Manipulation of Arabidopsis bromodomain-containing proteins by a YopJ family acetyltransferase from Ralstonia solanacearum

Virginie Comorge

THÈSE

En vue de l'obtention du
DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE
Délivré par l'Université Toulouse 3 - Paul Sabatier

Présentée et soutenue par

Virginie COMORGE

Le 15 juin 2022

Manipulation de protéines à bromodomaine d'Arabidopsis par une acetyltransférase de la famille YopJ de chez Ralstonia solanacearum

---

Ecole doctorale : **SEVAB - Sciences Ecologiques, Vétérinaires, Agronomiques et Bioingenieries**

Spécialité : **Developpement des plantes, interactions biotiques et abiotiques**

Unité de recherche :
LIPME - Laboratoire des Interactions Plantes-Microbes-Environnement

Thèse dirigée par
Laurent DESLANDES

Jury

M. Bruno FAVERY, Rapporteur
Mme Isabelle FUDAL, Rapporteure
Mme Mélanie HAMON, Rapporteure
Mme Sylvie GERMAN-RETANA, Examinatrice
M. Laurent DESLANDES, Directeur de thèse
M. Jean-Philippe GALAUD, Président

Table of contents

Résumé

Les mécanismes épigénétiques contribuent à réguler l'expression des gènes sans en changer leur séquence, en influençant la structure de la chromatine. De plus en plus d'études montrent que les agents pathogènes ont développé des stratégies de virulence pour interférer avec les mécanismes épigénétiques de l'hôte. Bien décrits chez les modèles animaux, de tels mécanismes d'interférence sur l'épigénome de cellules hôtes végétales demeurent encore méconnus, et plus particulièrement en réponse à des bactéries.

Ralstonia solanacearum est la bactérie responsable du flétrissement bactérien, qui affecte plus de 250 espèces végétales dont des grandes cultures et des plantes modèles comme *Arabidopis thaliana*. En tant que facteur de virulence majeur de *R. solanacearum*, PopP2 est une acetyltransférase de la famille YopJ qui atténue la résistance basale d'Arabidopsis en ciblant des facteurs de transcription WRKY. Pour mieux comprendre les fonctions de virulence de PopP2, des interacteurs ont été recherchés par une approche double hybride. Les protéines GTE9 et GTE11 de la famille GTE (General Transcription factor, group E) ont ainsi été identifiées. Ces protéines possèdent un bromodomaine connu pour interagir avec des résidus lysine acétylés, notamment présents chez des histones, suggérant que ces protéines pourraient être impliquées dans des processus épigénétiques. Précédemment, des travaux réalisés dans l'équipe ont révélé que GTE9 et GTE11 (i) co-localisent et interagissent avec PopP2 dans le noyau de cellules végétales, et (ii) sont acétylées par PopP2. De plus, GTE9 et GTE11 interagissent *in planta* avec l'Histone H4 via leur bromodomaine, suggérant que ce sont des lecteurs épigénétiques ciblés par PopP2.

Dans ce contexte, les principaux objectifs de ma thèse furent de mieux comprendre la fonction de GTE9 et GTE11 en essayant de déterminer la façon dont PopP2 pourrait les manipuler et si ces protéines jouent un rôle dans la réponse d'*A. thaliana* vis-à-vis de *R. solanacearum*.

Des analyses de spectrométrie de masse nous ont permis de cartographier les résidus lysine de GTE9 et GTE11 modifiés par PopP2. Plusieurs de ces résidus sont conservés entre les deux protéines et situés autour de leur bromodomaine. Par une approche de FRET-FLIM semi-quantitatif *in vivo*, nous avons montré que l'interaction GTE9-H4 est altérée par l'activité acetyltransferase de PopP2 suggérant que l'acétylation de GTE9 par PopP2 le dissocie de la chromatine. En sus de GTE9 et GTE11, PopP2 acétyle plusieurs autres protéines GTE. Concernant le rôle de GTE9 et GTE11 dans la réponse de la plante à *R. solanacearum*, des lignées d'*A. thaliana* sur-exprimant *GTE9* et *GTE11* sont plus sensibles à *R. solanacearum* et cela dépend de l'activité enzymatique de PopP2. Collectivement, nos données indiquent que GTE9 et GTE11 s'apparentent à des lecteurs épigénétiques qui sont ciblés par une bactérie phytopathogène à l'aide d'une acétyltransférase de la famille YopJ. Les GTEs pourraient être des cibles clefs de virulence car nous avons également identifié PopP1, une autre acetyltransferase YopJ de *R. solanacearum*, comme interagissant aussi avec certaines GTEs.

Il reste à déterminer comment le ciblage des protéines GTEs par PopP2 facilite l'infection chez Arabidopsis par *R. solanacearum*. Pour répondre à cette question, une approche ChIP-seq visant à identifier les régions chromatiniennes ciblées par GTE9 et GTE11 a été initiée (approche en cours de réalisation). En parallèle, nous voulions identifier les sites de la chromatine visités par PopP2 chez Arabidopsis. Pour cela, une seconde analyse ChIP-seq a été entreprise en générant divers outils moléculaires, incluant des versions étiquetées de PopP2 délivrées *in planta* via un système de sécrétion de type III bactérien.

De façon générale, ce projet de thèse a permis de progresser sur la compréhension d'une stratégie de virulence développée par une bactérie phytopathogène qui manipule des composantes épigénétiques pour favoriser l'infection.

Abstract

Epigenetic mechanisms contribute to the regulation of gene expression without changing its sequence by influencing the chromatin structure. Increasing evidence reveals that pathogens display virulence strategies that can interfere with host epigenetic mechanisms. This is particularly well described in animal pathogens but less in plant pathogens. Especially, very little evidence relate such virulence strategies used by plant pathogenic bacteria.

Ralstonia solanacearum is the causal agent of the bacterial wilt disease, which can affect more than 250 plant species including major crops and model plants such as *Arabidopsis thaliana*. As a potent *R. solanacearum* virulence factor, PopP2 is an acetyltransferase from the YopJ family that dampens basal immune responses by targeting defensive WRKY transcription factors. In order to better understand the virulence activities of PopP2, we searched for PopP2-interacting proteins using a yeast two hybrid assay, and identified the GTE9 and GTE11 proteins from the GTE family (General Transcription factor, group E). GTE proteins possess a bromodomain, a specific protein module allowing interaction with acetylated lysine residues, notably on histones tails suggesting that they could be involved in epigenetic-related processes. GTE9 and GTE11 were previously shown to (i) co-localise and interact with PopP2 in the plant nucleus, and (ii) to be acetylated by PopP2. Also, GTE9 and GTE11 were shown to interact *in planta* with Histone H4 through their bromodomain, suggesting that they function as epigenetic readers whose manipulation by PopP2 would promote *R. solanacearum* virulence.

In this context, the main objectives of my PhD were to better understand the the function of GTE9 and GTE11, by trying to determine how they can be manipulated by PopP2 and whether these proteins play a role in the plant response to *R. solanacearum*.

Mass-spectrometry-based analysis enabled us to map the lysine residues modified by PopP2 in GTE9 and GTE11. Several of these residues are conserved between the two proteins and localised on either side of their bromodomain. By semi-quantitative FRET-FLIM assay performed *in vivo*, we demonstrated that GTE9 interaction with Histone H4 is altered by PopP2 acetyltransferase activity suggesting that PopP2 uses acetylation to dissociate GTE9 from chromatin. In addition to GTE9 and GTE11, PopP2 acetylates several other GTE members. Regarding the role of GTE9 and GTE11 in the plant response to *R. solanacearum*, *GTE9* and *GTE11* over-expressing lines displayed an enhanced disease response to *R. solanacearum* that depended on PopP2 enzymatic activity. Overall, these data indicate that GTE9 and GTE11 behave as epigenetic readers that are manipulated by a plant bacterial pathogen through their targeting by a YopJ family acetyltransferase. GTE proteins could represent key virulence targets for *R. solanacearum* since PopP1, an additional YopJ family acetyltransferase that belongs to its effector repertoire, also interacts with several of these proteins.

How the targeting of GTE proteins is mechanistically impacting the overall course of *R. solanacearum* infection remains elusive. To answer this question, we undertook a ChIP-seq analysis aimed at identifying the chromatin regions targeted by GTE9 and GTE11 (approach in progress). In addition to this approach, we wanted to identify more globally the chromatin sites visited by PopP2 in Arabidopsis. For this, we have initiated a second ChIP-seq analysis using various molecular tools including tagged versions of PopP2 for *in planta* delivery through a bacterial type III secretion system.

Overall, this PhD work allows to progress on the understanding of a virulence strategy used by a plant bacterial pathogen that consist in manipulating host epigenetic components to promote infection.

Acknowledgments

Je tiens à remercier infiniment Laurent Deslandes. Tu m'a épaulée depuis le stage de master jusqu'à la fin de ma thèse, toujours présent pour répondre à mes questions et m'aiguiller dans ce projet, et m'apprendre les ficelles du métier. Même dans les moments où tu étais moins disponibles je savais que je pouvais compter sur toi. Ma thèse s'est bien passée en grande partie grâce à ton encadrement, tu es un encadrant en or !

Un grand merci à Françoise Jardinaud, pour m'avoir aider à comprendre comment analyser mes données en RT-qPCR.

Merci à toutes les personnes qui ont été gentilles avec moi pendant mon passage au LIPME.

Merci à mes amis, qui m'ont suivie de près ou de loin pendant ma thèse. Merci à Estelle et Aymeric, pour tous les bons moments de détente passés ensemble ; à Beubeu et Erla pour m'avoir sauvé de la dépression lors du 1er confinement ; à Julie pour notre soutien mutuel de thésardes parfois en souffrance ; et petite dédicace à Amélie, pour m'avoir souvent dit d'être moins dure avec moi-même et ramenée vers la voie de l'optimisme tant de fois.

Papa, merci de m'avoir soutenue toutes ces années, jusqu'à mon dernier diplôme (normalement) le voilà tu l'attendais avec fierté, et m'y voilà ! Je ne le dis pas souvent mais tu es dans mon cœur papa.

Mamounette, sans les journées et les soirées à me forcer à me faire faire mes devoirs en étant petite, je n'en serais probablement pas là. Tu suis peut être de loin mon parcours désormais mais je pense souvent que c'est grâce à ta persévérance à me forcer d'être moins paresseuse que j'y suis arrivée (je suis un caillou mais pas trop finalement hein ?). Je t'aime très fort Mamounette.

Merci aussi à toute ma famille, pour m'avoir soutenue et toujours compris lorsque je me renfermais et ne voulais pas parler de ma thèse, alors que vous vouliez en savoir plus.

And last but not least, I want to deeply thank you, Bram. We began sharing our lives at the end of both our PhDs and I know the end of mine has been difficult with my rollercoaster mood. Thank your for not holding it against me, never reproaching it to me, and for supporting me through all of it. Thank you for loving me.

Abbreviations

[Ca2+]cyt: Cytosolic Ca2+ concentration
35S: Cauliflower mosaic virus 35S
aa: Amino acid
ABA: Abscissic acid
acetyl-CoA: acetyl co-enzyme A
ACIP1: ACETYLATED INTERACTING PROTEIN 1
ADA2: Alteration/Deficiency in Activation 2
ADC: Arginine decarboxylase
ADR1: ACTIVATED DISEASE RESISTANCE 1
AS1: ASYMMETRIC LEAVES 1
BAK1: BRI1-ASSOCIATED RECEPTOR KINASE
BET: Bromodomain and Extra-Terminal domain
bHLH: basic Helix-Loop-Helix
BIK1: BOTRYTIS-INDUCED KINASE 1
BRD: Bromodomain
BSK1: BR-SIGNALLING KINASE 1
bZIP/TGA: basic leucine zipper subfamily TGA
CBP60g: Calmodulin Binding Protein 60g
CC: Coiled-Coil
CDC5: Cell division cycle 5
CERK1: CHITIN ELICITOR RECEPTOR KINASE 1
ChIP-seq: Chromatin Immunoprecipitation followed by sequencing
CHR5: Chromatin-Remodelling Factor 5
Col-0: Columbia-0
CRC: Chromatin Remodelling Complex
C-term: Carboxy terminus
DAMP: Damage-Associated Molecular Pattern
Dpi: Day post infiltration/inoculation
DRM2: Domains rearranged methyltransferase 2
EAR: Ethylene-responsive element binding factor associated Amphiphilic Repression
EBE: Effector binding element
EDS1: Enhanced disease susceptibility 1
EFR : EF-Tu receptor
EF-Tu: (protein) Elongation Factor Tu
ELP2: Elongator subunit 2
EPS: Exopolysaccharide
NPR1: Nonexpresser of PR genes 1
NRC: NLR required for cell death
ERF: Ethylene-responsive factor
ET: Ethylene
ETI: Effector-Triggered Immunity
ETS: Effector-Triggered Susceptibility
FLS2: Flagellin-sensing 2
GCN5: General Control Non-depressive 5
GTE: General Transcription factor, group E
HAT: Histone Acetyltransferase
HDAC: Histone Deacetylase
HIPP: HMA isoprenylated plant protein
HMA: Heavy Metal Associated
hNLR: helper NLR
HP1γ: Heterochromatin protein 1 γ
Hpi: Hours post infiltration/inoculation
HR: Hypersensitive Response
ID: Integrated domains
InsP6: Inositol-6-phosphate
JA: Jasmonic acid
kDa: kilodalton
lncRNA: Long non-coding RNA
LRR: Leucin Rich Repeat
LTP: Lipid transfer protein
LYK: LysM receptor-like Kinase
LysM: Lysine Motif
MAPK: Mitogen activated protein kinase
MAPKK: Mitogen activated protein kinase kinase
MAPKKK: Mitogen activated protein kinase kinase kinase
miRNA: micro RNA
NAD+: Oxidised form nicotinamide dinucleotide
NADH: Reduced form nicotinamide dinucleotide
NB-ARC: Nucleotide-binding APAF1, R gene products and CED-4 domain
ncRNA: non-coding RNA
Nd-1: Niederzenz-1
NLR: NOD-like receptor
NLS: Nulear Localisation Signal
Type2 ACS: Type 2 1-aminocyclopropane-1-carboxylate synthase
Ws-2: Wassilewskija-2

NRG1: N REQUIREMENT GENE 1
nt: Nucleotide
N-term: Amino terminus
OST1: OPEN STOMATA 1
PAMP: Pathogen-Associated Molecular Pattern
PR: PATHOGENESIS RELATED
PRR: Pattern-Recongition Receptor
Pst: Pseudomonas syringae pv. tomato
PTI: PAMP-Triggered Immunity/PRR-Triggered Immunity
PTM: Post-Translational Modification
RdDM: RNA dependent DNA methylation
RIPK: RPM1-interacting protein kinase
RLCK: Receptor-Like Cytoplasmic Kinase
RLK: Receptor-Like Kinase
RLP: Receptor-Like Protein
ROQ1: RECOGNITION OF XOPQ1
ROS: Reactive Oxygen Species
RPS4: RESISTANCE TO PSEUDOMONAS SYRINGAE4
RPT6: Regulatory particule triple-A ATPase subunit 6
RRS1: RESISTANCE TO RALSTONIA SOLANACEARUM1
RSSC: Ralstonia solanacearum species complex
SA: Salicylic acid
SAGA: Spt-ADA-Gcn5-Acetyltransferase
SARD1: SYSTEM ACQUIRED DEFICIENT 1
siRNA: Small interfering RNA
sRNA: Small RNA
SUMM2: SUPPRESSOR OF MKK1 MKK2 2
T2SS: Type 2 Secretion System
T3E: Type 3 Effector
T3SS: Type 3 Secretion System
T4SS: Type 4 Secretion System
TAD: Topologically Associated Domain
TALE: Transcription Activator-Like Effector
TF: Transcription factor
TIR: Toll/Interleukin1 receptor
WT: Wild type
Xcc: Xanthomonas campestris pv. campestris
Y2H: Yeast-two-hybrid
YopJ: Yersinia outer protein J
ZAR1: HOPZ-ACTIVATED RESISTANCE 1
ZED1: HOPZ-ETI-DEFICIENT 1

Figures and Tables list

Introduction

Results and Discussion

Chapter 1

Manuscript Comorge et al., in prep.

Figure 1. PopP2 interacts with Arabidopsis GTE9 and GTE11 in yeast and in the nucleus of N. benthamiana cells.

Figure 2. PopP2 acetylates GTE9 and GTE11.

Figure 3. The bromodomain of GTE9 confers preferential binding to acetylated histone H4.

Figure 4. PopP2 targets the bromodomain of GTE9 and interferes with its ability to bind histone H4.

Figure 5. Targeting of GTE9 and GTE11 by PopP2 promotes wilt disease development in Arabidopsis.

Figure 6. GTE9 and GTE11 are not required for RPS4/RRS1-R-mediated immunity.

Figure 7. PopP2 targets several members of the GTE family.

Supplemental Figure 1. GTE11 colocalises with wild-type PopP2 and PopP2-C321A catalytic mutant in plant cell nuclei.

Supplemental Figure 2. GTE9-YFP and GTE11-YFP are acetylated by PopP2.

Supplemental Figure 3. The bromodomain of GTE9 and GTE11 share conserved structural features with the second bromodomain of human BRD2 (Brd2(2)).

Supplemental Figure 4. Immunodetection of Lys-acetylated GTE11 co-expressed with PopP2 is favoured by the N206A mutation in GTE11 bromodomain.

Supplemental Figure 5. Molecular characterisation of GTE9 and GTE11 knock-out and overexpressing lines.

Supplemental Figure 6. Molecular characterisation of Col-0, gte9-1 and gte11-1 complemented with RPS4g/RRS1-Rg.

Supplemental Figure 7. PopP2 interacts with and acetylates several members of the GTE protein family.

Supplementary Table 1. FRET-FLIM measurements showing that GTE9 and GTE11 interact with PopP2 and histone H4 in nuclei of N. benthamiana cells.

Supplementary Table 2. Acetylation sites reproducibly identified by MS-based proteomics in GT9 and GTE11 co-expressed eitherwith PopP2 or the catalytically inactive PopP2-C321A mutant.

Supplementary Table 3: Peptides of GTE9 and GTE11 identified in individual samples analysed by MS-based proteomics.

Supplementary Table 4: Histone peptide array (raw data, Excel file).

Complements to the manuscript

Chapter 2

Chapter 3

Chapter 4

Conclusions and Perspectives

Figure 32. Targeting of GTE proteins by PopP2.

Materials and Methods

Table 3. Primers used in this study.

Introduction

1- Molecular bases of plant-pathogen interactions

A. Generalities

Plants possess several ways of protecting themselves from various biotic stresses. As one of the most important criteria in crop production is yield, crop diseases are a major concern in one's mind as they can cause yield loss (Savary et al., 2019). However, we should remember that most plants are resistant to most pathogens and that only the most successul pathogens infect plants and cause disease. To summarise the different mechanisms underlying plant-pathogen interactions, several concepts were introduced in the literature and in particular related to plant defence responses to pathogens. Non-host resistance describes the concept in which all members of a plant species are resistant to all variants of a pathogen. The plant species is then considered as non-host and is, therefore, fully resistant to this pathogen. Otherwise, if a pathogen is able to infect a plant species and only some genotypes of that species are resistant to the pathogen, we consider it to be host-resistance. In addition, if a pathogen fails to infect a plant genotype, the interaction is considered "incompatible" and if the pathogen succeeds infecting the plant genotype and causes symptoms, the interaction is considered "compatible" (Heath, 1981). Nowadays, a plant genotype is said "susceptible" to a pathogen when the interaction is compatible while it is said "resistant" to a pathogen when the interaction is incompatible.

To prevent pathogens from infecting them, plants possess various protection mechanisms. Plants protect themselves from outside threats with reinforced surfaces like tree bark; a wax layer called cuticle that covers leaves, fruits, flowers and non-woody stems; and cell wall that gives both structure and protection against pathogens and pests. Despite these mechanical barriers, pathogens can invade plants through wounds or degrade these barriers, resulting in disease. Interaction with microbes also triggers the activation of an innate immune system in plants and constitute another level of protection from infection. To summarise

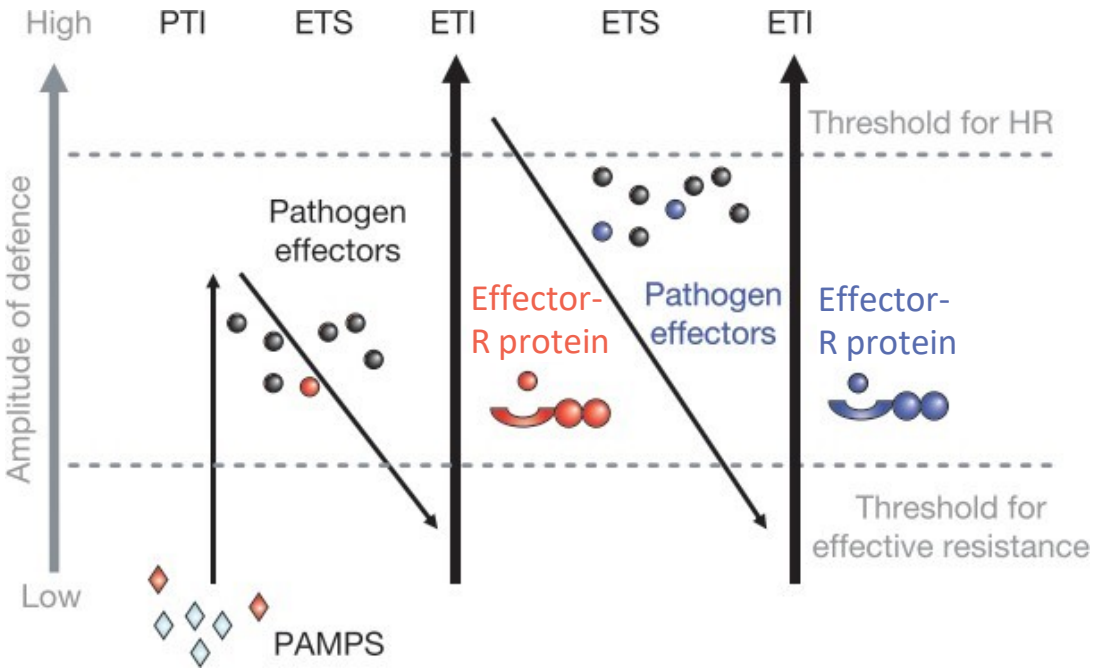


Figure 1. The Zig-zag model (adapted from Jones and Dangl, 2006).
The zig-zag model describes the arms-race that takes place between plants and pathogens upon interaction. Plants can detect conserved molecules from pathogens, PAMPs, through cell surface receptors, PRRs. The PAMP recognition by PRRs triggers PTI responses. During evolution, pathogens have evolved effectors capable of disturbing cellular processes to promote infection, known as ETS. Some effectors can be detected by plants through NLR (R) proteins leading to ETI responses, often associated with a programmed cell death (Hypersentive Response (HR) response). To counteract activation of host immune responses and evade recognition, pathogens evolve new effectors or delete/modify others. In turn, plants can also evolve new recognition mechanisms/capabilities to improve pathogen recognition and immunity activation.

the mechanisms underlying plant-pathogen interactions, the zig-zag model proposed by Jones and Dangl in 2006 integrated a great deal of knowledge about these mechanisms into a simple and elegant model described below. This model has the benefit of including a large number of known mechanisms related to plant-pathogen interactions and of taking into account the molecular arms race between plants and pathogens. However, this model suffers from certain limitations that will also be discussed later in this introductory chapter and that takes into account recent advances in the understanding of the mechanisms involved in the activation of plant immune responses.

B. The zig-zag model: a model to summarise the molecular bases of plant-pathogen interactions molecular bases

- **B.1. Presentation of the model**

The zig-zag model describes plant-induced immune responses against pathogens in several layers (Figure 1) (Jones and Dangl, 2006). The first layer that corresponds to basal immune responses is based on the recognition of conserved molecular patterns of the pathogen called Pathogen-Associated Molecular Patterns (PAMPs), by plant receptors located on the cell surface, called Pattern-Recognition Receptors (PRRs). PAMP recognition by a PRR leads to PAMP-Triggered Immunity or PRR-triggered Immunity (PTI). To overcome PTI, pathogens have evolved virulence factors called effector proteins that interfere with PTI. Pathogen effectors can interfere with PTI by altering various cellular mechanisms to promote infection. PTI interference by effectors is denominated Effector-Triggered Susceptibility (ETS). On the plant side, plants have evolved specific immune receptors, the R proteins, that are able to specifically recognise effectors directly or indirectly, leading to Effector-Triggered Immunity (ETI). A successful pathogen is a “discrete” pathogen that will not be defeated by the plant’s immune responses. Therefore, evolution selects pathogens that evade recognition by the plant immune system, so ones that do not possess a recognised effector anymore or a mutated effector

variant that evades recognition, or that possess new effectors that counteracts the recognition of other effectors. This new layer, called the 2nd ETS, is countered by mechanisms evolved in plants to prevent pathogens from infecting plants. PTI and ETI downstream signalling involves various events such as transcriptional reprogramming in plant cells with, for instance, up-regulation of defence-related genes and genes encoding proteins associated with hormone signalling (Jones and Dangl, 2006).

- **B.2. PAMP-Triggered Immunity (PTI)**

B.2.1. PAMPs are conserved microbial motifs

PAMPs are generally regarded as general elicitors perceived by apoplastic cell surface receptors. One reason for this is that some of them are well conserved among different pathogen genera. For instance, flagellin is one of the most studied PAMPs in plant and animal pathogenic bacteria. In plants, the conserved 22-amino-acid (aa) peptide flg22 that corresponds to a domain of the flagellin amino terminus (N-term) is a potent elicitor of immune responses in Arabidopsis and other plant species.

Known PAMPs include a large number of different relatively conserved molecules, such as motifs from bacterial or fungal cell wall proteins, elongation protein factors, proteases, cell-wall degradation enzymes… Some specific exemples include peptidoglycan fragments, lipopolysaccharides, protein elongation factor Tu (EF-Tu) from bacterial pathogens, chitin from fungal pathogens, as well as β-1,3 or β-1,6-glucans from oomycetes (Saijo et al., 2018).

We tend to think that PAMPs are highly conserved patterns and are widely recognised by PRRs, but this may not be so strict, as evidence in the literature show examples of PAMPs recognised by a narrower range of plant species. Indeed, the flgII-28 peptide is a flagellin peptide distinct from flg22 that elicits responses in some Solanaceae species but not in Arabidopsis (Cai et al., 2011). Another example is the peptide elf18 from EF-Tu, which is sufficient to elicit defence responses in

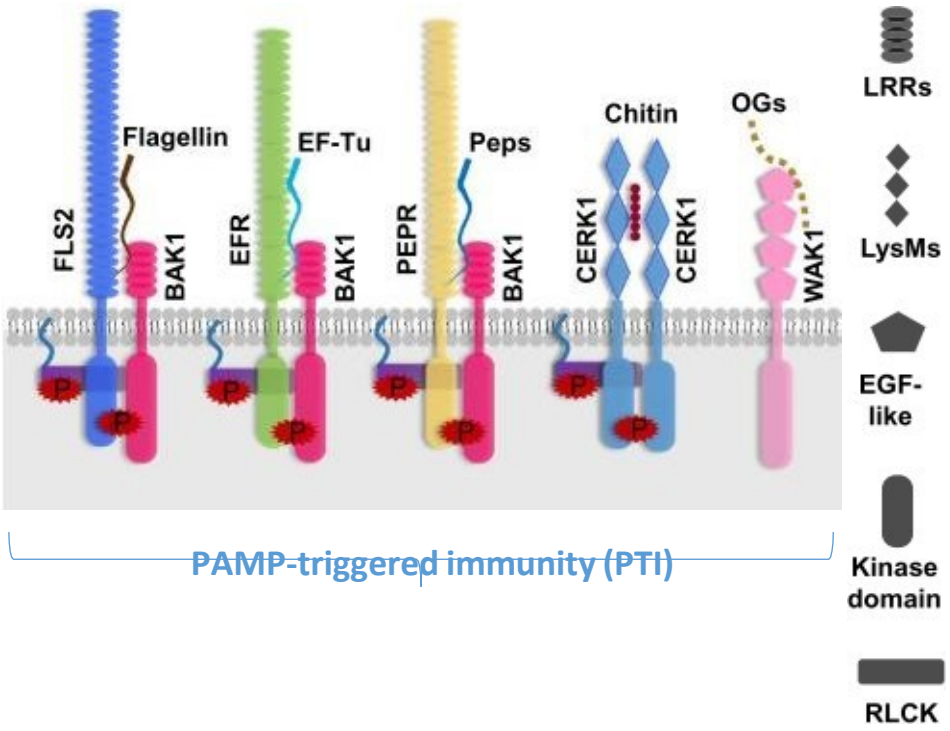


Figure 2. PAMPs and DAMPs recognition by PRRs (adapted from He and Wu, 2016).
During plant-microbe interactions, PAMPs are recognised by PRRs triggering PTI responses. Well-known PAMPs are derived from bacterial flagellin and elongation factor Tu (EF-Tu) and fungal chitin, which are recognised by the PRRs FLS2, EFR and CERK1, respectively. Plant elicitor proteins (Peps) and oligogalacturonides (OGs) are plant-derived molecules resulting from damage caused during pathogen infection or herbivore attack, called DAMPs. Peps and OGs are perceived by the PRRs PEPR and WAK1, respectively. Upon perception of PAMPs/DAMPs, PRRs often recruit BAK1, an adaptator playing a central role in the regulation of PTI. Then, PRRs autophosphorylate and transphosphorylate, triggering PTI responses, mediated by, among others, receptor like cytoplasmic kinases (RLCKs).

Brassicaceae species but not in plants outside this family. This suggests that PAMP diversification facilitates PTI evasion and that, despite the role of PAMPs as general elicitors, evolutionary pressure may increase the host-pathogen specificity of PAMP perception (Saijo et al., 2018; Yu et al., 2017).

B.2.2. PRRs are extracellular receptors that recognise PAMPs

Most PRRs are divided into two categories: Receptor-Like Kinases (RLKs) and Receptor-Like Proteins (RLPs). They are transmembrane receptors with an extracellular domain, a transmembrane domain and an intracellular domain. The main difference between RLKs and RLPs resides is the presence of an intracellular kinase domain in RLKs but not in RLPs. RLKs are involved in various plant growth and developmental processes, such as cell expansion, cell division and cell proliferation, but also in disease resistance and responses to abiotic stresses. RLPs are mainly associated with the detection of pathogenic threats. The extracellular domains of PRRs determine the specificity of ligand binding. These domains include leucine-rich repeats (LRRs), lysine motifs (LysMs), lectin motifs and epidermal growth-factor-like domains (Tang et al., 2017; Yu et al., 2017) (Figure 2). The two more common extracellular domains are LRR and LysM that can be found in both RLKs and RLPs. LRR-RLKs and LRR-RLPs are involved in the perception and signalling of peptide ligands. They include the well-studied Flagellin-sensing 2 (FLS2) and the EF-Tu receptor (EFR). LysM-RLKs and LysM-RLPs function in the perception and signalling of microbial patterns containing acetylated glucosamine. For instance, chitin perception is possible through the LysM-RLK CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) in Arabidopsis (Tang et al., 2017). One of the first signalling steps after PAMP perception by RLKs is the phosphorylation of downstream proteins by the kinase domain of RLKs. Since RLPs lack a kinase domain, signalling following PAMP perception by RLPs involves the association of RLPs with RLKs. However, RLKs also tend to act as complexes with other RLKs, triggering signalling processes (Saijo et al., 2018). For instance, LRR-RLKs have been shown to recruit BRI1-ASSOCIATED RECEPTOR KINASE (BAK1),

a LRR-RLK and other members of the SOMATIC EMBRYOGENESIS RECEPTOR KINASES family (Tang et al., 2017). After ligand perception, many LRR-RLPs form a complex with the adaptor LRR-RLK SOBIR1 and then with BAK1 (Saijo et al., 2018).

B.2.3. PRR/PAMP examples

flg22 recognition by the LRR-RLK FLS2 in Arabidopsis is well described in the literature (Figure 2). This receptor was discovered by screening flg22-insensitive Arabidopsis mutant plants (Boller and Felix, 2009). In the resting state, this receptor does not form a stable complex with BAK1 but interacts with several Receptor-Like Cytoplasmic Kinases (RLCKs) including BOTRYTIS-INDUCED KINASE 1 (BIK1) and BR-SIGNALLING KINASE 1 (BSK1) which will be involved in early signalling (He and Wu, 2016). Upon perception of flg22 by FLS2, FLS2 and BAK1 associate and this association is required to mediate immune responses (Tang et al., 2017). The formation of this RLK-RLK complex induces a rapid phosphorylation of BAK1 and FLS2 and allows initiation of intracellular signalling (Yu et al., 2017). This includes the release of BIK1 and BSK1 which will positively regulate some PTI responses including the production of Reactive Oxygen Species (ROS) (Tang et al., 2017; Yu et al., 2017).

A chitin receptor was also identified by screening Arabidopsis mutants with altered responses to chitin leading to the identification of the LysM-RLK CERK1. It belongs to the LysM receptor-like kinase (LYK) family which comprises five members in Arabidopsis including CERK1, also known as LYK1. Upon chitin perception, CERK1 heterodimerises with LYK5, resulting in homodimerisation and phosphorylation of CERK1 in response to chitin. Although CERK1 was the first PRR identified that perceives chitin, LYK5 has been shown to have a higher affinity for chitin than CERK1, suggesting that LYK5 is the primary chitin-binding protein in Arabidopsis instead of CERK1 (Cao et al., 2014). CERK1 interacts with BIK1 in a similar way than FLS2, and phosphorylated CERK1 phosphorylates BIK1 upon chitin perception leading to the activation of ROS production. CERK1 also interacts with the RLCK

PBL27 and phosphorylates it, leading to the activation of the Mitogen Activated Protein Kinases (MAPKs) cascade (Yu et al., 2017). These different interactions and responses following chitin perception provide an insight into the signalling events triggered by chitin perception. Surprisingly, CERK1, in association with the two RLPs LYM1 and LYM3, recognises bacterial peptidoglycans and induce cellular responses, suggesting that a PRR can mediate the recognition of different PAMPs depending on its association with other proteins (He and Wu, 2016; Tang et al., 2017).

- **B.3. Effector-Triggered Immunity (ETI)**

B.3.1. Intracellular receptors are able to recognise effector proteins

Plant R proteins are intracellular receptors that trigger ETI after perception of effector proteins. These R proteins are called NOD-like receptors (NLRs) because they share similarities with Nucleotide Oligomerisation Domain receptors from mammals. In general, NLRs have three different parts: at the N-term part of the NLRs there is either a coiled-coil (CC) domain or a Toll/Interleukin1 receptor (TIR) domain that is supposed to act as a signalling domain, a nucleotide-binding APAF1, R gene products and CED-4 domain (NB-ARC) proposed to act as a molecular switch, cycling between ADP (repressed) and ATP (active) bound forms, and the C-term LRR domain that determines recognition specifity (Sun et al., 2020). Most NLRs are structured with these three domains and are commonly classified based on their N-term part, the CC-NLRs or TIR-NLRs. Other NLRs display a different structure such as additional domains or truncated NLRs lacking the LRR domain or both the NB-ARC and the LRR domain (Baggs et al., 2017). NLRs are found in different cellular compartments: they are present in the cytoplasm, the nucleus, or also at the plasma membrane. Furthermore, their location can change after activation (Song et al., 2021b). In resting state, the amount of NLRs is kept at a basal level and/or in an inactive state in order to avoid unnecessary activation of immunity to maintain the trade-off between development and immunity, both

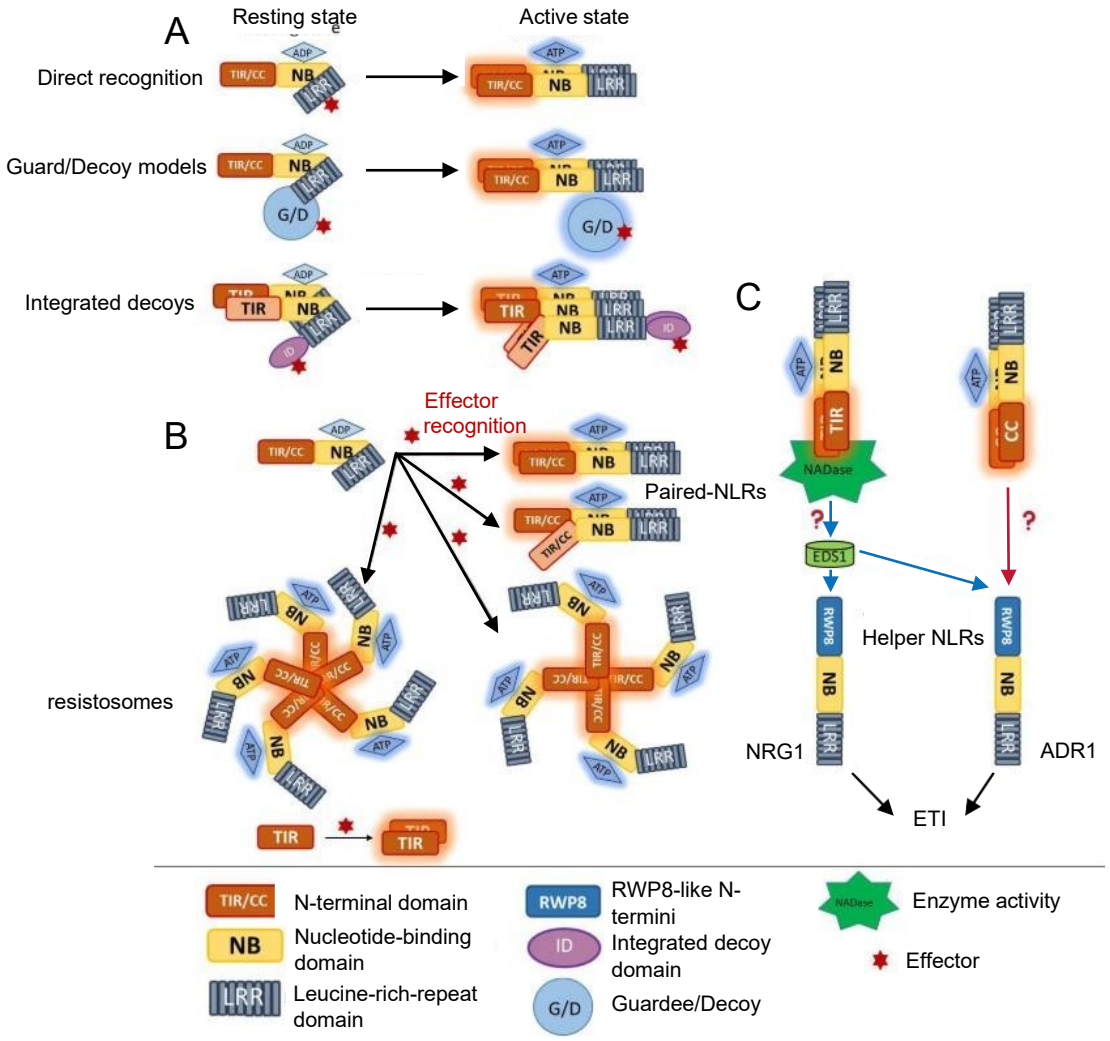


Figure 3. Effector recognition mediated by NLRs (adapted from Nguyen et al., 2021).
During plant-microbe interaction, microbes deliver effectors into plant tissue to alter diverse cellular processes. (A) Plants have evolved specialised intracellular immune receptors, the NLRs, which can specifically recognise some effectors by direct of indirect interaction. In indirect recognition mechanisms, NLRs generally guard an effector-targeted protein, whose modification triggers NLR activation. This host protein can be a guardee or a decoy. (B) & (C) NLRs tend to act cooperatively. Upon effector recognition, NLRs activate and tend to oligomerise into larger structures known as resistosomes, thereby activating ETI responses. ETI activation also depends on helper NLRs such as NRG1 and ADR1.

processes requiring a lot of energy. Controlling the amount of NLRs and their inactive state requires tight control through different mechanisms including transcriptional regulation, post-transcriptional regulation via alternative splicing, and post-translational regulation via stability control by chaperones and degradation of NLRs by the proteasome degradation complex (Sun et al., 2020; van Wersch et al., 2020). NLR activation is triggered by the recognition of effector proteins which can be direct or indirect.

B.3.1.a. Direct recognition of effectors

The first proposed mechanism of effector recognition was based on the gene-for-gene hypothesis (Flor, 1971). According to this hypothesis, the outcome of a plant-pathogen interaction depends on two factors: the pathogen's avirulence genes, now known as effector-encoding genes, and the plant's R genes, now known as NLR-encoding genes. The gene-for-gene hypothesis suggested that for every avirulence gene in a pathogen there was a corresponding R gene in plants and that the product of an avirulence gene could correspond to a ligand interacting with its matching plant R protein. The absence or presence of one or the other determined the success or failure of the interaction (Flor, 1971). Direct effector recognition has been documented for some NLRs: AvrL567 from the fungus Melampsora lini is directly recognised by the flax NLRs L5, L6, and L7 (Ellis et al., 2008), and the barley NLR MLA directly recognises the effector AVR_A from the fungal pathogen Blumeria graminis f. sp. hordei (Saur et al., 2019) (Figure 3A). Nevertheless, scientists tend to think that direct effector recognition is not the major recognition mechanism.

B.3.1.b. Indirect recognition of effectors: guardee and decoy models

Indirect recognition can occur when a NLR guards a host component and modification of this guarded component by an effector triggers activation of the NLR (Figure 3A). This is what we call the "guard model" (van Wersch et al., 2020). In detail, we distinguish the guard model from the decoy model. In the guard model, a NLR guards a plant protein that has a specific biological function, whereas the decoy mimics a host effector target, but has no real biological function. The decoy

only serves as a warning signal to trigger immune responses (van Wersch et al., 2020). For instance, the Arabidopsis NLR HOPZ-ACTIVATED RESISTANCE1 (ZAR1) guards several host proteins from different effectors of Pseudomonas syringae and Xanthomonas camprestris that are either guardees or decoys. For example, PBL2 is a RLCK targeted by the X. campestris effector AvrAC. Uridylation of PBL2 by AvrAC triggers ZAR1 activation (Wang et al., 2015b). In that case, PBL2 is a guardee as this RLCK is also required for the perception of PAMPs like flg22 and elf18 (Zhang et al., 2010). Another example is the targeting of the RLCK HOPZ-ETI-DEFICIENT1 (ZED1) by the effector HopZ1a of P. syringae. HopZ1a is an acetyltransferase that can acetylate ZED1 and trigger ZAR1-mediated immune responses. ZAR1 guards ZED1 but in this case ZED1 is considered as a decoy because in absence of ZAR1, ZED1 does not contribute to plant immunity or susceptibility in response to P. syringae (Lewis et al., 2013). It is not always clear whether a guarded protein is a guardee or a decoy but in both cases the recognition mechanism is indirect.

B.3.1.c. A particular type of decoy: the Integrated Decoys (ID)

Some NLRs harbour unusual domains called integrated domains (IDs) and are present in many plant species (NLR-IDs) (Grund et al., 2019; Kroj et al., 2016; Sarris et al., 2016). Some IDs from NLR-IDs mimick virulence targets of effectors making these domains integrated decoys but for most of NLR-IDs we do not know whether their IDs serve as decoys (Figure 3A). Well-characterised examples of NLR-IDs include the Arabidopsis RRS1 (RESISTANCE TO RASTONIA SOLANACEARUM1) and the rice NLRs RGA5 and Pik-1. RRS1 will be detailed later in this introduction in a specific section. In rice, both the RGA5 and Pik-1 NLRs harbour a C-term Heavy-Metal-Associated (HMA) domain, a domain orignally found in a Saccharomyces cerevisiae copper chaperone. The HMA domain of RGA5 interacts directly with the effectors AVR-PiA and AVR1-CO39 from the fungus Magnaporthe oryzae (Césari et al., 2014). Are HMA domain-containing proteins targeted by effectors? This is not yet known, but Pi21, a HMA-containing protein, is an

important factor for total susceptibility to M. oryzae (Fukuoka et al., 2009; Zhang et al., 2016b). However, it has been recently documented that the effector AVR-Pik from the same fungus, which is recognised by the NLR-ID Pik-1 which possesses a HMA domain, targets HMA domain-containing proteins. Indeed, AVR-Pik variants have been demonstrated to target proteins from the HMA isoprenylated plant protein family (HIPP) (Oikawa et al., 2020). Various variants of AVR-Pik have been demonstrated to interact with the HMA domains of OsHIPP19 and OsHIPP20, and OsHIPP20 was shown to be important for M. oryzae infection since a Oshipp20 knock-out mutant is less susceptible to the fungus than the WT (Maidment et al., 2021; Oikawa et al., 2020).

B.3.2. NLRs work often in cooperation

NLRs have frequently been observed to act in cooperation with other NLRs (Figure 3B). Interestingly, the first hints of cooperation between NLRs might be found in the location of the genes on chromosomes as in vascular plants, NLR-encoding genes are often arranged in pairs or located in complex multi-gene clusters. An NLR cluster is defined by a genomic area of less than 200 kb and containing less than eight non-NLR-encoding genes within the cluster. Across different vascular plant species, while the number of NLRs varies, the proportion of NLR clusters is relatively constant at around 60% (van Wersch and Li, 2019). It is tempting to imagine that NLR clustering is beneficial in activating plant immunity at a regulatory level or in eliminating some fitness costs, but this requires further investigation. The clustering of NLR encoding genes could allow them to be co-transcriptionally regulated, thus facilitating the assembly of NLRs into oligomeric complexes that mediate activation of immune responses;

B.3.2.a. Self-association of NLRs

Several NLRs have been reported to form homodimers and for some of them this homodimerisation plays a role in NLR activation and/or signalling function (Li et al., 2015a; Nguyen et al., 2021). For instance, the TIR domain of the flax NLR L6 was found to mediate L6 self-association and immune signalling was abolished

when L6 homodimerisation was disabled (Bernoux et al., 2011). Similar results were obtained on the CC domain of the barley NLR MLA10 (Maekawa et al., 2011).

B.3.2.b. Heterodimerisation of NLRs

Homodimerisation is not the only way NLR can associate. Some NLRs form heterodimers and participate in the regulation of their immune functions (Nguyen et al., 2021). Two well-studied heterodimer-forming pairs of NLRs are the TIR-NLRs RESISTANCE TO PSEUDOMONAS SYRINGAE 4 (RPS4) with RRS1 from Arabidopsis, and the CC-NLRs RGA4/RGA5 from rice. In Arabidopsis, RRS1 and RPS4 heterodimerise due to a strong affinity of their TIR domains that can be compromised if the domains are mutated (Williams et al., 2014). The specific mechanisms of RPS4/RRS1 interaction will be detailed later in this introduction. In rice, RGA4 and RGA5 form a heterodimer via their CC domains that prevents cell death activation by RGA4. RGA4 is released from RGA5 after recognition of the M. oryzae effector AVR-Pia, leading to activation of cell-death (Césari et al., 2014).

B.3.2.c. NLRs can associate in higher structure shapes: the resistosomes

Recent breakthroughs have revealed interesting complex assocation structures of NLRs known as resistosomes that largely ressemble the inflammasomes described in animal immunity activation (Ma et al., 2020; Martin et al., 2020; Wang et al., 2019).

In 2019, Wang and colleagues revealed the switching mechanism of the Arabidopsis CC-NLR ZAR1. In a resting state, ZAR1 interacts with the pseudokinase RKS1. Upon infection with Xanthomonas campestris pv. campestris, the secreted AvrAC effector uridylates the RLCK PBL2 which will activate ZAR1. Indeed, uridylated-PBS2 interacts with the ZAR1-RKS1 complex and changes its conformation leading to a pentamerisation in a ring-like structure of the ZAR1-RKS1-PBL2 complex, called the ZAR1 resistosome (Wang et al., 2019). This resistosome forms a funnel-shaped structure that can insert into the plasma

membrane to form a pore, like a calcium-channel, which triggers plant immune signalling (Bi et al., 2021).

Furthermore, it was shown in 2020 that the TIR-NLR RECOGNITION OF XopQ 1 (ROQ1) of Nicotiana benthamiana and the TIR-NLR RPP1 of Arabidopsis form tetrameric resistosomes after activation by the Xanthomonas euvesicatoria effector XopQ, and the Hyaloperonospora arabidopsidis effector ATR1, respectively (Martin et al., 2020; Ma et al., 2020). These new results hint that cooperation of NLRs through oligomerisation, either in pairs or through higher structures like resistosomes, is more common than previously thought and is important for NLR activation and signalling. It is also quite interesting to notice that these oligomeric structures in plant NLRs are similar to the ones described in animal NLRs, for which this type or higher level of oligomerisation has already been found several years ago (van Wersch et al., 2020).

B.3.2.d. NLRs cooperate with helper NLRs

In addition to homo- or hetero-oligomerisation of NLRs, NLRs can also cooperate with another type of NLR known as helper NLRs (hNLRs) (Figure 3C). The presence of hNLRs is necessary for the activation of NLR-mediated immune responses. The hNLRs do not function in effector recognition but rather in downstream signalling of NLR activation (Jubic et al., 2019). Three important families of helper NLRs have been reported: the ACTIVATED DISEASE RESISTANCE 1 (ADR1) family, the N REQUIRED GENE 1 (NRG1) family and the NLR required for cell death (NRC) family which is specific to Solanaceae (Castel et al., 2019; Wu et al., 2017). Which family is involved depends on the type of NLRs with which it cooperates but they all contribute to the activation of ETI-mediated defence responses (Saile et al., 2020). For instance, TIR NLR signalling pathways involve the ADR1 and NRG1 family of hNLR and require another protein called Enhanced disease susceptibility 1 (EDS1) (Feehan et al., 2020).

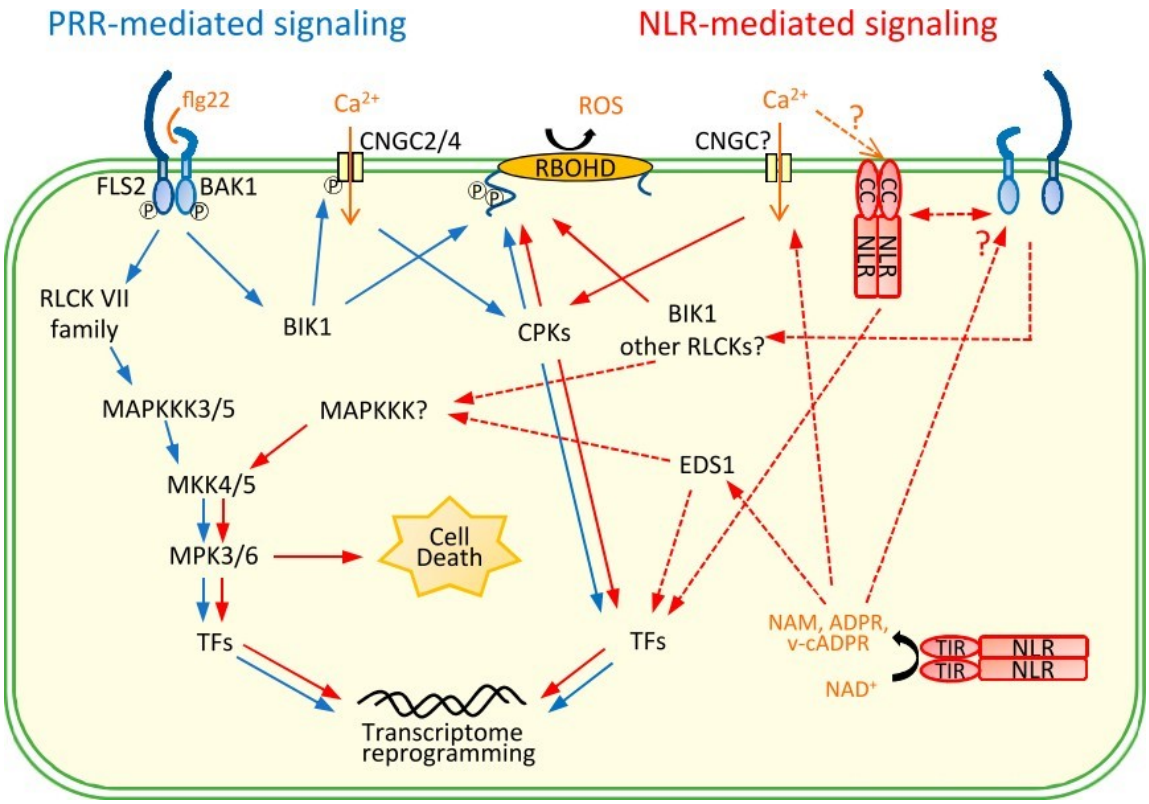


Figure 4. PTI and ETI downstream signalling events (adapted from Lu and Tsuda, 2021).
After microbe perception, cellular responses will be triggered leading to activation of plant immune responses. These cellular responses include calcium influx, ion fluxes, ROS burst, activation of MAPK cascades, activation of phytohormone signalling, transcriptional reprogramming, and sometimes programmed cell death. Solid arrows indicate known mechanisms. Dashed arrows indicate signalling mechanism remaining elusive. Question marks show connections that have not been characterised experimentally.

- **B.4. How is pathogen perception translated into immune responses?**

PAMP recognition induces the formation of a PRR complex that will lead to a series of cellular responses summarised in Figure 4. These events start from transphosphorylation of PRRs after PAMP-induced complex formation to downstream signalling comprising calcium signalling, plasma membrane depolarisation, activation of MAPK-signalling cascades, ROS burst, activation of hormonal pathways, and transcriptional reprogramming. It also involves RLCKs, heteromeric G proteins, plasmodesmata and stomata closure, and callose deposition (Yu et al., 2017).

While the recognition phenomena between PTI and ETI are distinct, the signalling events following PAMP or effector perception overlap for many signalling events. Some signalling pathways are triggered by both PAMP or effector perception but differ in intensity or duration (Yuan et al., 2021a).

B.4.1. Ca^{2+} influx and other ion fluxes

During PTI, one of the earliest responses after PAMP treatment is an increase in cytosolic Ca^{2+} concentration ($[Ca^{2+}]cyt$). This increase starts after 30-40 seconds, peaks at 2-6 minutes and lasts for about 30 minutes before returning to the resting state. The intensity of the increase in $[Ca^{2+}]cyt$ depends on the dose and the type of PAMP perceived but in any case, this $[Ca^{2+}]cyt$ induces the opening of ion channels (H^+ influx, K^+, Cl^- and NO^- efflux) leading to membrane depolarisation and apoplastic alkanisation (Bigeard et al., 2015; Yu et al., 2017).

Calcium signalling is also triggered in ETI via NLR signalling but the influx of $[Ca^{2+}]cyt$ is slower and more sustained than the one triggered during PTI. Recent discoveries about NLR resistosomes that can act as channels on the plasma membrane lead scientists to wonder whether these resistosomes might be involved in $[Ca^{2+}]cyt$ influx but this requires further investigation (Yuan et al., 2021a).

B.4.2. ROS burst

ROS can act as signalling molecules, toxic components against pathogenic infections and are involved in the reinforcement of plant cell walls through oxidative cross-linking of polymers. It is not surprising that ROS generation is a hallmark of PAMP-induced signalling responses. Indeed, a transient and rapid generation of apoplastic ROS is initiated within 5 minutes after PAMP treatment, peaking after 10-15 minutes and returning to resting state after 30 minutes. ROS interact with other responses triggered by PAMPs. For instance, ROS production depends on increased $[Ca^{2+}]$cyt and is required for stomatal closure and callose deposition (Tang et al., 2017; Yu et al., 2017).

ROS production is also induced during ETI, with the difference that the ROS burst is biphasic with two peaks and the second peak is usually much stronger and sustained than the first. Interestingly, the ETI-triggered ROS burst requires exposure to PAMPs, suggesting that ETI-associated ROS bursts depends on PRR signalling (Ngou et al., 2020; Yuan et al., 2021b). This shows that in addition to common signalling pathways, PTI and ETI appear to depend on each other for full potentiation of responses.

B.4.3. MAPK signalling cascades

MAPK cascades are involved in many biological processes including PTI and ETI. A MAPK cascade consists of MAPK Kinase Kinases (MAPKKKs/MEKKs), MAPK Kinases (MAPKKs/MKKs) and MAPKs/MPKs. The signalling cascade is triggered by the phosphorylation of MAPKKK/MEKKK, which activates MAPKK/MKK by phosphorylation which then activates MAPK/MPK by phosphorylation (Thulasi Devendrakumar et al., 2018).

Two MAPK cascades have been extensively studied in Arabidopsis immune responses: the MAPKKK3/5-MKK4/5-MPK3/6 cascade and the MEKK1-MKK1/2-MPK4 cascade (Thulasi Devendrakumar et al., 2018). The MAPKKK3/5-MKK4/5-MPK3/6 cascade is involved in the activation of ethylene, camalexin, and indole glucosinolate biosynthesis, which are important in plant defence against

pathogens. This cascade is also required for stomatal immunity (Thulasi Devendrakumar et al., 2018). The MEKK1-MKK1/2-MPK4 cascade was thought to have a negative role on plant defence as disruption of this cascade resulted in autoimmunity (Thulasi Devendrakumar et al., 2018). However, reports have shown that this MAPK is guarded by SUPPRESSOR OF MKK1 AND MKK2 2 (SUMM2), a CC-NLR that monitors phosphorylation of a substrate of MPK4 (Bigeard et al., 2015; Thulasi Devendrakumar et al., 2018). This suggests that autoimmunity in the disrupted mutant of the MEKK1-MKK1/2-MPK4 cascade may be due to SUMM2 monitoring mechanisms. Challenging summ2 mekk1 and summ2 mkk1 mkk2 mutant plants with Pseudomonas syringae DC3000 led to a greater susceptibility than in summ2 mutants, suggesting that MEKK1-MKK1/2-MPK4 cascade promotes plant defence against pathogens. Thus, it seems that MEKK1-MKK1/2-MPK4 cascade also positively regulates immune responses against pathogens and is guarded by SUMM2 that will trigger immunity activation when the cascade is disrupted (Thulasi Devendrakumar et al., 2018).

The activation of MAPK cascade upon treatment with PAMP takes a few minutes. Indeed, in A. thaliana, treatment with flg22 triggers the activation of four MAPKs (MPK3, MPK4, MPK6 and MPK11) within 1-2 minutes and MAPK activity reaches its peak at 10-15 minutes (Bigeard et al., 2015). During ETI, MAPK activation is slower but more sustained. Although MAPK cascades activation following PAMP perception is known to be mediated by RLCKs, how NLR signalling activates MAPK cascades remains unknown (Yuan et al., 2021a). Surprisingly, MAPK cascades cannot be triggered by the RRS1/RPS4 and RPP4 TIR-NLRs after inducible expression of AvrRps4 or AvrRpp4 in Arabidopsis, revealing that NLR-mediated activation of MAPK cascade cannot be triggered without PRR signalling (Ngou et al., 2020).

B.4.4. Phytohormones

Phytohormones play a central role in plants as they are involved in both development and immunity. The main hormones involved in immunity are salicylic

acid, jasmonic acid, ethylene and abscisic acid. We can also include cytokinins, auxin, gibberellins and brassinosteroids to a lesser extent (Shigenaga and Argueso, 2016). While hormonal pathways related to plant immunity promote plant resistance to pathogens, this is not without a fitness cost to the plants. Hence, fine-tuning of strong hormone-mediated immune responses is necessary to maintain the trade-off between growth and immunity (Ballaré and Austin, 2019; van Butselaar and Van den Ackerveken, 2020; Vos et al., 2015).

B.4.4.a. Salicylic acid (SA)

Despite its roles in plant development and resistance to abiotic stresses, the phenolic compound SA is primarily known as a key plant hormone required to promote plant defence responses against many biotrophic and hemibiotrophic pathogens. SA accumulation at pathogen infection sites correlates with plant resistance to pathogens (Shigenaga and Argueso, 2016). SA or related metabolites are involved in regulating HR and System Acquired Resistance, which represents a kind of systemic induced resistance. Several genes are required for SA response including Nonexpresser of PR genes 1 (NPR1), identified by forward genetic screens for mutants insensitive to SA induction. NPR1, together with other NPR proteins, seem to operate as hubs mediating the expression of SA-responsive genes, including the PATHOGENESIS RELATED (PR) genes that encode several proteins with antimicrobial activities promoting plant resistance to a wide range of pathogens (Ding and Ding, 2020; Verma et al., 2016).

B.4.4.b. Jasmonic acid (JA) and ethylene (ET)

JA controls the production of defence compounds that confer resistance to several pathogens and vertebrate herbivores. Pathogen perception and tissue injury trigger JA synthesis that will promote the expression of several secondary metabolites and proteins involved in plant immunity including alkaloids, terpenoids, anti-nutritional proteins and some PR proteins (Campos et al., 2014).
While JA-mediated defence mechanisms were long thought to be specific for necrotrophs, this assumption is now questioned. Originally, this assumption was

based on studies performed on a limited number of dicot plants, including Arabidopsis, tomato and tobacco. However, there is increasing evidence showing the importance of JA-induced defence mechanisms in monocot and gymnosperm species in response to pathogens and herbivores showing that JA is not always associated with resistance against necrotrophs (Campos et al., 2014). Indeed, JA elicits immunity in rice against biotrophic and hemibiotrophic parasites such as the root-knot nematode Meloidogyne graminicola and the bacterial pathogen Xanthomonas oryzae. As such JA is involved in defence mechanisms against different types of pathogens but it depends on the pathosystem (Nahar et al., 2011; De Vleesschauwer et al., 2013).

After pathogen perception, ET biosynthesis is stimulated and is then sensed by its receptors triggering a signalling cascade involving the Ethylene-responsive factor (ERF) transcription factor (TF) family, which plays an important role in defence regulation. In dicots, ET is best known to act synergistically with JA to promote plant defence against necrotrophic pathogens (Broekgaarden et al., 2015; Pieterse et al., 2012).

B.4.4.c. Abscisic acid (ABA)

ABA is a major hormone associated with various stages of plant growth but also with abiotic stresses including drought and salinity. There is also a growing body of research on the role of ABA in plant responses to biotic stresses. One of the best known roles of ABA in plant responses to biotic stresses is the control of stomatal closure in response to pathogen attack to prevent or restrict pathogen entry (Chen et al., 2020). ABA can induce the opening of Ca^{2+} channels to close stomatal pores and activate Open Stomata1 (OST1), a key regulator of stomatal closure. PAMP-induced stomatal closure can be triggered in an OST1-dependent or -independent manner (Su et al., 2017; Zheng et al., 2018). However, ABA has a broader role in plant defence against pathogens than the control of stomatal closure to prevent pathogen entry.

In many pathosystems, ABA has been reported to negatively affect plant defences. In some studies, a host plant species pretreated with ABA could lead to an increased susceptibility to a pathogen. This has been demonstrated in rice infected with the fungus Magnaporthe grisea (Jiang et al., 2010), in wheat in response to the fungus Fusarium graminearum (Buhrow et al., 2016), but also in barley in response to the fungus M. oryzae (Ulferts et al., 2015). Other studies have investigated the response of plants mutated in the ABA pathway to pathogens. For instance, an ABA-hypersensitive mutant showed increased susceptibility to P. syringae (Goritschnig et al., 2008). Another example is the infection of barley mutants in ABA synthesis with M. oryzae which led to a lower susceptibility implying a negative role of ABA in barley defence responses to M. oryzae (Ulferts et al., 2015).

In other pathosystems ABA seems to play a beneficial role in plant defence. In A. thaliana, infection by the oomycete Pythium irregular of ABA-insensitive mutants or mutants impaired in ABA biosynthesis revealed increased susceptibility compared to the wild-type (WT), suggesting that ABA has a positive role in plant defence in this pathosystem (Adie et al., 2007). Furthermore, exogenous application of ABA to rice prior to infection by the fungus Cochliobolus muyabeanus increased basal resistance and limited pathogen progression (de Vleesschauwer et al., 2010). Finally, Arabidopsis mutants impaired in ABA biosynthesis were shown to be more susceptible to Bamboo mosaic virus (BaMV), suggesting that ABA pathway has a positive role in Arabiopsis defences against this virus (Alazem et al., 2014). Thus, ABA seems to be involved in plant response to pathogens and its role is pathosystem-dependent.

B.4.4.e. Hormonal crosstalk

All of the plant hormones described above are involved in mediating immune responses against various biotic stresses. It is clear that there is no single master hormone that governs the activation of immunity in plants. Instead, plant immunity is modulated by a complex hormonal network called hormonal crosstalk.

In dicots, one parameter influencing the type of hormonal response against a given pathogen is the pathogen's lifestyle. SA is generally required for defence against biotrophic or hemi-biotrophic pathogens, whereas JA is mostly required for defence against necrotrophic pathogens, making these two pathways act antagonistically to each other. Other hormones, like cytokinins, auxin, gibberellins, and brassinosteroids play an important role in plant development, but also play a role in plant immune responses and are often involved in SA or JA/ET pathways, adjusting hormone signalling to adapt immune responses to the invading pathogen (Shigenaga and Argueso, 2016). It is also worth mentioning that hormone-mediated immune responses require a lot of energy that cannot be used for growth at the same time. Thus, hormonal crosstalk might be essential to adapt the immune response to invading pathogen, but it might also be cost saving in fitness to combine different pathways to promote defence to pathogens (Shigenaga and Argueso, 2016; Vos et al., 2015; Yang et al., 2012).

B.4.5. Transcriptional reprogramming

Plant-pathogen interactions induce a massive and dynamic reprogramming of plant gene expression, referred to as transcriptional reprogramming. Transcriptional reprogramming is the main link between signal transduction and the activation of defense mechanisms.

At the top of plant immunity transcriptional regulation, the SARD1 (System Acquired Resistance Deficient 1) and CBP60g (Calmodulin binding protein 60g) TFs act as a convergent point in PTI and ETI signalling (Peng et al., 2018). Originally shown to be involved in the SA signalling pathway, chromatin-immunoprecipitation sequencing (ChIP-seq) analysis demonstrated that SARD1 and CBP60g directly regulate the expression of a large number of plant immunity-related components. Since they regulate many positive and negative regulators of immune responses, they are considered as master regulators of plant immunity (Sun et al., 2015).

In addition to these master regulators, several families of TFs are involved in the transcriptional reprogramming associated with plant immunity. TFs of the basic

leucine-zipper subfamily TGA (bZIP/TGA) are positive regulators of SA-mediated gene expression and defence against biotrophs. The basic-Helix-Loop-Helix (bHLH) TF family includes members that are key regulators in JA responses and crosstalk with other phytohormones (Tsuda and Somssich, 2015).

Another important family of defensive TFs is the WRKY family. WRKY TFs are characterised by a conserved 60-aa DNA-binding domain called the WRKY domain, containig a highly conserved WRKYGQK heptad motif responsible for DNA binding activity. These TFs bind to the cis-regulatory “W-box” motifs in their DNA target sequences via their WRKY domain(s). W-boxes have been identified in the promoters of many genes associated with PTI or ETI and WRKY TFs have been shown to act as positive and negative regulators of ETI and PTI. For instance, AtWRKY33 is a negative regulator of SA-mediated plant defences but is a positive regulator of plant resistance against the necrotroph Botrytis cinerea; and AtWRKY70, AtWRKY46 and AtWRKY53 are involved in the positive regulation of plant defence against P. syringae (Garner et al., 2016; Pandey and Somssich, 2009; Tsuda and Somssich, 2015).

B.4.6. Hypersensitive Response and Systemic Acquired Resistance

After the perception of a pathogen, one way to stop the progression of the pathogen in plant is to condemn the site of infection. This phenomenon is called the Hypersensitive Response (HR) and is characterised by a rapid and localised cell death at the site of pathogen penetration. HR is often associated with ETI notably (Balint-Kurti, 2019).

While HR is a way of stopping pathogen invasion at the site of infection, plants can also protect distal tissues from present and future infections. This systemic defence is called system acquired resistance (SAR) and relies on the activation of systemic immune responses conferring broad-spectrum resistance at the whole plant level. It involves mobile signals generated at the site of infection, which then translocate to distal tissues to prepare the plant for future infections. Mobile

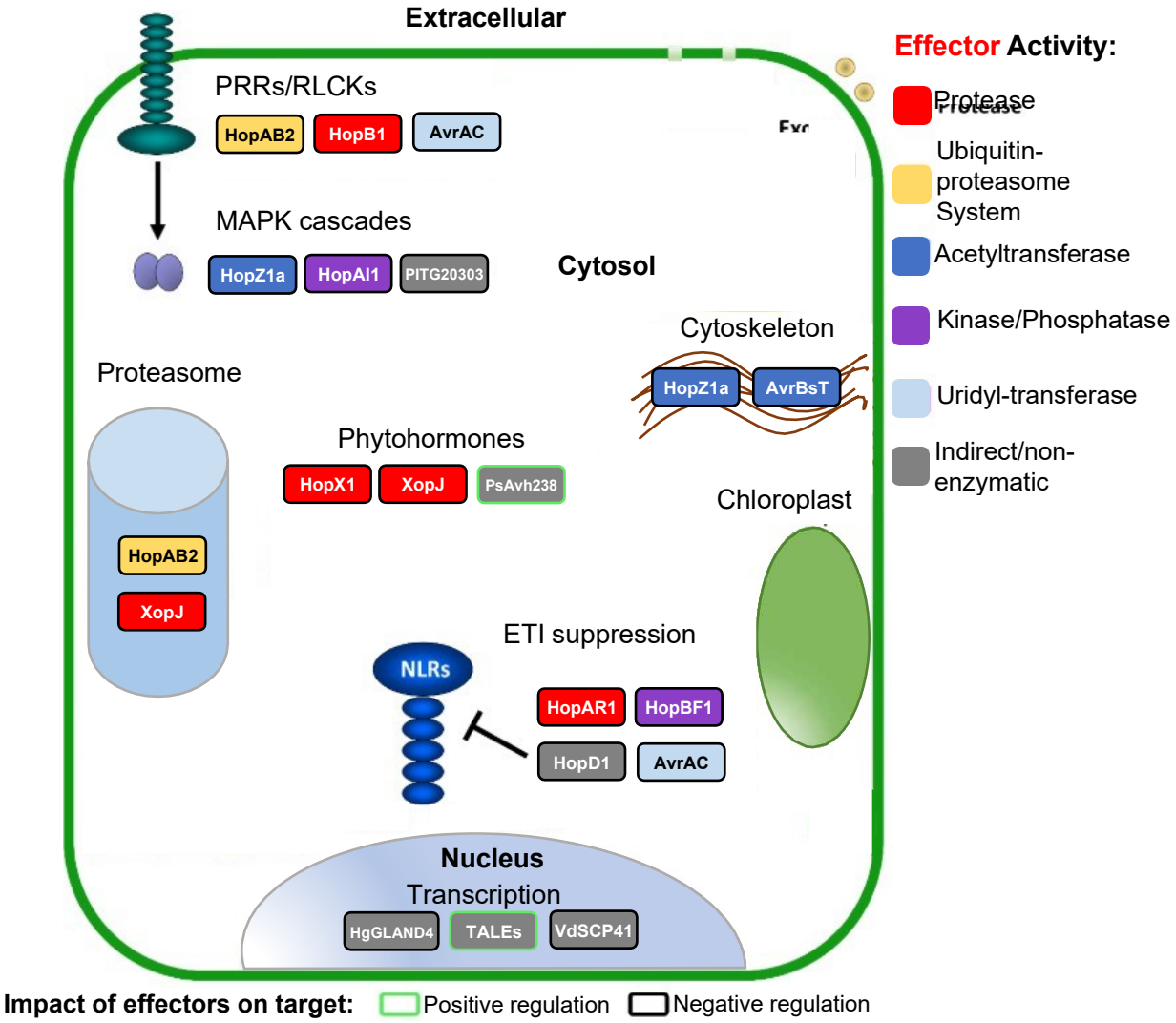


Figure 5. Pathogen effectors target various cellular processes (adapted from Schreiber et al., 2021).
To counteract PTI and promote infection, pathogens deliver effectors into plant cells to modify cellular activities to their advantage. Effectors can manipulate cellular processes via their enzymatic activities or simply by interacting with host components, regulating them positively or negatively. In this thesis manuscript, I have focused on some examples of host processes targeted by effectors such as PTI components, MAPK cascades, cytoskeleton, transcription, proteasome and ETI-related components.

signals include SA, pipecolic acid (a methylated derivative of SA) and ROS to name a few (Shine et al., 2019).

- **B.5. How phytopathogens facilitate infection: focus on Effector-Triggered Susceptibility**

To counteract basal immune responses and promote infection of their host, adpated pathogens have developed specialised proteins called effectors that promote infection by interfering with various plant cellular processes (Figure 5). In the zig-zag model, this pathogen interference is referred to ETS. I will introduce effector proteins and give several examples of manipulation of host processes by pathogens to promote infection. I will deliberately avoid examples concerning the bacterium Ralstonia solanacearum and the manipulation of the host epigenome by pathogens, as they will be detailed in later parts of this Introduction section.

B.5.1. Effectors, pathogen secreted proteins aiming at disturbing cell homeostasis to favour infection

Pathogen effectors can either be secreted into the apoplast or translocated into the host cytoplasm. Bacterial pathogens use complex multiprotein secretion systems such as type 3 and type 4 secretion systems (T3SS, T4SS) which are complex needle-like structures allowing translocation of effectors and effectors with DNA into the host cell (Costa et al., 2015; Hayes et al., 2010). Filamentous pathogens do not have these needle-like secretion systems but use different secretion systems via specialised structures like haustoria (Bozkurt and Kamoun, 2020). Once inside the cell, effectors will target various host components to make the environment beneficial to the pathogen, or to counter immune responses to promote infection. Some effectors can be recognised or detected by the infected plant leading to ETI responses (cf. B.3., Figure 3).

B.5.2. Effectors can manipulate various cellular processes

In this part, I will present some examples of effectors and how they can manipulate host cellular processes. This section intends on describing the virulence activities of diverse pathogen effectors but is by no means complete.

B.5.2.a. Effectors can dampen PTI responses by targeting PRRs

Some effectors can directly target PTI components to suppress basal immune responses. The P. syringae effector HopB1 is a threonine protease that interacts in Arabidopis with FLS2 and cleaves the RLK BAK1 when phoshorylated. The proposed model describes that upon perception of P. syringae, flg22 triggers the recruitment of BAK1 to FLS2, which is in complex with HopB1, and then, after BAK1 phosphorylation, HopB1 cleaves phosphorylated-BAK1 leading to the alteration of early PTI responses like ROS production (Li et al., 2016).
Another effector, HopAB2 (formerly AvrPtoB), from the same bacterium also targets PTI components in Arabidopsis and contributes to pathogen virulence (Göhre et al., 2008). HopAB2 is an E3 ubiquitin ligase that can ubiquitinate and catalyse the degradation of different PTI components like the RLKs FLS2 and CERK1 and this participates in promoting bacterial growth (Gimenez-Ibanez et al., 2009; Göhre et al., 2008).

B.5.2.b. Effectors can interfere with MAPK signalling cascades

Interference with MAPK cascades is an effective strategy as they constitute a node transducing signal perception into intracellular responses.
There is increasing evidence of plant pathogen effectors targeting MAPK cascades. For instance, the P. syringae HopAI1 effector targets MPK3 and MPK6 in Arabidopsis. Indeed, in presence of HopAI1, these MAPKs are permanently dephosphorylated on a key threonine residue, indicating that HopAI1 is a phosphothreonine lyase, an enzyme that covalently modifies phosphorylated threonines, leading to permament dephosphorylation of this residue and impairment of PTI responses (Zhang et al., 2007a).

Another example is the effector PITG20303 from the oomycete Phytophthora infestans. Du and colleagues demonstrated that PITG20303 dampens PTI responses and promotes pathogen colonisation by stabilising StMKK1, a potato MAPK kinase kinase acting as a negative regulator of PTI responses (Du et al., 2021).

Instead of stabilising a negative regulator of plant immunity, the P. syringae HopZ1a acetyltransferase targets a MAPK kinase kinase that acts as a positive regulator to suppress plant immunity in Arabidopsis. HopZ1a interacts with MKK7, a positive regulator of PTI responses, acetylates it and this induces the suppression of all MKK7-dependent signalling, suppressing local and systemic plant immunity (Rufián et al., 2021).

B.5.2.c. Effectors manipulate hormonal pathways

Since hormone signalling pathways are important for the regulation of plant immune responses, it makes sense that some effectors target these pathways.

One way to manipulate hormone pathways is to play on antagonistic pathways. In Arabidopsis, the effector HopX1 from P. syringae pv. tabaci 11528 is a cysteine protease that targets JAZ transcriptional repressors. HopX1 associates with and degrades several JAZ proteins. Degradation of JAZ proteins derepresses JA signalling pathways which antagonises the SA signalling pathway, a hormonal pathway detrimental to P. syringae, promoting susceptibility (Gimenez-Ibanez et al., 2014).

Another way to manipulate hormone signalling pathways is to disrupt the turnover of key actors. The Xanthomonas euvesicatoria XopJ effector is an acetyltransferase with a protease activity. XopJ was shown to degrade a subunit of the pepper (Capsicum annuum) proteasome, Regulatory particule triple-A APTase subunit 6 (RPT6) (Üstün and Börnke, 2015). Degradation of RPT6 decreases proteasome activity and impairs the turnover of NPR1, a major regulator of SA-dependent responses (Üstün and Börnke, 2015; Üstün et al., 2013).

Effectors can also interfere with phytohormone synthesis. The Phythophthora sojae effector PsAvh238 is a virulence factor promoting disease in soybean (Glycine max). PsAvh238 interacts with soybean type 2 1-aminocyclopropane-1-carboxylate synthases (Type2 ACSs), and trigger the destabilisation of these enzymes which are involved in ethylene biosynthesis, promoting infection by P. sojae (Yang et al., 2019).

B.5.2.d. Effectors can interfere with plant cytoskeleton

Some effectors have been reported to interfere with the plant cytoskeleton that is essential for many cellular processes including cell division and growth, vesicle trafficking, endocytosis, organelle movement, but is also important in plant defence responses (Li and Day, 2019).
The effector HopZ1a from P. syringae, among all its functions, has also been shown to target microtubule networks in Arabidopsis. Lee and colleagues demonstrated that HopZ1a acetylates tubulin and causes a strong decrease in microtubule networks, triggering alteration of the secretory pathway and suppression of a cell-wall mediated defense, callose deposition (Lee et al., 2012).
Disturbing the localisation of a protein can also contribute to disturbing the organisation of the cytoskeleton. Indeed, AvrBsT from Xanthomonas euvesicatoria is a YopJ family acetyltransferase that can target a component of the cytoskeleton in Arabidopsis. AvrBsT has been shown to interact both in vitro and in yeast with ACETYLATED INTERACTING PROTEIN 1 (ACIP1), a microtubule-associated protein. AvrBsT acetylates ACIP1 and disrupts ACIP1 localisation by promoting the accumulation of large ACIP1 aggregates. ACIP1 seems to be involved in plant immune responses to pathogens but the impact of ACIP1 targeting by AvrBsT on plant immune responses remains unclear (Cheong et al., 2014).

B.5.2.e. Effectors can alter host gene transcription

Considering that the plant transcriptional machinery is vast and complex, targeting master regulators could be a potent virulence strategy for blocking immune responses. The effector VdSCP41 from the plant pathogenic fungus Verticillium

dahliae contributes to pathogen virulence and has been shown to interact with the Arabidopsis master regulators CBP60g and SARD1 and cotton GhCBP60b. Binding of VdSCP41 to CBP60g compromises its transcriptionnal activity and may subsequently interfere with PTI and other defence pathways (Qin et al., 2018). Another virulence strategy consists in deploying effectors that can directly affect gene expression. In Arabidopsis, the nematode Heterodera glycines HgGLAND4 effector binds genomic DNA and was shown to repress the expression of a reporter gene in planta. A DNA-binding site is located at defence-related genes encoding lipid transfer proteins (LTPs), and these LTP genes were found to be down-regulated by HgGLAND4, suggesting that this effector might act as a transcriptional repressor to dampen LTP-triggered defences (Barnes et al., 2018). Acting in a similar way, the well-known transcription activator-like effectors (TALEs) from Xanthomonas spp. are known to target specific DNA sequences as well. These effectors contain series of tandem aa repeats between 33 and 35 residues thanks to which they can bind to specific host DNA sequences, called effector binding elements (EBEs), and activate gene transcription (Boch et al., 2014). To activate transcription, TALEs interact with the general plant transcription factor TFIIA γ, and coordinate the transcription of susceptibility genes (Hui et al., 2019; Yuan et al., 2016). TALEs targets include various genes encoding sugar transporters, abscisic acid synthesis hormones, and sulphate transporters (Boch et al., 2014; Cernadas et al., 2014; Peng et al., 2019).

B.5.2.f. Effectors can counteract ETI responses

To evade host recognition, pathogens can use effectors that block NLR-mediated activation of immune responses triggered by other effectors. HopAR1 (AvrPphB) from P. syringae can counter NLR RPM1-mediated immunity in Arabidopsis. In detail, the effector AvrB can induce phosphorylation of the RPM1-guarded protein RIN4 by RPM1-interacting protein kinase (RIPK) and this leads to the activation of RPM1-mediated immunity (Liu et al., 2011). HopAR1 can prevent this event by

cleaving RIPK, preventing phosphorylation of RIN4 and activation of RPM1-mediated immunity (Russell et al., 2015).

ETI can also be countered by targeting the regulation of NLR proteins. In Nicotiana benthamiana, the P. syringae effector HopBF1 acts as a host client of the plant chaperone Hsp90 by exhibiting a minimal protein kinase folding, resulting in HopBF1-Hsp90 interaction and Hsp90 phosphorylation by HopBF1. Phosphorylation of Hsp90 results in a reduction in its ATPase activity used to catalyse the folding of its target proteins, such as NLRs. As Hsp90 phosphorylation delays and reduces HR elicited by an autoactive variant of RPM1, it has been suggested that HopBF1 causes NLR destabilisation by inhibiting Hsp90 chaperon activity, thus attenuating ETI (Lopez et al., 2019).

Finally, another mean to alter ETI-related transcriptional responses can be by interfering with the function of TFs. The P. syringae HopD1 effector contributes to virulence in Arabidopsis and has been shown to target the NAC transcription factor NTL9, a positive regulator of plant immunity, resulting in the dampening of ETI responses (Block et al., 2014).

B.5.2.g. AvrAC, an effector that can disturb both PTI and ETI

Increasing evidence suggests the existence of intricate interactions between PTI and ETI (Yuan et al., 2021a). In this context, a potent virulence strategy would consist in interfering with both PTI and ETI using a single effector. The AvrAC (XopAC) effector from Xanthomonas campestris pv. campestris (Xcc) is an uridyltransferase. In Arabidopsis, AvrAC uridylates two kinases, the RLK BIK1, known for its role in PTI activation, and RIPK, involved in RPM1-mediated responses (Feng et al., 2012b). Uridylation of these two kinases by AvrAC compromises PTI and ETI responses. Indeed, transgenic lines expressing avrAC are strongly impacted in PTI responses normally triggered upon flg22 treatment such as MAPK activation and ROS burst. In these lines, increased bacterial growth of the type III secretion mutant strains Xcc ΔhrcV and Pst ΔhrcC was also observed. In addition, these transgenic lines supported greater growth of Pst delivering AvrB,

which normally triggers RPM1-triggered immunity in Arabidopsis (Feng et al., 2012b). However, plants evolved the decoy PBL2, a paralog of BIK1, enabling the detection of AvrAC activity by the ZAR1 immune complex (Wang et al., 2015b).

B.5.3. Effectors target in their own way different pathways in a redundant manner

To conclude, we have seen that plant pathogens have evolved different virulence strategies by using various effectors to subvert host processes related to defence, metabolism, and transcription. They can act by inducing modifications on host proteins like phosphorylation, uridylation, acetylation, or ubiquitination. These modifications can inactivate or degrade targeted proteins favouring pathogen infection. They can also target higher and larger levels of host protein regulation such as protein folding, protein turnover, master regulators of plant responses or even hormone biosynthesis. All these ways of manipulating plant processes are aimed at redirecting plant responses to promote pathogen infection.

Interestingly, it is frequently observed that several effectors from the same pathogen target similar pathways. From the examples I have described, we can see that P. syringae uses (i) HopAB2 and HopB1 to target PTI components, (ii) HopAI1 and HopZ1a to interfere with MAPK cascades and (iii) HopZ1a and AvrBsT to disturb the plant cytoskeleton. Diversifying effectors while staying redundant in the targeted pathway reflects how pathogens can prevent effector recognition while targeting key pathways to promote infection. We also note that a single effector can target several pathways, as HopZ1a which targets the cytoskeleton, PTI components and MAPK cascades. Finally, we can also notice hubs of action between different pathogens. For instance, AvrAC from Xcc and HopAR1 from P. syringae both target the kinase RIPK. Several pathogens can also specifically target particular pathways, which are essential for plant defence and/or for pathogen thriving in plants.

C. Adjustments and limitations to the zig-zag model

The zig-zag model has the benefit of summarising the evolutionnary arms race between plants and pathogens, but it was developed at a time when knowledge about plant responses to pathogens was mainly obtained from a few pathosystems. Indeed, as our knowledge of other pathosystems and the underlying molecular mechanisms that govern these interactions increase, the limitations and restrictions of the zig-zag model become even more apparent. The following section will briefly describe the limitations and adjustments of the zig-zag model.

One of the first adjustment made to the zig-zag model was to expand the definition of patterns. Originally, patterns were only motifs from conserved molecules found on pathogens. This definition was too restrictive, as host motifs generated upon damage caused by a pathogen or modified “self” can also be recognised in planta to trigger PTI: Damage Associated Molecular Patterns (Choi and Klessig, 2016; Erb and Reymond, 2019) (Figure 2). Nowadays, the term PAMPs is as commonly used as MAMPs (Microbe Associated Molecular Patterns) and other terms are used depending on the type of patterns referred to, like Herbivore-Associated Molecular Patterns or Nematode-Associated Molecular Patterns (Choi and Klessig, 2016; Erb and Reymond, 2019).

Other definitions and concepts from the original model were challenged by results indicating that there is a blurred dichotomy between ETI and PTI (Cook et al., 2015; Thomma et al., 2011). For instance, PAMPs were often considered to be highly conserved motifs in pathogens, whereas effectors were more likely to be less conserved as they experience greater evolutionary pressure. Whilst this definition is accurate in many cases, several examples have shown that some effectors are in fact highly conserved within and between different pathogen species and that some PAMPs are instead narrowly distributed (Cook et al., 2015; Thomma et al., 2011).

The blurred dichotomy is also reflected in the distinction between PTI and ETI responses. For a long time, scientists have observed that many of the responses

induced during PTI and ETI overlap, and that PTI and ETI differ mainly in intensity and duration of these responses (Lu and Tsuda, 2021; Tsuda and Katagiri, 2010). This overlap already hinted that ETI and PTI might not be strictly seperate pathways, and recently, evidence has shown a real interplay and interdependence between PTI and ETI (Lu and Tsuda, 2021). Indeed, two recent studies have demonstrated that PTI and ETI potentiate each other and that NLR signalling requires PRR signalling for full activation (Ngou et al., 2021; Yuan et al., 2021b). In detail, mutation of the PTI-related components BAK1 and BKK1, altered RPS2-, RPS4-, and RPS5-mediated resistance in A. thaliana (Ngou et al., 2021; Yuan et al., 2021b). Conversely, ETI-related responses also appear to be PTI-dependent. This is well illustrated with the RPS4/RRS1-dependent cell death response triggered by AvrRps4 which is aborted when this effector is transgenically expressed in A. thaliana instead of being delivered by a bacterial T3SS (Ngou et al., 2020). Also, AvrRpt2-induced HR is compromised in PTI defective mutants (Yuan et al., 2021b). These results show that ETI-mediated resistance and induction of cell death are dependent on PTI. Furthermore, Tian and colleagues showed that TIR-NLR signalling seems to be required in PTI, as mutation of the TIR NLR-signalling pathways EDS1/PAD4/ADR1 and EDS1/SAG101/NRG1 resulted in attenuated PTI responses, like SA accumulation or induction of defense-related genes after PAMP treatment (Tian et al., 2020). This could also mean that PTI also relies on signalling mechanisms we thought specific to ETI (Pruitt et al., 2020). In any case, either PTI requires ETI for full activation of responses, and/or these two pathways have much more in common than we thought. Overall, these adjustments and new findings revealed that ETI and PTI are not as distinct as previously thought and have prompted researchers to think of adjusted or alternative models to the zig-zag model. Among the various alternative models imagined by researchers are the invasion model, its variation the spatial invasion model, the danger model and its variation the spatial immunity model (van der Burgh and Joosten, 2019; Cook et al., 2015; Gust et al., 2017; Kanyuka and Rudd, 2019). To keep it simple, this

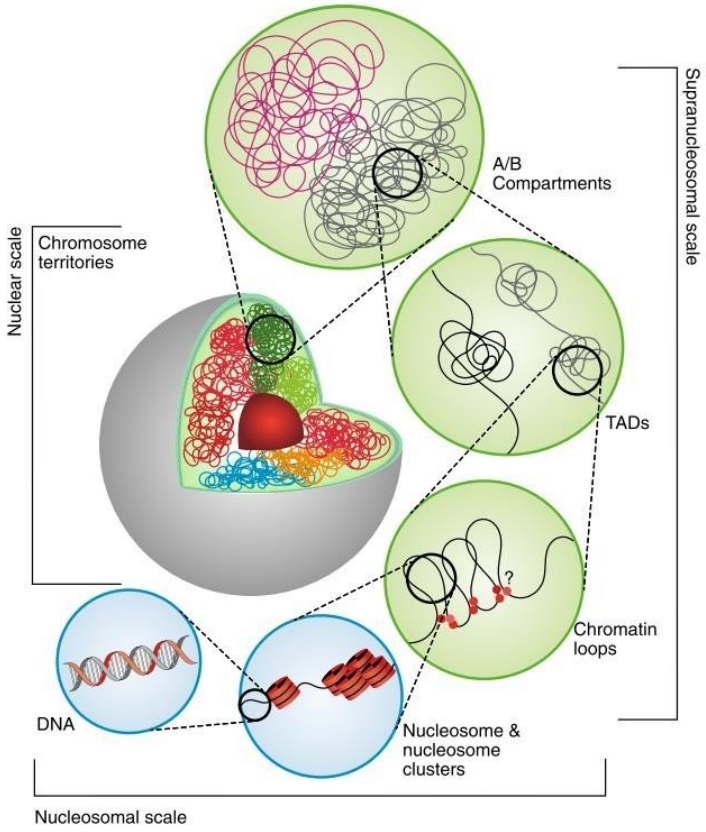


Figure 6. Eukaryotic chromatin structure (adapted from Doğan and Liu, 2018).
Eukaryotic genomic DNA is wrapped around histone octamers to form nucleosomes. Chromatin loops are the result of the interaction of nucleosomes from distant regions. They are parts of Topologically Associated Domains (TADs), regions of tens of kilobase pairs that represent areas of enhanced chromatin interaction within TADs but suppressed between TADs. The TADs together constitute A/B compartments, areas enriched in active or repressed chromatin. They are themselves sorted into distinct nuclear spaces, called chromosome territories.

manuscript will mainly consider the zig-zag model, its adjustments and limits keeping in mind that ETI and PTI are not as distinct as previously described.

2- The epigenome, an emerging player of plant-pathogen interactions

Since my PhD project focused on the functional characterisation of Arabidopsis histone readers targeted by a YopJ family acetyltransferase effector, I thought it would be useful to present in this introductory chapter a non-exhaustive overview of current knowledge about the regulation of plant defence responses by epigenetic mechanisms and the virulence strategies used by animal and plant pathogens to subvert the host epigenome.

A. Chromatin is a dynamic environment regulated by epigenetic factors

- **A.1. Chromatin structure influences gene expression**

In eukaryotes, genomic DNA is tightly condensed in the nucleus at different levels (Figure 6). The first level of DNA compaction is chromatin. Chromatin consists of nucleosomes which are units of 146 base pairs of DNA wrapped around histone octamers composed of the H2A, H2B, H3, H4 histones and the linker histone H1 (Luger et al., 1997). Beyond the nucleosomal scale, chromatin loops allow interactions between a regulatory component and its target gene even if they are distant in the genome (Doğan and Liu, 2018). Chromatin loops are themselves part of larger structures of tens of kilobase pairs called Topologically Associated Domains (TADs). TADs are chromatin regions that exhibit suppressed interactions with neighbouring TADs and enhanced chromatin interactions within the TAD. They allow distant chromatin areas to interact, but with spatial constraints that may confer target specificity of cis-regulatory elements (Doğan and Liu, 2018). TADs are part of structures of hundreds to thousands of kilobase-pairs called A/B compartments. A/B compartments are spatially distinct compartments of chromatin that are enriched in active chromatin, known as euchromatin, and

repressed chromatin, known as heterochromatin. Finally, the highest level of chromatin organisation is at the level of chromosomes. During cell replication, DNA tightly condense in the chromosomes to protect the genetic information during replication. During interphase, when DNA is not as condensed as during replication, chromosomes still occupy distinct nuclear spaces called chromosome territories (Doğan and Liu, 2018).

Despite the complex organisation of genetic information, chromatin is a highly dynamic structure that varies at different stages of the plant life cycle such as development or in response to stress (Pikaard and Scheid, 2014; Probst and Mittelsten Scheid, 2015). Importantly, chromatin structure influences the accessibility of genes for transcription machinery and is de facto involved in the regulation of gene transcription. Indeed, at the nucleosomal scale, DNA wrapped around histone octamers is less accessible to the transcription machinery than DNA located between nucleosomes (Workman and Kingston, 1998). During plant development and in response to stress, chromatin structure varies and this correlates with differential regulation of gene transcription (Lauria and Rossi, 2011; Li et al., 2007a). Changes in chromatin structure are regulated by a variety of mechanisms involving epigenetic factors.

- **A.2. Epigenetics, or how to study the regulation of chromatin condensation status**

Historically, “epigenetics” was defined by Waddington as “the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being” (Waddington, 1968). The word “epigenetics” itself refers to everything that is “in addition to changes in the genetic sequence” (Weinhold, 2006). In this manuscript, we will use a derived definition that is somewhat broader: “a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence” (Goldberg et al., 2007). These phenomena largely include all processes that modify chromatin condensation status.

Table 1. Epigenetic factors are involved in plant defence against pathogens.
There is increasing evidence in the literature that epigenetic regulators play a positive or negative role in plant defence against bacterial, fungal and oomycete pathogens. These include maintenance of DNA methylation, DNA demethylases, histone methyltransferases, histone demethylases, histone acetyltransferases, histone deacetylases, chromatin remodelling complexes and non-coding RNAs. lncRNA: long non-coding RNA; miRNA: microRNA.

Name	Related epigenetic mechanism	Host species	Pathogen	Mutant phenotype	Positive or negative regulator of plant defences?	Function in plant defence	Reference
DNA methylation maintenance	DNA methylation	*Arabidopsis thaliana*	*Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000	More resistant	Negative	Regulate *PR1* expression	Dowen et al., 2012
DNA demethylases (ROS1, DML1, DML3)	DNA demethylation	*A. thaliana*	*Fusarium oxysporum*	More susceptible	Positive	Regulate stress-responsive genes	Le et al., 2014
SDG8	Histone methylation	*A. thaliana*	*Botrytis cinerea* and *Alternaria brassicicola*	More susceptible	Positive	H3K36me3 activation of JA/ET-related genes	Berr et al., 2010
			P. syringae	More susceptible	Positive	H3K36me3 activation of SA-related genes	Zhang et al., 2020
						Maintain the NLR *LAZ5* in active state through H3K36me3	Palma et al., 2010
IBM1	Histone demethylation	*A. thaliana*	*P. syringae*	More susceptible	Positive	Activation of *PR1*, *PR2* and *FRK1* defence genes by controlling H3K9me2 and H3K4me3 levels	Chan and Zimmerli 2019
JMJ704	Histone demethylation	*Oryza sativa*	*Xanthomonas oryzae* pv. *oryzae*	More susceptible	Positive	Repression of negative regulators of rice immunity like *NRR*, *OsWRKY62* and *Os-11N3*	Hou et al., 2015
ELP2	Histone acetylation	*A. thaliana*	*B. cinerea* and *A. brassicicola*	More susceptible	Positive	Control levels of H3K9/14ac at defence-related genes like *NPR1*, *PR2*, *EDS1* and *PAD4*	Wang et al., 2015
HDA19	Histone deacetylation	*A. thaliana*	*A. brassicicola*	More susceptible	Positive	Positive control of JA/ET-related plant defence	Zhou et al., 2005
			P. syringae	More resistant	Negative	Negative control of SA-related plant defence by favouring repressive chromatin at loci like *PR1* and *PR2*	Choi et al., 2012
HDA9	Histone deacetylation	*A. thaliana*	*P. syringae*	More resistant	Negative	Negative regulation of *SNC1* expression upon infection	Yang et al., 2020
CHR5	Chromatin remodelling	*A. thaliana*	Avirulent *Pst* DC3000 strains and *P. syringae* pv. *maculicola*	More susceptible	Positive		Zou et al., 2017
SWP73A	Chromatin remodelling	*A. thaliana*	avirulent *Pst* DC3000 strains	More resistant	Negative	Suppresses directly or indirectly the transcription of several NLRs like *RPS2*, *RRS1*, *RPS4* and *ZAR1*	Huang et al., 2021
TAR-191, TAR197, TAR-212 and TAR-224	lncRNA	*A. thaliana*	*F. oxysporum*	More susceptible	Positive		Zhu et al., 2014
ELENA1	lncRNA	*A. thaliana*	*Pst* DC3000	More susceptible	Positive	Positive regulation of *PR1* expression	Seo et al., 2017
miR472	miRNA	*A. thaliana*	*Pst* DC3000	More resistant	Negative	Negatively regulates both PTI and ETI	Boccara et al., 2014

Changes in chromatin structure are regulated by a variety of epigenetic mechanisms, such as DNA methylation, histone post-translational modifications, chromatin remodelling complexes and non-coding RNAs. All these epigenetic marks and associated epigenetic factors design the fate of chromatin structure and help regulate gene transcription in many stages of the plant life cycle, including growth, development and response to biotic and abiotic stresses. In Table 1 are listed some epigenetic factors with a role in plant-pathogen interactions. They will be detailed in the section below.

B. Epigenetic mechanisms regulate plant defence against pathogens

- **B.1. DNA methylation mainly represses plant defence**

B.1.1. What is DNA methylation?

DNA methylation is one of the major epigenetic marks associated with gene repression. In plants, this mark is mainly found on the 5th carbon in the pyrimidine ring of cytosine nucleotides in the CG, CHG and CHH sequence contexts (where H is A, C or T) but this mark also exists on the 6th carbon of the purine ring of adenine in much lower abundance (Liang et al., 2018; Ouyang et al., 2020). De novo DNA methylation is catalysed by the RNA-dependent DNA methylation pathway (RdDM) that involves small interfering RNAs (siRNAs), and various proteins including ARGONAUTE proteins, RNA polymerases, DICER-LIKE proteins and the methylase Domains rearranged methylase 2 (DRM2) (Deleris et al., 2016; Zhang et al., 2018). Methylated DNA must be maintained by various DNA methyltransferases such as METHYLTRANSFERASE 1, DRM1, or CHROMOMETHYLASE3 (Zhang et al., 2018).
If not maintained, DNA methylation can be passively erased but there are also glycosylases that are responsible for active DNA demethylation by excising the methylated cytosine nucleotide (Zhang et al., 2018).
Mechanistically, DNA methylation is involved in the regulation of gene expression, transposon silencing and chromosome interactions (Zhang et al., 2018). Regarding gene expression regulation, DNA methylation can occur in the promoter or within

the gene. When in the promoter, DNA methylation is usually associated with repression of gene transcription, as it does prevent binding of transcription factors or promote other epigenetic marks associated with gene repression (Domcke et al., 2015; Zhang et al., 2018). However, this is not always the case, and the relationship between DNA methylation and transcription factors may depend on the cellular/biological context (Zhu et al., 2016a).
DNA methylation occurs at different stages of the plant life cycle, including growth, development, fruit ripening, but also in response to biotic and abiotic stress (Zhang et al., 2018).

B.1.2. DNA methylation mainly represses plant immunity

DNA methylation has been shown to be involved in the regulation of plant defences in some pathosystems. Dowen and colleagues showed that A. thaliana mutants impaired in the maintainance of DNA methylation were more resistant to Pst DC3000 compared to the WT line suggesting that DNA methylation is negatively correlated with plant defence against this pathogen (Dowen et al., 2012). They also showed that DNA methylation appears to directly or indirectly modulate some defence-related genes like PR1 since mutants in DNA methylation maintainance showed constitutive and inducible misexpression of this gene (Dowen et al., 2012).
During the interaction between A. thaliana and the fungal pathogen Fusarium oxysporum, the DNA demethylase triple mutant rdd (ros1 dml1 dml3) was found to be more susceptible than wild-type plants (Le et al., 2014). This suggests that DNA hypermethylation is linked to plant defence against F. oxysporum. Moreover, many stress-responsive genes were down-regulated, and that correlated with an enrichment for transposable elements in their promoters (Le et al., 2014).
Together, these studies show that DNA methylation and its regulators are directly or indirectly involved in regulating plant defences against fungal and bacterial pathogens, mainly by repressing stress-responsive genes.

- **B.2. Histone marks influence chromatin condensation state leading to differential regulation of defence-related genes in response to pathogens**

B.2.1. Histone marks: Post-Translational Modifications on histone tails influence chromatin condensation status

Post-Translational Modifications (PTMs) on histone tails, also known as histone marks, are another major epigenetic mark that influences chromatin structure. Histones consist of a structured core and an unstructured tail at their N-term that can carry various PTMs. The different types of histone marks include acetylation, methylation, phosphorylation, ubiquitylation and sumoylation. Some histone marks are associated with gene activation or repression such as histone methylation, histone acetylation and histone ubiquitylation (Kouzarides, 2007; Roudier et al., 2011).

Histone marks are dynamically regulated by various enzymes and proteins that can directly or indirectly modify histone marks and lead to changes in chromatin condensation state and transcription activity. The addition of histone marks is catalysed by enzymes called “writers”, and enzymes that can erase PTMs are called “erasers”. In addition, proteins carrying a domain that can detect and bind specific histone marks are called “readers”. It is important to keep in mind that although different epigenetic marks are sometimes studied separately, several marks are present at a certain locus and it is the combination of the different histone marks that determines the outcome in terms of chromatin condensation state or gene activity. Thus, whether it be writers, erasers or readers, depending on the chromatin state and histone mark environment, these histone mark regulators can be positively or negatively involved in gene transcription (Jenuwein and Allis, 2001; Roudier et al., 2011). In this introduction, histone methylation and acetylation will be detailed as well as their implications in plants.

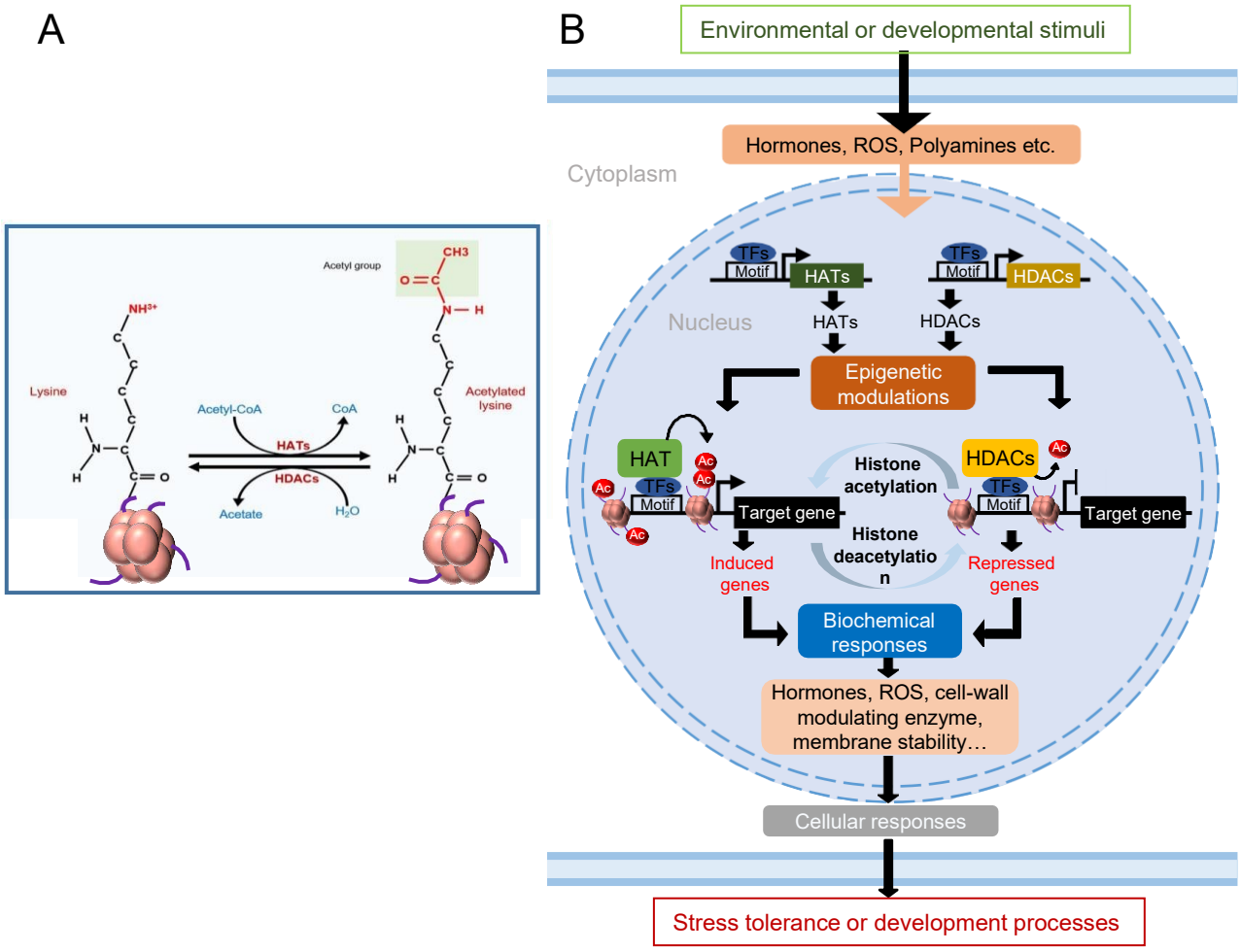


Figure 7. Histone acetylation correlates with active transcription (adapted from Kumar et al., 2021).

A. Acetylation of lysine residues on histone tails is catalysed by histone acetyltransferases (HATs) and removed by histone deacetylases (HDACs). Lysine acetylation neutralises the positive charge of the amine group, resulting in chromatin relaxation.

B. The response of plants to development and environmental stimuli is epigenetically regulated in part by histone acetylation. Under stimuli, HATs and HDACs are induced and lead to fine regulation of their target genes by acetylation or deacetylation, promoting or decreasing their transcription, resulting in biochemical responses that will be transduced into cellular responses and trigger developmental processes or stress tolerance responses.

TF: Transcription Factor.

B.2.1.a. Histone methylation

Histone methylation is catalysed by histone methyltransferases and can be erased by histone demethylases. In plants, histone methylation can be associated with both gene activation and gene silencing, depending on the number of methyl groups present, the residue involved, and the localisation. For instance, mono- and tri-methylation of lysine 27 of histone 3 (H3K27me1 and H3K27me3) in Arabidopsis are associated with repressed chromatin in heterochromatin and euchromatin respectively (Jacob et al., 2009; Zhang et al., 2007c). On the other hand, H3K4me3 is associated with active transcription (Zhang et al., 2009).

B.2.1.b. Histone acetylation, marker of active transcription

In Arabidopsis, histone acetylation occurs on the lysine residues on H2A, H2B, H3 and H4 tails (Zhang et al., 2007b). In general, histone acetylation, catalysed by histone acetyltransferases, is associated with permissive chromatine and active gene transcription, whereas histone deacetylation, catalysed by histone deacetylases, is associated with repression of gene transcription (Eberharter and Becker, 2002). Indeed, the addition of an acetyl group on the amine of a lysine residue neutralises the positive charge of the amine residue, leading to chromatin relaxation as the DNA-histone interaction is weakened (Figure 7A) (Eberharter and Becker, 2002; Kumar et al., 2021). Plant development and response to environmental stimuli/stress is regulated in part epigenetically, notably by histone acetylation. Upon environmental or developmental stimulus, a signalling cascade is activated and leads to the expression of histone acetyltransferases (HATs) and histone deacetylases (HDACs), enzymes responsible for histone acetylation and histone deacetylation respectively. In cooperation with other co-activators or co-repressors, HATs and HDACs target the genome, facilitating gene activation or repression respectively, leading to a fine-tuning of cellular processes (Figure 7B) (Kumar et al., 2021).

B.2.2. Histone marks regulators have different roles in plant response to pathogens

B.2.2.a. Histone methylation and plant-pathogen interactions

In Arabidopsis, the histone methyltransferase SDG8 regulates the tri-methylation of H3K36 (Li et al., 2015b). SDG8 has been shown to be involved in the plant response to B. cinerea and Alternaria brassicicola as well as to P. syringae (Berr et al., 2010; Zhang et al., 2020). The sdg8-1 mutant infected with B. cinerea, A. brassicicola or P. syringae had reduced resistance, suggesting that SDG8 plays a role in plant defence against these pathogens (Berr et al., 2010; Zhang et al., 2020). SDG8 seems to act via H3K36me3-related activation of various genes in the JA/ET and SA pathways, involved in plant defence against necrotrophs and biotrophs respectively (Berr et al., 2010; Zhang et al., 2020). It has also been shown that SDG8 is required to maintain the NLR-encoding gene LAZ5 in an active transcriptional state through H3K36me3 (Palma et al., 2010).

In addition, the histone demethylase IBM1 has recently been reported to be a positive regulator of Arabidopsis immunity against P. syringae. Infection of ibm1 mutants with P. syringae resulted in greater symptom development compared to the WT and the up-regulation of defense marker genes such as PR1, and PR2, normally induced upon bacterial infection, was abolished. In ibm1 mutants, these gene loci displayed enrichment of the inactive mark H3K9me2 and a reduction of the active mark H3K4me3. Since IBM1 can also bind directly to the gene body of PR1, and PR2 , it has been suggested that IBM1 facilitates the activation of these defence genes by directly or indirectly controlling the level of H3K9me2 and H3K4me3 (Chan and Zimmerli, 2019).

In rice, the histone lysine demethylase JMJ704 acts as a positive regulator of rice immunity against bacterial blight caused by Xanthomonas oryzae pv. oryzae. Expression of JMJ704 is induced upon infection with X. oryzae pv. oryzae and knock-down of JMJ704 led to reduced resistance of rice to bacterial blight (Hou et al., 2015). In this study, Hou and colleagues also demonstrated that JMJ704

participated in the repression of negative regulators of rice immunity against X. oryzae pv. oryzae such as NRR, OsWRKY62 and Os-11N3 by reducing active H3K4me2/3 (Hou et al., 2015).

B.2.2.b. Histone acetylation and plant response to pathogens

Since HATs catalyse histone acetylation, which correlates with active transcription, it is tempting to imagine that, in general, HATs are involved in the plant response to pathogens by activating transcription of immunity-related genes.

In Arabidopsis, the Elongator subunit 2 (ELP2) possesses a HAT domain and positively contributes to plant immunity against the necrotrophs B. cinerea and A. brassicicola (Wang et al., 2015a). Indeed, upon infection by these two necrotrophs, elp2 mutant was found to be more susceptible than WT plants and the induction of some JA/ET signaling pathway marker genes, such as PDF1.2 and WRKY33, was reduced or delayed (Wang et al., 2015a). In addition, the levels of histone H3K9/14ac in the defence-related genes NPR1, PR2, PR5, EDS1 and PAD4 were lower than in WT plants which could partly explain the delay in defence gene induction in the mutant (Wang et al., 2013). Thus, ELP2 participates in the regulation of histone acetylation levels at some defence genes to promote efficient defence induction.

HDACs are also involved in plant responses to pathogens. For instance, in Arabidopsis, HDA19 is involved in both JA- and SA-mediated defence responses. An early study revealed that HDA19 expression was induced upon infection with A. brassicicola, JA treatment and wounding (Zhou et al., 2005). Infection of the hda19 knock-down mutant with A. brassicicola led to an increased susceptibility associated with decreased transcription of several JA/ET pathway-related genes. Also, HDA19 overexpressing line showed increased resistance to the pathogen associated with upregulation of PR genes (Zhou et al., 2005). Another study showed that HDA19 expression was also induced in response to Pst DC3000 and infection of hda19 mutants or HDA19 over-expressing lines with this pathogen led to reduced and increased susceptible response, respectively, associated with

altered PR1 and PR2 expression (Choi et al., 2012). By ChIP assays, Choi and colleagues also demonstrated that under unchallenged conditions, HDA19 associates directly with PR1 and PR2 promoter regions and deacetylates histones at these loci. This suggests that HDA19 acts as a negative regulator of SA-related defences by favouring repressive chromatin under unchallenged conditions at loci such as PR1 and PR2, preventing unnecessary defence activation (Choi et al., 2012). Thus, HDA19 functions as a positive and a negative regulator of JA- and SA-related defence responses, respectively.

Histone acetylation also influences the regulation of NLR-encoding gene expression. HDA9, an Arabidopsis HDAC regulating H3 acetylation, including H3K9ac, seems to be a negative regulator of plant immunity against Pst DC3000, as infection of the hda9-1 mutant resulted in enhanced disease resistance to the pathogen (Yang et al., 2020). Upon pathogen infection, SNC1 is strongly up-regulated in hda9-1 mutant and more than in WT plants, suggesting that HDA9 has a role in the regulation of SNC1 expression in response to pathogen infection. Furthermore, levels of H3K9ac at the SNC1 locus were higher in the hda9-1 mutant than in the WT upon infection but not under normal conditions. This suggests that HDA9 regulates negatively SNC1 expression upon infection, likely by reducing H3K9 acetylation levels at this locus (Yang et al., 2020).

Altogether, these different studies demonstrate how histone mark regulators can modulate in various ways plant defence responses. Expression of some of them is increased during infection. They can also act by partially regulating hormonal pathways, NLR-encoding gene or defence gene expression. Some of them potentiate the activation of plant defence by enhancing the activation of defence genes, rendering immune responses fully effective. Others promote the expression of positive regulators of plant defences, or conversely, the repression of negative regulators. Whether histone mark regulators positively or negatively affect defences against a pathogen also depends on the nature of the pathosystem.

- **B.3. Chromatin remodelling complexes modulate chromatin density by playing with nucleosomes**

B.3.1. What are chromatin remodelling complexes?

We have seen that chromatin modifiers can add or remove chemical groups from DNA or histones, but there is another type of epigenetic regulator that can affect chromatin condensation: the chromatin remodelling complexes (CRCs). CRCs are protein complexes comprising enzymes that modify the DNA-histone interaction in a non-covalent manner, since there is no change in chemical groups. CRCs are also referred to as chromatin remodeling ATPases, as they use the energy of ATP to modify the contact between DNA and histone octamers, displace histone octamers along DNA, and exchange or remove nucleosomes (Clapier and Cairns, 2009). Conserved from yeast to humans and plants, there are four sub-families of chromatin remodelling ATPases : INO80/SWR1, CHD, ISWI and SWI/SNF (Han et al., 2015). They differ in the domains they harbour, and in their biochemical activities. They are involved in various chromatin-related mechanisms including DNA damage repair, homologous recombination, maintainance of other epigenetic marks, control of gene expression, or antagonise with specific chromatin silencing mechanisms (Han et al., 2015).

B.3.2. Chromatin remodelling complexes regulate plant responses to pathogens

Chromatin-Remodelling Factor 5 (CHR5) from A. thaliana is necessary for accurate plant immune responses against bacterial pathogens. chr5 mutant infected with different strains of P. syringae displayed higher bacterial growth than WT plants, notably for the strains P. syringae pv. maculicola, and the avirulent strains Pst DC3000 (avrRpt2) and Pst DC3000 (avrRps4), delivering the effectors AvrRpt2 and AvrRps4 that are recognised by the NLRs RPS2 and RPS4, respectively. This result suggests that CHR5 is a positive regulator of plant immunity in response to various P. syringae strains. Moreover, CHR5 was shown to be required for the up-regulation

of the NLR SNC1 and associated autoactivation of immune responses in the bon1 auto-immune mutant (Zou et al., 2017).

The Arabidopsis chromatin-remodelling component SWP73A functions as a negative regulator of NLR expression (Huang et al., 2021). SWP73A is an ortholog of BAF60 from mammals, which is known to be involved in inflammatory responses during infection. The swp73a mutant showed a higher expression of the NLR RPS2 and the defence-related PR1 gene (Huang et al., 2021). Infecting these mutants with Pst DC3000 (AvrRpt2) and Pst DC3000 (AvrRps4) avirulent strains led to a reduction in bacterial growth suggesting that SWP73A negatively regulates ETI. This was confirmed by the analyses carried on SWP73A over-expressing lines, which had lower expression of RPS2 and PR1 genes and increased bacterial growth compared to WT plants when infected with Pst DC3000 (AvrRpt2) or Pst DC3000 (AvrRps4). In details, SWP73A affects the transcription of several NLRs such as RPS2, RRS1, RPS4 and ZAR1 (Huang et al., 2021). By ChIP-seq, the authors showed that SWP73A can bind directly to the promoters of several NLRs including RPS2 and ZAR1 but not RPS4 and RRS1. This implies that SWP73A directly regulates the expression of some NLRs but for others, its regulatory action might be indirect. In fact, the regulation of RPS4 and RRS1 by SWP73A seems to occur via another component, Cell division cycle 5 (CDC5), a key regulator of alternative splicing (Palma et al., 2007). It appears that SWP73A directly down-regulates CDC5 probably leading to a splicing defect in RPS4, an important factor for RPS4 activity (Huang et al., 2021). Furthermore, binding of SWP73A to promoters is dependent on the repressive mark H3K9me2, which is reduced on RPS2 and CDC5 promoters upon infection with Pst DC3000 (AvrRps4) or Pst DC3000 (AvrRpt2). Thus, this study showed that under normal conditions, the chromatin-remodelling component SWP73A participates in the repression of some NLRs by binding direcly to their promoters thanks to the repressive mark H3K9me2. SWP73A can also negatively and indirectly regulate the expression of NLRs like RRS1 and RPS4 via down-regulation of CDC5, a key regulator of alternative splicing, important for NLR activity. However, under pathogen infection, the repression of CDC5 and RPS2 is

abolished/reduced correlating with a decrease in H3K9me2 levels which reduces the association of SWP73A with the CDC5 and RPS2 promoters. Interestingly, under infection with avirulent strains Pst DC3000 (AvrRpt2) and Pst DC3000 (AvrRps4), two small-RNAs were induced, mi3440 and siRNA-SWP73A which target SWP73A for down-regulation, presumably to prevent this negative regulator from inhibiting immune responses.

These small-RNAs are regulatory non-coding RNAs, a type of epigenetic regulator derived from RNAs that do not encode proteins but regulate gene expression at various levels in different biological processes, including in response to biotic stresses.

- **B.4. Non-coding RNAs**

B.4.1. Long and short non-coding RNAs are regulatory non-coding RNAs involved in many biological processes

Non-coding RNAs (ncRNAs) are parts of the genome that are transcribed into RNA but that do not encode proteins. They include housekeeping ncRNAs, like ribosomal RNAs and transfer RNAs, and regulatory RNAs. Regulatory ncRNAs consist of small RNAs (sRNAs) like microRNAs (miRNAs), small interfering RNAs (siRNAs) and long ncRNAs (lncRNAs) (Cech and Steitz, 2014; Morris and Mattick, 2014). Regulatory ncRNAs regulate gene expression at the transcriptional, post-transcriptional and epigenetic levels.

LncRNAs are transcripts of more than 200 nucleotides that modulate many cellular processes. In mammals, lncRNAs regulate gene silencing, protein activity, messenger RNA stability, mRNA processing, transcription; they serve as scaffold for higher-order complexes or as decoys or enhancers of protein-encoding gene promoters (Geisler and Coller, 2013). In plants, lncRNAs have similar function than those in mammals. They are involved notably in transcription regulation, gene silencing via the RdDM pathway, alternative splicing and chromatin structure (Wang and Chekanova, 2017).

miRNAs and siRNAs are small ncRNAs of 20-24 nucleotides. In most eukaryotic organisms, including plants, they are generated by DICER-LIKE proteins or the ribonuclease III-like enzyme DICER and induce gene silencing in a sequence-specific way (Baulcombe, 2004). miRNAs are derived from single-stranded RNA precursors encoded by MIR genes. They can repress gene expression by transcript cleavage, or translation repression (Yu et al., 2019). Most siRNAs are processed from long double-stranded RNAs from repeats and transposable elements in heterochromatin. They are involved in gene silencing by DNA methylation mediated by the RdDM pathway (Yu et al., 2019).

B.4.2. LncRNAs, miRNAs and siRNAs do also their part during plant infection by a pathogen

In different pathosystems, various lncRNAs have been shown to be induced upon infection or PAMP treatment such as in A. thaliana in response to the fungal pathogen Fusarium oxysporum or to Pst DC3000, or in wheat in response to the fungus Blumeria graminis f.sp. tritici (Seo et al., 2017; Xin et al., 2011; Zhu et al., 2014). Functional analyses using knock-down or knock-out lines revealed that some of these lncRNAs have a role in the plant defence responses to pathogens. For instance, during infection of Arabidopsis by F. oxysporum, knock-out or knock-down mutants of the lncRNAs TAR-191, TAR-197, TAR-212 and TAR-224 showed earlier and stronger symptom development compared to WT plants (Zhu et al., 2014). In the Arabidopsis-Pst DC3000 pathosystem, knock-down of the lncRNA ELENA1 also showed increased susceptibility (Seo et al., 2017). These studies suggested that lncRNAs are positively involved in plant defence against pathogens. However, much remains to be discovered about the mechanisms underlying their roles in the regulation of plant defence. The most documented example is ELENA1. Indeed, ELENA1 was shown to participate in the up-regulation of PR1 expression in challenged conditions, likely through an interaction with Mediator Subunit 19a, a component of the transcriptional coactivator complex Mediator that connects TFs to RNA polymerase II (Seo et al., 2017).

There is increasing evidence of a regulatory role of miRNAs in plant immunity in response to pathogens (Song et al., 2021a). Upon pathogen infection, miRNAs expression can be altered, resulting in differential regulation of immune responses. Since miRNAs function mainly by repressing gene expression, they positively or negatively regulate immune responses by silencing positive or negative regulators of immune responses like hormone receptors, TFs, proteins involved in ROS burst activation or NLRs (Song et al., 2021a). As an example, the Arabidopsis miR472 is a negative regulator of PTI and ETI against Pst DC3000 (Boccara et al., 2014). Regarding PTI regulation, flg22-induced ROS production and callose deposition was increased in the miR472 mutant suggesting that miR472 has a negative role in PTI regulation. Moreover, in the miR472 over-expressing lines, fewer transcripts of several NLRs, including RPS5, were detected. In addition, RPS5-mediated immunity was dampened in these over-expressing lines, suggesting that miR472 negatively regulates RPS5-mediated resistance (Boccara et al., 2014). miRNAs work also cooperatively with siRNAs, including endogenous siRNAs or phase secondary interfering RNA, a specific type of siRNAs generated by cleavage of a miRNA target (Song et al., 2021a). siRNAs influence immune responses to pathogens by silencing components involved in hormonal pathways, TF signalling, NLRs, or ETI (Huang et al., 2019; Song et al., 2021a).

In pathogens, epigenetic mechanisms also participate in the regulation of gene expression. In the above section, I deliberately focused on the role of epigenetic mechanisms in plant immunity, but the regulation of gene expression by epigenetic mechanisms in pathogens could deserve its own detailed section. Very briefly, epigenetic mechanisms are involved in pathogen development and pathogenesis (Dubey and Jeon, 2017; Sánchez-Romero and Casadesús, 2020). While the main epigenetic mechanism in bacteria is DNA methylation, genes from fungal pathogens can be regulated by DNA methylation, histone modifications, sRNAs and Transposable Elements (Sánchez-Romero and Casadesús, 2020; Sánchez-Vallet et al., 2018). Notably, it has been shown that DNA methylation might play a role in R.

Table 2. Pathogens can manipulate the host epigenome to favour infection.
Several animal pathogens are known to manipulate the host epigenome to their benefit, mainly bacteria while there are fewer examples in plant parasites, mainly from viruses, oomycetes and nematodes. Parasites and pathogens can use effectors or other proteins to manipulate epigenetic mechanisms by modifying histones or DNA, interfering with histone writers, erasers or readers, and chromatin-remodelling complexes.

Name	Host type	Pathogen	Epigenetic mechanism targeted	Targeting mechanism	Reference
OspF	Animal	*Shigella flexneri*	Epigenetic readers	Blocks MAPK signalling cascade, preventing phosphorylation of the epigenetic reader HP1γ leading to the alteration of the transcription of pro-inflammatory genes	Harouz et al., 2014
OspF	Animal	*Shigella flexneri*	Histone phosphorylation	Blocks MAPK signalling cascade, preventing H3 phosphorylation at promoters of chemokines and cytokines, altering their transcription	Arbibe et al., 2007
NUE	Animal	*Chlamydia trachomatis*	Histone methylation	Methylates histones H2B, H3 and H4 *in vitro*	Pennini et al., 2010
BaSET	Animal	*Bacillus anthracis*	Histone methylation	Methylates histone H1 in infected cells	Mujtaba et al., 2014
RomA	Animal	*Legionella pneumophilia*	Histone methylation	Methylates H3K14 in mammalian cells leading to a genome-wide decrease in acetylation levels notably at immune-related genes	Rolando et al., 2013
LpqH	Animal	*Mycobacterium tuberculosis*	Chromatin remodelling	Prevents the recruitment of a SWI/SNF remodelling complex component at the promoter of an immune-related gene	Pennini et al., 2006
NleC	Animal	*Escherichia coli*	Histone acetylation	Likely degrades the HAT p300 resulting in the alteration of Interleukin-8 secretion	Shames et al., 2011
Rv2966c	Animal	*Mycobacterium tuberculosis*	DNA methylation	Methylates DNA on cytosines outside CpG contexts	Sharma et al., 2015
Rv3423	Animal	*Mycobacterium tuberculosis*	Histone acetylation	Acetylates H3K9 and K3K14 *in vitro*	Jose et al., 2016
TrAP	Plant	Geminiviruses	Histone methylation	Inhibit the histone methyltransferase SUVH4/KYP activity leading to a decrease in H3K9 methylation levels in the host and viral genome methylation	Castillo-Gonzalez et al., 2015
PsAvh23	Plant	*Phytophthora sojae*	Histone acetylation	Competitively binds to a subunit of the SAGA HAT complex, causing a decrease in acelytation levels at the promoters of various genes including immunity-related genes correlated with their down-regulation upon infection	Kong et al., 2017
32E03	Plant	*Heterodera schachtii*	Histone acetylation	Inhibits HDAC activities, which correlates with decreased acetylation levels on ribosomal chromatin and favours parasitism by the nematode	Vijayapalani et al., 2018
PsAvh52	Plant	*Phytophthora sojae*	Histone acetylation	Relocates an acetyltransferase into the nucleus triggering histone acetylation at susceptibility genes favouring their transcription and infection	Li et al., 2018

solanacearum pathogenesis (Erill et al., 2017). In addtion, epigenetic mechanisms are important for fugal pathogenesis (Zhu et al., 2016b). For instance, genes encoding recognised effectors can be silenced by sRNAs, leading to the promotion of virulence (Sánchez-Vallet et al., 2018). Thus, plant-pathogen interactions are characterised by an important regulation of gene expression, involving notably epigenetic mechanisms from both sides of the interaction.

C. Parasites and pathogens can manipulate the host epigenome to their benefit

Considering that more and more evidence reveals a key role of the plant epigenome in the regulation of immune responses against pathogens and that it acts upstream of the regulation of gene expression, it seems obvious that pathogens have evolved virulence strategies that consist in bypassing or counteracting host epigenetic mechanisms to promote infection. While for plant pathogens few examples are documented, more studies describe the manipulation of the host epigenome by animal pathogens. The following paragraphs provide an overview of the diverse and sophisticated virulence strategies used by animal pathogens to subvert the host epigenome and present the current state of knowledge about the manipulation of host epigenome by plant pathogens. I will focus in this section on the action of nucleomodulins, which are bacterial proteins targeted to the nucleus (Table 2, Figure 8).

- **C.1. Numerous animal pathogens have been shown to subvert host epigenome**

C.1.1. Pathogen targeting of host DNA, CRCs or epigenetic readers

C.1.1.a. Manipulation of DNA methylation by Mycobacterium tuberculosis

The effector Rv2966c from M. tuberculosis, the causal agent of tuberculosis, is a functional DNA methyltransferase (Sharma et al., 2015). Once secreted into the

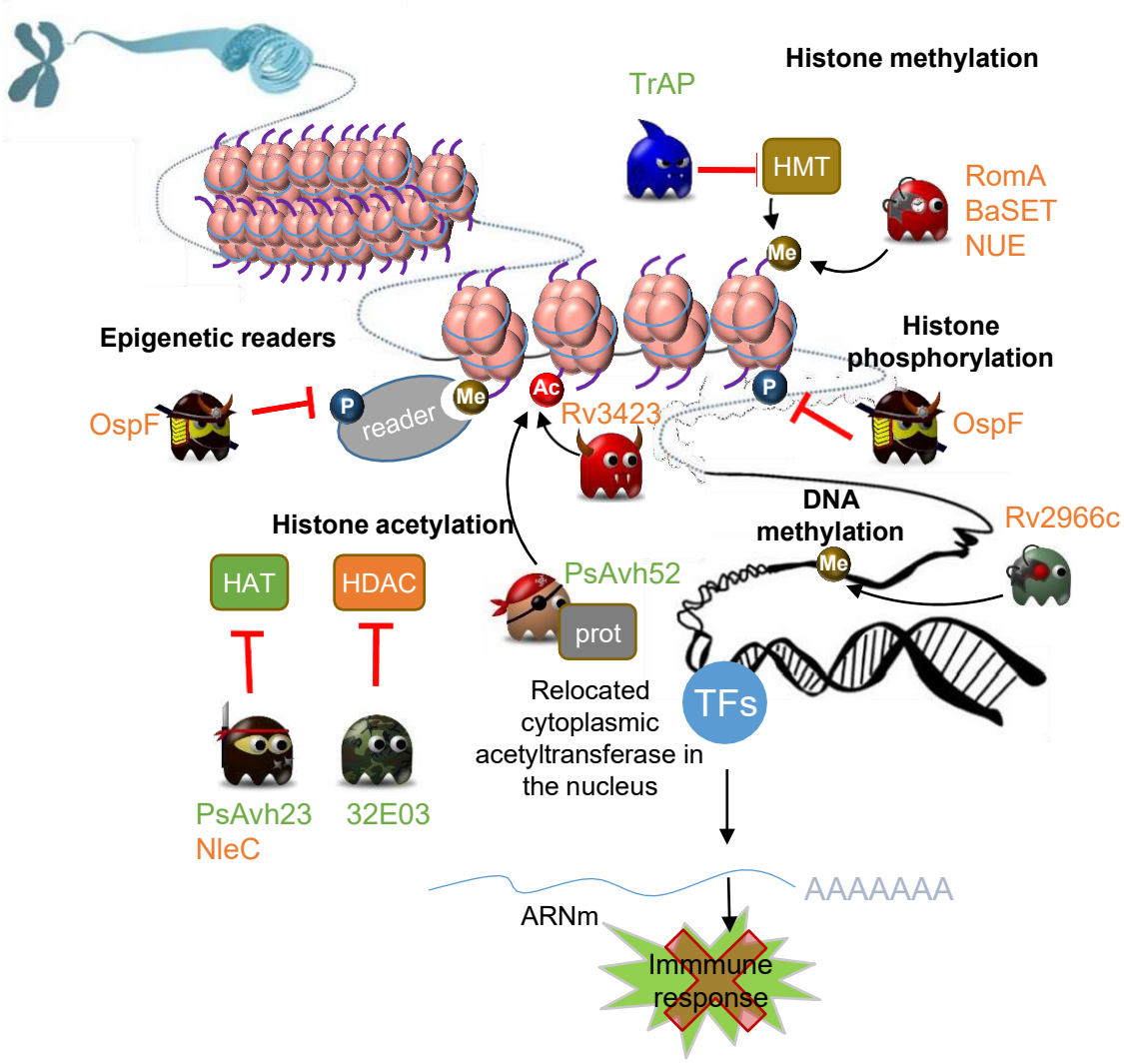


Figure 8. Pathogens manipulate the host epigenome by targeting/interfering with diverse mechanisms.
Various effectors from plant and animal pathogens and parasites were shown to interefere with the host epigenome, supposedly to favour infection. Some can directly or indirectly modify DNA methylation and histone marks such as acetylation, methylation or phosphorylation. Others target histone marks by manipulating cytoplasmic acetyltransferase of histone mark regulators such as histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), or epigenetic readers.
Orange: effectors from animal pathogens; green: effectors from plant parasites or pathogens.

host cell, Rv2966c localises into the nucleus where it binds to specific DNA sequences and methylates cytosines outside CpG contexts, in which cytosines are followed by guanines. It can also bind to histone H3 and H4 harbouring specific PTMs. Since it binds to specific DNA sequences and the transcription of these sites is altered upon mycobacterial infection, Rv2966c was hypothesised to cause the down-regulation of these loci by methylating their DNA sequence (Sharma et al., 2015). This effector introduces a way of manipulating the host epigenome, presumably to the benefit of the pathogen, although much remains to be elucidated about its mode of action and the consequences of DNA methylation on host transcription and immune responses.

C.1.1.b. Targeting chromatin remodelling to prevent transcription

The LpqH lipoprotein from M. tuberculosis alters host immunity by inhibiting the expression of several immunity-related genes. The expression of one of these immunity-related genes, CIITA, is affected by this lipoprotein, possibly via an alteration of its epigenetic regulation (Pennini et al., 2006). Indeed, in animal cells treated with LpqH, histone acetylation at CIITA promoter region was altered, together with the recruitment to this promoter of Brahma-related gene 1, a component of the SWI/SNF chromatin remodelling complex. This indicated that expression of the immune-related CIITA gene was likely inhibited by manipulating the accessibility of chromatin to the transcription machinery through changes in histone acetylation levels and chromatin remodelling. However, the underlying molecular mechanisms remain elusive (Pennini et al., 2006).

C.1.1.c. Interfering with epigenetic readers to hijack host transcription

Epigenetic readers contribute to the regulation of gene transcription by protecting the sites they recognise from adding or removing histone marks, or by helping to recruit epigenetic regulators, thus facilitating the switch of epigenetic marks. They indirectly participate in gene transcription regulation, making them a virulence target of choice for pathogens.

The animal pathogenic bacterium Shigella flexneri manipulates a host epigenetic reader by using the effector OspF (Harouz et al., 2014). OspF is a phosphothreonine lyase that catalyses eliminylation on its targets, a reaction that transforms a phosphothreonine residue into a dehydrobutyrine, permanently blocking phosphorylation of that residue. OspF was shown to target MAPKs by causing their permanent dephosphorylation, and thus their permanent inactivation (Li et al., 2007b). This permanent inactivation has various consequences on the proteins that are normally regulated directly or indirectly by MAPK-mediated phosphorylation. This includes Heterochromatin protein 1 γ (HP1γ), an epigenetic reader that recognises methylated H3K9. HP1γ is normally phosphorylated downstream of the MAPK ERK by the kinase MSK1, but whose phosphorylation is impaired because OspF blocks the ERK phosphorylation cascade. As a result, non-phosphorylated HP1γ accumulates at the promoters of target genes, possibly retained by interaction with OspF, impeding active transcription of pro-inflammatory genes in response to the bacterial pathogen (Harouz et al., 2014).

C.1.2. Pathogen interference with histone marks

C.1.2.a. OspF indirectly alters H3 phosphorylation

In addition to altering the phosphorylation status of an epigenetic reader, OspF also indirectly causes an alteration of H3 phosphorylation (Arbibe et al., 2007). By blocking MAPKs in an inactive conformation via eliminylation, OspF alters the subsequent phoshorylation of H3 at the promoters of immune-related genes and the recruitment of the transcription machinery to these promoters, preventing the transcription of pro-inflammatory chemokines and cytokines, important components of animal immune response to infection (Arbibe et al., 2007).

C.1.2.b. Several effectors can directly methylate host histones

As histone methylation is involved in gene activation and repression, targeting this mark would represent a potent strategy to alter pathogen-induced transcriptional reprogramming. Surprisingly, several effectors from different pathogens are

methyltransferases capable of directly modifying histones in vitro and/or in vivo (Rolando et al., 2015). These include BaSET from Bacillus anthracis, NUE from Chlamydia trachomatis, and RomA from Legionella pneumophilia. Remarkably, these effectors all contain a SET domain, a domain found in eukaryotic histone methyltransferases responsible for the methylation of histone H3 and H4 tails on lysine residues. This suggests that pathogens have evolved effectors mimicking host histone methyltransferases, possibly to disrupt the regulation of gene transcription (Rolando et al., 2015). However, for many of these effectors, the link between histone methyltransferase activity and their function in infection remains elusive. RomA is among the effectors for which we know a little more. The RomA type 4 effector from L. pneumophilia methylates mammalian H3K14 in vitro and in vivo, resulting in a genome-wide decreased acetylation at this residue. This reveals a potential switch between an active and a repressive histone mark (Rolando et al., 2013). The authors also identified a large set of H3K14 methylated promoter regions upon L. pneumophilia infection, including immunity-related genes (Rolando et al., 2013). Strikingly, H3K14 methylation has never been observed before in mammalian cells and therefore represents a particularly ingenious strategy used by L. pneumophilia to manipulate the host epigenome.

C.1.2.c. Manipulation of histone acetylation by animal bacterial pathogens

Similar to effectors capable of methylating histones, the M. tuberculosis effector Rv3423 acetylates H3K9 and H3K14 in vitro (Jose et al., 2016). Considering that acetylation is involved in the regulation of gene transcription, Rv3423 might disturb host gene transcription to promote the intracellular survival of M. tuberculosis. However, the role of Rv3423 during infection and its impact on gene expression by triggering histone acetylation remain uncharacterised (Jose et al., 2016).
Instead of directly modifying histone acetylation levels, effectors can also target regulators of histone acetylation such as HATs. For instance, the effector NleC from enteropathogenic and enterohaemorrhagic Escherichia coli is a protease that targets the HAT p300 and likely promotes its degradation in infected cells.

Consequently, p300 degradation correlates with altered secretion of Interleukin-8, a major cytokine involved in immune responses against enteropathogenic and enterohaemorrhagic Escherichia coli (Shames et al., 2011).

- **C.2. Plant pathogens can also alter the host epigenome**

C.2.1. Some phytopathogen effectors interfere with histone methylation

While some animal pathogens have evolved effectors that can directly modify histone methylation status through their methyltransferase activity, there is no evidence yet that effectors from plant pathogens act in the same way. However, this does not mean that plant pathogens cannot disturb histone methylation in plants. They can target regulators of histone methylation, such as histone methyltransferases.

TrAP proteins, encoded by the geminiviruses Tomato Golden Virus and Cabbage Leaf Curl Virus, inhibit the activity of the Arabidopsis histone methyltransferase SUVH4/KYP (Castillo-González et al., 2015). By luciferase complementation imaging, co-immunoprecipitation and acceptor bleaching FRET, the authors demonstrated that TrAP proteins interact with this histone methyltransferase that catalyses the dimethylation of H3K9, among other histone methyltransferases. Furthermore, SUVH4/KYP H3K9 methyltransferase activity was inhibited by TrAP proteins in vitro. Overexpression of TrAP led to a decrease of H3K9me2 marks at specific loci in Arabidopsis. The authors also demonstrated that SUVH4/KYP is involved in viral genome methylation. Considering that host-mediated methylation of viral chromatin can limit viral replication, altering SUVH4/KYP activity by TrAP proteins could represent a virulence strategy to prevent inhibition of viral replication by SUVH4/KYP-mediated methylation (Castillo-González et al., 2015; Pumplin and Voinnet, 2013).

C.2.2. Some phytopathogen effectors interfere with histone acetylation

C.2.2.a. by inhibiting HAT activity through competitive binding to a subunit of a HAT complex

Some effectors from the oomycete Phytophthora sojae can interfere with histone acetylation via various strategies. One of them, PsAvh23, is targeted to the nucleus where it can interfere with the subunit Alteration/Deficiency in Activation 2 (ADA2) from the HAT Spt-ADA-Gcn5-Acetyltransferase (SAGA) complex (Kong et al., 2017). Interestingly, the competitive binding of ADA2 to PsAvh23 alters the formation of the ADA2-General Control Non-depressive 5 (GCN5) subcomplex that mediates acetylation of nucleosomal histones. Also, PsAvh23 decreases H3K9ac levels upon its interaction with ADA2 in soybean. In addition, in PsAvh23-expressing roots, various genes involved in plant defence against P. sojae are down-regulated; and this correlated with decreased levels of H3K9ac marks at their promoters (Kong et al., 2017).

C.2.2.b. Inhibiting HDAC activities

Instead of targeting HAT, the effector 32E03 from the sugar beet cyst nematode Heterodera schachtii targets HDAC activities (Vijayapalani et al., 2018). HDT1, identified among the interacting partners of 32E03, is a HDAC that deacetylates H3K9 and is involved in the regulation of ribosomal RNA gene expression via chromatin modifications. Assays to determine HDACs activity in seedlings expressing 32E03 revealed a decrease in HDACs activity, as did similar assays performed on nuclear extracts from WT plants treated with recombinant 32E03, suggesting that this effector manipulates host histone deacetylation. At low levels, 32E03 was shown to participate in the derepression of some ribosomal RNA genes, which correlates with histone modifications on ribosomal chromatin including an increase in acetylated H3K9 levels, and appears to favour H. schachtii parasitism. This suggests that this effector can manipulate histone acetylation levels at ribosomal RNA genes to promote their derepression and successful parasitism of the nematode. However, at high levels, 32E03 triggers RdDM-mediated ribosomal DNA methylation, which correlates with repression of the tested ribosomal RNA

genes, indicating that successful nematode infection might rely on the appropriate dosage of the effector 32E03, as different dosage may trigger antagonistic effects on the expression of ribosomal RNA gene and the outcome of the nematode-plant interaction (Vijayapalani et al., 2018).

C.2.2.c. Favouring histone acetylation by relocating a cytoplasmic acetyltransferase into the nucleus

Another P. sojae effector, PsAvh52, affects host histone acetylation by manipulating a host acetyltransferase (Li et al., 2018). By co-immunoprecipitations and pull-down assays PsAvh52 was shown to interact with GmTAP1, a soybean acetyltransferase that is normally localised in the cytoplasm. Nuclear relocalisation of GmTAP1 triggered by PsAvh52 correlates with enhanced susceptible response to Phytophthora infection. Also, GmTAP1, although not being a HAT, can acetylate lysine residues on histone tails such as H3K9 and H2AK5, two marks associated with active transcription (Berger, 2007; Li et al., 2018). In vivo, transient expression in N. benthamiana of a version of GmTAP1 forced into plant nuclei revealed increased acetylation levels on H3, and H2K5 notably. Moreover, PsAvh52 induces increased acetylation levels of H3K9 and H2AK5 in soybean roots and this is dependent on the ability of PsAvh52 to interact with GmTAP1. Finally, in soybean roots expressing PsAvh52, the transcription of several susceptibility genes was increased, like SWEET, lypoxygenase and a polygalacturonase encoding genes. These genes showed an increase in H3K9 and H2AK5 acetylation levels 6 hours after P. sojae infection in soybean roots expressing PsAvh52. Together, these data highlight how P. sojae affects histone acetylation to control host gene expression and promote infection (Li et al., 2018).

- **C.3. From animal to plant parasites, effectors can affect the host epigenome in similar ways and yet differently**

This section has provided an overview of animal and plant parasites that can alter the host epigenome, notably via effector activities (Figure 8).

It is quite impressive to note the diversity of strategies used by parasites, including pathogens, to alter the host epigenome. They include the targeting of DNA methylation, histone marks (phosphorylation, methylation and acetylation), but also epigenetic readers. Interestingly, evidence in the literature describe the manipulation of histone methylation and acetylation by animal and plant parasites. This suggests that there has probably been evolutionary selection of virulence mechanisms in both animal and plant pathogens targeting epigenetic regulatory mechanisms that are common to both kingdoms.

Althought they sometimes target similar epigenetic mechanisms, the means in which pathogens manipulate these mechanisms can be quite diverse, particularly with regard to the manipulation of histone marks, since some effectors can directly or indirectly modify histones, or manipulate epigenetic regulators to alter histone mark levels, resulting in differential gene transcription, which is thought to benefit the pathogen.

Targeting the host epigenome appears to be a powerful and profitable strategy, as some pathogens have evolved several effectors and/or proteins that can target the host epigenome, such as the effectors Rv3423 and Rv2966c and the lipoprotein LpqH from M. tuberculosis and the effectors PsAvh23 and PsAvh52 from P. sojae. There also appears to be a co-evolution of some pathogens towards the development of effectors with similar functions, such as effectors with a SET domain, domain usually found in eukaryotic histone methyltransferases and found functional in the effectors NUE, BaSET and RomA from C. trachomatis, B. anthracis and L. pneumophilia, respectively.

However, it should be noted that it is not always easy to assess the actual role of an effector on an epigenetic mechanism for two main reasons. On one hand, the link between levels of epigenetic marks, transcription and functional

characterisation of targets is often indirect and mainly relies on correlations, more than on causalities. On the other hand, it is quite challenging to determine, from a given experiment, whether the observed effect on an epigenetic mechanism is directly caused by the function/activity of the effector or whether it is a side effect of the considered epigenetic mechanism or its interconnection with other epigenetic mechanisms. For instance, in the Geminiviruses-Arabidopsis interaction, given that KYP proteins are required for the maintenance of DNA methylation, it is difficult to link their manipulation by viral TrAP proteins and its putative consequences on histone and DNA methylation. This is also why these studies propose working hypotheses based on correlations, due to limititations that are inherent to their experimental model. Another limitation is that despite the importance of the epigenome in plant-pathogen interactions, its influence is vast and extremely complex, and far from being fully understood.

Finally, while most of the effectors or proteins described as being capable of subverting the host epigenome (and especially histone PTMs) are produced by bacterial animal pathogens, such examples of pathogen interference on plant epigenomes by bacterial plant pathogens and parasites are missing, with all documented studies involving only viruses, oomycetes and nematodes. During my PhD, I investigated the targeting of A. thaliana epigenetic readers by PopP2, a type III effector from the plant bacterial pathogen Ralstonia solanacearum species complex (RSSC). The following section presents the R. solanacearum species complex and focuses on the available knowledge concerning PopP2 and its biological functions.

3- The Ralstonia solanacearum species complex (RSSC)

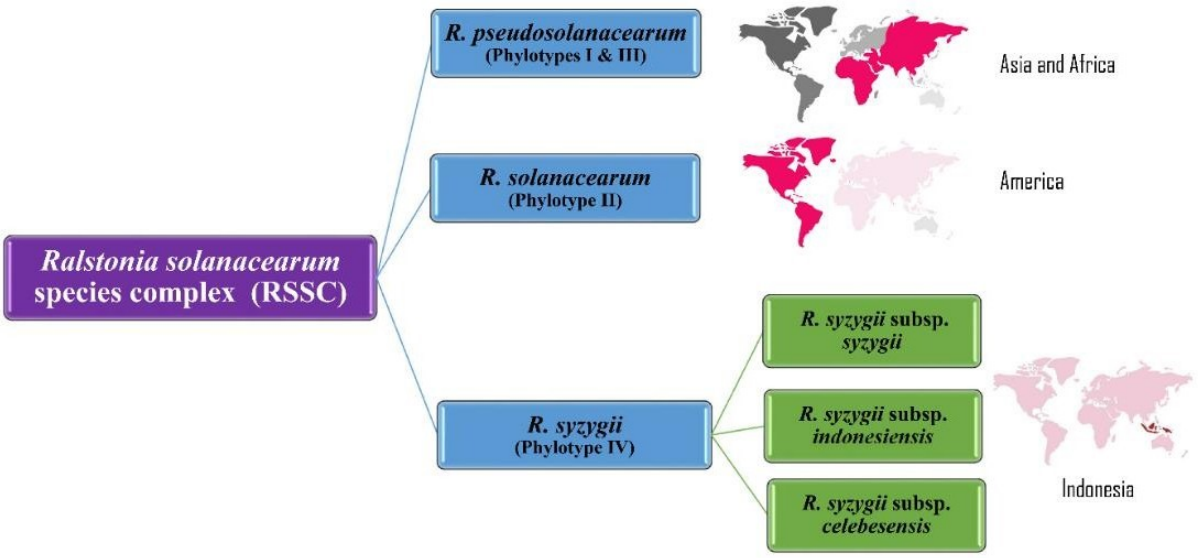


Figure 9. The RSSC consists of three species (adapted from Paudel et al., 2020).
After the 2014 taxonomic revision, the RSSC was defined as comprising three species: *Ralstonia pseudosolanacearum* which includes phylotypes I and III (strains originating in Asia and Africa respectively), *Ralstonia solanacearum* which corresponds to phylotype II (strains originating in America), and *Ralstonia syzygii* which corresponds to phylotype IV (strains originating in Indonesia and Japan), and subdivided into three subspecies *R. syzygii* subsp. *syzigii*, *R. syzygii* subsp. *indonesiensis* and *R. syzygii* subsp. *celebesensis*.

A. Pathogen description, classifications and taxonomy

- **A.1. Pathogen description**

Ralstonia solanacearum is a species complex of gram-negative β-proteobacteria of the Burkholderiales order and the Ralstoniaceae family. RSSC bacteria cause bacterial wilt disease, which can be called by different names depending on the host such as potato brown rot or the Moko disease affecting banana species. The bacterial cells of R. solanacearum are 0.5-1.5µm long, rod-shaped and harbour one polar flagellum. On growth medium, spread R. solanacearum bacteria have a fluid aspect, forming flat, irregular, pearly cream-white colonies (Osdaghi, 2020). Most strains generally grow in a moist environment with an optimum temperature of 35-37° C, but others can grow at a lower temperature.

- **A.2. Classifying the different strains of the species complex**

Due to the intraspecific diversity within the RSSC, several attempts have been made to classify the different RSSC strains, either by host range, biochemical types or geographical distribution. The concept of races was introduced in 1962 and allowed the classification of the different strains according to their pathogenicity to different hosts and the phenotypic characteristics of the strains (Buddenhagen and Kelman, 1964). A complementary classification system was introduced, defining biovars, sorting the RSSC strains by their ability to oxidise different carbohydrate sources (Hayward, 1964). However, the classification race/biovar was not fully satisfying as it did not provide sufficient discrimating features to effectively represent the genetic diversity within the species complex, even though the concept of a species complex was not official yet. Later, Prior and Fegan introduced a classification based on genetic differentiation, distinguishing strains into phylotypes (Prior and Fegan, 2005). Interestingly, these phylotypes correlated with the geographical origins of the strains: phylotype I corresponds to strains originating from Asia, phylotype II from America, phylotype III from Africa and surrounding islands and phylotype IV from Indonesia and Japan (Figure 9). For

many years and until today, this phylotype classification system is mostly used, although R. solanacearum has undergone various taxonomic changes, even in recent years before being considered as a species complex.

- **A.3. A complicated taxonomy**

The taxonomy of R. solanacearum is quite complex as this pathogen changed genus several times before entering the genus Ralstonia in 1995. While it was first described in 1986 as Bacillus solanacearum, it was transferred in different genera, from Bacterium to Pseudomonas and Xanthomonas genera and passing through Burkholderia before settling on Ralstonia in 1995. Today, R. solanacearum is now referred to as a species complex due to the high genetic diversity among R. solanacearum strains (Paudel et al., 2020).
More recently, in 2014, a taxonomic revision was carried out leading to the division of R. solanacearum phylotypes into different species (Prior et al., 2016; Safni et al., 2014). Indeed, phylogenetic analyses based on DNA-DNA hybridisation, DNA base composition, intergenic spacer region sequences, partial endoglucanase gene sequences, and other analyses based on denitrification assays and proteomics allowed to distinguish three different species (Prior et al., 2016; Safni et al., 2014). Phylotype I and III represent the species Ralstonia pseudosolanacearum, phylotype II remains R. solanacearum and phylotype IV is a distinct species, Ralstonia syzygii, subdivided in three subspecies names R. syzygii subsp. syzygii, R. syzygii subsp. indonesiensis and R. syzygii subsp. celebesensis (Figure 9).

B. Pathogenicity of the RSSC and its virulence determinants

- **B.1. The RSSC is an important threat for crop production due to its wide host range and persistence in the field**

RSSC strains are the causal agent of bacterial wilt disease, which can infect more than 250 plant species from 54 plant families, including Solanaceae, Cucurbitaceae, Musaceae, Zingiberaceae and Asteraceae (Hayward, 1991). Of

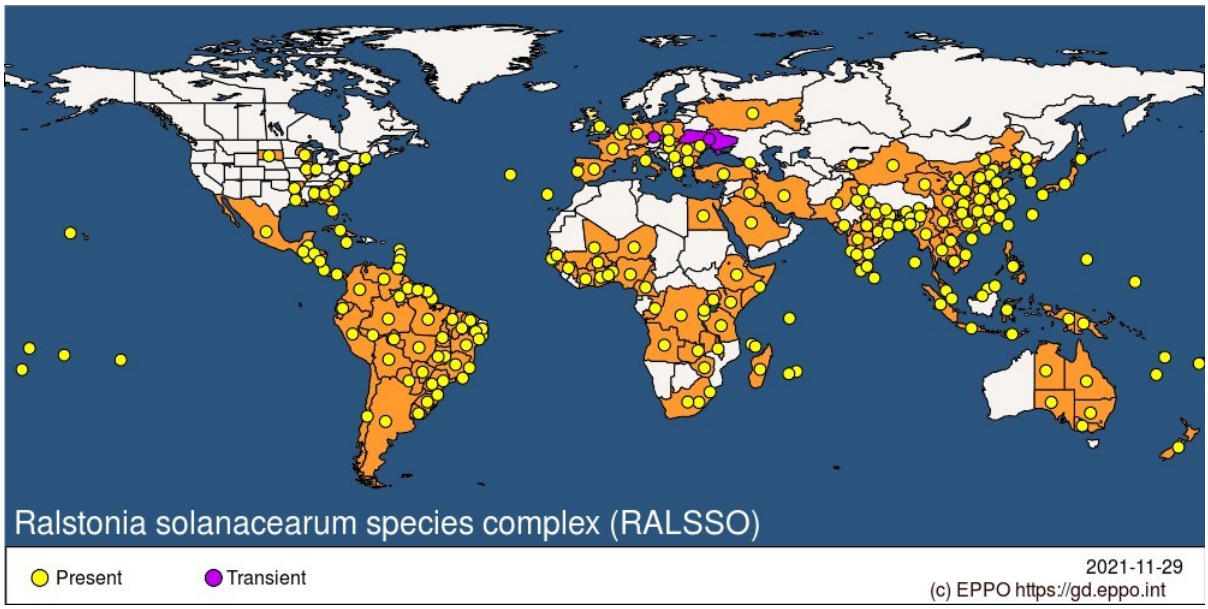


Figure 10. Worldwide distribution of the RSSC (extracted from EPPO, 2021).
The RSSC is a devastating plant bacterial pathogen with a worldwide distribution. Many strains thrive in equatorial regions but other strains are adapted to more temperate climates. While in many areas the RSSC is endemic, its presence in Europe is less pronounced and for this reason it is considered a quarantine pathogen in Europe.

these 250 plant species, many crops can be affected by RSSC bacteria including tomato, tobacco, eggplant, potato, banana, and ginger. It has also been reported that RSSC bacteria can infect or be hosted by weeds and ornamentals such as the weeds Urtica dioica and Solanum dulcamara and the ornamental species Rosa spp., Anthurium spp. and Pelargonium spp. (Bergsma-Vlami et al., 2018; Champoiseau et al., 2009; Poussier et al., 1999; Wenneker et al., 1999; Wicker et al., 2007).

With its genetic diversity and wide host range, the RSSC is considered the second most important pathogenic bacteria in terms of economic impact (Mansfield et al., 2012). While assessing the overall yield loss caused by RSSC bacteria is extremely complicated given the numerous factors that play a role in the calculation, local yield loss and incidence reports have been published at the country level with a focus on one host species (Bragard et al., 2019; Mamphogoro et al., 2020). For instance, yield loss in potato production has been reported in different countries: with 15% in Bangladesh, 95% in Nepal and 50% in Kenya (Karim et al., 2018). However, these yield loss estimates might vary depending on the host plant, infecting strains, soil management, year and weather conditions.

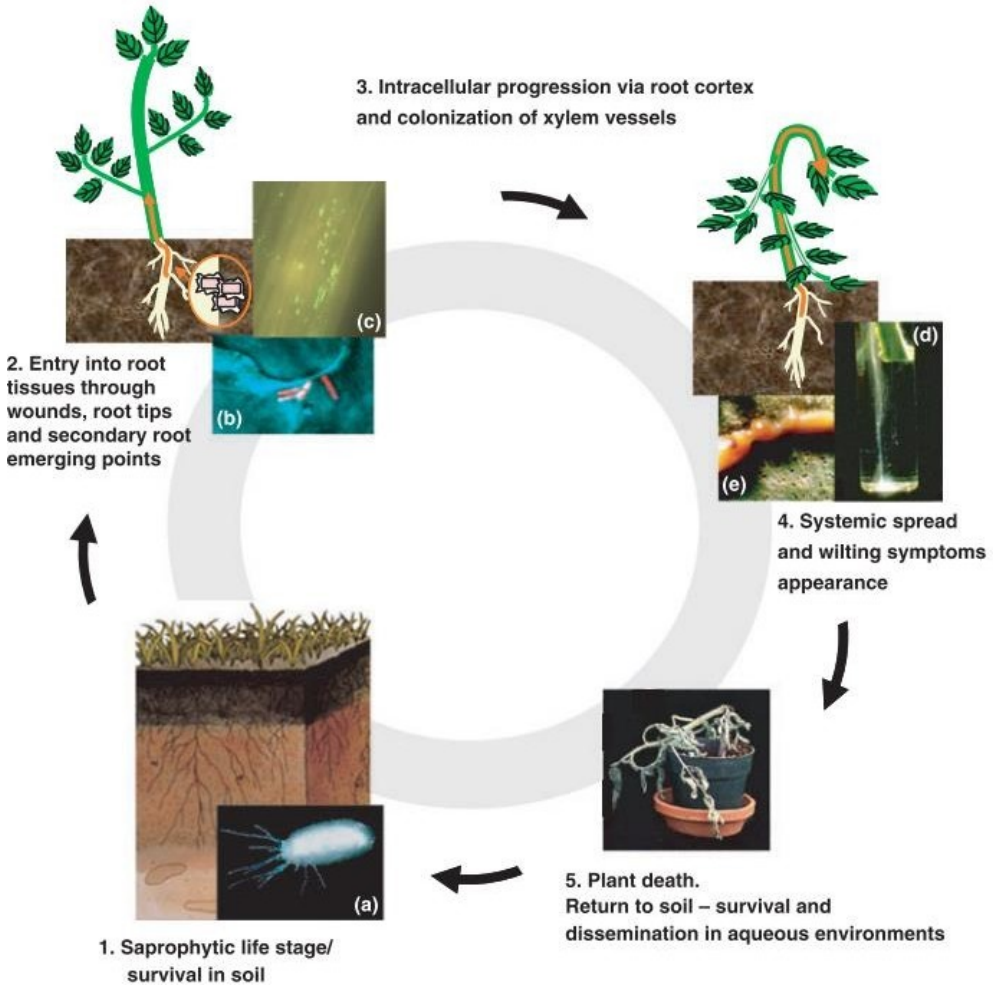
Nonetheless, the RSSC is a threat to crop production because it can affect many crops from various families, and because of the extreme persistence of the pathogen in the soil, as it can survive several years in the soil or infect weeds, creating a reservoir of pathogen that can be transmitted to subsequently sown crops (Elphinstone, 1996). Moreover, its genetic diversity favoured its presence worldwide, with a major presence in tropical areas although the presence of some strains of R. solanacearum have been reported in Europe (Figure 10). With a relatively low occurrence in Europe, R. solanacearum is considered as a quarantine pathogen by the European and mediterranean Plant Protection Organisation, in order to prevent the endemic establishment of this pathogen on the continent which could cause significant yield and economic losses (Bragard et al., 2019).

- **B.2. The control methods against the RSSC are diverse but not entirely satisfactory**

Due to the major economic and yield impact of the RSSC, researchers and companies are continually trying to find a way to control and breed cultivars resistant to the bacterial wilt disease. Many control strategies have been experimented in the laboratory, or to a lesser extent in the field, with varying degrees of success and associated limitations, making the management of bacterial wilt a complex challenge (Huet, 2014; Mamphogoro et al., 2020; Yuliar et al., 2015). Chemical control, although being one of the most effective control methods against bacterial wilt, is not considered as a sustainable control method as ignorance and incorrect application of pesticides can be harmful to the environment, farmers and consumers. Other known control methods to reduce the presence of the RSSC in soils are physical measures including soil solarisation, biofumigation, crop rotation or prophylactic measures that rely on the use of healthy seeds, water and soil free of the RSSC (Huet, 2014; Mamphogoro et al., 2020).

Alternative control methods are based on the use of biological agents, natural antimicrobial compounds, or the use/selection of resistant cultivars to minimise the incidence of bacterial wilt. The use of biological agents such as non-pathogenic strains of the RSSC, antagonistic bacteria, or bacteriophages has been and is still being studied in an attempt to reduce the virulence of RSSC strains (Álvarez and Biosca, 2017; Hanemian et al., 2013; Mamphogoro et al., 2020). For instance, pre-inoculation of A. thaliana with a T3SS-deficient RSSC strain results in increased resistance that is likely due to competition between the strains (Feng et al., 2012a). Other studies have focused on the use of bacteriophages that can infect various RSSC species. For example, it has been shown that the Jumbo phage RsoM2USA can infect strains of three different RSSC species and decrease the virulence of one RSSC strain in tomato plants when co-inoculated with that strain (Ahmad et al., 2021). However, although these control methods represent powerful strategies to limit the development of bacterial wilt, most of these studies were carried out

A



B

Figure 11. Life cycle and infection process of the RSSC (adapted from Genin 2010).
(A) The RSSC is a soil-borne vascular pathogen that can infect host plants through wounds in the roots or secondary roots emerging points, and then reach the xylem vessels, where it develops. Clogging of the xylem vessels induces wilting symptoms that will eventually cause the death of the host plants. After plant death, the RSSC bacteria return to the soil where it can survive for a long period (2-3 years) or colonise weeds, thus maintaining a bacterial reservoir in the soil.
(B) Wilting symptoms on Arabidopsis plant. A healthy Arabidopsis plant on the left, compared to a totally wilted Arabidopsis plant on the right.

under laboratory conditions and would require further investigation like conducting similar experiments on crops under field conditions and assessing the long-term effects of these treatments on the environment. Another recent study assessed the antimicrobial properties of three hydroxycoumarins against RSSC bacteria, which are natural secondary metabolites that accumulate in plants when challenged with pathogens (Yang et al., 2021). The authors showed that these hydroxycoumarins induce transcriptomic changes in a strain of R. pseudosolanacearum, resulting in cell membrane destabilisation and bacterial cell death (Yang et al., 2021). While the antimicrobial effects of hydroxycoumarins could represent a sustainable natural resource for the control of bacterial wilt, many factors remain to be investigated before these metabolites can be used in the field. Currently, the most efficient disease control method against RSSC remains the use of resistant or tolerant cultivars even though this method is geographically and climatically restricted (Huet, 2014). Indeed, the selection of resistant cultivars to RSSC strains is limited because it has to combine sustainable resistance with specific agronomic traits such as yield and most of the time, resistance is negatively correlated with yield. Thus, the control of bacterial wilt remains a great challenge and for the time being, the best measures remain the local use of resistant cultivars and prophylactic measures to prevent the establishment of the RSSC in the soil, but methods using biocontrol agents seem promising and could represent potent alternative disease control measures to fight bacterial wilt disease.

- **B.3. Infection process and virulence determinants of the RSSC**

RSSC bacteria have a relatively simple life cycle with an infection phase in planta and a saprophytic phase in the soil (Figure 11A). Present in the soil, the bacteria are attracted to root exudates by chemotaxis, attach to roots, enter the roots via lateral root emergence points and wounds and move to the xylem vessels where they thrive (Caldwell et al., 2017; Lowe-Power et al., 2018; Vasse, 1995). Once they reach the xylem, which occurs about 24 hours after inoculation in tomato, the

bacteria form a biofilm and secrete exopolysaccharides (EPS) that can obstruct the xylem sap flow (Caldwell et al., 2017; Kang et al., 2002). In tomato, typical infection symptoms include wilting of aerial parts, brown discolouration of the xylem, and oozing from cut stems (Genin, 2010). Plants continue to wilt until they die, resulting in the return of bacteria to the soil, where they can survive for 2-3 years as saprophytes, and/or spread via irrigation water. In potato, spread can also occur through the transport of infected tubers (Bragard et al., 2019). In Arabidopsis, it takes ~3 days for wilting symptoms on leaves to be visible (Figure 11B).

Several factors that play a role during infection are called virulence determinants. Virulence determinants are factors promoting pathogen infection. Among the virulence determinants of RSSC bacteria are motility, chemotaxis, EPS, the Type 2 Secretion System (T2SS) and its associated cell-wall degrading enzymes, as well as the T3SS and associated effectors (Genin and Denny, 2012). It seems quite logical that motility and chemotaxis are virulence determinants of RSSC bacteria, since the bacteria need to locate potential hosts in the soil, move towards them, and reach the xylem vessels. To do this, they can use swimming motility with their polar flagellum to locate roots in the soil, and twitching motility, which requires Type IV pili, to attach to and move along surfaces notably in xylem vessels (Lowe-Power et al., 2018). Once in contact with the host roots, the bacteria penetrate through the cell layers to reach the xylem vessels, with the help of their T2SS and Type II cell-wall degradation enzymes. Notably, mutants deficient in cellulolytic enzymes and T2SS induced delayed symptoms in tomato plants, suggesting the importance of penetrating plant tissues to reach xylem vessels for successful infection (Liu et al., 2005). In addition, secreted EPS are also a major virulence factor for RSSC bacteria. Indeed, compared to the WT strain, EPS-defective mutants are less virulent on eggplant and tobacco and spread less efficiently in tomato stems, suggesting that EPS may be involved in plant colonisation by the pathogen (Kao et al., 1992; Saile et al., 1997).

C. Focus on key virulence determinants of the RSSC: theType III effectors

- **C.1. The RSSC possesses a large Type III effector (T3E) repertoire**

Another main virulence determinant of the RSSC is the T3SS without which colonisation and symptom development of the host plant infected with the pathogen is strongly impacted (Vasse et al., 2000). The T3SS allows the secretion of T3Es in host cells, which can alter specific cellular processes to promote pathogen infection. RSSC strains have the particularity to possess a wide range of T3Es, with an average of 64 T3E encoding genes compared to 20-30 in P. syringae or X. campestris (Landry et al., 2020; Sabbagh et al., 2019). The RSSC T3Es repertoire was catalogued under a unified nomemclature in 2013, assigning a name starting with Rip for Ralstonia injected proteins, and an online repertoire (EffectorK) compiling T3Es from different RSSC strains is available with tools to compare the presence, absence and copy number of T3E encoding genes between different strains (Peeters et al., 2013). Landry and colleagues have provided a good synthesis of what has been described in the litterature on the different T3Es in the RSSC until recent years. Among the different T3Es categorised, about 50 of them have been characterised with a relative level of detail, from virulence or avirulence capacity in host species to functional and molecular characterisation (Landry et al., 2020). Redundancy has been noted between different T3Es of the RSSC, which could favour adaptation of pathogens to changing environments while maintaining an efficient infection capacity (Chen et al., 2014; Ghosh and O'Connor, 2017; Solé et al., 2012).

It has long been assumed that T3Es are important mainly for the virulence of the pathogen in the early stages of infection (Alfano and Collmer, 2004). However, recent studies tend to show that T3Es might be important throughout the whole infection process, even in late stages of the disease (Monteiro et al., 2012; de Pedro-Jové et al., 2021). Indeed, one of these recent studies demonstrated the dynamic expression of RSSC virulence determinants during infection in potato, including T3Es, and compared their expression pattern at different infection stages

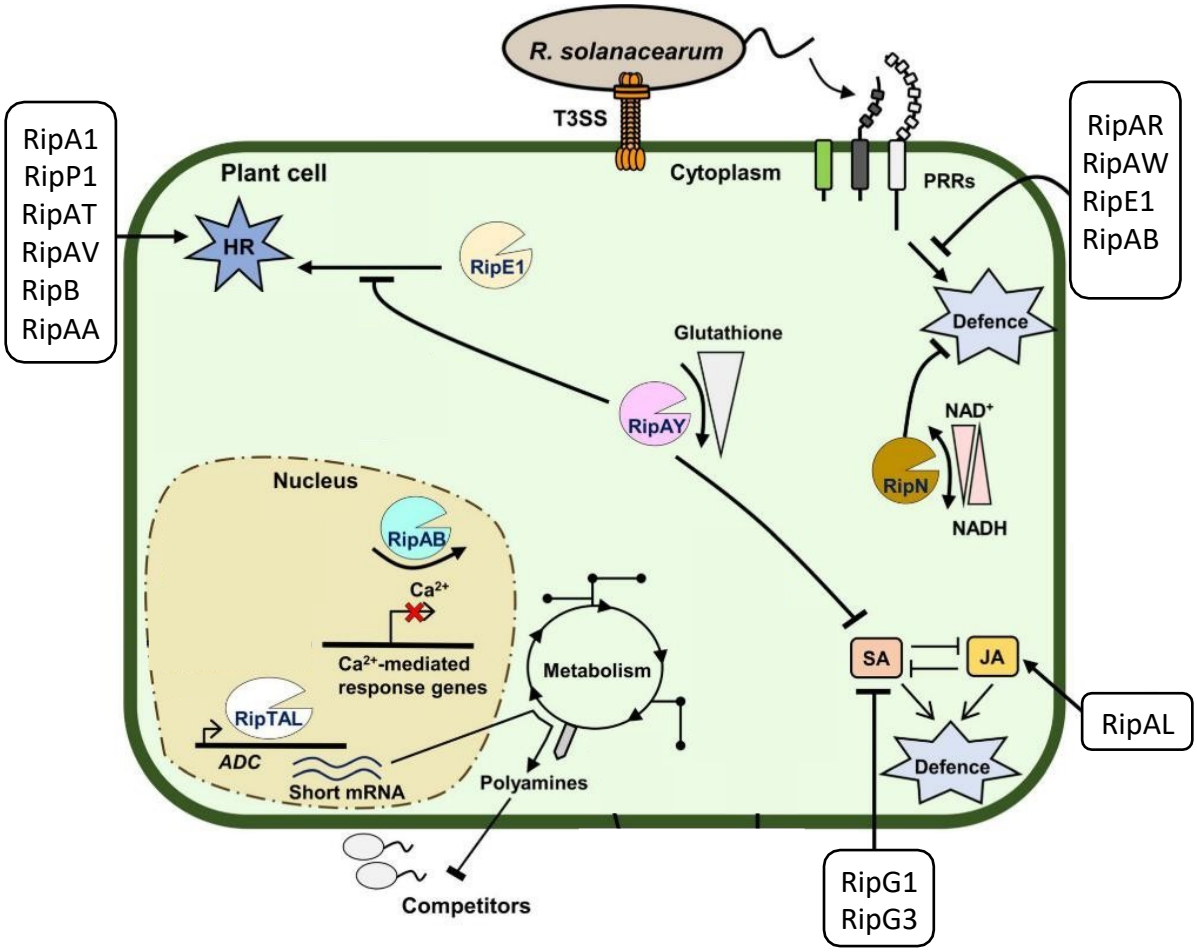


Figure 12. Effectors from the RSSC interfere with plant immune responses and affect host cellular processes (adapted from Landry et al., 2020).
RSSC bacteria translocate T3Es into the plant cell to subvert the plant defences and accommodate the bacterial needs. T3Es act on different host pathways. RipAY and RipN alter the glutathione level and NADH/NAD+ ratio, respectively. RipAL, RipG1, and RipG3 target the hormone synthesis and signalling level. RipTAL binds to the plant DNA, activating the expression of shorter and more efficiently translated transcripts of arginine decarboxylase (ADC) genes, key enzymes in the biosynthesis of polyamines. This boost in the polyamine level could prevent the proliferation of Ralstonia niche competitors. The nuclear T3E RipAB inhibits the expression of Ca2+- related defence genes. In addition to these functionally characterised RSSC T3Es, other effectors involved in dampening of basal defence through as yet unknown mechanisms have been identified including RipAR, RipAW, RipAB, and RipE1. RSSC T3Es can also be perceived *in planta* by NLRs, leading to the activation of specific defence mechanisms, often associated with an HR. RipE1, RipAA, RipP1, RipAT, RipAV, RipAB, and RipB induce HR on several hosts. Some T3Es can modulate the activity of others and prevent their recognition by the plant surveillance system. For instance, RipAY inhibit RipE1-mediated HR.

(de Pedro-Jové et al., 2021). They showed that virulence determinants are differentially expressed in the apoplast, early or late xylem. In particular, T3Es were predominantly expressed in xylem vessels at both early and late stages with a few exceptions, showing that many effectors play a role during infection once in the xylem vessels, but others are considered “early effectors” or present at all stages of infection (de Pedro-Jové et al., 2021). It is likely that the expression pattern of T3Es between apoplast and xylem is similar in other host plants, but it remains to be determined whether the expression of individual T3Es in apoplast and xylem follows the same pattern.

- **C.2. RSSC T3Es, diverse weapons to manipulate the host plant for the benefit of the pathogen**

Of the ~50 RSSC T3Es that have been characterised, some are simply known to contribute to virulence in certain host species, others have been shown to be avirulence factors, and others are known to affect specific host cellular processes for the benefit of the pathogen (Figure 12) (Landry et al., 2020).

C.2.1. Effectors known to be avirulence factors

One of the easiest characteristics of an effector is whether the effector is recognised or not in a given host species. As being an avirulence factor is synonymous with triggering ETI responses, the avirulence function of effectors can be studied by examining their ability to trigger a cell death response (Hypersentitve response, HR) in certains host species/cultivars once expressed or delivered in planta, or by inoculating host plants with RSSC strains mutated in the effector to assess the impact of the absence of the effector on disease development. For instance, in an attempt to characterise eight RSSC effectors, RipA1 was shown to trigger HR in N. benthamiana after transient expression via Agrobacterium tumefaciens (Jeon et al., 2020). Interestingly, a single effector can be an avirulence factor in various host species or cultivars. This is the case for RipP1 (formerly

PopP1), which triggers HR responses in three different Nicotiana species but is also known to be an avirulence factor in Petunia, since the cultivar St40 loses its resistance to GMI1000 when lacking RipP1 (Lavie et al., 2002; Poueymiro et al., 2009). Other examples are RipAT and RipAV, whose transient expression via A. tumefaciens in planta revealed that these two effectors elicit HR in many lettuce cultivars, and in some pepper and tomato cultivars (Wroblewski et al., 2009). It can be noted that these two effectors elicit HR in common lettuce and pepper cultivars indicating that several effectors can be avirulence factors in the same cultivar. When several RSSC effectors are avirulence factors in the same host species or cultivars, it can create difficulties for functional characterisation of the effectors, as there is a redundancy in triggering ETI. Typical example of RSSC effectors that are avirulent factors in the same species are RipB, RipP1 and RipAA (formerly AvrA) which are avirulent factors in N. benthamiana (Nakano and Mukaihara, 2019; Poueymiro et al., 2009). RSSC strains mutated in ripP1 or ripAA triggered a weaker HR than that induced by the WT strain, and a ΔripP1 ripAA double mutant triggered almost no HR (Poueymiro et al., 2009). Later, another study revealed that another effector, RipB, acts as a major avirulence factor in N. benthamiana and that RipP1 and RipAA are minor avirulence factors (Nakano and Mukaihara, 2019). Indeed, inoculation with a ΔripB mutant strain led to wilt symptoms in contrast to the tobacco-avirulent WT strain for which N. benthamiana is resistant. However, wilt symptoms induced by a ΔripB mutant were much reduced compared to a tobacco-virulent strain, suggesting that other factors are involved in avirulence, but to a lesser extent. This prompted the authors of the study to investigate the virulence of a ΔripB ripP1 ripAA triple mutant in N. benthamiana, which recovered full virulence compared to the tobacco-virulent strain (Nakano and Mukaihara, 2019). Thus, in N. benthamiana, RipB, RipP1 and RipAA are avirulence factors that contribute to ETI to a different extent. This implies that working on these effectors is more difficult due to the redundancy in their ETI activation function and therefore requires working on multiple mutants to avoid such redundancy.

C.2.2. Effectors that inhibit plant immune responses

C.2.2.a. Inhibiting ETI responses

We have seen that some RSSC effectors can trigger ETI responses in a given host species, leading to a strong plant defence activation that will restrict plant colonisation and result in plant resistance. It is therefore not beneficial for RSSC bacteria to let ETI responses be activated. To prevent this, the RSSC has evolved some effectors that can prevent ETI triggered by other effectors. For instance, RipE1 triggers ETI responses in N. benthamiana and upon its ectopic expression in transgenic A. thaliana, which in turn can be inhibited by the effector RipAY (Sang et al., 2020). RipAY can suppress plant immune responses by degrading plant glutathione, a regulator of plant redox homeostasis with a role in plant response to environmental and biotic stresses (Sang et al., 2018). In addition to triggering ETI responses, RipE1 expression in N. benthamiana is also accompanied with glutathione accumulation in plant leaves prior to immunity activation. Interestingly, transient co-expression of RipAY and RipE1 in N. benthamiana inhibited ion leakage and associated cell-death, as well as the expression of SA-related genes such as PR1, normally triggered by RipE1, revealing that RipAY suppresses RipE1-triggered immunity. Considering that RipE1-triggered immunity is inhibited by catalytically active RipAY only, it appears that RipAY may prevent RipE1-triggered immunity activation likely via its glutathione degradation activity (Sang et al., 2020).

C.2.2.b. Inhibiting plant basal defences

Some of the RSSC T3Es are able to suppress plant basal defences, PTI, in various ways. For instance, RipN is a conserved T3E in the RSSC that has been shown to inhibit PTI via its enzymatic activity (Sun et al., 2019). When RipN was transgenically expressed in A. thaliana, pathogen multiplication in planta was promoted and PTI responses were inhibited, such as callose deposition and activation of defence-related genes, including CBP60g, suggesting that RipN could inhibit PTI responses. This effector has a Nudix hydrolase domain, which supposedly catalyses the hydrolysis of some substrates such as the reduced and oxidised forms of nicotinamide adenine dinucleotide (NADH and NAD+

respectively), and ADP-ribose among others. In this study, the authors showed that RipN possesses pyrophosphorylase activity of ADP-ribose and NADH in vitro and destabilises NADH/NAD+ ratios in planta, which depends on RipN enzymatic activity. Furthermore, PTI inhibition also seems linked to RipN enzymatic activity since transgenic expression of a catalytically inactive version of RipN in A. thaliana did not alter callose deposition or defence-gene activation under flg22 activation (Sun et al., 2019). Thus, RipN inhibits PTI responses likely through its hydrolase activity.

Other examples of RSSC T3Es that can suppress PTI responses are RipAR and RipAW, cytoplasmic T3Es possessing an atypical E3 ubiquitin ligase domain, domain that catalyses protein ubiquitination (Nakano et al 2017). Both of these T3Es have been shown to possess functional E3 ubiquitin ligase activity in vitro. In addition, RipAW and RipAR can act as PTI suppressors since their Agrobacterium-mediated transient expression in N. benthamiana suppressed flg22-induced PTI responses, like ROS burst and activation of defence genes. Also, RipAW PTI-suppression effect seems to depend on its ubiquitin ligase activity, as transient expression of a RipAW catalytic mutant did not induce suppression of these PTI responses (Nakano et al., 2017).

With the examples of RipAR, RipAW, and RipN, we saw that, some RSSC T3Es can suppress PTI responses via different enzymatic activities, and presumably by acting at different levels within the plant cell. For other effectors, we know more about their effects on specific signalling pathways and on particular cellular mechanisms.

C.2.3. RSSC effectors affecting hormone signalling pathways

During infection, RSSC can face plant defences, some of which are controlled by hormone signalling pathways, including SA-related pathway. Therefore, to promote infection, manipulation of hormone signalling pathways by T3Es represents a potent virulence startegy.

For instance, RipAL is a chloroplastic T3E that can manipulate JA biosynthesis likely to inhibit SA-triggered defences (Nakano and Mukaihara, 2018). This effector has been demonstrated to suppress PTI responses in N. benthamiana leaves such as ROS burst and expression of PTI-related genes. Moreover, RipAL possesses a putative lipase domain that presents homology with the lipase domain of a plant chloroplastic phospholypase known to be involved in JA biosynthesis. Surprisingly, suppression of PTI responses depends on its enzymatic domain as a RipAL catalytic mutant no longer inhibits PTI responses. The authors also demonstrated that transient expression of RipAL in N. benthamiana induced JA accumulation and decreases SA levels, which depends on RipAL catalytic activity. In addition, this effector behaves as a virulence factor in pepper. Indeed, inoculation of pepper leaves with a RRSC strain mutated in ripAL led to lower bacterial growth and leaves did not accumulate JA compared to leaves inoculated with the WT strain (Nakano and Mukaihara, 2018). This study then revealed that the RSSC uses RipAL lipase activity to stimulate JA production that in turn antagonistically represses SA signalling, resulting in the dampening of plant immune responses.

Other effectors, RipG1 and RipG3, also alter SA signalling pathway to dampen plant defence responses (Medina-Puche et al., 2020). These RSSC T3Es are chloroplastic effectors belonging to the RipG family (former GALA family, comprising 7 members) and whose transient expression in N. benthamiana leaves seems to dampen the cytoplasmic calcium burst following flg22 treatment. Moreover, transgenic A. thaliana expressing RipG1 and RipG3 had reduced expression levels of SA-responsive genes after flg22 treatment and were more susceptible to Pst DC3000, suggesting that these effectors alter SA-related defences. However, they did not suppress flg22-triggered immunity since ROS burst in these transgenic A. thaliana was not altered (Medina-Puche et al., 2020). While these T3Es seem to manipulate SA-related defences, further research is needed to better understand the precise mechanisms of the SA signalling pathway manipulation and the benefit to RSSC bacteria, although this might be challenging as the RipG family is functionnally redundant (Cunnac et al., 2004).

C.2.4. Cellular processes altered through the nuclear activities of RSSC effectors

Some RSSC T3Es exert their virulence functions in the plant nucleus. RipAB is a nuclear targeted effector acting as a major virulence factor for the RSSC in potato (Zheng et al., 2019). In addition, transgenic expression of RipAB in potato plants led to a transcriptional reprogramming that resulted in the down-regulation of many genes including Ca^{2+} signalling genes like calmodulin-encoding genes. Also, these transgenic lines showed attenuated PAMP-triggered ROS burst associated with an enhanced suceptibility. The authors hypothesised that RipAB could act either as a TF-like protein that could repress specific Ca^{2+} signalling-related genes or by interacting with Ca^{2+} signalling components, leading to indirect down-regulation of Ca^{2+} signalling related genes (Zheng et al., 2019). However, many uncertainties remain regarding the precise action of RipAB in the nucleus and require further investigation.

The RipTAL effectors are known to alter cellular processes by binding DNA and inducing transcription of target genes. Brg11, also called RSc1815 or RipTALI-1, is a RipTAL from the RSSC strain GMI1000 that has been best documented in litterature so far (de Lange et al., 2013; Macho et al., 2010; Schandry et al., 2016; Wu et al., 2019). This effector participates in the virulence of the RSSC in eggplant and presents sequence similarity to TALEs found in Xanthomonas spp., which made researchers curious as to test whether this RipTAL could act in the same way as the TALEs, i.e. activate the transcription of susceptibility genes by binding to specific EBEs on host DNA (de Lange et al., 2013; Macho et al., 2010). Indeed, Brg11 has been demonstrated to bind to the EBE of two tomato genes encoding Arginine decarboxylases (ADC) and increase their transcript accumulation in tomato, eggplant, N. benthamiana and N. tabacum, suggesting that Brg11 functions in different host plants. ADCs are enzymes involved in polyamine biosynthesis by catalysing the conversion of arginine to agmatine which can be subsequently converted into putrescine and longer polyamines like spermine and spermidine. Putrescine and other polyamines are known to participate in ROS burst activation

after catabolition and have been shown to potentiate ROS burst during PTI (O'Neill et al., 2018). RSSC-mediated delivery of Brg11 in tomato increases agmatine and putrescine levels in planta (Wu et al., 2019). This raises the following question: why a component presumably linked to plant defences could benefit RSSC bacteria? The authors noticed that inoculation of plants with RSSC bacteria lacking brg11 did not affect RSSC bacterial growth and that tomato plants defective in the Brg11-targeted ADCs favour Pst DC3000 growth in planta but not RSSC bacterial growth. Considering that Brg11 inhibits Pst DC3000 growth but not RSSC bacterial growth, the authors hypothesised that RSSC bacteria might use Brg11 to manipulate transcription and translation of polyamine biosynthesis regulators to compete with other pathogens that might not tolerate polyamine-related defence (Wu et al., 2019).

D. PopP2, a major virulence factor of the RSSC

Another effector acts at the nuclear level and has been shown to be a major virulence factor of the RSSC. This effector, well studied in the team, is PopP2 (RipP2) which deserves its own section as it is part of my thesis project.

- **D.1. PopP2 is a major virulence factor for the RSSC with an acetyltransferase activity**

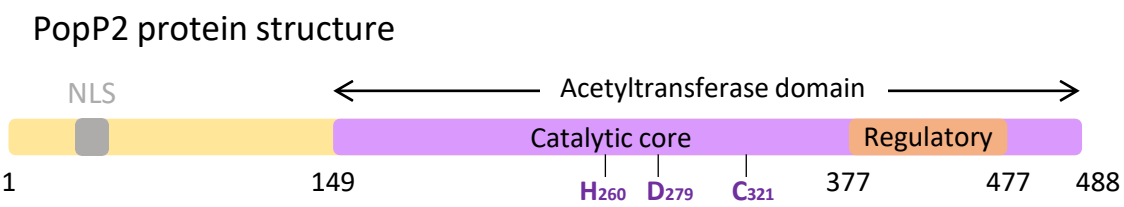
D.1.1. PopP2 is important for some RRSC strain fitness or virulence in different host species

PopP2 (RipP2) is a T3E belonging to the PopP (RipP) family in RSSC which comprises 3 members, PopP1 (RipP1), PopP2 and PopP3 (RipP3). The three effectors share about 17-20% protein identity when compared in pairs, and they share around 42% similarity overall, suggesting that they are evolutionarily distant (Lavie et al., 2004). While the representation of popP genes in the RSSC reveals that they are present mainly in R. pseudosolanacearum strains (phylotypes I and

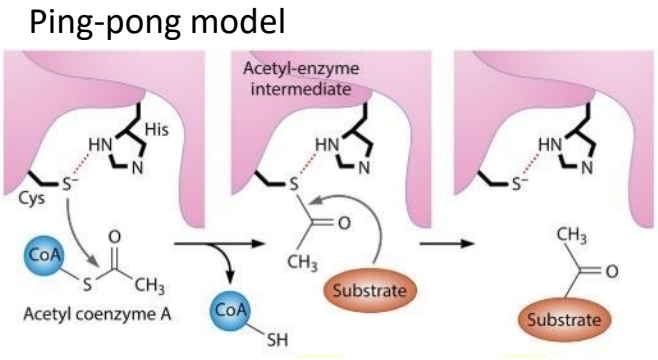
III) rather than in R. solanacearum strains (phylotype II), the representation of PopP2 among the different strains in the RSSC is more dispersed among the different species (Lavie et al., 2004). Indeed, PopP2 is either present or absent in strains belonging to the same species. For instance, PopP2 is present in R. pseudosolanacearum strain GMI1000 (phylotype I), originally isolated from tomato in Guyana but is absent from strain Rd15 which was originally isolated from radish in Taiwan and belongs to the same species and phylotype as GMI1000 (Lavie et al., 2004). In R. solanacearum phylotype IIB, PopP2 can be found in the Po82 strain isolated from potato in Mexico, but is not present in the MOLK2 strain, isolated from banana in Indonesia (Lavie et al., 2004; Peeters et al., 2013). However, host range does not condition the possibility of finding PopP2 in a RSSC strain. For instance, various strains originally isolated from tomato do or do not possess PopP2, like R. pseudosolanacearum CMR15 (phylotype III) from Cameroun and R. solanacearum K60 (phylotype IIA) from the United States, which do and do not possess PopP2, respectively (Lavie et al., 2004; Peeters et al., 2013). Even in a restricted geographical area, PopP2 is not necessarily found in all strains present there. For example, in the Republic of Korea, among several strains isolated in the early 2000s that infect pepper and tomato, some strains do not possess this effector (Segonzac et al., 2017).

While PopP2 was first shown to be an avirulence factor in some host species/cultivars (see section D.2.1.), it also contributes to the virulence of RSSC bacteria in others. In particular, PopP2 has been shown to contribute to the virulence of GMI1000 in A. thaliana as a popP2-mutated strain induces fewer symptoms than the WT strain (Le Roux et al., 2015). Also, PopP2 contributes to bacterial fitness, i.e. the ability of the pathogen to grow in a competitive environment, in different crops such as tomato, bean and eggplant (Macho et al., 2010). So, PopP2 represents a major virulence factor for RSSC bacteria in many strains, and in various host species and its virulence functions are mainly based on its enzymatic activity.

A



B



C

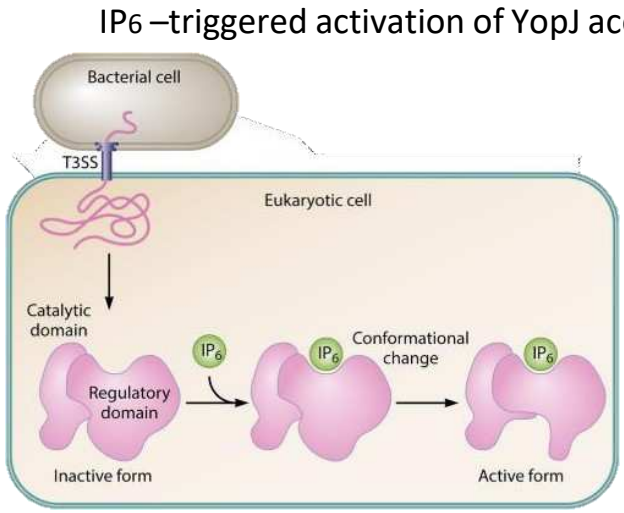


Figure 13. PopP2 is a YopJ family acetyltransferase (adapted from Xia et al., 2021 and Ma and Ma, 2016).
(A) PopP2 is a RSSC T3E with a NLS (grey box) and an acetyltransferase domain consisting of a catalytic core, conserved among all YopJ family members, and a regulatory domain. (B) Acetylation of PopP2 susbrates likely follows the ping-pong model, in which PopP2 auto-acetylates using acetyl-CoA as an acetyl donor, and then transfers the acetyl group onto its substrates. (C) In host cells, YopJ effectors activation likely occurs via interaction of the catalytic domain with the eukaryotic Inositol 6-phosphate ($InsP_6$) co-factor, resulting in conformational changes that switch the effectors from inactive to active form.

D.1.2. PopP2 is a YopJ family acetyltransferase targeted to the plant nucleus

Besides being part of the popP family, PopP2 also belongs to the Yersinia outer protein J (YopJ) effector family, a family of effectors found in animal and plant bacterial pathogens, which includes Salmonella enterica, Yersinia spp., Vibrio parahaemolyticus, Pseudomonas syringae, Xanthomonas campestris and the RSSC (Ma and Ma, 2016). The protein sequences of YopJ effectors are not highly conserved between species but their enzymatic domain sequence is. They possess an enzymatic domain that shares similarities with a cysteine protease domain, but YopJ effectors have been reported to exhibit acetyltransferase activity rather than protease activity. While the core of the catalytic triad of YopJ effectors consists of histidine, glutamic acid and cysteine amino acids, PopP2 harbours histidine, aspartic acid and cysteine residues instead (Figure 13A).

Several YopJ effectors are known to acetylate specific targets to promote infection in their host. For instance, YopJ effectors from animal pathogens can suppress the activation of inflammation by interfering with MAPK signalling cascades by acetylating MAPKKs, thereby preventing their activation by phosphorylation and subsequent signalling. In plants, YopJ effectors from plant pathogens can acetylate various cellular components in the cytoplasm or nucleus promoting disease development or triggering ETI responses (Ma and Ma, 2016). As an important virulence factor for the RSSC, the enzymatic activity and how PopP2 targets host components have been well studied. PopP2 is a nuclear targeted effector whose enzymatic function is necessary for its virulence and avirulence activities (Le Roux et al., 2015; Tasset et al., 2010). As an acetyltransferase, the acetylation process used by PopP2 and the other YopJ acetyltransferase effectors has been envisioned as a “ping-pong model” (Figure 13B, Ma and Ma, 2016). In this model, the enzyme acetylates its substrate in the presence of the acetyl-coenzyme A (acetyl-CoA) co-factor in two steps. First, the enzyme uses acetyl-CoA to auto-acetylate and then transfer the acetyl group to the substrate. To activate their acetyltransferase activity, some YopJ effectors need the host cofactor inositol hexaphosphate

(InsP6), an abundant metabolite in eukaryotic cells, which was hypothesised to induce changes in the effector conformation (Ma and Ma, 2016). Recent studies based on the analysis of crystal structures of PopP2 catalytic unit in complex or not with InsP6, acetyl-CoA or one of its substrates, revealed that InsP6 could enhance the acetyltransferase activity of PopP2 (Xia et al., 2021; Zhang et al., 2017b). In fact, PopP2 acetylation process could follow the ping-pong model. Indeed, it has been shown that PopP2 auto-acetylates on a specific lysine residue and this auto-acetylation is required for its activities (Tasset et al., 2010). Moreover, the reaction intermediate between PopP2 and acetyl-CoA was crystalised, supporting the two-step mechanism described in the ping-ping model (Zhang et al., 2017b). Also, crystal structures of the PopP2 catalytic unit in complex with InsP6 and/or its substrate revealed that InsP6 induces conformational changes in PopP2 including (i) opening of the binding pocket for Ac-CoA and (ii) structural rearrangement in a fold-switch motif located between PopP2 regulatory domain and its substrate recognition helix (Xia et al., 2021; Zhang et al., 2017b). This secondary–structure switch triggered by InsP6 enables PopP2 to become active and stabilises the interaction with its substrate (Xia et al., 2021) (Figure 13C). It is still unclear why such an activation process was evolved by bacterial pathogens. The authors hypothesised that the YopJ family effectors in their active state may be toxic to the bacteria producing them. To avoid potentially deleterious effects due to their enzymatic activity, these secreted effectors are completely latent until they are delivered into host cells where the InsP6 co-factor makes the enzyme active and regulates binding to its substrates.

- **D.2. PopP2 targets nuclear components involved in plant immunity**

D.2.1. In A. thaliana, PopP2-triggered immunity is mediated by the NLR pair RRS1-R/RPS4

In A. thaliana, some ecotypes are resistant to the RSSC GMI1000 strain and it has been shown that this resistance is conferred by the NLR-encoding gene RRS1-R,

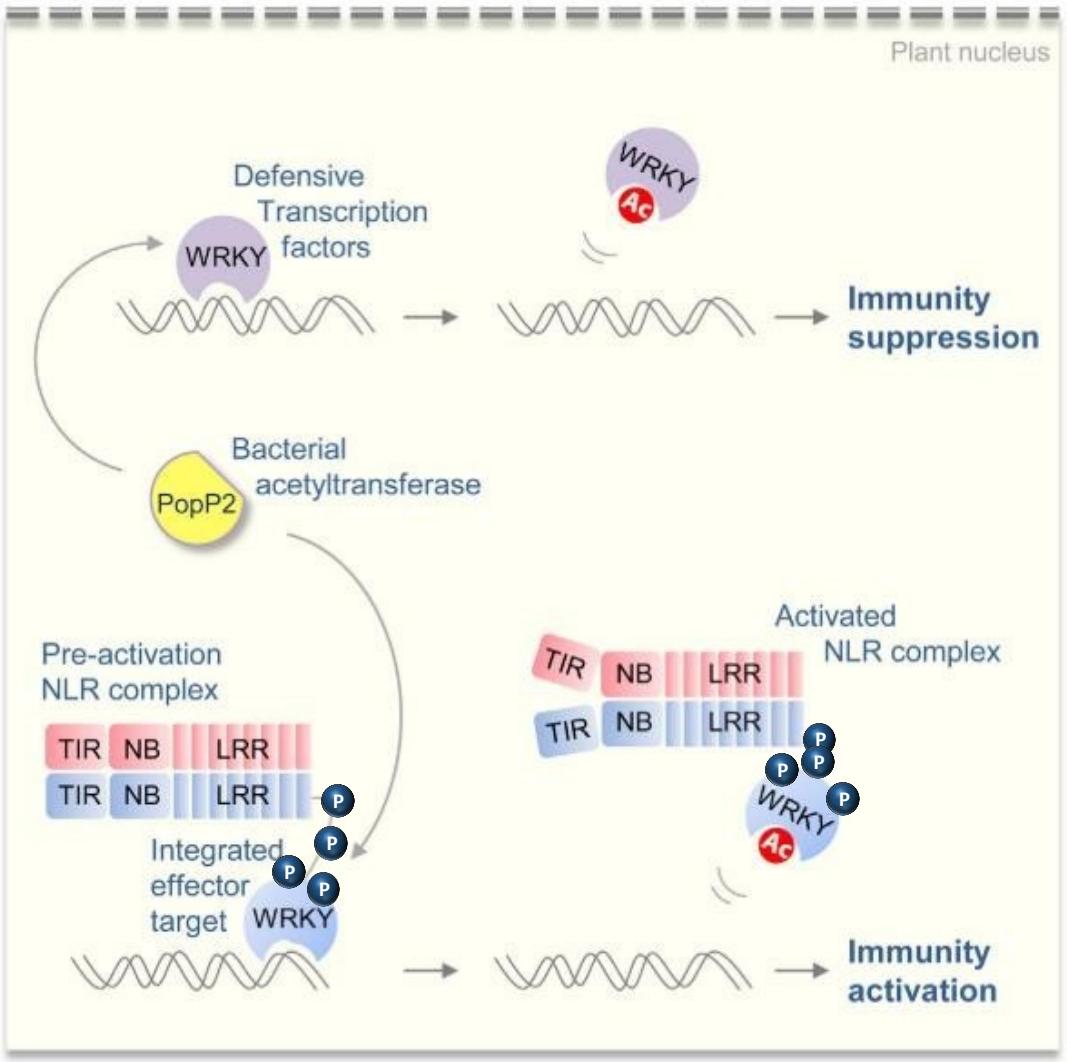


Figure 14. PopP2 acetylates WRKY domain-containing proteins (adapted from Le Roux et al., 2015 and Guo et al., 2020).
PopP2 targets WRKY domain-containing proteins, including various defensive WRKY TFs and the TIR-containing NLR RRS1-R. In susceptible plants, where RRS1-R is not present, PopP2 suppresses PTI by acetylating WRKY TFs, dislodging them from DNA, thereby disturbing their *trans*-regulating functions required for activation of basal immune responses. In resistant plants, where RRS1-R is present, PopP2 uses the same Lys-acetylation strategy to acetylate the WRKY domain of RRS1-R, which is in complex with RPS4. This dislodges the NLR complex from DNA and leads to its activation and the initiation of ETI. Phosphorylation of specific serine and threonine residues in RRS1-R is required for recognition of PopP2 by the NLR complex RRS1-R/RPS4.

behaving as a recessive resistance gene, and present in Nd-1 and Ws-2 accessions (Deslandes et al., 2002). In the Col-0 susceptible accession, the RRS1 protein is coded by the dominant RRS1-S allele. Later, it was shown that RRS1-R-mediated resistance was triggered by the effector PopP2, which interacts with and relocates RRS1 proteins in the nucleus (Deslandes et al., 2003). Actually, RRS1-R works in cooperation with RPS4, a second TIR NLR. The RPS4/RRS1 NLR pair confers resistance to 3 different pathogens: the bacterial pathogens RSSC producing PopP2, P. syringae expressing AvrRps4, and the fungal pathogen Colletotrichum higginsianum (Narusaka et al., 2009). The crystal structures of the TIR domains of RPS4 and RRS1 revealed that these two NLRs form a heterodimer via interaction of their TIR domains which is necessary for recognising two unrelated effectors, PopP2 and AvrRps4 (Williams et al., 2014). The main particularity of RRS1 resides in its C-terminus that contains a WRKY DNA-binding domain, which is found in WRKY TFs and known to bind specific cis-regulatory elements called the W-boxes (Deslandes et al., 2002; Rushton et al., 2010). Both RRS1-S and RRS1-R have a WRKY domain at the C-term but RRS1-R C-term has 90 additional residues compared to RRS1-S C-term (Deslandes et al., 2002).

Moreover, PopP2 recognition mechanism by RRS1-R/RPS4 has been elucidated (Figure 14). Indeed, after relocating RRS1-R to the nucleus, PopP2 was shown to acetylate the WRKY domain of RRS1-R on a specific lysine residue, the second lysine in the conserved WRKYGQK heptad motif of the WRKY domain, leading to a loss of affinity between the WRKY domain and DNA. PopP2 acetylation of RRS1-R WRKY domain is the trigger for activation of RPS4-dependent immunity (Le Roux et al., 2015; Sarris et al., 2015). Activation of RRS1-R/RPS4-mediated resistance depends on the acetyltransferase activity of PopP2 since a catalytic mutant of PopP2 fails to acetylate RRS1-R and does not trigger resistance in planta (Le Roux et al., 2015; Tasset et al., 2010). Surprisingly, PopP2 also acetylates RRS1-S on the same key Lysine residue and this leads to its dislodging from DNA, suggesting that the inability of RRS1-S to activate a robust immune response in presence of PopP2 is not linked to a change in its ability to bind DNA (Le Roux et al., 2015; Sarris et

al., 2015). Thus, at this stage, while the mechanism of PopP2 recognition by RRS1-R became clearer, the precise activation process of the immune-complex RRS1-R/RPS4 and downstream signalling remained elusive.

Subsequent studies have attempted to better understand the activation of this immune complex, including the involvment of particular PopP2 subdomains or the role of RRS1 PTMs in the activation of the immune complex. For instance, PopP2 possesses an Ethylene-responsive element binding factor associated Amphiphilic Repression (EAR) motif (LxLxLxL) within its catalytic unit, which is involved in PopP2 protein stability in planta and that is required in its avirulence functions since mutation of this motif results in a loss of recognition by RPS4/RRS1-R (Segonzac et al., 2017). This motif is normally involved in the recruitment of transcriptional co-repressors but it remains to be determined whether PopP2 can recruit such proteins.

Regarding RRS1-R, a recent study reported how phosphorylation of RRS1 might regulate its function (Guo et al., 2020). Indeed, RRS1-R but not RRS1-S C-terminus is phosphorylated in planta. This phosphorylation might participate in RRS1-R auto-inhibition and in PopP2 recognition. Activation of the RRS1-R/RPS4 complex seems to rely on increased proximity between RRS1 N-term and C-term triggered by interaction with matching effectors, inducing a decrease in affinity between the TIR domains of RRS1 and RPS4, which are known to be responsible for the inhibition of the complex. Phosphorylation of RRS1-R C-term at a specific Threonine, Thr1214, seems important for RRS1-R repression, and acetylation by PopP2 at nearby sites including Lys1221 seems to compete with Thr1214 phosphorylation and explain the derepression of RRS1-R/RPS4 complex (Guo et al., 2020). Thus, the fact that PopP2 is recognised by RRS1-R but not by RRS1-S might be due to phosphorylation of RRS1-R C-term but not of RRS1-S. Indeed, phosphorylation of RRS1-R C-term confers a greater proximity between RRS1-R N-term and C-term, which is then enhanced by interaction with PopP2 leading to derepression of the RRS1-R/RPS4 complex. In RRS1-S, as the C-term part is not phosphorylated, the proximity between RRS1-R N-term and C-term is less

pronounced, and the interaction with PopP2 does not increase this proximity sufficiently to activate the complex. The difference in phosphorylation status between RRS1-R and RRS1-S might lie in the 90 residues lacking in RRS1-S that could act as kinase docking domain (Guo et al., 2020).

D.2.2. PopP2 is also an avirulence factor in crops

Interestingly, PopP2 has been shown to be an avirulence factor also in crop species such as tomato, bean and eggplant (Macho et al., 2010; Pensec et al., 2015). While in bean and tomato the mechanisms of PopP2 recognition are still unclear, a NLR-encoding gene has been identified in eggplant that could mediate PopP2-triggered immunity (Xi'ou et al., 2015). The NLR-encoding gene is RE-bw, which encodes a NLR protein containing an integrated WRKY domain that shares 77.8% identity with RRS1-R based on protein sequence comparison. RE-bw NLR protein was shown to physically interact with PopP2 by Yeast-two-hybrid (Y2H) and bimolecular fluorescence complementation assay performed in eggplant protoplasts and also with ETI-related components such as EDS1, PAD4, NPR1, and SGT1. Plants producing RE-bw also displayed increased expression levels of these ETI-related genes. Moreover, transgenic tomato lines over-expressing RE-bw and eggplant resistant plant silenced in RE-bw gene expression revealed that RE-bw is an important factor for bacterial wilt resistance that seems involved in the promotion of SA-related defences, ROS burst, and cell wall fortification in roots. It can be noted that the protein sequence of RE-bw shares 60 to 70% identity with NLR proteins from pepper or potato, suggesting that there might be more RRS1-R homologs in crops than previously expected (Xi'ou et al., 2015).

To conclude, PopP2 has long been known as an avirulence factor in many crops as well as in the model plant A. thaliana, where the underlying molecular mechanisms involved in its recognition have been elucidated. It was revealed that PopP2 interacts with the RPS4/RRS1-R immune complex. Upon acetylation by PopP2, RRS1-R is detached from DNA, leading to the activation of the immune complex

and downstream signalling that trigger ETI responses. Considering that RRS1-R homologs exist in crop species, it would be interesting to further investigate whether they can confer PopP2-triggered resistance to improve crop resistance, even though PopP2 is not present in all RSSC strains. Another way to improve crop resistance to RSSC could be to better understand what the virulence functions of PopP2 are, i.e. by identfying which host components are manipulated by this effector.

D.2.3. PopP2 manipulates defensive WRKY TFs to dampen basal immune responses

So far, PopP2 was known to target WRKY domains of the TIR NLR RRS1-R and possibly from the TIR NLR RE-bw. When studying the targeting of RRS1-R, the discovery that PopP2 specifically targets the WRKY domain of RRS1-R prompted the authors to check whether PopP2 could also manipulate defensive WKRY TFs. Interestingly, other WRKY TFs, including WRKY8, WRKY18, WRKY28, WRKY22 and WRKY53 were acetylated by PopP2 (Le Roux et al., 2015; Sarris et al., 2015). In the absence of RRS1-R/RPS4 recognition, lysine-acetylation of multiple WRKY transcription factors by PopP2 dislodges them from their DNA-binding sites and disables their trans-activating functions needed for defence gene expression (Figure 14) (Le Roux et al., 2015). This essentially dampens host basal resistance, allowing rapid spread of the pathogen inside tissues and leading to the complete wilting of infected plants.

Therefore, RRS1-R with its integrated WRKY domain serves as a direct sensor of PopP2 virulence activity upon infection. This WRKY domain in RRS1-R can be viewed as an effector target ‘decoy’ which betrays the resistance-suppressing actions of PopP2 on its operational targets, the defensive WRKY transcription factors. Molecular integration of a WRKY decoy domain within the RRS1-R/RPS4 receptor complex creates an effective ‘radar’ for a powerful pathogen virulence activity which cannot be easily dispensed with by the pathogen. The observed fusion of further potential effector target decoy domains with NLR receptors in different plant species suggests a fundamental mechanism in plants for increasing

receptor recognition ‘space’ by integrating in immune receptors molecular decoys that mimick the true virulence targets of pathogen effectors (Grund et al., 2019; Kroj et al., 2016). This also provides promising avenues for engineering receptors that could effectively intercept disease-promoting activities of agronomically important crop pathogens.

4- Apart from WRKY defensive TFs, PopP2 also targets bromodomain-containing Arabidopsis epigenetic readers.

To elucidate other virulence activities of PopP2, a YopJ family acetyltransferase that is exclusivey targeted to the plant nucleus, a Y2H screening was undertaken several years ago to identify Arabidopsis proteins other than WRKY proteins that could be manipulated by PopP2. This screening was performed using a A. thaliana cDNA library composed of all genes expressed in resistant and susceptible seedlings challenged for 24 hours with the RSSC strain GMI1000 (Deslandes, unpublished data). After several rounds of screening, among the various candidate PopP2-interacting partners identified was a protein containing a bromodomain (BRD) and an extra-terminal domain: GTE11 from the General Transcription factor group E (GTE) family.

A. Arabidopsis GTE proteins contain a bromodomain, a domain that allows recognition of acetylated lysine residues

GTE11, and its closest member regarding BRD protein sequence GTE9, are members of the GTE family, also sometimes referred to as the Bromodomain and Extra-Terminal domain (BET) family, as members of this family harbour two particular protein domains: a N-term bromodomain and a C-term extra-terminal domain (Pandey, 2002). The bromodomain (BRD) is a specific module of ~110 aa that allows the recognition of acetylated lysines mainly on histone tails but also on

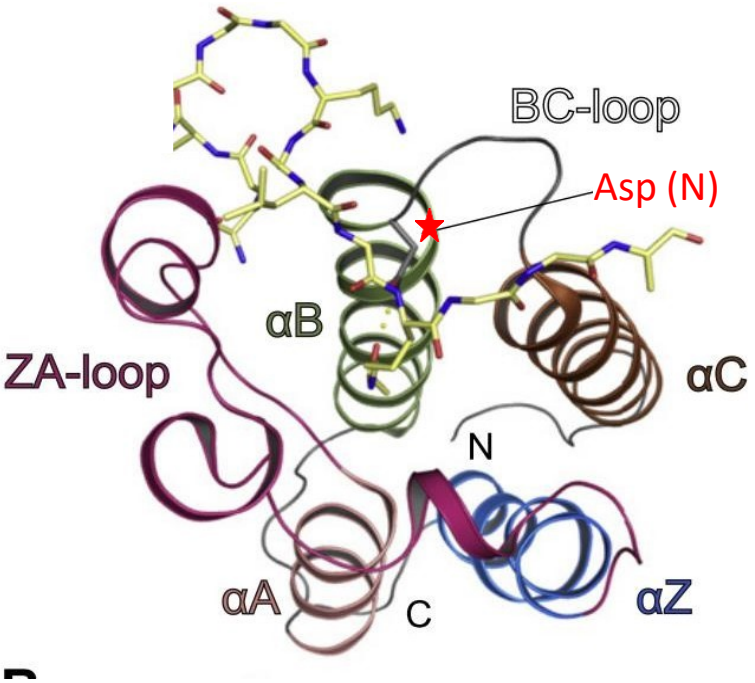


Figure 15. The bromodomain, a domain that recognises acetyl-lysine residues (adapted from Filippakopoulos and Knapp, 2012).
Structure of the first BRD from human BRD2 binding to acetylated H4K12. Bromodomains allows the recognition of acetylated lysine residues via a central hydrophobic pocket and mediated by anchoring to a conserved asparagine (N) residue represented with a red star. They share a similar folding with a left-handed bundle of four α-helices (αZ, αA, αB, and αC), linked by loops (ZA and BC loops).

other proteins; and the extra-terminal domain is a domain allowing protein-protein interactions (Filippakopoulos and Knapp, 2012; Wang et al., 2021). Mechanistically, BRD (whose crystal structure from many animal BRD-containing proteins was solved (see Figure 15)) can recognise acetylated lysine residues via a central hydrophobic pocket in which the acetylated lysine can anchor to a conserved asparagine residue (Wang et al., 2021). The specificity of the bromodomain for a type of acetylation is protein dependent (Zaware and Zhou, 2019).

BRD-containing proteins have been extensively studied in animals since they were shown to be involved in the development of various human diseases, including cancers (Wang et al., 2021). Thanks to this knowledge, we know that animal BRD-containing proteins can be found in different types of proteins such as HATs, histone methyltransferases, chromatin remodelling complex subunits, transcriptional regulators or ubiquitin ligases (Zaware and Zhou, 2019). Also, most BRD-containing proteins contain other domains that can function with or independently of the BRD to mediate protein functions. As they can bind acetylated histones, BRD-containing proteins are considered to be epigenetic readers and participate in PTM modification of histones, chromatin remodelling or also in transcriptional regulation as they can recruit components of the transcriptional machinery to specific gene regions (Zaware and Zhou, 2019).

Surprisingly, animal BRD-containing proteins possess between one and four BRDs while plant BRD-containing proteins possess only one (Wang et al., 2021; Zhao et al., 2018). A recent study profiled A. thaliana histone readers and found the presence of 28 BRD-containing proteins encoded by the A. thaliana genome and they all contained a single BRD (Zhao et al., 2018). The authors showed that BRDs from A. thaliana and animals shared conserved critical residues that are important for recognising acetylated lysines on histones and that two BRD-containing proteins from Arabidopsis were able to bind acetylated lysines on histone H4, suggesting that BRD function is quite conserved between animals and plants. Furthermore, they showed that the BRD of two Arabidopsis BRD-containing proteins had a higher affinity for diacetylated peptides on histone H4 than for

monoacetylated ones, suggesting that their BRD preferentially bind a combination of acetylated residues on histone tails, similar to animal BET proteins (Wang et al., 2021; Zhao et al., 2018). About ten Arabidopsis BRD-containing proteins profiled by Zhao and colleagues have been characterised and some of them are known to be subunits of chromatin remodelling complexes, such as BRM, BRD1, BRD2 and BRD13 from the BRAHMA chromatin remodelling complex or MBD9 and NPX1 from the SWR1 chromatin remodelling complex (Farrona et al., 2007; Nie et al., 2019; Yu et al., 2021). Others are HATs or components of the transcriptional machinery, like the HAT GCN5 and the RNA-polymerase II pre-initiation complex component TAF1 (Benhamed et al., 2008; Waterworth et al., 2015). Interestingly, for some of these proteins, it has been shown that their BRD can bind to acetylated histone H3 or H4 reinforcing the hypothesis that BRD is a conserved domain between animals and plants in its abilities to recognise acetylated histone tails (Nie et al., 2019; Zhang et al., 2016a; Zhao et al., 2018). All of these BRD-containing proteins appear to be involved in a variety of biological processes including flowering, ABA signalling pathway, DNA damage repair, environmental stress response, incorporation of the histone variant H2A.Z… (Farrona et al., 2011; Grasser et al., 2021; Luo et al., 2020; Peirats-Llobet et al., 2016; Waterworth et al., 2015; Zhang et al., 2017a).

Thus, BRDs, domains that allow recognition of acetylated lysine residues on histone tails in particular, are quite conserved between animal and plants, and can be found in a variety of proteins ranging from members of the chromatin remodelling complex, to components of the transcriptional machinery, to HATs. As they possess only one BRD, plant BRD-containing proteins have been assumed to act in complex to perform their functions, for example by addressing a given protein to specific promoters, or helping to recruit other components of a complex.

Among the BRD-containing proteins from A. thaliana are also members of the GTE family, to which the PopP2-targets GTE9 and GTE11 belong. Current knowledge about the biological roles of different members of this family, including GTE9 and GTE11, will be discussed in the section below.

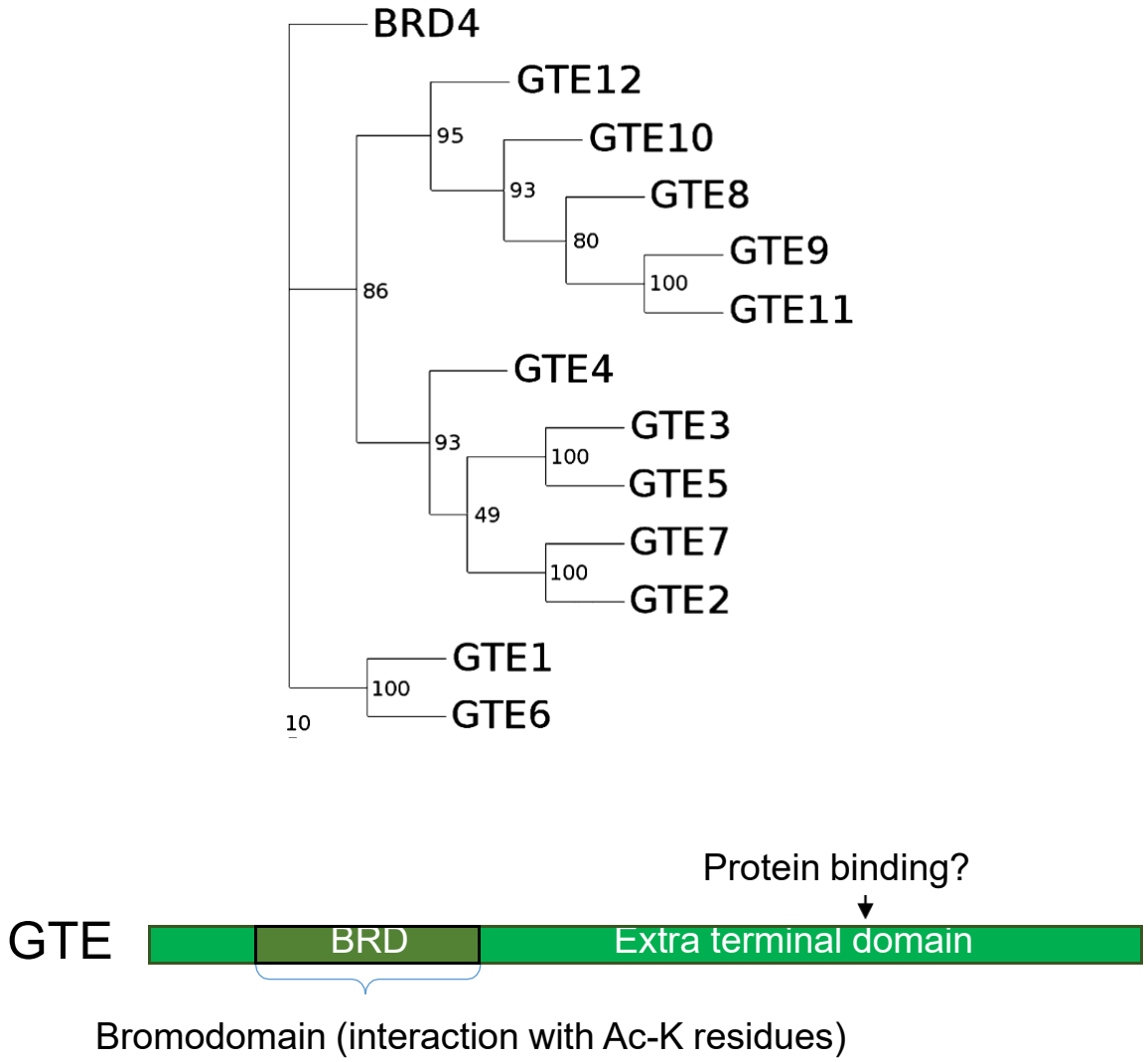


Figure 16. The GTE family in Arabidopsis counts twelve BRD-containing proteins (adapted from Airoldi et al., 2010).
The General Transcription factor from group E family is a family of BRD-containing proteins that counts 12 members in *A. thaliana*. They belong to the BET family, characterised by a BRD, domain allowing the recognition of acetylated lysine residues, and an extra-terminal domain, thought to be important for protein-protein interactions.

B. GTE9 and GTE11 belong to the GTE family, a family involved in various biological and cellular processes in A. thaliana, but whose molecular functions remain unclear

The GTE family, which includes GTE9 and GTE11, is sometimes known as BET family because the members of this family possess a BRD and an extra-terminal domain like the animal BET proteins (Pandey, 2002). In Arabidopsis, the GTE family counts twelve members (Figure 16), which appear to be involved in a variety of biological processes (Figure 17), although little is known about their precise biological functions.

- **B.1. GTE family proteins are involved in a variety of biological processes**

GTE1, also known as IMB1, is the first GTE member characterised and is involved in seed germination in A. thaliana (Duque and Chua 2003). Seed germination is controlled by pathways involving notably ABA and light, which is perceived by plant phytochromes (Yadukrishnan and Datta, 2021). In this context, by studying Arabidopsis gte1 mutant, GTE1 was shown to be a negative regulator of ABA-mediated responses and a positive regulator of phytochrome A-related responses during germination (Duque and Chua, 2003). However, the mechanistic details of the role of GTE1 in these pathways remain unknown.

Subsequently, a study characterised Arabidopsis plants defective in GTE4 and showed its importance in maintaining the mitotic cell cycle (Airoldi et al., 2010). Indeed, gte4 mutant plants are small, with scraggy leaves and fewer cells in organs than WT plants. Further investigations revealed that these mutants had a delayed activation of the cell cycle during germination, cell proliferation stopped sooner than in WT plants, which impacted on the whole plant development (Airoldi et al., 2010). The authors could then conclude that GTE4 has a role in cell cycle regulation, but the precise molecular functions of this protein require further investigation.

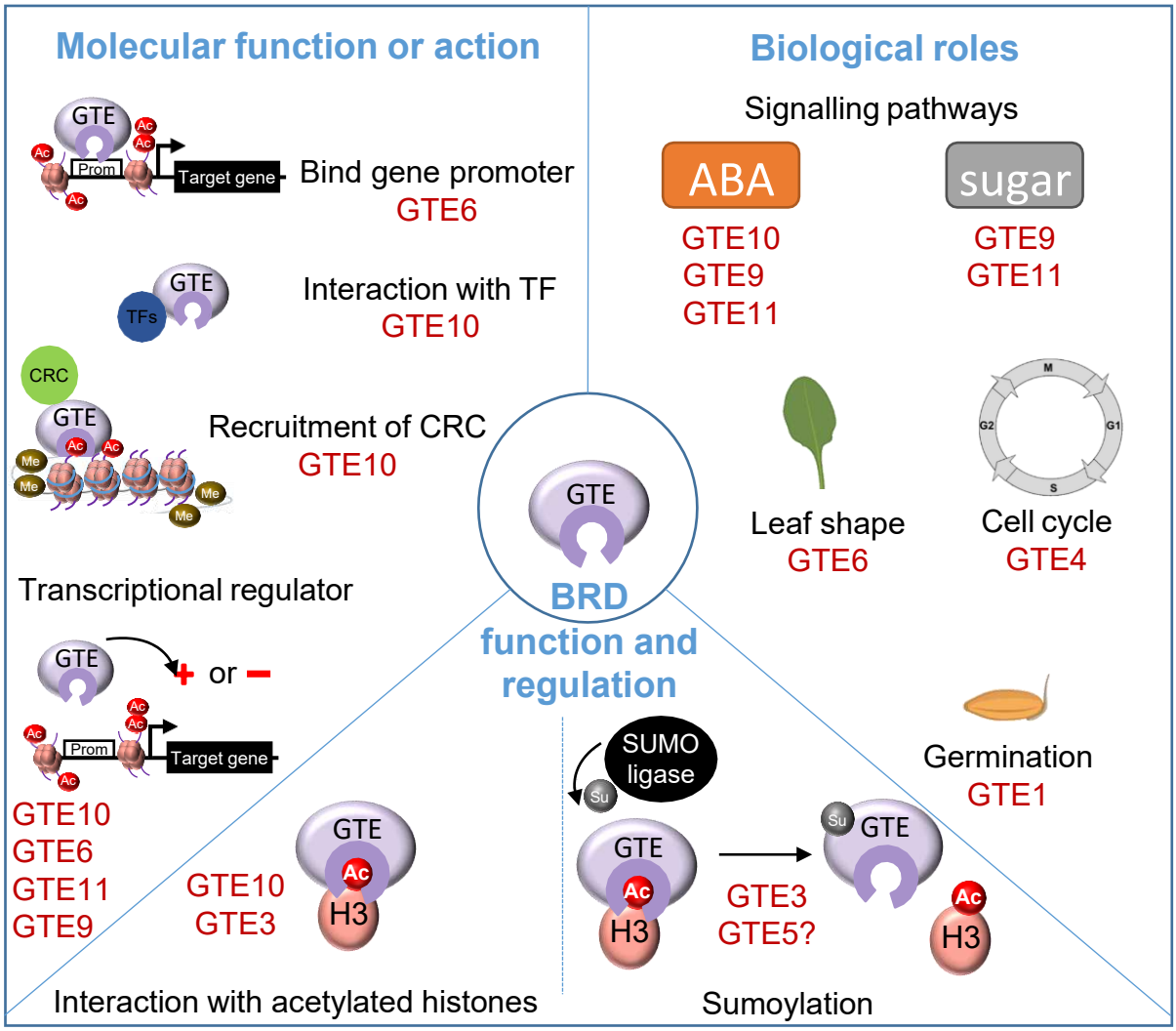


Figure 17. Arabidopsis GTEs are involved in various biological processes.
Members of the GTE family are involved in various biological processes such as ABA and sugar signalling pathways, control of leaf shape, cell cycle regulation or germination. The molecular functions of the different GTEs remain mostly unknown. However, GTE6 was shown to bind to the promoter of a target gene and promotes acetylation in that region, GTE10 interacts with TFs but can also recruit CRC subunits to methylated DNA regions. GTE6 and GTE10 regulate the transcription of various target genes likely indirectly. GTE9 and GTE11 are involved in the activation of a 35S enhancer-dependent gene. Regarding the GTE BRD, the BRDs of GTE3 and GTE10 have been shown to interact with histone H3. The GTE3 BRD binding to acetylated histones, and likely GTE5 BRD, might be regulated by sumoylation.
CRC: chromatin-remodelling complex

GTE6 was shown to be involved in leaf development (Chua et al., 2005). Indeed, gte6 mutant displayed abnormally shaped leaves in mature leaves, and GTE6 over-expressing lines had elongated young leaves. It seems that the link between GTE6 and leaf shape control relies on the positive regulation by GTE6 of ASYMMETRIC LEAVES1 (AS1), a protein that controls leaf shape, and this may be due to the binding of GTE6 to AS1 promoter which correlates with an increase in histone acetylation at this promoter. Indeed, AS1 transcripts were more abundant in the GTE6 over-expressing line and less abundant in the RNAi knockdown lines and ChIP analyses revealed that GTE6 can bind AS1 promoter transcription starting site and these regions were hyperacetylated on histones H3 and H4 in the GTE6 over-expressing line compared to WT (Chua et al., 2005). Thus, GTE6 seems to regulate leaf shape via positive regulation of AS1 expression by creating a favourable environment for histone acetylation, a marker of active transcription.

Lastly, GTE10, also known as NPX1, was found to participate in the regulation of ABA-related responses (Kim et al., 2009). GTE10 is up-regulated under stress and ABA treatment, and plants mutated or over-expressing GTE10 showed altered responses to ABA compared to WT plants. Indeed, the GTE10 over-expressing line was less sensitive to ABA as seed germination and root growth of 6-day-old seedlings were not impaired on a medium containing ABA, and ABA treatment did not induce stomatal closure contrary to WT plants. On the other hand, gte10 mutant is more sensitive to ABA suggesting that GTE10 is a negative regulator of ABA-mediated responses. Moreover, GTE10 over-expressing lines are less tolerant to drought stress and conversely, gte10 mutant is more resistant, which correlates with the fact that GTE10 over-expression alters stomatal closure. In addition, GTE10 seems to be a negative regulator of ABA-related responses notably by regulating ABA-related genes expression, including TFs (Kim et al., 2009).

In conclusion, members of the GTE family appear to be involved in various biological processes ranging from seed germination, cell cycle regulation, to leaf development and ABA signalling (Figure 17). However, for most of them, their role and their molecular functions remain elusive as studies have mainly focused on

phenotypical characterisation of mutants. For others, some more details are available regarding their molecular function or regulation, which will be described below.

- **B.2. Nuclear activities of particular GTE proteins**

As BRD-containing proteins, GTE members are expected to be nuclear proteins, and this has been demonstrated for GTE1, GTE6 and GTE10 (Chua et al., 2005; Duque and Chua, 2003; Kim et al., 2009). Moreover, two GTE members, GTE10, and GTE3, have been shown to interact with acetylated histone H3, suggesting that their BRD is functional to recognise acetylated histone tails (Figure 17) (Garcia-Dominguez et al., 2008; Nie et al., 2019).

From a mechanistic point of view, the molecular functions of GTE6 and GTE10 have been described in more detail (Figure 17) (Chua et al., 2005; Kim et al., 2009; Nie et al., 2019). As described in the precedent section, GTE6-mediated regulation of leaf development has been shown to be linked to AS1 up-regulation promoted by histone acetylation on AS1 promoter (Chua et al., 2005). Regarding GTE10-mediated regulation of ABA-related responses, it seems that GTE10 is involved in the up- or down-regulation of various ABA-related genes since the GTE10 over-expressing line showed differential expression of several ABA-responsive genes, including TFs and genes involved in ABA biosynthesis (Kim et al., 2009). Furthermore, as with some animal BRD-containing proteins, GTE10 has been shown to interact with a NAC TF called TIP, and to be a transcriptional repressor of this TF when co-expressed in yeast. Thus, it seems that GTE10 negatively regulates the ABA signalling pathway by acting notably as a transcriptional repressor of ABA-inducible TFs (Kim et al., 2009).

Recently, another role of GTE10 has been described, related to chromatin remodelling and incorporation of the histone variant H2A.Z. GTE10 has been shown to associate with the chromatin remodelling complex SWR1, which mediates the incorporation of the histone variant H2A.Z into nucleosomes (Lei and Berger, 2020; Nie et al., 2019). Indeed, when screening for interacting partners of the SWR1

subunit PIE1 by pull-down assay, various proteins were identified including GTE10 (Nie et al., 2019). Interestingly, another BRD-containing protein, MBD9, interacts with PIE1 and seems to function redundantly with GTE10 as gte10 and mbd9 single mutants do not show a distinct phenotype from WT but a gte10/mbd9 double mutant displays longer roots than WT. This phenotype seems to depend on the BRD of GTE10 since complementation with GTE10 restores the WT phenotype but complementation with a version of GTE10 that encodes a GTE10 version mutated in its BRD does not. Also, the gte10/mbd9 double mutant exhibited increased DNA methylation, similar to mutants defective in subunits of the SWR1 chromatin remodelling complex or in components involved in DNA demethylation, suggesting that these two BRD-containing proteins antagonise transcriptional silencing and prevent hypermethylation of specific genomic regions (Nie et al., 2019). Since GTE10 interacts with a subunit of the SWR1 remodelling complex, and in the gte10/mbd9 double mutant DNA methylation is increased and H2A.Z deposition at DNA hypermethylated loci is decreased, it is hypothesised that GTE10 and MBD9, by recognising acetylated histones can recruit the SWR1 chromatin remodelling complex at specific methylated genomic loci, leading to the incorporation of H2A.Z in the nucleosomes which can lead to DNA demethylation (Nie et al., 2019).

Finally, although the biological function of GTE3 and GTE5 remains unknown, a study related a way to regulate the binding of these GTEs to acetylated histones (Garcia-Dominguez et al., 2008). Indeed, GTE3 and GTE5 have been demonstrated to interact with an Arabidopsis SUMO ligase SIZ1 by Y2H. Of the twelve members from this family in Arabidopsis, only GTE3 and GTE5 interacted with SIZ1 in yeast and were sumoylated by SIZ1 in vitro. Interestingly, after showing that GTE3 binds to acetylated histone H3, sumoylation of GTE3 led to the alteration of GTE3 binding to acetylated histone H3 in vitro, suggesting that sumoylation could be a way to regulate GTE3 binding to acetylated histone H3, and likely for GTE5 which also binds acetylated histone H3 (Figure 17) (Garcia-Dominguez et al., 2008).

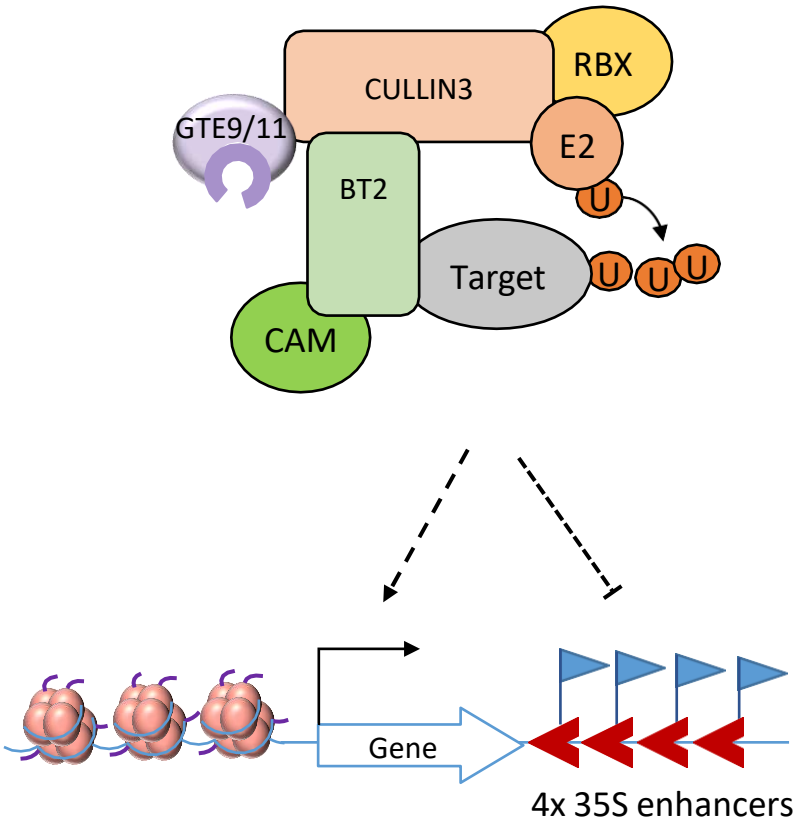


Figure 18. Hypothetical model: GTE9 and GTE11 participate in the regulation of multimerised 35S enhancers with BT2 (adapted from Irigoyen et al., 2021).
BT2 interacts with GTE9/GTE11 and with CULLIN3 to form a ubiquitin ligase complex. With the other components of the complex, BT2 may interact with and mark transcriptional regulators (target) for destruction by polyubiquitination or activation by monoubiquitination, resulting in reduced methylation of multimerised 35S enhancers, increasing their transcriptional activity. Other components of the ubiquitin ligase complex include UBIQUITIN (U), a UBIQUITIN conjugating enzyme (E2), a RING-box protein (RBX) that interact with CULLIN3 and CALMODULIN (CAM) protein. 35S enhancers are shown by red arrowheads, DNA methylation marks with blue flags.

- **B.3. GTE9 and GTE11 are negative regulators of sugar and ABA signalling pathways and involved in BT2 functions**

Few studies mention the role of GTE9 and GTE11 members, formerly called BET9 and BET10, in Arabidopsis but it seems that these GTEs are involved in BT2-mediated sugar and ABA responses (Figure 17) (Misra et al., 2018). Both GTE9 and GTE11 interact in vitro with BT2, a BTB/POZ and TAZ domain protein that is thought to be part of a ubiquitin ligase complex and mediates responses to various stresses (Figure 18) (Du and Poovaiah, 2004; Mandadi et al., 2009). Characterisation of the gte9 and gte11 mutants revealed similar phenotypic responses to bt2 mutant, i.e. greater sensitivity to ABA- and sugar-mediated inhibition of germination, and over-expression of BT2 in gte9 or gte11 mutants does not lead to resistance to ABA- and sugar-mediated germination inhibition in contrast to BT2 over-expression in the WT background, suggesting that both GTE9 and GTE11 are required for BT2 functions (Misra et al., 2018). Considering that GTE9 interacts with BT2 in vivo, that loss of function of GTE9 or GTE11 mimics the loss of BT2 in response to exogenous application of sugar or ABA by increasing ABA- and sugar-mediated inhibition of germination, it appears that GTE9 and GTE11 are negative regulators of sugar and ABA signalling pathways. In addition, GTE9 and GTE11 do not seem to affect the transcription of BT2 or ABA-responsive genes, suggesting that they may not play a role in regulating the transcription of these genes and/or that they act downstream in the signalling pathways (Misra et al., 2018). However, the molecular mechanisms underlying the role of GTE9 and GTE11 as negative regulators of ABA and sugar signalling pathways remain to be investigated.

Although GTE9 and GTE11 do not appear to be transcriptional regulators of the BT2-mediated ABA and sugar signalling pathways, this does not exclude them from acting as transcriptional regulators (Figure 17). Indeed, it has been reported in a yeast transcription activation assay that GTE11 might have a transcriptional activation activity (Du and Poovaiah, 2004). Moreover, GTE9 and GTE11 seem involved in BT2-mediated transcription activation of genes that depend on 35S

enhancers (Figure 18) (Irigoyen et al., 2021). Indeed, this study investigated the impact of BT2, GTE9 and GTE11 on the expression of YUCCA1, a gene involved in auxin biosynthesis, in the A. thaliana yucca1d line, which constitutively expresses YUCCA1 through a T-DNA insertion with four copies of the 35S enhancer near the YUCCA1 gene. By characterising crosses between yucca1d lines and mutants defective in BT2, GTE9 or GTE11, it was revealed that BT2, GTE9 and GTE11 were necessary for 35S-mediated activation of YUCCA1, meaning that GTE9 and GTE11 might be transcriptional regulators acting in complex with other transcription regulators such as BT2 (Irigoyen et al., 2021). However, whether there is a link between GTE9 and GTE11 role in ABA and sugar signalling pathways and their potential transcriptional regulatory activities remains unknown. It would also be interesting to investigate whether GTE9 and GTE11 have other transcriptional regulation activities and to find out which biological processes they are linked to.

Thus, even though the functional characterisation of the different members of the GTE family in Arabidospis remains limited, we have some insight into the different biological processes in which they participate, including ABA hormone signalling, regulation of germination, cell cycle and leaf shape. Interestingly, some of them, but not many, provide information on their molecular function and how they could contribute to the regulation of different biological processes. For instance, some GTEs interact with various nuclear processes including TFs, transcriptional regulators, or subunits of chromatin remodelling complexes, and others seem to be able to bind promoter and transcription starting regions. In any case, many of them seem to participate indirectly to the regulation of gene transcription, probably by promoting a transcriptionally active environment at specific loci, or by recruiting other nuclear components that could influence the transcriptional status of these specific regions. Also, some of the GTE members have been shown to bind acetylated histones via their BRD, suggesting that they are epigenetic readers like BET proteins in animals. In this context, GTE9 and GTE11, two GTE members

behaving as negative regulators of ABA and sugar signalling pathways, were found to be targeted by PopP2. These PopP2 targets have been the subject of preliminary studies in the team, notably by Alice Delga, a former PhD student, who started to characterise these PopP2 targets, work that I continued during my PhD.

C. Preliminary results: PopP2 targets GTE9 and GTE11, two histone readers that preferentially bind tetra-acetylated H4

Alice Delga worked on the characterisation of GTE11, a potential target of PopP2 identified from a Y2H cDNA library screening using PopP2 as bait. Alice also studied the potential targeting of GTE9 as it is the closest member of GTE11 in terms of their BRD protein sequences (Figure 16) (Delga, 2015).

During her PhD, Alice D. showed that :
- Both GTE9 and GTE11 co-localise with PopP2 or its catalytic mutant PopP2-C321A in A. thaliana or N. benthamiana nuclei.
- By using a FRET-FLIM assay performed in N. benthamiana, PopP2 was found to physically interact with GTE11 and GTE9.
- Also, PopP2 and PopP2-C321A were found to promote the protein accumulation of GTE9 and GTE11 in planta, as previously observed with some WRKY TFs (Le Roux et al., 2015).
- immunoprecipitation of Lys-acetylated proteins revealed that GTE9 and GTE11 co-expressed in planta with active PopP2 are modified by acetylation. These two proteins therefore behave as additional substrates of PopP2.
- a SPOT peptide array assay performed with the BRD of GTE9 expressed as a recombinant protein revealed its preferential binding to tetra-acetylated histone H4 and that this binding was dependent on the integrity of the conserved Asparagine residue present in the BRD of GTE9 in position 214 (N214).
- The ability of GTE9 and GTE11 to interact with histone H4 was further validated in planta using FRET-FLIM assay. Strikingly, GTE9-N214A and GTE11-N206A mutants, both mutated in the conserved N residue of their BRD, were unable to

physically interact with histone H4, confirming the critical role of this residue for BRD function, as previously described in bromodomain-containing animal proteins.

Thus, at this stage, we knew that PopP2 could target the BRD-containing proteins GTE9 and GTE11, which seem to behave as histone readers since they interact with histone H4 (Figure 19). Alice started to investigate the role of GTE9 and GTE11 in the plant response to the GMI1000 strain and to see whether GTE9 and GTE11 were the only GTEs targeted by PopP2. It was in this context that I started my PhD which aimed at further characterise the targeting of GTE9 and GTE11 by PopP2.

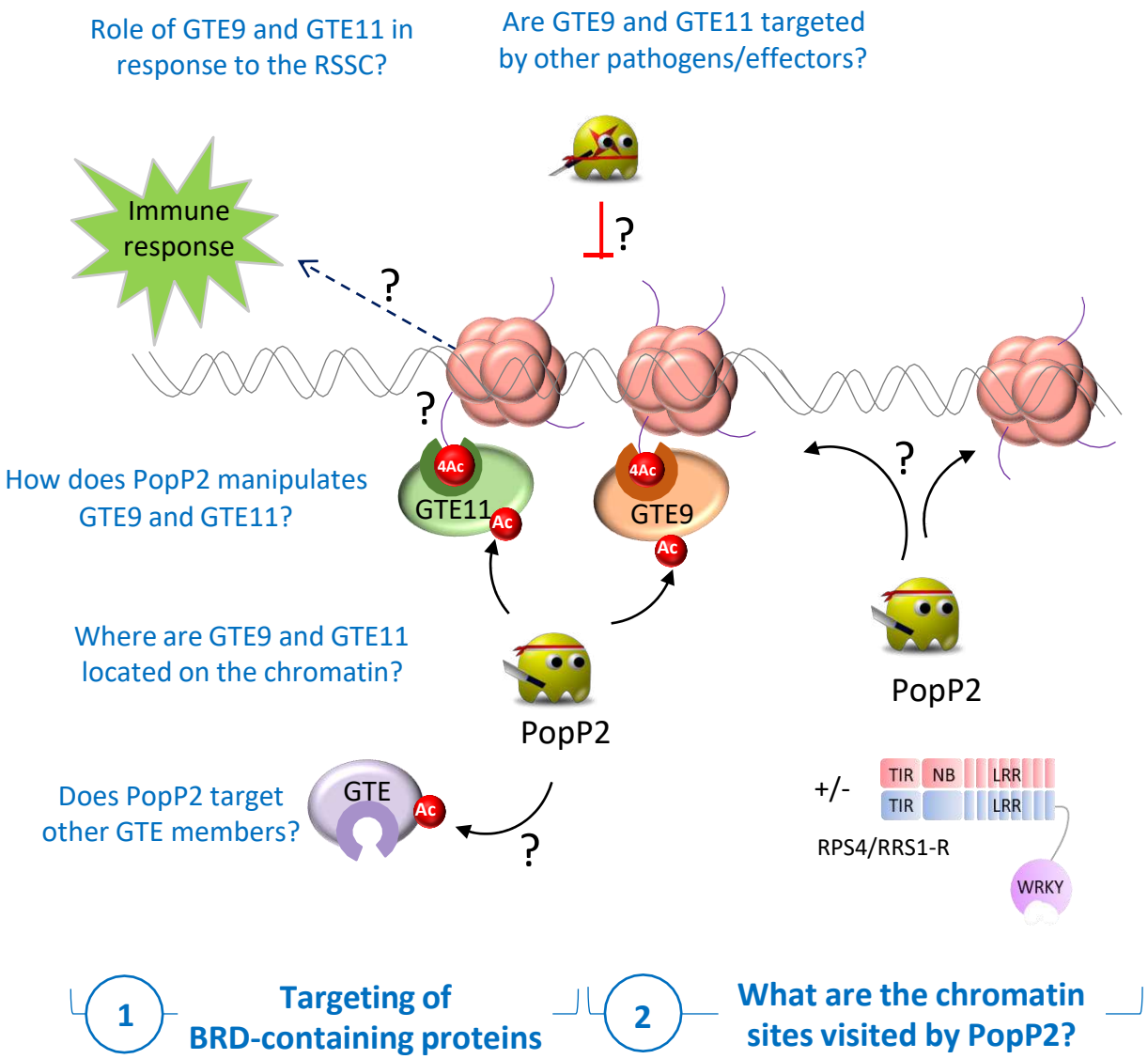


Figure 19. How does PopP2 target the histone readers GTE9 and GTE11?
Previous work carried by former PhD student Alice Delga allowed to know that PopP2 targets the BRD-containing proteins GTE9 and GTE11 by acetylation, that these GTEs interact with H4 and that their BRDs have preferential binding to tetra-acetylated H4, suggesting that they behave as histone readers. My PhD project continued to characterise the manipulation of GTE9 and GTE11 by PopP2 but also tried to study which chromatin sites are visited by PopP2 in plants that possess or not the immune receptor pair of proteins RPS4/RRS1-R.

PhD project

My PhD project aimed at further characterising the targeting of the A. thaliana BRD-containing proteins GTE9 and GTE11 by the RSSC T3E PopP2. This project was structured around two axes. The main axis was to follow up on Alice Delga's work, to determine how PopP2 can manipulate GTE9 and GTE11, whether these proteins play a role in the plant response to the RSSC, but also to better characterise their function. The second axis aimed at studying the chromatin sites visited by PopP2 in Arabidopsis, in the presence or not of the RPS4/RRS1-R immune receptor complex (Figure 19).

In the first axis, I tried to answer different questions to further characterise the targeting of GTE9 and GTE11 by PopP2:

- Does PopP2 manipulate GTE9 and GTE11, likely by altering their association with histone H4?
- Do GTE9 and GTE11 function as virulence targets playing a role in the plant response to the RSSC?
- Can PopP2 target other members of the GTE protein family?
- Can GTE9 and GTE11 be targeted by other effectors or pathogens?
- What is the role of GTE9 and GTE11 at the chromatin level?

The second axis will aim at investigating which chromatin sites are visited by PopP2 by developing molecular and genetic tools which will presented and discussed.

Results and Discussion

Chapter 1: PopP2 manipulates the BRD-containing proteins GTE9 and GTE11 to promote infection

Prior to Alice Delga's and my PhD, GTE11 had been identified as a potential target of PopP2 in a Y2H screening using PopP2 as a bait against a cDNA library from A. thaliana seedlings. Alice Delga started to investigate the targeting of GTE11 by PopP2, and of GTE9, its closest member regarding the BRD protein sequence, and the potential role of GTE9 and GTE11 as histone readers (Delga, 2015).

Although Alice D. generated a large amount of data, there was still a lot of uncertainty about the manipulation of GTE9 and GTE11 by PopP2 and their possible involvement in the plant response to the RSSC GMI1000 strain. I therefore continued the work already undertaken and the data obtained during my thesis were integrated into a manuscript that will be (re)submitted very soon (Comorge et al., in prep.). This manuscript is included in this Chapter 1.

To summarise the results shown in the article, many of which are the result of my thesis work, we show that PopP2 co-localises and interacts with GTE9 and GTE11 in plant cell nuclei in N. benthamiana. In addition, GTE9 BRD was found to bind preferentially to tri- and tetra-acetylated Histone H4 in vitro. This interaction with Histone H4 was further confirmed in vivo, as GTE9 and GTE11 interact with Histone H4. This interaction depends on the integrity of the BRD of GTE9 and GTE11 since substitution of the conserved asparagine residue, known to be responsible for BRD recognition of acetylated lysine residues in animal BRD-containing proteins, abrogated this interaction with Histone H4. A mass spectrometry-based proteomic analysis of GTE9 and GTE11 demonstrated both proteins behave as PopP2 substrates since they are both modified by PopP2 acetyltransferase activity on several lysine residues, some of which are located on either side of their BRD. In addition, by using a semi-quantitative FRET-FLIM assay, we demonstrated that PopP2 alters the binding of GTE9 to Histone H4 in vivo and this depends on its acetyltransferase activity. We also demonstrated that GTE9 and GTE11 over-expressing lines are more susceptible to the RSSC strain GMI1000, suggesting that

GTE9 and GTE11 are PopP2 virulence targets whose manipulation promote pathogen infection. Finally, we also showed that PopP2 can modify other members of the GTE family by acetylation (Comorge et al., in prep.). For greater clarity, I specify hereafter the parts of this article to which I contributed.

My contribution to the results presented in the article ranges from the result showing the alteration of GTE9 binding to H4 by PopP2, to the targeting of different GTE members by PopP2. In details, I developed in collaboration with the TRI imaging platform on campus molecular tools aimed at monitoring in living plant cells how PopP2 could alter GTE9 binding to H4 via its acetyltransferase activity. I also generated all the data related to the involvement of GTE9 and GTE11 in the plant response to the RSSC, as virulence targets and likely not involved in the RPS4/RRS1-R-mediated immunity. Finally, I also participated in the fine analysis of the mass spectrometry-related data, which allowed the precise identification of the Lys residues in GTE9 and GTE11 that are acetylated by PopP2.

Manuscript Comorge et al., in prep.

A bacterial pathogen manipulates Arabidopsis BET bromodomain-containing epigenetic readers using a YopJ family acetyltransferase

Authors

Virginie Comorge[1,†], Alice Delga[1,†], Gaëlle Huet [1], Cécile Pouzet[2], Alain Jauneau[2], Alexandra Kraut[3], Marbella Fonseca[4,5,6], Stefan Knapp[4,5], Yohann Couté[3], and Laurent Deslandes[1,*]

Affiliations

[1]Laboratoire des Interactions Plantes-Microbes-Environnement (LIPME), INRAE, CNRS, Université de Toulouse, F-31326 Castanet-Tolosan, France

[2]FRAIB-TRI Imaging Platform Facilities, FR AIB, Université de Toulouse, CNRS, 31320 Castanet-Tolosan, France

[3]Univ. Grenoble Alpes, INSERM, CEA, UMR BioSanté U1292, CNRS, CEA, FR2048 38000, Grenoble, France

[4]Institute of Pharmaceutical Chemistry, Goethe University, Max-von-Laue-Strasse 9, 60438 Frankfurt am Main, Germany

[5]Structural Genomics Consortium, Buchman Institute for Life Sciences, Goethe University, Max-von-Laue-Strasse 15, 60438 Frankfurt am Main, Germany

[6]Laboratório de Biologia Molecular e Genômica, Universidade Federal do Rio Grande do Norte, Natal, Brazil

[†]Co-first author

[*]Correspondence: laurent.deslandes@inrae.fr

Summary

Microbial pathogens have developed sophisticated strategies to defeat host immune responses and promote infection, among which manipulation of host epigenetic-related processes plays a prominent role. Here, we identify GTE9 and GTE11, two Arabidopsis Bromodomain-containing proteins with an extra-terminal domain (BET), as substrates of the YopJ family PopP2 acetyltransferase from the soil-borne bacterium *Ralstonia solanacearum*. We show that GTE9 and GTE11 function as epigenetic readers that interact in planta with histone H4 through their bromodomain. PopP2 acetylates several lysine residues flanking the bromodomain of GTE9 and GTE11 and, remarkably, alters the ability of GTE9 to bind histone H4 in vivo. Moreover, bacterial manipulation of GTE9 and GTE11 transgenically overexpressed in Arabidopsis promotes wilt disease development in a PopP2-dependent manner. Taken together, our study highlights a virulence strategy employed by a bacterial plant pathogen that manipulates host epigenetic readers using a YopJ family acetyltransferase.

Keywords

typeIII effector, acetyltransferase, bromodomain, Ralstonia solanacearum

INTRODUCTION

Upon interaction between plants and pathogenic bacteria, conserved pathogen-derived molecules called Pathogen Associated Molecular Patterns (PAMPs) are recognised by plant Pattern Recognition Receptors and trigger basal immune responses known as PAMP-Triggered Immunity (PTI) (Bigeard et al., 2015; Mott et al., 2014). PTI involves a signalling cascade leading to the induction of defence-responsive genes preventing host infection. However, PTI can be inhibited by virulence factors called effectors that are injected into host cells by adapted pathogens. PTI inhibition by such virulence strategies is called Effector-Triggered Susceptibility (ETS). To counteract these virulence strategies, plants have evolved immune receptors (resistance proteins encoded by *R* genes) that can specifically detect directly or indirectly effector activities and trigger a stronger activation of immune responses called Effector-Triggered Immunity (ETI) (Cui et al., 2015). This ETI is often associated with a programmed cell death (hypersensitive response) that restricts the pathogen growth around the site of infection.

A major consequence of both PTI and ETI signalling is a rapid and massive transcriptional reprogramming with overlapping sets of defence-related genes differing both in kinetics and intensity (Cui et al., 2015; Katagiri and Tsuda, 2010; Thomma et al., 2011). In Arabidopsis, these transcriptional changes involve epigenetic modifications in chromatin composition and remodelling (Ding and Wang, 2015; Latrasse et al., 2017; Ramirez-Prado et al., 2018b, 2018a; Zhu et al., 2016b). From a bacterial pathogen's perspective, subversion of host epigenome represents a potent virulence strategy to take control of gene expression. Several animal bacterial pathogens (e.g., *Listeria monocytogenes*, *Chlamydia trachomatis* or *Shigella flexneri*) target the host epigenome by interfering with the fine-tuned regulations of chromatin modifications (Dong and Hamon, 2020; Silmon De Monerri and Kim, 2014). They can favour infection by inducing histone modifications to alter the accessibility of gene promoters (Bierne and Cossart, 2012; Rolando et al., 2015).

Although host epigenome modification by animal pathogens is well documented, such mechanisms remain poorly characterised in plants. To date, only few examples of pathogen interference on epigenome reprogramming have been reported, among which suppression of small RNA silencing by the oomycete *Phytophthora sojae* (Xiong et al., 2014) and inhibition of defence responses through modulation of histone methylation by Geminiviruses (Castillo-González et al., 2015). The *P. sojae* PsAvh23 effector prevents the formation of a Histone Acetyltransferase complex required for immunity activation (Kong et al., 2017). These rare

examples highlight effective counter-defence mechanisms used by plant pathogens to suppress activation of host defence genes by interfering with epigenetic-related processes. However, while plant bacterial pathogens can modulate host cell reprogramming by interfering with various transcriptional and posttranscriptional regulation processes, whether they also subvert the host epigenome remains elusive.

Here, we investigated the nuclear activities of the type III effector PopP2 expressed by the root-infecting *Ralstonia solanacearum*. PopP2 belongs to the YopJ family of acetyltransferases (Deslandes et al., 2003; Tasset et al., 2010). PopP2 dampens basal immune responses by acetylating a key lysine residue in the WRKY DNA-binding domain of WRKY defensive transcription factor, thereby inhibiting their transactivating functions needed for defence gene expression (Le Roux et al., 2015; Sarris et al., 2015). In Arabidopsis, PopP2 is recognised by the NLR pair RPS4/RRS1-R (RESISTANCE TO *PSEUDOMONAS SYRINGAE*4/RESISTANCE TO *RALSTONIA SOLANACEARUM*1) that molecularly interact to form a pre-activation complex that is activated by PopP2 acetylation of RRS1-R WRKY domain behaving as an integrated decoy mimicking PopP2 true virulence targets (Le Roux et al., 2015; Sarris et al., 2015; Williams et al., 2014). For better understanding of PopP2 nuclear activities promoting its virulence functions, we searched for additional host components targeted by this bacterial effector. By yeast two-hybrid, we isolated GTE11 (Global Transcription Factor with Extra-terminal domain 11) as a PopP2-interacting partner candidate. GTE11 belongs to the Bromodomain and Extra-Terminal domain (BET) family also referred to as the Global Transcription Factor with Extra-terminal domain (GTE) family (Pandey et al., 2002). Bromodomains are conserved structural modules that bind acetylated lysine residues. They can serve as epigenetic readers by recognising specific acetylated lysine motifs on histone tails. Here, we showed that GTE11 and its closest homolog GTE9 interact with PopP2 in the plant nucleus. PopP2 acetylates several lysine residues located on either side of GTE9 and GTE11 bromodomain that binds histone H4 *in vivo*. Remarkably, enzymatically active PopP2 affects GTE9 binding to histone H4, indicating that PopP2 uses acetylation to regulate the association of GTE9 to chromatin. Moreover, transgenic overexpression of GTE9 and GTE11 in Arabidopsis enhances bacterial wilt disease development in a PopP2-dependent manner. This study identifies a virulence strategy employed by a plant pathogenic bacterium that promotes infection through manipulation of BET bromodomain-containing proteins using a typeIII-secreted acetyltransferase.

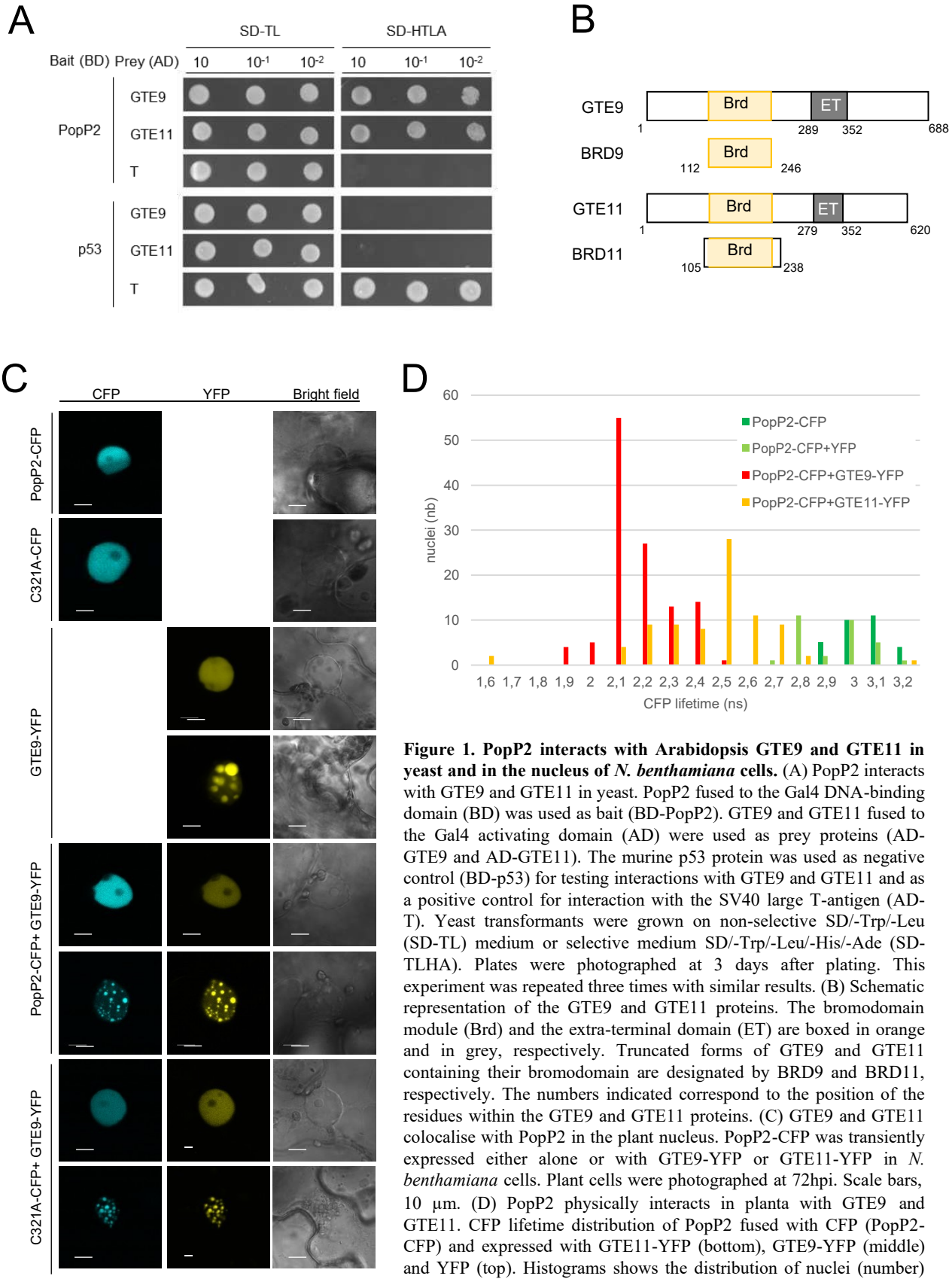


Figure 1. PopP2 interacts with Arabidopsis GTE9 and GTE11 in yeast and in the nucleus of *N. benthamiana* cells. (A) PopP2 interacts with GTE9 and GTE11 in yeast. PopP2 fused to the Gal4 DNA-binding domain (BD) was used as bait (BD-PopP2). GTE9 and GTE11 fused to the Gal4 activating domain (AD) were used as prey proteins (AD-GTE9 and AD-GTE11). The murine p53 protein was used as negative control (BD-p53) for testing interactions with GTE9 and GTE11 and as a positive control for interaction with the SV40 large T-antigen (AD-T). Yeast transformants were grown on non-selective SD/-Trp/-Leu (SD-TL) medium or selective medium SD/-Trp/-Leu/-His/-Ade (SD-TLHA). Plates were photographed at 3 days after plating. This experiment was repeated three times with similar results. (B) Schematic representation of the GTE9 and GTE11 proteins. The bromodomain module (Brd) and the extra-terminal domain (ET) are boxed in orange and in grey, respectively. Truncated forms of GTE9 and GTE11 containing their bromodomain are designated by BRD9 and BRD11, respectively. The numbers indicated correspond to the position of the residues within the GTE9 and GTE11 proteins. (C) GTE9 and GTE11 colocalise with PopP2 in the plant nucleus. PopP2-CFP was transiently expressed either alone or with GTE9-YFP or GTE11-YFP in *N. benthamiana* cells. Plant cells were photographed at 72hpi. Scale bars, 10 µm. (D) PopP2 physically interacts in planta with GTE9 and GTE11. CFP lifetime distribution of PopP2 fused with CFP (PopP2-CFP) and expressed with GTE11-YFP (bottom), GTE9-YFP (middle) and YFP (top). Histograms shows the distribution of nuclei (number) according to PopP2-CFP lifetime classes in the absence (green bars) or presence (orange and red bars) of the co-expressed YFP fusion proteins. The scanned nuclei correspond to those used for FRET-FLIM measurements presented in Supplementary Table 1.

RESULTS

PopP2 interacts with GTE11 and GTE9 in yeast

To progress in the elucidation of the functions of PopP2 *in planta*, we searched for Arabidopsis components interacting with PopP2. To this end, we performed a yeast-two-hybrid (Y2H) screening using PopP2 as bait for a prey cDNA library derived from Arabidopsis Col-0 seedlings. We identified one positive prey clone (designated as Pip8 for PopP2-interacting partner 8) corresponding to a partial cDNA encoding for the Arabidopsis protein GTE11 protein (At3g01770, also designated by BET10) lacking its 26 N-terminal residues. The full-length GTE11 cDNA that encodes for a protein of 620 amino acids was amplified and used as a prey clone to reconfirm interaction with PopP2 in Y2H (Fig 1A-B). GTE11 (for General Transcription factor with an Extra-terminal domain 11) belongs to a family of 12 members in Arabidopsis (GTE1 to GTE12). Also referred as Bromodomain and Extra Terminal domain (BET) proteins, GTEs are characterised by the presence of two types of domains, the bromodomain (Brd) and the extra-terminal domain (ET) (Fig 1B). Present in a broad range of nuclear proteins comprising histone acetyltransferases, methyltransferases, helicases, transcriptional coactivators or nuclear-scaffolding proteins, BRDs are protein modules that exclusively recognise acetylation motifs (Muller et al., 2011). As previously published (Airoldi et al., 2010), a phylogenetic analysis of the GTE members indicates that GTEs can be divided into three groups with GTE11 belonging to a group with four yet uncharacterised *GTE* genes (GTE9, GTE8, GTE10, and GTE12). This group includes the GTE9 protein whose sequence has 63% identity and 72% similarity with GTE11. Since GTE9 represents the closest paralog of GTE11, we therefore included GTE9 in our study to determine whether it might also be targeted by PopP2. Use of full-length GTE9 cDNA (encoding for a protein of 688 amino acids) as prey clone in yeast-two-hybrid revealed it can also interact with PopP2 (Fig 1A-B).

GTE11 and GTE9 co-localise and physically interact with PopP2 in the plant cell nucleus

As bona fide interactors of PopP2, GTE9 and GTE11 were hypothesised to localise with PopP2 in the plant nucleus where they should interact. We first investigated the subcellular localisation of GTE9 and GTE11. As expected, GTE11 and GTE9 C-terminally fused with a yellow fluorescent protein (GTE9-YFP and GTE11-YFP, respectively) and transiently expressed in *N. benthamiana* were found to co-localise with PopP2-CFP as well as with catalytically inactive

PopP2-C321A-CFP in the plant nucleus (Fig 1C and Supp Fig1A-B). Note that in a certain proportion of nuclei (with a rate of about 20 to 50% depending on the experiments), PopP2-CFP or C321A-CFP were found to be relocalized in subnuclear foci containing GTE9-YFP and GTE11-YFP (Fig 1C and Supp Fig 1A).

The nuclear localisation of GTE11 and GTE9 and our Y2H data strongly suggest they can physically interact *in planta* with PopP2. To test this hypothesis, we used a Förster resonance energy transfer (FRET) analysed by fluorescence lifetime imaging microscopy (FLIM) approach aimed at monitoring protein interactions in living cells. PopP2-CFP was used as a FRET donor and transiently expressed in *N. benthamiana* cells either alone or with GTE11-YFP or GTE9-YFP, both fusion proteins serving as FRET acceptors. In case of interaction, the fluorescence lifetime of PopP2-CFP was expected to decrease significantly due to close proximity with its interacting partners fused with YFP, producing transfer of energy (FRET) between the donor (CFP) and acceptor (YFP) fluorophores. In the presence of GTE11-YFP or GTE9-YFP, a significant decrease of the PopP2-CFP lifetime was observed, indicating a physical interaction between the tested proteins (Fig 1D, Supp Table 1). Notably, these interactions could only be detected when the co-expressed proteins accumulated in subnuclear foci. To verify that the decrease in PopP2-CFP lifetime was not due to a change in PopP2 nuclear environment triggered by GTE9 or GTE11 or influenced by their nuclear over-accumulation, PopP2-CFP was co-expressed either with GTE11-3HA or YFP alone, the former being theoretically able to interact with PopP2 but unable to serve as acceptor and vice versa for the latter. No decrease in PopP2-CFP lifetime was observed neither upon co-expression with GTE11-3HA nor YFP in *N. benthamiana* (Supp Table 1), thus confirming the physical associations detected between GTE9 and GTE11 with PopP2 in the nucleus. The catalytically inactive PopP2-C321A mutant (PopP2-C321A-CFP) was found also to interact with these two GTE proteins, indicating that the main Cys321 catalytic residue of PopP2 is dispensable for the interaction (Supp Table 1). Note that similar FLIM-FRET measurements were obtained in *A. thaliana* seedlings, confirming the protein interactions detected in *N. benthamiana* used as a heterologous expression system (Supp Table 1).

PopP2 acetylates GTE9 and GTE11

Considering that GTE9 and GTE11 physically interact with PopP2 in the nucleus, these two therefore represent putative substrates of PopP2 acetyltransferase activity. We first hypothesise that, similar to what was previously observed with WRKY transcription factors (Le Roux et al.,

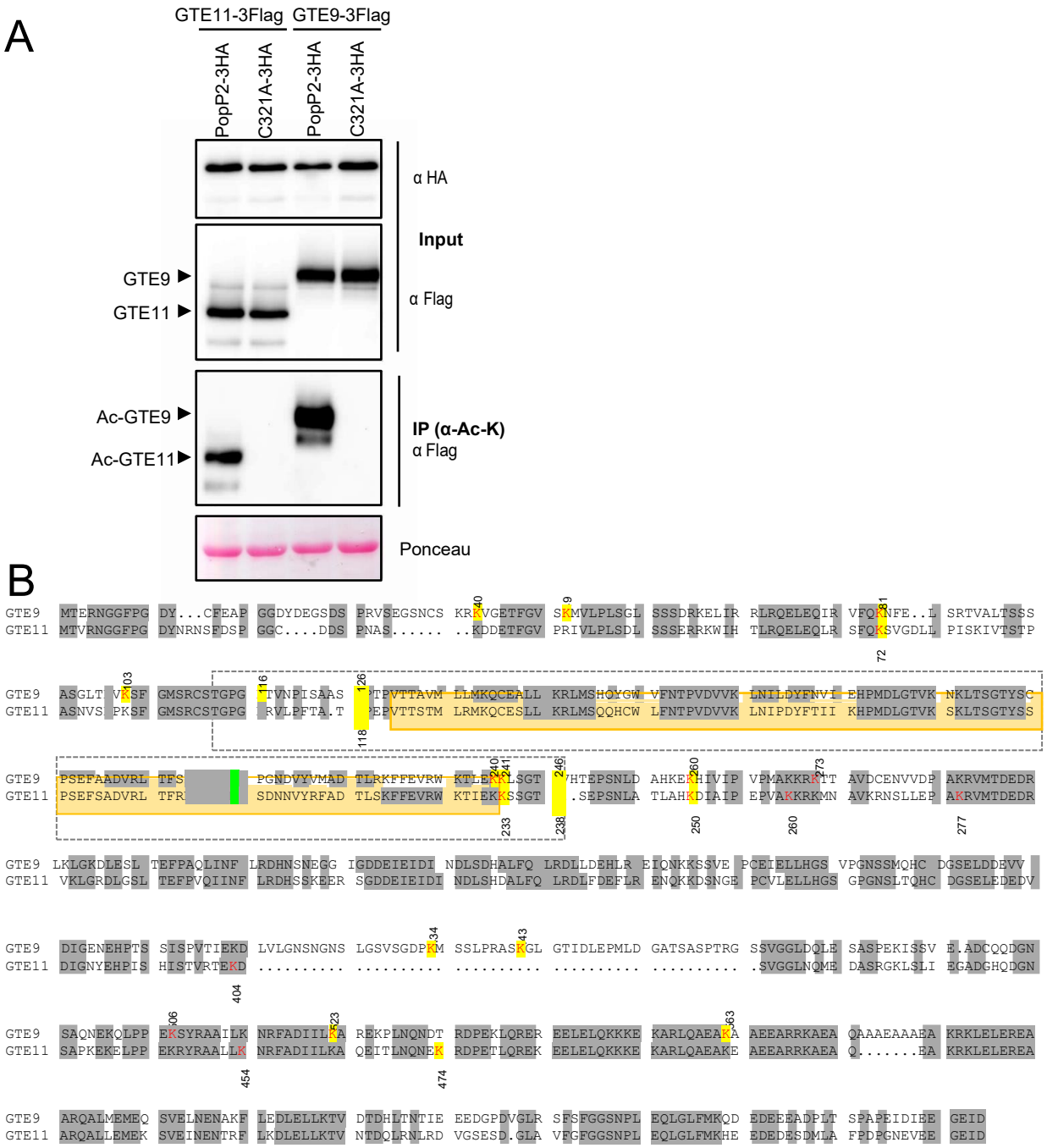


Figure 2. PopP2 acetylates GTE9 and GTE11. (A) GTE11-3Flag and GTE9-3Flag were transiently expressed with 3HA-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Samples were harvested at 48hpi. Protein extracts were immunoblotted with anti-Flag (α-Flag) and anti-HA antibodies (α-HA) (Input). Immunoprecipitation of Lys-acetylated proteins was done in presence of an anti-AcK antibody on protein A-agarose beads (IP α-Ac-K). The immuno-precipitated GTE9 and GTE11 proteins were detected after immunoblotting with α-Flag antibodies (Ac-GTE9 and Ac-GTE11, respectively). Ponceau S staining of total proteins indicates equal loading of the samples in the input. This experiment was repeated three times with similar results. (B) Mapping of the PopP2-acetylated lysine residues in GTE9 and GTE11 protein sequences. Conserved residues between GTE9 and GTE11 are shaded in grey. The bromodomain module of GTE9 and GTE11 is boxed in orange (BD^{GTE9}: position 130-240; BD^{GTE11}: position 122-232). The truncated forms of GTE9 and GTE11 containing the bromodomain are surrounded by a dotted line (BRD9: position 112-246 in GTE9; BRD11: position 105-238 in GTE11). The conserved Asp residue lying in the acetyl–lysine binding pocket of GTE9 and GTE11 bromodomain in boxed in green. The acetylated lysine residues reproducibly identified by MS-based proteomics in GTE9 and GTE11 in presence of active PopP2 are in red and boxed in yellow. The MS-based proteomic data used to design panel B are presented in Supplementary Tables 2 and 3.

2015; Sarris et al., 2015), PopP2 could also acetylate GTE9 and GTE11, likely on Lys residues. To check this hypothesis, GTE9 and GTE11 fused to a triple Flag epitope (GTE9-3Flag and GTE11-3Flag, respectively) were co-expressed transiently in *N. benthamiana* with triple hemagglutinin (HA) epitope-tagged PopP2 (PopP2-3HA) or the catalytically PopP2-C321A (PopP2-C321A-3HA) (Fig 2A). Total protein extracts were then subjected to immunoprecipitation using an anti-acetyl-lysine antibody. Immunoblotting of immune complexes captured on protein A-coupled agarose beads with anti-Flag antibodies led to the detection of GTE9-3Flag and GTE11-3Flag co-expressed with catalytically active PopP2 but not with PopP2-C321A, indicating that PopP2 causes acetylation of Lys residues in GTE9 and GTE11 (Fig 2A).

To identify the Lys residues of GTE9 and GTE11 modified by PopP2, purified GTE9 and GTE11 either fused to a 3Flag or a YFP tag and co-expressed with PopP2 or PopP2-C321A (Supp Fig 2) were used for mass spectrometry (MS)-based proteomic analysis. The Lys-acetylation sites identified with a localisation probability ≥ 75% in both 3Flag- and YFP-tagged proteins co-expressed with wild-type PopP2 are listed in Supp Table 2 (with the sequences of acetylated peptides presented in Supp Table 3). This analysis revealed the acetylation of 16 and 10 Lys residues in GTE9 and GTE11, respectively. Notably, of the five acetylated Lys residues shared by GTE9 and GTE11, three of them are located on either side of their Brd (position 130-240 for BD^{GTE9} and position 122-232 for BD^{GTE11}) (Fig 2B). Since PopP2 physically interacts with and acetylates multiple Lys residues of GTE9 and GTE11, these two GTE proteins therefore represent substrates of PopP2 enzymatic activity.

The bromodomain of GTE9 preferentially interacts with tri- and tetra-acetylated histone H4 N-terminal tails in vitro

PopP2 acetylation of conserved Lys residues in GTE9 and GTE11 and in particular at Lys residues flanking their bromodomain (designated Brd hereafter) was intriguing. We reasoned that this targeted Lys acetylation could reflect a pathogen virulence strategy aimed at interfering with the functions of these two proteins. In an attempt to elucidate the molecular properties of GTE9 and GTE11, we scrutinised their Brd to determine whether this protein module was capable of binding acetyl-Lys residues and if so, with what specificity.

The bromodomain is a conserved region of ~110 amino acids that structurally forms four α-helices (αZ, αA, αB, αC) and two variable loops that connect helices αZ–αA (ZA loop) and αB–αC (BC loop), shaping the acetyl–lysine binding pocket and thus, contributing to substrate

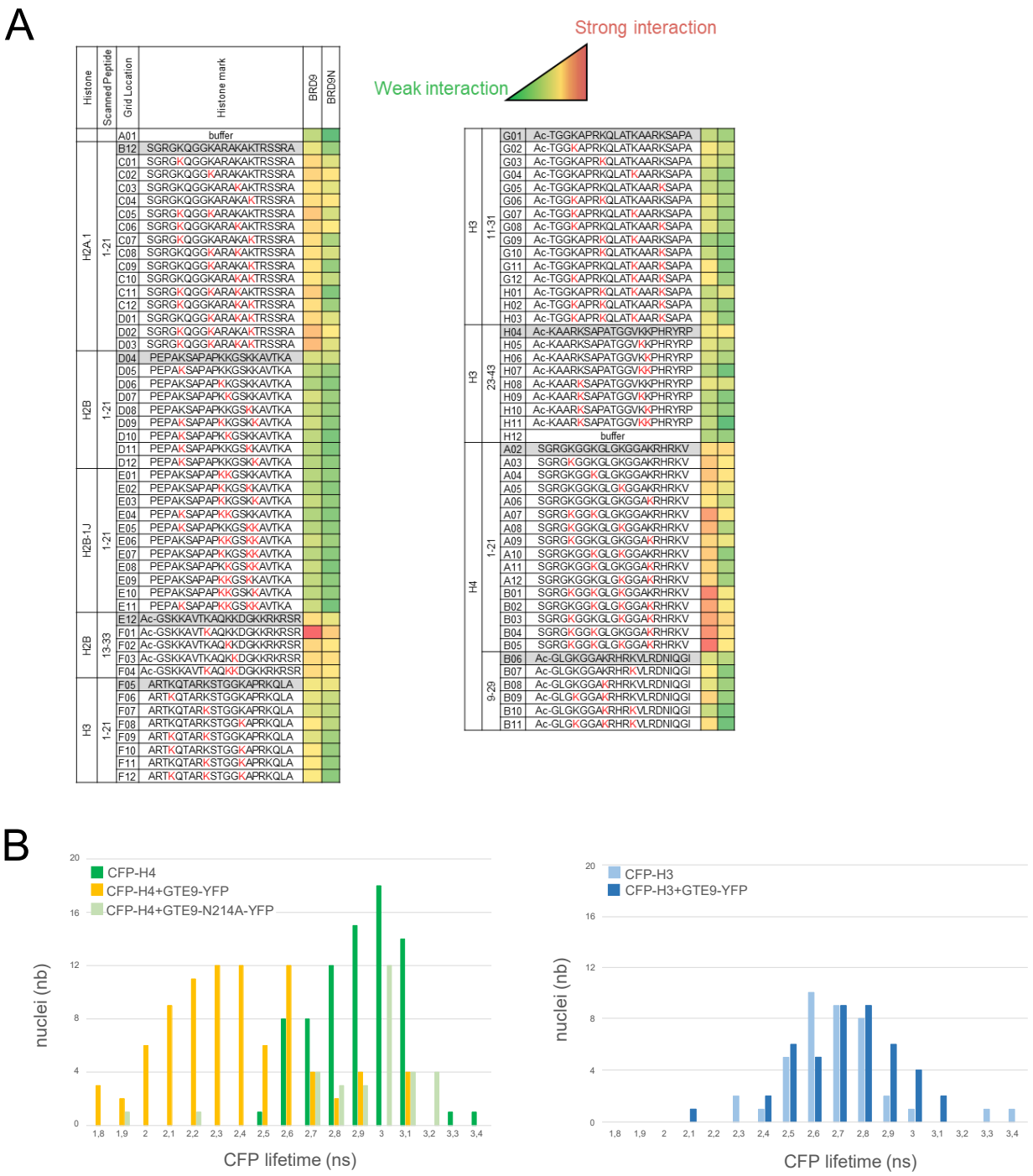


Figure 3. The bromodomain of GTE9 confers preferential binding to acetylated histone H4. (A) BRD9 binds preferentially to tetra-acetylated histone peptides from H4. A recombinant His_6-tagged protein corresponding to a truncated form of GTE9 containing its bromodomain (BRD9) was used for screening against an array of single/multiple acetylated peptides that cover all possible acetylation sites in H2A, H2B, H3 and H4. Non-acetylated peptides are indicated in grey. Values are representative of two independent experiments. The BRD9-N214A mutant (in which the conserved Asparagine residue of the acetyl-lysine binding pocket was substituted with an Alanine) was used as a negative control (6His-BRD9-N214A). See also Supplementary Table 4. (B) GTE9 binds to histone H4 *in vivo*, but not to histone H3. On the left, CFP lifetime distribution of histone H4 fused with CFP (CFP-H4) and transiently expressed either alone or with GTE9-YFP or GTE9-N214A-YFP. Histograms shows the distribution of nuclei (number) according to CFP-H4 lifetime classes in the absence (green bars) or presence of co-expressed GTE9-YFP (orange bars) or GTE9-N214A-YFP (light green bars). On the right, CFP lifetime distribution of histone H3 fused with CFP (CFP-H3) and transiently expressed either alone or with GTE9-YFP. Histograms shows the distribution of nuclei (number) according to CFP-H3 lifetime classes in the absence (light blue bars) or presence of co-expressed GTE9-YFP (dark blue bars).The scanned nuclei in (B) correspond to those used for FRET-FLIM measurements presented in Supplementary Table 1.

specificity (Dhalluin et al., 1999; Filippakopoulos et al., 2012) The *in silico* modelling of Brd^{GTE9} and Brd^{GTE11} using Phyre 2.0 revealed a strong homology with the solved crystal structure of second bromodomain of Brd2 (Brd2(2) hereafter) that belongs to the cluster II of human Brd families (Filippakopoulos et al., 2012). Overall, the structural elements of canonical Brds are well conserved in Brd^{GTE9} and Brd^{GTE11} modules (position 130 to 240 and position 122 to 232, respectively) (Supp Fig 3A). Notably, the canonical asparagine residue present in most Brds and that is required for the recognition of acetyl-lysine (Kac) residues (Owen et al., 2000) is also present in Brd^{GTE9} and Brd^{GTE11} modules (residues N214 in GTE9 and N206 in GTE11, respectively, see Fig 2B). Together, these structural data based on homology modelling strongly suggest that Brd^{GTE9} and Brd^{GTE11} are capable of interacting with acetyl-Lys residues, and more specifically with acetylated histone tails.

To identify potential substrate(s) of Brd^{GTE9} and Brd^{GTE11}, we used a SPOT peptide array approach that relies on the measurement of the affinity of recombinant Brds to all the possible combinations of acetylated histone tails. Because the four core histones (H2A, H2B, H3 and H4) are highly conserved across eukaryotes in terms of sequence and structure, we used histone-peptide arrays covering all possible Kac sites of the human histones and which have already been successfully used to identify interactions sites for a large sets of human Brds (Filippakopoulos et al., 2012). In this assay, truncated forms of GTE9 and GTE11 encompassing their Brd module (position 112-246 for GTE9 and position 105-238 for GTE11, hereinafter called BRD9 and BRD11, respectively, Figs 1B-2B) were subcloned into a bacterial expression system in frame with a N-terminal His_6 tag. We focused our investigation on His_6-BRD9 since His_6-BRD11 soluble form was more difficult to produce. As a negative control, we also produced the recombinant His_6-BRD9-N214A mutant in which the highly conserved N214 residue of GTE9 predicted to be necessary for interaction with Kac was substituted with an Alanine residue (Supp Fig 3B). Ni-NTA affinity purified His_6-BRD9 and His_6-BRD9-N214A were sequentially incubated on a spot peptide array containing the various combinations of acetylated histone peptides. We identified preferential binding of His_6-BRD9 with peptides corresponding to tri- and tetra-acetylated H4 (AcK5, AcK8 and AcK12 for tri-acetylated H4 and AcK5, AcK8, AcK12 and AcK16 for tetra-acetylated H4, respectively) (Fig 3A and Supp Table 4). On the contrary, the His_6-BRD9-N214A mutant showed a much reduced affinity for tri- and tetra-acetylated H4 peptides. These data demonstrate the functionality of the Brd module of GTE9 that preferentially binds to acetylated tails of histone H4 and, in particular, that the conserved residue N214 is required for these interactions, as previously described for yeast and human BRDs (Filippakopoulos et al., 2012) .

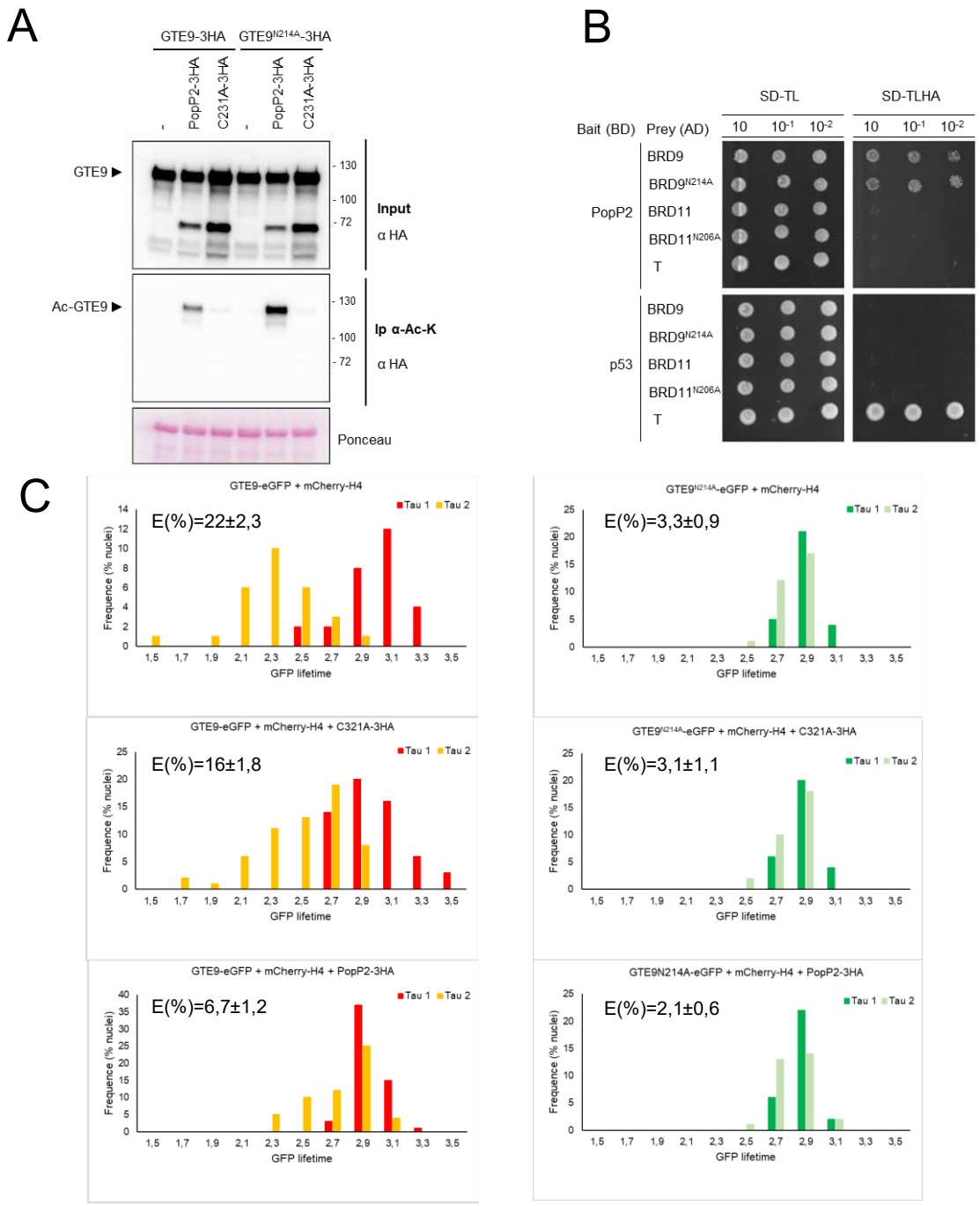


Figure 4. PopP2 targets the bromodomain of GTE9 and interferes with its ability to bind histone H4. (A) GTE9-3HA and GTE9-N214A-3HA were transiently expressed with 3HA-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Samples were harvested at 48hpi. Protein extracts were immunoblotted with anti-HA (α-HA) (Input). Immunoprecipitation of Lys-acetylated proteins was done in presence of an anti-AcK antibody on protein A-agarose beads (IP α-Ac-K). The immunoprecipitated GTE9 and GTE9-N214A proteins were detected after immunoblotting with an α-HA antibody. Ponceau S staining of total proteins indicates equal loading of the samples in the input. This experiment was repeated three times with similar results. (B) (A) PopP2 interacts with the bromodomain of GTE9 (BRD9). PopP2 fused to the Gal4 DNA-binding domain (BD) was used as bait (BD-PopP2). BRD9, BRD9-N214A, BRD11 and BRD11-N206A fused to the Gal4 activating domain (AD) were used as prey proteins. The murine p53 protein was used as negative control (BD-p53) for testing interactions with the different prey proteins and as a positive control for interaction with the SV40 large T-antigen (AD-T). Yeast transformants were grown on non-selective SD/-Trp/-Leu (SD-TL) medium or selective medium SD/-Trp/-Leu/-His/-Ade (SD-TLHA). Plates were photographed at 4 days after plating. This experiment was performed three times with similar results. (C) PopP2 alters the physical interaction between GTE9 and Histone 4. On the left, GFP lifetime distribution of GTE9 fused with eGFP (GTE9-eGFP) and transiently co-expressed with mCherry-Histone4 in absence or in presence of PopP2-3HA or C321A-3HA. Histograms shows the frequency of nuclei according to GTE9-eGFP lifetime classes with Tau1 corresponding to the lifetime of the free donor and Tau2 corresponding to the lifetime of the donor bound to the acceptor. The scanned nuclei in (C) correspond to those used for FRET-FLIM measurements presented in Supplementary Table 5.

GTE9 and GTE11 physically interact with histone H4 in planta

BRD11, given its high similarity with BRD9 (85.9%), most likely has similar binding capabilities to acetylated H4 peptides. To further validate these properties *in vitro* and to probe their biological relevance, we next examined whether the interaction of histone H4 with full-length GTE9 and GTE11 proteins could be recapitulated *in planta*.

As was successfully demonstrated for human Brds in living cells (Kanno et al., 2004), we performed a FRET-FLIM assay using H4 N-terminally fused with CFP (CFP-H4) and transiently expressed in *N. benthamiana* cells either alone or with GTE9-YFP and GTE11-YFP. As a FRET donor, CFP-H4 expressed alone displayed a mean CFP lifetime of 2.945 ns (Fig 3B, Supp Table 1). Co-expression of CFP-H4 with GTE9-YFP or GTE11-YFP led to a significant decrease of the CFP lifetime (2.432 ns and 2.455 ns, respectively). By contrast, no decrease of the CFP lifetime could be monitored when CFP-H4 was co-expressed either with GTE9-N214A-YFP or GTE11-N206A-YFP, indicating that these two mutant proteins were unable to physically interact with histone H4 (Fig 3B, Supp Table 1), confirming the interaction data obtained *in vitro* (Fig 3A). To determine the specificity of the interaction detected between histone H4 and GTE9 *in vivo*, we used histone H3 fused to CFP (CFP-H3) as control. The mean CFP lifetime of CFP-H3 expressed alone was not statistically different from that measured in the presence of GTE9-YFP, indicating that GTE9 did not interact with H3 (Fig 3B, Supp Table 1). This is consistent with our SPOT peptide array data that showed that His_6-BRD9 did not bind to acetylated H3 peptides (Fig 3A). Together, these results confirm the ability of GTE9 and GTE11 to interact with histone H4 *in vivo* and highlighted the critical role of the conserved Asp residue in their Brd module for this interaction.

PopP2 targets the Brd module of GTE9 and GTE11

We exploited the inability of GTE9-N214A and GTE11-N206A to bind H4 to determine whether the acetylation of GTE9 and GTE11 by PopP2 requires a functional Brd module. Thus, the GTE9-N214A and GTE11-N206A mutants were C-terminally fused with a 3HA tag and expressed either alone or with PopP2-3HA or C321A-3HA in *N. benthamiana*. Immunoprecipitations were carried out using an anti-Ac-K antibody and immunoblots were probed with an anti-HA. In the presence of PopP2, but not C321A, a signal corresponding to GTE9-N214A-3HA and GTE11-N206A-3HA proteins was detected, indicating these two mutants, despite their inability to interact with H4, were acetylated by PopP2 (Fig 4A and Supp Fig 4A). Interestingly, in a reproducible manner, the signal corresponding to the acetylated

forms of GTE9-N214A and GTE11-N206 was stronger than that obtained with wild-type GTE9 and GTE11 (Fig 4A, Supp Fig 4A). Furthermore, this signal intensity did not correlate to the accumulation level of the co-expressed proteins. One explanation is that PopP2 acetylation of GTE9 and GTE11 may be favoured when their bromodomain is unable to bind acetylated Lys residues, making it more easily accessible to PopP2. In such a scenario, we hypothesised that the Brd module of GTE9 and GTE11 is a target of PopP2, similar to the WRKY domain present in WRKY transcription factors that has been characterised as a direct substrate of PopP2 enzymatic activity (Le Roux et al., 2015; Sarris et al., 2015). As hypothesised, a physical interaction between PopP2 and BRD9 was detected in Y2H by PopP2 as bait and BRD9 as prey (AD-BRD9) (Fig 4B). This indicated that the Brd module of GTE9 was sufficient for interaction with PopP2. Unexpectedly, no interaction was detected between BRD11 and PopP2 in yeast. We next checked whether BRD9 was modified by PopP2 acetylation *in planta*. MS-based proteomic analysis of immuno-purified BRD9-YFP co-expressed with catalytically active PopP2 led to the identification of three acetylated Lys residues that were previously detected in full-length GTE9 (Supp Tables 2 and 3). In a similar assay performed on BRD11-N206A-YFP, these three Lys residues that are conserved between GTE9 and GTE11 were also found to be acetylated only in presence of active PopP2 (Supp Tables 2-3, Fig 2B). Together, our data demonstrated that PopP2 targets specific Lys residues at conserved positions on either side of the bromodomain of GTE9 and GTE11 ($GTE9^{K126/K241/K246}$ and $GTE11^{K118/K233/K238}$), and provided compelling evidence this functional domain represents a molecular target of PopP2.

PopP2 alters the ability of GTE9 to interact with Histone H4 in planta

Given that PopP2 acetylates several Lys residues located on either side of the bromodomain of GTE9, we hypothesised that PopP2 could interfere with its ability to interact with H4. To test this hypothesis, we used a FRET-FLIM imaging approach to quantitatively measure GTE9-histone H4 interactions in the nucleus of living plant cells in presence or absence of PopP2. The mono-exponential lifetime of the eGFP used as a donor allows the measurement of a FRET efficiency containing quantitative information about the fraction of the donor bound to the acceptor that can be directly extracted from FRET-FLIM data (Albertazzi et al., 2009) . GTE9-eGFP transiently expressed with mCherry-H4 in *N. benthamiana* led to a FRET efficiency of 22% (Fig 4C, Supp Fig 4B and Supp Table 5), indicating this FRET pair can reproduce the GTE9-histone H4 interaction previously detected (Fig 3B and Supp Table 1). In presence of PopP2, the FRET efficiency measured between GTE9-eGFP and mCherry-H4 dropped

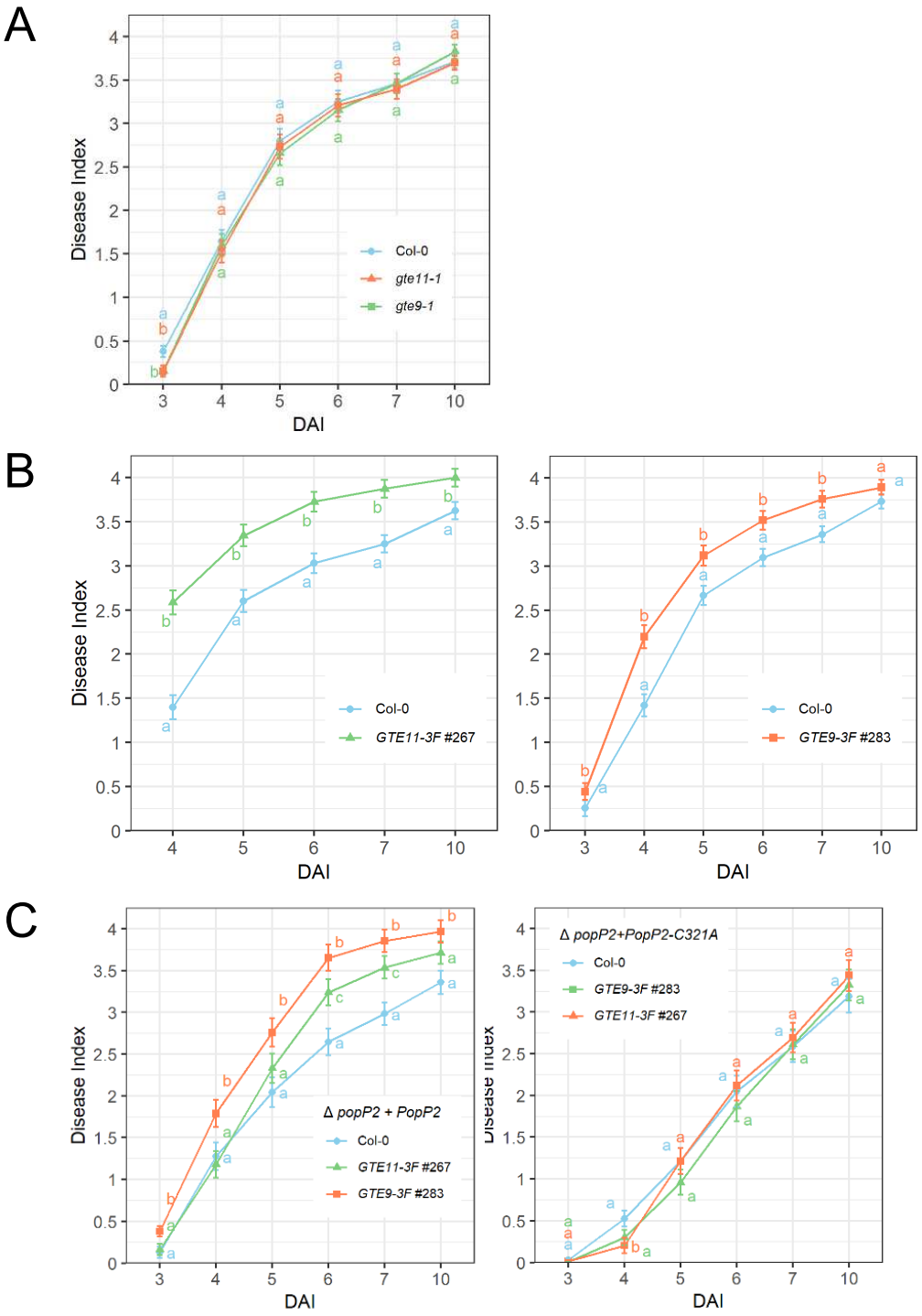


Figure 5. Targeting of GTE9 and GTE11 by PopP2 promotes wilt disease development in Arabidopsis. (A) *gte9-1* and *gte11-1* null mutant are not affected in their phenotypical response the GMI1000 strain of *R. solanacearum*. The graph shows least-square means +/- Standard Error of the LS means from 4 independent inoculations of ~24 plants (n total = 93 plants for Col-0 and 96 plants for *gte9-1* and *gte11-1*). (B) Transgenic overexpression of *GTE11-3Flag* or *GTE9-3Flag* in Col-0 plants enhances the development of bacterial wilt disease symptoms. The graph shows least-square means +/- Standard Error of the LS means from 2 and 3 independent inoculations of ~24-32 plants for *GTE11-3F* #265 and *GTE9-3F* #283, respectively (n total = 88 plants for Col-0, 64 plants for *GTE11-3F* #265 and 82 plants for *GTE9-3F* #283). (C) The enhanced susceptible response of *GTE11-3Flag* and *GTE9-3Flag* overexpressing lines to GMI1000 strain is dependent on catalytically active PopP2. The graph shows least-square means +/- Standard Error of the LS means from 3 independent inoculations of ~24 plants respectively (inoculation with Δ*popP2+PopP2*: n total = 69 plants for Col-0 and 71 plants for *GTE11-3F* #265 and *GTE9-3F* #283; inoculation with Δ*popP2+PopP2-C321A*: n total = 66 plants for Col-0 and 72 plants for *GTE11-3F* #265 and *GTE9-3F* #283. In A, B, and C, a, b and c denote significant difference between two lines at a certain DAI (one-way ANOVA performed on the mixed model described in the methods, p-value < 0.05).

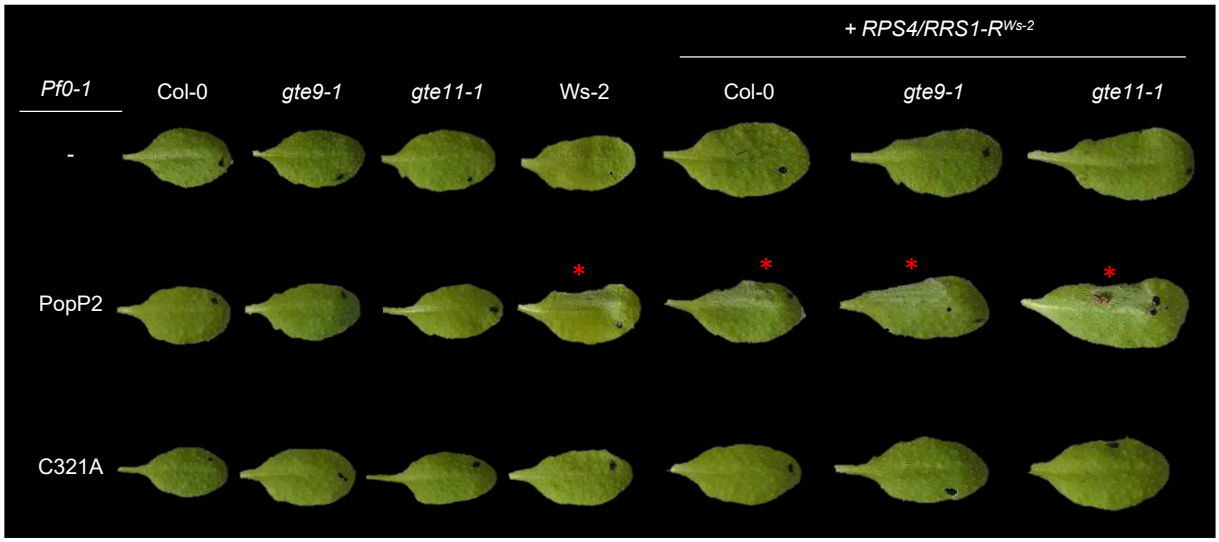
drastically to 6.6%, reflecting a significant decrease in the proportion of GTE9 interacting with histone H4 (p-value<0.05) (Fig 4C, Supp Table 5). Notably, this reduction is dependent on PopP2 enzymatic activity since only a slight reduction of FRET efficiency was observed with catalytically inactive PopP2-C321A, likely due to the binding of PopP2-C321A to GTE9 that could interfere, to a lesser extent than active PopP2, with the GTE9/H4 association. As control, we used GTE9-N214A which behaves as a PopP2 substrate but is unable to interact with histone H4 (Figs 4A and 3B). We thus checked that the GFP lifetime of GTE9-N214A-eGFP co-expressed with mCherry-H4 was not influenced by the presence of active PopP2 (Fig 4C, Supp Table 5). Together, these data demonstrate that active PopP2 affects the ability of GTE9 to interact with histone H4 *in vivo*, and therefore indicate that PopP2 uses acetylation to regulate GTE9 bromodomain histone-binding activity.

Overexpression of GTE9 and GTE11 contributes to PopP2-mediated virulence in Arabidopsis

Given that both GTE9 and GTE11 behave as epigenetic readers whose acetylation by PopP2 alters their association with Histone H4, they likely represent virulence targets of this bacterial effector. To determine whether GTE9 and GTE11 are required for PopP2-mediated virulence in Arabidopsis, we studied the phenotypical response of *gte9-1* and *gte11-1* knockout lines (previously described in Misra et al., 2018) to the *R. solanacearum* GMI1000 strain (Fig 5A). Note that these two mutant lines are in Col-0 susceptible background carrying the *RRS1-S* allele that is unable to recognise PopP2 and activate the RPS4/RRS1-R-dependent immunity. The altered gene expression of *GTE9* and *GTE11* in the corresponding null mutant lines was confirmed by RT-qPCR (Supp Fig 5A). Kinetic of wilting disease symptom development in *gte9-1* and *gte11-1* was indistinguishable from that of Col-0 control plants, indicating that loss of either *GTE9* or *GTE11* gene expression does not affect the plant response to the GMI1000 strain (Fig 5A). However, we cannot rule out that the lack of altered phenotypic response of *gte9-1* and *gte11-1* mutants to the GMI1000 strain may be due to functional redundancy between members of the GTE protein family.

Next, we studied the effect of the overexpression of *GTE9* and *GTE11* in Col-0. For this experiment, *GTE9* and *GTE11* cDNAs were fused either to a C-terminal 3Flag or 3HA epitope tag sequence for transgenic overexpression in Col-0. For each construct, one transgenic T2 line was selected for phenotypical characterisation. Overexpression of 3HA and 3Flag epitope-tagged GTE9 and GTE11 was confirmed by RT-qPCR and immunoblot analysis (Supp Fig 5B-

A



B

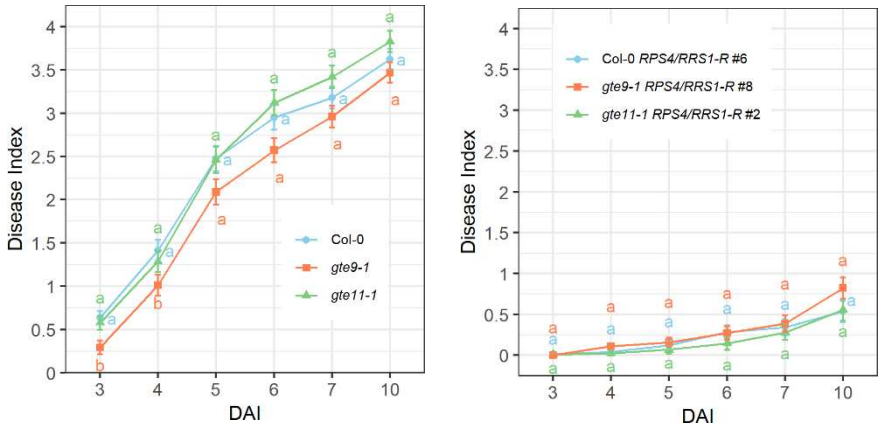


Figure 6. GTE9 and GTE11 are not required for RPS4/RRS1-R-mediated immunity. (A) *Pseudomonas fluorescens* (*Pf0-1*)-delivered wild-type PopP2 triggers a cell death response in Col-0, *gte9-1* and *gte11-1* plants transgenically expressing the *RPS4* and *RRS1-R* NLR genes from the Ws-2 ecotype. Leaves of four week-old Arabidopsis plants were infiltrated with *Pf0-1* strain delivering either PopP2 or PopP2-C321A or no effector. The photographs were taken at 48 hours post infection (hpi). The red * shows leaves with cell death response triggered by PopP2 recognition. This experiment was conducted three times with similar results. (B) The RPS4/RRS1-R-dependent immunity in response to the GMI1000 strain of *R. solanacearum* is not affected by *gte9-1* or *gte11-1* null mutations. Four week-old Col-0, *gte9-1* and *gte11-1* plants complemented with the *RPS4*/*RRS1-R* genomic clone from Ws-2 display a resistant phenotype upon root-inoculation with the GMI1000 strain of *R. solanacearum*, compared to that of wild-type Col-0, and untransformed *gte9-1* and *gte11-1* plants showing pronounced wilting disease symptoms. A disease score was attributed to every plant for 10 days after inoculation (DAI). 0 = no symptoms; 1 = 25% wilted leaves; 2 = 50% wilted leaves, 3 = 75% wilted leaves and 4 = 100% wilted leaves. The graph shows least-square means +/- Standard Error of the LS means from 3 independent inoculation assays. a and b denote significant difference between two lines at a certain DAI (one-way ANOVA performed on the mixed model described in the methods, p-value < 0.05). Left panel, *gte9-1* and *gte11-1* mutant lines in susceptible Col-0 background with ~24 inoculated plants in each independent experiment (n = 64 plants for Col-0, 69 plants for *gte9-1* and *gte11*-1). Right panel, *gte9-1* and *gte11-1* mutant lines complemented with the genomic clone *RPS4/RRS1-R* from the Ws-2 resistant accession, with ~24 inoculated plants in each independent experiment (n = 68 plants for Col-0, *gte9-1* and *gte11*-1).

D). After root-inoculation with the GMI1000 strain, the selected *GTE9* and *GTE11* overexpressing lines showed increased susceptibility compared to Col-0, indicating these two PopP2 targets can modulate the plant response to *R. solanacearum* (Fig 5B and Supp Fig 5E). To determine whether this increased susceptible response was dependent on PopP2 acetyltransferase activity, one representative 3Flag-tagged line for GTE9 and GTE11 was root-inoculated with a GMI1000 *popP2* knock-out strain expressing either wild-type PopP2 or the PopP2-C321A catalytic mutant (Δ*popP2+PopP2* and Δ*popP2+PopP2-C321A*, respectively) (Fig 5C). The GTE9-3Flag and GTE11-3Flag overexpressing lines remained more susceptible to Δ*popP2+PopP2* but responded to Δ*popP2+PopP2-C321A* similarly to wild-type Col-0 plants. This result demonstrates that the enhanced susceptibility of GTE9 and GTE11 overexpressing lines relies on PopP2 acetyltransferase activity, and is therefore biologically relevant to the virulence function of PopP2. Overall, these results indicate that GTE9 and GTE11 are virulence targets of PopP2 and whose manipulation promotes wilt disease development in Arabidopsis.

GTE9 and GTE11 are not involved in the RPS4/RRS1-R-dependent immunity triggered by PopP2

We also tested whether GTE9 and GTE11 could play a role in the PopP2-triggered immunity which involves the RPS4/RRS1-R NLR pair in Arabidopsis. Since the *gte9-1* and *gte11-1* null mutants are in Col-0 accession which carries the *RRS1-S* natural variant that does not trigger resistance to PopP2 (Deslandes et al., 2002), these two mutants were transformed with a genomic clone encompassing the *RPS4* and *RRS1-R* 5' and 3' regulatory sequences from the resistant Ws-2 accession (*RPS4/RRS1-R*$^{Ws-2}$). Wild-type PopP2 and its catalytic mutant PopP2-C321A were delivered in plant leaves through the TT3S of the non-pathogenic *Pseudomonas fluorescens Pf0-1* strain. In Ws-2, PopP2 but not its catalytic mutant triggers a cell death response which represents a proxy for activation of RPS4/RRS1-R-dependent immunity in Arabidopsis (Sohn et al., 2014). In Col-0, *gte9-1* and *gte11-1* plants transgenically expressing the RPS4/RRS1-R^{Ws-2} NLR pair, PopP2 delivered from *Pf0-1* triggered a cell death response similar to that observed in Ws-2, whereas the untransformed genotypes did not show any response (Fig 6A). This indicates that GTE9 and GTE11 are not genetically required for RPS4/RRS1-R-dependent immunity. Note that expression levels of transgenic RRS1-R^{Ws-2} was checked by RT-qPCR in the different genotypes considered (Supp Fig S6). We further tested if GTE9 or GTE11 may play a role in *Ralstonia*-delivered PopP2 recognition. To do so, the *gte9-*

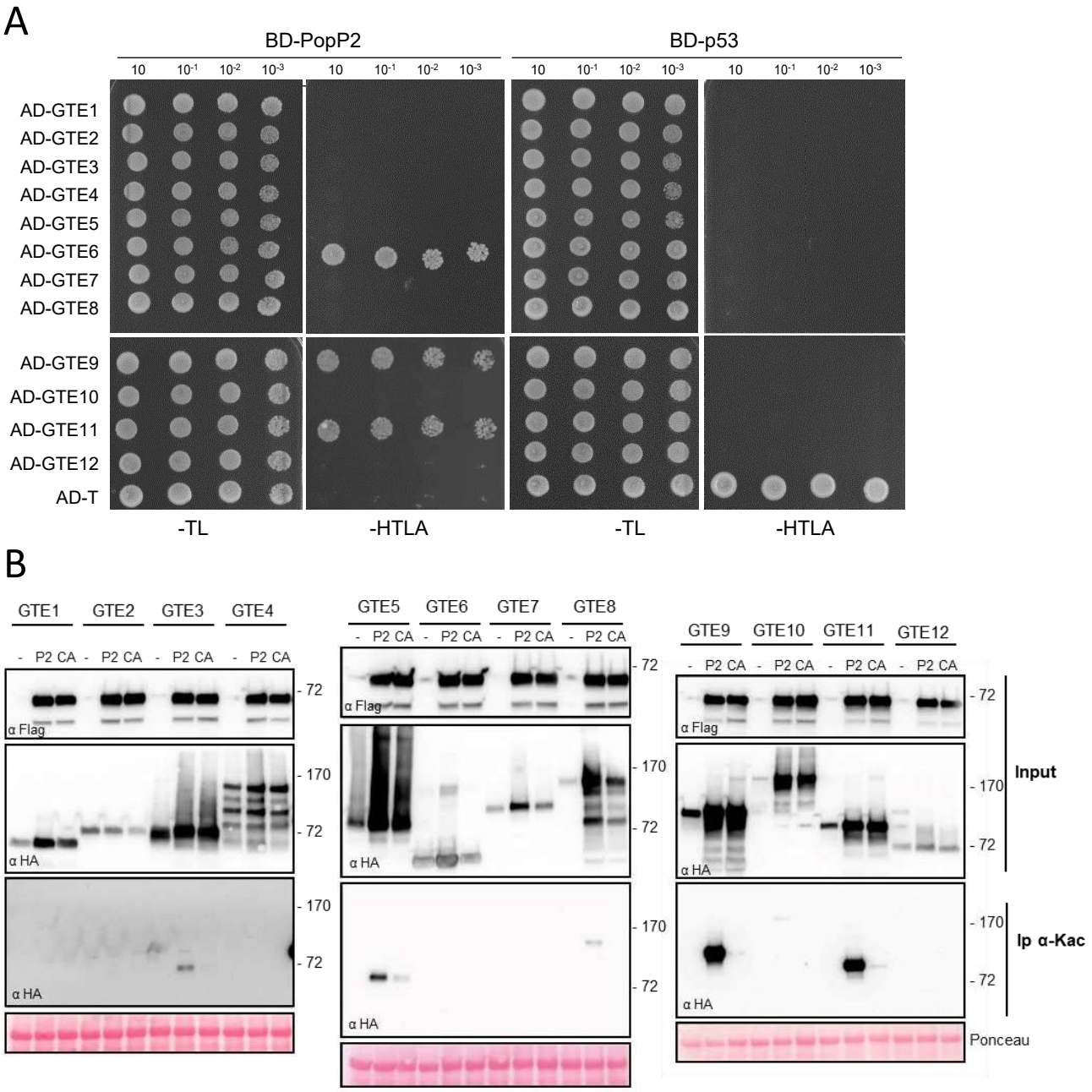


Figure 7. PopP2 targets several members of the GTE family. (A) Yeast two-hybrid assay to test the interaction between PopP2 and the different GTE proteins from *Arabidopsis thaliana*. Yeast cells transformed with different bait (BD-fusion) and prey (AD-fusion) plasmid combinations were spread on non-selective (-TL) or selective media (-HTLA). Growth on SD–HTLA medium indicate an interaction between the tested proteins. BD-p53 (bait) and AD-T (prey) were used as a positive control. This experiment was conducted at least two times with similar results. (B) In addition to GTE9 and GTE11, PopP2 acetylates GTE3, GTE5, and GTE8 *in planta*. All GTE protein members (GTE1 to GTE12) tagged with a 3HA epitope tag were transiently expressed with 3Flag-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Samples were harvested at 48hpi. Protein extracts were immunoblotted with anti-HA (α-HA) and anti-Flag (α-Flag) antibodies (Input). Ponceau S staining of total proteins indicates equal loading of the samples in the input. Lys-acetylated proteins were immunoprecipitated with an anti-Ac-K antibody (IP α-Ac-K). Immunoblot analysis of immunoprecipitated proteins with an anti-HA antibody reveals the presence of Lys-acetylated GTE3, GTE5, GTE8, GTE9 and GTE11 in presence of active PopP2 but not C321A. Immunoblots corresponding to GTE1/GTE2/GTE3/GTE4, GTE5/GTE6/GTE7GTE8, and GTE9/GTE10/GTE11/GTE12 represent, respectively, three independent experiments carried out under identical conditions. This experiment was conducted three times with similar results.

1 and *gte11-1* mutant lines expressing the RPS4/RRS1-R^{WS-2} NLR pair were root-inoculated with the GMI1000 strain. Similar to the complemented Col-0 line, these two transgenic lines displayed a resistance phenotype, with almost no wilting symptoms at 10 dpi (Fig 6B). Taken together, these data indicated that GTE9 and GTE11 are not required for RPS4/RRS1-R-dependent immunity, although we cannot exclude that *GTE9* and *GTE11* inactivation in *gte9-1* and *gte11-1,* respectively, could be compensated by other members of the GTE gene family with overlapping functions.

PopP2 targets multiple members of the GTE family

Considering that PopP2 targets both GTE9 and GTE11 by acetylating several lysine residues in the close environment of their bromodomains and that bromodomains of GTE members are well conserved, we wondered if PopP2 targets other members of the GTE family in Arabidopsis. For this, we first performed a yeast two-hybrid interaction test. The full-length cDNA of each of the other ten GTE members was cloned into a prey-plasmid for testing interaction pairs with PopP2 as bait. In addition to GTE9 and GTE11, a protein interaction was detected only between PopP2 and GTE6, although all prey proteins were correctly expressed in the yeast cells (Fig 7A and Supp Fig 7A). GTE6 belongs to subgroup I of GTE members and shares only 13.2% of identity and 24.9% of similarity with GTE11.

Since some protein interactions can be missed in Y2H due to inherent limitations in the technique – e.g. improper folding of the hybrid proteins, we took advantage of our *in planta* acetylation assay to test for acetylation of the different GTE members by PopP2. All the GTE members C-terminally fused to a 3HA epitope tag were transiently expressed in *N. benthamiana* either alone or with 3HA-tagged PopP2 or PopP2-C321A. Immunoprecipitations were performed with an anti-Ac-K antibody and purified proteins were subjected to immunoblot analysis with an anti-HA antibody. A signal corresponding to acetylated forms of GTE3, GTE5 and GTE8 was detected only in presence of active PopP2, indicating they also behave as PopP2 substrates (Fig 7B). For GTE10, a very weak signal was detected but not reproducibly in the three independent acetylation assays performed. Taken together, our data show that at least 5 of the 12 GTE family members can be modified by PopP2 enzymatic activity. Notably, several of the Lys residues acetylated by PopP2 in GTE9 and GTE11 (GTE9$^{K81/K126/K241}$ and GTE11$^{K72/K118/K233}$) (Supp Tables 2 and 3) are also present on either side of the bromodomain of GTE3, GTE5, and GT8 (GTE3$^{K79/K114/-,}$ GTE5$^{K87/K127/-}$, and GTE8$^{K95/-/K280}$) (Supp Fig 7B). They could therefore represent key lysine residues targeted by PopP2 to interfere with the

molecular functions of these GTEs. Interestingly, protein accumulation level of GTE1, GTE3, GTE5, GTE8, GTE9, GTE10, and GTE11 show a significant increase in presence of PopP2 or PopP2 catalytic mutant (Fig 7B), as previously observed with several WRKY transcription factors targeted by PopP2 (Le Roux et al., 2015), suggesting that PopP2, independently of its enzymatic activity, might promotes their accumulation.

DISCUSSION

Plants have evolved a sophisticated and robust immune system to protect themselves from infection by a vast majority of pathogens present in their environment. To evade host immune responses and promote infection, pathogens have developed various virulence strategies, among which subversion of host epigenetic mechanisms plays a prominent role. In this study, we describe a previously unknown mechanism used by the bacterial type III effector PopP2 acetyltransferase to promote infection in Arabidopsis by targeting members of the "Bromo- and Extra Terminal domains" (BET) family (also known as General Transcription factor Group E, GTE). Within the BET/GTE family, GTE9 and GTE11 behave as histone readers via their single bromodomain that represents a substrate for PopP2 enzymatic activity (Figs 1-4). Our study showed that catalytically active PopP2 alters the ability of GTE9 to interact with histone H4 *in vivo* (Fig 4C). Thus, PopP2 might represent a bacterial epigenetic regulator that uses acetylation to dissociate bromodomain-containing proteins from chromatin.

All members of the Arabidopsis GTE proteins are characterised by the presence of a single bromodomain and an extra-terminal (ET) domain, the latter likely serving as a platform for anchoring different proteins or complexes to chromatin (Florence and Faller, 2001; Pandey et al., 2002). Evolutionary conserved between plants and animals, bromodomains are structural modules found in many chromatin- and transcription-associated proteins that have the ability to recognise acetylated lysine residues on histone tails and other transcription-associated proteins, such as transcription factors and co-factors. This activity allows bromodomains to play a key role in many acetylation-mediated protein–protein interactions, ranging from recruiting substrate for histone acetyltransferases (HATs) to assisting in the assembly of multi-protein complexes in chromatin involved in the transcriptional activation or repression of genes (Smith and Zhou, 2016).

Recently, the systemic profiling of a wide variety of histone readers in Arabidopsis showed that the recognition of acetylated histone peptides by the bromodomain is highly conserved between

plants and humans, suggesting the conserved structural basis for hyper-acetylation chromatin signal readout (Zhao et al., 2018). By virtue of their association with histone H4 *in vivo* (Fig 3B) which requires the conserved Asparagine residue located in the BC loop of the Bromodomain of GTE9 and GTE11 ($GTE9^{N214}$ and $GTE11^{N206}$, Supp Fig 3A), these two proteins can be considered as epigenetic readers of histone lysine acetylation, an epigenetic mark that is central to epigenetic control of gene transcription.

Over the past decade, the subversion of host epigenetic mechanisms has emerged as an effective virulence strategy employed by various pathogens to defeat host immunity. Inhibition of defence gene expression relies on pathogen interference with various histones marks, including histone methylation (Castillo-González et al., 2015; Rolando et al., 2013) and histone acetylation. Although various animal pathogens effectors were found to cause changes in epigenetic histone acetylation marks (Grabiec and Potempa, 2018), examples from plant pathogens are rarer. The PsAvh23 effector from *Phytophthora sojae* was shown to repress the expression of defence-related genes and promote infection in soybean by modulating GmGCN5-mediated histone acetylation. PsAvh23, competitively binds ADA2, a member of the Histone acetyltransferase complex Spt-ADA-GCN5-Acetyltransferase (SAGA). This disrupts the SAGA complex formation leading to a decrease in H3K9 acetylation levels at defence-related gene loci (Kong et al., 2017). One other effector, PsAvh52 from *Phytophthora sojae*, binds to the soybean GmTAP1 transacetylase and relocates it into the nucleus where it could acetylate histones H2A and H3 to promote host susceptibility (Li et al., 2018). In Arabidopsis, the 32E03 effector from the sugar beet cyst nematode (*Heterodera schachtii*) inhibits histone deacetylases including HDT which is involved in the regulation of rRNA gene expression through chromatin modifications (Vijayapalani et al., 2018). From a pathogen's point of view, manipulation of bromodomain-containing proteins acting as chromatin adaptors could also represent a potent virulence strategy to interfere with host transcriptional reprogramming. To date, the only example of manipulation of a bromodomain-containing protein by a pathogen is the hijacking of the Bromodomain-containing protein 4's activity by Papillomaviruses (PVs). Brd4 from human is a dynamic cellular chromatin-binding factor and transcriptional regulator that recruits sequence-specific transcription factors and chromatin modulators to control target gene transcription (Rahman et al., 2011; Wu et al., 2013) . PVs have evolved to exploit the activity of Brd4 to create a facilitating environment for the viral life cycle. Through physical interaction with the major viral regulatory protein E2 of PVs, Brd4

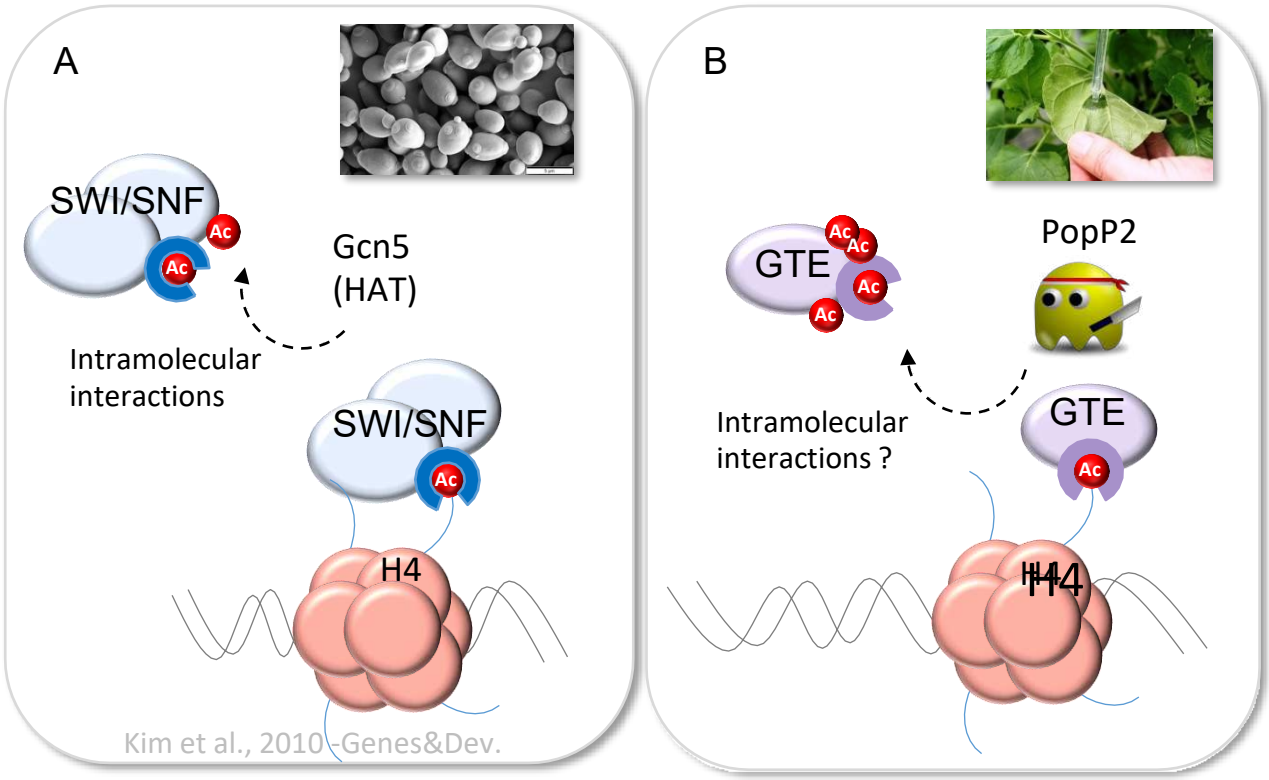


Figure 20. Intramolecular interactions between BRD and acetylated lysines regulating the binding of BRD-containing proteins to acetylated histones.
The binding of BRD-containing proteins to acetylated histones can be regulated by acetylation of lysine residues flanking their BRD, causing intramolecular interactions and altering their interaction with acetylated histones.
(A) In yeast, the SWI/SNF chromatin remodelling complex association to acetylated histones can be regulated by acetylation. The Snf2 subunit of this complex can be acetylated by the Gcn5 HAT on lysine residues flanking its BRD likely facilitating intramolecular interactions, inhibiting its ability to interact with H4 (Kim et al., 2010).
(B) We propose a similar mechanism used by PopP2 to alter the association GTE9-H4. Indeed, PopP2 acetylates lysines residues flanking GTE9 BRD which might cause intramolecular interactions and the alteration of its interaction with H4.

contributes to replication initiation, viral gene transcription, and viral genome segregation and maintenance of PVs (Iftner et al., 2017).

In this study, we identified GTE9 and GTE11 as substrates of PopP2 (Fig 2). Within these proteins, their bromodomains represent a molecular target of this bacterial effector (Figs 4B, Supp Tables 2-3). Remarkably, PopP2 acetylation of GTE9 is indeed accompanied with an alteration of GTE9 binding to histone H4 *in vivo* (Fig 4C). Among the acetyl residues identified in GTE9 and GTE11, several of them are also present in three other members of the GTE family, GTE3, GTE5 and GTE8, which behave as additional PopP2 substrates (Fig 7B and Supp Fig 7B). We therefore predict a scenario in which PopP2 uses acetylation to cause dissociation of GTE proteins from chromatin by allowing intramolecular interactions between their own bromodomain and adjacent acetyl-lysine residues. Such mechanism of bromodomain inactivation through acetylation-mediated intramolecular interactions was already reported (Kim et al., 2010; VanDemark et al., 2007). For example, the Gcn5 histone acetyltransferase regulates the dissociation of the SWI/SNF chromatin remodelling complex from acetylated histones by acetylating the Snf2 subunit at two lysine residues. This facilitates intramolecular interaction between the two acetyl lysine residues and the Snf2 bromodomain, inhibiting its ability to interact with acetylated histones (Figure 20) (Kim et al., 2010). It remains to determine whether the bromodomain of GTE proteins represents a ubiquitous substrate of PopP2 enzymatic activity, as was previously described with the WRKY DNA-binding domain of WRKY transcription factors (le Roux et al, 2015). In addition, the protein sequences surrounding the bromodomain of GTE proteins could mediate selective targeting by PopP2.

Overall, the molecular functions of the different GTE family members remain elusive. Some of them were already shown to be involved in important physiological and developmental processes. GTE1 (as known as IMB1) plays a role in the promotion of seed germination by both negatively and positively regulating the abscisic acid (ABA) and phytochrome A (phyA) transduction pathways, respectively (Duque and Chua, 2003). GTE4 is involved in the activation and the maintenance of cell division in the meristems (Airoldi et al., 2010), while GTE6 controls leaf development (Chua et al., 2005). Previously, GTE9 and GTE11 have been described as essential for BT2-mediated sugar and ABA responses. BT2 is a BTB-domain protein that regulates responses to various stress, metabolic conditions and hormones in Arabidopsis. Through physical association with BT2, GTE9 and GTE11 were hypothesised to mediate responses to ABA and sugar signals (Misra et al., 2018). More recently, both GTE9 and GTE11 were shown to be required for BT2-mediated regulation of cauliflower mosaic virus

35S enhancer activity in Arabidopsis (Irigoyen et al., 2021). Given that GTE11 activates transcription via its C-terminus extra-terminal domain in yeast (Du and Poovaiah, 2004), and as evidenced by genetic studies, authors proposed that BT2 assembles in a complex containing GTE9, GTE11 and Cullin3-based ubiquitin ligase that may serve as a scaffold and promote interactions among transcriptional regulators including the GTE proteins.

Our study revealed a previously undescribed role for GTE9 and GTE11, both of which represent PopP2 virulence targets since their transgenic overexpression in Col-0 plants enhances wilting disease development in response to *R. solanacearum* infection (Fig 5B and Supp Fig 5E). In Arabidopsis, transgenic overexpression of GTE6 increases the acetylation states of histones H3 and H4 in the promoter region of one of its target gene (Chua et al., 2005). GTE6 is thought to locally increase histone acetylation by binding to acetylated histones and preventing the action of histone deacetylases. Similarly, GTE9 and GTE11 could also shield acetylated histone 4 from deacetylation or increase the level of acetylation by interacting with histone acetyltransferases. Rather than the deregulation of as yet uncharacterised signalling pathways caused by overexpression of GTE9 and GTE11, our data indicate that it is the targeting of these two bromodomain-containing proteins by catalytically active PopP2 that creates a favourable context for infection by the pathogen (Fig 5C). How manipulation of GTE9 and GTE11 by PopP2 can promote pathogen infection remains to be elucidated. PopP2-triggered dissociation of GTE9 and GTE11 from chromatin might locally affect transcriptional regulation of gene expression, for example by preventing activation of defence-related genes. However, such a mechanism would not explain how GTE over-expression could increase plant's susceptibility to infection. An alternative hypothesis could thus be that over-accumulation of GTE9 and GTE11 at specific loci may help PopP2 to target these chromatin regions more efficiently or make them more accessible to the effector.

Of the 12 GTE members considered in this study, only GTE3, GTE5, GTE8, GTE9 and GTE11 could be detected as Lys-acetylated by PopP2 (Figs 2 and 7B). GTE6, which does not seem to be acetylated by PopP2, is however able to interact with (Fig 7A). From this observation, we infer that PopP2 might target GTE family members in different ways. Some of them would be dissociated from the chromatin by acetylation whereas others could serve as chromatin adaptors allowing PopP2 to anchor at specific loci. From these targeted chromatin sites, PopP2 could affect host gene expression, probably through the recruitment of negative transcriptional regulators thanks to its transcriptional repressor motif (Segonzac et al., 2017). In the future, an

investigation of the chromatin sites hosting GTE proteins should reveal key genetic loci whose transcriptional regulation is affected by PopP2 to promote pathogen infection.

The ability of PopP2 to target GTE and to modify their ability to bind histones makes this bacterial effector an enzyme acting as a regulator of bromodomain activities. Further investigation on the crystal structure of protein complexes containing PopP2 and GTE proteins or their bromodomain and/or acetylated histone peptides will help explain how PopP2 interferes with the activity of these epigenetic readers. Such studies could also pave the way for the engineering and/or optimisation of protein enzymes capable of selectively regulating the association of bromodomain-containing proteins to chromatin, as done by bromodomain inhibitors.

Acknowledgments

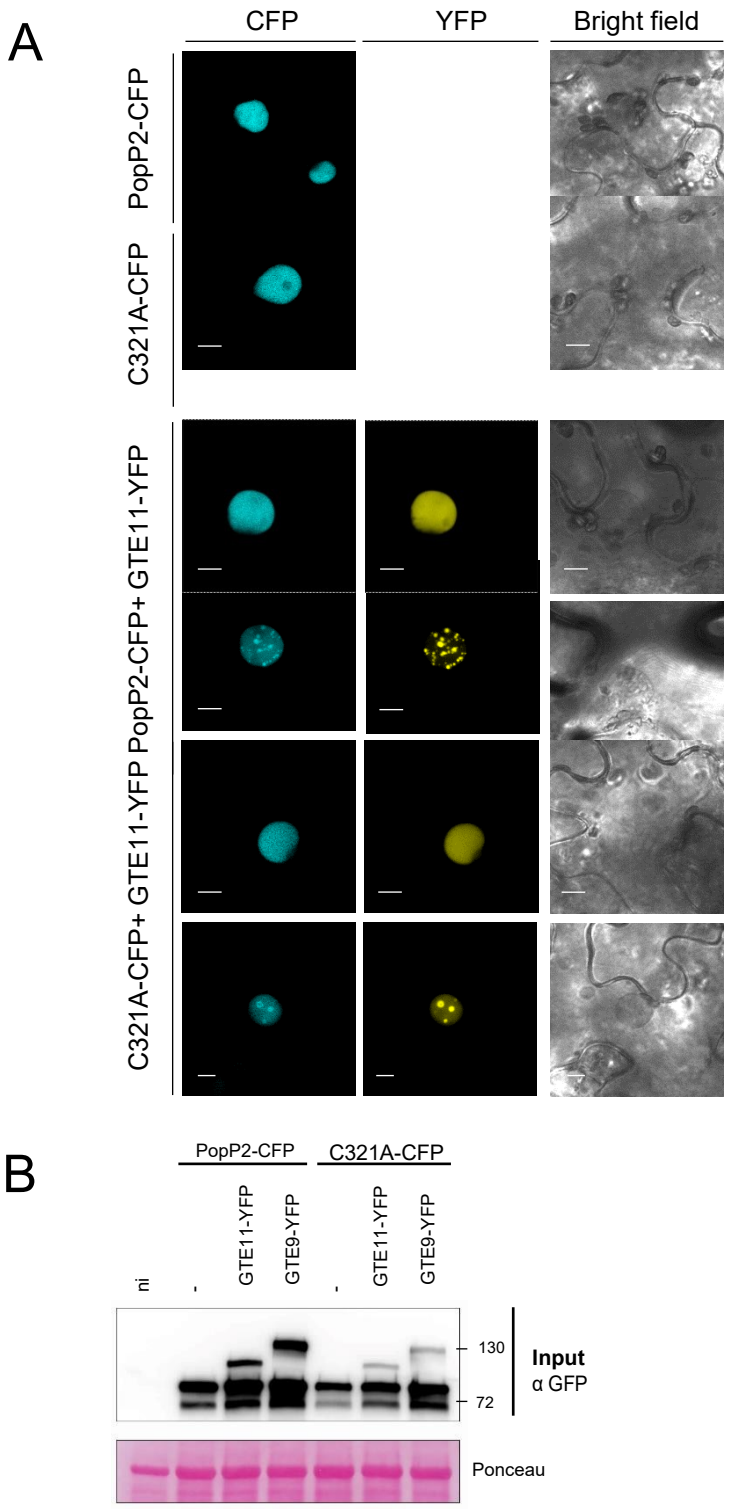
VC was supported by a doctoral fellowship co-funded by the Agence Nationale pour la Recherche (ANR-18-CE20-001) and the INRAE SPE department. AD and GH were supported by a research grant from the Agence Nationale pour la Recherche (ANR-10-JCJC-1706-01 (PercepTOME)). LD was supported by research grants from the Agence Nationale pour la Recherche (ANR15-CE20-0016-01 (RADAR) and ANR-18-CE20-0015 (EpiCLIPSE)). This study is set within the framework of the "Laboratoires d Excellences (LABEX)" TULIP (ANR 10 LABX 41) and of the "École Universitaire de Recherche (EUR)" TULIP GS (ANR 18 EURE 0019). The proteomic experiments were partially supported by Agence Nationale de la Recherche under projects ProFI (Proteomics French Infrastructure, ANR-10-INBS-08) and GRAL, a program from the Chemistry Biology Health (CBH) Graduate School of University Grenoble Alpes (ANR-17-EURE-0003). SK and MF are grateful for support by the SGC, a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA), Janssen, Merck KGaA Darmstadt Germany, MSD, Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome. We thank Dr Cécile Raynaud for critical reading of the manuscript.

Author Contributions

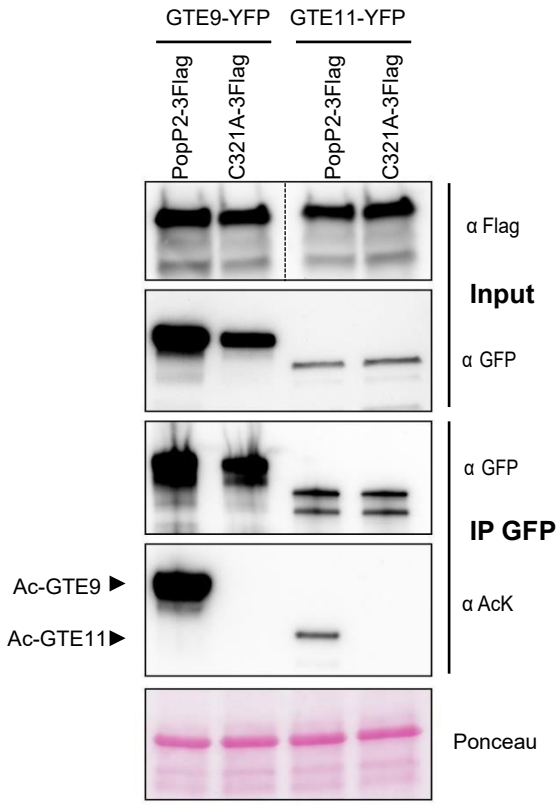
VC, AD, and LD designed the project. AK and YC performed MS-based proteomic analysis; VC, AD, AJ, CP and LD performed FRET-FLIM studies and confocal microscopic observations; VC, AD, GH and LD performed all in planta-related experiments. AD, MF and SK performed the histone peptide array; VC, AD, MF, SK, AJ, CP, AK, YC and LD analysed data; VC and LD wrote the manuscript with comments from all authors.

Declaration of interest

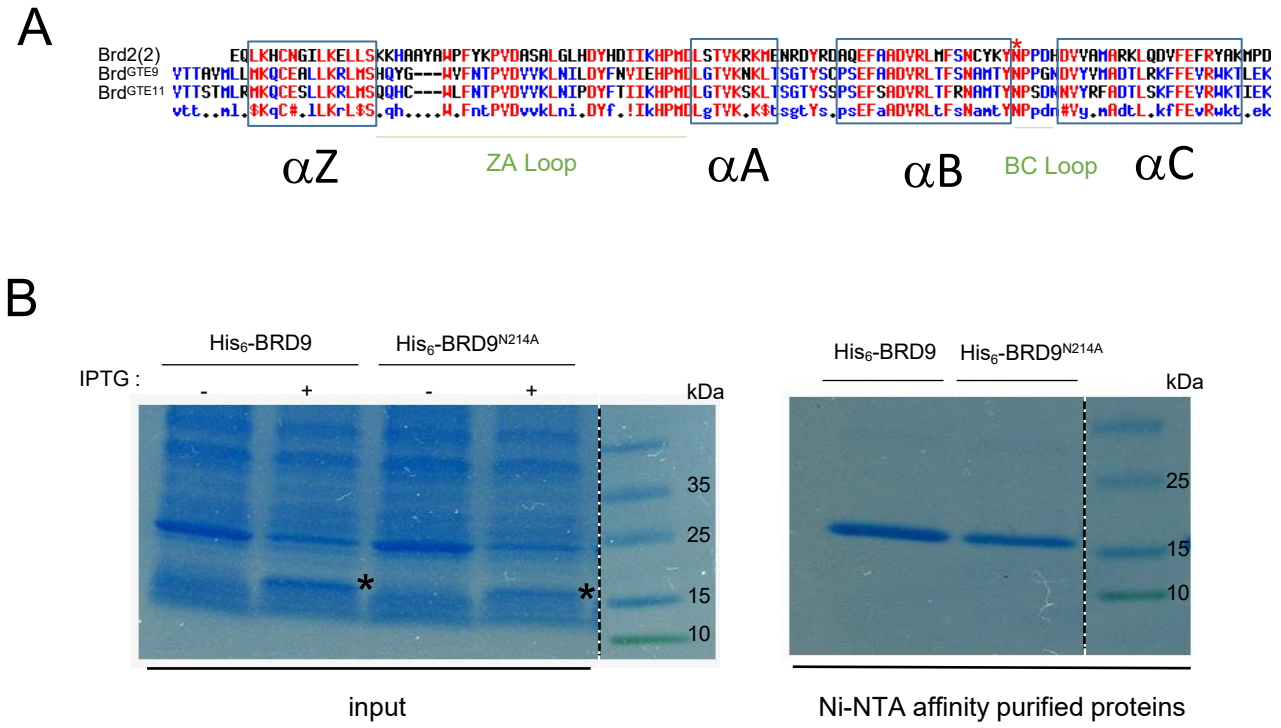
The authors declare no competing interests



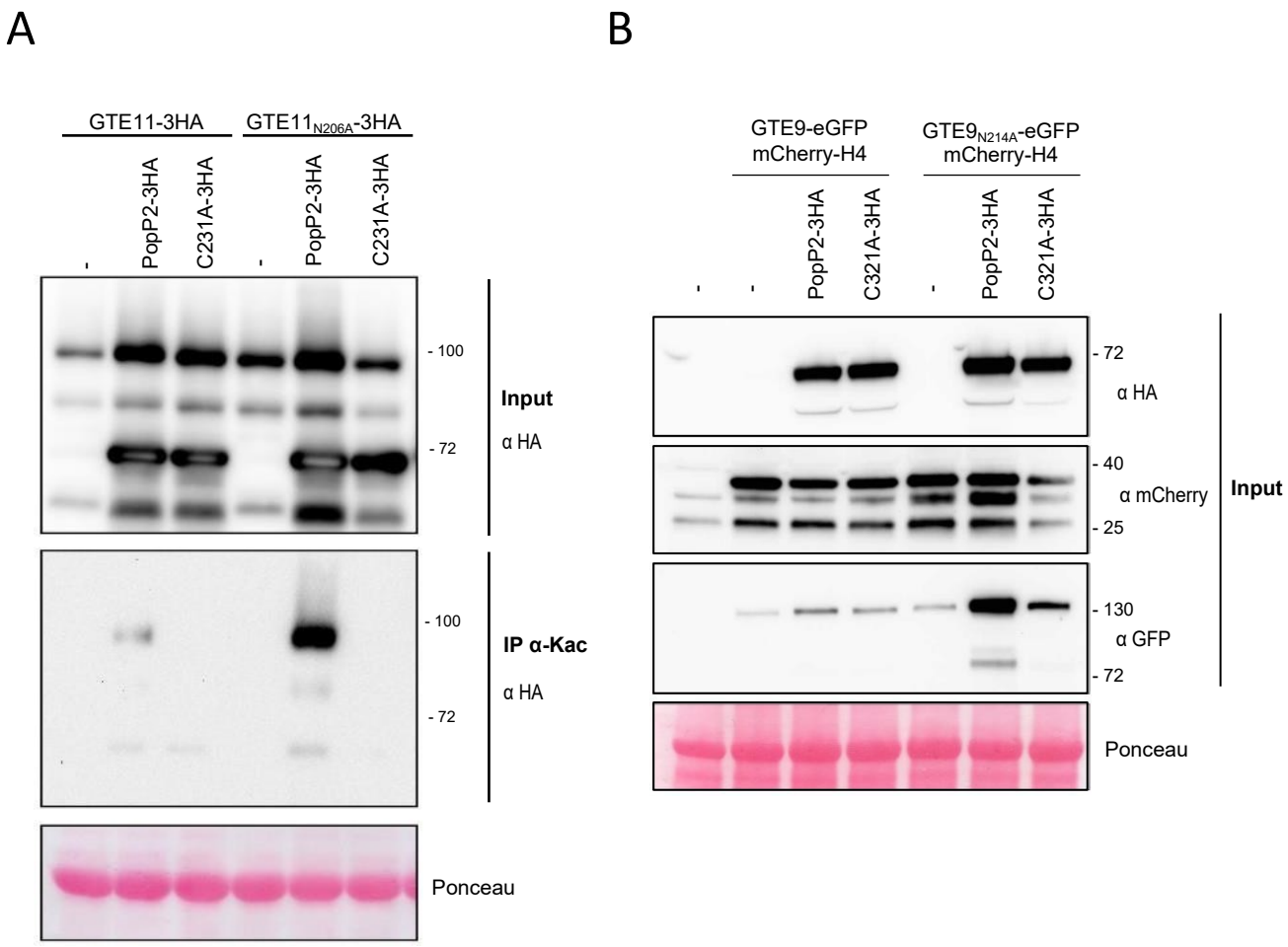
Supplemental Figure 1. GTE11 colocalises with wild-type PopP2 and PopP2-C321A catalytic mutant in plant cell nuclei. (A) GTE11 colocalises with PopP2 in the plant nucleus. PopP2-CFP and PopP2-C321A-CFP were transiently expressed either alone or with GTE11-YFP in *N. benthamiana* cells. Photographs were taken between 48 and 72 hpi. Scale bars, 10 µm. (B) Immunodetection of PopP2-CFP and PopP2-C321A-CFP transiently expressed either alone or with GTE9-YFP or GTE11-YFP in *N. benthamiana*. Leaf samples were harvested at 48 hpi. CFP- and YFP-tagged proteins were detected using an anti-GFP antibody form crude extract (Input). Ponceau S staining of total proteins indicates equal loading of the samples. This immunoblot was carried out in an independent transient expression assay.



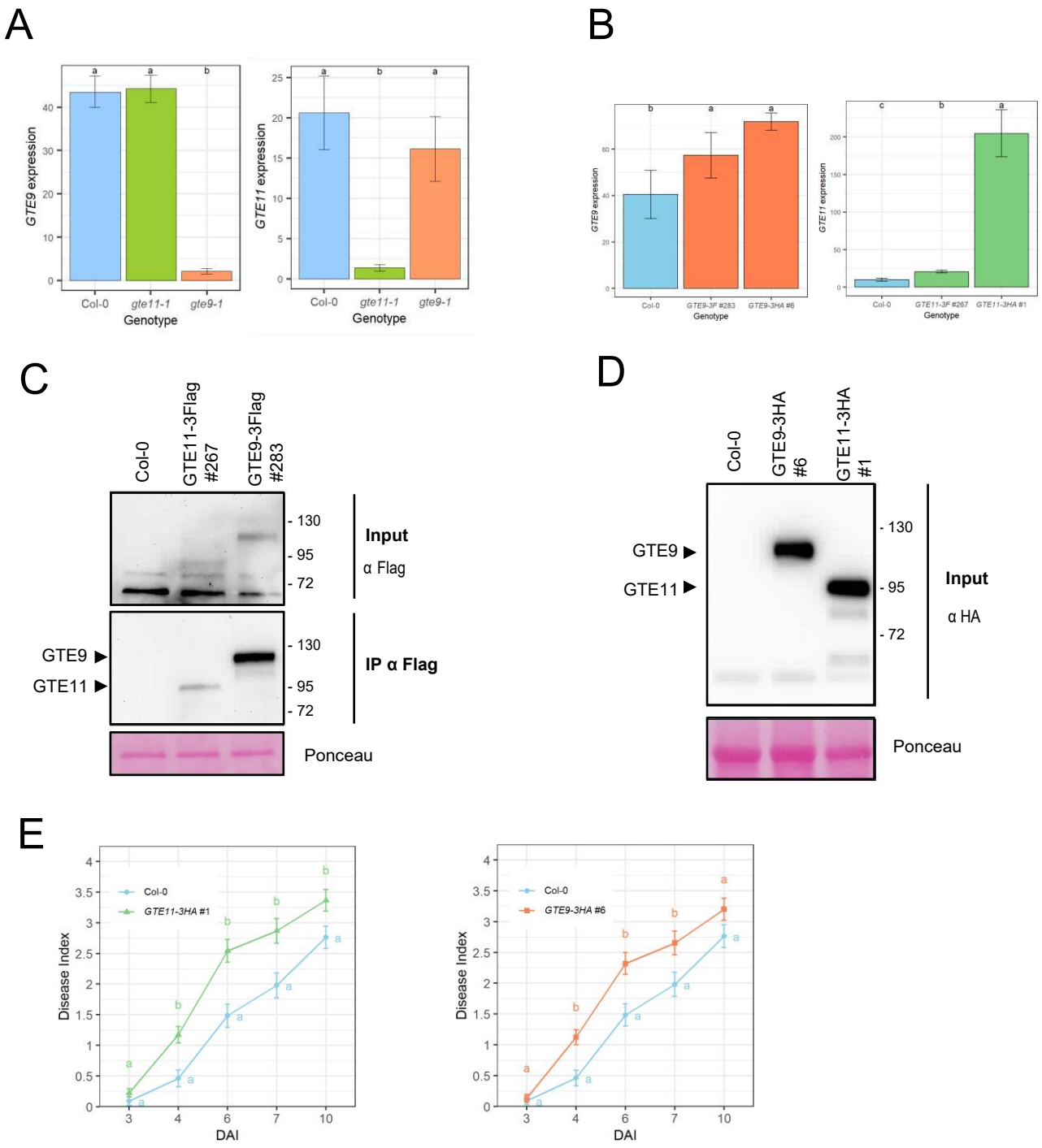
Supplemental Figure 2. GTE9-YFP and GTE11-YFP are acetylated by PopP2. (A) GTE9-YFP and GTE11-YFP were transiently expressed with 3Flag-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Samples were harvested at 48hpi. Protein extracts were immunoblotted with anti-Flag (α-Flag) and anti-GFP (α-GFP) antibodies (Input). GTE9-YFP and GTE11-YFP were immunoprecipitated on GFP agarose beads (IP GFP) and analysed by immunoblotting with anti-GFP (α-GFP) and anti-acetylated-lysine (α-Ac-K) antibodies. Ponceau S staining of total proteins indicates equal loading of the samples in the input. The dotted line indicates that the lanes from two membranes resulting from the same experiment have been combined. Immunopurified proteins were subjected to MS-based proteomic analysis (See Supplementary Tables 2 and 3).



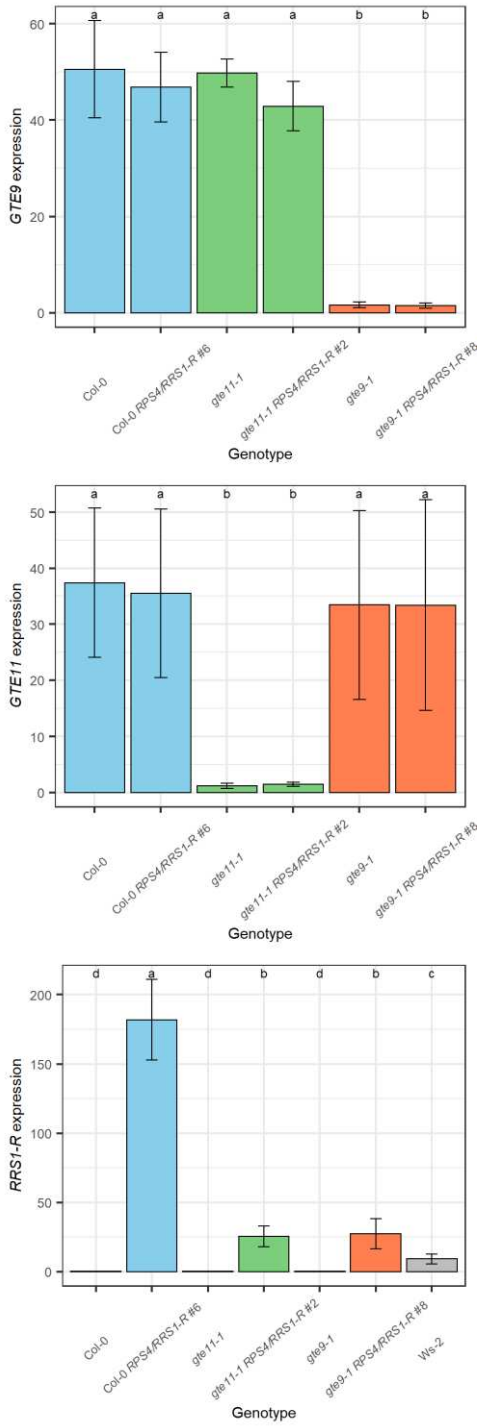
Supplemental Figure 3. The bromodomain of GTE9 and GTE11 share conserved structural features with the second bromodomain of human BRD2 (Brd2(2)). (A) Protein alignment of the bromodomain of GTE9 (Brd^{GTE9}) and GTE11 (Brd^{GTE11}) with Brd2(2) showing the conservation of the four α-helices (αZ, αA, αB, αC) and of the two variable loops that connect helices αZ–αA (ZA loop) and αB–αC (BC loop), shaping the acetyl–lysine binding pocket. The canonical Asparagine (N) residue present in most Brds and that is required for the recognition of acetyl-lysine (Kac) residues is indicated with a red star. (B) Expression and purification of recombinant His_6-BRD9 and His_6-BRD9-N214A proteins in *E. coli* Rosetta (DE3) cells. Bacteria were grown without (-) or with 200 µM IPTG (+). Total protein extracts were separated on SDS-PAGE and gels were stained with Coomassie blue. The stars indicate the band corresponding to the His_6-tagged proteins induced by IPTG (left). Affinity purified His_6-BRD9 and His^6-BRD9-N214A on Ni-NTA resin (right). The dotted lines indicate image splicing to remove unnecessary lanes. This pilot experiment was performed to check proper expression and purification of His_6-tagged recombinant proteins used for Histone peptide array.



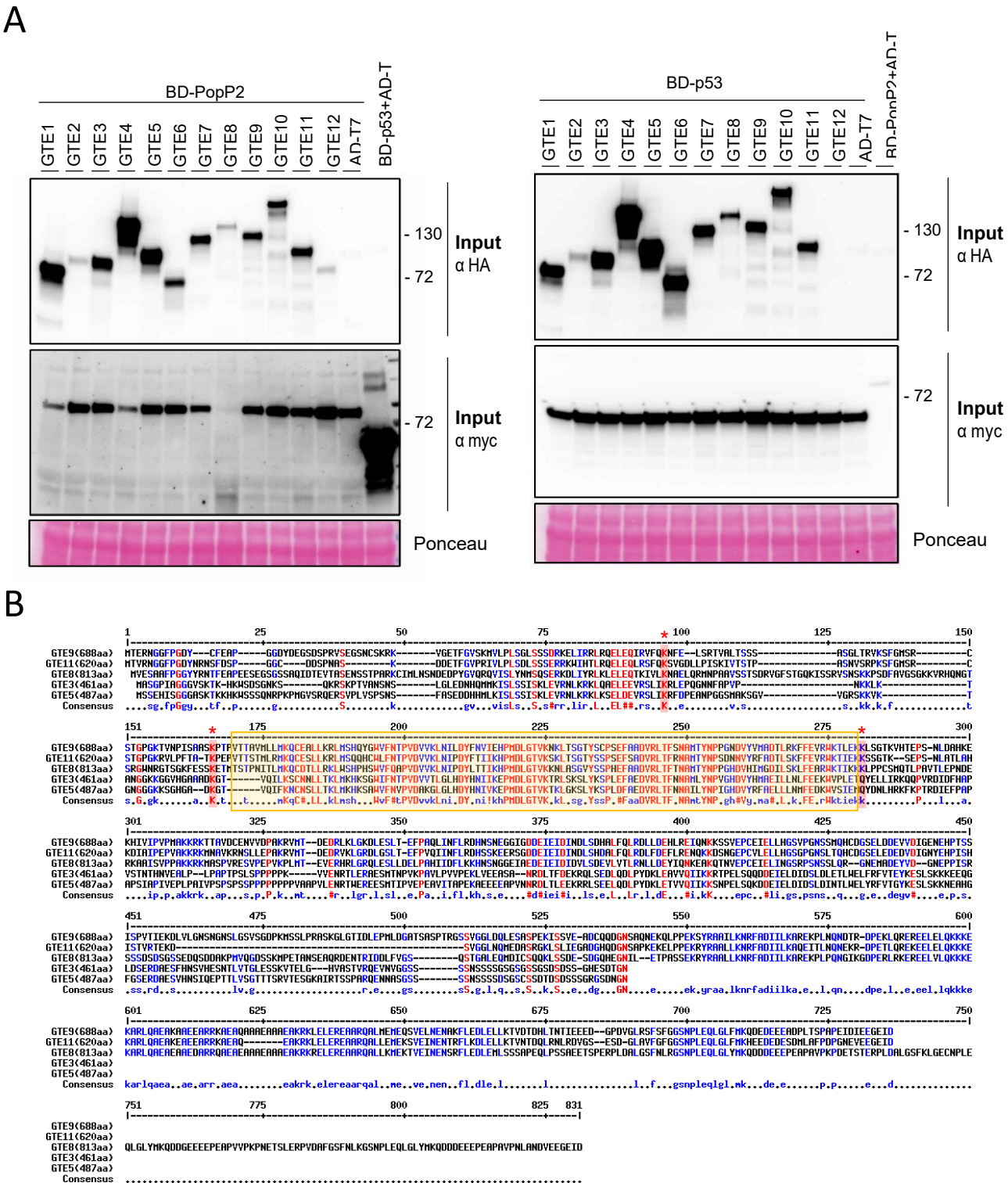
Supplemental Figure 4. Immunodetection of Lys-acetylated GTE11 co-expressed with PopP2 is favoured by the N206A mutation in GTE11 bromodomain. (A) GTE11-3HA and GTE11-N206A-3HA were transiently expressed with 3HA-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Samples were harvested at 48hpi. Protein extracts were immunoblotted with an anti-HA (α-HA) antibody (Input). Immunoprecipitation of Lys-acetylated proteins was done in presence of an anti-AcK antibody on protein A-agarose beads (IP α-Ac-K). The immunoprecipitated GTE11-3HA and GTE11-N206A-3HA were detected after immunoblotting with an anti-HA antibody. Ponceau S staining of total proteins indicates equal loading of the samples in the input. This experiment was repeated two times with similar results. (B) Accumulation of GTE9-eGFP, GTE9-N214A-eGFP, mCherry-H4, PopP2-3HA, and C321A-3HA proteins used for quantitative FRET-FLIM measurements was verified in an independent transient expression assay in *N. benthamiana.*



Supplemental Figure 5. Molecular characterisation of *GTE9* and *GTE11* knock-out and overexpressing lines. (A) Expression level of *GTE9* and *GTE11* in Col-0, *gte9-1* and *gte11-1* null mutants. The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants and normalised to the expression level of *AtEF1-α*. The primer pairs used were, respectively, GTE9-q-2-F/GTE9-q-2&3-R for GTE9, and GTE11-q-2-F/GTE11-q-2-R for GTE11. Mean values +/- SD (n = 3; p-value = 0.05, a and b show significative difference in mean ΔCt based on the one-way ANOVA or the kruskal-test). (B) Expression levels of GTE9 and GTE11 transgenically overexpressed in the indicated transgenic lines (Col-0 background). (C) Immuno-detection of GTE9-3Flag and GTE11-3Flag proteins immunoprecipitated from GTE9-3Flag #283 and GTE11-3Flag #267 seedlings using anti-Flag beads. Proteins were detected with an anti-Flag antibody. Ponceau S staining of total proteins indicates equal loading of the samples in the input. This experiment was repeated three times with similar results. (D) Immuno-detection of GTE9-3HA and GTE11-3HA proteins from crude protein extracts of GTE9-3HA #6 and GTE11-3HA #1 seedlings. (E) Transgenic overexpression of GTE9-3HA or GTE11-3HA enhances the development of wilting disease symptom in response to the GMI1000 strain. The graph shows least-square means +/- Standard Error of the LS means from 2 and 3 independent inoculations of ~24 plants for GTE11-3HA #1 and GTE9-3HA #6, respectively (n total = 69 plants for Col-0, and 72 plants for GTE11-3HA #1 and GTE9-3HA #6). a and b denote significant difference between two lines at a certain DAI (one-way ANOVA performed on the mixed model described in the methods, p-value < 0.05).



Supplemental Figure 6. Molecular characterisation of Col-0, *gte9-1* and *gte11-1* complemented with *RPS4g/RRS1-Rg*. Expression level of *GTE9, GTE11, and RRS1-R* (top, middle and bottom, respectively) in Col-0, *gte9-1* and *gte11-1* mutant lines complemented or not with a genomic clone containing the coding and regulatory sequences for expression of *RRS1-R* and *RPS4* genes from the Ws-2 accession. The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants and normalised to the transcript accumulation of *AtEF1-α*. Primers used were respectively GTE9-q-2-F and GTE9-q-2&3-R for *GTE9,* GTE11-q-2-F and GTE11-q-2-R for *GTE11,* and RRS1-R-ex5-q-F and RRS1-R-ex5-q-R for *RRS1-R*. Mean values +/- SD (n = 3; p-value = 0.05).



Supplemental Figure 7. PopP2 interacts with and acetylates several members of the GTE protein family. (A) Confirmation of protein expression in the yeast two-hybrid assay shown in Figure 7A. Yeast cells co-expressing the bait and prey constructs were grown in nutritional selection media (SD-TL). The yeast suspensions were from the same experiment depicted in Figure 7A. Bait and prey proteins were detected with an α-Myc and an α-HA antibody, respectively. Staining with Ponceau S was used as a loading control. This experiment was performed two times with similar results. (B) Protein alignment between GTE3, GTE5, GTE8, GTE9, and GTE11 that all behave as substrates of PopP2 enzymatic activity. The bromodomain is boxed in orange and the acetyl-lysine residues identified in GTE9 and GTE11 by MS-based spectrometry analysis also present in GTE3, GTE5 and/or GTE8 are boxed in red with a red star. This multiple sequence alignment was done by using Multalin program, with the first amino acid as the methionine from GTE9.

STAR METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-GFP from mouse IgG1k (clones 7.1 and 13.1)	Roche	Cat#11814460001
anti-HA-peroxydase, High affinity	Roche	Cat#12013819001
monoclonal anti-FLAG M2-peroxydase from mouse	Sigma-Aldrich	Cat#A8592
acetylated-Lysine Mouse mAb (Ac-K-103)	Cell Signaling	Cat#9681
monoclonal anti-mCherry antibody produced in mouse	Sigma-Aldrich	Cat#SAB2702291
goat anti-mouse IgG(H+L)-HRP conjugate	Bio-Rad	Cat#170-6516
goat anti mouse IgG2a-HRP	Bio-Rad	Cat#STAR133P
Bacterial and yeast strains		
AH109 *Saccharomyces cerevisiae*	Clontech	N/A
Agrobacterium tumefaciens GV3101	(Holsters et al., 1980)	N/A
Agrobacterium tumefaciens GV3103	(Holsters et al., 1980)	N/A
Agrobacterium tumefaciens C58	(Wood et al., 2001)	N/A
DH5α	ThermoFisher	Cat#18265017
Rosetta™ (DE3)	Sigma-Aldrich	Cat#70954
Pseudomonas fluorescens (Pf0-1)	(Le Roux et al., 2015)	N/A
Pseudomonas fluorescens (Pf0-1, PopP2)	(Le Roux et al., 2015)	N/A
Pseudomonas fluorescens (Pf0-1, C321A)	(Le Roux et al., 2015)	N/A
Rs GMI1000	(Salanoubat et al., 2002)	N/A
Rs ΔpopP2	(Deslandes et al., 2003)	N/A
Rs ΔpopP2(PopP2)	(Tasset et al., 2010)	N/A
Rs ΔpopP2(PopP2-C321A)	(Tasset et al., 2010)	N/A
Chemicals, Peptides		
Protein A-agarose	Roche	Cat#05015979001
GFP-Trap® Agarose	Chromotek	Cat#gta-20
ANTI-FLAG® M2 Affinity Gel	Sigma-Aldrich	Cat#A2220
Protease inhibitor cocktail	Sigma-Aldrich	Cat#P9599
Clarity™ Western ECL substrate	Bio-Rad	Cat#1705060
PageRuler™ Prestained Protein Ladder, 10 to 180 kDa	ThermoFisher	Cat#26617
gentamicin sulphate	Duchefa Biochemie	Cat#1405-41-0
carbenicillin disodium	Duchefa Biochemie	Cat#4800-94-6
spectinomycin pentahydrate	Duchefa Biochemie	Cat#22189-32-8
chloramphenicol	Duchefa Biochemie	Cat#56-75-7
kanamycine sulphate monohydrate	Duchefa Biochemie	Cat#25389-94-0
tetracyclin hydrochloride	Sigma-Aldrich	Cat#T7660
Phosphinotricin (PPT)	Duchefa Biochemie	Cat#77182-82-2
2,3,5-Triphenyltetrazolium chloride (TTC)	Sigma-Aldrich	Cat#T8877
Hexadecyltrimethylammonium bromide (CTAB)	Sigma-Aldrich	Cat#H6269
3,5-Dimethoxy-4′-hydroxyacetophenone (acetosyringone)	Sigma-Aldrich	Cat#D134406
Tween® 20	Sigma-Aldrich	Cat#P9416
SD/-Ade/-His/-Leu/-Trp Broth	Takarabio	Cat#630322
Lithium acetate dihydrate	Sigma-Aldrich	Cat#L6883
Sodium butyrate	Sigma-Aldrich	Cat#303410
Commercial cloning components		
Gateway™ BP Clonase™ II Enzyme mix	ThermoFisher	Cat#11789020
Gateway™ LR Clonase™ II Enzyme mix	ThermoFisher	Cat#11791100
Gateway™ pDONR™207 Vector	Invitrogen	N/A
PrimeStar® Max DNA polymerase	Takarabio	Cat#R045A
Experimental Models		
Nicotiana benthamiana	(Sarris et al., 2015)	

Arabidopsis thaliana gte9-1	(Misra et al., 2018)	N/A
Arabidopsis thaliana gte11-1	(Misra et al., 2018)	N/A
Arabidopsis:RPS4g/RRS1-Rg	This paper	N/A
Arabidopsis:35S-GTE9-3Flag	This paper	N/A
Arabidopsis:35S-GTE9-3HA	This paper	N/A
Arabidopsis:35S-GTE11-3Flag	This paper	N/A
Arabidopsis:35S-GTE11-3HA	This paper	N/A

Oligonucleotides		
Primers used in this study see Supplementary Table S6		

Recombinant DNA		
pB7FWG2-35S-GWY-eGFP	on-line	gatewayvectors.vib.be/collection/
pB7FWG2-D35S-GWY-eGFP	(Le Roux et al., 2015)	N/A
pBIN-35S-GWY-3Flag	(Tasset et al., 2010)	N/A
pBIN-35S-GWY-3HA	(Tasset et al., 2010)	N/A
pBIN-35S-GWY-YFP	(Tasset et al., 2010)	N/A
pAM-PAT-35S-GWY-3HA	(Tasset et al., 2010)	N/A
pAM-PAT-35S-GWY-CFP	(Tasset et al., 2010)	N/A
pAM-PAT-35S-CFP-GWY	(Tasset et al., 2010)	N/A
pGAD-GWY	This paper	N/A
pGBG-GWY	This paper	N/A
pDUET-6his-GWY	(Le Roux et al., 2015)	N/A
pENTR207-GTE1	This paper	N/A
pENTR207-GTE1	This paper	N/A
pENTR207-GTE1	This paper	N/A
pENTR207-GTE2	This paper	N/A
pENTR207-GTE3	This paper	N/A
pENTR207-GTE4	This paper	N/A
pENTR207-GTE5	This paper	N/A
pENTR207-GTE6	This paper	N/A
pENTR207-GTE7	This paper	N/A
pENTR207-GTE8	This paper	N/A
pENTR207-GTE9	This paper	N/A
pENTR207-GTE9-N214A	This paper	N/A
pENTR207-GTE10	This paper	N/A
pENTR207-GTE11	This paper	N/A
pENTR207-GTE11-N206A	This paper	N/A
pENTR207-GTE12	This paper	N/A
pENTR207-BRD9	This paper	N/A
pENTR207_BRD9-N214A	This paper	N/A
pENTR207-BRD11	This paper	N/A
pENTR207-BRD11-N206A	This paper	N/A
pENTR207-Histone3.3	This paper	N/A
pENTR207-Histone4	This paper	N/A
pENTR207-mCherry-Histone4	This paper	N/A
pGAD-GTE1	This paper	N/A
pGAD-GTE2	This paper	N/A
pGAD-GTE3	This paper	N/A
pGAD-GTE4	This paper	N/A
pGAD-GTE5	This paper	N/A
pGAD-GTE6	This paper	N/A
pGAD-GTE7	This paper	N/A
pGAD-GTE8	This paper	N/A
pGAD-GTE9	This paper	N/A
pGAD-GTE10	This paper	N/A
pGAD-GTE11	This paper	N/A
pGAD-GTE12	This paper	N/A
pGAD-BRD9	This paper	N/A
pGAD-BRD9-N214A	This paper	N/A
pGAD-BRD11	This paper	N/A

pGAD-BRD11-N206A	This paper	N/A
pGBG-BD-PopP2	This paper	N/A
pGBKT7-p53	Takarabio	Cat.#630489
pGADT7-T	Takarabio	Cat.#630489
pAM-PAT-35S-PopP2-CFP	(Tasset et al., 2010)	N/A
pAM-PAT-35S-C321A-CFP	(Tasset et al., 2010)	N/A
pAM-PAT-35S-YFP	(Tasset et al., 2010)	N/A
pBIN-35S-GTE9-YFP	This paper	N/A
pBIN-35S-GTE9-N214A-YFP	This paper	N/A
pBIN-35S-GTE11-YFP	This paper	N/A
pBIN-35S-GTE11-N206A-YFP	This paper	N/A
pBIN-35S-BRD9-YFP	This paper	N/A
pBIN-35S-BRD11-N206A-YFP	This paper	N/A
pAM-PAT-35S-CFP-Histone4	This paper	N/A
pAM-PAT-35S-CFP-Histone3	This paper	N/A
pAM-PAT-35S-PopP2-3HA	This paper	N/A
pAM-PAT-35S-C321A-3HA	This paper	N/A
pBIN-35S-PopP2-3Flag	This paper	N/A
pBIN-35S-C321A-3Flag	This paper	N/A
pAM-PAT-35S-GTE9-3Flag	This paper	N/A
pAM-PAT-35S-GTE11-3Flag	This paper	N/A
pDUET-His6-BRD9	This paper	N/A
pDUET-His6-BRD9-N214A	This paper	N/A
pBIN-35S-GTE9-3HA	This paper	N/A
pBIN-35S-GTE9-N214A-3HA	This paper	N/A
pBIN-35S-GTE11-3HA	This paper	N/A
pBIN-35S-GTE11-N206A-3HA	This paper	N/A
pB7-35S-GTE9-eGFP	This paper	N/A
pB7-35S-GTE9-N214A-eGFP	This paper	N/A
pB7-35S-mCherry-Histone4	This paper	N/A
pBIN-35S-GTE1-3HA	This paper	N/A
pBIN-35S-GTE2-3HA	This paper	N/A
pBIN-35S-GTE3-3HA	This paper	N/A
pBIN-35S-GTE4-3HA	This paper	N/A
pBIN-35S-GTE5-3HA	This paper	N/A
pBIN-35S-GTE6-3HA	This paper	N/A
pBIN-35S-GTE7-3HA	This paper	N/A
pBIN-35S-GTE8-3HA	This paper	N/A
pBIN-35S-GTE10-3HA	This paper	N/A
pBIN-35S-GTE12-3HA	This paper	N/A

Softwares		
R	On-line	https://www.r-project.org/
TAU_POGRAPHY-FLIM software v. 3.1		https://trigenotoul.com/
Mascot (version 2.7.0.1, Matrix Science)	Matrix Science Inc.	http://www.matrixscience.com/
Proline	(Bouyssié et al., 2020)	

LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents used in this study should be directed to and will be fulfilled by the Lead Contact, Laurent Deslandes (laurent.deslandes@inrae.fr).

EXPERIMENTAL MODELS

Arabidopsis

Arabidopsis thaliana Col-0 and Ws-2 plants, and null mutants (*gte9-1* (Salk_0119044C) *gte11-1* (Salk_059327C), previously described by Misra et al., 2018 were grown in short days (8h light/16h dark cycle) at 22°C (60% relative humidity, 125 µE/M2/s fluorescent illumination). Transgenic lines were generated by using the floral dip transformation method (Clough and Bent, 1998).

Nicotiana benthamiana

Nicotiana benthamiana (Nb) plants were sown on soil and grown at 24°C under long day photoperiod (16 h light/8 h dark) with 60% relative humidity. Leaves of 4 to 5 week-old plants were used for *Agrobacterium tumefaciens*-mediated transient expression for Immunoblotting, colocalisation, or FRET-FLIM assays.

Bacterial strains

Ralstonia solanacearum (*RSSC*) strains were spread on solid φ medium containing 5 g/L glucose and 0.004% TTC and grown for 3 days at 28°C. Bacteria were grown in liquid φ medium at 28°C under shaking overnight with appropriate antibiotics (Δ*popP2+PopP2* and Δ*popP2+PopP2-C321A* were grown in presence of gentamicin (5 µg/mL) and tetracyclin (5 µg/mL) (Tasset et al., 2010). *Pseudomonas fluorescens* (*Pf0-1*) expressing the PopP2 variants (wild-type and catalytically inactive mutant) were grown on King's B plate supplemented with antibiotics (chloramphenicol 30 µg/mL, tetracycline 5 µg/mL and gentamicin 15 µg/mL) at 28°C overnight. Bacterial cells were centrifugated and resuspended in infiltration buffer (10 mM $MgCl_2$) at OD_{600}=0.2.

METHODS

Plasmid construction

The oligonucleotides used in the experiments presented in this article are listed in Supp Table 6. The full-length *GTE1* (At2g34900), *GTE2* (At5g10550), *GTE3* (At1g73150), *GTE4* (At1g06230), *GTE5* (At1g17790), *GTE6* (At3g52280), *GTE7* (At5g65630), *GTE8* (At3g27260), *GTE9* (At5g14270), *GTE10* (At5g63320), *GTE11* (At1g01770) and *GTE12* (At5g46550) cDNA clones were PCR amplified from Col-0 cDNA using PrimeSTAR Max DNA polymerase (Takara Bio). The coding sequences of Histone 4 and Histone 3 were

amplified from Col-0 genomic DNA using PrimeSTAR Max DNA polymerase (Takara Bio) and PCR products were recombined in pDONR207 to obtain pENTR207-Histone4 and pENTR207-Histone3, respectively. The sequence corresponding to mCherry-Histone4 was generated by two-step PCR. Briefly, overlapping mCherry and Histone4 fragments were amplified using mCherry-Fw/mCherry-H4R and mCherry-H4F/H4-Rev primer pairs, respectively. Full-length mCherry-Histone4 sequence was generated by using the two PCR fragments as templates with AttB1-mCherry-H4 and AttB2-mCherry-H4. All PCR products flanked with AttB1 and AttB2 recombination sites were recombined in pENTR plasmid to generate the relevant pENTR clones. To generate *GTE9-N214A* and *GTE11-N206A* mutants, the corresponding mutations were introduced in pENTR207-GTE9 and pENTR207-GTE11 plasmids by site-directed mutagenesis using the PrimeStar HS DNA polymerase (Takara Bio Inc., Otsu Japan). The truncated cDNA clones corresponding to BRD9, BRD9N-N214A, BRD11, and BRD11N-206A were PCR amplified using pENTR207-GTE9, pENTR207-GTE9-N214A, pENTR207-GTE11 and pENTR207-GTE11-N206A as templates. All DNA constructs were sequence-verified. The inserts cloned in pENTR vectors were then recombined in pDEST vectors via LR reaction (Invitrogen). The expressed proteins *in planta* labelled with a -3HA, -3Flag, -CFP, or -YFP tag were generated by LR reaction in the following plasmid constructs: (i) pAM-PAT-35S-GWY-3HA, -3Flag, -YFP, or -CFP or (ii) pBIN-35S-GWY-3HA, -3Flag, -YFP or -CFP.

A. thaliana stable transformation

Agrobacterium tumefaciens strains carrying the binary vectors (pAM-PAT-35S-GTE9-3Flag and pAM-PAT-35S-GTE11-3Flag in GV3103 strain*;* pBIN-35S-GTE9-3HA, pBIN-35S-GTE11-3HA in GV3101 strain*;* RPS4g/RRS1-Rg in pB7FWG2-D35S-GWY-eGFP) were grown on solid LB medium with appropriate antibiotics for 2 days at 28°C. Bacteria were then cultured in YEB liquid medium (supplemented with antibiotics; gentamicin (15 µg/mL) and carbenicillin (25 µg/mL) for pAM-PAT-based vectors, tetracyclin (5 µg/mL) and kanamycin (25 µg/mL) for pBIN-based vectors, gentamicin (15 µg/mL) and spectinomycin (50 µg/mL) for pB7-based vectors) at 28°C for 12 hours. Cells were centrifugated and resuspended in a sucrose solution (50 g/L) with 0.02% Silwet-L77.

A. thaliana Col-0, *gte9-1* (Salk_0119044C) and *gte11-1* (Salk_059327C) were transformed with *Agrobacterium tumefaciens* using the floral dip method (Clough and Bent, 1998). Primary transformant (T1) were selected on MS medium supplemented with phosphinothricin (10 µg/mL) (Duchefa) for pAM-PAT and pB7-based binary vectors and with Kanamycin (50

ug/mL) for pBIN-based binary vectors. T2 transgenic lines were genotyped by PCR and accumulation of transgenically expressed proteins was verified by immunoblot.

Genomic DNA extraction

Genomic DNA extractions were done using ~100 mg of plant material. After grinding, samples were incubated at 65°C 15 min in CTAB extraction buffer (100 mM Tris-HCl pH7.5; 1.4 M NaCl; 20 mM EDTA pH 7.5; 2% CETAB (Hexadecyltrimethyammonium bromide)). Tubes were agitated 15 min with 500 µL of chloroform and centrifugated 5 min at 13200 rpm. Supernatant (500-700 µL) was transfered in a new tube and mixed with 0.8 volume isopropanol. After centrifugation (13200 rpm, 1 min), pelleted DNA was washed twice with 700 µL ethanol 70%, centrifugated 13200 rpm 3 min, dried and resuspended in 50 µL water with RNAse (1 µg/mL).

RNA extraction, reverse transcription and RT-qPCR

RNA extractions were performed using the Nucleospin RNAII kit (Macherey-Nagel) and following manufacturer's instructions. Reverse transcription was made with Transcriptor Reverse Transcriptase (TRT, Roche). A pre-mix of 7.25 µL (1 µL Oligo $(dT)_{17}$ at 1 µg/µL; 0.25 µL TRT ; 4 µL TRT buffer; 2 µL dNTP-Mix at 10 mM) was added to a final volume of 20 µL containing 1 µg of total RNA. Samples were incubated for 30 minutes at 55°C. Reverse transcription was stopped by incubating the samples at 85°C for 10 minutes. After 5 min on ice, samples were stored at -20°C. RT-qPCR were performed with a LightCycler 480 II machine (Roche Diagnostics) with Roche reagents. Expression of *AtEF1-α* (At5g60390) was used to normalise the expression values. The comparative Ct method ($2^{\Delta Ct}$) was used to represent the data (Schmittgen and Livak, 2008). Mean ΔCt were calculated from three technical replicates for three biological replicates (three independently grown sets of plants). The mean relative expressions from the biological replicates +/- SD was represented with ggplot2 package from R. Statistical analyses were performed on ΔCt values to assess the significance of the difference in the mean ΔCt between the different genetic lines. We applied either a one-way ANOVA when the assumptions about the underlying distribution of the data were fulfilled or a kruskal-test when they were not.

Root-inoculation assays in Arabidopsis

For root-inoculation assays, bacteria (*Rs*) were grown in a liquid culture at 28°C overnight and diluted in water at DO_{600}=0.01. Four-week-old *A. thaliana* plants were soaked in this bacterial solution for 10 min, transferred on soil and incubated in a growth chamber (12h day/12h night, 27°C during the day/26°C at night, 75% relative humidity). Symptoms were scored daily with the following disease scale: 0 = no symptoms, 1 = 25% leaves wilt, 2 = 50%, 3 = 75% and 4 = 100%. For each independent inoculation, between 24 and 32 plants from different lines in *Arabidopsis thaliana* ecotypes were root-inoculated with GMI1000 strain or mutant strains of *R. solanacearum* and wilting symptom developments were scored for 10 days.

For each presented graph, disease scoring data were analysed from two to four independent inoculations with R software as previously described (Aoun et al., 2020). Briefly, we used a mixed model (MIXED procedure in SAS v. 9.4; SAS Institute Inc.) as follow:

$$disease\ index_{ijc} = \mu + block_i + accession_j + covCol_c + \varepsilon_{ijc}$$

Where μ is the overall mean of the phenotypic data, "block" considers environmental conditions between the independent experiments included in the model, "accession" correspond to the tested genetic lines, "covCol" is a covariate accounting for tray effects within blocks and "ε" is the residual term. "block" was considered as a fixed factor and "accession" as a random factor. We tested the significance of the different accessions by likelihood ratio test of model with and without this effect. With p-value<0.05, the lines were considered to have a different disease index reflecting differential response to the pathogen.

Transient expression in *N. benthamiana* and in *A. thaliana*

For transient expression in *N. benthamiana* leaf epidermal cells, *A. tumefaciens* strains grown in liquid YEB medium containing appropriate antibiotics. Cells were harvested by centrifugation and resuspended in infiltration medium (10 mM MES pH5.6, 10 mM $MgCl_2$, 150 μM acetosyringone) at OD_{600}=0.25. For co-expression, each bacterial suspension carrying individual constructs was adjusted to OD_{600} = 0.25 in the final mix for infiltration. After incubation at room temperature for 1 h, bacteria were infiltrated into the leaves of 4-week-old *N. benthamiana* plants using a needleless syringe. Plants were incubated for 36-48h in growth chambers under controlled conditions. Agrobacterium-based transformation for transient gene expression in Arabidopsis Col-0 seedlings was performed as previously described (Li et al., 2009).

Protein Extraction and Immunoblot Analysis

Plant material (4 discs of 8 mm diameter harvested 48 hours post-infiltration for *N. benthamiana* samples; 20 seedlings (10 day-old) for Arabidopsis samples) were grinded and resuspended in 300 µL of Laemmli Buffer (2X). Protein extracts were then denatured for 3 min at 95°C, centrifugated at 13000 rpm for 1 min and then analysed by immunoblotting (SDS-PAGE). Transferred proteins were visualised by Ponceau S red staining. Membranes were blocked in a 2% milk TBS-T (Tris Buffer Saline-Tween 20; 50 mM Tris-HCl pH7.5, 150 mM NaCl, and 0.2% Tween-20) solution before incubation. The following primary antibodies were used in this study: anti-Acetylated Lysine (Ac-K-103, Cell Signaling Technology; dilution 1:2000), anti-HA-HRP (3F10; Roche; dilution 1:5000), anti-GFP (mouse monoclonal; Roche; dilution 1:3000), anti-mCherry (mouse monoclonal; Sigma; dilution 1:2000), anti-Flag-HRP (Sigma; dilution 1:5000). The appropriate horseradish peroxidase-conjugated secondary antibody was applied to the membranes: goat anti-mouse IgG-HRP (Bio-Rad; dilution 1:10000) for detection of anti-GFP and anti-mCherry; goat anti-mouse IgG2a-HRP (Bio-Rad; dilution 1:5000 for detection of anti-Ac-K antibody). Immunodetections were performed using Clarity Western ECL substrate reagent (Bio-Rad).

Fluorescence microscopy

Colocalisation assays were performed as described previously (Tasset et al., 2010). Briefly, *A. tumefaciens* strains carrying CFP- and YFP-tagged constructs were co-infiltrated in the leaves of 4-week-old *N. benthamiana* plants. Leaf samples were taken for imaging between 36-48 hpi. CFP and YFP fluorescence were analysed with laser confocal microscopy (TCS SP2-AOBS and CLSM SP8, Leica, Germany) using a water-immersed objective (63x/1.20 Numerical Aperture, PL APO or 25x/0.95 Numerical Aperture, Fluotar VISIR). A laser excited CFP fluorescence at $\lambda = 458$ nm and recorded in one channel of the confocal microscope (emission spectra: 470-510 nm). YFP was excited at $\lambda = 514$ nm and recorded (emission spectra: 525-585 nm). Images were acquired sequentially with the software Leica LCS version 2.61 and LAS X.

Yeast two-hybrid

Constructs for yeast two-hybrid analysis were prepared in the MatchMaker GAL4 two-hybrid system (Clontech) using the vectors pGBKT7- and pGAD-derived vectors to express bait and prey proteins, respectively. The pDEST pGAD-GWY vector was generated by ligating a Gateway cassette (Frame C) in the pGADT7 vector blunt-ended (klenow fill-in) after

linearisation with NdeI and XhoI restriction enzymes. The pDEST pGBG-GWY vector was generated by ligating a Gateway cassette (Frame C) in the pGBKT7 vector blunt-ended (klenow fill-in) after linearisation with NdeI and SalI restriction enzymes. The cDNA corresponding to *GTE1* to *GTE12* cloned into the relevant pENTR plasmids were recombined in the pGAD-GWY vector. The coding sequence of *PopP2* in pENTR-PopP2 was recombined in pGBG-GWY (bait) vector by LR reaction (Gateway, Invitrogen). All the pDEST plasmids were introduced in *E. coli* cells (DH5α, Stratagene) by electroporation (1mm tank, 1.8 kV, 25 µF and 200 Ohms). Transformed cells were spread on solid LB medium with appropriate antibiotic selection (carbenicillin 50 µg/mL for pGAD vectors, kanamycin 50 µg/mL for pGBG vectors). Plasmid DNAs were extracted with the Wizard DNA plasmid purification kit (Promega). Co-expression of BD-p53 (bait, pGBKT7-p53 vector, Clontech) and AD-T (prey, pGADT7-T vector, Clontech) was used as positive control on selective medium. Bait and prey proteins were tagged with a c-Myc and an HA epitope tag, respectively. Bait and prey constructs were introduced in AH109 *Saccharomyces cerevisae* strain (Clontech) according to the manufacturer's instructions. Transformed cells were spread on SD-TL medium and incubated 3 days at 30°C. Colonies were then dropped by dilution gradient on different media: non-selective (SD-TL) and selective (SD-TLHA) and incubated between 3-5 days at 30°C. Protein expression in yeast was evaluated following incubation of cultures under shaking at 30°C overnight in SD-TL media. Cultures were processed for protein extraction according to a lithium acetate (Liac)/NaOH pre-treatement protocol as previously described (Zhang et al., 2011). Samples were denatured 3 min at 95°C in Laemmli buffer (2X) before separation by SDS-PAGE and immunoblotting with anti-c-Myc-HRP and anti-HA-HRP conjugated antibodies.

FRET-FLIM assays

The FRET-FLIM system we used is made of a mode-locked Ti:sapphire IR femto laser (Tsunami; Spectra Physics, model n°3941) which is the IR laser excitation beam. A Diode-pumped laser (Spectra Physics, model Millenia-Pro 10sJ) modulates the energy of the laser. The laser emits pulses of light thanks to a lock-to-clock electronics module (Spectra Physics, model n°3955) and a pulse selector combined with its electronics module (Spectra Physics, model n°3980 and 3986) adjust the pulse frequency at 2 MHz. The laser system is linked to an inverted microscope acquiring images via the streak camera system. It consists of a streak camera (Hamamatsu, model n°C4334-21) and a CCD camera (Hamamatsu, model C8800-53C).

FRET-FLIM data were obtained by leaf observation of *N. benthamiana* or *A. thaliana* in which transient expression was carried out (leaf samples were harvested at 48 to 72 hpi). Data were analysed with the TAU_POGRAPHY-FLIM software v. 3.1 allowing the acquisition of the fluorescence lifetime of the donor fluorophore, the fluorescence intensity over the time and the proportion of nuclei in relation with the fluorescence lifetime. The measured fluorescence decay is either fit with a mono-exponential fit function for cells expressing donor alone or cells that have a negligible FRET level (Equation 1) or with a bi-exponential fit function for cells expressing both donor and acceptor, revealing FRET (Equation 2):

Equation 1 : $I(t) = \alpha_D \cdot e^{-(t-t_0)/\tau D} + B$

Equation 2 : $I(t) = \alpha_D \cdot e^{-(t-t_0)/\tau D} + \alpha_{DA} \cdot e^{-(t-t_0)/\tau DA} + B$

t_0 is the time origine and B a constant that takes in account the background level of photons. τ_D is the lifetime of the donor alone and τ_{DA} the lifetime of the donor in presence of the acceptor.

α_D and α_{DA} represent the contribution of each exponential in the fluorescence decay. It is then possible to calculate the mean lifetime (τ_m), the apparent FRET efficiency (EF) and the fraction of donor bound to the acceptor (β): $\tau_m = \Sigma\ \alpha_i\ \tau_i^2\ /\ \Sigma\ \alpha_i\ \tau_i$; $EF = 1 - (\tau_{DA}/\tau_D)$; $\beta = \alpha_{DA} / \alpha_{DA} + \alpha_D$

Immunoprecipitation of GTE proteins acetylated on lysine residues

For immunoprecipitation of lysine-acetylated 3HA-tagged GTE proteins in denaturing conditions, plant protein samples were obtained from *N. benthamiana* leaves (4 discs of 8 mm diameter harvested 48 hours post-infiltration) transiently expressing the relevant constructs. Samples were grinded and homogenised in 300 µL of denaturing Buffer (50 mM Tris-HCl pH7.5, 10 mM sodium butyrate, 5 mM EDTA, 2 mM DTT, 1x Protease Inhibitor Cocktail, 1% SDS) and denatured 3 min at 95°C. The extract was centrifuged at 13000 rpm for 2 min at room temperature. 25µL of the supernatant (crude extract) were denatured in 25 µL of Laemmli buffer (4X). The remaining supernatant was mixed with 10 mL of immunoprecipitation buffer (IP1 buffer: 50 mM Tris-HCl pH7.5, 150 mM NaCl, 10 mM sodium butyrate, 5 mM EDTA, 2 mM DTT, 1% Triton). This mix was incubated for 1 hour at 4°C under stirring with 1 µL of anti-acetylated lysine antibody (Cell Signalling). Then, 25 µL of agarose protein A agarose were added to the mix and incubated for 2 hours at 4°C under stirring. Agarose beads were washed three times with 800 µL of IP1 buffer and subsequently denatured for 3 min at 95°C in 40 µL of Laemmli buffer (2X) before analysing the immunoprecipitated proteins by immunoblotting (SDS-PAGE).

For immunoprecipitation of lysine-acetylated GTE9/GTE11 in non-denaturing conditions, plant protein samples were obtained from *N. benthamiana* leaves (4 discs of 8 mm diameter harvested 48 hours post-infiltration) transiently expressing the relevant constructs. Samples were grinded and homogenised in IP2 buffer (50 mM Tris-HCl pH7.5; 150 mM NaCl; 10 mM EDTA; 0.2% Triton; Protease Inhibitor Cocktail 1X; 10 mM sodium butyrate; 2 mM DTT). Tubes were centrifugated 1 min at 14000 rpm at 4°C and 50 µL of the supernatant (crude extract) was denatured in 50 µL of Laemmli buffer (2X) at 95°C for 3 min. A pre-clearing was done by adding 25 µL of Agarose protein A to the remaining supernatant for 10 minutes at 4°C under stirring. After centrifugation (13200 rpm, 1 min), the supernatant was transferred in a new tube and incubated with 1 µL of anti-acetylated lysine antibody (Cell Signaling) for 1 hour at 4°C under stirring. Then, 25 µL of agarose protein A were added to the mix and incubated for 2 hours at 4°C under stirring. Beads were washed twice with 800 µL of IP2 buffer and subsequently denatured for 3 min at 95°C in 40 µL of Laemmli buffer (2X) before analysing the IPs by immunoblotting (SDS-PAGE).

MS-based proteomic analyses

For immunoprecipitation of 3Flag- and YFP-tagged proteins, between 3g and 5g of *N. benthamiana* leaves transiently expressing the relevant constructs were harvested 48 hours after infiltration. Leaf samples were grinded in liquid nitrogen and homogenised in 30-50 mL of extraction buffer (50 mM Tris-HCl pH7.5; 150 mM NaCl; 10 mM EDTA; 10 mM sodium butyrate; 1X Protease Inhibitor Cocktail; 1% triton). After centrifugation (14000 rpm, 10 min, 4°C), the supernatant was filtered with miracloth to get rid of the waste material. 50 µL of the supernatant (crude extract) was denatured 3 min at 95°C. The remaining supernatant was incubated 2 hours at 4°C with 100 µL of anti-Flag M2 affinity gel (Sigma) or GFP-trap agarose (Chromotek). After centrifugation (13200 rpm, 5 min, 4°C) we discarded the supernatant. Beads were washed 3 times in new 1.5 mL-tubes with 1 mL of extraction buffer (with 0.2% triton). Fixed proteins on the beads were then denatured 3 min at 95°C.

Immunoprecipitated proteins were separated by SDS-PAGE before Coomassie blue staining. The bands containing the protein of interest were cut out and submitted to in-gel digestion using trypsin (modified, sequencing purity, Promega), as previously described (Casabona et al., 2013). The resulting peptides were analysed by online nanoliquid chromatography coupled to MS/MS (respectively Ultimate U3000, Dionex, and LTQ-Orbitrap Velos pro, Thermo Fisher Scientific, for the 3FLAG constructs, and UltiMate 3000 RSLCnano and Q-Exactive Plus, Thermo Fisher Scientific, for the YFP constructs). For this purpose, peptides were sampled on

a precolumn (300 µm x 5 mm PepMap C18, ThermoFisher Scientific) and separated by a 25-min gradient in a 75 µm x 150 mm column (PepMap C18, 3µm, Thermo Scientific) for the 3FLAG constructs, or a 60-min gradient in a 75 µm x 250 mm column (Reprosil-Pur 120 C18-AQ, 1.9 µm, Dr. Maisch), for the YFP constructs. The MS and MS/MS data were acquired by Xcalibur (Thermo Fisher Scientific). Peptides and proteins were identified by Mascot (version 2.7.0.1, Matrix Science) through concomitant searches against the Uniprot database (*Nicotiana benthamiana* taxonomy, June 2021 download), specific sequences of tagged proteins (homemade), and a homemade database containing the sequences of classical contaminant proteins found in proteomic analyses (human keratins, trypsin, etc.). Trypsin/P was chosen as the enzyme and two missed cleavages were allowed. Precursor and fragment mass error tolerances were set at respectively at 10 ppm and 0.6 Da for LTQ-Orbitrap Velos Pro data, and 10 and 20 ppm for Q-Exactive Plus data. Peptide modifications allowed during the search were: Carbamidomethyl (C, fixed), Acetyl (Protein N-term, variable), Acetyl (K, variable), Phospho (S/T/Y, variable), and Oxidation (M, variable). The Proline software (Bouyssié et al., 2020) was used for the compilation, grouping, and filtering of the results (conservation of rank 1 peptides, peptide length ≥ 6 amino acids, peptide score ≥ 25, and minimum of one specific peptide per identified protein group). Proline was then used to perform a compilation, grouping and spectral counting-based comparison of the protein groups identified in the different samples. MS data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (Perez-Riverol et al., 2019) with the dataset identifier PXD029367. The acetylation sites were taken into account if their localisation probability was above 75%.

SPOT peptide array

Recombinant proteins were expressed in Rosetta cells (Novagen) transformed with pDUET-His_6-BRD9 or pDUET-His_6-BRD9-N214A. Briefly, for each construct, bacteria were precultured in 50 mL liquid L medium with appropriate antibiotics (carbenicillin (50µg/mL) and chloramphenicol (30 µg/mL)) at 37°C for 12 hours under stirring (180 rpm). The precultures were diluted in 1L liquid L medium (ration 1:100, with antibiotics) at 37°C under stirring (180 rpm). When the OD_{600nm} reaches 0.6, incubator temperature was decreased at 18°C. One hour later, protein expression was induced with 0.2 mM IPTG and cells were incubated overnight at 18°C under stirring. Cultures were centrifuged (6500 rpm, 20 min, 4°C) and pellets were resuspended in lysis buffer (500 mM NaCl, 20 mM HEPES pH7.5, 10 mM Imidazole, 1 mM TCEP, 1:1000 protease inhibitor cocktail) (for 10 g of bacterial pellet, 100 mL lysis buffer was added). After bacterial resuspension, cells were sonicated for 21 min (35%

amplitude, 5 seconds ON and 10 seconds OFF) at 4°C. Samples were then centrifuged (16000 rpm, 1 hour, 4°C). For each sample, 5 mL Ni (Nickel) beads beforehand balanced were added to the supernatant. Samples were stirred 1 hour at 20 rpm at 4°C and then poured in affinity column. 50 µL of the flow-through was denatured 3 min at 95°C in 50 µL Laemmli buffer (4X). Proteins bound to the Ni beads were eluted with buffer A (500 mM NaCl, 5% glycerol, 20 mM HEPES pH7.5) added with different Imidazole concentrations (1st fraction: 30 mM, 2nd fraction: 60 mM, 3rd fraction: 90 mM, 4th fraction: 120 mM, 5th fraction: 240 mM, 6th fraction: 300 mM) at 4°C. For all the eluted fractions, 50 µL aliquots were mixed with 50 µL Laemmli buffer (4X) and denatured at 95°C for 3 min. Samples were loaded on SDS-PAGE gel. After migration, the gel was stained with Coomassie Blue for 1 hour and washed overnight in sterile nanopure water. Fractions with the most concentrated and the purest proteins were selected for the next steps. Purified proteins were then concentrated with successive centrifugations (3500 rpm, 3 min, 4°C) using an Amicon Ultra-15 centrifugal spin (10 kDa). Concentrated proteins were then separated according to their size by exclusion chromatography, and collected fractions were analysed with Q-TOF. 50 µl of the fractions containing the protein of interest were mixed with 50 µL Laemmli buffer (4X) and denatured at 95°C for 3 min. After migration on SDS-PAGE gel and Coomassie Blue staining, fractions in which our protein was detected were selected for Q-TOF analysis. The SPOT array was performed as previously described (Filippakopoulos et al., 2012). Briefly, peptides corresponding to the N-terminal tails of the histones H2A, H2B, H3 and H4 (non-acetylated or acetylated on different lysine residues) were synthetised on a cellulose membrane thanks to a MultiPep SPOT peptide arrayer (Intavis). His_6-tagged BRD9 and BRD9-N214A were added at a 1 mM final concentration and blots were developed with the ECL kit (Thermo Scientific) following the manufacturer's instructions.

Pseudomonas fluorescens-mediated delivery of PopP2 in Arabidopsis

For *Pf0-1*-mediated delivery, full-length PopP2 and PopP2-C321A recombined in the pBBR-AvrRps4prom-GWY-3HA gateway destination vector were used as previously described (Le Roux et al., 2015). Transformed *Pf0-1* cells were spread on King's B agar supplemented with 6mM MgS04 and with appropriate antibiotics (tetracycline 5 µg/mL, chloramphenicol 30 µg/mL, and gentamicin 15 µg/mL). Bacteria were resuspended in 500 µL $MgCl_2$ (10 mM) and centrifuged (8000 rpm, 1 min). Bacteria were resuspended in 500 µL $MgCl_2$ (10 mM) and centrifuged again (8000 rpm, 1 min) to wash out antibiotics. Bacterial pellet was resuspended in 500 µL $MgCl_2$ (10 mM) solution and bacterial density was adjusted to OD600=0,2 (OD_{600nm} = 1 means 1.75 x 10^8 bacteria/mL). Three-week-old plants were placed in a humid environment

14 hours before infiltration to facilitate stomata opening. Bacteria were infiltrated in the plant leaves with a needleless syringe. ~12 leaves were infiltrated for each plant tested and the PopP2-tiggered cell death was observed 48 hours after infiltration.

Quantification and statistical analysis

For FRET-FLIM analyses, significance of the difference between the donor lifetimes in the absence and in the presence of acceptor was assessed by a Student's *t* test. Mass-spectrometry based quantitative proteomics to assess acetylated residues on peptides parameters regarding precursor and fragment mass error tolerances were set at respectively at 10 ppm and 0.6 Da for LTQ-Orbitrap Velos Pro data, and 10 and 20 ppm for Q-Exactive Plus data. For the compilation, grouping, and filtering of the results (conservation of rank 1 peptides, peptide length ≥ 6 amino acids, peptide score ≥ 25, and minimum of one specific peptide per identified protein group), the Proline software (Bouyssié et al., 2020) was used. Proline was then used for compilation, grouping and spectral counting-based comparison of the protein groups identified in the different samples. The acetylation sites were considered accurate when their localisation probability was above 75%. For the *Rs* inoculations, disease symptom scoring was analysed and plot with R software. Graphs show least-square means +/- Standard Error of the LS means represented as linear plots with points at each day when symptoms were scored. Significance of the difference of disease index LS means at a given day between different genotypes was assessed with a one-way ANOVA performed on a mixed model generated from the data as previously used (Aoun et al., 2020). At a given day, significance of the difference between LS means was set at p-value<0.05. For RT-qPCR, data were analysed and represented with R software using the comparative Ct method ($2^{\Delta Ct}$) (Schmittgen and Livak, 2008).

Mean ΔCt were calculated from three technical replicates for three biological replicates (three independently grown sets of plants). The mean relative expressions from the biological replicates +/- SD was represented in barplots. ΔCt values were used to assess the significance of the difference in ΔCt between the different genetic lines. A one-way ANOVA was applied when the assumptions about the underlying distribution of the data were fulfilled, or a kruskal-test when they were not.

Data and code availability

All originally unprocessed photos of immunoblots and yeast two-hybrid assays presented in this study can be accessed through https://data.mendeley.com/datasets/f34jsmktfs/draft?a=3290f3f8-c230-4d8a-8f36-

ff4f2789a26f in Mendeley Data. MS-based proteomic data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository (Perez-Riverol et al., 2019) with the dataset identifier PXD029367. Before acceptance, the data can be accessed using the following link : https://www.ebi.ac.uk/pride/login (Username: reviewer_pxd029367@ebi.ac.uk ; Password: YaSg2asZ).

Supplementary Tables 3 and 4 can be accessed via the following link in Mendeley Data: https://data.mendeley.com/datasets/zysnkkp8vz/1

Transient assay in	Donor	Acceptor	τ (ns)*	Δ (ps)(a)	SD (b)	sem (c)	N (d)	p-value	E (%) (e)
N. benthamiana	PopP2-CFP	-	3,097	-	0,078	0,014	30	-	-
N. benthamiana	PopP2-CFP	GTE11-YFP	2,5	597	0,23	0,025	83	5.10^{-28}	19,3
N. benthamiana	PopP2-CFP	GTE9-YFP	2,224	873	0,115	0,011	119	$1,6.10^{-3}$	28,2
N. benthamiana	PopP2-CFP	YFP	2,993	104	0,123	0,022	30	2.10^{-1}	-
N. benthamiana	C321A-CFP	-	3,08	-	0,055	0,01	29	-	-
N. benthamiana	C321A-CFP	GTE11-YFP	2,294	786	0,247	0,025	93	$1,5.10^{-33}$	25,5
N. benthamiana	C321A-CFP	GTE9-YFP	2,462	618	0,202	0,026	60	$5,4.10^{-28}$	20,1
N. benthamiana	CFP-H4	-	2.945	-	0.177	0.020	78	-	-
N. benthamiana	CFP-H4	GTE9-YFP	2.432	513	0.300	0.032	87	2 10-27	17.4
N. benthamiana	CFP-H4	GTE9-N214A-YFP	2.965	20	0.276	0.049	32	0.65	-
N. benthamiana	CFP-H4	GTE11-YFP	2.455	490	0.200	0.047	18	4 10-17	16.3
N. benthamiana	CFP-H4	GTE11-N206A-YFP	2.864	81	0.107	0.036	9	0.18	-
N. benthamiana	CFP-H3	-	2.743	-	0.212	0.033	40	-	-
N. benthamiana	CFP-H3	GTE9-YFP	2.773	31	0.198	0.030	44	0.49	-

Supplementary Table 1. **FRET-FLIM measurements showing that GTE9 and GTE11 interact with PopP2 and histone H4 in nuclei of *N. benthamiana* cells.**

(a) Mean lifetime, τ, in nanoseconds (ns). For each nucleus, average fluorescence decay profiles were plotted and fitted with exponential function using a non linear square estimation procedure and the mean lifetime was calculated according to $t = \Sigma a_i t_i^2 / \Sigma a_i ti$ with $I(t) = \Sigma a_i e^{-t/ti}$, (b) Δt=tD – tDA (in ns), (c) Standard error of the mean, (d) Total number of measured nuclei, (e) % FRET efficiency: E=1 – (τDA/τD), and (f) p-value of the difference between the donor lifetimes in the absence and in the presence of acceptor (Student's *t* test). The statistical test used was two-sided. The lifetime measurements were carried out from at least two independent expression assays performed in *N. benthamiana* (leaf samples were taken between 36 and 48 hours after infiltration with *A. tumefaciens*).

Site	GTE9-3xFLAG		GTE9-YFP		GTE9-BRD-YFP	
	SC PopP2 C321A	SC PopP2 WT	SC PopP2 C321A	SC PopP2 WT	SC PopP2 C321A	SC PopP2 WT
K40	0	1	0	1		
K49	0	2	0	7		
K81	**0**	**1**	**0**	**3**		
K103	0	1	0	4		
K116	0	3	1	21	2	3
K126	**0**	**2**	**0**	**11**	**0**	**1**
K240	0	1	0	3		
K241	**0**	**1**	**0**	**3**	**0**	**2**
K246	**0**	**1**	**0**	**5**	**0**	**1**
K260	**0**	**1**	**0**	**6**		
K273	0	2	1	4		
K434	0	1	0	2		
K443	0	2	0	7		
K506	0	1	0	7		
K523	0	1	0	1		
K563	0	1	0	2		

Site	GTE11-3xFLAG		GTE11-YFP		GTE11-BRDmut-YFP	
	SC PopP2 C321A	SC PopP2 WT	SC PopP2 C321A	SC PopP2 WT	SC PopP2 C321A	SC PopP2 WT
K72	**0**	**1**	**0**	**2**		
K118	**0**	**5**	**1**	**9**	**0**	**1**
K179					0	1
K233	**0**	**1**	**0**	**4**	**0**	**1**
K238	**0**	**3**	**0**	**5**	**0**	**1**
K250	**0**	**3**	**0**	**4**		
K260	0	1	0	3		
K277	0	1	0	1		
K404	0	2	0	2		
K454	0	1	0	1		
K474	0	1	0	1		

Supplementary Table 2. Acetylation sites reproducibly identified by MS-based proteomics in GT9 and GTE11 co-expressed either with PopP2 or the catalytically inactive PopP2-C321A mutant. GTE9 and GTE11, or the bromodomains of GT9 (BRD9) and GTE11 (BRD11-N206A), were transiently co-expressed with either wild type PopP2 or PopP2-C321A in N. benthamiana and immunopurified before MS-based proteomic analyses. The table lists the acetylation sites (localisation probability ≥ 75%) identified in both 3xFLAG- and YFP-tagged constructs for full-length proteins and YFP-tagged bromodomain-containing constructs (SC = spectral counts). The Lys-acetylated residues whose position is conserved between GTE9 and GTE11 proteins are shaded in grey. The total list of identified peptides and sites is given in Supplemental Table 3.

Donor	Acceptor	In presence of	Ƭ1 (ns)[a]	$SD_{Ƭ1}$[b]	$sem_{Ƭ1}$[c]	Ƭ2 (ns)[a]	$SD_{Ƭ2}$[b]	$sem_{Ƭ2}$[c]	Δ (ps)[d]	Ƭm (ns) [e]	$SD_{Ƭm}$[b]	$sem_{Ƭm}$[c]	N[f]	p-value[g]	E (%)[h]
GTE9-eGFP	mCherry-H4	-	2.895	0.199	0.037	2.229	0.263	0.049	665	2.633	0.149	0.028	28	-	22 +/- 2.3%
GTE9-eGFP	mCherry-H4	PopP2-3HA	2.842	0.221	0.028	2.645	0.280	0.036	197	2.691	0.101	0.013	60	2.2×10^{-9}	6.7 +/-1.2%
GTE9-eGFP	mCherry-H4	C321A-3HA	2.887	0.105	0.014	2.413	0.205	0.027	474	2.754	0.080	0.011	56	2.7×10^{-2}	16 +/- 1.8%
GTE9-N214A-eGFP	mCherry-H4	-	2.784	0.084	0.015	2.689	0.090	0.017	95	2.738	0.052	0.009	30	-	3.3 +/- 0.9%
GTE9-N214A-eGFP	mCherry-H4	PopP2-3HA	2.766	0.081	0.015	2.706	0.093	0.017	60	2.737	0.072	0.013	30	4.7×10^{-1}	2.1 +/- 0.6%
GTE9-N214A-eGFP	mCherry-H4	C321A-3HA	2.777	0.103	0.019	2.682	0.106	0.019	95	2.731	0.046	0.008	30	8.1×10^{-1}	3.1 +/- 1.1%

Supplementary Table 5. Quantitative FRET-FLIM measurements showing that catalytically active PopP2 alters the ability of GTE9 to interact with histone H4 in nuclei of *N. benthamiana* cells.

[a] Estimated values of fluorescence lifetime (in nanoseconds, ns) when there is no FRET (Ƭ1) and when there is FRET (Ƭ2). These parameters were calculated from the fluorescence intensity data after the observation of N nuclei for each combination. [b] Standard deviation, [c] Standard error of the mean, [d] Δ=Ƭ1 – Ƭ2, [e] Ƭm, the mean lifetime lifetime in nanoseconds (ns), [f] N is the number of measured nuclei, [g] p-value of the difference between the donor lifetimes and in the presence of acceptor (Student's *t* test) , [h] % FRET efficiency: E=(1 – (Ƭ2/Ƭ1))*100. For each nucleus, average fluorescence decay profiles were plotted and fitted with exponential function using a non-linear square estimation procedure and the mean lifetime was calculated according to Ƭm=Σαie-t/Ƭi.

Table S6. Primers used in this study.

Primer	Sequence (5' to 3')	Cloning/sequencing/RT-qPCR
AttB1-GTE1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCTGTACATGTCAAGGAA	GTE1 cDNA (At2g34900)
AttB2-GTE1	GGGACCACTTTGTACAAGAAAGCTGGGTCAGCTTTCTTAGCTCTTTTAATTG	
AttB1-GTE2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGATCGGGCAACGTCATT	GTE2 cDNA (At5g10550)
AttB2-GTE2	GGGACCACTTTGTACAAGAAAGCTGGGTCACACTCAAAATCTTTTCTTTCGA	
GTE2-500	TCAGGGAGCTGAAGAAGCGTCTCAA	sequencing primer
GTE2-1000	CAAAGGGTCAAGATGTTTATTTGAT	sequencing primer
GTE2-1500	CGTGAATCTACAAGAATTGCCTCCT	sequencing primer
AttB1-GTE3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGTCTGGTCCTATAGC	GTE3 cDNA (At1g73150)
AttB2-GTE3	GGGACCACTTTGTACAAGAAAGCTGGGTCATTACCGGTATCAGATTCATGT	
GTE3-500	TATTAAGGAGCCTATGGATTTAGGA	sequencing primer
AttB1-GTE4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTTCGGAGCCTGTTAA	GTE4 cDNA (At1g06230)
AttB2-GTE4	GGGACCACTTTGTACAAGAAAGCTGGGTCGGTCTGATCTGATCCAGATGATGA	
GTE4-500	TTGATGAAAACTCTATCAAGGAACC	sequencing primer
GTE4-1000	AATGGAGGAGGGAGGATTCTGTCAG	sequencing primer
GTE4-1500	TGCTATGACTTACAACCCAGAAGGA	sequencing primer
AttB1-GTE5	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCTTCTGAACATATATCAG	GTE5 cDNA (At1g17790)
AttB2-GTE5	GGGACCACTTTGTACAAGAAAGCTGGGTCATTACCATTATCTGATCCACGT	
GTE5-500	AAGCACCTGTTAACAACAGGGACCT	sequencing primer
AttB1-GTE6	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTGACTCAGTGCCAG	GTE6 cDNA (At3g52280)
AttB2-GTE6	GGGACCACTTTGTACAAGAAAGCTGGGTCTATGCGTGACCGCTTCGTTT	
AttB1-GTE7	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTCCGGCTGTTTTCG	GTE7 cDNA (At5g65630)
AttB2-GTE7	GGGACCACTTTGTACAAGAAAGCTGGGTCACATTGGGCTTCTTTTGCTTC	
GTE7-500	TCCGTCGGATCCGGAGTCGGAGAAA	sequencing primer
GTE7-1000	GAAGCTTGATTCAGTGAAGCCGCCA	sequencing primer
AttB1-GTE8	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTTGAAAGTGCTGCGTT	GTE8 cDNA (At3g27260)
AttB2-GTE8	GGGACCACTTTGTACAAGAAAGCTGGGTCGATTTCACCTTCTTCCACGTC	
GTE8-500F	GTACCTCAGGGAAGTTTGAGTCTTC	sequencing primer
GTE8-1000F	AGAACCGGTGAAGCCACTTATGACA	sequencing primer
GTE8-1500F	TTGTTGGGAGTCAATCTACTGGTGC	sequencing primer
GTE8-2000F	TACCAAGCTCAGCCGAAGAAACCAG	sequencing primer
AttB1-GTE9	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACAGAGAGAAACGGTG	GTE9 cDNA (At5g14270)
AttB2-GTE9	GGGGACCACTTTGTACAAGAAAGCTGGGTCATCAATCTCTCCCTCTTCTA	
GTE9-500F	GTGTACATACATGAATGAACCT	sequencing primer
GTE9-1000F	CGCTTCAGTTGGATCATCGATT	sequencing primer
GTE9-1500F	AGTTGATTCGTAGGCTAAGACA	sequencing primer
GTE9-2000F	TCATACTGAACCGAGTAACTTA	sequencing primer
GTE9-2500F	TCTATTGCAGCTTCTGCATGGA	sequencing primer
GTE9-3000F	CTGGGTTGTACTACTGATTGCT	sequencing primer
GTE9-3500F	CAACAAGAGGATGTGAGTGGAT	sequencing primer
GTE9-4000F	TAAATCAACTGTAGTTGGTGAC	sequencing primer
GTE9-1350-R	TCGGTAAACTTGACATTTTAGGATCA	sequencing primer
AttB1-GTE10	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGTAAAGCGCGGAAACA	GTE10 cDNA (At5g63320)
AttB2-GTE10	GGGACCACTTTGTACAAGAAAGCTGGGTCAATTTCTACATCTATATCTTCCT	
GTE10-500	AGTGATGAAGGAGTGTGAGACGTTA	sequencing primer
GTE10-1000	CAAGATCTGATGGCATTAGAAGAAG	sequencing primer
GTE10-1500	AGAGGATGATAGCAACAGTGAGAAG	sequencing primer
GTE10-2000	AGACCTCCAGATGCTTAGAGCTACA	sequencing primer
GTE10-2500	GATATGGGAGTGGAAGAAGTACATC	sequencing primer
AttB1-GTE11	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACTGTGAGGAACGGTG	GTE11 cDNA (At1g01770)
AttB2-GTE11	GGGGACCACTTTGTACAAGAAAGCTGGGTCGTCGATTTCACCCTCTTCTA	
GTE11-500F	GCGCGTGCCGTTCCTGCGAT	sequencing primer
GTE11-1000F	CATATTGAGTGTTCACTGCT	sequencing primer
GTE11-1500F	AGAGCCAGTCACCACTTCCA	sequencing primer
GTE11-2000F	ATGACAGATGAGGATAGAGT	sequencing primer
GTE11-2500F	ACATCTCAACTGTCAGAACA	sequencing primer
GTE11-3000F	CAGATGGTCATCAAGATGGT	sequencing primer
GTE11-3500F	CCAAGGAAGCTGAAGAAGCT	sequencing primer
GTE11-1350g-R	TGAGATGTGAGATATAGGGTGC	sequencing primer
AttB1-GTE12	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTTGCGATACCCAATATT	GTE12 cDNA (At5g46550)
AttB2-GTE12	GGGACCACTTTGTACAAGAAAGCTGGGTCAAGGATCTCTCCTTCCTCAAG	
GTE12-500	TTGAGGTTAGATGGGAATCACTCAT	sequencing primer
GTE9-N214A-F	CAATGACGTATGCTCCACCTGGTAATGATGTTTAT	GTE9 N214A mutant
GTE9-N214A-R	TACCAGGTGGAGCATACGTCATTGCATTGCTGAAG	
GTE11-N206A-F	TGCTATGACTTATGCCCCGTCAGATAATAATGTGTATC	GTE11 N206A
GTE11-N206A-R	TTATCTGACGGGGCATAAGTCATAGCATTGCGGAAA	
AttB1-BRD9	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACGGGTCCAGGAAAGACG	BRD9
AttB2-BRD9	GGGGACCACTTTGTACAAGAAAGCTGGGTCATTTAGTTCCGGATAATTTCTT	
AttB1-BRD11	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGACTGGCCCTGGAAAAAGG	BRD11
AttB2-BRD11	GGGGACCACTTTGTACAAGAAAGCTGGGTCATTTGGTTCCGGATGACTTCT	
AttB1-Histone4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTCAGGAAGAGGAAAAG	Histone 4 (At2g28740)
AttB2-Histone4	GGGACCACTTTGTACAAGAAAGCTGGGTCAACCACCAAATCCATATAGAG	
AttB1-Histone3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTCGTACCAAGCAAA	Histone 3.3 (At4g40030)
AttB1-Histone3	GGGACCACTTTGTACAAGAAAGCTGGGTCTTAAGCGCGTTCACCTCTGAT	
mCherry-Fw	ATGGTGAGCAAGGGCGAGGAGGATAA	mCherry-Histone H4
mCherry-H4R	TCCTCTTCCTGACATGGACCCTCCTCGAGATCTGAGTCCGGA	
mCherry-H4F	ACTCAGATCTCGAGGAGGGTCCATGTCAGGAAGAGGAAAAGGA	
H4-Rev	AACCACCAAATCCATATAGAGTTCTTCCTTGT	
AttB1-mCherry-H4	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGTGAGCAAGGGCGA	
AttB2-mCherry-H4	GGGACCACTTTGTACAAGAAAGCTGGGTCAACCACCAAATCCATATAGA	
AtEF1-F	CTGGAGGTTTTGAGGCTGGTAT	RT-qPCR primer
AtEF1-R	CCAAGGGTGAAAGCAAGAAGA	RT-qPCR primer
GTE11-q-2-F	CGATTTGCGGATATAATCTTGA	RT-qPCR primer
GTE11-q-2-R	TTCCTTCTCACGTTGCAGTG	RT-qPCR primer
GTE9-q-2&3-R	CTTGACATTTTAGGATCACC	RT-qPCR primer
GTE9-q-2-F	CATTGGTGAAAATGAACACC	RT-qPCR primer
RRS1-R-ex5-q-F	GGGCAAAGAAATCCTCCAT	RT-qPCR primer
RRS1-R-ex5-q-R	CTTTCGCGATGTTTCTT	RT-qPCR primer

REFERENCES

Adie, B.A.T., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., and Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell *19*, 1665–1681.

Ahmad, A.A., Addy, H.S., and Huang, Q. (2021). Biological and Molecular Characterization of a Jumbo Bacteriophage Infecting Plant Pathogenic Ralstonia solanacearum Species Complex Strains. Front. Microbiol. *12*, 1–15.

Airoldi, C.A., Rovere, F. Della, Falasca, G., Marino, G., Kooiker, M., Altamura, M.M., Citterio, S., and Kater, M.M. (2010). The Arabidopsis BET Bromodomain Factor GTE4 Is Involved in Maintenance of the Mitotic Cell Cycle during Plant Development. PLANT Physiol. *152*, 1320–1334.

Alazem, M., Lin, K.Y., and Lin, N.S. (2014). The abscisic acid pathway has multifaceted effects on the accumulation of Bamboo mosaic virus. Mol. Plant-Microbe Interact. *27*, 177–189.

Albertazzi, L., Arosio, D., Marchetti, L., Ricci, F., and Beltram, F. (2009). Quantitative FRET analysis with the E0GFP-mCherry fluorescent protein pair. Photochem. Photobiol. *85*, 287–297.

Alfano, J.R., and Collmer, A. (2004). Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. Annu. Rev. Phytopathol. *42*, 385–414.

Álvarez, B., and Biosca, E.G. (2017). Bacteriophage-based bacterial wilt biocontrol for an environmentally sustainable agriculture. Front. Plant Sci. *8*, 1–7.

Aoun, N., Desaint, H., Boyrie, L., Bonhomme, M., Deslandes, L., Berthomé, R., and Roux, F. (2020). A complex network of additive and epistatic quantitative trait loci underlies natural variation of Arabidopsis thaliana quantitative disease resistance to Ralstonia solanacearum under heat stress. Mol. Plant Pathol. *21*, 1405–1420.

Arbibe, L., Kim, D.W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C., and Sansonetti, P.J. (2007). An injected bacterial effector targets chromatin access for transcription factor NF-κB to alter transcription of host genes involved in immune responses. Nat. Immunol. *8*, 47–56.

Baggs, E., Dagdas, G., and Krasileva, K. V. (2017). NLR diversity, helpers and integrated domains: making sense of the NLR IDentity. Curr. Opin. Plant Biol. *38*, 59–67.

Balint-Kurti, P. (2019). The plant hypersensitive response: concepts, control and consequences. Mol. Plant Pathol. *20*, 1163–1178.

Ballaré, C.L., and Austin, A.T. (2019). Recalculating growth and defense strategies under competition: Key roles of photoreceptors and jasmonates. J. Exp. Bot. *70*, 3425–3436.

Barnes, S.N., Wram, C.L., Mitchum, M.G., and Baum, T.J. (2018). The plant-parasitic cyst nematode effector GLAND4 is a DNA-binding protein. Mol. Plant Pathol. *19*, 2263–2276.

Baulcombe, D. (2004). RNA silencing in plants. Nature *431*, 356–363.

Benhamed, M., Martin-Magniette, M.L., Taconnat, L., Bitton, F., Servet, C., De Clercq, R., De Meyer, B., Buysschaert, C., Rombauts, S., Villarroel, R., et al. (2008). Genome-scale Arabidopsis promoter array identifies targets of the histone acetyltransferase GCN5. Plant J. *56*, 493–504.

Berger, S.L. (2007). The complex language of chromatin regulation during transcription. Nature *447*, 407–412.

Bergsma-Vlami, M., van de Bilt, J.L.J., Tjou-Tam-Sin, N.N.A., Westenberg, M., Meekes, E.T.M., Teunissen, H.A.S., and van Vaerenbergh, J. (2018). Phylogenetic Assignment of Ralstonia pseudosolanacearum (Ralstonia solanacearum Phylotype I) Isolated from Rosa spp. Plant Dis. *102*, 2258–2267.

Bernoux, M., Ve, T., Williams, S., Warren, C., Hatters, D., Valkov, E., Zhang, X., Ellis, J.G., Kobe, B., and Dodds, P.N. (2011). Structural and functional analysis of a plant resistance protein TIR domain reveals interfaces for self-association, signaling, and autoregulation. Cell Host Microbe *9*, 200–211.

Berr, A., McCallum, E.J., Alioua, A., Heintz, D., Heitz, T., and Shen, W.H. (2010). Arabidopsis histone methyltransferase SET DOMAIN GROUP8 mediates induction of the jasmonate/ethylene pathway genes in

plant defense response to necrotrophic fungi. Plant Physiol. *154*, 1403–1414.

Bi, G., Su, M., Li, N., Liang, Y., Dang, S., Xu, J., Hu, M., Wang, J., Zou, M., Deng, Y., et al. (2021). The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. Cell *184*, 3528–3541.

Bierne, H., and Cossart, P. (2012). When bacteria target the nucleus: The emerging family of nucleomodulins. Cell. Microbiol. 622–633.

Bigeard, J., Colcombet, J., and Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). Mol. Plant *8*, 521–539.

Block, A., Toruño, T.Y., Elowsky, C.G., Zhang, C., Steinbrenner, J., Beynon, J., and Alfano, J.R. (2014). The Pseudomonas syringae type III effector HopD1 suppresses effector-triggered immunity, localizes to the endoplasmic reticulum, and targets the Arabidopsis transcription factor NTL9. New Phytol. *201*, 1358–1370.

Boccara, M., Sarazin, A., Thiébeauld, O., Jay, F., Voinnet, O., Navarro, L., and Colot, V. (2014). The Arabidopsis miR472-RDR6 Silencing Pathway Modulates PAMP- and Effector-Triggered Immunity through the Post-transcriptional Control of Disease Resistance Genes. PLoS Pathog. *10*, 1–16.

Boch, J., Bonas, U., and Lahaye, T. (2014). TAL effectors - pathogen strategies and plant resistance engineering. New Phytol. *204*, 823–832.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. *60*, 379–407.

Bouyssié, D., Hesse, A.M., Mouton-Barbosa, E., Rompais, M., MacRon, C., Carapito, C., Gonzalez De Peredo, A., Couté, Y., Dupierris, V., Burel, A., et al. (2020). Proline: An efficient and user-friendly software suite for large-scale proteomics. Bioinformatics *36*, 3148–3155.

Bozkurt, T.O., and Kamoun, S. (2020). The plant-pathogen haustorial interface at a glance. J. Cell Sci. *133*, 1–6.

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Miret, J.A.J., Justesen, A.F., Macleod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., et al. (2019). Pest categorisation of the ralstonia solanacearum species complex. EFSA J. *17*, 1–28.

Broekgaarden, C., Caarls, L., Vos, I.A., Pieterse, C.M.J., and Van Wees, S.C.M. (2015). Ethylene: Traffic controller on hormonal crossroads to defense. Plant Physiol. *169*, 2371–2379.

Buddenhagen, I., and Kelman, A. (1964). Biological and Physiological Aspects of Bacterial Wilt Caused by Pseudomonas Solanacearum. Annu. Rev. Phytopathol. *2*, 203–230.

Buhrow, L.M., Cram, D., Tulpan, D., Foroud, N.A., and Loewen, M.C. (2016). Exogenous abscisic acid and gibberellic acid elicit opposing effects on Fusarium graminearum infection in wheat. Phytopathology *106*, 986–996.

van der Burgh, A.M., and Joosten, M.H.A.J. (2019). Plant Immunity: Thinking Outside and Inside the Box. Trends Plant Sci. *24*, 587–601.

van Butselaar, T., and Van den Ackerveken, G. (2020). Salicylic Acid Steers the Growth–Immunity Tradeoff. Trends Plant Sci. *25*, 566–576.

Cai, R., Lewis, J., Yan, S., Liu, H., Clarke, C.R., Campanile, F., Almeida, N.F., Studholme, D.J., Lindeberg, M., Schneider, D., et al. (2011). The plant pathogen pseudomonas syringae pv. tomato is genetically monomorphic and under strong selection to evade tomato immunity. PLoS Pathog. *7*, 1–15.

Caldwell, D., Kim, B.S., and Iyer-Pascuzzi, A.S. (2017). Ralstonia solanacearum differentially colonizes roots of resistant and susceptible tomato plants. Phytopathology *107*, 528–536.

Campos, M.L., Kang, J.-H., and Howe, G.A. (2014). Jasmonate-Triggered Plant Immunity. J. Chem. Ecol. *40*, 657–675.

Cao, Y., Liang, Y., Tanaka, K., Nguyen, C.T., Jedrzejczak, R.P., Joachimiak, A., and Stacey, G. (2014). The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. Elife *3*, 1–19.

Casabona, M.G., Vandenbrouck, Y., Attree, I., and Couté, Y. (2013). Proteomic characterization of

Pseudomonas aeruginosa PAO1 inner membrane. Proteomics *13*, 2419–2423.

Castel, B., Ngou, P.M., Cevik, V., Redkar, A., Kim, D.S., Yang, Y., Ding, P., and Jones, J.D.G. (2019). Diverse NLR immune receptors activate defence via the RPW8-NLR NRG1. New Phytol. *222*, 966–980.

Castillo-González, C., Liu, X., Huang, C., Zhao, C., Ma, Z., Hu, T., Sun, F., Zhou, Y., Zhou, X., Wang, X.-J., et al. (2015). Geminivirus-encoded TrAP suppressor inhibits the histone methyltransferase SUVH4/KYP to counter host defense. Elife *4*, 1–31.

Cech, T.R., and Steitz, J.A. (2014). The noncoding RNA revolution - Trashing old rules to forge new ones. Cell *157*, 77–94.

Cernadas, R.A., Doyle, E.L., Niño-Liu, D.O., Wilkins, K.E., Bancroft, T., Wang, L., Schmidt, C.L., Caldo, R., Yang, B., White, F.F., et al. (2014). Code-Assisted Discovery of TAL Effector Targets in Bacterial Leaf Streak of Rice Reveals Contrast with Bacterial Blight and a Novel Susceptibility Gene. PLoS Pathog. *10*, 1–24.

Césari, S., Kanzaki, H., Fujiwara, T., Bernoux, M., Chalvon, V., Kawano, Y., Shimamoto, K., Dodds, P., Terauchi, R., and Kroj, T. (2014). The NB - LRR proteins RGA 4 and RGA 5 interact functionally and physically to confer disease resistance . EMBO J. *33*, 1941–1959.

Champoiseau, P.G., Jones, J.B., and Allen, C. (2009). Ralstonia solanacearum Race 3 Biovar 2 Causes Tropical Losses and Temperate Anxieties . Plant Heal. Prog. *10*, 35.

Chan, C., and Zimmerli, L. (2019). The Histone Demethylase IBM1 Positively Regulates Arabidopsis Immunity by Control of Defense Gene Expression. Front. Plant Sci. *10*, 1–10.

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K., and Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. J. Integr. Plant Biol. *62*, 25–54.

Chen, L., Shirota, M., Zhang, Y., Kiba, A., Hikichi, Y., and Ohnishi, K. (2014). Involvement of HLK effectors in Ralstonia solanacearum disease development in tomato. J. Gen. Plant Pathol. *80*, 79–84.

Cheong, M.S., Kirik, A., Kim, J.G., Frame, K., Kirik, V., and Mudgett, M.B. (2014). AvrBsT Acetylates Arabidopsis ACIP1, a Protein that Associates with Microtubules and Is Required for Immunity. PLoS Pathog. *10*, 1–15.

Choi, H.W., and Klessig, D.F. (2016). DAMPs, MAMPs, and NAMPs in plant innate immunity. BMC Plant Biol. *16*, 1–10.

Choi, S.M., Song, H.R., Han, S.K., Han, M., Kim, C.Y., Park, J., Lee, Y.H., Jeon, J.S., Noh, Y.S., and Noh, B. (2012). HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. Plant J. *71*, 135–146.

Chua, Y.L., Channelière, S., Mott, E., and Gray, J.C. (2005). The bromodomain protein GTE6 controls leaf development in Arabidopsis by histone acetylation at ASYMMETRIC LEAVES1. Genes Dev. *19*, 2245–2254.

Clapier, C.R., and Cairns, B.R. (2009). The biology of chromatin remodeling complexes. Annu. Rev. Biochem. *78*, 273–304.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. *16*, 735–743.

Cook, D.E., Mesarich, C.H., and Thomma, B.P.H.J. (2015). Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol. *53*, 541–563.

Costa, T.R.D., Felisberto-Rodrigues, C., Meir, A., Prevost, M.S., Redzej, A., Trokter, M., and Waksman, G. (2015). Secretion systems in Gram-negative bacteria: Structural and mechanistic insights. Nat. Rev. Microbiol. *13*, 343–359.

Cui, H., Tsuda, K., and Parker, J.E. (2015). Effector-Triggered Immunity: From Pathogen Perception to Robust Defense. Annu. Rev. Plant Biol. *66*, 487–511.

Cunnac, S., Occhialini, A., Barberis, P., Boucher, C., and Genin, S. (2004). Inventory and functional analysis of the large Hrp regulon in Ralstonia solanacearum: Identification of novel effector proteins translocated to plant host cells through the type III secretion system. Mol. Microbiol. *53*, 115–128.

Deleris, A., Halter, T., and Navarro, L. (2016). DNA Methylation and Demethylation in Plant Immunity. Annu. Rev. Phytopathol. *54*, 579–603.

Delga, A. (2015). L'effecteur PopP2 de Ralstonia solanacearum cible GTE9 et GTE11, deux lecteurs épigénétiques d'Arabidopsis thaliana. Université Toulouse III - Paul Sabatier.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D.X., Bittner-Eddy, P., Beynon, J., and Marco, Y. (2002). Resistance to Ralstonia solanacearum in Arabidopsis thaliana is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. Proc. Natl. Acad. Sci. *99*, 2404–2409.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., and Marco, Y. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. *100*, 8024–8029.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491–496.

Ding, B., and Wang, G.-L. (2015). Chromatin versus pathogens: the function of epigenetics in plant immunity. Front. Plant Sci. *6*, 1–8.

Ding, P., and Ding, Y. (2020). Stories of Salicylic Acid: A Plant Defense Hormone. Trends Plant Sci. *25*, 549–565.

Doğan, E.S., and Liu, C. (2018). Three-dimensional chromatin packing and positioning of plant genomes. Nat. Plants *4*, 521–529.

Domcke, S., Bardet, A.F., Adrian Ginno, P., Hartl, D., Burger, L., and Schübeler, D. (2015). Competition between DNA methylation and transcription factors determines binding of NRF1. Nature *528*, 575–579.

Dong, W., and Hamon, M.A. (2020). Revealing eukaryotic histone-modifying mechanisms through bacterial infection. Semin. Immunopathol. *42*, 201–213.

Dowen, R.H., Pelizzola, M., Schmitz, R.J., Lister, R., Dowen, J.M., Nery, J.R., Dixon, J.E., and Ecker, J.R. (2012). Widespread dynamic DNA methylation in response to biotic stress. Proc. Natl. Acad. Sci. *109*, 2183–2191.

Du, L., and Poovaiah, B.W. (2004). A novel family of Ca 2+/calmodulin-binding proteins involved in transcriptional regulation: Interaction with fsh/Ring3 class transcription activators. Plant Mol. Biol. *54*, 549–569.

Du, Y., Chen, X., Guo, Y., Zhang, X., Zhang, H., Li, F., Huang, G., Meng, Y., and Shan, W. (2021). Phytophthora infestans RXLR effector PITG20303 targets a potato MKK1 protein to suppress plant immunity. New Phytol. *229*, 501–515.

Dubey, A., and Jeon, J. (2017). Epigenetic regulation of development and pathogenesis in fungal plant pathogens. Mol. Plant Pathol. *18*, 887–898.

Duque, P., and Chua, N.H. (2003). IMB1, a bromodomain protein induced during seed imbibition, regulates ABA- and phyA-mediated responses of germination in Arabidopsis. Plant J. *35*, 787–799.

Eberharter, A., and Becker, P.B. (2002). Histone acetylation: A switch between repressive and permissive chromatin. Second in review on chromatin dynamics. EMBO Rep. *3*, 224–229.

Ellis, J.G., Dodds, P.N., and Lawrence, G.J. (2008). Flax rust resistance gene specificity is based on direct resistance-avirulence protein interactions. Annu. Rev. Phytopathol. *45*, 289–306.

Elphinstone, J.G. (1996). Survival and possibilities for extinction of Pseudomonas solanacearum (Smith) Smith in cool climates. Potato Res. *39*, 403–410.

Erb, M., and Reymond, P. (2019). Molecular Interactions between Plants and Insect Herbivores. Annu. Rev. Plant Biol. *70*, 527–557.

Erill, I., Puigvert, M., Legrand, L., Guarischi-Sousa, R., Vandecasteele, C., Setubal, J.C., Genin, S., Guidot, A., and Valls, M. (2017). Comparative analysis of ralstonia solanacearum methylomes. Front. Plant Sci. *8*, 1–16.

Farrona, S., Hurtado, L., and Reyes, J.C. (2007). A Nucleosome Interaction Module Is Required for Normal Function of Arabidopsis thaliana BRAHMA. J. Mol. Biol. *373*, 240–250.

Farrona, S., Hurtado, L., March-Díaz, R., Schmitz, R.J., Florencio, F.J., Turck, F., Amasino, R.M., and Reyes, J.C. (2011). Brahma Is Required for Proper Expression of the Floral Repressor FLC in Arabidopsis. PLoS One *6*, 1–11.

Feehan, J.M., Castel, B., Bentham, A.R., and Jones, J.D. (2020). Plant NLRs get by with a little help from their friends. Curr. Opin. Plant Biol. *56*, 99–108.

Feng, D.X., Tasset, C., Hanemian, M., Barlet, X., Hu, J., Trémousaygue, D., Deslandes, L., and Marco, Y. (2012a). Biological control of bacterial wilt in Arabidopsis thaliana involves abscissic acid signalling. New Phytol. *194*, 1035–1045.

Feng, F., Yang, F., Rong, W., Wu, X., Zhang, J., Chen, S., He, C., and Zhou, J.M. (2012b). A Xanthomonas uridine 5′-monophosphate transferase inhibits plant immune kinases. Nature *485*, 114–118.

Filippakopoulos, P., and Knapp, S. (2012). The bromodomain interaction module. FEBS Lett. *586*, 2692–2704.

Filippakopoulos, P., Picaud, S., Mangos, M., Keates, T., Lambert, J., Barsyte-Lovejoy, D., Felletar, I., Volkmer, R., Müller, S., Pawson, T., et al. (2012). Histone Recognition and Large-Scale Structural Analysis of the Human Bromodomain Family. Cell *149*, 214–231.

Flor, H.H. (1971). Current status of the gene-for-gene concept. 275–296.

Florence, B., and Faller, D. V. (2001). You bet-cha: a novel family of transcriptional regulators. Front. Biosci. *6*, 1008–1018.

Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K., et al. (2009). Loss of function of a proline-containing protein confers durable disease resistance in Rice. Science (80-.). *325*, 998–1001.

Garcia-Dominguez, M., March-Diaz, R., and Reyes, J.C. (2008). The PHD domain of plant PIAS proteins mediates sumoylation of bromodomain GTE proteins. J. Biol. Chem. *283*, 21469–21477.

Garner, C.M., Kim, S.H., Spears, B.J., and Gassmann, W. (2016). Express yourself: Transcriptional regulation of plant innate immunity. Semin. Cell Dev. Biol.

Geisler, S., and Coller, J. (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat. Rev. Mol. Cell Biol. *14*, 699–712.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. New Phytol. *187*, 920–928.

Genin, S., and Denny, T.P. (2012). Pathogenomics of the ralstonia solanacearum species complex. Annu. Rev. Phytopathol. *50*, 67–89.

Ghosh, S., and O'Connor, T.J. (2017). Beyond paralogs: The multiple layers of redundancy in bacterial pathogenesis. Front. Cell. Infect. Microbiol. *7*, 1–14.

Gimenez-Ibanez, S., Hann, D.R., Ntoukakis, V., Petutschnig, E., Lipka, V., and Rathjen, J.P. (2009). AvrPtoB Targets the LysM Receptor Kinase CERK1 to Promote Bacterial Virulence on Plants. Curr. Biol. *19*, 423–429.

Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J.P., and Solano, R. (2014). The Bacterial Effector HopX1 Targets JAZ Transcriptional Repressors to Activate Jasmonate Signaling and Promote Infection in Arabidopsis. PLoS Biol. *12*, 1–15.

Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W., and Robatzek, S. (2008). Plant Pattern-Recognition Receptor FLS2 Is Directed for Degradation by the Bacterial Ubiquitin Ligase AvrPtoB. Curr. Biol. *18*, 1824–1832.

Goldberg, A.D., Allis, C.D., and Bernstein, E. (2007). Epigenetics: A Landscape Takes Shape. Cell *128*, 635–638.

Goritschnig, S., Weihmann, T., Zhang, Y., Fobert, P., McCourt, P., and Li, X. (2008). A novel role for protein farnesylation in plant innate immunity. Plant Physiol. *148*, 348–357.

Grabiec, A.M., and Potempa, J. (2018). Epigenetic regulation in bacterial infections: targeting histone deacetylases. Crit. Rev. Microbiol. *44*, 336–350.

Grasser, K.D., Rubio, V., and Barneche, F. (2021). Multifaceted activities of the plant SAGA complex. Biochim. Biophys. Acta - Gene Regul. Mech. *1864*, 194613.

Grund, E., Tremousaygue, D., and Deslandes, L. (2019). Plant NLRS with integrated domains: Unity makes strength. Plant Physiol. *179*, 1227–1235.

Guo, H., Ahn, H.-K.K., Sklenar, J., Huang, J., Ma, Y., Ding, P., Menke, F.L.H., and Jones, J.D.G. (2020). Phosphorylation-Regulated Activation of the Arabidopsis RRS1-R/RPS4 Immune Receptor Complex Reveals Two Distinct Effector Recognition Mechanisms. Cell Host Microbe *27*, 1–13.

Gust, A.A., Pruitt, R., and Nürnberger, T. (2017). Sensing Danger: Key to Activating Plant Immunity. Trends Plant Sci. *22*, 779–791.

Han, S.K., Wu, M.F., Cui, S., and Wagner, D. (2015). Roles and activities of chromatin remodeling ATPases in plants. Plant J. *83*, 62–77.

Hanemian, M., Zhou, B., Deslandes, L., Marco, Y., and Trémousaygue, D. (2013). Hrp mutant bacteria as biocontrol agents: Toward a sustainable approach in the fight against plant pathogenic bacteria. Plant Signal. Behav. *8*, 1–5.

Hannan Parker, A., Wilkinson, S.W., and Ton, J. (2022). Epigenetics: a catalyst of plant immunity against pathogens. New Phytol. *233*, 66–83.

Harouz, H., Rachez, C., Meijer, B.M., Marteyn, B., Donnadieu, F., Cammas, F., Muchardt, C., Sansonetti, P., and Arbibe, L. (2014). Shigella flexneri targets the HP 1γ subcode through the phosphothreonine lyase O sp F . EMBO J. *33*, 2606–2622.

Hayes, C.S., Aoki, S.K., and Low, D.A. (2010). Bacterial contact-dependent delivery systems. Annu. Rev. Genet. *44*, 71–90.

Hayward, A.C. (1964). Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. *27*, 265–277.

Hayward, A.C. (1991). BIOLOGY AND EPIDEMIOLOGY OF BACTERIAL WILT CAUSED BY PSEUDOMONAS SOLANACEARUM.

He, K., and Wu, Y. (2016). Receptor-Like Kinases and Regulation of Plant Innate Immunity. Enzymes *40*, 105–142.

Heath, M.C. (1981). A Generalized Concept of Host-Parasite Specificity. Phytopathology *71*, 1121.

Henry, E., Toruño, T.Y., Jauneau, A., Deslandes, L., and Coaker, G. (2017). Direct and indirect visualization of bacterial effector delivery into diverse plant cell types during infection. Plant Cell *29*, 1555–1570.

Holsters, M., Silva, B., Vliet, F., Genetello, C., and Schell, J. (1980). The Functional Organization of the Nopaline Plasmid pTiC58. Plasmid *3*, 212–230.

Hou, Y., Wang, L., Wang, L., Liu, L., Li, L., Sun, L., Rao, Q., Zhang, J., and Huang, S. (2015). JMJ704 positively regulates rice defense response against Xanthomonas oryzae pv. oryzae infection via reducing H3K4me2/3 associated with negative disease resistance regulators. BMC Plant Biol. *15*, 1–13.

Hu, J., Barlet, X., Deslandes, L., Hirsch, J., Feng, D.X., Somssich, I., and Marco, Y. (2008). Transcriptional Responses of Arabidopsis thaliana during Wilt Disease Caused by the Soil-Borne Phytopathogenic Bacterium, Ralstonia solanacearum. PLoS One *3*, 1–10.

Huang, C.-Y.Y., Rangel, D.S., Qin, X., Bui, C., Li, R., Jia, Z., Cui, X., and Jin, H. (2021). The chromatin-remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs. Cell Host Microbe *29*, 1–10.

Huang, C.Y., Wang, H., Hu, P., Hamby, R., and Jin, H. (2019). Small RNAs – Big Players in Plant-Microbe Interactions. Cell Host Microbe *26*, 173–182.

Huet, G. (2014). Breeding for resistances to Ralstonia solanacearum. Front. Plant Sci. *5*, 1–5.

Hui, S., Liu, H., Zhang, M., Chen, D., Li, Q., Tian, J., Xiao, J., Li, X., Wang, S., and Yuan, M. (2019). The host basal transcription factor IIA subunits coordinate for facilitating infection of TALEs-carrying bacterial pathogens in rice. Plant Sci. *284*, 48–56.

Iftner, T., Haedicke-Jarboui, J., Wu, S.Y., and Chiang, C.M. (2017). Involvement of Brd4 in different steps of

the papillomavirus life cycle. Virus Res. *231*, 76–82.

Irigoyen, S., Ramasamy, M., Misra, A., McKnight, T.D., and Mandadi, K.K. (2021). A BTB-TAZ protein is required for gene activation by Cauliflower mosaic virus 35S multimerized enhancers. Plant Physiol. *188*, 397–410.

Jacob, Y., Feng, S., LeBlanc, C.A., Bernatavichute, Y. V, Stroud, H., Cokus, S., Johnson, L.M., Pellegrini, M., Jacobsen, S.E., and Michaels, S.D. (2009). ATXR5 and ATXR6 are H3K27 monomethyltransferases required for chromatin structure and gene silencing. Nat. Struct. Mol. Biol. *16*, 763–768.

Jenuwein, T., and Allis, C.D. (2001). Translating the Histone Code. Science (80-.). *293*, 1074–1080.

Jeon, H., Kim, W., Kim, B., Lee, S., Jayaraman, J., Jung, G., Choi, S., Sohn, K.H., and Segonzac, C. (2020). Ralstonia solanacearum Type III Effectors with Predicted Nuclear Localization Signal Localize to Various Cell Compartments and Modulate Immune Responses in Nicotiana spp. Plant Pathol. J. *36*, 303–303.

Jiang, C.J., Shimono, M., Sugano, S., Kojima, M., Yazawa, K., Yoshida, R., Lnoue, H., Hayashi, N., Sakakibara, H., and Takatsuji, H. (2010). Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-magnaporthe grisea interaction. Mol. Plant-Microbe Interact. *23*, 791–798.

Jones, J.D.G., and Dangl, J.L. (2006). The plant immune system. Nature *444*, 323–329.

Jose, L., Ramachandran, R., Bhagavat, R., Gomez, R.L., Chandran, A., Raghunandanan, S., Omkumar, R.V., Chandra, N., Mundayoor, S., and Kumar, R.A. (2016). Hypothetical protein Rv3423.1 of Mycobacterium tuberculosis is a histone acetyltransferase. FEBS J. *283*, 265–281.

Jubic, L.M., Saile, S., Furzer, O.J., El Kasmi, F., and Dangl, J.L. (2019). Help wanted: helper NLRs and plant immune responses. Curr. Opin. Plant Biol. *50*, 82–94.

Kang, Y., Liu, H., Genin, S., Schell, M.A., and Denny, T.P. (2002). Ralstonia solanacearum requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol. Microbiol. *46*, 427–437.

Kanno, T., Kanno, Y., Siegel, R.M., Jang, M.K., Lenardo, M.J., and Ozato, K. (2004). Selective Recognition of Acetylated Histones by Bromodomain Proteins Visualized in Living Cells. Mol. Cell *13*, 33–43.

Kanyuka, K., and Rudd, J.J. (2019). Cell surface immune receptors: the guardians of the plant's extracellular spaces. Curr. Opin. Plant Biol. *50*, 1–8.

Kao, C.C., Barlow, E., and Sequeira, L. (1992). Extracellular polysaccharide is required for wild-type virulence of Pseudomonas solanacearum. J. Bacteriol. *174*, 1068–1071.

Karim, Z., Hossain, M., and Begum, M. (2018). Ralstonia solanacearum: A Threat to Potato Production in Bangladesh. Fundam. Appl. Agric. *3*, 407–421.

Katagiri, F., and Tsuda, K. (2010). Understanding the Plant Immune System. Mol. Plant-Microbe Interact. *23*, 1531–1536.

Kim, J.H., Saraf, A., Florens, L., Washburn, M., and Workman, J.L. (2010). Gcn5 regulates the dissociation of SWI/SNF from chromatin by acetylation of Swi2/Snf2. Genes Dev. *24*, 2766–2771.

Kim, M.J., Shin, R., and Schachtman, D.P. (2009). A nuclear factor regulates abscisic acid responses in Arabidopsis. Plant Physiol. *151*, 1433–1445.

Kong, L., Qiu, X., Kang, J., Wang, Y., Chen, H., Huang, J., Qiu, M., Zhao, Y., Kong, G., Ma, Z., et al. (2017). A Phytophthora Effector Manipulates Host Histone Acetylation and Reprograms Defense Gene Expression to Promote Infection. Curr. Biol. *27*, 981–991.

Kouzarides, T. (2007). Chromatin Modifications and Their Function. Cell *128*, 693–705.

Kroj, T., Chanclud, E., Michel-Romiti, C., Grand, X., and Morel, J.B. (2016). Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. New Phytol. *210*, 618–626.

Kumar, V., Thakur, J.K., and Prasad, M. (2021). Histone acetylation dynamics regulating plant development and stress responses. Cell. Mol. Life Sci. *78*, 4467–4486.

Landry, D., González-Fuente, M., Deslandes, L., and Peeters, N. (2020). The large, diverse, and robust arsenal of

Ralstonia solanacearum type III effectors and their in planta functions. Mol. Plant Pathol. *21*, 1377–1388.

de Lange, O., Schreiber, T., Schandry, N., Radeck, J., Braun, K.H., Koszinowski, J., Heuer, H., Strauß, A., and Lahaye, T. (2013). Breaking the DNA-binding code of Ralstonia solanacearum TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease. New Phytol. *199*, 773–786.

Latrasse, D., Jégu, T., Li, H., de Zelicourt, A., Raynaud, C., Legras, S., Gust, A., Samajova, O., Veluchamy, A., Rayapuram, N., et al. (2017). MAPK-triggered chromatin reprogramming by histone deacetylase in plant innate immunity. Genome Biol. *18*, 1–19.

Lauria, M., and Rossi, V. (2011). Epigenetic control of gene regulation in plants. Biochim. Biophys. Acta - Gene Regul. Mech. *1809*, 369–378.

Lavie, M., Shillington, E., Eguiluz, C., Grimsley, N., and Boucher, C. (2002). PopP1, a new member of the YopJ/AvrRxv family of type III effector proteins, acts as a host-specificity factor and modulates aggressiveness of Ralstonia solanacearum. Mol. Plant-Microbe Interact. *15*, 1058–1068.

Lavie, M., Seunes, B., Prior, P., and Boucher, C. (2004). Distribution and sequence analysis of a family of type III-dependent effectors correlate with the phylogeny of Ralstonia solanacearum strains. Mol. Plant-Microbe Interact. *17*, 931–940.

Le, T.N., Schumann, U., Smith, N.A., Tiwari, S., Khang Au, P.C., Zhu, Q.H., Taylor, J.M., Kazan, K., Llewellyn, D.J., Zhang, R., et al. (2014). DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in Arabidopsis. Genome Biol. *15*, 1–18.

Lee, A.H.-Y., Hurley, B., Felsensteiner, C., Yea, C., Ckurshumova, W., Bartetzko, V., Wang, P.W., Quach, V., Lewis, J.D., Liu, Y.C., et al. (2012). A Bacterial Acetyltransferase Destroys Plant Microtubule Networks and Blocks Secretion. PLoS Pathog. *8*, 1–14.

Lei, B., and Berger, F. (2020). H2A Variants in Arabidopsis: Versatile Regulators of Genome Activity. Plant Commun. *1*, 100015.

Lewis, J.D., Lee, A.H.Y., Hassan, J.A., Wana, J., Hurleya, B., Jhingree, J.R., Wang, P.W., Lo, T., Youn, J.Y., Guttman, D.S., et al. (2013). The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the Pseudomonas syringae type III effector HopZ1a. Proc. Natl. Acad. Sci. U. S. A. *110*, 18722–18727.

Li, P., and Day, B. (2019). Battlefield Cytoskeleton: Turning the Tide on Plant Immunity. Mol. Plant-Microbe Interact. *32*, 25–34.

Li, B., Carey, M., and Workman, J.L. (2007a). The Role of Chromatin during Transcription. Cell *128*, 707–719.

Li, H., Xu, H., Zhou, Y., Zhang, J., Long, C., Li, S., Chen, S., Zhou, J.-M., and Shao, F. (2007b). The Phosphothreonine Lyase Activity of a Bacterial Type III Effector Family. Science (80-.). *315*, 1000–1003.

Li, H., Wang, H., Jing, M., Zhu, J., Guo, B., Wang, Y., Lin, Y., Chen, H., Kong, L., Ma, Z., et al. (2018). A phytophthora effector recruits a host cytoplasmic transacetylase into nuclear speckles to enhance plant susceptibility. Elife *7*, 1–23.

Li, J.-F., Park, E., von Arnim, A.G., and Nebenführ, A. (2009). The FAST technique: a simplified Agrobacterium-based transformation method for transient gene expression analysis in seedlings of Arabidopsis and other plant species. Plant Methods *5*, 1–15.

Li, L., Kim, P., Yu, L., Cai, G., Chen, S., Alfano, J.R., and Zhou, J.M. (2016). Activation-Dependent Destruction of a Co-receptor by a Pseudomonas syringae Effector Dampens Plant Immunity. Cell Host Microbe *20*, 504–514.

Li, X., Kapos, P., and Zhang, Y. (2015a). NLRs in plants. Curr. Opin. Immunol. *32*, 114–121.

Li, Y., Mukherjee, I., Thum, K.E., Tanurdzic, M., Katari, M.S., Obertello, M., Edwards, M.B., McCombie, W.R., Martienssen, R.A., and Coruzzi, G.M. (2015b). The histone methyltransferase SDG8 mediates the epigenetic modification of light and carbon responsive genes in plants. Genome Biol. *16*, 1–15.

Liang, Z., Geng, Y., and Gu, X. (2018). Adenine Methylation: New Epigenetic Marker of DNA and mRNA. Mol. Plant *11*, 1219–1221.

Liu, H., Zhang, S., Schell, M.A., and Denny, T.P. (2005). Pyramiding unmarked deletions in Ralstonia solanacearum shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. Mol. Plant-Microbe Interact. *18*, 1296–1305.

Liu, J., Elmore, J.M., Lin, Z.J.D., and Coaker, G. (2011). A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. Cell Host Microbe *9*, 137–146.

Lopez, V.A., Park, B.C., Nowak, D., Sreelatha, A., Zembek, P., Fernandez, J., Servage, K.A., Gradowski, M., Hennig, J., Tomchick, D.R., et al. (2019). A Bacterial Effector Mimics a Host HSP90 Client to Undermine Immunity. Cell *179*, 205–218.

Lowe-Power, T.M., Khokhani, D., and Allen, C. (2018). How Ralstonia solanacearum Exploits and Thrives in the Flowing Plant Xylem Environment. Trends Microbiol. *26*, 929–942.

Lu, Y., and Tsuda, K. (2021). Intimate Association of PRR- and NLR-Mediated Signaling in Plant Immunity. Mol. Plant-Microbe Interact. *34*, 3–14.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251–260.

Luo, Y., Hou, X., Zhang, C., Tan, L., Shao, C., Lin, R., Su, Y., Cai, X., Li, L., Chen, S., et al. (2020). A plant-specific SWR1 chromatin-remodeling complex couples histone H2A.Z deposition with nucleosome sliding. EMBO J. *39*, 1–16.

Ma, K.-W., and Ma, W. (2016). YopJ Family Effectors Promote Bacterial Infection through a Unique Acetyltransferase Activity. Microbiol. Mol. Biol. Rev. *80*, 1011–1027.

Ma, S., Lapin, D., Liu, L., Sun, Y., Song, W., Zhang, X., Logemann, E., Yu, D., Wang, J., Jirschitzka, J., et al. (2020). Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. Science (80-.). *370*.

Macho, A.P., Guidot, A., Barberis, P., Beuzón, C.R., and Genin, S. (2010). A Competitive Index Assay Identifies Several Ralstonia solanacearum Type III Effector Mutant Strains with Reduced Fitness in Host Plants. MPMI *23*, 1197–1205.

Maekawa, T., Cheng, W., Spiridon, L.N., Töller, A., Lukasik, E., Saijo, Y., Liu, P., Shen, Q.H., Micluta, M.A., Somssich, I.E., et al. (2011). Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. Cell Host Microbe *9*, 187–199.

Maidment, J.H.R., Franceschetti, M., Maqbool, A., Saitoh, H., Jantasuriyarat, C., Kamoun, S., Terauchi, R., and Banfield, M.J. (2021). Multiple variants of the fungal effector AVR-Pik bind the HMA domain of the rice protein OsHIPP19, providing a foundation to engineer plant defense. J. Biol. Chem. *296*, 1–13.

Mamphogoro, T.P., Babalola, O.O., and Aiyegoro, O.A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (Capsicum annuum) and other Solanaceous crops. J. Appl. Microbiol. *129*, 496–508.

Mandadi, K.K., Misra, A., Ren, S., and McKnight, T.D. (2009). BT2, a BTB Protein, Mediates Multiple Responses to Nutrients, Stresses, and Hormones in Arabidopsis. Plant Physiol. *150*, 1930–1939.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M.A., et al. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. *13*, 614–629.

Martin, R., Qi, T., Zhang, H., Liu, F., King, M., Toth, C., Nogales, E., and Staskawicz, B.J. (2020). Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. Science (80-.). *370*, 1–7.

Medina-Puche, L., Tan, H., Dogra, V., Wu, M., Rosas-Diaz, T., Wang, L., Ding, X., Zhang, D., Fu, X., Kim, C., et al. (2020). A Defense Pathway Linking Plasma Membrane and Chloroplasts and Co-opted by Pathogens. Cell *182*, 1109–1124.

Misra, A., McKnight, T.D., and Mandadi, K.K. (2018). Bromodomain proteins GTE9 and GTE11 are essential for specific BT2-mediated sugar and ABA responses in Arabidopsis thaliana. Plant Mol. Biol. *96*, 393–402.

Monteiro, F., Genin, S., van Dijk, I., and Valls, M. (2012). A luminescent reporter evidences active expression of Ralstonia solanacearum type III secretion system genes throughout plant infection. Microbiol. (United Kingdom) *158*, 2107–2116.

Morris, K. V., and Mattick, J.S. (2014). The rise of regulatory RNA. Nat. Rev. Genet. *15*, 423–437.

Mott, G.A., Middleton, M.A., Desveaux, D., and Guttman, D.S. (2014). Peptides and small molecules of the plant-pathogen apoplastic arena. Front. Plant Sci. *5*, 1–12.

Mukhtar, M.S., Carvunis, A.-R., Dreze, M., Epple, P., Steinbrenner, J., Moore, J., Tasan, M., Galli, M., Hao, T., Nishimura, M.T., et al. (2011). Independently Evolved Virulence Effectors Converge onto Hubs in a Plant Immune System Network. Science (80-.). *333*, 596–601.

Muller, S., Filippakopoulos, P., and Knapp, S. (2011). Bromodomains as therapeutic targets. Expert Rev. Mol. Med. *13*, 1–21.

Nahar, K., Kyndt, T., de Vleesschauwer, D., Höfte, M., and Gheysen, G. (2011). The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiol. *157*, 305–316.

Nakano, M., and Mukaihara, T. (2018). Ralstonia solanacearum type iii effector ripal targets chloroplasts and induces jasmonic acid production to suppress salicylic acid-mediated defense responses in plants. Plant Cell Physiol. *59*, 2576–2589.

Nakano, M., and Mukaihara, T. (2019). The type III effector RipB from Ralstonia solanacearum RS1000 acts as a major avirulence factor in Nicotiana benthamiana and other Nicotiana species. Mol. Plant Pathol. *20*, 1237–1251.

Nakano, M., Oda, K., and Mukaihara, T. (2017). Ralstonia solanacearum novel E3 ubiquitin ligase (NEL) effectors RipAW and RipAR suppress pattern-triggered immunity in plants. Microbiol. (United Kingdom) *163*, 992–1002.

Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., and Narusaka, Y. (2009). RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. Plant J. *60*, 218–226.

Navarro, L., Jay, F., Nomura, K., Sheng, Y.H., and Voinnet, O. (2008). Suppression of the MicroRNA pathway by bacterial effector proteins. Science (80-.). *321*, 964–967.

Ngou, B.P.M., Ahn, H., Ding, P., Redkar, A., Brown, H., Ma, Y., Youles, M., Tomlinson, L., and Jones, J.D.G. (2020). Estradiol-inducible AvrRps4 expression reveals distinct properties of TIR-NLR-mediated effector-triggered immunity. J. Exp. Bot. *71*, 2186–2197.

Ngou, B.P.M., Ahn, H.K., Ding, P., and Jones, J.D.G. (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. Nature *592*, 110–115.

Nguyen, Q.-M., Iswanto, A.B.B., Son, G.H., and Kim, S.H. (2021). Recent Advances in Effector-Triggered Immunity in Plants: New Pieces in the Puzzle Create a Different Paradigm. Int. J. Mol. Sci. *22*.

Nie, W.F., Lei, M., Zhang, M., Tang, K., Huang, H., Zhang, C., Miki, D., Liu, P., Yang, Y., Wang, X., et al. (2019). Histone acetylation recruits the SWR1 complex to regulate active DNA demethylation in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. *116*, 16641–16650.

O'Neill, E.M., Mucyn, T.S., Patteson, J.B., Finkel, O.M., Chung, E.H., Baccile, J.A., Massolo, E., Schroeder, F.C., Dangl, J.L., and Li, B. (2018). Phevamine A, a small molecule that suppresses plant immune responses. Proc. Natl. Acad. Sci. U. S. A. *115*, E9514–E9522.

Oikawa, K., Fujisaki, K., Shimizu, M., Takeda, T., Saitoh, H., Hirabuchi, A., Hiraka, Y., Białas, A., Langner, T., Kellner, R., et al. (2020). The blast pathogen effector AVR-Pik binds and stabilizes rice heavy metal-associated (HMA) proteins to co-opt their function in immunity. BioRxiv.

Osdaghi, E. (2020). Ralstonia solanacearum (bacterial wilt of potato). Invasive Species Compendium.

Ouyang, W., Cao, Z., Xiong, D., Li, G., and Li, X. (2020). Decoding the plant genome: From epigenome to 3D organization. J. Genet. Genomics *47*, 425–435.

Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Filetici, P., and Travers, A.A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. EMBO J. *19*, 6141–6149.

Palma, K., Zhao, Q., Yu, T.C., Bi, D., Monaghan, J., Cheng, W., Zhang, Y., and Li, X. (2007). Regulation of plant innate immunity by three proteins in a complex conserved across the plant and animal kingdoms. Genes

Dev. *21*, 1484–1493.

Palma, K., Thorgrimsen, S., Malinovsky, F.G., Fiil, B.K., Nielsen, H.B., Brodersen, P., Hofius, D., Petersen, M., and Mundy, J. (2010). Autoimmunity in Arabidopsis acd11 Is Mediated by Epigenetic Regulation of an Immune Receptor. PLoS Pathog. *6*, 1–12.

Pandey, R. (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*, 5036–5055.

Pandey, S.P., and Somssich, I.E. (2009). The Role of WRKY Transcription Factors in Plant Immunity. Plant Physiol. *150*, 1648–1655.

Pandey, R., Müller, A., Napoli, C.A., Selinger, D.A., Pikaard, C.S., Richards, E.J., Bender, J., Mount, D.W., and Jorgensen, R.A. (2002). Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversi®cation of chromatin modi®cation among multicellular eukaryotes. Nucleic Acids Res. *30*, 5036–5055.

Park, E., Lee, H.-Y., Woo, J., Choi, D., and Dinesh-Kumar, S.P. (2017). Spatiotemporal Monitoring of Pseudomonas syringae Effectors via Type III Secretion Using Split Fluorescent Protein Fragments . Plant Cell *29*, 1571–1584.

Paudel, S., Dobhal, S., Alvarez, A.M., and Arif, M. (2020). Taxonomy and phylogenetic research on ralstonia solanacearum species complex: A complex pathogen with extraordinary economic consequences. Pathogens *9*, 1–26.

de Pedro-Jové, R., Puigvert, M., Sebastià, P., Macho, A.P., Monteiro, J.S., Coll, N.S., Setúbal, J.C., and Valls, M. (2021). Dynamic expression of Ralstonia solanacearum virulence factors and metabolism-controlling genes during plant infection. BMC Genomics *22*, 1–19.

Peeters, N., Carrère, S., Anisimova, M., Plener, L., Cazalé, A.-C., and Genin, S. (2013). Repertoire, unified nomenclature and evolution of the Type III effector gene set in the Ralstonia solanacearum species complex. BMC Genomics *14*, 1–18.

Peirats-Llobet, M., Han, S.K., Gonzalez-Guzman, M., Jeong, C.W., Rodriguez, L., Belda-Palazon, B., Wagner, D., and Rodriguez, P.L. (2016). A Direct Link between Abscisic Acid Sensing and the Chromatin-Remodeling ATPase BRAHMA via Core ABA Signaling Pathway Components. Mol. Plant *9*, 136–147.

Peng, Y., Van Wersch, R., and Zhang, Y. (2018). Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. Mol. Plant-Microbe Interact. *31*, 403–409.

Peng, Z., Hu, Y., Zhang, J., Huguet-Tapia, J.C., Block, A.K., Park, S., Sapkota, S., Liu, Z., Liu, S., and White, F.F. (2019). Xanthomonas translucens commandeers the host rate-limiting step in ABA biosynthesis for disease susceptibility. Proc. Natl. Acad. Sci. U. S. A. *116*, 20938–20946.

Pennini, M.E., Pai, R.K., Schultz, D.C., Boom, W.H., and Harding, C. V. (2006). Mycobacterium tuberculosis 19-kDa Lipoprotein Inhibits IFN-γ-Induced Chromatin Remodeling of MHC2TA by TLR2 and MAPK Signaling . J. Immunol. *176*, 4323–4330.

Pensec, F., Lebeau, A., Daunay, M.C., Chiroleu, F., Guidot, A., and Wicker, E. (2015). Towards the identification of type III effectors associated with ralstonia solanacearum virulence on tomato and eggplant. Phytopathology *105*, 1529–1544.

Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D.J., Inuganti, A., Griss, J., Mayer, G., Eisenacher, M., et al. (2019). The PRIDE database and related tools and resources in 2019: Improving support for quantification data. Nucleic Acids Res. *47*, D442–D450.

Pieterse, C.M.J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. *28*, 489–521.

Pikaard, C.S., and Scheid, O.M. (2014). Epigenetic regulation in plants. Cold Spring Harb. Perspect. Biol. *6*, 1–31.

Poueymiro, M., Cunnac, S., Barberis, P., Deslandes, L., Peeters, N., Cazale-Noel, A.C., Boucher, C., and Genin, S. (2009). Two type III secretion system effectors from Ralstonia solanacearum GMI1000 determine host-range Specificity on Tobacco. Mol. Plant-Microbe Interact. *22*, 538–550.

Poussier, S., Vandewalle, P., and Luisetti, J. (1999). Genetic diversity of African and worldwide strains of Ralstonia solanacearum as determined by PCR-restriction fragment length polymorphism analysis of the hrp gene region. Appl. Environ. Microbiol. *65*, 2184–2194.

Prior, P., and Fegan, M. (2005). Recent developments in the phylogeny and classification of ralstonia solanacearum. Acta Hortic. *695*, 127–136.

Prior, P., Ailloud, F., Dalsing, B.L., Remenant, B., Sanchez, B., and Allen, C. (2016). Genomic and proteomic evidence supporting the division of the plant pathogen Ralstonia solanacearum into three species. BMC Genomics *17*, 1–11.

Probst, A. V., and Mittelsten Scheid, O. (2015). Stress-induced structural changes in plant chromatin. Curr. Opin. Plant Biol. *27*, 8–16.

Pruitt, R.N., Zhang, L., Saile, S.C., Karelina, D., Fröhlich, K., Wan, W., Rao, S., Weigel, D., Parker, J.E., Kasmi, F. El, et al. (2020). Arabidopsis cell surface LRR immune receptor signaling through the EDS1-PAD4-ADR1 node. BioRxiv *1*, 391516.

Pumplin, N., and Voinnet, O. (2013). RNA silencing suppression by plant pathogens: Defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. *11*, 745–760.

Qin, J., Wang, K., Sun, L., Xing, H., Wang, S., Li, L., Chen, S., Guo, H.S., and Zhang, J. (2018). The plant-specific transcription factors CBP60G and SARD1 are targeted by a verticillium secretory protein VDSCP41 to modulate immunity. Elife *7*, 1–25.

Rahman, S., Sowa, M.E., Ottinger, M., Smith, J.A., Shi, Y., Harper, J.W., and Howley, P.M. (2011). The Brd4 Extraterminal Domain Confers Transcription Activation Independent of pTEFb by Recruiting Multiple Proteins, Including NSD3. Mol. Cell. Biol. *31*, 2641–2652.

Ramirez-Prado, J.S., Abulfaraj, A.A., Rayapuram, N., Benhamed, M., and Hirt, H. (2018a). Plant Immunity: From Signaling to Epigenetic Control of Defense. Trends Plant Sci. *23*, 833–844.

Ramirez-Prado, J.S., Piquerez, S.J.M., Bendahmane, A., Hirt, H., Raynaud, C., and Benhamed, M. (2018b). Modify the Histone to Win the Battle: Chromatin Dynamics in Plant–Pathogen Interactions. Front. Plant Sci. *9*, 1–18.

Rolando, M., Sanulli, S., Rusniok, C., Gomez-Valero, L., Bertholet, C., Sahr, T., Margueron, R., and Buchrieser, C. (2013). Legionella pneumophila effector RomA uniquely modifies host chromatin to repress gene expression and promote intracellular bacterial replication. Cell Host Microbe *13*, 395–405.

Rolando, M., Gomez-Valero, L., and Buchrieser, C. (2015). Bacterial remodelling of the host epigenome: Functional role and evolution of effectors methylating host histones. Cell. Microbiol. *17*, 1098–1107.

Roudier, F., Ahmed, I., Bérard, C., Sarazin, A., Mary-Huard, T., Cortijo, S., Bouyer, D., Caillieux, E., Duvernois-Berthet, E., Al-Shikhley, L., et al. (2011). Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. EMBO J. *30*, 1928–1938.

Le Roux, C., Huet, G., Jauneau, A., Camborde, L., Trémousaygue, D., Kraut, A., Zhou, B., Levaillant, M., Adachi, H., Yoshioka, H., et al. (2015). A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. Cell *161*, 1074–1088.

Rufián, J.S., Rueda-Blanco, J., López-Márquez, D., Macho, A.P., Beuzón, C.R., and Ruiz-Albert, J. (2021). The bacterial effector HopZ1a acetylates MKK7 to suppress plant immunity. New Phytol. *231*, 1138–1156.

Rushton, P.J., Somssich, I.E., Ringler, P., and Shen, Q.J. (2010). WRKY transcription factors. Trends Plant Sci. *15*, 247–258.

Russell, A.R., Ashfield, T., and Innes, R.W. (2015). Pseudomonas syringae effector AvrPphB suppresses AvrB-induced activation of RPM1 but not AvrRpm1-induced activation. Mol. Plant-Microbe Interact. *28*, 727–735.

Sabbagh, C.R.R., Carrere, S., Lonjon, F., Vailleau, F., Macho, A.P., Genin, S., and Peeters, N. (2019). Pangenomic type III effector database of the plant pathogenic Ralstonia spp. PeerJ *7*, 1–21.

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., and Kappler, U. (2014). Polyphasic taxonomic revision of the Ralstonia solanacearum species complex: Proposal to emend the descriptions of Ralstonia solanacearum and Ralstonia syzygii and reclassify current R. syzygii strains as Ralstonia syzygii subsp. syzygii subsp. nov., R.

s. Int. J. Syst. Evol. Microbiol. *64*, 3087–3103.

Saijo, Y., Loo, E.P. iian, and Yasuda, S. (2018). Pattern recognition receptors and signaling in plant–microbe interactions. Plant J. *93*, 592–613.

Saile, E., McGarvey, J.A., Schell, M.A., and Denny, T.P. (1997). Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by Ralstonia solanacearum. Phytopathology *87*, 1264–1271.

Saile, S.C., Jacob, P., Castel, B., Jubic, L.M., Salas-Gonzáles, I., Bäcker, M., Jones, J.D.G., Dangl, J.L., and El Kasmi, F. (2020). Two unequally redundant "helper" immune receptor families mediate Arabidopsis thaliana intracellular "sensor" immune receptor functions. PLoS Biol. *18*, 1–28.

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottiert, P., Camus, J.C., Cattolico, L., et al. (2002). Genome sequence of the plant pathogen ralstonia solanacearum. Nature *415*, 497–502.

Sánchez-Romero, M.A., and Casadesús, J. (2020). The bacterial epigenome. Nat. Rev. Microbiol. *18*, 7–20.

Sánchez-Vallet, A., Fouché, S., Fudal, I., Hartmann, F.E., Soyer, J.L., Tellier, A., and Croll, D. (2018). The Genome Biology of Effector Gene Evolution in Filamentous Plant Pathogens. Annu. Rev. Phytopathol. *56*, 21–40.

Sang, Y., Wang, Y., Ni, H., Cazalé, A.C., She, Y.M., Peeters, N., and Macho, A.P. (2018). The ralstonia solanacearum type III effector ripay targets plant redox regulators to suppress immune responses. Mol. Plant Pathol. *19*, 129–142.

Sang, Y., Yu, W., Zhuang, H., Wei, Y., Derevnina, L., Yu, G., Luo, J., and Macho, A.P. (2020). Intra-strain Elicitation and Suppression of Plant Immunity by Ralstonia solanacearum Type-III Effectors in Nicotiana benthamiana. Plant Commun. *1*, 100025.

Sarris, P.F., Duxbury, Z., Huh, S.U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S.B., et al. (2015). A plant immune receptor detects pathogen effectors that target WRKY transcription factors. Cell *161*, 1089–1100.

Sarris, P.F., Cevik, V., Dagdas, G., Jones, J.D.G., and Krasileva, K. V. (2016). Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. BMC Biol. *14*, 1–18.

Saur, I., Bauer, S., Kracher, B., Lu, X., Ku, F., Franzeskakis, L., Mu, M.C., Panstruga, R., Maekawa, T., Schulze-lefert, P., et al. (2019). Multiple pairs of allelic MLA immune receptor-powdery mildew AVR. Elife 1–31.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. *3*, 430–439.

Schandry, N., De Lange, O., Prior, P., and Lahaye, T. (2016). TALE-Like effectors are an ancestral feature of the ralstonia solanacearum species complex and converge in DNA targeting specificity. Front. Plant Sci. *7*, 1–16.

Schmittgen, T.D., and Livak, K.J. (2008). Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. *3*, 1101–1108.

Schreiber, K.J., and Lewis, J.D. (2021). Identification of a putative DNA-binding protein in arabidopsis that acts as a susceptibility hub and interacts with multiple Pseudomonas syringae effectors. Mol. Plant-Microbe Interact. *34*, 410–425.

Schreiber, K.J., Chau-Ly, I.J., and Lewis, J.D. (2021a). What the wild things do: Mechanisms of plant host manipulation by bacterial type III-secreted effector proteins. Microorganisms *9*, 1–48.

Schreiber, K.J., Hassan, J.A., and Lewis, J.D. (2021b). Arabidopsis Abscisic Acid Repressor 1 is a susceptibility hub that interacts with multiple Pseudomonas syringae effectors. Plant J. *105*, 1274–1292.

Schultink, A., Qi, T., Bally, J., and Staskawicz, B. (2019). Using forward genetics in Nicotiana benthamiana to uncover the immune signaling pathway mediating recognition of the Xanthomonas perforans effector XopJ4. New Phytol. *221*, 1001–1009.

Segonzac, C., Newman, T.E., Choi, S., Jayaraman, J., Choi, D.S., Jung, G.Y., Cho, H., Lee, Y.K., and Sohn,

K.H. (2017). A Conserved EAR Motif Is Required for Avirulence and Stability of the Ralstonia solanacearum Effector PopP2 In Planta. Front. Plant Sci. *8*, 1–13.

Seo, J.S., Sun, H.X., Park, B.S., Huang, C.H., Yeh, S.D., Jung, C., and Chua, N.H. (2017). ELF18-INDUCED LONG-NONCODING RNA associates with mediator to enhance expression of innate immune response genes in arabidopsis. Plant Cell *29*, 1024–1038.

Shames, S.R., Bhavsar, A.P., Croxen, M.A., Law, R.J., Mak, S.H.C., Deng, W., Li, Y., Bidshari, R., de Hoog, C.L., Foster, L.J., et al. (2011). The pathogenic Escherichia coli type III secreted protease NleC degrades the host acetyltransferase p300. Cell. Microbiol. *13*, 1542–1557.

Sharma, G., Upadhyay, S., Srilalitha, M., Nandicoori, V.K., and Khosla, S. (2015). The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. Nucleic Acids Res. *43*, 3922–3937.

Shigenaga, A.M., and Argueso, C.T. (2016). No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. Semin. Cell Dev. Biol. *56*, 174–189.

Shine, M.B., Xiao, X., Kachroo, P., and Kachroo, A. (2019). Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. Plant Sci. *279*, 81–86.

Silmon De Monerri, N.C., and Kim, K. (2014). Pathogens hijack the epigenome: A new twist on host-pathogen interactions. Am. J. Pathol. *184*, 897–911.

Smith, S.G., and Zhou, M.M. (2016). The Bromodomain: A New Target in Emerging Epigenetic Medicine. ACS Chem. Biol. *11*, 598–608.

Sohn, K.H., Segonzac, C., Rallapalli, G., Sarris, P.F., Woo, J.Y., Williams, S.J., Newman, T.E., Paek, K.H., Kobe, B., and Jones, J.D.G. (2014). The Nuclear Immune Receptor RPS4 Is Required for RRS1SLH1-Dependent Constitutive Defense Activation in Arabidopsis thaliana. PLoS Genet. *10*, e1004655.

Solé, M., Popa, C., Mith, O., Sohn, K.H., Jones, J.D.G., Deslandes, L., and Valls, M. (2012). Type III Effectors Displaying Virulence and Avirulence Activities. Mol. Plant-Microbe Interact. *25*, 941–953.

Song, L., Fang, Y., Chen, L., Wang, J., and Chen, X. (2021a). Role of non-coding RNAs in plant immunity. Plant Commun. *2*, 100180.

Song, W., Forderer, A., Yu, D., and Chai, J. (2021b). Structural biology of plant defence. New Phytol. *229*, 692–711.

Su, J., Zhang, M., Zhang, L., Sun, T., Liu, Y., Lukowitz, W., Xu, J., and Zhang, S. (2017). Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid. Plant Cell *29*, 526–542.

Sun, T., Zhang, Y., Li, Y., Zhang, Q., Ding, Y., and Zhang, Y. (2015). ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. Nat. Commun. *6*, 1–12.

Sun, Y., Li, P., Shen, D., Wei, Q., He, J., and Lu, Y. (2019). The Ralstonia solanacearum effector RipN suppresses plant PAMP-triggered immunity, localizes to the endoplasmic reticulum and nucleus, and alters the NADH/NAD + ratio in Arabidopsis. Mol. Plant Pathol. *20*, 533–546.

Sun, Y., Zhu, Y.X., Balint-Kurti, P.J., and Wang, G.F. (2020). Fine-Tuning Immunity: Players and Regulators for Plant NLRs. Trends Plant Sci. *25*, 695–713.

Tang, D., Wang, G., and Zhou, J.M. (2017). Receptor kinases in plant-pathogen interactions: More than pattern recognition. Plant Cell *29*, 618–637.

Tasset, C., Bernoux, M., Jauneau, A., Pouzet, C., Brière, C., Kieffer-Jacquinod, S., Rivas, S., Marco, Y., and Deslandes, L. (2010). Autoacetylation of the Ralstonia solanacearum Effector PopP2 Targets a Lysine Residue Essential for RRS1-R-Mediated Immunity in Arabidopsis. PLoS Pathog. *6*, 1–14.

Thomma, B.P.H.J., Nürnberger, T., and Joosten, M.H.A.J. (2011). Of PAMPs and Effectors: The Blurred PTI-ETI Dichotomy. Plant Cell *23*, 4–15.

Thulasi Devendrakumar, K., Li, X., and Zhang, Y. (2018). MAP kinase signalling: interplays between plant PAMP- and effector-triggered immunity. Cell. Mol. Life Sci. *75*, 2981–2989.

Tian, H., Chen, S., Wu, Z., Ao, K., Yaghmaiean, H., Sun, T., Huang, W., Xu, F., Zhang, Y., Wang, S., et al. (2020). Activation of TIR signaling is required for pattern-triggered immunity. BioRxiv.

Tsuda, K., and Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr. Opin. Plant Biol. *13*, 459–465.

Tsuda, K., and Somssich, I.E. (2015). Transcriptional networks in plant immunity. New Phytol. *206*, 932–947.

Ulferts, S., Delventhal, R., Splivallo, R., Karlovsky, P., and Schaffrath, U. (2015). Abscisic acid negatively interferes with basal defence of barley against Magnaporthe oryzae. BMC Plant Biol. *15*, 1–13.

Üstün, S., and Börnke, F. (2015). The Xanthomonas campestris type III effector XopJ proteolytically degrades proteasome subunit RPT6. Plant Physiol. *168*, 107–119.

Üstün, S., Bartetzko, V., and Börnke, F. (2013). The Xanthomonas campestris Type III Effector XopJ Targets the Host Cell Proteasome to Suppress Salicylic-Acid Mediated Plant Defence. PLoS Pathog. *9*, 1–22.

VanDemark, A.P., Kasten, M.M., Ferris, E., Heroux, A., Hill, C.P., and Cairns, B.R. (2007). Autoregulation of the Rsc4 Tandem Bromodomain by Gcn5 Acetylation. Mol. Cell *27*, 817–828.

Vasse, J. (1995). Microscopic Studies of Intercellular Infection and Protoxylem Invasion of Tomato Roots by Pseudomonas solanacearum. Mol. Plant-Microbe Interact. *8*, 241–251.

Vasse, J., Genin, S., Frey, P., Boucher, C., and Brito, B. (2000). The hrpB and hrpG regulatory genes of Ralstonia solanacearum are required for different stages of the tomato root infection process. Mol. Plant-Microbe Interact. *13*, 259–267.

Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K.G., Kim, S.K., Crespi, M., Hirt, H., Bergounioux, C., Raynaud, C., et al. (2016). LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. PLoS One *11*.

Verma, V., Ravindran, P., and Kumar, P.P. (2016). Plant hormone-mediated regulation of stress responses. BMC Plant Biol. *16*, 1–10.

Vijayapalani, P., Hewezi, T., Pontvianne, F., and Baum, T.J. (2018). An effector from the cyst nematode heterodera schachtii derepresses host rRNA genes by altering histone acetylation. Plant Cell *30*, 2795–2812.

de Vleesschauwer, D., Yang, Y., Cruz, C.V., and Höfte, M. (2010). Abscisic acid-induced resistance against the brown spot pathogen Cochliobolus miyabeanus in rice involves MAP kinase-mediated repression of ethylene signaling. Plant Physiol. *152*, 2036–2052.

De Vleesschauwer, D., Gheysen, G., and Höfte, M. (2013). Hormone defense networking in rice: Tales from a different world. Trends Plant Sci. *18*, 555–565.

Vos, I.A., Moritz, L., Pieterse, C.M.J., and Van Wees, S.C.M. (2015). Impact of hormonal crosstalk on plant resistance and fitness under multi-attacker conditions. Front. Plant Sci. *6*, 1–13.

Waddington, C.H. (1968). Towards a Theoretical Biology. Nat. 1968 2185141 *218*, 525–527.

Wang, H.L. V., and Chekanova, J.A. (2017). Long noncoding RNAs in plants. Adv. Exp. Med. Biol. *1008*, 133–154.

Wang, C., Ding, Y., Yao, J., Zhang, Y., Sun, Y., Colee, J., and Mou, Z. (2015a). Arabidopsis Elongator subunit 2 positively contributes to resistance to the necrotrophic fungal pathogens Botrytis cinerea and Alternaria brassicicola. Plant J. *83*, 1019–1033.

Wang, G., Roux, B., Feng, F., Guy, E., Li, L., Li, N., Zhang, X., Lautier, M., Jardinaud, M.F., Chabannes, M., et al. (2015b). The Decoy Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-Self Recognition and Immunity in Plants. Cell Host Microbe *18*, 285–295.

Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H.-W., Zhou, J.-M., and Chai, J. (2019). Reconstitution and structure of a plant NLR resistosome conferring immunity. Science (80-.). *364*, 1–11.

Wang, N., Wu, R., Tang, D., and Kang, R. (2021). The BET family in immunity and disease. Signal Transduct. Target. Ther. *6*, 1–22.

Wang, Y., An, C., Zhang, X., Yao, J., Zhang, Y., Sun, Y., Yu, F., Amador, D.M., and Moua, Z. (2013). The

Arabidopsis elongator complex subunit2 epigenetically regulates plant immune responses. Plant Cell *25*, 762–776.

Waterworth, W.M., Drury, G.E., Blundell-Hunter, G., and West, C.E. (2015). Arabidopsis TAF1 is an MRE11-interacting protein required for resistance to genotoxic stress and viability of the male gametophyte. Plant J. *84*, 545–557.

Weinhold, B. (2006). Epigenetics: the science of change. Environ. Health Perspect. *114*, 160–167.

Wenneker, M., Verdel, M.S.W., Groeneveld, R.M.W., Kempenaar, C., Van Beuningen, A.R., and Janse, J.D. (1999). Ralstonia (Pseudomonas) solanacearum race 3 (biovar 2) in surface water and natural weed hosts: First report on stinging nettle (Urtica dioica). Eur. J. Plant Pathol. *105*, 307–315.

van Wersch, S., and Li, X. (2019). Stronger When Together: Clustering of Plant NLR Disease resistance Genes. Trends Plant Sci. *24*, 688–699.

van Wersch, S., Tian, L., Hoy, R., and Li, X. (2020). Plant NLRs: The Whistleblowers of Plant Immunity. Plant Commun. *1*, 1–18.

Weßling, R., Epple, P., Altmann, S., He, Y., Yang, L., Henz, R., Mcdonald, N., Wiley, K., Bader, K.C., Gläßer, C., et al. (2015). Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. Cell Host Microbe *16*, 364–375.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. (2007). Ralstonia solanacearum strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Appl. Environ. Microbiol. *73*, 6790–6801.

Williams, S.J., Sohn, K.H., Wan, L., Bernoux, M., Sarris, P.F., Segonzac, C., Ve, T., Ma, Y., Saucet, S.B., Ericsson, D.J., et al. (2014). Structural basis for assembly and function of a heterodimeric plant immune receptor. Science (80-.). *344*, 299–303.

Wood, D.W., Setubal, J.C., Kaul, R., Monks, D.E., Kitajima, J.P., Okura, V.K., Zhou, Y., Chen, L., Wood, G.E., Almeida, J., et al. (2001). The genome of the natural genetic engineer Agrobacterium tumefaciens C58. Science (80-.). *294*, 2317–2323.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. *67*, 545–579.

Wroblewski, T., Caldwell, K.S., Piskurewicz, U., Cavanaugh, K.A., Xu, H., Kozik, A., Ochoa, O., Mchale, L.K., Lahre, K., Jelenska, J., et al. (2009). Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of pseudomonas and Ralstonia. Plant Physiol. *150*, 1733–1749.

Wu, C., Abd-El-Haliem, A., Bozkurt, T.O., Belhaj, K., Terauchi, R., Vossen, J.H., and Kamoun, S. (2017). NLR network mediates immunity to diverse plant pathogens. Proc. Natl. Acad. Sci. *114*, 8113–8118.

Wu, D., von Roepenack-Lahaye, E., Buntru, M., de Lange, O., Schandry, N., Pérez-Quintero, A.L., Weinberg, Z., Lowe-Power, T.M., Szurek, B., Michael, A.J., et al. (2019). A Plant Pathogen Type III Effector Protein Subverts Translational Regulation to Boost Host Polyamine Levels. Cell Host Microbe *26*, 638–649.

Wu, S.Y., Lee, A.Y., Lai, H.T., Zhang, H., and Chiang, C.M. (2013). Phospho switch triggers brd4 chromatin binding and activator recruitment for gene-specific targeting. Mol. Cell *49*, 843–857.

Xi'ou, X., Bihao, C., Guannan, L., Jianjun, L., Qinghua, C., Jin, J., and Yujing, C. (2015). Functional Characterization of a Putative Bacterial Wilt Resistance Gene (RE-bw) in Eggplant. Plant Mol. Biol. Report. *33*, 1058–1073.

Xia, Y., Zou, R., Escouboué, M., Zhong, L., Zhu, C., Pouzet, C., Wu, X., Wang, Y., Lv, G., Zhou, H., et al. (2021). Secondary-structure switch regulates the substrate binding of a YopJ family acetyltransferase. Nat. Commun. *12*, 1–10.

Xin, M., Wang, Y., Yao, Y., Song, N., Hu, Z., Qin, D., Xie, C., Peng, H., Ni, Z., and Sun, Q. (2011). Identification and characterization of wheat long non-protein coding RNAs responsive to powdery mildew infection and heat stress by using microarray analysis and SBS sequencing. BMC Plant Biol. *11*, 1–13.

Xiong, Q., Ye, W., Choi, D., Wong, J., Qiao, Y., Tao, K., Wang, Y., and Ma, W. (2014). Phytophthora

Suppressor of RNA Silencing 2 is a Conserved RxLR Effector that Promotes Infection in Soybean and Arabidopsis thaliana. Mol. Plant-Microbe Interact. *27*, 1379–1389.

Yadukrishnan, P., and Datta, S. (2021). Light and abscisic acid interplay in early seedling development. New Phytol. *229*, 763–769.

Yang, B., Wang, Y., Guo, B., Jing, M., Zhou, H., Li, Y., Wang, H., Huang, J., Wang, Y., Ye, W., et al. (2019). The Phytophthora sojae RXLR effector Avh238 destabilizes soybean Type2 GmACSs to suppress ethylene biosynthesis and promote infection. New Phytol. *222*, 425–437.

Yang, D.-L., Yao, J., Mei, C.-S., Tong, X.-H., Zeng, L.-J., Li, Q., Xiao, L.-T., Sun, T., Li, J., Deng, X.-W., et al. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. *109*, 1192–1200.

Yang, L., Chen, X., Wang, Z., Sun, Q., Hong, A., Zhang, A., Zhong, X., and Hua, J. (2020). HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. New Phytol. *226*, 507–522.

Yang, L., Guan, D., Valls, M., and Ding, W. (2021). Sustainable natural bioresources in crop protection: antimicrobial hydroxycoumarins induce membrane depolarization-associated changes in the transcriptome of <scp> *Ralstonia solanacearum* </scp>. Pest Manag. Sci. *77*, 5170–5185.

Yu, X., Feng, B., He, P., and Shan, L. (2017). From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition. Annu. Rev. Phytopathol. *55*, 109–137.

Yu, Y., Zhang, Y., Chen, X., and Chen, Y. (2019). Plant noncoding RNAs: Hidden players in development and stress responses. Annu. Rev. Cell Dev. Biol. *35*, 407–431.

Yu, Y., Fu, W., Xu, J., Lei, Y., Song, X., Liang, Z., Zhu, T., Liang, Y., Hao, Y., Yuan, L., et al. (2021). Bromodomain-containing proteins BRD1, BRD2, and BRD13 are core subunits of SWI/SNF complexes and vital for their genomic targeting in Arabidopsis. Mol. Plant *14*, 888–904.

Yuan, M., Ke, Y., Huang, R., Ma, L., Yang, Z., Chu, Z., Xiao, J., Li, X., and Wang, S. (2016). A host basal transcription factor is a key component for infection of rice by TALE-carrying bacteria. Elife *5*, 1–17.

Yuan, M., Ngou, B.P.M., Ding, P., and Xin, X.F. (2021a). PTI-ETI crosstalk: an integrative view of plant immunity. Curr. Opin. Plant Biol. *62*, 1–11.

Yuan, M., Jiang, Z., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.M., He, S.Y., and Xin, X.F. (2021b). Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature *592*, 105–109.

Yuliar, Asi Nion, Y., and Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ. *30*, 1–11.

Zaware, N., and Zhou, M.-M. (2019). Bromodomain biology and drug discovery. Nat. Struct. Mol. Biol. *26*, 870–879.

Zhang, C.J., Hou, X.M., Tan, L.M., Shao, C.R., Huang, H.W., Li, Y.Q., Li, L., Cai, T., Chen, S., and He, X.J. (2016a). The Arabidopsis acetylated histone-binding protein BRAT1 forms a complex with BRP1 and prevents transcriptional silencing. Nat. Commun. *7*, 1–13.

Zhang, D., Li, Y., Zhang, X., Zha, P., and Lin, R. (2017a). The SWI2/SNF2 Chromatin-Remodeling ATPase BRAHMA Regulates Chlorophyll Biosynthesis in Arabidopsis. Mol. Plant *10*, 155–167.

Zhang, H., Lang, Z., and Zhu, J.K. (2018). Dynamics and function of DNA methylation in plants. Nat. Rev. Mol. Cell Biol. *19*, 489–506.

Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., Zou, Y., Long, C., Lan, L., Chai, J., et al. (2007a). A Pseudomonas syringae Effector Inactivates MAPKs to Suppress PAMP-Induced Immunity in Plants. Cell Host Microbe *1*, 175–185.

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., et al. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted by a Pseudomonas syringae effector. Cell Host Microbe *7*, 290–301.

Zhang, K., Sridhar, V. V., Zhu, J., Kapoor, A., and Zhu, J.-K. (2007b). Distinctive Core Histone Post-

Translational Modification Patterns in Arabidopsis thaliana. PLoS One *2*, 1–11.

Zhang, T., Lei, J., Yang, H., Xu, K., Wang, R., and Zhang, Z. (2011). An improved method for whole protein extraction from yeast Saccharomyces cerevisiae. Yeast *28*, 795–798.

Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y. V., Pellegrini, M., Goodrich, J., and Jacobsen, S.E. (2007c). Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. PLoS Biol. *5*, 1026–1035.

Zhang, X., Bernatavichute, Y. V., Cokus, S., Pellegrini, M., and Jacobsen, S.E. (2009). Genome-wide analysis of mono-, di- and trimethylation of histone H3 lysine 4 in Arabidopsis thaliana. Genome Biol. *10*, 1–14.

Zhang, X., Ménard, R., Li, Y., Coruzzi, G.M., Heitz, T., Shen, W.H., and Berr, A. (2020). Arabidopsis SDG8 Potentiates the Sustainable Transcriptional Induction of the Pathogenesis-Related Genes PR1 and PR2 During Plant Defense Response. Front. Plant Sci. *11*, 1–14.

Zhang, Y., Zhao, J., Li, Y., Yuan, Z., He, H., Yang, H., Qu, H., Ma, C., and Qu, S. (2016b). Transcriptome analysis highlights defense and signaling pathways mediated by rice pi21 gene with partial resistance to Magnaporthe oryzae. Front. Plant Sci. *7*, 1–17.

Zhang, Z.M., Ma, K.W., Gao, L., Hu, Z., Schwizer, S., Ma, W., and Song, J. (2017b). Mechanism of host substrate acetylation by a YopJ family effector. Nat. Plants *3*.

Zhao, C., Wang, H., Lu, Y., Hu, J., Qu, L., Li, Z., Wang, D., He, Y., Valls, M., Coll, N.S., et al. (2019). Deep sequencing reveals early reprogramming of arabidopsis root transcriptomes upon ralstonia solanacearum infection. Mol. Plant-Microbe Interact. *31*, 813–827.

Zhao, S., Zhang, B., Yang, M., Zhu, J., and Li, H. (2018). Systematic Profiling of Histone Readers in Arabidopsis thaliana. Cell Rep. *22*, 1099–1102.

Zheng, X., Kang, S., Jing, Y., Ren, Z., Li, L., Zhou, J.M., Berkowitz, G., Shi, J., Fu, A., Lan, W., et al. (2018). Danger-associated peptides close stomata by OST1-independent activation of anion channels in guard cells. Plant Cell *30*, 1132–1146.

Zheng, X., Li, X., Wang, B., Cheng, D., Li, Y., Li, W., Huang, M., Tan, X., Zhao, G., Song, B., et al. (2019). A systematic screen of conserved Ralstonia solanacearum effectors reveals the role of RipAB, a nuclear-localized effector that suppresses immune responses in potato. Mol. Plant Pathol. *20*, 547–561.

Zhou, C., Zhang, L., Duan, J., Miki, B., and Wu, K. (2005). Histone Deacetylase19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell *17*, 1196–1204.

Zhu, H., Wang, G., and Qian, J. (2016a). Transcription factors as readers and effectors of DNA methylation. Nat. Rev. Genet. *17*, 551–565.

Zhu, Q.-H., Shan, W.-X., Ayliffe, M.A., and Wang, M.-B. (2016b). Epigenetic Mechanisms: An Emerging Player in Plant-Microbe Interactions. Mol. Plant-Microbe Interact. *29*, 187–196.

Zhu, Q.H., Stephen, S., Taylor, J., Helliwell, C.A., and Wang, M.B. (2014). Long noncoding RNAs responsive to Fusarium oxysporum infection in Arabidopsis thaliana. New Phytol. *201*, 574–584.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D.G., Felix, G., and Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. Nature *428*, 764–767.

Zou, B., Sun, Q., Zhang, W., Ding, Y., Yang, D.L., Shi, Z., and Hua, J. (2017). The arabidopsis chromatin-remodeling factor CHR5 regulates plant immune responses and nucleosome occupancy. Plant Cell Physiol. *58*, 2202–2216.

In this section, I briefly summarises the main progresses made on the identification of GTE9 and GTE11 as targets of the RSSC T3E PopP2. I also present some additional data obtained during my thesis and which, although not included in the manuscript, allow to progress on the functional characterisation of GTE9 and GTE11 and their involvement in bacterial wilt disease.

Main conclusions from the manuscript (Comorge et al., in prep.)

From the article, the following conclusions can be drawn:

- PopP2 co-localises and interacts with both GTE9 and GTE11 in plant cells.
- PopP2 acetylates GTE9 and GTE11 in planta.
- GTE9 and GTE11 bind preferentially to tri- and tetra-acetylated lysine residues on histone H4 and interact with histone H4 in vivo, suggesting that they could behave as epigenetic readers.
- PopP2 acetylates multiple lysine residues in GTE9 and GTE11, some of which are located on either side of their BRD.
- PopP2 alters the association GTE9-H4 through its acetyltransferase activity.
- GTE9 and GTE11 over-expression contributes to the virulence of the GMI1000 strain of the RSSC, and it depends on PopP2-acetyltransferase activity.
- PopP2 acetylates various members of the GTE family.

Complements to the manuscript

- **gte9-1 and gte11-1 mutants behave similarly to WT Col-0 plants in response to RSSC producing or not PopP2**

As a complement to the inoculations of gte9-1 and gte11-1 mutants with GMI1000, we also inoculated these lines with RSSC strains either lacking PopP2 (ΔpopP2) or expressing WT PopP2 (ΔpopP2+PopP2) or PopP2 catalytic mutant (Δ

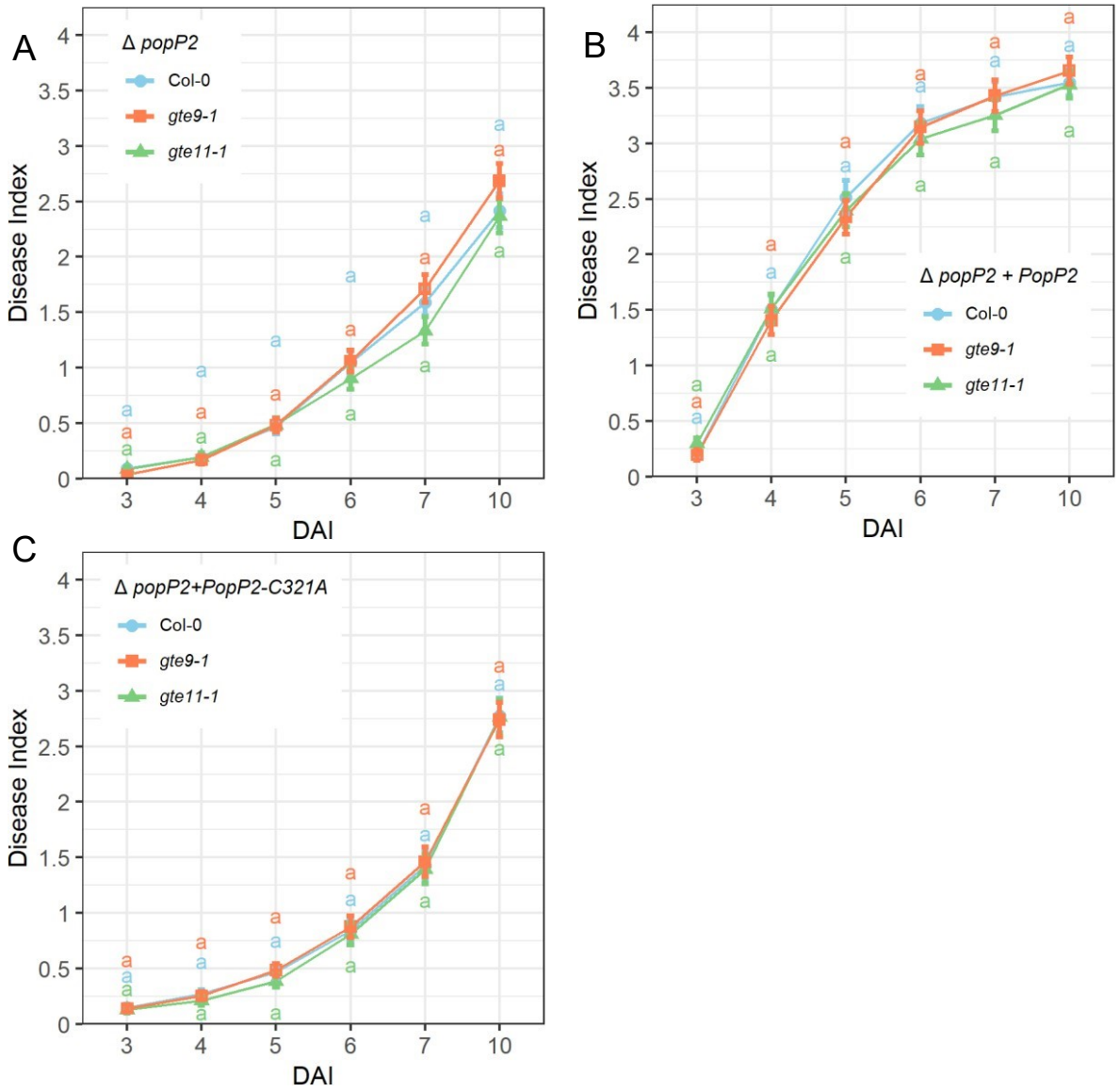


Figure 21. *gte9-1* and *gte11-1* mutants show similar disease development as Col-0 in presence or absence of PopP2.

The graphs show least-square means +/- Standard Error of the LS means from 3 independent inoculations of ~24 plants respectively (inoculation with ΔPopP2: n total = 72 plants for Col-0 and *gte11-1* and 70 for *gte9-1*; with ΔPopP2+PopP2: n total = 72 plants for Col-0 and *gte11-1* and 70 for *gte9-1;* with ΔPopP2+PopP2- C321A: n total = 72 plants for Col-0, 71 for *gte9-1* and 70 for *gte11-1)*. In A, B and C, a denotes no significant difference between two lines at a certain DAI (one-way ANOVA performed on the mixed model described in the methods, p-value < 0.05).

popP2+PopP2-C321A) (Figure 5A, Comorge et al., in prep.), Figure 21). As previously observed in response to the reference strain GMI1000, inoculation of gte9-1 and gte11-1 mutants with a ΔpopP2+PopP2 strain resulted in similar symptom development compared to the WT line (Figure 21). This confirms that the ΔpopP2+PopP2 strain behaves like GMI1000. Furthermore, the gte9-1 and gte11-1 mutants also display similar phenotypes to the WT Col-0 line in response ΔpopP2 or ΔpopP2+PopP2-C321A (Figure 21). Overall, this suggests that these two knockout mutants behave like the WT line in response to R. solanacearum strains producing or not the PopP2 effector. This could support the hypothesis of functional redundancy, likely between different GTE members. However, another possible explanation could be related to the position of the T-DNA present in gte9-1 and gte11-1 mutants. Indeed, the T-DNA insertion in these mutants is located after the second exon of GTE9 and GTE11, downstream of the BRD coding region (Figure 22A). This means that gte9-1 and gte11-1 could still produce shorter transcripts encoding for truncated forms of GTE9 and GTE11 that would contain the BRD protein module. Such truncated proteins could therefore be partially functional (if they are still able to bind acetylated histone tails) and thus potentially manipulated by PopP2. To test this hypothesis, we investigated whether such truncated transcripts could be detected by RT-qPCR in gte9 -1 and gte11-1 mutants. For this, we used primer pairs that could amplify a region located upstream the BRD of GTE9 and GTE11 (pairs in darker color in Figure 22A). Interestingly, in the gte9-1 mutant, such truncated GTE9 transcripts could be detected, even though relatively less expressed than in the WT line (Figure 22B). These data indicate gte9-1 should not be considered a null mutant since it does produce shorter GTE9 transcripts that could lead to the production of a truncated GTE9 protein lacking its last 328 residues but still containing a BRD, and designated by $GTE9_{1\text{-}370}$. Interestingly, transient expression of 3Flag-tagged GTE9-

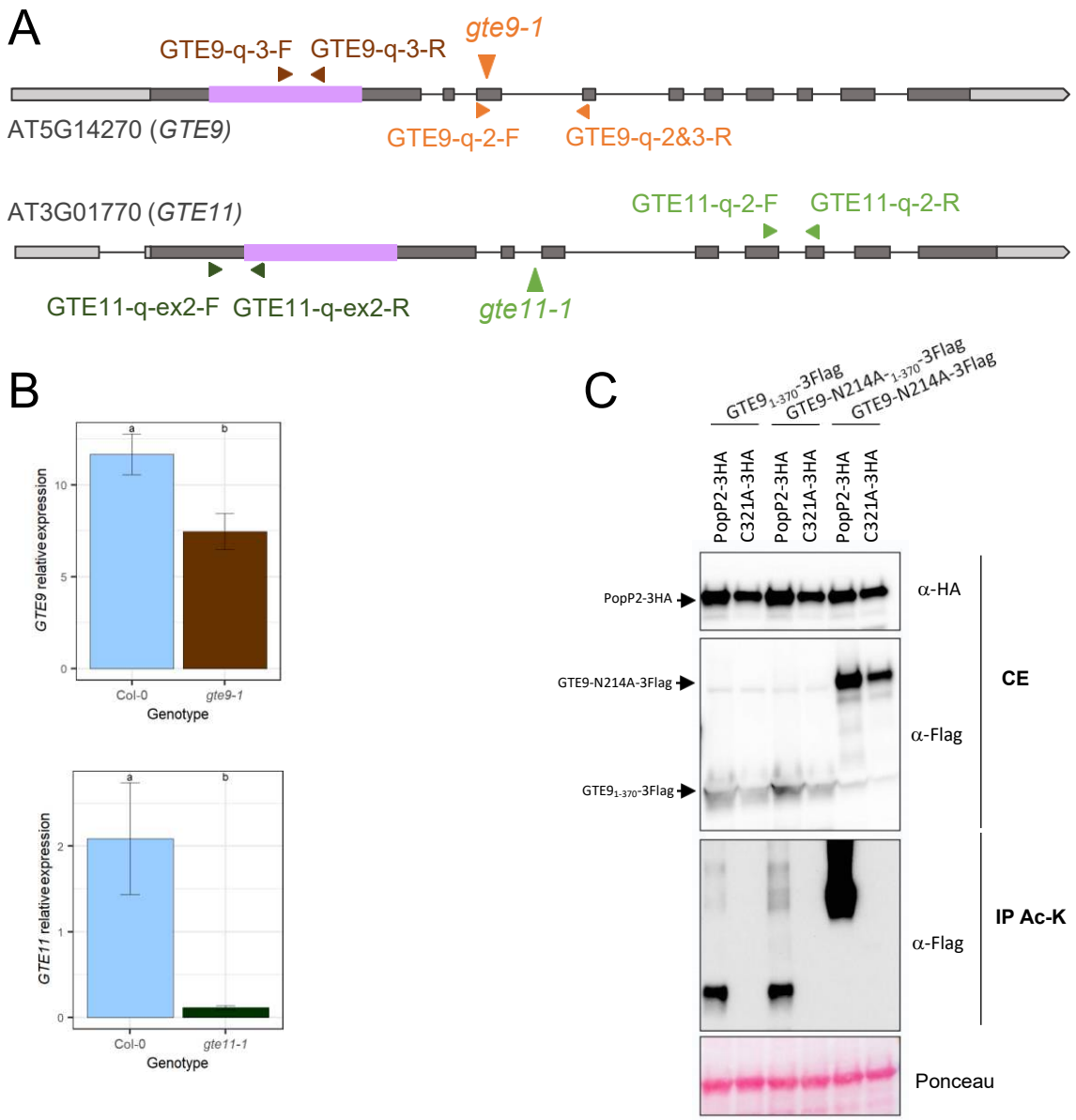


Figure 22. Expression analysis of GTE9 and GTE11 truncated transcripts in *gte9-1* and *gte11-1* T-DNA insertion mutant lines.

The T-DNA insertion in *gte9-1* and *gte11-1* mutants (orange and green triangle, respectively) is located downstream the exon encoding for the BRD module.

(A) *GTE9* and *GTE11* gene structure in *A. thaliana* and the position of the T-DNA insertion for *gte9-1* and *gte11-1* mutants. Exons are the rectangles and introns lines. The pink rectangle is the approximate region encoding for the BRD. Primers used for checking the T-DNA insertion are shown as the same color of the T-DNA insertion and primers usptream the T-DNA insertion are in a darker color.

(B) Expression level of *GTE9* and *GTE11* in Col-0, *gte9-1* and *gte11-1* mutants. The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants and normalised to the expression level of AtEF1-α. The primer pairs used were, respectively, GTE9-q-3-F and GTE9-q-3-R for *GTE9* and GTE11-q-ex2-F and GTE11-q-ex2-R for *GTE11*. Mean values +/- SD (n = 3; p-value = 0.05, a and b show significative difference in mean ΔCt based on the one-way ANOVA or the kruskal-test).

(C) $GTE9_{1-370}$-3Flag, $GTE9\text{-}N214A_{1-370}$-3Flag and GTE9-N214A-3Flag were transiently expressed with 3HA-tagged active PopP2 or C321A catalytic mutant in *N. benthamiana* leaves. Protein extracts were immunoblotted with anti-HA and anti-Flag antibodies (Input). Immunoprecipitation of Lys-acetylated proteins was done in presence of an anti-AcK antibody on protein A-agarose beads (IP a-Ac-K). The immunoprecipitated $GTE9_{1-370}$-3Flag, $GTE9\text{-}N214A_{1-370}$-3Flag and GTE9-N214A-3Flag proteins were detected after immunoblotting with an anti-Flag antibody. Ponceau S staining of total proteins indicates equal loading of the samples in the input. This experiment was repeated two times with similar results.

$_{1\text{-}370}$ (or a mutant containing the N214A substitution, and designated by GTE9-N214A-$_{1\text{-}370}$-3Flag) with active PopP2 in N. benthamiana led to the detection of an acetylated form of this truncated GTE9 protein, indicating that GTE9-$_{1\text{-}370}$ behaves as a PopP2 substrate, like full-length GTE9 (Figure 22C). Therefore, it is highly likely that the gte9-1 mutant produces a truncated GTE9 protein with a functional BRD and that is acetylated by PopP2. Regarding the gte11-1 mutant, it is more difficult to conclude since the selected primers amplified less GTE11 short transcripts in both gte11-1 and WT plants. Indeed, the primer pair used for amplifying the upstream region of GTE11 had a relatively low efficiency (89% compared to ~110% for the GTE11-q-2-F and GTE11-q-2-R pair), making difficult to assess properly the amplification even in the WT and to conclude for the presence of transcripts upstream the region of GTE11 encoding the BRD (Figure 22A). Nonetheless, it seems that in the gte11-1 mutant, GTE11 transcripts accumulate much less than in the WT (Figure 22B).

- **Does PopP2 regulate the expression of GTE family genes?**

Apart from GTE9 and GTE11, our data demonstrate that PopP2 can target other members of the GTE family by acetylation (Figure 7B, Comorge et al., in prep.) which could thus also represent PopP2 virulence targets. We next wondered whether the expression level of the GTE gene family was modulated in presence of PopP2. To investigate this question, 3-week-old A. thaliana Col-0 leaves were infiltrated with Pseudomonas fluorescens (Pf0-1) delivering or not active PopP2 or its catalytic mutant the expression level of the different GTE genes was measured by RT-qPCR in plant leaf samples at 0, 3 and 6 hpi (hours after infiltration). Figure 23 shows the mean GTE expression of the biological replicates from the two independent experiments, at the different time points and per infiltrated strain. The top panel represents GTE genes for which their proteins were acetylated by PopP2, and the bottom panel those that were not acetylated by PopP2 (Figure 23). Note

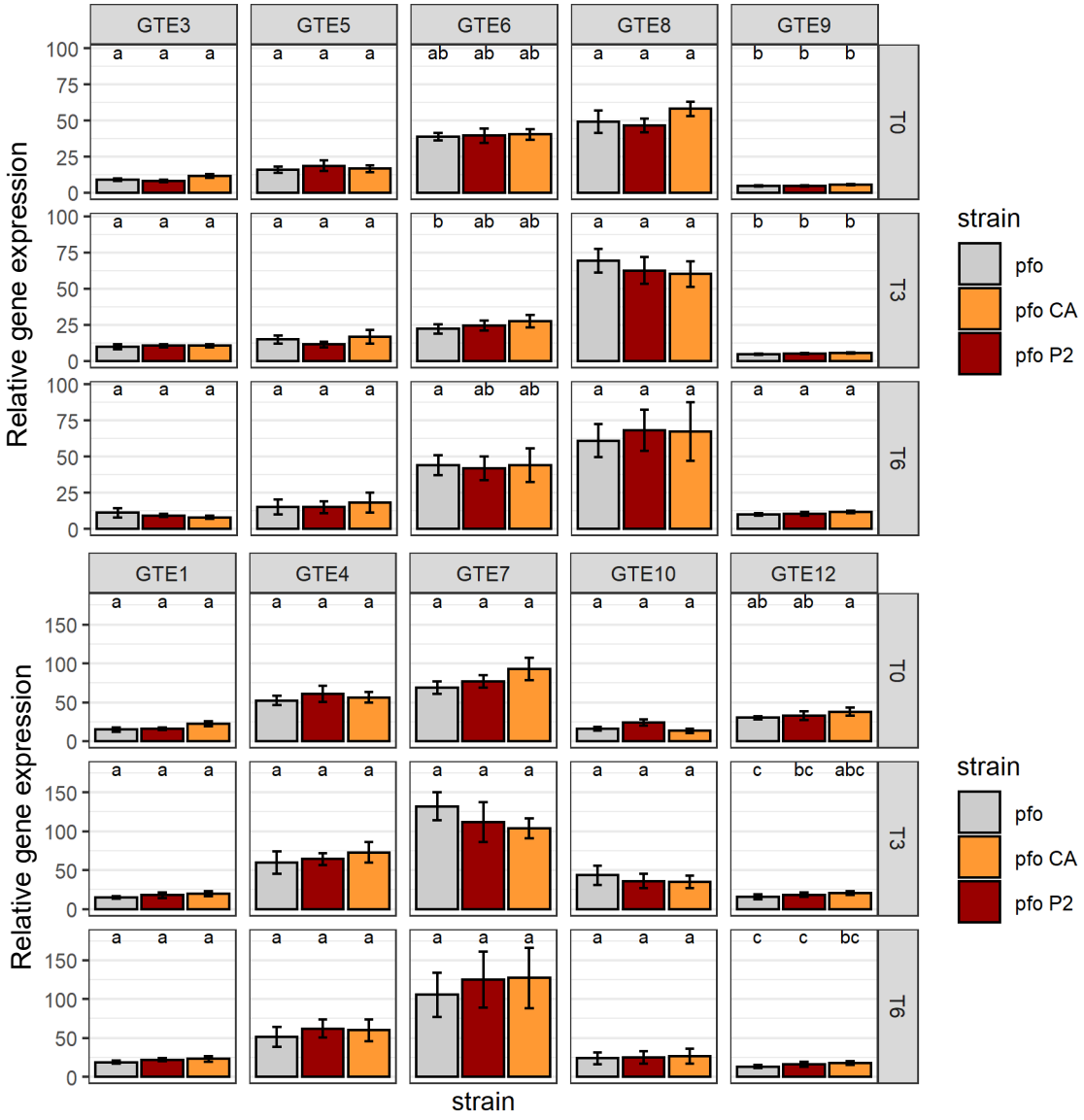


Figure 23. Expression level of *GTE* genes family is not affected by the presence of active PopP2 or PopP2-C321A catalytic mutant.

Expression level of *GTE* genes in Col-0 leaves infiltrated with *Pf0-1* (pfo), *Pf0-1 PopP2-3HA* (pfo P2) or *Pf0-1 PopP2-C321A-3HA* (pfo CA). The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants at 0, 3 or 6 hours post-infiltration and normalised to the expression level of *AtEF1-α*. Mean values +/- SD from 2 independent experiments of 3 biological replicates (n=6; p-value=0.05, a, b, and c show significative difference in mean ΔCt for a given gene at each combination of T and strain, based on two-way ANOVA or kruskal-test). Primers used for RT-qPCR analyses are listed in Table 3.

also that only 10 GTE genes are represented on Figure 23 because for GTE2 and GTE11, analyses could not be performed on data combined from the two independent experiments. Analyses for these two genes were performed for each independent experiment separately and represented in Figure 24. Overall, at 3 or 6 hpi, there is no significant changes in the GTE expression levels when PopP2 is delivered or not by Pf0-1 cells in plant leaves, for all the GTE genes, encoding for the GTEs acetylated by PopP2 or not (Figures 23 and 24). It can be only noted that for some GTE like GTE9 and GTE12, their expression is modulated over the time. Similar conclusions can be drawn for GTE2 and GTE11 (Figure 24). Indeed, in both repetitions, GTE2 and GTE11 expression levels do not seem to be affected by PopP2 or its catalytic mutant at all, but GTE11 expression varies over time after infiltration with Pf0-1 cells. To conclude, delivering PopP2 or its catalytic mutant in plant leaves does not seem to affect the expression of the GTE genes, suggesting that these genes are not transcriptionally regulated in response to PopP2.

Conclusions

In this first chapter, we demonstrated that PopP2 acetylates GTE9 and GTE11 on several lysine residues, including residues flanking their BRD. This acetylation correlates with an alteration of their ability to interact with histone H4. Moreover, over-expression of GTE9 and GTE11 in the susceptible A. thaliana ecotype Col-0 resulted in an increased susceptibility in response to the RSSC strain GMI1000 and this increased susceptibility of the GTE9 and GTE11 over-expressing lines depends on the acetyltransferase activity of PopP2. Together, these data indicate that GTE9 and GTE11 represent virulence targets of PopP2. Considering that GTE9 and GTE11 are histone readers that appear to be virulence targets PopP2, it would be interesting to determine whether the increased susceptibility of the GTE9 and GTE11 over-expressing lines to GMI1000 depends on the ability of overexpressed

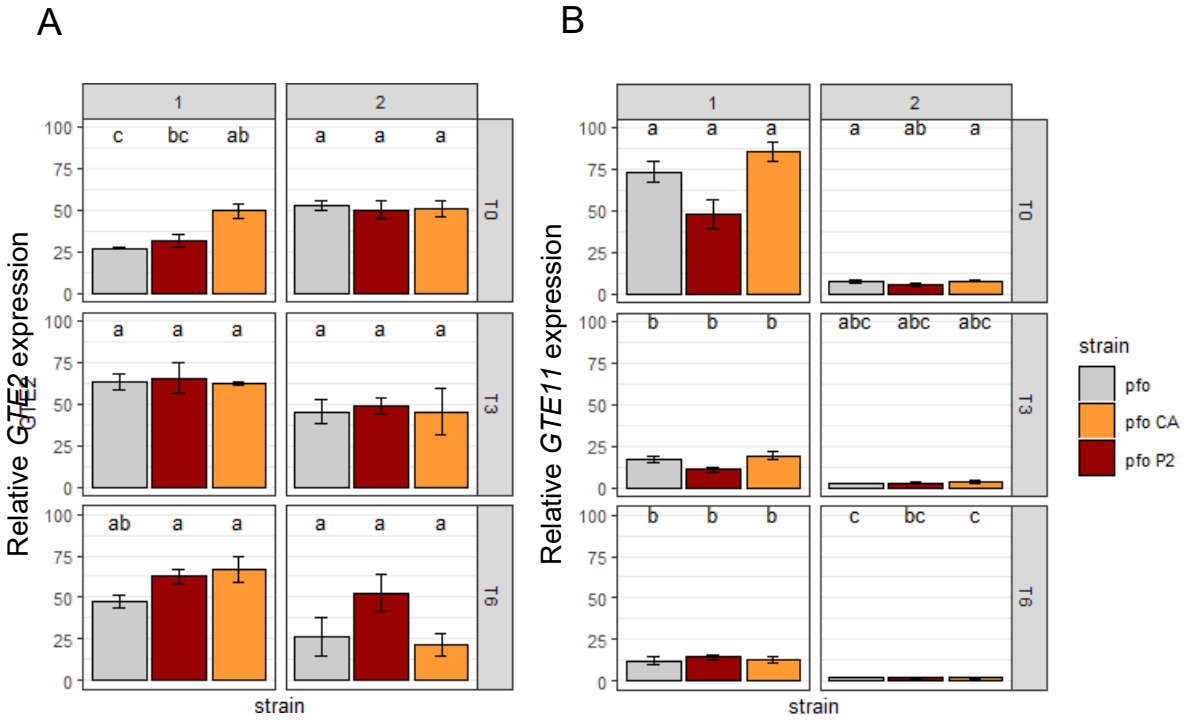


Figure 24. Expression level of GTE2 and GTE11 is not modulated by the presence of active and catalytically inactive PopP2.
Expression level of *GTE2* and *GTE11* genes in Col-0 leaves infiltrated with *Pf0-1* (pfo), *Pf0-1 PopP2-3HA* (pfo P2) or *Pf0-1 PopP2-C321A-3HA* (pfo CA) (A and B respectively). The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants at 0, 3 or 6 hours post-infiltration and normalised to the expression level of AtEF1-α. Mean values +/- SD (n = 3; p-value = 0.05, a, b, and c show significative difference in mean ΔCt for a given gene at each combination of T and strain, based of the two-way ANOVA or the kruskal-test). Data shown from the two independent experiments seperately (1 and 2) because they could not be combined. Primers used for RT-qPCR analyses are listed in Table 3.

GTE9 and GTE11 to bind acetylated histones via their BRD. Knowing this, we could link the alteration of GTE-H4 interaction by PopP2-mediated acetylation and the role of GTE9 and GTE11 as virulence targets.

Also, the fact that gte9-1 and gte11-1 mutants behave as Col-0 in response to GMI1000 or popP2-deficient strains and/or complemented with PopP2 or its catalytic mutant suggested functional redundancy between GTE9 and GTE11, as they regulate similar pathways, the ABA and sugar signalling pathways (Misra et al., 2018). This functional redundancy is even more likely as PopP2 seems to target several members of the GTE family. To overcome this functional redundancy, it would have been very informative to study the phenotypical response of a gte9-1/gte11-1 double mutant to RSSC strains producing or not PopP2. Unfortunately, despite several attempts, it was not possible to select such a gte9-1/gte11-1 double mutant from independent genetic crosses between the gte9-1 and gte11-1 single mutant lines. Nonetheless, it would not have been guaranteed that a gte9-1/gte11-1 double mutant could have overcome the potential functional redundancy since PopP2 can target additional GTE members (GTE3, GTE5 and GTE8). Furthermore, GTE9 and GTE11 are regulators of the ABA signalling pathway, but two other GTE members, GTE1 and GTE10, appear to be also involved in the regulation of this pathway. Therefore, the double gte9-1/gte11-1 mutation may not be sufficient to overcome potential functional redundancy (Duque and Chua, 2003; Kim et al., 2009).

In addition, we formulated another hypothesis to explain why the phenotypical response gte9-1 and gte11-1 mutants to R. solanacearum was not affected, as compared to WT Col-0 plants. Since these two mutants carry a T-DNA insertion downstream of the BRD coding region, it is possible that these mutants still express truncated forms of GTE9 and GTE11 able to bind acetylated Histones thanks to their BRD (Figure 22A). We tried to assess the presence of corresponding truncated

GTE9 and GTE11 transcripts in these mutant lines. We found that that there could indeed be such truncated transcripts in the gte9-1 mutant, although it is less likely in gte11-1 (Figure 22B). Consequently, and considering that a $GTE9_{1-370}$ truncated version can be acetylated by PopP2 (Figure 22C), it is possible that PopP2 targets these truncated forms. So, to determine whether the phenotype observed in the mutants is due to functional redundancy or to the presence of truncated forms of GTE9 and GTE11 that would be able to complement the mutation resulting from the T-DNA insertion, other mutants with a T-DNA insertion upstream of the BRD coding region should be tested in interaction with R. solanacearum strains. Alternatively, we could also try to mutate both the 5' part of GTE9 and GTE11 coding regions by CRISPR/Cas9-based genome editing which is now commonly used in model plants such as A. thaliana.

In addition to GTE9 and GTE11, we have also shown that PopP2 acetylates other GTEs such as GTE3, GTE5, and GTE8 but it may affect these epigenetic readers only at a post-translational level protein level since PopP2 does not seem to modulate the expression level of the GTEs upon Pf0-1-mediated delivery of PopP2 in Arabidopsis (Figures 23 and 24). However, such a conclusion should be taken with caution. Indeed, to assess whether PopP2 modulates the transcription of the different GTE genes, we used an engineered pathogen effectors delivery assay (Pf0-1-mediated delivery) allowing the delivery of PopP2 or its variants in plant cells through a T3SS and we measured the expression of the different GTE genes at 3 hpi and 6 hpi. During the natural infection process used by the RSSC, PopP2 is likely secreted and injected in root and leaf tissues which are colonised by this bacterial pathogen. In other words, the spatio-temporal delivery of PopP2 by the RSSC in root-inoculated Arabidopsis plants might be quite different from that obtained with Pf0-1 cells infiltrated in leaf cells. So, to determine if PopP2 does

affect or not the transcription of GTE members, we should study the expression of the different GTE genes in A. thaliana Col-0 plants over the kinetic of infection with RSSC strains producing or not PopP2. Transcriptomic analyses of A. thaliana roots and leaves from susceptible ecotypes in response to GMI1000 have already been published, and none of the GTE genes were found to be differentially expressed (Hu et al., 2008; Zhao et al., 2019). Another study investigated the changes in transcriptomes of the resistant ecotype Ws-2 and Ws-2 rrs1-1 mutated line after delivering PopP2 or its catalytic mutant by Pf0-1 (Sohn et al., 2014). However, their analyses were focused on transcriptional changes linked to RRS1-R and PopP2 acetyltransferase activity, but not on the presence or absence of PopP2 itself. Thus, to determine whether PopP2 affects or not the transcription of GTEs, it would still be interesting to analyse the expression of the different GTE genes in A. thaliana Col-0 plants after infection by RRSC strains producing or not PopP2.

Finally, an important question to be answered is to what extent PopP2 uses its acetyltransferase activity to control/modulate the biological activitiy(ies) of GTEs. Our data clearly demonstrate that active PopP2 alters the ability of GTE9 to interact with Histone H4 in planta. It is highly likely that PopP2 compromises GTE9 BRD binding abilities by acetylating critical Lysine residues around this protein module, promoting intramolecular interactions that in turn inhibits the BRD and promotes the dissociation of GTE9 from chromatin (see Discussion section in Comorge et al., in prep.). Is it true for the other GTEs members we identified as PopP2 substrates? This remains to be determined. In this context, it would be very interesting to identify by a mass spectrometry-based analysis which lysine residues are acetylated by PopP2 in GTE3, GTE5 and GTE8. This could lead to the identification of key conserved Lysine residues

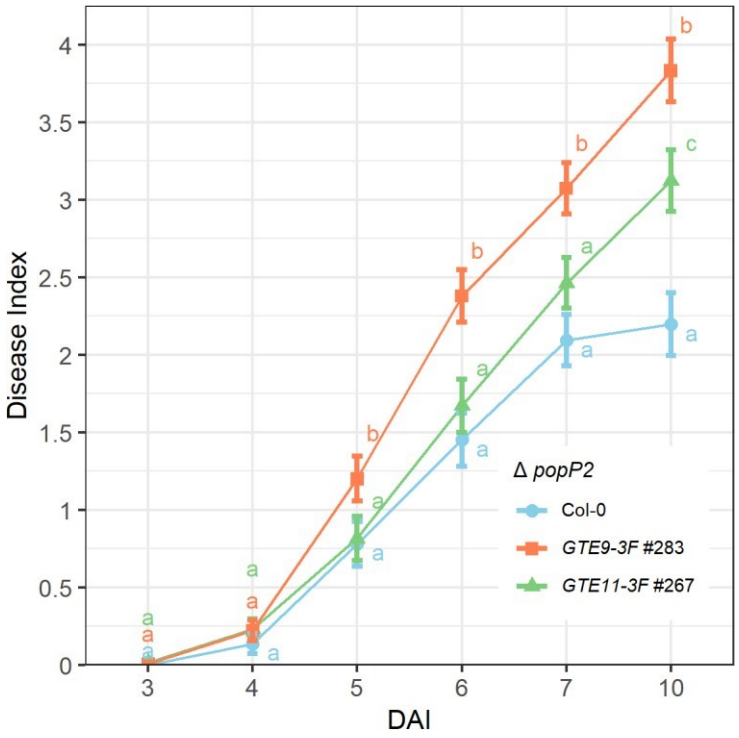


Figure 25. Disease wilting symptom developement is accelarated in *GTE9* and *GTE11* overexpressing lines in response to a RSSC strain lacking the *popP2* gene.
GTE9-3Flag and *GTE11-3Flag* over-expressing lines are more susceptible to a RSSC strain lacking *popP2* in late stages of disease.
The graphs show least-square means +/- Standard Error of the LS means from 3 independent inoculations of ~24 plants respectively (n total = 70 plants for Col-0, *GTE9-3Flag* and *GTE11-3Flag*). a, b and c denote significant difference between two lines at a certain DAI (one-way ANOVA performed on the mixed model described in the methods, p-value < 0.05).

in GTEs that would be specifically targeted by PopP2 to manipulate their function. This is reminiscent of PopP2's targeting of a particular Lysine residue within the DNA-binding domain of WRKY TFs and which results in their inability to bind to their target DNA (ie defence-related genes), and consequently in the dampening of basal immune responses (Le Roux et al., 2015; Sarris et al., 2015) .

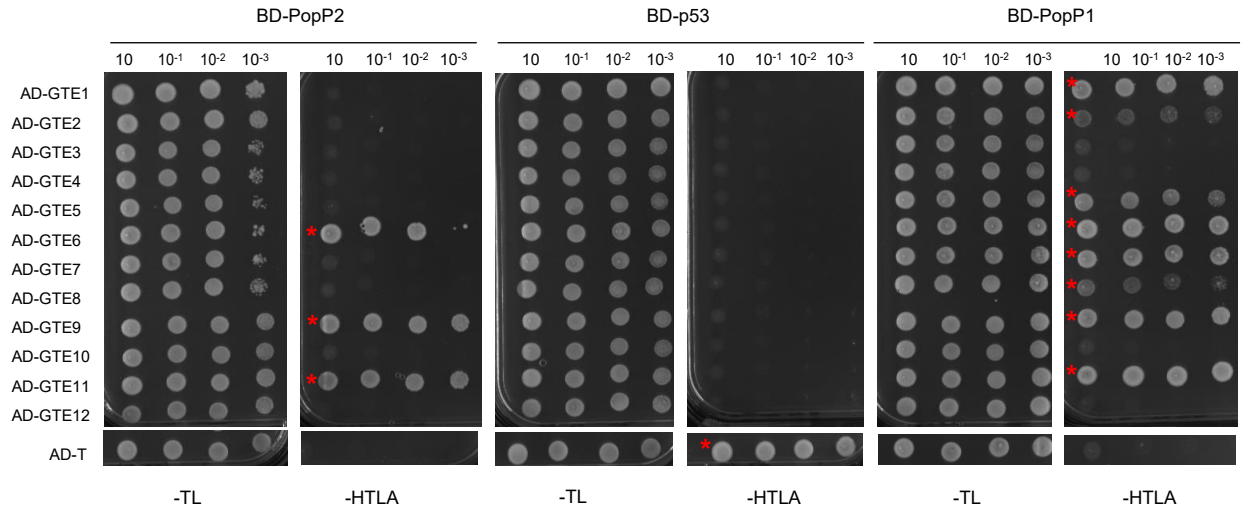
Chapter 2: Can other effectors target GTE9 and GTE11?

GTE9 and GTE11 appear to behave as histone readers and because epigenetic mechanisms regulate a wide variety of cellular processes, including immune responses, these chromatin-associated components represent promising virulence targets that can be manipulated by pathogens.

We were able to show in Chapter 1 that GTE9 and GTE11 represent PopP2 virulence targets since (i) they are acetylated by PopP2 and that (ii) over-expression of GTE9 and GTE11 leads to enhanced disease response to R. solanacearum, which depends on the acetyltransferase activity of PopP2 (Comorge et al., in prep.).

If PopP2 were the only effector responsible for the increased susceptibility observed in the over-expressing lines in response to GMI1000, inoculation with a popP2-deficient strain (ΔpopP2 strain) should result in comparable wilting symptom development between the over-expressing lines and the WT. Strikingly, upon root-inoculation of GTE9 and GTE11 over-expressing lines with a R. solanacearum strain lacking popP2, these transgenic lines also displayed an enhanced disease response compared to WT plants (Figure 25). Therefore, the absence of PopP2 does not seem to abrogate the increased susceptibility of the GTE9 and GTE11 over-expressing lines to the RSSC. To explain this, one scenario

A



B

```
PopP2            MKVSSANAGVPASSADN SARPSQTN  TTPLGR  RA  DAPGSP AFR RQDS E SA    60
PopP1            MKRLFRALGVSQPTRTY PTTDTTSP  ATPESS  SS  SSRSPS VRS SRNH D --    57

PopP2            QTMFRRAGMTS LPPSPATSEHVPLLDNRPTLERMGVDHPLPGRTWYETGHTTASLADRTS  12 0
PopP1            ----------LSDLP------------------------------------------------ - 62

PopP2             TASAAQVASSSRSAGPATA ARFQPTRTSAGQQATVGRLRTQVTGFLSGALGK QA SAQN 180
PopP1 ------------------------------SRPRRKAEMLAESIQSA D SVDPE   AETT  N ASH-  102

PopP2            MDPEL ACFRVLDVDRAIMPLLIVAENA RNPGLNLVPLHMDMAEDEE  TQPPMAGSRHIA   240
PopP1            -----TQPS VMETNLD RH QTIVQSY  NNLNIQAFNS---REAF ALVEGQGSGRAE   154

PopP2            EFVASARPGRYRAVIDDGS TRAADI  DAS     VV  RKEKD  SA VY D V   297
PopP1            RGVVRDYPN---------L HFAADV  HED  T  IL  AGNQ N PG T L SL   205

PopP2            NME RGEHAKCAFTPVDIQKSFFDCRILS LA MHD-KDDAFAAFHET NG DPSHHV   356
PopP1            RYN LGSQCRMVVEAEAQKSLSDCVAFA DFA AYQERRTTFDQ WHEN AY  IADNG   265

PopP2            SRAQQTEELGATLVL D-------GAPLVDARMM QGQAASSVSRYLGN H QSTVP  KR   409
PopP1            VQDKKYGPFDRGLYH NYGIHLIKSNGVLPPVFY SAHSRETLKGVEKRQ GLETD S G   325

PopP2            NET LGERTTRHLVKRKVRNR ADSEGRVTSGETKEIT NSVEQKR  LLNRA SYVNSAP 469
PopP1            RNK DGAES----LEERMEAFS NH ---------- E----PRN SEASR R--------360

PopP2            PPVVMRMAK LLQDSLLDTN  488
PopP1            -----KIRHA ES -------368
```

Figure 26. PopP1 interacts with various GTE members in yeast cells.
(A).Yeast two-hybrid assay to test the interaction between PopP1 and the different GTE proteins from *A. thaliana*. Yeast cells transformed with different bait (BD-fusion) and prey (AD-fusion) plasmid combinations were spread on non-selective (-TL) and selective media (-HTLA). Growth on –HTLA selective medium indicates an interaction between the tested proteins. Photographs were taken at 5 days post-dilution. PopP2 (left), p53 (middle) and PopP1 (right) fused with the BD domain were used as bait proteins, respectively. White stars indicate interaction between the bait and prey proteins tested in yeast cells. This experiment has been carried out twice.
(B) Protein alignement between PopP2 (488 residues) and PopP1 (388 residues). Identical residues are boxed in black. The three residues of PopP2 and PopP1 catalytic triad are boxed in red. The PopP2 Lys residue targeted by PopP2 autoacetylation activity (K383) is boxed in green (corresponding residue in PopP1 is at position 299)

predicts that these epigenetic readers could be also targeted by other T3Es produced by the GMI1000 strain.

PopP2 belongs to the PopP family of RSSC effectors, which is composed of PopP1, PopP2 and PopP3. In the reference strain GMI1000, only popP1 and popP2 are functional genes, since popP3 is inactivated by the presence of an insertion sequence within the gene (Lavie et al., 2004). Given its similarities with PopP2 (24% identity, 39.5% similarity), we hypothesised that PopP1 which could represent a promising effector candidate also able to physically interact with GTE proteins, like PopP2, and responsible for the enhanced disease response of the GTE9 and GTE11 over-expressing lines to a RSSC strain lacking popP2. To test this hypothesis, PopP1 was used as a bait in a Y2H interaction assay with the twelve different A. thaliana GTE members used as prey proteins (Figure 26A). As negative control, we used the BD-p53 protein as bait. Strikingly, PopP1 was found to interact with various GTE members in yeast cells including GTE1, GTE2, GTE5, GTE6, GTE7, GTE8, GTE9 and GTE11. These data suggest that within the GTE family, PopP1 and PopP2 could interact with specific subsets of GTE proteins. The nucleocytoplasmic localisation of PopP1 (unpublished data) is consistent with a physical interaction between PopP1 and GTEs that would occur in the plant nucleus, as described with PopP2. The targeting of different GTEs by PopP1, including GTE9 and GTE11, would explain why the GTE9 and GTE11 over-expressing lines inoculated with GMI1000 deleted from popP2 but still producing PopP1 were still more susceptible that the WT line in later stages of disease. In N. benthamiana, PopP1 recognition is mediated by the NLR NbZAR1 (Deslandes, unpublished data) but the virulence targets of PopP1 remain largely unknown, both in N. benthamiana and in A. thaliana. The identification of GTEs proteins as putative interacting partners and substrates of PopP1 should help elucidate the virulence functions of this effector.

These findings prompted us to investigate whether GTE9 and GTE11 could represent a cellular hub targeted by effectors from different plant bacterial pathogens that would have independently evolved virulence strategies converging

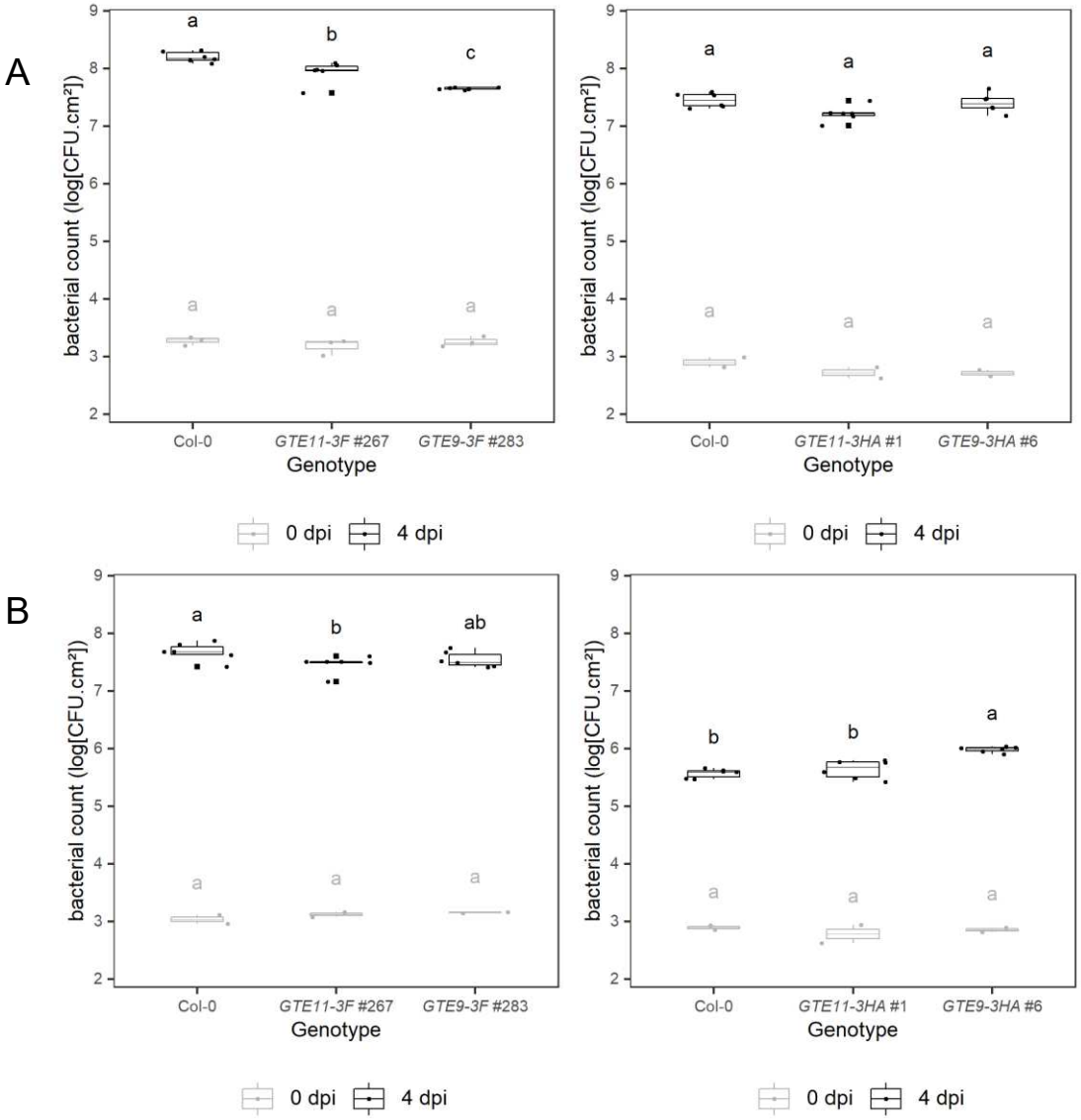


Figure 27. The *in planta* growth of *Pst* DC3000 is not impacted by overexpression of *GTE9* and *GTE11*.

Bacterial growth assay in the *GTE9* and *GTE11* over-expressing lines.

The graphs show boxplots of *Pst* DC3000 bacterial count (log[CFU.cm²]) in Col-0, *GTE9-3Flag* #283 and *GTE11-3Flag* #267 (left panel); and Col-0, *GTE9-3HA* #6 and *GTE11-3HA* #1 (right panel) at 0 dpi and 4 dpi. Graphs represent bacterial growth from two independent experiments (A and B), performed on 6 biological replicates per genotype at 4 dpi and 2-3 biological replicates par genotype at 0 dpi. a, b and c denote significant difference between the mean bacterial growth between genotypes at a same day post infiltration (one-way ANOVA or kruskal test, p-value <0.05).

onto the manipulation of GTEs proteins to promote infection. To address this question, GTE9 and GTE11 over-expression lines were challenged with the virulent bacterial pathogen Pst DC3000 which contains a repertoire of T3Es with well-described virulence activities (Schreiber et al., 2021a). In detail, 3-week-old Arabidopsis Col-0 and lines over-expressing GTE9 or GTE11, previously used for inoculations with R. solanacearum strains in Chapter 1, were leaf-inoculated by infiltration of a Pst DC3000 suspension in order to assess their bacterial multiplication in planta at 4 dpi (Figure 27). For each genotype this experiment has been repeated independently twice (Figure 27A and 27B). Taking the results of the two independent repetitions, the conclusions are unclear. Indeed, according to the results presented in Figure 27A, the 3HA-tagged GTE9 and GTE11 over-expressing lines (GTE9-3HA and GTE11-3HA) displayed similar bacterial growth to that of Col-0, but in the second independent experiment, the GTE9-3HA line seemed to be more susceptible than Col-0 (Figure 27B). Similarly, in the second independent experiment, the GTE9-3Flag over-expressing line showed similar bacterial multiplication to Col-0; whereas in the first experiment, this line contained less bacteria than Col-0 (Figure 27B and 27A). Note also that the significant differences in bacterial loads between genotypes at 4 dpi are not 1 log difference, but closer to 0.5 log or less. To conclude, since DC3000 bacterial growth in the GTE9 and GTE11 over-expressing lines did not show a reproducible trend, it is not clear whether the GTE9 or GTE11 over-expressing lines are more susceptible to this virulent pathogen. Additional rounds of this bacterial multiplication assay will be performed soon in order to determine if the overexpression of GTE9 and GTE11 also promote infection by DC3000.

Conclusions

To conclude this chapter, it seems that GTE9 and GTE11 are not only targeted by PopP2 but also by at least one other effector from the RSSC which also belong to the YopJ family of acetyltransferase and corresponding to PopP1 (Figure 26A and 26B). The interaction between PopP1 and GTE9 and GTE11 remains to be confirmed in planta (e.g. by FRET-FLIM assay or co-immunoprecipitations). Such assays performed in N. benthamiana through Agrobacterium-mediated transient expression could be quite challenging since PopP1 triggers a strong cell death response in Nicotiana spp. (Lavie et al., 2002; Poueymiro et al., 2009), involving the NLR NbZAR1 in N. benthamiana (Deslandes, unpublished data). To circumvent this problem, the use of the zar1-1 mutant line of N. benthamiana (Schultink et al., 2019), which is unable to recognise the Xanthomonas perforans XopJ4 effector (which shares ~61% identity with PopP1), should make possible the transient expression of PopP1, and of its putative GTE interacting partners, without triggering of cell death.

It would be also interesting to know whether GTE9 and GTE11 are virulence targets of other effectors from the RSSC. To check this, we could first study the phenotypical response of the GTE9 and GTE11 over-expressing lines to a RSSC strain lacking both PopP2 and PopP1 (ΔpopP1 ΔpopP2 strain). If the loss of both popP1 and popP2 suppresses the hyper-susceptible phenotype of the GTE9 and GTE11 over-expressing lines in response to R. solanacearum, this would mean that PopP1 and PopP2 are likely the only GMI1000 effectors manipulating these histone readers. Otherwise, it would mean that other GMI1000 effectors could target GTE9 and/or GTE11 and this could be tested by a Y2H screening using GTE9 and GTE11 as baits against a library containing the different GMI1000 effectors cloned as prey constructs (and vice versa).

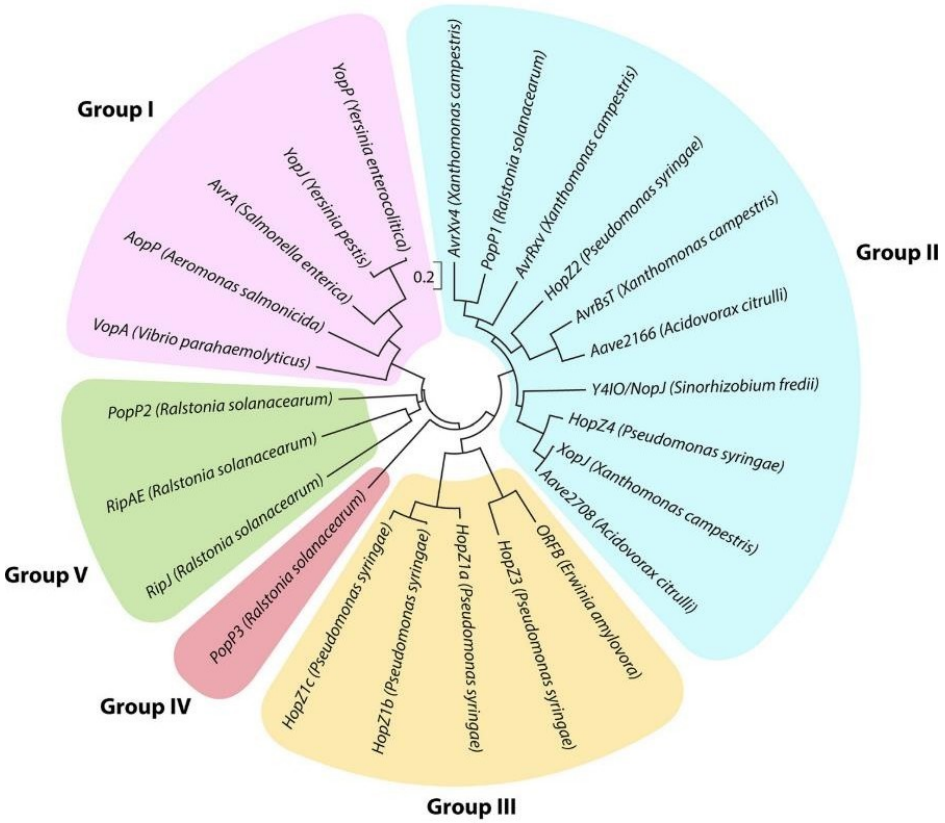


Figure 28. Phylogeny of the YopJ family effectors (Extracted from Ma and Ma, 2016)**.**
The phylogenetic tree was generated from the protein sequences of 24 YopJ family effectors. YopJ effectors from animal pathogens are clustered in group I. YopJ family effectors from plant pathogens are found in different groups. AvrXv4, AvrRxv, AvrBsT, and XopJ come from *Xanthomonas campestris* and are clustered in group II. Also in group II, Aave2166 and Aave2708 are found in *Acidovorax citrulli*. HopZ1, HopZ2, HopZ3, and HopZ4 are effectors from *Pseudomonas syringae*, and are distributed in groups II and III. *Erwinia amylovora* produces ORFB and is found in group III. *Ralstonia solanacearum* produces PopP1, PopP2, PopP3, RipAE, and RipJ. PopP2, PopP3, RipAE and RipJ are clustered in group IV and group V while PopP1 is found in group II (and is closely related to AvrXv4/XopJ4 from *X. campestris*). Only one YopJ effector is produced by a symbiotic bacterium, NopJ which is produced by the nonpathogenic symbiotic bacterium *Sinorhizobium fredii* strain NGR234.

We also saw that PopP1 and PopP2 interact in yeast cells with several GTE proteins, and they both interact with GTE6, GTE9 and GTE11 (Figure 26A). Interestingly, despite both belonging to the YopJ effector family, PopP1 and PopP2 belong to different phylogenetic groups in this family (group II and group V, respectively) (Figure 28) (Ma and Ma, 2016). This raises the question of whether other effectors from the YopJ family could target GTE proteins and this could be tested by Y2H.

As GTE9 and GTE11 are epigenetic components that probably act on a wide range of biological mechanisms, we wondered whether GTE9 and GTE11 could be targeted by other pathogen effectors. As this was not the priority of my PhD, we simply tried to see if GTE9 and GTE11 could be virulence targets of a different bacterial pathogen such as the well-described Pst DC3000. Unfortunately, in planta bacterial growth assays performed on the GTE9 and GTE11 over-expressing lines led to unclear results since the two independent experiments did not display reproducible trends (Figure 27A and 27B). In order to draw conclusions, it is necessary to repeat this experiment again. In addition, it could be interesting to study the phenotypical response of the GTE9 and GTE11 over-expressing lines challenged with other pathogens available in the lab, including bacteria and fungi. This should help to better understand how GTE9 and GTE11 could play a role in modulating host immune responses.

Although we know that GTE11 and GTE9 are negative regulators of ABA and sugar signalling pathways and appear to be involved in the regulation of 35S enhancer activity (Irigoyen et al., 2021; Misra et al., 2018), we lack information on the precise role of GTE11 and GTE9 as epigenetic readers at the chromatin level. Identifying the chromatin regions hosting GTE9 and GTE11 could help us to elucidate (i) what

are the function(s) of these epigenetic readers, and (ii) how their manipulation by PopP2 can impact the course of the infection by RSSC in A. thaliana.

Chapter 3: What is the role of GTE9 and GTE11 at the chromatin level?

It has been described in the literature that GTE9 and GTE11 behave as negative regulators of BT2-mediated sugar and ABA signalling pathways, and are involved in the activation of genes by Cauliflower mosaic virus 35S (35S) multimerised enhancers (Irigoyen et al., 2021; Misra et al., 2018). However, we still lack information on (i) their precise involvement in these mechanisms, (ii) the link with their histone reader activity, and (iii) the reason of their targeting by an acetyltransferase like PopP2. To try to gather more information about the role of GTE9 and GTE11 as histone readers at the molecular level, we decided to explore their role at the chromatin level. Our objective was to identify the chromatin regions targeted by GTE9 and GTE11 thanks to a Chromatin Immunoprecipitation followed by sequencing (ChIP-seq) approach (Veluchamy et al., 2016). This work was performed in the frame of an ANR project (ANR EpiCLIPSE) involving as scientific partners Drs Cécile Raynaud and Moussa Benhamed from Institut Paris Saclay 2 (IPS2) in Paris, as they are experts in techniques related to epigenetics in plants, including ChIP-seq.

As a pre-requisite for this ChIP-seq analysis, we generated some transgenic lines expressing tagged versions of GTE9 and GTE11 under the control of their endogenous promoter regions. To this end, gte9-1 and gte11-1 mutant lines were complemented with GTE9 and GTE11 genomic clone, respectively, both fused to a triple hemagglutinin tag (GTE9prom::GTE9g-3HA and GTE11prom::GTE11g-3HA). As negative controls, similar genomic constructs corresponding to GTE9 and GTE11 mutated in their BRD module (GTE9prom::GTE9g-N214A-3HA and GTE11prom::GTE11g-N206A-3HA) and making them theoretically unable to bind acetylated histone tails were also introduced in gte9-1 and gte11-1, respectively.

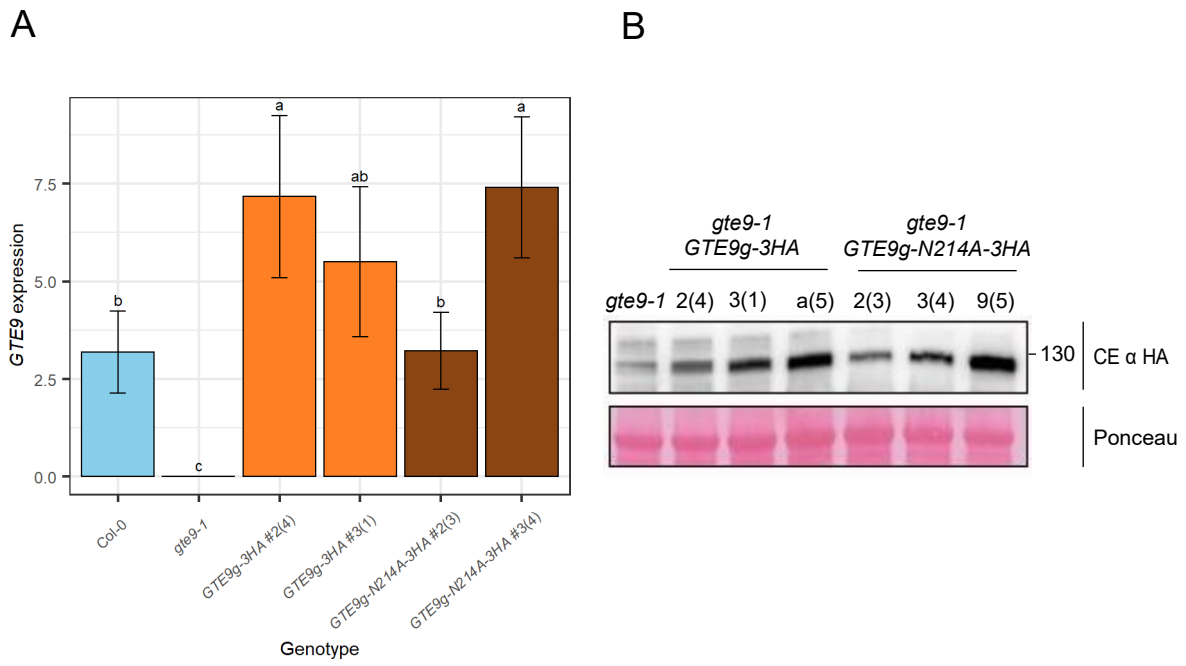


Figure 29. Genetic complementation of the *gte9-1* mutant with genomic clones encoding for GTE9-3HA and GTE9-N214A-3HA.

Generation of *gte9-1 GTE9g-3HA* and *gte9-1 GTE9g-N214A-3HA* complemented lines for ChIP-seq analyses.

(A) Expression level of *GTE9* in Col-0, *gte9-1 GTE9g-3HA* and *gte9-1 GTE9g-N214A-3HA* complemented lines. The relative gene expression levels were measured by RT-qPCR on leaf discs from 3-week-old plants and normalised to the transcript accumulation of *AtEF1-α.* Mean values +/- SD (n = 3; p-value = 0.05, a and b show significative difference in mean ΔCt based on the one-way ANOVA or the kruskal-test). Primers used were GTE9-q-2-F/GTE9-q-2&3-R.

(B) Immuno-detection of GTE9-3HA or GTE9-N214A-3HA proteins from crude protein extracts of *gte9-1* transgenically expressing the relevant 3HA-tagged proteins. Proteins were detected with an anti-HA antibody. Ponceau S staining of total proteins indicates equal loading of the samples in the input.

(n=1).

In Figure 29 and hereafter in the text, these lines are referred to as GTE9g-3HA, GTE11g-3HA, GTE9g-N214A-3HA, GTE11g-N206A-3HA lines.
For a given construct, complemented lines (hemizygous T1 lines and subsequent homozygous T3 lines) were selected. As the selection of the GTE11g-3HA homozygous lines was trickier than for GTE9g-3HA, we prioritised this chapter on the GTE9g-3HA lines. Among the homozygous lines available, two independent lines were selected for GTE9g-3HA and GTE9g-N214A-3HA (in gte9-1 genetic background) based on GTE9 expression level and immunodetection of the 3HA-tagged proteins (Figure 29A and 29B, respectively). Originally, our plan was to select two lines with comparable gene expression to the WT. Unfortunately, among the available homozygous lines, only one line for GTE9g-3HA and GTE9g-N214A-3HA fulfilled this condition. The second independent line for GTE9g-3HA and GTE9g-N214A-3HA was chosen for having a relatively close GTE9 expression level to the one in the WT, but they are slightly over-expressed. These conditions are not optimal but since we plan to cross the ChIP-seq data obtained from two independent lines and we will focus on GTE9 targets identified in the two independent lines.

I generated the transgenic plants (T3 progeny) and the material (seed stock for GTE9g-3HA) was sent to our collaborators in Paris, who routinely perform ChIP-seq analyses on A. thaliana seedlings. This ChIP-seq analysis should lead to the identification of peaks corresponding to particular genomic DNA regions hosting transgenically expressed 3HA-tagged GTE9. This analysis will be performed on unchallenged seedlings (uninfected context). Information provided by these results will allow a better understanding on the role of GTE9 in planta, about their importance in specific cellular processes, but also if they seem associated with genes involved in plant response to pathogens. After obtaining the possible chromatin regions associated with GTE9, the next step will be to confirm that the

targeted loci are really associated with GTE9. To do that, the idea will be to select various candidate genes and check that they are bound by GTE9 by ChIP-qPCR in the GTE9g-3HA lines. Similar analyses will be performed on the GTE9g-N214A-3HA lines in which mutated GTE9-N214A should be unable to bind those loci.

These ChIP-seq data will be crossed with PopP2-dependent RNA-seq data (showing differentially expressed genes induced by PopP2) in order to identify candidates genes hosting GTE9 and transcriptionally deregulated by PopP2 (Sohn et al., 2014). Considering that PopP2 alters the GTE9-H4 interaction likely by acetylating GTE9 around its bromodomain, further experiments could be conducted to check whether PopP2 dissociates GTE9 from particular loci and triggers its relocalisation to other genomic regions. To do so, we could consider performing ChIP-seq analyses on leaf cells expressing GTE9g-3HA and in which PopP2 or its catalytic mutant PopP2-C321A would be delivered by Pf0-1 cells. Finally, it would be very interesting to perform similar analyses on GTE11g-3HA lines and compare the different loci associated with GTE9 and with GTE11, and how PopP2 could influence their association with particular chromatin regions.

Chapter 4: Where is PopP2 addressed at the chromatin level?

Considering that PopP2 targets chromatin-associated proteins, such as WRKY TFs, GTE9 and GTE11, we wondered whether we could have a global overview of the chromatin sites visited by PopP2, and to see whether these sites differ in contexts where PopP2 is recognised or not by the immunoreceptor complex RPS4/RRS1-R (Le Roux et al., 2015).

To unravel the chromatin sites visited by PopP2, we initially planned to perform a ChIP-seq analysis by focusing only on plant cells in which PopP2 was delivered through a T3SS. To achieve this goal, we decided to use a GFP strand system which has been previously successfully used for the detection of plant nuclei hosting PopP2 delivered in a T3SS-dependent manner (Henry et al., 2017). Combined with Fluorescence-Activated Cell Sorting, plant nuclei hosting PopP2 could be separated from nuclei that do not host PopP2. Then, we would be able to perform ChIP-seq analyses on nuclei specifically hosting PopP2 to unravel the chromatin sites visited by PopP2 and determine if there are differences between genetic backgrounds that recognise or not PopP2.

I spent several months to try to set up the GFP strand system to perform ChIP-seq analyses on PopP2, but we were not able to use it in the end, as described hereafter, and we decided to use more conventional approaches to carry the ChIP-seq analyses. This work was also performed in collaboration with Cécile Raynaud and Moussa Benhamed from Institut Paris Saclay 2 (IPS2) in Paris.

A

B

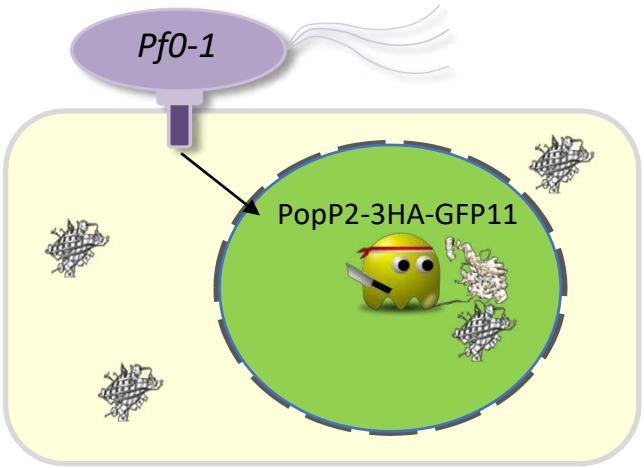


Figure 30. Monitoring of the spatio-temporal delivery of PopP2 in Arabidopsis cells by using the GFP strand system (principle).
The GFP strand system can be used to monitor effector delivery in plant cells or focus on cells that contain the effector when associated with Fluorescence-Associated Cell Sorting.
(A) The GFP strand system consists in reconstituting fluorescence in cells from transgenic plants expressing GFP_{1-10} in which was delivered an effector fused to GFP_{11} (Adapted from Henry et al., 2017).
(B) We intended on using the GFP strand system for delivering PopP2-3HA-GFP_{11} in *A. thaliana* leaves via the T3SS of *Pf0-1*, followed by Fluorescence-Associated Cell Sorting to find the chromatin sites visited by PopP2 with ChIP-seq analyses performed on nuclei hosting PopP2.

Principle of the GFP-strand system and its uses for the detection of plant cells hosting PopP2

The GFP strand system relies on the use of a truncated GFP, with on one side the GFP first 10 beta strands, GFP_{1-10}, and on the other side its last beta strand, GFP_{11}. The GFP_{1-10} is not fluorescent and can be stably expressed in plants. After delivering an effector fused to GFP_{11}, plant cells containing the effector show reconstituted green fluorescence (Figure 30A). This system can be used for monitoring the spatio-temporal delivery of pathogen effectors in plant cells, and has been successfully implemented to various effectors from Pseudomonas syringae in A. thaliana and N. benthamiana (Henry et al., 2017; Park et al., 2017). This system has also been successfully tested for delivering PopP2 fused to GFP_{11} (PopP2-GFP_{11}) by a RSSC strain (Henry et al., 2017). Indeed, a strain mutated in popP2 (ΔpopP2) and complemented with PopP2-GFP_{11} was able to deliver PopP2-GFP_{11} in transgenic A. thaliana expressing 35S::GFP_{1-10}, leading to the reconstitution of fluorescence in root and petiole cells hosting PopP2. Moreover, the 35S::GFP_{1-10} Arabidopsis transgenic line showed similar symptom development to the WT following infection with a ΔpopP2 strains or complemented with PopP2 (popP2+PopP2-3HA), its catalytic mutant PopP2-C321A (ΔpopP2+PopP2-C321A-3HA), or PopP2-GFP_{11} (ΔpopP2+PopP2-GFP_{11}). In addition, the ΔpopP2+PopP2-GFP_{11} complemented strain was comparable to the ΔpopP2+PopP2-3HA complemented strain in terms of infection on susceptible or resistant A. thaliana ecotypes (Henry et al., 2017). Thus, the GFP strand system seems to be compatible with the A. thaliana – R. solanacearum pathosystem, and more precisely for studying the secretion and injection of PopP2 in plant cells. However, ChIP-seq analyses performed on root cells in which PopP2 has been injected by the RSSC is quite challenging since ChIP-seq analyses require a relatively large amount of plant material, which might not be easily obtainable with roots. Hence, we decided to use an alternative GFP strand system-based strategy

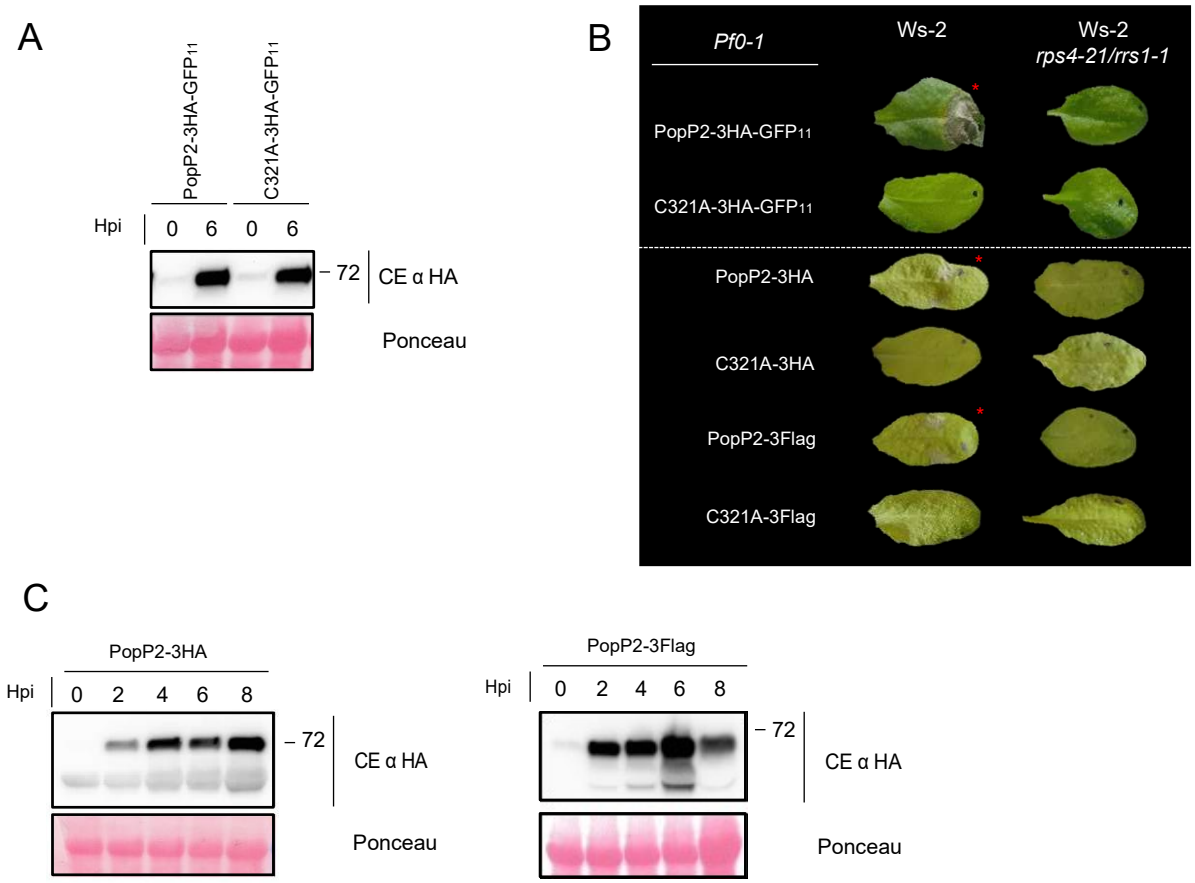


Figure 31. WT PopP2 C-terminally tagged with various epitopes retains its avirulence activity in Ws-2 accession.

(A) PopP2-3HA-GFP11 and C321A-3HA-GFP11 seem to be delivered by *Pf0-1* in *N. benthamiana* leaves 6 hours after infiltration. Immunodetection of PopP2-3HA-GFP11 and C321A-3HA-GFP11 from crude protein extracts of *N. benthamiana* leaves infiltrated with *Pf0-1* delivering these fusion proteins.

(B) PopP2-3HA-GFP11, PopP2-3HA and PopP2-3Flag trigger HR in the Ws-2 *A. thaliana* ecotype. Leaves of four-week-old *A. thaliana* plants were infiltrated with *Pf0-1* strains delivering various PopP2 fusions. The photographs were taken 48 hours after infection. The dashed line indicates independent infiltrations.

(C) Kinetics of the delivery of PopP2-3HA and PopP2-3Flag by *Pf0-1* in *A. thaliana* Ws-2 and *N. benthamiana* leaves respectively, every 2 hours after infiltration. Immunodetection of PopP2-3HA and PopP2-3Flag from crude protein extracts of plant leaves infiltrated with *Pf0-1* delivering these fusion proteins.

by exploiting the ability of Pf0-1 cells to deliver PopP2 through its T3SS in leaf cells. For this strategy, I generated a Pf0-1 strain producing the PopP2 effector C-terminally fused with a triple hemagglutinin epitope followed by the 11th beta strand of GFP (PopP2-3HA-GFP$_{11}$) (Figure 30B). These two tags are useful for protein immunoprecipitation and detection, respectively.

Setting up the GFP-strand system for the detection of plant cells hosting PopP2 and be compatible for ChIP-seq analyses

We generated Pf0-1 strains that can deliver PopP2-3HA-GFP$_{11}$ or its catalytic mutant PopP2-C321A-3HA-GFP$_{11}$. These protein fusions seem to be delivered by the T3SS of Pf0-1 since when infiltrating N. benthamiana leaves with these Pf0-1 strains, we could detect the proteins by immunoblot 6 hours after infiltration (Figure 31A). In addition, we checked that PopP2-3HA-GFP$_{11}$ retained its avirulence function. Indeed, in wild type Ws-2 expressing the immunoreceptor pair of proteins RPS4/RRS1-R, Pf0-1-mediated delivery of GFP$_{11}$-tagged PopP2, but not PopP2 catalytic mutant, triggered a cell death response, as previously observed with WT PopP2 tagged with only a 3HA epitope. By contrast, in rps4-21/rrs1-1 double mutant, none of the PopP2 fusion delivered by Pf0-1 were able to trigger such a cell death response (Figure 31B). together, these data indicate that PopP2-3HA-GFP$_{11}$ retains its avirulence function in triggering activation of the RPS4/RRS1-R immune receptor complex.

The next step was to check that PopP2-3HA-GFP$_{11}$ delivered by Pf0-1 in transgenic Arabidopsis expressing GFP$_{1-10}$ could be detected. Despite multiple attempts, and even though PopP2-3HA-GFP$_{11}$ seems to be delivered in plant cells, it was not possible to detect any GFP fluorescence signal, 6 hours after infiltration of Pf0-1 cells. Since this GFP strand system approach was not successful and time-consuming, we decided to switch to a more classical ChIP-seq approach.

Alternatives to the GFP-strand system by using a more classical ChIP-seq approach

This approach is based on the use of tagged versions of PopP2 that would be still delivered by Pf0-1 cells and the immunoprecipitations will be performed on the whole infiltrated leaf material (containing a mixture of cells hosting PopP2 and cells that do not host PopP2 at all). In order to generate robust ChIP-seq data, two different tagged versions of PopP2 have been generated (PopP2-3HA and PoPP2-3Flag), so that we could cross the outputs from two ChIP-seq performed independently using different affinity resins (HA vs Flag). This would increase the reliability of the results provided by the ChIP-seq analyses. That is why we planned to use Pf0-1 strains expressing WTPopP2 or its catalytic mutant, both of them C-terminally fused either with 3HA or 3Flag epitope tag. The strains expressing PopP2-3HA or PopP2-C321A-3HA were already available in the lab, and I generated the strains expressing PopP2-3Flag or PopP2-C321A-3Flag. Both PopP2-3HA and PopP2-3Flag retained their avirulence functions as evidenced by the cell death response they triggered in Ws-2 plants (Figure 31B). We could also follow the kinetics of PopP2-3HA and PopP2-3Flag accumulation in A. thaliana and N. benthamiana leaves (Figure 31C). Both fusion proteins were detected by immunoblot already 2 hours after infiltration. For the ChIP-seq experiments, we decided to consider the “4 hours after infiltration” (hpi) timepoint since previously performed RNA-seq analyses revealed a large subset of differentially expressed genes in presence of PopP2 (Sohn et al., 2014). Unfortunately, these ChiP-seq analyses have been delayed and we hope to have the results available in the near future.

Once/if the system to deliver PopP2 in A. thaliana plantlets is set up, we will be able to investigate the chromatin sites visited by PopP2 in Ws-2 and in rps4-21/rrs1-1. ChIP-seq analyses will reveal peaks associated with PopP2 in a context when it is recognised in planta or not, and will show chromatin regions and genes

visited by PopP2 after mapping these peaks on A. thaliana genome. Crossing these data with RNA-seq data obtained from the same material would allow us to identify the chromatin regions associated with PopP2 and for which genes are differentially expressed because of PopP2 in a resistant or susceptible background. In addition, by comparing data obtained with the catalytic mutant of PopP2, we should be able to determine whether its acetyltransferase activity enables PopP2 to reach specific chromatin regions.

Altogether, these results could allow us to draw a picture of the impact of PopP2 on the host transcriptome. It could allow us to better understand how PopP2 could influence transcriptome reprogramming through the targeting of specific chromatin regions, likely by manipulating the activity of Transcription Factors (ie defensive WRKY TFs) or epigenetic readers (ie GTE proteins).

Conclusions and Perspectives

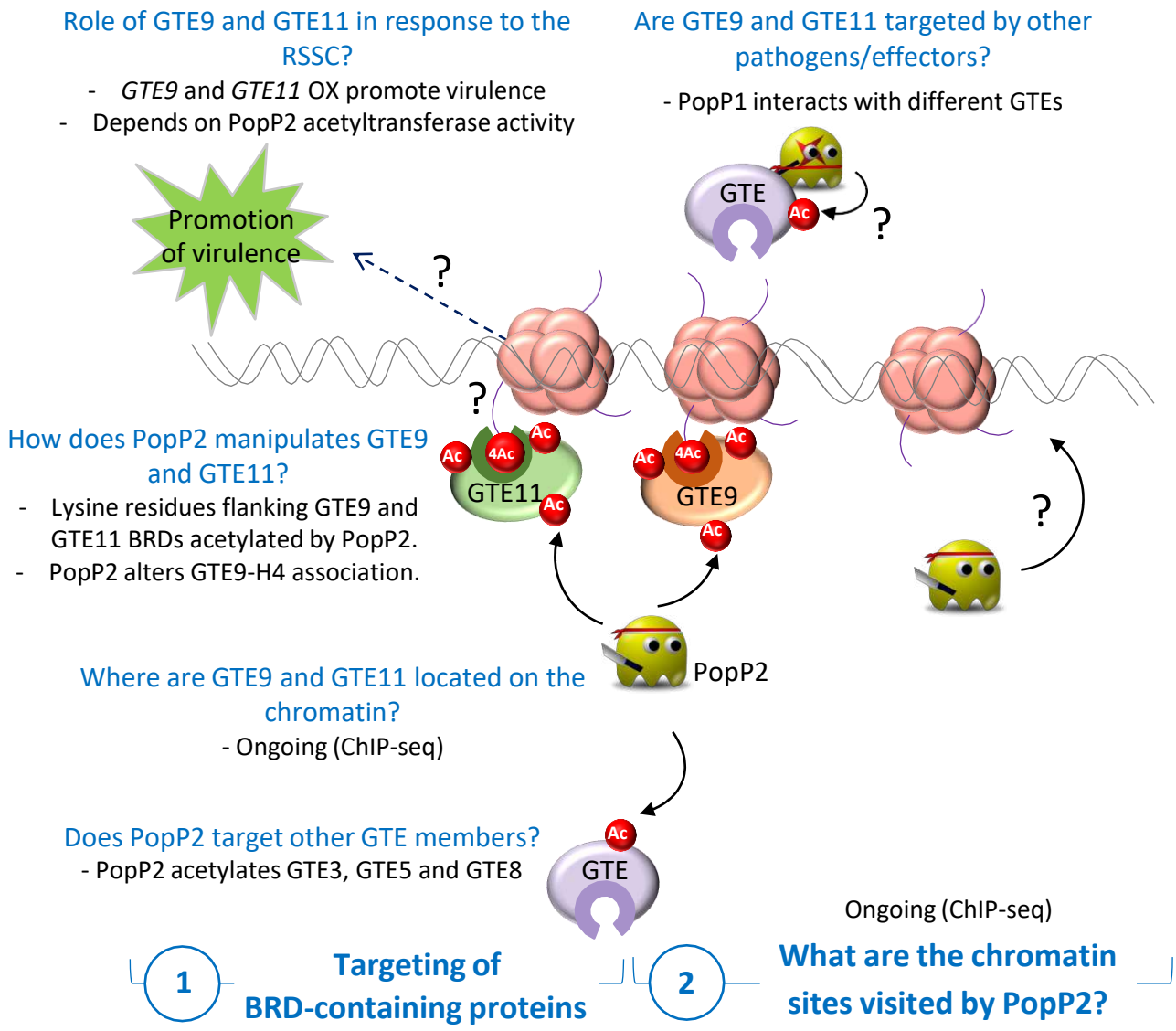


Figure 32. Targeting of GTE proteins by PopP2.
This schema illustrates the two main objectives of my PhD and the main results obtained. During my PhD (and based also on A. Delga's data), we showed that PopP2 can acetylate GTE9 and GTE11 on several lysine residues notably flanking their BRD. PopP2 alters GTE9-H4 association through its acetyltransferase activity. *GTE9* and *GTE11* over-expression contribute to virulence of the RSSC, and it depends on PopP2 acetyltransferase activity too. PopP2 can also acetylate other GTE: GTE3, GTE5 and GTE8. PopP1 could also target GTE proteins as it can interact with various GTE proteins. Where GTE9 and GTE11 are located at the chromatin level remains to be determined, as well as the chromatin sites visited by PopP2 (ongoing).

Recent studies related the importance of the plant epigenome in the regulation of plant defences (Ding and Wang, 2015; Hannan Parker et al., 2022; Zhu et al., 2016b). As a way to counter the activation of host defence, parasites have evolved ingenious ways to manipulate the host epigenome to promote infection via effectors. It is quite well documented for animal pathogens, for which some effectors have been described to manipulate histone readers, histone marks and chromating remodelling (Dong and Hamon, 2020; Ramirez-Prado et al., 2018b). While less examples are documented in plant pathogens, some effectors from plant parasites were shown to manipulate the plant epigenome. They include mainly diverse effectors from viruses, fungi and nematodes (Castillo-González et al., 2015; Kong et al., 2017; Li et al., 2018; Vijayapalani et al., 2018). Examples of manipulation of the plant epigenome by bacterial pathogens are scarce, even though some effectors from P. syringae have been shown to inhibit plant RNA silencing to promote infection (Navarro et al., 2008).

In this context, my PhD project focused on characterising the targeting of A. thaliana histone readers by a T3E from a plant bacterial pathogen. More precisely, pursuing the work from Alice Delga, my project aimed at further characterising the targeting of the BRD containing proteins GTE9 and GTE11 by the RSSC T3E PopP2 acetyltransferase by trying to answer several questions (Figure 32). We wondered how PopP2 could manipulate GTE9 and GTE11 and demonstrated that PopP2 acetylates both of them on several lysine residues, including residues flanking their BRD, among which common residues shared by the two proteins (Figure 2B, Comorge et al., in prep.). We also showed that PopP2 alters the interaction between GTE9 and H4, involving its acetyltransferase activity (Figure 4C, Comorge et al., in prep.). As GTE9 and GTE11 are targeted by PopP2, we next wondered whether they could represent PopP2 virulence targets playing a role in the plant response to the RSSC. Whereas gte9 and gte11 mutant line did not show any alteration of their phenotypical response to the GMI1000 strain, we showed that A. thaliana GTE9 and GTE11 over-expressing lines were more susceptible than WT plants to the

RSSC producing active PopP2 (Figure 5B and 5C, Comorge et al., in prep.). This suggests that RRSC uses PopP2 to intefere with GTE9 and GTE11 functions and promote infection. As GTE9 and GTE11 belong to a family of 12 proteins in Arabidopsis, we investigated the potential targets of other GTE members by PopP2 and found that PopP2 can acetylate other GTE members such as GTE3, GTE5 and GTE8 (Figure 7B, Comorge et al., in prep.). Therefore, GTE proteins represents additional host components whose targeting by a YopJ family acetyltransferase helps the pathogen infect its host plant. Could GTEs be targeted by other effectors/pathogens? Notably, we were able to demonstrate that PopP1, an other YopJ family member from the RSSC, can interact with several GTE members in yeast (GTE1, GTE2, GTE5, GTE6, GTE7, GTE8, GTE9 and GTE11) (Figure 26). I also developped molecular and genetic tools aimed at unraveling the chromatin binding sites of GTE9 and GTE11 (Figure 29, ongoing).

Altogether, this work reveals that PopP2 targets by acetylation the BRD-containing proteins GTE9 and GTE11, promoting virulence of the RSSC. To our knowledge, this represents the first example of a T3E from a plant pathogenic bacteria manipulating histone readers. This work adds more evidence about pathogens targeting host epigenetic components in plants to promote virulence. Interestingly, it seems that PopP2 can target various GTE proteins and so might PopP1. Similarly to some virulence targets that represent a susceptibility hub for P. syringae (Schreiber and Lewis, 2021; Schreiber et al., 2021b), the targeting of the GTE proteins may also represent a susceptibility hub for the RSSC.

While this project paves the way towards the characterisation of the manipulation of histone readers by a plant bacterial pathogen, more questions arise and would be worth investigating. We demonstrated that PopP2 alters the interaction between GTE9 and histone H4 and that it depends on PopP2 acetyltransferase activity and we know that PopP2 acetylates lysine residues surrounding GTE9 and GTE11 BRD. Similarly to the regulation of the yeast SWI/SNF subunit Snf2 by intramolecular

interaction of its BRD with flanking Lysine residues acetylated by a HAT (Kim et al., 2010), we proposed that PopP2 could trigger similar intramolecular interaction between GTE9 BRD and surrounding acetylated lysine residues (Figure 20). It would be interesting to unravel which acetylated lysine residues are responsible for the alteration of the GTE9-H4 interaction. For that, we could generate GTE9 variants mutated on some of the lysine residues normally targeted by PopP2 that could become insentive to PopP2 manipulation. The ability of these “refractory” GTE9 variants to bind in planta Histone H4 in presence of PopP2 could be easily monitored by using FRET-FLIM assays.

In addition, we showed that GTE9 and GTE11 over-expression promotes the RSSC virulence, but the link between the alteration of the interaction GTE-H4 by acetylation by PopP2 and its impact on the overall course of infection with RSSC is still not established. One way to answer this question would be to study the phenotypical response to RSSC of A. thaliana transgenic lines over-expressing GTE9-N214A and GTE11-N206A mutants, both of which being impaired in their histone reader activity. Alternatively, if we identify “refractory” GTE9 variants that could not be manipulated anymore by PopP2, we could generate corresponding A. thaliana over-expressing lines and test their phenotypical response to the RSSC.

We also discovered that PopP2 can acetylate other GTE proteins. This suggests that PopP2 could target various members of the GTE family, as previously observed for many WRKY factors that belong to the same family of TFs. It’s then tempting to imagine that PopP2 could target GTE3, GTE5 and GTE8 in a similar way than GTE9 and GTE11, by acetylating residues flanking their BRDs. It would be informative to investigate by MS/MS-based analyses which lysine residues are acetylated by PopP2 in the other targeted GTEs, and see whether key conserved lysine(s) can be identified. In complement, since GTE3 was found to interact with acetylated H3, we could assess whether PopP2 is also able to compromise the ability of GTE3 to interact with H3 in planta (Garcia-Dominguez et al., 2008).

But more than being a protein family targeted by a bacterial effector, GTE proteins could represent a susceptibility hub. It has already been described in the litterature that some host proteins could represent a hub for different effectors from the same pathogen, or from different pathogens (Mukhtar et al., 2011; Schreiber and Lewis, 2021; Schreiber et al., 2021b; Weßling et al., 2015). Here, we showed that PopP1 also interacts in yeast with several GTE proteins, suggesting that GTE proteins could represent a susceptibility hub for different effectors from the RSSC. Considering that PopP1 is also a YopJ family acetyltransferase, assessing if PopP1 can acetylate certain GTE proteins (in planta acetylation assays) would allow us to determine whether PopP1 and PopP2 could cooperate together for efficient manipulation of histone readers. Moreover, as PopP1 and PopP2 belong to the YopJ family of effectors, but in different subgroups, the fact that both effectors can target or interact with GTE proteins raises the question of whether other YopJ family effectors from plant pathogenic bacteria, such as P. syringae or X. campestris, also manipulate GTE proteins too (Figure 28). This raises the question of whether YopJ family acetyltransferases produced by animal pathogenic bacteria could also exert their virulence functions by manipulating epigenetic readers.

Material and Methods

EXPERIMENTAL MODELS

Arabidopsis

Arabidopsis thaliana Col-0 and Ws-2 plants, and null mutants (gte9-1 (Salk_0119044C) gte11-1 (Salk_059327C), previously described by Misra et al., 2018 were grown in short days (8h light/16h dark cycle) at 22° C (60% relative humidity, 125 µE/M2/s fluorescent illumination). Transgenic lines were generated by using the floral dip transformation method (Clough and Bent, 1998).

Nicotiana benthamiana

Nicotiana benthamiana plants were sown on soil and grown at 24° C under long day photoperiod (16 h light/8 h dark) with 60% relative humidity. Leaves of 4 to 5 week-old plants were used for Agrobacterium tumefaciens-mediated transient expression for Immunoblotting, colocalisation, or FRET-FLIM assays.

Bacterial strains

Ralstonia solanacearum (RSSC) strains were spread on solid ϕ medium containing 5 g/L glucose and 0.004% TTC and grown for 3 days at 28° C. Bacteria were grown in liquid ϕ medium at 28° C under shaking overnight with appropriate antibiotics (ΔpopP2+PopP2 and ΔpopP2+PopP2-C321A were grown in presence of gentamicin (7.5 µg/mL) and tetracyclin (5 µg/mL) (Tasset et al., 2010). Pseudomonas fluorescens (Pf0-1) expressing the PopP2 variants (wild-type and catalytically inactive mutant) were grown on King's B plate supplemented with antibiotics (chloramphenicol 30 µg/mL, tetracycline 5 µg/mL and gentamicin 15 µg/mL) at 28° C overnight. Bacterial cells were centrifugated and resuspended in infiltration buffer (10 mM $MgCl_2$) at OD_{600}=0.2.

METHODS

Plasmid construction

The oligonucleotides used in the experiments presented in this article are listed in Table 3. The full-length GTE1 (At2g34900), GTE2 (At5g10550), GTE3 (At1g73150), GTE4 (At1g06230), GTE5 (At1g17790), GTE6 (At3g52280), GTE7 (At5g65630), GTE8 (At3g27260), GTE9 (At5g14270), GTE10 (At5g63320), GTE11 (At1g01770) and GTE12 (At5g46550) cDNA clones were PCR amplified from Col-0 cDNA using PrimeSTAR Max DNA polymerase (Takara Bio). The full-length GTE9prom::GTE9g and GTE11prom::GTE11g genomic clones were amplified from Col-0 genomic DNA using PrimeSTAR Max DNA polymerase (Takara Bio). PopP2-3Flag and PopP2-C321A-3Flag coding sequences have been generated by chimeric PCR from pENTR-PopP2 and pENTR-PopP2-C321A vectors using PrimeSTAR Max DNA polymerase (Takara Bio). All PCR products flanked with AttB1 and AttB2 recombination sites were recombined in pENTR plasmid to generate the relevant pENTR clones. To generate GTE9-N214A and GTE11-N206A mutants, the corresponding mutations were introduced in pENTR207-GTE9 and pENTR207-GTE11 plasmids by site-directed mutagenesis using the PrimeStar HS DNA polymerase (Takara Bio Inc., Otsu Japan). All DNA constructs were sequence-verified. The inserts cloned in pENTR vectors were then recombined in pDEST vectors via LR reaction (Invitrogen). The expressed proteins in planta labelled with a -3HA, or -3Flag tag were generated by LR reaction in the following plasmid constructs: (i) pAM-PAT-35S-GWY-3HA, or -3Flag or (ii) pBIN-35S-GWY-3HA, -3Flag.

A. thaliana stable transformation

Agrobacterium tumefaciens strains carrying the binary vectors (pAM-PAT-35S-GTE9-3Flag and pAM-PAT-35S-GTE11-3Flag in GV3103 strain; pBIN-35S-GTE9-3HA, pBIN-35S-GTE11-3HA in GV3101 strain; pAM-PAT-Δ35S-GTE9prom::GTE9g-3HA-GTE9term, pAM-PAT-Δ35S-GTE9prom::GTE9g-N214A-3HA-GTE9term, pAM-PAT-D35S-GTE11prom::GTE11g-3HA-GTE11term, and pAM-PAT-D35S-GTE11prom::GTE11g-N206A-3HA-GTE11term in GV3103 strain) were grown on solid LB medium with appropriate antibiotics for 2 days at 28° C. Bacteria were then cultured in YEB liquid medium (supplemented with antibiotics; gentamicin (15 µg/mL) and carbenicillin (25 µg/mL) for pAM-PAT-based vectors, tetracyclin (5 µg/mL) and kanamycin (25 µg/mL) for pBIN-based vectors) at 28° C for 12 hours. Cells were centrifugated and resuspended in a sucrose solution (50 g/L) with 0.02% Silwet-L77.

A. thaliana Col-0, gte9-1 (Salk_0119044C) and gte11-1 (Salk_059327C) were transformed with Agrobacterium tumefaciens using the floral dip method (Clough and Bent, 1998). Primary transformant (T1) were selected on MS medium supplemented with phosphinothricin (10 µg/mL) (Duchefa) or with Kanamycin (50 µg/mL) for pAM-PAT-and pBIN-based binary vectors, respectively. T2 transgenic lines were genotyped by PCR and accumulation of transgenically expressed proteins was verified by immunoblot.

RNA extraction, reverse transcription and RT-qPCR

RNA extractions were performed using the Nucleospin RNAII kit (Macherey-Nagel) and following manufacturer's instructions. Reverse transcription was made with Transcriptor Reverse Transcriptase (TRT, Roche). A pre-mix of 7.25 µL (1 µL Oligo $(dT)_{17}$ at 1 µg/µL; 0.25 µL TRT ; 4 µL TRT buffer; 2 µL dNTP-Mix at 10 mM) was added to a final volume of 20 µL containing 1 µg of total RNA. Samples were

incubated for 30 minutes at 55° C. Reverse transcription was stopped by incubating the samples at 85° C for 10 minutes. After 5 min on ice, samples were stored at -20° C. RT-qPCR were performed with a LightCycler 480 II machine (Roche Diagnostics) with Roche reagents. Expression of AtEF1-α (At5g60390) was used to normalise the expression values. The comparative Ct method ($2^{\Delta Ct}$) was used to represent the data (Schmittgen and Livak, 2008). Mean ΔCt were calculated from three technical replicates for three biological replicates (three independently grown sets of plants). The mean relative expressions from the biological replicates +/- SD was represented with ggplot2 package from R. Statistical analyses were performed on ΔCt values to assess the significance of the difference in the mean ΔCt between the different genetic lines or conditions. We applied either a one-way ANOVA when the assumptions about the underlying distribution of the data were fulfilled or a kruskal-test when they were not.

Root-inoculation assays in Arabidopsis

For root-inoculation assays, bacteria (Rs) were grown in a liquid culture at 28° C overnight and diluted in water at DO_{600}=0.01. Four-week-old A. thaliana plants were soaked in this bacterial solution for 10 min, transferred on soil and incubated in a growth chamber (12h day/12h night, 27° C during the day/26° C at night, 75% relative humidity). Symptoms were scored daily with the following disease scale: 0 = no symptoms, 1 = 25% leaves wilt, 2 = 50%, 3 = 75% and 4 = 100%. For each independent inoculation, between 24 and 32 plants from different lines in Arabidopsis thaliana ecotypes were root-inoculated with GMI1000 strain or mutant strains of R. solanacearum and wilting symptom developments were scored for 10 days.

For each presented graph, disease scoring data were analysed from three independent inoculations with R software as previously described (Aoun et al.,

2020). Briefly, we used a mixed model (MIXED procedure in SAS v. 9.4; SAS Institute Inc.) as follow:

$$disease\ index_{ijc} = \mu + block_i + accession_j + covCol_c + \varepsilon_{ijc}$$

Where μ is the overall mean of the phenotypic data, "block" considers environmental conditions between the independent experiments included in the model, "accession" correspond to the tested genetic lines, "covCol" is a covariate accounting for tray effects within blocks and " ε " is the residual term. "block" was considered as a fixed factor and "accession" as a random factor. We tested the significance of the different accessions by likelihood ratio test of model with and without this effect. With p-value<0.05, the lines were considered to have a different disease index reflecting differential response to the pathogen.

Bacterial growth quantification of Pst DC3000

The Pst DC3000 strain was cultured on King B solid medium with 50 µg/mL Rifampicin 2 days prior inoculation, and respread on a new King B plate with 50 µg/mL Rifampicin 1 day before inoculation. For disease assay, we proceded similarly to what described (Zipfel et al., 2004). Briefly, bacteria were collected by centrifugation and washed once with a 10mM $MgCl_2$ solution, and adjusted to a cell density of OD_{600}=0.0003. Bacteria were infiltrated into leaves with a needleless syringe, and inoculated plants were kept under at ambiant humidity for 2h to let evaporate excess water from the leaf and then were covered with a holed transparent plastic foil to keep humidity for facilitating disease development. As a control at 0 day post-infiltration, 4 leaf discs of 4mm diameter were harvested as one biological repeat, and 2-3 repeats were taken for each genetic line.

For quantification of bacteria 4 days after DC3000 infiltration, four leaf discs from 4 different leaves of 4mm diameter were harvested as one biological repeat, and 6 repeats were taken for each genetic line. Leaf discs were ground and diluted in

sterile water, and the extraction solutions were then plated on King B + 50 µg/mL Rifampicin agar plates. Colonies were counted 24h after incubation at 28° C. The whole experiment has been repeated twice.

Transient expression in N. benthamiana

For transient expression in N. benthamiana leaf epidermal cells, A. tumefaciens strains grown in liquid YEB medium containing appropriate antibiotics. Cells were harvested by centrifugation and resuspended in infiltration medium (10 mM MES pH5.6, 10 mM $MgCl_2$, 150 μM acetosyringone) at OD_{600}=0.25. For co-expression, each bacterial suspension carrying individual constructs was adjusted to OD_{600} = 0.25 in the final mix for infiltration. After incubation at room temperature for 1 h, bacteria were infiltrated into the leaves of 4-week-old N. benthamiana plants using a needleless syringe. Plants were incubated for 36-48h in growth chambers under controlled conditions.

Protein Extraction and Immunoblot Analysis

Plant material (4 discs of 8 mm diameter harvested 48 hours post-infiltration for N. benthamiana samples; 20 seedlings (10 day-old) or 8 leaf discs of 4mm diameter for Arabidopsis samples) were grinded and resuspended in 300 µL of Laemmli Buffer (2X). Protein extracts were then denatured for 3 min at 95° C, centrifugated at 13000 rpm for 1 min and then analysed by immunoblotting (SDS-PAGE). Transferred proteins were visualised by Ponceau S red staining. Membranes were blocked in a 2% milk TBS-T (Tris Buffer Saline-Tween 20; 50 mM Tris-HCl pH7.5, 150 mM NaCl, and 0.2% Tween-20) solution before incubation. The following primary antibodies were used in this study: anti-HA-HRP (3F10; Roche; dilution 1:5000), anti-Flag-HRP (Sigma; dilution 1:5000). Immunodetections were performed using Clarity Western ECL substrate reagent (Bio-Rad).

Yeast two-hybrid

Constructs for yeast two-hybrid analysis were prepared in the MatchMaker GAL4 two-hybrid system (Clontech) using the vectors pGBKT7- and pGAD-derived vectors to express bait and prey proteins, respectively. The pDEST pGAD-GWY vector was generated by ligating a Gateway cassette (Frame C) in the pGADT7 vector blunt-ended (klenow fill-in) after linearisation with NdeI and XhoI restriction enzymes. The pDEST pGBG-GWY vector was generated by ligating a Gateway cassette (Frame C) in the pGBKT7 vector blunt-ended (klenow fill-in) after linearisation with NdeI and SalI restriction enzymes. The cDNA corresponding to GTE1 to GTE12 cloned into the relevant pENTR plasmids were recombined in the pGAD-GWY vector. The coding sequence of PopP2 and PopP1 in pENTR-PopP2 and pENTR-PopP1 were recombined in pGBG-GWY (bait) vector by LR reaction (Gateway, Invitrogen). All the pDEST plasmids were introduced in E. coli cells (DH5α, Stratagene) by electroporation (1mm tank, 1.8 kV, 25 µF and 200 Ohms). Transformed cells were spread on solid LB medium with appropriate antibiotic selection (carbenicillin 50 µg/mL for pGAD vectors, kanamycin 50 µg/mL for pGBG vectors). Plasmid DNAs were extracted with the Wizard DNA plasmid purification kit (Promega). Co-expression of BD-p53 (bait, pGBKT7-p53 vector, Clontech) and AD-T (prey, pGADT7-T vector, Clontech) was used as positive control on selective medium. Bait and prey proteins were tagged with a c-Myc and an HA epitope tag, respectively. Bait and prey constructs were introduced in AH109 Saccharomyces cerevisae strain (Clontech) according to the manufacturer's instructions. Transformed cells were spread on SD-TL medium and incubated 3 days at 30° C. Colonies were then dropped by dilution gradient on different media: non-selective (SD-TL) and selective (SD-TLHA) and incubated between 3-5 days at 30° C.

Pseudomonas fluorescens-mediated delivery of PopP2 in Arabidopsis

For Pf0-1-mediated delivery, full-length PopP2 and PopP2-C321A recombined in the pBBR-AvrRps4prom-GWY-3HA gateway destination vector were used as previously described (Le Roux et al., 2015). For PopP2-3Flag and PopP2-C321A-3Flag delivery (both ending with a stop codon), pENTR207-PopP2-3Flag and pENTR207-PopP2-C321A-3Flag were used for LR recombination with the pBBR-AvrRps4prom-GWY-3HA gateway destination vector. For PopP2-3HA-GFP_{11} or PopP2-C321A-3HA-GFP_{11} delivery, full-length PopP2 and PopP2-C321A were introduced in the pBBR-AvrRps4prom-GWY-3HA-GFP_{11} gateway destination vector by using LR reaction. pBBR-derived contructs were introduced in Pf0-1 cells by tri-parental mating. Transformed Pf0-1 cells were selected on King's B agar supplemented with 6mM MgS04 and with appropriate antibiotics (tetracycline 5 µg/mL, chloramphenicol 30 µg/mL, and gentamicin 15 µg/mL). For infiltration in Arabidopsis leaves, bacteria were resuspended in 500 µL $MgCl_2$ (10 mM) and centrifuged (8000 rpm, 1 min). Bacteria were resuspended in 500 µL $MgCl_2$ (10 mM) and centrifuged again (8000 rpm, 1 min) to wash out antibiotics. Bacterial pellet was resuspended in 500 µL $MgCl_2$ (10 mM) solution and bacterial density was adjusted to OD600=0,2 (OD_{600nm} = 1 means 1.75 x 10^8 bacteria/mL). Three-week-old plants were placed in a humid environment 14 hours before infiltration to facilitate stomata opening. Bacteria were infiltrated in the plant leaves with a needleless syringe. ~12 leaves were infiltrated for each plant tested and the PopP2-tiggered cell death was observed 48 hours after infiltration.

Quantification and statistical analysis

For the Rs inoculations, disease symptom scoring was analysed and plot with R software. Graphs show least-square means +/- Standard Error of the LS means

represented as linear plots with points at each day when symptoms were scored. Significance of the difference of disease index LS means at a given day between different genotypes was assessed with a one-way ANOVA performed on a mixed model generated from the data as previously used (Aoun et al., 2020). At a given day, significance of the difference between LS means was set at p-value<0.05.

For RT-qPCR, data were analysed and represented with R software using the comparative Ct method ($2^{\Delta Ct}$) (Schmittgen and Livak, 2008). Mean ΔCt were calculated from three technical replicates for three biological replicates (three independently grown sets of plants). The mean relative expressions from the biological replicates +/- SD was represented in barplots. ΔCt values were used to assess the significance of the difference in ΔCt between the different genetic lines. A one-way ANOVA was applied when the assumptions about the underlying distribution of the data were fulfilled, or a kruskal-test when they were not.

For DC3000 bacterial growth analyses, data were analysed and represented with R software. Graphs show boxplots of the biological replicates from one experiment. Significance of the difference of bacterial growth between genetic lines at 0 dpi and 4 dpi was assessed on R. A one-way ANOVA was applied when the assumptions about the underlying distribution of the data were fulfilled, or a kruskal-test when they were not.

References

Adie, B.A.T., Pérez-Pérez, J., Pérez-Pérez, M.M., Godoy, M., Sánchez-Serrano, J.J., Schmelz, E.A., and Solano, R. (2007). ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in Arabidopsis. Plant Cell 19, 1665–1681.

Ahmad, A.A., Addy, H.S., and Huang, Q. (2021). Biological and Molecular Characterization of a Jumbo Bacteriophage Infecting Plant Pathogenic Ralstonia solanacearum Species Complex Strains. Front. Microbiol. 12, 1–15.

Airoldi, C.A., Rovere, F. Della, Falasca, G., Marino, G., Kooiker, M., Altamura, M.M., Citterio, S., and Kater, M.M. (2010). The Arabidopsis BET Bromodomain Factor GTE4 Is Involved in Maintenance of the Mitotic Cell Cycle during Plant Development. PLANT Physiol. 152, 1320–1334.

Alazem, M., Lin, K.Y., and Lin, N.S. (2014). The abscisic acid pathway has multifaceted effects on the accumulation of Bamboo mosaic virus. Mol. Plant-Microbe Interact. 27, 177–189.

Albertazzi, L., Arosio, D., Marchetti, L., Ricci, F., and Beltram, F. (2009). Quantitative FRET analysis with the E0GFP-mCherry fluorescent protein pair. Photochem. Photobiol. 85, 287–297.

Alfano, J.R., and Collmer, A. (2004). Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. Annu. Rev. Phytopathol. 42, 385–414.

Álvarez, B., and Biosca, E.G. (2017). Bacteriophage-based bacterial wilt biocontrol for an environmentally sustainable agriculture. Front. Plant Sci. 8, 1–7.

Aoun, N., Desaint, H., Boyrie, L., Bonhomme, M., Deslandes, L., Berthomé, R., and Roux, F. (2020). A complex network of additive and epistatic quantitative trait loci underlies natural variation of Arabidopsis thaliana quantitative disease resistance to Ralstonia solanacearum under heat stress. Mol. Plant Pathol. 21, 1405–1420.

Arbibe, L., Kim, D.W., Batsche, E., Pedron, T., Mateescu, B., Muchardt, C., Parsot, C., and Sansonetti, P.J. (2007). An injected bacterial effector targets chromatin access for transcription factor NF-κB to alter transcription of host genes involved in immune responses. Nat. Immunol. 8, 47–56.

Baggs, E., Dagdas, G., and Krasileva, K. V. (2017). NLR diversity, helpers and integrated domains: making sense of the NLR IDentity. Curr. Opin. Plant Biol. 38, 59–67.

Balint-Kurti, P. (2019). The plant hypersensitive response: concepts, control and consequences. Mol. Plant Pathol. 20, 1163–1178.

Ballaré, C.L., and Austin, A.T. (2019). Recalculating growth and defense strategies under competition: Key roles of photoreceptors and jasmonates. J. Exp. Bot. 70, 3425–3436.

Barnes, S.N., Wram, C.L., Mitchum, M.G., and Baum, T.J. (2018). The plant-parasitic cyst nematode effector GLAND4 is a DNA-binding protein. Mol. Plant Pathol. 19, 2263–2276.

Baulcombe, D. (2004). RNA silencing in plants. Nature 431, 356–363.

Benhamed, M., Martin-Magniette, M.L., Taconnat, L., Bitton, F., Servet, C., De Clercq, R., De Meyer, B., Buysschaert, C., Rombauts, S., Villarroel, R., et al. (2008). Genome-scale Arabidopsis promoter array

identifies targets of the histone acetyltransferase GCN5. Plant J. 56, 493–504.

Berger, S.L. (2007). The complex language of chromatin regulation during transcription. Nature 447, 407–412.

Bergsma-Vlami, M., van de Bilt, J.L.J., Tjou-Tam-Sin, N.N.A., Westenberg, M., Meekes, E.T.M., Teunissen, H.A.S., and van Vaerenbergh, J. (2018). Phylogenetic Assignment of Ralstonia pseudosolanacearum (Ralstonia solanacearum Phylotype I) Isolated from Rosa spp. Plant Dis. 102, 2258–2267.

Bernoux, M., Ve, T., Williams, S., Warren, C., Hatters, D., Valkov, E., Zhang, X., Ellis, J.G., Kobe, B., and Dodds, P.N. (2011). Structural and functional analysis of a plant resistance protein TIR domain reveals interfaces for self-association, signaling, and autoregulation. Cell Host Microbe 9, 200–211.

Berr, A., McCallum, E.J., Alioua, A., Heintz, D., Heitz, T., and Shen, W.H. (2010). Arabidopsis histone methyltransferase SET DOMAIN GROUP8 mediates induction of the jasmonate/ethylene pathway genes in plant defense response to necrotrophic fungi. Plant Physiol. 154, 1403–1414.

Bi, G., Su, M., Li, N., Liang, Y., Dang, S., Xu, J., Hu, M., Wang, J., Zou, M., Deng, Y., et al. (2021). The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. Cell 184, 3528–3541.

Bierne, H., and Cossart, P. (2012). When bacteria target the nucleus: The emerging family of nucleomodulins. Cell. Microbiol. 622–633.

Bigeard, J., Colcombet, J., and Hirt, H. (2015). Signaling mechanisms in pattern-triggered immunity (PTI). Mol. Plant 8, 521–539.

Block, A., Toruño, T.Y., Elowsky, C.G., Zhang, C., Steinbrenner, J., Beynon, J., and Alfano, J.R. (2014). The Pseudomonas syringae type III effector HopD1 suppresses effector-triggered immunity, localizes to the endoplasmic reticulum, and targets the Arabidopsis transcription factor NTL9. New Phytol. 201, 1358–1370.

Boccara, M., Sarazin, A., Thiébeauld, O., Jay, F., Voinnet, O., Navarro, L., and Colot, V. (2014). The Arabidopsis miR472-RDR6 Silencing Pathway Modulates PAMP- and Effector-Triggered Immunity through the Post-transcriptional Control of Disease Resistance Genes. PLoS Pathog. 10, 1–16.

Boch, J., Bonas, U., and Lahaye, T. (2014). TAL effectors - pathogen strategies and plant resistance engineering. New Phytol. 204, 823–832.

Boller, T., and Felix, G. (2009). A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–407.

Bouyssié, D., Hesse, A.M., Mouton-Barbosa, E., Rompais, M., MacRon, C., Carapito, C., Gonzalez De Peredo, A., Couté, Y., Dupierris, V., Burel, A., et al. (2020). Proline: An efficient and user-friendly software suite for large-scale proteomics. Bioinformatics 36, 3148–3155.

Bozkurt, T.O., and Kamoun, S. (2020). The plant-pathogen haustorial interface at a glance. J. Cell Sci. 133, 1–6.

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Miret, J.A.J., Justesen, A.F., Macleod, A., Magnusson, C.S., Milonas, P., Navas-Cortes, J.A., et al. (2019). Pest categorisation of the ralstonia solanacearum species complex. EFSA J. 17, 1–28.

Broekgaarden, C., Caarls, L., Vos, I.A., Pieterse, C.M.J., and Van Wees, S.C.M. (2015). Ethylene: Traffic controller on hormonal crossroads to defense. Plant Physiol. 169, 2371–2379.

Buddenhagen, I., and Kelman, A. (1964). Biological and Physiological Aspects of Bacterial Wilt Caused by Pseudomonas Solanacearum. Annu. Rev. Phytopathol. 2, 203–230.

Buhrow, L.M., Cram, D., Tulpan, D., Foroud, N.A., and Loewen, M.C. (2016). Exogenous abscisic acid and gibberellic acid elicit opposing effects on Fusarium graminearum infection in wheat. Phytopathology 106, 986–996.

van der Burgh, A.M., and Joosten, M.H.A.J. (2019). Plant Immunity: Thinking Outside and Inside the Box. Trends Plant Sci. 24, 587–601.

van Butselaar, T., and Van den Ackerveken, G. (2020). Salicylic Acid Steers the Growth–Immunity Tradeoff. Trends Plant Sci. 25, 566–576.

Cai, R., Lewis, J., Yan, S., Liu, H., Clarke, C.R., Campanile, F., Almeida, N.F., Studholme, D.J., Lindeberg, M., Schneider, D., et al. (2011). The plant pathogen pseudomonas syringae pv. tomato is genetically monomorphic and under strong selection to evade tomato immunity. PLoS Pathog. 7, 1–15.

Caldwell, D., Kim, B.S., and Iyer-Pascuzzi, A.S. (2017). Ralstonia solanacearum differentially colonizes roots of resistant and susceptible tomato plants. Phytopathology 107, 528–536.

Campos, M.L., Kang, J.-H., and Howe, G.A. (2014). Jasmonate-Triggered Plant Immunity. J. Chem. Ecol. 40,657–675.

Cao, Y., Liang, Y., Tanaka, K., Nguyen, C.T., Jedrzejczak, R.P., Joachimiak, A., and Stacey, G. (2014). The kinase LYK5 is a major chitin receptor in Arabidopsis and forms a chitin-induced complex with related kinase CERK1. Elife 3, 1–19.

Casabona, M.G., Vandenbrouck, Y., Attree, I., and Couté, Y. (2013). Proteomic characterization of Pseudomonas aeruginosa PAO1 inner membrane. Proteomics 13, 2419–2423.

Castel, B., Ngou, P.M., Cevik, V., Redkar, A., Kim, D.S., Yang, Y., Ding, P., and Jones, J.D.G. (2019). Diverse NLR immune receptors activate defence via the RPW8-NLR NRG1. New Phytol. 222, 966–980.

Castillo-González, C., Liu, X., Huang, C., Zhao, C., Ma, Z., Hu, T., Sun, F., Zhou, Y., Zhou, X., Wang, X.-J., et al. (2015). Geminivirus-encoded TrAP suppressor inhibits the histone methyltransferase SUVH4/KYP to counter host defense. Elife 4, 1–31.

Cech, T.R., and Steitz, J.A. (2014). The noncoding RNA revolution - Trashing old rules to forge new ones. Cell 157, 77–94.

Cernadas, R.A., Doyle, E.L., Niño-Liu, D.O., Wilkins, K.E., Bancroft, T., Wang, L., Schmidt, C.L., Caldo, R., Yang, B., White, F.F., et al. (2014). Code-Assisted Discovery of TAL Effector Targets in Bacterial Leaf Streak of Rice Reveals Contrast with Bacterial Blight and a Novel Susceptibility Gene. PLoS Pathog. 10, 1–24.

Césari, S., Kanzaki, H., Fujiwara, T., Bernoux, M., Chalvon, V., Kawano, Y., Shimamoto, K., Dodds, P., Terauchi, R., and Kroj, T. (2014). The NB - LRR proteins RGA 4 and RGA 5 interact functionally and

physically to confer disease resistance . EMBO J. 33, 1941–1959.

Champoiseau, P.G., Jones, J.B., and Allen, C. (2009). Ralstonia solanacearum Race 3 Biovar 2 Causes Tropical Losses and Temperate Anxieties . Plant Heal. Prog. 10, 35.

Chan, C., and Zimmerli, L. (2019). The Histone Demethylase IBM1 Positively Regulates Arabidopsis Immunity by Control of Defense Gene Expression. Front. Plant Sci. 10, 1–10.

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K., and Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. J. Integr. Plant Biol. 62, 25–54.

Chen, L., Shirota, M., Zhang, Y., Kiba, A., Hikichi, Y., and Ohnishi, K. (2014). Involvement of HLK effectors in Ralstonia solanacearum disease development in tomato. J. Gen. Plant Pathol. 80, 79–84.

Cheong, M.S., Kirik, A., Kim, J.G., Frame, K., Kirik, V., and Mudgett, M.B. (2014). AvrBsT Acetylates Arabidopsis ACIP1, a Protein that Associates with Microtubules and Is Required for Immunity. PLoS Pathog. 10, 1–15.

Choi, H.W., and Klessig, D.F. (2016). DAMPs, MAMPs, and NAMPs in plant innate immunity. BMC Plant Biol. 16, 1–10.

Choi, S.M., Song, H.R., Han, S.K., Han, M., Kim, C.Y., Park, J., Lee, Y.H., Jeon, J.S., Noh, Y.S., and Noh, B. (2012). HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. Plant J. 71, 135–146.

Chua, Y.L., Channelière, S., Mott, E., and Gray, J.C. (2005). The bromodomain protein GTE6 controls leaf development in Arabidopsis by histone acetylation at ASYMMETRIC LEAVES1. Genes Dev. 19, 2245–2254.

Clapier, C.R., and Cairns, B.R. (2009). The biology of chromatin remodeling complexes. Annu. Rev. Biochem. 78, 273–304.

Clough, S.J., and Bent, A.F. (1998). Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16, 735–743.

Cook, D.E., Mesarich, C.H., and Thomma, B.P.H.J. (2015). Understanding Plant Immunity as a Surveillance System to Detect Invasion. Annu. Rev. Phytopathol. 53, 541–563.

Costa, T.R.D., Felisberto-Rodrigues, C., Meir, A., Prevost, M.S., Redzej, A., Trokter, M., and Waksman, G. (2015). Secretion systems in Gram-negative bacteria: Structural and mechanistic insights. Nat. Rev. Microbiol. 13, 343–359.

Cui, H., Tsuda, K., and Parker, J.E. (2015). Effector-Triggered Immunity: From Pathogen Perception to Robust Defense. Annu. Rev. Plant Biol. 66, 487–511.

Cunnac, S., Occhialini, A., Barberis, P., Boucher, C., and Genin, S. (2004). Inventory and functional analysis of the large Hrp regulon in Ralstonia solanacearum: Identification of novel effector proteins translocated to plant host cells through the type III secretion system. Mol. Microbiol. 53, 115–128.

Deleris, A., Halter, T., and Navarro, L. (2016). DNA Methylation and Demethylation in Plant Immunity. Annu.

Rev. Phytopathol. 54, 579–603.

Delga, A. (2015). L'effecteur PopP2 de Ralstonia solanacearum cible GTE9 et GTE11, deux lecteurs épigénétiques d'Arabidopsis thaliana. Université Toulouse III - Paul Sabatier.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D.X., Bittner-Eddy, P., Beynon, J., and Marco, Y. (2002). Resistance to Ralstonia solanacearum in Arabidopsis thaliana is conferred by the recessive RRS1-R gene, a member of a novel family of resistance genes. Proc. Natl. Acad. Sci. 99, 2404–2409.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S., and Marco, Y. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. 100, 8024–8029.

Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K., and Zhou, M.M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature 399, 491–496.

Ding, B., and Wang, G.-L. (2015). Chromatin versus pathogens: the function of epigenetics in plant immunity. Front. Plant Sci. 6, 1–8.

Ding, P., and Ding, Y. (2020). Stories of Salicylic Acid: A Plant Defense Hormone. Trends Plant Sci. 25, 549–565.

Doğan, E.S., and Liu, C. (2018). Three-dimensional chromatin packing and positioning of plant genomes. Nat. Plants 4, 521–529.

Domcke, S., Bardet, A.F., Adrian Ginno, P., Hartl, D., Burger, L., and Schübeler, D. (2015). Competition between DNA methylation and transcription factors determines binding of NRF1. Nature 528, 575–579.

Dong, W., and Hamon, M.A. (2020). Revealing eukaryotic histone-modifying mechanisms through bacterial infection. Semin. Immunopathol. 42, 201–213.

Dowen, R.H., Pelizzola, M., Schmitz, R.J., Lister, R., Dowen, J.M., Nery, J.R., Dixon, J.E., and Ecker, J.R. (2012). Widespread dynamic DNA methylation in response to biotic stress. Proc. Natl. Acad. Sci. 109, 2183–2191.

Du, L., and Poovaiah, B.W. (2004). A novel family of Ca 2+/calmodulin-binding proteins involved in transcriptional regulation: Interaction with fsh/Ring3 class transcription activators. Plant Mol. Biol. 54, 549–569.

Du, Y., Chen, X., Guo, Y., Zhang, X., Zhang, H., Li, F., Huang, G., Meng, Y., and Shan, W. (2021). Phytophthora infestans RXLR effector PITG20303 targets a potato MKK1 protein to suppress plant immunity. New Phytol. 229, 501–515.

Dubey, A., and Jeon, J. (2017). Epigenetic regulation of development and pathogenesis in fungal plant pathogens. Mol. Plant Pathol. 18, 887–898.

Duque, P., and Chua, N.H. (2003). IMB1, a bromodomain protein induced during seed imbibition, regulates ABA- and phyA-mediated responses of germination in Arabidopsis. Plant J. 35, 787–799.

Eberharter, A., and Becker, P.B. (2002). Histone acetylation: A switch between repressive and permissive

chromatin. Second in review on chromatin dynamics. EMBO Rep. 3, 224–229.

Ellis, J.G., Dodds, P.N., and Lawrence, G.J. (2008). Flax rust resistance gene specificity is based on direct resistance-avirulence protein interactions. Annu. Rev. Phytopathol. 45, 289–306.

Elphinstone, J.G. (1996). Survival and possibilities for extinction of Pseudomonas solanacearum (Smith) Smith in cool climates. Potato Res. 39, 403–410.

Erb, M., and Reymond, P. (2019). Molecular Interactions between Plants and Insect Herbivores. Annu. Rev. Plant Biol. 70, 527–557.

Erill, I., Puigvert, M., Legrand, L., Guarischi-Sousa, R., Vandecasteele, C., Setubal, J.C., Genin, S., Guidot, A., and Valls, M. (2017). Comparative analysis of ralstonia solanacearum methylomes. Front. Plant Sci. 8, 1–16.

Farrona, S., Hurtado, L., and Reyes, J.C. (2007). A Nucleosome Interaction Module Is Required for Normal Function of Arabidopsis thaliana BRAHMA. J. Mol. Biol. 373, 240–250.

Farrona, S., Hurtado, L., March-Díaz, R., Schmitz, R.J., Florencio, F.J., Turck, F., Amasino, R.M., and Reyes, J.C. (2011). Brahma Is Required for Proper Expression of the Floral Repressor FLC in Arabidopsis. PLoS One 6, 1–11.

Feehan, J.M., Castel, B., Bentham, A.R., and Jones, J.D. (2020). Plant NLRs get by with a little help from their friends. Curr. Opin. Plant Biol. 56, 99–108.

Feng, D.X., Tasset, C., Hanemian, M., Barlet, X., Hu, J., Trémousaygue, D., Deslandes, L., and Marco, Y. (2012a). Biological control of bacterial wilt in Arabidopsis thaliana involves abscissic acid signalling. New Phytol. 194, 1035–1045.

Feng, F., Yang, F., Rong, W., Wu, X., Zhang, J., Chen, S., He, C., and Zhou, J.M. (2012b). A Xanthomonas uridine 5′ -monophosphate transferase inhibits plant immune kinases. Nature 485, 114–118.

Filippakopoulos, P., and Knapp, S. (2012). The bromodomain interaction module. FEBS Lett. 586, 2692–2704.

Filippakopoulos, P., Picaud, S., Mangos, M., Keates, T., Lambert, J., Barsyte-Lovejoy, D., Felletar, I., Volkmer, R., Müller, S., Pawson, T., et al. (2012). Histone Recognition and Large-Scale Structural Analysis of the Human Bromodomain Family. Cell 149, 214–231.

Flor, H.H. (1971). Current status of the gene-for-gene concept. 275–296.

Florence, B., and Faller, D. V. (2001). You bet-cha: a novel family of transcriptional regulators. Front. Biosci. 6, 1008–1018.

Fukuoka, S., Saka, N., Koga, H., Ono, K., Shimizu, T., Ebana, K., Hayashi, N., Takahashi, A., Hirochika, H., Okuno, K., et al. (2009). Loss of function of a proline-containing protein confers durable disease resistance in Rice. Science (80-.). 325, 998–1001.

Garcia-Dominguez, M., March-Diaz, R., and Reyes, J.C. (2008). The PHD domain of plant PIAS proteins mediates sumoylation of bromodomain GTE proteins. J. Biol. Chem. 283, 21469–21477.

Garner, C.M., Kim, S.H., Spears, B.J., and Gassmann, W. (2016). Express yourself: Transcriptional regulation

of plant innate immunity. Semin. Cell Dev. Biol.

Geisler, S., and Coller, J. (2013). RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat. Rev. Mol. Cell Biol. 14, 699–712.

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in Ralstonia solanacearum. New Phytol. 187, 920–928.

Genin, S., and Denny, T.P. (2012). Pathogenomics of the ralstonia solanacearum species complex. Annu. Rev. Phytopathol. 50, 67–89.

Ghosh, S., and O'Connor, T.J. (2017). Beyond paralogs: The multiple layers of redundancy in bacterial pathogenesis. Front. Cell. Infect. Microbiol. 7, 1–14.

Gimenez-Ibanez, S., Hann, D.R., Ntoukakis, V., Petutschnig, E., Lipka, V., and Rathjen, J.P. (2009). AvrPtoB Targets the LysM Receptor Kinase CERK1 to Promote Bacterial Virulence on Plants. Curr. Biol. 19, 423–429.

Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J.P., and Solano, R. (2014). The Bacterial Effector HopX1 Targets JAZ Transcriptional Repressors to Activate Jasmonate Signaling and Promote Infection in Arabidopsis. PLoS Biol. 12, 1–15.

Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W., and Robatzek, S. (2008). Plant Pattern-Recognition Receptor FLS2 Is Directed for Degradation by the Bacterial Ubiquitin Ligase AvrPtoB. Curr. Biol. 18, 1824–1832.

Goldberg, A.D., Allis, C.D., and Bernstein, E. (2007). Epigenetics: A Landscape Takes Shape. Cell 128, 635–638.

Goritschnig, S., Weihmann, T., Zhang, Y., Fobert, P., McCourt, P., and Li, X. (2008). A novel role for protein farnesylation in plant innate immunity. Plant Physiol. 148, 348–357.

Grabiec, A.M., and Potempa, J. (2018). Epigenetic regulation in bacterial infections: targeting histone deacetylases. Crit. Rev. Microbiol. 44, 336–350.

Grasser, K.D., Rubio, V., and Barneche, F. (2021). Multifaceted activities of the plant SAGA complex. Biochim. Biophys. Acta - Gene Regul. Mech. 1864, 194613.

Grund, E., Tremousaygue, D., and Deslandes, L. (2019). Plant NLRS with integrated domains: Unity makes strength. Plant Physiol. 179, 1227–1235.

Guo, H., Ahn, H.-K.K., Sklenar, J., Huang, J., Ma, Y., Ding, P., Menke, F.L.H., and Jones, J.D.G. (2020). Phosphorylation-Regulated Activation of the Arabidopsis RRS1-R/RPS4 Immune Receptor Complex Reveals Two Distinct Effector Recognition Mechanisms. Cell Host Microbe 27, 1–13.

Gust, A.A., Pruitt, R., and Nürnberger, T. (2017). Sensing Danger: Key to Activating Plant Immunity. Trends Plant Sci. 22, 779–791.

Han, S.K., Wu, M.F., Cui, S., and Wagner, D. (2015). Roles and activities of chromatin remodeling ATPases in plants. Plant J. 83, 62–77.

Hanemian, M., Zhou, B., Deslandes, L., Marco, Y., and Trémousaygue, D. (2013). Hrp mutant bacteria as biocontrol agents: Toward a sustainable approach in the fight against plant pathogenic bacteria. Plant Signal. Behav. 8, 1–5.

Hannan Parker, A., Wilkinson, S.W., and Ton, J. (2022). Epigenetics: a catalyst of plant immunity against pathogens. New Phytol. 233, 66–83.

Harouz, H., Rachez, C., Meijer, B.M., Marteyn, B., Donnadieu, F., Cammas, F., Muchardt, C., Sansonetti, P., and Arbibe, L. (2014). Shigella flexneri targets the HP 1 γ subcode through the phosphothreonine lyase O sp F . EMBO J. 33, 2606–2622.

Hayes, C.S., Aoki, S.K., and Low, D.A. (2010). Bacterial contact-dependent delivery systems. Annu. Rev. Genet. 44, 71–90.

Hayward, A.C. (1964). Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27, 265–277.

Hayward, A.C. (1991). BIOLOGY AND EPIDEMIOLOGY OF BACTERIAL WILT CAUSED BY PSEUDOMONAS SOLANACEARUM.

He, K., and Wu, Y. (2016). Receptor-Like Kinases and Regulation of Plant Innate Immunity. Enzymes 40, 105–142.

Heath, M.C. (1981). A Generalized Concept of Host-Parasite Specificity. Phytopathology 71, 1121.

Henry, E., Toruño, T.Y., Jauneau, A., Deslandes, L., and Coaker, G. (2017). Direct and indirect visualization of bacterial effector delivery into diverse plant cell types during infection. Plant Cell 29, 1555–1570.

Holsters, M., Silva, B., Vliet, F., Genetello, C., and Schell, J. (1980). The Functional Organization of the Nopaline Plasmid pTiC58. Plasmid 3, 212–230.

Hou, Y., Wang, L., Wang, L., Liu, L., Li, L., Sun, L., Rao, Q., Zhang, J., and Huang, S. (2015). JMJ704 positively regulates rice defense response against Xanthomonas oryzae pv. oryzae infection via reducing H3K4me2/3 associated with negative disease resistance regulators. BMC Plant Biol. 15, 1–13.

Hu, J., Barlet, X., Deslandes, L., Hirsch, J., Feng, D.X., Somssich, I., and Marco, Y. (2008). Transcriptional Responses of Arabidopsis thaliana during Wilt Disease Caused by the Soil-Borne Phytopathogenic Bacterium, Ralstonia solanacearum. PLoS One 3, 1–10.

Huang, C.-Y.Y., Rangel, D.S., Qin, X., Bui, C., Li, R., Jia, Z., Cui, X., and Jin, H. (2021). The chromatin-remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs. Cell Host Microbe 29, 1–10.

Huang, C.Y., Wang, H., Hu, P., Hamby, R., and Jin, H. (2019). Small RNAs – Big Players in Plant-Microbe Interactions. Cell Host Microbe 26, 173–182.

Huet, G. (2014). Breeding for resistances to Ralstonia solanacearum. Front. Plant Sci. 5, 1–5.

Hui, S., Liu, H., Zhang, M., Chen, D., Li, Q., Tian, J., Xiao, J., Li, X., Wang, S., and Yuan, M. (2019). The host basal transcription factor IIA subunits coordinate for facilitating infection of TALEs-carrying bacterial pathogens in rice. Plant Sci. 284, 48–56.

Iftner, T., Haedicke-Jarboui, J., Wu, S.Y., and Chiang, C.M. (2017). Involvement of Brd4 in different steps of the papillomavirus life cycle. Virus Res. 231, 76–82.

Irigoyen, S., Ramasamy, M., Misra, A., McKnight, T.D., and Mandadi, K.K. (2021). A BTB-TAZ protein is required for gene activation by Cauliflower mosaic virus 35S multimerized enhancers. Plant Physiol. 188, 397–410.

Jacob, Y., Feng, S., LeBlanc, C.A., Bernatavichute, Y. V, Stroud, H., Cokus, S., Johnson, L.M., Pellegrini, M., Jacobsen, S.E., and Michaels, S.D. (2009). ATXR5 and ATXR6 are H3K27 monomethyltransferases required for chromatin structure and gene silencing. Nat. Struct. Mol. Biol. 16, 763–768.

Jenuwein, T., and Allis, C.D. (2001). Translating the Histone Code. Science (80-.). 293, 1074–1080.

Jeon, H., Kim, W., Kim, B., Lee, S., Jayaraman, J., Jung, G., Choi, S., Sohn, K.H., and Segonzac, C. (2020). Ralstonia solanacearum Type III Effectors with Predicted Nuclear Localization Signal Localize to Various Cell Compartments and Modulate Immune Responses in Nicotiana spp. Plant Pathol. J. 36, 303–303.

Jiang, C.J., Shimono, M., Sugano, S., Kojima, M., Yazawa, K., Yoshida, R., Lnoue, H., Hayashi, N., Sakakibara, H., and Takatsuji, H. (2010). Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-magnaporthe grisea interaction. Mol. Plant-Microbe Interact. 23, 791–798.

Jones, J.D.G., and Dangl, J.L. (2006). The plant immune system. Nature 444, 323–329.

Jose, L., Ramachandran, R., Bhagavat, R., Gomez, R.L., Chandran, A., Raghunandanan, S., Omkumar, R.V., Chandra, N., Mundayoor, S., and Kumar, R.A. (2016). Hypothetical protein Rv3423.1 of Mycobacterium tuberculosis is a histone acetyltransferase. FEBS J. 283, 265–281.

Jubic, L.M., Saile, S., Furzer, O.J., El Kasmi, F., and Dangl, J.L. (2019). Help wanted: helper NLRs and plant immune responses. Curr. Opin. Plant Biol. 50, 82–94.

Kang, Y., Liu, H., Genin, S., Schell, M.A., and Denny, T.P. (2002). Ralstonia solanacearum requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol. Microbiol. 46, 427–437.

Kanno, T., Kanno, Y., Siegel, R.M., Jang, M.K., Lenardo, M.J., and Ozato, K. (2004). Selective Recognition of Acetylated Histones by Bromodomain Proteins Visualized in Living Cells. Mol. Cell 13, 33–43.

Kanyuka, K., and Rudd, J.J. (2019). Cell surface immune receptors: the guardians of the plant's extracellular spaces. Curr. Opin. Plant Biol. 50, 1–8.

Kao, C.C., Barlow, E., and Sequeira, L. (1992). Extracellular polysaccharide is required for wild-type virulence of Pseudomonas solanacearum. J. Bacteriol. 174, 1068–1071.

Karim, Z., Hossain, M., and Begum, M. (2018). Ralstonia solanacearum: A Threat to Potato Production in Bangladesh. Fundam. Appl. Agric. 3, 407–421.

Katagiri, F., and Tsuda, K. (2010). Understanding the Plant Immune System. Mol. Plant-Microbe Interact. 23, 1531–1536.

Kim, J.H., Saraf, A., Florens, L., Washburn, M., and Workman, J.L. (2010). Gcn5 regulates the dissociation of

SWI/SNF from chromatin by acetylation of Swi2/Snf2. Genes Dev. 24, 2766–2771.

Kim, M.J., Shin, R., and Schachtman, D.P. (2009). A nuclear factor regulates abscisic acid responses in Arabidopsis. Plant Physiol. 151, 1433–1445.

Kong, L., Qiu, X., Kang, J., Wang, Y., Chen, H., Huang, J., Qiu, M., Zhao, Y., Kong, G., Ma, Z., et al. (2017). A Phytophthora Effector Manipulates Host Histone Acetylation and Reprograms Defense Gene Expression to Promote Infection. Curr. Biol. 27, 981–991.

Kouzarides, T. (2007). Chromatin Modifications and Their Function. Cell 128, 693–705.

Kroj, T., Chanclud, E., Michel-Romiti, C., Grand, X., and Morel, J.B. (2016). Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. New Phytol. 210, 618–626.

Kumar, V., Thakur, J.K., and Prasad, M. (2021). Histone acetylation dynamics regulating plant development and stress responses. Cell. Mol. Life Sci. 78, 4467–4486.

Landry, D., González-Fuente, M., Deslandes, L., and Peeters, N. (2020). The large, diverse, and robust arsenal of Ralstonia solanacearum type III effectors and their in planta functions. Mol. Plant Pathol. 21, 1377–1388.

de Lange, O., Schreiber, T., Schandry, N., Radeck, J., Braun, K.H., Koszinowski, J., Heuer, H., Strauß, A., and Lahaye, T. (2013). Breaking the DNA-binding code of Ralstonia solanacearum TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease. New Phytol. 199, 773–786.

Latrasse, D., Jégu, T., Li, H., de Zelicourt, A., Raynaud, C., Legras, S., Gust, A., Samajova, O., Veluchamy, A., Rayapuram, N., et al. (2017). MAPK-triggered chromatin reprogramming by histone deacetylase in plant innate immunity. Genome Biol. 18, 1–19.

Lauria, M., and Rossi, V. (2011). Epigenetic control of gene regulation in plants. Biochim. Biophys. Acta - Gene Regul. Mech. 1809, 369–378.

Lavie, M., Shillington, E., Eguiluz, C., Grimsley, N., and Boucher, C. (2002). PopP1, a new member of the YopJ/AvrRxv family of type III effector proteins, acts as a host-specificity factor and modulates aggressiveness of Ralstonia solanacearum. Mol. Plant-Microbe Interact. 15, 1058–1068.

Lavie, M., Seunes, B., Prior, P., and Boucher, C. (2004). Distribution and sequence analysis of a family of type III-dependent effectors correlate with the phylogeny of Ralstonia solanacearum strains. Mol. Plant-Microbe Interact. 17, 931–940.

Le, T.N., Schumann, U., Smith, N.A., Tiwari, S., Khang Au, P.C., Zhu, Q.H., Taylor, J.M., Kazan, K., Llewellyn, D.J., Zhang, R., et al. (2014). DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in Arabidopsis. Genome Biol. 15, 1–18.

Lee, A.H.-Y., Hurley, B., Felsensteiner, C., Yea, C., Ckurshumova, W., Bartetzko, V., Wang, P.W., Quach, V., Lewis, J.D., Liu, Y.C., et al. (2012). A Bacterial Acetyltransferase Destroys Plant Microtubule Networks and Blocks Secretion. PLoS Pathog. 8, 1–14.

Lei, B., and Berger, F. (2020). H2A Variants in Arabidopsis: Versatile Regulators of Genome Activity. Plant Commun. 1, 100015.

Lewis, J.D., Lee, A.H.Y., Hassan, J.A., Wana, J., Hurleya, B., Jhingree, J.R., Wang, P.W., Lo, T., Youn, J.Y., Guttman, D.S., et al. (2013). The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the Pseudomonas syringae type III effector HopZ1a. Proc. Natl. Acad. Sci. U. S. A. 110, 18722–18727.

Li, P., and Day, B. (2019). Battlefield Cytoskeleton: Turning the Tide on Plant Immunity. Mol. Plant-Microbe Interact. 32, 25–34.

Li, B., Carey, M., and Workman, J.L. (2007a). The Role of Chromatin during Transcription. Cell 128, 707–719.

Li, H., Xu, H., Zhou, Y., Zhang, J., Long, C., Li, S., Chen, S., Zhou, J.-M., and Shao, F. (2007b). The Phosphothreonine Lyase Activity of a Bacterial Type III Effector Family. Science (80-.). 315, 1000–1003.

Li, H., Wang, H., Jing, M., Zhu, J., Guo, B., Wang, Y., Lin, Y., Chen, H., Kong, L., Ma, Z., et al. (2018). A phytophthora effector recruits a host cytoplasmic transacetylase into nuclear speckles to enhance plant susceptibility. Elife 7, 1–23.

Li, J.-F., Park, E., von Arnim, A.G., and Nebenführ, A. (2009). The FAST technique: a simplified Agrobacterium-based transformation method for transient gene expression analysis in seedlings of Arabidopsis and other plant species. Plant Methods 5, 1–15.

Li, L., Kim, P., Yu, L., Cai, G., Chen, S., Alfano, J.R., and Zhou, J.M. (2016). Activation-Dependent Destruction of a Co-receptor by a Pseudomonas syringae Effector Dampens Plant Immunity. Cell Host Microbe 20, 504–514.

Li, X., Kapos, P., and Zhang, Y. (2015a). NLRs in plants. Curr. Opin. Immunol. 32, 114–121.

Li, Y., Mukherjee, I., Thum, K.E., Tanurdzic, M., Katari, M.S., Obertello, M., Edwards, M.B., McCombie, W.R., Martienssen, R.A., and Coruzzi, G.M. (2015b). The histone methyltransferase SDG8 mediates the epigenetic modification of light and carbon responsive genes in plants. Genome Biol. 16, 1–15.

Liang, Z., Geng, Y., and Gu, X. (2018). Adenine Methylation: New Epigenetic Marker of DNA and mRNA. Mol. Plant 11, 1219–1221.

Liu, H., Zhang, S., Schell, M.A., and Denny, T.P. (2005). Pyramiding unmarked deletions in Ralstonia solanacearum shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. Mol. Plant-Microbe Interact. 18, 1296–1305.

Liu, J., Elmore, J.M., Lin, Z.J.D., and Coaker, G. (2011). A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. Cell Host Microbe 9, 137–146.

Lopez, V.A., Park, B.C., Nowak, D., Sreelatha, A., Zembek, P., Fernandez, J., Servage, K.A., Gradowski, M., Hennig, J., Tomchick, D.R., et al. (2019). A Bacterial Effector Mimics a Host HSP90 Client to Undermine Immunity. Cell 179, 205–218.

Lowe-Power, T.M., Khokhani, D., and Allen, C. (2018). How Ralstonia solanacearum Exploits and Thrives in

the Flowing Plant Xylem Environment. Trends Microbiol. 26, 929–942.

Lu, Y., and Tsuda, K. (2021). Intimate Association of PRR- and NLR-Mediated Signaling in Plant Immunity. Mol. Plant-Microbe Interact. 34, 3–14.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature 389, 251–260.

Luo, Y., Hou, X., Zhang, C., Tan, L., Shao, C., Lin, R., Su, Y., Cai, X., Li, L., Chen, S., et al. (2020). A plant - specific SWR1 chromatin - remodeling complex couples histone H2A.Z deposition with nucleosome sliding. EMBO J. 39, 1–16.

Ma, K.-W., and Ma, W. (2016). YopJ Family Effectors Promote Bacterial Infection through a Unique Acetyltransferase Activity. Microbiol. Mol. Biol. Rev. 80, 1011–1027.

Ma, S., Lapin, D., Liu, L., Sun, Y., Song, W., Zhang, X., Logemann, E., Yu, D., Wang, J., Jirschitzka, J., et al. (2020). Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. Science (80-.). 370.

Macho, A.P., Guidot, A., Barberis, P., Beuzón, C.R., and Genin, S. (2010). A Competitive Index Assay Identifies Several Ralstonia solanacearum Type III Effector Mutant Strains with Reduced Fitness in Host Plants. MPMI 23, 1197–1205.

Maekawa, T., Cheng, W., Spiridon, L.N., Töller, A., Lukasik, E., Saijo, Y., Liu, P., Shen, Q.H., Micluta, M.A., Somssich, I.E., et al. (2011). Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. Cell Host Microbe 9, 187–199.

Maidment, J.H.R., Franceschetti, M., Maqbool, A., Saitoh, H., Jantasuriyarat, C., Kamoun, S., Terauchi, R., and Banfield, M.J. (2021). Multiple variants of the fungal effector AVR-Pik bind the HMA domain of the rice protein OsHIPP19, providing a foundation to engineer plant defense. J. Biol. Chem. 296, 1–13.

Mamphogoro, T.P., Babalola, O.O., and Aiyegoro, O.A. (2020). Sustainable management strategies for bacterial wilt of sweet peppers (Capsicum annuum) and other Solanaceous crops. J. Appl. Microbiol. 129, 496–508.

Mandadi, K.K., Misra, A., Ren, S., and McKnight, T.D. (2009). BT2, a BTB Protein, Mediates Multiple Responses to Nutrients, Stresses, and Hormones in Arabidopsis. Plant Physiol. 150, 1930–1939.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M.A., et al. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13, 614–629.

Martin, R., Qi, T., Zhang, H., Liu, F., King, M., Toth, C., Nogales, E., and Staskawicz, B.J. (2020). Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. Science (80-.). 370, 1–7.

Medina-Puche, L., Tan, H., Dogra, V., Wu, M., Rosas-Diaz, T., Wang, L., Ding, X., Zhang, D., Fu, X., Kim, C., et al. (2020). A Defense Pathway Linking Plasma Membrane and Chloroplasts and Co-opted by Pathogens. Cell 182, 1109–1124.

Misra, A., McKnight, T.D., and Mandadi, K.K. (2018). Bromodomain proteins GTE9 and GTE11 are essential for specific BT2-mediated sugar and ABA responses in Arabidopsis thaliana. Plant Mol. Biol. 96, 393–402.

Monteiro, F., Genin, S., van Dijk, I., and Valls, M. (2012). A luminescent reporter evidences active expression of Ralstonia solanacearum type III secretion system genes throughout plant infection. Microbiol. (United Kingdom) 158, 2107–2116.

Morris, K. V., and Mattick, J.S. (2014). The rise of regulatory RNA. Nat. Rev. Genet. 15, 423–437.

Mott, G.A., Middleton, M.A., Desveaux, D., and Guttman, D.S. (2014). Peptides and small molecules of the plant-pathogen apoplastic arena. Front. Plant Sci. 5, 1–12.

Mukhtar, M.S., Carvunis, A.-R., Dreze, M., Epple, P., Steinbrenner, J., Moore, J., Tasan, M., Galli, M., Hao, T., Nishimura, M.T., et al. (2011). Independently Evolved Virulence Effectors Converge onto Hubs in a Plant Immune System Network. Science (80-.). 333, 596–601.

Muller, S., Filippakopoulos, P., and Knapp, S. (2011). Bromodomains as therapeutic targets. Expert Rev. Mol. Med. 13, 1–21.

Nahar, K., Kyndt, T., de Vleesschauwer, D., Höfte, M., and Gheysen, G. (2011). The jasmonate pathway is a key player in systemically induced defense against root knot nematodes in rice. Plant Physiol. 157, 305–316.

Nakano, M., and Mukaihara, T. (2018). Ralstonia solanacearum type iii effector ripal targets chloroplasts and induces jasmonic acid production to suppress salicylic acid-mediated defense responses in plants. Plant Cell Physiol. 59, 2576–2589.

Nakano, M., and Mukaihara, T. (2019). The type III effector RipB from Ralstonia solanacearum RS1000 acts as a major avirulence factor in Nicotiana benthamiana and other Nicotiana species. Mol. Plant Pathol. 20, 1237–1251.

Nakano, M., Oda, K., and Mukaihara, T. (2017). Ralstonia solanacearum novel E3 ubiquitin ligase (NEL) effectors RipAW and RipAR suppress pattern-triggered immunity in plants. Microbiol. (United Kingdom) 163, 992–1002.

Narusaka, M., Shirasu, K., Noutoshi, Y., Kubo, Y., Shiraishi, T., Iwabuchi, M., and Narusaka, Y. (2009). RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. Plant J. 60, 218–226.

Navarro, L., Jay, F., Nomura, K., Sheng, Y.H., and Voinnet, O. (2008). Suppression of the MicroRNA pathway by bacterial effector proteins. Science (80-.). 321, 964–967.

Ngou, B.P.M., Ahn, H., Ding, P., Redkar, A., Brown, H., Ma, Y., Youles, M., Tomlinson, L., and Jones, J.D.G. (2020). Estradiol-inducible AvrRps4 expression reveals distinct properties of TIR-NLR-mediated effector-triggered immunity. J. Exp. Bot. 71, 2186–2197.

Ngou, B.P.M., Ahn, H.K., Ding, P., and Jones, J.D.G. (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. Nature 592, 110–115.

Nguyen, Q.-M., Iswanto, A.B.B., Son, G.H., and Kim, S.H. (2021). Recent Advances in Effector-Triggered

Immunity in Plants: New Pieces in the Puzzle Create a Different Paradigm. Int. J. Mol. Sci. 22.

Nie, W.F., Lei, M., Zhang, M., Tang, K., Huang, H., Zhang, C., Miki, D., Liu, P., Yang, Y., Wang, X., et al. (2019). Histone acetylation recruits the SWR1 complex to regulate active DNA demethylation in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 116, 16641–16650.

O'Neill, E.M., Mucyn, T.S., Patteson, J.B., Finkel, O.M., Chung, E.H., Baccile, J.A., Massolo, E., Schroeder, F.C., Dangl, J.L., and Li, B. (2018). Phevamine A, a small molecule that suppresses plant immune responses. Proc. Natl. Acad. Sci. U. S. A. 115, E9514–E9522.

Oikawa, K., Fujisaki, K., Shimizu, M., Takeda, T., Saitoh, H., Hirabuchi, A., Hiraka, Y., Białas, A., Langner, T., Kellner, R., et al. (2020). The blast pathogen effector AVR-Pik binds and stabilizes rice heavy metal-associated (HMA) proteins to co-opt their function in immunity. BioRxiv.

Osdaghi, E. (2020). Ralstonia solanacearum (bacterial wilt of potato). Invasive Species Compendium.

Ouyang, W., Cao, Z., Xiong, D., Li, G., and Li, X. (2020). Decoding the plant genome: From epigenome to 3D organization. J. Genet. Genomics 47, 425–435.

Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Filetici, P., and Travers, A.A. (2000). The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase Gcn5p. EMBO J. 19, 6141–6149.

Palma, K., Zhao, Q., Yu, T.C., Bi, D., Monaghan, J., Cheng, W., Zhang, Y., and Li, X. (2007). Regulation of plant innate immunity by three proteins in a complex conserved across the plant and animal kingdoms. Genes Dev. 21, 1484–1493.

Palma, K., Thorgrimsen, S., Malinovsky, F.G., Fiil, B.K., Nielsen, H.B., Brodersen, P., Hofius, D., Petersen, M., and Mundy, J. (2010). Autoimmunity in Arabidopsis acd11 Is Mediated by Epigenetic Regulation of an Immune Receptor. PLoS Pathog. 6, 1–12.

Pandey, R. (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, 5036–5055.

Pandey, S.P., and Somssich, I.E. (2009). The Role of WRKY Transcription Factors in Plant Immunity. Plant Physiol. 150, 1648–1655.

Pandey, R., Müller, A., Napoli, C.A., Selinger, D.A., Pikaard, C.S., Richards, E.J., Bender, J., Mount, D.W., and Jorgensen, R.A. (2002). Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversi®cation of chromatin modi®cation among multicellular eukaryotes. Nucleic Acids Res. 30, 5036–5055.

Park, E., Lee, H.-Y., Woo, J., Choi, D., and Dinesh-Kumar, S.P. (2017). Spatiotemporal Monitoring of Pseudomonas syringae Effectors via Type III Secretion Using Split Fluorescent Protein Fragments . Plant Cell 29, 1571–1584.

Paudel, S., Dobhal, S., Alvarez, A.M., and Arif, M. (2020). Taxonomy and phylogenetic research on ralstonia

solanacearum species complex: A complex pathogen with extraordinary economic consequences. Pathogens 9, 1–26.

de Pedro-Jové, R., Puigvert, M., Sebastià, P., Macho, A.P., Monteiro, J.S., Coll, N.S., Setúbal, J.C., and Valls, M. (2021). Dynamic expression of Ralstonia solanacearum virulence factors and metabolism-controlling genes during plant infection. BMC Genomics 22, 1–19.

Peeters, N., Carrère, S., Anisimova, M., Plener, L., Cazalé, A.-C., and Genin, S. (2013). Repertoire, unified nomenclature and evolution of the Type III effector gene set in the Ralstonia solanacearum species complex. BMC Genomics 14, 1–18.

Peirats-Llobet, M., Han, S.K., Gonzalez-Guzman, M., Jeong, C.W., Rodriguez, L., Belda-Palazon, B., Wagner, D., and Rodriguez, P.L. (2016). A Direct Link between Abscisic Acid Sensing and the Chromatin-Remodeling ATPase BRAHMA via Core ABA Signaling Pathway Components. Mol. Plant 9, 136–147.

Peng, Y., Van Wersch, R., and Zhang, Y. (2018). Convergent and divergent signaling in PAMP-triggered immunity and effector-triggered immunity. Mol. Plant-Microbe Interact. 31, 403–409.

Peng, Z., Hu, Y., Zhang, J., Huguet-Tapia, J.C., Block, A.K., Park, S., Sapkota, S., Liu, Z., Liu, S., and White, F.F. (2019). Xanthomonas translucens commandeers the host rate-limiting step in ABA biosynthesis for disease susceptibility. Proc. Natl. Acad. Sci. U. S. A. 116, 20938–20946.

Pennini, M.E., Pai, R.K., Schultz, D.C., Boom, W.H., and Harding, C. V. (2006). Mycobacterium tuberculosis 19-kDa Lipoprotein Inhibits IFN-γ-Induced Chromatin Remodeling of MHC2TA by TLR2 and MAPK Signaling . J. Immunol. 176, 4323–4330.

Pensec, F., Lebeau, A., Daunay, M.C., Chiroleu, F., Guidot, A., and Wicker, E. (2015). Towards the identification of type III effectors associated with ralstonia solanacearum virulence on tomato and eggplant. Phytopathology 105, 1529–1544.

Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D.J., Inuganti, A., Griss, J., Mayer, G., Eisenacher, M., et al. (2019). The PRIDE database and related tools and resources in 2019: Improving support for quantification data. Nucleic Acids Res. 47, D442–D450.

Pieterse, C.M.J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S.C.M. (2012). Hormonal modulation of plant immunity. Annu. Rev. Cell Dev. Biol. 28, 489–521.

Pikaard, C.S., and Scheid, O.M. (2014). Epigenetic regulation in plants. Cold Spring Harb. Perspect. Biol. 6, 1–31.

Poueymiro, M., Cunnac, S., Barberis, P., Deslandes, L., Peeters, N., Cazale-Noel, A.C., Boucher, C., and Genin, S. (2009). Two type III secretion system effectors from Ralstonia solanacearum GMI1000 determine host-range Specificity on Tobacco. Mol. Plant-Microbe Interact. 22, 538–550.

Poussier, S., Vandewalle, P., and Luisetti, J. (1999). Genetic diversity of African and worldwide strains of Ralstonia solanacearum as determined by PCR-restriction fragment length polymorphism analysis of the hrp gene region. Appl. Environ. Microbiol. 65, 2184–2194.

Prior, P., and Fegan, M. (2005). Recent developments in the phylogeny and classification of ralstonia solanacearum. Acta Hortic. 695, 127–136.

Prior, P., Ailloud, F., Dalsing, B.L., Remenant, B., Sanchez, B., and Allen, C. (2016). Genomic and proteomic evidence supporting the division of the plant pathogen Ralstonia solanacearum into three species. BMC Genomics 17, 1–11.

Probst, A. V., and Mittelsten Scheid, O. (2015). Stress-induced structural changes in plant chromatin. Curr. Opin. Plant Biol. 27, 8–16.

Pruitt, R.N., Zhang, L., Saile, S.C., Karelina, D., Fröhlich, K., Wan, W., Rao, S., Weigel, D., Parker, J.E., Kasmi, F. El, et al. (2020). Arabidopsis cell surface LRR immune receptor signaling through the EDS1-PAD4-ADR1 node. BioRxiv 1, 391516.

Pumplin, N., and Voinnet, O. (2013). RNA silencing suppression by plant pathogens: Defence, counter-defence and counter-counter-defence. Nat. Rev. Microbiol. 11, 745–760.

Qin, J., Wang, K., Sun, L., Xing, H., Wang, S., Li, L., Chen, S., Guo, H.S., and Zhang, J. (2018). The plant-specific transcription factors CBP60G and SARD1 are targeted by a verticillium secretory protein VDSCP41 to modulate immunity. Elife 7, 1–25.

Rahman, S., Sowa, M.E., Ottinger, M., Smith, J.A., Shi, Y., Harper, J.W., and Howley, P.M. (2011). The Brd4 Extraterminal Domain Confers Transcription Activation Independent of pTEFb by Recruiting Multiple Proteins, Including NSD3. Mol. Cell. Biol. 31, 2641–2652.

Ramirez-Prado, J.S., Abulfaraj, A.A., Rayapuram, N., Benhamed, M., and Hirt, H. (2018a). Plant Immunity: From Signaling to Epigenetic Control of Defense. Trends Plant Sci. 23, 833–844.

Ramirez-Prado, J.S., Piquerez, S.J.M., Bendahmane, A., Hirt, H., Raynaud, C., and Benhamed, M. (2018b). Modify the Histone to Win the Battle: Chromatin Dynamics in Plant–Pathogen Interactions. Front. Plant Sci. 9, 1–18.

Rolando, M., Sanulli, S., Rusniok, C., Gomez-Valero, L., Bertholet, C., Sahr, T., Margueron, R., and Buchrieser, C. (2013). Legionella pneumophila effector RomA uniquely modifies host chromatin to repress gene expression and promote intracellular bacterial replication. Cell Host Microbe 13, 395–405.

Rolando, M., Gomez-Valero, L., and Buchrieser, C. (2015). Bacterial remodelling of the host epigenome: Functional role and evolution of effectors methylating host histones. Cell. Microbiol. 17, 1098–1107.

Roudier, F., Ahmed, I., Bérard, C., Sarazin, A., Mary-Huard, T., Cortijo, S., Bouyer, D., Caillieux, E., Duvernois-Berthet, E., Al-Shikhley, L., et al. (2011). Integrative epigenomic mapping defines four main chromatin states in Arabidopsis. EMBO J. 30, 1928–1938.

Le Roux, C., Huet, G., Jauneau, A., Camborde, L., Trémousaygue, D., Kraut, A., Zhou, B., Levaillant, M., Adachi, H., Yoshioka, H., et al. (2015). A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. Cell 161, 1074–1088.

Rufián, J.S., Rueda - Blanco, J., López - Márquez, D., Macho, A.P., Beuzón, C.R., and Ruiz - Albert, J. (2021).

The bacterial effector HopZ1a acetylates MKK7 to suppress plant immunity. New Phytol. 231, 1138–1156.

Rushton, P.J., Somssich, I.E., Ringler, P., and Shen, Q.J. (2010). WRKY transcription factors. Trends Plant Sci. 15, 247–258.

Russell, A.R., Ashfield, T., and Innes, R.W. (2015). Pseudomonas syringae effector AvrPphB suppresses AvrB-induced activation of RPM1 but not AvrRpm1-induced activation. Mol. Plant-Microbe Interact. 28, 727–735.

Sabbagh, C.R.R., Carrere, S., Lonjon, F., Vailleau, F., Macho, A.P., Genin, S., and Peeters, N. (2019). Pangenomic type III effector database of the plant pathogenic Ralstonia spp. PeerJ 7, 1–21.

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., and Kappler, U. (2014). Polyphasic taxonomic revision of the Ralstonia solanacearum species complex: Proposal to emend the descriptions of Ralstonia solanacearum and Ralstonia syzygii and reclassify current R. syzygii strains as Ralstonia syzygii subsp. syzygii subsp. nov., R. s. Int. J. Syst. Evol. Microbiol. 64, 3087–3103.

Saijo, Y., Loo, E.P. iian, and Yasuda, S. (2018). Pattern recognition receptors and signaling in plant–microbe interactions. Plant J. 93, 592–613.

Saile, E., McGarvey, J.A., Schell, M.A., and Denny, T.P. (1997). Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by Ralstonia solanacearum. Phytopathology 87, 1264–1271.

Saile, S.C., Jacob, P., Castel, B., Jubic, L.M., Salas-Gonzáles, I., Bäcker, M., Jones, J.D.G., Dangl, J.L., and El Kasmi, F. (2020). Two unequally redundant "helper" immune receptor families mediate Arabidopsis thaliana intracellular "sensor" immune receptor functions. PLoS Biol. 18, 1–28.

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottiert, P., Camus, J.C., Cattolico, L., et al. (2002). Genome sequence of the plant pathogen ralstonia solanacearum. Nature 415, 497–502.

Sánchez-Romero, M.A., and Casadesús, J. (2020). The bacterial epigenome. Nat. Rev. Microbiol. 18, 7–20.

Sánchez-Vallet, A., Fouché, S., Fudal, I., Hartmann, F.E., Soyer, J.L., Tellier, A., and Croll, D. (2018). The Genome Biology of Effector Gene Evolution in Filamentous Plant Pathogens. Annu. Rev. Phytopathol. 56, 21–40.

Sang, Y., Wang, Y., Ni, H., Cazalé, A.C., She, Y.M., Peeters, N., and Macho, A.P. (2018). The ralstonia solanacearum type III effector ripay targets plant redox regulators to suppress immune responses. Mol. Plant Pathol. 19, 129–142.

Sang, Y., Yu, W., Zhuang, H., Wei, Y., Derevnina, L., Yu, G., Luo, J., and Macho, A.P. (2020). Intra-strain Elicitation and Suppression of Plant Immunity by Ralstonia solanacearum Type-III Effectors in Nicotiana benthamiana. Plant Commun. 1, 100025.

Sarris, P.F., Duxbury, Z., Huh, S.U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S.B., et al. (2015). A plant immune receptor detects pathogen effectors that target WRKY transcription factors. Cell 161, 1089–1100.

Sarris, P.F., Cevik, V., Dagdas, G., Jones, J.D.G., and Krasileva, K. V. (2016). Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. BMC Biol. 14, 1–18.

Saur, I., Bauer, S., Kracher, B., Lu, X., Ku, F., Franzeskakis, L., Mu, M.C., Panstruga, R., Maekawa, T., Schulze-lefert, P., et al. (2019). Multiple pairs of allelic MLA immune receptor-powdery mildew AVR. Elife 1–31.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439.

Schandry, N., De Lange, O., Prior, P., and Lahaye, T. (2016). TALE-Like effectors are an ancestral feature of the ralstonia solanacearum species complex and converge in DNA targeting specificity. Front. Plant Sci. 7, 1–16.

Schmittgen, T.D., and Livak, K.J. (2008). Analyzing real-time PCR data by the comparative CT method. Nat. Protoc. 3, 1101–1108.

Schreiber, K.J., and Lewis, J.D. (2021). Identification of a putative DNA-binding protein in arabidopsis that acts as a susceptibility hub and interacts with multiple Pseudomonas syringae effectors. Mol. Plant-Microbe Interact. 34, 410–425.

Schreiber, K.J., Chau-Ly, I.J., and Lewis, J.D. (2021a). What the wild things do: Mechanisms of plant host manipulation by bacterial type III-secreted effector proteins. Microorganisms 9, 1–48.

Schreiber, K.J., Hassan, J.A., and Lewis, J.D. (2021b). Arabidopsis Abscisic Acid Repressor 1 is a susceptibility hub that interacts with multiple Pseudomonas syringae effectors. Plant J. 105, 1274–1292.

Schultink, A., Qi, T., Bally, J., and Staskawicz, B. (2019). Using forward genetics in Nicotiana benthamiana to uncover the immune signaling pathway mediating recognition of the Xanthomonas perforans effector XopJ4. New Phytol. 221, 1001–1009.

Segonzac, C., Newman, T.E., Choi, S., Jayaraman, J., Choi, D.S., Jung, G.Y., Cho, H., Lee, Y.K., and Sohn, K.H. (2017). A Conserved EAR Motif Is Required for Avirulence and Stability of the Ralstonia solanacearum Effector PopP2 In Planta. Front. Plant Sci. 8, 1–13.

Seo, J.S., Sun, H.X., Park, B.S., Huang, C.H., Yeh, S.D., Jung, C., and Chua, N.H. (2017). ELF18-INDUCED LONG-NONCODING RNA associates with mediator to enhance expression of innate immune response genes in arabidopsis. Plant Cell 29, 1024–1038.

Shames, S.R., Bhavsar, A.P., Croxen, M.A., Law, R.J., Mak, S.H.C., Deng, W., Li, Y., Bidshari, R., de Hoog, C.L., Foster, L.J., et al. (2011). The pathogenic Escherichia coli type III secreted protease NleC degrades the host acetyltransferase p300. Cell. Microbiol. 13, 1542–1557.

Sharma, G., Upadhyay, S., Srilalitha, M., Nandicoori, V.K., and Khosla, S. (2015). The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and histone H3/H4 binding. Nucleic Acids Res. 43, 3922–3937.

Shigenaga, A.M., and Argueso, C.T. (2016). No hormone to rule them all: Interactions of plant hormones during the responses of plants to pathogens. Semin. Cell Dev. Biol. 56, 174–189.

Shine, M.B., Xiao, X., Kachroo, P., and Kachroo, A. (2019). Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. Plant Sci. 279, 81–86.

Silmon De Monerri, N.C., and Kim, K. (2014). Pathogens hijack the epigenome: A new twist on host-pathogen interactions. Am. J. Pathol. 184, 897–911.

Smith, S.G., and Zhou, M.M. (2016). The Bromodomain: A New Target in Emerging Epigenetic Medicine. ACS Chem. Biol. 11, 598–608.

Sohn, K.H., Segonzac, C., Rallapalli, G., Sarris, P.F., Woo, J.Y., Williams, S.J., Newman, T.E., Paek, K.H., Kobe, B., and Jones, J.D.G. (2014). The Nuclear Immune Receptor RPS4 Is Required for RRS1SLH1-Dependent Constitutive Defense Activation in Arabidopsis thaliana. PLoS Genet. 10, e1004655.

Solé, M., Popa, C., Mith, O., Sohn, K.H., Jones, J.D.G., Deslandes, L., and Valls, M. (2012). Type III Effectors Displaying Virulence and Avirulence Activities. Mol. Plant-Microbe Interact. 25, 941–953.

Song, L., Fang, Y., Chen, L., Wang, J., and Chen, X. (2021a). Role of non-coding RNAs in plant immunity. Plant Commun. 2, 100180.

Song, W., Forderer, A., Yu, D., and Chai, J. (2021b). Structural biology of plant defence. New Phytol. 229, 692–711.

Su, J., Zhang, M., Zhang, L., Sun, T., Liu, Y., Lukowitz, W., Xu, J., and Zhang, S. (2017). Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid. Plant Cell 29, 526–542.

Sun, T., Zhang, Y., Li, Y., Zhang, Q., Ding, Y., and Zhang, Y. (2015). ChIP-seq reveals broad roles of SARD1 and CBP60g in regulating plant immunity. Nat. Commun. 6, 1–12.

Sun, Y., Li, P., Shen, D., Wei, Q., He, J., and Lu, Y. (2019). The Ralstonia solanacearum effector RipN suppresses plant PAMP-triggered immunity, localizes to the endoplasmic reticulum and nucleus, and alters the NADH/NAD + ratio in Arabidopsis. Mol. Plant Pathol. 20, 533–546.

Sun, Y., Zhu, Y.X., Balint-Kurti, P.J., and Wang, G.F. (2020). Fine-Tuning Immunity: Players and Regulators for Plant NLRs. Trends Plant Sci. 25, 695–713.

Tang, D., Wang, G., and Zhou, J.M. (2017). Receptor kinases in plant-pathogen interactions: More than pattern recognition. Plant Cell 29, 618–637.

Tasset, C., Bernoux, M., Jauneau, A., Pouzet, C., Brière, C., Kieffer-Jacquinod, S., Rivas, S., Marco, Y., and Deslandes, L. (2010). Autoacetylation of the Ralstonia solanacearum Effector PopP2 Targets a Lysine Residue Essential for RRS1-R-Mediated Immunity in Arabidopsis. PLoS Pathog. 6, 1–14.

Thomma, B.P.H.J., Nürnberger, T., and Joosten, M.H.A.J. (2011). Of PAMPs and Effectors: The Blurred PTI-ETI Dichotomy. Plant Cell 23, 4–15.

Thulasi Devendrakumar, K., Li, X., and Zhang, Y. (2018). MAP kinase signalling: interplays between plant PAMP- and effector-triggered immunity. Cell. Mol. Life Sci. 75, 2981–2989.

Tian, H., Chen, S., Wu, Z., Ao, K., Yaghmaiean, H., Sun, T., Huang, W., Xu, F., Zhang, Y., Wang, S., et al. (2020). Activation of TIR signaling is required for pattern-triggered immunity. BioRxiv.

Tsuda, K., and Katagiri, F. (2010). Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity. Curr. Opin. Plant Biol. 13, 459–465.

Tsuda, K., and Somssich, I.E. (2015). Transcriptional networks in plant immunity. New Phytol. 206, 932–947.

Ulferts, S., Delventhal, R., Splivallo, R., Karlovsky, P., and Schaffrath, U. (2015). Abscisic acid negatively interferes with basal defence of barley against Magnaporthe oryzae. BMC Plant Biol. 15, 1–13.

Üstün, S., and Börnke, F. (2015). The Xanthomonas campestris type III effector XopJ proteolytically degrades proteasome subunit RPT6. Plant Physiol. 168, 107–119.

Üstün, S., Bartetzko, V., and Börnke, F. (2013). The Xanthomonas campestris Type III Effector XopJ Targets the Host Cell Proteasome to Suppress Salicylic-Acid Mediated Plant Defence. PLoS Pathog. 9, 1–22.

VanDemark, A.P., Kasten, M.M., Ferris, E., Heroux, A., Hill, C.P., and Cairns, B.R. (2007). Autoregulation of the Rsc4 Tandem Bromodomain by Gcn5 Acetylation. Mol. Cell 27, 817–828.

Vasse, J. (1995). Microscopic Studies of Intercellular Infection and Protoxylem Invasion of Tomato Roots by Pseudomonas solanacearum. Mol. Plant-Microbe Interact. 8, 241–251.

Vasse, J., Genin, S., Frey, P., Boucher, C., and Brito, B. (2000). The hrpB and hrpG regulatory genes of Ralstonia solanacearum are required for different stages of the tomato root infection process. Mol. Plant-Microbe Interact. 13, 259–267.

Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K.G., Kim, S.K., Crespi, M., Hirt, H., Bergounioux, C., Raynaud, C., et al. (2016). LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. PLoS One 11.

Verma, V., Ravindran, P., and Kumar, P.P. (2016). Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16, 1–10.

Vijayapalani, P., Hewezi, T., Pontvianne, F., and Baum, T.J. (2018). An effector from the cyst nematode heterodera schachtii derepresses host rRNA genes by altering histone acetylation. Plant Cell 30, 2795–2812.

de Vleesschauwer, D., Yang, Y., Cruz, C.V., and Höfte, M. (2010). Abscisic acid-induced resistance against the brown spot pathogen Cochliobolus miyabeanus in rice involves MAP kinase-mediated repression of ethylene signaling. Plant Physiol. 152, 2036–2052.

De Vleesschauwer, D., Gheysen, G., and Höfte, M. (2013). Hormone defense networking in rice: Tales from a different world. Trends Plant Sci. 18, 555–565.

Vos, I.A., Moritz, L., Pieterse, C.M.J., and Van Wees, S.C.M. (2015). Impact of hormonal crosstalk on plant resistance and fitness under multi-attacker conditions. Front. Plant Sci. 6, 1–13.

Waddington, C.H. (1968). Towards a Theoretical Biology. Nat. 1968 2185141 218, 525–527.

Wang, H.L. V., and Chekanova, J.A. (2017). Long noncoding RNAs in plants. Adv. Exp. Med. Biol. 1008, 133–

154.

Wang, C., Ding, Y., Yao, J., Zhang, Y., Sun, Y., Colee, J., and Mou, Z. (2015a). Arabidopsis Elongator subunit 2 positively contributes to resistance to the necrotrophic fungal pathogens Botrytis cinerea and Alternaria brassicicola. Plant J. 83, 1019–1033.

Wang, G., Roux, B., Feng, F., Guy, E., Li, L., Li, N., Zhang, X., Lautier, M., Jardinaud, M.F., Chabannes, M., et al. (2015b). The Decoy Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-Self Recognition and Immunity in Plants. Cell Host Microbe 18, 285–295.

Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H.-W., Zhou, J.-M., and Chai, J. (2019). Reconstitution and structure of a plant NLR resistosome conferring immunity. Science (80-.). 364, 1–11.

Wang, N., Wu, R., Tang, D., and Kang, R. (2021). The BET family in immunity and disease. Signal Transduct. Target. Ther. 6, 1–22.

Wang, Y., An, C., Zhang, X., Yao, J., Zhang, Y., Sun, Y., Yu, F., Amador, D.M., and Moua, Z. (2013). The Arabidopsis elongator complex subunit2 epigenetically regulates plant immune responses. Plant Cell 25, 762–776.

Waterworth, W.M., Drury, G.E., Blundell-Hunter, G., and West, C.E. (2015). Arabidopsis TAF1 is an MRE11-interacting protein required for resistance to genotoxic stress and viability of the male gametophyte. Plant J. 84, 545–557.

Weinhold, B. (2006). Epigenetics: the science of change. Environ. Health Perspect. 114, 160–167.

Wenneker, M., Verdel, M.S.W., Groeneveld, R.M.W., Kempenaar, C., Van Beuningen, A.R., and Janse, J.D. (1999). Ralstonia (Pseudomonas) solanacearum race 3 (biovar 2) in surface water and natural weed hosts: First report on stinging nettle (Urtica dioica). Eur. J. Plant Pathol. 105, 307–315.

van Wersch, S., and Li, X. (2019). Stronger When Together: Clustering of Plant NLR Disease resistance Genes. Trends Plant Sci. 24, 688–699.

van Wersch, S., Tian, L., Hoy, R., and Li, X. (2020). Plant NLRs: The Whistleblowers of Plant Immunity. Plant Commun. 1, 1–18.

Weßling, R., Epple, P., Altmann, S., He, Y., Yang, L., Henz, R., Mcdonald, N., Wiley, K., Bader, K.C., Gläßer, C., et al. (2015). Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. Cell Host Microbe 16, 364–375.

Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. (2007). Ralstonia solanacearum strains from Martinique (French West Indies) exhibiting a new pathogenic potential. Appl. Environ. Microbiol. 73, 6790–6801.

Williams, S.J., Sohn, K.H., Wan, L., Bernoux, M., Sarris, P.F., Segonzac, C., Ve, T., Ma, Y., Saucet, S.B., Ericsson, D.J., et al. (2014). Structural basis for assembly and function of a heterodimeric plant immune receptor. Science (80-.). 344, 299–303.

Wood, D.W., Setubal, J.C., Kaul, R., Monks, D.E., Kitajima, J.P., Okura, V.K., Zhou, Y., Chen, L., Wood, G.E.,

Almeida, J., et al. (2001). The genome of the natural genetic engineer Agrobacterium tumefaciens C58. Science (80-.). 294, 2317–2323.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. 67, 545–579.

Wroblewski, T., Caldwell, K.S., Piskurewicz, U., Cavanaugh, K.A., Xu, H., Kozik, A., Ochoa, O., Mchale, L.K., Lahre, K., Jelenska, J., et al. (2009). Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of pseudomonas and Ralstonia. Plant Physiol. 150, 1733–1749.

Wu, C., Abd-El-Haliem, A., Bozkurt, T.O., Belhaj, K., Terauchi, R., Vossen, J.H., and Kamoun, S. (2017). NLR network mediates immunity to diverse plant pathogens. Proc. Natl. Acad. Sci. 114, 8113–8118.

Wu, D., von Roepenack-Lahaye, E., Buntru, M., de Lange, O., Schandry, N., Pérez-Quintero, A.L., Weinberg, Z., Lowe-Power, T.M., Szurek, B., Michael, A.J., et al. (2019). A Plant Pathogen Type III Effector Protein Subverts Translational Regulation to Boost Host Polyamine Levels. Cell Host Microbe 26, 638–649.

Wu, S.Y., Lee, A.Y., Lai, H.T., Zhang, H., and Chiang, C.M. (2013). Phospho switch triggers brd4 chromatin binding and activator recruitment for gene-specific targeting. Mol. Cell 49, 843–857.

Xi'ou, X., Bihao, C., Guannan, L., Jianjun, L., Qinghua, C., Jin, J., and Yujing, C. (2015). Functional Characterization of a Putative Bacterial Wilt Resistance Gene (RE-bw) in Eggplant. Plant Mol. Biol. Report. 33, 1058–1073.

Xia, Y., Zou, R., Escouboué, M., Zhong, L., Zhu, C., Pouzet, C., Wu, X., Wang, Y., Lv, G., Zhou, H., et al. (2021). Secondary-structure switch regulates the substrate binding of a YopJ family acetyltransferase. Nat. Commun. 12, 1–10.

Xin, M., Wang, Y., Yao, Y., Song, N., Hu, Z., Qin, D., Xie, C., Peng, H., Ni, Z., and Sun, Q. (2011). Identification and characterization of wheat long non-protein coding RNAs responsive to powdery mildew infection and heat stress by using microarray analysis and SBS sequencing. BMC Plant Biol. 11, 1–13.

Xiong, Q., Ye, W., Choi, D., Wong, J., Qiao, Y., Tao, K., Wang, Y., and Ma, W. (2014). Phytophthora Suppressor of RNA Silencing 2 is a Conserved RxLR Effector that Promotes Infection in Soybean and Arabidopsis thaliana. Mol. Plant-Microbe Interact. 27, 1379–1389.

Yadukrishnan, P., and Datta, S. (2021). Light and abscisic acid interplay in early seedling development. New Phytol. 229, 763–769.

Yang, B., Wang, Y., Guo, B., Jing, M., Zhou, H., Li, Y., Wang, H., Huang, J., Wang, Y., Ye, W., et al. (2019). The Phytophthora sojae RXLR effector Avh238 destabilizes soybean Type2 GmACSs to suppress ethylene biosynthesis and promote infection. New Phytol. 222, 425–437.

Yang, D.-L., Yao, J., Mei, C.-S., Tong, X.-H., Zeng, L.-J., Li, Q., Xiao, L.-T., Sun, T., Li, J., Deng, X.-W., et al. (2012). Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. 109, 1192–1200.

Yang, L., Chen, X., Wang, Z., Sun, Q., Hong, A., Zhang, A., Zhong, X., and Hua, J. (2020). HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. New Phytol. 226, 507–522.

Yang, L., Guan, D., Valls, M., and Ding, W. (2021). Sustainable natural bioresources in crop protection: antimicrobial hydroxycoumarins induce membrane depolarization - associated changes in the transcriptome of <scp> Ralstonia solanacearum </scp>. Pest Manag. Sci. 77, 5170–5185.

Yu, X., Feng, B., He, P., and Shan, L. (2017). From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition. Annu. Rev. Phytopathol. 55, 109–137.

Yu, Y., Zhang, Y., Chen, X., and Chen, Y. (2019). Plant noncoding RNAs: Hidden players in development and stress responses. Annu. Rev. Cell Dev. Biol. 35, 407–431.

Yu, Y., Fu, W., Xu, J., Lei, Y., Song, X., Liang, Z., Zhu, T., Liang, Y., Hao, Y., Yuan, L., et al. (2021). Bromodomain-containing proteins BRD1, BRD2, and BRD13 are core subunits of SWI/SNF complexes and vital for their genomic targeting in Arabidopsis. Mol. Plant 14, 888–904.

Yuan, M., Ke, Y., Huang, R., Ma, L., Yang, Z., Chu, Z., Xiao, J., Li, X., and Wang, S. (2016). A host basal transcription factor is a key component for infection of rice by TALE-carrying bacteria. Elife 5, 1–17.

Yuan, M., Ngou, B.P.M., Ding, P., and Xin, X.F. (2021a). PTI-ETI crosstalk: an integrative view of plant immunity. Curr. Opin. Plant Biol. 62, 1–11.

Yuan, M., Jiang, Z., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.M., He, S.Y., and Xin, X.F. (2021b). Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature 592, 105–109.

Yuliar, Asi Nion, Y., and Toyota, K. (2015). Recent trends in control methods for bacterial wilt diseases caused by Ralstonia solanacearum. Microbes Environ. 30, 1–11.

Zaware, N., and Zhou, M.-M. (2019). Bromodomain biology and drug discovery. Nat. Struct. Mol. Biol. 26,870–879.

Zhang, C.J., Hou, X.M., Tan, L.M., Shao, C.R., Huang, H.W., Li, Y.Q., Li, L., Cai, T., Chen, S., and He, X.J. (2016a). The Arabidopsis acetylated histone-binding protein BRAT1 forms a complex with BRP1 and prevents transcriptional silencing. Nat. Commun. 7, 1–13.

Zhang, D., Li, Y., Zhang, X., Zha, P., and Lin, R. (2017a). The SWI2/SNF2 Chromatin-Remodeling ATPase BRAHMA Regulates Chlorophyll Biosynthesis in Arabidopsis. Mol. Plant 10, 155–167.

Zhang, H., Lang, Z., and Zhu, J.K. (2018). Dynamics and function of DNA methylation in plants. Nat. Rev. Mol. Cell Biol. 19, 489–506.

Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., Zou, Y., Long, C., Lan, L., Chai, J., et al. (2007a). A Pseudomonas syringae Effector Inactivates MAPKs to Suppress PAMP-Induced Immunity in Plants. Cell Host Microbe 1, 175–185.

Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., et al. (2010). Receptor-like cytoplasmic kinases integrate signaling from multiple plant immune receptors and are targeted

by a Pseudomonas syringae effector. Cell Host Microbe 7, 290–301.

Zhang, K., Sridhar, V. V., Zhu, J., Kapoor, A., and Zhu, J.-K. (2007b). Distinctive Core Histone Post-Translational Modification Patterns in Arabidopsis thaliana. PLoS One 2, 1–11.

Zhang, T., Lei, J., Yang, H., Xu, K., Wang, R., and Zhang, Z. (2011). An improved method for whole protein extraction from yeast Saccharomyces cerevisiae. Yeast 28, 795–798.

Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y. V., Pellegrini, M., Goodrich, J., and Jacobsen, S.E. (2007c). Whole-genome analysis of histone H3 lysine 27 trimethylation in Arabidopsis. PLoS Biol. 5, 1026–1035.

Zhang, X., Bernatavichute, Y. V., Cokus, S., Pellegrini, M., and Jacobsen, S.E. (2009). Genome-wide analysis of mono-, di- and trimethylation of histone H3 lysine 4 in Arabidopsis thaliana. Genome Biol. 10, 1–14.

Zhang, X., Ménard, R., Li, Y., Coruzzi, G.M., Heitz, T., Shen, W.H., and Berr, A. (2020). Arabidopsis SDG8 Potentiates the Sustainable Transcriptional Induction of the Pathogenesis-Related Genes PR1 and PR2 During Plant Defense Response. Front. Plant Sci. 11, 1–14.

Zhang, Y., Zhao, J., Li, Y., Yuan, Z., He, H., Yang, H., Qu, H., Ma, C., and Qu, S. (2016b). Transcriptome analysis highlights defense and signaling pathways mediated by rice pi21 gene with partial resistance to Magnaporthe oryzae. Front. Plant Sci. 7, 1–17.

Zhang, Z.M., Ma, K.W., Gao, L., Hu, Z., Schwizer, S., Ma, W., and Song, J. (2017b). Mechanism of host substrate acetylation by a YopJ family effector. Nat. Plants 3.

Zhao, C., Wang, H., Lu, Y., Hu, J., Qu, L., Li, Z., Wang, D., He, Y., Valls, M., Coll, N.S., et al. (2019). Deep sequencing reveals early reprogramming of arabidopsis root transcriptomes upon ralstonia solanacearum infection. Mol. Plant-Microbe Interact. 31, 813–827.

Zhao, S., Zhang, B., Yang, M., Zhu, J., and Li, H. (2018). Systematic Profiling of Histone Readers in Arabidopsis thaliana. Cell Rep. 22, 1099–1102.

Zheng, X., Kang, S., Jing, Y., Ren, Z., Li, L., Zhou, J.M., Berkowitz, G., Shi, J., Fu, A., Lan, W., et al. (2018). Danger-associated peptides close stomata by OST1-independent activation of anion channels in guard cells. Plant Cell 30, 1132–1146.

Zheng, X., Li, X., Wang, B., Cheng, D., Li, Y., Li, W., Huang, M., Tan, X., Zhao, G., Song, B., et al. (2019). A systematic screen of conserved Ralstonia solanacearum effectors reveals the role of RipAB, a nuclear-localized effector that suppresses immune responses in potato. Mol. Plant Pathol. 20, 547–561.

Zhou, C., Zhang, L., Duan, J., Miki, B., and Wu, K. (2005). Histone Deacetylase19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell 17, 1196–1204.

Zhu, H., Wang, G., and Qian, J. (2016a). Transcription factors as readers and effectors of DNA methylation. Nat. Rev. Genet. 17, 551–565.

Zhu, Q.-H., Shan, W.-X., Ayliffe, M.A., and Wang, M.-B. (2016b). Epigenetic Mechanisms: An Emerging Player in Plant-Microbe Interactions. Mol. Plant-Microbe Interact. 29, 187–196.

Zhu, Q.H., Stephen, S., Taylor, J., Helliwell, C.A., and Wang, M.B. (2014). Long noncoding RNAs responsive to Fusarium oxysporum infection in Arabidopsis thaliana. New Phytol. 201, 574–584.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E.J., Jones, J.D.G., Felix, G., and Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. Nature 428, 764–767.

Zou, B., Sun, Q., Zhang, W., Ding, Y., Yang, D.L., Shi, Z., and Hua, J. (2017). The arabidopsis chromatin-remodeling factor CHR5 regulates plant immune responses and nucleosome occupancy. Plant Cell Physiol. 58, 2202–2216.