The role of effector proteins on suppression of the plant immune system.

A case study: 'HaRxL21'

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by

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List of publications

1. Sarah Harvey*, **Priyanka Kumari***, Dmitry Lapin, Thomas Griebel, Richard Hickman, Wenbin Guo, Runxuan Zhang,Jane E. Parker, Jim Beynon, Katherine Denby, Jens Steinbrenner. (2020) *****shared first author
Downy Mildew effector HaRxL21 interacts with the transcriptional repressor TOPLESS to promote pathogen susceptibility. PLoS Pathogens, 16(8), e1008835

2. Hazel McLellan, Sarah Harvey, Jens Steinbrenner, Miles Armstrong, Qin He, Rachel Clewes, Leighton Pritchard, Wei Wang, Shumei Wang, Thomas Nussbaumer, Bushra Dohai, Qingquan Luo, **Priyanka Kumari**, Hui Duan, Ana Roberts, Petra C Boevink, Christina Neumann, Nicolas Champouret, Ingo Hein, Pascal Falter-Braun, Jim Beynon, Katherine Denby, Paul R. J. Birch
Breakdown in nonhost effector-target interactions to boost host disease resistance. PNAS 119 (35) e2114064119

3. QQ Luo et al.
Plant pathogen interaction network 3: Effectors of the pathogen *Phytophthora infestans* show similar targeting signatures on the non-host *Arabidopsis thaliana* as effectors of adapted pathogens. Manuscript in preparation

4. Aneesha Singh, Kruti Jani, **Priyanka Kumari**, Pradeep Kumar Agarwal. "Effect of $MgCl_2$ and double concentration of Murashige and Skoog medium on *in vitro* plantlet and root cultures generation in halophytic grasswort *Salicornia brachiata*", Plant Cell, Tissue and Organ Culture (PCTOC),120, pages 563–570(2015).

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1 Introduction

1.1 Plant immunity

Plants have a multi-layered defense system that aids them to fight off phytopathogens. Plants use their cell wall, waxy cuticular layers, actin microfilaments, and antimicrobial compounds known as phytoanticipins (Nürnberger & Lipka, 2005) as basal barriers, acting together these prevent the entry of pathogens. However, these defensive structures and chemical obstacles might not be effective against specific pathogens, such as fungi that directly enter epidermal plant cells or pathogenic bacteria via wounds or pores. Therefore, signaling cascades and inducible defense mechanisms become critical and play essential steps to fight against disease and establish resistance against pathogens. These inducible defenses are divided into two branches (Fig. 1.1) according to the plant cell's ability to recognize and respond to the pathogen: Pattern-Triggered immunity (PTI) and Effector-Triggered Immunity (ETI) (J. Jones; J. D. Jones & Dangl, 2006).

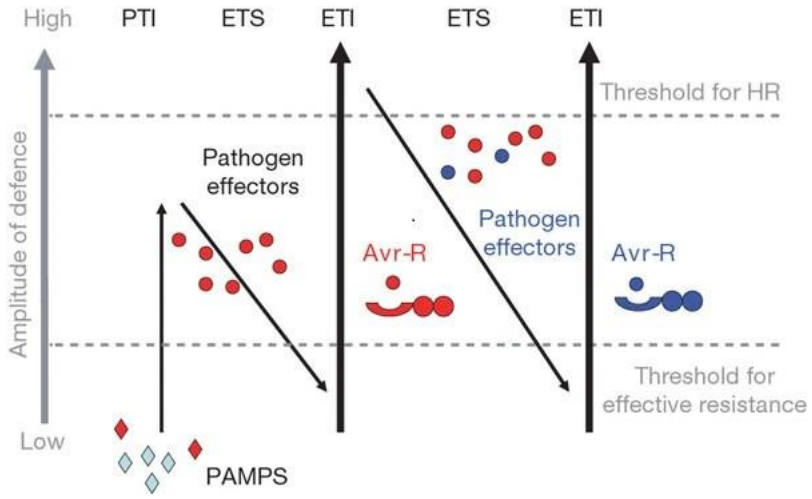

Figure 1.1 A zigzag model illustrates the quantitative output of the plant immune system (J. D. Jones & Dangl, 2006). In phase 1, when the plant encounters PAMPs/MAMPs (red diamonds), PTI is triggered by PRRs. In phase 2, successful pathogens secrete effectors to interfere with PTI or help in pathogen nutrition and dispersal, leading to effector-triggered susceptibility (ETS). In phase 3, ETI is activated by R protein recognition of the effector (red). In phase 4, only pathogens that can deploy new effectors (in blue) will succeed in penetration to suppress ETI.

1.1.1 Pattern Triggered Immunity (PTI)

PTI is the first line of the defense system in plants. It is triggered by recognizing pathogen or microbe-associated molecular patterns (PAMPs or MAMPs) by pattern recognition receptors (PRRs). Among several identified PAMPs, the most studied are flg22 (an evolutionarily conserved 22-residue peptide of bacterial flagellin), chitin (a component of fungal cell walls), and the elf18 epitope of the bacterial elongation factor-Tu (EF-Tu) (Bigeard, Colcombet, & Hirt, 2015; Hayafune et al., 2014; Kunze et al., 2004). In addition to PAMP/MAMP, the damage-associated molecular patterns (DAMPs), like systemin in Solanaceae species and

*At*PEP1 in *Arabidopsis thaliana* (*A. thaliana*) may also be detected by the host plant PRRs and activate PTI (Huffaker, Pearce, & Ryan, 2006; Lotze et al., 2007).

In plants, PRRs are surface localized and include receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Boutrot & Zipfel, 2017; Couto & Zipfel, 2016). RLKs are composed of a variable N-terminal extracellular domain (ECD) that mediates ligand recognition, a transmembrane domain, and a short intracellular kinase domain (KD) that is activated by the ECD to transduce the signals to downstream immune components (J. Wang & Chai, 2020). RLPs have an overall similar structure to RLKs, but they lack the intracellular kinase domain, so they require a partner co-receptor for signaling, mainly RLKs (Jamieson, Shan, & He, 2018; G. Wang et al., 2008). The ECD domains of PRR can contain leucine-rich repeats (LRRs), lysine motifs (LysMs), lectin motifs, and epidermal growth factor (EGF)-like domains (Couto & Zipfel, 2016).

There are some well-studied PRRs in Arabidopsis, such as the LRR-RLK FLAGELLIN SENSING 2 (FLS2) recognizing flg22 and the LRR-RLK EF-TU RECEPTOR (EFR) recognizing the elongation factor thermo-unstable from *Agrobacterium tumefaciens*, one of the most abundant proteins of the bacterial cell (Kunze et al., 2004; Zipfel et al., 2006). Following ligand perception, FLS2 and EFR associate in a complex with brassinosteroid insensitive 1 (BRI1)-associated receptor kinase 1 (BAK1) to phosphorylate Botrytis-induced kinase 1 (BIK1), leading to reactive oxygen species (ROS) production (Lu et al., 2010; J. Zhang & Zhou, 2010). In addition to the Arabidopsis FLS2 and EFR, LRR-RLKs have also been characterized in rice and solanaceous plants. In rice, the cell-surface receptor Xa21 binds to bacterial tyrosine-sulfated protein RaxX21-sY (Pruitt et al., 2015), whereas the csp22 cell-surface receptor from tomato (CORE) and tobacco (NbCSPR) recognize conserved epitopes derived from bacterial cold shock protein (Felix & Boller, 2003; L. Wang et al., 2016). Similarly, the conserved peptide Necrosis- and ethylene-inducing-like protein (NLP)-20, derived from ethylene-inducing peptide1-like originating from bacterial and filamentous pathogen proteins, is perceived by Arabidopsis RLP23 (Albert et al., 2015). LysM-RLP, chitin-elicitor binding protein (OsCEBiP), and LysM-RLK, chitin-elicitor receptor kinase 1 (OsCERK1) of rice recognize fungal chitin, mediating chitin-induced plant defenses (Hayafune et al., 2014; Miya et al., 2007; Shimizu et al., 2010).

PTI comprises multiple signaling events like extracellular alkalization, increase in cytoplasmic Ca^{2+}, ROS accumulation, activation of mitogen-activated protein kinases (MAPKs), defense hormone activation, and callose deposition as early and late responses, respectively (Boller & Felix, 2009).

1.1.2 Effector Triggered Immunity (ETI)

As plant and their pathogens have a long coevolutionary history, specialized pathogens have evolved mechanisms to suppress PTI. Pathogenic microorganisms with different lifestyles and evolutionary origins have developed a diverse repertoire of effector proteins. These effector proteins are translocated into the host apoplastic space or cytoplasm to promote disease by affecting host protein or gene activity (Cui, Tsuda, & Parker, 2015). In turn, plants deploy intracellular receptors, so-called resistance (R) proteins, to detect these effectors

leading to ETI. This leads to amplified defense responses that may result in a hypersensitive response (HR) to limit the spread of infection and systemic acquired resistance (SAR) in the host (Thomma, Nürnberger, & Joosten, 2011). Most R proteins belong to the Nucleotide-binding Leucine-rich Repeat (NLR) protein family. They have two major subclasses with distinct N-terminal domains: Toll–interleukin 1 receptor (TIR) domain-containing NLRs (TNLs) and coiled-coil (CC) domain-containing NLRs (CNLs) (Cui et al., 2015; Maekawa, Kufer, & Schulze-Lefert, 2011). Three different models of effector recognition have been proposed; (1) the guard model: effectors modify a host protein that is guarded by an NLR protein, and (2) the decoy model: in which effectors target proteins evolved into decoy proteins guarded by host NLRs, (3) integrated decoy model: decoy protein is integrated into the R protein structure of NLR pair allowing direct recognition of avirulence (AVR) effectors. For example, the Arabidopsis R protein, RRS1-R (resistance to *Ralstonia solanacearum*), directly binds to the effector protein PopP2 from *R. solanacearum*, leading to ETI (Bernoux et al., 2008). RPM1 (resistance to *Pseudomonas syringae* pv. maculicola 1) and RPS2 (resistance to *Pseudomonas syringae* 2) NLR proteins guard the RPM1-interacting protein 4 (RIN4) and recognize the presence of *P. syringae* effectors AvrB, AvrRpm1, and AvrRpt2 (Axtell & Staskawicz, 2003; Mackey, Belkhadir, Alonso, Ecker, & Dangl, 2003). RIN4 is targeted by multiple bacterial effectors for manipulation and acts as a negative regulator of basal resistance in the absence of RPM1 and RPS2 (Katagiri & Tsuda, 2010; J. Liu et al., 2009). In rice, the effector AvrXa10 activates the Xa10 gene, resulting in induced endoplasmic reticulum Ca^{2+} release and HR (D. Tian et al., 2014). Transcription activator-like (TAL) effector AvrBs4 induces expression of Bs4c-R to elicit resistance against the *X. campestris* in pepper (Schornack et al., 2004; Strauß et al., 2012).

The activated NLRs trigger an array of immune responses, such as ROS production, MAPK cascade activation, Ca^{2+} spikes, transcriptional reprogramming, and phytohormone production (Buscaill & Rivas, 2014; Cui et al., 2015). Although the immune pathway framework is shared in PTI and ETI, it has been proposed that immune responses in ETI occur more quickly, more prolonged, and more robust than those in PTI, which suggests that PTI is a weak variant of ETI (Tao et al., 2003; Tsuda & Katagiri, 2010; Tsuda, Sato, Stoddard, Glazebrook, & Katagiri, 2009).

1.1.3 Effector proteins

Plant pathogens utilize effectors to suppress host immunity for successful colonization, particularly in biotrophs, to obtain nutrients from living plant tissue. They are used by a wide range of phytopathogens, including bacteria, fungi, oomycetes, and nematodes. The bacterial effectors may be directly injected into the host cell via a type III or IV secretion system, whereas phytonematode effectors are secreted through the stylet (Byndloss, Rivera-Chávez, Tsolis, & Bäumler, 2017; Mitchum et al., 2013). Filamentous pathogens like fungi and oomycetes use haustoria and appressoria to secret and translocate effector proteins to suppress the plant immune system (Petre & Kamoun, 2014).

A successful pathogen overcomes stomatal defense for colonization; e.g., *P. syringae* (*Pst*) strains containing effector HopX1 can induce stomatal reopening, indicating that HopX1 may

be sufficient to induce JA signaling (Gimenez-Ibanez et al., 2014). Arabidopsis plants expressing the bacterial effector HopZ1a can also suppress stomatal defense (Ma et al., 2015) and the bacterial HopM1 effector suppresses stomatal defense by targeting the Arabidopsis 14-3-3 protein GRF8/AtMIN10 (*Arabidopsis thaliana* HopM interactor 10) (Lozano-Durán, Bourdais, He, & Robatzek, 2014). Pathogen effectors also suppress immunity by inhibiting PRR complex's activity and interfering with their translation or other downstream responses (Toruño, Stergiopoulos, & Coaker, 2016). For example, HopU1 inhibits the ability of GRP7 (glycine-rich RNA binding protein 7) to bind PRR mRNAs, leading to a decreased amount of PRRs at the plasma membrane (Nicaise et al., 2013). The *P. syringae* effector AvrB and the *Xanthomonas campestris* effector AvrAC enhance virulence by targeting BIK1 and RIPK (two cytoplasmic RLKs which are involved in immune signaling) (Shang et al., 2006; R.-Q. Xu et al., 2008). The fungal effector Ecp6 also prevents activation of host immunity by sequestering chitin fragments that are released during the chitin-triggered host immunity (De Jonge et al., 2010). *P. syringae* effectors, such as AvrPto, AvrPtoB, and HopAO1 suppress plant immunity by targeting the MAPK cascade, which interrupts signaling upstream of MAPKKKs or inhibits MAPKs (Büttner, 2016; He et al., 2006). Expression of specific effectors during distinct pathogenic stages, like during appressorial penetration, biotrophic growth in host cells, and the conversion from biotrophy to necrotrophy, suggest that particular sets of effectors function in each colonization stage. For instance, cell death-inhibiting effectors were expressed during the biotrophic phase, whereas cell death-inducing effectors were expressed during the switch to necrotrophy (Kleemann et al., 2012). Apart from this, effectors have been shown to be able to move from cell to cell to enhance colonization (Toruño et al., 2016). Some *M. oryzae* effectors were observed to move up to two cells away from hyphae, probably via plasmodesmata (Khang et al., 2010).

1.2 Role of phytohormones in plant immune system

Upon pathogen attack, plants synthesize a complex blend of hormones and activate defense-related genes (Pieterse, Van der Does, Zamioudis, Leon-Reyes, & Van Wees, 2012). Plant hormones, including salicylic acid (SA), jasmonic acid (JA), ethylene (ET), brassinosteroid (BR), abscisic acid (ABA), and auxin (AUX), play important roles in the precise regulation of plant immune responses and coregulate the trade-offs between plant growth and immunity (Denancé, Sánchez-Vallet, Goffner, & Molina, 2013; W. Liu, Liu, Triplett, Leach, & Wang, 2014). SA is primarily induced by and involved in defense against biotrophic pathogens, whereas necrotrophic pathogens upregulate both the JA and ET pathways (Glazebrook, 2005). Activation of PTI and ETI dramatically elevates endogenous levels of SA and its conjugates, leading to the induction of pathogenesis-related (PR) proteins and the onset of local and systemic acquired resistance (SAR) (Dempsey, Vlot, Wildermuth, & Klessig, 2011; Tsuda et al., 2009). It has been shown that SA contributes to pathogen resistance mediated by NLRs like SNC1, RPP4, and RPS2 (Nawrath & Métraux, 1999; Van Der Biezen, Freddie, Kahn, Parker¶, & Jones, 2002; Y. Zhang, Goritschnig, Dong, & Li, 2003). EDS1 and PAD4 are involved upstream of SA synthesis and are positively feedback regulated to trigger ETI and basal immunity against biotrophic pathogens (Jirage et al., 1999; Lipka et al., 2005).

The JA pathway mainly protects plants from necrotrophic pathogens and wounding. The F-box protein CORONATINE INSENSITIVE1 (COI1), a key regulator of the JA pathway, functions as a receptor for Jasmonoyl isoleucine (JA-Ile) leading to ubiquitinylation and subsequent degradation of JAZ (JASMONATE ZIM-DOMAIN) repressor proteins via the 26S proteasome (Pauwels & Goossens, 2011; Sheard et al., 2010). Downstream of COI1-JAZ perception, the JA signaling pathway functions via two distinct branches: the MYC-branch and the ERF-branch. The MYC branch, controlled by MYC-type transcriptional factors, like MYC2, MYC3, and MYC4, regulates and modulates the JA pathway (Fernández-Calvo et al., 2011). The ERF branch controls the JA-responsive gene plant defensin 1.2 (PDF1.2) using the ethylene response factor 1 (ERF1) and octadecanoid-responsive Arabidopsis 59 (ORA59) transcription factors. Both branches are antagonistic to each other, as the MYC branch is suppressed by ORA59 overexpression and MYC2 negatively regulates genes of ERF branch-like, ERF1, ORA59, and PDF1.2 by an unknown mechanism (Howe, Major, & Koo, 2018; Verhage et al., 2011).

ET is also known as a modulator of disease resistance in plants and can act both positively and negatively on plant immunity (Adie, Chico, Rubio-Somoza, & Solano, 2007). The transcriptional activity of EIN3/EIL1 is enhanced in JA- and ET-stimulated cells during COI1-dependent degradation of JAZ proteins, resulting in the activation of ERF1 and its downstream target genes, like PDF1.2 (Ziqiang Zhu et al., 2011). An extensive cross-talk between ET, SA and JA signaling has been revealed in analyses of global gene expression profiles of pathogen-infected wild-type and hormone-signaling-defective mutant plants (Glazebrook et al., 2003; Sato et al., 2010).

Compared with the classic defensive hormones SA, JA, and ET, other hormones also play a role in plant defense. The ABA pathway is known to trigger the closure of stomata to stop the entry of the pathogen. For example, through the plant–pathogen interaction specific manner ABA, both positively and negatively regulates resistance to the necrotrophic fungi *A. brassicicola* and *B. cinerea*, respectively (Adie et al., 2007). Pathogens use stomatal openings for entry; in turn, for defense, the plant closes the stomata (Melotto, Underwood, Koczan, Nomura, & He, 2006). Moreover, SA and ABA synergistically mediate the complete closure of the stomata, although the synergistic mechanism remains unknown. When produced in combination with JA, such as after wounding or herbivore attack, ABA positively regulates the MYC branch of the JA response, while it antagonizes the ERF branch (Abe et al., 2003; J. P. Anderson et al., 2004). ABA signaling also antagonizes plant immunity by suppressing SA-dependent defenses (Berens, Berry, Mine, Argueso, & Tsuda, 2017). Auxin, a major phytohormone operating in plant development, can repress SA levels and signaling, and biotrophic pathogens exploit auxin-mediated suppression of SA to enhance host's susceptibility (Z. Chen et al., 2007; Robert-Seilaniantz, Grant, & Jones, 2011). Gibberellin (GAs) signaling negatively influence the JA pathway by regulating the degradation of growth-repressing DELLA proteins. Degradation of DELLA proteins enhances JAZ-mediated suppression of JA-responsive gene expression via MYC branch (Robert-Seilaniantz et al., 2011).

1.2.1 Hormone crosstalk

The complex network of communication between phytohormones is often referred to as hormone crosstalk and is employed not only in immune responses but also in many plant processes. However, the interplay between plant hormones makes it challenging to differentiate their individual effects. The mutually antagonistic effect of the SA and JA pathways are well documented (X. Dong, Jiang, Peng, & Zhang, 2015; Wei Zhang et al., 2018). The nonexpressor of pathogenesis-related genes 1 (NPR1), considered a hormone cross-talk hub, plays a key role in SA / JA antagonism (Shigenaga, Berens, Tsuda, & Argueso, 2017). *A. thaliana* mutant npr1-1 showed enhanced expression of the JA-responsive gene upon *P. syringae* infection (Spoel et al., 2003). It has been shown that SA and MeJA treatment leads to the repression of JA responsive gene PDF1.2 (Koornneef & Pieterse, 2008). Many other regulators, such as the WRKY TFs, including WRKY70, WRKY50, WRKY51, WRKY33, and WRKY75, have been implicated in cross-talk of SA and JA pathways exerting antagonistic influences on SA–JA communication (Birkenbihl, Diezel, & Somssich, 2012; Gao, Venugopal, Navarre, & Kachroo, 2011; J. Li, Brader, Kariola, & Tapio Palva, 2006; Vlot, Dempsey, & Klessig, 2009). When applied exogenously, the low concentrations of both SA and JA can synergistically upregulate both the SA and JA pathways (Mur, Kenton, Atzorn, Miersch, & Wasternack, 2006). DELLAs, enable crosstalk between JA and GA by actively competing with MYC2 in the absence of GA and bind to the negative regulators of JA signaling Jasmonate-ZIM-domain proteins (JAZ) (Hou, Lee, Xia, Yan, & Yu, 2010). JAZs-MYC2 also participates in the cross-talk between JA and ABA signaling pathways, affecting plant growth and defense (Q. Chen et al., 2011).

1.3 Plant pathogen Oomycetes

Oomycetes are among the most notorious plant pathogens and are responsible for many devastating diseases in plants. Several oomycete genera like Albugo, Phytophthora, Peronospora, and Plasmopara cause downy mildew and white rusts on many crops, impacting agriculture and natural ecosystems (Kamoun, 2003). Apart from these, many Pythium species also cause root rot on many glasshouse crop plants (Coates & Beynon, 2010). The most notable and studied pathogenic oomycete *Phytophthora infestans* (*Pi*) is known to cause enormous economic damage to important crops like potato and tomato by late blight disease (Fry & Goodwin, 1997). Apart from crop plants, oomycetes also cause disease in model plants like *A. thaliana* or *N. benthamiana*, making them tractable pathosystems for studying effectors in the laboratory. The downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) and white rust pathogen Albugo (*A. laibachii* and *A. candida*) are two naturally occurring oomycete pathogen of *A. thaliana* (E. Holub & Beynon, 1997).

1.3.1 *Hpa* pathosystem

Hpa (formally known as *Peronospora parasitica*) was the first documented eukaryotic pathogen of Arabidopsis (Koch & Slusarenko, 1990). It is a frequently occurring pathogen in natural Arabidopsis populations and shows the natural variation in host specificity (E. Holub et al., 1995; E. B. Holub, 2008). *Hpa* was adopted as a reference due to abundant genetic polymorphism in the host and the pathogen (Crute, 1994; Dangl et al., 1992). Although *Hpa*

itself does not cause disease in any crop, its closely and distantly related downy mildew species are a serious problem for crops like cabbage, brassica grape, cucurbit, lettuce, spinach, sunflower, and basil (Clark & Spencer-Phillips, 2000). Thus, for a group of oomycete pathogens with significant economic impact, *Hpa* is a reference species.

Hpa is primarily a foliar pathogen, and its asexual phase of the life cycle begins when asexual spores land and germinate on the plant surfaces. The infection hypha then penetrates between the epidermal cells and establishes haustoria, lobed feeding structures, which invaginate in the host mesophyll cells (Fig. 1.2) (Mims, Richardson, Holt Iii, & Dangl, 2004; Soylu, Keshavarzi, Brown, & Mansfield, 2003). Haustoria are thought to be used to extract nutrients from host cells and to secret and translocate the effector proteins into host cells (Spencer-Phillips, 1997; S. Wang et al., 2017). The hypha emerges from stomata and forms asexual fruiting bodies (sporangiophores), culminating in the asexual cycle. High numbers of sporangiospores show the downy appearance at the exterior of colonized leaves (Kamoun et al., 2015). *Hpa* can also produce sexual spores (oospores), which are produced within the plant tissue and can also tissue and persist in the soil after the host plant dies and decays. The infection cycle is initiated by germinating oospores through infection of adjacent host plant roots.

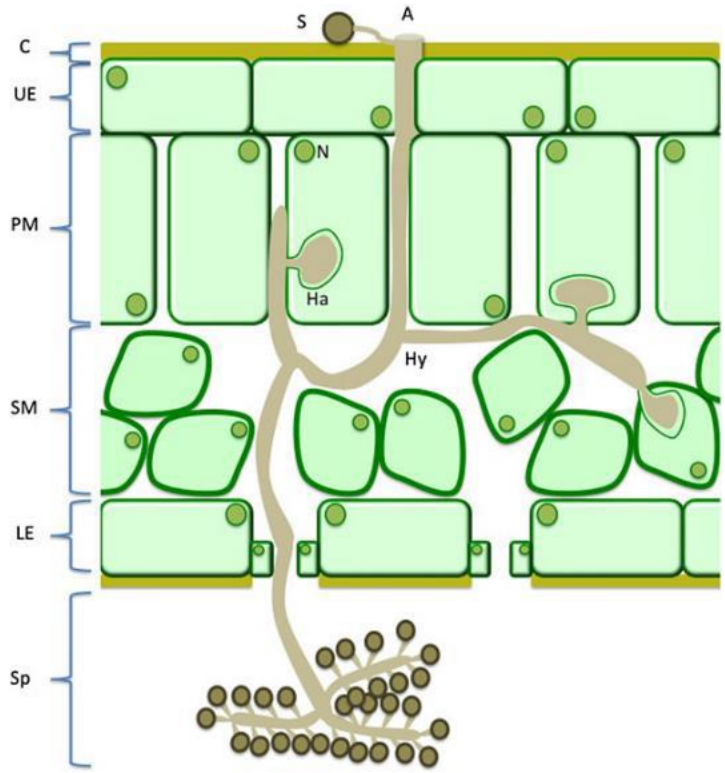

Figure 1.2 Diagram depicting the infection cycle of *Hpa* on Arabidopsis (Kamoun et al., 2015). A, appressorium; C, cuticle; Ha, haustorium; Hy, hyphae; LE, lower epidermis; N, nucleus; PM, palisade mesophyll cells; S, sporangiospore; SM, spongy mesophyll cells; Sp, mature sporangiophore; UE, upper epidermis.

Hpa has several advantages for laboratory experiments together with the greatest limitation due to its obligate lifestyle: *Hpa* can only propagate on its host, which impairs the genetic

transformation of the pathogen (McDowell, 2011). Genetic mapping populations have been generated in successful support of map-based cloning efforts to identify *Hpa* avirulence genes, encoding host-recognized effectors (Rehmany et al., 2003; Woods-Tör et al., 2018). The gene-for-gene model fits with genetic analysis of avirulence in *Hpa* (Flor, 1971), and Arabidopsis resistance genes against *Hpa* are known as RPP genes (Resistance to *Peronospora parasitica*). The first resistant gene to be cloned was RPP5 from the *A. thaliana* ecotype Lansberg erecta (Parker et al., 1997). About 27 RPP genes have been mapped (reviewed in (Slusarenko & Schlaich, 2003), but only eight RPP genes encoding the cytoplasmic NLRs have been cloned and/or characterized so far: RPP1, RPP2, RPP4, RPP5, RPP7, RPP8, RPP13, and RPP39 (reviewed in (Herlihy, Ludwig, van den Ackerveken, & McDowell, 2019)

1.3.2 Oomycete RxLR effectors

Since *Hpa* is a natural bona fide pathogen of Arabidopsis, it has been co-evolving with its host (E. Holub et al., 1995), allowing for the cloning of host resistance genes and the corresponding effector/avirulence genes from the pathogen (E. B. Holub, 2001). Initially, four oomycete AVR effector proteins were reported: AVR1b from the soybean pathogen *P. sojae* (Shan, Cao, Leung, & Tyler, 2004), ATR1 (Rehmany et al., 2005), and ATR13 (Allen et al., 2004) from *Hpa* and AVR3a from *Pi* (Armstrong et al., 2005). Alignment of the amino acid sequences of these proteins showed that they all have in common a signal peptide for secretion from the pathogen, followed by the consensus sequence RxLR motif and an acidic region, often ending in EER (aspartate, glutamate, glutamate, arginine) (Cabral et al., 2011). The most common host-translocated effectors in many oomycetes, such as Phytophthora spp. and downy mildews, are the RxLR-type proteins that contain a C-terminal domain carrying the effector activity and an N-terminal signal peptide and a RxLR (or RxLR-EER) motif (Fig. 1.3) involved in secretion and host uptake (Bos et al., 2006; Dou et al., 2008; Whisson et al., 2007).

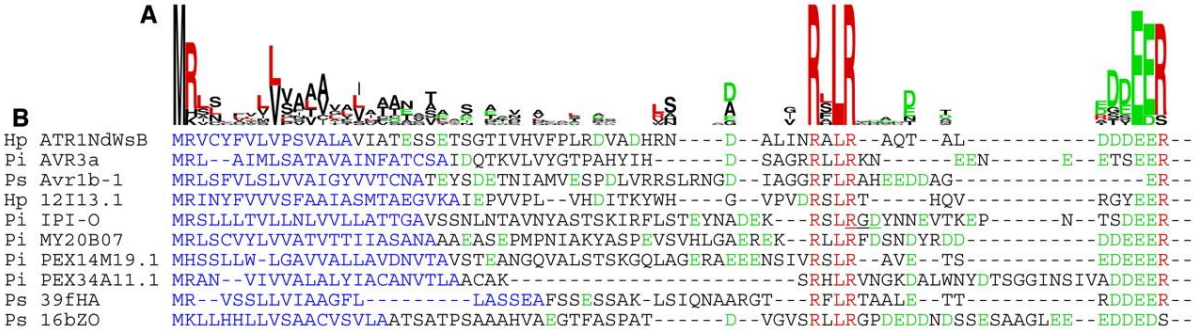

Figure 1.3: The RxLR-DEER Motif (Rehmany et al., 2005). RxLR-DEER motif was identified by the alignment of ten oomycete effector proteins. The RxLR motif is displayed in red, acidic amino acids are shown in green.

The RxLR motif shares some similarities with the host-targeting signal (RxLxE/D/Q) of Plasmodium spp., which is required to export effector proteins across both a pathogen-

derived membrane and an invaginated host membrane (Hiller et al., 2004). Therefore, it was postulated that this motif might play a role in translocating secreted oomycete proteins into the host plant cell (Rehmany et al., 2005). The RxLR motif was shown to be required for translocation of Avr3a into host cells in potato leaves because without the RxLR-EER motif, the protein was secreted into the haustoria and extrahaustorial matrix but not into the host cell (Whisson et al., 2007).

RxLR effectors from *Phytophthora sojae* (*P. sojae*), *Pi,* and *Hpa* have been shown to play major roles in the suppression of plant immunity (R. G. Anderson et al., 2012; Fabro et al., 2011; Q. Wang et al., 2011; Yin et al., 2017; Zheng et al., 2014). A total of 134 high-confidence effector candidates (*Ha*RxL genes) were predicted *in silico* by genome analysis of the *Hpa* isolate Emoy2 (Baxter et al., 2010). However, about 672 and 531 RxLR effector candidates have been predicted in *P. sojae* and *P. ramorum*, respectively (Tyler et al., 2006), which are higher compared to *Hpa,* and this could be because *Hpa* only infects *A. thaliana* whereas *P. sojae* and *P. ramorum* have much broader host ranges. *Ha*RxLs can localize to different subcellular compartments, and a majority of them contribute positively to pathogen fitness against host immunity (Badel et al., 2013; M. C. Caillaud et al., 2012). Additionally, various plant protein targets of *Hpa* and *P. syringae* effectors were identified; subsequently, an Arabidopsis interactome map was generated by mapping binary protein-protein interactions between 552 immune and pathogen proteins and ~8000 full-length Arabidopsis proteins (Mukhtar et al., 2011).

Several RxLs candidate effectors from *Hpa* isolate *Emoy2* were able to manipulate host defense by enhancing the *Pst*/*Hpa* growth, and the majority of effectors that increased bacterial growth also suppressed the accumulation of callose deposition (R. G. Anderson, Deb, Fedkenheuer, & McDowell, 2015; Fabro et al., 2011). When expressed in Arabidopsis, the membrane-associated effector *Ha*RxL17 confers enhanced susceptibility and localizes to *Hpa* haustoria upon infection (M. C. Caillaud et al., 2012). *Ha*RxL96 and its homologous effectors *Ps*Avh163 from *P. sojae* can suppress one or both ETI and PTI (R. G. Anderson et al., 2012). Some effectors, such as *Ha*RxL62 and *Ha*RxL44, manipulate host responsiveness to the hormone SA. *Hpa* effector RxLR44 causes proteasomal degradation of MED19a (subunit of the mediator transcriptional complex), leading to the de-repression of JA-responsive genes, which antagonistically represses SA signaling resulting in a compromised plant immune response (M.-C. Caillaud et al., 2013). Similarly, *Ha*RxL62 also manipulates host responsiveness to the hormone SA by suppressing the SA-inducible PR-1 transcription, and therefore enhances host susceptibility for *Hpa* (Asai et al., 2014). *Ha*RxL23 and the RxLR effector *Ps*Avh73 from *P. sojae* exhibit PTI suppression and enhancement of bacterial and oomycete virulence (Deb, Anderson, How-Yew-Kin, Tyler, & McDowell, 2018). *Ha*RxL106 has been shown to alter plant growth responses to light by binding to radical-induced cell death 1 and suppressing SA-induced defense (Wirthmueller et al., 2018). The virulence mechanisms used by RxLR effectors to manipulate their host can be elucidated by identifying their targets *in planta*.

1.3.3 EAR Motif containing proteins

Ethylene-responsive element-binding factor-associated Amphiphilic (EAR) motifs, which were initially identified in members of the Arabidopsis ethylene response factor (ERF) family and are known as predominant transcriptional repression motifs in plants (Ohta, Matsui, Hiratsu, Shinshi, & Ohme-Takagi, 2001). Aux/IAA proteins contain a repression domain (LxLxL), which showed similarity to the L/FDLNL/F(x)P motif in ERF genes and the 'EAR-like' sequence identified in SUPERMAN at the C terminal region (Hiratsu, Ohta, Matsui, & Ohme-Takagi, 2002; Tiwari, Hagen, & Guilfoyle, 2004). It was shown that the DLELRL motif of SUPERMAN was necessary and sufficient for transcriptional repression in Arabidopsis, in particular, the Leu residues within this motif (Hiratsu, Mitsuda, Matsui, & Ohme-Takagi, 2004). EAR motifs are present in a large fraction of all transcriptional regulatory proteins which are involved in major plant hormone signaling pathways like salicylic acid, jasmonate, ethylene, and abscisic acid (McGrath et al., 2005; Weigel, Pfitzner, & Gatz, 2005; Yang, Tian, Latoszek-Green, Brown, & Wu, 2005). In *Arabidopsis thaliana*, EAR motif-containing protein play important roles in regulating plants growth and development by interacting with co-suppressors, such as TOPLESS (TPL) and SAP18 to perform transcriptional repression functions (Causier, Lloyd, Stevens, & Davies, 2012; Hill, Wang, & Perry, 2008; Song & Galbraith, 2006). The transcriptional repression mechanism used by EAR motifs is becoming clearer due to the discovery of EAR motifs mediating protein-protein interactions. For example, IAA12 interacts via its EAR domain with the CTLH (C-terminal to LisH lissencephaly type 1like homology) domain of TPL for its repression activity (Szemenyei, Hannon, & Long, 2008). Similarly, the Novel Interactor of JAZ (NINJA) interacts with TPL, connecting it to jasmonate signaling for transcriptional repression. This interaction was abolished when Leu residues in the EAR motif were mutated to Ala (Pauwels et al., 2010). JAZ8 binds with TPL via its EAR motif and recruits it for transcription repression mediating jasmonate responses (Shyu et al., 2012).

In another example, MYB44 forms a complex with TPR co-repressors and recruits histone deacetylases to suppress PP2C gene transcription (Nguyen & Cheong, 2018). EAR domains have been identified in many Arabidopsis proteins involved in stress, such as NIMIN1 and its related proteins interact with NPR1 and regulate defense responses by PR gene expression (Weigel et al., 2005), whereas RAP2.1 is involved in the regulation of response to cold stress (C.-J. Dong & Liu, 2010). Effector XopD contains two tandemly repeated EAR motifs which are required for XopD-dependent virulence in tomato (J.-G. Kim et al., 2008). However, in Arabidopsis, XopD represses host defenses by targeting the Arabidopsis transcription factor MYB30, but the EAR motifs are not sufficient for this process and suggest their involvement in targeting different components of the host defense response (Canonne et al., 2011). Consequently, the growing evidence in the literature collectively supports that EAR motif-mediated repression may be considered one of the key mechanisms of gene regulation in plants that evolved during evolution to control gene expression.

1.3.4 TOPLESS and related co-repressor

The regulation of transcription involves transcription factors (TFs) that bind to regulatory sequences and either activate or repress gene expression. In *Arabidopsis thaliana*, LEUNIG (LUG) and TOPLESS (TPL) are the founding members of the GRO/TUP family of co-repressors (Lee & Golz, 2012; Z. Liu & Karmarkar, 2008). TPL was identified after isolating a mutant *tpl-1* from the *A. thaliana* ecotype Landsberg erecta (Ler) (Long, Woody, Poethig, Meyerowitz, & Barton, 2002). TPL and four TPL-related proteins (TPRs) comprise a family of co-repressors interacting with numerous repressors, transcription factors, and adaptors to modulate plant development and signaling (Kieffer et al., 2006; Pauwels et al., 2010; Szemenyei et al., 2008). Among TPR proteins, TPR1 showed the most similarity to TPL, which shares 92% sequence identity and 95% similarity at the amino acid level (Long, Ohno, Smith, & Meyerowitz, 2006). The most closely related to TPL and TPR1 is TPR4, which shares 69% sequence identity and 81% similarity to TPL at the amino acid level (Zhaohai Zhu et al., 2010).

TPL encodes a 124 kDa protein, with a predicted C-terminal to LisH (lissencephaly type 1like homology) (CTLH) domain at the N-terminus, which might be necessary for protein-protein interactions or self-dimerization (Emes & Ponting, 2001). Y-2-H screens using TPL as bait resulted in the identification of multiple AUX/IAAs (transcriptional regulators that degrade during the auxin response (Worley et al., 2000). These interactions were also confirmed *in planta* using co-immunoprecipitation (Szemenyei et al., 2008). Aux/IAA proteins contain the EAR motif (LxLxL), which is essential for mediating transcriptional repression (Tiwari et al., 2004). Mutations to this motif were found to weaken the interaction between IAA12/BDL and TPL; also the CTLH domain of TPL has been shown to be necessary and sufficient for this interaction (Szemenyei et al., 2008). TPL and its homologues function redundantly in plant defense; as double mutants *tpr1-tpl* and triple mutants *tpr1-tpl-tpr4* have been found to increase susceptibility to *Pst* (Zhaohai Zhu et al., 2010). JAZs are suppressors of JA-induced transcriptional response, and their mechanism repression of JA-signaling in the absence of JA-Ile has been characterized. In the absence of JA, JAZ proteins recruit the transcriptional co-repressor TPL/TPRs via interaction with the accessory protein NINJA (Fig. 1.4) (Pauwels et al., 2010). Upon stress, accumulated JA-Ile binds to the F-box protein COI1 to form the COI1-JAZs complex, resulting in ubiquitination and the ultimately 26S proteasomal degradation of JAZ repressors (Pauwels & Goossens, 2011).

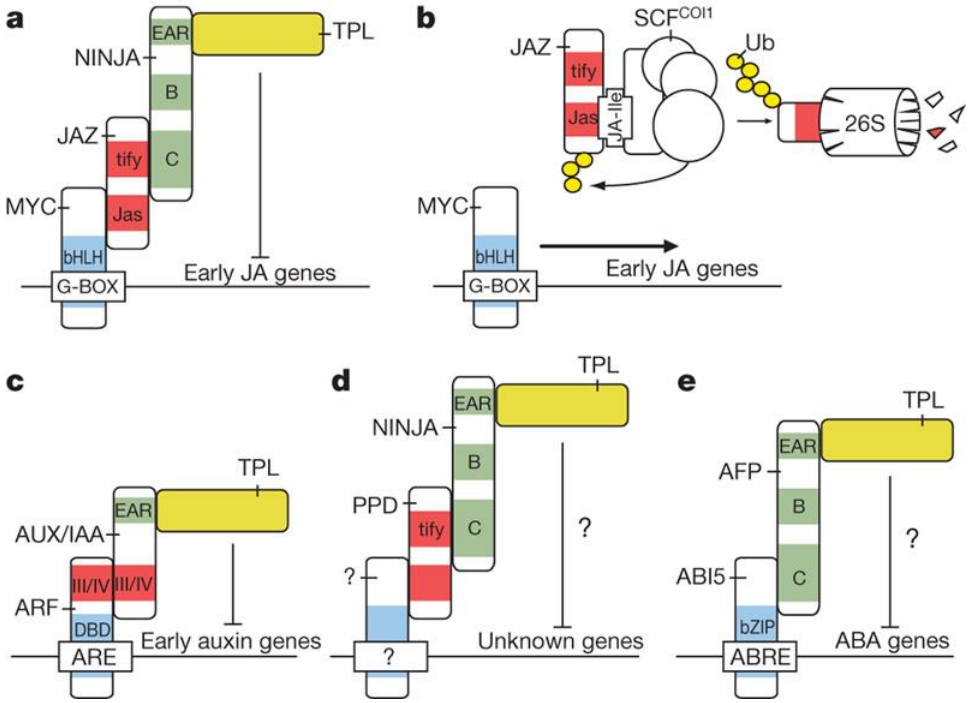

Figure 1.4. Mechanism of JA signaling (Pauwels et al., 2010). (a) in the absence of jasmonates, MYC transcription factors interact with JAZ proteins, which in turn recruit TPL via the co-repressor NINJA, leading to the repression of JA signaling. (b) In the presence of JA-Ile, JAZ proteins interact with SCFCOI1 and are targeted for degradation by the 26S proteosome, relieving repression of early JA genes.

TPL and TPR proteins are involved in a wide range of biological processes like hormone signaling pathways, response to stress, and developmental pathways. For example, TPL regulates JA signaling by interacting with the co-repressor NINJA or directly with JAZ5 or JAZ8, whereas interactions with NIMIN2 or NIMIN3 and ERF9 lead to the regulation of SA signaling and ET signaling, respectively (Consortium, 2011). Interestingly, TPL and TPR proteins not only interact with the EAR motif LxLxL but also interact with other repression domains (DLNxxP, R/KLFGV, and TLxLF), which are enriched amongst transcription factors (Causier, Lloyd, et al., 2012; Ikeda & Ohme-Takagi, 2009; Ohta et al., 2001).

1.4 Context of this work

As shown in the introduction, successful plant pathogens can deliver effector proteins into their hosts. However, the molecular mechanism used by effectors within the plant cell is still unclear to many effectors. So, understanding the pathogen effector's function, host targets, and ecological strategies of interaction with the host protein will allow us to better understand how pathogens successfully colonize their hosts. In this Ph.D. work, the *Hpa*–Arabidopsis model pathosystem was used to shed light on how communication works between both organisms and how *Hpa* effectors suppress host immunity. Previous reports indicate that the *Hpa* effector HaRxL21 is a promising candidate and suggest the putative interactions of HaRxL21 with TPL, TCP14 (TEOSINTE BRANCHED1, CYCLOIDEA, and PCF 14), SWAP (SUPPRESSOR OF WHITE APRICOT), and OBE1 (OBERON 1) generated by a matrix-two hybrid screen (Fabro et al., 2011; Mukhtar et al., 2011). Several findings support the role of TPL/TPRs as 'master regulators or general repressors in *A. thaliana*. Therefore,

they are an essential target if pathogens can manipulate its mode of action. These data are particularly interesting in the context of HaRxL21, which also contains an EAR motif. Therefore, it is reasonable to hypothesize that the interaction of HaRxL21 with TPL is mediated by the C-terminal EAR motif of the effector and the CTLH domain of TPL. In this thesis, we mainly focus on determining the function of HaRxL21, its host targets, and the mechanism used by this effector to promote pathogen virulence.

2. Material and Methods

2.1 Materials

2.1.1 Plant materials and growth conditions

The *Hpa* effector HaRxL21 expressing transgenic lines were generated using *A. thaliana* ecotype Col-0, under the control of β-estradiol inducible (pER8) promoter. All the transgenic lines used in this study are mentioned below in table 2.1.

Table 2.1 Plant material used in the study

Transgenic lines	Stock number	Disposition	Source
WS-eds1		Pathogen maintenance	(Parker et al., 1996)
Est: EV	floral dip transformation	Pathogen assay, q-PCR, western blot, Co-IP, ChIP	(Harvey et al., 2020)
Est:21#2			
Est:21#6			
Est:21#9			
Est:21ΔEAR#1			JLU, Giessen
Est:21ΔEAR#2			
Est:21ΔEAR#3			
tpr1-tpl-tpr4		Pathogen assays	(Zhaohai Zhu et al., 2010)
myb44	GK-197F10.05		(Kleinboelting, Huep, Kloetgen, Viehoever, & Weisshaar, 2012)
myb77	SALK_067655		Salk Institute Genomic Analysis Laboratory(Alonso et al., 2003)
myb73	SALK_023478		
MYB73-1			(Park et al., 2015)
MYB73-2			

Before performing experiments, effector HaRxL21, HaRxL21ΔEAR (deletion of EAR motif) expressing homozygous lines were selected using Hygromycin. The homozygosity of all the Arabidopsis T-DNA insertional lines were confirmed by PCR genotyping (primer listed in Table S8.1). Arabidopsis Col-0 WT and transgenic plants were grown in a climate chamber with 8 h photoperiod at 18°C with 60% relative humidity or on ½ MS media in a growth chamber (CLF Plant Climatics, Germany) under short-day conditions (8 h of photoperiod at 22°C, 16 h of the dark at 18°C, and 60% humidity). For floral dip transformation, the flowering of Arabidopsis plants was induced by switching to long-day conditions at 16 h photoperiod with 60% relative humidity at 22°C. *Nicotiana benthamiana* (*Nb*) plants were grown under long-day conditions at 24°C with a 16 h photoperiod and 70% relative humidity in the growth chamber.

2.1.2 Microbial material

Escherichia coli (*E. coli*) was grown on lysogeny broth (LB) agar plates or LB liquid culture with appropriate antibiotics at 37°C. *Agrobacterium tumefaciens* was grown on yeast extract broth (YEB) agar plates or as liquid culture (Table S8.2) using the appropriate antibiotics at 28°C. Yeast strains Y8800 and Y8930 were cultured on yeast peptone dextrose adenine (YPDA) (Table S8.2) media at 28°C. *Hpa* isolates Noks1 was grown on susceptible Arabidopsis for maintenance and pathogen assay. *Botrytis cinerea* (*B.cinerea.*) was grown on HA-Agar medium plate at 25°C in an incubator.
Microbial strains and plasmids used for this study are listed below in Tables 2.2 and 2.3

Table 2.2 Microbial strains used in the study

Organism	Strain	Source	Disposition
Escherichia coli	DH5α	(Thermo Scientific, Germany)	Cloning
Agrobacterium tumefaciens	GV3101	(Koncz & Schell, 1986)	Protein transient expression
H. arabidopsidis	Noks1	(Tomé, Steinbrenner, & Beynon, 2014)	Pathogen assay
Botrytis cinerea	B 05.10	(Van Kan, Van't Klooster, Wagemakers, Dees, & Van der Vlugt-Bergmans, 1997)	
Saccharomyces cerevisiae	Y8800, Y8930	(Dreze et al., 2010)	Y-2-H

Table 2.3 List of plasmids used in the study

Plasmid	Source	Disposition
pDONRTM/Zeo	Thermo Scientific	Gateway cloning
pER8	(Zuo, Niu, & Chua, 2000)	Arabidopsis transformation and protein expression in *Nb*
pCsVMV: HA3-N-1300	(Jin et al., 2016)	Protein expression in *Nb*
Yeast vector	(Dreze et al., 2010)	Y-2-H,
Bifc vector	(Gehl, Waadt, Kudla, Mendel, & Hänsch, 2009)	Pathogen assays

2.2 Methods

2.2.1 Polymerase chain reaction (PCR)

PCR for yeast transformants, genotyping of transgenic Arabidopsis lines, and colony PCR for selection of bacterial transformants were performed using the DCS-Taq DNA Polymerase (DNA cloning service). Standard 20 µl PCR reaction and temperature protocol are shown below (Table 2.4). The annealing temperature and elongation time of PCR were adjusted

according to the primer melting temperature and length of the required PCR product, respectively.

Table 2.4 Standard 20 µl PCR reaction and temperature protocol for DNA amplifications with the DCS-Taq DNA Polymerase

Component	amount (µl)
10x BD buffer	2
dNTPs (2 mM)	2
$MgCl_2$	1.6
Primer forward (10 pmol)	1
Primer reverse (10 pmol)	1
DCS Taq	0.3
Template (50-200 ng)	x µl
MilliQ H_2O	add up to 20 µl

Thermocycler (Bio-Rad, Germany) **setup (35 cycles)**

	Initial denaturation	Denaturation	Annealing	Extension	Final ext.	Cooling
Temperature	98°C	98°C	50 °C	72°C	72°C	4°C
Time	5 min	30 sec	30 sec	1 min/Kb	5 min	∞

For vector cloning, PCR was performed using the Phusion High-Fidelity DNA polymerase (Thermo Scientific). The standard 50 µl PCR approach and temperature protocol are shown below (Table 2.5). Annealing temperature and elongation time for PCR were adjusted according to primer melting temperature and length of PCR product, respectively.

Table 2.5 Standard 50 µl PCR approach and temperature protocol for DNA amplifications with the Phusion High-Fidelity DNA Polymerase

Component	amount (µl)
5x HF/GC buffer	10
dNTPs (2 mM)	4
Primer forward (10 pmol)	2.5
Primer reverse (10 pmol)	2.5
Phusion	0.6
Template (50-250 ng)	x µl
MilliQ H_2O	add up to 50 µl

Thermocycler setup (35 cycles)

	Initial denaturation	Denaturation	Annealing	Extension	Final ext.	Cooling
Temperature	98°C	98°C	50-60°C	72°C	72°C	4°C
Time	30 sec	10 sec	30 sec	30 sec/Kb	5 min	∞

2.2.2 Vector cloning by restriction digestion and ligation

For restriction enzyme cloning, insert (PCR product), and vector was digested in a 20 µl reaction according to the manufacturer's instructions (New England BioLab (NEB) or

Thermo Scientific). The digested PCR product and vector were purified with a PCR clean-up kit (Promega Wizard™, Germany) before the ligation reaction. The equimolar ratio of vector and insert was calculated with the following formula. For ligation, reaction ratios of 3:1 of the insert to vector were used.

$$\frac{ng\ vector\ x\ size\ of\ insert\ (bp)}{size\ of\ vector\ (bp)} = ng\ insert$$

Ligation was performed for one h at room temperature (RT) or overnight at 4°C in a 20 µl reaction using the T4 DNA ligase (Thermofisher Scientific) as shown in (Table 2.6).

Table 2.6 Ligation reaction

Component	amount (µl)
10x T4 ligase buffer	2
Vector	100 ng
Insert	3:1 ratio over vector
T4 ligase	1
MilliQ H_2O	add up to 20 µl

2.2.3 Gateway vector cloning of Yeast and BiFC vectors

The yeast vector for Y-2-H and BiFC vectors for transient protein expression in *Nicotiana benthamiana* were obtained by Gateway cloning (Invitrogen™). The genes were amplified by a two-step PCR protocol. In the first PCR step, genes were amplified using gene-specific containing only half of the attB sites of attB1/2. In the second PCR, these fragments, after purification by the Wizard PCR purification kit (Promega), were further amplified using primers that contain the 5' end of attB sites. The attB site containing PCR fragments were transferred into the entry vector pDONRTM/Zeo (Thermo Scientific, Germany), which requires a BP reaction to generate entry clones. BP reaction was performed as shown below in a 10 µl reaction overnight at RT (Table 2.7). After the reaction, 1 µl Proteinase K was added to denaturate BP clonase and incubated for 10 min at 37°C.

Table 2.7 BP reaction for the generation of entry clones

Component	amount (µl)
5x BP clonase reaction buffer	1
vector	100 ng
attB-PCR product	150-300 ng
BP clonase	1
TE buffer	add up to 10 µl

Afterward 5 µl of BP reaction were transformed into competent *E. coli* DH5α cells by using the heat-shock transformation method (2.2.5), and cells were plated on selective LB agar plates at 37°C. Grown colonies were analysed by colony PCR (2.2.1) using the M13 primer

pair (Table S8.1) for the selection of positive plasmids. Positive plasmid clones were isolated and verified by Sanger sequencing (2.2.4). Afterward, LR reaction was performed in a 16 µl reaction overnight at RT (Table 2.8). Before the transformation of 5-10 µl of the reaction into competent *E. coli* DH5α cells, LR clonase was denatured by adding 1 µl proteinase K and incubated at 37°C for 10 min. Positive transformants were selected as described above, and positive clones were verified by Sanger sequencing (2.2.4).

Table 2.8 LR reaction between entry clone and destination vector

Component	amount (µl)
5x LR clonase reaction buffer	4
Destination vector	100 ng
Entry clone	150-300 ng
LR clonase	1
TE buffer	add up to 16 µl

2.2.4 DNA sequencing

For sequencing, plasmid DNA was sent to LGC Genomics (Germany) for Sanger sequencing according to the manufacturer's instructions.

2.2.5 Heat shock transformation of chemically component *Escherichia coli* DH5α cells.

For the transformation, 50-80 µl of competent *E. coli* cells were thawed on ice for 20 min. 30-50 ng DNA was used for the retransformation of plasmids. In the case of plasmids generated by Gateway cloning or ligated plasmids; 5-10 µl of BP/LR reaction or 5 µl the ligation reaction was used. Plasmid DNA and thawed *E. coli* cells were mixed carefully and incubated on ice for 20 min. Heat shock was performed in a water bath at 42°C for 45 sec, 2 times with 2 min ice incubation in between. After that, cells were cooled on ice shortly and mixed with 450 µl sterile liquid LB media. Transformed cells were regenerated for 60 min at 37°C with agitation on a shaker and plated on LB-agar plates containing the appropriate antibiotics. Plates were incubated at 37°C overnight, growing colonies were analyzed by colony PCR, and positive clones were sent for Sanger sequencing.

2.2.6 Heat-shock transformation of *Agrobacterium tumefaciens* (*A. tumefaciens*)

For the transformation of *A. tumefaciens* a modified freeze-thaw method was used (Rainer & Willmitzer, 1988). 100 µl competent cells were thawed on ice and mixed with 100 ng plasmid DNA. After mixing cells and DNA by flicking the tube, incubated on ice for 5 min. Afterward, cells were incubated for 5 min in liquid nitrogen and then 5 min in a water bath at 37°C. Cells were resuspended in 1 ml YEB media and incubated at 28°C for 2 h with agitation. 100-150 µl of the cell suspension were plated on YEB agar plates containing the appropriate antibiotics. Plates were incubated at 28°C for two days, and grown colonies were analyzed by colony PCR (2.2.1) for the selection of successfully transformed bacteria. Positive clones were sent for Sanger sequencing for further confirmation.

2.2.7 Isolation of genomic DNA from plant leaves

Arabidopsis genomic DNA was extracted with the method (Y. Lu, 2011) with slight modifications. About 150 mg of plant leaves were harvested in 2.0 ml reaction tubes containing a glass bead, frozen in liquid nitrogen, and crushed to a fine powder using tissue lyzer II (Qiagen, Germany). Then 500 µl Edwards DNA extraction buffer (200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA, 0.5% w/v SDS) (Edwards et al., 1991) was added to the frozen powder, mixed vigorously, and incubated for 10 min at RT. 500 µl chloroform was added to samples, and after mixing for 10-15 sec, samples were centrifuged for 10 min at 15,000 g. The upper phase was transferred to a new 1.5 ml reaction tube, and DNA was precipitated by adding 500 µl isopropanol at RT for 10 min and centrifuged for 10 min at 15,000 g at 4°C. The pellet was washed with 70% ethanol and dried completely before resuspension in 30-50 µl ddH_2O. DNA concentration was determined using NanoDrop 1000 (Thermo Scientific, Germany), and DNA was stored at 4°C.

2.2. 8 RNA extraction from plant leaves

Arabidopsis leaves or around ten seedlings, were crushed to a fine powder using tissue lyzer II (Qiagen, Germany). 1 ml GENEzol (Geneaid) was added to the frozen plant powder and resuspended by vortexing vigorously. After 5 min incubation, ice cold 200 µl Chloroform was added, mixed vigorously by shaking, and incubated for 5 min at RT. After centrifugation at 12000 g for 15 min at 4°C, upper phase (~ 500 µl) was transferred to a new 1.5 ml tube, mixed with 500 µl isopropanol, and incubated for 15 min at RT. RNA was precipitated by centrifugation at 12000 g for 10 min, the supernatant was removed carefully, and the pellet was washed with 1 ml ice-cold 70% ethanol. After final centrifugation at 12,000 g for 5 min at 4°C and removing the supernatant, the pellet was dried completely at RT. For resuspension, 50 µl DEPC ddH_2O was added to the pellet and mixed well. RNA concentration was determined using Nanodrop 1000 (Thermo Scientific, Germany), and RNA was stored at -80°C.

2.2.9 DNAse I digest and cDNA synthesis

Before cDNA synthesis, the remaining genomic DNA in RNA was digested by DNAse I (Thermo Scientific) using RiboLock RNAse Inhibitor (Thermo Scientific) for 30 min at 37°C as shown below in (Table 2.9).

Table 2.9 Reaction mixture for DNAse I digest

Component	amount (µl)
10x DNAse I buffer	1
RNA	2 µg
DNAse I	1
RiboLock (40 U/µl)	0.5
DEPC-ddH_2O	add up to 10 µl

After 30 minutes, 2 µl EDTA (50 mM) was added and incubated at 70°C for 10 minutes to stop the reaction.

For cDNA synthesis, 1 µg digested RNA was used.

cDNA synthesis was performed using the RevertAid Reverse Transcriptase (Thermo Scientific, Germany), the manufacturer's protocol (Table 2.10). The reaction mix was incubated at 25°C for 10 min and followed by incubation at 42° for 60 minutes and 72°C for 10 minutes. Before using the cDNA for qRT-PCR, the reaction was diluted using 80 µl ddH_2O and stored at -20°C.

Table 2.10 Reaction for cDNA synthesis using RevertAid Reverse Transcriptase kit

Component	amount (µl)
Digested RNA (1 µg)	5
5x buffer	4
OligodT Primer (100pmol)	1
Random Hexamer Primer (100pmol)	1
dNTPs (10mM)	2
RiboLock (40 U/µl)	0.25
DEPC-ddH_2O	add up to 20 µl

2.2.10 Real-Time Quantitative PCR (qRT-PCR)

Quantitative Real-Time PCR (qRT-PCR) was performed with freshly synthetized cDNA using SYBR® green JumpStart™ Taq ReadyMix™ kit (Sigma-Aldrich, Germany) in the QuantStudio™ 5 Real-Time PCR system (Applied Biosystems, Germany) in 384-well plates. For each sample, three technical replicates were used, and target transcript levels were determined via the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) by normalizing the amount of target transcript to the amount of reference transcripts tubulin beta chain 4 (TUB4, AT5G44340). Reaction assembly is shown in (Table 2.11). Primers used for qRT-PCR are shown in (Table S1).

Table 2.11 Reaction assembly and temperature protocol for qRT-PCR in 384-well plates

Component	amount (µl)
SYBR® Mix	5
Primer forward	0.25
Primer reverse	0.25
cDNA (10 ng)	1.5
ddH_2O	add up to 10 µl

Thermocycler setup (40 cycles)

	Initial denaturation	Denaturation	Annealing	Extension	Final ext.	Cooling
Temperature	98°C	98°C	50-60°C	72°C	72°C	4°C
Time	30 sec	10 sec	30 sec	30 sec/Kb	5 min	∞

2.2.11 Agrobacterium tumefaciens mediated transformation of *Arabidopsis thaliana*

Plasmids for the transformation of *Arabidopsis* were introduced into the *A. tumefaciens* strain GV3101 by the heat shock method (2.2.6). Transformation of Arabidopsis was performed with the floral dip method as described (Bechtold & Pelletier, 1998), and transgenic plants

were selected on rockwool containing ½ MS medium with 30 µg/ml Hygromycin (Invitrogen, Germany)

2.2.12 Seeds sterilization

Arabidopsis seeds were sterilized 2 mL Eppendorf tube using 50% Ethanol with 0.5% Triton X-100, and the tube was agitated for 2-3 mins to submerge the seeds in solution. The supernatant was removed after 30 seconds and centrifuged at 5000 g. Afterword, a quick rinse of 100% ethanol was given to the seeds, and after flicking the tube, seeds were collected on sterile filter paper under a clean bench. Once dried out, the seeds were collected in a sterile 1.5 ml Eppendorf tube and stored at 4°C.

2.2.13 Hygromycin segregation

Primary transformants (T1) were collected after floral dip transformation of Arabidopsis ecotype Col-0. Seeds were selected for Hygromycin resistance by growing on rock wool containing ½ MS medium without sugar, 30 µg/ml Hygromycin B and cefotaxime antibiotics for agrobacteria. Sterile seeds of the T2 generation were again selected for homozygous T3 lines by growing on rockwool containing ½ MS medium with 30 µg/ml Hygromycin B. At least three independent HaRxL21 and HaRxL21ΔEAR overexpressing transgenic lines were selected and used in this study

2.2.14 Pathogen infection assay

2.2.14.1 *Hpa* pathogen assay

Hpa isolate Noks1 was used for the pathogen infection assay. Noks1 was recovered and maintained on Arabidopsis ecotype Col-0 and Ws-eds1 lines, according to (Tomé et al., 2014). In transgenic effector expressing lines, protein expression was induced about 18 h before infection by spraying 30 µM β-estradiol with .01% tween. For Mock control, seedlings were sprayed with water with .01% tween.

For infection assays, conidiophores were harvested by collecting and vortexing infected leaves in ice-cold water. The spore concentration was adjusted to 3×10^4 spores/ml and spray inoculated on 2-week-old Arabidopsis seedlings. The sprayed seedlings were incubated under short-day conditions for four days, as described above (2.1.1). Sporangiophores on the first two true leaves of each seedling were counted using the dissecting microscope to assess *Hpa* infection.

2.2.14.2 *B. cinerea* infection assay

B. cinerea was grown on HA-Agar medium (Table S7.2) plates for 2-3 weeks at 25°C. The spores were washed from the surface of the plate using a PDB medium. Spores were diluted to a concentration of 2×10^5spores/mL in ½ PDB medium. Detached leaf assay (Zimmerli, Métraux, & Mauch-Mani, 2001) with drop inoculation of *B. cinerea* was performed on 5-6-week-old Arabidopsis plants. In transgenic effector expressing lines, protein expression was induced about 18 h before infection by spraying 30 µM β-estradiol with .01% tween, and for Mock control, seedlings were sprayed with water with .01% tween. Detached leaves were placed on 0.5% w/v water agar square plates, and two drops of 3-µl were inoculated on the

adaxial surface of leaves at both left and right sides of the midvein. Control leaves were spotted with droplets of PDB and plates were incubated at RT. Infected leaves were imaged 72 hpi, and the lesion area was measured using the ImageJ software (Schneider, Rasband, & Eliceiri, 2012).

2.2.15 Protein-Protein interaction

2.2.15.1 Yeast-2-Hybrid

Yeast-2-Hybrid (Y-2-H) screening was performed as described in (Dreze et al., 2010). To identify HaRxL21 interacting proteins in Arabidopsis, a Y-2-H screen was carried out using HaRxL21 as bait against a library of 12500 individually cloned CDSs of *A. thaliana* (Weßling et al., 2014). The yeast strains Y8800 and Y8930 harboring AD-X and DB-X constructs respectively, were mated on yeast-extract, peptone, dextrose, Adenine (YPDA) medium. YPDA plates were replica plated onto Synthetic complete (SC) media lacking combinations of -leucine (Leu) -tryptophan (Trp) -Histidine (His) -Adenine (Ade) + 1 mM 3-AT medium. Plates were cleaned using sterile velvets after 1 day and imaged after 4 days. All vectors were checked for autoactivation and confirmed by sequencing (2.2.4) before experiments were performed.

2.2.15.2 Bimolecular Fluorescence Complementation (BiFC) assay

For the bimolecular fluorescence complementation assay (BiFC), HaRxL21 effector protein, TPL, and MYB73 were cloned into the gateway compatible destination vectors (Gehl et al., 2009). Agrobacterium GV3101 cells containing the desired construct were co-infiltrated in *N.b.* leaves. Infiltrated leaf area was infiltrated with ddH_2O at 48 hpi, and the leaf disc was taken for microscopic analysis. The subcellular localization of the interaction was visualized using a confocal laser-scanning microscope (Leica, Germany). Images were processed with Leica Application Suite X (LAS X) software.

2.2.15.3 Co-immunoprecipitation by nuclear enrichment

For the Co-IP assay, proteins were expressed transiently in *N. benthamiana*. Leaves of about four-week-old plants were infiltrated with Agrobacteria using infiltration buffer (2.2.16). TPL and HaRxL21 were expressed in pCsVMV-HA3-N-1300 (C-terminal HA tag) and Per8 (N-terminal myc tag) vectors (Table 2.3), respectively. In the case of the Per8 construct, after 24 hpi, leaves were sprayed with 30 µM β-estradiol to induce the expression of HaRxL21 and subsequent mutants. 48 hrs post infiltration, about 2 g of tissue from the infiltrated area was collected and frozen with liquid nitrogen. Before Co-IP, leaves were tested for protein expression by the protein extraction method (2.2.17). All steps were carried out either on ice or in a 4°C cold room. For nuclear enrichment, approximately 1.5 g of frozen leaves were ground with liquid nitrogen using a cool mortar and pestle. Pulverized tissue was again grounded with 7 ml of ice-cold Nuclear Isolation Buffer (NIB) with protease inhibitor cocktail (PIC) on ice for a few minutes. The mixture was filtered through 2 layers of Miracloth into a chilled 50 ml falcon tube. **Sample 1** (100 µl) was collected, and the filtrate was centrifuged at 3320 g for 15 min. Pellet was resuspended in 2 ml NIB with PIC with gentle pipetting and transferred into a chilled 2 ml tube. **Sample 2** (100 µl) was collected

resuspended pellet was centrifuged at 1900 g for 5 minutes at 4ºC and carefully decanted the supernatant. This was repeated with 500 µl ice-cold NIB with PIC, and the pellet was gently resuspended by pipetting. Three or more washes were performed until the pellet (nuclei) looked whitish with little if any green surface and the supernatant was very light green. The supernatant was removed after the last wash. The pellet was resuspended in 1 ml lysis buffer by gentle pipetting, and **sample 3** (100 µl) was collected. The resuspended pellet was then sonicated with 60% amplitude and five times for a 10-second cycle with 2 min intervals in between on ice. Sonicated nuclei were centrifuged at maximum speed for 30 min, and the supernatant was transferred to a new 1.5 ml tube and used as input for IP. **Sample 4** (50 µl input) was collected, and IP was performed using a µMACS kit (130-091-123; Miltenyi Biotech) of magnetic microbeads conjugated to an anti-c-myc monoclonal antibody, according to the manufacturer's instructions. All samples were mixed with 4x SDS buffer, heated at 95ºC for 5 min, and analyzed by western blot using HRP-conjugated anti-myc antibody (130-092-113; Miltenyi Biotec), or an anti-HA antibody (3F10; Roche) with 1:15000 or 1:8000 dilution, respectively.

2.2.16 Transient expression in *N. benthamiana*

Agrobacteria harboring vector of interest was cultured overnight in a YEB liquid medium containing appropriate antibiotics at 28°C. RNA silencing suppressor P19 (Voinnet, Rivas, Mestre, & Baulcombe, 2003) was always used during co-infiltration. Agrobacterial cells were harvested in 50 ml falcon tube by centrifugation at 2500 g for 20 min at RT and washed with infiltration buffer (10 mM MES, 10 mM $MgCl_2$, and 100 µM acetosyringone). The pellet was resuspended in infiltration buffer, and the optical density (OD) was measured at 600 nm (OD600) by BioSpectrometer (Eppendorf, Germany). Agrobacterium suspensions with appropriate OD (p19, pCSVMV, pER8 at 0.3, 0.2, and 0.1 final $OD_{600,}$ respectively) were mixed according to the required co-infiltration and infiltrated into 4-6-week-old *N. benthamiana* leaves (abaxial) by using 1 ml needless syringe. Infiltrated plants were kept at RT, and the leaves were harvested after 48 hpi for further experiments. In case of estradiol induction, infiltrated area of leaves was sprayed or brushed with 30 µM β-estradiol after 24 hpi.

2.2.17 Protein extraction from plant leaves

Protein extraction was done using 4x SDS-buffer (125 mM Tris-HCl pH 6.8, 4% w7v SDS, 50% glycerol, 0.02% w/v bromophenol blue). Before usage, 100 mM fresh DTT was added to the buffer. Arabidopsis leaves/seedlings or N.B. leaf discs were crushed to a fine powder by using tissue lyzer II (Qiagen, Germany) and mixed with 50-100 µl 4x SDS buffer and heated for 5-10 min at 95°C. Insoluble particles were separated by centrifugation at 15000 g for 5 min, and the supernatant was transferred to a new 1.5 ml reaction tube. The samples were used directly for western blot (2.2.19).

2.2.18 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

For the separation of proteins, discontinuous polyacrylamide gels were used containing a 10% resolving gel and a 3% stacking gel. The protocol for two gels is shown below (Tab 2.12).

Protein extracts were mixed with 4x SDS-buffer (1M Tris pH 6.8, 80% Glycerol, 4% (w/v) SDS, 0.05% (w/v) bromophenol blue) and denatured in a 95°C heating block for 5 min before loading into the wells of the SDS gel. Separation of proteins was done at 100 volts for two h or depending on the protein's size until the buffer's blue colour came out.

Table 2.12 10% SDS-gels for the 1 mm BioRad Mini-Protein gel

Recipe for two SDS-gels (12%) for 1-mm BioRad Mini-protein gel system component	**Resolving gel**	**Stacking gel**
Component	amount	amount
30% acrylamide mix	4 ml	1 ml
1.5 M Tris-HCl pH 8.8	2.5 ml	-
1 M Tris-HCl pH 6.8	-	600 µl
H_2O	3.4 ml	3.6 ml
10% w/v SDS	100 µl	100 µl
10% w/v Ammonium Persulfate (APS)	100 µl	50 µl
TEMED	10 µl	10 µl

2.2.19 Western Blot

For the separation of proteins, SDS-PAGE was used (2.2.18). Protein was transferred on PVDF membrane (Roth, Germany) by making the blot sandwich built from bottom to top by 7x whatman paper (GE Healthcare), PVDF membrane, SDS-gel, and 7x whatman paper using the Trans-Blot Turbo Transfer System (Bio-Rad). Before, Whatman paper was soaked in towbin buffer (25 mM Tris, 192 mM glycin, 20% methanol) and the PVDF membrane in methanol and washed with towbin buffer. After blotting, the PVDF membrane was directly incubated for blocking in 5% w/v milk powder in TBS-T (100 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% tween-20) for 1 h at RT or overnight at 4°C. The membrane was then washed 3x with TBS-T for around 20 min and incubated with HRP-conjugated anti-myc antibody with 1:15000 dilution in 1% milk powder or an anti-HA antibody with 1:8000 dilution in 5% milk powder in TBS-T for 3 h at RT or overnight at 4°C. The membrane was washed 5x with TBS-T for 5 min, and bound antibodies were detected using the Amersham ECL™ Prime western blotting detection reagent (GE Healthcare, UK). The developing chemiluminescence was detected with the ChemiDoc MP imaging system (Bio-Rad, Germany) using the ImageLab Software.

2.2.20 Chromatin immunoprecipitation assay (ChIP)

ChIP PCR assay was performed as described in (Gendrel, Lippman, Martienssen, & Colot, 2005). The two-week-old Arabidopsis Seedlings (around 2 grams) were harvested in a 50 mL falcon tube and washed with ddH_2O. For the cross-linking, 37 ml of 1% formaldehyde (Sigma-Aldrich, Germany) solution was added and incubated under vacuum for 16 min (2X8 min) at RT. Cross-linking reaction was stopped by adding 2 M glycine and again vacuumed for 5 min. Plant seedlings were washed three times with ddH_2O, and water was removed using paper towels and snap-frozen in liquid nitrogen. For chromatin isolation, tissue was

ground in fine powder (using prechilled mortars and pestles) and resuspended in 30 ml of cold (4°C) extraction buffer 1 (Table 2.13) and incubated for 5 min on ice. The resuspended solution was filtered through 2 layers of Miracloth, centrifuged at 3000 g for 20 min, and discarded the supernatant. The remaining pellet was resuspended in 1 ml of extraction buffer 2 (Table 2.14) and transferred to a 1.5 tube, centrifuged again at 12000 g at 4°C for 10 min to gain white pellet with an overlay of chlorophyll. The pellet was resuspended in 300 µl of cold extraction buffer 3 (Table 2.15) (avoid foaming during pipetting). In a new tube, 500 µl of extraction buffer 3 was added and overlaid with a resuspended pellet and centrifuged at 16000 g at 4°C for 1h. The supernatant was removed, and the chromatin pellet was resuspended in 300 µl of nuclei lysis buffer (Table 2.16). 5 µl of extracted chromatin can be set aside for comparison. Nuclei were then sheared by sonication (Bioruptor, Diagenode, Ougrée, Belgium) five times with 2.5 min at medium level to reduce the average DNA fragment size to around 500 base pair. Sheared nuclei were centrifuged for 5 min at 12000 g at 4°C. The supernatant was transferred to a new tube, and 5 µl was set aside to compare sheared DNA using agarose gel electrophoresis and 5-10 µl for INPUT control.

2.2.20.1 Immunoprecipitation

Samples were diluted with ChIP dilution buffer (Table 2.17) and divided equally into 2 tubes (one with antibodies + one without antibody control). The protein A magnetic beads (Biolabs) were washed with ChIP-buffer. 40 µl of pre-washed beads were added to the sample and incubated for one h at 4°C with gentle rotation. Beads were magnetic pulled down at 4°C and discarded. 5 µl of the Myc Antibody (ab9132; ChIP-grade, Abcam, Cambridge, UK) were added and incubated overnight at 4°C with gentle rotation. Afterward, 40 µl pre-washed (ChIP-buffer) protein A magnetic beads were added and continued incubation for 2h at 4°C with gentle rotation. Beads were magnetic pulled down at 4°C, and the supernatant was discarded. Magnetic beads were washed for 5 min with gentle rotation at 4°C with 1 ml of the following buffers and magnetic pull-down at 4°C

a) 1x low salt wash buffer b) 1x high salt wash buffer c) 1x LiCl wash buffer d) 2x TE Buffer (Table 2.18) (each time a quick wash, then a second wash for 5 min)

The immuno-complex was eluted from magnetic beads by adding 250 ml freshly prepared elution buffer, vortexed, incubated for 15 min 65°C, and reversed the tubes every 3 min. After magnetic pull-down, the supernatant was transferred into new Eppi. This elution was repeated with 250µl elution buffer, and both eluates were combined.

2.2.20.2 Reverse cross-linking and protein digestion

For the reverse cross-linking, 20 µl of 5M NaCl was added to each sample and reversed the cross-linking by incubation at 65°C for at least 6 h to overnight. The Input control was also included (add elution buffer to 500 µl and 20 µl of 5 M NaCl and incubate at 65°C overnight).

For protein digestion following were added to all samples:

10 µl of 0.5 M EDTA, 20 µl of 1M Tris-HCl (pH 6.5)

2 µl of 10 mg/ml proteinase k and incubated for 1h at 45°C

2.2.20.3 DNA precipitation

To precipitate the DNA, an equal volume (550 µl) of phenol/chloroform/isoamyl alcohol was added to each tube and vortexed briefly. Centrifugation was performed at 13,800 g for 15 min at 4°C, and the supernatant was transferred into 2 ml Eppi. Subsequently, following solutions were added to each tube: 2.5 volume of 100% ethanol, 1/10 volume of 3 M Sodium acetate (pH 5.2), 2µl glycogen (10 mg/ml) and incubated for 1h at -80°C to precipitate DNA and centrifuged at 13,800 g for 15 min on 4°C. The supernatant was discarded, and the pellet was washed with 500 µl of 70% ethanol and centrifuged again at 13,800 g for 10 min 4°C. DNA was resuspended in Milli-Q water, and diluted DNA was analysed by qPCR.

Table 2.13 Extraction buffer 1:

Component	amount
0.4 M sucrose	20 ml of 2 M
10 mM Tris-HCl pH8	1 ml of 1 M
10 mM $MgCl_2$	1 ml of 1 M
DTT	500 µl of 1 M
0.1 mM PMSF	50 µl of 0.2 M
PI; Mini (Roche)	2 tablets
H_2O	up to 100 ml

Table 2.14 Extraction buffer 2:

Component	amount
0.25 M sucrose	1.25 ml of 2 M
10 mM Tris-HCl pH8	100 µl of 1 M
10 mM $MgCl_2$	100 µl of 1 M
1% Triton X-100	0.5 ml of 20% w/v
DTT	50 µl of 1 M
PI; Mini (Roche)	1 tablet
water	up to 10 ml

Table 2.15 Extraction buffer 3:

Component	amount
1.7 M sucrose	8.5 ml of 2 M
10 mM Tris-HCl pH8	100 µl of 1 M
2 mM $MgCl_2$	20 µl of 1 M
0.15% Triton X-100	75 µl of 20% w/v
DTT	50 µl of 1 M

0.1 mM PMSF	5 µl of 0.2 M
PI; Mini (Roche)	1 tablet
water	up to 10 ml

Table 2.16 Nuclei lysis buffer:

Component	amount
50 mM Tris-HCl pH8	0.5 ml of 1 M
10 mM EDTA	200 µl of 0.5 M
1% SDS	1 ml of 10% w/v
PI; Mini (Roche)	1 tablets
water	up to 10 ml

Table 2.17 ChIP dilution buffer:

Component	amount
16.7 mM Tris-HCl pH8	334 µl of 1 M
167 mM NaCl	668 µl of 5 M
1.1% Triton X-100	1.1 ml of 20% w/v
1.2 mM EDTA	48 µl of 0.5 M
water	up to 20 ml

Table 2.18 Wash buffer:

low salt wash buffer	**high salt wash buffer**	**LiCl wash buffer**	
Component	amount	Component	amount
20 mM Tris-HCl pH 8.0	20 mM Tris-HCl pH 8.0	LiCl	0.25 M
150 mM NaCl	500 mM NaCl	NP-40	1%
0.1 % w/v SDS	0.1 % w/v SDS	Sodium deoxycholate	1% w/v
1% w/v Triton X-100	1% w/v Triton X-100	Tris-HCl pH 8.0	10 mM
2 mM EDTA	2 mM EDTA	EDTA	1 mM

TE buffer		**Elution buffer**
10 mM	Tris-HCl pH 8.0	1 ml of 20% w/v SDS
1 mM	EDTA	0.168 g $NaHCO_3$
		Add water up to 20 ml

3. Results

3.1 HaRxL21 characterisation

HaRxL21 is a 45 kDa effector protein identified from the genome of *Hpa*. It is conserved across multiple *Hpa* isolates and contains a 'RLLR-DEER' motif at the N-terminal and an EAR (LxLxL) motif (amino acid sequence LMLTL) at the very C-terminal. Alleles of HaRxL21 have been reported in the *Hpa* isolates Cala2, Emco5, Emoy2, Emwa1, Hind2, Maks9, Noks1 and Waco9 (Asai et al., 2014) and BioProject PRJNA298674 (Noks1). To determine the defining features of HaRxL21 in different isolates, the amino acid sequences of HaRxL21 were aligned using ESPript 3.0 multiple sequence alignment program (Robert & Gouet, 2014) shown in Figure 3.1. The signal peptide cleavage site was predicted to be between positions 16 and 17 (SignalP-5.0). The RxLR and DEER motifs are conserved across all alleles and the EAR motif at the C-terminus is also conserved between all aligned alleles with the exception of Noks1 (truncated due to Serine 197 changed to a stop codon). Therefore, it does not have an EAR motif at the C-terminus and would be expected to not interact with TPL.

3.2 HaRxL21 interacts with transcriptional co-repressors and transcription factors

In initial studies, HaRxL21 has been shown to interact with TCP14, OBE1, SWAP and TPL (Mukhtar et al., 2011) by mapping the effector against a collection of 8,500 individually cloned open reading frames (ORFs) of *A. thaliana* using Y-2-H. Due to the availability of an expanded library of 12,500 individually cloned CDSs (Weßling et al., 2014), we carried out an additional Y-2-H screen to find more interacting proteins. In this screen, together with TPL and TCP14, four additional interacting proteins (TCP13, TPR2, TPR3, MYB73) were identified. Teosinte Branched Cycloidea and PCF (TCP 13/14) transcription factors (TF) have a bHLH (basic helix-loop-helix) motif, facilitating DNA binding and protein-protein interactions. The loss-of-function mutants of many TCP family members cause developmental defects (Martín-Trillo & Cubas, 2010). TCP14 is known as a 'hub' protein in the *A. thaliana* immune network because large number of interactions with both pathogen effectors and *A. thaliana* proteins involved in defense have been reported (Mukhtar et al., 2011). MYB73 is a R2R3 MYB TF and has been shown to be involved in *NPR1*-mediated SA and JA signaling pathways (Jiao, Xing, Dong, Han, & Liu, 2011). TPL/TPRs play key roles in different hormonal signaling pathways (JA, Auxin) and developmental processes. TPL protein family members contain the CTLH domain, which is necessary and sufficient for its transcriptional repression activity (Szemenyei et al., 2008).

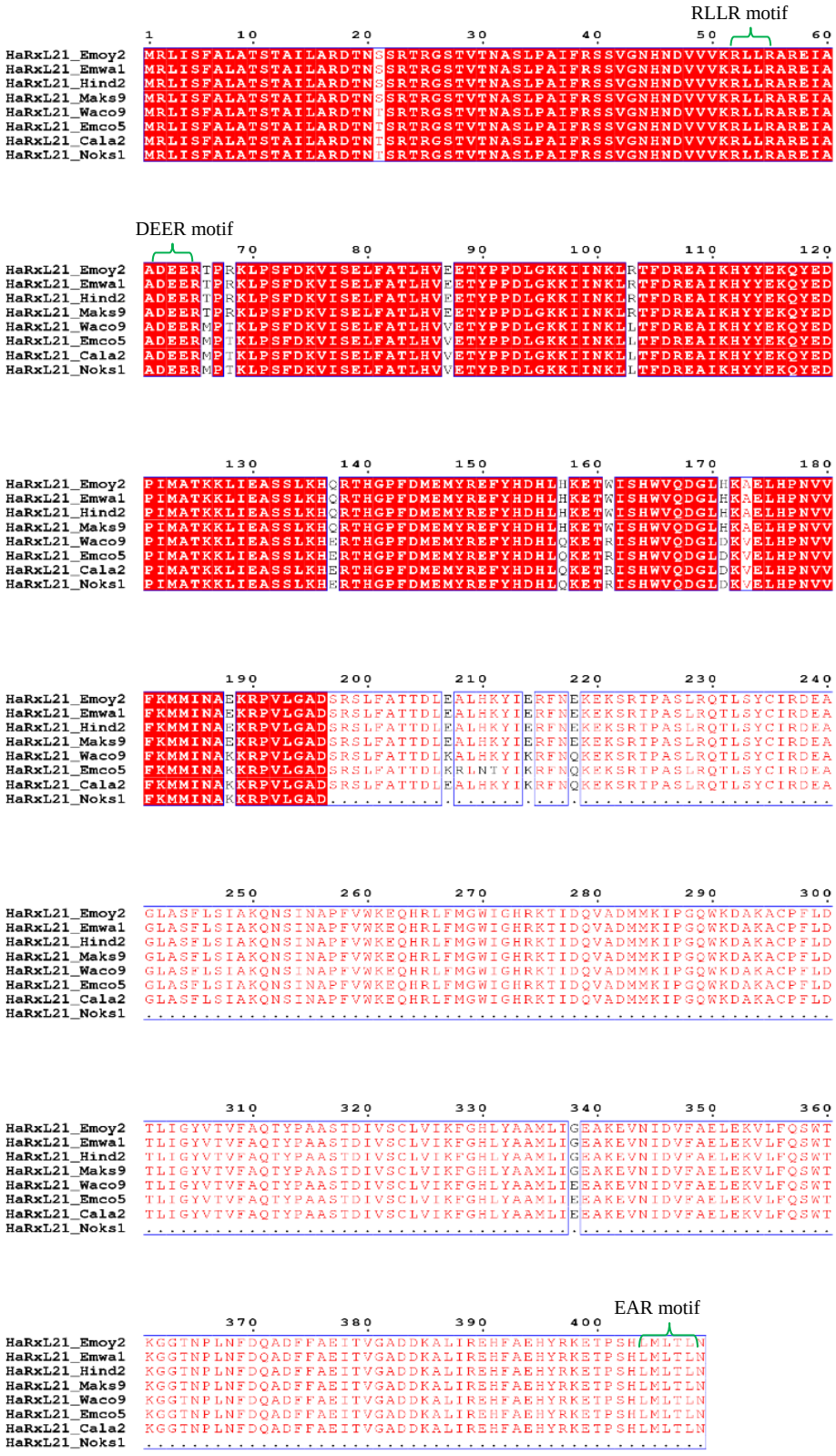

Figure 3.1 Amino acid sequence alignment of HaRxL21 alleles from *Hpa* isolates Cala2, Emco5, Emoy2, Emwa1, Hind2, maks9, Waco9 and Noks1. Multiple sequence alignment was performed using ESPript 3.0 (Robert & Gouet, 2014). Identical and high-similarity amino acid residues are in red background and red color, respectively. The RLLR, DEER and EAR motifs are conserved across alleles except EAR motif in Noks1.

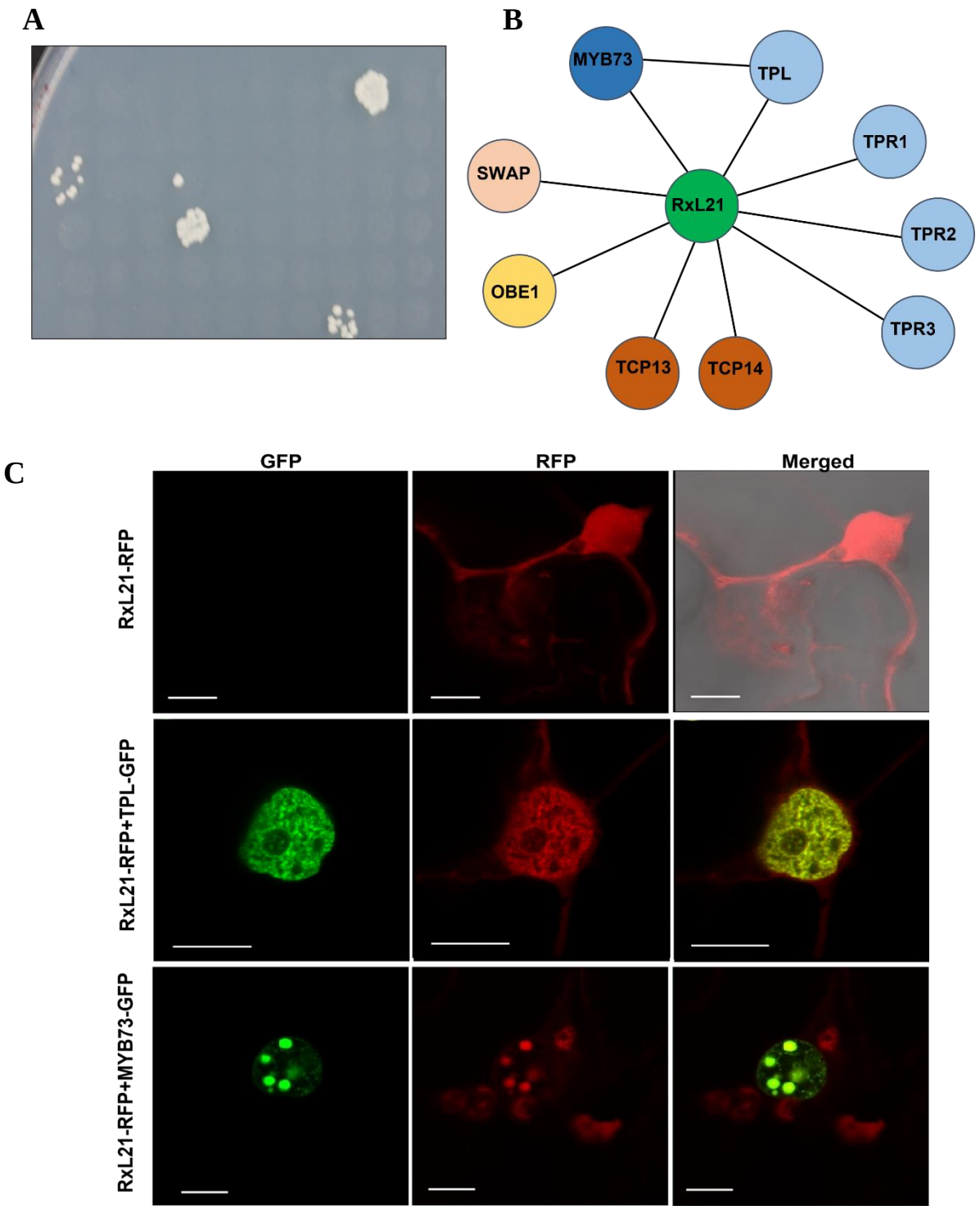

Figure 3.2 Yeast two-hybrid assay and localization of HaRxL21. (A)Yeast culture was spotted onto the synthetic complete (SC) mating (SC/-Leu-Trp) and selection (SC/-Leu-Trp-His-Ade+1mM 3-AT) plates. The interaction between pDB-RxL21 as bait and a pAD-CDS library as prey was assessed. (B) HaRxL21 interacts with TFs (MYB73, TCP 13/14, SWAP and OBE1) and Topless family members (TPL, TPR1, TPR2, TPR3). (C) For localization of HaRxL21, *N. benthamiana* leaves were infiltrated with RFP-RxL21 and co-infiltrated with GFP-TPL/MYB73 construct in the presence of the silencing suppressor p19. Infiltrated tissues were imaged at 48 hpi by confocal scanning laser microscopy for GFP or RFP fluorescence. Re-localization of HaRxL21 in nuclear foci was observed when co-expressed with TPL or MYB73. Scale bars 10 μm.

3.3 HaRxL21 co-localized in sub-nuclear foci with TPL and MYB73

HaRxL21 is a cytoplasmic and nuclear-localized effector (Fig. 3.2C, row1). Since we observed that HaRxL21 interacts with nuclear proteins like TPL and MYB73, we checked if the localization of HaRxL21 is affected when expressed together with its interacting proteins. RFP tagged HaRxL21 (35S:RFP-RxL21) was co-infiltrated with GFP tagged TPL or MYB73 (35S:GFP-TPL/MYB73) in the presence of the silencing suppressor p19 (Voinnet et al., 2003). We observed re-localization of HaRxL21 when co-expressed with either TPL or MYB73 (Fig. 3.2C, rows 2 and 3), HaRxL21 shows co-localization into sub-nuclear foci. These results indicate that HaRxL21 interacts with the partner proteins in the nucleus and converges onto the host target proteins.

3.4 BiFC assay confirms the interaction of HaRxL21 with MYB73 and TPL

To further validate the protein-protein interactions *in planta*, bimolecular fluorescence complementation (BiFC) was used. The two halves (N terminal half and C terminal half) of the yellow fluorescent protein (YFP) were fused to each of the interacting proteins. If the proteins interact with each other, the N and C-terminal halves of YFP will be complemented, resulting in fluorescence.

To determine the subcellular localization and *in planta* interactions between HaRxL21 and interacting proteins, the partner proteins were transiently co-expressed in *N. benthamiana* epidermal cells. The nuclear presence of TPL is required to act as a transcriptional corepressor. We first determined that TPL homodimerizes in the nucleus (Fig. 3.3A, row 1) as the N-terminus of TPL has been shown to dimerize in the nucleus (Martin-Arevalillo et al., 2017). Additionally, HaRxL21 was also tested for homodimerization, but no fluorescence was observed (Fig. 3.3A, row 4), indicating that HaRxL21 is a monomeric protein. We observed a strong BiFC nuclear signal when HaRxL21 and TPL were co-expressed (Fig. 3.3A, row 2), confirming our Y-2-H results. As it has been shown that TPL requires its CTLH domain for interactions (Szemenyei et al., 2008), we co-expressed HaRxL21 with TPL-delCTLH (TPL lacking the CTLH domain). No fluorescence was observed in the case of TPL-delCTLH, indicating that HaRxL21 interacts with TPL via the CTLH domain (Fig. 3.3A, row 3).

Interaction between HaRxL21-MYB73 was also verified via BifC, and fluorescence was observed in the nucleus confirming the interaction observed in Y-2-H (Fig. 3.3B, row 1). We further checked whether MYB73 interaction is EAR motif dependent. Strong nuclear fluorescence was observed when MYB73 was transiently co-expressed with HaRxL21ΔEAR construct (Fig. 3.3B, row 2), indicating that MYB73 interacts with HaRxL21, and this interaction is independent of the EAR motif of HaRxL21. Moreover, MYB73 homodimerizes in the nucleus and interacts with TPL (Fig. 3.3B, row 3,4).

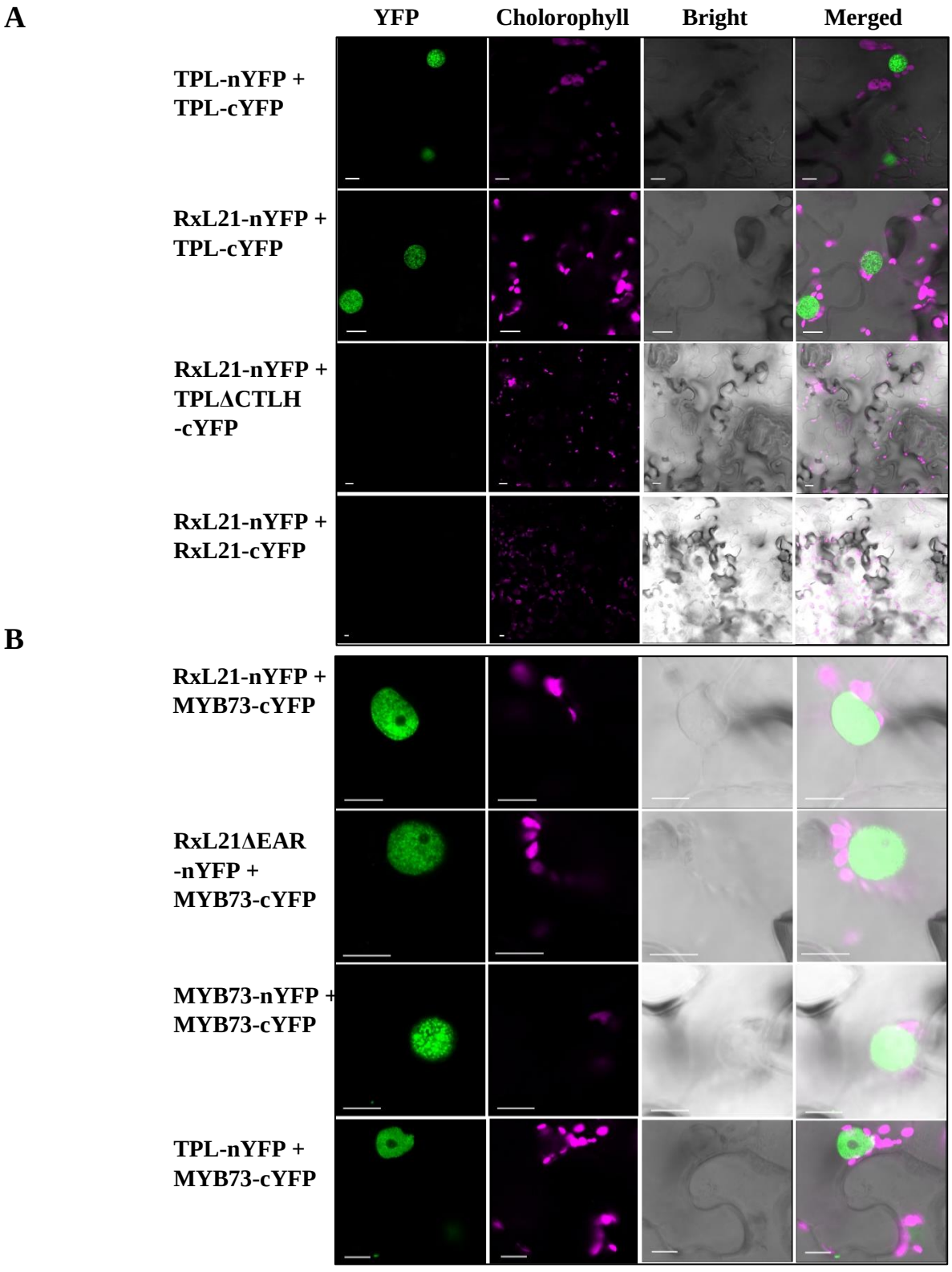

Figure 3.3. TPL and MYB 73 interact with HaRxL21 in the BiFC assay (A) Agrobacteria harboring TPL-n/c YFP, RxL21-n/cYFP, TPLΔCTLH-nYFP were co-infiltration in epidermal cells of *Nb* leaves in the presence of p19. Infiltrated tissues were imaged at 48 hpi by confocal scanning laser microscopy for YFP fluorescence. The interaction between HaRxL21 and TPL was lost with the deletion of the CTLH motif of TPL. TPL homodimerization was used as a positive control and HaRxL21 does not appear to form dimers unlike TPL where strong fluorescence was observed in the nucleus. (B) MYB73-cYFP was co-expressed with RxL21-nYFP or RxL21ΔEAR-nYFP and strong fluorescence was observed. Scale bar = 10 μm.

3.5 Co-IP confirms *in planta* HaRxL21-TPL interaction and both CTLH & EAR motifs are necessary for this interaction

BiFC assay might give false-positive results due to a high concentration of proteins forced into close proximity (Kudla & Bock, 2016), thus for the specific *in planta* interaction of HaRxL21 with MYB73 and TPL Co- Immunoprecipitation (Co-IP) was performed. As in BiFC system, TPL and MYB73 have been shown to interact with HaRxL21 in the nucleus (Fig. 3.3) so to get an enriched amount of nuclear protein, we performed Co-IP with the nuclear enrichment method (as described in section 2.2.15.3). A Myc tag was fused to the N-terminus of HaRxL21 and HA-tagged MYB73 was co-expressed in *N. benthamiana* plants. However, we were unable to prove this interaction via Co-IP. To validate HaRxL21-TPL interaction and requirement of EAR/CTLH motif for this interaction HaRxL21, HaRxL21ΔEAR or its subsequent mutant variants (RxL21-FMFTF or RxL21-AMATA) in which the Leu residues in the EAR motif were mutated to Phe or Ala) were co-expressed in *N. benthamiana* with C-terminal HA-tagged TPL or TPLΔCTLH. Immunoprecipitation of HaRxL21 using an anti-Myc antibody resulted in Co-IP of TPL (Fig. 3.4 A), confirming direct protein-protein interaction occurring *in planta*, whereas Myc-tagged HaRxL21ΔEAR was unable to pull down TPL. Furthermore, TPLΔCTLH was not immunoprecipitated by Myc-tagged RxL21. This demonstrated that, as in Y-2-H and BiFC, the EAR motif and CTLH domain are required for HaRxL21-TPL interaction. TPL was also not immunoprecipitated when using variants of HaRxL21ΔEAR (RxL21-FMFTF or RxL21-AMATA), again confirming the observations in yeast (Harvey et al., 2020) that the Leu residues in the EAR motif of HaRxL21 are necessary for interaction with TPL.

3.6 HaRxL21 also interacts with TPRs

TPL and its family member TPRs have been shown both redundancy and specificity with their interactors. For example, all family members interact with multiple IAAs or several ERF TFs interact with one or a subset of the co-repressor TPL/TPR family members (Causier, Lloyd, et al., 2012). We looked if HaRxL21 also interacts with the TPRs. The N terminally Myc tagged HaRxL21 was co-expressed in *N. benthamiana* with C-terminal HA-tagged TPR1 and TPR3. Immunoprecipitation of HaRxL21 using an anti-Myc antibody resulted in Co-IP of both TPR1 and TPR3, confirming that direct protein-protein interaction occurs *in planta* (Fig. 3.4B).

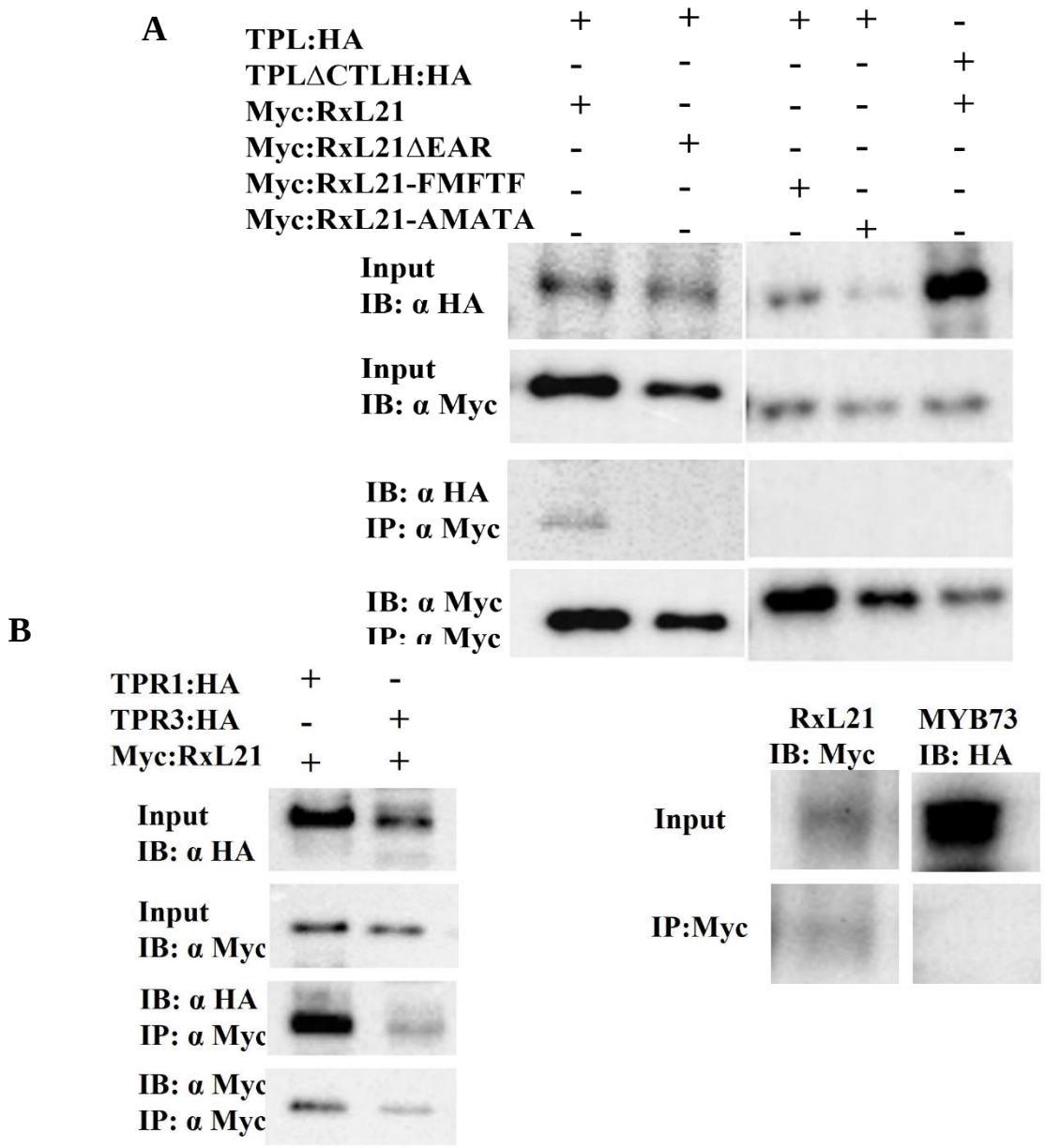

Figure 3.4 TPL interacts with HaRxL21 and requires its EAR motif in planta. (A) Co-IP of Myc:RxL21 and TPL:HA. *N. benthamiana* plants were grown in 16 hrs light/8 hrs dark. TPL:HA, TPLΔCTLH:HA (Δ25-91) and Myc:RxL21 and respective EAR motif mutant were transiently co-expressed in leaves and harvested after 48 hrs. HaRxL21 expression was induced by 30μM β-estradiol 24 hrs prior to harvesting. μMACs c-myc magnetic beads were used for immunoprecipitations (IP). HA antibody was used to detect TPL and TPLΔCTLH immunoblots (IB) and c-myc antibody was used to detect HaRxL21, HaRxL21ΔEAR and respective EAR motif mutants immunoprecipitates. (B) Co-IP of Myc:RxL21 and TPR1/3:HA. Myc:RxL21 was transiently co-expressed TPR1/3:HA and μMACs c-myc magnetic beads were used for immunoprecipitations (IP). HA antibody was used to detect TPRs immunoblots (IB), and c-myc antibody was used to detect HaRxL21. (C) Co-IP of Myc:RxL21 and MYB73:HA. In *N. benthamiana* plant leaves both proteins were co-expressed and μMACs c-myc magnetic beads were used for immunoprecipitations (IP). HA antibody was used to detect MYB73 immunoblots (IB), and c-myc antibody was used to detect HaRxL21.

3.7 Pathology impacts of protein-protein interactions

As target candidates of the *Hpa* effector HaRxL21, we speculated that interacting proteins might be involved in plant immunity against *Hpa*. To evaluate the importance of interaction targets of HaRxL21, we further determined whether knocking out interacting proteins or preventing interaction in the absence of the target protein could also prevent the effector from providing a susceptibility advantage. Col-0 *tpr1-tpl-tp4* triple knockout plants show enhanced susceptibility to the bacterial pathogen *Pst*. (Zhaohai Zhu et al., 2010) so we checked the impact of the biotrophic pathogen on TPL and TPRs triple knockout lines by spray inoculating fourteen-day old seedlings of Col-0 *tpr1-tpl-tp4* and Col-0 WT with *Hpa* isolate Noks1 and sporangiophores were counted 4 days post-infection (method described in section 2.2.6.3).

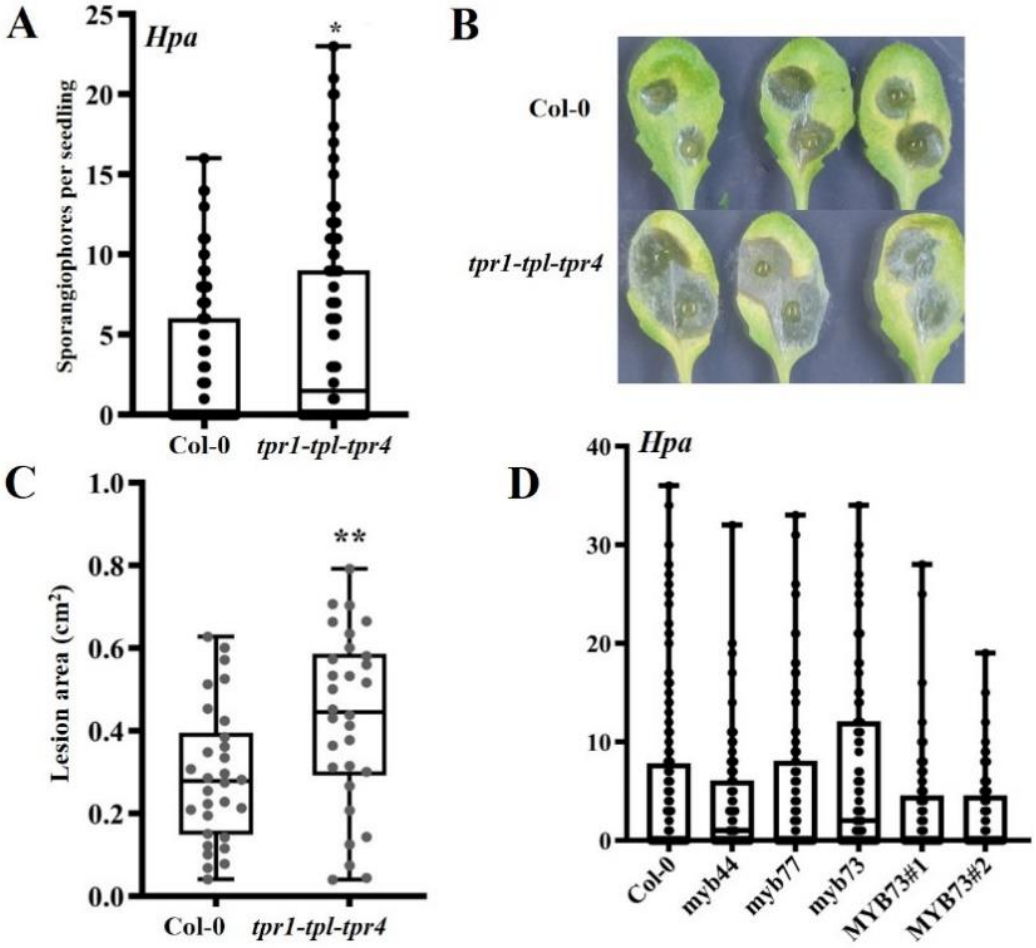

Figure 3.5 Triple k/0 lines of *tpr1-tpl-tpr4* showed susceptibility phenotype for *Hpa* and *B.C.* but MYB73 lines do not show any phenotype (A)Triple k/0 lines of *tpr1-tpl-tpr4* were infected with *Hpa* isolate Noks1 (n=96 per treatment) and compared to Col-0 WT. Asterisk indicate significant difference between treatments using non-parametric Mann-witney test $P < 0.05$. Whiskers show data range. Experiments were repeated with similar results. **(B, C)** Leaves were infected with *B. cinerea* spores and lesion area was measured at 72_hpi (n=30). Asterisks indicate significant difference between lines using welch's t test $P>0.05$. Whiskers show data range. **(D)** MYB O/E and T-DNA insertion lines of MYB TF were spray inoculated with *Hpa* spores of isolate Noks1. and compared to Col-0 WT. Letters indicate significant difference between treatments using a Kruskal-wallis and Dunn's multiple comparison test. $P < 0.05$. Whiskers show data range.

TPL has been shown to be involved in JA signaling, so we further checked the impact of necrotrophic pathogen on these lines. In the case of Botrytis, six-week-old plants leaves were drop inoculated with Botrytis spore solution and the lesion area was measured at 48 hpi. Triple knock out *tpr1-tpl-tpr4* plants showed enhance susceptibility to *Hpa* when compared to the wild type (Fig. 3.5A) and showed significantly increased lesion area (Fig. 3.5B) with the visual symptoms of sporulation (Fig. 3.5C). These results suggest that transcriptional regulation by TPL and family corepressor members is a crucial part of immune signaling against multiple pathogens.
We observed HaRxL21 and MYB73 interaction via both Y-2-H and BiFC assays, but we could not validate the interaction in Co-IP experiments (Fig. 3.4C), so to check if MYB73 not directly but indirectly associated with HaRxL21, so we tested *Hpa* susceptibility in MYB73 lines. We checked MYB73 overexpressing lines, homozygous T-DNA insertion lines and homozygous T-DNA insertion lines of its closely related MYB transcription factors like MYB77 and MYB44 for *Hpa* susceptibility. All lines were sprayed with Noks1 sporangiophores suspension and checked for disease phenotypes. None of these lines show any significant difference in susceptibility compared to control Col-0 (Fig. 3.5D). These results support our Co-IP results for MYB73 where we were unable to see the HaRxL21 and MYB73 interaction.

3.8 HaRxL21 expression in Arabidopsis plants using an estradiol-inducible system

A previous study has shown that when delivered by a bacterial translocation system, *Hpa* effector HaRxL21 causes enhanced susceptibility to host immunity (Fabro et al., 2011). Since *Hpa* is an obligate biotroph and it cannot be genetically modified to determine the involvement of a particular effector in pathogenicity, we expressed HaRxL21 in Arabidopsis. We generated transgenic lines expressing HaRxL21 and HaRxL21ΔEAR in Col-0 background under the control of an estradiol-inducible promoter (XVE) fused to a N-terminal Myc tag (Est:21#2/6/9) Est:21ΔEAR#1/2/3). We used estradiol-inducible promoter to have controlled expression of effector in plants as constitutively expressing the effector in plants throughout their lifetime may arise physiological or developmental changes in plants. To examine and distinguish the effects of HaRxL21 expression, we transformed the col-0 line with the same empty vector and used these lines as control. Homozygous lines were selected using hygromycin (as described in 2.2.13). Homozygous plants were grown in short-day conditions for 5-6 weeks, and expression of HaRxL21 was verified by qPCR 18 hrs after estradiol induction. Protein accumulation was checked by western blot (before and after estradiol-induction) (Fig. 3.6).

3.8.1 HaRxL21 expression in Arabidopsis causes enhanced susceptibility to biotroph *Hpa* EAR motif is necessary for HaRxL21 virulence function

To test whether the effector could contribute to virulence in Arabidopsis, two independent transgenic lines expressing HaRxL21 (Est:21#2/9), two independent Rx21ΔEAR (Est:21ΔEAR#1/2) lines, Est:EV (Empty vector) and WT Col-0, were tested for susceptibility

to *Hpa* isolate Noks1. After estradiol induction, Est:21 lines showed enhanced susceptibility to *Hpa* in both lines compared to Col-0, EV and Est:21ΔEAR (Mock and Estradiol) measured by the number of sporangiophores per seedling at 4 dpi (Fig. 3.7A). In contrast, Est:21ΔEAR lines did not show enhanced susceptibility to *Hpa*, which confirm the requirement of the EAR motif for virulence.

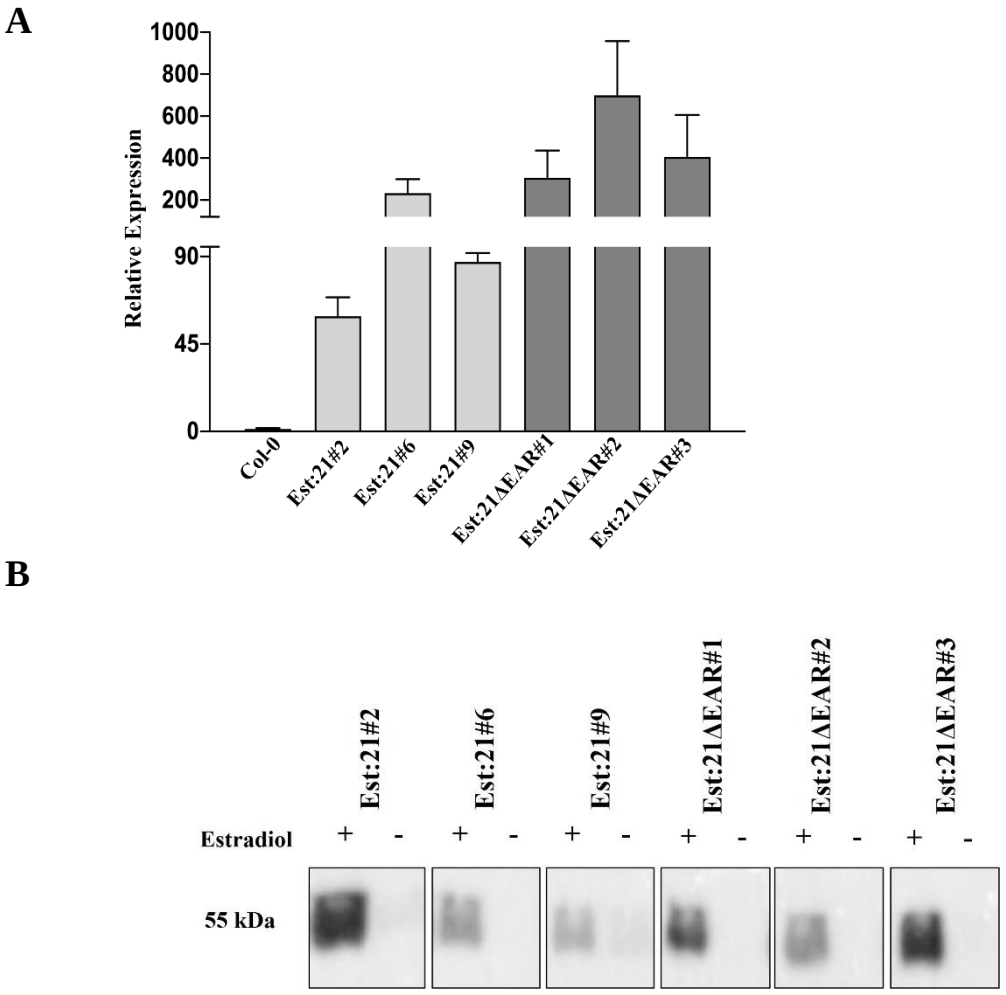

Figure 3.6 HaRxL21 expression in HaRxL21 and HaRxL21ΔEAR estradiol inducible lines. (A) Relative expression of HaRxL21 in Arabidopsis plants expressing Myc:RxL21 and Myc:RxL21ΔEAR (under an estradiol (Est) inducible promoter) was determined by qRT-PCR 18 h after induction with 30 µM estradiol. The expression level was normalized to Arabidopsis *tubulin 4*. Error bars show standard error between 3 biological replicates. (B) Anti Myc immunoblot showing Est-inducible expression of HaRxL21 and HaRxL21ΔEAR in Arabidopsis lines. Samples were collected 18 h after induction with 30 µM estradiol.

3.8.2 HaRxL21 expression in Arabidopsis causes enhanced susceptibility to necrotroph Botrytis and EAR motif is necessary for HaRxL21 virulence function

We confirmed HaRxL21 interaction with TPL/TPRs (TPR1, TPR3) and TPL proteins function as negative regulators of jasmonate responses (Pauwels et al., 2010). We also observed that *tpr1-tpl-tpr4* knockout lines showed enhanced susceptibility for necrotrophic

pathogen Botrytis, so we further tested whether HaRxL21 expression has any impact on necrotrophic pathogen Botrytis. To verify HaRxL21 impact and whether the EAR motif was also important for providing a susceptibility advantage to necrotrophic pathogens, transgenic lines Est:21#2/9, Est:21ΔEAR#1/2, Est: EV and Col-0 were tested for *B. cinerea* growth promotion. Six-week-old *A. thaliana* leaves were drop inoculated with a suspension of *B. cinerea* spores. HaRxL21 expression resulted in increased lesion size caused by *B. cinerea* infection compared to control lines (Fig. 3.7B). Enhanced sporulation was also clearly visible on leaves from the Est:21#2/9 lines compared to Est:21ΔEAR#1/2, Est: EV and the wildtype (Col-0) control line (Fig. 3.7C). These data demonstrate that the presence of the effector conferred enhanced susceptibility to both biotrophic and necrotrophic pathogens. Moreover, those results indicate that HaRxL21 expression shortly before the infection is sufficient to enhance host susceptibility. In contrast, HaRxL21ΔEAR did not show enhanced susceptibility to either pathogen, confirming that the EAR motif of HaRxL21 is necessary for enhanced susceptibility to pathogens and this susceptibility advantage is most likely due to the interaction with TPL/TPRs.

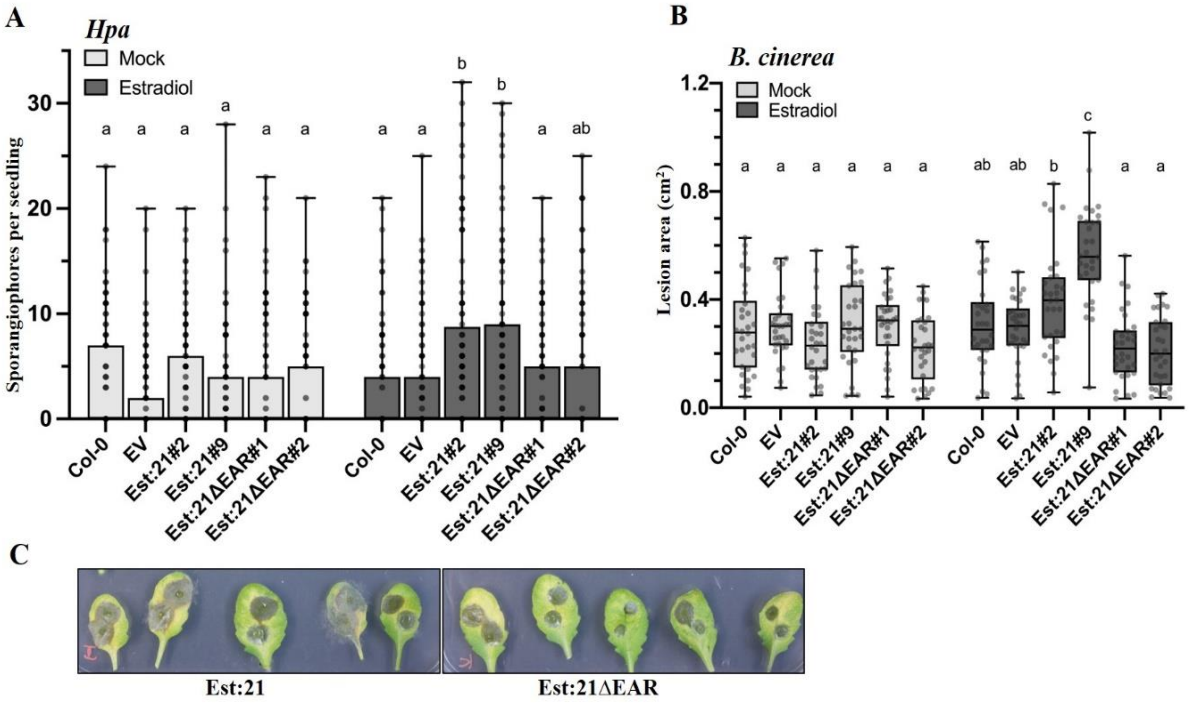

Figure 3.7 HaRxL21 expression causes enhanced susceptibility to biotrophic pathogen and EAR motif is necessary for HaRxL21 virulence function *in planta*. (A) Transgenic lines expressing HaRxL21 under control of a XMV promoter and N-terminal fused Myc tag (Est:21#2/9 and Est:21ΔEAR#1/2) were compared to Col-0 WT and Est:EV (Col-0 background) controls. Lines were infected with *Hpa* isolate Noks1 18 hours after induction with 30 µM β-estradiol or mock treatment (n=100 per treatment). Letters indicate significant difference between treatments using a 2-way ANOVA and Bonferroni's multiple comparison test. $P < 0.05$. Whiskers show data range. Experiments were repeated with similar results. (B, C) Leaves were infected with *B. cinerea* spores, 18 hours after induction with 30 µM β-estradiol or mock treatment and the lesion area was measured at 72 hpi (n=30). Letters indicate a significant difference between treatments using a 2-way ANOVA and Bonferroni's multiple comparison test. $P < 0.05$. Whiskers show data range.

3.9 RNA sequencing of HaRxL21 expressing Arabidopsis lines

We observed that HaRxL21 expression in Arabidopsis leads to susceptibility against biotrophic and necrotrophic pathogens. A comprehensive comparison of transcriptome changes due to HaRxL21 expression might give us an insight into molecular mechanism used by the effector to suppress plant immunity. We applied RNA-seq, a powerful tool to analyse transcriptome changes, to monitor the differential response of Arabidopsis seedlings expressing HA:21 and HA:21ΔEAR lines. We selected a comparison between HaRxL21 and HaRxL21ΔEAR to specifically determine whether HaRxL21 interaction with TPL/TPRs due to EAR motif is driving alterations in the host transcriptome and also to eliminate transcriptional effects due to the presence of any other domains of the effector. Arabidopsis seedlings were grown on MS plates, transferred to liquid MS media either mock-treated or 100 nm flg22 treated before harvesting pooled seedlings after 2 hours. In RNA seq 676,059,072 reads of 75 bp paired-end were generated from in total 24 samples (Harvey et al., 2020). Transcript abundance was calculated by using pseudoalignment of reads to AtRTD2 (Arabidopsis reference transcript dataset) using Kallisto. Genes with low expression were removed from the dataset and TMM normalization was performed (Robinson & Oshlack, 2010).

3.9.1 HaRxL21 expression does not interrupt PTI response.

After 2 hours of treatment, flg22 caused dramatic transcriptome changes in HaRxL21 expressing lines. A large number of genes were upregulated and down regulated in both HA:21 and HA:21ΔEAR lines after Flg22 treatment. 2275 genes were up regulated in HaRxL21, and 1023 down regulated whereas 2435 genes were upregulated, and 778 genes were down regulated in HA:21ΔEAR upon Flg22 treatment (Fig. 3.8A). However, the scale of transcriptional reprogramming in both the HA:21 and HA:21ΔEAR lines in response to flg22 treatment is similar (Fig. 3.8B), and indeed the vast majority of the flg22-induced DEGs (2681 genes) are conserved across the two genotypes (with the direction of differential expression also conserved: 2090 upregulated and 591 downregulated). When we compared the DEGs induced upon flg22 treatment in HA:21 and HA:21ΔEAR lines (as compared to Mock) with the DEGs in Col-0 at 120 minutes post flg22 treatment as compared to water treatment from Rallapalli et al. (2014), we observed a strong positive correlation (Harvey et al., 2020); (Pearson correlation coefficients of 0.841 and 0.844 respectively). This comparison indicates that the majority of the flg22 induced PTI response in HA:21 lines is intact, and that the pathogen phenotype caused by *in planta* expression of HaRxL21 but not HaRxL21ΔEAR is therefore not due to large scale transcriptional changes during induction of PTI or the failure of these plants to induce PTI.

3.9.2 HaRxL21 causes differential expression of defense-related genes

To identify genes causing the susceptibility phenotype (Fig. 3.7) between full length and HaRxL21ΔEAR, we next looked at genes that were differentially expressed between both genotypes when mock-treated or when both were induced with flg22. There were only 417 genes differentially expressed between HaRxL21 and HaRxL21ΔEAR lines under mock treatment, whereas after flg22 treatment total of 240 DEGs were identified. Among these,

113 genes were differentially expressed in both comparisons, all of which responded in the same direction (Fig. 3.8C). We further analysed the DEGs in two groups: the subset of DEGs between HaRxL21 and HaRxL21ΔEAR under mock conditions (417 genes, 316 up and 101 down) and DEGs specific to flg22 treatment when HaRxL21 compared to HaRxL21ΔEAR (127; 68 up and 59 down). We further looked if the differentially expressed genes of HaRxL21 are important for the plant defense responses. Eighty-two genes that were misregulated by the effector, either before or after flg22 treatment were also differentially expressed in response to flg22 in WT col-0 plants (Rallapalli et al., 2014). In this comparison, we observed fifteen genes that were induced post flg22 treatment in WT Arabidopsis but were downregulated by the full-length effector compared to control after flg22 induction (including AVRPPHB SUSCEPTIBLE 3 (PBS3), CRKs (24,38) and MYB85).

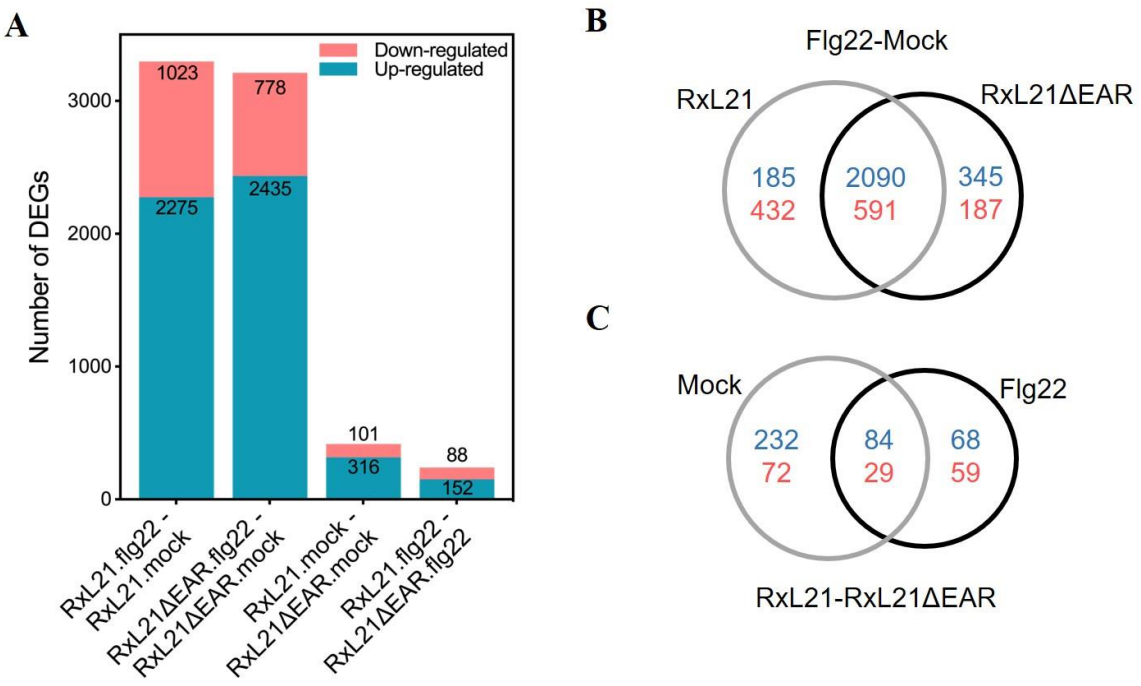

Figure 3.8 Differentially expressed genes in HaRxL21 compared to HaRxL21ΔEAR transgenic lines. RNAseq was performed on HA-tagged RxL21 and RxL21ΔEAR-expressing transgenic lines. (A) The number of up- and down-regulated genes among differentially expressed genes under mock and flg22 treatment. DEGs were defined as having a $\log_2$ fold change ≥ 1 or ≤ 1, and a BH adjusted p-value of < 0.05. (B) Venn diagram shows differentially expressed genes in HaRxL21 and HaRxL21ΔEAR lines between mock and flg22 treatment with an overlap of 2090 upregulated and 591 down regulated genes. (C) Venn diagram shows differentially expressed genes between HaRxL21 and HaRxL21ΔEAR after mock and flg22 treatment with an overlap of 84 upregulated and 29 down regulated genes.

Since HaRxL21 expression showed enhanced susceptibility against both a biotrophic and a nectrophic pathogen, we looked whether HaRxL21 DEGs were also differentially expressed during infection by a biotrophic pathogen *Pst*; (Lewis et al., 2015) and a necrotrophic pathogen *B. cinerea*; (Windram et al., 2012) (Harvey et al., 2020).The *Pst* data set profiles the gene expression in both virulent strain *Pst* DC3000, which use the Type III secretion system to secret effector proteins directly into plant cells as well as a non-pathogenic mutant

DC3000HrpA- which lacks the Type III secretion system. Hence, we can distinguish between suppression of PTI due to host response and the activities of pathogen effectors to manipulate host gene expression. 213 genes that were differentially expressed in response to HaRxL21 are also differentially expressed during *Pst* infection (DC3000 and/or DC3000HrpA compared to mock) (Lewis et al., 2015), whereas 142 genes are differentially expressed during *B. cinerea* infection (Windram et al., 2012). Twenty defense related genes that were suppressed by *Pst* effectors are also regulated in a similar manner by HaRxL21 (i.e. both *Pst* effectors and HaRxL21 downregulated or upregulated expression compared to respective controls). Moreover, 18 genes that were specifically expressed in response to *Pst* effectors and are not part of the host PTI response are also differentially expressed in response to HaRxL21. These data indicate that a significant proportion of the genes whose expression is perturbed by the HaRxL21 effector are associated with plant immunity. In summary, the *Pst* effectors and the *Hpa* effector HaRxL21 seem to share similar strategies of host manipulation.

3.9.3 GO analysis

Singular Enrichment Analysis was performed by agriGO (T. Tian et al., 2017) to identify the enrichment of Gene Ontology (GO) terms. We looked for GO terms of DEGs under MOCK condition and DEGS under only flg22 treatment. There were 101 transcripts downregulated in HaRxL21 compared to HaRxL21ΔEAR (mock). For these downregulated genes, we observed an enrichment of the GO terms: response to stimulus, biotic stimulus and response to other organisms. In case of upregulated transcripts by HaRxL21, we found GO terms defense response and extracellular region, whereas GO terms involved in secondary metabolism were over-represented in both up and down regulated genes.

3.10 Validation of DEGs by q-PCR-

Considering that HaRxL21 doesn't influence PTI after flg22 treatment via transcriptional reprogramming, we focused on transcripts that were influenced by HaRxL21 compared to HaRxL21ΔEAR under MOCK condition. To confirm the RNA-Seq results, we selected several DEGs to quantify expression via q-PCR in the independent lines expressing Est:21, Est:21ΔEAR and wildtype (Col-0) control. Eight of the downregulated genes (HaRxL21 compared to HaRxL21ΔEAR) were selected based on their putative functions in plant immunity or growth regulation; these genes code for two TFs (WRKY63 and NAC019), three receptor-like proteins (RLP 6,20,23), two calmodulins and a protein of unknown function (Table S8.4). Seven out of these eight genes were significantly downregulated in lines expressing Est:21 compared to Est:21ΔEAR lines (Fig. 3.10), whereas six of these genes also showed significantly reduced expression in Est:21 lines as compared to wild-type Col-0. However, NAC019 did not show difference between Col-0 and Est:21 but was downregulated in Est:21 compared to Est:21ΔEAR, whereas WRKY63 showed an intermediate expression level in Est:21ΔEAR between Est:21 and Col-0. In general, the qPCR results showed a similar pattern of log2 fold change for the selected eight genes between HaRxL21 and HaRxL21ΔEAR lines compared to RNA-seq results (Fig. 3.11). Thus,

the consistency of the qRT-PCR data with the RNA sequencing data indicates the reliability of our RNA-Seq results.

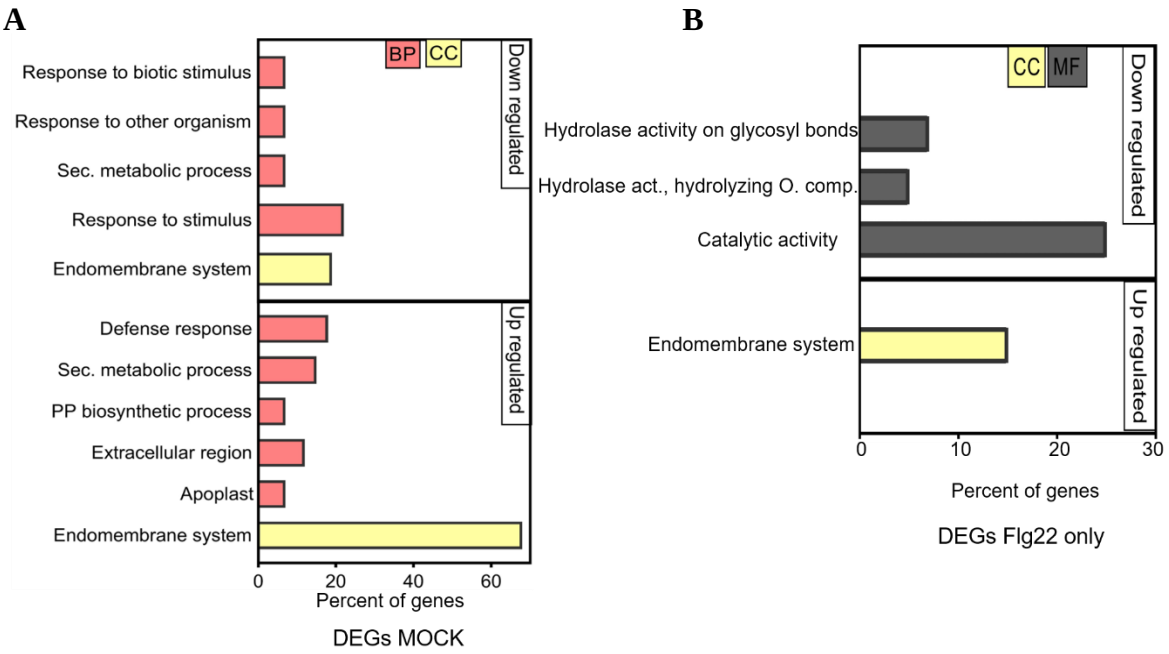

Figure 3.9 GO-term analysis for biological process, molecular function and cellular component of genes differentially expressed in Arabidopsis lines expressing HaRxL21and HaRxL21ΔEAR. (**A**) Go-term analysis for the up/down-regulated genes in lines expressing HaRxL21 compared to HaRxL21ΔEAR in mock condition (**B**) GO analysis for the up/down-regulated genes in HaRxL21 expressing lines compare to HaRxL21ΔEAR only upon Flg22 treatment. The percentage of genes were calculated for each category related to the total number of genes. All GO categories showed P values < 0.05.

3.11 HaRxL21-repressed genes are enriched for TPR1 binding targets

Since HaRxL21 has been shown to interact with TPL/TPRs (TPR1, TPR3) corepressors, we hypothesised that HaRxL21 modulates host gene expression using these interactions. HaRxL21 expression *in planta* can influence transcriptional programming, possibly by two approaches. One, HaRxL21 could manipulate the repression process via recruitment of these corepressor proteins to new sites on the genome for repression (by either binding to the DNA itself or to TFs). Second, HaRxL21 might sustain TPL/TPRs mediated repression when it would typically be diminished during infection. Using iDNA-Prot (Lin, Fang, Xiao, & Chou, 2011), we found no evidence to suggest that HaRxL21 contains a DNA binding motif. In the motif analysis, promoters of DEGs under mock condition shows significant enrichment for WRKY TF binding motifs in both up and down-regulated genes under mock conditions (Harvey et al., 2020). In the DEGs only evident upon flg22 treatment, we observed significant enrichment for CAMTA (Calmodulin-binding transcription activator) binding motifs in the genes downregulated and MYB TF binding motifs and a WRKY binding motif in the upregulated genes by HaRxL21. These results suggest that HaRxL21 may exert at least some of its effects via modulating TPL/TPR interaction with MYB, WRKY and CAMTA TFs.

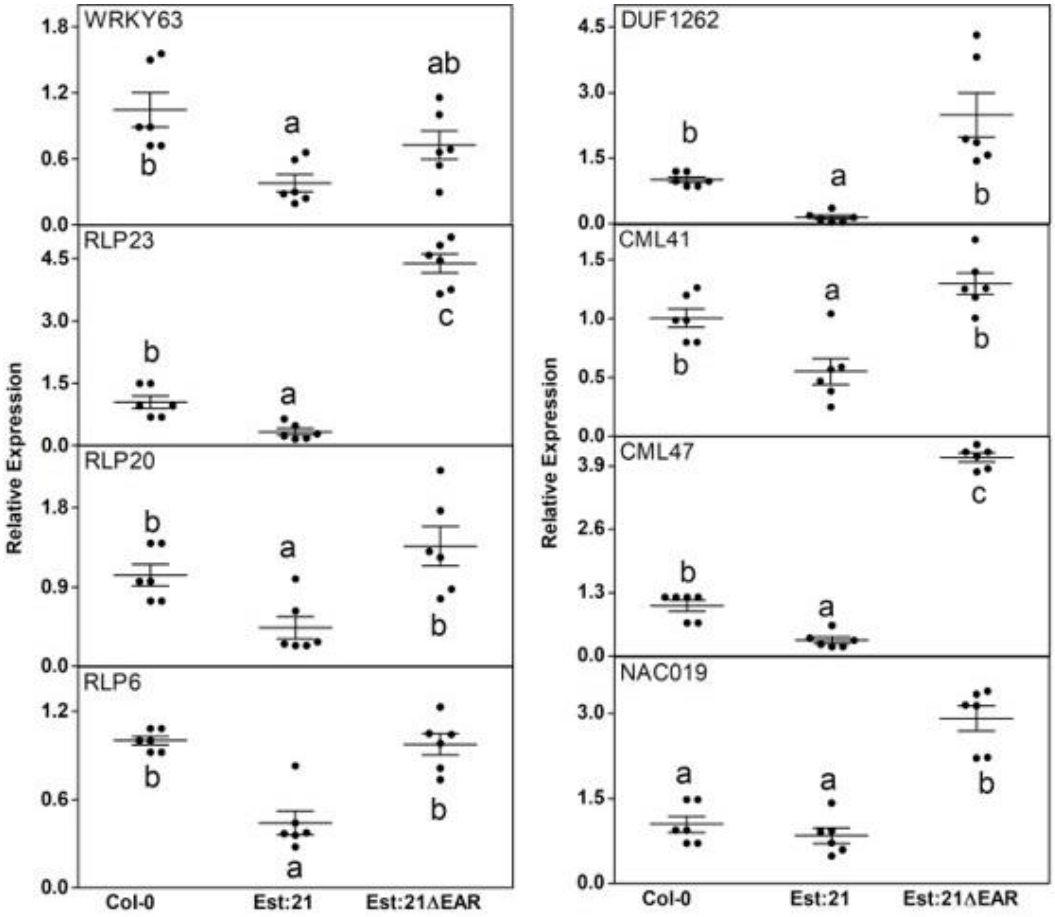

Figure 3.10 RNAseq validation differentially expressed genes in HaRxL21 compared to HaRxL21ΔEAR transgenic lines. Eight genes were selected for validation in estradiol (Est) inducible HaRxL21 and HaRxL21ΔEAR expressing lines and Col-0 WT. We treated Arabidopsis seedlings with 30 μM of β-estradiol for 18 hours. For Est:21 and Est:21ΔEAR, data were obtained from 2 independent lines each with three biological replicates and expression was normalised to Arabidopsis tubulin gene. Black circles are individual data points and bars denote the mean ± SE of target gene expression. Letters indicate significant differences ($P < 0.05$) (One-way ANOVA with Tukey's honest significance difference).

Next, we wanted to understand why HaRxL21 interaction with TPL is responsible for changes in gene expression. To address this, we further compared the DEGs derived from HaRxL21-RNAseq analysis with the TPR1 target/bound genes derived from ChIP-seq data of *pTPR1*:*TPR1*:*GFP* col-0 lines (Griebel et al., 2020). These plants display an autoimmune phenotype and show constitutive activation of defense responses (Zhaohai Zhu et al., 2010). They can therefore inform us about TPR1 binding to chromatin during the defense response. In this comparison, we observed that genes repressed by HaRxL21 compared to HaRxL21ΔEAR (both under mock and flg22 treatment) showed enrichment for the TPR1 targets with binding observed immediately upstream of the transcription start site (TSS) (Fig. 3.12A, purple and orange lines). However, HaRxL21-repressed DEGs showed lower TPR1 ChIP signal level when compared to the group of all genes defined as TPR1 targets in the ChIP-seq data.

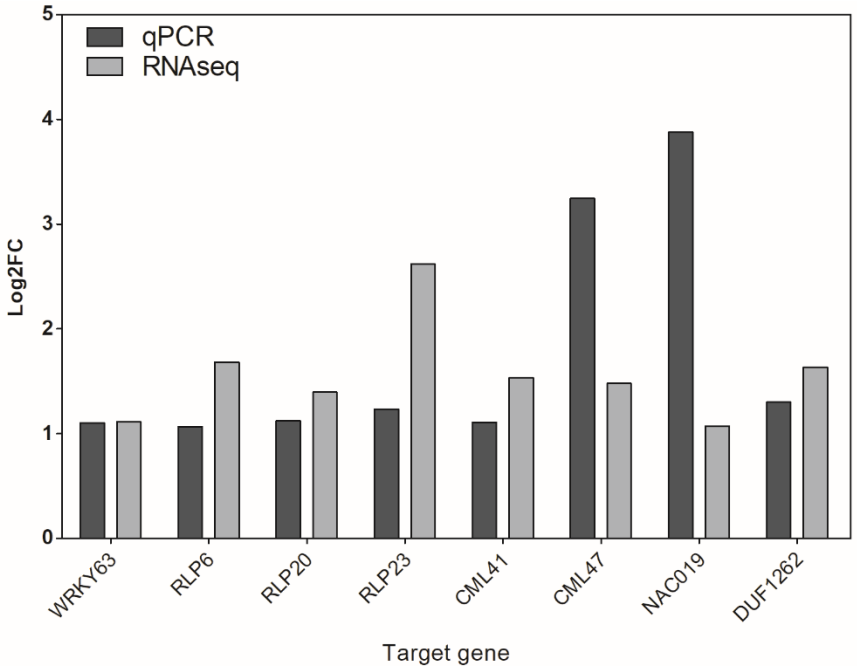

Figure 3.11 Comparison of RNA-seq expression data and qPCR data. Comparison of $\log_2$ fold change ($\log_2$FC) for the 8 selected downregulated genes between HaRxL21 and HaRxL21ΔEAR lines by qPCR compared to RNA-seq read counts. Mean fold change of three biological replicates is shown.

This analysis indicates that HaRxL21 impairs the expression of at least some of its DEGs, possibly by the interaction with TPR1 upstream of the TSS as in HaRxL21 repressed genes (compared to HaRxL21ΔEAR in mock or after flg22 induced condition) 20 genes were identified as TPR1 targets. Among these genes, several are known positive regulators of immunity against biotrophic pathogens, like *PBS3*, *ICS1* and *RLP20*. Some of them, like *WRKY46*, *WRKY63*, *NAC019*, function in abiotic stress tolerance (Table S8.5). The WRKY binding motifs were enriched in the HaRxL21 DEGs promoters, and WRKYs (46 and 63) are also identified as possible direct targets of HaRxL21 suppression via the TPR1 corepressor. These results suggest that these two TFs could be one key mechanism underlying HaRxL21-induced pathogen susceptibility. However, two enzymes (ICS1 and PBS3), which are required for SA accumulation, are also likely to be targeted by HaRxL21/TPR1 mediated repression. So, it appears that HaRxL21 might be involved in mis-regulation of salicylic acid (SA) signaling as well.

Genes that were not responsive to the effector expression or did not respond to the effector (genes that showed no difference between HaRxL21 and HaRxL21ΔEAR) were used as control (HaRxL21 control in Fig. 3.12A; Table S8.6) and these genes do not show any enrichment for TPR1 binding. However, interestingly, HaRxL21 upregulated genes (compared to HaRxL21ΔEAR in mock or after flg22 treatment; Fig 3.12A, yellow and brown lines) showed depletion of TPR1 binding around the TSS. These results suggest that promoters of genes induced by HaRxL21 are not TPR1 targets and mis-regulation of these genes by the effector is likely to be an indirect effect rather than through mis-placing bound TPR1.

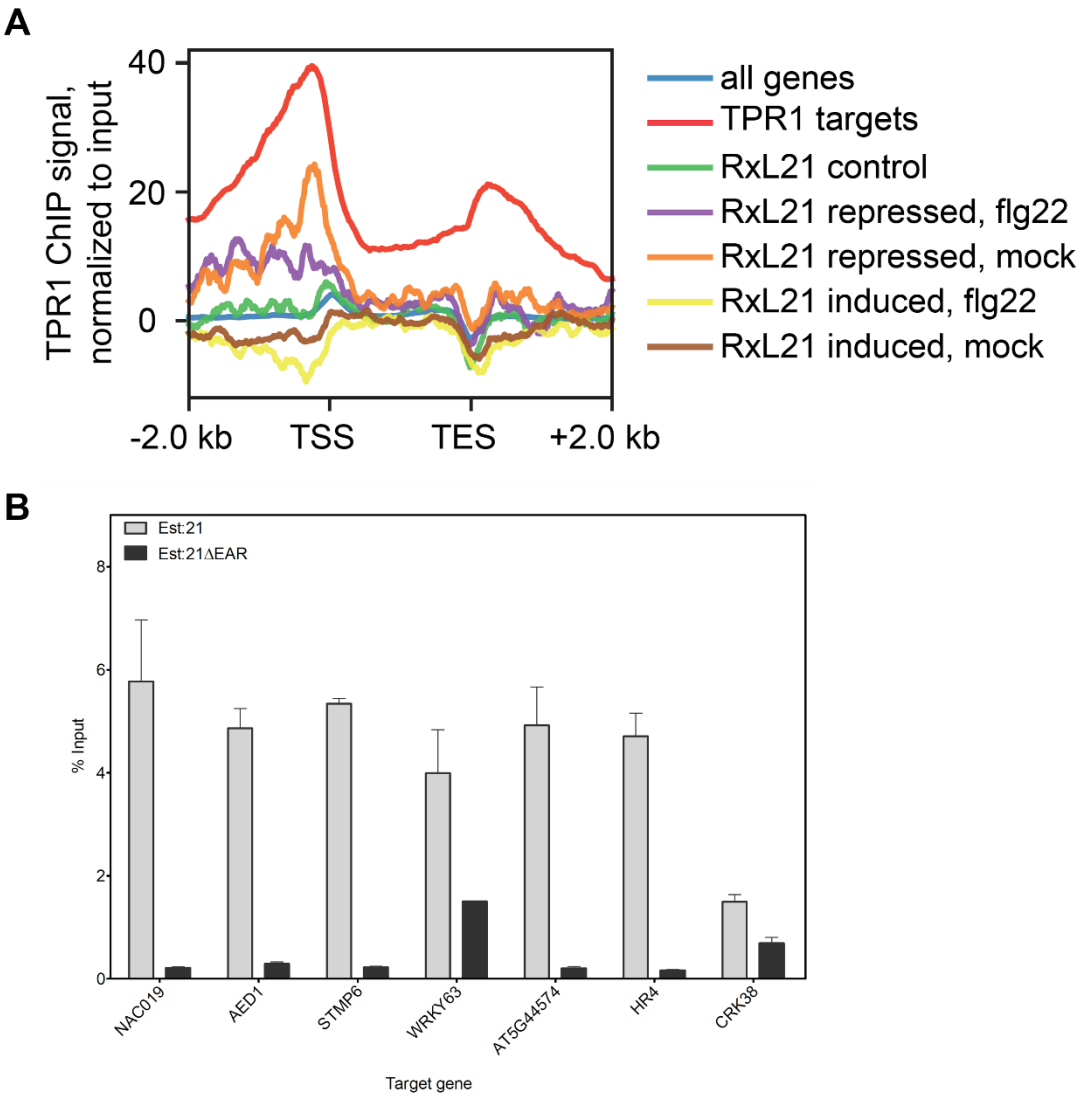

Figure 3.12 HaRxL21 repressed genes are overrepresented in genes that are bound by TPR1. (A) Arabidopsis genes with repressed expression in HA:RxL21 lines compared to HA:21ΔEAR lines show weak TPR1 binding. Metaplots display the TPR1 enrichment around the transcription start site (TSS) on genes regulated by HaRxL21 (BH adjusted p-value < 0.05 and log2-fold-change ≥1, with or without flg22) or control genes without evidence for HaRxL21 dependent expression regulation (HaRxL21 control). TPR1 bound genes defined in Griebel et al. (2020) were used as a positive control (red line). On the y-axis is mean read count for the TRP1-GFP ChIP samples normalized to the input samples. TPR1 ChIP and input samples were scaled based on the number of mapped reads. TES = transcription end site. (B) ChIP-qPCR of HaRxL21-repressed genes. Two-week old seedlings, overexpressing HaRxL21 or HaRxL21ΔEAR with an N-terminal myc tag and under the control of an estradiol inducible promoter, were treated with 30 µM β-estradiol to induce HaRxL21 expression 18 hrs before harvesting and cross-linking with 1% formaldehyde. ChIP assays were performed with an anti-Myc antibody. In the ChIP–qPCR, the enrichment of immunoprecipitated DNA was normalized by the percent input method (signals obtained from ChIP samples were divided by signals obtained from an input sample). Error bar represents ± SE of four technical repeats. Arabidopsis *Actin 2* was included as a control, but no amplification was detected after 40 cycles. The experiment was repeated with similar results.

Next, we performed independent ChIP-qPCR in the Est:21 and Est:21ΔEAR expressing lines for the confirmation of the observed overlap of HaRxL21 repressed genes with TPR1 binding sites. Total seven genes (labelled bold in Table S8.5) were selected for ChIP-qPCR. Promoter regions encompassing 500 bp upstream and 500 bp downstream of the TSS for five of these genes (*NAC019*, *AED1*, *STMP6*, *AT5G44574* and *HR4*) showed approximately 5-fold enrichment in HaRxL21 immunoprecipitated samples compared to HaRxL21ΔEAR samples (Fig. 3.12B). Whereas, in the case of the promoter regions of *CRK38* and *WRKY63*, no strong enrichment was observed however, in our RNA seq. data set, *CRK38* was repressed by HaRxL21 only after flg22 induction. *AtActin2* was also amplified as negative control which was undetermined in ChIP samples after 40 qPCR cycles. Therefore, HaRxL21 and TPR1 both can be directly bound to the Promoter regions of these enriched five genes, but HaRxL21ΔEAR cannot bind. Furthermore, HaRxL21ΔEAR samples showed a lack of enrichment in the ChIP-qPCR assays; this indicates that HaRxL21 binds to these promoter regions through TPR1 and maintain TPR1 mediated repression when it would normally be relieved instead of recruiting TPR1 to new repression locations.

4 Discussion

4.1 HaRxL21 interacts with TPL repressor and mimics a host gene regulation mechanism

For successful colonization plant, pathogens use a subset of their effector repertoire to manipulate the host transcription. This Ph.D. project aimed to investigate the role of the *Hpa* effector HaRxL21 in the suppression of the plant immune system of *A. thaliana*. In initial studies, HaRxL21 was shown to confer *Hpa/Pst* susceptibility (Fabro et al., 2011) and has been shown to interact with TCP14, OBE1, SWAP, and TPL (Mukhtar et al., 2011). To understand the role of HaRxL21 in host susceptibility and how does it contribute to pathogen colonization, we first performed Y-2-H test to check the interaction partner of the effector. In this screen, TPL, TPR2, TPR3, TCP13, TCP14, and MYB73 were identified (Fig. 3.2). The TCP TF (13, 14, and 19) are targeted by *Pst* and *Hpa* effectors and the single mutants of *tcp13*, *tcp14* and *tcp19* exhibit enhanced disease susceptibility to 2 different avirulent *Hpa* isolates (Emwa1 and Emoy2) (S. Li, 2015). So, these TF could be interesting targets for further study. The MYB73 TF belongs R2R3 gene family, and its loss-of-function mutant showed increased susceptibility to *Bipolaris oryzae* (Jiao et al., 2011). Other HaRxL21-interacting proteins, TPL/TPRs, play a role in defense, and TPL-mediated transcriptional repression is involved in a variety of processes in *A. thaliana*, and its mechanism is conserved throughout the plant kingdom (Causier, Lloyd, et al., 2012). In response to multiple stresses, the transcriptional regulation is regulated by chromatin remodeling, particularly the role of histone deacetylases which deacetylate chromatin and therefore repress gene expression (L.-T. Chen & Wu, 2010; van den Burg & Takken, 2009). TPL-mediated transcriptional repression mechanism is also linked to chromatin remodeling (Kagale & Rozwadowski, 2011), and TPL has been shown to interact with HDA19 (Krogan, Hogan, & Long, 2012).

4.2 MYB73 and TPL interaction re-localize HaRxL21 into sub-nuclear foci

We further validated Y-2-H results using BiFC for MYB73 and TPL. HaRxL21 shows interaction with MYB73, and this interaction was independent of EAR motif (Fig. 3.3B). TPL-HaRxL21 was also confirmed by BiFC, and here the interaction was dependent on the CTLH domain of TPL (Fig. 3.3A). TCP14 interacts with HaRxL21 and shifts it into subnuclear foci (Weßling et al., 2014), so we further looked at whether HaRxL21 localization is changed due to this interaction. HaRxL21 is localized to the cytoplasm and the nucleus (Fig. 3.2C). When the effector was co-expressed with MYB73 and TPL, we observed that HaRxL21 co-localized into sub-nuclear foci (Fig. 3.2C). These results indicate that the effector interacts host targets which is consistent with earlier findings, where HaRxL21 and TCP14 re-localized to sub-nuclear foci (Weßling et al., 2014). Although MYB73 showed an interaction with HaRxL21 in Y-2-H and BiFC assays (Fig. 3.2, 3.3), we could not verify this interaction by Co-IP (Fig. 3.4C). Also, the MYB73 O/E lines, its T-DNA insertion lines, and its closely related MYB family members, did not show any phenotype when sprayed with the

Hpa isolate Noks1, which supports our Co-IP results. It might be possible that MYB73 does not interact with HaRxL21 *in planta*. We also checked the *Hpa* and Botrytis phenotype in the Arabidopsis plants lacking proteins of TPL, TPR1 and TPR4. Here, we observed enhanced susceptibility to both pathogens (Fig. 3.2), which supports earlier findings by (Zhaohai Zhu et al., 2010).

Hormone signaling pathways use robust mechanisms of transcriptional regulation and this mechanism employs a co-repressor protein that is degraded by phytohormones. This co-repressor protein then interacts with a protein carrying an EAR motif, recruiting TPL (Pérez & Goossens, 2013; Thines et al., 2007; Weigel et al., 2005). However, the EAR domain remains a constant feature in all described complexes but sometimes only one protein is required, like in the circadian transcription, where TPL interacts with PSEUDO RESPONSE REGULATOR (PRR) proteins that contain both an EAR motif and a DNA binding domain which directly targets the promoter region of the target gene (L. Wang, Kim, & Somers, 2013). Similar to this mechanism, the effector HaRxL21 also contains an EAR motif at the C-terminus to interact with TPL. In our study, we confirmed that TPL requires the CTLH domain to interact with HaRxL21 via the EAR motif (Fig. 3.3A and Fig. 3.4A). Remarkably, the effector HaRxL21 also interacts with TPR1 and TPR3 (Fig. 3.4B). The EAR-motif mediated interaction of TPL is well characterised in the literature, where TPL interacts with Aux/IAA and JAZ proteins (via NINJA), and this complex prevents the transcription of IAA or JA responsive genes (Pauwels et al., 2010; Robert-Seilaniantz et al., 2011). It is likely that HaRxL21 mimics the action of plant EAR motif-containing repressor proteins and recruits TPL to sites of transcriptional initiation to bring about repression through histone deacetylation.

4.3 EAR domain is required by the effector for host susceptibility

EAR motif-containing proteins play key roles in plant development and growth regulation by interacting with co-suppressors, like TPL and SAP18 (Causier, Lloyd, et al., 2012; Hill et al., 2008; Song & Galbraith, 2006). We confirmed that the EAR motif of HaRxL21 is required to interact with TPL and deletion/mutation in EAR motif abolish this interaction (Fig. 3.4). Among other EAR motif-containing RxL effectors of *Hpa* only HaRxL21 was identified to interact with TPL in a Y-2-H screen by Mukhtar et al. (2011). It might be due to the possibility that HaRxL21 is the only effector to contain an EAR motif at the absolute C-terminus and only one amino acid between the EAR motif and the stop codon. This could be the reason that none of the other RxLs tested were found to interact with TPL using Y-2-H. To validate the contribution of HaRxL21 in plant susceptibility against pathogens we generated HaRxL21 and HaRxL21ΔEAR expressing lines under the control of an estradiol inducible promoter. HaRxL21 expressing lines do not show any phenotypical and developmental changes compared to the control lines. The results from three independent biological experiments (Fig. 3.3) clearly show that expression of HaRxL21 in *A. thaliana* results in strongly enhanced *Hpa* susceptibility, whereas HaRxL21ΔEAR did not show any difference compared to control lines. Apart from *Hpa* we also tested if the effector HaRxL21 is also involved in mediating susceptibility to pathogens that follow a different lifestyle. To

elucidate this, HaRxL21 expressing lines were inoculated with the necrotrophic pathogen *B. cinerea*. Similar to *Hpa*, HaRxL21 expression results in higher susceptibility compared to control and HaRxL21ΔEAR-expressing lines (Fig. 3.4), indicating that HaRxL21 also supports the growth of necrotrophic pathogens. TPL and its closest family member TPR1 and TPR4 loss of function mutant show enhanced susceptibility to *Pst* (Zhaohai Zhu et al., 2010); here, we reveal that the TPL triple mutant also exhibits enhanced susceptibility to *Hpa* and *B. cinerea*. Taken together, our data confirm the pivotal role of TPL and TPRs at the core of plant immunity and highlight that they are a key target for pathogen effector proteins. It is, therefore, possible that HaRxL21 hijacks the host machinery and recruits TPL/TPRs into a complex to repress transcription of defense related genes, therefore promoting the growth of pathogens. There are a few examples of other EAR motifs containing effectors, such as the bacterial XopD effector family (J. G. KIM, Taylor, & Mudgett, 2011) and an effector from fungi that contains an EAR motif and interacts with TPR4 (Petre et al., 2015).

4.4 HaRxL21 expression in Arabidopsis alters a subset of the host defense related genes

To investigate the transcriptional effects caused by *in planta* HaRxL21 expression as compared to HaRxL21ΔEAR (effector lacking the EAR motif), we performed RNA-seq in the seedlings under mock and 2 hrs flg22 treatment to induce PTI. Our results showed that there is no large-scale change in gene expression in response to the presence of the effector under mock conditions. In the case of the flg22 induction, broad range genes were up/down regulated in both HA:21 and HA:21ΔEAR expressing lines, but the vast majority of the flg22-induced DEGs are conserved across the two genotypes. So, from RNA-seq analysis, we concluded that effector expression did not cause huge transcriptome reprogramming but still delivers a strikingly enhanced susceptibility to the host plant. A similar situation occurred with *Pst*, where blocking delivery of effectors through the Type III Secretion System only 872 PTI-regulated genes expression was perturbed, and the majority (5350) did not show any amplification or a change in expression (Lewis et al., 2015). These findings indicate that pathogen effectors possibly target specific components of the plant defense response to cause a massive effect on the plant immune system.

When we compared the DEGs between HaRxL21 and HaRxL21ΔEAR we observed mis-regulation of several important components of plant defense responses. Among these several receptor-like protein (RLP) genes were down regulated like; *RLP6*, *RLP22*, *RLP23* and *RLP35*, with *RLP20* specifically after flg22 treatment. RLPs play roles in plant innate immunity, stress response and many development processes. As a part of the second-largest family of Arabidopsis leucine-rich repeat-containing receptors, several RLP members function as PAMP receptors and RLPs also play a critical role in pathogen recognition (Jamieson et al., 2018). RLPs have been shown to involved in fungal and oomycete resistance (Albert et al., 2015; Jiang et al., 2013; Ramonell et al., 2005; Shen & Diener, 2013; Weiguo Zhang et al., 2013). Consistent with this, increased expression was observed for many *RLP* genes like *RLP6, 22, 23* and *35* during pathogen infection and/or hormone treatment (Jamieson et al., 2018; Wu et al., 2016). Apart from RLPs, there are several other genes downregulated by HaRxL21 and are known components of the defense response. For example, Calmodulin-like *CML41* is upregulated by flg22 and regulates flg22-induced

stomatal closure by facilitating rapid callose deposition at plasmodesmata (B. Xu et al., 2017). NAC019 play a role in JA mediated defense signaling as the *anac019 anac055* double mutant showed increased resistance to *B. cinerea* and decreased expression of MeJA-induced *VSP1* and *LOX2* (Bu et al., 2008).
Another gene downregulated by HaRxL21, the *accelerated cell death 6* (*ACD6*) has impact on resistance against biotrophic pathogens (Todesco et al., 2010), whereas the lipid transfer protein AZI3 (Chassot, Nawrath, & Métraux, 2007) contribute to defense against necrotrophic pathogen. The disease resistance protein (TIR-NBS-LRR class) family member AT3G04220 is targeted by miR825 target (Nie et al., 2019). We observed downregulation of few Cysteine-rich receptor-like kinase (CRK 6,13,39) by HaRxL21. *CRK13* leads to hypersensitive response-associated cell death and induces defense against pathogens by causing increased accumulation of salicylic acid (Acharya et al., 2007), whereas the lectin receptor kinase LecRK4.1 positively regulates Arabidopsis PTI and resistance against both biotrophic and necrotrophic pathogens (Singh et al., 2012). HaRxL21 expressing lines showed downregulation of several genes which are involved in resistance against biotroph and/or necrotrophic pathogens, and similar to this, we observed misregulation of many genes by HaRxL21, which were also differentially expressed during *Pst* infection (Lewis et al., 2015) and *B. cinerea* infection (Windram et al., 2012). Reduced expression or mis regulation of these immune related genes could be contributing to the observed enhanced susceptibility of HaRxL21 over-expressing plants to both a biotrophic and a necrotrophic pathogen.

4.5 Recruitment of HaRxL21 to TPL/TPRs transcriptional complexes

Based on our findings, HaRxL21 suppresses plant immunity by interacting with the TPL/TPRs co-repressors via its C-terminal EAR motif. Therefore, it is likely that HaRxL21 suppresses plant immunity by modulating the actions of TPL or TPRs and to confirm this, we looked if TPR1 targets genes were also differentially expressed in HaRxL21 expressing lines. Interestingly, in the ChIP seq and RNA seq comparison, twenty genes were identified as direct TPR1 targets, which were repressed by HaRxL21. Among these 20 genes, seven were specifically repressed after flg22 treatment. The tetraspanin9 (*TET9*) is 2.6-fold downregulated by HaRxL21 compared to HaRxL21ΔEAR. TET8/9 has been shown to promote the formation of exosome-like extracellular vesicles to deliver host sRNAs into *B. cinerea* to decrease fungal virulence (Cai et al., 2018). The *tet9* loss-of-function mutants display weak but consistently enhanced susceptibility towards *B. cinerea,* whereas TET9 accumulation was seen around fungal infection sites after *B. cinerea* infection; these results suggest the *B. cinerea* susceptibility we observed in HaRxL21-expressing lines could be due to *TET9* repressed expression.
Another direct TPR1 targets were WRKY TFs (WRKY46 and WRKY63), and they are repressed in HaRxL21, expressing lines in comparison to the HaRxL21ΔEAR variant. WRKY TFs have been shown to regulate defense responses against both biotrophic and necrotrophic pathogens (Birkenbihl et al., 2012; S. Liu, Ziegler, Zeier, Birkenbihl, & Somssich, 2017). WRKY46 is known to be involved in the SA-signaling pathway, and its overexpressing plants are more resistant to *Pst*. (Hu, Dong, & Yu, 2012). Apart from this, WRKY46 and WRKY54 regulate the non-host resistance against *Erwinia amylovora* via

EDS1 (Moreau et al., 2012). WRKY46 and 63 binds to the promoter region of the *SID2* (SA biosynthesis gene), and they may control the level of SA by regulating the expression of *SID2* (S. Zhang et al., 2017). Consistent with this, *SID2* (also known as *ICS1*) is downregulated by HaRxL21 after flg22 treatment; however, this might be due to direct repression via association with TPR1 as *SID2* is also a target of TPR1. Moreover, WRKY46 is a transcriptional activator of *PBS3* expression (van Verk, Bol, & Linthorst, 2011), and consistent with this, *PBS3* expression is also repressed in lines expressing HaRxL21 in comparison to HaRxL21ΔEAR lines. Recently, it has been shown that PBS3 binds to and protects EDS1 from proteasomal degradation (Chang et al., 2019); hence reductions in PBS3 expression could lead to lower levels of EDS1 protein and enhanced susceptibility to biotrophic pathogens (Parker et al., 1996).

In the promoters of HaRxL21 DEGs, we observed an overrepresentation of WRKY TF binding motifs, which indicates that many of the DEGs are downstream targets of WRKY TFs. Therefore, it is possible that WRKY46 and WRKY63 TFs are directly repressed by effector HaRxL21, which in turn results in the changed expression of their target genes. The CAMTA motif was also overrepresented in genes that were specifically downregulated by HaRxL21 upon flg22 treatment in an EAR-dependent manner, indicating that target genes of CAMTA TFs are showing differential expression. Though there is no evidence to date that CAMTA TFs are targets of TPL/TPR (Causier, Ashworth, Guo, & Davies, 2012) but these TFs have been shown to play a role in immune regulation through suppressing pathogen-responsive genes and, therefore, these could be important targets for HaRxL21 manipulation via TPL/TPRs (Jacob et al., 2018; Y. Kim, Gilmour, Chao, Park, & Thomashow, 2020; Yuan, Du, & Poovaiah, 2018).

Taking together, our finding indicates that there are several possible ways how HaRxL21 suppresses its host plant immunity. One possible scenario is that HaRxL21 might interact with a TF and mediate subsequent TPL/TPR1 binding to specific sites in a manner like NINJA or JAZ proteins (Pauwels et al., 2010). As evidence for an interaction of HaRxL21 with the TF we found TCPs and MYB73 TFs in Y-2-H screen. However, we were unable to find MYB73 interaction with HaRxL21 in Co-IP. However, if HaRxL21 was binding with TFs independently of TPL/TPR1, then in our ChIP-PCR experiment, it would be expected to observe a similar enrichment in the HaRxL21 and HaRxL21ΔEAR at the tested loci. On the other hand, an alternative model for HaRxL21 mediated suppression is that it could interfere with the normal repression mechanism of existing TPL/TPRs targets or lift of repression of genes.

5 Conclusion and future prospective

As effector proteins play key roles in promoting infection, the identification of effector action and targets can uncover vital components of plant defense and promote our understanding of how pathogens manipulate their hosts. The aim of this work was to gain a better understanding of the role of effector proteins of *Hpa* in suppressing the plant immune system of *A. thaliana*. We focused on a case study of the *Hpa* effector HaRxL21. We found that HaRxL21 interacts with some TFs and TPL co-repressor family members. TPL/TPRs are involved in a variety of biological processes, such as the transcriptional regulation of hormone signaling pathways via EAR motif-mediated repression. In HaRxL21-overexpressing transgenic Arabidopsis lines, we observed that HaRxL21 expression *in planta* caused immune suppression that supported the growth of both the biotrophic and the necrotrophic pathogen *Hpa* and *Botrytis cinerea*, respectively. This immune suppression mediated by HaRxL21 is dependent on its C-terminal EAR motif.

Furthermore, we confirmed that HaRxL21 interacts with TPL/TPRs via its EAR motif, and therefore HaRxL21 most likely suppresses immunity by modifying the mode of action of TPL and TPRs. However, it is still unclear how HaRxL21 suppresses immunity in detail. Our findings indicate several possible scenarios. Since we have observed HaRxL21 interaction with some TFs in Y-2-H assays, it is possible that HaRxL21 interacts with a TF and mediates subsequent TPL/TPRs binding to specific genomic loci in a manner similar to NINJA or JAZ proteins (Pauwels et al., 2010). Interaction of HaRxL21 with the TCP14 TF was also seen in Y-2-H experiments (Mukhtar et al., 2011), and it was shown that TCP14 shifts HaRxL21 into subnuclear foci (Weßling et al., 2014), similarly we also observed re-localization of HaRxL21 when it was co-expressed with MYB73 and TPL. However, if HaRxL21 were to bind to the TFs independently of TPL/TPRs, then similar enrichment of HaRxL21 and HaRxL21ΔEAR would be expected to be observed at the tested loci in our ChIP-PCR experiments. An alternative model for HaRxL21 mediated suppression is that HaRxL21 might interfere with the repression (or lifting of repression) of existing TPL/ TPRs targets. In our study, we provide evidence to support this assumption, at least for a few genes, that HaRxL21 appears to bind to TPL/TPRs within transcriptional complexes at plant gene promoters and prevents transcriptional de-repression. First, we observed a significant overrepresentation in TPR1 binding sites upstream of genes that are repressed by full-length HaRxL21 compared to HaRxL21ΔEAR. Second, promoters of some HaRxL21-repressed genes were shown to be not only binding targets of TPR1 but also binding targets of effector HaRxL21 (and not of HaRxL21ΔEAR). Thus, at least in these cases, it is indicated that HaRxL21 probably binds to the TPR1 protein already bound to the gene promoter (since without the EAR motif, there is no binding of HaRxL21 to these promoters) and consequently exerts its activity on the TPL/TPR1 complex.

The Groucho (Gro) / Tup1 family co-repressors in animals and fungi (Z. Liu & Karmarkar, 2008) show structural similarity to TPL and TPRs, specifically the presence of WD-repeat motifs (consisting of tryptophan (W) and aspartate (D) residues). They have been shown to form tetrameric structures, enabling the recruitment of multiple TFs to a single complex

(Martin-Arevalillo et al., 2017), and the binding of the EAR motif does not prevent tetramerization (G. Chen, Nguyen, & Courey, 1998; Martin-Arevalillo et al., 2017; Nuthall, Husain, McLarren, & Stifani, 2002). Therefore, it is possible that HaRxL21 is able to bind TPL/TPRs via its EAR domain, while other epitopes of the TPL oligomer are still binding with other proteins such as TFs within the transcriptional complex. TPR1 activity has been shown to be regulated by the (SUMO) E3 ligase SIZ1. Probably via SUMOylation, TPR1 interaction with HDA19 is inhibited to suppress the corepressor activity of TPR1 (Niu et al., 2019). However, it is not clear how does TPL/TPRs-bound HaRxL21 behave, but still, there is the possibility that HaRxL21 enhances the TPL/TPR co-repressors activity by shielding the SUMO attachment sites K282 and K721 in TPR1 and/or preventing (SUMO) E3 ligase activity.

In RNA-seq analysis of HaRxL21 expressing lines, we observed a downregulation of several defense-related genes, but many genes were upregulated as well. In the comparison of these upregulated genes with more than 1500 genes that were specifically upregulated in response to *Pst* effectors during *Pst* infection, an overlap of 15 genes was observed. So, it is not evident that HaRxL21 use the only mechanism through maintained repression of direct TPL/TPRs targets as there are many DEGs that were upregulated in the presence of HaRxL21. However, apart from immune suppression, pathogen effectors are also known to directly manipulate plant gene expression, and hence plant physiology, to aid pathogen infection (L.-Q. Chen et al., 2010; Fatima & Senthil-Kumar, 2015). Therefore, it is plausible that upregulated genes could be downstream targets of TFs or other regulatory genes which were targeted by HaRxL21. This seems to be the most likely explanation since the HaRxL21 upregulated genes were not enriched for TPR1 binding in wildtype plants and that the promoter regions of these genes have a high concentration of WRKY TF binding motifs.

We showed that the pathogen effector HaRxL21 alone is able to enhance susceptibility to pathogens with different virulence strategies and lifestyles. To our knowledge, there are rarely, if there are, any, effectors that exhibit such activity. HaRxL21 is one of several effectors that mimics the action of plant EAR motif-containing repressor proteins and recruits TPL/TPRs to sites of transcriptional initiation to actively initiate and/or maintain repression of immune-related gene expression. We have shown here that the HaRxL21 EAR motif is essential for interaction with TPL co-repressor family members, its virulence function, and for modifying the expression of crucial host immune-related genes.

Future work will be to determine the HaRxL21 mode of action on TPL/TPRs transcriptional repression complexes and investigate which effector manipulated defense ultimately result in enhanced susceptibility of the host plant.

6 Summary (Zusammenfassung)

6.1 Summary

Plant diseases result from the interaction between the pathogen and the host plant, where pathogens deploy effector proteins to invade the host immune system to aid colonization. The oomycete *Hyaloperonospora arabidopsidis* (*Hpa*) is the causal agent of downy mildew of *Arabidopsis thaliana*. The oomycete effectors have a conserved 'RxLR' motif, which is required for their translocation into the host cell. Here we characterised 'HaRxL21', which is an EAR motif-containing *Hpa* effector that can suppress plant immunity. HaRxL21 expressing lines show enhanced susceptibility against *Hpa* and *Botrytis cinerea*. The interaction targets of HaRxL21 were identified, and we confirmed that HaRxL21 interacts with the Arabidopsis transcriptional co-repressor TPL. We have established that HaRxL21 and TPL interact via an EAR motif at the C-terminus of the effector. We show that the EAR motif is required for the interaction with TPL and is necessary for the virulence function of the effector. RNA-seq analysis has revealed the effects of HaRxL21 on host transcription, mainly through the down-regulation of defense-related genes. Hence, this effector mimics the host plant mechanism of EAR motif mediated repression by recruiting TPL to the transcriptional repression site. Moreover, we have provided evidence that HaRxL21 uses the interaction with TPL, and its close relative TPR1, in order to manipulate the plant immunity and enhance susceptibility to both a biotrophic and a necrotrophic pathogen.

6.2 Zusammenfassung

Pflanzenkrankheiten entstehen, wenn ein Pathogen das Immunsystem der Wirtspflanze erfolgreich unterdrückt. Zu diesem Zweck setzen viele biotrophe Krankheitserreger Proteine (Effektoren) ein, um die Besiedlung zu fördern. Der Eipilz (Oomycet) *Hyaloperonospora arabidopsidis* (*Hpa*) ist der Erreger des Falschen Mehltaus auf *Arabidopsis thaliana*. In der vorliegende Arbeit haben wir den Proteineffektor 'HaRxL21' charakterisiert, welcher die pflanzliche Immunität erfolgreich unterdrücken kann; HaRxL21-exprimierende Arabidopsis-Pflanzen weisen eine erhöhte Anfälligkeit für *Hpa* und den nekrotrophen Pilz *Botrytis cinerea* auf. Um mehr über die Wirkungsweise der *Hpa* Effektoren zu erfahren, haben wir Wirtsproteine identifiziert, die mit HaRxL21 interagieren. Wir bestätigten, dass HaRxL21 mit dem transkriptionellen Co-Repressor Topless (TPL) aus Arabidopsis interagiert. Wir fanden zudem heraus, dass HaRxL21 und TPL über ein EAR-Motiv am C-Terminus des Effektors interagieren, welches auch für die Virulenzfunktion des Effektors erforderlich ist. RNA-Seq-Studien zeigen, dass HaRxL21 die Transkription der Wirtspflanze beeinflusst, vor allem durch die Herunterregulierung von Genen, die an der Pflanzenimmunität beteiligt sind. Somit ahmt dieser Effektor den Mechanismus der durch das EAR-Motiv vermittelten Unterdrückung in der Wirtspflanze nach und kann so die transkriptionelle Unterdrückung von Immungenen vermitteln.

7 References

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2003). Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *The Plant Cell, 15*(1), 63-78.

Acharya, B. R., Raina, S., Maqbool, S. B., Jagadeeswaran, G., Mosher, S. L., Appel, H. M., Raina, R. (2007). Overexpression of CRK13, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to Pseudomonas syringae. *The Plant Journal, 50*(3), 488-499.

Adie, B., Chico, J. M., Rubio-Somoza, I., & Solano, R. (2007). Modulation of plant defenses by ethylene. *Journal of Plant Growth Regulation, 26*(2), 160-177.

Albert, I., Böhm, H., Albert, M., Feiler, C. E., Imkampe, J., Wallmeroth, N., Zhang, H. (2015). An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. *Nature plants, 1*(10), 1-9.

Allen, R. L., Bittner-Eddy, P. D., Grenville-Briggs, L. J., Meitz, J. C., Rehmany, A. P., Rose, L. E., & Beynon, J. L. (2004). Host-parasite coevolutionary conflict between Arabidopsis and downy mildew. *Science, 306*(5703), 1957-1960.

Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Cheuk, R. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. *Science, 301*(5633), 653-657.

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. *The Plant Cell, 16*(12), 3460-3479.

Anderson, R. G., Casady, M. S., Fee, R. A., Vaughan, M. M., Deb, D., Fedkenheuer, K., McDowell, J. M. (2012). Homologous RXLR effectors from Hyaloperonospora arabidopsidis and Phytophthora sojae suppress immunity in distantly related plants. *The Plant Journal, 72*(6), 882-893.

Anderson, R. G., Deb, D., Fedkenheuer, K., & McDowell, J. M. (2015). Recent progress in RXLR effector research. *Molecular plant-microbe interactions, 28*(10), 1063-1072.

Armstrong, M. R., Whisson, S. C., Pritchard, L., Bos, J. I., Venter, E., Avrova, A. O., Cherevach, I. (2005). An ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding a protein that is recognized in the host cytoplasm. *Proceedings of the National Academy of Sciences, 102*(21), 7766-7771.

Asai, S., Rallapalli, G., Piquerez, S. J., Caillaud, M.-C., Furzer, O. J., Ishaque, N., Jones, J. D. (2014). Expression profiling during Arabidopsis/downy mildew interaction reveals a highly-expressed effector that attenuates responses to salicylic acid. *PLoS Pathogens, 10*(10), e1004443.

Axtell, M. J., & Staskawicz, B. J. (2003). Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. *Cell, 112*(3), 369-377.

Badel, J. L., Piquerez, S. J., Greenshields, D., Rallapalli, G., Fabro, G., Ishaque, N., & Jones, J. D. (2013). In planta effector competition assays detect Hyaloperonospora arabidopsidis effectors that contribute to virulence and localize to different plant subcellular compartments. *Molecular plant-microbe interactions, 26*(7), 745-757.

Baxter, L., Tripathy, S., Ishaque, N., Boot, N., Cabral, A., Kemen, E., Badejoko, W. (2010). Signatures of adaptation to obligate biotrophy in the Hyaloperonospora arabidopsidis genome. *Science, 330*(6010), 1549-1551.

Bechtold, N., & Pelletier, G. (1998). In planta Agrobacteriummediated transformation of adult Arabidopsis thaliana plants by vacuum infiltration. In *Arabidopsis protocols* (pp. 259-266): Springer.

Berens, M. L., Berry, H. M., Mine, A., Argueso, C. T., & Tsuda, K. (2017). Evolution of hormone signaling networks in plant defense. *Annual review of phytopathology, 55*, 401-425.

Bernoux, M., Timmers, T., Jauneau, A., Briere, C., de Wit, P. J., Marco, Y., & Deslandes, L. (2008). RD19, an Arabidopsis cysteine protease required for RRS1-R–mediated resistance, is relocalized to the nucleus by the Ralstonia solanacearum PopP2 effector. *The Plant Cell, 20*(8), 2252-2264.

Bigeard, J., Colcombet, J., & Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plant, 8*(4), 521-539.

Birkenbihl, R. P., Diezel, C., & Somssich, I. E. (2012). Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward Botrytis cinerea infection. *Plant physiology, 159*(1), 266-285.

Boller, T., & Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual review of plant biology, 60*, 379-406.

Bos, J. I., Kanneganti, T. D., Young, C., Cakir, C., Huitema, E., Win, J., Kamoun, S. (2006). The C-terminal half of Phytophthora infestans RXLR effector AVR3a is sufficient to trigger R3a-mediated hypersensitivity and suppress INF1-induced cell death in Nicotiana benthamiana. *The Plant Journal, 48*(2), 165-176.

Boutrot, F., & Zipfel, C. (2017). Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annual review of phytopathology, 55*, 257-286.

Bu, Q., Jiang, H., Li, C.-B., Zhai, Q., Zhang, J., Wu, X., Li, C. (2008). Role of the Arabidopsis thaliana NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses. *Cell research, 18*(7), 756-767.

Buscaill, P., & Rivas, S. (2014). Transcriptional control of plant defence responses. *Current opinion in plant biology, 20*, 35-46.

Büttner, D. (2016). Behind the lines–actions of bacterial type III effector proteins in plant cells. *FEMS microbiology reviews, 40*(6), 894-937.

Byndloss, M. X., Rivera-Chávez, F., Tsolis, R. M., & Bäumler, A. J. (2017). How bacterial pathogens use type III and type IV secretion systems to facilitate their transmission. *Current opinion in microbiology, 35*, 1-7.

Cabral, A., Stassen, J. H., Seidl, M. F., Bautor, J., Parker, J. E., & Van den Ackerveken, G. (2011). Identification of Hyaloperonospora arabidopsidis transcript sequences expressed during infection reveals isolate-specific effectors. *PLoS One, 6*(5), e19328.

Cai, Q., Qiao, L., Wang, M., He, B., Lin, F.-M., Palmquist, J., Jin, H. (2018). Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. *Science, 360*(6393), 1126-1129.

Caillaud, M.-C., Asai, S., Rallapalli, G., Piquerez, S., Fabro, G., & Jones, J. D. (2013). A downy mildew effector attenuates salicylic acid–triggered immunity in Arabidopsis by interacting with the host mediator complex. *PLoS biology, 11*(12), e1001732.

Caillaud, M. C., Piquerez, S. J., Fabro, G., Steinbrenner, J., Ishaque, N., Beynon, J., & Jones, J. D. (2012). Subcellular localization of the Hpa RxLR effector repertoire identifies a tonoplast-associated protein HaRxL17 that confers enhanced plant susceptibility. *The Plant Journal, 69*(2), 252-265.

Canonne, J., Marino, D., Jauneau, A., Pouzet, C., Brière, C., Roby, D., & Rivas, S. (2011). The Xanthomonas type III effector XopD targets the Arabidopsis transcription factor MYB30 to suppress plant defense. *The Plant Cell, 23*(9), 3498-3511.

Causier, B., Ashworth, M., Guo, W., & Davies, B. (2012). The TOPLESS interactome: a framework for gene repression in Arabidopsis. *Plant physiology, 158*(1), 423-438.

Causier, B., Lloyd, J., Stevens, L., & Davies, B. (2012). TOPLESS co-repressor interactions and their evolutionary conservation in plants. *Plant signaling & behavior, 7*(3), 325-328.

Chang, M., Zhao, J., Chen, H., Li, G., Chen, J., Li, M., Liu, F. (2019). PBS3 protects EDS1 from proteasome-mediated degradation in plant immunity. *Molecular Plant, 12*(5), 678-688.

Chassot, C., Nawrath, C., & Métraux, J. P. (2007). Cuticular defects lead to full immunity to a major plant pathogen. *The Plant Journal, 49*(6), 972-980.

Chen, L.-T., & Wu, K. (2010). Role of histone deacetylases HDA6 and HDA19 in ABA and abiotic stress response. *Plant signaling & behavior, 5*(10), 1318-1320.

Chen, Q., Sun, J., Zhai, Q., Zhou, W., Qi, L., Xu, L., Qi, J. (2011). The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in Arabidopsis. *The Plant Cell, 23*(9), 3335-3352.

Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J., & Kunkel, B. N. (2007). Pseudomonas syringae type III effector AvrRpt2 alters Arabidopsis thaliana auxin physiology. *Proceedings of the National Academy of Sciences, 104*(50), 20131-20136.

Clark, J. S., & Spencer-Phillips, P. (2000). Downy mildews. *Encyclopaedia of microbiology, 2*, 117-129.

Coates, M. E., & Beynon, J. L. (2010). Hyaloperonospora arabidopsidis as a pathogen model. *Annual review of phytopathology, 48*, 329-345.

Consortium, A. I. M. (2011). Evidence for network evolution in an Arabidopsis interactome map. *Science, 333*(6042), 601-607.

Couto, D., & Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature Reviews Immunology, 16*(9), 537.

Crute, I. R. (1994). Gene-for-gene recognition in plant—pathogen interactions. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 346*(1317), 345-349.

Cui, H., Tsuda, K., & Parker, J. E. (2015). Effector-triggered immunity: from pathogen perception to robust defense. *Annual review of plant biology, 66*, 487-511.

Dangl, J. L., Ritter, C., Gibbon, M. J., Mur, L., Wood, J. R., Goss, S., Vivian, A. (1992). Functional homologs of the Arabidopsis RPM1 disease resistance gene in bean and pea. *The Plant Cell, 4*(11), 1359-1369.

De Jonge, R., Van Esse, H. P., Kombrink, A., Shinya, T., Desaki, Y., Bours, R., Thomma, B. P. (2010). Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. *Science, 329*(5994), 953-955.

Deb, D., Anderson, R. G., How-Yew-Kin, T., Tyler, B. M., & McDowell, J. M. (2018). Conserved RxLR effectors from oomycetes Hyaloperonospora arabidopsidis and Phytophthora sojae suppress PAMP-and effector-triggered immunity in diverse plants. *Molecular plant-microbe interactions, 31*(3), 374-385.

Dempsey, D. M. A., Vlot, A. C., Wildermuth, M. C., & Klessig, D. F. (2011). Salicylic acid biosynthesis and metabolism. *The Arabidopsis book/American Society of Plant Biologists, 9*.

Denancé, N., Sánchez-Vallet, A., Goffner, D., & Molina, A. (2013). Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. *Frontiers in plant science, 4*, 155.

Dong, C.-J., & Liu, J.-Y. (2010). The Arabidopsis EAR-motif-containing protein RAP2. 1 functions as an active transcriptional repressor to keep stress responses under tight control. *BMC plant biology, 10*(1), 1-15.

Dong, X., Jiang, Z., Peng, Y.-L., & Zhang, Z. (2015). Revealing shared and distinct gene network organization in Arabidopsis immune responses by integrative analysis. *Plant physiology, 167*(3), 1186-1203.

Dou, D., Kale, S. D., Wang, X., Chen, Y., Wang, Q., Wang, X., Thakur, P. B. (2008). Conserved C-terminal motifs required for avirulence and suppression of cell death by Phytophthora sojae effector Avr1b. *The Plant Cell, 20*(4), 1118-1133.

Dreze, M., Monachello, D., Lurin, C., Cusick, M. E., Hill, D. E., Vidal, M., & Braun, P. (2010). High-quality binary interactome mapping. *Methods in enzymology, 470*, 281-315.

Emes, R. D., & Ponting, C. P. (2001). A new sequence motif linking lissencephaly, Treacher Collins and oral–facial–digital type 1 syndromes, microtubule dynamics and cell migration. *Human molecular genetics, 10*(24), 2813-2820.

Fabro, G., Steinbrenner, J., Coates, M., Ishaque, N., Baxter, L., Studholme, D. J., Rougon-Cardoso, A. (2011). Multiple candidate effectors from the oomycete pathogen Hyaloperonospora arabidopsidis suppress host plant immunity. *PLoS Pathogens, 7*(11), e1002348.

Felix, G., & Boller, T. (2003). Molecular sensing of bacteria in plants: the highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. *Journal of Biological Chemistry, 278*(8), 6201-6208.

Fernández-Calvo, P., Chini, A., Fernández-Barbero, G., Chico, J.-M., Gimenez-Ibanez, S., Geerinck, J., Franco-Zorrilla, J. M. (2011). The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *The Plant Cell, 23*(2), 701-715.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual review of phytopathology, 9*(1), 275-296.

Fry, W. E., & Goodwin, S. B. (1997). Re-emergence of potato and tomato late blight in the United States. *Plant disease, 81*(12), 1349-1357.

Gao, Q.-M., Venugopal, S., Navarre, D., & Kachroo, A. (2011). Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. *Plant physiology, 155*(1), 464-476.

Gehl, C., Waadt, R., Kudla, J., Mendel, R.-R., & Hänsch, R. (2009). New GATEWAY vectors for high throughput analyses of protein–protein interactions by bimolecular fluorescence complementation. *Molecular Plant, 2*(5), 1051-1058.

Gendrel, A.-V., Lippman, Z., Martienssen, R., & Colot, V. (2005). Profiling histone modification patterns in plants using genomic tiling microarrays. *Nature methods, 2*(3), 213-218.

Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J. P., & Solano, R. (2014). The bacterial effector HopX1 targets JAZ transcriptional repressors to activate jasmonate signaling and promote infection in Arabidopsis. *PLoS Biol, 12*(2), e1001792.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol., 43*, 205-227.

Glazebrook, J., Chen, W., Estes, B., Chang, H. S., Nawrath, C., Métraux, J. P., Katagiri, F. (2003). Topology of the network integrating salicylate and jasmonate signal

transduction derived from global expression phenotyping. *The Plant Journal*, *34*(2), 217-228.

Griebel, T., Lapin, D., Kracher, B., Concia, L., Benhamed, M., & Parker, J. E. (2020). Genome-wide chromatin binding of transcriptional corepressor Topless-related 1 in Arabidopsis. *BioRxiv*.

Harvey, S., Kumari, P., Lapin, D., Griebel, T., Hickman, R., Guo, W., Denby, K. (2020). Downy Mildew effector HaRxL21 interacts with the transcriptional repressor TOPLESS to promote pathogen susceptibility. *PLoS Pathogens, 16*(8), e1008835.

Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., Tokuyasu, K. (2014). Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proceedings of the National Academy of Sciences, 111*(3), E404-E413.

He, P., Shan, L., Lin, N.-C., Martin, G. B., Kemmerling, B., Nürnberger, T., & Sheen, J. (2006). Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. *Cell, 125*(3), 563-575.

Herlihy, J., Ludwig, N. R., van den Ackerveken, G., & McDowell, J. M. (2019). Oomycetes used in Arabidopsis research. *The Arabidopsis Book, 17*.

Hill, K., Wang, H., & Perry, S. E. (2008). A transcriptional repression motif in the MADS factor AGL15 is involved in recruitment of histone deacetylase complex components. *The Plant Journal, 53*(1), 172-185.

Hiller, N. L., Bhattacharjee, S., van Ooij, C., Liolios, K., Harrison, T., Lopez-Estrano, C., & Haldar, K. (2004). A host-targeting signal in virulence proteins reveals a secretome in malarial infection. *Science, 306*(5703), 1934-1937.

Hiratsu, K., Mitsuda, N., Matsui, K., & Ohme-Takagi, M. (2004). Identification of the minimal repression domain of SUPERMAN shows that the DLELRL hexapeptide is both necessary and sufficient for repression of transcription in Arabidopsis. *Biochemical and biophysical research communications, 321*(1), 172-178.

Hiratsu, K., Ohta, M., Matsui, K., & Ohme-Takagi, M. (2002). The SUPERMAN protein is an active repressor whose carboxy-terminal repression domain is required for the development of normal flowers. *FEBS letters, 514*(2-3), 351-354.

Holub, E., & Beynon, J. (1997). Symbiology of mouse-ear cress (Arabidopsis thaliana) and oomycetes. *Advances in Botanical Research, 24*, 227-273.

Holub, E., Brose, E., Tör, M., Clay, C., Crute, I., & Beynon, J. (1995). Phenotypic and genotypic variation in the interaction between Arabidopsis thaliana and Albugo candida. *Molecular plant-microbe interactions: MPMI, 8*(6), 916-928.

Holub, E. B. (2001). The arms race is ancient history in Arabidopsis, the wildflower. *Nature Reviews Genetics, 2*(7), 516-527.

Holub, E. B. (2008). Natural history of Arabidopsis thaliana and oomycete symbioses. *The Downy Mildews-Genetics, Molecular Biology and Control*, 91-109.

Hou, X., Lee, L. Y. C., Xia, K., Yan, Y., & Yu, H. (2010). DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Developmental cell, 19*(6), 884-894.

Howe, G. A., Major, I. T., & Koo, A. J. (2018). Modularity in jasmonate signaling for multistress resilience. *Annual review of plant biology, 69*, 387-415.

Hu, Y., Dong, Q., & Yu, D. (2012). Arabidopsis WRKY46 coordinates with WRKY70 and WRKY53 in basal resistance against pathogen Pseudomonas syringae. *Plant science, 185*, 288-297.

Huffaker, A., Pearce, G., & Ryan, C. A. (2006). An endogenous peptide signal in Arabidopsis activates components of the innate immune response. *Proceedings of the National Academy of Sciences, 103*(26), 10098-10103.

Ikeda, M., & Ohme-Takagi, M. (2009). A novel group of transcriptional repressors in Arabidopsis. *Plant and cell physiology, 50*(5), 970-975.

Jacob, F., Kracher, B., Mine, A., Seyfferth, C., Blanvillain-Baufumé, S., Parker, J. E., Maekawa, T. (2018). A dominant-interfering camta3 mutation compromises primary transcriptional outputs mediated by both cell surface and intracellular immune receptors in Arabidopsis thaliana. *New Phytologist, 217*(4), 1667-1680.

Jamieson, P. A., Shan, L., & He, P. (2018). Plant cell surface molecular cypher: Receptor-like proteins and their roles in immunity and development. *Plant science, 274*, 242-251.

Jiang, Z., Ge, S., Xing, L., Han, D., Kang, Z., Zhang, G., Cao, A. (2013). RLP1. 1, a novel wheat receptor-like protein gene, is involved in the defence response against Puccinia striiformis f. sp. tritici. *Journal of experimental botany, 64*(12), 3735-3746.

Jiao, J., Xing, J.-H., Dong, J.-G., Han, J.-M., & Liu, J.-S. (2011). Functional analysis of MYB73 of Arabidopsis thaliana against Bipolaris oryzae. *Agricultural Sciences in China, 10*(5), 721-727.

Jin, L., Ham, J. H., Hage, R., Zhao, W., Soto-Hernández, J., Lee, S. Y., Coplin, D. L. (2016). Direct and indirect targeting of PP2A by conserved bacterial type-III effector proteins. *PLoS Pathogens, 12*(5), e1005609.

Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feys, B. J., Parker, J. E., Glazebrook, J. (1999). Arabidopsis thaliana PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proceedings of the National Academy of Sciences, 96*(23), 13583-13588.

Jones, J. Dangl,. JL (2006). *The plant immune system. nature, 444*, 323-329.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*(7117), 323-329.

Kagale, S., & Rozwadowski, K. (2011). EAR motif-mediated transcriptional repression in plants: an underlying mechanism for epigenetic regulation of gene expression. *Epigenetics, 6*(2), 141-146.

Kamoun, S. (2003). Molecular genetics of pathogenic oomycetes. *Eukaryotic cell, 2*(2), 191-199.

Kamoun, S., Furzer, O., Jones, J. D., Judelson, H. S., Ali, G. S., Dalio, R. J., Panabières, F. (2015). The Top 10 oomycete pathogens in molecular plant pathology. *Molecular plant pathology, 16*(4), 413-434.

Katagiri, F., & Tsuda, K. (2010). Understanding the plant immune system. *Molecular plant-microbe interactions, 23*(12), 1531-1536.

Khang, C. H., Berruyer, R., Giraldo, M. C., Kankanala, P., Park, S.-Y., Czymmek, K., Valent, B. (2010). Translocation of Magnaporthe oryzae effectors into rice cells and their subsequent cell-to-cell movement. *The Plant Cell, 22*(4), 1388-1403.

Kieffer, M., Stern, Y., Cook, H., Clerici, E., Maulbetsch, C., Laux, T., & Davies, B. (2006). Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. *The Plant Cell, 18*(3), 560-573.

Kim, J.-G., Taylor, K. W., Hotson, A., Keegan, M., Schmelz, E. A., & Mudgett, M. B. (2008). XopD SUMO protease affects host transcription, promotes pathogen growth, and delays symptom development in Xanthomonas-infected tomato leaves. *The Plant Cell, 20*(7), 1915-1929.

KIM, J. G., Taylor, K. W., & Mudgett, M. B. (2011). Comparative analysis of the XopD type III secretion (T3S) effector family in plant pathogenic bacteria. *Molecular plant pathology, 12*(8), 715-730.

Kim, Y., Gilmour, S. J., Chao, L., Park, S., & Thomashow, M. F. (2020). Arabidopsis CAMTA transcription factors regulate pipecolic acid biosynthesis and priming of immunity genes. *Molecular Plant, 13*(1), 157-168.

Kleemann, J., Rincon-Rivera, L. J., Takahara, H., Neumann, U., van Themaat, E. V. L., van der Does, H. C., Schmalenbach, W. (2012). Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen Colletotrichum higginsianum. *PLoS Pathog, 8*(4), e1002643.

Kleinboelting, N., Huep, G., Kloetgen, A., Viehoever, P., & Weisshaar, B. (2012). GABI-Kat SimpleSearch: new features of the Arabidopsis thaliana T-DNA mutant database. *Nucleic acids research, 40*(D1), D1211-D1215.

Koch, E., & Slusarenko, A. (1990). Arabidopsis is susceptible to infection by a downy mildew fungus. *The Plant Cell, 2*(5), 437-445.

Koncz, C., & Schell, J. (1986). The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of Agrobacterium binary vector. *Molecular and General Genetics MGG, 204*(3), 383-396.

Koornneef, A., & Pieterse, C. M. (2008). Cross talk in defense signaling. *Plant physiology, 146*(3), 839-844.

Krogan, N. T., Hogan, K., & Long, J. A. (2012). APETALA2 negatively regulates multiple floral organ identity genes in Arabidopsis by recruiting the co-repressor TOPLESS and the histone deacetylase HDA19. *Development, 139*(22), 4180-4190.

Kudla, J., & Bock, R. (2016). Lighting the way to protein-protein interactions: recommendations on best practices for bimolecular fluorescence complementation analyses. *The Plant Cell, 28*(5), 1002-1008.

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., & Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. *The Plant Cell, 16*(12), 3496-3507.

Lee, J. E., & Golz, J. F. (2012). Diverse roles of Groucho/Tup1 co-repressors in plant growth and development. *Plant signaling & behavior, 7*(1), 86-92.

Lewis, L. A., Polanski, K., de Torres-Zabala, M., Jayaraman, S., Bowden, L., Moore, J., Baxter, L. (2015). Transcriptional dynamics driving MAMP-triggered immunity and pathogen effector-mediated immunosuppression in Arabidopsis leaves following infection with Pseudomonas syringae pv tomato DC3000. *The Plant Cell, 27*(11), 3038-3064.

Li, J., Brader, G., Kariola, T., & Tapio Palva, E. (2006). WRKY70 modulates the selection of signaling pathways in plant defense. *The Plant Journal, 46*(3), 477-491.

Li, S. (2015). The Arabidopsis thaliana TCP transcription factors: a broadening horizon beyond development. *Plant signaling & behavior, 10*(7), e1044192.

Lin, W.-Z., Fang, J.-A., Xiao, X., & Chou, K.-C. (2011). iDNA-Prot: identification of DNA binding proteins using random forest with grey model. *PLoS One, 6*(9), e24756.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Scheel, D. (2005). Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. *Science, 310*(5751), 1180-1183.

Liu, J., Elmore, J. M., Fuglsang, A. T., Palmgren, M. G., Staskawicz, B. J., & Coaker, G. (2009). RIN4 functions with plasma membrane H+-ATPases to regulate stomatal apertures during pathogen attack. *PLoS Biol, 7*(6), e1000139.

Liu, S., Ziegler, J., Zeier, J., Birkenbihl, R. P., & Somssich, I. E. (2017). Botrytis cinerea B05. 10 promotes disease development in Arabidopsis by suppressing WRKY33-mediated host immunity. *Plant, cell & environment, 40*(10), 2189-2206.

Liu, W., Liu, J., Triplett, L., Leach, J. E., & Wang, G.-L. (2014). Novel insights into rice innate immunity against bacterial and fungal pathogens. *Annual review of phytopathology, 52*, 213-241.

Liu, Z., & Karmarkar, V. (2008). Groucho/Tup1 family co-repressors in plant development. *Trends in plant science, 13*(3), 137-144.

Long, J. A., Ohno, C., Smith, Z. R., & Meyerowitz, E. M. (2006). TOPLESS regulates apical embryonic fate in Arabidopsis. *Science, 312*(5779), 1520-1523.

Long, J. A., Woody, S., Poethig, S., Meyerowitz, E. M., & Barton, M. K. (2002). Transformation of shoots into roots in Arabidopsis embryos mutant at the TOPLESS locus.

Lotze, M. T., Zeh, H. J., Rubartelli, A., Sparvero, L. J., Amoscato, A. A., Washburn, N. R., Billiar, T. (2007). The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. *Immunological reviews, 220*(1), 60-81.

Lozano-Durán, R., Bourdais, G., He, S. Y., & Robatzek, S. (2014). The bacterial effector HopM1 suppresses PAMP-triggered oxidative burst and stomatal immunity. *New Phytologist, 202*(1), 259-269.

Lu, D., Wu, S., Gao, X., Zhang, Y., Shan, L., & He, P. (2010). A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. *Proceedings of the National Academy of Sciences, 107*(1), 496-501.

Ma, K. W., Jiang, S., Hawara, E., Lee, D., Pan, S., Coaker, G., Ma, W. (2015). Two serine residues in P seudomonas syringae effector HopZ1a are required for acetyltransferase activity and association with the host co-factor. *New Phytologist, 208*(4), 1157-1168.

Mackey, D., Belkhadir, Y., Alonso, J. M., Ecker, J. R., & Dangl, J. L. (2003). Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. *Cell, 112*(3), 379-389.

Maekawa, T., Kufer, T. A., & Schulze-Lefert, P. (2011). NLR functions in plant and animal immune systems: so far and yet so close. *Nature immunology, 12*(9), 817-826.

Martin-Arevalillo, R., Nanao, M. H., Larrieu, A., Vinos-Poyo, T., Mast, D., Galvan-Ampudia, C., Parcy, F. (2017). Structure of the Arabidopsis TOPLESS corepressor provides insight into the evolution of transcriptional repression. *Proceedings of the National Academy of Sciences, 114*(30), 8107-8112.

Martín-Trillo, M., & Cubas, P. (2010). TCP genes: a family snapshot ten years later. *Trends in plant science, 15*(1), 31-39.

McDowell, J. M. (2011). Genomes of obligate plant pathogens reveal adaptations for obligate parasitism. *Proceedings of the National Academy of Sciences, 108*(22), 8921-8922.

McGrath, K. C., Dombrecht, B., Manners, J. M., Schenk, P. M., Edgar, C. I., Maclean, D. J., .Kazan, K. (2005). Repressor-and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. *Plant physiology, 139*(2), 949-959.

Melotto, M., Underwood, W., Koczan, J., Nomura, K., & He, S. Y. (2006). Plant stomata function in innate immunity against bacterial invasion. *Cell, 126*(5), 969-980.

Mims, C., Richardson, E., Holt Iii, B., & Dangl, J. (2004). Ultrastructure of the host pathogen interface in Arabidopsis thaliana leaves infected by the downy mildew Hyaloperonospora parasitica. *Canadian Journal of Botany, 82*(7), 1001-1008.

Mitchum, M. G., Hussey, R. S., Baum, T. J., Wang, X., Elling, A. A., Wubben, M., & Davis, E. L. (2013). Nematode effector proteins: an emerging paradigm of parasitism. *New Phytologist, 199*(4), 879-894.

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. *Proceedings of the National Academy of Sciences, 104*(49), 19613-19618.

Moreau, M., Degrave, A., Vedel, R., Bitton, F., Patrit, O., Renou, J.-P., Fagard, M. (2012). EDS1 contributes to nonhost resistance of Arabidopsis thaliana against Erwinia amylovora. *Molecular plant-microbe interactions, 25*(3), 421-430.

Mukhtar, M. S., Carvunis, A.-R., Dreze, M., Epple, P., Steinbrenner, J., Moore, J., . . . Nishimura, M. T. (2011). Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science, 333*(6042), 596-601.

Mur, L. A., Kenton, P., Atzorn, R., Miersch, O., & Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant physiology, 140*(1), 249-262.

Nawrath, C., & Métraux, J.-P. (1999). Salicylic acid induction–deficient mutants of Arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *The Plant Cell, 11*(8), 1393-1404.

Nguyen, N. H., & Cheong, J.-J. (2018). AtMYB44 interacts with TOPLESS-RELATED corepressors to suppress protein phosphatase 2C gene transcription. *Biochemical and biophysical research communications, 507*(1-4), 437-442.

Nicaise, V., Joe, A., Jeong, B. r., Korneli, C., Boutrot, F., Westedt, I., Zipfel, C. (2013). Pseudomonas HopU1 modulates plant immune receptor levels by blocking the interaction of their mRNAs with GRP7. *The EMBO journal, 32*(5), 701-712.

Nie, P., Chen, C., Yin, Q., Jiang, C., Guo, J., Zhao, H., & Niu, D. (2019). Function of miR825 and miR825* as Negative Regulators in Bacillus cereus AR156-elicited Systemic Resistance to Botrytis cinerea in Arabidopsis thaliana. *International journal of molecular sciences, 20*(20), 5032.

Nürnberger, T., & Lipka, V. (2005). Non-host resistance in plants: new insights into an old phenomenon. *Molecular plant pathology, 6*(3), 335-345.

Ohta, M., Matsui, K., Hiratsu, K., Shinshi, H., & Ohme-Takagi, M. (2001). Repression domains of class II ERF transcriptional repressors share an essential motif for active repression. *The Plant Cell, 13*(8), 1959-1968.

Park, S., Lee, C. M., Doherty, C. J., Gilmour, S. J., Kim, Y., & Thomashow, M. F. (2015). Regulation of the Arabidopsis CBF regulon by a complex low-temperature regulatory network. *The Plant Journal, 82*(2), 193-207.

Parker, J. E., Coleman, M. J., Szabò, V., Frost, L. N., Schmidt, R., van der Biezen, E. A., Jones, J. (1997). The Arabidopsis downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6. *The Plant Cell, 9*(6), 879-894.

Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D., & Daniels, M. J. (1996). Characterization of eds1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes. *The Plant Cell, 8*(11), 2033-2046.

Pauwels, L., Barbero, G. F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A. C., . . . Gil, E. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. *Nature, 464*(7289), 788-791.

Pauwels, L., & Goossens, A. (2011). The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *The Plant Cell, 23*(9), 3089-3100.

Pérez, A. C., & Goossens, A. (2013). Jasmonate signalling: a copycat of auxin signalling? *Plant, cell & environment, 36*(12), 2071-2084.

Petre, B., & Kamoun, S. (2014). How do filamentous pathogens deliver effector proteins into plant cells? *PLoS Biol, 12*(2), e1001801.

Petre, B., Saunders, D. G., Sklenar, J., Lorrain, C., Win, J., Duplessis, S., & Kamoun, S. (2015). Candidate effector proteins of the rust pathogen Melampsora larici-populina target diverse plant cell compartments. *Molecular plant-microbe interactions, 28*(6), 689-700.

Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. (2012). Hormonal modulation of plant immunity. *Annual review of cell and developmental biology, 28*, 489-521.

Pruitt, R. N., Schwessinger, B., Joe, A., Thomas, N., Liu, F., Albert, M., Chen, H. (2015). The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. *Science advances, 1*(6), e1500245.

Rallapalli, G., Kemen, E. M., Robert-Seilaniantz, A., Segonzac, C., Etherington, G. J., Sohn, K. H., Jones, J. D. (2014). EXPRSS: an Illumina based high-throughput expression-profiling method to reveal transcriptional dynamics. *BMC genomics, 15*(1), 1-18.

Ramonell, K., Berrocal-Lobo, M., Koh, S., Wan, J., Edwards, H., Stacey, G., & Somerville, S. (2005). Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen Erysiphe cichoracearum. *Plant physiology, 138*(2), 1027-1036.

Rehmany, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., Beynon, J. L. (2005). Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two Arabidopsis lines. *The Plant Cell, 17*(6), 1839-1850.

Rehmany, A. P., Grenville, L. J., Gunn, N. D., Allen, R. L., Paniwnyk, Z., Byrne, J., Beynon, J. L. (2003). A genetic interval and physical contig spanning the Peronospora parasitica (At) avirulence gene locus ATR1Nd. *Fungal Genetics and Biology, 38*(1), 33-42.

Robert-Seilaniantz, A., Grant, M., & Jones, J. D. (2011). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual review of phytopathology, 49*, 317-343.

Robert, X., & Gouet, P. (2014). Deciphering key features in protein structures with the new ENDscript server. *Nucleic acids research, 42*(W1), W320-W324.

Robinson, M. D., & Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome biology, 11*(3), 1-9.

Sato, M., Tsuda, K., Wang, L., Coller, J., Watanabe, Y., Glazebrook, J., & Katagiri, F. (2010). Network modeling reveals prevalent negative regulatory relationships between signaling sectors in Arabidopsis immune signaling. *PLoS Pathog, 6*(7), e1001011.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods, 9*(7), 671-675.

Schornack, S., Ballvora, A., Gürlebeck, D., Peart, J., Ganal, M., Baker, B., Lahaye, T. (2004). The tomato resistance protein Bs4 is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. *The Plant Journal, 37*(1), 46-60.

Shan, W., Cao, M., Leung, D., & Tyler, B. M. (2004). The Avr1b locus of Phytophthora sojae encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene Rps 1b. *Molecular plant-microbe interactions, 17*(4), 394-403.

Shang, Y., Li, X., Cui, H., He, P., Thilmony, R., Chintamanani, S., Zhou, J.-M. (2006). RAR1, a central player in plant immunity, is targeted by Pseudomonas syringae effector AvrB. *Proceedings of the National Academy of Sciences, 103*(50), 19200-19205.

Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., Browse, J. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature, 468*(7322), 400-405.

Shen, Y., & Diener, A. C. (2013). Arabidopsis thaliana RESISTANCE TO FUSARIUM OXYSPORUM 2 implicates tyrosine-sulfated peptide signaling in susceptibility and resistance to root infection. *PLoS genetics, 9*(5), e1003525.

Shigenaga, A. M., Berens, M. L., Tsuda, K., & Argueso, C. T. (2017). Towards engineering of hormonal crosstalk in plant immunity. *Current opinion in plant biology, 38*, 164-172.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Kaku, H. (2010). Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *The Plant Journal, 64*(2), 204-214.

Shyu, C., Figueroa, P., DePew, C. L., Cooke, T. F., Sheard, L. B., Moreno, J. E., Howe, G. A. (2012). JAZ8 lacks a canonical degron and has an EAR motif that mediates transcriptional repression of jasmonate responses in Arabidopsis. *The Plant Cell, 24*(2), 536-550.

Singh, P., Kuo, Y.-C., Mishra, S., Tsai, C.-H., Chien, C.-C., Chen, C.-W., Chinchilla, D. (2012). The lectin receptor kinase-VI. 2 is required for priming and positively regulates Arabidopsis pattern-triggered immunity. *The Plant Cell, 24*(3), 1256-1270.

Slusarenko, A. J., & Schlaich, N. L. (2003). Downy mildew of Arabidopsis thaliana caused by Hyaloperonospora parasitica (formerly Peronospora parasitica). *Molecular plant pathology, 4*(3), 159-170.

Song, C.-P., & Galbraith, D. W. (2006). AtSAP18, an orthologue of human SAP18, is involved in the regulation of salt stress and mediates transcriptional repression in Arabidopsis. *Plant molecular biology, 60*(2), 241-257.

Soylu, S., Keshavarzi, M., Brown, I., & Mansfield, J. W. (2003). Ultrastructural characterisation of interactions between Arabidopsis thaliana and Albugo candida. *Physiological and molecular plant pathology, 63*(4), 201-211.

Spencer-Phillips, P. T. (1997). Function of fungal haustoria in epiphytic and endophytic infections. *Advances in Botanical Research, 24*, 309-333.

Spoel, S. H., Koornneef, A., Claessens, S. M., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., . . . Kazan, K. (2003). NPR1 modulates cross-talk between salicylate-and jasmonate-dependent defense pathways through a novel function in the cytosol. *The Plant Cell, 15*(3), 760-770.

Strauß, T., van Poecke, R. M., Strauß, A., Römer, P., Minsavage, G. V., Singh, S., Lee, H.-A. (2012). RNA-seq pinpoints a Xanthomonas TAL-effector activated resistance gene in a large-crop genome. *Proceedings of the National Academy of Sciences, 109*(47), 19480-19485.

Szemenyei, H., Hannon, M., & Long, J. A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science, 319*(5868), 1384-1386.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.-S., Han, B., Katagiri, F. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae. *The Plant Cell, 15*(2), 317-330.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Browse, J. (2007). JAZ repressor proteins are targets of the SCFCOI1 complex during jasmonate signalling. *Nature, 448*(7154), 661-665.

Thomma, B. P., Nürnberger, T., & Joosten, M. H. (2011). Of PAMPs and effectors: the blurred PTI-ETI dichotomy. *The Plant Cell, 23*(1), 4-15.

Tian, D., Wang, J., Zeng, X., Gu, K., Qiu, C., Yang, X., Murata-Hori, M. (2014). The rice TAL effector–dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *The Plant Cell, 26*(1), 497-515.

Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., Su, Z. (2017). agriGO v2. 0: a GO analysis toolkit for the agricultural community, 2017 update. *Nucleic acids research, 45*(W1), W122-W129.

Tiwari, S. B., Hagen, G., & Guilfoyle, T. J. (2004). Aux/IAA proteins contain a potent transcriptional repression domain. *The Plant Cell, 16*(2), 533-543.

Todesco, M., Balasubramanian, S., Hu, T. T., Traw, M. B., Horton, M., Epple, P., Lanz, C. (2010). Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. *Nature, 465*(7298), 632-636.

Tomé, D. F., Steinbrenner, J., & Beynon, J. L. (2014). A growth quantification assay for Hyaloperonospora arabidopsidis isolates in Arabidopsis thaliana. In *Plant-Pathogen Interactions* (pp. 145-158): Springer.

Toruño, T. Y., Stergiopoulos, I., & Coaker, G. (2016). Plant-pathogen effectors: cellular probes interfering with plant defenses in spatial and temporal manners. *Annual review of phytopathology, 54*, 419-441.

Tsuda, K., & Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. *Current opinion in plant biology, 13*(4), 459-465.

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., & Katagiri, F. (2009). Network properties of robust immunity in plants. *PLoS Genet, 5*(12), e1000772.

Tyler, B. M., Tripathy, S., Zhang, X., Dehal, P., Jiang, R. H., Aerts, A., Beynon, J. L. (2006). Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science, 313*(5791), 1261-1266.

van den Burg, H. A., & Takken, F. L. (2009). Does chromatin remodeling mark systemic acquired resistance? *Trends in plant science, 14*(5), 286-294.

Van Der Biezen, E. A., Freddie, C. T., Kahn, K., Parker¶, J. E., & Jones, J. D. (2002). Arabidopsis RPP4 is a member of the RPP5 multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. *The Plant Journal, 29*(4), 439-451.

Van Kan, J., Van't Klooster, J., Wagemakers, C., Dees, D., & Van der Vlugt-Bergmans, C. (1997). Cutinase A of Botrytis cinerea is expressed, but not essential, during penetration of gerbera and tomato. *Molecular plant-microbe interactions, 10*(1), 30-38.

van Verk, M. C., Bol, J. F., & Linthorst, H. J. (2011). WRKY transcription factors involved in activation of SA biosynthesis genes. *BMC plant biology, 11*(1), 1-12.

Verhage, A., Vlaardingerbroek, I., Raaijmakers, C., Van Dam, N., Dicke, M., Van Wees, S., & Pieterse, C. M. (2011). Rewiring of the jasmonate signaling pathway in Arabidopsis during insect herbivory. *Frontiers in plant science, 2*, 47.

Vlot, A. C., Dempsey, D. M. A., & Klessig, D. F. (2009). Salicylic acid, a multifaceted hormone to combat disease. *Annual review of phytopathology, 47*, 177-206.

Voinnet, O., Rivas, S., Mestre, P., & Baulcombe, D. (2003). Retracted: An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *The Plant Journal, 33*(5), 949-956.

Wang, G., Ellendorff, U., Kemp, B., Mansfield, J. W., Forsyth, A., Mitchell, K., Zipfel, C. (2008). A genome-wide functional investigation into the roles of receptor-like proteins in Arabidopsis. *Plant physiology, 147*(2), 503-517.

Wang, J., & Chai, J. (2020). Structural insights into the plant immune receptors PRRs and NLRs. *Plant physiology, 182*(4), 1566-1581.

Wang, L., Albert, M., Einig, E., Fürst, U., Krust, D., & Felix, G. (2016). The pattern-recognition receptor CORE of Solanaceae detects bacterial cold-shock protein. *Nature plants*, *2*(12), 1-9.

Wang, L., Kim, J., & Somers, D. E. (2013). Transcriptional corepressor TOPLESS complexes with pseudoresponse regulator proteins and histone deacetylases to regulate circadian transcription. *Proceedings of the National Academy of Sciences*, *110*(2), 761-766.

Wang, Q., Han, C., Ferreira, A. O., Yu, X., Ye, W., Tripathy, S., Sui, Y. (2011). Transcriptional programming and functional interactions within the Phytophthora sojae RXLR effector repertoire. *The Plant Cell*, *23*(6), 2064-2086.

Wang, S., Boevink, P. C., Welsh, L., Zhang, R., Whisson, S. C., & Birch, P. R. (2017). Delivery of cytoplasmic and apoplastic effectors from Phytophthora infestans haustoria by distinct secretion pathways. *New Phytologist*, *216*(1), 205-215.

Weigel, R. R., Pfitzner, U. M., & Gatz, C. (2005). Interaction of NIMIN1 with NPR1 modulates PR gene expression in Arabidopsis. *The Plant Cell*, *17*(4), 1279-1291.

Weßling, R., Epple, P., Altmann, S., He, Y., Yang, L., Henz, S. R., Gläßer, C. (2014). Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell host & microbe*, *16*(3), 364-375.

Whisson, S. C., Boevink, P. C., Moleleki, L., Avrova, A. O., Morales, J. G., Gilroy, E. M., Chapman, S. (2007). A translocation signal for delivery of oomycete effector proteins into host plant cells. *Nature*, *450*(7166), 115-118.

Windram, O., Madhou, P., McHattie, S., Hill, C., Hickman, R., Cooke, E., Breeze, E. (2012). Arabidopsis defense against Botrytis cinerea: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. *The Plant Cell*, *24*(9), 3530-3557.

Wirthmueller, L., Asai, S., Rallapalli, G., Sklenar, J., Fabro, G., Kim, D. S., Kangasjärvi, J. (2018). Arabidopsis downy mildew effector HaRxL106 suppresses plant immunity by binding to RADICAL-INDUCED CELL DEATH1. *New Phytologist*, *220*(1), 232-248.

Woods-Tör, A., Studholme, D. J., Cevik, V., Telli, O., Holub, E. B., & Tör, M. (2018). A suppressor/avirulence gene combination in Hyaloperonospora arabidopsidis determines race specificity in Arabidopsis thaliana. *Frontiers in plant science*, *9*, 265.

Worley, C. K., Zenser, N., Ramos, J., Rouse, D., Leyser, O., Theologis, A., & Callis, J. (2000). Degradation of Aux/IAA proteins is essential for normal auxin signalling. *The Plant Journal*, *21*(6), 553-562.

Wu, J., Liu, Z., Zhang, Z., Lv, Y., Yang, N., Zhang, G., Joosten, M. H. (2016). Transcriptional regulation of receptor-like protein genes by environmental stresses and hormones and their overexpression activities in Arabidopsis thaliana. *Journal of experimental botany*, *67*(11), 3339-3351.

Xu, B., Cheval, C., Laohavisit, A., Hocking, B., Chiasson, D., Olsson, T. S., Gilliham, M. (2017). A calmodulin-like protein regulates plasmodesmal closure during bacterial immune responses. *New Phytologist*, *215*(1), 77-84.

Xu, R.-Q., Blanvillain, S., Feng, J.-X., Jiang, B.-L., Li, X.-Z., Wei, H.-Y., Chen, B. (2008). AvrACXcc8004, a type III effector with a leucine-rich repeat domain from Xanthomonas campestris pathovar campestris confers avirulence in vascular tissues of Arabidopsis thaliana ecotype Col-0. *Journal of bacteriology*, *190*(1), 343-355.

Yang, Z., Tian, L., Latoszek-Green, M., Brown, D., & Wu, K. (2005). Arabidopsis ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. *Plant molecular biology*, *58*(4), 585-596.

Yin, J., Gu, B., Huang, G., Tian, Y., Quan, J., Lindqvist-Kreuze, H., & Shan, W. (2017). Conserved RXLR effector genes of Phytophthora infestans expressed at the early stage of potato infection are suppressive to host defense. *Frontiers in plant science, 8*, 2155.

Yuan, P., Du, L., & Poovaiah, B. (2018). Ca2+/Calmodulin-dependent AtSR1/CAMTA3 plays critical roles in balancing plant growth and immunity. *International journal of molecular sciences, 19*(6), 1764.

Zhang, J., & Zhou, J.-M. (2010). Plant immunity triggered by microbial molecular signatures. *Molecular Plant, 3*(5), 783-793.

Zhang, S., Li, C., Wang, R., Chen, Y., Shu, S., Huang, R., Yao, N. (2017). The Arabidopsis mitochondrial protease FtSH4 is involved in leaf senescence via regulation of WRKY-dependent salicylic acid accumulation and signaling. *Plant physiology, 173*(4), 2294-2307.

Zhang, W., Fraiture, M., Kolb, D., Löffelhardt, B., Desaki, Y., Boutrot, F. F., Brunner, F. (2013). Arabidopsis receptor-like protein30 and receptor-like kinase suppressor of BIR1-1/EVERSHED mediate innate immunity to necrotrophic fungi. *The Plant Cell, 25*(10), 4227-4241.

Zhang, W., Zhao, F., Jiang, L., Chen, C., Wu, L., & Liu, Z. (2018). Different pathogen defense strategies in Arabidopsis: more than pathogen recognition. *Cells, 7*(12), 252.

Zhang, Y., Goritschnig, S., Dong, X., & Li, X. (2003). A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in suppressor of npr1-1, constitutive 1. *The Plant Cell, 15*(11), 2636-2646.

Zheng, X., McLellan, H., Fraiture, M., Liu, X., Boevink, P. C., Gilroy, E. M., Birch, P. R. (2014). Functionally redundant RXLR effectors from Phytophthora infestans act at different steps to suppress early flg22-triggered immunity. *PLoS Pathogens, 10*(4), e1004057.

Zhu, Z., An, F., Feng, Y., Li, P., Xue, L., Mu, A., Li, W. (2011). Derepression of ethylene-stabilized transcription factors (EIN3/EIL1) mediates jasmonate and ethylene signaling synergy in Arabidopsis. *Proceedings of the National Academy of Sciences, 108*(30), 12539-12544.

Zhu, Z., Xu, F., Zhang, Y., Cheng, Y. T., Wiermer, M., Li, X., & Zhang, Y. (2010). Arabidopsis resistance protein SNC1 activates immune responses through association with a transcriptional corepressor. *Proceedings of the National Academy of Sciences, 107*(31), 13960-13965.

Zimmerli, L., Métraux, J.-P., & Mauch-Mani, B. (2001). β-Aminobutyric acid-induced protection of Arabidopsis against the necrotrophic fungus Botrytis cinerea. *Plant physiology, 126*(2), 517-523.

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D., Boller, T., & Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. *Cell, 125*(4), 749-760.

Zuo, J., Niu, Q. W., & Chua, N. H. (2000). An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. *The Plant Journal, 24*(2), 265-273.

8 Own work

Experiments, data analysis and writing of the present thesis, unless otherwise indicated, were all done by myself, except RNA seq was performed by Sarah Harvey.

9 Supplemental data

Table S8.1 Primer list

Primer name	Sequence (5'-3')	Use
AD_Fw	CGCGTTTGGAATCACTACAGGG	Validation for Y-2-H PCR
DB_Fw	GGCTTCAGTGGAGACTGATATGCCTC	
Term_Rv	GGAGACTTGACCAAACCTCTGGCG	
M13_N_F	GTAAAACGACGGCCAGTC	Gateway cloning validation
M13_N_R	AACAGCTATGACCATG	
oligoDt	TTTTTTTTTTTTTTTTTTT	cDNA synthesis
RanHex	NNNNNN	
HaRxL21_delEAR_SpeI_Rev	CGACTAGTTTCACGGCGTTTCTTTACGATAG	Cloning Primer Per8 vector
HaRxl21_FMFTF_SpeI_Rev	CGACTAGTTTCAGTTGAACGTGAACATAAAATG	
HaRxl21_AMATA_SpeI_Rev	CGACTAGTTTCAGTTGATCGTTATCATAATATG	
TPL_KpnI_Fwd	GGGGGGTACCATGTCTTCTCTTAGTAGAGAGCTCG	Cloning Primer pCSVMV1300HA
TPL_XmaI_Rev(-stop)	GGGGCCCGGGACTCTCTGAGGCTGATCAGATGCGA	
Myb73_Rev(X)_GW	AGAAAGCTGGGTCTCACTACTCCATCTTCCCAATTC	Gateway cloning
Myb73_Rev(O)_GW	AGAAAGCTGGGTCCTACTCCATCTTCCCAATTC	
Myb73_For_GW	AAAAAGCAGGCTCCACCATGTCAAACCCGACCCGTAAG	
216SK_023478_LP	CTCTCCACGCTCGATGTTAAC	Genotyping of myb knockout lines
216SK_023478_RP	CCATGTTTCTGAACAAGCCTC	
220SK_067655_LP	TCAGTTTGGTAACAAGTGGGC	
220SK_067655_RP	AAAATCCCCAATCGATTCAAG	
233-38GK-197F10_LP	TTATCAATGAATCCTCCGACG	
233-38GK-197F10_RP	TCGTCTTCTCAACGGTCGTAC	
LBb1.3_Salk	ATTTTGCCGATTTCGGAAC	
LB_GABI	CGCTGCGGACATCTACATTTTTG	
HaRxL21_For	GTATTCGCCCAAACGTACCC	qPCR Primer
HaRxL21_Rev	TGTTCACCTCCTTCGCTTCT	
UBQ5_RT_F	AAGAAGACTTACACCAAGCCGAAG	qPCR Primer (ref gene)
UBQ5_RT_R	ACAGCGAGCTTAACCTTCTTATGC	qPCR Primer (ref gene)
At-Act2_F	ACCTTGCTGGACGTGACCTTACTGAT	qPCR Primer (ref gene)
At-Act2_R	GTTGTCTCGTGGATTCCAGCAGCTT	qPCR Primer (ref gene)
DUF1262_For	TGCCACTTCTGTTGCTCCTAACG	qPCR Primer for RNAseq
DUF1262_Rev	AGATGTCGAGTACTCAGCGTTCC	
RLP23_For	AGGCGGCTATGGTTATACAGATGC	
RLP23_Rev	AAGTGAGGGCCTTAGCTTGCTC	
CML47_For	AGTTGTTGTCCCAAATCGAGCAG	

CML47_Rev	TCCGAGTCATCGATTATCGCCTTG	
CML41_For	AAAGAGTCACCAGGCCTCCAAAG	
CML41_Rev	TCGCTGTCGAAATGGCTGAAGAC	
WRKY63_For	AACATCGATCACAAGGCTGTGG	
WRKY63_Rev	TCTTGAGGATGTTAGCGCATCCC	
RLP6_For	ACTCGGGACTCTCTCTTCTCT	
RLP6_Rev	TGAAACTGTGTGCCTTGTGG	
RLP20_For	TGTGTCTCCCCTTCAAGCTT	
RLP20_Rev	TGTGGGGATGACAACGTACT	
NAC019_For	ACGGAGGAGGAAGTCGAGAG	
NAC019_Rev	CAACTTGCCCCGAATACCCA	
TUB4_For	AGGGAAACGAAGACAGCAAG	
TUB4_Rev	GCTCGCTAATCCTACCTTTGG	
NAC019_For	GTATCCAAGAAACTGACCCGTTAAC	ChIP-qPCR Primer
NAC019_Rev	GAAGAGAGAAATCGTAACCAGCTG	
AED1_For	AATGACATCAACATCTAGCTGACG	
AED1_Rev	CGCGTACTAAAGGGGTTGTAAC	
STMP6_For	CGACTAATATGAAATGGCAAAGAAGTC	
STMP6_Rev	ACCTACAAGCCTACAAGATTCC	
WRKY63_For	GTAGGGCCGAAGTGGGAAAG	
WRKY63_Rev	GGAAACTTCAATATTTGAAAGTTCCC	
AT5G44574_For	CCTGGTTTGAATTTAATTAAAACCG	
AT5G44574_Rev	CCAATAGATAAGGATACACAGTTGGG	
HR4_For	CCACCATTTTCCTCTTCTGCTTC	
HR4_Rev	GCGTCACTTTCACTCTCACTG	
CRK38_For	CCCTTAGTTCATACGAAGTGATTAAG	
CRK38_Rev	GTAATGGTTGACTTCAAAGATGGCC	
ACT2_For	CGTTTCGTTTCCTTAGTGTTAGCT	
ACT2_Rev	AGCGAACGGATCTAGAGACTCACCTTG	

Table S8.2 Medium used in this study

Medium	Recipes per liter
HA-Agar	10 g malt extract, 4 g glucose, 4 g yeast extract, 15 g agar; adjust to pH5.5.
LB	10 g tryptone peptone, 5 g yeast extract, 10 g NaCl, 10 g agar (for solid medium). For using with zeocin, use 5 g NaCl instead of 10 g.
YEB	5 g beef extract, 1 g yeast extract, 5 g peptone, 5 g sucrose, 0.5 g $MgCl_2$, 15 g agar (for solid medium).
YPDA	10 g yeast extract, 20 g peptone, 50 ml 40% glucose, 15 mL 65mM adenine, 20 g agar.
SC	6.7 g yeast nitrogen base without amino acids and with ammonium sulfate, 20 g agar, 20 g glucose, amino acid mix as needed.
½ MS	2.2g MS salt including vitamin, 10g Sucrose, 0.5g MES; adjust to pH5.4 with KOH; add 3~4 g gelrite.

½ PDB	12 g Potato dextrose broth

Table S8.3 GO term for RxL21 DEGs

Go term for down regulated genes in RxL21 in Mock

GO term	Ontology	Description	Number in input list	p-value	FDR
GO:0019748	P	secondary metabolic process	7	0.0003	0.031
GO:0050896	P	response to stimulus	22	0.00055	0.031
GO:0051707	P	response to other organism	7	0.00096	0.037
GO:0009607	P	response to biotic stimulus	7	0.0014	0.04
GO:0012505	C	endomembrane system	19	0.001	0.043

Go term for up-regulated genes in RxL21 in Mock

GO term	Ontology	Description	Number in input list	p-value	FDR
GO:0019748	P	secondary metabolic process	15	1.70E-05	0.0059
GO:0006952	P	defense response	18	7.70E-05	0.013
GO:0009699	P	phenylpropanoid biosynthetic process	7	0.00021	0.024
GO:0019825	F	oxygen binding	14	4.70E-08	5.20E-06
GO:0003824	F	catalytic activity	122	3.10E-08	5.20E-06
GO:0050660	F	FAD binding	7	6.30E-06	0.00046
GO:0016491	F	oxidoreductase activity	28	3.20E-05	0.0018
GO:0016740	F	transferase activity	48	7.40E-05	0.0032
GO:0048037	F	cofactor binding	9	0.00024	0.0089
GO:0046872	F	metal ion binding	30	0.001	0.022
GO:0043169	F	cation binding	31	0.001	0.022
GO:0043167	F	ion binding	31	0.001	0.022
GO:0046914	F	transition metal ion binding	26	0.00087	0.022
GO:0050662	F	coenzyme binding	7	0.0012	0.023
GO:0012505	C	endomembrane system	68	4.60E-12	3.60E-10
GO:0005576	C	extracellular region	12	6.00E-06	0.00023
GO:0048046	C	apoplast	7	0.00089	0.023

Go term for down regulated genes in RxL21 upon Flg22 treatment only

GO term	Ontology	Description	Number in input list	p-value	FDR
GO:0016798	F	hydrolase activity, acting on glycosyl bonds	7	1.20E-05	6.00E-04
GO:0004553	F	hydrolase activity, hydrolyzing O-glycosyl compounds	5	0.00067	1.70E-02
GO:0003824	F	catalytic activity	25	0.0011	1.80E-02

Go term for up regulated genes in RxL21 upon Flg22 treatment only

O term	Ontology	Description	Number in	p-value	FDR

			input list		
GO:0012505	C	endomembrane system	15	0.00049	2.70E-02

Table S8.4 Genes used for qPCR for RNAseq validation

Details of the genes used for qPCR for RNAseq verification. Gene descriptions are from Araport 11. Adj.pval indicates significance after limma-voom pipeline and Benjamini Hochberg false discovery rate correction. Log2 fold change (FC) indicates expression in HA:RxL21 lines compared to HA:RxL21ΔEAR lines. Flg22 specific DEGs only show significant differential expression after flg22 induction. Mock / flg22 independent DEGs are DE under mock and/or mock and flg22 conditions. Comparison is shown to B. cinerea responsive DEGs from Windram et al. (2012) and DEGs during Pst infection (Lewis et al. 2015), including (Column 8) description of Pst expression type from Figure 4 (Lewis et al, 2015) where applicable.

Gene identi	Gene name and description.	Comment	log2FC	log2FC	*B.C.*	*Pst*	*Pst* (2)
AT1G66600	AtWRKY63. A member of WRKY Transcription Factor; Group III. Involved in the regulation of plant responses to ABA and drought stress.	Mock / flg22 independent DEG	-1.111				
AT1G52890	NAC DOMAIN CONTAINING PROTEIN 19 (NAC019). Encodes a NAC transcription factor whose expression is induced by drought, high salt, and	Mock / flg22 independent DEG	-1.074		up	Differentially Expressed between all three treatments	Amplification of Defense Response by Up-Regulation
AT2G32680	RECEPTOR LIKE PROTEIN 23 (RLP23). Known to improve plant resistance to fungal and oomycete pathogens.	Mock / flg22 independent DEG	-2.619		down	Differentially Expressed between Mock and DC3000, and between DC3000hrpA and DC3000 but not between Mock and DC3000hrpA treatment.	DC3000-Specific Down-Regulation
AT2G25440	RECEPTOR LIKE PROTEIN 20 (RLP20)	flg22 specific DEG		-1.39			
AT1G45616	RECEPTOR LIKE PROTEIN 6 (RLP6).	Mock / flg22 independent DEG	-1.682	-1.42	up		
AT3G50770	CALMODULIN-LIKE 41 (CML41). Calcium ion binding protein.	Mock / flg22 independent DEG	-1.533	-1.40	up		
AT3G47480	Y CALMODULIN-LIKE 47 (CML47) Calcium-binding EF-hand family protein.	Mock / flg22 independent DEG	-1.479	-1.59	up	Differentially Expressed between Mock and DC3000hrpA, and between Mock and DC3000 but not between DC3000hrpA and DC3000.	
AT1G13470	hypothetical protein (DUF1262).	Mock / flg22 independent DEG	-1.632	-1.80		Differentially Expressed between DC3000hrpA and DC3000 but not between any other treatments	

Table S8.5 Overlap between genes associated with TPR1 binding sites and genes differentially regulated in Arabidopsis plants expressing the RxL21 effector.

RxL21 induced" or "RxL21 repressed" indicates genes which show significantly higher or lower expression respectively in HA:21-expressing plants compared to HA:21ΔEAR-expressing plants. Number of genes for each comparison is shown with overlap to TPR1 targets in brackets. P values indicate significance of overlap between each group of genes with TPR1 targets. Genes selected for verification by ChIP-PCR are indicated in bold (Column 4). Gene descriptions taken from Araport 11.

Group of genes	Total genes in the set	p-value from Fisher's exact test	GeneIDs which Overlap with TPR1 targets	Gene Name and Description
RxL21 induced flg22	152 (3)	0.222	AT5G47990	CYTOCHROME P450, FAMILY 705, SUBFAMILY A, POLYPEPTIDE 5 (CYP705A5), THALIAN-DIOL DESATURASE (THAD1). Encodes an endomembrane system-expressed member of the CYP705A family of cytochrome P450 enzymes.
			AT4G17490	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 6 (ERF6). Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ATERF-6). It is involved in the response to reactive oxygen species and light stress.
			AT5G60760	P-loop containing nucleoside triphosphate hydrolases superfamily protein.
RxL21 induced mock	316 (12)	0.888	AT5G57620	MYB DOMAIN PROTEIN 36. MYB36 is a transcriptional regulator that acts to promote differentiation of the endodermis during root development.
			AT3G46700	0UDP-Glycosyltransferase superfamily protein.
			AT4G27260	WES1; encodes an IAA-amido synthase that conjugates Asp and other amino acids to auxin in vitro. It is involved in camalexin biosynthesis via conjugating indole-3-carboxylic acid (ICA) and cysteine (Cys).
			AT4G17490	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 6 (ERF6). Encodes a member of the ERF (ethylene response factor) subfamily B-3 of ERF/AP2 transcription factor family (ATERF-6). It is involved in the response to reactive oxygen species and light stress.
			AT1G26410	ATBBE6; FAD-binding Berberine family protein.
			AT5G65600	L-TYPE LECTIN RECEPTOR KINASE IX.2 (LECRK-IX.2) Concanavalin A-like lectin protein kinase family protein.
			AT5G50300	*ARABIDOPSIS THALIANA* AZA-GUANINE RESISTANT2 (ATAZG2). Encodes a homolog of the adenine-guanine-hypoxanthine transporter AzgA of Aspergillus nidulans. Function as a plant adenine-guanine transporter.
			AT1G14540	PEROXIDASE 4 (PER4). Peroxidase superfamily protein.
			AT1G02440	ADP-RIBOSYLATION FACTOR D1A (ARFD1A). A member of ARF GTPase family, known to be essential for vesicle coating and uncoating and functions in GTP-binding. Gene encoding ADP-ribosylation factor and similar to other ARFs and ARF-like proteins.

			AT5G47990	CYTOCHROME P450, FAMILY 705, SUBFAMILY A, POLYPEPTIDE 5 (CYP705A5), THALIAN-DIOL DESATURASE (THAD1). Encodes an endomembrane system-expressed member of the CYP705A family of cytochrome P450 enzymes.
			AT2G28850	CYTOCHROME P450, FAMILY 710, SUBFAMILY A, POLYPEPTIDE 3 (CYP710A3).
			AT1G16150	WALL ASSOCIATED KINASE-LIKE 4 (WAKL4). Encodes a WAK-like receptor-like kinase with a cytoplasmic Ser/Thr protein kinase domain and an extracellular domain with EGF-like repeats. Likely involved in Arabidopsis root mineral responses to Zn2+, Cu2+, K+, Na+ and Ni+.
RxL21 repressed flg22	88 (9)	0.017	AT5G13320	AVRPPHB SUSCEPTIBLE 3 (PBS3) Encodes an enzyme capable of conjugating amino acids to 4-substituted benzoates. This enzyme is involved in disease-resistance signaling. It is required for the accumulation of salicylic acid, activation of defense responses, and resistance to *Pseudomonas syringae*.
			AT4G04510	CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 38 (CRK38)
			AT2G25440	RECEPTOR LIKE PROTEIN 20 (RLP20)
			AT4G30430	TETRASPANIN9 (TET9)
			AT5G44575	Hypothetical protein.
			AT2G46400	WRKY DNA-BINDING PROTEIN 46 (WRKY46). Encodes a WRKY transcription factor that contributes to the feedforward inhibition of osmotic/salt stress-dependent LR inhibition via regulation of ABA signaling and auxin homeostasis.
			AT1G74710	ENHANCED DISEASE SUSCEPTIBILITY TO ERYSIPHE ORONTII 16 (EDS16) Also called ICS1 and SID2. Encodes a protein with isochorismate synthase activity. Mutants fail to accumulate salicylic acid. Its function may be redundant with that of ICS2 (AT1G18870).
			AT1G01480	AT-ACC2; a member of the 1-aminocyclopropane-1-carboxylate (ACC) synthase gene family.
			AT5G44574	Transmembrane protein.
RxL21 repressed mock	101 (13)	0.001	AT4G18540	Transmembrane protein.
			AT4G23140	CYSTEINE-RICH RLK (RECEPTOR-LIKE PROTEIN KINASE) 6.
			AT3G28580	P-loop containing nucleoside triphosphate hydrolases superfamily protein.
			AT3G51860	CATION EXCHANGER 3 (CAX3) cation exchanger 3.
			AT5G10760	APOPLASTIC, EDS1-DEPENDENT 1 (AED1). Eukaryotic aspartyl protease family protein.
			AT1G31173	MICRORNA167D (MIR167D). Encodes a microRNA that targets ARF family members ARF6 and ARF8.
			AT1G66600	WRKY63; A member of WRKY Transcription Factor; Group III. Involved in the regulation of plant responses to ABA and drought stress.
			AT1G65500	STMP6; Secreted peptide which functions in plant growth and pathogen defense.
			AT4G38560	Phospholipase-like protein (PEARLI 4) family protein.

			AT3G50480	HOMOLOG OF RPW8 4 (HR4).
			AT1G52890	NAC DOMAIN CONTAINING PROTEIN 19 (NAC019) encodes a NAC transcription factor whose expression is induced by drought, high salt, and abscisic acid.
			AT5G44574	Transmembrane protein.
			AT5G44575	Hypothetical protein.
Control set	150 (6)	1	AT2G40670	RESPONSE REGULATOR 16 (RR16).
			AT3G60966	ATL91; RING/U-box superfamily protein.
			AT5G64320	MITOCHONDRIAL TRANSLATION FACTOR 1 (MTL1) MTL1 is a mitochondria localized PRR protein involved in mitochondrial protein translation and group II intron splicing.
			AT3G49210	WS /DGAT 6 (WSD6); can function in vitro as wax ester synthase but does not appear to be essential for cuticular wax biosynthesis.
			AT5G24660	RESPONSE TO LOW SULFUR 2 (LSU2).
			AT1G07280	Tetratricopeptide repeat (TPR)-like superfamily protein.

Table S8.6 Genes used as control set for comparison to TPR1 chipseq.

150 genes were selected based on having no significant difference in expression between HA:21 and HA:21ΔEAR lines.

	GeneID		GeneID		GeneID
1	AT5G04810	51	AT5G10910	101	AT1G29530
2	AT2G01070	52	AT4G00440	102	AT3G08740
3	AT2G07648	53	AT3G62080	103	AT5G45540
4	AT3G59440	54	AT1G15010	104	AT1G26690
5	AT3G01515	55	AT4G28080	105	AT5G66815
6	AT5G67580	56	AT5G12230	106	AT1G05610
7	AT1G10585	57	AT3G03750	107	AT1G80860
8	AT5G24318	58	AT3G01795	108	AT5G09635
9	AT2G02510	59	AT4G28330	109	AT5G26660
10	AT2G39720	60	AT1G43850	110	AT1G75890
11	AT5G34851	61	AT3G27340	111	AT1G26762
12	AT1G54830	62	AT3G15351	112	AT1G12750
13	AT3G09160	63	AT1G72660	113	AT1G54680
14	AT2G40670	64	AT2G42070	114	AT2G03330
15	AT3G58380	65	AT5G62710	115	AT3G26600
16	AT5G24105	66	AT5G42080	116	AT4G07825
17	AT1G01860	67	AT3G49210	117	AT4G35800
18	AT4G20270	68	AT3G16020	118	AT2G45350
19	AT5G60290	69	AT1G15190	119	AT2G04080
20	AT1G12430	70	AT3G02360	120	AT2G43445
21	AT2G29080	71	AT2G35500	121	AT1G06977
22	AT3G60966	72	AT3G20870	122	AT3G54085

23	AT1G76800	73	AT1G76260	123	AT1G63640
24	AT4G17140	74	AT1G50020	124	AT3G54680
25	AT4G32160	75	AT5G19980	125	AT3G43828
26	AT3G07390	76	AT5G13460	126	AT3G03810
27	AT2G46040	77	ATCG00550	127	AT5G13220
28	AT3G06670	78	AT5G59970	128	AT4G32360
29	AT1G10500	79	AT1G35910	129	AT1G69390
30	AT1G74250	80	AT1G69020	130	AT5G24660
31	AT5G20050	81	AT3G44730	131	AT1G07823
32	AT3G55030	82	AT2G43550	132	AT5G65760
33	AT4G02760	83	AT1G35516	133	AT2G05075
34	AT3G01150	84	AT5G52550	134	AT3G01640
35	AT1G79610	85	AT5G06080	135	AT3G58570
36	AT5G49800	86	AT5G25930	136	AT1G05860
37	AT1G24350	87	AT5G61660	137	AT5G43560
38	AT2G20540	88	AT5G01020	138	AT1G13195
39	AT4G16620	89	AT5G58020	139	AT2G27580
40	AT1G51355	90	AT1G07290	140	AT1G63310
41	AT2G38010	91	AT3G16010	141	AT4G18300
42	AT4G37210	92	AT3G01590	142	AT3G52561
43	AT5G64320	93	AT1G28380	143	AT1G16680
44	AT3G57170	94	AT5G00760	144	AT1G07280
45	AT2G28950	95	AT3G01770	145	AT4G33630
46	AT3G53350	96	AT5G00765	146	AT5G59330
47	AT1G48120	97	AT5G45360	147	AT3G11773
48	AT3G27320	98	AT5G09410	148	AT1G09200
49	AT5G46400	99	AT3G59980	149	AT1G04140
50	AT4G10570	100	ATMG00560	150	AT4G10360

10 Abbreviations

cDNA	complementary DNA
ddH_2O	double-distilled water
DEPC	Diethylpyrocarbonate
dNTP	Deoxyribonucleosidtriphosphate
dpi	days past infection
DTT	Dithiothreitol
eds	enhanced disease susceptibility
EDTA	Ethylenediaminetetraacetic acid
E. coli	*Escherichia coli*
GFP	Green fluorescent protein
h	hours
hpi	hours post infection
HEPES	N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid
kDa	Kilodalton
LB	Lysogeny broth
LRR	Leucin-rich-repeat
mRNA	messenger RNA
MS	Murashige & Skoog Medium
MYB	myb domain protein
Nb	*Nicotiana benthamiana*
OBE	OBERON
PBS	phosphate buffered saline
PR	pathogenesis-related protein
PRR	pattern recognition receptor
Pst	*Pseudomonas syringae* pv. *tomato*
qRT-PCR	quantitative real-time PCR
RFP	Red fluorescent protein
RNA	Ribonucleic acid

RT room temperature

RXLR arginine, any, leucine, argenine (motif)

SA Salicylic Acid

SAR Systemic Acquired Resistance

SC Synthetic complete (media)

SDS sodium dodecyl sulfate

SDS-PAGE SDS polyacrylamide gel electrophoresis

SWAP SUPPRESSOR OF WHITE APRICOT

T3SS type III secretion system

TBS Tris-buffered saline

TCP TEOSINTE BRANCHED1, CYCLOIDEA, and PCFTE

Temed Tetramethylethylendiamin

Y-2-H Yeast-two-hybrid

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