



# Effects of the *Xanthomonas campestris* pv *campestris* type III effectors on *Arabidopsis* physiology

Noe Arroyo Velez

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# THÈSE

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

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**Présentée et soutenue par  
Noe ARROYO VELEZ**

Le 1 avril 2022

**Effets des effecteurs de type III de *Xanthomonas campestris* pv  
*campestris* dans la physiologie d'*Arabidopsis*.**

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## Abstract

*Xanthomonas campestris* pv. *campestris* (*Xcc*) causes black rot disease on Brassicaceae species including cabbages, radish, mustard, and the model species *Arabidopsis thaliana*. During pathogenesis, *Xcc* secretes Type 3 Effector (T3E) proteins via the Type 3 Secretion System (T3SS) into plant cells to modulate host physiology and promote pathogenicity. The repertoire of T3Es present in a given strain largely influences its niche, host range and lifestyle. In the *Xcc* strain 8004, twenty-eight genes have been predicted to encode proteins secreted by the T3SS. The functions of most Type 3 Secreted Proteins (T3SPs) within plant cells remain elusive. In this project, different strategies were approached to characterize the biological functions of the T3SPs of *Xcc* strain 8004 in plant cells.

In the first chapter, we showed that the loss of individual T3SPs did not cause a significant effect on *Xcc* virulence on *Arabidopsis*. Yet, the heterologous expression of individual T3SPs in *Arabidopsis* plants revealed many T3SPs with marked effects on plant growth and transcriptome. Several T3SPs also triggered plant immune responses and some exhibited ambivalent activities by simultaneously inhibiting flg22-triggered phosphorylation of MPK3/6.

In the second chapter, we conducted a comparative analysis of the *in planta* functions of the T3E XopAG and RipO1 which are encoded by orthologous genes in *Xcc* strain 8004 and *Ralstonia pseudosolanacearum* strain GMI1000 respectively. In our experiments, XopAG showed a significant contribution to *Xcc* pathogenicity that was not related to the suppression of some basal immune responses. XopAG and RipO1 exhibited functional similarities. Indeed, both T3E affected the expression of genes responsive to auxin, jasmonic acid, and ethylene suggesting that both effectors inhibit plant growth. Finally, we made some efforts to identify the plant target of XopAG. An *in silico* search followed by pathogenicity assays posits BRG3 (BOI-RELATED GENE 3) as a candidate target of XopAG. In a parallel approach, we performed a suppressor screen to identify suppressor mutations that alleviate the growth defect induced by XopAG in *Arabidopsis* plants, resulting in eight suppressor lines. These provide a valuable opportunity to identify the pathways targetted by XopAG in *Arabidopsis*.

Altogether, this project contributes to the better comprehension of the biological activities exerted by the *Xcc* strain 8004 T3SPs *in planta*.

## Résumé

*Xanthomonas campestris* pv. *campestris* (*Xcc*) est responsable de la nervation noire sur les Brassicaceae, notamment les choux, les radis, la moutarde et l'espèce modèle *Arabidopsis thaliana*. Au cours de l'infection, *Xcc* transloque des protéines effectrices de type 3 (T3E) dans les cellules végétales via le système de sécrétion de type 3 (T3SS), pour moduler la physiologie de l'hôte et favoriser la maladie. Le répertoire des T3E présents dans une souche donnée influence largement sa niche, sa gamme d'hôtes et son mode de vie. Dans la souche *Xcc* 8004, vingt-huit gènes ont été prédits pour coder des protéines sécrétées par le T3SS (T3SP).

Dans un premier chapitre à l'échelle de l'effectome, nous montrons que la plupart des fonctions des T3SP au sein des cellules végétales restent inconnues. Dans ce projet, différentes stratégies ont été abordées pour caractériser les fonctions biologiques des T3SPs de la souche *Xcc* 8004 dans les cellules végétales. Bien que la délétion des gènes individuels codant des T3SP n'ait pas eu d'effet significatif sur la virulence de *Xcc* chez *Arabidopsis*, l'expression hétérologue de T3SP individuelles chez *Arabidopsis* a montré des effets marqués sur la physiologie de la plante pour de nombreuses T3SP. De manière surprenante, plusieurs T3SP sont capables de déclencher des réponses immunitaires et certaines ont présenté des effets ambivalentes en inhibant simultanément la phosphorylation déclenchée par flg22 de MPK3/6.

Dans une deuxième partie, nous avons réalisé une analyse comparative des fonctions *in planta* des T3SPs XopAG et RipO1 qui sont codées par des gènes orthologues dans *Xcc* souche 8004 et *Ralstonia pseudosolanacearum* souche GMI1000, respectivement. Dans nos expériences, XopAG a montré une contribution significative à la pathogénicité de *Xcc* qui ne semble pas liée à la suppression de certaines réponses immunitaires basales. XopAG et RipO1 présentent des similitudes fonctionnelles. En effet, les deux T3SP affectent l'expression de gènes connus de réponse à l'auxine, à l'acide jasmonique et à l'éthylène, ce qui est cohérent avec l'inhibition de la croissance des plantes induite par ces deux effecteurs chez *Arabidopsis*. Une recherche *in silico* de cibles végétales potentielles de XopAG suivie d'essais du pouvoir pathogène sur des mutants d'*Arabidopsis* a permis d'identifier BRG3 (BOI-RELATED GENE 3) comme cible putative de XopAG. Dans une approche parallèle, nous avons effectué un criblage de supresseurs pour identifier les mutations qui atténuent le phénotype d'arrêt de croissance induit par XopAG chez *Arabidopsis*, résultant en l'identification de huit lignées supresseurs. Ces lignées fournissent une occasion précieuse d'identifier les voies affectées par XopAG chez *Arabidopsis*.

De manière générale, ce projet contribue à la compréhension des activités biologiques exercées par les T3SP de la souche *Xcc* 8004 dans les cellules végétales.

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## List of abbreviations

<b>ABA:</b>	abscisic acid	<b>NASC:</b>	Nottingham Arabidopsis Stock Centre
<b>AUX:</b>	auxin	<b>NLR:</b>	NOD-like receptor
<b>Avr:</b>	Avirulence	<b>OD:</b>	Optical density
<b>BAK1:</b>	BRI1 Associated Receptor Kinase 1	<b>P. syringae:</b>	<i>Pseudomonas syringae</i>
<b>CDPK:</b>	Calcium Dependent Protein Kinase	<b>PAMP:</b>	Pathogen-associated molecular pattern
<b>CERK1:</b>	Chitin Elicitor Receptor Kinase 1	<b>PR:</b>	Pathogenesis related
<b>CFU:</b>	Colony-forming unit	<b>PRR:</b>	Pattern recognition receptor
<b>Col-0:</b>	Columbia 0	<b>PTI:</b>	PAMP-triggered immunity
<b>CWDE:</b>	Cell wall-degrading enzymes	<b>pv:</b>	Pathovar
<b>Cyp:</b>	Cyclophilin	<b>R:</b>	Resistance
<b>DAMP:</b>	Danger-associated molecular pattern	<b>RBOHD:</b>	Respiratory burst oxidase homolog D
<b>DMSO:</b>	Dimethyl sulfoxide	<b>Rip:</b>	Ralstonia injected protein
<b>dpi:</b>	Days post inoculation	<b>RLK:</b>	Receptor-like protein.
<b>EF-Tu:</b>	Elongation factor Tu	<b>RLP:</b>	Receptor-like kinase
<b>EMS:</b>	Ethyl methanesulfonate	<b>ROS:</b>	Reactive oxygen species
<b>EPS:</b>	Exopolysaccharides	<b>SA:</b>	Salicylic acid
<b>ET:</b>	Ethylene	<b>SAR:</b>	Systemic acquired resistance
<b>ETI:</b>	Effector-triggered immunity	<b>Sf-2:</b>	San Feliu 2
<b>ETS:</b>	Effector-triggered susceptibility	<b>sxg:</b>	Suppressor of <i>xopAG</i> -mediated phenotype
<b>flf22</b>	flagellin 22	<b>T3E:</b>	Type III effector
<b>FLS2:</b>	Flagellin Sensitive 2	<b>T3SS:</b>	Type III secretion system
<b>HR:</b>	Hypersensitive response	<b>Vir:</b>	Virulence
<b>hrc:</b>	hrp-conserved	<b>Xcc:</b>	<i>Xanthomonas campestris</i> pv. <i>campestris</i>
<b>hrp:</b>	Hypersensitive reaction and pathogenicity	<b>Xop:</b>	<i>Xanthomonas</i> outer protein
<b>ISR:</b>	Induced systemic resistance	<b>Y2H:</b>	Yeast two-hybrid
<b>JA:</b>	Jasmonic acid		
<b>LPS:</b>	Lipopolysaccharide		
<b>LYS1:</b>	Lysozyme 1		
<b>LysM:</b>	Lysine Motifs		
<b>MAPK:</b>	Mitogen-activated protein kinase		
<b>MAPKK:</b>	MAPK kinase		
<b>MEKKs:</b>	MAPK kinase kinase		

# Chapter 1: Introduction



## **1.1 Plant and microbes are essential for terrestrial ecosystems**

Plants are key components of terrestrial ecosystems; they influence all aspects of the land environments including weather, humidity, food chain, landscape, etc. During photosynthesis, plants produce large amounts of organic molecules and a big proportion of the total atmospheric oxygen from sunlight and inorganic matter, introducing chemical energy to the ecosystems. A vast proportion of living beings, ranging from microorganisms to large mammals such as whales or elephants, directly or indirectly use the organic compounds generated by plant cells as a primary carbon source. Terrestrial plants not only supply food to other species, they also regulate and maintain the physicochemical characteristics of the terrestrial ecosystems making them more stable and suitable for the survival of other species. The abiotic factors influenced by land plants include soil humidity, minerals bioavailability, temperature, soil acidity and many others. Additionally, land plants provide a physical surface to live and shelter to thousands of species, providing them protection against potentially dangerous factors such as solar radiation, drought, wind, rain, etc (Hull, 2008; E.-D. Schulze et al., 2005).

In natural conditions, plants maintain ecological interactions with most of the species that inhabit terrestrial ecosystems including the ubiquitous microorganisms that are widely distributed throughout the entire earth surface. The microorganisms are defined as “living things that on their own are too small to be seen without a microscope” (Cambridge University Press, 2009) and they include among other taxonomic groups: protists, oomycets, bacteria and some fungal species. The microorganisms are other relevant regulators of natural ecosystems with similar or greater influence than plants. The microorganisms have broad-spectrum metabolic capabilities that range from heterotrophs to autotrophs. Microbial species can be found in both aerobic and anaerobic environments and survive in extremely harsh conditions in terms of temperature, salinity, acidity, osmolarity, humidity, etc. Their physiological plasticity and adaptability allow microbes to colonize virtually the entire planet, from inside the lithosphere to the troposphere including the most extreme regions where most species cannot survive such as volcanoes or glaciers (Pommerville, 2017). Considering all these, it is not surprising that land plants and microorganisms can establish such close relationships. Furthermore, those interactions are of great relevance for the maintenance of the biological cycles and consequently for the survival of all the living beings on the planet, including us humans.

## **1.2 Plant-microbes interaction: an ancient story**

Fossil evidence suggests that plant-microbe associations took place since the very first appearance of terrestrial plants. It is very likely that land plants' evolution from aquatic green algae was facilitated by intimate associations with fungal cells more than 700 million years ago (Heckman et al., 2001).

The term symbiosis originates from the Greek and means "living together". This word designates the interdependence of two or more organisms of different species. All plants in natural conditions establish symbiotic interactions with many microbial species. Symbiotic microorganisms may colonize plant apoplastic spaces, plant surface, or areas adjacent to the plant surface like the rhizosoil (Schirawski & Perlin, 2018). All microorganisms sharing a given environment are referred to as microbiota (Trivedi et al., 2020). Plant microbiota are complex communities composed by different microbial species with a diverse range of lifestyles and metabolic capabilities. Several plant-derived compounds attract and feed the associated microbes. The associated microbes may in turn secrete compounds that alter not only plant physiology but also other microorganisms and the surrounding environment (Schirawski & Perlin, 2018). Briefly, the plants and its associated microbiota form an intricate system where each of the participants acquire and exchange metabolites, in order to shape the ecological interaction and the surrounding environment.

## **1.3 Good or bad neighbors?**

Microbes benefit from symbiotic interactions by exploiting plant-derived resources in order to survive, multiply and spread. However, from the plant side, symbiotic interactions are not always beneficial. Whereas some microbes promote plant growth, facilitate nutrient acquisition and tolerance to stress, other microbes are detrimental and cause diseases to their host. Microbial symbionts are often classified as mutualists, commensals or pathogens based on the balance between the costs and benefits for the host plant involved in the symbiotic interaction (Trivedi et al., 2020). Mutualistic microbes demand resources from their host. However, this cost is compensated by the benefits provided to the plant during the interaction. The cooperative interactions allow plants and their partner microbes to exploit novel resources and expand their niche in order to gain fitness (Schirawski & Perlin, 2018). The microorganisms that find their niche in the association with plants but have no detrimental, neither positive effects on their hosts are referred to as commensals. The third class of microbial symbionts



include all the organisms that take resources from the host while causing detrimental effects which leads to reduced host fitness. This class of microbial parasites known as pathogens or phytopathogens may destroy plant tissues, decrease fertility and/or kill the host plant. Phytopathogens possess diverse strategies to acquire nutrients from their hosts. Necrotrophic pathogens employ degrading enzymes, necrosis-inducing proteins and toxins to produce plant cell death and then consume its contents. On the other hand, biotrophic pathogens evolved mechanisms to exploit resources while keeping the host alive, feeding on living tissues. Some pathogens known as hemibiotrophs, exhibit biotrophic strategies during early infection stages then cause cell death and feed from dead tissue (Toruño et al., 2016).

During infection process most of the phytopathogens enter plant tissues by either wounds or natural openings and invade the apoplast or the xylem to manipulate the host cell physiology for nutrient release from the surrounding cells (Fatima & Senthil-Kumar, 2015). All microbial symbionts profit from the host plant resources. Ultimately, what defines the kind of symbiotic relationship is the balance between benefits and costs from the plant perspective. However, microbes can behave differently according to the host species and the environmental conditions; even if they show no detrimental effects on some plant species in specific conditions, they may exhibit pathogenicity on other host species or in different environmental circumstances. This behavioral diversity challenges strict categorizations. Yet, for practical reasons hereafter I will refer to phytopathogens as microbial species causing harm to a specific host in specific environmental conditions. In the following pages, I will describe some aspects of the plants and microbes that have been related with the establishment of pathogenic interactions, with special attention in molecular and physiological mechanisms.

#### **1.4 First encounters occur at plant surface**

The total surface of a plant, colonized by microorganisms is referred to as the phyllosphere if we consider the aerial part of the plant or rhizosphere if we focus on the belowground portion. The microbes present in the plant surface can be vertically transmitted to the seeds or horizontally acquired from the soil, the air, the rain or transported by other organisms such as insects (Müller et al., 2016).

The initial steps that lead to pathogenic interactions take place on the plant surface. First microbes must find a way to contact the plant host; they pass through an epiphytic phase and move to reach specific entry points to the internal plant tissues (Brader et al., 2017). The

epiphytic survival of microbial communities largely depends on nutrient and water availability on leaf surfaces, where they are exposed to harsh environmental conditions like solar radiation or drastic and frequent changes in temperature, humidity, osmolarity, etc. The microbiome composition in the phyllosphere is extremely diverse and includes bacteria, yeasts, filamentous fungi, protozoans and nematodes, bacteria being considered as the most abundant inhabitants of the leaves. The activities of the plant microbiota largely influence plant health and growth since the prevalence of mutualistic and commensal species could restrict pathogenic species proliferations (Sivakumar et al., 2020; Müller et al., 2016).

In order to protect their internal tissues from microbial intruders and abiotic factors, land plants developed an extracellular hydrophobic layer that covers the aerial epidermis, named cuticle. The plant cuticle main components are the polymer cutin and cuticular waxes that limit the permeability of the leaf surface, protecting plant tissues of desiccation and restricting diffusion of nutrients from plants' interior to the phyllosphere (Yeats & Rose, 2013). Low humidity conditions and scarce nutrients in leaf surfaces hinder microbes' survival. However, some species have developed strategies to cope with such challenges like the epiphytic bacterium *Pseudomonas syringae* (*P. syringae*) strain B728a that produces the bio surfactant syringafactin which increases liquid water availability and reduces the water stress experienced on leaf surface (Hernandez & Lindow, 2019). In addition to the cuticle, the plant cell wall, which mainly consists of polysaccharides such as cellulose, hemicelluloses, pectin and glycosylated proteins, also protects plants against external factors. In their search for nutrients, successful pathogens overcome the plant physical barriers and gain access to the plant interior. Many fungal pathogens can directly penetrate the plant epidermis by mechanical rupture or secrete an array of enzymes, capable of depolymerize the cuticle (cutinases) or the cell wall (Cell Wall-Degrading Enzymes [CWDEs]) (Purdy & Kolattukudy, 1975; Kubicek et al., 2014). Pathogenic fungi can utilize the molecules resulting from plant tissue degradation as a direct carbon source. In response, plant cells produce proteins that inhibit microbial enzymes such as inhibitors of pectin methylesterases, pectin lyases, endoxylanases and xyloglucan endoglucanases (Juge, 2006).

Many bacterial species use a different strategy to reach the inner plant tissues: they enter through natural opertures in plant epidermis such as hydathodes, lenticels or stomata, or they enter directly through wounds caused by biotic (insects, animals or other plants) or abiotic (wind, rain, etc.) forces (Brader et al., 2017).

Pathogens proliferation is highly detrimental for plants fitness; they not only capture nutrients but also perturb physiological processes, hamper growth and inflict tissue damage to their host. In consequence, plants evolved a battery of immune strategies to hinder pathogens progression, for example the secretion of chitinases and glucanases that degrade the cell wall of microbial invaders (Vorwerk et al., 2004). Plants also produce antimicrobial metabolites to protect their tissues from intruders: phytoanticipins are constitutively produced and include saponins, cyanogenic glucosides, glucosinolates, fatty acid derivatives, and terpenoids whereas phytoalexins are synthesized only in response to microbial presence (VanEtten et al., 1994).

## **1.5 Plants perceive guests and mount immune responses**

Plant cells have the intrinsic capacity to detect microbial presence and discern beneficial species from pathogens; these are key steps to mount proper immune responses that restrain harmful species while promoting proliferation of mutualistic microbes (Lapin & Van den Ackerveken, 2013). Microbes are recognized by immune receptors located in the plasma membrane and the cytosolic space that monitor pathogen-associated molecules in the extracellular environment or the intracellular space.

### **1.5.1 MAMPs and DAMPs betray microbes to plant receptors**

The plant immune surveillance system perceives molecules belonging to essential structures for pathogens; these molecules are usually well conserved in entire classes of microbes such as chitin for fungi or peptidoglycan for bacteria. The conserved microbe-specific molecules are known as Pathogen Associated Molecular Patterns (PAMPs). Beneficial microbes also present molecules that can be recognized by the plant immune receptors so in a broader sense the microbial immunogenic molecules are known as Microbe-Associated Molecular Patterns (MAMPs) (Newman et al., 2013).

In order to facilitate the release of soluble molecules that serve as MAMPs, plants produce lytic enzymes that degrade bacterial structures. A good example of this is the Lysozyme 1 (LYS1), produced by *Arabidopsis thaliana* (hereafter referred to as *Arabidopsis*) to degrade the bacterial cell wall, releasing peptidoglycan fragments that are recognized by plant cells to further activate immune responses (Liu et al., 2014). During host colonization, many microbes produce degradative enzymes that result in breakdown of plant tissues releasing fragments of plant components to the apoplastic space such as pectin fragments, extracellular ATP, cellobiose fragments, etc. Such self-derived molecules can also be perceived by the plant

immune receptors and trigger immune responses. These immunogenic molecules are known as Danger-Associated Molecular Patterns (DAMPs) (Zhou & Zhang, 2020) and since they are passively released upon danger, they are considered as primary endogenous danger signals (Gust et al., 2017).

Other sets of immunogenic host-derived molecules have been described in plants: the phytocytokines. These peptides are produced, processed and/or secreted upon wounding during microbial colonization to activate plant immune responses. Consequently they are considered by some authors as secondary danger signals (Gust et al., 2017). Systemin is one of the most studied endogen signaling peptides. This proteinase inhibitor is produced by tomato plants (*Solanum lycopersicum*) in response to wounding (Pearce et al., 1991). Phytocytokines production can be stimulated by plant hormones, PAMPs, DAMPs or phytocytokines themselves establishing a positive feed-back system as was discovered for the Arabidopsis plant elicitor peptide 1 (AtPeps) that induces alkalization in plant cell cultures. AtPep1 is encoded by PROPEP1 gene and is induced by wounding, methyl jasmonate, ethylene, flg22, and AtPep1 itself (Huffaker et al., 2006). Damage in localized portions of plant tissues result in the release of immunogenic signals including DAMPs and phytocytokines, which serve to activate or enhance the immune responses in the surrounding cells. Then molecules diffuse to more distant tissues to activate and promote immune responses in long-distant organs, establishing a systemic immune response (T. Sun & Zhang, 2021).

### **1.5.2 Pathogens perception is mediated by surface receptors (PRRs)**

Plant cells present no adaptive immune responses and rely only on innate immunity to cope with pathogens. As part of this innate immunity, plants possess a diverse set of cell-surface receptors to sense the surrounding biotic environment, which allows the recognition of immunogenic molecular patterns associated with infection, commonly referred to as pattern recognition receptors (PRRs). The interaction of the PRRs with their cognate ligands leads to the activation of many immune-related proteins inside the plant cells to ultimately mount a battery of immune responses known as pattern-triggered immunity (PTI) (Gust et al., 2017). As PRRs ectodomains directly bind ligands, they determine the nature of the molecule recognized. Leucine-rich-repeat (LRR) containing PRRs bind proteins or peptides, such as bacterial flagellin (Gómez-Gómez & Boller, 2000). PRRs containing Lysine Motifs (LysM) bind carbohydrate-based molecules, such as fungal chitin or bacterial peptidoglycan. Lectin-type PRRs recognize extracellular ATP or bacterial lipopolysaccharides (LPS). PRRs with epidermal growth factor

(EGF)-like ectodomains recognize plant cell-wall derived oligogalacturonides (Couto & Zipfel, 2016) (Figure 1).

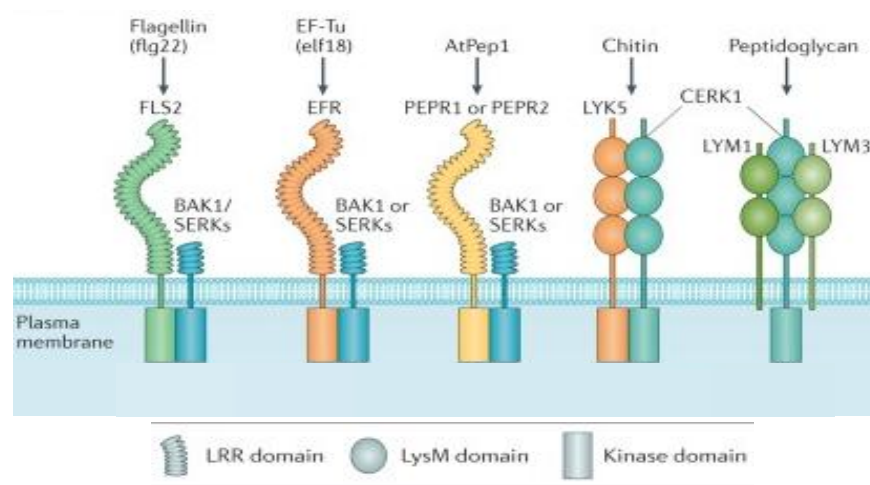
Plant PRRs are structurally classified in receptor-like kinases (RLKs) and receptor-like proteins (RLPs). RLKs present an ectodomain involved in ligand binding, a single transmembrane domain and an intracellular kinase domain. RLPs have a similar ectodomain and a single transmembrane domain but they lack an intracellular kinase domain. This is why RLPs are thought to depend on regulatory receptor kinases to transduce ligand perception into intracellular signaling (Macho & Zipfel, 2014).

Upon ligand binding, PRRs experience conformational rearrangements that trigger downstream signaling pathways. Plant genomes encode a large repertoire of PRRs. However, their cognate ligands and the mechanisms by which PRRs induce signaling cascades remain elusive for many of them. Fortunately, some PRRs have been largely characterized and their signaling mechanisms are to some extent clear. These paradigmatic discoveries revealed some components of the so-called PTI response that is activated after MAMP perception. The most studied PRR in plants is the Arabidopsis LRR-RLK Flagellin Sensitive 2 (FLS2). FLS2 is involved in the recognition of the flagellin epitope flg22, a main component of bacterial flagellum (Gómez-Gómez & Boller, 2000). FLS2 orthologs have been identified in Arabidopsis, tomato, *Nicotiana benthamiana* and rice and play a fundamental role in the induction of the PTI responses (Chinchilla et al., 2006). A few years later, the receptor EFR was discovered in a reverse-genetic approach to identify the receptor kinase responsible for perceiving the bacterial Elongation factor Tu (EF-Tu) in Arabidopsis (Zipfel et al., 2006). This was preceded by the observation that the N-acetylated peptide comprising the first 18 amino acids of EF-Tu termed elf18 induces an oxidative burst and biosynthesis of ethylene (Kunze et al., 2004). The EF-Tu protein is highly conserved in several bacterial species and elicits a set of defense responses highly similar to that induced by flagellin (Zipfel et al., 2006) suggesting the involvement of common mechanisms during activation of both receptors.

Perception of elicitors (regardless of their origin) by the host, leads to rapid activation of defense mechanisms. PRRs activation triggers different signaling mechanisms to communicate to the rest of the cell not only the presence of danger but also the nature (abiotic factors, biotrophs, and necrotrophs), intensity and localization of the harmful agents.

### 1.5.3 PRRs are included in signaling complexes to activate immune responses

The plant cell membrane is a highly dynamic structure and includes diverse kinds of proteins that interact with each other. A recent study on hundreds of *Arabidopsis* LRR-RLKs uncovered a complex cell-surface interaction network in which diverse LRR-RLKs are connected through other short LRR-RLKs (Smakowska-Luzan et al., 2018). Upon ligand stimulation, PRRs recruit specific co-receptors or adaptor kinases to form stable signaling complexes. PRRs activation and signaling is entirely dependent on the dynamic protein associations/dissociations in the PRR complexes (Zipfel & Oldroyd, 2017). For instance in *Arabidopsis* cells the activation of the LRR-RLK FLS2 after flg22 perception requires the association of BRI1-Associated Receptor Kinase 1 (BAK1) that acts as a co-receptor by recognizing the C terminus of FLS2. The heteromeric complex formation triggered by flg22 induces phosphorylation of both FLS2 and BAK1 within 15s of stimulation; these are key steps to start signal transduction (B. Schulze et al., 2010). BAK1 belongs to the Somatic Embryogenesis Receptor Kinase (SERK) protein family that includes five related SERK proteins. Upon ligand stimulation SERK proteins are recruited to PRRs of the LRR-RLK type (Couto & Zipfel, 2016). In plant membranes, several PRRs other than FLS2 form tight complexes with BAK1 after ligand binding to assemble functional PRR complexes. Subsequent phosphorylation of both BAK1 and the corresponding PRR is required to initiate signaling cascades and activate immune responses (Heese et al., 2007) (Figure 1).



**Figure 1. Pattern recognition receptors (PRRs) recruit different regulatory receptor kinases according to their ectodomain.** In *Arabidopsis thaliana*, BRI1-ASSOCIATED RECEPTOR KINASE 1(BAK1) related SERKs and CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1) are recruited upon ligand perception by leucine-rich repeat (LRR)-receptor kinases and LysM-receptor kinases or receptor-like proteins (RLPs), respectively. Taken and adapted from Couto & Zipfel, 2016.

PRRs of the type RLPs that lack a signaling kinase domain depend on other proteins containing cytoplasmic kinase domains to transduce signaling and activate downstream pathways. The Suppressor Of BIR1-1 (SOBIR1) is a membrane LRR-RLK that is required for the function of diverse PRR complexes containing LRR-RLPs. Upon ligand perception, SOBIR1 recruits BAK1 to RLP-SOBIR1 complexes to trigger immune responses. Recent findings in tomato plants suggest that a receptor complex formed by the LRR-RLP Cf-4, SOBIR1 and BAK1 mediates the recognition of the Avr4 molecule from the fungus *Cladosporium* in tomato. Upon Avr4 recognition, SOBIR1 and BAK1 perform trans- and auto-phosphorylation activities to achieve further downstream signaling.

The relevant role of BAK1 as a general regulator in several membrane-associated PRR complexes together with the essential regulatory functions of the co-receptors CERK1 and SOBIR1 suggest that PRRs activation and signaling, whether RLK or RLP depend on dynamic interactions with regulatory proteins of the type LRR-RKs and most likely also with other types of proteins or molecules. The specificity of the recognition of particular PAMPs or DAMPs is dictated by multiprotein receptor-complexes instead of single receptors, this fact expands the variety of epitopes and conditions recognized by the plant cells given the large number of possible combinations between PRRs and regulatory co-receptors.

The mere perception of pathogens by plant cells is not sufficient to mount effective immune responses, it is also necessary to identify the nature of the threat to develop responses with appropriate intensity. If plants develop a low intensity response in front of a great danger then the intruder will succeed and colonize the host. On the other hand, an exacerbated response to face a low threatening invader would cause a waste of valuable resources and even affect plant-self tissues (autoimmunity). In both scenarios plants fitness is affected and in extreme cases, inadequate responses could lead to plant death. The membrane-associated regulatory co-receptors provide the plants with an effective mechanism to fine-tune the plant immune response through the regulation of the signals emitted from PRR complexes to the rest of the cell. Many LRR-RLKs are known to regulate immune activation as is suggested by the fact that Arabidopsis plants mutated in both genes BAK1 and BKK1 exhibit a seedling-lethality phenotype due to constitutive defense-gene expression, callose deposition and reactive oxygen species (ROS) accumulation, all characteristic PTI responses (K. He et al., 2007).

#### 1.5.4 The RLCKs are important relays in immune signal transduction downstream of PRRs

It is well known that PRR complexes activation upon ligand binding leads to major downstream immune responses that affect intruders' survival and restrains pathogens colonization, such as calcium influx, a burst of ROS, production of immunogenic peptides and defense hormones, etc. However, many of the intermediate relays connecting danger perception with immune responses and their regulatory mechanisms remain elusive. In this section, I will summarize some of the most relevant aspects known so far.

After PRR complex activation upon ligand binding, another kind of proteins with kinase activity appear on stage: the Receptor-Like Cytoplasmic Kinases (RLCKs) that function as a link between extracellular ligand perception and downstream signaling. Many RLCKs suffer from N-myristoylation or palmitoylation that anchor them to the plasma membrane where they are direct substrates of the activated PRR complexes (Liang & Zhou, 2018). One of the first discovered RLCKs is the *Botrytis*-Induced Kinase 1 (BIK1) on Arabidopsis; this protein was initially proven to be required for disease resistance to the fungal pathogen *Botrytis cinerea* (Lin et al., 2014). BIK1 together with BAK1 and FLS2 form a protein kinase complex essential to initiate and transduce immune signaling in response to flg22. In this process BIK1 is auto-phosphorylated and trans-phosphorylated by BAK1 (Lin et al., 2014). A subsequent study with RLCKs of the subfamily group VII including BIK1, AvrPpHB Susceptible 1 (PBS1) and PBS1-Like (PBL) proteins -1 and -2 revealed that these RLCKs are associated with FLS2 in the resting state. The activation of FLS2 by flg22 perception induces the phosphorylation and release of the RLCKs of the PRR complex which is related with immune responses activation (Zhang et al., 2010).

The characterization of the BIK1 functions was fundamental to understand the role of the RLCKs as a link between activated PRR complexes and downstream responses since activation of BIK1 through phosphorylation leads to diverse plant responses. One of the mechanisms employed by RLCKs to transduce signals is the direct phosphorylation of other plant proteins. For instance the membrane-bound NADPH oxidase Respiratory Burst Oxidase Homolog D (RBOHD) is directly phosphorylated by BIK1 in its activated state to activate ROS burst (L. Li et al., 2014). Other mechanisms employed by the RLCKs to induce plant responses were revealed when Lal and collaborators showed that BIK1 after being phosphorylated by the PRR EFR, localizes to the nucleus where it interacts with WRKY transcription factors to regulate defense hormones signaling during plant immunity (Lal et al., 2018).



The RLCKs present variable affinities for different PRR complexes, and activate distinct branches of PTI signaling depending on the nature of the stimuli and the regulatory conditions. This is exemplified by the diverse array of PRRs that rely on BIK1 or/and PBL1 for immune signaling. For this reason the RLCKs represent another efficient mechanism for plant cells to regulate immunity. The large repertoire of RLCKs together with the diversity of regulatory LRR-RLKs confers robustness and flexibility to the plant immune system.

### **1.5.5 Plants deploy a battery of responses to restrict pathogen colonization**

PAMPs/DAMPs recognition by their cognate PRRs complexes triggers a battery of immune responses to cope with the intruders including ion fluxes at the plasma membrane, a rapid production of ROS, phosphorylation of Mitogen Activated Protein Kinases (MAPKs) and Calcium-Dependent Protein Kinases (CDPKs), transcriptional reprogramming and regulation of hormones production. Upon danger perception, multiple responses are mounted simultaneously, often with redundant mechanisms ensuring robust defenses. This is proven by the fact that the activation of FLS2 by flg22 leads to the activation of both BIK1 and PBL1. BIK1 and PBL1 are redundantly involved in the transient bursts of cytosolic calcium, apoplastic ROS, and disease resistance to multiple pathogens (J. Qi et al., 2017).

#### **1.5.5.1 Calcium influx is an important component of the immune-signaling pathways**

One of the first responses following MAMPs/DAMPs perception is a rapid increase of cytosolic calcium as was proven by Jeworutzki and collaborators for Arabidopsis plants that experienced rapid membrane potential depolarization in response to either flg22 or elf18, followed by an increase in cytosolic calcium concentration (Jeworutzki et al., 2010). Cytosolic  $\text{Ca}^{2+}$  concentrations are regulated by plasma membrane and endomembrane  $\text{Ca}^{2+}$  channels that mediate the influx of  $\text{Ca}^{2+}$  from the extracellular portion and the vacuole, respectively. Plants possess multiple types of calcium channel proteins with possible redundant functions, which makes it difficult to identify the specific calcium channels driving the PTI-triggered calcium influx (Seybold et al., 2014). However, recent evidence points to proteins of the Glutamate-Like Receptor (GLR) family and the Cyclic-Nucleotide Gated Channels (CNGCs) as responsible for the  $\text{Ca}^{2+}$  influx in plant membranes (Seybold et al., 2014). While the Two-Pore-Channel 1 (TPC1) seems to control the  $\text{Ca}^{2+}$  flux in endomembranes (Seybold et al., 2014). Increased cytosolic calcium concentrations have multiple effects in plant immunity. It can activate calcium-dependent metacaspases that cleave protein precursor Pro-Peps to release mature Peps into

the apoplast. Peps are recognized by membrane PRRs in near and distant cells. This is an effective mechanism to amplify signaling and enhance immune responses (Zhou & Zhang, 2020). The rapid increase of  $\text{Ca}^{2+}$  in cell cytosol also activates RBOHD in cell membranes to accumulate ROS. Furthermore, free cytosolic  $\text{Ca}^{2+}$  is an effective second messenger involved in the activation of various plants  $\text{Ca}^{2+}$  sensors such as Calcium Calmodulins (CaM), Calmodulin-Like proteins (CMLs) and CDPKs that are important regulators of transcriptional reprogramming and redundantly activate RBOHD.  $\text{Ca}^{2+}$  variations in response to stress differ in their spatio-temporal properties according to the nature of the stimulus; consequently, plant responses vary in accordance. In order to have proper immune responses  $\text{Ca}^{2+}$  transients should return to basal levels within minutes. Therefore, plants possess  $\text{Ca}^{2+}$  efflux transporters that reestablish homeostasis.

#### **1.5.5.2 The production of reactive oxygen species activate several immune responses**

In addition to cytosolic  $\text{Ca}^{2+}$  increase, the production and accumulation of ROS is another early PTI response. ROS species include oxygen ( $\text{O}_2$ ), superoxide anion ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radicals (radical OH). In the context of plant immunity the best-studied process is the production of apoplastic  $\text{H}_2\text{O}_2$  by plasma membrane localized Respiratory Burst Oxidase Homologs (RBOHs). RBOHs transfer electrons from cytosolic NADPH or FAD to apoplastic oxygen to form  $\text{O}_2^-$  radicals, which are then converted to  $\text{H}_2\text{O}_2$  by superoxide dismutases. (J. Qi et al., 2017). ROS are powerful oxidants that can produce severe damage to lipids, proteins, and nucleic acids. They have been proposed to act as potent antimicrobial molecules, inducers of lignin formation and mediators of cross-linking of cell wall components to limit pathogens entry (del Río, 2015). ROS play a critical role in PTI by acting as an intercellular and intracellular secondary messenger to trigger additional immune responses, such as changes in gene expression or induction of stomatal closure (J. Qi et al., 2017).

In Arabidopsis, ROS production induced by PRRs activation promotes the deposition of callose between the plasma membrane and the cell wall at sites of pathogen attack at the plasmodesmata, and on other plant tissues to slow pathogen invasion and spread. ROS production by RBOHD in plasma membranes is regulated by diverse mechanisms. Upon PAMPs/MAMPs perception, BIK1 after being primed by activated PRR complexes, directly interacts with and phosphorylates RBOHD. This process is essential for ROS production (Kadota et al., 2015). Additionally  $\text{Ca}^{2+}$  ions are major regulators of RBOHD activity through direct binding to EF-hand motifs and indirectly through phosphorylation of the RBOHD N-

terminus by the  $\text{Ca}^{2+}$ -dependent protein kinase CPK5 (Dubiella et al., 2013). Interestingly ROS accumulation increases intracellular calcium concentration, the  $\text{Ca}^{2+}$  influx at the cytosol is recognized by CDPKs, which further phosphorylate RBOHD leading to boosted ROS production (Kadota et al., 2015). This positive feedback between ROS and calcium signals is an efficient mechanism to amplify specific signals and confer robustness to the plant immune mechanisms.

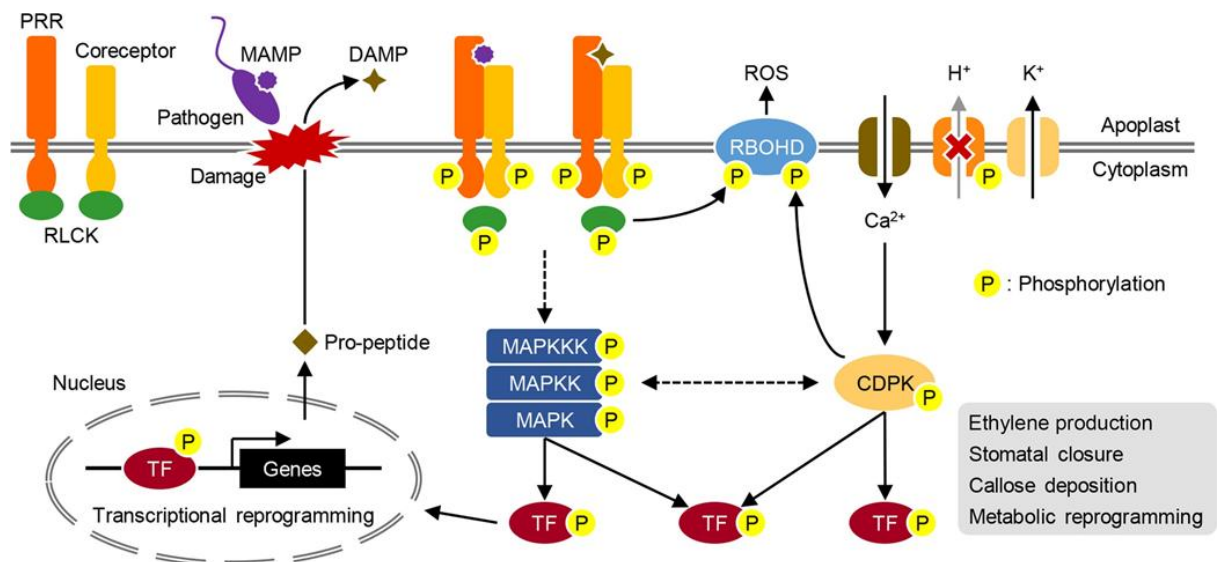
#### **1.5.5.3. MAPK and CDPK cascades are important relays in immune signaling pathways.**

Following PAMPs/DAMPs perception many signaling mechanisms are activated, among them the signaling cascades formed by MAPKs and CDPKs are some of the earliest events and they play fundamental roles in the PTI activation (Figure 2). CDPK and MAPK cascades activation regulates the synthesis and activation of several defense proteins such as PRRs, Nucleotide Binding Leucine Rich Repeats (NLRs), immune signaling proteins, hormones and antimicrobial compounds. This regulatory process occurs mainly through phosphorylation and activation of key transcription factors like those belonging to the WRKY family (Zipfel & Oldroyd, 2017).

MAPK cascades are signaling modules that transduce and convert the signals generated by many PRRs including the most paradigmatic in plant science: FLS2, EFR and CERK1 (Meng & Zhang, 2013). Activation signals in MAPK cascades pass through three kinds of protein kinases: first a MAPK kinase kinase (MAPKKK also known as MEKKs) that phosphorylate a MAPK kinase (MAPKK) which in turn phosphorylate a third protein kinase, a MAPK. In Arabidopsis two kinases cascades have been widely recognized as regulators of cell immunity. One cascade employs MAPKKK3/MAPKKK5 to activate MAPKK4/MAPKK5 to further activate MPK3 and MPK6 (T. Sun et al., 2018). The other is composed by MEKK1, MAPKK1/MAPKK2 and culminates in the phosphorylation of MPK4 and MPK11 (Gao et al., 2008). It is not fully understood yet how PRRs transduce immune signals to the MAPK cascades. Some evidence shows that the receptor-like cytoplasmic kinase PBL27 connects the chitin receptor complex CERK1-LYK5 with the MAPKs cascades by phosphorylating the MAPKKK5 in response to chitin perception (Yamada et al., 2016). Another vein of information proved that BSK1 can phosphorylate the N terminus of MAPKKK5 which is required for disease resistance functions of MAPKKK5 (H. Yan et al., 2018). These works suggest that upon danger perception, a set of membrane bound RLCK relays immune signaling from PRR complexes to MKKKs by direct phosphorylation to activate MAPKs cascades.

In a similar way, CDPK cascades activate several PTI responses by phosphorylating various substrates including ion channels, transcription factors and metabolic enzymes, but unlike MAPK cascades, the CDPK signaling cascades are responsive to calcium concentration fluctuations. Active CDPKs have been implicated in the regulation of several immune responses: CDPK 4, 5, 6 and 11 phosphorylate RBOHD and induce flg22-mediated production of ROS, CDPK1 can phosphorylate the Phenylalanine Ammonia-Lyase (PAL) to promote phytoalexin biosynthesis, CDPK4, 5 and 11 have been shown to directly phosphorylate and activate WRKY transcription factors. Additionally CDPK5 and CDPK6 regulate ethylene production through the modulation of ethylene biosynthesis enzyme ACC synthase (Yip Delormel & Boudsocq, 2019).

Like many other plant immune mechanisms, CDPKs and MAPKs are tightly regulated to avoid autoimmunity. In Arabidopsis, DUSPs, as well as Protein Tyr Phosphatases (PTPs) and protein Ser/Thr phosphatases target PRR-activated MAPKs. This was evidenced for the PP2C-type phosphatase AP2C1 that suppress the activation of MAPK3 and MAPK6 in response to flg22 and oligogalacturonides (Galletti et al., 2011). Concerning CDPKs, it has been discovered that CDPK28 phosphorylates the U-box type E3 ubiquitin ligases PUB25 and PUB26 to enhance their activity in promoting BIK1 degradation to negatively regulate immunity.



**Figure 2: Scheme representing an overview of the Pattern-Triggered Immunity (PTI).** Perception of MAMPs/DAMPs by cognate PRRs involves dynamic association/dissociation with co-receptors and RLCKs, and trans-phosphorylation within the PRR complexes to initiate the downstream signaling. PRR-derived signals are transmitted via further phosphorylation cascades including MAPKs and CDPKs to the downstream targets such as the NADPH oxidase RBOHD, the Plasma Membrane (PM)-resident H<sup>+</sup>-ATPases and Transcriptional Factors (TFs) during PTI. Taken from Saijo et al., 2018.

#### 1.5.5.4 Hormone pathways regulate plant immunity.

The plant cells produce signalling molecules called hormones to regulate many physiological processes including immunity against pathogens. The plant immune system is regulated by a complex network of hormones that integrates both external and internal signals of danger to mount proper responses against specific types of invaders. The main hormones produced by plant cells to modulate immune responses are ethylene (ET), jasmonic acid (JA) and salicylic acid (SA). Plants respond differently according to the nature of the faced pathogen, the JA signaling cascade is triggered against herbivores and necrotrophic pathogens, whereas biotrophic pathogens activate pathways controlled by SA. These two hormone pathways act antagonistically and the prevalence of one or the other is fine-tuned by other hormones, such as ethylene, abscisic acid (ABA), gibberellins (GAs), and auxin (AUX). This is why plants mount resistance to herbivores or biotrophs, but not both at the same time (Bürger & Chory, 2019). In natural conditions, plants are exposed to multiple stimuli. Often, biotrophic and necrotrophic pathogens colonize simultaneously the same plant, placing the existence of such antagonistic dichotomy between JA and SA as a paradox. Intriguing questions arise from this paradox: how do plants deal with simultaneous invasion of necrotrophic and biotrophic pathogens? Why are these pathways mutually exclusive? Colonization by necrotrophic pathogens inhibit biotrophs proliferation and *vice versa*?

SA is produced upon PAMP/DAMP perception by PRR complexes. Cytokines, chitin and flagellin stimulate SA synthesis from the metabolite chorismate after being converted to isochorismate by the enzyme Isochorismate Synthase (ICS). During PTI activation, the protein NPR1 perceives SA accumulation and then relocates to the nucleus. Nuclear NPR1 is recruited to the promoters of SA-responsive genes such as Pathogenesis-Related 1 (PR-1) by the transcription factors TGA2, TGA5, and TGA6 (Ding et al., 2018). The transcriptional reprogramming caused by SA upon danger perception enhances the expression of immune-related molecules such as antimicrobial compounds, other hormones, PRRs and their co-receptors, Nucleotide Binding Leucine-rich repeat proteins (NLRs), etc. As illustration, SA positively regulates basal FLS2 levels enhancing the flg22-triggered responses such as ROS burst, callose deposition, stomatal closure, etc. (Yi et al., 2014). Defensive hormones accumulate in cells maintaining direct contact with MAMPs/DAMPs but they also diffuse to distant plant tissues to activate immune responses. This phenomenon is known as Systemic Acquired Resistance (SAR) and is typically based on the movement of SA through the apoplast

from infected cells to neighboring unprimed tissues leading to the expression of Pathogenesis-Related (PR) genes.

## **1.6 Successful pathogens overcome plant responses**

The plant pre-formed defense mechanisms namely physical barriers and phytoanticipins together with an inducible defense system including phyto cytokines production and PTI responses, allow plants to withstand the majority of the plant pathogens (non-adapted species). This phenomenon is known as non-host resistance and leads to incompatible interactions. However, some microbial species evolved efficient strategies to circumvent plant primary recognition and/or reduce immune responses to survive and feed from the host tissues; these kinds of interactions are often referred to as compatible interactions (Reignault & Sancholle, 2005).

All the different determinants contributing to the pathogens proliferation inside the plant host are known as virulence factors (Reignault & Sancholle, 2005). Some of them are well conserved across microbial species, while others are found only in few strains and are related with highly specialized strategies to colonize specific niches.

### **1.6.1 Microbes deploy multiple mechanisms to avoid recognition and/or suppress plant immunity**

In a broad sense, the pathogenicity or virulence factors induce susceptibility in an otherwise resistant or tolerant host. Microbial species have developed a wide variety of virulence factors to achieve host colonization; one of the most conserved strategies is the production and secretion of polysaccharides such as exopolysaccharides (EPS) and lipopolysaccharides (LPS). These molecules are often exposed at the surface of many microbes and help to protect them from harsh conditions caused by environmental factors or by the plant immune responses such as the production of apoplastic ROS or antimicrobial compounds (Pontes et al., 2020). The xanthan gum produced by the bacterium *Xanthomonas campestris* is a good example of protective EPS (Crossman & Dow, 2004), likewise amylovoran produced by *Erwinia amylovora* whose role in pathogenicity is associated with its higher viscosity that impairs the passage of water by the vascular system of their host (Piqué et al., 2015).

Some microbes actively modify the physicochemical conditions of the surrounding environment making them more appropriate to their colonization. One example is the host

acidification mediated by *Penicillium* species to promote virulence in apple trees (Prusky et al., 2004). Some pathogens use nucleic acids such as small RNAs to silence genes involved in plant immunity (Weiberg et al., 2013). And others achieve the direct detoxification of the plant-produced antimicrobial compounds through enzymatic catalysis (Pontes et al., 2020).

One of the most prevalent strategies used by pathogens to reduce plant defenses is the production and secretion of metabolites. Fungi and bacteria produce a vast number of metabolites that play crucial roles as virulence factors and are collectively known as phytotoxins (Strange, 2007). The detailed description of the phytotoxins action mode is out of the scope of this thesis, but suffice to say that plant pathogen phytotoxins present countless actions and effects in plant cells (Strange, 2007). Importantly one same microbial species can present different phytotoxins as has been proven for *P. syringae* that produces syringomycin, syringopeptin, coronatine, phaseolotoxin and tabtoxin (Bender et al., 1999).

Successful plant pathogens often rely on multiple secretion systems to secrete molecules into the extracellular milieu or translocate them directly into the host cell cytosol. In Gram-negative bacteria, six secretion systems (types I–VI) have been described to date with a wide range of substrates including phytotoxins, nucleic acids, polysaccharides and proteins. These substrates have key roles in bacterial adhesion, pathogenicity, adaptation and survival in their host environments (Costa et al., 2015).

#### **1.6.1.1 The T3SS: an efficient machine to govern plant immunity**

The Type III Secretion System (T3SS) is the main virulence factor for various microbial pathogens such as *Salmonella*, *Shigella*, *Erwinia*, *Yersinia* and *Pseudomonas*. This is evidenced by the marked decrease or complete loss of virulence of T3SS mutants. Pathogens harboring T3SS infect a great diversity of eukaryotic host groups ranging from animals to plants. Mutualistic and commensal microbes also use the T3SS to mediate host interactions, reflecting the relevance and effectiveness of this secretion system in microbial ecology (Ghosh, 2004; Alfano & Collmer, 2004). The T3SS resembles a molecular syringe that translocates a set of proteins known as Type III effectors (T3Es) directly inside the host cell cytosol aiming to disrupt the plant immune system and optimize the host environment for its growth. The components of the T3SS present extensive sequence and functional similarities among the gram-negative bacterial species (Alfano & Collmer, 2004). In plant pathogens the T3SS is composed of two distinct parts: an elongated, extracellular needle-like structure termed Hypersensitive response

and pathogenicity (Hrp) pilus and a cylindrical base which possesses a pair of rings, embedded in the inner and outer membrane, providing a continuous path from the bacterial environment to the host cytosol (Ghosh, 2004).

Phytopathogens harboring a functional T3SS deploy large and diverse sets of T3Es; the complete repertoire of T3Es secreted by a given strain is designated as the T3 effectome (Arroyo-Velez et al., 2020). The T3SS machinery exhibits a high degree of similarity among plant pathogens. Often bacterium of a certain species can secrete effectors from another distantly related species, suggesting that the T3SS machinery itself may have little role in host specificity. Instead, virulence capabilities are rather conferred by the functions of the T3Es delivered by each T3SS-harboring strain (Alfano & Collmer, 2004). T3Es main functions are carried inside the eukaryotic host cells. Surprisingly, recent studies found that some effectors can have important intra-bacterial biochemical activities. The T3E NleB of *Escherichia coli* mediates microbial survival in oxidative stress conditions by glycosylating the glutathione synthase GshB, proving that the T3Es also might play an important role in regulating bacterial physiology.(El Qaidi et al., 2020).

Collectively, the T3Es protect the pathogens from harsh environments and modulate the host physiology to achieve proliferation, and nutrient acquisition inside host tissues. Therefore, they determine many aspects of the pathogens lifestyle such as host range and tissue specificity. Revealing the T3Es action modes is key to fully describe the plant-pathogen interactions.

#### **1.6.1.2 Many T3Es dampen PTI activation**

Given their relevance in microbial recognition, the plant PRRs and their associated co-receptors and adaptor kinases are common targets of many T3Es; impairments in their functions impede the activation of the PTI responses and induce host susceptibility to microbial colonization.

Some T3Es directly block the recognition of MAMPs and DAMPs in host membranes by causing the degradation of the PRRs. For example, the *P. syringae* effector AvrPtoB ubiquitinates FLS2 (Göhre et al., 2008) and CERK1 (Gimenez-Ibanez et al., 2009) to induce their proteasome-mediated degradation in *A. thaliana* plants. Other effectors with proteolytic activity directly degrade plant proteins involved in PTI activation, like the *P. syringae* effector AvrPphB that is a cysteine protease and cleave several RLCKs including PBS1, BIK1, PBL1,



and PBL2 (Zhang et al., 2010). Other T3Es rather affect the accumulation of the PRRs in plant cells like the *P. syringae* effector HopQ1 that repress the FLS2 accumulation in *A. thaliana* (Hann et al., 2014).

T3Es hijack downstream immune signaling pathways by changing the phosphorylation status of some PRR: *P. syringae* effector, HopAO1, displays phosphatase activity to inhibit EFR phosphorylation and consequently block the elf18-triggered immunity (Macho et al., 2014).

### **1.7 Plants perceive and counteract T3Es to restrict pathogens**

The T3Es were first identified as potent elicitors of plant immunity and were named Avirulence (Avr) factors. Bacterial isolates carrying Avr genes failed to cause disease on specific hosts carrying a cognate resistance (R) genes (Staskawicz et al., 1984). Later studies with Avr genes revealed many of the components and mechanisms of the plant immune system: the so-called R gene-mediated disease resistance, currently known as Effector-Triggered Immunity (ETI).

ETI signaling is initiated following direct or indirect recognition of pathogen effectors by some specialized intracellular receptors called NOD-Like receptors or Nucleotide-Binding domain Leucine-Rich Repeat containing receptors (NLRs or NB-LRR). The activation of the ETI results in enhanced resistance and is often associated with the hypersensitive response (HR) in which the cells surrounding the site of infection experience programmed cell death to contain any spread of the disease. NLRs are commonly classified in three major groups based on their N-terminal domain: the coiled-coil (CC-NLRs or CNLs), Toll/interleukin-1 receptor (TIR-NLRs or TNLs) and the Resistance to powdery mildew 8-like domain (RPW8-NLR or RNLs)(Zhou & Zhang, 2020). The NLRs responsible for the recognition of microbial T3Es are known as sensor NLRs. Some of them recognize T3Es through direct protein–protein interactions. However, most of the NLRs studied so far recognize the T3Es indirectly by perceiving effector-induced modifications on host proteins, thereby triggering ETI. The monitored host proteins are crucial plant proteins (guardees) or plant decoy proteins that mimic the actual host target (Jones et al., 2016).

A second type of NLRs known as helpers, contribute to signal relay and amplification. The molecular mechanisms that link the activation of NLRs with the immune response are not completely clear yet (H. Cui et al., 2015). The canonical ETI responses include ROS burst, Ca<sup>2+</sup> spikes, MAPK cascades activation, transcriptional reprogramming and production of

phytohormones. These are qualitatively similar to the PTI responses but ETI responses are greater in terms of intensity and duration. Thus, ETI signaling appears to boost the PTI basal immune responses (H. Cui et al., 2015).

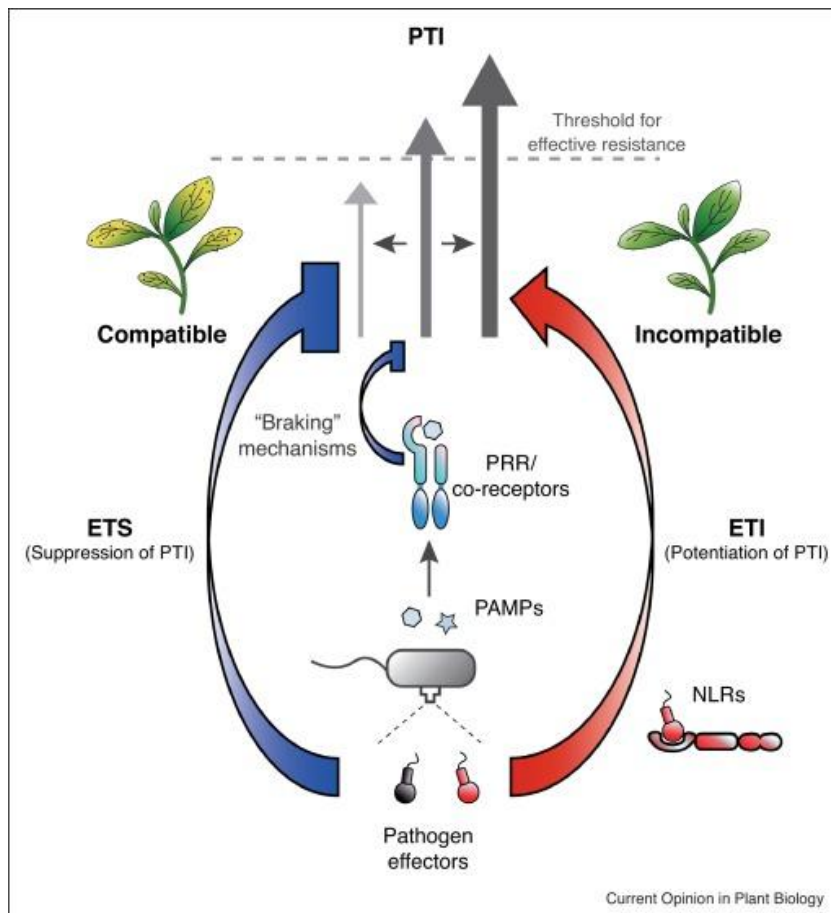
Plants and microbes are together in an intricate evolutive race. Each organism must adapt or evolve new strategies to modulate the interaction to its favor. In this context, plants evolved intracellular receptors to recognize the T3Es translocated by microbes and activate immune responses. However, pathogens may evade ETI through loss or mutation of recognized effectors or by suppression of ETI using novel effectors. This kind of red queen effect was formalized as a zig-zag phenomenon in which the plant restores ETI by evolving new R genes and the pathogen causes disease by overcoming recognition (Jones & Dangl, 2006).

### **1.7.1 PTI/ETI form an integrated immune system**

PTI and ETI pathways require different mechanisms for their activation and signaling . However, they lead to many convergent responses. Recent evidence suggests that the simultaneous activation of both pathways is required for full disease resistance. Activation of the NLRs by their cognate T3Es, triggers transcript and protein accumulation of multiple PRR signaling components, including BAK1, SOBIR1, BIK1, RBOHD and MPK3 (Ngou et al., 2021). In addition, the ETI activation potentiates ROS production and cell death triggered by the fungal PAMP chitin (Yuan, Ngou, et al., 2021). These results suggest that the ETI activation mediates enhanced PTI responses that ultimately leads to enhanced disease resistance. On the other hand, the correct activation of the ETI signaling pathway requires the proper activity of the PTI pathway. We can infer this from the fact that the ETI-associated resistance against microbial pathogens is seriously compromised in absence of several PRR and its co-receptors (Yuan, Ngou, et al., 2021). Additionally, multiple PTI-related RLCKs serve as ‘decoys’ or ‘adaptors’ in the NLR complexes to initiate ETI such as PBS1, PBL2 and ZED1/ZRKs (Yuan, Ngou, et al., 2021).

The ROS burst during ETI activation presents a biphasic response where the second pulse is higher and longer than the first. The normal development of the ETI-triggered ROS burst requires the concomittent stimulation with PAMPs (Ngou et al., 2021). PTI-ETI crosstalk also is evident in other immune responses. The NLRs of the type TNLs cannot trigger MAPK activation in Arabidopsis plants in absence of the PRR signaling, suggesting that TNL-associated MAPK phosphorylation signals through the PTI pathway.

The border between PTI and ETI is increasingly blurred; plant immunity works as an integrated system that requires the coordinated functions of many plant molecules that were previously thought as exclusive of one pathway or the other. Effective immune responses require the simultaneous activation of both, PTI and ETI, each one provides feedback and enhances the other. It has been proposed a new model to describe this unified vision of the plant immune system (Figure 3). The future studies in the field of pathogenic microbes must take this into account since the study of the PTI or ETI as isolated pathways could lead to erroneous conclusions. Thus, it is necessary to approach the immune system as a whole with the simultaneous treatment of plant cells with PAMPs and Effectors to fully understand the plant pathogens interactions.



**Figure 3. Updated model of the plant immune system.** PTI is the basal defense mechanism against microbes, and its components are under negative control by endogenous 'braking' mechanisms of plants to prevent over activation and by microbial effectors (blue blunt arrows). Activated NLRs trigger ETI, which potentiates PTI through upregulation of PTI components (red arrow). The final resistance output is the combination of i) inhibition of PTI by ETS or endogenous 'braking' mechanisms and ii) potentiation of PTI by ETI. ETS, effector triggered susceptibility. 'PTI + ETS' is usually associated with compatible interactions (on the left) and 'PTI + ETI' with incompatible interactions (on the right). Taken from Yuan, Ngou, et al., 2021.

### 1.7.1.1 MAPK cascades are important hinges in the PTI-ETI crosstalk and are targeted by multiple T3Es

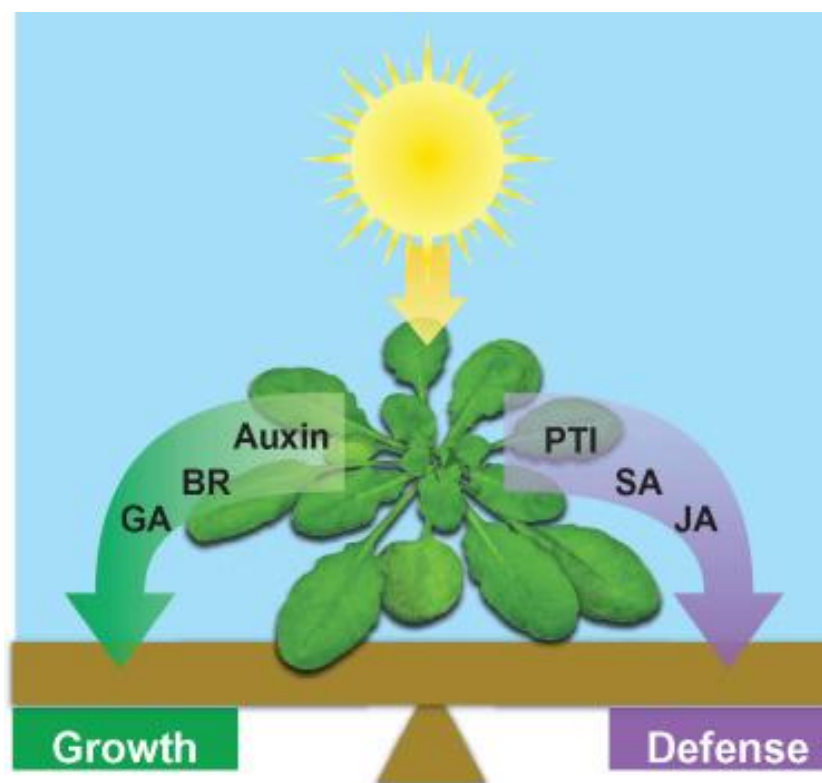
The MAPK cascades are key components of the signaling pathways activated in response to biotic stresses. These cascades relay signals by sequential phosphorylation ending with the activation of MAPKs (mainly MAPK3, MAPK4 and MAPK6) that promote a set of immune responses. PTI activation has been related with the transient phosphorylation of the MAPKs whereas ETI activation leads to a delayed and prolonged phosphorylation (Lang & Colcombet, 2020). Recent evidence suggest that MAPKs cascades are important hinges in the positive PTI-ETI crosstalk (Ngou et al., 2021, Yuan, Jiang, et al., 2021). Expectedly, many T3Es disrupt different levels of the MAPK signaling pathways to promote pathogenicity. In Arabidopsis, the phosphorylation of MPK3 and MPK6 has been related with the activation of many immune responses. One of the best known is the phosphorylation of the WRKY transcription factor WRKY33 to regulate the transcription of several immune-related genes. These MAPKs are targeted by several T3Es to promote pathogenicity. For instance, HopA11 from *P. syringae* presents phosphothreonine lyase activity and can suppress the activation of the MPK3 and MPK6 by threonine dephosphorylation (Zhang et al., 2007). Also, HopF2 of *P. syringae* blocks flg22-induced PTI responses up-stream of MPK3 and MPK6 by adding ADP-ribose moieties to MKK5, blocking its kinase domain and preventing signal relay (Y. Wang et al., 2010). Some T3Es promote the phosphorylation of certain components of the MAPKs cascades. For instance AvrB from *P. syringae* induces MPK4 phosphorylation to induce JA responses, which presumably suppress other immune-signaling pathways by negative crosstalk (H. Cui et al., 2010). MEKK1 and MKK1/2 kinases are upstream of MPK4. The T3E XopAU of *Xanthomonas euvesicatoria* directly phosphorylates MKK2, presumably leading to MPK4 phosphorylation. The specific contribution of MAPK4 activation to pathogenicity is not fully clear yet. However, the fact that Arabidopsis cells possess an intricate system to guard the activation of the MAPK4, including the NLR SUMM2 and the Calmodulin-binding Receptor-like Cytoplasmic Kinase 3 (CRCK3), suggest that the signaling cascade MEKK1-MKK1/2-MPK4 play a relevant role in plant immunity (Lang & Colcombet, 2020).

Many other T3Es modulate MAPKs cascades in yet unknown ways (Teper et al., 2015). Further studies might reveal novel strategies employed by microbes to suppress the immune-activating activities or promote the immune-suppressing activities of the MAPKs cascades.

### 1.7.2 Immunity-Growth Interference

The production and maintenance of immune-related molecules have energetic costs for plants; in absence of pathogens, these costs have a negative impact on plant fitness (Tian et al., 2003). Besides, activation of plant immunity and the consequent immune responses restrict plant growth and yield, resulting in a growth-immunity trade-off (Eichmann & Schäfer, 2015). It has been proposed that this trade-off arises from the limited availability of nutrients and resources that cannot be allocated to both processes simultaneously. However, previous evidence suggests that the growth-immunity trade-off is the result of mutually antagonistic activities of the plant hormone pathways (Eichmann & Schäfer, 2015). Under basal conditions, certain hormone combinations maintain cell proliferation. Upon biotic stress, changes in hormone balance redirect plants physiology towards immunity (Eichmann & Schäfer, 2015) (Figure 4). It has been reported that the signaling pathways activated by the growth-promoting hormone brassinosteroid (BR) have a suppressor effect in the PTI activation thus producing enhanced disease susceptibility to bacterial pathogens in *Arabidopsis* (Lozano-Durán & Zipfel, 2015). The immunity-growth trade-off is also evident in the growth effects induced by the hormone gibberellic acid (GA) that can be suppressed by the activity of the defense-promoting hormone jasmonic acid (JA), and vice versa (H. Huang et al., 2017). As mentioned in previous sections, salicylic acid (SA) has a major role in inducing immune responses to face biotrophic pathogens. In parallel SA accumulation actively suppresses plant growth and developmental processes. Conversely, growth processes as those induced by the auxin Indole-Acetic Acid (IAA) actively suppress SA responses and therefore immunity and disease resistance to biotrophic microbes (Huot et al., 2014). The negative crosstalk between growth and immunity seems to be mediated by shared molecules between antagonistic pathways as is suggested by the fact that the signaling pathway of the main BR Receptor (BR11) share many components with the FLS2 signaling pathway namely BAK1, BSK1, and BIK1 (Lozano-Durán & Zipfel, 2015). Plant cells need to tightly balance the immunity-growth dichotomy to maximize fitness; they must restrict pathogens proliferation in the most optimal way to save resources for other fundamental activities such as growth or reproduction. The molecular basis of these regulatory mechanisms are just starting to be unraveled, still they are of great importance for plant survival as failure to regulate plant immunity compromises plant growth, development and often leads to cell death (van Wersch et al., 2016). The regulatory mechanisms of the growth-immunity crosstalk might have a prominent role in plant-microbe interactions since the balance between immunity and growth is continuously challenged by the microbes' virulence strategies. In

consequence, plants must respond with appropriate intensity to restrict pathogens but without compromising growth and survival in order to gain fitness.



**Figure 4. Scheme depicting the growth-defense tradeoffs.** Plants allocate resources towards growth or defense, depending on the presence or absence of specific stresses. This process is mediated by the differential accumulation of different hormones. Auxin, brassinosteroid (BR) and gibberellin (GA) are related with resources allocation towards growth while PTI activation and the associated hormones salicylic acid (SA) and jasmonates (JA) direct plant resources towards defensive responses (Huot et al., 2014).

#### 1.7.2.1 Microbes use T3Es to manipulate hormonal balance and exploit the negative growth-immunity crosstalk

Many T3Es of diverse pathogens convergently exploit the negative crosstalk established between SA and JA pathways. The biotrophic pathogen *Ralstonia pseudosolanacearum* uses RipAL to induce JA production and suppress SA signaling presumably by releasing JA precursors (Nakano & Mukaihara, 2019). Alternatively other effectors of the same pathogen and other biotrophic species, degradate the Jasmonate-ZIM-domain (JAZ) proteins that are transcriptional repressors of the JA signaling pathway, such as RipE1 (*Ralstonia pseudosolanacearum*), HopZ1a and HopX1 (both of *P. syringae*) (Gimenez-Ibanez et al., 2014).

Conversely, the necrotrophic microbial species *Phytophthora sojae* and *Verticillium dahliae* encode their own Isochorismate Synthase (ICS) to induce SA production and antagonize the JA signaling pathway (Bürger & Chory, 2019).

The heterologous expression of the *X. campestris* pv. *campestris* effector AvrXccC/XopAH enhance accumulation, biosynthesis and signaling of the phytohormone Absciscic Acid (ABA) which is related with enhanced disease susceptibility to *X. campestris* (Ho et al., 2013). Another example of negative crosstalk exploited by microbes is the *P. syringae* HopAM1 that makes Arabidopsis cells hypersensitive to ABA which is related to immune defenses suppression and bacterial proliferation. Some microbes stimulate growth-promoting hormones pathways to inhibit immunity. The T3E AvrRpt2 of *P. syringae*, promotes auxin accumulation by stimulating the turnover of the key negative regulators of auxin signaling: the Aux/IAA proteins. The auxin accumulation promotes pathogen's virulence in Arabidopsis cells (F. Cui et al., 2013). *Agrobacterium tumefaciens* also manipulate auxin synthesis and signaling in host plants to promote pathogenesis, auxin accumulation in host cells lead to growth enhancement and tumor formation that serve the pathogen to feed (Zupan et al., 2000).

All the examples exposed above suggest that microbial pathogens convergently manipulate the negative crosstalk between different signaling pathways, by promoting the pathways that suppress plant immunity. The molecular mechanisms responsible for such antagonistic effects are not fully understood, As T3Es target many of such regulatory mechanisms, the study of the T3Es' functions might unveil the molecules that serve as crossroads in the antagonistic signaling pathways. Despite the fact that many pathogens manipulate the negative cross talks of plant signaling pathways, still these antagonistic mechanisms are maintained in modern plants. This means that they confer adaptive advantages that are greater than the fitness costs due to pathogen's colonization. Intriguing questions remain open such as: What advantages do negative cross talks confer to plant fitness? What mechanisms have plants evolved to prevent the manipulation of these cross talks by microbes? Can we manipulate those regulatory mechanisms to increase plants yield?

## 1.8 All for one and one for all: the collective nature of the T3Es

### 1.8.1 Effectome composition shapes pathogens lifestyle

The T3SS is fundamental to mediate the interaction with the host for a large proportion of plant-feeding microbes. This prominent role in pathogenicity relies on the translocations of T3Es with immune-suppressor activity. However some translocated T3Es can be recognized by some plant specialized receptors and trigger immune responses which limit pathogen's proliferation, survival and fitness. Therefore the repertoire of T3Es (T3 effectome) deployed by a plant-pathogen defines its niche, lifestyle, host range and virulence. In some cases, the presence of a single avirulence effector is sufficient to trigger plant immune responses and confer disease resistance to plant ecotypes harboring the cognate resistance (R) protein. This has been proven in lettuce plants that exhibit resistance to all isolates of the oomycete *Bremia lactucae* expressing the *avr3* gene. This effective disease resistance is mediated solely by the R gene *dm3* which fits with the gene-for-gene hypothesis proposed by Henry Flor (Flor, 1971). In literature we can find many other cases where plant disease resistance is mediated by R/Avr interactions. On the contrary, little is known about complete susceptibility induced by one single effector, individual effectors have rather discreet contributions to global pathogenicity and small groups of effectors, collectively induce plant susceptibility. Therefore, the effectome composition (presence of Avr and/or Vir effectors) is a main determinant of pathogens host range and must be carefully considered to fully understand and predict the outcomes of specific plant-microbe interactions.

Microbes use a combination of different secretion systems other than the T3SS to secrete small molecules in the host cell environment to ensure efficient bacterial multiplication and disease progression (Lo Presti et al., 2015). In a broader sense, all the molecules secreted by a pathogen with influence in the host machinery can be referred to as effectors and the full repertoire of effectors present in a given strain as the effectome. Many of the principles governing the T3Es are shared among all the different classes of effectors. Therefore, in the following sections I will use indistinctly the terms effectors and effectome to describe some of the properties shared among the diverse types of effectors although it is worthy to note that some exceptions may exist.

Usually effectomes composition is characterized by *in silico* approaches. Effectors are predicted based on sequence similarity to already known effectors, or the presence of



characteristic domains, such as pip boxes in some bacterial phytopathogens, or RxLR domains in some species of oomycetes (Lanver et al., 2017). A large proportion of the effectors predicted by the second approach are completely novel, as they do not contain any annotated domains. Unexplored effectors could be useful tools for discovering novel resistance genes, which highlights the importance of sequencing different pathogenic field isolates to identify novel effectors. The expression and translocation of candidate effectors should be subsequently validated by experimental means since *in silico* predicted effectors might not be expressed *in planta* conditions (Schechter et al., 2006). Predicted and confirmed effectors are classified in families according to their sequence similarity. Some effector families are conserved among several microbial species while other accessory families are only present in few species or even in a strain-specific manner.

#### **1.8.1.1 Effectome size is diverse among microbes.**

Effectomes size and composition are highly variable among plant pathogens. Some strains of *Erwinia amylovora* present as little as a dozen of effectors (Nissinen et al., 2007) whereas other species of nematodes, oomycetes and fungi can produce up to many hundreds of effectors per strain (Rojas-Estevez et al., 2020). All living pathogens can successfully colonize at least one plant ecotype, otherwise, they would go extinct. The large variability in effectomes size suggest that effectomes contribution to pathogenicity is predominantly qualitative instead of quantitative, since pathogens with small effectomes are as efficient in colonizing certain ecotypes as pathogens with large effectomes in colonizing other ecotypes.

The effectomes of several model pathosystems like *Pseudomonas syringae*, *Xanthomonas campestris*, *Ralstonia pseudosolanacerum* or *Ustilago maydis*, range between 20 to 70 effectors (Lanver et al., 2017; Guy et al., 2013; Kvitko et al., 2009). This bearable number of effectors allows the implementation of full effectome approaches that provide vast amounts of valuable information as has been done previously with *P. syringae* effectome (Kvitko et al., 2009). A large-scale analysis of the reactions of 59 diverse plant accessions to 171 effectors of four strains of *P. syringae*, and one strain of *Ralstonia pseudosolanacearum*, revealed that the effectors produced by a strain are more frequently recognized by non-host species, as compared to host species. Implying that the pathogen's host-specificity is guided by the specific recognition of some Avr effectors (Wroblewski et al., 2009).

Effectors influence on host-specificity is also dictated by the presence of different polymorphisms: effectors could have greater or lesser affinity for their cognate plant-interactors (whether they are R proteins or true effector's targets) depending on their specific polymorphisms. It has been proven that avirulent effectors can escape R recognition by specific single mutations such as AvrLm4-7 of *Leptosphaeria maculans* that escaped the Rlm7-mediated resistance in *Brassica napus* (Plissonneau et al., 2017). Likewise, certain polymorphisms could enhance the affinity of one effector for its target, enhancing in turn the global pathogenicity. In terms of effectomes size and composition, microbes have adopted different strategies that often lead to successful colonization of a specific niche. Overall, the composition of the effectomes is dictated by its evolutive history in close relation with their cognate host.

#### **1.8.1.2 Effectomes are prone to evolution**

The evolutive race between plants and pathogens impose a large selective pressure on the effectomes composition. Plants possess a large repertoire of immune-receptor proteins that are constantly evolving to increase the recognition of the pathogens. In response, pathogens modify their virulence strategies to escape recognition. Since plants immune receptors often mimic effectors targets, effector's evolution faces a trade-off between escaping from being detected and optimizing their virulence functions. The need to escape from plant recognition seems to be the reason behind the accelerated evolutive pace of the effectors as has been seen in some fungal pathogens that present higher levels of positive selection in genes encoding secreted proteins as compared to genes encoding non-secreted proteins (Lo Presti et al., 2015). Additionally, the emergence of novel effectors to capture new host targets might contribute to pathogenicity. Effectors can evolve by sequential addition of several synonymous mutations followed by more rare non-synonymous mutations. However, this process is very long and requires several generations to fix relevant changes in the populations. Genetic recombination by sexual means is rare in microbes. Instead, they achieve fast acquisition of novel genes by Horizontal Gene Transfer (HGT). Effectors are rapidly dispersed inter- and intra-specifically through HGT; further selective forces imposed by the host ambience fix the advantageous ones while pushing for the suppression of the disadvantageous Avr genes. Gene loss and/or pseudogenization occur mainly through insertions of transposable elements. The microbes that succeed in suppressing avirulent-gene's expression are able to sustain pathogenic interactions, survive and proliferate.

In terms of genetic environment, effectors are often clustered in flexible genome compartments associated with mobile genetic elements. Such clusters arose through gene duplications and represent genomic islands with accelerated evolutive rates (Lanver et al., 2017). The high evolvability present in the genes that code for effectors originated a great diversity of effectors with a wide range of activities and targets. This phenotypic plasticity is advantageous in the frame of a co-evolutive war, since the acquisition of novel strategies increase the range of potential niches colonized by a given strain. Each new strategy implies energetic costs that must be compensated by the benefits. Often, mutations of single effectors do not have detectable effects in pathogenicity, as has been proven in several studies on fungal species responsible for the barley powdery mildew, which suggested that only 20 out of 80 tested effectors contribute significantly to virulence (Thordal-Christensen, 2020). This raises an important question: why do pathogens evolve and maintain those apparently “expendable” effectors considering the energetic cost that this entails?

### **1.8.2 The effectors are collectively essential but individually dispensable**

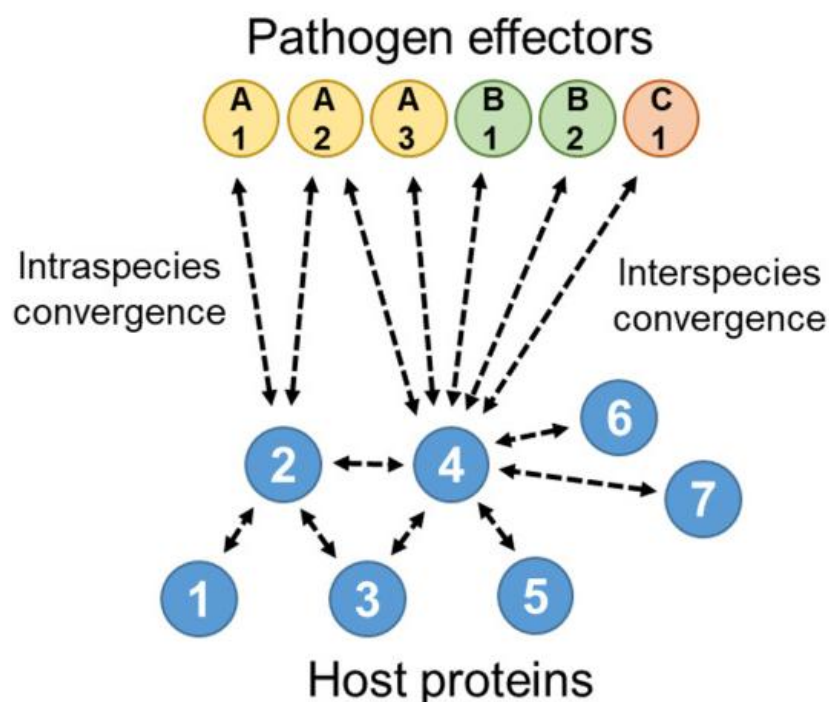
Henry Flor proposed the gene-for-gene model, where the presence or absence of a pair of matching genes (Avr in the pathogen and R in the host) determine the compatibility or incompatibility between plant and microbe species (Flor, 1971). Since then, many monogenic Avr-R interplays have been discovered. However, the vast majority of the plant-microbe interactions are driven by the discrete contribution of multiple genes (Corwin & Kliebenstein, 2017). In many studies deletions of single or few effectors didn't lead to obvious effects in pathogenicity (Hao et al., 2020) whereas mutations disabling secretion systems often leads to strong virulence impairments (Boucher et al., 1987) as a result of the deficient translocation of the effectors. Thus, effectors are collectively essential but individually dispensable for pathogenic progression. As effectomes are major determinants of microbes' pathogenicity, deciphering their functions and composition is imperative to understand the molecular basis of the plant-pathogen interactions. A key step to predict and prevent disease in economically important species. The functional characterization of microbial effectomes should consider the repertoire of effectors that compose it and their coordinated functions related to functional redundancy, differential expression patterns and epistatic interactions (Arroyo-Velez et al., 2020).

### 1.8.2.1 Effectors within an effectome are functionally redundant

The effectors can target multiple host proteins and display simultaneously different biochemical activities. These "multi-tasking" properties have been found in the effector AvrPto of *P. syringae* that possesses two virulence domains and exert different biochemical functions (Wei & Collmer, 2018). If this multi-tasking property is specific to AvrPto or rather is conserved in other effectors is still unknown. Nevertheless the pleiotropic effects exhibited by AvrPto proves the complexity of the functions performed by the effectors. However, full effectomes exhibit even greater complexity levels, as is proven by the fact that AvrPto is dispensable when other functionally redundant effectors are present. A series of studies in *P. syringae* pv. *tomato* DC3000 using combinatorial deletions of twenty effector genes revealed that some deletions diminished growth in *N. benthamiana* only in combination with deletions of other effectors. Two redundant groups were discovered that appear to separately target two relevant processes in plant defense, one composed of AvrPto and AvrPtoB, and the other formed by AvrE, HopM1 and HopR1 (Kvitko et al., 2009). Redundancy can be seen either when multiple effectors target one same molecule or when multiple effectors target different molecules involved in the same pathway. Like AvrPto, AvrPtoB has multiple targets and deploy diverse biochemical activities. Both effectors redundantly affect the PRRs activation and signaling which are key steps to mount proper plant immune responses (Wei & Collmer, 2018). Besides, redundancy is a common strategy among many if not all the species of microbial pathogens. For example in the fungal species *Phytophthora infestans* AVR2, AVR3a and Pi02860 redundantly suppress INF1-triggered immunity via different mechanisms (Thordal-Christensen, 2020). Effectors of human-colonizing pathogens also present redundant activities as has been seen for the T3Es EspG and EspG2 of *Escherichia coli* that convergently disrupt the tight junctions between the epithelial cells of human intestine. In the same pathosystem, Map and EspF effectors redundantly target the host mitochondria to alter the organelle shape and cause dysfunction (Dean & Kenny, 2009).

Some plant molecules can be redundantly targeted by multiple effectors of the same strain (intraspecies) or between multiple effectors of different microbial species (interspecies) (Figure 5). This interspecific redundancy becomes relevant if we consider that disease processes in many cases involve complex communities within a pathobiome (Mannaa & Seo, 2021). Large-scale screenings to identify the universe of interactions between effectors and host proteins demonstrated that multiple effectors of diverse pathogens tend to interact with a group

of highly interconnected host proteins (intrator-hubs) that rather behave as a network (González-Fuente et al., 2020; Mukhtar et al., 2011; Weßling et al., 2014).



**Figure 5. Scheme representing the convergent interactions between pathogen's effectors and host's targets.** The blue circles represent host proteins; the yellow, green and red circles represent pathogen effectors,. Differently colored effector proteins originate from different pathogen species. Broken lines denote protein–protein interactions. Intraspecies (left) and interspecies (right) convergence of pathogen effectors on host proteins. Host proteins 2 and 4 can be considered as 'hubs'. Taken and modified from (Thordal-Christensen et al., 2018).

Genetic redundancy is prevalent in many biological systems to enhance robustness and evolvability of certain relevant pathways. Multiple effectors targeting a single host pathway could buffer against the loss of function of individual effector genes originated either by the emergence of R genes in the host or genetic perturbations in the pathogen's genome (by mutations, rearrangements, pseudogenization etc.). Future studies, focused in host molecules redundantly targeted by multiple effectors could reveal paramount mechanisms for pathogenicity progression.

#### 1.8.2.2 Effectors expression in the right place at the right time

To become pathogens, microbes pass through different stages: they survive as epiphytes; they penetrate plant tissues and survive inside plant ambience to finally proliferate

and move to another host. At each stage, pathogens face specific challenges; in order to survive they employ specific virulence tools in a tissue- and stage-specific manner. This spatio-temporal specificity is evident in maize colonization by the fungus *Ustilago maydis*, where different effectors are needed in different stages and/or plant organs: Pep1 and Tin2 effectors are required during initial penetration and early infection phases; later Pit2 is needed to induce tumor formation and maintain the biotrophic interaction (Toruño et al., 2016). Although it is clear that the effectors are differentially required, little is known about their expression patterns during pathogenicity progression. Ros1 was discovered as a master transcription factor during the late stages of *U. maydis* pathogenesis: Ros1 downregulated the expression of 128 genes encoding secreted effectors involved in the establishment of biotrophic development while upregulating a set of 70 late effectors. This suggests that microbes use active mechanisms to tightly regulate different sets of effectors during different stages of pathogenicity (Tollot et al., 2016). Some evidence found in other hemibiotrophic pathogens suggests that the effectors are differentially expressed in successive waves during infection. Hemibiotrophic microbes present two different pathogenic stages. They behave as biotrophs during early stages, then they acquire necrotrophic characteristics in later stages. Different effectors are expressed during each phase. The biotrophic phase is related with the expression of immune-suppressor effectors whereas cell-death inducing effectors are rather expressed during the necrotrophic phase (Toruño et al., 2016). The current evidence suggests that the expression of the correct effectors in the proper time and tissue is key for microbial virulence. However, further studies are needed to confirm this hypothesis.

The evidence suggests that different effectors are required in a spatio-temporal specific manner, but their levels of expression might also be key for successful pathogenicity. Experiments done with the effectors SnToxA and SnTox2 of the pathogenic fungus *Stagonospora nodorum* showed that the expression levels of the effectors *in planta* largely influence its specific contribution to pathogenicity; higher expression levels of SnToxA correlated with a larger contribution of SnToxA to pathogenicity in wheat plants (Faris et al., 2011). This effect could be explained by the fact that necrotrophic pathogens use effectors to induce cell death in plants, to feed from the dead tissues. Higher expression of necrosis-inducing effectors might correlate with higher rates of cell death *in planta* and consequently to increased disease symptoms.

Pathogens possess elaborated mechanisms to ensure the proper secretion and translocation of the effectors. Some species like *Shigella flexneri*, use a cytoplasmic Sorting

Platform (SP) composed of several proteins that regulate the substrate selection and energize the secretion of the effectors (Tachiyama et al., 2021). Some phytopathogenic species use chaperons to regulate and promote the hierarchical translocation of the effectors through the T3SS. For example in *Xanthomonas euvesicatoria* (Xev) the chaperone HpaB and HpaA regulate and direct the secretion of many Xev T3Es (Prochaska et al., 2018). While regulatory mechanisms controlling the expression and translocation of the effectors are not directly involved with the interference of the host cells physiology, they are nonetheless vital for the biological functions of the secretion systems and consequently for pathogenicity. The chaperon HpaA has an apparent dual role since in addition to its regulatory role it is also translocated itself into the plant cell (Prochaska et al., 2018). It is not clear yet if this dual properties are shared by many effectors. However, the evidence showed by HpaA suggest that effectors take active part in the regulation of the hierarchical expression of the effectome.

All in all, the need for diverse effectors in a specific spatio-temporal manner, together with the existence of relevant mechanisms ensuring the hierarchical and selective secretion of the effectors suggest that the effectome entails a superior level of organization and complexity. The collective activities of the effectors and more importantly their organized expression and translocation are key aspects for the virulence of the plant-colonizing microbes.

### **1.8.2.3 Effectors are modified by other proteins to complete their activities**

In order to be translocated into host cells, some effectors suffer molecular or structural modifications, accomplished by diverse microbial proteins. For instance, in some species, chaperons mediate effectors folding and addressing to the secretion system (Prochaska et al., 2018). In effectors translocated by the T3SS, hexameric ATPase mediates chaperon release and effector's unfolding, to achieve the pass of the effectors through the T3SS channel that is as narrow as 2nm (LeBlanc et al., 2021). Other examples are the effectors of multiple species of fungus, which are translated as precursor proteins, and suffer proteolytic processing by unknown microbial or plant proteases, to generate mature effectors. For instances Avr4 and Avr9 from *Cladosporium fulvum* or SnToxA from *Stagonospora nodorum* (Lo Presti et al., 2015).

Inside host ambience, many effectors undergo post-translational modifications to become active. The effector NopT of *Rhizobium* and ORF4 and AvrPphb effectors of *Pseudomonas pv. phaseolicola* are processed by autoproteolytic cleavage upon translocation into the host cell (Tahir et al., 2019). Post-translational modifications also help the effectors to

escape the recognition by host resistance proteins as has been seen for the effector PsXEG1 of *Phytophthora sojae* that undergoes N-glycosylation to avoid recognition and degradation by the aspartic protease GmAP5 secreted by soybean plants (Xia et al., 2020). Some effectors dimerize after being translocated into the host cells. For example, AvrBs3 of *Xanthomonas euvesicatoria* that works as a transcription activator, homo-dimerizes in the plant cytosol prior to nuclear import (Xia et al., 2020). We can see the same phenomenon with the Avr2 effector of *Fusarium oxysporum sp. lycopersici* that function as virulence factor in some plant species. Avr2 proteins homodimerize *in vivo* when it is transiently expressed in *Nicotiana benthamiana* leaves (Ma et al., 2013). Six5 effector is also secreted by *Fusarium oxysporum sp. lycopersici* during host colonization; it has been proven that Six5 physically interacts *in planta* with Avr2. Interestingly both effectors are required for full virulence on susceptible tomato plants. Both effectors are also required for resistance in resistant tomato plants, showing a functional link between the two effectors that is not related with their accumulation levels but rather suggest complex physical interactions between them (Ma et al., 2015).

The T3Es translocated by *Xanthomonas oryzae pv. oryzae* during colonization of rice plants also present a complex interplay; it has been proven that the effector XopX interacts with the plant proteins 14-3-3 to suppress host immunity. However, when XopX is ectopically expressed in rice cells together with XopQ, it results in activation of plant immune responses that are not observed when either protein is individually delivered. Subsequent Yeast two-hybrid, bimolecular fluorescence complementation and co-immunoprecipitation assays indicated that XopQ and XopX physically interact with each other. Furthermore another five *X. oryzae pv. oryzae* effectors were found to individually suppress the immune responses induced by XopQ-XopX simultaneous expression (Deb et al., 2020). The examples mentioned above indicate that some emergent functions arise from the complex interactions between different effectors. These emergent properties increase the repertoire of possible activities exerted by individual effectors and highlight the relevance of the collective properties of the effectomes studies.

#### **1.8.2.4 Epistasis expand the functions of the effectors**

Often the functional interaction between genes is referred to as epistasis. This term is in constant debate and many authors differ in its exact definition. However in a broad sense epistasis refers to “the non-additive genetic interaction that results from the activity of one gene masking the phenotype or effect caused by another gene” (Eckardt, 2008). Epistasis is a common phenomenon among the microbial effectors as can be seen for some of the examples



provided above (functional redundancy and hetero-dimerization). Several effectors of *P. syringae* also present epistatic interactions as has been seen for HopPtoE, AvrPphE, AvrPpiB1, AvrPtoB, and HopPtoF that can suppress the programmed cell death induced by the effector HopPsyA in tobacco and Arabidopsis plants (Jamir et al., 2004). A similar effect was found in the interaction of *Leptosphaeria maculans* with oilseed rape plants where the effector AvrLm4-7 suppresses the recognition of AvrLm3 by the R protein Rlm3 (Petit-Houdenot & Fudal, 2017). An intriguing study in *Phytophthora sojae* evidenced the complex interactions that regulate microbial pathogenicity. *Phytophthora sojae* strains expressing the Avr3a gene are avirulent on plants carrying the resistance gene *rps3a*. In natural populations of this pathogen, the Avr3a gene displays allelic variation in messenger RNA transcript levels; some strains lack Avr3a mRNA escape detection by *rps3a* and are virulent. The naturally occurring silencing of Avr3a is mediated by unknown means but can be trans-generationally transferred. Additionally gene-silenced strains accumulate abundant small RNA molecules covering the Avr3a genomic region (Qutob et al., 2013). All in, it is clear that effector's properties (including patterns and levels of expression, translocation, and epistatic interactions) largely rely on their genetic and proteomic environment and confer emergent properties to the effectome.

#### **1.8.2.5 The host genetic repertoire defines the properties of the effectors.**

As discussed previously, many effectors require interaction with additional host molecules to become active. For instance, AvrRpt2 of *P. syringae* require eukaryotic cyclophilins to get activated (Popa, Tabuchi, et al., 2016). Interactions with the host cell machinery often leads to post-translational modifications in the effectors, including phosphorylation, ubiquitination, SUMOylation, myristoylation and acylation. These modifications seem to target the effectors to specific subcellular compartments and/or regulate their biochemical activities. Many effectors require these host-mediated modifications to exhibit full virulence or avirulence properties (Tahir et al., 2019; Popa et al., 2016). Effectors are classified according to their contribution to pathogenicity in avr factors if their activities in plant molecules (resistance molecules) leads by any means to the activation of immune responses. Conversely, effectors behave as virulence factors if their activities in host molecules (susceptibility molecules) contribute by any means to pathogenicity. Thus effector functions are dependent on the host repertoire as they acquire their “functional sense” only in association with their plant cognate interactors (Arroyo-Velez et al., 2020). Effectors behave differently among different plant cultivars depending on the presence or absence of specific resistance/susceptibility

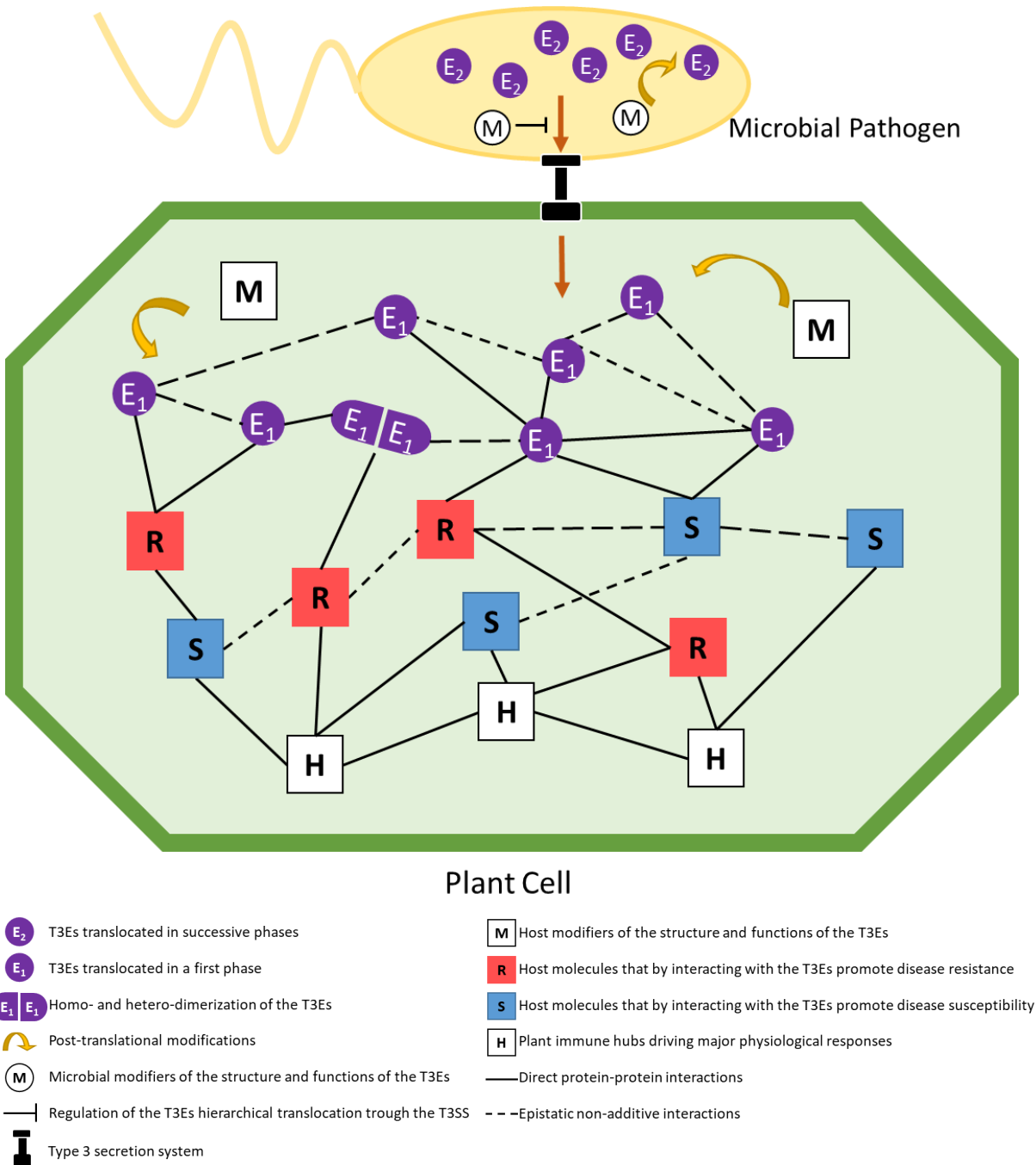
molecules, meaning that host specificity would result from the compatible or incompatible interactions between repertoires of microbial virulence genes and repertoires of genes involved in host defenses (resistance and susceptibility genes). Sometimes host specificity is determined by the presence of one or few pairs of cognate avr/R and vir/S genes (qualitative contribution). However, the quantitative contribution of many genes with moderate effects governs the outcome of the vast majority of host-pathogen interactions. Most of the current plant pathology research has focused on the qualitative basis of the plant-pathogen interactions since those are amenable to detailed molecular analysis. By contrast, the quantitative foundations involve many genes with small effects that are rather difficult to detect and analyze (Corwin & Kliebenstein, 2017).

The plant immune system is highly complex, thanks to its numerous components and more importantly to the numerous interactions among them. It has been reported that multiple resistance genes can redundantly recognize one effector, for instance Rlm1 and LepR3 from oilseed rape plant convergently recognize AvrLm1 of the ascomycete *Leptosphaeria maculans* (Petit-Houdenot & Fudal, 2017). In another example of complexity, Arabidopsis plants have an intricate mechanism to guard the functions of the MAPK4, composed of SUMM1, SUMM2 and SUMM3. It has been proposed that SUMM2 monitors elevated levels of activity of MAPK4 through the phosphorylation levels of SUMM3, and then SUMM2 provokes the upregulation of SUMM1, which in turn decreases MAPK4 activity (Nitta et al., 2020). Interestingly MAPK4 is targeted by several effectors with diverse effects; HopA11 is related with MPK4 inactivation while AvrB induces its phosphorylation (Lang & Colcombet, 2020). Concluding, the components of the plant immune system maintain intricate interactions that regulate their functions and responses to diverse microbial effectors.

### **1.8.3 The effectomes exert a wide range of activities**

As exposed above, effectomes are key for plant colonizing microbes. They mediate the interactions with the host and shape pathogen's host-range, lifestyle and niche. The range of activities that the effectome of a given pathogen exert within a given host is dictated mainly by:

- i) The repertoire of effectors coded in the microbe genome.
- ii) Emergent properties originated from the interaction with effector-modulating proteins derived from the microbe and the host (hierarchical expression and secretion, dimerization, redundancy, posttranslational modifications, etc.)
- iii) Repertoire of host genes (Figure 6).



**Figure 6. Scheme depicting the main factors driving the effectomes activities and properties.** Range of activities exerted by the effectome within the plant cell results from a given host is dictated mainly by: i) the repertoire of effectors coded in the microbe genome. ii) Emergent properties originated from the interaction with effector-modulating proteins derived from the microbe and the host (hierarchical expression and secretion, dimerization, redundancy, posttranslational modifications, etc.) iii) Repertoire of host genes.

#### 1.8.4 What is the best approach to tackle the properties of the effectome?

Conceptually the most intuitive approach to decipher the contribution of each effector to pathogenicity is to mutate single effector genes and quantify their impact in pathogenicity. This strategy has provided successful results in some particular cases, especially in fungal species (Pliego et al., 2013). However, the functional characterization of most of the known effectors has required alternative approaches.

In order to tackle the collective properties of the T3 effectome, many studies have reduced the number of effectors to characterize by constructing polymutant strains devoid of multiple effector genes. In *P. syringae* pv. *tomato* strain DC3000, the simultaneous and random mutation of multiple T3Es lead to the discovery that AvrE/HopR1/HopM1 and AvrPto/AvrPtoB form functional redundant groups that make major contributions to DC3000 growth in *N. benthamiana* plants (Kvitko et al., 2009). Strains of the pathogen *Yersinia enterocolitica* mutated in single or multiple T3Es were required to prove the implications of the T3E YopP in the suppression of tumor necrosis factor alpha (TNF- $\alpha$ ) release by infected macrophages (Boland & Cornelis, 1998). Sequential deletion of multiple effectors is a useful method to characterize T3Es. However, many of the properties exhibited by the T3Es depend on multiple complex factors that should be carefully considered such as the host genetic repertoire. For instance Castañeda et al. (2005) found that the simultaneous mutagenesis of eight Avr genes of the pathogen *Xanthomonas campestris* pv. *campestris* strain 528T had no detected effect on bacterial virulence in six cruciferous species, while the deletion of only one Avr gene enabled the mutants to become virulent on Florida Mustard plants.

In another attempt to avoid redundancy and epistasis in microbial effectomes, many T3Es have been heterologously expressed individually or collectively in different classes of organisms, producing a vast amount of information about their biochemical activities and physiological targets in host cells. For instance, Multiple T3Es have been expressed in the yeast *Saccharomyces cerevisiae* to reveal their plant-interactors by large-scale Yeast Two-Hybrid (Y2H) screens (Mukhtar et al., 2011; González-Fuente et al., 2020). Also, based on the assumption that the T3Es target key cellular processes conserved among eukaryotes, the ectopic expression of single T3Es in yeast revealed common targets inside host cells. Such as cytoskeleton components, GTPases of the Rho family, components of the MAPKs signaling pathways, proteins related with vesicle trafficking and membrane structures (Popa, Coll, et al., 2016; Salomon et al., 2012; Salomon & Sessa, 2010).

Genetically engineered strains of the bacterial species *Agrobacterium tumefaciens* are routinely used to mediate the transient expression of multiple T3Es *in planta*. Adlung et al., (2016) explored this strategy to express ectopically twenty one T3Es from different Xev strains in leaves of 86 non-host *Solanaceae* lines, allowing the recognition of the effector XopQ as a host range determinant in many *Nicotiana* species. Another strategy to deliver single or few effectors into plant hosts is by using nonpathogenic T3SS-proficient strains. This technique was explored with a nonpathogenic *Pseudomonas fluorescens* (*P. fluorescens*) strain that was genetically engineered to express the functional T3SS of *P. syringae* together with the effector HopA1. This modified strain was used to prove that many *P. syringae* effectors can suppress the HR response induced by HopA1 *in planta* (Guo et al., 2009). Similarly, in another study, the same T3SS-proficient *P. fluorescens* strain was used to test the ability of several *P. syringae* pv. *tomato* DC3000 effectors to suppress the reduced vascular flow associated with basal resistance in *N. benthamiana* leaves (Oh & Collmer, 2005).

In the phytopathology research field, numerous strategies have been developed to decipher the individual and collective properties of the microbial effectors. Undoubtedly, the studies exploring diverse and complementary approaches have provided the vast majority of the information that is known so far. The recent molecular characterization of the effector XopL of *Xanthomonas campestris* pv. *campestris* illustrates the conveniences of using multiple approaches: first, the mutation of the *xopL* gene in the strain 8004 affected pathogenicity progression in eight cultivars of Chinese cabbage. Then, the heterologous expression of XopL in *A. thaliana* plants from the inducible vector pER8, demonstrated that XopL promotes bacterial infection, affects plant development and suppresses several immune responses such as production of ROS, callose deposition and the expression of defense-related gene markers. Finally, the same inducible vector (pER8-*xopL*) was used to achieve the accumulation of XopL in *N. benthamiana* plants proving that *xopL* does not trigger immune responses *in planta* (X. Yan et al., 2019). A different study in the same pathogen and host species (*Xcc*) combined *in silico* predictions of candidate T3Es, translocation assays with the HR-inducing domain of AvrBs1 as a reporter and pathogenicity assays in Chinese radish to recognize six T3Es with functional translocation signals and active incidence in the virulence of the pathogen (W. Jiang et al., 2009).

Some studies are based on the culture of microbes in specific growth media to stimulate the production and secretion of the T3Es. For instance minimal medium (MMX) that is used to induce the expression of virulence-related genes in *Xanthomonas campestris* pv. *campestris*

(G.-F. Jiang et al., 2014). Although this strategy facilitates the experimental manipulations, it must be carefully considered since *in vitro* growth conditions do not always mimic the natural invasion as was evidenced in some experiments carried out in *Magnaporthe oryzae* (Mosquera et al., 2009). These results highlight the relevance of the approaches carried *in planta* to decipher the processes that truly occur in natural infections.

Deciphering the full range of activities deployed by microbial effectomes is crucial to understand and predict the outcomes of the plant-pathogen interactions. Considering the large range of potential activities deployed by the effectomes and the numerous factors tuning their properties, the most fruitful studies combined multiple approaches including *in silico* and *in vivo* strategies. The physiological and genetic background of the bacterial and host species were carefully considered to choose the growth and experimental conditions adequate to mimic natural infections. The future strategies to explore the complexity of the effectomes should continue under these guidelines.

#### **1.8.5 “From effectors to effectomes: Are functional studies of individual effectors enough to decipher plant pathogen infectious strategies?”**

During the completion of my thesis, in a joint effort, several members of the LIPME synthesized our knowledge and ideas around an opinion piece named “From effectors to effectomes: Are functional studies of individual effectors enough to decipher plant pathogen infectious strategies?”. In these lines, we discuss some of the limitations and challenges faced during the study of the T3Es as a result of their collective nature. In addition, we propose the sequential deconstruction of the effectome and the construction of synthetic effectomes as promising strategies to access the full range of activities exerted by the effectomes.

The opinion piece was published in the journal PLOS Pathogens (<https://doi.org/10.1371/journal.ppat.1009059>) on December 3<sup>rd</sup>, 2020. I actively contributed to the planning, preparation, and writing of the manuscript, which is presented in its entirety on the following pages.

## OPINION

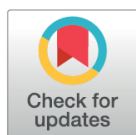
# From effectors to effectomes: Are functional studies of individual effectors enough to decipher plant pathogen infectious strategies?

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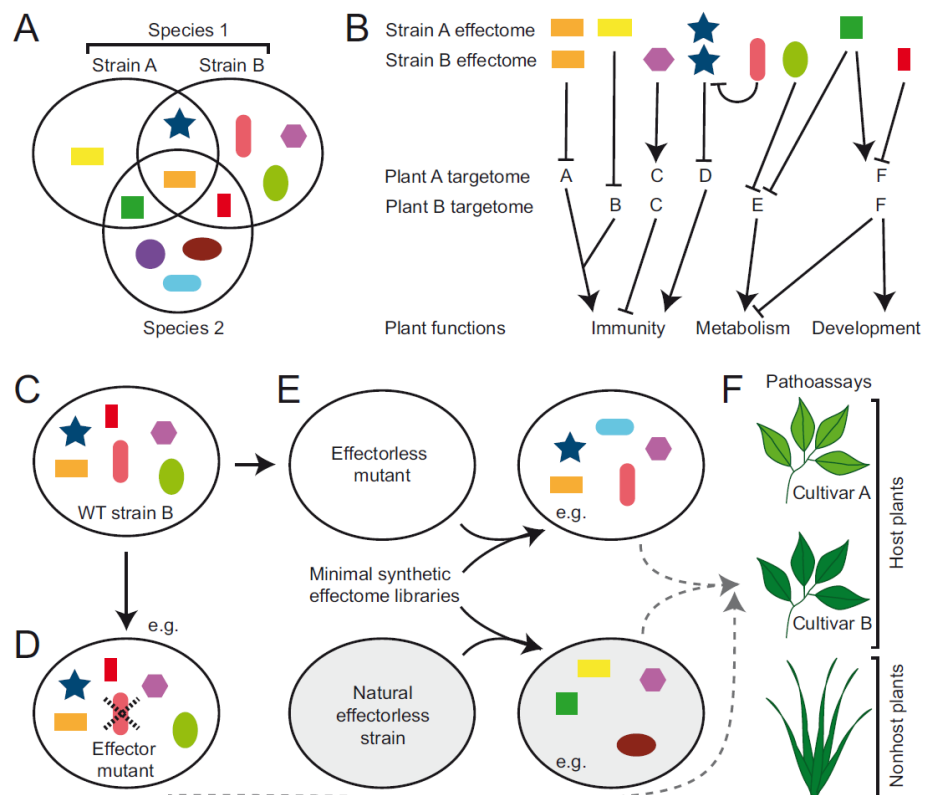
Effector proteins of plant pathogens are key virulence determinants which can be secreted in the apoplast or translocated inside plant cells where they subvert host immunity and physiology to the pathogen's benefit [1]. In some specific plant accessions, effector proteins may also be detected by plant immune receptors and trigger strong specific resistance [2–4].

## Achievements and limits of current effectors studies in plant pathogens

A pathogen's effectome (sometimes also referred to as effectorome) is the repertoire of all its effector proteins (Fig 1A). To date, most effector proteins are studied individually, omitting the broader context in which they function as the effectome. Size and composition of effectomes vary greatly between pathogens, including at the intraspecific level, ranging from as little as 4 in *Erwinia amylovora* to hundreds of effector proteins per isolate in some fungi, nematodes, and oomycetes (Fig 2A) [5–14]. These differences influence pathogen's virulence, life-style, and host range [15–17]. Known effector functions are the result of a combination of experimental approaches, often low throughput and based on *in vitro* or heterologous systems (Figs 1B, 2C and 2D) [18–21]. Some effectome-scale screens have been conducted, but these are still a compilation of individual effectors studies and thus present the same limitations as smaller-scale studies [22–28].

## Evidences for effector–effector interferences within effectomes

Studies of individual effector proteins intrinsically overlook their coordinated functions due to functional redundancy [29–31], expression patterns dependent on infection stages or plant organ [32–34], and epistatic interactions within effectomes [35–40] (Fig 2B). Therefore, effectome functions are usually not the sum of the individual effector functions (Fig 1C), and dedicated experimental approaches would be needed to determine how effectomes function as a whole. Prerequisites are the knowledge of the effectome composition, an experimentally manageable effectome size, and a genetically amenable pathogen. Consequently, functional characterization of effectomes is most advanced in bacteria [30,35,41,42] and developing at an ever increasing pace in fungi or nematodes thanks to powerful genome-editing tools [43,44] and



**Fig 2. Diversity of both the microbial effectome and the plant target repertoire impacts the function of the effectome.** (A) Effectomes are diverse at the intra- and interspecific levels. (B) Individual effectors can have 1 or multiple plant targets with either positive (arrowheads) or negative (blunt arrows) impacts. Hubs are plant proteins or functions which are targeted by multiple effectors. Some effectors might directly or indirectly affect the function of other effectors. To date, plant functions targeted by effectors are immunity, physiology, and metabolism. Distinct plant targets are affected depending on the pathogen effectome. Target diversity implies that different plant accessions will respond differently to distinct effectomes. (C–E) Schematic representation of possible genetic manipulations of effectomes. The effectome of WT strain B (C) can be genetically manipulated by deleting individual (D) or multiple effector genes yielding an effectome mutant (E). Effectorless strains found in the environment can also be used and complemented with the appropriate effector secretion-translocation machinery if missing. Examples of random or informed libraries corresponding to an effector combinatorial originating from strain B or any other strain could be reintroduced in an effectorless strain (E) and tested for functional complementations on host or nonhost plants of multiple cultivars (F). Each symbol represents a distinct effector produced by the pathogen. Members of a given effector protein family are represented with the same shape but different colors. WT, wild-type.

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sensitive high-throughput pathogenicity assays on automated phenotyping infrastructures [62–65].

### Synthetic effectomes to understand how effectomes really function

Effectome mutants open the possibility not only to identify avirulence genes which recognition can be masked by other effectors [35–40] but also to reconstruct synthetic effectomes and test for their function. The choice of the receiver strain and the composition and size of the minimal effectomes to be tested in infection tests have to be carefully considered (Fig 2E). While natural effectorless strains often require the introduction of a functional effector secretion-translocation machinery [66,67], effectome mutants might still express and translocate yet



unidentified effectors that could interfere with the characterization of synthetic effectomes [35,41]. Because of functional redundancy between effectors, functional synthetic effectomes can include only a portion of an original effectome (e.g., [41]). Effectors originating from other strains, species, genera, or even kingdoms could also be studied by such approaches as long as effector secretion-translocation happens (e.g., [68–70]). Though sometimes random [41], effector combinations have, up to now, been mostly based on gene families [60], gene clusters [41], or functional categories [30]. Yet, the combination of synthetic biology, next-generation sequencing technologies, and high-throughput phenotyping methods now opens the avenue for the generation of large random effector libraries to be tested in minimal strains and their functional characterization on host or nonhost plants (Fig 2F).

Similar limitations hindering effectomes characterization also apply to animal pathogens. Though these effectomes are also major virulence determinants (e.g., [71–74]) and the first effectome polymutant was generated in *Yersinia enterocolitica* [75], effectomes studies are also extremely limited in animal pathogens. We believe that the proposed holistic genetic approaches applied to effectomes should greatly advance our understanding of 2 basic questions: How do pathogens evolve and adapt to new hosts?

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## References

1. Toruno TY, Stergiopoulos I, Coaker G. Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners. *Annu Rev Phytopathol*. 2016; 54:419–41. Epub 2016 Jul 1. <https://doi.org/10.1146/annurev-phyto-080615-100204> PMID: 27359369; PubMed Central PMCID: PMC5283857.
2. Jones JD, Dangl JL. The plant immune system. *Nature*. 2006; 444(7117):323–9. Epub 2006 Nov 17. <https://doi.org/10.1038/nature05286> PMID: 17108957.
3. Kourelis J, van der Hoorn RAL. Defended to the Nines: 25 Years of Resistance Gene Cloning Identifies Nine Mechanisms for R Protein Function. *Plant Cell*. 2018; 30(2):285–99. Epub 2018 Feb 1. <https://doi.org/10.1105/tpc.17.00579> PMID: 29382771; PubMed Central PMCID: PMC5868693.
4. Spoel SH, Dong X. How do plants achieve immunity? Defence without specialized immune cells. *Nat Rev Immunol*. 2012; 12(2):89–100. Epub 2012 Jan 26. <https://doi.org/10.1038/nri3141> PMID: 22273771.
5. Kim KT, Jeon J, Choi J, Cheong K, Song H, Choi G, et al. Kingdom-Wide Analysis of Fungal Small Secreted Proteins (SSPs) Reveals their Potential Role in Host Association. *Front Plant Sci*. 2016; 7:186. Epub 2016 Mar 1. <https://doi.org/10.3389/fpls.2016.00186> PMID: 26925088; PubMed Central PMCID: PMC4759460.
6. Nissinen RM, Ytterberg AJ, Bogdanove AJ, KJ VANW, Beer SV. Analyses of the secretomes of *Erwinia amylovora* and selected *hrp* mutants reveal novel type III secreted proteins and an effect of HrpJ on extracellular harpin levels. *Mol Plant Pathol*. 2007; 8(1):55–67. Epub 2007 Jan 1. <https://doi.org/10.1111/j.1364-3703.2006.00370.x> PMID: 20507478.
7. Bogdanove AJ, Kim JF, Wei ZM, Kolchinsky P, Charkowski AO, Conlin AK, et al. Homology and functional similarity of an *hrp*-linked pathogenicity locus, *dspEF*, of *Erwinia amylovora* and the avirulence locus *avrE* of *Pseudomonas syringae* pathovar *tomato*. *Proc Natl Acad Sci U S A*. 1998; 95(3):1325–30. <https://doi.org/10.1073/pnas.95.3.1325> PMID: 9448330
8. Zhao Y, He SY, Sundin GW. The *Erwinia amylovora* *avrRpt2EA* gene contributes to virulence on pear and *AvrRpt2EA* is recognized by Arabidopsis RPS2 when expressed in *pseudomonas syringae*. *Mol Plant Microbe Interact*. 2006; 19(6):644–54. Epub 2006 Jun 17. <https://doi.org/10.1094/MPMI-19-0644> PMID: 16776298.
9. Schuster M, Schweizer G, Kahmann R. Comparative analyses of secreted proteins in plant pathogenic smut fungi and related basidiomycetes. *Fungal Genet Biol*. 2018; 112:21–30. Epub 2017 Jan 17. <https://doi.org/10.1016/j.fgb.2016.12.003> PMID: 28089076.

10. Dillon MM, Almeida RND, Laflamme B, Martel A, Weir BS, Desveaux D, et al. Molecular Evolution of *Pseudomonas syringae* Type III Secreted Effector Proteins. *Front Plant Sci.* 2019; 10:418. Epub 2019 Apr 27. <https://doi.org/10.3389/fpls.2019.00418> PMID: [31024592](#); PubMed Central PMCID: PMC6460904.
11. Vieira P, Gleason C. Plant-parasitic nematode effectors—insights into their diversity and new tools for their identification. *Curr Opin Plant Biol.* 2019; 50:37–43. Epub 2019 Mar 29. <https://doi.org/10.1016/j.pbi.2019.02.007> PMID: [30921686](#).
12. Roux B, Bolot S, Guy E, Denance N, Lautier M, Jardinaud MF, et al. Genomics and transcriptomics of *Xanthomonas campestris* species challenge the concept of core type III effectome. *BMC Genomics.* 2015; 16:975. <https://doi.org/10.1186/s12864-015-2190-0> PMID: [26581393](#); PubMed Central PMCID: PMC4652430.
13. Sabbagh CRR, Carrere S, Lonjon F, Vaillau F, Macho AP, Genin S, et al. Pangenomic type III effector database of the plant pathogenic *Ralstonia* spp. *PeerJ.* 2019; 7:e7346. Epub 2019 Oct 4. <https://doi.org/10.7717/peerj.7346> PMID: [31579561](#); PubMed Central PMCID: PMC6762002.
14. Badet T, Oggenfuss U, Abraham L, McDonald BA, Croll D. A 19-isolate reference-quality global pan-genome for the fungal wheat pathogen *Zymoseptoria tritici*. *BMC Biol.* 2020; 18(1):12. Epub 2020 Feb 13. <https://doi.org/10.1186/s12915-020-0744-3> PMID: [32046716](#); PubMed Central PMCID: PMC7014611.
15. Sarkar SF, Gordon JS, Martin GB, Guttman DS. Comparative genomics of host-specific virulence in *Pseudomonas syringae*. *Genetics.* 2006; 174(2):1041–56. Epub 2006 Sep 5. <https://doi.org/10.1534/genetics.106.060996> PMID: [16951068](#); PubMed Central PMCID: PMC1602070.
16. Liao J, Huang H, Meusnier I, Adreit H, Ducasse A, Bonnot F, et al. Pathogen effectors and plant immunity determine specialization of the blast fungus to rice subspecies. *Elife.* 2016; 5. Epub 2016 Dec 23. <https://doi.org/10.7554/eLife.19377> PMID: [28008850](#); PubMed Central PMCID: PMC5182064.
17. Gaulin E, Pel MJC, Camborde L, San-Clemente H, Courbier S, Dupouy MA, et al. Genomics analysis of *Aphanomyces* spp. identifies a new class of oomycete effector associated with host adaptation. *BMC Biol.* 2018; 16(1):43. Epub 2018 Apr 20. <https://doi.org/10.1186/s12915-018-0508-5> PMID: [29669603](#); PubMed Central PMCID: PMC5907361.
18. Dalio RJD, Herlihy J, Oliveira TS, McDowell JM, Machado M. Effector Biology in Focus: A Primer for Computational Prediction and Functional Characterization. *Mol Plant Microbe Interact.* 2018; 31(1):22–33. Epub 2017 Oct 13. <https://doi.org/10.1094/MPMI-07-17-0174-FI> PMID: [29023190](#).
19. Rehman S, Gupta VK, Goyal AK. Identification and functional analysis of secreted effectors from phyto-parasitic nematodes. *BMC Microbiol.* 2016; 16:48. Epub 2016 Mar 24. <https://doi.org/10.1186/s12866-016-0632-8> PMID: [27001199](#); PubMed Central PMCID: PMC4802876.
20. Kanja C, Hammond-Kosack KE. Proteinaceous effector discovery and characterization in filamentous plant pathogens. *Mol Plant Pathol.* 2020. Epub 2020 Aug 9. <https://doi.org/10.1111/mpp.12980> PMID: [32767620](#).
21. Varden FA, De la Concepcion JC, Maidment JH, Banfield MJ. Taking the stage: effectors in the spotlight. *Curr Opin Plant Biol.* 2017; 38:25–33. Epub 2017 May 2. <https://doi.org/10.1016/j.pbi.2017.04.013> PMID: [28460241](#).
22. Mukhtar MS, Carvunis AR, Dreze M, Eppe P, Steinbrenner J, Moore J, et al. Independently evolved virulence effectors converge onto hubs in a plant immune system network. *Science.* 2011; 333(6042):596–601. Epub 2011 Jul 30. <https://doi.org/10.1126/science.1203659> PMID: [21798943](#); PubMed Central PMCID: PMC3170753.
23. Chen S, Songkumarn P, Venu RC, Gowda M, Bellizzi M, Hu J, et al. Identification and characterization of *in planta*-expressed secreted effector proteins from *Magnaporthe oryzae* that induce cell death in rice. *Mol Plant Microbe Interact.* 2013; 26(2):191–202. Epub 2012 Oct 6. <https://doi.org/10.1094/MPMI-05-12-0117-R> PMID: [23035914](#).
24. Popov G, Fraiture M, Brunner F, Sessa G. Multiple *Xanthomonas euvesicatoria* Type III Effectors Inhibit flg22-Triggered Immunity. *Mol Plant Microbe Interact.* 2016; 29(8):651–60. Epub 2016 Aug 17. <https://doi.org/10.1094/MPMI-07-16-0137-R> PMID: [27529660](#).
25. Robin GP, Kleemann J, Neumann U, Cabre L, Dallery JF, Lapalu N, et al. Subcellular Localization Screening of *Colletotrichum higginsianum* Effector Candidates Identifies Fungal Proteins Targeted to Plant Peroxisomes, Golgi Bodies, and Microtubules. *Front Plant Sci.* 2018; 9:562. Epub 2018 May 18. <https://doi.org/10.3389/fpls.2018.00562> PMID: [29770142](#); PubMed Central PMCID: PMC5942036.
26. González-Fuente M, Carrère S, Monachello D, Marsella BG, Cazalé A-C, Zischek C, et al. EffectorK, a comprehensive resource to mine for *Ralstonia*, *Xanthomonas* and other published effector interactors in the Arabidopsis proteome. *Mol Plant Pathol.* 2020; 21(10):1257–70. <https://doi.org/10.1111/mpp.12965>

27. Wessling R, Eppe P, Altmann S, He Y, Yang L, Henz SR, et al. Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell Host Microbe*. 2014; 16(3):364–75. Epub 2014 Sep 12. <https://doi.org/10.1016/j.chom.2014.08.004> PMID: 25211078; PubMed Central PMCID: PMC4191710.
28. Wroblewski T, Caldwell KS, Piskurewicz U, Cavanaugh KA, Xu H, Kozik A, et al. Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathogens of *Pseudomonas* and *Ralstonia*. *Plant Physiol*. 2009; 150(4):1733–49. Epub 2009 Jul 3. <https://doi.org/10.1104/pp.109.140251> PMID: 19571308; PubMed Central PMCID: PMC2719141.
29. Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD. Characterization of the interaction of a novel *Stagonospora nodorum* host-selective toxin with a wheat susceptibility gene. *Plant Physiol*. 2008; 146(2):682–93. Epub 2007 Dec 11. <https://doi.org/10.1104/pp.107.108761> PMID: 18065563; PubMed Central PMCID: PMC2245837.
30. Kvitko BH, Park DH, Velasquez AC, Wei CF, Russell AB, Martin GB, et al. Deletions in the repertoire of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. *PLoS Pathog*. 2009; 5(4):e1000388. Epub 2009 Apr 22. <https://doi.org/10.1371/journal.ppat.1000388> PMID: 19381254; PubMed Central PMCID: PMC2663052.
31. Thordal-Christensen H, Birch PRJ, Spanu PD, Panstruga R. Why did filamentous plant pathogens evolve the potential to secrete hundreds of effectors to enable disease? *Mol Plant Pathol*. 2018; 19(4):781–5. Epub 2018 Mar 15. <https://doi.org/10.1111/mpp.12649> PMID: 29536647; PubMed Central PMCID: PMC6638121.
32. Kleemann J, Rincon-Rivera LJ, Takahara H, Neumann U, Ver Loren van Themaat E, van der Does HC, et al. Sequential delivery of host-induced virulence effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. *PLoS Pathog*. 2012; 8(4):e1002643. Epub 2012 Apr 13. <https://doi.org/10.1371/journal.ppat.1002643> PMID: 22496661; PubMed Central PMCID: PMC3320591.
33. Wang Q, Han C, Ferreira AO, Yu X, Ye W, Tripathy S, et al. Transcriptional programming and functional interactions within the *Phytophthora sojae* RXLR effector repertoire. *Plant Cell*. 2011; 23(6):2064–86. Epub 2011 Jun 10. <https://doi.org/10.1105/tpc.111.086082> PMID: 21653195; PubMed Central PMCID: PMC3160037.
34. Skibbe DS, Doehlemann G, Fernandes J, Walbot V. Maize tumors caused by *Ustilago maydis* require organ-specific genes in host and pathogen. *Science*. 2010; 328(5974):89–92. Epub 2010 Apr 3. <https://doi.org/10.1126/science.1185775> PMID: 20360107.
35. Wei HL, Chakravarthy S, Mathieu J, Helmann TC, Stodghill P, Swingle B, et al. *Pseudomonas syringae* pv. *tomato* DC3000 Type III Secretion Effector Polymutants Reveal an Interplay between HopAD1 and AvrPtoB. *Cell Host Microbe*. 2015; 17(6):752–62. Epub 2015 Jun 13. <https://doi.org/10.1016/j.chom.2015.05.007> PMID: 26067603; PubMed Central PMCID: PMC4471848.
36. Phan HT, Rybak K, Furuki E, Breen S, Solomon PS, Oliver RP, et al. Differential effector gene expression underpins epistasis in a plant fungal disease. *Plant J*. 2016; 87(4):343–54. Epub 2016 May 3. <https://doi.org/10.1111/tj.13203> PMID: 27133896; PubMed Central PMCID: PMC5053286.
37. Guo X, Zhong D, Xie W, He Y, Zheng Y, Lin Y, et al. Functional Identification of Novel Cell Death-inducing Effector Proteins from *Magnaporthe oryzae*. *Rice (N Y)*. 2019; 12(1):59. Epub 2019 Aug 8. <https://doi.org/10.1186/s12284-019-0312-z> PMID: 31388773; PubMed Central PMCID: PMC6684714.
38. Wei HL, Zhang W, Collmer A. Modular Study of the Type III Effector Repertoire in *Pseudomonas syringae* pv. *tomato* DC3000 Reveals a Matrix of Effector Interplay in Pathogenesis. *Cell Rep*. 2018; 23(6):1630–8. Epub 2018 May 10. <https://doi.org/10.1016/j.celrep.2018.04.037> PMID: 29742421.
39. Bourras S, McNally KE, Ben-David R, Parlange F, Roffler S, Praz CR, et al. Multiple Avirulence Loci and Allele-Specific Effector Recognition Control the *Pm3* Race-Specific Resistance of Wheat to Powdery Mildew. *Plant Cell*. 2015; 27(10):2991–3012. Epub 2015 Oct 11. <https://doi.org/10.1105/tpc.15.00171> PMID: 26452600; PubMed Central PMCID: PMC4682313.
40. Thordal-Christensen H. A holistic view on plant effector-triggered immunity presented as an iceberg model. *Cell Mol Life Sci*. 2020. Epub 2020 Apr 12. <https://doi.org/10.1007/s00018-020-03515-w> PMID: 32277261.
41. Cunnac S, Chakravarthy S, Kvitko BH, Russell AB, Martin GB, Collmer A. Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. *Proc Natl Acad Sci U S A*. 2011; 108(7):2975–80. Epub 2011 Feb 2. <https://doi.org/10.1073/pnas.1013031108> PMID: 21282655; PubMed Central PMCID: PMC3041132.
42. Wei HL, Collmer A. Defining essential processes in plant pathogenesis with *Pseudomonas syringae* pv. *tomato* DC3000 disabled polymutants and a subset of key type III effectors. *Mol Plant Pathol*. 2018; 19(7):1779–94. Epub 2017 Dec 27. <https://doi.org/10.1111/mpp.12655> PMID: 29277959; PubMed Central PMCID: PMC6638048.

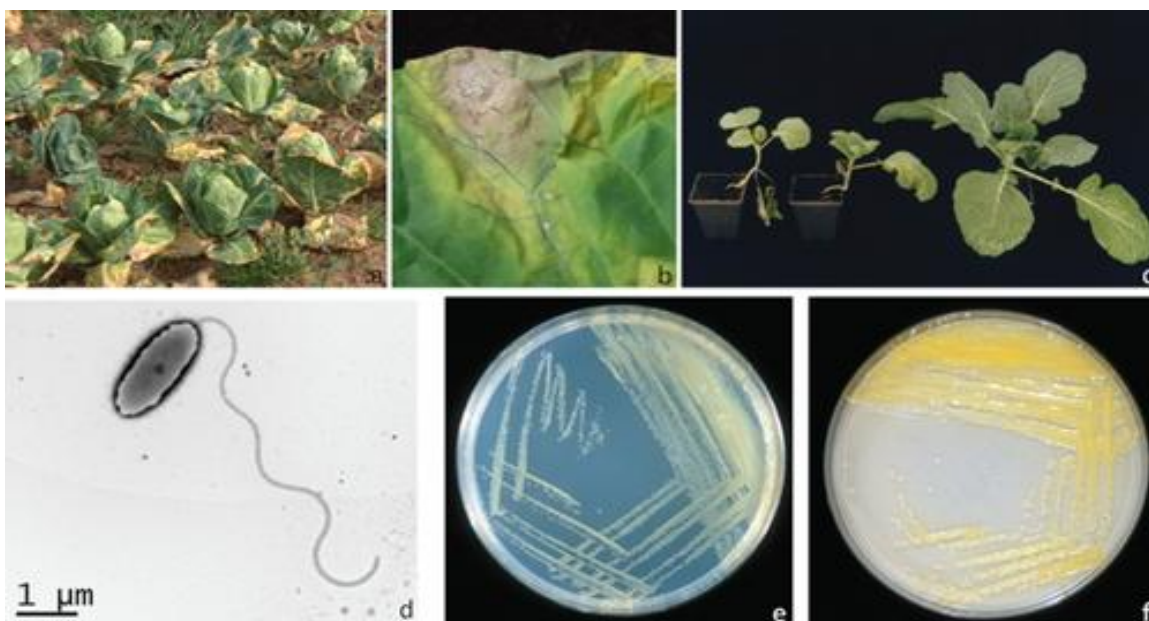


43. Nodvig CS, Nielsen JB, Kogle ME, Mortensen UH. A CRISPR-Cas9 System for Genetic Engineering of Filamentous Fungi. *PLoS ONE*. 2015; 10(7):e0133085. Epub 2015 Jul 16. <https://doi.org/10.1371/journal.pone.0133085> PMID: 26177455; PubMed Central PMCID: PMC4503723.
44. Friedland AE, Tzur YB, Esvelt KM, Colaiacovo MP, Church GM, Calarco JA. Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nat Methods*. 2013; 10(8):741–3. Epub 2013 Jul 3. <https://doi.org/10.1038/nmeth.2532> PMID: 23817069; PubMed Central PMCID: PMC3822328.
45. Kamper J, Kahmann R, Bolker M, Ma LJ, Brefort T, Saville BJ, et al. Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature*. 2006; 444(7115):97–101. Epub 2006 Nov 3. <https://doi.org/10.1038/nature05248> PMID: 17080091.
46. Brefort T, Tanaka S, Neidig N, Doehlemann G, Vincon V, Kahmann R. Characterization of the largest effector gene cluster of *Ustilago maydis*. *PLoS Pathog*. 2014; 10(7):e1003866. Epub 2014 Jul 6. <https://doi.org/10.1371/journal.ppat.1003866> PMID: 24992561; PubMed Central PMCID: PMC4081774.
47. Eisermann I, Weihmann F, Krijger JJ, Kroling C, Hause G, Menzel M, et al. Two genes in a pathogenicity gene cluster encoding secreted proteins are required for appressorial penetration and infection of the maize anthracnose fungus *Colletotrichum graminicola*. *Environ Microbiol*. 2019; 21(12):4773–91. Epub 2019 Oct 11. <https://doi.org/10.1111/1462-2920.14819> PMID: 31599055.
48. Li H, Zhou Y, Zhang Z. Network Analysis Reveals a Common Host-Pathogen Interaction Pattern in Arabidopsis Immune Responses. *Front Plant Sci*. 2017; 8:893. Epub 2017 Jun 15. <https://doi.org/10.3389/fpls.2017.00893> PMID: 28611808; PubMed Central PMCID: PMC5446985.
49. Ahmed H, Howton TC, Sun Y, Weinberger N, Belkadir Y, Mukhtar MS. Network biology discovers pathogen contact points in host protein-protein interactomes. *Nat Commun*. 2018; 9(1):2312. Epub 2018 Jun 15. <https://doi.org/10.1038/s41467-018-04632-8> PMID: 29899369; PubMed Central PMCID: PMC5998135.
50. Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*. 2009; 326(5959):1509–12. Epub 2009 Nov 26. <https://doi.org/10.1126/science.1178811> PMID: 19933107.
51. Moscou MJ, Bogdanove AJ. A simple cipher governs DNA recognition by TAL effectors. *Science*. 2009; 326(5959):1501. Epub 2009 Nov 26. <https://doi.org/10.1126/science.1178817> PMID: 19933106.
52. de Jonge R, van Esse HP, Kombrink A, Shinya T, Desaki Y, Bours R, et al. Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. *Science*. 2010; 329(5994):953–5. Epub 2010 Aug 21. <https://doi.org/10.1126/science.1190859> PMID: 20724636.
53. Mentlak TA, Kombrink A, Shinya T, Ryder LS, Otomo I, Saitoh H, et al. Effector-mediated suppression of chitin-triggered immunity by *magnaporthe oryzae* is necessary for rice blast disease. *Plant Cell*. 2012; 24(1):322–35. Epub 2012 Jan 24. <https://doi.org/10.1105/tpc.111.092957> PMID: 22267486; PubMed Central PMCID: PMC3289562.
54. Salomon D, Guo Y, Kinch LN, Grishin NV, Gardner KH, Orth K. Effectors of animal and plant pathogens use a common domain to bind host phosphoinositides. *Nat Commun*. 2013; 4:2973. Epub 2013 Dec 19. <https://doi.org/10.1038/ncomms3973> PMID: 24346350; PubMed Central PMCID: PMC4981085.
55. Hajri A, Brin C, Hunault G, Lardeux F, Lemaire C, Manceau C, et al. A "repertoire for repertoire" hypothesis: repertoires of type three effectors are candidate determinants of host specificity in *Xanthomonas*. *PLoS ONE*. 2009; 4(8):e6632. Epub 2009 Aug 15. <https://doi.org/10.1371/journal.pone.0006632> PMID: 19680562; PubMed Central PMCID: PMC2722093.
56. Casadevall A, Fang FC, Pirofski LA. Microbial virulence as an emergent property: consequences and opportunities. *PLoS Pathog*. 2011; 7(7):e1002136. Epub 2011 Aug 5. <https://doi.org/10.1371/journal.ppat.1002136> PMID: 21814511; PubMed Central PMCID: PMC3141035.
57. Angot A, Peeters N, Lechner E, Vailleau F, Baud C, Gentzbittel L, et al. *Ralstonia solanacearum* requires F-box-like domain-containing type III effectors to promote disease on several host plants. *Proc Natl Acad Sci U S A*. 2006; 103(39):14620–5. Epub 2006 Sep 20. <https://doi.org/10.1073/pnas.0509393103> PMID: 16983093; PubMed Central PMCID: PMC1600009.
58. Khrunyk Y, Munch K, Schipper K, Lupas AN, Kahmann R. The use of FLP-mediated recombination for the functional analysis of an effector gene family in the biotrophic smut fungus *Ustilago maydis*. *New Phytol*. 2010; 187(4):957–68. Epub 2010 Aug 3. <https://doi.org/10.1111/j.1469-8137.2010.03413.x> PMID: 20673282.
59. Sole M, Popa C, Mith O, Sohn KH, Jones JD, Deslandes L, et al. The awr gene family encodes a novel class of *Ralstonia solanacearum* type III effectors displaying virulence and avirulence activities. *Mol Plant Microbe Interact*. 2012; 25(7):941–53. Epub 2012 Mar 15. <https://doi.org/10.1094/MPMI-12-11-0321> PMID: 22414437.
60. Kay S, Boch J, Bonas U. Characterization of AvrBs3-like effectors from a Brassicaceae pathogen reveals virulence and avirulence activities and a protein with a novel repeat architecture. *Mol Plant*

- Microbe Interact. 2005; 18(8):838–48. Epub 2005 Sep 2. <https://doi.org/10.1094/MPMI-18-0838> PMID: 16134896.
61. Lei N, Chen L, Kiba A, Hikichi Y, Zhang Y, Ohnishi K. Super-Multiple Deletion Analysis of Type III Effectors in *Ralstonia solanacearum* OE1-1 for Full Virulence Toward Host Plants. Front Microbiol. 2020; 11:1683. Epub 2020 Aug 28. <https://doi.org/10.3389/fmicb.2020.01683> PMID: 32849353; PubMed Central PMCID: PMC7409329.
62. Fichman Y, Miller G, Mittler R. Whole-Plant Live Imaging of Reactive Oxygen Species. Mol Plant. 2019; 12(9):1203–10. Epub 2019 Jun 21. <https://doi.org/10.1016/j.molp.2019.06.003> PMID: 31220601.
63. Pieruschka R, Schurr U. Plant Phenotyping: Past, Present, and Future. Plant Phenomics. 2019; 2019:7507131. <https://doi.org/10.34133/2019/7507131>
64. Tisne S, Serrand Y, Bach L, Gilbault E, Ben Ameur R, Balasse H, et al. Phenoscope: an automated large-scale phenotyping platform offering high spatial homogeneity. Plant J. 2013; 74(3):534–44. Epub 2013 Mar 5. <https://doi.org/10.1111/tpj.12131> PMID: 23452317.
65. Yang W, Feng H, Zhang X, Zhang J, Doonan JH, Batchelor WD, et al. Crop Phenomics and High-Throughput Phenotyping: Past Decades, Current Challenges, and Future Perspectives. Mol Plant. 2020; 13(2):187–214. Epub 2020 Jan 26. <https://doi.org/10.1016/j.molp.2020.01.008> PMID: 31981735.
66. Collmer A, Badel JL, Charkowski AO, Deng WL, Fouts DE, Ramos AR, et al. *Pseudomonas syringae* Hrp type III secretion system and effector proteins. Proc Natl Acad Sci U S A. 2000; 97(16):8770–7. Epub 2000 Aug 2. <https://doi.org/10.1073/pnas.97.16.8770> PMID: 10922033; PubMed Central PMCID: PMC34010.
67. Meline V, Delage W, Brin C, Li-Marchetti C, Sochard D, Arlat M, et al. Role of the acquisition of a type 3 secretion system in the emergence of novel pathogenic strains of *Xanthomonas*. Mol Plant Pathol. 2019; 20(1):33–50. Epub 2018 Aug 5. <https://doi.org/10.1111/mpp.12737> PMID: 30076773; PubMed Central PMCID: PMC6430459.
68. Upadhyaya NM, Mago R, Staskawicz BJ, Ayliffe MA, Ellis JG, Dodds PN. A bacterial type III secretion assay for delivery of fungal effector proteins into wheat. Mol Plant Microbe Interact. 2014; 27(3):255–64. Epub 2013 Oct 26. <https://doi.org/10.1094/MPMI-07-13-0187-FI> PMID: 24156769.
69. Sohn KH, Lei R, Nemri A, Jones JD. The downy mildew effector proteins ATR1 and ATR13 promote disease susceptibility in *Arabidopsis thaliana*. Plant Cell. 2007; 19(12):4077–90. Epub 2008 Jan 1. <https://doi.org/10.1105/tpc.107.054262> PMID: 18165328; PubMed Central PMCID: PMC2217653.
70. Fabro G, Steinbrenner J, Coates M, Ishaque N, Baxter L, Studholme DJ, et al. Multiple candidate effectors from the oomycete pathogen *Hyaloperonospora arabidopsidis* suppress host plant immunity. PLoS Pathog. 2011; 7(11):e1002348. Epub 2011 Nov 11. <https://doi.org/10.1371/journal.ppat.1002348> PMID: 22072967; PubMed Central PMCID: PMC3207932.
71. Coburn B, Sekirov I, Finlay BB. Type III secretion systems and disease. Clin Microbiol Rev. 2007; 20(4):535–49. Epub 2007 Oct 16. <https://doi.org/10.1128/CMR.00013-07> PMID: 17934073; PubMed Central PMCID: PMC2176049.
72. Marcos CM, de Oliveira HC, de Melo WC, da Silva JF, Assato PA, Scorzoni L, et al. Anti-Immune Strategies of Pathogenic Fungi. Front Cell Infect Microbiol. 2016; 6:142. Epub 2016 Nov 30. <https://doi.org/10.3389/fcimb.2016.00142> PMID: 27896220; PubMed Central PMCID: PMC5108756.
73. Gan J, Giogha C, Hartland EL. Molecular mechanisms employed by enteric bacterial pathogens to antagonise host innate immunity. Curr Opin Microbiol. 2020; 59:58–64. Epub 2020 Aug 31. <https://doi.org/10.1016/j.mib.2020.07.015> PMID: 32862049.
74. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of parasite secreted proteins in modulating host immunity. Mol Biochem Parasitol. 2009; 167(1):1–11. Epub 2009 May 2. <https://doi.org/10.1016/j.molbiopara.2009.04.008> PMID: 19406170; PubMed Central PMCID: PMC2706953.
75. Boyd AP, Grosdent N, Totemeyer S, Geuijen C, Bleves S, Iriarte M, et al. *Yersinia enterocolitica* can deliver Yop proteins into a wide range of cell types: development of a delivery system for heterologous proteins. Eur J Cell Biol. 2000; 79(10):659–71. Epub 2000 Nov 23. <https://doi.org/10.1078/0171-9335-00098> PMID: 11089914.

### 1.9 *Xanthomonas campestris*: a useful pathosystem to unravel the T3 effectome

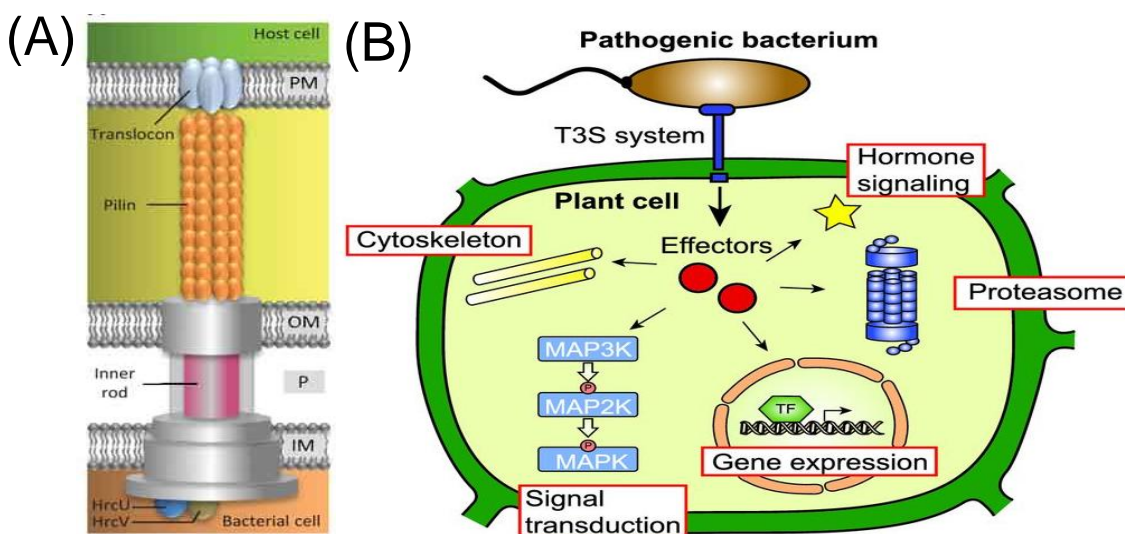
*Xanthomonas campestris* pv. *campestris* (*Xcc*) is a Gram-negative bacterium of the *Xanthomonadaceae* family that causes the black rot disease in a large number of edible species belonging to the Brassicaceae group such as cabbage, cauliflower, broccoli, Brussels sprouts and kale. Therefore, it is recognized as one of the major plant pathogenic bacteria from an economic, agricultural and academic perspective (Mansfield et al., 2012). *Xcc* also colonizes ornamental plant species and many other weeds including *Arabidopsis thaliana*. Usually this phytopathogen is vertically transferred from parental plants to seeds, although it can also be spread horizontally by biotic (animals and insects) and abiotic factors (rain, irrigation water, infested soil, plant debris, wind etc.)(Vicente & Holub, 2013). The bacteria persist as epiphytes on the plant surface and access to plant inner tissues through hydathodes or wounds, then colonize xylem vessels and spread systemically (Cerutti et al., 2017). Typical disease symptoms are V-shaped chlorotic lesions extending from leaf margins to the vascular system. In later stages *Xcc* cause the blackening of the veins and necrosis of plant tissues (Figure 7). The disease is favored by warm and humid conditions, although it is world-wide distributed, it is more prevalent in tropical and subtropical regions (Vicente & Holub, 2013).



**Figure 7. *Xcc* characteristics and symptoms.** Symptoms of black rot on cabbage (a). Typical black rot V-shaped lesion on a cabbage leaf (b). Plants of Savoy cabbage with symptoms of *Xcc* systemic infection (left) and a healthy control plant (right) (c). Electron microscopy image of a *X. campestris* pv. *campestris* rod-shaped cell (d). *Xanthomonas campestris* pv. *campestris* culture growing on King's medium B (e) or Yeast Dextrose Calcium Carbonate medium (f). Adapted from Vicente & Holub, 2013.



*Xanthomonas* species deploy a battery of virulence factors to modulate plant physiology, which is essential for host colonization (Büttner & Bonas, 2010). One of the best studied is the production of the polymer xanthan that protects bacteria from environmental stress and have been related with the suppression of basal plant defense responses such as callose deposition (Büttner & Bonas, 2010). The key determinant of *Xcc* pathogenicity is the secretion of effector molecules. To date six types of protein secretion systems (type I to type VI) have been recognized in *Xanthomonas* species, although the most decisive is the T3SS. Most of genes encoding the T3SS are included in the Hypersensitive Response and Pathogenicity (*hrp*) gene cluster. Its sequence is conserved among the different *Xanthomonas* species and its expression is tightly regulated by the master regulators HrpG and HrpX. The transcription regulator HrpG promotes the expression of HrpX that directly binds to the plant-inducible promoter (PIP) box, (TTCGB-N15/N8-TTCGB) found in the promoter region of five of the six structural T3SS operons and of multiple T3Es to induce their transcription (Teper et al., 2021). The T3SS of *Xanthomonas* consists from inside to outside of peripheral cytoplasm components, Inner Membrane (IM) components (HrcUVRST), outer membrane (OM) component (HrcC), extracellular pilin subunits that form the pilus and translocon proteins that form a pore in the host plasma membrane (PM) (Figure 8).



**Figure 8. Components and functions of the T3SS.** (A) Schematic representation of the T3SS of *Xanthomonas euvesicatoria*. The basal body of the T3SS spans the innermembrane (IM), the periplasm (P) and the outermembrane (OM) of the bacterium. Pilin subunits form the pilus and translocon proteins form a pore in the host plasma membrane (PM) (Adapted from Lohou et al., 2013). (B) Overview on known plant targets of type III effectors. Translocated effector proteins interfere with the assembly of the cytoskeleton, MAPK cascades, gene expression, proteasome-dependent protein degradation or hormone signaling pathways (Adapted from Büttner, 2016).

The T3Es deployed by *Xanthomonas* species are designated as Xop's (*Xanthomonas* outer proteins) with some exceptions like AvrBs1, AvrBs2, AvrXccA1 and AvrXccA2 among others. Currently, more than 50 Xop families have been identified, with an alphabetical nomenclature from XopA to XopBA (<http://Xanthomonas.org>). Identification of putative T3Es has relied on homology-based searches and more recently in machine-learning approaches to identify characteristic patterns of T3Es such as secretion signals, amino acid composition, conserved motifs, structural disorder, regulation by HrpX and HrpG, GC content, codon usage and homology to known and validated T3Es (Teper et al., 2016). In some cases subsequent functional reporter assays confirm translocation of the putative T3Es into the plant cell (Timilsina et al., 2020).

In an early effort to clarify the species, *Xcc* strains were grouped in races based on their host range (Fargier & Manceau, 2007); further phylogenetic analysis found a significant clade/race correlation suggesting that the host shaped the evolution of *Xcc* (Guy et al., 2013).

*In silico* studies on genomes of three *Xcc* strains (B100, ATCC 33913, and 8004) identified twenty nine different genes encoding putative type 3 secreted proteins (T3SPs). Fifteen of those were shared in a large number of *Xcc* strains while the presence of the fourteen remaining was variable among the strains analyzed, suggesting that *Xcc* isolates present an important natural genetic diversity (Guy et al., 2013).

The *Xcc* strain 8004 presents one of the most diverse T3 effectomes among the different isolates of *Xcc* sequenced so far (Guy et al., 2013) while maintaining an amenable size for extensive molecular studies. The *Xcc* strain 8004 was originated as a spontaneous Rif<sup>R</sup> mutant of the *Xcc* strain NCPPB 1145 (Turner et al., 1984). This strain has been used to characterize many *Xcc* effectors; below (Table 1) are summarized all the T3Es described so far in *Xcc* strain 8004 (<http://Xanthomonas.org>).

Table 1. T3Es described so far in *Xcc* strain 8004 ([xantho.org](http://xantho.org)).

Family	Current knowedlege
AvrBs1	It has been better characterized in <i>Xanthomonas euvesicatoria</i> (Xev). In <i>N. benthamiana</i> , localizes to the plant cell cytoplasm, and induces an enlargement of the central vacuole and the cell wall; causes an increased ion efflux (Gürlebeck et al., 2009).
AvrBs2	An <i>A. thaliana</i> mesophyll protoplast-based assay identified that <i>Xcc</i> 8004 AvrBs2 inhibits expression of the flg22-inducible gene FRK1 (Y. Huang et al., 2020).
AvrXccA1	Also known as AvrXca. An orthologous gene in <i>Xanthomonas campestris</i> pv. <i>raphani</i> was transferred



	into Xcc8004 and strongly reducing symptom development and bacterial growth in <i>A. thaliana</i> plants (Parker et al., 1993).
AvrXccA2	Only predicted by <i>in silico</i> approaches, might not be a true T3E (xantho.org)
HpaA	Characterized in <i>Xev</i> . HpaA interacts with HpaB to promote secretion of pilus, translocon and effector proteins, HpaA seems to be secreted and translocated itself by the T3S system (Lorenz et al., 2008).
HrpW	A hairpin protein. An orthologous gene was first described in <i>Erwinia amylovora</i> (J. F. Kim & Beer, 1998). An <i>A. thaliana</i> mesophyll protoplast-based assay identified that Xcc8004 HrpW inhibit expression of the flg22-inducible gene FRK1 (Y. Huang et al., 2020)
XopA	Characterized in <i>Xev</i> . XopA is secreted by the T3SS. Mutations in <i>xopA</i> resulted in reduced bacterial growth <i>in planta</i> and delayed plant reactions in susceptible and resistant host plants (Noël et al., 2002).
XopAC	Formerly known as AvrAC. Uridylylates BIK1 kinase and the decoy protein PBL2 to suppress and trigger plant basal immunity respectively (G. Wang et al., 2015).
XopAG	An Arabidopsis mesophyll protoplast-based assay identified that Xcc8004 XopAG inhibits expression of the flg22-inducible gene <i>frk1</i> (Y. Huang et al., 2020). Additionally <i>Xanthomonas fuscans ssp. aurantifolii</i> possesses AvrGf2 that belongs to the XopAG effector family and induces disease resistance in grapefruit (Gochez et al., 2017).
XopAH	Also known as AvrXccC. XopAH of Xcc8004 conferred avirulence on mustard and Chinese cabbage (Y.-Q. He et al., 2007). Manipulates ABA homeostasis (Ho et al., 2013), induces PR1 expression and confers resistance in Arabidopsis plants in a RAR1- and SGT1b-dependent manner (Rong et al., 2010).
XopAL	No information available
XopAM	XopAM of Xcc8004 functions as an avirulence gene on Arabidopsis plants of the ecotype Col-0 (Guy et al., 2013).
XopAR	Formed by 132 aminoacids. Present in <i>Xcc</i> and <i>Raltonia solanacearum</i> . (Emanuelle Lauber, personal communication)
XopAY	No information available
XopAZ	No information available
XopD	XopD of Xcc8004 triggers plant disease tolerance in Arabidopsis plants by targeting DELLA proteins (L. Tan et al., 2014) and elicit the accumulation of host defense-response genes dependent on salicylic acid and desumoylate the transcription factor HFR1 in photomorphogenesis in Arabidopsis plants (C. M. Tan et al., 2015).
XopE	Characterized in <i>Xev</i> . XopE2 of <i>Xev</i> inhibits flg22-induced callose deposition <i>in planta</i> and enhances disease symptoms caused by attenuated <i>Pseudomonas syringae</i> bacteria (Popov et al., 2016).
XopF	In <i>Xev</i> XopF1 is secreted and translocated by the T3SS in a HpaB and HpaH dependent manner (Büttner et al., 2007).
XopG	XopG of Xcc8004 inhibit expression of the flg22-inducible gene WRKY33 in Arabidopsis mesophyll protoplast (Y. Huang et al., 2020).
XopH	XopH of <i>Xev</i> possesses phytase activity and dephosphorylates phytate (myo-inositol-hexakisphosphate, InsP6), the major phosphate storage compound in plants, which is also involved in pathogen defense (Blüher et al., 2017).
XopJ	Characterized in <i>Xev</i> . <i>Xev</i> XopJ degrades RPT6, leading to reduced proteasome activity impairing the proteasomal turnover of Nonexpressor of Pathogenesis-Related1 (NPR1), the master regulator of

	salicylic acid responses, leading to the accumulation of ubiquitinated NPR1 which interferes with the full induction of NPR1 target genes (Üstün & Börnke, 2015).
XopK	XopK of <i>Xcc8004</i> inhibits the expression of the flg22-inducible genes FRK1 and WRKY33 and affects the activation of the MAPK3 and MAPK6 in Arabidopsis mesophyll protoplasts (Y. Huang et al., 2020). In <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> XopK inhibits PTI responses in rice by ubiquitinating OsSERK2 that results in its degradation (Qin et al., 2018).
XopL	<i>Xcc8004</i> XopL promotes bacterial infection, affects plant development and suppresses ROS production, callose deposition and the expression of several defense-related gene markers (X. Yan et al., 2019).
XopM	In <i>Xev</i> , XopM was proven to be translocated by the T3SS and its heterologous expression causes cell death in <i>N. benthamiana</i> leaves (S. Schulze et al., 2012). No information available in <i>Xcc</i> .
XopN	XopN secretion by the T3SS has been validated and is required for <i>Xcc8004</i> full virulence in Chinese radish (B.-L. Jiang et al., 2008). Additionally XopN of <i>Xcc8004</i> inhibits the expression of the flg22-inducible gene FRK1 in Arabidopsis mesophyll protoplasts (Y. Huang et al., 2020).
XopP	No references found in <i>Xcc</i> . Studies in <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> revealed that XopP can suppress the rice immune response induced by the heterologous expression of both XopQ-XopX (Deb et al., 2020). Additionally XopP directly inhibits the positive regulator of rice immunity OsPUB44 (Ishikawa et al., 2014).
XopQ	XopQ of <i>Xcc8004</i> inhibits the expression of the flg22-inducible genes FRK1 and WRKY33 and affect the activation of the MAPK3 and MAPK6 in Arabidopsis mesophyll protoplasts (Y. Huang et al., 2020). In <i>Xev</i> XopQ is recognized by dozens of <i>Nicotiana</i> spp. This recognition is dependent on EDS1 and induces cell death (Adlung et al., 2016).
XopR	XopR of <i>Xcc8004</i> manipulates the host actin cytoskeleton by forming a macromolecular complex with actin-binding proteins at the host cell cortex and progressively subverts multiple steps of actin assembly, including formin-mediated nucleation, crosslinking of F-actin, and actin depolymerization (H. Sun et al., 2021).
XopX1	XopX1 of <i>Xcc8004</i> inhibits the expression of the flg22-inducible genes FRK1 and WRKY33 and affects the activation of the MAPK3 and MAPK6 in Arabidopsis mesophyll protoplast (Y. Huang et al., 2020). <i>Xev</i> presents one effector belonging to the XopX family with ambiguous effects in plant immunity. It suppresses flagellin-induced ROS production while promotes ethylene production, accumulation of pattern-triggered immunity (PTI) gene transcripts and plant cell death in tomato and <i>N. benthamiana</i> plants (Stork et al., 2015).
XopX2	No evidence of previous studies on this effector. XopX2 orthologue in <i>Xev</i> has been studied previously (See on XopX1 section)
XopZ	XopZ of <i>Xcc8004</i> inhibits the expression of the flg22-inducible genes FRK1 and affects the activation of the MAPK3 and MAPK6 in Arabidopsis mesophyll protoplasts (Y. Huang et al., 2020). In <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> XopZ suppresses the peptidoglycan-triggered MAPK activation (Long et al., 2018) and the rice immune response induced by the simultaneous ectopic expression of XopQ-XopX (Deb et al., 2020).

## 1.10 PhD project description

### Objectives and structure.

As explained above, the T3Es play a major role in the virulence of many phytopathogens, as they adapt the host ambience towards favorable conditions for microbial survival. However, most of their biological functions remain elusive. Interestingly, the size and composition of the T3 effectome is highly variable among microbes. Some pathogens present hundreds of T3Es while others require only few T3Es to achieve plant colonization. All this raise importante questions such as:

What is the biological role of the T3Es in the host cell?

Which T3Es are dispensable and which are essential to modulate plant physiology?

During this work, we studied the pathosystem formed by *Xcc* and Arabidopsis to anwer the questions above. Our results are exposed in **Chapter 2** and **Chapter 3**. In **Chapter 2**, we heterologously expressed single *Xcc* strain 8004 T3Es in Arabidopsis to decihier their effects in plant growth, immunity and transcriptome. As the nature of plant responses to each effector could reveal their molecular functions. In **Chapter 3** we conducted a comparative analysis of the *in planta* functions of the T3Es XopAG and RipO1 wich encoded by orthologous genes in the hemibiotrophic pathogens *Xcc*<sub>8004</sub> and *Ralstonia pseudosolanacearum*<sub>GMI1000</sub> respectively. With this strategy, we aimed to decipher i) if these conserved T3Es exhert similar *in planta* functions ii) the biological role of these T3Es for the virulence of their respective pathogens. Both parts are presented in an article format to facilitate their future publication.

In **Chapter 4** we discuss the main contributions of this project to the understanding of the T3Es' role inthe modulation of plant physiology during the plant-microbe interactions.



## **Chapter 2: Multiple *Xcc*<sub>8004</sub> type III effectors affect plant growth and immunity in Arabidopsis.**



## **Multiple Xcc8004 type III effectors affect plant growth and immunity in Arabidopsis.**

### **Context and contribution:**

During plant-pathogen encounter, the nature and intensity of the responses activated on host cells reveal multiple characteristics of the pathogen such as lifestyle, virulence strategies, host range and preferred niche. Consequently, in this chapter we described our efforts to characterize the responses induced by each of the *Xcc*<sub>8004</sub> T3Es on plants with particular emphasis in *Arabidopsis thaliana* ecotype Col-0. Our experiments are exposed in an article format, to facilitate its future publication in a peer-reviewed journal.

The work described here was developed within the facilities of the LIPME as part of a more ambitious project that seeks to decipher the mechanisms by which the T3Es enable *Xcc* pathogenicity in different host contexts. Upon my arrival to the SIX team, part of the biological material that served as basis for the experiments realized during my PhD had already been generated, including *Xcc*<sub>8004</sub> mutant strains lacking one or two effectors. The genes encoding all *Xcc*<sub>8004</sub> T3Es had been cloned into pENTRY and pER8 vectors. In a joint effort with the staff of the greenhouse and the transgenesis service of the LIPME and the PhD student Manuel Gonzalez Fuente, we achieved the generation and validation of hundreds of Arabidopsis transgenic lines expressing single effector genes. I participated in the validation of the expression of T3E genes in the transgenic lines by qPCR and the subsequent experiments with these transgenic plants. My participation also included pathogenicity assays in the Arabidopsis susceptible ecotype Sf-2 with *Xcc*<sub>8004</sub> effector mutant strains and the generation and functional validation of vectors for the transient expression of single T3Es in *N. benthamiana* plants. The statistical and bioinformatic analyzes of RNAseq data was performed by Marie-Françoise Jardinaud and Sébastien Carrère from the LIPME biostatistics service. Finally, the drafted manuscript found below was also part of my contribution to the project.





# Multiple *Xcc*<sub>8004</sub> type III effectors affect plant growth and immunity in *Arabidopsis*.

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## Abstract

*Xanthomonas campestris* pv. *campestris* (*Xcc*) causes the black rot disease in *Brassicaceae* plants. During pathogenesis, *Xcc* secrete Type 3 Effector (T3E) proteins into the host cell environment to modulate plant physiology and promote pathogenicity. The T3 effectome of *Xcc* strain 8004 has been predicted to be composed of 28 T3Es. Although some of the activities of few effectors have been revealed before, most of their functions in plant physiology remain elusive. In this work, we characterized some of the *in planta* functions of each of the T3E predicted in *Xcc*<sub>8004</sub> genome. We found that the pathogenicity function of T3 effectome is highly resilient to the loss of single or pairs of T3Es. Furthermore, we evidenced multiple *Xcc*<sub>8004</sub> T3Es either affecting or promoting aspects of *Arabidopsis* physiology such as development or phosphorylation of Mitogen-Activated Protein (MAP) kinases MPK3/MPK6 in response to the immunogenic peptide flg22. By transcriptomic approaches, we found evidence of the previously identified avirulence functions of XopAC and XopAH on *Arabidopsis* plants and found that XopL, XopK and AvrXccA1 induce similar responses. These results reveal the complexity in the functions exerted by the T3 effectome of *Xcc* strain 8004 *in planta*, and provide solid basis for future studies unraveling the contribution of the T3Es to *Xcc* pathogenicity.

## Introduction

Plant immunity relies in two different recognition systems. The first one is formed by cell surface-localized pattern recognition receptors (PRRs) and initiates the so-called pattern-triggered immunity (PTI) (Bigeard et al., 2015). The second recognition system detects directly or indirectly the presence of microbial effector molecules by the action of intracellular Nucleotide-Binding domain Leucine-Rich Repeat containing receptors (NLRs or NB-LRR) and is known as effector triggered immunity (Bonas, 1998; Steinbrenner et al., 2012). PTI and ETI pathways lead to many convergent responses including a rapid increase in  $\text{Ca}^{2+}$  concentration, production of reactive oxygen species (ROS), biosynthesis of hormones and antimicrobial compounds, expression of defense related genes, activation of MAPK signaling cascades, among others (Zhou & Zhang, 2020). Recent reports indicate a synergistic effect between PTI and ETI signaling pathways leading to strong immune responses (Ngou et al., 2021, Yuan et al., 2021). Upon microbial molecules perception, plants reallocate nutrients and resources towards the generation of defensive responses (Eichmann & Schäfer, 2015). This process often restrict plant growth resulting in a growth-immunity trade-off directed by mutually antagonistic activities of the plant hormone pathways (Lozano-Durán & Zipfel, 2015).

Many bacterial phytopathogens secrete Type 3 Effector proteins (T3Es) through the Type 3 Secretion System (T3SS) to modulate plant physiology and promote pathogenicity (Alfano & Collmer, 2004). The T3Es can affect multiple host pathways, including the signaling cascade formed by the Mitogen-Activated Protein Kinases (MAPKs) that constitute an important relay for plant immune-signaling pathway (Lang & Colcombet, 2020). T3Es activities often affect the transcriptome of host cells, providing hints of the T3Es functions (Gangadharan et al., 2013).

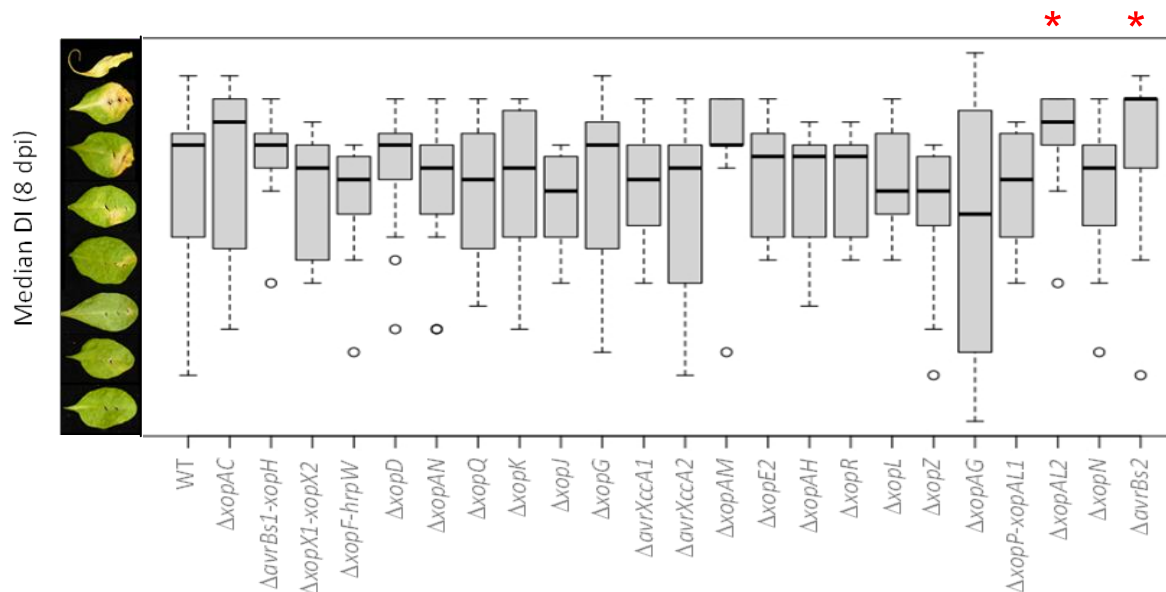
T3Es either promote or restrict pathogenicity by triggering or dampening plant immunity, respectively. In consequence, the repertoire of T3Es present in a given strain (known as the T3 effectome), dictates its host-range, lifestyle and virulence intensity (Anderson et al., 2017; Hajri et al., 2009; Li et al., 2020). Thus, deciphering the composition and functions of the T3Es is fundamental to understand and predict the plant-pathogen interactions. Effectomes size and composition is highly variable among microbial species, ranging from few in *Erwinia amylovora* (Nissinen et al., 2007) to hundreds in some species of nematodes, oomycetes and fungi (Rojas-Estevez et al., 2020).

The Gram-negative bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*) represents a practical model to study the functions of an entire effectome *in planta* since it presents an effectome of manageable size and infects the model plant *Arabidopsis thaliana* (Vicente & Holub, 2013). *Xcc* causes the black rot disease in crops of the *Brassicaceae* family and is considered as one of the most devastating bacterial pathogens at global scale (Mansfield et al., 2012). *In silico* studies predicted the presence of 28 proteins putatively secreted by the T3SS in the genome of *Xcc* strain 8004, these will be hereafter referred to as the T3Es of *Xcc*<sub>8004</sub> (Guy et al., 2013). To date, the activities of multiple *Xcc*<sub>8004</sub> T3Es on *Arabidopsis* plants have been explored: XopAH has been proven to confer disease resistance on *Arabidopsis* plants in a RAR1- and SGT1b-dependent manner (Rong et al., 2010). XopAC uridylylates the BIK1 kinase and the decoy protein PBL2 to suppress and trigger plant immunity respectively (G. Wang et al., 2015). XopD triggers plant disease tolerance in *Arabidopsis* plants by targeting DELLA proteins (L. Tan et al., 2014). XopL promotes bacterial infection and inhibits PTI responses (X. Yan et al., 2019). XopN is required for full virulence of *Xcc* on Chinese radish (B.-L. Jiang et al., 2008). XopR manipulates host actin cytoskeleton (H. Sun et al., 2021). Although several effectors have been individually characterized, only one study focuses on the *Xcc* T3Es in a full-T3 effectome context. Huang et al., 2020 achieved the individual expression of 24 T3Es in *Arabidopsis* protoplasts, showing that twelve effectors suppress PTI responses. In this work, we analyzed the effects induced by each of the *Xcc*<sub>8004</sub> T3E *in planta* by multiple approaches. We evidenced that the molecular activities of some T3Es vary among different hosts. Subsequently, we identified multiple T3Es which affect development, flg22-triggered MAPK activity and transcriptional patterns of *Arabidopsis* Col-0 plants. Unexpectedly, various effectors triggered plant-immune responses. This comprehensive characterization of the activities of the *Xcc* T3 effectome *in planta* contributes to decipher part of the molecular mechanisms that govern *Xcc*-*Arabidopsis* interactions.

## Results

### *Xcc* single or double mutants in T3Es are not affected in pathogenicity on *Arabidopsis thaliana* ecotype Sf-2

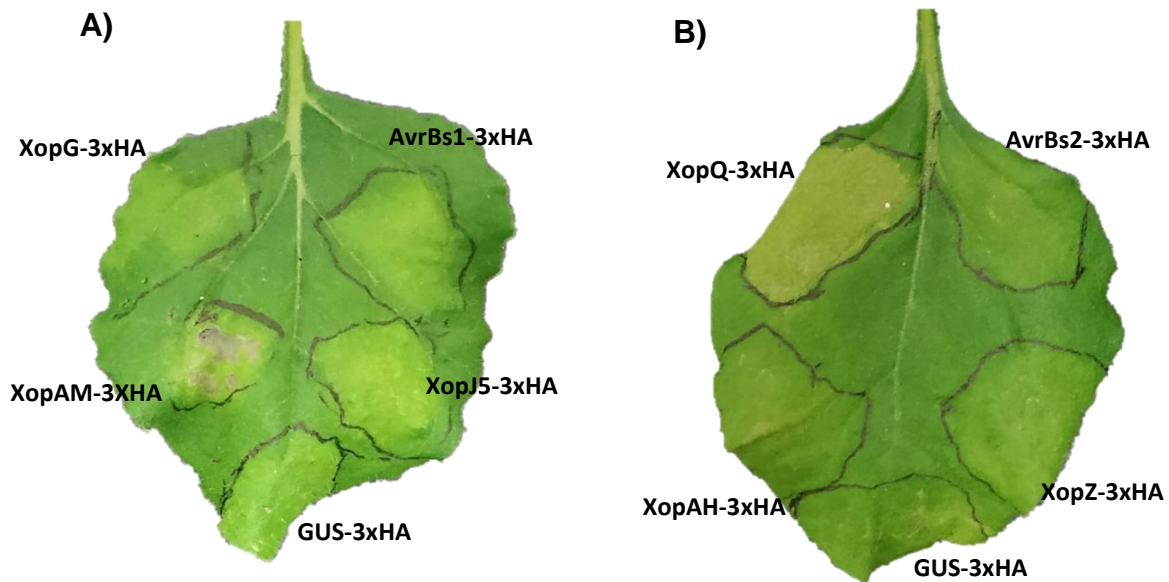
One of the approaches to discover the contribution of the T3Es to pathogens virulence in a given host is the use of mutant strains lacking of one or few effectors, to subsequently quantify the impact of these single mutations on the pathogenic abilities of the studied bacteria by means of pathogenicity assays. Previous studies in *Xcc* strain 8004 following this strategy in a full-effectome context, succeeded at identifying *xopAC*, and *xopAM* as avirulence factors in the host *A. thaliana* ecotype Col-0 (Guy et al., 2013). We took advantage of those T3E single or double mutant strains (Guy et al., 2013) (Supplementary Table S1) to investigate their involvement in *Xcc* strain 8004 pathogenicity in the susceptible host *A. thaliana* ecotype Sf-2, aiming to identify effectors with a predominant role in plant susceptibility. Only the mutation of the T3E genes *avrBs2* and *xopAL2* significantly increased the aggressiveness of the *Xcc* mutant strains on Sf-2 plants inoculated by wound in the central vein, as compared to the virulence shown by the wild type (WT) strain (Figure 1).



**Figure 1. Pathogenicity of T3E mutants in *Xcc* strain 8004 inoculated on *A. thaliana* Sf-2 plants.** Bacterial strains were inoculated by piercing the central vein of leaves of 4-weeks-old plants, and disease symptoms were scored after 8 days. The disease index scale was measured as 0 to 1, no symptoms; 1 to 2, weak chlorosis; 2 to 3, strong chlorosis; and 3 to 4, necrosis. \* Significant differences as compared to Sf-2 WT plants determined by Kruskal Wallis test ( $p < 0.05$ ).

## Transient expression in *N. benthamiana*: a prospective tool to study the *Xcc*<sub>8004</sub> T3Es

In order to investigate the activities of the *Xcc* strain 8004 T3Es in a different host species, we cloned individually each of the T3E genes fused to 3xHA in the expression vector pGWB614 or pGWB615 (Nakamura et al., 2010) as indicated (Supplementary Table S2). The plasmids were transformed in the *A. tumefaciens* strain C58C1 and delivered transiently in *N. benthamiana* leaves. We observed that the expression of the effector XopAM caused cell death in *N. benthamiana* cells four days after infiltration (Figure 2A). Additionally, *N. benthamiana* cells heterologously expressing the *Xcc*<sub>8004</sub> effector XopQ exhibited strong chlorosis four days after infiltration (Figure 2B). It is important to note that the accumulation of XopQ proteins could not be detected *in planta* by western blot (data not shown). No other *Xcc* strain 8004 T3E caused visible effects in *N. benthamiana* leaves.



**Figure 2. Agrobacterium-mediated transient expression of multiple *Xcc*<sub>8004</sub> T3Es in *N. benthamiana* leaves.** Transient expression of *xopAM* caused necrosis in transformed tissues (A). Plant cells transiently expressing *xopQ* turned chlorotic (B). Plants were infiltrated with *Agrobacterium* strains at OD<sub>600</sub> = 0.5. Pictures were taken 4 days after infiltration.

## Construction of transgenic Arabidopsis lines expressing single *Xcc* T3Es.

In order to study the *in planta* activities of each *Xcc* strain 8004 T3E independently of infection and to avoid functional redundancy with other effectors, we expressed heterologously single *Xcc*<sub>8004</sub> T3Es in Arabidopsis Col-0 plants. Briefly we cloned each of the *Xcc*<sub>8004</sub> T3E genes previously described by Guy et al., 2013 and mentioned in <http://Xanthomonas.org/> in the  $\beta$ -estradiol-inducible expression vector pER8 (Zuo et al., 2000). The expression vectors (see Supplementary Table S3) were transformed in *A. thaliana* Col-0 plants by Agro-mediated delivery and floral dip method (Clough & Bent, 1998). The presence of the expression vectors in transformed plants was validated by hygromycin-resistance selection and PCR. To ensure single-copy-insertion, we selected transgenic lines with 3:1 hygromycin-resistance segregation in T<sub>2</sub> generation and homozygous T<sub>3</sub> lines. Transgenic lines were constructed for 26 *Xcc*<sub>8004</sub> T3Es (Table 1). Several attempts were made to generate transgenic lines harboring the effector genes *avrBs2* and *xopAM* without success, reflecting a possible toxic activity *in planta*. No molecular tags were fused to the effectors and their expression was validated in all transgenic lines by qPCR (Supplementary Table S4). We generated multiple independent lines for 22 effectors, while for four T3Es namely *hrpW*, *xopAC*, *xopAL1* and *xopK* only one line was constructed. Interestingly, the qPCR analysis to validate the expression of the T3Es in the transgenic lines revealed that independently generated lines transformed with the same vector, accumulate different levels of the T3E transcript (Supplementary Table S4).

## Multiple *Xcc*<sub>8004</sub> T3Es affect plant development.

Sometimes, the heterologous expression of T3Es affects plant growth and development upon immunity or metabolism misregulation (Degraeve et al., 2013; Block et al., 2010). To study the effect of each *Xcc*<sub>8004</sub> T3E on plant development, we induced the expression of the T3E genes in the Col-0 transgenic lines mentioned above during seed germination. Strikingly the ectopic expression of ten *Xcc*<sub>8004</sub> T3E genes resulted in seedling growth inhibition (Figure 3), while the ectopic expression of the effector *xopJ5* produced bigger seedlings.

152 Table 1. List describing the T3E genes studied during this work, the resultant vectors and their effects  
153 upon heterologous expression in Col-0.

Gene	Code	Vector	No. of lines generated	Effects in development	Effects in MAPK activation	No. of DEG
<i>avrBs1</i>	XC_2081	pER8- <i>avrBs1</i>	2	-	Enhancer	-
<i>avrBs2*</i>	XC_0052	Nd	0	Nd	Nd	Nd
<i>avrXccA1</i>	XC_4318	pER8- <i>avrXccA1</i>	2	Dwarf (2/2)	Suppressor	3736
<i>avrXccA2</i>	XC_1716	pER8- <i>avrXccA2</i>	2	-	-	-
<i>HpaA</i>	XC_3018	pER8- <i>hpaA</i>	2	-	-	-
<i>hrpW</i>	XC_3023	pER8- <i>hrpW</i>	1	-	-	37
<i>xopA</i>	XC_3002	pER8- <i>xopA</i>	2	-	Enhancer	-
<i>xopAC</i>	XC_1553	pER8- <i>xopAC</i>	1	Dwarf (1/1)	Suppressor	3398
<i>xopAG</i>	XC_0563	pER8- <i>xopAG</i>	2	Dwarf (2/2)	-	46
<i>xopAH</i>	XC_2004	pER8- <i>xopAH</i>	2	Dwarf (2/2)	Enhancer	805
<i>xopAL1</i>	XC_2995	pER8- <i>xopAL1</i>	1	-	-	-
<i>xopAL2</i>	XC_3915-6	pER8- <i>xopAL2</i>	2	-	Enhancer	-
<i>xopAM*</i>	XC_3160	Nd	0	Nd	Nd	Nd
<i>xopD</i>	XC_1213	pER8- <i>xopD</i>	2	-	-	-
<i>xopE2</i>	XC_2602	pER8- <i>xopE2</i>	2	-	-	-
<i>xopF</i>	XC_3024	pER8- <i>xopF</i>	2	-	-	-
<i>xopG</i>	XC_0967	pER8- <i>xopG</i>	2	-	-	-
<i>xopH</i>	XC_2082	pER8- <i>xopH</i>	2	-	-	-
<i>xopJ</i>	XC_3802	pER8- <i>xopJ</i>	2	Enlarged (2/2)	Enhancer	-
<i>xopK</i>	XC_1210	pER8- <i>xopK</i>	1	Dwarf	-	502
<i>xopL</i>	XC_4273	pER8- <i>xopL</i>	2	Dwarf (2/2)	Suppressor	680
<i>xopN</i>	XC_0241	pER8- <i>xopN</i>	2	Dwarf (2/2)	Enhancer	-
<i>xopP</i>	XC_2994	pER8- <i>xopP</i>	2	-	-	-
<i>xopQ</i>	XC_3177	pER8- <i>xopQ</i>	2	-	-	-
<i>xopR</i>	XC_0268	pER8- <i>xopR</i>	2	Dwarf (2/2)	Suppressor	-
<i>xopX1</i>	XC_0541	pER8- <i>xopX1</i>	2	-	-	-
<i>xopX2</i>	XC_0542	pER8- <i>xopX2</i>	2	Dwarf (2/2)	-	22
<i>xopZ</i>	XC_2210	pER8- <i>xopZ</i>	2	Dwarf (2/2)	-	14

154 \* No lines obtained

155 Nd: No determined



Col-0 (WT)

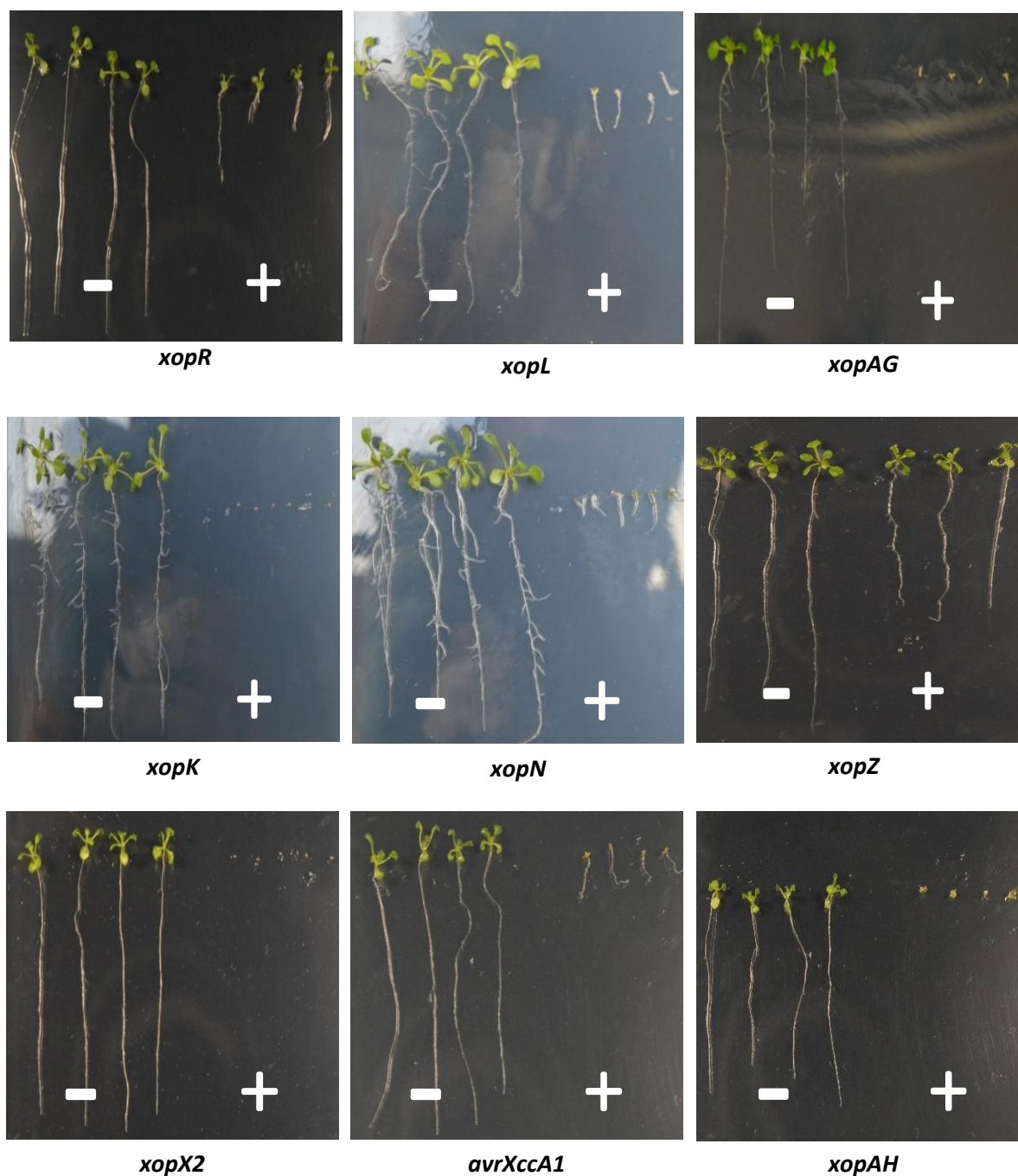


*xopJ5*



*xopAC*





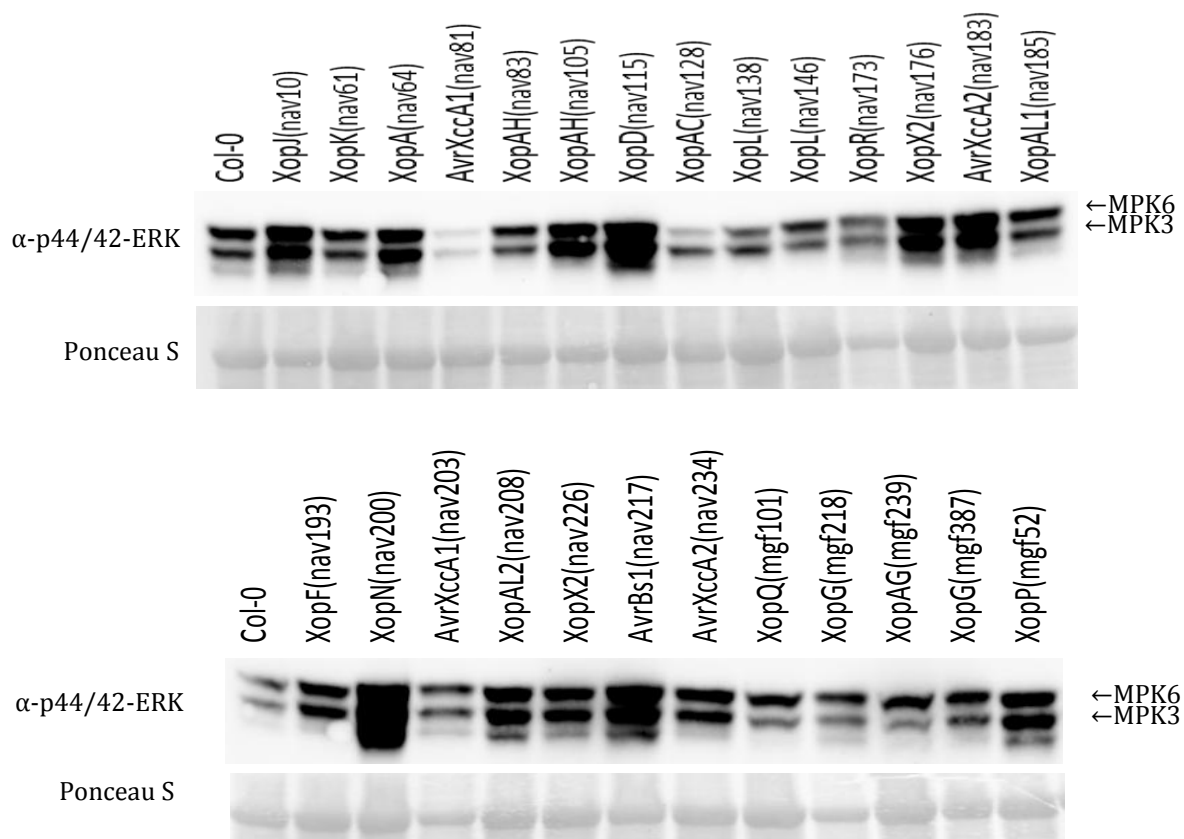
**Figure 3. The heterologous expression of eleven *Xcc* strain 8004 T3Es alters plant development.** Transgenic plants were grown for two weeks *in vitro* in MS solid medium supplemented with either 5μM β-estradiol (+) or the polar solvent DMSO (-) in equal proportion as indicated. Col-0 wild type (WT) plants were used as negative control.



Undoubtedly, the fact that eleven effectors have a marked effect on plant development is an intriguing finding. However, this result does not provide much information about their particular contribution to the *Xcc* pathogenicity since the plant immune system is connected to nearly all processes within the host cell (P. Li *et al.*, 2020) and any effect on its components to either activate or suppress immune responses could alter plant development.

### **T3Es influence MAPKs activation**

In the following assay, we investigated the influence of the heterologous expression of each effector in the activation of the MAPKs in response to flg22 by western blot using the phospho-p44/42-ERK antibody (Cell signalling) that recognize the MAPK3 and MAPK6 only in their phosphorylated state. The ectopic expression of four effectors (XopR, XopL, AvrXccA1 and XopAC) in Arabidopsis Col-0 plants consistently decreased the activation of the MAPK cascades in response to flg22 in three independent experiments as compared to wild-type (WT) seedlings (Figure 4). For AvrXccA1 two independent lines were tested; in one line (nav81) the flg22-triggered phosphorylation of the MAPKs was decreased whereas in the second line (nav203) there were no obvious differences. This correlated with the expression levels of *avrXccA1* in each line, since the nav81 line accumulated 10 times more *avrXccA1* transcript than the line nav203 (Supplementary Table S4). This indicates that T3E functions are dose-dependent. Interestingly, the ectopic expression of other six T3E genes (*xopJ5*, *xopA*, *xopAH*, *xopN*, *xopAL2* and *avrBs1*), consistently enhanced the activation of the MAPKs in response to the peptide flg22, indicating that some *Xcc*<sub>8004</sub> T3Es have synergistic effects on plant PTI-responses as has been proven for T3Es of *Pseudomonas syringae* (Ngou *et al.*, 2021, Yuan *et al.*, 2021). We couldn't detect MAPKs phosphorylation by the sole expression of any *Xcc*<sub>8004</sub> T3E (Data not shown). The number of effectors enhancing the flg22-activated immune response is surprising, considering that *Xcc* strain 8004 only requires the mutation of a single effector (XopAC) to achieve significant rates of pathogenicity in Arabidopsis Col-0.

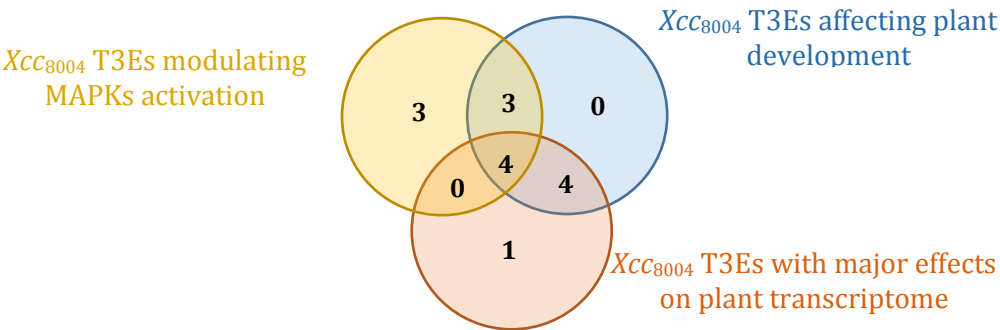


**Figure 4. Effect of the *Xcc* strain 8004 T3Es expression on flg22-induced activation of the MAPK cascade in *Arabidopsis* plants.** Two-weeks old seedlings of multiple transgenic lines or the *Arabidopsis* ecotype Col-0 were treated overnight with 1ml of liquid MS medium supplemented with 5 $\mu$ M  $\beta$ -estradiol and then treated with 100 nM flg22 for 15 min. Total protein extracts were separated on SDS-PAGE (10%) and transferred to PVDF membrane. Activated MAPKs were detected by immunoblots using  $\alpha$ -p44/42-ERK antibody. Ponceau staining of Rubisco indicates protein loading.

#### Multiple effectors caused a relevant impact in *Arabidopsis* transcriptome

To get more insights about the activities of the *Xcc*<sub>8004</sub> T3Es within host cells, we used the T3E gene transgenic *Arabidopsis* lines to investigate the effect of each T3E in plant transcriptome. Briefly, two-weeks-old seedlings of each transgenic line were incubated for 16h in 1ml of MS liquid media supplemented with 5 $\mu$ M  $\beta$ -estradiol to induce the expression of the T3Es. Then, plants were collected and used to isolate total RNA that was subsequently analyzed by RNAsequencing. Seedlings of *Arabidopsis* Col-0 wild-type plants were used as negative controls. Considering the large number of transgenic lines to be analyzed, we limited our number of replicates to two per effector, which allowed us to detect only major changes. Whenever possible, independent transgenic lines were used as replicates to minimize the effect

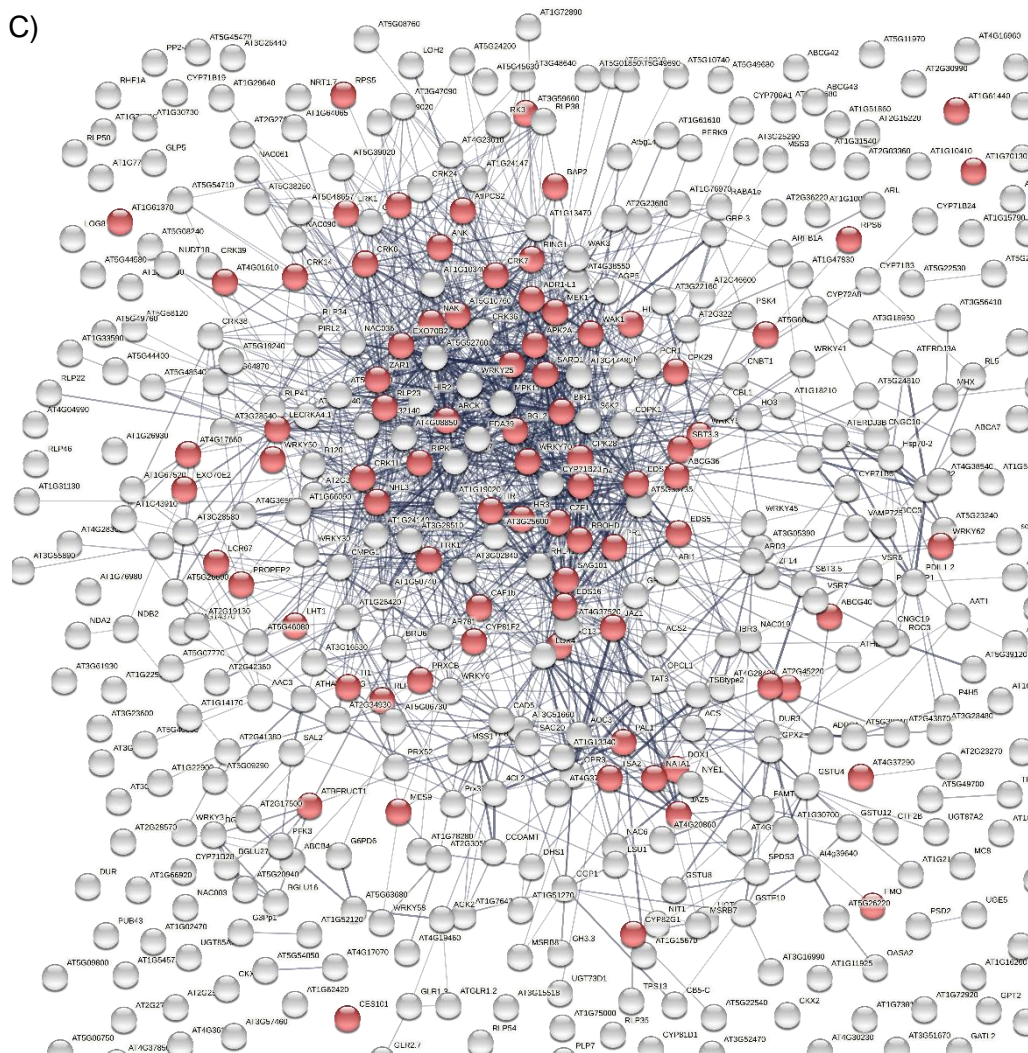
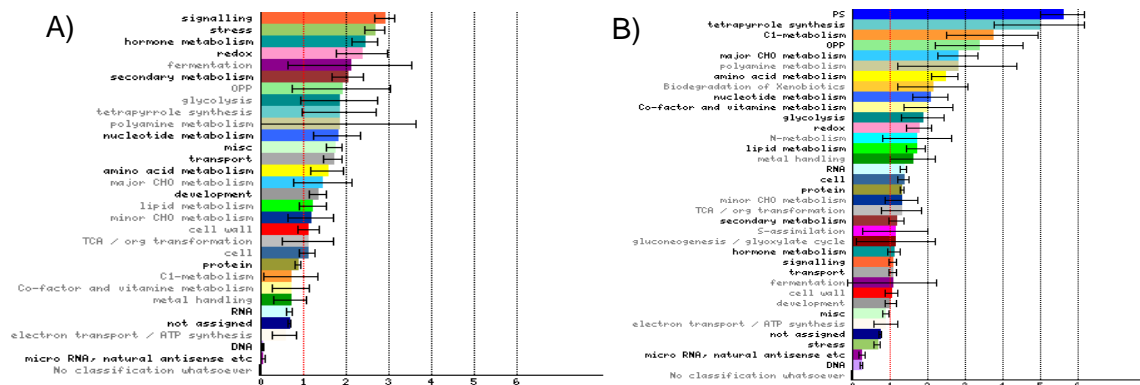
due to the positional insertion of the T-DNA. By comparing the transcriptomic profiles induced by each T3E with that of Col-0 wild-type plants, we found that nine effectors (AvrXccA1, XopAH, HrpW, XopAC, XopAG, XopK, XopL, XopX2 and XopZ) significantly altered the expression of multiple plant genes (false discovery rate < 0.05 and fold change > 5) as indicated (Table 1). Coincidentally, most of these genes also affected plant development and/or MAPKs phosphorylation (Figure 5) suggesting a strong impact on plant physiology.



**Figure 5. Venn diagram depicting the T3Es affecting different aspects of Arabidopsis physiology.** Four T3Es showed significant effects in all the plant processes analyzed (XopAC, XopL, XopAH and AvrXccA1)

#### Four effectors promoted the expression of immune-related genetic pathways similarly to XopAC

XopAC is an elicitor of ETI responses in Arabidopsis by uridylylating the decoy protein PBL2 (G. Wang et al., 2015). We analyzed the effects induced by XopAC on the plant transcriptome. During our experiments, *xopAC* induced the differential expression of more than 3000 plant genes (Full list in Supplementary Table S5). 1153 were up-regulated while 2245 were down-regulated. The functional classification of these genes revealed that *xopAC* essentially promoted genetic pathways related to signalling, stress, hormone metabolism (Figure 6A), while suppressing genetic pathways related to photosynthesis and metabolism (Figure 6B). Additionally, we analyzed the genes up-regulated by XopAC in the STRING (<https://string-db.org/cgi/>) web tool. Due to visualization constraints, we only considered DEGs with FDR < 0.001 (448 genes). As expected *xopAC* promoted the expression of a highly interconnected genetic network composed of immune-genes (red spheres in Figure 6C) such as *zar1*, *ripk*, *eds1*, *crk11*, *rboh*, *pad4*, *pr1*, *sard1*, *rps5*, etc. Our results evidence a clear activation of plant immune responses by *xopAC*, validating the functionality of our transcriptomic approach.



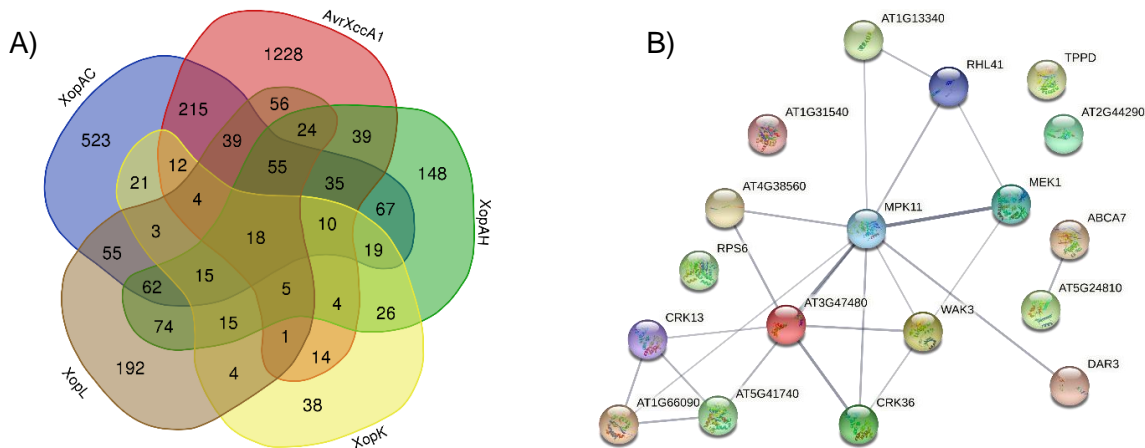
**Figure 6. Functional classification of DEG regulated by XopAC.** Genes upregulated (A) and downregulated (B). Identified by the Classification SuperViewer Tool w/ Bootstrap (Provart & Zhu, 2003) based on MapMan classification. Functions with a p-value < 0.05 are in bold letters. Bars represent the normalized frequency of each category ( $\pm$  StdDev of 1000 bootstrap). (C) Predicted interaction network of the genes up-regulated by XopAC in STRING webtool. Red spheres represent genes related with plant defense response. Line thickness indicates the strength of data support.

By comparing the transcriptomic responses induced by different *Xcc*<sub>8004</sub> T3Es *in planta*, we found that the effects elicited by *avrXccA1*, *xopK*, *xopL* and *xopAH* were highly similar to those elicited by *xopAC* in terms of DEG (Table 2). Surprisingly a large proportion of the genes commonly up-regulated by *XopAC* and either *AvrXccA1* (Supplementary Figure S1), *XopK* (Supplementary Figure S2), *XopAH* (Supplementary figure S3) or *XopL* (Supplementary Figure S4) were involved in defense response, including genes with fundamental roles in ETI pathways such as *sag101*, *wky70*, *cerk1*, *sard1*, *zar1*, *pad4*, *eds1*, etc. These results indicate that *AvrXccA1*, *XopK*, *XopL* and *XopAH* induce plant immune responses similarly to *xopAC*, suggestive of avirulent activities which are not detectable by simple loss of function approach in *Xcc* strain 8004.

Table 2. Superposition of the DEG caused by the expression of *xopL*, *xopAH*, *avrXccA1* or *xopK* with the DEG induced by the expression of *xopAC* in Arabidopsis Col-0 plants.

T3E	DEG total	DEG shared with <i>XopAC</i>	Percentage
Genes up-regulated			
<i>XopK</i>	209	102	48.80
<i>AvrXccA1</i>	1750	388	22.17
<i>XopL</i>	622	251	40.35
<i>XopAH</i>	616	281	45.62
Genes down-regulated			
<i>XopK</i>	293	103	35.15
<i>AvrXccA1</i>	1976	718	36.34
<i>XopL</i>	188	30	15.96
<i>XopAH</i>	55	17	30.91

We identified a set of 18 plant genes that are commonly up-regulated by the five immune-triggering effectors *XopL*, *XopAH*, *AvrXccA1*, *XopK*, and *XopAC* (Figure 7A). This cluster includes multiple proteins involved in disease resistance, belonging to the protein families TIR-NBS-LRR, Receptor-like protein kinases (RLK) and other kinases (Described in Supplementary Table S5). The analysis of this group of 18 genes in the STRING web tool showed a well-connected network with multiple putative associations (Figure 7B). Interestingly the protein kinase MPK11 appeared as a central node and established putative associations with most of the analyzed genes. This suggests that MPK11 plays a relevant role in the organization of the genetic network commonly promoted by *xopL*, *xopAH*, *avrXccA1*, *xopK*, and *xopAC*.

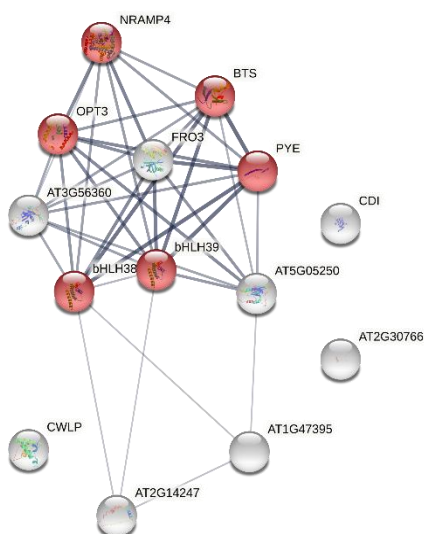


**Figure 7. Five effectors up-regulated 18 core plant genes related to plant defense responses.** (A) Venn diagram depicting the superposition of the genes commonly up-regulated by the expression of *xopL*, *xopAH*, *avrXccA1*, *xopK*, or *xopAC* expression in Arabidopsis Col-0 plants. (B) Predicted interaction network with the STRING web tool. Line thickness indicates the strength of data support.

#### Four other *Xcc*<sub>8004</sub> effectors have distinct effects in plant transcriptome

The expression of four other effector genes, *xopZ*, *xopAG*, *xopX2* and *hrpW*, caused significant changes (FDR<0.05 and fold change>5) in the transcriptome of Arabidopsis Col-0 plants. None of these effectors caused significant alterations on immune related pathways but affected the functions of many other pathways.

The expression of *xopZ* altered the expression of 14 plant genes. All affected genes were up-regulated and they were mainly related to iron homeostasis (Figure 8 and Table 3).



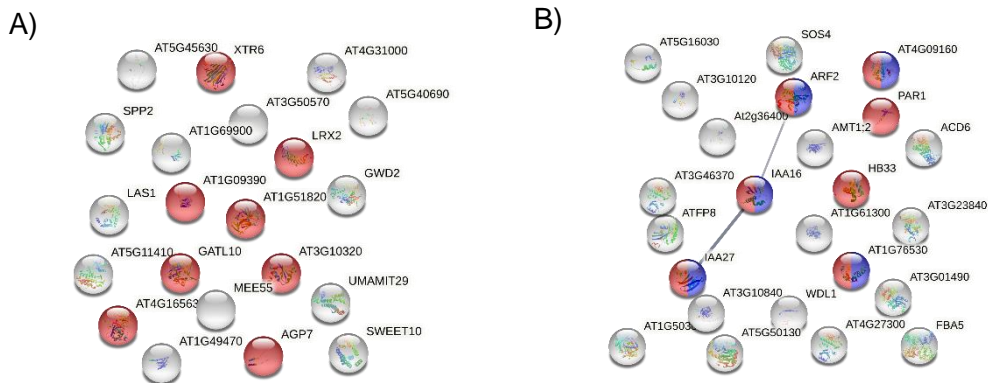
**Figure 8. Prediction of the interaction network of DEG induced by the heterologous expression of *xopZ* done with the STRING web tool. Red spheres represent genes involved in iron homeostasis. (GO:0055072, FDR 5.06e-08). Line thickness indicates the strength of data support.**



Table 3. Annotation of genes up-regulated by the expression of *xopZ* in STRING web tool.

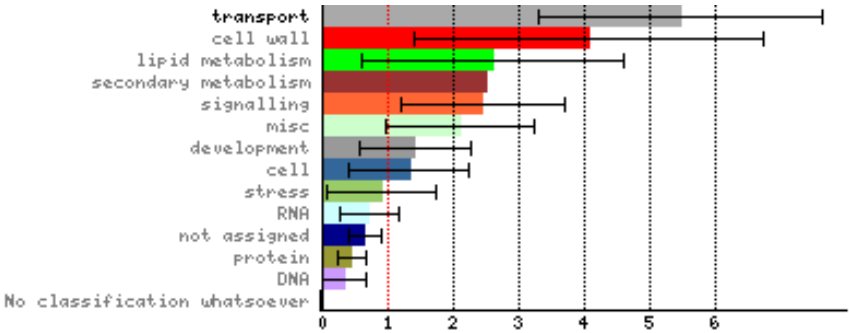
Name	Annotation
BTS	Negatively regulates the response to iron deficiency and homeostasis. Exhibits E3 ubiquitin-protein ligase activity <i>in vitro</i> . Facilitates 26S proteasome-mediated degradation of PYEL proteins in the absence of iron.
NRAMP4	Vacuolar metal transporter involved in intracellular metal homeostasis. Regulates metal accumulation under Fe starvation.
FRO3	Ferric reduction oxidase 3. May participate in the transport of electrons to a Fe(3+) ion via FAD and heme intermediates.
AT3G56360	Uncharacterized protein.
CDI	Nucleotide-diphospho-sugar transferases superfamily protein.
PYE	Encodes POPEYE (PYE), a bHLH transcription factor regulating response to iron deficiency in Arabidopsis roots.
OPT3	Oligopeptide transporter 3. Acts also as a metal transporter that could be a component of the copper transport machinery.
CWLP	Cell wall-plasma membrane linker protein homolog (CWLP).
AT5G05250	Unknown protein.
bHLH39	Basic helix-loop-helix (bhlh) dna-binding superfamily protein.
AT1G47395	Uncharacterized protein.
AT2G30766	Unknown protein.
bHLH38	Basic helix-loop-helix (bhlh) dna-binding superfamily protein.
AT2G14247	Uncharacterized protein.

Twenty-one plant genes were up-regulated in response to *xopAG* expression. Many of these are annotated as glycoproteins in Uniprot database (red spheres in Figure 9A) and are involved in diverse functions. *xopAG* expression down-regulated 25 plant genes mainly related to hormone response (red spheres in Figure 9B) particularly response to auxin (blue spheres in Figure 9B).



**Figure 9. Functional classification and interaction network prediction of DEG induced by the heterologous expression of *xopAG*** done with the STRING web tool. (A) Up-regulated genes, red spheres represent genes coding for glycoproteins (Uniprot). (B) Down-regulated genes, red spheres represent hormone-response genes; blue spheres represent auxin-response genes.

*hrpW* ectopic expression up-regulated thirty-one genes mainly related to transport of molecules (Figure 10) (Full list in Supplementary Table S6). Only six genes were down-regulated by the expressions of *hrpW* (Table 4).



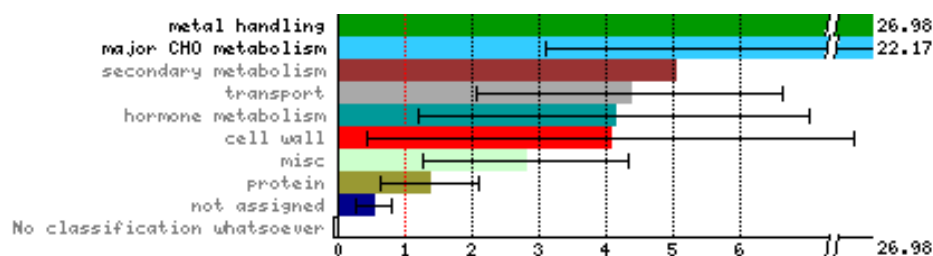
**Figure 10. Functional classification of genes up-regulated by the heterologous expression of *hrpW* in Col-0 plants** done with the Classification SuperViewer Tool w/ Bootstrap (Provart & Zhu, 2003) based on MapMan classification. Ontology groups with a p-value < 0.05 are in bold letters. Bars represent the normalized frequency of each category ( $\pm$  StdDev of 1000 bootstrap).

Table 4. Annotation of genes down-regulated by the expression of *hrpW* in STRING web tool.

Gen ID	Name	Annotation
AT1G12010	AT1G12010	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein, involved in the ethylene biosynthesis.
AT5G45080	PP2-A6	Phloem protein 2-A6 (PP2-A6); Toll-Interleukin receptor, involved in signal transduction, defense response, innate immune response.
AT5G63560	FACT	Fatty alcohol:caffeoyl-coa caffeoyl transferase; Involved in the synthesis of alkyl hydroxycinnamates in root waxes.
AT1G80050	APT2	Adenine phosphoribosyl transferase 2; May contribute to the recycling of adenine into adenyate nucleotides and the inactivation of cytokinins by phosphoribosylation.
AT2G38390	AT2G38390	Peroxidase superfamily protein, removal of H <sub>2</sub> O <sub>2</sub> , oxidation of toxic reductants, biosynthesis and degradation of lignin, suberization, auxin catabolism, response to stres such as wounding, pathogen attack and oxidative stress.
AT1G67865	AT1G67865	Unknown protein

*xopX2* expression up-regulated the expression of sixteen genes. Metal handling and major CHO metabolism were the most represented pathways (Figure 11, full list in Supplementary Table S7). *XopX2* down-regulated six plant genes that are described in Table 5.





**Figure 11. Functional classification of genes up-regulated by the heterologous expression of *xopX2* in Col-0 plants** done with the Classification SuperViewer Tool w/ Bootstrap (Provar & Zhu, 2003) based on MapMan classification. Ontology groups with a p-value < 0.05 are in bold letters. Bars represent the normalized frequency of each category ( $\pm$  StdDev of 1000 bootstrap).

Table 5. Annotation of genes down-regulated by the expression of *xopX2* in STRING web tool.

Gene	Name	Annotation
AT5G01320	AT5G01320	Thiamine pyrophosphate dependent pyruvate decarboxylase family protein;
AT4G30170	AT4G30170	Peroxidase family protein; Removal of $H_2O_2$ , oxidation of toxic reductants, biosynthesis and degradation of lignin, suberization, auxin catabolism, response to environmental stresses such as wounding, pathogen attack and oxidative stress.
AT3G62470	AT3G62470	Pentatricopeptide repeat-containing protein.
AT1G63780	IMP4	Putative u3 small nucleolar ribonucleoprotein protein; involved in ribosomal RNA processing. Located in nucleolus and cajal bodies.
AT1G54630	ACP3	Acyl carrier protein 3, chloroplastic; Encodes an acyl carrier protein expressed in leaves, roots, and dry seeds.
AT3G20000	TOM40	Translocase of the outer mitochondrial membrane 40; Central component of the receptor complex responsible for the recognition and translocation of cytosolically synthesized mitochondrial preproteins. Facilitates the movement of preproteins into the translocation pore.

## Discussion

### Reverse-genetics approaches revealed the avirulence activities of multiple T3Es.

Our reverse-genetics approach suggested that *xopAL2* and *avrBs2* genes confer partial avirulence to *Xcc* 8004 on Arabidopsis ecotype Sf-2. Both T3Es are part of the variable type III secretome of *X. campestris* pv. *campestris* (Guy et al., 2013). The contribution of *AvrBs2* to *Xcc* pathogenicity depends on the host genetic context, as revealed previous studies indicating that *avrBs2* mutation in *Xcc* strain 8004 reduced bacterial pathogenicity on Chinese radish (Rongqi et al., 2006), while, *avrBs2* conferred avirulence to *Xcc* strain 512/2 on *Brassica juncea* and *Brassica oleracea* (Ignatov et al., 2003). On the other hand, previous genome-wide association studies with 45 *Xcc* strains suggested that *xopAL2* is associated with variation in *Xcc*

pathogenicity on *Arabidopsis* Col-0, but it was not significant for *Xcc* virulence on the *Arabidopsis* ecotype Kas (Guy et al., 2013). Future comparative-genomics studies with different *Arabidopsis* ecotypes could reveal the genetic basis behind the *in planta* recognition of XopAL2, which could be useful to develop strategies of breeding resistance in economically important species.

*xopAM* is another *Xcc* 8004 T3E gene that has been associated with avirulence in Col-0 (Guy et al., 2013). This could explain why Col-0 plants were recalcitrant to *xopAM* transformation. Furthermore, *xopAM* induced evident phenotypic-alterations upon transient expression in *N. benthamiana* suggesting that this T3E can induce immune responses in different host species. Col-0 plants were also recalcitrant to *avrBs2* transformation and the reverse-genetics approach conducted in this work suggested that it also confers partial avirulence to *Xcc* in the Sf-2 *Arabidopsis* ecotype. Additionally, the transient expression of *xopQ* in *N. benthamiana* leaves caused chlorosis after four days which is consistent with previous reports showing that the NLR protein Recognition of XopQ1 (Roq1) is the mediator of XopQ recognition in *N. benthamiana* (Schultink et al., 2017). Strikingly, we were unable to detect XopQ expression by western blot in any of our experiments despite the appearance of strong symptoms in the transformed cells. This suggests that XopQ accumulation *in planta* is extremely low resulting undetectable in western blot assays.

### **The heterologous expression of the *Xcc* T3Es in *Arabidopsis* Col-0 revealed their effects in the plant physiology**

The *Xcc* T3Es fulfill diverse and complex functions within host cells, which are difficult to access in their natural context due to their epistatic interactions. In this work, the heterologous expression of individual T3Es in *Arabidopsis* Col-0, revealed part of their role in the modulation of plant physiology. We identified eleven effectors whose heterologous expression affected the plant development. However, deciphering the molecular basis behind these marked effects entails a complicated task considering that developmental effects might result from multiple reasons. The activation of plant immunity by avirulence genes often leads to plant growth impairments as a consequence of the negative growth-immunity crosstalk (Eichmann & Schäfer, 2015; Wang et al., 2015). Effectors causing an imbalance in plant hormone pathways could be another reason of growth impairments (Huot et al., 2014). Alternatively, *Xcc* effectors can interfere with relevant cell-structures as has been evidenced for HopG1 of *Pseudomonas syringae* that affects mitochondria and cytoskeleton to promote pathogenicity, resulting in plant

growth impairments (Block et al., 2010; Shimono et al., 2016). Subsequently, we analyzed the transcriptomic responses induced by the expression of each *Xcc*<sub>8004</sub> T3E in Arabidopsis. This allowed us to identify groups of effectors based on the nature of their effects on plant physiology.

### **Twelve T3Es didn't cause significant effects in plant physiology.**

The heterologous expression of twelve T3Es didn't have a significant effect in the plant phenotype, the plant transcriptome or the flg22-triggered phosphorylation of MAPKs in Arabidopsis. This suggests that the contribution of this group of effectors to the modulation of the plant physiology by *Xcc* is discrete. However, this raises the question. Why does *Xcc* maintain numerous effectors that do not appear to contribute significantly to the modulation of plant physiology? One possible explanation is that these T3Es have a quantitative contribution to *Xcc* pathogenicity and the addition of several small effects could lead to the partial or total modulation of host immunity. Another possible hypothesis is that these T3Es don't find their biological targets in the genetic background of Arabidopsis, as it is not a natural host of *Xcc*, but they might exert important functions in other plant species that are naturally colonized by *Xcc* such as *Brassica napus* or *Brassica oleracea*.

### **Five T3Es strongly affect plant physiology and trigger plant immune responses.**

We identified five effectors promoting the expression of many immune-related genes. One of these is the known avirulence gene *xopAC* (G. Wang et al., 2015). The fact that a large proportion of the immune-genes promoted by XopAC were also up-regulated by XopAH, XopL, XopK and AvrXccA1, insinuates that these four latter T3Es also present avirulence functions in Arabidopsis. This is in line with previous reports showing XopAH as a promoter of plant disease resistance on Arabidopsis in a RAR1- and SGT1b-dependent manner (Rong et al., 2010). Furthermore, these T3Es strongly affected plant growth, probably as a consequence of the activation of the plant immune system.

Previous studies revealed that the mutation of *xopAC* increased the virulence of *Xcc* in Arabidopsis Col-0 (Guy et al., 2013). However, in the same studies, the mutation of *xopL*, *xopAH*, *xopK*, or *avrXccA1* had no significant effect on *Xcc* pathogenicity. This suggests that *Xcc* possesses mechanisms to bypass the recognition of these four effectors. Previous studies have reported that some T3Es can block the recognition of other effectors. For instance, the delivery of twenty-nine T3Es singly and in pairs by an effectorless polymutant strain of

*Pseudomonas syringae*, showed that eight T3Es can suppress the cell death caused by six others in *N. benthamiana* leaves (Wei et al., 2018). It has also been shown that the T3Es HopPtoE, AvrPphE, AvrPpiB1, AvrPtoB, and HopPtoF of *Pseudomonas syringae* can suppress the programmed cell death induced by HopPsyA in Arabidopsis (Jamir et al., 2004). Likely, during *Xcc* natural infections, the immune responses activated by XopL, AvrXccA1, XopAH and XopK are suppressed by the activities of other T3Es. This hypothesis could be tested by delivering sets of effectors in Arabidopsis either by heterologous expression or with an effectorless *Xcc* strain, to discover if any combination of T3Es suppress the immune responses triggered by XopL, AvrXccA1, XopAH and XopK.

On the other hand, our results also revealed that all the T3Es with immune-triggering activities promoted the expression of the signalling cascade formed by the kinases MPK11 and MEK1. Previous reports showed that MPK11 expression is also increased upon treatment with PAMPs such as flg22 (Bethke et al., 2012) or elf18 (Eschen-Lippold et al., 2012). Additionally, recent evidence showed that AvrRpt2 of *P. syringae* suppresses the activation of MPK4 and MPK11 which correlates with reduced expression of plant defense genes and enhanced pathogen infection (Eschen-Lippold et al., 2016). All these point to the signalling cascade formed by MPK11-MEK1 as a relevant player in Arabidopsis immune system. Therefore, it should be considered as a candidate for future research.

### **Some T3Es affected plant physiology without triggering the plant immune system**

The T3Es XopZ, XopX2, HrpW and XopAG had significant effects on the transcriptional profile of Arabidopsis. The genes affected by these T3Es are not related to defense responses, which insinuates that they are not recognized by the plant immune system. However, the constitutive expression of XopZ, XopX2 and XopAG in Arabidopsis, strongly affect plant growth suggesting a big impact in plant physiology. XopZ and XopX2 altered the expression of pathways related to iron and metal homeostasis, respectively. It is known that problems in metal homeostasis lead to nutritional deficiencies or toxicity in plants (Clemens, 2001), which could explain why these T3Es affected *Arabidopsis* growth. On the other hand, the expression of *xopAG* in Arabidopsis repressed the expression of several auxin response genes, which could explain the growth impairments in these plants since auxins are major regulators of plant growth and development (Gomes & Scortecci, 2021). HrpW promoted the expression of genes related to the transport of molecules such as galactose or proline, which did not correlate with important effects on plant development.

On the other hand, the T3Es XopJ5, XopR and XopN affected the plant phenotype without having a significant impact on the transcriptome. Our results suggest that XopR has a modulatory effect on the flg22-triggered activation of MAPK and previous reports mention that XopR hijacks the host actin cytoskeleton (H. Sun et al., 2021); if these two things are related is unknown. The constitutive expression of XopJ5 promoted the growth of *Arabidopsis*. Interestingly, the XopJ5 orthologous gene in *Xanthomonas euvesicatoria* reduces the salicylic acid pools by targeting the cell proteasome in pepper plants (Üstün et al., 2013). XopJ5 of *Xcc* strain 8004 probably has a similar activity in *Arabidopsis*, which could explain why it causes an increase in plant size, if we take into account that SA pathways are known to have a negative crosstalk with growth and mutant plants impeded in the accumulation of salicylic acid present an increased size (Abreu & Munné-Bosch, 2009). Finally, XopN affected *Arabidopsis* growth, and strongly promoted MAPK activation in response to flg22. However, we couldn't detect significant effects in plant transcriptome.

The fact that the constitutive expression of XopJ5, XopR and XopN affect plant phenotype, but had no significant impact on the transcriptome is intriguing. This could mean that these T3Es have a greater effect in early stages of development or that their effects are rather discrete and accumulate over time. However, these ideas only have speculative value and require further study to be validated.

### **The *Xcc* T3Es have diverse roles in the modulation of *Arabidopsis* physiology**

In summary, the results of this work suggest that the *Xcc* T3Es have diverse roles in the modulation of *Arabidopsis* physiology: several T3Es have discrete effects in plant physiology. Others may be recognized by the plant immune machinery and affect the virulence of *Xcc* (such as XopAC). The bacterium can bypass the recognition of some T3Es and sustain pathogenicity (such as XopL, XopAH, XopK and AvrXccA1). Finally, other T3Es have a significant effect on plant physiology without inducing plant immune responses (XopZ, XopR, XopAG, XopJ, HrpW and XopN and XopX2). These last probably have a significant role on the virulence of *Xcc*, and therefore should be considered for future research.

## 470 **List of supplemental material**

471 Supplementary Table S1. Strains used in this work

472 Supplementary Table S2. List of vectors used for transient delivery of the T3Es in *N.*  
473 *benthamiana*.

474 Supplementary Table S3. List of vectors used to construct the Arabidopsis transgenic lines.

475 Supplementary Table S4. *A. thaliana* Col-0 transgenic lines and validation by qPCR

476 Supplementary Table S5. List of genes commonly regulated by XopAC and either XopL,  
477 XopAH, XopK or AvrXccA1.

478 Supplementary Table S6. Plant genes up-regulated by the heterologous expression of hrpW

479 Supplementary Table S7. Plant genes up-regulated by the heterologous expression of *xopX2*

480 Supplementary Table S8. Annotation color code.

481 Supplementary Table S9. Primers used for qPCR assays

482 Supplementary Figure S1. Prediction of the interaction network of DEG commonly up-regulated  
483 by the heterologous expression of *xopAC* and *avrXccA1*

484 Supplementary Figure S2. Prediction of the interaction network of DEG commonly up-regulated  
485 by the heterologous expression of *xopAC* and *xopK*

486 Supplementary Figure S3. Prediction of the interaction network of DEG commonly up-regulated  
487 by the heterologous expression of *xopAC* and *xopAH*

488 Supplementary Figure S4. Prediction of the interaction network of DEG commonly up-regulated  
489 by the heterologous expression of *xopAC* and *xopL*

490

## 491 **Methods**

### 492 **Plant growth conditions**

493 For growth *in vitro*, *A. thaliana* seeds were surfaced-sterilized with bleach diluted in tap water  
494 [1:3] for 15 min. Then, seeds were washed with sterile water and grown in sterile conditions in  
495 plates of Murashige & Skoog [MS] [Sigma-Aldrich] solid medium for two weeks [16h light; 20°C].  
496 *A. thaliana* plants used for pathogenicity assays were grown in Jiffy pellets for four weeks [9h  
497 light; 22°C]. *N. benthamiana* plants were grown in Jiffy pellets for three weeks, then the plants

498 were transplanted to plastic pots filled with soil and grown for one to two weeks [16h light; 21°C;  
499 relative humidity 70%].

#### 500 **Bacterial culture conditions**

501 *E. coli* strains were grown in Luria-Bertani (LB) medium (5 g/l yeast extract; 10 g/l bacto tryptone  
502 and 10 g/l NaCl. For solid medium: 15 g/l agar) at 37°C. *Xcc* strains were grown in Moka rich  
503 medium (4 g/l yeast extract; 8 g/l casamino acids; 2 g/l K<sub>2</sub>HPO<sub>4</sub> and 0.3 g/l Mg<sub>2</sub>SO<sub>4</sub> · 7 H<sub>2</sub>O. For  
504 solid medium: 15 g/l agar) at 28°C. *A. tumefaciens* strains were grown in LB medium. Liquid  
505 cultures of all strains were grown in constant shaking. Antibiotics concentrations were as  
506 follows: 100 mg/l carbenicillin or chloramphenicol; 50 mg/l kanamycin or rifampicin; 40 mg/l  
507 spectinomycin; 30 g/l pimaricin; or 10 mg/l gentamycin or tetracycline. For *Xcc* and *A.*  
508 *tumefaciens* strains 10 g/l chloramphenicol or 5 g/l tetracycline.

#### 509 **Pathogenicity assays**

510 Four-weeks-old plants were used. Four leaves of healthy plants were pierced three times in the  
511 central vein with a syringe needle dipped in *Xcc* bacterial suspension at 10<sup>8</sup> CFU/ml (~  
512 OD<sub>600</sub>=0.1) on 1 mM MgCl<sub>2</sub>. In each experiment, six plants were inoculated with each strain.  
513 After inoculation, plants were placed in plastic tray and grown in chamber conditions (9h light;  
514 22°C). Symptoms development was scored at 7 and 10 days post inoculation. Symptoms scale  
515 were as follows: 0-1, no symptoms; 1-2, weak chlorosis around the inoculation sites; 2-3,  
516 extended chlorosis; and 3-4, necrosis. Results from independent experiments were combined  
517 and one-tailed Wilcoxon signed-rank tests were conducted on R version 3.5.2 ([https://www.r-](https://www.r-project.org/)  
518 [project.org/](https://www.r-project.org/)).

#### 519 ***A. thaliana* transformation.**

520 Plants were transformed by floral dip method (Clough & Bent, 1998) with *Agrobacterium*  
521 *tumefaciens* strain GV3101 pMP90 harboring the desired vector. Plants were grown in  
522 greenhouse conditions. Transformed plants were screened on Murashige-Skoog (Sigma) agar  
523 plates added with 50 µg/ml hygromycin. pER8 insertion was validated by PCR. For all  
524 transformed lines, resistance segregation rates were registered in T2 and T3 generations to  
525 validate single plasmid insertions and homozygosity.

526

## **Transcript quantification by qPCR**

Two weeks-old transgenic seedlings were incubated overnight in 1ml of Murashige-Skoog (Sigma) liquid media under constant agitation. Then plant samples were frozen in liquid nitrogen and total RNA was isolated with the “Nucleospin RNA Plus kit” (Macherey-Nagel) as manufacturers’ instructions. Reverse transcription was performed using 500 ng of total RNA with the “Transcriptor Reverse Transcriptase” (Roche) and oligo(dT) primer as manufacturers’ instruction. Real-time quantitative PCR (qRT-PCR) was performed on the Light Cycler 480 II system (Roche Diagnostics), using Roche reagents as manufacturers’ instructions. Primers used are presented in Supplementary Table S9. Relative expression was calculated with the  $\Delta C_p$  method in reference to the genes encoding the Glyceraldehyde 3-phosphate dehydrogenase C2, (*gapc2*; AT1G13440) (Czechowski et al., 2005) and Oxidase Assembly 1 (*oxa1*; AT5G62050) (Quentin et al., 2009).

## **MAPK activation immunoblot**

Two weeks-old transgenic seedlings were incubated overnight in 1ml of Murashige-Skoog (Sigma) liquid media under constant agitation. Three plants of each line were frozen in liquid nitrogen and grinded using a tissue lyser and glass beads. The ground material was resuspended in 100  $\mu$ L of protein isolation buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM DTT (Sigma-Aldrich chemicals), protease inhibitor cocktail (Roche), 2 mM NaF, 2 mM  $\text{Na}_3\text{VO}_4$  and 1% v/v Triton X-100. The suspension was centrifuged at 12,000 g for 15 min at 4°C and the supernatant was collected. Protein concentration was measured with Quick Start™ Bradford reagent (Bio-Rad) as manufacturers’ instructions. Protein extracts were diluted in Laemmli buffer 2X containing 4% (w/v) SDS, 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 mM dithiothreitol (DTT). Samples were denatured at 95°C for 5 min. Equal amounts of proteins were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes (Bio-Rad). Blots were blocked with 5% (w/v) BSA (Sigma-Aldrich) in TBST for 2h. Blots were incubated overnight at 4°C in primary antibody incubation buffer containing TBST, 5% (w/v) BSA (Sigma-Aldrich) and rabbit anti-phospho-p44/42 (Erk1/2)(Thr202/Tyr204) monoclonal antibody (Cell Signaling, #9101) at a dilution of 1:1,500. Then, blots were washed with TBST and incubated for 2h at 4°C in secondary antibody incubation buffer containing TBST, 5% (w/v) non-fat dry milk, 1:10,000 dilution of HRP-conjugated anti-rabbit IgG (Pierce, #31460). HRP activity was detected with ECL system (Bio-Rad) using the ChemiDoc imaging system (Bio-Rad).



## 559 **RNAseq analysis**

560 Plants were grown *in vitro* on full MS solid medium for 2 weeks (20°C, 16h light). Subsequently  
561 3 plantlets of each treatment were transferred to 1ml of Murashige-Skoog (Sigma) liquid media  
562 added with 5µM β-estradiol, and incubated in a growth chamber (20°C, 16h light) under  
563 constant agitation. Plantlets were harvested 18 hours post β-estradiol treatment and frozen in  
564 liquid nitrogen. Total RNA was isolated with the mirVana miRNA Isolation kit (Invitrogen)  
565 according to manufacturers' instructions. RNAseq was performed at the GeT-PlaGe core  
566 facilities (INRAE Toulouse). RNA-seq libraries were prepared according to Illumina's protocols  
567 using the Illumina TruSeq Stranded mRNA sample prep kit to analyze mRNA. Briefly, mRNA  
568 was selected using poly-T beads. Subsequently RNA was fragmented and used to generate  
569 double stranded cDNA and adaptators were ligated to be sequenced. Eleven cycles of PCR  
570 were applied to amplify libraries. Library quality was assessed using a Fragment Analyzer and  
571 libraries were quantified by qPCR using the Kapa Library Quantification Kit (Roche). RNA-seq  
572 experiments were done on the Illumina NovaSeq6000 system using a paired-end read length of  
573 2x150 pb with the Illumina NovaSeq6000 sequencing kit.

## 574 **RNAseq data analysis and bioinformatics approaches**

575 Transcript pseudo-mapping and quantification were done using the pipeline nf-core/rnaseq v3.0  
576 with Araport11 as annotation reference. Special parameters were set as reverseStranded,  
577 pseudo-mapping and quantification with SALMON. Transcripts count normalization and  
578 characterization of Differentially Expressed Genes (DEG) was done using EdgeR package with  
579 GLM Fit in R version 4.1.1. A gene was considered to be differentially regulated between two  
580 conditions when the gene showed. log fold-change > 0.7 and an FDR-adjusted p value < 0.05 or  
581 0.005 as indicated.

582 The intersection between different lists of DEGs to construct Venn diagrams, was calculated in  
583 the online tool <https://bioinformatics.psb.ugent.be/webtools/Venn/>.

584 Interaction networks were constructed in the STRING database version 11.5 (Szklarczyk et al.,  
585 2021) that can be found in (<https://string-db.org/cgi/>), parameters were set as default. Functional  
586 categories were consider only when FDR<0.001.

587 The functional classifications on sets of DEG were done in the Classification SuperViewer Tool  
588 w/ Bootstrap that can be found in ([http://bar.utoronto.ca/ntools/cgi-bin/ntools\\_](http://bar.utoronto.ca/ntools/cgi-bin/ntools_)

classification\_superviewer.cgi) based on MapMan classification with all the other parameters set as default. Only groups with p-value < 0.05 were considered. All functional classifications on sets of DEG were done in the Classification SuperViewer Tool w/ Bootstrap that can be found in ([http://bar.utoronto.ca/ntools/cgi-bin/ntools\\_classification\\_superviewer .cgi](http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi)) based on MapMan classification with all the other parameters set as default. Only groups with p-value < 0.05 were considered. The frequency of genes falling into each category (Norm. Freq.) is normalized to the frequency of genes assigned to the category in a reference set of *A. thaliana* genes by the tool according to Provar & Zhu, 2003 and is calculated as: (Number\_in\_Classinput\_set/Number\_Classifiedinput\_set)/(Number\_in\_Classreference\_set/Number\_Classifiedreference\_set).

### **Construction of vectors for the transient expression of T3E in *N. benthamiana* plants.**

Genes of 28 T3E genes of *Xcc* strain 8004 were recombined into pGWB614 or pGWB615 (Nakamura et al., 2010) as indicated in Supplementary Table S2 from several pENTRY vectors (González-Fuente et al., 2020) using L/R-Clonase (Invitrogen) as manufacturer's instructions. T3E insertion was validated in resultant vectors by endpoint PCR. Expression vectors were electroporated in *A. tumefaciens* strain C58C1 using the "Gene Pulser Xcell Electroporation System" (Bio-Rad). Transformed strains were validated by colony PCR (Z. Sun et al., 2014).

### **Transient expression in *N. benthamiana***

For infiltration of *N. benthamiana* leaves, *A. tumefaciens* strains harboring the desired expression vector were infiltrated into the abaxial side of leaves of four weeks-old plants, using a needleless 1-ml syringe. *A. tumefaciens* strains were cultured overnight at 28°C in the presence of appropriate antibiotics. The cultures were harvested by centrifugation, the pellets were washed and resuspended in infiltration buffer (10 mM 2-(N-morpholino)-ethanesulfonic acid (MES); 10 mM MgCl<sub>2</sub>; 150 µM acetosyringone) to a final optical density at (OD<sub>600</sub>) of 0.5. Samples were incubated in darkness for two hours. Then cells were used for infiltration. Infiltrated plants were cultured in growth chamber conditions (16h light; 21°C; 70% relative humidity).

### **Protein extraction and detection by Western blot**

Four leaf disks (8mm diameter) from transformed *N. benthamiana* leaf tissue were harvested and frozen in liquid nitrogen two days after inoculation. Leaf disks were grinded in a tissue lyser. Samples were added with 300 µl of extraction buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 5

619 mM DTT, 0.5% triton X-100) and centrifuged at 12 500 g for 10 min at 4°C. 100 µl of resultant  
620 sample were diluted with 100µl of 2X loading buffer (4% SDS, 5 % 2-mercaptoethanol, 20%  
621 glycerol, 0.004% bromophenol blue, 0.125 M Tris HCl pH 6.8). Samples were denatured at  
622 95°C for 5 min. Proteins were separated on “Mini-PROTEAN TGX Stain Free gel 10%” (Bio-  
623 Rad) and transferred onto PVDF membranes (Bio-Rad). Protein loading was confirmed by Stain  
624 Free Blot technology (Bio-Rad) as manufacturer's instructions. Blots were blocked with 5% (w/v)  
625 non-fat dry milk in TBST for 2h then incubated from 4h to overnight at 4°C in antibody  
626 incubation buffer containing TBST, 5% (w/v) non-fat dry milk and HRP conjugated anti-HA  
627 antibody (Sigma) at a dilution of 1:10,000. Then, blots were washed with TBST and HRP activity  
628 was detected with ECL system (Bio-Rad) using the ChemiDoc imaging system (Bio-Rad).

629

## Bibliography

- Abreu, M. E., & Munné-Bosch, S. (2009). Salicylic acid deficiency in NahG transgenic lines and *sid2* mutants increases seed yield in the annual plant *Arabidopsis thaliana*. *Journal of Experimental Botany*, 60(4), 1261–1271. <https://doi.org/10.1093/jxb/ern363>
- Alfano, J. R., & Collmer, A. (2004). TYPE III SECRETION SYSTEM EFFECTOR PROTEINS: Double Agents in Bacterial Disease and Plant Defense. *Annual Review of Phytopathology*, 42(1), 385–414. <https://doi.org/10.1146/annurev.phyto.42.040103.110731>
- Anderson, J. P., Sperschneider, J., Win, J., Kidd, B., Yoshida, K., Hane, J., Saunders, D. G. O., & Singh, K. B. (2017). Comparative secretome analysis of *Rhizoctonia solani* isolates with different host ranges reveals unique secretomes and cell death inducing effectors. *Scientific Reports*, 7(1), 10410. <https://doi.org/10.1038/s41598-017-10405-y>
- Bethke, G., Pecher, P., Eschen-Lippold, L., Tsuda, K., Katagiri, F., Glazebrook, J., Scheel, D., & Lee, J. (2012). Activation of the *Arabidopsis thaliana* mitogen-activated protein kinase MPK11 by the flagellin-derived elicitor peptide, flg22. *Molecular Plant-Microbe Interactions: MPMI*, 25(4), 471–480. <https://doi.org/10.1094/MPMI-11-11-0281>
- Bigeard, J., Colcombet, J., & Hirt, H. (2015). Signaling Mechanisms in Pattern-Triggered Immunity (PTI). *Molecular Plant*, 8(4), 521–539. <https://doi.org/10.1016/j.molp.2014.12.022>
- Block, A., Guo, M., Li, G., Elowsky, C., Clemente, T. E., & Alfano, J. R. (2010). The *Pseudomonas syringae* type III effector HopG1 targets mitochondria, alters plant development and suppresses plant innate immunity. *Cellular Microbiology*, 12(3), 318–330. <https://doi.org/10.1111/j.1462-5822.2009.01396.x>
- Bonas, U. (1998). 5.4 Avirulence Genes. In P. Williams, J. Ketley, & G. Salmond (Eds.), *Methods in Microbiology* (Vol. 27, pp. 149–155). Academic Press. [https://doi.org/10.1016/S0580-9517\(08\)70278-2](https://doi.org/10.1016/S0580-9517(08)70278-2)
- Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, 212(4), 475–486. <https://doi.org/10.1007/s004250000458>
- Clough, S. J., & Bent, A. F. (1998). Floral dip: A simplified method for *Agrobacterium* -mediated transformation of *Arabidopsis thaliana*. *The Plant Journal*, 16(6), 735–743. <https://doi.org/10.1046/j.1365-313x.1998.00343.x>
- Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K., & Scheible, W.-R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiology*, 139(1), 5–17. <https://doi.org/10.1104/pp.105.063743>
- Degrave, A., Moreau, M., Launay, A., Barny, M., Brisset, M., Patrit, O., Taconnat, L., Vedel, R., & Fagard, M. (2013). The bacterial effector DspA/E is toxic in *Arabidopsis thaliana* and is required for multiplication and survival of fire blight pathogen. *Molecular Plant Pathology*, 14(5), 506–517. <https://doi.org/10.1111/mpp.12022>
- Eichmann, R., & Schäfer, P. (2015). Growth versus immunity—A redirection of the cell cycle? *Current Opinion in Plant Biology*, 26, 106–112. <https://doi.org/10.1016/j.pbi.2015.06.006>

668 Eschen-Lippold, L., Bethke, G., Palm-Forster, M. A. T., Pecher, P., Bauer, N., Glazebrook, J.,  
669 Scheel, D., & Lee, J. (2012). MPK11-a fourth elicitor-responsive mitogen-activated protein  
670 kinase in *Arabidopsis thaliana*. *Plant Signaling & Behavior*, 7(9), 1203–1205.  
671 <https://doi.org/10.4161/psb.21323>

672 Eschen-Lippold, L., Jiang, X., Elmore, J. M., Mackey, D., Shan, L., Coaker, G., Scheel, D., &  
673 Lee, J. (2016). Bacterial AvrRpt2-Like Cysteine Proteases Block Activation of the Arabidopsis  
674 Mitogen-Activated Protein Kinases, MPK4 and MPK11[OPEN]. *Plant Physiology*, 171(3),  
675 2223–2238. <https://doi.org/10.1104/pp.16.00336>

676 Gangadharan, A., Sreerekha, M.-V., Whitehill, J., Ham, J. H., & Mackey, D. (2013). The  
677 *Pseudomonas syringae* pv. Tomato Type III Effector HopM1 Suppresses Arabidopsis  
678 Defenses Independent of Suppressing Salicylic Acid Signaling and of Targeting AtMIN7.  
679 *PLOS ONE*, 8(12), e82032. <https://doi.org/10.1371/journal.pone.0082032>

680 Gomes, G. L. B., & Scortecci, K. C. (2021). Auxin and its role in plant development: Structure,  
681 signalling, regulation and response mechanisms. *Plant Biology (Stuttgart, Germany)*, 23(6),  
682 894–904. <https://doi.org/10.1111/plb.13303>

683 González-Fuente, M., Carrère, S., Monachello, D., Marsella, B. G., Cazalé, A.-C., Zischek, C.,  
684 Mitra, R. M., Rezé, N., Cottret, L., Mukhtar, M. S., Lurin, C., Noël, L. D., & Peeters, N. (2020).  
685 EffectorK, a comprehensive resource to mine for Ralstonia, Xanthomonas, and other  
686 published effector interactors in the Arabidopsis proteome. *Molecular Plant Pathology*,  
687 21(10), 1257–1270. <https://doi.org/10.1111/mpp.12965>

688 Guy, E., Genissel, A., Hajri, A., Chabannes, M., David, P., Carrere, S., Lautier, M., Roux, B.,  
689 Boureau, T., Arlat, M., Poussier, S., & Noël, L. D. (2013). Natural genetic variation of  
690 *Xanthomonas campestris* pv. *Campestris* pathogenicity on arabidopsis revealed by  
691 association and reverse genetics. *MBio*, 4(3), e00538-00512.  
692 <https://doi.org/10.1128/mBio.00538-12>

693 Hajri, A., Brin, C., Hunault, G., Lardeux, F., Lemaire, C., Manceau, C., Boureau, T., & Poussier,  
694 S. (2009). A "Repertoire for Repertoire" hypothesis: Repertoires of type three effectors are  
695 candidate determinants of host specificity in *Xanthomonas*. *PLoS ONE*, 4(8), 21 p.

696 Huang, Y., Li, T., Xu, T., Tang, Z., Guo, J., & Cai, Y. (2020). Multiple *Xanthomonas campestris*  
697 pv. *Campestris* 8004 type III effectors inhibit immunity induced by flg22. *Planta*, 252(5), 88.  
698 <https://doi.org/10.1007/s00425-020-03484-1>

699 Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth–Defense Tradeoffs in Plants:  
700 A Balancing Act to Optimize Fitness. *Molecular Plant*, 7(8), 1267–1287.  
701 <https://doi.org/10.1093/mp/ssu049>

702 Ignatov, A., Monakhos, G., Dzhililov, F., & Pozmogova, G. (2003). [A virulence gene from  
703 *Xanthomonas campestris* pv. *Campestris* homologous to the *avrBs2* locus is recognized in  
704 race-specific reaction by two different resistance genes in Brassica plant species]. *Genetika*,  
705 38, 1656–1662.

706 Jamir, Y., Guo, M., Oh, H.-S., Petnicki-Ocwieja, T., Chen, S., Tang, X., Dickman, M. B.,  
707 Collmer, A., & Alfano, J. R. (2004). Identification of *Pseudomonas syringae* type III effectors

708 that can suppress programmed cell death in plants and yeast. *The Plant Journal: For Cell*  
709 *and Molecular Biology*, 37(4), 554–565. <https://doi.org/10.1046/j.1365-313x.2003.01982.x>

710 Jiang, B.-L., He, Y.-Q., Cen, W.-J., Wei, H.-Y., Jiang, G.-F., Jiang, W., Hang, X.-H., Feng, J.-X.,  
711 Lu, G.-T., Tang, D.-J., & Tang, J.-L. (2008). The type III secretion effector XopXccN of  
712 *Xanthomonas campestris* pv. *Campestris* is required for full virulence. *Research in*  
713 *Microbiology*, 159(3), 216–220. <https://doi.org/10.1016/j.resmic.2007.12.004>

714 Lang, J., & Colcombet, J. (2020). Sustained Incompatibility between MAPK Signaling and  
715 Pathogen Effectors. *International Journal of Molecular Sciences*, 21(21), 7954.  
716 <https://doi.org/10.3390/ijms21217954>

717 Li, J., Cornelissen, B., & Rep, M. (2020). Host-specificity factors in plant pathogenic fungi.  
718 *Fungal Genetics and Biology*, 144, 103447. <https://doi.org/10.1016/j.fgb.2020.103447>

719 Li, P., Lu, Y.-J., Chen, H., & Day, B. (2020). The Lifecycle of the Plant Immune System. *Critical*  
720 *Reviews in Plant Sciences*, 39(1), 72–100. <https://doi.org/10.1080/07352689.2020.1757829>

721 Lozano-Durán, R., & Zipfel, C. (2015). Trade-off between growth and immunity: Role of  
722 brassinosteroids. *Trends in Plant Science*, 20(1), 12–19.  
723 <https://doi.org/10.1016/j.tplants.2014.09.003>

724 Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier,  
725 V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant  
726 pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, 13(6), 614–629.  
727 <https://doi.org/10.1111/j.1364-3703.2012.00804.x>

728 Nakamura, S., Mano, S., Tanaka, Y., Ohnishi, M., Nakamori, C., Araki, M., Niwa, T., Nishimura,  
729 M., Kaminaka, H., Nakagawa, T., Sato, Y., & Ishiguro, S. (2010). Gateway Binary Vectors  
730 with the Bialaphos Resistance Gene, *bar*, as a Selection Marker for Plant Transformation.  
731 *Bioscience, Biotechnology, and Biochemistry*, 74(6), 1315–1319.  
732 <https://doi.org/10.1271/bbb.100184>

733 Ngou, B. P. M., Ahn, H.-K., Ding, P., & Jones, J. D. G. (2021). Mutual potentiation of plant  
734 immunity by cell-surface and intracellular receptors. *Nature*, 592(7852), 110–115.  
735 <https://doi.org/10.1038/s41586-021-03315-7>

736 Nissinen, R. M., Ytterberg, A. J., Bogdanove, A. J., Van Wijk, K. J., & Beer, S. V. (2007).  
737 Analyses of the secretomes of *Erwinia amylovora* and selected hrp mutants reveal novel type  
738 III secreted proteins and an effect of HrpJ on extracellular harpin levels. *Molecular Plant*  
739 *Pathology*, 8(1), 55–67. <https://doi.org/10.1111/j.1364-3703.2006.00370.x>

740 Provart, N., & Zhu, T. (2003). A Browser-based Functional Classification SuperViewer for  
741 *Arabidopsis* Genomics. *Currents in Computational Molecular Biology*, 2003.

742 Quentin, M., Allasia, V., Pegard, A., Allais, F., Ducrot, P.-H., Favery, B., Levis, C., Martinet, S.,  
743 Masur, C., Ponchet, M., Roby, D., Schlaich, N. L., Jouanin, L., & Keller, H. (2009).  
744 Imbalanced Lignin Biosynthesis Promotes the Sexual Reproduction of Homothallic Oomycete  
745 Pathogens. *PLOS Pathogens*, 5(1), e1000264. <https://doi.org/10.1371/journal.ppat.1000264>

- 746 Rojas-Estevez, P., Urbina-Gómez, D. A., Ayala-Usma, D. A., Guayazan-Palacios, N., Mideros,  
747 M. F., Bernal, A. J., Cardenas, M., & Restrepo, S. (2020). Effector Repertoire of  
748 *Phytophthora betacei*: In Search of Possible Virulence Factors Responsible for Its Host  
749 Specificity. *Frontiers in Genetics*, 11, 579. <https://doi.org/10.3389/fgene.2020.00579>
- 750 Rong, W., Feng, F., Zhou, J., & He, C. (2010). Effector-triggered innate immunity contributes  
751 Arabidopsis resistance to *Xanthomonas campestris*. *Molecular Plant Pathology*, 11(6), 783–  
752 793. <https://doi.org/10.1111/j.1364-3703.2010.00642.x>
- 753 Rongqi, X., Xianzhen, L., Hongyu, W., Bole, J., Kai, L., Yongqiang, H., Jiaxuan, F., & Jiliang, T.  
754 (2006). Regulation of eight avr by hrpG and hrpX in *Xanthomonas campestris* pv. *Campestris*  
755 and their role in pathogenicity. *Progress in Natural Science*, 16(12), 1288–1294.  
756 <https://doi.org/10.1080/10020070612330143>
- 757 Schultink, A., Qi, T., Lee, A., Steinbrenner, A. D., & Staskawicz, B. (2017). Roq1 mediates  
758 recognition of the *Xanthomonas* and *Pseudomonas* effector proteins XopQ and HopQ1. *The*  
759 *Plant Journal*, 92(5), 787–795. <https://doi.org/10.1111/tpj.13715>
- 760 Shimono, M., Lu, Y.-J., Porter, K., Kvitko, B. H., Henty-Ridilla, J., Creason, A., He, S. Y., Chang,  
761 J. H., Staiger, C. J., & Day, B. (2016). The *Pseudomonas syringae* Type III Effector HopG1  
762 Induces Actin Remodeling to Promote Symptom Development and Susceptibility during  
763 Infection. *Plant Physiology*, 171(3), 2239–2255. <https://doi.org/10.1104/pp.16.01593>
- 764 Steinbrenner, A. D., Goritschnig, S., Krasileva, K. V., Schreiber, K. J., & Staskawicz, B. J.  
765 (2012). Effector Recognition and Activation of the *Arabidopsis thaliana* NLR Innate Immune  
766 Receptors. *Cold Spring Harbor Symposia on Quantitative Biology*, 77, 249–257.  
767 <https://doi.org/10.1101/sqb.2012.77.014860>
- 768 Sun, H., Zhu, X., Li, C., Ma, Z., Han, X., Luo, Y., Yang, L., Yu, J., & Miao, Y. (2021).  
769 *Xanthomonas* effector XopR hijacks host actin cytoskeleton via complex coacervation.  
770 *Nature Communications*, 12(1), 4064. <https://doi.org/10.1038/s41467-021-24375-3>
- 771 Sun, Z., Huang, Y., Wang, Y., Zhao, Y., & Cui, Z. (2014). Potassium hydroxide-ethylene  
772 diamine tetraacetic acid method for the rapid preparation of small-scale PCR template DNA  
773 from actinobacteria. *Molekuliarnaia Genetika, Mikrobiologiya i Virusologiya*, 1, 38–40.
- 774 Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T.,  
775 Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). The STRING  
776 database in 2021: Customizable protein-protein networks, and functional characterization of  
777 user-uploaded gene/measurement sets. *Nucleic Acids Research*, 49(D1), D605–D612.  
778 <https://doi.org/10.1093/nar/gkaa1074>
- 779 Tan, L., Rong, W., Luo, H., Chen, Y., & He, C. (2014). The *Xanthomonas campestris* effector  
780 protein XopDXcc8004 triggers plant disease tolerance by targeting DELLA proteins. *The New*  
781 *Phytologist*, 204(3), 595–608. <https://doi.org/10.1111/nph.12918>
- 782 Turner, P., Barber, C., & Daniels, M. (1984). Behaviour of the transposons Tn5 and Tn7 in  
783 *Xanthomonas campestris* pv. *Campestris*. *Molecular and General Genetics MGG*, 195(1),  
784 101–107. <https://doi.org/10.1007/BF00332731>

- Üstün, S., Bartetzko, V., & Börnke, F. (2013). The *Xanthomonas campestris* Type III Effector XopJ Targets the Host Cell Proteasome to Suppress Salicylic-Acid Mediated Plant Defence. *PLOS Pathogens*, 9(6), e1003427. <https://doi.org/10.1371/journal.ppat.1003427>
- Vicente, J. G., & Holub, E. B. (2013). *Xanthomonas campestris* pv. *Campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. *Molecular Plant Pathology*, 14(1), 2–18. <https://doi.org/10.1111/j.1364-3703.2012.00833.x>
- Wang, G., Roux, B., Feng, F., Guy, E., Li, L., Li, N., Zhang, X., Lautier, M., Jardinaud, M.-F., Chabannes, M., Arlat, M., Chen, S., He, C., Noël, L. D., & Zhou, J.-M. (2015). The Decoy Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-Self Recognition and Immunity in Plants. *Cell Host & Microbe*, 18(3), 285–295. <https://doi.org/10.1016/j.chom.2015.08.004>
- Wei, H.-L., Zhang, W., & Collmer, A. (2018). Modular Study of the Type III Effector Repertoire in *Pseudomonas syringae* pv. *Tomato* DC3000 Reveals a Matrix of Effector Interplay in Pathogenesis. *Cell Reports*, 23(6), 1630–1638. <https://doi.org/10.1016/j.celrep.2018.04.037>
- Yan, X., Tao, J., Luo, H.-L., Tan, L.-T., Rong, W., Li, H.-P., & He, C.-Z. (2019). A type III effector XopLXcc8004 is vital for *Xanthomonas campestris* pathovar *campestris* to regulate plant immunity. *Research in Microbiology*, 170(3), 138–146. <https://doi.org/10.1016/j.resmic.2018.12.001>
- Yuan, M., Jiang, Z., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.-M., He, S. Y., & Xin, X.-F. (2021). Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature*, 592(7852), 105–109. <https://doi.org/10.1038/s41586-021-03316-6>
- Zhou, J.-M., & Zhang, Y. (2020). Plant Immunity: Danger Perception and Signaling. *Cell*, 181(5), 978–989. <https://doi.org/10.1016/j.cell.2020.04.028>
- Zuo, J., Niu, Q. W., & Chua, N. H. (2000). Technical advance: An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. *The Plant Journal: For Cell and Molecular Biology*, 24(2), 265–273. <https://doi.org/10.1046/j.1365-313x.2000.00868.x>



## 2.1 Supplementary material

Supplementary Table S1. Strains used in this work

Strain	Relevant modification	Origin
<i>Xcc</i> 8004	$\Delta xopAN$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopD$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta avrXccA1$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopL$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopK$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopAH$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopQ$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopJ$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopAM$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopR$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopE2$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopN$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopX1-xopX2$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta hrpW-xopF$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopZ$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta avrXccA2$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopP-xopAL1$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta xopG$	Guy et al., 2013.
<i>Xcc</i> 8004	$\Delta avrBs1$	Guy et al., 2013.
<i>Xcc</i> 8004	Wild-type	Turner et al., 1984.
<i>A. tumefaciens</i> GV3101	Wild type	
<i>A. tumefaciens</i> C58C1	Wild type	

Supplementary Table S2. List of vectors used for transient delivery of the T3Es in *N. benthamiana*.

Plasmid	Gene insert	Accession No.	Destination vector	Validation by WB
pGWB615- <i>avrBs1</i>	<i>avrBs1</i>	XC_2081	pGWB615	Yes
pGWB615- <i>avrBs2</i>	<i>avrBs2</i>	XC_0052	pGWB615	-
pGWB615- <i>avrXccA1</i>	<i>avrXccA1</i>	XC_4318	pGWB615	-
pGWB615- <i>avrXccA2</i>	<i>avrXccA2</i>	XC_1716	pGWB615	Yes
pGWB615- <i>hpaA</i>	<i>hpaA</i>	XC_3018	pGWB615	Yes
pGWB615- <i>hrpW</i>	<i>hrpW</i>	XC_3023	pGWB615	Yes
pGWB615- <i>xopA</i>	<i>xopA</i>	XC_3002	pGWB615	-
pGWB614- <i>xopAC</i>	<i>xopAC</i>	XC_1553	pGWB614	Yes
pGWB615- <i>xopAG</i>	<i>xopAG</i>	XC_0563	pGWB615	Yes

pGWB614- <i>xopAH</i>	<i>xopAH</i>	XC_2004	pGWB614	Yes
pGWB615- <i>xopAL1</i>	<i>xopAL1</i>	XC_2995	pGWB615	Yes
pGWB615- <i>xopAL2</i>	<i>xopAL2</i>	XC_3915-6	pGWB615	-
pGWB614- <i>xopAM</i>	<i>xopAM</i>	XC_3160	pGWB614	Yes
pGWB615- <i>xopD</i>	<i>xopD</i>	XC_1213	pGWB615	Yes
pGWB614- <i>xopE2</i>	<i>xopE2</i>	XC_2602	pGWB614	Yes
pGWB615- <i>xopF</i>	<i>xopF</i>	XC_3024	pGWB615	Yes
pGWB615- <i>xopG</i>	<i>xopG</i>	XC_0967	pGWB615	-
pGWB615- <i>xopH</i>	<i>xopH</i>	XC_2082	pGWB615	Yes
pGWB614- <i>xopJ</i>	<i>xopJ</i>	XC_3802	pGWB614	Yes
pGWB615- <i>xopK</i>	<i>xopK</i>	XC_1210	pGWB615	Yes
pGWB615- <i>xopL</i>	<i>xopL</i>	XC_4273	pGWB615	Yes
pGWB615- <i>xopN</i>	<i>xopN</i>	XC_0241	pGWB615	Yes
pGWB615- <i>xopP</i>	<i>xopP</i>	XC_2994	pGWB615	Yes
pGWB615- <i>xopQ</i>	<i>xopQ</i>	XC_3177	pGWB615	-
pGWB615- <i>xopR</i>	<i>xopR</i>	XC_0268	pGWB615	Yes
pGWB615- <i>xopX2</i>	<i>xopX1</i>	XC_0541	pGWB615	Yes
pGWB615- <i>xopX1</i>	<i>xopX2</i>	XC_0542	pGWB615	Yes
pGWB615- <i>xopZ</i>	<i>xopZ</i>	XC_2210	pGWB615	-

Supplementary Table S3. List of vectors used to construct the Arabidopsis transgenic lines.

Plasmid	Gene insert	Accession No.	Destination vector
pER8- <i>avrBs1</i>	<i>avrBs1</i>	XC_2081	pER8
pER8- <i>avrBs2</i>	<i>avrBs2</i>	XC_0052	pER8
pER8- <i>avrXccA1</i>	<i>avrXccA1</i>	XC_4318	pER8
pER8- <i>avrXccA2</i>	<i>avrXccA2</i>	XC_1716	pER8
pER8- <i>hpaA</i>	<i>HpaA</i>	XC_3018	pER8
pER8- <i>hrpW</i>	<i>hrpW</i>	XC_3023	pER8
pER8- <i>xopA</i>	<i>xopA</i>	XC_3002	pER8
pER8- <i>xopAC</i>	<i>xopAC</i>	XC_1553	pER8
pER8- <i>xopAG</i>	<i>xopAG</i>	XC_0563	pER8
pER8- <i>xopAH</i>	<i>xopAH</i>	XC_2004	pER8
pER8- <i>xopAL1</i>	<i>xopAL1</i>	XC_2995	pER8
pER8- <i>xopAL2</i>	<i>xopAL2</i>	XC_3915-6	pER8
pER8- <i>xopAM</i>	<i>xopAM</i>	XC_3160	pER8
pER8- <i>xopAN</i>	<i>xopAN</i>	XC_3176	pER8
pER8- <i>xopD</i>	<i>xopD</i>	XC_1213	pER8
pER8- <i>xopE2</i>	<i>xopE2</i>	XC_2602	pER8
pER8- <i>xopF</i>	<i>xopF</i>	XC_3024	pER8
pER8- <i>xopG</i>	<i>xopG</i>	XC_0967	pER8

pER8- <i>xopH</i>	<i>xopH</i>	XC_2082	pER8
pER8- <i>xopJ</i>	<i>xopJ</i>	XC_3802	pER8
pER8- <i>xopK</i>	<i>xopK</i>	XC_1210	pER8
pER8- <i>xopL</i>	<i>xopL</i>	XC_4273	pER8
pER8- <i>xopN</i>	<i>xopN</i>	XC_0241	pER8
pER8- <i>xopP</i>	<i>xopP</i>	XC_2994	pER8
pER8- <i>xopQ</i>	<i>xopQ</i>	XC_3177	pER8
pER8- <i>xopR</i>	<i>xopR</i>	XC_0268	pER8
pER8- <i>xopX1</i>	<i>xopX1</i>	XC_0541	pER8
pER8- <i>xopX2</i>	<i>xopX2</i>	XC_0542	pER8
pER8- <i>xopZ</i>	<i>xopZ</i>	XC_2210	pER8

Supplementary Table S4. *A. thaliana* Col-0 transgenic lines and validation by qPCR.

Insert	Line ID	Cp values			$\Delta$ ct (OXA1)	$\Delta$ ct (GAPC2)
		T3E	OXA1	GAPC2		
pER8- <i>AvrBs1</i>	NAV217	28.51	28.13	23.93	0.77	0.04
pER8- <i>AvrBs1</i>	NAV220	28.04	26.44	22.01	0.33	0.02
pER8- <i>AvrXccA1</i>	NAV203	29.55	27.32	21.87	0.21	0.00
pER8- <i>AvrXccA1</i>	NAV81	23.87	24.84	20.17	1.96	0.08
pER8- <i>AvrXccA2</i>	NAV 183	22.67	29.15	24.65	89.26	3.94
pER8- <i>AvrXccA2</i>	NAV 234	19.47	27.12	23.22	200.85	13.45
pER8- <i>HpaA</i>	N333	26.87	26.26	21.00	0.66	0.02
pER8- <i>HpaA</i>	N367	27.10	26.58	20.65	0.70	0.01
pER8- <i>HrpW</i>	N479	20.40	25.86	21.50	44.17	2.15
pER8- <i>XopA</i>	N375	26.68	25.86	21.24	0.57	0.02
pER8- <i>XopA</i>	NAV76	26.24	25.21	20.84	0.49	0.02
pER8- <i>XopAC</i>	N403	20.64	25.01	20.48	20.75	0.90
pER8- <i>XopAG</i>	MGF 221	24.36	27.03	22.52	6.36	0.28
pER8- <i>XopAH</i>	NAV105	24.64	25.05	19.70	1.33	0.03
pER8- <i>XopAH</i>	NAV 83	25.53	29.31	24.68	13.74	0.55
pER8- <i>XopAL1</i>	NAV 185	30.61	24.93	19.92	0.02	0.00
pER8- <i>XopAL2</i>	NAV129	25.25	25.06	20.44	0.88	0.04
pER8- <i>XopAL2</i>	NAV208	20.89	28.55	22.30	202.25	2.66
pER8- <i>XopD</i>	NAV115	20.99	23.71	19.47	6.59	0.35
pER8- <i>XopD</i>	NAV117	25.34	23.24	18.87	0.23	0.01
pER8- <i>XopE2</i>	NAV135	23.16	28.93	23.12	54.57	0.97
pER8- <i>XopE2</i>	NAV225	22.86	24.87	20.71	4.03	0.23
pER8- <i>XopF</i>	NAV 162	27.82	26.28	21.12	0.34	0.01
pER8- <i>XopF</i>	NAV 193	25.90	24.68	19.10	0.43	0.01
pER8- <i>XopG</i>	MGF 218	23.75	24.78	20.13	2.04	0.08

pER8- <i>XopG</i>	MGF 387	19.34	27.56	22.43	298.17	8.51
pER8- <i>XopH</i>	NAV245	27.94	26.11	21.54	0.28	0.01
pER8- <i>XopH</i>	NAV261	19.72	25.32	19.88	48.67	1.12
pER8- <i>XopI</i>	N361	23.30	-	19.88	-	0.09
pER8- <i>XopI</i>	N362	26.58	-	20.68	-	0.02
pER8- <i>XopK</i>	NAV370	19.38	27.59	22.09	296.11	6.54
pER8- <i>XopL</i>	NAV138	18.59	24.56	19.47	62.68	1.84
pER8- <i>XopL</i>	NAV146	22.01	25.86	20.06	14.42	0.26
pER8- <i>XopN</i>	NAV 199	23.71	25.10	19.12	2.62	0.04
pER8- <i>XopN</i>	NAV200	27.77	26.97	22.83	0.57	0.03
pER8- <i>XopP</i>	MGF 52	32.51	27.07	21.17	0.02	0.00
pER8- <i>XopP</i>	MGF 77	27.96	25.92	21.76	0.24	0.01
pER8- <i>XopQ</i>	MGF 101	24.81	24.59	19.70	0.86	0.03
pER8- <i>XopQ</i>	MGF 90	28.08	26.80	21.73	0.41	0.01
pER8- <i>XopR</i>	NAV173	25.02	28.28	23.51	9.58	0.35
pER8- <i>XopR</i>	N328	20.28	29.51	21.29	600.49	2.01
pER8- <i>XopX1</i>	NAV226	29.30	27.68	23.48	0.33	0.02
pER8- <i>XopX1</i>	NAV228	24.51	25.10	21.43	1.51	0.12
pER8- <i>XopX2</i>	NAV119	17.73	25.69	20.03	249.00	4.92
pER8- <i>XopX2</i>	NAV 176	22.49	28.91	22.99	85.63	1.41
pER8- <i>XopZ</i>	NAV269	22.28	25.93	20.88	12.55	0.38
pER8- <i>XopZ</i>	NAV271	24.77	25.83	20.81	2.08	0.06

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Supplementary Table S5. List of genes commonly regulated by XopAC and either XopL, XopAH, XopK or AvrXccA1. Annotation color code in Supplementary Table S8.

DEG commonly down-regulated		DEG commonly up-regulated	
Gen ID	Annotation	Gen ID	Annotation
<b>AvrXccA1, XopAC, XopAH, XopK</b>		<b>AvrXccA1, XopAC, XopAH, XopK, XopL</b>	
At5g20160	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein	At1g31540	Disease resistance protein (TIR-NBS-LRR class) family
<b>AvrXccA1, XopAC, XopK, XopL</b>		At5g41740	Disease resistance protein (TIR-NBS-LRR class) family
At5g47550	CYS5_Cystatin/monellin superfamily protein	At1g66090	Disease resistance protein (TIR-NBS class)
<b>AvrXccA1, XopAC, XopAH</b>		At5g46470	RP56_disease resistance protein (TIR-NBS-LRR class) family
At1g54690	G-H2AX_GAMMA-H2AX_H2AXB_HTA3_gamma histone variant H2AX	At2g44290	Bifunctional inhibitor/lipid-transfer protein/seed storage 25 albumin superfamily protein
At3g53190	Pectin lyase-like superfamily protein	At5g59820	ATZAT12_RHL41_ZAT12_C2H2-type zinc finger family protein
At2g01830	AHK4_ATCRE1_CRE1_WOL_WOL1_CHASE domain containing histidine kinase protein	At1g21240	WAK3_wall associated kinase 3
At2g42190		At4g04490	CRK36_cysteine-rich RLK (RECEPTOR-like protein kinase) 36
At5g23010	GSM1_IMS3_MAM1_methylthioalkylmalate synthase 1	At4g23210	CRK13_cysteine-rich RLK (RECEPTOR-like protein kinase) 13
At5g13930	ATCH5_CHS_TT4_Chalcone and stilbene synthase family protein	At3g47480	Calcium-binding EF-hand family protein
At3g23390	RPL36aA_Zinc-binding ribosomal protein family protein	At4g26070	ATMEK1_MEK1_MKK1_NMAPKK_MAP kinase/ ERK kinase 1
<b>AvrXccA1, XopAC, XopK</b>		At1g01560	ATMPK11_MPK11_MAP kinase 11
At5g02610	Ribosomal L29 family protein	At5g24810	ABC1 family protein
At5g64140	RPS28_ribosomal protein S28	At5g66640	DAR3_DAI-related protein 3
At3g53890	EVR1_RPS21B_Ribosomal protein S21e	At4g38560	Arabidopsis phospholipase-like protein (PEARL1 4) family
At1g01540	Protein kinase superfamily protein	At1g13340	ISTL6_Regulator of Vps4 activity in the MVB pathway protein
At2g36490	ATROS1_DML1_ROS1_demeter-like 1	At3g47780	ABCA7_ATATH6_ATH6_ABC2 homolog 6
At5g08130	BIM1_basic helix-loop-helix (bHLH) DNA-binding superfamily protein	At1g35910	ATTPD_TPD_Haloacid dehalogenase-like hydrolase (HAD) superfamily protein
At3g05730		<b>AvrXccA1, XopAC, XopAH, XopK</b>	
At1g28290	AGP31_arabinogalactan protein 31	At2g17040	anac036_NAC036_NAC domain containing protein 36
At1g70310	SPD52_spermidine synthase 2	At5g64000	ATSAL2_SAL2_Inositol monophosphatase family protein
At5g19530	ACL5_S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	At3g61390	RING/U-box superfamily protein
At1g60810	ACLA-2_ACLA2_ATP-citrate lyase A-2	At1g14200	RING/U-box superfamily protein
At1g23720	Proline-rich extensin-like family protein	At2g41100	ATCAL4_CML12_TH3_Calcium-binding EF hand family protein
At4g15640		At2g46600	Calcium-binding EF-hand family protein
At1g49975		At3g35230	AGP5_arabinogalactan protein 5
At3g16670	Pollen Ole e 1 allergen and extensin family protein		
At1g05960	ARM repeat superfamily protein	At1g51270	structural molecules;transmembrane receptors;structural molecules
At1g16170		At4g23610	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
At2g34770	ATFAH1_FAH1_fatty acid hydroxylase 1	<b>AvrXccA1, XopAC, XopAH, XopL</b>	
At3g14310	ATPME3_OZS2_PME3_pectin methyltransferase 3	At1g67970	AT-HSFA8_ATHSFA8_HSFA8_heat shock transcription factor A8
At5g06160	ATO_splicing factor-related	At4g31800	ATWRKY18_WRKY18_WRKY DNA-binding protein 18
At1g63470	AHL5_at hook motif DNA-binding family protein	At3g10815	RING/U-box superfamily protein
At2g02070	ATIDD5_IDD5_RVN_indeterminate(ID)-domain 5	At3g19580	AZF2_ZF2_zinc-finger protein 2
At1g77060	Phosphoenolpyruvate carboxylase family protein	At3g13380	BRL3_BR1-like 3
At5g04600	RNA-binding (RRM/RBD/RNP motifs) family protein	At1g76580	Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein
At4g36180	Leucine-rich receptor-like protein kinase family protein	At3g61190	BAP1_BON association protein 1
At4g35950	ARAC6_ATRAC6_ATROP5_RAC2_RAC6_ROP5_RAC-like 6	At1g02580	EMB173_F1S1_MEA_SDG5_SET domain-containing protein
At4g20270	BAM3_Leucine-rich receptor-like protein kinase family protein	At1g02220	ANAC003_NAC003_NAC domain containing protein 3
<b>AvrXccA1, XopAC, XopL</b>		At5g14930	SAG101_senescence-associated gene 101
At3g61430	ATPIP1_PIP1_PIP1_P1P1A_plasma membrane intrinsic protein 1A	At5g17650	glycine/proline-rich protein
At4g19820	Glycosyl hydrolase family protein with chitinase insertion domain	At3g51660	Tautomerase/MIF superfamily protein
At2g33180		At3g22160	JAV1_VQ motif-containing protein
<b>XopAC, XopAH, XopK</b>		At1g24147	
At4g30170	Peroxidase family protein	At5g45000	Disease resistance protein (TIR-NBS-LRR class) family
At2g20490	EDA27_NOP10_nucleolar RNA-binding Nop10p family protein	At1g24145	
At1g63780	IMP4_Ribosomal RNA processing Brix domain protein	At1g50740	Transmembrane proteins 14C
<b>XopAC, XopK, XopL</b>		At3g26440	Protein of unknown function (DUF707)
At1g29950	SACL3_basic helix-loop-helix (bHLH) DNA-binding superfamily protein	At5g45480	Protein of unknown function (DUF594)
At1g76080	ATCDSP32_CDSP32_chloroplastic drought-induced stress protein of 32 kD	At1g29640	Protein of unknown function, DUF584
At3g58020	Chaperone DnaJ-domain superfamily protein	At5g22520	
<b>AvrXccA1, XopAC</b>		At1g73810	Core-2/1-branched beta-1,6-N-acetylglucosaminyltransferase family protein
At5g03650	SBE2.2_starch branching enzyme 2.2	At4g22305	SOBER1_alpha/beta-Hydrolases superfamily protein
At1g11720	ATSS3_SS3_starch synthase 3	At2g14610	AICAPE9_ATPR1_PR_1_PR1_pathogenesis-related gene 1
At4g19170	CCD4_NCED4_nine-cis-epoxycarotenoid dioxygenase 4	At4g19520	disease resistance protein (TIR-NBS-LRR class) family
At1g69700	ATHVA22C_HVA22C_HVA22 homologue C	At3g25510	disease resistance protein (TIR-NBS-LRR class), putative
At5g23360	GRAM domain-containing protein /ABA-responsive protein-related	At3g44400	disease resistance protein (TIR-NBS-LRR class) family
At1g74520	ATHVA22A_HVA22A_HVA22 homologue A	At4g16960	SRK1C3_Disease resistance protein (TIR-NBS-LRR class) family
At5g67030	ABA1_ATABA1_ATZEP_IBS3_LOS6_NPQ2_ZEP_zeaxanthin epoxidase (ZEP) (ABA1)	At1g57650	ATP binding
At1g70940	ATPIN3_PIN3_Auxin efflux carrier family protein	At3g44630	Disease resistance protein (TIR-NBS-LRR class) family
At2g34680	AIR9_Outer arm dynein light chain 1 protein	At2g17220	Kin3_Protein kinase superfamily protein
At4g38860	SAUR16_SAUR-like auxin-responsive protein family	At4g25910	ATMKX2_MK1_MK2_MAP kinase kinase 2
At2g33310	IAA13_auxin-induced protein 13	At4g11890	AROK1_CRK45_Protein kinase superfamily protein
At3g52940	ELL1_FK_HYD2_Ergosterol biosynthesis ERG4/ERG24 family	At5g60280	LecRK1_8_Concanavalin A-like lectin protein kinase family protein
At1g62960	ACS10_ACC synthase 10	At5g7025	U-box domain-containing protein kinase family protein
At5g61590	DEWAX_ERF107_Integrase-type DNA-binding superfamily protein	At3g61960	Protein kinase superfamily protein
At5g07580	DEWAX2_ERF106_Integrase-type DNA-binding superfamily protein	At3g22940	ATSBT3_5_SB3T3.5_Subtilase family protein
At1g74670	GAS46_Gibberellin-regulated family protein	At3g35620	Transducin/GD40 repeat-like superfamily protein
At5g16400	ATF2_TRF2_thioredoxin F2	At1g24140	AT3-AMMP_Matrixin family protein
At4g09010	APX4_TL29_ascorbate peroxidase 4	At1g08050	Zinc finger (C3HC4-type RING finger) family protein
At5g21100	Plant L-ascorbate oxidase	At5g19980	GFT1_CONSTA_golgi nucleotide sugar transporter 4
At2g25080	ATGPX1_GPX1_GPX11_glutathione peroxidase 1	At5g48400	ATGLR1.2_GL1R1.2_Glutamate receptor family protein
At5g20140	ATHBP5_HBP5_SOUL heme-binding family protein	At4g04540	CRK39_cysteine-rich RLK (RECEPTOR-like protein kinase) 39
At5g06290	2-Cys Prx B_2CPB_2-cysteine peroxidase B	At4g11890	AROK1_CRK45_Protein kinase superfamily protein
At4g25100	ATFSD1_FSD1_Fe superoxide dismutase 1	At5g01550	LecRK-Vi.3_LECRKA4.2_lectin receptor kinase a4.1
At1g15390	ATDEF1_PDF1A_peptide deformylase 1A	At5g28210	LRK10L3_Protein kinase family protein
At5g14640	ATDEF2_DEF2_PDF1B_peptide deformylase 1B	At3g25600	CML16_Calcium-binding EF-hand family protein
At4g13770	CYP83A1_REF2_cytochrome P450, family 83, subfamily A, polypeptide 1	At4g34150	Calcium-dependent lipid-binding (CaLB domain) family protein
At3g14660	CYP72A13_cytochrome P450, family 72, subfamily A, polypeptide 13	At5g54490	PBP1_pinoid-binding protein 1
At4g00360	ATT1_CYP86A2_cytochrome P450, family 86, subfamily A, polypeptide 2	At4g09570	ATCPK4_CPK4_calcium-dependent protein kinase 4
At3g00190	Peroxidase superfamily protein	At1g18890	ATCDPK1_ATCPK10_CDPK1_CP100_calcium-dependent protein kinase 1
At5g51890	Prx66_Peroxidase superfamily protein	At4g29810	ATMKK2_MK1_MK2_MAP kinase kinase 2
At1g51470	BGLU35_TGG5_beta glucosidase 35	At5g41610	ATOHX18_CHX18_cat1ion/H+ exchanger 18
At3g19720	ARCS_DRP5B_P-loop containing nucleoside triphosphate hydrolases superfamily protein	At3g17700	ATONGC20_CNBT1_CNGC20_cyclic nucleotide-binding transporter 1
At5g64620	ATC/VIF2_CVIF2_cell wall/vacuolar inhibitor of fructosidase 2	At2g38290	AMT2_AMT2.1_ATAMT2_ammonium transporter 2
At1g27120	GALT4_Galactosyltransferase family protein	At4g13510	AMT1.1_ATAMT1_ATAMT1.1_ammonium transporter 1;1
At2g31790	UDP-Glycosyltransferase superfamily protein	At4g18253	
At2g31750	UGT74D1_UDP-glucosyl transferase 74D1	<b>AvrXccA1, XopAC, XopK, XopL</b>	
At5g05890	UGT76C5_UDP-Glycosyltransferase superfamily protein	At5g52760	Copper transport protein family
At3g55710	UDP-Glycosyltransferase superfamily protein	At5g45630	Protein of unknown function, DUF584
At1g24120	GATL8_LGT9_Nucleotide-diphospho-sugar transferases superfamily protein	At2g32210	ATHCYSTM6
At1g55260	LTPG6_bifunctional inhibitor/lipid-transfer protein/seed storage 25 albumin superfamily protein	At1g73805	SARD1_Calmodulin binding protein-like
At3g43720	LTPG2_bifunctional inhibitor/lipid-transfer protein/seed storage 25 albumin superfamily protein	<b>XopAC, XopAH, XopK, XopL</b>	
At2g45180	Bifunctional inhibitor/lipid-transfer protein/seed storage 25 albumin superfamily protein	At5g48657	defense protein-related

At1g62500	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	At1g53625	
At3g22142	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	At3g50950	ZAR1__HOP2-ACTIVATED RESISTANCE 1
At5g48490	DIR1-like__Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily	At5g58120	Disease resistance protein (TIR-NBS-LRR class) family
At3g08920	Rhodanese/Cell cycle control phosphatase superfamily protein	At4g18880	AT-HSFA4A_HSF A4A__heat shock transcription factor A4A
At2g42220	Rhodanese/Cell cycle control phosphatase superfamily protein	At5g66210	CPK28__calcium-dependent protein kinase 28
At2g39000	ATNAA70__Acyl-CoA N-acyltransferases (NAT) superfamily protein	At1g78290	SNRK2-8_SNRK2-8_SRK2C__protein kinase superfamily protein
At4g18970	GDSL-like Lipase/Acylhydrolase superfamily protein	At1g16420	ATMCR8_AtMCP2e_MCR8_MCP2e__metacaspase 8
At4g14890	FdC1__2Fe-2S ferredoxin-like superfamily protein	At5g54720	Ankyrin repeat family protein
At5g42100	ATBG_PPAP_BG_PPAP__beta-1,3-glucanase_putative	At1g24150	ATFH4_FH4__formin homologue 4
At5g58480	O-Glycosyl hydrolases family 17 protein	At4g23170	CRK9_EP1__receptor-like protein kinase-related family protein
At4g18340	Glycosyl hydrolase superfamily protein	At5g66210	CPK28__calcium-dependent protein kinase 28
At3g12270	ATPRMT3_PRMT3__protein arginine methyltransferase 3	At3g46090	ZAT7__C2H2 and C2HC zinc fingers superfamily protein
At5g49020	ATPRMT4A_PRMT4A__protein arginine methyltransferase 4A	At3g46080	C2H2-type zinc finger family protein
At4g29720	AtPAOS_PAO5__polyamine oxidase 5	At3g56400	ATWRKY70_WRKY70__WRKY DNA-binding protein 70
At5g66920	skt17_SKU5 similar 17	At2g18660	ATPNP-A_PNP-A__plant natriuretic peptide A
At1g23740	AOR__Oxidoreductase, zinc-binding dehydrogenase family protein	<b>AvrXcca1,XopAC,XopAH</b>	
At3g04630	WDL1__WVD2-like 1	At1g48210	Protein kinase superfamily protein
At1g71790	AtCPB_CPB__Subunits of heterodimeric actin filament capping protein Capz superfamily	At1g48260	CPK17_SnRK3.21__CBL-interacting protein kinase 17
At3g19050	POK2__phragmoplast orienting kinesin 2	At2g26290	ARSK1__root-specific kinase 1
At4g15930	Dynein light chain type 1 family protein	At3g28580	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At4g26760	MAP65-2__microtubule-associated protein 65-2	At5g49570	ATPNG1_PNG1__peptide-N-glycanase 1
At5g55230	ATMAP65-1_MAP65-1_MAP65-1__microtubule-associated proteins 65-1	At5g18780	F-box/RN1-like superfamily protein
At1g15730	Cobalamin biosynthesis CobW-like protein	At5g45090	ATPP2-A7_PP2-A7__phloem protein 2-A7
At5g27550	P-loop containing nucleoside triphosphate hydrolases superfamily protein	At2g31990	Exostoin family protein
At4g34160	CYCD3_CYCD3;1__CYCLIN D3;1	At1g15220	Plant basic secretory protein (BSP) family protein
At1g15660	CENP-C_CENP-C__centromere protein C	At1g72930	ATTN10_TIR_TN10__toll/interleukin-1 receptor-like
At2g36250	ATFTS2-1_FT52-1__Tubulin/ftsZ family protein	At5g46520	ACQOS_VICTR__Disease resistance protein (TIR-NBS-LRR class) family
At5g13840	CCS52B_FZR3_FIZZY-related 3	At1g47890	ATRLP7_RLP7__receptor like protein 7
At5g67260	CYCD3;2__CYCLIN D3;2	At1g72900	Toll-Interleukin-Resistance (TIR) domain-containing protein
At1g15570	CYA2;3__CYCLIN A2;3	At1g48320	DHNAT1__Thioesterase superfamily protein
At3g15520	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	At4g18250	receptor serine/threonine kinase, putative
At1g74070	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	At4g23150	CRK7__cysteine-rich RLK (RECEPTOR-like protein kinase) 7
At4g15780	ATVAMP724_VAMP724__vesicle-associated membrane protein 724	At1g34420	leucine-rich repeat transmembrane protein kinase family protein
At1g08560	ATSYPI11_KN_SYP11__syntaxin of plants 111	At1g11050	Protein kinase superfamily protein
At2g28740	HIS4__histone H4	At4g04695	CPK31__calcium-dependent protein kinase 31
At4g24790	AAA-type ATPase family protein	At2g17290	ATCDPK3_ATCPK6_CPK6__Calcium-dependent protein kinase family protein
At2g37025	TRFL8__TRF-like 8	At3g52870	IQ calmodulin-binding motif family protein
At5g63960	GIS5__DNA binding;nucleotide binding;nucleic acid binding;DNA-directed DNA polymerases;DNA-directed DNA polymerases	At4g08500	ARAKIN_ATMEKK1_MAPKK8_MEKK1__MAPK/ERK kinase kinase 1
At1g66730	ATUG6_UG6__DNA LIGASE 6	At2g26560	PLA IIA_PLA2A_PLAII alpha_PLP2_PLP2__phospholipase A 2A
At3g20540	POLGAMMA1_PolB__polymerase gamma 1	At1g34180	anac016_NAC016__NAC domain containing protein 16
At3g10690	GYRA__DNA GYRASE A	At1g14880	ATPCR1_PCR1__PLANT CADMIUM RESISTANCE 1
At1g05470	CVP2__DNase I-like superfamily protein	At4g09770	TRAF-like family protein
At4g09730	RH39__RH39	At1g13550	Protein of unknown function (DUF1262)
At5g46580	SOT1__pentatricopeptide (PPR) repeat-containing protein	At3g15536	
At5g02820	BIN5_RHL2__Spo11/DNA topoisomerase VII, subunit A protein	At3g15770	
At2g42320	nucleolar protein gar2-related	At1g15790	
At3g24320	ATMSH1_CHM_CHM1_MSH1__MUTL protein homolog 1	At2g28570	
At3g50100	SDN1__small RNA degrading nuclease 1	At5g17350	
At3g02060	DEAD/DEAH box helicase, putative	At4g19970	
At1g12244	Polynucleotidyl transferase, ribonuclease H-like superfamily protein	At2g30660	ATP-dependent caseinolytic (Cip) protease/crotonase family protein
At3g35970	P-loop containing nucleoside triphosphate hydrolases superfamily protein	At3g07390	AIR12__auxin-responsive family protein
At4g08110	ATGLY12_GLXI-3__lactoylglutathione lyase family protein / glyoxalase I family protein	<b>AvrXcca1,XopAC,XopK</b>	
At4g01593	other RNA	At4g22690	CYP706A1__cytochrome P450, family 706, subfamily A, polypeptide 1
At3g54690	SETH3__Sugar isomerase (SIS) family protein	At1g30730	ATBBE11__FAD-binding Berberine family protein
At2g22740	ATCSLA02_ATCSLA2_CSLA02_CSLA2__cellulose synthase-like A02	At1g67980	CCOAMT__caffeoyl-CoA 3-O-methyltransferase
At5g60920	C0B__COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family	At2g46750	AtGuLO2_GuLO2__D-arabinono-1,4-lactone oxidase family protein
At4g18780	ATCESA8_CESA8_IRX1_LEW2__cellulose synthase family protein	At3g48640	
At2g35650	ATCSLA07_ATCSLA7_CSLA07_CSLA07_CSLA7__cellulose synthase like	At3g46930	Raf43__Protein kinase superfamily protein
At2g15880	LRX10__Leucine-rich repeat (LRR) family protein	At5g02490	ATHsp70-2_Hsp70-2__Heat shock protein 70 (Hsp 70) family protein
At2g14890	AGP9__arabinogalactan protein 9	At2g32680	ATRLP23_RLP23__receptor like protein 23
At4g16980	arabinogalactan-protein family	At5g64890	PROPEP2__elicitor peptide 2 precursor
At3g11700	FLA18__FASCLIN-like arabinogalactan protein 18 precursor	At3g48090	ATEDS1_EDS1__alpha/beta-Hydrolases superfamily protein
At5g06390	FLA17__FASCLIN-like arabinogalactan protein 17 precursor	At4g39640	GGT1__gamma-glutamyl transpeptidase 1
At2g47930	AGP26_ATAGP26__arabinogalactan protein 26	At3g10985	ATWI-12_SAG20_W112__senescence associated gene 20
At2g04780	FLA7__FASCLIN-like arabinogalactan 7	<b>AvrXcca1,XopAC,XopL</b>	
At3g06770	Pectin lyase-like superfamily protein	At2g01650	PUX2__plant UBX domain-containing protein 2
At1g23460	Pectin lyase-like superfamily protein	At5g46350	ATWRKY8_WRKY8__WRKY DNA-binding protein 8
At2g37640	ATEXP3_ATEXP3_ATHXP ALPHA 1.9_EXP3_EXPA3__Barwin-like endoglucanases superfamily	At1g66600	ABO3_ATWRKY63_WRKY63__ABA overly sensitive mutant 3
At2g28950	ATEXP6_ATEXP6_ATHXP ALPHA 1.8_EXPA6__expansin A6	At3g57390	AGL18__AGAMOUS-like 18
At5g09760	Plant invertase/pectin methyltransferase inhibitor superfamily	At1g78280	transferases, transferring glycosyl groups
At4g33220	ATPME44_PME44__pectin methyltransferase 44	At4g17720	RNA-binding (RRM/RBD/RNP motifs) family protein
At1g49430	LACS2_LRD2__long-chain acyl-CoA synthetase 2	At1g03850	ATGRXS13_GRXS13_ROXY18__Glutaredoxin family protein
At4g31780	EMB2797_MGD1_MGDA_UGT81A1__monogalactosyl diacylglycerol synthase 1	At3g10300	Calcium-binding EF-hand family protein
At2g22230	Thioesterase superfamily protein	At4g37640	ACA2__calcium ATPase 2
At2g26640	KCS11__3-ketoacyl-CoA synthase 11	At2g43290	ATCMLS_MSS3__Calcium-binding EF-hand family protein
At4g24510	CER2_VC-2_VC2__HXXXD-type acyl-transferase family protein	At4g34390	XLG2__extra-large GTP-binding protein 2
At2g15090	KCS8__3-ketoacyl-CoA synthase 8	At3g13610	F6H1__2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
At2g29980	AtFAD3_FAD3__fatty acid desaturase 3	At4g11840	PLDGAMMA3__phospholipase D gamma 3
At4g00400	AtGPAT8_GPAT8__glycerol-3-phosphate acyltransferase 8	At2g05940	RIPK__Protein kinase superfamily protein
At1g64720	CP5__Polyketide cyclase/dehydrase and lipid transport superfamily protein	At4g40180	ATHPP2C5_PP2C5__phosphatase 2C5
At1g27950	LTPG1__glycosylphosphatidylinositol-anchored lipid protein transfer 1	At1g78280	transferases, transferring glycosyl groups
At5g43270	SPL2__squamosa promoter binding protein-like 2	At2g05840	PAA2__20S proteasome subunit PAA2
At1g27370	SPL10__squamosa promoter binding protein-like 10	At1g19270	DA1__DA1
At3g04630	WDL1__WVD2-like 1	At1g22510	RING/U-box protein with domain of unknown function (DUF 1232)
At1g27470	transducin family protein / WD-40 repeat family protein	At5g47120	ATBI-1_ATBI1_BI-1_BI1__BAX inhibitor 1
At1g63260	TET10__tetraspanin10	At1g33560	ADR1__Disease resistance protein (CC-NBS-LRR class) family
At3g07430	ATYLMG1-1_EMB1990_YLMG1-1__YGGT family protein	At4g03450	Ankyrin repeat family protein
At5g22330	ATTIP49A_RIN1__P-loop containing nucleoside triphosphate hydrolases superfamily protein	At1g76970	Target of Myb protein 1
At1g75500	UMAMIT5_WAT1__Walls Are Thin 1	At3g49530	ANAC062_NAC062_NTL6__NAC domain containing protein 62
At3g18390	EMB1865_CRS1 / YhbY (CRM) domain-containing protein	At3g10500	AIF_ANAC053_NAC053_NTL4__NAC domain containing protein 53
At3g49670	BAM2__Leucine-rich receptor-like protein kinase family protein	At1g48370	YSL8__YELLOW STRIPE like 8
At1g49510	DPG1_emb1273__embryo defective 1273	At2g25520	Drug/metabolite transporter superfamily protein
At4g21190	emb1417__Pentatricopeptide repeat (PPR) superfamily protein	At2g36330	CASPLA43__Uncharacterised protein family (UPFO497)
At4g33400	Vacuolar import/degradation, Vid27-related protein	At1g23550	SRO2__similar to RCD one 2
At2g45190	AFO_FIL_YAB1__Plant-specific transcription factor YABBY family protein	At2g17120	CL-1_LYM2_LYP1__lysm domain GPI-anchored protein 2 precursor
At2g34680	AIR9__Outer arm dynein light chain 1 protein	At5g52720	Copper transport protein family
At1g18080	ATARCA_ATTRACK1_RACK1A_RACK1A_AT_SAC53__Transducin/WD40 repeat-like superfamily protein	At5g08660	PSI3__Protein of unknown function (DUF668)
At4g26370	antitermination NusB domain-containing protein	At1g77890	ATG14a__DNA-directed RNA polymerase II protein
At1g32580	MORF5__plastid developmental protein DAG, putative	At5g37480	
At1g29900	CARB_VEN3__carbamoyl phosphate synthetase B	At5g16170	Core-2/l-branching beta-1,6-N-acetylglucosaminyltransferase family protein
At2g35040	AICARFT/IMPChase bienzyme family protein	At1g13470	Protein of unknown function (DUF1262)
At3g21110	ATPURC_PUR7_PURC__purin 7	At1g76980	
At1g72040	AtDNK_dnk__P-loop containing nucleoside triphosphate hydrolases superfamily protein	At5g65300	

At3g53900	PYRR_UPP__uracil phosphoribosyltransferase	At3g53150	UGT73D1__UDP-glucosyl transferase 73D1
At3g09820	ADK1__ATADK1__adenosine kinase 1	At2g43080	AT-P4H-1__P4H isoform 1
At5g63310	ATNDPK2_NDPK1A_NDPK1A1A_NDPK1A_NDPK2__nucleoside diphosphate kinase 2	<b>XopAC, XopAH, XopK</b>	
At1g01920	SET domain-containing protein	At2g19190	FRK1__SIRK__FLG22-induced receptor-like kinase 1
At3g52140	FMT_FRIENDLY_NOXY38__tetratricopeptide repeat (TPR)-containing protein	At5g01540	LecRK-Vi.2__LECRKA4.1__lectin receptor kinase a4.1
At1g67870	glycine-rich protein	At2g41410	Calcium-binding EF-hand family protein
At2g02150	EMB2794__Tetratricopeptide repeat (TPR)-like superfamily protein	At4g33050	AtIQM1_EDA39_IQM1__calmodulin-binding family protein
At4g35850	Pentatricopeptide repeat (PPR) superfamily protein	At5g54710	Ankyrin repeat family protein
At5g60960	PNM1_RPPR9__Pentatricopeptide repeat (PPR) superfamily protein	At5g06320	NHL3__NDR1/HIN1-like 3
At5g13770	Pentatricopeptide repeat (PPR-like) superfamily protein	At4g14370	Disease resistance protein (TIR-NBS-LRR class) family
At1g15510	ATECB2_FCB2_VAC1__Tetratricopeptide repeat (TPR)-like superfamily protein	At2g27660	Cysteine/Histidine-rich C1 domain family protein
At3g02650	Tetratricopeptide repeat (TPR)-like superfamily protein	At3g01290	AtHIR2_HIR2__SPFH/Band 7/PHB domain-containing membrane-associated protein family
At2g32230	PRORP1__proteinaceous RNase P 1	At2g23680	Cold acclimation protein WCOR413 family
At4g21190	emb1417__Pentatricopeptide repeat (PPR) superfamily protein	At5g44580	
At2g42920	Pentatricopeptide repeat (PPR-like) superfamily protein	At5g19240	Glycoprotein membrane precursor GPI-anchored
At1g61870	PPR336_rPPR1__pentatricopeptide repeat 336	At4g38550	Arabidopsis phospholipase-like protein (PEARL1 4) family
At5g15280	Pentatricopeptide repeat (PPR) superfamily protein	At5g64870	SPFH/Band 7/PHB domain-containing membrane-associated protein family
At1g14620	DECOY__decoy	At1g07000	ATEXO70B2_EXO70B2__exocyst subunit exo70 family protein B2
At5g15550	atPEIP2_AtPEP2__Transducin/WD40 repeat-like superfamily protein	At5g49520	ATWRKY48_WRKY48__WRKY DNA-binding protein 48
At2g34460	NAD(P)-binding Rossmann-fold superfamily protein	At2g40140	AtSZF2_CZF1_SZF2_TZF10_ZFAR1__zinc finger (CCCH-type) family protein
At3g23940	DHAD__dehydratase family	At2g45220	AtPME17_PME17__Plant invertase/pectin methylesterase inhibitor superfamily
At2g22170	PLAT2__Lipase/lipoxygenase, PLAT/LH2 family protein	At5g10380	AtTRING1_RING1__RING/U-box superfamily protein
At4g34290	SWIB3__SWIB/MDM2 domain superfamily protein	<b>XopAC, XopAH, Xopl</b>	
At5g57170	Tautomerase/MIF superfamily protein	At5g62150	peptidoglycan-binding LysM domain-containing protein
At5g53500	Transducin/WD40 repeat-like superfamily protein	At1g72890	Disease resistance protein (TIR-NBS class)
At1g70280	NHL domain-containing protein	At4g36150	Disease resistance protein (TIR-NBS-LRR class) family
At5g61020	ECT3__evolutionarily conserved C-terminal region 3	At3g11010	AtRLP34_RLP34__receptor like protein 34
At1g17200	CASPL2A1__Uncharacterised protein family (UPF0497)	At5g66910	NRG1.2__Disease resistance protein (CC-NBS-LRR class) family
At2g35450	catalytic/hydrolases	At2g16870	Disease resistance protein (TIR-NBS-LRR class) family
At3g02690	nodulin MtN21_EamA-like transporter family protein	At1g33950	IAN7__Avirulence induced gene (AIG1) family protein
At1g68780	RNI-like superfamily protein	At1g31580	CXC750_ECS1__ECS1
At3g58220	TRAF-like family protein	At4g33300	ADR1-L1__ADR1-like 1
At4g02530	MPH2__chloroplast thylakoid lumen protein	At2g32140	transmembrane receptors
At3g16260	TRZ4__tRNase Z4	At3g50470	HR3_MLA10__homolog of RPW8 3
At1g52670	BAD_C2_BLP1__Single hybrid motif superfamily protein	At1g09560	GLP5_PDLGP1__germin-like protein 5
At2g41050	PQ-loop repeat family protein / transmembrane family protein	At3g62600	ATERDJB8_ERDJB3__DNAJ heat shock family protein
At1g15330	Cystathionine beta-synthase (CBS) protein	At3g54100	O-fucosyltransferase family protein
At5g11240	AtGHS40_GHS40_NuGWD1__transducin family protein / WD-40 repeat family protein	At5g48410	ATGLR1.3_GLR1.3__glutamate receptor 1.3
At1g79880	AtLa2_La2__RNA recognition motif (RRM)-containing protein	At1g70690	HW11_PDLP5__Receptor-like protein kinase-related family protein
At4g12830	alpha/beta-Hydrolases superfamily protein	At3g59700	ATHLECRK_HLECRK_LecRK-V.5_LecRK-V.5_LECRK1__lectin-receptor kinase
At3g61080	Protein kinase superfamily protein	At5g48380	BIR1__BAK1-interacting receptor-like kinase 1
At5g52970	thylakoid lumen 15.0 kDa protein	At3g21630	AtCERK1_AtLYK1_CERK1_LYK1_LYSM RLK1__chitin elicitor receptor kinase 1
At5g56750	NDL1__N-MYC downregulated-like 1	At1g21250	AtWAK1_PRO25_WAK1__cell wall-associated kinase
At1g75200	flavodoxin family protein / radical SAM domain-containing protein	At1g21270	WAK2__wall-associated kinase 2
At5g23430	KTN80.4__Transducin/WD40 repeat-like superfamily protein	At4g23200	CRK12__cysteine-rich RLK (RECEPTOR-like protein kinase) 12
At3g53530	NAKR3__Chloroplast-targeted copper chaperone protein	At4g04500	CRK37__cysteine-rich RLK (RECEPTOR-like protein kinase) 37
At3g56900	ALADIN__Transducin/WD40 repeat-like superfamily protein	At1g76040	CPK29__calcium-dependent protein kinase 29
At1g65370	TRAF-like family protein	At5g42380	AtCML37_CML37__calmodulin like 37
At3g20820	Leucine-rich repeat (LRR) family protein	At1g08920	ESL1__ERD (early response to dehydration) six-like 1
At1g63980	D111/G-patch domain-containing protein	At5g60800	HIPP3__Heavy metal transport/detoxification superfamily protein
At5g48360	FH9__Actin-binding FH2 (formin homology 2) family protein	At1g30900	BP80-3;3_VSR3;3_VSR6__VACUOLAR SORTING RECEPTOR 6
At3g48500	PDE312_PTAC10_TAC10__Nucleic acid-binding, OB-fold-like protein	At4g20110	BP80-3;1_VSR3;1_VSR7__VACUOLAR SORTING RECEPTOR 7
At5g49960		At3g17420	GPK1__glyoxysomal protein kinase 1
At4g35920	MCA1__PLAC8 family protein	At5g02290	NAK__Protein kinase superfamily protein
At5g20600		At1g72540	Protein kinase superfamily protein
At3g57180	BPG2_YL1__P-loop containing nucleoside triphosphate hydrolases superfamily protein	At3g28540	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At1g08300	NVL__no vein-like	At5g57480	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At2g25480	TPX2 (targeting protein for Xkfp2) protein family	At3g21810	Zinc finger C-x8-C-x5-C-x3-H type family protein
At1g28530	ANU10	At3g25882	NIMIN-2__NIM1-interacting 2
At5g58930	OPL4__Protein of unknown function (DUF740)	At1g02450	NIMIN-1_NIMIN1__NIM1-interacting 1
At4g33625		At2g40750	ATWRKY54_WRKY54__WRKY DNA-binding protein 54
At3g07860	Ubiquitin-like superfamily protein	At5g26170	ATWRKY50_WRKY50__WRKY DNA-binding protein 50
At5g20130		At5g09290	Inositol monophosphatase family protein
At2g36410	Family of unknown function (DUF662)	At4g11655	CASPL4A4__Uncharacterised protein family (UPF0497)
At1g06240	Protein of unknown function DUF455	At2g38830	Ubiquitin-conjugating enzyme/RWD-like protein
At5g38150	PMI15__Plant protein of unknown function (DUF827)	At1g69840	AtHIR1__SPFH/Band 7/PHB domain-containing membrane-associated protein family
At5g14410		At1g03660	Ankyrin-repeat containing protein
At3g25805		At3g18250	Putative membrane lipoprotein
At1g14345	NAD(P)-linked oxidoreductase superfamily protein	At5g05190	EDR4__Protein of unknown function (DUF3133)
At4g39040	CFM4__RNA-binding CRS1 / YhbY (CRM) domain protein	At2g37940	AtPC32_ERH1__Arabidopsis Inositol phosphorylceramide synthase 2
At3g63170	AtFAP1_FAP1__Chalcone-flavanone isomerase family protein	At4g37030	
At2g21070	FIO1__methyltransferases	At3g60966	RING/U-box superfamily protein
At1g07970		At1g21520	
At1g29700	Metallo-hydrolase/oxidoreductase superfamily protein	At5g62770	Protein of unknown function (DUF1645)
At1g09340	CRB_CSP41B_HIP1.3__chloroplast RNA binding	At5g22530	
At4g30720	PDE327__FAD/NAD(P)-binding oxidoreductase family protein	At5g45470	Protein of unknown function (DUF594)
At3g08030	Protein of unknown function, DUF642	At3g26210	CYP71B23__cytochrome P450, family 71, subfamily B, polypeptide 23
At1g55370	NDP5__NDH-dependent cyclic electron flow 5	At3g09020	alpha 1,4-glycosyltransferase family protein
At5g08720	HCF145_HCF145_PPP1	At5g52810	SARD4__NAD(P)-binding Rossmann-fold superfamily protein
At4g37110	Zinc-finger domain of monoamine-oxidase A repressor R1	At5g50340	ATP-dependent peptidases;nucleotide binding;serine-type endopeptidases;DNA helicases;ATP binding;damaged DNA binding;nucleoside-triphosphatases
At2g11910		At5g45110	ATNPR3_NPR3__NPR1-like protein 3
At5g27400	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	At3g23280	XBAT35__XB3 ortholog 5 in Arabidopsis thaliana
At3g06150		At3g53230	AtCDC48B__ATPase, AAA-type, CDC48 protein
At1g50450	Saccharopine dehydrogenase	At4g16957	
At4g14870	SECE1__secE/secE1-gamma protein transport protein	At4g09215	
At5g52370		<b>XopAC, XopK, Xopl</b>	
At4g15790		At1g10340	Ankyrin repeat family protein
At3g22210		At1g21550	Calcium-binding EF-hand family protein
At3g11770	RICE1__Polynucleotidyl transferase, ribonuclease H-like superfamily protein	At1g31130	
At2g21385	AtCGLD11_BFA3_CGLD11	<b>AvrXccA1, XopAC</b>	
At1g44000	SGR1	At4g05390	ATRFNR1_RFRN1__root FNR 1
At3g43850		At4g30530	GGP1__Class I glutamine amidotransferase-like superfamily protein
At1g67910		At1g26440	ATUP55_UPS5_UPS5__ureide permease 5
At1g22850	SNARE associated Golgi protein family	At3g57550	AGK2_GK-2__guanylate kinase
At2g40640	RING/U-box superfamily protein	At1g47510	SPTASE11_ATSPTASE11__inositol polyphosphate 5-phosphatase 11
At4g29905		At3g50900	
At3g01860		At5g63680	Pyruvate kinase family protein
At1g33810		At2g36580	Pyruvate kinase family protein
At3g49990		At5g56350	Pyruvate kinase family protein
At3g49290	ABIL2__ABL interactor-like protein 2	At3g47960	AtNPF2.10_GTR1_NPF2.10__Major facilitator superfamily protein
At3g51510		At5g51710	ATKEA5_KEA5__K+ efflux antiporter 5
At5g17670	alpha/beta-Hydrolases superfamily protein	At1g15520	ABCG40_ATABCG40_ATPDR12_PDR12__pleiotropic drug resistance 12



At2g24290	Protein of unknown function (DUF1068)	At4g15233	ABCG42__ABC-2 and Plant PDR ABC-type transporter family protein
At1g67865		At4g15236	ABCG43__ABC-2 and Plant PDR ABC-type transporter family protein
At2g30350	HIGLE__Excinuclease ABC, C subunit, N-terminal	At1g55730	ATCAX5_CAX5__cation exchanger 5
At1g22790		At1g61800	ATGPT2_GPT2__glucose-6-phosphate/phosphate translocator 2
At2g20020	ATCAF1_CAF1__RNA-binding CRS1 / YhbY (CRM) domain-containing protein	At3g47420	ATG3Pp1_ATP53_G3Pp1_P53__phosphate starvation-induced gene 3
At5g66090		At1g23800	ALDH2B_ALDH2B7__aldehyde dehydrogenase 2B7
At2g30170	PBCP__Protein phosphatase 2C family protein	At2g24180	CYP71B6__cytochrome p450 71b6
At1g35780		At3g14620	CYP72A8__cytochrome P450, family 72, subfamily A, polypeptide 8
At1g16520	NAIP2	At2g45570	CYP76C2__cytochrome P450, family 76, subfamily C, polypeptide 2
At2g42130	Plastid-lipid associated protein PAP / fibrillin family protein	At3g26170	CYP71B19__cytochrome P450, family 71, subfamily B, polypeptide 19
At4g08555		At5g36220	CYP81D1_CYP91A1__cytochrome p450 81d1
At5g25240		At4g22710	CYP70A2__cytochrome P450, family 706, subfamily A, polypeptide 2
At4g24750	Rhodanese/Cell cycle control phosphatase superfamily protein	At4g37520	Peroxidase superfamily protein
At2g05120	NUP133__Nucleoporin, Nup133/Nup155-like	At1g62760	AtPMEI10__Plant invertase/pectin methylesterase inhibitor superfamily protein
At1g16000	OEP9.1	At4g26940	Galactosyltransferase family protein
At5g22390	Protein of unknown function (DUF3049)	At1g353160	UGT73C7__UDP-glucosyl transferase 73C7
At3g54260	TBL36__TRICHOME BIREFRINGENCE-LIKE 36	At1g62790	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
At3g43540	Protein of unknown function (DUF1350)	At2g47130	AtSDR3_SDR3__NAD(P)-binding Rossmann-fold superfamily protein
At3g45160	Putative membrane lipoprotein	At1g65610	ATGH9A2_ATKOR2_KOR2__Six-hairpin glycosidases superfamily protein
At5g56850		At3g60130	BGLU16__beta glucosidase 16
At1g64680		At5g20940	Glycosyl hydrolase family protein
At5g52110	CCB2_HCF208__Protein of unknown function (DUF2930)	At5g44400	AtBBE26__FAD-binding Berberine family protein
At4g39300		At2g29350	SAG13__senescence-associated gene 13
At2g36145		At1g30700	AtBBE8__FAD-binding Berberine family protein
At1g13670	BGL2	At1g69920	ATGSTU12_GSTU12__glutathione S-transferase TAU 12
At1g69160	BGL1	At1g69930	ATGSTU11_GSTU11__glutathione S-transferase TAU 11
At3g10525	LGO_SMR1__LOSS OF GIANT CELLS FROM ORGANS	At2g29460	ATGSTU4_GST22_GSTU4__glutathione S-transferase tau 4
At1g77270		At5g19140	AtLIP1_ATAILP1__Aluminium induced protein with YGL and LRDR motifs
At5g41190	AtNOB1_NOB1	At3g09940	AtMDAR3_Mdar3__monodehydroascorbate reductase
At4g15710		At5g03630	MDAR2__Pyridine nucleotide-disulphide oxidoreductase family protein
At1g53450		At4g19230	CYP707A1__cytochrome P450, family 707, subfamily A, polypeptide 1
At1g15980	NDF1_NDH48_PnsB1__NDH-dependent cyclic electron flow 1	At4g37390	AUR3_BRU6_GH3-2_GH3.2_YDK1__Auxin-responsive GH3 family protein
At3g29185	BFA1__Domain of unknown function (DUF3598)	At3g44300	AtNIT2_NIT2__nitrilase 2
At2g03420		At3g44310	AtNIT1_NIT1_NITI__nitrilase 1
At5g64460	Phosphoglycerate mutase family protein	At2g31230	ATERF15_ERF15__ethylene-responsive element binding factor 15
At4g27010	EMB2788	At4g17500	AtERF100_ATERF-1_ERF-1_ERF1A__ethylene responsive element binding factor 1
At3g60360	EDA14_UTP11__embryo sac development arrest 14	At3g04580	EIN4__Signal transduction histidine kinase, hybrid-type, ethylene sensor
At2g24020	STIC2__Uncharacterised BCR, YbaB family COG0718	At2g23240	AtERF092_ERF1_ERF1B__ethylene response factor 1
At4g34260	AXY8_FUC5A__1,2-alpha-L-fucosidases	At1g02400	ATGA20X4_ATGA20X6_DTA1_GA20X6__gibberellin 2-oxidase 6
At4g38440	IYO	At2g06050	AtOPR3_DDE1_OPR3__oxophytodienoate-reductase 3
At1g34010		At1g19180	AJAZ1_JAZ1_TIFY10A__jasmonate-zim-domain protein 1
At5g12900		At1g72520	AtLOX4_LOX4__PLAT/LH2 domain-containing lipooxygenase family protein
At1g01430	MOAT3_TBL25__TRICHOME BIREFRINGENCE-LIKE 25	At1g51420	AtSP1_SPP1__sucrose-phosphatase 1
At1g14300	ARM repeat superfamily protein	At2g31390	FRK1_FRK2__pfk8-like carbohydrate kinase family protein
At5g19190		At5g38530	TSBtype2__tryptophan synthase beta type 2
At1g73090		At3g54640	TRP3_TSA1__tryptophan synthase alpha chain
At3g54080	Concanavalin A-like lectin family protein	At3g10370	SDP6__FAD-dependent oxidoreductase family protein
At5g42765		At1g75000	ELO3_GNS1/SUR4 membrane protein family
At1g65230	Uncharacterized conserved protein (DUF2358)	At1g77420	MAGL5__alpha/beta-Hydrolases superfamily protein
At2g26110	Protein of unknown function (DUF761)	At1g54890	Late embryogenesis abundant (LEA) protein-related
At1g78915	Tetrapeptide repeat (TPR)-like superfamily protein	At1g52890	ANAC019_ANAC19_NAC019__NAC domain containing protein 19
At4g30990	ARM repeat superfamily protein	At5g50520	Major facilitator superfamily protein
At1g75120	RRA1__Nucleotide-diphospho-sugar transferase family protein	At4g12250	GAE5__UDP-D-glucuronate 4-epimerase 5
At3g15480	Protein of unknown function (DUF1218)	At5g16910	ATCSLD2_CSLD2__cellulose-synthase like D2
At2g04790		At5g60950	COBL5__COBRA-like protein 5 precursor
At4g24972	TPD1_tapetum determinant 1	At5g15650	AtTRGP2_MUR5_RGP2__reversibly glycosylated polypeptide 2
At4g37080	Protein of unknown function, DUF547	At1g21310	AtEXT3_EXT3_RSH__extensin 3
At5g27390	Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein	At3g07970	QRT2__Pectin lyase-like superfamily protein
At4g32330	WDL5__TPX2 (targeting protein for Xlp2) protein family	At4g30280	AtXTH18_XTH18__xyloglucan endotransglucosylase/hydrolase 18
At4g23870		At4g25810	XTH23_XTR6__xyloglucan endotransglucosylase 6
At1g05070	Protein of unknown function (DUF1068)	At3g55410	2-oxoglutarate dehydrogenase, E1 component
At4g19100	PAM68__Protein of unknown function (DUF3464)	At4g36640	Sec14p-like phosphatidylinositol transfer family protein
At5g43750	NDH18_PnsB5__NAD(P)H dehydrogenase 18	At1g22180	Sec14p-like phosphatidylinositol transfer family protein
At5g03830	CDK inhibitor P21 binding protein	At3g60540	Preprotein translocase Sec, Sec61-beta subunit protein
At1g50040	Protein of unknown function (DUF1005)	At1g52600	Peptidase S24/S26A/S26B/S26C family protein
At1g32220	NAD(P)-binding Rossmann-fold superfamily protein	At3g56170	PATL6__SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein
At4g16141	AtGATA17L_GATA17L__GATA type zinc finger transcription factor family protein	At4g12120	AtSEC18_SEC18__Sec1/munc18-like (SM) proteins superfamily
At1g50900	GDCL1_LTD__Ankyrin family protein	At2g17790	VPS35A_ZIP3__VPS35 homolog A
At5g67370	CGLD27__Protein of unknown function (DUF1230)	At3g09830	PCRK1__Protein kinase superfamily protein
At5g39600		At3g26020	PP2AB'ETA__Protein phosphatase 2A regulatory B subunit family protein
At1g66840	PMI2_WEB2__Plant protein of unknown function (DUF827)	At5g14640	ATSK13_SK13__shaggy-like kinase 13
At3g51140	Protein of unknown function (DUF3353)	At5g01850	Protein kinase superfamily protein
At4g27030	FAD4_FADA__fatty acid desaturase A	At3g05580	AUN1_TOPP9__Calcineurin-like metallo-phosphoesterase superfamily protein
At3g13510	Protein of Unknown Function (DUF239)	At1g61360	S-locus lectin protein kinase family protein
At5g39210	CRR7__chlororespiratory reduction 7	At3g15260	Protein phosphatase 2C family protein
At3g07460	Protein of unknown function, DUF538	At5g51290	ACD5__Diacylglycerol kinase family protein
At3g04160		At2g15310	ARFB1A_ATARFB1A__ADP-ribosylation factor B1A
At3g12345		At1g61610	S-locus lectin protein kinase family protein
At2g35790		At5g65500	U-box domain-containing protein kinase family protein
At2g42870	HLH1_PAR1__phy rapidly regulated 1	At3g26980	MUB4__membrane-anchored ubiquitin-fold protein 4 precursor
At2g45000	AtNUP62_EMB2766_NUP62__structural constituent of nuclear pore	At1g67800	RLG5__Copine (Calcium-dependent phospholipid-binding protein) family
At5g27330	Prefoldin chaperone subunit family protein	At5g63970	RLG3__Copine (Calcium-dependent phospholipid-binding protein) family
At2g18220	AtNOC2_NOC2__Noc2p family	At4g01610	AtcatbB3__Cysteine proteinases superfamily protein
At3g23760		At1g26930	Galactose oxidase/kelch repeat superfamily protein
At1g53800		At2g27310	F-box family protein
At2g34510	Protein of unknown function, DUF642	At1g70170	At2-MMP_MMP__matrix metalloproteinase
At1g28395		At5g57500	Galactosyltransferase family protein
At5g50150	LOTR1__Protein of Unknown Function (DUF239)	At4g19990	FRS1__FAR1-related sequence 1
At4g38280		At5g38240	Protein kinase family protein
At1g63240		At5g38250	Protein kinase family protein
At5g25475	AP2/B3-like transcriptional factor family protein	At3g47090	Leucine-rich repeat protein kinase family protein
At2g33855		At2g23200	Protein kinase superfamily protein
At3g17160		At5g38990	MDS1__Malectin/receptor-like protein kinase family protein
At5g57930	APO2_emb1629__Arabidopsis thaliana protein of unknown function (DUF794)	At4g21380	ARK3_RK3__receptor kinase 3
At5g13630	ABAR_CCH_CCH1_CHLH_GUN5__magnesium-chelatase subunit chlH, chloroplast, putative / Mg-protoporphyrin IX chelatase, putative (CHLH)	At1g65800	ARK2_ATARK2_RK2__receptor kinase 2
At3g25660	Amidase family protein	At1g65790	ARK1_RK1__receptor kinase 1
At3g51820	ATG4_CHLG_G4_PDE325__UbiA prenyltransferase family protein	At4g23320	CRK24__cysteine-rich RLK (RECEPTOR-like protein kinase) 24
At5g40850	AtUPM12_UPM1__urophorin methylase 1	At4g23230	CRK15__cysteine-rich RLK (RECEPTOR-like protein kinase) 15
At4g03205	hemf2__Coproporphyrinogen III oxidase	At3g55950	AtCRR3_CCR3__CRINKLY4 related 3
At1g30440	Phototropic-responsive NPH3 family protein	At5g45800	MEEG2__Leucine-rich repeat protein kinase family protein
At4g31820	ENP_MAB4_NPY1__Phototropic-responsive NPH3 family protein	At1g51820	SIF4__Leucine-rich repeat protein kinase family protein
At4g29080	IAA27_PAP2__phytochrome-associated protein 2	At5g61900	BON1_BON1_CPN1__Calcium-dependent phospholipid-binding Copine family protein
At4g37590	MEL1_NPY5__Phototropic-responsive NPH3 family protein	At1g65540	AtLETM2_LETM2__LETM1-like protein
At2g02950	PKS1__phytochrome kinase substrate 1	At1g08450	AtCRT3_CRT3_EBS2_PSL1__calreticulin 3
At3g18890	AtTic62_Tic62__NAD(P)-binding Rossmann-fold superfamily protein	At2g15760	Protein of unknown function (DUF1645)



At5g61480	PXY_TDR__Leucine-rich repeat protein kinase family protein	At3g14590	NTMC2T6.2_NTM2TYPE6.2__Calcium-dependent lipid-binding (CaLB domain) family protein
At3g28040	Leucine-rich receptor-like protein kinase family protein	At4g31000	Calmodulin-binding protein
At2g41820	PXC3__Leucine-rich repeat protein kinase family protein	At1g76650	CML38__calmodulin-like 38
At2g26330	ER_QRP1__Leucine-rich receptor-like protein kinase family protein	At2g43490	Ypt/Rab-GAP domain of gyp1p superfamily protein
At2g02780	Leucine-rich repeat protein kinase family protein	At3g49350	Ypt/Rab-GAP domain of gyp1p superfamily protein
At1g34210	ATSERK2_SERK2__somatic embryogenesis receptor-like kinase 2	At3g02520	GF14 NU_GRF7__general regulatory factor 7
At1g11280	S-locus lectin protein kinase family protein	At1g18720	Protein of unknown function (DUF962)
At3g58690	Protein kinase superfamily protein	At5g13860	ELC-Like_Vps23B_VPS32.1__ELCH-like
At3g57830	Leucine-rich repeat protein kinase family protein	At5g16360	NC domain-containing protein-related
At4g23740	Leucine-rich repeat protein kinase family protein	At3g55890	Yippee family putative zinc-binding protein
At5g58300	Leucine-rich repeat protein kinase family protein	At3g44100	MD-2-related lipid recognition domain-containing protein
At5g16590	LRR1__Leucine-rich repeat protein kinase family protein	At5g21090	Leucine-rich repeat (LRR) family protein
At1g48480	RKL1__receptor-like kinase 1	At5g44568	
At2g27060	Leucine-rich repeat protein kinase family protein	At1g78410	VQ motif-containing protein
At2g26730	Leucine-rich repeat protein kinase family protein	At3g16990	Haem oxygenase-like, multi-helical
At1g53730	SRF6__STRUBBELIG-receptor family 6	At4g26910	Dihydrolipoamide succinyltransferase
At1g74690	IQD31__IQ-domain 31	At4g16600	PGSIP8__Nucleotide-diphospho-sugar transferases superfamily protein
At3g49260	iqd21__IQ-domain 21	At3g29075	glycine-rich protein
At4g05520	ATEHD2_EHD2__EPS15 homology domain 2	At4g08230	glycine-rich protein
At4g38430	ATROPGEF1_ROPGEF1__rho guanyl-nucleotide exchange factor 1	At3g11402	Cysteine/Histidine-rich C1 domain family protein
At1g50920	Nog1-1__Nucleolar GTP-binding protein	At4g37290	
At5g17790	OZ1_VAR3__zinc finger (Ran-binding) family protein	At2g21180	
At5g67070	RALF34_RALFL34__ralf-like 34	At3g26090	ATRG51_RGS1__G-protein coupled receptors;GTPase activators
At1g16390	ATOCT3_OCT3__organic cation/carnitine transporter 3	At5g11950	LOG8_MOBP2__Putative lysine decarboxylase family protein
At1g78560	BASS1__Sodium Bile acid symporter family	At4g36500	
At1g60160	KT12__Potassium transporter family protein	At1g25370	Protein of unknown function (DUF1639)
At2g40540	ATKT2_ATKUP2_KT2_KUP2_SHY3_TRK2__potassium transporter 2	At1g73650	Protein of unknown function (DUF1295)
At5g15100	ABCG12_ATABCG12_ATWBC12_CER5_D3_WBC12__ABC-2 type transporter family protein	At1g67850	Protein of unknown function (DUF707)
At3g21250	ABCC8_ATMRP6_MRP6__multidrug resistance-associated protein 6	At5g07820	Plant calmodulin-binding protein-related
At1g17840	ABCG11_ATABCG11_ATWBC11_COF1_D50_WBC11__white-brown complex homolog protein 11	At4g38260	Protein of unknown function (DUF833)
	ABCB19_ATABCB19_ATMDR1_ATMDR11_ATPGP19_MDR1_MDR11_PGP19__ATP binding cassette subfamily B19	At4g04972	
At2g47160	AtBOR1_BOR1__HCO3- transporter family	At3g27210	
At2g39010	PIP2;6_PIP2E__plasma membrane intrinsic protein 2E	At2g18680	
At2g37170	PIP2;2_PIP2B__plasma membrane intrinsic protein 2	At2g43320	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At4g17340	DELTA-TIP2_TIP2;2__tonoplast intrinsic protein 2;2	At5g12010	
At5g27360	SFP2__Major facilitator superfamily protein	At2g29950	ELF4-L1__ELF4-like 1
At4g35300	TMT2__tonoplast monosaccharide transporter2	At5g56250	HAP8__hapless 8
At2g38170	ATCAX1_CAX1_RC4__cation exchanger 1	At3g13910	Protein of unknown function (DUF3511)
At2g21050	LAX2__like AUXIN RESISTANT 2	At4g28460	
At5g10180	AST68_SULTR2;1__sulfate transporter 2;1	At3g61930	
At5g26200	Mitochondrial substrate carrier family protein	At1g69610	Protein of unknown function (DUF1666)
At5g49990	Xanthine/uracil permease family protein	At3g49210	WSD6__O-acyltransferase (WSD1-like) family protein
At2g38120	AtAUX1_AUX1_MAP1_PIR1_WAV5__Transmembrane amino acid transporter family protein	At5g23510	
At2g27810	ATNAT12_NAT12__nucleobase-ascorbate transporter 12	At2g28400	Protein of unknown function, DUF584
At5g24420	PGLS__6-phosphogluconolactonase 5	At1g64065	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
At1g31230	AK-HSDH_AK-HSDH1__aspartate kinase-homoserine dehydrogenase I	At4g26060	Ribosomal protein L18ae family
At1g74040	IMS1_IPMS2_MAML-3__2-isopropylmalate synthase 1	At5g42530	
At5g10920	L-Aspartase-like family protein	At5g54850	
At5g52100	CRR1__Dihydrodipicolinate reductase, bacterial/plant	At1g13360	
At1g31180	ATIMD3_IMD3_IPMDH1__isopropylmalate dehydrogenase 3	At4g22980	
At1g31860	AT-E_HISN2__histidine biosynthesis bifunctional protein (HISIE)	At2g03360	Glycosyltransferase family 61 protein
At5g65010	ASN2__asparagine synthetase 2	At1g10040	alpha/beta-Hydrolases superfamily protein
At3g19710	BCAT4__branched-chain aminotransferase4	At2g25625	CV
At1g68010	ATHPR1_HPR__hydroxypyruvate reductase	At5g44820	Nucleotide-diphospho-sugar transferase family protein
At1g11860	Glycine cleavage T-protein family	At1g17830	Protein of unknown function (DUF789)
At1g08110	AtGLY12_GLXI-3__lactoyglutathione lyase family protein / glyoxalase I family protein	At5g41860	
At1g50575	Putative lysine decarboxylase family protein	At4g28420	Tyrosine transaminase family protein
At3g44740	Class II aaRS and biotin synthetases superfamily protein	At4g36220	CYP84A1_FAH1__ferulic acid 5-hydroxylase 1
At4g33760	tRNA synthetase class II (D, K and N) family protein	At1g35190	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
At2g39140	PDE328_SVR1__pseudouridine synthase family protein	At1g74010	Calcium-dependent phosphotriesterase superfamily protein
At4g34620	SSR16__small subunit ribosomal protein 16	At3g44300	AtNIT2_NIT2__nitrilase 2
At3g49010	AtBBBC1_BBBC1_RSU2__breast basic conserved 1	At3g44310	AtNIT1_NIT1_NITI__nitrilase 1
At5g04800	Ribosomal S17 family protein	At5g39050	PMAT1__HXXXD-type acyl-transferase family protein
At2g21580	Ribosomal protein S25 family protein	At1g17020	AtSRG1_SRG1__senescence-related gene 1
At3g62870	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein	At3g61890	ATHB-12_ATHB12_HB-12_HB12__homeobox 12
At2g47610	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein	At4g26680	ATHB-7_ATHB7_HB-7__homeobox 7
At4g00100	ATRPS13A_PFL2_RPS13_RPS13A__ribosomal protein S13A	At4g22070	ATWRKY53_WRKY31__WRKY DNA-binding protein 31
At3g60245	Zinc-binding ribosomal protein family protein	At4g23810	ATWRKY53_WRKY53__WRKY family transcription factor
At5g24490	30S ribosomal protein, putative	At2g38470	ATWRKY33_WRKY33__WRKY DNA-binding protein 33
At2g43030	PRPL3__Ribosomal protein L3 family protein	At5g56960	basic helix-loop-helix (bHLH) DNA-binding family protein
At3g16780	RPL19B__Ribosomal protein L19e family protein	At5g49620	AtMYB78_MYB78__myb domain protein 78
At3g55280	RPL23A2_RPL23AB__ribosomal protein L23AB	At1g48000	AtMYB112_MYB112__myb domain protein 112
At3g39460	ATRLP23A_RPL23A_RPL23A1_RPL23AA__ribosomal protein L23AA	At1g77920	TGA7__bZIP transcription factor family protein
At4g17560	Ribosomal protein L19 family protein	At5g10760	AED1__Eukaryotic aspartyl protease family protein
At1g43170	ARP1_emb2207_RP1_RPL3A__ribosomal protein 1	At3g48760	DHHC-type zinc finger family protein
At2g42740	RPL16A__ribosomal protein large subunit 16A	At2g38250	Homeodomain-like superfamily protein
At2g44120	Ribosomal protein L30/L7 family protein	At5g56260	Ribonuclease E inhibitor RraA/Dimethylmenaquinone methyltransferase
At1g50920	Nog1-1__Nucleolar GTP-binding protein	At5g54610	ANK_BDA1__ankyrin
At1g41880	Ribosomal protein L35Ae family protein	At2g24600	Ankyrin repeat family protein
At3g55750	Ribosomal protein L35Ae family protein	At4g11000	Ankyrin repeat family protein
At2g27530	PGY1__Ribosomal protein L1p/L10e family	At2g16600	AtCYP19-1_CYP19_ROC3__rotamase CYP 3
At3g22660	EBP2__rRNA processing protein-related	At5g50380	ATEXO70F1_EXO70F1__exocyst subunit exo70 family protein F1
At3g51270	protein serine/threonine kinases;ATP binding;catalytics	At1g47830	AP25__SNARE-like superfamily protein
At4g16720	Ribosomal protein L23/L15e family protein	At4g05020	NDB2__NAD(P)H dehydrogenase B2
At4g17390	Ribosomal protein L23/L15e family protein	At2g29990	NDA2__alternative NAD(P)H dehydrogenase 2
At3g28900	Ribosomal protein L34e superfamily protein	At1g19180	AJAZ21_JAZ21_TIFY10A__jasmonate-zim-domain protein 1
At4g31700	AtRPS6_RPS6_RPS6A__ribosomal protein S6	At4g13920	AtRLP50_RLP50__receptor like protein 50
At5g03850	Nucleic acid-binding, OB-fold-like protein	At3g23010	AtRLP36_RLP36__receptor like protein 36
At5g38290	Peptidyl-tRNA hydrolase family protein	At2g15042	Leucine-rich repeat (LRR) family protein
At1g48920	ATNUC-L1_NUC-L1_NUC1_PARL1__nucleolin like 1	At3g04220	Disease resistance protein (TIR-NBS-LRR class) family
At3g06530	ARM repeat superfamily protein	At1g64160	AtDIR5 DIR5__Disease resistance-responsive (dirigent-like protein) family protein
At3g57150	AtCBF5_ATNAP57_CBF5_NAP57__homologue of NAP57	At5g36970	NHL25__NDR1/HIN1-like 25
At5g66540		At1g27900	Disease resistance-responsive (dirigent-like protein) family protein
At3g13120	PRPS10__Ribosomal protein S10p/S20e family protein	At4g39030	EDSS_SCORD3_SID1__MATE efflux family protein
At5g09770	Ribosomal protein L17 family protein	At3g04720	AtPR4_HEL_PR-4_PR4__pathogenesis-related 4
At2g18020	EMB2296__Ribosomal protein L2 family	At1g75830	LCR67_PDF1.1_PR12__low-molecular-weight cysteine-rich 67
At2g37270	ATRPS5B_RPS5B__ribosomal protein 5B	At3g02840	ARM repeat superfamily protein
At5g16130	Ribosomal protein S7e family protein	At3g01420	ALPHA-DOX1_DIOX1_DOX1_PADOX-1__Peroxidase superfamily protein
At3g56340	RPS26e__Ribosomal protein S26e family protein	At5g39120	RmlC-like cupins superfamily protein
At3g49910	Translation protein SH3-like family protein	At2g00410	
At5g58420	Ribosomal protein S4 (RPS4A) family protein	At3g00850	
At2g17360	Ribosomal protein S4 (RPS4A) family protein		
At4g13170	Ribosomal protein L13 family protein		
At5g19510	Translation elongation factor EF1B/ribosomal protein S6 family protein		
		XopAC_XopAH	
		At3g18950	Transducin/WD40 repeat-like superfamily protein
		At5g46180	DELTA-OAT__ornithine-delta-amino transferase

At5g12110	Glutathione S-transferase, Translation elongation factor EF1B/ribosomal protein S6	At4g32810	ATCCD8_CCD8_MAX4__carotenoid cleavage dioxygenase 8
At4g20360	ATRA8B0_ATRA8E1B_RABE1b__RAB GTPase homolog E1B	At4g26080	AB11_AtAB11__Protein phosphatase 2C family protein
At3g12390	Nascent polypeptide-associated complex (NAC), alpha subunit family protein	At4g26200	ACCS7_ACS7_ATACS7__1-amino-cyclopropane-1-carboxylate synthase 7
At2g19740	Ribosomal protein L31e family protein	At4g34120	CBSX2_CDCP1_LEJ1__Cystathionine beta-synthase (CBS) family protein
At4g26230	Ribosomal protein L31e family protein	At4g34380	Transducin/WD40 repeat-like superfamily protein
At4g30930	NFD1__Ribosomal protein L21	At1g60470	AtGolS4_GolS4__galactinol synthase 4
At5g41520	RPS10B__RNA binding Plectin/S10 domain-containing protein	At1g56600	AtGolS2_GolS2__galactinol synthase 2
At5g52650	RNA binding Plectin/S10 domain-containing protein	At3g48080	alpha/beta-Hydrolases superfamily protein
At4g25740	RNA binding Plectin/S10 domain-containing protein	At3g44480	cog1_RPP1__Disease resistance protein (TIR-NBS-LRR class) family
At1g07210	Ribosomal protein S18	At1g72920	Toll-Interleukin-Resistance (TIR) domain family protein
At5g47700	RPP1_3_RPP1C__60S acidic ribosomal protein family	At3g11080	AtRLP35_RLP35__receptor like protein 35
At5g60670	RPL12C__Ribosomal protein L11 family protein	At5g45240	Disease resistance protein (TIR-NBS-LRR class)
At5g39740	OLI7_RPL5B__ribosomal protein L5 B	At3g25010	AtRLP41_RLP41__receptor like protein 41
At3g25520	ATL5_OU5_PGY3_RPL5A__ribosomal protein L5	At5g39130	RmlC-like cupins superfamily protein
At3g48930	EMB1080__Nucleic acid-binding, OB-fold-like protein	At2g30550	DALL3__alpha/beta-Hydrolases superfamily protein
At5g53070	Ribosomal protein L9/RNase H1	At3g01175	Protein of unknown function (DUF1666)
At1g57860	Translation protein SH3-like family protein	At3g08720	ATPK19_ATPK2_ATS6K2_S6K2__serine/threonine protein kinase 2
At4g28360	Ribosomal protein L22p/L17e family protein	At3g54030	BSK6__Protein kinase protein with tetratricopeptide repeat domain
At4g18100	Ribosomal protein L32e	At3g16030	CES101_RFO3__lectin protein kinase family protein
At2g27720	60S acidic ribosomal protein family	At4g21840	ATMSR88_MSRB8__methionine sulfoxide reductase B8
At2g24090	PRPL35__Ribosomal protein L35	At5g11410	Protein kinase superfamily protein
At2g42710	Ribosomal protein L1p/L10e family	At2g40270	Protein kinase family protein
At2g33800	EMB3113_PRP55_RP55_SCA1__Ribosomal protein S5 family protein	At5g40000	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At3g22300	RPS10__ribosomal protein S10	At3g12220	scpl16__serine carboxypeptidase-like 16
At1g33120	Ribosomal protein L6 family	At4g25110	AtMC2_AtMCP1c_MC2_MCP1c__metacaspase 2
At3g46040	RPS15AD__ribosomal protein S15A D	At2g44180	MAP2A__methionine aminopeptidase 2A
At5g13510	EMB3136__Ribosomal protein L10 family protein	At2g02360	AtPP2-B10_PP2-B10__phloem protein 2-B10
At2g38140	PSRP4__plastid-specific ribosomal protein 4	At1g03370	C2 calcium/lipid-binding and GRAM domain containing protein
At1g68590	PSRP3/1__Ribosomal protein PSRP-3/Ycf65	At5g07770	AtFH16_FH16__Actin-binding FH2 protein
At5g27850	RPL18C__Ribosomal protein L18e/L15 superfamily protein	At3g26600	ARO4__armadillo repeat only 4
At3g05590	RPL18__ribosomal protein L18	At1g52200	PLAC8 family protein
At1g70600	Ribosomal protein L18e/L15 superfamily protein	At2g39530	CASPL4D1__Uncharacterised protein family (UPF0497)
At4g01560	MEE49__Ribosomal RNA processing Brk domain protein	At3g26500	PRL2__plant intracellular ras group-related LRR 2
At5g55140	ribosomal protein L30 family protein	At3g52470	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
At3g57190	PrfB3__peptide chain release factor, putative	At5g11650	MAGL13__alpha/beta-Hydrolases superfamily protein
At2g40010	Ribosomal protein L10 family protein	At2g26400	ARD_ARD3_ATARD3__acireductone dioxygenase 3
At2g44860	Ribosomal protein L24e family protein	At2g04495	
At1g68660	AtClpS1_ClpS1__Ribosomal protein L12/ ATP-dependent Clp protease adaptor protein ClpS family protein	At5g08240	
At1g18540	Ribosomal protein L6 family protein	At1g70160	
At4g25730	ATTRM7b_TRM7b__FtsI-like methyltransferase family protein	At2g14560	LURP1__Protein of unknown function (DUF567)
At2g33370	Ribosomal protein L14p/L23e family protein	At5g12930	
At1g72370	AP40_P40_RP40_RPSAA__40s ribosomal protein SA	At5g18490	Plant protein of unknown function (DUF946)
At2g32060	Ribosomal protein L7Ae/L30e/S12e/Gad45 family protein	At2g25510	
At3g22230	Ribosomal L27e protein family	At1g77145	Protein of unknown function (DUF506)
At5g27395	Mitochondrial inner membrane translocase complex, subunit Tim44-related protein	At3g15518	
At3g57350	Nucleoporin interacting component (Nup93/Nic96-like) family protein	At1g73610	GDSL-like Lipase/Acylhydrolase superfamily protein
At5g52280	Myosin heavy chain-related protein	At2g19570	AT-CD4A1_CDA1_DES2__cytidine deaminase 1
At1g48240	ATNPSN12_NPSN12__novel plant snare 12	At5g63030	GRXC1__Thioredoxin superfamily protein
At3g23710	AtTic22-III_Tic22-III__Tic22-like family protein	At1g51860	Leucine-rich repeat protein kinase family protein
At2g28900	ATOEP16-1_ATOEP16-L_OEP16_OEP16-1__outer plastid envelope protein 16-1	At1g16260	Wall-associated kinase family protein
At5g03940	5ACP_CPSRP54_FFC_SRP54CP__chloroplast signal recognition particle 54 kDa subunit	At4g23220	CRK14__cysteine-rich RLK (RECEPTOR-like protein kinase) 14
At1g20650	ASG5__Protein kinase superfamily protein	At3g45860	CRK4__cysteine-rich RLK (RECEPTOR-like protein kinase) 4
At1g09440	Protein kinase superfamily protein	At1g68690	AtPERK9_PERK9__Protein kinase superfamily protein
At1g56720	Protein kinase superfamily protein	At4g18430	ATRABA1e_RABA1e__RAB GTPase homolog A1E
At5g50000	Protein kinase superfamily protein	At1g64280	ATNPR1_NIM1_NPR1_SAI1__regulatory protein (NPR1)
At5g58140	ATPHOT2_NPL1_PHOT2__phototropin 2	At2g25000	ATWRK60_WRKY60__WRKY DNA-binding protein 60
At1g49580	Calcium-dependent protein kinase (CDPK) family protein	At5g61010	ATEX070E2_EXO70E2__exocyst subunit exo70 family protein E2
At5g28290	ATNEK3_NEK3__NIMA-related kinase 3	At1g09930	ATOPT2_OPT2__oligopeptide transporter 2
At2g36350	AGC1-9__Protein kinase superfamily protein	At3g13080	ABCC3_ATMRP3_MRP3_MRP3__multidrug resistance-associated protein 3
At5g22840	Protein kinase superfamily protein	At3g17690	ATCNGC19_CNGC19__cyclic nucleotide gated channel 19
At3g53380	LecRK-VIII.1__Concanavalin A-like lectin protein kinase family protein	At4g28390	AAC3_ATAAC3__ADP/ATP carrier 3
At5g16480	ATPFA-DSP5_PFA-DSP5__Phosphotyrosine protein phosphatases superfamily protein	At2g39210	Major facilitator superfamily protein
At1g80640	Protein kinase superfamily protein	At1g63245	CLE14__CLAVATA3/ESR-RELATED 14
At3g04340	emb2458_FtsH5__FtsH extracellular protease family	At5g22380	anac090_NAC090__NAC domain containing protein 90
At4g34980	SLP2__subtilisin-like serine protease 2	At3g21305	
At5g51750	ATSBT1_3_SBT1_3__subtilase 1.3	<b>XopAC, XopK</b>	
At5g56530	Protein of Unknown Function (DUF239)	At1g65510	
At4g18370	DEG5_DEGP5_HHOA__DEGP protease 5	At5g25260	flot2__SPFH/Band 7/PHB domain-containing membrane-associated protein family
At5g26110	Protein kinase superfamily protein	At4g21920	
At1g49630	ATPREP2_PREP2__presequence protease 2	At5g41100	
At4g03190	AFB1_ATGRH1_GRH1__GRR1-like protein 1	At1g19020	
At4g10400	F-box/RN1-like/FBD-like domains-containing protein	At2g36220	
At1g50490	UBC20__ubiquitin-conjugating enzyme 20	At2g04450	ATNUDT6_ATNUDX6_NUDT6_NUDX6__nudix hydrolase homolog 6
At4g17740	Peptidase S41 family protein	At1g61370	S-locus lectin protein kinase family protein
At3g02110	scpl25__serine carboxypeptidase-like 25	At5g43190	Galactose oxidase/kelch repeat superfamily protein
At1g11750	CLPP6_NCLPP1_NCLPP6__CLP protease proteolytic subunit 6	At1g43910	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At5g64580	EMB3144_FtsHi4__AAA-type ATPase family protein	At1g74710	ATICS1_EDS16_CSI1_SID2__ADC synthase superfamily protein
At1g23030	PUB11__ARM repeat superfamily protein	At2g37040	ATPAL1_PAL1__PHE ammonia lyase 1
At1g74370	RING/U-box superfamily protein	At1g66920	Protein kinase superfamily protein
At4g22290	SHOU4L2__Ubiquitin-specific protease family C19-related protein	At2g23190	At-RLK3_CRK11__cysteine-rich RLK (RECEPTOR-like protein kinase) 11
At4g26555	FKBP-like peptidyl-prolyl cis-trans isomerase family protein	At2g23170	GH3.3__Auxin-responsive GH3 family protein
At1g26230	Cpn60beta4_CPNB4__TCP-1/cpn60 chaperonin family protein	At1g01480	ACS2_AT-ACC2__1-amino-cyclopropane-1-carboxylate synthase 2
At4g39710	FKBP16-2_PnsL4_FK506-binding protein 16-2	At2g41380	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At3g10060	FKBP-like peptidyl-prolyl cis-trans isomerase family protein	At3g23250	ATMYB15_ATY19_MYB15__myb domain protein 15
At5g49510	PDF3__prefoldin 3	At3g52430	ATPAD4_PAD4__alpha/beta-Hydrolases superfamily protein
At1g14980	CPN10__chaperonin 10	At5g24200	alpha/beta-Hydrolases superfamily protein
At5g45680	ATFKBP13_FKBP13__FK506-binding protein 13	At5g44567	
At2g15620	ATHNIR_NIR_NIR1__nitrite reductase 1	<b>XopAC, Xopl</b>	
At5g04140	FD-GOGAT_GLS1_GLU1_GLU5__glutamate synthase 1	At5g53120	ATSPD3_SPD3_SPMS__spermidine synthase 3
At3g55200	ATSAP130a_SAP130a__Cleavage and polyadenylation specificity factor (CPSF) A subunit protein	At5g13200	GER5__GRAM domain family protein
At3g30330	PCP-like_SME2__Small nuclear ribonucleoprotein family protein	At3g25290	Auxin-responsive family protein
At4g34730	RBF1__ribosome-binding factor A family protein	At5g35735	Auxin-responsive family protein
At2g34750	RNA polymerase I specific transcription initiation factor RRN3 protein	At3g20600	NDR1__Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
At1g03530	ATNAF1_NAF1__nuclear assembly factor 1	At1g17600	SOC3__Disease resistance protein (TIR-NBS-LRR class) family
At3g03710	PDE326_PNP_RIF10__polyribonucleotide nucleotidyltransferase, putative	At5g36930	Disease resistance protein (TIR-NBS-LRR class) family
At2g41500	ATPRP4_AtSAP60_EMB2776_LIS__WD-40 repeat family protein / small nuclear ribonucleoprotein Prp4p-related	At3g53040	late embryogenesis abundant protein, putative / LEA protein, putative
At3g07750	RRP42__3'-5'-exoribonuclease family protein	At5g18270	ANAC087__Arabidopsis NAC domain containing protein 87
At3g21300	ATRM2a_TRM2a__RNA methyltransferase family protein	At5g51640	TBL17_YLS7__Plant protein of unknown function (DUF828)
At5g14610	RH46__DEAD box RNA helicase family protein	At1g70520	ASG6_CRK2__cysteine-rich RLK (RECEPTOR-like protein kinase) 2
At5g08620	ATRH25_STRS2__DEA(D/H)-box RNA helicase family protein	At1g16670	CRPK1__Protein kinase superfamily protein
At5g26742	ATRH3_emb1138_RH3__DEAD box RNA helicase (RH3)	At5g47140	GATA27__GATA transcription factor 27
At1g12770	EMB1586_ISE1__P-loop containing nucleoside triphosphate hydrolases superfamily protein	At1g42990	ATBZIP60_BZIP60_BZIP60__basic region/leucine zipper motif 60
At1g68990	MGP3__male gametophyte defective 3	At5g64810	ATWRKY51_WRKY51__WRKY DNA-binding protein 51

At3g49500	AtDR6_RDR6_SDE1_SGS2_RNA-dependent RNA polymerase 6	At2g21900	ATWRKY59_WRKY59_WRKY DNA-binding protein 59
At2g24120	PDE319_SCA3_DNA/RNA polymerases superfamily protein	At2g33490	hydroxyproline-rich glycoprotein family protein
At1g76110	HMG (high mobility group) box protein with ARID/BRIGHT DNA-binding domain	At4g28570	Long-chain fatty alcohol dehydrogenase family protein
At5g15310	ATMIXTA_ATMYB16_MYB16_myb domain protein 16	At1g78210	alpha/beta-Hydrolases superfamily protein
At4g27950	CRF4__cytokinin response factor 4	At1g65690	NHL6__Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family
At2g45820	Rem1.3__Remorin family protein	At5g26690	HIPP02__Heavy metal transport/detoxification superfamily protein
At2g47790	GT51__Transducin/WD40 repeat-like superfamily protein	At3g57950	
At5g27120	NOP56-like pre RNA processing ribonucleoprotein	At2g32190	AthCYSTM4
At1g80270	PPR596__PENTATRICOPEPTIDE REPEAT 596	At1g56060	AthCYSTM3
At1g15910	FDM1_IDNL1_IDP1__XH/XS domain-containing protein	At1g07220	Arabidopsis thaliana protein of unknown function (DUF821)
At3g52170	DNA binding	At5g10695	
At3g13000	Protein of unknown function, DUF547	At5g56050	
At2g18850	SET domain-containing protein	At3g58600	Adaptin ear-binding coat-associated protein 1 NECAP-1
At1g78930	Mitochondrial transcription termination factor family protein	At1g14370	APK2A_Kin1_PBL2__protein kinase 2A
At1g61970	Mitochondrial transcription termination factor family protein	At5g47070	PBL19__Protein kinase superfamily protein
At5g41060	DHHC-type zinc finger family protein	At2g17760	Eukaryotic aspartyl protease family protein
At3g60400	SHOT1__Mitochondrial transcription termination factor family protein	At2g40880	ATCYSA_CYSA_FL3-27__cystatin A
At1g5810	ATRM1b_TRM1b__N2,N2-dimethylguanosine tRNA methyltransferase	At3g57460	catalytics; metal ion binding
At1g07840	Sas10/Utp3/C1D family	At2g46620	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At5g12440	CCH-type zinc fingerfamily protein with RNA-binding domain	At3g28510	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At3g52150	PSRP2__RNA-binding (RRM/RBD/RNP motifs) family protein	At1g76390	AtPUB43_PUB43__ARM repeat superfamily protein
At2g21710	EMB2219__Mitochondrial transcription termination factor family protein	At1g77810	Galactosyltransferase family protein
At1g23280	MAK16 protein-related	At1g71140	DTX14__MATE efflux family protein
At5g38140	NF-YC12__nuclear factor Y, subunit C12	At3g29360	UGD2__UDP-glucose 6-dehydrogenase family protein
At4g32880	ATHB-8_ATHB8_HB-8__homeobox gene 8	At1g30620	HSR8_MUR4_UXE1__NAD(P)-binding Rossmann-fold superfamily protein
At4g07950	DNA-directed RNA polymerase, subunit M, archaeal	At5g11920	AtcwlINV6_cwlINV6__6-&1-fructan exohydrolase
At5g44560	VPS2.2__SNF7 family protein	At5g02620	ANK1_ATANK1__ankyrin-like 1
At1g72440	EDA25_SWA2__CCAAT-binding factor	At2g31200	ADF6_ATADF6__actin depolymerizing factor 6
At3g24520	At-HSFC1_HSFC1__heat shock transcription factor C1	At3g57090	BIGVIN_FIS1A__Tetratricopeptide repeat (TPR)-like superfamily protein
At2g26580	YAB5__plant-specific transcription factor YABBY family protein	At5g61210	ATSNAP33_ATSNAP33B_SNAP33_SNP33__soluble N-ethylmaleimide-sensitive factor adaptor protein 33
At5g43700	ATAUX2-11_IAA4__AUX/IAA transcriptional regulator family protein	At1g13090	CYP71B28__cytochrome P450, family 71, subfamily B, polypeptide 28
At2g22670	IAA8__indoleacetic acid-induced protein 8	At1g53100	Core-2/1-branching beta-1,6-N-acetylglucosaminyltransferase family protein
At1g04550	BDL_IAA12__AUX/IAA transcriptional regulator family protein	At3g28480	Oxoglutarate/iron-dependent oxygenase
At3g23050	AXR2_IAA7__indole-3-acetic acid 7	At2g17720	P4H5__2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
At1g33310	IAA13__auxin-induced protein 13	At3g09270	ATGSTU8_GSTU8__glutathione S-transferase TAU 8
At3g14980	IDM1_ROS4__Acyl-CoA N-acyltransferase with RING/FYVE/PHD-type zinc finger protein	At2g24850	TAT_TAT3__tyrosine aminotransferase 3
At1g68190	BBX27__B-box zinc finger family protein	At2g13810	ALD1_ATALD1_EDT55__AGD2-like defense response protein 1
At2g31380	BBX25_STH__salt tolerance homologue	At1g77510	ATPDI6_ATPDI1L-2_PD16_PD1L1-2__PDI-like 1-2
At2g47890	B-box type zinc finger protein with CCT domain	At2g21750	ATPDI5_ATPDI1L-1_PD15_PD1L1-1__PDI-like 1-1
At3g22780	ATTSO1_TSO1__Tesmin/TSO1-like CXC domain-containing protein	At2g31570	ATGPX2_GPX2_GPX2L__glutathione peroxidase 2
At5g44190	ATGLK2_GLK2_GPRI2__GOLDEN2-like 2		
At2g34640	HMR_PTAC12_TAC12__plastid transcriptionally active 12		
At2g34900	GTE01_GTE1_IMB1__Transcription factor GTE6		
At5g23280	AtTCP7_TCP7__TCP family transcription factor		
At4g38890	FMN-linked oxidoreductases superfamily protein		
At3g02790	MB51__zinc finger (C2H2 type) family protein		
At5g20220	zinc knuckle (CCHC-type) family protein		
At3g17100	AIF3__sequence-specific DNA binding transcription factors		
At2g46810	MYC70__basic helix-loop-helix (bHLH) DNA-binding superfamily protein		
At1g12860	ICE2_SCRM2__basic helix-loop-helix (bHLH) DNA-binding superfamily protein		
At3g47500	CDF3__cycling DOF factor 3		
At3g17600	HYH__HY5-homolog		
At5g24800	ATBZIP9_BZIP9_BZO2H2__basic leucine zipper 9		
At5g65410	ATHB25_HB25_ZFHD2_ZHD1__homeobox protein 25		
At2g37220	RNA-binding (RRM/RBD/RNP motifs) family protein		
At4g24770	ATRBP31_ATRBP33_CP31_RBP31__31-kDa RNA binding protein		
At5g54580	ORRM2__RNA-binding (RRM/RBD/RNP motifs) family protein		
At3g52380	CP33_PDE322__chloroplast RNA-binding protein 33		
At3g13180	ATRM4e_TRM4e__NOL1/NOP2/sun family protein / antitermination NusB domain-containing protein		
At2g46780	RNA-binding (RRM/RBD/RNP motifs) family protein		
At5g47210	Hyaluronan / mRNA binding family		
At3g53460	CP29__chloroplast RNA-binding protein 29		
At5g14740	BETA_CA2_CA18_CA2__carbonic anhydrase 2		
At3g01500	ATBCA1_ATSABP3_CA1_SABP3__carbonic anhydrase 1		
At5g10170	ATMIP33_MIP33__myo-inositol-1-phosphate synthase 3		
At2g27680	NAD(P)-linked oxidoreductase superfamily protein		
At2g37790	AKR4C10__NAD(P)-linked oxidoreductase superfamily protein		
At1g04420	NAD(P)-linked oxidoreductase superfamily protein		
At5g61130	AtPDCB1_PDCB1__plasmodesmata callose-binding protein 1		
At5g09660	PMDH2__peroxisomal NAD-malate dehydrogenase 2		
At2g44050	COS1_COS1__6,7-dimethyl-8-ribitylumazine synthase / DMRL synthase / lumazine synthase / riboflavin synthase		
At5g46510	VICTL__Disease resistance protein (TIR-NBS-LRR class) family		
At4g37000	ACD2_ATRCCR__accelerated cell death 2 (ACD2)		
At1g72500			
At5g64940	abc1k8_ATATH13_ATH13_ATOSA1_OSA1__ABC2 homolog 13		
At1g78040	Pollen Ole e 1 allergen and extensin family protein		
At1g33170	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein		
At5g27240	DNAI heat shock N-terminal domain-containing protein		
At5g48030	GFA2__gametophytic factor 2		
At4g37910	mtHsc70-1__mitochondrial heat shock protein 70-1		
At2g04030	AtHsp90.5_AtHsp90C_CR88_EMB1956_Hsp88.1_HSP90.5__Chaperone protein htpG family protein		
At5g42480	ARC6__Chaperone DnaJ-domain superfamily protein		
At2g15970	ATCOR413-PM1_ATCYP19_COR413-PM1_FL3-5A3_WCOR413_WCOR413-LIKE__cold regulated 413 plasma membrane 1		
At4g38680	ATCSP2_CSDP2_CSP2_GRP2__glycine rich protein 2		
At1g20950	Phosphofructokinase family protein		
At5g45040	CYT6A__Cytochrome c		
At1g19150	LHCA2*1_Lhca6__photosystem I light harvesting complex gene 6		
At1g61520	LHCA3__photosystem I light harvesting complex gene 3		
At3g16250	ND4_Pns83__NDH-dependent cyclic electron flow 1		
At1g60950	ATFD2_FD2_FED A__2Fe-2S ferredoxin-like superfamily protein		
At4g10340	LHCB5__light harvesting complex of photosystem II 5		
At2g05100	LHCB2_LHCB2.1__photosystem II light harvesting complex gene 2.1		
At1g29920	AB165_CAB2_LHCB1.1__chlorophyll A/B-binding protein 2		
At1g76570	AtLHCB7_LHCB7__Chlorophyll A-B binding family protein		
At4g37920			
At4g37925	NDH-M_NdhM__subunit NDH-M of NAD(P)H:plastoquinone dehydrogenase complex		
At2g01590	CRR3__chlororespiratory reduction 3		
At2g46820	CURT1B_PSA_P_PSI-P_P_TAC8_TMP14__photosystem I P subunit		
At4g28660	PSB28__photosystem II reaction center PSB28 protein		
At1g14150	PnstL2_PQL1_PQL2__PsbQ-like 2		

At3g55330	PPL1__PsbP-like protein 1
At2g01918	PQL3__PsbQ-like 3
At5g02120	OHP_OHP1_PDE335__one helix protein
At4g22890	PGR5-LIKE A__PGR5-LIKE A
At1g32470	Single hybrid motif superfamily protein
At1g68010	ATHPR1_HPR__hydroxypyruvate reductase
At5g36700	ATPGLP1_PGLP1__2-phosphoglycolate phosphatase 1
At1g11860	Glycine cleavage T-protein family
At3g14420	GOX1__Aldolase-type TIM barrel family protein
At3g55800	SBPASE__sedoheptulose-bisphosphatase
At1g55490	CPN60B_Cpn60beta1_CPNB1_LEN1__chaperonin 60 beta
At2g39730	RCA__rubisco activase
At4g38970	AtFBA2_FBA2__fructose-bisphosphate aldolase 2
At3g54050	cfbp1_HCEF1__high cyclic electron flow 1
At5g64380	Inositol monophosphatase family protein
At5g49740	ATFRO7_FRO7__ferric reduction oxidase 7
At3g56090	ATFER3_FER3__ferritin 3
At1g78970	ATLUP1_LUP1__lupeol synthase 1
At1g74470	Pyridine nucleotide-disulphide oxidoreductase family protein
At2g26930	ATCDPMEK_CDPMEK_CMEK_CMK_ISPE_PDE277__4-(cytidine 5'-phospho)-2-C-methyl-D-erythritol kinase
At4g39330	ATCAD9_CAD9__cinnamyl alcohol dehydrogenase 9
At3g19710	BCAT4__branched-chain aminotransferase4
At4g13770	CYP83A1_REF2__cytochrome P450, family 83, subfamily A, polypeptide 1
At2g31790	UDP-Glycosyltransferase superfamily protein
At1g31180	ATIMD3_IMD3_IPMDH1__isopropylmalate dehydrogenase 3
At5g57800	CER3_FLP1_WAX2_YRE__Fatty acid hydroxylase superfamily
At1g25450	CER60_KCS5__3-ketoacyl-CoA synthase 5
At1g68530	AtCER6_AtCUT1_CER6_CUT1_G2_KCS6_POP1__3-ketoacyl-CoA synthase 6
At5g57590	BIO1__adenosylmethionine-8-amino-7-oxononanoate transaminases
At4g36250	ALDH3F1__aldehyde dehydrogenase 3F1
At4g06410	
At5g00430	
<b>XopAC, XopAH</b>	
At3g62270	BOR2__HCO3- transporter family
At2g38380	Peroxidase superfamily protein
At3g21350	MED6__RNA polymerase transcriptional regulation mediator-related
At2g24570	ATWRKY17_WRKY17__WRKY DNA-binding protein 17
At5g20510	AL5__alfin-like 5
At1g75540	AtBBX21_BBX21_LHUS_STH2__salt tolerance homolog2
At1g77690	LAX3__like AUX1 3
At2g01950	BRL2_VH1__BRI1-like 2
At1g64190	PGD1__6-phosphogluconate dehydrogenase family protein
At2g01530	MLP329_ZCE2__MLP-like protein 329
At4g04955	ALN_ATALN__allantoinase
At5g23535	KOW domain-containing protein
At5g08180	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
At5g47930	Zinc-binding ribosomal protein family protein
At3g22790	NET1A__Kinase interacting (KIP1-like) family protein
At2g28130	ASAP1
At4g09630	Protein of unknown function (DUF616)
At1g12080	Vacuolar calcium-binding protein-related
At3g35880	TPX2 (targeting protein for Klp2) protein family
<b>XopAC, XopK</b>	
At2g33810	SPL3__squamosa promoter binding protein-like 3
At3g05680	EMB2016_VIR__embryo defective 2016
At1g28330	AtDRM1_DRM1_DYL1__dormancy-associated protein-like 1
At1g74910	KIC1__ADP-glucose pyrophosphorylase family protein
At2g20360	NAD(P)-binding Rossmann-fold superfamily protein
At4g37830	cytochrome c oxidase-related
At1g54630	ACP3_ATACP3__acyl carrier protein 3
At2g38040	CAC3__acetyl Co-enzyme a carboxylase carboxyltransferase alpha subunit
At1g14290	AtSBH2_SBH2__sphingoid base hydroxylase 2
At5g24400	EMB2024_PGL3_NagB/RpiA/CoA transferase-like superfamily protein
At5g47780	GAUT4__galacturonosyltransferase 4
At3g56650	PPD6__Mog1/PsbP/DUF1795-like photosystem II reaction center PsbP family protein
At4g20130	PTAC14_TAC14__plastid transcriptionally active 14
At2g01910	ATMAP65-6_MAP65-6__Microtubule associated protein (MAP65/ASE1) family protein
At2g34560	AtCCP1_CCP1__P-loop containing nucleoside triphosphate hydrolases superfamily protein
At3g02450	FtsH13__cell division protein ftsH, putative
At5g06110	AtGlsA1_AtGlsA2_ZRF1b__DnaJ domain ;Myb-like DNA-binding domain
At5g64960	CDK2_CDKC;2__cyclin dependent kinase group C2
At5g42560	Abscisic acid-responsive (TB2/DP1, HVA22) family protein
At1g52980	AtNug2_Nug2__GTP-binding family protein
At1g20090	ARAC4_ATRAC4_ATROP2_ROP2__RHO-related protein from plants 2
At2g42880	ATMPK20_MPK20__MAP kinase 20
At2g47580	U1A__spliceosomal protein U1A
At2g36930	zinc finger (C2H2 type) family protein
At4g15080	DHHC-type zinc finger family protein
At5g14170	BAF60_CHC1_SWP73B__SWIB/MDM2 domain superfamily protein
At3g14100	RNA-binding (RRM/RBD/RNP motifs) family protein
At1g61100	disease resistance protein (TIR class), putative
At2g02100	LCR69_PDF2.2__low-molecular-weight cysteine-rich 69
At3g02560	Ribosomal protein S7e family protein
At4g33865	Ribosomal protein S14p/S29e family protein
At1g04480	Ribosomal protein L14p/L23e family protein
At3g18740	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
At1g52980	AtNug2_Nug2__GTP-binding family protein
At1g10840	TIF3H1__translation initiation factor 3 subunit H1
At2g19790	AP45__SNARE-like superfamily protein
At3g49560	HP30_Tric1__Mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein
At5g62810	AtPED2_ATPEX14_PED2_PEX14__peroxin 14
At4g12060	ClpT2__Double Clp-N motif protein
At1g24510	TCP-1/cpn60 chaperonin family protein
At2g44900	ARABIDILLO-1_ARABIDILLO1__ARABIDILLO-1
At3g20060	UBC19__ubiquitin-conjugating enzyme19
At5g02240	NAD(P)-binding Rossmann-fold superfamily protein
At5g64350	ATFKBP12_FKBP12_FKP12__FK506-binding protein 12
At3g18190	TCP-1/cpn60 chaperonin family protein
At4g04770	ABC1_ABCI8_ATABC1_ATNAP1_LAF6__ATP binding cassette protein 1
At5g48230	AACT2_ACAT2_EMB1276__acetoacetyl-CoA thiolase 2
At1g75330	OTC__ornithine carbamoyltransferase
At3g10050	OMR1__L-O-methylthreonine resistant 1
At5g62050	ATOXA1_OXA1_OXA1a_OXA1AT__homolog of yeast oxidase assembly 1 (OXA1)

At4g37210	Tetratricopeptide repeat (TPR)-like superfamily protein
At3g28220	TRAF-like family protein
At5g52882	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At5g23890	
At5g19390	PHGAP2__Rho GTPase activation protein (RhoGAP) with PH domain
At5g19330	ARIA__ARM repeat protein interacting with ABF2
At5g53620	
At4g14000	Putative methyltransferase family protein
At3g27770	
At4g30010	
At1g27150	Tetratricopeptide repeat (TPR)-like superfamily protein
At5g06220	LETM1-like protein
At3g11560	LETM1-like protein
At4g18740	Rho termination factor
At2g48070	RPH1__resistance to phytophthora 1
At3g03150	
At1g69070	
At3g19680	Protein of unknown function (DUF1005)
At5g49820	RUS6__Protein of unknown function, DUF647
<b>XopAC, XopL</b>	
At3g19800	DUF177B__Protein of unknown function (DUF177)
At5g14970	
At5g58650	PSY1__plant peptide containing sulfated tyrosine 1
At4g34265	
At4g28025	
At3g62650	
At3g01790	Ribosomal protein L13 family protein
At3g46820	TOPP5__type one serine/threonine protein phosphatase 5
At5g28020	ATCYSD2_CYSD2__cysteine synthase D2
At1g10960	ATFD1_FD1__ferredoxin 1

Supplementary Table S6. Plant genes up-regulated by the heterologous expression of *hrpW*

<i>ID</i>	<i>Annotation</i>
<a href="#">At3g45130</a>	LAS1__lanosterol synthase 1
<a href="#">At2g21140</a>	ATPRP2_PRP2__proline-rich protein 2
<a href="#">At3g23730</a>	XTH16__xyloglucan endotransglucosylase/hydrolase 16
<a href="#">At3g54400</a>	Eukaryotic aspartyl protease family protein
<a href="#">At5g49520</a>	ATWRKY48_WRKY48__WRKY DNA-binding protein 48
<a href="#">At2g38870</a>	Serine protease inhibitor, potato inhibitor I-type family protein
<a href="#">At4g13345</a>	MEE55__Serinc-domain containing serine and sphingolipid biosynthesis protein
<a href="#">At4g00360</a>	ATT1_CYP86A2__cytochrome P450, family 86, subfamily A, polypeptide 2
<a href="#">At1g62510</a>	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein
<a href="#">At4g38420</a>	sks9__SKU5 similar 9
<a href="#">At1g74790</a>	Catalytic
<a href="#">At2g02810</a>	ATUTR1_UTR1__UDP-galactose transporter 1
<a href="#">At1g14360</a>	ATUTR3_UTR3__UDP-galactose transporter 3
<a href="#">At4g32650</a>	ATKC1_AtLKT1_KAT3_KC1__potassium channel in <i>Arabidopsis thaliana</i> 3
<a href="#">At2g13610</a>	ABCG5__ABC-2 type transporter family protein
<a href="#">At2g36590</a>	ATPROT3_ProT3__proline transporter 3
<a href="#">At5g11410</a>	Protein kinase superfamily protein
<a href="#">At4g16563</a>	Eukaryotic aspartyl protease family protein
<a href="#">At4g21620</a>	glycine-rich protein
<a href="#">At2g16630</a>	FOCL1__Pollen Ole e 1 allergen and extensin family protein
<a href="#">At1g65845</a>	
<a href="#">At1g69900</a>	Actin cross-linking protein
<a href="#">At5g58570</a>	
<a href="#">At3g05727</a>	S locus-related glycoprotein 1 (SLR1) binding pollen coat protein family

<u>At1g55980</u>	FAD/NAD(P)-binding oxidoreductase family protein
<u>At3g18950</u>	Transducin/WD40 repeat-like superfamily protein
<u>At1g27950</u>	LTPG1__glycosylphosphatidylinositol-anchored lipid protein transfer 1
<u>At1g51820</u>	SIF4__Leucine-rich repeat protein kinase family protein
<u>At1g66920</u>	Protein kinase superfamily protein
<u>At4g31000</u>	Calmodulin-binding protein
<u>At2g00790</u>	

Supplementary Table S7. Plant genes up-regulated by the heterologous expression of *xopX2*

<i>ID</i>	<i>Annotation</i>
<u>At5g17450</u>	HIPP21__Heavy metal transport/detoxification superfamily protein
<u>At4g24450</u>	At4g24450_ATGWD2_GWD3_PWD__phosphoglucan, water dikinase
<u>At4g39955</u>	alpha/beta-Hydrolases superfamily protein
<u>At3g02240</u>	GLV4_RGF7
<u>At1g55980</u>	FAD/NAD(P)-binding oxidoreductase family protein
<u>At1g34060</u>	TAR4__Pyridoxal phosphate (PLP)-dependent transferases superfamily protein
<u>At1g26770</u>	AT-EXP10_ATEXP10_ATEXPA10_ATHEXP ALPHA 1.1_EXP10_EXPA10__expansin A10
<u>At5g45380</u>	ATDUR3_DUR3__solute:sodium symporters;urea transmembrane transporters
<u>At2g47000</u>	ABCB4_AtABCB4_ATPGP4_MDR4_PGP4__ATP binding cassette subfamily B4
<u>At4g37310</u>	CYP81H1__cytochrome P450, family 81, subfamily H, polypeptide 1
<u>At5g57140</u>	ATPAP28_PAP28__purple acid phosphatase 28
<u>At4g37390</u>	AUR3_BRU6_GH3-2_GH3.2_YDK1__Auxin-responsive GH3 family protein
<u>At2g39380</u>	ATEXO70H2_EXO70H2__exocyst subunit exo70 family protein H2
<u>At5g11410</u>	Protein kinase superfamily protein
<u>At2g18193</u>	P-loop containing nucleoside triphosphate hydrolases superfamily protein
<u>At3g06355</u>	



Supplementary Table S8. Annotation color code.

Color	Related function
	amino acid metabolism
	Biodegradation of Xenobiotics
	cell
	cell wall
	Co-factor and vitamine metabolism
	development
	DNA
	fermentation
	gluconeogenesis / glyoxylate cycle
	glycolysis
	hormone metabolism
	lipid metabolism
	major CHO metabolism
	metal handling
	micro RNA, natural antisense etc
	minor CHO metabolism
	misc
	mitochondrial electron transport / ATP synthesis
	not assigned
	nucleotide metabolism
	OPP
	protein
	PS
	redox
	RNA
	S-assimilation
	secondary metabolism
	signalling
	stress
	TCA / org transformation
	tetrapyrrole synthesis
	transport

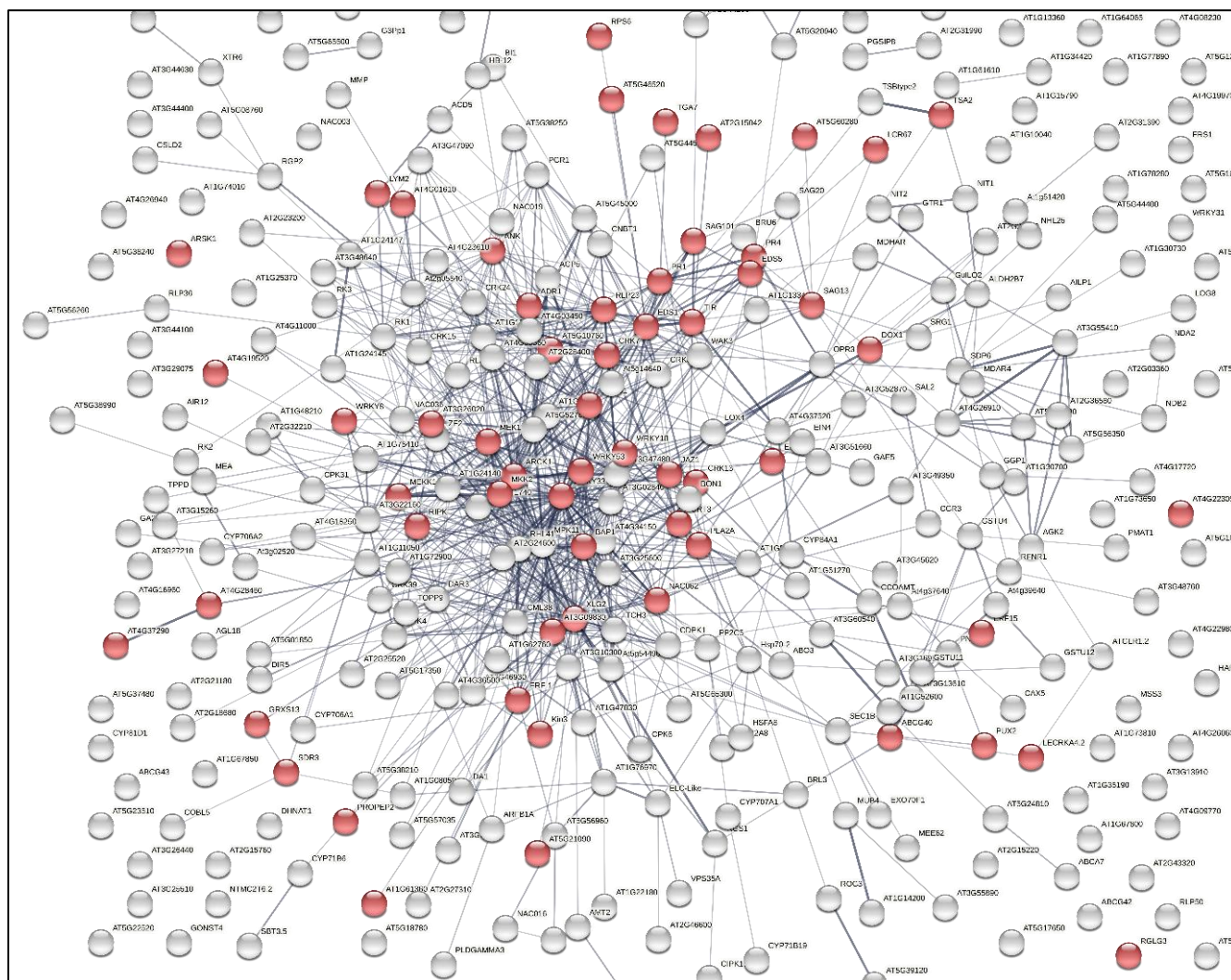
Supplementary Table S9. Primers used for qPCR assays

Primer	Sequence
AvrBs1-F	CGAAATCTGGACTGGTGGCG
AvrBs1-R	ACGATGAGGTGTGCAAAGCA
AvrXccA1-F	TGGTGAACCTACCCGCACCTG
AvrXccA1-R	GGCGTATCCGTAGCGGTTCT
AvrXccA2-F	AGTATTCAGGGCGCCACTCC
AvrXccA2-R	GCTCGAACTGATGGCCGTTG
HpaA-F	TCTGGAGGTTGATGCGCGAG
HpaA-R	AGGCGATGTATCCGGTTGCG
HrpW-F	CCGGCAAGGTCTTTCGTACC
HrpW-R	CGGAACACCGCTTCCTTGAC
XopAC-F	AAATGCGTGAGGGTGCATCG
XopAC-R	TCCTCTGCCCAACTGCTGAC
XopA-F	CCGATCAGGAGTGCGGAGAC
XopA-R	CGCCTTGGTTCTGCATCAGC

XopAG-F	CCAACCTTGCCTGCCTCAAG
XopAG-R	CGGGCTTCTACTCAGGTCCG
XopAH-F	GGACGCTCCTGCCGATATGT
XopAH-R	TGGA CTGCTTGAGAGTGCGA
XopAL1-F	CGACGCAGATGAAGACCATGC
XopAL1-R	GTCACCCACGCATCCACAAC
XopAL2-F	TGGCGCGATAGAACGGTAGG
XopAL2-R	TCAAGGGGATTGGAGTGGAGC
XopD-F	CGGCCTCATCACGGATCGTC
XopD-R	CTGACGCTGCTTTCTGCTGG
XopE2-F	GAGGATGGCGGGCAGATGAT
XopE2-R	GA CTCTCCGCCTCTATCGC
XopF-F	GGTCTGGAATGCGCTCAAGG
XopF-R	CCTGAAGCGTGCGTTCGATG
XopG-F	GA ACTGGAGCCCTGGTGTTG
XopG-R	AGCCCCACAAATGCCTGTTC
XopH-F	CGGCTCAATTGCACCATCGG
XopH-R	GCGGGAACCGCTTGATGAAC
XopJ-F	GAACCGCTGGAGGAGAGCTT
XopJ-R	CAGCGCATCCCGTAGGTACT
XopK-F	ACCACGCAACTGCATCAAGC
XopK-R	CAGCTCCTCGTTTGCCATGC
XopL-F	CCCTTCCACTCCCCAGGTTG
XopL-R	CGATCTTCTGACGCGCTTGC
XopN-F	CGATGAAACTGCCGGACACC
XopN-R	CTCAATCCCTGCGCCTGGTA
XopP-F	ACCCTCCGACCACGACAATG
XopP-R	GTGTACGAACCATGGCGCAG
XopQ-F	GTACCGCAAGGCACAGCAAC
XopQ-R	TCGTAGAACGAGGGCGACAC
XopR-F	AAGCAGTTGCCGTTGACGTG
XopR-R	CTGCGTTCCGTGCTGTTGTT
XopX1-F	TCATGCGGGTGTTCAAGGGA
XopX1-R	TTCGCCGTGTCTTGACGGT
XopX2-F	GAGCAGCCTCAGCAAAC TCG
XopX2-R	CTGCGCCTGGTTGTT CAGTC
XopZ-F	CAGCCGCTTTCTGCATGAGG
XopZ-R	GCCATTCCAGCCGTTCCATC

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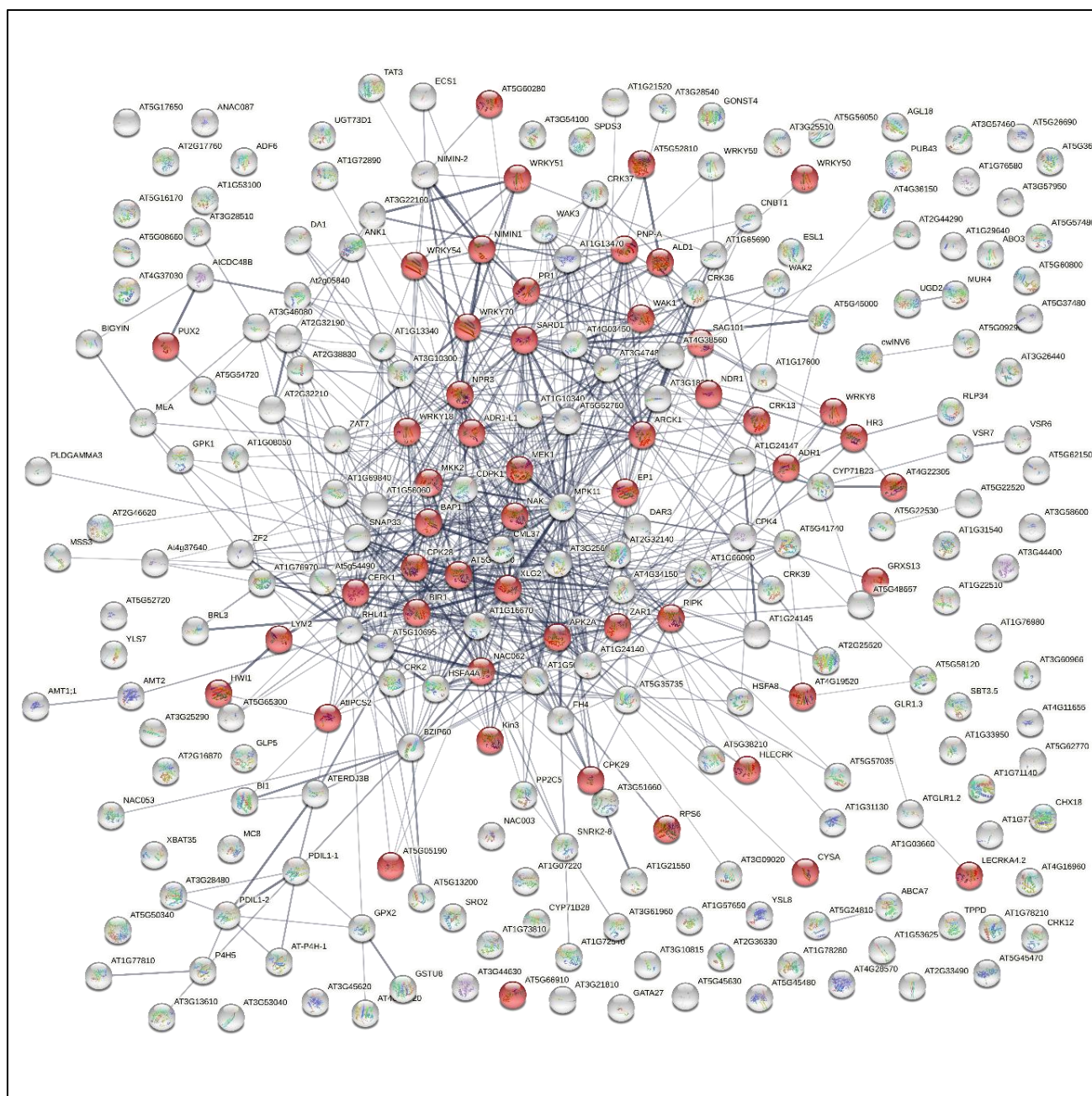




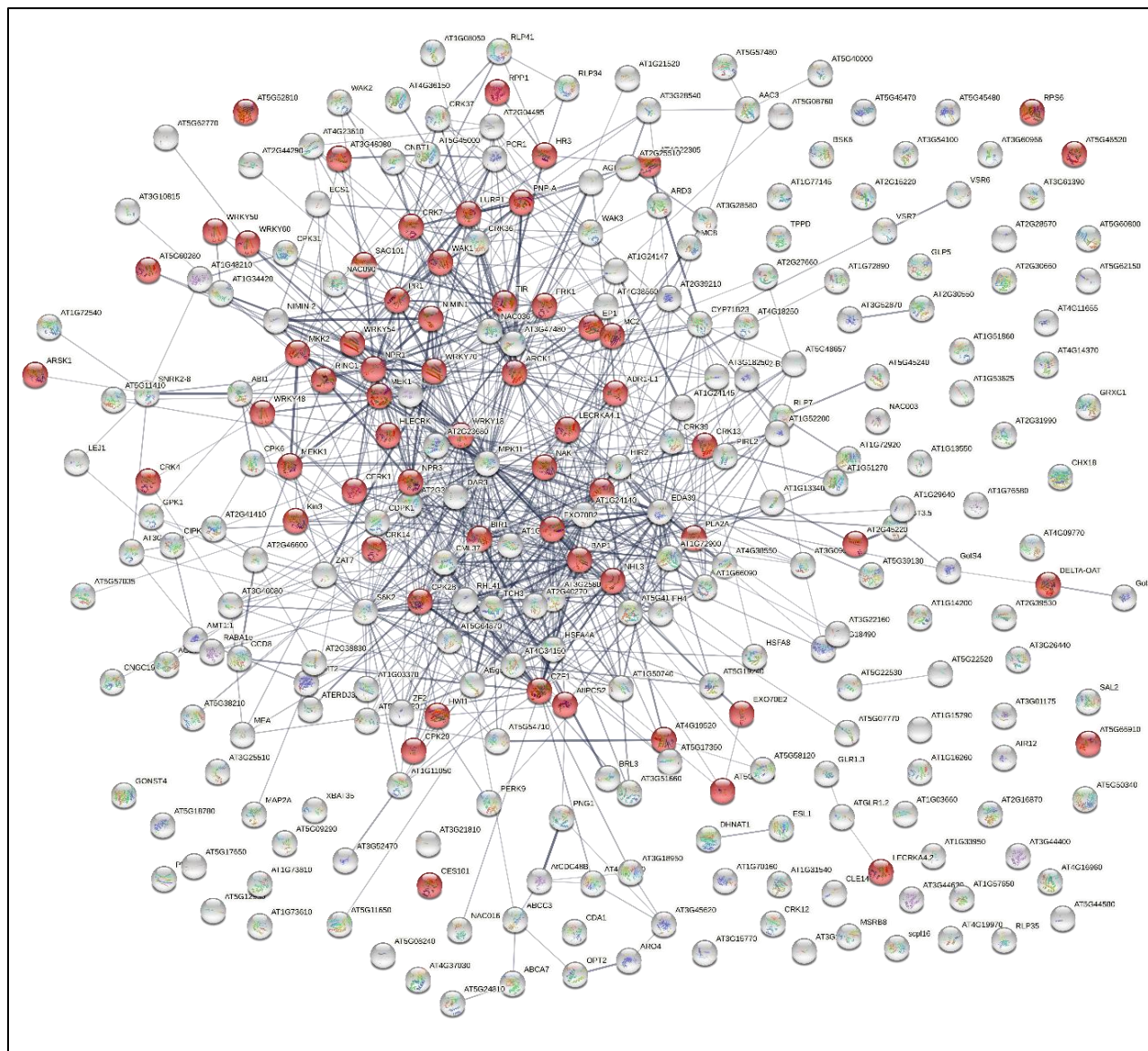
**Supplementary Figure S1. Prediction of the interaction network of DEG commonly up-regulated by the heterologous expression of *xopAC* and *avrXccA1* done with the STRING webtool. Red spheres represent genes involved in immune responses.**







**Supplementary Figure S3. Prediction of the interaction network of DEG commonly up-regulated by the heterologous expression of *xopAC* and *xopAH* done with the STRING webtool. Red spheres represent genes involved in immune responses.**



**Supplementary Figure S4. Prediction of the interaction network of DEG commonly up-regulated by the heterologous expression of *xopAC* and *xopL* done with the STRING webtool. Red spheres represent genes involved in immune responses.**

## **Chapter 3: The orthologous effectors XopAG and RipO1 alter plant growth and hormone-response pathways on Arabidopsis.**



## **The orthologous effectors XopAG and RipO1 alter plant growth and hormone-response pathways on Arabidopsis.**

### **Context and contribution:**

In the first part of this chapter, we studied some of the characteristics of the *Xcc* T3Es in a full-effectome context. In this second chapter, we moved our focus to a pair of orthologous T3Es, XopAG from *Xcc* strain 8004 and RipO1 from *Ralstonia pseudosolanacearum* strain GM1000. Considering that these pathogens colonize similar tissues within the plant host and display similar life habits, we explored whether orthologous T3Es in these two species present similar functions within the plant cell. We used multiple approaches to characterize the functions of the T3Es in plant cells, including pathogenicity assays with mutant strains and transcriptomic analysis in transgenic plants ectopically expressing single T3Es. Additionally, we conducted *in silico* analysis and forward genetic approaches to identify the biological target of XopAG.

This work was developed in the facilities of the LIPME on the frame of two consecutive PhD projects. In a first effort, Manuel Gonzales-Fuente identified the effects of XopAG and RipO1 in plant development and the relevance of XopAG for *Xcc* pathogenicity. When I joined this project, the characterization of the *in planta* molecular functions of XopAG and RipO1 had already started. I contributed to the analysis of the effects of *xopAG* in the basal immune responses of Col-0 plants by qPCR and western blot experiments. I also participated in the characterization of the effects induced by both effectors in the plant transcriptome by RNAseq. I screened previously EMS-mutagenized pER8-*xopAG* lines to identify XopAG-insensitive plants and I participated in the subsequent validation of *xopAG* sequence and expression in these suppressor mutants. Finally, I contributed to the writing of the manuscript that is presented hereafter.





# The orthologous effectors XopAG and RipO1 alter plant growth and hormone-response pathways on *Arabidopsis thaliana*.

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## Abstract

The phytopathogens *Xanthomonas campestris* pv. *campestris* (*Xcc*) and *Ralstonia pseudosolanacearum* (*Rps*) present several similarities during their infectious cycles. Including the secretion of type 3 effector proteins (T3Es) to modulate the plant physiology, a biotrophic lifestyle, and a preference for the vascular tissues within the host plant. In this work, we identified a pair of orthologous T3Es formed by the *Xcc* XopAG and the *Rps* RipO1. In a comparative analysis of the effects caused by these T3Es in *Arabidopsis*, we identified that both effectors affected the auxin-response pathway and repressed plant growth. XopAG also decreased the expression of defense-response genes involved in the jasmonic acid (JA)-pathway. Additionally, the mutation of XopAG decreased the pathogenicity of *Xcc* in *Arabidopsis*. Data obtained suggest that the plant defense responses regulated by JA are relevant for *Arabidopsis* resistance to *Xcc*, and XopAG can suppress such defense responses by an unknown mechanism.

## Introduction

Plant pathogens are classified according to their mode of nutrition in biotrophs, necrotrophs, and hemibiotrophs. Biotrophs derive nutrients and energy from living cells, while necrotrophs derive their energy from dead tissues (Rajarammohan, 2021). Hemibiotrophs transition from biotrophs in early stages to a necrotrophic lifestyle in later phase (Chowdhury et al., 2017). Regardless of their lifestyle, many phytopathogens secrete type 3 effector proteins (T3E) via the type 3 secretion system (T3SS) to modulate plant physiology and promote pathogenicity (Büttner & Bonas, 2010). As effectors are tools that redirect host metabolism, pathogens with different nutritional requirements, deploy different effectors (Uhse & Djamei, 2018). The T3Es deployed by necrotrophic pathogens promote susceptibility by inducing host cell death, while biotrophic pathogens produce effectors that promote host colonization by suppressing host defense surveillance system (Pradhan et al., 2021). Increasing evidence suggest that hemibiotrophic pathogens secrete distinct classes of effectors that first suppress plant defense responses, and later induce cell death in host tissues (S.-J. Lee & Rose, 2010; Jupe et al., 2013).

Pathogens are perceived in plant cells by two different recognition systems, one initiate the so-called pattern-triggered immunity (PTI) and relay in cell surface-localized pattern recognition receptors (PRRs) (Bigeard et al., 2015). The second recognition system detect directly or indirectly the presence of microbial effectors by intracellular Nucleotide-Binding domain Leucine-Rich Repeat containing receptors (NLRs or NB-LRR) (Steinbrenner et al., 2012). Upon pathogens perception, plants deploy multiple defense mechanisms that usually repel intruder's attacks. Plants respond differently to distinct classes of pathogens. Defense responses against biotrophic pathogens often culminate in programmed cell death (PCD) of the infected tissues to avoid microbial survival (Dickman & Fluhr, 2013). On the contrary, plant responses to necrotrophic pathogens, involve the accumulation of antifungal proteins (plant defensins) and defensive secondary metabolites (phytoalexins) as well as the avoidance of PCD (Pandey et al., 2016). The divergence in plant defense responses is directed by hormone signalling pathways. Responses against biotrophic pathogens are stimulated by salicylic acid (SA) (Peng et al., 2021), whereas jasmonic acid (JA), and ethylene (ET) promote immune responses against necrotrophic pathogens (Mengiste, 2012). A negative crosstalk between SA pathway and JA/ET pathway direct immunity towards one direction or the other (N. Li et al., 2019). However, plant responses to hemibiotrophic pathogens that employ both infection strategies involve a complex and highly regulated transition from SA-mediated responses at the early stages of the interaction to JA/ET mediated responses at later stages of infection (Glazebrook, 2005).

Increasing evidence suggests that regulation of the immune responses is rather a complex process that involves the activity of other hormones such as auxins, cytokinins, abscisic acid, gibberellins, brassinosteroids, and strigolactones (S. Huang et al., 2020). JA and auxin signaling pathways present positive crosstalk, since JA induces the expression of auxin synthase gene (ASA1) (J. Yang et al., 2019). On the other hand, the activation of the auxin response factors ARF6/ARF8 increase the level of endogenous JA promoting petal and stamen growth (Reeves et al., 2012). Additionally, Auxin is known to regulate nearly all developmental processes and has been recognized as an important negative regulator of SA-mediated immune responses (D.-L. Yang et al., 2013). The molecular mechanisms by which auxin modulates plant immunity have not been fully described yet. However, it has been reported that some plant pathogens promote the auxin-signaling pathway to promote plant disease susceptibility such as the *Pseudomonas syringae* AvrRpt2 that promotes pathogenicity by increasing free IAA levels and auxin sensitivity in *A. thaliana* (Chen et al., 2007; D.-L. Yang et al., 2013).

Among the most important species for phytopathology, there are several species of biotrophs including *Ralstonia pseudosolanacearum* and *Xanthomonas campestris* (Mansfield et al., 2012). In addition to having the same lifestyle, both pathogens colonize vascular vessels. *Xanthomonas campestris* pv. *campestris* (*Xcc*) causes the black rot disease in numerous species of *Brassicaceae* plants including economically important crops such as cabbage, cauliflower and broccoli (Dow & Daniels, 1994). *Xcc* accesses the inner tissues of susceptible plants through hydrotodes or wounds to colonize the xylem vessels and spread systemically (Cerutti et al., 2017). *Ralstonia pseudosolanacearum* (*Rps*) causes bacterial wilt disease on many economically important species such as tomato or potato. *Rps* penetrates plant tissues through wounds in roots or emergence points of lateral roots, and similarly to *Xcc*, colonizes xylem vessels (Denny, 2006).

In this work, we identified the pair of orthologous effectors XopAG and RipO1 from the hemibiotrophs *Xcc* and *Rps* respectively. Considering that these pathogens present similar lifestyle, we hypothesized that XopAG and RipO1 could exert similar functions in *Arabidopsis*. Therefore, we carried out a comparative study of the effects of these T3Es on some aspects of plant physiology such as phenotype, transcriptome, susceptibility to pathogenesis, among others.

## Results

### *Xcc* and *Rps* have similar virulence factors

The T3E repertoire of a given strain, largely influence its lifestyle, host range and niche. To explore whether the similarity in lifestyle of the hemibiotrophic pathogens *Xanthomonas campestris pv campestris* (*Xcc*) and *Ralstonia pseudosolanacearum* (*Rps*) can be explained by similar repertoires of T3Es, we compared the sequences of the T3Es of the reference strains *Rps* GMI1000 (Sabbagh et al., 2019) and *Xcc* 8004 (Roux et al., 2015). We found nine *Rps* T3Es with significant similarity to six *Xcc* T3Es (Table 1).

<i>Xcc</i> T3E	<i>Xcc</i> <sub>8004</sub> accession	<i>Rps</i> T3E	<i>Rps</i> <sub>GMI1000</sub> accession	blastp E-value	Identity	Query coverage
XopAG	XC_0563	RipO1	RSp0323	$7.5 \cdot 10^{-149}$	46.4%	88.2%
XopQ	XC_3177	RipB	RSc0245	$8.2 \cdot 10^{-130}$	47.4%	94.1%
XopP	XC_2994	RipH1	RSc1386	$4.1 \cdot 10^{-93}$	34.6%	80.9%
		RipH2	RSp0215	$1.0 \cdot 10^{-77}$	29.9%	96.1%
		RipH3	RSp0160	$4.0 \cdot 10^{-110}$	35.8%	84.6%
XopAM	XC_3160	RipR	RSp1281	$5.8 \cdot 10^{-96}$	27.2%	76.7%
HrpW	XC_3023	RipW	RSc2775	$1.3 \cdot 10^{-64}$	47.9%	71.7%
XopG	XC_0967	RipAX1	RSc3290	$2.1 \cdot 10^{-21}$	48.3%	88.8%
		RipAX2	RSp0572	$2.0 \cdot 10^{-21}$	44.4%	96.9%

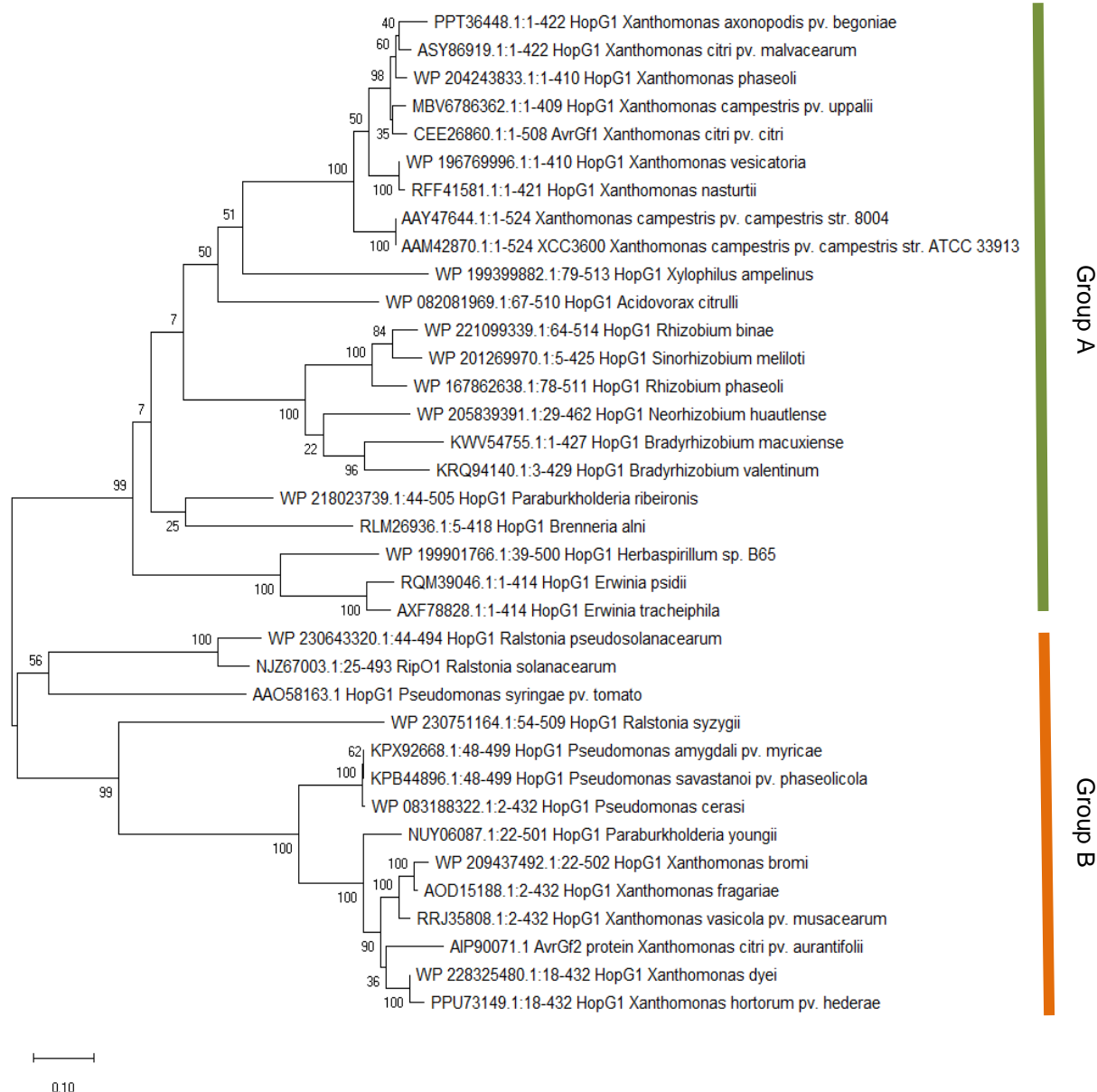
Table 1. Orthology relationship between shared T3Es from *Xcc*<sub>8004</sub> and *Rps*<sub>GMI1000</sub>. Table summarizing the orthology relationship between T3Es from *Xcc*<sub>8004</sub> and *Rps*<sub>GMI1000</sub> (blastp E-value <  $10^{-20}$ ) as defined by full-length protein sequence blastp querying the full effectome of *Xcc*<sub>8004</sub> against the full effectome of *Rps*<sub>GMI1000</sub>.

In parallel, we used the EffectorK database ([www.effectork.org](http://www.effectork.org)) to retrieve the putative plant-interactors of the full repertoire of T3Es of both strains. We identified 19 common interactors of T3Es of both strains (Supplementary Table 1). Among these, nine were common to T3Es of other pathogenic species (*Agrobacterium tumefaciens*, *Glovinomyces orontii*, *Hyaloperonospora arabidopsidis* or *Pseudomonas syringae*) whereas ten were exclusive to T3Es of *Xcc* and *Rps*. Interestingly a pair of orthologous T3Es formed by XopAG<sub>8004</sub> and RipO1<sub>GMI1000</sub>, presented three common putative plant-interactors: AT4G17680, BRG3 (AT3G12920) and KLCR2 (AT3G27960).

### **XopAG and RipO1 belong to a family of T3Es widely distributed among microbial species.**

XopAG<sub>8004</sub> and RipO1<sub>GMI1000</sub> protein sequences are formed by 524 aminoacids and 512 aminoacids respectively. There are no annotations available in NCBI database regarding

conserved domains. By a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), we detected many orthologous genes to both T3Es, those belonged to a wide range of bacterial species, including mutualist and phytopathogens. To explore the evolutionary relationships among the orthologs of this family, we selected 35 T3Es from diverse species with more than 40% of similarity to XopAG<sub>8004</sub>. The selected list is mainly formed by T3Es from pathogens (e.g., *Xanthomonas*, *Ralstonia*, *Pseudomonas*, *Erwinia*, *Acidovorax*, etc.) and nitrogen-fixing microbes (e.g., *Rhizobium*, *Sinorhizobium*, *Mesorhizobium* or *Bradyrhizobium*, *Paraburkholderia*). The protein sequences of the selected T3Es were retrieved from the NCBI database and used to construct a cladogram of the XopAG family (Figure 1). Two main groups were clearly defined. The species included in Group B are phytopathogens except for *Paraburkholderia youngii*. The group A presents two main subdivisions. In one of these subdivisions are grouped several nitrogen-fixing bacteria including species of *Rhizobium*, *Neorhizobium*, *Sinorhizobium* and *Bradyrhizobium* suggesting that XopAG was acquired by a common ancestor to all these symbiotic species. The second subdivision of group A comprises phytopathogenic bacteria except for *Herbaspirillum* and *Paraburkholderia ribeironis*. The XopAG orthologs of many species of *Xanthomonas* (including Xcc<sub>8004</sub>) are associated in a monophyletic clade in Group A. Intriguingly, other *Xanthomonas* species (e.g., *dyei*, *bromi*, *fragariae*, *citri*) are included in Group B, more related to *Pseudomonas* and *Paraburkholderia youngii* orthologs. This suggests that XopAG was acquired independently in each group by different processes of horizontal gene transfer (HGT). RipO1<sub>GMI1000</sub> and its orthologue in *Ralstonia pseudosolanacearum* grouped with HopG1 of the hemibiotrophic pathogen *Pseudomonas syringae* in a monophyletic branch in Group B, relatively far from Xcc<sub>8004</sub>XopAG. Previous studies identified common features in AvrGf1 of *X. citri* pv. *citri* and AvrGf2 of *X. citri* pv. *aurantifolii* including the HR elicitation in grapefruit and a characteristic cyclophilin-binding motif (GPxL) (Gochez et al., 2017). Surprisingly, these orthologs appeared in different phylogenetic groups being AvrGf1 closer to Xcc<sub>8004</sub>XopAG. Further comparative studies revealed that XopAG, RipO1 and HopG1 share the cyclophilin(Cyp)-binding motif (GPxL) present in AvrGf1 and AvrGf2 (Figure 2). This suggests that all these related effectors might target proteins of the Cyclophilin gene family (CYPs).



**Figure 1. Phylogenetic tree showing the evolutionary relationships of the XopAG orthologs of diverse bacterial species.** The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model, bootstrap support values (1000 replicates) were applied. The tree with the highest log likelihood (-11529.53) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The GenBank accession numbers of the full-length proteins used in the alignment are preceded by the name of the protein and the species to which it belongs. Analyses conducted in MEGA X software (Kumar et al., 2018).

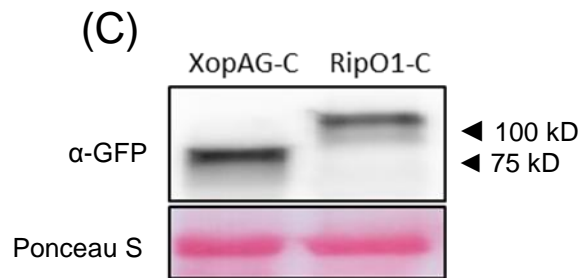
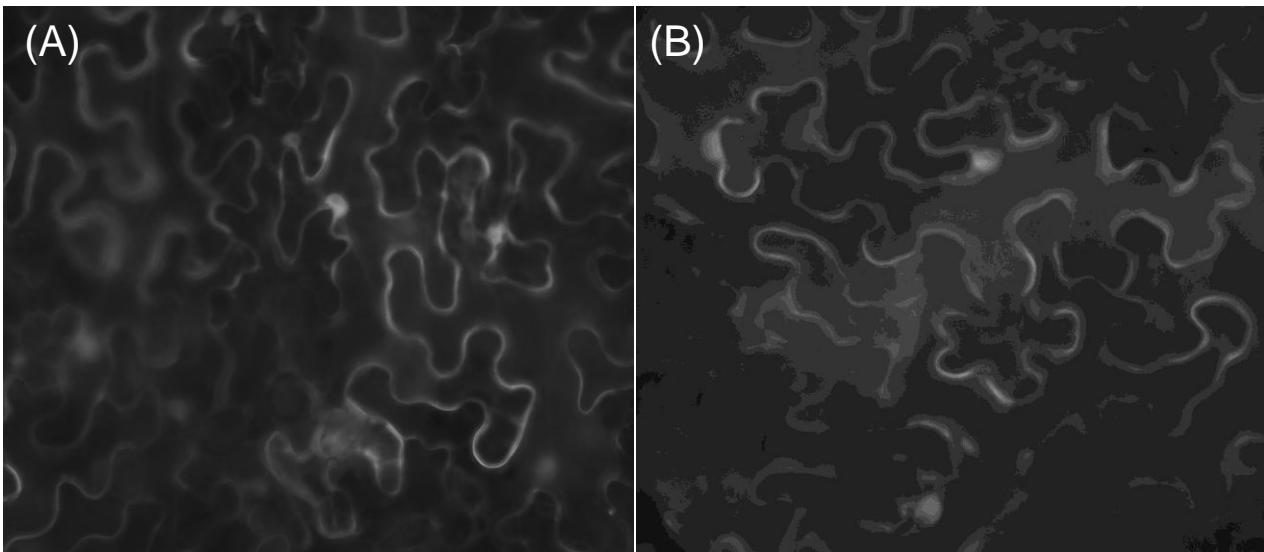
XopAG	MISVLHSRRPIMRTKTSPLATVQRLLTPGTSTGLSTPASASATPCAETTAGLLGALPTR
HopG1	-----MQIKNSHLYSASRMVQNTFNASPKMEVTNAIAKN
AvrGf2	-----MRVAKHNVPVSVLLVNEASKNSPTSQTIGGANSLN
AvrGf1	-----MRTKAQLPLTAIQRFLAHDAASTQAPSASASTSLHKNETAGLLAALPAR
RipO1	-----MPKIPKNLFHAIGSVTQHINETRAPLRS
XopAG	KNKQKQQSQRPNTQDGTTPKNGRDHGGQWATRAAKYALGIAGAGY---VADNFFLSTTS
HopG1	NEPAALSATQTAKTHEGDSKGQSSNNSKLPFRAMRYAAYLAGSAYLYDKTANFFLSTTS
AvrGf2	EILSGLPSFSKNANRTSSSRKTPRRLASLAKTAMKYVAGTAGAAYVYDNTANRFFLSTTS
AvrGf1	N--ARQGAQRKSKEKEGARQNNNGRGGQWASRAAKYALGIAGAGY---VADNFVLSTTS
RipO1	VQTARERSAPLPKAPKTASEGQRSQGSQFQSRVMKGILGAGAMAYTYDTIANHFFLSTTA
XopAG	LRDGKAGFSSNDRLEKACVKAESYHARYHSATEGERASHSRPFVPIRTCGSNQFATMSDY
HopG1	LHDGKGGFTSDARLNDADQDKARKRYQNNHSSSTLENK---NSLLSPLRLCGENQFLTMIDY
AvrGf2	LHDGKQGFTSDARLQEAEEKAEAIYAEYHAHECPDKIEVKRTSLWPKLVGENAFVTMLDF
AvrGf1	LVDGKGGFTSNDRLDKACAKAETTYARYHSATEDERASHSRPFVPIRTCGSNQFATMTDY
RipO1	LHDSKYGFTSDERLDKARPEAEENYQIYQSRTPVEQRAHNKSVNPFRLCGENHFVTMTDY
XopAG	RAATKVHIGHLFDSQHARQSLLTNLACLKGERIRDECIAQYAPTHVPANPDLSRSPLYET
HopG1	RAATKIYLSLDVDEQAHTSILKNIMCLKGELTNEEAIKKLNPEKTPKDYDLTNSAYIS
AvrGf2	RSATKVHLKELINTKEARDSIALNISILGERIKPALLTEHGVVQAPVAFDITKQDDFEL
AvrGf1	RAATKVHVHGLFDSQAARESLVTNLACLKGERIKQECIIRYAPAQVPADPDLSKSELYDR
RipO1	RVATRVYLSRLVDSKAAHALMSNNVQCLKGTTHVKKEAVEQFNPTKLPKNFDLTQSAAYDR
XopAG	KNKYSLTGVPNAQTGASGYTSRSITQPFINRGMQHFQDSQSDRALSCLKQCMELLERTLE
HopG1	KNKYSLTGVKNEETGSTGYTSRSITKPFVEKGLKHFIIKATHGEKALTPKQCMETLDNLLR
AvrGf2	KNKYSLLGVPNNDTGSYGYASRSILNPFIEKGEKHHQAIAISDQALAPRDCVAALQPMK
AvrGf1	KNKYSLVGMPNAQTGASGYTSRSITQPFINRGMHFQASQSDKALSRLQCMQSLERALQ
RipO1	KNKYSLIGVRNEETGSFGYTSRSATHPFVAEGWDHFFEATRGENGVTPKQCVETLEALLE
XopAG	GDDKLGKQAQHAAGQAILNFRQVYAADDEHWGHPKIVIMKTLIANGLLSQEQTDRIDATLM
HopG1	KSITLNSDSQFAAGQALLVFRQVYAGEDAWGDAERVLKSHYNRGTVLQDEADKIELSRP
AvrGf2	NSQSLIPEAQFRAGQALLILRPLYCGPKTWGDAHKVLMFPFLESKGLASTRENQRLGETRP
AvrGf1	DTDKLGKQAQHAAGQAILNFRQVYAADDEHWGHPKIVIMKTLIANGLLSQEQTDRIDATLM
RipO1	RGDTLSAEAQFAAGQLLLIYRQAYADDENWNGNAESVVLADLYRHGLASKAEADKIELTRP
XopAG	F--EDPSISVLKNTSVA <b>GPVL</b> QHLETKFQSRRLQDRPEALADFMEMAKQKNMEGLPIVH
HopG1	FSEQDLAKNMFKRNTSIA <b>GPVL</b> YHAYIYIQEKIFKLPPDKIED-LKHKSMADLKNLPLTH
AvrGf2	FTPSDMEKGVARRNTSVA <b>GPVL</b> HELNMLIQKSIYKKDEEGMSD-LRSKNLREMKYIPISH
AvrGf1	F--EDPSISVLKNTSIA <b>GPVL</b> QKLETKIQSRRLQDQPETLADFMEMAKQKNMEGLPIAH
RipO1	PYQEDLDMGWARRNTSLI <b>GPML</b> QHTDIWWQEHILRKDPETIKS-INNLHVADLQYTPLSH
XopAG	FKLNAQNGGFEDCSGLGDSFTSANAVACINHARLMSGEPRLSKQDVGVVVACINAVYDDA
HopG1	VKLSNSGVGFEDASGLGDSFTALNATSCVNHARIMSGEPPLSKDDVVILIGCLNAVYDNS
AvrGf2	FRMNDCTGFEDSSGLADSFTGYNVAAVINHARLLSGEDRLSKQDVVAVVGVCLNAVYDNA
AvrGf1	FKLNAEGTGFEDCSGLGDSFTSANAVACINHARLMSGEPRLSKEDVGVVVACINAVYDDA
RipO1	FKLNEQGNGGFEDCSGLGDSFTSLNATSCLNHARLMSGRERLSRDEVIVLIACINAVYDNA
XopAG	SSVRHTLHEIARGCFVGAGYTTEDADVIFYENVCKDAAQAFYAGRSMTRNA-----
HopG1	SGIRHSLREIARGCFVGAGFTVQDGDFFYKQICKNASKQFYNG-----
AvrGf2	SSERHTLREIAHGCFVGAGYTVDAEAFYKDVCKDSAREFYGGALRAAKSN-----
AvrGf1	SSIRHSLHEIARGCFVGAGYTTEDADAFYEQICKDAARAFYAGKSMTSSD-----
RipO1	GGIRHTLQEIARGCFVGAGYTVAEADDFYRRLCKNAAEMFYGGRNLKPLVQPERPEVISV

154 **Figure 2. The orthologs of XopAG in multiple bacterial species present a common cyclophilin-**  
 155 **binding motif (GPxL).** Protein sequences of *X. citri* pv. *citri* AvrGf1 (CEE26860.1), *X. citri* pv.  
 156 *aurantifolii* AvrGf2 (AIP90071.1), *P. syringae* HopG1 (AA058163.1), *Ralstonia pseudosolanacearum*  
 157 RipO1(NJ267003.1) and *X. campestris* pv. *campestris* strain 8004 XopAG (AAY47644.1) were  
 158 aligned with free gap ends in Geneious software (Cost matrix: BLOSUM 62, gap open/extension  
 159 penalties: 12/3). The conserved cyclophilin-binding domain (GPxL) is boxed in red.



**XopAG and RipO1 present nucleocytoplasmic localization in *N. benthamiana* cells.**

Often, effectors' localization *in planta* hint their molecular functions. With this in mind, we determined the subcellular localization of XopAG and RipO1 within plant cells. For that purpose, we transiently expressed either XopAG or RipO1 fused to the fluorescent protein CFP in *N. benthamiana* leaves and tracked their localization by confocal microscopy. Strikingly, both effectors distributed throughout the cytoplasm and the nucleus of the transformed cells, without a clear pattern of specific localization to any cell compartment (Figure 3).



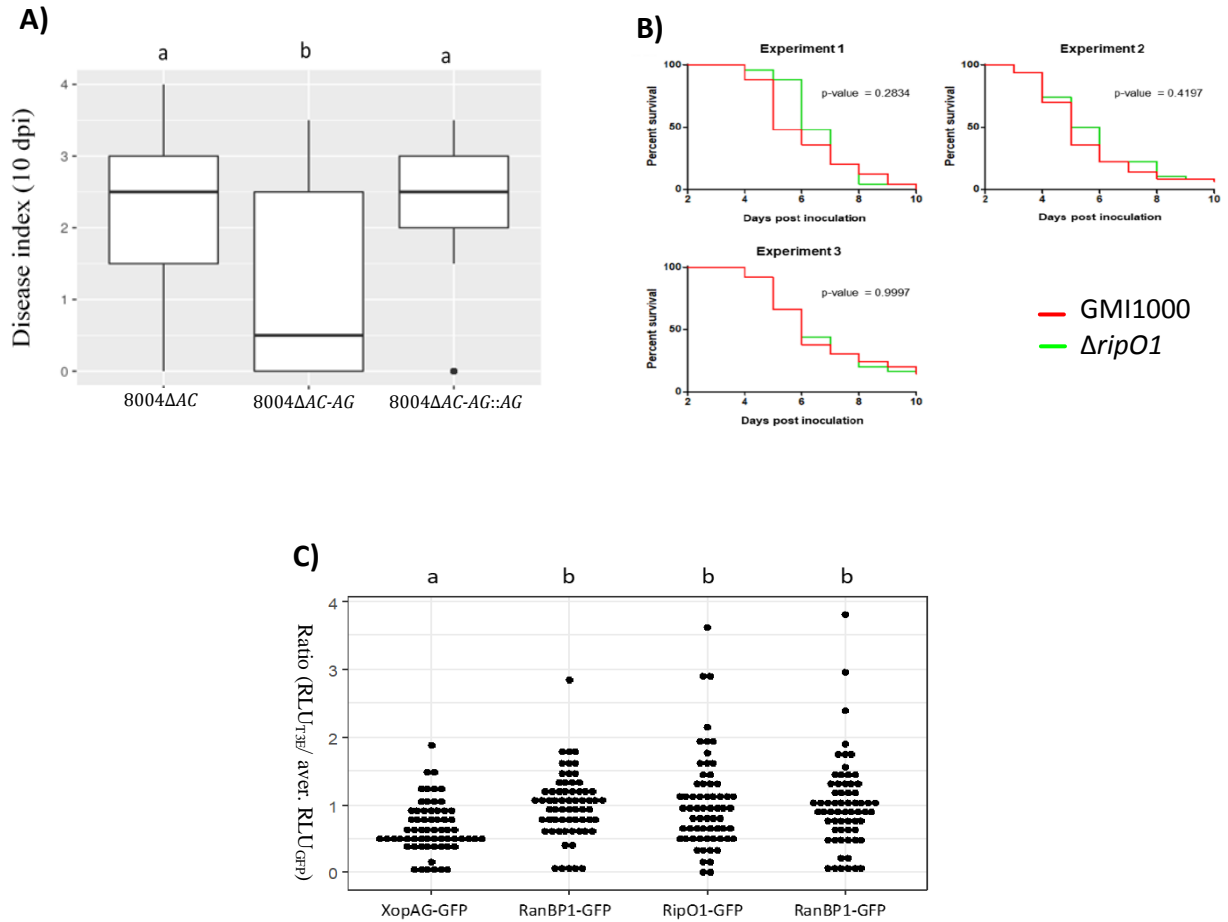
**Figure 3. XopAG and RipO1 present nucleocytoplasmic localization in *N. benthamiana* cells.** XopAG-CFP (A) and RipO1-CFP (B) were transiently expressed in *N. benthamiana* cells upon *A. tumefaciens* transformation. Cellular localization was determined by confocal microscopy. XopAG (57 kDa) and RipO1 (57 kDa) expression was confirmed by western blot assays using anti-GFP antibody (C). Three independent experiments showed similar results



**XopAG promotes pathogenicity *in planta* whereas RipO1 has no significant effect.**

In order to investigate the role of XopAG<sub>8004</sub> and RipO1<sub>GMI1000</sub> in the virulence of the their respective pathogen, we generate KO-mutant strains for *xopAG* and *ripO1* genes in *Rps*<sub>GMI1000</sub> and *Xcc*<sub>8004</sub> respectively. Since the wild-type (WT) *Xcc*<sub>8004</sub> is avirulent on *A. thaliana* Col-0 plants due to the presence of the T3E *avrAC/xopAC* that uridylyates the plant decoy PBL2 in turn activating host immune responses, the *Xcc*<sub>8004</sub> $\Delta$ *xopAC* strain was thus used in this experiment. The double mutant *Xcc*<sub>8004</sub> $\Delta$ *xopAC-xopAG* presented decreased pathogenicity when wound inoculated in Col-0 leaves, as compared to the compatible strain *Xcc*<sub>8004</sub> $\Delta$ *xopAC* (Figure 4A). The *xopAG* gene and the upstream genomic region including its native promoter were re-introduced in the genome of the double mutant *Xcc*<sub>8004</sub> $\Delta$ *xopAC-xopAG*. The complemented strain *Xcc*<sub>8004</sub> $\Delta$ *xopAC-xopAG::xopAG* exhibited virulence levels similar to those presented by the virulent strain *Xcc*<sub>8004</sub> $\Delta$ *xopAC*. On the other hand, the mutation of *ripO1* on *Rps*<sub>GMI1000</sub> did not affect pathogenicity on Arabidopsis Col-0 plants, as compared to the wild-type (WT) *Rps*<sub>GMI1000</sub> (Figure 4B). All the strains used in this experiment showed growth levels similar to the wild-type reference strain for each pathogen when cultured in rich and poor medium (Supplementary Figure S1), suggesting that the differences observed in *Xcc* pathogenicity are caused by the deletion of *xopAG* and not by general alterations in bacterial fitness.

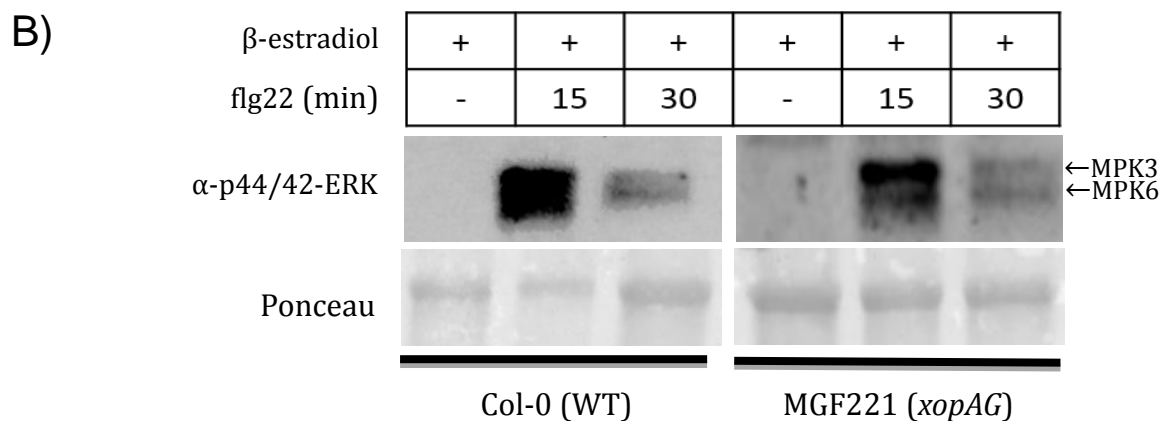
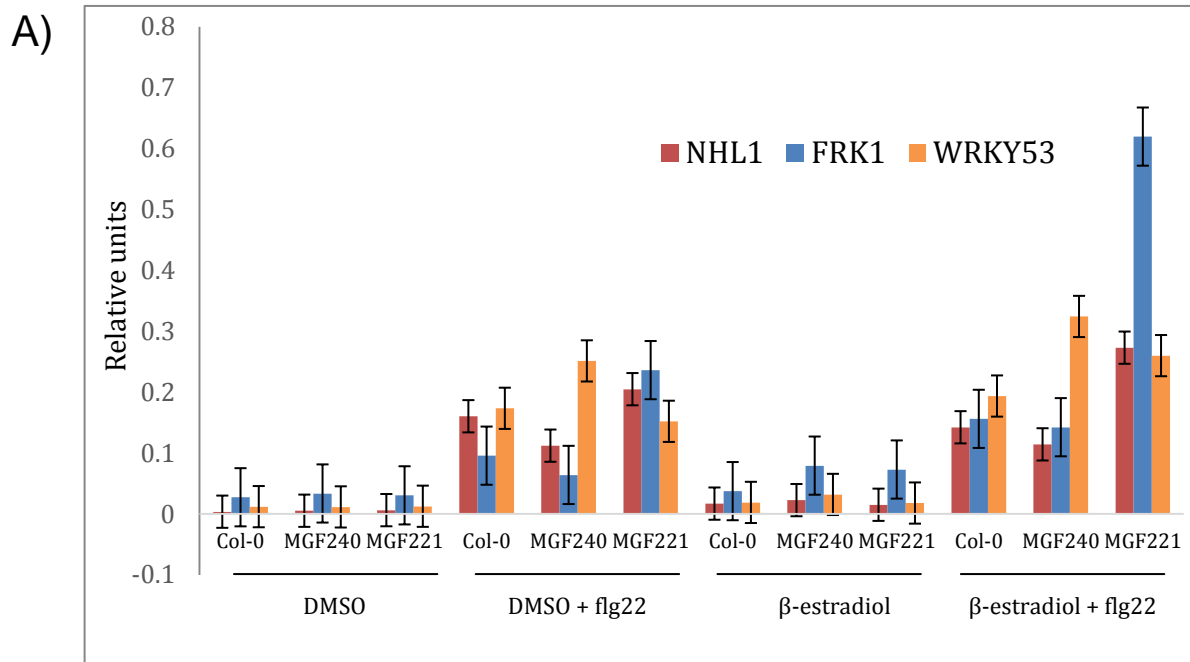
Often T3Es contribute to microbial pathogenicity by suppressing the host immune responses (Yan et al., 2019; Block et al., 2010). In order to evaluate the influence of XopAG and RipO1 in the plant basal defenses, XopAG, RipO1 and the immunologically innocuous protein RanBP1 were transiently expressed in *Nicotiana benthamiana* leaves. Then, the production of Reactive Oxygen Species (ROS) in response to the peptide flg22 was measured by luminometry as previously described (Sang & Macho, 2017). *xopAG* expression caused a statistically significant reduction of ROS accumulation upon flg22-treatment as compared to the expression of the control protein RanBP1 (Figure 4C), suggesting that XopAG plays an active role in the suppression of this plant immune response. On the other hand, *ripO1* expression didn't cause significant effects in the accumulation of ROS upon flg22-treatment as compared to the expression of the control protein RanBP1.



**Figure 4. XopAG promotes *Xcc* pathogenicity on *Arabidopsis* Col-0 plants.** A) Box plot of the disease index scored 10 days after inoculation (dpi) on *A. thaliana* Col-0 plants inoculated by piercing with the strains *Xcc*8004Δ*xopAC* *Xcc*8004Δ*xopAC*-AG *Xcc*8004Δ*xopAC*-AG::AG as indicated in methods. Results of three independent experiments. B) Survival curves scored for 10 days after soil-drenching inoculation of *A. thaliana* ecotype Col-0 with wild-type *Rps*<sub>GMI1000</sub> (red) and *Rps*<sub>GMI1000</sub>Δ*ripO1* at 10<sup>8</sup> CFU/ml. Twenty plants were inoculated per line and experiment and the results of three independent experiments are shown. Statistically significant differences are indicated in each graph (Mantel-Cox logrank test p-value < 0.05). C) Integrated ROS production over 1 hour after 50 nM flg22 treatment by luminometry on *N. benthamiana* leaves upon transient expression of XopAG-GFP, RipO1-GFP or RanBP1-GFP as indicated in methods. Results of four independent experiments. For A) and C) statistical groups are indicated by letters “a” and “b” on the top (One-tailed Wilcoxon signed-rank [*p*-value < 0.05]).

## **XopAG does not affect PTI responses in Col-0 plants**

The previous experiment indicated the ability of *xopAG* to suppress a PTI response in *N. benthamiana* leaf tissue. However, the biochemical activities of the T3Es are host-dependent and often vary among different plant species. To test whether XopAG promotes *Xcc* pathogenicity on *Arabidopsis* Col-0 by suppressing basal immune responses, we constructed *Arabidopsis* Col-0 transgenic lines harboring the expression vector pER8-*xopAG*. Transcript accumulation of *xopAG* in transgenic plants was corroborated by RT-qPCR (Supplementary Table S2). Subsequently, we quantified the influence of the ectopic expression of *xopAG* in the expression of some PTI-marker genes in response to the immunogenic peptide flg22 by RT-qPCR. Briefly, two-weeks-old pER8-*xopAG* transgenic plants were treated overnight with 5 $\mu$ M  $\beta$ -estradiol to induce the accumulation of *xopAG* transcripts. Then, plants were treated either with flg22 or with the polar solvent DMSO for 1h. In parallel, Col-0 wild-type plants received identical treatment. Total RNA was extracted from all samples and used to quantify the relative expression of the PTI-marker genes NHL1, FRK1 and WRKY53. The gene GAPC2 was used as reference. Two independent transgenic lines were used (MGF240 and MGF221). Surprisingly, the ectopic expression of *xopAG* did not significantly alter the expression of the PTI-marker genes in response to flg22 (Figure 5A). The activation of the MAPKs was also monitored by immunoblot using an anti-p44/42-ERK antibody (Cell-Signaling) on Col-0 and pER8-*xopAG* transgenic plants, either untreated or treated with flg22 for 15 min. or 30 min. (Figure 5B). The heterologous expression of *xopAG* in *A. thaliana* Col-0 plants did not affect the flg22-induced activation of the immune-relevant MAPK3 and MAPK6. All together, these results suggest that, though XopAG contributes to *Xcc* pathogenicity on *Arabidopsis thaliana* Col-0, it does not seem to be related with the modulation of some archetypal PTI-triggered responses.



**Figure 5. Arabidopsis PTI responses are not affected by XopAG.** Transcript accumulation of the PTI marker-genes *nhl1*, *frk1* and *wrky33* in Col-0 and pER8-*xopAG* seedlings treated with 5μM β-estradiol or DMSO in response to a 1 hour flg22 (100 nM) treatment. Transcript accumulation is expressed relative to the transcript level of the reference gene GAPC2. Bars represent means of three independent biological replicates ± SE. Differences weren't statistically significant (Kruskal wallis test)(A). Phosphorylation of MAPK3/MAPK6 induced by flg22 in Col-0 wild-type (WT) and pER8-*xopAG* (MGF221) plants. Samples treated for 0 to 30 min. with 5μM β-estradiol and 100 nM flg22 as indicated. Activated MAPKs were detected by immunoblots using α-p44/42-ERK antibody. Protein loading is indicated by the Ponceau staining of Rubisco.

## **The heterologous expression of *xopAG* and *ripO1* alter multiple pathways in Col-0 plants.**

To further characterize the molecular functions of both T3Es *in planta*, we followed a transcriptomic approach. First, we constructed pER8-*ripO1* transgenic plants (expression of *ripO1* validated by RT-qPCR (Supplementary Table S2). Subsequently, we characterized by RNAseq the transcriptomic profile of pER8-*xopAG* and pER8-*ripO1* transgenic plants previously treated overnight with 5 $\mu$ M  $\beta$ -estradiol. By independently comparing those with the transcriptomic profile of similarly treated Col-0 wild-type plants, we were able to identify Differentially Expressed Genes in response to either XopAG or RipO1. We considered as DEGs only those with an absolute expression fold change higher than five and a False Discovery Rate (FDR) smaller than 0.001. When mentioned, DEGs were functionally classified with the Classification SuperViewer Tool w/ Bootstrap (<http://bar.utoronto.ca/>) based on MapMan classification. The frequency of genes falling into each category (Norm. Freq.) was normalized to the frequency of genes assigned to the category in a reference set of *A. thaliana* genes as previously described (Provat & Zhu, 2003).

XopAG upregulated the expression of 131 plant genes while downregulated the expression of 112 genes (Supplementary Table S3). Upregulated genes were predominantly related with mitochondrial electron transport/ATP synthesis, metal handling, and cell wall among others (Table 2). Whereas XopAG-downregulated genes were primarily related to gluconeogenesis/glyoxylate cycle and sulfur assimilation among others (Table 2). Interestingly six plant genes showed expression levels ten thousand or more times lower when *xopAG* was heterologously expressed *in planta*. Five of those genes are related with defense responses against necrotrophic pathogens including ACD6 (Accelerated Cell Death 6), ACT (Anthocyanin 5-aromatic acyltransferase 1), PCC1 (Pathogen and circadian controlled 1) the defensin-like protein PDF1.2 and the Cysteine-Rich Transmembrane Module 8 (ATHCYSTM8).

In order to make a more detailed analysis, we predicted the interaction networks formed independently by the sets of plant genes upregulated and downregulated in response to *xopAG* by using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) version 11.5 (Szklarczyk et al., 2021). The predicted network with the set of genes upregulated by XopAG resulted in associations between proteins with catalytic activity and proteins localized in the plant membrane and the cell wall (Figure 6) suggesting that XopAG induces the re-arrangement of the plant membrane and the surrounding cell wall. In contrast, the interaction network predicted with the set of 112 downregulated genes showed many interactors involved in

response to external stimuli, followed by proteins related to interactions with other organisms, and response to hormones particularly to auxins (Figure 7).

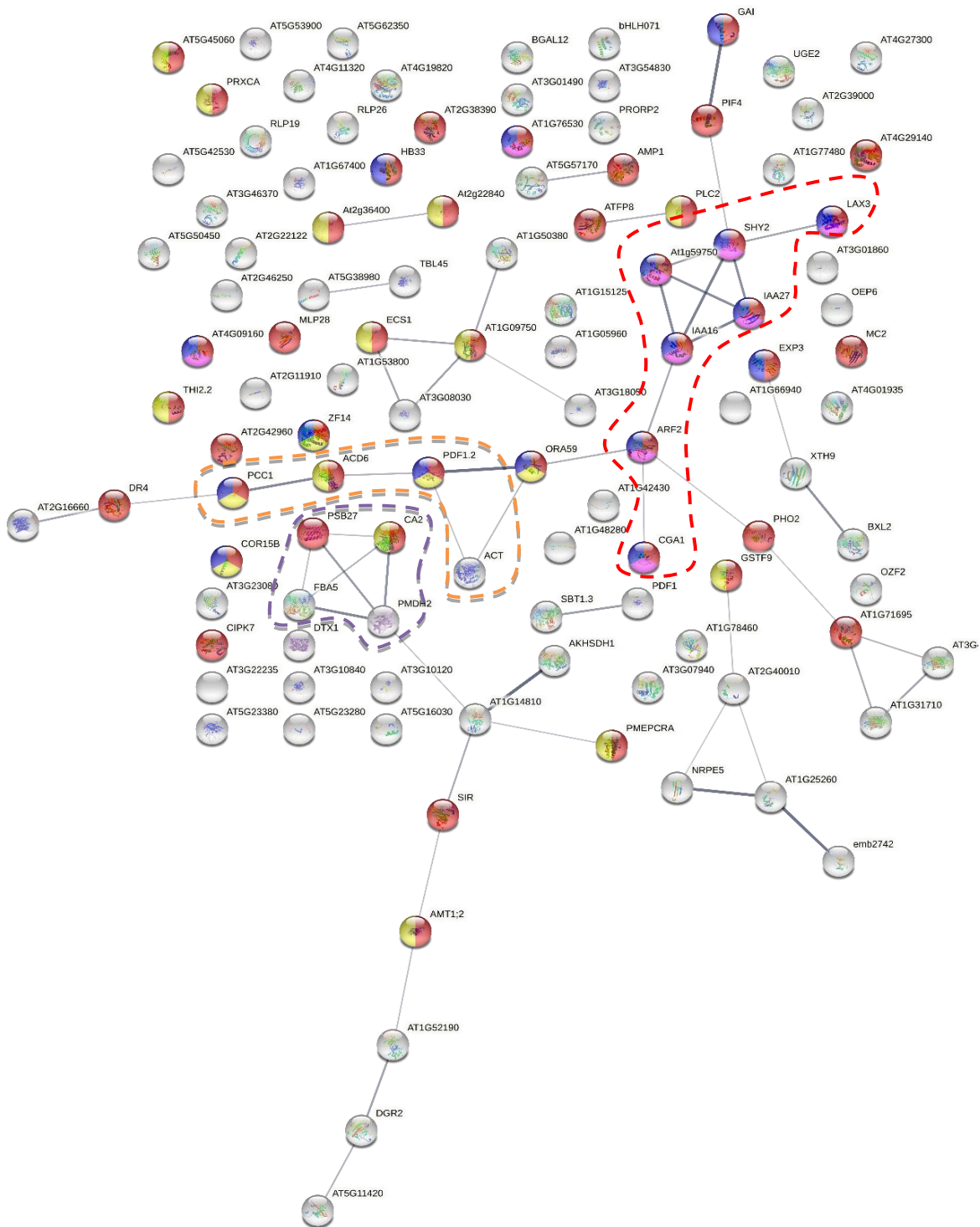
Table 2. Functional classification of DEG by *Xcc*<sub>8004</sub>*xopAG* heterologous expression in Arabidopsis.

Ontology group	Norm. Freq.	p-value
<b>Genes upregulated.</b>		
Mitochondrial electron transport / ATP synthesis	6.84	2.56E-03
Metal handling	6.22	0.037
Cell wall	4.71	4.69E-05
Redox	3.69	0.039
Hormone metabolism	2.86	0.014
Micro RNA, natural antisense etc.	2.8	0.024
Development	2.62	8.13E-03
Miscellaneous	2.11	5.28E-03
Signalling	1.7	0.044
Not assigned	0.7	2.85E-03
RNA	0.5	0.025
Protein	0.48	3.92E-03
<b>Genes downregulated.</b>		
Gluconeogenesis/glyoxylate cycle	23.07	0.042
S-assimilation	23.07	0.042
Cell wall	2.73	0.027
Stress	2.68	1.87E-03
Transport	2.05	0.034
Miscellaneous	1.88	0.023
RNA	1.47	0.036

In this set of downregulated genes, three clusters can be clearly recognized; one is formed by proteins related to defense against necrotrophic pathogens namely ACD6, ACT, PCC1 and PDF1.2 (yellow dashed line in Figure 7) that happen to be some of the genes whose expression levels are the most affected by XopAG as mentioned above. The second evident cluster (red dashed line) comprises proteins related to auxin response including the auxin influx carrier LAX3, the indole-3-acetic acid inducible protein IAA3 (also known as SHY2), IAA27 and IAA16; the GATA transcription factor CGA1; and the auxin response factors ARF1 (AT1G59750) and ARF2. In the middle of both clusters is localized the ethylene- and Jasmonic Acid (JA)-responsive transcription factor ORA59 that regulates defense against necrotrophic pathogens (Aerts et al., 2021). The third cluster (purple dashed line) is formed by proteins involved in photosynthesis and energy transport namely the Beta Carbonic Anhydrase 2 (CA2), the Malate dehydrogenase (PMDH2), the Fructose-bisphosphate, aldolase 5 (FBA5) and the Photosystem II lipoprotein (PSB27).







348  
 349 **Figure 7. PPI network predicted in STRING database with the genes downregulated in**  
 350 **response to *xopAG* expression in *planta*.** Proteins involved in response to stimulus [0.32] (red),  
 351 interspecies interaction between organisms (yellow), response to auxin [0.84] (purple) and  
 352 response to hormones [0.5] (blue). Strength of enrichment in brackets. Line thickness indicates the  
 353 strength of data support.



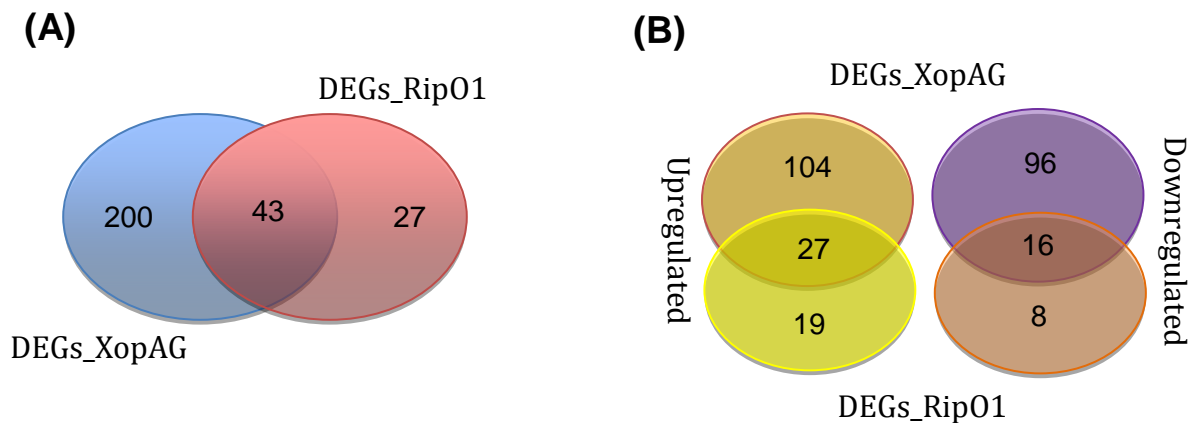
The ectopic expression of *ripO1* in Arabidopsis Col-0 transgenic lines altered the expression of 70 plant genes (Full list in Supplementary Table S4) as compared to Col-0 wild-type plants. 24 of these were downregulated while the expression of 46 plant genes was upregulated in response to RipO1. The functional characterization of these genes showed that RipO1 affected genes involved in photosynthesis, stress and regulation of RNA transcription (Table 3), whereas promoted the expression of genes related to development and cell wall (Table 3).

Table 3. Functional classification of the DEG by the ectopic expression of *ripO1* in *A. thaliana* Col-0.

Ontology group	Norm. Frec.	p-value
<b>Genes downregulated by RipO1</b>		
Photosynthesis	13.59	9.028e-03
Stress	3.42	0.045
RNA	2.75	0.013
<b>Genes upregulated by RipO1</b>		
Development	5.68	5.304e-04
Cell wall	4.08	0.031
Miscellaneous	3.28	3.786e-03

**XopAG and RipO1 affect the auxin-response pathway and photosynthetic machinery, and promote early flowering genes in Arabidopsis.**

The number of plant genes affected by *ripO1* expression *in planta* was more than three times smaller than the number of genes affected by *xopAG*. Interestingly, a large overlap in the genes affected by the T3Es was evident: the number of genes commonly affected by both effectors represents 61% of the number of genes affected by *ripO1* (Figure 8A). The proportion slightly varies when considering separately the genes upregulated (58%) and downregulated (66%) by both effectors (Figure 8B).



**Figure 8. Venn diagrams illustrating the overlap in the transcriptomic responses induced by *xopAG* and *ripO1*.** Considering the total number of DEG (A) or DEG upregulated and downregulated separately (B).

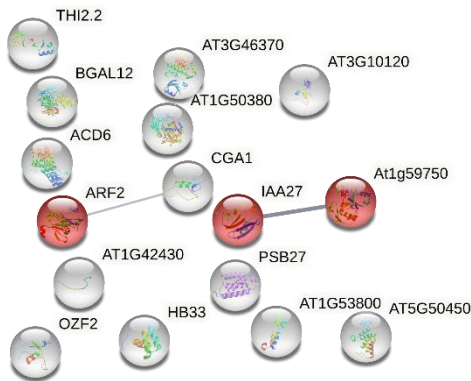
Both effectors repressed the expression of sixteen plant genes (Figure 9A) (Full list in Supplementary Table 5) that are mainly involved in regulation of RNA transcription (Table 4) and include multiple auxin-response genes such as IAA27, ARF1 (AT1G59750) and ARF2. Interestingly, in this group are included CGA1 and PSB27, that modulate chlorophyll biosynthesis and repair the damaged on the photosystem II respectively. This suggests that both T3Es affect the photosynthetic machinery.

Twenty-seven plant genes were upregulated by both effectors (Supplementary Table 5). However, the analysis of this group in the STRING database did not reveal significant associations with each other. Still, functional classification revealed that multiple genes are related to development and cell wall (Figure 9B). In the former group are included the proteins SINA2 (At3g13672) and SWEET10 that promote autophagy and early flowering respectively (H. Qi et al., 2020; Andrés et al., 2020). In the later classification are included the pectin lyases PGL1 (At1g56710) and At5g19730 that are related to flower organ abscission and siliques formation respectively (Kim et al., 2006; Louvet et al., 2006). All these suggest that RipO1 and XopAG promote the flowering phase on Arabidopsis plants.

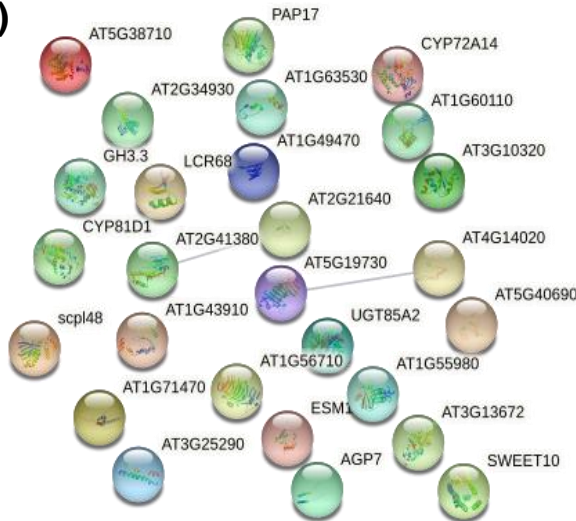
Table 4. Functional classification of DEG commonly modulated by XopAG and RipO1 in *A. thaliana* Col-0.

Ontology group	Norm. Frec.	p-value
<b>Genes downregulated</b>		
RNA	2.75	0.013
<b>Genes upregulated</b>		
Development	6.81	8.521e-03
Cell wall	4.73	0.021
Miscellaneous	4.69	1.190e-03

(A)



(B)

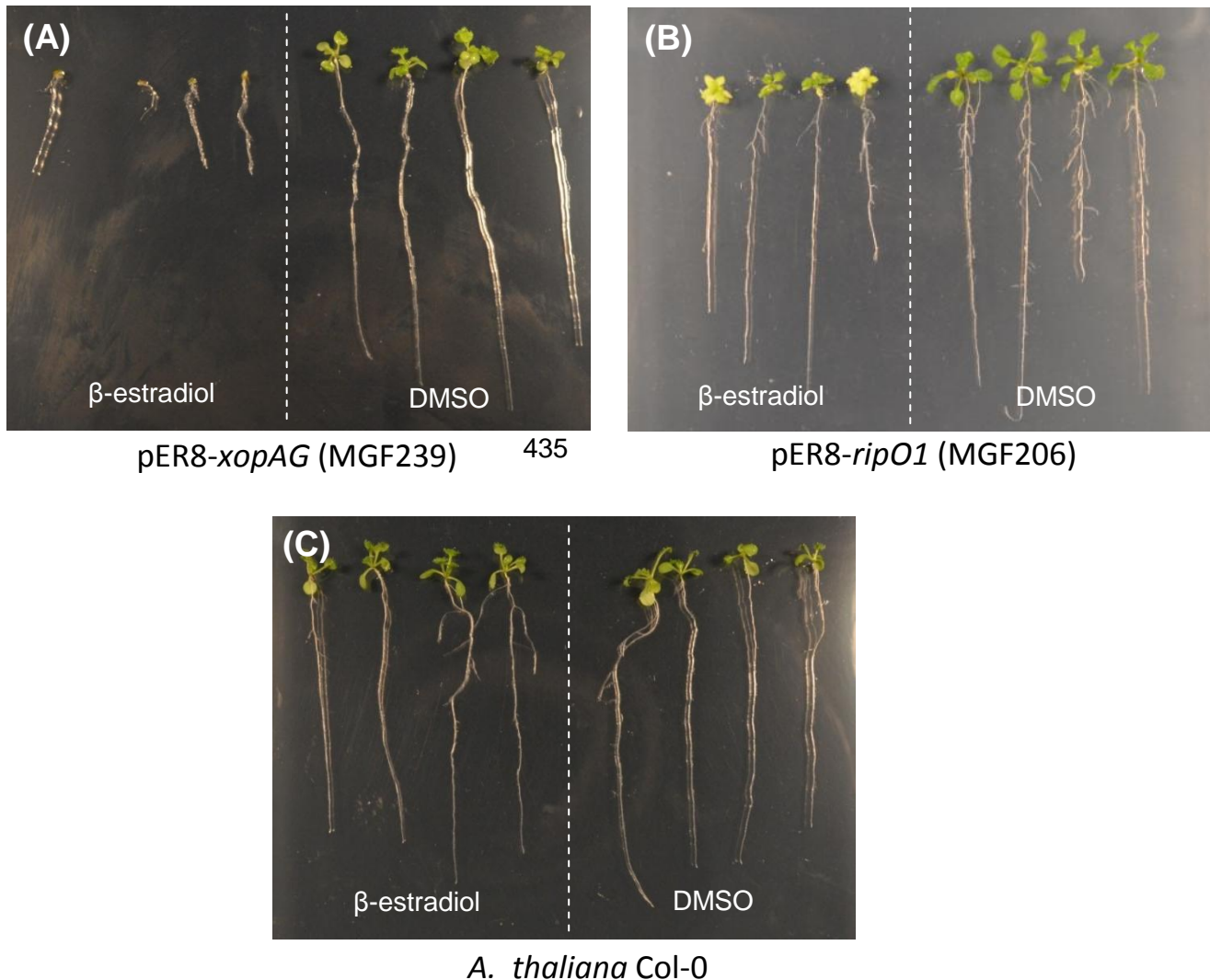


**Figure 9. PPI network predicted in STRING database with DEGs in response to RipO1 and XopAG in Col-0 plants.** (A) DEGs downregulated by both T3Es. Auxin-response genes are represented in red. (B) DEGs up-regulated by both T3Es. Line thickness indicates the strength of data support.

**Constitutive expression of XopAG and RipO1 affect plant growth.**

pER8-*xopAG* and pER8-*ripO1* transgenic plants were used to explore the effect of the constitutive expression of XopAG or RipO1 in Arabidopsis development. Both effectors altered plants phenotype. pER8-*xopAG* transgenic plants turned brownish and dwarf upon growth on MS medium supplemented with 5μM β-estradiol (Figure 10A), whereas upon the same treatment, pER8-*ripO1* plants presented yellow rosettes and are reduced in size (Figure 10B) as

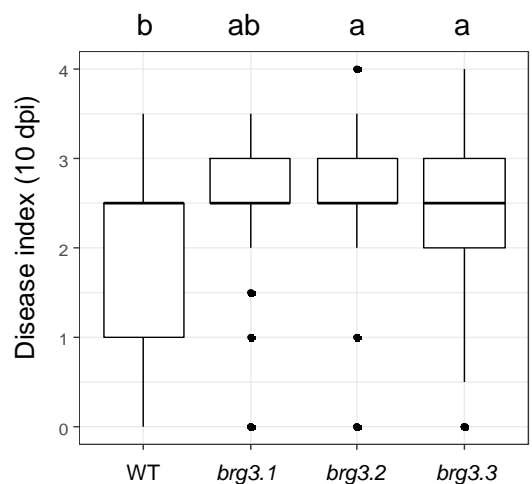
compared to seedlings treated with the polar solvent DMSO (Figure 10C). The effects of XopAG heterologous expression in Arabidopsis plant growth are significantly stronger than the effects caused by the expression of RipO1. This is consistent with the greater effect of XopAG on Arabidopsis transcriptome as compared to the effect caused by RipO1 showed in the previous experiment.



**Figure 10. Heterologous expression of *xopAG* or *ripO1* alters plant development.** Plants grown for two weeks in MS solid medium supplemented with either  $\beta$ -estradiol (5 $\mu$ M) or the polar solvent DMSO. pER8-*xopAG* transgenic plants (A), pER8-*ripO1* transgenic plants (B) or Wild-type Arabidopsis Col-0 plants (C).

**BRG3: a putative plant interactor of XopAG and RipO1 contributes to plant disease susceptibility.**

Considering the similarities exhibited by XopAG and RipO, we explored the EffectorK database ([www.effectork.org](http://www.effectork.org)) (González-Fuente et al., 2020), to identify putative interactors common to both T3Es. Three proteins resulted putative interactors of both T3Es, BRG3 (AT3G12920), KLCR2 (AT3G27960) and AT4G17680. Brg3 is a RING E3 ligase known to contribute to Arabidopsis resistance against *Botrytis cinerea* and control plant cell-death in response to *Pseudomonas syringae* (Luo et al., 2010). Consequently, we analyzed the role of the Brg3 in Arabidopsis resistance against *Xcc*. Three independent lines with a T-DNA insertion causing loss-of-function mutation in *brg3* were obtained from the NASC public collection (GK-661B07.01, SAIL\_261\_G05 and SAIL\_302\_F07). After challenge with *Xcc*<sub>8004</sub> $\Delta$ *xopAC* compared with Col-0 wild-type plants, a statistically significant increase in disease lesions was observed for two *brg3* mutants (*brg3.2* and *brg3.3*) (Figure 11). The remaining mutant (*brg3.1*) showed a tendency toward increased susceptibility although it was not statically significant.

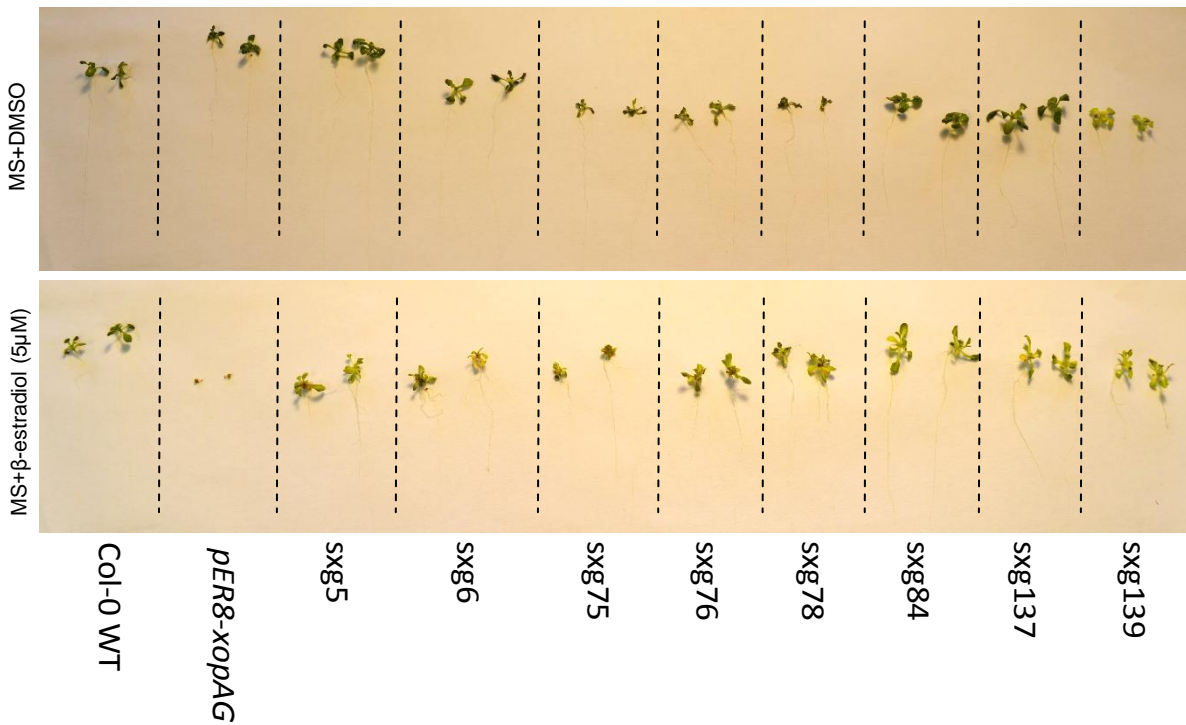


**Figure 11. Col-0 *brg3* mutant plants present increased resistance to *Xcc*<sub>8004</sub> $\Delta$ *xopAC* infection.**

Disease index scored at 10 days post-inoculation on *A. thaliana* Col-0 (WT) or *brg3* mutant lines *brg3.1* (GK-661B07.01), *brg3.2* (SAIL\_261\_G05) and *brg3.3* (SAIL\_302\_F07) plants. Disease symptoms were recorded as no symptoms (0-1), weak chlorosis (1-2), strong and extended chlorosis (2-3) and necrosis (3-4). Six plants (four leaves per plant) were wound inoculated with the strain *Xcc*<sub>8004</sub> $\Delta$ *xopAC* at OD<sub>600 nm</sub> = 0.1. Results of five independent experiments were grouped for statistical analysis. Statistical groups are indicated by letters “a” and “b” in the top part (One-tailed Wilcoxon signed-rank [p-value < 0.05]).

**Genetic screen to identify the molecular targets of XopAG in *A. thaliana* Col-0 plants.**

Although the above results allowed us to speculate that Brg3 is the effective target of XopAG and RipO1, this has not been validated and requires further experiments. Consequently, we conducted an alternative strategy to identify the molecular targets of XopAG. Previous studies have succeed at identifying two molecular targets of the *Xcc*<sub>8004</sub>XopAC effector by a suppressor screen approach (G. Wang et al., 2015). Following a similar strategy, we screened for suppressors of the *xopAG*-induced phenotype (*sxg*) in an Ethyl methanesulfonate (EMS)-mutagenized population of more than 3264 pER8-*xopAG* transgenic plants. Eight mutant lines (*sxg5*, *sxg6*, *sxg75*, *sxg76*, *sxg78*, *sxg137* and *sxg139*) were able to grow in presence of 5µM β-estradiol (Figure 12). Expression and sequence of *xopAG* were validated by sanger sequencing and RT-qPCR respectively in all the *sxg* lines (Data not shown).



**Figure 12. Identification of suppressor lines insensitive to XopAG.** Photographs of Col-0 wild-type (WT), pER8-*xopAG* and EMS-mutagenized (*sxg*) plants grown *in vitro* for two weeks on solid MS medium added with the solvent Dimethyl sulfoxide (DMSO) or 5 µM of β-estradiol as indicated in the image.

EMS-mutagenesis causes multiple mutations in plants genome. In order to characterize the mutations causing the suppression of the XopAG-induced phenotype in *sxg* lines, we have done allelism tests by doing combinatorial crosses between the different *sxg* lines. The rational is that if two recessive suppressor-mutations are allelic, they would fail to complement each other in the F1 hybrids showing affected phenotypes. In case that F1 hybrids show parental phenotypes, the parental lines would be considered to have allelic suppressor-mutations. Common mutations in allelic *sxg* lines revealed by whole genome sequencing assays would be candidate targets of XopAG. To date, we have analyzed descendants for seven crosses comprising the eight *sxg* lines (Table 5). Our crosses suggest an allelic group of six lines in which the crossing of any line of this group with another line of this same group originates offspring with XopAG-induced suppressor phenotype (Supplementary Figure S2). *sxg6* x *sxg137* cross originated descendants with phenotype similar to pER8-*xopAG* line, suggesting not allelic mutation with the former group. A similar result was observed for descendants of the cross *sxg6* x *sxg5* suggesting this two lines as no allelic. However, we ignore if *sxg5* is allelic to the group of six allelic *sxg* lines.

Table 5. Allelism test for the suppressor lines of the phenotype induced by XopAG (*sxg*)

Lines crossed	Phenotype + $\beta$ est.
<i>sxg6</i> x <i>sxg5</i>	Affected
<i>sxg139</i> x <i>sxg76</i>	Suppressor
<i>sxg137</i> x <i>sxg76</i>	Suppressor
<i>sxg84</i> x <i>sxg75</i>	Suppressor
<i>sxg78</i> x <i>sxg137</i>	Suppressor
<i>sxg84</i> x <i>sxg139</i>	Suppressor
<i>sxg6</i> x <i>sxg137</i>	Affected

In parallel, we are conducting experiments to identify the XopAG-supressor mutation following an approach of mapping-by-sequencing (James et al., 2013). In this strategy, *sxg* lines are backcrossed with the parental line pER8-*xopAG* (BC x F1). Within F2 hybrids (BC x F2) all EMS-caused mutations will segregate, except for the XopAG-suppressor mutation, then F2 hybrids showing *sxg* phenotype will be selected. This process will fix the XopAG-suppressor mutation while reducing the number of EMS-caused mutations. Pools of these plants will be sequenced, the mutant allele (as compared to pER8-*xopAG* line) with the highest allelic frequency will reveal the biological target of XopAG. To date we have F1 hybrids exhibiting 100% descendants with phenotype similar to pER8-*xopAG* would reveal *sxg* lines with

recessive suppressor mutations. These lines will be selected and self-crossed. In the F2 generation, 25% will be homozygous mutant, 25% will be homozygous wild-type and 50% will be heterozygous. F2 homozygous mutants will be used for whole genome sequencing. By comparing their genomic sequence to the genomic sequence of pER8-*xopAG*, we will be able to identify putative targets of XopAG. To date, all *sxg* lines have been backcrossed with the pER8-*xopAG* parental line. The phenotype in presence of  $\beta$ -estradiol of two F1 hybrid lines have been analyzed (Table 6) (Supplementary Figure S3)

Table 6. Crosses of *sxg* lines with pER8-*xopAG* parental line.

<b>Lines crossed</b>	<b>Phenotype + <math>\beta</math> est.</b>
<i>sxg</i> 137 x MGF240	Affected
<i>sxg</i> 75 x MGF240	Affected



## Discussion

Orthologous genes of RipO1 and XopAG are present in diverse species of pathogens and rhizobes, suggesting that the T3Es of the XopAG family play a relevant role in the plant-bacteria interactions. As expected, based on their sequence similarity, RipO1 and XopAG caused similar effects in growth and transcriptome of Arabidopsis although they differ in intensity, since XopAG caused stronger effects.

### **XopAG and RipO1 effects in plant growth correlate with altered expression of genes involved in auxin-response and photosynthetic machinery.**

Our transcriptomic approach revealed that XopAG and RipO1 affected the expression of genes related to the photosynthetic machinery and auxin-responses, and promoted the expression of genes related to early flowering and siliques formation. Interestingly, both T3Es deeply affected the expression of the transcription factor CGA1 which is known as a master regulator of chloroplast development (Zubo et al., 2018). In previous reports, Arabidopsis *cga1* mutants displayed an early flowering phenotype and decreased contents of chlorophyll (Hudson et al., 2011). This suggests that the reduced accumulation of CGA1 transcripts might cause the chlorotic phenotypes in pER8-*xopAG* and pER8-*ripO1* lines. CGA1 expression is directly repressed by the Auxin-Response Factor 2 (ARF2) (Richter et al., 2013). Strikingly, XopAG and RipO1 significantly decreased the transcript levels of ARF2 in our transgenic lines. This is contradictory since *arf2* mutant plants present delayed senescence and enlarged rosettes (Ellis et al., 2005). Previous reports indicated that ARF2 protein represses the expression of the auxin response genes including its own transcription as part of a negative feedback regulatory loop (Richter et al., 2013; Choi et al., 2018). Additionally, ARF2 is known to inhibit the growth of root cells (Choi et al., 2018) and inhibition of auxin signaling components is related to plant-growth defects (Takei et al., 2015). Hence, an increase in the accumulation of the ARF2 protein could explain the simultaneous decrease in *cga1* and *arf2* transcript accumulation as well as growth impairments and increased expression of senescence related genes in pER8-*xopAG* and pER8-*ripO1* lines. In this study we did not analyze the protein accumulation of ARF2 but this emerges as an interesting option for future studies.

XopAG and RipO1 altered the expression of multiple auxin response genes including the auxin response factor 1 (ARF1) and the Indole-3-acetic acid-amido synthetase GH3.3. Previous studies in Arabidopsis have shown that mutations in auxin signaling components increase

susceptibility to the necrotrophic fungi *Plectosphaerella cucumerina* and *Botrytis cinerea* (Llorente et al., 2008). Additionally, the accumulation of the conjugated form of indole-3-acetic acid (IAA)-Asp in Arabidopsis, promoted disease progression of *Botrytis cinerea* and the biotroph *Pseudomonas syringae* DC3000 (González-Lamothe et al., 2012). Considering this, it is likely that XopAG and RipO1 mediated effects in auxin-response pathways might contribute to Arabidopsis tolerance to bacterial colonization.

**XopAG affects the expression of the JA/ET-responsive genes ORA59, PDF1.2 and ACT, which might be related with increased plant susceptibility.**

Our transcriptomic analysis revealed that XopAG strongly repressed the expression of a genetic pathway formed by the Plant Defensin 1.2 (PDF1.2), the Agmatine coumaroyltransferase (ACT), and their transcriptional regulator ORA59. This pathway is considered as a classic marker of the ET/JA pathways and is decisive in plant resistance to necrotrophic pathogens (Zarei et al., 2011; J. Li et al., 2018). In previous studies, the overexpression of PDF1.2 upon treatment with Cell Wall Protein (CWP) fractions correlated with enhanced resistance to the hemibiotrophic pathogens *R. solanacearum* and *Pseudomonas syringae* in Arabidopsis (Kawamura et al., 2009). Additionally, in *Brassica olearacea*, PDF1.2 expression is related to plant resistance to *Xcc* colonization (H. Jiang et al., 2011). Other studies with different varieties of *Brassica napus*, either susceptible or resistant to *Xcc*, suggested that reduced ratios of SA/JA and ABA/JA as well as decreased expression of PDF1.2 is indicative of susceptibility to *Xcc* (Islam et al., 2017; Islam et al., 2021). The synergistic interplay between JA and ET pathways in plant resistance to necrotrophs has been well documented (J. Yang et al., 2019). Increasing evidence link auxin and JA pathways at multiple levels (Nagpal et al., 2005; Cai et al., 2014) and auxin pathway has been found to cooperate with JA to mount plant immune responses. For instance the application of IAA and MeJA synergistically induced the expression of PDF1.2 and increased plant disease resistance to the necrotroph *Alternaria brassicicola* (L. Qi et al., 2012). On the other hand, two distinct phases of *Xcc* infection in *B. napus* have been recognized: an early asymptomatic biotrophic phase and a subsequent necrotrophic phase. During necrotrophic phase, plant resistance is mediated by JA-related responses (Islam et al., 2017; Islam et al., 2021). The present study identified that XopAG affected simultaneously the auxin-response pathway and suppressed JA-related defense genes. Additionally XopAG mutation decreased *Xcc* virulence in Arabidopsis. This suggests that XopAG modulate the molecular hinges in the JA-auxin crosstalk to promote plant susceptibility during late infection (necrotrophic phase) of *Xcc*.

RipO1 similarly repressed auxin-responses in *Arabidopsis* although expression levels of PDF1.2, ACT and ORA59 were not significantly affected. This could explain the absence of effects on *Rps* virulence upon *ripO1* mutation. RT-qPCR assays showed that *xopAG* transcript accumulation in pER8-*xopAG* plants was several times higher than *ripO1* transcript accumulation in pER8-*ripO1* plant (Supplementary Table S2). Probably, T3Es effects in plant physiology are dosage-dependent and the differences observed between the two effectors are due to the lower expression of RipO1 in the transgenic plants. Future experiments using transgenic lines with similar T3E expression levels could reveal greater functional similarity between both T3Es.

#### **The CPxL motif might be relevant for XopAG and RipO1 processing.**

Our comparative analyzes evidenced a Cyclophilin-binding motif (GPxL) in RipO1 and XopAG that was conserved in XopAG orthologs of different pathogen species, including the T3E AvrGf2 of *X. citri* pv. *aurantifolii*. Cyclophilins are involved in the correct folding of the proteins by catalyzing the cis-trans isomerization of the peptide bond preceeding a proline residue (Singh et al., 2020). Previous studies identified that the interaction of the grapefruit cyclophilin GfCyp with the GPxL motif in AvrGf2 is a prerequisite for the HR-triggering activity of the T3E (Gochez et al., 2017). The authors proposed that GfCyp promotes a conformational change in AvrGf2. A similar process was identified for the effector AvrRpt2 of *Pseudomonas syringae* that is activated upon binding of the Arabidopsis cyclophilin ROC1 to its GPxL motif (Coaker et al., 2006). The presence of a GPxL motif and its similarity to AvrGf2, suggest that XopAG and RipO1 are equally modified by a cyclophilin in Arabidopsis. However a BLAST search for the GfCyp (Gene ID: GQ853548.1) ortholog in Arabidopsis yielded no significant results. The fact that XopAG and RipO1 had effects *in planta* suggests that an alternative cyclophilin processes both T3Es. Interestingly, GfCyp present highly similar orthologs (Identity > 80%) in the *Xcc* natural hosts *Brassica rapa* (LR031571.1) and *Brassica napus* (XM\_013875590.2). While the natural hosts of *Rps*, *Solanum lycopersicum* and *Solanum tuberosum* did not yield any significant match in the BLAST database. The GPxL motif and the five aminoacids upstream in XopAG are identical to those of AvrGf2 (NTSVAGPLL) while the GPxL motif of RipO1 and its upstream aminoacids differ slightly (NTSLLGPML). Likely, the GPxL motif of RipO1 suffered a process of pathoadaptation to be recognized by an alternative cyclophilin in its natural hosts. Additionally, given the differences in the GPxL motif, it is likely that RipO1 and XopAG present different cognate cyclophilins in Arabidopsis or different affinities for the same cyclophilin and therefore different levels of processing. This could be the reason for the lower effects of RipO1

on Arabidopsis transcriptome and phenotype as compared to the effects caused by XopAG. Nevertheless, this is only speculative and further experiments are required to validate this hypothesis. The fact that the GPxL motif is conserved among different species of pathogens suggests an important role in the functions of the T3Es belonging to the XopAG family. Future studies analyzing point mutations in the (Cyp) binding motif of XopAG and RipO1 followed by co-immunoprecipitation assays could uncover its relevance for the functions of the T3Es, and reveal its cognate cyclophilin in Arabidopsis respectively.

## **Deciphering the molecular targets of XopAG and RipO1**

A search in the EffectorK database, pointed to the BOI Related Gene 3 (BRG3) as a common putative interactor of XopAG and RipO1. In this work, *brg3* mutant plants presented enhanced susceptibility to *Xcc*. This is in line with previous reports mentioning BRG3 as a positive regulator of Arabidopsis resistance to the necrotrophic fungus *Botrytis cinerea*, and a negative regulator of cell death upon *Pseudomonas syringae* inoculation (Luo et al., 2010). Though the molecular mechanisms behind the contribution of BRG3 to plant disease resistance remain obscure, the evidence allows us to speculate that XopAG targets BRG3 to promote plant disease susceptibility during the necrotrophic phase of *Xcc* colonization. Brg3 is an E3 ubiquitin ligase ([Uniprot.org](https://www.uniprot.org/)) and has been associated with functions as diverse as mediating the turnover of the CC-NLR L5 (J. Huang et al., 2021) or repressing the gibberellin responses by binding to DELLA proteins in Arabidopsis (Park et al., 2013). The functional diversity of Brg3 prevents us from speculating on the consequences of its possible interaction with XopAG or RipO1. Further studies are required to validate the *in vivo* interactions of Brg3 with XopAG or RipO1 and their relevance for the virulence of *Xcc* and *Rps* respectively.

## **Methods**

### **Plant growth conditions**

For growth *in vitro*, *A. thaliana* seeds were surfaced-sterilized with bleach 36° diluted in tap water [1:3] for 15 min. Then, seeds were washed with sterile water and grown in sterile conditions in plates of Murashige & Skoog [MS] [Sigma-Aldrich] solid medium for two weeks [16h light; 20°C]. *A. thaliana* plants used for pathogenicity assays were grown in Jiffy pellets for four weeks [9h light; 22°C]. *N. benthamiana* plants were grown in Jiffy pellets for three weeks, and repotted into plastic pots filled with soil and grown for one to two weeks [16h light; 21°C; relative humidity 70%].

## Bacterial culture conditions

*E. coli* strains were grown in Luria-Bertani (LB) medium (5 g/l yeast extract; 10 g/l bacto tryptone and 10 g/l NaCl. For solid medium 15 g/l agar was added) at 37°C. *Xcc* strains were grown in Moka medium (4 g/l yeast extract; 8 g/l casamino acids; 2 g/l K<sub>2</sub>HPO<sub>4</sub> and 0.3 g/l Mg<sub>2</sub>SO<sub>4</sub> · 7 H<sub>2</sub>O. For solid medium 15 g/l agar was added) at 28°C. *A. tumefaciens* strains were grown in LB medium. Liquid cultures of all strains were grown in constant shaking. Antibiotics concentrations were as follows: 100 mg/l carbenicillin or chloramphenicol; 50 mg/l kanamycin or rifampicin; 40 mg/l spectinomycin; 30 g/l pimaricin; or 10 mg/l gentamycin or chloramphenicol. Tetracycline: 5 g/l for *Xcc* and *A. tumefaciens*, 10 g/l for *E. coli*.

## RNAseq

Plants were grown *in vitro* on full MS solid medium for two weeks (20°C, 16h light). Subsequently three plantlets of each treatment were transferred to 1ml of liquid full MS medium supplemented with 5µM β-estradiol, and incubated in a growth chamber (20°C, 16h light) under constant shaking. Plantlets were harvested 18 hours post β-estradiol treatment and frozen in liquid nitrogen. Prior to harvest, some samples were treated for 1h with flg22 under growth chamber conditions as indicated in Results section. Total RNA was isolated with the mirVana miRNA Isolation kit (Invitrogen) according to manufacturers' instructions. RNAseq was performed at the GeT-PlaGe core facilities (INRAE Toulouse). RNA quality was assayed with Agilent 2100 bioanalyzer as manufacturers' instructions. RNA-seq libraries were prepared according to Illumina's protocols using the Illumina TruSeq Stranded mRNA sample prep kit to analyze mRNA. Briefly, mRNA was selected using poly-T beads. Subsequently RNA was fragmented and used to generate double stranded cDNA and adaptators were ligated. Eleven cycles of PCR were applied to amplify libraries. Library quality was assessed using a Fragment Analyzer and libraries were quantified by qPCR using the Kapa Library Quantification Kit (Roche). RNA-seq experiments were done on the Illumina NovaSeq6000 system using a paired-end read length of 2x150 pb with the Illumina NovaSeq6000 sequencing kit.

## RNAseq data analysis and bioinformatics approaches

Transcript pseudo-mapping and quantification were done using the pipeline nf-core/rnaseq v3.0 with Araport11 as annotation reference. Special parameters were set as reverseStranded, pseudo-mapping and quantification with SALMON. Transcripts count normalization and characterization of Differentially Expressed Genes (DEG) was done using EdgeR package with

690 GLM Fit in R version 4.1.1. A gene was considered to be differentially regulated between two  
691 conditions when the gene showed. log fold-change > 0.7 and an FDR-adjusted p value < 0.05 or  
692 0.005 as indicated.

693 The intersection between different lists of DEGs to construct Venn diagrams, was calculated in  
694 the online tool <https://bioinformatics.psb.ugent.be/webtools/Venn/>.

695 PPI networks were constructed in the STRING database version 11.5 (Szklarczyk et al., 2021;  
696 <https://string-db.org/cgi/>), default parameters. Functional categories were consider only when  
697 FDR<0.001

698 The functional classifications on sets of DEG were done in the Classification SuperViewer Tool  
699 w/ Bootstrap ([http://bar.utoronto.ca/ntools/cgi-bin/ntools\\_classification\\_superviewer.cgi](http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi)) based  
700 on MapMan classification with all the other parameters set to default. Only groups with p-value  
701 < 0.05 were considered. The frequency of genes falling into each category (Norm. Freq.) is  
702 normalized to the frequency of genes assigned to the category in a reference set of *A. thaliana*  
703 genes by the tool (Provar & Zhu, 2003) and is calculated as: (Number\_in\_Classinput\_  
704 set/Number\_Classifiedinput\_set)/(Number\_in\_Classreference\_set/Number\_Classifiedreference  
705 \_set).

706 For phylogenetic and comparative analysis, the protein sequences were obtained from the  
707 public database NCBI. All protein sequence alignments were done with the Geneious software  
708 version 11.1.4 using the BLOSUM62 scoring matrix with gap open penalty of 12 and gap  
709 extension penalty of 3. To construct phylogenetic trees we used the Maximum Likelihood  
710 method and JTT matrix-based model, whit bootstrap support values of 1000 replicates.

## 711 **Plasmids construction**

712 Different Gateway clonings were performed using “Gateway Cloning kit” (Thermo) following the  
713 manufacturer’s instructions. BP reaction was used to generate an entry plasmid for *A. thaliana*  
714 *BRG3* using pDONR207. LR reactions were used to generate expression plasmids using  
715 different *xop* and *rip* entry plasmids (González-Fuente et al., 2019), and pER8-GW (Zuo et al.,  
716 2000) for  $\beta$ -estradiol-inducible expression *in planta*, pMDC43 (Curtis & Grossniklaus, 2003) for  
717 N-terminal GFP-fusions and pBIN-GW-CFP (for C-terminal CFP fusions) as destination vectors.

718 The plasmid for the complementation of *xopAG* was constructed by ligation of *Bam*HI/*Hind*III  
719 (Promega) digestion products of pCZ1013, an *Xcc* integrative plasmid, and PCR-amplified  
720 *xopAG* full gene. The ligation was carried out with T4 DNA Ligase (Promega).

#### 721 **Measurment of *Xcc* pathogenicity in *A. thaliana* Col-0 plants**

722 Four weeks old plants were used. Four leaves of healthy plants were pierced three times with a  
723 syringe needle dipped in *Xcc* bacterial suspension at  $10^8$  CFU/ml ( $\sim OD_{600}=0.1$ ) in 1 mM  $MgCl_2$ .  
724 In each experiment, six plants were inoculated with each strain. After inoculation, plants were  
725 placed in plastic trays and grown in controlled conditions (9h light; 22°C). Symptoms  
726 development was scored at 7 and 10 days post inoculation. Symptoms scale was as follows: 0-  
727 1, no symptoms; 1-2, weak chlorosis around the inoculation sites; 2-3, extended chlorosis; and  
728 3-4, necrosis. Results from independent experiments were combined and one-tailed Wilcoxon  
729 signed-rank tests were conducted on R version 3.5.2 (<https://www.r-project.org/>)

#### 730 **Measurment of *Rps* pathogenicity in *A. thaliana* Col-0 plants**

731 For soil-drenching inoculation, four weeks-old Arabidopsis plants grown on Jiffys were drenched  
732 in 2l of *Rps* bacterial suspension at  $10^8$  CFU/ml ( $\sim OD_{600}=0.1$ ) in water for 20 minutes. Then, soil  
733 was added to soak the remaining bacterial suspension and Jiffys were placed over. Plants were  
734 grown under long day conditions (12h light; 27°C; relative humidity 80-85%). Different treated  
735 plants were randomized within trays to minimize position biases. Disease symptom appearance  
736 was scored daily 3 to 10 days post inoculation following this index: 0, no symptoms; 1, first  
737 wilting symptoms; 2, wilting of half of the leaves; 3, wilting of more than half of the leaves; and 4,  
738 complete wilting. For survival analyses, plants are considered alive when disease index is lower  
739 than 2 and dead when greater or equal than 2. Results from independent experiments were  
740 analyzed separately and the survival curves, Mantel-Cox logrank tests and hazard ratios were  
741 calculated with GraphPad Prism 5.03 (<https://www.graphpad.com>).

#### 742 ***A. thaliana* transformation with pER8- vectors**

743 Plants were transformed by floral dip method (Clough & Bent, 1998) with *Agrobacterium*  
744 *tumefaciens* strain GV3101 pMP90 harboring the desired vector. Transformed plants were  
745 grown in greenhouse conditions. Transformants were screened on solid MS medium (Sigma)  
746 supplemented with 50  $\mu$ g/ml hygromycin. Plasmid presence was validated by PCR. For all

transformed lines, resistance segregation rates were registered in T2 and T3 generations to select single plasmid insertions and homozygous lines.

#### **Transient expression in *Nicotiana benthamiana* plants.**

Leaves of four weeks old *N. benthamiana* plants were infiltrated with *A. tumefaciens* strains carrying the desired expression vector. Bacterial suspension at OD<sub>600</sub> = 0.25 in infiltration buffer (10 mM 2-(N-morpholino)-ethanesulfonic acid (MES); 10 mM MgCl<sub>2</sub>; and 150 µM acetosyringone) were incubated in darkness for 2 hours and mesophyll infiltrated in plants. Plants were grown in controlled conditions (16h light; 21°C; relative humidity 70%) for two days before sampling.

#### **Transcript quantification by qPCR**

Three plants (two weeks old) per treatment were frozen in liquid nitrogen and total RNA was isolated with the “Nucleospin RNA Plus kit” (Macherey-Nagel) following manufacturers’ instructions. Reverse transcription was performed using 1 µg of total RNA with the “Transcriptor Reverse Transcriptase” (Roche) and oligo(dT)<sub>18</sub> primer. Real-time quantitative PCR (qPCR) was performed on the Light Cycler 480 II system (Roche Diagnostics), using Roche reagents following manufacturers’ instructions. Relative expression was calculated with the ΔCt method in reference to the gene of the Glyceraldehyde 3-phosphate dehydrogenase C2, (GAPC2; AT1G13440) (Czechowski et al., 2005).

#### **ROS measurement by luminometry**

The flg22-induced ROS production was measured by luminometry as previously described (Sang & Macho, 2017). Briefly, 4mm leaf disks were harvested and placed in OptiPlate-96-well microplates (Perkin Elmer) containing 100 µl water and washed for 24 hours. In the case of pER8 transgenic lines, the water was supplemented with 5 µM β-estradiol or equivalent volume of DMSO for induction. Then, the washing medium was substituted by 100 µl of elicitation medium (20 µg/ml horseradish peroxidase (HRP); 100 µM luminol; and 50 nM (unless otherwise noted) flg22) and luminescence was measured every minute for 50 minutes on a GloMax Multimode Multiplate Reader (Promega).

#### **Protein extraction and detection by Western blot**



Four leaf disks (diameter: 8 mm) from transformed *N. benthamiana* plants were harvested and frozen in liquid nitrogen 24 to 48 hours post inoculation. Leaf disks were grinded and the proteins extracted in Laemmli buffer 2X at 95°C for 5 minutes. Immunodetection of proteins were performed by loading 5-15 µl of protein extract on precast gels SDS-PAGE (4-15%, Biorad). Migrated proteins were transferred to a nitrocellulose membrane by Transblot turbo (Biorad). Total proteins were evidenced with Ponceau S staining. GFP and CFP proteins were detected with anti-GFP-HRP antibody (1:3000, Roche).

#### **MAPKs activation immunoblot**

Three two-weeks-old *A. thaliana* plants for each treatment were frozen in liquid nitrogen and grinded using a tissue lyser and glass beads. The ground material was resuspended in 100 µL of protein isolation buffer containing 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM DTT (Sigma-Aldrich chemicals), protease inhibitor cocktail (Roche), 2 mM NaF, 2 mM Na<sub>3</sub>VO<sub>4</sub> 1% v/v Triton X-100. The suspension was centrifuged at 12,000 g for 15 min at 4°C and the supernatant was collected. Protein concentration was measured with Quick Start™ Bradford reagent (Bio-Rad) as manufacturers' instructions. Protein extracts were diluted in Laemmli buffer 2X containing 4% (w/v) SDS, 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 mM dithiothreitol (DTT). Samples were denatured at 95°C for 5 min. Equal amount of proteins were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes (Bio-Rad). Blots were blocked with 5% (w/v) BSA (Sigma-Aldrich) in TBST for 2h. Blots were incubated overnight at 4°C in primary antibody incubation buffer containing TBST, 5% (w/v) BSA (Sigma-Aldrich) and rabbit anti-phospho-p44/42 (Erk1/2)(Thr202/Tyr204) monoclonal antibody (Cell Signaling, #9101) at a dilution of 1:1,500. Blots were washed with TBST and incubated for 2h at 4°C in secondary antibody incubation buffer containing TBST, 5% (w/v) non-fat dry milk, 1:10,000 dilution of HRP-conjugated anti-rabbit IgG (Pierce, #31460). HRP activity was acquired with ECL kit (Bio-Rad) using the ChemiDoc imaging system (Bio-Rad).

#### **Suppressor screen**

Seeds from pER8-*xopAG* line were mutagenized with ethyl methanesulfonate (EMS) as previously described (Weigel & Glazebrook, 2002). Individually harvested M2 seeds (3264 lines) were sterilized with chlorine gas (Lindsey et al., 2017) and screened for suppression of the *xopAG*-mediated growth arrest on 5 µM β-estadiol and 30 mg/l pimarinol solid MS medium (Sigma). Lines insensitive to *xopAG* expression were selected and grown to produce M3 seeds

806 that were subsequently screened to validate insensitivity to *xopAG*. In the resultant lines the  
807 sequence and expression of *xopAG* was validated by Sanger sequencing and qPCR  
808 respectively.

## 809 **Microscopy**

810 CFP fluorescence was analyzed with a confocal laser scanning microscope (TCS SP8; Leica)  
811 using a x25 water immersion objective lens (numerical aperture 0.95; HCX PL APO CS2). CFP  
812 fluorescence was excited with the 458 nm ray line of the argon laser and recorded in one of the  
813 confocal channels in the 465-520 emission range. The images were acquired in the mode using  
814 Leica LAS X software (version 3.0).

## 815 **Supplementary material:**

816 Supplementary Figure S1. *In vitro* growth curve of *Xcc* 8004 wild type,  $\Delta xopAC$  and  $\Delta xopAC$ -  
817 *xopAG*.

818 Supplementary Figure S2. Allelism test for the suppressor lines of the phenotype induced by  
819 XopAG (*sxg*).

820 Supplementary Figure S3. Phenotype of BC x F1 lines in presence of  $\beta$ -estradiol.

821 Supplementary Table S1. Common putative targets of type III effectors (T3Es) from  
822 *Xanthomonas campestris* pv. *campestris* (*Xcc*) and *Ralstonia pseudosolanacearum* (*Rps*).

823 Supplementary Table S2. T3Es' transcript accumulation in pER8-*xopAG* and pER8-*ripO1*  
824 transgenic lines by RT-qPCR.

825 Supplementary Table S3. List of plant genes differentially expressed in response to XopAG.

826 Supplementary Table S4. List of plant genes differentially expressed in response to RipO1.

827 Supplementary Table S5. List of plant genes differentially expressed in response to XopAG and  
828 RipO1.

829 Supplementary Table S6. Color code of the functional groups classification.

830 Supplementary Table S7. Bacterial strains used in this work.

831 Supplementary Table S8. Plasmids used in this work.

832 Supplementary Table S9. Primers used in this work.

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## 834 Bibliography

- 835 Aerts, N., Pereira Mendes, M., & Van Wees, S. C. M. (2021). Multiple levels of crosstalk in  
836 hormone networks regulating plant defense. *The Plant Journal*, 105(2), 489–504.  
837 <https://doi.org/10.1111/tpj.15124>
- 838 Andrés, F., Kinoshita, A., Kalluri, N., Fernández, V., Falavigna, V. S., Cruz, T. M. D., Jang, S.,  
839 Chiba, Y., Seo, M., Mettler-Altmann, T., Huettel, B., & Coupland, G. (2020). The sugar  
840 transporter SWEET10 acts downstream of FLOWERING LOCUS T during floral transition of  
841 *Arabidopsis thaliana*. *BMC Plant Biology*, 20(1), 53. [https://doi.org/10.1186/s12870-020-](https://doi.org/10.1186/s12870-020-2266-0)  
842 2266-0
- 843 Bigeard, J., Colcombet, J., & Hirt, H. (2015). Signaling Mechanisms in Pattern-Triggered  
844 Immunity (PTI). *Molecular Plant*, 8(4), 521–539. <https://doi.org/10.1016/j.molp.2014.12.022>
- 845 Block, A., Guo, M., Li, G., Elowsky, C., Clemente, T. E., & Alfano, J. R. (2010). The  
846 *Pseudomonas syringae* type III effector HopG1 targets mitochondria, alters plant  
847 development and suppresses plant innate immunity. *Cellular Microbiology*, 12(3), 318–330.  
848 <https://doi.org/10.1111/j.1462-5822.2009.01396.x>
- 849 Büttner, D., & Bonas, U. (2010). Regulation and secretion of *Xanthomonas* virulence factors.  
850 *FEMS Microbiology Reviews*, 34(2), 107–133. [https://doi.org/10.1111/j.1574-](https://doi.org/10.1111/j.1574-6976.2009.00192.x)  
851 6976.2009.00192.x
- 852 Cai, X.-T., Xu, P., Zhao, P.-X., Liu, R., Yu, L.-H., & Xiang, C.-B. (2014). *Arabidopsis* ERF109  
853 mediates cross-talk between jasmonic acid and auxin biosynthesis during lateral root  
854 formation. *Nature Communications*, 5, 5833. <https://doi.org/10.1038/ncomms6833>
- 855 Cerutti, A., Jauneau, A., Auriac, M.-C., Lauber, E., Martinez, Y., Chiarenza, S., Leonhardt, N.,  
856 Berthomé, R., & Noël, L. D. (2017). Immunity at Cauliflower Hydathodes Controls Systemic  
857 Infection by *Xanthomonas campestris* pv *campestris*. *Plant Physiology*, 174(2), 700–716.  
858 <https://doi.org/10.1104/pp.16.01852>
- 859 Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J., & Kunkel, B. N. (2007).  
860 *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology.  
861 *Proceedings of the National Academy of Sciences*, 104(50), 20131–20136.  
862 <https://doi.org/10.1073/pnas.0704901104>
- 863 Choi, H.-S., Seo, M., & Cho, H.-T. (2018). Two TPL-Binding Motifs of ARF2 Are Involved in  
864 Repression of Auxin Responses. *Frontiers in Plant Science*, 9, 372.  
865 <https://doi.org/10.3389/fpls.2018.00372>
- 866 Chowdhury, S., Basu, A., & Kundu, S. (2017). Biotrophy-necrotrophy switch in pathogen evoke  
867 differential response in resistant and susceptible sesame involving multiple signaling  
868 pathways at different phases. *Scientific Reports*, 7(1), 17251. [https://doi.org/10.1038/s41598-](https://doi.org/10.1038/s41598-017-17248-7)  
869 017-17248-7
- 870 Clough, S. J., & Bent, A. F. (1998). Floral dip: A simplified method for *Agrobacterium* -mediated  
871 transformation of *Arabidopsis thaliana*. *The Plant Journal*, 16(6), 735–743.  
872 <https://doi.org/10.1046/j.1365-313x.1998.00343.x>

873 Coaker, G., Zhu, G., Ding, Z., Van Doren, S. R., & Staskawicz, B. (2006). Eukaryotic cyclophilin  
874 as a molecular switch for effector activation. *Molecular Microbiology*, 61(6), 1485–1496.  
875 <https://doi.org/10.1111/j.1365-2958.2006.05335.x>

876 Curtis, M. D., & Grossniklaus, U. (2003). A Gateway Cloning Vector Set for High-Throughput  
877 Functional Analysis of Genes in Planta. *Plant Physiology*, 133(2), 462–469.

878 Czechowski, T., Stitt, M., Altmann, T., Udvardi, M. K., & Scheible, W.-R. (2005). Genome-wide  
879 identification and testing of superior reference genes for transcript normalization in  
880 *Arabidopsis*. *Plant Physiology*, 139(1), 5–17. <https://doi.org/10.1104/pp.105.063743>

881 Denny, T. (2006). Plant pathogenic *Ralstonia* species. In S. S. Gnanamanickam (Ed.), *Plant-*  
882 *Associated Bacteria* (pp. 573–644). Springer Netherlands. [https://doi.org/10.1007/978-1-](https://doi.org/10.1007/978-1-4020-4538-7_16)  
883 4020-4538-7\_16

884 Dickman, M. B., & Fluhr, R. (2013). Centrality of host cell death in plant-microbe interactions.  
885 *Annual Review of Phytopathology*, 51, 543–570. [https://doi.org/10.1146/annurev-phyto-](https://doi.org/10.1146/annurev-phyto-081211-173027)  
886 081211-173027

887 Dow, J. M., & Daniels, M. J. (1994). Pathogenicity Determinants and Global Regulation of  
888 Pathogenicity of *Xanthomonas campestris* pv. *Campestris*. In J. L. Dangi (Ed.), *Bacterial*  
889 *Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms* (pp. 29–41).  
890 Springer. [https://doi.org/10.1007/978-3-642-78624-2\\_2](https://doi.org/10.1007/978-3-642-78624-2_2)

891 Ellis, C. M., Nagpal, P., Young, J. C., Hagen, G., Guilfoyle, T. J., & Reed, J. W. (2005). AUXIN  
892 RESPONSE FACTOR1 and AUXIN RESPONSE FACTOR2 regulate senescence and floral  
893 organ abscission in *Arabidopsis thaliana*. *Development*, 132(20), 4563–4574.  
894 <https://doi.org/10.1242/dev.02012>

895 Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic  
896 pathogens. *Annual Review of Phytopathology*, 43, 205–227.  
897 <https://doi.org/10.1146/annurev.phyto.43.040204.135923>

898 Gochez, A. M., Shantharaj, D., Potnis, N., Zhou, X., Minsavage, G. V., White, F. F., Wang, N.,  
899 Hurlbert, J. C., & Jones, J. B. (2017). Molecular characterization of XopAG effector AvrGf2  
900 from *Xanthomonas fuscans* ssp. *Aurantifolii* in grapefruit. *Molecular Plant Pathology*, 18(3),  
901 405–419. <https://doi.org/10.1111/mpp.12408>

902 González-Fuente, M., Carrère, S., Monachello, D., Marsella, B. G., Cazalé, A.-C., Zischek, C.,  
903 Mitra, R. M., Rezé, N., Cottret, L., Mukhtar, M. S., Lurin, C., Noël, L. D., & Peeters, N. (2020).  
904 EffectorK, a comprehensive resource to mine for *Ralstonia*, *Xanthomonas*, and other  
905 published effector interactors in the *Arabidopsis* proteome. *Molecular Plant Pathology*,  
906 21(10), 1257–1270. <https://doi.org/10.1111/mpp.12965>

907 González-Lamothe, R., El Oirdi, M., Brisson, N., & Bouarab, K. (2012). The Conjugated Auxin  
908 Indole-3-Acetic Acid–Aspartic Acid Promotes Plant Disease Development[C][W]. *The Plant*  
909 *Cell*, 24(2), 762–777. <https://doi.org/10.1105/tpc.111.095190>

910 Huang, J., Wu, X., & Gao, Z. (2021). A nucleocytoplasmic-localized E3 ligase affects the NLR  
911 receptor stability. *Biochemical and Biophysical Research Communications*, 583, 1–6.  
912 <https://doi.org/10.1016/j.bbrc.2021.10.052>

- 913 Huang, S., Zhang, X., & Fernando, W. G. D. (2020). Directing Trophic Divergence in Plant-  
914 Pathogen Interactions: Antagonistic Phytohormones With NO Doubt? *Frontiers in Plant*  
915 *Science*, 11. <https://www.frontiersin.org/article/10.3389/fpls.2020.600063>
- 916 Hudson, D., Guevara, D., Yaish, M. W., Hannam, C., Long, N., Clarke, J. D., Bi, Y.-M., &  
917 Rothstein, S. J. (2011). GNC and CGA1 Modulate Chlorophyll Biosynthesis and Glutamate  
918 Synthase (GLU1/Fd-GOGAT) Expression in Arabidopsis. *PLOS ONE*, 6(11), e26765.  
919 <https://doi.org/10.1371/journal.pone.0026765>
- 920 Islam, M. T., Mamun, M. A., Lee, B.-R., La, V. H., Jung, W.-J., Bae, D.-W., & Kim, T.-H. (2021).  
921 Role of salicylic acid signaling in the biotrophy-necrotrophy transition of *Xanthomonas*  
922 *campestris* pv. *Campestris* infection in *Brassica napus*. *Physiological and Molecular Plant*  
923 *Pathology*, 113, 101578. <https://doi.org/10.1016/j.pmpp.2020.101578>
- 924 Islam, Md. T., Lee, B.-R., Park, S.-H., La, V. H., Bae, D.-W., & Kim, T.-H. (2017). Cultivar  
925 Variation in Hormonal Balance Is a Significant Determinant of Disease Susceptibility to  
926 *Xanthomonas campestris* pv. *Campestris* in *Brassica napus*. *Frontiers in Plant Science*, 8.  
927 <https://www.frontiersin.org/article/10.3389/fpls.2017.02121>
- 928 James, G. V., Patel, V., Nordström, K. J., Klasen, J. R., Salomé, P. A., Weigel, D., &  
929 Schneeberger, K. (2013). User guide for mapping-by-sequencing in Arabidopsis. *Genome*  
930 *Biology*, 14(6), R61. <https://doi.org/10.1186/gb-2013-14-6-r61>
- 931 Jiang, H., Song, W., Li, A., Yang, X., & Sun, D. (2011). Identification of genes differentially  
932 expressed in cauliflower associated with resistance to *Xanthomonas campestris* pv.  
933 *Campestris*. *Molecular Biology Reports*, 38(1), 621–629. [https://doi.org/10.1007/s11033-010-](https://doi.org/10.1007/s11033-010-0148-5)  
934 0148-5
- 935 Jupe, J., Stam, R., Howden, A. J., Morris, J. A., Zhang, R., Hedley, P. E., & Huitema, E. (2013).  
936 *Phytophthora capsici*-tomato interaction features dramatic shifts in gene expression  
937 associated with a hemi-biotrophic lifestyle. *Genome Biology*, 14(6), R63.  
938 <https://doi.org/10.1186/gb-2013-14-6-r63>
- 939 Kakei, Y., Yamazaki, C., Suzuki, M., Nakamura, A., Sato, A., Ishida, Y., Kikuchi, R., Higashi, S.,  
940 Kokudo, Y., Ishii, T., Soeno, K., & Shimada, Y. (2015). Small-molecule auxin inhibitors that  
941 target YUCCA are powerful tools for studying auxin function. *The Plant Journal*, 84(4), 827–  
942 837. <https://doi.org/10.1111/tpj.13032>
- 943 Kawamura, Y., Takenaka, S., Hase, S., Kubota, M., Ichinose, Y., Kanayama, Y., Nakaho, K.,  
944 Klessig, D. F., & Takahashi, H. (2009). Enhanced defense responses in Arabidopsis induced  
945 by the cell wall protein fractions from *Pythium oligandrum* require SGT1, RAR1, NPR1 and  
946 JAR1. *Plant & Cell Physiology*, 50(5), 924–934. <https://doi.org/10.1093/pcp/pcp044>
- 947 Kim, J., Shiu, S.-H., Thoma, S., Li, W.-H., & Patterson, S. E. (2006). Patterns of expansion and  
948 expression divergence in the plant polygalacturonase gene family. *Genome Biology*, 7(9),  
949 R87. <https://doi.org/10.1186/gb-2006-7-9-r87>
- 950 Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular  
951 Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and*  
952 *Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>

- 953 Lee, S.-J., & Rose, J. K. C. (2010). Mediation of the transition from biotrophy to necrotrophy in  
954 hemibiotrophic plant pathogens by secreted effector proteins. *Plant Signaling & Behavior*,  
955 5(6), 769–772. <https://doi.org/10.4161/psb.5.6.11778>
- 956 Li, J., Zhang, K., Meng, Y., Hu, J., Ding, M., Bian, J., Yan, M., Han, J., & Zhou, M. (2018).  
957 Jasmonic acid/ethylene signaling coordinates hydroxycinnamic acid amides biosynthesis  
958 through ORA59 transcription factor. *The Plant Journal: For Cell and Molecular Biology*, 95(3),  
959 444–457. <https://doi.org/10.1111/tpj.13960>
- 960 Li, N., Han, X., Feng, D., Yuan, D., & Huang, L.-J. (2019). Signaling Crosstalk between Salicylic  
961 Acid and Ethylene/Jasmonate in Plant Defense: Do We Understand What They Are  
962 Whispering? *International Journal of Molecular Sciences*, 20(3), 671.  
963 <https://doi.org/10.3390/ijms20030671>
- 964 Lindsey, B. E., Rivero, L., Calhoun, C. S., Grotewold, E., & Brkljacic, J. (2017). Standardized  
965 Method for High-throughput Sterilization of Arabidopsis Seeds. *Journal of Visualized*  
966 *Experiments: JoVE*, 128, 56587. <https://doi.org/10.3791/56587>
- 967 Llorente, F., Muskett, P., Sánchez-Vallet, A., López, G., Ramos, B., Sánchez-Rodríguez, C.,  
968 Jordá, L., Parker, J., & Molina, A. (2008). Repression of the auxin response pathway  
969 increases Arabidopsis susceptibility to necrotrophic fungi. *Molecular Plant*, 1(3), 496–509.  
970 <https://doi.org/10.1093/mp/ssn025>
- 971 Louvet, R., Cavel, E., Gutierrez, L., Guénin, S., Roger, D., Gillet, F., Guerineau, F., & Pelloux, J.  
972 (2006). Comprehensive expression profiling of the pectin methylesterase gene family during  
973 silique development in *Arabidopsis thaliana*. *Planta*, 224(4), 782–791.  
974 <https://doi.org/10.1007/s00425-006-0261-9>
- 975 Luo, H., Laluk, K., Lai, Z., Veronese, P., Song, F., & Mengiste, T. (2010). The Arabidopsis  
976 Botrytis Susceptible1 Interactor Defines a Subclass of RING E3 Ligases That Regulate  
977 Pathogen and Stress Responses. *Plant Physiology*, 154(4), 1766–1782.  
978 <https://doi.org/10.1104/pp.110.163915>
- 979 Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier,  
980 V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant  
981 pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, 13(6), 614–629.  
982 <https://doi.org/10.1111/j.1364-3703.2012.00804.x>
- 983 Mengiste, T. (2012). Plant immunity to necrotrophs. *Annual Review of Phytopathology*, 50, 267–  
984 294. <https://doi.org/10.1146/annurev-phyto-081211-172955>
- 985 Nagpal, P., Ellis, C. M., Weber, H., Ploense, S. E., Barkawi, L. S., Guilfoyle, T. J., Hagen, G.,  
986 Alonso, J. M., Cohen, J. D., Farmer, E. E., Ecker, J. R., & Reed, J. W. (2005). Auxin  
987 response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation.  
988 *Development (Cambridge, England)*, 132(18), 4107–4118. <https://doi.org/10.1242/dev.01955>
- 989 Pandey, D., Rajendran, S. R. C. K., Gaur, M., Sajeesh, P. K., & Kumar, A. (2016). Plant  
990 Defense Signaling and Responses Against Necrotrophic Fungal Pathogens. *Journal of Plant*  
991 *Growth Regulation*, 35(4), 1159–1174. <https://doi.org/10.1007/s00344-016-9600-7>

992 Park, J., Nguyen, K. T., Park, E., Jeon, J.-S., & Choi, G. (2013). DELLA Proteins and Their  
 993 Interacting RING Finger Proteins Repress Gibberellin Responses by Binding to the  
 994 Promoters of a Subset of Gibberellin-Responsive Genes in Arabidopsis[C][W]. *The Plant*  
 995 *Cell*, 25(3), 927–943. <https://doi.org/10.1105/tpc.112.108951>

996 Peng, Y., Yang, J., Li, X., & Zhang, Y. (2021). Salicylic Acid: Biosynthesis and Signaling. *Annual*  
 997 *Review of Plant Biology*, 72, 761–791. [https://doi.org/10.1146/annurev-arplant-081320-](https://doi.org/10.1146/annurev-arplant-081320-092855)  
 998 092855

999 Pradhan, A., Ghosh, S., Sahoo, D., & Jha, G. (2021). Fungal effectors, the double edge sword  
 1000 of phytopathogens. *Current Genetics*, 67(1), 27–40. [https://doi.org/10.1007/s00294-020-](https://doi.org/10.1007/s00294-020-01118-3)  
 1001 01118-3

1002 Provart, N., & Zhu, T. (2003). A Browser-based Functional Classification SuperViewer for  
 1003 Arabidopsis Genomics. *Currents in Computational Molecular Biology*, 2003.

1004 Qi, H., Li, J., Xia, F.-N., Chen, J.-Y., Lei, X., Han, M.-Q., Xie, L.-J., Zhou, Q.-M., & Xiao, S.  
 1005 (2020). Arabidopsis SINAT Proteins Control Autophagy by Mediating Ubiquitylation and  
 1006 Degradation of ATG13[OPEN]. *The Plant Cell*, 32(1), 263–284.  
 1007 <https://doi.org/10.1105/tpc.19.00413>

1008 Qi, L., Yan, J., Li, Y., Jiang, H., Sun, J., Chen, Q., Li, H., Chu, J., Yan, C., Sun, X., Yu, Y., Li, C.,  
 1009 & Li, C. (2012). *Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and  
 1010 transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. *The*  
 1011 *New Phytologist*, 195(4), 872–882. <https://doi.org/10.1111/j.1469-8137.2012.04208.x>

1012 Rajarammohan, S. (2021). Redefining Plant-Necrotroph Interactions: The Thin Line Between  
 1013 Hemibiotrophs and Necrotrophs. *Frontiers in Microbiology*, 12.  
 1014 <https://www.frontiersin.org/article/10.3389/fmicb.2021.673518>

1015 Reeves, P. H., Ellis, C. M., Ploense, S. E., Wu, M.-F., Yadav, V., Tholl, D., Chételat, A., Haupt,  
 1016 I., Kennerley, B. J., Hodgens, C., Farmer, E. E., Nagpal, P., & Reed, J. W. (2012). A  
 1017 Regulatory Network for Coordinated Flower Maturation. *PLOS Genetics*, 8(2), e1002506.  
 1018 <https://doi.org/10.1371/journal.pgen.1002506>

1019 Richter, R., Behringer, C., Zourelidou, M., & Schwechheimer, C. (2013). Convergence of auxin  
 1020 and gibberellin signaling on the regulation of the GATA transcription factors GNC and GNL in  
 1021 *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences*, 110(32), 13192–  
 1022 13197. <https://doi.org/10.1073/pnas.1304250110>

1023 Roux, B., Bolot, S., Guy, E., Denancé, N., Lautier, M., Jardinaud, M.-F., Fischer-Le Saux, M.,  
 1024 Portier, P., Jacques, M.-A., Gagnevin, L., Pruvost, O., Lauber, E., Arlat, M., Carrère, S.,  
 1025 Koebnik, R., & Noël, L. D. (2015). Genomics and transcriptomics of *Xanthomonas campestris*  
 1026 species challenge the concept of core type III effectome. *BMC Genomics*, 16(1), 975.  
 1027 <https://doi.org/10.1186/s12864-015-2190-0>

1028 Sabbagh, C. R. R., Carrere, S., Lonjon, F., Vailleau, F., Macho, A. P., Genin, S., & Peeters, N.  
 1029 (2019). Pangenomic type III effector database of the plant pathogenic *Ralstonia* spp. *PeerJ*,  
 1030 7, e7346. <https://doi.org/10.7717/peerj.7346>

- 1031 Sang, Y., & Macho, A. P. (2017). Analysis of PAMP-Triggered ROS Burst in Plant Immunity.  
1032 *Methods in Molecular Biology (Clifton, N.J.)*, 1578, 143–153. [https://doi.org/10.1007/978-1-](https://doi.org/10.1007/978-1-4939-6859-6_11)  
1033 4939-6859-6\_11
- 1034 Singh, H., Kaur, K., Singh, M., Kaur, G., & Singh, P. (2020). Plant Cyclophilins: Multifaceted  
1035 Proteins With Versatile Roles. *Frontiers in Plant Science*, 11, 1558.  
1036 <https://doi.org/10.3389/fpls.2020.585212>
- 1037 Steinbrener, A. D., Goritschnig, S., Krasileva, K. V., Schreiber, K. J., & Staskawicz, B. J.  
1038 (2012). Effector Recognition and Activation of the *Arabidopsis thaliana* NLR Innate Immune  
1039 Receptors. *Cold Spring Harbor Symposia on Quantitative Biology*, 77, 249–257.  
1040 <https://doi.org/10.1101/sqb.2012.77.014860>
- 1041 Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T.,  
1042 Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). The STRING  
1043 database in 2021: Customizable protein-protein networks, and functional characterization of  
1044 user-uploaded gene/measurement sets. *Nucleic Acids Research*, 49(D1), D605–D612.  
1045 <https://doi.org/10.1093/nar/gkaa1074>
- 1046 Uhse, S., & Djamei, A. (2018). Effectors of plant-colonizing fungi and beyond. *PLOS Pathogens*,  
1047 14(6), e1006992. <https://doi.org/10.1371/journal.ppat.1006992>
- 1048 Wang, G., Roux, B., Feng, F., Guy, E., Li, L., Li, N., Zhang, X., Lautier, M., Jardinaud, M.-F.,  
1049 Chabannes, M., Arlat, M., Chen, S., He, C., Noël, L. D., & Zhou, J.-M. (2015). The Decoy  
1050 Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-  
1051 Self Recognition and Immunity in Plants. *Cell Host & Microbe*, 18(3), 285–295.  
1052 <https://doi.org/10.1016/j.chom.2015.08.004>
- 1053 Weigel, D., & Glazebrook, J. (2002). *Arabidopsis: A laboratory manual*. Cold Spring Harbor  
1054 Laboratory Press.
- 1055 Yan, X., Tao, J., Luo, H.-L., Tan, L.-T., Rong, W., Li, H.-P., & He, C.-Z. (2019). A type III effector  
1056 XopLXcc8004 is vital for *Xanthomonas campestris* pathovar *campestris* to regulate plant  
1057 immunity. *Research in Microbiology*, 170(3), 138–146.  
1058 <https://doi.org/10.1016/j.resmic.2018.12.001>
- 1059 Yang, D.-L., Yang, Y., & He, Z. (2013). Roles of Plant Hormones and Their Interplay in Rice  
1060 Immunity. *Molecular Plant*, 6(3), 675–685. <https://doi.org/10.1093/mp/sst056>
- 1061 Yang, J., Duan, G., Li, C., Liu, L., Han, G., Zhang, Y., & Wang, C. (2019). The Crosstalks  
1062 Between Jasmonic Acid and Other Plant Hormone Signaling Highlight the Involvement of  
1063 Jasmonic Acid as a Core Component in Plant Response to Biotic and Abiotic Stresses.  
1064 *Frontiers in Plant Science*, 10. <https://www.frontiersin.org/article/10.3389/fpls.2019.01349>
- 1065 Zarei, A., Körbes, A. P., Younessi, P., Montiel, G., Champion, A., & Memelink, J. (2011). Two  
1066 GCC boxes and AP2/ERF-domain transcription factor ORA59 in jasmonate/ethylene-  
1067 mediated activation of the PDF1.2 promoter in *Arabidopsis*. *Plant Molecular Biology*, 75(4–5),  
1068 321–331. <https://doi.org/10.1007/s11103-010-9728-y>
- 1069 Zubo, Y. O., Blakley, I. C., Franco-Zorrilla, J. M., Yamburenko, M. V., Solano, R., Kieber, J. J.,  
1070 Loraine, A. E., & Schaller, G. E. (2018). Coordination of Chloroplast Development through



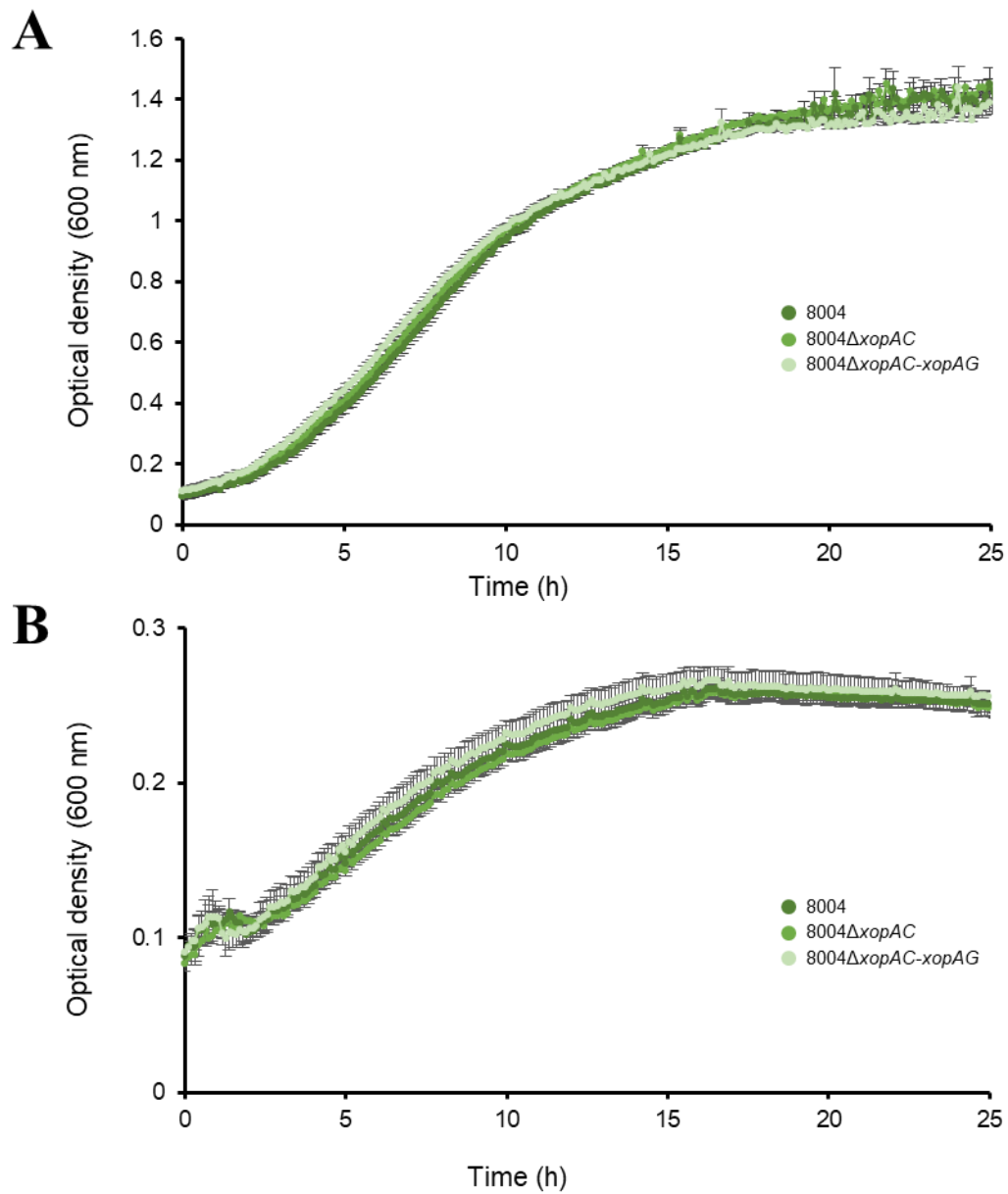
1071 the Action of the GNC and GLK Transcription Factor Families. *Plant Physiology*, 178(1),  
1072 130–147. <https://doi.org/10.1104/pp.18.00414>

1073 Zuo, J., Niu, Q. W., & Chua, N. H. (2000). Technical advance: An estrogen receptor-based  
1074 transactivator XVE mediates highly inducible gene expression in transgenic plants. *The Plant*  
1075 *Journal: For Cell and Molecular Biology*, 24(2), 265–273. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-313x.2000.00868.x)  
1076 313x.2000.00868.x

1077

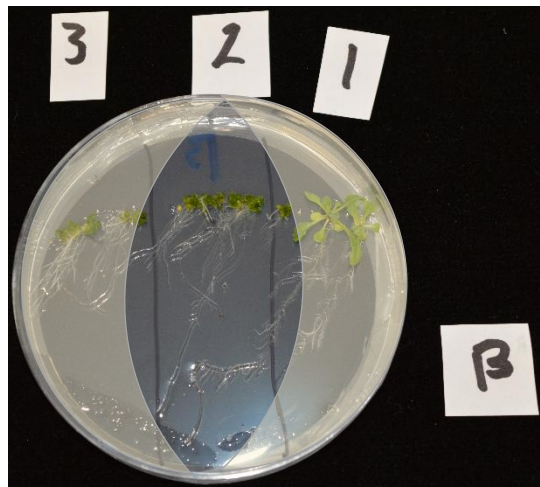
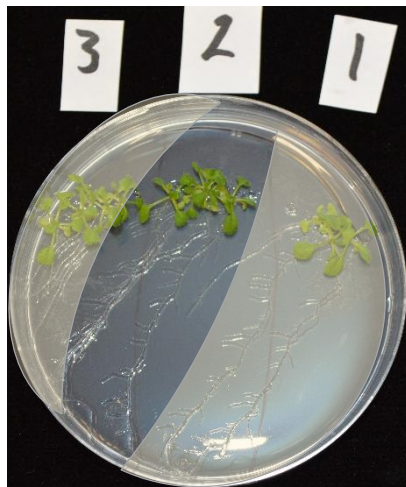
### 3.1 Supplementary material

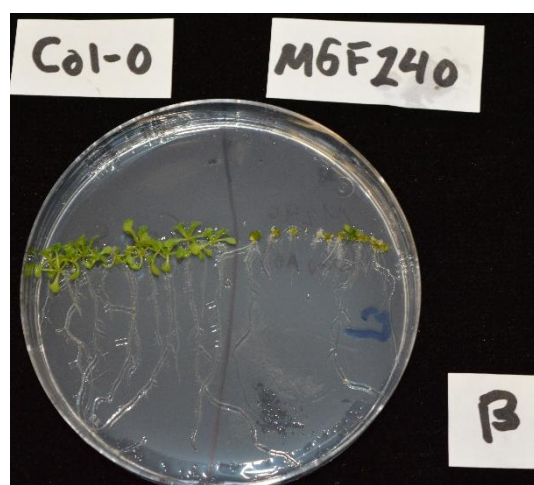
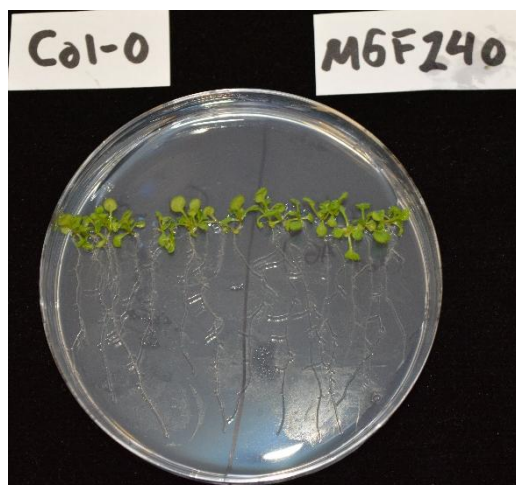
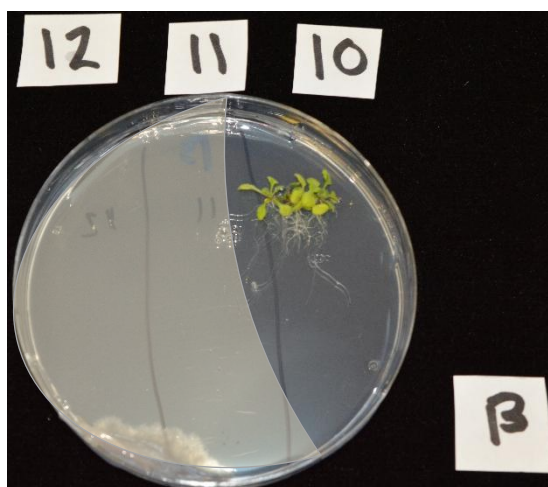
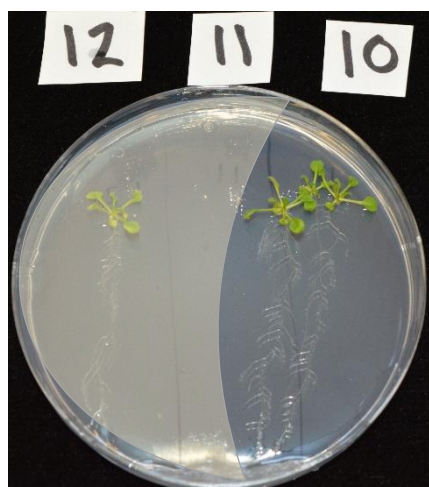
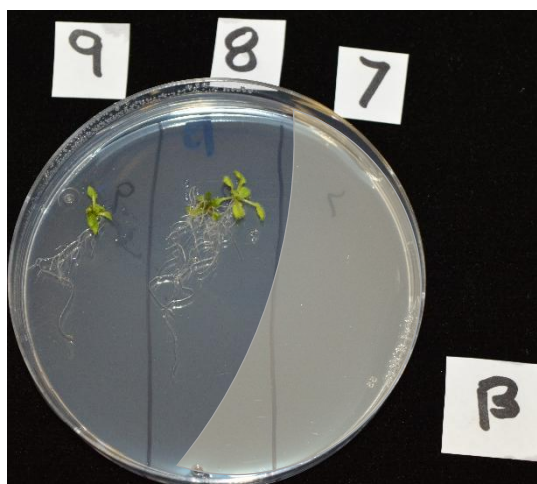
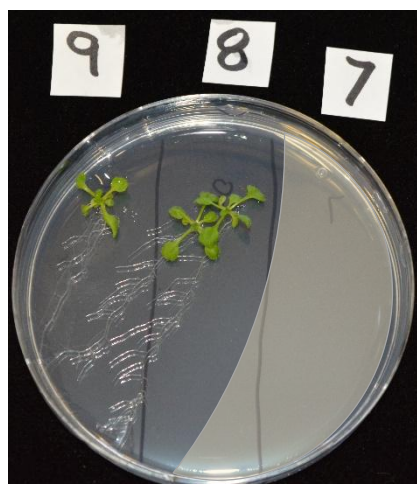
**Supplementary Figure S1. *In vitro* growth curve of *Xcc* 8004 wild type,  $\Delta xopAC$  and  $\Delta xopAC$ -*xopAG*.** Bacterial growth measured spectrophotometrically (optical density at a wavelength of 600 nm) over 25 hours on Moka rich medium (A) and MME minimal medium (B). Each point represents the mean of eight replicates coming from two independent bacterial pre-cultures. Error bars indicate the standard error of the mean.



**Supplementary Figure S2. Allelism test for the suppressor lines of the phenotype induced by XopAG (sxg).** Plants grown for two weeks in MS solid medium supplemented with either  $\beta$ -estradiol (5 $\mu$ M) or the solvent Dimethyl sulfoxide (DMSO).

Lines crossed	Phenotype + $\beta$ est.	ID.
<i>sxg6</i> x <i>sxg5</i>	Affected	2
<i>sxg139</i> x <i>sxg76</i>	Suppressor	5
<i>sxg137</i> x <i>sxg76</i>	Suppressor	6
<i>sxg84</i> x <i>sxg75</i>	Suppressor	8
<i>sxg78</i> x <i>sxg137</i>	Suppressor	9
<i>sxg84</i> x <i>sxg139</i>	Suppressor	10
<i>sxg6</i> x <i>sxg137</i>	Affected	16

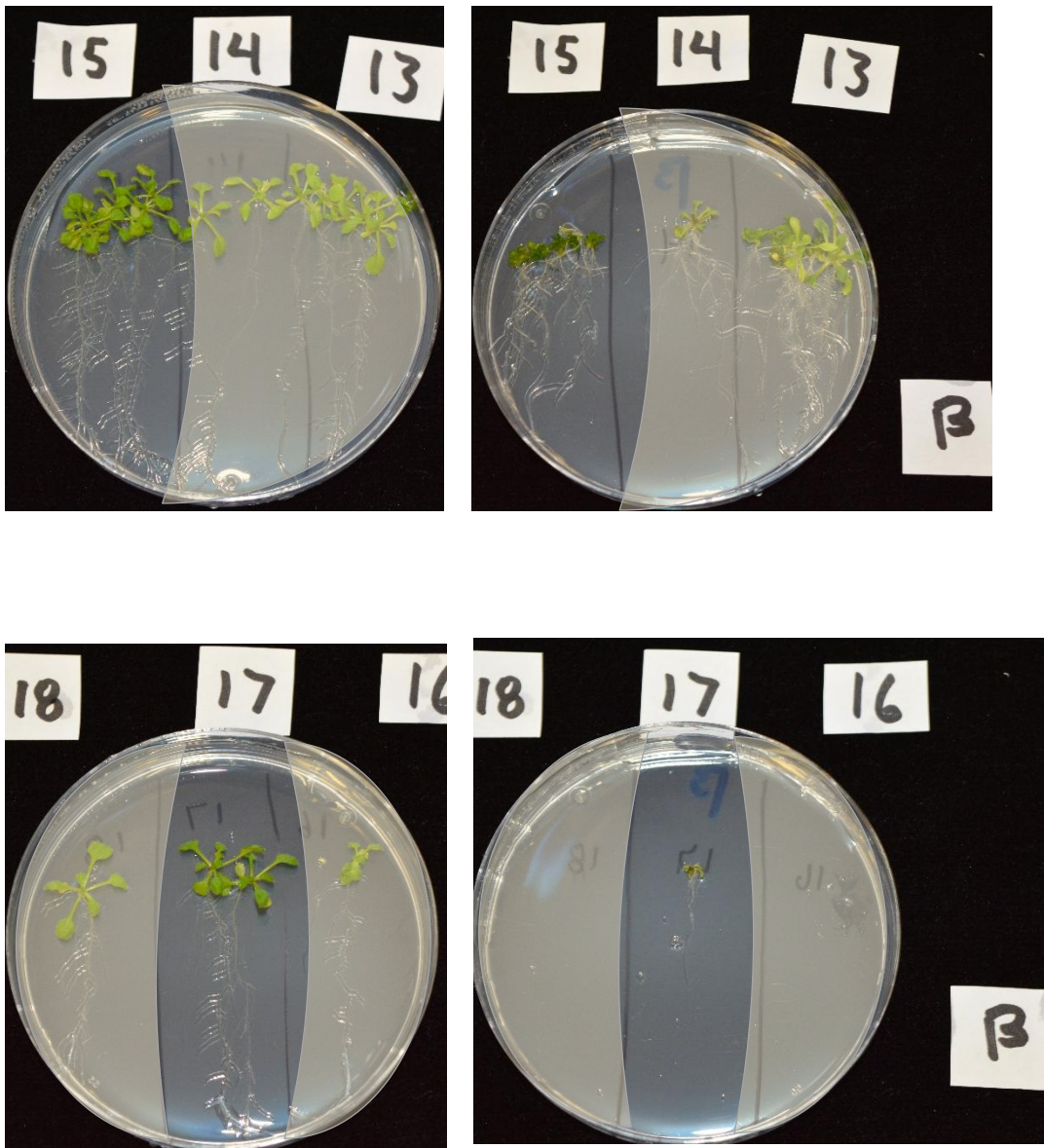






**Supplementary Figure S3. Phenotype of BC x F1 lines in presence of  $\beta$ -estradiol.** Plants grown for two weeks in MS solid medium supplemented with either  $\beta$ -estradiol (5 $\mu$ M) or the solvent Dimethyl sulfoxide (DMSO).

Lines crossed	Phenotype + $\beta$ est.	ID.
<i>sxg137</i> x pER8- <i>xopAG</i>	Affected	17
<i>sxg75</i> x pER8- <i>xopAG</i>	Affected	15



Supplementary Table S1. Common putative targets of type III effectors (T3Es) from *Xanthomonas campestris* pv. *campestris* (*Xcc*) and *Ralstonia pseudopseudosolanacearum* (*Rps*).

Accession	Gene symbol and description	Degree <sup>a</sup>	Interacting Xop(s) <sup>b</sup>	Interacting Rip(s) <sup>b</sup>	Other interacting effector(s) <sup>c</sup>	Immune phenotype <sup>d</sup>	Reference(s)
AT1G13320	(PP2AA3) Protein phosphatase 2A subunit A3	3	XopAC, XopJ	RipAJ	-	-	-
AT1G22920	(CSN5A) COP9 signalosome 5A	37	XopAC	RipAJ, <b>Rip01</b>	( <i>Atu</i> ) VirE3, ( <i>Hpa</i> ) ATR1, ATR13, HARXL10, HaRxL145, HARXL16, HARXL62, HARXL68, HARXL69, HARXLL108, HARXLL445, HARXLL445_A, HARXLL445_B, HaRxLL518, ( <i>Psy</i> ) AvrB1, AvrB2, AvrB4-1, AvrPto1, AvrPto5, AvrRpm1, HopAO1, HopAR1, HopAT1, HopBF1, HopF3, HopO1-2, HopP1, ( <i>Gor</i> ) OEC21, OEC25, OEC61, OEC67, OEC70, OEC71, OEC78, OEC85, OEC89	EDR to <i>Hpa</i> .	Mukhtar et al. (2011)
AT1G25490	(RCN1) Roots curl in NPA 1	7	XopAC	RipAJ	( <i>Hpa</i> ) ATR1, HaRxL145, HARXL16, ( <i>Gor</i> ) OEC119, OEC70	Impaired in stomatal closure.	Saito et al. (2008)
AT1G71230	(CSN5B) COP9 signalosome 5B	8	AvrBs1, XopAC, <b>XopG</b> , XopK	RipAJ, RipAM, RipBJ, <b>Rip01</b>	-	-	-
AT2G45680	(TCP9) TCP domain protein 9	15	XopAL1, XopK	RipAE, RipAJ, RipAK, RipAW, RipG4, <b>Rip01</b> , RipP2	( <i>Gor</i> ) OEC27, OEC39, OEC49, OEC67, OEC70, OEC76	EDS to <i>Psy</i> .	Wang et al. (2015)
AT3G08530	(CHC2) Clathrin heavy chain 2	11	XopL, XopR	RipAD, RipAE, RipP2	( <i>Hpa</i> ) ATR13, HARXL73, HaRxLL515, ( <i>Gor</i> ) OEC45, OEC63	EDR to <i>Gci</i> .	Wu et al. (2015)
AT3G12920	(BRG3) BOI-related gene 3	7	XopAC, <b>XopAG</b> , XopAL1	RipA1, RipAE, <b>Rip01</b> , RipV1	-	EDS to <i>Bci</i> .	Luo et al. (2010)
AT3G25800	(PP2AA2) Protein phosphatase 2A subunit A2	3	XopAC	RipAJ	( <i>Gor</i> ) OEC119	-	-
AT3G27960	(KLCR2) Kinesin light chain-related 2	21	XopAC, <b>XopAG</b> , XopF, XopZ	RipA1, RipA2, RipA4, RipA5, RipAD, RipAE, RipG4, <b>Rip01</b> , RipS3, RipS6, RipV1	( <i>Hpa</i> ) ATR13, HARXL30, HARXL73, HARXL79, HARXLL60, ( <i>Psy</i> ) HopAB1	EDS to <i>Hpa</i> .	Mukhtar et al. (2011)
AT3G54000	TIP41-like protein	3	XopAL1	RipAK, <b>Rip01</b>	-	-	-
AT4G01090	Hypothetical protein	9	<b>XopAG</b> , XopR	RipA2, RipAE, RipP2, RipS3, RipV1	( <i>Hpa</i> ) HaRxLL515, ( <i>Gor</i> ) OEC45	-	-
AT4G09060	Hypothetical protein	3	XopK	RipAE, <b>Rip01</b>	-	-	-
AT4G17680	SBP (S-ribonuclease binding protein) family protein	16	XopAC, <b>XopAG</b> , XopAL1, <b>XopP</b>	RipAD, RipAE, RipAK, <b>Rip01</b>	( <i>Hpa</i> ) ATR1, ( <i>Psy</i> ) AvrB1, AvrB2, AvrB4-1, HopAB1, HopR1, AvrC, ( <i>Gor</i> ) OEC61	EDR to <i>Hpa</i> .	Mukhtar et al. (2011)
AT4G26660	Kinesin-like protein	2	XopR	RipAE	-	-	-
AT5G02020	(SIS) Salt induced serine rich	2	XopR	<b>Rip01</b>	-	-	-

AT5G08070	(TCP17) TCP domain protein 17	7	XopAC, XopK, XopR	RipAE, RipAJ, RipAK, <b>Rip01</b>	-	-	-
AT5G26720	Ubiquitin carboxyl-terminal hydrolase-like protein	4	XopAC, XopJ, XopK	RipAJ	-	-	-
AT5G51110	(SDIRIP1) SDIR1-interacting protein 1	3	XopK	<b>Rip01</b>	( <i>Hpa</i> ) ATR1	-	-
AT5G51440	(HSP23.5) HSP20-like chaperones superfamily protein	7	XopAC, XopAL1	RipAE, RipAK, RipAW, <b>RipH3</b> , <b>Rip01</b>	-	-	-

<sup>a</sup> Effector degree (number of interacting effector proteins) in EffectorK database.

<sup>b</sup> Effectors with orthology between *Xcc*<sub>8004</sub> and *Rps*<sub>GMI1000</sub> are highlighted in bold. When both orthologous effectors interact with a same Arabidopsis protein, the effectors are underlined and the full row is colored in grey.

<sup>c</sup> Other plant pathogen effectors interacting with the same putative target of *Xcc* and *Rps* effectors preceded by the species in brackets. *Atu*, *Agrobacterium tumefaciens*; *Gor*, *Glovinomyces orontii*; *Hpa*, *Hyaloperonospora arabidopsidis*; and *Psy*, *Pseudomonas syringae*.

<sup>d</sup> Published immune-related phenotypes characterized on Arabidopsis T-DNA insertion mutant lines. EDR, enhanced diseases resistance; EDS, enhanced disease susceptibility; *Bcy*, *Botrytis cinerea* and *Gci*, *Glovinomyces cichoracearum*.

Supplementary Table S2. T3Es' transcript accumulation in pER8-*xopAG* and pER8-*ripO1* transgenic lines by RT-qPCR.

Construct	Line	Cp (Transgene)	Cp ( <i>OXA1</i> )	Cp ( <i>GAPC2</i> )	$\Delta$ ct ( <i>Oxa1</i> )	$\Delta$ ct ( <i>GAPC2</i> )
pER8- <i>ripO1</i>	MGF185	21.8	28.3	21.4	90.51	0.76
pER8- <i>xopAG</i> a	MGF240	16.0	24.6	20.8	388.02	27.86

Supplementary Table S3. List of plant genes differentially expressed in response to XopAG (Color code in Supplementary Table S6)

ID	Annotation
DEGs up-regulated by XopAG	
At3g22370	AOX1A_ATAOX1A_AtHSR3_HSR3_alternative oxidase 1A
ATMG00160	COX2_cytochrome oxidase 2
At2g07698	ATPase, F1 complex, alpha subunit protein
ATMG00640	ORF25_hydrogen ion transporting ATP synthases, rotational mechanism;zinc ion binding
At1g77510	ATPDI6_ATPDIL1-2_PDI6_PDIL1-2_PDI-like 1-2
At1g04980	ATPDI10_ATPDIL2-2_PDI10_PDIL2-2_PDI-like 2-2
At3g06050	ATPRXIIIF_PRXIIF_peroxiredoxin IIF
At3g28180	ATCSLC04_ATCSLC4_CSLC04_CSLC4_Cellulose-synthase-like C4
At2g32530	ATCSLB03_ATCSLB3_CSLB03_cellulose synthase-like B3
At5g44130	FLA13_FASCICLIN-like arabinogalactan protein 13 precursor
At5g65390	AGP7_arabinogalactan protein 7
At5g06860	ATPGIP1_PGIP1_polygalacturonase inhibiting protein 1
At1g56710	PGL1_Pectin lyase-like superfamily protein
At3g23730	XTH16_xyloglucan endotransglucosylase/hydrolase 16
At3g45970	ATEXLA1_ATEXPL1_ATHEXP BETA 2.1_EXLA1_EXPL1_expansin-like A1
At4g14130	XTH15_XTR7_xyloglucan endotransglucosylase/hydrolase 15
At5g19730	Pectin lyase-like superfamily protein
At5g67488	other RNA
At5g60022	other RNA
At2g06002	other RNA
At1g31173	MIR167D_MIR167D; miRNA
At1g09421	other RNA
At4g08950	EXO_Phosphate-responsive 1 family protein
At5g20050	Protein kinase superfamily protein
At1g51790	Leucine-rich repeat protein kinase family protein
At1g51820	SIF4_Leucine-rich repeat protein kinase family protein
At4g04490	CRK36_cysteine-rich RLK (RECEPTOR-like protein kinase) 36
At4g31000	Calmodulin-binding protein
At3g18820	ATRAB7B_AtRabG1f_ATRABG3F_RAB71_RAB7B_RAB7B_RABG3F_RAB GTPase homolog G3F
At5g59840	AtRabE1b_Ras-related small GTP-binding family protein
At5g58350	WNK4_ZIK2_with no lysine (K) kinase 4
At5g41080	AtGDPD2_GDPD2_PLC-like phosphodiesterases superfamily protein
At4g26260	MIOX4_myoinositol oxygenase 4
At5g50460	secE/sec61-gamma protein transport protein
At1g59660	DRAL_Nucleoporin autopeptidase
At3g45010	scpl48_serine carboxypeptidase-like 48
At4g16563	Eukaryotic aspartyl protease family protein
At1g65040	AtHrd1B_Hrd1B_RING/U-box superfamily protein



At1g76390	AtPUB43_PUB43_ARM repeat superfamily protein
At1g14200	RING/U-box superfamily protein
At3g23880	F-box and associated interaction domains-containing protein
At1g43910	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At5g26740	Protein of unknown function (DUF300)
At5g54100	AtSLP2_SLP2_SPFH/Band 7/PHB domain-containing membrane-associated protein family
At1g23550	SR02_similar to RCD one 2
At4g14020	Rapid alkalization factor (RALF) family protein
At3g51540	
At1g15040	GAT_GAT1_2.1_Class I glutamine amidotransferase-like superfamily protein
At5g07080	HXXXD-type acyl-transferase family protein
At5g67470	ATFH6_FH6_formin homolog 6
At1g72790	hydroxyproline-rich glycoprotein family protein
At5g09530	PELPK1_PRP10_hydroxyproline-rich glycoprotein family protein
At3g50570	hydroxyproline-rich glycoprotein family protein
At5g48790	Domain of unknown function (DUF1995)
At5g11810	
At3g54880	
At4g25690	
At2g23790	Protein of unknown function (DUF607)
At1g59710	Protein of unknown function (DUF569)
At1g49470	Family of unknown function (DUF716)
At5g05190	EDR4_Protein of unknown function (DUF3133)
At2g41730	
At1g24095	Putative thiol-disulphide oxidoreductase DCC
At1g55980	FAD/NAD(P)-binding oxidoreductase family protein
At5g40690	
At2g25770	Polyketide cyclase/dehydrase and lipid transport superfamily protein
At1g63530	
At3g10320	MUCI21_MUM5_MUM5_Glycosyltransferase family 61 protein
At5g45630	Protein of unknown function, DUF584
At3g58150	Optic atrophy 3 protein (OPA3)
At2g23110	Late embryogenesis abundant protein, group 6
At4g12735	
At2g21640	
At1g71470	
At3g52340	ATSP2_SPP2_sucrose-6F-phosphate phosphohydrolase 2
At4g24450	At4g24450_ATGWD2_GWD3_PWD_phosphoglucan, water dikinase
At5g36220	CYP81D1_CYP91A1_cytochrome p450 81d1
At3g19270	CYP707A4_cytochrome P450, family 707, subfamily A, polypeptide 4
At3g14680	CYP72A14_cytochrome P450, family 72, subfamily A, polypeptide 14
At3g17790	ATACP5_ATPAP17_PAP17_purple acid phosphatase 17
At1g73680	ALPHA DOX2_alpha-DOX2_alpha dioxygenase
At1g60110	Mannose-binding lectin superfamily protein
At1g22360	AtUGT85A2_UGT85A2_UDP-glucosyl transferase 85A2
At5g02540	NAD(P)-binding Rossmann-fold superfamily protein
At5g14450	GDSL-like Lipase/Acylhydrolase superfamily protein
At3g14210	ESM1_epithiospecifier modifier 1
At3g03640	BGLU25_GLUC_beta glucosidase 25
At1g65610	ATGH9A2_AtKOR2_KOR2_Six-hairpin glycosidases superfamily protein
At5g62480	ATGSTU9_GST14_GST14B_GSTU9_glutathione S-transferase tau 9
At1g58190	AtRLP9_RLP9_receptor like protein 9
At3g50480	HR4_homolog of RPW8 4
At2g02130	LCR68_PDF2.3_low-molecular-weight cysteine-rich 68
At2g34930	disease resistance family protein / LRR family protein

At1g13700	PGL1_6-phosphogluconolactonase 1
At2g02810	ATUTR1_UTR1_UDP-galactose transporter 1
At4g25450	ABCB28_ATNAP8_NAP8_non-intrinsic ABC protein 8
At2g13610	ABCG5_ABC-2 type transporter family protein
At3g13080	ABCC3_ATMRP3_MRP3_MRP3_multidrug resistance-associated protein 3
At3g55130	ABCG19_ATWBC19_WBC19_white-brown complex homolog 19
At2g38940	ATPT2_PHT1;4_phosphate transporter 1;4
At1g29310	SecY protein transport family protein
At3g02250	RRT2_O-fucosyltransferase family protein
At3g25290	Auxin-responsive family protein
At2g23170	GH3.3_Auxin-responsive GH3 family protein
At2g03760	AtSOT1_AtSOT12_ATST1_AtSULT202A1_RAR047_SOT12_ST_ST1_SULT202A1_sulphotransferase 12
At1g22400	ATUGT85A1_UGT85A1_UDP-Glycosyltransferase superfamily protein
At4g26200	ACCS7_ACS7_ATACS7_1-amino-cyclopropane-1-carboxylate synthase 7
At2g38400	AGT3_alanine:glyoxylate aminotransferase 3
At5g38710	PDH2_Methylenetetrahydrofolate reductase family protein
At4g08870	ARGAH2_Arginase/deacetylase superfamily protein
At1g32870	ANAC013_ANAC13_NAC13_NAC domain protein 13
At5g18270	ANAC087_Arabidopsis NAC domain containing protein 87
At5g45830	ATDOG1_DOG1_GAAS5_GSQ5_delay of germination 1
At2g41380	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At5g20240	PI_K-box region and MADS-box transcription factor family protein
At5g50790	AtSWEET10_SWEET10_Nodulin MtN3 family protein
At1g09380	UMAMIT25_nodulin MtN21 /EamA-like transporter family protein
At3g13672	SINA2_SINAT6_TRAF-like superfamily protein
At2g31200	ADF6_ATADF6_actin depolymerizing factor 6
At1g74790	catalytics
At5g59890	ADF4_ATADF4_actin depolymerizing factor 4
At5g56350	Pyruvate kinase family protein
At1g23020	ATFR03_FRO3_ferric reduction oxidase 3
At5g17450	HIPP21_Heavy metal transport/detoxification superfamily protein
At3g14210	ESM1_epithiospecifier modifier 1
At2g39980	HXXXD-type acyl-transferase family protein
At1g32870	ANAC013_ANAC13_NAC13_NAC domain protein 13
At4g36900	DEAR4_RAP2.10_related to AP2 10
At2g01930	ATBPC1_BBR_BPC1_basic pentacysteine1
At3g01080	ATWRKY58_WRKY58_WRKY DNA-binding protein 58
At2g36080	ABS2_NGAL1_AP2/B3-like transcriptional factor family protein
At3g02150	A-PTF-1_bHLHb1_PTF1_TCP13_TFPD_plastid transcription factor 1
At4g03005	
DEGs down-regulated by XopAG	
At2g38390	Peroxidase superfamily protein
At3g49110	ATPCA_ATPRX33_PRX33_PRXCA_peroxidase CA
At1g71695	Peroxidase superfamily protein
At5g62350	Plant invertase/pectin methylesterase inhibitor superfamily protein
At2g39000	AtNAA70_Acyl-CoA N-acyltransferases (NAT) superfamily protein
At4g26140	BGAL12_beta-galactosidase 12
At1g15125	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At1g31710	Copper amine oxidase family protein
At3g43670	Copper amine oxidase family protein
At2g30860	ATGSTF7_ATGSTF9_GLUTTR_GSTF9_glutathione S-transferase PHI 9
At1g77690	LAX3_like AUX1 3
At1g06160	ERF59_ORA59_octadecanoid-responsive Arabidopsis AP2/ERF 59
At1g14920	GAI_RGA2_GRAS family transcription factor family protein
At4g23920	ATUGE2_UGE2_UDP-D-glucose/UDP-D-galactose 4-epimerase 2

At1g02640	ATBXL2_BXL2_beta-xylosidase 2
At4g03210	XTH9_xyloglucan endotransglucosylase/hydrolase 9
At2g37640	ATEXP3_ATEXPA3_ATHEXP ALPHA 1.9_EXP3_EXPA3_Barwin-like endoglucanases superfamily protein
At1g11580	ATPMEPCRA_PMEPCRA_methylesterase PCR A
At1g31230	AK-HSDH_AK-HSDH I_aspartate kinase-homoserine dehydrogenase i
At1g14810	semialdehyde dehydrogenase family protein
At5g09660	PMDH2_peroxisomal NAD-malate dehydrogenase 2
At5g61160	AACT1_ACT_anthocyanin 5-aromatic acyltransferase 1
At2g46250	myosin heavy chain-related
At4g14400	ACD6_ankyrin repeat family protein
At1g52190	AtNPF1.2_NPF1.2_NRT1.11_Major facilitator superfamily protein
At4g29140	ABS3_ADP1_ADS1_MATE efflux family protein
At3g54830	Transmembrane amino acid transporter family protein
At1g64780	AMT1;2_ATAMT1;2_ammonium transporter 1;2
At1g76530	PILS4_Auxin efflux carrier family protein
At1g58340	ABS4_BCD1_ZF14_ZRZ_MATE efflux family protein
At2g04040	ATDTX1_DTX1_TX1_MATE efflux family protein
At5g04590	SIR_sulfite reductase
At3g57080	DMS12_NRPE5_RPB5B_Eukaryotic rpb5 RNA polymerase subunit family protein
At1g75240	AtHB33_HB33_ZHD5_homeobox protein 33
At4g26150	CGA1_GATA22_GNL_cytokinin-responsive gata factor 1
At3g07940	Calcium-dependent ARF-type GTPase activating protein family
At5g23280	AtTCP7_TCP7_TCP family transcription factor
At1g59750	ARF1_auxin response factor 1
At5g62000	ARF1-BP_ARF2_AtARF2_HSS_ORE14_auxin response factor 2
At5g50450	HCP-like superfamily protein with MYND-type zinc finger
At1g09750	Eukaryotic aspartyl protease family protein
At5g46690	bHLH071_beta HLH protein 71
At2g36400	AtGRF3_GRF3_growth-regulating factor 3
At2g22840	AtGRF1_GRF1_growth-regulating factor 1
At1g04240	IAA3_SHY2_AUX/IAA transcriptional regulator family protein
At3g04730	IAA16_indoleacetic acid-induced protein 16
At4g29190	AtC3H49_AtOZF2_AtTZF3_OZF2_TZF3_Zinc finger C-x8-C-x5-C-x3-H type family protein
At2g15080	AtRLP19_RLP19_receptor like protein 19
At5g45060	Disease resistance protein (TIR-NBS-LRR class) family
At1g73330	ATDR4_DR4_drought-repressed 4
At5g36910	THI2.2_thionin 2.2
At3g22231	PCC1_pathogen and circadian controlled 1
At1g31580	CXC750_ECS1_ECS1
At4g19820	Glycosyl hydrolase family protein with chitinase insertion domain
At2g33050	AtRLP26_RLP26_receptor like protein 26
At5g44420	LCR77_PDF1.2_PDF1.2A_plant defensin 1.2
At2g42530	COR15B_cold regulated 15b
At1g70830	MLP28_MLP-like protein 28
At4g01593	other RNA
At5g14740	BETA CA2_CA18_CA2_carbonic anhydrase 2
At4g29080	IAA27_PAP2_phytochrome-associated protein 2
At2g43010	AtPIF4_PIF4_SRL2_phytochrome interacting factor 4
At3g46370	Leucine-rich repeat protein kinase family protein
At4g27300	S-locus lectin protein kinase family protein
At3g08510	ATPLC2_PLC2_phospholipase C 2
At3g11730	ATFP8_ATRABD1_RABD1_Ras-related small GTP-binding family protein
At2g16660	Major facilitator superfamily protein
At2g42840	PDF1_protodermal factor 1
At3g12670	CTPS2_emb2742_CTP synthase family protein

At2g40010	Ribosomal protein L10 family protein
At1g25260	Ribosomal protein L10 family protein
At4g09160	PATL5_SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein
At2g42960	Protein kinase superfamily protein
At3g01490	Protein kinase superfamily protein
At1g66940	protein kinase-related
At3g23000	ATSR2_ATSRPK1_CIPK7_PKS7_SnRK3.10_CBL-interacting protein kinase 7
At4g25110	AtMC2_AtMCP1c_MC2_MCP1c_metacaspase 2
At1g50380	Prolyl oligopeptidase family protein
At3g54720	AMP1_AtAMP1_COP2_HPT_MFO1_PT_Peptidase M28 family protein
At1g77480	Eukaryotic aspartyl protease family protein
At5g51750	ATSBT1.3_SBT1.3_subtilase 1.3
At4g11320	AtCP2_CP2_Papain family cysteine protease
At2g33770	ATUBC24_PHO2_UBC24_phosphate 2
At1g48280	hydroxyproline-rich glycoprotein family protein
At1g67400	ELMO/CED-12 family protein
At3g10840	alpha/beta-Hydrolases superfamily protein
At1g78460	SOUL heme-binding family protein
At5g57170	Tautomerase/MIF superfamily protein
At3g23080	Polyketide cyclase/dehydrase and lipid transport superfamily protein
At3g22235	AthCYSTM8
At5g42530	
At3g08030	Protein of unknown function, DUF642
At2g22122	
At5g16030	
At3g63160	OEP6_OEP7.2
At3g18050	
At3g01860	
At3g10120	
At5g11420	Protein of unknown function, DUF642
At5g53900	Serine/threonine-protein kinase WNK (With No Lysine)-related
At1g42430	ESV1
At5g25460	DGR2_Protein of unknown function, DUF642
At2g16650	PRORP2_proteinaceous RNase P 2
At2g30010	TBL45_TRICHOME BIREFRINGENCE-LIKE 45
At5g38980	
At1g53800	
At2g11910	
At4g01935	
At5g23380	Protein of unknown function (DUF789)
At1g05960	ARM repeat superfamily protein
At1g03600	PSB27_photosystem II family protein
At4g26530	AtFBA5_FBA5_Aldolase superfamily protein

Supplementary Table S4. List of plant genes differentially expressed in response to RipO1

ID	Annotation
DEG up-regulated by RipO1	
At3g60160	ABCC9_ATMRP9_MRP9_multidrug resistance-associated protein 9
At3g05400	Major facilitator superfamily protein
At4g25000	AMY1_ATAMY1_alpha-amylase-like
At5g36220	CYP81D1_CYP91A1_cytochrome p450 81d1
At3g14680	CYP72A14_cytochrome P450, family 72, subfamily A, polypeptide 14
At3g17790	ATACP5_ATPAP17_PAP17_purple acid phosphatase 17

At1g60110	Mannose-binding lectin superfamily protein
At1g22360	AtUGT85A2_UGT85A2__UDP-glucosyl transferase 85A2
At3g14210	ESM1__epithiospecifier modifier 1
At1g62580	NOGC1__Flavin-binding monooxygenase family protein
At2g34930	disease resistance family protein / LRR family protein
At2g02130	LCR68_PDF2.3__low-molecular-weight cysteine-rich 68
At3g45130	LAS1__lanosterol synthase 1
At3g14210	ESM1__epithiospecifier modifier 1
At3g25290	Auxin-responsive family protein
At2g23170	GH3.3__Auxin-responsive GH3 family protein
At3g45010	scpl48__serine carboxypeptidase-like 48
At1g43910	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At4g04460	AtPaspA3_PaspA3__Saposin-like aspartyl protease family protein
At4g14020	Rapid alkalization factor (RALF) family protein
At2g31560	Protein of unknown function (DUF1685)
At2g21640	
At1g23150	
At1g49470	Family of unknown function (DUF716)
At5g40690	
At2g06255	ELF4-L3__ELF4-like 3
At2g41230	ARL2__OSR1
At1g55980	FAD/NAD(P)-binding oxidoreductase family protein
At1g63530	
At3g10320	MUCI21_MUM5_MUM5__Glycosyltransferase family 61 protein
At1g02470	Polyketide cyclase/dehydrase and lipid transport superfamily protein
At1g03170	FAF2_FTM5__Protein of unknown function (DUF3049)
At1g71470	
At2g47190	ATMYB2_MYB2__myb domain protein 2
At5g43500	ARP9_ATARP9__actin-related protein 9
At1g32350	AOX1D__alternative oxidase 1D
At4g13345	MEE55__Serinc-domain containing serine and sphingolipid biosynthesis protein
At2g41380	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At4g01430	UMAMIT29__nodulin MtN21 /EamA-like transporter family protein
At1g52890	ANAC019_ANAC19_NAC019__NAC domain containing protein 19
At5g50790	AtSWEET10_SWEET10__Nodulin MtN3 family protein
At3g13672	SINA2_SINAT6__TRAF-like superfamily protein
At5g65390	AGP7__arabinogalactan protein 7
At1g56710	PGL1__Pectin lyase-like superfamily protein
At5g19730	Pectin lyase-like superfamily protein
At5g38710	PDH2__Methylenetetrahydrofolate reductase family protein
At4g02795	

#### DEG down-regulated by RipO1

At3g10120	
At4g13575	
At1g42430	ESV1
At1g53800	
At5g23250	Succinyl-CoA ligase, alpha subunit
At1g50380	Prolyl oligopeptidase family protein
At2g39850	Subtilisin-like serine endopeptidase family protein
At1g27370	SPL10__squamosa promoter binding protein-like 10
At4g26140	BGAL12__beta-galactosidase 12
At1g03600	PSB27__photosystem II family protein
At1g14150	PnsL2_PQL1_PQL2__PsbQ-like 2
At1g61300	LRR and NB-ARC domains-containing disease resistance protein
At5g36910	THI2.2__thionin 2.2

At1g66100	Plant thionin
At4g14400	ACD6_ankyrin repeat family protein
At4g26150	CGA1_GATA22_GNL_cytokinin-responsive gata factor 1
At5g50450	HCP-like superfamily protein with MYND-type zinc finger
At1g59750	ARF1_auxin response factor 1
At5g62000	ARF1-BP_ARF2_AtARF2_HSS_ORE14_auxin response factor 2
At4g29190	AtC3H49_AtOZF2_AtTZF3_OZF2_TZF3_Zinc finger C-x8-C-x5-C-x3-H type family protein
At1g75240	AtHB33_HB33_ZHD5_homeobox protein 33
At4g29080	IAA27_PAP2_phytochrome-associated protein 2
At3g46370	Leucine-rich repeat protein kinase family protein
At1g11280	S-locus lectin protein kinase family protein

Supplementary Table S5. List of plant genes differentially expressed in response to XopAG and RipO1

ID	Annotation
Plant genes up-regulated by XopAG and RipO1	
At4g14020	Rapid alkalization factor (RALF) family protein
At1g71470	
At1g63530	
At2g21640	
At1g55980	FAD/NAD(P)-binding oxidoreductase family protein
At5g40690	
At3g10320	MUCI21_MUM5_MUM5_Glycosyltransferase family 61 protein
At1g49470	Family of unknown function (DUF716)
At3g14210	ESM1_epithiospecifier modifier 1
At3g13672	SINA2_SINAT6_TRAF-like superfamily protein
At5g50790	AtSWEET10_SWEET10_Nodulin MtN3 family protein
At2g41380	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
At5g65390	AGP7_arabinogalactan protein 7
At1g56710	PGL1_Pectin lyase-like superfamily protein
At5g19730	Pectin lyase-like superfamily protein
At5g38710	PDH2_Methylenetetrahydrofolate reductase family protein
At3g25290	Auxin-responsive family protein
At2g23170	GH3.3_Auxin-responsive GH3 family protein
At1g43910	P-loop containing nucleoside triphosphate hydrolases superfamily protein
At3g45010	scpl48_serine carboxypeptidase-like 48
At5g36220	CYP81D1_CYP91A1_cytochrome p450 81d1
At3g14680	CYP72A14_cytochrome P450, family 72, subfamily A, polypeptide 14
At3g17790	ATACP5_ATPAP17_PAP17_purple acid phosphatase 17
At1g60110	Mannose-binding lectin superfamily protein
At1g22360	AtUGT85A2_UGT85A2_UDP-glucosyl transferase 85A2
At3g14210	ESM1_epithiospecifier modifier 1
At2g34930	disease resistance family protein / LRR family protein
At2g02130	LCR68_PDF2.3_low-molecular-weight cysteine-rich 68
Plant genes down-regulated by XopAG and RipO1	
At5g36910	THI2.2_thionin 2.2

At4g29080	IAA27_PAP2__phytochrome-associated protein 2
At3g46370	Leucine-rich repeat protein kinase family protein
At1g50380	Prolyl oligopeptidase family protein
At4g14400	ACD6__ankyrin repeat family protein
At1g75240	AtHB33_HB33_ZHD5__homeobox protein 33
At4g26150	CGA1_GATA22_GNL__cytokinin-responsive gata factor 1
At4g29190	AtC3H49_AtOZF2_AtTZF3_OZF2_TZF3__Zinc finger C-x8-C-x5-C-x3-H type family protein
At5g50450	HCP-like superfamily protein with MYND-type zinc finger
At5g62000	ARF1-BP_ARF2_AtARF2_HSS_ORE14__auxin response factor 2
At1g59750	ARF1__auxin response factor 1
At4g26140	BGAL12__beta-galactosidase 12
At1g03600	PSB27__photosystem II family protein
At1g42430	ESV1
At1g53800	
At3g10120	

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Supplementary Table S6. Color code of the functional groups classification

Color	Related function
	amino acid metabolism
	Biodegradation of Xenobiotics
	cell
	cell wall
	Co-factor and vitamine metabolism
	development
	DNA
	fermentation
	gluconeogenesis / glyoxylate cycle
	glycolysis
	hormone metabolism
	lipid metabolism
	major CHO metabolism
	metal handling
	micro RNA, natural antisense etc
	minor CHO metabolism
	misc
	mitochondrial electron transport / ATP synthesis
	not assigned
	nucleotide metabolism
	OPP
	protein
	PS
	redox
	RNA
	S-assimilation
	secondary metabolism
	signalling
	stress
	TCA / org transformation
	tetrapyrrole synthesis
	transport

Supplementary Table S7. Bacterial strains used in this work

Strain	Genotype	Resistance	Stock Id.
<i>Xanthomonas campestris</i> pv. <i>campestris</i> 8004	Wild type (WT)	Rif	MC40
<i>Xanthomonas campestris</i> pv. <i>campestris</i> 8004	$\Delta xopAC$	Rif	MC44
<i>Xanthomonas campestris</i> pv. <i>campestris</i> 8004	$\Delta xopAC\Delta xopAG$	Rif	MGF195
<i>Xanthomonas campestris</i> pv. <i>campestris</i> 8004	$\Delta xopAC\Delta xopAG::xopAG$	Rif	MGF284



Supplementary Table S8. Plasmids used in this work

Plasmid	Resistance	Stock Id.
pER8- <i>xopAG</i>	Rif/Gm/Spc	LN1313
pER8- <i>ripO1</i>	Rif/Gm/Spc	MGF42
pMDC43- <i>ranBP1</i>	Rif/Tc/Kan	NP120
pMDC43- <i>xopAG</i>	Rif/Tc/Kan	MGF182
pBIN- <i>xopAG</i> -CFP	Tc/Kan	MGF249
pBIN- <i>ripO1</i> -CFP	Tc/Kan	MGF244
pAM-PAT-35S-GFP	Gm/Cb	MGF283

Supplementary Table S9. Primers used in this work

Primer	Sequence (5' to 3')	Source	Stock Id.
GAPC2-Fw	AGGTCAAGCATTTTCGATGC	This work	MGF47
GAPC2-Rv	AACGATAAGGTCAACGACACG	This work	MGF48
<i>xopAG</i> -Fw	TCAAGCGTGC GTGGTTGATGC	This work	MGF40
<i>xopAG</i> -Rv	GCAGACTTCATGGAGATGGCG	This work	MGF41
<i>ripO1</i> -Fw	TCGGTCAATCCCTTCAGGCTG	This work	MGF51
<i>ripO1</i> -Rv	GGCACTGAACGTTGTTCGAC	This work	MGF23
AttB1-BRG3	GGGGACAAGTTTGTACAAAAAGCAGGCTTCATGGCCGTTGAAGCTCACC	This work	MGF229
AttB2-BRG3	GGGGACCACTTTGTACAAGAAAGCTGGGTCAGAGGAAAGATTAAACATGTAGAC	This work	MGF230
Before <i>xopAG</i> +BamHI	CGGAGGATCCCTGCAGCACACCTGGATCG	This work	MGF214
After <i>xopAG</i> +HindIII	GCCTAAGCTTCCAGGAAAAGGTGCCTAAAGACG	This work	MGF215
FRK1_Fw	ATCTTCGCTTGGAGCTTCTC	Boutrot et al. 2010 PNAS	MGF301
FRK1_Rv	TGCAGCGCAAGGACTAGAG	Boutrot et al. 2010 PNAS	MGF302
NHL10_Fw	TTCCTGTCCGTAACCCAAAC	Singh et al. 2014 Plant Cell	MGF303
NHL10_Rv	CCCTCGTAGTAGGCATGAGC	Singh et al. 2014 Plant Cell	MGF304
WRKY53_Fw	CACCAGAGTCAAACCAGCCATTAC	Singh et al. 2014 Plant Cell	MGF305
WRKY53_Rv	CTTTACCATCATCAAGCCCATCGG	Singh et al. 2014 Plant Cell	MGF306



## **Chapter 4: General discussion**



## 4.1 Context

In this study, we characterized some of the effects caused by individual T3Es in plant physiology. Similarities and differences in plant responses to such effectors provided hints about their individual and collective activities. The general implications of many of the results obtained during this work were discussed at the end of each chapter. Consequently, in this section, I will focus on the implications that emerge from the functional redundancy exhibited by the different T3Es, at interspecific and intraspecific levels. First, I will discuss the knowledge gained from the comparison of the activities *in planta* of two orthologous effectors from different hemibiotrophic pathogens. Then, I will discuss about the numerous *Xcc* T3Es with immune-triggering activities and how this may be compatible with *Xcc* pathogenicity. Finally, I will summarize in a model some of the knowledge gained through this work about the contribution of the T3Es to *Xcc* pathogenicity.

## 4.2 Do similar T3Es have similar functions *in planta*?

In this work, we identified that RipO1 effector of *Ralstonia pseudosolanacearum* presents a large extent of sequence similarity to the *Xcc* effector XopAG. Previous reports have shown orthologous T3Es with similar *in planta* functions. For instance, the XopQ of *Xanthomonas* sp and HopQ1 of *Pseudomonas* sp. are recognized by the tobacco R protein Roq1 (Schultink et al., 2017). Considering that both pathogens colonize vascular tissues and exhibit hemibiotrophic lifestyles, we hypothesized that the T3Es RipO1 and XopAG could exert similar *in planta* functions. Interestingly, the effects elicited by XopAG and RipO1 in *Arabidopsis* Col-0 plants were largely similar. For instance, both effectors had a negative impact on plant development, promoted the expression of genes related to early flowering, and repressed the expression of genes related to auxin-response and photosynthesis. Yet, XopAG, in contrast to RipO1 was important for virulence in *Arabidopsis*. Additionally, in all my experiments, RipO1 induced subtle effects as compared to XopAG. Subsequent qPCR analysis revealed differences in the level of expression of both effectors, which could explain the functional differences, though we cannot discard possible biological divergence. Despite this, RipO1 and XopAG showed many functional similarities suggesting a common origin for both T3Es. However, the differences in their induced responses in plant cells indicate a process of diversification and adaptation of the effector to the pathosystem that contains it. Our phylogenetic analysis indicated that the *xopAG/ripO1* gene family is widely distributed in both, rhizobium and pathogen species, suggesting that these T3Es play a relevant role during plant colonization. Among the *xopAG* orthologs analyzed in this work,

*ripO1* was phylogenetically distant. Surprisingly the effects caused *in planta* by both effectors were globally similar. This raises important questions such as “Do all the T3Es in the *xopAG/ripO1* gene family have similar functions? What is their relevance for the plant-microbe interactions? Do they have the same importance for pathogen species as for rhizobium species?”. These questions remain open for future research. The XopAG/RipO1 family is found in a wide variety of species with different lifestyles such as the pathogen *Xanthomonas phaseoli* or the rhizobial microbe *Paraburkholderia youngi*. Further evolutionary ecology approaches in these species could yield important insights about the functional diversification of the XopAG/RipO1 gene family due to pathoadaptation processes.

#### **4.3 Multiple Xcc T3Es redundantly activated plant immune responses.**

##### **4.3.1 Six Xcc T3Es enhance PTI responses**

During this work, we decided to evaluate the effects of Xcc T3Es on the activities of MAPK3 and MAPK6 due to their key signal transduction functions in PTI responses (Meng & Zhang, 2013). Six Xcc effectors enhanced the activation of such MAPK in response to flg22 but strikingly none of the Xcc T3Es activated MAPK by their own. Recent reports based mainly on the immunogenic activities of T3Es of *P. syringae*, proposing that ETI pathways participate predominantly as enhancers of PTI by promoting the expression of PTI-related genes, being the PTI components the main activators of plant immune responses (Ngou et al., 2021; Yuan et al., 2021). Our results are the first report of this phenomenon on T3Es of Xcc suggesting that the role of ETI as a PTI enhancer could be rather a general phenomenon in the plant response to T3Es and not specific to T3Es of certain species.

Importantly, flg22 is just one of the multiple MAMPs and DAMPs that activate plant PTI pathways and different PTI-elicitors could induce specific defense profiles (Martin et al., 2020). Additionally, Xcc strains usually do not present a form of the peptide flg22 recognized by FLS2. The fact that *XccΔhrcV* is unable to infect Arabidopsis plants (Cerutti et al., 2017) suggests the existence of MAMP/DAMP of Xcc that elicit plant basal defense. Plant immune pathways activated by the Xcc MAMP/DAMP might differ from those activated by flg22. Studying the effect of Xcc T3Es on the immune pathways activated by such MAMP/DAMP could reveal other unknown activities or pathways of great interest.

#### **4.3.2 Five *Xcc* T3Es promote the expression of immune-related genes.**

Our transcriptomic approaches revealed that five *Xcc* T3Es including XopAC promoted the expression of similar immune-related genes. XopAC has been extensively recognized as an ETI-triggering effector (avirulence protein). This suggests that the other four T3Es could also have avirulence activities. This cluster of immune-related genes will be interesting for future studies, as they could provide us with clues about the interplay between the ETI and PTI responses. For instance, CRK36 has been found to be associated *in vivo* with FLS2 and BIK1, enhancing the flg22-triggered phosphorylation of BIK1 (D. S. Lee et al., 2017). CRK13 overexpression leads to hypersensitive response-associated cell death, and induces defense against *P. syringae* by causing increased accumulation of salicylic acid which is characteristic of PTI activation (Acharya et al., 2007). Future studies are needed to test whether the overexpression of these genes enhances the flg22-triggered activities of the MAPK. Interestingly, the five immunogenic T3Es also induced the overexpression of MKK1 and MPK11. Furthermore, MEKK1 was overexpressed in response to XopAC, XopAH and AvrXccA1, suggesting that the signaling cascade formed by MEKK1, MKK1 and MPK11 play a relevant role in ETI. Strikingly, the transcript levels of MAPK3, MAPK6, MKK4 and MKK5 genes, which form a parallel signaling cascade, were not affected by any of the five immunogenic effectors. It is known that the signaling cascade formed by MAPK3/MAPK6 and MKK4/MKK5 is very important for PTI pathways (Meng & Zhang, 2013). Our results suggest that the signaling cascade formed by MEKK1, MKK1 and MPK11 is promoted by the ETI. However, whether and how these signaling cascades are related, as well as if this is relevant for plant disease resistance, is still unknown but rise exiting questions for further studies.

#### **4.4 Multiple avirulence T3Es in the virulent pathogen *Xcc* suggest that the collective properties of the T3 effectome are epistatic on individual T3E functions**

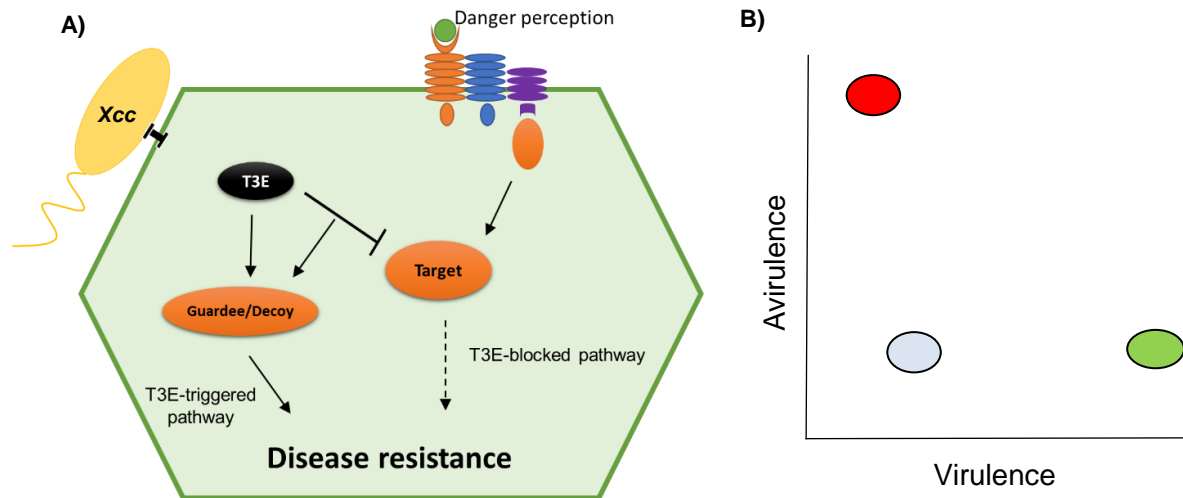
Strikingly, our work revealed that ten *Xcc* T3Es primed plant immunity by inducing the expression of immune-genes and/or enhancing MAPK activities. This number is astonishing considering that the *xopAC* mutation is sufficient to render *Xcc* 8004 virulent in Arabidopsis Col-0 accession (Guy et al., 2013) . This raises the question: How can *Xcc* overcome the immune responses triggered by the T3Es on Col-0 while successfully colonizing plant tissues? Likely, the collective properties of the T3Es allow *Xcc* to evade host cell recognition and/or defensive responses. The following paragraphs will describe how our results support this hypothesis.

#### 4.4.1 Xcc T3Es have opposite activities within host cells.

Our results demonstrated that the functions of the T3Es within plant cells are complex and sometimes activate simultaneously antagonistic responses. For instance, XopJ5 promoted the flg22-triggered activation of MAPK and plant growth. Results presented by XopL, XopAC and AvrXccA1 are other clear examples of “ambivalent activities” since those effectors suppressed the flg22-triggered activity of MAPK while promoting the expression of plant immune-genes characteristic of ETI responses. However, AvrXccA1 deserves special attention since its secretion by the T3SS has not yet been validated. AvrXccA1 (also known as AvrXca) was first identified as a *X. campestris* pv. *raphani* avirulence gene in many Arabidopsis ecotypes (Parker et al., 1993) and orthologs have been described in strains of many bacterial species. Nevertheless, it possesses a signal motive for type 2 secretion in its N-terminal portion indicating that AvrXccA1 might not be a T3E. Future studies are needed to validate AvrXccA1 secretion and the secretion system that mediates it.

It is known that XopAC uridylylates the BIK1 kinase and the decoy protein PBL2 to suppress PTI and trigger ETI respectively (G. Wang et al., 2015). Evidence of ambivalent activities can be found in T3Es of other pathosystems such as AvrRps4 and PopP2 from *P. syringae* and *R. solanacearum*, respectively, which interfere with WRKY transcription factors to inhibit their defense-promoting activities while activating the plant complex formed by RRS1 and RPS4 to activate plant defense (Sarris et al., 2015; Le Roux et al., 2015). The ambivalence in the activities of the T3Es seems to be a common phenomenon in effectors of several pathogenic species that originates from the mechanisms of indirect recognition of the plant cell. This means that a T3E with ambivalent functions exert a molecular activity by which it deactivates a molecule/module of the plant immune system, while in parallel, such molecular activity modifies a secondary plant protein known as guardee or decoy (Dangl & Jones, 2001; van der Hoorn & Kamoun, 2008) that triggers immune responses (Figure 1A). Therefore, the functions of the effectors could be considered as a result of two components, one describing the suppressing activity of the T3E on its intended target and how it contributes to pathogenicity (X axis in figure 1B). The second component describes the recognition of the T3E by the plant immune-molecules and how it contributes to disease resistance (Y-axis in figure 1B).

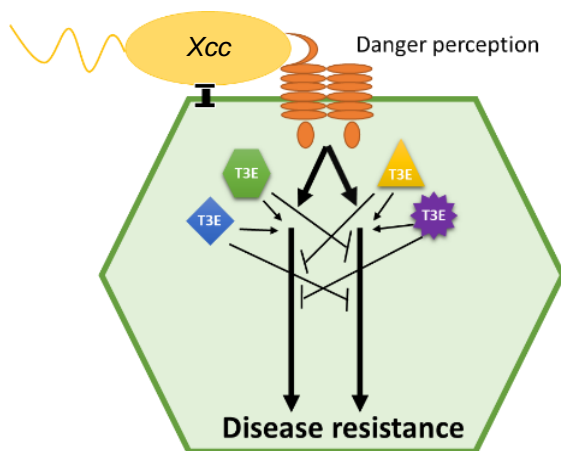




**Figure 1. T3Es have ambivalent effects in plant cell.** T3Es activities block specific modules or pathways of plant immunity and simultaneously trigger secondary immune pathways (A). T3Es activities can be represented in terms of Avirulence and Virulence with basis in their contribution to pathogenicity or plant disease resistance respectively. T3E with strong avirulence activities is in red, T3E with subtle effects in plant physiology in gray and T3E with strong virulence activity in green (B).

#### 4.4.2 The emergent properties of the effectors define *Xcc* pathogenic features.

The ambivalent functions of the T3Es become relevant for pathogenesis if we consider previous studies showing that the activity of one effector can suppress the recognition of a second effector. For instance five *X. oryzae* pv. *oryzae* T3Es individually suppressed the immune responses induced by XopQ and XopX (Deb et al., 2020). The *P. syringae* effectors HopPtoE, AvrPphE, AvrPpiB1, AvrPtoB, and HopPtoF suppress the programmed cell death induced by HopPsyA in tobacco and Arabidopsis plants (Jamir et al., 2004). Similarly, the effector AvrLm4-7 of *Leptosphaeria maculans* suppresses the recognition of AvrLm3 by the R protein Rlm3 of oilseed rape (Petit-Houdenot & Fudal, 2017). For *Xcc*, its virulence can only be explained if the activities of some effectors inhibit the recognition of others (Figure 2), otherwise, the strain would



**Figure 2. The recognition of some T3Es is blocked by the activities of other T3Es.** Membrane bound receptors triggering PTI responses are in orange. Activating functions represented as →. Suppressing functions represented as T.

simply be avirulent considering the presence of numerous effectors with immune-triggering activities. Since we analyzed the effects caused *in planta* by individual T3Es, we probably detected their avirulence function unraveled by the absence of their epistatic T3Es. Although the suppression of the recognition of one effector by another effector has been demonstrated in different pathogenic species, to our knowledge, this phenomenon has not been validated in *Xcc*. Further studies are required to verify our hypothesis. For example, our transgenic *Arabidopsis* lines expressing single T3Es could be crossed to generate descendant lines expressing simultaneously pairs or groups of different effectors.

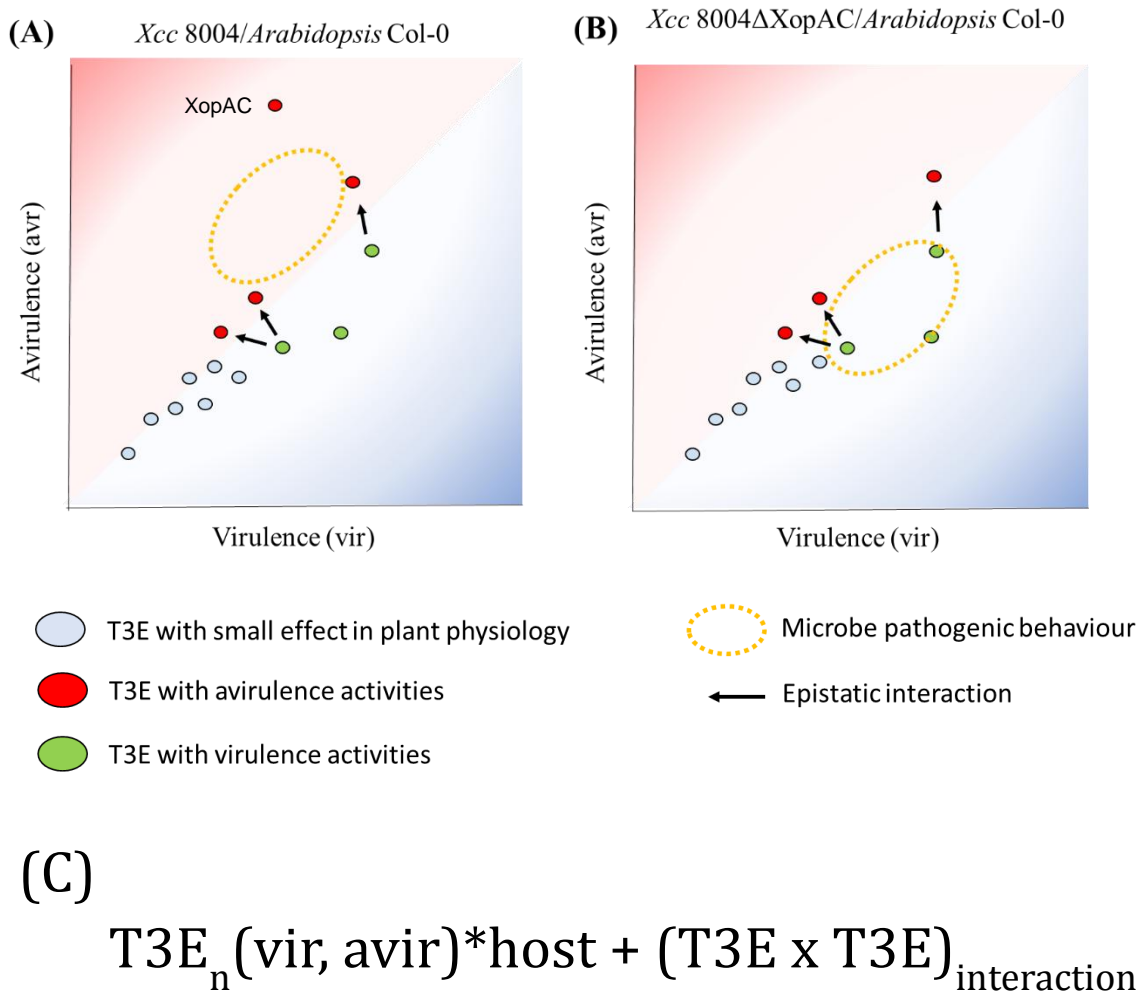
#### **4.4.3 The *Xcc* T3 effectome is resilient to the loss of single T3Es thanks to its collective properties.**

Our pathogenicity assays in *Arabidopsis* Sf-2 plants with *Xcc* strains deleted of single or pairs of T3Es evidenced that the *Xcc* T3 effectome is highly resilient to the loss of few effectors. Similar experiments, using the same collection of mutant strains in *Arabidopsis* plants of the Col-0 accession, support similar characteristics (Guy et al., 2013). The resilience shown by the T3 effectome of *Xcc* likely arises from the functional redundancy. Previous studies achieving combinatorial deletions of more than twenty T3Es of *P. syringae* revealed that much of the virulence capabilities exhibited by *P. syringae* on *N. benthamiana* relied on five T3Es clustered in two redundant-effector groups (REGs), which separately targeted two plant pathways (Kvitko et al., 2009). Later studies using the same experimental system proved that no single T3E is essential nor sufficient for virulence of *P. syringae* on *N. benthamiana* plants; instead, T3Es in small groups with reduced redundancy allow pathogenicity (Cunnac et al., 2011). These studies suggest that redundancy only confer robustness to the activities of the T3 effectome. Our experiments showing multiple effectors commonly suppressing MAPK activities indicate that the T3 effectome of *Xcc* exhibit some degree of redundancy. Additionally, many T3Es may have weak effects on host physiology and may therefore not have a detectable contribution to *Xcc* pathogenicity. Taken together, our results suggest that the resilience of the *Xcc* T3 effectome to the loss of single or pairs of T3Es arises from the functional redundancy of T3Es with discrete contributions to pathogenicity. Our research group has initiated approaches to reconstruct synthetic effectomes in natural and engineered effector-less strains that would be useful to decipher the extent of robustness and redundancy of the *Xcc* T3 effectome (Arroyo-Velez et al., 2020).

#### 4.5. What is the contribution of the T3Es to *Xcc* pathogenicity in *Arabidopsis*.

The ideas and results of this work can be summarized in a model representing the contribution of the T3Es to the pathogenicity of *Xcc* in *Arabidopsis* Col-0, although it can be valid for other pathosystems (Figure 3A). The repertoire of functions that the *Xcc* T3E effectome displays in *Arabidopsis* (dashed yellow line) is dictated by the actions of the T3Es that compose it, which dictate the existence of compatible (blue area) or incompatible (red area) interactions. As T3Es can have opposite functions, blocking certain immune modules while promoting others (G. Wang et al., 2015), each effector is represented by an avirulence component and a virulence component. T3Es eliciting strong (Effector triggered immunity [ETI]) responses (Lewis et al., 2013) have a major contribution to Avr (red spheres) stretching the T3 effectome towards incompatible interactions, for instance *Xcc* 8004 XopAC. However, the recognition of some T3Es may be masked by the actions of other effectors (Effector triggered susceptibility [ETS]), reducing their avirulence component. In *Xcc* 8004, the recognition of the T3Es XopL, AvrXccA1, XopAH and XopK is probably masked by other unknown T3E, as *Xcc* strains mutants for any of these T3E genes don't have any increase in pathogenicity (Guy et al., 2013). T3Es contributing to virulence stretch the T3 effectome towards a compatible interaction (green spheres). *Xcc* 8004 XopAG could be considered in this group as our results suggested that its mutation decrease *Xcc* pathogenicity in *Arabidopsis*. One T3E may block the recognition of multiple T3Es, thus its contribution to pathogenicity will be multiplied (Figure 3C). Instead, different T3Es may have redundant functions, so their contribution to pathogenicity will be reduced. T3Es with discrete activities in both components (Avr and Vir) will not have a significant effect in the total activity of the T3 effectome (gray spheres) but the additive action of many effectors with subtle activities will confer robustness to the system. In our experiments, multiple *Xcc* 8004 T3Es did not cause significant effects in plant physiology. Although, it is not known if these T3Es have a quantitative contribution to *Xcc* pathogenicity in *Arabidopsis*. This model is useful to explain why the deletion of *xopAC* increases the virulence of *Xcc* strain 8004 in *Arabidopsis* Col-0 (Figure 3B), despite having four T3Es with immune-triggering activities. At the same time, the individual deletion of most of its T3Es does not have a significant effect on *Xcc* pathogenicity. It is also important to consider that the functions of the T3 effectome depend on the host genetic context. As the T3Es contribute to virulence or avirulence depending on the presence or absence of their targets and cognate R-proteins on the genetic repertoire of the host plant (Figure 3C). The genetic diversity present in plants can cause a T3 effectome to generate compatible interactions

in certain hosts while other plant species or ecotypes exhibit incompatibility and remain aside of the ecological niche of such pathogen.



**Figure 3. Model representing the contribution of the T3Es to the pathogenicity of *Xcc* in *Arabidopsis* Col-0.** The repertoire of functions of the *Xcc* T3 effectome on *Arabidopsis* Col-0 is dictated by the activities of the T3E that compose it (A). Shift in the pathogenic behavior of *Xcc* upon *xopAC* mutation (B). Mathematical representation of the model (C).

## Bibliography

- Acharya, B. R., Raina, S., Maqbool, S. B., Jagadeeswaran, G., Mosher, S. L., Appel, H. M., Schultz, J. C., Klessig, D. F., & Raina, R. (2007). Overexpression of CRK13, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to *Pseudomonas syringae*. *The Plant Journal*, 50(3), 488–499. <https://doi.org/10.1111/j.1365-313X.2007.03064.x>
- Adlung, N., Prochaska, H., Thieme, S., Banik, A., Blüher, D., John, P., Nagel, O., Schulze, S., Gantner, J., Delker, C., Stuttmann, J., & Bonas, U. (2016). Non-host Resistance Induced by the Xanthomonas Effector XopQ Is Widespread within the Genus *Nicotiana* and Functionally Depends on EDS1. *Frontiers in Plant Science*, 7, 1796. <https://doi.org/10.3389/fpls.2016.01796>
- Alfano, J. R., & Collmer, A. (2004). TYPE III SECRETION SYSTEM EFFECTOR PROTEINS: Double Agents in Bacterial Disease and Plant Defense. *Annual Review of Phytopathology*, 42(1), 385–414. <https://doi.org/10.1146/annurev.phyto.42.040103.110731>
- Arroyo-Velez, N., González-Fuente, M., Peeters, N., Lauber, E., & Noël, L. D. (2020). From effectors to effectomes: Are functional studies of individual effectors enough to decipher plant pathogen infectious strategies? *PLOS Pathogens*, 16(12), e1009059. <https://doi.org/10.1371/journal.ppat.1009059>
- Bender, C. L., Alarcón-Chaidez, F., & Gross, D. C. (1999). *Pseudomonas syringae* Phytotoxins: Mode of Action, Regulation, and Biosynthesis by Peptide and Polyketide Synthetases. *Microbiology and Molecular Biology Reviews*, 63(2), 266–292.
- Blüher, D., Laha, D., Thieme, S., Hofer, A., Eschen-Lippold, L., Masch, A., Balcke, G., Pavlovic, I., Nagel, O., Schonsky, A., Hinkelmann, R., Wörner, J., Parvin, N., Greiner, R., Weber, S., Tissier, A., Schutkowski, M., Lee, J., Jessen, H., ... Bonas, U. (2017). A 1-phytase type III effector interferes with plant hormone signaling. *Nature Communications*, 8(1), 2159. <https://doi.org/10.1038/s41467-017-02195-8>
- Boland, A., & Cornelis, G. R. (1998). Role of YopP in Suppression of Tumor Necrosis Factor Alpha Release by Macrophages during *Yersinia* Infection. *Infection and Immunity*, 66(5), 1878–1884. <https://doi.org/10.1128/IAI.66.5.1878-1884.1998>
- Boucher, C. A., Van Gijsegem, F., Barberis, P. A., Arlat, M., & Zischek, C. (1987). *Pseudomonas solanacearum* genes controlling both pathogenicity on tomato and hypersensitivity on tobacco are clustered. *Journal of Bacteriology*, 169(12), 5626–5632.
- Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., & Sessitsch, A. (2017). Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology*, 55, 61–83. <https://doi.org/10.1146/annurev-phyto-080516-035641>
- Bürger, M., & Chory, J. (2019). Stressed Out About Hormones: How Plants Orchestrate Immunity. *Cell Host & Microbe*, 26(2), 163–172. <https://doi.org/10.1016/j.chom.2019.07.006>
- Büttner, D. (2016). Behind the lines—actions of bacterial type III effector proteins in plant cells. *FEMS Microbiology Reviews*, 40(6), 894–937. <https://doi.org/10.1093/femsre/fuw026>

- Büttner, D., & Bonas, U. (2010). Regulation and secretion of *Xanthomonas* virulence factors. *FEMS Microbiology Reviews*, 34(2), 107–133. <https://doi.org/10.1111/j.1574-6976.2009.00192.x>
- Büttner, D., Noël, L., Stuttmann, J., & Bonas, U. (2007). Characterization of the nonconserved hpaB-hrpF region in the hrp pathogenicity island from *Xanthomonas campestris* pv. *Vesicatoria*. *Molecular Plant-Microbe Interactions: MPMI*, 20(9), 1063–1074. <https://doi.org/10.1094/MPMI-20-9-1063>
- Cambridge University Press. (2009). *Cambridge academic content dictionary*. Cambridge University Press.
- Castañeda, A., Reddy, J. D., El-Yacoubi, B., & Gabriel, D. W. (2005). Mutagenesis of all eight avr genes in *Xanthomonas campestris* pv. *Campestris* had no detected effect on pathogenicity, but one avr gene affected race specificity. *Molecular Plant-Microbe Interactions: MPMI*, 18(12), 1306–1317. <https://doi.org/10.1094/MPMI-18-1306>
- Cerutti, A., Jauneau, A., Auriac, M.-C., Lauber, E., Martinez, Y., Chiarenza, S., Leonhardt, N., Berthomé, R., & Noël, L. D. (2017). Immunity at Cauliflower Hydathodes Controls Systemic Infection by *Xanthomonas campestris* pv. *campestris*. *Plant Physiology*, 174(2), 700–716. <https://doi.org/10.1104/pp.16.01852>
- Chinchilla, D., Bauer, Z., Regenass, M., Boller, T., & Felix, G. (2006). The Arabidopsis Receptor Kinase FLS2 Binds flg22 and Determines the Specificity of Flagellin Perception. *The Plant Cell*, 18(2), 465–476. <https://doi.org/10.1105/tpc.105.036574>
- Corwin, J. A., & Kliebenstein, D. J. (2017). Quantitative Resistance: More Than Just Perception of a Pathogen. *The Plant Cell*, 29(4), 655–665. <https://doi.org/10.1105/tpc.16.00915>
- Costa, T. R. D., Felisberto-Rodrigues, C., Meir, A., Prevost, M. S., Redzej, A., Trokter, M., & Waksman, G. (2015). Secretion systems in Gram-negative bacteria: Structural and mechanistic insights. *Nature Reviews Microbiology*, 13(6), 343–359. <https://doi.org/10.1038/nrmicro3456>
- Couto, D., & Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature Reviews Immunology*, 16(9), 537–552. <https://doi.org/10.1038/nri.2016.77>
- Crossman, L., & Dow, J. M. (2004). Biofilm formation and dispersal in *Xanthomonas campestris*. *Microbes and Infection*, 6(6), 623–629. <https://doi.org/10.1016/j.micinf.2004.01.013>
- Cui, F., Wu, S., Sun, W., Coaker, G., Kunkel, B., He, P., & Shan, L. (2013). The *Pseudomonas syringae* Type III Effector AvrRpt2 Promotes Pathogen Virulence via Stimulating Arabidopsis Auxin/Indole Acetic Acid Protein Turnover. *Plant Physiology*, 162(2), 1018–1029. <https://doi.org/10.1104/pp.113.219659>
- Cui, H., Tsuda, K., & Parker, J. E. (2015). Effector-triggered immunity: From pathogen perception to robust defense. *Annual Review of Plant Biology*, 66, 487–511. <https://doi.org/10.1146/annurev-arplant-050213-040012>
- Cui, H., Wang, Y., Xue, L., Chu, J., Yan, C., Fu, J., Chen, M., Innes, R. W., & Zhou, J.-M. (2010). A *Pseudomonas syringae* protein perturbs Arabidopsis hormone signaling by

- activating MAP KINASE 4. *Cell Host & Microbe*, 7(2), 164–175. <https://doi.org/10.1016/j.chom.2010.01.009>
- Cunnac, S., Chakravarthy, S., Kvitko, B. H., Russell, A. B., Martin, G. B., & Collmer, A. (2011). Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. *Proceedings of the National Academy of Sciences*, 108(7), 2975–2980. <https://doi.org/10.1073/pnas.1013031108>
- Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature*, 411(6839), 826–833. <https://doi.org/10.1038/35081161>
- Dean, P., & Kenny, B. (2009). The effector repertoire of enteropathogenic *E. coli*: Ganging up on the host cell. *Current Opinion in Microbiology*, 12(1–3), 101–109. <https://doi.org/10.1016/j.mib.2008.11.006>
- Deb, S., Ghosh, P., Patel, H. K., & Sonti, R. V. (2020). Interaction of the *Xanthomonas* effectors XopQ and XopX results in induction of rice immune responses. *The Plant Journal*, 104(2), 332–350. <https://doi.org/10.1111/tpj.14924>
- del Río, L. A. (2015). ROS and RNS in plant physiology: An overview. *Journal of Experimental Botany*, 66(10), 2827–2837. <https://doi.org/10.1093/jxb/erv099>
- Ding, Y., Sun, T., Ao, K., Peng, Y., Zhang, Y., Li, X., & Zhang, Y. (2018). Opposite Roles of Salicylic Acid Receptors NPR1 and NPR3/NPR4 in Transcriptional Regulation of Plant Immunity. *Cell*, 173(6), 1454–1467.e15. <https://doi.org/10.1016/j.cell.2018.03.044>
- Dubiella, U., Seybold, H., Durian, G., Komander, E., Lassig, R., Witte, C.-P., Schulze, W. X., & Romeis, T. (2013). Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. *Proceedings of the National Academy of Sciences of the United States of America*, 110(21), 8744–8749. <https://doi.org/10.1073/pnas.1221294110>
- Eckardt, N. A. (2008). Epistasis and Genetic Regulation of Variation in the Arabidopsis Metabolome. *The Plant Cell*, 20(5), 1185–1186. <https://doi.org/10.1105/tpc.108.061051>
- Eichmann, R., & Schäfer, P. (2015). Growth versus immunity—A redirection of the cell cycle? *Current Opinion in Plant Biology*, 26, 106–112. <https://doi.org/10.1016/j.pbi.2015.06.006>
- El Qaidi, S., Scott, N. E., Hays, M. P., Geisbrecht, B. V., Watkins, S., & Hardwidge, P. R. (2020). An intra-bacterial activity for a T3SS effector. *Scientific Reports*, 10(1), 1073. <https://doi.org/10.1038/s41598-020-58062-y>
- Fargier, E., & Manceau, C. (2007). Pathogenicity assays restrict the species *Xanthomonas campestris* into three pathovars and reveal nine races within *X. campestris* pv. *Campestris*. *Plant Pathology*, 56(5), 805–818. <https://doi.org/10.1111/j.1365-3059.2007.01648.x>
- Faris, J. D., Zhang, Z., Rasmussen, J. B., & Friesen, T. L. (2011). Variable Expression of the *Stagonospora nodorum* Effector SnToxA Among Isolates Is Correlated with Levels of Disease in Wheat. *Molecular Plant-Microbe Interactions*, 24(12), 1419–1426. <https://doi.org/10.1094/MPMI-04-11-0094>

- Fatima, U., & Senthil-Kumar, M. (2015). Plant and pathogen nutrient acquisition strategies. *Frontiers in Plant Science*, 6. <https://doi.org/10.3389/fpls.2015.00750>
- Flor, H. H. (1971). Current Status of the Gene-For-Gene Concept. *Annual Review of Phytopathology*, 9(1), 275–296. <https://doi.org/10.1146/annurev.py.09.090171.001423>
- Galletti, R., Ferrari, S., & De Lorenzo, G. (2011). Arabidopsis MPK3 and MPK6 play different roles in basal and oligogalacturonide- or flagellin-induced resistance against *Botrytis cinerea*. *Plant Physiology*, 157(2), 804–814. <https://doi.org/10.1104/pp.111.174003>
- Gao, M., Liu, J., Bi, D., Zhang, Z., Cheng, F., Chen, S., & Zhang, Y. (2008). MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. *Cell Research*, 18(12), 1190–1198. <https://doi.org/10.1038/cr.2008.300>
- Ghosh, P. (2004). Process of Protein Transport by the Type III Secretion System. *Microbiology and Molecular Biology Reviews*, 68(4), 771–795. <https://doi.org/10.1128/MMBR.68.4.771-795.2004>
- Gimenez-Ibanez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J. P., & Solano, R. (2014). The Bacterial Effector HopX1 Targets JAZ Transcriptional Repressors to Activate Jasmonate Signaling and Promote Infection in Arabidopsis. *PLoS Biology*, 12(2), e1001792. <https://doi.org/10.1371/journal.pbio.1001792>
- Gimenez-Ibanez, S., Hann, D. R., Ntoukakis, V., Petutschnig, E., Lipka, V., & Rathjen, J. P. (2009). AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. *Current Biology: CB*, 19(5), 423–429. <https://doi.org/10.1016/j.cub.2009.01.054>
- Gochez, A. M., Shantharaj, D., Potnis, N., Zhou, X., Minsavage, G. V., White, F. F., Wang, N., Hurlbert, J. C., & Jones, J. B. (2017). Molecular characterization of XopAG effector AvrGf2 from *Xanthomonas fuscans* ssp. *Aurantifolii* in grapefruit. *Molecular Plant Pathology*, 18(3), 405–419. <https://doi.org/10.1111/mpp.12408>
- Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J. W., & Robatzek, S. (2008). Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Current Biology: CB*, 18(23), 1824–1832. <https://doi.org/10.1016/j.cub.2008.10.063>
- Gómez-Gómez, L., & Boller, T. (2000). FLS2: An LRR Receptor-like Kinase Involved in the Perception of the Bacterial Elicitor Flagellin in Arabidopsis. *Molecular Cell*, 5(6), 1003–1011. [https://doi.org/10.1016/S1097-2765\(00\)80265-8](https://doi.org/10.1016/S1097-2765(00)80265-8)
- González-Fuente, M., Carrère, S., Monachello, D., Marsella, B. G., Cazalé, A.-C., Zischek, C., Mitra, R. M., Rezé, N., Cottret, L., Mukhtar, M. S., Lurin, C., Noël, L. D., & Peeters, N. (2020). EffectorK, a comprehensive resource to mine for *Ralstonia*, *Xanthomonas*, and other published effector interactors in the Arabidopsis proteome. *Molecular Plant Pathology*, 21(10), 1257–1270. <https://doi.org/10.1111/mpp.12965>
- Guo, M., Tian, F., Wamboldt, Y., & Alfano, J. R. (2009). The Majority of the Type III Effector Inventory of *Pseudomonas syringae* pv. *Tomato* DC3000 Can Suppress Plant Immunity.



- Molecular Plant-Microbe Interactions*: *MPMI*, 22(9), 1069–1080.  
<https://doi.org/10.1094/MPMI-22-9-1069>
- Gürlebeck, D., Jahn, S., Gürlebeck, N., Szczesny, R., Szurek, B., Hahn, S., Hause, G., & Bonas, U. (2009). Visualization of novel virulence activities of the *Xanthomonas* type III effectors AvrBs1, AvrBs3 and AvrBs4. *Molecular Plant Pathology*, 10(2), 175–188.  
<https://doi.org/10.1111/j.1364-3703.2008.00519.x>
- Gust, A. A., Pruitt, R., & Nürnberger, T. (2017). Sensing Danger: Key to Activating Plant Immunity. *Trends in Plant Science*, 22(9), 779–791.  
<https://doi.org/10.1016/j.tplants.2017.07.005>
- Guy, E., Genissel, A., Hajri, A., Chabannes, M., David, P., Carrere, S., Lautier, M., Roux, B., Boureau, T., Arlat, M., Poussier, S., & Noël, L. D. (2013). Natural genetic variation of *Xanthomonas campestris* pv. *Campestris* pathogenicity on arabidopsis revealed by association and reverse genetics. *MBio*, 4(3), e00538-00512.  
<https://doi.org/10.1128/mBio.00538-12>
- Hann, D. R., Domínguez-Ferreras, A., Motyka, V., Dobrev, P. I., Schornack, S., Jehle, A., Felix, G., Chinchilla, D., Rathjen, J. P., & Boller, T. (2014). The *Pseudomonas* type III effector HopQ1 activates cytokinin signaling and interferes with plant innate immunity. *The New Phytologist*, 201(2), 585–598. <https://doi.org/10.1111/nph.12544>
- Hao, G., McCormick, S., Usgaard, T., Tiley, H., & Vaughan, M. M. (2020). Characterization of Three *Fusarium graminearum* Effectors and Their Roles During *Fusarium* Head Blight. *Frontiers in Plant Science*, 11, 1886. <https://doi.org/10.3389/fpls.2020.579553>
- He, K., Gou, X., Yuan, T., Lin, H., Asami, T., Yoshida, S., Russell, S. D., & Li, J. (2007). BAK1 and BKK1 Regulate Brassinosteroid-Dependent Growth and Brassinosteroid-Independent Cell-Death Pathways. *Current Biology*, 17(13), 1109–1115.  
<https://doi.org/10.1016/j.cub.2007.05.036>
- He, Y.-Q., Zhang, L., Jiang, B.-L., Zhang, Z.-C., Xu, R.-Q., Tang, D.-J., Qin, J., Jiang, W., Zhang, X., Liao, J., Cao, J.-R., Zhang, S.-S., Wei, M.-L., Liang, X.-X., Lu, G.-T., Feng, J.-X., Chen, B., Cheng, J., & Tang, J.-L. (2007). Comparative and functional genomics reveals genetic diversity and determinants of host specificity among reference strains and a large collection of Chinese isolates of the phytopathogen *Xanthomonas campestris* pv. *Campestris*. *Genome Biology*, 8(10), R218. <https://doi.org/10.1186/gb-2007-8-10-r218>
- Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., & Hedges, S. B. (2001). Molecular Evidence for the Early Colonization of Land by Fungi and Plants. *Science*, 293(5532), 1129–1133. <https://doi.org/10.1126/science.1061457>
- Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M. E., He, K., Li, J., Schroeder, J. I., Peck, S. C., & Rathjen, J. P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences*, 104(29), 12217–12222. <https://doi.org/10.1073/pnas.0705306104>
- Hernandez, M. N., & Lindow, S. E. (2019). *Pseudomonas syringae* Increases Water Availability in Leaf Microenvironments via Production of Hygroscopic Syringafactin. *Applied and Environmental Microbiology*, 85(18), e01014-19. <https://doi.org/10.1128/AEM.01014-19>

- Ho, Y.-P., Tan, C. M., Li, M.-Y., Lin, H., Deng, W.-L., & Yang, J.-Y. (2013). The AvrB\_AvrC domain of AvrXccC of *Xanthomonas campestris* pv. *Campestris* is required to elicit plant defense responses and manipulate ABA homeostasis. *Molecular Plant-Microbe Interactions: MPMI*, 26(4), 419–430. <https://doi.org/10.1094/MPMI-06-12-0164-R>
- Huang, H., Liu, B., Liu, L., & Song, S. (2017). Jasmonate action in plant growth and development. *Journal of Experimental Botany*, 68(6), 1349–1359. <https://doi.org/10.1093/jxb/erw495>
- Huang, Y., Li, T., Xu, T., Tang, Z., Guo, J., & Cai, Y. (2020). Multiple *Xanthomonas campestris* pv. *Campestris* 8004 type III effectors inhibit immunity induced by flg22. *Planta*, 252(5), 88. <https://doi.org/10.1007/s00425-020-03484-1>
- Huffaker, A., Pearce, G., & Ryan, C. A. (2006). An endogenous peptide signal in *Arabidopsis* activates components of the innate immune response. *Proceedings of the National Academy of Sciences*, 103(26), 10098–10103. <https://doi.org/10.1073/pnas.0603727103>
- Hull, J. C. (2008). Plant Ecology. In S. E. Jørgensen & B. D. Fath (Eds.), *Encyclopedia of Ecology* (pp. 2818–2824). Academic Press. <https://doi.org/10.1016/B978-008045405-4.00843-0>
- Huot, B., Yao, J., Montgomery, B. L., & He, S. Y. (2014). Growth–Defense Tradeoffs in Plants: A Balancing Act to Optimize Fitness. *Molecular Plant*, 7(8), 1267–1287. <https://doi.org/10.1093/mp/ssu049>
- Ishikawa, K., Yamaguchi, K., Sakamoto, K., Yoshimura, S., Inoue, K., Tsuge, S., Kojima, C., & Kawasaki, T. (2014). Bacterial effector modulation of host E3 ligase activity suppresses PAMP-triggered immunity in rice. *Nature Communications*, 5, 5430. <https://doi.org/10.1038/ncomms6430>
- Jamir, Y., Guo, M., Oh, H.-S., Petnicki-Ocwieja, T., Chen, S., Tang, X., Dickman, M. B., Collmer, A., & Alfano, J. R. (2004). Identification of *Pseudomonas syringae* type III effectors that can suppress programmed cell death in plants and yeast. *The Plant Journal: For Cell and Molecular Biology*, 37(4), 554–565. <https://doi.org/10.1046/j.1365-313x.2003.01982.x>
- Jeworutzki, E., Roelfsema, M. R. G., Anschutz, U., Krol, E., Elzenga, J. T. M., Felix, G., Boller, T., Hedrich, R., & Becker, D. (2010). Early signaling through the *Arabidopsis* pattern recognition receptors FLS2 and EFR involves Ca-associated opening of plasma membrane anion channels. *The Plant Journal: For Cell and Molecular Biology*, 62(3), 367–378. <https://doi.org/10.1111/j.1365-313X.2010.04155.x>
- Jiang, B.-L., He, Y.-Q., Cen, W.-J., Wei, H.-Y., Jiang, G.-F., Jiang, W., Hang, X.-H., Feng, J.-X., Lu, G.-T., Tang, D.-J., & Tang, J.-L. (2008). The type III secretion effector XopXccN of *Xanthomonas campestris* pv. *Campestris* is required for full virulence. *Research in Microbiology*, 159(3), 216–220. <https://doi.org/10.1016/j.resmic.2007.12.004>
- Jiang, G.-F., Jiang, B.-L., Yang, M., Liu, S., Liu, J., Liang, X.-X., Bai, X.-F., Tang, D.-J., Lu, G.-T., He, Y.-Q., Yu, D.-Q., & Tang, J.-L. (2014). Establishment of an inducing medium for type III effector secretion in *Xanthomonas campestris* pv. *Campestris*. *Brazilian Journal of Microbiology*, 44(3), 945–952.

- Jiang, W., Jiang, B.-L., Xu, R.-Q., Huang, J.-D., Wei, H.-Y., Jiang, G.-F., Cen, W.-J., Liu, J., Ge, Y.-Y., Li, G.-H., Su, L.-L., Hang, X.-H., Tang, D.-J., Lu, G.-T., Feng, J.-X., He, Y.-Q., & Tang, J.-L. (2009). Identification of Six Type III Effector Genes with the PIP Box in *Xanthomonas campestris* pv. *Campestris* and Five of Them Contribute Individually to Full Pathogenicity. *Molecular Plant-Microbe Interactions*, 22(11), 1401–1411. <https://doi.org/10.1094/MPMI-22-11-1401>
- Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, 444(7117), 323–329. <https://doi.org/10.1038/nature05286>
- Jones, J. D. G., Vance, R. E., & Dangl, J. L. (2016). Intracellular innate immune surveillance devices in plants and animals. *Science*, 354(6316), Article 6316. <https://doi.org/10.1126/science.aaf6395>
- Juge, N. (2006). Plant protein inhibitors of cell wall degrading enzymes. *Trends in Plant Science*, 11(7), 359–367. <https://doi.org/10.1016/j.tplants.2006.05.006>
- Kadota, Y., Shirasu, K., & Zipfel, C. (2015). Regulation of the NADPH Oxidase RBOHD During Plant Immunity. *Plant & Cell Physiology*, 56(8), 1472–1480. <https://doi.org/10.1093/pcp/pcv063>
- Kim, J. F., & Beer, S. V. (1998). HrpW of *Erwinia amylovora*, a new harpin that contains a domain homologous to pectate lyases of a distinct class. *Journal of Bacteriology*, 180(19), 5203–5210. <https://doi.org/10.1128/JB.180.19.5203-5210.1998>
- Kubicek, C. P., Starr, T. L., & Glass, N. L. (2014). Plant Cell Wall–Degrading Enzymes and Their Secretion in Plant-Pathogenic Fungi. *Annual Review of Phytopathology*, 52(1), 427–451. <https://doi.org/10.1146/annurev-phyto-102313-045831>
- Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., & Felix, G. (2004). The N Terminus of Bacterial Elongation Factor Tu Elicits Innate Immunity in Arabidopsis Plants. *The Plant Cell*, 16(12), 3496–3507. <https://doi.org/10.1105/tpc.104.026765>
- Kvitko, B. H., Park, D. H., Velásquez, A. C., Wei, C.-F., Russell, A. B., Martin, G. B., Schneider, D. J., & Collmer, A. (2009). Deletions in the repertoire of *Pseudomonas syringae* pv. *Tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. *PLoS Pathogens*, 5(4), e1000388. <https://doi.org/10.1371/journal.ppat.1000388>
- Lal, N. K., Nagalakshmi, U., Hurlburt, N. K., Flores, R., Bak, A., Sone, P., Ma, X., Song, G., Walley, J., Shan, L., He, P., Casteel, C., Fisher, A. J., & Dinesh-Kumar, S. P. (2018). The Receptor-like Cytoplasmic Kinase BIK1 Localizes to the Nucleus and Regulates Defense Hormone Expression during Plant Innate Immunity. *Cell Host & Microbe*, 23(4), 485-497.e5. <https://doi.org/10.1016/j.chom.2018.03.010>
- Lang, J., & Colcombet, J. (2020). Sustained Incompatibility between MAPK Signaling and Pathogen Effectors. *International Journal of Molecular Sciences*, 21(21), 7954. <https://doi.org/10.3390/ijms21217954>
- Lanver, D., Tollot, M., Schweizer, G., Lo Presti, L., Reissmann, S., Ma, L.-S., Schuster, M., Tanaka, S., Liang, L., Ludwig, N., & Kahmann, R. (2017). *Ustilago maydis* effectors and their

- impact on virulence. *Nature Reviews Microbiology*, 15(7), 409–421. <https://doi.org/10.1038/nrmicro.2017.33>
- Lapin, D., & Van den Ackerveken, G. (2013). Susceptibility to plant disease: More than a failure of host immunity. *Trends in Plant Science*, 18(10), 546–554. <https://doi.org/10.1016/j.tplants.2013.05.005>
- Le Roux, C., Huet, G., Jauneau, A., Camborde, L., Trémousaygue, D., Kraut, A., Zhou, B., Levaillant, M., Adachi, H., Yoshioka, H., Raffaele, S., Berthomé, R., Couté, Y., Parker, J. E., & Deslandes, L. (2015). A Receptor Pair with an Integrated Decoy Converts Pathogen Disabling of Transcription Factors to Immunity. *Cell*, 161(5), 1074–1088. <https://doi.org/10.1016/j.cell.2015.04.025>
- LeBlanc, M.-A., Fink, M. R., Perkins, T. T., & Sousa, M. C. (2021). Type III secretion system effector proteins are mechanically labile. *Proceedings of the National Academy of Sciences of the United States of America*, 118(12), e2019566118. <https://doi.org/10.1073/pnas.2019566118>
- Lee, D. S., Kim, Y. C., Kwon, S. J., Ryu, C.-M., & Park, O. K. (2017). The Arabidopsis Cysteine-Rich Receptor-Like Kinase CRK36 Regulates Immunity through Interaction with the Cytoplasmic Kinase BIK1. *Frontiers in Plant Science*, 8. <https://www.frontiersin.org/article/10.3389/fpls.2017.01856>
- Lewis, J. D., Lee, A. H.-Y., Hassan, J. A., Wan, J., Hurley, B., Jhingree, J. R., Wang, P. W., Lo, T., Youn, J.-Y., Guttman, D. S., & Desveaux, D. (2013). The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. *Proceedings of the National Academy of Sciences*, 110(46), 18722–18727. <https://doi.org/10.1073/pnas.1315520110>
- Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., Cai, G., Gao, L., Zhang, X., Wang, Y., Chen, S., & Zhou, J.-M. (2014). The FLS2-Associated Kinase BIK1 Directly Phosphorylates the NADPH Oxidase RbohD to Control Plant Immunity. *Cell Host & Microbe*, 15(3), 329–338. <https://doi.org/10.1016/j.chom.2014.02.009>
- Liang, X., & Zhou, J.-M. (2018). Receptor-Like Cytoplasmic Kinases: Central Players in Plant Receptor Kinase-Mediated Signaling. *Annual Review of Plant Biology*, 69, 267–299. <https://doi.org/10.1146/annurev-arplant-042817-040540>
- Lin, W., Li, B., Lu, D., Chen, S., Zhu, N., He, P., & Shan, L. (2014). Tyrosine phosphorylation of protein kinase complex BAK1/BIK1 mediates Arabidopsis innate immunity. *Proceedings of the National Academy of Sciences*, 111(9), 3632–3637. <https://doi.org/10.1073/pnas.1318817111>
- Liu, X., Grabherr, H. M., Willmann, R., Kolb, D., Brunner, F., Bertsche, U., Kühner, D., Franz-Wachtel, M., Amin, B., Felix, G., Ongena, M., Nürnberger, T., & Gust, A. A. (2014). Host-induced bacterial cell wall decomposition mediates pattern-triggered immunity in Arabidopsis. *ELife*, 2014(3). Scopus. <https://doi.org/10.7554/eLife.01990.001>
- Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M., Zuccaro, A., Reissmann, S., & Kahmann, R. (2015). Fungal effectors and plant susceptibility. *Annual*

*Review of Plant Biology*, 66, 513–545. <https://doi.org/10.1146/annurev-arplant-043014-114623>

- Lohou, D., Lonjon, F., Genin, S., & Vaillau, F. (2013). Type III chaperones & Co in bacterial plant pathogens: A set of specialized bodyguards mediating effector delivery. *Frontiers in Plant Science*, 4, 435. <https://doi.org/10.3389/fpls.2013.00435>
- Long, J., Song, C., Yan, F., Zhou, J., Zhou, H., & Yang, B. (2018). Non-TAL Effectors From *Xanthomonas oryzae* pv. *Oryzae* Suppress Peptidoglycan-Triggered MAPK Activation in Rice. *Frontiers in Plant Science*, 9, 1857. <https://doi.org/10.3389/fpls.2018.01857>
- Lorenz, C., Kirchner, O., Egler, M., Stuttmann, J., Bonas, U., & Büttner, D. (2008). HpaA from *Xanthomonas* is a regulator of type III secretion. *Molecular Microbiology*, 69(2), 344–360. <https://doi.org/10.1111/j.1365-2958.2008.06280.x>
- Lozano-Durán, R., & Zipfel, C. (2015). Trade-off between growth and immunity: Role of brassinosteroids. *Trends in Plant Science*, 20(1), 12–19. <https://doi.org/10.1016/j.tplants.2014.09.003>
- Ma, L., Cornelissen, B., & Takken, F. (2013). A nuclear localization for Avr2 from *Fusarium oxysporum* is required to activate the tomato resistance protein I-2. *Frontiers in Plant Science*, 4, 94. <https://doi.org/10.3389/fpls.2013.00094>
- Ma, L., Houterman, P. M., Gawehns, F., Cao, L., Sillo, F., Richter, H., Clavijo-Ortiz, M. J., Schmidt, S. M., Boeren, S., Vervoort, J., Cornelissen, B. J. C., Rep, M., & Takken, F. L. W. (2015). The AVR2–SIX5 gene pair is required to activate I-2-mediated immunity in tomato. *New Phytologist*, 208(2), 507–518. <https://doi.org/10.1111/nph.13455>
- Macho, A. P., Schwessinger, B., Ntoukakis, V., Brutus, A., Segonzac, C., Roy, S., Kadota, Y., Oh, M.-H., Sklenar, J., Derbyshire, P., Lozano-Durán, R., Malinovsky, F. G., Monaghan, J., Menke, F. L., Huber, S. C., He, S. Y., & Zipfel, C. (2014). A bacterial tyrosine phosphatase inhibits plant pattern recognition receptor activation. *Science (New York, N.Y.)*, 343(6178), 1509–1512. <https://doi.org/10.1126/science.1248849>
- Macho, A. P., & Zipfel, C. (2014). Plant PRRs and the Activation of Innate Immune Signaling. *Molecular Cell*, 54(2), 263–272. <https://doi.org/10.1016/j.molcel.2014.03.028>
- Mannaa, M., & Seo, Y.-S. (2021). Plants under the Attack of Allies: Moving towards the Plant Pathobiome Paradigm. *Plants*, 10(1), 125. <https://doi.org/10.3390/plants10010125>
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, 13(6), 614–629. <https://doi.org/10.1111/j.1364-3703.2012.00804.x>
- Martin, R. L., Boulch, P. L., Clin, P., Schwarzenberg, A., Yvin, J.-C., Andrivon, D., Nguema-Ona, E., & Val, F. (2020). A comparison of PTI defense profiles induced in *Solanum tuberosum* by PAMP and non-PAMP elicitors shows distinct, elicitor-specific responses. *PLOS ONE*, 15(8), e0236633. <https://doi.org/10.1371/journal.pone.0236633>

- Meng, X., & Zhang, S. (2013). MAPK Cascades in Plant Disease Resistance Signaling. *Annual Review of Phytopathology*, 51(1), 245–266. <https://doi.org/10.1146/annurev-phyto-082712-102314>
- Mosquera, G., Giraldo, M. C., Khang, C. H., Coughlan, S., & Valent, B. (2009). Interaction Transcriptome Analysis Identifies *Magnaporthe oryzae* BAS1-4 as Biotrophy-Associated Secreted Proteins in Rice Blast Disease. *The Plant Cell*, 21(4), 1273–1290. <https://doi.org/10.1105/tpc.107.055228>
- Mukhtar, M. S., Carvunis, A.-R., Dreze, M., Eppele, P., Steinbrenner, J., Moore, J., Tasan, M., Galli, M., Hao, T., Nishimura, M. T., Pevzner, S. J., Donovan, S. E., Ghamsari, L., Santhanam, B., Romero, V., Poulin, M. M., Gebreab, F., Gutierrez, B. J., Tam, S., ... Dangl, J. L. (2011). Independently Evolved Virulence Effectors Converge onto Hubs in a Plant Immune System Network. *Science*, 333(6042), 596–601. <https://doi.org/10.1126/science.1203659>
- Müller, D. B., Vogel, C., Bai, Y., & Vorholt, J. A. (2016). The Plant Microbiota: Systems-Level Insights and Perspectives. *Annual Review of Genetics*, 50, 211–234. <https://doi.org/10.1146/annurev-genet-120215-034952>
- Nakano, M., & Mukaihara, T. (2019). Comprehensive Identification of PTI Suppressors in Type III Effector Repertoire Reveals that *Ralstonia solanacearum* Activates Jasmonate Signaling at Two Different Steps. *International Journal of Molecular Sciences*, 20(23), 5992. <https://doi.org/10.3390/ijms20235992>
- Newman, M.-A., Sundelin, T., Nielsen, J., & Erbs, G. (2013). MAMP (microbe-associated molecular pattern) triggered immunity in plants. *Frontiers in Plant Science*, 4, 139. <https://doi.org/10.3389/fpls.2013.00139>
- Ngou, B. P. M., Ahn, H.-K., Ding, P., & Jones, J. D. G. (2021). Mutual potentiation of plant immunity by cell-surface and intracellular receptors. *Nature*, 592(7852), 110–115. <https://doi.org/10.1038/s41586-021-03315-7>
- Nissinen, R. M., Ytterberg, A. J., Bogdanove, A. J., Van Wijk, K. J., & Beer, S. V. (2007). Analyses of the secretomes of *Erwinia amylovora* and selected hrp mutants reveal novel type III secreted proteins and an effect of HrpJ on extracellular harpin levels. *Molecular Plant Pathology*, 8(1), 55–67. <https://doi.org/10.1111/j.1364-3703.2006.00370.x>
- Nitta, Y., Qiu, Y., Yaghmaiean, H., Zhang, Q., Huang, J., Adams, K., & Zhang, Y. (2020). MEKK2 inhibits activation of MAP kinases in Arabidopsis. *The Plant Journal*, 103(2), 705–714. <https://doi.org/10.1111/tpj.14763>
- Noël, L., Thieme, F., Nennstiel, D., & Bonas, U. (2002). Two novel type III-secreted proteins of *Xanthomonas campestris* pv. *Vesicatoria* are encoded within the hrp pathogenicity island. *Journal of Bacteriology*, 184(5), 1340–1348. <https://doi.org/10.1128/JB.184.5.1340-1348.2002>
- Oh, H.-S., & Collmer, A. (2005). Basal resistance against bacteria in *Nicotiana benthamiana* leaves is accompanied by reduced vascular staining and suppressed by multiple *Pseudomonas syringae* type III secretion system effector proteins. *The Plant Journal*, 44(2), 348–359. <https://doi.org/10.1111/j.1365-313X.2005.02529.x>

- Parker, J. E., Barber, C. E., Fan, M. J., & Daniels, M. J. (1993). Interaction of *Xanthomonas campestris* with *Arabidopsis thaliana*: Characterization of a gene from X. c. pv. raphani that confers avirulence to most A. thaliana accessions. *Molecular Plant-Microbe Interactions: MPMI*, 6(2), 216–224. <https://doi.org/10.1094/mpmi-6-216>
- Pearce, G., Strydom, D., Johnson, S., & Ryan, C. A. (1991). A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science (New York, N.Y.)*, 253(5022), 895–897. <https://doi.org/10.1126/science.253.5022.895>
- Petit-Houdenot, Y., & Fudal, I. (2017). Complex Interactions between Fungal Avirulence Genes and Their Corresponding Plant Resistance Genes and Consequences for Disease Resistance Management. *Frontiers in Plant Science*, 8, 1072. <https://doi.org/10.3389/fpls.2017.01072>
- Piqué, N., Miñana-Galbís, D., Merino, S., & Tomás, J. M. (2015). Virulence Factors of *Erwinia amylovora*: A Review. *International Journal of Molecular Sciences*, 16(6), 12836–12854. <https://doi.org/10.3390/ijms160612836>
- Pliego, C., Nowara, D., Bonciani, G., Gheorghe, D. M., Xu, R., Surana, P., Whigham, E., Nettleton, D., Bogdanove, A. J., Wise, R. P., Schweizer, P., Bindschedler, L. V., & Spanu, P. D. (2013). Host-Induced Gene Silencing in Barley Powdery Mildew Reveals a Class of Ribonuclease-Like Effectors. *Molecular Plant-Microbe Interactions®*, 26(6), 633–642. <https://doi.org/10.1094/MPMI-01-13-0005-R>
- Plissonneau, C., Blaise, F., Ollivier, B., Leflon, M., Carpezat, J., Rouxel, T., & Balesdent, M.-H. (2017). Unusual evolutionary mechanisms to escape effector-triggered immunity in the fungal phytopathogen *Leptosphaeria maculans*. *Molecular Ecology*, 26(7), 2183–2198. <https://doi.org/10.1111/mec.14046>
- Pommerville, J. C. (2017). *Fundamentals of Microbiology*.
- Pontes, J. G. de M., Fernandes, L. S., dos Santos, R. V., Tasic, L., & Fill, T. P. (2020). Virulence Factors in the Phytopathogen–Host Interactions: An Overview. *Journal of Agricultural and Food Chemistry*, 68(29), 7555–7570. <https://doi.org/10.1021/acs.jafc.0c02389>
- Popa, C., Coll, N. S., Valls, M., & Sessa, G. (2016). Yeast as a Heterologous Model System to Uncover Type III Effector Function. *PLOS Pathogens*, 12(2), e1005360. <https://doi.org/10.1371/journal.ppat.1005360>
- Popa, C., Tabuchi, M., & Valls, M. (2016). Modification of Bacterial Effector Proteins Inside Eukaryotic Host Cells. *Frontiers in Cellular and Infection Microbiology*, 6, 73. <https://doi.org/10.3389/fcimb.2016.00073>
- Popov, G., Fraiture, M., Brunner, F., & Sessa, G. (2016). Multiple *Xanthomonas euvesicatoria* Type III Effectors Inhibit flg22-Triggered Immunity. *Molecular Plant-Microbe Interactions: MPMI*, 29(8), 651–660. <https://doi.org/10.1094/MPMI-07-16-0137-R>
- Prochaska, H., Thieme, S., Daum, S., Grau, J., Schmidtke, C., Hallensleben, M., John, P., Bacia, K., & Bonas, U. (2018). A conserved motif promotes HpaB-regulated export of type III effectors from *Xanthomonas*. *Molecular Plant Pathology*, 19(11), 2473–2487. <https://doi.org/10.1111/mpp.12725>

- Prusky, D., McEvoy, J. L., Saftner, R., Conway, W. S., & Jones, R. (2004). Relationship Between Host Acidification and Virulence of *Penicillium* spp. On Apple and Citrus Fruit. *Phytopathology*, 94(1), 44–51. <https://doi.org/10.1094/PHYTO.2004.94.1.44>
- Purdy, R. E., & Kolattukudy, P. E. (1975). Hydrolysis of plant cuticle by plant pathogens. Properties of cutinase I, cutinase II, and a nonspecific esterase isolated from *Fusarium solani* pisi. *Biochemistry*, 14(13), 2832–2840. <https://doi.org/10.1021/bi00684a007>
- Qi, J., Wang, J., Gong, Z., & Zhou, J.-M. (2017). Apoplastic ROS signaling in plant immunity. *Current Opinion in Plant Biology*, 38, 92–100. <https://doi.org/10.1016/j.pbi.2017.04.022>
- Qin, J., Zhou, X., Sun, L., Wang, K., Yang, F., Liao, H., Rong, W., Yin, J., Chen, H., Chen, X., & Zhang, J. (2018). The *Xanthomonas* effector XopK harbours E3 ubiquitin-ligase activity that is required for virulence. *The New Phytologist*, 220(1), 219–231. <https://doi.org/10.1111/nph.15287>
- Qutob, D., Patrick Chapman, B., & Gijzen, M. (2013). Transgenerational gene silencing causes gain of virulence in a plant pathogen. *Nature Communications*, 4(1), 1349. <https://doi.org/10.1038/ncomms2354>
- Reignault, P., & Sancholle, M. (2005). Plant–pathogen interactions: Will the understanding of common mechanisms lead to the unification of concepts? *Comptes Rendus Biologies*, 328(9), 821–833. <https://doi.org/10.1016/j.crv.2005.07.002>
- Rojas-Estevez, P., Urbina-Gómez, D. A., Ayala-Usma, D. A., Guayazan-Palacios, N., Mideros, M. F., Bernal, A. J., Cardenas, M., & Restrepo, S. (2020). Effector Repertoire of *Phytophthora betacei*: In Search of Possible Virulence Factors Responsible for Its Host Specificity. *Frontiers in Genetics*, 11, 579. <https://doi.org/10.3389/fgene.2020.00579>
- Rong, W., Feng, F., Zhou, J., & He, C. (2010). Effector-triggered innate immunity contributes Arabidopsis resistance to *Xanthomonas campestris*. *Molecular Plant Pathology*, 11(6), 783–793. <https://doi.org/10.1111/j.1364-3703.2010.00642.x>
- Saijo, Y., Loo, E. P., & Yasuda, S. (2018). Pattern recognition receptors and signaling in plant–microbe interactions. *The Plant Journal*, 93(4), 592–613. <https://doi.org/10.1111/tpj.13808>
- Salomon, D., Bosis, E., Dar, D., Nachman, I., & Sessa, G. (2012). Expression of *Pseudomonas syringae* type III effectors in yeast under stress conditions reveals that HopX1 attenuates activation of the high osmolarity glycerol MAP kinase pathway. *Microbiology (Reading, England)*, 158(Pt 11), 2859–2869. <https://doi.org/10.1099/mic.0.062513-0>
- Salomon, D., & Sessa, G. (2010). Identification of growth inhibition phenotypes induced by expression of bacterial type III effectors in yeast. *Journal of Visualized Experiments: JoVE*, 37, 1865. <https://doi.org/10.3791/1865>
- Sarris, P. F., Duxbury, Z., Huh, S. U., Ma, Y., Segonzac, C., Sklenar, J., Derbyshire, P., Cevik, V., Rallapalli, G., Saucet, S. B., Wirthmueller, L., Menke, F. L. H., Sohn, K. H., & Jones, J. D. G. (2015). A Plant Immune Receptor Detects Pathogen Effectors that Target WRKY Transcription Factors. *Cell*, 161(5), 1089–1100. <https://doi.org/10.1016/j.cell.2015.04.024>



- Schechter, L. M., Vencato, M., Jordan, K. L., Schneider, S. E., Schneider, D. J., & Collmer, A. (2006). Multiple approaches to a complete inventory of *Pseudomonas syringae* pv. Tomato DC3000 type III secretion system effector proteins. *Molecular Plant-Microbe Interactions: MPMI*, 19(11), 1180–1192. <https://doi.org/10.1094/MPMI-19-1180>
- Schirawski, J., & Perlin, M. H. (2018). Plant–Microbe Interaction 2017—The Good, the Bad and the Diverse. *International Journal of Molecular Sciences*, 19(5). <https://doi.org/10.3390/ijms19051374>
- Schultink, A., Qi, T., Lee, A., Steinbrenner, A. D., & Staskawicz, B. (2017). Roq1 mediates recognition of the *Xanthomonas* and *Pseudomonas* effector proteins XopQ and HopQ1. *The Plant Journal*, 92(5), 787–795. <https://doi.org/10.1111/tpj.13715>
- Schulze, B., Mentzel, T., Jehle, A. K., Mueller, K., Beeler, S., Boller, T., Felix, G., & Chinchilla, D. (2010). Rapid Heteromerization and Phosphorylation of Ligand-activated Plant Transmembrane Receptors and Their Associated Kinase BAK1. *Journal of Biological Chemistry*, 285(13), 9444–9451. <https://doi.org/10.1074/jbc.M109.096842>
- Schulze, E.-D., Beck, E., & Müller-Hohenstein, K. (2005). *Plant Ecology* (D. Lawlor, K. Lawlor, & G. Lawlor, Trans.).
- Schulze, S., Kay, S., Büttner, D., Egler, M., Eschen-Lippold, L., Hause, G., Krüger, A., Lee, J., Müller, O., Scheel, D., Szczesny, R., Thieme, F., & Bonas, U. (2012). Analysis of new type III effectors from *Xanthomonas* uncovers XopB and XopS as suppressors of plant immunity. *New Phytologist*, 195(4), 894–911. <https://doi.org/10.1111/j.1469-8137.2012.04210.x>
- Seybold, H., Trempel, F., Ranf, S., Scheel, D., Romeis, T., & Lee, J. (2014). Ca<sup>2+</sup> signalling in plant immune response: From pattern recognition receptors to Ca<sup>2+</sup> decoding mechanisms. *New Phytologist*, 204(4), 782–790. <https://doi.org/10.1111/nph.13031>
- Sivakumar, N., Sathishkumar, R., Selvakumar, G., Shyamkumar, R., & Arjunekumar, K. (2020). Phyllospheric Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. *Plant Microbiomes for Sustainable Agriculture*, 25, 113–172. [https://doi.org/10.1007/978-3-030-38453-1\\_5](https://doi.org/10.1007/978-3-030-38453-1_5)
- Smakowska-Luzan, E., Mott, G. A., Parys, K., Stegmann, M., Howton, T. C., Layeghifard, M., Neuhold, J., Lehner, A., Kong, J., Grünwald, K., Weinberger, N., Satbhai, S. B., Mayer, D., Busch, W., Madalinski, M., Stolt-Bergner, P., Provart, N. J., Mukhtar, M. S., Zipfel, C., ... Belkhadir, Y. (2018). An extracellular network of *Arabidopsis* leucine-rich repeat receptor kinases. *Nature*, 553(7688), 342–346. <https://doi.org/10.1038/nature25184>
- Staskawicz, B. J., Dahlbeck, D., & Keen, N. T. (1984). Cloned avirulence gene of *Pseudomonas syringae* pv. *Glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proceedings of the National Academy of Sciences of the United States of America*, 81(19), 6024–6028. <https://doi.org/10.1073/pnas.81.19.6024>
- Stork, W., Kim, J.-G., & Mudgett, M. B. (2015). Functional Analysis of Plant Defense Suppression and Activation by the *Xanthomonas* Core Type III Effector XopX. *Molecular Plant-Microbe Interactions: MPMI*, 28(2), 180–194. <https://doi.org/10.1094/MPMI-09-14-0263-R>

- Strange, R. N. (2007). Phytotoxins produced by microbial plant pathogens. *Natural Product Reports*, 24(1), 127–144. <https://doi.org/10.1039/B513232K>
- Sun, H., Zhu, X., Li, C., Ma, Z., Han, X., Luo, Y., Yang, L., Yu, J., & Miao, Y. (2021). Xanthomonas effector XopR hijacks host actin cytoskeleton via complex coacervation. *Nature Communications*, 12(1), 4064. <https://doi.org/10.1038/s41467-021-24375-3>
- Sun, T., Nitta, Y., Zhang, Q., Wu, D., Tian, H., Lee, J. S., & Zhang, Y. (2018). Antagonistic interactions between two MAP kinase cascades in plant development and immune signaling. *EMBO Reports*, 19(7), e45324. <https://doi.org/10.15252/embr.201745324>
- Sun, T., & Zhang, Y. (2021). Short- and long-distance signaling in plant defense. *The Plant Journal: For Cell and Molecular Biology*, 105(2), 505–517. <https://doi.org/10.1111/tpj.15068>
- Tachiyama, S., Skaar, R., Chang, Y., Carroll, B. L., Muthuramalingam, M., Whittier, S. K., Barta, M. L., Picking, W. L., Liu, J., & Picking, W. D. (2021). Composition and Biophysical Properties of the Sorting Platform Pods in the Shigella Type III Secretion System. *Frontiers in Cellular and Infection Microbiology*, 11, 682635. <https://doi.org/10.3389/fcimb.2021.682635>
- Tahir, J., Rashid, M., & Afzal, A. J. (2019). Post-translational modifications in effectors and plant proteins involved in host–pathogen conflicts. *Plant Pathology*, 68(4), 628–644. <https://doi.org/10.1111/ppa.12983>
- Tan, C. M., Li, M.-Y., Yang, P.-Y., Chang, S. H., Ho, Y.-P., Lin, H., Deng, W.-L., & Yang, J.-Y. (2015). Arabidopsis HFR1 is a potential nuclear substrate regulated by the Xanthomonas type III effector XopD(Xcc8004). *PloS One*, 10(2), e0117067. <https://doi.org/10.1371/journal.pone.0117067>
- Tan, L., Rong, W., Luo, H., Chen, Y., & He, C. (2014). The Xanthomonas campestris effector protein XopDXcc8004 triggers plant disease tolerance by targeting DELLA proteins. *The New Phytologist*, 204(3), 595–608. <https://doi.org/10.1111/nph.12918>
- Teper, D., Burstein, D., Salomon, D., Gershovitz, M., Pupko, T., & Sessa, G. (2016). Identification of novel Xanthomonas euvesicatoria type III effector proteins by a machine-learning approach. *Molecular Plant Pathology*, 17(3), 398–411. <https://doi.org/10.1111/mpp.12288>
- Teper, D., Pandey, S. S., & Wang, N. (2021). The HrpG/HrpX Regulon of Xanthomonads—An Insight to the Complexity of Regulation of Virulence Traits in Phytopathogenic Bacteria. *Microorganisms*, 9(1), 187. <https://doi.org/10.3390/microorganisms9010187>
- Teper, D., Sunitha, S., Martin, G. B., & Sessa, G. (2015). Five Xanthomonas type III effectors suppress cell death induced by components of immunity-associated MAP kinase cascades. *Plant Signaling & Behavior*, 10(10), e1064573. <https://doi.org/10.1080/15592324.2015.1064573>
- Thordal-Christensen, H. (2020). A holistic view on plant effector-triggered immunity presented as an iceberg model. *Cellular and Molecular Life Sciences*, 77(20), 3963–3976. <https://doi.org/10.1007/s00018-020-03515-w>

- Thordal-Christensen, H., Birch, P. R. J., Spanu, P. D., & Panstruga, R. (2018). Why did filamentous plant pathogens evolve the potential to secrete hundreds of effectors to enable disease? *Molecular Plant Pathology*, 19(4), 781–785. <https://doi.org/10.1111/mpp.12649>
- Tian, D., Traw, M. B., Chen, J. Q., Kreitman, M., & Bergelson, J. (2003). Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*. *Nature*, 423(6935), 74–77. <https://doi.org/10.1038/nature01588>
- Timilsina, S., Potnis, N., Newberry, E. A., Liyanapathirana, P., Iruegas-Bocardo, F., White, F. F., Goss, E. M., & Jones, J. B. (2020). Xanthomonas diversity, virulence and plant–pathogen interactions. *Nature Reviews Microbiology*, 18(8), 415–427. <https://doi.org/10.1038/s41579-020-0361-8>
- Tollot, M., Assmann, D., Becker, C., Altmüller, J., Dutheil, J. Y., Wegner, C.-E., & Kahmann, R. (2016). The WOPR Protein Ros1 Is a Master Regulator of Sporogenesis and Late Effector Gene Expression in the Maize Pathogen Ustilago maydis. *PLOS Pathogens*, 12(6), e1005697. <https://doi.org/10.1371/journal.ppat.1005697>
- Toruño, T. Y., Stergiopoulos, I., & Coaker, G. (2016). Plant-Pathogen Effectors: Cellular Probes Interfering with Plant Defenses in Spatial and Temporal Manners. *Annual Review of Phytopathology*, 54, 419–441. <https://doi.org/10.1146/annurev-phyto-080615-100204>
- Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: From community assembly to plant health. *Nature Reviews Microbiology*, 18(11), 607–621. <https://doi.org/10.1038/s41579-020-0412-1>
- Turner, P., Barber, C., & Daniels, M. (1984). Behaviour of the transposons Tn5 and Tn7 in *Xanthomonas campestris* pv. *Campestris*. *Molecular and General Genetics MGG*, 195(1), 101–107. <https://doi.org/10.1007/BF00332731>
- Üstün, S., & Börnke, F. (2015). The *Xanthomonas campestris* Type III Effector XopJ Proteolytically Degrades Proteasome Subunit RPT6. *Plant Physiology*, 168(1), 107–119. <https://doi.org/10.1104/pp.15.00132>
- van der Hoorn, R. A. L., & Kamoun, S. (2008). From Guard to Decoy: A New Model for Perception of Plant Pathogen Effectors. *The Plant Cell*, 20(8), 2009–2017. <https://doi.org/10.1105/tpc.108.060194>
- van Wersch, R., Li, X., & Zhang, Y. (2016). Mighty Dwarfs: Arabidopsis Autoimmune Mutants and Their Usages in Genetic Dissection of Plant Immunity. *Frontiers in Plant Science*, 7, 1717. <https://doi.org/10.3389/fpls.2016.01717>
- VanEtten, H. D., Mansfield, J. W., Bailey, J. A., & Farmer, E. E. (1994). Two Classes of Plant Antibiotics: Phytoalexins versus “Phytoanticipins.” *The Plant Cell*, 6(9), 1191–1192. <https://doi.org/10.1105/tpc.6.9.1191>
- Vicente, J. G., & Holub, E. B. (2013). *Xanthomonas campestris* pv. *Campestris* (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. *Molecular Plant Pathology*, 14(1), 2–18. <https://doi.org/10.1111/j.1364-3703.2012.00833.x>

- Vorwerk, S., Somerville, S., & Somerville, C. (2004). The role of plant cell wall polysaccharide composition in disease resistance. *Trends in Plant Science*, 9(4), 203–209. <https://doi.org/10.1016/j.tplants.2004.02.005>
- Wang, G., Roux, B., Feng, F., Guy, E., Li, L., Li, N., Zhang, X., Lautier, M., Jardinaud, M.-F., Chabannes, M., Arlat, M., Chen, S., He, C., Noël, L. D., & Zhou, J.-M. (2015). The Decoy Substrate of a Pathogen Effector and a Pseudokinase Specify Pathogen-Induced Modified-Self Recognition and Immunity in Plants. *Cell Host & Microbe*, 18(3), 285–295. <https://doi.org/10.1016/j.chom.2015.08.004>
- Wang, Y., Li, J., Hou, S., Wang, X., Li, Y., Ren, D., Chen, S., Tang, X., & Zhou, J.-M. (2010). A *Pseudomonas syringae* ADP-ribosyltransferase inhibits Arabidopsis mitogen-activated protein kinase kinases. *The Plant Cell*, 22(6), 2033–2044. <https://doi.org/10.1105/tpc.110.075697>
- Wei, H.-L., & Collmer, A. (2018). Defining essential processes in plant pathogenesis with *Pseudomonas syringae* pv. Tomato DC3000 disarmed polymutants and a subset of key type III effectors. *Molecular Plant Pathology*, 19(7), 1779–1794. <https://doi.org/10.1111/mpp.12655>
- Weiberg, A., Wang, M., Lin, F.-M., Zhao, H., Zhang, Z., Kaloshian, I., Huang, H.-D., & Jin, H. (2013). Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNA Interference Pathways. *Science (New York, N.Y.)*, 342(6154), 118–123. <https://doi.org/10.1126/science.1239705>
- Weßling, R., Eppe, P., Altmann, S., He, Y., Yang, L., Henz, S. R., McDonald, N., Wiley, K., Bader, K. C., Gläßer, C., Mukhtar, M. S., Haigis, S., Ghamsari, L., Stephens, A. E., Ecker, J. R., Vidal, M., Jones, J. D. G., Mayer, K. F. X., van Themaat, E. V. L., ... Braun, P. (2014). Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell Host & Microbe*, 16(3), 364–375. <https://doi.org/10.1016/j.chom.2014.08.004>
- Wroblewski, T., Caldwell, K. S., Piskurewicz, U., Cavanaugh, K. A., Xu, H., Kozik, A., Ochoa, O., McHale, L. K., Lahre, K., Jelenska, J., Castillo, J. A., Blumenthal, D., Vinatzer, B. A., Greenberg, J. T., & Michelmore, R. W. (2009). Comparative Large-Scale Analysis of Interactions between Several Crop Species and the Effector Repertoires from Multiple Pathovars of *Pseudomonas* and *Ralstonia*. *Plant Physiology*, 150(4), 1733–1749. <https://doi.org/10.1104/pp.109.140251>
- Xia, Y., Ma, Z., Qiu, M., Guo, B., Zhang, Q., Jiang, H., Zhang, B., Lin, Y., Xuan, M., Sun, L., Shu, H., Xiao, J., Ye, W., Wang, Y., Wang, Y., Dong, S., Tyler, B. M., & Wang, Y. (2020). N-glycosylation shields *Phytophthora sojae* apoplastic effector PsXEG1 from a specific host aspartic protease. *Proceedings of the National Academy of Sciences*, 117(44), 27685–27693. <https://doi.org/10.1073/pnas.2012149117>
- Yamada, K., Yamaguchi, K., Shirakawa, T., Nakagami, H., Mine, A., Ishikawa, K., Fujiwara, M., Narusaka, M., Narusaka, Y., Ichimura, K., Kobayashi, Y., Matsui, H., Nomura, Y., Nomoto, M., Tada, Y., Fukao, Y., Fukamizo, T., Tsuda, K., Shirasu, K., Kawasaki, T. (2016). The Arabidopsis CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. *The EMBO Journal*, 35(22), 2468–2483. <https://doi.org/10.15252/emboj.201694248>

- Yan, H., Zhao, Y., Shi, H., Li, J., Wang, Y., & Tang, D. (2018). BRASSINOSTEROID-SIGNALING KINASE1 Phosphorylates MAPKKK5 to Regulate Immunity in Arabidopsis. *Plant Physiology*, 176(4), 2991–3002. <https://doi.org/10.1104/pp.17.01757>
- Yan, X., Tao, J., Luo, H.-L., Tan, L.-T., Rong, W., Li, H.-P., & He, C.-Z. (2019). A type III effector XopLXcc8004 is vital for *Xanthomonas campestris* pathovar *campestris* to regulate plant immunity. *Research in Microbiology*, 170(3), 138–146. <https://doi.org/10.1016/j.resmic.2018.12.001>
- Yeats, T. H., & Rose, J. K. C. (2013). The Formation and Function of Plant Cuticles. *Plant Physiology*, 163(1), 5–20. <https://doi.org/10.1104/pp.113.222737>
- Yi, S. Y., Shirasu, K., Moon, J. S., Lee, S.-G., & Kwon, S.-Y. (2014). The Activated SA and JA Signaling Pathways Have an Influence on flg22-Triggered Oxidative Burst and Callose Deposition. *PLOS ONE*, 9(2), e88951. <https://doi.org/10.1371/journal.pone.0088951>
- Yip Delormel, T., & Boudsocq, M. (2019). Properties and functions of calcium-dependent protein kinases and their relatives in *Arabidopsis thaliana*. *New Phytologist*, 224(2), 585–604. <https://doi.org/10.1111/nph.16088>
- Yuan, M., Jiang, Z., Bi, G., Nomura, K., Liu, M., Wang, Y., Cai, B., Zhou, J.-M., He, S. Y., & Xin, X.-F. (2021). Pattern-recognition receptors are required for NLR-mediated plant immunity. *Nature*, 592(7852), 105–109. <https://doi.org/10.1038/s41586-021-03316-6>
- Yuan, M., Ngou, B. P. M., Ding, P., & Xin, X.-F. (2021). PTI-ETI crosstalk: An integrative view of plant immunity. *Current Opinion in Plant Biology*, 62, 102030. <https://doi.org/10.1016/j.pbi.2021.102030>
- Zhang, J., Li, W., Xiang, T., Liu, Z., Laluk, K., Ding, X., Zou, Y., Gao, M., Zhang, X., Chen, S., Mengiste, T., Zhang, Y., & Zhou, J.-M. (2010). Receptor-like Cytoplasmic Kinases Integrate Signaling from Multiple Plant Immune Receptors and Are Targeted by a *Pseudomonas syringae* Effector. *Cell Host & Microbe*, 7(4), 290–301. <https://doi.org/10.1016/j.chom.2010.03.007>
- Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., Zou, Y., Long, C., Lan, L., Chai, J., Chen, S., Tang, X., & Zhou, J.-M. (2007). A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants. *Cell Host & Microbe*, 1(3), 175–185. <https://doi.org/10.1016/j.chom.2007.03.006>
- Zhou, J.-M., & Zhang, Y. (2020). Plant Immunity: Danger Perception and Signaling. *Cell*, 181(5), 978–989. <https://doi.org/10.1016/j.cell.2020.04.028>
- Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., & Felix, G. (2006). Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation. *Cell*, 125(4), 749–760. <https://doi.org/10.1016/j.cell.2006.03.037>
- Zipfel, C., & Oldroyd, G. E. D. (2017). Plant signalling in symbiosis and immunity. *Nature*, 543(7645), 328–336. <https://doi.org/10.1038/nature22009>

Zupan, J., Muth, T. R., Draper, O., & Zambryski, P. (2000). The transfer of DNA from *Agrobacterium tumefaciens* into plants: A feast of fundamental insights. *The Plant Journal*, 23(1), 11–28. <https://doi.org/10.1046/j.1365-313x.2000.00808.x>