), and pICH41414 (35S terminator, AddGene #50337) into pICSL22026. Purified RAR1 fragments or the GUS control (Addgene #50327), were assembled with one of two promoters, pICSL13004 (Promoter mas, (*A. tumefaciens*) + 5' UTR, Ω (Tobacco Mosaic Virus), TSL Synbio) or pICSL13010 (Promoter Nos, (*A. tumefaciens*) + 5' UTR, Ω (Tobacco Mosaic Virus), TSL Synbio), pICSL30055 (VP16 activation domain; TSL SynBio), and pICH41414 (35S terminator, AddGene #50337) into pICSL22027. The final constructs were verified by Sanger sequencing.

The plasmids for split GAL4 Ruby assays were electroporated into *A. tumefaciens* GV3101. For infiltration, equal amounts of three independent *A. tumefaciens* cultures carrying the *RAR1* construct, the *SGT1* construct, and p19 driven by promoter 35S were mixed at a 1:1:1 ratio to the desired final OD₆₀₀. Final OD₆₀₀ was set to 0.1 for mas–mas combinations and 0.5 for Nos–Nos combinations. Prior to syringe infiltration, the mixed *A. tumefaciens* cultures were incubated in induction buffer (10 mM MES pH 5.6, 10 mM MgCl₂, and 150 µM acetosyringone) for 1 h in the dark at 4 °C. Leaves of

4-week-old *N. benthamiana* plants were infiltrated with *A. tumefaciens* mixtures carrying binary expression plasmids.

Quantification of betalain accumulation was performed as previously described (Chen et al., 2023) with minor modifications as follows. *Agrobacterium*-infiltrated *N. benthamiana* leaves were harvested and bleached overnight in 100% ethanol. An 8 mm-diameter leaf disc was excised from the centre of each infiltration site. For each *RAR1*–*SGT1* combination, leaf discs were collected from six independent leaves and pooled. The pooled discs were extracted overnight in 0.6 mL of water. Betalain absorbance of the clean supernatant was measured at 538 nm wavelength using a spectrophotometer (CLARIOstar Plus, BMG Labtech) with three technical replicates.

2.4 Structural and computational analyses

2.4.1 Protein structure modelling and visualization

The amino acid sequence of AtRAR1, AtSGT1b, CrRAR1, and CrSGT1 was obtained from the dataset used in prior phylogenetic analyses. Protein structure predictions were performed using AlphaFold2 (Jumper et al., 2021), via the open-source platform ColabFold (Mirdita et al., 2022). All predicted RAR1–SGT1 binding interfaces were visualized by Chimera X (Pettersen et al., 2020). To assess model quality, the predicted AtRAR1–AtSGT1b binding interface was overlaid with the crystal structure of the AtRAR1–AtSGT1a binding interface (PDB: 2XCM).

2.4.2 Ancestral state reconstruction

Based on aligned 60 fern SGT1 sequences and an outgroup species bearing a single-copy SGT1, the bryophyte *M. polymorpha*, a fern SGT1 phylogenetic tree was created using IQ-TREE multicore version 2.3.2 (Nguyen et al., 2014). The “MG+F3X4+R5” model was chosen by ModelFinder as the best-fit model according to the BIC (Kalyaanamoorthy et al., 2017). The maximum likelihood tree for ancestral state reconstruction was built with 1,000 ultrafast bootstrap replicates (Hoang et al., 2018). To generate a codon-based nucleotide sequence alignment input, the nucleotide sequences were threaded onto the amino acid alignment using phykit v 2.0.1 (Steenwyk et al., 2021), and the resulting codon-based alignment was verified by back-translation of each sequence. Ancestral state reconstruction was conducted using the CODON model of the “ancseq” pipeline (<https://github.com/YuSugihara/ancseq>; Sugihara et al., 2025). Briefly, the pipeline integrates IQ-TREE’s empirical Bayes method with a Jukes-Cantor type binary model to reconstruct both ancestral sequences and indels. The results were visualized in iTOL v6 (Letunic and Bork, 2024) with annotated node numbers.

2.5 Infection assays

2.5.1 Agar plug infection assay

For agar plug assay, agar discs (4.762 mm in diameter) were collected from the actively growing edge of 1-week-old cultures. Four *Phytophthora* agar discs were placed per plant, around the dichotomous branching points of each 24-day-old *M. polymorpha* thalli (Figure 2.1). Eight *M. polymorpha* plants were used for each *Phytophthora* strain. Disease phenotypes were scored at 7 dpi (days after infection) on a subjective 0-3 scale, from 0 (no infection) to 3 (strong infection) (Figure 2.2). Quantified disease phenotypes were visualized by GraphPad Prism version 9.0.1.

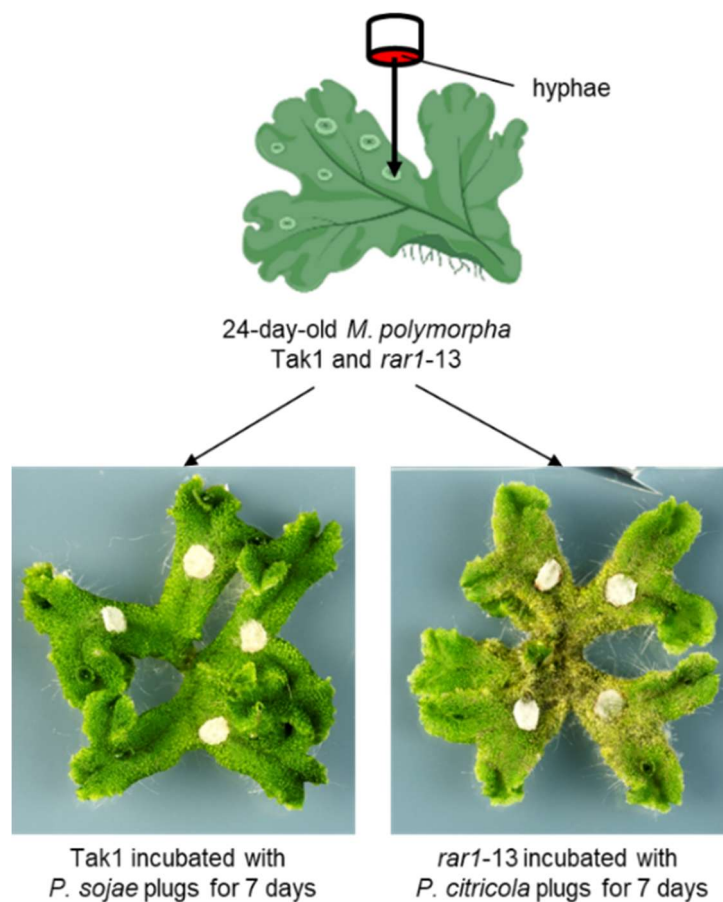


Figure 2.1 Schematic description of the agar plug assay. Agar plugs were excised from the actively growing edge of a 1-week-old culture plate.

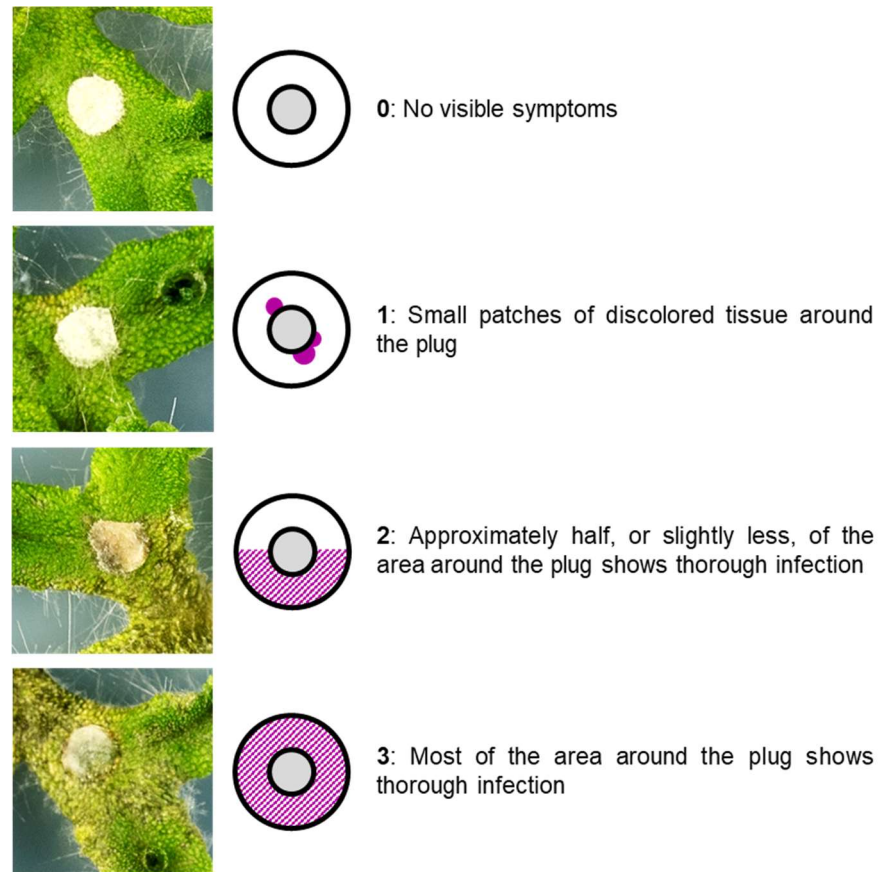


Figure 2.2 Subjective scoring scale used in the agar plug assay. Disease phenotypes were quantified on a scale from 0 to 3, where 0 = no infection, 1 = weak infection, 2 = moderate infection, and 3 = strong infection.

2.5.2 Infection assays for time-course RNA-seq

Phytophthora palmivora ARI-td was cultivated on V8 medium at 25 °C and maintained by zoospore inoculation. *P. palmivora* ARI-td zoospores were collected as follows. Briefly, 1-week-old cultures were flooded with 4 °C distilled water, incubated at 4 °C for 30 minutes, then at room temperature for 30 minutes (Le Fevre et al., 2016). For infection assays, a single 10 µl droplet of zoospore suspension (2×10^5 zoospores per ml) was inoculated at the center of 2-week-old *C. purpureus* protonemal colonies and on thalli of 3-week-old *A. agrestis*. Pathogen growth was monitored by epifluorescence microscopy using a Zeiss Axio Zoom V16 II with RFP filter. Images were processed with Zeiss ZEN Blue software.

For the second set, agar plugs from the actively growing edge of *Phytophthora capsici* LT1534, *Phytophthora cactorum* Pc10300, and *Phytophthora parasitica* 310 were inoculated on thalli of 24-day-old *M. polymorpha* and leaves of 4-week-old *N. benthamiana* plants, with the mycelium side of the plug in direct contact with the plant tissue.

2.6 Infection time-course RNA-seq and data analysis

2.6.1 RNA isolation, library preparation and sequencing

mRNA was purified from DNase-treated total RNA (as described above) of mock-inoculated (water) or *P. palmivora* ARI-td-infected *C. purpureus* and *A. agrestis* at 1, 2, 3 and 4 dpi using poly(A) selection, and then fragmented. For the second set, samples were from mock-inoculated (plain medium plug) or *Phytophthora*-infected (agar plug with pathogen) *M. polymorpha* and *N. benthamiana* at 4 dpi. cDNA library preparation and sequencing were performed by Genewiz (UK). Briefly, cDNA libraries were prepared using the TruSeq® RNA Sample Preparation Kit (Illumina) following the manufacturer's instructions. Sequencing was performed on the Illumina NovaSeq X in 150 bp paired-end mode. De-multiplexed samples were used for downstream expression analyses. Raw fastq data will be made available at NCBI-SRA.

2.6.2 RNA-sequencing read processing and gene expression analysis

Raw sequencing reads were quality-checked using FastQC v0.11.9 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Processed reads were aligned to reference coding sequences of the species: *M. polymorpha* (MpTak1_v5.1; https://marchantia.info/about_genome/; Montgomery et al., 2020), *C. purpureus* (GG1 v1.1; https://phytozome-next.jgi.doe.gov/info/CpurpureusGG1_v1_1; Carey et al., 2021), *A. agrestis* (Oxford; <https://www.hornworts.uzh.ch/en.html>; Li et al., 2020), and *N. benthamiana* (NbLAB; <https://bioweb01.qut.edu.au/benthTPM/download.html>; Ranawaka et al., 2023), using HiSAT2 (Kim et al., 2019). Differentially expressed genes (DEGs) were analyzed using a pairwise approach with all data, using DESeq2 (Love et al., 2014). DEGs passed a strict threshold (absolute log2 fold change (LFC) ≥ 2 , and adjusted *P*-value $\leq 10^{-3}$) with an exception for *A. agrestis*, whose DEGs were screened with LFC ≥ 1.5 and the same adjusted *P*-value cutoff. when performing hierarchical clustering of samples. Heatmaps of DEGs were generated for each plant-pathogen combination using the R package pheatmap (<https://www.rdocumentation.org/packages/pheatmap/versions/1.0.12>), with variance stabilized counts.

2.6.3 PR gene identification

Putative PR genes in the *C. purpureus* and *A. agrestis* genomes were predicted from the annotated proteomes by profiling PR family-specific domain signatures (Table 2.2) using HMMER v3.4. For cross-reference, the same set of Pfam motif queries were run on *A. thaliana* (Araport11; https://phytozome-next.jgi.doe.gov/info/Athaliana_Araport11; Cheng et al., 2016) and rice (*Oryza*

sativa) (IRGSP release 7; <https://rice.uga.edu/>; Kawahara et al., 2013) proteomes to compare domain structures with well-characterized PR proteins from these species.

Candidates identified as PR-4 were further classified by domain structure: class I when a Barwin domain (PF00967) co-occurred with a hevein-like chitin-binding domain (PF00187) on the same polypeptide, or class II when only the Barwin domain was found. For each PR family, preliminary HMM hits were filtered based on two criteria: protein sequence alignment by MUSCLE (Edgar, 2004), and maximum-likelihood trees generated by FastTree 2 (Price et al., 2010) including PR proteins from *A. thaliana* and rice.

Table 2.2 List of Pfam motifs to identify PR genes by HMMER profiling

Pfam motif	Description	PR family
PF00188	Cysteine-rich secretory protein family	PR1
PF00332	Glycosyl hydrolases family 17	PR2
PF00182	Chitinase class I (GH19)	PR3
PF00967	Barwin family	PR4
PF00187	Chitin recognition protein (CBM18/hevein-like)	PR4
PF00314	Thaumatococcus family	PR5
PF00280	Potato type I proteinase inhibitor family	PR6
PF02428	Potato type II proteinase inhibitor family	PR6
PF00014	Kunitz/Bovine pancreatic trypsin inhibitor domain	PR6
PF00228	Bowman-Birk serine protease inhibitor family	PR6
PF00079	Serpin (serine protease inhibitor)	PR6
PF00082	Subtilase	PR7
PF00704	GH18 (chitinase)	PR8
PF00141	Peroxidase	PR9
PF00407	Pathogenesis-related protein Bet v 1 family	PR10
PF00304	γ -thionin family (plant defensin)	PR12
PF00321	Plant thionin	PR13
PF00234	Protease inhibitor/seed storage/LTP family	PR14
PF00190	Cupin	PR15/16
PF04450	Peptidase of plants and bacteria	PR17

2.6.4 Gene enrichment analysis

GO annotations of *M. polymorpha* v7.1 genome were obtained from Ensembl Biomart (<https://www.ensembl.org/info/data/biomart/index.html>). For the test set, the 436 genes commonly upregulated at 4 dpi across the four *Phytophthora* infections with the same DEG cutoff described above were used. Enrichment analysis was performed with clusterProfiler v.4.16.0 (Yu et al., 2012; Wu et al., 2021) using a hypergeometric test and Benjamini–Hochberg correction (False Discovery Rate < 0.05). Terms were ranked by adjusted p value and presented the top 20 enriched terms (covering 157 genes) in the main figure.

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CHAPTER 3. Lineage-specific divergence of RAR1–SGT1 binding interface in ferns

3.1 Introduction

Precise protein interactions are essential for a variety of key cellular processes across the tree of life. Protein–protein interactions are often stabilized by coevolution at their binding interfaces, ensuring functional compatibility while lineages diversify (Lovell and Robertson, 2010). Consistent with this, many studies have demonstrated that coevolving residues are located at binding interfaces and spatially coupled (Liu et al., 2007; Yeang and Haussler, 2007; Skerker et al., 2008). Because of this pervasive signal, coupled residues have even been used to predict unknown protein–protein interactions (PPIs) and reconstruct protein networks *in silico* (Hopf et al., 2012; Green et al., 2021).

3.1.1 Chaperones and co-chaperones

Chaperones provide robustness against environmental perturbations by buffering protein folding and stability (Jarosz et al., 2010). Core chaperones, such as heat shock proteins (HSPs), assist client proteins by maintaining their native conformations through correct folding and refolding (Kim et al., 2013). Their functions are fine-tuned by co-chaperones, which act as non-client partners that redirect or modulate chaperone capacity toward specific molecular and cellular processes. Co-chaperones achieve this by two modes of action: chaperone–client coupling (e.g. SGT1, Cdc37, and Hop) and regulating ATPase cycles of core chaperones (e.g. RAR1, Aha1, and p23) (Caplan, 2003; Jackson et al., 2004; Shirasu, 2009; Taipale et al., 2010). In this context, binding interfaces within the chaperone complex, whether between core chaperone and co-chaperones or between co-chaperones themselves, must preserve PPIs, although residues in the binding interface sometimes diverge during evolution. This mandatory balance between divergence of residues and consistency of PPI makes chaperone complexes valuable systems to study molecular coevolution of interacting proteins, especially when their PPIs can be assayed directly *in vitro* or when functional outputs of the disturbed PPI are readable.

3.1.2 RAR1–SGT1–HSP90 chaperone complex in plant immunity

RAR1 and SGT1 are conserved co-chaperones that form a robust complex in flowering plants, where their interaction contributes to immune responses by stabilizing NLR proteins (Shirasu, 2009). This interaction has been demonstrated across multiple angiosperm species and experimental systems (Wang et al., 2008; Fu et al., 2009; Cantu et al., 2013; Tai, 2015; Yuan et al., 2021; Wang et al., 2024). Furthermore, due to its reproducibility, AtRAR1–AtSGT1 interaction in *Arabidopsis thaliana* is

frequently used as a benchmark in evaluating novel *in planta* PPI assays (Chen et al., 2008; Chen et al., 2023). Consistent with their role in plant immunity and nonhost resistance, disturbance of the RAR1–SGT1 interaction provides a clear functional readout: *rar1* and *sgt1* mutants in angiosperms consistently show enhanced disease susceptibility to non-adapted pathogens. These readouts can be quantified by measuring symptom or pathogen growth (Shirasu et al., 1999; Austin et al., 2002; Muskett et al., 2002; Tör et al., 2002; Liu et al., 2002a, 2002b). Their importance in immunity and co-evolutionary relationships makes RAR1 and SGT1 an important and interesting system to dissect the history of their PPI during land plant evolution. However, the evolutionary history of the RAR1–SGT1 interaction outside flowering plants remains unexplored.

Land plants (Embryophyta) are a monophyletic clade that includes many familiar flowering and non-flowering plants. This group comprises several major lineages, including bryophytes (non-seed and non-vascular plants), lycophytes and monilophytes (non-seed and vascular plants), and vascular seed plants including gymnosperms and angiosperms (One Thousand Plant Transcriptomes Initiative, 2019). Despite the growing availability of genome sequences from non-flowering plants, only one *RAR1* homolog has been described outside angiosperms, Pp*RAR1* in the model moss *Physcomitrium patens*. Pp*RAR1* has a conserved domain structure, but its functional interaction with SGT1 from the same species has not yet been tested (Shirasu, 2009; Mayorga-López et al., 2018). SGT1 homologs have been functionally characterized in yeast and human (Kitagawa et al., 1999; da Silva Correia et al., 2007), and *SGT1* from *A. thaliana* rescues yeast *sgt1* mutants (Azevedo et al., 2002). Yet no *SGT1* homologs from non-flowering plants have been identified or functionally studied, leaving a major gap in understanding RAR1–SGT1 coevolution in land plants.

3.1.3 Aims of this chapter

RAR1 and SGT1 provide an ideal platform to study the molecular coevolution of interacting proteins in land plants: both genes are conserved between distantly related species, and their binding interface is well-established in angiosperms. By comparing phylogenetic relationships, and interaction properties across major plant lineages, it becomes possible to trace how this conserved complex evolved (Figure 3.1). In this chapter, I show that *RAR1*, *SGT1*, and the RAR1–SGT1 interaction are broadly conserved in land plants while a clade of vascular non-seed plants, monilophytes (ferns), has experienced an atypical divergence of the RAR1^{CHORD II}–SGT1^{CS} binding interface through a promiscuous intermediate state.

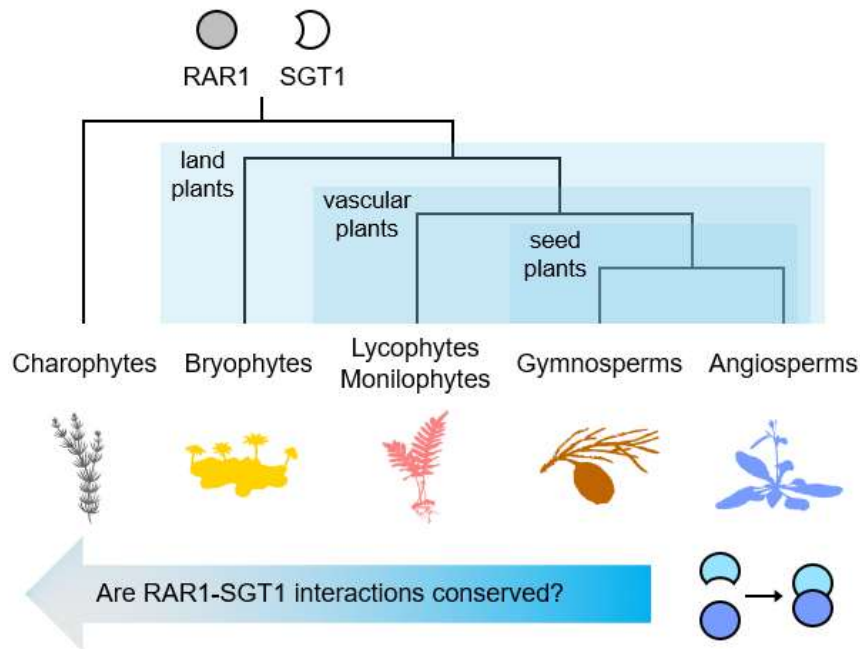


Figure 3.1 Cartoon about conservation of *RAR1*, *SGT1* and RAR1–SGT1 interaction.

3.2 Results

3.2.1 Identifying *RAR1* and *SGT1* in land plants

Genes encoding *RAR1* and *SGT1* with intact domain structures have been identified in many angiosperm species and in *P. patens* (Pp*RAR1*) (Azevedo et al., 2002; Wang et al., 2008; Fu et al., 2009; Shirasu, 2009; Cantu et al., 2013; Tai, 2015). However, a large gap between angiosperms and *P. patens* remains, particularly in non-flowering plants. To address this, I first collected protein sequences of *RAR1* and *SGT1* homologs from 364 species across diverse land plants and streptophyte algae using BLAST and pHMMER. In total, I identified 330 *RAR1* homologs from 330 species (6 algae and 324 land plants) and 419 *SGT1* sequences from 333 species (5 algae and 328 land plants), respectively (Table S2).

3.2.2 *Ceratopteris richardii* genome encodes a single copy of *RAR1* and *SGT1*

Ceratopteris richardii is a model fern species with chromosome-scale genome assembly (Marchant et al., 2022). Recent studies have incorporated *C. richardii* as a model fern for evo-MPMI research, demonstrating that it encodes functionally conserved NLR N-terminal domains and that it is susceptible to broad host bacterial pathogen *P. syringae* (Grenz et al., 2025). The search for *RAR1*–*SGT1* homologs in *C. richardii* (reciprocal BLAST with *A. thaliana* *RAR1* and *SGT1* homologs as queries) identified one Cr*RAR1* and two fragmented Cr*SGT1* homologs, designated Cr*SGT1N* and

CrSGT1C. SGT1 proteins typically consist of three domains: an N-terminal TPR domain, a central CS domain, and a C-terminal SGS domain. CrSGT1N encodes the TPR domain and CS domain, whereas CrSGT1C encodes the SGS domain. Interestingly, an intact CrSGT1 homolog containing all three domains was identified from an independently performed *de novo* transcriptome assembly (Geng et al., 2021).

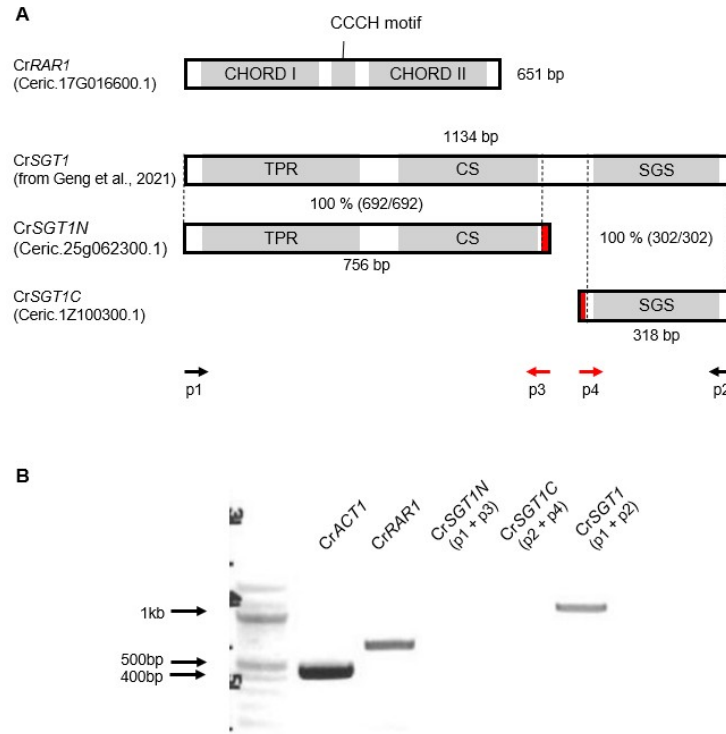


Figure 3.2 Cloning of CrRAR1 and CrSGT1 from *C. richardii* cDNA. **(A)** Schematic representation of putative CrRAR1 and CrSGT1 coding sequences. Red patches indicate unique region of CrSGT1N and CrSGT1C. Primers (p1–p4) were designed on either common (p1 and p2) or unique (p3 and p4) sequences. **(B)** RT-PCR analysis using a *C. richardii* gametophyte cDNA. The RT-PCR forward primer for CrACT1 (Ceric.08g028400.1) was adopted from Plackett et al., (2018), and the reverse primer was designed based on the *C. richardii* v2.1 genome (Phytozome genome ID: 676 • NCBI taxonomy ID: 49495).

The three putative CrSGT1 homologs show nearly complete sequence homology, except that the variable region between the CS domain and the SGS domain is absent in both CrSGT1N or CrSGT1C (Figure 3.2A). Considering their nearly 100% sequence homology, and the fact that CrSGT1C is not annotated on a chromosome unlike CrSGT1N, CrSGT1N and CrSGT1 may be the result of misannotation in the genome assembly. To test this, primers were designed to amplify the intact

CrSGT1, CrSGT1N, and CrSGT1C from *C. richardii* frond cDNA. Consistent with my hypothesis, CrSGT1N and CrSGT1C were not detected while transcripts for CrACT1, CrRAR1, and an intact CrSGT1 were successfully amplified (Figure 3.2B). In summary, *C. richardii* encodes a single copy of RAR1 and SGT1.

3.2.3 Phylogenetic analyses of RAR1 and SGT1 across land plants

Next, I performed phylogenetic analyses of RAR1 and SGT1, using streptophyte algal homologs as outgroups. In both phylogenies, algal homologs were not clustered with other land plant homologs as a previous study about RAR1 (Mayorga-López et al., 2018). Both RAR1 and SGT1 phylogenies mirrored known species relationships in most clades (Figure 3.3–4).

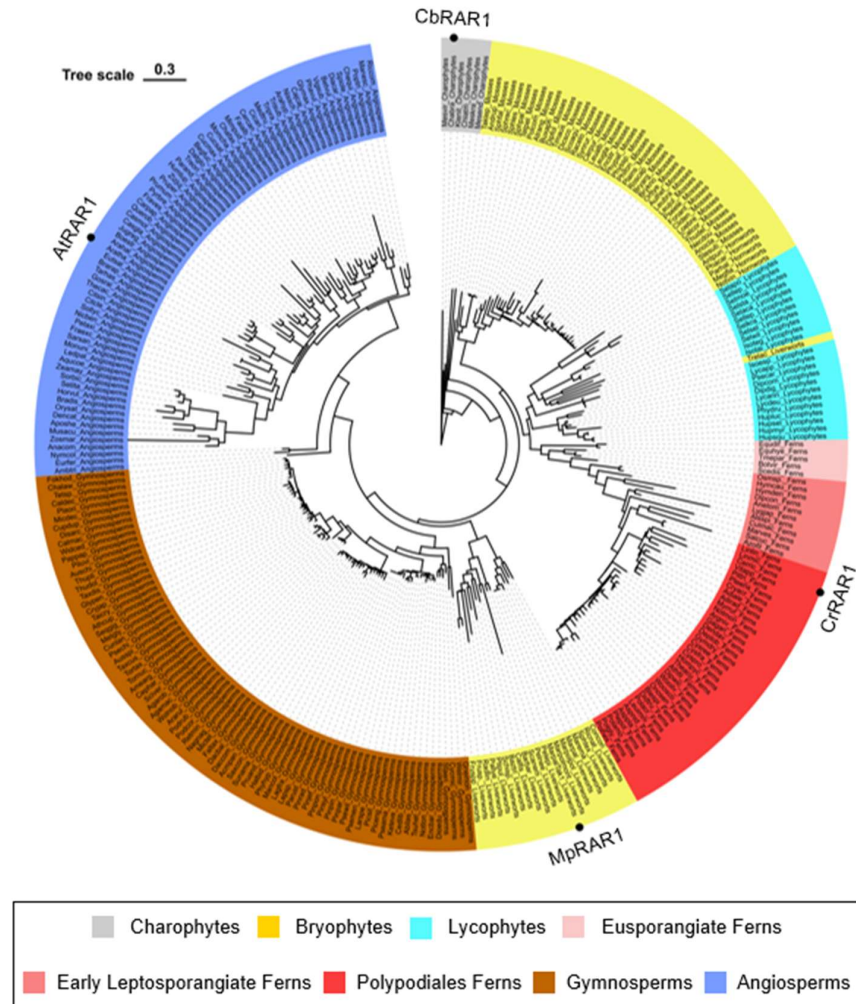


Figure 3.3 Maximum likelihood phylogenetic trees of *RAR1* across land plants. Dot marks indicate species selected for yeast two-hybrid assays shown in Figure 3.6.

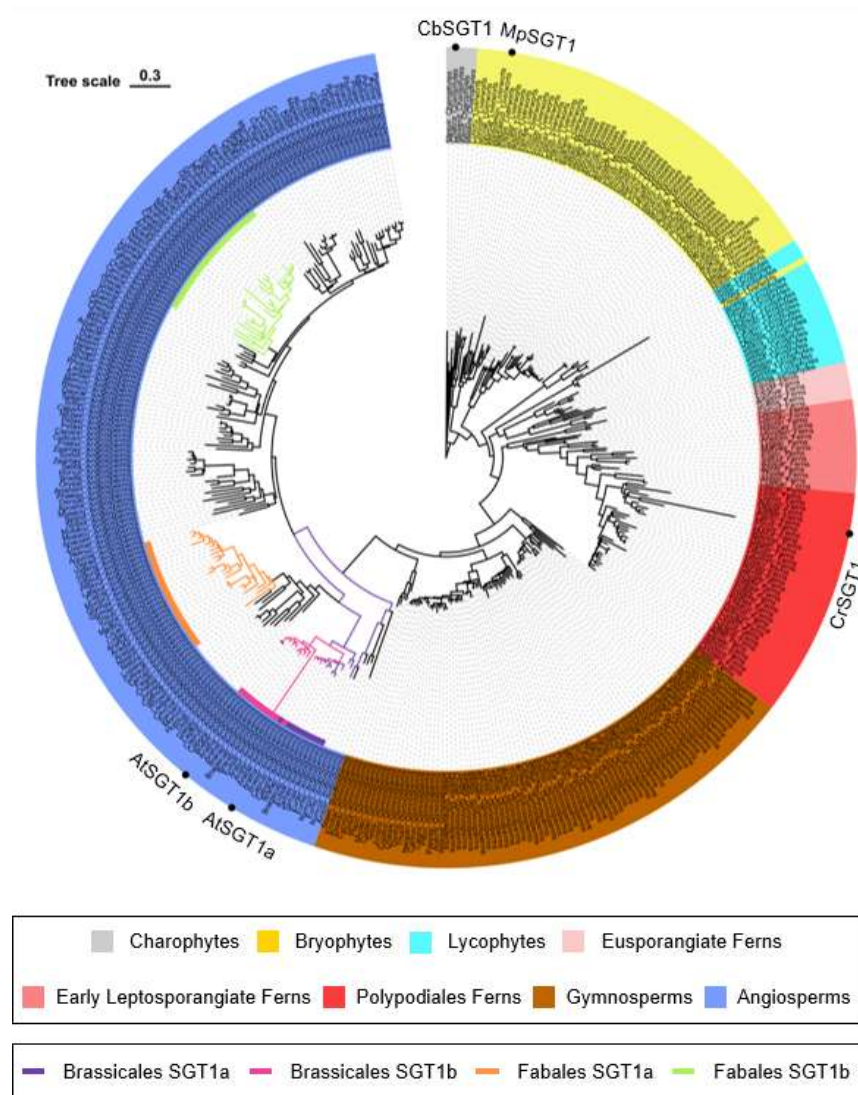


Figure 3.4 Maximum likelihood phylogenetic trees of *SGT1* across land plants. Dot marks indicate species selected for yeast two-hybrid assays shown in Figure 3.6.

Interestingly, the evolutionary trajectories of RAR1 and SGT1 differed in certain taxonomic groups. In the case of RAR1, branch lengths were notably shorter in gymnosperms, Polypodiales ferns, and a small subset of mosses compared to other groups (Figure 3.3). Although the same pattern was less clear in the SGT1 phylogeny, gymnosperm homologs again displayed shorter branch lengths than other taxa (Figure 3.4).

Unexpected clustering patterns were also observed. Homologs from hornworts were grouped with lycophyte homologs rather than other bryophytes (mosses and liverworts), which is consistent with the view that liverworts and mosses are more closely related in bryophytes and form the Setaphyta clade (Puttick et al., 2018; Donoghue et al., 2021). The leafy liverwort *Treubia lacunosa* was

consistently clustered together with lycophytes in both *RAR1* and *SGT1* phylogeny. Notably, *RAR1* homologs of liverworts were placed between gymnosperms and ferns while their *SGT1* homologs grouped with other bryophyte clades.

3.2.3.1 *SGT1* is expanded in angiosperms

Consistent with the known duplications of *SGT1* in *A. thaliana* and other Brassicaceae model species, I observed higher numbers of *SGT1* copies in several clades, particularly in angiosperms. In contrast, *RAR1* was always present as a single copy gene in every species. More than half of the angiosperm species in the dataset had at least two *SGT1* paralogs, whereas the ratio was much lower in all other clades and even liverworts and mosses persistently have a single copy of *SGT1*. Multiplication events (three or more *SGT1* paralogs) occurred exclusively in angiosperms (Figure 3.5A, Table 3.1).

Two specific orders in angiosperms, Fabales and Brassicales, showed especially high frequencies of expansion (Figure 3.5B, Table 3.1). The pattern was more dramatic in Fabales; for example, soybean (*Glycine max*) encodes five annotated *SGT1* paralogs with one *RAR1* homolog. These results suggest that while *RAR1* is constrained as single-copy gene across land plants, *SGT1* has undergone lineage-specific expansions in angiosperms.

Table 3.1 Number of species with different *SGT1* copy numbers across land plant lineages.

Clade	Counts of species with <i>SGT1</i> paralog copy numbers			Total
	1	2	3 or more	
Hornworts	6	1	0	7
Liverworts	22	0	0	22
Mosses	41	0	0	41
Lycophytes	18	1	0	19
Monilophytes	52	5	0	57
Gymnosperms	82	1	0	83
Angiosperms	55	52	11	118
– Brassicales	0	9	1	10
– Fabales	2	17	4	23
– Other angiosperms	53	26	6	85

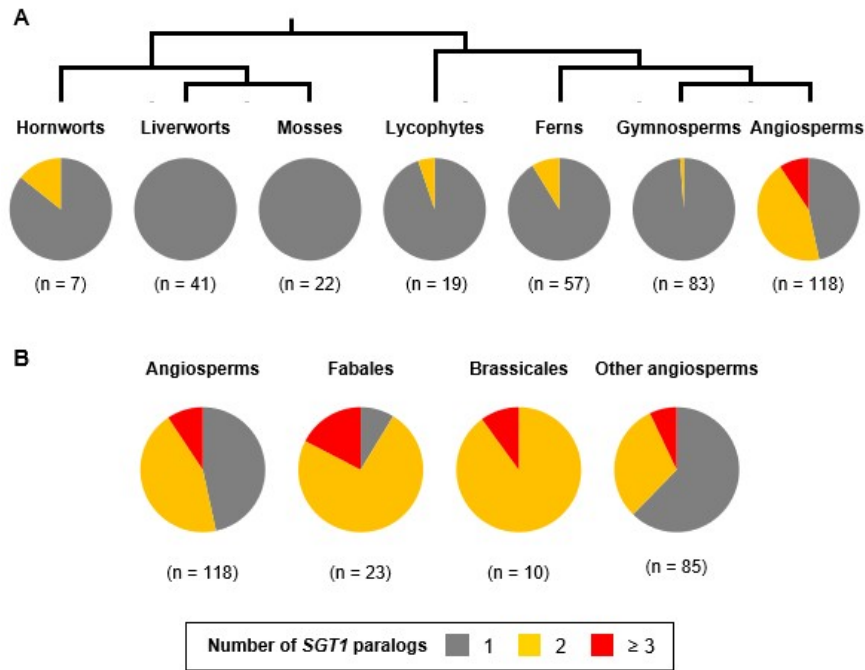


Figure 3.5 Number of *SGT1* paralogs varies by clade in land plants. Pie charts show the proportion of species with different *SGT1* copy numbers in **(A)** major land plant clades and **(B)** selected angiosperm clades. The actual counts of species in each category are provided in Table 3.1.

3.2.4 RAR1–SGT1 interaction is conserved across land plants

To assess whether the RAR1–SGT1 interaction is conserved in land plants and streptophyte algae (collectively streptophytes), I chose four representative species with accessible genome resources from diverse lineages for yeast two-hybrid (Y2H) assays: the charophyte alga *Chara braunii*, the bryophyte *Marchantia polymorpha* (non-vascular and non-seed plant) and the fern *C. richardii* (vascular and non-seed plant). In angiosperms (vascular seed plants), *A. thaliana* was chosen as its RAR1 and SGT1 are the most studied homologs. *A. thaliana* harbors two *SGT1* paralogs, *AtSGT1a* and *AtSGT1b*, and both were included in my initial PPI analyses.

Y2H assays were used given the historical utility in characterizing RAR1–SGT1 interactions in angiosperms, including cross-species RAR1–SGT1 interactions (Azevedo et al., 2002; Liu et al., 2002b; Wang et al., 2008; Fu et al., 2009; Cantu et al., 2013). Yeast colony growth on interaction-specific (His auxotrophy) plates showed that the RAR1–SGT1 interactions were conserved in these representative species, while empty vector (EV) controls failed to interact (Figure 3.6A). The same pattern was observed on Ura counter-selection plates that every RAR1–SGT1 interaction observed in His auxotrophic selection plate failed to grow on the counter-selection (Figure 3.6B). RAR1–SGT1 interactions between homologs of distantly related species, including mixed RAR1–SGT1 pairs from the alga *C. braunii*, liverwort *M. polymorpha*, and angiosperm *A. thaliana* were also

observed. For instance, homologs between *C. braunii* and *A. thaliana* interacted, indicating strong conservation of both genes across streptophytes. Interestingly, CbRAR1 failed to interact with MpSGT1 while MpRAR1 and AtRAR1 could.

Notably, the fern *C. richardii* CrRAR1 failed to interact with SGT1 homologs outside of its species. Moreover, CrSGT1 also displayed unique incompatibility compared to other SGT1 homologs, as it was the only SGT1 homolog that failed to interact with AtRAR1. Together, these results suggest that the RAR1–SGT1 interaction is highly conserved across land plants and even streptophytes but has diverged to become highly specific in the fern *C. richardii*.

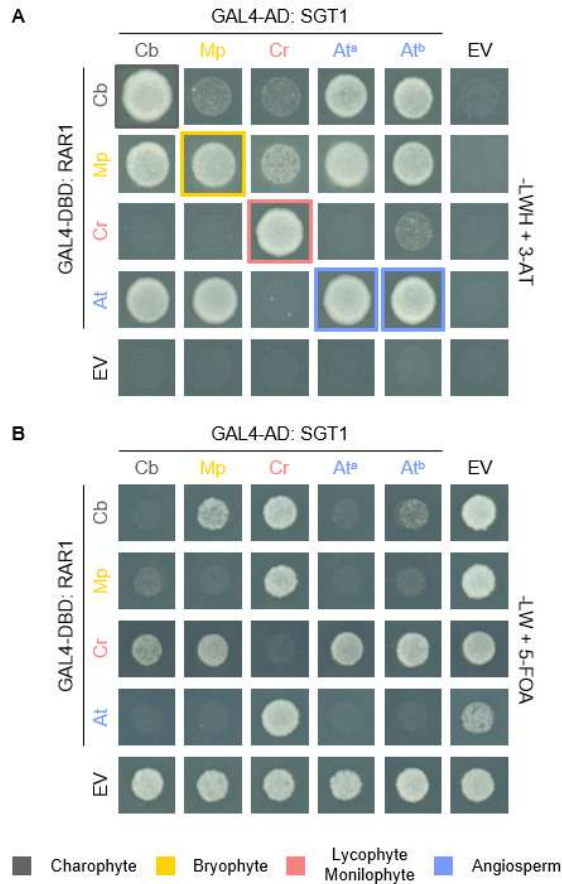


Figure 3.6 RAR1–SGT1 interaction is conserved across streptophyte lineages. Yeast two-hybrid assays testing interactions between RAR1 and SGT1 from the representative species: from *Chara braunii* (Cb, gray), *Marchantia polymorpha* (Mp, yellow), *Ceratopteris richardii* (Cr, salmon pink), and *Arabidopsis thaliana* (At, blue). At^a and At^b correspond to AtSGT1a and AtSGT1b, respectively. Each interaction was tested in two to four independent replicates with similar results. **(A)** Growth on His selection plates. **(B)** Growth on Ura counter-selection plates. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting.

3.2.5 A single mutation in the CHORD II domain of CrRAR1 determined the incompatibility

The interaction between AtRAR1 and AtSGT1a is mediated by the CHORD II domain of AtRAR1 and the CS domain of AtSGT1a (Azevedo et al., 2002; Zhang et al., 2010). I therefore hypothesized that the specificity of CrRAR1 is a consequence of sequence variation within its CHORD II domain. To test this, I generated two chimeric RAR1 proteins that swap the CHORD II domain of CrRAR1 and AtRAR1 and examined their interactions by Y2H assays (Figure 3.7A).

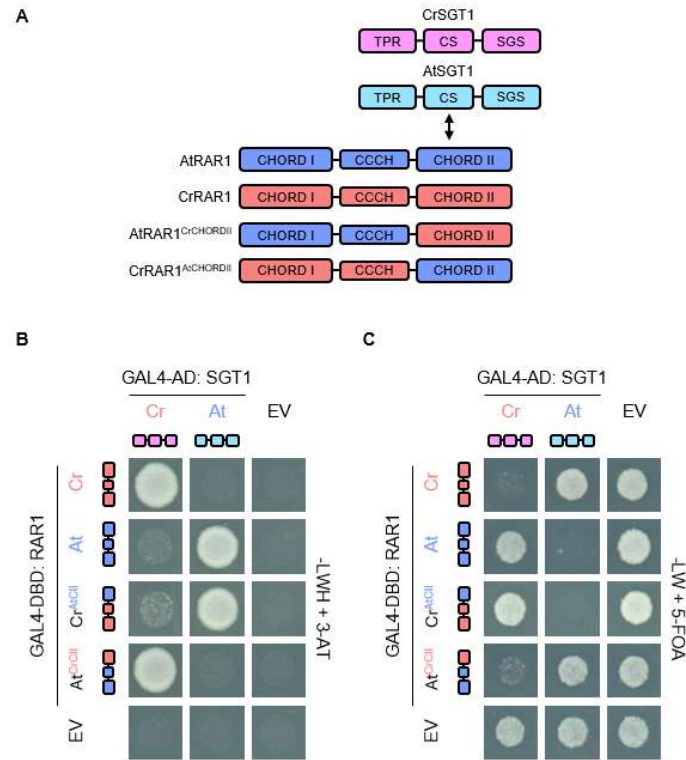


Figure 3.7 The CHORD II domain of RAR1 determines the incompatibility between *C. richardii* and *A. thaliana* homologs. (A–C) CHORD II domain swapping between CrRAR1 and AtRAR1 (A) Schematic explaining two chimeric RAR1 proteins (AtRAR1^{CrCHORD II} and CrRAR1^{AtCHORD II}) (B–C) Yeast two-hybrid assays testing interactions of the chimeric proteins. Each interaction was tested in two to four independent replicates with similar results. (B) Growth on His selection plates. (C) Growth on Ura counter-selection plates. AtRAR1^{CrCH} = AtRAR1^{CrCHORD II}; CrRAR1^{AtCH} = CrRAR1^{AtCHORD II}. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting.

As positive controls, CrRAR1–CrSGT1 and AtRAR1–AtSGT1b interaction were included together with combinations of the chimeric proteins. Domain swapping resulted in reciprocal incompatibility, where CrRAR1^{AtCHORD II} interacted only with AtSGT1b and AtRAR1^{CrCHORD II} interacted only with CrSGT1 (Figure 3.7B). No differences in interaction strength were observed as the yeasts with

interacting RAR1 and SGT1 did not grow on Ura counter-selection plates (Figure 3.7C). Together, these results confirm that the incompatibility is determined by CHORD II domain identity.

I further analyzed CHORD II domain sequences from representative plant and algal species, including five additional fern species with available genome sequences, to identify single amino acid polymorphisms (SAPs) specific to ferns. Amino acid sequence alignments revealed six fern-specific differences within the CHORD II domain: Ile153Phe (I153F), Lys162 Δ 2 (K162 Δ 2), Gly165Lys (G165K), Arg171Ser (R171S), Ala185Pro (A185P), and Asp198Asn (D198N), designated based on comparisons between AtRAR1 and CrRAR1 sequences (Figure 3.8). Two of these variants, I153F and R171S, occur in AtSGT1-interacting residues of AtRAR1 that were previously resolved by crystal structure (Zhang et al., 2010: PDB; 2XCM), whereas the four other variants did not (Figure 3.8). Moreover, previous studies have shown that AtRAR1–AtSGT1a interaction is greatly reduced when the Ile153 residue of AtRAR1 is mutated to Glu (Zhang et al., 2010). For this reason, the I153F mutation was included into the set of ‘fern-specific differences’, although it was found only in three species out of six analyzed fern species. In addition, two other critical residues in AtRAR1–AtSGT1a interactions, Glu170 and Trp217, are conserved across all homologs including CrRAR1.

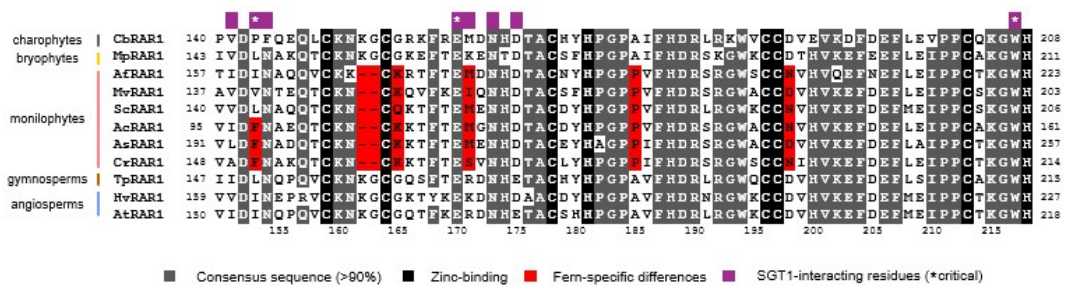


Figure 3.8 CHORD II domain of fern RAR1 homologs harbors six lineage-specific differences. Amino acid sequence alignment of CHORD II domains from representative RAR1 homologs. Fern-specific single amino acid polymorphisms are highlighted in red and known SGT1-interacting residues of AtRAR1 are marked with purple boxes. Asterisks indicate critical residues tested in AtRAR1–AtSGT1a interaction studies by site-directed mutagenesis from Zhang et al., (2010). Species abbreviations: Af = *Azolla filliculoides*; Mv: *Marsilea vestita*; Sc: *Salvinia cucullata*; Ac: *Adiantum capillus-veneris*; As: *Alsophila spinulosa*; Tp: *Thuja plicata*, Hv: *Hordeum vulgare*. Other abbreviations are the same as in Figure 3.6.

To understand the molecular basis of fern-specific RAR1–SGT1 interaction, I generated AtRAR1 variants possessing each of the fern-specific differences (Figure 3.9A). As an additional negative control for AtRAR1–AtSGT1b interaction, I included the AtRAR1^{I153Q} variant, which is known to disrupt AtRAR1–AtSGT1a binding (Zhang et al., 2010). Wildtype AtRAR1 interacted with AtSGT1b,

whereas both AtRAR1^{I153Q} and CrRAR1 failed to interact with AtSGT1b. Individual AtRAR1 variants carrying the fern-specific mutations K162Δ, G165K, A185P, and D198N all interacted with AtSGT1b but not CrSGT1, indicating that these mutations do not contribute to the CrRAR1–AtSGT1 incompatibility. By contrast, the I153F substitution fully reversed the specificity, such that AtRAR1^{I153F} interacted with CrSGT1 instead of AtSGT1b (Figure 3.9B). AtRAR1^{I153F}–CrSGT1 interaction resulted in growth on Ura counter-selection plates, indicating a weak interaction. Considering that CrRAR1–CrSGT1 interaction also grew on the counter-selection plates, AtRAR1^{I153F}–CrSGT1 interaction appears to be a genuine protein-protein interaction (Figure 3.9C). These results underscore the importance of the isoleucine residue in position 153 of the CHORD II domain, as both I153Q and I153F mutations profoundly altered AtRAR1–AtSGT1b interactions.

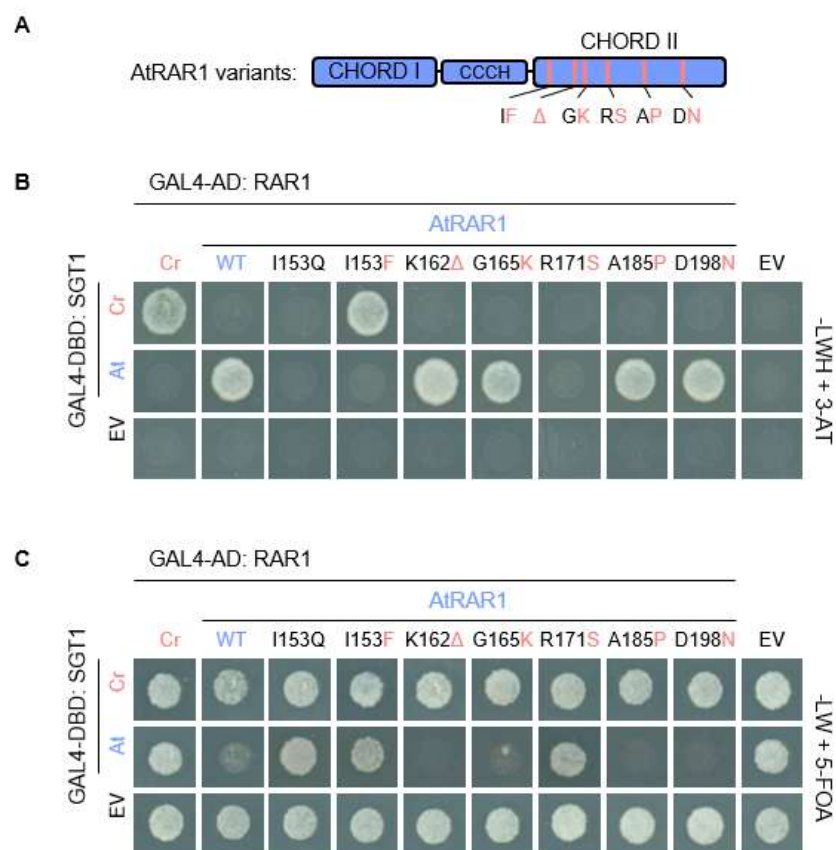


Figure 3.9 A fern-specific mutation in CrRAR1, I153F, switches the incompatibility between AtRAR1 and CrSGT1. **(A)** Schematic representation of AtRAR1 variants with fern-specific mutations **(B–C)** Yeast two-hybrid assays testing interactions of AtRAR1 variants. Each interaction was tested in two to four independent replicates with similar results. **(B)** Growth on His selection plates. **(C)** Growth on Ura counter-selection plates. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting.

To better visualize the impact of the I153F mutation at this position, I modelled the AtRAR1–AtSGT1b and CrRAR1–CrSGT1 binding interface using AlphaFold-Multimer based structure prediction. The predicted AtRAR1–AtSGT1b binding interface showed that isoleucine residues AtRAR1:I153 and AtSGT1b:I193 are located at the center of a hydrophobic interaction similar to the AtRAR1–AtSGT1a crystal structure (Zhang et al., 2010). By contrast, the CrRAR1–CrSGT1 binding interface includes a bulky phenylalanine (CrRAR1:F151), which is facing a smaller serine residue of CrSGT1 (CrSGT1:S209), maintaining distance between these residues. Considering different physicochemical properties between isoleucine, phenylalanine, and serine, these fern-specific mutations likely have rewired in the interface while maintaining the RAR1–SGT1 interaction (Figure 3.10). Collectively, this data show that the substitution from a small isoleucine to a bulky phenylalanine residue in RAR1 underlies the shift in RAR1–SGT1 specificity in ferns.

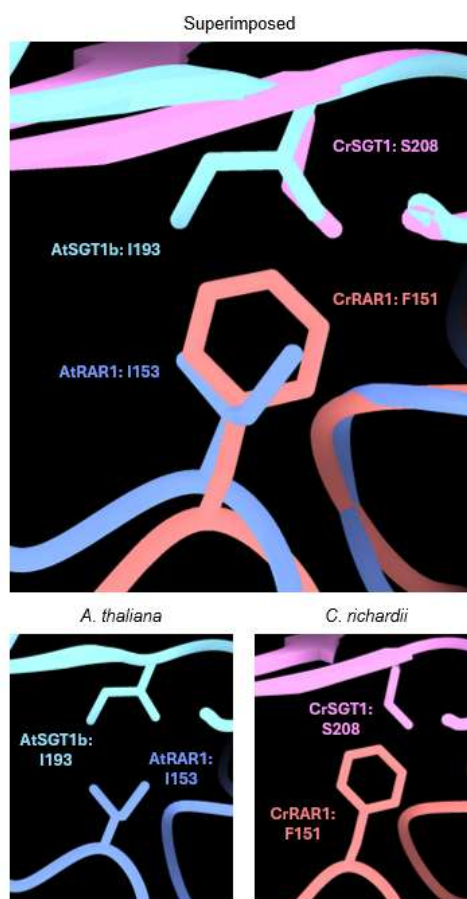


Figure 3.10 The I153F mutation on CrRAR1 is compensated by I193S mutation of CrSGT1. AlphaFold modeling of the RAR1^{CHORD II}–SGT1^{CS} binding interface. (A–C) Predicted structures of the protein complexes. The superimposed structures (top), AtRAR1–AtSGT1b binding interface (bottom left), and CrRAR1–CrSGT1 binding interface (bottom right).

3.2.6 CS domain of CrSGT1 harbors three fern-specific differences to accommodate CrRAR1

To investigate whether compensatory changes in CrSGT1 evolved to accommodate divergence in CrRAR1, I examined CS domain sequences from representative SGT1 orthologs to identify fern-specific variations. Protein sequence alignments revealed four fern-specific SAPs: Glu191Ile (E191I), Ile193Ser (I193S), Ser195Lys (S195K), and Glu203Gln (E203Q) (numbering according to AtSGT1b) (Figure 3.11). Among these lineage-specific differences, three fern-specific mutations (E191I, I193S, and S195K) lie within the AtRAR1–AtSGT1a binding interface (Zhang et al., 2010). Notably, earlier work demonstrated that altering Ile193 to Asp disrupts RAR1–SGT1 binding (Botër et al., 2007). In contrast, Gly190, another residue shown to be essential in the previous study, is conserved across all land plant homologs (Figure 3.11).

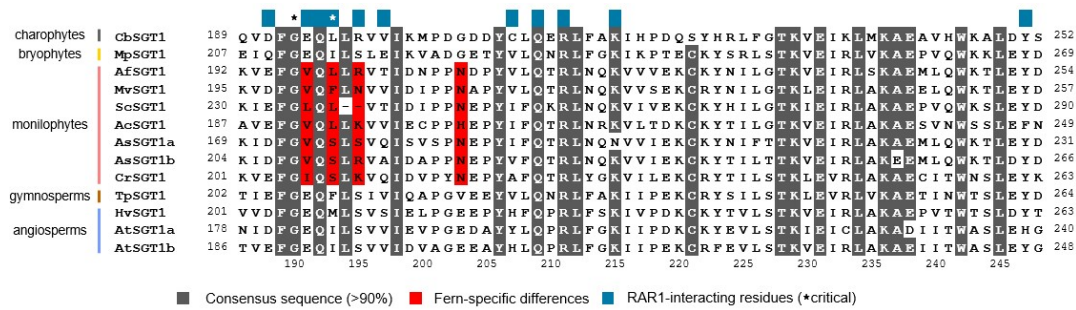


Figure 3.11 CS domain of fern SGT1 homologs harbors four lineage-specific differences. Amino acid sequence alignment of CS domains from representative SGT1 homologs. Fern-specific single amino acid polymorphisms are highlighted in red and known RAR1-interacting residues of AtSGT1a are marked with teal boxes. Asterisks indicate critical residues tested in AtRAR1–AtSGT1a interaction studies by site-directed mutagenesis from Botër et al., (2007). Species abbreviations are the same as in Figure 3.8.

To assess their contribution to the specificity of the PPI, I generated AtSGT1b variants carrying each of the fern-specific mutations, both individually and in combination (Figure 3.12A). Their interactions with AtRAR1 and CrRAR1 were examined by Y2H assays. As an additional negative control, I included the AtSGT1b^{I193D}, previously shown to lose binding ability to AtRAR1 (Botër et al., 2007). Consistent with previous results, wildtype AtSGT1b and CrSGT1 interacted only with corresponding RAR1 orthologs from their own species, and the AtSGT1b^{I193D} variant failed to interact with AtRAR1 (Figure 3.12B). All interacting RAR1–SGT1 pairs failed to grow on the counter-selection plates, indicating strong PPIs in yeast (Figure 3.12C).

Introducing all four fern-specific substitutions into AtSGT1b, the AtSGT1b^{Cr-like} variant was sufficient to reverse the incompatibility that the variant interacted with CrRAR1 but not with

AtRAR1. Single substitutions had distinct effects. AtSGT1b^{E203N} behaved like wild-type AtSGT1b, leaving the specificity unchanged. The E191I mutation broadened binding capacity, allowing interactions with both AtRAR1 and CrRAR1. By contrast, the I193S and the S195K mutations disrupted binding with both RAR1 homologs. Since E203N mutation had no impact on the specificity, I193S and S195K mutation were combined with E191I mutation. When combined, I193S or S195K with E191I retained the broad binding capacity of AtSGT1b^{E191I}, whereas the AtSGT1b^{EI+IS} variant was incompatible with both partners. Strikingly, the variant with these three mutations, AtSGT1b^{EI+IS+SK}, reproduced fern-like specificity, interacting only with CrRAR1 but not with AtRAR1 (Figure 3.12C).

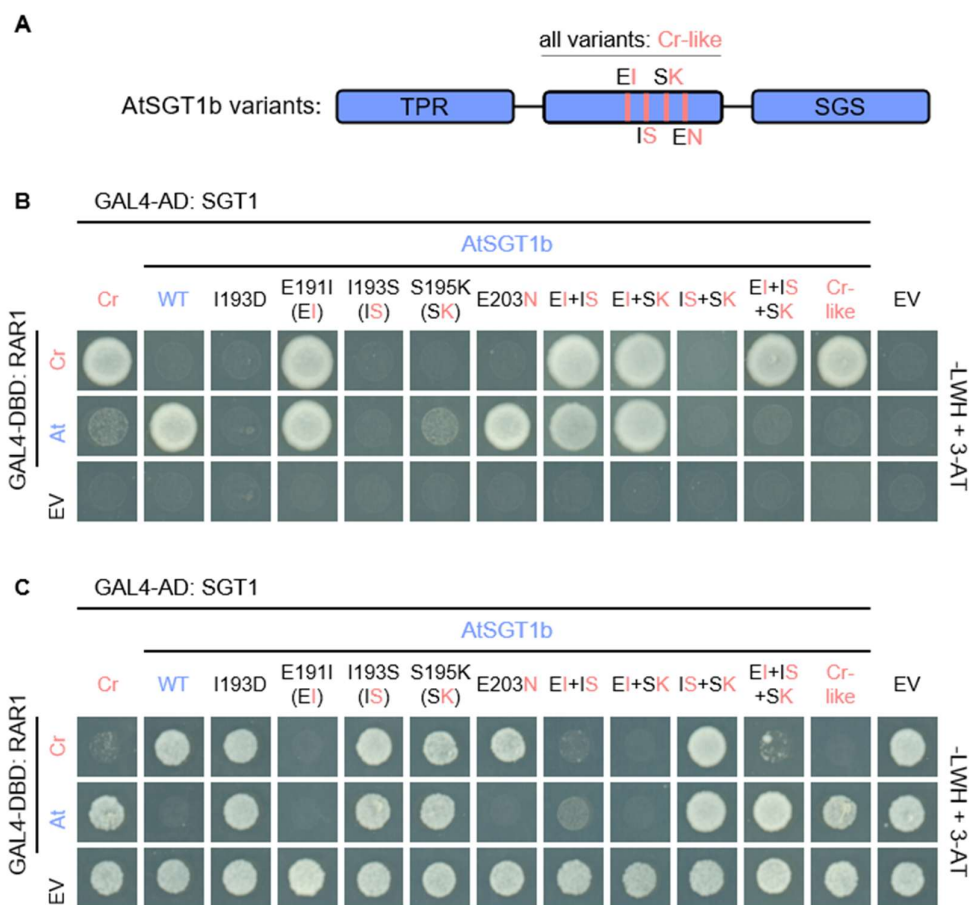


Figure 3.12 CS domain of CrSGT1 harbors mutations corresponding to CrRAR1. Three fern-specific mutations in CrSGT1 cooperatively accommodate the I153F mutation in CrRAR1. **(A)** Schematic representation of AtSGT1b variants with fern-specific mutations **(B–C)** Yeast two-hybrid assays testing interactions of AtSGT1b variants. Each interaction was tested in two to four independent replicates with similar results. **(B)** Growth on His selection plates. **(C)** Growth on Ura counter-selection plates. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting. This complete reversal in binding specificity demonstrates that all three mutations in CrSGT1 are

required to maintain interaction with CrRAR1. To visualize these residues within the RAR1–SGT1 binding interface, I modeled AtRAR1–AtSGT1b and CrRAR1–CrSGT1 complexes using AlphaFold-Multimer. The models revealed that the three fern-specific substitutions in CrSGT1 cluster in close proximity to the I153F substitution in the CHORD II domain of CrRAR1 (Figure 3.13). Collectively, these results define the molecular basis in CrSGT1 that coevolved with CrRAR1 to maintain RAR1–SGT1 interaction in ferns.

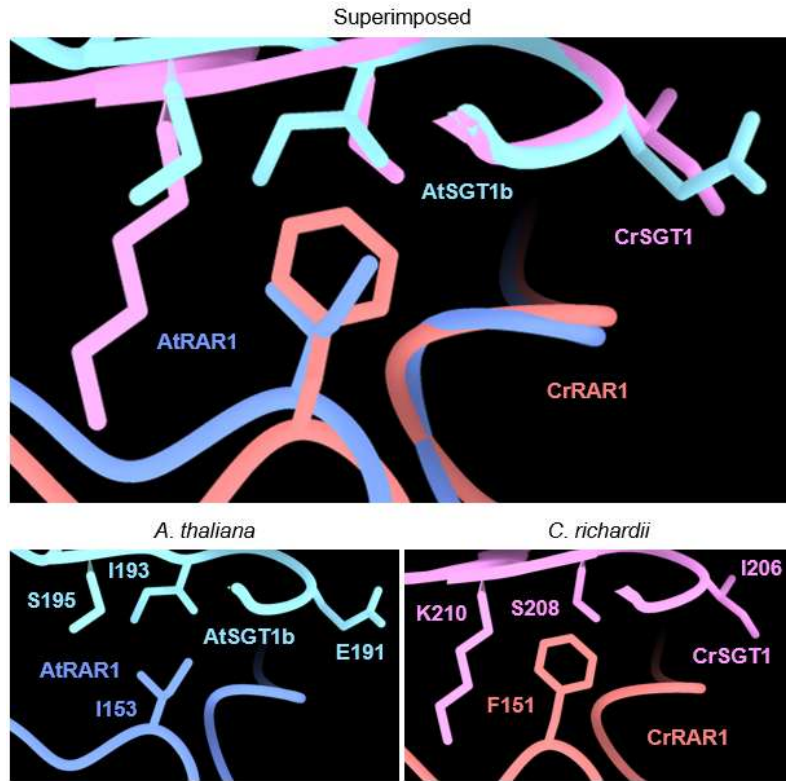


Figure 3.13 Four critical fern-specific residues are located in proximity at the binding interface. AlphaFold modeling of the RAR1^{CHORD II}–SGT1^{CS} binding interface. (A–C) Predicted structures of the protein complexes. The superimposed structures (top), AtRAR1–AtSGT1b binding interface (bottom left), and CrRAR1–CrSGT1 binding interface (bottom right).

3.2.7 Four fern-specific mutations can mimic the CrRAR1–CrSGT1 binding interface in angiosperms

To examine whether the four fern-specific mutations (three in SGT1 and one in RAR1) are sufficient to rewire the RAR1–SGT1 interface, I performed Y2H assays with fern-like AtRAR1 variant (AtRAR1^{I153F}) and fern-like AtSGT1b variant (AtSGT1b^{EI+IS+SK}) (Figure 3.14). The AtSGT1b^{EI+IS+SK} showed strong colony growth when paired with CrRAR1 and AtRAR1^{I153F}, with comparable strength

to CrSGT1. Interestingly, AtRAR1–AtSGT1b^{EI+IS+SK} interaction resulted in growth both on selective and counter-selective media, indicating a weaker interaction than CrRAR1–AtSGT1b^{EI+IS+SK} and CrRAR1–CrSGT1 (Figure 3.14). These observations likely reflect minor contributions of other residues to AtRAR1–AtSGT1b interaction or inherent stickiness of AtRAR1 in Y2H assays.

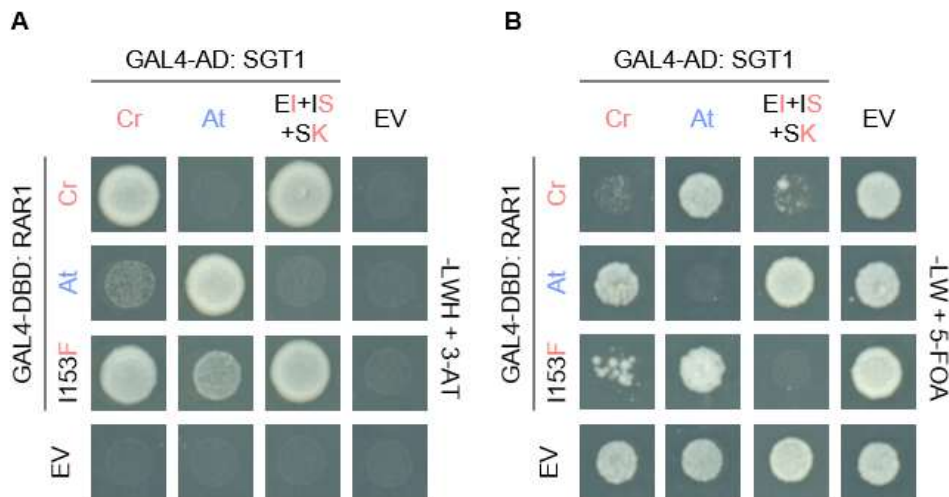


Figure 3.14 Four critical fern-specific residues mimic the CrRAR1–CrSGT1 binding interface in yeasts. **(A–B)** Yeast two-hybrid assays testing interactions of fern-like AtRAR1 and AtSGT1b variants. Each interaction was tested in two to four independent replicates with similar results. **(A)** Growth on His selection plates. **(B)** Growth on Ura counter-selection plates. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting.

To further test whether these four fern-specific substitutions are sufficient to define binding specificity, I performed split GAL4 RUBY assays to quantify the RAR1–SGT1 interaction *in planta*. In this assay, two proteins of interest are transiently co-expressed in *Nicotiana benthamiana* leaves by *Agrobacterium*, each fused to the GAL4 DNA-binding domain (GAL4DBD) or the VP16 activation domain. If the proteins interact, it induces activation of the betalain biosynthesis reporter, RUBY. The resulting pigmentation provides a visible readout of PPI strength and can be quantified by pigment extraction and spectrophotometry. A previous study validated this system by showing that AtSGT1b–GAL4DBD and VP16–AtRAR1 co-expression induced strong betalain accumulation (Chen et al., 2023).

To optimize RUBY reporter output, different promoters were tested. When AtSGT1b was used as a bait, the *mas* promoter yielded the strongest pigmentation, which is consistent with known RAR1–SGT1 interactions. Under this promoter, wildtype AtRAR1–AtSGT1b produced strong betalain signals, whereas AtRAR1^{I153Q}, a negative control for AtRAR1–AtSGT1b interactions used in Y2H

experiments, failed to activate the reporter in the presence of AtSGT1b (Figure 3.15). By contrast, CrRAR1–CrSGT1 interactions gave the strongest pigmentation when both constructs were driven by the *Nos* promoter, which is weaker than *mas* promoter (Figure 3.16).

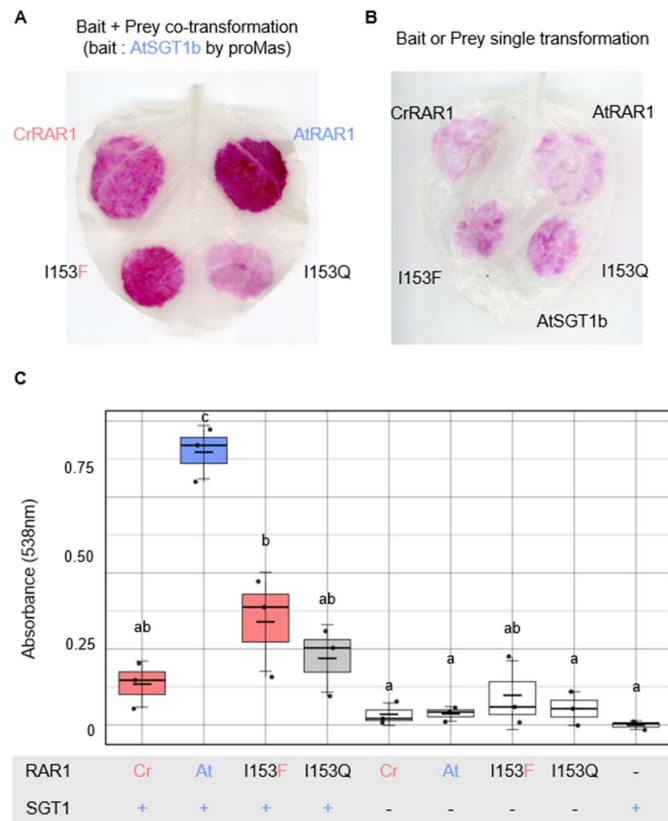


Figure 3.15 AtSGT1b selectively bound to AtRAR1 in *planta*. **(A–C)** Split GAL4 Ruby assays testing interactions between AtRAR1 variants and AtSGT1b. Every protein was expressed under *mas* promoter. Each interaction was tested in three independent replicates. Different letters indicate statistically significant differences between groups (Tukey's HSD test, $p < 0.05$). **(A)** Representative bleached *N. benthamiana* leaf showing four RAR1–AtSGT1b interactions. **(B)** Representative bleached *N. benthamiana* leaf infiltrated with individual RAR1 or AtSGT1b constructs. **(C)** Measured absorbance at 538 nm for each interaction. *N. benthamiana* leaves were collected at 5 dpi.

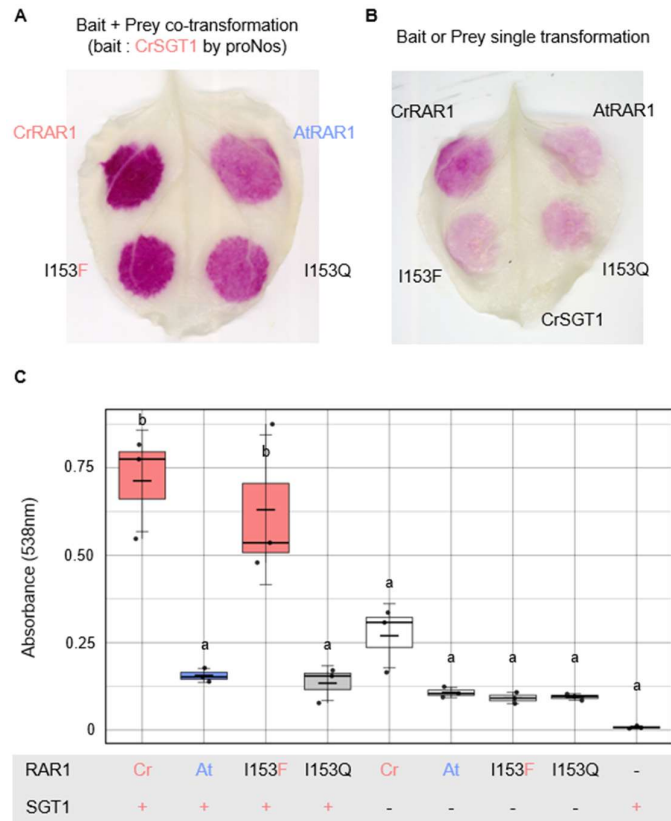


Figure 3.16 The I153F mutation in AtRAR1 mimicked CrRAR1 *in planta*. **(A–C)** Split GAL4 Ruby assays testing interactions between AtRAR1 variants and CrSGT1. Every protein was expressed under Nos promoter. Each interaction was tested in three independent replicates. Different letters indicate statistically significant differences between groups (Tukey's HSD test, $p < 0.05$). **(A)** Representative bleached *N. benthamiana* leaf showing four RAR1–CrSGT1 interactions. **(B)** Representative bleached *N. benthamiana* leaf infiltrated with individual RAR1 or CrSGT1 constructs. **(C)** Measured absorbance at 538 nm for each interaction. *N. benthamiana* leaves were collected at 5 dpi.

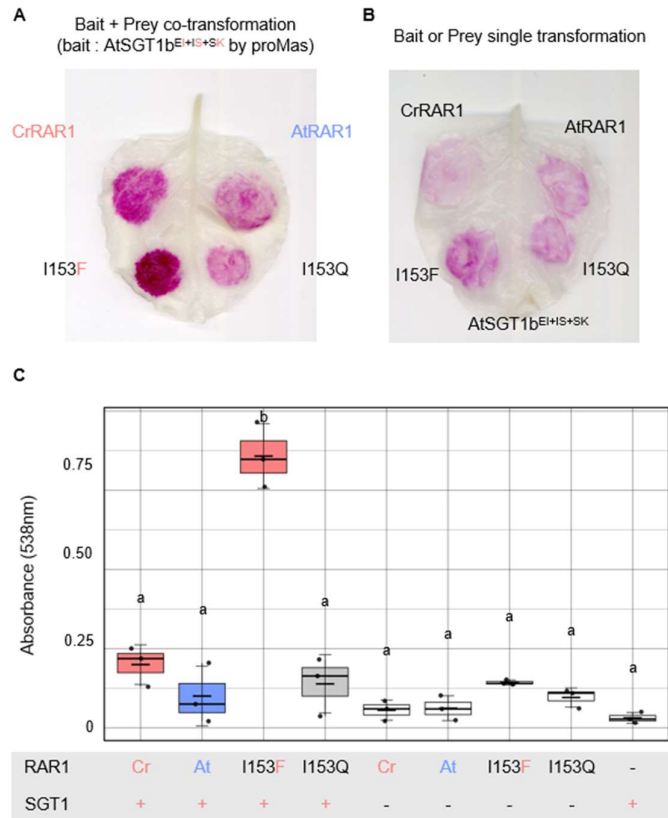


Figure 3.17 AtSGT1b^{EI+IS+SK} bound to AtRAR1^{I153F} but not to CrRAR1 *in planta* when driven by mas promoter. **(A–C)** Split GAL4 Ruby assays testing interactions between AtRAR1 variants and AtSGT1b^{EI+IS+SK}. Every protein was expressed under mas promoter. Each interaction was tested in three independent replicates. Different letters indicate statistically significant differences between groups (Tukey’s HSD test, $p < 0.05$). **(A)** Representative bleached *N. benthamiana* leaf showing four RAR1–AtSGT1b^{EI+IS+SK} interactions. **(B)** Representative bleached *N. benthamiana* leaf infiltrated with individual RAR1 or AtSGT1b^{EI+IS+SK} constructs. **(C)** Measured absorbance at 538 nm for each interaction. *N. benthamiana* leaves were collected at 5 dpi.

As expected, CrSGT1 interacted with CrRAR1 and AtRAR1^{I153F}, while AtSGT1b exclusively interacted with wildtype AtRAR1 (Figure 3.15–16). The AtSGT1b^{EI+IS+SK} variant showed strong affinity only to AtRAR1^{I153F} when expressed by *mas* promoter but interacted with both CrRAR1 and AtRAR1^{I153F} when driven by *Nos* promoter (Figure 3.17–18). Considering that AtSGT1b^{EI+IS+SK} did not show appreciable betalain pigmentation when co-expressed with AtRAR1 under either promoter combination, I concluded that its binding affinity was successfully shifted towards CrRAR1 rather than the original AtRAR1. Altogether, these assays support the hypothesis that four key residues are the minimum requirements to alter binding specificity of RAR1–SGT1 interface in plants.

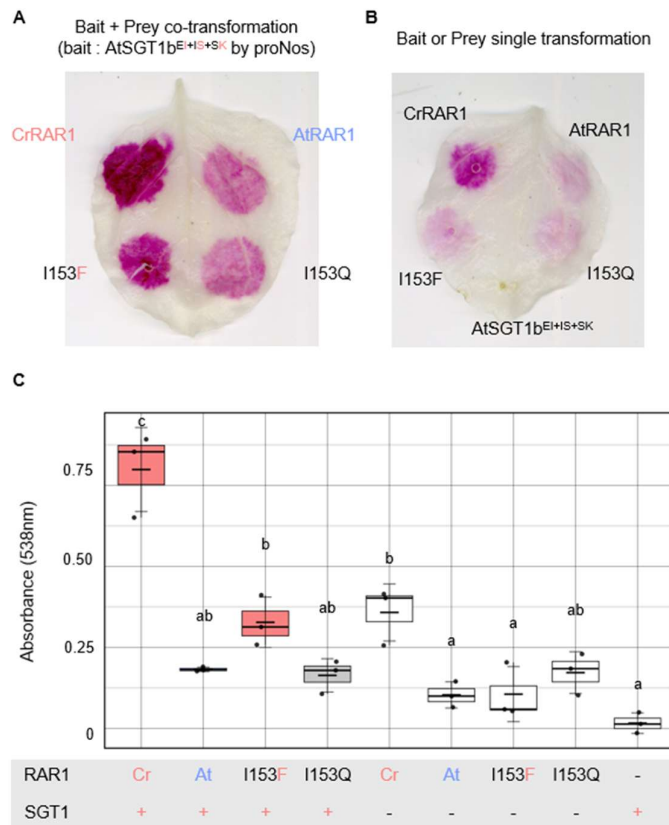


Figure 3.18 AtSGT1b^{EI+IS+SK} bound to AtRAR1^{I153F} and CrRAR1 *in planta* when driven by Nos promoter. **(A–C)** Split GAL4 Ruby assays testing interactions between AtRAR1 variants and AtSGT1b^{EI+IS+SK}. Every protein was expressed under Nos promoter. Each interaction was tested in three independent replicates. Different letters indicate statistically significant differences between groups (Tukey’s HSD test, $p < 0.05$). **(A)** Representative bleached *N. benthamiana* leaf showing four RAR1–AtSGT1b^{EI+IS+SK} interactions. **(B)** Representative bleached *N. benthamiana* leaf infiltrated with individual RAR1 or AtSGT1b^{EI+IS+SK} constructs. **(C)** Measured absorbance at 538 nm for each interaction. *N. benthamiana* leaves were collected at 5 dpi.

3.2.8 Stepwise divergence in the SGT1 precedes a compensatory mutation in RAR1 of leptosporangiate ferns

To determine when RAR1–SGT1 binding interface began to diverge, I surveyed critical interface residues in RAR1 (Ile153) and SGT1 (Glu191, Ile193, and Ser195) across a wide diversity of extant ferns from both eusporangiate to leptosporangiate lineages (Figure 3.19). This revealed that the fern-specific I153F substitution in RAR1 is restricted to Cyatheales and Polypodiales, the latter of which includes the model fern *C. richardii*. Expanding the survey to other land plant lineages confirmed that bulky amino acids at position 153 are absent outside ferns (Figure 3.20). The most common amino acid at the Ile153 position was leucine, which is encoded by six possible codons (TTA, TTG,

and CTN). The most common replacement across plants is leucine, encoded by six codons (TTA, TTG, and CTN). In eusporangiate ferns, an early-diverging lineage relative to leptosporangiate ferns (Pteridophyte Phylogeny Group I, 2016), the TTG codon encodes leucine at position 153. Notably, leptosporangiate ferns, which include the Cyatheales and the Polypodiales, typically encode phenylalanine at this site, most frequently via TTC and less commonly via TTT (Figure 3.20). These patterns suggest that a single G-to-C polymorphism in the ancestor of Cyatheales and Polypodiales introduced the isoleucine-to-phenylalanine substitution, which underlies the fern-specific RAR1–SGT1 binding specificity (Figure 3.19–20).

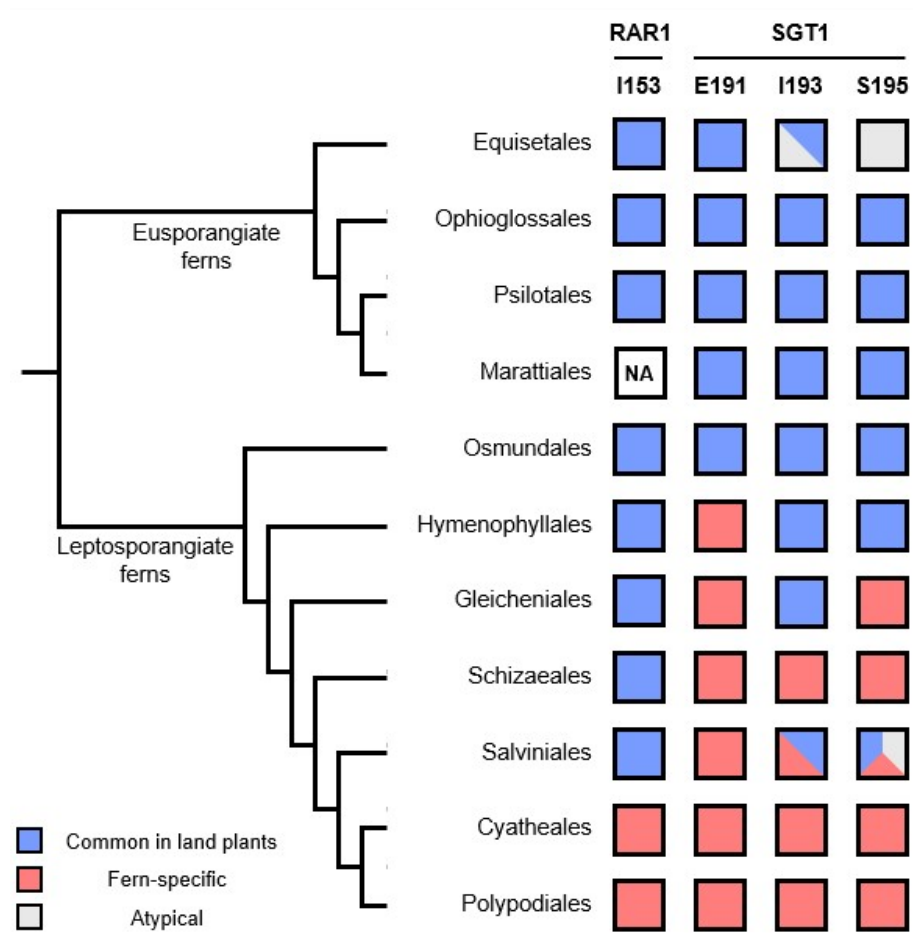


Figure 3.19 Critical fern-specific differences appear first in SGT1. Summary of the presence of critical fern-specific mutations across extant fern species is shown next to a fern phylogenetic tree. *C. richardii* is in Polypodiales order. The phylogenetic tree is modified from Nitta et al., (2022).

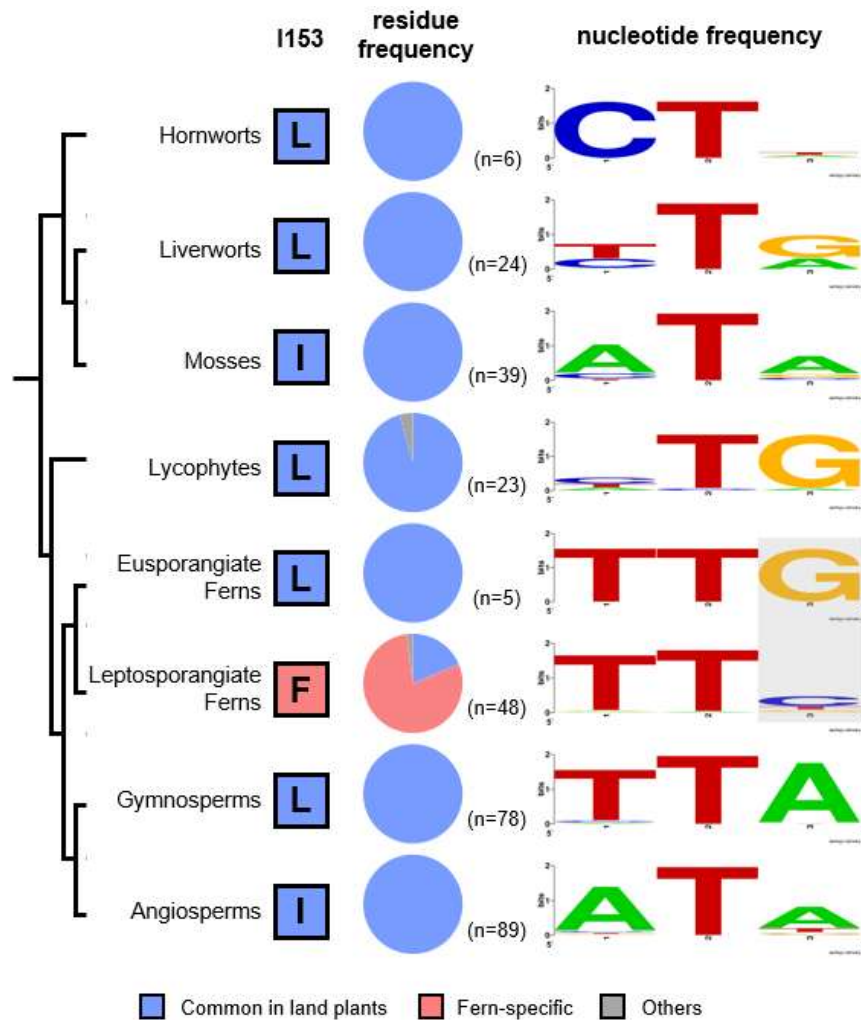


Figure 3.20 A G-to-C single nucleotide polymorphism occurred in leptosporangiate ferns, resulting in the I153 mutation. The most prevalent amino acid at the I153 position, the proportion of residues at this position, and the proportion of codons encoding the residue are shown. The consensus nucleotide sequence of the codon is illustrated using WebLogo (<https://weblogo.berkeley.edu/logo.cgi>).

On the SGT1 side of the interface, the three fern-specific mutations in the CS domain appear earlier than the one in RAR1. They appear to have accumulated after Osmundales diverged from other leptosporangiate ferns. Among these, the E191I substitution, previously shown to confer the binding versatility, emerged first, followed by substitutions at positions 193 and 195, which likely added binding specificity in an additive manner (Figure 3.12; 3.19; 3.21). The relative order of the I193S and S195K substitutions remains unresolved due to apparent reversions in Salviniales (Figure 3.19).

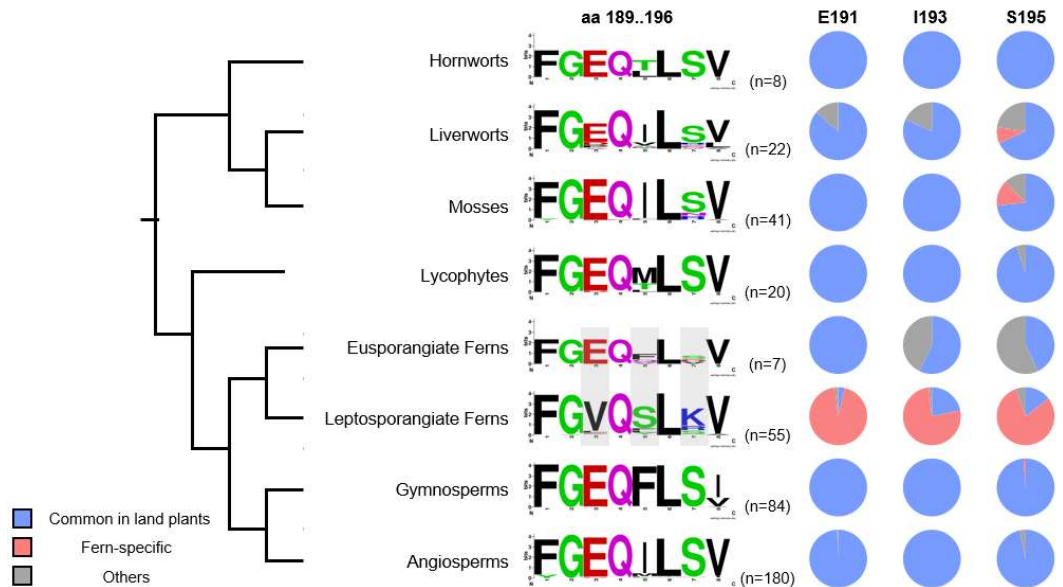


Figure 3.21 Critical lineage-specific mutations in fern SGT1 are prevalent in leptosporangiate ferns. Residues at positions E191, I193, and S195 are shown by clade together with the conserved neighboring residues. The proportions of residues at these positions are indicated alongside. The consensus amino acid sequence is illustrated using WebLogo (<https://weblogo.berkeley.edu/logo.cgi>).

To clarify the timing of these trajectories, we performed ancestral sequence reconstruction (ASR) of 60 SGT1 homologs from extant fern species and *M. polymorpha* SGT1 as an outgroup (Figure 3.22). ASR is a powerful tool to rebuild and trace the evolutionary trajectory of proteins and PPIs using present biochemistry techniques (Brayer et al., 2011; Hochberg and Thornton, 2017; Laursen et al., 2021). ASR clearly supports a sequential acquisition of the three fern-specific SGT1 substitutions. After divergence from Osmundales, glutamic acid at position 191 (E191) was first substituted by a non-charged residue, valine (ancestral state B), consistent with the variation observed in extant ferns (Figure 3.21; 3.23). This was followed by the phenylalanine-to-serine substitution at position 193 (ancestral state C), which is isoleucine in angiosperms but phenylalanine in gymnosperms and other ferns (Figure 3.21; 3.23). Finally, the serine-to-arginine substitution at position 195 (ancestral state D) arose in the common ancestors of Cyatheaales and Polypodiales. ASR further suggests that Salviniales later experienced a reversion at this site to a bulky residue, most likely phenylalanine (Figure 3.23).

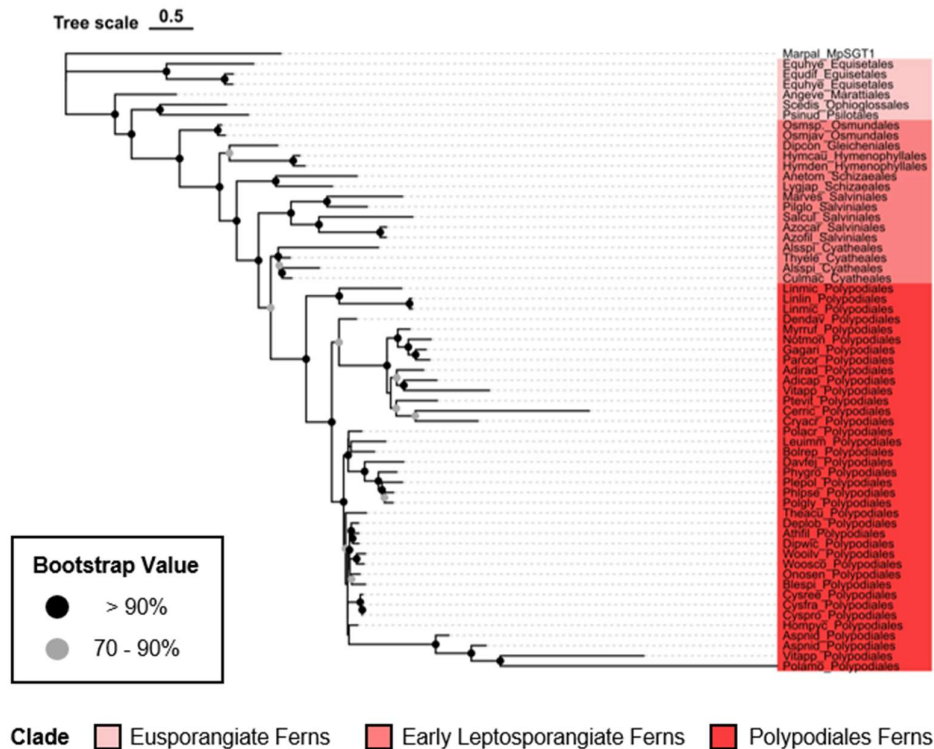


Figure 3.22 Maximum likelihood phylogenetic trees of *SGT1* in ferns. MpSGT1 was used as the outgroup sequence.

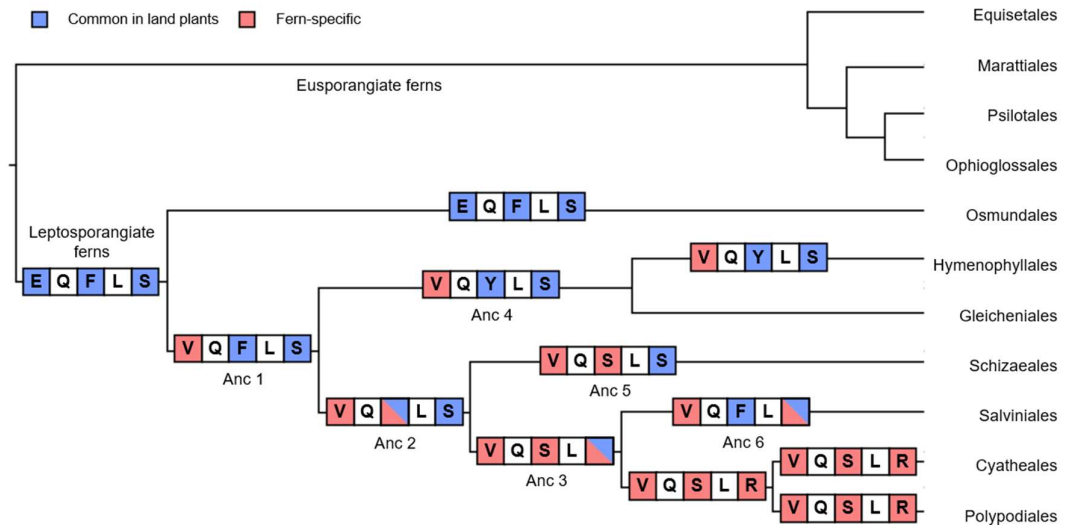


Figure 3.23 Ancestral state reconstruction confirms the sequential mutation accumulation in fern *SGT1*. Summary of ancestral state reconstruction for fern *SGT1*. If multiple residues were predicted with > 5% probability at a given position, the state was labeled as 'Anc' 1–6. If two or more residues had >40% probability, the position was labeled with both colors. Predicted codons and amino acids with their probabilities in ancestral states Anc 1–6 are provided in Table 3.2.

Table 3.2 Predicted codon and amino acids from ancestral state reconstruction

Anc No.	Position	Predicted codon			Predicted amino acid		
		Codon 1	Codon 2	Codon 3	Amino acid 1	Amino acid 2	Amino acid 3
1	193I	TTT	0.4208	TAT	0.4019	TCT	0.1721
2	193I	TCT	0.5882	TTT	0.3433	TAT	0.0549
3	193I	TCT	0.6254	TTT	0.352		
	195S	AGT	0.5711	AGG	0.4238		
4	193I	TAT	0.8982	TTT	0.0744		
5	195S	TCT	0.6499	TTT	0.1465	TGT	0.1232
6	195S	AGT	0.5813	AGG	0.4084		

Phylogenetic and ASR analyses predict that divergence of the RAR1–SGT1 binding interface was initiated within the CS domain of SGT1, creating the first ‘promiscuous’ intermediate. The second substitution resulted in another promiscuous intermediate, with no further change in binding capacity. After acquiring the third substitution in SGT1, fern SGT1 lost compatibility with RAR1, which was compensated by the I-to-F substitution in RAR1. Given the broadened interaction capacity observed for the initial E-to-I mutation in yeast, these data support a model that molecular coevolution of the RAR1–SGT1 interface in ferns was facilitated by the stepwise emergence of promiscuous SGT1 intermediates (Figure 3.24).

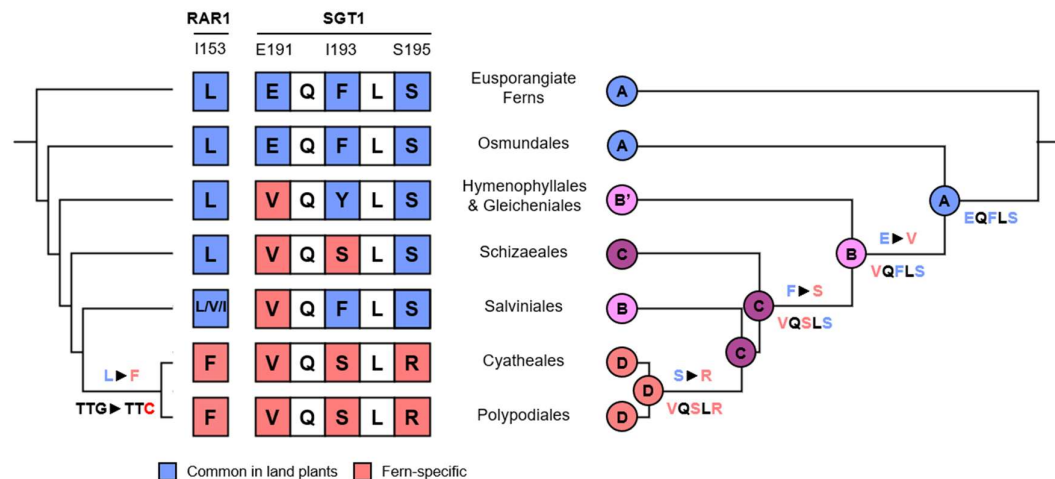


Figure 3.24 The I153F mutation in RAR1 is a potential compensatory mutation adapting to *SGT1*-driven RAR1–SGT1 divergence in ferns. Ancestral state reconstruction revealed the order of how four critical fern-specific differences accumulated in fern RAR1 and SGT1.

3.2.9 Shift of RAR1–SGT1 specificity is observed in extant fern species

Ancestral state reconstruction in ferns suggested a stepwise divergence in the CS domain of SGT1, concluded by a compensatory substitution within the CHORD II domain of RAR1. To test this predicted evolutionary trajectory supported by promiscuous intermediates, I examined RAR1–SGT1 compatibility using homologs from four extant fern species representing key evolutionary lineages: *C. richardii* (Pol; Polypodiales), *Azolla filliculoides* (Sal; Salviniales), *Hymenophyllum dentatum* (Hym; Hymenophyllales), and *Osmunda* sp. (Osm; Osmundales) alongside *A. thaliana* (Ang; Angiosperm) (Figure 3.25).

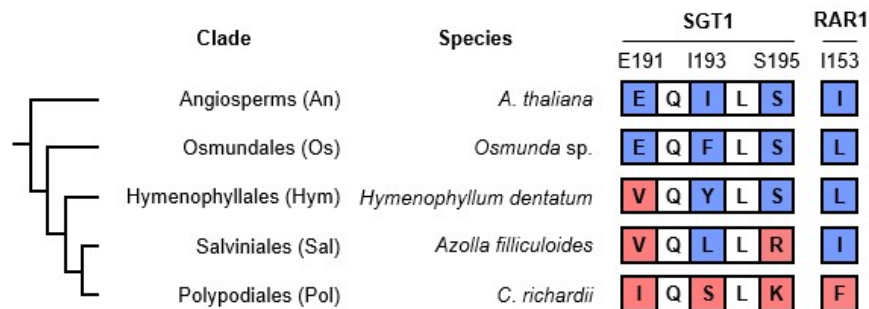


Figure 3.25 Extant fern RAR1 and SGT1 homologs representing ancestral states.

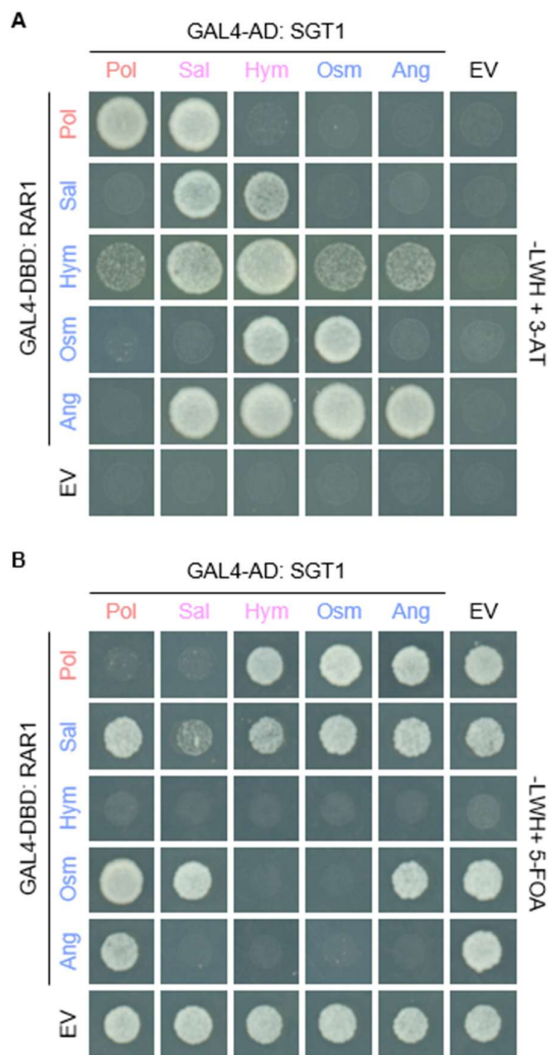


Figure 3.26 RAR1–SGT1 compatibility shifted during fern evolution. **(A–B)** Yeast two-hybrid assays testing compatibility between selected fern RAR1 and SGT1 homologs. **(A)** Growth on His selection plates. **(B)** Growth on Ura counter-selection plates. Colonies were spotted at $OD_{600} = 0.1$ and imaged 3 days after spotting. A different 3-AT concentration (50 mM) was used for HdRAR1.

Consistent with earlier Y2H assays with representative streptophyte species, RAR1–SGT1 interactions were robust within species pairs. Similar to experiments with AtSGT1b variants, two SGT1 homologs representing promiscuous intermediates, HdSGT1 (Hym) and AfSGT1 (Sal), interacted with more RAR1 homologs than AtSGT1b or CrSGT1 (Figure 3.26), although HdRAR1 had to be titrated with different OD₆₀₀ values and concentration of 3-AT due to autoactivity (Figure 3.27). By contrast, clades representing ancestral states (Osm and Ang) or the fully diverged state (Pol) interacted only with one or two closely related RAR1 homologs. These results are consistent with the stepwise trajectory predicted by ancestral state reconstruction (section 3.2.8) and are consistent with the promiscuous binding observed in AtSGT1b variant assays (section 3.2.6).

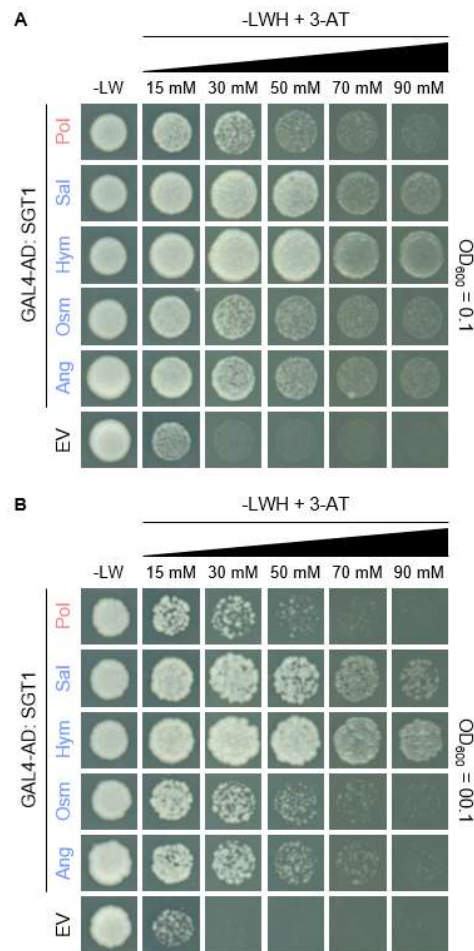


Figure 3.27 HdRAR1 interacted with AfSGT1 and HdSGT1 when autoactivation was reduced. Due to high autoactivation of HdRAR1, HdRAR1–SGT1 interactions were tested with different OD₆₀₀ and 3-AT concentration. **(A–B)** Yeast two-hybrid assays on His selection plates with increasing 3-AT concentrations. Colonies were spotted at **(A)** OD₆₀₀ = 0.1 and **(B)** OD₆₀₀ = 0.01 and imaged 3 days after spotting.

3.2.10 Reversion of the compensatory mutation in CrRAR1 restored its ancestral state

As a final proof of concept to test the compensatory mutation (I-to-F) in fern RAR1, I generated a CrRAR1 variant whose phenylalanine at position 151 was reverted to isoleucine (CrRAR1^{F151I}, hereafter Cr^{FI}) to test if it would lose the ability to interact with CrSGT1 (Figure 3.28A). Strikingly, Cr^{FI} failed to interact with CrSGT1, or with the fern-like AtSGT1b^{EI+IS+SK} triple variant. Instead, this variant interacted specifically with wildtype AtSGT1b, mirroring the interaction pattern of wildtype AtRAR1 (Figure 3.28B–C). Furthermore, the Phe151Ile mutation made Cr^{FI} interact with fern SGT1 homologs representing ancestral state (Osm) and transition state (Sal and Hym) during the RAR1–SGT1 divergence (Figure 3.28D). These results indicate that the I-to-F substitution in RAR1 functions as a compensatory mutation, enabling adaptation to structural changes in the RAR1–SGT1 interface caused by divergence in SGT1.

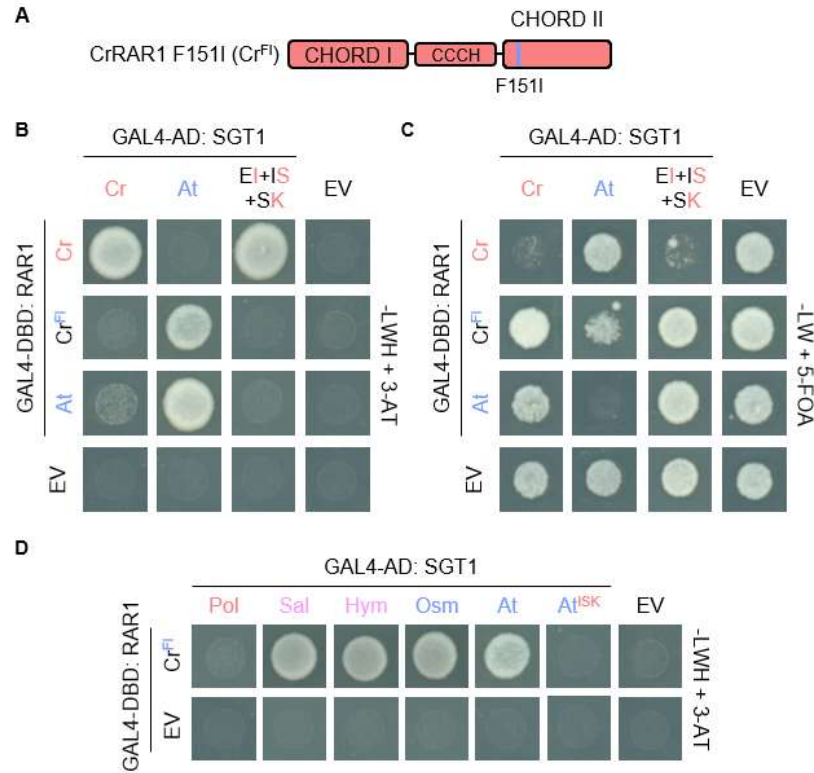


Figure 3.28 Reversion of the compensatory mutation in CrRAR1 restored its ancestral state. **(A)** Schematic representation of Cr^{FI}, a CrRAR1 variant with the reverted compensatory mutation (F151I) **(B–C)** Yeast two-hybrid assays testing compatibility of Cr^{FI} with different SGT1 variants. **(B)** Growth on His selection plates. **(C)** Growth on Ura counter-selection plates. **(D)** Interaction between Cr^{FI} and different SGT1 homologs and variants. At^{ISK} stands for AtSGT1b^{EI+IS+SK}. Colonies were spotted at OD₆₀₀ = 0.1 and imaged 3 days after spotting.

3.3 Conclusion and discussion

In this chapter, I report two major findings. First, the RAR1–SGT1 interaction is highly conserved across land plants, and even in the streptophyte alga *C. braunii*. Cross-species interactions were observed even between distantly related species such as *C. braunii* and *A. thaliana*, and protein sequence alignments showed high sequence homology. Second, the RAR1–SGT1 binding interface in leptosporangiate ferns diverged through promiscuous SGT1 intermediates, allowing the RAR1–SGT1 interaction to be maintained during the divergence. *C. richardii* RAR1 and SGT1 homologs showed strict incompatibility with homologs outside of its lineage, interacting only with homologs from the Salviniiales and Polypodiales. By leveraging protein sequences of RAR1 and SGT1 from extant land plants, I identified lineage-specific polymorphisms that underlie this divergence and determined the minimal set of changes required to explain the observed RAR1–SGT1 compatibility. Using both phylogenomic analyses of extant sequences and *in silico* ancestral sequence reconstruction, I discovered stepwise divergence, with promiscuous SGT1 intermediates preceding the compensatory mutation in RAR1. This highlights how binding promiscuity can facilitate molecular coevolution, a principle observed across diverse protein systems.

Strikingly, molecular coevolution of interacting proteins through promiscuous intermediate has been reported in bacteria. In the ParD–ParE toxin–antitoxin system, precise protein–protein interaction is essential because the free toxin kills the cell. When ParD–ParE interaction was rewired by experimental evolution, the coevolution was initiated by a promiscuous state of ParD, then followed by accumulation of additional mutations in ParD and specificity-narrowing mutation in ParE. This pattern of molecular coevolution is possibly to avoid nonfunctional ‘dead’ intermediates (Aakre et al., 2015). An analogous constraint likely has shaped the RAR1–SGT1 binding interface in ferns. Mutations at the RAR1–SGT1 binding interface result in compromised immunity (Shirasu, 2009; Zhang et al., 2010). Thus, while a nonfunctional RAR1–SGT1 intermediate may not be immediately lethal, it poses a conditional fitness cost to plants by increasing disease susceptibility. This provides a plausible explanation for RAR1–SGT1 divergence via promiscuous SGT1 intermediates, and why such divergence has not become common across land plant lineages.

SGT1-binding effectors offer a possible driver of the RAR1–SGT1 divergence. Several studies have identified effectors that bind to SGT1 and disrupt the RAR1–SGT1 interaction, including RipAC from the bacterial pathogen *Ralstonia solanacearum* and PstSIE1 from the wheat yellow rust (*Puccinia striiformis* f. sp. *tritici*) (Nakano et al., 2020; Yu et al., 2020; Wang et al., 2022). Effector-driven divergence of host targets is well illustrated by potyviral VPg (viral genome-linked protein) and eIF4E (eukaryotic translation initiation factor 4E) of *Capsicum* plant hosts. Polymorphism analysis on both sides revealed that host eIF4E bears substitutions that confer recessive resistance while counter-mutations in VPg restore VPg–eIF4E interaction (Charron et al., 2008). Similar escape mechanisms also occur to immune genes, such as divergence of a helper NLR, NRC3 (NLR-required

for cell death 3) to evade immunosuppression by a binding effector (Sugihara et al., 2025). These observations support the idea that the RAR1–SGT1 divergence could have initiated by a SGT1-binding effector from an ancient fern pathogen (Figure 3.29).

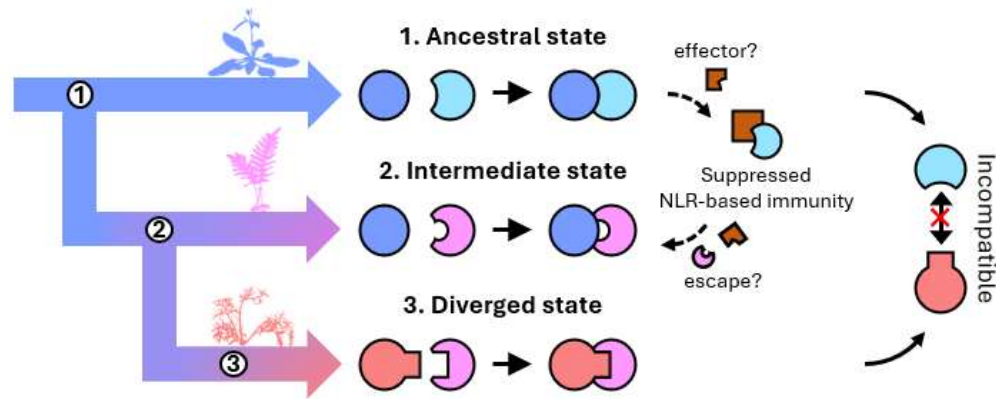


Figure 3.29 The RAR1–SGT1 binding interface diverged through promiscuous intermediates in ferns. Sequential mutations in SGT1 and a compensatory mutation in RAR1 shaped RAR1–SGT1 molecular coevolution in ferns. The divergence of fern SGT1 may have been initiated by SGT1-binding effectors, genetic drift, or both.

This work focused on land plants, leaving open questions about RAR1 and SGT1 evolution in other green plant (Viridiplantae) clades. A limited number of RAR1 and SGT1 homologs were identified in charophytes from the small dataset, and unexpectedly, the tested *C. braunii* homologs possess the canonical RAR1 domain structure, two tandem CHORD domains and the CCCH motif in the middle. This raises the question of when CHORD-containing proteins (CHPs) became RAR1-like and specialized for immunity rather than general development, since many CHORD-containing proteins in non-plant species are involved in development (Shirasu et al., 1999; Brancaccio et al., 2002; Groza et al., 2023). It had been assumed that the CCCH motif of RAR1 was specific to land plants, since it was observed in bryophytes and angiosperms but not in other eukaryotes. Interestingly, I found that charophyte RAR1 also harbors the CCCH motif, suggesting RAR1 arose from CHPs in charophytes or earlier.

I identified four critical residues that largely explain the specificity differences between CrRAR1–CrSGT1 and AtRAR1–AtSGT1 interactions. However, these residues did not fully explain all observed compatibility patterns. For instance, AfSGT1 (Salviniales) and HdSGT1 (Hymenophyllales) were both predicted to be promiscuous intermediates. They interacted with more RAR1 homologs than other fern SGT1 homologs, but their interaction pattern was not identical: AfSGT1 interacted with CrRAR1, while HdSGT1 failed. Similarly, SGT1 from ‘ancestral’ states (AtSGT1b and

OsmSGT1) should have interacted with most RAR1 homologs except CrRAR1. However, AtSGT1b interacted only with AtRAR1 and OsmSGT1 failed to bind HdRAR1 or AfRAR1. These discrepancies suggest that additional ‘hidden’ residues outside the RAR1^{CHORD II}–SGT1^{CS} interface make minor contributions to the binding specificity but can make different readouts during the screening.

Overall, this work shows that RAR1–SGT1 coevolution in ferns followed an evolutionary trajectory enabled by promiscuous intermediates in SGT1, which buffered interaction compatibility until a compensatory mutation in RAR1 restored the specificity. These findings not only provide a molecular explanation for divergence in fern RAR1–SGT1 interactions but also illustrate a general evolutionary principle: promiscuous PPI can act as an intermediate state that permits protein–protein interfaces to diverge without disrupting essential cellular processes.

3.4 Research contributions

I thank Dr. Yu Sugihara at The Sainsbury’s Laboratory for helping me to do ancestral state reconstruction. Y2H in Figure 3.28D was performed by Dr. Philip Carella at Cell and Developmental Biology department in John Innes Centre. I appreciate advice for split GAL4 Ruby assay from Dr. Indira Saado at Banfield group in John Innes Centre.

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CHAPTER 4. RAR1-mediated nonhost resistance in *Marchantia polymorpha*

4.1 Introduction

Oomycetes (Oomycota) are one of the two major eukaryotic plant pathogens alongside fungi. Although both are filamentous pathogens that share many morphological traits, including hyphal mycelia and spore-based reproduction, they are distantly related. Oomycetes belong to the Stramenopiles, thus they are more closely related to brown algae and diatoms than to fungi (Latijnhouwers *et al.*, 2003). Both groups also form specialized penetrative structures in plant cells, called haustoria, during infection. The shape of haustoria varies, and each haustorium is enveloped by a host-derived membrane, leaving a narrow apoplastic space between pathogen and host (Voegelé and Mendgen, 2003). Haustoria are key sites during infection where pathogen effector proteins are delivered, although precise translocation mechanisms remain less characterized than the bacterial effector translocation apparatus (Petre and Kamoun, 2014). Oomycota include many important angiosperm pathogens, such as *Aphanomyces euteiches* (pea pathogen), *Hyaloperonospora arabidopsidis* (a natural pathogen of *Arabidopsis thaliana*), *Phytophthora capsici* (infecting peppers, tomatoes, and cucurbits), and the most extensively studied species, *Phytophthora infestans* (potato pathogen) (Kamoun *et al.*, 2015).

4.1.1 *Phytophthora* species known as phytopathogens

Phytophthora is arguably the most popular oomycete genus, as it infects a wide range of economically important staple and ornamental crops, causing severe economic and food security losses (Brasier *et al.*, 2022). The *Phytophthora* genus includes some of the most notorious plant pathogens (Kamoun *et al.*, 2015). For example, *P. infestans* causes potato late blight, the disease responsible for the Great Irish Famine. This pathogen remains as a major threat to potato and tomato production worldwide (Haas *et al.*, 2009). *P. sojae* has a relatively narrow host range, infecting primarily soybeans. However, it is the second most destructive pathogen as soybeans are one of the world's most widely cultivated and traded crops (Tyler *et al.*, 2006). *P. ramorum* poses a significant threat to forests by infecting not only many tree species, including oak and larch, but also shrubs that are other important components of forest ecosystems. It causes rapid decline of several tree species, referred to as sudden oak death in the United States, and sudden larch death in the United Kingdom. In perspective of industry, it also causes severe damage in nurseries (Rizzo *et al.*, 2005). Similarly, *P. cinnamomi* damages native forests in Australia and Mediterranean regions and is a serious problem in commercial nurseries (Brasier *et al.*, 2022).

4.1.1.1 *Phytophthora palmivora*

Phytophthora palmivora is a hemibiotrophic pathogen primarily found in the tropics and subtropics. Its extremely broad host range, more than 170 different species across monocots and dicots, poses a severe threat to many tropical crops, including cacao, citrus, rubber, oil palm, and coconut (Ashby, 1929; Torres et al., 2016). A previous study has replicated *P. palmivora* infection and haustorium formation in coconut secondary roots under laboratory conditions (Harris et al., 1984). Haustorium formation following successful infection has also been documented across diverse model species: barley leaves and roots (Le Fevre et al., 2016), the temperate legume *Medicago truncatula* roots (Huisman et al., 2015; Rey et al., 2015), and even the bryophyte *Marchantia polymorpha*, which lacks true leaves and roots (Carella et al., 2018). In *M. polymorpha*, *P. palmivora* primarily infects the photosynthetic layer. It triggers cellular and molecular immune responses such as, callose deposition, localization of membrane-associated proteins (Rab GTPases and syntaxins), and upregulation of PR (pathogenesis-related) genes and phenylpropanoid pathway genes. Interestingly, these responses mirror the immune responses of compatible angiosperm hosts like *Nicotiana benthamiana* (Carella et al., 2018; Carella et al., 2019).

4.1.2 RAR1-mediated nonhost resistance

The RAR1–SGT1–HSP90 immune chaperone components contribute to nonhost resistance (NHR) by stabilizing NLR (nucleotide binding and leucine rich repeat) proteins, and the chaperone-mediated NHR is frequently compromised when this complex is disturbed (Shirasu, 2009). Each component presents advantages and limitations to study the chaperone-mediated NHR. *rar1* mutants do not show any developmental defects, making disease phenotype interpretation cleaner (Muskett et al., 2002; Tornero et al., 2002). However, when genetic knockouts are not available, RNAi (RNA interference)-based knockdown approaches can give ambiguous readouts due to low endogenous expression of *RAR1*. Partial depletion of RAR1 may not cross a functional threshold and thus demands more quantitative methods (Situ et al., 2020). For *SGT1*, silencing in *N. benthamiana* causes stunted growth (Peart et al., 2002), which complicates disentangling developmental and immune phenotypes. In *A. thaliana*, *AtSGT1a* and *AtSGT1b* have partially redundant functions in plant immunity with a predominant role of *AtSGT1b*. However, *Atsgt1a Atsgt1b* mutants are embryo-lethal (Austin et al., 2002; Azevedo et al., 2006). Plant genome encodes multiple HSP90 isoforms, and each isoform responds differently to infection, indicating there is a specialized HSP90 isoform for the chaperone-mediated NHR (Takahashi et al., 2003). Chemical inhibitor of HSP90, geldanamycin, can be used to suppress HSP90s (Takahashi et al., 2003), but the results may be pleiotropic in this context. Moreover, growing evidence indicate that HSP90 also has roles in plant immunity outside the canonical RAR1–SGT1–HSP90 complex (Chen et al., 2010; Zhang et al., 2015; Wei et al., 2021). In summary, when

genetic knockout is feasible, *RAR1* often offers the cleanest genetics to dissect the chaperone-mediated NHR.

4.1.3 Aims of this chapter

In the previous chapter, I showed that RAR1–SGT1 interaction is conserved across land plants including *M. polymorpha*. This suggests that *M. polymorpha* possesses a functional RAR1–SGT1–HSP90 complex and the chaperone-mediated NHR is conserved in this species (Figure 4.1). CRISPR/Cas9-based genome editing (Sugano et al., 2018) enabled the generation of *M. polymorpha rar1* knockout mutants. In this chapter, I investigated whether RAR1 mediates NHR in *Marchantia–Phytophthora* interactions by screening *M. polymorpha* Tak1 (wild type, WT) and *rar1*-13 mutants with 33 diverse *Phytophthora* strains. These results highlight possible interactions between MpNLR proteins and *Phytophthora* effectors. Furthermore, I examine HSP90-interacting residues in MpRAR1 and MpSGT1 and argue that the RAR1–SGT1–HSP90 complex can assemble and function in *M. polymorpha*. This suggests that *M. polymorpha* is an exciting new resource for discovering R genes against important *Phytophthora* species that are understudied and less accessible.

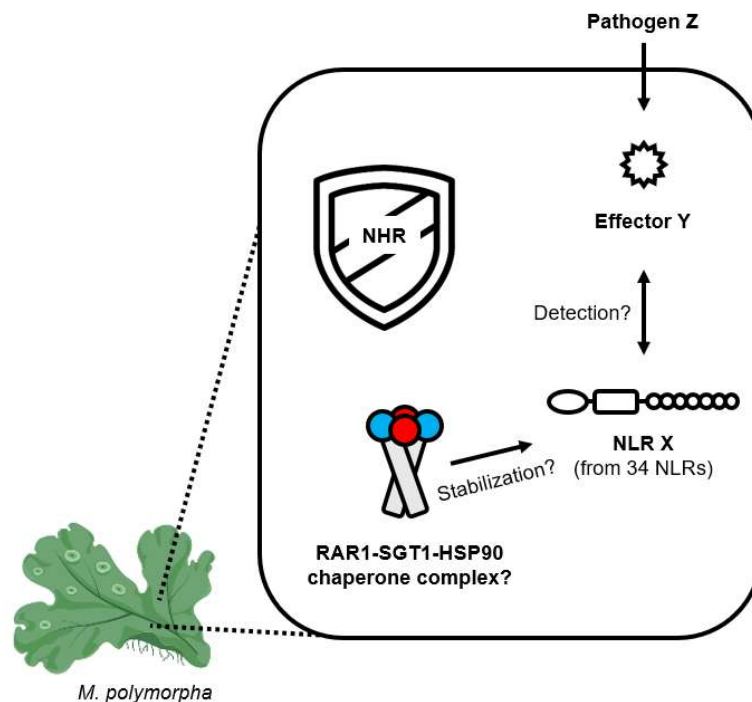


Figure 4.1 Is RAR1-mediated NHR conserved in *Marchantia polymorpha*? If RAR1–SGT1–HSP90 complex is functionally conserved in *M. polymorpha*, the mechanism defined in angiosperms may also exist in this species. To test this, 33 *Phytophthora* strains were screened to identify a suitable pathogen showing enhanced infection in *Mprar1* mutants.

4.2 Results

4.2.1 Interrogating RAR-mediated NHR in *M. polymorpha* with diverse *Phytophthora* pathogens

Pathogenic interactions between *M. polymorpha* and microbial pathogens result in macroscopically visible disease symptoms including progressive chlorosis and tissue maceration. Correlations between these visual cues and the underlying pathogenesis have been addressed by microscopic and molecular biological approaches (Carella et al., 2018; Gimenez-Ibanez et al., 2019; Redkar et al., 2022a).

To determine whether *Marchantia* liverworts harbor RAR1-mediated NHR against *Phytophthora*, I performed an agar plug-based *Phytophthora* infection screen of *M. polymorpha* WT (Tak-1) and *rar1*-13 mutants as described in chapter 2 (Figure 2.1; Figure 2.2). Briefly, macroscopic visual cues were used to quantify disease phenotypes over a 7-day time course. First, lesion severity was scored at 7 days post infection (dpi) based on the proportion of thallus tissue showing visible symptoms (e.g. chlorosis, maceration, and water-soaking) around the agar plug. Second, infection efficiency was calculated based on number of inoculation sites with successful colonization out of 32 sites (= 8 plants). Environmental conditions and inoculation procedures were kept constant: standardized agar plug sizes were used and always inoculation onto the dorsal plant surface using the hyphal side of the plug.

The 33 *Phytophthora* strains tested in this experiment belong to 16 distinct species and span five phylogenetic clades (Figure 4.2). Each strain showed distinct disease phenotypes, and differences were even observed among strains of the same species. In the following sections, I briefly describe the macroscopic phenotypes observed for each of the 33 *Phytophthora* strains at 7 dpi by subclade or clade, noting lesion severity (0–3, where 3 is the most severe), infection efficiency (out of 32 sites), and distinctive features. The subsequent section summarizes these observations into figures and statistics based on quantitative data.

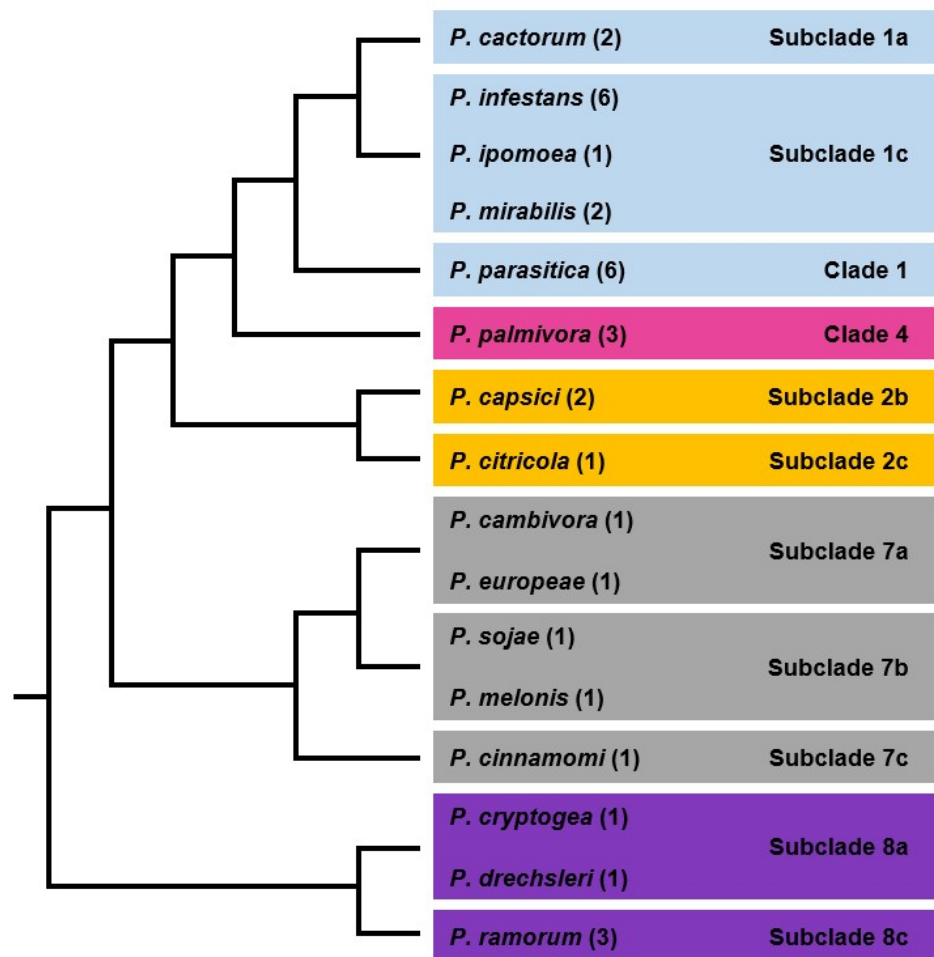


Figure 4.2 Phylogenetic relationships among 33 *Phytophthora* strains used in this chapter. The number of strains per species are indicated next to the species name, and the clade where each species belongs is shown on the right. The phylogenetic tree is modified from Yang et al., (2017).

4.2.2 *rar1* mutation enabled infection of three non-adapted *Phytophthora* strains

To address the breakdown of RAR-mediated NHR in *M. polymorpha*, I analyzed quantitative data for each strain, lesion severity and infection efficiency, in a pairwise comparison between the two host genotypes (WT and *rar1* mutants) (Figure 4.3–4; Table S4–5).

Both infectious strains and non-adapted strains were identified from the analyses. Five strains, two *P. capsici* strains, two *P. parasitica* strains, and *P. palmivora* MAZI, consistently scored 3 in lesion severity and 100% in infection efficiency. Eleven strains completely failed to colonize WT and *rar1*-13 mutants (Figure 4.3; Table S4). The ‘no infection’ group included subclade 1c strains (except *P. infestans* 88069-td), *P. cambivora* (subclade 7a), *P. sojae* (subclade 7b), and *P. cryptogea* (subclade 8a).

Six strains exhibited greater lesion severity in *rar1*-13 mutants than in WT, indicating that RAR1-mediated NHR contributes to resistance against these *Phytophthora* strains (Table S5). Among them,

three strains showed more distinct differences in lesion severity between wildtype and *rar1* mutants: *P. cinnamomi*, *P. ramorum* 128, and *P. citricola*. Interestingly, these three strains differed in infection efficiency: *P. citricola* displayed no differences in infection efficiency between two genotypes (i.e. all agar plugs lead to some amount of infection), while two other strains exhibited higher infection efficiency in *rar1*-13 mutants than in WT (Figure 4.4; Table S5).

Pathogenicity of species varied by strain, consistent with the previous report about *P. palmivora* infection in *M. polymorpha* (Carella et al., 2018). For example, *P. palmivora* LILIfp barely colonized *M. polymorpha* while *P. palmivora* ARI-td and MAZI colonized the host successfully. In *P. parasitica*, two strains, R0 and 329, had weaker symptoms compared to the other four strains. Notably, two *P. ramorum* strains varied not only in lesion severity, but also in infection efficiency by loss of *RAR1* (Figure 4.3; Figure 4.18).

Taken together, these data revealed that NHR of *M. polymorpha* to six non-adapted *Phytophthora* strains may be dependent on the RAR1-SGT1-HSP90 complex, while pathogenicity varied by strain.

Table 4.1 *Phytophthora* strains exhibited a breakdown of RAR1-mediated NHR in *M. polymorpha*

Clade	Species	Strain	Plant genotype	Lesion severity	Infection degree	p value	Infection efficiency
7c	<i>P. cinnamomi</i>		Tak1	0.3±0.4	Weak	<0.0001	28.13%
			<i>rar1</i> -13	1.9±1.1	Moderate		84.38%
8c	<i>P. ramorum</i>	128	Tak1	0.6±0.6	Weak	<0.0001	53.13%
			<i>rar1</i> -13	1.2±0.5	Moderate		96.88%
2c	<i>P. citricola</i>		Tak1	1.5±0.6	Moderate	<0.0001	100.00%
			<i>rar1</i> -13	2.7±0.5	Strong		100.00%
8c	<i>P. ramorum</i>	cbs 101328	Tak1	0.8±0.5	Weak	0.0017	75.00%
			<i>rar1</i> -13	1.2±0.4	Moderate		100.00%
1a	<i>P. cactorum</i>	Pc404	Tak1	1.3±0.5	Moderate	0.0066	96.88%
			<i>rar1</i> -13	1.7±0.7	Moderate		100.00%
1	<i>P. parasitica</i>	329	Tak1	1.9±0.3	Moderate	0.0066	100.00%
			<i>rar1</i> -13	2.3±0.6	Strong		100.00%

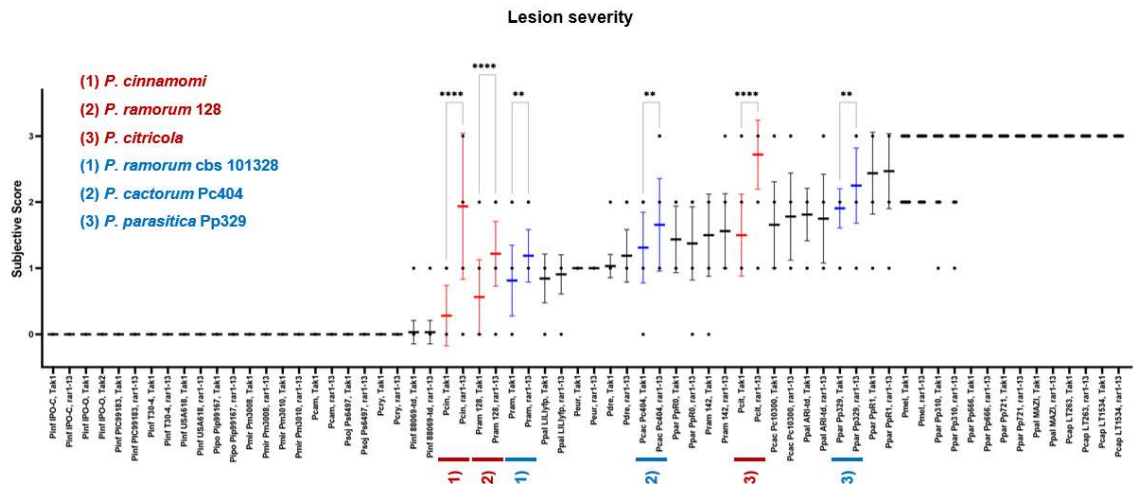


Figure 4.3 Lesion severity of 33 *Phytophthora* strains in *M. polymorpha*. Disease phenotypes were assessed for each plant genotype–pathogen combination in 32 independent tests. Lesion severity was quantified by subjective scoring. Pathogens showing differential infection between WT and *rar1* mutants are highlighted in red or blue (red: ****, $p < 0.0001$; blue: $0.0001 < p < 0.01$). Strains with $p > 0.05$ are not highlighted. Names of selected candidate strains are indicated at the top left.

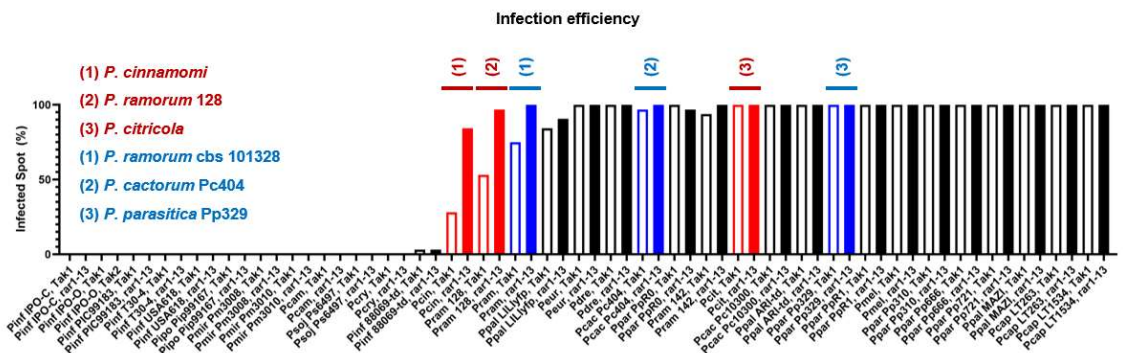


Figure 4.4 Infection efficiency of 33 *Phytophthora* strains in *M. polymorpha*. Infection efficiency was quantified from the same experiment described in Figure 4.3. Pathogens identified as differentially infecting in the lesion severity analysis (Figure 4.3) are labeled with the same color scheme.

4.2.2.1 Disease phenotypes of two *P. cactorum* strains in subclade 1a

Two *P. cactorum* strains, Pc404 and Pc10300, were tested. Both strains infected WT and *rar1-13* mutants similarly by visual inspection, and the lesion severity fell into the same category; moderate ($1 < \text{score} \leq 2$). However, one-way ANOVA analysis on *P. cactorum* Pc404 disease phenotypes revealed that this pathogen infected *rar1-13* mutants significantly better than WT (Figure 4.5). Interestingly, I observed water-soaking around the inoculation site, accompanied by discoloration, which is also reported from strawberry crown rot caused by *P. cactorum* (Tuomainen et al., 2024). These disease phenotypes suggest that *P. cactorum* successfully colonizes both plant species using similar pathogenesis strategy and then progress to a necrotrophic phase.

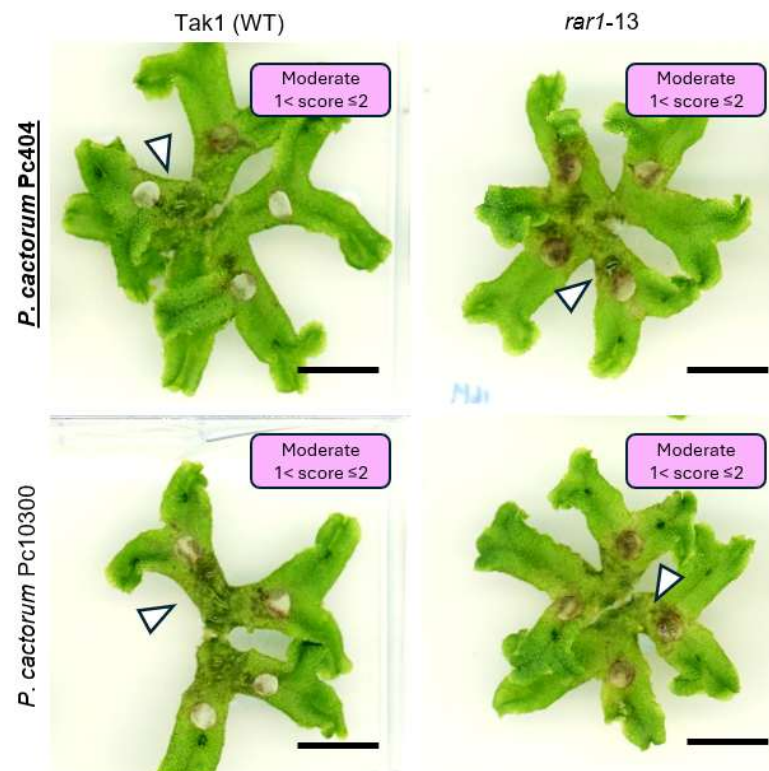


Figure 4.5 Disease phenotypes of two *P. cactorum* strains in subclade 1a. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Underline indicates pathogen strains that showed differential infection between two plant genotypes (WT and *rar1*). White arrowheads indicate sites with water-soaking. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.2. Disease phenotypes of *Phytophthora* species in subclade 1c

Three *Phytophthora* species in subclade 1c were included in the screen. A total of nine strains were tested: six strains of *P. infestans*, one strain of *P. ipomoeae*, and two strains of *P. mirabilis*. All nine strains failed to infect WT or *rar1-13* mutants, and they were classified as the no infection (score = 0) category (Figure 4.6–4.8). This is consistent with a previous study tested *P. infestans* 88069-td, which was re-tested and showed the same result in this screen (Carella et al., 2018). The other five *P. infestans* strains also failed to infect either genotype, suggesting that NHR against *P. infestans* in *M. polymorpha* may not rely on RAR1-dependent NLR signaling.

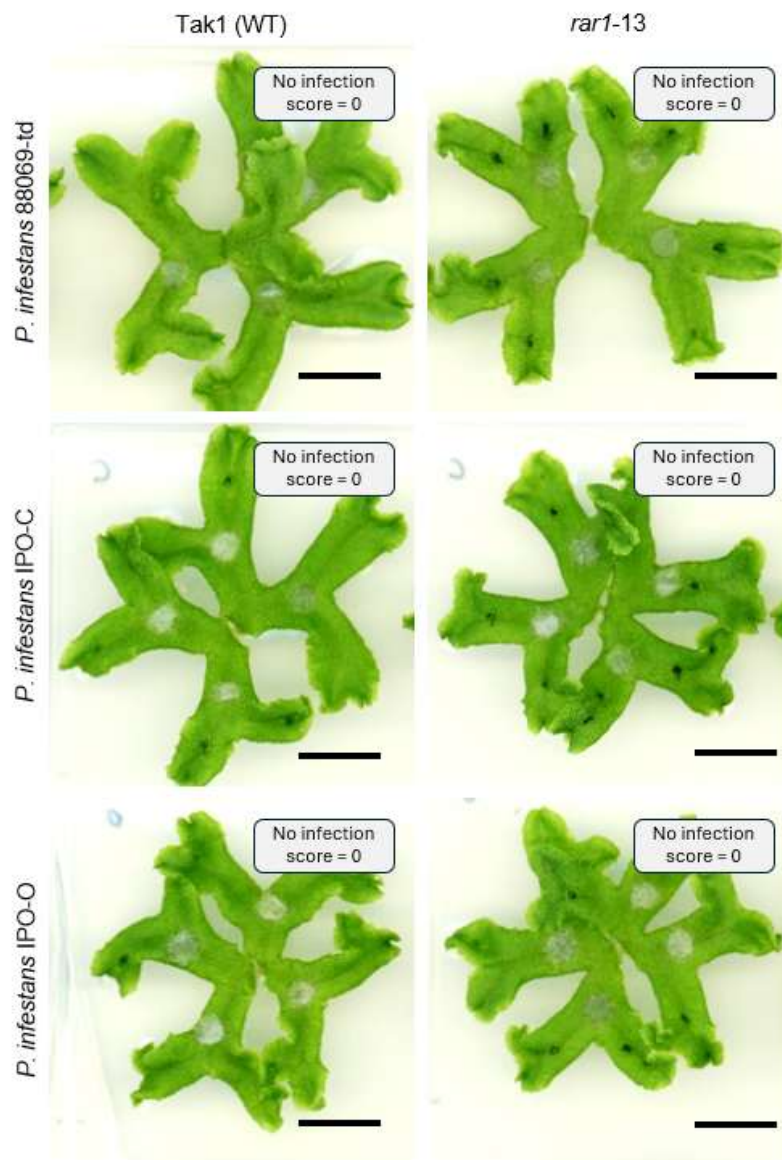


Figure 4.6 Disease phenotypes of three *P. infestans* strains in subclade 1c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Plants were imaged at 7 dpi. Bar = 1 cm.

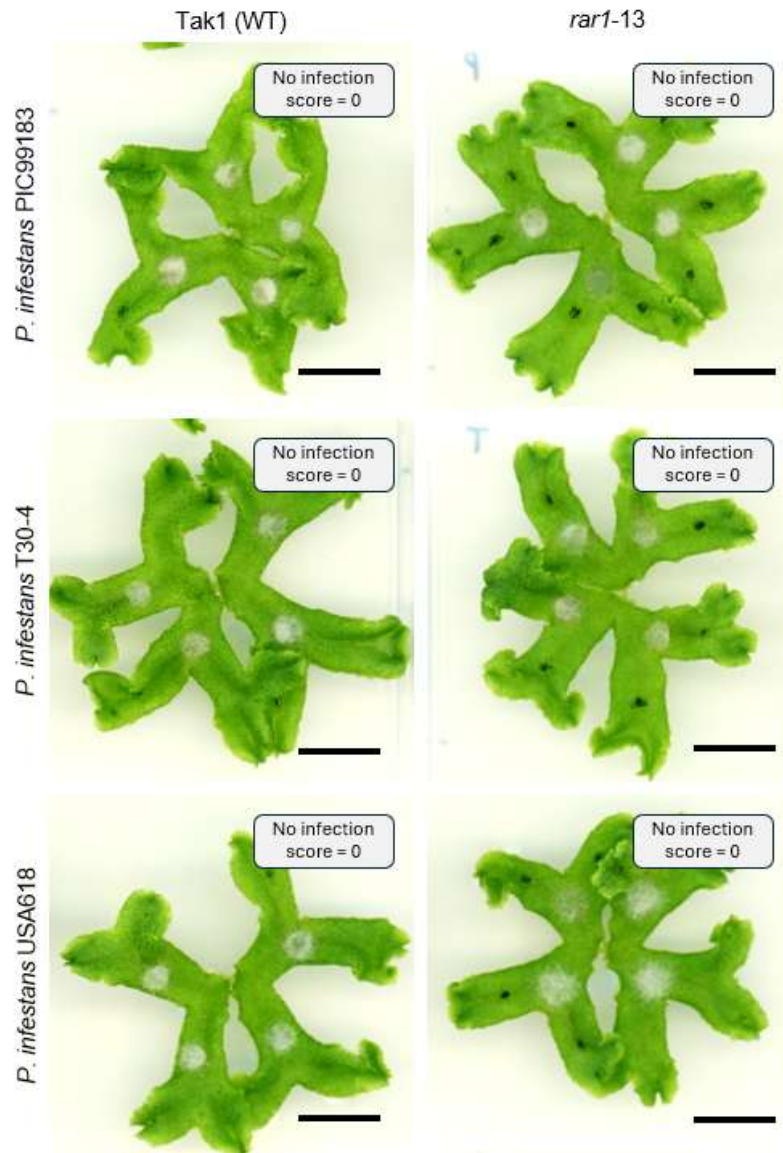


Figure 4.7 Disease phenotypes of three other *P. infestans* strains in subclade 1c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Plants were imaged at 7 dpi. Bar = 1 cm.

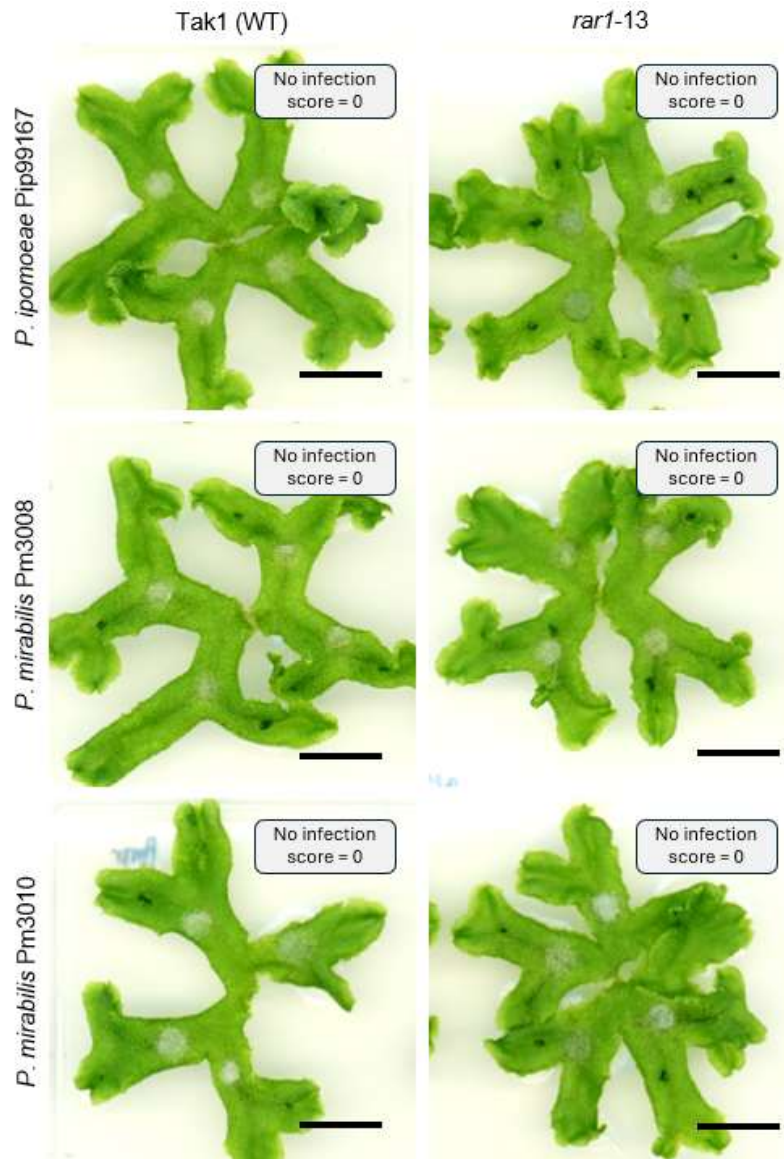


Figure 4.8 Disease phenotypes of *P. mirabilis* and *P. ipomoeae* in subclade 1c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.3 Disease phenotypes of six *P. parasitica* strains in clade 1

All six strains of *P. parasitica* infected both WT and *rar1-13* mutants, ranging from moderate ($1 < \text{score} \leq 2$) to strong ($2 < \text{score} \leq 3$). One strain (R0) showed moderate infection, while four strains (R1, 310, 666 and 721) showed strong infection. Interestingly, the 329 strain showed stronger infection in WT than *rar1-13* mutants in terms of lesion severity, although there was no difference in infection efficiency between them (Figure 4.9–10; Table S5). I observed water-soaking with three strains, R1, 310, and 666, which is a symptom that often precedes necrosis of roots in angiosperms (Meng et al., 2014). Strain 721 exhibited strong hyphal growth on the thalli, appearing fluffy white, alongside

chlorosis (Figure 4.10).

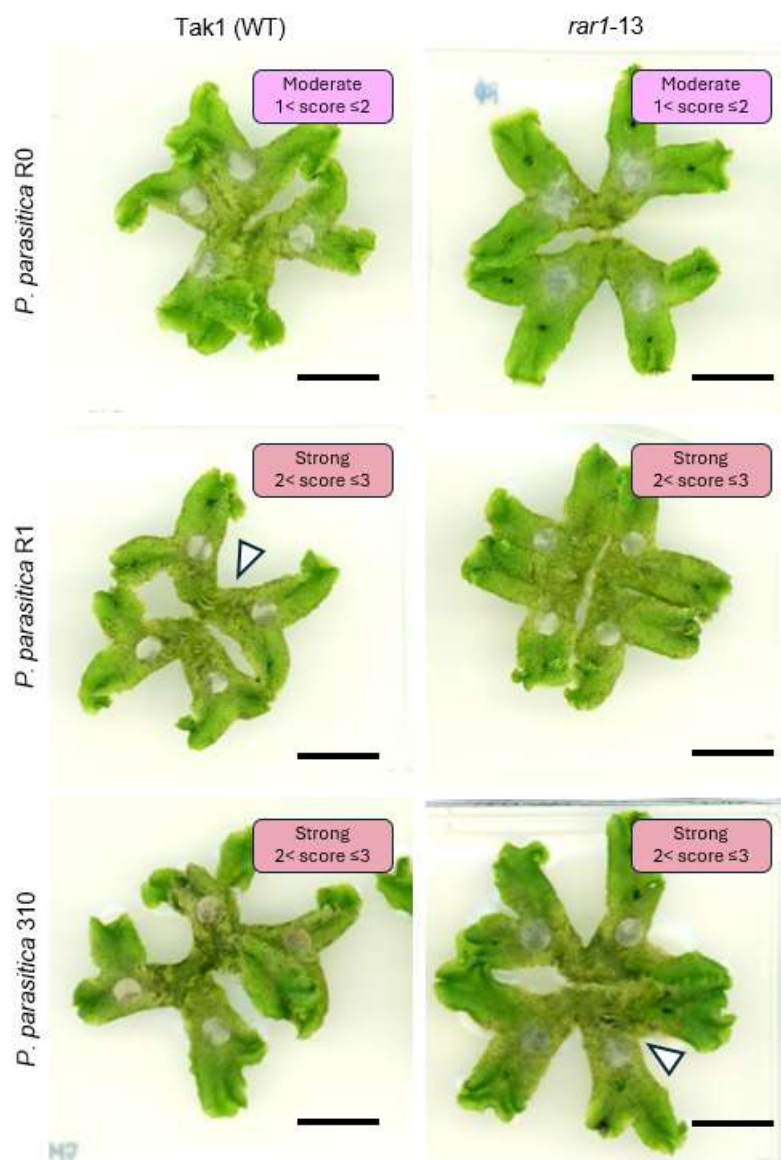


Figure 4.9 Disease phenotypes of three *P. parasitica* strains in clade 1. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. White arrowheads indicate sites with water-soaking. Plants were imaged at 7 dpi. Bar = 1 cm.

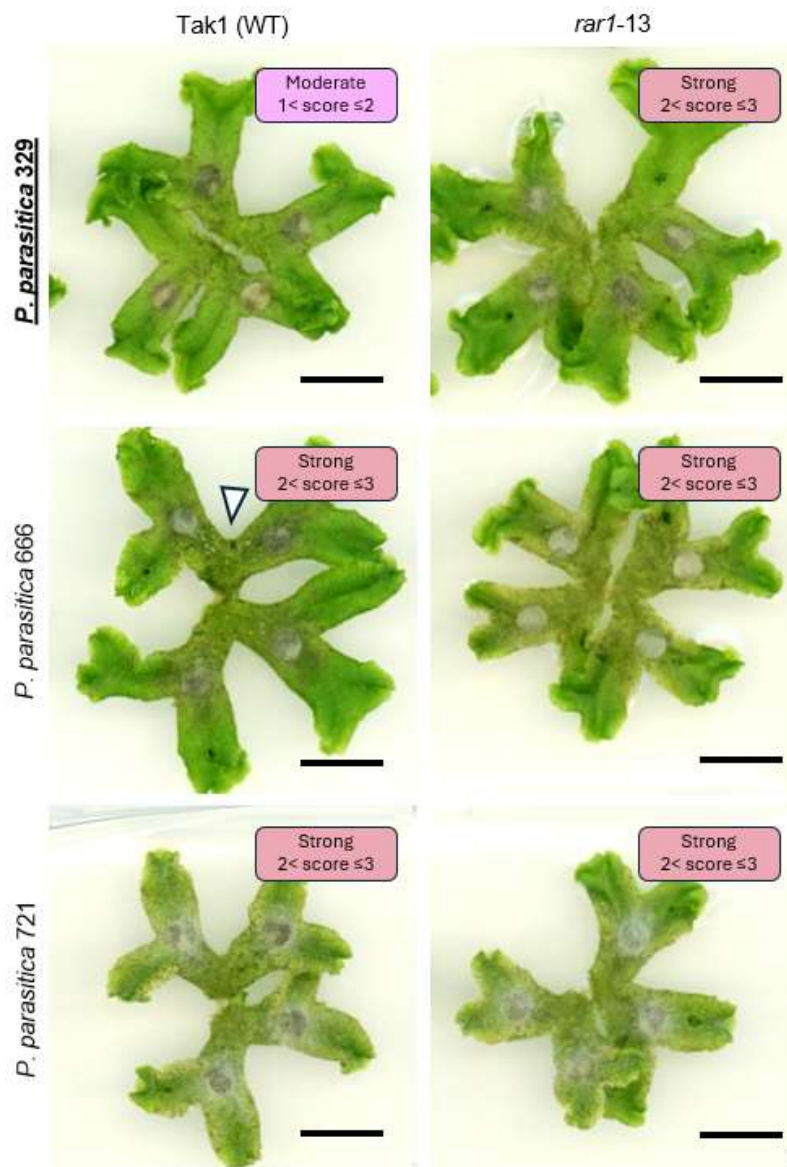


Figure 4.10 Disease phenotypes of three other *P. parasitica* strains in clade 1. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Underline indicates pathogen strains that showed differential infection between two plant genotypes (WT and *rar1*). White arrowheads indicate sites with water-soaking. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.4 Disease phenotypes of three *P. palmivora* strains in clade 4

Three *P. palmivora* strains, ARI-td, LILIfp, and MAZI, displayed strain-specific differences in pathogenicity. LILIfp showed weak infection ($0 < \text{score} \leq 1$), ARI-td showed moderate infection ($1 < \text{score} \leq 2$), and MAZI showed strong infection ($2 < \text{score} \leq 3$) (Figure 4.11). This is consistent with a previous study that reported varying aggressiveness of eleven *P. palmivora* strains, including ARI-td and MAZI, in *M. polymorpha* (Carella et al., 2018). There was no differential infection between

two genotypes, but MAZI showed several interesting disease phenotypes in both genotypes. MAZI infection resulted in generally smaller size, indicating rapid disease progression, and accompanied with pronounced darkening and water-soaking. Strong darkening was particularly observed in the notches, the meristematic regions of *M. polymorpha* (Figure 4.11).

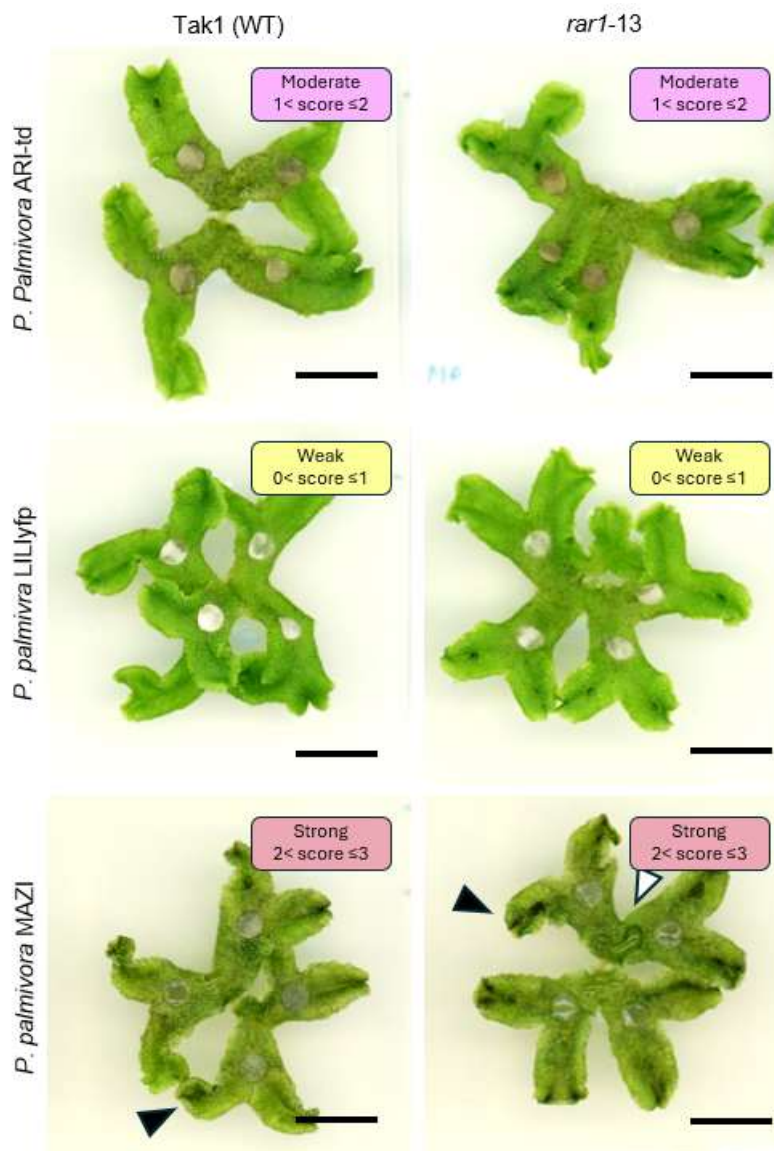


Figure 4.11 Disease phenotypes of three *P. palmivora* strains in clade 4. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. White arrowheads indicate sites with water-soaking. Black arrowheads indicate darkening in notches. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.5 Disease phenotypes of three *Phytophthora* strains in clade 2

Two strains of *P. capsici* and one strain of *P. citricola* successfully colonized WT and *rar1-13* mutants. Two *P. capsici* strains displayed strong infection ($2 < \text{score} \leq 3$) equally in both genotypes, with distinct darkening in notches and the ‘midvein’ area. The disease progression was sometimes visible macroscopically, such that a diseased area with tissue darkening appeared next to a healthy green area and was separated by thin zone of chlorosis (Figure 4.12). *P. citricola* caused chlorosis in both genotypes and resulted in greater lesion severity in *rar1-13* mutants than in WT, without any difference in infection efficiency (Figure 4.13; Table S5).

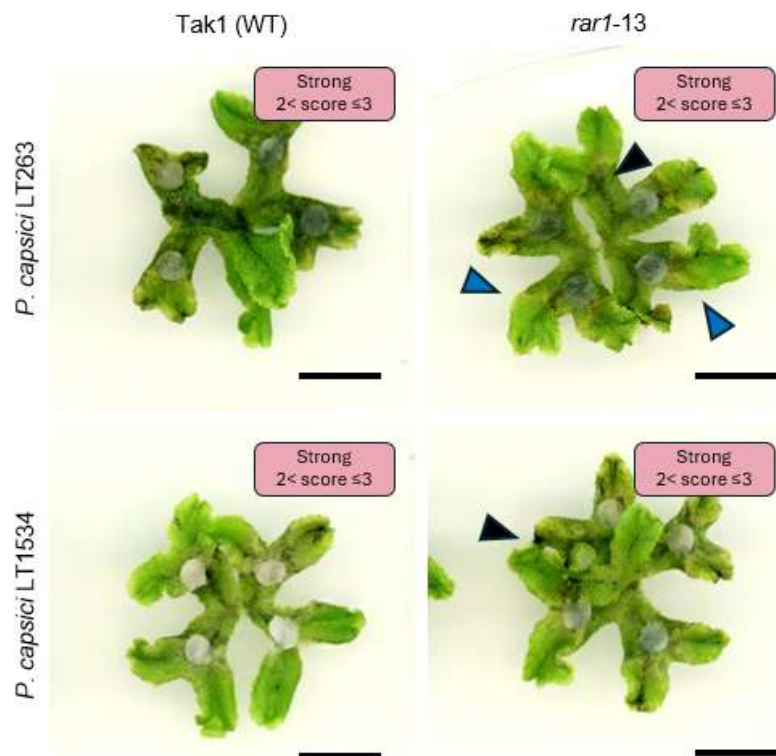


Figure 4.12 Disease phenotypes of two *P. capsici* strains in subclade 2b. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Black arrowheads indicate darkening in notches. Blue arrowheads indicate visible disease progression. Plants were imaged at 7 dpi. Bar = 1 cm.

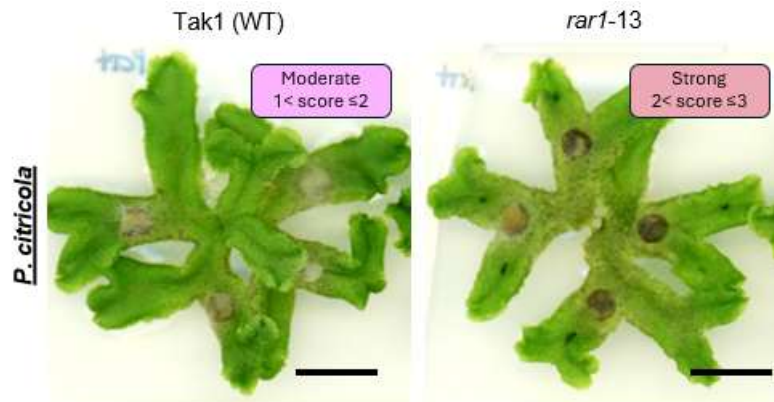


Figure 4.13 Disease phenotypes of *P. citricola* in subclade 2c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Underline indicates pathogen strains that showed differential infection between two plant genotypes (WT and *rar1*). Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.6 Disease phenotypes of five *Phytophthora* species in clade 7

Five species were examined in clade 7: *P. cambivora* (subclade 7a), *P. sojae* (subclade 7b), *P. europaea* (subclade 7a), *P. melonis* (subclade 7b), and *P. cinnamomi* (subclade 7c). Two strains, *P. cambivora* and *P. sojae* Ps6497, caused no infection in either genotype (score = 0) (Figure 4.14; 4.16), whereas other three strains induced visible discoloration.

P. europaea showed robust hyphal growth like *P. parasitica* 721. However, only occasional speckles around inoculation sites were observed, and it was classified as weak infection ($0 < \text{score} \leq 1$) group in both genotypes (Figure 4.14). *P. melonis* caused strong infection ($2 < \text{score} \leq 3$) with clear disease progression, but without the pronounced darkening seen with *P. palmivora* MAZI or *P. capsici* (Figure 4.16).

P. cinnamomi showed differential infection between genotypes. *M. polymorpha* WT exhibited only occasional brown spots with chlorosis, whereas *rar1-13* mutants displayed broad discoloration and more extensive colonization. Moreover, *P. cinnamomi* infection efficiency was higher in *rar1-13* mutants (Figure 4.15; Table S5).

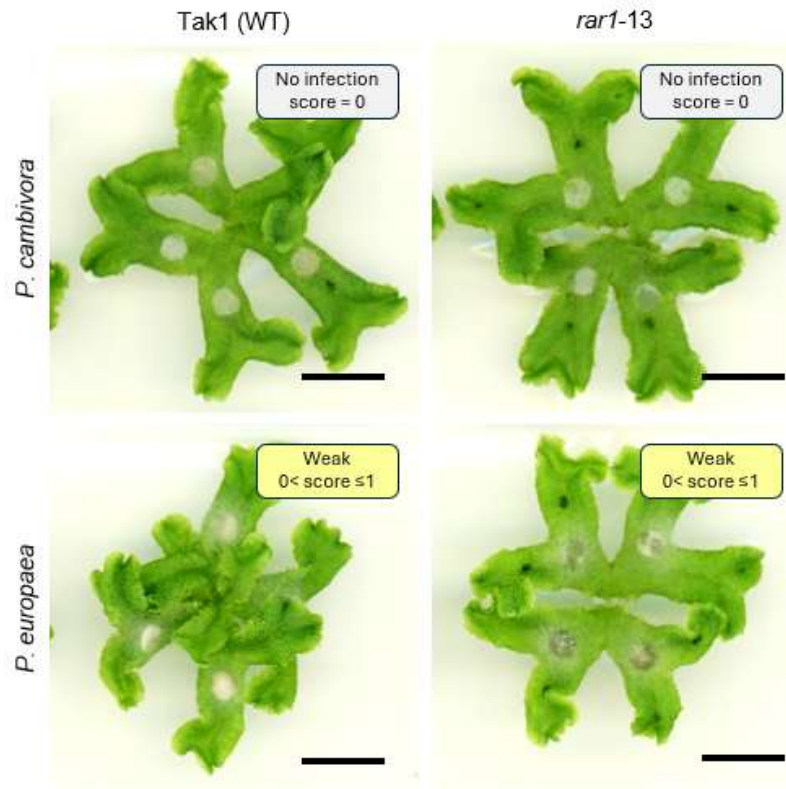


Figure 4.14 Disease phenotypes of two *Phytophthora* species in subclade 7a. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Plants were imaged at 7 dpi. Bar = 1 cm.

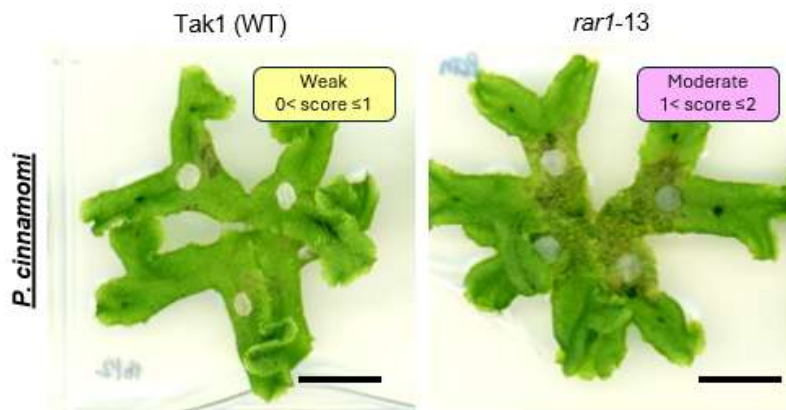


Figure 4.15 Disease phenotypes of *P. cinnamomi* in subclade 7c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Underline indicates pathogen strains that showed differential infection between two plant genotypes (WT and *rar1*). Plants were imaged at 7 dpi. Bar = 1 cm.

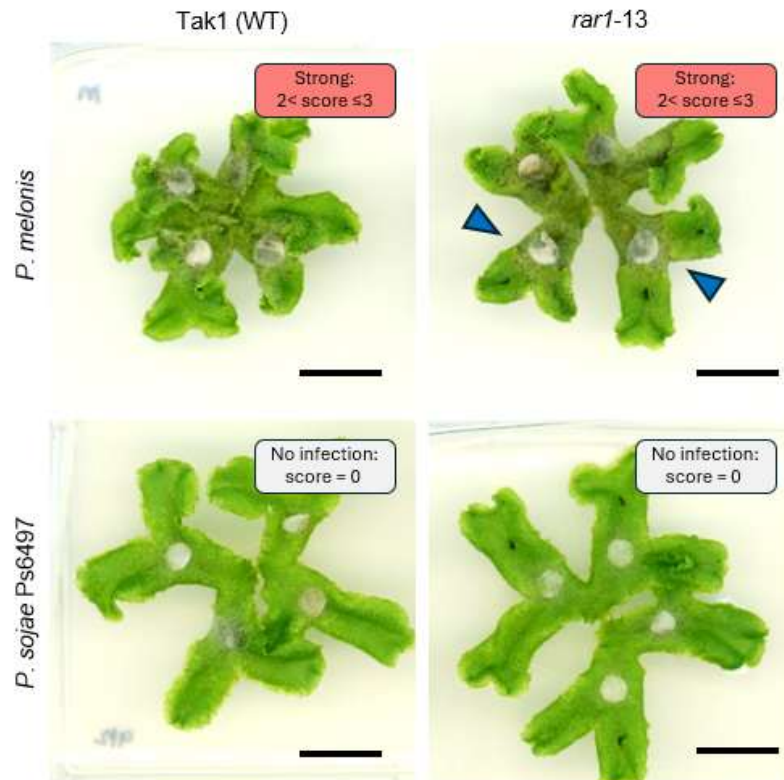


Figure 4.16 Disease phenotypes of two *Phytophthora* species in subclade 7b. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Blue arrowheads indicate visible disease progression. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.7 Disease phenotypes of two *Phytophthora* species in subclade 8a

Two species in subclade 8a, *P. cryptogea* and *P. drechsleri*, were examined. *P. cryptogea* caused no infection in either genotype (score = 0). *P. drechsleri* colonized both WT and *rar1-13* mutants. Lesions in WT were classified as weak ($0 < \text{score} \leq 1$), while lesions in *rar1-13* were moderate ($1 < \text{score} \leq 2$). However, the difference between genotypes was not statistically significant (Figure 4.17; Table S4).

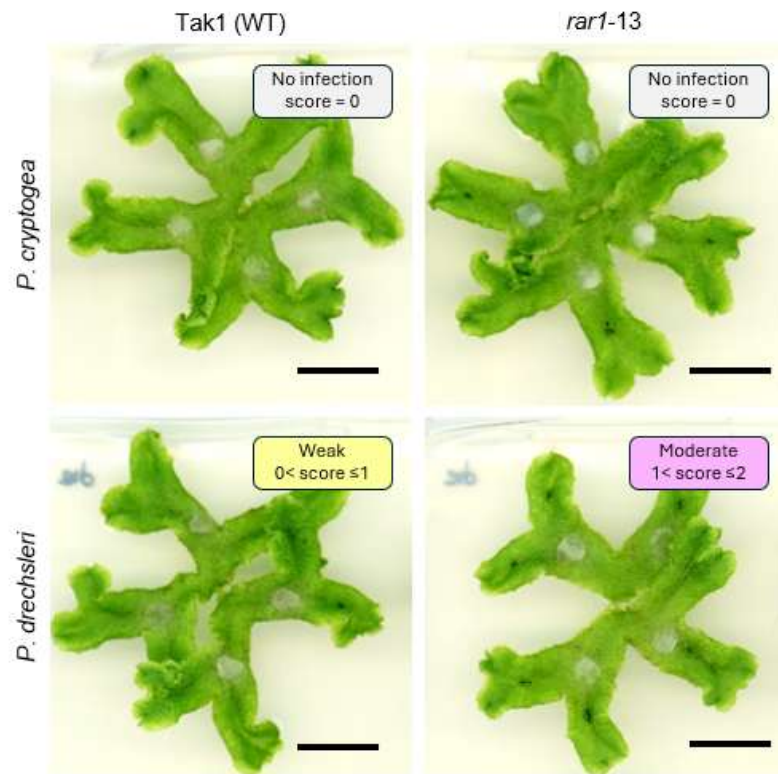


Figure 4.17 Disease phenotypes of two *Phytophthora* species in subclade 8a. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.2.8 Disease phenotypes of three *P. ramorum* strains in subclade 8c

Three *P. ramorum* strains were tested and all caused weak ($0 < \text{score} \leq 1$) to moderate infection ($1 < \text{score} \leq 2$). Two strains, *P. ramorum* cbs 101328 (hereafter, *P. ramorum* cbs) and *P. ramorum* 128 showed greater lesion severity and infection efficiency on *rar1*-13 mutants compared to WT (Table 4.1; Figure 4.18). Interestingly, *P. ramorum* 142 showed no genotype-dependent difference (Figure 4.3). These results indicate that RAR1 confers resistance to at least a subset of *P. ramorum* isolates.

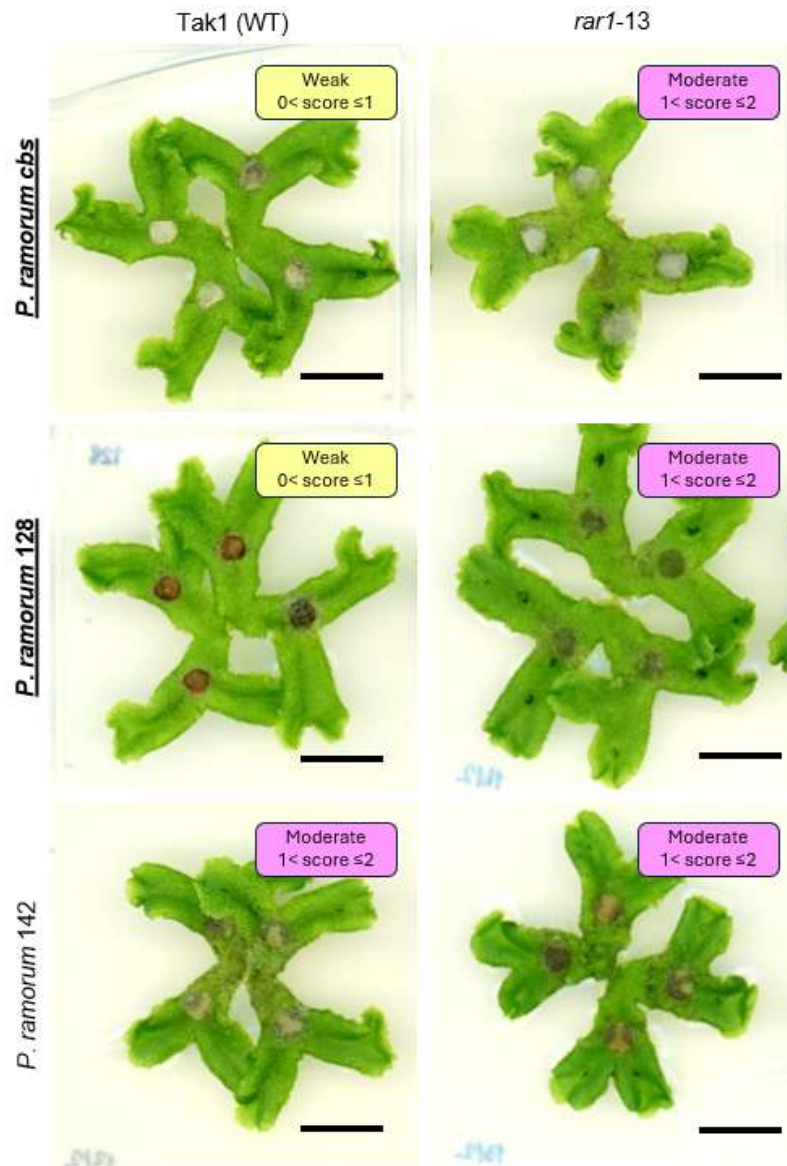


Figure 4.18 Disease phenotypes of three *P. ramorum* strains in subclade 8c. The most representative image among 8 plants (32 infection sites) is shown. Quantified lesion severity is indicated at the top right of each image. Underline indicates pathogen strains that showed differential infection between two plant genotypes (WT and *rar1*). Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.3 *Marchantia*–*Phytophthora* compatibility was not related to known host range

Diverse outcomes from interactions between *M. polymorpha* and 33 *Phytophthora* strains show that closely related isolates can produce markedly different disease phenotypes, such as visible symptoms, lesion severity, and infection efficiency (Figure 4.19). For instance, all strains in subclade 1c failed to infect *M. polymorpha*, whereas *P. parasitica* strains, which are in a different clade of clade 1, completely colonized the host. Likewise, two species in subclade 7b, *P. sojae* and *P. melonis*, exhibited strikingly different phenotypes. *P. sojae*-infected *M. polymorpha* thalli remained green and symptomless until 7 dpi, whereas *P. melonis* caused large discoloration and tissue maceration. This pattern is consistent with an extensive survey of 196 culturable *Phytophthora* species showing no clear correlation between clade and host range (Brasier et al., 2020). Therefore, strain- and species-level genetics are likely more critical determinants for *Marchantia*–*Phytophthora* compatibility.

To test this possibility, I surveyed host range lists that are widely accepted by plant pathologists for the *Phytophthora* species used here and compiled them into a reference set. For one species, *P. europaea*, its host range has not been tested extensively since the original description. Therefore, its host range was described the species where it was isolated (Jung et al., 2002; Balci et al., 2006). The resulting table was aligned with the disease phenotype of the *Phytophthora* strains in *M. polymorpha* WT (Table 4.2). Overall, no clear relationship was found between reported host range and the capacity to infect *M. polymorpha*. Many broad host range *Phytophthora* failed to infect or weakly infected *M. polymorpha*, such as *P. cambivora* (no infection), *P. cryptogea* (no infection), and *P. drechsleri* (weak infection). By contrast, *P. melonis*, which has a relatively restricted host range (primarily cucurbits), was one of the most aggressive strains in *M. polymorpha*.

Table 4.2 Comparison between disease phenotypes in *M. polymorpha* WT (Tak1) and host range

Clade	Species	Infection in Tak1	Host range	References
1a	<i>P. cactorum</i>	Moderate	broad	Erwin and Ribeiro, 1996
	<i>P. infestans</i>	No	Solanaceae	Becktell et al., 2006
1c	<i>P. ipomoeae</i>	No	species-specific	Flier et al., 2002
	<i>P. mirabilis</i>	No	species-specific	Goodwin and Fry, 1994
1	<i>P. parasitica</i>	Moderate-Strong	broad	Meng et al., 2014
4	<i>P. palmivora</i>	Weak-Strong	broad	Erwin and Ribeiro, 1996
2b	<i>P. capsici</i>	Strong	broad	Lamour et al., 2012
2c	<i>P. citricola</i>	Moderate	broad	Jung and Burgess, 2009
7a	<i>P. cambivora</i>	No	broad	Mullett et al., 2023
	<i>P. europaea</i>	Weak	Oak trees (<i>Quercus</i>)	Jung et al., 2002
7b	<i>P. melonis</i>	Strong	Cucurbits	Erwin and Ribeiro, 1996
	<i>P. sojae</i>	No	species-specific	Tyler, 2006
7c	<i>P. cinnamomi</i>	Weak	broad	Hardham and Blackman, 2017
8a	<i>P. cryptogea</i>	No	broad	Cooper et al., 2025
	<i>P. drechsleri</i>	Weak	broad	Olson and Benson, 2012
8c	<i>P. ramorum</i>	Weak-Moderate	broad	Grünwald et al., 2008

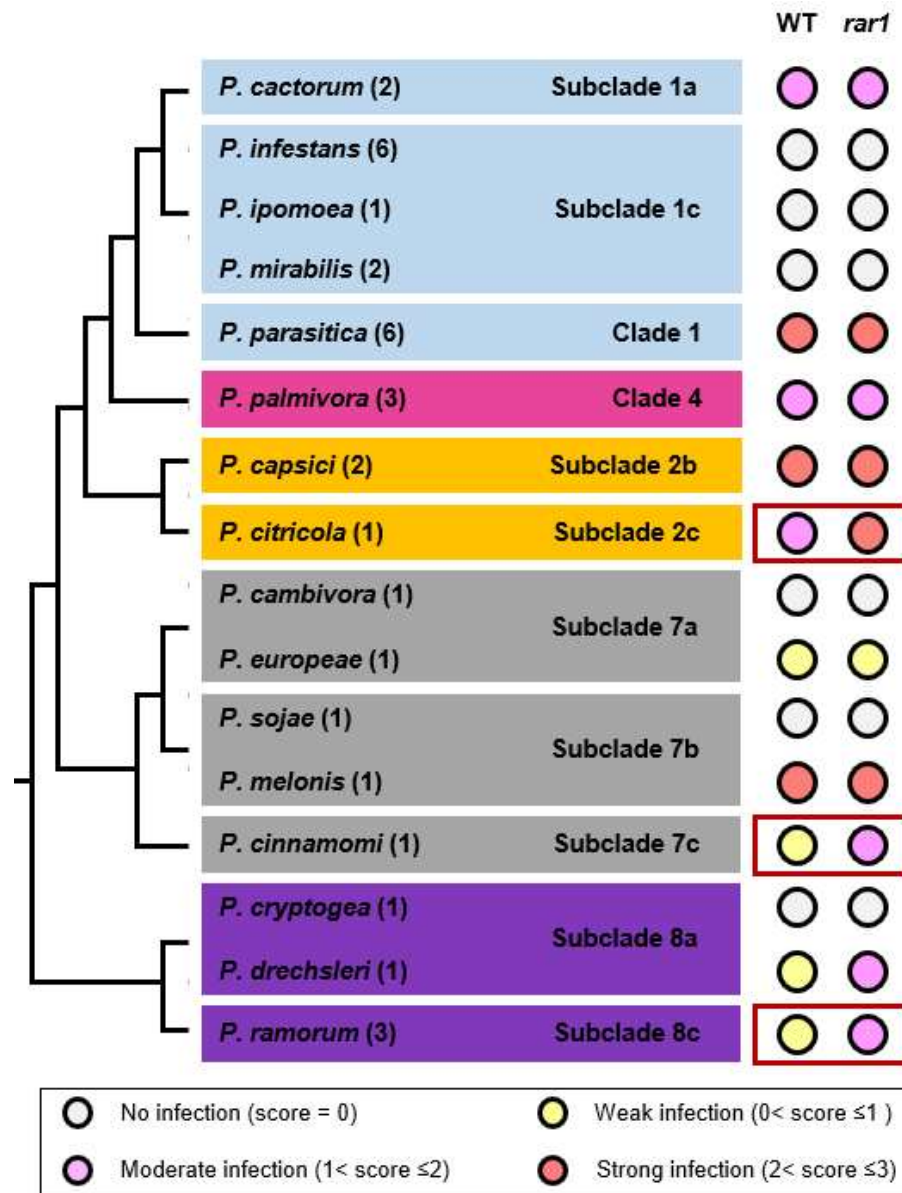


Figure 4.19 Summary of *Marchantia*–*Phytophthora* infection screening. Species with the top three strains showed significant differential infection between Tak1 and *rar1*-13 mutants were marked with red boxes. Average lesion severity for each plant genotype–pathogen combination is indicated by color: light gray = no infection (0), pale yellow = weak infection (0–1), pink = moderate infection (1–2), and salmon pink = strong infection (2–3). When there are multiple strains in a species, the average is illustrated. The phylogenetic relationships among *Phytophthora* strains are modified from Yang et al. (2017).

4.2.4 The chaperone-mediated nonhost resistance is conserved in *M. polymorpha*

4.2.4.1 HSP90-dependency of *P. ramorum* and *P. cinnamomi* resistance in *M. polymorpha*

To identify whether other components of the RAR1-SGT1-HSP90 chaperone complex are involved in the nonhost resistance of *M. polymorpha*, I challenged *Mphsp90.2-71* mutants with two candidate pathogens, *P. ramorum* 128 and *P. cinnamomi*, alongside WT and *Mprarl-13* mutants. In a preliminary experiment, both pathogens colonized *Mphsp90.2-71* more readily than WT. For *P. ramorum* 128, WT showed no visible symptoms, whereas occasional darkening was observed in *Mphsp90.2-71* and *Mprarl-13* mutants. For *P. cinnamomi*, its infection was enhanced in *Mphsp90.2-71* and *Mprarl-13* mutants, with larger area of water-soaking and chlorosis (Figure 4.20). Together, these results suggest that MpRAR1–MpSGT1–MpHSP90.2 complex contributes to NLR-mediated NHR against these pathogens.

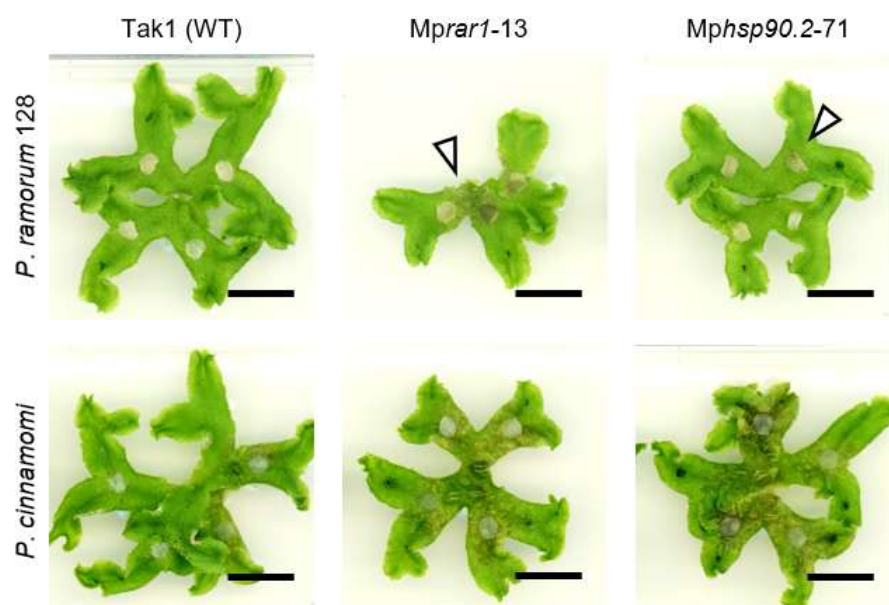


Figure 4.20 HSP90-dependency of *P. ramorum* and *P. cinnamomi* resistance in *M. polymorpha*. White arrowheads indicate discoloration by *P. ramorum*. Plants were imaged at 7 dpi. Bar = 1 cm.

4.2.4.2 HSP90-interacting residues in RAR1 are conserved across land plants

RAR1 interacts with HSP90 through two CHORD domains, CHORD I and CHORD II, although their stoichiometry and dynamics in the immune chaperone complex remain incompletely defined (Takahashi et al., 2003; Chen et al., 2008; Zhang et al., 2010). In yeast two-hybrid (Y2H) assays, *A. thaliana* RAR1 (AtRAR1) and barley (*Hordeum vulgare*) RAR1 (HvRAR1) interact with barley HSP90 (HvHsp90). Interestingly, the CHORD I domain fragment of AtRAR1 interacted with HSP90, but the CHORD II domain did not (Takahashi et al., 2003). A similar trend was observed for

AtRAR1-AtHSP90.1 interactions using split luciferase assay, which is an *in planta* protein-protein interaction (PPI) assay. Consistent with the Y2H study, the CHORD I domain generated a strong chemiluminescence signal, whereas the CHORD II domain fragment produced only a marginal signal (Chen et al., 2008).

Structural work has identified HSP90-interacting residues in CHORD domains (Zhang et al., 2010). A crystal structure of the AtRAR1^{CHORD II}-AtSGT1a^{CS}-HvHsp90^{N-terminal} ternary complex revealed HSP90-interacting residues in the CHORD II domain of AtRAR1. The AtRAR1^{CHORD II}-HvHSP90^{N-terminal} interaction is mainly mediated by hydrophobic patches in each domain and reinforced by polar and solvent-bridged interactions. Key HSP90-interacting residues of the AtRAR1 CHORD II domain are generally conserved in the CHORD I domain, while two notable SAPs exist in each branch of PPI: (i) an alanine-proline polymorphism exists at the hydrophobic patch (Pro38 in the CHORD I domain and Ala185 in the CHORD II domain) and (ii) a lysine-arginine polymorphism lies at the polar interaction (Lys45 in the CHORD I domain and Arg192 in the CHORD II domain) (Figure 4.21A). Structure-guided mutagenesis on these sites of AtRAR1 CHORD I domain resulted in disruption of AtRAR1^{CHORD I}-HvHsp90^{N-terminal} interactions (Zhang et al., 2010). These observations suggest that physicochemical properties, such as hydrophobicity (alanine↔proline) or positive charge (lysine↔arginine), are more critical than exact residue identity.

To survey whether these two SAPs are consistent across land plants, I performed multiple sequence alignments of CHORD I and CHORD II domains. These two SAPs were completely conserved in CHORD I domains, except at position 61 (Figure 4.21B). This third SAP between CHORD domains, leucine↔methionine SAP, even exist between *A. thaliana* CHORD domains (Zhang et al., 2010). Considering its location within the hydrophobic patch, this leucine↔methionine SAP is unlikely to have a substantial impact on RAR1-HSP90 interactions. This leucine↔methionine SAP was also observed in the CHORD II domains and is not discussed further here.

In the CHORD II domain, one of the fern-specific differences identified in the previous chapter overlapped with the alanine↔proline SAP. Only fern homologs carry proline at this position in the CHORD II domain (Figure 4.21C). Another fern-specific difference, AtRAR1:Asp198Asn, did not overlap known HSP-interacting residues, consistent with that CHORD domains utilize different sets of amino acids to interact with SGT1 and HSP90 on the same domain (Zhang et al., 2010). Interestingly, MpRAR1 encodes lysine instead of arginine at position 192, where the lysine↔arginine SAP was observed, while other homologs had arginine at this position (Figure 4.21C).

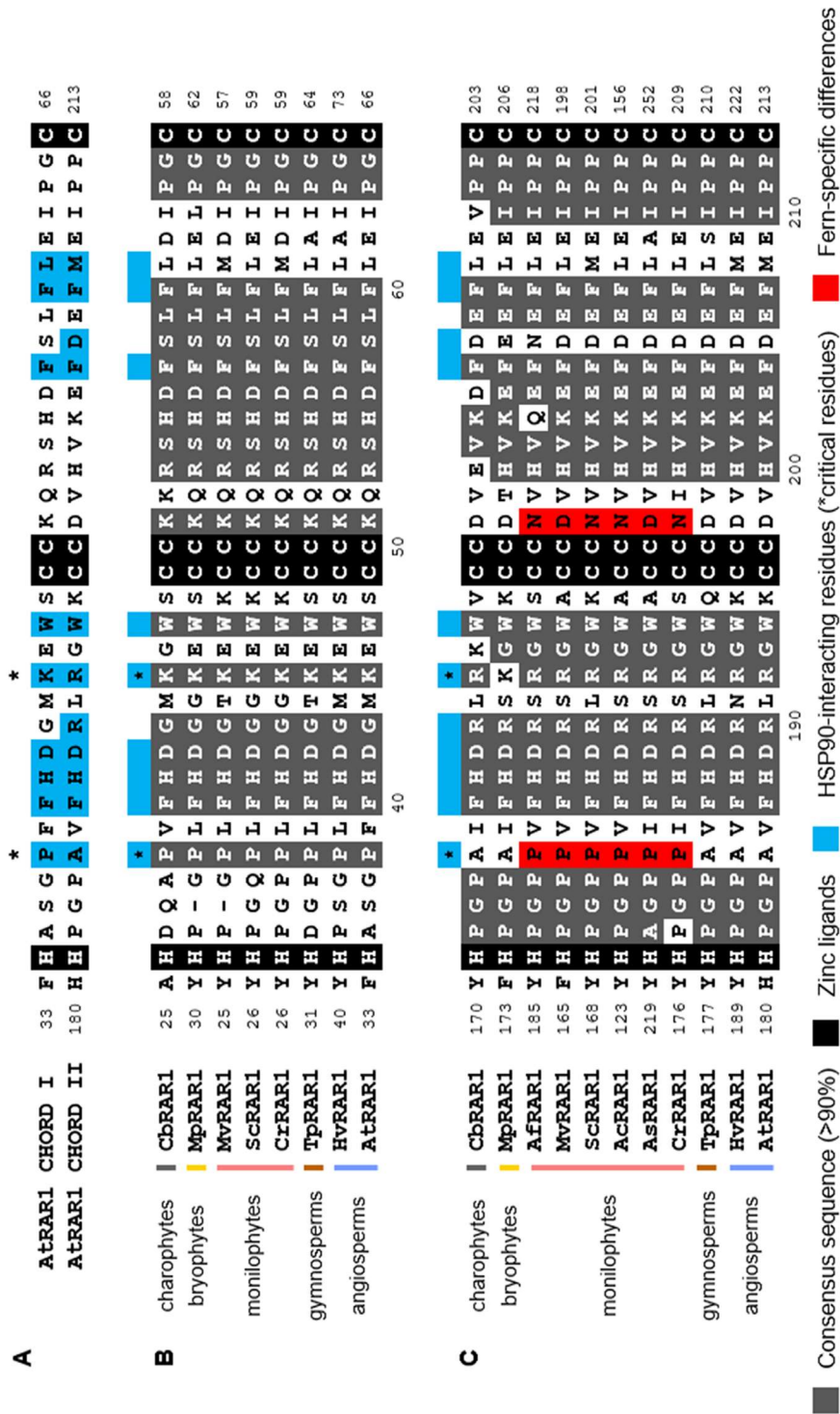


Figure 4.21 HSP90-interacting residues in CHORD domains are conserved across land plants. HSP90-interacting residues and critical residues of AtRAR1 were defined by Zhang et al., (2010). Residues were numbered according to AtSGT1b. (A) Protein sequence alignment of CHORD domains from *A. thaliana*. (B) Protein sequence alignment of CHORD I domains from representative species. (C) Protein sequence alignment of CHORD II domains from representative species.

4.2.4.3 HSP90-interacting residues in SGT are conserved across land plants

Two conserved aromatic residues make major contributions in AtSGT1a^{CS}–HvHsp90^{N-terminal} interactions. A conserved tyrosine, Tyr157 in AtSGT1a or Tyr165 in AtSGT1b, is involved in two interactions: a hydrogen bond with charged residues in the HvHsp90^{N-terminal} and aromatic ring stacking with hydrophobic recess on HvHsp90^{N-terminal}. Another aromatic residue, Phe168 in AtSGT1a (equivalent to Phe176 in AtSGT1b) is at the center of the hydrophobic interaction (Zhang et al., 2008). Unlike the tyrosine, this position showed a SAP to leucine in some plant homologs (Figure 4.22). Considering that this position is methionine in metazoans (Zhang et al., 2008), hydrophobicity of residue at this site may be more important than other physical properties, such as aromatic ring or size. Except this phenylalanine↔leucine SAP, critical residues tested by site-directed mutagenesis in the previous study were completely conserved across the chosen species (Figure 4.22).

MpSGT1 shares the SAP patterns described above with AtSGT1 and HvSGT1. MpRAR1 carries lysine instead of arginine at the lysine↔arginine SAP site in the CHORD II domain (Figure 4.21–22). Given that this SAP does not impair AtRAR1^{CHORD I}–HvHsp90^{N-terminal} interactions, it is likely that MpRAR1–MpHSP90 and MpSGT1–MpHSP90 can interact in *M. polymorpha*.

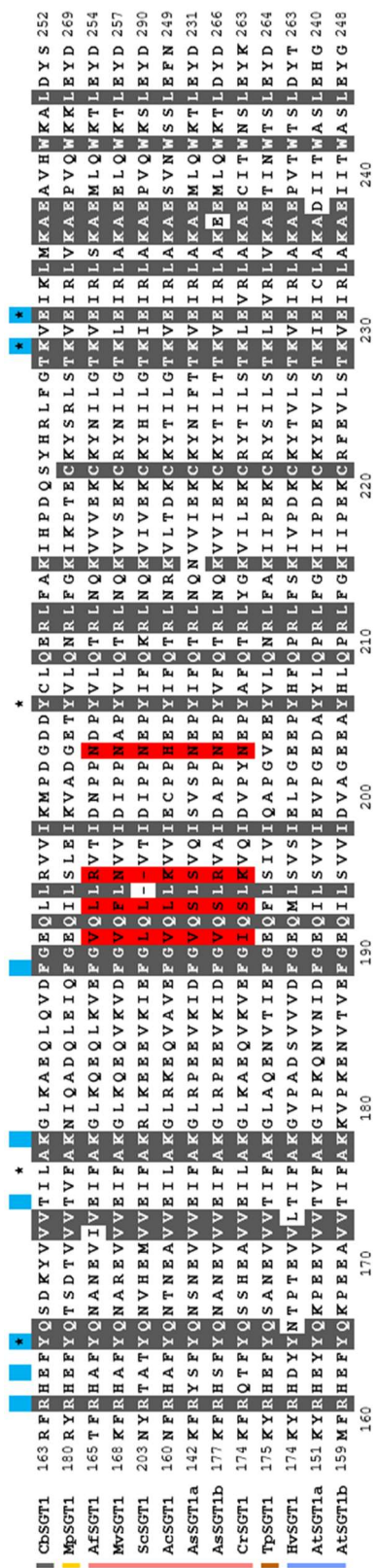


Figure 4.22 HSP90-interacting residues in CS domain are conserved across land plants. HSP90-interacting residues of AtSGT1a were defined by Zhang et al., (2008). Residues were numbered according to AtSGT1b. The same color scheme was used to illustrate each streptophyte lineages as Figure 4.20.

4.3 Conclusion and discussion

Across the *Marchantia–Phytophthora* infection screen, loss of *RAR1* revealed breakdowns of NHR. Three non-adapted strains, *P. cinnamomi*, *P. ramorum* 128, and *P. citricola*, showed the clearest increases in lesion severity on *rar1-13* mutants compared to WT (Figures 4.3–4; Tables 4.1). Notably, infection efficiency did not always increase on *rar1-13* mutants for three strains: *P. cinnamomi* and *P. ramorum* 128 exhibited higher infection efficiency in *rar1-13*, whereas *P. citricola* consistently initiated infection at similar rates on both genotypes (Figures 4.4; Table 4.1). At the same time, infection outcomes and the presence of RAR1-mediated NHR were distributed sporadically across *Phytophthora* phylogeny (Figure 4.19), underscoring the strain-dependent nature of RAR1-mediated NHR in the *Marchantia–Phytophthora* pathosystems.

In *Phytophthora*, two major classes of effectors are known to be translocated into hosts: RXLRs (named after the RXLR motif, is a conserved sequence required for translocation) and Crinklers (CRNs, named for the ‘leaf-crinkling’ phenotype upon expression in plants) (Stassen and van den Ackerveken, 2011). Previous genomics and bioinformatics studies identified both classes of effectors in the genomes of *P. citricola* (Srivastava et al., 2022) and *P. ramorum* (Jiang et al., 2008; Goss et al., 2013), although their biological functions remain largely untested. *P. cinnamomi* also encodes these effectors (Engelbrecht et al., 2021), and recent functional studies implicate roles of both RXLR and CRN effectors in virulence. The *P. cinnamomi* RXLR effector PcAvh87 binds to PIP113 (plant immune protein 113) and attenuates immune responses of *N. benthamiana* (He et al., 2024). In one of its hosts, avocado (*Persea americana*), multiple PcCRN effectors trigger host cell death in a partially resistant rootstock, suggesting that resistance involves recognition of these CRN effectors (Midgley et al., 2024).

Hauatoria are characteristic of many *Phytophthora* infections and are major sites of effector delivery (Petre and Kamoun, 2014; Whisson et al., 2016). The previous study demonstrated that *P. palmivora* forms hauatoria-like structures and its effector-encoding genes are upregulated in *M. polymorpha* (Carella et al., 2018). Considering hauatoria or haustorium-like structures *in planta* have been reported for *P. cinnamomi* and *P. ramorum* (Oßwald et al., 2014; Redondo et al., 2015), the three candidate *Phytophthora* strains may also form hauatoria and secrete effectors in *M. polymorpha*, thereby it potentially engages the chaperone-mediated NHR. In turn, it implies that *M. polymorpha* harbors chaperone-dependent NLR proteins capable of detecting the effector. *M. polymorpha* encodes fewer NLR proteins compared to other land plants (Bowman et al., 2017; Chia et al., 2024). Thus, this low genetic redundancy can facilitate identification of NLR–effector interactions in these *Marchantia–Phytophthora* pathosystems and the discovery of novel R genes against these pathogens (Figure 4.23).

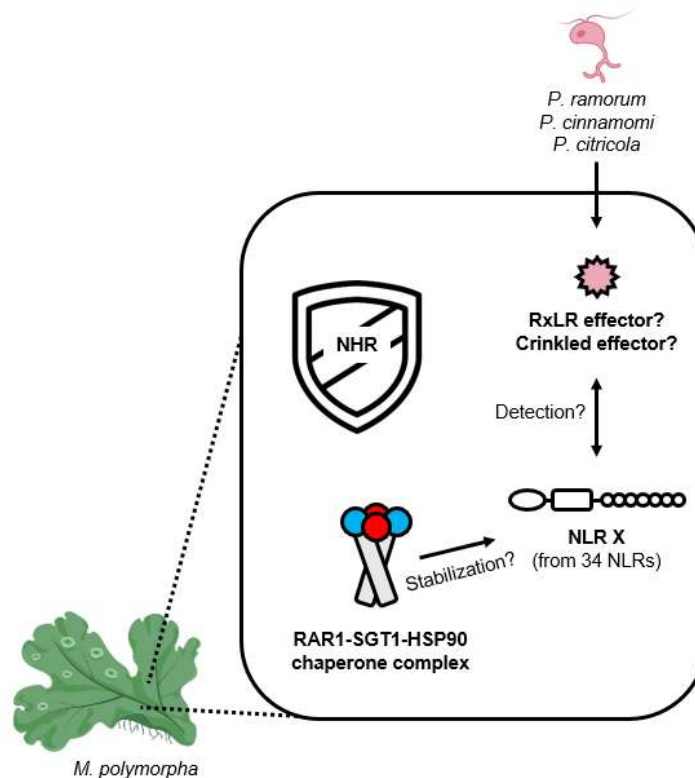


Figure 4.23 RAR1-mediated NHR of *M. polymorpha* against *Phytophthora*. Three candidate *Phytophthora* species, encodes two families of effectors, RXLR and CRN effectors. Next task will be identification of NLR X and functional analyses of its interaction with effectors from these pathogens.

Although *P. cinnamomi* and *P. ramorum* were listed in the top 10 oomycete pathogens voted by plant pathologists (Kamoun et al., 2015), their molecular plant-microbe interactions are largely unexplored, especially for *P. ramorum*. Most *P. ramorum* studies have been focused on genomics, epidemiology, and ecology. To our knowledge, robust laboratory infection systems for *P. ramorum* in model angiosperms, *Nicotiana benthamiana* or *Arabidopsis thaliana*, have not been established. In this context, *M. polymorpha* can be useful not only as genomic resources to discover the first R gene against *P. ramorum*, but also as a feasible system to dissect molecular mechanisms of *P. ramorum* effectors. Many genetic tools, such as CRISPR/Cas9-based knockout mutation, β -estradiol inducible expression, gene silencing by artificial microRNA, and transient gene expression by *Agrobacterium tumefaciens*, are available in this species (Flores-Sandoval et al., 2016; Sugano et al., 2018; Iwakawa et al., 2021).

Subjective lesion scoring was useful for the initial screening across thirty-three different strains, but other quantitative methods will be important for the follow-up. Lesion severity was scored on a 0–3 ordinal scale due to heterogeneity of disease phenotypes. *P. ramorum* 142 was the only strain in this species that did not show a breakdown of RAR1-mediated NHR by this scoring system. However,

qRT-PCR of *Phytophthora* biomass and sporulation marker genes can examine this in quantitative manner, as shown in previous studies with *P. palmivora* (Carella et al., 2018; Carella et al., 2019). Accordingly, the breakdowns of RAR1-mediated NHR to *P. ramorum*, *P. cinnamomi*, and *P. citricola* will need to be measured by this method to validate the scoring-based results.

The known host range of *Phytophthora* species was not predictive of successful infection in this system. It is also observed in angiosperms: *P. drechsleri* is often labelled as a broad host range pathogen in floriculture, but the host range of each isolate can vary dramatically from broad to species-specific (Olson and Benson, 2012). Two species that did not cause infection in my study, *P. infestans* (a Solanaceae pathogen) and *P. sojae* (a soybean pathogen) are generally considered to have narrower host range than many other *Phytophthora* (Chen et al., 2023). In a previous bryophyte-based study, *P. sojae* failed to infect the model moss *Physcomitrium patens*. Strikingly, *P. infestans* can colonize *P. patens*, and its effector AVR1 targets the same host protein as in potato (Overdijk et al., 2016; Overdijk et al., 2021). Together, these observations suggest that the strain-dependent manner of plant-pathogen compatibility may need to be interrogated at the molecular level including effectors and ETI. Transcriptomics and proteomics in the *M. polymorpha*–*P. palmivora* pathosystem have successfully revealed both conserved and lineage-specific aspects of immune responses in this plant (Carella et al., 2019). Extending such multi-omics approaches will be valuable for the *Marchantia*–*Phytophthora* pathosystems discovered here.

4.4 Research contributions

I thank Dr. Philip Carella at Cell and Developmental Biology department at the John Innes Centre for generating *Mprar1*-13 and *Mphsp90.2*-71 mutants for this research. *In planta* interaction between MpRAR1 and MpHSP90.2 was also tested by Dr. Philip Carella.

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5. Molecular dialogues in bryophyte–*Phytophthora* interactions

5.1 Introduction

5.1.1 Questioning host range in the light of EvoMPMI

Plant–pathogen interactions are powerful systems for studying co-evolutionary dynamics, as hosts and pathogens are tightly locked in an evolutionary arms race. Historically, mechanistic research on plant–pathogen interactions has focused on identifying molecular components that determine host–pathogen compatibility, such as R and Avr genes, in both plants and their pathogens. As more non-model species, genomic resources, and phylogenetic methodologies become available, the molecular dialogue between plants and pathogens can now be examined across multiple timescales within the framework of evolutionary molecular plant–microbe interactions (EvoMPMI) (Upson et al., 2018; Betsuyaku, 2020; Schornack and Kamoun, 2023).

Recent EvoMPMI studies have broadened our understanding of plant–pathogen coevolution by dissecting the underlying molecular mechanisms. Mechanistic examples highlight effectors as key determinants of host specificity and host shifts. For instance, orthologous *Phytophthora* protease inhibitor effectors from *P. infestans* and *P. mirabilis* show biochemical adaptation to the target protein from each pathogen’s host, accompanied with a host jump (Dong et al., 2014). A single amino acid polymorphism in a conserved effector of the rice blast fungus can expand its host range across Poaceae grasses (Bentham et al., 2021). On the host side, the rice NLR immune receptors, Pikm-1 and Pikp-1, show convergent evolution in the same integrated domain to acquire higher binding affinity to the same effector (Białas et al., 2021). Moreover, helper NLR proteins can evolve to evade pathogen suppression, exemplified by NRC3 (NLR-required for cell death 3) variants that escape binding and inhibition by a nematode effector (Sugihara et al., 2025). Together, these studies, which were enabled by expanding genomic resources and phylogenetic methodology like ancestral state reconstruction, underscore how integrated evolutionary, biochemical, and comparative approaches illuminate the origins and limits of host range.

As more non-seed plants are introduced into the EvoMPMI field, it has become clear that pathogens isolated from angiosperms can infect non-angiosperms, expanding their experimental host range across diverse land plant lineages. The *Pseudomonas syringae* species complex of bacterial pathogens gives striking examples. Many isolates can cause disease on the angiosperm *Nicotiana benthamiana*, the fern *Ceratopteris richardii*, and the bryophyte *Marchantia polymorpha*, with the necrotizing lipopeptide toxin syringomycin facilitating these infections (Grenz et al., 2025). On the other hand, *Pseudomonas viridiflava* strains from wild *M. polymorpha* retain virulence on *N. benthamiana* in an effector-dependent manner (Robinson et al., 2025). Cross-lineage *Pseudomonas* bacterial diseases also occur. A *Pseudomonas* isolate from the diseased fern *Asplenium nidus*, *P.*

asplenii, can infect gladiolus, whereas the gladiolus pathogen *P. gladioli* can colonize *A. nidus* (Chase, 1984). Host range is also expanded in filamentous eukaryotic pathogens. The tropical crop pathogen *Phytophthora palmivora* has a broad host range, and an isolate from mango can complete a full infection cycle in barley and *M. polymorpha* (Le Fevre et al., 2016; Carella et al., 2018). A tomato-infecting isolate of the vascular fungal pathogen *Fusarium oxysporum* successfully colonizes *M. polymorpha* and causes disease symptoms, including tissue maceration and chlorosis (Redkar et al., 2022a). Collectively, these observations support the expanding concept of host range beyond traditional angiosperm systems (Baltrus and Kvitko, 2025).

5.1.2 Conserved plant defense responses and pathogen infection strategies

Multi-omics approaches highlight conserved defense responses between non-seed plants and angiosperms. Both the liverwort *M. polymorpha* and the angiosperm *N. benthamiana* are compatible hosts of *P. palmivora*. During *P. palmivora* infection, a set of orthologous genes is induced, including genes encoding signaling pathway components such as receptor-like kinases (RLKs) and mitogen-activated protein kinases (MAPKs). Other typical hallmarks of defense responses include genes associated with the phenylpropanoid pathway and genes encoding PR (Pathogenesis-related) proteins, which are inducible proteins in defense responses with diverse functions, typically small secretory proteins with antimicrobial activities (Carella et al., 2019). Transcriptome analysis of pathogenic interactions between the moss *Physcomitrium patens* and the fungal pathogen *Botrytis cinerea* also identified activation of PR genes and genes involved in phenylpropanoid pathway (Reboledo et al., 2021), highlighting conserved molecular signatures of defense responses across land plants.

Interestingly, disease resistance conferred by a PR protein can transfer across lineages. Infection by the oomycete pathogen *Pythium irregulare* strongly induces expression of the *P. patens* PR10 gene (*PpPR-10*). *P. irregulare* also infects the model angiosperm *Arabidopsis thaliana*, and overexpression of *PpPR-10* enhances resistance to this pathogen in both *P. patens* and *A. thaliana* (Castro et al., 2016). Together, these omics and genetic approaches demonstrate the conserved function of PR proteins despite evolutionary distances.

5.1.3 Bryophyte models in EvoMPMI

Bryophytes consist of three subdivisions: liverworts (Marchantiophyta), mosses (Bryophyta), and hornworts (Anthocerotophyta). Species in these clades share several physiological and ecological features. They do not produce seeds but instead propagate via spores. Their life cycles are dominated by a haploid gametophyte stage, and their diploid stage, the sporophyte, is relatively short-lived and nutritionally as well as physically dependent on gametophytic tissue. Bryophytes do not have true

vascular tissues or the structural polymers to support them, such as lignin and suberin (Cox, 2018; Puttick et al., 2018; Renault et al., 2019). Interestingly, some moss species possess specialized structures that are functionally analogous to vascular tissues (Brodribb et al., 2020). Together, these traits make bryophytes powerful systems to interrogate the adaptability of pathogens and core pathogenesis strategies. For example, *M. polymorpha* does not possess vascular tissues that are typical propagation routes of *F. oxysporum* in angiosperms, yet these pathogens still infect this host (Carella et al., 2018; Redkar et al., 2022a).

The moss *P. patens* and the liverwort *M. polymorpha* have been used to probe conserved and lineage-specific mechanisms in plant immunity (Ponce de León, 2011; Bowman et al., 2022; Jeong et al., 2023). There are other bryophyte model species with potential to broaden our understanding of EvoMPMI. The cosmopolitan moss *Ceratodon purpureus* has been used across disciplines in plant science, from developmental biology to ecological genetics (Cove and Quatrano, 2006; Tavernier et al., 2024). Like *P. patens*, *C. purpureus* is a genetically tractable model, with homologous recombination-based precise genome editing and an established neutral locus for knock-in transformation (Tavernier et al., 2024). Interestingly, *C. purpureus* secretes small molecules with antibacterial activity against Gram-positive bacteria in a strain-specific manner, implying adaptation to the local microbial context (Dague et al., 2023).

Hornworts possess several unique features for land plants. A pyrenoid, a specialized large chloroplast organelle found in many algae, is also present in hornworts. Interestingly, these species establish symbiotic relationships with endophytic cyanobacteria, as do a few other land plants like the water fern *Azolla filiculoides* and cycads (Frangedakis et al., 2020). This relationship can be found in the wild (Bouchard et al., 2020; Nelson et al., 2021) and can be replicated under laboratory conditions (Chatterjee et al., 2022). Several hornwort species in the genus *Anthoceros*, such as *A. agrestis* and *A. punctatus*, have available genetic tools (Frangedakis et al., 2021; Waller et al., 2023). Pathogenic interactions in this clade have not been studied yet, but these findings put hornworts in a unique position in EvoMPMI research.

5.1.4 Aims of this chapter

In this chapter, I established additional bryophyte–*P. palmivora* pathosystems using two model species, the moss *C. purpureus* and the hornwort *A. agrestis*, together with *P. palmivora* (Figure 5.1A). I assessed transcriptional responses and identified the induction of PR genes during infection in both hosts as well as a core set of *P. palmivora* effectors across different plant lineages. These findings support a model that pathogens deploy a conserved ‘master key’ infection program rather than a bundle of host-specific keys, and highlight conserved transcriptome shifts across bryophyte clades.

Building on the *Marchantia–Phytophthora* interactions established in the previous chapter, I further analyzed *M. polymorpha* challenged with multiple *Phytophthora* species to identify common host defense responses (Figure 5.1B). Enrichment analysis of the commonly upregulated genes pointed to apoplast immunity and the phenylpropanoid pathway as conserved components in bryophyte immunity.

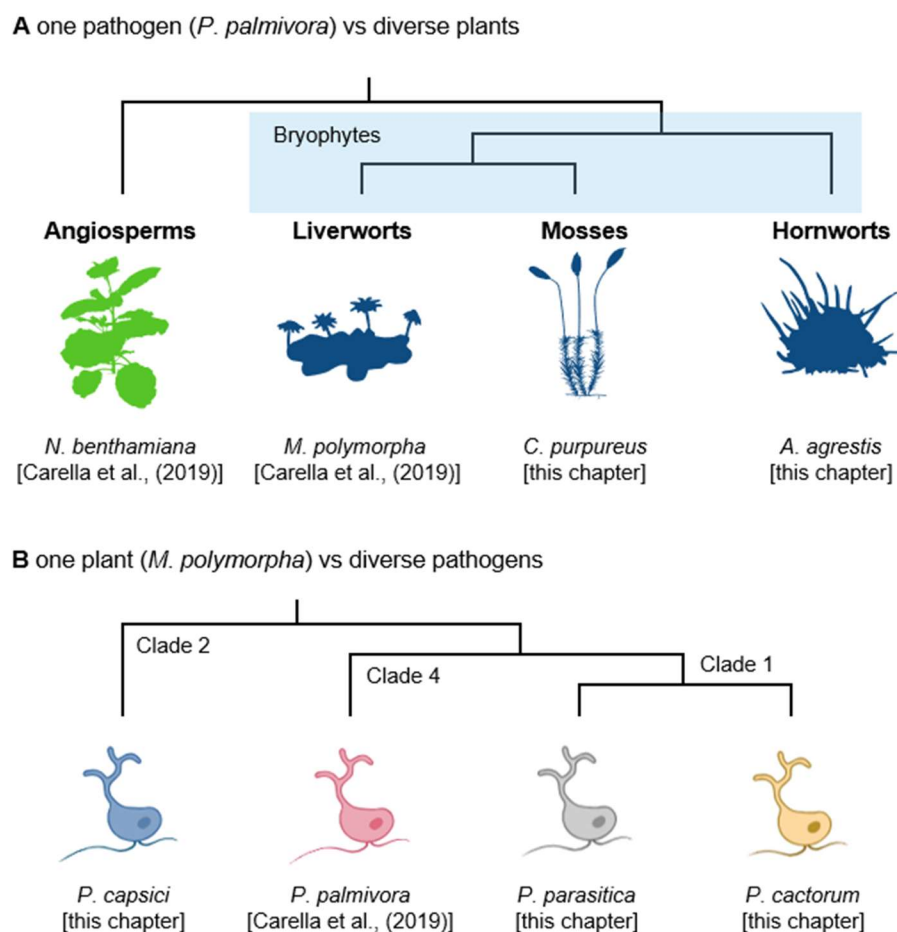


Figure 5.1 Expansion of *M. polymorpha–P. palmivora* system for comparative analyses. *M. polymorpha–P. palmivora* pathosystem from Carella et al., (2019) was expanded in two directions to explore conserved defense responses of plants and common infection strategies of pathogens. **(A)** Two model bryophytes, *C. purpureus* and *A. agrestis*, were infected by *P. palmivora*. **(B)** Three *Phytophthora* species, *P. capsici*, *P. parasitica*, and *P. cactorum* were introduced to *M. polymorpha*.

5.2 Results

Two bryophyte species, the moss *C. purpureus* and the hornwort *A. agrestis*, were challenged with the broad host range pathogen *P. palmivora* (Figure 5.1A). Pathogen growth was monitored using a red fluorescence protein reporter in the transgenic line *P. palmivora*, ARI-tdTomato (hereafter ARI-td). Subsequently, I performed time-course RNA-seq to dissect molecular signatures during the plant-pathogen interactions.

5.2.1 *P. palmivora* infects the moss *C. purpureus*

C. purpureus protonemal colonies were inoculated with a single 10 μ l droplet of *P. palmivora* ARI-td zoospore suspension (2×10^5 zoospores/ml). In both mock-treated and pathogen-infected samples, I observed visible growth of moss colonies in both conditions during the given time window. However, clear disease symptoms, such as chlorosis and visible hyphal growth, began to appear in pathogen-infected samples from 3 dpi. The fluorescence signal was detectable from 2 dpi. At 2 dpi, there was typically a very weak fluorescence signal at the inoculation spot. The location was confirmed with bright field imaging because liquid inoculation, both mock and zoospore suspensions, always resulted in a collapse of protonemal filaments at the center, leaving an ‘inoculation’ crater. The tdTomato signal increased markedly from 3 dpi, with diffused fluorescence alongside brighter punctate signals. Such condensed and ovoid signals are characteristic sporangia and have also been reported in other fluorescence-tagged *Phytophthora* species (Si-Ammour et al., 2003; Ah-Fong and Judelson, 2011; Dai et al., 2021). By 4 dpi, the whole colony was covered with numerous puncta. These observations indicate that *P. palmivora* completed its infection cycle by ~3 dpi, producing bright dots corresponding to sporangia due to their high cell density, and fully colonized the moss colony at 4 dpi (Figure 5.2). Since developmental stages from germination to sporulation were evident in this setting, such as hyphal propagation (2–3 dpi) and sporangium formation (3–4 dpi), these time points were used for the time-course RNA-seq.

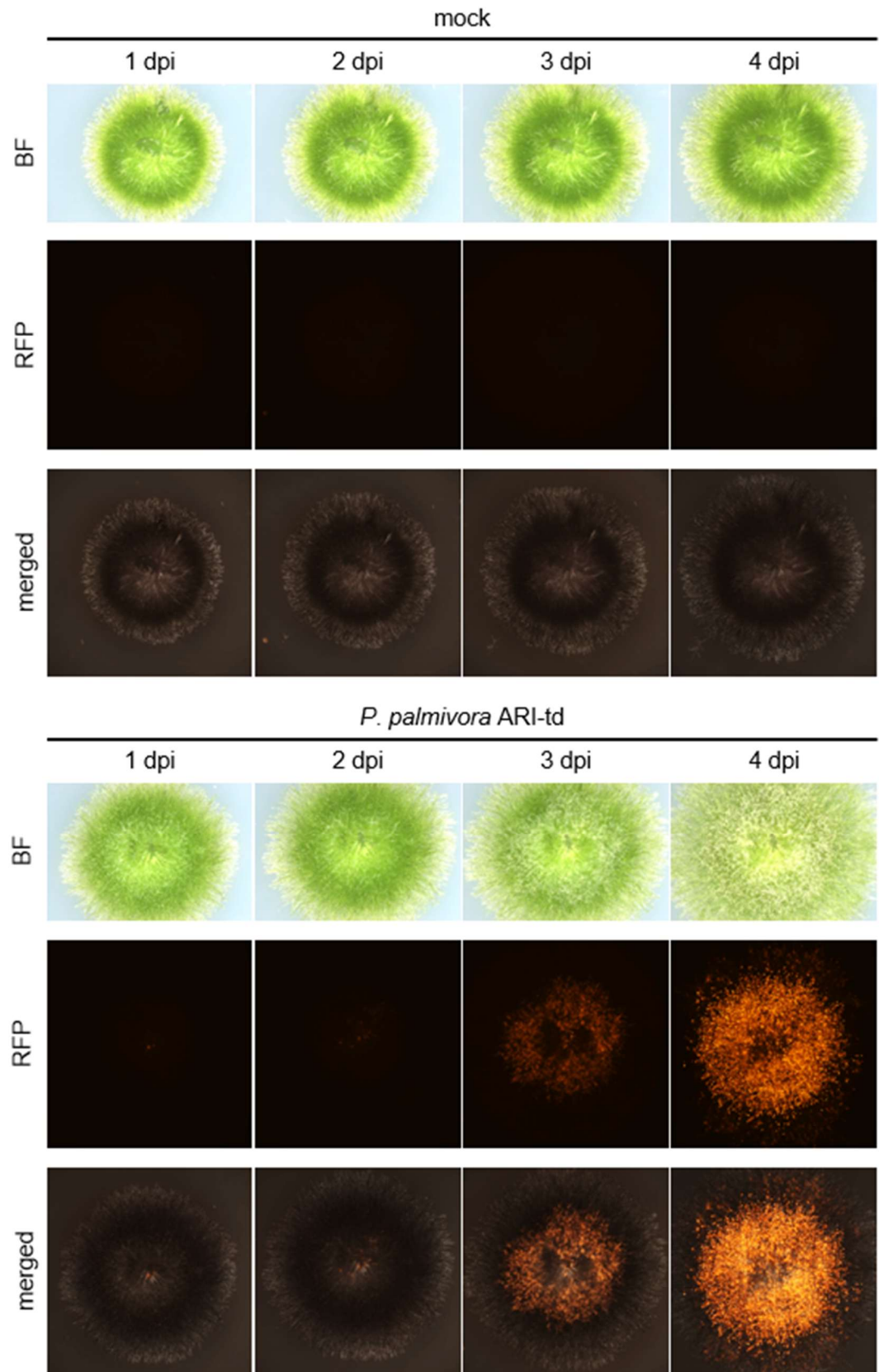


Figure 5.2 Time-course imaging of *P. palmivora* infection in the model moss *C. purpureus*. Mock treatment is distilled water. BF: bright field. Merged: overlay of black/white and RFP channels. Exposure times: BF = 150 ms, BW = 10 ms, RFP = 5000 ms.

5.2.1.1 Later stages of *P. palmivora* infection show the largest transcriptome shift

To assess how *C. purpureus* transcriptome is reprogrammed during infection, I performed time-course RNA-seq analysis comparing the transcription profiles of 2-week-old GG1 (female) protonemal colonies inoculated with either water (mock) or *P. palmivora* ARI-td zoospores. Differentially expressed genes (DEGs) were defined using the following thresholds: $\log_2$ fold change $[\text{LFC}] \geq 2$ and adjusted $p < 10^{-3}$, comparing mock versus infected colonies sampled daily from 1 to 4 dpi. Hierarchical clustering and principal component analysis (PCA) of transcriptome profiles showed that the later infection stages (3–4 dpi) exhibit the most pronounced transcriptome shift compared to earlier stages (1–2 dpi) and mock-treated samples across the full time course (1–4 dpi) (Figure 5.3A). Interestingly, the mock samples and early infection stage samples shared several clusters of differentially expressed genes. Although no further analysis was performed on these clusters, it might reflect ongoing protonemal growth and possible senescence when the colonies were saturated in the plates. In the PCA plot, infected samples showed a stronger time-dependent transcriptome shift, progressing along PC1 (82% variance explained), whereas mock samples varied primarily along PC2 (11%) (Figure 5.3B). Together, these patterns indicate that *C. purpureus* responds to the *P. palmivora* infection, especially at the later infection stages.

5.2.1.2 Time-resolved DEG analyses reveal large-scale transcriptional reprogramming

Volcano plots summarized pairwise differential expression between mock-treated and infected samples at each time point. It showed an increase of DEGs across the time course. At 4 dpi, I detected 1,135 upregulated genes and 304 downregulated genes in the set thresholds. DEG counts increased from early (1–2 dpi) to late stages (3–4 dpi), with a transient decrease of downregulated genes from 2 to 3 dpi (Figure 5.3C).

UpSet analysis was performed separately to examine the number of upregulated and downregulated genes shared between timepoints. For upregulated genes, the largest intersection comprises genes specific to 4 dpi ($n = 605$), followed by genes shared between 3 and 4 dpi ($n = 421$). This trend was consistent with the previous study on *Marchantia-Phytophthora* transcriptomics (Carella et al., 2019). All other intersections were smaller than 35 genes. 24 genes were constantly upregulated across all time points (1–4 dpi). By contrast, no genes were commonly downregulated genes across 1–4 dpi, possibly because only one gene was downregulated at 1 dpi (Figure 5.3D). These patterns support that the presence of a conserved core response accompanied by stage-specific transcriptional program emerging at later infection stages.

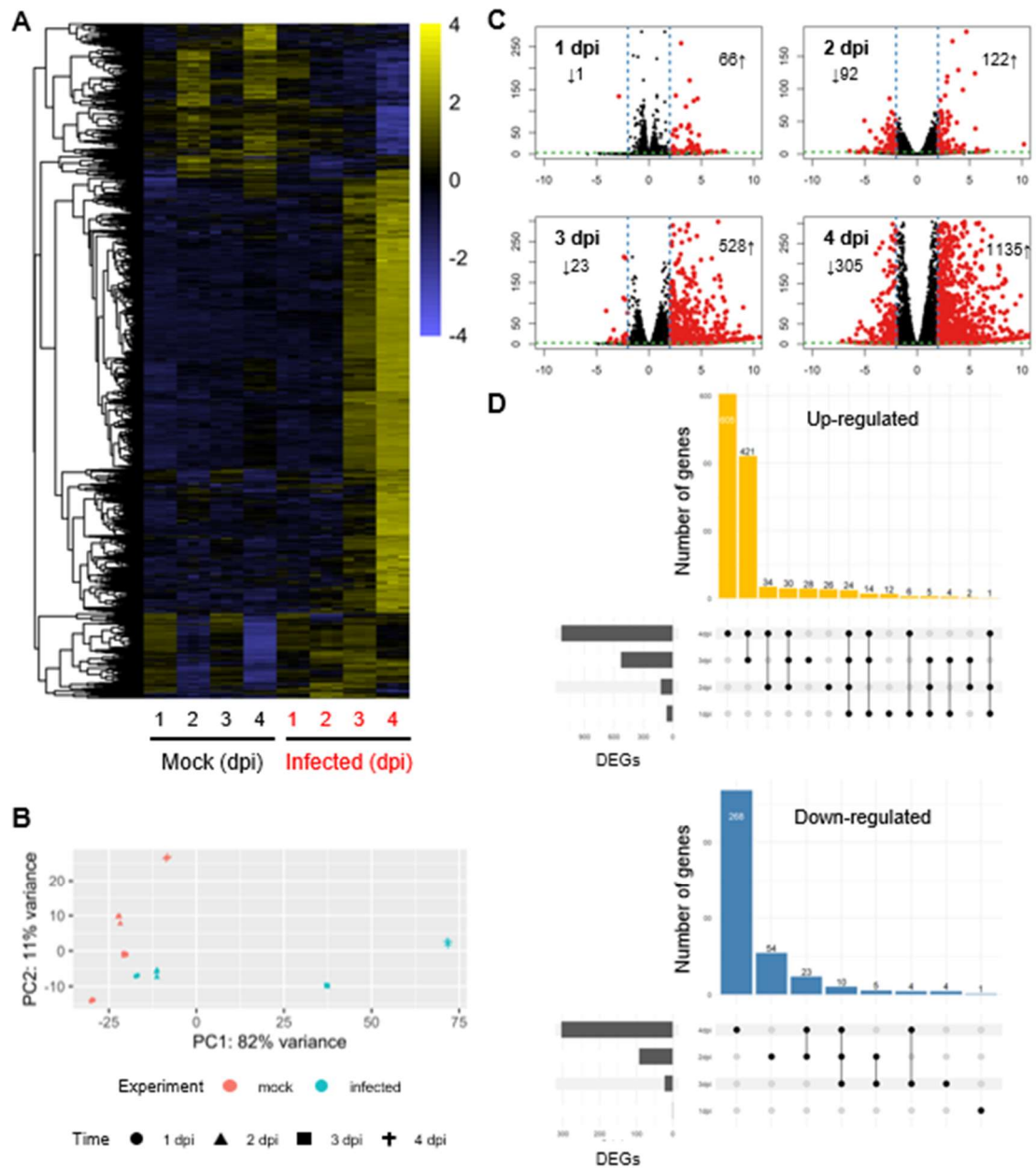


Figure 5.3 Transcriptional responses of *C. purpureus* during *P. palmivora* infection. DEGs were defined by the following thresholds: adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. **(A)** Hierarchical clustering of DEGs. **(B)** PCA of transcriptome profiles between two treatments (mock vs infected). **(C)** Volcano plots displaying pairwise differential expression analysis at each time point. **(D)** UpSet plots showing shared and time point-specific DEGs.

5.2.1.3 Induction of conventional PR families and PR17-like genes during infection

PR gene families are typically induced by infection in plants, including *M. polymorpha* (Carella et al., 2019). To assess whether *C. purpureus* employs PR genes against pathogens, I profiled potential PR genes in the genome. PR12 (γ -thionin/plant defensin) and PR13 (thionin) were not detected, consistent with reports that these PR families are absent from non-flowering plants, such as *Pinus tecunumanii* (gymnosperm) and *M. polymorpha* (bryophyte) (Visser et al., 2018; Carella et al., 2019). In addition, I failed to detect the Barwin domain, the characteristic domain of PR4 proteins, in the annotated proteome of *C. purpureus* using HMMER profiling. While the presence of PR4 cannot be completely excluded due to potential misannotation or missing annotation, PR4 was not detected under my search criteria (Table 5.1).

Table 5.1 List of potential PR genes identified in *C. purpureus* by HMMER profiling

Pfam motif	Description	PR family	Presence
PF00188	Cysteine-rich secretory protein family	PR1	Yes
PF00332	Glycosyl hydrolases family 17	PR2	Yes
PF00182	Chitinase class I (GH19)	PR3	Yes
PF00967	Barwin family	PR4	No
PF00187	Chitin recognition protein (CBM18/hevein-like)*	PR4	Yes
PF00314	Thaumatococcus family	PR5	Yes
PF00280	Potato type I proteinase inhibitor family	PR6	Yes
PF02428	Potato type II proteinase inhibitor family	PR6	No
PF00014	Kunitz/Bovine pancreatic trypsin inhibitor domain	PR6	No
PF00228	Bowman-Birk serine protease inhibitor family	PR6	No
PF00079	Serpin (serine protease inhibitor)	PR6	Yes
PF00082	Subtilase	PR7	Yes
PF00704	GH18 (chitinase)	PR8	Yes
PF00141	Peroxidase	PR9	Yes
PF00407	Pathogenesis-related protein Bet v 1 family	PR10	Yes
PF00304	γ -thionin family (plant defensin)	PR12	No
PF00321	Plant thionin	PR13	No
PF00234	Protease inhibitor/seed storage/LTP family	PR14	Yes
PF00190	Cupin	PR15/16	Yes
PF04450	Peptidase of plants and bacteria	PR17	Yes

*Some PR4 proteins harbor this domain alongside Barwin domain

I analyzed whether PR genes were enriched among upregulated genes at 4 dpi. A number of candidate PR genes from well-characterized families were identified: PR1 (cysteine-rich secretory protein), PR2 (β -glucanase), PR3 and PR8 (chitinase), PR5 (thaumatin-like), PR7 (subtilase), PR9 (peroxidase), PR14 (lipid transfer proteins), and PR15 (cupin) (Table 5.2). Genes encoding PR6 proteins with Pin I (potato inhibitor type I) and serpin (serine proteinase inhibitor) domains were present in the genome but were not detected among the upregulated genes at 4 dpi (Table 5.1; 5.2). *C. purpureus* possessed more putative PR9 and PR15 genes than *M. polymorpha* (Carella et al., 2019), which may reflect the whole genome duplication event in *C. purpureus* (Szövényi et al., 2014).

Table 5.2 Putative *C. purpureus* PR genes induced by *P. palmivora*

PR family	Description	Number of genes *
PR1	Cysteine-rich secretory protein	4
PR2	β -1,3-glucanase, GH17	1
PR3	Chitinase, GH19	5
PR5	Thaumatin-like proteins	6
PR7	Subtilisin-like proteases	6
PR8	Chitinase, class III (GH18)	2
PR9	Peroxidase, class III	22
PR14	Lipid transfer proteins	2
PR15	Cupin/germin	26
PR17-like	Peptidase	3

* = upregulated during infection at 4 dpi

Interestingly, genes encoding PR17 proteins, one of the least well-characterized PR proteins with putative aminopeptidase activity, were present in *C. purpureus* genome and appeared among the upregulated genes at 4 dpi (Table 5.1; 5.2). A few previous studies identified PR17 genes in angiosperms, including tobacco and barley (Okushima et al., 2000; Christensen et al., 2002; Han and Schneider, 2024). Notably, five conserved motifs described in barley PR17 homologs were also present in the CpPR17-like proteins (Figure 5.4), although biochemical and genetic studies will be needed to validate their roles as PR proteins in *C. purpureus*.

CepurGG1.11G028800.1.p	-----MYQS-KDSKMGALRTLVLFLVNFVTLHVSATTFVQNLAPDTPGGKRYA	50
CepurGG1.4G008700.1.p	-----MGALRALSVLLLLVNMATLHVTATTFVQNLAPNTGGGKRYT	42
CepurGG1.11G140000.1.p	MARMRAGSPKFEDAMSSSR-VSVCVLLLTVLLATVHPTSAAVYKVTNAVPNTPGGKRFD	59
HvPR-17a HORVU.MOREX.r3.1HG0046330.1	-----MK-ISIAA-AAVLLLLAATASAVTFDVTNEASSTAGGQRFD	40
HvPR-17b HORVU.MOREX.r3.6HG0550040.1	-----MK-PQVATVSFLLLVMTAATARAFTFDASNTASGTAGGQRFD	41
	:: . . *.. . * . * **::	
CepurGG1.11G028800.1.p	AEMGDWTIQLQDVKTDFVNA-----LSLSPEKPVHDITFIVEOTDAAASNSNNEIHVG	105
CepurGG1.4G008700.1.p	AEMGDWTIQLQEVKTDFVNA-----LSLSPEKPVHDITFIEOTDIAA-YSNNEIHVG	96
CepurGG1.11G140000.1.p	QVLGASNMKRVLQDAANFCRGKA----FSLSTPKQIATVTLIVESFSGVAFTAGDTIHLS	115
HvPR-17a HORVU.MOREX.r3.1HG0046330.1	REYGAAYAKQVLSDASSFTWGFNQDPDSRRPADGDTVLAVROTNGIASSTSGSTIELS	100
HvPR-17b HORVU.MOREX.r3.6HG0550040.1	NAVGLAYSKVLSDASTFIWNTFNQRAAADRKPVDA--VTLVVEDIGGVAFASGGIHLIS	99
	* ::*:. . * . : : : * : . . * : . . *	
CepurGG1.11G028800.1.p	ARFLNGITG----DLRFEIKGLMFHEVCHSFQNNRGDYGNDAHFRGVVEGIATYMEIAG	161
CepurGG1.4G008700.1.p	ANFLNGITG----DLRFEIKGLMFHEVCHSFQNNRGDYGNDAHFRGVVEGIATYMEIAD	152
CepurGG1.11G140000.1.p	AEYVAGYNPGDSAAALRREITGVLYHESAHVWQNGDKGSG--QFFGGVIEGIADWVRLRSG	173
HvPR-17a HORVU.MOREX.r3.1HG0046330.1	ARSVGGITG---DNLKEQVDGVLYHEVHVWQWGLQDYH---EHHGIFEIADIYVRLKAG	154
HvPR-17b HORVU.MOREX.r3.6HG0550040.1	AKYVGGYSG---DV-KKEVTGVLYHEATHVWQWNGRGT---ANGGLIEGIADYVRLKAG	151
	*. : . . : : : *::** * : * . . *:.*** :. : *	
CepurGG1.11G028800.1.p	YGIGPKV---KGGNWYAGYETTAHFFAWIDQTQISSFVNRLNQRMGLLDWNONFFVFITS	218
CepurGG1.4G008700.1.p	YGVGPKV---KGGNWFQGYHTTAHFFAWIDQTQISAFVNRLNQRMGLLDWNONFFTSITG	209
CepurGG1.11G140000.1.p	FAAAHWTRQRGGNW-DGYETTAFFLDWIQINKKKNFVNLLNQKMQQAQWSNDFRQIVG	232
HvPR-17a HORVU.MOREX.r3.1HG0046330.1	YVAANWVKEGGGSRWDEGYDTARFLDYCDLR-KPGFVAEMNGKLK-DGYNDQYFVQILG	212
HvPR-17b HORVU.MOREX.r3.6HG0550040.1	LAPGHMRPQSGSRWQGYDTARFLDYCDLR-MPGFVAQLNAKMK-SGYSDOFFAQILG	209
	. * . * ** * * : : : * * : * : : : : * *	
CepurGG1.11G028800.1.p	KQVDTLWSLYQAEF--	233
CepurGG1.4G008700.1.p	KQVDTLWSLYQADSF--	224
CepurGG1.11G140000.1.p	VSDQLWQVQNSI---	246
HvPR-17a HORVU.MOREX.r3.1HG0046330.1	TSADQLWQDYKAKYSQG	229
HvPR-17b HORVU.MOREX.r3.6HG0550040.1	KNVQQLWKDYKAKFGG-	225
	.. : * * * : *	

Figure 5.4 Putative PR17 proteins in *C. purpureus* share conserved motifs with cereal PR17 proteins. Red boxes indicate highly conserved motifs in PR17 proteins identified by Christensen et al., (2002).

5.2.2 *P. palmivora* infects the hornwort *A. agrestis* and *A. punctatus*

A single 10 µl droplet of *P. palmivora* ARI-td zoospore suspension (2×10^5 zoospores/ml) was applied on the *A. agrestis* and *A. punctatus* thalli to test compatibility with this pathogen. Infected samples of both species showed visible disease progression including browning and chlorosis compared to mock-treated samples. At 4 dpi, the tdTomato signal was stronger in *A. agrestis* so subsequent analyses were conducted with this species (Figure 5.5; 5.6).

In *A. agrestis*, discoloration of infected thalli was observed from 2 dpi and hyphal growth on plants was visible from 3 dpi, whereas the mock-treated sample remained green across all time points. The disease progression in *A. agrestis* showed a pattern similar to *C. purpureus* but faster. At 2 dpi, diffused tdTomato signal and a few puncta were observed on the surface. More puncta appeared from 3 dpi, and the hornwort was fully colonized by puncta (sporangia) at 4 dpi (Figure 5.6). Because disease progression was clearly resolved at 2–4 dpi, these time points were selected for the time-course RNA-seq.

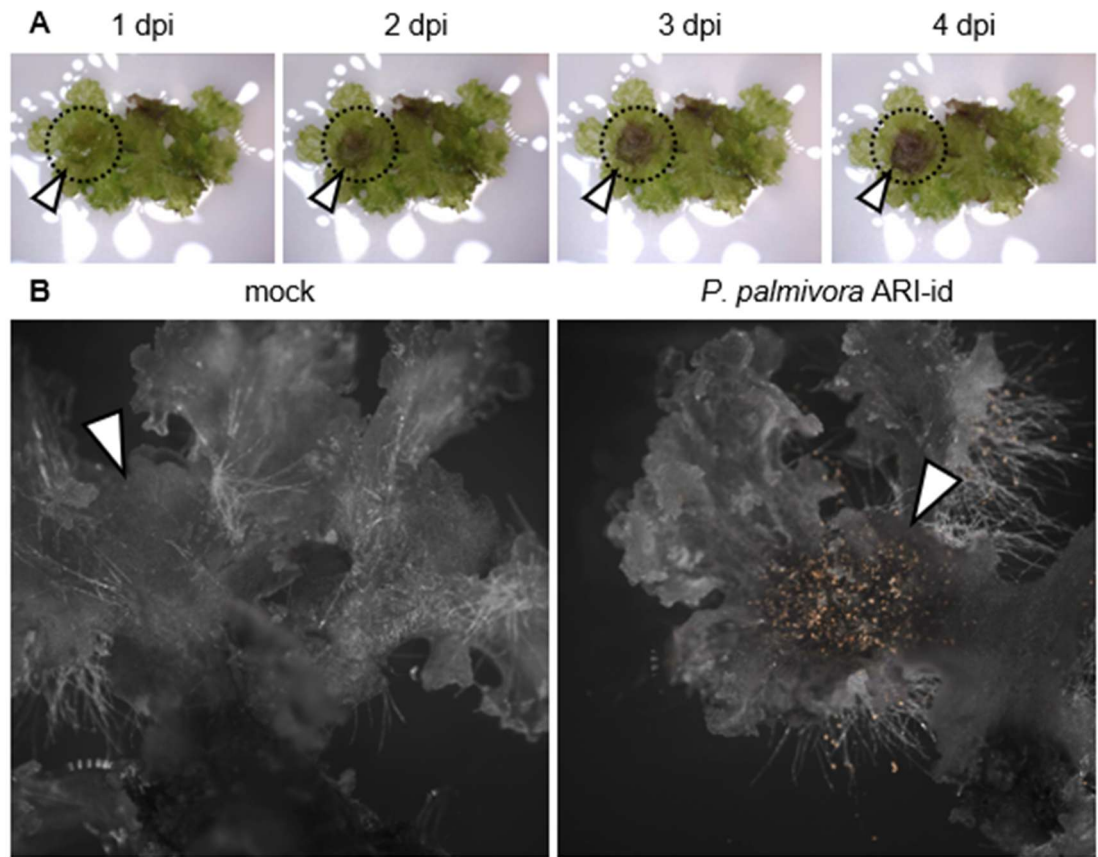


Figure 5.5 *P. palmivora* can infect the model hornwort *A. punctatus*. **(A)** Time-course images *P. palmivora* infection in *A. punctatus*. **(B)** *P. palmivora* growth was visualized using epifluorescence scopy at 4 dpi. Mock treatment is distilled water. Shown images are overlay of black/white and RFP channels (exposure times: BW = 50 ms, RFP = 5000 ms). A single 10 μ l droplet of zoospore suspension (2×10^5 zoospores/ml) was applied on *A. punctatus* thalli. The inoculation spot is labelled with white arrowheads.

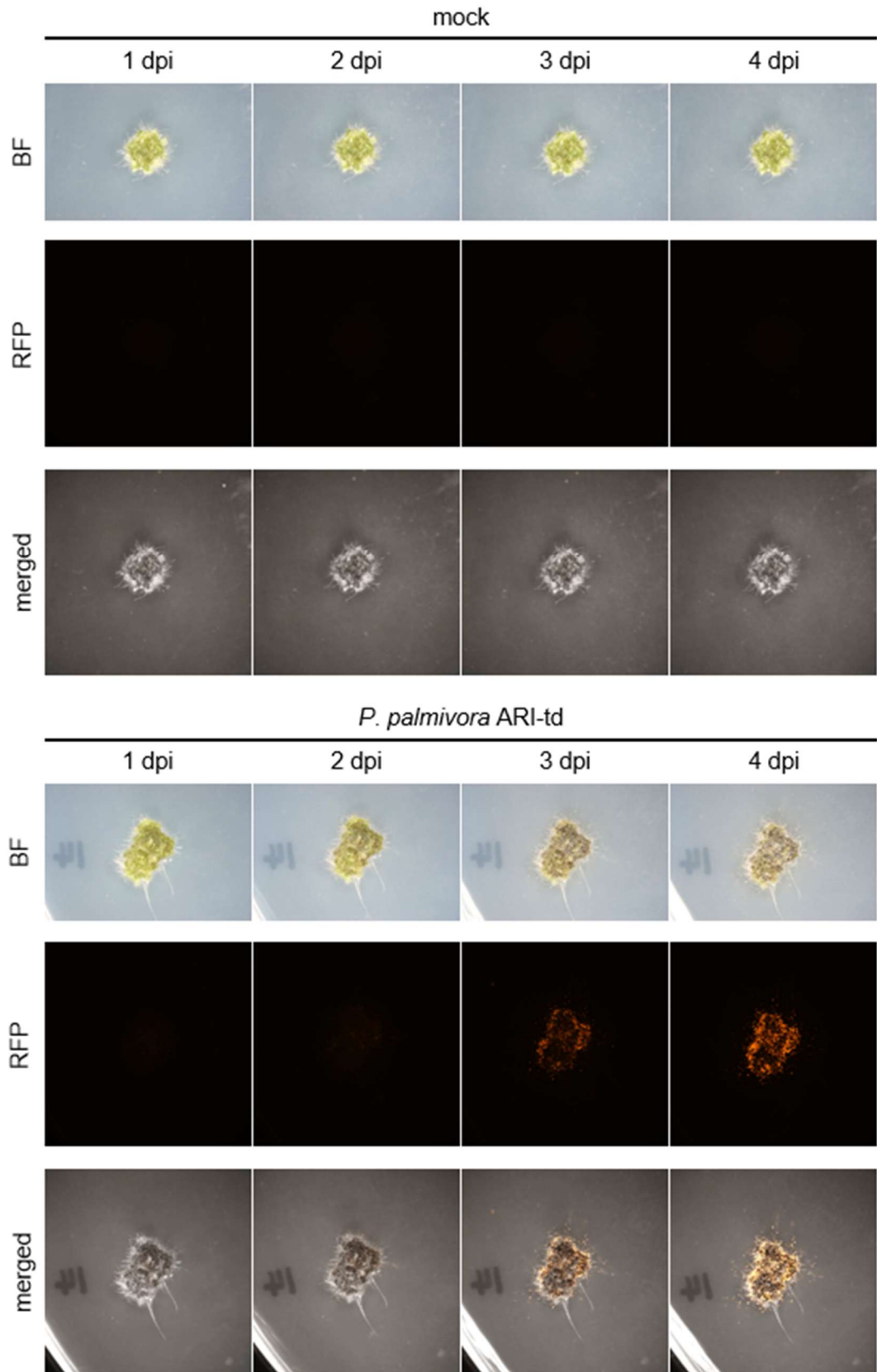


Figure 5.6 Time-course imaging of *P. palmivora* infection in the model hornwort *A. agrestis*. Mock treatment is distilled water. BF: bright field. Merged: overlay of black/white and RFP channels. Exposure times: BF = 40 ms, BW = 5 ms, RFP = 5000 ms.

5.2.2.1 Transcriptome shifts during *P. palmivora* infection appear more evident in *A. agrestis* than in *C. purpureus*

To understand how *A. agrestis* responds to infection, I performed time-course RNA-seq analysis comparing the transcription profiles of 3-week-old Oxford strains spot inoculated with water (mock) or a suspension of *P. palmivora* ARI-td zoospores. DEGs were defined using these thresholds, log2 fold change $[|LFC|] \geq 1.5$ and adjusted $p < 10^{-3}$, of mock versus infected thalli sampled daily from 1 to 4 dpi. In *A. agrestis*, mock-treated and infected samples were clearly separated by treatment in hierarchical clustering, with all mock-treated samples across 1–4 dpi clustering together. Among infected samples, the later infection stages (3 and 4 dpi) clustered together, and the earlier stages (1 and 2 dpi) clustered together; the early-stage clusters partially overlapped with mock, an effect that was weaker by 2 dpi (Figure 5.7A).

The same pattern was displayed in the PCA plot. All mock-treated samples clustered tightly and showed minimal dispersion on both PC1 (92% variance) and PC2 (7% variance), whereas infected samples separated by time point along the infection trajectory (Figure 5.7B). Together, these observations indicate a rapid and time-ordered transcriptome shift during *P. palmivora* infection in *A. agrestis*, consistent with faster disease progression tracked by the tdTomato signal. They also support the idea that continued vegetative growth in *C. purpureus* may have impacted early transcriptome shifts.

5.2.2.2 Identification of a core infection-induced gene set in *A. agrestis*

Both upregulated and downregulated genes defined by the set thresholds increased over time across the sampled time points, with an exemption that the number of upregulated genes peaked at 3 dpi (1,283 genes) and slightly decreased at 4 dpi (1,219 genes). The number of downregulated genes became almost equivalent to upregulated genes at 3 dpi (1,264 genes) and exceeded it at 4 dpi (1,361 genes) (Figure 5.7C).

For upregulated genes, UpSet analysis identified the three largest intersections as follows: genes common to 1–4 dpi ($n = 418$), genes shared between 3 and 4 dpi ($n = 342$), and common genes between 2–4 dpi ($n = 283$). The fourth and fifth largest intersections were 4 dpi-specific ($n = 171$) and 3 dpi-specific ($n = 164$) genes, respectively. All other intersections were smaller than 40. These patterns indicate the presence of a core set of responding genes in *A. agrestis*, that is augmented by genes that are specific in later infection stages (Figure 5.7D).

The largest intersection for downregulated genes consisted of genes shared between 3 and 4 dpi ($n = 564$), followed by genes common to 2–4 dpi ($n = 450$). By contrast to upregulated genes, genes downregulated across all time points were few ($n = 41$) (Figure 5.7D). These results suggest that

infection-associated downregulation in *A. agrestis* is more time- or disease progression- dependent, with a limited number of persistently repressed genes.

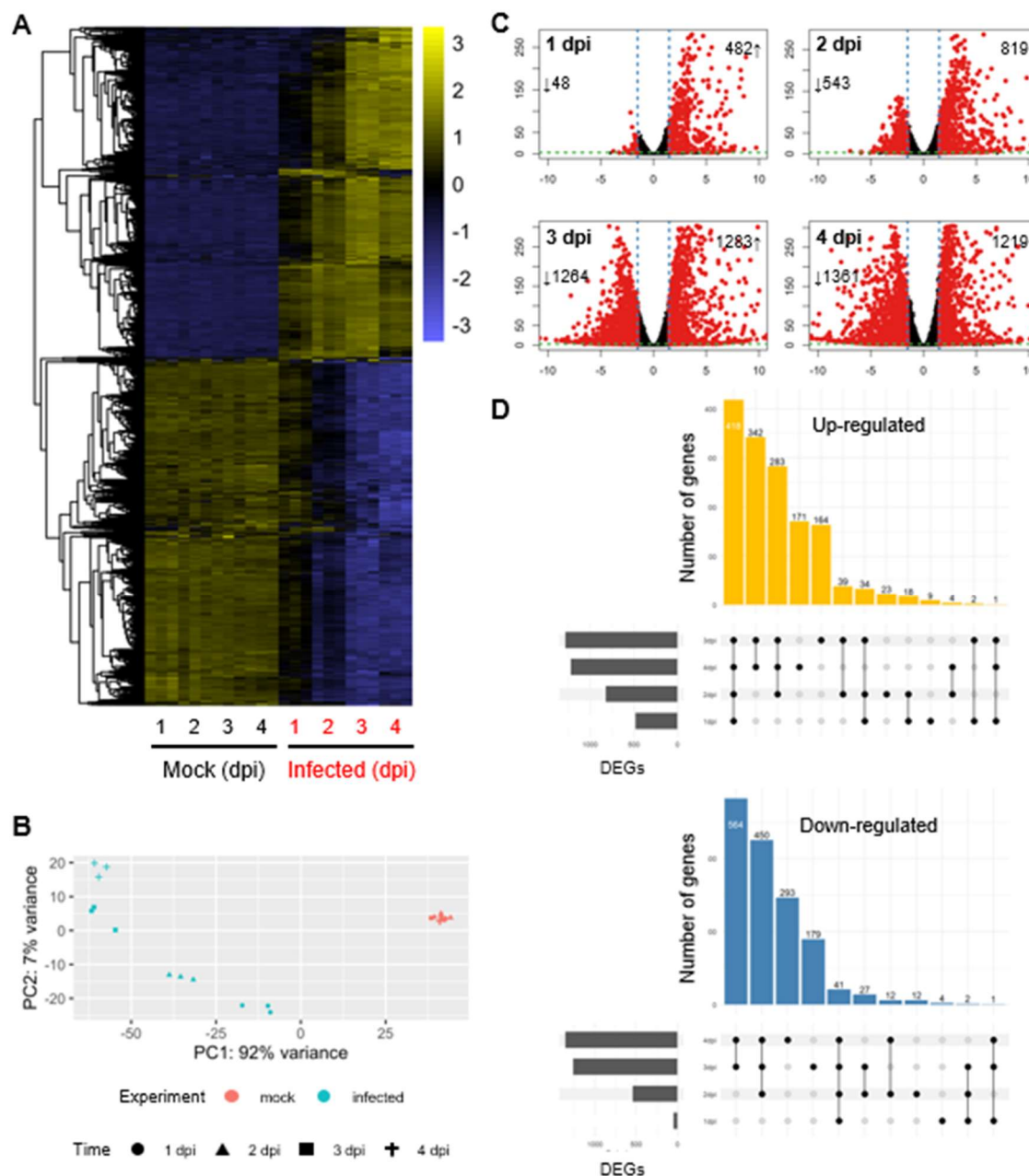


Figure 5.7 Transcriptional responses of *A. agrestis* during *P. palmivora* infection. DEGs were defined by the following thresholds: adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. **(A)** Hierarchical clustering of DEGs. **(B)** PCA of transcriptome profiles between two treatments (mock vs infected). **(C)** Volcano plots displaying pairwise differential expression analysis at each time point. **(D)** UpSet plots showing shared and time point-specific DEGs.

5.2.2.3 Putative *A. agrestis* PR genes are induced during infection

Potential PR genes in most families were detected by HMMER profiling in the *A. agrestis* genome. As in *C. purpureus* and *M. polymorpha*, *A. agrestis* lacked PR12 (γ -thionin/plant defensin) and PR13 (thionin) (Carella et al., 2019). One Barwin domain protein was detected in the annotated proteome. A separate hevein-like chitin binding domain (CBM18) was also detected. However, these domains did not occur on the same protein. Class I PR4 proteins harbor a C-terminal Barwin domain and an N-terminal CBM18 domain, whereas class II PR4 proteins are absent of the CBM18 domain (Dabravolski and Frenkel, 2021). Therefore, this Barwin only protein was classified as class II PR4 protein. In addition, PR17-like genes were not found in this (Table 5.3).

Table 5.3 List of potential PR genes identified in *A. agrestis* by HMMER profiling

Pfam motif	Description	PR family	Presence
PF00188	Cysteine-rich secretory protein family	PR1	Yes
PF00332	Glycosyl hydrolases family 17	PR2	Yes
PF00182	Chitinase class I (GH19)	PR3	Yes
PF00967	Barwin family	PR4	Yes
PF00187	Chitin recognition protein (CBM18/hevein-like)*	PR4	Yes
PF00314	Thaumatococcus family	PR5	Yes
PF00280	Potato type I proteinase inhibitor family	PR6	Yes
PF02428	Potato type II proteinase inhibitor family	PR6	No
PF00014	Kunitz/Bovine pancreatic trypsin inhibitor domain	PR6	No
PF00228	Bowman-Birk serine protease inhibitor family	PR6	No
PF00079	Serpin (serine protease inhibitor)	PR6	Yes
PF00082	Subtilase	PR7	Yes
PF00704	GH18 (chitinase)	PR8	Yes
PF00141	Peroxidase	PR9	Yes
PF00407	Pathogenesis-related protein Bet v 1 family	PR10	Yes
PF00304	γ -thionin family (plant defensin)	PR12	No
PF00321	Plant thionin	PR13	No
PF00234	Protease inhibitor/seed storage/LTP family	PR14	Yes
PF00190	Cupin	PR15/16	Yes
PF04450	Peptidase of plants and bacteria	PR17	No

*Some PR4 proteins harbor this domain alongside Barwin domain

I next asked if potential PR genes overlapped with the upregulated genes at 4 dpi. A number of candidate PR genes were identified, such as: PR2 (β -glucanase), PR3 and PR8 (chitinase), PR5 (thaumatin-like), PR6 (protease inhibitor), PR7 (subtilase), PR9 (peroxidase), PR14 (lipid transfer proteins), and PR15 (cupin). Interestingly, putative PR1 proteins were detected by the profiling, but were not enriched in the upregulated genes (Table 5.4). Numbers of enriched *A. agrestis* PR9 and PR15 genes were similar to *M. polymorpha* compared to *C. purpureus* (Carella et al., 2019), consistent with the absence of whole genome duplication in both species (Li et al., 2020).

Table 5.4 Putative *A. agrestis* PR genes induced by *P. palmivora*

PR family	Description	Number of genes *
PR2	β -1,3-glucanase, GH17	1
PR3	Chitinase, GH19	3
PR4	Barwin domain protein**	1
PR5	Thaumatin-like proteins	5
PR6	Protease inhibitor, Pin-I type	3
PR7	Subtilisin-like proteases	2
PR8	Chitinase, class III (GH18)	2
PR9	Peroxidase, class III	16
PR15	Cupin/germin	9

* = upregulated during infection at 4 dpi

**PR4, class II: lacking hevein-like chitin binding domain

5.2.3 *M. polymorpha* is a compatible host of three broad host range *Phytophthora* species

In the previous chapter, I showed that many broad host range *Phytophthora* strains can infect *M. polymorpha*, including *P. parasitica* 310, *P. cactorum* Pc10300, and *P. capsici* LT1534 (Figure 5.1B). To compare host responses to these distantly related *Phytophthora* species, I also performed RNA-seq on *M. polymorpha* infected with each pathogen at 4 dpi, matching the time point used to capture full infection in *P. palmivora*.

5.2.3.1 Three *Phytophthora* species induced transcriptional reprogramming of *M. polymorpha*

To assess *M. polymorpha* responses to different pathogens, I called DEGs using the thresholds (adjusted $p < 10^{-3}$ and $|\log_2 \text{ fold change}| \geq 2$) between mock-treated (plain V8 agar plugs) and infected 24-day-old *M. polymorpha* thalli. Hierarchical clustering displayed clustering of

transcriptome shifts by each pathogen, although there were several pathogen-specific differences. Interestingly, two *Phytophthora* species in clade 1, *P. parasitica* and *P. cactorum*, clustered more closely than *P. capsici* (clade 2) (Figure 5.8A). In PCA, infected samples with each pathogen separated from mocks along PC1 (81% variance) but showed dispersion in PC2 (17% variance) (Figure 5.8B). These results indicate both shared transcriptional reprogramming induced by pathogens, and pathogen-specific signatures.

Pairwise comparisons between mock-treated and infected samples showed that the three *Phytophthora* species induced more DEGs than *P. palmivora* in most cases. At 4 dpi, significantly downregulated genes were more abundant in *P. cactorum* (n = 841), *P. capsici* (n = 663), and *P. parasitica* (n = 652) than in *P. palmivora* (n = 435). For upregulated genes, two species, *P. cactorum* (n = 1082) and *P. capsici* (n = 1297), exceeded *P. palmivora* (n = 954), whereas *P. parasitica* induced a slightly smaller set of downregulated genes (n = 925) (Figure 5.8C). Together, these comparisons indicate that all three *Phytophthora* species trigger robust transcriptional reprogramming in *M. polymorpha*, with species-differences in the induction profiles.

5.2.3.2 *M. polymorpha* induces a shared set of genes against three *Phytophthora* species

I analyzed three infection transcriptomes acquired at 4 dpi together with the publicly available *P. palmivora* infection transcriptome from the same host and time point. All datasets were processed with the same DEG thresholds ($\log_2$ fold change $|\text{LFC}| \geq 2$ and adjusted p value $< 10^{-3}$) prior to analyses. Notably, UpSet analysis recapitulated the pattern observed when the single broad host pathogen was challenged to different plant species (Figure 5.8D; 5.9). The largest intersection comprised genes commonly upregulated by all four pathogens (n = 436). Three pathogen-specific intersections followed the largest intersection with similar numbers to one another: *P. cactorum* (n = 276), *P. capsici* (n = 275), and *P. palmivora* (n = 248). On the other hand, *P. parasitica*-specific genes formed the smallest set (n = 14).

For downregulated genes, the common intersection across the pathogens (n = 101) was smaller than any pathogen-specific set: *P. cactorum* (n = 312), *P. capsici* (n = 263), *P. palmivora* (n = 162), and *P. parasitica* (n = 128) (Figure 5.8D; 5.9). Together, the parallel patterns between the broad host range pathogen and the broadly susceptible host suggest that both players in plant–pathogen interactions have a ‘to-go’ core gene set, while the detailed strategies are modulated in context-dependent manner.

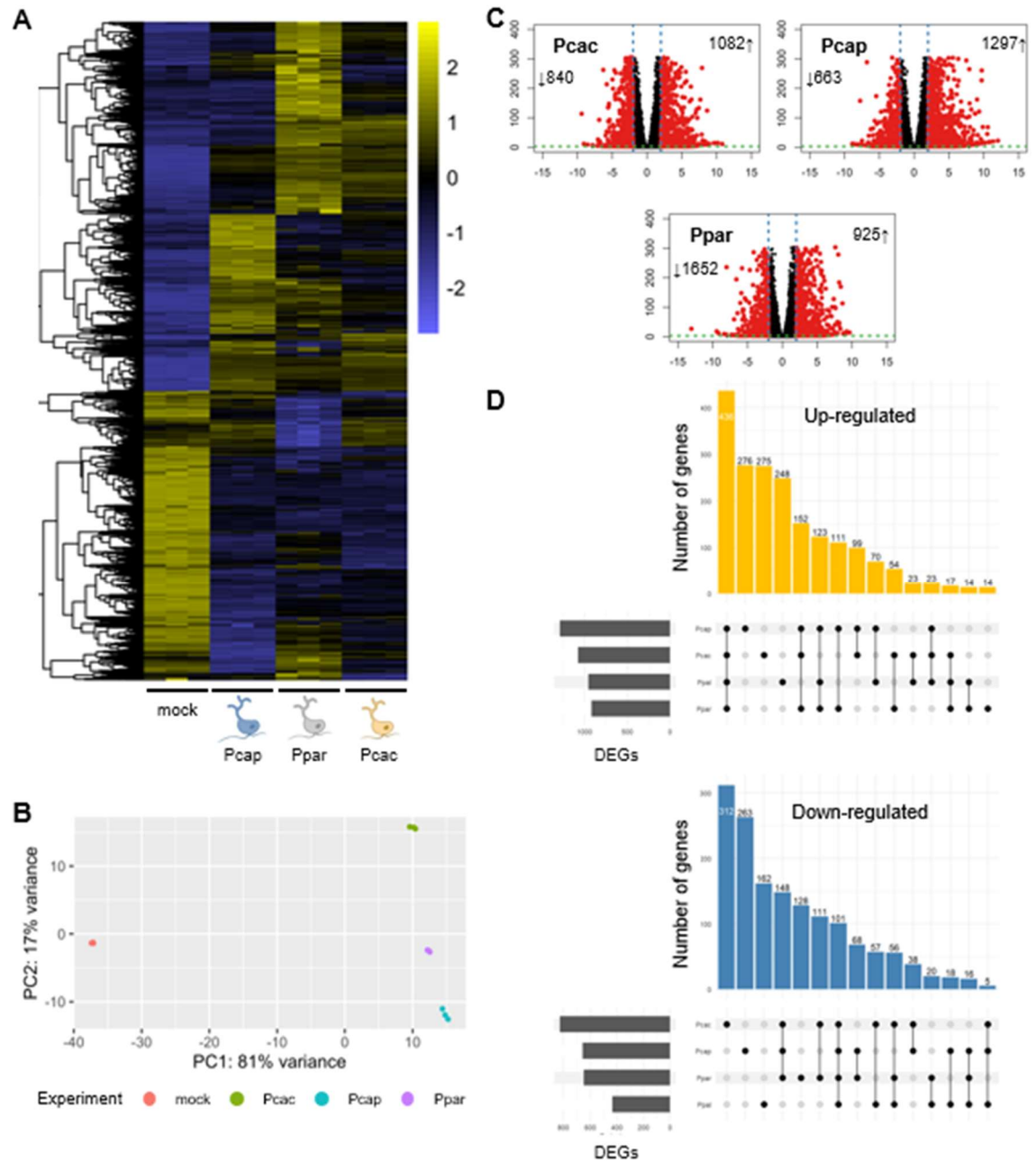


Figure 5.8 Transcriptional responses of *M. polymorpha* during *Phytophthora* infection. DEGs were defined by the following thresholds: adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. Abbreviations: Pcap = *P. capsici*, Ppar = *P. parasitica*, Pcac = *P. cactorum*, Ppal = *P. palmivora*. **(A)** Hierarchical clustering of DEGs. **(B)** PCA of transcriptome profiles between two treatments (mock vs infected). **(C)** Volcano plots displaying pairwise differential expression analysis at each time point. **(D)** UpSet plots showing shared and time point-specific DEGs.

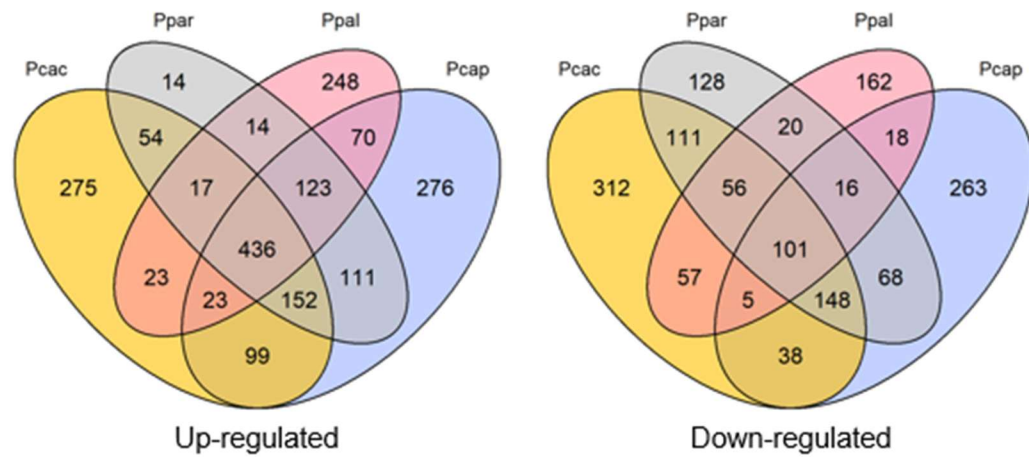


Figure 5.9 Shared and pathogen-specific DEGs of *M. polymorpha* during infection of four *Phytophthora* species. Time point is 4 dpi. Abbreviations: Pcap = *P. capsici*, Ppar = *P. parasitica*, Pcac = *P. cactorum*, Ppal = *P. palmivora*.

5.2.3.3 Enrichment analysis of commonly upregulated genes highlight apoplast immunity and phenylpropanoids

To profile members of the core *M. polymorpha* defense gene set, I conducted enrichment analysis of the 436 commonly upregulated genes. The top twenty significantly enriched gene ontology (GO) terms included 157 genes and encompassed a variety of metabolic and stress response processes (Figure 5.10). These terms can be categorized into two broader themes: (i) apoplastic immunity, and (ii) phenylpropanoid pathway.

Apoplast-linked terms were enriched, including peroxidase activity (GO:0004601), hydrogen peroxide catabolic process (GO:0042744), response to oxidative stress (GO:0006979), heme binding (GO:0020037), manganese ion binding (GO:0030145), and signaling receptor activity (GO:0038023). Together these indicate cell surface signal perception and a reactive oxygen species (ROS) burst, a hallmark of pattern-triggered immunity. ROS production of *M. polymorpha* in response to pathogen-associated molecular patterns (PAMPs) has been demonstrated (Yotsui et al., 2023), and the membrane-associated oxidase required for the apoplastic ROS burst, RBOHD (RESPIRATORY BURST OXIDASE HOMOLOG D), is also functionally conserved in this species (Chu et al., 2023).

Enzymatic activity characteristics of two PR families were enriched: for PR6 (serine type endopeptidase inhibitor activity, GO:0004867) and for PR9 (peroxidase activity; GO:0004601 and heme binding; GO:0020037). This mirrors prior work showing that these families are the most highly induced during *P. palmivora* infection in *M. polymorpha* (Carella et al., 2019).

Terms tied to entry and supply into phenylpropanoid pathway were detected, including ammonia-

lyase activity (GO:0016841), L-phenylalanine catabolism (GO:0006559), and aromatic amino acid biosynthetic process (GO:0009073), consistent with roles of phenylpropanoid metabolism during *M. polymorpha* defense (Carella et al., 2019). The pathway's first step is catalyzed by PAL (phenylalanine ammonia-lyase), converting phenylalanine to cinnamic acid. Notably, the term wounding response (GO:0009611) was enriched, and prior work has demonstrated that wounding induces MpPAL genes and promotes phenylpropanoid accumulation (Yoshikawa et al., 2018).

Two overlapping terms about acylation, acyltransferase activity (GO:0016746) and acyltransferase activity, transferring groups other than amino-acyl groups (GO:0016747), were also enriched. Since acyltransferases participate in diverse processes, it may reflect multiple and layered biochemical processes. Interestingly, plant BAHD (BEAT, AHCT, HCBT, and DAT) acyltransferases modify secondary metabolites, providing a link to phenylpropanoid pathway (D'Auria, 2006).

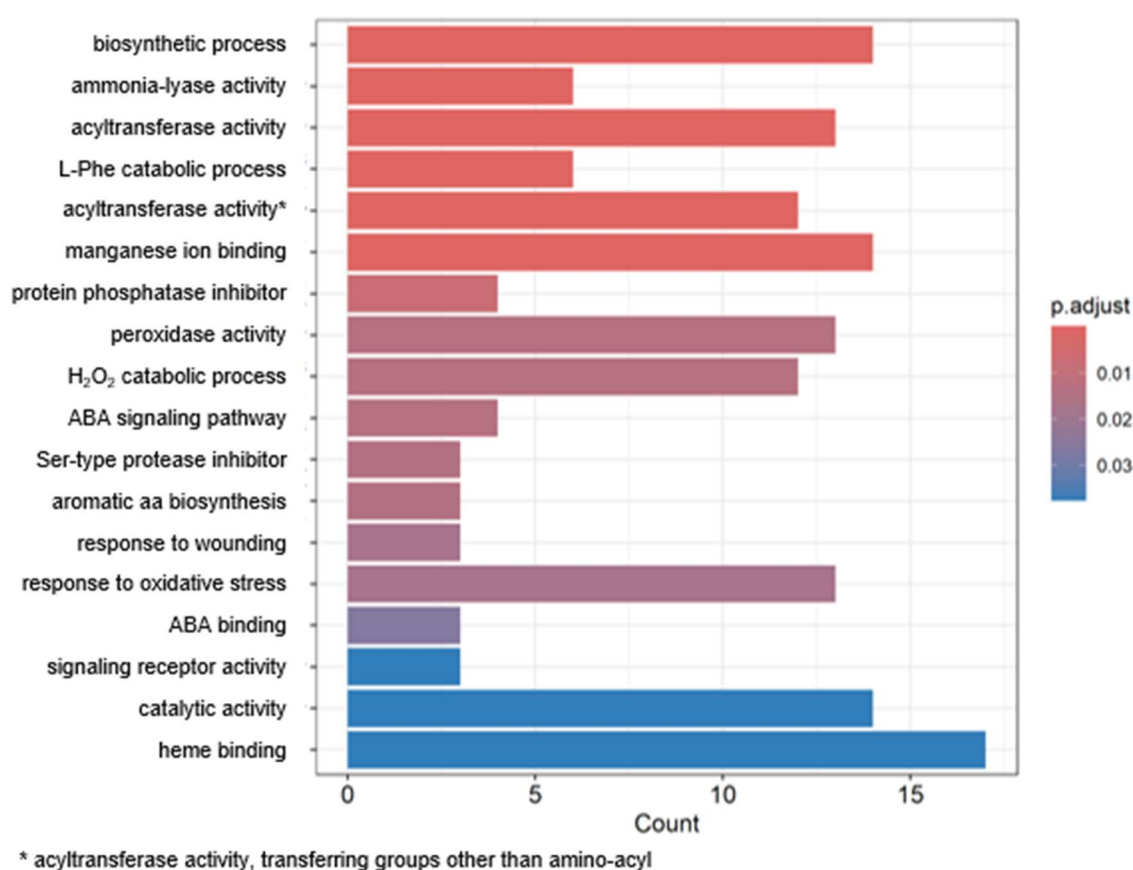


Figure 5.10 Enrichment analysis of commonly upregulated genes of *M. polymorpha* during infection by four *Phytophthora* infections. Adjust p values are shown in color with a gradient scale on the right. Significant GO terms were defined as false discovery rate (FDR) < 0.05.

5.2.4 *P. palmivora* has a core set of pathogenesis genes to infect different hosts

I analyzed the *P. palmivora* pathogen transcriptome during infection of the moss *C. purpureus* and the hornwort *A. agrestis* and integrated these data with publicly available infection transcriptomes from the liverwort *M. polymorpha* and the angiosperm *N. benthamiana* after remapping all reads to the current *P. palmivora* reference genome (Carella et al., 2019). Because the disease progression may not be synchronized across hosts, I focused on the 4 dpi time point to capture an active and ongoing infection in all systems, as confirmed in previous microscopy (Figure 5.2; 5.6). All datasets were processed with the same DEG thresholds ($\log_2$ fold change $|LFC| \geq 2$ and adjusted p value $< 10^{-3}$), comparing pathogen in axenic growth in liquid culture (mock) and in hosts.

Strikingly, an UpSet analysis of upregulated genes at 4 dpi revealed that the largest intersection comprised genes commonly upregulated across four hosts ($n = 2,296$), a substantial fraction given the total gene number in this species (37,363) (Figure 5.11). Host-specific sets were smaller than the common set: *M. polymorpha* ($n = 1,697$), *A. agrestis* ($n = 1,423$), *N. benthamiana* ($n = 1,423$), and *C. purpureus* ($n = 240$). This order mirrored the total number of upregulated genes detected in each host: *M. polymorpha* ($n = 6,371$), *A. agrestis* ($n = 5,996$), *N. benthamiana* ($n = 4,301$), and *C. purpureus* ($n = 4,093$), suggesting that intersection sizes of host-specific genes were influenced by the total DEGs in each host. For downregulated genes, sizes of host-specific intersections followed the same ordering by total DEG counts. However, the size of the intersection of commonly downregulated genes ($n = 171$) was between the second largest (*N. benthamiana*, $n = 229$) and the third largest species-specific intersection (*C. purpureus*, $n = 107$) (Figure 5.11).

To profile candidate pathogenicity factors, I extracted the top twenty commonly upregulated *P. palmivora* genes based on the average $\log_2$ fold change across hosts. Five were annotated as putative effectors by EffectorP, including three predicted apoplastic effectors and two predicted cytoplasmic effectors without canonical RXLR or CRN motifs. Notably, almost half of the top twenty proteins (9/20), including one potential elicitor, were predicted to be secreted (Table 5.5). Together, these results suggest that *P. palmivora* tends to deploy a conserved transcriptional program to infect diverse plant species. Considering that sizes of host-specific intersections were not trivial, the core pathogenicity program may be fine-tuned by these host-specific modules.

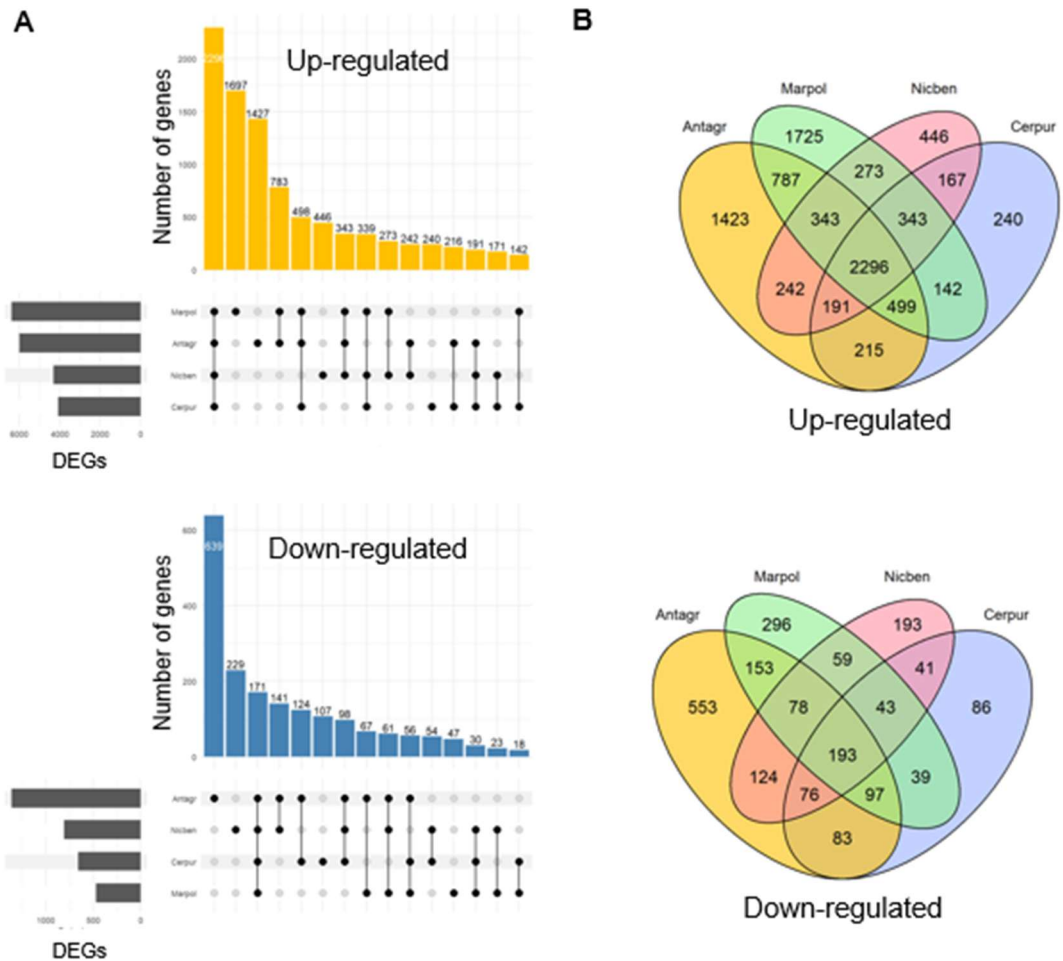


Figure 5.11 Transcriptional responses of *P. palmivora* during infection across all four plants. DEGs were defined by the following thresholds across all four time points (1–4 dpi) : adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. Abbreviations: Cerpur = *C. purpureus*, Antagr = *A. agrestis*, Marpol = *M. polymorpha*, Nicben = *N. benthamiana*. **(A)** UpSet plots showing shared and host-specific DEGs in *P. palmivora* during infection **(B)** Comparison of differentially regulated *P. palmivora* genes identified at 4 dpi.

Table 5.5 Predicted function of the top twenty commonly upregulated *P. palmivora* genes during infection

Gene ID	log2 fold change				Predicted function	effectorP prediction
	Antagr	Cerpur	Marpol	Nicben		
ARI_23624	16.5	15.7	15.9	14.4	hypothetical protein	Non-effector
ARI_29818	13.7	13.2	14.3	11.9	secretory protein	Cytoplasmic effector
ARI_23560	13.9	13.3	13.5	12.1	secretory protein	Non-effector
ARI_27422	15.1	13.1	14.4	10.2	NLI interacting factor-like phosphatase	N/A
ARI_23134	11.0	14.3	11.4	15.8	elicitin	Non-effector
ARI_17805	14.6	12.8	13.5	11.2	hypothetical protein	N/A
ARI_03561	14.8	12.2	14.7	10.1	secretory protein	Non-effector
ARI_28268	14.8	13.2	12.9	10.8	hypothetical protein	N/A
ARI_17804	14.8	12.1	13.7	10.7	hypothetical protein	N/A
ARI_05118	14.8	12.4	13.1	10.9	type 1 membrane protein	N/A
ARI_19491	13.2	12.2	12.9	11.2	secretory protein	Apoplasic effector
ARI_15180	14.2	11.7	12.8	10.5	secretory protein	Cytoplasmic effector
ARI_28267	14.8	12.2	12.4	9.8	hypothetical protein	N/A
ARI_23868	13.7	12.0	12.8	10.6	CBM1 family cellulase, secretory	Apoplasic effector
ARI_31171	14.8	11.7	12.1	10.0	type 1 membrane protein, secretory	Apoplasic effector
ARI_01561	14.3	11.9	13.2	9.2	NLI interacting factor-like phosphatase	N/A
ARI_11824	12.0	12.9	10.7	12.9	mitochondrial aldehyde dehydrogenase	N/A
ARI_08397	10.3	12.7	11.7	13.7	hypothetical protein	N/A
ARI_29814	14.2	11.2	12.4	10.6	membrane-associated fatty acid hydroxylase (dioxygenase), secretory	Non-effector
ARI_27590	14.1	11.7	12.4	10.2	kinase-like protein	N/A

I next focused on upregulated genes encoding effectors that are known to be translocated into the host, RXLR and Crinkler (CRN) effectors (Stassen and van den Ackerveken, 2011). To include stage-specific expression, I aggregated upregulated effector-encoding genes across 1–4 dpi. UpSet analysis of induced effector genes recapitulated the global trend seen in the upregulated genes across four hosts. The largest intersection consisted of effectors commonly upregulated in all hosts ($n = 51$), followed by host-specific intersections: *C. purpureus* ($n = 45$), *N. benthamiana* ($n = 23$), *A. agrestis* ($n = 22$). The size of the *M. polymorpha*-specific gene set was 8 (Figure 5.12A). Among the shared effectors, most (48 out of 51) were annotated as RXLR or potential RXLR effectors, with the remainder of potential CRN effectors (Figure 5.12B; Table 5.6).

To further dissect effector dynamics, I examined temporal expression profiles of 51 commonly upregulated effector genes. Although a small subset of effectors (ARI_08766, ARI_25545, ARI_30231, ARI_36198, and ARI_37418) were consistently expressed in all hosts and at all time points, the majority were not. Most effector-encoding genes showed peak induction early in infection followed by a decline, suggesting that later infection does not rely on effectors as much as earlier stages (Figure 5.13). Notably, a few effectors, such as ARI_23107 in *A. agrestis* and ARI_36198 in *N. benthamiana*, maintained or even increased expression toward later stages, hinting at roles in sustaining colonization rather than initial steps.

Taken together, these analyses support a model in which *P. palmivora* infection relies on a core set of pathogenesis genes across phylogenetically distant hosts, with RXLR effectors likely playing a central role in this conserved program.

Table 5.6 List of commonly upregulated *P. palmivora* RXLR and CRN effectors

Gene ID	Prediction by EffectorP	Predicted class
ARI_01783	Cytoplasmic effector	potential RXLR
ARI_02135	Cytoplasmic effector	RXLR
ARI_02590	Cytoplasmic effector	RXLR
ARI_03037	Cytoplasmic effector	potential RXLR
ARI_03093	Cytoplasmic effector	RXLR
ARI_03209	Cytoplasmic effector	RXLR
ARI_03394	Cytoplasmic effector	potential CRN
ARI_03612	Cytoplasmic effector	potential RXLR
ARI_03670	Cytoplasmic effector	RXLR
ARI_04952	Cytoplasmic effector	potential RXLR
ARI_06236	Cytoplasmic effector	RXLR
ARI_06716	Cytoplasmic effector	RXLR
ARI_08766	Cytoplasmic effector	RXLR
ARI_10052	Cytoplasmic effector	potential RXLR
ARI_10115	Cytoplasmic effector	RXLR
ARI_10180	Cytoplasmic effector	RXLR
ARI_10594	Cytoplasmic effector	RXLR
ARI_13011	Cytoplasmic effector	potential RXLR
ARI_13019	Cytoplasmic effector	RXLR
ARI_14039	Cytoplasmic effector	RXLR
ARI_14041	Cytoplasmic effector	RXLR
ARI_14920	Cytoplasmic effector	potential RXLR
ARI_15250	Cytoplasmic effector	potential RXLR
ARI_15610	Apoplastic effector	potential CRN
ARI_19382	Cytoplasmic effector	RXLR
ARI_20178	Cytoplasmic effector	potential RXLR
ARI_21189	Cytoplasmic effector	RXLR
ARI_21772	Apoplastic effector	potential RXLR
ARI_22001	Cytoplasmic effector	RXLR
ARI_22025	Cytoplasmic effector	potential RXLR
ARI_22179	Cytoplasmic effector	RXLR
ARI_22640	Cytoplasmic/apoplastic effector	potential RXLR
ARI_23017	Cytoplasmic effector	potential RXLR
ARI_24428	Cytoplasmic effector	RXLR
ARI_24459	Cytoplasmic effector	RXLR
ARI_24469	Cytoplasmic effector	RXLR
ARI_24555	Cytoplasmic effector	potential CRN
ARI_25545	Cytoplasmic effector	RXLR
ARI_26468	Cytoplasmic/apoplastic effector	potential RXLR
ARI_27687	Cytoplasmic effector	RXLR
ARI_27916	Cytoplasmic effector	potential RXLR
ARI_28780	Cytoplasmic/apoplastic effector	potential RXLR
ARI_30231	Cytoplasmic effector	RXLR
ARI_30282	Cytoplasmic effector	potential RXLR
ARI_32713	Cytoplasmic effector	RXLR
ARI_34421	Cytoplasmic effector	RXLR
ARI_35881	Cytoplasmic effector	potential RXLR
ARI_35996	Cytoplasmic effector	RXLR
ARI_36094	Apoplastic effector	potential RXLR
ARI_36198	Cytoplasmic effector	RXLR
ARI_37418	Cytoplasmic effector	RXLR

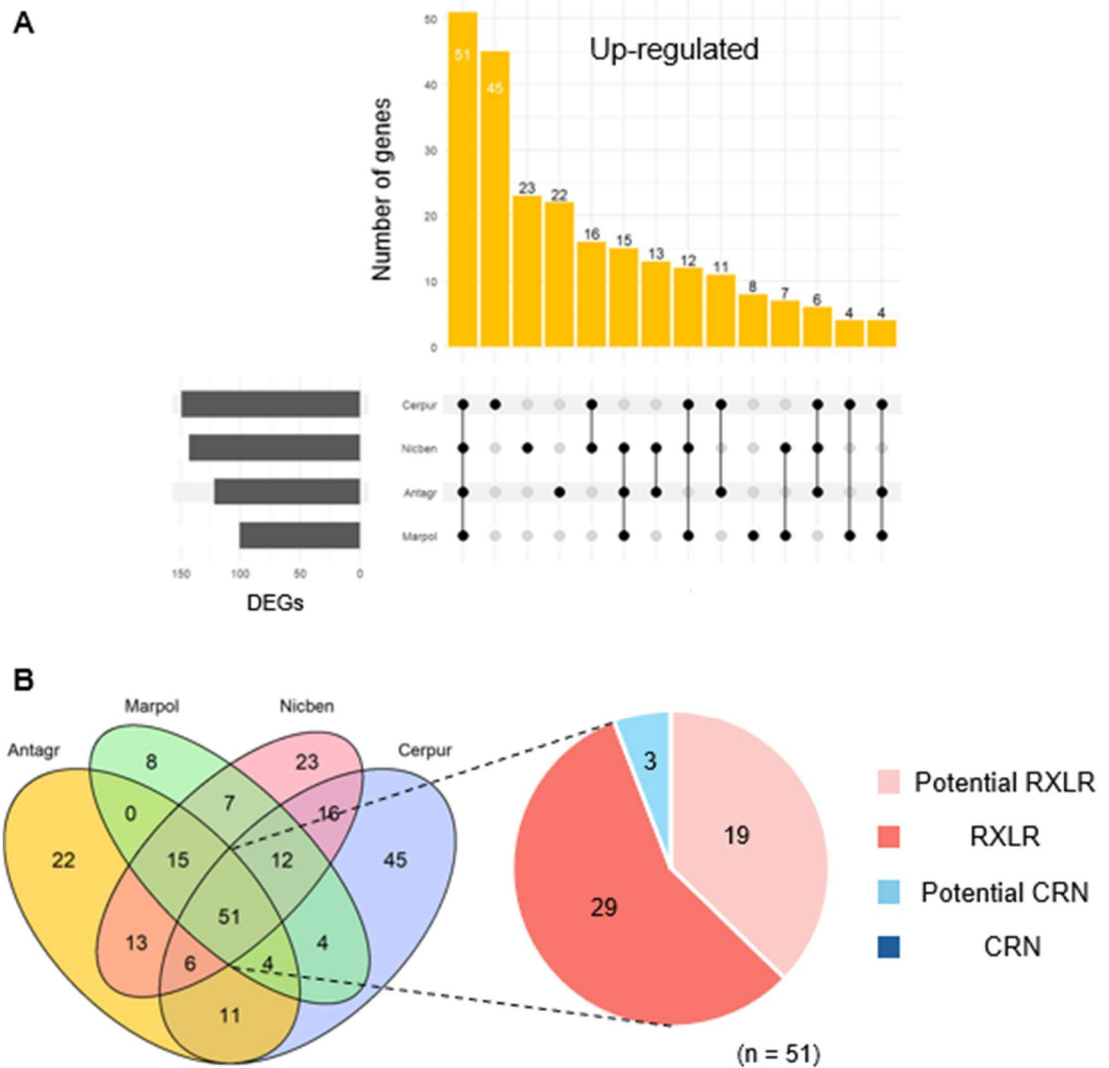


Figure 5.12 Commonly upregulated effectors of *P. palmivora* all four plants. DEGs were defined by the following thresholds across all four time points (1–4 dpi) : adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. Abbreviations: Cerpur = *C. purpureus*, Antagr = *A. agrestis*, Marpol = *M. polymorpha*, Nicben = *N. benthamiana*. **(A)** UpSet plots showing shared and host-specific upregulated genes in *P. palmivora* during infection. **(B)** Ratio of effector classes among commonly upregulated *P. palmivora* effectors.

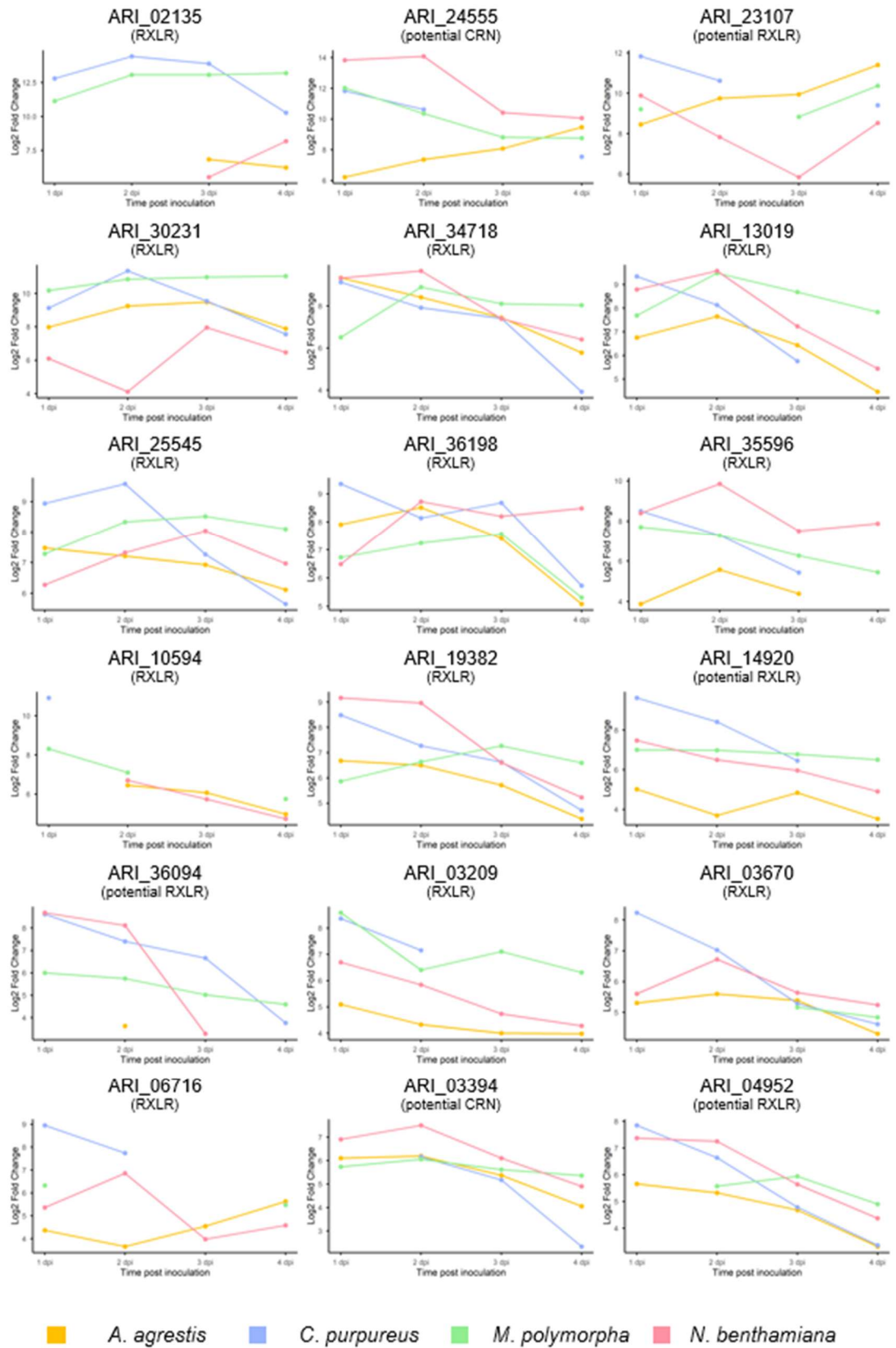


Figure 5.13 Expression profiles of commonly upregulated *P. palmivora* effectors. DEGs were defined by the following thresholds across all four time points (1–4 dpi) : adjusted $p < 10^{-3}$ and $|\log_2 \text{fold change}| \geq 2$. Top eighteen effectors were chosen based on average log₂ fold change.

5.3 Conclusion and discussion

In this chapter, I established two additional bryophyte–*P. palmivora* pathosystems to interrogate molecular determinants of its broad host range. *P. palmivora* successfully colonized the model moss *C. purpureus* and the model hornworts *A. agrestis* and *A. punctatus*. Infection progression and completion in *C. purpureus* and *A. agrestis* were tracked by tdTomato fluorescence in transgenic *P. palmivora*, alongside visible macroscopic symptoms. *C. purpureus* continued growth, whereas *A. agrestis* did not. This physiological difference between two species may have impacted the hosts' early transcriptome shifts.

Most PR families were identified in both species, with notable differences: *C. purpureus* appeared to lack PR4 proteins under my criteria but encode PR17-like genes that were induced by infection. Putative CpPR6 genes were present but not induced by infection. In *A. agrestis*, HMMER profiling and transcriptome analysis identified candidates of most well-characterized PR gene families, including PR4 and PR6, but not PR1. Although their roles in bryophyte immunity remain to be examined by functional studies, these observations suggest that PR gene-based defense mechanisms are conserved across three bryophyte subdivisions (liverworts, mosses, and hornworts), as well as across angiosperms.

When *M. polymorpha* was infected by diverse *Phytophthora* species, host responses were dominated by commonly induced genes rather than pathogen-specific genes. Enrichment analysis underscored two conserved defense mechanisms in land plants: apoplast immunity and phenylpropanoid pathway. Abscissic acid (ABA)-related terms were also enriched. Although ABA is best known for its role in gemmae dormancy (Eklund et al., 2018), several lines of evidence link ABA to defense mechanisms, particularly via secondary metabolites. Exogenous ABA promotes biosynthesis of phenolic compounds that provide UV-C tolerance (Kageyama et al., 2015). Furthermore, components of the ABA signaling pathway contribute to abiotic stress tolerance (Tougane et al., 2010; Jahan et al., 2019). Therefore, enrichment of ABA-related terms may reflect indirect support of phenylpropanoid-linked defenses by ABA and/or crosstalk between ABA pathway and immune signaling.

P. palmivora transcriptome shifts in different hosts revealed that commonly upregulated pathogen genes outnumbered host-specific sets; the same pattern was observed for upregulated effector genes, with RXLRs dominating as commonly induced effectors. This is consistent with observations in other broad host range pathogens such as the necrotroph *Sclerotinia sclerotiorum*. When it infects two different hosts, canola (*Brassica napus*) and lupin (*Lupinus angustifolius*), PCA separated *in planta* samples from *in vitro* axenic culture while most DEGs did not differ between two species (Allan et al., 2019). Interestingly, several effector-encoding genes were consistently induced across four angiosperm species, including both susceptible and resistant ecotypes of *A. thaliana* (Guyon et al., 2014). Taken together, it suggests that *S. sclerotiorum* also primarily deploys its 'master key' program

across hosts.

The ‘master key’ underlying such generalism includes both effectors and non-effectors. For example, the *S. sclerotiorum* effector SsSSVP1 (small secreted virulence-related protein 1) perturbs host mitochondria by targeting the cytochrome-b-c₁ complex, a ubiquitous component of the respiratory chain (Lyu et al., 2016). In parallel, *S. sclerotiorum* produces oxalate to create a favorable environment. Cereal crops, which are normally nonhost, harbors oxalate oxidases, and transgenic expression of these enzymes in susceptible crops enhances resistance (Newman and Derbyshire, 2020). Consistently, within Sclerotiniaceae family, broad host range species including *S. sclerotiorum* express higher level of *oah* (oxaloacetate acetylhydrolase), which is required for oxalic acid biosynthesis, than narrow host range relatives (Andrew et al., 2012). These examples hint that non-effectors in the top 20 commonly expressed genes (15/20) may be involved in constructing permissive environment.

Temporal dynamics of *P. palmivora* effectors support a hemibiotrophic transcriptome trajectory. In my data, most of the 51 commonly upregulated *P. palmivora* effector genes showed decreased expression over time (Figure. 5.13). Similarly, in *P. infestans*, expression of RXLR effector genes decline as it enters necrotrophy phase, while necrosis-associated genes rise (Zuluaga et al., 2015). *P. capsici* transcriptome also shifts during biotrophy-to-necrotrophy transition, including decline of a subset of RXLR effectors (Jupe et al., 2013). Thus, the decline that I observed likely reflects that most of the conserved *P. palmivora* effector toolkit are involved in biotrophic interactions and biotrophy-to-necrotrophy transition.

Cross-lineage compatibility may help to understand how *P. palmivora* manipulate diverse hosts. For example, the potato pathogen *P. infestans* deploys an effector AVR1, which binds the same conserved host target in both potato and the moss *P. patens* (Overdijk et al., 2021). In angiosperms, the bacterial pathogen *P. syringae* effector AvrPto targets pattern recognition receptors, FLS2 (FLAGELLIN SENSING 2), EFR (EF-TU RECEPTOR) and the co-receptor BAK1 (BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1), to suppress pattern-triggered immunity (Xiang et al., 2008; Shan et al., 2008; Xiang et al., 2011). *M. polymorpha*, a compatible host of *P. syringae*, lacks clear FLS2 and EFR orthologs but encodes BAK1 ortholog. AvrPto binds to MpBAK1 and is required for full virulence (Bowman et al., 2017; Gimenez-Ibáñez et al., 2019). Although I cannot exclude additional AvrPto targets with structural similarity to FLS2/EFR, the compatibility conferred by AVR1 and AvrPto suggests that homologous counterparts in different species can help to understand the effector function.

Beyond effectors, modulating ROS burst and metabolic flexibility are also considered to contribute generalism (Newman and Derbyshire, 2020). To dissect the broad host range of *P. palmivora*, the conserved genes and effector toolkit will need to be tested in different perspectives.

5.4 Contribution

I thank Dr. Martin Vickers at Computational and Systems Biology Department in John Innes for helping to process raw reads and map them on the reference genomes. The reference genome of *P. palmivora* ARI-td was kindly shared by Dr. Edouard Evangelisti at Côte d'Azur University. The putative effector list of *P. palmivora* ARI-td predicted by effectorP was provided by Dr. Philip Carella at Cell and Developmental Biology Department in John Innes Centre. I thank Kayla Robinson at Carella group, John Innes Centre for growing *C. purpureus*, *A. agrestis*, and *A. punctatus* for infection experiments.

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6. Discussion

6.1 Introduction

Despite recent advances in evolutionary molecular plant–microbe interactions (EvoMPMI), most of our understanding is derived from two model bryophytes, the liverwort *Marchantia polymorpha* and the moss *Physcomitrium patens* (Ponce De León and Montesano, 2017; Jeong et al., 2023; Castel et al., 2024). However, land plant lineages are deeply divergent, and no extant species uniquely preserves the features of the last common ancestor of land plants (Harris et al., 2022). This highlights the need to expand EvoMPMI research into understudied land plant lineages, including hornworts, lycophytes, monilophytes (ferns), and gymnosperms, as well as less-characterized angiosperms, to reconstruct the evolution of plant immunity with higher resolution.

In this thesis, I leveraged recent genomic resources and emerging non-seed plant models to address this gap. In Chapter 3, I demonstrated lineage-specific divergence of the RAR1–SGT1 interface in ferns, by integrating comparative genomics, computational biological approaches, and protein–protein interaction assays. With recently established pathosystems using diverse fern species (Castel et al., 2025), this work provides a foundation for understanding fern immunity within the land plant EvoMPMI framework. In Chapter 4 and 5, I established pathosystems in the liverwort *M. polymorpha*, the moss *Ceratodon purpureus*, and the hornwort *Anthoceros agrestis*. Using these systems, I showed that RAR1-mediated nonhost resistance (NHR) is conserved in *M. polymorpha* and is likely widespread across non-flowering plants, supported by the conservation of key residues required to form the RAR1–SGT1–HSP90 immune chaperone complex. Furthermore, RNA-seq analyses across these pathosystems revealed conserved defense responses in *M. polymorpha*, including a potential role of abscisic acid in biotic stress responses in this species, and identified core virulence factors of the broad host range pathogen *Phytophthora palmivora*.

6.2 Promiscuity of SGT1 during RAR1–SGT1 divergence in ferns

Protein promiscuity often drives evolutionary innovation. Many well-characterized cases involve protein binding to small molecules, such as enzymes or receptors. Ancestral proteins typically show broader binding or catalytic capacities that later diverge into descendants with refined specificity (Wheeler et al., 2018; Shomar et al., 2024). In plants, the MADS-box protein SEP3 (SEPALLATA3) exemplifies this trajectory. SEP3 acts as a hub of MADS-box proteins during angiosperm development. The previous analysis showed that SEP3 appears to have shifted from an ancestrally broader interaction repertoire to greater specificity during evolution (Alhindi et al., 2017). Several intrinsic properties of *SGT1* may have supported a promiscuous intermediate state that enabled rewiring of the RAR1–SGT1 binding interface in leptosporangiate ferns.

6.2.1 *SGT1* is frequently a single copy gene

With no paralog to buffer loss of function, a single copy gene must maintain its original function while exploring new functions. *LEAFY* (*LFY*) is typically present as a single copy gene in most land plants. In flowering plants, *LFY* encodes a key transcription factor determining floral meristem identity. In a non-flowering plant, the moss *P. patens*, *LFY* plays a distinct role in early embryo development, implying shifts in binding DNA sequences to produce different biological outputs during evolution (Tanahashi et al., 2005). Consistent with this, *LFY* from different land plant lineages binds distinct DNA motifs. Notably, hornwort *LFY* proteins exhibited a promiscuous binding capability recognizing all DNA motifs. Ancestral state reconstruction predicted that the ancestral land plant *LFY* had a hornwort-like binding state, highlighting how promiscuity can enable innovation in single copy regulator without losing its functions (Sayou et al., 2014). Because *SGT1* is commonly single copy in non-flowering plants, a similar permissive nature of *SGT1* is a possible route to the *RAR1*–*SGT1* divergence. Notably, single copy genes are often related to essential cellular processes (Gu et al., 2003; Conant and Wagner, 2004; De Smet et al., 2013), so the copy number of *SGT1* provides a direct link to its essentiality, as discussed in the next section

6.2.2 *SGT1* is an essential gene

SGT1 is broadly essential across eukaryotes. It is required for kinetochore function in yeasts and animals, although its role in kinetochore assembly is less clear. However, its essentiality in plants is evidenced by embryo lethal phenotypes of *Arabidopsis thaliana sgt1a sgt1b* double mutants (Kitagawa et al., 1999; Azevedo et al., 2006; Shirasu, 2009). Essentiality constrains evolutionary change without versatile intermediates, as shown in bacterial ParD–ParE system (Aakre et al., 2015). A clear analogy comes from gp120 protein from HIV (human immunodeficiency virus). gp120 is the external envelope protein that is indispensable for full life cycle as it mediates host cell entry. During later stages of disease progression, some HIV lineages switch their co-receptor possibly due to depletion of cells with the first co-receptor. Co-receptor switching proceeds through dual-usage stage, which means HIV can use both co-receptors to start infection cycle in this stage. Dual-usage stage is facilitated by mutations in gp120, which makes gp120 promiscuous to bind both host proteins (Lu et al., 1998). An *in vivo* study using SHIV (Simian/Human Immunodeficiency Virus) and macaques, similarly documented switching through a promiscuous, dual-usage phase (Ho et al., 2007; Ren et al., 2013). Although the precise reason of *SGT1* essentiality remains unsolved, there are two possible contributors: (i) immune functions mediated by the *RAR1*–*SGT1*–HSP90 chaperone complex via the CS domain, and/or (ii) developmental roles in SCF^{TIR1}-mediated auxin signaling, potentially involving the TPR domain.

6.2.3 Genetic drift driven by protein stability

Maintaining protein stability can shape protein evolution. Stability-driven protein evolution can produce changes at distant sites to compensate for destabilizing mutations. For example, the evolutionary trajectory of influenza nucleoprotein highlights that compensatory mutations can even arise between domains (Gong et al., 2013).

In plants, *A. thaliana* AtSGT1b is involved in auxin signaling and development through the SCR^{TIR1} E3 ubiquitin ligase complex (Gray et al., 2006). Although exact domain(s) of AtSGT1b engaging SCR^{TIR1} complex remains unresolved, yeast and animal homologs interact with SCF components with the TPR domain (Bansal et al., 2004; Catlett and Kaplan, 2006; Willhoft et al., 2017). In this context, the first mutation in the CS domain of fern SGT1 may have been compensatory, in response to prior changes in TPR domain induced by non-immune functions.

More broadly, protein evolution is heavily context-dependent as exemplified by the previous study using two interacting bacterial proteins, PhoQ–PhoP. When PhoQ–PhoP interaction was rewired, it revealed that there are > 1,000 mutational solutions to achieve the same result (Podgornaia and Laub, 2015). Collectively, these considerations suggest that a stability-mediated route of the RAR1–SGT1 binding interface divergence (6.1.3) cannot be excluded, although intrinsic properties of SGT1 likely facilitated promiscuity during the binding interface rewiring in ferns (6.1.1–2).

6.3 Expanded framework to identify broad host range factors of *Phytophthora*

EvoMPMI (evolutionary molecular plant-microbe interaction) work on bryophyte–*Phytophthora* interactions in chapter 5 indicates that three *Phytophthora* species deploy a core infection program that underpins their broad host ranges. The framework in Chapter 5 can be extended by incorporating known angiosperm host of these *Phytophthora* species. *Nicotiana benthamiana* is an established host for *P. cactorum*, *P. parasitica* and *P. capsici* (Figure 6.1) (Chen et al., 2014; Shen et al., 2016; Vega-Arreguín et al., 2017). This sets up a comparative framework to define conserved pathogenesis or resistance mechanisms using bioinformatic approaches. As shown previously by other studies, genome scale orthogroup profiling can pull out ortholog sets to test without predefining genes of interest (Emms and Kelly, 2015; Carella et al., 2019). The three *Phytophthora* species used in this chapter can colonize the model angiosperm *N. benthamiana*, which was used in the *P. palmivora* study. This will provide a cross-pathogen and cross-lineage framework to compared common host defense mechanisms in distantly related plants. Furthermore, the challenge of low sequence homology among effectors in sequence-based approaches can be mitigated via structure-based homology searches and fold clustering (Seong and Krasileva, 2023). Together, orthogroup- and structure-based bioinformatic approaches can advance our understanding of host–pathogen

compatibility in a macroevolutionary perspective.

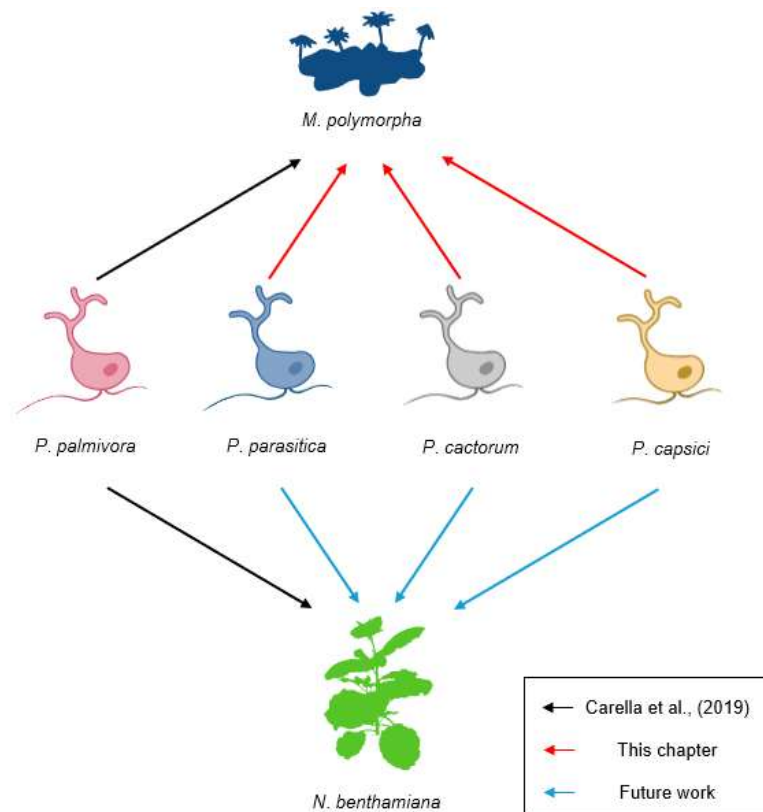


Figure 6.1 How *Marchantia–Phytophthora* interaction can be leveraged to understand host range? Three *Phytophthora* species used in this chapter are also able to infect *N. benthamiana* and their interactions can be facilitated orthogroup- and structure-based bioinformatics approaches.

At the same time, effector requirements are context-dependent, varying with effector class and host physiology. In *Fusarium oxysporum*, ERC (early root colonization) effectors that promotes infection in tomato also aid colonization of *Marchantia polymorpha*, whereas Six (secreted in xylem) effectors only essential in tomato infection. Considering the canonical destination of Six effectors is xylem, mislocation of these effectors in the non-vascular plant potentially resulted in the incompatibility between *F. oxysporum* six mutants and *M. polymorpha* (Redkar et al., 2022a; Redkar et al., 2022c). Thus, considering bryophyte physiology will help to distinguish universally required infection factors.

6.4 Potential role of RAR1 and SGT1 in heterosis

Newly formed hybrids can display enhanced traits, including disease resistance, and this phenomenon is called heterosis (Wu et al., 2021). At the same time, hybridization tests compatibility

between parental immune systems. In *A. thaliana*, the cluster of NLR genes, *DM2* (*DANGEROUS MIX2*), can trigger hybrid necrosis in F1 hybrids, reflecting immunity incompatibilities (Chae et al., 2015).

The RAR1–SGT1–HSP90 chaperone complex has a broad client range, possibly even across species. The maize NLR protein Rxo1 recognizes the bacterial rice pathogen *Xanthomonas oryzae* pv. *oryzicola* and confers nonhost resistance in maize. Transgenic rice expressing Rxo1 acquires the resistance (Zhao et al., 2005). Although RAR1- or SGT1- dependency of Rxo1 has not been tested yet, the cross-species functionality of Rxo1 is consistent with the idea that the RAR1–SGT1–HSP90 complex can stabilize many NLR proteins in a generic manner.

Given the conservation of RAR1–SGT1 interaction and the broad client range of this immune chaperone complex, it may be plausible to hypothesize that hybrids can achieve cross-subgenome NLR buffering. Cultivated peanut (*Arachis hypogaea*) is an allotetraploid hybrid derived from two wild peanut species, *A. duranensis* and *A. ipaensis* (Zhuang et al., 2019). Strikingly, a systemic survey in *A. hypogaea* identified multiple RAR1, SGT1, and HSP90 homologs in each subgenome and showed extensive interactions among components in each RAR1–SGT1–HSP90 complex (Yuan et al., 2021). In principle, such compatibility would allow AdSGT1 and AiSGT1 to recruit NLR proteins to both AdHSP90 and AiHSP90, by forming heterologous RAR1–SGT1–HSP90 complex in the hybrid background. Considering that NLR proteins can confer nonhost resistance between closely related plant species, such as maize and rice as discussed above (Panstruga and Moscou, 2020), cross-subgenome chaperoning could result in double-layered nonhost resistance that might contribute to disease resistance heterosis.

6.5 EvoMPMI's next top models

Model organisms are at the center of biological research. In plant science, many ‘non-model’ species have become indispensable because their unique traits address questions that traditional models cannot. The liverwort *M. polymorpha*, which was selected as one of ‘plant science’s next top models’ (Cesarino et al., 2020), now underpins macroevolutionary works in reproductive biology, epigenetics, and EvoMPMI (Jeong et al., 2023). Then which non-seed plants can expand our understanding of MPMI?

6.5.1 The moss *Ceratodon purpureus*

The fire moss *C. purpureus* has been a model in plant biology, from classic developmental studies to ecological genetics. Uniquely, its genome carries UV sex-determination system, enabling tractable

analyses of sexual dimorphism. (Carey et al., 2021). In mammals, sexual dimorphism shapes immunity and microbiomes (Gal-Oz et al., 2019; Stoffel et al., 2020; Koliada et al., 2021). Role of sexual dimorphism in plant immunity is less understood than mammals, but in dioecious plants, males and females can differ in growth, abiotic stress tolerance, and immunity, resulting in sex-biased distributions across environments (Guo et al., 2023).

C. purpureus provides a parallel platform to study sexual dimorphism in plant immunity alongside *M. polymorpha*. Recent work revealed sex-biased defense biochemistry in this species. *C. purpureus* R40 (male) exudates exhibit antibacterial activity to Gram-positive bacteria, while GG1 (female) does not (Dague et al., 2023). Genome resources are available for standard *C. purpureus* male and female lab strains. Together with stable transformation tools, including homologous recombination-based gene targeting and CRISPR/Cas9 editing, *C. purpureus* enables precise reverse genetics in sexual dimorphism and plant immunity (Tavernier et al., 2024).

C. purpureus sits occupies a unique position in NLR biology. In land plants, five types of N-terminal domains have been identified: three common domains (CC, TIR, and RPW8) and two atypical domains (α/β -hydrolase and protein kinase). Only two known species encode all five types of NLR proteins, and *C. purpureus* is one of them. The other species, peat moss (*Sphagnum*), currently offers only transient protoplast transformation (Lüth et al., 2023). This positions *C. purpureus* as a powerful testing platform for non-seed plant NLR proteins, especially those with uncommon N-terminal domains.

Finally, population-scale genetics is possible with this species. For example, a *M. polymorpha* pangenome now enables GWAS (genome-wide association study) in this species (Beaulieu et al., 2025). *C. purpureus* is a cosmopolitan species that has been used in ecological genetics. Together with genetic tools described above, it opens a possibility of *C. purpureus* as another bryophyte model for EvoMPMI following *M. polymorpha*.

6.5.2 The hornwort *Anthoceros agrestis*

The hornwort *A. agrestis* is an emerging model species in plant evolutionary developmental biology with reference genomes and stable *Agrobacterium*-mediated transformation method (Li et al., 2020; Frangedakis et al., 2021d; Waller et al., 2024). The hornworts have unique features among land plants, including the pyrenoid, a large photosynthetic compartment typically found in algae, and symbiotic relationships with cyanobacteria (Frangedakis et al., 2021a). Chloroplasts contribute to plant immunity through reactive oxygen species (ROS) production and morphological responses such as elongated stromules (Littlejohn et al., 2020; Liu et al., 2024). The role of the pyrenoid in plant immunity remains completely explored due to limited alga-based pathosystems. In this context,

hornworts may offer a unique opportunity to investigate pyrenoid-linked immunity beyond canonical chloroplast immunity.

Hornworts offer the most tractable system for studying plant–cyanobacteria symbiosis, whereas other plant hosts such as cycads are less accessible in laboratory conditions. Molecular mechanisms of hornwort–cyanobacteria symbiosis remain largely underexplored, but a recent transcriptome study using *A. agrestis* and the symbiotic cyanobacterium *Nostoc punctiforme* showed upregulation of genes involved in nutrient transport during association. It resembles that angiosperms feed their symbionts (Chatterjee et al., 2022). In nodule symbiosis, host legumes can also sanction low-performing cheaters while keeping efficient partners to reduce harmful interactors (Westhoek et al., 2021). Plant–cyanobacteria interactions also can be beneficial and harmful depending on contexts (Nowruzi et al., 2021) but sanctioning in cyanobacterial symbiosis has not yet been studied. Hornworts will be an ideal system to probe cyanobacterial symbiosis and sanctioning mechanisms across land plants.

Considering that hornworts form arbuscular mycorrhizal symbioses (Desirò et al., 2013), these species provide a chance to study multiple plant-microbe interactions altogether in one place: cyanobacterial symbiosis, arbuscular mycorrhizal symbiosis, and pathogenic interactions with microbes. Prior work suggests that immunity and symbiosis are not separate and antagonistic programs, but rather a continuum that adapts fluctuating microbial contexts (Zhang et al., 2025). Therefore, hornworts may be the system of choice to dissect the immunity–symbiosis interface with multiple microbial players involved.

6.6 Conclusion

In this thesis, I aimed to elucidate the molecular mechanisms underlying NHR across diverse land plant lineages. This work highlights what non-seed plants can advance our understanding of EvoMPMI and plant evolution within a more complete framework. The lineage-specific divergence of the RAR1–SGT1 interface in ferns exemplified that “conserved” immune mechanisms may not be universally conserved in understudied lineages. Furthermore, the possible role of the RAR1–SGT1–HSP90 complex in heterosis may contribute to a better understanding of land plant evolution, as hybridization also occurs in ferns and gymnosperms (Wan et al., 2022; Tseng et al., 2024).

At a broader scale, comparative analyses across bryophytes and angiosperms with broad host range pathogens *Phytophthora* show that both hosts and pathogens deploy core molecular during infection. These findings not only provide molecular details in these host–pathogen interactions description in improve plant health, but also suggest that understudied pathogens, particularly those lacking feasible and compatible angiosperm hosts, can be investigated in genetically tractable non-seed plant systems.

Scope of EvoMPMI is being broadened by developing novel model systems in understudied land plant lineages. Considering the deep divergence of land plant from their last common ancestor, these systems will provide opportunities to address questions that cannot be answered using existing models. Such approaches will not only improve fundamental understanding of plant immune systems but may also uncover novel sources of resistance genes against emerging pathogens driven by climate change. Ultimately, establishing a more comprehensive framework for EvoMPMI across diverse land plant lineages will help reconstruct the origin of plant immunity and inform novel strategies for sustainable agriculture.

6.7 References

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APPENDICES

Table S1 List of *Phytophthora* strains used in this study

Species	Abbreviation	pathovar/strain	Stock ID	Transgene	Antibiotic selection	Media	Temperature (°C)
<i>Phytophthora cactorum</i>	Pcac	Pc404				V8	25
<i>Phytophthora cactorum</i>	Pcac	Pc10300				V8	25
<i>Phytophthora cambivora</i>	Pcam		cbs 114085			RSA	25
<i>Phytophthora capsici</i>	Peap	LT263				V8	25
<i>Phytophthora capsici</i>	Pcap	LT1534				V8	25
<i>Phytophthora cinnamomi</i>	Pcin		cbs 144.22			RSA	25
<i>Phytophthora citricola</i>	Pcit		cbs 221.88			RSA	25
<i>Phytophthora cryptogea</i>	Pcry		cbs 113.19			RSA	25
<i>Phytophthora drechsleri</i>	Pdre		cbs 292.35			RSA	25
<i>Phytophthora europaea</i>	Peu					V8	25
<i>Phytophthora infestans</i>	Pinf	88069-td	88069	tdTomato	G418	RSA	18
<i>Phytophthora infestans</i>	Pinf	USA618				RSA	18
<i>Phytophthora infestans</i>	Pinf	T30-4				RSA	18
<i>Phytophthora infestans</i>	Pinf	IPO-C				RSA	18
<i>Phytophthora infestans</i>	Pinf	IPO-O				RSA	18
<i>Phytophthora infestans</i>	Pinf	PIC99183				RSA	18
<i>Phytophthora ipomea</i>	Pipo	Pip99167				RSA	18

Table S1 List of *Phytophthora* strains used in this study (continued)

Species	Abbreviation	pathovar/strain	Stock ID	Transgene	Antibiotic Selection	Media	Temperature (°C)
<i>Phytophthora melonis</i>	Pmel					V8	25
<i>Phytophthora mirabilis</i>	Pmir	Pm3010				RSA	18
<i>Phytophthora mirabilis</i>	Pmir	Pm3008				RSA	18
<i>Phytophthora palmivora</i>	Ppal	ARI-td		tdTomato	G418	V8	25
<i>Phytophthora palmivora</i>	Ppal	LILlyfp		eYFP	G418	V8	25
<i>Phytophthora palmivora</i>	Ppal	MAZI-WT				V8	25
<i>Phytophthora parasitica</i>	Ppar	Pp721				V8	25
<i>Phytophthora parasitica</i>	Ppar	PpR0				V8	25
<i>Phytophthora parasitica</i>	Ppar	Pp329				V8	25
<i>Phytophthora parasitica</i>	Ppar	Pp666				V8	25
<i>Phytophthora parasitica</i>	Ppar	Pp310				V8	25
<i>Phytophthora parasitica</i>	Ppar	PpR1				V8	25
<i>Phytophthora ramorum</i>	Pram		cbs 101328			RSA	25
<i>Phytophthora ramorum</i>	Pram	128	cc2176			RSA	25
<i>Phytophthora ramorum</i>	Pram	142				RSA	25
<i>Phytophthora sojae</i>	Psoj	Ps6497				RSA	25

Table S2.List of species used in the phylogenetic analyses

Taxonomic clade	Species	Source
Charophytes	<i>Mesostigma viride</i> NIES-296	Liang et al., 2020, DOE-JGI, Phycocosm, https://phycocosm.jgi.doe.gov/phycocosm/home
Charophytes	<i>Chara braunii</i>	Sterck et al., 2012, ORCAE, http://bioinformatics.psb.ugent.be/orcae/
Charophytes	<i>Klebsormidium nitens</i> NIES-2285	Hori et al., 2014, v1.1, DOE-JGI, Phycocosm, https://phycocosm.jgi.doe.gov/phycocosm/home
Charophytes	<i>Chlorokybus atmophyticus</i> CCAC 0220	Wang et al., 2020, DOE-JGI, Phycocosm, https://phycocosm.jgi.doe.gov/phycocosm/home
Charophytes	<i>Mesotaenium endlicherianum</i> SAG 12.97	Cheng et al., 2019, DOE-JGI, Phycocosm, https://phycocosm.jgi.doe.gov/phycocosm/home
Charophytes	<i>Mesotaenium kramstae</i> Lemmermann NIES-657	v3.0, DOE-JGI, Phycocosm, https://phycocosm.jgi.doe.gov/phycocosm/home
Hornworts	<i>Anthoceros punctatus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Anthoceros agrestis</i> Oxford	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Megaceros flagellaris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Nothoceros aenigmaticus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Nothoceros vincentianus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Paraphymatoceros hallii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Hornworts	<i>Phaeoceros carolinianus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Treubia lacunosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Pellia cf. epiphylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Pellia neesiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Pallavicinia lyellii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Schistochila sp.</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Scapania nemorosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Barbilophozia barbata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Calypogeia fissa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Bazzania trilobata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Odontoschisma prostratum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Frullania sp.</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Frullania spp.</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Porella pinnata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Porella navicularis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Radula lindenbergiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Ptilidium pulcherrimum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Sphaerocarpos texanus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Lunularia cruciata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Conocephalum conicum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Ricciocarpos natans</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Marchantia emarginata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Marchantia paleacea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Marchantia polymorpha</i> ssp. <i>montivagans</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Marchantia polymorpha</i> ssp. <i>polymorpha</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Liverworts	<i>Marchantia polymorpha</i> ssp. <i>ruderalis</i> Tak1	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Takakia lepidozioides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Mosses	<i>Sphagnum fallax</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Sphagnum lescurii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Sphagnum palustre</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Sphagnum recurvum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Andreaea rupestris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Tetraphis pellucida</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Polytrichum commune</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Atrichum angustatum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Buxbaumia aphylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Diphyscium foliosum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Physcomitrium patens</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Physcomitrium sp.</i>	Bi et al., 2024. Physcomitrum patens v6, DOE-JGI, https://phytozome-next.jgi.doe.gov/
Mosses	<i>Encalypta streptocarpa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Philonotis fontana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Timmia austriaca</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Scouleria aquatica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Racomitrium elongatum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Racomitrium varium</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Syntrichia ruralis</i>	Zhang et al., 2024
Mosses	<i>Ceratodon purpureus</i>	Carey et al., 2021, Ceratodon purpureus GG1 v1.1, DOE-JGI, https://phytozome-next.jgi.doe.gov/
Mosses	<i>Dicranum scoparium</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Leucobryum albidum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Leucobryum glaucum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Hedwigia ciliata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Bryum argenteum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Ptychostomum knowltonii</i> (<i>Bryum knowltonii</i>)	Ma et al., 2025
Mosses	<i>Rosulabryum cf. capillare</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Plagiomnium insigne</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Fontinalis antipyretica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Orthotrichum lyellii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Aulacomnium heterostichum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Anomodon attenuatus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Rhynchostegium serrulatum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Calliergon cordifolium</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Climacium dendroides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Claopodium rostratum</i> (<i>Anomodon rostratus</i> , <i>Anocla</i>)	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Leucodon brachypus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Leucodon julaceus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Loeskeobryum brevirostre</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Neckera douglasii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Pseudotaxiphyllum elegans</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Stereodon subimponens</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Mosses	<i>Thuidium delicatulum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Lycopodiella appressa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Lycophytes	<i>Pseudolycopodiella caroliniana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Diphasiastrum complanatum</i>	Diphasiastrum complanatum v3.1, DOE-JGI, Phytozome, http://phytozome-next.jgi.doe.gov/
Lycophytes	<i>Diphasiastrum digitatum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Lycopodium annotinum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Lycopodium deuterodensum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Dendrolycopodium obscurum</i> (<i>Lycopodium obscurum</i>)	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Huperzia lucidula</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Huperzia myrsinites</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Huperzia selago</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Huperzia squarrosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Phylloglossum drummondii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella acanthonota</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella apoda</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella cf. pallescens</i> (Sellep)	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella lepidophylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella kraussiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella moellendorffii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella selaginoides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella moellendorffii</i> (<i>S. stauntoniana</i>)	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Selaginella willdenowii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Isoetes</i> sp.	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Lycophytes	<i>Isoetes taiwanensis</i>	Wickell et al., 2021, FernBase, https://fernbase.org/
Lycophytes	<i>Isoetes tegetiformans</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Equisetum diffusum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Equisetum hyemale</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Botrypus virginianus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Sceptridium dissectum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Tmesipteris parva</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Psilotum nudum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Angiopteris evecta</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Osmunda</i> sp.	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Osmunda javanica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Hymenophyllum dentatum</i>	Ostria-Gallardo et al., 2020
Monilophytes	<i>Hymenophyllum caudiculatum</i>	Ostria-Gallardo et al., 2020
Monilophytes	<i>Dipteris conjugata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Anemia tomentosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Lygodium japonicum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Marsilea vestita</i>	Rahmatpour et al., 2022, FernBase, https://fernbase.org/
Monilophytes	<i>Pilularia globulifera</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Salvinia cucullata</i>	Li et al., 2018, FernBase, https://fernbase.org/
Monilophytes	<i>Azolla cf. caroliniana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Azolla filliculoides</i>	Li et al., 2018, FernBase, https://fernbase.org/
Monilophytes	<i>Alsophila spinulosa</i>	Huang et al., 2022
Monilophytes	<i>Culcita macrocarpa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Monilophytes	<i>Thyrsopteris elegans</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Lindsaea linearis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Lindsaea microphylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Adiantum aleuticum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Adiantum capillus-veneris</i>	Fang et al., 2022
Monilophytes	<i>Adiantum raddianum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Argyrochosma nivea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Ceratopteris richardii</i>	Geng et al., 2021; Marchant et al., 2022, Ceratopteris richardii v2.1, DOE-JGI, Phytozome, http://phytozome-next.jgi.doe.gov/
Monilophytes	<i>Cryptogramma acrostichoides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Gaga arizonica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Myriopteris rufa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Notholaena montieliae</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Pityrogramma trifoliata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Parahemionitis cordata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Pteris ensiformis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Pteris vittata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Vittaria appalachiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Dennstaedtia davallioides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Leucostegia immersa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Didymochlaena truncatula</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Bolbitis repanda</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Polystichum acrostichoides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Phlebodium pseudoaureum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Phymatosorus grossus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Pleopeltis polypodioides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Polypodium amorphum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Polypodium glycyrrhiza</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Polypodium hesperium</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Davallia fejeensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Cystopteris fragilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Cystopteris protrusa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Cystopteris reevesiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Gymnocarpium dryopteris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Homalosorus pycnocarpus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Asplenium nidus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Asplenium platyneuron</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Thelypteris acuminata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Woodsia ilvensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Woodsia scopulina</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Athyrium filix-femina</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Deparia lobato-crenata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Diplazium wichurae</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Onoclea sensibilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Monilophytes	<i>Blechnum spicant</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Ginkgo biloba</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Gymnosperms	<i>Cycas micholitzii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Dioon edule</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Encephalartos barteri</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Stangeria eriopus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Gnetum montanum</i>	Wan et al., 2018
Gymnosperms	<i>Ephedra sinica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Welwitschia mirabilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Abies lasiocarpa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cedrus libani</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Keteleeria evelyniana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Larix speciosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Nothotsuga longibracteata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cathaya argyrophylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Picea abies</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Picea engelmannii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Picea sitchensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus jeffreyi</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus lambertiana</i>	Pinus lambertiana v1.0, PlantGenIE, https://plantgenie.org/
Gymnosperms	<i>Pinus parviflora</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus pinaster</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus ponderosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus radiata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pinus taeda</i>	Pinus taeda v2.01, PlantGenIE, https://plantgenie.org/
Gymnosperms	<i>Pinus sylvestris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pseudolarix amabilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pseudotsuga menziesii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pseudotsuga wilsoniana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Tsuga heterophylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Agathis macrophylla</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Agathis robusta</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Araucaria rulei</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Wollemia nobilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Acmopyle pantheri</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Dacrydium balansae</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Dacrycarpus compactus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Falcatifolium taxoides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Halocarpus bidwillii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Lagarostrobos franklinii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Microstrobos fitzgeraldii</i> (<i>Lepidothamnus</i> sp.)	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Manoao colensoi</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Microcachrys tetragona</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Nageia nagi</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Parasitaxus usta</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Phyllocladus hypophyllus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Podocarpus coriaceus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Gymnosperms	<i>Podocarpus rubens</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Prumnopitys andina</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Retrophyllum minus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Saxegothaea conspicua</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Sundacarpus amarus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Sciadopitys verticillata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Athrotaxis cupressoides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Austrocedrus chilensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Calocedrus decurrens</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Callitris gracilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Callitris macleayana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Chamaecyparis lawsoniana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cryptomeria japonica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cunninghamia lanceolata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cupressus dupreziana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Diselma archeri</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Fokienia hodginsii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Juniperus scopulorum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Glyptostrobus pensilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Metasequoia glyptostroboides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Microbiota decussata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Papuacedrus papuana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pilgerodendron uviferum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Platycladus orientalis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Sequoiadendron giganteum</i> Glaucum	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Taiwania cryptomerioides</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Taxodium distichum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Tetraclinis</i> sp.	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Thujopsis dolabrata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Thuja plicata</i>	Shalev et al., 2022, <i>Thuja plicata</i> v3.1, DOE-JGI, Phytozome, http://phytozome-next.jgi.doe.gov/
Gymnosperms	<i>Widdringtonia cedarbergensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Neocallitropsis pancheri</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Amentotaxus argotaenia</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Austrotaxus spicata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Pseudotaxus chienii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Taxus baccata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Taxus cuspidata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Torreya nucifera</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Torreya taxifolia</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Gymnosperms	<i>Cephalotaxus harringtonia</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Amborella trichopoda</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Eurylae ferox</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Nymphaea colorata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Spirodela polyrhiza</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Zostera marina</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Angiosperms	<i>Apostasia shenzhenica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Dendrobium catenatum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Phalaenopsis equestris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Ananas comosus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Brachypodium distachyon</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Oryza sativa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Hordeum vulgare</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Setaria italica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Sorghum bicolor</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Zea mays PH207</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Musa acuminata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Aquilegia coerulea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Nelumbo nucifera</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Manihot esculenta</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Ricinus communis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Populus trichocarpa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cephalotus follicularis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Arachis duranensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Arachis hypogaea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Arachis ipaensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cajanus cajan</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Castanospermum australe</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cicer arietinum ICC4958</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Glycine max</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Lablab purpureus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Lotus japonicus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Lupinus angustifolius</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Medicago truncatula</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Mucuna pruriens</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Nissolia schottii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Phaseolus vulgaris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Trifolium pratense</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Trifolium subterraneum Dailak</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Vigna angularis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Vigna subterranea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Vigna unguiculata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Vigna radiata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cercis canadensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Chamaecrista fasciculata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Faidherbia albida</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Mimosa pudica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Dryas drummondii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Fragaria x ananassa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Fragaria vesca</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Malus domestica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Prunus mume</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Angiosperms	<i>Prunus persica</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Pyrus x bretschneideri</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Pyrus communis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Rosa chinensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Rubus occidentalis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Discaria trinervis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Ziziphus jujuba</i> cv. Dongzao	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Humulus lupulus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Parasponia andersonii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Trema orientalis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Morus notabilis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Castanea mollissima</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Quercus robur</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Juglans regia</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Alnus glutinosa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Casuarina glauca</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Citrullus lanatus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cucumis melo</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cucumis sativus</i> PI183967	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cucurbita maxima</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cucurbita moschata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Curcubita pepo</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Lagenaria siceraria</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Momordica charantia</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Datisca glomerata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Begonia fuchsiodes</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Sclerocarya birrea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Citrus clementina</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Citrus sinensis</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Arabidopsis halleri</i>	Arabidopsis halleri v2.1.0, DOE-JGI, Phytozome, http://phytozome-next.jgi.doe.gov/
Angiosperms	<i>Arabidopsis lyrata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Arabidopsis thaliana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Boechera stricta</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Brassica oleraceae capitata</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Brassica rapa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Capsella grandiflora</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Capsella rubella</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Carica papaya</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Eutrema salsugineum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Tarenaya hassieriana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Theobroma cacao</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Gossypium raimondii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Beta vulgaris</i> ssp <i>vulgaris</i> KWS2320	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Amaranthus hypochondriacus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Spinacia oleracea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Angiosperms	<i>Dianthus caryophyllus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Chenopodium quinoa</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cavendishia cuatrecasasii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Ledum palustre</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Monotropa uniflora</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Rhododendron fortunei</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Rhododendron scopulorum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Sarcodes sanguinea</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Helianthus annuus</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Daucus carota</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Fraxinus excelsior</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Cuscuta campestris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Capsicum annuum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Nicotiana benthamiana</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Petunia axillaris</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Solanum lycopersicum</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/
Angiosperms	<i>Solanum pennellii</i>	SymDB, https://www.polebio.lrsv.ups-tlse.fr/symdb/web/

Table S3 Primers used in this study

Primer Name	Primer Sequence (5' to 3')	Purpose
CrRAR1-B1f	AAAAAGCAGGCTtcATGGCGGTACGGTGCCAGAGA	Cloning CrRAR1 from <i>C. richardii</i> cDNA library
CrRAR1-B2s	AGAAAGCTGGGTCTAGCTTCCACAATGCCATCC	
CrSGT1-B1f	AAAAAGCAGGCTtcATGATGGAGAAAGTCAGAGTTATGG	
p1 (CrSGT1-Nfrag-B2s)	AGAAAGCTGGGTCTACTTTCCAAAGAGAAAGTTTCCATTTC	Examining and Cloning CrSGT1 paralogs from <i>C. richardii</i> cDNA library
p3 (CrSGT1-Cfrag-B1f)	AAAAAGCAGGCTcATGTTTCAATTTTCAGGCCATTACAAAGTAC	
p2 (CrSGT1-B2s)	AGAAAGCTGGGTCTAGGTCTCCCACTTCTTCAATTTCC	
p4 (CrACT1-RT-F)	AATGAGTTACGTGTGGCACC	To amplify CrACT1 from <i>C. richardii</i> cDNA library; positive control for RT-PCR
CrACT1-qR	AGGAAATTTCGTAACTCTTCTCCAA	
AtSGT1b-intlS_Fw	aatGGTCTCaTCTCTGAGTGTGTCATTGATGTT	
AtSGT1b-intlS_Rv	aatGGTCTCagagaCTGAATACCAAACTCGACAGT	Primers to introduce I193S mutation into AtSGT1b E191I variant
AtSGT1b-intSK_Fw	aatGGTCTCaaaggTTGTCAATTGATGTTGCTGG	Forward primer to introduce S195K mutation into AtSGT1b variants
AtSGT1b-ElintSK_Rv	aatGGTCTCaccttCAGAAATCTGAATACCAAACTC	Reverse primer to introduce S195K mutation into AtSGT1b E191I variant
AtSGT1b-ISintSK_Rv	aatGGTCTCaccttCAGAGACTGCTCACCAAAAC	Reverse primer to introduce S195K mutation into AtSGT1b I193S variant
AtSGT1b_EIISintSK_Rv	aatGGTCTCaccttCAGAGACTGAATACCAAACTC	Reverse primer to introduce S195K mutation into AtSGT1b E191I I193S variant
AtSGT1b_Bsal-Rv2	aatGGTCTCaCGAAATCAATACTCCCACTTCTTGAG	Reverse primer for Golden Gate-based mutation introduction into AtSGT1b variants

Table S3 Primers used in this study (continued)

Primer Name	Primer Sequence (5' to 3')	Purpose
AtSGT1b_B1f	AAAAAGCAGGCTtcATGGCCCAAGGAATTAGCAGAG	Primers to subclone AtSGT1b multiple variants into Gateway system
AtSGT1b_B2s	AGAAAGCTGGGTTCaATACTCCCACTTCTTGAG	
CrR-FI_Bsal-Fw	aatGGTCTCaattaATGCAAAAGCAACATGCAAG	Primers to introduce F151I mutation into CrRAR1
CrR-FI_Bsal-Rv	aatGGTCTCataatATCCGCCCACTCGGGG	
Bsal-CrRAR1_Fw	aatGGTCTCaAATGGCGGTACGGTGC	Primers to clone CrRAR1 into Golden Gate-based split GAL4 Ruby assay system
Bsal-CrRAR1_Rv	aatGGTCTCaAAGCCTAGCTTCCACAAATGCCATCC	
Bsal-AtRAR1-Fw	aatGGTCTCaAATGGAAAGTAGGATCTGCAACG	Primers to clone AtRAR1 into Golden Gate-based split GAL4 Ruby assay system
Bsal-AtRAR1-Rv	aatGGTCTCaAAGCTCAGACCCGCCGGATCAG	
CrSGT1_Bsal-Fw	aatGGTCTCaAATGATGGAGAAGGTCAGAGTTATG	Primers to clone CrSGT1 into Golden Gate-based split GAL4 Ruby assay system
CrSGT1_Bsal-Rv	aatGGTCTCaCGAAaccGGTTTCCCACTTCTTCATTTCATGC	
AtSGT1b_Bsal-Fw	aatGGTCTCaAATGGCCCAAGGAATTAGCAGAG	Primers to clone AtSGT1b into Golden Gate-based split GAL4 Ruby assay system

Table S4 Lesion severity of 33 *Phytophthora* strains in *M. polymorpha*

Clade	Species	Strain	Plant genotype	Lesion severity	Degree of infection	p value
1a	<i>P. cactorum</i>	Pc404	Tak1	1.3±0.5	Moderate	0.0066
			<i>rar1</i> -13	1.7±0.7	Moderate	
		Pc10300	Tak1	1.7±0.6	Moderate	>0.05
			<i>rar1</i> -13	1.8±0.6	Moderate	
1c	<i>P. infestans</i>	88069-td	Tak1	0±0.2	No	>0.05
			<i>rar1</i> -13	0±0.2	No	
		IPO-C	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
		IPO-O	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
		PIC99183	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
		T30-4	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
		USA618	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
	<i>P. ipomoeae</i>	Pip99167	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
	<i>P. mirabilis</i>	Pm3008	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
		Pm3010	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
1	<i>P. parasitica</i>	R0	Tak1	1.4±0.5	Moderate	>0.05
			<i>rar1</i> -13	1.4±0.5	Moderate	
		R1	Tak1	2.4±0.6	Strong	>0.05
			<i>rar1</i> -13	2.5±0.6	Strong	
		310	Tak1	2.7±0.5	Strong	>0.05
			<i>rar1</i> -13	2.7±0.5	Strong	
		329	Tak1	1.9±0.3	Moderate	0.0066
			<i>rar1</i> -13	2.3±0.6	Strong	
		666	Tak1	3±0	Strong	>0.05
			<i>rar1</i> -13	3±0	Strong	
		721	Tak1	3±0	Strong	>0.05
			<i>rar1</i> -13	3±0	Strong	

Table S4 Lesion severity of 33 *Phytophthora* strains in *M. polymorpha* (continued)

Clade	Species	Strain	Plant genotype	Lesion severity	Degree of infection	p value
4	<i>P. palmivora</i>	ARI-td	Tak1	1.8±0.4	Moderate	>0.05
			<i>rar1</i> -13	1.8±0.7	Moderate	
		LILIfp	Tak1	0.8±0.4	Weak	>0.9999
			<i>rar1</i> -13	0.9±0.3	Weak	
2b	<i>P. capsici</i>	MAZI	Tak1	3±0	Strong	>0.05
			<i>rar1</i> -13	3±0	Strong	
		LT263	Tak1	3±0	Strong	>0.05
			<i>rar1</i> -13	3±0	Strong	
		LT1534	Tak1	3±0	Strong	>0.05
			<i>rar1</i> -13	3±0	Strong	
2c	<i>P. citricola</i>		Tak1	1.5±0.6	Moderate	<0.0001
			<i>rar1</i> -13	2.7±0.5	Strong	
7a	<i>P. cambivora</i>		Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
	<i>P. europaea</i>		Tak1	1±0	Weak	>0.05
			<i>rar1</i> -13	1±0	Weak	
7b	<i>P. melonis</i>		Tak1	2.5±0.5	Strong	>0.05
			<i>rar1</i> -13	2.6±0.5	Strong	
	<i>P. sojae</i>	Ps6497	Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
7c	<i>P. cinnamomi</i>		Tak1	0.3±0.4	Weak	<0.0001
			<i>rar1</i> -13	1.9±1.1	Moderate	
8a	<i>P. cryptogea</i>		Tak1	0±0	No	>0.05
			<i>rar1</i> -13	0±0	No	
	<i>P. drechsleri</i>		Tak1	1±0.2	Weak	>0.05
			<i>rar1</i> -13	1.2±0.4	Moderate	
8c	<i>P. ramorum</i>	cbs 101328	Tak1	0.8±0.5	Weak	0.0017
			<i>rar1</i> -13	1.2±0.4	Moderate	
		128	Tak1	0.6±0.6	Weak	<0.0001
			<i>rar1</i> -13	1.2±0.5	Moderate	
		142	Tak1	1.5±0.6	Moderate	>0.05
			<i>rar1</i> -13	1.6±0.6	Moderate	

Table S5 Infection efficiency of 33 *Phytophthora* strains in *M. polymorpha*

Clade	Species	Strain	Plant genotype	Infection efficiency
1a	<i>P. cactorum</i>	Pc404	Tak1	96.88%
			<i>rar1</i> -13	100.00%
		Pc10300	Tak1	100.00%
			<i>rar1</i> -13	100.00%
1c	<i>P. infestans</i>	88069-td	Tak1	3.13%
			<i>rar1</i> -13	3.13%
		IPO-C	Tak1	0.00%
			<i>rar1</i> -13	0.00%
		IPO-O	Tak1	0.00%
			<i>rar1</i> -13	0.00%
		PIC99183	Tak1	0.00%
			<i>rar1</i> -13	0.00%
		T30-4	Tak1	0.00%
			<i>rar1</i> -13	0.00%
		USA618	Tak1	0.00%
			<i>rar1</i> -13	0.00%
	<i>P. ipomoeae</i>	Pip99167	Tak1	0.00%
			<i>rar1</i> -13	0.00%
	<i>P. mirabilis</i>	Pm3008	Tak1	0.00%
			<i>rar1</i> -13	0.00%
		Pm3010	Tak1	0.00%
			<i>rar1</i> -13	0.00%
1	<i>P. parasitica</i>	R0	Tak1	100.00%
			<i>rar1</i> -13	96.88%
		R1	Tak1	100.00%
			<i>rar1</i> -13	100.00%
		310	Tak1	100.00%
			<i>rar1</i> -13	100.00%
		329	Tak1	100.00%
			<i>rar1</i> -13	100.00%
		666	Tak1	100.00%
			<i>rar1</i> -13	100.00%
		721	Tak1	100.00%
			<i>rar1</i> -13	100.00%

Table S5 Infection efficiency of 33 *Phytophthora* strains in *M. polymorpha* (continued)

Clade	Species	Strain	Plant genotype	Infection efficiency
4	<i>P. palmivora</i>	ARI-td	Tak1	100.00%
			<i>rarI</i> -13	100.00%
		LILlyfp	Tak1	84.38%
			<i>rarI</i> -13	90.63%
		MAZI	Tak1	100.00%
			<i>rarI</i> -13	100.00%
2b	<i>P. capsici</i>	LT263	Tak1	100.00%
			<i>rarI</i> -13	100.00%
		LT1534	Tak1	100.00%
			<i>rarI</i> -13	100.00%
2c	<i>P. citricola</i>		Tak1	100.00%
			<i>rarI</i> -13	100.00%
7a	<i>P. cambivora</i>		Tak1	0.00%
			<i>rarI</i> -13	0.00%
	<i>P. europaea</i>		Tak1	100.00%
			<i>rarI</i> -13	100.00%
7b	<i>P. melonis</i>		Tak1	100.00%
			<i>rarI</i> -13	100.00%
	<i>P. sojae</i>	Ps6497	Tak1	0.00%
			<i>rarI</i> -13	0.00%
7c	<i>P. cinnamomi</i>		Tak1	28.13%
			<i>rarI</i> -13	84.38%
8a	<i>P. cryptogea</i>		Tak1	0.00%
			<i>rarI</i> -13	0.00%
	<i>P. drechsleri</i>		Tak1	100.00%
			<i>rarI</i> -13	100.00%
8c	<i>P. ramorum</i>	cbs 101328	Tak1	75.00%
			<i>rarI</i> -13	100.00%
		128	Tak1	53.13%
			<i>rarI</i> -13	96.88%
		142	Tak1	93.75%
			<i>rarI</i> -13	100.00%