Characterization of Receptor Kinases and Downstream Signalling Components Involved in Fusarium Head Blight Resistance

by

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Abstract

Fusarium Head Blight (FHB) is a devastating disease that affects wheat, barley and other small grain crops. Despite huge economic losses, current measures have not yielded desirable resistance to FHB. Resistance to *F. graminearum* is quantitative and thus identification of putative transmembrane receptors and signalling components involved in plant immunity will help combat this disease. A reverse genetic screen using 249 T-DNA *Arabidopsis* mutant plants identified two putative leucine-rich transmembrane localized receptors, RLK7 and APEX and a downstream signalling component, RbohF as contributors to FHB resistance. The expression analysis suggested that RLK7 and APEX activated distinct hormone signalling pathways, ethylene and salicylic acid, to mediate the resistance. Our analyses indicated that as an intracellular signalling component, RbohF integrates signals from these hormone signalling pathways to regulate stomatal pore closures, a potential entry point for the pathogen. Overall, our findings identified key genes involved in mediating quantitative resistance to *F. graminearum*.

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List of Abbreviations

FHB - Fusarium Head Blight

DON - deoxynivalenol

DMI - Demethylation Inhibitors

MAMP - Microbe-Associated Molecular Patterns

PTI - Pattern-Triggered Immunity

PRR - Plant Recognition Receptor

NHR - Non-Host Resistance

NBS-LRR - Nucleotide-Binding Site Leucine Rich Repeat

ETI - Effector Triggered Immunity

SA - Salicylic Acid

SAR - Systemic Acquired Resistance

PIP - Pipecolic Acid

ICS1 - Isochorismate Synthase 1

ALD1 - AGD2-like Defense response protein 1

FMO1 - Flavin-dependent Mono-oxygenase 1

TF - Transcription Factor

SARD1 - SAR Deficient 1

CBP60g – Calmodulin-Binding Protein 60-like G

qRT-PCR – Quantitative Real Time- Polymerase Chain Reaction

DAMP – Damage Associated Molecular Pattern

MAPK – Mitogen Activated Protein Kinase

RLK – Receptor Like Kinase

RLP – Receptor-Like Protein

LRR – Leucine Rich Repeat

RLCK – Receptor Like Cytoplasmic Kinase

CPK – Calcium-dependent Protein Kinase

MEKK1 – Mitogen-Activated Protein Kinase Kinase 1

QDR – Quantitative Disease Resistance

QTL – Quantitative Trait Loci

EFR – Elongation Factor-Tu Receptor

ROS – Reactive Oxygen Species

CERK1 – Chitin Elicitor Receptor Kinase 1

WGA – Wheat Germ Agglutinin

Rboh – Respiratory burst oxidase homolog

NADPH –Nicotinamide Adenine Dinucleotide Phosphate

FAD – Flavin Adenine Dinucleotide

MYB51 – Myeloblastosis 51

UPOX – Up-regulated by oxidative stress

JAZ10 – Jasmonate ZIM- domain

ABA – Abscisic acid

PR – Pathogenesis Related

PAL – Phenylalanine

NPR – Non-expressor of PR genes

ET – Ethylene

ETR – Ethylene Receptor

ERS – Ethylene Response Sensor

EIN – Ethylene Insensitive

IOS1 - Impaired Oomycete Susceptibility 1

SDS-PAGE – Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis

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Chapter 1: Introduction

1.1 Fusarium Head Blight (FHB)- An Overview

1.1.1 Description of FHB disease and its impact on society

Fusarium Head Blight (FHB) is a devastating disease that affects wheat, barley and other small grain crops (McMullen *et al.,* 1997). Although many *Fusarium* species can cause FHB, *F. graminearum* is the predominant pathogen in many countries including North and South America, China and Eastern Europe (Bai and Shaner, 2004). Given favorable humid and warm environmental conditions, *F. graminearum* has the ability to destroy a potentially high-yielding field within a few weeks of harvest (McMullen *et al.,* 1997). FHB infects wheat kernels resulting in discoloration with a characteristic chalky white or pink color (Peiris *et al.,* 2009). Apart from discoloration of wheat heads, the disease also results in significant reduction of seed quality and yield through the accumulation of toxins (O'Donnell *et al.,* 2000; Desjardins *et al.,* 1996).

FHB has been listed as the worst plant disease after stem rust epidemics of the 1950s. In the 1990s, FHB led to an estimated economic loss of $200 million in Quebec and Ontario and around $300 million in Manitoba (Windels, 2000). FHB has also been a threat to wheat and barley in many other countries. For example, FHB resulted in loss of more than one million tons of yields during severe epidemics in China during 1951-1985 (Bai and Shaner, 2004; Zhuping, 1994). In the United States, several severe FHB outbreaks occurred between 1991-1997 on wheat and barley that resulted in cumulative economic loss of $4.8 billion (Bai and Shaner, 2004). The epidemic of 1993 was the worst of all epidemics recorded in United States history, where it alone led to loss of

seventy million tons of barley yield with an estimated economic loss of $122 million (Bai and Shaner, 2004).

F. graminearum infection results in the accumulation of a mycotoxin called deoxynivalenol (DON) in wheat heads (McMullen *et al.,* 1997). DON is categorized in the group of trichothecenes that belongs to a large group of sesquiterpenoid secondary metabolites (Khaneghah *et al.,* 2018). DON ingestion can lead to both acute and chronic toxic effects. The acute symptoms include diarrhea, abdominal discomfort and vomiting (Khaneghah *et al.,* 2018). The chronic toxic effects include altered nutritional efficiency, weight loss and anorexia (Khaneghah *et al.,* 2018). At the molecular level, trichothecenes bind to ribosome 60S, which then interacts with peptidyl transferase enzyme to inhibit protein synthesis (Khaneghah *et al.,* 2018). Thus, contaminated grains exacerbate economic losses to the farmers.

1.1.2 Life cycle of *Fusarium graminearum*

FHB disease lifecycle can be initiated with different types of inoculum such as macroconidia produced by sporodochia (cushion-shaped hyphal structures) or ascospores produced inside perithecia from mycelia surviving on maize or wheat residues (Ireta and Gilchrist, 1994; Trail, 2009). Primary infections arise from either ascospores or macroconidia deposited by wind or rain on glumes or extruded anthers of wheat (Ireta and Gilchrist, 1994). If anthers have not emerged yet, spores can remain viable for several days until anthesis and initiate germination later to penetrate anther tissues (Ireta and Gilchrist, 1994). Penetration usually occurs through natural openings such as the base of the lemma and the palea or through degenerating anther tissues (Figure 1) (Trail, 2009). At the infection site, fungus grows intracellularly without any symptoms of

infection (the biotrophic phase) (Trail, 2009). Later, the fungus spreads through xylem and pith and eventually initiates necrosis and colonizes the entire wheat head (Trail, 2009). At this stage, the colonized wheat head becomes bleached, a typical sign of FHB disease (Trail, 2009). The spread of the disease from florets within the wheat head is facilitated by the mycotoxin DON, which is synthesized almost immediately after infection (Trail, 2009). Along with the synthesis of mycotoxin, *F. graminearum* also destroys starch granules, protein storage and cellular wall in the host, which augments spread of the fungus (Ireta and Gilchrist, 1994).

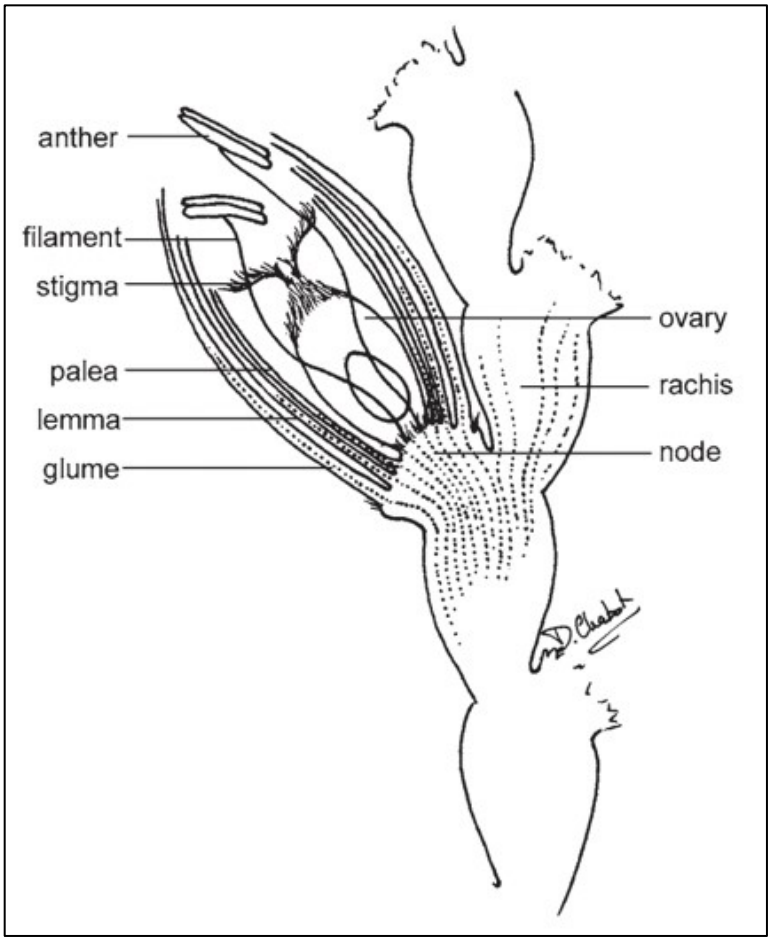


Figure 1. Diagram of a wheat floret with indicated glume, lemma, palea, stigma, filament, anther, node, rachis and ovary. Reprinted by permission from [the Licensor]: [Taylor & Francis] [Canadian Journal of Plant Pathology] [Use of a Fusarium Graminearum strain transformed with green fluorescent protein to study infection in wheat (Triticum aestivum), S. Shea Miller, Denise M.P. Chabot, et al.] [2004].

1.1.3 Current measures to mitigate FHB disease

Several agronomic and cultural practices are being followed by farmers across the world to tackle FHB. Agricultural practices such as tillage has shown to reduce initial inoculum as tillage helps to bury host crop residues (Leplat *et al.,* 2013). Buried *F. graminearum* infested residues decompose faster than non-buried residues, which leads to reduced available nutrients for *F. graminearum*'s development (Leplat *et al.,* 2013). Crop rotation is another agricultural technique that also helps reduce the initial inoculum (Leplat *et al.,* 2013). It was discovered that FHB symptoms were severe when wheat planting was followed by maize, but symptoms were less severe when planting was followed by soybean (Dill-Macky and Jones, 2000).

Application of fungicides is one of the other strategies used to tackle the disease (Dweba *et al.,* 2017). The most widely used fungicides are in the class of demethylation inhibitors (DMI) (Wegulo *et al.,* 2015). These inhibitors include metconazole, propiconazole, prothioconazole and tebuconazole (Wegulo *et al.,* 2015). These fungicides suppress FHB and DON, but do not completely eradicate the fungus. There are also many caveats associated with fungicides' use. They are most effective when they are sprayed during the time of anthesis and up to six days after anthesis (Wegulo *et al.,* 2015). This gives a very small window for farmers to spray the fields as not all crops flower at the same time. It might be challenging, time consuming and expensive for farmers to spray multiple times at specific targeted sites. Unfavorable weather conditions can also create a problem for newly sprayed fields as rainfall shortly after application could wash off fungicides from wheat heads and be less efficient at controlling FHB infection and DON accumulation (Wegulo *et al.,* 2015).

Biological control such as the use of microbial antagonists is one of the other strategies to reduce FHB infection and toxin contamination. Many bacterial species have been tested with significant reduction in FHB severity. Examples include *Bacillus subtilis* strain AS 43.3 that showed a 90% decrease in FHB severity, *Pseudomonas flourescens* strains MKB 158 and MKB 249 that showed more than 23% reduction in both FHB severity and DON contamination and *Streptomyces* sp. BRC87B that showed a potential of 100% reduction in DON production on wheat spikes (Schisler *et al.,* 2002; Khan and Doohan, 2009; Palazzini *et al.,* 2007). Fungi have also been tested to reduce FHB symptoms including *Cryptococcus* sp. OH 71.4 and *C. nodaensis* OH 182.9 that showed a 57% reduction in disease severity, but showed no reduction in DON contamination. Moreover, efficacy of these biocontrol agents is questionable as they were shown to be effective in one field trial and failed to protect crops in other field trials (Schisler *et al.,* 2002). There are many other challenges associated with the use of biological controls to tackle FHB. Some of the challenges include the time of application, best and cheapest way to apply to fields and a medium that will preserve and prolong shelf-life of the microbes (Wegulo *et al.,* 2015).

There are established and proven measures to reduce FHB severity that include conventional breeding (Bai and Shaner, 2004). Breeding programs to obtain commercial resistant FHB crops started in early 1960s and resulted in moderate resistant varieties including Wanning 2, Wumai 1, Yangmai 1 and Yangmai 2. These varieties were commercially grown for many years and provided protection during moderate epidemics (Bai and Shaner, 2004). However, not all resistant varieties can be used as commercial crops. For example, Sumai 3 is a highly resistant wheat cultivar but deployed alone

results in a decrease in overall yield. Therefore, it is only used in crosses as a resistant parent in breeding programs (Bai and Shaner, 2004). Nevertheless, Sumai 3 has been very beneficial in obtaining many resistant varieties such as Ning 7840 (Avrora/Anhui11//Sumai 3), which results in higher yield than Sumai 3 and resistance to other wheat pathogens including leaf rust, stripe rust and stem rust (Bai and Shaner, 2004). The resistant variety Ning 7840 has not been commercialized because it does not possess desired agronomic traits. Moreover, all the commercial "resistant" varieties only result in partial resistance to FHB. Despite all the preventive measures described above, no single measure can completely eradicate FHB infection. Hence, various breeding programs and molecular techniques need to be combined to achieve desirable resistance to FHB infection.

1.2 Plant defense

1.2.1 Host resistance vs. non-host resistance

Plants interact with many pathogenic microbes on a daily basis. Evolution has driven plants to develop multiple strategies to overcome pathogen attack. Pathogen resistance in plants can be divided into two layers. The first layer is mediated by the perception of evolutionary conserved microbe-associated molecular patterns (MAMPs), termed as pattern-triggered immunity (PTI) (Boller and Felix, 2009). Plants use cell surface localized pattern recognition receptors (PRRs) to detect MAMPs (Boller and Felix, 2009).

PTI is generally effective against non-adapted pathogens in a phenomenon called non-host resistance (NHR) (Dodds and Rathjen, 2010). NHR protects plants from pathogens that are adapted to other plant species (Fan and Doerner, 2012). *Arabidopsis*

has been widely used as a model to study NHR with respect to many non-adapted pathogens such as obligate biotrophic fungus *Blumeria graminis* f. sp. *Hordei* and hemibiotrophic pathogen *Magnaporthe oryzae* (Lipka *et al.,* 2005; Stein *et al.,* 2006; Nakao *et al.,* 2011). *Blumeria graminis* and *Magnaporthe oryzae* are host-adapted pathogens on barley and rice, respectively.

Arabidopsis mediates resistance to both pathogens via limiting their penetration into epidermal cells (pre-invasive resistance) and then later inhibits hyphal growth (post-invasive resistance) (Fan and Doerner, 2012). The genes mediating pre-invasive resistance include *Penetration 1 (PEN1), PEN2 and PEN3* (Lipka *et al.,* 2005; Stein *et al.,* 2006; Nakao *et al.,* 2011). *PEN1* encodes a plasma membrane-anchored syntaxin with a soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) domain, which is involved in transport of vesicle-mediated secretion of unknown cargo to pathogen contact sites (Collins *et al*., 2003). *PEN2* encodes an atypical myrosinase involved in indole gluosinolate and tryptophan-derived secondary metabolism (Lipka *et al.,* 2005). *PEN3* encodes a pleiotropic drug resistance (PDR) ATP-binding cassette (ABC) transporter, which is involved in secretion of antimicrobial products at the infection sites (Stein *et al.,* 2006). The genes mediating post-invasive resistance include *Enhanced Disease Susceptibility 1 (EDS1), Phytoalexin Deficient 4 (PAD4) and Senescence Associated Gene 101 (SAG101)* (Lipka *et al.,* 2005). EDS1 along with its interacting partners PAD4 and SAG101 restrict the growth of pathogens (Lipka *et al.,* 2005). The above examples present a complex defense network that integrates many MAMP signalling components and quantitatively contributes towards non-host resistance. NHR is very durable and protects plants from broad range of potential

pathogens and thus has been proposed as a mechanism to protect crop plants from host-adapted pathogens (Fan and Doerner, 2012).

However, adapted pathogens inhibit PTI via the secretion of "effector" proteins into the cell (Boller and Felix, 2009). The effectors compromise host resistance by interfering with many aspects of plant defense. The MAPK (Mitogen-Activated Protein Kinase) module is one of the effector targets, probably due to their central role in defense signalling. For example, the *Pseudomonas syringae* effector HopF2b ADP-ribosylates the plant MKK5 kinase and inactivates it and inhibits PTI responses (Wang *et al.,* 2010). However, to overcome PTI inhibition, plants have evolved polymorphic nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins that either directly recognize effectors or indirectly recognize these effectors and activate the second layer of immunity, which is termed as effector-triggered immunity (ETI) (Boller and Felix, 2009). Compared to PTI, ETI results in stronger defense response, which sometimes leads to programmed cell death (Boller and Felix, 2009). ETI is a cornerstone of host resistance, defined as resistance of a given plant species against certain races of pathogens that can normally colonize the host (Dodds and Rathjen, 2010). During ETI, effectors are recognized (directly or indirectly) by corresponding resistance genes in the host (Felix *et al.,* 1999). This type of resistance is referred to as 'gene-for-gene' resistance, often leads to localized cell death resulting in the death of pathogen.

A combined action of PTI and ETI result in the activation of systemic acquired resistance (SAR; Figure 2) (Hartmann and Zeier, 2019). SAR is a mechanism where previously infected plants are better able to resist any future infection. Hallmark characteristics of SAR include development of enhanced resistance in distal and

uninoculated tissues and provide long-lasting protection (can last for weeks to months and sometimes throughout entire season) against a broad spectrum of pathogens (Conrath, 2006). The plant hormone salicylic acid (SA) is a central and critical component of SAR (Singh *et al.,* 2017). Besides SA, the pipecolic acid (PIP) pathway has been recently shown to contribute to SAR maintenance (Figure 2) (Hartmann and Zeier, 2019). The *Isochorismate Synthase 1 (ICS1)*-mediated SA biosynthesis and *AGD2-like Defense response protein 1 (ALD1),* and *Flavin-dependent Mono-oxygenase 1 (FMO1)* mediated biosynthesis of pipecolic acid share common elements towards establishment of SAR (Figure 2) (Jogelkar *et al.,* 2018; Hartmann and Zeier, 2019). Two pathogen responsive transcription factors (TFs), SAR- Deficient 1 (SARD1), and Calmodulin-Binding Protein 60-like G (CBP60g) bind to the promoters of *ICS1, ALD1* and *FMO1* to activate SA and PIP biosynthesis (Wang *et al.,* 2011; Sun *et al.,* 2015). As a result of common regulatory elements, SA and PIP both combine to establish SAR (Figure 2).

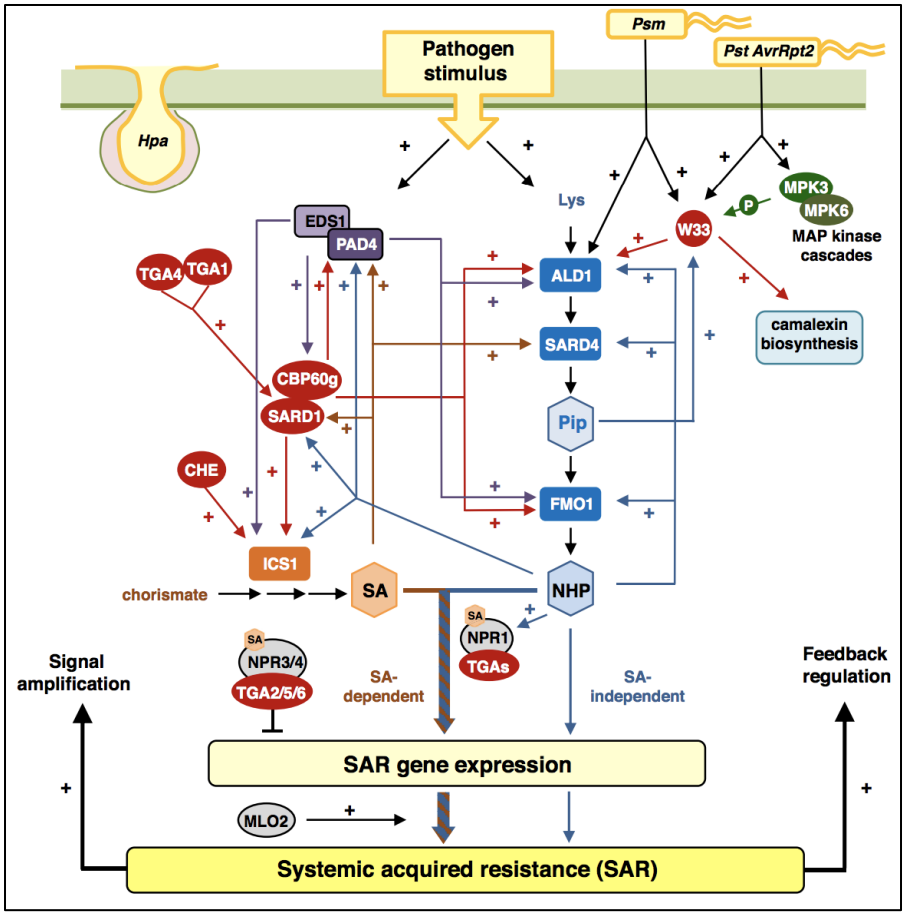


Figure 2. Systemic acquired resistance is mediated by salicylic acid and pipcolic acid. Upon pathogen attack, there is SA and NHP accumulation which are regulated by common genes such as *EDS1/PAD4* and *CBP60g/SARD1*. These genes positively regulate *ICS1, ALD1, SARD4* and *FMO1* to ultimately turn on SAR gene expression. SA: salicylic acid; NHP: *N*-hydroxypipecolic acid; EDS1: Enhanced Disease Susceptibility 1; PAD4: Phytoalexin-Deficient 4; CBP60g: Calmodulin-Binding Protein 60-like G; SARD1: SAR- Deficient 1; ICS1: Isochorismate synthase 1; ADL1: AGD2-like Defense response protein 1; FMO1: Flavin-dependent Mono-oxygenase 1; SAR: Systemic Acquired Resistance; NPR: Non-expressor of PR gene and W33: WRKY33. Reprinted by permission from [the Licensor]: [Elseiver] [Current Opinion in Plant Biology] [N-hydroxypipecolic acid and salicylic acid: a metabolic duo for systemic acquired resistance, Michael Hartmann and Jürgen Zeier] [2019].

Plant defense against *F. graminearum* is currently unknown, however, due to the absence of 'gene-for-gene' resistance between *F. graminearum* and its host, it is hypothesized that resistance to FHB is quantitative. As a result, research focus has been to identify quantitative disease resistance genes (QDRs) encoded within quantitative trait loci (QTL) and to date, genetic mapping has revealed over 100 QTL conferring resistance to *Fusarium*, distributed across all wheat chromosomes (Dweba *et al.,* 2017). One of the QTL, *fhb1* confers resistance to a broad range of *Fusarium* species, however, the mechanism by which it confers resistance is not known. Recently, one of the genes in this QTL was cloned and the sequence identified it to encode a chimeric protein with lectin and agglutinin domains, resembling a member of a PRR receptor family (Rawat *et al.,* 2016). Since many of the previously identified fungal MAMPs have lectin binding domains, this newly identified resistance protein may be a novel PRR that may potentially interact with fungal cell wall components and activate a PTI response, offering partial resistance to FHB. Recently, another gene, *TaHRC* was cloned from the same QTL with sequence identity to histidine-rich calcium-binding proteins (Su *et al.,* 2019). *TaHRC* is likely a signalling component and functions downstream of activated PRR. A link between PRR encoded by *Fhb1* and TaHRC has not been established. These new data validate the need to conduct research on identification of other QDRs involved in FHB resistance. Availability of genomics resources will greatly facilitate identification of genes in other QTL including, PRRs and signalling components.

Cloning genes from QTL poses a challenge due to large and complex genome of wheat. On the other hand, due to small, diploid, fully sequenced genome and enormous genetic resources, non-host model plant *Arabidopsis thaliana* can be used to identify

candidate QDR genes involved in *F. graminearum* resistance. This information could then be transferred to a host plant and can provide successful resistance to the pathogen. In this regard, an effort was made to introduce a *Brassicaceae*-specific PRR called Elongation factor-Tu receptor (EFR) into *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*) (Lacombe *et al.,* 2010), which provided resistance to bacteria belonging to diverse genera such as *Pseudomonas*, *Agrobacterium*, *Xanthomonas* and *Ralstonia*. The resistance provided by transgenic PRR was not limited to dicots but also shown to be applicable to monocots. According to Schoonbeek *et al.,* a transgenic activation of the PRR *AtEFR* from *Arabidopsis* by the MAMP elf18 (bacterial elongation factor-Tu) in wheat conferred resistance to the pathogen *Pseudomonas syringae* pv. *Oryzae* (Schoonbeek *et al.,* 2015). This demonstrated that immune signalling is highly conserved across distant phyla and underscores the importance of PTI and its application to developing broad-spectrum plant disease resistance in crops.

1.3 Molecular mechanism of plant defense

1.3.1 Pattern Recognition Receptors/PRR: role and location

As sessile organisms, plants have developed effective strategies to overcome pathogen attack. Plants use cell-surface localized PRRs to detect MAMPs to activate PTI (Boller and Felix, 2009). Plant PRRs fall under two categories: surface-localized receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Zipfel, 2014). RLKs have a ligand-binding ectodomain with a single-pass transmembrane domain, and an intracellular kinase domain. To date, two different types of intracellular kinase domains have been reported: Serine/Threonine kinases and Histidine kinases (Shiu and Bleecker, 2003). RLPs have a similar structure as RLKs except that lack an intracellular kinase

domain (Zipfel, 2014). Since RLPs lack a kinase domain for signalling, they function in conjunction with one or several RLKs to transduce signals (Zipfel, 2014).

The RLK family is further subdivided into transmembrane localized RLKs and cytoplasm localized RLCKs (receptor like cytoplasmic kinases- lack extracellular domain) (Shiu and Bleecker, 2001b). Evidence support the idea that post-PRR activation, transmembrane localized RLKs bind and phosphorylate RLCK to transduce MAMP signals from receptor complexes to other downstream signalling components (Lu *et al.,* 2010). RLCKs have a common monophyletic origin with RLKs and together form one of the largest gene families in the *Arabidopsis* genome with more than 610 members (Shiu and Bleecker, 2001a; Shiu and Bleecker, 2003).

The transmembrane RLKs are classified into sixteen different RLK types based on sequence variation in their extracellular domains, which are further divided into sub-families (Table 1) (Shiu and Bleecker, 2001b). Members within the same subfamily are denoted with Roman numerals for example, receptors belonging to the first subfamily with leucine-rich repeats in the extracellular domain will be identified as LRR I (Supplement Table 1). RLKs rich in leucine repeats consist of 223 members, which is the largest group of RLK members (Mott *et al.,* 2016). Since RLPs have similar extracellular domains as RLKs, the greatest number of RPLs also comes from leucine–rich domain, where 57 members are known to date (Mott *et al.,* 2016).

Table 1. Classification of RLK family members based on variations in the extracellular domain.

RLKs with different extracellular domains	Number of subfamilies
C-lectin	1
CR4-like (CRINKLY4-like)	1
CrRLK1-like (*Catharanthus roseus* RLK1 also known as malectin-like receptor kinases)	2
Extensin-like (proline rich)	1
L-lectin (Legume lectin a/b)	1
LRK10-like (Leaf rust 10 disease-resistance locus receptor-like protein kinase)	2
LRR I (Leucine Rich Repeat)	15
LysM (Lysin Motif)	1
PERK (Proline Extensin Receptor Kinase)	1
RKF3-like (Receptor-like Kinase in Flowers)	1
S-domain (Agglutinine-rich)	3
Thaumatin	1
URK1 (Unknown Receptor Kinase1)	1
WAK-like (Wall Associated Kinase)	1
RLCK (Receptor-like Cytoplasmic Kinase)	11

1.3.2 MAMPs and their function

MAMPs are microbial components that include proteins and small molecules perceived by PRRs. These MAMPs are highly conserved molecular signatures found in entire groups of microbes/pathogens and play an important role in activating plant defense (Zhang *et al.,* 2014). Evolutionary conserved, MAMPs are under high negative selection pressure, despite being recognized by host and reducing their chances of survival. As a result, they are passed on from generation to generation and stay evolutionarily consistent over a wide range of species. Due to invariant characteristic features, MAMP recognition by corresponding PRRs can be exploited to develop broad-spectrum resistance against pathogens. Listed below are examples of MAMP/PRR pairs known to date (Table 2). *Arabidopsis* genome encodes 610 receptor proteins, whereas only a handful MAMP/PRR pairs have been identified. Therefore, there is a need to identify more such pairs to develop resistant crops.

Table 2. Examples of known MAMP/PRR pairs.

MAMP	PRR	Co-receptor	Host	Origin of MAMP	References
flg22	FLS2 (LRR-RLK)	BAK1 (LRR-RLK)	*A. thaliana*	Bacteria (*P. syringae)*	Felix *et al.,*1999; Gómez-Gómez *et al.,* 2001
elf18	EFR (LRR-RLK)	BAK1 (LRR-RLK)	*Brassicaceae* species	Bacteria (*Escherichia coli*)	Kunze *et al.,* 2004
Peptidoglycans (PGNs)	LYM1/3 (LysM-RLP)	CERK1 (LysM-RLK)	*A. thaliana*	Gram-positive and gram-negative bacteria	Gust *et al.,* 2007; Erbs *et al.,* 2008; Willmann *et al.,* 2011
	LYP4/6 (LysM-RLP)	OsCERK1 (LysM-RLK)	*Oryza sativa* (rice)	Gram-positive and gram-negative bacteria	Akamatsu *et al.,* 2016
Ax21	XA21 (LRR-RLK)	SERK2	*Oryza sativa* (rice)	Bacteria (*Xanthomas oryzae* pv. *Oryzae*)	Song *et al.,* 1995; Wang *et al.,* 1998; Lee *et al.,* 2009
eMax	RLP1 (LRR-RLP)	unknown	*A. thaliana*	Bacteria (*Xanthomonas*)	Jehle *et al.,* 2013
Lipopolysaccharide (LPS)	LORE (S-lectin RLK)	unknown	*A. thaliana*	Bacteria	Ranf *et al.,* 2015
Xylanase	EIX1 and EIX2 (LRR-RLP)	BAK1 and SOBIR	*Solanum lycopersicum* (tomato)	Fungal	Bailey *et al.,* 1990; Ron and Avni, 2004

Cold shock protein (CSP22)	unknown	unknown	*Nicotiana tabacum* (tobacco)	Bacteria (*M. lysodeikticus*)	Felix and Boller, 2003
Chitin	LYK5 (LsyM-RLK)	CERK1 (LysM-RLK)	*A. thaliana*	Fungus	Felix *et al.,* 1993; Kaku *et al.,* 2006; Miya *et al.,* 2007; Shimizu *et al.,* 2010; Cao *et al.,* 2014
	CEBiP (LysM-RLP)	OsCERK1 (LysM-RLK)	*Oryza sativa* (rice)	Fungus	Kaku *et al.,* 2006; Shimizu *et al.,* 2010
Endopolygalacturonases (PGs)	RBGP1 (LRR-RLP)	unknown	*A. thaliana*	Fungus (*Botrytis cinerea*)	Zhang *et al.,* 2014
Unknown	RLP30 (LRR-RLP)	SOBIR1 (LRR-RLK)	*A. thaliana*	Fungus (*Sclerotinia sclerotorium*)	Zhang *et al.,* 2013
nlp20	RLP23 (LRR-RLP)	SOBIR1 (LRR-RLK) and BAK1 (LRR-RLK)	*A. thaliana*	Fungus	Albert *et al.,* 2015
Ave1	Ve1 (LRR-RLP)	unknown	*Solanum lycopersicum* (tomato)	Fungus	de Jonge *et al.,* 2012
pep13	unknown	unknown	*Solanum tuberosum* (potato)	Oomycete (*Phytophthora sojae*)	Brunner *et al.,* 2002
INF1	ELR (LRR-RLP)	BAK1 (LRR-RLK)	*Solanum tuberosum* (potato)	Oomycete (*Phytophthora infestans*)	Du *et al.,* 2015

1.3.3 Early defense responses during pattern induced immunity

1.3.3.1 Production of Reactive Oxygen Species (ROS)

After the perception of MAMPs, PRRs along with their co-receptors and cytoplasmic kinases turn on a cascade of signalling events leading to defense responses (Figure 3). One of the very well characterized MAMP/PRR pair is flg22/FLS2 (Zipfel, 2014). FLS2 (Flagellin sensing 2) is a leucine rich receptor kinase (LRR-RLK subfamily XII) and flg22 is a 22-amino acid domain in the bacterial flagellin protein (Zipfel *et al.,* 2004). The binding of flg22 to FLS2 initiates an interaction with a co-receptor BAK1 (BRI1 associated receptor kinase 1), which belongs to SERK (somatic embryogenesis receptor like kinase) LRR-RLK subfamily II (Chinchilla *et al.,* 2007). The receptor interactions between FLS2 and BAK1 initiates a series of phosphorylation events facilitating PTI signal transduction (Figure 3) (Lu *et al.,* 2010). Another example of MAMP/PRR pair is chitin/LYK5. Chitin (a fungal cell wall component) is perceived by LYK5 with LysM motif, which forms a complex with another co-receptor CERK1 (Chitin Elicitor Receptor Kinase 1) (Cao *et al.,* 2014). Both FLS2/BAK1 and LYK5/CERK1 complex share a common cytoplasmic kinase belonging to RLCK VII subfamily called BIK1 that plays an important role in the regulation of NADPH oxidase enzyme complex involved in the production of reactive oxygen species (ROS) (Figure 3 and Figure 4) (Couto and Zipfel, 2016). The production of ROS (H_2O_2) peaks around 10-12 minutes following MAMP perception and is involved in intracellular signal transduction process (Bigeard *et al.,* 2015). *A. thaliana* genome consists of ten NADPH oxidases or *Atrboh* (*respiratory bust oxidase homolog*) genes, sequentially named *Rboh A-J* (Suzuki *et al.,* 2011). Typically, Rbohs have six transmembrane (TM) helices, C-

terminal intracellular FAD/NADPH-binding domains and extended N-terminal region containing two Ca^{2+}-binding EF-hand motifs (Kawarazaki *et al.,* 2013). In plant pathogen interactions, RbohD has been identified as the dominant player in the production of ROS. After dissociation of BIK1 from FLS2/BAK1 or LYK5/CERK1 complex, RbohD is phosphorylated at multiple sites in a calcium independent manner (Figure 3 and Figure 4) (Kadota *et al.,* 2014). The activation of RbohD is further fine-tuned by calcium dependent protein kinases (CPKs) to produce ROS (Figure 3 and Figure 4) (Qi *et al.,* 2017).

RbohF is another respiratory burst oxidase homolog that has been identified to contribute to ROS production (Torres *et al.,* 2002). RbohF is regulated by another Ca^{2+} sensor family called calcineurin B-like protein (CBL), which plays a key role in decoding transient calcium signals and is phosphorylated by a family of protein kinases called calcineurin B-like interacting protein kinases (CIPKs) (Figure 4) (Han *et al.,* 2018). Upon MAMP perception, the plant hormone abscisic acid (ABA) signals to increase cytosolic calcium levels and activates Open Stomata 1 (OST1) kinase that phosphorylates RbohF resulting in the production of ROS (Han *et al.,* 2018). Therefore, RbohF contributes to fine tuning of ROS production through Ca^{2+} activated CBL/CIPK26 complex and ABA-activated OST1 kinase (Figure 4) (Han *et al.,* 2018). In summary, studies show that RbohD contributes more to the ROS pool than RbohF, however, the latter contributes to initiating local cell death via positively regulating salicylic acid (SA) levels (Figure 4) (Chaouch *et al.,* 2012).

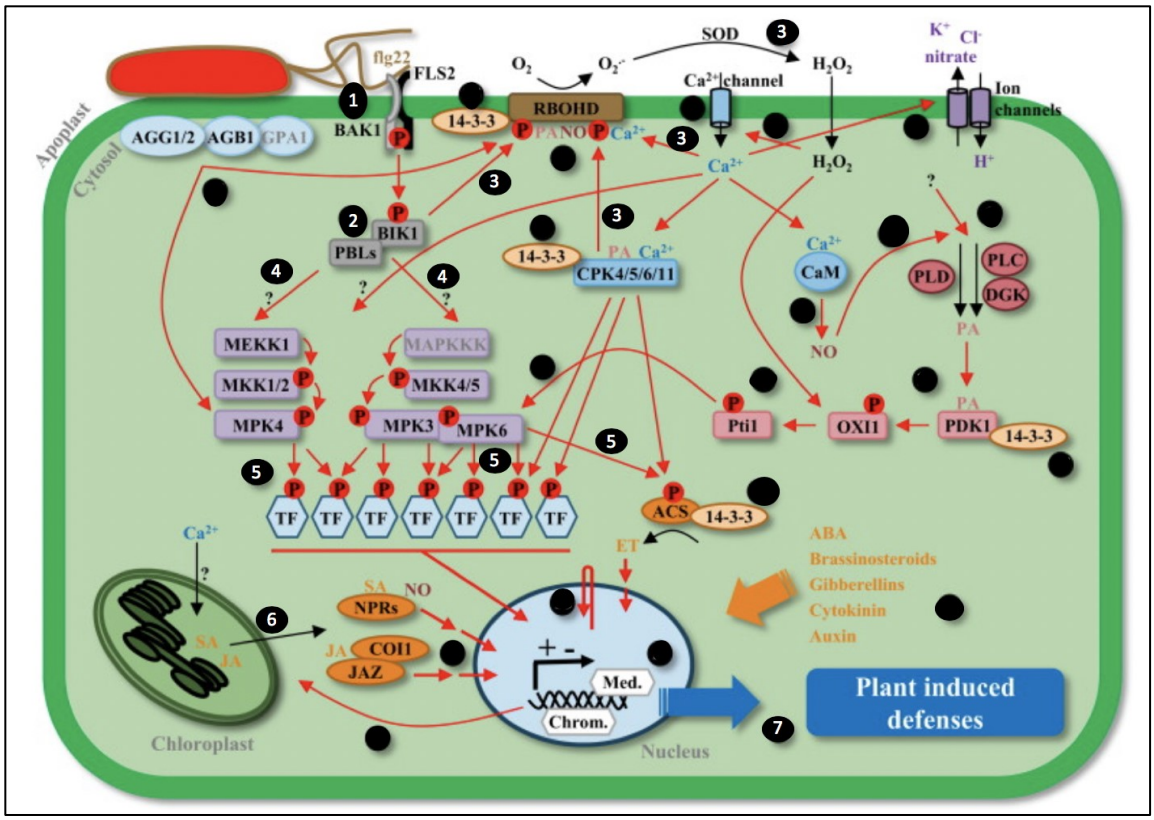


Figure 3. Illustration of defense cascade activated during PTI. (1) FLS2 perceives flg22 and recruits BAK1 kinase. FLS2 heterodimerizes with BAK1 leading to activation through phosphorylation. The activated BAK1 phosphorylates BIK1, which transphosphorylates FLS2-BAK1 complex. (2) After transphosphorylation, BIK1 dissociates from complex. (3) RbohD gets phosphorylated by BIK1, Ca^{2+} ions and Ca-dependent CDPKs, which leads to the production of ROS. (4) MAPK cascade is turned on via unknown RLCKs. (5) MPK3/6 phosphorylates TFs and regulates *ACS* gene via phosphorylation to activate ET synthesis. MPK4 also phosphorylates TFs. (6) SA is synthesized in chloroplast and is perceived by its receptor (NPRs). (7) Through transcriptional regulation, there is an induction of defense genes. Reprinted by permission from [the Licensor]: [Elsevier] [Molecular Plant] [Signalling mechanisms in patter-triggered immunity (PTI), Jean Bigeard, Jean Colcombet and Heribert Hirt] [2015].

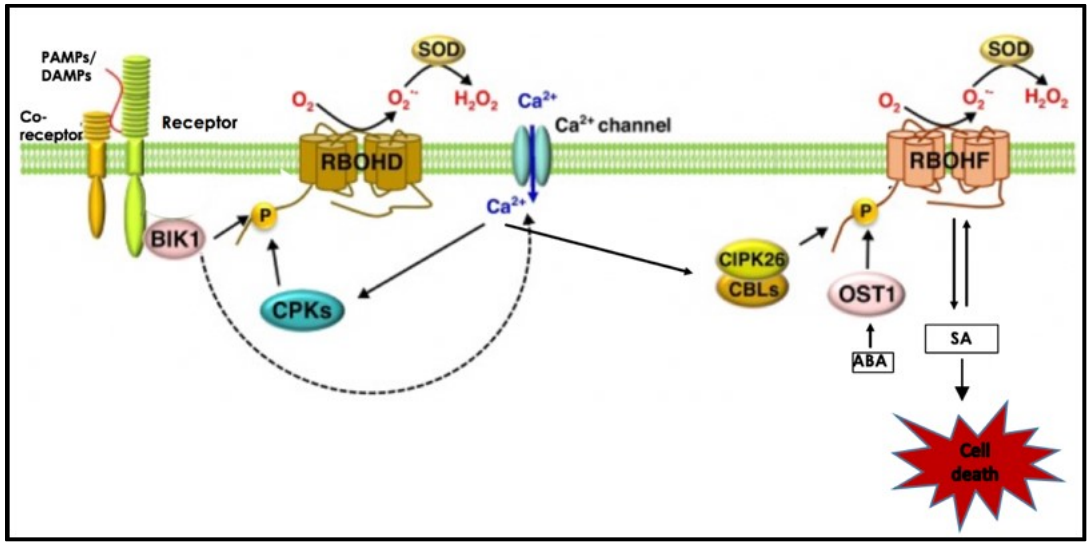


Figure 4. Regulation of ROS by RbohD and RbohF. Perception of MAMPs by its receptor activates the cytoplasmic protein kinase BIK1, which simultaneously activates an unknown calcium channel and phosphorylates RbohD in the N terminus. Intracellular influx of calcium ions leads to activation of calcium scavenging kinases such as CPKs and CBLs/CIPK26, which phosphorylates RbohD and RbohF, respectively. BIK1 and CPKs work towards fine tuning of ROS production by RbohD. On the other hand, ABA and CBL/CIPK26 work together towards fine tuning of ROS production by RbohF. RbohF also regulates SA through a positive feedback loop, which might result in cell death. RbohD: Respiratory Burst Oxidase Homolog D, SOD: Superoxide Dismutase, BIK1: Botrytis-Induced Kinase 1, CPK: Calcium dependent Protein Kinases, CBL: Calcineurin B-like Protein, CIPK26: Calcineurin B-like Interacting Protein Kinases, OST1: Open Stomata 1, ABA: Abscisic acid and SA: Salicylic Acid (modified from Qi *et al.,* 2017).

1.3.3.2 Mitogen-Activated Protein Kinases (MAPK)

Mitogen-activated protein kinases (MAPKs) are highly conserved signalling pathway that play a major role in signal transduction. MAPK pathway is activated within minutes of MAMP perception and reaches its peak at 15 minutes (Bigeard *et al.,* 2015). MAPK consists of three tier cascade components MAPKKKs, MAPKKs and MAPKs, where phosphorylation occurs from upstream receptors to their downstream targets (Figure 3) (Jalmi and Sinha, 2015). There are two different MAPK cascades that are activated in *Arabidopsis* after MAMP perception. The first cascade involves MAPKs, MPK3/6 activated by the upstream MAPKKs, MKK4/5, while the second cascade involves MPK4 along with its homolog MPK11, activated by upstream MKK1/2 (Figure 3) (Asai et al., 2002). These two cascades play a significant role in triggering transcriptional changes during PTI. The MAPKs MPK3/4/6 are the most intensively studied plant MAPKs that have been shown to be activated by MAMPs flg22, elf18 and chitin (Asai et al., 2002).

The first cascade MPK3/6 mediates plant defense responses by regulating hormone biosynthesis. One of the hormones regulated by MPK3/6 is the induction of ethylene (ET) production. ET biosynthesis involves a two-step reaction: conversion of S-adenosyl-methionine (SAM), a common metabolic precursor, to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase (ACS) and oxidative cleavage of ACC to form ethylene by ACC oxidase (ACO) (Wang *et al.,* 2002). MPK3/6 phosphorylates ACS2/6 proteins *in vivo*, which stabilizes ACS activity and increases ET production (Figure 3) (Han *et al.,* 2010; Liu *et al.,* 2004). Phosphorylation of ACS6 also protects it from ubiquitin-proteasome degradation and thus further increases its stability (Joo *et al.,* 2008).

In addition to exhibiting control at post-transcriptional level, *ACS* is also regulated at transcriptional level. MPK3/6 phosphorylates the transcription factor WRKY23, which binds to promoters of *ACS6* and regulates its transcription (Li *et al.,* 2012). MPK3/6 also phosphorylate another transcription factor WRKY33, which leads to upregulation of genes *PAD2* (phytoalexin deficient 2), *CYP71A13* (Cytochrome P450 71A13-Indoleacetaldoxime dehydratase), and *PAD3*, collectively involved in camalexin biosynthesis pathway (Mao *et al.,* 2011). Camalexin is an antimicrobial compound that is produced in plants in response to pathogen attack.

The second MAPK cascade that involves MPK4 is also known to regulate production of aforementioned camalexin upon flg22 treatment (Qiu *et al.,* 2008). MPK4 exists in nuclear complex with transcription factors WRKY33 and MSK1 (a target of MPK4). Upon MAMP perception, MPK4 phosphorylates MKS1 and induces the release of the MKS1-WRKY33 complex from MPK4, which then activates *PAD3* gene expression (Qiu *et al.,* 2008). MPK4 also acts as a negative regulator of salicylic acid biosynthesis and defense genes upon MAMP perception. Upon perception with pathogen, *mpk4* mutant *Arabidopsis* plants over-accumulate salicylic acid, resulting in the expression of pathogenesis related genes *PR1* (Gao *et al.,* 2008).

1.3.3.3 Ethylene signalling

Ethylene (ET) is a plant hormone with diverse roles in plant development, growth and immune responses. During the interaction with the hemibiotroph pathogen (*Phytophthora parasitica*), ET is activated in a biphasic manner. In the first phase, ET starts to accumulate within the first hour of pathogen perception and peaks at 3 hpi and the second phase occurs between 48 hpi and 72 hpi (Wi *et al.,* 2012). ET is perceived by

endoplasmic reticulum localized receptor proteins ETR1 (Ethylene Receptor 1), ETR2, ERS1 (Ethylene Response Sensor 1), ERS2 and EIN4 (Ethylene Insensitive 4) (Merchante *et al.,* 2013). The ethylene perception releases inhibition on EIN2 by CTR1 (Constitutive Triple Response 1), where the C-terminus end of EIN2 is cleaved and translocated to the nucleus. The C-terminus end of EIN2 stabilizes EIN3/EIL1(Ethylene Insensitive 3-like 1) transcription factors and results in transcriptional initiation of ET responsive genes (Merchante *et al.,* 2013).

ET plays a very important role in the formation of structural barriers such as accumulation of cell wall strengthening hydroxyproline-rich proteins upon pathogen perception (Broekaert *et al.,* 2006). It was demonstrated that ET was essential to inhibit the spread of *Fusarium oxysporum* f.sp. *lycopersici* through plant's vascular system (Broekaert *et al.,* 2006). ET also results in the production of antimicrobial secondary metabolities, phytoalexins derived specifically from phenylpropanoid pathway (Broekaert *et al.,* 2006). There are mixed reports in the literature regarding specific roles of ET in mediating susceptibility (Chen *et al.,* 2009) or resistance to FHB (Foroud *et al.,* 2018). Genetic studies revealed that RNAi-mediated silencing of *EIN2* transcripts in susceptible wheat cultivar (Bobwhite) resulted in reduced susceptibility to FHB, but enhanced susceptibility to FHB upon exogenous application of ethephon (ET enhancer) (Chen *et al.,* 2009). In contrast, another study demonstrated an enhanced resistance to FHB upon exogenous application of ethephon to susceptible wheat cultivars (Awesome, Roblin and Superb) (Foroud *et al.,* 2018). These results suggest that plant genotypes and timing of treatments and crosstalk between other hormone pathways could have an impact on the final (disease or resistance) outcome.

Ethylene has been shown to work synergistically with another plant hormone, jasmonic acid (JA) to mediate resistance against necrotrophic pathogens (Glazebrook *et al.,* 2005). Activation of JA signalling degrades JAZ (Jasmonate ZIM-domain) proteins, which releases its inhibition on ET-stabilized transcription factors EIN3/EIL1 (Ethylene Insensitive 1/ Ethylene Insensitive3-Like 1), involved in the regulation of pathogenicity-related (*PR*) genes like *PR4* and *PDF1.2* to promote resistance against necrotrophic pathogens (Song *et al.,* 2014).

1.3.4 Late defense responses during pattern induced immunity

1.3.4.1 Salicylic acid

The phytohormone salicylic acid (SA) is an important signalling molecule in plants. The hormone starts to accumulate between 3 h and 6 h and reaches a peak at 9 h during PTI response (Tsuda *et al.,* 2008). The biosynthesis of SA occurs via two branches of the shikimic acid pathway (Singh *et al.,* 2017). Both branches use chorismic acid as the substrate. In one branch, chorismic acid is converted to SA via phenylalanine conversion to cinnamic acid by phenylalanine ammonia lyase (PAL) (Singh *et al.,* 2017). In the second branch, chorismic acid is converted to SA via isochorismic acid, catalyzed by isochorismate synthase (ICS), localized in chloroplasts (Singh *et al.,* 2017). The primary pathway contributing towards pathogen-induced SA in *Arabidopsis* is mediated by the *ICS* gene (Singh *et al.,* 2017). The ICS pathway contributes towards 90% of total SA produced during pathogen attack (Vlot *et al.,* 2009).

SA is recognized by its receptors encoded by *Non- expressor of PR genes (NPR)* (Figure 3) (Yan and Dong, 2014). In addition to regulating defense gene expression of *pathogenesis-related (PR)* genes, SA treatment can also induce ROS production through

extracellular superoxide generation by NADPH oxidases (Kawano *et al.,* 1998). These responses are very rapid (occurs within 10 min) and suggests of yet unknown SA receptors mediating early SA responses.

Downstream of NPR, several WRKY transcription factors are known to regulate SA-mediated defense responses. For example, the *Arabidopsis* WRKY70 was shown to mediate antagonistic interaction between SA and another plant hormone jasmonic acid (JA) (Euglem and Somssich, 2007). The overexpression of *WRKY70* resulted in constitutive expression of *PR* genes and enhanced resistance to biotrophic pathogen *Erysiphe cichoracearum* (Li *et al.,* 2004; Li *et al.,* 2006). On the other hand, overexpression of *WRKY70* repressed JA-responsive marker gene *PDF1.2* and compromised resistance to necrotrophic pathogen *Alternaria brassicicola* (Li *et al.,* 2004; Li *et al.,* 2006). Recently, the wheat *WRKY70* was shown to be required for basal resistance to FHB (Kage *et al.,* 2017). Interestingly, this gene is associated with the *QTL-2DL*, which limits the fungal spread (Kage *et al.,* 2017). These results indicate that WRKY70 plays a very important role in determining a balance between SA and JA-dependent defense pathways. SA signalling also plays an important role in mediating resistance to *F. graminearum* during early infection stages as evident from enhanced resistance upon exogenous exposure to SA in *Arabidopsis* (Makandar *et al.,* 2010). In contrast, due to antagonistic behavior, exogenous exposure of JA resulted in enhanced susceptibility to *F. graminearum* during early infection stages (Makandar *et al.,* 2010).

1.3.4.2 Role of stomata in plant defense

Stomata are microscopic pores present in the epidermis of plants that regulates gas exchange and transpiration required for photosynthesis and water homeostasis

(Montillet and Hirt, 2013). Plants control opening and closure of stomata by regulating osmotic pressure in guard cells (Montillet and Hirt, 2013). Guard cell movements require regulation of plasma membrane-located ion channels and H^+-ATPases, where synthesis of these proteins is known to be dependent on the plant hormone abscisic acid (ABA) (Joshi-Saha *et al.,* 2011).

Besides regulating gas exchange, stomata also act as sites of entry for multiple pathogens. For example, it was shown that *Pseudomonas syringae* pv. *tomato* DC3000 selectively moved towards open stomata on *Arabidopsis* leaves and was able to decrease stomatal aperture within 1 to 2 h after inoculation (Melotto *et al.,* 2006). *Fusarium graminearum* has also been shown to target stomata as sites of entry on *Arabidopsis* cotyledons (Schreiber *et al.,* 2011). In PTI response, MAMPs such as flg22 and chitin induce stomata closure within 1 h of its perception (Melotto *et al.,* 2006; Lee *et al.,* 1999). As mentioned earlier, both ABA and flg22 trigger H_2O_2 production by the plasma-membrane localized NADPH oxidase RbohF and RbohD, respectively (Kwak *et al.,* 2003; Kadota *et al.,* 2014). The presence of ABA results in stomata closure through activation of the Snf1-related protein kinase OST1 (Open Stomata 1), which facilitates K^+ efflux through the function of outward rectifying channels and results in the decrease of guard cell turgor pressure leading to stomata closure (Figure 5) (Sawinski *et al.,* 2013). Besides ABA and H_2O_2, SA has also been assigned as a key component in regulating stomata closure (Khokon *et al.,* 2011). Mutant plants such as *eds16-2* that reduce SA levels or *NahG* that converts SA to catechol are compromised in their ability to close stomata in response to infection by *P. syringae* (Melotto *et al.,* 2006) (Figure 5).

Despite multiple components regulating stomata closure, ABA remains as a key regulator in mediating stomata closure during plant pathogen interaction. This is based on the observations that *Arabidopsis* carrying *aba3-1* or *open stomata 1 (ost1-2)* mutation were more susceptible to *Pseudomonas syringae* pv. *tomoto* (*Pst*) and also *Pst* or flg22 were no longer able to induce stomata closure (Melotto *et al.,* 2006).

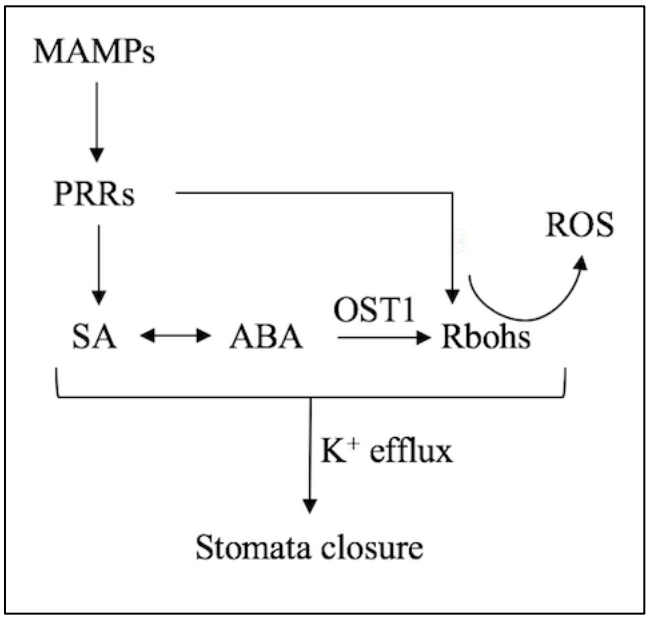


Figure 5. Regulation of stomata closure upon MAMP perception. MAMP perception triggers ROS activation and results in stomata closure. MAMP perception also triggers SA accumulation and ABA activation. ABA activation further results in ROS production through activation of the snf1-related protein kinase OST1, which facilitates K^+ efflux through the function of outward rectifying channels and results in the decrease of guard cell turgor pressure leading to stomata closure. MAMPs: Microbe-Associated Molecular Patterns; PRRs: Pattern Recognition Receptors; SA: Salicylic acid; ABA: Abscisic acid; OST1: Open Stomata 1; Rboh: Respiratory burst oxidase homolog.

1.4 Project Outline

My thesis project is based on our current knowledge of FHB resistance that is largely quantitative. Thus, we used *Arabidopsis*, a non-host to the pathogen *F. graminearum* to identify QDR genes. My thesis conducted a high throughput screen of 249 T-DNA insertion mutant lines in *Arabidopsis* that included a combination of PRR receptors and downstream signalling components involved in PTI. A total of seven genes were identified as significantly susceptible and two genes as significantly resistant to *F. graminearum* infection when compared to infected WT seedlings. The resistant lines characterized in detail in my thesis are the two PRRs, namely line# 206 (*RLK7* - *AT1g09970*) and line# 420 (*APEX*- *AT5g63710*). *RLK7* belongs to LRR XI subfamily and *APEX* belongs to LRR II subfamily (Pitorre *et al.,* 2010; Shiu and Bleecker, 2001a; Mott *et al.,* 2016). My overall goal was to characterize the role of RLK7 and APEX and elucidate downstream signalling components involved in FHB resistance.

Chapter 2: Methods

2.1 Fungal strains and inoculum preparation

F. graminearum wild-type strain DAOM 233423 and *F. graminearum*-GFP strain DAOM 227650 (Miller *et al.,* 2004) were obtained from Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa. Fungi were grown on potato dextrose agar (PDA) media for routine culture and analyses. To prepare conidiophores for pathogenicity assays, a three-mm plug was extracted from a fresh PDA culture plate and transferred to 100 ml of carboxymethylcellulose (CMC) media (Cappellini and Peterson, 1965). Cultures were shaken at 28°C for three to four days to generate conidiophores. Mycelial solids were separated from conidia by passing through four layers of sterile cheesecloth. Spores were then washed with sterile water twice by centrifugation at 5,000 rpm for 5 min at room temperature. The pelleted spores were re-suspended in sterile water and then stored at 4°C. Prior to use, spores were quantified by haemocytometer.

2.2 *Arabidopsis* growth conditions

Wild type (WT) *Arabidopsis thaliana* (Col-0) and 249 T-DNA insertion lines were obtained from *Arabidopsis* Biological Resource Center (ABRC) (Supplement Table 1). Experimental assays including ROS activation, MAPK activation and stomata assay were conducted using four to five-week-old plants grown in Cornell mix (Boodley and Sheldrake, 1977) at 22°C with 18h/6h, day/night cycle. All other assays were conducted using five-day-old seedlings grown in 96-well plates (Schreiber *et al.,* 2011). Seeds were sterilized in 30% bleach with 0.1% tween for 10 min and washed with sterile water three times. Six-seven seeds were pipetted into each well of a 96-well plate with liquid 1/20

MS (Murashige and Skoog) media (where in 1L of 1/2 MS liquid media, MS basal mix of 2.16g and MES monohydrate of 0.28g was added with final pH between 5.7 - 5.8). The lid of the plate was taped with surgical tape and was stratified for two to four days at 4°C with shaking (600 rpm). The seeds were grown in a chamber for five days without shaking at 22°C with 18h/6h, day/night cycle. The plate was tilted from time to time to eliminate any ethylene build-up.

2.3 *Arabidopsis* seedling infection assay

Arabidopsis seedlings were grown as described above. Prior to inoculation, five-day-old seedlings were washed with 0.5% sucrose and then inoculated with *F. graminearum* wild-type strain DAOM 233423. Seedlings were inoculated with 40 spores (concentration adjusted in 0.5% sucrose) and placed in similar growth conditions without shaking for three days. Phenotypic observations were qualitatively monitored by fluorescence microscope (Olympus SZX16) and quantified at 3 dpi.

2.4 Genotyping of the *apex-1* and *rlk7* mutant plants

Genomic DNA was extracted from four to five-week-old plant leaves of *apex-1* and *rlk7* mutants using the Nucleon Phytopure DNA extraction kit (Amersham Bioscience, Quebec, Canada) according to manufacturer's recommendations. The T-DNA insertions in each mutant were identified by PCR (a final concentration of 0.2 μM primers were used with 1 min denaturation at 95°C, 30 sec annealing at 56°C, 2 min extension at 72°C repeated for a total of 40 times, followed by a 10 min extension at 72°C) using the T-DNA left border primer LB1.3 along with forward and reverse primers (Supplement Table 4). The precise location of T-DNA insert is indicated in Figure 6.

2.5 Fungal biomass quantification

Infected cotyledons (3dpi) were excised from roots of infected seedlings. The DNA was extracted using Phytopure Plant DNA extraction kit (Amersham Bioscience, Quebec, Canada) according to manufacturer's recommendations. An equal amount of DNA (40 ng) and primers (500 nM) were used for qPCR experiments (15 sec denaturation at 95°C, 15 sec annealing at 60°C and 1 min extension at 72°C repeated for a total of 40 times) using PowerUp Syber green master mix (Applied Biosystems). *EF-1α (FGSG_08811)* was used as a reference gene for quantification of fungal biomass and *PP2A (AT1G69960)* was used as primer set to normalize amount of plant DNA. The primer sequence used is listed in Supplement Table 4.

2.6 Measurement of ROS generation

Two four-mm leaf disks from six to eight plants per line (wildtype Col-0, *rlk7, apex-1, rbohF* and *cerk1*) were collected from four to five-week-old plants and placed adaxial side up in deionized water overnight in a 96-well plate. The following day, the water was replaced with 100 µL of detection buffer (40 ug/ml of chemiluminescent reagent L-012 and 40 ug/mL of horseradish peroxidase from Sigma-Aldrich) containing 100 µg/ml chitin or 5 mg/ml ground *F. graminearum* mycelia (5mg/ml of mycelia equivalent to 100ug/ml chitin calculated according to Ayliffe *et al.,* 2013) (Zhang *et al.,* 2017). The luminescence was measured at 420 nm for 60 min in an Infinite 200 PRO microplate reader (Tecan).

2.7 Stomata aperture measurements

Four-mm leaf disks from four to five-week-old plants were exposed to white light for 2h while submerged abaxial side down in buffer (50mM KCl, 10µM $CaCl_2$, 0.01% Tween 20 and 10 mM MES-KOH pH 6.15), to open maximum number of stomata.

Subsequently, 10 μM of ABA and 5mg/ml of ground *F. graminearum* mycelia or mock solution was added to the buffer and the samples were incubated under same conditions for 1 h for mycelia treatment and 3 h for ABA treatment. Abaxial leaf surfaces were observed under a microscope (Leica Zeiss) and the stomata aperture was measured using ImageJ software.

2.8 RNA extraction and Quantitative Reverse Transcriptase-PCR

RNA was isolated for gene expression analysis from five-day-old seedlings by Trizol (Invitrogen, Carlsbad, CA, USA) followed by RNase-free DNase (Qiagen, Missisauga, Canada) treatment and purified using the RNeasy Mini kit (Qiagen) according to manufacturer's instructions. RNA was converted into cDNA by the Applied Biosystems cDNA synthesis kit according to manufacturer's recommendations (Applied Biosystems, Burlington, ON, Canada). Biological replicates contained 18 to 21 seedlings and all of the qRT-PCR reactions (15 sec denaturation at 95°C, 15 sec annealing at 60°C and 1 min extension at 72°C repeated for a total of 40 times) were performed in triplicate by Applied Biosystems Power SYBR Green kit (Life Technologies) and the QuantStudio3 Real-Time PCR System according to manufacturer's instructions (ThermoFisher Scientific). For relative quantification, a standard curve for each primer set was created. *PP2A (AT1G69960)* was used as the internal standard between samples. Primers used for qRT-PCR are listed in Supplement Table 4.

2.9 Salicylic acid measurement

Salicylic acid was extracted using a protocol modified from Li *et al.,* 1999. Approximately 0.4 g of *Arabidopsis* seedlings infected with *F. graminearum* spores or mock solution for 3 days were collected and ground to powder in liquid nitrogen. Three

to four biological replicates for each genotype were collected and analyzed. A volume of 1 ml of 90% methanol was added to each sample, vortexed and sonicated for 20 min. After centrifugation at 16,000g for 20 min, the supernatant was collected and the sample was re-extracted with 0.5 ml of 100% methanol. The supernatants from both extractions were combined and dried under continuous stream of nitrogen gas. The dried samples were re-suspended in 0.5 ml of 5% trichloroacetic acid (TCA) followed by sonication and centrifugation. The supernatant was collected and pellet was re-extracted three times using 0.5 ml of extraction medium: ethylacetate: cyclopentane: isopropanol in a ratio of 100:99:1 (vol). The top organic phase was removed and dried under continuous stream of nitrogen gas. The dried extract was suspended in 250 µl of mobile phase (10% acetonitrile, 90% H_2O, 0.1% tri-fluoroacetic acid), vortexed, sonicated for 5 min and centrifuged. To measure the amount of extracted salicylic acid, samples were separated through a 5 micron C18 Hypersil Reverse Phase Column (ThermoFisher Scientific Inc) using a one-step gradient from 5 to 95% acetonitrile in water over 25 min at a flow rate of 1 ml/min. The eluted salicylic acid was detected by UV at 300 nm and quantified in mAU*min using peak integration with the Unicorn software (Version 5.01, GE Healthcare, Canada). Salicylic acid retention time was recorded at 16.29 min.

2.10 Detection of MAPK activation by Western blot analysis

Four to five-week-old *Arabidopsis* plant leaves were infiltrated with 1 µM flg22 or 5 mg/ml ground *F. graminearum* mycelia and frozen in liquid nitrogen. The protein was extracted using extraction buffer (100 mM Tris-HCl pH 7.5, 100 mM NaCl, 5 mM EDTA, 5 mM EGTA, 10% glycerol, 0.10% Triton X-100, 1 mM DTT, 1 mM PMSF, 1x complete protease inhibitors [Roche] and PhosStop [Roche]). The extracted protein was

quantified using the Bradford assay. Equal amount (10μg) of total protein was separated using 16% SDS-PAGE gel and transferred to nitrocellulose membrane using semi-dry buffer (48 mM tris base pH 8.3, 39 mM glycine, 0.037% SDS) for 45 min at constant current of 50mA. After transfer, membrane was stained with PonceauS to monitor equal protein loading. The membrane was rinsed thrice for 5 min with distilled water to wash off stain. The membrane was incubated in the blocking reagent buffer that included 5%BSA (Bovine Serum Albumin) in TBS-T (1.25 mM NaCl, 250 mM Tris-HCl pH 8 and 0.1% Tween-20) for 1 h and was washed twice with TBS-T for 5 min. The membrane was probed with primary antibodies, anti-p42/44 MAPK antibodies (1:1000 diluted in blocking reagent; Cell Signalling Technology, Danvers, MA) overnight at 4°C, and was washed thrice for 10 min with TBS-T. The membrane was incubated with secondary antibodies, anti-rabbit-HRP antibodies (1:2000 diluted in blocking reagent; Cell Signalling Technology, Danvers, MA) for 1 h and was washed thrice with TBS-T for 10 min. The membrane was incubated with Super Signal West Femto chemiluminescent substrate and Super Signal Pico chemiluminescent substrate (ThermoFisher Scientific) in a 1:9 ratio and the emitted light was detected by photographic film (SRX-101A, DynaTech 2000 Inc.).

Chapter 3: Results

3.1 *Arabidopsis* mutant screen identified receptor kinases and signalling genes involved in FHB resistance

Arabidopsis is a non-host to *F. graminearum*, however, at seedling stage, the plant is susceptible to this pathogen (Schreiber *et al.,* 2011). We initiated a reverse genetic screen to identify genes involved in FHB resistance in *Arabidopsis*. Two hundred and forty-nine *Arabidopsis* T-DNA insertion lines were infected with GFP-labelled *F. graminearum* strain (*Fg*-GFP) and monitored the infection by counting the number of infected cotyledons by GFP-fluorescence and compared them to non-infected cotyledons in each well (Table 3 and Supplement Table 1). The experiments were performed three times and we identified nine mutant lines that showed consistent phenotypes compared to wild-type (WT) *Arabidopsis* (Figure 7a). Among the nine mutant lines, two mutant lines *apex-1* (line 420) and *rlk7* (line 206) showed lower infection than WT. Whereas, seven lines: *588, psy1r, rbohD/F, 528, lyk4/5, 333,* and *sobir1-12* showed more infection than WT. Since the mutants *apex-1* and *rlk7* were used for further analysis, we confirmed the location of the T-DNA insert in exon 1 of the *apex-1* and in the sequence spanning leucine-rich repeat 1of the *rlk7*, respectively (Figure 6a-d). We also demonstrated that expression of these genes was reduced in the respective mutant lines, compared to WT (Figure 6e-f).

Table 3. Total number of *Arabidopsis* T-DNA lines used to screen for potential candidates for providing resistance to FHB. The unshaded region represents plasma membrane localized RLKs (with different extracellular domains) and RLPs. The shaded region represents downstream signalling components included in the screen.

LRR	131
LRK10L-2	8
LysM	4
Thaumatin	1
malectin	2
s-domain	11
DUF 26	7
L- lectin	14
PERK	3
unidentified RLK type	9
RLPs	29
RLCK	5
GRP1 (AT1G77960- endoplasmic reticulum localized protein was used a negative control)	1
NADPH subunit	1
Ion gated channel	10
MAPK related protein	3
Isopentyl transferase	1
Intracellular RAS group related LRR	2
RAF related kinase	1
Acyl amino acid synthethase	1
Protease inhibitor	1
Nuclear transcription factor	2
Salicylic acid associated protein	2
Total	249

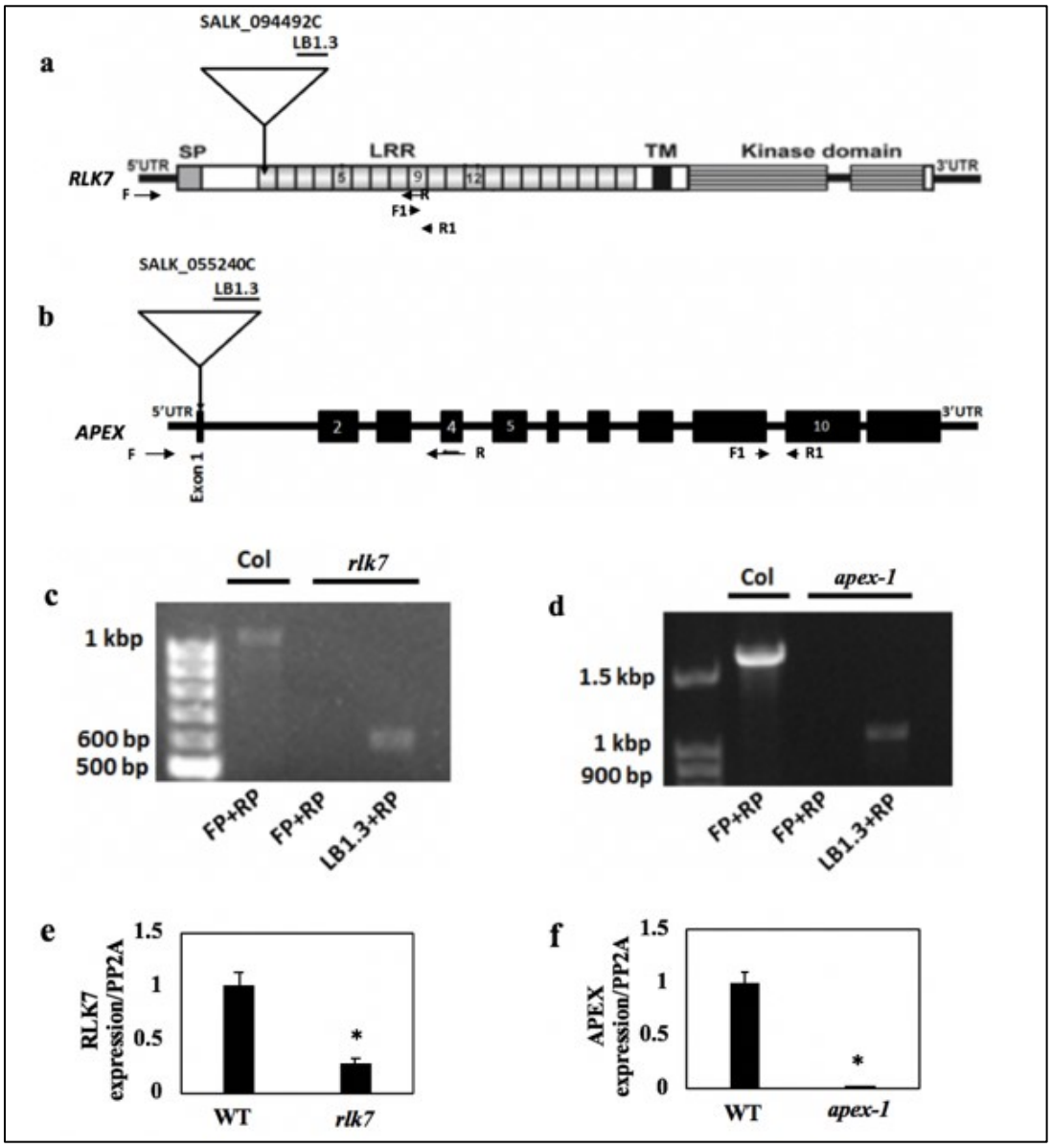

a
SALK_094492C
LB1.3
SP
LRR
TM
Kinase domain
RLK7
5'UTR
3'UTR
F
F1
R
R1
b
SALK_055240C
LB1.3
APEX
5'UTR
3'UTR
F
Exon 1
R
F1
R1
c
Col
rlk7
1 kbp
600 bp
500 bp
FP+RP
FP+RP
LB1.3+RP
d
Col
apex-1
1.5 kbp
1 kbp
900 bp
FP+RP
FP+RP
LB1.3+RP
e
RLK7 expression/PP2A
1.5
1
0.5
0
WT
rlk7
f
APEX expression/PP2A
1.5
1
0.5
0
WT
apex-1

Figure 6. Confirmation of T-DNA inserts and reduced gene expression in *rlk7* and *apex-1* mutant alleles. (a) The diagram shows the location of T-DNA insertion in *RLK7* gene in *rlk7* (SALK_094492C) mutant. Line indicates non-coding sequence. Boxes indicate coding sequence. The domain organization of predicted RLK7 protein is indicated. Primer set F/R and F1/R1 was used for molecular characterization of mutant using PCR and qRT-PCR, respectively. SP: Signal Peptide, UTR: Untranslated Region, TM: Transmembrane Domain, LB1.3: Left Border, LRR: Leucine Rich Repeat (modified from Pitorre *et al.,* 2010). (b) The diagram shows the location of T-DNA insertion in *APEX* gene in *apex-1* (SALK_055240C) mutant. Line indicates non-coding region (introns). Boxes indicate coding sequence. Primer set F/R and F1/R1 was used for molecular characterization of mutant using PCR and qRT-PCR, respectively. UTR: Untranslated Region, LB1.3: Left Border. (c-d) PCR experiment demonstrates that *rlk7* and *apex-1* mutants are homozygous. Primer combinations for PCR reaction are shown below each lane. (e-f) qRT-PCR analyses shows fold reduction of *RLK7* and *APEX* transcript levels in mutant *rlk7* and *apex-1 Arabidopsis* lines. Gene expression levels were normalized to *PP2A* gene expression levels. n=3 biologically independent mRNA samples from three different four to five-week-old plants were included for each genotype. Asterisks (*) indicate significant difference from WT. Statistical analysis were determined using Student's *t* test, where $p \leq 0.05$. This experiment was performed once. Reprinted by permission from [the Licensor]: [Springer Nature] [Planta] [RLK7, a leucine-rich repeat receptor-like kinase, is required for proper germination speed and tolerance to oxidative stress in Arabidopsis thaliana, Delphine Pitorre, Christel Llauro, Edouard Jobet et al] [2010].
See previous page for figure.

The screen was based on characterizing phenotypes visually and hence, qualitative. In order to verify the phenotype of the identified mutants quantitatively, DNA was extracted from the leaf of cotyledons (roots were separated from leaves) after 3dpi and fungal biomass was quantified by qPCR analysis (Figure 7b). The results corroborated our qualitative data and showed that both *apex-1* and *rlk7* resistant lines had significantly lower fungal biomass accumulation than infected WT seedlings and the susceptible mutant lines had higher fungal biomass accumulation (Figure 7b). The qualitative screen also included the double mutant *rbohD/F*, downstream signalling components of PTI and the result showed that the double mutant was susceptible to *F. graminearum* infection (Figure 7a). As previously mentioned, both *RbohD* and *RbohF* are involved in ROS production upon pathogen attack, however, are differentially regulated (Figure 4) (Qi *et al.,* 2017). In order to determine the individual contribution of the two genes, single *rbohD* and *rbohF* mutants were also included in the quantitative qPCR analysis (Figure 7b). In comparison to the double mutant *rbohD/F* that accumulated three times more fungal biomass, the single mutant *rbohF* accumulated five times more fungal biomass than infected WT seedlings (Figure 7b). These results suggest that *rbohD* is suppressing fungal growth in double mutant *rbohD/F*. Based on the biomass quantification results, we selected two resistant lines *rlk7* and *apex* and two susceptible lines *rbohF* and *cerk1* for further characterization. *CERK1* was included because it is a known chitin co-receptor and would serve as control for fungal infection.

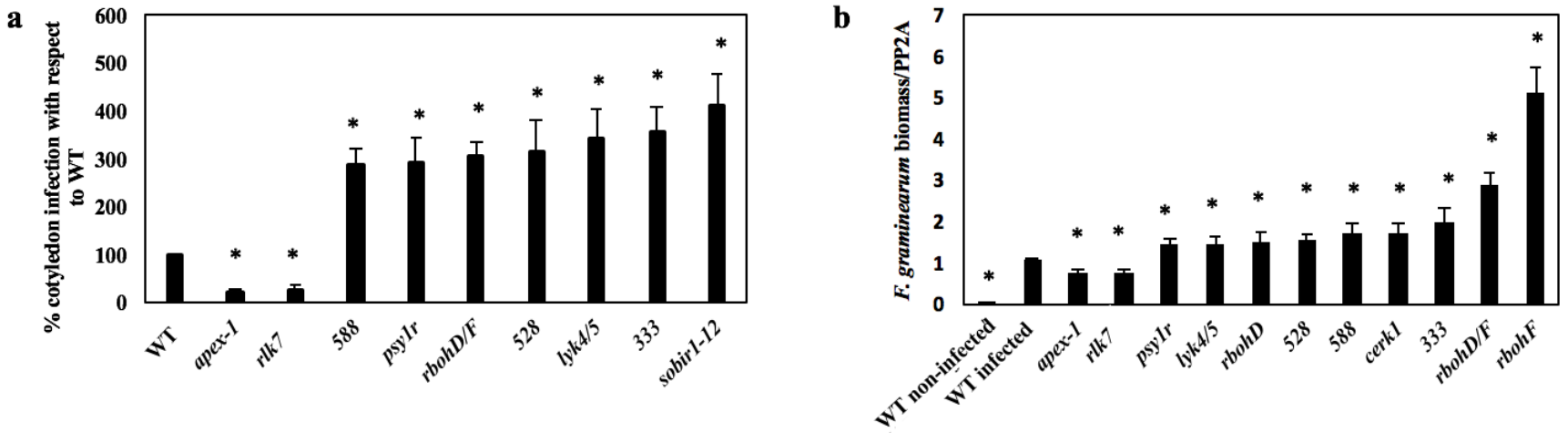


Figure 7. Identification and assessment of candidate *Arabidopsis* genes involved in resistance/susceptibility to *F. graminearum* infection. (a) Cotyledons infected by GFP-labelled *F. graminearum* were visualized 3dpi under fluorescence microscope (Olympus SZX16). The experiment was performed three times with similar results. Error bars denote standard error. Here, WT as a control has been set to 100%. Statistical analysis was performed using Student's *t* test with $p \leq 0.05$, where asterisks (*) indicate significant difference relative to WT. (b) Quantitative assessment of relative *F. graminearum* biomass in candidate *Arabidopsis* genes involved in resistance/susceptibility to *F. graminearum* infection. The relative *F. graminearum* biomass in *A. thaliana* cotyledons was estimated using qPCR on genomic DNA, where *EF-1α* served as a measure for fungal biomass quantification and *PP2A* was used for normalization. The experiment was performed three times with similar results. Here control (WT infected) was set to 1. Error bars denote standard error (n=11-12 biological replicates, where each replicate contained cotyledons from 60-70 seedlings). Statistical analysis was performed using Student's *t* test with $p \leq 0.05$, where asterisks (*) indicate significant difference relative to WT infected.

In summary, the PRR RLK7 identified in the screen belongs to LRR-RLK subfamily XI with an extracellular 20 leucine rich repeats, a single pass transmembrane domain and an intracellular kinase domain (Pitorre *et al.,* 2010). The extracellular LRR domain binds to the MAMP-induced secreted peptide (PIP1), which is secreted by plants upon MAMP perception (Hou *et al.,* 2014). The activation of RLK7 by the DAMP, PIP1 results in similar downstream defense responses as observed with the MAMP, flg22 (Hou *et al.,* 2014). It is known that a combination of exogenous MAMPs and endogenous DAMPs amplify PTI response and increases resistance (Hou *et al.,* 2014). The second identified PRR, APEX also belongs to receptors with leucine rich extracellular domain. It is in the same subfamily as BAK1 (a LRR-II family member) (Shiu and Bleecker, 2001a). Similar to BAK1, it consists of four leucine rich repeats and due to its short extracellular domain, is believed to act as a co-receptor. Functionally, APEX is similar to BAK1 and is sensitive to plant hormone brassinolide, where reduced hypocotyl length was observed in both single *apex-1* and double mutant *apex/bak1-5,* suggesting functional redundancy between APEX and BAK1 (Smakowska-Luzan *et al.,* 2018). The *bak1-5* mutant has a single amino-acid substitution in its kinase domain with reduced kinase activity (Schwessinger *et al.,* 2011), whereas, the *apex* mutant used in my screen has a T-DNA insertion in the first exon and is functionally not competent.

We included CERK1 as a positive control because it is involved in mediating signalling in response to MAMPs derived from fungi. Chitin is a polymer that is found in cell walls of all fungi and is perceived by plants as a MAMP and activates defense responses that include ROS production, MAPK activation and defense gene induction (Cao *et al.,* 2014). As a co-receptor, CERK1 forms a complex with a PRR LYK5 to

perceive chitin leading to activation of defense responses (Cao *et al.,* 2014). LYK5 belongs to RLK subfamily with extracellular domain consisting of lysin motif (LYK) (Cao *et al.,* 2014).

Our screen also identified receptor kinases that were susceptible to *Fusarium* infection that significantly accumulated more fungal biomass when compared to WT (Figure 7b). PSY1R (AT1G72300) (Plant peptide containing Sulfated Tyrosine 1 Receptor) belongs to LRR-RLK subfamily X and consists of a single exon with extracellular 23 leucine rich repeats and an intracellular kinase domain (Amano *et al.,* 2007). The extracellular LRR domain binds to an 18-amino acid sulfated and glycosylated secreted peptide called PSY1 (Amano *et al.,* 2007). PSY1 is widely expressed in various *Arabidopsis* tissues, including shoot apical meristem and is highly upregulated upon wounding (Amano *et al.,* 2007). Upon wounding, PSY1 is released, processed and sulfated by Golgi-membrane localized tyrosyltransferase (TPST) enzyme (Mosher *et al.,* 2013). Activation of the receptor PSY1R leads to diverse responses such as increase in cell proliferation and expansion, enhanced resistance against necrotrophic fungal pathogen (*Alternaria brassicicola*) and enhanced susceptibility to the bacterial pathogen (*Pseudomonas syringae* pv. *tomato* DC3000) (Mosher *et al.,* 2003).

The receptor line 333 (AT3G46340) belongs to LRR-RLK subfamily I and has 13 exons, 3 leucine-rich repeats and also harbors an extracellular malectin-like domain (Shiu and Bleecker, 2001a). Malectin is a conserved animal protein located in the lumen of endoplasmic reticulum that binds to maltose and related oligosaccharides (Boisson-Dernier *et al.,* 2011). The receptor has not been characterized yet, but another gene *IOS1* belonging to same subfamily as line 333 has been very well characterized. The IOS1

(Impaired Oomycete Susceptibility 1) harbors a malectin-like extracellular domain with 3 leucine rich repeats (Hok *et al.,* 2011; Yeh *et al.,* 2016). IOS1 acts as a positive regulator of PTI mediated defense responses, where deletion results in reduced callose deposition and MAPK activation upon flg22, and elf18 treatments (Yeh *et al.,* 2016).

The receptor line 528 (AT5G38260) belongs to RLK subfamily with extracellular domain as Leaf rust 10 disease-resistance locus receptor-like protein kinase 2 (LRK10L-2) (Feuillet *et al.,* 1997). The receptor line 528 consists of 2 exons, signal peptide and transmembrane domain (Shiu and bleecker 2001a). This gene has not been functionally characterized, but another receptor, AtLRK10L1 that belongs to same subfamily as 528 has been shown to be involved in ABA-mediated signalling and drought resistance (Lim *et al.,* 2015). The receptor line 588 (AT4G05200) belongs to RLK subfamily called Domain of Unidentified Function 26 (DUF26) (Shiu and bleecker, 2001a). It consists of cysteine-rich extracellular domain, signal peptide and transmembrane domain (Shiu and bleecker, 2001a). The expression of *AT4G05200* is known to be upregulated upon abiotic stress (Wrzaczek *et al.,* 2010). No further functional characterization has been conducted on this gene.

Overall, the genetic screen revealed a complex genetic pathway involved in *Fusarium* resistance; we identified both gain of function and loss of function mutant alleles. It is well established that perception of a pathogen by a host is multifactorial and our genetic screen reaffirms that view. It is also well established that many of the permutations and combinations involved in receptor complex formation eventually lead to activation of common conserved downstream signalling pathways.

3.2 Characterization of *Arabidopsis* receptors and downstream signalling components

3.2.1 Production of ROS by *Arabidopsis* after *F. graminearum* infection

(Quantification of reactive oxygen species was performed by Dr. Elizabeth Kalinda Brauer)

One of the initial molecular responses during PTI response is the accumulation of reactive oxygen species (ROS) that peaks within 10-12 minutes after MAMP perception (Torres *et al.,* 2006). In order to test if ROS production is compromised in the mutant lines, leaf disks from four to five-week-old plants were treated with 100 μg/ml chitin or 5 mg/ml of *F. graminearum* ground mycelia, representing 100 μg/ml chitin. Chitin content in ground *F. graminearum* mycelia was determined using a dye, which uses lectin wheat germ agglutinin (WGA) conjugated to a fluorophore Alexa488 (Ayliffe *et al.,* 2013). WGA possess a high binding affinity to trimers of N-acetylglucosamine, a component of chitin (Ayliffe *et al.,* 2013). The concentration of chitin in ground mycelia of *F. graminearum* was determined from a standard curve with pure chitin (Figure 8). As previously shown, chitin exposure led to the production of ROS in WT plants and the chitin receptor mutant *cerk1* produced less ROS than WT plants (Figure 9a) (Cao *et al.,* 2014). The mutant lines *rlk7* and *apex-1* produced similar ROS level as WT treated with chitin (Figure 9a). On the other hand, *rbohF* plants were compromised in ROS production compared to WT (Figure 9a). The treatment of plants with ground mycelia resulted in ROS production, however, we did not observe any differences in any of the mutant lines (Figure 9b). Our results suggest that ROS production induced by either ground mycelia or chitin is not mediated through the receptors RLK7 and APEX.

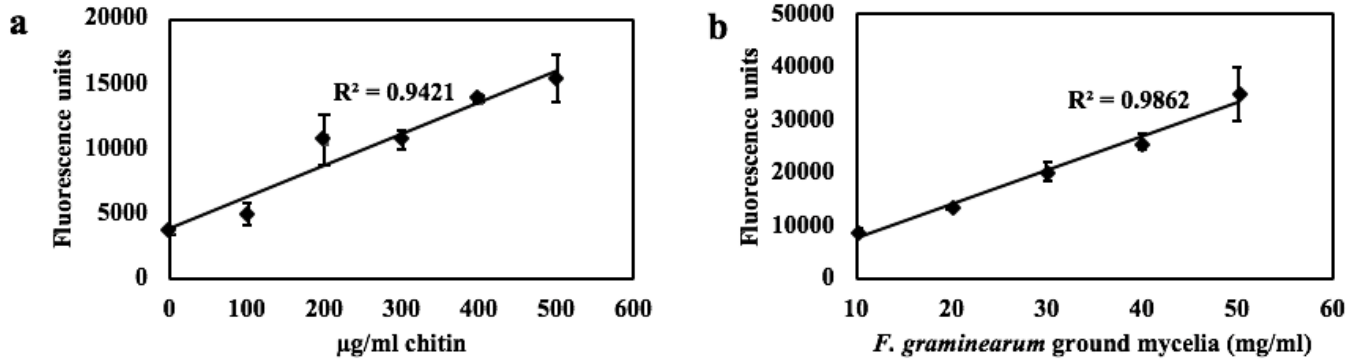


Figure 8. Quantification of chitin in ground *F. graminearum* mycelia. (a) Chitin standard curve generated by wheat germ agglutinin (WGA) chitin (WAC) assay. The concentration of chitin is shown along x-axis and fluorescence units are shown along y-axis. Each point is average of three technical replicates. Error bars denote standard deviation. (b) A directly proportional relationship is seen between *F. graminearum* ground mycelia (x-axis) and fluorescence (y-axis). Each point is average of three technical replicates. Error bars denote standard deviation. The experiment was repeated twice with similar results.

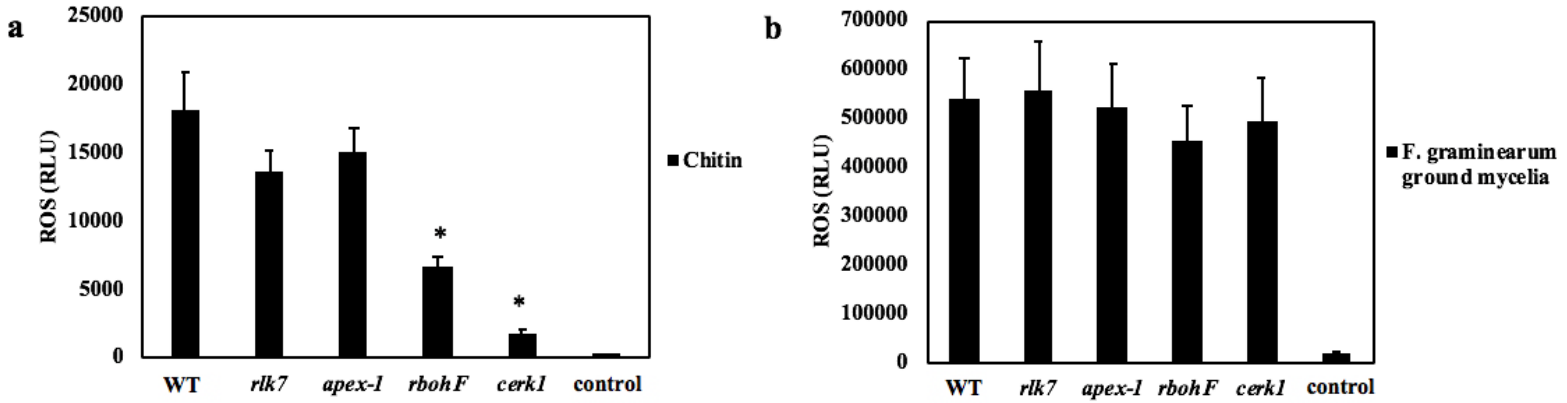


Figure 9. Mutants *rbohF* and *cerk1* produce lower ROS relative to chitin treated Col-0 WT plants. ROS was measured from four to five-week-old Col-0 WT, *rlk7, apex-1, rbohF* and *cerk1* plants for 60 min after treatment with (a) 100 ug/ml chitin and (b) 5mg/ml of ground *F. graminearum* mycelia. Data are mean±SE (n=8 leaf disks). Asterisks (*) indicate significant difference from WT ($p \leq 0.05$, Student's *t* test). Control here denotes background chemiluminescence from detection buffer. The experiment was repeated three times with similar results. The relative light units were added together for time period between 0-60 minutes during chitin and mycelia treatment in order to capture maximum signal over a range of time. RLU- relative light units. (These set of experiments were performed by Dr. Elizabeth Kalinda Brauer).

3.2.2 *F. graminearum* activates MAPKs in *Arabidopsis* plants

Temporally, activation of MAPK cascades follow ROS production, reaching peak at 15 minutes after MAMP perception (Boller and Felix, 2009). MAMPs such as flg22 and chitin activate MAPKs 3 and 6 in *Arabidopsis* (Cao *et al.,* 2014; Bi *et al.,* 2018). In order to test if *F. graminearum* can activate MAPKs 3 and 6, four-five-week-old leaves were infiltrated with 5 mg/ml *F. graminearum* ground mycelia. We demonstrated that ground mycelia was able to activate both MAPKs, detected by activated MAPK antibodies within 15 minutes after exposure in WT plants (Figure 10a). The timeline of activation by *F. graminearum* followed a similar trend as flg22 (Figure 10a) (Suarez-Rodriguez *et al.,* 2007). Upon exposure to *F. graminearum* ground mycelia, all the mutant lines (*rlk7, apex-1, rbohF* and *cerk1*) showed a reduction in the MAPK activation when compared to WT (Figure 10b). It should be noted that this experiment was performed only once. These results implicate MAPK3/6 module in response to *F. graminearum* and more importantly is mediated through the two PRR receptors RLK7 and APEX. Since *rlk7* and *apex-1* lines show enhanced resistance to *F. graminearum* infection, we predicted an increase in MAPK activation. Our results show the opposite trend suggesting that temporal activation of MAPK3/6 are different from other MAMP perception. Thus, experiments will be performed to assess differential activation at earlier time points.

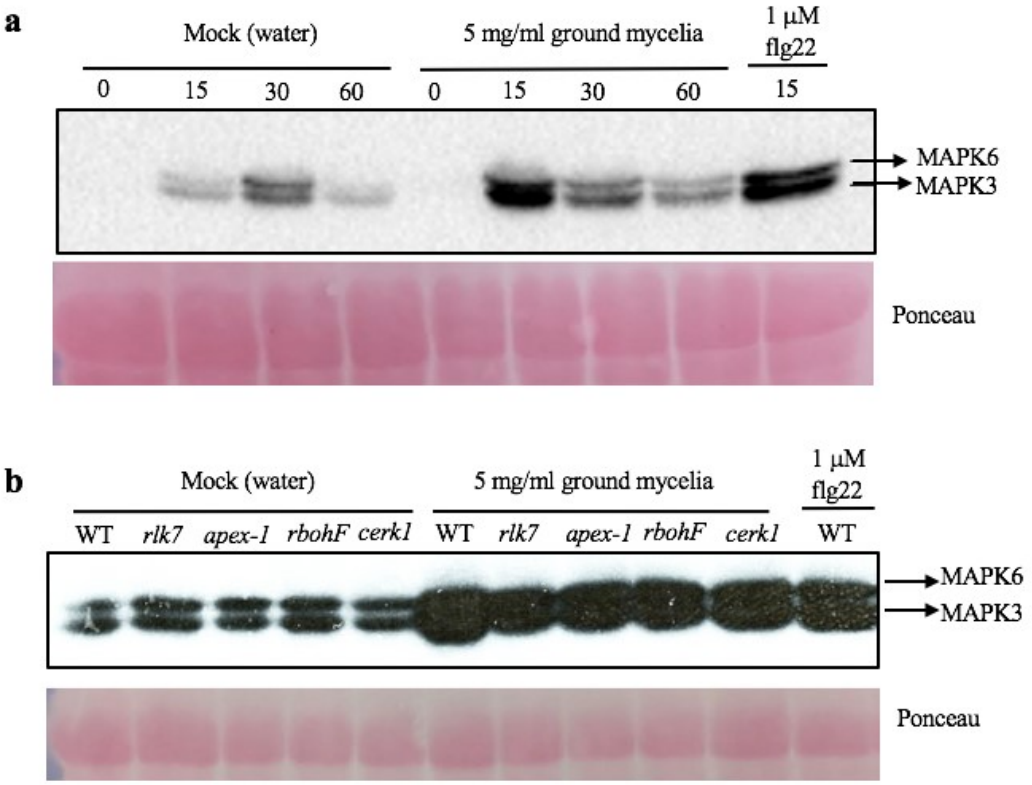


Figure 10. Activation of MAPK cascades by *F. graminearum* (a) Adult four to five-week-old Col-0 WT *Arabidopsis* leaves were infiltrated with either mock (water) or 5 mg/ml ground *F. graminearum* mycelia and samples were collected at 0, 15, 30 or 60 minutes or with 1 µM flg22 at 15 minutes post infiltration. Western blot analysis was performed with the phospho-p44/42 MAPK antibodies. This experiment was performed once. (b) Mutant *rlk7, apex-1, rbohF* and *cerk1 Arabidopsis* lines show a reduced MAPK activation after 15 minutes of ground *F. graminearum* mycelia perception. Adult four to five-week-old Col-0 WT, *rlk7, apex-1, rbohF* and *cerk1 Arabidopsis* leaves were infiltrated with either mock (water) or 5 mg/ml ground *F. graminearum* mycelia and samples were collected at 15 minutes post infiltration. Col-0 WT leaves infiltrated with 1 µM flg22 was used as positive control to MAPK activation, where samples were collected at 15 minutes post infiltration. Western blot analysis was performed with the phospho-p44/42 MAPK antibodies. This experiment was performed once.

3.2.3 Entry points of *F. graminearum* through stomata is regulated by RbohF

Plant stomata are tiny pores on the epidermis of leaves, which besides regulating gas exchange can act as natural openings for pathogen entry (Montillet and Hirt, 2013). It is known that during PTI response, exposure to MAMP such as chitin can lead to stomata closure (Khokon *et al.,* 2010). Based on published work, germinating *F. graminearum* hyphae can enter through plant stomata (Schreiber *et al.,* 2011). In order to determine if resistance to *Fusarium* involve stomata and regulated by the receptor kinases RLK7 and APEX, leaf disks from five-week-old plants were treated with 5 mg/ml of ground mycelia, representing 100 μg/ml of chitin. Ground mycelia was used as a proxy to trigger stomatal closure as it is difficult to monitor stomata aperture with *F. graminearum* spores (spores germinate at different rates). We discovered that exposure to mycelia led to 33% closure of stomata in WT when compared to mock treated WT samples (Figure 11, Supplement Figure 1). Since stomata act as a potential entry point of *Fusarium,* we hypothesized that both *apex-1* and *rlk7* mutants being resistant to *Fusarium* infection must be more responsive to mycelia treatment and result in higher percentage of stomata closure, whereas *rbohF* being susceptible must be less responsive to same treatment, resulting in more stomatal opening. We observed no significant differences between WT and the two *RLK* mutants with 26% and 32% of stomatal closure in *rlk7* and *apex-1* mutants, respectively. However, we did observe a significant difference in the signalling mutant *rbohF* with only 9% stomatal closure (Figure 11). These results suggest that stomatal aperture is regulated through RbohF, downstream of the two RLKs.

Abscisic acid (ABA) is a plant hormone involved in many important cellular responses and upon biotic or abiotic stress regulates stomata closure (Kwak *et al.,* 2003).

Since ABA is known to regulate RbohF, the next research question was to identify if RLK7 and APEX are responsive to ABA treatments (Zhang *et al.,* 2009). It was found that exposure to ABA led to 49% closure of stomata in WT when compared to mock treated WT samples (Figure 11, Supplement Figure 1). We observed no significant difference between WT and the two RLK mutants with 46% and 50% of stomata closure in *rlk7-1* and *apex-1* mutants, respectively (Figure 11, Supplement Figure 1). However, we did observe significance in the signalling mutant *rbohF* with 35% stomatal closure (Figure 11, Supplement Figure 1). These results suggest that ABA regulation of RbohF results in activation of ROS, which might ultimately lead to stomata closure.

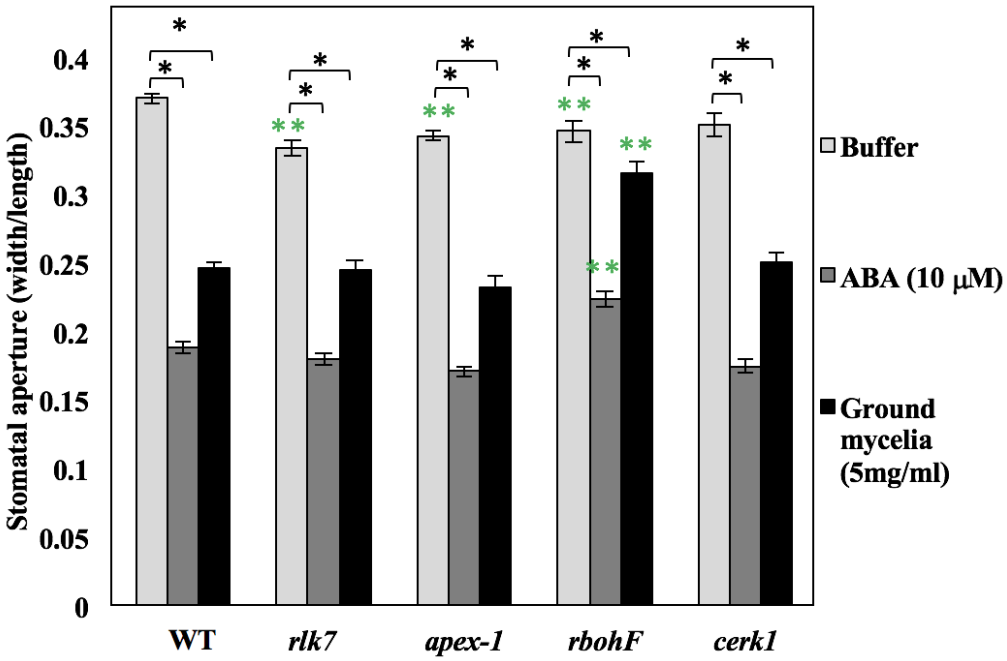


Figure 11. RbohF regulates entry points to *F. graminearum* through stomata. Stomatal aperture for wild-type Col-0, *rlk7*, *apex-1*, *rbohF* and *cerk1* mutant five-week-old plants was measured 1 h after mock (buffer) and 5mg/ml ground mycelia treatments and 3 h after 10 μM ABA treatment. Values are averages ± SE (n=18-24 leaf disks, where 30 stomata/replicate were measured). Statistical analysis was performed using Student's *t* test with $p \leq 0.05$. Single asterisk (*) indicates significant difference within mutant lines and double asterisks (**) indicate significant difference relative to WT within same treatment. This experiment was performed three times with similar results.

3.2.4 APEX and RLK7 regulate expression of defense genes

(Dr. Elizabeth Kalinda Brauer assisted me in establishing results presented in Table 5)

In order to get insight into the genes that may be regulated by the perception of *F. graminearum*, we took advantage of the 17 transgenic lines that expresses genes involved in various aspects of PTI signalling (https://bar.utoronto.ca/). The promoter of each gene is fused to the fluorescent marker mVenus (Table 4) and expression of the genes could be monitored by fluorescence microscopy after various treatments (Poncini *et al.,* 2017). The lines were obtained from Dr. Niko Geldner, University of Lausanne.

Table 4. List of 17 promoter: mVenus fusion lines in *Arabidopsis*.

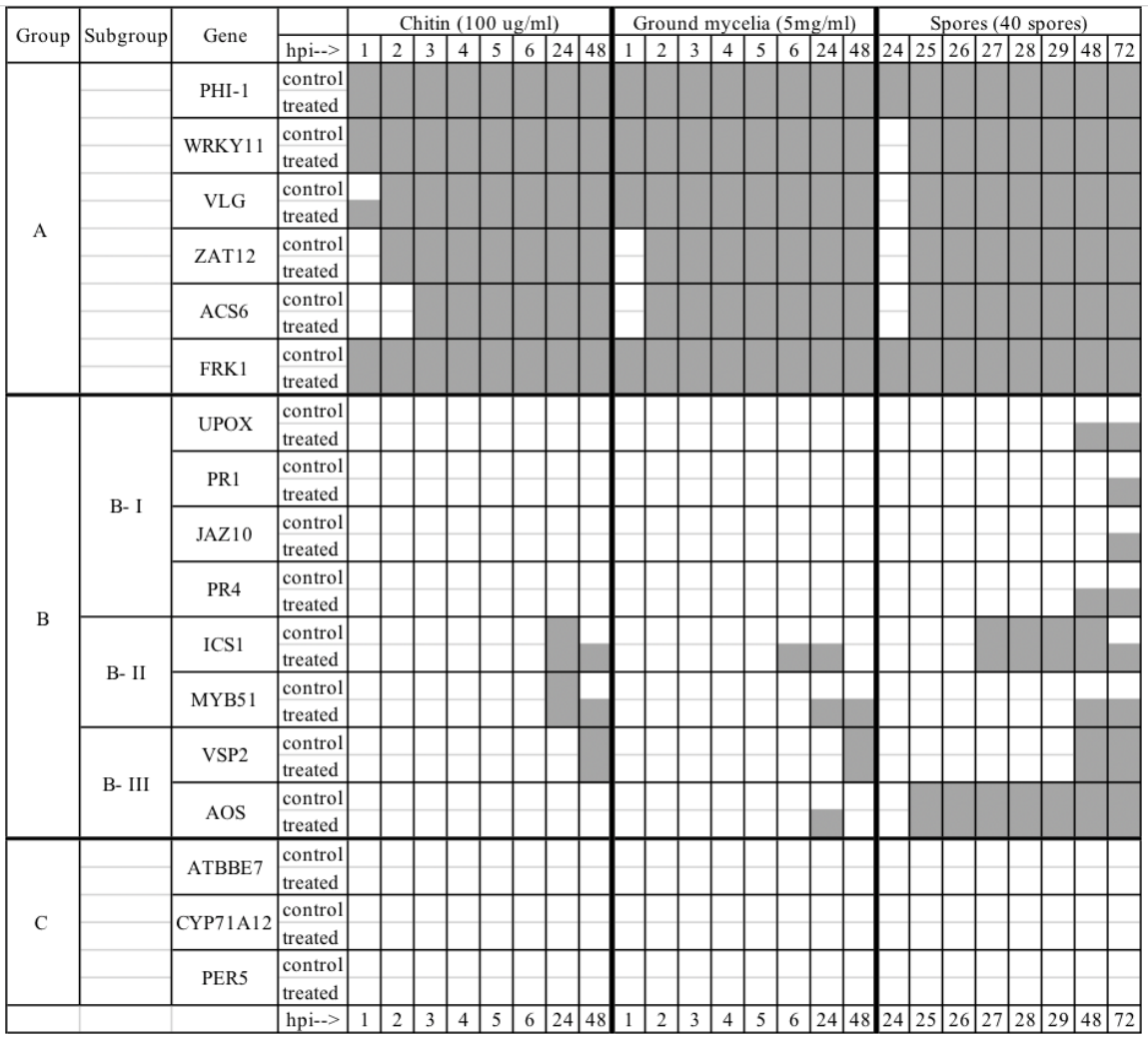
Promotor	**Locus**	**Marker for**	**Hormone/pathway**
ICS1	At1g74710	SA synthesis	Salicylic acid (SA)
PR1	At2g14610	Downstream marker for SA	
AOS	At5g42650	JA synthesis	Jasmonic acid (JA)
VSP2	At5g24770	Downstream marker for JA	
JAZ10	At5g13220	Early downstream marker for JA	
ACS6	At4g11280	ET biosynthesis	Ethylene (ET)
PR4/HEL	At3g04720	Downstream marker for ET	
MYB51	At1g18570	Regulator of camalexin and glucosinolate pathway	MAMP signalling
CYP71A12	At2g30750	Camalexin biosynthetic enzyme	
ATBBE7	At1g26420	FAD-binding Berberine family protein	
PER5	At1g14550	Peroxidase superfamily	
WRKY11	At4g31550	Early MAMP downstream target	
FRK1	At2g19190	Receptor-like protein kinase	
VLG	At2g17740	MAMP marker	
PHI-1	At1g35140	MAMP response	Pi response
ZAT12	At5g59820	Marker for ROS	ROS
UPOX	At2g21640	Marker for ROS	

The 17 PTI mVenus lines were tested for their responses to chitin, ground mycelia from *F. graminearum* and *F. graminearum* spores. Chitin was chosen as a MAMP to compare gene expression pattern as a result of its interaction with *Arabidopsis* seedlings (Figure 9a). Based on the mVenus fluorescence observations, the PTI genes were categorized into three groups. Group A consisted of six genes that constitutively expressed in both control and fungal treatments at all recorded time points (*PHI-1, WRKY11, VLG, ZAT12, ACS6* and *FRK1*, Table 5). Group B consisted of eight genes that showed induced expression after fungal treatments relative to control (*ICS1, MYB51, UPOX, PR1, JAZ10, PR4, VSP2* and *AOS*). Group C consisted of three genes *ATBBE7, CYP71A12* and *PER5* that did not induce expression at any observed time points.

The group B genes were induced by one or all fungal treatments after 24 hours. These genes are involved in a variety of defense pathways such as *ICS1 (Isochorismate Synthase 1)*, involved in the biosynthesis of SA (Wildermuth *et al.,* 2001), MYB51 is a transcription factor that regulates camalexin and glucosinolate pathway (Frerigmann *et al.,* 2015), *UPOX (up-regulated by oxidative stress)* is a stress-responsive gene, which is induced in response to H_2O_2 and SA (Ho *et al.,* 2008). It is also induced in response to trichothecene toxin, specifically T-2 toxin produced by *Fusarium* species (Masuda *et al.,* 2007). *PR1 (Pathogenesis-Related 1)* is a downstream marker for the induction of SA signalling pathway (Wildermuth *et al.,* 2001), *JAZ10 (JASMONATE ZIM-domain)* is an early downstream marker for jasmonic acid (JA) signalling pathway, where it has been shown to bind to transcription factors to repress JA-responsive genes (Moreno *et al.,* 2013), *PR4 (Pathogenesis-Related 4)* is a downstream gene expression marker for both ethylene and JA signalling pathways (Gu *et al.,* 2002; Van Loon and Van Strein, 1999;

Yang *et al.,* 2017), *VSP2 (Vegetative Storage Protein 2)* acts as a downstream gene expression marker of JA (Van Wees *et al.,* 1999) and *AOS (Allene Oxide Synthase)* is involved in the biosynthesis of JA (Park *et al.,* 2002). Interestingly, out of eight genes from group B, there were four genes that were specifically induced as a result of *F. graminearum* infection that included *UPOX, PR1, PR4* and *JAZ10* (Table 5). Rest of the genes *ICS1, MYB51, VSP2* and *AOS* were induced after 24 h in all three treatments. We also observed differences in expression of *MYB51* with respect to type of treatment. This gene was induced at 24 hpi with chitin and mycelia treatment, but later at 48 hpi with *F. graminearum* spores.

Table 5. Summary of expression of PTI genes over time in response to chitin, ground *F. graminearum* mycelia and spores. Expression pattern has been divided into three groups A, B and C. Grey box indicates fluorescence and white box indicates a lack of fluorescence in cotyledons.



Next, we were interested in determining if any of the genes in group B were differentially expressed in the mutants *rlk7, apex-1, rbohF* and *cerk1* during infection. Within group B, genes were divided into subgroups based on similar trends. The genes *UPOX, PR1, JAZ10* and *PR4* (subgroup B- I) showed induced expression relative to control with a single treatment (*F. graminearum* spores) (Table 5). Whereas, *ICS1* and *MYB51* (subgroup B- II) showed induced expression relative to control in all three treatments and induced at different time points (Table 5). On the other hand, *VSP2* and *AOS* (Group B- III) induced expression in both control and treated samples after 48hpi and 24hpi, respectively (Table 5). Therefore, based on variation in gene expression relative to control, genes from subgroup B- I (*UPOX, PR1, JAZ10* and *PR4*) and subgroup B- II (*ICS1* and *MYB51*) were chosen for validation by quantitative (qRT-PCR) analysis (Figure 12a-b).

Based on qRT-PCR analysis, we discovered that all genes in subgroup B- I *UPOX, PR1* and *PR4* except for *JAZ10* were upregulated in WT infected with *F. graminearum* spores compared to non-infected WT seedlings (Figure 12a). When the expression of this group was monitored in the mutants *rlk7, apex-1, rbohF* and *cerk1* seedlings, we observed differential expression with respect to each of the genes. We noticed that both *apex-1* and *cerk1* had a significantly lower basal *PR1* expression relative to WT non-infected seedlings (Figure 12a). As a result, *apex-1* and *cerk1* showed a ~ 6-fold and ~ 3-fold increase in *PR1* expression, respectively relative to WT infected seedlings (Supplement Table 2). This suggests that APEX plays an important role in SA perception and signalling. In contrast, the expression of *JAZ10* was significantly affected with higher expression in the *apex-1* and *rbohF* mutant seedlings infected with *F.*

graminearum (Figure 12a and Supplement Table 2). The expression pattern of *JAZ10* followed a similar trend as *PR1* expression profile in *apex-1* mutant plants and suggested that JA signalling is suppressed in this mutant line by the induction of *JAZ10* expression (Figure 12a). It is well established that JAZ10 is a negative regulator of JA signalling and moreover, SA signalling work antagonistically to the JA signalling pathway in plant defense (Moreno *et al.,* 2013; Euglem and Somssich, 2007). A similar trend was observed with *UPOX* gene where only the *apex-1* and *rbohF* mutants showed a significantly lower expression (0.4-fold and 0.7-fold, respectively) when compared to WT infected seedlings (Figure 12a and Supplement Table 2). On the other hand, mutant *rlk7* and *cerk1* seedlings displayed a similar *UPOX* gene expression as WT infected but had significantly variable basal *UPOX* expression relative to WT non-infected (Figure 12a).

The expression of *PR4* gene serves as a marker for ET and JA responsive pathways. We observed that its expression is strongly induced by the infection, but remained unchanged in the *apex-1, rbohF* and *cerk1* mutant seedlings relative to WT infected whereas, *rlk7* showed a significant 1.6-fold reduction in *PR4* expression relative to WT infected (Figure 12a and Supplement Table 2). Cumulatively, the expression of genes in subgroup B- I (induced by a single treatment of *F. graminearum* spores), suggest that SA and ET signalling pathways are responsive to *F. graminearum* infection. Specifically, activation of APEX will lead to SA signalling along with JA inhibition, while RLK7 activation might intersect with both JA and ET pathways.

The genes (*ICS1* and *MYB51*) from subgroup B- II (induced by all three treatments) were both upregulated in WT infected seedlings relative to WT non-infected

seedlings (Figure 12b). Similarly, all mutant lines except for *rbohF* displayed increased *ICS1* expression with respect to their non-infected controls (Figure 12b). When we compared the expression of the mutants to the WT after infection, both *apex-1* (0.5-fold) and *rbohF* (0.2-fold) showed a significant decrease in *ICS1* expression, whereas *cerk1* showed a significant increase (2.2-fold) compared to WT infected seedlings (Figure 12b and Supplement Table 2). Similar to *ICS1*, all mutant lines showed significant increase in *MYB51* gene expression relative to their non-infected controls and also displayed a significantly reduced expression compared to WT infected (Figure 12b and Supplement Table 2). The mutant *apex-1, rbohF* and *cerk1* seedlings also expressed significant reduction in basal *MYB51* gene expression compared to WT non-infected (Figure 12b). The results from the expression of *ICS1* of group B- II genes suggest that both APEX and RbohF might influence SA biosynthesis, originating from chloroplasts. Whereas, the expression of *MYB51*, which has pleiotropic functions is influenced by a range of activation from multiple receptors.

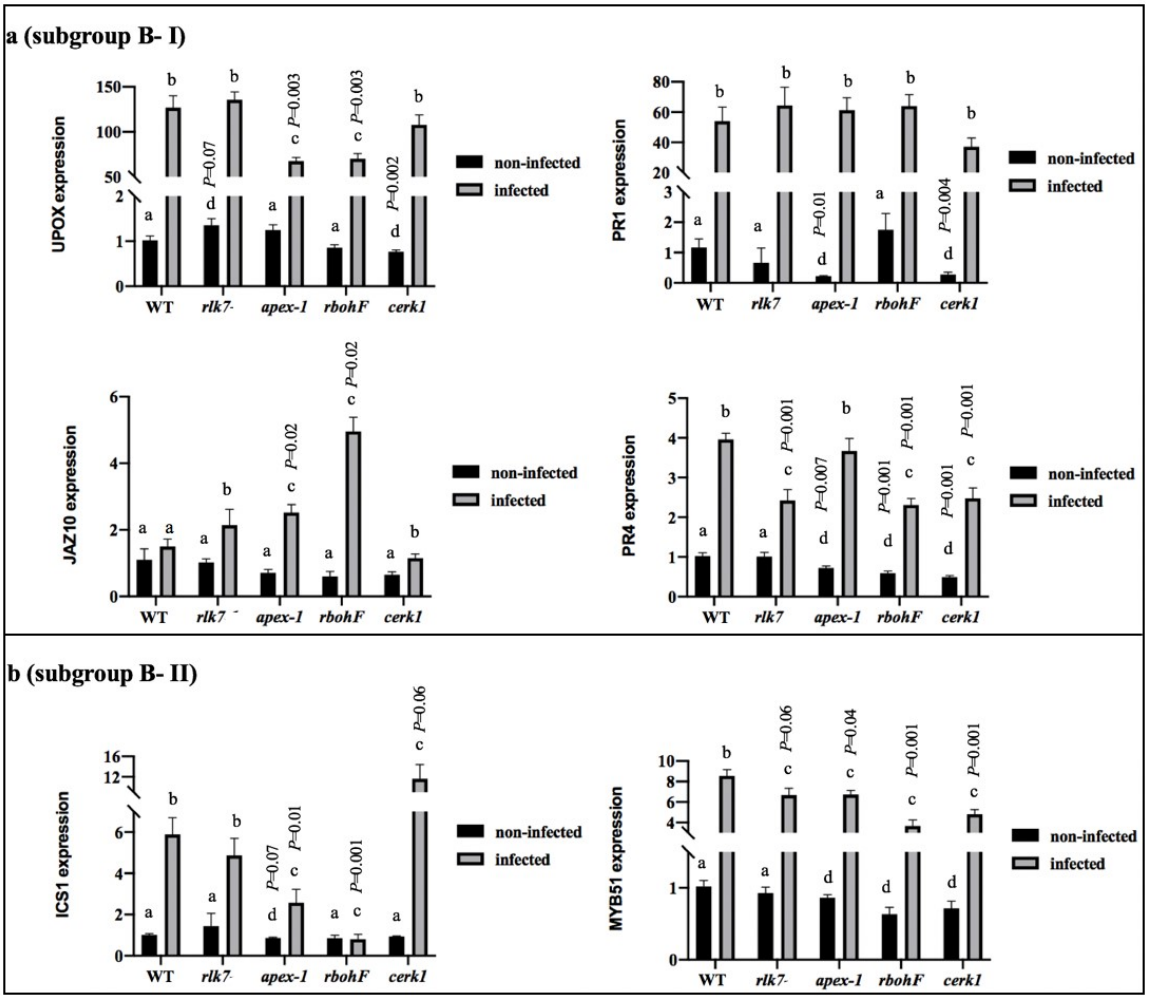


Figure 12. Defense gene expression is altered in *rlk7, apex-1, rbohF* and *cerk1* upon *F. graminearum* perception. (a-b) Expression of defense genes was measured using qRT-PCR following mutant seedlings growth in mock (non-infected) or 3dpi with *F. graminearum* spores. WT non-infected expression was set to 1 and defense gene expression was normalized to *PP2A* gene expression. Values are averages ± SE (n= 4-8 bio-replicates, where each bio-replicate contains 18-21 seedlings). Different letters indicate significant difference from WT plants. Statistical analysis was performed using one-way ANOVA and Tukey test, where $p \leq 0.1$.

3.2.5 RbohF mediates resistance to *F. graminearum* infection by regulating salicylic acid levels in *Arabidopsis*

The hormone salicylic acid (SA) promotes immunity in plants upon pathogen attack (Kim *et al.,* 2014). SA signalling contributes to basal resistance to FHB in both wheat and barley (Makandar *et al.,* 2012). Increase in SA levels in *Arabidopsis* have also been shown to reduce *F. graminearum* infection (Makandar *et al.,* 2015). Based on changes in *ICS1* gene expression in *apex-1* and *rbohF* mutant lines, we hypothesized that there will be comparable changes in the accumulation of SA in these two mutants (Figure 12b). To test this hypothesis, *apex-1* and *rbohF* seedlings were inoculated with *F. graminearum* spores and infected tissues were harvested 3dpi. The *rbohF* mutant significantly accumulated lower levels of SA relative to WT infected, whereas, in the *apex-1* mutant, no significant changes in SA accumulation was observed (Figure 13). The expression of *PR1* serves as a marker for the activation of SA signalling pathway. As demonstrated before, the expression is significantly increased in the mutant lines *apex-1* and *rbohF* following infection (Figure 12a). Overall, these results suggest that APEX influences SA signalling downstream of SA biosynthesis that is independent of *ICS* expression, while RbohF mediates both SA biosynthesis and perception.

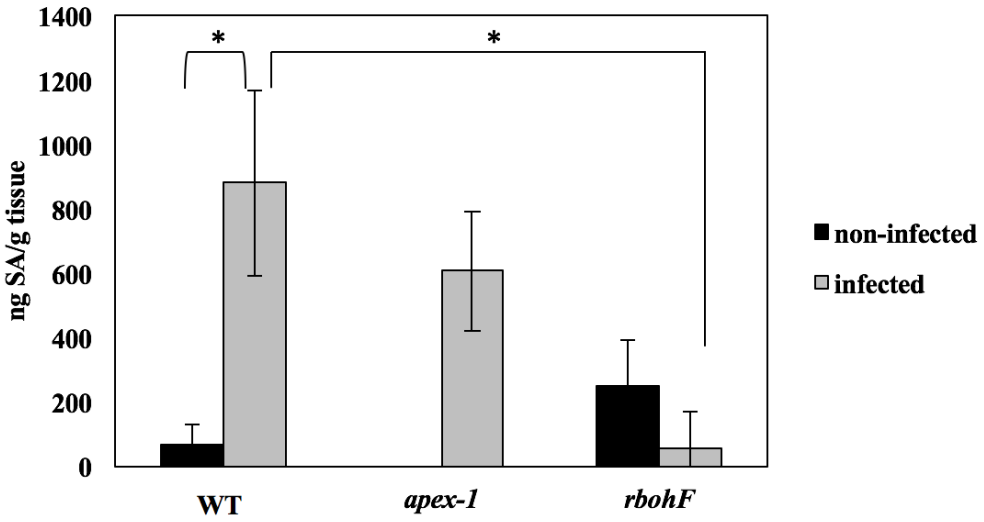


Figure 13. RbohF mediates resistance to *F. graminearum* via the regulation of salicylic acid levels. SA content in Col-0 WT, *apex-1* and *rbohF Arabidopsis* seedlings was quantified by HPLC, where samples were either untreated (non-infected) or collected 3dpi by *F. graminearum* spores. Values are averages ± standard deviation (n=3-4 biological replicates consisting of non-infected or infected seedlings). Statistical analysis was done using Student's *t* test where $p \leq 0.05$, where asterisks (*) indicate significant difference relative to as indicated in the figure. No SA was quantified from non-infected *apex-1* mutant plants. The experiment was done once.

3.2.6 *Fusarium graminearum* upregulates Damage-Associated Molecular Patterns (DAMPs) expression upon infection

Upon pathogen attack, plants activate defense responses via recognition of MAMPs/DAMPs. MAMPs are considered as "non-self" structures derived from attacking pathogen, while DAMPs are considered as "self" structures derived from plants (Gust *et al.,* 2017). DAMPs can be further categorized into two groups: those that are either passively released upon cell damage (for example Pant Elicitor Peptide 1 [PEP1], oligogalacturonides, cellobiose and cutin) or peptides that are processed and/or secreted upon MAMP perception (for example, PIP1; MAMP-Induced Peptide 1) (Gust *et al.,* 2017). PIP1 is recognized by receptor RLK7 and amplifies flg22-induced responses in *Arabidopsis* (Hou *et al.,* 2014). PEP1 is recognized by receptor PEPR1, which interacts with receptor APEX in a ligand-independent manner and regulates DAMP signalling (Smakowska-Luzan *et al.,* 2018).

In order to test our hypothesis that DAMPs are activated following *F. graminearum* infection, we monitored the expression of the precursors *proPEP1* and *proPIP1* by qRT-PCR. We showed that precursors of both genes were significantly upregulated (5.9-fold and 570-fold, respectively) in WT seedlings infected with *F. graminearum* spores relative to non-infected WT seedlings (Figure 14and Supplement Table 3). The mutant *apex-1* seedlings displayed a consistent significant reduction in *proPEP1* (2-fold) and *proPIP1* (1.2-fold) expression relative to WT infected (Figure 14). Whereas, mutant *rlk7* seedlings were significantly upregulated in *proPIP1* expression (1.7-fold) relative to WT infected and displayed a similar trend in *proPEP1* expression compared to WT infected (Figure 14). In the mutant *rbohF* seedlings, *ProPEP1*

expression was also upregulated (2.4-fold) and displayed a similar trend with respect to *ProPIP1* expression relative to WT infected seedlings (Figure 14 and Supplement Table 3). On the other hand, mutant *cerk1* seedlings did not display any significant variation in either *ProPEP1* or *ProPIP1* expression pattern relative to WT infected tissues (Figure 14). Overall, APEX as a co-receptor upregulates expression of both DAMP precursors *ProPEP1* and *ProPIP1* upon *F. graminearum* infection and whereas, RLK7 as a receptor specifically regulates expression of a single DAMP (*PIP1*). Furthermore, our results strongly suggest that DAMP gene *PEP1* and *PIP1* are not regulated and likely do not participate in the well characterized fungal chitin co-receptor CERK1 response pathway.

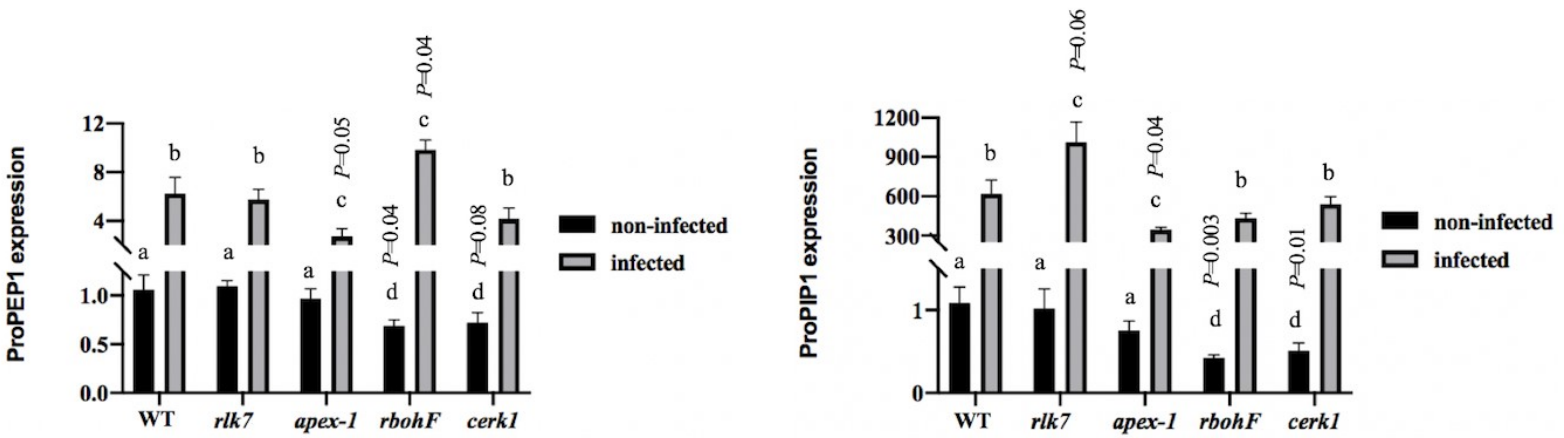


Figure 14. APEX regulates expression of DAMPs following *F. graminearum* infection. Expression of DAMP genes was measured using qRT-PCR following mutant seedlings growth in mock (non-infected) or 3dpi with *F. graminearum* spores. WT non-infected expression was set to 1 and gene expression was normalized to *PP2A* gene expression. Values are averages ± SE (n= 4-8 bio-replicates, where each bio-replicate contains 18-21 seedlings). Different letters indicate significant difference from WT plants. Statistical analysis was performed using one-way ANOVA and Tukey test, where $p \leq 0.1$.

Chapter 4: Discussion

To identify receptors and downstream signalling components mediating quantitative resistance against *F. graminearum*, a reverse genetics approach was used. Two hundred and forty-nine mutant *Arabidopsis* lines were subjected to *F. graminearum* infection and were quantified for differences relative to WT (Figure 7 and Supplement Table 1). The screen included a total of 219 transmembrane localized receptors along with 30 downstream PTI signalling components (Table 3). The transmembrane receptors included in the screen belonged to subfamilies with different extracellular domains such as leucine-rich domain, malectin domain, LysM domain, L-lectin domain, etc. (Table 3 and Supplement Table 1). The downstream signalling components included NADPH oxidases, ion gated channels, MAPK-related proteins, protease inhibitors, transcription factors, etc. (Table 3 and Supplement Table 1). Since it is known that resistance against *F. graminearum* is mediated quantitatively and through activation of PTI, identification of receptors and the downstream signalling components will help decipher key players of defense against *F. graminearum*.

Our screen identified nine mutant lines with significant difference from WT during infection with *F. graminearum*, where eight mutant lines were transmembrane localized receptors (*rlk7, apex-1, 588, psy1r, 528, lyk4/5, 333* and *sobir1-12*) and one mutant line (*rbohD/F*) was involved in downstream signalling (Figure 7a). We discovered that except for *rlk7* and *apex-1*, rest of the mutant receptor lines were associated with increased susceptibility (Figure 7b). Upon further analysis of the double mutant *rbohD/F*, we discovered that the signalling component RbohF was mainly responsible for the phenotype observed in the double mutant (Figure 7b). As a result, the

mutant lines *rlk7, apex-1* and *rbohF* were selected for further characterization.

The receptor RLK7 belongs to LRR-RLK subfamily XI with an extracellular 20 leucine rich repeats along with an intracellular kinase domain (Pitorre *et al.,* 2010). The long extracellular LRR domain binds to the MAMP-induced secreted peptide called PIP1 (Hou *et al.* 2014). PIP1 is a DAMP and as such DAMPs are characterized as "self" structures derived from plants, which are either released passively upon cell damage or processed upon MAMP perception (Gust *et al.,* 2017). PIP1 has been shown to be secreted as pro-peptide by plants after flg22 treatment and is subsequently targeted to the endoplasmic reticulum/Golgi-dependent secretory pathway where its signal peptide is removed. The resulting peptide is secreted into apoplast where it undergoes further proteolytic processing (Hou *et al.,* 2014). While another DAMP, PEP1 (Plant Elicitor Peptide 1) is passively released upon tissue damage as it lacks N-terminal signal peptide for secretion (Gust *et al.,* 2017). The activation of RLK7 by exogenous application of PIP1 has been reported to trigger similar downstream defense responses as with the bacterial MAMP flg22 (Hou *et al.,* 2014). In fact, it has also been shown that PIP1 signalling amplifies MAMP-triggered PTI immune response and promotes additive resistance against the bacterial pathogen *Pst*DC3000 (Hou *et al.,* 2014). A role of DAMPs in *F. graminearum* infection process is affirmed by the observations that expression of both *proPEP1* and *proPIP1* are significantly increased following infection (Figure 14). Moreover, the expression is also influenced by both RLK7 and APEX, suggesting a link between the PRRs and DAMP signalling. The mechanism by which these DAMPs influence the defense response require further research.

4.1 Relationship between RLK7 and APEX in defense response signalling

A recent study established a relationship between all the LRR receptor kinases in *Arabidopsis*, based on ligand independent protein interactions. The study resulted in the construction of cell surface interaction network (CSI) that consisted of 567 interactions (reproduced in Figure 15) (Smakowska-Luzan *et al.,* 2018). The study showed that in addition to a direct interaction between RLK7 and APEX, they also form an important protein hub that links various sub-networks- an articulation point (Smakowska-Luzan *et al.,* 2018). Articulation points are nodes whose removal results in formation of at least two disconnected subnetworks (Smakowska-Luzan *et al.,* 2018). As protein hubs, RLK7 and APEX interact with seven and nineteen other LRRs, respectively in the network (Figure 15). Analysis revealed that APEX also interacted with one of the other receptors identified in our screen, namely, the receptor line 333 (AT3G46340). A direct interaction between proteins suggest that they may have a common purpose regarding cellular functions. Thus, a direct interaction between a MAMP receptor (#333), and a co-receptor (APEX) and another direct interaction between the same co-receptor and DAMP receptor (RLK7), strongly suggests that all three receptors may form a complex. It should be noted that the interaction studies were performed without a ligand and the foremost question is how does the receptor complex behave in the presence of a ligand such as *F. graminearum*?

We propose a model, where the MAMP receptor 333 is kept inactive by the co-receptor APEX and the DAMP receptor RLK7 (Figure 16a). The MAMP receptor 333 has a small leucine-rich repeat with a malectin-like domain in its extracellular domain. It belongs to the subfamily CrRLK1-like (*Catharanthus roseus* RLK1) (Shiu and Bleecker, 2001a). Malectin-like domain in plants was named after homologous sequences found in

a carbohydrate-binding domain of MALECTIN protein characterized in *Xenopus laevis* that binds specifically to N-acetylglucosamine, a major component of chitin (Dernier *et al.,* 2011). Based on homology, it has been hypothesized that malectin-like extracellular domain of CrRLK1L is involved in binding to carbohydrates including oligo or polysaccharides from cell-wall constituents released upon cell wall degradation or glycosylated proteins (Dernier *et al.,* 2011). As suggested by the model, a mutation in the MAMP receptor will prevent it from perceiving any signals from *F. graminearum* and as such should result in increased susceptibility. In support, we identified the mutant line (333) as a loss of function allele in our screen. This supports the notion that this receptor is likely a positive regulator of PTI response, directly recognizing a MAMP with a carbohydrate moiety, originating from *F. graminearum* (Figure 16a).

After perception, we propose that the MAMP receptor 333 is activated with the disassociation of the two negative regulators in the complex (Figure 16b). In this regard, the model suggests that a gain of function mutations of either APEX or RLK7 will result in activation of PTI signalling. This is supported by the observation that mutations in both co-receptors APEX and the DAMP receptor RLK7 show a decrease in susceptibility to *F. graminearum* infection (Figure 7). APEX has been shown previously as a negative regulator of the flg22-FLS2 complex, where flg22 treatment of *apex-1* mutant plants resulted in the formation of complex between FLS2 and the co-receptor BAK1. The complex formation resulted in enhanced MAPK activation, enhanced ROS production and upregulated expression of defense marker gene FRK1 (Smakowska-Luzan *et al.,* 2018).

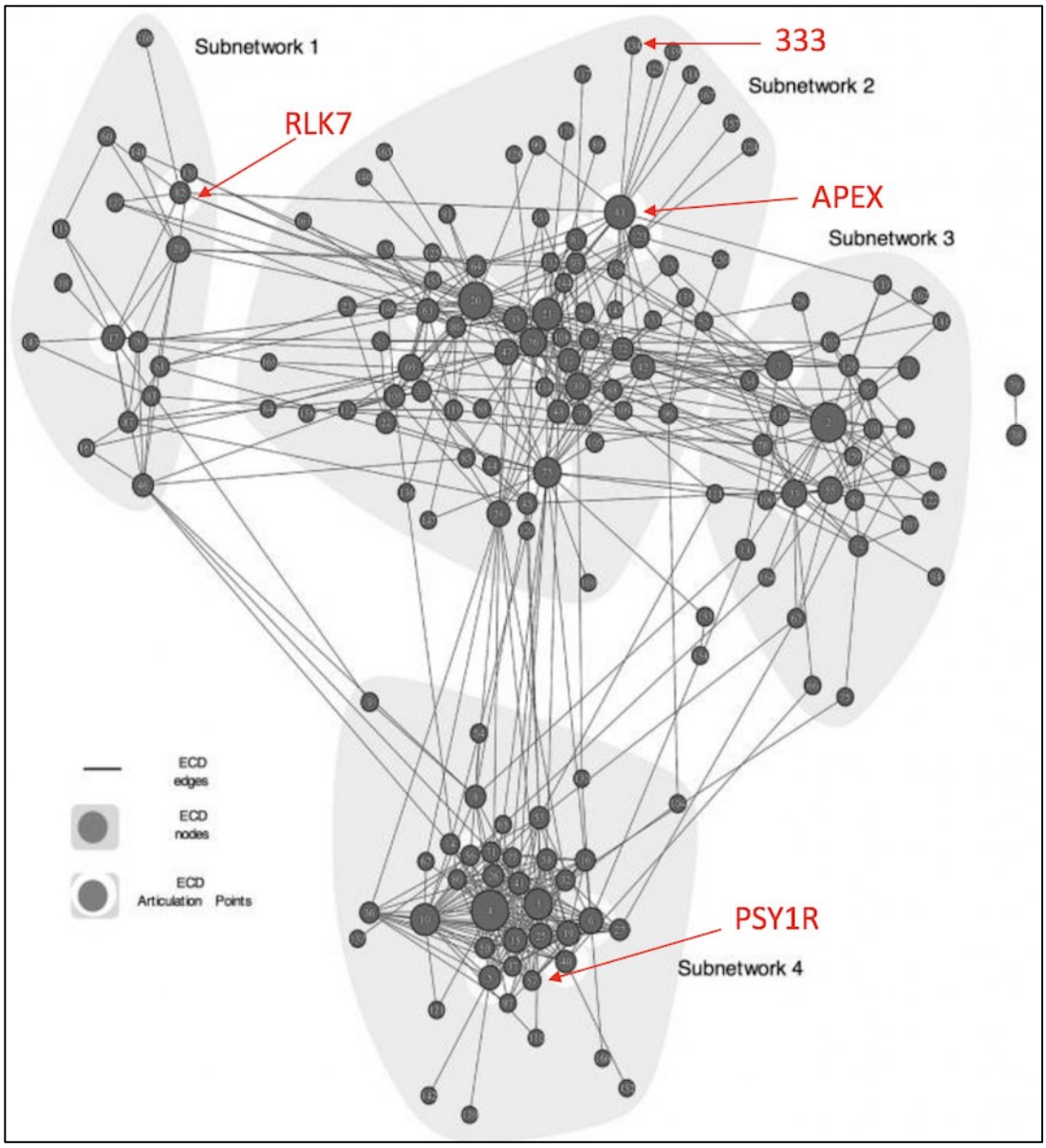


Figure 15. Leucine-rich repeat receptor-like kinase network depicts interaction between receptor line 333 and APEX and between APEX and RLK7. The network also shows additional LRR-RLK PSY1R that belongs to a different subnetwork than APEX and RLK7. Here interaction is depicted with black lines. Reprinted by permission from [the Licensor]: [Springer Nature] [An extracellular network of Arabidopsis leucine-rich repeat receptor kinases, Elwira Smakowska-Luzan, G. Adam Mott, Katarzyna Parys, Martin Stegmann, Timothy C Howton et al.] [2018].

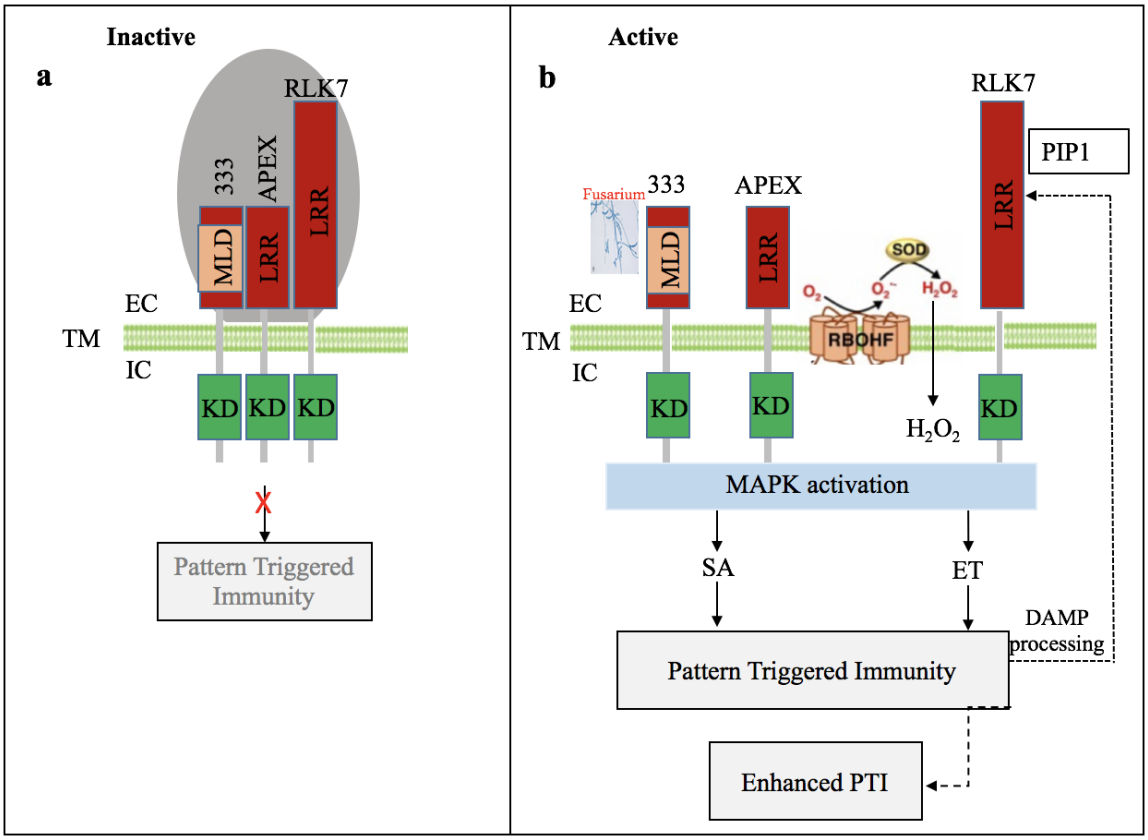


Figure 16. A model representing association of APEX and RLK7 during *F. graminearum* infection process. (a) Inactive receptor complex: In the absence of *F. graminearum*, the receptor 333 encoded by *AT3G46340*, APEX and RLK7 exists in a complex where APEX and RLK7 inhibit onset of MAMP-triggered immunity. (b) Active receptor complex: Upon *F. graminearum* infection, the receptor line 333 binds to a yet unidentified MAMP from the pathogen leading to the dissociation of the receptor complex and their activation. The activation triggers numerous downstream responses that include ROS production via the RbohF complex, MAPK activation and two hormone signalling pathways, namely salicylic acid (SA) and ethylene (ET) resulting in PTI. The receptor activation also leads to synthesis of DAMPs such as PIP1, which potentiates the signalling response by binding to the RLK7 receptor. Together both MAMP and DAMP signalling leads to an enhanced PTI response. TM: Transmembrane domain; EC: Extracellular domain; IC: Intracellular domain; MLD: Malectin-Like Domain; KD: Kinase Domain; LRR: Leucine Rich Repeat; MAMP: Microbe Associated Molecular Pattern; PTI: Pattern Triggered Immunity; SA: Salicylic Acid; ET: Ethylene; SOD: Superoxide dismutase and MAPK: Mitogen Activated Protein Kinase.

4.2 Downstream responses during *F. graminearum* infection process in *Arabidopsis*

PTI is characterized by the induction of early defense responses such as calcium burst, K^+ efflux, ROS activation, MAPK activation, callose deposition, and ethylene production and late defense responses include stomata regulation, salicylic acid accumulation, and defense gene activation (Bigeard *et al.,* 2015). In our study, we characterized the role of *RLK7, APEX* and *RbohF* during early defense responses that included ROS production, and MAPK activation and late defense responses that included SA accumulation, stomata regulation, and defense genes activation. During early defense responses, the mutant *rlk7, apex-1* and *rbohF* plants produced similar ROS levels relative to WT infected when treated with either ground *F. graminearum* mycelia or chitin (Figure 9b), but genes responsive to ROS such as *UPOX* was significantly affected both in the *apex-1* and *rbohF* mutant lines. This suggested that similar to other plant-pathogen interactions, ROS is a predictive response to *F. graminearum* perception, however, the response to ROS is not linked to either RLK7 or APEX.

Similarly, MAPK module 3/6 is responsive to *F. graminearum*, however, their activation in the mutants was not predicted (Figure 10). Since both APEX and RLK7 receptors are proposed as negative regulators, we expected the mutation of these receptors would result in increased activation of the MAPK module. We speculate that temporal differences may account for this discrepancy and experiments with earlier time points should be performed to answer this question.

The two receptors RLK7 and APEX and signalling component RbohF were also characterized during late PTI-induced responses including stomata regulation, SA

accumulation and defense gene activation. Based on previous published work, it is known that *F. graminearum* hyphae can enter through plant stomata (Schreiber *et al.,* 2011). As expected, *rbohF* mutant plants were less responsive to stomata closure with both ground mycelia and ABA treatments, whereas, *rlk7* and *apex-1* mutant plants were not responsive to either of the treatments (Figure 11). This suggested that initial entry of *F. graminearum* into *Arabidopsis* leaves is likely not mediated by RLK's regulation of stomata. Since the primary inoculum activates ROS production, stomatal entry points may play a role later in the infection cycle when the plants encounter new spores and restrict the entry of the pathogen.

In order to get insight into defense genes that may be regulated by the perception of *F. graminearum*, we took advantage of the 17 transgenic lines that expresses genes involved in various aspects of PTI signalling such as SA, JA, ET and ROS activation (Poncini *et al.,* 2017). The microscopy results provided information to conduct gene expression analysis. The results suggested that among the four genes *UPOX, PR1, JAZ10* and *PR4* that are induced by *F. graminearum* spores treatment, differential expression of *PR1* and *JAZ10* provided clearer insight into the signalling pathways involved in *F. graminearum* resistance (Figure 12). *PR1* is a downstream marker for SA signalling (Wildermuth *et al.,* 2001) and results suggested that APEX has significant impact on the SA signalling pathway. In support of this, we showed that the expression of *JAZ10*, which is a suppressor of jasmonic acid (JA) pathway has increased expression in the *apex-1* mutant. It is known that SA and JA pathways work antagonistically and our results support this established paradigm. Although, our results implicate that SA signalling pathway is regulated by APEX, we did not find evidence to support that SA

biosynthesis is regulated by this receptor. In fact, we observed that the expression of *ICS1* was lower in the *apex-1* mutant with no changes in SA accumulation (Figure 12 and Figure 13). This suggested that there is alternate source of SA, possibly via PAL (phenylalanine) mediated shikimate pathway. This view is supported by a study with the necrotrophic pathogen (*Botrytis cineria*), where *ICS1* pathway mediated mutants (*sid2* and *npr1*) did not exhibit enhanced susceptibility against necrotrophic pathogen (*Botrytis cineria*) but inhibitors that block PAL activity resulted in enhanced susceptibility against this pathogen (Ferrari *et al.,* 2003).

It is generally recognized that SA signalling protects plants from biotrophic pathogens and that JA signalling protects plants from necrotrophic pathogens (Glazebrook, 2005). Previous studied have shown that both SA and JA signalling contribute to *Arabidopsis* defense against *F. graminearum* (Makandar *et al.,* 2010). Since *F. graminearum* is a hemibiotroph, SA pathway is purported to be involved in the early stage (biotrophic) of infection and JA protects plants during the necrotrophic phase of the pathogen. In this regard, APEX might be mediating resistance via the SA signalling.

A recent study also reported that ET-signalling can mediate FHB resistance to *F. graminearum* in different wheat backgrounds, where exogenous application of ET-enhancers (ACC; 1-aminocyclopropane-1-carboxylic acid and ETp; ethephon) resulted in reduced susceptibility in three susceptible wheat (Roblin, Awesome and Superb) cultivars (Foroud *et al.,* 2018). *PR4* is a downstream marker of ET, where ET-stabilized transcription factors EIN3/EIL1 (Ethylene Insensitive 1/ Ethylene Insensitive3-Like 1) regulate *PR4* expression (Yang *et al.,* 2017). ET has been shown to exhibit a biphasic activation step in the hemibiotroph pathogen (*Phytophthora parasitica*) where the first

phase peaks at 3 hpi and second phase peaks between 48 hpi and 72 hpi (Wi *et al.,* 2012). The mutant *rlk7* presented a significant 1.2-fold reduction in *PR4* expression relative to WT-infected seedlings suggesting that RLK7 might be mediating resistance via the second phase of ET signalling (Supplement Table 2).

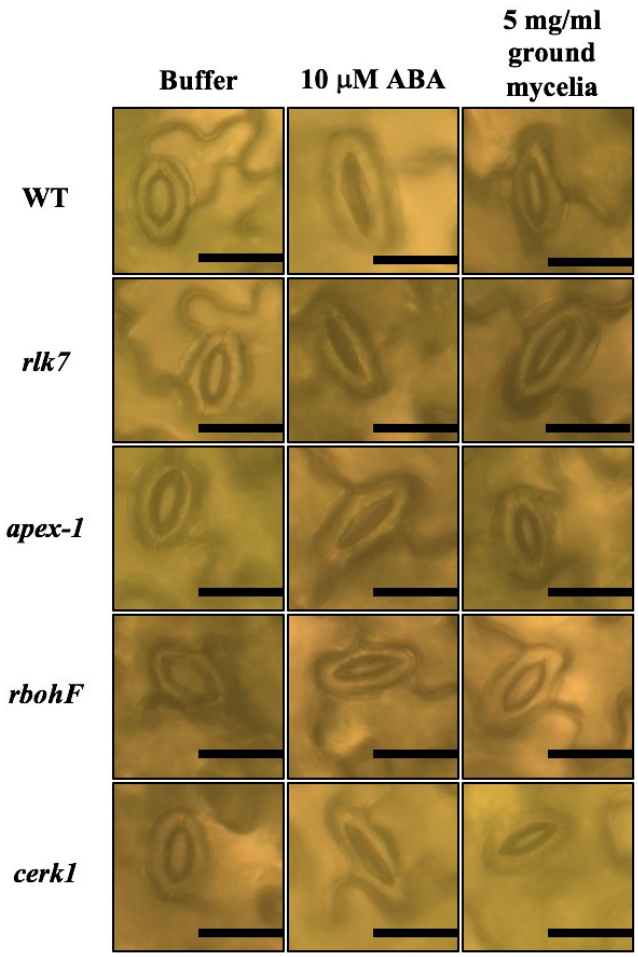
4.3 Conclusions and Perspectives

Overall, the above results suggest that APEX and RLK7 might be targeting distinct defense pathways to mediate resistance to *F. graminearum*. We propose that APEX, along with the MAMP receptor 333 trigger SA signalling early in the infection process, while the DAMP receptor is activated later to trigger the ET signalling pathways. We also propose that RbohF mediates resistance to *Fusarium* by regulating stomata closure and salicylic acid levels upon *F. graminearum* perception. In conclusion, we were able to identify key receptors and downstream signalling components that play a critical role in mediating quantitative resistance against FHB. This knowledge can be used to generate resistant wheat varieties as there are homologous genes present in wheat for the receptors RLK7 and APEX. Through breeding programs, many recombinant wheat populations are available that have been phenotyped for FHB resistance. By screening of these mutant population, we can identify phenotypes associated with mutations in the receptors identified in my thesis. Once plants with desired mutation are identified, they can be crossed with commercial varieties.

Appendices

Appendix A

A.1 Supplement Figure 1



Supplement Figure 1. Visual representation of stomatal aperture in wild-type Col-0, *rlk7*, *apex-1*, *rbohF* and *cerk1* mutant plants, where leaf disks from four to five-week-old plants were taken and measurements were recorded 1 h after mock (buffer) and 5mg/ml ground mycelia treatments and 3 h after 10 μM ABA treatment. Black bar represents 25 mm length.

Appendix B

B.1 Supplement Table 1

Supplement Table 1. List of T-DNA *Arabidopsis* mutants used in the study.

	Locus_tag	Common name	RLK/RLP/signalling component	Subfamily
1	AT2G31880	sobir1-12	LRR-RLK	LRR XI
2	AT4G33430/ AT2G13790	bak1-5/bkk1	LRR-RLK (SERK3)/LRR-RLK (SERK4)	LRR II/ LRR II
3	AT5G47910/ AT1G64060	rbohD/F	NADPH sub-unit	
4	AT5G46330	fls2	LRR-RLK	LRR XII
5	AT2G39940	coi1	LRR with F-box domain	
6	AT2G46370	jar1	Gretchen Hagen 3 (GH3) subfamily acyl acid amido synthetases	
7	AT3G21630	cerk1	lysM	
8	AT2G23770/A T2G33580	lyk4/lyk5	lysM/lysM both RLK	
9	AT5G44190	glk2 ko	nuclear transcription factor	
10	AT2G20570	GLK1 OE (5-7 line)	nuclear transcription factor	
11	Transgenic line expressing bacterial gene that encodes for hydroxylase enzyme to suppress SA accumulation	nahG	salicylate hydroxylase that converts salicylic acid to catechol	
12	AT1G64280	npr1	salicylic acid regulator-transcriptional inhibitor	
13	AT4G33430	bak1-4	LRR-RLK	LRR II
14	AT5G20480	efr-A	LRR-RLK	LRR XII
15	AT5G20480	efr-B	LRR-RLK	LRR XII
16	AT1G05700	200	LRR-RLK	LRR I
17	AT1G06840	201	LRR-RLK	LRR VIII-1
18	AT1G07550	202	LRR-RLK	LRR I

19	AT1G07650	204	LRR-RLK	LRR VIII-2
20	AT1G08590	205	LRR-RLK	LRR XI
21	AT1G09970	206 (RLK7)	LRR-RLK	LRR XI
22	AT1G10850	207	LRR-RLK	
23	AT1G12460	210	LRR-RLK	LRR VII
24	AT1G14390	211	LRR-RLK	LRR VI
25	AT1G17230	212	LRR-RLK	LRR XI
26	AT1G24650	214	LRR-RLK	LRR IX
27	AT1G27190	216	LRR-RLK	LRR X
28	AT1G28440	217	LRR-RLK	LRR XI
29	AT1G31420	225	LRR-RLK	LRR XIII
30	AT1G51800	234	LRR-RLK	LRR I
31	AT1G51805	235	LRR-RLK	
32	AT1G53730	249	LRR-RLK	LRR V
33	AT1G56140	252	LRR-RLK	LRR VIII-2
34	AT1G60630	254	LRR-RLK	LRR III
35	AT1G66830	260	LRR-RLK	LRR III
36	AT1G69270	263	LRR-RLK	
37	AT1G69990	264	LRR-RLK	LRR X
38	AT1G72300	267 (PSY1R)	LRR-RLK	LRR X
39	AT1G74360	271	LRR-RLK	LRR X
40	AT1G78980	274	LRR-RLK	LRR V
41	AT2G01210	276	LRR-RLK	LRR III
42	AT2G01820	278	LRR-RLK	LRR IX
43	AT2G01950	279	LRR-RLK	LRR X
44	AT2G02220	280	LRR-RLK	LRR X
45	AT2G02780	281	LRR-RLK	LRR XI
46	AT2G26730	300	LRR-RLK	LRR III
47	AT2G27060	301	LRR-RLK	LRR III
48	AT2G28960	303	LRR-RLK	LRR I
49	AT2G28970	304	LRR-RLK	LRR I
50	AT2G29000	307	LRR-RLK	
51	AT2G33170	309	LRR-RLK	LRR XI
52	AT2G36570	311	LRR-RLK	LRR III
53	AT2G37050	312	LRR-RLK	LRR I
54	AT2G41820	313	LRR-RLK	LRR X
55	AT2G45340	315	LRR-RLK	LRR IV

56	AT3G02130	316	LRR-RLK	
57	AT3G03770	317	LRR-RLK	LRR VI
58	AT3G14350	320	LRR-RLK	LRR V
59	AT3G24660	327	LRR-RLK	LRR III
60	AT3G25560	328	LRR-RLK	LRR II
61	AT3G28040	329	LRR-RLK	LRR VII
62	AT3G42880	331	LRR-RLK	LRR III
63	AT3G46330	332	LRR-RLK	LRR I
64	AT3G46340	333	LRR-RLK	LRR I
65	AT3G46350	334	LRR-RLK	LRR I
66	AT3G46370	335	LRR-RLK	LRR I
67	AT3G46400	336	LRR-RLK	LRR I
68	AT3G46420	337	LRR-RLK	LRR I
69	AT3G51740	341	LRR-RLK	LRR III
70	AT3G53590	342	LRR-RLK	LRR VIII-1
71	AT3G56100	343	LRR-RLK	LRR III
72	AT4G20940	354	LRR-RLK	LRR III
73	AT4G28650	360	LRR-RLK	LRR XI
74	AT4G33430	366	LRR-RLK	LRR II
75	AT4G36180	368	LRR-RLK	LRR VII
76	AT5G01890	372	LRR-RLK	LRR III
77	AT5G01950	373	LRR-RLK	LRR VIII-1
78	AT5G07150	376	LRR-RLK	LRR VI
79	AT5G10020	379/380	LRR-RLK	LRR III
80	AT5G14210	381	LRR-RLK	LRR VI
81	AT5G16000	382	LRR-RLK	LRR II
82	AT5G44700	390	LRR-RLK	LRR XI
83	AT5G45780	391	LRR-RLK	LRR II
84	AT5G48380	394	LRR-RLK	LRR X
85	AT5G48740	395	LRR-RLK	LRR I
86	AT5G48940	397	LRR-RLK	LRR XI
87	AT5G51350	403	LRR-RLK	
88	AT5G51560	404	LRR-RLK	LRR IV
89	AT3G14840	406	LRR-RLK	LRR VIII-2
90	AT5G59650	409/410	LRR-RLK	LRR I
91	AT5G59670	412/413	LRR-RLK	LRR I
92	AT5G59680	414	LRR-RLK	LRR I

93	AT5G61480	415	LRR-RLK	LRR XI
94	AT5G62230	416	LRR-RLK	LRR XIII
95	AT5G62710	417/418	LRR-RLK	LRR XIII
96	AT5G63410	419	LRR-RLK	LRR VI
97	AT5G63710	420 (APEX)	LRR-RLK	LRR II
98	AT5G65700	422	LRR-RLK	LRR XI
99	AT1G04210	481	RAF related kinase	
100	AT3G13380	482	LRR-RLK	LRR X
101	AT1G66920	521	RLK	LRK10L-2
102	AT1G66930	522	protein kinase subfamily (RLK)	
103	AT1G66980	523	RLK	LRK10L-2
104	AT1G67000	524	RLK	LRK10L-2
105	AT4G18250	525	thaumatuin-like RLK	thaumatin
106	AT5G38240	526	RLK	LRK10L-2
107	AT5G38250	527	RLK	LRK10L-2
108	AT5G38260	528	RLK	LRK10L-2
109	AT5G38260	529	RLK	LRK10L-2
110	AT5G39020	531	malectin RLK	CrRLK1L-2
111	AT5G39020	532	malectin RLK	CrRLK1L-2
112	AT2G13800	534	LRR-RLK	LRR II
113	AT5G05160	535	LRR-RLK	LRR III
114	AT4G26540	537	LRR-RLK	LRR XI
115	AT5G56040	538	LRR-RLK	LRR XI
116	AT2G24130	539	LRR-RLK	LRR XII
117	AT3G47090	540	LRR-RLK	LRR XII
118	AT3G47570	541	LRR-RLK	LRR XII
119	AT3G47580	542	LRR-RLK	LRR XII
120	AT5G39390	543	LRR-RLK	LRR XII
121	AT5G39390	544	LRR-RLK	LRR XII
122	AT3G26700	545		RLCK IX
123	AT2G45910	546		RLCK IX
124	AT2G30940	547	protein kinase superfamily (RLK)	
125	AT2G45590	549		RLCK XI

126	AT4G25390	550	protein kinase superfamily (RLK)	
127	AT5G51770	551		RLCK XI
128	AT1G33260	552	protein kinase superfamily (RLK)	
129	AT1G52540	554	protein kinase superfamily (RLK)	
130	AT3G15890	555	protein kinase superfamily (RLK)	
131	AT5G20050	556	protein kinase superfamily (RLK)	
132	AT1G34300	557	S-lectin protein kinase family protein	
133	AT2G19130	558	S-locus lectin protein kinase family protein	
134	AT2G19130	559	S-locus lectin protein kinase family protein	
135	AT4G32300	561	S-domain-2 5 (SD2-5) (RLK)	SD-2
136	AT4G32300	562	S-domain-2 5 (SD2-5) (RLK)	SD-2
137	AT5G35370	563	S-locus lectin protein kinase family protein	
138	AT1G66910	564	RLK	LRK10L-2
139	AT5G24080	565	S-domain-2 5 (SD2-5) (RLK)	SD-2
140	AT5G24080	566	S-domain-2 5 (SD2-5) (RLK)	SD-2
141	AT1G70250	567	Protease inhibitor/seed storage/LTP family protein	
142	AT1G68400	568	LRR-RLK	LRR III
143	AT3G47110	569	LRR-RLK	LRR XII
144	AT2G45910	570	U-box domain-containing protein kinase family protein	RLCK IX
145	AT3G47110	571	LRR-RLK	LRR XII
146	AT2G17440	572	PIRL5, plant intracellular ras group-related LRR 5	
147	AT2G17440	573	PIRL5, plant intracellular ras group-related LRR 5	
148	AT1G56120	574	LRR-RLK	LRR VIII-2
149	AT1G56120	575	LRR-RLK	LRR VIII-2
150	AT1G10620	578	proline-rich extensin-like receptor kinase	PERK
151	At3g24550	579	proline-rich extensin-like receptor kinase	PERK
152	AT3G18810	580	proline-rich extensin-like receptor kinase	PERK
153	At4g29050	584	L-lectin kinse protein	
154	At5g01560	585	L-lectin kinse protein	

155	At5g55830	586	L-lectin kinse protein	
156	AT4G21400	587	cysteine-rich RLK	DUF26
157	AT4G05200	588	cysteine-rich RLK	DUF26
158	AT4G38830	589	cysteine-rich RLK	DUF26
159	AT4G23280	591	cysteine-rich RLK	DUF26
160	AT4G27300	592	s-locus lectin RLK	SD-1
161	AT4G23140	593	cysteine-rich RLK	DUF26
162	AT4G21410	594	cysteine-rich RLK	DUF26
163	AT4G23220	595	cysteine-rich RLK	DUF26
164	AT4G27290	596	s-locus lectin RLK	SD-1
165	At1g11330	598	s-locus lectin RLK	SD-1
166	AT2G23770	599	lysM motif RLK (LYK4)	
167	AT2G23950	600	LRR-RLK	LRR II
168	AT5G06740	601	L-lectin kinse protein	
169	AT2G35620	602	LRR-RLK	LRR XIII
170	AT2G33580	603	lysM motif RLK (LYK5)	
171	AT1G48480	604	LRR-RLK	LRR III
172	AT5G08160	605	serine/threonine protein kinase (RLK)	
173	AT4G02420	606	L-lectin RLK	
174	AT2G43700	607	L-lectin RLK	
175	AT3G59700	608	L-lectin RLK	
176	AT3G59740	609	L-lectin RLK	
177	AT5G03140	610	L-lectin RLK	
178	AT5G10540	611	L-lectin RLK	
179	AT5G65600	612	L-lectin RLK	
180	AT1G15530	613	L-lectin RLK	
181	AT5G13130	614	L-lectin RLK	
182	AT5G42120	615	L-lectin RLK	
183	AT1G45616	617	RLP6	
184	AT1G47890	618	RLP7	
185	AT1G71390	619	RLP11	
186	AT1G71400	620	RLP12	
187	AT2G32660	624	RLP22	
188	AT2G32680	625	RLP23	
189	AT2G33020	626	RLP24	
190	AT2G33050	627	RLP26	
191	AT2G33060	628	RLP27	
192	AT2G33080	629	RLP28	

193	AT3G05360	630	RLP30	
194	AT3G05370	631	RLP31	
195	AT3G05650	632	RLP32	
196	AT3G05660	633	RLP33	
197	AT3G11010	634	RLP34	
198	AT3G11080	635	RLP35	
199	AT3G23010	636	RLP36	
200	AT3G23120	638	RLP38	
201	AT3G24900	639	RLP39	
202	AT3G24982	640	RLP40	
203	AT3G25010	641	RLP41	
204	AT3G28890	643	RLP43	
205	AT4G04220	644	RLP46	
206	AT4G13810	645	RLP47	
207	AT4G13880	646	RLP48	
208	AT4G13920	648	RLP50	
209	AT5G25910	649	RLP52	
210	AT5G27060	650	RLP53	
211	AT5G40170	651	RLP54	
212	AT1G17750	652	LRR RLK	LRR XI
213	AT1G35710	654	LRR RLK	LRR XII
214	AT1G55610	655	LRR RLK	LRR X
215	AT1G72180	656	LRR RLK	LRR XI
216	AT1G73080	657 (PEPR1)	LRR-RLK	LRR XI
217	AT2G25790	658	LRR RLK	
218	AT3G19700	659	LRR RLK	LRR XI
219	AT3G24240	660	LRR RLK	LRR XI
220	AT4G08850	661	LRR RLK	LRR XII
221	AT4G36180	662	LRR RLK	LRR VII
222	AT5G06940	663	LRR RLK	
223	AT5G07180	664	LRR RLK	LRR XIII
224	AT5G49660	665	LRR RLK	LRR XI
225	AT5G63930	666	LRR RLK	LRR XI
226	AT2G26730 (additonal T-DNA for receptor #300)	667	LRR RLK	LRR III
227	AT2G26730 (additonal T-	668	LRR RLK	LRR III

	DNA for receptor #300)			
228	MKK4	669	downstream signalling protein (mitogen-activated protein kinase kinase)	
229	MPK6	670	downstream signalling protein	
230	MKK5	671	downstream signalling protein	
231	KCO1/TPK1	672	Ca^{2+} activated outward rectifying K^{+} channel	
232	AT1G25410	673	isopentyl transferase	
233	AT3G02850	674	potassium ion channel	
234	AT1G56140	675	LRR-RLK	LRR VIII-2
235	AT2G17260	679	glutamate receptor (Ca^{2+} programmed stomatal closure) GLR2	
236	AT3G51480	680	glutamate receptor (ion gated channel) GLR3.6	
237	AT1G67510	681	LRR-RLK	LRR III
238	AT3G07520	682	GLR 1.4	
239	AT5G54250	683	cngc4 (cyclic-nucleotide gated channel)	
240	cngc12	684	cngc12 (cyclic-nucleotide gated channel)	
241	cngc2/dnd	686	cyclic nucleotide gated ion channel	
242	cngc11/12 (Ws, heterozygous)	687	cyclic nucleotide gated ion channel	
243	AT3G17840	689	LRR-RLK (RLK 902)	LRR III
244	AT3G17840	690	LRR-RLK (RLK 902)	LRR III
245	AT3G17840	691	LRR-RLK (RLK 902)	LRR III
246	AT3G17840	692	LRR-RLK (RLK 902)	LRR III
247	AT1G42540	693	glutamate receptor (ion gated channel) GLR3.3	
248	AT1G08590	695	LRR-RLK	LRR XI
249	AT1G77960	GRP1	negative control – a downstream signalling component, it was shown to be resistant, it is located in the nucleus and endoplasmic reticulum	

B.2 Supplement Table 2

Supplement Table 2. qRT-PCR analysis of the defense genes upon *F. graminearum* infection, where fold change is calculated with their respective to non-infected controls and with respect to WT infected.

Mutants	Non-infected (Avg ± SE)	Infected (Avg ± SE)	Fold change (compared to non-infected controls)	Fold change (compared to WT infected)	Non-infected (Avg ± SE)	Infected (Avg ± SE)	Fold change (compared to non-infected controls)	Fold change (compared to WT infected)
	UPOX				*PR1*			
WT	1.02 ± 0.09	126.9 ± 13.3	124	1.0	1.17 ± 0.28	54.0 ± 9.3	46	1.0
rlk7	1.36 ± 0.15	135.6 ± 8.6	100	0.8	0.66 ± 0.49	64.4 ± 11.9	97	2.1
apex-1	1.25 ± 0.12	67.7 ± 4.0	54	0.4	0.22 ± 0.02	61.4 ± 8.2	285	6.1
rbohF	0.86 ± 0.06	70.3 ± 5.6	82	0.7	1.75 ± 0.54	64.1 ± 7.4	37	0.8
cerk1	0.77 ± 0.04	107.9 ± 10.8	141	1.1	0.28 ± 0.07	37.2 ± 5.7	133	2.9
	JAZ10				*PR4*			
WT	1.10 ± 0.33	1.5 ± 0.2	1.4	1.0	1.02 ± 0.09	3.9 ± 0.16	3.9	1.0
rlk7	1.02 ± 0.11	2.1 ± 0.5	2.1	1.5	1.01 ± 0.11	2.4 ± 0.3	2.4	0.6
apex-1	0.71 ± 0.10	2.5 ± 0.2	3.5	2.6	0.73 ± 0.04	3.7 ± 0.3	5.0	1.3
rbohF	0.60 ± 0.15	5.0 ± 0.4	8.3	6.1	0.59 ± 0.06	2.3 ± 0.2	3.9	1.0
cerk1	0.65 ± 0.09	1.2 ± 0.1	1.8	1.3	0.50 ± 0.03	2.5 ± 0.3	5.0	1.3
	ICS1				*MYB51*			
WT	1.01 ± 0.06	5.9 ± 0.8	5.8	1.0	1.02 ± 0.08	8.6 ± 0.6	8.4	1.0
rlk7	1.44 ± 0.62	4.9 ± 0.8	3.4	0.6	0.93 ± 0.08	6.7 ± 0.7	7.2	0.9
apex-1	0.87 ± 0.03	2.6 ± 0.7	3.0	0.5	0.86 ± 0.04	6.7 ± 0.4	7.8	0.9
rbohF	0.86 ± 0.13	0.81 ± 0.23	0.9	0.2	0.64 ± 0.10	3.7 ± 0.6	5.8	0.7
cerk1	0.93 ± 0.03	11.6 ± 2.8	12.5	2.2	0.72 ± 0.10	4.8 ± 0.5	6.7	0.8

B.3 Supplement Table 3

Supplement Table 3. qRT-PCR analysis of precursors of DAMP-related genes upon *F. graminearum* infection, where fold change is calculated with respective to their non-infected controls and with respect to WT infected.

Mutants	Non-infected (Avg ± SE)	Infected (Avg ± SE)	Fold change (compared to non-infected controls)	Fold change (compared to WT infected)
	ProPEP1			
WT	1.06 ± 0.15	6.2 ± 1.3	5.9	1.0
rlk7	1.10 ± 0.06	5.8 ± 0.8	5.2	0.9
apex-1	0.97 ± 0.10	2.7 ± 0.6	2.8	0.5
rbohF	0.69 ± 0.06	9.8 ± 0.8	14.2	2.4
cerk1	0.72 ± 0.10	4.2 ± 0.9	5.8	1.0
	ProPIP1			
WT	1.08 ± 0.19	618.6 ± 105.2	570	1.0
rlk7	1.02 ± 0.24	1012.0 ± 153.5	996	1.7
apex-1	0.75 ± 0.11	346.7 ± 16.9	460	0.8
rbohF	0.43 ± 0.03	433.3 ± 36.3	1019	1.8
cerk1	0.51 ± 0.09	539.9 ± 57.2	1054	1.8

B.4 Supplement Table 4

Supplement Table 4. List of primers used in the study.

Gene	Use	Forward (5'--> 3')	Reverse (5'--> 3')
PP2A (AT1G69960)	Fungal biomass quantification (DNA)	AGTTCCAGAATCCAAACCAAC	CCTAGAGGCAACACAAACATC
EF-1α (FGSG_08811)	Fungal biomass quantification (DNA)	CCATTGATATCGCCCTCTGGAA	TGACAGCAGTGGTGACAACATA
rlk7 (SALK_094492C)	Genotyping (PCR)	TTCTCGCGCCCGCCTCTCTCT (labelled as F in Figure 6)	TTCACCAAACTCCAGCGGAATCTCA (labelled as R in Figure 6)
apex-1 (SALK_055240C)	Genotyping (PCR)	ACGCCCTTTTGCATTGTCATCGTCC (labelled as F in Figure 6)	GTGTTCCTGTGAATCCACTCGAGGC (labelled as R in Figure 6)
RLK7 (AT1G09970)	Gene expression (qRT-PCR)	ACCGATAGAGGCAGAGTTTGG (labelled as F1 in Figure 6)	TGTCCACAATCTCCATCACACT (labelled as R1 in Figure 6)
APEX (AT5G63710)	Gene expression (qRT-PCR)	CAGCTCATAAGCGTTGCGGTTC (labelled as F1 in Figure 6)	CTTCCTCTCCCGCTTTCAAATCTC (labelled as R1 in Figure 6)
proPEP1 (AT5G64900)	Gene expression (qRT-PCR)	GGATTCCTCTTCAGTGCCTCG	TGCTTTGCCTTGACCTTTGTG
prePIP1 (AT4G28460)	Gene expression (qRT-PCR)	AATCGGGAGAATGGAAGTGC	GACGCCAAACGCTGAAAC
UPOX (AT2G21640)	Gene expression (qRT-PCR)	GCAAGTGCCTGCATCATC	CCATGTGATCCTTGAGGTTTG
PR1 (AT2G14610)	Gene expression (qRT-PCR)	GTGCCAAAGTGAGGTGTAAC	GGCTTCTCGTTCACATAATTCC
JAZ10 (AT5G13220)	Gene expression	CCAAGTGTCTCGTAACAAAGC	ACCGAAAGATCTGTCTCCATC

	(qRT-PCR)		
PR4 (AT3G04720)	Gene expression (qRT-PCR)	CAACGGAGGCTTGGATTTG	GGTAGTCAACAATGAGATGGC
ICS1 (AT1G74710)	Gene expression (qRT-PCR)	GTGTCTGCAGTGAAGCTTTG	TCGTCTTTCGGACTGGTTAG
MYB51 (AT1G18570)	Gene expression (qRT-PCR)	ATCTCTCTTCACGCCCTTC	ATCGGTTCTTCCTGGTAGTC
PP2A (AT1G69960)	Gene expression (qRT-PCR)	TAACGTGGCCAAAATGATGC	GTTCTCCACAACCGCTTGGT

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