Genetic bases of natural variation of Arabidopsis thaliana response to Ralstonia solanacearum in a global warming context

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To cite this version:

Nathalie Aoun. Genetic bases of natural variation of Arabidopsis thaliana response to Ralstonia solanacearum in a global warming context. Cell Behavior [q-bio.CB]. Université Paul Sabatier - Toulouse III, 2019. English. ⟨NNT : 2019TOU30219⟩. ⟨tel-05215634⟩

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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

Présentée et soutenue par

NATHALIE AOUN

Le 18 décembre 2019

Identification des bases génétiques de la variation naturelle d'Arabidopsis thaliana à Ralstonia solanacearum dans le contexte de réchauffement climatique

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

Spécialité : **Interactions plantes-microorganismes**

Unité de recherche :
LIPM - Laboratoire des Interactions Plantes-Microorganismes

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Acknowledgments

I would like to take the opportunity to thank everyone who guided me, helped me and believed in me during these three years and yet before.

I would like to thank first the jury of my thesis who accepted to read and evaluate my work. I would also like to thank Antoine Gravot and Jean-Philippe Galaud for their advice and interest in my project during both of my thesis committees. Finally, I would like to thank the ministry of higher education, research and innovation and INRA for funding my three-years PhD project.

Most importantly, I would like to thank my incredible supervisors, Richard and Fabrice. I was lucky having you supervise my project. Your patience and motivation for this project was the core of its progress. Thank you for your open spirit, support, motivation and encouragements. Richard, you trusted my work since I first started with this project during my master 2 internship, you guided me and taught me a lot about research work. I really enjoyed working on this project with you, thank you for your generous input in it. Fabrice, I also really enjoyed working with you, especially when we had to spend long days on preparing a paper, I learned so much from you during these days. You were supportive and I am still amazed by your dedication to science. I also learned so much from you by watching you manage your team, your students and all the projects you were committed to.

I am thankful for TULIP Labex who gave me the once in a lifetime opportunity to participate in the “Biological interactions: from genes to ecosystems” summer school. More particularly, I would like to thank Dominique Roby, Etienne Danchin and Guillaume Cassiede-Berjon.

This PhD would not have been enjoyable without the members of the REACH team. Laurent, thank you for teaching me about cloning and all your advices on molecular biology. Dominique, you’ve been very sweet. Also, it was really interesting to see your dedication for science popularization. Patrick, thank you for the “haribo aux réglisses” supply! And for taking care of my plants when I was away. Maud, it has been nice having you in the team and to discuss with you, about Australia mostly. Céline, thank you for always caring about everyone in the team. Henri, thank you for all the advices on R and the interesting discussions about genetics. Virginie, thank you for always smiling and the positivity and your advices on infiltration. Maxime, thank you for your help when I had questions on certain protocols . I would also like to thank Eline, who I supervised during my last year of PhD, for her help in the project.

I would also like to thank people I worked with during this thesis:

- Thank you Fabrice Devoilles, Claudette Icher and Marine Pinsard, for taking care of my plants in the greenhouse.
- Bioinformatics service: Jérôme Gouzy, Sébastien Carrere, Ludovic Legrand and Erika Sallet. Thank you for the work you have done on genome assembly and annotation and for your time whenever I had a question.
- Thank you for Dominique Senac, Christian Pince and Florian Delhommeau from the “laverie”, you made our lives easier.
- Marie Dideron, Mehdi Khafif and Sylvain Raffaele, thank you for providing me with the growth chambers for my last experiment.
- Baptiste, thank you for your help in extracting DNA with high-molecular weight.
- Thank you Nemo Peeters for giving me the access to the TPMP to do some HR tests.
- Thank you for the administration for always being efficient with our demands. Specially Christophe Sartori, thank you for all the paper work you did for all the congresses we had to attend.
- I would like to thank also our neighbors in the corner, the ENOD team: Agnès, Andréas, Fernanda and Lisa, it’s been really nice exchanging with you.
- Thank you Xavier for taking care of the L2.
- Fabienne Vailleau, thank you for orienting me before getting into the research field and for recommending me for the internship at the beginning of this project.

Behind this thesis, there’s also a big moral support that comes from family and friends; Gracia, Maro, Jaishree, Nargess, Carolina, Laura, Choghag, Richard, Hélène. I thank you for that. My warm gratitude goes certainly to Elli and Rekha for taking care of me during the good and bad days.

I want to thank Charly, who was my biggest support through all these years. You taught me to be stronger than anything in life, to be patient and hard-willing. In my toughest times you were my solid rock.

Finally, I would like to thank my family for permanently believing in me and supporting me at all levels. Thank you mom for being the first who encouraged me to pursue my career at a “different geographical scale”. Your patience and strong will in life made me who I am today. Thank you dad for teaching me that I can reach anything I want in life with hard work and dedication. Your support made me stronger. I want to thank my sister for also encouraging me to pursue my career abroad. Laurent, thank you for being the best brother anyone could wish for. You taught me patience and you have always had my back.

“I did then what I knew how to do. Now that I know better, I do better.”

Maya Angelou

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Abbreviations

ABA = abscisic acid
ABRC = Arabidopsis Biological Resource Center
ADP = Adenosine diphosphate
AI-RIL = Advanced Intercross – Recombinant Inbred Lines
AmiRNA = artificial microRNA
APHIS = Animal and Plant Health Inspection Service
ATP = Adenosine triphosphate
AUX = auxins
avr = avirulence proteins
BAR = Bio-Analytic Resource for Plant Biology
BDB = Blood Disease Bacterium
BLUP = Best Linear Unbiased Prediction
BR = Brassinosteroids
BSA = Bulk Segregant Approach
cfu = colony forming unit
CC = coiled-coil
CDPK = Calcium Dependent Protein Kinases
CK = cytokinins
CoIP = Co-Immunoprecipitation
Col-0 = Columbia-0
Col-5 = Columbia-5
dai = days after inoculation
DAMPs = Danger-Associated Molecular Patterns
DI = disease index
DNA = Deoxyribonucleic acid
DsDNA = double strand DNA
EC = European Commission
EDS1 = Enhanced Disease Susceptibility 1
Egl = Endonucleases
EMS = Ethyl methanesulfonate
ET = Ethylene
ETI = Effector Triggered Immunity
ETS = Effector Triggered Susceptibility
FRET = Förster resonance energy transfer
GA = Giberellins
GMI1000 = Génétique des Microorganismes INRA 1000
GW-LS = genome-wide local score approach

GWA = Genome Wide Association
HR = Hypersensitive Response
HRP = Horseradish peroxidase
HSC70/HSP70 = Heat shock cognate 71 kDa protein/Heat shock protein 70 family
INRA = Institut National de la Recherche Agronomique
JA = Jasmonic acid
JAZ9 = jasmonate 9
JAX1 = JACALIN-TYPE LECTIN REQUIRED FOR POTEXIVIRUS RESISTANCE1
HIF = Heterogeneous Inbred Family
JAX1 = JACALIN-TYPE LECTIN REQUIRED FOR POTEXIVIRUS RESISTANCE1
Ler-0 = Landsberg-0
LIPM = Laboratory of Plant-Microorganisms Interactions
LD = Linkage Disequilibrium
LRR = Leucine-Rich Repeat domain
MAGIC = Multiparent Advanced Generation Intercross
MAPKs = Mitogen-Activated Protein Kinases
MARF = minor allele relative frequency
Mb = Megabasis
MLST = MultiLocus Sequence Typing
MOS12 = Modifier of SNC1, 12
MOS4 = Modifier of SNC1, 4
mRNA = messenger RNA
MS = Murashige and Skoog
NASA = National Aeronautics and Space Administration
NASC = Nottingham Arabidopsis Stock Centre
NB = nucleotide-binding domain
NB-LRR = nucleotide-binding leucine-rich repeat
NBS= Nucleotide Binding Site
Nd-1 = Niedersen-
NDR1 = Non-Race-Specific Disease resistance 1
Ne = not estimated
NILs = Near-Isogenic Lines
NLR = Nod-like Receptr
NOAA = National Oceanic and Atmospheric
ns = non-significant
OD = optical density
PAMP = pathogen associated molecular pattern
Pfo-1 = Pseudomonas fluorescens strain
PIAMV = Plantago asiatica mosaic virus
PopP2 = Putative outer protein 2

PRR = pathogen recognition receptor
PTI = PAMP-triggered immunity
QDR = Quantitative Disease Resistance
QRS = Quantitative Resistance to *Ralstonia solanacearum*
QTL = Quantitative Trait Loci
RCBD = a randomized complete block design
RFO1 = RESISTANCE TO FUSARIUM OXYSPORUM 1
RNA = Ribonucleic acid
RNAse A = ribonuclease A
rRNA = ribosomal ribonucleic acid
ROS = Reactive Oxygen Species
RILs = Recombinant Inbred Lines
RH = relative humidity
RRS1 = Resistance to Ralstonia Solanacearum 1
RPS4 = Resistance to Pseudomonas syringae 4
RSSC = Ralstonia Solanacearum Species Complex
SA = Salicylic acid
SNP = Single Nucleotide Polymorphism
SDS = cyclin-like protein SOLO DANCERS
SPL = SQUAMOSA PROMOTER BINDING PROTEIN-LIKE
SSL4 = Strictosidine synthase-like 4
SSL5 = Strictosidine synthase-like 5
STAND = signal transduction ATPases with Numerous Domains
TAIR = The *Arabidopsis* International Resource
TAE = Tris Acetate-EDTA
TF = transcription factor
TIR = Toll, inerleukin-1 receptor
T3E = Type III Effector
T2SS = type II secretion system
T3SS = type III secretion system
USDA = United States Department of Agriculture
Ws-2 = Wassilewskija-2
Ws-4 = Wassilewskija-4
Xcc = Xanthomonas campestris pv. *Campestris*
Y2H = Yeast two-hybrid
YopJ = *Yersinia* outer protein J

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Chapter I. General introduction

A. General overview

Climate change has been in the center of scientific interest since the end of 19th century and is gaining more attention nowadays due to its impact on a global scale. In the 1860s, John Tyndall, an Irish physicist, was the first to report the Earth's greenhouse effect and hypothesized that slender changes in the atmospheric composition can lead to variations in the climate (Tyndall, 1861). Svante Arrhenius (1896), a Swedish chemist, was the first to speculate on the extent to which carbon dioxide can influence Earth’s surface temperature and contribute to long-term variations in climate. Following him, an American scientist, Charles David Keepling (1961) was the first to alert about the contribution of human activity to global warming. In this context and until now, research devoted to climate warming has predicted scenarios among which a rise of temperature from 1°C to 4.8°C is expected by the end of the century (IPCC, 2018). Temperature anomaly has been fluctuating since 1880 with an increasing tendency (figure 1; Lenssen et al., 2019).

Current climate change is likely to favor conditions for development as well as higher/faster migration and adaptation potentials of pathogens (Bergot et al., 2004; Garrett et al., 2006; Tylianakis et al., 2008). Accordingly, climate change scenarios predict a poleward geographic expansion of pathogen distribution and an increase in epidemics severity in plants (Bebber et al., 2013; Bergot et al., 2004; Evans et al., 2008). An increase in the frequency and strength of epidemics will undoubtedly lead to important crop yield losses, and therefore threatens food security (figure 2; Bebber, 2015; IPCC 2014, 2018).

A large range of means can control pests and pathogens. The chemical control or the use of pesticides is possible, but it can be harmful to the environment. Using eco-friendly agricultural practices such as crop rotation, certified seeds and plantlets, use of biopesticides, etc., is also an important mean of pathogen control but its efficiency is generally limited to specific pathogenic species. Another way to fight pathogens is the use of plant genetic control by exploiting robust plant defenses. Using natural plant defense against microbial pathogens has been shown to be highly successful in both wild plants and crops (Dropkin, 1969; Narusaka et al., 2013; Onaga and Asea, 2016).

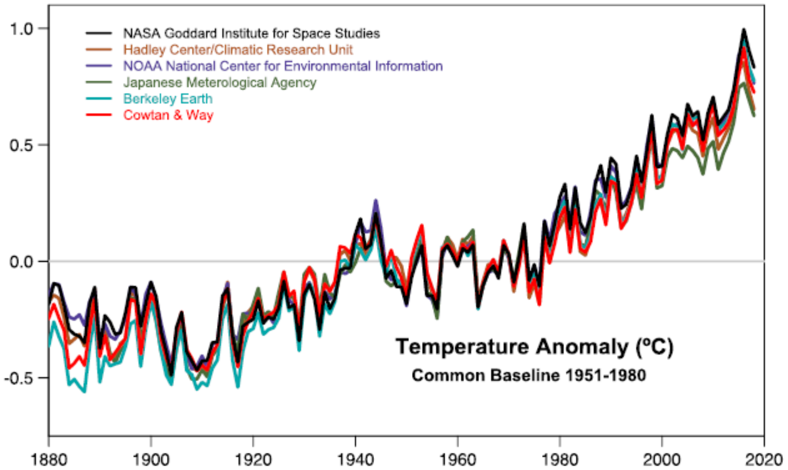

Figure 3. Annual global surface temperature anomaly through 2018, compared from six analyses. NASA = National Aeronautics and Space Administration; NOAA = National Oceanic and Atmospheric (Lenssen et al., 2019).

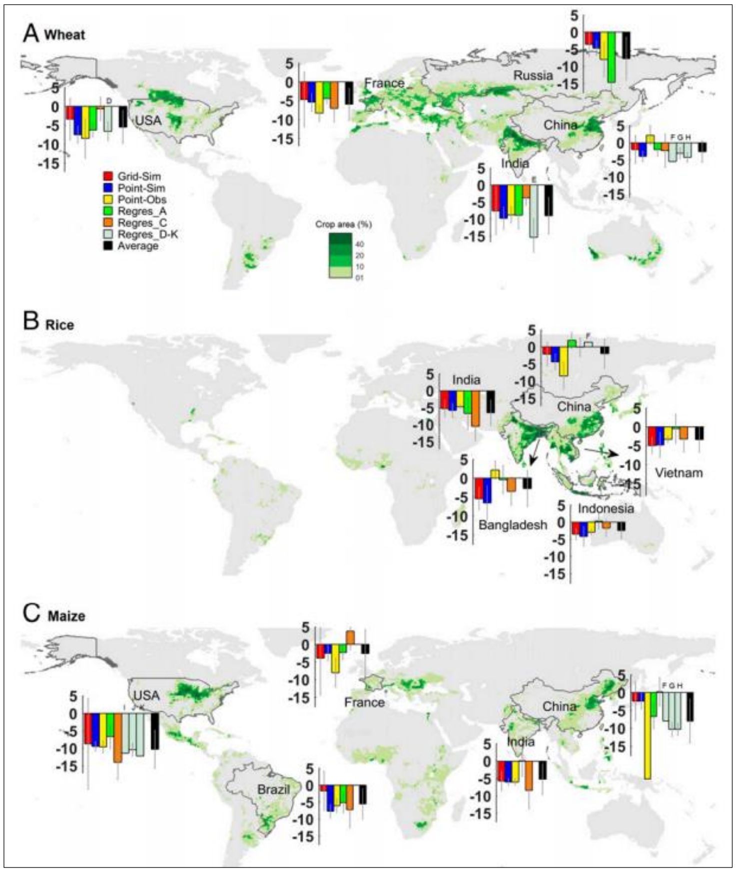

Figure 4. Estimated yield of wheat, rice and maize, in the five major producing countries, upon 1°C increase of global temperature. Colors represent different methods of simulations. The y-axis represents the impact of temperature on crop yield in percent per degree Celsius increase (Zhao et al., 2017).

B. Physical barriers and immune signaling responses as defense mechanisms against microbial pathogens

The main plant defence mechanisms against a microbial pathogen attack have been studied for a long time and are well characterized. These works helped to build and update a conceptual model of plant defence and pathogens recognition, which are detailed in many reviews (Dangl and Jones, 2001; Cohn et al., 2001; Chisholm et al., 2006; Jones and Dangl, 2006; Dodds and Rathjen, 2010; Schneider and Collmer, 2010; Muthamilarasan and Prasad, 2013; Miller et al., 2017). However, I must mention that a bibliometric analysis realized by Gimenez et al. (2018) on papers published in that field between 1979 and 2016 revealed that a majority of the studies were mainly performed on *A. thaliana*, using either lab mutants obtained through a mutant screening or a single (or few) natural accessions. In addition, most studies were carried out on a limited number of pathogens, mainly on the bacterium *Pseudomonas syringae*. However, since 2017 the dynamic seems to be reversed, with a decrease of studies on *A. thaliana* and an increase of studies on crop species (tomato and wheat). In this part, I will give a short overview of the main known mechanisms.

Preformed barriers found on the surface of the plant organs, constitute a first strategy to prevent pathogens entry. They correspond to wax layers, rigid cell walls, cuticle lipids (Reina-Pinto and Yephremov, 2009), antimicrobial enzymes (Habib and Fazili, 2007) or secondary metabolites (Ahuja et al., 2012; Bednarek, 2012). Pathogens that overcome these first barriers will systematically be confronted to a defence mechanism based on two layers of immune response well known as the zig-zag model (Jones and Dangl, 2006; Dodds and Rathjen, 2010). The first layer relies on the recognition of conserved pathogen associated molecular patterns (PAMPs) by transmembrane recognition receptors (PRRs) leading to PAMP-triggered immunity (PTI). This defence response is usually described as non-specific and confers weak resistance to a broad spectrum of pathogens (Dangl and Jones, 2001; Shiu and Bleecker, 2001; Jones and Dangl, 2006; Boller and Felix, 2009; Monaghan and Zipfel, 2012). Successful pathogens have evolved strategies to bypass PTI by injecting a battery of effector proteins (T3E) into the cytoplasm through a type III secretion system (T3SS) (Cornelis and Van Gijsegem, 2000; Grant et al., 2006; Deslandes and Rivas, 2012). These T3E promote the Effector Triggered Susceptibility (ETS) through different manipulation of the host cell (Feng and Zhou, 2012). The second layer of defence is based on the recognition of these effectors by highly specific and specialized receptors inside the cell leading to an Effector Triggered Immunity (ETI) (Dodds and Rathjen, 2010; Muthamilarasan and Prasad, 2013; Miller et al., 2017). ETI, also designated as qualitative resistance, leads to complete disease resistance phenotypes. ETI is generally species- and strain-specific, which results in a strong selective pressure on strains to overcome this response (Roux et al., 2014). ETI often induces a hypersensitive responses (HR) characterized by the cell death of infected cells and the surrounding areas to limit pathogens propagation (Mur et al., 2008). The receptor proteins, encoded by *R* genes, belong in majority to the NLR protein family characterized by a nucleotide-binding domain (NB) and a Leucine-Rich-Repeat domain (LRRs) (Jones and Dangl, 2006). Depending on the amino acid domain localized at their N-termini, these immune-receptors are classified into CC-NB-LRR (Coil-coiled) and TIR-NB-LRR (Toll, inerleukin-1 receptor) proteins (Eitas and Dangl, 2010). NLR proteins recognize effectors either by their direct physical interaction with an effector or through indirect interaction mediated by accessory-proteins (Muthamilarasan and Prasad, 2013). The nature of these accessory proteins is at the origin of different defence response models. In the guard model, these proteins are real targets of effectors. In the decoy model, they correspond to proteins, whether or not integrated into the NLR, which have evolved to mimic effector targets (Dangl and Jones, 2001; van der Hoorn and Kamoun, 2008; Dodds and Rathjen, 2010; Le Roux et al., 2015).

The signalling pathways operating immediately downstream of PRR and NLR protein activation is not yet fully elucidated. PTI signalling is linked to the activation of Mitogen-Activated Protein Kinases (MAPKs) cascade or Calcium Dependent Protein Kinases (CDPK) and some genes were also identified as part of ETI signalling, such as EDS1 (Enhanced Disease Susceptibility 1) and NDR1 (Non-Race-Specific Disease resistance 1) (Dodds and Rathjen, 2010). Therefore, PTI and ETI downstream events present a significant overlap, the distinction being mainly in term of response magnitude and robustness (Dodds and Rathjen, 2010; Tsuda and Katagiri, 2010, Göhre et al., 2012). The first response triggered by both mechanisms is the activation of membrane-localized ion channels leading to cytoplasmic calcium levels increase. Following stages correspond to the production of Reactive Oxygen Species (ROS), transcriptional reprogramming, callose deposition on the cell wall at sites of infection (Dodds and Rathjen, 2010; Muthamilarasan and Prasad, 2013). Phytohormomes such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play also an important role in the downstream responses of ETI and PTI (Gimenez et al., 2018). They seem to act antagonistically to some extent since SA pathway is involved in resistance to biotrophic and hemibiotrophic pathogens while JA-ET pathway is involved in response to necrotrophic pathogens and chewing insects (Dodds and Rathjen, 2010; De Vleesschauwer et al., 2014; Gimenez-Ibanez et al., 2018). Other hormones such as abscisic acids (ABA), auxins (AUX), brassinosteroids (BR), cytokinins (CK) and gibberellins (GA) have also been reported as important regulators of immune responses (Bari and Jones, 2009).

As many incomplete resistances are frequently observed in natural habitats or crop fields, several studies proposed that such phenomenon is explained by another defence mechanism called quantitative disease resistance (QDR). QDR is characterized by a continuous distribution of resistance phenotypes within a population rather than by a total absence of disease and a polygenic origin (Roux et al., 2014; French et al., 2016). Knowing that the QDR provides a broad-spectrum resistance, it appears to be much more prevalent than ETI in natural plant populations and crop fields (Young, 1996). Accordingly, by establishing a first list of 11 cloned genes contributing to QDR, French et al. (2016) highlighted that these QDR genes are often effective against a wide range of pathogens but also specific to the developmental stage, spatiotemporally regulated and dependent on environment. Importantly, only few cloned QDR genes correspond to nucleotide-binding leucine-rich repeat (NB-LRR) genes (Broglie et al., 2011; Fukuoka et al., 2014; Staal et al., 2006). On the contrary, most QDR genes encode for a broad range of molecular functions (Balint-Kurti and Holland, 2015; Roux et al., 2014), including (i) the kinase-START WKS1 conferring resistance to wheat stripe rust (Fu et al., 2009) (ii) the putative ABC transporter LR34 conferring resistance to multiple fungal pathogens in wheat (Krattinger et al., 2009), (iii) the serine hydroxymethyltransferase RGH4 conferring cyst nematode resistance in soybean (Liu et al., 2012), and (iv) the atypical kinase RKS1 conferring resistance to several races of the bacterial pathogen *Xanthomonas campestris* pv *campestris (Huard-Chauveau et al., 2013; Debieu et al., 2016)*. Together, these studies suggest that the molecular mechanisms underlying QDR may be more diverse than anticipated (Roux et al., 2014).

C. Impact of heat stress on plant immunity

Heat stress is one of the major abiotic stresses and a main component of climate warming that can affect plant growth and development. A temperature increase is considered as a heat stress when temperature shifts above the optimal growth temperature of plants and causes damages (Zaidi et al., 2014; Liu et al., 2015). A heat stress is also defined according to the applied temperature range while depending on the species. For instance, in the case of *Arabidopsis thaliana*, temperatures are considered warm between 22°C and 27°C, high between 27°C and 30°C and extremely high between 37°C and 42°C (Liu et al., 2015).

Numerous studies have assessed plant response to heat stress at the phenotypic, physiological and molecular levels (Saidi et al., 2011; Bita and Gerats, 2013; Hasanuzzaman et al., 2013; Hatfield and Prueger, 2015; Gray and Brady, 2016; Nievola et al., 2017). Importantly, all the major forms of immunity described in the previous section can be drastically affected by heat stress. Regardless of the plant pathosystem considered, numerous studies reported that an increase in temperature inhibits several major defense mechanisms induced following pathogen attack, notably by suppressing ETI-HR related phenotypes (de Jong et al., 2002; Xiao et al., 2003; Yang and Hua, 2004; Wang et al., 2009; Cheng et al., 2013; Menna et al., 2015; Aoun et al., 2017).

However, most of these studies have focused on the impact of more than 5°C of temperature increase, which does not corroborate with the expected increase of global surface temperature means in the climate changes scenarios. Thus, what is the impact of a lower increase of temperature on plant resistance? It is therefore highly important and urgent to find efficient mechanisms of resistance maintained under realistic heat stress.

The main findings on the impact of heat stress on plants, pathogens and their interactions are summarized in the following review co-authored with Henri Desaint, another PhD student from the team.

Review article: Fight hard or die trying: plants *versus* pathogens under heat stress

Authors

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Abstract

In their natural environment, plants are exposed to biotic or abiotic stresses that can occur simultaneously or sequentially. However, plant responses to these stresses have been widely studied and well characterized in simple systems involving the interaction of one single plant species with only one stress. Because temperature elevation is one of the main abiotic drivers of climate change and scenarios predict an increase in the number and severity of epidemics, we reviewed here our current knowledge on the effects of heat stress on plant-pathogenic microorganism interactions. In this review, we investigated 46 available studies on model or crop species. We discussed the possible implications of the optimal growth temperature of partners, mode of stress application and temperature variation magnitude on resistance modulations. The verdict is worrying: a majority of the identified resistances appears to be altered by a temperature elevation, regardless of the plant and pathogen species. Thereby, we listed current knowledge on the plant's heat-dependent immune mechanisms and on the thermos-sensory processes predominantly studied in mammals and human pathogens, which could help to understand the outcome of plant/pathogen/elevated temperature interactions. Finally, we provide recommendations enabling to obtain a general overview of the mechanisms involved in the plant's response to a pathogen, integrating multiple interactions with its biotic environment in order to optimize plant immune response and identify resistance mechanisms remaining efficient under elevated temperature conditions.

Keywords

Plant-pathogen-abiotic stress interactions, combined stress, resistance, temperature elevation, pathogen infection

Introduction

To survive and reproduce, wild and cultivated plants have to deal with multiple constraints often occurring simultaneously during their life cycle. The complex sequence of molecular, genetic and physiological mechanisms leading to plant responses is defined as a stress that can be positive or negative (Brussaard et al., 2007; Suzuki et al., 2014; Pandey et al., 2017; Engelberth and Engelberth, 2019). Depending on the nature of the triggering factor, stresses are classified in two categories, biotic and abiotic. The first category is induced by other living organisms such as weeds, insects, bacteria, fungi and viruses, which may be either beneficial (symbiotic interactions) or harmful (i.e. competitive and pathogenic interactions). The second category, caused by non-living factors, involves for example drought, pollution, soil salinity, non-optimal temperatures and variations in irradiation or in water or in nutrients availability. Due to their sessile nature, the ability of plants to adapt to these stresses is crucial. Until recently, the identification and the study of physiological, genetic and molecular mechanisms involved in plant response have mainly focused on stresses applied separately, while the number of studies on combined stresses remains surprisingly small (Suzuki et al., 2014; Pandey et al., 2017; Zhang and Sonnewald, 2017). Studies are even scarcer when it comes to plant-pathogen-abiotic factors interactions. In all cases, it appears that involved mechanisms, studied mainly at the plant level, are complex and significantly different from the response to an individual stress (Pandey et al., 2015; Kissoudis et al., 2016; Zhang and Sonnewald, 2017; Bai et al., 2018). Plants developed different strategies to face pathogens attacks. Preformed components on the surface of plant organs such as wax layer, rigid cell walls, cuticular lipids (Reina-Pinto and Yephremov, 2009), antimicrobial enzymes (Habib and Fazili, 2007) or secondary metabolites (Ahuja et al., 2012; Piasecka et al., 2015) constitute a first barrier to the entry of pathogens. Pathogens overcoming these first impediments will systematically confront defense responses induced in the plant following their perception. They have been studied for a long time and are well characterized (Jones and Dangl, 2006; Boller and Felix, 2009; Dodds and Rathjen, 2010; Monaghan and Zipfel, 2012; Muthamilarasan and Prasad, 2013; Miller et al., 2017). Briefly, two layers of defense have been proposed and constitute the core of the zig-zag model (Jones and Dangl, 2006). The first layer relies on the recognition of conserved pathogen associated molecular patterns (PAMPs) by transmembrane recognition receptors (PRRs) leading to PAMP-triggered immunity (PTI). This immune response is non-specific and confers weak resistance to a broad spectrum of pathogens. Successful pathogens have evolved strategies to bypass PTI by injecting a battery of effector proteins (T3E) into the cytoplasm thanks to a type III secretion system (T3SS), promoting the Effector Triggered Susceptibility (ETS) (Cornelis and Van Gijsegem, 2000; Grant et al., 2006; Deslandes and Rivas, 2012). The second layer of defense is based on the recognition of these effectors by highly specialized receptors, encoded by *R* genes, inside the cell, leading to an Effector Triggered Immunity (ETI). These receptor proteins belong in majority to the NLR protein family characterized by a nucleotide-binding domain (NB) and a Leucine-Rich-Repeat domain (LRRs) (Jones and Dangl, 2006). Depending on the amino acid domain localized at their N-termini, these immunoreceptors are classified into CC-NB-LRR (Coil-coiled) and TIR-NB-LRR (Toll, inerleukin-1 receptor) proteins (Eitas and Dangl, 2010). ETI, also designated as qualitative resistance, leads to complete disease resistance phenotypes. ETI often induces a hypersensitive response (HR) characterized by cell death of infected cells and the surrounding areas to limit pathogens propagation (Mur et al., 2008). ETI is generally species- and strain-specific, which results in a strong selective pressure of strains to overcome this response (Roux et al., 2014). As many incomplete resistances are more frequently observed than ETI in natural populations and crop fields (Young, 1996; Bartoli and Roux, 2017), several studies proposed that such phenomenon is explained by another defense mechanism called quantitative disease resistance (QDR). QDR is characterized by a continuous distribution of resistance phenotypes within a population rather than a total absence of disease and by a polygenic architecture (Roux et al., 2014; French et al., 2016; Bartoli and Roux, 2017). In addition, QDR confers broad-spectrum resistance. Accordingly, French et al. (2016) highlighted that QDR genes are often effective against a wide range of pathogens but are also specific to a developmental stage, spatially and temporally regulated and dependent on the environment.

In the context of climate change, scenarios predict variations in all components of the climate system, resulting in more intense, frequent and long lasting extreme weather events worldwide (Pachauri et al., 2015; Allen et al., 2019). The speed, brutality and severity of projected changes represent a major threat of unknown magnitude, which would increase the likelihood of altering species distribution areas and ecosystems functioning (Bebber, 2015), thereby affecting natural biodiversity (Pimm et al., 2014; Ocampo-Penuela et al., 2016) and global food security (Pachauri et al., 2015; Allen et al., 2019). Amongst climatic risks, an increase of mean temperatures is one of the main abiotic fluctuation to which plants must adapt (Bita and Gerats, 2013; Suzuki et al., 2014; André C. Velásquez et al., 2018). Based on climatic models, average global surface temperature is predicted to rise from 1.5 up to 4.8°C by the end of the century (Pachauri et al., 2015; Allen et al., 2019). While a shift of the geographic expansion of pathogens poleward have already been demonstrated (Bebber et al., 2013), temperature elevation is also expected to favour the emergence of new pathogens and to increase the occurrence and severity of epidemics (Garrett et al., 2006; Evans et al., 2008; Elad and Pertot, 2014; Bebber, 2015; McDonald and Stukenbrock, 2016; Bebber et al., 2019).

Interestingly, a growing number of studies describe that a temperature elevation can balance the plant immune response in different ways. Meanwhile the mechanisms involved remain poorly understood. The purpose of this review is to gather and discuss studies describing the effect of temperature elevation (TpE) on plant/pathogenic microorganisms (nematodes, fungi, oomycetes, bacteria, viruses) interactions. Since mechanisms involved in plant-pathogens interactions are well studied, this review focuses on the knowledge acquired on the physiological and molecular effects of TpE 1) on plants or pathogens 2) on both partners during an interaction and 3) on the outcome of the pathogen's attack on the plant. Based on forty-three available studies that described the effect of TpE on resistance to pathogens, we found a predominantly negative impact of TpE on the main known resistance mechanisms. We discuss implications of the diversity of experimental conditions used, the application mode of combined stresses, the level of genetic diversity explored and the cellular, genetic and molecular mechanisms modulated by a TpE in plants and pathogens. Finally, we provide some recommendations on research directions to improve our understanding on the combinatorial effect of TpE and pathogen attack on the plant, to maintain plant resistance or to uncover novel resistance mechanisms in order to minimize crop failure in changing temperature conditions.

Round one: impact of temperature elevation on plants

Higher plants with an optimal growth in a thermal niche ranging from 10°C to 30°C are classified as mesophilic (Nievola et al., 2017). Depending on the plant species, incidence of temperature elevation is defined according to the applied temperature range. For example, in *Arabidopsis thaliana*, temperatures are considered warm between 22°C and 27°C, high between 27°C and 30°C and extremely high between 37°C and 42°C (Liu et al., 2015). The effect of TpE on plants has been widely studied and has been recently reviewed in several articles (Bita and Gerats, 2013;

Hasanuzzaman et al., 2013; Hatfield and Prueger, 2015; Gray and Brady, 2016; Nievola et al., 2017). We will briefly describe the main findings and invite readers to consult the listed reviews for more information. However, we should highlight that most studies addressed the consequences of high and extremely high TpE on plants and the mechanisms involved. On the other hand, studies assessing the impact of a temperature increase below 5°C while still above the optimal growth temperature are lacking.

TpE severely affects plant homeostasis and main vital functions. First, at the phenotypic level, plant architecture is altered at different levels: i) leaf and stem scorching as well as hypocotyl and petiole elongation, ii) abscission of leaves and reproductive organs, iii) inflorescence abortion and fruit damage, iv) shoot and root growth inhibition, v) shortened lifespan and reduced plant productivity due to altered respiration and photosynthesis (Christiansen and Lewis, 1982; Tian et al., 2009; Zinn et al., 2010; Hatfield et al., 2011; Gray and Brady, 2016; Zhao et al., 2017; Wang et al., 2018a; Yang et al., 2018b). Second, plants exposed to elevated temperatures are affected at the physiological level during all stages of plant development (Saidi et al., 2011; Bita and Gerats, 2013). TpE increases membranes fluidity and permeability leading to (i) lipid-based signaling cascades that modulate heat-sensing factors into membranes, and (ii) a re-organization of cellular structures such as microtubules, organelles and cytoskeleton, thereby affecting cellular differentiation, elongation and expansion (Saidi et al., 2011). TpE also triggers a specific transient influx of extracellular Ca^{2+} across the plasma membrane. Perception of Ca^{2+} fluctuations by readers such as calmodulins (CaMs) and the activation of specific mitogen-activated protein (MAP) kinase or CaM-binding protein kinase (CBK) are part of the Ca^{2+} dependent heat specific downstream signaling pathway regulating the activity of heat shock factors (HSF) and heat shock protein expression (HSP) (Saidi et al., 2011). Production of reactive oxygen species also contributes to the transduction of the heat signal into expression of HSP genes known to participate in thermotolerance process (Königshofer et al., 2008). Signaling of TpE also leads to the production of osmolytes which are small organic compounds (e.g sugars, polyols, amino acids) (Bita and Gerats, 2013). They were proposed to participate in heat tolerance helping the stabilization of proteins and the structure of the membrane bilayer (Sung et al., 2003; Mirzaei et al., 2012) and stimulating the accumulation of phenolic compounds (flavonoids, anthocyanins, steroids) (Wahid et al., 2007). The biosynthesis and compartmentalization of metabolites is disturbed (Maestri et al., 2002) and the level of several key hormones increases [abscisic acid (ABA), salicylic acid (SA) and ethylene] while others decreases (cytokinin, auxin and gibberellic acids). Existing proteins are denaturized while newly synthetized proteins are misfolded (Ellis, 1990). Third, at the molecular level, TpE is known to promote chromatin remodeling leading to up-regulation of genes involved in primary and secondary metabolism, translation, transcription regulation, response to environmental stresses; calcium, phytohormone, sugar and lipid signaling and protein phosphorylation ((Larkindale and Vierling, 2008; Saidi et al., 2011). TpE leads to transient activation of repetitive elements or silenced gene clusters as well as the transient loss of epigenetic gene silencing (Lang-Mladek et al., 2010; Pecinka et al., 2010; Smith and Workman, 2012). It also represses the expression of genes involved in plant defense response (NLR protein) and cell growth, such as histones and DNA polymerases (Larkindale and Vierling, 2008). Moreover, Kumar and Wigge (2010) showed that an alternative form of a histone, H2A.Z, is essential for direct perception of ambient temperature and coordinate the temperature-dependent gene transcription. Recently, studies suggested the connection of a class of bHLH transcription factors (TF), the phytochrome interacting factors (PIFs), to heat related signaling mechanisms. Interestingly, PIF4 may both coordinate temperature-dependent growth of plants and act as a negative regulator of plant immunity (Gangappa et al., 2017).

In case of a non-lethal dose of TpE, plants can enhance their ability to cope with and respond more efficiently to a repeated heat stress. This phenomenon commonly refers to “priming” or acquisition of thermotolerance. This rapid, highly conserved and actively maintained response leads to heat stress memory, recently reviewed in Bäurle (2016) and Friedrich et al. (2019). This priming induces HSFs and HSPs expression and stabilization, which in turns enhance protein homeostasis (Finka et al., 2015; Haslbeck and Vierling, 2015). Underlying molecular mechanisms are still not well understood. However, *HsfA2* was shown to regulate heat stress memory genes encoding small HSPs such as HEAT STRESS-ASSOCIATED 32kD Protein (Hsa32) or ASCORBATE PEROXIDASE 2 (APX2) (Charng et al., 2007; Liu and Charng, 2012; Lämke et al., 2016). Expression of these genes remains induced even after heat stress exposure or rapidly activated after a second stress (Lämke et al., 2016; Liu et al., 2018). *APX2A* and *HPS22* loci can be di- and trimethylated with histone H3 lysine 4 (H3K4me2 and H3K4me3) upon heat stress priming, regulating their ability to be transcribed (Lämke et al., 2016). This phenomenon is linked to HsfA2 and shows the impact of epigenetics marks following heat stress.

Round 2: impact of temperature elevation on pathogens

The overall effect of climate changes on pathogens is difficult to determine as the optimal infection conditions, host specificity and plant defense mechanisms greatly differ from one pathogen to another (Elad and Pertot, 2014). Temperature is one of the most important climatic variable for phytopathogens infection along with relative humidity (RH) and leaf wetness for foliar pathogens (Colhoun, 1973; Huber and Gillespie, 1992). For pathogens that have evolved at higher latitudes, warming is predicted to improve their fitness and increase the risk of epidemics due to their adaptation to temperatures below their physiological optimum (Deutsch et al., 2008). The effect of temperature also depends on the pathogen trophic behavior. Indeed, elevated temperature increases tissue necrosis and encourages colonization by necrotrophic pathogens (Elad and Pertot, 2014). Furthermore, elevated temperatures modify the physiology of a host species that deeply alters the colonization of host tissues by biotrophic pathogens (Agrios, 2005). Temperature and relative humidity govern the rate of reproduction of many pathogens (Caffarra et al., 2012). Longer growing seasons due to climate warming will increase the length of pathogen reproduction and dissemination periods. For instance, studying the impact of temperature on life-history traits of *Podosphaera planteginis*, the powdery mildew agent of *Plantago lanceolate*, showed that this fungus strains germinate faster and produce more spores at higher temperatures, suggesting that all the asexual traits perform better at higher temperatures in contrary to the sexual traits (Vaumourin and Laine, 2018). At the epidemiological level, prolonged periods of optimal temperature, precipitation and/or humidity conditions during pathogen development lead to increased damage on crops (Agrios, 2005). The strongest consequences of climate warming on pathogens dissemination are expected in tropical regions and on pathogens fitness in temperate regions, because species with a narrow temperature growth range are assumed to have increased sensitivity to extreme temperature fluctuations (Elad and Pertot, 2014).

Several mechanisms involved in the sensing of thermal fluctuations have been extensively studied in fungal and bacterial pathogenic species, mainly in humans and mammals. We will present them briefly, since they have been well discussed in Shapiro and Cowen (2012) and because they are relevant examples that could help to understand the mechanisms involved in the plant-pathogen interactions at TpE (Figure 1A). Temperature affects developmental transitions promoting virulence of fungi and influences replication and growth properties of viruses infecting human and mice. For mammal’s bacteria, temperature is an indicator of successful infection of the host leading to the expression of bacterial virulence regulators such as T3E and adhesins (Konkel and Tilly, 2000). The cellular membrane is one of the first elements enabling to detect temperature fluctuations for many microorganisms. Extreme temperatures alter membrane properties, fatty acid composition and the level of unsaturated lipids, thus allowing membranes themselves to act as a thermosensor (Digel, 2011). Proteins modulating the lipid composition of the membrane in response to temperature have been identified in bacteria and cyanobacteria (Suzuki et al., 2000; Mikami et al., 2002; Albanesi et al., 2004; Albanesi et al., 2009; Li et al., 2012). The second step of this regulation involves thermosensors such as transcriptional regulators, kinases and chaperones (Shapiro and Cowen, 2012). In the case of bacteria, a two-component regulatory system (TCS), composed of a membrane sensor and a cytoplasmic regulator, has a key role in the environmental fluctuations perception and can influence the expression of virulence genes. By contrast, for phytopathogens, the TCS system is the only one that has been studied so far in *Agrobacterium tumefaciens* and *Pseudomonas syringae* (Jin et al., 1993; Braun et al., 2007).

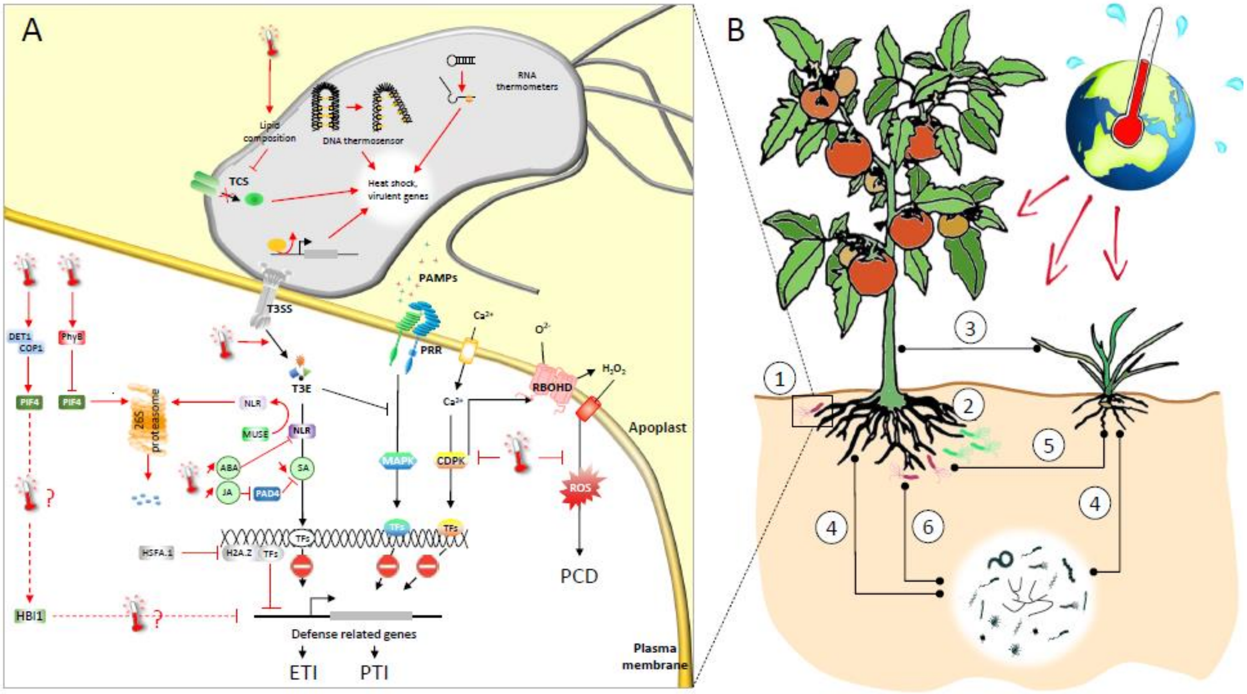

Figure 1: Avenues for understanding the effect of temperature elevation on plant/biotic environment interactions.

A Model of known TpE-dependent molecular mechanisms modulating plant-defense and bacteria responses (adapted from Shapiro and Cowen, 2012; Venkatesh and Kang, 2019). The red lines indicate elevated temperature depend regulations. Blunt and pointed arrows indicate inhibition and activation, respectively. Microbes first sense temperature fluctuations through cellular membrane saturated/unsaturated fatty acids ratio modifications. Upon temperature exposition, cellular membrane altered fluidity modify the TCS, resulting in its inactivation and modulation of virulence genes transcription. Key temperature sensors such as transcriptional repressors dissociate from DNA upon elevated temperature perception allowing transcription of heat shock and/or virulent genes. Temperature elevation can influence DNA supercoiling and histone-like nucleoid structuring protein (H-NS) DNA binding, modulating transcription efficiency and allowing the regulation of many virulence and heat shock genes. Temperature responsive RNA thermometers form a hairpin structure in mRNA transcripts at the level of the translation initiation site. Increasing temperature destabilizes these structures, allowing the ribosome-binding site to become accessible and facilitating the translation initiation of many heat shock and virulence factors. Elevated temperatures also promote T3E translocation into plant cells. At the plant level, elevated temperatures trigger specific transient influx of extracellular Ca2+ across the plasma membrane as well as production of reactive oxygen species. Temperature elevation could counteract both signaling pathways known to be also involved in plant defense activation upon perception of PAMPs by PRRs, leading to transcriptional repression of defense related genes. Enhanced translocation of T3E combined to the absence of expression of genes involved in PTI and SA accumulation are responsible for increase susceptibility at elevated temperature. PIF4 accumulation negatively modulates plant defense genes via the activation of HBI 1. Contrasting results do not allow to conclude on the temperature dependent regulation of this pathway. However, the ubiquitin E3 ligase activity of COP1 with DET1 participate in the stabilization of PIF4 at elevated temperature. Alternatively, at elevated temperature, defense genes can be expressed through the activation of PhyB that interacts with PIF4 and leads to its proteasomal degradation. MUSE proteins were recently proposed to be responsible for NLR elevated temperatures-dependent destabilization and degradation. In addition, elevated temperature promotes the rapid replacement of H2A.Z, mediated by HSFA1, allowing stress-responsive regulators and defense genes to be transcriptionally regulated. Elevated temperatures inhibit SA biosynthesis and SA responsive genes expression and upregulates JA signaling and ABA biosynthesis genes. Furthermore, the PAD4 and EDS5 regulated SA-mediated defense responses are suppressed at elevated temperature by JA. Enhanced accumulation of ABA at elevated temperature is correlated to NLR mediated resistance alteration and inhibition of NLR nuclear localization. ABA, abscissic acid; CDPKs, calcium-dependent protein kinases; JA, jasmonic acid; MAPKs, mitogen-activated protein kinases; PCD, programmed cell death; RBOHD, RESPIRATORY BURST OXIDASE HOMOLOG B; SA, salicylic acid; T3E, type III effector; T3SS, type III secretion system; TFs, transcription factors.

B- Multiple interactions, which could determine the plant response under pathogen attack
In natural conditions, the outcome of a plant's infection by a pathogen will be determined by genetic basis related to its immune system (1) influenced by its ability to respond to direct interactions (beneficial or harmful) with: (2) other pathogens (co-infections) and (3) its microbiota (here root microbiota). Neighboring plants can also alter pathogens (4). The species constituting the microbiota can compete, cooperate or coexist with each other and with phytopathogens (5) and be manipulated by neighboring plants (3), indirectly shaping the result of the pathogenic attack. In turn, all plant interactions with living organisms can be affected by abiotic variations in the environment (here temperature elevation). Green bacteria: other pathogens.

Above 32°C in *A.tumefaciens*, the autophosphorylation of VirA (sensor) and phosphorylation of VirG (regulator) are abolished, leading to an impaired virulence (Jin et al., 1993). In *P. syringae*, temperature-dependent conformation changes of CorS (sensor kinase) lead to the inactivation of CorR (regulator) at elevated temperature (Braun et al., 2007). A third mechanism involves RNA thermometers considered as RNA control elements located at the 5' UTR of bacterial genes. Temperature can either modulate their expression or destabilize them, making ribosome-binding site accessible and facilitating the initiation of translation (Klinkert and Narberhaus, 2009; Rinnenthal et al., 2010; Rinnenthal et al., 2011; Kortmann and Narberhaus, 2012). Finally, the structure and topology of DNA can act as a thermosensor through its super-coiling (Kataoka et al., 1996; López-García and Forterre, 1997; Mizushima et al., 1997; López-García and Forterre, 2000) or the temperature-dependent accessibility of promoter regions occupied by histone-type proteins (Prosseda et al., 2004; Duong et al., 2007; White-Ziegler and Davis, 2009).

Round three: Impact of temperature elevation on plant-pathogen interactions

In agreement with the observed effects of global warming and the prediction of its impact on living organisms and ecosystems, the number of studies reporting an alteration of plant disease resistances under TpE largely increased in recent years. Therefore, we decided to review current available knowledge to assess the impact of a TpE on either model plants or crops/pathogenic microorganism's interactions. Using "high temperature, temperature elevation, pathogens, plants, resistance, immune response and combined stresses" keywords, we performed our bibliographic searches on the Web of Science, google scholar and PubMed-NCBI websites. We selected 43 studies in which the effect of TpE on 123 resistances to pathogens were described (Tables 1 and 2). First, these studies involve 25 pathosystems corresponding to a combination of ten plant species (including nine crop species) with 25 pathogen species (including eight fungi, seven viruses, four oomycetes, three bacteria and three nematodes). Second, of all the temperature elevation applied, only five correspond to variations less than or equal to 5°C above the initial temperature. Third, depending on the method of application of temperature elevation and pathogen attack, we classified the studies as follow: i) a continuous or intermittent and repeated application of the first stress, several days before the second stress, is considered as an acclimatization, ii) an application of the first stress just before (one day or hours) and/or after the second stress is defined as sequential, and iii) an application of both stresses at the same time is qualified as simultaneous.

Among the 43 studies, 28 and 9 studies reported a sequential or simultaneous (with a permanent TpE applied) application of stresses, respectively. Two studies correspond to acclimatization (Wang et al., 2009; Menna et al., 2015) while the remaining studies do not specify the mode of application of stresses. There is only one study in which the thermosensitivity of soybean resistance to *Phytophtora sojae* was assessed using two methods of TpE (Gijzen et al., 1996). Fourth, most of the resistances were evaluated in controlled conditions (41 studies). Only two resistances related to *Mi-1* and *Xa-7* genes were assessed in both controlled (Jablonska et al., 2007; Cohen et al., 2017) and field conditions over several years (Dropkin, 1969; Webb et al., 2010). Additionally, two other resistances were also evaluated in both controlled and field conditions, in the same study (Uauy et al., 2005a; Plotnikova and Stubei, 2013a). Carrying out experiments under controlled conditions has the advantage of supervising precisely specific abiotic and biotic factors. On the other hand, while field experiments (especially when conducted over several years) increase the level of complexity, they allow the evaluation of robustness and transferability of resistances into more ecologically realistic conditions.

Alarmingly, we found that TpE resulted in an increased plant susceptibility or an inhibition of plant defenses for 60% of the studied resistances (Table 1). All the remaining studies reported no significant effect of TpE on the level of plant resistance (Table 2) and none reported a positive effect of TpE on plant resistance. The alteration of plant resistance is not dependent on plant species, pathogens species or the nutrition mode of pathogens. A primary explanation for this high frequency of resistance alteration in presence of TpE may rely on the adaptation of most pathogens to temperatures above the optimal growth temperature of plant species. Accordingly, all the resistances observed in wheat in presence of TpE, were observed for pathogens that have an optimal growth temperature below the TpE applied (Table 2). Noteworthy, the effect of TpE on plant resistance is highly dependent on the mode of stress application. For instance, while Cheng et al. (2013) applied TpE and promoted AvrRpt2 expression simultaneously in *A. thaliana* for 3 to 6 h, Menna et al. (2015) primed *A. thaliana* plants 24h at elevated temperature prior to inoculation with *Pst* strains expressing either HopZ1a or AvRpt2 avirulent factors and harvested samples 4 days after infection. In both cases, plant resistance was altered. Cheng et al. (2013) clearly showed that elevated temperature inhibits AvrRpt2 related ETI response. However, in Menna et al. (2015), HR was suppressed but the bacterial multiplication remained repressed at 28°C, suggesting the resistance is still active even if bacteria multiplication repression appears to be less effective than at 22°C. Thus, TpE treatment before or after inoculation could influence the output of an interaction. Acclimatization and priming effects following a chronic and intermittent abiotic stress exposure are also known to help plants resist against biotic stresses (Hilker et al., 2015). When wheat was exposed at 15°C or 25°C until the booting stage before inoculation with *Blumeria graminis f. sp. Tritici,* plants were resistant and the level of expression of *Pm4a* and *Pm4b* resistance genes was correlated to the inoculation temperature applied before the inoculation (Ge et al., 1998a). Wang et al. (2001) also speculate that the expression of some race specific *R* genes such as *Pib* rice-blast resistance genes could be primed by specific environmental conditions.

Table 1. List of studies carried on plant-pathogens interactions under heat excess with a resistance alteration. In this table the optimal temperature for plant and pathogen species is given along with the resistance gene if studied and the treatment applied conditions with the type of study.

Plant species	Optimal vegetative growth temperature	Optimal temperature requirement (plant cycle)	Pathogen species	Pathogen optimal growth temperature	Pathogen type	Trophic state [a]	Resistance genes	Δ in temperature	Time of HT exposure	Type of stress application [b]	Conditions [c]	Mechanisms	Type of study [d]	References
Arabidopsis (*Arabidopsis thaliana*)	22°C-23°C (Rivero et al., 2014)	16°C-30°C (Yang and Hua., 2004)	*Peronospora parasitica*	20°C (Achar, 2008)	Oomycete	B	*SNC1*	22°C→28°C	22°C or 28°C for weeks, before inoculation	Seq (Tp→P)	C	*R* gene expression regulated by HT	M	Yang and Hua., 2004
			Ralstonia solanacearum	28°C (Huerta et al., 2015)	Bacterium	H	*RPS4/RRS1-R*	27°C→30°C	10 days	Sim	C	QDR conferred by *SSL4*	M	Aoun et al., 2017
			Pseudomonas syringae pv. *tomato* DC3000	28°C (Young et al., 1977)	Bacterium	N	*RPS2, RPM1, RPS4*	22 →28°C	1 week	Acc (Tp→P)	C	HR suppressed at HT	M	Wang et al., 2009
							RPS2, RPM1	16°C-22°C →28°C & 32°C	PTI: 3h, ETI: 6h	Sim	C	ETI inhibition + HR suppressed at HT	M	Cheng et al., 2013
							RPS2, ZAR1	21°C-24°C→30°C	4 days	Acc (P→Tp)	C	HR suppressed at HT, higher bacterial multiplication at HT	M	Menna et al., 2015
			Golovinomyces cichoracearum	20°C (Ward and Manners, 1974)	Ascomycete	B	*RPW8*	22°C→30°C	30 days	Seq (P→Tp)	C	HR suppressed at HT	M	Xiao et al., 2003
Pepper (*Capsicum annuum*)	20°C-25°C (Saha et al., 2010)	15°C-30°C (https://www.starkeayres.co.za)	*Phytophthora capsici*	25°C-28°C (Hausbeck and Lamour, 2004)	Oomycete	H	na	25°C→37°C	0 to 7 days	Sim	C	Higher expression of HSP, inhibited expression of Ca^{2+} signaling pathways in resistant germplasm	M	Lu et al., 2017
Pepper (*Capsicum chinense*)			*Tomato Spotted Wilt Virus (TSWV)*	na	Virus	B	*Tsw*	22°C→32°C	3/5/9/12 days till 1 month	Seq (P↔Tp & Tp→P→Tp)	C	Destabilization of HR	D	Moury et al., 1998
Soybean (*Glycine max*)	12°C-20°C (Hopper et al., 1997)	12°C-25°C (Hopper et al., 1997)	*Phytophthora sojae*	25°C-30°C (Dorrance et al., 2012)	Oomycete	H	*Rps1-a,Rps1-b,Rps1-c,Rps1-d,Rps1-k,Rps2,Rps3-a,Rps4,Rps5, Rps6*	33°C 44°C →25°C	48h	Sim Seq (Tp→P+Tp)	C	Thermosensitive resistances, depending on oomycete isolate	D	Gijzen et al., 1996
Tobacco (*Nicotiana benthamiana*)	23.5°C (Yang et al., 2018)	20°C-30°C (https://www.cabi.org/isc/datasheet/36326)	*Potato virus X (PVX)*	28°C (Choi et al., 2017)	Virus	B	*Rx*	22°C→22°C & 28°C & 30°C	4 days	Acc (P→Tp), Acc (P→Tp)	C	HR suppressed at HT	D	Wang et al., 2009
Tobacco (*Nicotiana rustica, N. acuminata, N. glutinosa*)			*Tobacco Mosaïc Virus (TMV)*	24°C (Lebeurier and Hirth, 1966)	Virus	B	*N*	22°C→22°C & 28°C & 30°C 21°C, 28°C→35°C	3 days	Seq (P→Tp)	C	No necrosis at HT (no HR)	D	Wang et al., 2009 Samuel, 1931
								21°C→ >28°C	3 days	Seq (P+Tp (30°C)→ Tp (21°C))	C	Loss of HR at HT	M	Whitham et al., 1994; Zhu et al., 2010
Tobacco (*Nicotiana tabacum*)			*Cucumber Mosaïc Virus (CMV)*	na	Virus	B	*na*	18°C, 24°C→28°C	28 days	Sim	C	Higher virus replication	M	Zhao et al., 2016

Tomato (*Lycopersicum esculentum*)	18°C-25°C (Hussey, 1965; Schwarz et al., 2014)	10°C-35°C (Shamshiri et al., 2018)	*Cladosporium fulvum*	22°C (Small, 1930)	Ascomycete	B	*Cf4, Cf9*	20°C→33°C	3 weeks	Seq (Tp→P)	C	Loss of HR at HT	D	de Jong et al. 2002
Tomato (*Solanum lycopersicum*)			*Meloidogyne javanica, M. incognita, M. arenaria*	22°C-28°C (Small, 1930, Tyler, 1933)	Root-knot Nematodes	B	*Mi-1*	24°C→32°C	Until 6 days	Seq (P→Tp)	C	Loss of HR at HT	M	Hwang et al. 2000
Tomato (*Solanum arcanum, Solanum*			*Meloidogyne incognita*	22°C-28°C (Small, 1930, Tyler, 1933)	Root-knot Nematode	B	*Mi-1*	22°C, 26°C→32°C	2-3 days till 4 weeks	Seq (Tp→P→Tp(22°C-26°C))/Nat	C/F	*Mi-9* is a Mi-1 homolog	D	Dropkin, 1969 Jablonska et al., 2007
Tomato (*Lycopersicum esculentum*)			*Ralstonia solanacearum*	28°C (Huerta et al., 2015)	Bacterium	H	*na*	24°C→32°C	10-12 days	Sim	C	Loss of resistance	D	Krausz and Thurston, 1975
Maize (*Zea mays*)	20°C-25°C (Hardacre and Turnbull, 1986)	11°C-30°C (Wang et al., 2018)	*Puccinia sorghi*	25°C (Dey et al., 2015)	Basidiomycete	B	*Rp1, D21*	18°C, 22°C, 26°C→ >30°C	Until 3 weeks	Seq (P→Tp)	C	Suppressed expression of *Rp1-D21*	D	Negeri et al., 2013
Oat (*Avena sativa*)	20°C-25°C (Hopper et al., 1997)	20°C-25°C (Hopper et al., 1997)	*Puccinia graminis* f. sp. *avenae*	20°C-25°C (Martens et al., 1967)	Basidiomycete	B	*H, F, B, E*	15°C, 20°C, 25°C→ 30°C	*na*	Seq (P→Tp)	C	Resistance is thermal sensitive	D	Martens et al., 1967
Rice (*Oryza sativa*)	20°C-27°C (Wang et al., 2018)	22°C-31°C (Wang et al., 2018)	*Xanthomonas oryzae* pv. *oryzae*	25°C-30°C (Sullivan et al., 2011)	Bacterium	B / H	*Xa3, Xa4, Xa5, Xa10*	Controlled: 29°C→35°C Field: Cool season 22.4°C→30°C Hot season 22.4°C→31.3°C	Two seasons / 48 hpi	Nat/Seq (Tp→P)	C/F	Better recognition of *Xa7 by (to) the pathogen, loss of avrXa7 function*	D/M	Webb et al., 2010 Cohen et al., 2017
Rice (*Oryza sativa* L.ssp. Japonica cv. Nipponbare)			*Magnaporthe oryzae*	28°C (Uddin et al., 2002)	Oomycete	B	*Pi5(t), Pi7(t), Pia, Pik-s, Pik, Pik-p, Pi-h, Piz, Piz-5, Pita, Pib, Pit, Pish, Pi1, Pl3, Pi12(t), Pi19, Pik-m, Pi20, Pita2 (Pi No.4), Pita, Pi11(t), Piz5*	28°C→35°C	14 days (7 days + 7 days)	Seq (Tp→P→Tp)	C	Susceptibility	M	Onaga et al., 2017
Wheat (*Triticum aestivum*)	20°C-25°C (Acevedo et al., 2002)	12°C-25°C (Acevedo et al., 2002)	*Puccinia recondita also known as P. triticina*	10°C -22°C (Kramer and Eversmeyer, 1992)	Basidiomycete	B	*Lr10, LrB, LrEch, Lr22a, Lr30, RL6057, RL6058, RL6059, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr18*	10°C→25°C	*na*	Seq (P→Tp)	C	Phenotypic observation of loss of resistance at HT, depending on the fungal isolate	D	Dyck and Johnson., 1983 Wang et al., 2016
							Lr22b, Lr34	Controlled: 14°C→23°C Field: HT vs MT (3 years)	Until 9 days	*na*	C/F	*Lr37* resistance is unaffected by temperature conditions	D	Plotkinova and Stubei, 2013
			Puccinia graminis f. sp. *tritici*	15°C-23°C (Burrage, 1970)	Basidiomycete	B	*Sr6*	26°C→19°C	48 hours	Seq (Tp(26°C)→P→ Tp(19°C))	C	Incompatible reaction at 19°C, susceptible reaction at 26°C	D	Mayama et al., 1975 Harder et al., 1979
							Sr15	Ambiant→15°C, 18°C, 22°C, 26°C, 30°C	*na*	Seq (P→Tp)	C	More symptoms at HT	D	Gousseau et al., 1985
			Wheat streak mosaic virus (WSMV)	na	Virus	B	*Wsm1,Wsm3*	18°C→24°C 18°C→27°C, 30°C, 33°C, 35°C	21 days 28 days	Sim	C	Symptoms on plants Isolate dependent desease response	D	Liu et al., 2011 Seifers et al., 2013
			Triticum mosaic virus (TriMV)	na	Virus	B	*Wsm1, Wsm3*	18°C→24°C,27°C, 30°C, 33°C	28 days	Sim	C	Symptoms on plants	D	Liu et al., 2011

ETI = Effector Triggered Immunity, Hpi = Hours post inoculation, HR = Hypersensitive Response, HSP = Heat Shock Proteins, HT= High Temperature, IL = Isogenic Line, MT = Moderate Temperature, na = not available, NIL = Near Isogenic Line, P = Pathogen, PTI = Pathogen associated molecular patterns (PAMPs) Triggered Immunity, QDR = Quantitative Disease Resistance, Tp = Temperature. [a] Trophic state = B, Biotrophic; H, Hemibiotrophic; N, Necrotropic. [b] Type of stress application = Seq, Sequential (Tp, Temperature stress; P, Pathogen stress); Sim, Simultaneous stress; Acc, Acclimatization; Nat, Natural; Comb, Combined. [c] Conditions = C, Controlled; F, Field. d Type of study = M, Mechanistic; D, Descriptive.

Table 2. List of studies carried on plant-pathogen interactions under heat excess with a resistance maintenance. In this table the optimal temperature for plant and pathogen species is given along with the resistance gene if studied and the treatment applied conditions with the type of study.

Plant species	**Optimal vegetative growth temperature**	**Optimal temperature requirement (plant cycle)**	**Pathogen species**	**Pathogen optimal growth temperature**	**Pathogen type**	**Trophic state [a]**	**Resistance genes**	**Δ in temperature**	**Time of HT exposure**	**Type of stress application [b]**	**Conditions [c]**	**Mechanisms**	**Type of study [d]**	**References**
Arabidopsis (*Arabidopsis thaliana*)	22°C-23°C (Rivero et al., 2014)	16°C-30°C (Yang and Hua, 2004)	*Ralstonia solanacearum*	28°C (Huerta et al., 2015)	Bacterium	H	*SSL4-SSL5*	27°C→30°C	10 days	Sim	C	QDR conferred by *SSL4*	M	Aoun et al., 2017
Chinese cabbage (*Brassica campestris*)	13°C-18°C (Rural Development Administration, 2005)	7.2°C-29.4°C (Burt et al., 2006)	*Turnip mosaic virus (TuMV)*	18°C-28°C (Chung et al., 2015)	Virus	B	*na*	Ambiant→13°C, 18°C, 23°C, 28°C, 33°C	24 hours	Sim	C	No symptoms at HT due to the low accumulation of virus coat protein	D	Chung et al., 2015
Tomato (*Solanum arcanum*, *Solanum lycopersicum*)	18°C-25°C (Hussey, 1965; Schwarz et al., 2014)	10°C-35°C (Shamshiri et al., 2018)	*Meloidogyne incognita*	22°C-28°C (Small, 1930, Tyler, 1933)	Root-knot Nematode	B	*Mi-9*	22°C, 26°C→32°C	2-3 days → 4 weeks	Seq (Tp→P→Tp(22°C-26°C))	C	*Mi-9* is a homolog of *Mi-1*	D	Jablonska et al. 2007
Soybean (*Glycine max*)	12°C-20°C (Hopper et al., 1997)	12°C-25°C (Hopper et al., 1997)	*Phytophthora sojae*	25°C-30°C (Dorrance et al., 2012)	Oomycete	H	*Rps1-c, Rps2, Rps5*	33°C	48h	Sim	C	Thermosensitive resistances, depending on oomycete isolate	D	Gijzen et al., 1996
Rice (*Oryza sariva*)	20°C-27°C (Wang et al., 2018)	22°C-31°C (Wang et al., 2018)	*Xanthomonas oryzae* pv. *oryzae*	25°C-30°C (Sullivan et al., 2011)	Bacterium	B / H	*Xa7*	Controlled: 29°C→35°C Field: Cool season 22.4°C→30°C Hot season 22.4°C→31.3°C	Two seasons / 48 hpi	Nat/Seq (Tp→P)	C/F	Enhanced resistance at HT, loss of *AvrXa7* function	D/M	Webb et al., 2010 Cohen et al., 2017
Rice (*Oryza sativa-indica, O. sativa-japonica*)			*Magnaporthe oryzae*	28°C (Uddin et al., 2002)	Oomycete	B	*Pi54*	28°C→35°C	17 days (7 days + 10 days)	Seq (Tp→P→Tp)	C	Enhanced resistance at HT, lower expression of *Pi54* at HT	M	Onaga et al., 2017a
Rice (*Oryza sativa* L.ssp. Japonica cv. Nipponbare)							*Piz-t, Pita2, Pi9, Pii*	28°C→35°C	14 days (7 days + 7 days)	Seq (Tp→P→Tp)	C	Increased resistance	M	Onaga et al., 2017b
Oat (*Avena sativa*)	20-25°C (Hopper et al., 1997)	20°C-25°C (Hopper et al., 1997)	*Puccinia graminis* f. sp. *avenae*	20°C-25°C (Martens et al., 1967)	Basidiomycete	B	*A, D*	15°C, 20°C, 25°C→ 30°C	*na*	Seq (P→Tp)	C	Resistance is thermal sensitive	D	Martens et al., 1967
Wheat (*Triticum aestivum*)	20°C-25°C (Acevedo et al., 2002)	12°C-25°C (Acevedo et al., 2002)	*Puccinia recondita also known as P. triticina*	10°C -22°C (Kramer and Eversmeyer, 1992)	Basidiomycete	B	*Lra, Lr2a, Lr2b, Lr2c, Lr3,Lr3bg, Lr1, Lr3ka, Lr9, Lr15, Lr16, Lr17, Lr19, Lr21, Lr23, Lr24*	10°C→25°C	*na*	Seq (P→Tp)	C	Phenotypic observation of loss of resistance at HT, depending on the fungal isolate	D	Dyck and Johnson, 1983
							Lr37	Controlled: 14°C→23°C Field: HT vs MT (3 years)	Until 9 days	*na*	C/F	*Lr37* resistance is unaffected by temperature conditions	D	Plotkinova and Stubei, 2013
							LrZH22	18°C→25°C	14 days	Seq (P→Tp (18°C for 24 hours→22°C/25°C)	C	Effective against most chinese races	D	Wang et al., 2016

Wheat (*Triticum aestivum*)	20°C-25°C (Acevedo et al., 2002)	12°C-25°C (Acevedo et al., 2002)	*Puccinia striiformis* f. sp. *tritici*	13°C-16°C (Newton and Johnson, 1936)	Basidiomycete	B	*Yr18, Yr29, Yr36, Yr39*	4°C→20°C (seedling test) 10°C→30°C (adult test)	20 days	*na*	C	HTAP resistance	M	Chen et al., 2013
							Yr36	18°C→25°C	18 days	Seq (P→Tp(25°C→18°C))	C	HTAP resistance	D	Bryant et al., 2014
							TaXa21	15°C→20°C	24 hours	Seq (P→Tp 15°C, 20°C or 15°C→21°C→15°C)	C	HTSP resistance	M	Toa et al., 2018 Wang et al., 2019
							Yr79	4°C/20°C→10°C/30°C	22 days	Seq (P→Tp (10°C for 24 hours→20°C/30°C)	C	HTAP resistance	D	Feng et al., 2018
							Yr62	4°C/20°C→10°C/30°C	20-25 days	Seq (P→Tp (10°C for 24 hours→20°C/30°C)	C	HTAP resistance	D	Lu et al., 2014
							Yr59	4°C/20°C→10°C/30°C	18-21 days	Seq (P→Tp (10°C for 24 hours→20°C/30°C)	C	HTAP resistance	D	Zhou et al., 2014
							Yr52	4°C/20°C→10°C/30°C	18-22 days	Seq (P→Tp (10°C for 24 hours→20°C/30°C)	C	HTAP resistance	D	Ren et al., 2012
Wheat (*Triticum turgidum* ssp. *dicoccoides*)			*Puccinia striiformis* f. sp. *tritici*	13°C-16°C (Newton and Johnson, 1936)	Basidiomycete	B	*Yr36*	Controlled: 4°C→18°C-20°C (seedling experiment), 35°C (adult-plant test) Field: High diurnal temperatures followed by cool nights	20 days	Seq (P→Tp)/Nat	C/F	HTAP resistance	D	Uauy et al., 2005
Wheat (*Triticum aestivum*)			*Puccinia graminis* f. sp. *tritici*	15°C-23°C (Burrage, 1970)	Basidiomycete	B	*Sr14, Sr9b*	Ambiant→15°C, 18°C, 22°C, 26°C, 30°C	*na*	Seq (P→Tp)	C	Less symptoms at HT	D	Gousseau et al., 1985
			Blumeria graminis f. sp. *tritici*	20°C (Ward and Manners, 1974)	Ascomycete	B	*Pm1, Pm8, Pm4a, Pm4b*	15°C→15°C, 25°C/25°C→15°C/25°C	4 weeks (prior inoculation), up to 17 days (post inoculation)	Seq (Tp→P+Tp)	C	*Pm4a* expression is temperature-dependent	D	Ge et al., 1998
			Wheat streak mosaic virus (WSMV)	na	Virus	B	Temperature-sensitive resistance (TSR)	18°C→24°C	21 days	Sim	C	Isolate dependent desease response	D	Seifers et al., 2013

Hpi = Hours post inoculation, HT = High Temperature, HTAP = High Temperature Adult-Plant, HTSP = High Temperature, Seedling-Plant, IL = Isogenic Line, na = not available, MT = Moderate Temperature, NIL = Near Isogenic Line, P = Pathogen, QDR = Quantitative Disease Resistance, RIL = Recombinant Inbred Line, Tp = Temperature. ETI = Effector Triggered Immunity, Hpi = Hours post inoculation, HR = Hypersensitive Response, HSP = Heat Shock Proteins, HT= High Temperature, IL = Isogenic Line, MT = Moderate Temperature, na = not available, NIL = Near Isogenic Line, P = Pathogen, PTI = Pathogen associated molecular patterns (PAMPs) Triggered Immunity, QDR = Quantitative Disease Resistance, Tp = Temperature. [a] Trophic state = B, Biotrophic; H, Hemibiotrophic; N, Necrotropic. [b] Type of stress application = Seq, Sequential (Tp, Temperature stress; P, Pathogen stress); Sim, Simultaneous stress; Acc, Acclimatization; Nat, Natural; Comb, Combined. [c] Conditions = C, Controlled; F, Field. d Type of study = M, Mechanistic; D, Descriptive.

Mechanisms involved in the thermosensitivity of the plant-pathogen interaction

While TpE alters all plant resistance categories (PTI, ETI and QDR), the underlying mechanisms remain poorly understood. Several studies started to decipher the molecular mechanisms underlying TpE sensitivity of the basal and *R* gene-mediated resistances in plant-pathogens interactions. On one hand, the majority were performed at the transcriptomic level with three studies on *A. thaliana* (Rasmussen et al., 2013; Sham et al., 2015; Huot et al., 2017) and five studies on either wheat, rice or tobacco (Király et al., 2008; Chen et al., 2013; Cohen et al., 2017; Onaga et al., 2017a; Tao et al., 2018a). On the other hand, few studies focused on known genes involved in immunity, mainly in the context of *A. thaliana* interactions with *Pst* (Zhu et al., 2010; Mang et al., 2012; Cheng et al., 2013; Menna et al., 2015; MacQueen and Bergelson, 2016; Huot et al., 2017). In particular, NLR proteins such as RPS2 (RESISTANCE TO PSEUDOMONAS SYRINAGEA 2), RPM1 (RESISTANCE TO PSEUDOMONAS SYRINGAE PV MACULICOLA 1), RPS4 (RESISTANCE TO PSEUDOMONAS SYRINAGAE 4), SNC1 (SUPPRESSOR of nrp1-1 CONSTITUTIVE), ZAR1 (HOPZ-ACTIVATED RESISTANCE 1) and regulators of plant defense response including *EDS1*, *PAD4*, ABA, SA and JA synthesis or signaling have been studied for their role in plant immune sensitivity to TpE.

Transcriptome specificities of combined TPE and pathogenic stresses

In *A. thaliana*, some experiments were conducted to compare plant response to individual or combined stresses (Rasmussen et al., 2013; Huot et al., 2017) or to analyze the overlap among publically available transcriptomic studies carried out on individual stresses (Sham et al., 2015). Other transcriptomic studies were conducted to decipher mechanisms involved in (i) stable *Xa7*- and *Pi54*-mediated resistance to *Xanthomonas oryzae pv. oryzae* (*Xoo) or Magnaporthe oryzae (Mo)* in rice, respectively (Cohen et al., 2017; Onaga et al., 2017a), (ii) high temperature seedling plant (HTSP) *or* in *Yr39*-mediated high temperature adult-plant (HTAP) resistances to *Puccinia striiformis f.sp. tritici* in wheat (Chen et al., 2013; Tao et al., 2018a), and (iii) TpE inhibition of the N-mediated resistance to the Tobacco mosaïc virus (TMV) in Tobacco (Király et al., 2008). For each transcriptomic study, pathogen or PAMP types, stress application modes and plant growth conditions were different. Together, three main observations could be drawn from these eight transcriptomics studies. First, as previously proposed for other stresses combinations (Rizhsky et al., 2002; Rizhsky et al., 2004; Voelckel and Baldwin, 2004; Mittler and Blumwald, 2010;

Atkinson and Urwin, 2012), plant response for both sequential and simultaneous applied stresses is specific and unpredictable from an individual stress application. Second, among the few common responses identified between individual and combined stresses, they are suggested to be involved in conserved mechanisms (Chen et al., 2013; Rasmussen et al., 2013; Pandey et al., 2015; Sham et al., 2015; Huot et al., 2017; Onaga et al., 2017a; Suzuki and Katano, 2018). Finally, another transcriptome analysis dedicated to study the *A. thaliana* response to a triple combination of stresses applied sequentially, implying turnip mosaic virus infection, mild drought and TpE stresses, previously showed that plant response is more similar to the response to the most severe individual stress or to the latest stress applied (Prasch and Sonnewald, 2013). Onaga et al. (2017a) drew similar conclusion in his study of heat stress effect on *Pi54*-mediated resistance to *Mo* in two genetic backgrounds of rice. Indeed, they observed that the transcriptome responses of a rice genotype pretreated either at 28°C or 35°C seven days prior *Mo* infection were close, suggesting that plant response was mostly driven by the last *Mo* infection stress (Onaga et al., 2017a).

Several reviews listed commonalities and differences among transcriptome results on plant response to individual and combined stresses (Atkinson and Urwin, 2012; Suzuki et al., 2014; Pandey et al., 2015; Zhang and Sonnewald, 2017). Here, we only provide a general overview of the main regulated pathways while focusing on TpE and pathogen attack combinations. Overall, the resistance alteration is associated with the down-regulation of redox pathways and plant defense signaling (NLR, protein kinase, signaling) and the up-regulation of ABA metabolism and TFs expression (Király et al., 2008; Rasmussen et al., 2013; Cohen et al., 2017; Onaga et al., 2017a; Suzuki and Katano, 2018). With regard to the resistances maintained under TpE, the down-regulation of primary carbon metabolism appears to be a general shared response. Moreover, it seems that redox and hormonal signaling are the most important nodes regulated, being induced and suppressed respectively (Cohen et al., 2017; Onaga et al., 2017a). Recently, Wang et al. (2019) demonstrated the involvement of the *LRR-RLK* gene *TaXa21* in HTSP to *Pst* in wheat. Interestingly, TaXa21 interacts with the two WRKY TFs, TaWRK76 and TaWRK62, which are positive regulators of the HTSP resistance. Transcriptional regulation by this category of TFs appears to be particularly important in plant response to a combined TpE and pathogen attack. Similarly, CaWRKY40 and CaWRKY46 TFs, for which expression is induced in *Caspicum annuum* upon *R. solanacearum* infection or TpE (at 42°C for 24h) were demonstrated to promote the expression of genes encoding HR-associated and pathogenesis-related proteins as well as heat shock proteins such as *NtHSF2,* thus suggesting they are positive regulators of both defense responses to *R. solanacearum* and tolerance to TpE (Dang et al., 2013; Cai et al., 2015).

Impact of TpE on known plant defense mechanisms and pathogens

First, when stresses are combined, several studies suggest that regulation of the genes expression involved in immune responses would be a part of the causal factors leading to the thermosensitivity of the plant immunity. For instance, upon TpE acclimatization during seven days before inoculation with pathogens, *EDS1* (*A. thaliana* defense regulator genes) and *Pi54* (conferring resistance to *Mo in rice*) are down-regulated (Wang et al., 2009; Onaga et al., 2017a). Furthermore, direct culture under high temperature conditions of tomato plants expressing the *R/AvR* gene pairs *cf4/AvR4* and *Cf9/AvR9*, mediating resistance to *Cladosporium fulvum* resistance in tomato, results in HR suppression and repression of known *Cf/Avr*-induced defense related genes (de Jong et al., 2002). Other studies reported that the expression of *NLRs* can be also specifically regulated only under combined TpE and pathogen stresses (Chen et al., 2013; Lu et al., 2017a; Onaga et al., 2017a). Interestingly, MacQueen and Bergelson, (2016) explored the expression profile of thirteen *R* genes in twelve natural *A. thaliana* accessions, grown in eight different environmental conditions, including TpE, before and after infection by several strains of *Pst.* They showed that *R* gene expression is in average increased for the 12 accessions tested in all environments prior to infection with *Pst*. Furthermore, they observed TpE dependent *R* gene expression regulation was related to geographical origin and historical climate as accessions from dry climate down-regulate *R* genes expression more than accessions from wet climate (MacQueen and Bergelson, 2016). By contrast, Cheng et al. (2013) applied short-term TpE treatments simultaneously with *Pst* infection. However, they did not observe any change in the transcript level of defense related genes. Together, these results suggest an effect of the TpE application method. Second, the TpE-dependent suppression of immune response correlates with the altered localization of three nuclear SNC1, RPS4 and N NLR proteins (Zhu et al., 2010; Mang et al., 2012). Third, the hormonal cross-talk between MeJ/JA, ET and ABA clearly participates in the regulation of plant response under combined stresses, with sometimes opposite effects depending on the pathosystem studied (de Jong et al., 2002; Mang et al., 2012; Chen et al., 2013; Cohen et al., 2017). Nonetheless, some results support that ABA plays a central regulatory role. ABA responsive cis-regulatory elements were found in genes specifically down regulated in genotype expressing the stable *Xa7*-mediated resistance (Cohen et al., 2017). In addition, ABA deficiency stabilizes the nuclear localization of both SNC1 and RPS4 and antagonizes the immune response inhibition at TpE (Zhu et al., 2010; Mang et al., 2012). Third, a possible link between TpE-dependent immune sensitivity and chromatin remodeling was highlighted by the study of *arp6* (ACTIN-RELATED PROTEIN6) mutant (Cheng et al., 2013). ARP6 is a component of SWR1 complex (SWR1c) that promotes the histone H2A (HTA) replacement by the H2A.Z variant in nucleosomes. A recent transcriptomic analysis (Cortijo et al., 2017) showed that H2A.Z-containing nucleosomes are specifically evicted from TpE-sensitive target genes by Heat stress **factor A**1 class transcription factors (HSFA1), thus facilitating induction of downstream stress-responsive transcriptional regulators. Interestingly, *arp6* displays a constitutive expression of TpE-responsive genes and an enhanced resistance to *Pst* (Kumar and Wigge, 2010; Cheng et al., 2013).

Recently, Venkatesh and Kang (2019) extensively reviewed the *R* gene-mediated defense sensitivity under TpE and proposed a relevant general model focusing on the well-studied NLR protein SNC1 (Venkatesh and Kang, 2019). In Figure 1A, we gathered TpE-dependent regulators associated with SNC1-mediated response including the known mechanisms involved in TpE sensing on the pathogen side (Shapiro and Cowen, 2012). In addition to TpE impact on the hormonal interplay and the NLR proteins subcellular localization, the TpE-dependent effect relies on NLR protein stabilization, specific transcriptional reprogramming and chromatin remodeling. First, at elevated temperatures, stabilization of NLR proteins is strictly regulated by highly conserved chaperone such as HSP90 (Heatshock protein 90), SGT1 (SUPPRESSOR of G-TWO ALLELE OF SKP1) and RAR1 (REQUIRED FOR MLA12 RESISTANCE) (Zhou et al., 2007; Shirasu, 2009). The stabilization of SNC1 is determined by temperature regulation of the ubiquitin proteasome system (UPS) through (i) the E3 ligase complex members such as SCF (SKP1-CULLIN1-F-BOX) or SAUL1 (SENESCENCE-ASSOCIATED E3 UBIQUITIN LIGASE1) (Burg et al., 2008; Cheng et al., 2011; Gou et al., 2012; Tong et al., 2017), or (ii) temperature-sensitive interactions of MUTANT SNC1-ENHANCING (MUSE) proteins with UPS (Huang et al., 2014; Copeland et al., 2016; Huang et al., 2016). Second, the formation of signaling module with ubiquitin E3 ligase activity, involving DET1 (DE-ETIOLATED 1) and COP1 (CONSTITUTIVE PHOTOMORPHOGENIC1) (Jung et al., 2016; Legris et al., 2016), participate in the stabilization of PIF4 TF at elevated temperature and would be required in the transcriptional repression of SNC1 (Gangappa et al., 2017). A TpE-dependent inhibition of the interaction between PIF4 and the Phytochrome B (PhyB), a canonical light receptor involved in thermosensing, would lead to the PIF4 accumulation and the negative modulation of plant defense genes via the activation of the bHLH TF HOMOLOG OF BEE2 INTERACTING WITH IBH 1 (HBI1) (Gangappa et al., 2017). However, because Huot et al. (2017) found no evidence of TpE-dependent PIF4 involvement in plant defense response, its role is still a matter of debate. Venkatesh and Kang (2019) suggested that these contrasting findings might be due to differences in experimental designs and to the mode of stress application.

Few studies investigated the effect of TpE on phytopathogens during interactions with their hosts. Apart from promoting the multiplication of bacteria or virus (Menna et al., 2015; Zhao et al. 2016, Huot et al., 2017), they showed that the secretion of the avirulent factors AvrB, AvrRpm1 and AvrRpt2, necessary for the induction of resistances linked respectively to RPM1 and RPS2, was not affected under TpE in *A. thaliana*. By contrast, using the rice cultivar Nipponbare becoming susceptible to *Mo* at elevated temperature, Onaga et al., (2017b) showed by a transcriptome analysis that genes encoding putative effectors in *Mo* were more strongly expressed under TpE, suggesting that elevated temperature favors pathogen infection. Similarly, Huot et al. (2017) showed that the increased susceptibility of *A. thaliana* at elevated temperature was associated with an increased multiplication of *Pst* in plant tissues associated with an increase of T3E secretion (Huot et al., 2017).

TpE and plant-pathogen interactions: role of the genetic background?

TpE-dependent modulation of resistance could rely on the plasticity and adaptability of different plant genetic backgrounds to TpE. In wheat, HTAP and HTSP resistances conferred by genes such as *Yr36 or Yr39* are non-race specific, durable and influenced by specific environmental conditions (Chen et al., 2013; Bryant et al., 2014). Interestingly, studying the mechanisms involving *Yr36* resistance, Bryant et al. (2014) demonstrated that the instability of temperature-dependent resistance was specific to the plant genotype. Similar results were obtained for different soybean isolines carrying different *Rps* genes, which confer resistance to *Phytophthora sojae* (Gijzen et al., 1996). Onaga et al., (2017a) also showed that the genetic background of *Oryza sativa - japonica* improved the function of the *Pi54* resistance gene under TpE conditions against *Mo* more than the genetic background of *Oryza sativa – indica*.

Round 4: future avenues for robust resistances in the context of global warming

Climate change is already affecting ecosystems worldwide. Among the components of climate change, TpE is one of the main factors that alter both plant development and plant-pathogen interactions. Adapting agricultural systems to minimize crop yield losses while limiting the use of pesticides and fertilizers is even more challenging under climate warming. To achieve these objectives, the combination of complementary approaches (Figure 1A, 1B) while considering plants and pathogens from the individual to the population level, should lead us to a global vision of the mechanisms involved in plant defense response during interactions under TpE conditions.

Understanding mechanisms involved in resistance alteration under elevated temperatures

Genetic sources of resistance are often the most effective and environment-friendly mean of controlling plant diseases. One of the first current challenges is to investigate the robustness and the spectrum of new resistance mechanisms identified under TpE, either in controlled or in field conditions. Secondly, dedicated understanding of the physiological, metabolic, molecular, genetic and epigenetic basis of plant defense response modulation under TpE is essential to propose alternative solutions (i.e. use of natural allelic variants or editing methods) to maintain efficiency of already identified resistances. So far, these mechanisms have been mostly investigated in few plant species. Furthermore, during combined stresses, the response of plants depends not only on their ability to adapt to TpE but also on the effect that TpE might have on pathogens and plant-pathogen interactions. It is therefore important to study at the pathogen level the mechanisms underlying their perception and regulation of response to TpE conditions. Although this domain is well studied in mammalian's and human's pathogens (Shapiro and Cowen, 2012) (Figure 1A), it remains poorly explored for plant pathogens. Third, the genetic diversity of both interacting partners should be better considered to help i) in the evaluation of the immune response robustness and ii) in the study of mechanisms that could promote it. Indeed, the majority of studies listed in this review were mostly carried-out on a limited number of genotypes, both within plant and pathogen species. This observation is not limited to the impact of TpE on plant-pathogen interactions. A bibliometric analysis realized by Gimenez et al. (2018) on papers published in the field of plant pathology between 1979 and 2016 revealed that a majority of the studies was realized on a single (or few) genotype(s) of *A. thaliana* and on a limited number of pathogens, mainly the bacterium *P. syringae*. However, since 2017, this situation seems to be reversed, with a decrease of studies on *A. thaliana* and an increase of studies on crop species, in particular tomato and wheat.

Identification and study of uncovered robust resistance mechanisms

GWA mapping approaches, developed on either model plants or crops proved their efficiency in identifying already known resistance mechanisms as well as the genetic bases of many new QDRs mechanisms to bacteria, fungi and oomycetes (French et al., 2016; Bartoli and Roux, 2017; Bruessow et al., 2019). Therefore, such strategy could be developed to uncover robust resistance mechanisms under TpE and more broadly considering the changing abiotic environment. For instance, in this project, we successfully identified and functionally validated several defense response mechanisms that remain efficient under TpE (Aoun et al., 2017; Aoun et al., submitted). Furthermore, the development of a new statistical method allowing simultaneous GWA mapping on two interacting species makes it possible to map a phenotypic trait on a pair of genomes (Wang et al., 2018c). Its application, taking into account the genetic diversity of both the plant and the pathogen for a given pathosystem, should facilitate the identification of molecular actors ruling the interaction under TpE conditions. Implementing such strategies directly on model crop species in field conditions over several years and monitoring the climatic parameters is another major challenge that could be addressed.

Consider the natural complexity of interactions

Although they proved their accuracy in the characterization of the mechanisms involved in plant immunity, studies of plant-pathogen interactions are still generally performed on simple pathosystems composed of a single host plant interacting with a single pathogen. However, in nature, plants are often co-infected by several pathogens (i.e. Pathobiota) (Bartoli et al., 2018). Therefore, in order to predict and optimize plant responses to pathogens under abiotic stresses, it is crucial to investigate how the plant manage its interactions with all the living micro-organisms in its environment. Recent studies on plant-multi-pathogenic systems showed that interactions between pathogens could be based on coexistence, cooperation or competition, resulting in very different outcomes for the hosts (Abdullah et al., 2017). They also reported the importance of microbiota to help plants cope with biotic or abiotic stresses. Indeed, plant's "beneficial" microbiota can improve and even contribute in extending the defense response to diseases by i) the direct modulation of plant immunity (IMM) or ii) the competition between members of the microbiota that can indirectly influence the host (DCM) (Vannier et al., 2019). For example, in the case of our *R. solanacearum* study model, the transplantation of microbiota from the rhizosphere of the Hawaii tomato variety, tolerant to *R. solanacearum*, to a susceptible tomato variety contributes to the suppression of disease symptoms in susceptible plants. Interestingly a flavobacterium, more abundant in the plant resistant rhizosphere, was demonstrated to inhibit the *R. solanacearum* development (Kwak et al., 2018).

Authors contributions

HD, NA and RB conceived and wrote de review. All authors, including LD, VL and FR read, corrected and approved the manuscript.

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D. The lofty goal of identifying uncovered resistance mechanisms under heat stress

Due to the importance and necessity to find durable and robust mechanisms of resistance to pathogens under heat stress, my PhD project aims to identify and characterize the genetic and molecular basis of robust resistance mechanisms to pathogens under heat stress conditions. The novelty of this study is the exploitation of plant natural genetic diversity, and its screening for resistance during the infection process to stresses applied simultaneously, i.e. an infection of a plant species by a bacterial pathogen combined with temperature elevation of 3°C above the standard growth conditions. Specifically, the general questions from my PhD project are:

i. Is it possible to find resistance mechanisms to bacterial pathogens that remains efficient under heat stress in natural plant populations?
ii. If yes, what is the nature of these mechanisms? Are they different from those already studied? Are they interconnected?

To address these questions, we focused on the *Arabidopsis thaliana* - *Ralstonia solanacearum* pathosystem. This pathosystem is well studied at the LIPM. Resistance mechanisms are already identified and well characterized in standard growth conditions. In addition, extensive genomic and genetic resources are available for both biotic partners.

1. The pathosystem *Arabidopsis thaliana – Ralstonia solanacearum*

This pathosystem was established in the LIPM laboratories (1998) in order to study resistance mechanisms of the plant model *A. thaliana* to *R. solanacearum*, a devastating bacterial pathogen (Deslandes et al., 1998).

a. *Arabidopsis thaliana*

A. thaliana is still the flagship species in plant physiology, genetics and molecular biology. Since more than a decade, *A. thaliana* is also widely used to study the genetics of natural variation and eco-evolutionary responses to biotic and abiotic factors (Gaut, 2012; Roux and Bergelson, 2016).

Description and life cycle

A. thaliana, also called the mouse ear cress or wild thale, is an annual species and belongs to the Brassicaceae family (also known as the mustard family) (Shindo et al., 2007). *A. thaliana* is widely used because of its small size, ease of cultivation, short life cycle (in greenhouse conditions) and ability to self-fertilize, thus allowing to maintain homozygous lines and to phenotype them infinitely (Weigel and Nordborg, 2005). These characteristics, combined with the small size of its genome (5 chromosomes, ~ 119 Mb), led to the complete sequencing of its genome, the first in higher plants, completed in 2000 (Col-0 line, The Arabidopsis Genome Initiative 2000). The reference sequence made it possible to annotate all the genes. Currently, the database TAIR 10 (The Arabidopsis Information Resource, http://www.arabidopsis.org/) has 33,518 genes including 27,379 encoding proteins (Swarbreck et al., 2007). *A. thaliana* has two main types of life cycle: i) winter annuals with seeds germinating in autumn, plants overwintering as rosettes and then growing and maturing in spring, and ii) spring annuals with seeds germinating in early spring and then growing and maturing in the same season (Griffith et al., 2004). *A. thaliana* can produce a high number of seeds per plant, between 10,000 and 40,000 seeds (figure 3).

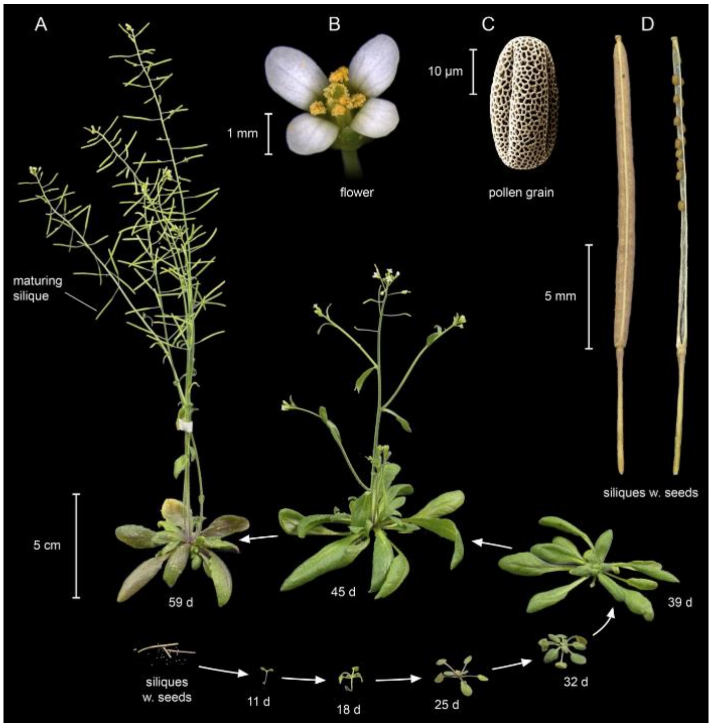

Figure 5. Life cycle of *Arabidopsis thaliana*. From seed to seed (Krämer, 2015).

Geographic distribution and native habitats

Native from Eurasia and North Africa, *A. thaliana* is now distributed worldwide (figure 4), highlighting its strong ability to colonize and inhabit contrasted environments. For instance, in south-west of France, *A. thaliana* is naturally found in diverse habitats that are contrasted for climate and edaphic factors as well as biotic interactions such as the level of interspecific interaction (figure 5; Bartoli et al., 2018).

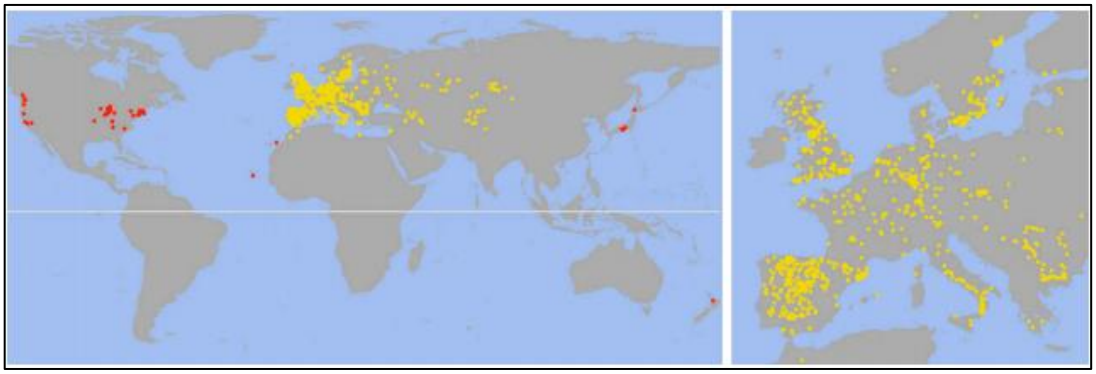

Figure 6. Worldwide distribution of *Arabidopsis thaliana*'s accessions. Yellow dots show the native distribution of A. thaliana and red dots show the areas where A. thaliana have been introduced (Weigel, 2012).

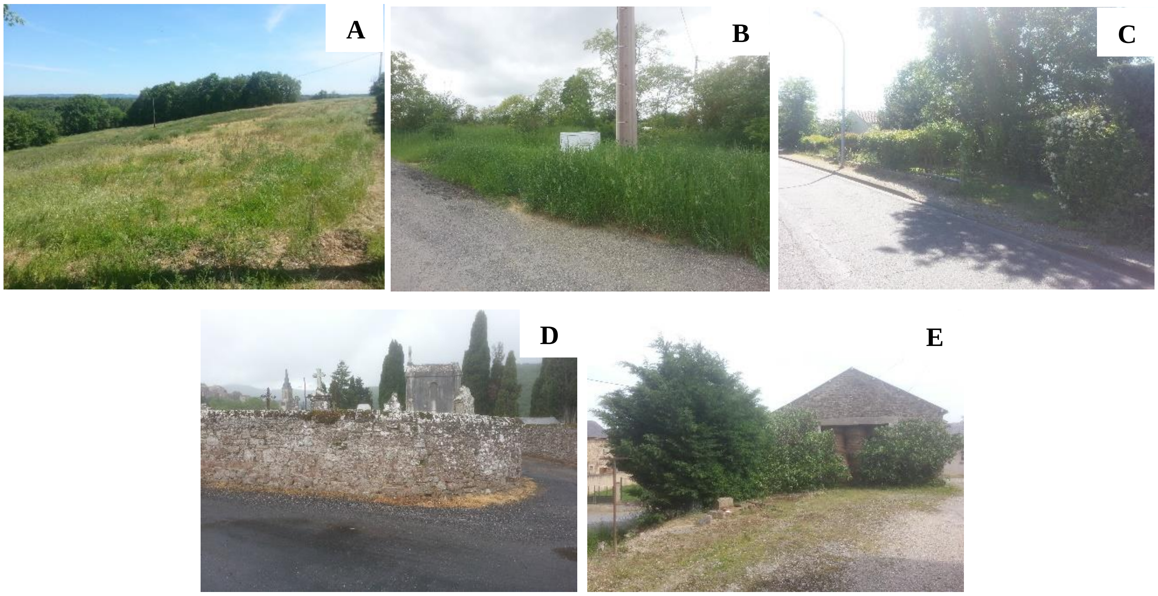

Figure 8. Examples illustrating the diversity of habitats occupied by *Arabidopsis thaliana* in south-west of France. A. Organic field **B.** Road sides with intense interspecific competition **C.** Bare grounds **D.** Stone walls **E.** Farm yard (Bartoli et al., 2018).

Available genetic and genomic resources

Impressive genetic resources for detecting the genetic basis of artificial phenotypic variation are publicly available in *A. thaliana* (random insertion and gene disruption: EMS mutagenesis, T-DNA mutants, specific gene disruption: artificial microRNA gene silencing, amiRNA) (Alonso and Ecker, 2006; O'Malley and Ecker, 2010), making functional validation by quantitative complementation or quantitative silencing much easier (figure 6).

To explore the genetic basis of natural phenotypic variation, the joint effort of several laboratories working on *A. thaliana* has led to a worldwide collection of several thousand accessions (Platt et al., 2010); Horton et al., 2012). The selfing rate of 98% described in the 1970s has long suggested that natural populations of *A. thaliana* were predominantly monomorphic. However, it was found later on that populations can be highly polymorphic both at the genetic and phenotypic levels (Corre, 2005; Bomblies et al., 2010; Platt et al., 2010; Kronholm et al., 2012; Brachi et al., 2013). It is therefore possible to look at patterns of evolution of phenotypic traits and their genetic bases across the distribution range of the species, but also at a very local scale. This collection of natural accessions allows performing (i) traditional linkage mapping (QTL mapping) by creating diverse experimental mapping populations such as segregating F2 population that derived from two parents contrasted for a phenotypic trait of interest, Near-Isogenic Lines (NILs), Recombinant Inbred Lines (RILs) or Multiparent Advanced Generation Intercross lines (MAGIC) (see more details in section D2), and (ii) Genome-Wide Association (GWA) mapping at different geographical scales (Bergelson and Roux, 2010)with notably the recent characterization of more than 1,000 accessions of *A. thaliana* at the genomic, epigenomic and transcriptomic levels in the frame of the 1001 Genome Consortium (https://1001genomes.org; Kawakatsu et al., 2016) (see more details in section D2).

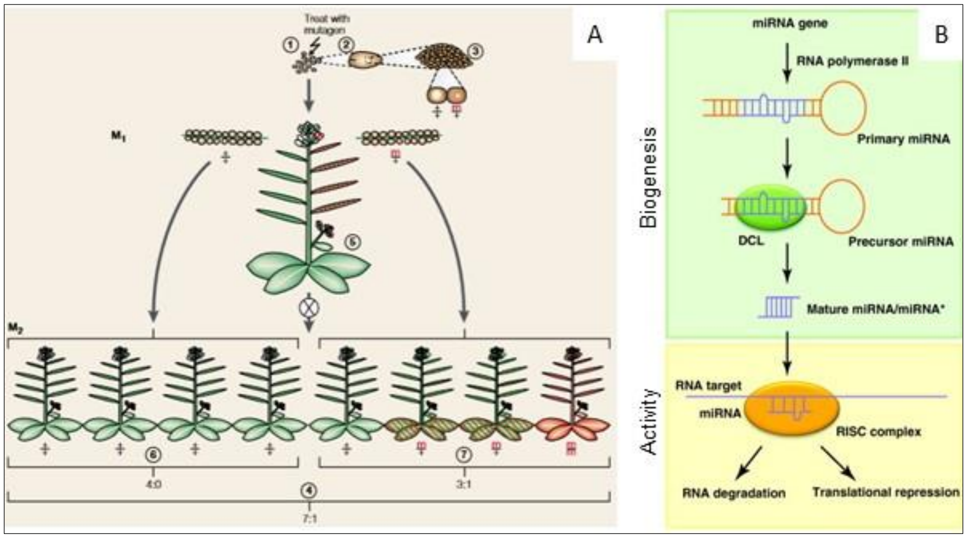

Figure 6. Examples of EMS and amiRNA strategies for *A. thaliana* mutagenesis. A. Seeds are treated with EMS and the progeny growing from mutation carrying seeds will be screened for mutation (Page and Grossniklaus, 2002). **B.** amiRNA sequences are used to target a specific gene to cause RNA degradation and translational repression (Molesini et al., 2012).

All these resources mentioned in this section have been made publicly available through international seed stock centers. The main ones are the Nottingham Arabidopsis Stock Centre (NASC, http://arabidopsis.info), the Arabidopsis Biological Resource Center (ABRC, https://abrc.osu.edu) and the Versailles Arabidopsis Stock Center of INRA (http://publiclines.versailles.inra.fr). There are also several database platforms that provide genetic and molecular data in *A. thaliana* such as the *Arabidopsis* International Resource (TAIR 10, https://www.arabidopsis.org), the Bio-Analytic Resource for Plant Biology (BAR, https://www.bar.utoronto.ca) and the plant ontology database 'Planteome' (http://planteome.org).

A model to understand the genetic and molecular mechanisms of resistance to pathogens

Although initially viewed as a poor choice to study plant–pathogen interactions because of the belief that *A. thaliana* was not attacked by pathogens in nature (Roux and Bergelson, 2016), *A. thaliana* is now considered as a great model to study plant-pathogen interactions at the physiological, genetic and molecular levels. As previously mentioned, our knowledge on the genetic and molecular mechanisms of pathogen resistance in *A. thaliana* is however mainly based on both the use of artificial phenotypic variation and the interaction with the bacterial pathogen *P. syringae* (in particular the strain DC3000) (Gimenez et al., 2018).

In a complementary way, exploiting natural genetic variation in *A. thaliana* for resistance to various pathogens led to the cloning of >40 genes involved in plant-pathogen interactions (Roux and Bergelson, 2016; Bartoli and Roux, 2017), which in turn allows the discovery of uncovered defense mechanisms. For instance, a map-based positional cloning strategy revealed a lectin gene *JACALIN-TYPE LECTIN REQUIRED FOR POTEXIVIRUS RESISTANCE1* (*JAX1*) associated with *A. thaliana* natural variation of resistance response to the *Plantago asiatica* mosaic virus (PIAMV) (Yamaji et al., 2012). Similarly, the gene *RESISTANCE TO FUSARIUM OXYSPORUM 1* (*RFO1*) was cloned as conferring resistance against the fungus *Fusarium oxysporum* (Diener and Ausubel, 2005). More recently, combining GWA mapping of 384 natural accessions of *A. thaliana* with functional analysis helped to clone three genes (the atypical kinase RKS1, the gene *At5g22540* with unknown function and the immune receptor pair RPS4/RRS1) involved in QDR to the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*) (Huard-Chauveau et al., 2013; Debieu et al., 2016).

b. *Ralstonia solanacearum*

Description

R. solanacearum is a soil borne bacterium that was first described in 1896 by Erwin F. Smith as a bacterial wilt causal agent in *Solanaceous* species. During the 20th century, *R. solanacearum* was previously known as *Bacillus solanacearum*, *Pseudomonas solanacearum* and *Burkholderia solanacearum*. It is a gram-negative soil bacterium with a hemibiotrophic life style and belongs to the class of β-proteobacteria (Elphinstone, 2005). *R. solanacearum* has a rod-shape of 0.5-1.5 μm length and has one to four polar flagellum that makes it very mobile (figure 7; Kelman and Hruschka, 1973).

Figure 7. Electron microscopy image of *Ralstonia solanacearum* (Tasset et al., 2010).

R. solanacearum is characterized by a large host spectrum. *R. solanacearum* can infect more than 200 plant species belonging to 53 botanical families (Genin, 2010) including monocotyledons and dictoyledons as well as perennial and woody species. Among the host plants, we can find potatoes, tomatoes, eggplants, ginger, bananas, peanuts, olive trees, Eucalyptus, *A. thaliana*, *Medicago truncatula*, *Pelargonium* as well as roses (Hayward, 1991; Tjou-Tam-sin et al., 2017).

R. solanacearum is classified among the ten most important devastating phytopathogens in the world, causing serious and inestimable economic losses (Mansfield et al., 2012). For instance, *R. solanacearum* was responsible for an estimated worldwide loss in 2005 of US$1 billion of potato yields per year (Elphinstone, 2005). In 1998, the European Union has officially considered *R. solanacearum* as a quarantine pathogen under the directive 98/57/EC. It is also considered as a bioterrorist agent in the United States of America (USDA/APHIS, 2005).

Life cycle and symptoms

When *R. solanacearum* is in its saprophytic state, it is able to survive in the soil for a long period, from 10 to 12 months (Dirk Van Elsas et al., 2000). Once the bacterium is in contact with a host plant, it is able to penetrate through roots wounds and ruptures or root tips and secondary root emerging points (Champoiseau et al., 2009). Then, *R. solanacearum* colonizes the root cortex and invade xylem vessels. Due to its high mobility, bacteria will systemically propagate towards the aerial parts of the plant through the xylem, where it will reproduce (10^7-10^9cfu / g of fresh tissue). Therefore, a massive colonization of vascular tissues and a high production of exopolysaccharides takes place and leads to variable symptoms.

The main characteristic symptoms caused by *R. solanacearum* are leaf roll, xylem browning and lethal wilting of the plant (figure 8). Wilting probably results from exopolysaccharides secretion that alters the vascular system of the plant. For instance, *R. solanacearum* will lead to the complete wilting of potato plants along with brown rot on tubers and a secretion of milky exudates at the level of the vascular ring (El-habbaa et al., 2016). In tomato, symptoms are represented by leaf roll and wilting (figure 8A; F et al., 2009)). In *A. thaliana*, similar symptoms to tomato are observed (figure 8B). When the plant is dead, *R. solanacearum* returns to its saprophytic state into the soil and is able to reach aquatic spots when possible (figure 9; Genin, 2010). *R. solanacearum* can also persist in debris and weeds (Genin, 2010).

This bacterium is known to be able to survive in ultra-pure water, until 132 days in nutrient deficient conditions while still keeping its infectious function. Together, it indicates the ability of *R. solanacearum* to adapt to strong abiotic constraints.

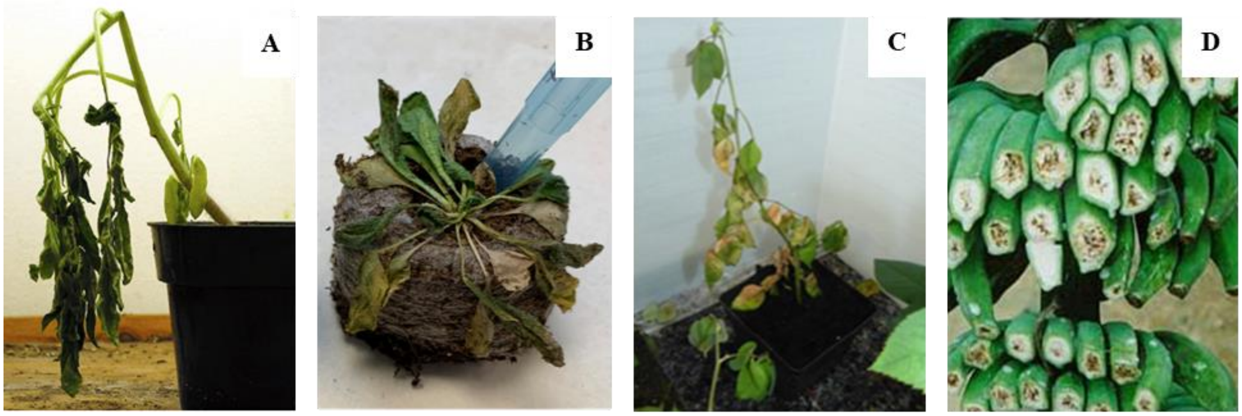

Figure 8. Symptoms caused by *Ralstonia solanacearum* on different host plants. A shows a wilted tomato plant (source: P. Champoiseau, University of Florida). **B** corresponds to a complete wilting of *A. thaliana* (picture taken during one of the PhD experiments). **C** picture of a dead *Rosa spp.* plant following infection with *R. solanacearum* (Tjou-Tam-sin et al., 2017). **D** shows the Moko disease on banana (source: Dr Luadir Gasparotto, Embrapa).

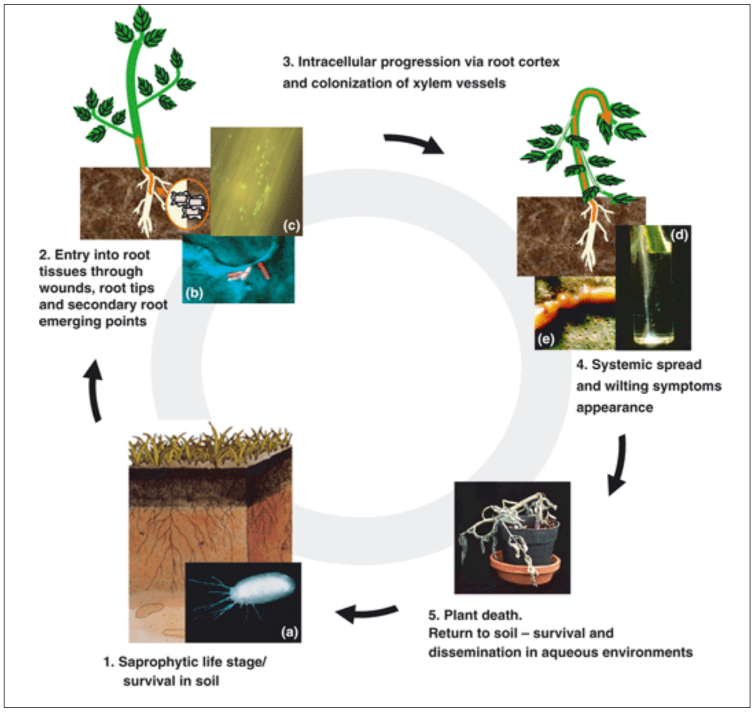

Figure 9. Life cycle of *Ralstonia solanacearum* (Genin, 2010).

Phylotypes and geographic distribution

After its discovery, *R. solanacearum* was classified in the *Bacterium* genus by Erwin F. Smith in 1896. After using classification methods based on *in situ* DNA hybridization, Ralstonia was moved to the *Burkholderia* genus in 1992 and then to the *Ralstonia* genus in 1995 (Yabuuchi et al., 1995). Later in 1998, it was demonstrated that *R. solanacearum* strains have a DNA-DNA relatedness level of 70%, making them members of different sub-species. Therefore, this bacterium was considered as a species complex, named RSSC for Ralstonia Solanacearum Species Complex, and composed of strains that are close but genetically different (Safni et al., 2014). The population structure of RSSC and its ability to adapt to new hosts or resistant accessions are probably driven by its genomic characteristics of recombination and horizontal genes transfer within RSSC (Bertolla et al., 1999; Fall et al., 2007; Coupat et al., 2008; Guidot et al., 2009; Remenant et al., 2010; Wicker et al., 2012).

Several approaches have been adopted to classify the strains of *R. solanacearum*. In 1962, the first one, mainly based on host range and geographical origin of strains, classifies this bacterial species into five races (Buddenhagen et al., 1962). The second approach classified *R. solanacearum* into biovars based on the medium acidification during the metabolization of six different carbohydrates: maltose, cellobiose, lactose, mannitol, sorbitol and dulcitol (Hayward, 1964). The third approach corresponds to a more recent phylogenetic classification system. It is based on the MultiLocus Sequence Typing (MLST) strategy that consists on (A) sequence polymorphism analysis of (i) introns of genes coding for ribosomal RNA (rRNA) of the 16S and 23S subunits and (ii) *hrpB* genes coding for a type three secretion system (T3SS) formation regulator and endonucleases (*Egl*); and (B) comparative genomic hybridization (Poussier et al., 2000; Fegan and Prior, 2005). This approach has classified *R. solanacearum* into four phylotypes, each divided into sequevars or related strains, depending on *egl* sequence similarities (Fegan and Prior, 2005). *R. solanacearum* has a wide geographical distribution and is present in tropical, sub-tropical and warm temperate regions (figure 10). This approach allows to group Ralstonia strains from Asia, America and Africa in phylotypes I, II and III regroup, respectively ; while Phylotype IV regroups strains from Indonesia and Australia (Castillo and Greenberg, 2007; Wicker et al., 2012). Phylotype IV is highly diverse because it is composed of *R. solanacearum* strains, *R. syzgii* and the Blood Disease Bacterium (BDB, causal agent of Moko disease) (Genin, 2010; Safni et al., 2014).

One way to track the origin of evolution and diversity of a worldwide collection of *R. solanacearum* strains is by applying the MLST approach on nine genes among which seven correspond to chromosome housekeeping genes (gdhA, mutS, ppsA, adk, leuS, rplB and gyrB) and two correspond to virulence-associated genes (egl and fliC). This analysis demonstrated that in most of the detected introgression events, the phylotype IV seems to be a gene donor for all of the other phylotypes (Wicker et al., 2012).

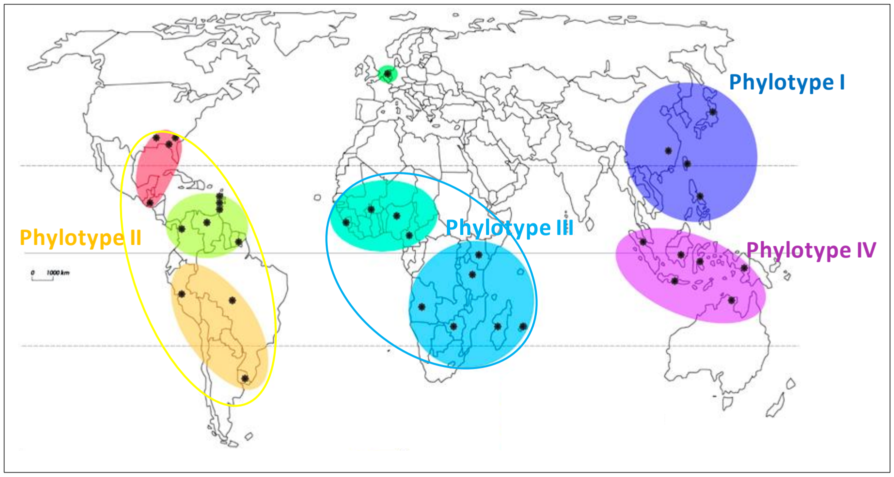

Figure 10. Worldwide distribution of *Ralstonia solanacearum* phylotypes. Phylotype I (in purple) comes from North and Eastern Asia. Phylotype II englobes three origins (in pink, light green and orange) from Central America and Southern Latin America, Northern Latin America and from Carribean, and Austral Latin America. Phylotype III with two origins (in green and blue) coming from Central and Western Africa and from Austral and Eastern Africa and Indian Ocean. Phylotype IV (in pink) comes from Indonesia and Northern Australia (adapted from (Wicker et al., 2012).

Genomic features and virulence determinants

GMI1000 (Génétique des Microorganismes INRA 1000) is the first strain of *R. solanacearum* for which the genome was sequenced. This strain was isolated from a tomato plant in French Guiana, showing bacterial wilt symptoms and was classified in the phylotype I, race 1, biovar 3 (Hong et al., 2012). The genome size of GMI1000 is 5.8 Mb. It encodes for 5,120 proteins and has a high GC-content (67%). It is divided into two circular replicons, one chromosome (3.7 Mb) and a megaplasmid (2.1 Mb) (Salanoubat et al., 2002). Around 80% of the housekeeping genes in the chromosome encodes proteins involved in basic cellular functions. Many of the genes present in the megaplasmid are essential for pathogenicity and bacterial plasticity. Until now, the genome of 155 strains of *R. solanacearum* has been sequenced and are publically available (https://iant.toulouse.inra.fr/bacteria/annotation/site/prj/T3Ev3).

The expression of *R. solanacearum*'s virulence factors is under the control of a complex and well defined network (Genin and Denny, 2012). One of the central regulators of this network is the transcription regulator PhcA, which once activated leads to the suppression of the T3SS, bacterial mobility and certain metabolic pathways. This also results in the transcription activation of genes encoding enzymes for cell wall degradation and exopolysaccharides synthesis (Genin and Denny, 2012; Huang and Schell, 1995). The second central regulator of virulence is the protein HrpG that regulates the expression of T3SS and T3E genes (Genin et al., 1992). During plant colonization, a non-diffusible signal in the cell wall is perceived by a cell membrane sensor called PrhA (Aldon et al., 2000). The PrhA protein activates a membrane regulator PrhR that in turn activates the PrhI protein, an extracytoplasmic sigma factor. This latter protein activates the transcription of *hrpG* genes (Genin et al., 2005; Yoshimochi et al., 2009). The HrpG protein can activate another gene of *hrp* family of genes, the *hrpB* gene. The HrpB protein is known to regulate the expression of T3SS and T3E (Genin et al., 1992). *hrp* genes are implicated in the induction of disease development in susceptible plants or in the hypersensitive response (HR) in non-host and resistant plants. They are able to regulate genes involved in host interactions such as adhesion factors, pectinases, cellulases and ethylene secreting enzymes (Valls et al., 2006).

Some genes in the genome of *R. solanacearum* encodes for molecular actors that are keys for pathogenicity and are involved in colonization and wilting of the host plants (figure 11). Among these genes, we can find: (i) flagella components essential for mobility and disease enhancement

(Genin and Denny, 2012), (ii) enzymes transported by the type II secretion system (T2SS) that can hydrolyze the cells of plants cell wall into nutrients and energy that are needed in the early stages of infection and colonization (Álvarez et al., 2010), (iii) genes encoding exopolysaccharides allowing for rapid colonization of the xylem, (iv) enzymes that are involved in ROS, phenolic compounds or in detoxification and tolerance to oxidative environments (Brown and Allen, 2004; Hernández-Romero et al., 2005), (v) T3E that are virulent molecules secreted by the T3SS and (vi) factors that are adhesive to the cell (Genin and Denny, 2012). Ralstonia species are able to secrete around 110 effectors in the plant (Peeters et al., 2013). However, the mode of action of most of these effectors is yet to be discovered.

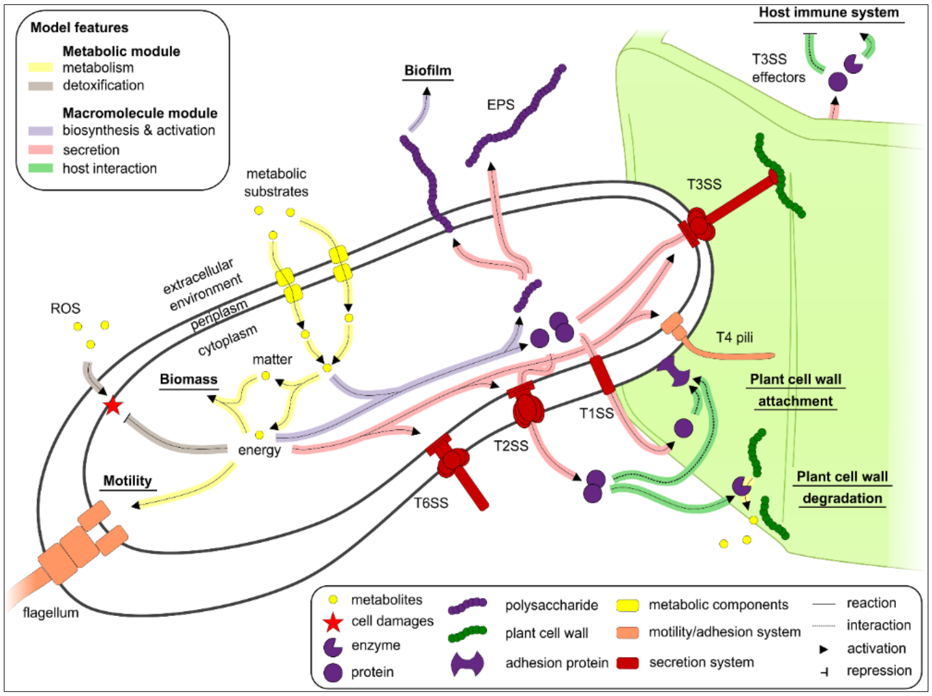

Figure 11. Metabolic and macromolecular network of *R. solanacearum* including the major virulence actors (Peyraud et al., 2016).

Dissemination and means of control

R. solanacearum can be transmitted by diverse means. Plant material is the first causal factor of disease propagation (Hayward, 1991). Agricultural practices also largely contribute to Ralstonia propagation by the means of infected tools and their exchange between farmers. At the plant level, root wounds caused by nematodes or agricultural practices facilitates bacteria penetration.

Chemical control of Ralstonia is limited because it consists of using environmentally dangerous pesticides such as chloropicrin, metam sodium, formalin, calcium hypochlorite and methyl bromide. Biocontrol is also restricted and mainly based on the use of a microbial antagonist that is a virulent strain of Ralstonia mutated in the *hrp* gene. Being more competitive, this mutant protects the plant from the phytopathogenic bacteria (Frey et al., 1994). Another mean of biocontrol is the use of bacteriophages (Fujiwara et al., 2011). A recent study reported that when susceptible plants to *R. solanacearum* were watered with a mix of bacterial strain (avirulent M4S strain and virulent U-10 strain) and lytic bacteriophages, most of them were not wilting (Álvarez and Biosca, 2017).

Plant breeding and the identification of resistance mechanisms to Ralstonia remain the only efficient mean of control against bacterial wilt disease. Until now, few mechanisms of resistance to *R. solanacearum* have been identified and characterized. In *A. thaliana*, it was shown that an immuno-receptor pair RPS4/RRS1 confers monogenic and recessive resistance to different *R. solanacearum* strains at 27°C (Deslandes et al., 2002). Another study demonstrated that 90% of phenotypic variation of resistance of the Col-0 *A. thaliana* accession to the 14.25 Ralstonia strain was governed by at least three QRS loci (Quantitative Resistance to *R. solanacearum*) on chromosomes I, II and V (Godiard et al., 2003). Similarly, resistance to Ralstonia in tomato is polygenic. Based on a RIL family issued from a cross between a tolerant line *Lycopersicum esculentum* cv. Hawaii 7996 and the susceptible line *L. pimpinellifolium* WVa700, four QTLs of resistance to the race 3 of phylotype II have been identified on chromosomes III, IV, VI and VIII (Carmeille et al., 2006).

c. *Arabidopsis thaliana* and *Ralstonia solanacearum* interactions

Molecular basis of the interaction

In 1998, two accessions of *A. thaliana* were crossed: the susceptible Columbia-5 (Col-5) and the resistant Niedersen-1 (Nd-1) to the *R. solanacearum* GMI1000 reference strain (Deslandes et al., 2003). Then, a phenotyping of the resulting F2 population was conducted with the GMI1000 strain and resulted in a 1 resistant : 3 susceptible segregating ratio, indicating that resistance to this strain was monogenic and recessive. After generating F_9 population of RILs, a gene playing a crucial role in plant resistance response to the GMI1000 strain was cloned on the lower arm of chromosome V and it was designated *RRS1* for Recognition of *R. solanacearum* 1. RRS1 encodes a Toll Interleukin 1 Receptor – Nucleotide Binding Site – Leucine Rich Repeat resistance protein (TIR-NBS-LRR) harboring a C-terminal extension containing a domain representative of WRKY transcription factors (group III) (figure 12). This latter domain belongs to a large family of transcription factors in plants and is involved in the expression of signaling pathways leading to either resistance or susceptibility. RRS1 has a signal of nuclear localization suggesting that its interaction with avirulence proteins (*avr*) take place in the nucleus (Deslandes et al., 2002). Two alleles that are 98% identical were identified: RRS1-S responsible for Col-5 susceptibility and RRS1-R responsible for Nd-1 resistance. These alleles differ at the position of a stop codon leading to the formation of RRS1-S (Col-5 genetic background) protein truncated by 90 amino acids (figure 12).

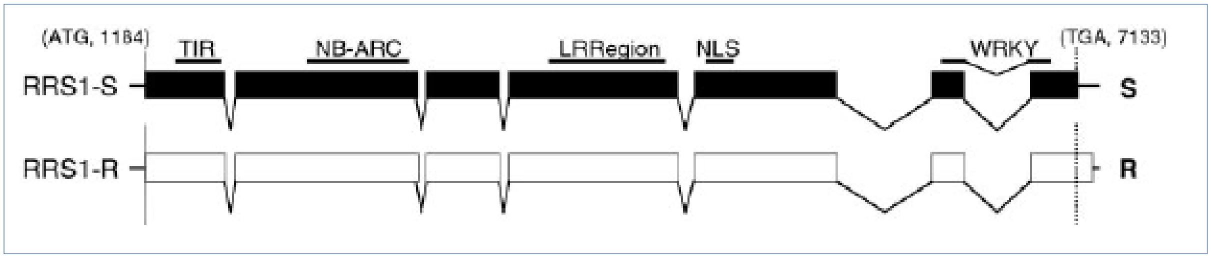

Figure 12. Allelic structures of RRS1-S and RRS1-R in Col-5 and Nd-1, respectively. Filled and empty boxes correspond to exons in Col-5 and Nd-1 genetic backgrounds, respectively. Gaps correspond to introns (Deslandes et al., 2002).

One of the T3Es secreted by the GMI1000 in *A. thaliana* is PopP2, a putative outer protein. This effector belongs to the YopJ family (*Yersinia* outer protein J) and has an acetyltransferase activity (Deslandes et al., 2003). Interestingly, PopP2 plays an important role in Ralstonia virulence because the mutation of this T3E (ΔPopP2) makes the bacteria less virulent. Importantly, it was demonstrated that RRS1-R cooperates with another TIR-NB-LRR protein, RPS4 in an inverted physical position nearby RRS1, for resistance induction to *R. solanacearum* (Birker et al., 2009). Once PopP2 is injected inside the plant, it is recognized by the immuno-receptor pair RPS4/RRS1-R. Thus, PopP2 will acetylate a lysine residue in the WRKY domain of RRS1-R leading to the separation of this latter from DNA, by altering its electrostatic potential. This phenomenon leads to the immunity activation in resistant genotypes (i.e. Nd-1, Wassilewskija (Ws-2)). In susceptible genetic backgrounds, PopP2 will acetylate the lysine residue of other WRKY defensive transcription factors (Tasset et al., 2010) leading to basal resistance suppression and plant disease development (figure 13; Le Roux et al., 2015). The susceptible accession Col-0 harbors the RPS4/RRS1-S pair, known for recognition of AvrRps4, a *Pseudomonas syringae* T3E, but not for PopP2, thus leading to resistance to *P. syringae* but not to *R. solanacearum* (Sarris et al., 2015). Beside the RPS4/RRS1-R, another molecular mechanism of resistance has been identified in *A. thaliana* and involves the LRR receptor-like kinase ERECTA gene, localized near the QRS1 (Quantitative Resistance to *R. solanacearum* 1) locus that confers resistance to the *R. solanacearum* 14.25 strain (Godiard et al., 2003).

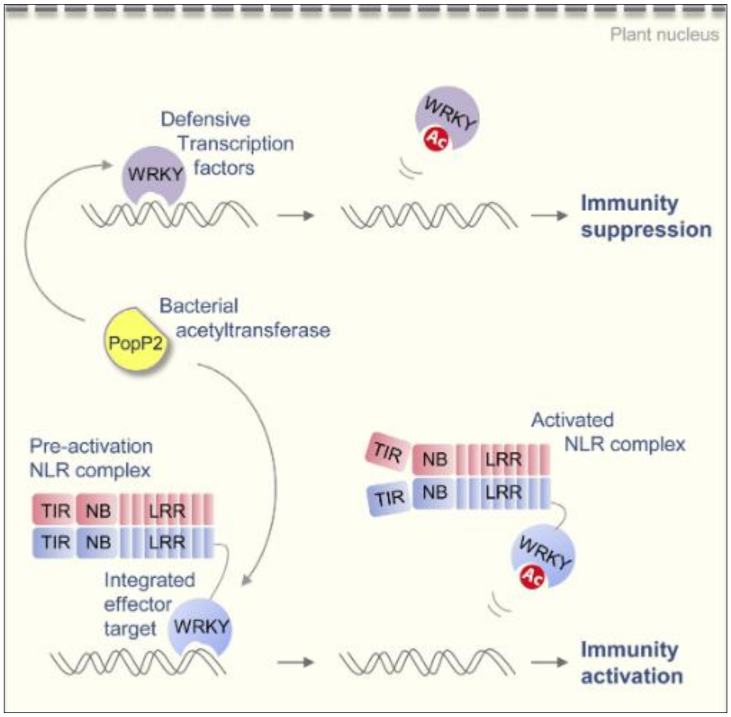

Figure 13. Activity of PopP2 acetyltransferase (Le Roux et al., 2015).

Impact of heat stress on the interaction

As presented in section C ('Impact of heat stress on plant immunity'), studies carried out on the impact of heat stress on plant resistance mechanisms reflect a preponderant tendency of an alteration of these mechanisms. The impact of heat stress on *A. thaliana* following *R. solanacearum* has not been studied before the initiation of this project. In 2014, a project was developed in the team to identify robust resistance mechanisms to *R. solanacearum* at elevated temperatures by exploiting the natural variation of plant response to *R. solanacearum* under heat stress. We demonstrated that a temperature increase of 3°C has a drastic impact on *A. thaliana* response to the GMI1000 strain and on RRS1-R, the major resistance mechanism against *R. solanacearum (Aoun et al., 2017).*

2. Exploring natural diversity of plants to identify the genetic basis of resistance

During the past three decades, genetic tools have been developed to assist in the traditional mapping of QTLs in experimental populations, but these tools remain laborious (Bergelson and Roux, 2010). Almost one decade ago, the first GWA study was reported in plants (*A. thaliana*; (Atwell et al., 2010), bringing a breath of fresh air to the area of gene discovery associated with natural phenotypic variation. The high resolution conferred by GWA mapping facilitates mapping of the genetic basis of a large range of phenotypes, including pathogen resistance. Long considered the privilege of human mapping studies, GWA mapping is now established as a powerful alternative approach to finely dissect the intraspecific genetic variation that underlies phenotypic variation in plants (Bergelson and Roux, 2010). Most of these tools are presented below.

a. Traditional QTL mapping

Traditional QTL mapping can be achieved by complementary experimental populations (Bergelson and Roux, 2010):

- **F2 population**: a segregating population coming from selfing of F1 plants resulting from a cross between two phenotypically contrasted parents (figure 14). In *A. thaliana*, F2 populations are generally used for preliminary mapping and QTL dominance estimation. Fine mapping with a

F2 population is limited because F2 plants are largely heterozygous and can therefore only be phenotyped once (with the exception of species with a vegetative reproduction system; (Collard and Mackill, 2008). Nonetheless, F2 populations can also be used for the Bulk Segregant Analysis (BSA) mapping approach. BSA consists in (i) pooling F2 individuals according to the extreme phenotypes observed in the F2 population, (ii) whole-genome sequencing of phenotypically contrasted pools, and (iii) testing differences in allele frequency across the genome (figure 15; (Wenger et al., 2010).

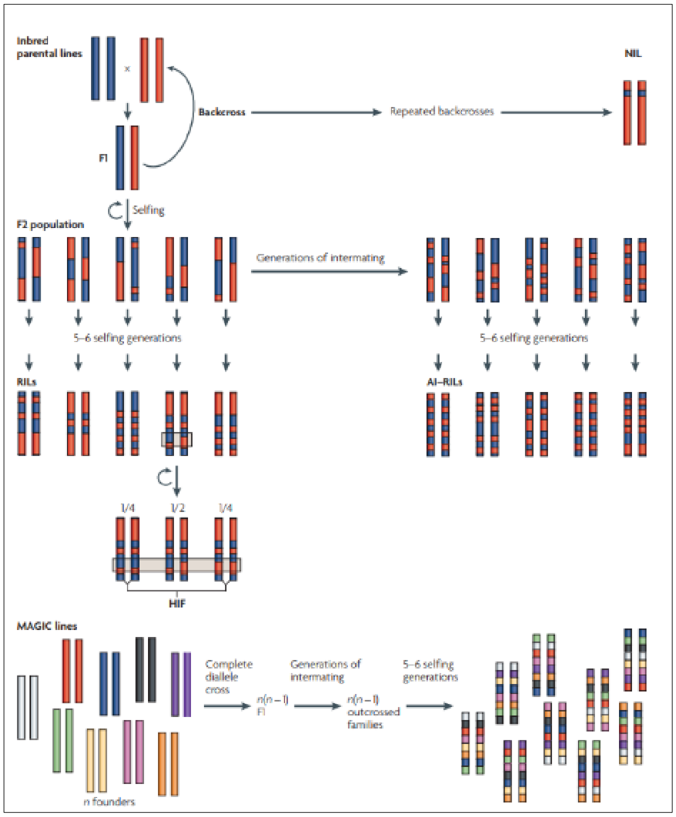

Figure 14. Different QTL mapping populations in *Arabidopsis thaliana*. AI-RILs, Advanced Intercross–Recombinant Inbred Lines; **HIF**, Heterogeneous Inbred Family; **MAGIC lines**, Multiparent Advanced Generation Intercross Lines; **NIL**, Near-Isogenic Line; **RILs**, Recombinant Inbred Lines (Bergelson and Roux, 2010).

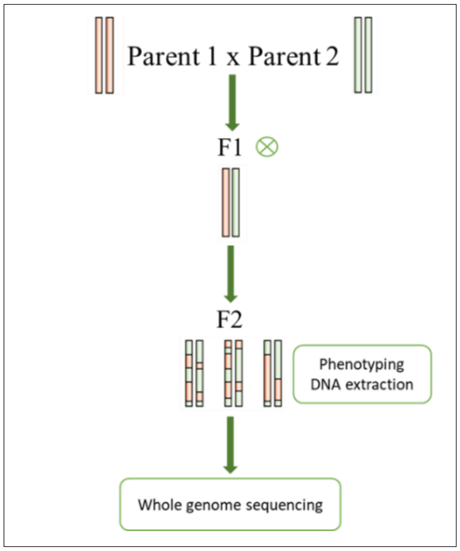

Figure 15. Principle of the Bulk Segregant Approach.

● **Near Isogenic Lines (NILs)** result from repeated backcrosses of the F1 with one of the two parental lines (figure 14), leading to the introgression of a small genomic fragment of one of the two parental lines into the genome of the other parental line. NILs are generally used to validate the physical position of QTLs with small allelic effects (Keurentjes et al., 2007).

● **Recombinant Inbred Lines (RILs)** correspond to the progeny of repeated selfing of F2 plants over several generations (figure 14). In contrary to a F2 population, RILs are almost completely homozygous, thereby allowing replicating RILs with an experiment and/or among several environmental conditions. Mapping resolution of RILs is however coarse, with the identification of QTL regions of a few megabases covering hundreds to thousands of genes. A related experimental population correspond to Advanced Intercross-Recombinant Inbred Lines (AI-RILs). AI-RILs are generated from successive generations of random intermating between F2 plants, followed by several generations of selfing (figure 15; Darvasi and Soller, 1995). This phase of random intermating increases the number of recombination events, thereby increasing mapping resolution.

● **Multiparent Advanced Generation Intercross lines (MAGIC lines):** an experimental population that is based on intermating between a certain numbers of founders for several generations (figure 15). Similar to AI-RILs, this phase of random intermating followed by several generations of selfing increases mapping resolution (figure 14; Cavanagh et al., 2008; Kover et al., 2009; Bergelson and Roux, 2010).

Together, mapping resolution and genetic diversity in linkage mapping populations is determined by the number of founders, the number of generations of intermating and the number of generations of selfing (Bergelson and Roux, 2010).

b. Genome wide association mapping (GWA)

Principle

GWA mapping aims to identify genetic polymorphisms associated with phenotypic variation measured in a set of natural genotypes (Bush and Moore, 2012; Read and Massey, 2014). GWA was first developed for medical and pharmacological purposes (Haines et al., 2005; Klein et al., 2005; Cooper et al., 2008; Bergelson and Roux, 2010) and since more than a decade, it has also been used in plants *(Aranzana et al., 2005; Zhao et al., 2007; Atwell et al., 2010)*. GWA mapping is a powerful tool that relies on the short linkage disequilibrium (LD) observed in natural populations, resulting from tens thousands of generations of recombination. LD is an estimate of the relationship between two genetic polymorphisms and the physical distance between them (Bush and Moore, 2012). For a more precise mapping resolution in GWA mapping population, it is better to have a short LD and a genetically very diverse population (Sauvage et al., 2014). Typically, estimates of LD decay in *A. thaliana* is between 5 and 10kb, but can largely vary among geographical regions. For instance, LD decay is longer in regions recently colonized by *A. thaliana* such as Northern Europe and USA.

However, GWA has two important disadvantages, that is, the presence of false positives and genetic and/or allelic heterogeneity. The production of false positives corresponds to a false genotype-phenotype association due to the impact of the species demographical history. The effect of population structure in GWA mapping is particularly important in *A. thaliana* at the European scale. Indeed, after the last glacial period, the colonization of Central and North Europe from refuges located at the Mediterranean perimeter led to a strong differentiation at the genome level (Nordborg et al., 2005). To reduce the rate of false-positives, mixed models including a kinship matrix can be used to control for confounding by population structure (Kang et al., 2010). However, this statistical correction can lead to an increase of the rate of false negatives; which are causative genetic markers that are lost after correcting for population structure (Bergelson and Roux, 2010). Therefore, to limit the rate of false positive and false negatives, GWA mapping can be combined with traditional linkage mapping, which is prone for identifying rare alleles and is not subject to the effect of population structure (Bergelson and Roux, 2010). Nonetheless, this combined approach remains time consuming due to the requested phenotyping of thousands experimental lines (Brachi et al., 2010). An alternative approach to limit the drawbacks of GWA mapping performed at a worldwide scale is to work at a small geographical scale, thereby limiting the degree of differentiation at the genome level (Bergelson and Roux, 2010).

The second major disadvantage of GWA is the presence of genetic and/or allelic heterogeneity. This happens when the same observed phenotypic value within a mapping population results from different QTLs or different alleles of the same QTL (figure 16; Bergelson and Roux, 2010). Genetic and/or allelic heterogeneity largely diminishes the statistical power of GWA mapping, since it increases the level of asymmetry between intra-allelic variances to a given genetic marker (figure 16). To solve this problem, it is recommended to work at a small geographical scale in order to limit the number of rare alleles, while maintaining a relative high genetic diversity in comparison to the worldwide scale (Bergelson and Roux, 2010).

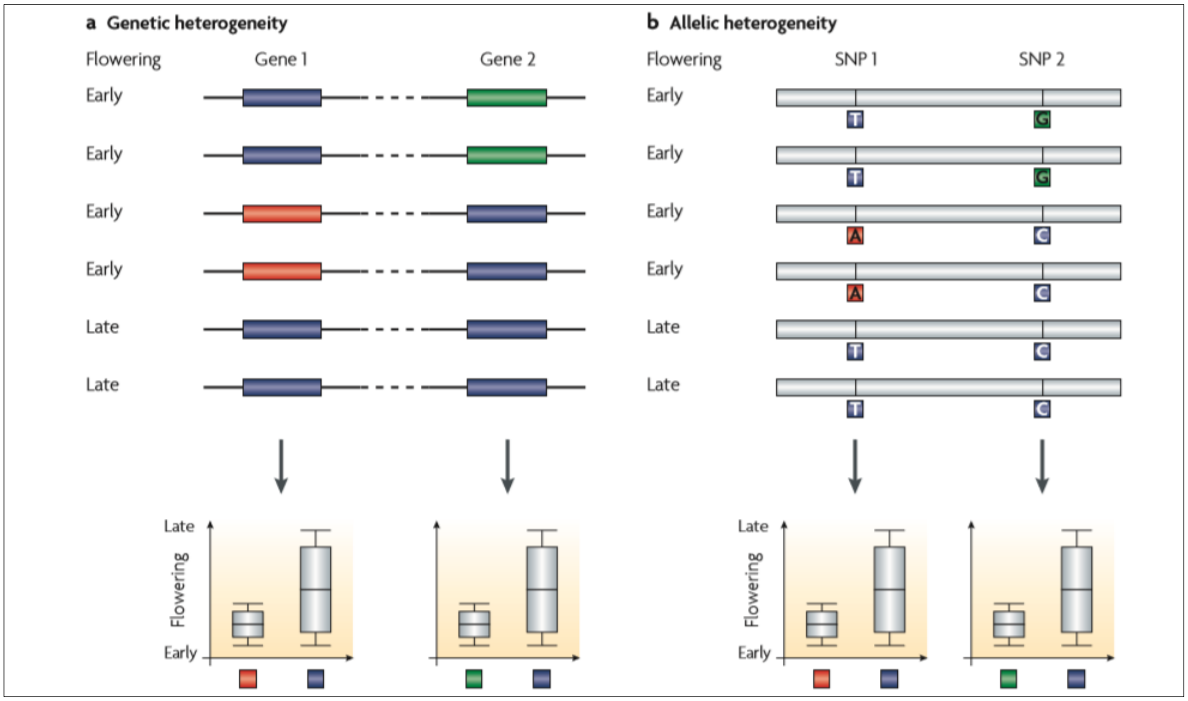

Figure 16. Schematic representation of the effect of genetic and allelic heterogeneity on QTL detection in GWA mapping in the case of flowering time (Bergelson and Roux, 2010).

GWA mapping applications to unravel the genetics of natural variation of pathogen resistance under heat stress

To our knowledge, studying the genetic basis of the natural variation of the response of wild and cultivated plants to pathogens under heat stress has never been done before. GWA mapping has been proven to be highly successful to fine map genes conferring pathogen resistance in *A. thaliana* and crops at normal temperatures (Bartoli and Roux, 2017). Therefore, we find it relevant to use GWA mapping both on worldwide and local collections of *A. thaliana* to identify resistance mechanisms to *R. solanacearum* under heat stress.

E. Thesis project objectives

Observed climate change scenarios are becoming more alerting and frequent with heat stress, thereby threatening crop yields and food security (Zhao et al., 2017). To find efficient and durable resistances to phytopathogens, one solution is to explore the natural diversity of plant response to look for stable resistances under temperature elevation. Therefore, in this project, I aimed to identify resistance mechanisms to *R. solanacearum* remaining efficient under heat stress in *A. thaliana*. I started working on this project during my master 2 internship (six months) where I had the opportunity to work with two different natural collections of *A. thaliana*, a worldwide and a local collection (figure 17).

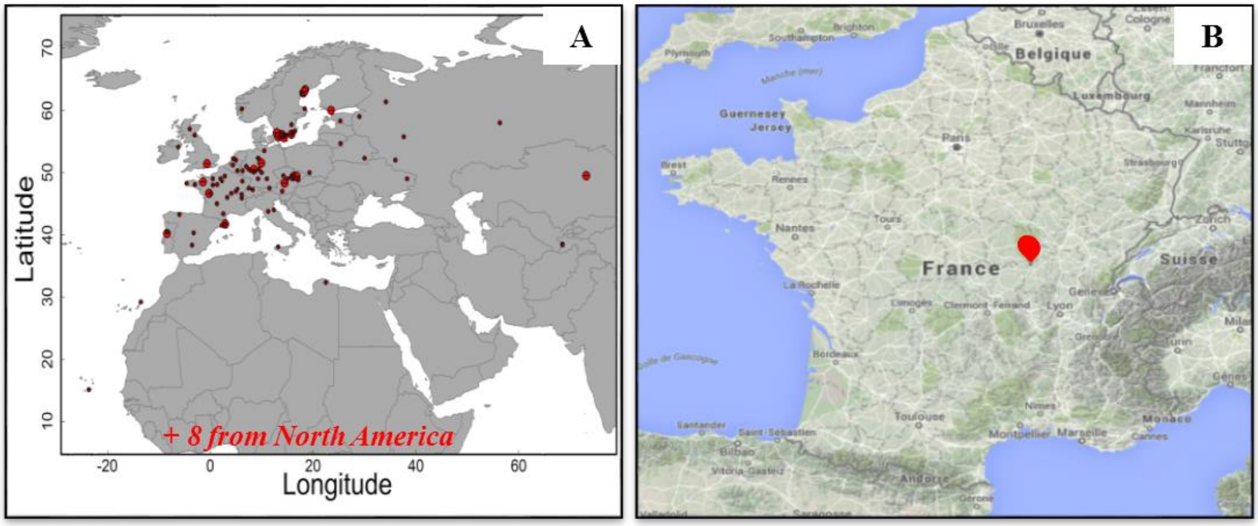

Figure 17. Geographical distribution of *A. thaliana* natural accessions of the worldwide and local collections used in this study. A. Represents the origin of accessions of the worldwide collection. **B.** Represents the origin of accessions of the local collection located in Burgundy (France).

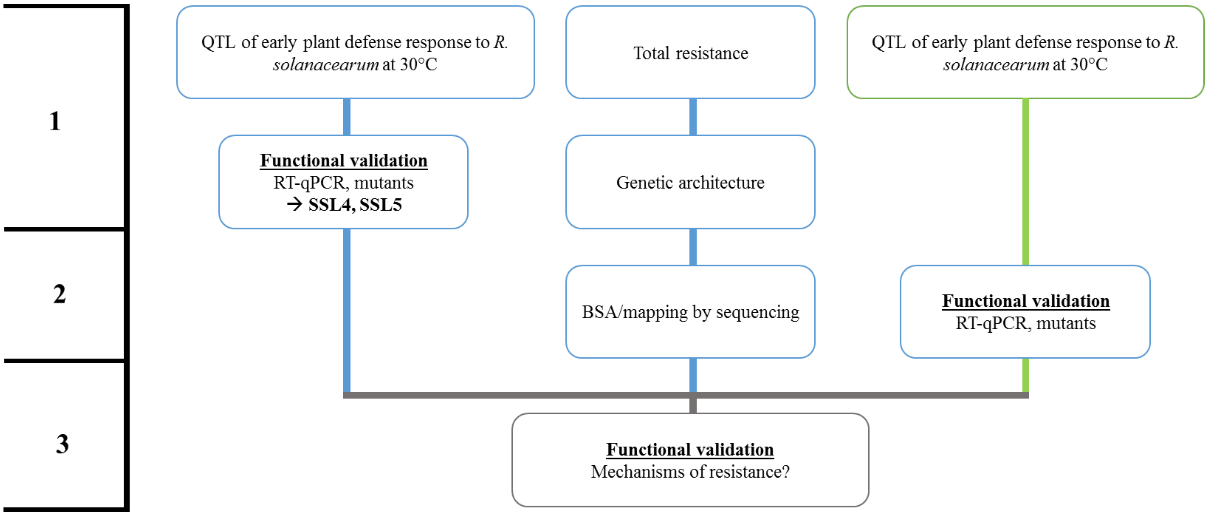

Figure 18. Overview of the PhD project work packages.

Following a GWA mapping experiment performed by a former intern (Laetitia Tauleigne) on the worldwide collection challenged with *R. solanacearum* GMI1000 strain, an association peak was detected at 27°C at the end of chromosome V. The most significantly associated SNP (top SNP) fall in the locus RPS4/RRS1 previously described as a major resistance mechanism to *R. solanacearum*, thus demonstrating the power of a GWA mapping approach to identify the genetic basis of already known defense mechanisms to this pathogen. At 30°C, we detected a QTL involved in early plant defense response to *R. solanacearum* GMI1000 strain on the chromosome III. Therefore, the first work package of my PhD was dedicated to functionally validate the genetic basis involved in early defense response to the GMI1000 strain under heat stress by adopting a reverse genetic approach (figure 18). The phenotyping of the worldwide collection has revealed a single accession, Eden-1, that remained symptomless during all infection stages. Thereby, the second work package was dedicated to the study of the genetic architecture underlying total resistance of Eden-1, the identification and the cloning of the underlying gene and its functional validation (figure 18). Concerning the third work package, we aimed to study the genetic basis of *A. thaliana* response to *R. solanacearum* GMI1000 strain in the highly genetically polymorph French local TOU-A population, under heat stress (figure 18).

The objectives of this PhD project can be resumed as follow:

1. Study the natural diversity of response to *R. solanacearum* under heat stress at two geographical scales.
2. Identify the genetic basis of resistance maintained under heat stress in two different collections of *A. thaliana*.
3. Functionally validate candidate genes involved in QDR to combined stresses (heat stress and *R. solanacearum*).
4. Functionally validate and characterize the genetic basis underlying the total resistance observed in Eden-1 accession.

Chapter II. Identification of the genetic basis of natural variation of plant response to *Ralstonia solanacearum* under elevated temperature in a worldwide population of Arabidopsis

A. Introduction

As previously explained in the part D of chapter I (‘The lofty goal of identifying new resistance mechanisms under heat stress’), exploring natural genetic diversity might be a great value to find durable mechanisms of plant resistance mechanisms maintained under heat stress. In this part of the project, we worked with a worldwide collection of *A. thaliana*, containing 176 natural accessions that have been genotyped for 214,051 SNPs (Horton et al., 2012). These accessions have been mainly collected across the European native area of *A. thaliana* and used to identify the genetic basis of local adaptation to climate across Europe (Fournier-Level et al., 2011; Hancock et al., 2011). In order to investigate the mechanisms of resistance to *R. solanacearum* under heat stress, this collection was inoculated with the GMI1000 reference strain at a standard temperature of inoculation (27°C) and applying a +3°C increase of temperature (30°C), thereby corroborating with the range of average temperature elevation predicted in climate change scenarios. Inoculation was done following two methods where the roots were either cut to give bacteria a direct access in the xylem (CUT) or left uncut to mimic a natural infection (UNCUT).

In the CUT condition of inoculation, accessions were on average more susceptible at 30°C than at 27°C. We also detected an important genetic variation in response to the GM1000 strain for each temperature treatment, with the presence of susceptible and resistant accessions to *R. solanacearum* at both temperatures. During all stages of infection, the broad-sense heritability estimates were high; suggesting that plant response to the *R. solanacearum* GMI1000 strain is genetically controlled even at early stages of infection. However, the level of quantitative disease response variability to the GMI1000 strain detected at 27°C was strongly decreased at 30°C, thus demonstrating the drastic impact of heat stress on this response. Interestingly, only one accession, Eden-1, was totally resistant to the bacteria during all the experiment at 30°C.

Using the phenotyping data generated by Laetitia Tauleigne (former bachelor intern), GWA mapping revealed at 27°C a major QTL with the top SNP falling in the *RPS4/RPS1-R* locus located on the long arm of chromosome V. As this immunoreceptor pair has long been known to confer resistance to several strains of *R. solanacearum* at 27°C (Deslandes et al., 2002), these results confirmed the power of GWA mapping to identify well-known resistance mechanisms to this bacterium. At 30°C, the QTL located in *RPS4/RRS1-R* locus was no longer detected. Instead, a new QTL was identified on the long arm of chromosome III at 5 dai. By adopting a reverse genetics approach, I have functionally validated two genes that belongs to the Strictosidine synthase-like family, S*SL4 and SSL5,* as genes of susceptibility. These results as well as results related to the UNCUT condition of inoculation were published in *Frontiers in Plant Science* in 2017 (chapter II part B). Some candidates found in the UNCUT condition are being validated by Henri Desaint in the frame of his PhD project.

Concerning the total resistant accession Eden-1, we first wanted to describe the underlying associated genetic architecture by phenotyping F2 plants resulting from a cross between Eden-1 and NW-77; an EMS sterile mutant of the accession Landsberg-0 (Ler-0) susceptible to Ralstonia. The F2 population was phenotyped with the *R. solanacearum* GMI1000 reference strain at 30°C in the CUT condition of inoculation. The segregating ratio '1 resistant : 3 susceptible' indicated that the genetic architecture responsible for total resistance is mainly monogenic and recessive. Based on this simple genetic architecture, we then decided to adopt a BSA approach to fine map the main QTL conferring total resistance in Eden-1 at 30°C. To do so, we sequenced one pool of resistant plants and seven pools of susceptible plants. Based on allele frequency differentiation between resistant and susceptible pools, we found that the most differentiated SNPs were localized within the *RPS4/RRS1* locus. Alongside with this, we also showed that Eden-1 total resistance was lost when inoculated with a GMI1000 strain mutated in the T3E PopP2 (RSΔPopP2) at 30°C. As *RPS4/RRS1-R* resistance is known to be monogenic and recessive and is located on chromosome V (Deslandes et al., 2002), we hypothesized that an allelic form of the *RPS4/RRS1-R* locus could be responsible for the total resistance phenotype in this accession. To test this, the *RPS4/RRS1-R* locus present in Eden-1 was cloned and sequenced. The generated sequence was aligned with other versions of *RPS4*/*RRS1* sequences previously obtained from a set of accessions that are either susceptible or resistant at 27°C, but all susceptible at 30°C. Interestingly, a SNP specific to the *RRS1-R* allelic version of Eden-1 accession was found in the LRR domain. This polymorphism is located in the same domain than an artificial EMS point mutation (*int102* mutant) created in the *snc1-1* mutant background by Zhu et al. (2010), known to confer heat-stability to the SNC1 NLR protein (Zhu et al., 2010). To functionally validate our results, we transformed plants of Ws-0 and the double mutant *rps4-21/rrs1-1* (Ws-0 background; Narusaka et al., 2009) with construction enabling the expression of the *RPS4/RRS1-R* allelic form of Eden-1 (*RPS4/RRS1-R* $^{\text{Eden-1}}$; resistant both at 27°C and 30°C) and Nd-1 (*RPS4/RRS1-R* $^{\text{Nd-1}}$; resistant at 27°C but not at 30°C) and also with the *RPS4/RRS1-R* sequence of Nd-1 in which the specific mutation identified in the *RRS1-R* sequence of the Eden-1 accession was introduced (*RPS4/RRS1-R*$^{\mathrm{A641T}}$; cloning realized in collaboration with L. Deslandes). Results will be presented in the chapter II part C under the format of an article in preparation.

B. Genetic basis of early resistance to *Ralstonia solanacearum* under elevated temperature

Article: Quantitative Disease Resistance Under Elevated Temperature: Genetic Basis of New Resistance Mechanisms to *Ralstonia solanacearum*

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Quantitative Disease Resistance under elevated temperature: genetic basis of new resistance mechanisms to *Ralstonia solanacearum*

Running title:

QDR to *R. solanacearum* at elevated temperature

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Word count: 7225

Number of tables: 2

Number of figures: 6

Number of supplemental tables: 8

Number of supplemental figures: 8

Abstract

In the context of climate warming, plants will be facing an increased risk of epidemics as well as the emergence of new highly aggressive pathogen species. Although a permanent increase of temperature strongly affects plant immunity, the underlying molecular mechanisms involved are still poorly characterized. In this study, we aimed to uncover the genetic bases of resistance mechanisms remaining efficient at elevated temperature to the *Ralstonia solanacearum* species complex (RSSC), one of the most harmful phytobacteria causing bacterial wilt. To start the identification of quantitative trait loci (QTLs) associated with natural variation of response to *R. solanacearum,* we adopted a genome wide association (GWA) approach using 176 worldwide natural accessions of *Arabidopsis thaliana* inoculated with the *R. solanacearum* GMI1000 strain. Following two different procedures of root-inoculation (root apparatus cut *vs* uncut), plants were incubated either at 27°C or 30°C, with the latter temperature mimicking a permanent increase in temperature. At 27°C, the *RPS4/RRS1-R* locus was the main QTL of resistance detected regardless of the method of inoculation used. This highlights the power of GWA mapping to identify functionally important loci for resistance to the GMI1000 strain. At 30°C, although most of the accessions developed wilting symptoms, several QTLs were identified depending on the inoculation method used. We focused on a QTL region associated with response to the GMI1000 strain in the early stages of infection and, by adopting a reverse genetic approach, and we functionally validated the involvement of a strictosidine synthase-like 4 (SSL4) protein that shares structural similarities with animal proteins known to play a role in animal immunity.

Keywords

Climate warming, Quantitative Disease Resistance, *Ralstonia solanacearum*, *Arabidopsis thaliana*, natural diversity, Genome Wide Association mapping

Introduction

Global changes are predicted to increase the frequency of extreme climate events and to accentuate abiotic stresses such as temperature increase, drought and water logging (Orlowsky and Seneviratne, 2012; IPCC, 2014), thereby impacting crop development (e.g. rate of photosynthetic carbon assimilation, rate of leaf initiation, leaf expansion, root architecture or reproduction) and ultimately yield (Hatfield et al., 2011; Gray and Brady, 2016). Amongst climatic risks, global surface temperature is predicted to rise by the end of the century, from 1.0 up to 4.8°C, with higher frequencies and longer periods of heat waves (IPCC, 2014). While an increase of mean temperature constitutes one of the major abiotic stresses that plants have to cope with (Bita and Gerats, 2013; Suzuki et al., 2014), it is also expected to favor the emergence of new pathogens and to increase the occurrence of epidemics (Garrett et al., 2006; Evans et al., 2008; Bebber et al., 2013). For example, recent studies reported that climate change likely increased the emergence of highly aggressive and invasive strains of rust *Puccina graminis*, *P. striiformis* and of the oomycete *Phytophthora infestans* (Hovmoller et al., 2008; Singh et al., 2011; Cooke et al., 2012; Bebber et al., 2013). These future outbreaks are a major concern for the maintenance of global food security. Therefore, identifying and studying the genetic and molecular basis of defense mechanisms allowing plants to cope with epidemics under higher temperature conditions is critical.

In their natural habitats, plants have evolved complex defense responses to deal with the simultaneous and/or sequential attacks from various bio-aggressors (e.g. viruses, bacteria, fungi, oomycetes and herbivores) (Roux and Bergelson, 2016). Defense responses include plant preformed physical barriers (e.g. rigid cell wall, presence of cuticles or trichomes, production of toxic or repellent compounds) (Osbourn, 1996; Nurnberger and Lipka, 2005) as well as immune signaling responses that are activated upon pathogen recognition. This latter type of resistance mechanism corresponds to a two-level defense system described as the zig-zag model (Jones and Dangl, 2006; Dodds and Rathjen, 2010). In the first level, microbial elicitors, called pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs) are perceived by plant cell surface and transmembrane pattern recognition receptors (PRRs) to initiate a signaling cascade leading to the MAMP- or PRR-triggered immunity (MTI/PTI), that are efficient against a broad spectrum of pathogens. To overcome PTI, pathogens produce virulence factors called effectors (T3Es) which can promote effector-triggered susceptibility (ETS) by interfering with host defense responses. In turn, effectors can be specifically recognized by plant intracellular resistance proteins, known as nucleotide binding site-leucine rich repeat containing proteins (NLRs), thereby activating a second level of plant defense, the effector-triggered immunity (ETI). ETI results in a strong defense response often associated with a hypersensitive response (HR), characterized by a rapid and a local cell death. However, this type of qualitative response is generally specific to a single pathogen species, and even strain-specific, thereby leading to a strong selective pressure of virulent strains that can bypass ETI (Roux et al., 2014). Another form of resistance called Quantitative Diseases Resistance (QDR) is characterized by a reduction rather than an absence of disease (St Clair, 2010; Mundt, 2014; Roux et al., 2014; French et al., 2016) and is typically polygenic (Poland et al., 2009). Knowing that QDR provides a durable and a broad-spectrum resistance, this form of resistance appears to be unsurprisingly much more prevalent than ETI in crops and natural plant populations (Young, 1996). The characterization of the main molecular mechanisms underlying PTI and ETI have paved the way to decipher basal and specific immune responses, which is not currently the case of QDR's molecular mechanisms that remain largely unknown (Roux et al., 2014).

Plants are exposed to multiple abiotic and biotic stresses either in crop fields or in natural populations. However, studies deciphering the molecular mechanisms governing plant response to these combined stresses are scarce (Mittler, 2006; Suzuki et al., 2014). Yet, several transcriptome studies recently demonstrated that despite a certain overlap, each combination of stress involves a unique response that could not be easily predicted by the study of each stress individually, and leading to either positive or negative effects on host immunity (Atkinson and Urwin, 2012; Atkinson et al., 2013; Prasch and Sonnewald, 2013; Rasmussen et al., 2013; Suzuki et al., 2014; Onaga et al., 2017). Remarkably, an increase in temperature (Δ+3-7°C) was demonstrated (i) to inhibit several major defense mechanisms induced following a pathogen attack, regardless of the pathogen and the plant species considered, or (ii) to suppress ETI-HR related phenotypes. For example, in *Arabidopsis thaliana*, the expression of two regulators of plant immunity, *PAD4* and *EDS1*, is down-regulated by a rise in temperature from 22°C to 28°C (Yang and Hua, 2004). RPS4- or RPM1-mediated resistance responses to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 strains are efficient at 22°C but inhibited at 28°C (Wang et al., 2009). In addition, the HR triggered either by *Pst* DC3000 strain containing the HopZ1A avirulent effector or by the *RPW8* gene conferring resistance against powdery mildew is suppressed by a temperature above 28°C and 30°C, respectively (Xiao et al., 2003; Menna et al., 2015). However, for *Pst* DC3000 strains carrying the *AvrRpt2* effector gene, contradictory results were observed. Indeed, elevated temperatures led to either a resistance inhibition (Wang et al., 2009) or a HR suppression without ETI-mediated virulence suppression (Menna et al., 2015). Similar examples of inactivation by an elevated temperature were also reported in other plant species. Resistance conferred by the *Mi-1* gene to root-knot nematodes, by the *N* gene to tobacco mosaic virus (TMV) and by the *Cf4* and *Cf9* genes to the fungal pathogen *Cladosporium fulvum* are inactivated in tomato at temperatures above 28, 30, and 33°C respectively (Whitham et al., 1996; Hwang et al., 2000; de Jong et al., 2002). An elevated temperature also negatively modulated plant defense response linked to *Rp1-D21* in maize (Negeri et al., 2013). By contrast, partial or more efficient *Yr36*, *Xa7* and *Pi54* genes-related resistance-related genes were also described against *P. graminis* in wheat (Fu et al., 2009), *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae* in rice, respectively (Webb et al., 2010; Onaga et al., 2017). Although an increase of temperature can differentially balance host immunity, the molecular mechanisms involved remain elusive.

The *Ralstonia solanacearum* species complex (RSSC) is responsible for the bacterial wilt; one of the most harmful disease causing tremendous yield losses in more than 200 plant species in tropical, subtropical and warm temperate areas worldwide (Elphinstone, 2005). The strains composing the RSSC are phylogenetically represented by four phylotypes according to their geographical origin (I: Asia, II: America, III: Africa, IV: Australia-Indonesia-Japan) (Ailloud et al., 2015). The ability of RSSC to quickly adapt to new host plants or to the universal resistant reference Hawaii7996 tomato cultivar (Wicker et al., 2007) is thought to be promoted by (i) many molecular determinants involved in pathogenicity and host-range specificity, (ii) large and variable repertoires of T3Es and (iii) a high evolutionary ability (Coupat et al., 2008; Remenant et al., 2010; Genin and Denny, 2012). In addition, climate warming has been recently proposed to be involved in the expansion of the potato brown rot disease caused by some RSSC strains in Bolivia (Castillo and Plata, 2016). Host resistance to RSSC, which remains the most efficient strategy of disease control, is generally controlled by a polygenic architecture (Mangin et al., 1999; Wang et al., 2000; Carmeille et al., 2006). In the model plant *A. thaliana,* the two molecular resistance mechanisms identified so far involve the LRR receptor-like kinase ERECTA (Godiard et al., 2003) and the *RPS4/RRS1*-R pair of immune receptors (Deslandes et al., 2002; Le Roux et al., 2015), respectively. Recently, Le Roux *et al.* (2015) demonstrated that RPS4 with RRS1-R represent a DNA-bound immune receptor complex with an integrated effector decoy that directly converts the virulence activity of the PopP2 effector into activation of immunity (Le Roux et al., 2015). Interestingly, this immune-receptor pair also confers resistance to various pathogens including the fungus *Colletotrichum higginsianum*, *Pst* DC3000 delivering the AvrRps4 effector, and the strain CFBP6943 of *Xanthomonas campestris* pv.

campestris (*Xcc*) (Narusaka et al., 2009; Debieu et al., 2016). In addition, in contrast to the well-characterized resistance against *R. solanacearum* GMI1000 strain identified in the *A. thaliana* accession Nd-1 (Deslandes et al., 1998; Deslandes et al., 2002), a tolerant phenotype to *R. solanacearum* BCCF402 strain in the *A. thaliana* Kil-0 accession, resulting in the absence of symptom despite a high bacterial multiplication *in planta*, was demonstrated to be also dependent on the PopP2 effector but with a specific allele of *RRS1-R* (Van der Linden et al., 2013). Thus, these results suggest that *RRS1-R* may also be involved in a QDR mechanism in the *A. thaliana* Kil-0 accession.

Despite the fact that a temperature increase is known to affect host resistance to *R. solanacearum* in several plant species (Hayward, 1991; Prior et al., 1996), no mechanism of resistance remaining efficient under a permanent increase in temperature has been identified yet. Moreover, studies reporting the genetic architecture and the molecular mechanisms underlying the genetic diversity of plant responses to RSSC are still lacking in the context of climate change. Therefore, exploring natural genetic variation can help to identify uncovered sources of resistance to RSSC that remain efficient under elevated temperature conditions. Thanks to the development of Next-Generation Sequencing (NGS) technologies and appropriate statistical methods (Bergelson and Roux, 2010), the method of genome-wide association (GWA) mapping recently emerged in plants and has been used so far in 11 species to fine map genomic regions associated with natural variation of response to a range of microbial enemies, including bacteria, fungi and oomycetes (Bartoli and Roux, 2017). Notably, five QTLs identified by GWA mapping have been subsequently functionally validated in *A. thaliana*, demonstrating the power of this method to precisely dissect the intraspecific genetic variation underlying pathogen resistance (Bartoli and Roux, 2017). Therefore, in this study, we adopted a GWA mapping approach to fine map quantitative trait loci (QTLs) associated with natural variations of response to the *R. solanacearum* GMI1000 strain among 176 worldwide natural accessions of *A. thaliana*. Two inoculation methods were used and root-inoculated plants were incubated at two different temperatures to mimic a permanent increase of temperature. Whatever was the method of inoculation used, GWA mapping confirmed the *RPS4/RRS1-R* locus as the main QTL of resistance detected at 27°C, demonstrating the power of this approach to functionally fine map important loci for resistance to *R. solanacearum*. At 30°C, most accessions developed wilting symptoms, confirming the drastic effect of the temperature increase (+3°C) on the mechanisms of resistance triggered by plants. Interestingly, we uncovered several QTLs, underlying natural variation for early plant defense response to *R. solanacearum*. A reverse genetic approach revealed that one of these specific QTLs at 30°C involves a strictosidine synthase-like 4 (SSL4) protein.

Material and Methods

1. Bacterial strain, plant material and growth conditions

The wild type *R. solanacearum* GMI1000 strain used in all inoculation experiments was grown in complete BG medium as described by Plener et al. (2010). A collection of 176 *A. thaliana* worldwide accessions was used in this study (Table S1). Five to ten seeds of each accession were directly sown on Jiffy pots (Jiffy Products International AS, Norway) and stratified for 48h at 4°C in order to release seed dormancy. Accessions were then grown in growth chamber under controlled conditions for four weeks (22°C, 70% relative humidity (RH), 9h of light) prior to phenotyping experiments. The 38 T-DNA insertion mutants (Ws-0 and Col-0 background) corresponding to 22 genes included in a 80 kb genomic region underlying the QTL of early plant defense response to *R. solanacearum* were identified using the online Arabidopsis gene mapping tool T-DNA express (http://signal.salk.edu/cgi-bin/tdnaexpress) (Table S2) and ordered from the Nottingham Arabidopsis Stock center (http://arabidopsis.info/).

Corresponding seeds were sown and stratified as described above and grown in greenhouse conditions (26.5°C +/- 1.5°C, 16h light). Progenies of genotyped homozygous of each mutant were harvested and grown for four weeks as described above before inoculation. The Col-0 accession (N60000), susceptible to the *R. solanacearum* GMI1000 strain, was used as a control in all experiments.

2. Plant inoculation and phenotyping

Four-week-old plants were used in all experiments. Plant response to *R. solanacearum* GMI1000 strain was assessed at 27°C and 30°C using two inoculation conditions: (i) the UNCUT condition previously described (Lohou et al., 2014), where the roots were not wounded thereby mimicking natural infection, and (ii) the CUT condition (Deslandes et al., 1998) where the roots where sectioned with scissors, approximately 1 cm from the bottom of the pot, giving the bacteria a direct access to the xylem vessels. Plants were soaked for 15 min in 2 L per tray of a bacterial suspension at 1.10^7 bacteria/mL and 1.10^8 bacteria/mL, for the CUT and the UNCUT conditions, respectively. Inoculated plants were then transferred in growth chambers with controlled conditions at 27°C or at 30°C (75% HR, 12h light, 100µmol $m^{-2}s^{-1}$). The wilting symptoms were scored on an established 0 to 4 disease index scale (Deslandes et al., 1998) with the score 0 and 4 corresponding to healthy and dead plants, respectively. Symptoms were monitored from 3 to 13 days after inoculation (dai), and from 3 dai to 10 dai for plants incubated at 27°C and 30°C, respectively.

3. Natural variation of QDR

3.1 Experimental design

For each 'CUT condition x temperature treatment' combination, an experiment of 1152 plants was set up using a randomized complete block design (RCBD) of two experimental blocks.

Each block was represented by six trays of 96 positions. Each block corresponded to 576 plants with three replicates per accession (n = 528 = 176 accessions * 3 replicates) and the control accession Col-0 placed in the same five positions within each tray (n = 30 = 6 trays * 5 replicates). In each block, the remaining 18 positions in the trays were left empty.

For each ‘UNCUT condition x temperature treatment’ combination, an experiment of 570 plants was set up using a RCBD of three experimental blocks. Each block was represented by three trays of 64 positions. Each block corresponded to 192 plants with one replicate per accession (n = 176) and the control accession Col-0 placed in 14 positions across the three trays (n = 14). In each block, the remaining two positions in the trays were left empty.

3.2 Statistical analyses

For each ‘CUT/UNCUT condition x temperature treatment’ combination, we used the following mixed model (MIXED procedure in SAS9.3; SAS Institute Inc., Cary, NC, USA) to explore the natural genetic variation of the disease index at each time point of phenotyping:

$$\text{disease index}_{ijc} = \mu + \text{block}_i + \text{accession}_j + \text{block}_i \times \text{accession}_j + \text{covCol}_c + \varepsilon_{ijc} \quad (1)$$

where μ is the overall mean of the phenotypic data, ‘block’ accounts for differences in micro-environmental conditions between the two or three experimental blocks; ‘accession’ corresponds to the genetic differences among the natural accessions; ‘block x accession’ accounts for genetic differences among the natural accessions depending on the block; covCol is a covariate accounting for tray effects within blocks (phenotypic mean of the four or -five Col-0 replicates per tray was used as a covariate); and ‘ε’ is the residual term. Normality of the residuals was not improved by transformation of the data. The factor ‘block’ was treated as a random factor, whereas the factor ‘accession’ was treated as a fixed factor. Significance of the random effect was determined by likelihood ratio tests of model with and without this effect. Least-square means (LSmeans) were obtained for each natural accession and were subsequently used for GWA mapping analyses. Broad-sense heritabilities (H^2) at each time point of phenotyping were estimated from the mean square (MS) of equation (1) using a formula adapted from Gallais (1990). Due to the absence of Col-0 control plants in some trays of the 'UNCUT condition x 27°C' combination, the term 'covCol$_c$' was not modeled in equation (1).

3.3 GWA mapping

The 176 natural accessions used in this study have been genotyped for 214,051 SNPs evenly spaced across the genome (Horton et al., 2012). In order to fine map the genomic regions associated with natural disease index variation at each time of phenotyping for each 'CUT/UNCUT condition x temperature treatment' combination, we ran a mixed model implemented in the software EMMAX [Efficient Mixed-Model Association eXpedited; (Kang et al., 2010)]. This mixed model includes a genetic kinship matrix K based on the 214,051 SNPs as a covariate to control for population structure in the mapping panel. Because of bias due to rare alleles, we only considered SNPs with minor allele relative frequency (MARF) > 10% (Brachi et al., 2010; Kang et al., 2010). Manhattan plots illustrating the results of phenotype-genotype associations at all stages of infection at 27°C and 30°C, in the CUT and UNCUT conditions of inoculation are presented in Figure S1, S2, S3 and S4, respectively. Corresponding Q-Q plots and lists of the most significantly associated SNPs (i.e. top SNPs with a $-\log_{10} p$-value > 4) are depicted in Figure S5, S6 and Table S8.

3.4 Gene ontology and biological pathways enrichment tests

To determine the biological processes involved in response to *R. solanacearum* GM1000 strain at 30°C and perform comparisons between the two inoculation methods used, we first tested for each 'CUT/UNCUT condition x time point of phenotyping' to determine whether SNPs in the 0.1% upper tail of the $-\log_{10}$ p-value distribution were over-represented in each of 736 Gene

Ontology Biological Processes from the GOslim set (Consortium, 2008). A total of 10,000 permutations were run to assess significance using the same methodology as described in Hancock *et al.* (2011). For each significant enriched biological process at a $P < 0.05$, we then retrieved the identity of all the genes containing SNPs in the 0.1% upper tail of the $-\log_{10}$ p-value distribution. Finally, each list of genes, corresponding to each phenotyping time point, was used after removal of duplicates, to identify biological pathways significantly over-represented ($P < 0.01$) with the classification superviewer tool on the university of Toronto website (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi) using the MAPMAN classification (Provart and Zhu, 2003).

4. T-DNA insertion mutants' validation, plant assays and statistical analyses

For each of the 38 T-DNA insertion mutants located in a QTL region of early *A. thaliana* defense responses to *R. solanacearum* (see *Results* section), 12 seedlings were genotyped to check the presence of the T-DNA insertion and to identify homozygous plants. For each seedling, one leaf was collected and used for genomic DNA extraction was adapted from QIAGEN DNeasy kit®as described in Mayjonade (Mayjonade et al., 2016). For genotyping, primer pairs were designed using the T-DNA primer design online tool (http://signal.salk.edu/tdnaprimers.2.html). All the primer sequences and corresponding PCR fragment sizes are listed in Table S3. For one PCR reaction, 2 µL of genomic DNA (10 ng/µL) were added to a PCR master mix composed of: 1 µL (10 pM) of each primer composing the LP+RP or RP+BP primer pairs (see Table S2), 0.5 µl (10 mM) dNTPs, 0.2 µl (10 u/µl) of GoTaq® DNA polymerase (Promega, Madison, WI, USA), 5 µL of 5X GoTaq buffer and 16.2 µL of sterilized water. The PCR cycling conditions were as follow: 95°C for 2 min; 10 cycles at 95°C for 30 sec, 62°C to 52°C for 30 sec (touch-down, 1°C decrease at each cycle) and 72°C for 1 min; 30 cycles at 95°C for 30 sec, 52°C for 30 sec and 72°C for 1 min; 72°C for 2 min.

T-DNA insertion mutants were inoculated using the CUT inoculation method with a bacterial suspension of 1.10^7 bacteria/mL and transferred at 30°C. Three to six independent experiments were made for each T-DNA insertion mutant. In all experiments, plants were organized according to a RCBD. To test whether the disease index was statistically different between the wild type Col-0 and each T-DNA mutants, we used a Kruskal-Wallis analysis under the *R* environment version 3.3.2 (R_Development_Core_Team, 2013).. The dynamics of T-DNA mutant lines response to the *R. solanacearum* GMI1000 strain was drawn using ggplot2 package (http://ggplot2.org/) showing the confidence interval.

5. RNA extractions and RT-qPCR

RNA extractions and RT-qPCR analyses were performed as previously described (Le Roux et al., 2015) using two leaves from healthy plants. Primer pairs used are listed in Table S3.

Results

Extensive genetic variation among worldwide accessions of *A. thaliana* for the response to *R. solanacearum*: effects of temperature and inoculation methods

Based on the phenotyping of 176 worldwide accessions of *A. thaliana,* we detected substantial genetic variation in the response to the GM1000 strain for each 'temperature treatment x inoculation method' (Figure 1, Table 1), with the presence of tolerant accessions to *R. solanacearum* at 30°C (Figure 1B and 1D). The high broad-sense heritability estimates observed at all stages of infection suggests that plant response to *R. solanacearum* is genetically controlled even at early stages of infection (Figure 1, Table 1). In both inoculation methods, accessions were on average more susceptible at 30°C compared to 27°C (27°C – 10 dai, $F = 136.9$, $P < 0.0001$; 30°C – 7 dai, $F = 227.1$, $P < 0.0001$) (Figure 1). At 27°C, accessions were on average more susceptible in the CUT conditions than in the UNCUT condition (13 dai, $F = 17.0$, $P < 0.0001$) (Figure 1). At 30°C, although the accessions had on average a similar disease index at the late stages of infection (6 dai: $F = 3.85$, $P = 0.0509$; 7 dai: $F = 0.02$, $P = 0.8889$; 10 dai: $F = 0.89$, $P = 0.3472$), the dynamics of disease induction was faster in the CUT condition compared to the UNCUT condition at the early stages of infection (3 dai: $F = 58.7$, $P < 0.0001$; 4 dai: $F = 88.7$, $P < 0.0001$; 5 dai: $F = 17.9$, $P < 0.0001$) (Figure 1).

In both methods of inoculation, variation in disease index at 30°C was significantly correlated with variation in disease index at 27°C (Figure 2). However, the correlation coefficient of Pearson was strongly different from 1 (CUT condition, 95% confidence intervals: 0.037 – 0.357; UNCUT condition, 95% confidence intervals: 0.087 – 0.383). The observation of crossing reaction norms between 27°C and 30°C suggests that the genetic architecture of the response of *A. thaliana* to the *R. solanacearum* GMI1000 strain is different between these two temperatures (Figure 2).

A major QTL corresponding to the immune receptor pair *RPS4/RRS1* locus confers QDR to the *R. solanacearum* GMI1000 strain at 27°C

In the CUT condition, GWA mapping revealed a unique peak of association on the long arm of chromosome 5 whose significance increased with the stages of infection (Figure S1). Thirteen days post-inoculation, the three most associated SNPs (SNP-5-18325032, $P = 5.37 \times 10^{-13}$, MARF = 0.125; SNP-5-18325565, $P = 3.55 \times 10^{-9}$, MARF = 0.237; SNP-5-18325915, $P = 8.65 \times 10^{-9}$, MARF = 0.151) were located in the *RPS4* gene (*At5g45250*) (Figures 3A-C), that encodes the RPS4 immune receptor. Previously described as cooperating genetically and molecularly with RRS1-R for resistance to *R. solanacearum* (Narusaka et al., 2009), *RPS4* and *RRS1* gene are localized near each other and are inserted in opposite directions. Therefore, the identification of the *RPS4/RRS1* locus as the major resistance QTL to *R. solanacearum* confirms the suitability of the GWAS approach to investigate the genetic bases of resistance responses to this bacterial pathogen.

In the UNCUT condition, we detected two neat peaks of association. The first association peak, at the beginning of chromosome IV, was detected at the late stages of infection (Figure S2), with the top SNP (SNP-4-5080256, $P = 4.93 \times 10^{-7}$, MARF = 0.429) located in a transposable element gene (*At4g08100*) belonging to the gypsy-like retrotransposon family (Figures 3D-E). The significance of the second association peak located on the chromosome V also increased with the stages of infection (Figure S2). Similarly to the CUT condition, the four most associated SNPs (SNP-5-18325565, $P = 1.60 \times 10^{-10}$, MARF = 0.252; SNP-5-18325032, $P = 2.80 \times 10^{-9}$, MARF = 0.123; SNP-5-18322558, $P = 1.19 \times 10^{-8}$, MARF = 0.301; SNP-5-18323844, $P = 4.85 \times 10^{-7}$, MARF = 0.295) were also found in *RPS4* at 13 dai (Figure 3D and 3F). These results suggest that although the major QTL was common for the two methods of inoculation, medium/minor QTLs can be specific to the inoculation method and/or to the stage of infection.

Different genetic architectures at 30°C highlight different plant defense response mechanisms

In agreement with the small correlation coefficients observed at the phenotypic level between 27°C and 30°C (Figure 2), GWA mapping revealed a different genetic architecture at 30°C, with no association peak located in the *RPS4/RRS1* locus or at its close vicinity (Figures S3 and S4). In addition, for each phenotyping time point, less than 2% of the 100 most significant associated SNPs (i.e. top SNPs) were shared between the two inoculation methods at 30°C (Figures S3 and S4), suggesting that the genetic basis of response to *R. solanacearum* largely differs between the CUT and UNCUT conditions. Different significantly over-represented functional classes assigned using the MapMan classification, (supported from >97.5%

bootstrap replicate), in which gene lists corresponding to the top SNPs for each ‘CUT/UNCUT condition x time point of phenotyping’ fall in, also support this observation. In the CUT condition, numerous genes involved in RNA processing and RNA regulation were found at 3 and 4 dai, whereas genes related to the perception and the regulation of plant response to biotic or abiotic stresses (i.e. leucine rich repeat protein, NBS-LRR protein, Cystein rich receptor like kinase protein, cytochrome P450) and protein degradation were mostly found at later stages of infection (Table S6, Figure 4A). By contrast, genes retrieved in the UNCUT condition were significantly over-represented in metabolic pathways at 3 to 5 dai, with genes having a role in hormone metabolism, defense response to biotic and abiotic stresses (CYP450 family 705 subfamily A; flavonoïd synthase), cell wall modification and signaling. Genes retrieved at 6 and 7 dai reflect an intense protein metabolism activity with numerous genes involved in protein synthesis and degradation (Table S7, Figure 4B).

Interestingly, for both inoculation methods, we observed playful dynamics of the association peaks along the infection stages (Figures S3 and S4). For example, in the CUT condition, we detected multiple association peaks specific to the 5 dai stage (Figure 5A). In particular, GWA mapping revealed an association peak located at the end of chromosome III supported by 12 SNPs with an association score above 3, embedded in a region of 80kb (Figure 5B). Similarly, in the UNCUT condition, GWA mapping revealed multiple association peaks specific to the 6 dai stage (Figure 6A). More specifically, two association peaks caught our attention as they are supported by numerous top SNPs, including those the most significantly associated to the plant response to *R. solanacearum* at the 6 dai stage (Table S8), and overlap with candidate genes known to be involved in plant response to different pathogens. The first one is located at the end of chromosome III, with the top SNP (SNP-3-22-260-125) located in the promoter region of the *EIF4G* (*At3g60240*) gene (Figure 6B). The second one is located at the beginning of chromosome V, with the six most associated SNPs all located within the *CesA3* (*At5g05170*) gene (Figure 6C).

QDR to the *R. solanacearum* GMI1000 strain at 30°C in the CUT condition is conferred by a gene encoding for a strictosidine synthase-like protein 4

Since neat association peaks at the early stages of infection could be detected (Figures 5B, 6B and 6C), we therefore investigated the molecular mechanisms underlying plant response to *R. solanacearum* at 30°C. To functionally validate one of those peaks, we focused on the QTL region identified at the bottom of chromosome III at 30°C in the CUT condition (Figure 5B). We screened this 80-kb QTL region (Figure 5C) by genotyping 38 T-DNA insertion mutants publically available and corresponding to 22 candidate genes (Table S2). T-DNA insertions were confirmed by genotyping and sequencing the T-DNA flanking sequences for eighteen mutants. Homozygous progenies were produced and their responses to *R. solanacearum* at 30°C were scored. Out of the 38 mutants tested, two allelic mutants of the *At3g51420* gene, named *ssl4-1* (N683907) and *ssl4-2* (N684981), for which the altered gene expression was confirmed by RT-qPCR (Figure S8), exhibited a significant higher level of tolerance from 4 to 7 dai compared to wild-type Col-0 (Figure 5D and E). These data indicate that *At3g51420* is a gene that increases the susceptibile response to the *R. solanacearum* GMI1000 strain at 30°C. This gene, that encodes for a strictosidine synthase-like protein 4 (SSL4), belongs to a family of four genes (SSL4, 5, 6 and 7) organized in tandem at this locus. Interestingly, knocking-down of one of the three other genes of the SSL family [*ssl5* mutant (N659319)], has also led to a higher level of tolerance from 4 to 7 dai (Figure 5C and F). Representative symptoms observed at 5 dai for *ssl4-1*, *ssl4-2* and *ssl5* mutants compared to Col-0 accession are shown in Figure S7. Together, our data reveal that both *SSL4* and *SSL5* can be considered as susceptibility genes since they promote the development of wilting symptoms of Col-0 plants in response to the GMI1000 strain.

Discussion

In this study, we explored the genetic diversity of responses to *R. solanacearum* in *A. thaliana* under elevated temperature conditions i) to estimate the extent of genetic diversity in response to this combined abiotic-biotic stress, ii) to describe the genetic architecture of plant response to the bacterial wilt disease, and iii) to unravel the genetic bases of QDR mechanisms remaining efficient under a permanent increase of 3°C. Because *R. solanacearum* is a soil borne pathogen that penetrates into the plants through the roots, we also aimed at studying how the infection process could modulate the response of *A. thaliana* to *R. solanacearum*. For this, two inoculation methods were used: (i) the CUT condition in which the roots were cut allowing the bacteria to access directly to the xylem vessels and (ii) the UNCUT condition, in which the roots were not injured to mimic natural infection.

Significant natural genetic variation is observed in *A. thaliana* challenged with *R. solanacearum*, at both 27°C and 30°C

In our study, we observed a drastic effect of elevated temperature on *A. thaliana* defense response to *R. solanacearum*. At 30°C, most accessions were on average more susceptible, with wilting disease progression being always faster regardless of the infection condition used. These results corroborate several previous studies describing that elevated temperatures generally inhibit resistance responses (Whitham et al., 1996; Hwang et al., 2000; de Jong et al., 2002; Xiao et al., 2003; Wang et al., 2009; Zhu et al., 2010; Menna et al., 2015). At the same time, we also observed an extensive genetic diversity of response in *A. thaliana* accessions challenged with the GMI1000 strain as well as high estimates of broad-sense heritability across the stages of infection at 30°C, as it was also the case at 27°C. These results reveal the existence of tolerant accessions to *R. solanacearum* with a genetically controlled response at the two temperatures tested. However, the crossing reaction norms observed between 27°C and 30°C suggest that the genetic architecture of *A. thaliana* for the response to *R. solanacearum* can largely depend on abiotic conditions. Such a temperature-dependent genetic architecture has been previously reported in *A. thaliana* for diverse phenotypic traits such as flowering time (Lempe et al., 2005) and fertility-related traits (Bac-Molenaar et al., 2015) (Thoen et al., 2017).

The major QTL associated to resistance to *R. solanacearum* at 27°C, underlying the *RPS4/RRS1* locus, is not detected at 30°C

Whatever was the method of inoculation, GWA mapping revealed a major QTL on the long arm of chromosome V with an increasing temporal significance at 27°C, suggesting that a mechanism with a simple genetic architecture governs the defense response to this strain. For both inoculation conditions, the most significant SNPs were found in the *RPS4/RRS1* locus, known to be involved in one of the most well characterized resistance mechanism to *R. solanacearum* in *A. thaliana* (Deslandes et al., 2002; Le Roux et al., 2015). Interestingly, a previous GWA study also identified this locus to be responsible for specific QDR in response to the *Xcc* CFBP6943 strain (race 6) (Debieu et al., 2016). Therefore, our results demonstrate the potential of GWAS to identify major loci associated with natural variation of resistance to *R. solanacearum,* as previously shown for several other bacterial pathogens, predominantly in *A. thaliana* (Huard-Chauveau et al., 2013; Debieu et al., 2016; Roux and Bergelson, 2016; Bartoli and Roux, 2017).

By contrast, at all phenotyping time points, no significant association peak underlying the *RPS4/RRS1* genomic region was detected at 30°C. This result supports, for the first time, that the resistance mechanism conferred by this immunoreceptor pair is impaired at 30°C. The thermosensitivity of basal resistance and *R*-gene-mediated defense for different pathogens in different plant species has been reported in several studies (Whitham et al., 1996; Hwang et al., 2000; de Jong et al., 2002; Xiao et al., 2003; Yang and Hua, 2004; Wang et al., 2009; Menna et al., 2015). In addition, the dwarf phenotype of several autoimmune mutants such as *bon1* and *snc4-1D* or *snc2-1D, mkk1 mkk2,* and *bir1-1* can be totally or partially suppressed above 28°C (van Wersch et al., 2016). This is also the case for the autoimmune response of *A. thaliana* transgenic lines constitutively expressing the *RPS4* immune receptor (Heidrich et al., 2013). To date, the mechanisms involved in such temperature-inhibition of immune responses remain largely uncharacterized. Interestingly, Cheng and colleagues (2013) proposed that plants preferably activate ETI signaling at low temperatures while they use PTI signaling at elevated temperatures.

In this first GWA study on *R. solanacearum*, we have identified at 27°C, in both inoculation methods, the *RRS1*-containing locus, originally mapped by a conventional positional cloning approach (Deslandes et al., 2002). Therefore, we clearly showed that the study of natural diversity of plant response to *R. solanacearum* is a powerful strategy to fine map genomic regions governing susceptible and resistance responses to bacterial wilt. We also showed that an increase in temperature strongly impacts plant defense responses to *R. solanacearum* preventing the detection of *RSP4/RRS1* locus at 30°C most likely due to a loss-of function of this immunoreceptor pair.

GWA analyses at 30°C highlight playful dynamics in the genetics of response to *R. solanacearum* depending on the inoculation procedure

Owing to GWA analyses carried out on the phenotypic data monitored every day after inoculation, a more pronounced playful dynamics of association peaks along the infection stages was observed at 30°C than at 27°C. Among the multiple peaks identified, comparison of the 100 top SNPs at each phenotyping time point revealed that less than 2% were shared between the CUT and UNCUT conditions, suggesting that the genetic basis of response to *R. solanacearum* largely depend on the infection process. Enrichment tests performed to determine the most important processes set up by the plant in response to *R. solanacearum* at 30°C for both inoculation conditions also support these specificities. Indeed, only few genes were found to be significantly over-represented in both conditions and correspond to the same miscellaneous functional class at 3 dai. Interestingly, they mainly encode for CYP450 subfamily 71 and 705 (CYP705) proteins as well as uridine diphosphate glycosyltransferases (UGT) known to be involved in plant response to biotic and abiotic stresses (Ross et al., 2001; Bak et al., 2011). In the CUT condition, at early infection stages, most genes underlying significantly over-represented biological processes correspond to regulatory genes. For instance, many genes encoding transcription factors (TF) were retrieved at 3 and 4 dai, suggesting a rapid transcriptional reprogramming in the early stages of infection. Interestingly, at later infection stages (i.e. from 5 to 7 dai), many genes are involved in calcium-dependent signaling, pathogen perception (TIR-NBS-LRR proteins) and regulation of biotic and abiotic stress responses (LRR- and cystein rich receptor like kinase proteins). For example, CRK28 and its interacting partner CRK29, were recently demonstrated to enhance plant immune responses (Yadeta et al., 2017). By contrast, genes retrieved with the UNCUT condition are predominantly involved in metabolic processes. In particular, genes underlying significantly over-represented biological processes at early infection stages correspond to Brassicaceae-specific CYP705 family and flavonol synthases. Even if the role of the CYP705 family is poorly characterized, the CYP705A1 was described as participating in the formation of a volatile homoterpene, (E)-4,8-dimethyl-1,3,7-nonatriene (DNMT), involved in the *A. thaliana* resistance to the root-rot pathogen *Pythium irregular* (Sohrabi et al., 2015). In addition, flavonoïds are well-described molecules known to be important for response to other organisms or environmental stresses (Mierziak et al., 2014). At later infection stages (i.e. from 5 to 6 dai), candidate genes have primarily a role in cell wall formation. Interestingly, among virulence factors used by *R. solanacearum*, cell wall-degrading enzymes such as pectinolytic and cellulytic enzymes, can promote invasion of roots and/or penetration of xylem vessels (Liu et al., 2005). Such specific processes could allow particular accessions to restrict bacterial penetration at the early stages of infection on intact roots. Indeed, through the inoculation of plants with intact or cut roots, Turner et al. (2009) dissected in *R. solanacearum* the steps involved in root colonization of the model legume plant *Medicago truncatula*. While two distinct T3Es (Gala7 and AvrA) are involved in the early stages of colonization, suggesting that specific basal defense mechanisms are rapidly manipulated by the bacteria, Gala7 alone was shown to play a major role in the later stages of infection on cut roots (Turner et al., 2009).

In addition to the association peak identified at 5 dai in the CUT condition, GWA analyses revealed two other neat association peaks in the UNCUT condition, with the top SNPs located in the promoter region of the *EIF4G* gene and within the *CesA3* gene. EIF4G is a translation initiation factor that belongs to protein families participating in the eukaryotic translation initiation complex. *EIF4G* was identified as a recessive resistance gene impairing multiplication of the cucumber mosaic virus (CMV) and of the turnip crinkle virus (TCV) in *A. thaliana* (Yoshii et al., 2004), or conferring high resistance to the rice yellow mottle virus in rice (Albar et al., 2006). The *CesA3* gene belongs to a family of 10 *CesA* genes identified in *A. thaliana* of which 9 encode cellulose synthase subunits with known function. CesA1, -2, -3(IXR1), -5 and -6(IXR2) were shown to participate in the cellulose production during the primary cell wall formation, while CesA4 (IRX5), -7 (IRX3), -8(IRX1) and -9 are involved in the secondary cell wall cellulose synthase complex (Endler and Persson, 2011). Hernandez-Blanco et al. (2007) demonstrated that mutation in CesA4, -7 and -8, involved in secondary cell wall formation, enhanced tolerance to *Botrytis cinerea* and *Plectosphaerella cucumerina* fungi as well as to *R. solanacearum*. Strikingly, performing inoculation at 27°C with plants for which roots were cut, the authors showed that the *ixr1-1/cev1* mutant, altered in *CesA3* expression, was susceptible to *R. solanacearum*. Clearly, further studies are needed to functionally validate the implication of these candidate genes in tolerance to *R. solanacearum* at elevated temperatures in the early infection stages.

As in many cases in which genetic mapping approaches were developed to identify resistance mechanisms to pathogens (Cook et al., 2012; Roux et al., 2014; Fukuoka et al., 2015), we found in our study that several QTLs control QDR to *R. solanacearum* at 30°C in a playful manner specific to the inoculation method. Interestingly, the CUT condition highlights different processes that could be related to the perception and regulation pathways, dependent on temperature, of well-conserved microbial signatures such as ubiquitous effectors and PAMPs. On the other hand, the UNCUT condition revealed processes associated to plant development and metabolism which could reflect strategies set up by the plant to limit bacteria penetration and propagation in root tissues at the early stages of infection. As demonstrated by (Turner et al., 2009) on the bacterial side and proposed by Roux et al. (2014), these processes could involve host components, sequentially manipulated by effectors during host colonization.

A QDR to *R. solanacearum* efficient at 30°C is conferred by a strictosidine synthase-like protein 4

At 5 and 6 dai in the CUT condition, a neat association peak was identified at the end of the chromosome III, corresponding to another QTL involved in an early defense response at 30°C. The phenotyping of several T-DNA insertion mutants corresponding to genes underlying this QTL allowed the functional validation of *At3g51420* (*AtSSL4*) as a gene of susceptibility to the *R. solanacearum* GMI1000 strain. Indeed, two allelic mutants were found to be significantly more tolerant compared to wild-type Col-0 susceptible plants. The *At3g51420* gene belongs to a subset of four genes (*AtSSL4-AtSSL7*), arranged in tandem, which shows a strong similarity with genes encoding for hemomucin membrane-anchored immune proteins from *Drosophila melanogaster.* These proteins contain strictosidine synthase-like (SSL) domain related to plant and *Caenorhabditis elegans* SSL proteins. Because hemomucin proteins from *Drosophila* have immune-related functions, Sohani et al. (2009) explored the possible role of AtSSL4-AtSSL7 in plant defense response. In contrary to other *AtSSLs* genes, *AtSSL4* appeared to be slightly up-regulated upon wounding stress but not induced following inoculation with CMV and *Alternaria brassicicola* (Sohani et al., 2009). Still, these data do not exclude the possibility that *AtSSL4* could be regulated by other pathogens, like *R. solanacearum*. Among the knockout mutants corresponding to the three other *AtSSLs* genes, the *ssl5* mutant was also found tolerant to *R. solanacearum* at 30°C, from 3 to 10 dai. This result also suggests that *AtSSL5* could be a gene of susceptibility to *R. solanacearum*. Interestingly, unlike *AtSSL4, AtSSL5* expression is induced by salicylic acid, known to be a defense signaling compound, and is strongly up-regulated upon CMV and *Alternaria brassicicola* inoculation (Sohani et al., 2009). In tomato, *SSL* gene expression is also up-regulated following the roots infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Manzo et al., 2016). Strictosidine synthase participates to the production of a wide range of monoterpenoid indole alkaloids important for plant defense response (Kibble et al., 2009) and Manzo et al (2016) proposed that SSL could be involved in the incompatible interaction with the fungus. Thus, our result is the first demonstration of a role of plant SSLs in the tolerance response to a bacterial pathogen. The enzymatic activity of proteins encoded by *AtSSL4-AtSSL7* has not been demonstrated yet (Kibble et al., 2009). Nonetheless, knowing that *SSLs* expression is highly regulated in different plant species upon pathogen attack, the tolerance spectrum conferred by these mutants to other RSSC strains and other pathogens will be investigated at elevated temperatures.

Conclusion

To our knowledge, this study is the first report of significant genetic variation in *A. thaliana* in response to *R. solanacearum* at elevated temperature. Our results also highlight that plant defense responses are strongly impacted at 30°C and differ according to the inoculation procedure. By combining a GWA mapping approach with the response to combined stresses, we identified three neat association peaks underlying QDR to *R. solanacearum* remaining efficient at elevated temperature. Among the most interesting genes underlying the QTLs identified, we functionally validated *AtSSL4* as a susceptibility gene that plays a role in *R. solanacearum* infection at 30°C. Apart from *AtSSL4*, other genes underlying additional QTLs also constitute good candidates for QDRs that might represent new sources of sustainable resistance to the RSSC in the context of climate warming. In addition, the inhibition of the *RPS4/RRS1* resistance signalling at 30°C represents an interesting tool to investigate the impact of elevated temperature on ETI-related processes.

Figure Legends

Table 1: **Natural variation among natural accessions for disease index in the CUT condition.** F: F value resulting from the test of fixed effect. LRT: LRT value resulting from the likelihood ratio test.

Table 2: Natural variation among natural accessions for disease index in the UNCUT condition. F: F value resulting from the test of fixed effect. LRT: LRT value resulting from the likelihood ratio test.

Figure 1: Phenotypic diversity of plant response to the *R. solanacearum* GMI1000 strain in CUT and UNCUT conditions of inoculation at 27°C and 30°C. The red line represents

the mean of disease index overall the accessions. Inoculation was performed at 27°C (**A**, **C**) and 30°C (**B**, **D**) on 4-week-old plants for which roots have been cut (**A**, **B**) or left uncut (**C**, **D**). dai: days after inoculation. DI: disease index.

Figure 2: Correlations of temperature conditions, 27°C and 30°C, in response to *R. solanacearum* inoculation of the worldwide collection of *A. thaliana*, at five days after inoculation. Inoculation was performed on 4-weeks-old plants for which roots have been cut (**A**) or left uncut (**B**). The red line represents the mean of disease index. dai: days after inoculation. DI: disease index.

Figure 3: The genetics of quantitative disease resistance to *R. solanacearum* GMI1000 strain at 27°C, identified by GWA mapping at 13 dai in CUT and UNCUT conditions of inoculation. Whole genome scan of 214,051 single-nucleotide polymorphisms (SNPs) for association with disease index at 13 dai across 152 accessions that have germinated for which roots have been cut (**A**). Zoom showing the absence of QTLs of resistance in the CUT condition of inoculation on the chromosome IV, compared with the UNCUT condition thereafter (**B**). Zoom spanning a genomic region on the chromosome V from 17.8 Mb to 18.8 Mb containing the QTL of resistance corresponding to the *RPS4/RRS1* locus (**C**). Whole genome scan of 214,051 single-nucleotide polymorphisms (SNPs) for association with disease index at 13 dai across 163 accessions that have germinated for which roots remained uncut (**D**) and focus on two genomic regions corresponding to new QTLs of resistance observed on chromosomes IV (D) and chromosome V (E). The red circle indicates the top SNP (SNP-4-5080256, $P = 4.93 \times 10^{-7}$, MARF = 0.429) corresponding to the major QTL of resistance detected at the beginning of chromosome IV in the UNCUT condition. The green circles highlight the top SNPs corresponding to the major QTL of resistance detected on the long arm of chromosome V in

the UNCUNT (SNP-5-18325032, $P = 5.37 \times 10^{-13}$, MARF = 0.125) and CUT (SNP-5-18325565, $P = 1.60 \times 10^{-10}$, MARF = 0.252) conditions.

Figure 4: Simplified representation of MapMan classification pathways found to be significantly over-represented (P< 0.01). Genes list were retrieved from enrichment tests performed using the SNPs in the 0.1% upper tail of the –log10 p-value distribution for each time point of phenotyping in the CUT (**A**) and UNCUT (**B**) conditions at 30°C (Table S6 and S7).

Figure 5: The genetics of quantitative disease resistance to *R. solanacearum* GMI1000 strain at 30°C, identified by GWA mapping at 5 dai in CUT conditions of inoculation. Whole genome scan of 214,051 single-nucleotide polymorphisms (SNPs) for association with disease index at 5 dai across 141 accessions having germinated for which roots have been cut (**A**). Zoom spanning a genomic region on the chromosome III from 18.5 Mb to 19.5 Mb containing the QTL of early plant defense response to *R. solanacearum* (**B**). Zoom showing the 80 Kb genomic region underlying the QTL of early plant defense response to *R. solanacearum*, containing 31 annotated genes. Red frames indicate genes coding for *AtSSL4* (*At3g51420*) and *AtSSL5* (*At3g51430*) (**C**). Dynamics of disease symptoms after inoculation with the GMI1000 strain in wild type Col-0 genetic background and *ssl4-1* mutant at 30°C. Means ± SD of the means (*ssl4-1* n=60; Col-0 n=40) from three independent inoculations (**D**). Dynamics of disease symptoms after inoculation with the GMI1000 strain in wild type Col-0 genetic background and *ssl4-2* mutant at 30°C. Means ± SD of the means (*ssl4-2* n=83; Col-0 n=59) from four independent inoculations (**E**). Dynamics of disease symptoms after inoculation with the *R. solanacearum* GMI1000 strain in wild type Col-0 genetic background and *ssl5* mutant at 30°C. Means ± SD of the means (*ssl5* n=124; Col-0 n=77) from six independent inoculations (**F**).

Symbols *, ** and *** denote significant difference observed between Col-0 and each mutant at P<0.05, P<0.01 and P<0.001 respectively, in Kruskal-Wallis analyses. The red circle indicates the top SNP (SNP-3-18972992, $P = 4.70 \times 10^{-7}$, MARF = 0.142) corresponding to the major QTL of resistance detected at the end of chromosome III.

Figure 6: The genetics of quantitative disease resistance to *R. solanacearum* GMI1000 strain at 30°C, identified by GWA mapping at 6 dai in UNCUT conditions of inoculation. Whole genome scan of 214,051 single-nucleotide polymorphisms (SNPs) for association with disease index at 6 dai across 158 accessions that have germinated (**A**) and focus on two genomic regions corresponding to new QTLs of early plant defense to *R. solanacearum* observed at the end of chromosome III (B) and at the beginning of chromosome V (C). The red circle indicates the top SNP (SNP-3-2260125, $P = 5.65 \times 10^{-8}$, MARF = 0.137) corresponding to the major QTL of resistance detected at the end of chromosome III. The green circle highlights the top SNP (SNP-5-1530992, $P = 4.04 \times 10^{-8}$, MARF = 0.189) corresponding to the major QTL of resistance detected at the beginning of chromosome V.

Authors and Contributors

N.A., F.V., F.R. and R.B. conceived the experiments. N.A., L.T., F.L., F.V. and R.B. performed the experiments. N.A. and F.R. performed GWA analyses. N.A., F.V. and R.B. analyzed the other data. N.A, F.R, L.D., F.V. and R.B. wrote and corrected the manuscript. All authors approved the manuscript.

Funding

N.A. benefited from a PhD Grant co-financed by the Occitanie Regional Council and the INRA Plant Health and Environment division (SPE). F.L. was funded by a grant from the French

Ministry of National Education and Research. This study was founded by INRA Plant Health and Environment division (SPE). This work was realized at the LIPM belonging to the Laboratoire d'Excellence (LABEX) entitled TULIP (ANR-10-LABX-41).

Acknowledgments

We thank the LIPM greenhouse service for their technical assistance and the Jiffy pots preparation.

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Table 1: **Natural variation among natural accessions for disease index in the CUT condition.** F: F value resulting from the test of fixed effect. LRT: LRT value resulting from the likelihood ratio test.

A. 27°C - CUT

Traits	**Symptoms D3**		**Symptoms D4**		**Symptoms D5**		**Symptoms D6**		**Symptoms D7**		**Symptoms D10**		**Symptoms D11**		**Symptoms D12**		**Symptoms D13**	
Model terms	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***
Block	1.70	0.1929	0.00	1.0000	1.30	0.2542	23.90	< 0.0001	23.60	< 0.0001	27.70	< 0.0001	27.50	< 0.0001	21.30	< 0.0001	16.50	< 0.0001
Accession	1.14	0.2250	1.82	0.0003	2.40	< 0.0001	2.99	< 0.0001	3.03	< 0.0001	3.65	< 0.0001	4.23	< 0.0001	4.60	< 0.0001	5.02	< 0.0001
*Accession*Block*	1.40	0.2367	4.70	0.0302	2.90	0.0886	4.00	0.0455	5.50	0.0190	12.00	0.0005	7.10	0.0077	5.70	0.0170	3.00	0.0833
Control Col-0	30.99	< 0.0001	149.27	< 0.0001	50.24	< 0.0001	21.42	< 0.0001	14.83	< 0.0001	0.28	0.5976	0.66	0.4180	0.10	0.7547	1.19	0.2761
Broad-sense heritability H^2	0.16^{ns}		0.61***		0.74***		0.79***		0.78***		0.81***		0.85***		0.86***		0.88***	

B. 30°C - CUT

Traits	**Symptoms D3**		**Symptoms D4**		**Symptoms D5**		**Symptoms D6**		**Symptoms D7**		**Symptoms D10**	
Model terms	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***
Block	0.00	1.0000	1.40	0.2367	2.50	0.1138	3.20	0.0736	1.60	0.2059	0.00	1.0000
Accession	2.04	< 0.0001	1.86	0.0002	1.93	0.0001	2.65	< 0.0001	2.98	< 0.0001	4.21	< 0.0001
*Accession*Block*	0.00	1.0000	3.10	0.0783	4.30	0.0381	0.90	0.3428	0.00	1.0000	0.50	0.4795
Control Col-0	24.95	< 0.0001	13.80	0.0006	11.59	0.0007	0.15	0.6950	0.02	0.8967	-	-
Broad-sense heritability H^2	0.71***		0.63***		0.63***		0.76***		0.82***		0.88***	

Table 2: Natural variation among natural accessions for disease index in the UNCUT condition. F: F value resulting from the test of fixed effect. LRT: LRT value resulting from the likelihood ratio test.

A. 27°C - UNCUT

Traits	**Symptoms D3**		**Symptoms D4**		**Symptoms D5**		**Symptoms D6**		**Symptoms D7**		**Symptoms D8**		**Symptoms D9**		**Symptoms D11**		**Symptoms D13**	
Model terms	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***
Block	3.50	0.0614	18.70	< 0.0001	44.40	< 0.0001	72.30	< 0.0001	63.70	< 0.0001	75.30	< 0.0001	58.40	< 0.0001	19.60	< 0.0001	19.40	< 0.0001
Accession	1.65	< 0.0001	2.62	< 0.0001	3.68	< 0.0001	5.22	< 0.0001	5.87	< 0.0001	6.84	< 0.0001	6.53	< 0.0001	5.30	< 0.0001	5.26	< 0.0001
Broad-sense heritability H^2	0.37^{ns}		0.57***		0.71***		0.81***		0.83***		0.86***		0.85***		0.81***		0.81***	

B. 30°C - UNCUT

Traits	**Symptoms D3**		**Symptoms D4**		**Symptoms D5**		**Symptoms D6**		**Symptoms D7**		**Symptoms D10**	
Model terms	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***	***F*** **or LRT**	***P***
Block	7.90	0.0049	11.60	0.0007	48.00	< 0.0001	0.60	0.4386	0.00	1.0000	0.00	1.0000
Accession	1.86	< 0.0001	1.86	< 0.0001	2.87	< 0.0001	2.55	< 0.0001	2.72	< 0.0001	1.28	0.0357
Control Col-0	1.08	0.2989	2.52	0.1136	0.01	0.9299	2.83	0.1582	0.10	0.7652	-	-
Broad-sense heritability H^2	0.44***		0.47***		0.67***		0.62***		0.65***		0.14^{ns}	

Supporting information

Genome-wide local score analysis

A new statistical approach based on the aggregation of GWA mapping results on short physical distances was recently adapted to plants by our colleague Maxime Bonhomme (LRSV, UPS Toulouse). This genome-wide local score approach (GW-LS) allows the detection of QTLs with minor effects (Fariello et al., 2017; Bonhomme et al., 2019). To improve our previous GWA analyses and strengthen the robustness of our results, we decided to apply this method on the GWA mapping results obtained by EMMAX in the CUT condition of inoculation (Aoun et al., 2017). By collaborating with Maxime Bonhomme, the GW-LS analysis revealed new QTLs and candidate genes that might be involved in defense response to the *R. solanacearum* GMI1000 strain either at 27°C or at 30°C (figure 19; table 1). Overall, 18 QTLs were detected among which the *RSP4/RRS-R's and SSL4's* QTLs have been identified again (QTL6 and QTL14 respectively in figure 19) with the same top SNPs previously identified by GWA mapping.

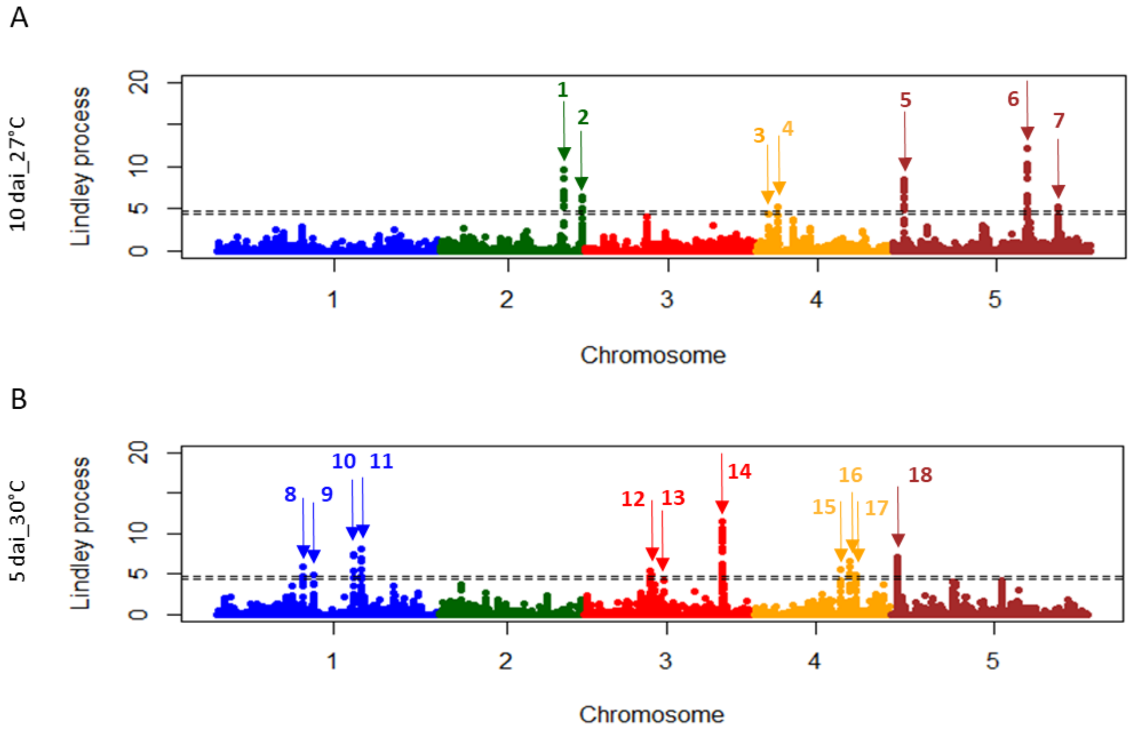

Figure 19. The genetics of quantitative disease resistance to *R. solanacearum* GMI1000 strain in the worldwide collection of *A. thaliana*, identified with GW-LS. A. Manhattan plot of the Lindley process at 13 dai and 27°C. **B.** Manhattan plot of the Lindley process at 5 dai and 30°C. Lindley process values on the y-axis range from 0 to 20. The number close to association peaks correspond to the 18 QTLs with a Lindley process value above 5.

Temperature_dai	QTL identity	chr	Start position_top SNP	End position_top SNP	Lindley process peak	Gene ID	Gene function
27°C_10 dai	QTL1	2	16894648	16896465	9,567266833	**AT2G40450**	**BTB/POZ domain-containing protein**
	QTL2		19373592	19374090	6,486124696	AT2G47190	myb domain protein 2
	QTL3	4	1609867	1610013	4,445041498	**AT4G03630**	**RNI-like superfamily protein**
	QTL4		2766072	2766414	5,173742464	**AT4G05470**	**RNI-like superfamily protein**
	QTL5	5	1530396	1535883	8,429578508	**AT5G05170**	**CESA3, cellulose synthase family protein**
	QTL6		18322558	18325915	12,20826828	**AT5G45250**	**RPS4, disease resistance protein (TIR-NBS-LRR class) family**
						AT5G45260	**RESISTANT TO RALSTONIA SOLANACEARUM 1 (RRS1-R)**
	QTL7		22531430	22539881	5,15885998	**AT5G55630**	**KCO1, outward rectifying potassium channel protein**
						AT5G55640	**Na-translocating NADH-quinone reductase subunit A**
						AT5G55650	**Transmembrane protein**
						AT5G55660	**DEK domain-containing chromatin associated protein**
30°C_5 dai	QTL8	1	11833706	11837433	5,829576858	**AT1G32710**	**Cytochrome c oxidase, subunit Vib family protein**
	QTL9		13282502	13289245	4,911369169	AT1G35780	N-lysine methyltransferase
	QTL10		18726662	18728221	7,426599803	**AT1G50570**	**Calcium-dependent lipid-binding (CaLB domain) family protein**
	QTL11		19832346	19835729	8,150215541	**AT1G53180**	**Hypothetical protein**
	QTL12	3	9169561	9170249	5,431750513	AT3G25180	CYP82G1, cytochrome P450, family 82, subfamily G, polypeptide 1
	QTL13		9379300	9380516	4,818249368	**AT3G25719**	**Hypothetical protein**
						AT3G25720	**RNA-directed DNA polymerase (reverse transcriptase)-related family protein**
	QTL14		19025591	19026913	11,44477822	**AT3G51240**	**F3H, flavanone 3-hydroxylase**
						AT3G51420	STRICTOSIDINE SYNTHASE-LIKE 4 (SSL4)
	QTL15	4	11564151	11564726	5,566817673	**AT4G21770**	**Pseudouridine synthase family protein**
	QTL16		12981948	12983372	6,55094837	**AT4G25400**	**Basic helix-loop-helix (bHLH) DNA-binding superfamily protein**
	QTL17		13832209	13837470	4,916504163	**AT4G27720**	**Major facilitator superfamily protein**
						AT4G27730	**Oligopeptide transporter 1**
	QTL18	5	847764	851547	7,18326401	**AT5G03420**	**5'-AMP-activated protein kinase-like protein**

Table 1. List of candidate genes in the identified QTLs with a Lindley process value above 5, 10 dai at 27°C and 5 dai at 30°C. For each QTL, the candidate gene in which the top SNP was located is in bold unlike the flanking gene.

Interestingly, at 27°C, we identified *CesA3* (*At5g05170*) as the main candidate gene underlying QTL5 at 10 dai. In Aoun et al. (2017), we also identified this gene but at 5 dai and 30°C in the UNCUT condition of inoculation. These results suggest that (i) primary cell wall can play an important role in plant response to *Ralstonia*, and (ii) *CesA3* can be spatio-temporally regulated depending on the phase of the Ralstonia root infection cycle and the abiotic stress to which the plant is subjected (here a temperature elevation). In addition, as mentioned in Desaint et al. (review submitted), heat stress modulates membranes fluidity and permeability and leads to cellular structures re-organization that alter cellular elongation, differentiation and expansion (Saidi et al., 2011). Such modifications might affect the process of infection at elevated temperature.

Mechanisms underlying SSL4 and SSL5

In order to understand the pathways in which *SSL4* is involved, we run GWA mapping on natural variation of *SSL4* expression using transcriptome data previously obtained on 665 natural accessions (Kawakatsu et al., 2016). Strikingly, we identified a neat association peak at the end of chromosome V, suggesting a distant gene regulation by trans-regulatory elements (figure 20).

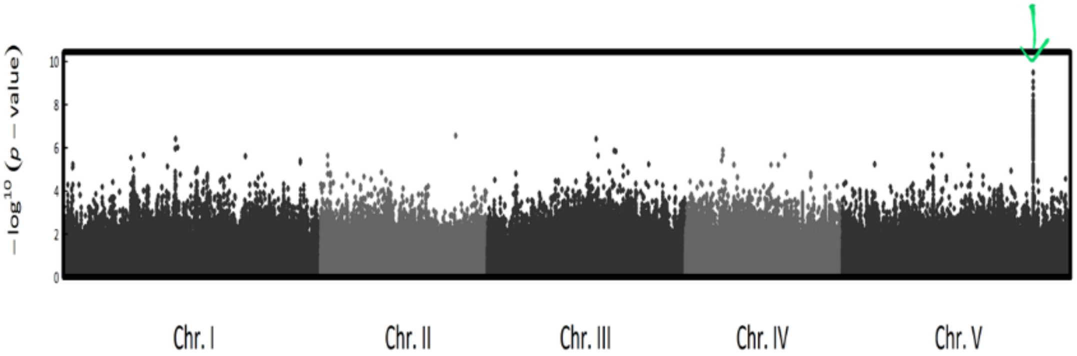

Figure 20. GWA mapping on expression variation of *SSL4* among 665 natural accessions of *A. thaliana*.

The association peak covers a 7kb genomic region corresponding to three genes (figure 20). First, *At5g55830* encodes the L-TYPE LECTIN RECEPTOR KINASE S.7 that belongs to trans-membrane proteins with a lectin-like domain believed to be involved in perception of ligands. They are thought to be important actors of environmental stimuli perception, involved in diverse functions ranging from plant growth and development to stress tolerance (Vaid et al., 2016). Second, MICRORNA156H (*At5g55835*) encodes for *miR156h. miRNA* isoforms were shown to regulate plant development and response to heat stress (Stief et al., 2014). The SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes, a family of transcription factors that negatively regulate memory genes expression, were identified as target genes of *miR156h*, *miR156a-f* and *miR156h* under heat stress condition (Stief et al., 2014). In our case, we can hypothesize that *miR156h* targets *SSL4* and *SSL5* and negatively regulates their expression (either by attenuating their translation or by directing mRNA cleavage) which in turn enhances plant resistance to the GMI1000 under heat stress. To test this hypothesis, it would be interesting to i) check the expression pattern of *miR156h* in our combined stress conditions, ii) phenotype corresponding mutants (if available) with the GMI1000 strain at 30°C, iii) identify putative *miR156h* target sites in *SSL4* and *SSL5* sequences, iv) express *miR156* resistant *SSL4* and *SSL5* transcripts (mutated in target sites) in *ssl4* and *ssl5* background to determine if the resistance observed to GMI1000 is abolished at 30°C, and v) overexpress *miR156h* or mimic its effect using miPEP156 (Lauressergues et al., 2015). Finally, although the role of the *At5g55840* gene is not precisely known, it belongs to large family of RNA-binding proteins known to modulate processing, splicing, editing, stability and translation of RNAs predominantly in organelles but also in the nucleus (Manna, 2015).

C. Naturally resilient: the genetic basis of total resistance to *Ralstonia solanacearum* remaining efficient at elevated temperature

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1. Introduction

Since plants are sessile, they are sentenced by the exposure to multiple stresses that appear at the same time or at different periods in their habitats. These stresses are grouped according to their nature, abiotic or biotic. Components of climate change are among the main abiotic factors predicted to vary in recent global change scenarios, resulting in a more frequent, diverse and longer periods of extreme weather events (IPCC, 2018). These changes represent a crucial menace on species distribution and ecosystem functioning that could dramatically affect natural biodiversity and food security (Bebber, 2015; Pimm et al., 2014). One of the predicted consequences of these climate changes is the increase of the global surface temperature from 1°C to 4.8°C by the end of the century (IPCC, 2014, 2018). Temperature fluctuation and elevation is a major factor of the climate warming with which plants have to cope. Temperature shifting above the optimal growth temperature of plants will be considered here as heat stress (Zaidi et al., 2014; Liu et al., 2015; Desaint et al., submitted). Besides the well-known effect of heat stress on plant development, there is mounting evidence that heat stress can also alter wild and crop species resistance to different pathogens (Desaint et al., submitted). Notably, heat stress mostly results in an increased plant susceptibility or an inhibition of plant defenses (Desaint et al., submitted). However, the genetic and molecular mechanisms underlying plant-pathogen interactions under heat stress remains poorly studied (Cheng et al., 2013; Menna et al., 2015; Huot et al., 2017), stressing the need to identify mechanisms involved in the maintenance of resistance under heat stress. This challenge is even more relevant in the context of climate warming since temperature elevation is predicted to favor the emergence of new pathogens and the incidence of epidemics (Garrett et al., 2006; Evans et al., 2008; Bebber et al., 2013; Elad and Pertot, 2014; Bebber, 2015; McDonald and Stukenbrock, 2016).

In order to face biotic stresses, plants have developed different types of responses including physical barriers (Osbourn, 1996; Nürnberger and Lipka, 2005) or immune signaling responses (Jones and Dangl, 2006; Dodds and Rathjen, 2010). These latter are based on two types of immune response shaping in a zig-zag model (Jones and Dangl, 2006; Dodds and Rathjen, 2010). The first type of immune response is called Pathogen-Triggered Immunity (PTI) that is activated when elicitors, originating either directly from pathogens (Pathogen- or Microbe-Associated Molecular Patterns; PAMPs or MAMPs) or corresponding to plant endogenous molecules resulting from pathogen invasion (Danger-Associated Molecular Patterns; DAMPs), are recognized in the extracellular compartment by membrane receptors called Pattern Recognition Receptors (PRRs). This response is efficient against a large spectrum of pathogens. However, pathogens have evolved to produce virulence factors called effectors (T3Es) in the intracellular compartment that interfere with PTI and results in effector-triggered susceptibility (ETS). The second type of immune response is the Effector-Triggered Immunity (ETI), also known as qualitative resistance. It takes place when effectors are specificallyrecognized in the plant intracellular compartment by resistance proteins called nucleotide binding domain-leucine rich repeat containing proteins (NLRs). In most cases, this recognition results in a hypersensitive response (HR). ETI is generally species- and strain-specific, which results in a strong selective pressure of strains to overcome this response (Roux et al., 2014). Another type of resistance is the Quantitative Diseases Resistance (QDR) that is polygenic, durable and broad-spectrum (Young, 1996; Poland et al., 2009). However, in comparison to PTI and ETI, the associated genetic and molecular mechanisms underlying QDR remain largely unknown (Bartoli and Roux, 2017).

NLR proteins are classified into the signal transduction ATPases with Numerous Domains (STAND) P-loop NTPases clade (Leipe et al., 2004). This clade contains regulators of immunity such as the P-loop nucleotide-binding domain (Bendahmane et al., 2002; Williams et al., 2011) and the apoptosome component APAF1 in the NBS domain of animals NLRs (Takken et al., 2006; Jones et al., 2016). NLRs are composed of an N-terminal domain (either a TIR (Toll-Interleukine-1 receptor) or a CC (coiled-coil) domain), a central nucleotide-binding site domain (NBS) and a C-terminal leucine-rich repeat domain (LRR) (Dodds and Rathjen, 2010). The NBS domain controls NLRs activity by binding to ADP or ATP, thus leading to inactive or active conformation, respectively (Bonardi et al., 2012). CC and TIR domains are involved in regulating effectors recognition and HR initiation and are suggested to transduce the activation signal to downstream pathways (Ellis et al., 1999; Lukasik and Takken, 2009; Nishimura et al., 2017; Swiderski et al., 2009). The LRR domain plays a positive role in specific signal recognition and a negative role in signaling auto-inhibition. It ensures the NB-LRR a competent state of signaling in the absence of effectors and a mobility in case of presence of effectors (Collier and Moffett, 2009; Lukasik and Takken, 2009). Effector-binding LRRs are exposed to diversifying selection, supposedly due to the pressure of the diverse panel of effectors (Goritschnig et al., 2016; Krasileva et al., 2010). In *Arabidopsis thaliana*, 38 genes associated with natural variation of resistance to virus, bacteria, fungi and oomycetes resistance have been cloned. Among them, 18 genes correspond to NB-LRRs, including 12 TIR-NB-LRRs and 6 CC-NB-LRRs (Roux and Bergelson, 2016).

Ralstonia solanacearum is a gram-negative bacterium from the tropical, sub-tropical and warm temperate regions and is the causal agent of the bacterial wilt disease. *R. solanacearum* is classified among the top 10 most destructive phytopathogens that is able to infect more than 200 plant species that belongs to 53 botanical families including important corps species such as tomato, potato, banana, etc. (Elphinstone, 2005). Host resistance is the only effective way of controlling this bacterium (Huet, 2014). Until now, two molecular mechanisms of resistance have been identified in *A. thaliana*, i.e. the LRR receptor-like kinase ERECTA (Godiard et al., 2003) and the RPS4/RRS1-R (RESISTANCE TO PSEUDOMONAS SYRINGAE 4 / RESISTANCE TO RALSTONIA SOLANACEARUM 1) immunoreceptor pair (Deslandes et al., 2002; Le Roux et al., 2015). RRS1-R protein contains has a WRKY DNA binding domain at its C terminus, conserved in defensive WRKY transcription factors (TFs) regulating plant response to biotic stresses (Rushton et al., 2010). RRS1-R cooperates genetically and molecularly with RPS4 and form a functional complex of receptor recognition/signaling complex (Birker et al., 2009; Narusaka et al., 2009; Williams et al., 2014). RPS4/RRS1-R complex is activated when RRS1-R WRKY domain is targeted by the T3Es PopP2 (member of the YopJ superfamily of acetyltransferase) and AvrRps4, secreted by *R. solanacearum* and *Pseudomonas syringae* pv. *pisi*, respectively (Deslandes et al., 2003; Tasset et al., 2010; Le Roux et al., 2015; Sarris et al., 2015). PopP2 acetylates a key lysine residue (K1221) in the invariant heptad of RRS1-R WRKY domain, thus disrupting the electrostatic potential of this domain and blocking its binding to W-box DNA sequences (Grund et al., 2019). Thereafter, the RPS4/RRS1-R dissociates and the resistance defense response is activated. However, in the absence of RPS4/RRS1-R, PoP2 disrupts defense genes expression and basal resistance by inhibiting the WRKY DNA binding TF activity and transactivation functions through their acetylation (Le Roux et al., 2015; Grund et al., 2019).

Resistance to *R. solanacearum* is largely impacted by heat stress (Desaint et al., submitted). To identify the resistance mechanisms remaining efficient at elevated temperature, we recently conducted a Genome-Wide Association study (GWAS) in *A. thaliana* to identify genes associated with natural variation of resistance to *R. solanacearum* under heat stress (Aoun et al., 2017). Accessions were on average more susceptible at 30°C than at 27°C. In addition, the level of quantitative disease response variability to the GMI1000 strain detected at 27°C was strongly decreased at 30°C. At 27°C, the genetic architecture of QDR was rather simple with the identification of a major association peak located on RPS4/RRS1. On the other hand, the genetic architecture of QDR at 30°C was highly polygenic and no association peak was detected near RPS4/RRS1. Interestingly, we identified at 30°C a single accession, Eden-1 that remained symptom-free during all infection stages (Aoun et al., 2017). A bulk segregant analysis (BSA) developed from a cross between Eden-1 and a susceptible sterile EMS mutant of Landsberg-0 (NW-77) showed that the genetic architecture of Eden-1 total resistance mainly corresponds to a single recessive nuclear locus. A mapping-by-sequencing approach using the two parental lines and the F2 progeny allowed the identification of a major QTL located onto the long arm of the chromosome V near the physical position of RPS4/RRS1. Interestingly, when Eden-1 was inoculated with a *R. solanacearum* strain mutated in PopP2, it started wilting albeit remaining more resistant than the susceptible control at 30°C. All these results suggested that an allelic form of the *RRS1* gene was responsible for this total resistance at elevated temperature. The cloning, the sequencing, the analysis of polymorphisms of Eden-1 *RPS4/RRS1* locus and its use to complement the double mutant of *rps4-21/rrs1-1* at 30°C (Ws-0 background, Narusaka et al., 2009) demonstrated that a specific amino acid change, naturally present in the LRR domain of Eden-1 *RRS1-R*, conditioned the maintenance of the RPS4/RRS1 immuno-receptor pair activity at elevated temperature.

2. Material and methods

Bacterial strains, plant materials and growth conditions

The *R. solanacearum* GMI1000 reference strain and mutated strain in PopP2 (RSΔPopP2) were used for inoculation experiments. These strains were grown at 28°C on either complete BG solid or liquid medium (Plener et al., 2010) containing gentamycine (15µg/mL) for RSΔPopP2. *Pseudomonas fluorescens* (Pfo-1) strains used, expressing either PopP2 native version or PopP2 C321A mutant under the control of AvrRps4 promoter sequence (Le Roux et al., 2015), were grown on King's B solid and liquid medium containing chloramphenicol (30 µg/mL), tetracycline (5 µg/mL) and gentamicin (15 µg/mL) at 28 °C.

In this study, we used (i) the natural accessions Col-0, Eden-1, Ws-0, (ii) a F2 population resulting from a cross between Eden-1 and an EMS mutant of Ler-0 (NW-77), and (iii) the WS *rps4-21/rrs1-1* mutant (Narusaka et al., 2009). T1 and T2 *A. thaliana* transgenic lines C1, N1 and A641 correspond to the WS *rps4-21/rrs1-1* mutant complemented respectively with *RPS4/RRS1* allele of Eden-1, *RPS4/RRS1* allele of Nd-1 and *RPS4/RRS1* allele of Nd-1 in which the specific mutation identified in the *RRS1-R* sequence of the Eden-1 accession was introduced. These lines were cultivated for seed production in greenhouse conditions (26.5°C +/- 1.5°C, 16h light). For selecting *A. thaliana* transgenic lines, seeds were sterilized for eight minutes with a mix of one capsule of bleach (1.5 g of active chlorine, C' SIMPLE ®), few drops of Tween® 20 (Sigma-Aldrich) and 50 mL of ethanol (96%) and then washed twice with ethanol (70%) before stratification. Then seeds were sown on MS medium (Murashige and Skoog, 1962) with Basta (10mg/L). One week after, seedlings were transferred on Jiffy pots (Jiffy Products International AS, Norway).

For inoculation and phenotyping experiments, seeds were directly sown on jiffy pots (Jiffy Products International AS, Norway), then put at 4°C for 48h for stratification, and grown in controlled growth chambers at 22°C, 70% relative humidity (RH), 125 µE/M2/s fluorescent illumination and 9h of light, for four weeks.

Plant inoculation and phenotyping

Four weeks old plants were inoculated with GMI1000 or RsΔPopP2 strains (OD600nm = 0,01) as described in Deslandes et al. (1998). Inoculated plants were transferred to a growth chamber (75% HR, 12h light, 100µmol $m^{-2}s^{-1}$) in standard (26°C night/27°C day) or elevated (29°C night/30°C day) temperature conditions. Wilting symptoms were scored based on a scale from 0 (healthy plants) to 4 (dead plants) (Deslandes et al., 1998). Symptoms were monitored from 3 to 10 days after inoculation (dai).

Genetic architecture, bulk segregant analysis, DNA extraction and sequencing

To estimate the genetic architecture associated with the total resistance of Eden-1, we adopted a BSA approach. We phenotyped 1,978 F2 plants resulting from a cross between Eden-1 and NW-77 (sterile EMS mutant in the Ler-0 susceptible background). One leaf of each labeled plant was sampled just before inoculation with the GMI1000 strain. Genomic DNA extraction protocol was adapted from QIAGEN DNeasy kit®. For DNA purification of the F2 plants, we used a buffer composed of guanidium chloride (8M) and ethanol (96%). Prior to quantification, DNA samples were treated with RNAse A (ribonuclease A) (Thermo Fisher Scientific™). DNA of F2 plants was quantified using the Quanti-iT™ Picogreen® dsDNA kit, according to manufacturer's instructions (Thermo Fisher Scientific™) in order to compose equimolar DNA pools of 167 individuals. One pool of resistant and seven pools of susceptible F2 plants were constituted. For DNA sequencing, libraries were prepared with TruSeq Nano DNA sample kits (Illumina). Pooled libraries were subjected to a 150-pb paired-end sequencing following the manufacturer's protocol (Illumina HiSeq3000). Demultiplexed FastQ files were generated using the bcl2fastq conversion software v2.17. This work was performed at the GeT-PlaGe platform (Toulouse, France).

de novo genomes for the parental lines Eden-1 and NW-77 were obtained by using the PacBio Single Molecule Real-Time technology. The SMRTBell library was prepared using a SMRTbell Express Template prep kit, following the "procedure and checklist -preparing >30kb librairies using SMRTbell Express Template preparation kit" protocol. Genomic DNA (5µg) was sheared with the 75kb program using a Diagenode Megaruptor (Diagenode) generating DNA fragments of approximately 25kb. A Femto Pulse (Agilent Technologies, Santa Clara, CA, USA) assay was used to assess the fragment size distribution. Sheared genomic DNA was carried into the first enzymatic reaction to repair any damages that may be present on the DNA backbone. A-tailing reaction followed by the overhang adapter ligation was conducted to generate the SMRT Bell template. After a 0.6X AMPure PB beads purification, the samples were size-selected using the BluePippin (Sage Science, Beverly, MA, USA) in order to recover all the material above 20kb. The eluted DNA was then purified with 1X AMPure PB Beads to obtain the final libraries around 25kb. The SMRTBell libraries were quality inspected and quantified on a Femto Pulse (Agilent Technologies) and a Qubit fluorimeter with Qubit dsDNA HS reagent Assay kit (Life Technologies). A ready-to-sequence SMRTBell Polymerase Complex was created using a Binding

Kit 2.1 (PacBio) and the primer V4. The diffusion loading protocol was used, according to the manufacturer's instructions. The PacBio Sequel instrument was programmed to load and sequence the sample on PacBio SMRT cells v2.0 (Pacific Biosciences, Menlo Park, CA, USA), acquiring one movie of 600 min per SMRTcell. The work was performed at the Gentyane Sequencing Platform (Clermont-Ferrand, France).

For genome assembly of each parental line, reads were corrected with CANU 1.8 and used as inputs in SMARTdenovo (Jue Ruan, https://github.com/ruanjue/smartdenovo) and wtdbg2.0 (Ruan and Li, 2019) software. The first wtdbg2.0 assembly was performed by using corrected reads only, while the second wtdbg2.0 assembly was performed by using both corrected reads and raw reads. The sequences of these four primary assemblies were transformed in pseudo long reads of 100kb with an overlap of 50kb. Then, the pseudo long reads longer than 20kb were assembled with CANU 1.8 (–trim-assemble –pacbio-corrected). After removal of spurious contigs (i.e. short contigs included in longer contigs; overlaps detected by minimap2 –x asm5, Li, 2018), the pseudo-chromosomes were obtained with ALLMAPS (Tang et al., 2015) by scaffolding the contigs using the *A. thaliana* Col-0 genome (TAIR Arabidopsis) as reference. Overlaps detected by minimap2 –x asm5 were transformed into pseudo-genetic markers (~1 marker per 30kb). Two iterations of polishing with arrows were performed on the raw genome sequences, including mitochondrial and chloroplast genomes. Protein coding genes were annotated by integrating five sources of information. Priority was successively given to: i) a BLASTp search of reciprocal best hits with 15,832 "taxonomy:3702" (*A. thaliana*) proteins tagged as reviewed in the Uniprot database (90% span, 80% identity) as of March 2019 (Uniprot C), ii) EC numbers assigned by using the protocol (Schläpfer et al., 2017, E2P2 v3.1) with BLAST e-value cutoff lowered to 1.e-5 and pathway-prediction-score set to 0.3 in pathway-tools, iii) the transcription factors and kinases identified by ITAK release 1.7 (Zheng et al., 2016), iv) the transcription factors identified by PlantTFCat (Dai et al., 2013) and v) the Interpro (release 72.0) search (Finn et al., 2017). The EC numbers were tested against the ENZYME (Bairoch, 2000) database (release March 2019), updated when deprecated and then used to get the description of the enzymes. Finally, the protein annotations were validated by the tbl2asn software (https://www.ncbi.nlm.nih.gov/genbank/tbl2asn2, March 2019). GO terms were assigned using the BLAST2GO pro software integrating blastp similarities with Genbank NR (Benson et al., 2013; March 2019, e-value < 1e-5, 20 best-scoring hits) and Interpro (release 72.0) results.

SNP mapping analysis

The analysis of sequences obtained for F2 plants was performed on the Galaxy platform following the "SNP detection and effect prediction" protocol by using the two *de novo* assembly genomes, Eden-1 and NW-77 (Enis et al., 2018). The depth of sequencing of the eight pools was on average 51X (min: 43X, max: 55X) when mapped on either Eden-1 or NW-77. Raw reads of each of the eight pools were mapped onto the two reference genomes with a maximum of four mismatches on at least 80 nucleotides. For SNP calling, the minimum position coverage of supporting reads was 20, the minimum variant coverage was 10, the minimum variant frequency was 0.2 and the minimum homozygous frequency was 0.75. Following the analysis, the allele read count matrix, for the reference and alternate alleles, was composed of 354,763 and 316,504 SNPs across the eight pools using Eden-1 and NW-77 reference genomes, respectively. Generated data was first filtered for i) non-nuclear SNPs, ii) for SNPs without mapped reads in at least two pools out of the seven pools of susceptible F2 plants, and iii) for missing data corresponding to unmapped genomic positions (number of remaining SNPs with Eden-1 and NW-77 reference genomes: 341,094 and 313,178 SNPs, respectively). Second, we filtered our data according to a distribution of the depth of raw reads per SNP obtained for each pool and we removed SNPs with extreme values. For resistance and susceptible pools, SNPs for which the depth of reads ranging between 26 and 65 were kept (number of remaining SNPs with Eden-1 and NW-77 reference genomes: 285,873 and 253,884 SNPs, respectively). Finally, to identify QTL regions underlying total resistance of Eden-1, we first calculated for each SNP the difference between the reference allele frequency obtained in the resistant pool and the average of the reference allele frequencies between the seven susceptible pools, hereafter named DIFF. Then, in order to aggregate DIFF results among physically linked SNPs, we calculated at each focal SNP position an average DIFF based on all the SNPs present in a 500kb window centered on that focal SNP. To illustrate our results, plots were computed under the *R* environment 3.6.1 (R_Core_Team, 2019).

RPS4/RRS1-R$^{\textit{Eden-1}}$ *locus cloning and analysis of polymorphisms*

Eden-1 *RPS4/RRS1* locus (*RPS4/RRS1*$^{\textit{Eden-1}}$) was cloned according to Gateway$^{\text{TM}}$ technology (Invitrogen, Carlsbad, CA, USA). DNA was extracted with a CTAB (hexadecyltrimethyl ammonium bromide; C19H42BrN) DNA extraction protocol adapted from Allen et al. (2006). PCR amplification of the locus was performed with ATT-GATE1 and ATT-GATE2 primers (table 2) using PrimeStar HS DNA polymerase from Takara Bio Inc. (Otsu, Japan) according to the following PCR program:

Step	Temperature	Time	Number of cycles
Intial Denaturation	98°C	20 seconds	1
Denaturation	98°C	2 minutes	30
Annealing	55°C	30 seconds	
Extension	72°C	4.5 minutes	
Final extension	72°C	2 minutes	1

Once the locus was amplified, the fragment was visualized on a 1% agarose TAE (1X) electrophoresis gel, extracted and purified with a QIAEX II, Gel Extraction Kit (Qiagen GmbH, Hilden, Germany). After a BP recombinant reaction using pDONR207 vector according manufacturer instructions (Invitrogen), the presence of *RPS4/RRS1*$^{\textit{Eden-1}}$ locus in the pENTR-RPS4/RRS1$^{\text{Eden-1.1}}$ and pENTR- RPS4/RRS1$^{\text{Eden-1.2}}$ was confirmed by digesting 1µg of plasmids with *Xba1*. Thereafter, this locus was sequenced with 28 primers (table 2) positioned every 500pb along *RPS4/RRS1* sequence, according to the Sanger method (Sanger et al., 1977). Both clones were sequenced. Generated sequences were aligned on clone manager©.

Sequences from pENTR- RPS4/RRS1$^{\text{Eden-1.1}}$ and pENTR- RPS4/RRS1$^{\text{Eden-1.2}}$ plasmids were aligned with the *RPS4*/*RRS1* sequence from five accessions susceptible to the GMI1000 strain at 27°C and 30°C (Col-0, Gre-0, Lip-0, Ms-0 and Sah-0) and seven accessions resistant at 27°C but susceptible at 30°C (Bl-1, Kondara, Pyl-1, Nd-1, Shadara, Sp-0, Stw-0). Alignment was performed using Bioedit 7.2.5 (Hall, 1999). As a result of sequence polymorphism analysis, several polymorphisms were identified and are listed in annexes 1 and 2. A specific polymorphism leading to an amino acid change (A641T) was identified at the nucleotide position 1,921 of Eden-1 *RRS1*-

R genomic sequence. Following a two-step PCR-based site-directed mutagenesis, using primers listed in table 2 and PrimeStar HS DNA polymerase from Takara Bio Inc. (Otsu, Japan), a *RRS1-R*^{A641T} variant was generated by mutating the genomic sequence of *RRS1-R*$^{Nd\text{-}1}$. We then introduced the KpnI-BamHI restriction fragment containing the A641T mutation in the pENTR-RPS4/RRS1-R$^{\text{Nd-1}}$ genomic clone (Le Roux et al., 2015) generating pB7-RPS4-RRS1$^{\text{A641T.}}$

Two binary vectors were generated. The RPS4/RRS1-R$^{\text{Eden-1.1}}$ fragment of the pENTR-RPS4/RRS1$^{\text{Eden-1.1}}$ was transferred in the pB7-D35S-GWY-GFP binary vectors using LR Gateway reaction according manufacturer instructions (Invitrogen) resulting in pB7-RPS4-RRS1$^{\text{Eden-1.1}}$. pENTR-RPS4-RRS1$^{\text{A641T}}$ was used to generate the pB7-RPS4-RRS1$^{\text{A641T}}$. The binary vector pB7-RPS4-RRS1$^{\text{Nd-1}}$ containing Nd-1 RPS4/RRS1 locus was already available (Deslandes et al., 1998). For complementation, all constructs were introduced in the *Agrobacterium tumefaciens* GV3101 strain and used for floral dip transformation (Clough and Bent, 1998) of five weeks old *A. thaliana rps4-21/rrs1-1* mutant (Ws-0 background; Narusaka et al., 2009). T1 and T2 progenies were selected using BASTA (7.5 µg/mL) and genotyped by PCR using GFP primers (table 2).

Primer name	**Sequence 5'→3'**	**Plasmid construct sequencing**
ATT-GATE1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATCAAATGTACTAGACTTTGGGG	ATT-GATE1-RPS4-RRS1$^{\text{Eden-1}}$-ATT-GATE2
ATT—GATE2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTCTTCAGACTATGGTTTTATTG	
SelA	TCGCGTTAACGCTAGCATGGATCTC	pENTR- RPS4/RRS1$^{\text{Eden-1.1}}$ and pENTR- RPS4/RRS1$^{\text{Eden-1.2}}$
SelB	GTAACATCAGAGATTTTGAGACAC	
RPS4-400F	ATTCCCAGACAGTCAAAATGA	
RPS4-500F	CGTGTGCATGATCACTGAATT	
RPS4-1000F	TCGTACTTTGTTTCAAGAGCA	
RPS4-1500F	GTAGCGTTTCCGAGCATATGA	
RPS4-2000F	AGTGTTGTGCAACCTTCAAGG	
RPS4-2500F	ACTGTAGGGCAGCTTAAGGTC	
RPS4-3000F	TCGAATTCCAATGATCCATAC	
RRS1-500F	GCACTCATTGCTTCAGCCGAACCAA	
RRS1-1000F	CCAGTCTAGTATCTCACGAAGAGCA	
RRS1-1500F	ACGCTGTCTTCACCGCCTTCACAAT	
RRS1-2000F	GGTTGACCTCTGTCTTCATAGTCGT	
RRS1-2500F	ACCTCTCTGAAGCTCTCCGTCGAAA	
RRS1-3000F	GAAGCTGCTGGAGATTGAAAACATG	
RRS1-3500F	AGCAGAATCTCCATGAGTTGTCAGT	
RRS1-4000F	AGAACCTAAAACAACCTTCAAACGT	
RRS1-4500F	CAACATGCCGTATAGTCAACTTCAG	
RRS1-5000F	GTTATATCTTGGTGGCACTGCAATA	
RRS1-5500F	CAACAAAGCTCCGACTTTCAGCTTC	
RRS1-6000F	AGTGTCTAAATGATAGGTTCACAGT	
RRS1-6500F	TTCCATGACCGAGAATTTGTCTGAC	
RRS1-7000F	TGAGAGACATATATGCATTATGAAA	
RRS1-7500F	GCTATAGACGAGGGAGATCTATGGA	
RRS1-8000F	GCGTGAAACTGTAAAATAGAAAGTT	
RRS1-8500F	CTCCTAGAAACGCGGCTGTCTTGTT	
RRS1-9700F	GTGTCGATAGCAAAAGAGGGT	
RRS1-10200F	GCTATTACTTACCTATCTGAG	
RRS1-R-A641T-Fw	TGCAGAATTTCCCAGCCACGGGTCGATTGCTACGTCTACGA	ATT-GATE1-RPS4-RRS1$^{\text{A641T}}$-ATT-GATE2
RRS1-R-A641T-Rev	AGACGTAGCAATCGACCCGTGGCTGGGAAATTCTGCAGTCT	
KpnI-Tir-Fw	CATAGATCTATCTACACTTGTAATCTAATCACTACTTTTGT	pENTR-RPS4-RRS1$^{\text{A641T}}$
Bam-Tir-rev	ATAGCTGACTCTCAATACTTCATAACCAGAAACCTCCACTGGA	
AttB1-Kpn-Tir	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATAGATCTATCTACACTTGT	pB7-RPS4-RRS1$^{\text{A641T}}$
AttB2-BamHI-Tir	GGGGACCACTTTGTACAAGAAAGCTGGGTCATAGCTGACTCTCAATACTT	

Table 2. List of primers used in this study.

Hypersensitive Response assays

For plant infiltration with *Pfo-1:*PopP2 and *Pfo-1*:PopP2-C321A strains, basal leaves of four-week old *A. thaliana* plants of T2 transgenics selected for Basta resistant and controls (Nd-1, Eden-1, Col-0, Ws-0 and *rps4-21/rrs1-1*) were infiltrated at the 1/3 top surface with bacterial suspensions at OD600nm = 0.2 in 10 mM MgCl2. Plants were then left overnight in a climatic cabinet (125 μE/M2/s fluorescent illumination and 9h of light) under the following temperature treatment: i) 22°C, ii) 26°C/27°C day (night/day) and iii) 29°C/30°C day (night/day). HR response was observed the following day.

3. Results

EDEN-1: robust resistance to GMI1000 at 30°C

The phenotyping of 176 worldwide accessions of *A. thaliana* revealed one fully resistant accession to the *R. solanacearum* GMI1000 strain, Eden-1, from 3 till 10 days after inoculation (dai) at 27°C and 30°C (Aoun et al., 2017). To confirm these results, we performed three additional independent inoculations by comparing at 30°C the response of Eden-1 to the GMI1000 strain with the response of two control lines, i.e. Col-0 (harboring the susceptible allele *RRS1*) and Nd-1 (harboring the resistance allele *RRS1-R*) that are susceptible and resistant at 27°C, respectively. While Col-0 was susceptible at both 27°C and 30°C, Eden-1 remains fully resistant at both temperatures (figure 21). On the other hand, Nd-1 was fully resistant at 27°C but susceptible at 30°C (figure 21).

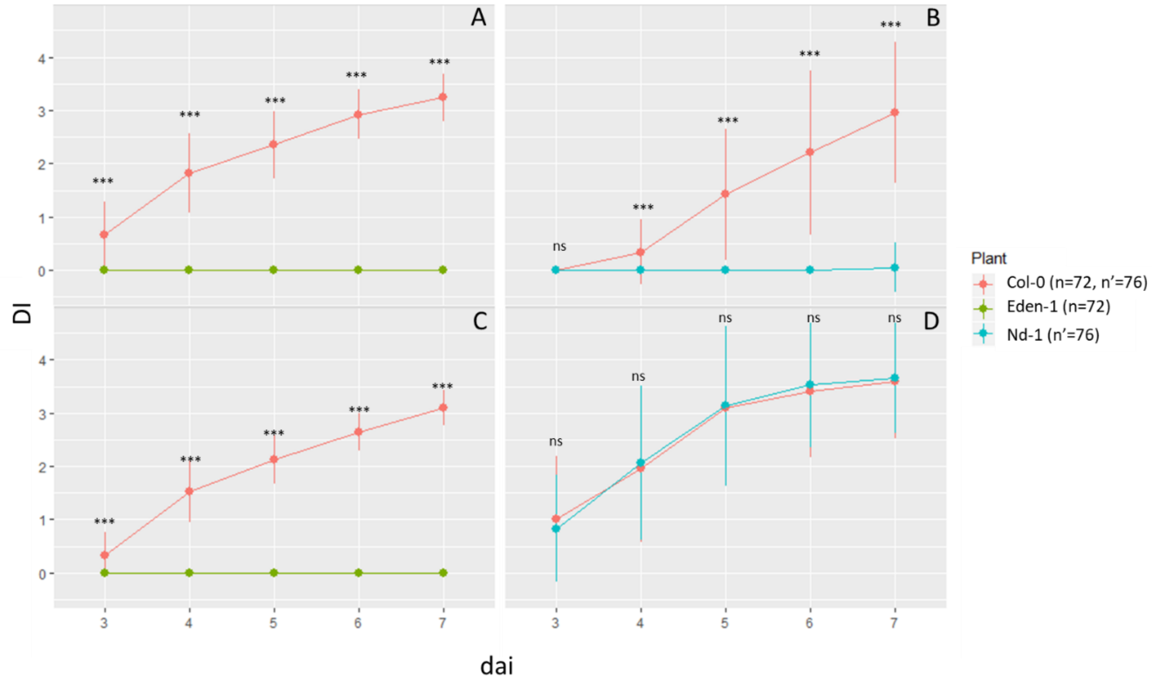

Figure 21. Disease progress in Col-0, Nd-1 and Eden-1 following *R. solanacearum* GMI1000 infection at 27°C and 30°C. Curves represent disease progression from 3 to 7 days after inoculation at 27°C (A, B) and 30°C (C, D) for Col-0 compared with Eden-1 (A, C) or with Nd-1 (B, D) accessions. Dynamics of disease symptoms were computed under *R* environment 3.6.1 (R_Core_Team, 2019) using ggplot2 package. Symbols * and *** denotes a significant difference observed between the two accessions in comparison at $P < 0.05$ and $P < 0.001$, respectively, in ANOVA analysis. The "ns" abbreviation correspond to a non-significant difference between the two accessions in comparison. dai: days after inoculation. DI: disease index. n and n' correspond to the number of plants used in Col-0 and Eden-1 or Col-0 and Nd-1 experiments, respectively.

Genetic architecture of Eden-1 total resistance to GMI1000 at 30°C

A F1 progeny originating from a cross between Eden-1 and NW-77, an EMS sterile mutant of Landsberg-0 (Ler-0) known to be susceptible to GMI1000 (Godiard et al., 2003) was available to develop a BSA strategy (F. Roux, LIPM) (figure 22A). The F2 plants resulting from F1 self-fertilization was generated, sown (n=1,978) and grown until 4 weeks old and then sampled for DNA extraction prior to inoculation with the *R. solanacearum* GMI1000 strain at 30°C. At 10 dai, plants with a disease index from 0 to 2 were considered as resistant and plants with a disease index strictly higher than 2 were considered as susceptible. 73.3% and 26.7% of the plants were susceptible and resistant, respectively (figure 22B). These relative fractions did not deviate significantly from the segregating ratio '1 resistant : 3 susceptible' (χ^2 = 3.03 < 3.84; α = 5%), thereby suggesting that the Eden-1 total resistance to *R. solanacearum* at 30°C is mainly driven by a single recessive nuclear locus.

In order to identify the main QTL associated with total resistance of Eden-1 to *R. solanacearum*, we proceeded with a BSA approach combined with a mapping-by-sequencing approach. One pool of resistant and seven pools of susceptible plants were prepared with equimolar DNA of 167 individuals per pool and sequenced using the Illumina technology. Eden-1 and NW-77 were sequenced using the PacBio technology to obtain high quality genome sequences, thereby facilitating mapping of the reads generated in the eight pools and estimation of allele frequencies across the genome within each pool. Estimating allelic frequency differentiation across the genome between resistant and susceptible pools revealed a major QTL region at the end of chromosome V, with the top SNP located (i) within the *RPS4*/*RRS1* locus when reads were mapped on NW-77 genome, and (ii) 100kb from the *RPS4*/*RRS1* locus when reads were mapped on Eden-1 genome (figure 22C).

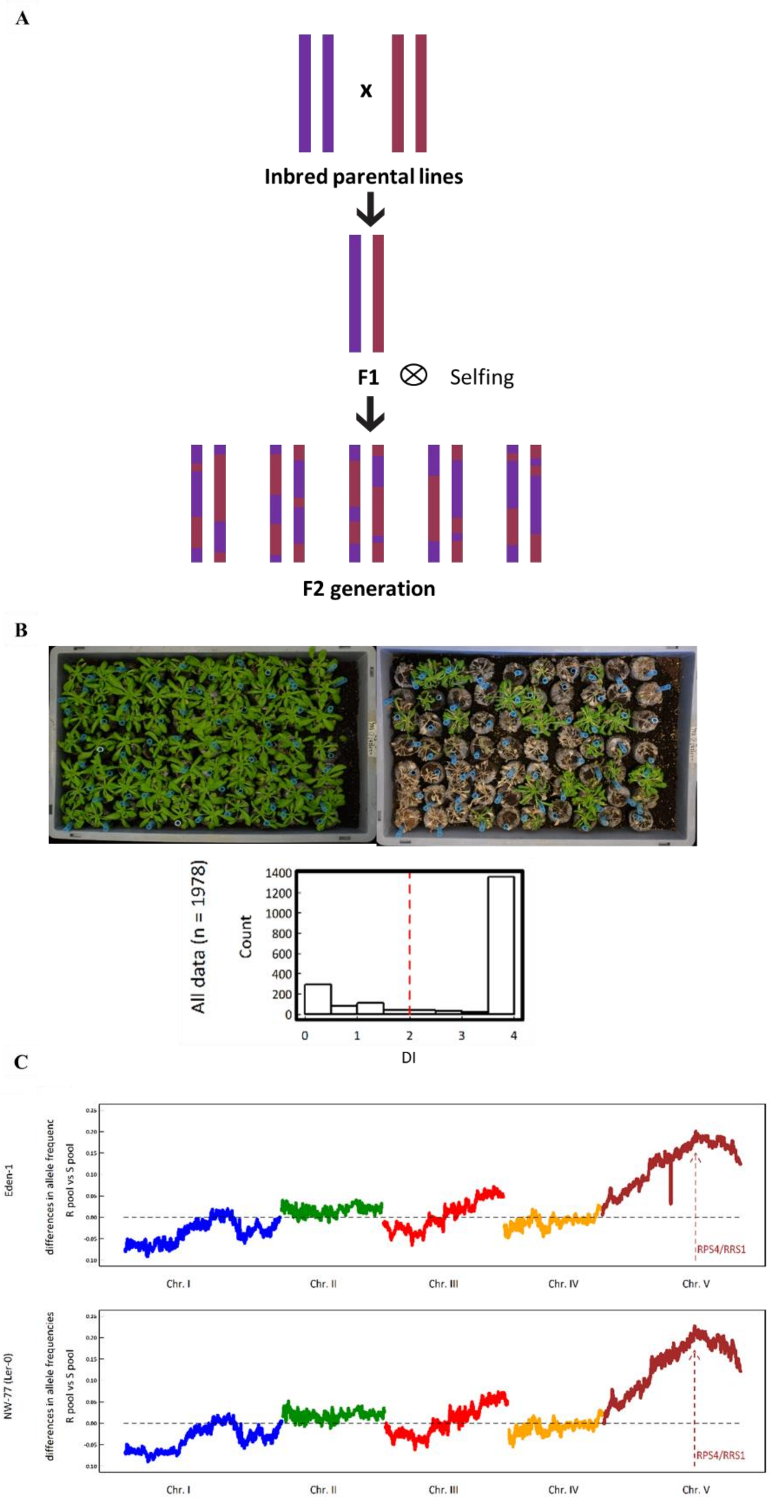

Figure 22. Genetic architecture of total resistance revealed by a BSA approach combined with a mapping-by-sequencing approach. **A.** Scheme representing the generation of the F2 population. **B.** F2 plants before inoculation (on the left) and after 24 days after inoculation (on the right) with the *R. solanacearum* GMI1000 strain and distribution of disease index (resistance: disease index between 0 and < 2, and susceptibility: disease index ≥ 2). **C.** Plot showing differentiation of allele frequency between resistance and susceptible pools across the genome of Eden-1 (top panel) and NW77 (bottom panel). The arrows highlight the physical position of the RPS4/RRS1 locus on each reference genome.

Heat-stable resistance of Eden-1 is dependent on perception of PopP2

The identification of the major QTL region near the *RPS4*/*RRS1* locus suggests that an allelic version of the *RPS4/RRS1-R*$^{Eden\text{-}1}$ locus can be responsible of Eden-1 total resistance under elevated temperature. Interestingly, the total resistance conferred in the Nd-1 natural accession at 27°C was previously demonstrated to be i) monogenic and recessive and ii) conferred by an allelic version of *RRS1-R*$^{Nd\text{-}1}$ (Deslandes et al., 1998). Therefore, to determine if the defense response was related to the immunoreceptor pair RSP4/RRS1-R and its activation by the perception of the T3E PopP2, Eden-1 and Col-0 accessions were inoculated with the RSΔPopP2 strain mutated in PopP2 at 30°C. Although three independent replicates were performed, phenotyping data are missing at 7 and 10 dai for one of them. Therefore, I only present the results of two replicates with all dai phenotyping points. Interestingly, at 30°C, Eden-1 plants developed symptoms, albeit still being less susceptible than Col-0 (figure 23). This strongly reinforced our choice of *RPS4/RRS1* as the candidate gene underlying heat-stable resistance.

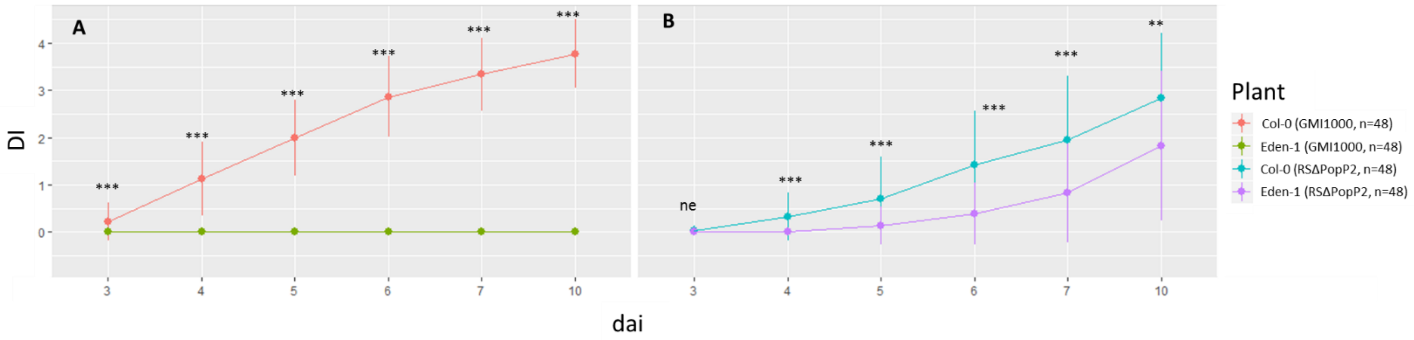

Figure 23. Dynamics of disease progression in inoculated Col-0 and Eden-1 with the GMI1000 and RSΔPopP2 strains at 30°C. Curves represent disease progression from 3 to 10 days after inoculation at 30°C for Col-0 and Eden-1 accessions inoculated with the GMI1000 (A) or with RSΔPopP2 (B). Dynamics of disease symptoms were computed under *R* environment 3.6.1 (R_Core_Team, 2019) using ggplot2 package. Symbols *, ** and *** denotes to significant difference observed between the two accessions in comparison at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, in ANOVA analysis. The "ns" abbreviation correspond to a non-significant difference between the two accessions in comparison. dai: days after inoculation. DI: disease index. 'n' correspond to the number of Col-0 and Eden-1 phenotyped plants.

Eden-1 genome contains a specific allelic version of RPS4-RRS1-R

Together, our results suggest *RPS4-RRS1-R*$^{Eden\text{-}1}$ locus as a candidate gene conferring resistance stability to *R. solanacearum* at elevated temperature. As a next step, we cloned the *RPS4-RRS1-R*$^{Eden\text{-}1}$ locus in two plasmids. Sequences of both clones were aligned with i) the *RPS4* loci of Col-0 and Nd-1 and ii) the *RPS4/RRS1* genomic sequences of seven resistant and five susceptible accessions of *A. thaliana* to the *R. solanacearum* GMI1000 strain at 27°C, but all susceptible at 30°C. We identified 16 polymorphisms between *RPS4* sequences of Eden-1 and Nd-1, with four of them falling in introns. By comparing the *RPS4* sequences between Eden-1 and Col-0, 33 polymorphisms were found with 13 of them falling in introns. For *RPS4/RRS1* genomic sequences alignment of the resistant and susceptible accessions, we identified 6 polymorphisms that were specific to Eden-1. These 6 polymorphisms correspond to 4 intronic polymorphisms and two exonic polymorphisms (Annex 1). Only one exonic polymorphism leads to a nonsynonymous mutation and corresponds to a G to A point mutation resulting in a change of Alanine to Threonine at amino acid residue 641 (A641T) at the start of the LRR domain of *RRS1* (figure 24). This substitution is a switch from a hydrophobic to a hydrophilic state of the amino acid residue.

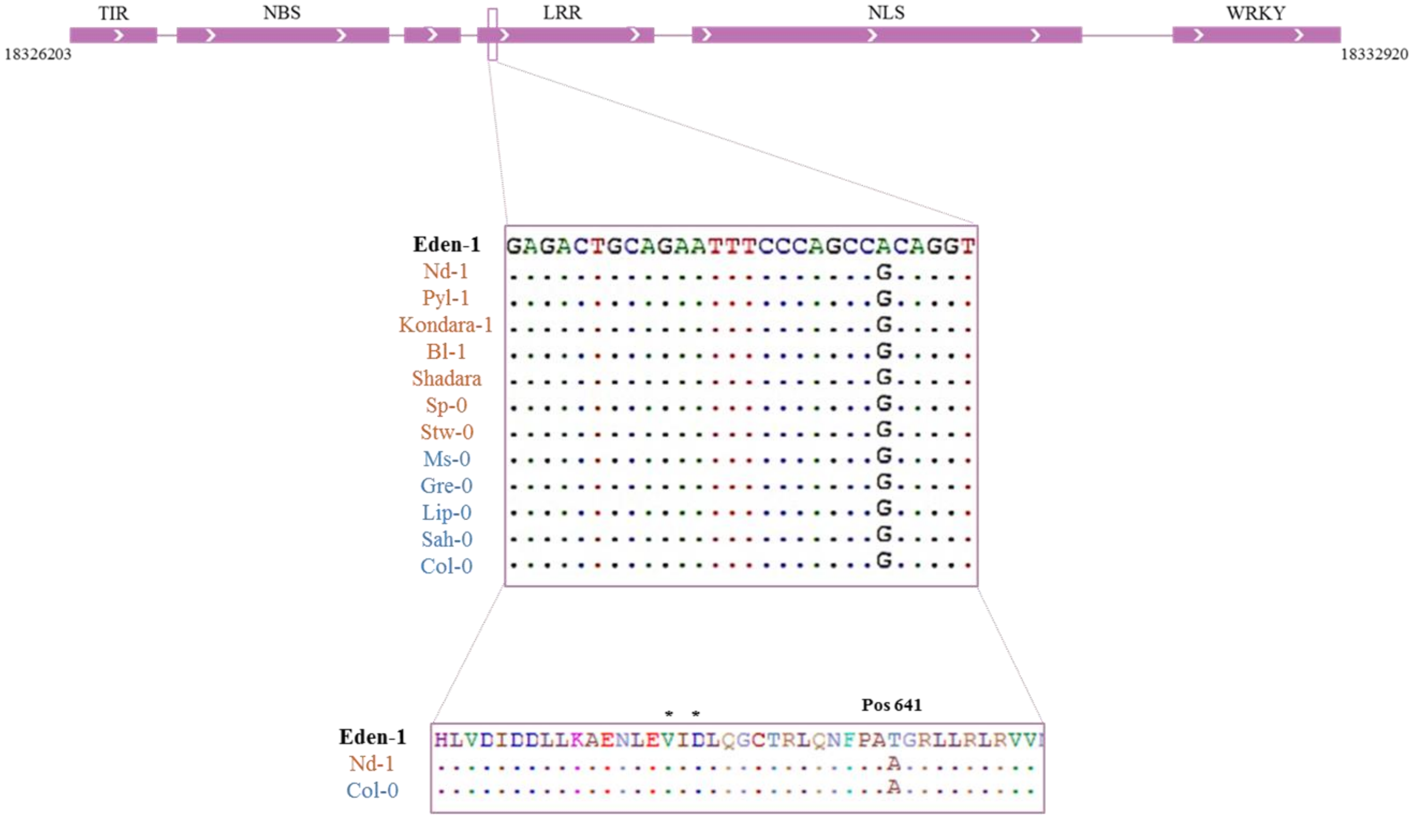

Figure 24. Identification of polymorphisms between the *RRS1* version of Eden-1 and other resistant and susceptible accessions. Accessions marked in brown and blue correspond to the resistant and susceptible accessions at 27°C, respectively. The asterisk corresponds to the artificial mutations generated in *SCN1* by Zhu et al. (2010).

Heat-stable resistance of Eden-1 is conferred by RPS4/RRS1-R$^{Eden\text{-}1}$

In order to determine if the amino acid change A641T is responsible for Eden-1 total resistance under heat stress, we transformed the homozygous *rps4-21/rrs1-1 A. thaliana* susceptible mutant (Deslandes et al., 2002) with different allelic forms of *RPS4/RRS1* genomic sequence: i) *RPS4/RRS1* of Nd-1, ii) *RPS4/RRS1* of Eden-1 and ii) *RPS4/RRS1* of Nd-1 in which the point mutation A641T was introduced. The second generation (T2) of transformed plants were i) identified using Basta selection, ii) inoculated with the GMI1000 strain at 27°C and 30°C for a preliminary phenotyping, and iii) infiltrated with *Pfo:*PopP2 and *Pfo*:PopP2-C321A at 22°C, 27°C and 30°C. However, due to technical problems in the quarantine laboratory (temperature stability), results obtained following inoculation with the GMI1000 strain at 27°C and 30°C could not be valorized. This experiment should be therefore repeated. At 22°C, induced visible HR in the presence of the elicitor PopP2 was observed in all constructs (figure 25). This suggests that i) the tested constructs are functional, ii) the locus version of *RPS4/RRS1* with which plants were transformed is expressed, and that iii) PopP2 perception induced an HR response. A 27°C, all constructs were functional and led to an HR upon perception of native PopP2 (figure 26). However, at 30°C, only *RPS4-RRS1-R*$^{Eden\text{-}1}$ and *RPS4-RRS1-R*$^{\mathrm{A641T}}$ constructs were functional and led to an HR upon perception of native PopP2. This response was also observed in their genetic background Eden-1 (figure 27). An absence of HR with *Pfo*:PopP2-C321A mutant confirmed that the HR observed is related to RPS4/RRS1-R (figures 25, 26, 27).

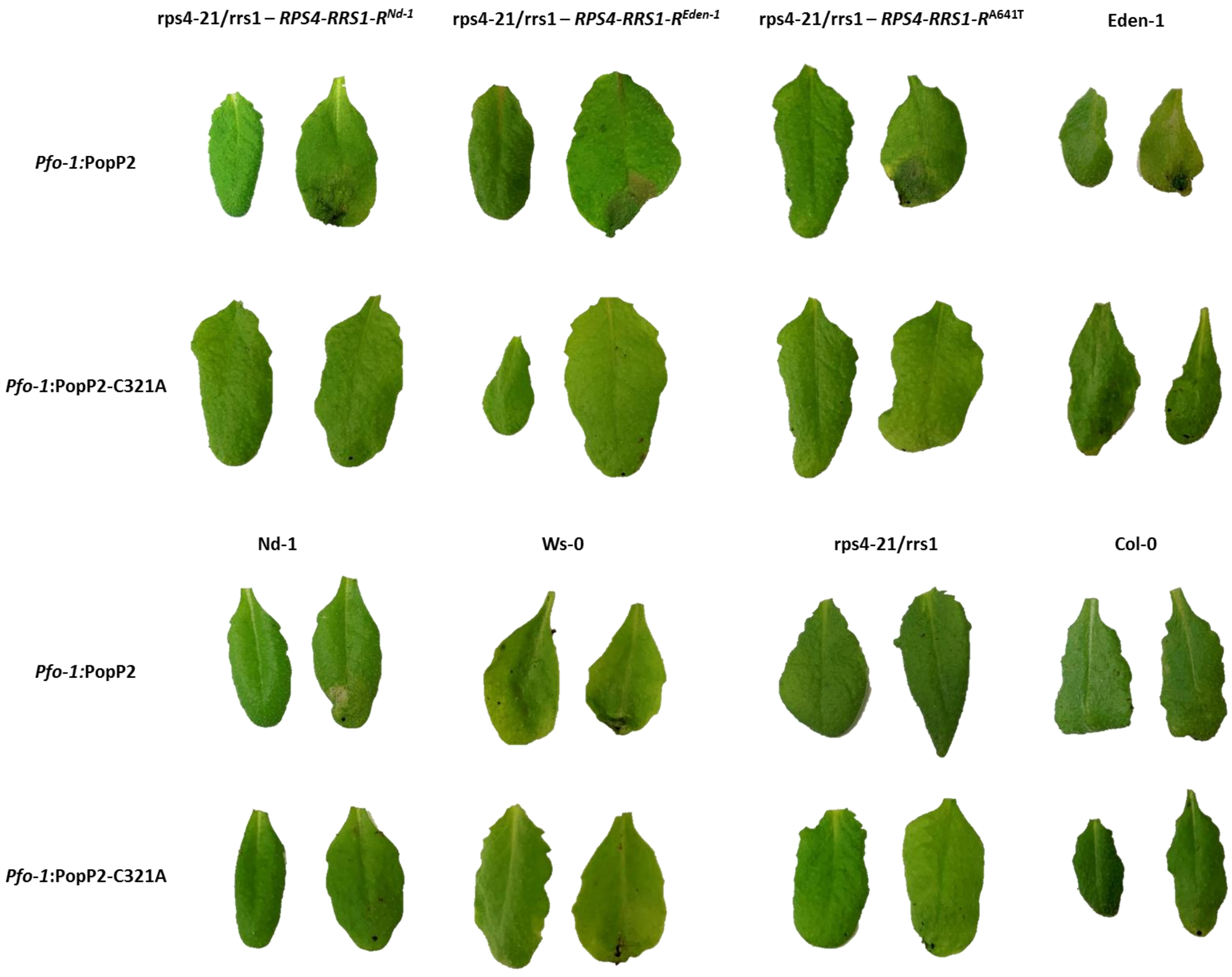

Figure 25. HR assay on transgenics of *A. thaliana* with *Pfo-1* at 22°C. Pictures were taken after 48h of leaves infiltration with *Pfo-1* on the transgenics and controls. For comparison purposes, a non-infiltrated and infiltrated leaves were put on the left and right side of the picture, respectively.

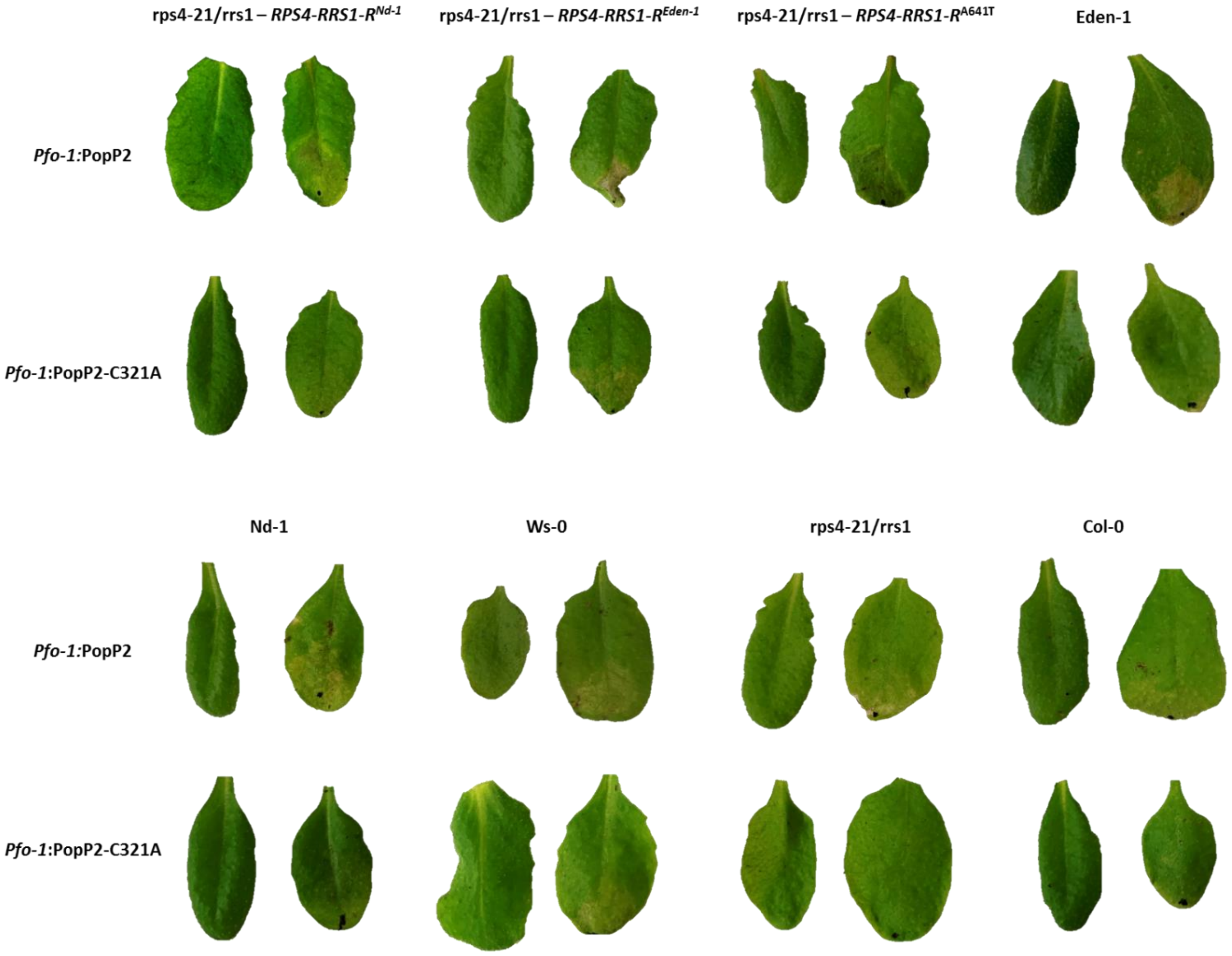

Figure 26. HR assay on transgenics of *A. thaliana* with *Pfo-1* at 27°C. Pictures were taken after 48h of leaves infiltration with *Pfo-1* on the transgenics and controls. For comparison purposes, a non-infiltrated and infiltrated leaves were put on the left and right side of the picture, respectively.

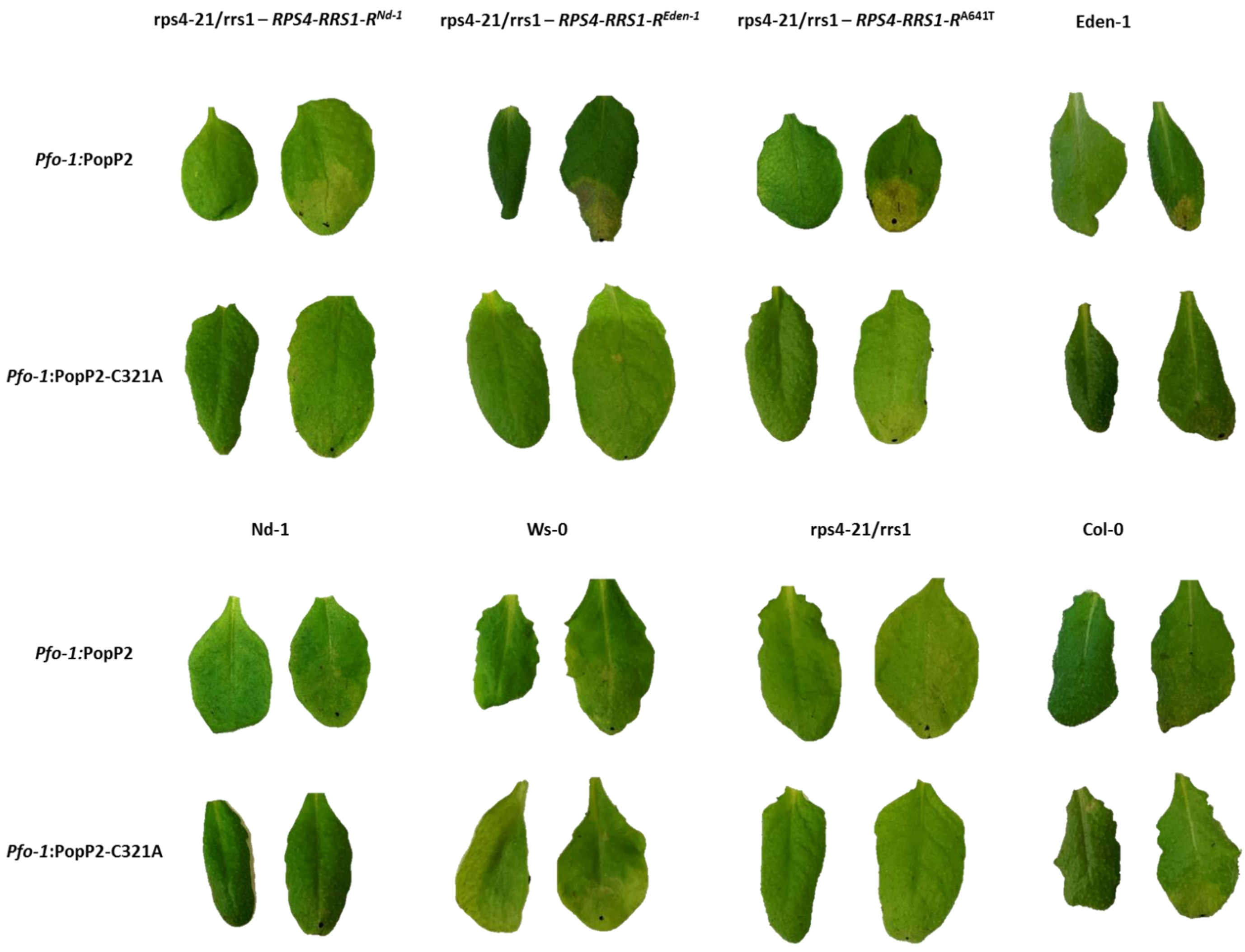

Figure 27. HR assay on transgenics of *A. thaliana* with *Pfo-1* at 30°C. Pictures were taken after 48h of leaves infiltration with *Pfo-1* on the transgenics and controls. For comparison purposes, a non-infiltrated and infiltrated leaves were put on the left and right side of the picture, respectively.

4. Discussion

Our screen of genetic variation in *A. thaliana* revealed a wild accession with a heat-stable total resistance to the GMI1000 strain of *R. solanacearum*: Eden-1. By characterizing the genetic architecture of this resistance, mapping, cloning and complementing a susceptible mutant, I found that Eden-1 allelic variant of RPS4/RRSI-R is potentially responsible for the heat-stable resistance. Furthermore, I identified at the beginning of the LRR domain of *RRS1*$^{\textit{Eden-1}}$ a unique point mutation that leads to a nonsynonymous substitution and likely maintains resistance at elevated temperature. This point mutation led to a configuration change from a hydrophobic (Alanine) to a hydrophilic (Threonine) state that might affect protein-protein or protein-ligand interactions. This model will therefore be used by the team to study the mechanisms that stabilize the activity of the immuno-receptor pair under heat stress. Interestingly, this mutation is genetically close to an artificial EMS point mutation (*int102* mutant) created in the *snc1-1* mutant background in the LRR domain of another NLR gene, *SNC1 (Zhu et al., 2010)*. This EMS mutation also confers pathogen resistance under heat stress. However, in that particular case, this mutation was identified through the heat sensitive autoimmune phenotype suppression of the *snc1-1* mutant (Zhu et al., 2010). Therefore, our results along with those of Zhu et al. (2010) suggest that this region of the LRR domain of NLR proteins has a key role in stabilizing their activity.

Yet, mechanisms underlying this RRS1-R$^{\text{Eden-1}}$ resistance still need to be deciphered. One of the avenues that can be explored is that heat stress downregulates many mRNAs and causes 5’-ribosome pausing characterized by (i) an upstream ribosomes accumulation, (ii) a downstream ribosomes depletion and (iii) a degradation of specific mRNAs, which are stored in the polysomes of stress granules in the nucleus (Liu et al., 2013; Shalgi et al., 2013). In addition, it was shown that in *A. thaliana*, heat stress led to 5’-ribosome pausing by blocking translation initiation and differentially affecting elongation rates of coding regions; thus leading to polysomal degradation of a high number of non-aberrant mRNAs (Merret et al., 2015). Thereby, further experiments are needed to check if during infection, RPS4/RRS1-R$^{\text{Eden-1}}$ is still present in polysomal fractions in heat granules at the nuclear level and whether their accumulation is affected by the unique mutation identified; by checking the amount of stable mRNAs engaged in polyribosomes (Matsuura et al., 2010; Muñoz and Castellano, 2012; Yángüez et al., 2013). It was also shown that mRNAs that code for amino terminal hydrophobic products contribute to 5’-ribosome pausing that is also dependent on the activity of HSC70/HSP70 chaperone (Liu et al., 2013; Merret et al., 2015; Shalgi et al., 2013). This raises the curiosity of studying the effect of the A614T amino acid change on RRS1 protein configuration that may save the corresponding mRNAs from translation inhibition or degradation. Currently, a collaboration is under construction with Jean-Marc Deragon (LGDP, Perpignan – France) to test at the mechanistic level the presence or not of ribosome pausing or mRNAs destabilization. Another interesting non-exclusive hypothesis to test is the existence of spliced isoforms of a *RPS4/RRS1-R*$^{Eden\text{-}1}$ due to the presence of mutations in the intronic regions of this gene. This has been shown in the case of *SNC1* and *RPS4* whose alternative splicing fine-tuned their expression due to the Modifier of SNC1, 12 (MOS12) (Xu et al., 2012).

Interestingly, Eden-1 is also resistant to (i) *Fusarium oxysporum*, which is also a soil-borne pathogen like *R. solanacearum (Lyons et al., 2015)*, and (ii) *Xanthomonas campestris* pv *campestris* (strain CFBP6943) which is a vascular foliar bacterial pathogen (Debieu et al., 2016). In the latter case, natural variation of QDR to the strain CFBP6943 is associated with *RPS4/RRS1* (Debieu et al., 2016). Whether the *RPS4-RRS1-R*$^{Eden\text{-}1}$ locus also maintains resistance against these two pathogens (and others) under heat stress is an open question but certainly deserves to be investigated.

D. Conclusion

In this chapter, by adopting a GWA mapping approach on 176 worldwide accessions, I have detected a major QTL at 27°C with the top SNP falling in the locus encoding the well characterized RPS4/RRS1-R immune-receptor pair (Deslandes et al., 2002). This result represents one of the few examples of identification of a typical immune receptor that can also be considered as a QDR gene (Roux et al., 2014). In agreement with several studies (Desaint el al., submitted), I also confirmed the impact of heat stress on resistance to *R. solanacearum* in the worldwide collection of *A. thaliana* at 30°C. However, at 30°C, no association peak was near *RPS4/RRS1*. Instead, the genetic architecture was highly polygenic. Among the multiple detected QTLs with a small effect, I identified one QTL associated with early plant defense response to *R. solanacearum* at 30°C and functionally validated two genes, *SSL4* and *SSL5*. Interestingly, expression of *SSL4* and *SSL5* genes was demonstrated in previous studies to be specifically up-regulated in plant response to abiotic (*SSL4)* or biotic (*SSL5*) stresses (Sohani et al., 2009; Manzo et al., 2016; . As previously mentioned, *SSL4* and *SSL5* correspond to genes of susceptibility rather than to classical resistance genes. More generally, the results based on a worldwide collection of *A. thaliana* show that the genetic architecture of plant response to *R. solanacearum* largely depends on temperature stress and infection process. In addition, the identification of several QDRs with minor effects as well as a total and stable resistance under elevated temperature support the high diversity of resistance mechanisms involved.

Using the GW-LS method, we refined my first GWA mapping analysis and detected more QTLs with top SNPs falling in or alongside genes encoding a broad range of molecular functions, not previously associated with plant disease resistance. For instance, *KCO1* (*At5g55630*) or *At4g03630* identified at 27°C, are annotated as an outward rectifying potassium channel protein or implicated in root nodule development, respectively. At 30°C, there are also several candidate genes for which a direct link with defense mechanisms is not established, such as *At3g51240* encoding a FLAVANONE 3-HYDROXYLASE involved in flavonoids and *At4g27730* annotated as oligopeptide transporter 1. The identification of molecular functions not previously associated with plant disease resistance has already been reported for other pathogens. For instance, in *A. thaliana*, some kinases have been found to participate in resistance response to *F. oxysporum* (*WALK22/RFO1*; Diener and Ausubel, 2005) and to *X campestris* (*RKS1*; *(Huard-Chauveau et al., 2013)*. Thus, the identification of these numerous dynamic QTLs, some of which with minor effects, highlight the multiple mechanisms involved in QDR to *R. solanacearum*. Whether these QTLs identified at the worldwide scale are conserved at smaller geographical scales is a question that I address in the next chapter.

Acknowledgments

We would like to thank Baptiste Mayjonade for his help and advices in the extraction of high-molecular-weight genomic DNA. We are also grateful for Marie Didelon, Mehdi Khafif and Sylvain Raffaele for providing us with growth chambers.

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Chapter III. Identification of the genetic basis of natural variation of plant response to *Ralstonia solanacearum* under elevated temperature in a local population of Arabidopsis

A. Introduction

The first part of my project focused on studying the genetic basis of natural variation of response to *R. solanacearum* using a worldwide mapping population of *A. thaliana*, under elevated temperature. GWA mapping revealed an association peak falling in the *RPS4/RRS1-R* locus at 27°C. At 30°C, using a method of inoculation in which we were cutting the roots to force the entry of the bacteria, a QTL was found on chromosome III. It involves a pair of genes (*SSL4* and *SSL5*), both encoding a strictosidine synthase-like protein, validated by a reverse genetic approach as susceptibility genes having a role in early defense response to *R. solanacearum.* While informative, the use of worldwide accessions may suffer from several limitations, including the effect of population structure and the presence of both genetic and allelic heterogeneities (Bergelson and Roux, 2010; Brachi et al., 2010). Highly powerful statistical methods such as mixed models including a kinship matrix as a covariate have been developed to limit the effects of population structure (i.e. EMMAX software; Kang et al., 2010). While these methods largely decrease the rate of false positive associations, they also increase the rate false negative associations. A common alternative to reduce the main limitations of working with worldwide accessions is to use a set of accessions collected at a smaller geographical scale while presenting extensive genetic diversity across the genome (Brachi et al., 2013).

Therefore, in the second part of my PhD project, I had the opportunity to use the French local mapping population TOU-A generated by F. Roux (LIPM) in order to identify other genes associated with the natural variation of response to *R. solanacearum* at 27°C and 30°C. The TOU-A population contains 195 natural accessions, collected from the Toulon-sur-Arroux village in Burgundy (France). The genome sequencing of these accessions revealed 1,902,592 SNPs, thereby representing almost one-sixth of the total number of SNPs identified in a set of 1,135 worldwide whole-genome sequenced accessions (Frachon et al., 2017). In addition, the short LD decay observed in this population (<3Kb) allowed fine mapping down to the gene level (Frachon et al., 2017). GWA mapping combined with a local score analysis revealed a different genetic architecture of resistance to *R. solanacearum* compared to the worldwide collection and uncovered a playful dynamics of 14 major QTLs; 2 QTLs at 27°C and 12 QTLs at 30°C. At 30°C, nine QTLs form a complex intra- and inter-chromosomal epistatic network. These results as well as the functional validation of *At1g14750*, encoding the atypical meiotic cyclin-like protein SOLO

DANCERS (SDS), as a susceptibility gene involved in QDR to *R. solanacearum* at elevated temperature, are described more in details in the manuscript presented below.

B. Article: Natural variation of *Arabidopsis thaliana* quantitative disease response to *Ralstonia solanacearum* is controlled by a complex genetic architecture involving additive and epistatic QTLs

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SUMMARY

1. Plant immunity is often negatively impacted by temperature elevation. However, the underlying molecular mechanisms remain poorly characterized. Based on a Genome Wide Association mapping approach, this study aims to identify in *Arabidopsis thaliana* the genetic basis of robust resistance mechanisms to the devastating pathogen *Ralstonia solanacearum* under heat stress.
2. A local mapping population was phenotyped against the *R. solanacearum* GMI1000 strain at 27°C and 30°C. To obtain a precise description of the genetic architecture underlying natural variation of quantitative disease resistance (QDR), we applied a genome-wide local score analysis. The candidate gene underlying a major additive QTL detected at 30°C was validated by a reverse genetic approach.
3. Alongside with an extensive genetic variation found in this local population at both temperatures, we observed a playful dynamics of QTLs along the infection stages. In addition, a complex genetic network of interacting loci could be detected at 30°C. Among those, the atypical meiotic cyclin *SOLO DANCERS* gene was identified as involved in QDR to *R. solanacearum* at 30°C.
4. In the context of climate change, the complex genetic architecture underlying QDR under heat stress in a local mapping population revealed candidate genes with diverse molecular functions.

Keywords: epistasis, GWA mapping, heat stress, local score, natural accessions, *Ralstonia solanacearum*, *SOLO DANCERS*

INTRODUCTION

Climate scenarios predict that extreme climate events will become more frequent by the end of the century (IPCC, 2018), alongside with an expected increase of global surface temperature from 1.5 to 4.8°C (IPCC, 2018). In such a context of climate warming, global food security is at risk with crop yields threatened by both the direct effect of increased temperature on plant development (Hatfield et al., 2011; Saidi et al., 2011; Bita and Geratz, 2013; Gray and Brady, 2016), and the indirect effect of increased temperature on the emergence of new pathogens and the number and severity of epidemics (Garett et al., 2006; Evans et al., 2008; Bebber et al., 2013; Bita and Gerats, 2013; Suzuki et al., 2014). Unraveling the genetic and molecular mechanisms allowing plants to face pathogen attacks under elevated temperature represents therefore a promising strategy for sustainable disease resistance.

Being sessile, plants have developed a wide range of immune responses to face simultaneous and/or sequential stresses caused by various bio-aggressors (Roux and Bergelson, 2016). Plant immunity relies on surveillance system involving plasma membrane-anchored pattern recognition receptors (PRRs) that perceive microbial elicitors, called pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). PRR-triggered immunity (PTI) is efficient against a broad spectrum of pathogens (Cook et al., 2015). Adapted pathogens such as phytopathogenic bacteria trigger susceptibility thanks to secreted virulence factors called effectors that can inhibit PTI and promote pathogen invasion (ETS for Effector-triggered susceptibility). The specific recognition of pathogen effectors by plant intracellular NLR (Nod-like Receptors) triggers a more robust immune response called effector-triggered immunity (ETI), often associated with a cell death response or hypersensitive response (HR) that restricts pathogen invasion to the infection site. ETI is specific to a single pathogenic species, and even to a single pathogenic strain. This specificity causes a strong selective pressure on virulent strains to bypass ETI, making this form of immunity not durable in crop field conditions (Roux et al., 2014). Another form of resistance represented by a reduction rather than an absence of disease refers to Quantitative Disease Resistance (QDR) (St Clair, 2010; Mundt, 2014; Roux et al., 2014; French et al., 2016). QDR is generally polygenic, durable and broad-spectrum (Young, 1996; Poland et al., 2009). Unlike PTI and ETI, molecular mechanisms underlying QDR remain largely unknown (Roux et al., 2014). Noteworthy is the alteration of all these major forms of immunity by temperature elevation. Numerous studies involving various pathosystems reported inhibition of ETI responses by a temperature increase (3-7°C) (de Jong et al. 2002; Xiao et al., 2003; Yang and Hua, 2004; Wang et al., 2009; Cheng et al., 2013; Menna et al., 2015; Aoun et al., 2017; Venkatesh & Kang, 2019).

Bacterial wilt, caused by the gram-negative bacteria *R. solanacearum,* is one of the most devastating bacterial diseases in the world. Indeed, this soil-borne pathogen affects more than 200 species including Solanaceae and Brassicaceae, and is responsible for dramatic yield losses not only in tropical and subtropical areas, but also in warm temperate regions (Elphinstone, 2005). In the model plant *Arabidopsis thaliana*, a broad-spectrum resistance response to *R. solanacearum* is conferred by the RPS4/RRS1-R locus that encodes for a pair of NLR receptors cooperating molecularly to form homodimers (Deslandes et al., 2002; Birker el al, 2009; Narusaka 2009, Williams et al., 2014). In addition, the LRR receptor-like kinase ERECTA was identified as gene underlying one of the three QTLs detected against the *R. solanacearum* 14.25 strain (Godiard et al., 2003)

The genetic architecture and the molecular mechanisms of plant responses to *R. solanacearum* in changing abiotic environments, and more particularly under elevated temperature conditions, remain elusive. Recently, a Genome-Wide Association Study (GWAS) performed in *A. thaliana* and aimed at exploring the genetic basis associated with the natural variation of plant response to GMI1000 strain at 30°C led to the identification of the *Strictosidine Synthase-Like protein 4* (*SSL4*) gene, although the underlying molecular mechanisms involved are still unknown (Aoun et al., 2017). This study was based on 176 natural accessions of *A. thaliana* from a worldwide collection. While being informative, a limitation of this mapping population-based approach resides in an increased effect of the demographic history on genotype-phenotype association at large geographical scales. Statistical methods controlling for confounding by population structure can reduce the inflation of false-positive associations, but to the detriment of a loss of detection power (i.e. causative genetic markers that are lost after correcting for population structure; Bergelson & Roux 2010, Brachi et al. 2010). In addition, due to the increased probability of the presence of rare alleles at large geographical scales, the power of GWAS can be strongly reduced by the effects of genetic and allelic heterogeneity (Bergelson & Roux 2010). To limit these drawbacks, GWA mapping can be combined with traditional linkage mapping (based on the use of experimental populations such as Recombinant Inbred Lines), which is prone for identifying rare alleles and not subjected to the effect of population structure (Bergelson & Roux 2010). Combining GWA mapping and traditional linkage mapping has been demonstrated to reduce the rates of false positives and negatives when applied to flowering time data in *A. thaliana* (Brachi et al. 2010), but remains time-consuming due to the need of phenotyping thousands experimental lines. To limit the drawbacks of GWA mapping performed at a worldwide scale, an alternative approach is to work at a small geographical scale (Bergelson & Roux 2010). As reported in a GWAS performed on flowering in *A. thaliana* from a worldwide to a local scale (by using two highly polymorphic French mapping populations), a great reduction of confounding by population structure was observed at the smaller geographical scales (Brachi et al. 2013). In addition, the genetic architecture was highly specific on the considered geographical scale (Brachi et al. 2013).

In the present study, we therefore investigated the genetic bases of QDR to *R. solanacearum* under elevated temperature by performing a GWAS at a small geographical scale using the TOU-A local mapping population. This local mapping population offers several advantages including (i) a high genetic diversity representing almost one-sixth of the genetic diversity observed at a worldwide scale, (ii) an extensive genetic variation for a large range of phenotypic traits including QDR to the bacterial vascular pathogen *Xanthomonas campestris* pv *campestris*, (iii) a linkage disequilibrium (LD) decay below 3kb allowing fine-mapping of genomic regions associated with phenotypic variation, (iv) a strongly reduced confounding effect by population structure, and (v) an adaptation to local warming in less than eight generations (Brachi et al. 2013, Huard-Chauveau et al. 2013, Baron et al. 2015, Debieu et al. 2016, Frachon et al. 2017).

Interestingly, this work revealed a genetic architecture of natural variation of QDR to *R. solanacearum* that totally differs from the one previously described at the worldwide scale (Aoun et al., 2017). In particular, at 30°C, we observed a playful dynamics of 12 QTLs along the disease symptom progression, with most QTLs displaying complex epistatic relationships. Using a reverse genetic approach, we identified *SOLO DANCERS* (*SDS*) encoding for a cyclin-like protein as the gene underlying one of the two additive QTLs detected at 30°C.

MATERIAL AND METHODS

1. Bacterial strain, plant material and growth conditions

The wild type *R. solanacearum* GMI1000 strain was grown on complete BG medium as previously described (Plener et al., 2010). GWAS was performed using 192 whole-genome sequenced natural accessions of the TOU-A population (France, Burgundy, 46° 38′ 57.302″ N, 4° 7′ 16.892″ E; Frachon et al., 2017) (Table S1). Around five seeds of each accession were directly sown on Jiffy pots (Jiffy Products International AS, Norway) and put for 48h at 4°C for stratification. Afterwards, plants were grown under controlled conditions for four weeks (22°C, 70% relative humidity (RH), 9h of light) before inoculation. The two homozygous *sds-2* and *sds-3* mutants (SAIL and FAG105 T-DNA insertion mutants in Columbia-0 (Col-0) and Wassilewskija (Ws-4) genetic backgrounds, respectively) were kindly provided by Raphaël Mercier (INRA of Versailles, France) (De muyt et al., 2009). An altered expression of *SDS* in these two mutants was confirmed in De muyt et al., 2009. The two null mutants were grown as described above.

2. Plant inoculation and phenotyping

Four-week-old plants were root-inoculated with the *R. solanacearum* GMI1000 strain. Before inoculation, roots were cut with scissors at 1 cm from the bottom of the Jiffy pot (Deslandes et al., 1998). This method gives the bacteria a direct access to the xylem vessels. During inoculation, plants were soaked in a bacterial suspension at 1.10^7 bacteria/mL for 15 min. Inoculated plants were incubated in growth chambers at 27°C or at 30°C (75% HR, 12h light, 100µmol $m^{-2}s^{-1}$). Disease symptom appearance was scored daily using a disease index scale from 0 to 4 as previously described (Deslandes et al., 1998), with the score 0 and 4 corresponding to healthy and fully wilted plants, respectively. Wilting symptoms were scored daily from 3 to 9 days after inoculation (dai).

3. Natural variation of QDR in the TOU-A population

Experimental design

For each temperature treatment, 624 plants were used and arranged by following a randomized complete block design (RCBD) of three temporal experimental blocks. Each block was represented by two trays of 104 positions, corresponding to one replicate per accession (n = 192 accessions) and the susceptible Col-0 accession was placed in the same three positions within each tray (n = 6). In each block, the remaining 10 positions in the trays were kept empty. Note that plants of the third block were not scored at 9 dai.

Statistical analyses

For each temperature treatment, a mixed model (MIXED procedure in SAS9.4; SAS Institute Inc., Cary, NC, USA) was used to explore the natural genetic variation of the disease index at each time point of phenotyping, as follow:

$$\text{disease index}_{ijc} = \mu + \text{block}_i + \text{accession}_j + \text{covCol}_c + \varepsilon_{ijc} \quad (1)$$

where μ is the overall mean of the phenotypic data, 'block' accounts for differences in micro-environmental conditions between the three experimental blocks; 'accession' corresponds to the genetic differences among the TOU-A natural accessions; covCol is a covariate accounting for tray effects within blocks (phenotypic mean of the three Col-0 replicates per tray was used as a covariate); and 'ε' is the residual term. The factor 'block' was considered as a fixed factor and the factor 'accession' as a random factor. Significance of the random effect was determined by likelihood ratio tests of model with and without this effect. Residuals were normally distributed so no transformation was applied on raw phenotypic data. For GWA mapping analyses, we used Best

Linear Unbiased Predictors (BLUPs) obtained for each natural accession. Because *A. thaliana* is a highly selfing species (Platt et al. 2010), BLUPs correspond to genotypic values. Using a formula adapted from Gallais (1990), broad-sense heritabilities (H^2) at each time point of phenotyping were estimated from the variance component estimates of the 'block' and 'accession' terms obtained with the VARCOMP procedure in SAS9.4 (SAS Institute Inc., Cary, NC, USA).

GWA mapping with local score analysis

To fine map the genomic regions associated with natural disease index variation at each time of phenotyping for each temperature treatment, a mixed model implemented in the software EMMAX was adopted [Efficient Mixed-Model Association eXpedited; (Kang et al., 2010)]. To control for the effect of population structure, we included as a covariate an identity-by-state kinship matrix K based on the 1,902,592 SNPs identified in the TOU-A population (Frachon et al., 2017). Because rare alleles increase the rate of false positives when included in mixed models, we considered a threshold of minor allele relative frequency (MARF) > 7% and ended up with 981,617 SNPs (Brachi et al., 2010; Kang et al., 2010).

In order to increase the probability to discover minor effect QTLs conferring QDR to *R. solanacearum* along the infection stages, we implemented a local score approach on the set of p-values provided by EMMAX. The local score allows detecting significant genomic segments by accumulating the statistical signals from contiguous markers such as SNPs (Fariello et al., 2017). In a given QTL region, the association signal, through the p-values, will cumulate locally due to LD between SNPs, which will then increase the local score (Bonhomme et al. 2019). Briefly, a sequence of scores is calculated along the chromosome as $X_i = -\log_{10}(p_i) - \xi$, where p_i is the p-value of marker i and ξ a tuning parameter with an optimal value that can be fixed at 2 or 3 in a

GWAS context (Bonhomme et al., 2019). Then, finding segments that accumulate strong signals is equivalent to finding peaks along a Lindley process defined as $h_i = \max(0, h_{i-1} + X_i)$ along the chromosome, with $h_0 = 0$. Significant SNP-phenotype associations was identified by estimating a chromosome-wide significance threshold for each chromosome (Bonhomme et al., 2019).

Detecting QTL epistasis

In order to detect epistatic interactions among our set of 14 candidate QTLs, we first identified within each QTL region the SNP with the highest association score estimated by EMMAX, hereafter named bait top SNP. For each of the 14 QTLs, we then computed LD estimates between the bait top SNP and all the other SNPs in the TOU-A population. LD between two bi-allelic (homozygous) SNPs was calculated using the absolute value of r statistic (correlation coefficient) between two SNP genotype vectors. For each QTL, we obtained a distribution of LD estimates between the bait top SNP and the other 981,616 SNPs of the population (MARF > 7%). In order to exclude strong LD values due to physical proximity, SNPs located in a 100kb window surrounding a bait top SNP were not included in the calculation. To estimate whether the bait top SNP of a given QTL (i.e. focal bait top SNP) was significantly in LD with the bait top SNPs of the remaining 13 QTLs, we estimated in the LD distribution (conditional on each focal bait top SNP) the quantile q for each bait top SNP of the 13 QTLs. A LD estimate between a focal bait top SNP and another bait top SNP was declared significant if $(1 - q) < 0.01$.

Estimates of allelic effect

To display the allelic effect of the bait SNPs, BLUPs estimated from model (1) were adjusted by fitting them with a kinship matrix. Kinship adjusted BLUPs were computed under the *R* environment 3.6.1 (R_Core_Team, 2019). In order to avoid pseudo-replication due to the presence of SNPs in stretches of LD, we first pruned the SNP data set with the snpgdspLD command using the following parameters 'ld.threshold = 0.8, slide.max.bp=500, maf =0.07 ('gdsfmt' and 'SNPRelate' packages), leaving 365,952 SNPs for the estimation of the kinship matrix. The kinship matrix was then estimated using the popkin function (allowing missing data in the SNP matrix) in the popkin package, with the subpopulation vector set to NULL. Because the resulting matrix was not positive semi definite, the function make.positive.definite() from the package lqmm was used. Finally, the kinship adjusted BLUPs were calculated with the function kin.blup from package rrBLUP. Keeping the notations from model (1), the parameters were: accession as geno, disease index as pheno, the above mentioned kinship matrix as K, GAUSS=F indicating that the genotypes are not independent and follow $G = K\ V_G$, block as fixed effect and covCol as covariate. The kinship adjusted BLUPs were then extracted using the command $pred.

4. Analysis of the *SDS* candidate gene

For each temperature treatment, an experiment of 288 plants was set up according to a RCBD of three temporal experimental blocks. Each block was represented by one tray of 96 positions, corresponding to 24 replicates of each genotype, i.e. the *sds-2* and *sds-3* mutants with their corresponding wild-type background Col-0 and Ws-4, respectively.

For each temperature treatment, we tested whether each null mutant differs from its corresponding wild-type background along the infection stages, by using the following mixed model (MIXED procedure in SAS9.4; SAS Institute Inc., Cary, NC, USA):

$$\text{disease index}_{ij} = \mu + \text{block}_i + \text{genotype}_j + \text{block}_i \text{ x } \text{genotype}_j + \varepsilon_{ij} \; (2)$$

where μ is the overall mean of the phenotypic data, 'block' accounts for differences in micro-environmental conditions between the three experimental blocks; 'genotype' corresponds to the genetic differences between the T-DNA mutant and its corresponding wild-type background; 'block x genotype' accounts for variation in between genotype differences among blocks; and 'ε' is the residual term. All factors were considered as fixed.

RESULTS

Impact of temperature on genetic variation for QDR to *R. solanacearum* among local *A. thaliana* accessions

No germination was observed for six accessions that were therefore discarded from the study (Table S1). The remaining 186 local accessions from the TOU-A population challenged with the GMI1000 strain were on average more susceptible at 30°C than at 27°C (Fig.1a, 1b). For each temperature treatment, we observed a large genetic variation at most infection stages, i.e. 5, 6 and 7 dai at 27 °C and 4, 5, 6 and 7 dai at 30°C (Table 1, Fig. 1), with broad-sense heritability estimates ranging from 0.34 to 0.41 at 27°C and from 0.29 to 0.39 at 30°C (Table 1). Based on genotypic values estimated for the 186 TOU-A accessions, cross-temperature genetic correlation was weak, albeit significant (5 dai: Pearson's $r = 0.23$, $P = 0.003$, 6 dai: Pearson's $r = 0.16$, $P = 0.033$, 7 dai: Pearson's $r = 0.20$, $P = 0.008$; Fig. 1c), suggesting a flexible genetic architecture of *A. thaliana.* response to the GMI1000 strain between 27°C and 30°C.

Table 1. Natural variation among TOU-A natural accessions for disease index at 27°C and 30°C.

		symptoms 3dpi		symptoms 4dpi		symptoms 5dpi		symptoms 6dpi		symptoms 7dpi		symptoms 9dpi	
Temperature	**Model terms**	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***	***F* or LRT**	***P***
27°C	Block	0.7	0.5262	1.7	0.2049	6.3	0.0036	36.4	0.0002	58.4	0.0002	50.9	0.0002
	Accession	0.0	1.0000	2.9	0.1076	9.0	0.0042	14.3	0.0004	7.9	0.0069	3.3	0.0906
	Control Col-0	-	-	54.8	0.0002	105.9	0.0002	122.8	0.0002	110.0	0.0002	87.1	0.0002
	H^2	0.00^{ns}		0.21^{ns}		0.35**		0.41***		0.34**		0.37^{ns}	
30°C	Block	3.7	0.0382	20.9	0.0004	18.2	0.0004	9.0	0.0004	5.8	0.0064	4.2	0.0602
	Accession	1.4	0.2874	9.5	0.0051	18.4	0.0004	8.7	0.0064	12.4	0.0014	0.2	0.6956
	Control Col-0	0.1	0.7862	12.2	0.0014	6.8	0.0158	0.4	0.5967	2.4	0.1595	-	-
	H^2	0.13^{ns}		0.29**		0.39***		0.3**		0.37**		0.11^{ns}	

F, *F* value resulting from the test of fixed effect. LRT, LRT value resulting from the likelihood ratio test. H^2, broad-sense heritability values. Italic terms indicate random effects. ns, non-significant; $^{**}P < 0.01$; $^{***}P < 0.001$.
ne: not estimated due to the absence of variation in disease symptoms among Col-0 control plants.

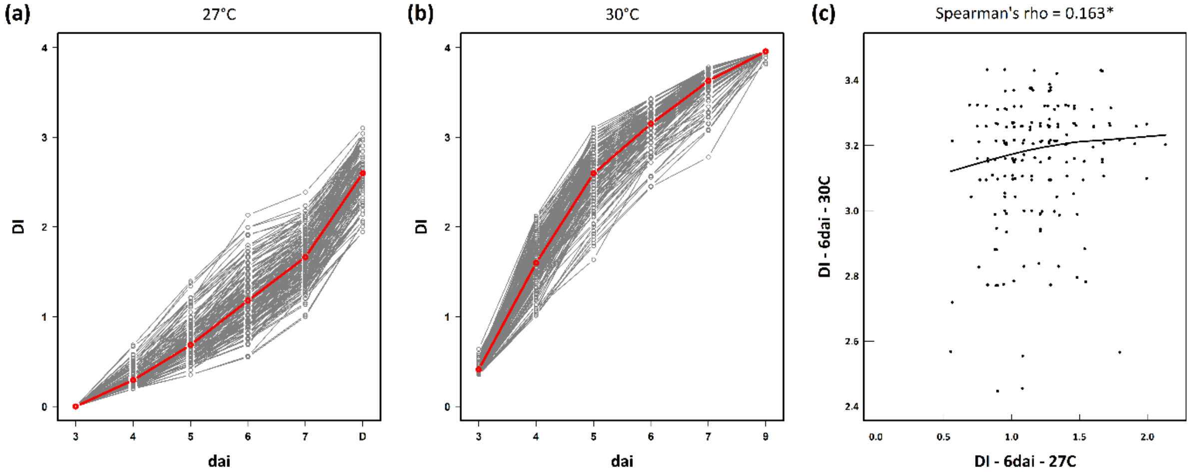

Figure 1. Genetic diversity of plant response to *R. solanacearum* GMI1000 strain in the local TOU-A mapping population. (**a**) Genetic variation of response dynamics at 27°C. (**b**) Genetic variation of response dynamics at 30°C. The red line represents the mean of disease index over all the accessions in (**a**) and (**b**). (**c**) Relationship between disease index at 6dai scored at 27°C and 30°C. dai, days after inoculation. DI, disease index.

Playful dynamics of QTLs a 27°C and 30°C

Using a genome-wide local score analysis (with tuning parameter $\xi = 2$), we detected over the kinetic of infection 215 and 738 significant unique SNPs (i.e. top SNPs) at 27°C and 30°C, respectively (Fig. 2). In agreement with weak cross-temperature genetic correlation, no single top SNP was common to both temperatures, indicating a contrasted genetic architecture for natural variation of response to *R. solanacearum* GMI1000 between 27°C and 30°C. Next, we focused on the 14 most highly significant QTLs (i.e. top QTL with a Lindley process > 10), each explaining at least 11.9% of phenotypic variation (Fig. 2, 3, Fig. S1). Two top QTLs were detected at 27°C while the remaining top QTLs were detected at 30°C (Fig. 2). Interestingly, all these top QTLs displayed playful dynamics, with two QTLs (i.e. QTL1 at 27°C and QTL3 at 30°C) and 12 QTLs showing a decrease and increase in significance with advanced infection stages, respectively (Fig. 2).

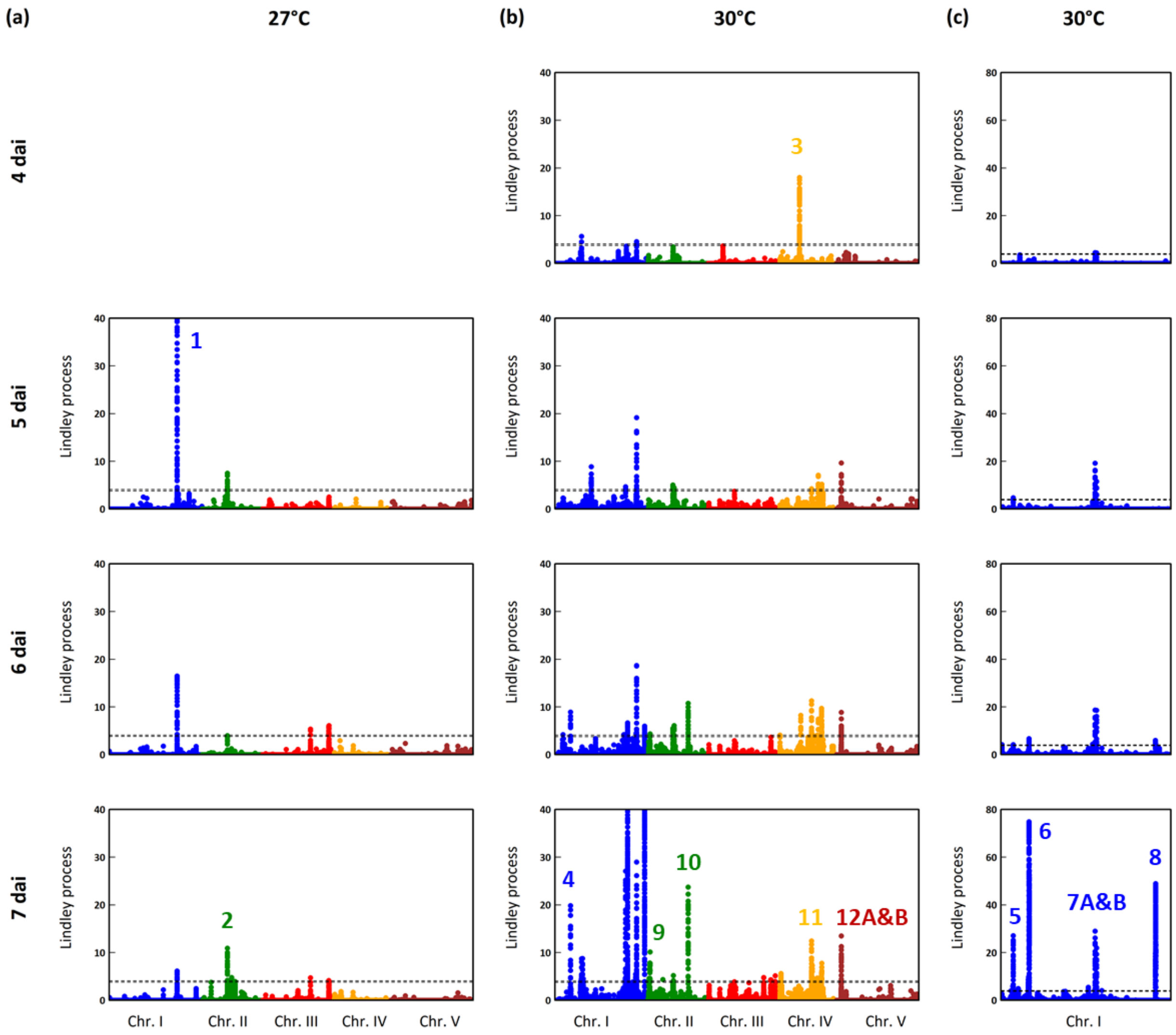

Figure 2. The genetics of quantitative disease resistance to *R. solanacearum* GMI1000 strain in the TOU-A population. (**a**) Manhattan plot of the Lindley process (local score method with a tuning parameter ξ = 2) at 5, 6 and 7 dai and 27°C. (**b**) Manhattan plot of the Lindley process (local score method with a tuning parameter ξ = 2) at 4, 5, 6 and 7 dai and 30°C. (**c**) Zoom spanning a genomic region at the end of chromosome I from 23Mb to 29.3Mb containing five QTLs. The two horizontal dashed lines indicate the minimum and maximum of the five chromosome-wide significance thresholds. To better highlight minor QTLs in (**a**) and (**b**), Lindley process values on the y-axis range from 0 to 40. Note that for the main association peak detected on chromosome 1 at 5 dai and 27°C, the highest local score value of is 58.6. The number close to association peaks correspond to the 14 QTLs with a Lindley process value above 10. '7A&B' corresponds to two QTLs on chromosome 1 separated by ~63.5kb. '12A&B' corresponds to two QTLs on chromosome 5 separated by ~27.1kb.

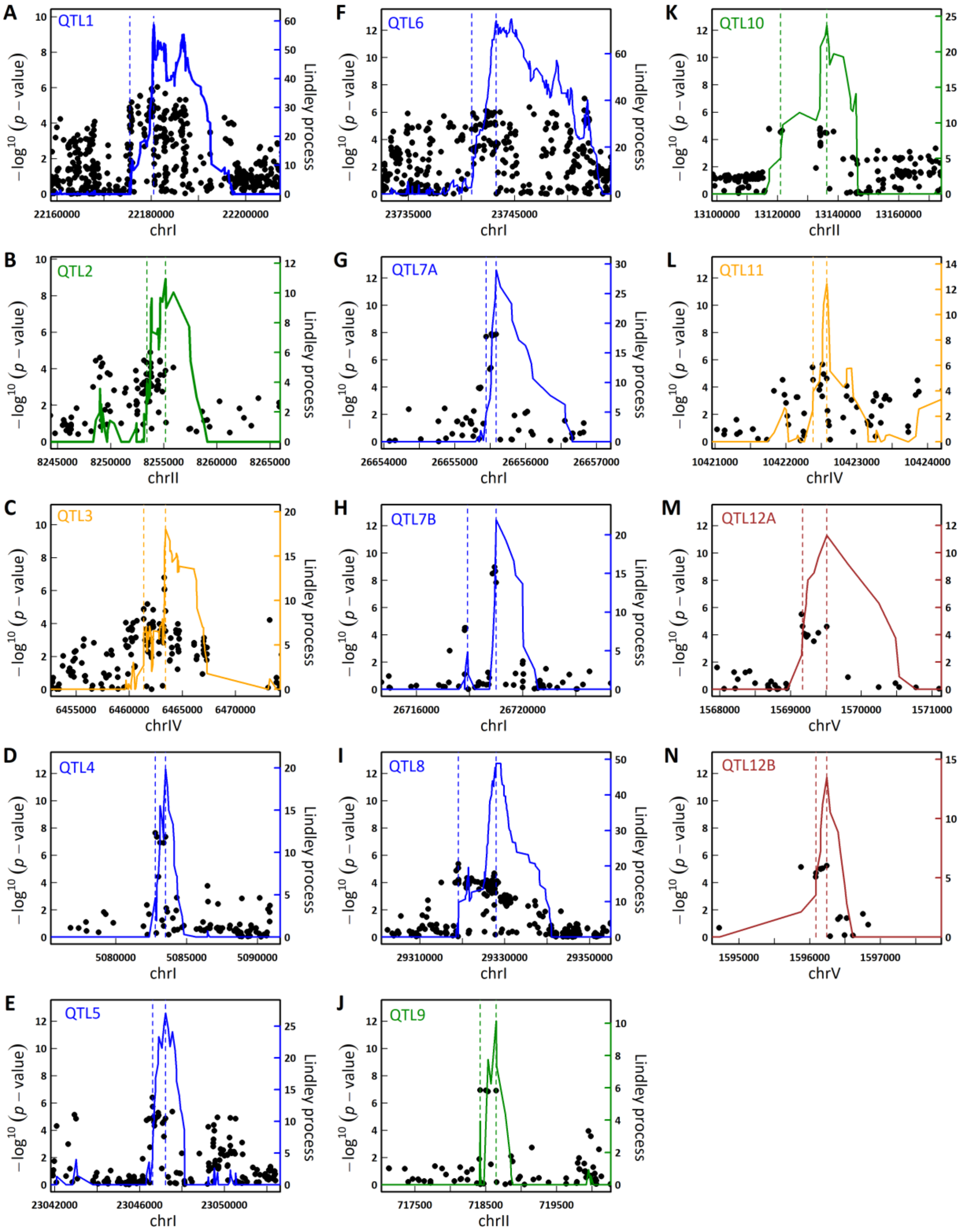

Figure 3. Zoom spanning the 14 QTLs with a Lindley process value above 10. Each of the 14 QTLs highlighted in Figure 2 are depicted from (**a**) to (**n**). The *x*-axis corresponds to the physical position of the SNPs. The dots correspond to the $-\log_{10}$ *p*-values of the SNPs obtained with the mixed model implemented in the EMMAX software (*y*-axis on the left). The solid colored curve indicates the Lindley process (local score method with $\xi = 2$) calculated from left to right (*y*-axis on the right). The two colored dashed vertical lines indicate the QTL intervals detected, without taking into account the right part of the curve (Fariello et al., 2017; Bonhomme et al., 2019).

Based on LD calculation, both QTLs detected at 27°C only present additive (i.e. independent) effects (Fig 4, Fig. S2). By contrast, at 30°C, nine out of the 12 top QTLs displayed epistatic interactions (Fig 4, Fig. S2) with the identification of three groups of epistatic QTLs (Table 2). The first one regroups five QTLs (QTL5 + QTL6 + QTL7A + QTL7B + QTL 9) with highly significant pairwise LD values ($P < 0.001$), in particular at the interchromosomal level (Table 2**,** Fig. 4). Based on the representative SNPs of the five QTLs, plants with the AACTC haplotype were more resistant (disease index = 2.98) than the plants with the GCTCG haplotype (disease index = 3.67) (Fig. S1). The second and third groups contain QTL10 + QTL11 and QTL12A + QTL12B, respectively (Table 2, Fig. S1). No significant epistatic relationship was detected for the three remaining QTLs detected at 30°C (i.e. QTL3, QTL4 and QTL8) (Fig. S2).

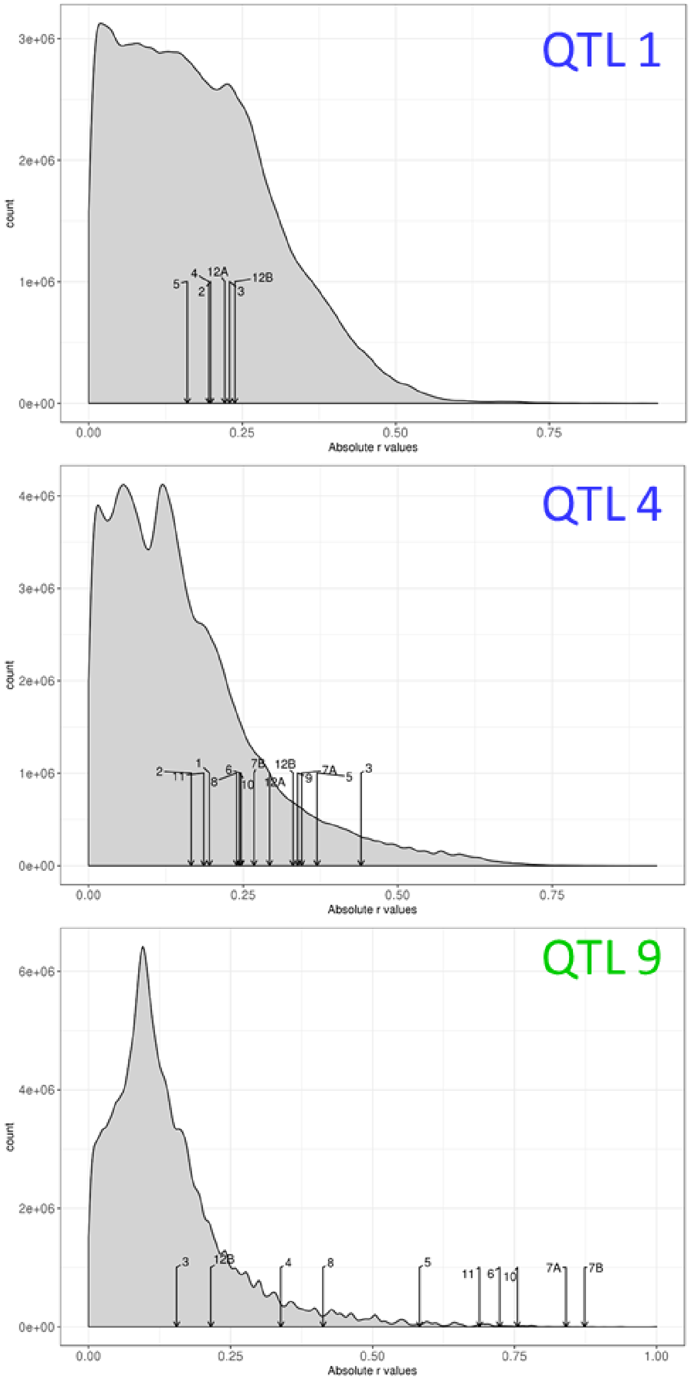

Figure 4. Detection of inter-QTLs epistasis for QTL1 (a), QTL4 (b) and QTL9 (c). For each QTL, a null distribution was established by calculating LD values (i.e. *r* correlation coefficient) between the bait top SNP and all the other SNPs across the genome (with the exception of the SNPs located within a 50kb window on each side of the bait top SNP). Only SNPs with a MARF > 0.07 were considered. In addition, LD values (above 0.1) between the bait top SNP for the corresponding QTL and the bait top SNPs from the other QTLs are represented by arrows. The *x*-axis corresponds to the LD estimates expressed in absolute values.

Table 2. Linkage Disequilibrium matrix among the 14 QTLs with a Lindley process value above 10.

QTL2	-0.20												
QTL3	-0.23	0.04											
QTL4	-0.20	0.17	0.44										
QTL5	0.16	0.08	0.17	0.37									
QTL6	0.07	0.05	0.14	0.25	**0.70**								
QTL7A	-0.01	0.00	0.14	0.34	**0.53**	**0.73**							
QTL7B	0.04	-0.05	0.12	0.27	**0.51**	**0.67**	**0.96**						
QTL8	0.05	-0.15	0.24	0.24	**0.39**	0.28	0.30	0.30					
QTL9	-0.01	0.04	0.15	0.34	**0.58**	**0.72**	**0.84**	**0.87**	**0.41**				
QTL10	-0.06	0.25	0.28	0.24	**0.60**	**0.40**	**0.48**	**0.47**	0.25	**0.75**			
QTL11	-0.06	0.06	0.15	0.19	**0.45**	**0.52**	**0.55**	**0.51**	0.26	**0.69**	**0.64**		
QTL12A	-0.22	-0.13	0.21	0.29	-0.10	-0.10	0.00	0.01	0.10	-0.07	-0.08	-0.08	
QTL12B	-0.24	-0.08	0.29	0.33	0.11	0.03	0.12	0.08	0.18	0.22	0.11	0.03	**0.89**
	QTL1	QTL2	QTL3	QTL4	QTL5	QTL6	QTL7A	QTL7B	QTL8	QTL9	QTL10	QTL11	QTL12A

LD values correspond to *r* correlation coefficients among the 14 top SNPs. Blue values: $P < 0.01$, red values: $P < 0.001$. The bait top SNPs are SNP-1-22180112 (QTL1), SNP-2-8253743 (QTL2), SNP-4-6463310 (QTL3), SNP-1-5082790 (QTL4), SNP-1-23021261 (QTL5), SNP-1-23742319 (QTL6), SNP- 1-26655520 (QTL7A), SNP-1-26718947 (QTL7B), SNP-1-29319094 (QTL8), SNP-2-718424 (QTL9), SNP-2-13134129 (QTL10), SNP-4-10422518 (QTL11), SNP- 5-1569170 (QTL12A) and SNP-5-1596241 (QTL12B).

Molecular functions of the candidate gene products underlying the QTLs identified

In agreement with the LD decay below 3kb observed in the TOU-A population, the average size of the 14 QTLs was 4,589bp (min = 141 bp, max = 26.07kb, Fig. 3), thereby limiting the number of candidate genes underlying each QTL. The AGI locus code and the corresponding predicted molecular function(s) of the candidate genes is indicated in Table 3. At 27°C, the top SNP of QTL1 (SNP-1-22180112) is located in the coding region of *TREHALOSE PHOSPHATE SYNTHASE 10* (*At1g60140*) (Fig. 3a). QTL2 covers a short region of ~ 1.77kb, with the top SNP SNP-2-8253743 located in a gene (*At2g19050*) belonging to a GDSL-like Lipase/Acylhydrolase protein superfamily (Fig. 3b).

At 30°C, the candidate genes underlying the 12 top QTLs encode for various molecular functions. Functional classification was performed with the classification superviewer tool on the university of Toronto website (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi) using the MAPMAN classification as source (Provart and Zhu, 2003) and the list of genes in which top SNPs were falling in or in the vicinity. In particular, two QTLs correspond to genes involved in abiotic stress signaling pathways, i.e. QTL3 with the top SNP SNP-4-6463310 and QTL7B with the top SNP SNP-1-26718947 falling within the *At4g10450* and *At1g70860* encoding for a Ribosomal protein L6 family and a cytokinin responsive lipid transport protein, respectively (Fig. 3c, 3h; Table 3). QTL4 covers a small region of 730 bp, with the top SNP SNP-1-5082790 falling within the promoter region of *SOLO DANCERS (At1g14750)* that encodes for an atypical meiotic cyclin-like protein (Fig. **3d**; Table **3**). The top SNPs SNP-1-29319094 and SNP-4-10422518 of QTL8 and QTL11 were respectively located in the genomic region of *At1g77990* (*SULPHATE TRANSPORTER 2;2*) and *At4g19030* (*NOD26-LIKE INTRINSIC PROTEN 1,1*), encoding for a sulfate and an aquaporin transporter, respectively (Fig. 3i, 3l; Table 3). QTL12A and QTL12B with the top SNP SNP-5-1569170 and SNP-5-1596241 are located within genes encoding for an EXPANSIN 2 (*At5g05290*) involved in cell wall modification and for a lignin peroxidase (*At5g05390*) involved in vascular development, respectively (Fig. 3m; 3n; Table 3). However, among the different biological pathways represented by the 18 candidate genes identified, only the hormonal metabolism was significantly over-represented ($P < 0.01$). For instance, the top SNPs of QTL7A (SNP-1-26655520) and QTL10 (SNP-2-13134129) are located in genes *At1g70700* (*JAZ9*) and *At2g30830* (or alongside *At2g30810*) that are involved in jasmonate (JA), ethylene (ET) and gibberellins (GAs) hormonal metabolisms, respectively (Fig. 3g, 3h, 3k; Table 3).

Table 3. List of candidate genes underlying the 14 QTLs with a Lindley process value above 10 at 27°C and 30°C. For each QTL, the candidate gene corresponding to the top SNP and the flanking gene are in bold and normal text, respectively.

Temperature (°C)	dai	QTL id	Chromosome	Bait SNP	*P* values	Genes	Genes description
27	5	QTL1	1	22 180 112	1.20 x 10^{-6}	**AT1G60140**	**TREHALOSE PHOSPHATE SYNTHASE 10**
	7	QTL2	2	8 253 743	1.27 x 10^{-5}	**AT2G19050**	**GDSL-like Lipase/Acylhydrolase superfamily protein**
30	4	QTL3	4	6 463 310	1.63 x 10^{-7}	AT4G10440	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein
						AT4G10450	**Ribosomal protein L6 family**
	7	QTL4	1	5 082 790	2.30 x 10^{-8}	**AT1G14750**	**SOLO DANCERS**
	7	QTL5	1	23 021 261	2.28 x 10^{-8}	AT1G62305	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
						AT1G62330	O-fucosyltransferase family protein
	7	QTL6	1	23 742 319	8.08 x 10^{-7}	**AT1G63980**	**D111/G-patch domain-containing protein**
	7	QTL7A	1	26 655 520	1.31 x 10^{-7}	**AT1G70700**	**JASMONATE-ZIM-DOMAIN PROTEIN 9**
	7	QTL7B	1	26 718 947	1.07 x 10^{-9}	**AT1G70860**	**Polyketide cyclase/dehydrase and lipid transport superfamily protein**
	7	QTL8	1	29 319 094	4.28 x 10^{-6}	**AT1G77990**	**SULPHATE TRANSPORTER 2**
	7	QTL9	2	718 424	1.15x 10^{-7}	**AT2G02620**	**Cysteine/Histidine-rich C1 domain family protein**
	7	QTL10	2	13 134 129	1.51x 10^{-5}	AT2G30800	HELICASE IN VASCULAR TISSUE AND TAPETUM
						AT2G30810	Gibberellin-regulated family protein
						AT2G30820	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit
						AT2G30830	**2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein**
						AT2G30840	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein
	7	QTL11	4	10 422 518	2.21 x 10-6	**AT4G19030**	**NOD26-like intrinsic protein 1**
	7	QTL12A	5	1 569 170	2.41 x 10-5	**AT5G05290**	**EXPANSIN A2**
	7	QTL12B	5	1 596 241	5.93 x 10-6	**AT5G05390**	**LACCASE 12**

SOLO DANCERS is the gene underlying QTL4 involved QDR to *R. solanacearum* GMI1000 strain at 30°C

Next, we investigated the molecular mechanisms underlying plant response to *R. solanacearum* at 30°C in the TOU-A population. For this, we focused on the additive QTL with the highest allelic effect, that is QTL4 located on the top of chromosome I and that encompasses the *SOLO DANCERS* locus (*SDS*, *At1g14750*) (Fig. 2b, Fig. S1). To check whether *SDS* was involved in this QDR, we monitored at 27°C and 30°C the phenotypical response of *sds-2* and *sds-3* null mutants, (in Col-0 and Ws-4 genetic background, respectively). Col-0 and Ws-4 are both susceptible to GMI1000 at 30°C while Ws-4, but not Col-0, is resistant at 27°C. At 27°C, the *sds-2* mutant response was not significantly different from Col-0 except at 6 dai (Fig. 5a; Table s3). At 30°C, the wilting of *sds-2* was significantly delayed compared to that of Col-0 from 3 dai to 5 dai (Fig. 5b; Table s3). As expected, *sds-3* mutant and Ws-4 plants remained symptomless at 27°C, from 3 dai to 7 dai (Fig. 5c; Table s3). By contrast, at 30°C, wilting of *sds-3* plants was strongly reduced during all infection stages (Fig. 5d; Table s3). Altogether, these data suggest that *SDS* plays a role in wilting disease development upon infection with the *R. solanacearum* GMI1000 strain at 30°C.

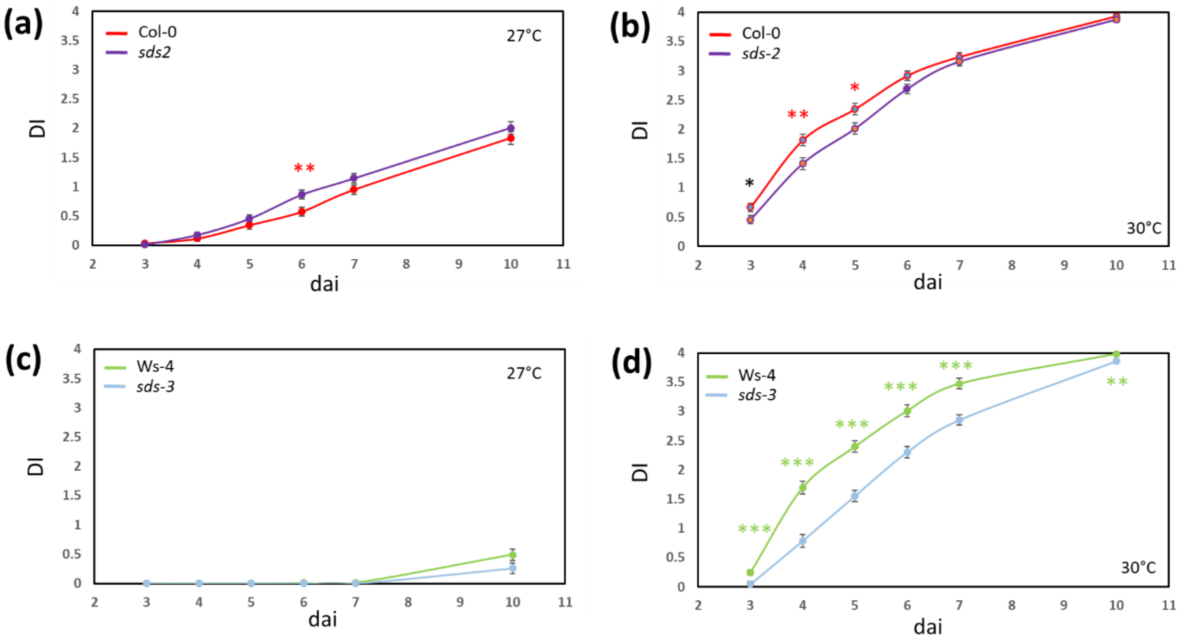

Figure 5. Effects of knock-down of *SDS* expression on the dynamics of disease symptoms after inoculation with the *R. solanacearum* GMI1000 strain in two wild-type genetic backgrounds at 27°C and 30°C. Dynamics of disease symptoms in Col-0 and *sds-2* mutant at 27°C (**a**) and 30°C (**b**). Dynamics of disease symptoms in Ws-4 and *sds-3* mutant at 27°C (**c**) and 30°C (**d**). Least-square means ± SE of the LS means from three independent inoculations (n = 72 plants per 'genetic line * temperature' combination. Symbols *, **, and *** denote significant difference observed between each wild-type background and its corresponding mutant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ respectively. Colored stars indicate significant differences after a False-Discovery Rate correction. DI: disease index, dai: days after inoculation.

DISCUSSION

Worldwide *vs* local genetic variation in *A. thaliana* facing *R. solanacearum*

In comparison with a temperature of 27°C, local Arabidopsis accessions exposed at 30°C were on average more susceptible to the GMI1000 strain as indicated with a faster wilting disease progression than that observed at 27°C. This observation is in line with the drastic impact of temperature elevation on Arabidopsis response to the GMI1000 strain previously monitored in a worldwide collection of *A. thaliana* (Aoun et al., 2017). This is in accordance with studies performed on crops that have described the drastic impact of temperature elevation on resistance response (Moury et al., 1998; Wang et al., 2009; Onaga et al., 2012; Negeri et al., 2013).

In previous studies, the level of genetic variation for diverse phenotypic traits such as flowering time and QDR to the bacterial pathogen *X. campestris* was similar between the TOU-A population and a set of worldwide accessions (Brachi et al. 2013; Huard-Chauveau et al. 2013; Debieu et al. 2016). Here, the level of genetic variation for QDR to *R. solanacearum* in the TOU-A population was limited compared to that of the worldwide collection (Aoun et al., 2017). This may be explained by the absence in the TOU-A population of fully resistant accessions at 27°C, which is consistent with the absence of any association peak located around the *RPS4/RRS1-R* locus on chromosome V (Fig. 2). By contrast, this locus was detected as the major association peak at 27°C in a set of worldwide accessions (Aoun et al., 2017).

Given that most of the *A. thaliana* natural populations located in France are genetically diverse (Le Corre, 2005; Platt et al., 2010; Brachi et al., 2013; Frachon et al., 2018; Frachon et al., 2019), it would be interesting to investigate the level of genetic variation of QDR to *R. solanacearum* GMI1000 strain (or to other strains) within those populations. This would provide valuable information on the local dynamics of QDR to a bacterial pathogen in *A.*

thaliana at the metapopulation level (Ding et al., 2007; Vetter et al., 2012; Karasov et al., 2014; Roux and Bergelson, 2016).

Complex genetic architecture of QDR to *R. solanacearum* at 27°C and 30°C

Combining GWA mapping related mixed models and genome-wide local score analysis increases the probability to discover minor effect QTLs (Fariello et al., 2017; Bonhomme et al., 2019). This is also well exemplified here with a more detailed characterization of the genetic determinants responsible for QDR to *R. solanacearum*. Since no top SNPs were common to both temperature treatments, our data illustrate how only a weak temperature increase of 3°C can drastically affect the genetic architecture of QDR to *R. solanacearum*. We next evaluated the effect of the geographic scale on the genetic architecture of this QDR, by applying a genome-wide local score approach to the EMMAX results previously obtained on a set of 176 worldwide *A. thaliana* accessions (Aoun et al., 2017). No top SNPs were shared between the local and worldwide scale (Data S1). Interestingly, similar results were obtained in a study investigating the genetic determinants of flowering time scored on both local and worldwide mapping populations of *A. thaliana,* in two environmental conditions simulating two seasonal germination cohorts (Brachi et al., 2013). The genetic architecture of flowering was highly dependent upon both the geographical scale and the considered season (Brachi et al., 2013). Together, data obtained from various phenotypic traits reinforce the need to account for the geographical scale of phenotypic variation when choosing accession panels for GWAS (Bergelson and Roux, 2010). Consequently, this would help to get a better view of the genetic architecture flexibility of phenotypic traits.

Theoretical predictions suggest that phenotypic changes in ontogenetic time (typically time-to-event or time-to-failure traits such as flowering time or death time) are often driven by the temporal regulation of QTLs (Johannes et al., 2007). In this study, all the 14 top QTLs control QDR to *R. solanacearum* GMI1000 strain in a playful manner at both 27°C and 30°C, suggesting that disease progression to *R. solanacearum* highly depends on the time specificity of the genetic effects. At 30°C, disease progression resulted also from a complex genetic network of interacting loci. Epistatic networks involving long-distance LD among physically unlinked loci was reported to represent the main fraction of phenotypic variance for herbivore resistance in *A. thaliana* at a worldwide scale (Brachi et al., 2015) and body weight in chicken (Carlborg et al., 2006). While complex epistatic relationships among QTLs may be therefore more frequent than anticipated, the functional validation of epistatic QTLs remains challenging but feasible if we consider a multi-CRISPR-Cas9 approach to create double, triple, quadruple, etc. mutants. Nonetheless, it would be interesting to determine whether such an epistatic network is restricted to the TOU-A population by estimating LD between these five QTLs in other local highly polymorphic populations or at a larger geographical scale.

Various molecular functions are involved in QDR to the GMI1000 strain at 30°C

Consistent with the molecular functions of previously cloned QDR genes (Roux et al. 2014), the nature of most candidate genes underlying the 14 major QTLs identified here is quite diverse and they do not correspond to typical resistance genes encoding for NLRs. Indeed, unlike a previous GWAS performed on worldwide *A. thaliana* accessions that led to the detection of the *RPS4/RRS1-R* NLR locus as the main genetic determinant for full resistance to GMI1000 at 27°C (Aoun et al., 2017), the two main QTLs identified at 27°C in the local TOU-

A population do not correspond to any *NLR* genes. For QTL1, the top SNP fall in *TREHALOSE PHOSPHATE SYNTHASE 10* (*At1g60140*), suggesting that the regulation of trehalose-6-phosphate synthesis participates in the plant response. This is consistent with previous data showing that the production of this metabolite by the *R. solanacearum* effector RipTPS plays an important role in pathogen virulence (Poueymiro et al., 2009). For QTL2, the top SNP fall in the *At2g19050* gene encoding a GDSL-like Lipase/Acylhydrolase superfamily protein. Interestingly, overexpression of *GLIP1* that also belongs to the Arabidopsis *GDSL LIPASE-LIKE* gene family was shown to confer enhanced resistance to several pathogens including *Alternaria brassicicola*, *Erwinia carotovora* and *Pseudomonas syringae* (*Pst*) (Kwon et al., 2009). Therefore, these proteins might also play a role in plant immunity against *R. solanacearum*.

The molecular functions of the candidate genes underlying the 12 major QTLs detected at 30°C are even more diverse. Interestingly, these functions may reflect different plant responses to face virulence strategies developed by the bacteria to colonize plant tissues and promote its multiplication within the xylem vessels. For instance, candidate genes underlying QTL7A and QTL10 are involved in the synthesis or signaling of hormones that may contribute positively to pathogen resistance and in plant response to combined biotic and abiotic stress. In particular, JA is known to interfere with GAs signaling through the degradation of transcriptional repressors such as JAZ9 (the candidate gene underlying QTL7A*)* to balance plant defense response and growth (Yang et al., 2012).

From 4 until 7 dai, QTL4 was detected with increasing significance on chromosome I. The corresponding candidate gene, *SDS*, encodes an atypical meiotic cyclin-like protein related to A and B-type cyclins, previously described as being required for DNA double-strand break (DSB) repair (Azumi et al., 2002; De Muyt et al., 2009). To our knowledge, *SDS* has never been associated with plant disease susceptibility. Interestingly, two allelic null *sds* mutants were found to be more resistant at both 27°C and 30°C to GMI1000, albeit the allelic effect was different between the two genetic backgrounds. The functional validation of *SDS* as a susceptibility gene represents the first demonstration of its involvement in plant defense response to a bacterial pathogen under heat stress. It is noteworthy that (i) SDS acts together with CYCB3;1 in suppressing unscheduled cell wall synthesis (Bulankova et al., 2013), and (ii) the two candidate genes underlying QTL12A and QTL12B encode respectively proteins involved in cell wall and lignin polymerization. Two cyclin-L type proteins, MOS12 (Modifier of SNC1, 12) and MOS4-associated complex (Modifier of SNC1, 4) have also been shown to participate in the alternative splicing of *SNC1* and *RPS4* genes, thereby enabling the fine-tuning of *NLR* gene expression (Xu et al., 2012). As several *NLR* genes have been described to be alternatively spliced without knowing the regulatory mechanism (Xu et al., 2012), it is tempting to hypothesize that SDS would participate in the regulation of NLR functions under combined *R. solanacearum* and elevated temperature conditions through the production of splicing variants. Because the top SNPs are located in the promoter region of *SDS*, the next step to decipher the underlying molecular mechanisms would be to investigate the natural variation of *SDS* expression in the TOU-A population and its link to the QDR.

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ACKNOWLEDGEMENTS

We are grateful to Raphaël Mercier for providing T-DNA insertion mutants seeds (INRA – Versailles, France).

AUTHOR CONTRIBUTION

R.B. and F.R. supervised the project. N.A., R.B and F.R. designed the experiments. N.A. conducted the phenotyping experiments. N.A. and F.R. analyzed the phenotypic traits. F.R. performed the GWA mapping. M.B. performed the genome-wide local score analysis. L.B. performed the LD analyses. H.D. estimated the allelic effects of the QTLs. N.A., L.D., R.B. and F.R. wrote the manuscript. All authors contributed to the revisions.

Supplementary information

Natural variation of *Arabidopsis thaliana* quantitative disease response to *Ralstonia solanacearum* is controlled by a complex genetic architecture involving additive and epistatic QTLs

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Number of supplemental tables: 2

Number of supplemental figures: 2

Table S1. List of the 192 natural accessions of the local TOU-A mapping population used in this study. This list shows the ecotype ID and name of the accessions, as well as their germination status at 27°C and 30°C.

Ecotype ID	Name	27°C	30°C	Ecotype ID	Name	27°C	30°C
71001	TOU-A1-10	✓	✓	71099	TOU-A6-115	✓	✓
71003	TOU-A1-106	✓	✓	71100	TOU-A6-116	✓	✓
71004	TOU-A1-109	✓	✓	71101	TOU-A6-117	✓	✓
71005	TOU-A1-110	✓	✓	71102	TOU-A6-118	✓	✓
71006	TOU-A1-111	✓	✓	71103	TOU-A6-12	✓	✓
71007	TOU-A1-112	✓	✓	71104	TOU-A6-13	✓	✓
71008	TOU-A1-113	✓	✓	71105	TOU-A6-14	✓	✓
71009	TOU-A1-114	✓	✓	71106	TOU-A6-15	✓	✓
71010	TOU-A1-115	✓	✓	71107	TOU-A6-16	✓	✓
71011	TOU-A1-116	x	x	71108	TOU-A6-17	✓	✓
71012	TOU-A1-117	✓	x	71109	TOU-A6-18	✓	✓
71013	TOU-A1-118	✓	✓	71110	TOU-A6-19	✓	✓
71014	TOU-A1-119	✓	x	71111	TOU-A6-2	✓	✓
71015	TOU-A1-12	✓	✓	71112	TOU-A6-20	✓	✓
71016	TOU-A1-120	✓	✓	71113	TOU-A6-21	✓	✓
71017	TOU-A1-122	✓	✓	71114	TOU-A6-22	✓	✓
71018	TOU-A1-124	✓	✓	71115	TOU-A6-23	✓	✓
71019	TOU-A1-125	✓	✓	71116	TOU-A6-24	✓	✓
71020	TOU-A1-128	✓	✓	71117	TOU-A6-25	✓	✓
71021	TOU-A1-129	✓	✓	71118	TOU-A6-26	✓	✓
71022	TOU-A1-13	✓	✓	71119	TOU-A6-27	✓	✓
71023	TOU-A1-131	x	x	71120	TOU-A6-28	✓	✓
71024	TOU-A1-133	✓	✓	71121	TOU-A6-29	✓	✓
71025	TOU-A1-134	✓	✓	71122	TOU-A6-3	✓	✓
71026	TOU-A1-137	✓	✓	71123	TOU-A6-30	✓	✓
71027	TOU-A1-138	✓	✓	71124	TOU-A6-31	✓	✓
71028	TOU-A1-14	✓	✓	71125	TOU-A6-32	✓	✓
71029	TOU-A1-141	✓	✓	71126	TOU-A6-33	✓	✓
71030	TOU-A1-143	✓	x	71127	TOU-A6-34	✓	✓
71031	TOU-A1-15	x	x	71128	TOU-A6-35	✓	✓
71032	TOU-A1-16	✓	✓	71129	TOU-A6-36	✓	✓
71033	TOU-A1-17	✓	✓	71130	TOU-A6-37	✓	✓
71034	TOU-A1-18	✓	✓	71131	TOU-A6-38	✓	✓
71035	TOU-A1-19	✓	✓	71132	TOU-A6-39	✓	✓
71036	TOU-A1-2	✓	✓	71133	TOU-A6-4	✓	✓
71037	TOU-A1-23	✓	✓	71134	TOU-A6-40	✓	✓
71038	TOU-A1-26	✓	✓	71135	TOU-A6-41	✓	✓
71040	TOU-A1-29	✓	✓	71136	TOU-A6-42	✓	✓
71041	TOU-A1-3	✓	✓	71137	TOU-A6-43	✓	✓
71042	TOU-A1-33	✓	✓	71138	TOU-A6-44	✓	✓
71043	TOU-A1-34	✓	✓	71139	TOU-A6-45	✓	✓
71044	TOU-A1-37	✓	✓	71140	TOU-A6-46	✓	✓
71045	TOU-A1-38	✓	✓	71141	TOU-A6-47	✓	✓
71046	TOU-A1-39	✓	✓	71142	TOU-A6-48	✓	✓
71047	TOU-A1-4	x	x	71143	TOU-A6-49	✓	✓
71048	TOU-A1-41	✓	✓	71144	TOU-A6-5	✓	✓
71049	TOU-A1-43	✓	✓	71145	TOU-A6-50	✓	✓
71050	TOU-A1-47	✓	✓	71146	TOU-A6-51	✓	✓
71051	TOU-A1-6	✓	✓	71147	TOU-A6-52	✓	✓
71052	TOU-A1-60	✓	✓	71148	TOU-A6-53	✓	✓
71053	TOU-A1-61	✓	✓	71150	TOU-A6-55	✓	✓

Table S1 (continued)

Ecotype ID	Name	27°C	30°C	Ecotype ID	Name	27°C	30°C
71054	TOU-A1-62	✓	✓	71151	TOU-A6-56	✓	✓
71055	TOU-A1-63	✓	✓	71152	TOU-A6-57	✓	✓
71056	TOU-A1-65	✓	✓	71153	TOU-A6-58	✓	✓
71057	TOU-A1-66	✓	✓	71154	TOU-A6-6	✓	✓
71058	TOU-A1-67	x	x	71155	TOU-A6-60	✓	✓
71059	TOU-A1-69	✓	✓	71156	TOU-A6-61	✓	✓
71060	TOU-A1-7	x	✓	71157	TOU-A6-62	✓	✓
71061	TOU-A1-73	✓	✓	71158	TOU-A6-63	✓	✓
71062	TOU-A1-74	✓	✓	71159	TOU-A6-64	✓	✓
71063	TOU-A1-75	✓	✓	71160	TOU-A6-65	✓	✓
71064	TOU-A1-77	✓	✓	71161	TOU-A6-66	✓	✓
71065	TOU-A1-78	✓	✓	71162	TOU-A6-67	✓	✓
71066	TOU-A1-79	✓	x	71163	TOU-A6-68	✓	✓
71067	TOU-A1-8	✓	✓	71164	TOU-A6-69	✓	✓
71068	TOU-A1-80	x	x	71165	TOU-A6-7	✓	✓
71069	TOU-A1-81	✓	✓	71166	TOU-A6-70	✓	✓
71070	TOU-A1-82	✓	✓	71167	TOU-A6-71	✓	✓
71071	TOU-A1-84	✓	✓	71168	TOU-A6-72	✓	✓
71072	TOU-A1-85	✓	✓	71169	TOU-A6-73	✓	✓
71073	TOU-A1-88	✓	✓	71170	TOU-A6-74	✓	✓
71074	TOU-A1-89	✓	✓	71171	TOU-A6-75	✓	✓
71075	TOU-A1-9	✓	✓	71172	TOU-A6-76	✓	✓
71076	TOU-A1-90	✓	✓	71173	TOU-A6-77	✓	✓
71077	TOU-A1-91	✓	✓	71174	TOU-A6-78	✓	✓
71078	TOU-A1-93	✓	✓	71175	TOU-A6-79	✓	✓
71079	TOU-A1-96	✓	✓	71176	TOU-A6-8	✓	✓
71080	TOU-A1-98	✓	✓	71177	TOU-A6-80	✓	✓
71081	TOU-A6-1	✓	✓	71178	TOU-A6-81	✓	✓
71082	TOU-A6-10	✓	✓	71179	TOU-A6-82	✓	✓
71083	TOU-A6-100	✓	✓	71180	TOU-A6-83	✓	✓
71084	TOU-A6-101	✓	✓	71181	TOU-A6-84	✓	✓
71085	TOU-A6-102	✓	✓	71182	TOU-A6-85	✓	✓
71086	TOU-A6-103	✓	✓	71183	TOU-A6-86	✓	✓
71087	TOU-A6-104	✓	✓	71184	TOU-A6-87	✓	✓
71088	TOU-A6-105	✓	✓	71185	TOU-A6-88	✓	✓
71089	TOU-A6-106	✓	✓	71186	TOU-A6-89	✓	✓
71090	TOU-A6-107	✓	✓	71187	TOU-A6-9	✓	✓
71091	TOU-A6-108	✓	✓	71188	TOU-A6-90	✓	✓
71092	TOU-A6-109	✓	✓	71189	TOU-A6-91	✓	✓
71093	TOU-A6-11	✓	✓	71190	TOU-A6-92	✓	✓
71094	TOU-A6-110	✓	✓	71191	TOU-A6-94	✓	✓
71095	TOU-A6-111	✓	✓	71192	TOU-A6-95	✓	✓
71096	TOU-A6-112	✓	✓	71193	TOU-A6-96	✓	✓
71097	TOU-A6-113	✓	✓	71194	TOU-A6-97	✓	✓
71098	TOU-A6-114	✓	✓	71195	TOU-A6-99	✓	✓

Table S2. Effects of knock-down of *SDS* expression at 3, 4, 5, 6, 7 and 10 dai against the *R. solanacearum* GMI1000 strain in two wild-type genetic backgrounds at 27°C and 30°C.

		27°C				30°C			
		Col-0 *vs* *sds-2*		Ws-0 *vs* *sds-3*		Col-0 *vs* *sds-2*		Ws-0 *vs* *sds-3*	
dai	model terms	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
3	replicate	1.63	0.2007	ne	ne	0.74	0.4807	0.19	0.8300
	line	0.78	0.3795	ne	ne	4.43	0.0371	11.50	**0.0009**
	replicate*line	0.25	0.7756	ne	ne	1.13	0.3246	0.47	0.6278
4	replicate	17.69	1.5E-07	ne	ne	7.36	0.0009	1.33	0.2674
	line	1.02	0.3136	ne	ne	8.14	**0.005**	35.34	**2.2E-08**
	replicate*line	1.91	0.1521	ne	ne	1.45	0.2387	0.68	0.5082
5	replicate	45.23	1.0E-15	ne	ne	3.84	0.024	0.43	0.6520
	line	1.25	0.2659	ne	ne	6.10	**0.0148**	35.40	**2.2E-08**
	replicate*line	1.82	0.1660	ne	ne	2.90	0.0582	1.85	0.1617
6	replicate	91.08	1.2E-25	1	0.3786	11.72	2.1E-05	2.54	0.0828
	line	7.44	**0.0072**	1	0.3262	3.71	0.0561	25.80	**1.2E-06**
	replicate*line	10.78	4.6E-05	1	0.3786	2.96	0.0552	2.58	0.0793
7	replicate	128	1E-31	2.1	0.1333	6.91	0.0014	0.70	0.4966
	line	2.75	0.0993	2	0.1565	0.64	0.4251	23.40	**3.5E-06**
	replicate*line	3.53	0.0320	2.1	0.1333	6.18	0.0027	2.60	0.0779
10	replicate	114	1.4E-29	9	0.0002	3.17	0.0453	1.36	0.2609
	line	1.31	0.2552	3.1	0.0821	0.76	0.3847	9.25	**0.0028**
	replicate*line	0.53	0.5884	5.1	0.0076	1.19	0.3082	1.57	0.2113

F, *F* value resulting from the test of fixed effect. * $P < 0.05$; **$P < 0.01$; ***$P < 0.001$, ne: not estimated due to the absence of phenotypic variation. Significant differences between each wild-type background and its corresponding mutant after a FDR correction are indicated in bold.

Figure S1. Box-plots illustrating the additive effects of each top SNP located in the 14 QTLs with a Lindley process value above 10 as well as the epistatic effects within the three groups of epistatic QTLs (QTLs 5+6+7A+7B+9, QTLs 10+11 and QTLs 12A+12B). Blue, green, orange and red boxes indicate that the QTLs are located on chromosome I, II, IV and V, respectively. White boxes illustrate epistatic interactions among QTLs.

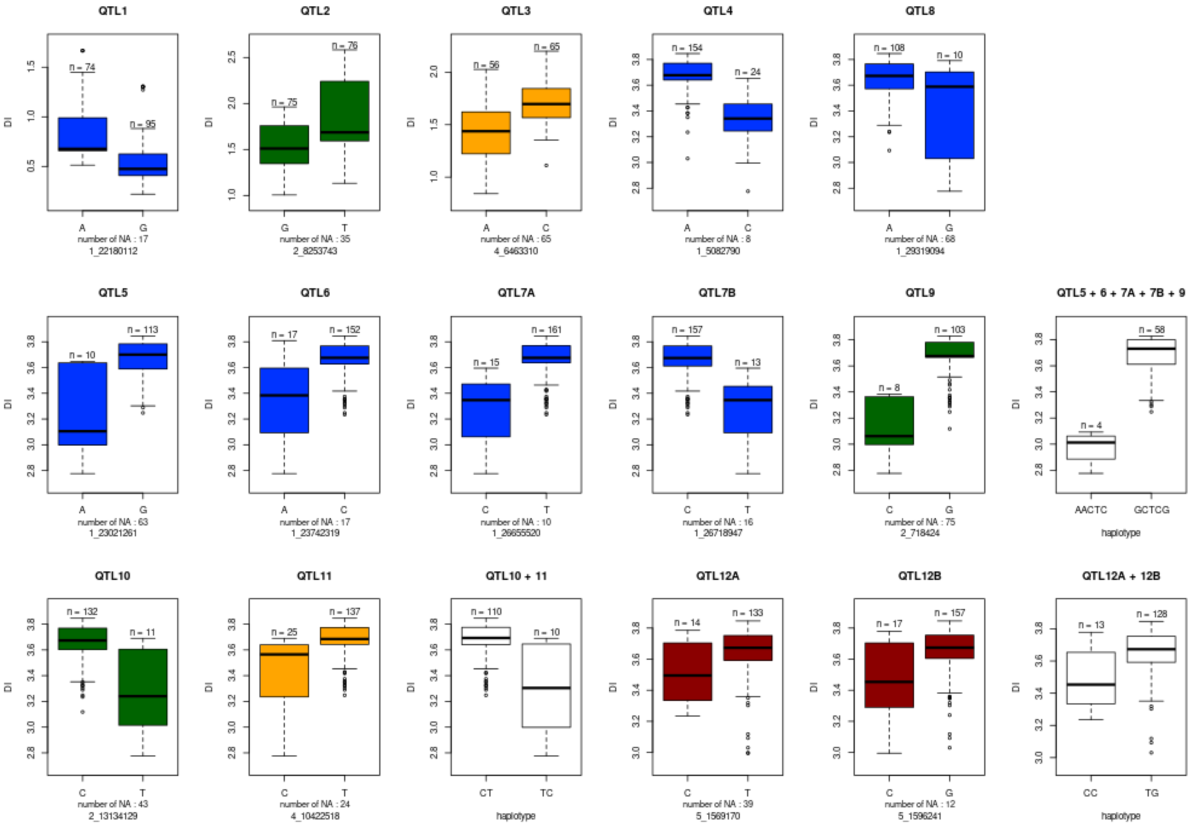


Figure S2. Detection of inter-QTLs epistasis for 11 out of the 14 QTLs with a Lindley process value above 10. For each QTL, a null distribution was established by calculating LD values (i.e. *r* correlation coefficient) between the bait top SNP and all the other SNPs across the genome (with the exception of the SNPs located within a 50kb window on each side of the bait top SNP). Only SNPs with a MARF > 0.07 were considered. In addition, LD values (above 0.1) between the bait top SNP for the corresponding QTL and the bait top SNPs from the other QTLs are represented by arrows. The *x*-axis corresponds to the LD estimates expressed in absolute values.

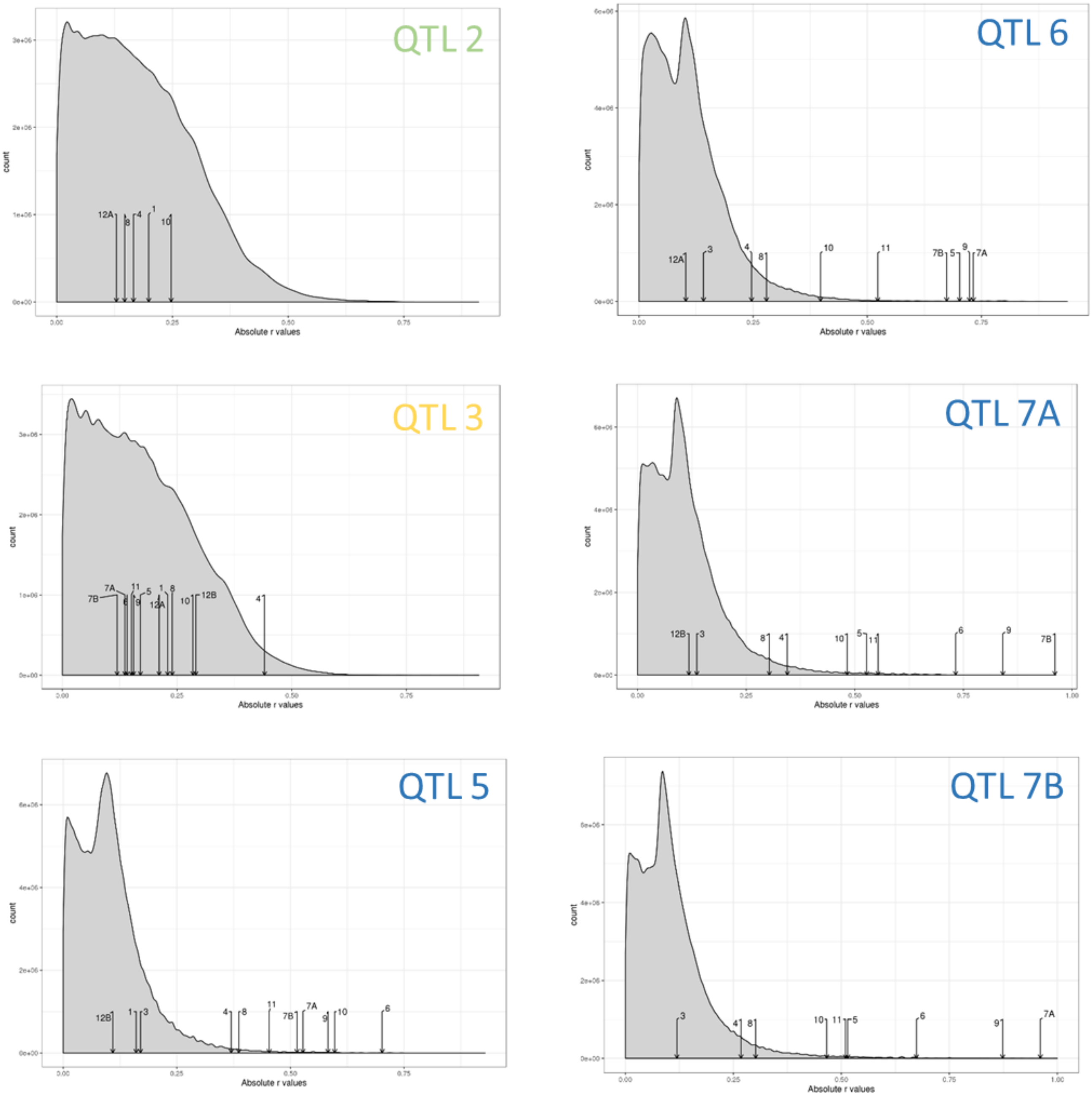
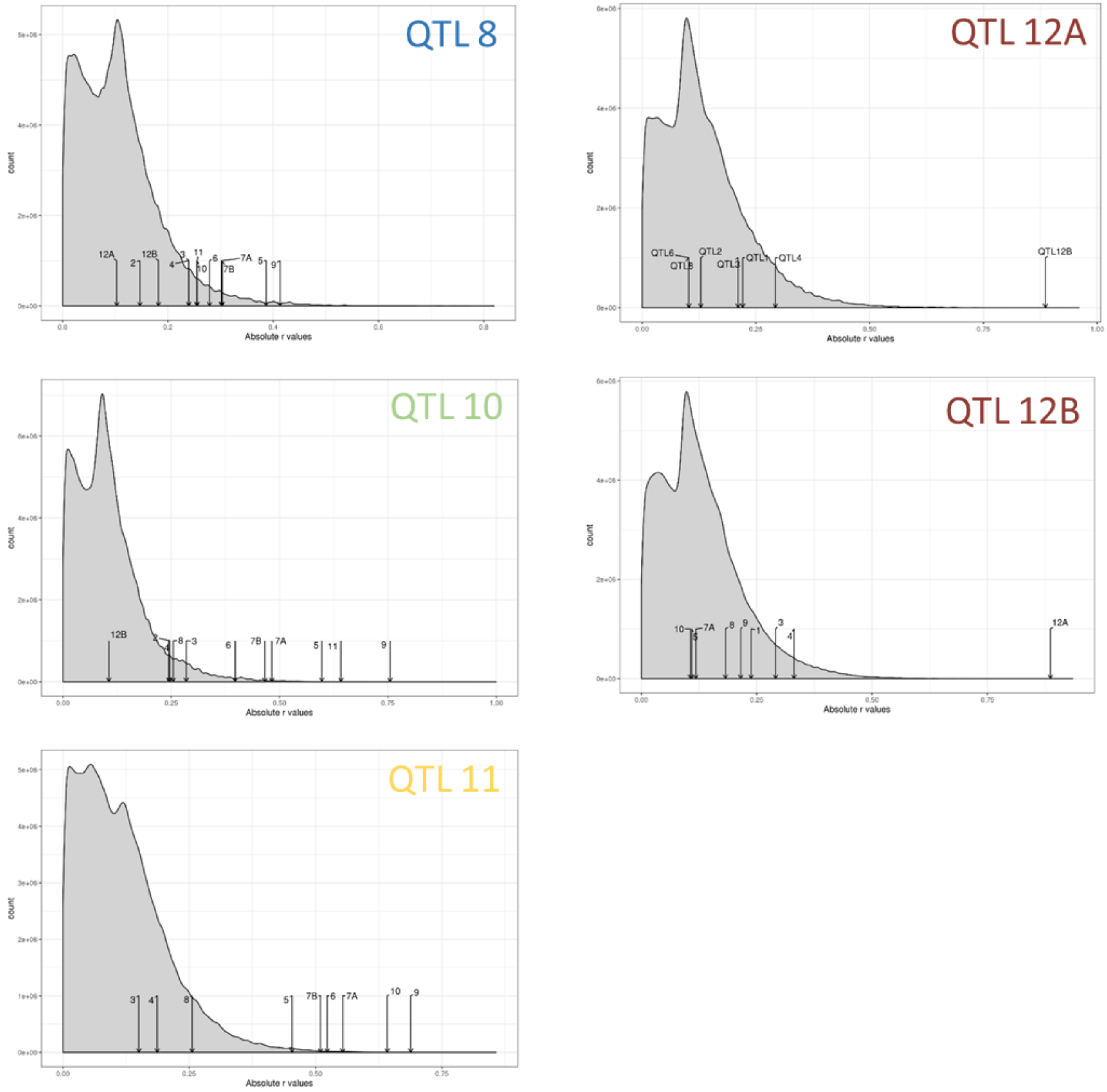


Figure S2 (continued)



C. Supporting information

In addition, of validating *SDS* at the gene underlying QTL4, I started the validation of candidate genes underlying other QTLs of plant response to *R. solanacearum* at 30°C in the TOU-A population. To do so I have screened 18 T-DNA insertion homozygous mutants corresponding to 11 candidate genes. For these mutants i) T-DNA insertion was confirmed by genotyping, ii) individual lines homozygous for the T-DNA insertion were selected and ii) homozygous progenies were produced for phenotyping with the GMI1000 strain at 30°C, with the wild type corresponding lines for each mutant as controls. Overall, except for *sds-2* and *sds-3* mutants, no obvious phenotype was obtained (figure 28). For instance, three homozygous allelic mutants of JAZ9 (Col-0 background) were obtained, *jaz9-1* (Salk_046563), *jaz9-2* (Salk_004872C) and *jaz9-3* (GK-265H05) (figure 28A) and inoculated with the GMI1000 strain (three replicates, n=72) at 30°C. Two mutants out of three did not show any significant difference to their wild type background Col-0 and only one mutant was significantly slightly less susceptible at 4, 5 and 6 dai ($P < 0.05$) (figure 28B).

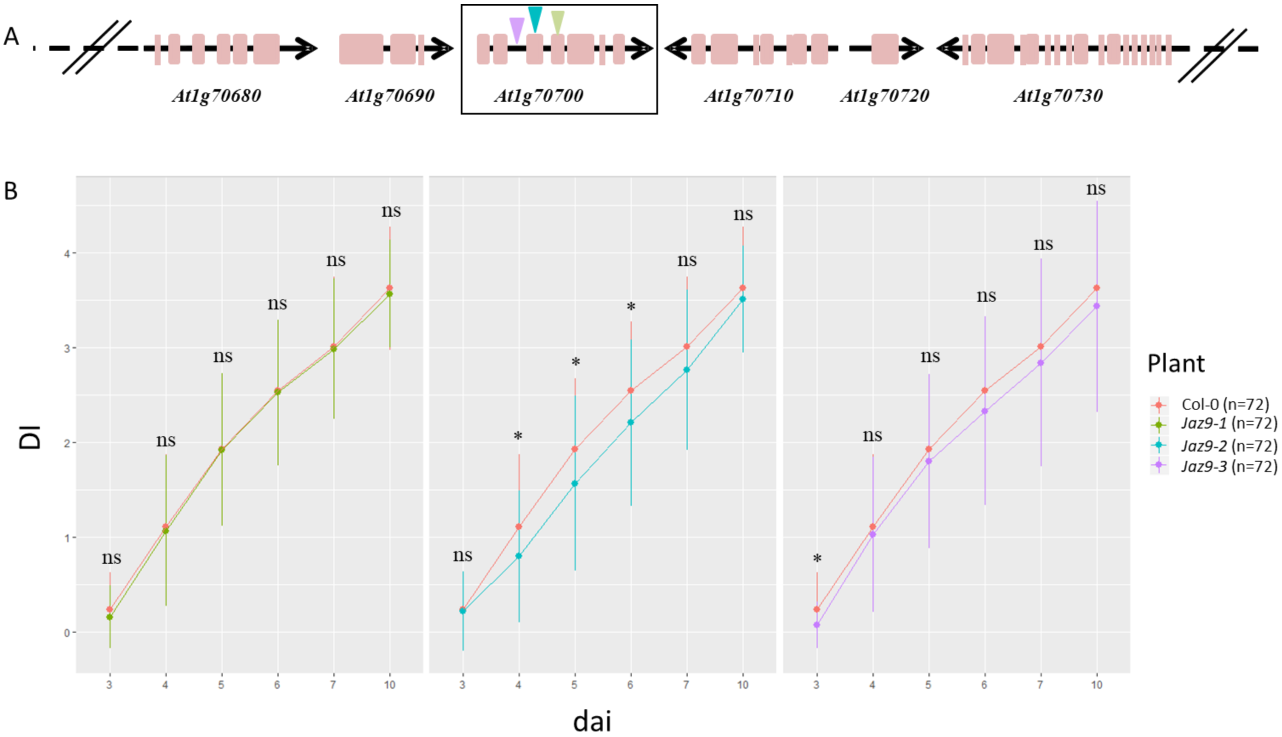


Figure 28. Dynamics of disease symptoms of *jaz9* mutants after inoculation with the GMI1000 at 30°C. A. Schematic representation of the structure of the *JAZ9* (*At1g70700*) gene on the chromosome. The pink boxes represent the exons, the black lines the introns, the arrow the orientation of the genes on the + or - strand of the DNA and the triangles the T-DNA insertion sites. **B.** Three allelic T-DNA insertion mutants *jaz9-1*, *jaz9-2* and *jaz9-3* and their corresponding genetic background Col-0 were inoculated with the GMI1000 in three independent biological replicates. The dynamics of disease symptoms were computed under *R* environment 3.6.1 (R_Core_Team, 2019) using ggplot2 package. Symbol '*' denotes a significant difference between Col-0 and each mutant at $P < 0.05$, using a Kruskal-Wallis analysis. The "ns" abbreviation correspond to the non-significant difference between Col-0 and each mutant.

D. Discussion and conclusion

Following the genome-wide local score analysis, we were able to obtain a better description of the genetic architecture of QDR to the GMI1000 strain by detecting a higher number of QTLs than by using solely our GWA mapping approach. In particular, GW-LS method allowed the detection of several QTLs with minor effects, which in turn allowed identifying a complex network of epistatic interactions (especially at 30°C). Only few studies already reported the identification of such complex epistatic networks, e.g. response to herbivores in *A. thaliana* (Brachi et al., 2015) and body weight in chicken (Carlborg et al., 2006). While complex epistatic relationships among QTLs may be therefore more frequent than anticipated, the functional validation of epistatic QTLs remains challenging but feasible if we consider a multi-CRISPR-Cas9 approach to create double, triple, quadruple, etc. mutants. Interestingly, no top SNP was shared between the worldwide and local collections of *A. thaliana*, reinforcing the need to consider different geographical scales if one wants to have a glimpse of the flexible genetic architecture underlying any quantitative trait.

The screening of 18 mutants with the GMI1000 was not fruitful except for *sds*. Several explanations can be proposed for this low rate of validated candidate genes. First, this could be associated with the effect of the T-DNA insertion on gene expression. An insertion in the promoter could lead do a downregulation or an overexpression of the gene, whereas an insertion in the coding sequence or an intron could lead to the synthesis of non- or partially functional truncated proteins by disrupting the transcript (Wang, 2008). Second, it could be also due to the genetic background of the T-DNA mutants. Indeed, most of T-DNA insertion mutants' collections have been generated in the Col-0 background, which is susceptible to *R. solanacearum* at 27°C and 30°C. Thus, such a susceptible background may not be appropriate to confirm resistance genes. Finally, the absence of phenotype may be related to epistatic relationships among QTLs. For instance, the absence of phenotype for the mutants *jaz9-1*, *jaz9-2* and *jaz9-3* may be due to the epistatic interactions detected between QTL7A (with *JAZ9* as a candidate gene) and QTL7B *(with the At1g70860* polyketide cyclase / dehydrase and lipid transport superfamily protein).

Genes underlying the identified QTLs with the GW-LS method are involved in diverse mechanisms. For instance, at 27°C, we identified a TREHALOSE PHOSPHATE SYNTHASE 10 (*At1g60140*), involved in trehalose biosynthesis, and a GDSL-like Lipase/Acylhydrolase protein superfamily (*At2g19050*) involved in lipid catabolic processes. However, at 30°C, a long list of genes participating in plant response to *R. solanacearum* was revealed. To date, these genes have different predicted molecular functions that have not directly been associated to defense mechanisms such as *At4g10450* involved in cytoplasmic translation and *At1g63980* annotated as D111/G-patch domain-containing protein. As previously shown in chapter II part D, this result shows once again the presence of a wide range of highly dynamic and complex molecular mechanisms involved in plant defense response under heat stress.

Chapter IV. Discussion and Perspectives

Discussion

During this PhD project, based on two geographically complementary collections of *A. thaliana*, we aimed at identifying and characterizing the genetic basis of natural variation of resistance mechanisms to *R. solanacearum* under heat stress. More precisely, we explored the variability of plant response in a worldwide and the local French TOU-A collections, to the GMI1000 strain at 27°C and 30°C. By combining a GWA mapping approach with a local score method, we identified a highly flexible genetic architecture of *A. thaliana* response to *R. solanacearum* between both temperature treatments and both *A. thaliana* collections. In addition, in the TOU-A collection, we have unraveled the presence of a dynamic and complex epistatic network of QTLs underlying QDR of *A. thaliana* to *R. solanacearum* under heat stress.

We also highlighted the high diversity of molecular functions involved in thermo-stable resistance mechanisms to *R. solanacearum.* In particular, we validated *SSL4* and *SDS* genes as susceptibility genes involved in QDR to *R. solanacearum* in the worldwide and local TOU-A collections, respectively. Furthermore, we identified in the worldwide collection an accession conferring total resistance to the GMI1000 strain at 27°C and 30°C. Results generated from BSA, mapping by sequencing and functional complementation strategies supported an allelic variant of RPS4/RRS1-R, with the identification a specific point mutation in the LRR domain of RRS1 to be responsible for this total resistance.

Together, although *A. thaliana* is not a natural host of *R. solanacearum*, these results demonstrate the power of combining quantitative genetics and molecular biology to identify key genetic and molecular resistance mechanisms in a wild plant species against a devastating crop pathogen.

A. Impact of heat stress on plant defense response

In our study, we demonstrated that, under elevated temperature, disease progression was faster in both collections of *A. thaliana* (Aoun et al., 2017; chapter III), highlighting the drastic impact of heat stress on immune response of *A. thaliana* to *R. solanacearum.* In agreement, in the worldwide collection, the power of GWA mapping revealed that the locus involved in the major resistance to *R. solanacearum*, *RPS4/RRS1-R*, was impaired following a temperature elevation of only 3°C. These findings are in line with our investigation on 45 studies carried out on the effect of temperature increase on plant resistance to pathogens, involving 123 resistance genes with 60% of them altered under heat stress. We also found that this effect is not specific neither to plant nor to pathogens species (Desaint et al., submitted). All these findings highlight the crucial need to identify and study robust mechanisms of resistance in a warming climate context.

The use of a GW-LS method revealed more accurately in both collections a higher number of QTLs (most of them with remarkable dynamics over the infection stages) at 30°C rather than at 27°C. The more complex genetic architecture observed at 30°C suggests that under heat stress, *A. thaliana* responds to *R. solanacearum* by engaging a complex network of defense mechanisms associated with time specificity of the genetic effects. Thus this may indicate that the higher the complexity of the environment is, in a real ecological context, the more the plant is challenged; and establish a response with a polygenetic architecture of different pathways involved. However, it would be interesting to check if this complexity of involved mechanism in the defense response is present in other pathosystems.

B. *A. thaliana / R. solanacearum* interaction under heat stress: natural diversity to be explored

In order to study the genetic basis of plant defense response to *R. solanacearum* and identify robust mechanisms of resistance under heat stress, we decided to explore the natural diversity of *A. thaliana* species by considering the following criteria:

i) The size of the mapping panel

Most studies investigated in the review (Desaint et al., submitted) were based on a restricted number of plant genotypes, thereby impeding the use of any QTL mapping approach. By largely increasing the size of the panel of genotypes, we were able to obtain in *A. thaliana* a better description of the level of genetic variation of resistance to *R. solanacearum*, as exemplified by the discovery of Eden-1 being totally resistant under heat stress to the GMI1000 *R. solanacearum* strain. The large size of our two collections of *A. thaliana* also increased the statistical power of GWA mapping, which in turn allowed a better description of the genetic architecture underlying resistance to *R. solanacearum*. In this PhD project, by phenotyping 176 and 192 accessions of *A. thaliana* from a worldwide and local geographical scale, respectively, we were able to finely map numerous QTLs involved in the defense response to *R. solanacearum*. Such a strategy has also proven its efficiency in the study of the genetic bases of natural variation of *A. thaliana* and other crops in response to viruses, bacteria, fungi, oomycetes (Bartoli and Roux, 2017).

ii) The geographical scale of collections

There is a belief that increasing the geographical scale at which genotypes are collected increases the extent of phenotypic variation. However, more and more studies reported, at least in *A. thaliana*, that the level of phenotypic variation could be similar from a worldwide to a local scale (Brachi et al., 2013). This is particularly well illustrated by natural variation of plant-pathogen interactions (Jorgensen and Emerson, 2008; Huard-Chauveau et al., 2013; Karasov et al., 2014; Debieu et al., 2016) and may result from the adaptive dynamics of plant pathosystems occurring at a local scale (Karasov et al., 2014). As previously advised, mapping populations used to identify genomic regions associated with natural phenotypic variation should therefore be chosen according to the geographical scale at which the corresponding selective pressures vary (Bergelson and Roux, 2010). Increasing the geographical scale at which genotypes are collected definitely increases the extent of genetic diversity but to the detriment of the large effects of population structure and genetic/allelic heterogeneities on the power of GWA mapping to unravel the genetic basis of natural phenotypic variation. These limitations may be in part resolved by working at small geographical scales (Bergelson and Roux, 2010). In our study, we explored the natural diversity of two geographically complementary collections of natural accessions of *A.*

thaliana response to *R. solanacearum* under heat stress. Doing so, in our case, helped to reveal different actors in the response to combined stresses and unwrapped the presence of a complex epistatic network in the local collection.

However, it would be interesting to test if the same level of flexibility for the genetic architecture between different geographic scales is also observed in other plant species (tomato, eggplant, etc.) under combined stress conditions (e.g. *R. solanacearum* and heat stress). Nevertheless, such a comparison might be impeded by the quasi-absence of local mapping populations in these species, stressing the need to go back to the wild to establish new genomic resources at a local scale.

C. Different plant defense mechanisms under heat stress

At 30°C and in both *A. thaliana* collections, we identified multiple QDR candidate genes that encode various molecular functions that are largely distinct from *NLR* resistance genes (figure 29). For instance, in the local collection, we validated a QTL falling in the *SDS* gene encoding a protein necessary for DNA double strand break repair (Azumi et al., 2002; De Muyt et al., 2009) and identified another QTL falling in a LACCASE12, involved in lignin catabolic processes (Turlapati et al., 2011) (figure 29). This quasi-absence of *NLR* genes underlying QDR to *R. solanacearum* is not surprising because i) under heat stress, *R* genes has been shown to be impaired (Aoun et al., 2017; Desaint et al., submitted), ii) the lack of *NLR* genes detected in response to combined stresses has been also previously shown in literature (Pilet-Nayel et al., 2017), and (iii) the lack of *NLR* genes underlying QDR has been reported in many other pathosystems (Bartoli and Roux, 2017).

In this project, we identified the involvement of different QDR genes in the response to *R. solanacearum*. In particular, we functionally validated *SDS* and *SSL4* as conferring QDR to *R. solanacearum* under heat stress (figure 29). Interestingly, these two genes correspond to susceptibility genes. We should however stress that these genes have been validated by adopting a reverse genetics approach. The limitation of this method is that the screening of mutants in a susceptible background does not allow the identification of resistance genes. Therefore, we could be missing interesting candidates involved in the defense response under heat stress. Nonetheless, our results could expand the list of the 16 validated genes, underlying identified QTLs of resistance in plants, established in Pilet-Nayel et al. (2017) study, by adding new genes involved in response to *R. solanacearum* at elevated temperature. Like for the other cloned QTLs, the remaining enigma is to investigate (i) in which genetic pathways our functionally validated QTLs are involved in, and (ii) whether these pathways are similar between cloned QTLs.

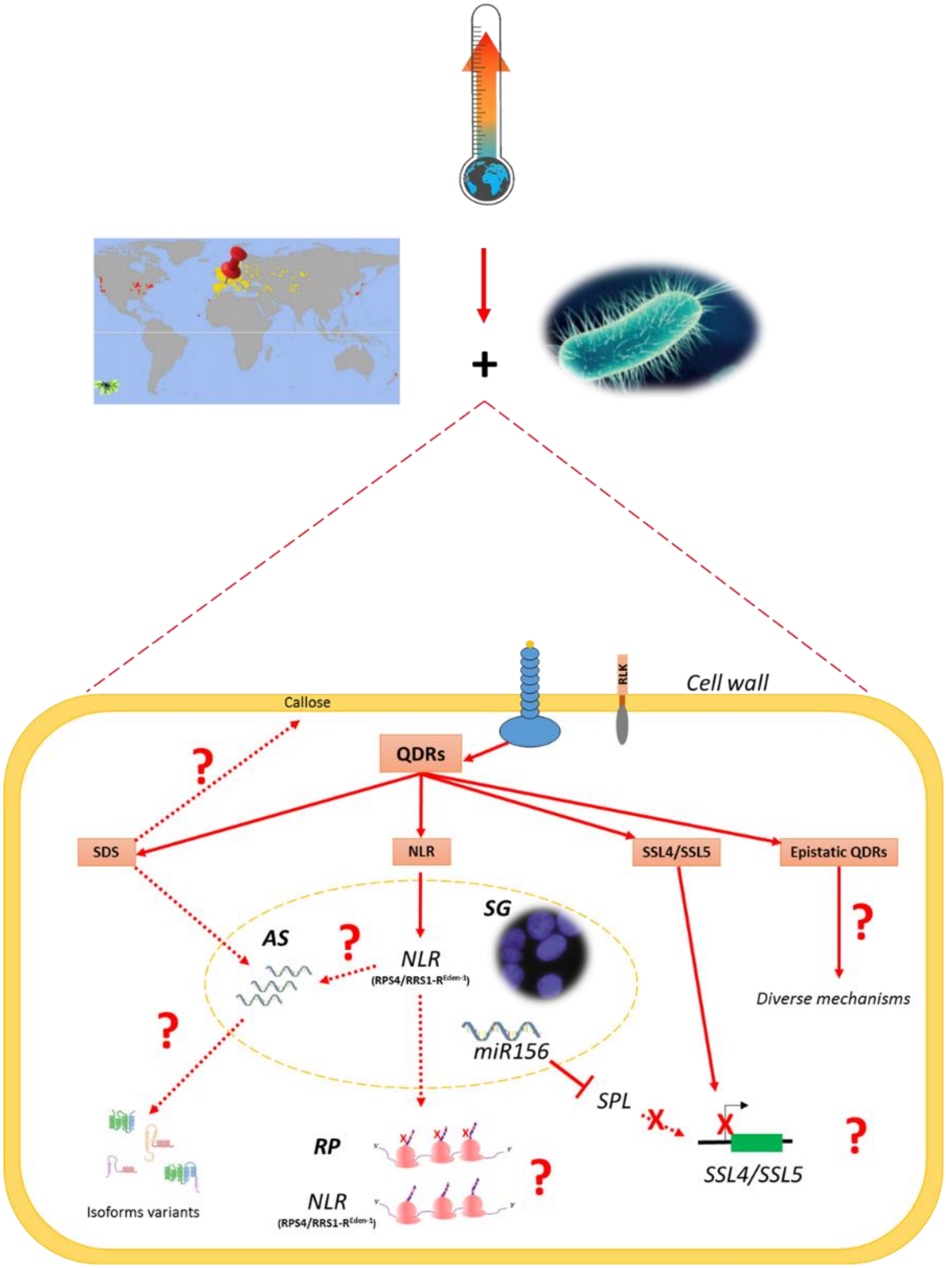


Figure 29. Schematic representation of the identified resistance mechanisms in response to *A. thaliana* under heat stress showing the avenues that could be addressed. AS: alternative splicing; QDR: Quantitative Disease Resistance; NLR: Nucleotide-binding/Leucine-Rich-repeat receptor; RP: 5'-ribosome pausing: SDS: SOLO DANCERS; SG: stress granules; SPL: SQUAMOSA PROMOTER BINDING PROTEIN-LIKE; SSL4/SSL5: Strictosidine synthase like 4/5 proteins. Blunt and pointed arrows indicate inhibition and activation, respectively. The symbols 'X' and refers to an alteration.

Last, most of the identified genes were previously known and studied for specific vital functions in the plant. However, in our study, we revealed these genes involvement in the defense response to *R. solanacearum* at both temperature conditions. For instance, the *TREHALOSE PHOSPHATE SYNTHASE 10* gene involved in trehalose-6-phosphate synthesis and the EXPANSIN 2 involved in cell wall modification and for a lignin peroxidase have never been found before to be associated with plant defense response (chapter III). Thus, more attention is needed on the functions of these genes: are they differentially regulated under stress conditions? Or are they just involved in a multitude of functions?

D. Nature of polymorphisms associated with resistance mechanisms

In this study, we found that the nature of candidate polymorphisms underlying resistance to *R. solanacearum* might be associated with regulation of gene expression. For instance, in the case of the validated gene *SDS*, the top SNPs are located in the promoter region, suggesting that natural variation of QDR to *R. solanacearum* at 30°C might be associated with natural variation in cis-regulation of *SDS*. A similar observation was found in the case of QDR to *X. campestris* involving the atypical kinase *RKS1* (Huard-Chauveau et al., 2013). In the case of *SSL4* and *SSL5* validated as genes of susceptibility, their natural variation of expression might be associated with trans-regulatory elements such as *miR156h* (figure 29).

Besides the identification of polymorphisms potentially involved in regulation of gene expression, we functionally validated a polymorphism in the LRR domain of RRS1-R of Eden-1 accession responsible of heat-stable total resistance to the GMI1000 strain. This polymorphism is close to an artificial EMS point mutation (*int102* mutant) created in the *snc1-1* mutant background, conferring heat-stable autoimmune phenotype for the *snc1-1* mutant (Zhu et al., 2010). Surprisingly, the point mutation identified in Eden-1 is not found neither in the worldwide collection of 1,305 accessions (The 1001 Genome Consortium, 2016), nor in a set of 168 natural populations from the south-west of France (Frachon et al., 2018)or in the local TOU-A population (Frachon et al., 2017). To test the uniqueness of this polymorphism, it would be interesting to sample additional accessions from the native population of Eden-1, which is located in Sweden. Interestingly, we recently found that other mutations are present in the NLR domain of RRS1-R in our set of 168 natural populations of *A. thaliana* from the south-west of France. Therefore, it would be interesting to test if they may also confer heat-stable resistance to *R. solanacearum*.

Perspectives

The functional validation of two QDR genes and a specific allelic from of *R* gene *RRS1-R*$^{\mathit{Eden-1}}$ opens up new avenues to explore at the molecular, functional and ecological levels.

Deciphering and understanding underlying molecular mechanisms of resistance

First, an effort has to be put on the understanding of the molecular functioning of Eden-1 resistance. As already mentioned in chapter II part B.4., testing first the presence of 5'-ribosome pausing to check if RRS1-R$^{\text{Eden-1}}$ were still translated under heat stress would be interesting (figure 29). In addition, it could be important to check if the configuration change from a hydrophilic to a hydrophobic state of the amino acid following the mutation in the LRR domain, evades RRS1-R$^{\text{Eden-1}}$ from 5'-ribosome pausing under the protection of HSC70/HSP70 chaperone.

Second, it would be interesting to check for the presence of spliced variants of RRS1-R$^{\text{Eden-1}}$ that could be involved in the expression of the gene (figure 29). Similar analysis should be done on selected *R* genes using *sds-2* and *sds-3* backgrounds, to test the participation of *SDS* in alternative splicing of *R* genes, as previously discussed in Aoun et al., 2019 (chapter III; figure 29).

Third, among the identified mechanisms, several candidates acting on the cell wall were found, such as EXPANSIN 2 involved in cell wall modification and synthesis and *SDS* involved in cell wall formation (chapter III). Together, it suggests that these genes could play a specific role in cell wall functions in a combined stress condition (figure 29). Additionally, because cell wall takes part in different biological processes (cell morphogenesis, response to biotic or abiotic stresses), it would be then interesting to study in *sds* mutants the cellular and biochemical modifications (i.e. cellulose, hemicellulose, lignin and pectin composition), in order to assess the role of *SDS* in the resistance response to the *R. solanacearum* under heat stress.

Finally, this project also holds interest in studying protein-protein or protein-DNA interactions, in order to identify the interactors that participate in the thermos-stability of resistances. Yeast two-hybrid (Y2H) or Co-Immunoprecipitation (CoIP) systems have been successfully used in the team to identify protein-protein interactions. Moreover, Förster resonance energy transfer (FRET) (Xing et al., 2016) could reveal DNA-binding property or transcriptional activity of our candidates. Because physical interactions between the plant and the bacteria take place in specific tissues/organs over the infection cycle and molecular interactions between actors leading to immune response are labile, other methods are currently adopted by the team and can be used to decipher mechanisms involved in stable resistance under heat stress. For instance, by adapting the INTACT system (Deal and Henikoff, 2010, 2011), we should be able to have access to the transcriptome of cells that would have been injected by the T3SS system of the bacteria, thus allowing to study the cell specific transcriptome reprogramming during interaction and at elevated temperature using different genetic backgrounds. By adopting a BioID system, we should also be able to detect proteins that transiently interact with our candidate proteins (Roux et al., 2018).

What happens on the pathogen side?

Although this project focused on the impact of heat stress on plant response to the *R. solanacearum* GMI1000 reference strain, little is known on the mechanisms underlying *R. solanacearum* response to heat stress during infection. In combined stress conditions, focus has been mostly given to plant immune response (Desaint et al., submitted). As already discussed in the review paper, in many cases where resistance is maintained under heat stress, applied temperature was lower than the optimal growth temperature of the pathogen. Thus, maintained resistance could be due either to i) the growth stage of the plant during infection resembling an incompatibility, because pathogens infect plants at a specific developmental stage or through a specific organ (Caldwell et al., 2017; Ziv et al., 2018), ii) the low level of pathogen virulence and iii) a higher efficiency of resistance on the plant side. To better understand and accurately decipher mechanisms underlying plant-pathogens interactions under heat stress, it is important to consider the impact of temperature elevation on plants as well as on bacteria behavior; an approach that is currently adopted by the team.

Beyond resistance to the R. solanacearum GMI1000 reference strain under heat stress

In this PhD project, all results were obtained using the *R. solanacearum* GMI1000 reference strain. Knowing that (i) the genetic architecture of resistance to *R. solanacearum* in *A. thaliana* largely depends on the identity of the *R. solanacearum* strain (Godiard et al., 2003) and (ii) *RPS4/RRS1-R* confers resistance to a broad spectrum of pathogens, including another soil-borne bacterium, *F. oxysporum*, and the vascular bacterium *X. campestris (Debieu et al., 2016; Lyons et al., 2015)*, it will be interesting to test our three candidate genes for resistance under heat stress against several *R. solanacearum* strains and pathogen species. .

Similarly, it could be interesting to study the natural variation of *A. thaliana* response to *R. solanacearum* to other abiotic stresses known to be important for the bacteria, either using single stress or combined stresses treatment (e.g. relative humidity, temperature, relative humidity x temperature).

Towards the understanding of an integrated system

In order to face the future challenges of climate warming and its impact on food security, it is now a priority to transfer the identified resistance genes in crop species, either by exploring the genetic diversity of crop species to identify polymorphisms associated with resistance or by investing in resistance mechanisms by genetic engineering. Until now, few studies have been carried out in field conditions to assess the impact of heat stress on plant immunity (Desaint et al., submitted). However, in order to study the genetic basis of plant response variation to the pathogen and to identify robust resistance mechanisms maintained under heat stress, it would be crucial to assess plant resistance to pathogens in more ecologically realistic conditions. In line to this, we propose the following interesting avenues that are being explored by the team:

i) Are the identified resistance genes at 30°C, in this study, still robust in field conditions? Because *R. solanacearum* is a quarantine bacterium in Europe, experiments in field conditions could only be possible by collaborating with other countries (i.e. Thailand).
ii) What is the impact of microbiota on the identified QDR genes?
iii) What is the impact of the microbiota on plant response to pathogens under heat stress?
iv) What is the impact of heat stress on plant-pathogen-microbiota interactions?

Considering the whole system of plant-pathogen-microbiota interactions would allow a solid evaluation of resistance mechanisms remaining efficient in a changing climate.

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Annex 1. List of identified polymorphisms in the RPS4/RRS1sequence of Eden-1, following its alignment with the resistant and susceptible accessions.

Loci	Genomic position	Region	Polymorphism	Accession(s) with polymorphism
RPS4	18321303	Intron	X→CAGTCCTGA	Bl-1, Col-0, Eden-1, Gre-0, Pyl-1, Sp-0
	18322161		G→A G→X	Col-0, Eden-1, Gre-0, Kondara Sp-0
	18321796		C→T	Bl-1, Eden-1
	18321957		C→A	Bl-1, Col-0, Eden-1, Gre-0,
	18322658		G→A	Bl-1, Col-0, Eden-1, Gre-0, Pyl-1, Sp-0
	18322781		C→A	Eden-1
	18323563		A→G	Bl-1, Eden-1
	18323703		T→C	Bl-1, Eden-1
	18324550		C→T	Bl-1, Eden-1
	18324643		T→G	Bl-1, Eden-1
	18324962		G→A	Bl-1, Eden-1
	18325851		T→A	Eden-1
	18325914		C→T	Bl-1, Eden-1, Gre-0, Pyl-1, Sp-0
	18325993		G→T	Bl-1, Eden-1
RRS1	18326624	Intron	T→G	Bl-1, Eden-1
	18327055	Exon	G→T	Eden-1
	18327305		T→A	Bl-1, Col-0, Eden-1, Gre-0, Pyl-1, Sp-0
	18328261	Intron	A→T	Bl-1, Eden-1, Pyl-1
	18328267		X→ATATG	Bl-1, Eden-1, Pyl-1
	18328276		T→G	Bl-1, Eden-1, Pyl-1
	18328282		ATC→CTT	Bl-1, Eden-1, Pyl-1
	18328298		AATT→CGAA	Bl-1, Eden-1, Pyl-1
	18328331		G→X	Bl-1, Eden-1, Pyl-1
	18328388	Exon	G→C	Bl-1, Col-0, Eden-1, Pyl-1
	18328479		**G→A**	**Eden-1**
	18329293		TC→CA	Bl-1, Eden-1, Pyl-1
	18329538		T→G	Bl-1, Eden-1, Pyl-1
	18330434	Intron	T→C	Bl-1, Col-0, Eden-1, Pyl-1
	18330469		T→C	Bl-1, Col-0, Eden-1, Pyl-1
	18330483		T→C	Bl-1, Eden-1, Pyl-1
	18330568		T→A	Bl-1, Col-0, Eden-1, Pyl-1
	18330684		C→T	Bl-1, Col-0, Eden-1, Pyl-1
	18330725		T→C	Bl-1, Col-0, Eden-1, Pyl-1
	18330888		AT→X	Bl-1, Col-0, Eden-1, Pyl-1
	18330899		AX→TT	Bl-1, Col-0, Eden-1, Pyl-1
	18330986		A→C	Bl-1, Eden-1, Pyl-1
	18331033		A→T	Bl-1, Eden-1, Pyl-1
	18331249		G→A	Bl-1, Eden-1
	18331285		T→A	Bl-1, Eden-1, Pyl-1
	18331356		G→C	Bl-1, Col-0, Eden-1, Gre-0, Lip-0, Pyl-1, Sp-0
	18331677		T→A	Bl-1, Col-0, Eden-1, Lip-0, Pyl-1
	18331756		T→G	Bl-1, Eden-1, Pyl-1
	18331843		C→T	Bl-1, Eden-1
	18332032		G→T	Bl-1, Col-0, Eden-1, Stw-0
	18332088		G→A	Bl-1, Eden-1
	18332204		G→C	Eden-1
	18332538		G→A	Bl-1, Eden-1, Gre-0, Sp-0
	18332739		A→G	Eden-1

The 'X’ and ‘_’ symbols refer to a deletion and an insertion of nucleotide(s), respectively. The polymorphism in bold correspond to the functionally validated mutation, involved in heat-stable total resistance the R. solanacearum GMI1000 strain.

Abstract

Genetic bases of natural variation of *Arabidopsis thaliana* response to *Ralstonia solanacearum* in a global warming context

In the context of climate warming, global surface temperature is predicted to increase from 1.5 to 4.8°C by the end of the century and extreme climatic events are expected to become more frequent. These changes will affect plant development and favor the poleward geographic migration of pathogens. Thus, plants will be facing an increased risk of epidemics as well as the emergence of new highly aggressive pathogen species. As a consequence, crop yields losses are anticipated and food security is put at risk. Alarmingly, several studies showed that an increase of temperature inhibits most of the major known resistance mechanisms. However, the underlying molecular mechanisms are still poorly characterized. In my thesis project, by exploiting natural genetic variation in *Arabidopsis thaliana*, I aimed to identify and characterize the genetic bases of resistance mechanisms remaining efficient at elevated temperature to *Ralstonia solanacearum*, one of the most harmful pathogenic phytobacteria causing bacterial wilt. To uncover quantitative trait loci (QTLs) associated with natural variation of response to *R. solanacearum* under heat stress, I adopted genome wide association (GWA) approaches using two geographically complementary collections of accessions of *A. thaliana*. In the worldwide collection, a single accession was totally resistant to the *R. solanacearum* GMI1000 strain under heat stress. Using a bulk segregant analysis approach, we identified the genetic basis of this total resistance that turned out to be controlled by a specific allelic form of the *RPS4/RRS1* immunoreceptor pair. Beyond this simple genetic architecture, GWA analysis revealed a polygenic architecture underlying disease symptom progression. By adopting a reverse genetic approach, I functionally validated two genes encoding strictosidine synthase-like proteins (SSL) involved in early plant defense response to *R. solanacearum.* Using a local French collection revealed a genetic architecture of quantitative disease resistance (QDR) to *R. solanacearum* that totally differs from the one obtained with the worldwide collection. In particular, a complex genetic network of interacting loci was detected under heat stress. Among the detected QTLs, I functionally validated the atypical meiotic cyclin *SOLO DANCERS* gene as involved in QDR to *R. solanacearum*. By adopting an interdisciplinary approach between quantitative genetics and molecular biology, I highlighted in *A. thaliana* the diversity of molecular functions underlying natural variation of resistance to *R. solanacearum* under stress, which may in turn provide candidate genes for crop resistance to pathogens in the context of climate warming.

Keywords: disease resistance, heat stress, natural variation, *Arabidopsis thaliana*, *Ralstonia solanacearum,* GWA mapping, functional validation, *RPS4/RRS1,* strictosidine synthase-like proteins, *SOLO DANCERS.*

Identification des bases génétiques de la variation naturelle d'*Arabidopsis thaliana* à *Ralstonia solanacearum* dans le contexte de réchauffement climatique

Dans le contexte du réchauffement climatique, la température moyenne devrait augmenter de 1,5 à 4,8°C d'ici la fin du siècle et les évènements climatiques extrêmes devraient devenir plus fréquents. Ces changements affectent d'ores et déjà le développement des plantes et favorisent la migration géographique des agents pathogènes vers les pôles. Ainsi, les plantes sont confrontées à un risque accru d'épidémies et à l'émergence de nouvelles espèces d'agents pathogènes extrêmement agressifs. Des pertes de rendements sont prédites, menaçant ainsi potentiellement la sécurité alimentaire mondiale. De manière alarmante, une augmentation permanente de la température inhibe la plupart des principaux mécanismes de résistance connus chez les plantes. Cependant, les mécanismes moléculaires sous-jacents sont encore mal caractérisés. Dans mon projet de thèse, en exploitant la variation génétique naturelle d'*Arabidopsis thaliana*, j'ai cherché à identifier et à caractériser les bases génétiques des mécanismes de résistance restant efficaces à température élevée contre *Ralstonia solanacearum*, une des phytobactéries pathogènes les plus nocives causant le flétrissement bactérien. Pour découvrir les QTLs associés à une variation naturelle de la réponse à *R. solanacearum* sous un stress thermique, j'ai utilisé deux collections d'accessions d'*A. thaliana* géographiquement complémentaires et des approches de GWA mapping. Au sein de la collection mondiale, une seule accession est restée totalement résistante à la souche GMI1000 de *R. solanacearum* à température élevée. En utilisant une approche de type Bulk Segregating Analysis, nous avons démontré que cette résistance totale était contrôlée par une forme allélique spécifique de la paire d'immuno-récepteurs *RPS4/RRS1.* Au-delà de cette architecture génétique simple, l'analyse GWA a révélé une architecture polygénique sous-jacente à la progression des symptômes de la maladie. En adoptant une approche de génétique inverse, j'ai validé fonctionnellement deux gènes codant pour des protéines de type strictosidine synthase (SSL) impliqués dans la réponse de défense précoce à la bactérie. L'utilisation d'une collection française locale a révélé une architecture génétique de quantitative disease resistance (QDR) à *R. solanacearum* totalement différente de celle obtenue avec la collection mondiale. En particulier, un réseau génétique complexe de QTLs épistatique a été détecté. Parmi les QTLs détectés, j'ai validé fonctionnellement la cycline méiotique atypique *SOLO DANCERS* comme étant impliqué dans la QDR à *R. solanacearum*. En adoptant une approche interdisciplinaire entre la génétique quantitative et la biologie moléculaire, j'ai mis en évidence chez *A. thaliana* la diversité des fonctions moléculaires sous-jacentes à la variation naturelle de la résistance à *R. solanacearum* en présence d'un stress thermique, identifiant ainsi des gènes candidats susceptibles d'être utilisés chez les espèces cultivées pour conférer des résistances robustes aux agents pathogènes dans le contexte du réchauffement climatique.

Mots clés: résistance aux maladies, stress thermique, variation naturelle, *Arabidopsis thaliana*, *Ralstonia solanacearum*, GWA mapping, validation fonctionnelle, *RPS4/RRS1,* strictosidine synthase-like proteins, *SOLO DANCERS.*