The Arabidopsis MYST Histone Acetyltransferase, HAG4, modulates root immunity responses

By

Alexia Tornesaki

Thesis

Submitted to the University of Warwick

for the degree of

Doctor of Philosophy in Life Sciences

School of Life Sciences, Gibbet Hill Campus, University of Warwick

March 2023

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to express my sincere gratitude to the University of Warwick and the Royal Society for the funding of this PhD project. I am extremely grateful to Professor Vardis Ntoukakis for the supervision of this thesis. He supported me through my ups and downs with immense patience and guided me through the research world making me a better scientist. He also encouraged and assisted me to attend international conferences which had a great impact in my career goals. The last 4.5 years being in his lab, he provided me with career and life advice.

I would like to acknowledge Prof. Sotirios Tjamos and Dr. Danai Gkizi for accepting me into their lab and passing me the knowledge of *Verticillium*. I would also like to express my gratitude to Prof. John Clarkson and Dr. Sascha Jenkins for providing me with *Fusarium* and the knowledge to use it. I would like to thank my advisory panel, Prof Eric Holub and Dr. Charlotte Allender for their support and advise in our annual meetings.

For the computational help: special thanks to Zoi Ioannidou, for the constant support and teaching me bioinformatics from scratch. I have been extremely fortunate to work with all the amazing people in C30 lab, especially Stefano, Lorenzo, Javier, Yang, Ana, Claudia, Despoina, Marianna, Richa, Najeeb and Jianan that tolerated me constantly complaining about work, shared lots of drinks, gatherings and have been a huge part of my life the last 4.5 years. Ultimately, I would like to thank my family and Rob Maple for the unconditional love and support all these years which made this work possible.

Declarations

This thesis is presented in accordance with the regulations of School of Life Sciences, University of Warwick for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree except where otherwise stated. The work in this thesis has been undertaken by myself except where otherwise stated.

Abstract

A major part of the immune responses to pathogens is reprogramming of gene expression. 10% of the plant genome is differentially expressed upon infection. Acetylation of the N-terminal tails of histones by histone acetyltransferases (Tran, Jones et al.) and deacetylation by histone deacetylases (HDACs) are linked to transcriptional changes. Histone acetyltransferases (Tran, Jones et al.) of the MYST family (MYST-HATs) carry out a significant proportion of histone acetylation and therefore play critical roles in transcription regulation.

In this study, we investigate the role of the MYST-HATs in regulating *Arabidopsis thaliana* transcriptional responses to pathogens. The *A. thaliana* genome contains two MYST-HATS*, AtHAG4* and *AtHAG5,* which are believed to work redundantly in gametophyte development and flowering time. In contrast, our results show that the two *A. thaliana* MYST-HATs interact with distinct sets of transcription factors and have organ specific functions in plant immunity. AtHAG4 regulates immunity against the root pathogens *Verticillium dahliae* and *Fusarium oxysporum* while AtHAG5 regulates immunity against the leaf pathogen *Pseudomonas syringae.* Thus, our results demonstrate how organ specific defence responses to pathogens are regulated by differential histone acetylation.

Interestingly, not all plant species have two homologues of MYST-HATs. For example, tomato has only one copy of MYST-HATs which raise the question if a single copy of MYST-HAT can perform both functions of Arabidopsis MYST family. To answer this question, a chemical approach was adopted to identify specific inhibitors of HAG4 and HAG5. Compounds with high docking scores and with desirable agrochemical properties have been found to be able to inhibit HAG5. To test these inhibitors, *HAG5* mutation developmental phenotypes (longer roots and bigger rosettes) and drought tolerance responses were explored in crop plants with only one copy of the MYST family (tomato, spinach, and lettuce) by adding the chemical compounds and confirmed that one copy (HAG4-like) of the MYST family can mirror the phenotypes of *hag5* mutants.

Abbreviations

35S	Cauliflower Mosaic Virus promoter
ABA	Abscisic Acid
A. thaliana	Arabidopsis thaliana
AT	Amino 1,2,4, Triazole
AUDPC	Area Under Disease Progress Curve
B. oleracea	Brassica oleracea
BGI	Beijing Genome Institute
bp	base pair
CBB	Coomassie Brilliant Blue
cDNA	Complementary deoxyribonucleic acid
CDPK	Calcium Dependent Protein Kinase
ChIP	Chromatin Immunoprecipitation
SEQ	Sequencing
DEG	Differentially Expressed Gene
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPI	Days Post Inoculation
ERF	Ethylene Response Factor
ET	Ethylene
ETI	Effector Triggered Immunity
ETS	Effector Triggered Susceptibility
EV	Empty Vector
EZ	Elongation Zone
FAO	Food and Agriculture Organisation
FLC	FLOWERING LOCUS C
F. oxysporum	*Fusarium oxysporum*
GA	Gibberelic Acid
GO	Gene Ontology
HAT	Histone Acetyl Transferase
HDAC	Histone Deacetylase
HIS3	Histidine
HPA	*Hyaloperonospora parasitica*
HR	Hyper Sensitive Response
IDT	Integrated DNA Technologies

IP	Immunoprecipitation
JA	Jasmonic Acid
KB	King's B
LUC	Luciferase
MAMP	Microbe-Associated Molecular Pattern
MAPK	Mitogen-Activated Protein Kinase
MS	Murashige and Skoog
MZ	Meristem Zone
NASC	Nottingham Arabidopsis Stock Centre
NBS-LRR	Nucleotide-Binding Site Leucine-Rich Repeat
NE	Nuclear Extract
OD	Optical Density
OE	Overespressor
PCR	Polymerase Chain Reaction
P. syringae	Pseudomonas syringae
PC	Principal Component
PR	Pathogenesis Related
PRR	Pathogen Recognition Receptor
PTI	Pathogen-Triggered Immunity
PTM	Post-Translational Modification
QC	Quiescent Center
qPCR	quantitative Polymerase Chain Reaction
R	Resistance
RNA	Ribonucleic acid
RNAi	RNA interference
RNAPII	RNA Polymerase II
ROS	Reactive Oxygen Species
RT-PCR	Reverse-Transcription PCR
SA	Salicylic Acid
SD	Standard Deviation
TEF	Transcription Elongator Factor
TSS	Transcription Start Site
V. dhaliae	Verticillum dhaliae
Y2H	Yeast-2-Hybrid
YFP	Yellow Fluorescent Protein

Chapter 1

Histone modifications and their role in plant immunity

Introduction

Climate change, food security and agriculture

Climate has been changing in the last three decades. By mid-21st century, temperatures are predicted to increase about 3-5 °C while precipitation patterns (rain and snow) are predicted to shift (Arnell 2004, Meehl, Arblaster et al. 2005) Climate change affects agriculture and food production in complex ways. Agriculture is highly climate-dependent activity and is one of the most vulnerable of sectors to these changes in climate (Thornton, Jones et al. 2011, Ramirez-Villegas, 2010 #19,). It is the basis for food security and economic sustainability and provides the necessary input for sustaining people's livelihoods, regardless of their economic status (FAO 2010). In developing countries, agriculture is a key driver of national and local economies, and the way households live largely depends on what they can grow and how efficiently they can do it.

The impact of a +3-5 °C warmer world, for example, in the tropics and subtropics, could be disastrous for current crop varieties of several crops that it would be unlikely to produce under extreme conditions (Challinor, Slingo et al. 2005, Byjesh, Naresh Kumar et al. 2010, Challinor, Simelton et al. 2010). As a result, some cultivated areas may become unsuitable for cropping and some tropical grassland may become increasingly arid. Importantly, temperature rise will also expand the range of many agricultural pests and increase the ability of pest populations to survive the winter and attack spring crops. Therefore, climate change will have a significant impact in three areas: in losses from plant diseases, in the efficacy of disease management strategies and in the geographical distribution of plant diseases, according to limited literature in this area S. Chakraborty et al, 2000). Climate change is predicted to exacerbate agricultural challenges by enhancing abiotic stress and by creating conditions in which novel plant diseases may emerge and become epidemic (Boyd, Freer-Smith et al. 2013).

It is difficult to predict the impact of the climate change on the behaviour of crop pathogens. Disease depends on what is known as the “disease triangle”, in which the pathogen is affected by its host and both are affected by environmental factors such as nutrient availability and climate (Fig. 1).

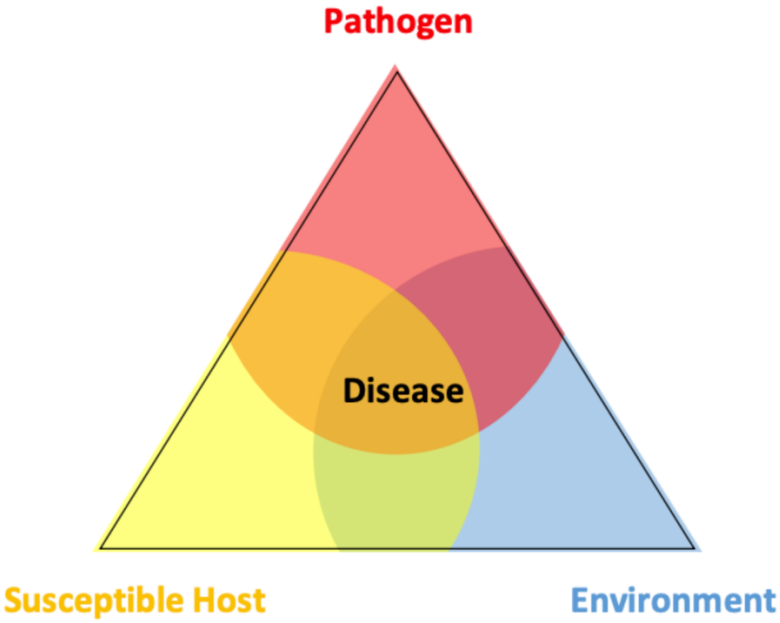


Figure 1. The "disease triangle" for plants. Disease relies on the pathogen, host plant and wider environmental factors and only develops where all three are conducive at the same time and in the same place. (Helen Fones at carbonbrief.org)

Crop losses due to plants pathogens are already a threat to food security. Economically important crops are exposed to a variety of pathogens such as viruses, bacteria, oomycetes and fungi that have different lifestyle modes and infection strategies. Therefore, the main scope of plant biologists is to achieve increased resistance against economically important pathogens, while maintaining complex agronomic traits such as yield, shape and flavour. Despite our advances in understanding the molecular mechanisms regulating plant growth and immunity, there are not enough sustainable strategies derived from this knowledge that are currently applicable.

Plant immune responses

Microbes have engaged in antagonistic associations with plants for hundreds of millions of years. Plants, in turn, have evolved sophisticated immune strategies to counteract microbial pathogens. Plant pathogens employ diverse strategies to attack plants and impair plant growth and reproduction. Unlike mammals, plants lack mobile immune cells and an adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from infection sites (Ausubel 2005).

Plants block a significant number of pathogens at point of contact through non-host resistance that relies on physical barriers. That involves waxy cuticles, rigid cell walls, and antimicrobial secondary metabolites. The microbes that successfully overcome this strategy, must then overcome the plant immune system in order to enable the progression of microbial colonization. Plants depend on each cell exerting innate immunity, with systemic signals emerging from the infected cells and the ability of plant cells to remember previous infections (Reimer-Michalski and Conrath 2016).

Plant pathogens adopt a wide range of life strategies and invasion tactics. Pathogenic bacteria propagate in intercellular spaces known as the apoplast after entering through stomata, hydathodes or via wounds. Fungi can extend hyphae through plant cells to enter plant epidermal cells. Regardless of their invasion strategy and lifestyle, all pathogens need to evade detection by the host and to suppress the plant immune responses. The plant immune system is two-layered. One uses cell-surface pattern recognition receptors (PRRs) in plants that recognize conserved pathogen- / damage- / microbe-/ herbivore-associated molecular patterns (PAMPs/DAMPs/MAMPs/HAMPs) and activate pattern-triggered immunity (Latrasse, Jégu et al.) (Zipfel and Felix 2005). PRRs are plasma membrane (PM)-associated and are usually either receptor-like kinases (RLKs) or receptor-like proteins (RLPs) that lack a protein kinase domain. Activation of PRRs leads to intracellular signalling, chromatin remodelling, transcriptional reprogramming, and biosynthesis of a complex output response (Monaghan and Zipfel 2012). Other markers for PTI include callose deposition (Hauck, Thilmony et al.), reduced uptake of vascular dyes into minor veins (Oh and Collmer), and the generation of reactive oxygen species (ROS) (Torres, Jones et al. , Chinchilla, Zipfel et al.).

The two most well studied PRRs are the plasma-membrane-localised leucine-rich-repeat receptor kinases (LRR-RK) FLAGELLIN SENSING 2 (FLS2) and ELONGATION FACTOR TU RECEPTOR (DeFraia, Zhang et al.). FLS2 and EFR recognise the bacterial PAMPs flagellin and EF-Tu, or their peptide epitopes flg22 and elf18, respectively (Nürnberger and Kemmerling 2006). To counteract the first layer of plant immunity, microbial pathogens deliver effector molecules (virulence

factors) into the apoplast and the plant cell in an effort to supress PTI and to enhance microbial fitness (Jones and Dangl 2006).

The second layer of immunity acts largely inside the cell. Plants have evolved intracellular nucleotide-binding (NB) leucine-rich repeat receptors (NLRs), often referred to as *R* genes, to detect the presence of effectors, and initiate effector-triggered immunity (ETI) (Dangl and Jones 2001). Pathogen secreted effector molecules are recognized by NB-LRR proteins and activate defence responses (Fig. 2).

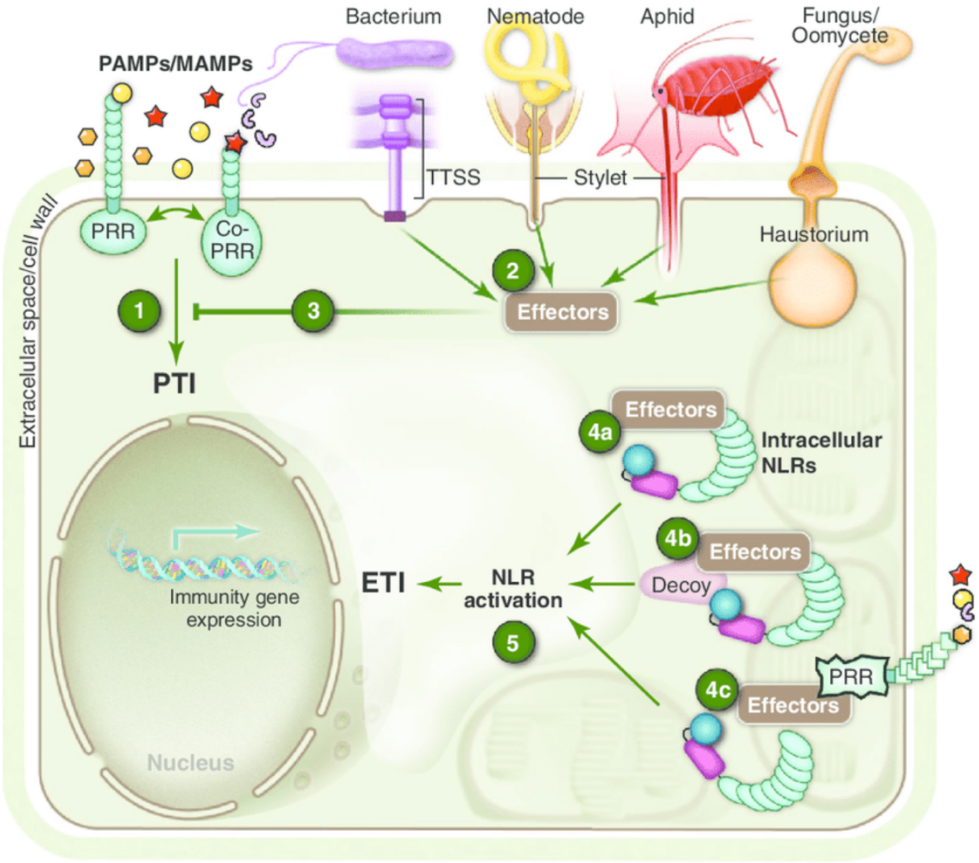


Figure 2. Schematic of the plant immune system. Pathogens of all lifestyle classes express PAMPs and MAMPs as they colonize plants. Plants perceive these via extracellular PRRs and initiate PRR-mediated immunity. Pathogens deliver virulence effectors to both the plant cell apoplast to block PAMP/MAMP perception and to the plant cell interior. These effectors are addressed to specific subcellular locations where they can suppress PTI and facilitate virulence. Intracellular NLR receptors can sense effectors first, by direct receptor ligand interaction, second, by sensing effector-mediated alteration in a decoy protein that structurally mimics an effector target and third, by sensing effector-

mediated alteration of a host virulence target, like the cytosolic domain of a PRR. (Dangl, Horvath et al. 2013).

The production of virulence effectors by pathogen leads to their recognition by plants encoding *R* genes. Recognition results in rapid activation of defence responses and consequent termination of pathogen colonisation. *R* gene–mediated resistance is usually accompanied by a rapid generation of ROS. ROS production is required for HR, a type of programmed cell death thought to limit the access of the pathogen to water and nutrients (Lamb and Dixon 1997). *R* gene–mediated resistance is also associated with activation of a salicylic acid (SA)-dependent signalling pathway that leads to expression of certain pathogenesis-related (PR) proteins thought to contribute to resistance.

The NLRs are classified into two major groups according to their N-terminal domains: TIR-NB-LRR (TNL) proteins carrying a Toll Interleukin-1 Receptor (TIR) domain, and CC-NB-LRR (CNL) proteins carrying a coiled coil (CC) domain. In general, the LRR domain mainly functions in the recognition of effectors, although exceptions exist (Cui, Tsuda et al. 2015). The Nucleotide Binding Domain (NBD) binds ADP in the resting state and ATP in the active state. The TIR and CC domains function in signalling (Jubic, Saile et al. 2019). TNLs and CNLs typically engage the ETI machinery through different regulators of plant defence (Feys, Moisan et al. 2001, Wiermer, Feys et al. 2005, Rietz, Stamm et al. 2011, Wagner, Stuttmann et al. 2013, Cui, Yan et al. 2017)

A distinct feature of NLR-triggered immunity is induction of a programmed cell death called hypersensitive response (HR) which is characterized by rapid cell death at the site of infection., among other defence responses. NLR- mediated disease resistance is effective against pathogens that can grow only on living host tissue (obligate biotrophs), or hemibiotrophic pathogens, but not against pathogens that kill host tissue during colonization (necrotrophs) (Glazebrook 2005).

Defence mechanisms against biotrophic and necrotrophic pathogens

Plant pathogens are often divided into biotrophs and necrotrophs, according to their feeding lifestyles. Biotrophs are the pathogens that have a sustained parasitic nutritional relationship with living plant tissue. Many biotrophs cause host cell death later in pathogenesis and they are referred to as hemibiotrophs. Necrotrophs kill the host and feed on the remains. In the case of biotrophic pathogens, *R* gene–mediated resistance and SA signalling result in resistance. The HR response would deprive such pathogens of a food source. However, in the case of necrotrophs, it seems that programmed cell death in the host would merely make life easier for the pathogen (Glazebrook 2005). Some pathogens that adopt to different feeding strategies and belong to different taxa will be described below as they were used in this study.

It is not surprising that pathogens with diverse lifestyles secrete different collection of virulence effectors. *P. syringae – Arabidopsis thaliana* is the most well studied pathosystem for plant-microbe interactions. *P. syringae* is a Gram-negative bacterium that colonizes the intercellular spaces in leaves and other aerial organs and causes diseases in a wide range of economical important crops. *P. syringae* releases PAMPs or MAMPs, such as flagellin, lipopolysaccharide, peptidoglycan, and elongation factor TU that elicit PTI. To supress PTI, *P. syringae* injects effector proteins into host cells via the T3SS. The entry of effectors inside plant cells can be recognized by *R* proteins and ETI is induced (Jones and Dangl , Boller and Felix).

The pangenome of *P. syringae* encodes 57 families of effectors injected via T3SS. The T3SS in *P. syringae* is encoded by *hrp* genes and is required for elicitation of the HR in nonhost or resistant host plants and for pathogenesis in susceptible plants. The model strain typically used in laboratories is *P. syringae pv. tomato* DC3000 (*Pto* DC3000), whose complete repertoire of effector genes has been deleted and experimentally reassembled (Cunnac, Chakravarthy et al. 2011). Several effectors were identified in various strains of pathovars glycinea, phaseolicola, pisi, and tomato as ‘Avr’ proteins based on the gain-of-function avirulence ETI phenotype they conferred on otherwise virulent strains in test plants with matching R genes (Keen 1990).

Pto DC3000 secrets 28 type III effectors which are classified as *Hop* (Hrp outer proteins) or *Avr* (Avirulence) proteins (Lindeberg, Cunnac et al. 2012). The effector

AvrPto is well studied for elucidating the multiple activities of effectors in defense suppression and elicitation. AvrPto inhibits the FLS2/BAK1 PRR complex important for recognition of the flagellin PAMP (Xiang, Zong et al. 2008 L. Shan et al., 2008), and when transgenically produced in Arabidopsis or translocated into plants via a T3SS heterologously expressed in a nonpathogen, AvrPto is sufficient to suppress PTI (Hauck, Thilmony et al. 2003)

Hyaloperonospora arabidopsidis (*Hpa*) is an obligate biotrophic oomycete pathogen. *Hpa* is the causal agent of the downy mildew of Arabidopsis and can cause economically important damage by killing seedlings or affecting the quality of produce intended for freezing. The symptoms of the disease include lesions on spikelet, fungal growth on leaves, and necrosis on stems and leaves. It is related to other oomycete phytopathogens that include several species of *Phytophthora*, including the causal agent of potato late blight. *Hpa* as a filamentous pathogen, form sophisticated intracellular feeding structures called haustoria in plant cells. Pathogen effectors are likely to play a role in the establishment and maintenance of haustoria in addition to their better-characterized role in suppressing plant defence. *Hpa* secretes their effectors from intercellular hyphae or haustoria and HR typically initiates after haustoria are formed (Torto, Li et al. 2003).

Oomycete effectors usually contain a secretory signal peptide and a conserved domain featuring the motif RxLR, followed by a motif with a high frequency of acidic (D/E) residues, and a C-terminal domain(s) associated with virulence function (Rehmany, Gordon et al. 2005, Lamour, Win et al. 2007, Birch, Armstrong et al. 2009). In the *Hpa-Arabidopsis* pathosystem, nine avirulent oomycete RxLR effectors have been identified, including ATR1 and ATR13 from Hpa (Allen, Samol et al. 2004, Rehmany, Gordon et al. 2005). The sequencing of the *Hpa* isolate *Emoy2* genome revealed its potential to encode at least 134 candidate effectors (HaRxLs) (Baxter, Tripathy et al. 2010). The main target of HaRxLs is PTI (Fabro, Steinbrenner et al. 2011). Additionaly, considering that *P. syringae* and *Hpa* are biotrophs, SA plays essential roles in resistance against these pathogens. SA has profound importance in the amplification of PRR- and NLR-mediated immune signaling (Zhang and Li 2019).

Necrotrophic fungi secrete toxins and enzymes that kill host cells and then take up nutrients released from the dead tissue (Horbach, Navarro-Quesada et al. 2011). Plant immune responses to necrotrophs may differ from plant immune responses to biotrophs depending on the pathogen species and the primary determinant of virulence. Interestingly, effectors of necrotrophic pathogens include phytotoxins and traditional virulence effectors. Phytotoxins can be either non-host specific toxins (HSTs) that affect a broad range of plant species or HSTs that affect only a particular plant species or more often genotypes of that species (Wolpert, Dunkle et al. 2002, Berestetskiy 2008). HSTs secreted by necrotrophic fungi activate R protein-mediated ETI to cause HR cell death.

Studies have showed that the plant resistance to necrotrophs also involves PRR perception of PAMPs. Chitin perception and signalling has been well characterized in Arabidopsis. Chitin perception depends on the lysin motif (LysM)-containing receptor-like kinases such as LysM RLK1/ CHITIN ELICITOR RECEPTOR KINASE 1 (AtLYK1/ AtCERK1) (Miya, Albert et al. 2007 2008). Fungal endopolygalacturonases (PGs) act as PAMPs that are recognized by the Arabidopsis LRRRLP RBPG1 (RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1) (Zhang, Kars et al. 2014). Other effectors from *Sclerotinia sclerotiorum* and *Rhizoctonia solani* have been identified and studied (Zhang, Fraiture et al. 2013, Zheng, Lin et al. 2013).

Fusarium oxysporum is a root-infecting pathogen that infects several plant species, including cotton, tomato, banana and Arabidopsis. *F. oxysporum* acts as a hemi-biotrophic pathogen in Arabidopsis because it begins its infection cycle as a biotroph but later changes to a necrotroph. During the biotrophic phase, *F. oxysporum* establishes infection via the roots and travels towards the vasculature. Once *F. oxysporum* enters the vascular system, it travels upwards in the plant and accumulation of fungal mycelia and defence related compounds in the xylem cause vascular wilting. As the infection progresses, *F. oxysporum* changes to a necrotrophic pathogen, causing foliar necrosis, lesion development and ultimately, plant death. *F. oxysporum* is thought to secrete phototoxic compounds which cause root cell collapse and veinal chlorosis in the leaves and is reported to reprogramme the host to induce senescence and facilitate disease during the necrotrophic phase of

infection (Schenk, Kazan et al. 2005, Jones and Dangl 2006, Dong, Xiong et al. 2014).

The PTI and its suppression by effector proteins is well studied by foliar pathogens. Roots, though, are exposed to both beneficial and pathogenic microbes, which has likely formed the immune system in roots (De Coninck, Timmermans et al. 2015, Hacquard, Spaepen et al. 2017). 14 candidate effectors from a tomato-infecting strain (*Fo f.sp. lycopersici*; Fol) were identified in the xylem sap of infected tomato plants named Secreted in xylem (Six) proteins (Schmidt, Houterman et al. 2013). Three of them are recognised as avirulence factors: Six3 (Avr2) is recognised by an NB-LRR, whereas Six1 (Avr3) and Six4 (Avr1) are recognised by an RLK and an RLP, respectively (Houterman, Ma et al. 2009, Catanzariti, Lim et al. 2015, Catanzariti, Do et al. 2017).

Another root-infecting fungus used in this study, *Verticillium dahliae*, is also hemibiotrophic pathogen as *F. oxysporum. V. dahliae* has a wide host range (more than 160 plant species) and worldwide distribution (Klosterman, Atallah et al. , Zhou, Feng et al.). *V. dahliae* is a soil-borne pathogenic fungus that colonises the root systems of host plans, invading the xylem and spreading towards the aerial parts of the plant through spores known as conidia (Barbara and Clewes 2003). The diseased plants display several symptoms, such as leaf flaccidity, chlorosis and necrosis, stunting and vascular discoloration in stems (Pegg and Young 1981).

V. dahliae has been well studied in cotton and tomato where *R* genes and its effectors have been identified. In cotton, Vd424Y is an important effector protein targeting the host nucleus to regulate and activate effector-triggered immunity in plants (Liu, Wang et al.). Effector proteins including VdNEP, PevD1, VdCP1 were secreted by *V. dahliae* into host, all of those can induce cotton cell death and trigger immunity responses (Wang, Cai et al. , Cui, Yan et al.). In tomato, *Ve1*, an *R* gene, provides resistance in tomato against *V. dahliae* (Fradin, Zhang et al. 2009).

Epigenetic regulation of plant growth and immunity

Epigenetic modification is a mechanism that regulates gene expression caused by changes in chromatin structure without changing the DNA sequence. Epigenetic

regulation of gene expression involves various components, including enzymes that catalyse or remove DNA methylation and histone post-translational modification (PTM), sRNAs, and chromatin remodellers. These components together influence the chromatin structure and, in turn, the accessibility of genetic information. Chromatin modifications regulate its structure, and they recruit remodelling enzymes that utilize the energy derived from the hydrolysis of ATP to reposition nucleosomes (Bannister and Kouzarides 2011).

Heterochromatin and euchromatin are two major categories of chromatin higher order structure. In brief, heterochromatin has condensed chromatin structure where the nucleosomes are packed tightly and is inactive for transcription. Euchromatin has loose chromatin structure and is active for transcription (Fig. 3).

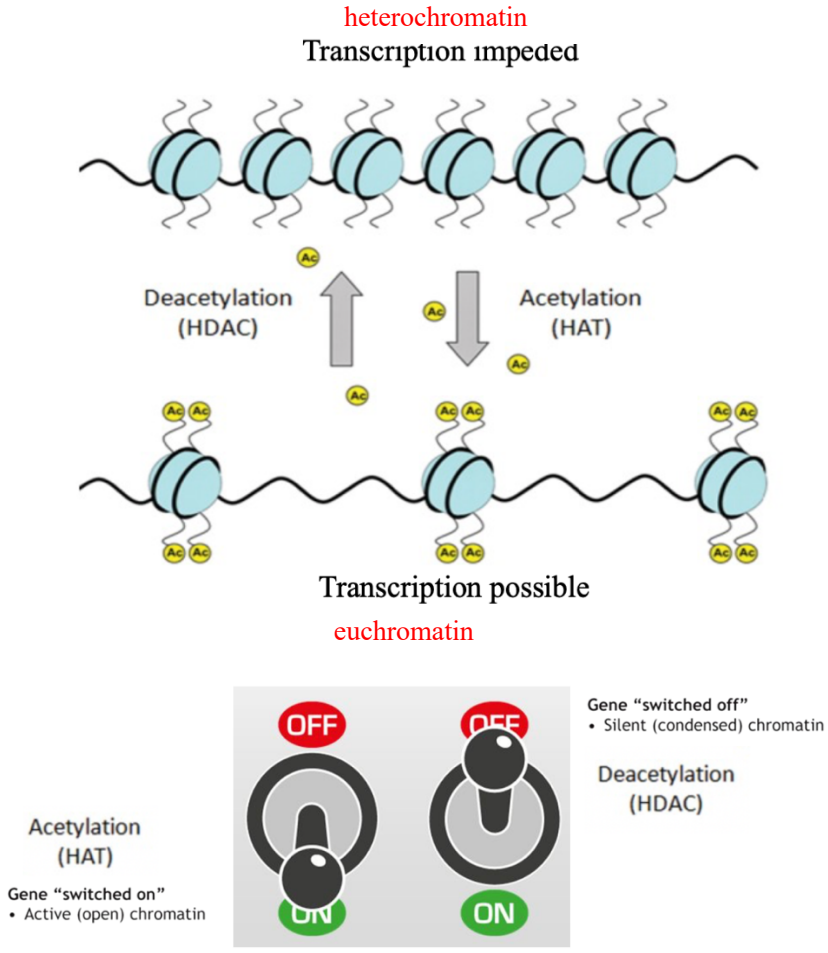


Figure 3. Heterochromatin and euchromatin formation regulate transcription. HATs acetylate the lysine of histone tails which results in open chromatin and the transcription is possible. When HDACs remove the acetylation mark, chromatin is condensed, and the transcription is inactive.

In *A. thaliana*, genome-wide DNA methylation is characterized by heavy methylation in heterochromatin, which is enriched with transposable elements (transposons) and other repetitive DNA sequences (Zhang, Yazaki et al. 2006, Henderson and Jacobsen 2007).

Modifications at histone residues mainly include methylation, acetylation, ubiquitination, and phosphorylation. In eukaryotes, chromatin consists of nucleosomes formed by DNA and histone H2A, H2B, H3, and H4 (Luger, Mäder et al. 1997). Acetylation of the N-terminal tails lysines is regulated by the opposing action of two families of enzymes, histone acetyltransferases (Tran, Jones et al.) and histone deacetylases (HDACs). The HATs utilize acetyl CoA as cofactor and catalyse the transfer of an acetyl group to the ε-amino group of lysine side chains. HATs and HDACs neutralize the lysine's positive charge and by doing so, they weaken the interactions between histones and DNA.

A growing number of studies have revealed that epigenetic regulation is crucial for plant immunity responses and phenotypic variations during plant-microbe interaction. Part of the immune responses to pathogens is reprogramming of gene expression. Transcriptional studies have shown that 10% of the plant genome is differentially expressed (Tao, Xie et al. 2003, Tsuda, Sato et al. 2009). The activity of HATs and HDACs is linked to transcriptional changes (Kurdistani and Grunstein 2003). HATs and HDACs function within multimolecular enzymatic complexes, recruited to their specific target promoters through a physical interaction with a sequence-specific transcription factor (Legube and Trouche 2003). HATs and HDACs have been previously implicated in the regulation of plant growth and immunity, being involved in a variety of biological processes including development, response to abiotic stress (Earley, Shook et al. 2007), flowering time (Xiao, Zhang et al. 2013). responses to light (Bertrand, Benhamed et al. 2005) and Jasmonic acid (JA)/ Ethylene (ET) and Salicylic acid (SA) signalling pathways (Servet, Conde e Silva et al. 2010).

The *Arabidopsis* genome contains 12 HATs which are subdivided into four families: GNAT (HAG1, HAG2, HAG3), p300/CBP (HAC1, HAC2, HAC4, HAC5,

HAC12), MYST (HAG4 and HAG5) and TAFII250 (HAF1 and HAF2) (Pandey, Muller et al. 2002) (Fig. 4).

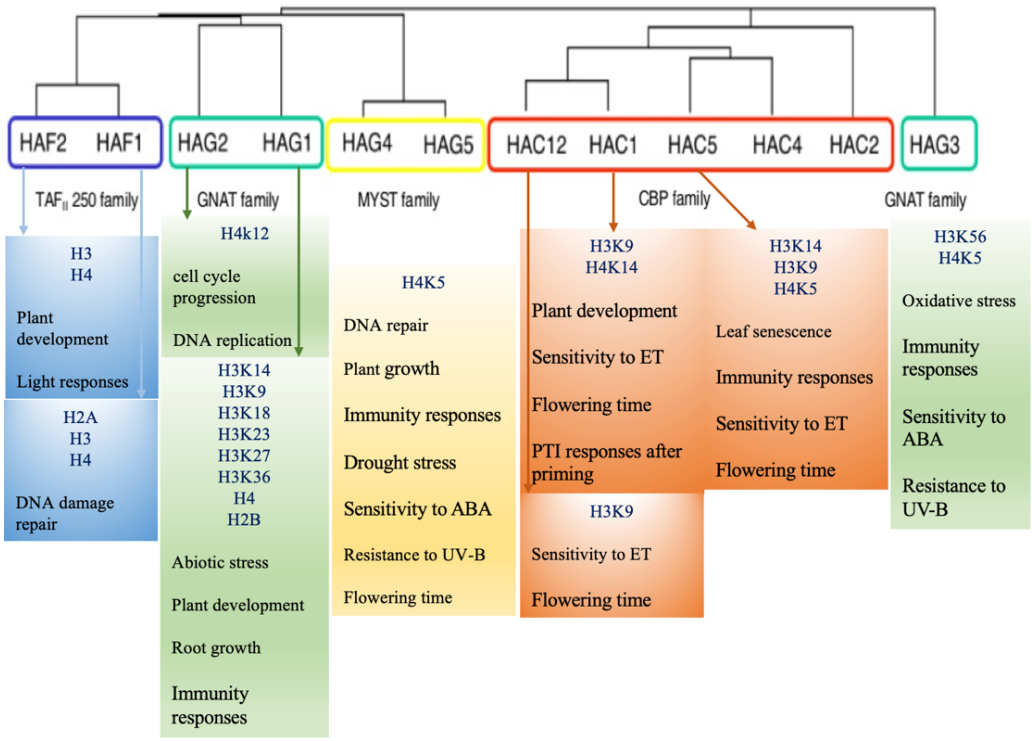


Figure 4. Arabidopsis HATs and their role in plants.

The GNAT family comprises ELP3/HAG2 (a transcriptional Elongator complex protein) and HAT1/HAG3 (Pandey, Muller et al.). GCN5, also known as HAG1, is the most studied of all *Arabidopsis* HAT. HAG1 is a member of the Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex, a transcriptional coactivator which is involved in various physiological programs through the regulation of histone modifications (Huisinga and Pugh 2004). HAG1 has been reported to be essential for heat stress responses (Hu, Song et al. 2015). HAG1 is also involved in root growth and root meristem maintenance (Kornet and Scheres 2009, Chen, Li et al. 2016). During embryogenesis, HAG1 suppresses TOPLESS (TPL) embryonic activity indicating that the polarity during embryogenesis is mediated by TPL and HAG1 genetic interaction (Long, Ohno et al. 2006). HAG1 is involved in defence responses against *Pto* DC3000 by repressing SA accumulation and SA-mediated immunity (Kim, Piquerez et al. 2020). The *hag1* mutation leads to a pleiotropic developmental phenotype as dwarfism (Vlachonasios, Thomashow et al. 2003).

HAG1 was first reported to target histone H3 lysine 14 (H3K14) however, later studies confirmed that it could also acetylate additional histone lysine residues, such as H3K9, H3K18, H3K23, H3K27, H3K36, and other histones such as H4 and H2B (Kuo, Brownell et al. 1996, Grant, Duggan et al. 1997, Morris, Rao et al. 2007). HAG2 acetylates H4K12 (Earley, Lawrence et al.) while *hag3* mutants are deficient in H3K56Ac and H4K5Ac (Xu, Huang et al.). HAG3 is a subunit of the Elongator complex, and mutations in different subunits of this complex result in hypersensitivity to ABA, resistance to oxidative stress, severely aberrant auxin phenotypes, disease susceptibility, altered cell cycle progression and is involved in the transcription of UV-B-regulated genes (Nelissen, Fleury et al. , Chen, Zhang et al. , Zhou, Hua et al. , DeFraia, Zhang et al. , Xu, Huang et al. , Fina and Casati 2015). HAG2 is regulated by E2F transcription factors that induce the transcription of genes required for cell cycle progression and DNA replication (Ramirez-Parra, Fründt et al. , Vandepoele, Vlieghe et al.).

In Arabidopsis, there are two HAF proteins, HAF1 or TAF1 and HAF2 or TAF1b (Pandey, Muller et al. 2002, Lago, Clerici et al. 2004). HAF2 is involved in plant development and mediates light responses by acetylating H3 and H4 (Bertrand, Benhamed et al. 2005, Benhamed, Bertrand et al. 2006). Interestingly, *haf2* mutant plants are viable but chlorotic while no major growth defects were observed in the *haf1* mutant line used in that same study (Bertrand, Benhamed et al. 2005). HAF1 was suggested to participate in DNA damage repair (Waterworth, Drury et al. 2015). HAF1 interacts with acetylated histones H4, H3 and H2A in vivo (Martinez 2002, Kanno, Kanno et al. 2004).

Regarding HATs from the p300/CBP family, HAC2 did not show any HAT activity in *in vitro* assays, whereas all the other 4 HACs showed HAT activity (Bordoli, Netsch et al. 2001). HAC1 specifically acetylates histone H4K14 while HAC1, HAC5, and HAC12 can acetylate H3K9 amongst other Lys residues, showing broad specificity in their activities (Earley, Shook et al. 2007). HAC1, HAC5, and HAC12 promote flowering regulating transcription of FLOWERING LOCUS C (FLC), a major floral repressor (Deng, Liu et al. 2007, Han, Song et al. 2007). *hac1* mutations lead to pleiotropic developmental defects, such as short primary roots and reduced fertility (Boycheva, Vassileva et al. 2014). Combination of *hac* mutants, *hac1*, *hac5*,

and *hac12*, unveils pleiotropic phenotypes associated with hypersensitivity to ethylene in both dark and light conditions (Li, Xu et al. 2014). HAC1 together with HAC5 participate in the MEDIATOR complex, a conserved multi-subunit complex that facilitates transcriptional initiation (Guo, Wei et al. 2021). Additionally, it has been reported that environmental history shapes PTI responses to bacteria in a HAC1-dependent manner and HAC1 is involved in translation control in yeast (Singh, Yekondi et al. 2014, Xia 2019). HAC4 lacks acetylase activity, and it has been suggested to be an expressed pseudogene (Chinchilla, Zipfel et al. 2007).

The MYST family of HATs (HAG4 and HAG5) have been shown to regulate diverse cellular processes such as DNA repair, stem cell homeostasis and cell-cycle regulation through acetylation of histone 4 (H4) lysine residues (Yuan, Rossetto et al. 2012). HAG4 together with its close homologue HAG5 are assumed to work redundantly to regulate gametophyte development and flowering time (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013). In work leading to this PhD project (Kancy, 2017), HAM2 (HAG5) was shown to act as a transcriptional repressor to moderate defence gene expression. *Hag5* mutant lines were more resistant to *Pseudomonas syringae* pv. *tomato* DC3000 and have enhanced leaf surface area, root length and normal gametophyte development. According to previous work in our group, HAG4 (HAM1) is involved in defence responses to the fungal pathogen *Verticillium dahliae. hag4* mutants are more resistant to infection with the root pathogen *V.dahliae* (Gkizi, González Gil et al. 2021).

Both HAG4 (AT5G64610) and HAG5 (AT5G09740) have been shown to specifically acetylate the same target (H4K5) *in vitro* (Earley, Shook et al. 2007) and share 84% amino acid sequence identity. The majority of sequence divergence is located at the N-terminus of the protein, close to and within the chromodomain suggesting that may interact with different transcriptional factors (TFs).

In plants, the involvement of HDACs in the immune response has extensively studied. In Arabidopsis, the 18 HDAC genes are divided into three different types – RPD3-like, HD-tuin and sirtuin – with two or more members in each type.

In Arabidopsis, HDA19 acts as a positive regulator of plant immunity, as overexpression of HDA19 enhances plant resistance to the necrotrophic pathogen *Alternaria brassicicola* (Zhou, Zhang et al.). However, later study revealed that HDA19 negatively regulated plant resistance to *P. syringae* by repressing SA-defence markers *PR1* and *PR2* (Choi, Song et al. 2012). Thus, the role of HDA19 in the regulation of plant immunity remains controversial. Other HDACs have been related to have a positive or negative role in plant immunity. MAP kinase MPK3 interacts with the histone deacetylase HD2B which has been shown to deacetylate the lysine 9 residue of histone 3 (H3K9) and occurs resistance against *Pto* DC300 (Latrasse, Jégu et al. 2017).

HDA6 is involved in the JA-pathway by downregulating JA responsive genes, including *PDF1.2*, *VSP2*, *JIN1* and *ERF1*. *HDA6* expression is induced by JA and ACC (1-aminocyclopropane-1-carboxylic acid, an ethylene precursor) and is important for the repression of defence genes involved in the SA pathway (Wu, Zhang et al. 2008). HDA6 is likely to function redundantly with HDA19 in the repression of development genes such as the *Flowering Locus C* (*FLC*) and embryo-specific genes. *hda6* mutants also display increased resistance to *Pto* DC3000 (Wu, Zhang et al. 2008). HDA6 controls stress responses as cold, drought and salt stress tolerance (Chen, Luo et al. 2010, Bannister and Kouzarides 2011, To, Kim et al. 2011). HDA6 is also required for ABA-mediated responses to drought or salt (Luo, Wang et al. 2012, Perrella, Lopez-Vernaza et al. 2013).

Aims of the thesis: A major part of plant immune responses to pathogens is reprogramming of gene expression. Histone acetyltransferases (Tran, Jones et al.) of the MYST family (MYST-HATs) carry out a significant proportion of histone acetylation and therefore play critical roles in transcription regulation. In this study, I aim to investigate the role of the MYST-HATs in regulating transcriptional responses to pathogens. To this end, this study began with a reverse genetics approach using Arabidopsis HATs T-DNA insertion lines investigating the immunity phenotypes resulting from down-regulating or knocking out particular HATs. *Arabidopsis thaliana* contains 2 MYST-HATs, AtHAG4 and AtHAG5, which are believed to work redundantly in gametophyte development and flowering time. I investigated the interactions of HATs with transcription factors to uncover distinct functions in the responses to biotic and abiotic stress. In this study, I

demonstrate that HAG4 mutation confers root-specific immunity responses while HAG5 is involved in plant growth and leaf-specific responses.

In Chapter 2, immunity phenotypes of *hag4* mutation show enhanced resistance against the root pathogens (*Verticilium dahliae* and *F. oxysporum*) whilst infections with the leaf pathogens *P. syringae*, *B. cinerea* and *H. arabidopsidis* show immune responses indistinguishable to Col-0 control plants. In order to investigate the molecular mechanism of these defence responses, the interaction of HAG4 and the Transcriptional Factor (TF), VFP4 was confirmed by Yeast Two Hybrid.

In Chapter 3, HAG4 associates with VFP4 and I hypothesise that it binds to/acetylate promoters of defence related genes to mediate the plant pathogen response. To test this hypothesis, I carried out histone ChIP seq for the histone mark H4K5ac and RNA seq to identify these target genes in leaves and roots.

In Chapter 4, plant growth and drought responses of *hag4* and *hag5* mutations in *Arabidopsis* were characterised. Interestingly, not all plant species have two homologues of MYST-HATs. Tomato (*Solanum lycopersicum*) has a single copy, but Brassica (*Brassica oleracea*) has two copies of MYST-HATs, which raise the question if a single copy of MYST-HAT can perform both functions of MYST family. To address this question, we have generated mutants of Brassica plants and performed an *in silico* screen to identify chemical inhibitors of tomato and Brassica MYST-HATs. The inhibitors were tested in tomato, spinach and lettuce.

Chapter 2

HAG4 interacts with VFP4 and modulates root-specific immunity

Alexia Tornesaki[1], Sotirios Tjamos[2], Vardis Ntoukakis[1]

[1]School of Life Sciences, University of Warwick

[2]Plant Pathology Lab, Agricultural University of Athens

Abstract

A major part of the immune responses to pathogens is reprogramming of gene expression. Histone acetyltransferases of the MYST family (MYST-HATs) carry out a significant proportion of histone acetylation and therefore play a critical role in transcription regulation. Here, the role of the MYST-HATs in regulating *Arabidopsis thaliana* defence responses to pathogens was investigated. The *A. thaliana* genome contains two MYST-HATS, *AtHAG4* and *AtHAG5*, which are suggested to work redundantly in gametophyte development and flowering time. In contrast, my results show that the two *A. thaliana* MYST-HATs interact with distinct sets of transcription factors and have organ specific functions in plant immunity. This study shows that *AtHAG4* regulates immunity against the root pathogens *Verticillium dahliae* and *Fusarium oxysporum* while *AtHAG5* regulates immunity against the leaf pathogen *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis*.

Introduction

Defence responses in leaves vs roots

Plants are constantly exposed to a wide range of microorganisms that affect crop production and food security. Plant roots, by growing in soil, interact with a plethora of microbes. In the last decades, substantial progress has been made to understand the molecular and cellular interactions between pathogens and plants. The defence responses of the upper part of the plants are well-studied whereas root–microbe interactions are less understood.

Plant roots represent an important opportunistic entryway for many soil pathogens. Plant pathogens can penetrate roots through natural apertures at the junction between the main and lateral roots as epidermal cracks (Perrine-Walker, Prayitno et al. 2007) and via young growing tissues that lack secondary cell walls (Okubara and Paulitz 2005). Thus, it is crucial for the roots to detect soil pathogens and initiate defence responses to limit pathogen infection.

Plants recognize conserved epitopes of micro molecules called microbe-associated molecular patterns (MAMPs), such as bacterial flagellin (Georg Felix* 1999), bacterial elongation factor Tu (Kunze, Zipfel et al. 2004), chitin, a major component of the fungal cell wall, lipopolysaccharides, and peptidoglycans (PGNs) (Felix, Regenass et al. 1993, Newman, Daniels et al. 1995, Meyer, Pühler et al. 2001, Gust, Biswas et al. 2007, Miya, Albert et al. 2007). MAMPs get recognised by pattern recognition receptors (PRRs) and plant innate immune responses gets activated. In leaves, MAMP recognition triggers an oxidative burst, ET and nitric oxide production, as well as a complex cascade of mitogen-activated protein kinases that leads to the activation of transcription factors (TFs) and defence response genes. MAMP recognition in leaves also triggers the deposition of callose (Aist and Bushnell 1991).

Many pathogens have evolved strategies to counteract the plant immune response, including, in the case of bacteria, the injection of virulence effectors directly into the plant cell using the type III secretion system (Xiang, Zong et al. 2008). In leaves, type III effectors play a key role in the virulence of pathogenic bacteria such as *P.*

syringae by suppressing the plant basal immune responses activated by MAMP recognition. So far, there is no evidence that pathogens suppress immunity in roots using the type III secretion system, although *Rhizobium* species use the type III secretion system for the delivery of nodulation proteins (Nops) to root cells (Kambara, Ardissone et al. 2009).

Three MAMPs, flg22, chitin, and PGN, trigger strong tissue-specific responses in *Arabidopsis* roots. In particular, the flg22 and PGN responses are limited to the elongation zone of the root tip, whereas the response to chitin is localized in the mature zone of the roots (Millet, Danna et al. 2010). Millet et al. also show that *P. syringae* suppress MAMP responses in the roots, but unlike in leaves, suppression is not dependent on the type III secretion system but rather on the production of COR, a low molecular weight phytotoxin coronatine (COR) that functions in leaves as a mimic of JA-Ile and triggers a mutually antagonistic interaction between the SA and JA signalling pathways and suppresses SA signalling, a key component in basal resistance against *P. syringae*. In the same study, they illustrate that MAMP-triggered callose deposition in roots is independent of SA signalling.

Treatment with flg22 was shown to enhance plant pathogen resistance in roots by inducing reactive oxygen species (ROS) accumulation, callose deposition and the production of antimicrobial compounds during PTI response (Millet, Danna et al. 2010, Tran, Jones et al. 2016). The PTI responses of *Arabidopsis fls2* mutant to flg22 were completely suspended, confirming the existence of MAMPs/PAMPs receptors in *Arabidopsis* roots (Gómez-Gómez and Boller 2000, Zipfel, Robatzek et al. 2004, Robatzek, Chinchilla et al. 2006, Millet, Danna et al. 2010). The intensity of these responses observed was more pronounced within the inner root tissues believed to be due to enriched density in the endodermis and stele cells and higher expression of Flg22 receptor (*FLS2*) gene (Beck, Wyrsch et al. 2014, Wyrsch, Domínguez-Ferreras et al. 2015).

Resistance proteins, including proteins encoded by classical resistance (*R*) genes and the pathogenesis-related (PR) proteins, are expressed in some root–pathogen interactions. The tomato *I-2* gene, conferring resistance to the wilt pathogen *Fusarium oxysporum f.sp. lycopersici*, encodes a member of the nucleotide-binding

site/leucine-rich repeats (NBS-LRR) family of resistance proteins (Alonso, Stepanova et al. 2003). *I-2* is expressed in lateral root primordia of young roots, and vascular regions of mature roots and foliar organs. The arrest of hyphal growth at the vascular interface is postulated to be the basis for *I-2*-mediated resistance against *F. oxysporum*. *RFO1* (RESISTANCE TO FUSARIUM OXYSPORUM 1), another resistance protein of *F. oxysporum,* confers resistance to a broad spectrum of Fusarium races and is identical to the previously named Arabidopsis gene *WAKL22* (*WALL-ASSOCIATED KINASE-LIKE KINASE 22*), which encodes a receptor-like kinase that does not contain an extracellular leucine-rich repeat domain (Diener and Ausubel 2005). Additionally, Fusarium wilt disease was enhanced in mutants with defects in salicylic acid (SA) accumulation (Diener and Ausubel 2005). In tomato (*Solanum lycopersicum*), the *Ve1* resistant gene, which encode extracellular leucine-rich repeat receptor-like proteins (eLRR-RLPs) (Wang, Ellendorff et al. 2008), was confirmed to provide *V. dahliae* resistance (Fradin, Zhang et al. 2009).

In this chapter, the role of the two MYST Histone Acetyltransferases, HAG4 and HAG5, in plant immunity was investigated using plant pathogens that belong to different taxa. First, the knock-out mutants of the HATs were inoculated with *P. syringae pv tomato DC3000*, *Botrytis cinerea* and *Hyaloperonospora arabidopsidis* as they all affect the leaves (pathogens described in chapter 1). I discovered that HAG5 is involved in plant immunity against biotrophic aerial pathogens as its mutation confers resistance. Then, the soil borne pathogens, *Verticullium dahliae* and *Fusarium oxysporum* (pathogens described in chapter 1) were used and the pathogenesis assays confirmed that HAG4 modulates defence responses to necrotrophic root pathogens. A potential explanation of these differential functions of these HATs could be the fact that they interact with distinct transcription factors (TFs). Yeast two-hybrid assays supported this hypothesis since in this chapter I show that HAG4 and HAG5 interact with different TFs, VFP4 and ARIA, respectively. Immunity phenotypes of VFP4 were explored as it is known to be involved in bacterial resistance.

Materials and methods

Plant lines

Arabidopsis thaliana

Arabidopsis thaliana ecotype Columbia (Col-0) is used as wild type. The seeds were sown on *Arabidopsis* mix (F2 compost, Intercept, grit) and stratified in darkness in cold room at 4°C for 2 days. Seedlings were germinated in a controlled environment chamber at 21°C, 60% humidity in short-day 10/14 hour (day/night). When 14 days old, seedlings were transferred to individual pots and maintained in the same growth chamber. For seeds collection, the adult plants were transferred to a long-day 16h light, 60% humidity chamber 4 weeks after germination.

The *Arabidopsis* T-DNA insertion lines were purchased from the Nottingham *Arabidopsis* Stock Centre (NASC, http://arabidopsis.org.uk/) and can be found in the Table 1.

Table 1. T-DNA insertion lines used in this study.

Arabidopsis line	AGI number	T-DNA insertion line
hag4-1	At5g64610	SALK_103726
hag4-2	At5g64610	SALK_027726
hag5-1	At5g09740	SALK_012086
hag5-2	At5g09740	SALK_106046
vfp4-1	At5g28040	SALK_129879
TF250	At4g03250	SALK_044445
MBD2	At5g35330	GK-650A05.01

Plant DNA extraction for genotyping PCR

DNA from *Arabidopsis* plants was extracted from leaf tissue with 100 µL 5% Chelex (Biorad). The samples were ground manually with pipette tips at room temperature. Samples were further mixed with a vortex machine and then heated at 95 °C for 10 minutes. The samples were spun down at maximum speed in centrifuge for 5 minutes. 30 µL of supernatant were collected and stored at -20°C for further testing.

Two PCR reactions were used per sample to amplify DNA fragments from T-DNA insertion lines. LP – RP primers, flanking the T-DNA insertion, in wild type plants produced a PCR fragment of 1000 ± 200 base pairs. LBb1.3 – RP primers in homozygous lines produced a band of an expected size of 500 ± 200 base pairs. Heterozygous lines produced bands in both PCR reactions (conditions mentioned in table 4).

Table 2. Conditions of genotyping PCR.

Step	Temperature (°C)	Time	Cycles
Initial denaturation	98	30sec	1
Denaturation	98	10sec	
Annealing	55	20sec	30
Elongation	72	30sec	
Final elongation	72	4min	1
Cooling	4	Hold	

The primer sequences were obtained through the software provided by the Salk institute, (http://signal.salk.edu/tdnaprimers.2.html). Primers were synthesised by IDT Integrated DNA Technologies, Inc. (Table 5).

Table 3. Primers used for genotyping SALK lines.

Name	Sequence	Description
LBb1.3	ATTTTGCCGATTTCGGAAC	Genotyping SALK lines
N655396-LP	ATGGTGTGCGAATCTATGACC	Genotyping *hag4-2* mutants
N655396-RP	ACGGAGAGGAAAGCTCAAGAC	Genotyping *hag4-2* mutants
N681550-LP	AGAATCAGCCACTTCAACACG	Genotyping hag4-1 mutants
N681550-RP	GATTCTGAATTCGTGAGAGCG	Genotyping hag4-1 mutants
vfp4-1-LP	TCCACTGCGTTTAAACCAGTC	Genotyping vfp4-1 mutants
vfp4-1-RP	AGTCATACGGTGCCATTTCTG	Genotyping vfp4-1 mutants

Gel electrophoresis

PCR products were separated by size through electrophoresis on 1% agarose (Sigma), 1 x TAE buffer (40mM Tris base, 1mM EDTA, 20mM acetic acid, pH 8.0) gels, with 1x GelRed (Biotium). Gel was run between 20 to 40 min at 100 V, 500 A and imaged using a UV transilluminator (Gel Doc 1000, Bio-Rad).

Plant pathogens used for the pathogenesis assays

Leaf pathogens

The fungus ***Botrytis cinerea var pepper*** (Denby, Kumar et al. 2004) was stored as a 5 x 105 spores/mL solution in 20% glycerol at -80 °C. Spores from glycerol stock was cultured on sterilised apricot halves at 25°C. Spores were collected 14 days post inoculation by scraping fungal material into 3 mL sterile H_2O in a Class II sterile cabinet. The spore solution was filtered through glass wool. Spores were counted using haemocytometer and the concentration was adjusted 400000 spores/mL. The solution was then diluted 1:1 to a final concentration of 200000 spores/mL with sterile grape juice.

The fungus was cultured in potato dextrose agar (PDA) plates. To maintain full virulence, spores were transferred to sterile apricots and grown there for two weeks prior to infection assays. Leaves of 4 weeks old plants were detached and placed on 0.8% Phytoagar trays. A minimum of 20 leaves per plant genotype were used and each genotype was distributed across several trays to reduce variability. The amount of 5 µL of 200000 spores/mL solution was placed in the middle of each leaf. 5 leaves per genotype were placed in each tray, 1 mock leaf (5 µl of solution of 1:1 sterile water and grape juice was placed) and 4 leaves treated with spores' solution. Trays were covered with lids and sealed with micropore tape to maintain approximately 90% humidity. Leaves were photographed every 24 hours post inoculation for 4 days and necrosis sizes were measured and analysed using ImageJ.

For the pathogenicity experiments of ***Hyaloperonospora arabidopsidis*** (*Hpa*), 5 week-old *A. thaliana* plants were sprayed to imminent run-off with a spore suspension of *H. arabidopsidis* isolate (5×104 spores ml−1). Subsequently, the plants were placed at 16°C with a photoperiod of 9-h day, 15-h night and 100% relative humidity for 7 days. At 7 days post inoculation (dpi), leaves of the different treatments were harvested and weighed. The number of the liberated *H. arabidopsidis* spores were counted under a light microscope and the infection severity was expressed as number of spores mg−1 fresh weight (FW). The experiment was replicated 3 times (21 plants per treatment).

P. syringae pv tomato DC3000 was grown in King's Broth medium (20g/L proteose peptone, 8.6mM K2HPO4, 163 mM glycerol, pH adjusted to 7.0 with HCl before autoclaving; liquid, or solid, with the addition of 1.5% agar (King et al., 1954) adding the required antibiotics for selection (Rifampicin 100 μg/mL, Gentamycin 100 μg/mL as mentioned by Cuppels, 1986. For single colony isolation, stocks were streaked onto solid plates and incubated over-night prior to inoculation in liquid medium. *P. syringae* was grown at 28°C. Liquid cultures were grown in an incubator with shaking at 220 rpm.

For infiltration, a single bacterial colony of *P. syringae* was inoculated in 15 mL of liquid culture (KB) with the corresponding antibiotics. The cultures were harvested by 3 X centrifugation (3000g for 10 min), washed and re-suspended in sterile 10mM $MgCl_2$. Bacterial suspension of OD600 0.001 (equivalent to 2x105 colony-forming units/mL) was prepared in 10mM $MgCl_2$. The optical density was measured with a Biochrom WPA CO8000 cell density meter (Biochrom Ltd., UK) at 600 nm (OD600). Three leaves of 5 week-old *Arabidopsis* plants (leaves 7, 8 and 9) were infiltrated using a needleless 1 mL syringe. Bacterial population sizes in infected leaves were quantified three days post-inoculation.

For sample collection, 0.5 cm^2 leaf discs from leaves number 6 and 7, were collected with a disc borer. Two leaf discs were collected per plant, resulting in a total of 12 leaf disks per genotype and condition. 2 leaf discs were added to 2mL tubes containing three metallic beads (3 mm diameter) and 200μL 10mM MgCl2. To grind the plant tissue, two pulses of 28hz for 30 seconds were applied with a mixer mill (Tissue Lyser MM300, Retsch). The plant and bacterial suspension was then diluted up to 1mL with 800μL 10mM MgCl2 and serial dilutions were plated on KB plates containing the selective antibiotics. After incubating the plates over-night at 28°C, bacterial colonies were counted at each dilution and the log of colony forming units (CFU) was calculated for each line. Each experiment, as described, was repeated 3 times on different days, or otherwise as stated.

Root pathogens

Verticillium dahliae isolated from *Raphanus sativus L.* (provided by E. Ligoxygakis, National Agricultural Research Institute, Crete, Greece), with known

pathogenicity against *A. thaliana* plants (Tjamos, Flemetakis et al. 2005), was used in the experiments. The fungal strain was cryopreserved by freezing a conidial suspension in 25% aqueous glycerol at 80ºC.

The fungus was transferred to potato dextrose agar (Merck) at 24ºC for 5 days. For the inoculation, 107 conidia per mL were prepared from a culture grown for 5 days at 24ºC and suspended in sucrose sodium nitrate liquid medium. Three weeks old plants were inoculated with *V. dahliae* by root drenching with 10mL of a suspension of 1 Å~ 10^7 conidia/ml of sterile distilled water (Tjamos, Flemetakis et al. 2005). Control plants were inoculated with 10mL of sterile distilled water. Disease severity was calculated from the number of leaves that showed wilting as a percentage of the total number of leaves of each plant and was periodically recorded for 25 days inoculation. Disease ratings were plotted over time to generate disease progression curves. AUDPC was calculated by the trapezoidal integration method (Campbell and Madden). Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment, which is referred to as the relative AUDPC. For this experiment, 30 plants were used per treatment and plant genotype.

Fusarium oxysporum was provided from Professor John Clarkson, Wellesbourne campus, University of Warwick and was treated as described for *V. dahliae*. For the identification, the genomic DNA of *F. oxysporum* was extracted as described in Jenkins S. et al., 2021.

Bacterial strains used for cloning of HAG4, HAG5, VFP4 and ARIA

Bacteria stocks were in 20%glycerol at -80°C. *Escherichia coli* and *Agrobacterium tumefaciens* were grown on Luria Broth (LB) medium (1% tryptone, 0.5% yeast extract, 1% NaCl liquid, or solid, with the addition of 1.5% agar) (Bertani 1951), adding the required selective antibiotics as shown at Table 2 once the medium was autoclaved and cooled below 65 °C. For single colony isolation, stocks were streaked on solid plates and incubated over-night prior to inoculation in liquid medium. *E. coli* strains were grown at 37°C. *A. tumefaciens* strains were grown at 28°C. Liquid cultures were grown in an incubator with shaking at 220 rpm.

Table 4. Bacterial strains and antibiotic resistance used in this study.

Species	Strain	Selection	Citation
E. coli	TOP10	-	Thermo Fisher, C404010
A. tumefaciens	GV3101	Rifampicin 100 µg/mL, Gentamycin 100 µg/mL	(Holsters, Silva et al. 1980)

Gateway cloning

For *in planta* expression of HAG4, the destination gateway vectors pEG202 (N-ter FLAG, 35S promoter) was used (Earley, Shook et al. 2007). HAG4 cDNA was cloned from cDNA library in pDONR-Zeocin. A BP reaction was performed using pDONR-Zeo as the target vector and BP clonase II enzyme mix (Invitrogen). The DNA insert, entry plasmid and BR clonase™ were incubated at 25 °C overnight. Plasmid DNA with selection in Zeocin was extracted with a mini-prep kit NucleoSpin® Plasmid (Macherey-Nagel), following manufacturer's specifications. DNA quality and concentration were determined with a NanoDrop ND- 1000 (Thermo Fisher Scientific). Plasmid sequence was verified by Sanger sequencing (Genewiz), following supplier's instructions (400 ng of DNA and 1 µM plasmid in 10 µL were sent to supplier).

After extracting plasmid DNA from positive clones, LR reactions were performed using 150ng of pEarleyGate vectors 202 (for cDNA), 150ng of pDONR-Zeo-HAG4 vector, supplemented with LR clonase II enzyme mix (Invitrogen). The DNA insert, entry plasmid and LR clonase™ were incubated at 25 °C overnight. Transformation and Sanger sequencing were performed as before with the difference that selection was made in Kanamycin (100µg/mL). pEG202-HAG4 was used to transform *A. tumefaciens* GV3101.

For *in plant* expression of VFP4, we selected the expression vector pEG104 (Nter YFP, 35S promoter). VFP4 in pDEST-DB were extracted from the transcription factor library in *E. coli*. A BP reaction was performed using pDONR-Zeo® as the target vector and BP clonase II enzyme mix (Invitrogen). The DNA insert, entry plasmid and BP clonase™ were incubated at 25 °C overnight. The resulting reaction

was transformed into *E. coli* TOP10 competent cells as described below and selected with Zeocin. Plasmid extraction and sequencing was performed as for HAG4.

A LR reaction was performed using LR clonase II enzyme mix (Invitrogen), 150ng of pEarleyGate104 and 150ng of pDONR/zeo- Interactor. The DNA insert, entry plasmid and LR clonase™ were incubated at 25 °C overnight. Transformation and Sanger sequencing were performed as before with the difference that selection was made in Kanamycin (100µg/mL). Plasmid was transformed into *A. tumefaciens* GV3101 as described below..

***E.coli* transformation**

20 µl of TOP10 *E. coli* cells (Invitrogen) were placed on ice with 2 µl of plasmid for 30 minutes. Then, the cells were placed at 42°C in a water bath for 2 minutes (heat shock). After the heat shock, the cells were transferred immediately on ice for 5 minutes. In a hood, 200 µl of liquid LB were added and the cells were placed in an incubator for 1h at 37ºC shaking at 215 rpm. LBA plates were prepared with the correct antibiotic for selection. 100 µl of each transformation was plated on the plates. The plates were sealed with Parafilm and placed upside down in a 37ºC incubator for 24h.

***A. tumefaciens* transformation**

50 µl of *A. tumefaciens* GV3101 electrocompetent cells were thawed, mixed with 1µg of plasmid. The cells were transferred to an electroporation cuvette and electroporated using the Agro program (1800V, capacity 25 µF and 200 Ω resistance) in a Micro Pulser Electroporator (Bio-Rad). Cells were re-suspended in 1ml of liquid LB, transferred to an 2 ml Eppendorf tube and incubated for 3h at 28ºC shaking at 215 rpm. In a hood, the cells were placed on LBA plates with the correct antibiotic selection. The plates were sealed with Parafilm and placed upside down in a 28ºC incubator for 24 h.

***A. tumefaciens* for *N. benthamiana* transient expression of the constructs**

Cells form overnight cultures were harvested by centrifugation at 3500 rpm and washed twice in Agro-infiltration buffer (10 mM MES pH 5.6, 10 mM MgCl2).

OD600 was adjusted to 0.5 for single or double construct infiltration. *N. benthamiana* leaves were infiltrated with the bacterial cultures using a 2mL syringe. Harvesting of leaf tissue occurred and frozen in liquid nitrogen in 3 days post-infiltration.

Protein immunoprecipitation with nuclear enrichment

Plant tissue was flash-frozen in liquid nitrogen and pulverised into a fine powder. Approximately 5 g of infiltrated *N. benthamiana* leaves were used per sample Grounded samples were resuspended in cold Honda Buffer containing 1.25% Ficoll (GE healthcare), 2.5% Dextran T40 (Sigma), 440 mM sucrose, 0.5% TritonX-100, 10 nM $MgCl_2$, 5 mM DTT, 1 mM PMSF and 1% Plant protease inhibitors. Tissue was incubated for 15 min at 4°C while shaking and then filtered through two layers of Miracloth (Merk Millipore). The extract was filtered again and stored on ice. Samples were centrifuged at 2000 g for 17 min at 4°C. Supernatant was removed. Pellet was resuspended in 1ml of Honda buffer. Samples were then centrifuged at 1500 g for 15 min at 4°C. Nuclei washing was repeated 3 times. Pellet was resuspended in 500 µl of Nuclei Lysis Buffer containing 1 mM PMSF, 10 mM EDTA, 50 mM Tris-HCl and 1% Plant protease inhibitors, and sonicated (High power, 6 x 30sec ON, 60 sec OFF) to break the chromatin. Samples were centrifuged in a bench-top centrifuge at 16,100g for 15 min at 4ºC to break the nuclei. A small aliquot of the supernatant (10% of the sample) was kept as an input before the IP. The remaining sample was diluted 10 X by adding 4.5 of IP Dilution Buffer (1.1% TritonX-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH8, 167 mM NaCl and 1% Plant protease inhibitors).

For the Immunoprecipitation, GFP-Trap agarose beads (ChromoTek) were added, and the samples were incubating for 2hours at 4ºC in a rotating wheel. Beads were washed thoroughly with beads-washing buffer containing 150 mM NaCl, 20 mM Tris HCl, 2 mM EDTA, 1 mM PMSF and 1% Plant protease inhibitors to remove non-specific binding.

Input and IP samples (beads/resin) were resuspended in 1x SDS-PAGE, incubated at 90ºC for 10 minutes. Samples were loaded on 10% SDS-PAGE gel (120 V, 40

min), transferred onto a PVDF membrane (30 V over night at 4ºC) and blotted with the corresponding antibodies (Table 3).

Table 5. Antibodies used for ChIP.

Target	Antigen	Source	Concentration
Primary antibody			
α-GFP-HRP	GFP/YFP tag	Santa Cruz Biotechnology	1:10000
α-FLAG (Mouse)	FLAG tag	Merck	1:2000
Secondary antibody			
α-mouse-HRP	Mouse IgG	Merck	1:10000
Affinity matrix			
GFP trap	GFP/YFP tag	ChromoTek	

Yeast two-Hybrid assay

Yeast transformation

The constructs of *HAG4, VFP4, HAG5* and *ARIA* were transformed in yeast in order to perform yeast two-hybrid assays and confirm the interactions. This experiment required S. cerevisiae strain Y8930 transformed with *HAG4* (sub-cloned in yeast 2-hybrid vector pDEST-DB). Transformation was performed by first suspending pelleted yeast culture (3mL grown overnight at 28°C with shaking) in 0.1M LiAc in a 1.5 mL Eppendorf tube, spinning at 2500 rpm for 5 min at room temperature, then resuspending again in 20 mL 0.1 M LiAc. 30mL 1 M LiAc, 40 mL 2 mg/ml ssDNA, 10mL sterile ddH_2O and ~200ng of miniprepped *HAG4* were added. The tube contents were inverted following addition of 200 mL of PEG 4000 (fresh and filter sterilised) then incubated for 1 hour at 42°C in a water bath.

Yeast two-hybrid screening of interactors

The Y2H system, used to test protein–protein interactions was pDEST-DB/pDEST-AD (Dreze, Monachello et al. 2010), where prey constructs *HAG4* and *HAG5* and empty vector control pDEST-DB were transformed into strain Y8930, with the transcription factor library having previously been cloned into strain Y8800 as bait constructs. Prey constructs were +Leucine, while bait constructs were +Tryptophan, with interactions permitting the synthesis of histidine, such that successfully mated

yeast contained both a bait and prey construct might grow on -LeuTrp but would only grow on -LeuTrpHis if the proteins encoded by bait and prey constructs interacted in yeast. The competitive inhibitor of the HIS3 enzyme 3- amino 1,2,4 triazole (3AT) was also used as a supplement to test the strength of interaction.

All yeast cultures were grown in Synthetic Complete (SC) media (6.8g Yeast Nitrogen Base, 20 g ammonium sulphate, 40g glucose, 5.2 amino acid drop out mix, ddH_2O to 1 L, adjusted to pH 5.9 with 1 M NaOH) lacking the appropriate amino acids to select for constructs of interest at 28°C for 2 days with shaking. Matted strains containing the bait and prey interaction pair to be tested were grown in -Leu-Trp then spotted onto selective solid media plates.

Y2H-inducible reporter gene expression levels can vary from weak to very strong, although these levels may not reflect the actual affinity of protein–protein interactions as they take place in their native environment. To help determine which candidate clones likely represent genuine biophysical interactors, five controls are added systematically to the master plates of Y2H experiments. Negative (C1) and positive controls (C2-C5) were included as described by Dreze et al. (2010).

Table 6. Y2H controls

	Plasmid pairs	Protein	Interaction strength
Control 1	pDEST-AD pDEST-DB	No insert No insert	None, background
Control 2	pDEST-AD-E2F1 pDEST-DB-*CYH2*-pRB	Human E2F1 aa 342–437 Human pRB aa 302–928	Weak (control for CHX control plates)
Control 3	pDEST-AD-Jun pDEST-DB-Fos	Mouse Jun aa 250–325 Rat Fos aa 132–211	Moderately strong
Control 4	pDEST-AD pDEST-DB-Gal4	No insert Yeast Gal4 aa 1–881	Very strong
Control 5	pDEST-AD-dE2F1 pDEST-DB-dDP	*Drosophila* E2F aa 225–433 *Drosophila* DP aa 1–377	Strong

RNA extraction, cDNA synthesis and qPCR

Plant tissue for RNA extraction was frozen in liquid nitrogen after harvesting and kept at -80°C. Plant tissue was ground in liquid nitrogen with a drill borer fitting a 1.5 or 2 ml micro-centrifuge tube. 1 ml of TRIzol® Reagent (Thermo Fisher Scientific) was added to the powder, mixed well by vortexing, and incubated at room temperature for 5 minutes. 200 µl of chloroform was added to the sample, mixed gently, and incubated for another 5 minutes at room temperature. Samples were spun down at 12,500 g for 20 minutes at 4°C. The aqueous supernatant was carefully transferred to RNase-free tubes avoiding carrying over contamination from the aqueous/organic interface. RNA was precipitated adding an equal volume of isopropanol, mixing gently and incubating for 3 h at -20°C. Samples were spun down at 16,800 g for 20 minutes at 4 °C. The supernatant was removed, and the RNA pellet was rinsed twice with 1 ml of 70% ethanol in DEPC water. Dried RNA pellets were resuspended in 50 µl nuclease-free water by incubating at 65°C for 5 minutes.

RNeasy® Mini kit (Quiagen) was also used for RNA extraction. *Arabidopsis* seedlings were stored in RNAse free tubes (3 seedlings per sample) and flash-frozen in liquid nitrogen. Samples were ground using a drill borer fitting a 2 mL micro-centrifuge tube. RNA extraction was performed following manufacturer instructions.

For Complementary DNA (cDNA) synthesis 2 µg of RNA were reverse transcribed with the SuperScriptTM II Reverse Transcriptase (18064, Thermo Fisher Scientific), following manufacturer's specifications and using a primer for polyA (TTTTTTTTTTTTTTTTTTTVN) tails. Final cDNA was diluted with 40 µL nuclease free water for a final volume of 60µL. cDNA was stored at -20°C.

Quantitative PCR (qPCR) was performed with SYBR®green JumpStartTM Taq ReadyMixTM (S4438, Sigma), following manufacturer's recommendations. The mix conditions are detailed in Table 6, and PCR conditions can be found in Table 7. The list of primers used for qPCR is displayed in Table 8 and 9. Three technical replicates were used for each sample. The thermocyclers used were the 384-well plate CFX384 Touch TM Real-Time PCR Detection System (Bio- Rad Laboratories), and a 96-well plate Mx3005P qPCR System (Agilent Technologies).

Table 7. Components used for qPCR with SYBR® Green JumpStart™ polymerase.

	Volume per triplicate	
Component	**66ml (96-well)**	**30ml (384-well)**
2 x JumpStart Taq ReadyMix	33 µl	15 µl
10 µM Forward Primer	2.96 µl	1.35 µl
10 µM Reverse Primer	2.96 µl	1.35 µl
Template DNA	5 µl	2.3 µl
Water	22.66 µl	10 µl

Table 8. Thermal cycling conditions used in qPCR with SYBR® Green JumpStart™ polymerase.

Step	Temperature (°C)	Time	Cycles
Initial denaturation	94	2 min	1
Denaturation	94	15 sec	
Annealing, elongation & fluorescence reading	*60/62 °C	60 sec	40
Dissociation curve	40 – 98 °C	10 seconds / 0.5 °C	1

Table 9. Primers used for used for testing expression levels of HAG4.

Code	Name	Sequence	Description
VN370	qHAG4-F	CCAGAATACAATGACTGCGTG	Forward qPCR primer for HAG4 (published by Xiao et al. 2013. Journal Plant Physiol)
VN371	qHAG4-R	TCTTCTTGCCATCCACCTCA	Reverse qPCR primer for HAG4 (published by Xiao et al. 2013. Journal Plant Physiol)
VN707	aTUB-F	TACACCAACCTCAACCGCCT	Forward qPCR primer for α-tubulin
VN708	aTUB-R	TGGGGCATAGGAGGAAAGCA	Reverse qPCR primer for α-tubulin

Table 10. Primers used for genes expression.

Gene	name	Gene ID	Sequence
FLC	FLC-qRT-F	At5g10140	TAACCTGGTCAAGATCCTTGAT
	FLC-qRT-R	At5g10140	CAAGTTCAAGTAGCTCATAGTGTGA
ATL31	ATL31-F	At5g27420	TGACCCGTATGCTTACAGCG
	ATL31_R	At5g27420	ACACTCCAACGCTCCTTTAC
PG5	PG5-F	AB256876	CGAGGGTAAGAGATGGTGGGATGG
	PG5-R	AB256876	GCCGCCGGTGAAGGTGATGT
PR1	PR1_F	At2g14610	TCACAACCAGGCACGAGGAG
	PR1_R	At2g14610	CACCGCTACCCCAGGCTAAG
PR2	PR2_F	At3g57260	GCTCTCCGTGGCTCTGACATC
	PR2_R	At3g57260	TACCGGAATCTGACACCATCTCTG
CHI/*PR3*	PR3_F	At3g12500	TTATCACCGCTGCAAAGTCCT
	PR3_R	At3g12500	TGGCGCTCGGTTCACAGTA
PDF1.2	PDF1.2 _F	At5g44420	CTGTTACGTCCCATGTTAAATCTACC
	PDF1.2 _R	At5g44420	CAACGGGAAAATAAACATTAAAACAG

For the qPCR analysis, data was extracted for CT values (theoretical cycle to overcome a threshold) accepting automatically calculated thresholds. Data was analysed with the ΔΔCT method (Livak and Schmittgen 2001). As controls, several genes with highly consistent expression levels at the studied conditions were used as a reference for the total messenger RNA concentration. a-TUBULIN (a-TUB) was used as housekeeping gene.

Results

HAG4 does not modulate leaf pathogen responses

Screening of *Arabidopsis* HATs for immunity responses against the leaf, hemibiotrophic, bacterial pathogen, *Pto DC3000*

Histone acetyltransferases (Tran, Jones et al.) are involved in various biological processes through transcriptional regulation of numerous genes. As mentioned in Chapter 1, the *Arabidopsis* genome encodes 12 HATs classified in different subfamilies (Pandey, MuÈller et al. 2002). In order to examine which HATs regulate plant immunity responses, previous members of the Ntoukakis group investigated the response of *Arabidopsis* HAT mutant lines to the hemibiotrophic bacterial pathogen *Pseudomonas syringae pv. tomato (Tran, Jones et al.) DC3000*. Nine homozygous T-DNA insertion lines from the 12 *Arabidopsis* HATs were obtained from the Nottingham *Arabidopsis* Stock Centre (NASC) (Alonso, Stepanova et al. 2003) and tested for their immunity phenotypes.

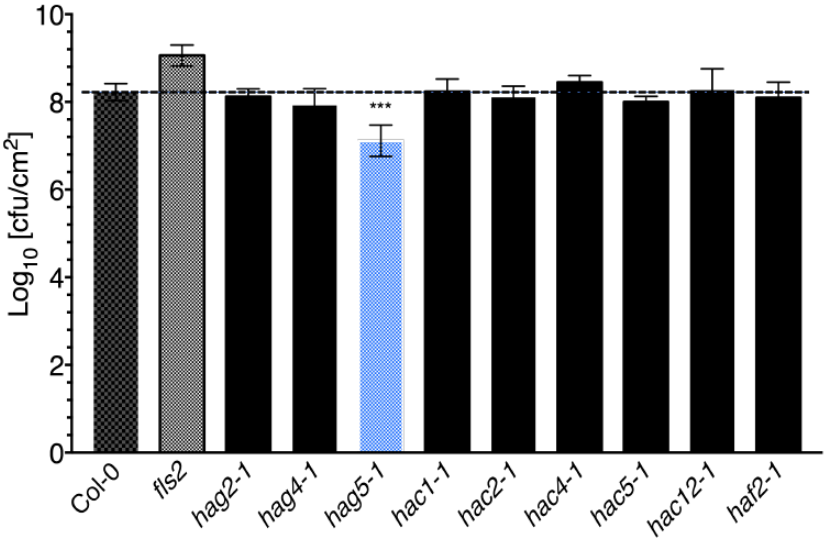


Figure 1. Screening of histone acetyltransferases for immunity phenotypes. *hag4-1* mutants perform as Col-0 when infected with *Pto DC3000* whereas *hag5-2* mutants are more tolerant to *Pto DC3000*. *fls2* is a susceptible control. 5-week-old plants were sprayed inoculated with *Pseudomonas syringae pv. tomato DC3000* (OD600 = 0.1). Samples were collected 3 days post-inoculation. Statistical significance versus Col-0 was determined by two-tailed t-test, n = 6, *** $P \leq 0.001$. Error bars indicate standard error. Genotyping for homozygosity was performed by Ntiana Mamafidou (an Erasmus student) and the bacterial growth assay was performed by previous members of Ntoukakis group, Dr Sophie Piquerez and Dr. Stephanie Kancy.

The *fls2* mutant was included as a positive control, since it has been previously shown that the lack of the FLS2 receptor results in increased susceptibility (Zipfel, Robatzek et al. 2004). This experiment highlighted that *hag5-1* is more tolerant to the bacterial pathogen, with a decreased bacterial growth after 3 dpi. However, HAG4 which is assumed to work redundantly with HAG5 in processes as flowering and gametophyte development (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013), seems not to be involved in the responses against the leaf pathogen *Pto DC3000*, a pathogen that *hag5-1* mutants are resistant to. These results caught our attention to further investigate whether HAG4 have separate functions than HAG5 in plant immunity responses.

In order to examine whether HAG4 contributes to defence responses, a T-DNA insertion mutant line from the SALK institute (NASC, http://arabidopsis.org.uk/) was selected for further characterisation.

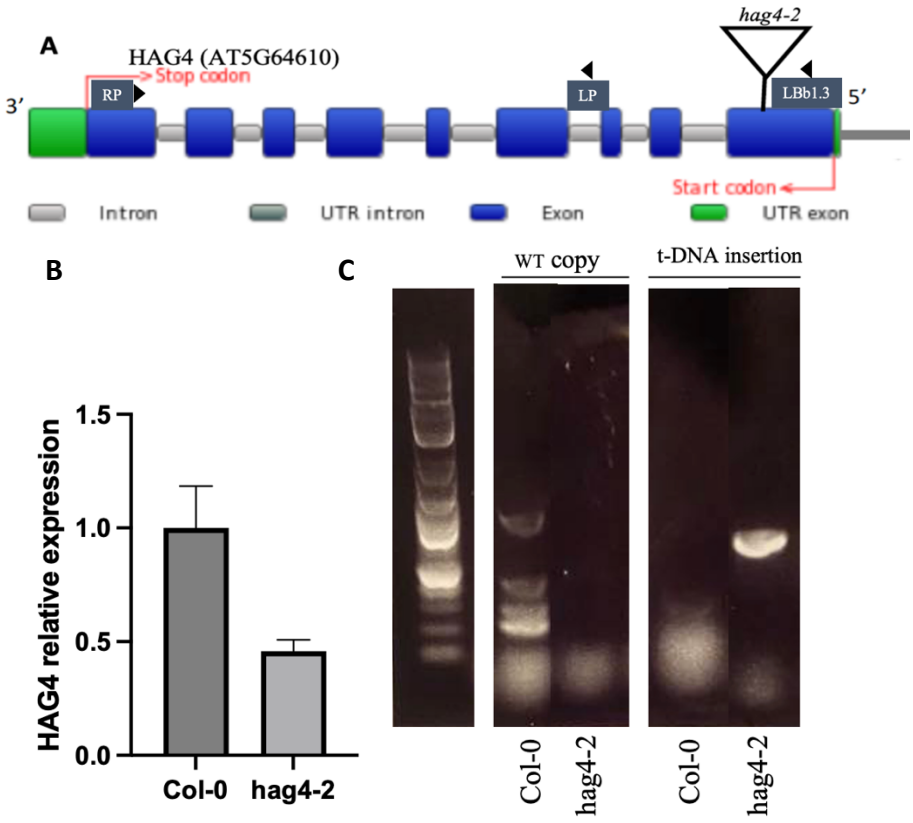


Figure 2. Schematic representation of *hag4-2* mutants. **A.** Diagram of *hag4-2* (SALK_027726), it is a loss-of-function mutant with a T-DNA insertion in the first exon. Triangles represent primers used for

section C. **B.** Expression of HAG4 in *Col-0* and *hag4-2* seedlings in basal conditions (14 days old seedlings grown in MS plates). Gene expression was normalised against the housekeeping gene αTubulin. **C.** PCR electrophoresis showing amplicons of one region of HAG4 cDNA in *Col-0* and *hag4-2* mutant. cDNA was reverse transcribed from RNA extracted form 14 days old seedlings (n = 3 seedlings per genotype).

The mutant line named as *hag4-2* (SALK_027726) has an insertion in the first exon (Fig. 2.A). As confirmed by qPCR assays, the expression of HAG4 is downregulated in the *hag4-2* mutant, which displays 42% of the HAG4 expression level found in Col-0 (Fig. 2.B). Unlike Figure 1, where the *HAG4* mutant lines used were the *hag4-1*, the *hag4-2* mutants were used in the following experiments as they express less *HAG4* and the phenotypes are stronger.

The mutant line named as *hag5-2* (SALK_106046C) was used in infection assays in order to compare the phenotypes of these two HATs. This SALK line has an insertion within the gene body and was used and genotyped by Dr. Anna Gil Gonzalez, a previous PhD student in Ntoukakis group. The *hag5-2* mutant is a knock-out, since HAG5 mRNA levels were not detected by qPCR and was used for the following assays.

Immunity responses against the leaf, necrotrophic, fungal pathogen, *B. cinerea*

Several pathogens that belong to different taxa and have distinct feeding strategies were assayed. *Botrytis cinerea*, a necrotrophic fungal pathogen which attacks foliage, stems, flowers, and fruits, infects more than 200 plant species, some of which are of high economic importance (e.g., grapes, strawberries, solanaceous vegetable) (Williamson, Duncan et al. 1995, Elad 1997, Guimaraes, Chetelat et al. 2004). In this study, *B. cinerea* was used as it is a leaf pathogen and it has different feeding strategies than *Pto DC3000*. *Col-0, hag4-2* and *hag5-2* mutant plants were inoculated. Both *HAG4* and *HAG5* are not involved in defence responses against *B. cinerea* (Fig. 3).

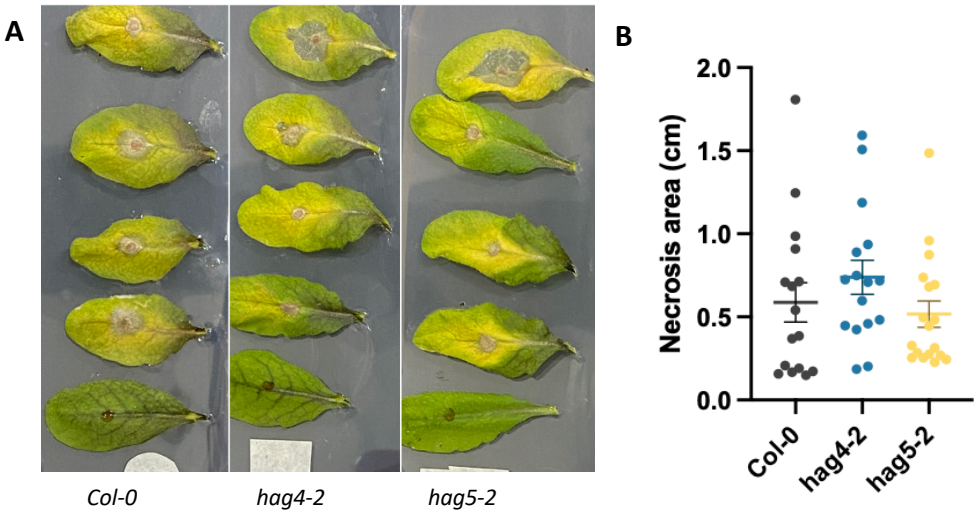


Figure. 3. *HAG4* and *HAG5* are not involved in defence responses against *B. cinerea.* Leaves of five-week-old plants of *Col-0, hag4-2* and *hag5-2* were inoculated with *B. cinerea*. **A.** Detached leaf assay symptoms 3 days post inoculation. The bottom row represents the control leaves. **B**. The necrotic areas of the leaves were measured using ImageJ. The experiment was repeated 3 times. Error bars represent the standard error of the mean, and the differences observed were not significant on a two-sided T-test (n = 20, p value >0.05)

Immunity responses against the leaf, biotrofic oomycete, *Hpa*

Hyaloperonospora arabidopsidis (*Hpa*) is another pathogen which affects foliage and causes downy mildew infection of *Arabidopsis*. *Hpa* is an obligate biotrophic oomycete. *Col-0, hag4-2* and *hag5-2* mutant plants were again infected. *HAG4* is not involved in defence responses against *Hpa* while *hag5* mutants have enhanced resistance against *Hpa* (Fig. 4).

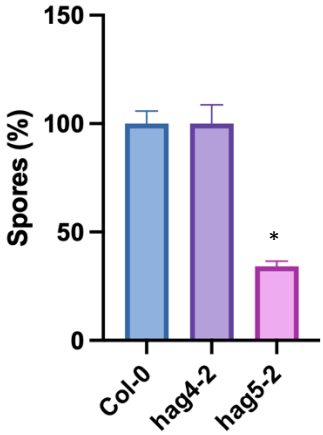


Figure 4. *HAG4* is not involved in responses against *Hpa*. Col-0*, hag4-2* and *hag5-2* mutant plants were inoculated with *Hpa*. *hag5-2* mutants are more resistant to *Hpa* since less spores were produced. *hag4-2* mutants performed as Col-0. The experiment was repeated 3 times. Error bars represent the

standard error of the mean. The differences observed were significant on a two-sided T-test (n = 20, p value <0.05 and represented with an *). This experiment was conducted by the Tjamos group at the Agricultural University of Athens.

HAG4 is a negative regulator of root pathogen responses

Defence responses against the root pathogen *Verticillium Dahliae*

Following the characterising the immunity performance of *hag4* mutants with the leaf pathogens *Pto DC3000, B. cinerea ver pepper* and *Hpa*, the next question was whether HAG4 had a role in defence to pathogens that affect different tissue as roots.

The fungal pathogen *Verticillium dahliae*, with known pathogenicity against *A. thaliana* plants, was selected for pathogenesis assays (Tjamos, Flemetakis et al. 2005).Col-0*, hag5* and *hag4* plants were inoculated with *V. dahliae* and disease severity was recorded every second day. Disease severity was calculated as a percentage of the number of leaves that showed wilting in the inoculated plants for the whole duration of the experiment (23 days), which is referred to as the relative AUDPC (area under disease progress curve). The experiment was repeated 3 times with 30 plants per treatment and plant genotype.

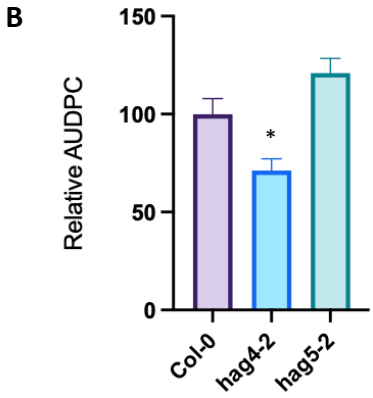


Figure 5. *hag4-2* mutants are resistant to the root pathogen *V. dahliae*. **A.** Symptoms of plants at the end of the experiment. **B.** Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment, which is referred to as the relative AUDPC. AUDPC was calculated by the trapezoidal integration method (Campbell and Madden). For this experiment, 30 plants were used per treatment and plant genotype. Statistical significance between genotypes was calculated with a two-sided T-test, *P < 0.05.

As seen in figure 5, *hag4* mutants are less sensitive to *V. dahliae* compared to Col-0 with an AUDPC value at 71%. *hag5* mutants have no significant increase in the relative AUDPC column (AUDPC value is 120%), which demonstrates that the wilted area produced by the fungus in the infected leaves was very similar to those in Col-0 plants. These pathogenesis assays show that *HAG4* is involved in root immunity against *V. dahliae* whereas *HAG5* seems to have a role in plant immunity responses in the upper plant of the plant. These results depict that *HAG4* and *HAG5* have distinct roles which argue with what was previously believed about these two MYST HATs working redundantly. According to Wolfe data (http://wolfe.gen.tcd.ie/), *HAG4* and *HAG5* occurred from a duplication event that took place during polyploidization on its *Brassicaceae* ancestor. This redundancy over time might have led to specialisation of these enzymes.

Defence responses against the root pathogen *Fusarium oxysporum*

Another root pathogen used to investigate the hypothesis of *HAG4* being involved in defence responses against root pathogens was *Fusarium oxysporum*. *F. oxysporum* is a root-infecting pathogen that causes wilt disease on several plant species including *A. thaliana*. *F. oxysporum* is a hemibiotrophic fungus as it begins its infection cycle as a biotroph and later changes to a necrotroph.

Col-0 and *hag4* plants were inoculated with the pathogen and disease severity was recorded every second day as previously described for *V. dahliae*. The experiment was repeated twice with 30 plants per treatment and plant genotype. Foliar symptoms were recorded for the next 21 days after inoculation. Disease severity in *hag4-2* plants was reduced by 25% compared to Col-0 plants (Fig. 6.B). Consequently, the relative AUDPC analysis revealed that *hag4-2* plants exhibited statistically less fusarium wilt symptoms compared to Col-0 plants (Fig. 6.B). Therefore, the pathogenicity tests revealed that *HAG4* has a key role in plant defence against *F. oxysporum.*

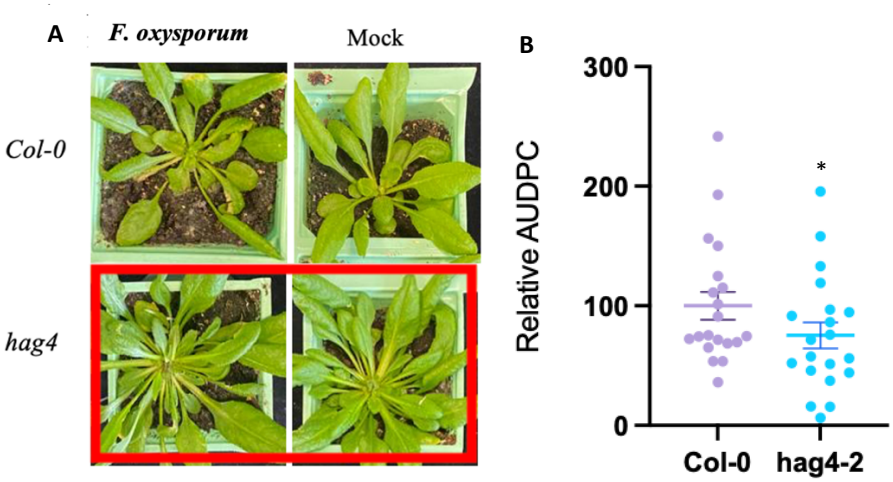


Figure 6. *hag4-2* mutants are resistant to the root pathogen *F. oxysporum*. **A.** Symptoms of plants at the end of the experiment (21 days post inoculation). **B.** Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment, which is referred to as the relative AUDPC. AUDPC was calculated by the trapezoidal integration method (Campbell and Madden). For this experiment, 30 plants were used per treatment and plant genotype. Statistical significance between genotypes was calculated with a two-sided T-test, *P < 0.05.

These results highlight the role of *HAG4* in tissue-specific immunity responses. In order to better understand the mechanism of *HAG4* in root defence responses and identify the separate functions of the MYST HATs in plant growth and immunity, the protein-protein interactions of HAG4 and HAG5 with transcriptional factors will be investigated in the following section.

Protein-protein interaction

A Yeast 2-Hybrid screening was performed in order to identify the interactors of HAG4 and HAG5. A library of ~1900 *Arabidopsis* transcription factors generated by (Dreze, Monachello et al. 2010 2014) was used for this experiment. This high-throughput experiment is a yeast-based screen for the identification of protein-protein interactions. To avoid false positives through autoactivation, we used increasing concentrations of 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product.

The Yeast 2-hybrid assay showed that HAG4 interacts with three transcriptional factors (TFs). These interactions were uncovered in liquid culture of yeast where the density was measured and then confirmed on solid media.

HAG4 interact with MBD2, a TF expressed during plant growth and development

The Yeast two-hybrid screening identified three potential interactors of HAG4. The first one, MBD2 (At5g35330), is a methyl-CpG-binding domain protein (Fig. 7). It has sequence similarity to human MBD proteins, functions in DNA binding and is expressed during growth and developmental stages of plants. It has been previously shown that MBD2 is a part of a protein complex containing HDA6, several Harbinger transposon-derived proteins (HHP1, SANT1, SANT2, SANT3, and SANT4), and other MBD domain containing proteins (MBD1 and MBD4). This protein complex regulates the expression of flowering repressors FLC, MAF4, and MAF5 through H3 deacetylation (Xiao, Zhang et al. 2013). In this study, close homologues of MBD2 were used in Y2H screening in order to examine the specificity of this interaction (Fig. 8).

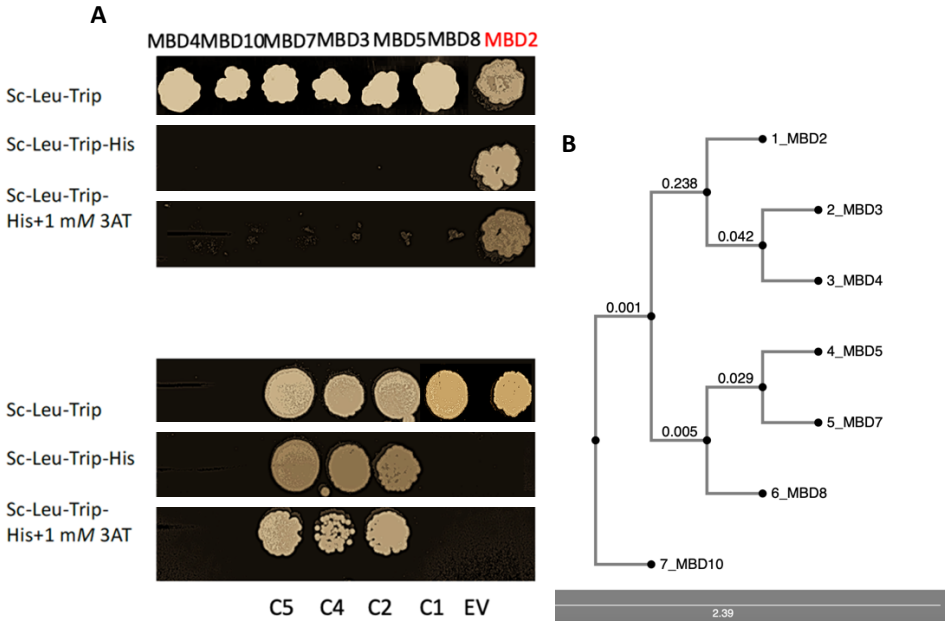


Figure 7. HAG4 interacts with the TF, MBD2 **A**. Yeast two hybrid confirmation on plates of HAG4 interaction with the TF, MBD2. The mated yeast is expressing both HAG4-DB and TF-AD vectors (mated). On each plate five controls were used. The controls consist of four strains C1–5, each containing a different pair of DB-X and AD-Y hybrid proteins. C1: negative control; C2, C4, C5: positive controls as described in (Dreze et al., 2010). The assay was repeated 3 times. To avoid false positives through autoactivation, we used 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product. **B**. Phylogenetic distances were calculated through ClustalW2.

HAG4 interacted with the TF, MBD2 and did not interact with other members of its family, hence, this interaction is specific (Fig. 7). HAG4 and HAG5 have been previously shown to be involved in flowering time by epigenetic modification of FLC and MAF3/4 chromatins at H4K5 acetylation (Xiao, Zhang et al. 2013). In the same study, the authors report that the double mutant *hag4/hag5* is lethal, and amiRNA-HAG4/5 transgenic plants showed early flowering and reduced fertility. They also demonstrate that HAG4 overexpression caused late flowering and elevated expression of FLC and MAF3/4 which suggests that HAG4 regulates flowering time depended on FLC.

This prompt us to investigate if HAG4 alone regulates flowering time. To this end, the *hag4-2* and *hag5-2* mutants were grown in a long day cabinet and the flowering time was evaluated (Fig. 8).

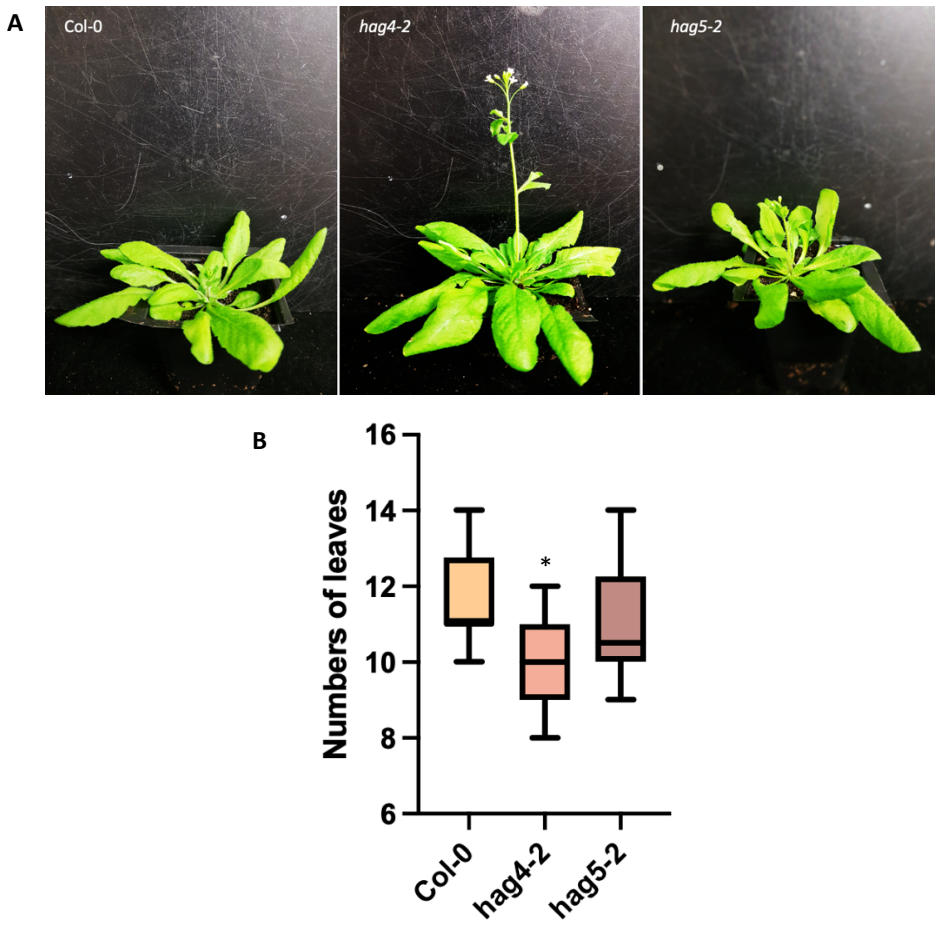


Figure 8. Early flowering phenotype of *hag4-2* mutants. **A.** Plants were 5-week-old in long day conditions (16h light) where pictures were taken. **B.** Number of leaves when the plants started bolting was calculated. Statistical significance between genotypes was calculated with two-sided T-test test, *P < 0.05. The experiment was repeated 3 times with 12 plants per genotype.

Eliminating HAG4 led to early flowering time, which suggests that HAG4 is a negative regulator of flowering (Fig.8). These results agree with the study of (Xiao, Zhang et al. 2013).

HAG4 interact with the TF, At4g03250

The Y2H screening revealed a second potential HAG4 interactor, the TF, At4g03250 (Fig. 9). This TF is a Homeodomain-like superfamily protein, that is been reported to interact with TOPLESS (TPL) and the four TPL-related (TPR) corepressors (Causier, Ashworth et al. 2012). The TPL/TPR family modulates gene expression in diverse processes, including hormone signalling, stress responses, and the control of flowering time repression of FLOWERING LOCUS T (Baxter, Tripathy et al.)

(Causier B et al., 2012. At4g03250, though, hasn't been reported for any function or phenotype.

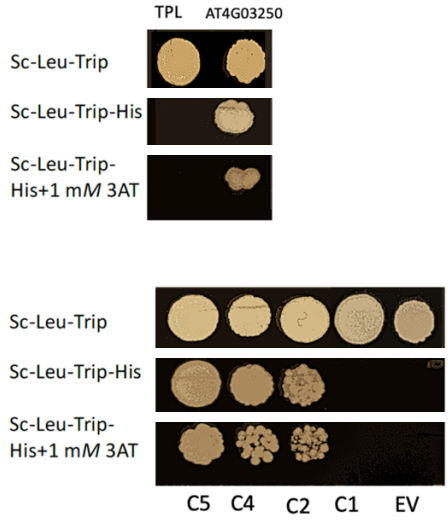


Figure 9. HAG4 interacts with the TF, At4g03250. Yeast two hybrid confirmation on plates of HAG4 with At4g03250 and its interactor TOPLESS (TPL). The mated yeast is expressing both HAG4-DB and TF-AD vectors (mated). On each plate five controls were used. The controls consist of four strains C1–5, each containing a different pair of DB-X and AD-Y hybrid proteins. C1: negative control; C2, C4, C5: positive controls as described in (Dreze et al., 2010). The assay was repeated 3 times. To avoid false positives through autoactivation, we used increasing concentrations of 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product.

The interaction of HAG4 with At4g03250 was confirmed by Y2H (Fig. 9). In order to confirm the specificity of the interaction, TPL which was available in the library was added as a negative control.

HAG4 interact with VFP4, a TF involved in plant immunity

The Y2H screening also revealed a third TF, VFP4 (AT5g28040) as an interactor of HAG4 (Fig. 10). VFP4 is a member of the GeBP/GPL family of leucine zipper TFs and interacts with the F-box proteins from *A. tumefaciens* VirF and VBF. As previously, we included the closest TFs to VFP4 available in the library as controls for the specificity of the interaction between VFP4 and HAG4 (Fig. 10).

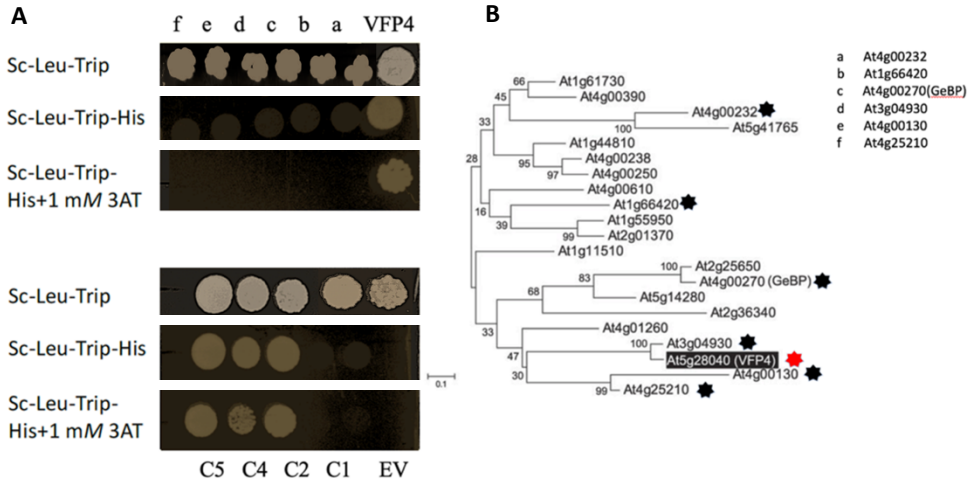


Figure 10. HAG4 interacts with the TF, VFP4. **A.** Yeast two hybrid confirmation on plates of HAG4 interaction with VFP4 including the closest TF to VFP4 available in the TF library. The mated yeast is expressing both HAG4-DB and TF-AD vectors (mated). On each plate five controls were used. The controls consist of four strains C1–5, each containing a different pair of DB-X and AD-Y hybrid proteins. C1: negative control; C2, C4, C5: positive controls as described in (Dreze et al., 2010). The assay was repeated 3 times. To avoid false positives through autoactivation, we used 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product. **B.** Phylogenetic tree of close family members of VFP4 (Garcia-Cano, Hak et al. 2018). To avoid false positives through autoactivation, we used increasing concentrations of 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product.

As HAG4 and HAG5 work redundantly but also distinct functions were reported in this study, it is important to examine whether HAG5 interacts with the same TFs. As seen in Figure 11A, HAG5 does not interact with VFP4, MBD2 or At4g03250, confirming that HAG4 interactions are a product of evolutionary diversification and acquirement of new functions of both paralogues. To further support this notion, a Y2H was performed to investigate whether HAG4 interacts with known interactors of HAG5. Dr. Anna Gil Gonzalez, a previous PhD student in Ntoukakis group, has identidied the TF, ARIA (AT5G19330) as HAG5 interactor. ARIA is an ARM repeat TF previously shown to act as a positive regulator of ABA responses (Kim, Kang et al. 2004) (Fig. 11). Consisted with our previously results, HAG4 does not interact with the interactor of HAG5.

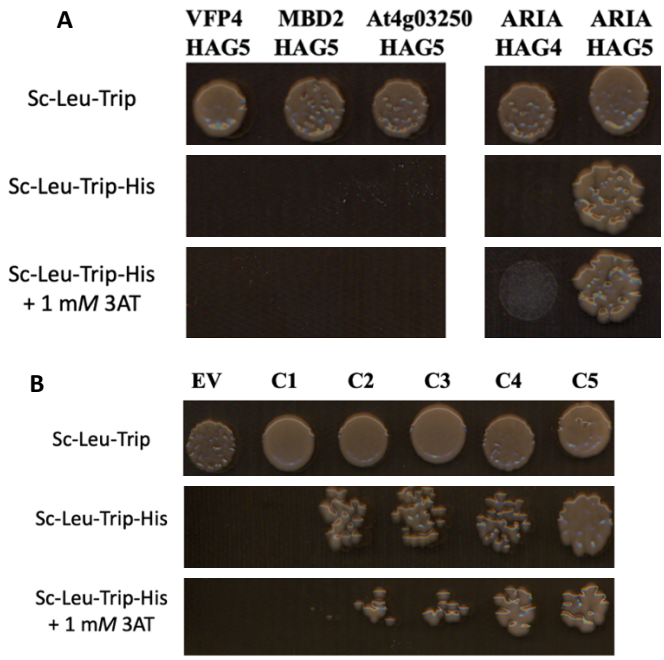


Figure 11. HAG4 and HAG5 do not interact with the same TFs. Yeast two-hybrid confirmation on plates. **A.** HAG5 does not interact with the TFs that HAG4 interact. HAG5 interacts with the TF, ARIA while HAG4 doesn't. The mated yeast is expressing both HAG4/5-DB and TFAD vectors (mated). **B**. On each plate five controls were used. The controls consist of four strains C1–5, each containing a different pair of DB-X and AD-Y hybrid proteins. C1: negative control; C2, C3, C4, C5: positive controls as described in (Dreze et al., 2010). The assay was repeated 3 times. To avoid false positives through autoactivation, we used 3AT (3-amino-1,2,4-triazole), a competitive inhibitor of the HIS3 gene product.

Recent findings revealed the role of VFP4, the interactor of HAG4, in plant immunity. García-Cano et al, 2018 investigated the role of VFP4 against *Agrobacterium tumefaciens* infection. In order to facilitate infection, *A. tumefaciens* exports the *F-box* effector *VirF* into the host cell, that interacts with VFP4 and targets it for proteasomal degradation. Furthermore, loss-of-function mutation in VFP4 resulted in differential expression of biotic stress response genes, suggesting that one of the functions of VFP4 is to control a spectrum of plant defences, including those against *A. tumefaciens*.

In these experiments, the authors used DESeq to identify differentially expressed genes (DEGs) and found statistically significant changes in the expression of 479 genes between wild-type Col-0 and the *vfp4-1* loss-of-function mutants. One of the DEGs identified is ATL31/CNI1 (At5g27420) encoding a RING-type ubiquitin ligase (Serrano, Parra et al.) shown to promote resistance to the bacterial pathogen

P. syringae (Maekawa, Sato et al.). Another DEG, At2g32030, encodes an acyl-CoA N-acyltransferase superfamily protein involved in abscisic acid response (Xin, Zhao et al.). The latter has no known involvement in bacterial infection and, thus, represents a useful control. The authors showed that the expression of *At3g32030* and *ATL31* decreased in the *vfp4-1* loss-of-function roots, showing that *VFP4* regulates the expression levels of these two genes.

The authors also show that loss-of-function *vfp4-1* plants exhibit no detectable changes in susceptibility to *Agrobacterium* tumorigenicity. However, gain-of-function VFP4 OE plants exhibit reduced susceptibility to *Agrobacterium* tumorigenicity. These results suggest that the role of HAG4 in plant immunity could be explained through its interaction with VFP4.

The relative expression of At2g32030 and ATL31, the 2 genes that we previously described that they are regulated by VFP4, was measured in *hag4-2* mutants. The expression level of At2g32030 was decreased significantly in *hag4-2* mutants compared to Col-0, although no significance changes in the expression of ATL31 were found in *hag4-2* mutants (Fig. 12).

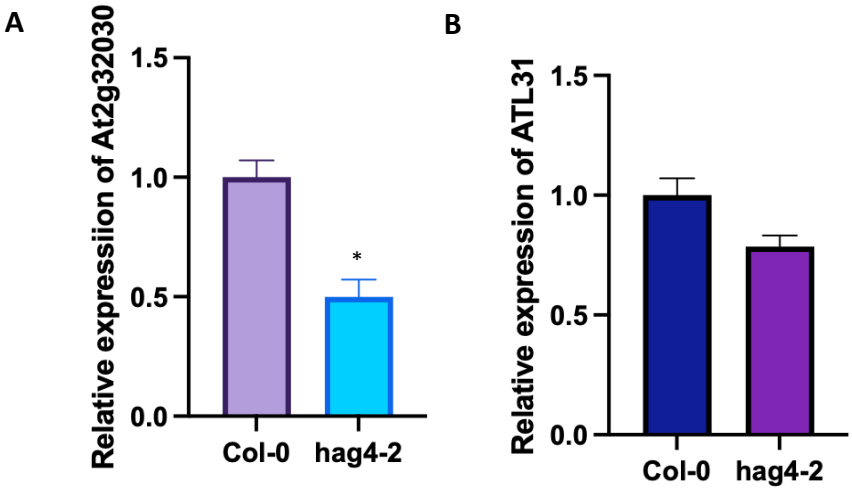


Figure.12. The expression of *ATL31* is not regulated by *HAG4*. Accumulation of **A.** At2g32030 and **B.** ATL31, two genes regulated by VFP4, was assessed by qPCR in 14 days old seedlings of Col-0 and *hag4-2* mutants. Seedlings were grown in ½ MS plates. RT-qPCR analysis shows that At2g32030 was significantly decreased in *hag4-2* mutants. The relative expression of ATL31 was not significantly decreased. Gene expression was normalised against the housekeeping gene αTubulin. Values are

average of three biological repeats ± SE calculated with two-sided T-test and presented as fold induction compared to Col-0. *P < 0.05.

Defence responses of *vfp4* mutants

In order to further investigate the role of HAG4 and its interactor VFP4 in plant immunity, T-DNA insertion mutants from the SALK institute (NASC, http://arabidopsis.org.uk/) were selected for further characterisation. The *vfp4-1* mutant line of Garcia-Cano et al., 2018, was used in this study in order to identify immunity phenotypes. The mutants were verified as homozygous (Fig. 13). The line *vfp4-1* is a loss-of-function mutant with a T-DNA insertion in the first exon of the gene.

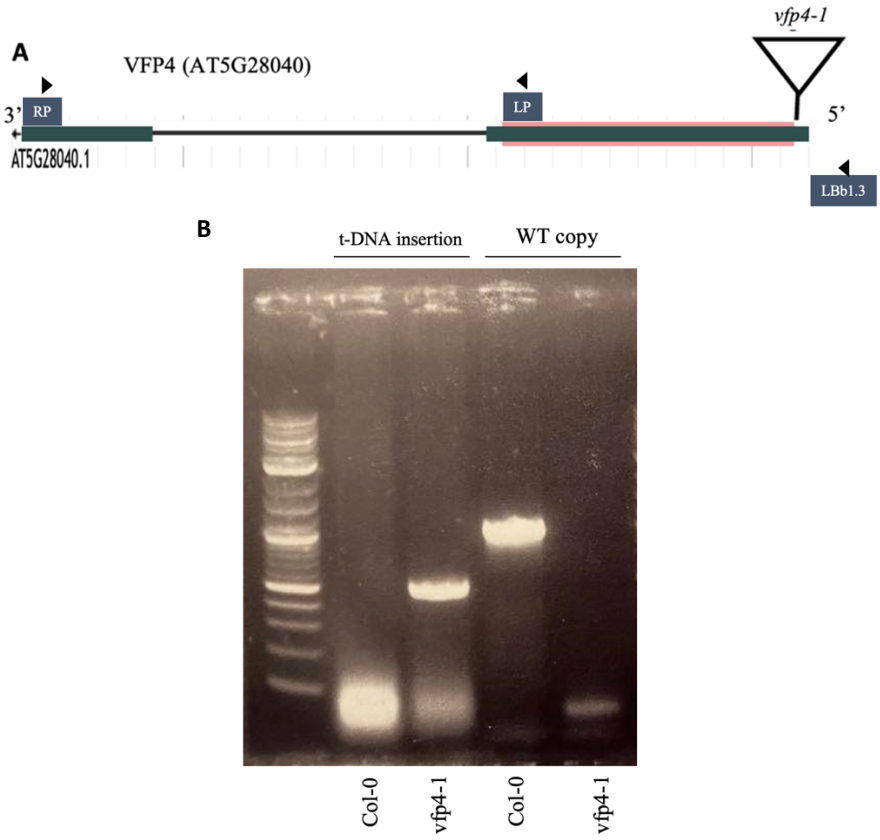


Figure 13. Schematic representations of *vfp4-1* mutants. **A.** Diagram of *vfp4-1* mutants (SALK 129879), it is a loss-of-function mutant with a T-DNA insertion in the first exon. Triangles represent primers used for section B. **B.** PCR electrophoresis showing amplicons of one region of VFP4 cDNA in *Col-0* and *vfp4-1* mutant. cDNA was reverse transcribed from RNA extracted form 14 days old seedlings (n = 3 seedlings per genotype).

The *vfp4-1* mutants were then infected with the leaf pathogens *Pto DC3000* and *B.cinerea* (Fig. 14). Consistent with the *hag4* phenotypes (Fig.1 and 4), *vfp4* immunity phenotypes where indistinguishable from Col-0 phenotypes (Fig. 14).

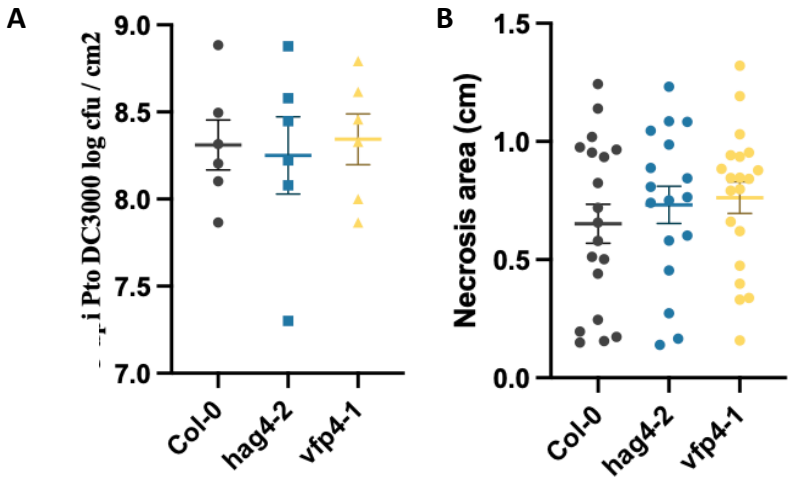


Figure 14. VFP4 is not involved in immunity responses against leaf pathogens. *vfp4-1* mutants were evaluated for leaf immunity phenotypes and perform as Col-0. Col-0, *hag4-1* and *vfp4*-1 mutants were infected with **A**. *Pto DC3000.* Samples were collected 3 days post-inoculation. Statistical significance versus Col-0 was determined by two-tailed t-test, n = 6, *** $P \leq 0.001$. **B**. Infection with *B.cinerea*. The necrotic areas of the leaves were measured using ImageJ. The experiment was repeated 3 times. The differences observed were not significant on a two-sided T-test (n = 20, $P > 0.05$). Error bars indicate standard error.

We next investigated the root immunity phenotype of the loss-of-function VFP4 mutants against the root pathogens *V. dahliae* and *F. oxysporum*. Foliar symptoms were recorded for Col-0, *hag4-2* and *vfp4-1* plants for 21 days after inoculation. When infected with *V. dahliae,* disease severity in *hag4-2* plants was 71% whereas in *vfp4-1* plants was 145% compared to *Col-0* wild type plants (Fig. 15). Similarly, when infected with *F. oxysporum,* disease severity in *hag4-2* plants is 75% while in *vfp4-1* plants is 118% compared to *Col-0* (Fig. 16). In order to determine the correlation between disease severity and degree of fungal proliferation, the expression of PG5, an extracellular endopolygalacturonase (endoPG) which is highly conserved among different formae speciales of *F. oxysporum,* was studied by qPCRs in *Col-0, hag4-2* and *vfp4-1* plants. The quantification of *F. oxysporum* (Fig. 17) confirmed the pathogenesis assays (Fig. 16).

Therefore, in comparison to Col-0 plants, *vfp4-1* plants are more susceptible while *hag4-2* plants are more resistance to both *V. dahliae* and *F. oxysporum* (Fig 15&16). Thus, despite their interaction HAG4 and VFP4 have opposite roles in root immunity.

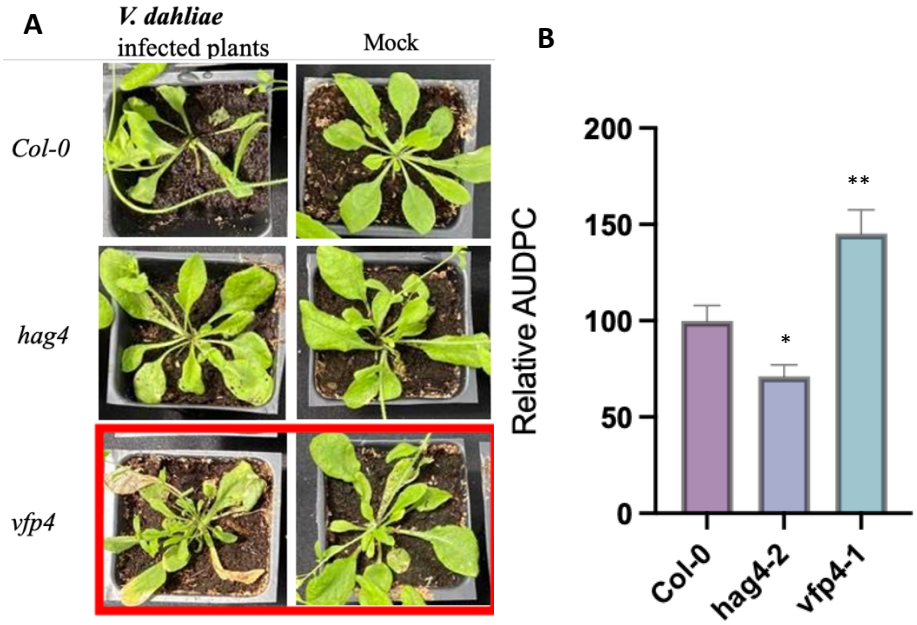


Figure 15. *vfp4-1* mutants are susceptible to *V.dahliae.* Infection by the root pathogen *V. dahliae*. **A.** Symptoms of plants at the end of the experiment (21dpi). **B.** Disease ratings were measured over time to generate disease progression curves. Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment, which is referred to as the relative AUDPC. AUDPC was calculated by the trapezoidal integration method (Campbell and Madden). For this experiment, 30 plants were used per treatment and plant genotype. Statistical significance between genotypes was calculated with a two-sided T-test, *P < 0.05, ** P < 0.001.

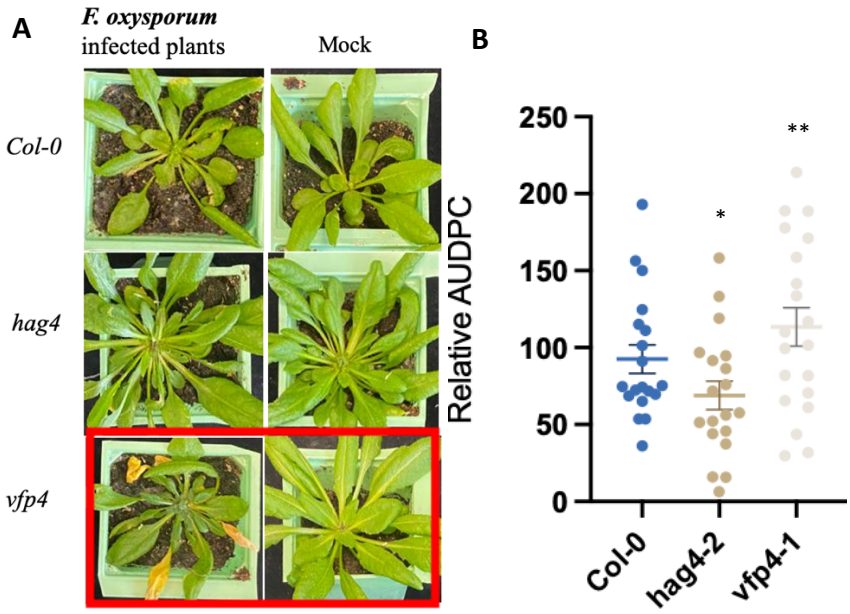


Figure 16. *vfp4-1* mutants are susceptible to *F. oxysporum.* Infection by the root pathogen *F. oxysporum*. **A.** Symptoms of plants at the end of the experiment (21days post inocullation). **B.** Disease ratings were measured over time to generate disease progression curves. Disease was expressed as a percentage of the maximum possible area for the whole period of the experiment, which is referred to as the relative AUDPC. AUDPC was calculated by the trapezoidal integration method (Campbell and Madden). For this experiment, 30 plants were used per treatment and plant genotype. Statistical significance between genotypes was calculated with a two-sided T-Test, *P < 0.05, ** P < 0.001.

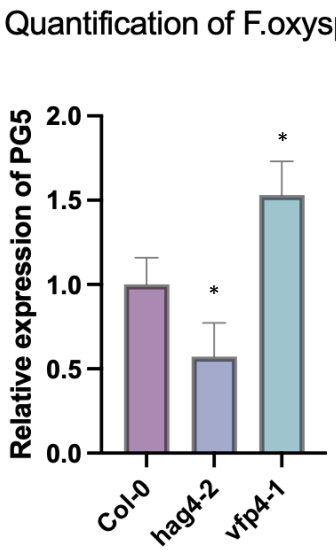


Figure 17. Quantification of *F. oxysporum* in Col-0*, hag4* and *vfp4* plants. Accumulation of PG5 transcripts was assessed by qPCRs in Col-0*, hag4* and *vfp4* plants. Quantification of *F. oxysporum* by measuring the relative expression of PG5 in 21 days old seedlings of Col-0*, hag4* and *vfp4* grown in soil and inoculated with *F. oxysporum.* 8 days post inoculation, the tissue was sampled. Gene expression was normalised against the housekeeping gene αTubulin. The experiment was repeated three times with 5 plants per treatment and replication. Values are average of three biological repeats ± SE presented as fold induction compared to Col-0. *P < 0.05.

Although in this study we show that the MYST HATs interact with different TFs and have distinct function in tissue-specific immunity, HAG4 and HAG5 have a major overlapping level of interactions according to the work of Lian-Mei Tan et al. (2018). The authors identified interacting proteins of HAG4 and HAG5 by affinity purification in combination with mass spectrometry and I revealed that HAG4 and HAG5 have 29 interacting proteins in common (Fig. 18A). Additionally, they are both expressed throughout the plant of Arabidopsis (Fig. 18B).

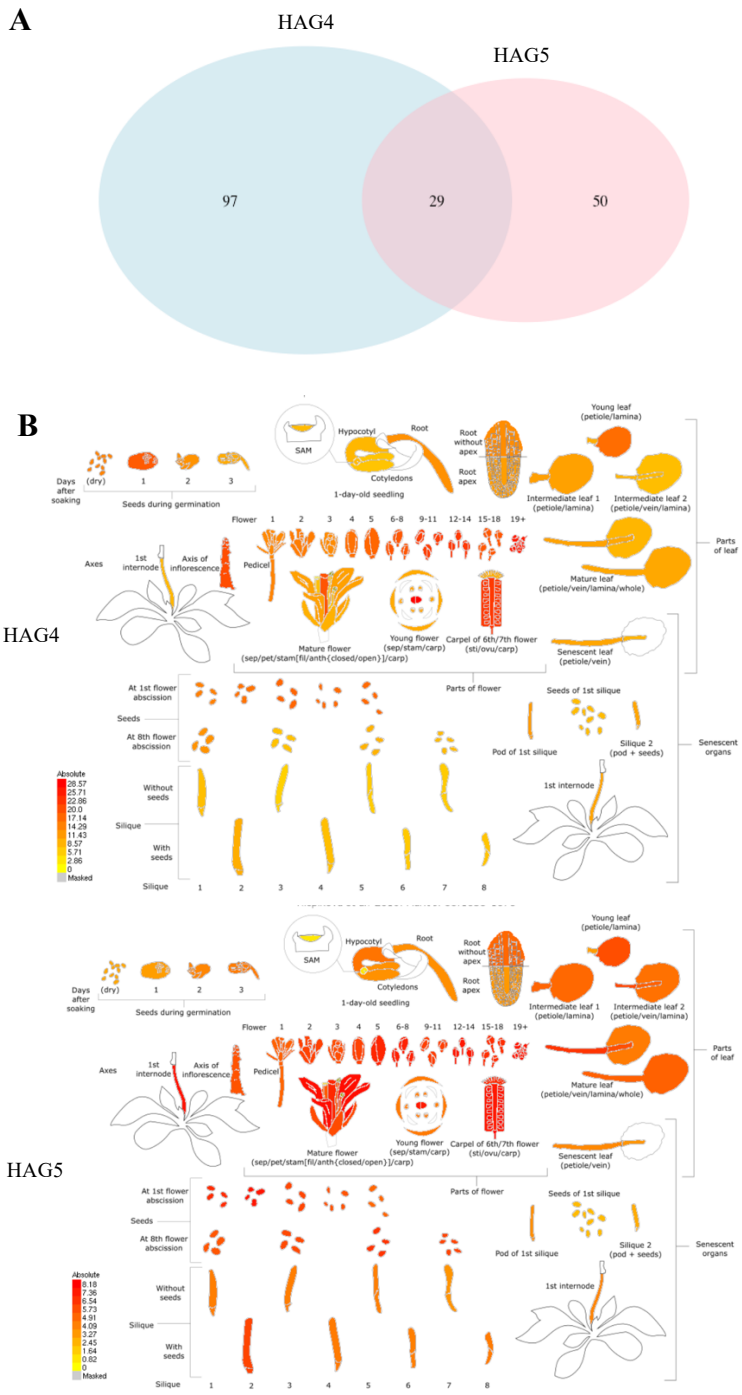


Figure 18. HAG4 and HAG5 have common interactors and they are expressed in the same tissues in Arabidopsis plants. **A.** Interactors of HAM1 and HAM2 by mass spectrometric analysis using R program. Tan, Zhang (Tan, 2018). **B.** Atlas presenting the expression levels of HAG4 and HAG5 throughout the whole plant of Arabidopsis (Klepikova et al., 2016). The HATs are expressed in the same tissues in the plant.

Discussion

Previously work has indicated that the two members of *Arabidopsis* HAT MYST family work redundantly or within the same complexes. HAG4 and HAG5 were shown to work together regulating flowering and gametophyte development (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013). Additionally, Lian-Mei Tan et al., 2018 show that HAG4 and HAG5 are part of the PEAT complex, composed of PWWP, EPCR, ARID, and TRB proteins, as well as HDA6 and HDA9 histone deacetylases. This repressor complex is recruited to individual loci and is involved in heterochromatin formation and transcriptional silencing through modulation of siRNAs and DNA methylation.

Our data suggest that the two MYST HATs have different immunity phenotypes. HAG5 modulates the responses against *Pto DC3000* (Fig. 1) and *Hpa* (Fig. 4), both biotrophic plant pathogens, which suggests that HAG5 plays a role in resistance against biotrophic aerial pathogens. That could be because the biotrophic pathogens, by keeping the tissue alive, use HAG5 to reprogram the cell to promote virulence. In contrast, HAG4 regulates defence responses to necrotrophic root pathogens (Fig 5&6) *V. dahliae* and *F. oxysporum. hag5* mutants performed as the WT plants when infected with *V. dahliae*(Fig. 5). The pathogenesis assay with *F. oxysporum* needs to be repeated as there was a germination issue with *hag5* mutants.

Both pathogens are considered hemibiotrophic where a biotrophic phase, within the root xylem without a visible disease phenotype, is followed by a necrotrophic phase in the aerial parts of the plant (Reusche, Thole et al. 2012). Potentially, these root pathogens use HAG4 the same way through the biotrophic phase. Another possible explanation could be a tissue specific expression of the MYST HATs. However, both HAG4 and HAG5 are expressed equally throughout the plant and that cannot explain the tissue specificity in immunity responses (Fig. 18B).

Furthermore, we were able to demonstrate that HAG4 and HAG5 interact with a distinct set of TFs further supporting that they have different functions in plant immunity. As mentioned earlier, HAG4 together with HAG5 regulate flowering time

by epigenetic modification of FLC and MAF3/4 chromatins at H4K5 acetylation and overexpressing HAG4 causes late flowering (Xiao, Zhang et al. 2013). The amiRNA-*HAG4/5* transgenic plants they used, showed early flowering and reduced fertility. In this study, an early flowering phenotype of *hag4* mutants has been reported (Fig. 8) which could be modulated by HAG4's interaction with MBD2 (Fig. 7), which is a part of a protein complex that regulates the expression of flowering repressors FLC, MAF4, and MAF5 through H3 deacetylation (Liu, Wang et al. 2021). However, HAG4 does not mediate H4K5 acetylation at the FLC locus based on our ChIP-seq data. Since the *hag4/hag5* knock out mutants are not viable, we created knock down mutants. There is no phenotype in flowering time though since the HATs are still expressed and they can still regulate some processes. Hence, these lines were not included in the pathogenesis assays.

Another interesting interactor identified in this work is *VFP4* (Fig. 10), a TF that is being targeted by the *VirF* and *VBF* effector proteins of *Agrobacterium tumefaciens* for degradation (Garcia-Cano, Hak et al. 2018). This published work also supports our root-specificity hypothesis, since *A. tumefaciens* colonises initially the roots. In detail, the authors demonstrate that *VFP4* renders plants less susceptible to *Agrobacterium* infection by regulating *ATL31*, a RING-type ubiquitin ligase, that is involved in plant immunity. *ATL31* enhances bacterial resistance (Garcia-Cano, Hak et al. 2018) and resistance to powdery mildew fungus (Maekawa, Inada et al. 2014). Thus, our hypothesis that HAG4 bind to VFP4 and together they regulate the expression of *ATL31* will be explained in chapter 3.

According to this study, VFP4 is not involved in defence responses to aerial pathogens (Fig. 14). Interestingly, *vfp4* knock-out mutants show susceptibility to root pathogens, the opposite responses compared to *hag4* mutants. In chapter 3, the root responses of *hag4* and *vfp4* mutants will be further characterized.

Hence, this study suggests that HAG4 and HAG5 interact with a distinct set of TFs further supporting that they have different functions in plant immunity and tissue-specificity. On the contrary, according to Lian-Mei Tan et al., (2018), the overlapping interacting proteins of HAG4 and HAG5 are 29 out of a total 196 interacting proteins (Fig 18A) and the two HATs are expressed throughout the whole

plant of Arabidopsis (Fig. 18B). Based on these data and our results, we cannot explain the tissue-specific immunity responses. In chapter 3, acetylation levels and transcriptomic responses will be explored before and after infection to answer this question.

HAG4 changes the chromatin landscape and transcriptional responses upon infection

Alexia Tornesaki[1], Zoi Ioannidou[1], Vardis Ntoukakis[1]

[1]School of Life Sciences, University of Warwick

Abstract

A major part of the plant immune responses to pathogens is reprogramming of gene expression. Histone acetyltransferases of the MYST family (MYST-HATs) carry out a significant proportion of histone acetylation and therefore play a critical role in plant transcription regulation. The *A. thaliana* MYST-HAT, *AtHAG4*, works redundantly with *AtHAG5* to regulate gametophyte development and flowering time. HAG4 interacts with the transcription factor, VFP4, and they regulate immunity against the root pathogens *Verticillium dahliae* and *Fusarium oxysporum* but they are not involved in responses against the leaf pathogen *Pseudomonas syringae, Hyaloperonospora arabidopsidis and Botrytis cinerea*. Interestingly, the phenotypes of *hag4* and *vfp4* mutants are contrary to each other. Here, we suggest that H4K5ac is important for resistance against *F. oxysporum* and this resistance is HAG4- and VFP4-dependent. Additionally, we support that the opposite phenotypes of *hag4* and *vfp4* mutants is result of changes in root architecture and cell homeostasis by *HAG4* mutation and not acetylation of immunity-related genes.

Introduction

Chromatin remodelling and gene regulation in plants

Chromatin remodelling and gene regulation are essential processes that govern how genes are expressed in living organisms. In plants, chromatin remodelling refers to the dynamic changes that occur to the structure of chromatin, which is the complex of DNA and associated proteins that compose chromosomes. These changes regulate gene expression and ultimately determine the traits and responses of a plant.

Chromatin remodelling can occur through a variety of mechanisms, including histone modification, nucleosome positioning, and DNA methylation. These changes can either activate or repress the expression of genes. The tight regulation of gene expression is critical for plant growth, development, and response to environmental cues. Plants have evolved complex mechanisms to control gene expression, including transcription factors and signalling pathways that activate or repress the expression of specific genes in response to internal and external stimuli. These mechanisms allow plants to respond to changes in their environment and to adapt to different conditions. One example of chromatin remodelling in plants is the role of the chromatin remodelling ATPase, *EMBRYO SAC DEVELOPMENT ARREST 16*, *EDA16* during immune responses through regulation of genes involved in redox homeostasis by nucleosome repositioning (Pardal, Piquerez et al., 2021).

Understanding the mechanisms of chromatin remodelling and gene regulation in plants is essential for developing strategies to improve crop yields, enhance plant resistance to disease and environmental stress, and to better understand the biology of plants.

Histone modifications during plant immunity

Histone modifications play a critical role in regulating gene expression in plants, including in immunity responses to pathogens. During plant immunity, histone modifications serve as a key mechanism for controlling the expression of genes involved in the defence response. Histone modifications such as acetylation, methylation, phosphorylation, and ubiquitination can alter the structure of chromatin

and regulate the accessibility of genes to transcription factors and other regulatory proteins (Bannister and Kouzarides, 2011).

Recent studies have revealed that specific histone modifications are associated with different stages of the plant immune response, including the recognition of microbe-associated molecular patterns (MAMPs) and the activation of defence responses. Understanding the role of histone modifications in plant immunity can provide insights into the molecular mechanisms underlying plant-pathogen interactions and may lead to the development of new strategies for crop protection.

One example of histone modifications being involved in defence responses is he deacetylation of the lysine 9 residue of histone 3 (H3K9) by histone deacetylase HD2B which is involved in the response to flg22 by interacting with MAP kinase MPK3 (Latrasse, Jégu et al. 2017). Additionally, histone acetylation by Arabidopsis CBP and MYST family regulates immune responses against *V. dahliae* (Gkizi, González Gil et al., 2021). GCN5, a member of the Arabidopsis GNAT family, mediates SA-mediated immunity by regulating H3K14ac levels (Kim, Piquerez et al.). Finally, HAC1-dependent histone acetylation regulates the expression of PTI-responsive genes and is associated with a primed Arabidopsis innate immunity and bacterial resistance (Singh, Yekondi et al., 2014). Overall, these studies demonstrate the importance of histone modifications in plant immunity and suggest that different modifications are involved in various stages of the defence response.

In this chapter, to better understand the role of HAG4 in regulating root immunity responses, and the contrary phenotypes of *hag4* and *vfp4* mutants, a transcriptomic analysis combined with a chromatin immunoprecipitation (ChIP) technique, will give us further insight into the molecular function of HAG4. This analysis provided evidence that H4K5ac is important for resistance against *F. oxysporum* and this resistance is HAG4- and VFP4-dependent. Additionally, looking at the transcriptomic differences between Col-0, *hag4-2* and *vfp4-2* plants, we support that the opposite phenotypes of *hag4* and *vfp4* mutants is a result of changes in root architecture and cell homeostasis by *HAG4* mutation and not acetylation of immunity-related genes upon *Fusarium oxysporum* infection.

Materials and methods

Sample preparation

The Arabidopsis lines used are described in chapter 2. Arabidopsis plants were grown for 5 weeks (60% humidity, 22ºC, 12 hours light/12 hours dark, 100 m mol m^{-2} s^{-1} light intensity) in soil. The inoculation with *F. oxysporum* was described in chapter 2.

RNA extraction

Plant tissue for RNA extraction was stored in RNAse free tubes (3 roots or 200mg of leaves per sample) and was frozen in liquid nitrogen immediately after harvesting and kept at -80°C. Plant tissue was ground in liquid nitrogen with a drill borer fitting a 2ml micro-centrifuge tube. RNeasy® Mini kit (Quiagen) was used for RNA extraction. RNA extraction was performed following manufacturer instructions. For quality control, RNA integrity was confirmed and quantified using the Agilent 2100 bioanalyser Plant Nano system (Agilent Biotechnologies). Multiplex RNA library construction was carried out at the Beijing Genome Institute (BGI).

RNAseq analysis

RNA libraries were checked for size quality with a Bioanalyzer and sequenced with an Ilumina sequencer. Sequnecing was prepared in Illumina HiSeq (4000) machine in paired mode. Trimmed data were received from BGI and checked for quality control with FastQC v0. 11.5. Trimmed data sequences were mapped to Arabidopsis TAIR10-55, downloaded from Ensembl (http://plants.ensembl.org/info/website/ftp/index.html) with STAR followed by read counting. Duplicates were marked with picard MarkDuplicates v2.26.6 (http://picard.sourceforge.net/). The data counts were normalised and analysed with the R package DEseq2 (Love et al., 2014). The PCA was computed with the plotPCA function over r-log normalized counts from DESeq2. The adjusted p-values accepted for significance were < 0.05 with a fold-change > 3, unless otherwise stated. To compare the treated and untreated samples a model accounting for a replicate effect was used: "Treatment + Genotype + Replicate". The package pheatmap was used to generate heatmaps. Venn diagrams between DEGs were prepared by https://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl. To determine functionality of DEGs, gene ontology (GO) enrichment analysis was performed using http://www.webgestalt.org/ and PantherDB.

Table 1. Summary statistics of RNAseq samples.

	Summary Statistics					
	ColCnt1	Col-0Cnt2	ColCnt3	Col-0ifn1	Col-0inf2	Col-0inf3
total reads	24253089	24068095	24061136	70485089	48377971	53517890
mapped reads	23658674	23121506	22977478	65408944	45774188	48268947
uniquely mapping reads	341426	337478	440342	3357875	1565962	4182610
	hag4Cnt1	hag4Cnt2	hag4Cnt3	hag4ifn1	hag4inf2	hag4inf3
total reads	24150077	24061709	24074972	74417717	57879622	50179580
mapped reads	23285431	23438699	23389869	70219230	54046304	45880459
uniquely mapping reads	372070	366696	431126	2823497	2892124	3149324
	vfp4Cnt1	vfp4Cnt2	vfp4Cnt3	vfp4ifn1	vfp4inf2	vfp4inf3
total reads	24014717	24079895	24007535	20255740	24147420	24112305
mapped reads	23464125	23509768	23416337	19166690	23519142	22921153
uniquely mapping reads	332442	358177	382949	776236	384306	810285

Chromatin immuno-precipitation (ChIP)

Plant tissue (leaves or roots) was cross-linked and immunoprecipitated as described by (Desvoyes, Vergara et al. 2018). The antibodies and the required concentration used can be found at Table 1. The DNA was extracted with the QIAquick PCR Purification Kit (QIAGEN) following manufacturer's instructions. Samples were eluted in 30µl elution buffer and DNA from the same IP sample were pooled together for a final volume of ~60 µl per sample. DNA samples were stored at -20 °C. Illumina sequencing was carried out at the Beijing Genome Institute (BGI).

Table 2. Antibodies used for ChIP.

Target	**Reference number**	**Volume used per IP**
Rabbit polyclonal to Histone H3	ab1791 Abcam	1 µl
Rabbit polyclonal to Histone H4K5ac	07-327	8 µl

ChIPseq analysis

DNA libraries were checked for size quality with a Bioanalyzer and sequenced with an Ilumina sequencer. Sequnecing was prepared in DNBSEQ ChIP-seq machine in unpaired mode. Trimmed data were received from BGI and checked for quality control with FastQC v0. 11.5. Trimmed data sequences were mapped to Arabidopsis TAIR10-55, downloaded from Ensembl (http://plants.ensembl.org/info/website/ftp/index.html) by Bowtie 2.5.1. Bam files

of the samples were log2 ratio-normalized against their controls with the bamCompare command from deepTools v3.5.2 creating the bigwig files. ComputeMatrix and plotHeatmap were used to generate the acetylation profile and the heatmap. The command grep -f was used to generate a subset of genes from the transcriptomic analysis (RNA-seq). Peaks were called using MACS v2.7.1 with options '--broad --bdg -t $file -f BAM -n. Intersections between peaks were performed using the merge command from bedtools v2.30.9 (59). The bed files were converted to saf format for feature counts to be visible to be read. Peak summits were annotated with genomic features and genes by intersecting them against the Araport11 annotation with the intersect command from bedtools v2.30.9 (59).

Results

In chapter 2, immunity responses of *hag4-2* mutants were investigated. In view of these results, HAG4 is involved in root-immunity responses and *hag4-2* mutants are resistant to *F. oxysporum*. Based on these findings, interactors of HAG4 were identified through a Y2H screening using a library of Arabidopsis TFs. VFP4, which belongs to GeBP/GPL family of leucine zipper TFs, was identified as an interactor of HAG4. VFP4 was previously shown to be involved in resistance against bacterial pathogens (Garcia-Cano, Hak et al.). Suprisingly, the immunity phenotypes of the knock-out plants of VFP4, were opposite to the ones of its interactor HAG4. To help us understand these opposite phenotypes, we performed an RNAseq analysis in order to reveal transcriptomic changes upon *F. oxysporum* infection in Col-0, *hag4-2* and *vfp4-1* plants.

PCA was used to cluster the treated and untreated samples. The samples Col-0 mock, *hag4-2* mock, *vfp4-1* mock and *vfp4-1* treated were grouped/aligned together within the first principal component (PC1), which accounted for 94% of the variation while they are separated within the PC2, which only accounts for 2% of the variation (Fig. 1). The treated samples of Col-0 and *hag4-2* plants were clustered together within the first principal component (PC1), which accounted for 94% of the variation and they are separated within the PC2, that only accounts for 2% of the variation as well (Fig. 1A). Intriguingly, the treated samples of *vfp4-1* mutants are not clustered together with Col-0 and *hag4-2* treated samples. These results are consistent with the immunity phenotypes (see chapter 2 for details) where Col-0 and *hag4-2* plants are partially resistant to *F. oxysporum,* hence, they both activate defence responses while *vfp4-1* mutants are susceptible and thus, *vfp4-1* treated samples cluster with the mock samples. In order to exclude the possibility of ineffective inoculation of *vfp4-1* treated samples with the soil-borne fungus, the samples from RNAseq were also annotated to the genome of the fungus and the ratio of FOXG_00661, a 40S ribosomal protein S15, and AT4G26410, known as RHIP1 (previously described as a stable reference gene (Czechowski et al., 2005)), was measured (Fig. 1B). The plants of *vfp4-1* mutants accumulate more fungus in their roots compared to Col-0.

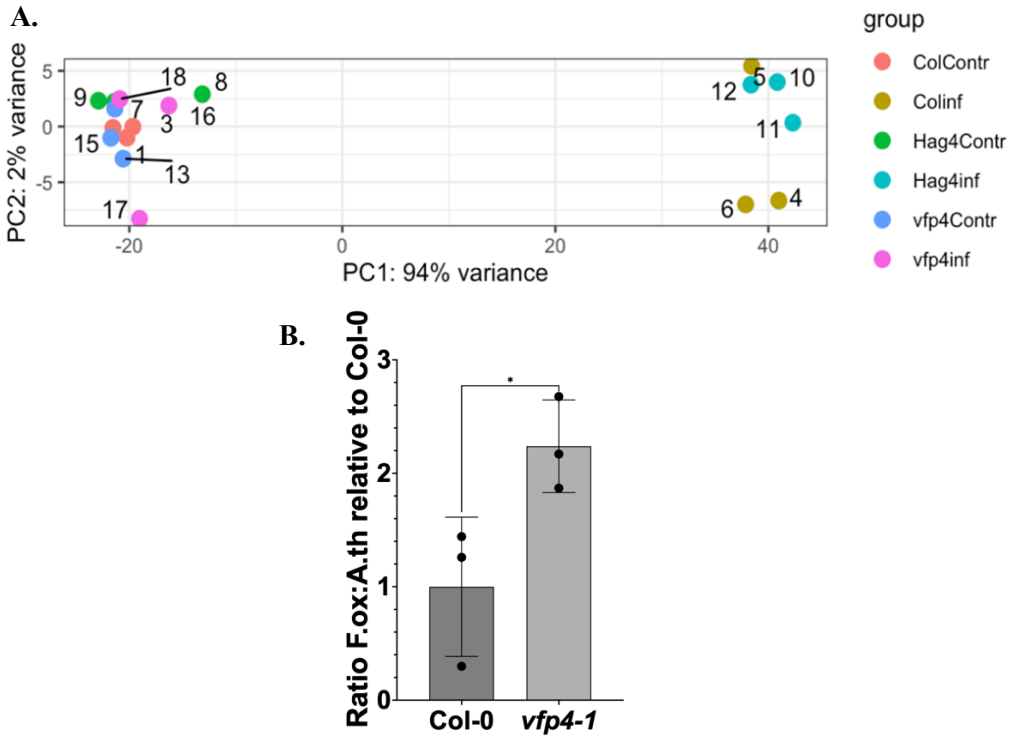


Figure 1. *hag4-2* and *vfp4-1* mutants have different transcriptional responses after *F.o* infection. **A.** Principal Component Analysis (PCA) of RNAseq of Col-0, *hag4-2* and *vfp4-1* mutants roots mock and *Fusarium oxysporum* treated. PCA was performed for all samples. The PC1 accounts for 94% of the variation, whilst PC2 accounts for 2% of the variation between samples. **B.** The quantification of *F.o.* in Col-0 and *vfp4-1* plants by counts ratio of FOXG_00661 and At4g26410 (RHIP1). The RNAseq was performed in 21 days old roots of Col-0, *hag4* and *vfp4* grown in soil, inoculated with *F. oxysporum* and collected 6 days post-inoculation. The experiment was repeated three times with 5 roots per replication. Values are average of three biological repeats ± SE presented as fold induction compared to Col-0. Two-talied T-test was used. *P < 0.05.

To estimate differential gene expression and fold change between the comparisons (table 1), average sequencing data from the three biological replicates sequenced per sample and compared with a fold change ≥ 3 and adjusted p-value ≤ 0.05. These three comparisons were created to reveal the *F. oxysporum* response genes per genotype.

Table 2. Table displaying the various comparisons of the RNAseq experiment between samples and their biological significance.

	Comparisons	Description	Significance
1	Col-0 vs Col-0 inf	DEGs in response to Fusarium	Transcriptional responses to Fusarium
2	*hag4-2* vs *hag4-2* inf	DEGs in response to Fusarium	HAG4 dependent transcriptional responses
3	*vfp4-1* vs *vfp4-1* inf	DEGs in response to Fusarium	VFP4 dependent transcriptional responses

F. oxysporum changes the transcription of ~7500 genes in Col-0 plants, most of these genes are up-regulated and overlap with the responses of *hag4-2* mutants upon infection (Fig. 2 and 3). The transcriptional responses to *F. oxysporum* result in ~6900 upregulated genes in Col-0, ~6500 for *hag4-2* and only 42 for *vfp4-1* (Fig.2). In detail, most of the upregulated differentially expressed genes (DEGs) of comparison 1 and 2 are common (~5000). Only the 560 genes (out of a total ~6500 genes) are differentially expressed exclusively in *hag4-2*. The transcriptional changes of *vfp4-1* plants after treatment reveal only one shared DEG with Col-0 and *hag4-2* mutants (Fig.1 and 2).

Gene ontology (GO) term enrichment of the DEGs from comparison 1 and 2 revealed that most of the up-regulated DEGs between Col-0 and *hag4-2* plants are categorised as cell killing, especially the overlaps between Col-0 and *hag4-2* and the ones specifically induced in *hag4-2* mutants (Fig. 2). According to http://amigo.geneontology.org/, cell killing categorises the genes associated with necrosis, any process in an organism that results in the killing of its own cells or those of another organism, including in some cases the death of the other organism. Additionally, in the shared DEGs and the unique DEGs of *hag4-2* mutants, genes related to interaction between organisms have been significantly upregulated (Fig. 2).

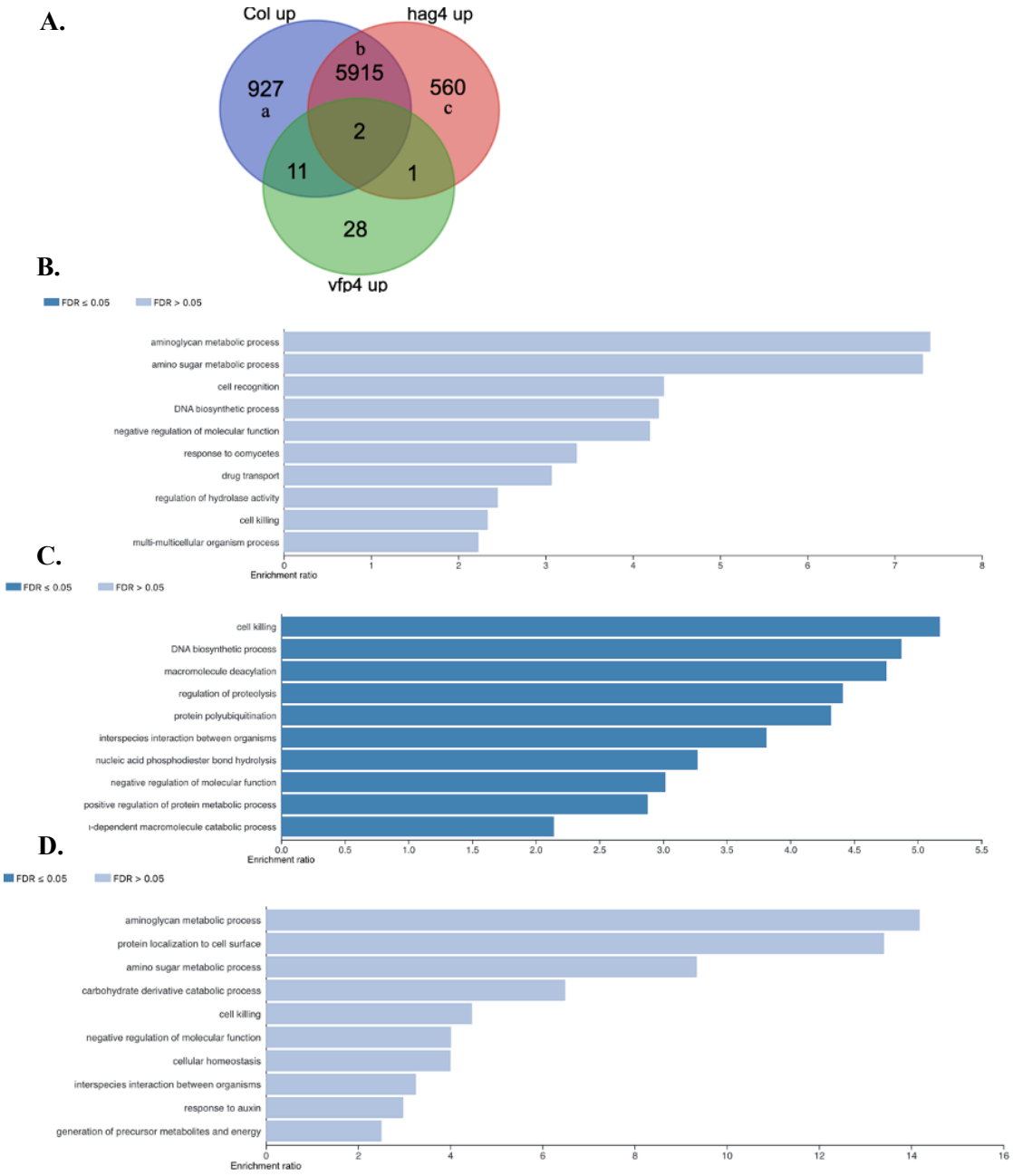


Figure 2. Upregulated genes upon *F. oxysporum* infection. **A.** Venn diagram of common and unique upregulated DEGs in response to *F. oxysporum* in Col-0, *hag4-2* and *vfp4-1* plants. **B**. biological process GO term enrichment of unique DEGs in Col-0 after treatment. **C.** biological process GO term enrichment of overlapping DEGs in Col-0 and *hag4-2* plants after treatment. **D.** biological process GO term enrichment of non-overlapping DEGs in *hag4-2* plants after treatment Fold enrichment was calculated by dividing the number of obtained DEGs for a particular GO term over the total number of random hits predicted for that GO term. The GO term enrichment was performed using webgestalt. Fold used for the DEGs was >3 and p value <0.05.

Of the 560 unique upregulated genes in *hag4-2* after infection, most of them are categorised as cell killing, interaction between organisms, response to auxin

(SAURs) and cellular homeostasis (Fig. 2). Auxin is a central regulator of plant growth and development as well as root gravitropism, root hair and lateral root formation (Grones and Friml 2015, Schaller, Bishopp et al. 2015). Thus, auxin signalling, and responses have a severe impact in plant defence. Many plant pathogens can directly synthesize auxin or invoke plant auxin biosynthesis as well as modulating auxin signalling to render the host more susceptible to infection (Kidd, Kadoo et al. 2011)

Unique upregulated DEGs in *hag4-2*, include induced cellular homeostasis responses (Fig. 2). Some of these genes are: AT4G15690 (*ROXY12*), AT5G18600 (*ROXY10*) and AT3G62960 (*ROXY8*). These CC-type glutaredoxins (*ROXYs*) are differentially expressed in response to nitrate deprivation. Nitrogen (N) is crucial factor for various cellular functions. C and N balance (C/N) plays an important role for plant growth and development. In addition, N availability has also been reported to be involved in the plant defence response (Mittelstraß, Treutter et al. 2006, Ros, Mohler et al. 2008). High availability of N, significantly increased potato susceptibility to *Phytophthora* infestans (Mittelstraß, Treutter et al. 2006). *ROXYs* also play a role in nutrient sensing through the regulation of chlorophyll content, root hair growth and ROS production in roots under nitrate starvation (Jung, Ahn et al. 2018).

The comparisons 1 and 2 exposed that the responses in treated *hag4-2* roots contain more downregulated genes (400) than Col-0 (~300) (Fig. 3). Most of the downregulated DEGs from comparison 1 are mutual with the ones from comparison 2 (261 genes) (Fig. 3).

Gene ontology (GO) term enrichment of the DEGs from comparison 1 and 2 displayed that genes involved protein ubiquitination are downregulated in Col-0 but not in *hag4-2* plants. Protein ubiquitination has been reported to have a significant impact on plant defence responses and signalling (Marino, Peeters et al. 2012).

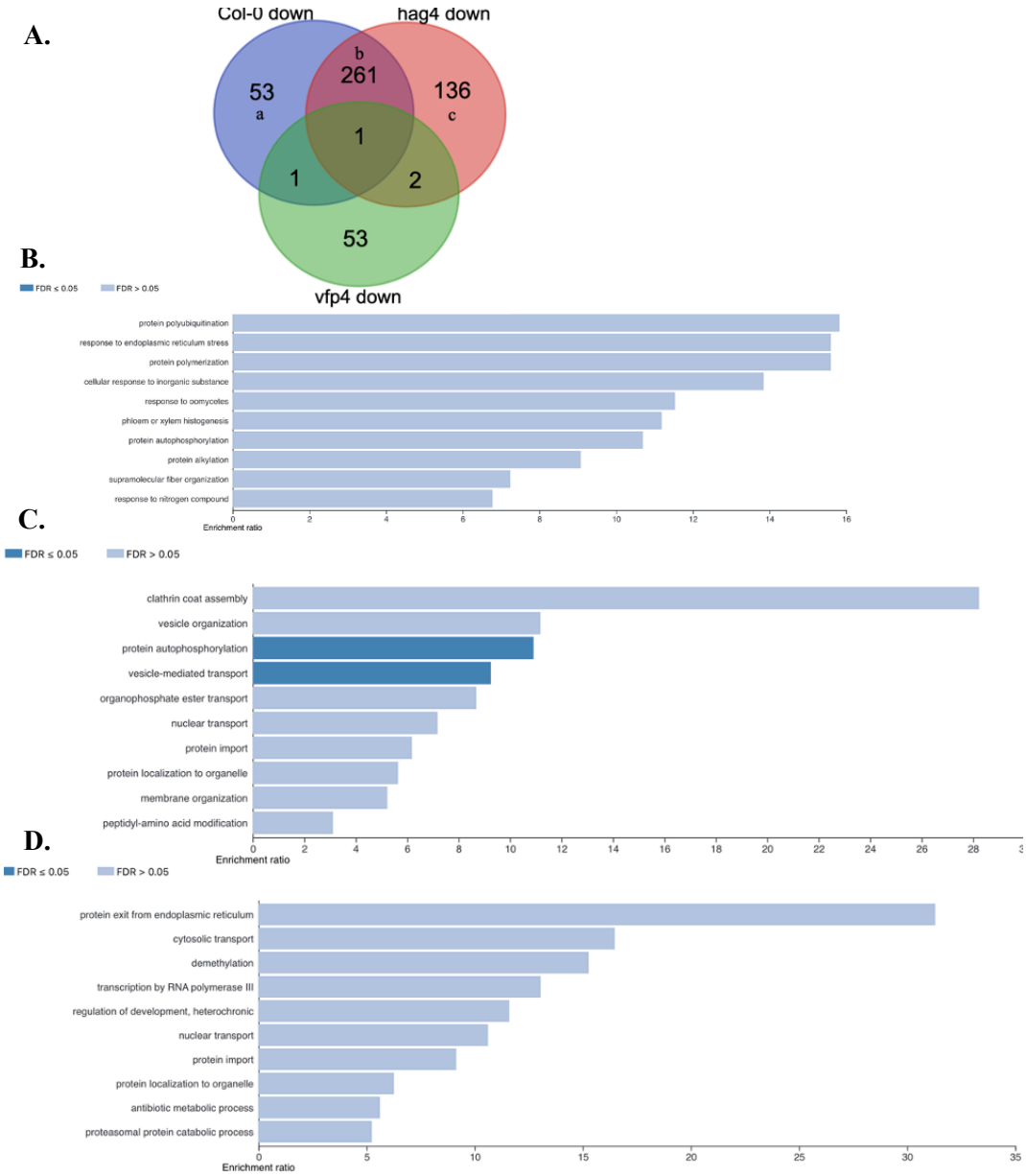


Figure 3. Downregulated genes upon *F. oxysporum* infection. **A.** Venn diagram of common and unique downregulated DEGs in response to *F. oxysporum* in Col-0, *hag4-2* and *vfp4-1* plants. **B.** biological process GO term enrichment of unique DEGs in Col-0 after treatment. **C.** biological process GO term enrichment of overlapping DEGs in Col-0 and *hag4-2* plants after treatment. **D.** biological process GO term enrichment of non-overlapping DEGs in *hag4-2* plants after treatment Fold enrichment was calculated by dividing the number of obtained DEGs for a particular GO term over the total number of random hits predicted for that GO term. The GO term enrichment was performed using webgestalt. Fold used for the DEGs was >3 and p value <0.05.

To have a more detailed insight of the up and down regulated genes in *hag4-2* plants in response to *F. oxysporum*, several genes involved in immunity responses or developmental processes were identified and described in table 1 and 2. Several immunity-related genes were upregulated while various significant downregulated

gene/regulators od plant development and root growth were distinguished in *hag4-2* plants.

Several CC-NBS-LRR plant disease resistance (*R*) genes were significantly upregulated in *hag4-2* plants upon infection (Table 2).

Table 3. Table displaying the unique upregulated genes *hag4-2* plants. PantherDB was used to identify these genes.

	DEGs only in *hag4-2*	Description	Significance	
1	AT1G58848 (RF9)	Disease resistance protein (CC-NBS-LRR class) family RF9	Antimicrobial response	upregulated
2	AT1G63360	Disease resistance protein (CC-NBS-LRR class) family	Antimicrobial response	upregulated
3	AT4G27190	NB-ARC domain-containing disease resistance protein	Antimicrobial response	upregulated
4	AT4G37810 (EPFL2)	controlling stomatal density and patterning in the plant epidermis.	Developmental processes	upregulated

Chromatin remodellers were significantly downregulated in *hag4-2* plants upon infection as HAC1, a histone acetyltrasferase of the Arabidopsis P300/CBP family (which is detailed described in chapter 1) and Chromatin remodelling factor 5 (CH5), an ATP-dependent chromatin-remodelling factor (Table 2). CH5 is positive regulator of plant immune responses including the expression of the intracellular immune receptor gene SNC1 (SUPPRESSOR OF npr1-1, CONSTITUTIVE1). Additionally, *chr5* mutant has increased nucleosome occupancy in the promoter region relative to the gene body region at the whole-genome level, suggesting a global role for CHR5 in remodelling nucleosome occupancy (Zou, Sun et al.).

Additionally, various genes that are downregulated in *hag4-2* plants upon infection as *AtELP1*, *PI4KBETA1* and *GIGANTEA* are involved in lateral root development and root growth (Table 3) suggesting that changes in root architecture might contribute to resistance against soil-borne pathogens as *F. oxysporum*.

Table 4. Table displaying the unique downregulated genes *hag4-2* plants. PantherDB was used to identify these genes.

	DEGs only in *hag4-2*	Description	Significance	
1	AT5G13680 (ATELP1, ELO2)	A subunit of Elongator, a histone acetyl transferase complex, consisting of six subunits (ELP1–ELP6)	Developmental processes (reduced root growth that results from a decreased cell division rate)	downregulated
2	AT1G79000 (HAC1)	Histone Acetyltransferase, a member of Arabidopsis P300/CBP family	Plant development and immunity responses	downregulated
3	AT5G64070 (PI4KBETA1)	PHOSPHATIDYLINOSITOL 4-OH KINASE BETA1	Lateral root formation/negative regulators of SA-mediated induction of pathogenesis-related gene expression	downregulated
4	AT1G22770	(GIGANTEA)	Flowering/Lateral root development/immunity responses	downregulated
5	AT2G13370	CHR5, CHROMATIN REMODELING 5	Immunity responses	downregulated

Next, a ChIPseq was performed of Col-0*, hag4-2* and *vfp4-1* plants before and after treatment with *F. oxysporum* to gain an insight into the changes of the acetylation levels of the chromatin post-infection. We show that the TSS is enriched with the epigenetic mark H4K5ac in Col-0 plants after the infection, especially in the upregulated genes in Col-0 after the infection by the soil-borne fungus (Fig. 4). In the same figure, we demonstrate that the H4K5 acetylation levels do not change after the infection in *hag4-2* and *vfp4-1* mutants (Fig. 4). Moreover, the upregulated genes in *hag4-2* mutants after infection have more H4K5ac at the TSS in Col-0 plants while in *hag4-2* and *vfp4-1* mutants the H4K5acetylation does not change after infection (Fig. 5).

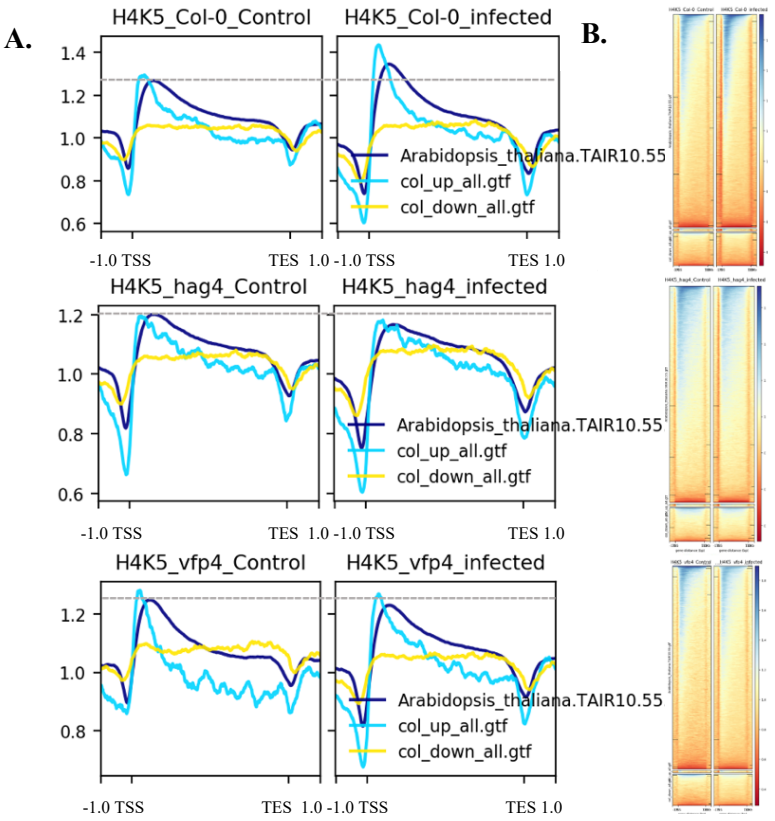


Figure 4. H4K5ac is an important epigenetic mark for resistance against *F. oxysporum*. **A.** Average occupancy of H4K5ac in Col-0, *hag4*-2 and *vfp4*-1 plants before and after infection over Arabidopsis genome and DEGs (upregulated and downregulated as specified) after infection by *F. oxysporum* in Col-0 plants. **B.** Heatmap showing gene expression levels in Col-0, *hag4*-2 and *vfp4*-1 plants before and after infection over Arabidopsis genome and DEGs (upregulated and downregulated as specified) after infection by *F. oxysporum* in Col-0 plants. Genes were sorted by mean signal intensity.

To answer the question why the *hag4-2* mutants have a resistant phenotype against soil-borne pathogens affecting primarily roots and not leaf pathogens, leaf and root samples were collected and RNAseq combined with ChIP seq were performed. The results support that the H4K5 acetylation levels in leaves are generally higher compared to roots (Fig. 6A). Additionally, the H4K5 acetylation levels are lower in leaves of *hag4-2* mutants than Col-0 leaves (Fig. 6) while the *F. oxysporum* defence responses-related genes in Col-0 plants are hyperacetylated at the TSS in both *hag4*-2 and Col-0 without infection (Fig. 6A). Interestingly, the downregulated genes in *hag4*-2 leaves compared to Col-0 leaves have low acetylation in *hag4*-2 mutants while in Col-0 plants the acetylation levels are higher (Fig 6B).

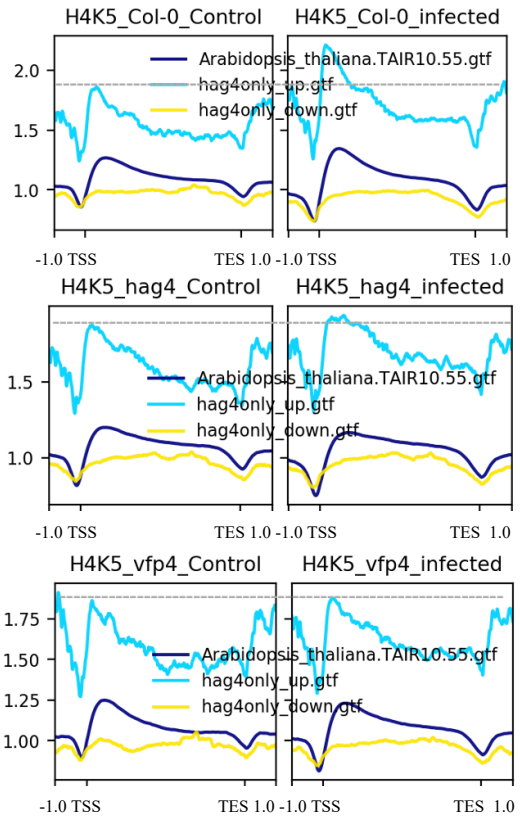


Figure 5. The expression of HAG4-regulated genes is not controlled by H4K5ac. The profiles show the average occupancy of H4K5ac in Col-0, *hag4*-2 and *vfp4*-1 plants before and after infection over Arabidopsis genome and DEGs (upregulated and downregulated as specified) after infection by *F. oxysporum* in *hag4*-2 mutants.

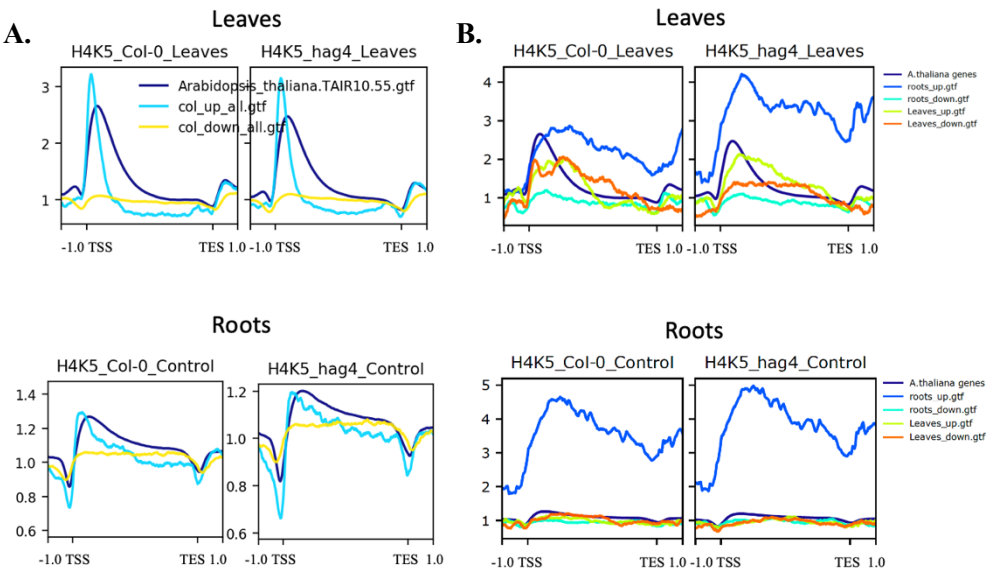


Figure 6. The H4K5 acetylation levels are higher in leaves compared to roots while the *F. oxysporum* defence responses-related genes in Col-0 roots are hyperacetylated in leaves without infection. Average occupancy of H4K5ac in Col-0, *hag4*-2 and *vfp4*-1 plants **A.** over Arabidopsis genome and DEGs (upregulated and downregulated as specified) after infection by *F. oxysporum* in Col-0 plants and **B.**

over Arabidopsis genome and DEGs (upregulated and downregulated as specified) in leaves and roots in *hag4*-2 mutants compared to leaves and roots of Col-0 plants.

Discussion

In chapter 2, the immunity responses of HAG4 were discussed. In details, HAG4 regulates immunity against the root pathogens *V. dahliae* and *F. oxysporum* but it is not involved in responses against the leaf pathogens *P. syringae* and *Hpa*. HAG4 interacts with the transcription factor VFP4 which is known to be involved in bacterial resistance (Garcia Cano et al., 2018). The loss of function mutants of *VFP4,* as well as the mutants of *HAG4,* have no phenotype against pathogens that affect the foliage of the plants. However, *VFP4* mutants were susceptible to both root pathogens *V. dahliae* and *F. oxysporum* while *HAG4* mutants were resistant.

Accordingly, plants of WT (Col-0), *HAG4* and *VFP4* mutants were infected with *F. oxysporum* for transcriptomic analysis and while treated samples of WT (Col-0), *HAG4* plants were grouped together, treated *VFP4* mutants were clustered with the mock samples (Fig. 1). The mutation of *VFP4* causes changes in the transcriptome even before the infection in comparison with WT plants. Col-0 plants are known to be partially resistant to *F. oxysporum* (Diener and Ausubel 2005) so that could be a possible explanation of the transcriptomic differences between Col-0 and *VFP4* mutants.

Responses to *F. oxysporum* promote the differential expression of ~7000 genes in Col-0 at 6 days post inoculation. Since Col-0 and *hag4-2* plants are resistant to *F. oxysporum*, they share most of the DEGs due to infection (Fig. 2). Most of the common DEGs are defence-related responses (Fig. 2). The responses against *F. oxysporum* in *hag4-2* plants are stronger since the unique upregulated genes include more defence-related genes as well as genes involved in cellular homeostasis and response to auxin (Fig. 2). Several *ROXYs* were found to be upregulated only in *hag4-2* plants. *ROXYs* are known to participate in N signalling and root development (Jung, Ahn et al. 2018). It has also been reported that *ROXYs* interact with the TFs, *TGAs* (Gutsche, Holtmannspötter et al. 2017). *TGAs* are responsible for the induction of pathogenesis-related (PR) genes (Kesarwani, Yoo et al., 2007) and confer resistance against the bacterial pathogen *Ralstonia solanacearum* in tomato (Qi, Huang et al., 2022).

SMALL AUXIN UPREGULATED RNAs (*SAURs*) were significantly upregulated as well in *hag4-2* plants 6 days post inoculation. *SAUR*s are members of a large gene family containing early-responsive auxin genes of largely unknown function (Hagen and Guilfoyle, 2002). SAURs were repressed by *F. oxysporum* in Arabidopsis in the leaves but they were not differentially expressed in roots at 6 days post inoculation (Lyons, Stiller et al. 2015). One possible reason could be the age, they used 2-week-old seedlings while in this study, 5-week-old Arabidopsis adult plants were used.

Treatment with auxin analogs enhance disease symptoms in Arabidopsis by biotrophic pathogens (*Pseudomonas syringae* pv. *tomato* (Pto) DC3000) and in rice (infected by *Xanthomonas oryzae* pv. *oryzae)* (Allen, Bittner-Eddy et al. 2004, Navarro, Dunoyer et al. 2006, Chen, Agnew et al. 2007). However, auxin can enhance disease resistance in Arabidopsis against some necrotrophic pathogens. For instance, treatment with auxin analogs recovers the susceptibility caused by *Botrytis cinerea* (Ferrari, Galletti et al. 2008).

The auxin-signalling and transport confers increased resistance to *F. oxysporum* and mutants of auxin signalling are known to have altered root auxin distributions as well as reduced lateral root development (Péret, De Rybel et al., 2009). The authors explain that the fungus may harness auxin transport and signalling to either evade detection by the host, suppress host defences, or enhance host processes that are advantageous to fungal colonization and disease development. Additionally, *F. oxysporum* infection alters root auxin homeostasis, especially in the regions such as root tips and lateral root initials.

As we mentioned before, only in *hag4-2* plants, root development-related genes were differentially expressed. Genes that are essential for root formation and lateral root development are downregulated (such as ROXYs). The infection by *F. oxysporum* begins with an initial, seemingly biotrophic phase of root colonization via xylem vessels. A potential explanation for this response could be that *hag4-2* plants alternate the root architecture in order to evade the colonisation by the fungus.

Interestingly, as we explain in the following chapter, the *hag4-2* mutants have a shorter primary root than Col-0 plants. These results suggest that HAG4 is responsible for transcriptional changes related to root architecture before and after the infection. The root developmental phenotype of *hag4-2* mutants can be used as a defensive strategy against soi-borne pathogens since plants reduce the size of their roots as a means of conserving water and resources, and to help them survive drought or water deficiency during dehydration (Sauter, Davies et al. 2001, Comas, Becker et al. 2013, Daszkowska-Golec 2016). Dehydration is caused by soi-borne pathogens during vascular colonization which affects transportation of water and nutrients.

HAG4 mutation upon *F. oxysporum* infection results in downregulation of important regulators of immunity and other chromatin modifiers. A potential reason for this could be that the HAG4 functions in the same complex with HAC1 and CH5 and the mutation of *HAG4* causes repression of these genes.

In general, resistance against *F. oxysporum*, is mediated through the JA-signalling pathway as the fungus colonises the roots through its necrotrophic phase. In the study of (Kidd, Kadoo et al.), the authors reported induction of the JA-responsive genes *PDF1.2* and *PR4* (hevein-like [HEL]) or induction of other JA-related genes such as those that encode JA biosynthesis enzymes (e.g., *LOX, JMT, AOS, AOC*, and *OPR*) after infection. However, the RNAseq analysis did not reveal any differentially expressed genes related to JA-signalling in *hag4-2* mutants neither *Fusarium* related resistance genes after infection as the *RESISTANCE TO FUSARIUM OXYSPORUM 1* (*RFO)* gene. Therefore, the resistance conferred to the plants by knocking out *HAG4*, is a result of tissue specificity architecture.

In this chapter, the acetylation levels of H4K5 (which is the target of HAG4) were also explored by ChIPseq. Here, we report that H4K5ac is important for defence responses against *F. oxysporum* and is correlated with transcription (Fig. 4). We also suggest that the resistance of WT plants is HAG4- and VFP4-dependent (Fig. 4). Combining transcriptional responses and chromatin immunoprecipitation after infection, we demonstrate that the unique DEGs after infection with the soil-borne fungus in *hag4-2* mutants are not regulated through H4K5ac change (Fig. 5).

In search of an answer about the tissue specificity of *hag4-2* mutants after infection by root pathogens, we performed RNAseq and ChIPseq including leaves samples. The results support that there is more acetylation in leaves of both transgenic and non-lines (Fig. 6). The DEGs after infection with *F. oxysporum* in WT plants already have higher H4K5 acetylation levels at the TSS prior the infection (Fig 6). Moreover, the downregulated genes in *hag4-2* leaves have less acetylation in *hag4-2* leaves and much higher in WT leaves (Fig 6). This subset of genes is regulated by HAG4 in leaves. Overall, the chromatin landscape of roots and leaves is different even before the infection.

However, the subset of genes (DEGs) of leaves vs roots and the DEGs after infection in in *hag4-2* roots are too small and deeptools give an error for plotting the acetylation profiles. For this reason, the acetylation profile of these subsets of genes may not be trustworthy. A way to test this is to perform ChIP assays on few genes in each subset to confirm the plots. Additionally, a ChIPseq including leaves after infection might give a further insight of the tissue specific immunity responses.

Chapter 4

Improvement of plant growth and immunity by impediment of MYST histone acetyltransferases

Alexia Tornesaki[1], Veselina Uzunova[1], Vardis Ntoukakis[1]

[1]School of Life Sciences, University of Warwick

Abstract

Acetylation of the N-terminal tails of histones by histone acetyltransferases (Tran, Jones et al.) and deacetylation by histone deacetylases (HDACs) can regulate transcriptional responses. Arabidopsis HAG4 together with its close homologue HAG5 belong to the MYST-HAT family. According to previous studies, AtHAG4 and AtHAG5 work redundantly to regulate gametophyte development and flowering time. We showed that the two MYST-HATs interact with distinct set of transcription factors and have different functions. AtHAG4 regulates immunity against root pathogens while AtHAG5 regulates plant growth and immunity against leaf pathogens. Interestingly, not all plant species have two homologues of MYST-HATs. Tomato (*Solanum lycopersicum*) has a single copy (HAG4-like), but Brassica (*Brassica oleracea*) has two copies of MYST-HATs, which raise the question if a single copy of MYST-HAT can perform both functions of MYST family. To address this question, we performed an *in silico* screen to identify chemical inhibitors of the HATs and then tested them on tomato, lettuce and brassica to explore whether they mirror *hag5* mutants phenotypes (longer primary root, bigger rosette and drought tolerance) on MS plates and soil. The selected compound provides the crop plant with the desirable phenotypes which proves our hypothesis that HAG4 alone can perform both HATs functions.

Introduction

The Arabidopsis MYST family of HATs has been shown to regulate diverse cellular processes such as DNA repair, stem cell homeostasis and cell-cycle regulation through acetylation of histone 4 (H4) lysine residues (Yuan et al. 2012). HAG4 and HAG5 occurred from a duplication event that took place during polyploidization on its Brassicaceae ancestor. They are believed to have redundant functions in Arabidopsis, since *hag4hag5* double mutation induced severe defects in the formation of male and female gametophyte, resulting in an arrest of mitotic cell cycle at early stages of gametogenesis (Latrasse, Benhamed et al. 2008).

In addition, HAG4 together with its close homologue HAG5 are assumed to work redundantly to regulate flowering (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013). The amiRNA-HAG4/5 transgenic plants showed early flowering and reduced fertility. Both members of *Arabidopsis* MYST family have been shown to specifically acetylate the same target (H4K5) *in vitro* (Earley et al. 2007) and share 84% amino acid sequence identity. In Chapter 2, the role of MYST HATs in plant immunity was investigated. HAG4 and HAG5 act as negative regulators of defence against root and aerial pathogens respectively. In detail, HAG4 modulates defence against the root necrotrophic pathogens, *V. dahliae* and *F. oxysporum* whereas HAG5 is involved in immunity responses against the leaf biotrophic pathogens, *Pto DC3000* and *Hpa*. These findings suggest that reducing the expression of *HAG4* and *HAG5*, could be beneficial to the plants regarding defence.

Due to the high conservation of HAG4 and HAG5 across plant lineages, targeting them in an agricultural context could be promising to translate these advantageous phenotypes to crop plants. Three different approaches for improving plant performance are implemented on this chapter. The first approach consists of evaluation of growth and developmental phenotypes of mutants of HAG4 and HAG5. The second approach includes screening of chemical inhibitors identified using *in silico* modelling of the MYST family by performing different phenotypic assays to evaluate whether inhibitors can replicate the mutation phenotypes when applied to Col-0 plants. The last approach comprises screening of multiple crop

plants (tomato, spinach, and lettuce) with the selected inhibitor to test whether the beneficial inhibition of MYST family can be translated to other plant species.

Materials and methods

Plant materials and growth conditions

Arabidopsis thaliana

Arabidopsis thaliana ecotype Columbia (Col-0) is used as wild type and treated as described in chapter 2. The Arabidopsis mutants used in this chapter are described in chapter 2. For *in vitro* work, *Arabidopsis* seeds were surface sterilised by chlorine gas exposure. Seeds in a seed storage (glassine) bag were incubated with chlorine gas for 4h inside a sealed desiccator. The chlorine gas was produced by adding 3mL of hydrochloric acid 37% to a beaker containing 100mL of 10% sodium hypochlorite. Seedlings were grown in sterile half (2.15g/L) Murashige and Skoog medium (1/2 MS, Duchefa Biochemie, 50 ml of the medium was placed), with 1% sucrose if indicated, pH adjusted with KOH 1 M at 5.80 and 0.5% Phytagel (Sigma). All plating procedures were carried out under sterile conditions using a Class II cabinet. Seedlings were stratified in darkness in cold room at 4ºC for 2 days and then placed vertically to a Sanyo cabinet set to 10h light, 21°C, 60% humidity conditions.

Brassica oleracea

B. oleracea DH1012 seeds were sown in FP7 pots containing F1 compost. Seeds were sown to twice the depth of the size of the seed, and grown in short photoperiod of 10h light, 21°C, 60% humidity. For *in vitro* work, *B. oleracea* seeds were sterilised with 70% ethanol for 1 minute, 10% bleach with a drop of Tween-20 detergent and regular shaking for 30 minutes, rinsing 3-4 times with sterile water and then fridge for 24 hours. The seeds were placed on ½ MS plates as described for *A. thaliana*.

Solanum lycopersicum

For *in vitro* work, *S. lycopersicum* seeds were sterilised with 70% ethanol for 1 minute, 10% bleach with a drop of Tween-20 detergent and regular shaking for 30 minutes, rinsing 3-4 times with sterile water and then at 4°C for 24 hours. After the sterilisation, the seeds were plated on ½ MS plates. The seeds were placed on ½ MS plates as described for *A. thaliana*. For growth in pots, *S. lycopersicum* seeds were sterilised and plated on ½ MS plates for a week and then were transferred to FP7 pots containing vermiculite (4/5 of the pot) and perlite on the top (1/5 of the pot).

Lactuca sativa

Seeds of *Lactuca sativa* were used for *in vitro* work. The seeds were sterilised with 70% ethanol for 1 minute, 10% bleach with a drop of Tween-20 detergent and regular shaking for 30 minutes, rinsing 3-4 times with sterile water and then at 4°C for 24 hours. After the sterilisation, the seeds were plated on ½ MS plates. The seeds were placed on ½ MS plates as described for *A. thaliana*.

Developmental assays

Leaf Surface area measurement

Pictures of Arabidopsis 24 5 weeks old plants rosettes were taken before plants attach each other using a ruler for setting the scale up. Leaf surface area was calculated using ImageJ (https://imagej.nih.gov/ij/). 24 plants were measured per genotype. The average leaf surface area was used to represent the size of each genotype.

Root apical meristem measurement

Arabidopsis seedlings were grown *in vitro*. Ten days after germination, ~1 cm root tips were cut and placed on glass slides. The root tips were immersed in a solution of 100µL of 10µg/µL of Propidium Iodide (Sigma P-4170) diluted in water (concetration 1%), covered with cover slip and left for 10 minutes incubation in the dark. Root tips were mounted on a microscope slide with water and imaged using confocal microscope Leica TSC SP5. At least 10 root tips were imaged per treatment/genotype. Root meristem size was expressed as the number of cells extending from the quiescent centre to the first elongated cortex cell. The number of cells was measured with ImageJ.

Primary root length measurement

Arabidopsis seedlings were grown in $^{1/2}$ MS plates. 15 days post germination, the plates were scanned using a HP PSC 2500 scanner, including a ruler to set the scale. Root length was measured with ImageJ (Schneider, Rasband et al. 2012), using the free-hand tool.

Drought stress assays

Drought tolerance in soil

24 plants per genotype were grown in soil as described at 2.1.1. and placed in Aralab chamber, 10h light, 22°C, 60% humidity. The plants were being watered normally until the age of 4 weeks and then they left unwatered for 2 weeks. After the stress, plants were re-watered and the trays were covered with a plastic cap for recovery for 2 days. Pictures of plants were taken during the stress and after the recovery. The percentage of recovery was calculated as the number of plants that had recovered / total number of plants x 100.

Drought tolerance in MS plates

Seedlings of each genotype were grown in MS plates for 10 days after germination. Then the plants were transferred under sterile conditions to plates with 25ml of ½ MS medium and left until they dry. Pictures of plates were taken when phenotypical differences in leaf turgor were observed (~5 weeks after sowing the seeds). For reduced water potential, 10 days old seedlings were transferred under sterile conditions to plates with 25ml of ½ MS medium and 3 different concentrations of PEG as described by (Kumar, Sahoo et al. 2015). The seedlings were left to dry and pictured were taken frequently. Three different concentrations of PEG were used. For -0.25 MPa, 0 gr of PEG was added per litre of media, for -0.5 MPa, 250 gr of PEG was added and for -0.7 MPa, 400 gr of PEG was added.

Screening of HAG4/HAG5 inhibitors

The screening of the inhibitors was based on root meristem inhibition assays. Arabidopsis seedlings were grown vertically *in vitro*. The seedlings were transferred to ½ MS plates supplemented with DMSO (10mg/mL) or 2μM of inhibitor (see Table 1) 7 days after stratification. The seedlings were then incubated vertically overnight and the root tips were imaged.

Table 1. HAG4/HAG5 inhibitor candidates from the ChemBridge library used in this study.

Name	Compound name
A	N-(4-{[5-(3-nitrophenyl)-2-furoyl]amino}phenyl)-1-benzofuran-2-carboxamide
B	3-(butyrylamino)-N-[1-(3-hydroxybenzyl)piperidin-3-yl]benzamide
C	2-[4-allyl-5-({2-[(4-methyl-3-nitrophenyl)amino]-2-oxoethyl}thio)-4H-1,2,4-triazol-3-yl]-N-(2-methylphenyl)acetamide
D	N-(2-methoxyethyl)-1-[1-(3-phenylpropanoyl)-4-piperidinyl]-1H-1,2,3-triazole-4-carboxamide
E	N,N'-(1,4-piperazinediyldi-3,1-propanediyl)bis(3-methylbenzamide)
F	2-[({5-[1-(5-methoxy-2-furoyl)piperidin-4-yl]-4-methyl-4H-1,2,4-triazol-3-yl}methyl)(methyl)amino]ethanol
G	N-{3-[4-({[3-(3,5-Dimethyl-1H-pyrazol-1-yl)propanoyl]amino}methyl)-5-methyl-1,3-oxazol-2-yl]phenyl}-2,5-dimethyl-3-furamide

Results

The findings presented in chapter 2 indicate that reducing the expression of *HAG4* benefits plant response to root pathogens while repressing *HAG5* expression results in beneficial phenotypes, both in the context of response to pathogens and tolerance to drought. As demonstrated in chapter 2, Dr Ana Gil Gonzalez, a previous PhD student in Ntoukakis group, reported that HAG5 acts as a negative regulator of defence and drought tolerance by modulating the transcriptional activation of ABA responsive genes. Targeting both HAG4 and HAG5 in an agricultural context has the potential to translate these advantageous phenotypes to crop plants. As mentioned above, both members of *Arabidopsis* MYST family have been shown to specifically acetylate the same target (H4K5) *in vitro* (Chinchilla, Zipfel et al. 2007) and share 84% amino acid sequence identity. The majority of sequence divergence is located at the N-terminus of the protein, close to and within the chromodomain suggesting that may interact with different transcriptional factors (TFs) (Fig. 1B).

Dr. Veselina Uzunova, a post-doctoral researcher at Ntoukakis Group, performed an *in silico* modelling of both proteins. In order to develop inhibitors of HAG4 and HAG5 catalytic activity, she characterised structural differences and demonstrated that these proteins have different TUDOR domain organisation while their acetyltransferase domains are almost identical (Fig. 1). The group member evaluated these chemical compounds to bind in the acetylation domain. Since this domain is almost identical (Fig 1B), the inhibitor should have the ability to bind, inhibit and affect potentially both of the proteins.

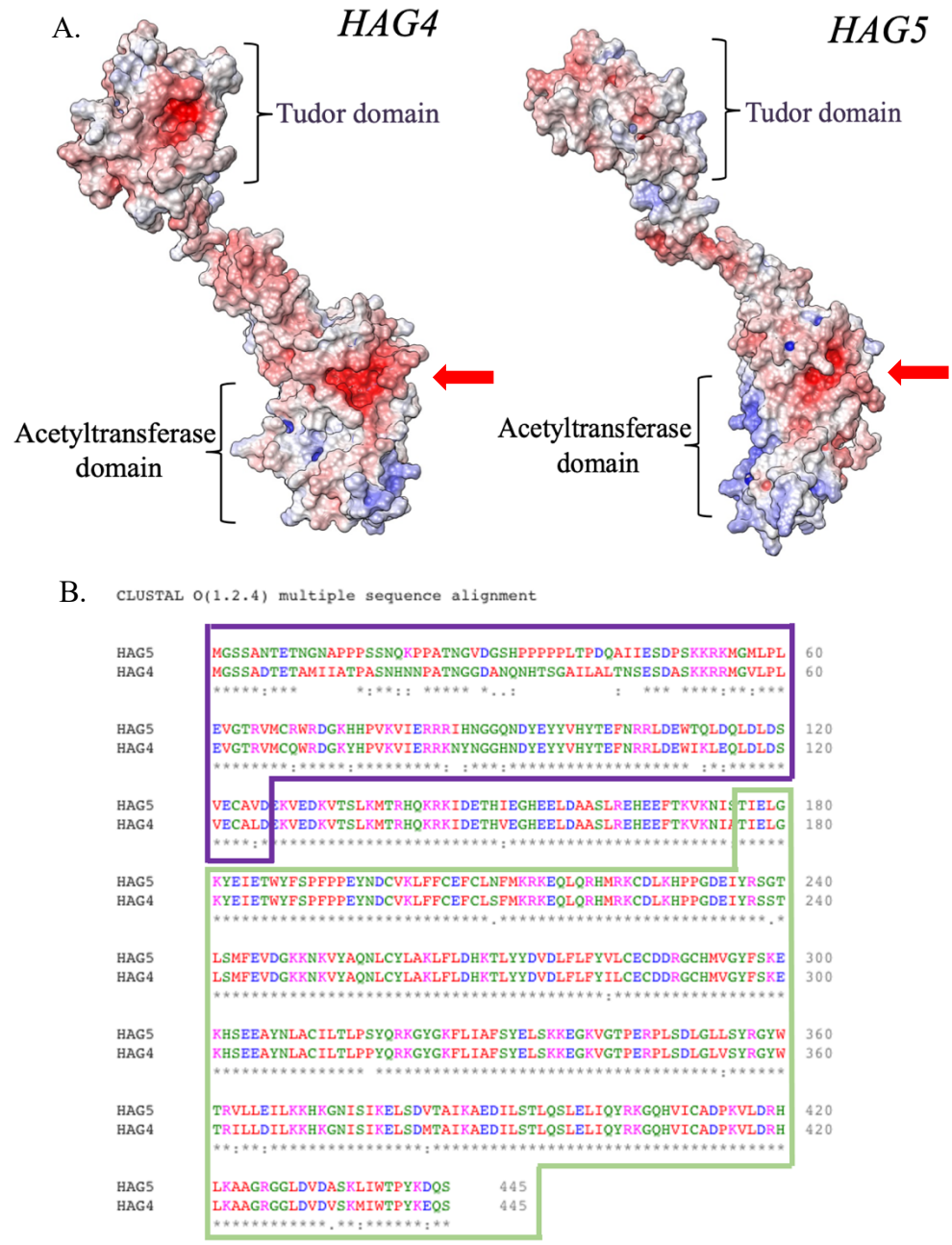


Figure 1. The Tudor domain of the two MYST HATs has the most sequence differences. **A.** Protein sequence alignment of Arabidopsis HAG4 and HAG5 using Clustal Omega (version 1.2.4). The sequence presented in purple represents the Tudor domain, the colour green represents the Acetylation domain and the sequence between them is the linker domain. The same colouring for aligned residues indicates conservation of amino acid chemical properties. (ClustalX colouring, blue: hydrophobic, red: positively charged, magenta: negative charged, green: polar, cyan: aromatic, pink: cysteine, yellow: proline, orange: glycine). The red arrows demonstrate where the inhibitors bind. **B.** 3D in silico modelling of HAG4 and HAG5 folded proteins. Hydrophobic pockets are represented in red, whilst hydrophilic ones are represented in blue. The three distinct domains (catalytic, disordered and Tudor) are shown by arrows. The homology modelling was done by Dr. Veselina Uzunova, a post-doctoral researcher at the Ntoukakis group.

Considering the immunity phenotypes of MYST HATs, developmental phenotypes were also explored in order test any other benefits of their inhibition. Indeed, developmental differences were found in adult plants (5-weeks-old) of *hag5* mutants. Mutants of *hag5-2* mutants have increased leaf surface area (Fig. 2A and B). On the other hand, *hag4-2* have the same leaf surface area as Col-0 plants (Fig. 3). Root length was also measured where *hag5-2* mutants were found to have longer while *hag4-2* has shorter primary roots in comparison with Col-0 plants (Fig. 2C).

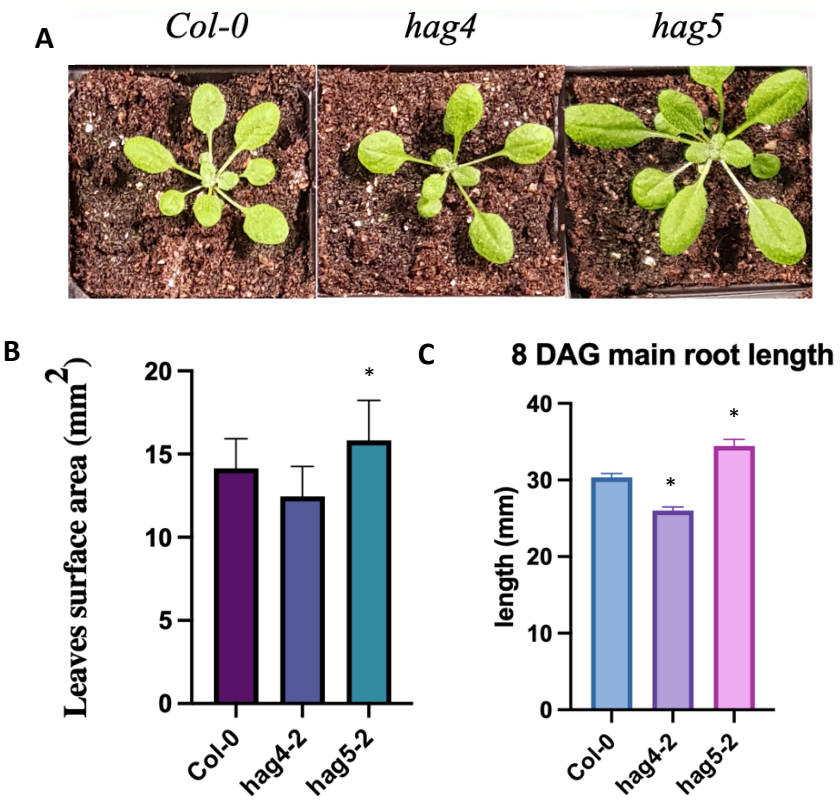


Figure 2. Developmental phenotypes of *Col-0, hag4-2* and *hag5- 2* plants. **A.** Representative pictures of the rosettes of 5-week-old *Col-0, hag4-2* and *hag5-2* mutants grown under short-day photoperiod. **B.** Measurement of the leaves of 4 weeks old plants surface area of *Col-0*, *hag4-2* and *hag5-2* mutants by ImageJ. **C.** Primary root length was measured 8 days after germination. The experiment was repeated 3 times with 10 seedlings per genotype. Statistical significance versus *Col-0* was determined by two-tailed t-test, * $P \leq 0.05$, n=30.

As described before, HAG5 interacts with the TF, ARIA, which is involved in ABA signalling. ARIA was initially discovered through its interaction with AREB1/ABF2 (Kim, Kang et al. 2004) a known bZIP transcription factor involved in abscisic acid (Morris, Rao et al.) signalling and osmotic stress responses (Kim, Kang et al. 2004). ABA regulates many development and growth processes, and it is the main regulator

of drought stress response. To investigate the role of HAG5 in drought tolerance, 4 week-old plants of *Col-0*, *hag5-2* and *hag4-2* mutants were exposed to drought stress. *hag5-2* mutants show a better performance under drought conditions compared to *Col-0* and *hag4* mutants (Fig. 3A). When we re-water the plants, *hag5* mutants seem to recover better that the rest (Fig. 3B).

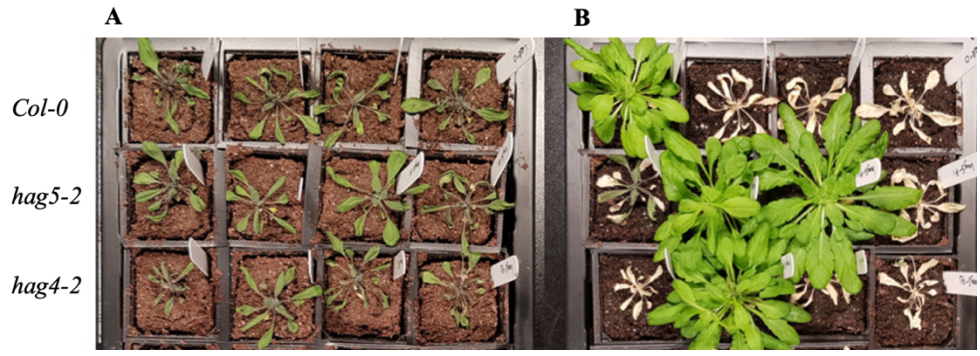


Figure 3. Drought responses of *hag5-2* mutants in soil. Representative pictures of **A.** 4 weeks old plants of *Col-0*, *hag4-2* and *hag5-2* under drought conditions (2 weeks) and **B.** after the non-watering period, plants were re-watered for 2 weeks. n=30.

To further investigate whether HAG4 and HAG5 are involved in drought tolerance, we tested the root elongation of the seedlings (7 days old plants) under different osmotic potential. Decreasing the water potential of the media (Ψw) by using polyethylene glycol (PEG)-infused plates (Kumar, Sahoo et al. 2015) is an accurate method to evaluate drought tolerance. Drought is defined as a decrease in soil water availability, which can be quantified as a decrease in water potential. Mathematically, Ψw is the chemical potential of water divided by the partial molar volume (Boyer and Kramer 1995). Decreased Ψw translates into higher difficulty of water uptake by the plant, which then starts an array of responses to either avoid water loss and facilitate water uptake or to tolerate reduced water content in the tissues (Kumar, Sahoo et al. 2015). Loss of water availability causes a number of rapid stress responses including high levels of ABA accumulation and induction of stress and ABA-regulated genes (Kumar et al., 2015).

At the water potential of Ψ_w is -0.5 MPa, *hag4-2* mutants are smaller than Col-0 and at Ψ_w of -0,7 MPa (higher concentration of PEG), *hag4-2* mutants stop growing. By contrast, *hag5-2* mutants show better performance than *Col-0* in all water potentials

(Fig. 4). The *hag4-2* mutants have significant less growth in mock condition (-0.25MPa) and in the highest stress (-0.7MPa) (Fig. 4B) compared to Col-0 plants while *hag5-2* mutants have longer roots than Col-0 in both stress level conditions (Fig. 4B). Where the ratios of root elongation of -0.25 MPa, -0.5 MPa and -0.7 MPa was measured, HAG4 mutation confers stop of root elongation in higher level of drought stress while HAG5 mutation confers longer roots upon less severe drought stress.

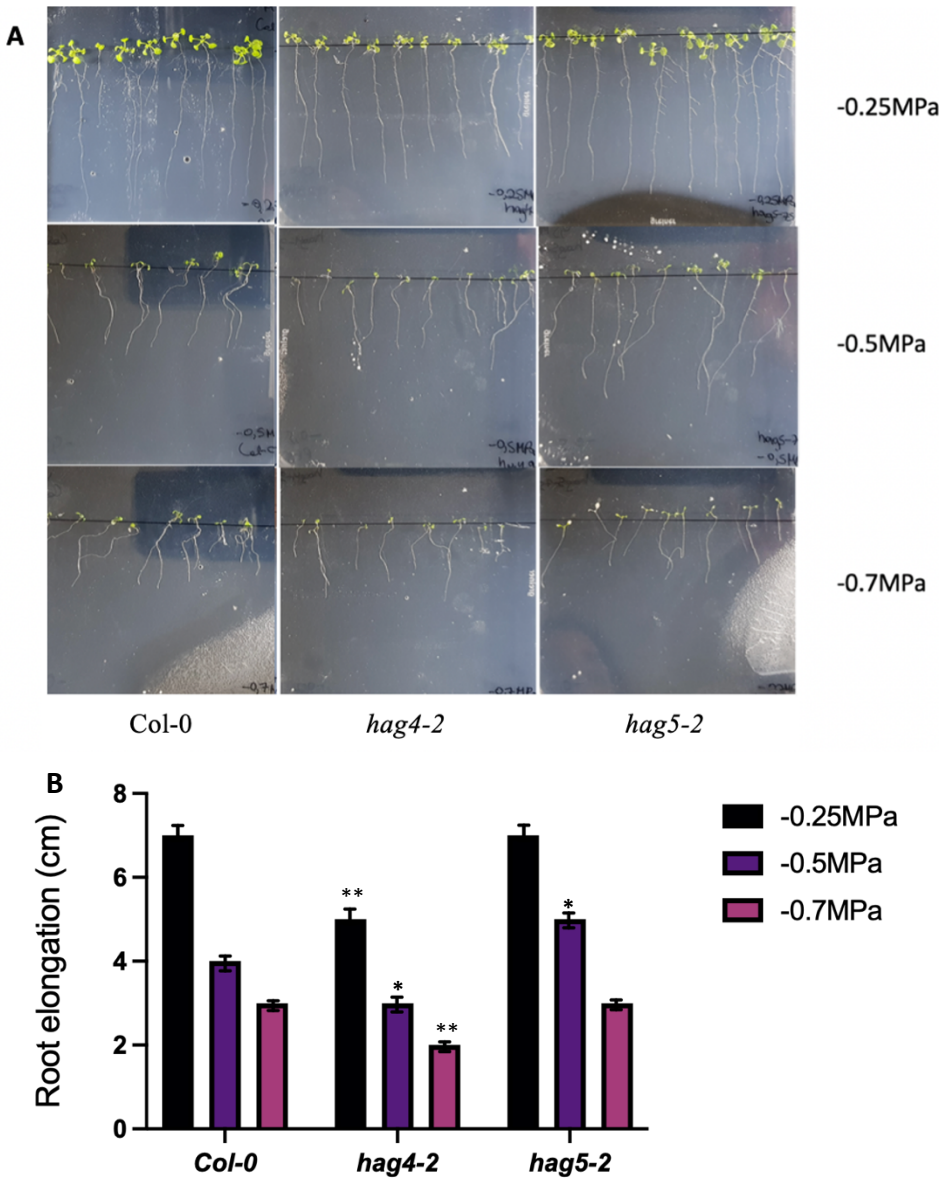


Figure 4. Drought responses of *hag5-2* mutants by dehydration assays using PEG-infused plates for low water potential treatment. **A.** Final water potential of the PEG-infused plates is -0.25 MPa, -0.5 MPa and -0.7 MPa. **B.** Root length (cm) of the seedlings during dehydration assays. The experiment was repeated 3 times. Statistical significance was measured versus Col-0 at each corresponding treatment and was determined by two-sided T-test, n = 10, * $P \leq 0.05$, **$P \leq 0.005$.

Developing *Arabidopsis* MYST family inhibitors to improve crop performance

The findings presented above indicate that reducing or repressing the expression of *HAG5* results in beneficial phenotypes, both in the context of response to pathogens and tolerance to drought. *hag5* loss-of-function mutants are bigger plants, with increased leaf area, longer roots and bigger root meristem. Targeting HAG5 in an agricultural context has the potential to translate these advantageous phenotypes to crop plants. Chemical inhibitors were identified by Dr. Stephanie Kancy and Dr. Veselina Uzunova (members of Ntoukakis group) using *in silico* modelling of HAG5. They created a homology model of HAG5. Once the model had been quality-evaluated, the ChemBridge library of structural data of over a million compounds was used to screen for compounds with agrochemical properties (foliar absorbed, able to translocate into the plant trans vascular system, able to translocate into the nucleus and have low reactivity towards non-plant HATs). Subsequently, an *in silico* docking was performed with the filtered 979,672 compounds to identify candidate inhibitors. The details of the homology model generated, and the computational docking performed against the AtHAG5 homology model can be found within Dr Kancy's PhD thesis.

Dr Uzunova and Dr. Kancy ran several docking simulations with the selected list of inhibitors against a homology model created for HAG4 following the same approach for modelling HAG5 in order to investigate whether the inhibitors were specific for MYST-histone acetyltraqnsferases. In addition, compounds were also filtered out for their affinity to bind to a homology model of the HAG5 human monologue HsKAT8, in order to select for compounds with low toxicity.

Screening of these inhibitors by performing different phenotypic assays are done in order to evaluate if inhibitors are able to replicate *hag5-2* mutant phenotypes when applied to Col-0 plants. All the inhibitors selected A-G were added on ½MS plates and Col-0 plants were growing for 4 weeks. Initial phenotypic observation suggested that inhibitor A protects seedlings from dehydration (Fig. 5).

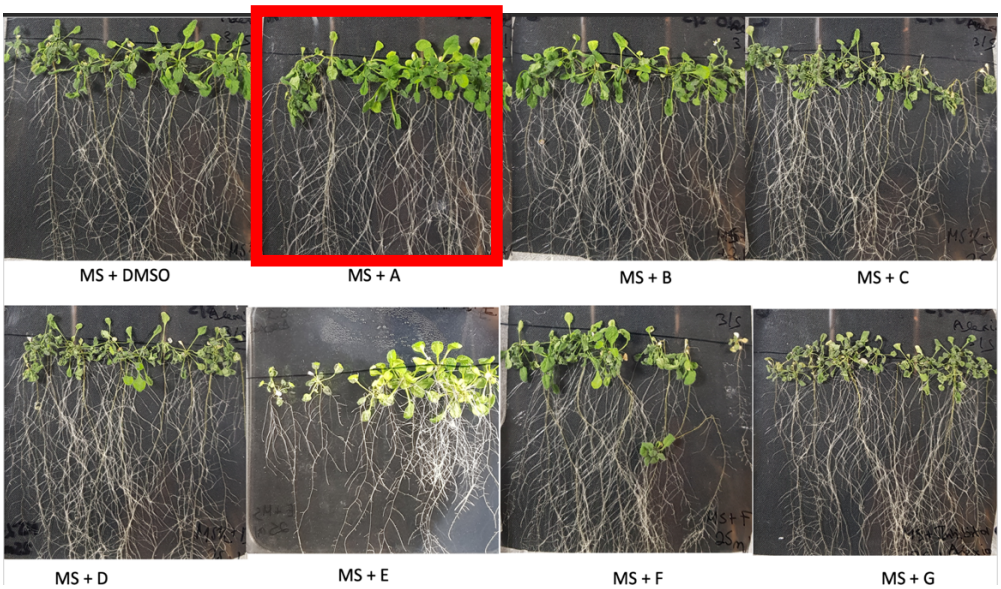


Figure 5. Inhibitor A protects Arabidopsis seedlings from dehydration. Dehydration plates with DMSO (control) and the 7 chemical compounds (Daszkowska-Golec). *Col-0.* 14 days old plants have been transferred to ½MS plates with the inhibitors A-G until they entirely dry. The experiment was repeated 3 times, n=15.

To further test the list of inhibitors, the root meristem size of Col-0 plants was measured with confocal microscopy after being transferred to normal ½ MS plates with the chemical compound in order to assess whether supplementing Col-0 plants with the inhibitors would confer increased tolerance to drought, as previously observed in *hag5-2* mutants. Plants reduce the size of their roots when they become dehydrated as a means of conserving water and resources, and to help them survive drought or water deficiency (Sauter, Davies et al. 2001, Comas, Becker et al. 2013, Daszkowska-Golec 2016). From all the inhibitor candidates, inhibitors A, B, C and G increased the meristem size of Col-0 plants after treatment (Fig. 6). Therefore, inhibitors D, E and F were discarded from further assays.

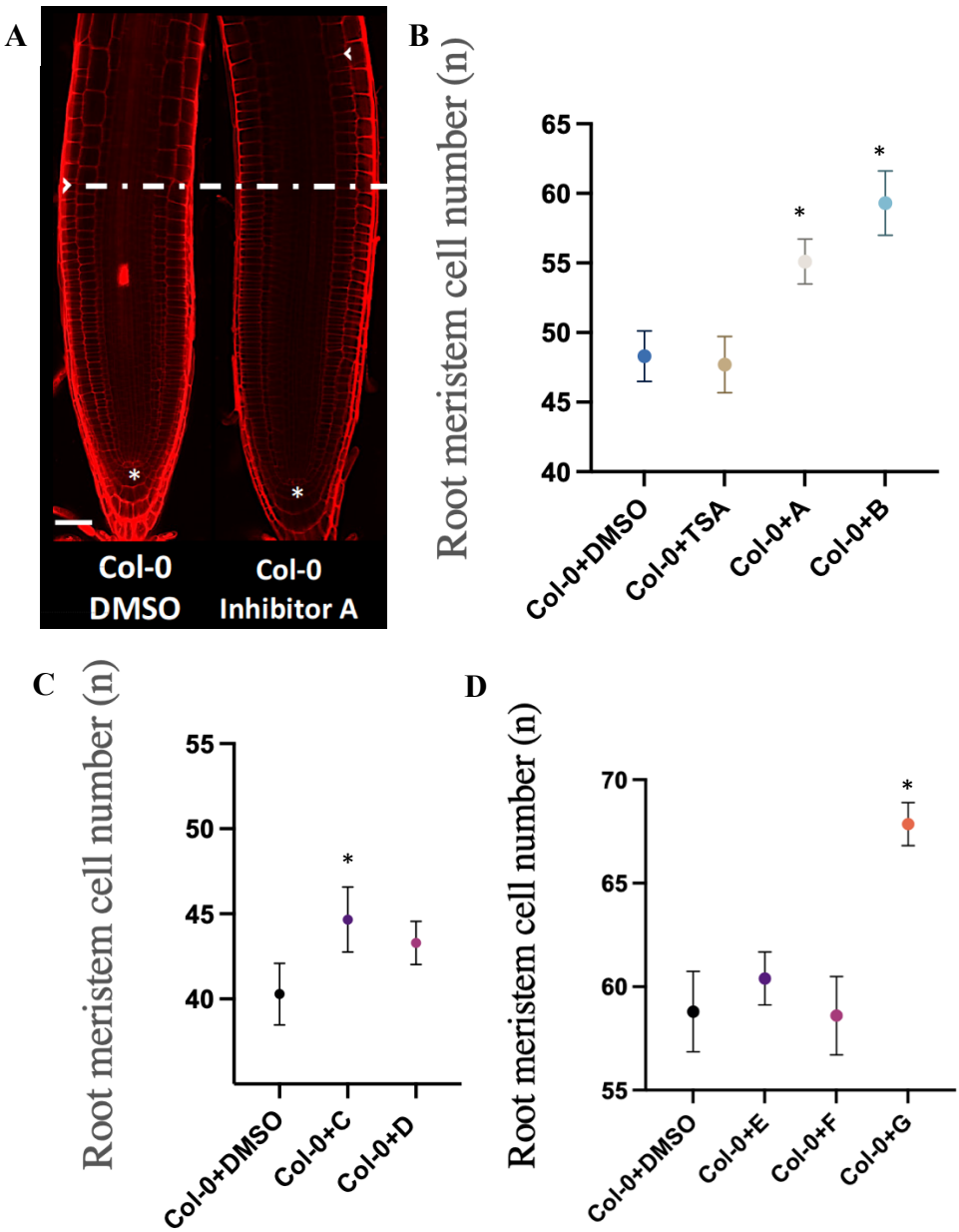


Figure 6. Meristem cell number is increased after the addition of the inhibitor A. **A.** Propidium iodide (PI) staining of 10 days old *Arabidopsis* roots. 10 root tips were imaged per treatment grown on ½ MS plates. The quiescent center (QC) is marked with an asterisk. The meristem size was determined as the number of meristematic cells form the QC to the first expanding cell (marked with arrows). DMSO is the control. Trichostatin A (TSA), a known class I and II HDAC inhibitor, is used as a positive control. **B-D.** *Col-0* plants were transferred to normal ½ MS plates (50ml of media per plate) with the chemical compounds A-G for 24 hours before imaging. The number of the cells in the room meristem between the QC and the elongation zone is measured. Asterisk indicates significant difference from mock treatment (DMSO) as determined by Student's two-tailed t test. *, $P < 0.05$.

Combining the drought assay (Fig. 5) and the root meristem measurements (Fig. 6), the drought tolerance and the increased meristem size conferred by inhibitor A

simulated HAG5 mutation. Thus, I proceeded to test the effects of this inhibitor to different crop species.

Interestingly, not all plant species have two homologues of MYST HATs. For instance, tomato (*Solanum lycopersicum*), spinach (*Spinacea oleracea*) and lettuce (*Lactuca sativa*) have only one copy of MYST HATs which raise the question if a single copy of MYST HATs can perform both functions of *Arabidopsis* MYST family (Fig. 7). *Brassica oleracea* has both copies of the HATs of MYST family as Arabidopsis.

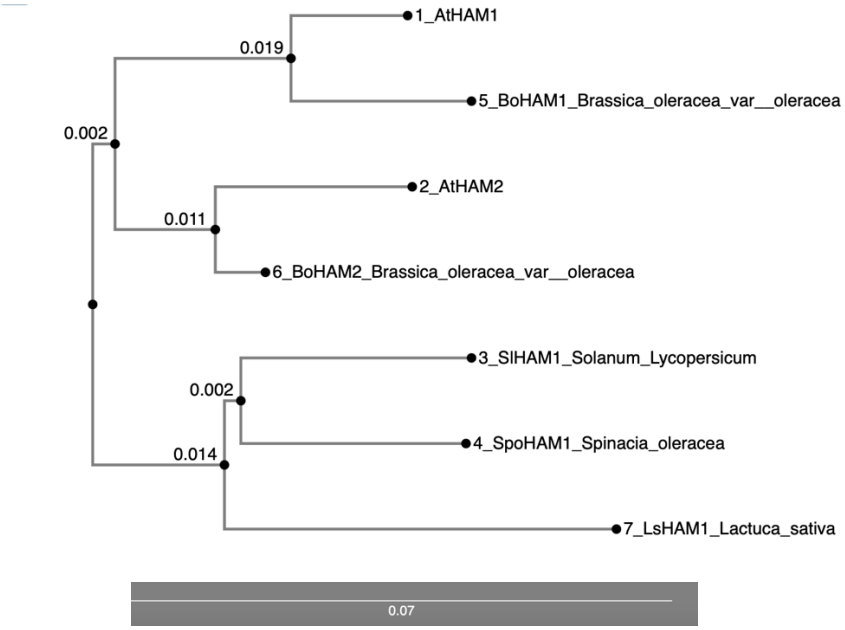
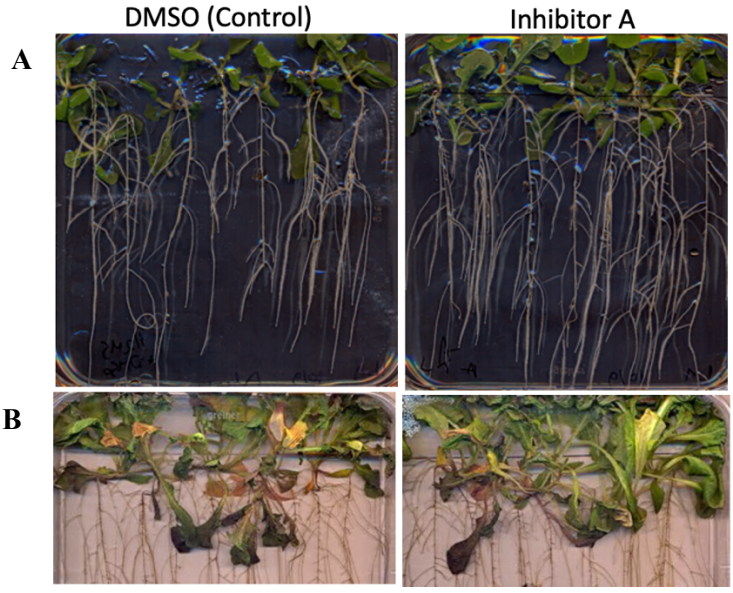


Figure 7. Phylogenetic tree of MYST family copies of *Spinacea oleracea, Brassica oleracea, Arabidopsis thaliana, Solanum lycopersicum* and *Lactuca sativa. Arabidopsis* and *B. oleracea* have two copies of MYSTs (HAM1 and HAM2), tomato and lettuce have a single copy.

In order to translate this research to crop plants, a collaboration was established with Enza Zaden, providing us with seeds of tomato, spinach and lettuce. Seedlings of tomato, spinach, lettuce as well as Brassica were screened to examine whether inhibitor A can enhance their drought tolerance. Indeed, the seedlings of all the aforementioned crop species were tested, and inhibitor A enhanced their drought tolerance when grown in ½ MS plates (Fig. 8, 9 and 10).

Figure 8. Inhibitor A enhances *Brassica oleracea* drought tolerance. Seedlings were sown on ½ MS plates. When 7 days old, the seedlings were transferred in ½ MS plates with DMSO (Control) or inhibitor A until they dry. The experiment was repeated 3 times.

Since *HAG5* mutation confers bigger size of plants, and longer roots, pictures of lettuce and tomato were taken 7 days and 30 days after the transfer to plates with the inhibitor A. The root length of lettuce and tomato was also measured before and after the addition of the inhibitor A as well as the secondary roots number. After the transfer to MS plates with the inhibitor A, lettuce plants grow more, were more drought tolerant and had longer roots and more secondary roots in comparison with the control plant (Fig. 9).



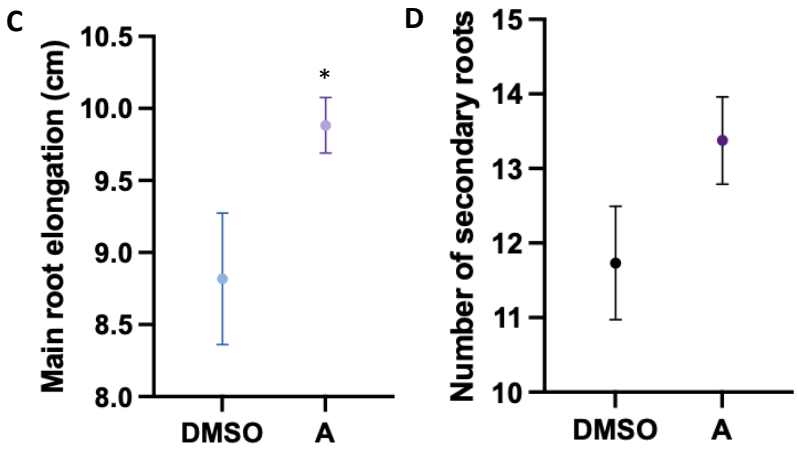


Figure 9. Testing inhibitor A on lettuce. Lettuce (Malami 2017.504880, Enza Zaden) seedlings were sown on ½ MS plates. When 7 days old, the seedlings were transferred on ½ MS plates with DMSO (Control) or inhibitor A and left until dry. Pictures were taken **A.** 7 days and **B.** 30 days after the transfer. **C, D**. The length of primary roots and the number of secondary roots were measured 30 days post germination. The experiment was repeated 3 times with 10 seedlings per treatment. Asterisk indicates significant difference from mock treatment (DMSO) as determined by Student's two-tailed t test. *, $P < 0.05$.

The addition of inhibitor A to tomato plants confers drought tolerance although the root length and the number of secondary roots were not significantly altered (Fig. 10). In order to further evaluate the drought tolerance in tomato after the addition of inhibitor A, tomato plants were grown in vermiculite/perlite pots. Pictures were taken 2 weeks and 1 month after the inhibitor was added, and 3 days after re-watering the pots. Tomato plants with inhibitor A were bigger plants, and they recovered after they were being re-watered in comparison to control plants (Fig.11).

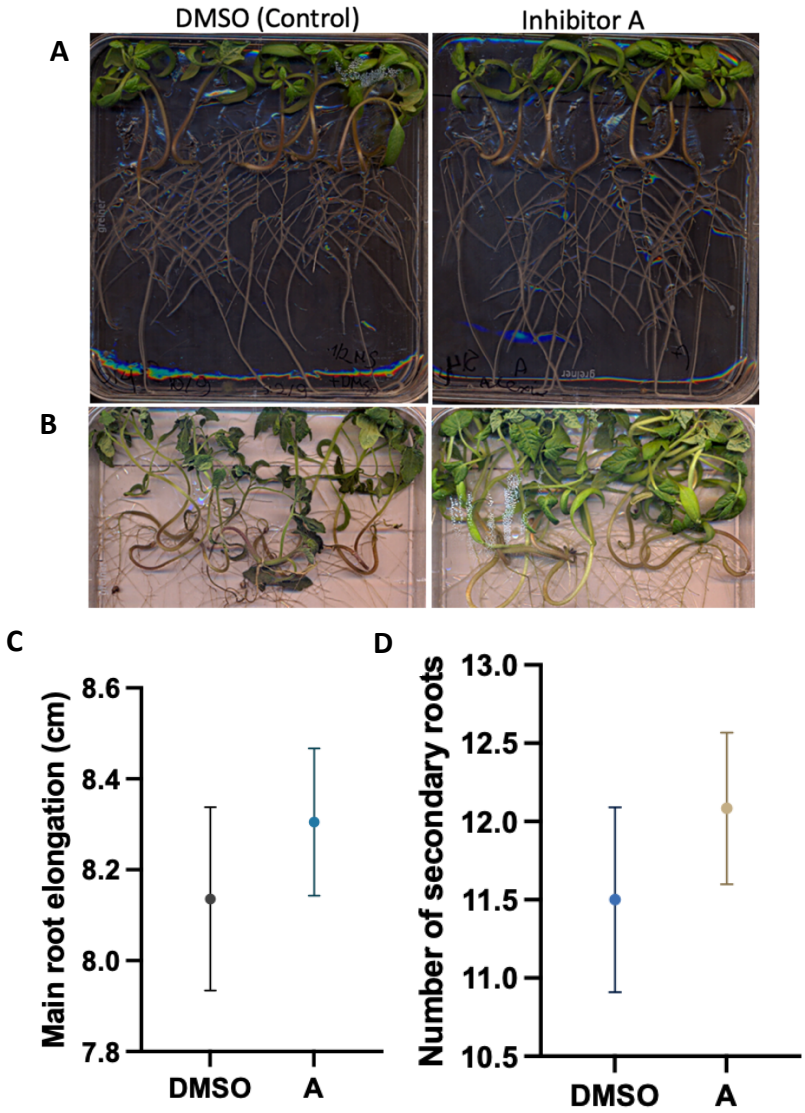


Figure 10. Inhibitor A does not significantly enhance drought tolerance on tomato plants grown on MS plates. **A.** Tomato (Batistuta 906845, Enza Zaden) seedlings were sown on ½ MS plates. When 7 days old, the seedlings were transferred on ½ MS plates with DMSO (Control) or inhibitor A and left until dry. Pictures were taken 7 days and **B.** 30 days after the transfer. **C, D**. The length of primary roots and the number of secondary roots were measured. The experiment was repeated 3 times with 10 seedlings per treatment. There is no significant difference from mock treatment (DMSO) as determined by Student's two-tailed t test.

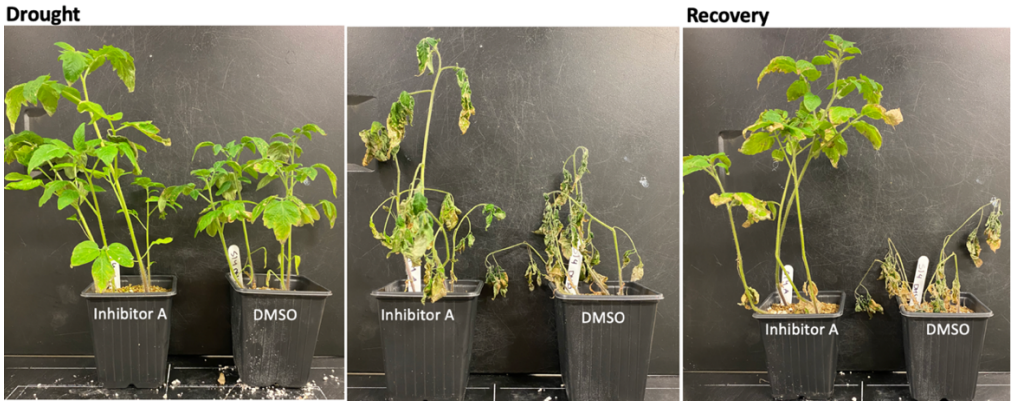


Figure 11. Inhibitor A enhances growth and drought tolerance in adult tomato plants. Tomato (Batistuta 906845, Enza Zaden) seedlings were sown on ½ MS plates. When 7 days old, the seedlings were transferred on vermiculite/perlite pots. After a month of regular watering, DMSO (Control) or inhibitor A was added and left until dry. **A.** Pictures were taken 2 weeks and **B.** 1 month after the last watering. The plants were watered again and **C**. picture was taken 3 days later. The experiment was performed once with 15 plants per treatment.

Discussion

The roles of the MYST HATs, HAG4 and HAG5 in regulating plant immunity was extensively explored in Chapter 2. In brief, HAG4 modulates defence against the root necrotrophic pathogens, *V. dahliae* and *F. oxysporum* whereas HAG5 is involved in immunity responses against the leaf biotrophic pathogens, *Pto DC3000* and *Hpa*. Since the mutation of these HATs is beneficial as confers better performing plants under stress, HAG4 and HAG5 comprise a good target for improving agricultural crops. Most of structural and sequencing differences of these HATs are located at the N-terminus of the protein (Fig 1 and 2) which explains their interaction with different TFs and have different function as explored in chapter 2.

Plant developmental and drought stress phenotypes were also explored since HAG5 is involved in ABA signalling which affects growth and drought responses. *hag5* mutants are bigger plants, with longer roots while *hag4* mutants show no differences to Col-0 plants (Fig. 3). Interestingly, HAG5 mutation also confers drought tolerance while *hag4* mutants were hypersensitive to drought (Fig. 4).

These findings highlight HAG5 as an excellent target for improving agricultural crops in plant development, immunity, and drought. Thus, 8 chemical compounds (Daszkowska-Golec) were selected as potential HAG5 inhibitors. The inhibitors were identified through an *in silico* screening and their efficacy was tested by *in planta* assay. Through these assays, we concluded that inhibitors A, B, C and G were able to mimic the *hag5* mutant phenotypes when applied to Col-0 seedlings. These inhibitors were tested for their ability to enhance drought tolerance, leaf surface area and root meristem size. Inhibitor A was selected as the better performing inhibitor for further experiments (Fig. 5 and 6). In Fig. 6, Trichostatin A (TSA), a known class I and II HDAC inhibitor, was used as a positive control, however it didn't work as expected. The fact, that Col-0 seedlings had the same root elongation rate in the control (DMSO) and in the positive control plates, could potentially be explained as HAG5's function at root elongation might not be cancelled/regulated by these HDACs or that the effect of TSA needs more time than 1 day. Additionally, *hag5* mutants should be included in Fig. 5 as this could show which inhibitor best mirrors its developmental phenotypes.

HAG5 has occurred from gene duplication, it is not present in most crop species (Fig. 8). *B. oleraceae* has two copies of the MYST HATs as Arabidopsis. However, lettuce, spinach and tomato have only one copy which at the sequence levels is more similar to *HAG4*. In order to answer the question whether HAG4 performs functions of HAG5, inhibitor A was tested on these plants for plant size, root length and drought tolerance. Interestingly, addition of inhibitor A results in drought tolerance in all the aforementioned species (Fig 9,10 and 11). In lettuce, which has only one copy of MYST family, the inhibitor A confers longer root and more secondary roots (Fig. 10). The inhibitor A was evaluated on adult tomato plants growing on vermiculite/perlite and the plants with the inhibitor were bigger and drought tolerant, which both are phenotypes of HAG5 (Fig. 12).

Consequently, this study highlights that HAG5 inhibition results in improving agricultural crops, since inhibiting this enzyme results in better performing plants under stress, without obvious developmental trade-offs. Additionally, the current study demonstrates that in the absence of HAG5, HAG4 performs HAG5 functions in plant growth and development as well as drought tolerance. We suggest a further investigation of the benefits of inhibitor A in plant immunity. Lastly, in order to test whether the inhibitor has the predicted molecular affect *in vivo*, RT-qPCRs of the HAG4/5-regulated DEGs (RNAseq in hag5 mutants was performed by another PhD student in Ntoukakis group) to investigate whether these genes are similarly affected when the inhibitor is applied. Alternatively, the whole genome levels of histone acetylation could be tested by bulk western blot.

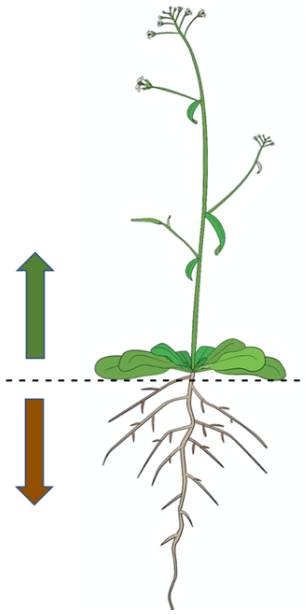
Histone acetyltransferases are significant for defence responses in plants

Studying plant immunity is crucial for human life and the planet's health. Plants provide us with food, oxygen, and serve as an essential component of the ecosystem. However, plants face constant threats from pathogens, such as bacteria, viruses, and fungi, as well as environmental stressors like drought and extreme temperatures. Understanding how plants defend themselves against these threats and how they adapt to stress is critical to ensure crop production and sustainable agriculture. A significant part of the immune responses to pathogens is reprogramming of gene expression. Transcriptional studies have shown that upon activation of immunity, 10% of the plant genome is differentially expressed (Tao, Xie et al. 2003, Tsuda, Sato et al. 2009). Various studies have demonstrated that chromatin regulation plays a major part in the expression of defence-related genes, and the establishment of a fast and efficient physiological immune response (Ding and Wang 2015). In chapter 1, the role of various histone acetyltransferases in plant immunity was discussed.

Arabidopsis MYST AtHAG4, together with its homologue AtHAG5 are assumed to work redundantly to regulate gametophyte development and flowering time (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013). In chapter 2, *hag4* and *hag5* mutants were screened for immunity phenotypes. The mutants of *HAG5* were resistant to the biotrophic leaf pathogens *Pto DC3000* and *Hpa* while mutants of *HAG4* were resistant to root pathogem *V. dahliae* and *F. oxysporum*. Therefore, HAG5 is a negative regulator of resistance against these two semi biotrophic and biotrophic leaf pathogens. These pathogens, by keeping the tissue alive, might use HAG5 to reprogram the cell to promote virulence. In contrast, HAG4 is a negative of two root pathogens which are hemibiotrophs, they enter the roots as biotrophs and they spread spores through the vessels where they reach the aerial part and they switch to the necrotrophic phase (Reusche, Thole et al. 2012).

The TF, VFP4, was revealed as a crucial interactor of HAG4 by Y2H assay since it regulates bacterial resistance genes and confers resistance against *Agrobacterium tumefaciens* infection (Garcia-Cano, Hak et al. 2018). HAG5 interacts with the TF, ARIA which is involved in ABA signalling (Kim, Kang et al.). These two HATs,

although they work together for flowering and gametophyte development, they interact with a distinct set of TFs and they operate different defence responses. Consequently, HAG4 and HAG5, which most likely occurred from a gene duplication event, they have gain separate functions. In figure 1, the immunity phenotypes of *hag4*, *vfp4* and *hag5* mutants are summarised.



Phenotypes of *hag4*, *vfp4* and *hag5* mutants

	hag4	*vfp4*	*hag5*
P. syringae	ns	ns	R
B. cinerea	ns	ns	ns
H. arabidopsidis	ns	?	R
V. dahliae	R	S	ns
F. oxysporum	R	S	?

(ns) not significant,
(R) resistant,
(S) susceptible,
(?) not determined

Figure 1. Phenotypes of *hag4, vfp4 and hag5* mutants against several against *P. syringae*, *B. cinerea*, *Hpa*, *V. dahliae* and *F. oxysporum*. HAG4 is a negative regulator of resistance against the root pathogens while *vfp4* mutants are susceptible. HAG5 is a negative regulator of immunity responses against the pathogens *P. syringae*, and *Hpa* which affect the aerial part of the plant.

Changes in the transcriptome and acetylation levels upon *F. oxysporum* infection

In order to examine the transcriptomic changes in *hag4* mutants, RNAseq was performed in roots, 6 days post inoculation. *F. oxysporum* as a hemibiotrophic fungus, uses the SA/JA pathway depending on which stage the fungus is. In this study, no SA or JA signalling, biosynthesis or responses-related genes were identified after infection. That could potentially be explained by the fact that the SA/JA responses mostly take place in the upper part of the plant (Badri, Loyola-Vargas et al. 2008, Attard, Gourgues et al. 2010, Lyons, Stiller et al. 2015). Additionally, after the *F.* oxysporum infection in *hag4* plants, genes significant for resistant to Fusarium as *Col1* or *Resistant to Fusarium 1 (ROF1)* were not differentially expressed in roots in comparison with Col-0 control plants. Instead,

genes related to root development and root hair and root lateral formation were differentially expressed in *hag4* plants as well as genes involved in cellular homeostasis.

The soil-borne pathogens penetrate the roots through the lateral roots and send the spores through the xylem vessels to the aerial part of the plant. Hence, the pathogen requires to keep the root alive (biotrophic phase) so it can expand its virulence. It is therefore possible that *hag4* mutants are resistant to root pathogens because the *HAG4* mutation alters the root structure and architecture as formation of root tip and root lateral development.

Additionally, cellular homeostasis related genes were upregulated in *hag4* plants after treatment as the *ROXY*s. *ROXY8*, *ROXY10* and *ROXY12* were differentially expressed upon *F. oxysporum* infection. The proteins encoded by these genes interact with the TFs called TGAs and they are regulated by nitrate and control primary root growth (Walters and Escobar , Jung, Ahn et al. 2018). *ROXY12* act as negative regulator of primary root growth by regulating root system architecture in response to nitrate availability in the soil (Patterson, Walters et al.). Studies have shown that Fusarium infection can alter the availability of soil nutrients, including nitrate by consuming or altering the availability of nitrate in the soil (Chaparro, Badri et al. 2014). Nitrogen limitation has been proposed as a key signal for activating the expression of virulence genes in plant pathogens (Snoeijers, Pérez-García et al. 2000). Overall, these studies suggest that *F. oxysporum* infection can have significant impacts on soil nitrate availability, which in turn can affect root development and nutrient uptake.

In this study we demonstrate that H4K5 acetylation is important for resistance against the soil-borne fungus and is correlated to transcription. While the H4K5 acetylation is higher at the TSS in WT plants, in both knockout mutants of *HAG4* and *VFP4* this acetylation is lost. This suggests that the resistance phenotype of Col-0 against *F.oxysporum* is HAG4 and VFP4-dependent. Moreover, in leaves the chromatin landscape appears to be different than the one of roots. The acetylation leves of H4K5 is generally much higher than the roots and the DEGs that confer this resistance to WT plants are already acetylated prior infection.

Inhibition of the MYST HATs can enhance crop performance

As mentioned in chapter 4, HAG5 occurred from a duplication event that took place during polyploidisation on the Brassicaceae ancestor. In fact, HAG4 and HAG5 share ~90% protein sequence identity (Latrasse, Benhamed et al. 2008), and both enzymes are assumed to acetylate H4K5 in vitro (Earley, Shook et al. 2007). Both HATs have identical acetylation domain while the most differences are located at the Tudor domain which provide an explanation for their interaction with different TFs.

Since the mutation of these HATs is beneficial as confers better performing plants under biotic (various root and leaf pathogens) and abiotic stress (drought), HAG4 and HAG5 comprise a great target for improving agricultural crops. To block enzymatic activity of these HATs, a chemical compound with high docking score was tested and selected for delivering drought tolerance to WT plants. To answer the question whether in plants like tomato, that carry one copy of the MYST HATs, HAG4 can perform both functions of HAG4 and HAG5, the selected inhibitor was tested for drought tolerance.

In all the crop plants tested which carry only one copy of the MYST family (HAG4-like) as lettuce, and tomato, the inhibitor provide drought tolerance to the WT crop plants by inhibiting HAG5 and mirroring the drought phenotypes of the Arabidopsis *hag5* mutants. These results suggests that in plants with a single copy of MYST HATs, the functions of HAG4 and HAG5 can be performed by a single protein and therefore, introducing the inhibitor to agrochemicals can be extremely beneficial for agriculture.

Molecular mechanism of HAG4 modulation in root-immunity responses

In chapter 2, immunity phenotypes of HAG4 and HAG5 were discovered. HAG5 immunity phenotypes were explored by Ntoukakis group and demonstrated that *HAG5* mutation confers resistance against the hemibiotrophic bacterium *P. syringae* and the biotrophic fungus *Hpa*. The interaction of HAG5 with the TF, ARIA which is involved in ABA signalling was also discovered. In this study, I demonstrated that

HAG4 is a negative regulator of root-specific immunity against *V. dahliae* and *F. oxysporum*. Since these HATs have different function in defence responses, a Y2H was performed and the TF, VFP4 was uncovered as an interactor of HAG4. A schematic representation of the model of how these HATs function is shown in figure 2. VFP4 is known to be involved in resistance against the root bacterium *A. tumefaciens* (Garcia-Cano, Hak et al. 2018). Hence, the TF, VFP4, has a role in defence responses against a root-infecting pathogens. Pathogenesis assays were performed with the mutants of *HAG4* and *VFP4* with these soil-borne pathogens and they had opposite phenotypes.

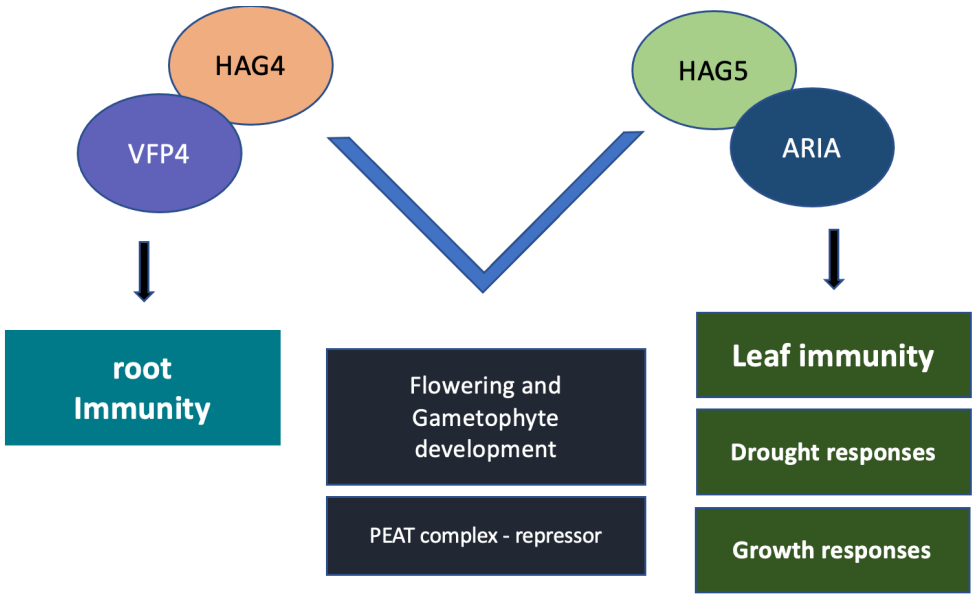


Figure 2. Schematic representation of the two MYST HATs, HAG4 and HAG5 and their functions. HAG4 and HAG5 work redundantly to regulate flowering and gametophyte development (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013) and they belong to the PEAT complex (Tan, Zhang et al.). HAG4 interacts with VFP4 and they regulate root immunity. HAG5 interacts with the TF, ARIA and it regulates drought responses, leaf immunity responses and plant growth.

As displayed in figure 3, in WT plants, HAG4 binds to VFP4 and VFP4 locates HAG4 in the promoters/gene bodies to potentially acetylate at H4K5 and regulate the expression of genes involved in defence responses/ root architecture. Once *VFP4* is mutated, HAG4 does not acetylate these regions, it potentially binds to the other two TFs, MBD2 and/or At4g03250 and they regulate acetylation in the promoters of genes related to different functions such as flowering. Finally, *HAG4* mutation compromises the acetylation levels of root development related genes and that leads to altered root architecture of the plant (Fig. 3). In order to test this model, a ChIPseq

of tagged lines of HAG4 including crossing this lines with *vfp4* mutants is recommending to prove if this is the way that HAG4 functions.

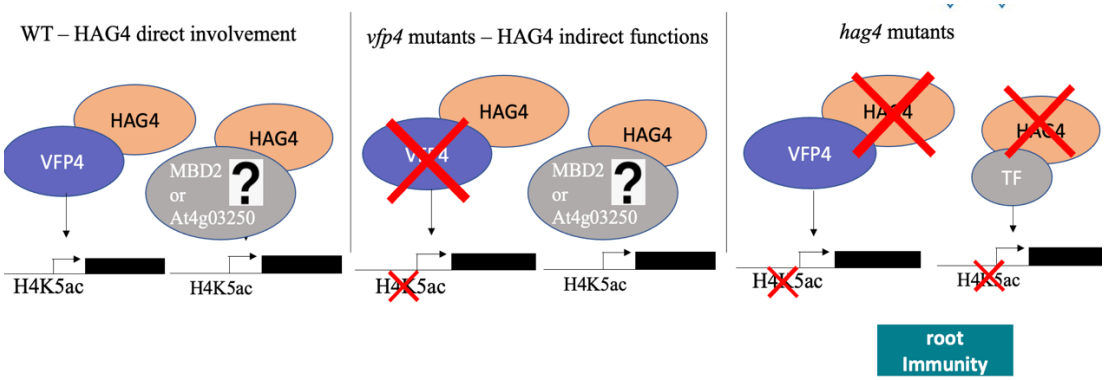


Figure 3. Working model of HAG4 and its interactor, VFP4.

Overall, HAG4 is a negative regulator of root immunity by regulating the nitrate availability and the root architecture by changing the primary root length, root hairs and lateral root development, areas where soil borne fungi can penetrate the plant and infect it. Moreover HAG4 is important for H4K5 acetylation which confers resistance by regulating specific genes.

Conclusions

Arabidopsis HAG4 and HAG5 are histone acetyltransferases that belong to the MYST family. They have high protein sequence similarity and are postulated to have the same acetylation target. Up to date, they are assumed to work redundantly to regulate flowering and gametophyte development (Latrasse, Benhamed et al. 2008, Xiao, Zhang et al. 2013). In this work, new roles of these HATs were discovered. These enzymes bind to a distinct set of TFs and have separate functions in plant immunity. AtHAG4 modulates root-specific immunity against *V. dahliae* and *F. oxysporum* by changing the root architecture of the plant and HAG5 regulates foliage-specific immunity against *P. syringae* and *Hpa*. HAG4 interacts with VFP4 and this complex changes the root architecture and development to promote resistance to soil borne pathogens. Chemical inhibition of these HATs can be exploited to improve crop performance upon challenging environmental stimuli. The diversification of HAG4 and HAG5 has provided the plant new toolkits to counteract plant pathogens affecting the root and aerial tissue.

Bibliography

Aist, J. R. and W. R. Bushnell (1991). Invasion of Plants by Powdery Mildew Fungi, and Cellular Mechanisms of Resistance. The Fungal Spore and Disease Initiation in Plants and Animals. G. T. Cole and H. C. Hoch. Boston, MA, Springer US**:** 321-345.

Allen, D. L., J. Samol, S. Benjamin, S. Verjee, A. Tusold and M. F. Murphy (2004). "Survey of the use and clinical effectiveness of HPA-1a/5b-negative platelet concentrates in proven or suspected platelet alloimmunization." Transfusion Medicine **14**(6): 409-417.

Allen, R. L., P. D. Bittner-Eddy, L. J. Grenville-Briggs, J. C. Meitz, A. P. Rehmany, L. E. Rose and J. L. Beynon (2004). "Host-Parasite Coevolutionary Conflict Between Arabidopsis and Downy Mildew." Science **306**(5703): 1957-1960.

Alonso, J. M., A. N. Stepanova, T. J. Leisse, C. J. Kim, H. Chen, P. Shinn, D. K. Stevenson, J. Zimmerman, P. Barajas, R. Cheuk, C. Gadrinab, C. Heller, A. Jeske, E. Koesema, C. C. Meyers, H. Parker, L. Prednis, Y. Ansari, N. Choy, H. Deen, M. Geralt, N. Hazari, E. Hom, M. Karnes, C. Mulholland, R. Ndubaku, I. Schmidt, P. Guzman, L. Aguilar-Henonin, M. Schmid, D. Weigel, D. E. Carter, T. Marchand, E. Risseeuw, D. Brogden, A. Zeko, W. L. Crosby, C. C. Berry and J. R. Ecker (2003). "Genome-wide insertional mutagenesis of Arabidopsis thaliana." Science **301**(5633): 653-657.

Arnell, N. W. (2004). "Climate change and global water resources: SRES emissions and socio-economic scenarios." Global Environmental Change **14**(1): 31-52.

Attard, A., M. Gourgues, N. Callemeyn-Torre and H. Keller (2010). "The immediate activation of defense responses in Arabidopsis roots is not sufficient to prevent Phytophthora parasitica infection." New Phytologist **187**(2): 449-460.

Ausubel, F. M. (2005). "Are innate immune signaling pathways in plants and animals conserved?" Nature Immunology **6**(10): 973-979.

Badri, D. V., V. M. Loyola-Vargas, J. Du, F. R. Stermitz, C. D. Broeckling, L. Iglesias-Andreu and J. M. Vivanco (2008). "Transcriptome analysis of Arabidopsis roots treated with signaling compounds: a focus on signal transduction, metabolic regulation and secretion." New Phytologist **179**(1): 209-223.

Bannister, A. J. and T. Kouzarides (2011). "Regulation of chromatin by histone modifications." Cell Research **21**(3): 381-395.

Barbara, D. J. and E. Clewes (2003). "Plant pathogenic Verticillium species: how many of them are there?" Molecular Plant Pathology **4**(4): 297-305.

Baxter, L., S. Tripathy, N. Ishaque, N. Boot, A. Cabral, E. Kemen, M. Thines, A. Ah-Fong, R. Anderson, W. Badejoko, P. Bittner-Eddy, J. L. Boore, M. C. Chibucos, M. Coates, P. Dehal, K. Delehaunty, S. Dong, P. Downton, B. Dumas, G. Fabro, C. Fronick, S. I. Fuerstenberg, L. Fulton, E. Gaulin, F. Govers, L. Hughes, S. Humphray, R. H. Y. Jiang, H. Judelson, S. Kamoun, K. Kyung, H. Meijer, P. Minx, P. Morris, J. Nelson, V. Phuntumart, D. Qutob, A. Rehmany, A. Rougon-Cardoso, P. Ryden, T. Torto-Alalibo, D. Studholme, Y. Wang, J. Win, J. Wood, S. W. Clifton, J. Rogers, G. Van den Ackerveken, J. D. G. Jones, J. M. McDowell, J. Beynon and B. M. Tyler (2010). "Signatures of adaptation to obligate biotrophy in the Hyaloperonospora arabidopsidis genome." Science **330**(6010): 1549-1551.

Beck, M., I. Wyrsch, J. Strutt, R. Wimalasekera, A. Webb, T. Boller and S. Robatzek (2014). "Expression patterns of FLAGELLIN SENSING 2 map to bacterial entry sites in plant shoots and roots." Journal of Experimental Botany **65**(22): 6487-6498.

Benhamed, M., C. Bertrand, C. Servet and D. X. Zhou (2006). "Arabidopsis GCN5, HD1, and TAF1/HAF2 interact to regulate histone acetylation required for light-responsive gene expression." Plant Cell **18**(11): 2893-2903.

Berestetskiy, A. (2008). "A review of fungal phytotoxins: from basic studies to practical use." Applied Biochemistry and Microbiology **44**: 453-465.

Bertani, G. (1951). "Studies on lysogenesis. I. The mode of phage liberation by lysogenic Escherichia coli." J Bacteriol **62**(3): 293-300.
Bertrand, C., M. Benhamed, Y. F. Li, M. Ayadi, G. Lemonnier, J. P. Renou, M. Delarue and D. X. Zhou (2005). "Arabidopsis HAF2 gene encoding TATA-binding protein (TBP)-associated factor TAF1, is required to integrate light signals to regulate gene expression and growth." J Biol Chem **280**(2): 1465-1473.
Birch, P. R. J., M. Armstrong, J. Bos, P. Boevink, E. M. Gilroy, R. M. Taylor, S. Wawra, L. Pritchard, L. Conti, R. Ewan, S. C. Whisson, P. van West, A. Sadanandom and S. Kamoun (2009). "Towards understanding the virulence functions of RXLR effectors of the oomycete plant pathogen Phytophthora infestans." Journal of Experimental Botany **60**(4): 1133-1140.
Boller, T. and G. Felix (2009). "A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors." Annu Rev Plant Biol **60**: 379-406.
Bordoli, L., M. Netsch, U. Lüthi, W. Lutz and R. Eckner (2001). "Plant orthologs of p300/CBP: conservation of a core domain in metazoan p300/CBP acetyltransferase-related proteins." Nucleic Acids Research **29**(3): 589-597.
Boycheva, I., V. Vassileva and A. Iantcheva (2014). "Histone acetyltransferases in plant development and plasticity." Curr Genomics **15**(1): 28-37.
Boyd, I. L., P. Freer-Smith, C. Gilligan and C. Godfray (2013). "The Consequence of Tree Pests and Diseases for Ecosystem Services." Science (New York, N.Y.) **342**: 1235773.
Boyer, J. S. and P. J. Kramer (1995). Water relations of plants and soils, Citeseer.
Byjesh, K., S. Naresh Kumar and P. K. Aggarwal (2010). "Simulating impacts, potential adaptation and vulnerability of maize to climate change in India." Mitigation and Adaptation Strategies for Global Change **15**: 413-431.
Campbell, C. L. and L. V. Madden (1990). Introduction to plant disease epidemiology. New York, John Wiley & Sons.
Catanzariti, A.-M., H. T. T. Do, P. Bru, M. de Sain, L. F. Thatcher, M. Rep and D. A. Jones (2017). "The tomato I gene for Fusarium wilt resistance encodes an atypical leucine-rich repeat receptor-like protein whose function is nevertheless dependent on SOBIR1 and SERK3/BAK1." The Plant Journal **89**(6): 1195-1209.
Catanzariti, A.-M., G. T. T. Lim and D. A. Jones (2015). "The tomato I-3 gene: a novel gene for resistance to Fusarium wilt disease." New Phytologist **207**(1): 106-118.
Causier, B., M. Ashworth, W. Guo and B. Davies (2012). "The TOPLESS interactome: a framework for gene repression in Arabidopsis." Plant physiology **158**(1): 423-438.
Challinor, A., J. Slingo, T. Wheeler and F. Doblas-Reyes (2005). "Probabilistic simulations of crop yield over western India using the DEMETER seasonal hindcast ensembles." Tellus, Series A: Dynamic Meteorology and Oceanography **57**.
Challinor, A. J., E. S. Simelton, E. D. G. Fraser, D. Hemming and M. Collins (2010). "Increased crop failure due to climate change: assessing adaptation options using models and socio-economic data for wheat in China." Environmental Research Letters **5**(3): 034012.
Chaparro, J. M., D. V. Badri and J. M. Vivanco (2014). "Rhizosphere microbiome assemblage is affected by plant development." The ISME Journal **8**(4): 790-803.
Chen, L.-T., M. Luo, Y.-Y. Wang and K. Wu (2010). "Involvement of Arabidopsis histone deacetylase HDA6 in ABA and salt stress response." Journal of Experimental Botany **61**(12): 3345-3353.
Chen, W.-Q., D.-X. Li, F. Zhao, Z.-H. Xu and S.-N. Bai (2016). "One additional histone deacetylase and 2 histone acetyltransferases are involved in cellular patterning of Arabidopsis root epidermis." Plant signaling & behavior **11**(2): e1131373.

Chen, Z., J. L. Agnew, J. D. Cohen, P. He, L. Shan, J. Sheen and B. N. Kunkel (2007). "Pseudomonas syringae type III effector AvrRpt2 alters Arabidopsis thaliana auxin physiology." Proceedings of the National Academy of Sciences of the United States of America **104**(50): 20131-20136.
Chen, Z., H. Zhang, D. Jablonowski, X. Zhou, X. Ren, X. Hong, R. Schaffrath, J. K. Zhu and Z. Gong (2006). "Mutations in ABO1/ELO2, a subunit of holo-Elongator, increase abscisic acid sensitivity and drought tolerance in Arabidopsis thaliana." Mol Cell Biol **26**(18): 6902-6912.
Chinchilla, D., C. Zipfel, S. Robatzek, B. Kemmerling, T. Nürnberger, J. D. Jones, G. Felix and T. Boller (2007). "A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence." Nature **448**(7152): 497-500.
Choi, S. M., H. R. Song, S. K. Han, M. Han, C. Y. Kim, J. Park, Y. H. Lee, J. S. Jeon, Y. S. Noh and B. Noh (2012). "HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis." Plant J **71**(1): 135-146.
Comas, L. H., S. R. Becker, V. M. V. Cruz, P. F. Byrne and D. A. Dierig (2013). "Root traits contributing to plant productivity under drought." Frontiers in plant science **4**: 442.
Cui, H., K. Tsuda and J. E. Parker (2015). "Effector-triggered immunity: from pathogen perception to robust defense." Annu Rev Plant Biol **66**: 487-511.
Cui, X., Q. Yan, S. Gan, D. Xue, D. Dou, N. Guo and H. Xing (2017). "Overexpression of gma-miR1510a/b suppresses the expression of a NB-LRR domain gene and reduces resistance to Phytophthora sojae." Gene **621**: 32-39.
Cunnac, S., S. Chakravarthy, B. H. Kvitko, A. B. Russell, G. B. Martin and A. Collmer (2011). "Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in Pseudomonas syringae." Proceedings of the National Academy of Sciences **108**(7): 2975-2980.
Dangl, J. L., D. M. Horvath and B. J. Staskawicz (2013). "Pivoting the plant immune system from dissection to deployment." Science **341**(6147): 746-751.
Dangl, J. L. and J. D. G. Jones (2001). "Plant pathogens and integrated defence responses to infection." Nature **411**(6839): 826-833.
Daszkowska-Golec, A. (2016). "The role of abscisic acid in drought stress: how ABA helps plants to cope with drought stress." Drought Stress Tolerance in Plants, Vol 2: Molecular and Genetic Perspectives: 123-151.
De Coninck, B., P. Timmermans, C. Vos, B. P. A. Cammue and K. Kazan (2015). "What lies beneath: belowground defense strategies in plants." Trends in Plant Science **20**(2): 91-101.
DeFraia, C. T., X. Zhang and Z. Mou (2010). "Elongator subunit 2 is an accelerator of immune responses in Arabidopsis thaliana." The Plant Journal **64**(3): 511-523.
Denby, K. J., P. Kumar and D. J. Kliebenstein (2004). "Identification of Botrytis cinerea susceptibility loci in Arabidopsis thaliana." The Plant Journal **38**(3): 473-486.
Deng, W., C. Liu, Y. Pei, X. Deng, L. Niu and X. Cao (2007). "Involvement of the Histone Acetyltransferase AtHAC1 in the Regulation of Flowering Time via Repression of FLOWERING LOCUS C in Arabidopsis." Plant Physiology **143**(4): 1660-1668.
Desvoyes, B., Z. Vergara, J. Sequeira-Mendes, S. Madeira and C. Gutierrez (2018). "A Rapid and Efficient ChIP Protocol to Profile Chromatin Binding Proteins and Epigenetic Modifications in Arabidopsis." Methods Mol Biol **1675**: 71-82.
Diener, A. C. and F. M. Ausubel (2005). "RESISTANCE TO FUSARIUM OXYSPORUM 1, a dominant Arabidopsis disease-resistance gene, is not race specific." Genetics **171**(1): 305-321.
Ding, B. and G.-L. Wang (2015). "Chromatin versus pathogens: the function of epigenetics in plant immunity." Frontiers in plant science **6**: 675.

Dong, X., Y. Xiong, N. Ling, Q. Shen and S. Guo (2014). "Fusaric acid accelerates the senescence of leaf in banana when infected by Fusarium." World Journal of Microbiology and Biotechnology **30**: 1399-1408.
Dreze, M., D. Monachello, C. Lurin, M. E. Cusick, D. E. Hill, M. Vidal and P. Braun (2010). High-Quality Binary Interactome Mapping. Guide to Yeast Genetics: Functional Genomics, Proteomics, and Other Systems Analysis**:** 281-315.
Earley, K., R. J. Lawrence, O. Pontes, R. Reuther, A. J. Enciso, M. Silva, N. Neves, M. Gross, W. Viegas and C. S. Pikaard (2006). "Erasure of histone acetylation by Arabidopsis HDA6 mediates large-scale gene silencing in nucleolar dominance." Genes Dev **20**(10): 1283-1293.
Earley, K. W., M. S. Shook, B. Brower-Toland, L. Hicks and C. S. Pikaard (2007). "In vitro specificities of Arabidopsis co-activator histone acetyltransferases: implications for histone hyperacetylation in gene activation." The Plant Journal **52**(4): 615-626.
Elad, Y. (1997). "Responses of plants to infection by Botrytis cinerea and novel means involved in reducing their susceptibility to infection." Biological Reviews **72**(3): 381-422.
Fabro, G., J. Steinbrenner, M. Coates, N. Ishaque, L. Baxter, D. J. Studholme, E. Körner, R. L. Allen, S. J. M. Piquerez, A. Rougon-Cardoso, D. Greenshields, R. Lei, J. L. Badel, M.-C. Caillaud, K.-H. Sohn, G. Van den Ackerveken, J. E. Parker, J. Beynon and J. D. G. Jones (2011). "Multiple Candidate Effectors from the Oomycete Pathogen Hyaloperonospora arabidopsidis Suppress Host Plant Immunity." PLOS Pathogens **7**(11): e1002348.
FAO (2010). "Food and Agriculture Organization of the United Nations."
Felix, G., M. Regenass and T. Boller (1993). "Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalinization, changes in protein phosphorylation, and establishment of a refractory state." The Plant Journal **4**(2): 307-316.
Ferrari, S., R. Galletti, D. Pontiggia, C. Manfredini, V. Lionetti, D. Bellincampi, F. Cervone and G. De Lorenzo (2008). "Transgenic expression of a fungal endo-polygalacturonase increases plant resistance to pathogens and reduces auxin sensitivity." Plant physiology **146**(2): 669.
Feys, B. J., L. J. Moisan, M.-A. Newman and J. E. Parker (2001). "Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4." The EMBO journal **20**(19): 5400-5411.
Fina, J. P. and P. Casati (2015). "HAG3, a histone acetyltransferase, affects UV-B responses by negatively regulating the expression of DNA repair enzymes and sunscreen content in Arabidopsis thaliana." Plant and Cell Physiology **56**(7): 1388-1400.
Fradin, E. F., Z. Zhang, J. C. Juarez Ayala, C. D. Castroverde, R. N. Nazar, J. Robb, C. M. Liu and B. P. Thomma (2009). "Genetic dissection of Verticillium wilt resistance mediated by tomato Ve1." Plant Physiol **150**(1): 320-332.
Garcia-Cano, E., H. Hak, S. Magori, S. G. Lazarowitz and V. Citovsky (2018). "The Agrobacterium F-Box Protein Effector VirF Destabilizes the Arabidopsis GLABROUS1 Enhancer/Binding Protein-Like Transcription Factor VFP4, a Transcriptional Activator of Defense Response Genes." Mol Plant Microbe Interact **31**(5): 576-586.
Georg Felix*, J. D. D., Sigrid Volko‡ and Thomas Boller (1999). "Plants have a sensitive perception system for the most conserved domain of bacterial flagellin." The Plant Journal **18(3), 265–276**.
Gkizi, D., A. González Gil, A. J. Pardal, S. J. M. Piquerez, C. Sergaki, V. Ntoukakis and S. E. Tjamos (2021). "The bacterial biocontrol agent Paenibacillus alvei K165 confers inherited resistance to Verticillium dahliae." Journal of Experimental Botany **72**(12): 4565-4576.

Glazebrook, J. (2005). "Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens." Annu Rev Phytopathol **43**: 205-227.
Gómez-Gómez, L. and T. Boller (2000). "FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis." Mol Cell **5**(6): 1003-1011.
Grant, P. A., L. Duggan, J. Côté, S. M. Roberts, J. E. Brownell, R. Candau, R. Ohba, T. Owen-Hughes, C. D. Allis and F. Winston (1997). "Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex." Genes & development **11**(13): 1640-1650.
Grones, P. and J. Friml (2015). "Auxin transporters and binding proteins at a glance." J Cell Sci **128**(1): 1-7.
Guimaraes, R. L., R. T. Chetelat and H. U. Stotz (2004). "Resistance to Botrytis cinerea in Solanum lycopersicoides is dominant in hybrids with tomato, and involves induced hyphal death." European journal of plant pathology **110**: 13-23.
Guo, J., L. Wei, S.-S. Chen, X.-W. Cai, Y.-N. Su, L. Li, S. Chen and X.-J. He (2021). "The CBP/p300 histone acetyltransferases function as plant-specific MEDIATOR subunits in Arabidopsis." Journal of Integrative Plant Biology **63**(4): 755-771.
Gust, A. A., R. Biswas, H. D. Lenz, T. Rauhut, S. Ranf, B. Kemmerling, F. Götz, E. Glawischnig, J. Lee and G. Felix (2007). "Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis." Journal of Biological Chemistry **282**(44): 32338-32348.
Gutsche, N., M. Holtmannspötter, L. Maß, M. O'Donoghue, A. Busch, A. Lauri, V. Schubert and S. Zachgo (2017). "Conserved redox-dependent DNA binding of ROXY glutaredoxins with TGA transcription factors." Plant Direct **1**(6): e00030.
Hacquard, S., S. Spaepen, R. Garrido-Oter and P. Schulze-Lefert (2017). "Interplay Between Innate Immunity and the Plant Microbiota." Annual Review of Phytopathology **55**(1): 565-589.
Hagen, G. and T. Guilfoyle (2002). "Auxin-responsive gene expression: genes, promoters and regulatory factors." Plant Mol Biol **49**(3-4): 373-385.
Han, S. K., J. D. Song, Y. S. Noh and B. Noh (2007). "Role of plant CBP/p300-like genes in the regulation of flowering time." The Plant Journal **49**(1): 103-114.
Hauck, P., R. Thilmony and S. Y. He (2003). "A Pseudomonas syringae type III effector suppresses cell wall-based extracellular defense in susceptible Arabidopsis plants." Proceedings of the National Academy of Sciences **100**(14): 8577-8582.
Henderson, I. R. and S. E. Jacobsen (2007). "Epigenetic inheritance in plants." Nature **447**(7143): 418-424.
Holsters, M., B. Silva, F. Van Vliet, C. Genetello, M. De Block, P. Dhaese, A. Depicker, D. Inzé, G. Engler and R. Villarroel (1980). "The functional organization of the nopaline A. tumefaciens plasmid pTiC58." Plasmid **3**(2): 212-230.
Horbach, R., A. R. Navarro-Quesada, W. Knogge and H. B. Deising (2011). "When and how to kill a plant cell: Infection strategies of plant pathogenic fungi." Journal of Plant Physiology **168**(1): 51-62.
Houterman, P. M., L. Ma, G. Van Ooijen, M. J. De Vroomen, B. J. C. Cornelissen, F. L. W. Takken and M. Rep (2009). "The effector protein Avr2 of the xylem-colonizing fungus Fusarium oxysporum activates the tomato resistance protein I-2 intracellularly." The Plant Journal **58**(6): 970-978.
Hu, Z., N. Song, M. Zheng, X. Liu, Z. Liu, J. Xing, J. Ma, W. Guo, Y. Yao, H. Peng, M. Xin, D.-X. Zhou, Z. Ni and Q. Sun (2015). "Histone acetyltransferase GCN5 is essential for heat stress-responsive gene activation and thermotolerance in Arabidopsis." The Plant Journal **84**(6): 1178-1191.
Huisinga, K. L. and B. F. Pugh (2004). "A genome-wide housekeeping role for TFIID and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae." Mol Cell **13**(4): 573-585.

Jones, J. D. G. and J. L. Dangl (2006). "The plant immune system." Nature **444**(7117): 323-329.
Jubic, L. M., S. Saile, O. J. Furzer, F. El Kasmi and J. L. Dangl (2019). "Help wanted: helper NLRs and plant immune responses." Curr Opin Plant Biol **50**: 82-94.
Jung, J.-Y., J. H. Ahn and D. P. Schachtman (2018). "CC-type glutaredoxins mediate plant response and signaling under nitrate starvation in Arabidopsis." BMC Plant Biology **18**(1): 281.
Kambara, K., S. Ardissone, H. Kobayashi, M. M. Saad, O. Schumpp, W. J. Broughton and W. J. Deakin (2009). "Rhizobia utilize pathogen-like effector proteins during symbiosis." Molecular Microbiology **71**(1): 92-106.
Kanno, T., Y. Kanno, R. M. Siegel, M. K. Jang, M. J. Lenardo and K. Ozato (2004). "Selective recognition of acetylated histones by bromodomain proteins visualized in living cells." Molecular cell **13**(1): 33-43.
Keen, N. T. (1990). "Gene-for-gene complementarity in plant-pathogen interactions." Annu Rev Genet **24**: 447-463.
Kesarwani, M., J. Yoo and X. Dong (2007). "Genetic Interactions of TGA Transcription Factors in the Regulation of Pathogenesis-Related Genes and Disease Resistance in Arabidopsis." Plant physiology **144**: 336-346.
Kidd, B. N., N. Y. Kadoo, B. Dombrecht, M. Tekeoglu, D. M. Gardiner, L. F. Thatcher, E. A. Aitken, P. M. Schenk, J. M. Manners and K. Kazan (2011). "Auxin signaling and transport promote susceptibility to the root-infecting fungal pathogen Fusarium oxysporum in Arabidopsis." Mol Plant Microbe Interact **24**(6): 733-748.
Kim, J. B., J. Y. Kang and S. Y. Kim (2004). "Over-expression of a transcription factor regulating ABA-responsive gene expression confers multiple stress tolerance." Plant Biotechnology Journal **2**(5): 459-466.
Kim, S., J.-y. Kang, D.-I. Cho, J. H. Park and S. Y. Kim (2004). "ABF2, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance." The Plant Journal **40**(1): 75-87.
Kim, S., S. J. M. Piquerez, J. S. Ramirez-Prado, E. Mastorakis, A. Veluchamy, D. Latrasse, D. Manza-Mianza, R. Brik-Chaouche, Y. Huang, N. Y. Rodriguez-Granados, L. Concia, T. Blein, S. Citerne, A. Bendahmane, C. Bergounioux, M. Crespi, M. M. Mahfouz, C. Raynaud, H. Hirt, V. Ntoukakis and M. Benhamed (2020). "GCN5 modulates salicylic acid homeostasis by regulating H3K14ac levels at the 5' and 3' ends of its target genes." Nucleic Acids Res **48**(11): 5953-5966.
Klosterman, S. J., Z. K. Atallah, G. E. Vallad and K. V. Subbarao (2009). "Diversity, pathogenicity, and management of verticillium species." Annu Rev Phytopathol **47**: 39-62.
Kornet, N. and B. Scheres (2009). "Members of the GCN5 histone acetyltransferase complex regulate PLETHORA-mediated root stem cell niche maintenance and transit amplifying cell proliferation in Arabidopsis." The Plant Cell **21**(4): 1070-1079.
Kumar, R., G. C. Sahoo, K. Pandey, V. Das and P. Das (2015). "Study the effects of PLGA-PEG encapsulated Amphotericin B nanoparticle drug delivery system against Leishmania donovani." Drug delivery **22**(3): 383-388.
Kunze, G., C. Zipfel, S. Robatzek, K. Niehaus, T. Boller and G. Felix (2004). "The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants." Plant Cell **16**(12): 3496-3507.
Kuo, M. H., J. E. Brownell, R. E. Sobel, T. A. Ranalli, R. G. Cook, D. G. Edmondson, S. Y. Roth and C. D. Allis (1996). "Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines." Nature **383**(6597): 269-272.
Kurdistani, S. K. and M. Grunstein (2003). "Histone acetylation and deacetylation in yeast." Nature Reviews Molecular Cell Biology **4**(4): 276-284.
Lago, C., E. Clerici, L. Mizzi, L. Colombo and M. M. Kater (2004). "TBP-associated factors in Arabidopsis." Gene **342**(2): 231-241.

Lamb, C. and R. A. Dixon (1997). "THE OXIDATIVE BURST IN PLANT DISEASE RESISTANCE." Annu Rev Plant Physiol Plant Mol Biol **48**: 251-275.
Lamour, K. H., J. Win and S. Kamoun (2007). "Oomycete genomics: new insights and future directions." FEMS Microbiology Letters **274**(1): 1-8.
Latrasse, D., M. Benhamed, Y. Henry, S. Domenichini, W. Kim, D.-X. Zhou and M. Delarue (2008). "The MYST histone acetyltransferases are essential for gametophyte development in Arabidopsis." BMC Plant Biology **8**(1): 121.
Latrasse, D., M. Benhamed, Y. Henry, S. Domenichini, W. Kim, D. X. Zhou and M. Delarue (2008). "The MYST histone acetyltransferases are essential for gametophyte development in Arabidopsis." BMC Plant Biol **8**: 121.
Latrasse, D., T. Jégu, H. Li, A. de Zelicourt, C. Raynaud, S. Legras, A. Gust, O. Samajova, A. Veluchamy, N. Rayapuram, J. S. Ramirez-Prado, O. Kulikova, J. Colcombet, J. Bigeard, B. Genot, T. Bisseling, M. Benhamed and H. Hirt (2017). "MAPK-triggered chromatin reprogramming by histone deacetylase in plant innate immunity." Genome Biology **18**(1): 131.
Latrasse, D., T. Jégu, H. Li, A. de Zelicourt, C. Raynaud, S. Legras, A. Gust, O. Samajova, A. Veluchamy, N. Rayapuram, J. S. Ramirez-Prado, O. Kulikova, J. Colcombet, J. Bigeard, B. Genot, T. Bisseling, M. Benhamed and H. Hirt (2017). "MAPK-triggered chromatin reprogramming by histone deacetylase in plant innate immunity." Genome Biol **18**(1): 131.
Legube, G. and D. Trouche (2003). "Regulating histone acetyltransferases and deacetylases." EMBO reports **4**(10): 944-947.
Li, C., J. Xu, J. Li, Q. Li and H. Yang (2014). "Involvement of Arabidopsis HAC family genes in pleiotropic developmental processes." Plant Signal Behav **9**(3): e28173.
Lindeberg, M., S. Cunnac and A. Collmer (2012). "Pseudomonas syringae type III effector repertoires: last words in endless arguments." Trends in Microbiology **20**(4): 199-208.
Liu, L., Z. Wang, J. Li, Y. Wang, J. Yuan, J. Zhan, P. Wang, Y. Lin, F. Li and X. Ge (2021). "Verticillium dahliae secreted protein Vd424Y is required for full virulence, targets the nucleus of plant cells, and induces cell death." Mol Plant Pathol **22**(9): 1109-1120.
Livak, K. J. and T. D. Schmittgen (2001). "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method." Methods **25**(4): 402-408.
Long, J. A., C. Ohno, Z. R. Smith and E. M. Meyerowitz (2006). "TOPLESS regulates apical embryonic fate in Arabidopsis." Science **312**(5779): 1520-1523.
Luger, K., A. W. Mäder, R. K. Richmond, D. F. Sargent and T. J. Richmond (1997). "Crystal structure of the nucleosome core particle at 2.8 Å resolution." Nature **389**(6648): 251-260.
Luo, M., Y.-Y. Wang, X. Liu, S. Yang, Q. Lu, Y. Cui and K. Wu (2012). "HD2C interacts with HDA6 and is involved in ABA and salt stress response in Arabidopsis." Journal of experimental botany **63**(8): 3297-3306.
Lyons, R., J. Stiller, J. Powell, A. Rusu, J. M. Manners and K. Kazan (2015). "Fusarium oxysporum Triggers Tissue-Specific Transcriptional Reprogramming in Arabidopsis thaliana." PLOS ONE **10**(4): e0121902.
Maekawa, S., N. Inada, S. Yasuda, Y. Fukao, M. Fujiwara, T. Sato and J. Yamaguchi (2014). "The carbon/nitrogen regulator ARABIDOPSIS TOXICOS EN LEVADURA31 controls papilla formation in response to powdery mildew fungi penetration by interacting with SYNTAXIN OF PLANTS121 in Arabidopsis." Plant physiology **164**(2): 879-887.
Maekawa, S., T. Sato, Y. Asada, S. Yasuda, M. Yoshida, Y. Chiba and J. Yamaguchi (2012). "The Arabidopsis ubiquitin ligases ATL31 and ATL6 control the defense response as well as the carbon/nitrogen response." Plant Mol Biol **79**(3): 217-227.

Marino, D., N. Peeters and S. Rivas (2012). "Ubiquitination during plant immune signaling." Plant Physiol **160**(1): 15-27.
Martinez, E. (2002). "Multi-protein complexes in eukaryotic gene transcription." Plant molecular biology **50**: 925-947.
Meehl, G. A., J. M. Arblaster and C. Tebaldi (2005). "Understanding future patterns of increased precipitation intensity in climate model simulations." Geophysical Research Letters **32**(18).
Meyer, A., A. Pühler and K. Niehaus (2001). "The lipopolysaccharides of the phytopathogen Xanthomonas campestris pv. campestris induce an oxidative burst reaction in cell cultures of Nicotiana tabacum." Planta **213**: 214-222.
Millet, Y. A., C. H. Danna, N. K. Clay, W. Songnuan, M. D. Simon, D. Werck-Reichhart and F. M. Ausubel (2010). "Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns." The Plant Cell **22**(3): 973-990.
Mittelstraß, K., D. Treutter, M. Plessl, W. Heller, E. Elstner and I. Heiser (2006). "Modification of Primary and Secondary Metabolism of Potato Plants by Nitrogen Application Differentially Affects Resistance to Phytophthora infestans and Alternaria solani." Plant biology (Stuttgart, Germany) **8**: 653-661.
Miya, A., P. Albert, T. Shinya, Y. Desaki, K. Ichimura, K. Shirasu, Y. Narusaka, N. Kawakami, H. Kaku and N. Shibuya (2007). "CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis." Proc Natl Acad Sci U S A **104**(49): 19613-19618.
Monaghan, J. and C. Zipfel (2012). "Plant pattern recognition receptor complexes at the plasma membrane." Curr Opin Plant Biol **15**(4): 349-357.
Morris, S. A., B. Rao, B. A. Garcia, S. B. Hake, R. L. Diaz, J. Shabanowitz, D. F. Hunt, C. D. Allis, J. D. Lieb and B. D. Strahl (2007). "Identification of histone H3 lysine 36 acetylation as a highly conserved histone modification." Journal of Biological Chemistry **282**(10): 7632-7640.
Navarro, L., P. Dunoyer, F. Jay, B. Arnold, N. Dharmasiri, M. Estelle, O. Voinnet and J. D. Jones (2006). "A plant miRNA contributes to antibacterial resistance by repressing auxin signaling." Science **312**(5772): 436-439.
Nelissen, H., D. Fleury, L. Bruno, P. Robles, L. De Veylder, J. Traas, J. L. Micol, M. Van Montagu, D. Inzé and M. Van Lijsebettens (2005). "The elongata mutants identify a functional Elongator complex in plants with a role in cell proliferation during organ growth." Proceedings of the National Academy of Sciences **102**(21): 7754-7759.
Newman, M.-A., M. J. Daniels and J. M. Dow (1995). "Lipopolysaccharide from Xanthomonas campestris induces defense-related gene expression in Brassica campestris." MPMI-Molecular Plant Microbe Interactions **8**(5): 778-780.
Nürnberger, T. and B. Kemmerling (2006). "Receptor protein kinases--pattern recognition receptors in plant immunity." Trends Plant Sci **11**(11): 519-522.
Oh, H. S. and A. Collmer (2005). "Basal resistance against bacteria in Nicotiana benthamiana leaves is accompanied by reduced vascular staining and suppressed by multiple Pseudomonas syringae type III secretion system effector proteins." Plant J **44**(2): 348-359.
Okubara, P. A. and T. C. Paulitz (2005). "Root defense responses to fungal pathogens: a molecular perspective." Root physiology: from gene to function: 215-226.
Pandey, R., A. MuÈller, C. A. Napoli, D. A. Selinger, C. S. Pikaard, E. J. Richards, J. Bender, D. W. Mount and R. A. Jorgensen (2002). "Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversification of chromatin modification among multicellular eukaryotes." Nucleic acids research **30**(23): 5036-5055.
Pandey, R., A. Muller, C. A. Napoli, D. A. Selinger, C. S. Pikaard, E. J. Richards, J. Bender, D. W. Mount and R. A. Jorgensen (2002). "Analysis of histone

acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversification of chromatin modification among multicellular eukaryotes." Nucleic Acids Res **30**(23): 5036-5055.
Pardal, A. J., S. J. M. Piquerez, A. Dominguez-Ferreras, L. Frungillo, E. Mastorakis, E. Reilly, D. Latrasse, L. Concia, S. Gimenez-Ibanez, S. H. Spoel, M. Benhamed and V. Ntoukakis (2021). "Immunity onset alters plant chromatin and utilizes EDA16 to regulate oxidative homeostasis." PLOS Pathogens **17**(5): e1009572.
Patterson, K., L. A. Walters, A. M. Cooper, J. G. Olvera, M. A. Rosas, A. G. Rasmusson and M. A. Escobar (2015). "Nitrate-Regulated Glutaredoxins Control Arabidopsis Primary Root Growth " Plant Physiology **170**(2): 989-999.
Pegg, G. and D. Young (1981). "Changes in glycosidase activity and their relationship to fungal colonization during infection of tomato by Verticillium albo-atrum." Physiological Plant Pathology **19**(3): 371-382.
Pennypacker, B. W. (2004). "Verticillium Wilts." CABI Publishing.
Péret, B., B. De Rybel, I. Casimiro, E. Benková, R. Swarup, L. Laplaze, T. Beeckman and M. J. Bennett (2009). "Arabidopsis lateral root development: an emerging story." Trends Plant Sci **14**(7): 399-408.
Perrella, G., M. A. Lopez-Vernaza, C. Carr, E. Sani, V. Gosselé, C. Verduyn, F. Kellermeier, M. A. Hannah and A. Amtmann (2013). "Histone deacetylase complex1 expression level titrates plant growth and abscisic acid sensitivity in Arabidopsis." The Plant Cell **25**(9): 3491-3505.
Perrine-Walker, F. M., J. Prayitno, B. G. Rolfe, J. J. Weinman and C. H. Hocart (2007). "Infection process and the interaction of rice roots with rhizobia." Journal of Experimental Botany **58**(12): 3343-3350.
Qi, P., M. Huang, X. Hu, Y. Zhang, Y. Wang, P. Li, S. Chen, D. Zhang, S. Cao, W. Zhu, J. Xie, J. Cheng, Y. Fu, D. Jiang, X. Yu and B. Li (2022). "A Ralstonia solanacearum effector targets TGA transcription factors to subvert salicylic acid signaling." The Plant Cell **34**(5): 1666-1683.
Ramirez-Parra, E., C. Fründt and C. Gutierrez (2003). "A genome-wide identification of E2F-regulated genes in Arabidopsis." The Plant Journal **33**(4): 801-811.
Rehmany, A. P., A. Gordon, L. E. Rose, R. L. Allen, M. R. Armstrong, S. C. Whisson, S. Kamoun, B. M. Tyler, P. R. J. Birch and J. L. Beynon (2005). "Differential Recognition of Highly Divergent Downy Mildew Avirulence Gene Alleles by RPP1 Resistance Genes from Two Arabidopsis Lines." The Plant Cell **17**(6): 1839-1850.
Reimer-Michalski, E. M. and U. Conrath (2016). "Innate immune memory in plants." Semin Immunol **28**(4): 319-327.
Reusche, M., K. Thole, D. Janz, J. Truskina, S. Rindfleisch, C. Drübert, A. Polle, V. Lipka and T. Teichmann (2012). "Verticillium infection triggers VASCULAR-RELATED NAC DOMAIN7–dependent de novo xylem formation and enhances drought tolerance in Arabidopsis." The Plant Cell **24**(9): 3823-3837.
Rietz, S., A. Stamm, S. Malonek, S. Wagner, D. Becker, N. Medina-Escobar, A. Corina Vlot, B. J. Feys, K. Niefind and J. E. Parker (2011). "Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in Arabidopsis immunity." New Phytologist **191**(1): 107-119.
Robatzek, S., D. Chinchilla and T. Boller (2006). "Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis." Genes & development **20**(5): 537-542.
Ros, B., V. Mohler, G. Wenzel and F. Thümmler (2008). "Phytophthora infestans-triggered response of growth- and defense-related genes in potato cultivars with different levels of resistance under the influence of nitrogen availability." Physiologia Plantarum **133**(2): 386-396.

Sauter, A., W. J. Davies and W. Hartung (2001). "The long-distance abscisic acid signal in the droughted plant: the fate of the hormone on its way from root to shoot." Journal of experimental botany **52**(363): 1991-1997.
Schaller, G. E., A. Bishopp and J. J. Kieber (2015). "The yin-yang of hormones: cytokinin and auxin interactions in plant development." Plant Cell **27**(1): 44-63.
Schenk, P. M., K. Kazan, A. G. Rusu, J. M. Manners and D. J. Maclean (2005). "The SEN1 gene of Arabidopsis is regulated by signals that link plant defence responses and senescence." Plant Physiology and Biochemistry **43**(10-11): 997-1005.
Schmidt, S. M., P. M. Houterman, I. Schreiver, L. Ma, S. Amyotte, B. Chellappan, S. Boeren, F. L. W. Takken and M. Rep (2013). "MITEs in the promoters of effector genes allow prediction of novel virulence genes in Fusarium oxysporum." BMC Genomics **14**(1): 119.
Schneider, C. A., W. S. Rasband and K. W. Eliceiri (2012). "NIH Image to ImageJ: 25 years of image analysis." Nature Methods **9**(7): 671-675.
Serrano, M., S. Parra, L. D. Alcaraz and P. Guzmán (2006). "The ATL gene family from Arabidopsis thaliana and Oryza sativa comprises a large number of putative ubiquitin ligases of the RING-H2 type." J Mol Evol **62**(4): 434-445.
Servet, C., N. Conde e Silva and D. X. Zhou (2010). "Histone acetyltransferase AtGCN5/HAG1 is a versatile regulator of developmental and inducible gene expression in Arabidopsis." Mol Plant **3**(4): 670-677.
Singh, P., S. Yekondi, P.-W. Chen, C.-H. Tsai, C.-W. Yu, K. Wu and L. Zimmerli (2014). "Environmental History Modulates Arabidopsis Pattern-Triggered Immunity in a HISTONE ACETYLTRANSFERASE1-Dependent Manner." The Plant cell **26**(6): 2676-2688.
Snoeijers, S. S., A. Pérez-García, M. H. A. J. Joosten and P. J. G. M. De Wit (2000). "The Effect of Nitrogen on Disease Development and Gene Expression in Bacterial and Fungal Plant Pathogens." European Journal of Plant Pathology **106**(6): 493-506.
Tan, L. M., C. J. Zhang, X. M. Hou, C. R. Shao, Y. J. Lu, J. X. Zhou, Y. Q. Li, L. Li, S. Chen and X. J. He (2018). "The PEAT protein complexes are required for histone deacetylation and heterochromatin silencing." The EMBO journal **37**(19): e98770.
Tao, Y., Z. Xie, W. Chen, J. Glazebrook, H.-S. Chang, B. Han, T. Zhu, G. Zou and F. Katagiri (2003). "Quantitative Nature of Arabidopsis Responses during Compatible and Incompatible Interactions with the Bacterial Pathogen Pseudomonas syringae." The Plant Cell **15**(2): 317-330.
Thornton, P., P. Jones, P. Ericksen and A. Challinor (2011). "Agriculture and food systems in Sub-Saharan Africa in a 4°C+ world." Philosophical transactions. Series A, Mathematical, physical, and engineering sciences **369**: 117-136.
Tjamos, S., E. Flemetakis, E. Paplomatas and P. Katinakis (2005). "Induction of Resistance to Verticillium dahliae in Arabidopsis thaliana by the Biocontrol Agent K-165 and Pathogenesis-Related Proteins Gene Expression." Molecular plant-microbe interactions : MPMI **18**: 555-561.
To, T. K., J.-M. Kim, A. Matsui, Y. Kurihara, T. Morosawa, J. Ishida, M. Tanaka, T. Endo, T. Kakutani, T. Toyoda, H. Kimura, S. Yokoyama, K. Shinozaki and M. Seki (2011). "Arabidopsis HDA6 Regulates Locus-Directed Heterochromatin Silencing in Cooperation with MET1." PLOS Genetics **7**(4): e1002055.
Torres, M. A., J. D. Jones and J. L. Dangl (2006). "Reactive oxygen species signaling in response to pathogens." Plant Physiol **141**(2): 373-378.
Torto, T. A., S. Li, A. Styer, E. Huitema, A. Testa, N. A. Gow, P. van West and S. Kamoun (2003). "EST mining and functional expression assays identify extracellular effector proteins from the plant pathogen Phytophthora." Genome Res **13**(7): 1675-1685.
Tran, T. M., M. B. Jones, A. Ongoiba, E. M. Bijker, R. Schats, P. Venepally, J. Skinner, S. Doumbo, E. Quinten, L. G. Visser, E. Whalen, S. Presnell, E. M. O'Connell, K. Kayentao, O. K. Doumbo, D. Chaussabel, H. Lorenzi, T. B. Nutman,

T. H. M. Ottenhoff, M. C. Haks, B. Traore, E. F. Kirkness, R. W. Sauerwein and P. D. Crompton (2016). "Transcriptomic evidence for modulation of host inflammatory responses during febrile Plasmodium falciparum malaria." Scientific Reports **6**(1): 31291.
Tsuda, K., M. Sato, T. Stoddard, J. Glazebrook and F. Katagiri (2009). "Network properties of robust immunity in plants." PLoS Genet **5**(12): e1000772.
Vandepoele, K., K. Vlieghe, K. Florquin, L. Hennig, G. T. S. Beemster, W. Gruissem, Y. Van de Peer, D. Inzé and L. De Veylder (2005). "Genome-Wide Identification of Potential Plant E2F Target Genes." Plant Physiology **139**(1): 316-328.
Vlachonasios, K. E., M. F. Thomashow and S. J. Triezenberg (2003). "Disruption mutations of ADA2b and GCN5 transcriptional adaptor genes dramatically affect Arabidopsis growth, development, and gene expression." Plant Cell **15**(3): 626-638.
Wagner, S., J. Stuttmann, S. Rietz, R. Guerois, E. Brunstein, J. Bautor, K. Niefind and Jane E. Parker (2013). "Structural Basis for Signaling by Exclusive EDS1 Heteromeric Complexes with SAG101 or PAD4 in Plant Innate Immunity." Cell Host & Microbe **14**(6): 619-630.
Walters, L. A. and M. A. Escobar (2016). "The AtGRXS3/4/5/7/8 glutaredoxin gene cluster on Arabidopsis thaliana chromosome 4 is coordinately regulated by nitrate and appears to control primary root growth." Plant Signaling & Behavior **11**(4): e1171450.
Wang, G., U. Ellendorff, B. Kemp, J. W. Mansfield, A. Forsyth, K. Mitchell, K. Bastas, C.-M. Liu, A. Woods-Tör, C. Zipfel, P. J. G. M. de Wit, J. D. G. Jones, M. Tör and B. P. H. J. Thomma (2008). "A Genome-Wide Functional Investigation into the Roles of Receptor-Like Proteins in Arabidopsis " Plant Physiology **147**(2): 503-517.
Wang, J.-Y., Y. Cai, J.-Y. Gou, Y.-B. Mao, Y.-H. Xu, W.-H. Jiang and X.-Y. Chen (2004). "VdNEP, an elicitor from Verticillium dahliae, induces cotton plant wilting." Applied and Environmental Microbiology **70**(8): 4989-4995.
Waterworth, W. M., G. E. Drury, G. Blundell-Hunter and C. E. West (2015). "Arabidopsis TAF1 is an MRE11-interacting protein required for resistance to genotoxic stress and viability of the male gametophyte." The Plant Journal **84**(3): 545-557.
Wiermer, M., B. J. Feys and J. E. Parker (2005). "Plant immunity: the EDS1 regulatory node." Curr Opin Plant Biol **8**(4): 383-389.
Williamson, B., G. H. Duncan, J. G. Harrison, L. A. Harding, Y. Elad and G. Zimand (1995). "Effect of humidity on infection of rose petals by dry-inoculated conidia of Botrytis cinerea." Mycological Research **99**(11): 1303-1310.
Wolpert, T. J., L. D. Dunkle and L. M. Ciuffetti (2002). "Host-selective toxins and avirulence determinants: what's in a name?" Annu Rev Phytopathol **40**: 251-285.
Wu, K., L. Zhang, C. Zhou, C.-W. Yu and V. Chaikam (2008). "HDA6 is required for jasmonate response, senescence and flowering in Arabidopsis." Journal of Experimental Botany **59**(2): 225-234.
Wyrsch, I., A. Domínguez-Ferreras, N. Geldner and T. Boller (2015). "Tissue-specific FLAGELLIN-SENSING 2 (FLS 2) expression in roots restores immune responses in A rabidopsis fls2 mutants." New Phytologist **206**(2): 774-784.
Xia, X. (2019). "Translation Control of HAC1 by Regulation of Splicing in Saccharomyces cerevisiae." International Journal of Molecular Sciences **20**(12): 2860.
Xiang, T., N. Zong, Y. Zou, Y. Wu, J. Zhang, W. Xing, Y. Li, X. Tang, L. Zhu, J. Chai and J.-M. Zhou (2008). "Pseudomonas syringae Effector AvrPto Blocks Innate Immunity by Targeting Receptor Kinases." Current Biology **18**(1): 74-80.
Xiao, J., H. Zhang, L. Xing, S. Xu, H. Liu, K. Chong and Y. Xu (2013). "Requirement of histone acetyltransferases HAM1 and HAM2 for epigenetic

modification of FLC in regulating flowering in Arabidopsis." J Plant Physiol **170**(4): 444-451.
Xin, Z., Y. Zhao and Z. L. Zheng (2005). "Transcriptome analysis reveals specific modulation of abscisic acid signaling by ROP10 small GTPase in Arabidopsis." Plant Physiol **139**(3): 1350-1365.
Xu, D., W. Huang, Y. Li, H. Wang, H. Huang and X. Cui (2012). "Elongator complex is critical for cell cycle progression and leaf patterning in Arabidopsis." Plant J **69**(5): 792-808.
Yuan, H., D. Rossetto, H. Mellert, W. Dang, M. Srinivasan, J. Johnson, S. Hodawadekar, E. C. Ding, K. Speicher, N. Abshiru, R. Perry, J. Wu, C. Yang, Y. G. Zheng, D. W. Speicher, P. Thibault, A. Verreault, F. B. Johnson, S. L. Berger, R. Sternglanz, S. B. McMahon, J. Cote and R. Marmorstein (2012). "MYST protein acetyltransferase activity requires active site lysine autoacetylation." Embo j **31**(1): 58-70.
Zhang, L., I. Kars, B. Essenstam, T. W. Liebrand, L. Wagemakers, J. Elberse, P. Tagkalaki, D. Tjoitang, G. van den Ackerveken and J. A. van Kan (2014). "Fungal endopolygalacturonases are recognized as microbe-associated molecular patterns by the arabidopsis receptor-like protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1." Plant Physiol **164**(1): 352-364.
Zhang, W., M. Fraiture, D. Kolb, B. Löffelhardt, Y. Desaki, F. F. G. Boutrot, M. Tör, C. Zipfel, A. A. Gust and F. Brunner (2013). "Arabidopsis RECEPTOR-LIKE PROTEIN30 and Receptor-Like Kinase SUPPRESSOR OF BIR1-1/EVERSHED Mediate Innate Immunity to Necrotrophic Fungi " The Plant Cell **25**(10): 4227-4241.
Zhang, X., J. Yazaki, A. Sundaresan, S. Cokus, S. W. Chan, H. Chen, I. R. Henderson, P. Shinn, M. Pellegrini, S. E. Jacobsen and J. R. Ecker (2006). "Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis." Cell **126**(6): 1189-1201.
Zhang, Y. and X. Li (2019). "Salicylic acid: biosynthesis, perception, and contributions to plant immunity." Current opinion in plant biology **50**: 29-36.
Zheng, A., R. Lin, D. Zhang, P. Qin, L. Xu, P. Ai, L. Ding, Y. Wang, Y. Chen, Y. Liu, Z. Sun, H. Feng, X. Liang, R. Fu, C. Tang, Q. Li, J. Zhang, Z. Xie, Q. Deng, S. Li, S. Wang, J. Zhu, L. Wang, H. Liu and P. Li (2013). "The evolution and pathogenic mechanisms of the rice sheath blight pathogen." Nature Communications **4**(1): 1424.
Zhou, C., L. Zhang, J. Duan, B. Miki and K. Wu (2005). "HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis." Plant Cell **17**(4): 1196-1204.
Zhou, J., Z. Feng, S. Liu, F. Wei, Y. Shi, L. Zhao, W. Huang, Y. Zhou, H. Feng and H. Zhu (2021). "CGTase, a novel antimicrobial protein from Bacillus cereus YUPP-10, suppresses Verticillium dahliae and mediates plant defence responses." Molecular plant pathology **22**(1): 130-144.
Zhou, X., D. Hua, Z. Chen, Z. Zhou and Z. Gong (2009). "Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis." The Plant Journal **60**(1): 79-90.
Zipfel, C. and G. Felix (2005). "Plants and animals: a different taste for microbes?" Current Opinion in Plant Biology **8**(4): 353-360.
Zipfel, C., S. Robatzek, L. Navarro, E. Oakeley, J. Jones, G. Felix and T. Boller (2004). "Bacterial disease resistance in Arabidopsis through flagellin perception." Nature **428**: 764-767.
Zipfel, C., S. Robatzek, L. Navarro, E. J. Oakeley, J. D. Jones, G. Felix and T. Boller (2004). "Bacterial disease resistance in Arabidopsis through flagellin perception." Nature **428**(6984): 764-767.

Zou, B., Q. Sun, W. Zhang, Y. Ding, D. L. Yang, Z. Shi and J. Hua (2017). "The Arabidopsis Chromatin-Remodeling Factor CHR5 Regulates Plant Immune Responses and Nucleosome Occupancy." Plant Cell Physiol **58**(12): 2202-2216.