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ORCHESTRATING PLANT DEFENCES IN RESPONSE TO *BOTRYTIS CINEREA* AND APOPLASTIC ROS: HORMONE INTERACTIONS, TRANSCRIPTIONAL AND POSTTRANSCRIPTIONAL REGULATION.

Katariina Vuorinen

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, in Auditorium 1041, Biocenter 2, on the 18th of August, 2023 at 12 o'clock.

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“Riippuu mistä roikkuu.”

-A Finnish proverb

ABSTRACT

Plants have complex defences against the countless threats they face. These complicated and fine-tuned defences are a necessity for an organism unable to escape its surroundings. Plant defences can be precisely and effectively raised for a particular stress. However, the underlying signalling networks and components, which are highly interconnected and in constant flux, are not yet fully understood. Hormones, transcription factors, signalling molecules, posttranslational modifications, and secondary metabolites interact in a complex network where correct timing and balance determine the survival of the plant. In addition, us humans depend on plants. Thus, understanding the intricacies of plant defences is an important facet in our quest to feed the growing global population while attempting to adjust to climate change.

New insights were uncovered into the complex interactions of stress hormones in grey mould infection and oxidative stress, into the important role of PP2A-βγ in glucosinolate metabolism and defence signalling, and into the complexity of WRKY transcription factor family. Jasmonic acid is crucial in defences to necrotrophic pathogens and salicylic acid in defences to biotrophic pathogens. Hormone interactions of jasmonic acid, salicylic acid, and ethylene, were examined by exposing signalling mutants of Arabidopsis thaliana to various treatments including necrotrophic fungal pathogen Botrytis cinerea and oxidative stress caused by ozone. It was uncovered that impairing salicylic acid signalling in addition to jasmonic acid rescued the sensitive phenotype of jasmonic acid signalling mutant to wild type level of resistance to Botrytis cinerea. As the balance of defences and growth is regulated at several levels, transcription factors and protein phosphorylation are key players in plant defences. Transcription factors function by either promoting or repressing the transcription of their target genes thus having an important role in regulating plant functions. WRKY transcription factors are abundant during stresses and examining their specificities revealed variation in binding sites but also shared roles. WRKY75 emerged as an important node connecting hormones, reactive oxygen species and protein phosphorylation. Protein phosphorylation is a key posttranslational modification where protein phosphatases remove phosphate groups from proteins counteracting kinases that add them. It became clear that PP2A-βγ has an important regulatory role in suppressing defences including affecting glucosinolate accumulation. Observing plant defences at different levels revealed how interconnected reactive oxygen species, salicylic acid and jasmonic acid, WRKYs, and protein phosphatase PP2A-βγ are. A better understanding of the defence network interactions is crucial in a world where food production and thus food security faces ever aggravated threats due to climate change.

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CONTENTS

ORIGINAL PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following publications:

I **Vuorinen**, **K**., Zamora, O., Vaahtera, L., Overmyer, K., and Brosché, M. 2021. Dissecting Contrasts in Cell Death, Hormone, and Defense Signaling in Response to *Botrytis cinerea* and Reactive Oxygen Species. Molecular Plant-Microbe Interactions. 34: 75-87.

II Durian, G., Jeschke, V., Rahikainen, M., **Vuorinen, K.**, Gollan, P. J., Brosché, M., Salojärvi, J., Glawischnig, E., Winter, Z., Li, S., Noctor, G., Aro, E. M., Kangasjärvi, J., Overmyer, K., Burow, M., and Kangasjärvi, S. 2020. PROTEIN PHOSPHATASE 2A-B9g controls *Botrytis cinerea* resistance and developmental leaf senescence. Plant Physiology. 182:1161–1181.

III Rahikainen, M., Trotta, A., Alegre, S., Pascual, J., **Vuorinen**, **K**., Overmyer, K., Moffatt, B., Ravanel, S., Glawischnig, E., Kangasjärvi, S. 2016. PP2A-B'γ modulates foliar trans-methylation capacity and the formation of 4-methoxy-indol-3-yl-methyl glucosinolate in Arabidopsis leaves. The Plant Journal. 89: 112-127.

IV Vaahtera, L., **Vuorinen, K**., Jolma, A., Brosché, M. The role of WRKY transcription factors in reactive oxygen species signaling. Manuscript.

The publications are referred to in the text by their roman numerals.

AUTHOR'S CONTRIBUTIONS

I K.V. performed all experiments, analysis, and visualization of experiments relating to GUS staining and performed all treatments where *B*. *cinerea* was used. K.V. analysed and visualized qPCR data. K.V wrote the paper in collaboration with K. Overmyer and M. Brosché.

II K.V. performed *B*. *cinerea* experiments and analysed the results.

III K.V. performed *B*. *cinerea* experiments including growing the plants and maintaining the fungal culture, treating the plants, and harvesting the samples.

IV K.V. performed the estradiol experiments, contributed to RNA-seq data analysis and contributed to editing the manuscript.

ABBREVIATIONS

12OH-JA-Ile	12-hydroxy-jasmonoyl-isoleucine
1MO-I3M	1-methoxy-indol-3-yl-methyl glucosinolate
13-HPL	13-hydroperoxylinoleic acid
3'-UTR	three prime untranslated region
4MO-I3M	4-methoxy-indol-3-yl-methyl glucosinolate
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC oxidase
ACP	ACYL CARRIER PROTEIN
ACS	1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE
AGI	Arabidopsis genome initiative
AIM1	ABNORMAL INFLORESCENCE MERISTEM 1
AOC	Allene oxide cyclase
AOS	Allene oxide synthase
AUX	Auxin
BA	Benzoic acid
BA2H	Benzoic acid-2-hydroxylase
BiFC	Bimolecular Fluorescence Complementation
BR	Brassinosteroids
CAMTA3	CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 3
CBP60g	CAM-BINDING PROTEIN 60-LIKE G
CK	Cytokinins
COI1	CORONATINE INSENSITIVE 1
CPK1	CALCIUM-DEPENDENT PROTEIN KINASE 1
CTR1	CONSTITUTIVE TRIPLE RESPONSE 1
CYP94B3	CYTOCHROME P450, FAMILY 94, SUBFAMILY B, POLYPEPTIDE 3
DAMP	Damage-associated molecular pattern
DDE2	DELAYED DEHISCENCE 2
DLO1	DMR6-LIKE OXYGENASE1
DMR6	DOWNY MILDEW RESISTANT 6
e.g.	Exempli gratia
eDNA	Extracellular DNA
EDS5	ENHANCED DISEASE SUSCEPTIBILITY 5
EIN2	ETHYLENE INSENSITIVE 2
EPS1	ENHANCED PSEUDOMONAS SUSCEPTIBILTY 1
ERF	ETHYLENE RESPONSE FACTOR
ERS1	ETHYLENE RESPONSE SENSOR 1
etc.	Et cetera
ETI	Effector-triggered immunity
ETR1	ETHYLENE RESPONSE 1
GA	Gibberellins
GO	Gene ontology
GSL	Glucosinolates

GUS	β-glucuronidase
H_2O_2	Hydrogen peroxide
hpi	Hours post infection
HR	Hypersensitive response
HT-SELEX	High-throughput systemic evolution of ligands by exponential enrichment
i.e.	Id est
I3M	Indol-3-yl-methyl glucosinolate
IC-9-Glu	Isocrosimate-9-glutamate
ICS	Isochorismate synthase
IGMT4	INDOLE GLUCOSINOLATE METHYLTRANSFERASE FORM 4
JA	Jasmonic acid
JAR1	JASMONATE RESISTANT 1
JAZ	JASMONATE-ZIM-DOMAIN
LOX	Lipoxygenases
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinases
MKP	MAP KINASE PHOSPHATASE
NAC	NAC DOMAIN CONTAINING PROTEIN
NADPH	Nicotinamide adenine dinucleotide phosphate
NAMP	Nematode-associated molecular pattern
NBD–TMD	Nucleotide-binding domain—transmembrane domain
NO	Nitric oxide
NPR	NONEXPRESSER OF PR GENES
O_3	Ozone
OG	Oligogalacturonide
OPDA	Oxo-phytodienoic acid
PAD4	PHYTOALEXIN DEFICIENT4
PAL	Phenylalanine ammonia lyase
PAMP	Pathogen-associated molecular pattern
PBS3	avrPphB SUSCEPTIBLE3
PCD	Programmed cell death
PDF	PLANT DEFENSIN
PDB	Potato dextrose broth
PME17	PECTIN METHYLESTERASE 17
PP2A	PROTEIN PHOSPHATASE 2A
PR	PATHOGENESIS-RELATED
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
qPCR	Quantitative real-time polymerase chain reaction
RBOH	Respitory burst oxidase homolog
ROS	Reactive oxygen species
SA	Salisylic acid
SAM	S-adenosyl-l-methionine
SAMS	SAM synthetase
SAR	Systemic acquired resistance
SARD1	SAR DEFICIENT 1

SID2	SALICYLIC ACID INDUCTION DEFICIENT 2
SINC	Salicylic acid-induced NPR1 condensate
SR1	SIGNAL RESPONSIVE 1
TF	Transcription factor
TGA	TGACG (TGA) MOTIF-BINDING PROTEIN
TGA3	TGA1A-RELATED GENE 3
WRKY	WRKY DNA-BINDING PROTEIN
WT	Wild type

1 INTRODUCTION

Understanding how plants deal with stress is of immense importance for human survival as our food production is based on plants. Plants need to allocate their resources in an efficient way for growth, reproduction, and survival. To balance between these, plants rely on sensing their surroundings and internal signalling to adjust their metabolism accordingly.

What is plant stress? Plant stress is in its core anything and everything that diverges from the plant's ideal surroundings or conditions. Plant stresses are often divided into two categories: biotic and abiotic stresses. Biotic stresses are caused by living organisms such as pathogens and pests. Abiotic stresses are caused by environmental factors such as temperature, nutrient levels, water availability, and air pollutants. Here the focus is on two stress factors, one biotic and the other abiotic, which share many similarities: *Botrytis cinerea* infection and ozone treatment leading to oxidative stress.

How plants deal with stress? Plants have innate defences, such as defensive structures like thorns and waxy layers and some of which are only activated when needed including ROS (reactive oxygen species) burst, stomatal closure, etc., and acquired immunity where previous stress makes them more robust against the next stressor. Also, plant defences can be dissected into many layers, each with its own characteristics and complexity. Working from bottom up; genes are the basis of everything, transcription factors regulate gene expression, hormones orchestrate signalling pathways, posttranslational modifications such as phosphorylation further define the proteins and their activity, secondary metabolites that can function as phytoalexins, and structural defences such as trichomes, thick waxy layers, and thorns, form yet another layer of defences. The main focus in this thesis is on the hormonal signalling pathways and the networks they form with each other and other parts of plant defences.

Plant defences are a complex system, which we still don't fully understand. Despite the tireless efforts of intense research, we are only starting to understand the enormous complexity of plant defences and the signalling networks driving them. Due to an incomplete picture, we are accustomed to depicting processes in plants as simple linear cascades of one thing leading to another. In reality those processes are far more complex, and it can be easy to miss or overlook a connection. As many of the players and interactions in plant defences are still unknown, we are still bound to the simplistic portrayals. Yet with each new peace of the puzzle, we can start to move towards a better understanding of the underlying complexity.

Population growth coupled with increase in living standards, loss of arable land, pathogen pressure, invasive species, pollution, and climate change all put pressure on agriculture and forestry. We have already witnessed the catastrophic outcomes of what crop failures can lead to. The Great Irish Famine caused by potato blight in the 1840's led to the death of at least a million people (Mokyr & Ó Gráda, 2002). Severe droughts in 1870's caused 9,5 – 13 million deaths in China (referred to in Ashton et al., 1984). Currently, Black Sigatoka (also known as Black Leaf Streak Disease caused by *Mycosphaerella fijiensis* Morelet) threatens various banana crops and its occurrence risk has risen over 44% due to climate change in the past 60 years (Bebber, 2019). Our survival depends on plants and thus understanding them is of great importance and not simply economic in nature.

2 REVIEW OF THE LITERATURE

2.1 Plant defences and pathogen stress recognition

Stress recognition could be considered the second line of defences against stress factors. The first line are unarguably the physical barriers including thorns, trichomes, morphology, waxy layers, and structures filled with deterring secondary metabolites. In the second line of defences, initial pathogen recognition is considered to take place through pattern recognition receptors (PRR's). They recognise pathogen-associated molecular patterns (PAMPs, also known as microbe-associated molecular patterns (MAMPs), such as chitin and flagellin) and damage-associated molecular patterns (DAMPs, fragments of the plant itself) as well as nematode-associated molecular patterns (NAMPs) (Choi & Klessig, 2016). Recognition of PAMP's through PRRs leads to PAMP-triggered immunity (PTI). To avoid this level of detection pathogens have evolved effectors which aim at silencing the initial recognition. In turn plants have evolved receptors to sense these effectors, nucleotide-binding domain leucine-rich repeat containing receptors (NLRs), which activates effector-triggered immunity (ETI) (Jones & Dangl, 2006). However, recent research indicates that division to PTI and ETI could be an oversimplification as both activate defences that overlap at several points (reviewed by Yuan et al., 2021). In fact, it might be that both PTI and ETI are required for full defence initiation (Zhang & Dong, 2022).

After the initial pathogen recognition plants need to adjust their metabolism to survive the infection. This is achieved through signalling which leads to defence reactions including alterations in transcription, posttranslational modifications, and adjustments in metabolism. Signalling components in plant defences include among others ROS, Ca^{2+}, NO (nitric oxide), hormones, mitogen-activated protein kinases (MAPKs), and transcription factors (TFs) (Mignolet-Spruyt et al., 2016). Different plant stresses tend to activate separate signalling pathways. Interestingly *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) infection (discussed further below) and oxidative stress caused by reactive oxygen species (ROS) have much in common at the transcriptional level (Xu et al., 2015a).

2.2 Reactive oxygen species, signalling and oxidative stress

Reactive oxygen species (ROS) include among others hydroxyl radical (HO•), hydrogen peroxide (H_2O_2), superoxide($O_2^{\bullet -}$), and ozone (O_3). Plants produce ROS in several organs during normal plant processes (e.g., photosynthesis in chloroplasts) as by-products which are then effectively scavenged before they can cause damage through oxidation. In lower atmosphere O_3 is a potent air pollutant capable of causing significant crop losses (Feng et al., 2022). ROS are not only harmful by-products of metabolism or air pollutants, they play an important part in plant signalling both within one cell and between cells (Vaahtera et al., 2014; Waszczak et al., 2018).

Early on during an infection an apoplastic ROS burst is produced by NADPH oxidases, known as respiratory burst oxidase homologs (RBOH) in plants, together with peroxidases (Torres,

2010). This burst of ROS can lead to hypersensitive response (HR) (Delledonne et al., 2001), which is rapid programmed cell death at the infection site. Altogether 10 RBOHs can be found in *Arabidopsis thaliana* (L.) Heynh. (Torres & Dangl, 2005) with different roles. These roles vary from pollen tube growth, seed ripening, and root hair formation to cell death and defences (Kadota et al., 2015). RBOHD and RBOHF share some functions in pathogen responses, cell death and stomatal closure (Kadota et al., 2015). RBOHD is essential in the ROS wave that leads to systemic acquired acclimation (Fichman & Mittler, 2020). After cell wall damage ROS is involved in lignification (Denness et al., 2011) which is a common reaction to pathogen infection (Moura et al., 2010). O_3 can be used in laboratory conditions to simulate apoplastic ROS signalling (Wohlgemuth et al., 2002). O_3 can activate programmed cell death (PCD), very similar to HR, a defensive measure that can be effective against biotrophic pathogens (Govrin & Levine, 2000; Overmyer et al., 2005). In contrast PCD can leave plants more vulnerable to necrotrophic pathogens (Govrin & Levine, 2000). Remarkably, even though late ROS accumulation can assist the pathogen during infection, early ROS can have an opposite effect (Asselbergh et al., 2007; Serrano et al., 2014; Survila et al., 2016) indicating that the timing of ROS production is important in successful defence reactions.

2.3 *Botrytis cinerea*

Plant pathogens are often divided into biotrophic and necrotrophic pathogens depending on their way of attaining nutrients from the host. Biotrophic pathogens require living host cells to thrive and aim to infect without raising plant defences. In turn necrotrophic pathogens thrive on dead and dying, necrotic, tissues and actively attempt to kill plant cells. Understandably, these two categories of pathogen require very different defence responses from the plant.

Botrytis cinerea is a necrotrophic fungal pathogen capable of infecting a wide range of hosts. This pathogen is the cause of grey mould and affects over 200 crop plants (including beans, strawberries, and grape) across different plant industries (agriculture and forestry) (Williamson et al., 2007). *B*. *cinerea* causes multiple symptoms including soft rots and grey mould. The pathogen is most often spread by air borne conidia and the pathogen can remain dormant until favourable conditions or until the host reaches a certain developmental stage (such as fruits ripening) (Williamson et al., 2007). Weiberg et al. (2013) mention that the economic losses due to *B*. *cinerea* can reach 10-100 billion dollars annually. *B*. *cinerea* is at the second place in a survey of fungal pathologists listing the most scientifically or economically significant fungal pathogens (Dean et al 2012). With a sequenced genome (Amselem et al., 2011) it is not surprising that *B*. *cinerea* is widely used as a model necrotrophic pathogen in plant sciences. In this thesis the focus is on the early defence regulation of *Arabidopsis* against *B*. *cinerea* infection.
As a necrotrophic pathogen *B*. *cinerea* thrives on dying tissues. There are several elements that can impact the success of the pathogen. B. *cinerea* genotype, in addition to host genotype, is an important factor in whether the infection is successful or not (W. Zhang et al., 2017). Also, infection success is strongly affected by environmental conditions (Ciliberti et al., 2015). In addition, the condition of the host plant has a strong effect. Sugar levels

(Lemonnier et al., 2014), developmental stage and environmental factors all play their part in infection success (Elad & Evensen, 1995).

Both the host plant and *B*. *cinerea* can produce ROS during an infection. *B*. *cinerea* is able to cause the host plant to produce a burst of ROS and the strength of this burst affects the infection severity; the higher the burst, the more effective the infection (Tiedemann, 1997). *B*. *cinerea* can trick the host plant into producing ROS and activate programmed cell death at the infection site (Torres, 2010). In addition to the host plant producing ROS, *B*. *cinerea* is able to produce ROS with extracellular enzymes (Rolke et al., 2004). *B*. *cinerea* NADPH oxidases have been expected to be involved in the ROS production by the pathogen during infection, but in turn seem to be important for development of penetration structures and lesion expansion (Segmüller et al., 2008). The HR, i.e., rapidly triggered cell death, is part of plant defences and is meant to slow down or stop an infection. Yet there is evidence that HR is necessary, and triggered, in successful *B*. *cinerea* infection (Govrin & Levine, 2000). Inhibiting ROS accumulation at the infection site reduces the lesion size and cell death (Asai & Yoshioka, 2009; Govrin & Levine, 2000). *B*. *cinerea* produces toxins as part of the infection strategy including botrydial. Botrydial requires light to cause lesions and the toxin aids in tissue penetration and tissue colonization (Colmenares et al., 2002).

2.4 Hormones

Phytohormones play crucial roles in plant development and defences. They orchestrate signalling pathways which entwine in a complex system. Central defence hormones discussed in this thesis are jasmonic acid (JA), ethylene, and salicylic acid (SA) due to their key regulatory roles in defences against pathogens. Generally, JA and ethylene are considered to be defence hormones against necrotrophic pathogens and salicylic acid has a key role in defences against biotrophic pathogens (Glazebrook, 2005; Stroud et al., 2022; Thomma et al., 2001). *De novo* production and reactivation of storage forms and then the degradation or inactivation of defence hormones at the right time are vital for successful defences and balancing defences with growth.

2.4.1 Jasmonic acid

JA and jasmonates (JAs, derivatives of JA), are oxylipins (oxidized fatty acids). In JA biosynthesis α-linoleic acid is converted into 12-oxo-phytodienoic acid (12-OPDA) in the chloroplast, transported out from the chloroplast by an outer membrane protein JASSY, transported into peroxisome where it gets synthesized into JA (Ghorbel et al., 2021; Guan et al., 2019) (Figure 1). Acyl carrier proteins (ACPs) are involved in building fatty acids. ACP1 is essential for linoleic acid biosynthesis in the chloroplast and thus to JA levels (Zhao et al., 2022). For example, wounding increases the amount of OPDA along with other oxylipins (Buseman et al., 2006). It is noteworthy that the precursor of JA, OPDA, can function as a signalling molecule on its own and regulate genes, for example some belonging to WRKY and MYB transcription factor families (Taki et al., 2005). In addition, OPDA is involved in increasing cellular redox capacity during stress (Park et al., 2013).

Increased levels of JA are required for activation of JA dependent defences. When the need for those defences subsides the degradation of JA is equally important. Cytochrome P450

gene *CYP94B3* has been found to play a key part in JA degradation. CYP94B3 converts JA into 12-hydroxy-jasmonoyl-isoleucine (12OH-JA-Ile) which no longer functions in JA signalling (Koo et al., 2011).

To activate signalling, JA binds to CORONATINE INSENSITIVE 1 (COI1) and forms a complex with JASMONATE-ZIM-DOMAIN (JAZ). This targets JAZ for degradation, which in turn allows downstream TFs to bind to their targets and to regulate JA dependent gene expression (the extensive literature is reviewed by Li et al., 2022, Figure 1). There appears to be two distinct branches of JA signalling, one in collaboration with ethylene and the other with abscisic acid (ABA) (Lorenzo et al., 2003, 2004). Defences against *B*. *cinerea* are raised via the ethylene response factor (ERF) branch, that is the JA and ethylene branch (Berrocal-Lobo et al., 2002; Lorenzo et al., 2003) while JA and ABA are related to wounding responses via the MYC branch (Lorenzo et al., 2004).

JA regulated genes are up-regulated within an hour of the stimuli and remain up-regulated for over 24 hours post infection (Zhang et al., 2020) even at 96 hpi when treated with *Alternaria brassicicola* (Schw.) Wilts. (Penninckx et al., 1996). JA accumulation in response to *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye & Wilkie and *A. brassicicola* infections and *Pieris rapae* L. feeding was fast and surprisingly high in *P*. *syringae* infection on *Arabidopsis* (De Vos et al., 2005). The accumulation of JA, gene expression, and proteins all follow a similar pattern, where the initial regulation is fast and remains up-regulated for several days (Stroud et al., 2022).

JA is involved in a plethora of plant processes both in plant development and in defences (Ghorbel et al., 2021). JA is known for its role in defences against necrotrophic pathogens but also plays important roles in timing of senescence (Kim et al., 2015) and stomatal closure (Ghorbel et al., 2021). In fact, even OPDA the precursor of JA is involved in stomatal closure (Savchenko et al., 2014). Other roles of JA include inhibition of lignin formation after cell wall damage (Denness et al., 2011). JA plays an essential role in defences against both apoplastic ROS and *B*. *cinerea* (Blomster et al., 2011; Méndez-Bravo et al., 2011; Overmyer et al., 2005; Xu et al., 2015a).

There is growing interest for pharmaceutical uses for JA. Jasmonates share structural similarities with prostaglandins which have anti-inflammatory effects. In addition to anti-inflammatory uses, there is interest for JA in cancer treatments due to promising results. The effects are possibly due to MAPK cascade activation or ROS production (Jarocka-Karpowicz et al., 2021) which are both significant parts of plant defences as well.

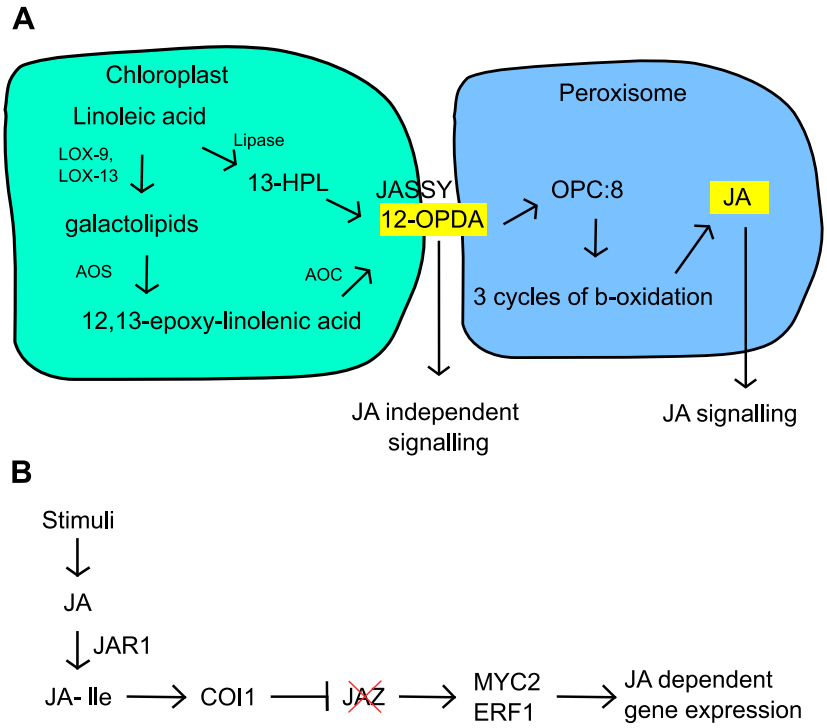

Figure 1. A. Two pathways of JA biosynthesis. Both pathways of JA synthesis use α-linoleic acid as the substrate. The pathways then diverge; in one pathway α-linoleic acid is converted into galactolipids by lipoxygenases (LOX), then into 12, 13-epoxy-linolenic acid by allene oxide synthase (AOS), and then into OPDA by allene oxide cyclase (AOC). In the other pathway α-linoleic acid is converted into 13-hydroperoxylinoleic acid (13-HPL) by lipases and then into OPDA by a still unknown process. At OPDA the pathways converge again and OPDA is transported out of the chloroplast. In order for OPDA to be converted into JA it is imported into peroxisome where it is finally oxidized into JA (Ghorbel et al., 2021; Guan et al., 2019). B. Simplified depiction of JA signalling. After stimuli JA is converted into the active form JA-Ile by JASMONATE RESISTANT 1 (JAR1). Pointed arrows indicate positive regulation and blunt arrows indicate degradation. JA signalling is dependent on release of inhibitory effect of JAZ proteins (Li et al., 2022).

2.4.2 Salicylic acid

SA is synthesised from chorismate in two different ways: through the isochorismate synthase (ICS) pathway (which is triggered by e.g., pathogens) and through phenylalanine ammonia lyase (PAL) pathway (Figure 2) (Peng et al., 2021). In the ICS pathway chorismate is converted to isochorismate by ISOCHORISMATE SYNTHASE 1 (ICS1) (Wildermuth et al., 2001) while the PAL pathway is less prominent in SA biosynthesis and uses phenylalanine as the substrate (Peng et al., 2021). In the ICS pathway ICS1 converts chorismate into

isochorismate which is transported from the plastid into cytosol by ENHANCED DISEASE SUSCEPTIBILITY5 (EDS5). In the cytosol avrPphB SUSCEPTIBLE3 (PBS3) converts isochorimate into isochorismate-9-glutamate which then spontaneously breaks down into SA and by-products (Rekhter et al., 2019).

Degradation or converting SA into storage forms appears to be an important regulatory step. As an example of further SA metabolism, DOWNY MILDEW RESISTANT 6 (DMR6) functions in SA degradation by hydroxylating it into 2,5-dihydroxybenzoic acid (2,5-DHBA)(Y. J. Zhang et al., 2017). DMR6-LIKE OXYGENASE1 (DLO1) appears to function similarly to DMR6 but localizes differently (Zeilmaker et al., 2015).

SA is one of the main hormones regulating plant stress responses. SA is involved in defences against biotrophic pathogens in particular but plays a role in abiotic stresses such as drought, cold, heat, and osmotic stress as well. In addition, SA has been found to play a role in plant growth and development such as to promote seed germination under stress conditions and in low concentrations (Rivas-San Vicente & Plasencia, 2011). SA is the precursor of acetyl salicylic acid, which is perhaps most commonly known for its pharmaceutical use in pain relief as aspirin (Madan & Levitt, 2014).

SA is required for systemic acquired resistance (SAR) (Gaffney et al., 1993), PTI, as well as ETI (Zhang & Li, 2019). Several NONEXPRESSER OF PR GENES (NPRs) play significant roles in SA signalling, where NPR3/NPR4 supress SA related genes and NPR1 functions as a positive regulator (Ding et al., 2018; Zhang et al., 2006, Figure 2). SA binding causes folding changes in NPR1 enabling its interaction with TFs. In the nucleus NPR1 dimerization allows it to connect two TGA1A-RELATED GENE 3 (TGA3) dimers to regulate SA mediated defences (Kumar et al., 2022). Redox change in cytoplasm by SA is considered to facilitate the monomerization of NPR1 from the oligomeric form and thus the movement of NPR1 from the cytosol to nucleus (Mou et al., 2003). Still, we might not yet have a full picture on how NPR1 is activated as the oligomer form of NPR1 might not exist *in vivo* (Ishihama et al., 2021). In addition, NPR1 has a role in the cytosol as well where it forms salicylic acid-induced NPR1 condensates (SINCs) (Zavaliev et al., 2020). These SINCs contain defence related proteins and target them for degradation (Zavaliev et al., 2020).

SA is involved in stomatal closure, an important defence mechanism against drought and pathogens, through NPR1 signalling (Wang et al., 2020; Zeng & He, 2010). SA can form a positive loop with ROS to promote cell death (Xu et al., 2015b), which can assist in defences against biotrophic pathogens. Along with other regulators, many NAC DOMAIN CONTAINING PROTEIN (NAC) and WRKY DNA-BINDING PROTEIN (WRKY) TFs play a role in the regulation of SA biosynthesis and signalling. ANAC019, ANAC055, ANAC072, ANAC061, ANAC090, WRKY18, WRKY40, WRKY58 function as negative regulators (Birkenbihl et al., 2017; Hickman et al., 2019; Wang et al., 2006; Zheng et al., 2012) and ANAC032, WRKY70, WRKY75, WRKY53, WRKY18 as positive regulators of SA signalling (Allu et al., 2016; Guo et al., 2017; Li et al., 2004; Wang et al., 2006). The dual role of WRKY18 as both negative and positive regulator of SA related defences could be an interesting topic to look into in future studies. WRKY70 and WRKY54 have been suggested to inhibit SA biosynthesis in non-stress conditions and after a stress subsides (Wang et al., 2006). The TFs CAM-BINDING PROTEIN

60-LIKE G (CBP60g) together with SAR DEFICIENT 1 (SARD1) are positive regulators of SA biosynthesis by binding to the promoter of *SALICYLIC ACID INDUCTION DEFICIENT 2* (*SID2*, also known as *ICS1*)(Wang et al., 2011).

SA responsive genes are up-regulated rapidly. The first set of up-regulated genes can be observed already 0,5 to 2 hours after the inducing treatment. However, the up-regulation is transient, between 12- and 16-hours a majority of up-regulated genes are back to the same level they were prior to treatment (Hickman et al., 2019; Zhang et al., 2020). Measurable accumulation of SA, as a result of *P. syringae* pv *tomato* infection, started at 3 hours post infection, the amount was at the maximum level at 24hpi and undetectable at 72hpi (De Vos et al., 2005). Perhaps, the earliest SA regulated genes are regulated by SA levels too low to be detected with current methodology or the early SA synthesis is under effective scavenging, which would need to be silenced or reduced for the accumulation of SA to occur.

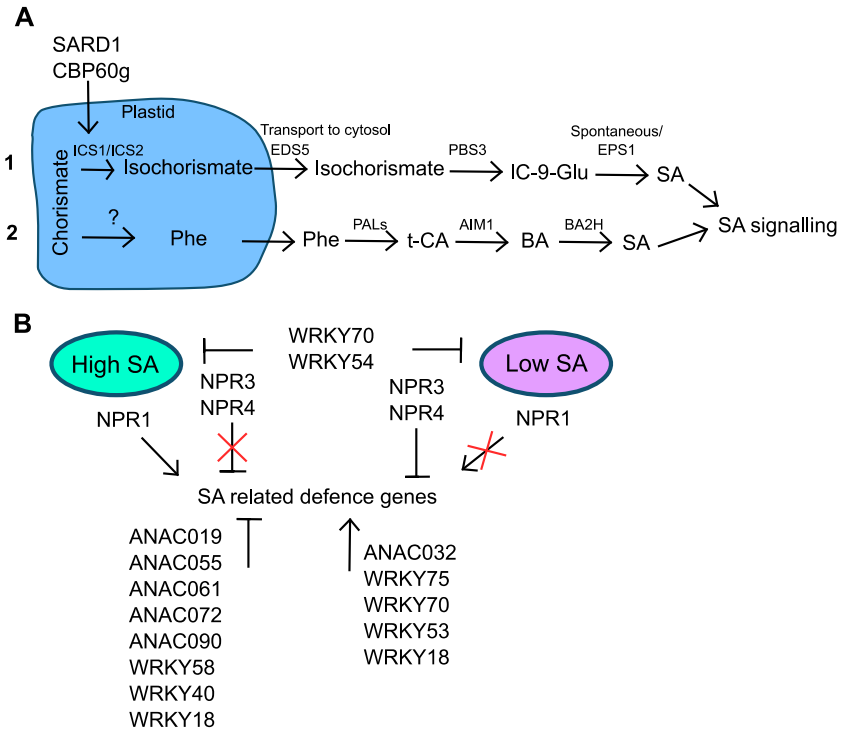

Figure 2. A. The two pathways of SA biosynthesis: phenylalanine ammonia lyase (PAL) and the isochorismate synthase (ICS) pathways (Peng et al., 2021). In the ICS pathway chorismite is converted into isochorismate by ICS1 and ICS2. SARD1 and CBD60g are TFs that bind to the promoter of ICS1. Isochorismate is converted into isocrosimate-9-glutamate (IC-9-Glu). IC-9-Glu can then spontaneously convert into SA, a process that ENHANCED PSEUDOMONAS SUSCEPTIBILTY 1 (EPS1) enhances. The PAL pathway is less prominent in *Arabidopsis*. Phenylalanine is converted into trans-cinnamic acid by PALs and then into

benzoic acid (BA) by ABNORMAL INFLORESCENCE MERISTEM 1 (AIM1). Finally, BA is converted into SA by benzoic acid-2-hydroxylase (BA2H). B. SA signalling, the effect different amount of SA has on the signalling, and TFs that play a significant role in the regulation of SA dependent gene expression. Sharp arrows indicate a positive regulatory effect on gene expression and blunt arrows indicate an inhibitory effect.

2.4.3 Ethylene

Ethylene is a small gaseous hormone and of great economic importance in agriculture, especially during post-harvest where ethylene affects storability and ripening of various crops (e.g., apples, tomatoes, cut flowers). Ethylene has multiple roles in plant development and defences. During development ethylene inhibits leaf growth and affects fruit ripening and senescence (Dubois et al., 2018). Ethylene is involved in the regulation of timing of senescence (Kim et al., 2015). In plant defence, ethylene is often coupled with JA against necrotrophic pathogens and PAMPs and DAMPs are connected to the up-regulation of ethylene production (Adie et al., 2007).

In the absence of ethylene, the ethylene receptors (ETHYLENE RESPONSE 1 (ETR1), ETR2, ETHYLENE RESPONSE SENSOR 1 (ERS1), ERS2 and ETHYLENE INSENSITIVE 4 (EIN4)) enable CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) to block ETHYLENE INSENSITIVE 2 (EIN2). In the opposite situation where ethylene is present the receptors are blocked by ethylene. This renders CTR1 unable to function and releases EIN2 (C-terminal re-localizes into nucleus) to activate the downstream components of the signalling pathway leading up to ethylene responses (Binder, 2020; Qiao et al., 2012, Figure 3). As with OPDA in JA synthesis, ACC (1-aminocyclopropane-1-carboxylic acid), a precursor of ethylene, can function as a signalling molecule on its own (Van de Poel, 2020). In *Arabidopsis* ACC is involved in several developmental processes including successful seed fertilization where it is involved in guiding pollen tube growth to the ovule (Mou et al., 2020) and it has an inhibiting regulatory role during early growth (Vanderstraeten et al., 2019). Ethylene receptors have been shown to affect the level of ethylene sensitivity where higher receptor protein levels lead to less sensitivity and vice versa (Binder, 2020).

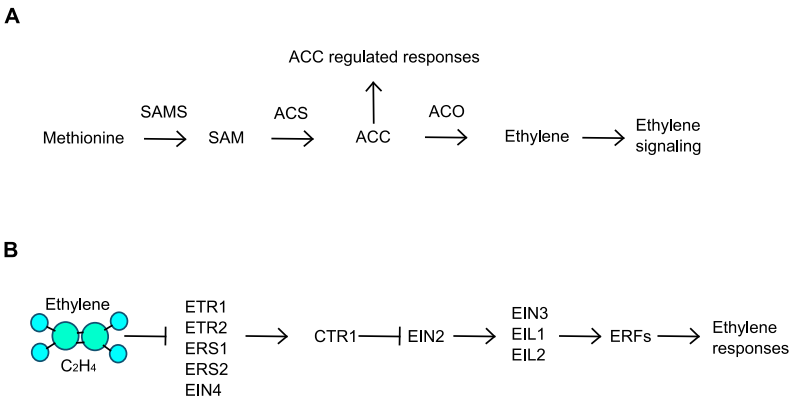

Figure 3. A. Ethylene biosynthesis splits into two signalling pathways: ACC signalling and ethylene signalling. Methionine is converted into S-adenosyl-l-methionine (SAM) by SAM synthetase (SAMS). SAM is then converted to ACC (1-aminocyclopropane-1-carboxylic acid) by acetyl-coA synthetase (ACS). ACC is in turn converted into ethylene by ACC oxidase (ACO). B. Simplified view of ethylene signalling. When present ethylene binds to receptors (ETRs, ERSs, EIN4) which renders CTR1 unable to phosphorylate EIN2. This removes the inhibitory effect CTR1 has on EIN2 allowing EIN2 to interact with its downstream targets leading to ethylene responses. Pointed arrows indicate a positive relation and blunt arrows indicate inhibitory effect.

2.5 Hormonal interactions

Hormone interactions are an integral part of orchestrating appropriate regulatory measures. There are indications of every type of interaction between hormones within the wealth of scientific literature (Overmyer et al., 2018). The many different growth conditions, experimental techniques and time scales used to study hormone interactions complicates their interpretation. Still, examining what is already known of hormone interactions in plant defences, especially when and how those interactions happen, can offer important insights into plant defences and can highlight what is still unknown (Figure 4).

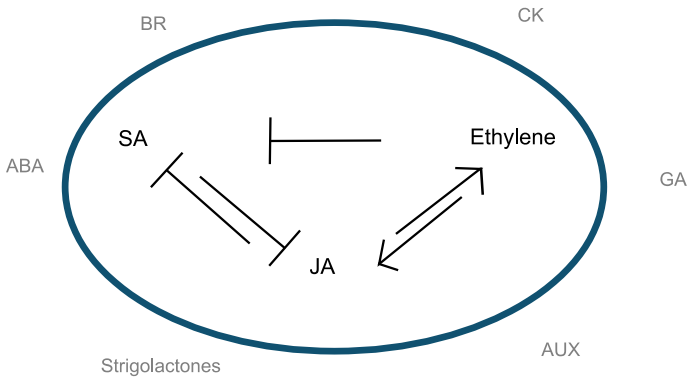

Figure 4. Hormonal interactions that are in focus of this thesis are depicted inside of the circle; SA, JA, ethylene and how they interact. Other hormones (brassinosteroids (BR), cytokinins (CK), gibberellins (GA), auxin (AUX), strigolactones, and ABA) outside of the circle (in grey font) interact with each other and with SA, JA, and ethylene, but these interactions are not discussed in this thesis. Pointed arrows indicate a positive interaction and blunt arrows indicate an inhibitory relationship.

JA and SA interactions are of particular interest as they are connected to two different defence strategies. JA has a crucial role in defences against necrotrophic pathogens, where avoiding cell death has a positive effect on survival. SA in turn has an important role in defences against biotrophs, which requires cell death. SA negatively affects JA signalling in several ways. Firstly, SA can suppress JA responsive genes via transcriptional regulators including NPR1 and TGACG (TGA) MOTIF-BINDING PROTEINs (TGAs) (Leon-Reyes et al., 2010; Spoel et al., 2003). TGAs may be positive indirect regulators of JA and ethylene responses when SA levels are low but functions as a negative regulator of JA when SA levels increase (Zander et al., 2010). Secondly, SA hinders JA regulated TFs by keeping them in the cytosol, possibly tying them into complexes (thus successfully blocking them from functioning), degrading them or promoting negative regulators (including WRKY's; WRKY70, WRKY62, WRKY50, WRKY51) (Caarls et al., 2015; Gao et al., 2011; Li et al., 2004; Mao et al., 2007; Spoel et al., 2003; Van der Does et al., 2013). Finally, SA is suggested to cause histone modifications, which in turn can repress JA dependent genes (Caarls et al., 2015).

Interactions between SA and JA are well documented. Yet, the extent of those interactions at the level of transcriptional regulation varies from study to study. SA treatment

dramatically affects, enhancing or repressing, the expression of almost 70% of JA responsive genes. The effect is not as strong reversed, JA has some effect on SA responsive genes but only on approximately 12% (Hickman et al., 2019). Meta-analysis of publicly available RNA-seq data suggested that the effect of JA on SA is higher. JA seemed to repress almost 40% of genes up-regulated by SA and the other way around, SA only seemed to repress 24% of genes up-regulated by JA (Zhang et al., 2020). Hickman et al. (2019) report that they saw a higher number of SA regulated genes than had been previously published. Different growth conditions and timepoints, differences in methods of analysis and which interactions were included (up-regulated or both up- and down-regulated) can all explain some of the differences between the two publications.

Plants can raise defences very locally so that the antagonistic relationship of SA and JA only affects the infection site. SA signalling and regulation of defences in infection with biotroph *P. syringae* rendered host plant vulnerable to infection with a necrotroph *A. brassicicola* but only at the same site or in very close proximity to the original infection site (Spoel et al., 2007).

Sometimes both SA and JA are needed to launch effective defences. In the case of nematodes on watermelon, red light promotes the accumulation of both JA and SA as well as the genes for their biosynthesis in the roots (Yang et al., 2018). Both SA and JA accumulation are induced in *P. syringae* infection (De Vos et al., 2005). This induction of both JA and SA can be due to spatial up-regulation where SA is produced at the infection site and JA around that infection site (Betsuyaku et al., 2018). Looking at the outcome of 96 isolates of *B. cinerea* on JA and SA deficient *Arabidopsis* mutants it was clear that blocking JA signalling led to infection in most cases. SA deficiency led to a varied outcome. Some of the 96 *B. cinerea* strains were able to cause an infection, some were not, and in some of the infections the SA deficient mutant behaved in a similar fashion to wild type (WT) (W. Zhang et al., 2017). This suggests that SA has a protective role against some *B. cinerea* strains, but its role is not as prominent as JA. Furthermore, when looking at the role of SA, JA, ethylene and PHYTOALEXIN DEFICIENT4 (PAD4) in necrotrophic infections (*P.syringae* and *A. brassicicola*) they all had a positive effect on resistance (Tsuda et al., 2009). Even though JA is crucial in *B. cinerea* infections, SA through PAL biosynthesis pathway seems to limit lesion size (Ferrari et al., 2003). Efficiency of synthesis of one hormone can reflect in the levels of another defence hormone. Lack of ACP1, an acyl carrier protein involved in building fatty acids, leads to lower JA levels but at the same time to increased SA levels in *P. syringae* pv. *tomato* DC3000 infection (Zhao et al., 2022).

Ethylene is involved in SA regulated ROS production in guard cells. SA signalling via NPR1 regulates the expression of three ACC synthetases, 1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE 2 (ACS2), ACS6, and ACS11, which leads to ethylene production. Ethylene signalling activates ROS production by ATRBOHD/F (Wang et al., 2020). Both ethylene and JA are required in regulating ERF1 (Lorenzo et al., 2003), which is a TF involved in pathogen defences. In fact, enhanced ERF1 expression leads to better *B. cinerea* resistance (Berrocal-Lobo et al., 2002). In addition, ERF1 represses genes involved in

defences to herbivory and wounding that are regulated by JA via MYC2 (Lorenzo et al., 2004). EIN3 can downregulate ICS1 activity thus repressing SA synthesis (Chen et al., 2009).

Transcription factors can play a different role in different signalling pathways, blocking one and promoting another. TGA2/5/6 TFs function at a SA/JA crossroads, positively regulating SA and negatively regulating JA (Xu et al., 2015a). WRKY70 has a very similar role to that of TGA2/5/6; it up-regulates SA responsive genes and represses JA responsive genes (Li et al., 2004). WRKY33 on the other hand positively regulates JA responsive genes and negatively regulates SA defences (Birkenbihl et al., 2012; Zheng et al., 2006). The transcription factor SIGNAL RESPONSIVE 1 (SR1) is involved in several defence pathways including SA responses (SR1 negatively regulates EDS1, which is involved in SA biosynthesis (Du et al., 2009)), ethylene signalling (SR1 binds to EIN2 and negatively affects its expression) (Nie et al., 2012) and SR1 regulates expression of early pathogen-responsive genes (Jacob et al., 2018).

The use of a quadruple mutant *dde2 ein2 sid2 pad4*, where the lack of DELAYED DEHISCENCE 2 (DDE2) impacts JA biosynthesis and the lack of PHYTOALEXIN DEFICIENT 4 (PAD4) impacts basal plant resistance, and corresponding double and triple mutants revealed interactions between hormone signalling pathways that become apparent only when several signalling pathways are compromised. This multilevel approach revealed that JA is needed in regulation of SA signalling (Hillmer et al., 2017). Another study with the *dde2 ein2 pad4 sid2* mutant indicated that the combined hormonal signalling network is required for correctly timed early defence initiation to *P. syringae* (Mine et al., 2018). During the early phase of defences, genes with the binding site for WRKY transcription factors were strongly up-regulated while the NAC and MYC TFs were more predominant during later timepoints (Mine et al., 2018). Overall, hormonal interactions play an integral role in tuning plant defences and new layers to those interactions are constantly being discovered.

2.6 Transcription factors: WRKYs in stress and hormone interactions

Transcription factors (TFs) are divided into families based on their DNA binding domains. TFs regulate gene expression by either promoting or decreasing their target gene's transcription. They are in turn also regulated via several mechanisms including other TFs and post-translational modifications (such as phosphorylation) (Moore et al., 2011). Understanding which TFs play a significant role in certain stresses can open new opportunities for crop improvement via engineering existing metabolism towards a desired outcome (Jirschitzka et al., 2013). Changes in TF activity has been identified as a major player in crop domestication of many main crops (Doebley et al., 2006) illustrating that TFs can be a powerful option for future crop improvements. For example, *SoyWRKY15a* was identified to be involved in seed size determination in wild soy (*Glycine soja* Siebold & Zucc.) (Gu et al., 2017).

One of the stress related TF families are WRKYs. WRKYs are a family of estimated 75 TFs (Riechmann & Ratcliffe, 2000), and they are characterized by containing a zinc-finger binding motif and the WRKYGQK motif (Eulgem et al., 2000). The WRKYGQK motif is conserved among WRKYs and binds to DNA at the W box element which has a conserved TGAC core

(Eulgem et al., 2000). WRKYs are further divided into three groups (I, II, III) based on the number of WRKY motifs found in them and by the type of zinc-finger like structures they have (Eulgem et al., 2000). The W-box is the most prevalent motif found among ozone regulated genes (Blomster et al., 2011; Xu et al., 2015a; Xu et al., 2015b). Often WRKYs have overlapping functions with other members of the same TF family making it difficult to determine their roles with single knockout mutants (Ülker & Somssich, 2004).

In addition to the earlier mentioned WRKYs in sections discussing JA (2.4.1), SA (2.4.2) and hormonal interactions (2.5), different roles of WRKY's include a wide range of processes from seed growth, root development, senescence, and flowering to abiotic and biotic stress responses (Chen et al., 2017). Focusing on stress responses, the three main stress hormones discussed in this thesis (SA, JA, and ethylene) can all regulate WRKY75 transcription (Guo et al., 2017). Ethylene regulates WRKY75 transcription as it is a target gene of EIN3 (Guo et al., 2017). WRKY75 forms a positive regulation loop with SA by binding to ICS1 (also known as SID2) enhancing SA production (S. Zhang et al., 2017). Also, WRKY75 represses *CAT2* transcription leading to increased ROS accumulation due to lower catalase activity (Guo et al., 2017). In turn, WRKY33 is required in controlling H_2O_2 levels as the lack of WRKY33 leads to elevated H_2O_2 concentration (Sun et al., 2020). Considering the timing of stress responses, WRKY33 seems to be part of the early defence reaction as its transcript levels are at highest at 1 and 2 hours after oxidative stress initiation (Blomster et al., 2011) and 14 hours after *B. cinerea* infection (Liu et al., 2015) indicating that WRKY33 plays an important role in early stress regulation.

2.7 Secondary metabolites: camalexin and glucosinolates

Secondary metabolites that have adverse effects on herbivores and harmful microbes are part of plant defences. An understanding of how they are metabolised and regulated is of great importance for both agriculture and for understanding ecological processes of a variety of plant-organism interactions. Glucosinolates (GSL) are important secondary metabolites in plant defences. There are at least 120 different GSLs found in plants (Fahey et al., 2001). The conversion of indol-3-yl-methyl glucosinolate (I3M) to 1-methoxy-I3M glucosinolate (1MO-I3M) is well conserved among Brassicaceae species yet their ratio to all GSLs differs between species (Bednarek et al., 2011; Fahey et al., 2001). Distribution of GSLs is different across different plant organs and tissues. Roots tend to have more GSL than shoots (Van Dam et al., 2009). In addition, which GSLs are most abundant, depends on the part of the plant: I3M tends to be the most prevalent GSL in leaves while overall levels of GSLs are highest in roots (Agerbirk et al., 2009). *B. cinerea* promotes the conversion of I3M into 4-methoxy-I3M glucosinolate (4MO-I3M) in *Arabidopsis* (J. Xu et al., 2016). JA treatment increases the level of some indole glucosinolates (I3M, 4-hydroxy-I3M) in *Brassica rapa* L., *B. napus* L. and *B. juncea* (L.) Czern. (Bodnaryk, 1994).

Camalexin, an indole phytoalexin, can play a protective role in *B. cinerea* infection, but it is dependent on the *B. cinerea* genotype (Kliebenstein et al., 2005). The same applies to glucosinolate breakdown products, some genotypes of *B. cinerea* are more sensitive than others (Buxdorf et al., 2013). Also, changes in secondary metabolites in the host plant, including camalexin and glucosinolates, vary between different *B. cinerea* strains

(Kliebenstein et al., 2005). Both camalexin and glucosinolates share a precursor indole-3-acetaldoxime (Glawischnig et al., 2004).

2.8 Protein phosphorylation: phosphatase PP2A

Protein phosphorylation is a reversible regulation system, where kinases add phosphate groups to proteins and phosphatases remove them. Protein phosphorylation is essential in signalling, both in perception and in transmission of signals thus regulating plant processes at the posttranslational level. Kinases and phosphatases are highly abundant and make up over 2% of protein encoding genes (Uhrig et al., 2013).

Mitogen-activated protein kinase (MAPK) cascades are conserved signalling pathway components that function downstream from receptors (Widmann et al., 1999). In the cascade, MAPK kinase kinase (MAPKKK) activates MAPK kinase (MAPKK) which in turn activates MAPK. In plant defences MPK3 and MPK6 are important MAPKs. During *B. cinerea* infection they are required for regulating camalexin synthesis (Ren et al., 2008) and regulating ethylene production by stabilizing ACS2 and ACS6 (Han et al., 2010). In addition, MPK3 and MPK6 through regulation of TF ERF6 are involved in indole glucosinolate synthesis by promoting I3M synthesis and its conversion into 4MO-I3M during *B. cinerea* infection (Xu et al., 2016). Kinases and phosphatases interact with each other regulating defence activation. For example, MAP KINASE PHOSPHATASE1 (MKP1) and MKP2 interact with MPK3 and MPK6 regulating their activation (Bartels et al., 2009; Lumbreras et al., 2010).

Phosphatases are divided into four groups: the phosphoprotein phosphatases (PPP), the phosphotyrosine phosphatases (PTP), the protein phosphatases dependent on Mg^{2+} or Mn^{2+} (PPM), and finally the aspartate dependent protein phosphatases (Uhrig et al., 2013). Protein phosphatase 2A (PP2A) is a subfamily of the PPP family and is built from three subunits. Subunit A is the structural subunit, B the regulatory subunit and C the catalytic subunit. *Arabidopsis* has three different A subunits, 17 B subunits and five C subunits enabling a wide range of variability (Booker & DeLong, 2017).

Plant defences require resources, which affects growth and development. Appropriate balancing of defence and growth ensure survival. Unnecessarily raised defences, although protecting the plant from one or several stressors, can lead to survival costs. For example, *pp2a-b'γ* mutants senescence and yellow faster than their wild type counterparts (Trotta et al., 2011). Another connection of PP2A-B'γ to defence regulation is that the lack of PP2A-B'γ leads to resistance to *Myzus persicae* Sulzer and *B. cinerea* (Rasool et al., 2014; Trotta et al., 2011). Certainly, phosphatases are required to regulate activity of proteins, but compared to kinases we still need to learn much more about their specific functions in plants.

3 AIMS AND HYPOTHESES

This thesis covers many layers of plant defences and circles around defence hormones, *B. cinerea* infection, and ROS. The focus is to reveal the similarities and differences between the stresses, and especially the interactions between hormone signalling pathways and the different layers of plant defences Examining interactions of defence hormones by using double and higher order mutants helps us better understand how plants determine the correct response to a stress and the nuanced effects signalling pathways have on each other. Transcription factors are key players in fine tuning defence reactions while protein phosphatases play an important role in posttranslational tuning of plant metabolism and signalling. Better understanding the specific roles of TFs and protein phosphatases in defence regulation as well as in secondary metabolism can reveal new insights and novel possibilities for crop improvements. Plant defences form a highly interconnected web of actors and connections between them, for which we are still missing many key players and interactions. The aims are to further:

- elucidate hormonal interactions between JA, SA and ethylene and their roles early on in *B. cinerea* infection and oxidative stress.
- increase our understanding of WRKY transcription factor family and their specificity.
- examine the specific regulatory roles and interactionsof PP2A-B'γ in plant defences.

4 MATERIALS AND METHODS

Materials and methods used in the publications I-IV are listed in this chapter. The method of performing a *B. cinerea* infection is described here in more detail than in the publication to pass on some of the silent knowledge that was not feasible or relevant to include in the publications.

4.1 Materials

4.1.1 Plant materials

Mainly one species of plant was used in the four publications: *Arabidopsis thaliana*. The model plant was used in all four publications. The different lines of *Arabidopsis* that were used are listed in Table 1. *Nicotiana benthamiana* Domin was used in publication II and III in BiFC assays.

Table 1. *Arabidopsis* material and the publication or manuscript they were used in.

Genotype	AGI	Annotation/Description	Publication
Columbia (Col-0)		Wild type	I, II, III, IV
axr1-3	At1g05180	auxin resistant 1	I
coi1-16	At2g39940	coronatine insensitive 1	I
ein2-1	At5g03280	ethylene insensitive 2	I
npr1-1	At1g64280	nonexpresser of pr genes 1	I, II
sid2-1	At1g74710	salicylic acid induction deficient 2	I, II
sr1-4D	At2g22300	signal responsive 1	I
coi1-16 ein2-1			I
coi1-16 npr1-1			I
coi1-16 sid2-1			I
ein2-1 sid2-1			I
coi1-16 ein2-1 sid2-1			I
coi1-16 npr1-1 sid2-1			I
pp2a-b'ϒ	At4g15415	B' regulatory subunit of protein phosphatase 2A (AtB'gamma)	II, III
pp2a-b'ζ	At3g21650	B' regulatory subunit of protein phosphatase 2A (AtB'zeta)	II
pp2a-b'ϒζ			II
pp2a-b'ϒ sid2-1			II
pp2a-b'ϒ P35S:PP2A-B'ϒ			II, III
pp2a-b'ϒ ore1			II
pp2a-b'ϒ sag12			II
pp2a-b'ϒ npr1-1			II
cpk1	At5g04870	calcium dependent protein kinase 1	II
sag12	At5g45890	senescence-associated gene 12	II
ore1	At5g39610		II
wrky75-25	At5g13080		IV
wrky75-1	At5g13080		IV

wrky25	At2g30250		IV
wrky33	At2g38470		IV
wrky25 wrky33			IV
estradiol inducible *WRKY75**			IV
estradiol inducible *WRKY75#*			IV
PR1:GUS	At2g14610	PATHOGENESIS-RELATED GENE 1	I
TIFY10A:GUS	At1g19180	JASMONATE-ZIM-DOMAIN PROTEIN 1	I
ICS1-GUS	At1g74710	ISOCHORISMATE SYNTHASE 1	I
RAP2.6-GUS	At1g43160	RELATED TO AP2 6	I
WRKY75:GUS	At5g13080		I
ABCG40:GUS	At1g15520	ATP-BINDING CASSETTE G40	I
PME17:GUS	At2g45220	PECTIN METHYLESTERASE 17	I
PDF1.1:GUS	At1g75830	PLANT DEFENSIN 1.1	I
At4g16260:GUS	At4g16260		I

* NASC line N2102362, # NASC line N2102363

4.1.2 *B. cinerea*

For infection assays the model fungal pathogen *B. cinerea* was used. The strain of *B*. *cinerea* used in all assays was B05.10. The strain was originally isolated from grapes (Shao et al., 2016).

4.2 Methods

Table 2. Methods and the publication or manuscript they were used in.

Method	Publication
O_3 treatment	I, IV
SA treatment	I
JA treatment	I
Wounding	I
Botrytis cinerea infection	I, II, III, IV
Estradiol treatment	IV
GUS-staining	I
Stomatal conductance measurement	I
Promoter-GUS line construction	I
Visualization of sub-cellular WRKY localization with YFP	IV
RT-qPCR	I, II, IV
RNA-seq sample preparation	II, IV
Protein interactions, Yeast Two Hybrid Screening, BiFC	II, III
In-Gel Kinase Assay	II
Immunoblotting	II
Microarray analysis	II
Metabolite analysis	II, III
Proteomic analysis	III
SELEX	IV

Analysis of RNA-seq data	I, II, IV
Statistical analysis, R, linear mixed effect model	I, II, III

Table 3. Genes used in qPCR and the paper they appear in.

Gene	AGI	Annotation	Publication
ABCG40	At1g15520	ATP-BINDING CASSETTE G40	I
ARGOS	At3g59900	AUXIN-REGULATED GENE INVOLVED IN ORGAN SIZE	I
At4g16260	At4g16260		I
FMO1	At1g19250	FLAVIN-DEPENDENT MONOOXYGENASE 1	I
ICS1	At1g74710	ISOCHORISMATE SYNTHASE 1	I
JAZ1	At1g19180	JASMONATE-ZIM-DOMAIN PROTEIN 1	I
PDF1.1	At1g75830	PLANT DEFENSIN 1.1	I
PDF1.2	At5g44420	PLANT DEFENSIN 1.2	I
PME17	At2g45220	PECTIN METHYLESTERASE 17	I
PR-1	At2g14610	PATHOGENESIS-RELATED GENE 1	I, II
RAP2.6	At1g43160	RELATED TO AP2 6	I
WRKY38	At5g22570	ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 38	I
WRKY75	At5g13080	ARABIDOPSIS THALIANA WRKY DNA-BINDING PROTEIN 75	I, IV
PR2	At3g57260	PATHOGENESIS-RELATED PROTEIN 2	II
PR5	At1g75040	PATHOGENESIS-RELATED PROTEIN 5	II
NUDX6	At2g04450	NUCLEOSIDE DIPHOSPHATE LINKED TO SOME MOIETY X6	II
CRK45	At4g11890	CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASE	II
CYP71A13	At2g30770	CYTOCHROME P450	IV
PAD3	At3g26830	PHYTOALEXIN DEFICIENT 3	IV

4.2.1 Detailed description of B. cinerea infection protocol

B. cinerea infections and propagation is often done with small differences (for example Ferrari et al., 2003, Zhang et al., 2017). Thus, the detailed description of how preparation and infection was done for the papers included in this thesis is included below.

4.2.1.1 Growing B. cinerea

In all publications and manuscripts of this thesis *B. cinerea* was grown on homemade potato dextrose agar. The base for 1000ml of media was made with 300 g of grated potato (with peels), 50 g of grated tomato (with peels), 25 g of grated carrot (peeled). The ingredients were slowly boiled in 750ml of MQ water for 20min. After the broth had cooked, it was filtered to remove largest solids. For rich growth media 10 g of dextrose, and 1.5 g of yeast extract was added into the broth. For poor media dextrose and yeast were not added. The volume was adjusted to 1000ml with MQ water. The broth was then divided into four 500ml bottles (to prevent boiling over during autoclaving) with 3,75g of agar for each bottle. The

potato dextrose agar was then autoclaved and poured onto plates while hot. For maintaining the culture and future propagation purposes of the *B. cinerea* culture normal round petri dishes were used. For best chance of sporulation larger plates are recommended. After cooling, the plates were ready for use. For storage, plates were sealed individually.

When inoculating new plates, a small piece of agar from a healthy growing *B. cinerea* plate (otherwise un-used) was transferred to a fresh plate. The inoculum used were small, approximately 2mm x 2mm, and contained hyphae and the media it is growing on. In other words, a small cube was cut from an old plate and the whole cube was transferred to a new plate. Instruments used in the process were sterilized at every step as the growth media is very well suited for many micro-organisms. The inoculated plates were sealed and kept in the dark at room temperature. For longer storage plates with *B. cinerea* were stored in a cold room or refrigerator.

4.2.1.2 Collecting spores and preparing infection solution

For experiments, spores of *B*. *cinerea* were collected into ½ strength commercial potato dextrose broth (PDB) in 2ml Eppendorf tubes or other easily closed vial. Spores were harvested with sterilized forceps from a plate with mature spores. Sporulating areas were identified by their dark fluffy mouldy appearance. When the area is perturbed while harvesting, a puff of released spores can be observed. Depending on the scale of the experiment a slight excess of sporulating mass was harvested. For a drop infection assay of ten *Arabidopsi*s genotypes a few picks with forceps was typically enough. When performing several infection assays within a close time period, the same plate of *B*. *cinerea* was used.

After the spores were harvested the tubes containing PDB, the hyphae and spores were vigorously shaken for 5min. The liquid was then strained through wet Miracloth to remove hyphae and other debris (pieces of the growing media). The purified liquid contains the *B*. *cinerea* spores. The concentration of the spore solution was determined by making dilutions (for example 1:10 and 1:100) and counting the spores on a counting chamber under a microscope. The spore solution was always mixed before pipetting while making the dilutions and before pipetting the liquid onto the counting chamber. From the counting chamber one whole medium square (identified by three edge lines) was counted with the assistance of a clicker counter. The medium square comprising of sixteen small squares which was counted one by one until the whole medium sized square had been counted. The correct concentration for the infection media was calculated from the spore count, dilution amount and the dimensions of the counting chamber. The infection solution was then mixed according to the spore count into ½ strength PDB.

4.2.1.3 Prepping plants for infection

Ideally plants used for infection experiments were grown on the infection trays. If this was not feasible plants were moved onto the infection trays couple of days prior the infection. Plants were handled with caution to avoid damaging leaves. *Arabidopsis* plants were not flowering, and all genotypes were of similar growth phase. While growing the plants they were sufficiently watered and stressed as little as possible. Plants used for infections were observed to be healthy.

4.2.1.4 Infecting plants

Infections were performed in a laminar hood to contain the *B. cinerea*. Plants were handled carefully during transferring to the hood and while infecting. During both drop and spray infections the inoculum was shaken often to ensure that the spores remained dispersed evenly in the liquid and that each plant was treated as similarly as possible. For drop infections a 5µl drop was pipetted onto 2-3 leaves per plant depending on the size of the *Arabidopsis* rosettes. In spray infection the inoculum covered the leaves but did not start to form large droplets or run-off. After applying the inoculum, plants were sealed in mini greenhouses with the lids sprayed with water to ensure high humidity and the sides were taped shut. Plants were infected in the afternoon in all experiments performed for the included papers and manuscripts. This yielded reliable and repeatable infections.

5 RESULTS AND DISCUSSION

Plant signalling and defences are both complex and extremely intertwined. The results are categorized into three main topics which group together findings from papers I, II, III, IV relating to those topics. First section discusses hormone interactions. The second topic relates to WRKYs and how they are connected to both the first section discussing hormones and to the last section, which focuses on post-translational matters including secondary metabolites (camalexin and glucosinolates) and protein phosphorylation.

5.1 Hormone interactions: SA, JA and ethylene

Hormones are essential in regulation of all types of biological processes in plants from seed germination to senescence and everything between. Dissecting how hormones are connected with different layers of plant defences and examining the intricacies of hormone signalling pathway interactions increases our understanding of plant defences. To expand on the vast amount of already available scientific literature, a wild-type and mutants deficient in hormone signalling pathways were subjected to several treatments (key focus on *B*. *cinerea* and ROS) and methods of analysis. JA has a key role in regulating defences against necrotrophic pathogens. This importance has been demonstrated with the *coi1* JA signalling mutant, which is very sensitive to the fungal pathogen *B*. *cinerea* (demonstrated in multiple publications, including Thomma et al., 1998, Fig. 1 in I). When examining mutants deficient in signalling pathways, the sensitivity of the *coi1* mutant to *B*. *cinerea* was evident not only as a single mutant (Fig. 1 in I), but also in double mutants when paired with impaired SA or ethylene signalling or biosynthesis (*coi1 sid2*, *coi1 npr1*, *coi1 ein2*) (Fig. 1 in I). Also, the triple mutant *coi1 ein2 sid2* (JA, ethylene and SA deficient) showed enhanced disease sensitivity (Fig. 1 in I). However, one of the key findings in this thesis was that the *B*. *cinerea* sensitive phenotype of *coi1* was restored to the wild type level of Col-0, when the mutations *npr1 sid2* were introduced into *coi1* (Figure 5) (Fig. 1 in I). In the triple mutant *coi1 npr1 sid2* both SA signalling (*npr1*) and biosynthesis (*sid2*, also known as ICS1) are blocked on top of JA signalling. Thus, the triple mutant should lack typical responses tied to the two key defence hormones. One possible reason for the surprising *B*. *cinerea* resistance of *coi1 npr1 sid2* could be nonconventional JA signalling where the function of COI1 is somehow circumvented. In ETI both JA and SA can accumulate (Liu et al., 2016), yet in the triple mutant SA biosynthesis is disrupted. Interestingly, NPR3 and NPR4(which in the absence or at low SA levels block SA signalling) degrade JAZ1, which then leads to JA signalling independent of COI1 (Liu et al., 2016). Therefore, in *coi1 npr1 sid2* there could be an expanded role for NPR3 or NPR4 mediated defences.

Another explanation for the *B*. *cinerea* resistance of the *coi1 npr1 sid2* mutant could be that while JA and SA are blocked, the JA precursor OPDA is enabled to take over some of the JA functions (Stintzi et al., 2001). As OPDA can induce genes from several defence related TF families including *ERF*s, *WRKY*s, *ZAT*s, and *MYB*s, it offers one plausible explanation for the resistance (Taki et al., 2005). Curiously, the triple mutant was still sensitive to ozone suggesting specificity to resistance to *B*. *cinerea* but not ROS (Fig. 2 in I). One possible

explanation for this specificity and sensitivity to ozone could be the positive loop of WRKY75 and ROS. Even though *WRKY75* transcript levels were lower in the triple mutant than in WT (Fig. 6 in I), these levels might be sufficient for WRKY75 to promote ROS production and cell death.

The *B*. *cinerea* resistance of the triple mutant *coi1 npr1 sid2* could be partially ethylene mediated. Or even with several plausible explanations related to hormones and interesting focuses for future research, the explanation for the *B*. *cinerea* resistance of the triple mutant can be some other mechanism altogether. Perhaps the lack of both JA and SA removes a repressor and thus allows a TF or a signalling component to function in their absence possibly in an unexpected way. For example, the overexpression of ERF1 (ERFs are part of ethylene signalling) can up-regulate pathogenesis-related (PR) genes (typically linked to SA regulated defences) in *coi1* and *ein2* mutants (Berrocal-Lobo et al., 2002; Lorenzo et al., 2003).

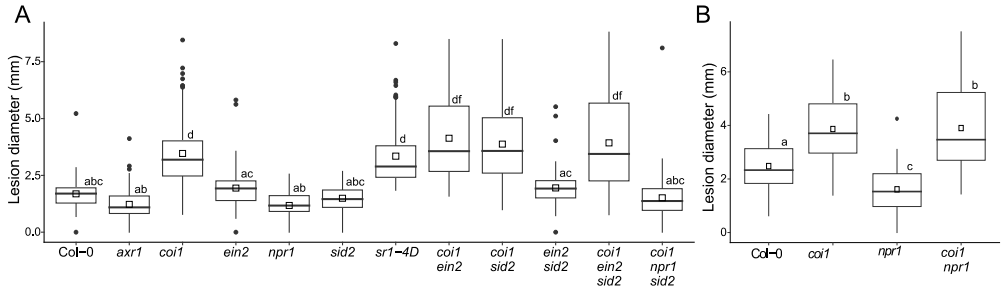

Figure 5. A. Size of lesions on *Arabidopsis* leaves caused by *B*. *cinerea* (drop-infection). Three week old wild type (Col-0) and signalling mutants were drop-infected and the lesion size was measured after 48hours (data: four independent biological repeats, n= 85 per genotype. Analysis: mixed linear model, Tukey test, $P < 0.05$). B. After the discovery of the resistant phenotype of *coi1 npr1 sid2* additional phenotyping was done to examine the effect of *coi1 npr1* on the resistance. Figure is based on Fig. 1 panels B and C in I.

Defence hormones often have antagonistic relationships if they are involved in defences against different stresses. The antagonistic relationship between SA and JA is complex and well documented (reviewed in Caarls et al., 2015; Stroud et al., 2022). Even though SA and JA interactions are relatively well known, an interesting connection between ethylene and SA could be seen when looking at the expression levels of *ICS1* (essential in SA biosynthesis via the ICS pathway) in *coi1*, *ein2* and *coi1 ein2* (Fig. 5 and Fig. 6 in I). *ICS1* was expressed higher in those mutants than in wild type under both ozone and *B*. *cinerea* treatments (Fig. 5 and Fig. 6 in I) indicating that not only JA but also ethylene might have inhibitory effect on SA. *PR-1* and *WRKY38* had increased expression levels in *ein2* and as they are connected to SA signalling it seems to indicate that ethylene inhibits SA dependent gene expression (Fig. 5 and Fig. 6 in I, Overmyer et al., 2018). Inhibition of defence mechanisms is equally important as raising them. The negative regulatory effect of both PP2a-B'γ (discussed in section 5.3) and ethylene on SA seem to play their part in the abundant arsenal of mechanisms of

keeping SA under control when SA mediated defences are not required (inhibitory mechanisms affecting SA reviewed by Stroud et al., 2022).

Specificity of stress responses is essential for plant survival. Revealing these specificities is thus of not only scientific interest but can benefit plant breeding. When comparing *B. cinerea* and ozone sensitivity or resistance, most genotypes (wild type and mutants, Fig. 2 in I) showed trends where sensitivity/resistance to one was coupled with matching sensitivity/resistance to the other (Fig. 2 in I). An exception to this was *axr1*, which was reported to be O_3 sensitive (Blomster et al 2011) but when subjected to *B. cinerea* exhibited a resistant phenotype (Fig. 2 in I). Previously *axr1* has been described to be both auxin and JA insensitive (Tiryaki & Staswick, 2002) making the *B. cinerea* resistance pecualiar. It might be that when it comes to *B. cinerea* infection there are other pathways that are linked to the auxin insensitivity that activate defences through other means than those triggered by O_3. Another possible explanation for the *B. cinerea* resistance and ozone sensitivity of *axr1* is that the JA insensitivity is not complete. Our qPCR data supports this possibility as *RAP2.6* and *PDF1.2* were closer to wild type expression levels in *axr1* compared to *coi1* (Fig. 5 and Fig. 6 in I). Yet another explanation could lie in expression of genes related to cell death (Kaurilind & Brosché, 2017), which could provide protection from *B. cinerea* but not from the ozone treatment.

Examining the spatial resolution of defence signalling histologically can reveal details of the signalling activation at the treatment site and if the signal is spreading and becoming systemic. To investigate the localization of defence signalling, plants with promoter:reporter constructs were subjected to various treatments (Figure 6) (Fig. 7 in I). Promoters for marker genes for different hormonal pathways and defence processes were used to drive expression of reporter genes. The reporter -*uidA* produces β-glucuronidase (GUS), which hydrolyzes 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) into a blue end product. The resulting blue colour (referred to later as GUS-staining) indicates where the promoter:reporter system was expressed. GUS-staining patterns of JA marker genes under SA treatment further emphasize the known connection between JA and SA (Fig. 7 in I). Even with evidence of activation of hormone signalling pathways by all treatments (Fig. 7 in I), no increase was detected in actual SA, JA or ACC levels in plants 24 hours after a spray infection with *B. cinerea* (II (in Supplemental Fig. S6)).

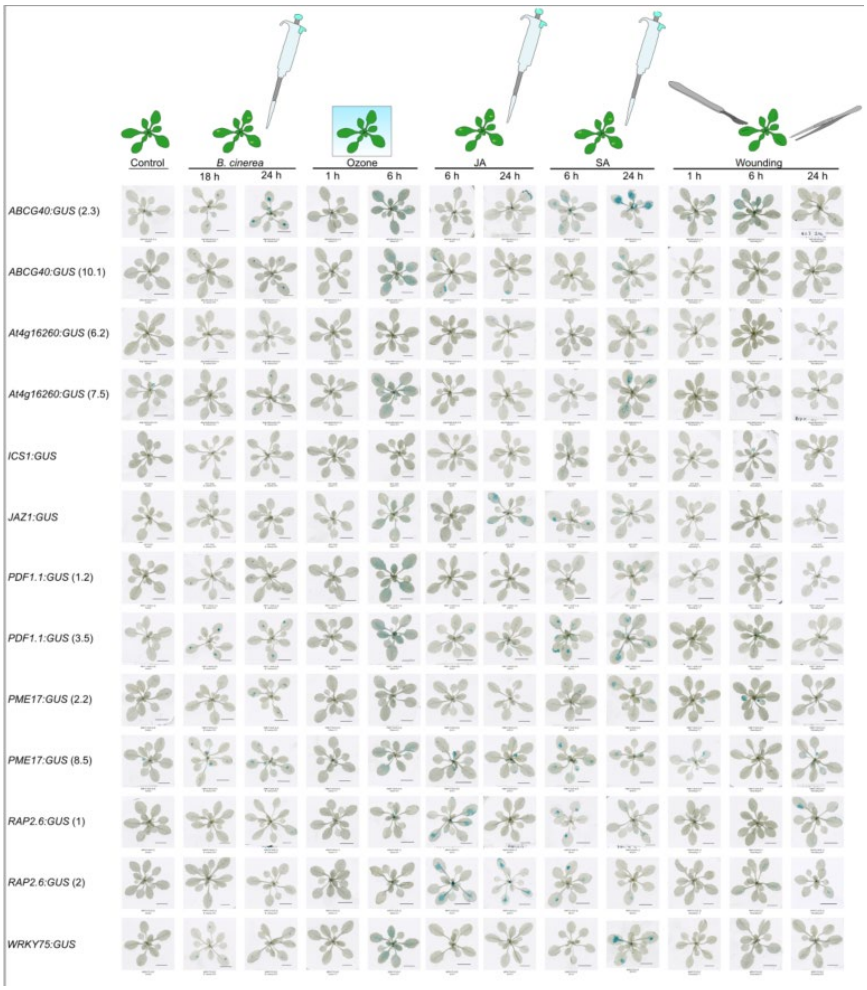

Figure 6. Combined grid of signalling localization visualized by GUS staining. Based on the results shown in Supplementary Figure S5 and Fig. 7 in I. The marker genes were: *JAZ1* and *RAP2.6* (*RELATED TO AP 2.6*) for JA, *ICS1* for SA. *At4g16260* (a putative beta-1,3-endoglucanase), *PDF1.1*, *PME17* (*PECTIN METHYLESTERASE 17*) and *WRKY75* were chosen as they all have an increased transcript levels in infections. *ABCG40* was considered a marker for ABA signalling but might be a camalexin transporter (see section 5.3).

SR1 (also known as CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 3 (CAMTA3)) is an example of a defence regulator that acts at in several different signalling pathways. Among its many roles, SR1 is involved in inhibiting SA production (Kim et al., 2019) and wounding responses (Benn et al., 2016). Wounding responses can link SR1 to JA signalling as wounding activates the MYC branch of JA signalling (Lorenzo et al., 2004). In addition, SR1 plays an important role as transcriptional regulator of PLANT DEFENSINs (PDFs)(Jacob et al., 2018) and as a cell death regulator (I). Examining gene expression, SR1 does not seem to control marker genes for SA, JA or ethylene (Fig. 5 and Fig. 6 in I). Thus in this thesis, the focus is mostly on the function of SR1 as a potential cell death regulator, using the dominant

negative allele *sr1-4D* (Nie et al., 2012). In this allele the function of SR1 is blocked. Observing *sr1-4D* in relation to *B*. *cinerea* infection and ozone treatment, we witnessed impaired *PDF1.1* and *PDF1.2* transcript levels (Fig. 5 and Fig. 6 in I). Examining the phenotype effect when crossed to lesion mimic *acd5* and constitutive defence mutant *mpk4*, we observed rescue of *acd5* and *mpk4* phenotypes (Fig. 8 in I). This indicates that SR1 is involved in cell death regulation as well as in the regulation of early pathogen-induced genes already reported by others (Jacob et al., 2018). Correct timing of cell death, i.e., preventing it when it is not beneficial and promoting it when appropriate, can be argued to play a significant role in defences against *B*. *cinerea* infection.

Overall, we can see evidence of hormonal interactions including JA and SA (e.g., the resistant *coi1 npr1 sid2* phenotype to *B*. *cinerea*) and the inhibitory effect of ethylene on SA regulated genes. Yet there are still gaps in our understanding of hormonal intercations (for example relating to the *coi1 npr1 sid2* triple mutant) and how defence responses are regulated. The answer to those questions might not be related to hormonal signalling pathways at all. One third of flg22 and 70% of ozone regulated genes are independent of SA, JA, and ethylene signalling (Hillmer et al., 2017; Xu et al., 2015a). This does not necessarily mean that there is no interplay with other hormone signalling pathways from the three discussed in this thesis, but it does raise a question if signalling components other than hormones play a larger role than is often considered.

With the evidence from both literature and from the papers (I-IV) included in this thesis it is clear that there are complex hormonal interactions as well as defence regulators that do not interact with defence hormone signalling pathways. Considering the new evidence gathered from triple (I) and quadruple mutants, including that JA is required in regulation of SA signalling and that several signalling pathways are needed in defences to *P*. *syringae* (Hillmer et al., 2017; Mine et al., 2018), we should consider examining higher order mutants more often. Higher order mutants can reveal new interactions and perspectives that would have been missed otherwise. In addition, in large gene families where there is redundancy among genes, several genes have to be knocked out to fully examine their functions. Pairing such gene combinations with the main signal regulator mutant of the pathway they function in could uncover new regulators and interactions. For example, pairing *wrky50*, *wrky51*, and *wrky75* (see 5.2) with SA mutants like *npr1* or *sid2* could reveal novel insights into which other pathways are involved in defences that are usually controlled by SA.

Even though hormones play an essential role in defence orchestration, a large portion of genes up-regulated in stress conditions are not hormone regulated. One key focus for future research would be to find those key regulators of hormone independent defence responses. In addition to increasing our knowledge on how defences are regulated, it could reveal new insights into how stress specificity works. One way to approach this could be to focus on the localization of defences by using single cell methods and spatial transcriptomics. Acquiring data with less noise from unaffected tissues could help unmask new interactions and key regulators.

5.2 WRKYs

Transcription factors play an important role in regulating defence responses as they either promote or repress the expression of their target genes. WRKY75 was one of the TFs that surfaced not only in study IV dedicated to WRKYs but in relation to all topics of this thesis. In fact, the W-box (the binding site of WRKYs) is the most enriched TF target motif of ozone (Blomster et al., 2011; Xu et al., 2015a; Xu, et al., 2015b) and *B. cinerea* regulated genes (Liu et al., 2015). Induction of the promoter WRKY75:GUS line was evident after SA treatment (Fig. 7 in I) along with *B. cinerea* and ozone treatments. This connection between WRKY75 and SA was evident also when looking at SA, WRKY75 and PP2A-B'γ (Figure 7) (II, III, , for further elaboration see section 5.3).

WRKYs are a significant TF family in defences with functionalities that often overlap (Ülker & Somssich, 2004). Further determining their specificity and pinpointing distinct roles is thus of importance to increase our understanding of how plants regulate their defences. One method to determine the DNA binding specificity for TF's is high-throughput systemic evolution of ligands by exponential enrichment (HT-SELEX). The TF is produced as a recombinant protein with a tag and is incubated with a random pool of DNA oligonucleotides, washed to remove unspecific binding, and finally after several cycles the TF bound oligonucleotides are sequenced. This reveals binding specificities of the examined TF which can help uncover the unique roles of individual TFs of a group of very similar TFs. Examining the binding specificity of different WRKYs (WRKY25/33/51/50/75) with HT-SELEX showed that WRKY25 and WRKY33 prefer the regular W-box (TTGAC[C/T]) (Figure 2 in IV). WRKY75 in turn prefers to bind to CGTTGACTTTT (IV). WRKY50 and WRKY51 share a very similar primary binding site to that of WRKY75. Furthermore, WRKY50/51 have another additional binding site (Figure 2 in IV), which has been connected to the regulation of *PR-1a* in *Nicotiana tabacum* (Van Verk et al., 2008). Also, when looking at the targets of WRKY75 two PATHOGENESIS RELATED GENEs were found: *PR-1* and *PR-2* (IV, Encinas-Villarejo et al., 2009). This raises the question if WRKY50/51 share some of the functions of WRKY75.

Finding target genes for TFs is essential when determining the role a TF has. In an effort to find targets for WRKY75 two estradiol inducible *WRKY75* lines and wild type (Col-0) plants were treated with estradiol. The estradiol inducible *WRKY75* lines showed that 112 genes were expressed higher in the inducible lines compared to wild type (included in Supplemental table 4 in IV). Among those genes were genes coding for JAZ proteins (IV) that negatively regulate JA signalling suggesting that WRKY75 is involved in the antagonistic relationship between JA and SA. The GO (gene ontology) categories that could be found from the 112 genes were 'Cell redox homeostasis', 'Response to JA stimulus', 'Transcription regulator activity' and 'Defence response'. All these categories can be tied to defence activities. Enrichment of W-box elements found in genes responsive to treatment was similar in both ozone and *B. cinerea* treatments suggesting that WRKY75 is not specifically linked to either of the stresses but involved in both. Enrichment of other binding sites suggested that MYB, NAC, TGA, AREB, and ERF play regulatory roles alongside the WRKYs (included in Supplemental table 2 in IV). These TF families are indeed involved in defences and for example TGAs are connected to SA regulated defences (see sections 2.4. and 2.5).

Continuing to seek the specificities of WRKYs and to examine the similarity and differences of ROS and *B. cinerea* several approaches were used. Comparison of RNA-seq data of wild type plants in 2h ozone treatment (IV, Xu et al., 2015a) and 14h *B. cinerea* infection (Liu et al., 2015) revealed very similar gene expression. This can be expected as ROS is heavily involved in signalling and responses to *B. cinerea*. There were 8278 genes regulated in a similar manner and only 262 genes having opposite regulation between the ozone and *B. cinerea* treatments (Figure 4 in IV). The GO categories that stand out for *B. cinerea* were Translation and DNA replication, and for ozone the categories were Cell death and Response to H_2O_2. Comparison of *wrky* mutants (*wrky33*, *wrky25 wrky33*, *wrky75*) revealed only a few differences between the two treatments. The GO categories included regulation of auxin signalling, DNA replication, and plastid and cytosolic ribosomes (Figure 5 in IV). Looking closer at the differentially expressed genes might reveal potential new candidates for future studies and interesting views of how the two stresses differentiate. Yet the specificity of WRKYs probably lies elsewhere. To elaborate, instead of considering transcriptional regulation as one step mechanism of simply promoting or repressing their target genes, there are likely many factors that can affect TF function or activity including the combination of tissue and stress, protein-protein interactions and posttranslational modifications (such as phosphorylation) (IV).

A significant difficulty we faced when trying to dissect WRKY specificity was that the main *wrky75* mutants were not complete knockouts in our conditions. The *wrky75-25* mutant has been reported to be a knockout by several publications (Encinas-Villarejo et al., 2009; Guo et al., 2017; Rishmawi et al., 2014; Chen et al., 2021) yet in our conditions this was not the case. As this mutant has a transposon insertion in the three prime untranslated region (3'-UTR) it might not be a complete knockout, or it requires specific conditions that we were unable to replicate. Thus, to proceed examining the functionality of WRKY75 we would need a *wrky75* knockout mutant that would behave as a true knockout in our growth conditions. In addition, as WRKYs seem to have lot of redundancy and as WRKY75 shares a DNA binding site with WRKY50 and WRKY51 a triple mutant *wrky75/50/51* could offer interesting insights into their specific roles in defences.

5.3 PP2A, glucosinolates and camalexin

Reversible protein phosphorylation is yet another important regulator of plant defences. In protein phosphorylation both kinases and phosphatases are needed. Phosphatases counterbalance phosphorylation by kinases and their interplay adjust defence responses. Protein phosphatases form a large group and yet not much is known about their specificities. Better understanding of how dephosphorylation affects defences as well as phytoalexin metabolism gives us new insights on how stress specific defences are built.

Phytoalexins are significant defensive compounds. Indole glucosinolates and camalexin are among the most important phytoalexins in *Arabidopsis* (He et al., 2019). In II (see Figure 5 in II) camalexin was found to accumulate in the tip halves of *pp2a-b'γ* mutant leaves. In III (see

Figure 2 in III) *B*. *cinerea* infection raised the amount of indole glucosinolate 4MO-I3M in all genotypes, particularly in *pp2a-b'γ* (Figure 7). Furthermore, the ratio of 4MO-I3M compared to all indole GSLs was higher in the *pp2a-b'γ* than in the wild type (Figure 2 in III). In addition to camalexin also SA was found to be accumulating in the tip halves of the *pp2a-b'γ* mutant (Figure 5 in II). Camalexin accumulation was reliant on SA and when *pp2a-b'γ* was crossed to *sid2* the increased accumulation of camalexin was no longer evident. Also, the protein levels of indole glucosinolate methyl transferase form 4 (IGMT4) were higher in the mutant compared to the wild type or complementation line. It is worthy to note that although there were no observable PP2A-B'γ-IGMT2/IGMT3 interactions, there could still be interactions under other conditions (Figure 1 in III).

As stated, phytoalexins are secondary metabolites essential in plant defences. When looking at the localization of marker genes with the promoter GUS lines we noticed that ABCG40:GUS (which we selected to represent ABA signalling based on the literature available at the time) had a strong local staining during most treatments and timepoints including *B*. *cinerea* infection (Figure 5) (Fig. 7 in I). ABC (ATP-binding cassette) genes are a large gene family characterized by their localization on the cell membrane and functions as transporters (either in or out of the cell) and are divided further into eight subgroups. ABCG40 is part of the G subgroup, characterized by reversed orientation of NBD–TMD (nucleotide-binding domain—transmembrane domain). Furthermore, ABCG40 is one of the PLEIOTROPIC DRUG RESISTANCE (PDR) genes, more specifically PDR12 (Verrier et al., 2008). Originally, ABCG40 was described as an ABA transporter (Kang et al., 2010) and thus we considered it as a marker to indicate local ABA signalling. Later, in 2019 ABCG40 (also known as PDR12), was reported to be a camalexin transporter alongside PEN3 (He et al., 2019). Considering the importance of camalexin in defences, our results with ABCG40:GUS showing strong GUS stain at the site of *B*. *cinerea* infection, are more consistent with ABCG40 having a role as a camalexin transporter than as an ABA transporter.

The *pp2a-b'γ* has been reported to be more resistant to *M*. *persicae* and to *B*. *cinerea* than wild type plants (Rasool et al., 2014; Trotta et al., 2011). This could be explained by some of the defences being constantly activated. In fact, the level of PATHOGENESIS RELATED GENE 2 (PR2) was elevated in *pp2a-b'γ* (Table 1 in III) compared to the wild type where PR2 was hardly expressed in non-stress conditions. PR1 was found to be accumulating in the apical halves of seven-week-old *pp2a-b'γ* mutants (Figure 4 in II, here see Figure 7). As mentioned before, both PR1 and PR2 were found to be regulated by WRKY75 (IV). This indicates that both PP2A- B'γ and WRKY75 act in SA and defence regulation. Without stress factors present PP2a-B'γ is required to reduce accumulation of SA related transcripts (Figure 5 in II). In addition to the connection to SA, the *pp2a-b'γ* mutant has an early senescence phenotype. RNA-seq showed usual senescence related gene expression demonstrating the importance of PP2A-B'γ in regulating the timing of cell death and senescence. As ethylene is involved in the timing of senescence (Kim et al., 2015) it could be interesting to investigate connections between ethylene and PP2A-B'γ more closely in the future.

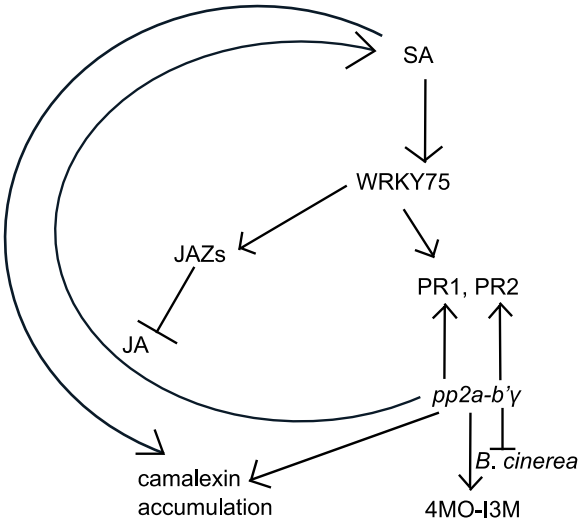

Figure 7. Interaction of SA, JA, WRKY75 and PP2A-B'γ based on the findings in papers I, II, II and IV. Pointed arrows indicate increase or positive association such as positive regulation of gene expression. Blunt arrows indicate repression or in the case of *B*. *cinerea* resistance to the pathogen.

Phosphorylation requires both phosphatases and kinases. Interactions between them is thus of particular interest. PP2A-B'γ interacts with CALCIUM-DEPENDENT PROTEIN KINASE 1 (CPK1) supressing its activation state (II). CPK1 plays a protective role against fungal pathogens (including *B*. *cinerea*) (Coca & San Segundo, 2010). As PP2A-B'γ interacts with CPK1 this interaction was further examined in *B*. *cinerea* infection. CPK1 abundance increased in both wild type and mutant plants treated with the pathogen but in particular in the *pp2a-b'γ* mutant indicating that CPK1 abundance is controlled by PP2A-B'γ during *B*. *cinerea* infection (Figure 3 in II). A notable curiosity was that the amount of CPK1 increased in both the *B*. *cinerea* treatment and in mock treatment. The increase of CPK1 protein abundance by the control media of potato dextrose broth (Figure 3 in II) could be due to DAMPs such as extracellular DNA (eDNA) still present in the control media as eDNA can cause CPK1 expression to increase (Rassizadeh et al., 2021). Interestingly the application of eDNA can lead to induced resistance to *B*. *cinerea* and can induce ROS production as well (Rassizadeh et al., 2021). Oligogalacturonides (OGs), products of pectin degradation, are another DAMP that can increase resistance towards *B*. *cinerea* even when JA, SA and ethylene are knocked out (Ferrari et al., 2007). OGs, eDNA and other DAMPs could very well be the signalling components that set apart the defence reactions to *B*. *cinerea* and ozone.

Although *pp2a-b'γ* has a defensive advantage against *B*. *cinerea* it comes at a cost (II). The leaves of the *pp2a-b'γ* mutant start to senescence earlier (Figure 4 in II) than leaves of the wild type indicating that there is a fitness cost. Overall, constitutive defences tend to come with a price. These costs can vary from a smaller seed yield, reduced growth, to difficulties dealing with the next stressor (Vos et al., 2013). In nature several stresses often occur at the same time which in turn results very differently when compared to the defences raised by

one stressor (Rasmussen et al., 2013; Zandalinas et al., 2018). Yet with functional defence pathways and crosstalk between them the fitness cost is minimized when faced with several stressors (Vos et al., 2015). On transcriptional level there is evidence of a large core group of repressed genes that are very similar across different pathogen stresses (Zhang et al., 2020). Still, the effect of several concurrent or consecutive stresses raises yet another level of complexity to understanding plant defences and the signalling networks driving them.

Finding the optimal level of improved defences without causing a significant fitness cost is one of the aims of crop improvement. Yet the existing yield gap of actual yields versus potential yields might offer an interesting view into balancing the fitness cost. The existing yield gaps vary, but the average yields can be as low as 20% of the potential yield (Lobell et al., 2009). This suggests that there is room for sacrificing some of the potential yield capacity of a crop variety into increased defences. In other words, breeding for hardier crops in the expense of the maximum yield would produce crops less reliant on intensive agricultural maintenance. At the same time, it is important to consider how readily results from the lab can be replicated in the field or across species. Even hormones play slightly different roles between *Arabidopsis* and rice (*Oryza sativa* L.)(De Vleesschauwer et al., 2014) and many genes that improve growth in *Arabidopsis* do not cause significant growth improvements in maize (*Zea mays* L.) (Inzé & Nelissen, 2022). Still, with keeping these limitations in mind, building an understanding of plant processes with model species such as *Arabidopsis* can help in providing candidate genes, protein interactions and pathways alongside new viewpoints for crop improvements.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

Considering that plants cannot generally move after germination, it makes sense that they have elaborate systems to balance growth, reproduction, and defence. Interacting signalling pathways enable plants to correctly respond to their environment where stresses can occur simultaneously or in rapid succession of each other. Uncovering and understanding these pathways and their interactions could help us in plant breeding and adapting agricultural practises during climate change.

Hormonal interactions were of particular interest in this thesis as well as seeking differences between the defence regulation of *B. cinerea* and ozone regulated stress. Most results confirm already known patterns of hormonal interactions and similarities between the two stresses. Yet some surprising details emerged. The single *coi1* mutant and double mutants *coi1 ein2, coi1 sid2* where all sensitive to *B. cinerea*. The triple mutant *coi1 npr1 sid2* was expected to be *B. cinerea* sensitive due to non-functional JA and SA signalling but instead was resistant. This unexpected tolerance could be further studied with for example RNAseq. A possible candidate for future studies comparing *B. cinerea* and ozone emerged in the form of *axr1*, which was ozone sensitive but *B. cinerea* resistant. Even with plausible explanations, such as incomplete JA insensitivity, defences released by compromised auxin signalling or differences in cell death regulation, the differential responses of this mutant might provide an interesting topic to look further into. Repression of SA signalling is essential to balance the function of this hormone. Here both ethylene and PP2A-B'γ were found to have an inhibitory effect on SA. WRKY75 emerged as the connecting node between different layers of defences. PP2A-B'γ inhibits SA, which up-regulates WRKY75 expression and results in up-regulation of PR genes that are relevant in *B. cinerea* defences.

It is noteworthy that all results that are observed in the lab should be considered as tentative. Not all findings can be replicated under field conditions or across several species. Also, if we only look at one time point and neither consider tissue specificities or the severity of the stress, we might make assumptions that are far from universal. The knowledge around individual genes, transcription factors, signalling components, and metabolites keeps growing but it remains a challenge to map all the knowledge into a comprehensive network. We need to look both closer and to take a step back**.** In order, to take a closer look, single cell methods should be used to pinpoint where, when and what is happening. This high-resolution data should patch some of the holes we have in our bigger picture. Connecting all the little bits of information that have been gathered could in turn reveal new patterns and raise our understanding of plant processes. The progress in data and computer sciences could offer interesting solutions in mapping and analysing data with the help of AI. Both ways of proceeding, looking closer and stepping back, could help us immensely in adapting agriculture to climate change. In our rapidly changing climatic and environmental conditions it is important to move into an innovative and especially collaborative era of science.

REFERENCES

Adie, B., Chico, J. M., Rubio-Somoza, I., & Solano, R. (2007). Modulation of Plant Defenses by Ethylene. *J. Plant Growth Regul.* 26, 160–177. https://doi.org/10.1007/s00344-007-0012-6

Agerbirk, N., De Vos, M., Kim, J. H., & Jander, G. (2009). Indole glucosinolate breakdown and its biological effects. *Phytochem Rev.* 8(1), 101–120. https://doi.org/10.1007/s11101-008-9098-0

Allu, A. D., Brotman, Y., Xue, G.-P., & Salma Balazadeh, &. (2016). Transcription factor ANAC032 modulates JA/SA signalling in response to Pseudomonas syringae infection. *EMBO Rep.* 17(11), 1578–1589. https://doi.org/10.15252/embr.201642197

Amselem, J., Cuomo, C. A., van Kan, J. A. L., Viaud, M., Benito E. P., et al. (2011). Genomic Analysis of the Necrotrophic Fungal Pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLOS Genet*. 7(8). https://doi.org/10.1371/journal.pgen.1002230

Asai, S., & Yoshioka, H. (2009). Nitric Oxide as a Partner of Reactive Oxygen Species Participates in Disease Resistance to Necrotrophic Pathogen Botrytis cinerea in Nicotiana benthamiana. *Mol. Plant-Microbe Interact*. 22(6), 619–629. https://doi.org/10.1094/MPMI-22-6-0619

Ashton, B., Hill, K., Piazza, A., & Zeitz, R. (1984). Famine in China, 1958-61 *Popul. Dev. Rev.* 10(4), 613–645. https://doi.org/10.2307/1973284

Asselbergh, B., Curvers, K., França, S. C., Audenaert, K., Vuylsteke, M., Van Breusegem, F., & Höfte, M. (2007). Resistance to Botrytis cinerea in sitiens, an abscisic acid-deficient tomato mutant, involves timely production of hydrogen peroxide and cell wall modifications in the epidermis. *Plant Physiol.* 144(4), 1863–1877. https://doi.org/10.1104/pp.107.099226

Bartels, S., Anderson, J. C., González Besteiro, M. A., Carreri, A., Hirt, H., Buchala, A., Métraux, J. P., Peck, S. C., & Ulm, R. (2009). MAP KINASE PHOSPHATASE1 and PROTEIN TYROSINE PHOSPHATASE1 Are Repressors of Salicylic Acid Synthesis and SNC1-Mediated Responses in Arabidopsis. *Plant Cell* 21(9), 2884–2897. https://doi.org/10.1105/TPC.109.067678

Bebber, D. P. (2019). Climate change effects on Black Sigatoka disease of banana. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, 374(1775). https://doi.org/10.1098/RSTB.2018.0269

Bednarek, P., Piślewska -Bednarek, M., Ver, E., Van Themaat, L., Kumar Maddula, R., Svatoš, A., & Schulze-Lefert, P. (2011). Conservation and clade-specific diversification of pathogen-inducible tryptophan and indole glucosinolate metabolism in Arabidopsis thaliana relatives. *New Phytol*. 192, 713–726. https://doi.org/10.1111/j.1469-8137.2011.03824.x

Benn, G., Bjornson, M., Ke, H., & Dehesh, K. (2016). Plastidial metabolite MEcPP induces a transcriptionally centered stress-response hub via the transcription factor CAMTA3. *Proc. Nat. Acad. Sci. U. S. A.* 113(31), 8855-8860. https://doi.org/10.1073/pnas.1602582113

Berrocal-Lobo, M., Molina, A., & Solano, R. (2002). Constitutive expression of Ethylene-Response-Factor1 in arabidopsis confers resistance to several necrotrophic fungi. *Plant J*. 29(1), 23–32. https://doi.org/10.1046/J.1365-313X.2002.01191.X

Betsuyaku, S., Katou, S., Takebayashi, Y., Sakakibara, H., Nomura, N., & Fukuda, H. (2018). Salicylic Acid and Jasmonic Acid Pathways are Activated in Spatially Different Domains Around the Infection Site During Effector-Triggered Immunity in Arabidopsis thaliana. *Plant and Cell Physiol*. 59(1), 8–16. https://doi.org/10.1093/PCP/PCX181

Binder, B. M. (2020). Ethylene signaling in plants. *J. Biol. Chem.* 295(22), 7710–7725.

https://doi.org/10.1074/jbc.REV120.010854

Birkenbihl, R. P., Diezel, C., & Somssich, I. E. (2012). Arabidopsis WRKY33 Is a Key Transcriptional Regulator of Hormonal and Metabolic Responses toward Botrytis cinerea Infection. *Plant Physiol*. 159(1), 266–285. https://doi.org/10.1104/PP.111.192641

Birkenbihl, R. P., Kracher, B., & Somssich, I. E. (2017). Induced Genome-Wide Binding of Three Arabidopsis WRKY Transcription Factors during Early MAMP-Triggered Immunity. *Plant Cell* 29(1), 20–38. https://doi.org/10.1105/TPC.16.00681

Blomster, T., Salojärvi, J., Sipari, N., Brosché, M., Ahlfors, R., Keinänen, M., Overmyer, K., & Kangasjärvi, J. (2011). Apoplastic reactive oxygen species transiently decrease auxin signaling and cause stress-induced morphogenic response in Arabidopsis. *Plant Physiol.*, 157(4), 1866–1883. https://doi.org/10.1104/pp.111.181883

Bodnaryk, R. P. (1994). Potent effect of jasmonates on indole glucosinolates in oilseed rape and mustard. *Phytochemistry* 35(2), 301–305. https://doi.org/10.1016/S0031-9422(00)94752-6

Booker, M. A., & DeLong, A. (2017). Atypical Protein Phosphatase 2A Gene Families Do Not Expand via Paleopolyploidization. *Plant Physiol*. 173(2), 1283–1300. https://doi.org/10.1104/PP.16.01768

Buseman, C. M., Tamura, P., Sparks, A. A., Baughman, E. J., Maatta, S., Zhao, J., Roth, M. R., Esch, S. W., Shah, J., Williams, T. D., & Welti, R. (2006). Wounding Stimulates the Accumulation of Glycerolipids Containing Oxophytodienoic Acid and Dinor-Oxophytodienoic Acid in Arabidopsis Leaves. *Plant Physiol*. 142(1), 28–39. https://doi.org/10.1104/PP.106.082115

Buxdorf, K., Yaffe, H., Barda, O., & Levy, M. (2013). The effects of glucosinolates and their breakdown products on necrotrophic fungi. PLoS One. 8(8), e70771. https://doi.org/10.1371/journal.pone.0070771

Caarls, L., Pieterse, C. M. J., & Van Wees, S. C. M. (2015). How salicylic acid takes transcriptional control over jasmonic acid signaling. *Front. Plant Sci.* 6(MAR). https://doi.org/10.3389/FPLS.2015.00170

Chen, F., Hu, Y., Vannozzi, A., Wu, K., Cai, H., Qin, Y., Mullis, A., Lin, Z., & Zhang, L. (2017). The WRKY Transcription Factor Family in Model Plants and Crops. *Crit. Rev. Plant Sci.* 36, 311–335. https://doi.org/10.1080/07352689.2018.1441103

Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., Cai, R., Zuo, J., Tang, X., Li, X., Guo, H., & Zhou, J.-M. (2009). ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 Repress SALICYLIC ACID INDUCTION DEFICIENT2 Expression to Negatively Regulate Plant Innate Immunity in Arabidopsis W OA. *Plant Cell* 21, 2527–2540. https://doi.org/10.1105/tpc.108.065193

Chen, L., Zhang, L., Xiang, S., Chen, Y., Zhang, H., & Yu, D. (2021). The transcription factor WRKY75 positively regulates jasmonate-mediated plant defense to necrotrophic fungal pathogens. *J. Exp. Bot.* 72(4), 1473-1489. https://doi.org/10.1093/jxb/eraa529

Choi, H. W., & Klessig, D. F. (2016). DAMPs, MAMPs, and NAMPs in plant innate immunity. *BMC Plant Biol.* 16, 232–239. https://doi.org/10.1186/s12870-016-0921-2

Ciliberti, N., Fermaud, M., Roudet, J., & Rossi, V. (2015). Environmental conditions affect Botrytis cinerea infection of mature grape berries more than the strain or transposon genotype. *Phytopathology* 105(8), 1090–1096. https://doi.org/10.1094/PHYTO-10-14-0264-R

Coca, M., & San Segundo, B. (2010). AtCPK1 calcium-dependent protein kinase mediates pathogen

resistance in Arabidopsis. *Plant J.*63(3), 526–540. https://doi.org/10.1111/J.1365-313X.2010.04255.X

Colmenares, A. J., Aleu, J., Durán-Patrón R., Collado I.G., Hernández-Galán R.. (2002). The Putative Role of Botrydial and Related Metabolites in the Infection Mechanism of Botrytis cinerea. *J. Chem. Ecol.* 28(5), 997–1005. https://doi.org/10.1023/A:1015209817830

De Vleesschauwer, D., Xu, J., & Höfte, M. (2014). Making sense of hormone-mediated defense networking: From rice to Arabidopsis. *Front. Plant Sci.* 5(November), 1–15. https://doi.org/10.3389/FPLS.2014.00611

De Vos, M., Van Oosten, V. R., Van Poecke, R. M. P., Van Pelt, J. A., Pozo, M. J., Mueller, M. J., Buchala, A. J., Métraux, J. P., Van Loon, L. C., Dicke, M., & Pieterse, C. M. J. (2005). Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Mol. Plant-Microbe Interact*. 18(9), 923–937. https://doi.org/10.1094/MPMI-18-0923

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G.D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.*, 13, 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.xDelledonne, M., Rgen Zeier, J., Marocco, A., & Lamb, C. (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. *Proc. Nat. Acad. Sci.* 98(23), 13454–13459. https://doi.org/10.1073/pnas.231178298

Denness, L., McKenna, J. F., Segonzac, C., Wormit, A., Madhou, P., Bennett, M., Mansfield, J., Zipfel, C., & Hamann, T. (2011). Cell wall damage-induced lignin biosynthesis is regulated by a reactive oxygen species- and jasmonic acid-dependent process in arabidopsis. *Plant Physiol.* 156(3), 1364–1374. https://doi.org/10.1104/pp.111.175737

Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X., & Zhang, Y. (2018). Opposite Roles of Salicylic Acid Receptors NPR1 and NPR3/NPR4 in Transcriptional Regulation of Plant Immunity. *Cell* 173(6), 1454-1467. https://doi.org/10.1016/j.cell.2018.03.044

Doebley, J. F., Gaut, B. S., & Smith, B. D. (2006). The Molecular Genetics of Crop Domestication. *Cell* 127, 1309–1321. https://doi.org/10.1016/j.cell.2006.12.006

Du, L., Ali, G. S., Simons, K. A., Hou, J., Yang, T., Reddy, A. S. N., & Poovaiah, B. W. (2009). Ca2+/calmodulin regulates salicylic-acid-mediated plant immunity. *Nature* 457, 1154–1158. https://doi.org/10.1038/nature07612

Dubois, M., Van Den Broeck, L., & Inzé, D. (2018). The Pivotal Role of Ethylene in Plant Growth. *Trends Plant Sci*. 23(4). https://doi.org/10.1016/j.tplants.2018.01.003

Elad, Y., & Evensen, K. (1995). Physiological aspects of resistance to Botrytis cinerea. *Phytopathology* 85(6), 637–643. https://doi.org/10.1094/PHYTO-85-637

Encinas-Villarejo, S., Maldonado, A. M., Amil-Ruiz, F., De Los Santos, B., Romero, F., Pliego-Alfaro, F., Muñ Oz-Blanco, J., & Caballero, J. L. (2009). Evidence for a positive regulatory role of strawberry (Fragaria3ananassa) Fa WRKY1 and Arabidopsis At WRKY75 proteins in resistance. *J. Exp. Bot.* 60(11), 3043–3065. https://doi.org/10.1093/jxb/erp152

Eulgem, T., Rushton, P. J., Robatzek, S., & Somssich, I. E. (2000). The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* 5(5), 1360–1385. https://doi.org/10.1016/S1360-1385(00)01600-9

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56(1), 5–51.

https://doi.org/10.1016/S0031-9422(00)00316-2

Feng, Z., Xu, Y., Kobayashi, K., Dai, L., Zhang, T., Agathokleous, E., Calatayud, V., Paoletti, E., Mukherjee, A., Agrawal, M., Park, R. J., Oak, Y. J., & Yue, X. (2022). Ozone pollution threatens the production of major staple crops in East Asia. *Nat. Food* 3(1), 47–56. https://doi.org/10.1038/s43016-021-00422-6

Ferrari, S., Galletti, R., Denoux, C., De Lorenzo, G., Ausubel, F. M., & Dewdney, J. (2007). Resistance to Botrytis cinerea Induced in Arabidopsis by Elicitors Is Independent of Salicylic Acid, Ethylene, or Jasmonate Signaling But Requires PHYTOALEXIN DEFICIENT3. *Plant Physiol.* 144(1), 367–379. https://doi.org/10.1104/PP.107.095596

Ferrari, S., Plotnikova, J. M., De Lorenzo, G., & Ausubel, F. M. (2003). Arabidopsis local resistance to Botrytis cinerea involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. *Plant J.* 35(2), 193–205. https://doi.org/10.1046/j.1365-313X.2003.01794.x

Fichman, Y., & Mittler, R. (2020). Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades? *Plant J.* 102, 887-896. https://doi.org/10.1111/tpj.14685

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., & Ryals, J. (1993). Requirement of Salicylic Acid for the Induction of Systemic Acquired Resistance. *Science*, 261, 754–756. https://doi.org/10.1126/SCIENCE.261.5122.754

Gao, Q.-M., Venugopal, S., Navarre, D., & Kachroo, A. (2011). Low Oleic Acid-Derived Repression of Jasmonic Acid-Inducible Defense Responses Requires the WRKY50 and WRKY51 Proteins . *Plant Physiol*. 155, 464–476. https://doi.org/10.1104/pp.110.166876

Ghorbel, M., Brini, F., Sharma, A., & Landi, M. (2021). Role of jasmonic acid in plants: the molecular point of view. *Plant Cell Rep*. 40(8), 1471–1494. https://doi.org/10.1007/s00299-021-02687-4

Glawischnig, E., Hansen, B. G., Olsen, C. E., & Halkier, B. A. (2004). Camalexin is synthesized from indole-3-acetaldoxime, a key branching point between primary and secondary metabolism in Arabidopsis. *Proc.Nat. Acad. Sci. U. S. A.* 101(21), 8245–8250. https://doi.org/10.1073/PNAS.0305876101

Glazebrook, J. (2005). Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. *Annu. Rev. Phytopathol.* 43, 205–227. https://doi.org/10.1146/ANNUREV.PHYTO.43.040204.135923

Govrin, E. M., & Levine, A. (2000). The hypersensitive response facilitates plant infection by the necrotrophic pathogen Botrytis cinerea. *Curr. Biol.* 10(13), 751–757. https://doi.org/10.1016/S0960-9822(00)00560-1

Gu, Y., Li, W., Jiang, H., Wang, Y., Gao, H., Liu, M., Chen, Q., Lai, Y., He, C., Leubner, G., & Holloway, R. (2017). Differential expression of a WRKY gene between wild and cultivated soybeans correlates to seed size. *J. Exp. Bot.* 68(11), 2717–2729. https://doi.org/10.1093/jxb/erx147

Guan, L., Denkert, N., Eisa, A., Lehmann, M., Sjuts, I., Weiberg, A., Soll, J., Meinecke, M., & Schwenkert, S. (2019). JASSY, a chloroplast outer membrane protein required for jasmonate biosynthesis. *Proc. Nat. Acad. Sci. U. S. A.* 116(21), 10568–10575. https://doi.org/10.1073/PNAS.1900482116

Guo, P., Li, Z., Huang, P., Li, B., Fang, S., Chu, J., & Guo, H. (2017). A Tripartite Amplification Loop Involving the Transcription Factor WRKY75, Salicylic Acid, and Reactive Oxygen Species Accelerates Leaf Senescence. *Plant Cell* 29(11), 2854–2870. https://doi.org/10.1105/TPC.17.00438

Han, L., Li, G. J., Yang, K. Y., Mao, G., Wang, R., Liu, Y., & Zhang, S. (2010). Mitogen-activated protein kinase 3 and 6 regulate Botrytis cinerea-induced ethylene production in Arabidopsis. *Plant Journal*, 64(1), 114–127. https://doi.org/10.1111/J.1365-313X.2010.04318.X

He, Y., Xu, J., Wang, X., He, X., Wang, Y., Zhou, J., Zhang, S., & Meng, X. (2019). The Arabidopsis Pleiotropic Drug Resistance Transporters PEN3 and PDR12 Mediate Camalexin Secretion for Resistance to Botrytis cinerea. *Plant Cell*, 31(9), 2206–2222. https://doi.org/10.1105/TPC.19.00239

Hickman, R., Mendes, M. P., Verk, M. C. Van, Dijken, A. J. H. Van, Sora, J. Di, Denby, K., Pieterse, C. M. J., & Wees, S. C. M. Van. (2019). Transcriptional Dynamics of the Salicylic Acid Response and its Interplay with the Jasmonic Acid Pathway. *BioRxiv* 742742. https://doi.org/10.1101/742742

Hillmer, R. A., Tsuda, K., Rallapalli, G., Asai, S., Truman, W., Papke, M. D., Sakakibara, H., Jones, J. D. G., Myers, C. L., & Katagiri, F. (2017). The highly buffered Arabidopsis immune signaling network conceals the functions of its components. *PLOS Genet*. 13(5), e1006639. https://doi.org/10.1371/JOURNAL.PGEN.1006639

Inzé, D., & Nelissen, H. (2022). The translatability of genetic networks from model to crop species: lessons from the past and perspectives for the future. *New Phytol.* 236(1), 43–48. https://doi.org/10.1111/NPH.18364

Ishihama, N., Choi, S. won, Noutoshi, Y., Saska, I., Asai, S., Takizawa, K., He, S. Y., Osada, H., & Shirasu, K. (2021). Oxicam-type non-steroidal anti-inflammatory drugs inhibit NPR1-mediated salicylic acid pathway. *Nat. Commun.* 12(1), 1–13. https://doi.org/10.1038/s41467-021-27489-w

Jacob, F., Kracher, B., Mine, A., Seyfferth, C., Blanvillain-Baufum, S., Parker, J. E., Tsuda, K., Schulze-Lefert, P., & Maekawa, T. (2018). A dominant-interfering camta3 mutation compromises primary transcriptional outputs mediated by both cell surface and intracellular immune receptors in Arabidopsis thaliana. *New Phytol.* 217, 1667–1680. https://doi.org/10.1111/nph.14943

Jarocka-Karpowicz, I., Markowska, A., & Zhang, H. (2021). Molecular Sciences Therapeutic Potential of Jasmonic Acid and Its Derivatives. *J. Mol. Sci*. 22,8437. https://doi.org/10.3390/ijms22168437

Jirschitzka, J., Mattern, D. J., Gershenzon, J., & Charles D'auria, J. (2013). Learning from nature: new approaches to the metabolic engineering of plant defense pathways. *Curr. Opin. Biotechnol.* 24, 320–328. https://doi.org/10.1016/j.copbio.2012.10.014

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature* 444(7117), 323–329. https://doi.org/10.1038/NATURE05286

Kadota, Y., Shirasu, K., & Zipfel, C. (2015). Regulation of the NADPH Oxidase RBOHD During Plant Immunity. *Plant Cell Physiol.* 56(8), 1472–1480. https://doi.org/10.1093/PCP/PCV063

Kang, J., Hwang, J. U., Lee, M., Kim, Y. Y., Assmann, S. M., Martinoia, E., & Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc. Nat. Acad. Sci. U. S. A.*, 107(5), 2355–2360. https://doi.org/10.1073/pnas.0909222107

Kaurilind, E., & Brosché, M. (2017). Stress marker signatures in lesion mimic single and double mutants identify a crucial leaf age-dependent salicylic acid related defense signal. *PLoS One* 12(1). https://doi.org/10.1371/journal.pone.0170532

Kim, J., Chang, C., & Tucker, M. L. (2015). To grow old: Regulatory role of ethylene and jasmonic acid in senescence. *Front. Plant Sci.* 6(JAN), 1–7. https://doi.org/10.3389/FPLS.2015.00020

Kim, Y., Gilmour, S. J., Chao, L., Park., S., & Thomashow, M. F. (2019). Arabidopsis CAMTA Transcription Factors Regulate Pipecolic Acid Biosynthesis and Priming of Immunity Genes. *Mol. Plant*. 13(1), 157-168. https://doi.org/10.1016/j.molp.2019.11.001

Kliebenstein, D. J., Rowe, H. C., & Denby, K. J. (2005). Secondary metabolites influence Arabidopsis/Botrytis interactions: Variation in host production and pathogen sensitivity. *Plant J.*44(1), 25–36. https://doi.org/10.1111/J.1365-313X.2005.02508.X

Koo, A. J. K., Cooke, T. F., & Howe, G. A. (2011). Cytochrome P450 CYP94B3 mediates catabolism and inactivation of the plant hormone jasmonoyl-L-isoleucine. *Proc. Nat. Acad. Sci. U. S. A.* 108(22), 9298–9303. https://doi.org/10.1073/PNAS.1103542108

Kumar, S., Zavaliev, R., Wu, Q., Zhou, Y., Cheng, J., Dillard, L., Powers, J., Withers, J., Zhao, J., Guan, Z., Borgnia, M. J., Bartesaghi, A., Dong, X., & Zhou, P. (2022). Structural basis of NPR1 in activating plant immunity. *Nature* 605, 561–566. https://doi.org/10.1038/s41586-022-04699-w

Lemonnier, P., Gaillard, C., Veillet, F., Verbeke, J., Lemoine, R., Coutos-Thévenot, P., & La Camera, S. (2014). Expression of Arabidopsis sugar transport protein STP13 differentially affects glucose transport activity and basal resistance to Botrytis cinerea. *Plant Mol. Biol*. 85, 473–484. https://doi.org/10.1007/s11103-014-0198-5

Leon-Reyes, A., Du, Y., Koornneef, A., Proietti, S., Körbes, A. P., Memelink, J., Pieterse, C. M. J., & Ritsema, T. (2010). Ethylene Signaling Renders the Jasmonate Response of Arabidopsis Insensitive to Future Suppression by Salicylic Acid. *Mol. Plant Microbe Interact.*, 23(2), 187–197. https://doi.org/10.1094/MPMI-23-2-0187

Li, C., Xu, M., Cai, X., Han, Z., Si, J., & Chen, D. (2022). Jasmonate Signaling Pathway Modulates Plant Defense, Growth, and Their Trade-Offs. *Int. J. Mol. Sci.* 23(7), 3945. https://doi.org/10.3390/IJMS23073945

Li, J., Brader, G., & Palva, E. T. (2004). The WRKY70 Transcription Factor: A Node of Convergence for Jasmonate-Mediated and Salicylate-Mediated Signals in Plant Defense. *Plant Cell* 16(2), 319–331. https://doi.org/10.1105/TPC.016980

Liu, L., Sonbol, F. M., Huot, B., Gu, Y., Withers, J., Mwimba, M., Yao, J., He, S. Y., & Dong, X. (2016). Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. *Nat. Comm.* 7(1), 1–10. https://doi.org/10.1038/ncomms13099

Liu, S., Kracher, B., Ziegler, J., Birkenbihl, R. P., & Somssich, I. E. (2015). Negative regulation of ABA Signaling By WRKY33 is critical for Arabidopsis immunity towards Botrytis cinerea 2100. *ELife*.4. https://doi.org/10.7554/ELIFE.07295

Lobell, D. B., Cassman, K. G., & Field, C. B. (2009). Crop Yield Gaps: Their Importance, Magnitudes, and Causes. *Annu. Rev. Environ. Resour.* 34, 179–204. https://doi.org/10.1146/ANNUREV.ENVIRON.041008.093740

Lorenzo, O., Chico, J. M., Sánchez-Serrano, J. J., & Solano, R. (2004). JASMONATE-INSENSITIVE1 Encodes a MYC Transcription Factor Essential to Discriminate between Different Jasmonate-Regulated Defense Responses in Arabidopsis. *Plant Cell.* 16(7), 1938–1950. https://doi.org/10.1105/TPC.022319

Lorenzo, O., Piqueras, R., Sánchez-Serrano, J. J., & Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 Integrates Signals from Ethylene and Jasmonate Pathways in Plant Defense. *Plant Cell.* 15(1), 165–178. https://doi.org/10.1105/TPC.007468

Lumbreras, V., Vilela, B., Irar, S., Solé, M., Capellades, M., Valls, M., Coca, M., & Pagès, M. (2010). MAPK phosphatase MKP2 mediates disease responses in Arabidopsis and functionally interacts with MPK3 and MPK6. *Plant J.* 63(6), 1017–1030. https://doi.org/10.1111/J.1365-313X.2010.04297.X

Madan, R. K., & Levitt, J. (2014). A review of toxicity from topical salicylic acid preparations. *J. Am. Acad. Dermatol.* 70(4), 788–792. https://doi.org/10.1016/J.JAAD.2013.12.005

Mao, P., Duan, M., Wei, C., & Li, Y. (2007). WRKY62 Transcription Factor Acts Downstream of Cytosolic NPR1 and Negatively Regulates Jasmonate-Responsive Gene Expression. *Plant Cell Physiol.* 48(6), 833–842. https://doi.org/10.1093/pcp/pcm058

Méndez-Bravo, A., Calderón-Vázquez, C., Ibarra-Laclette, E., Raya-González, J., Ramírez-Chávez, E., Molina-Torres, J., Guevara-García, A. A., López-Bucio, J., & Herrera-Estrella, L. (2011). Alkamides activate jasmonic acid biosynthesis and signaling pathways and confer resistance to botrytis cinerea in Arabidopsis thaliana. *PLoS ONE*. 6(11). https://doi.org/10.1371/journal.pone.0027251

Mignolet-Spruyt, L., Xu, E., Idänheimo, N., Hoeberichts, F. A., Mühlenbock, P., Brosche, M., Van Breusegem, F., & Kangasjärvi, J. (2016). Spreading the news: subcellular and organellar reactive oxygen species production and signalling. *J. Exp. Bot.* 67(13), 3831–3844. https://doi.org/10.1093/JXB/ERW080

Mine, A., Seyfferth, C., Kracher, B., Berens, M. L., Becker, D., & Tsuda, K. (2018). The Defense Phytohormone Signaling Network Enables Rapid, High-Amplitude Transcriptional Reprogramming during Effector-Triggered Immunity. *Plant Cell.* 30(6), 1199–1219. https://doi.org/10.1105/TPC.17.00970

Mokyr, J., & Ó Gráda, C. (2002). What do people die of during famines: the Great Irish Famine in comparative perspective. *Eur. Rev. Econ. Hist.* 6(3), 339–363. https://doi.org/10.1017/S1361491602000163

Moore, J. W., Loake, G. J., & Spoel, S. H. (2011). Transcription Dynamics in Plant Immunity. *Plant Cell* 23(8), 2809–2820. https://doi.org/10.1105/TPC.111.087346

Mou, W., Kao, Y. T., Michard, E., Simon, A. A., Li, D., Wudick, M. M., Lizzio, M. A., Feijó, J. A., & Chang, C. (2020). Ethylene-independent signaling by the ethylene precursor ACC in Arabidopsis ovular pollen tube attraction. *Nat. Comm.* 11(1), 1–11. https://doi.org/10.1038/s41467-020-17819-9

Mou, Z., Fan, W., & Dong, X. (2003). Inducers of Plant Systemic Acquired Resistance Regulate NPR1 Function through Redox Changes. *Cell* 113(7), 935–944. https://doi.org/10.1016/S0092-8674(03)00429-X

Moura, J. C. M. S., Bonine, C. A. V., de Oliveira Fernandes Viana, J., Dornelas, M. C., & Mazzafera, P. (2010). Abiotic and biotic stresses and changes in the lignin content and composition in plants. *J. Integr. Plant Biol* 52(4), 360–376. https://doi.org/10.1111/j.1744-7909.2010.00892.x

Nie, H., Zhao, C., Wu, G., Wu, Y., Chen, Y., & Tang, D. (2012). SR1, a calmodulin-binding transcription factor, modulates plant defense and ethylene-induced senescence by directly regulating NDR1 and EIN3. *Plant Physiol.* 158(4), 1847–1859. https://doi.org/10.1104/pp.111.192310

Overmyer, K., Vuorinen, K., & Brosché, M. (2018). Interaction points in plant stress signaling pathways. *Physiol. Plant.* 162(2). https://doi.org/10.1111/ppl.12639

Overmyer, Kirk, Brosché, M., Pellinen, R., Kuittinen, T., Tuominen, H., Ahlfors, R., Keinänen, M., Saarma, M., Scheel, D., & Kangasjärvi, J. (2005). Ozone-induced programmed cell death in the

Arabidopsis radical-induced cell death1 mutant. *Plant Physiol.* 137(3), 1092–1104. https://doi.org/10.1104/pp.104.055681

Park, S. W., Li, W., Viehhauser, A., He, B., Kim, S., Nilsson, A. K., Andersson, M. X., Kittle, J. D., Ambavaram, M. M. R., Luan, S., Esker, A. R., Tholl, D., Cimini, D., Ellerström, M., Coaker, G., Mitchell, T. K., Pereira, A., Dietz, K. J., & Lawrence, C. B. (2013). Cyclophilin 20-3 relays a 12-oxo-phytodienoic acid signal during stress responsive regulation of cellular redox homeostasis. *Proc. Nat. Acad. Sci. U. S. A.* 110(23), 9559–9564. https://doi.org/10.1073/PNAS.1218872110

Peng, Y., Yang, J., Li, X., & Zhang, Y. (2021). Salicylic Acid: Biosynthesis and Signaling. *Annu. Rev. Plant Biol.* 72, 761–791. https://doi.org/10.1146/annurev-arplant-081320-092855

Penninckx, I. A. M. A., Eggermont, K., Terras, F. R. G., Thomma, B. P. H. J., De Samblanx, G. W., Buchala, A., Métraux, J. P., Manners, J. M., & Broekaert, W. F. (1996). Pathogen-induced systemic activation of a plant defensin gene in Arabidopsis follows a salicylic acid-independent pathway. *Plant Cell* 8(12), 2309–2323. https://doi.org/10.1105/TPC.8.12.2309

Qiao, H., Shen, Z., Huang, S. S. C., Schmitz, R. J., Urich, M. A., Briggs, S. P., & Ecker, J. R. (2012). Processing and subcellular trafficking of ER-tethered EIN2 control response to ethylene gas. *Science*, 338(6105), 390–393. https://doi.org/10.1126/science.1225974

Rasmussen, S., Barah, P., Cristina Suarez-Rodriguez, M., Bressendorff, S., Friis, P., Costantino, P., Bones, A. M., Nielsen, H. B., & Mundy, J. (2013). Transcriptome Responses to Combinations of Stresses in Arabidopsis 1[W][OA]. *Plant Physiol.* 161, 1783–1794. https://doi.org/10.1104/pp.112.210773

Rasool, B., Karpinska, B., Konert, G., Durian, G., Denessiouk, K., Kangasjärvi, S., & Foyer, C. H. (2014). Effects of light and the regulatory B-subunit composition of protein phosphatase 2A on the susceptibility of Arabidopsis thaliana to aphid (Myzus persicae) infestation. *Front. Plant Sci.* 5(AUG), 405. https://doi.org/10.3389/FPLS.2014.00405

Rassizadeh, L., Cervero, R., Flors, V., & Gamir, J. (2021). Extracellular DNA as an elicitor of broad-spectrum resistance in Arabidopsis thaliana. *Plant Sci.* 312, 111036. https://doi.org/10.1016/J.PLANTSCI.2021.111036

Rekhter, D., Lüdke, D., Ding, Y., Feussner, K., Zienkiewicz, K., Lipka, V., Wiermer, M., Zhang, Y., & Feussner, I. (2019). Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. *Science* 365(6452), 498–502. https://doi.org/10.1126/SCIENCE.AAW1720

Ren, D., Liu, Y., Yang, K. Y., Han, L., Mao, G., Glazebrook, J., & Zhang, S. (2008). A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in Arabidopsis. *Proc. Nat. Acad. Sci. U. S. A.* 105(14), 5638–5643. https://doi.org/10.1073/PNAS.0711301105

Riechmann, J. L., & Ratcliffe, O. J. (2000). A genomic perspective on plant transcription factors. *Curr. Opin.Plant Biol.* 3(5), 423–434. https://doi.org/10.1016/S1369-5266(00)00107-2

Rishmawi, L., Pesch, M., Juengst, C., Schauss, A. C., Schrader, A., & Hülskamp, M. (2014). Non-Cell-Autonomous Regulation of Root Hair Patterning Genes by WRKY75 in Arabidopsis. *Plant Physiol.* 165(1), 186–195. https://doi.org/10.1104/PP.113.233775

Rivas-San Vicente, M., & Plasencia, J. (2011). Salicylic acid beyond defence: its role in plant growth and development. *J. Exp. Bot.* 62(10), 3321–3338. https://doi.org/10.1093/JXB/ERR031

Rolke, Y., Liu, S., Quidde, T., Williamson, B., Schouten, A., Weltring, K. M., Siewers, V., Tenberge, K. B., Tudzynski, B., & Tudzynski, P. (2004). Functional analysis of H2O2-generating systems in Botrytis cinerea: The major Cu-Zn-superoxide dismutase (BCSOD1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. *Mol. Plant Pathol*.5(1), 17–

27. https://doi.org/10.1111/j.1364-3703.2004.00201.x

Savchenko, T., Kolla, V. A., Wang, C.-Q., Nasafi, Z., Hicks, D. R., Phadungchob, B., Chehab, W. E., Brandizzi, F., Froehlich, J., & Dehesh, K. (2014). Functional Convergence of Oxylipin and Abscisic Acid Pathways Controls Stomatal Closure in Response to Drought. *Plant Physiol.* 164, 1151–1160. https://doi.org/10.1104/pp.113.234310

Segmüller, N., Kokkelink, L., Giesbert, S., Odinius, D., Van Kan, J., & Tudzynski, P. (2008). NADPH Oxidases Are Involved in Differentiation and Pathogenicity in Botrytis cinerea. *Mol. Plant Microbe Interact.* 21(6), 808–819. https://doi.org/10.1094/MPMI-21-6-0808

Serrano, M., Coluccia, F., Torres, M., L'Haridon, F., & Métraux, J. P. (2014). The cuticle and plant defense to pathogens. *Front. Plant Sci.* 5. https://doi.org/10.3389/fpls.2014.00274

Shao, W., Zhang, Y., Wang, J., Lv C., & Chen C. (2016). BcMtg2 is required for multiple stress tolerance, vegetative development and virulence in Botrytis cinerea. *Sci. Rep*. 6(28673) https://doi.org/10.1038/srep28673

Spoel, S. H., Johnson, J. S., & Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proc. Nat. Acad. Sci. U. S. A.* 104(47), 18842–18847. https://doi.org/10.1073/PNAS.0708139104

Spoel, S. H., Koornneef, A., Claessens, S. M. C., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., Buchala, A. J., Métraux, J.-P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X., & Pieterse, C. M. J. (2003). NPR1 Modulates Cross-Talk between Salicylate-and Jasmonate-Dependent Defense Pathways through a Novel Function in the Cytosol. *Plant Cell* 15, 760–770. https://doi.org/10.1105/tpc.009159

Stintzi, A., Weber, H., Reymond, P., Browse, J., & Farmer, E. E. (2001). Plant defense in the absence of jasmonic acid: The role of cyclopentenones. *Proc. Nat. Acad. Sci. U. S. A.* 98(22), 12837–12842. https://doi.org/10.1073/pnas.211311098

Stroud, E. A., Jayaraman, J., Templeton, M. D., & Rikkerink, E. H. A. (2022). Comparison of the pathway structures influencing the temporal response of salicylate and jasmonate defence hormones in Arabidopsis thaliana. *Front. Plant Sci.* 13. https://doi.org/10.3389/FPLS.2022.952301

Sun, Y., Liu, Z., Guo, J., Zhu, Z., Zhou, Y., Guo, C., Hu, Y., Li, J., Shangguan, Y., Li, T., Hu, Y., Wu, R., Li, W., Rochaix, J.-D., Miao, Y., & Sun, X. (2020). WRKY33-PIF4 loop is required for the regulation of H 2 O 2 homeostasis. *Biochem. Biophys. Res. Commun.* 527, 922-928. https://doi.org/10.1016/j.bbrc.2020.05.041

Survila, M., Davidsson, P. R., Pennanen, V., Kariola, T., Broberg, M., Sipari, N., Heino, P., & Palva, E. T. (2016). Peroxidase-Generated Apoplastic ROS Impair Cuticle Integrity and Contribute to DAMP-Elicited Defenses. *Front. Plant Sci.* 7. https://doi.org/10.3389/fpls.2016.01945

Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K. I., Shibata, D., Kobayashi, Y., & Ohta, H. (2005). 12-Oxo-Phytodienoic Acid Triggers Expression of a Distinct Set of Genes and Plays a Role in Wound-Induced Gene Expression in Arabidopsis. *Plant Physiol.* 139(3), 1268–1283. https://doi.org/10.1104/PP.105.067058

Thomma, B. P. H. J., Eggermont, K., Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A., & Broekaert, W. F. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in arabidopsis are essential for resistance to distinct microbial pathogens. *Proc. Nat. Acad. Sci. U. S. A.* 95(25), 15107–15111.

https://doi.org/10.1073/PNAS.95.25.15107

Thomma, B. P. H. J., Penninckx, I. A. M. A., Broekaert, W. F., & Cammue, B. P. A. (2001). The complexity of disease signaling in Arabidopsis. *Curr. Opin. Immunol.* 13(1), 63–68. https://doi.org/10.1016/S0952-7915(00)00183-7

Tiedemann, A. V. (1997). Evidence for a primary role of active oxygen species in induction of host cell death during infection of bean leaves withBotrytis cinerea. *Physiol. Mol. Plant Pathol.* 50(3), 151–166. https://doi.org/10.1006/PMPP.1996.0076

Tiryaki, I., & Staswick, P. E. (2002). An Arabidopsis Mutant Defective in Jasmonate Response Is Allelic to the Auxin-Signaling Mutant axr1. *Plant Physiol.*130(2), 887–894. https://doi.org/10.1104/PP.005272

Torres, M. A. (2010). ROS in biotic interactions. *Physiol. Plant.* 138, (4), 414–429. https://doi.org/10.1111/j.1399-3054.2009.01326.x

Torres, M. A., & Dangl, J. L. (2005). Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Curr. Opin. Plant Biol.* 8, 397–403. https://doi.org/10.1016/j.pbi.2005.05.014

Trotta, A., Wrzaczek, M., Scharte, J., Tikkanen, M., Konert, G., Rahikainen, M., Holmströ, M., Hiltunen, H.-M., Rips, S., Sipari, N., Mulo, P., Weis, E., Von Schaewen, A., Aro, E.-M., & Kangasjärvi, S. (2011). Regulatory Subunit B'γ of Protein Phosphatase 2A Prevents Unnecessary Defense Reactions under Low Light in Arabidopsis. *Plant Physiol.* 156, 1464–1480. https://doi.org/10.1104/pp.111.178442

Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., & Katagiri, F. (2009). Network properties of robust immunity in plants. *PLoS Genet.* 5(12). https://doi.org/10.1371/journal.pgen.1000772

Uhrig, R. G., Labandera, A. M., & Moorhead, G. B. (2013). Arabidopsis PPP family of serine/threonine protein phosphatases: many targets but few engines. *Trends Plant Sci.* 18(9), 505-513. https://doi.org/10.1016/j.tplants.2013.05.004

Ülker, B., & Somssich, I. E. (2004). WRKY transcription factors: from DNA binding towards biological function. *Curr. Opin. Plant Biol.* 7(5), 491–498. https://doi.org/10.1016/J.PBI.2004.07.012

Vaahtera, L., Brosché, M., Wrzaczek, M., & Kangasjä Rvi, J. (2014). Specificity in ROS Signaling and Transcript Signatures. *Antioxid. Redox Signal.* 21(9), 1422–1441. https://doi.org/10.1089/ars.2013.5662

Van Dam, N. M., Tytgat, T. O. G., & Kirkegaard, J. A. (2009). Root and shoot glucosinolates: A comparison of their diversity, function and interactions in natural and managed ecosystems. *Phytochem. Rev.* 8(1), 171–186. https://doi.org/10.1007/S11101-008-9101-9

Van de Poel, B. (2020). Ethylene's fraternal twin steals the spotlight. *Nat. Plants*, 6(11), 1309–1310. https://doi.org/10.1038/s41477-020-00796-8

Van der Does, D., Leon-Reyes, A., Koornneef, A., Van Verk, M. C., Rodenburg, N., Pauwels, L., Goossens, A., Körbes, A. P., Memelink, J., Ritsema, T., Van Wees, S. C. M., & Pietersea, C. M. J. (2013). Salicylic Acid Suppresses Jasmonic Acid Signaling Downstream of SCFCOI1-JAZ by Targeting GCC Promoter Motifs via Transcription Factor ORA59. *Plant Cell*, 25(2), 744–761. https://doi.org/10.1105/TPC.112.108548

Van Verk, M. C., Pappaioannou, D., Neeleman, L., Bol, J. F., & Linthorst, H. J. M. (2008). A Novel WRKY Transcription Factor Is Required for Induction of PR-1a Gene Expression by Salicylic Acid and Bacterial Elicitors. *Plant Physiol.*146(4), 1983–1995.

https://doi.org/10.1104/PP.107.112789

Vanderstraeten, L., Depaepe, T., Bertrand, S., & Van Der Straeten, D. (2019). The Ethylene Precursor ACC Affects Early Vegetative Development Independently of Ethylene Signaling. *Front. Plant Sci.* 10, 1591. https://doi.org/10.3389/FPLS.2019.01591

Verrier, P. J., Bird, D., Burla, B., Dassa, E., Forestier, C., Geisler, M., Klein, M., Ner Kolukisaoglu, U. ¨, Lee, Y., Martinoia, E., Murphy, A., Rea, P. A., Samuels, L., Schulz, B., Spalding, E. P., Yazaki, K., & Theodoulou, F. L. (2008). Plant ABC proteins-a unified nomenclature and updated inventory. *TrendsPlant Sci.* 13(4), 1360–1385. https://doi.org/10.1016/j.tplants.2008.02.001

Vos, I. A., Pieterse, C. M. J., & Van Wees, S. C. M. (2013). Costs and benefits of hormone-regulated plant defences. *Plant Pathol.* 62, 43–55. https://doi.org/10.1111/ppa.12105

Wang, D., Amornsiripanitch, N., & Dong, X. (2006). A Genomic Approach to Identify Regulatory Nodes in the Transcriptional Network of Systemic Acquired Resistance in Plants. *PLoS Pathog.* 2(11), e123. https://doi.org/10.1371/journal.ppat.0020123

Wang, H.-Q., Sun, L.-P., Wang, L.-X., Fang, X.-W., Li, Z.-Q., Zhang, F.-F., Hu, X., Qi, C., & He, J.-M. (2020). Ethylene mediates salicylic-acid-induced stomatal closure by controlling reactive oxygen species and nitric oxide production in Arabidopsis. *Plant Sci*. 294, https://doi.org/10.1016/j.plantsci.2020.110464

Wang, L., Tsuda, K., Truman, W., Sato, M., Nguyen, L. V., Katagiri, F., & Glazebrook, J. (2011). CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. *Plant J.* 67(6), 1029–1041. https://doi.org/10.1111/J.1365-313X.2011.04655.X

Waszczak, C., Carmody, M., & Kangasjärvi, J. (2018). Reactive Oxygen Species in Plant Signaling. *Annu. Rev. Plant Biol.* 69, 209-239. https://doi.org/10.1146/annurev-arplant-042817-040322

Weiberg, A., Wang, M., Lin, F. M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H. Da, & Jin, H. (2013). Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342(6154), 118–123. https://doi.org/10.1126/SCIENCE.1239705

Widmann, C., Gibson, S., Jarpe, M. B., & Johnson, G. L. (1999). Mitogen-activated protein kinase: Conservation of a three-kinase module from yeast to human. *Physiol. Rev.* 79(1), 143–180. https://doi.org/10.1152/PHYSREV.1999.79.1.143

Wildermuth, M. C., Dewdney, J., Wu, G., & Ausubel, F. M. (2001). *Isochorismate synthase is required to synthesize salicylic acid for plant defence*. Nature 414, 562-565. https://doi.org/10.1038/35107108

Williamson, B., Tudzynski, B., Tudzynski, P., & Van Kan, J. A. L. (2007). Botrytis cinerea: The cause of grey mould disease. *Mol. Plant Pathol.* 8(5), 561–580. https://doi.org/10.1111/j.1364-3703.2007.00417.x

Wohlgemuth, H., Mittelstrass, K., Kschieschan, S., Bender, J., Weigel, H.-J., Overmyer, K., Kangasjärvi, J., Sandermann, H., & Langebartels, & C. (2002). Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. *Plant Cell Environ.* 25, 717–726. https://doi.org/10.1046/j.1365-3040.2002.00859.x

Xu, E., Vaahtera, L., & Brosché, M. (2015a). Roles of Defense Hormones in the Regulation of Ozone-Induced Changes in Gene Expression and Cell Death. *Mol. Plant* 8(12), 1776–1794. https://doi.org/10.1016/j.molp.2015.08.008

Xu, E., Vaahtera, L., Hõrak, H., Hincha, D. K., Heyer, A. G., & Brosché, M. (2015b). Quantitative trait loci mapping and transcriptome analysis reveal candidate genes regulating the response to

ozone in Arabidopsis thaliana. *Plant CellEnviron.* 38, 1418–1433. https://doi.org/10.1111/pce.12499

Xu, J., Meng, J., Meng, X., Zhao, Y., Liu, J., Sun, T., Liu, Y., Wang, Q., & Zhang, S. (2016). Pathogen-Responsive MPK3 and MPK6 Reprogram the Biosynthesis of Indole Glucosinolates and Their Derivatives in Arabidopsis Immunity. *Plant Cell*, 28, 1144–1162. https://doi.org/10.1105/tpc.15.00871

Yang, Y. X., Wu, C., Ahammed, G. J., Wu, C., Yang, Z., Wan, C., & Chen, J. (2018). Red light-induced systemic resistance against root-knot nematode is mediated by a coordinated regulation of salicylic acid, jasmonic acid and redox signaling in watermelon. *Front. Plant Sci.* 9, 899. https://doi.org/10.3389/FPLS.2018.00899

Yuan, M., Ngou, B. P. M., Ding, P., & Xin, X. F. (2021). PTI-ETI crosstalk: an integrative view of plant immunity. *Curr. Opin. Plant Biol.*62, 102030. https://doi.org/10.1016/J.PBI.2021.102030

Zandalinas, S. I., Mittler, R., Balfagón, D., Arbona, V., & Gómez-Cadenas, A. (2018). Plant adaptations to the combination of drought and high temperatures. *Physiol. Plant.* 162, 2–12. https://doi.org/10.1111/ppl.12540

Zander, M., La Camera, S., Lamotte, O., Mé traux, J.-P., & Gatz, C. (2010). Arabidopsis thaliana class-II TGA transcription factors are essential activators of jasmonic acid/ethylene-induced defense responses. *Plant J.* 61, 200–210. https://doi.org/10.1111/j.1365-313X.2009.04044.x

Zavaliev, R., Mohan, R., Chen, T., & Dong, X. (2020). Formation of NPR1 Condensates Promotes Cell Survival during the Plant Immune Response. *Cell*, 182(5), 1093-1108. https://doi.org/10.1016/J.CELL.2020.07.016

Zeilmaker, T., Ludwig, N. R., Elberse, J., Seidl, M. F., Berke, L., Van Doorn, A., Schuurink, R. C., Snel, B., & Van Den Ackerveken, G. (2015). DOWNY MILDEW RESISTANT 6 and DMR6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis. *Plant J.* 81(2), 210–222. https://doi.org/10.1111/TPJ.12719

Zeng, W., & He, S. Y. (2010). A Prominent Role of the Flagellin Receptor FLAGELLIN-SENSING2 in Mediating Stomatal Response to Pseudomonas syringae pv tomato DC3000 in Arabidopsis. *Plant Physiol.* 153(3), 1188–1198. https://doi.org/10.1104/PP.110.157016

Zhang, N., Zhou, S., Yang, D., & Fan, Z. (2020). Revealing Shared and Distinct Genes Responding to JA and SA Signaling in Arabidopsis by Meta-Analysis. *Front. Plant Sci.*11, 908. https://doi.org/10.3389/FPLS.2020.00908

Zhang, S., Li, C., Wang, R., Chen, Y., Shu, S., Huang, R., Zhang, D., Li, J., Xiao, S., Yao, N., & Yang, C. (2017). The Arabidopsis Mitochondrial Protease FtSH4 Is Involved in Leaf Senescence via Regulation of WRKY-Dependent Salicylic Acid Accumulation and Signaling. *Plant Physiol.* 173(4), 2294–2307. https://doi.org/10.1104/PP.16.00008

Zhang, W., Corwinand, J. A., Copeland, D., Feusier, J., Eshbaugh, R., Chen, F., Atwell, S., & Kliebenstein, D. J. (2017). Plastic transcriptomes stabilize immunity to pathogen diversity: The jasmonic acid and salicylic acid networks within the Arabidopsis/Botrytis pathosystem open. *Plant Cell* 29(11), 2727–2752. https://doi.org/10.1105/tpc.17.00348

Zhang, X., & Dong, X. (2022). Life-or-death decisions in plant immunity. *Curr. Opin. Immunol.* 75, 102169. https://doi.org/10.1016/J.COI.2022.102169

Zhang, Y., Cheng, Y. T., Qu, N., Zhao, Q., Bi, D., & Li, X. (2006). Negative regulation of defense responses in Arabidopsis by two NPR1 paralogs. *Plant J.* 48(5), 647–656. https://doi.org/10.1111/J.1365-313X.2006.02903.X

Zhang, Y. J., Zhao, L., Zhao, J. Z., Li, Y. J., Wang, J. Bin, Guo, R., Gan, S. S., Liu, C. J., & Zhanga, K. W. (2017). S5H/DMR6 Encodes a Salicylic Acid 5-Hydroxylase That Fine-Tunes Salicylic Acid Homeostasis. *Plant Physiol.* 175(3), 1082–1093. https://doi.org/10.1104/PP.17.00695

Zhang, Y., & Li, X. (2019). Salicylic acid: biosynthesis, perception, and contributions to plant immunity. *Curr. Opin. Plant Biol.* 50, 29–36. https://doi.org/10.1016/J.PBI.2019.02.004

Zhao, Z., Fan, J., Yang, P., Wang, Z., Opiyo, S. O., Mackey, D., & Xia, Y. (2022). Involvement of Arabidopsis Acyl Carrier Protein 1 in PAMP-Triggered Immunity. *Mol. Plant Microbe Interact.* 35(8), 681-693. https://doi.org/10.1094/MPMI-02-22-0049-R

Zheng, X.-Y., Spivey, N. W., Zeng, W., Liu, P.-P., Fu, Z. Q., Klessig, D. F., Yang He, S., & Dong, X. (2012). Coronatine Promotes Pseudomonas syringae Virulence in Plants by Activating a Signaling Cascade that Inhibits Salicylic Acid Accumulation. *Cell Host Microbe* 11, 587–596. https://doi.org/10.1016/j.chom.2012.04.014

Zheng, Z., Qamar, S. A., Chen, Z., & Mengiste, T. (2006). Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant J.* 48(4), 592–605. https://doi.org/10.1111/J.1365-313X.2006.02901.X