Technische Universität München

TUM School of Life Sciences

Polymorphisms in defence responses of the wild tomato species *Solanum chilense* against *Phytophthora infestans* and *Cladosporium fulvum*: diversity in defence responses of a wild tomato species

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Leaf samples from *Solanum chilense,* seven days post-inoculation with *Phytophthora infestans* or 14 days post-inoculation with *Cladosporium fulvum* were press dried. Leaf samples from a plant from the southern highland population LA4330 show severe disease symptoms against both pathogens (top right and bottom right) compared to leave samples from the central population LA3111 (top left and bottom left).

Phytophthora infestans

Cladosporium fulvum

***Solanum chilense* inflorescence**

variation in disease symptoms

Summary

Solanum chilense is one of the wild tomato species found in extreme natural habitats, ranging from coastal deserts to high humidity mountain areas. Exposure to such different environments is expected to result in adaptations to various external stimuli. Different populations of *S. chilense* have been studied for abiotic stress tolerance as well as viral and bacterial resistance harbored in them. In this dissertation, resistance against two filamentous pathogens, *Phytophthora infestans* and *Cladosporium fulvum*, was analysed.

P. infestans resistance was evaluated in nine populations of *S. chilense* and quantitative variation in resistance was observed. Following that, underlying molecular cues were studied. To study the plant's immune response, elicitation with laminarin (a general glucan elicitor of plant immune responses) was used. Plants elicited with laminarin showed significant overlap in differentially expressed genes (DEGs) with plants inoculated with *P. infestans*. Screening of 83 plants from nine populations revealed high diversity in basal levels of immunity components as well as after elicitation. Upon elicitation with laminarin, the production of the phytohormone ethylene (ET) showed the strongest correlation with the observed resistance. However, using generalized linear mixed models (GLMMs), additive effects of elicitor-induced reactive oxygen species (ROS) production, elicitor-induced ethylene (ET) production, basal levels of abscisic acid (ABA) and basal levels of phaseic acid (PA) were found to best explain the resistance at the species level. The individual components showed a quantitatively different contribution to resistance in each of the geographically separated populations.

Qualitative resistance against the fungal pathogen *C. fulvum* was evaluated in 15 populations of *S. chilense*. Upon infiltration of fungal avirulence (Avr) proteins, presence-absence variations were observed in the hypersensitive response (HR), a typical qualitative defence response. Further, canonical regions of the major resistance genes *Cf-4*and *Cf-9* (Cf-4 and Cf-9 proteins are involved in Avr-recognition) showed presence-absence variations. In addition, complete loss of recognition of Avr proteins from *C. fulvum* race 5 was observed in the southern part of the species habitat.

Overall, the data shows polymorphisms in defense responses against *P. infestans* and *C. fulvum* within and between geographically distinct populations of *S. chilense*. Both the quantitative and qualitative resistance analyses highlight a link between resistance mechanisms and habitat adaptations.

Zusammenfassung

Solanum chilense gehört zu den Wildtomaten, die in extremen, natürlichen Lebensräumen vorkommen. Diese reichen von küstennahen Wüsten bis hin zu Berggebieten mit hoher Luftfeuchtigkeit. Es wird erwartet, dass die Exposition gegenüber solch unterschiedlichen Umgebungen zu Anpassungen an äußere Reize führt. Verschiedene Populationen von *S. chilense* wurden umfassend auf ihre abiotische Stresstoleranz sowie auf ihre viralen und bakteriellen Resistenzen untersucht. In dieser Dissertation wurde die Resistenz gegen zwei filamentöse Pathogene, *Phytophthora infestans* und *Cladosporium fulvum*, analysiert.

Die *P. infestans*-Resistenz wurde in neun Populationen von *S. chilense* ausgewertet, wobei eine quantitative Variation der Resistenz festgestellt wurde. Anschließend wurden die zugrundeliegenden molekularen Mechanismen untersucht. Um die Immunantwort der Pflanze zu untersuchen, wurde die Elizitierung mit Laminarin (einem allgemeinen Glucan-Elicitor für pflanzliche Immunantworten) verwendet. Pflanzen, die mit Laminarin elizitiert wurden, zeigten eine signifikante Überlappung in den differentiell exprimierten Genen (DEGs) mit Pflanzen, die mit *P. infestans* inokuliert wurden. Die Untersuchung von 83 Pflanzen aus neun Populationen ergab, dass schon auf basaler Ebene eine hohe Diversität verschiedener Immunitätskomponenten besteht, die ebenso divers nach Elizitorinduktion zu messen waren. Nach Elizitierung mit Laminarin zeigte die Produktion des Phytohormons Ethylen (ET) die stärkste Korrelation mit der beobachteten Resistenz. Unter Verwendung von generalisierten linearen gemischten Modellen (GLMMs) wurde jedoch festgestellt, dass additive Effekte der elizitor-induzierten Produktion reaktiver Sauerstoffspezies (ROS), der elizitor-induzierten Ethylen (ET)-Produktion, des basalen Gehalts an Abscisinsäure (ABA) und des basalen Gehalts an Phasensäure (PA) die Resistenz auf Speziesebene am besten erklären. Die einzelnen Komponenten zeigten quantitativ unterschiedliche Beiträge zur Resistenz von geographisch getrennten Populationen.

Die qualitative Resistenz gegen den pilzlichen Pathogen *C. fulvum* wurde in 15 Populationen von *S. chilense* untersucht. Nach Infiltration von pilzlichen Avirulenzproteinen (Avr) wurden An-/Abwesenheits-Variationen in der Hypersensitiven Reaktion (HR), einer typischen qualitativen Abwehrreaktion, beobachtet. Auch die kanonischen Regionen der Resistenzgene *Cf-4* und *Cf-9* (Cf- 4- und Cf-9-Proteine sind an der Avr-Erkennung beteiligt) zeigten An-/Abwesenheits-Variationen. Darüber hinaus wurde im südlichen Teil des Habitats von *S.*

chilense ein vollständiger Verlust der Erkennung von *C. fulvum* race 5 Avr-Proteinen beobachtet.

Insgesamt zeigen die Daten einen hohen Polymorphismus in den Abwehrreaktionen gegen *P. infestans* und *C. fulvum* innerhalb und zwischen geographisch unterschiedlichen Populationen von S. chilense. Sowohl die quantitativen als auch die qualitativen Resistenzanalysen weisen auf einen Zusammenhang zwischen Resistenzmechanismen und Habitatanpassungen hin.

List of Publications

Parvinderdeep S. Kahlon and Remco Stam (2021) Polymorphisms in plants to restrict losses to pathogens: from gene family expansions to complex network evolution. *Current Opinion in Plant Biology*, 62. DOI: 10.1016/j.pbi.2021.102040; ***peer-reviewed*** **(Publication I)**

Parvinderdeep S. Kahlon, Melissa Verin, Ralph Hückelhoven and Remco Stam (2021) Quantitative resistance differences between and within natural populations of *Solanum chilense* against the oomycete pathogen *Phytophthora infestans*. *Ecology and Evolution*, DOI:10.1002/ece3.7610; ***peer-reviewed*** **(Publication II)**

Parvinderdeep S. Kahlon and Remco Stam (2021). Protocol for chemiluminescence based detection of ROS production in tomato. *protocol.ios*, DOI: 10.17504/protocols.io.beeejbbe; ***non peer-reviewed*** **(Publication III)**

Parvinderdeep S. Kahlon, Andrea Förner, Michael Muser, Mhaned Oubounyt, Micahel Gigl, Richard Hammerl, Jan Baumbach, Ralph Hückelhoven, Corinna Dawid and Remco Stam (2021). Intraspecific diversity observed in the wild tomato species *Solanum chilense* in initial immune responses towards a glucan elicitor. *BioRxiv*, DOI: 10.1101/2021.06.25.449942; ***non peer-reviewed*** **(Publication IV)**

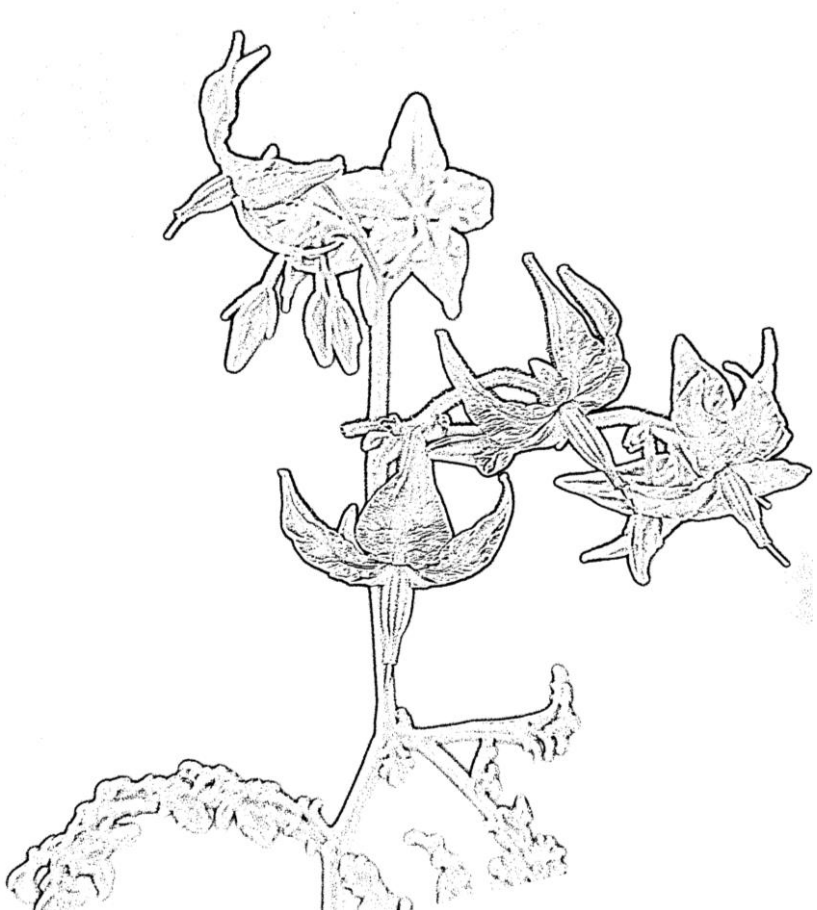
Parvinderdeep S. Kahlon, Shallet Mindih Seta, Gesche Zander, Daniela Scheikl, Ralph Hückelhoven, Matthieu H. A. J. Joosten and Remco Stam (2020) Population studies of the wild tomato species *Solanum chilense* reveal geographically structured major gene-mediated pathogen resistance. *Proceedings of the Royal Society B*, 287. DOI: 10.1098/rspb.2020.2723; ***peer-reviewed*** **(Publication V)**

Abbreviations

Avr	avirulent
Asr	ABA/water stress/ripening induced
ABA	abscisic acid
CEVd	Citrus Exocortis Viroid
Cf	*Cladosporium fulvum*
DPA	dihydrophaseic acid
ETI	effector triggered immunity
JA	jasmonic acid
ET	ethylene
GLMM	generalised linear mixed models
ICE	Inducer of CBF expression
IAA	indoleacetic acid
MAMPs	microbial-associated molecular patterns
MES	2-(N-morpholino) ethanesulfonic acid
MOPS	3-(N-morpholino) propanesulfonic acid
NLR	nucleotide-binding leucine-rich repeat receptors
PAMPs	pathogen-associated molecular patterns
PRRs	pattern recognition receptors
PTI	pattern triggered immunity
PA	phaseic acid
ROS	reactive oxygen species

RLK	receptor-like kinases
RLP	receptor-like proteins
RAD-seq	reduce-representation sequencing
SA	salicylic acid
ToMoV	Tomato mottle virus
TSWV	Tomato Spotted Wilt Virus

Contents



1. Introduction

1.1 Resistance in natural plant populations

In nature, plants are constantly challenged with multiple biotic and abiotic stresses. These challenges are strong forces leading to adaptations, speciation and extinction of a species (Darwin, 1859). The strong role of abiotic factors in triggering the macroevolution of a species and role of biotic interactions to drive evolution in shorter time scales has been widely discussed (Gould, 1985; Myers and Saupe, 2013). Further, a counter argument is emphasized by Vohe *et al.* (2015) regarding the importance of biotic interactions in macroevolution. Biotic interactions that lead to macroevolution are shown in complex food webs that can evolve due to biotic processes like predation and competition (Allhoff *et al.*, 2015). Plants are sessile organisms and the influence of environmental factors (biotic and abiotic) are stronger as they cannot escape it. For survival, plants can adapt to their surrounding environment based on structural and/or physiological mechanisms.

The interaction of a plant with pathogens (biotic stress) effects the individual's fitness and this can alter the evolutionary dynamics of the plant and pathogen populations. Populations can be defined as a group of individuals of the same species present in a geographically distinct location. When an interaction drives changes (and therefore imposes selection), on both plant and pathogen populations, the species are considered to be coevolving (Janzen, 1980). Coevolution of host-pathogen interactions are proposed to follow the trench warfare dynamics also called Red Queen dynamics (Van Valen, 1973) or arms race dynamics. At the genomic level, the trench warfare dynamic proposes that the interacting genetic loci will follow stable cycles of allele frequencies which will result in balanced polymorphisms. On the other hand, arms race dynamics show successive selective sweeps at the interacting loci, i.e., rapid evolution of the genes involved in the interaction and thus low standing genetic variations (Tellier *et al.*, 2014).

How plants resist pathogens in the wild can affect principles of the selection process in plant breeding programs. One of the first systematic reports to document differences in resistance in the natural populations was presented by Burdon in 1980. He showed significant differences in resistance in a population of white clover, *Trifolium repens,* against the fungal pathogens *Cymadothea trifolii* and *Pseudopeziza trifolii*. Plant defence mechanisms can be categorized in

two forms, quantitative and qualitative resistance. Quantitative resistance results in substantial reduction of the disease rather than complete absence of it. Quantitative resistance is driven by multiple underlying genes each with small effects in conferring resistance against pathogen attack and it depends largely on the plant genotype with minor effects of the pathogen genotype (Van der Plank, 1963). Several studies highlight quantitative resistance within a species and hypothesise or show the multigenic effects involved. For instance, *Chenopodium quinoa* resistance against the downy mildew pathogen *Peronospora variabilis* is largely variable among plant genotypes. The resistance was proposed to be a multigenic effect upon evaluation with genome-wide association mapping analysis (Colque-Little *et al.*, 2021).

Plant quantitative resistance mechanisms can be preformed or induced after pathogen recognition (Figure 1). Preformed mechanisms can include structural barriers (such as cell wall, cuticle) or chemical defence compounds like caffeine (for defence against various herbivory). Structural barriers are shown to undergo morphological changes upon contact with the pathogen (e.g., lignification, form of induced resistance mechanism). Lignin is a major component of cell walls of vascular plants and accumulation at the site of infection is a first line of defence (Vance *et al.*, 1980).

Apart from preformed defence and structural changes after pathogen attack, the activation of quantitative defence in plants is often associated with recognition of conserved microbial-associated molecular patterns (MAMPs) also referred to as pathogen-associated molecular patterns (PAMPs) by plant cell surface-localized pattern recognition receptors (PRRs) leading to pattern-triggered immunity (PTI). The activation of defence responses in plants after perceiving MAMPs leads to a complex signalling cascade and can be quantified in plants by measuring rapid production of reactive oxygen species (ROS), calcium influx and/or defence related phytohormone accumulations.

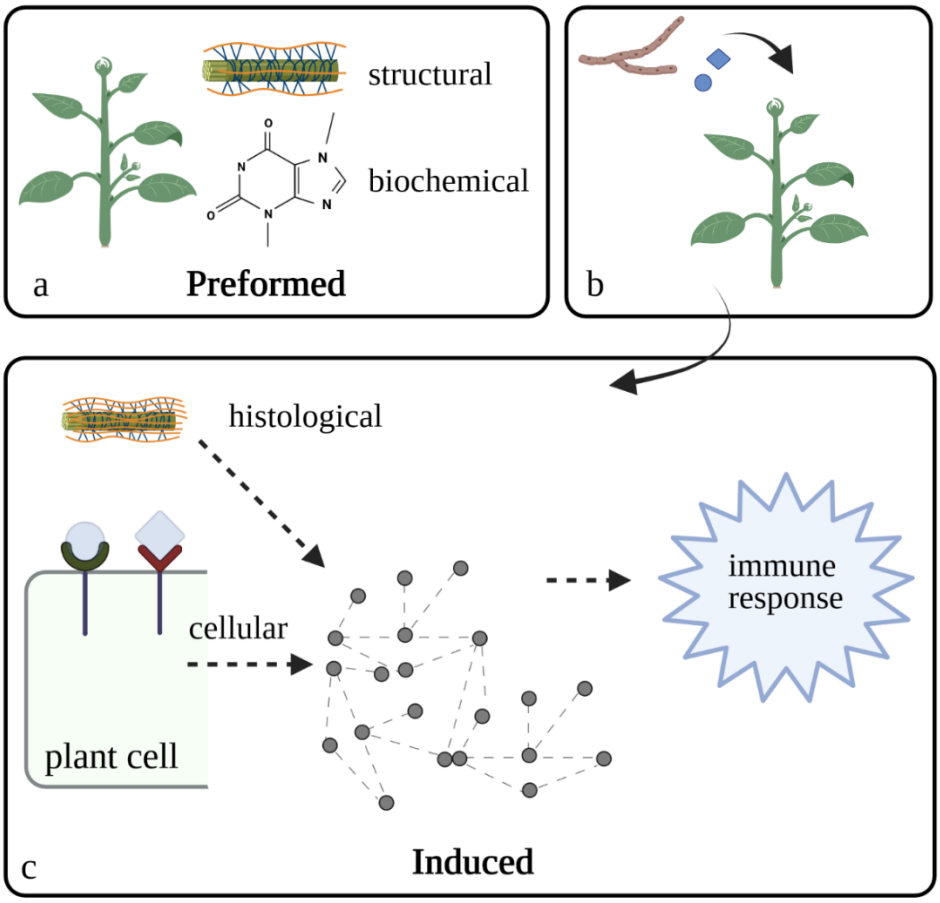


Figure 1: Schematic of components of quantitative resistance, plant survival strategies rely on preformed a) or/and induced defence mechanisms upon pathogen attack (b) and (c); preformed defence mechanisms include structural, such as plant cell wall, and/or biochemical defence mechanisms, like chemical compounds (e.g. caffeine) (a); Plants can sense pathogens and activate histological defence responses (e.g. lignification) or recognise pathogenic molecules via plant cell surface receptors and activate a cascade of signalling (e.g. production of phytohormones) leading to activation of basal immune responses (c).

The activation of defence can be counteracted by the pathogen by secreting effector proteins into the plant cells. Effectors are pathogenic proteins expressed inside the host cell and are often associated with host manipulation for successful survival and multiplication of a pathogen in the host. However, completely resistant plants have an additional set of receptors called resistance (R) proteins (most known ones are from the intracellular nucleotide-binding leucine-rich repeat receptors (NLR) family and receptor-like kinases/proteins (RLK/P) family), which can recognize various effector proteins from the pathogen. The effectors which can be recognised directly or indirectly by these R proteins are then called avirulence (Avr) proteins. Therefore, the recognition of Avr proteins by R proteins follow the so-called gene-for-gene interaction (proposed by Flor, 1942) and lead to activation of effector-triggered immunity (ETI; Figure 2). Such resistance is also denoted as qualitative resistance or major gene-mediated resistance.

In qualitative resistance, a plant relies on the recognition of a race-specific pathogenic protein and activation of resistance to completely halt the further multiplication and survival of the pathogen. It has been shown that presence of intragenic recombination among the homologs of a major-resistance gene family confer qualitative resistance in the natural populations of *Solanum pimpinellifolium* against the fungal pathogen *Cladosporium fulvum* and that this resistance is associated with the geographical location of the plant populations (Van der Hoorn *et al.*, 2001). Thrall *et al.* (2002) used the *Linum marginale–Melampsora lini* host-pathogen system which predominantly shows a gene-for-gene resistance/virulence structure and highlighted the role of local adaptation where the most resistant host harboured the most aggressive pathogen isolate. Similarly, resistance in the wild population of *Salix triandra* against *Melampsora amygdalinae* is reported to have resulted also from local adaptation where highly virulent pathogenic isolates were found on highly resistant plant populations (Niemi *et al.*, 2006).

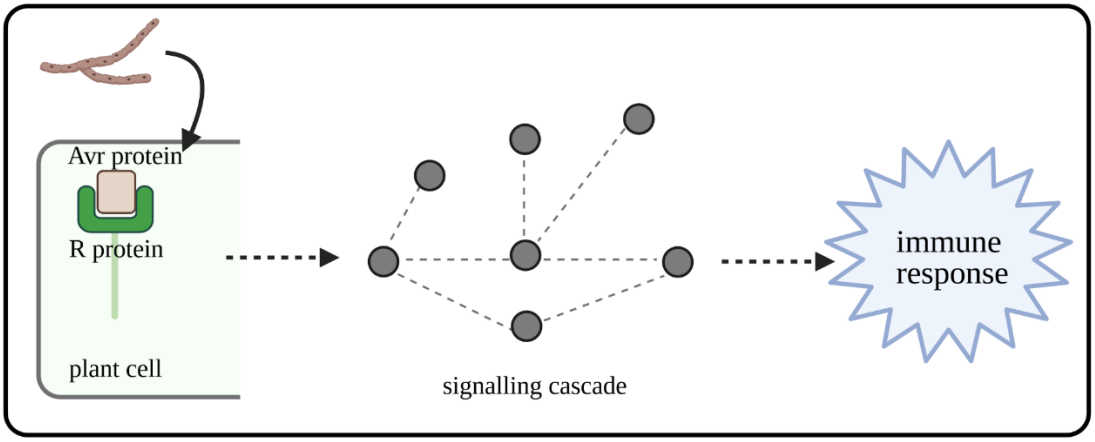


Figure 2: Schematic of qualitative resistance in plants; recognition of avirulence (Avr) proteins by a plant resistance (R) protein leads to activation of downstream signalling pathways leading to a immune response (e.g., hypertensive response; HR), a typical form of programmed cell death in plants, to halt further multiplication of the pathogen.

The plant immune system is often shown as a two-branched system, contrasting PTI and ETI (Jones and Dangl, 2006). Recent studies showed that resistance proteins involved in ETI work in a complex network to achieve the resistance in plants (Wu *et al.*, 2018, Wu *et al.*, 2019). In addition, the two branches PTI and ETI work not only independently but also in coordination to achieve effective defence responses (Ngou *et al.*, 2021; Yuan *et al.*, 2021).

1.2 Polymorphisms in molecular cues for delivering defence response

Polymorphisms in the plant-pathogen interactions hold a key to coevolutionary outcomes. Polymorphism in the perception of a bacterial peptide of flagellin, flg22 (representing a MAMP), across 22 tested species of Brassicaceae is reported (Vetter *et al.*, 2012). The authors showed flg22 binding specificity to the plant receptor kinase flagellin sensing 2 (FLS2) is highly variable between and within the species. This binding was further confirmed to be dependent on the protein abundance of the receptor FLS2 (Vetter *et al.*, 2012). Polymorphism in molecular cues delivering defence against the bacterial pathogen *Pseudomonas syringae* has been evaluated in 1041 accessions of *Arabidopsis thaliana*. Molecular mechanisms resulting in distinct responses in 14 resistant accessions were reported for delivering the resistance. This resistance was further categorized in to four mechanisms: accumulation of the phytohormone salicylic acid (SA) in three accessions, presence of surface barriers in the leaves of two accessions, a resistance gene mediated-like response in six accessions and an uncharacterized response in the remaining three accessions (Velásquez *et al.*, 2017).

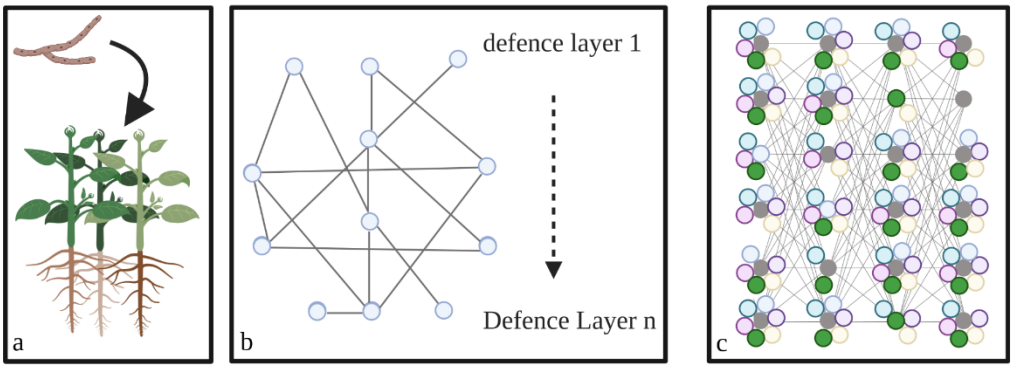


Figure 3: Polymorphisms in the plant defence network at the population level: Upon pathogen attack, plant defence relies on preformed or induced mechanisms (a); These mechanisms often work in intricate networks to achieve resistance (b); At the population scale, such networks can be depicted (c); where immunity component at different layers are expected to be of polymorphic nature.

The expected way to generate polymorphic defence responses in the natural population is depicted in Figure 3 (adapted from Publication I). A large amount of diversity is expected to be playing a role in defence mechanisms in wild populations. When plant populations encounter a pathogen (Figure 3a), plants can defend themselves with multiple layers of sophisticated surveillance such as physical barriers or chemical defences. In addition, plants can sense pathogenic molecules and activate defence pathways (Figure 3b) leading to physiological changes. The complexity increases when these immunity components are considered at the population level where most of the defence mechanisms can be expected to

be polymorphic (Figure 3c). Polymorphism in defence responses at the population scale are studied to elucidate evolutionary mechanisms and adaptations involved in the species.

1.3 Wild tomatoes are instrumental to study polymorphisms

Taxonomically, *Solanum* is one of the largest genera of angiosperms, consisting of approximately 1,500 species. Wild tomato species represent the section *Lycopersicon* in the genus *Solanum*. The known natural habitat of wild tomatoes ranges from Ecuador to northern Chile and the Galapagos Islands (Rick 1986; Taylor 1986; Spooner *et al.*, 2005). Due to the diverse habitats of wild tomatoes, they are used as a model system to study various adaptational processes. This in turn led to efforts of researchers to curate huge seed banks (such as C.M. Rick tomato genetic resource center, UC, Davis) and create rich genomic resources (such as Sol genomic consortia). 84 tomato accessions (54 crop accessions and 30 wild relative accessions) were sequenced in 2016 and wild species showed up to 20-fold higher number of single-nucleotide polymorphisms (SNPs) as compared to crop accessions (The 100 Tomato Genome Sequencing Consortium). Recently, Alonge *et al*. (2020) showed an enormous number of structural variants present in the evaluation of 100 tomato genomes. Included in these studies were the wild tomato species *S. pimpinellifolium, S. cheesmaniae*, *S. galapagense* and early domesticated form, vintage, and modern varieties of *S. lycopersicum*. High variation in copy number, presence-absence, duplication or rearrangements of structural variants in these species was shown to be prominent (Alonge *et al*., 2020). Due to the presence of this high occurrence of polymorphisms, wild tomato species are shown to be an important germplasm source for improvement in the disease resistance, yield and stability of tomato cultivars (Hajjar and Hodgkin, 2007).

1.3.1 Basal resistance observed in *Solanum* spp.

Due to *Solanum* spp. economic importance, they are used to study variation in disease responses. Quantitative resistance was observed in one accession of *S. habrochaites*, and two accessions of *S. arcanum* when compared with cultivated *S. lycopersicum* against two isolates of a bacterial canker causing pathogen, *Clavibacter michiganensis*. Interestingly, the authors showed that the bacteria can colonize all tested species, but wild tomatoes have reduced systematic spread (Peritore-Galve *et al.*, 2020). A panel of 216 accessions (consisting of wild tomato *S. pimpinellifolium* and accessions of cultivated tomato *S. lycopersicum*) were inoculated with three *P. syringae* pv. tomato (*Pst*) strains: NY15125 (*Pst25*) and two DC3000

strains lacking specific effectors. Novel infection phenotypes were observed: some atypical for this host-pathogen system, such as short holes, stem lesions, stem galls, necrotic spots, chlorosis, water soaking, necrosis, and abscission. In addition, variation in ROS production upon elicitation with the flagellin-derived peptides, flg22 and flgII-28 was reported (Robert *et al.*, 2019). Variation in quantitative resistance in domesticated and wild tomatoes has also been reported against the generalist fungal pathogen *Botrytis cinerea* and is driven by complex host and pathogen genotype interactions. Multiple loci from host and pathogen in combination determined the level of resistance (Soltis *et al.*, 2019).

1.3.2 Major gene-mediated resistance in *Solanum* spp.

Major gene-mediated resistance is well studied in wild tomato and has been used in crop protection programmes. Wild tomato species have been used to mine new unexplored resistance genes active against various fast-evolving pathogens on the crop. One popular example is resistance against the greenhouse pathogen *C. fulvum* is delivered by the product of *Cf* (*Cladosporium fulvum*) resistance genes in the form of hypersensitive response upon recognising Avr proteins. Cf proteins are members of the receptor-like proteins (RLP) family. *Cf* genes are shown to be present in many tested wild tomato species. Several functional allelic variants of *Cf-4* and *Cf-9* gene sequences from various wild tomato species have been reported (Krujit *et al.*, 2005). In *S. pimpinellifolium,* a recombinant variant of the *Cf-9* (*9C*) with it allele *9D* has been reported and was named *9DC* which was functionally active against pathogenic protein Avr9 (Van der Hoorn *et al.*, 2001). Additionally, 26 variants of *Cf-2* in *S. pimpinellifolium* have been reported with presence-absence variation at the genomic level (Caicedo and Schaal, 2004). Another important family for major gene-mediated resistance resistance is the NLR gene family. When evaluated in *S. pennellii,* NLR genes showed a low number of polymorphisms in the populations (Stam *et al.*, 2016). Surprisingly, in the same study some NLRs showed large variation within a population, a notable example was an ortholog of *RPP13,* which is also known to be highly polymorphic in *A. thaliana* (Rose *et al.*, 2004). *Pto is* another another major resistance gene which is shown to have sequence variation, which largely influences resistance in *S. pimpinellifolium* against the bacterial pathogen *P. syringae* (Rose *et al.*, 2005). Five genes from the *Pto* resistance pathway in *S. peruvianum* were studied in detail and depending on the placement of the gene product in the signalling pathways, the selection pressure on the genes varied. Genes encoding proteins which act rather upstream in the signalling pathway where under purifying selection and genes encoding downstream

components in the pathway showed balancing selection (Rose *et al.*, 2011). In summary, studying major resistance gene families in wild tomato species has been instrumental in exploring evolutionary pressures on different gene candidates.

1.4 *S. chilense* as a potential model to study polymorphism in context of plant-pathogen interactions

Wild tomato diversity in defence related genes make them a good model system to study plant evolution. *S. chilense* allows the possibility to study some aspects in detail, like diversity and adaptation processes that are based on the plants' demography. *S. chilense* genomes have shown to exhibit high nucleotide diversity and high recombination rates. It is also shown to outcross and possess high gene flow events within the populations (Arunyawat *et al.*, 2007). The *S. chilense* habitat ranges from southern Peru to northern Chile and the plant populations grow in deserts, mountains and coastal regions (Peralta *et al.*, 2008; Chetelat *et al.*, 2009).

Based on an elaborative study of 23 *S. chilense* populations, sequencing data from 30 genes was used to propose four genetic groups of the species (Böndel *et al.*, 2015). The four groups were described as northern, central and two southern groups. The study suggests the origin of the wild tomato species to be in the north of the species habitat with two independent colonization events towards the south, one to the coast of northern Chile and the other to the Chilean Andes. Northern populations showed high genetic diversity when compared to the southern populations. The two independent southward colonisations of *S. chilense* proposed by Böndel *et al*. (2015) were further supported by Stam *et al.* (2019a) with whole-genome resequencing data and a targeted sequencing approach for both NLRs and neutral genes to assess the demography. Further, Raduski and Igic (2021) were able to show evidence that the southern coast and the southern highland populations represent a separately evolving population based on reduce-representation sequencing (RAD-seq).

1.4.1 Abiotic stress tolerance observed in *S. chilense*

S. chilense is known to populate the most extreme environments among wild tomatoes. It can grow and thrive under the driest and coldest conditions when compared to other wild tomato species (Moyle, 2008). Different populations are reported to inhabit the extreme dry regions on the edges of the Atacama Desert as well as near rivers and creeks (Peralta *et al.*, 2008). Few examples include, the populations LA2750 and LA1958, which are present in the region with the lowest annual precipitation recorded among the wild tomato habitats. Populations LA3111,

LA4330 and LA4117A are found in the regions with the highest diurnal temperatures (Moyle 2008; Nakazato *et al.*, 2010). Due to its extreme condition habitats, *S. chilense* has served as a model system for many researchers to explore the signature of adaptations regarding drought, salt, and temperature stresses.

The *Asr* (absisic acid; ABA/water stress/ripening induced) gene family members were shown to be under different evolutionary forces. For *Asr1*, strong purifying selection was confirmed based on low sequence polymorphism in the coding regions, whereas the evaluation of *Asr4* showed patterns of local adaptations to dry climates (Fischer *et al.*, 2011). The presence of nucleotide diversity and cues of local adaptation was evident in two genes (*LeNCED1* and *pLC30*-15) that are involved in the phytohormone ABA biosynthesis (Xia *et al.*, 2010). The ABA pathway is involved in drought stress adaptation and these two genes showed distinct nucleotide diversity. *LeNCED1*, one of the genes involved in the pathway of ABA synthesis, showed extremely low nucleotide diversity suggesting strong purifying selection on the gene. However, *pLC30-15* which is acting downstream in the same signalling pathway as *LeNCED1* for drought tolerance, showed high genetic diversity as compared to the reference loci in the anaylsed populations which indicates a role in local adaptation. This study confirmed the hypothesis that upstream genes evolve rather slowly because of the higher number of functions as compared to downstream genes.

Cold stress tolerance has been another focus in studies looking into local adaptations of *S. chilense.* An association of tolerance to freezing with high altitude and low temperature is shown (Nosenko *et al.*, 2016). Signatures of positive selection were shown to be present in sequence diversity of the well-studied *ICE1* gene involved in the cold-stress responses in plants.

In 23 populations of *S. chilense*, 16 genes involved in abiotic responses such as salt, drought and cold stress were studied. High nucleotide diversity was observed at nonsynonymous sites of these genes when compared to reference genes, which indicates potential positive selection pressure. Local adaptation based on observed high nucleotide diversity at the edge of the species distribution was apparent at the southern coastal and highland populations with extreme environmental stresses (Böndel *et al.*, 2018).

1.4.2 Biotic stress tolerance reports on *S. chilense*

The extreme habitats of *S. chilense* also gives rise to the possibility of the species to encounter not only different abiotic stresses but also different biotic stresses. Different environments are expected to house different pathogens, in turn, generating diversity in the plants' response against these pathogens. *S. chilense* showed the highest level of resistance when compared to *S. pimpinellifolium, S. habrochaites* and *S. peruvianum* against tomato yellow leaf curl virus (Zakay, 1991). In breeding programs, three out of five widely exploited virus resistance genes (namely *Ty-1*, *Ty-3* and *Ty-4*) against yellow leaf curl virus originated from *S. chilense* (Zamir *et al.*, 1994; Hanson *et al.*, 2006; Ji *et al.*, 2007; Ji *et al.*, 2009; Anbinder *et al.*, 2009; Hutton *et al.*, 2012). However, a later study by Verlaan *et al.* (2013) showed *Ty-1* and *Ty-3* to be allelic. Tomato mottle virus (ToMoV) resistance was reported in 25 out of 36 tested *S. chilense* accessions (Scott and Schuster, 1991). Introgression lines obtained from the resistant accession of *S. chilense* crossed with cultivated tomato were proposed to deliver multigenic resistance (Scott *et al.*, 1996). The idea of multiple genes involved in ToMoV resistance was further confirmed by Griffiths and Scott (2001). The authors reported the involvement of at least two genes in the accession studied to confer resistance against ToMoV.

Phenotypic variation in resistance against three common filamentous pathogens of tomato, *Phytophtora infestans*, *Alternaria solani* and a *Fusarium* spp. were shown by Stam *et al.* (2017). The populations under study showed quantitative variation in resistance with no clear correlations to the habitat of the populations. Polymorphisms in resistance at the molecular level have been studied in *S. chilense.* For example, a high level of amino acid polymorphisms of the resistance protein Pto was observed when compared to other wild tomato species (Rose *et al.*, 2007). The nucleotide diversity in the NLR family in 14 populations of *S. chilense* consisting of 10 plants per population was evaluated (Stam *et al.*, 2019a). This study performed in-depth target resequencing of successfully enriched NLRs covering the whole species range. Interestingly, evidence was observed of variable selection pressure on individual NLRs in each population. NLRs showed positive or balancing selection depending on the population under consideration. Only a few NLRs consistently showed positive or balancing selection throughout the species. Based on these findings, the study concluded that the variety of selective pressure on the NLRs is potentially based on habitat and corresponding adaptation to the specific environment of *S. chilense*.

Recently, draft genome and transcriptome sequence assemblies of *S. chilense* were published (Stam *et al.*, 2019b) which provide an additional resource for genetic studies. Hence, *S. chilense* serves a favourable host system to explore host-pathogen interactions in a geographical context.

1.5 Objectives and methodology

This dissertation work aims to exploit *S. chilense* as a model system for studying the diversity in plant defence mechanisms at a species wide scale using natural populations from distinct geographic origins and to elucidate possible signatures of adaptations. In this dissertation the following objectives are addressed in detail:

1. Polymorphisms in defence responses of plants are reported at the phylogenetic and population scale. The recent studies highlighting such polymorphisms were presented in a review (Publication I) and importance of studying plant immunity in networks is discussed.

2. To access in detail, the role of plant and pathogen genotype in the previously observed phenotypic variation of *S. chilense* against *P. infestans* (Stam *et al.,* 2017), phenotypic screening of plants was performed. Repeated drop inoculations with one *P. infestans* isolate's sporangia solution were tested on detached leaves. In addition, sporangia solutions of seven *P. infestans* isolates were drop inoculated on detached leaves of one population (9 plants). Infection outcomes were recorded in the form of infection frequency, representing a ratio of the infected leaflets to inoculated leaflets per leaf. Further, using mathematical modelling, the role of plant and pathogen genotype on the resistance outcomes were evaluated. The findings from this study are presented in Publication II.

3. Characterisation of the responsible immunity components underlying quantitative resistance were observed in the populations of *S. chilense* (Publication II). Firstly, due to no reproducible measurement method for detecting ROS production in leaf discs of *S. chilense*, a robust measurement method was developed (Publication III). Secondly, different basal resistance components for all the tested plants used for objective 2 were measured upon elicitation with a glucan elicitor. Further, linear correlations and GLMM were generated to evaluate the effects of these measured components independently and in combination. The findings of this study are shown in Publication IV.

4. Assessment of the major-gene mediated resistance in a species-wide screening in geographically defined populations of *S. chilense* against the fungal pathogen *C. fulvum*. In this study, defence responses upon infiltration of different effectors secreted by the fungus as well as presence-absence variation of two major resistance genes were evaluated. The results are presented in Publication V.

Qualitative and quantitative resistance evaluation and documentation in natural populations in a systematic approach can further help to evaluate potential evolutionary pressures and adaptation processes in plant-microbe interactions. This dissertation provides three systematic research studies performed on natural populations of *S. chilense* against two common filamentous pathogens of tomato.

2. Results and Embedded Publications

2.1 Polymorphisms in plants restrict losses to pathogens: from gene family expansions to complex network evolution

Summary

In plants, polymorphisms have been shown to be a key in maintaining diversity in biological processes. Polymorphisms in defence related responses are important for the survival against certain enemies. This review discusses recent studies showing the presence of polymorphisms in defence-related components. The major focus of this study was on the model species *Arabidopsis thaliana* and some representatives of *Solanum* spp.

The immense diversity observed on phenotypic and genotypic levels exert evolutionary forces which varies depending on the host-pathogen interaction under study. On the genetic level, studies have shown that the evolutionary pressure can be different on different genes from the same gene family within a species. Studies in this review are discussed which show high diversity in disease symptoms on plants and polymorphism in microbial-associated molecular patterns (MAMPs)-mediated responses. In addition, cases of polymorphisms in major resistance gene families are also highlighted.

Recent studies highlight involvement of multiple genes working in networks in plant immune responses against certain pathogens. This makes the evaluation of evolutionary forces exerted on such immune networks complex. Further, using -omics studies and combining them with network biology and evaluating the evolutionary mechanisms will pose challenges in studying polymorphisms in resistance at a population scale in the future.

Work is published in *Current Opinion in Plant Biology*, (2021) 62. DOI: 10.1016/j.pbi.2021. 102040.

Authors: Parvinderdeep S. Kahlon and Remco Stam

Contributions

I drafted the initial review and figures. After critical evaluation and additions by Remco Stam, I finalised the review for submission.

2.2 Quantitative resistance differences between and within natural populations of *Solanum chilense* against the oomycete pathogen *Phytophthora infestans*

Summary

Quantitative resistance, often also called incomplete or basal resistance, is predominant in natural populations. In this study, 85 plants originating from nine distinct populations of *S. chilense* covering four geographical groups of the species were tested for their phenotypic differences in resistance against *P. infestans*. Detached leaf infection assays using drop inoculations were performed and the infection frequency of plants was calculated 7 days post inoculation with *P. infestans* isolate Pi100. Quantitative resistance diversity within and between populations was observed with no clear geographical patterns correlating to the resistance.

Further, the resistance of 9 plants from the central population LA3111 was tested against seven isolates of *P. infestans* with known phenotypic and genotypic diversity. Multi-way ANOVA evaluation confirmed no significant role of pathogen-host genotype interaction in the resistance outcome. Generalised linear mixed models (GLMMs) were generated to test the effect of plants and pathogen isolate on the infection frequency. A higher effect of the plants was observed compared to the effect of *P. infestans* isolate on the infection frequency outcome. The study showed high intraspecific variation in quantitative resistance in these nine tested natural populations of *S. chilense* against *P. infestans*. The observed resistance can be considered a basal resistance response against pathogens.

Work is published in *Ecology and Evolution* (2021), DOI:10.1002/ece3.7610

Authors: Parvinderdeep S. Kahlon, Melissa Verin, Ralph Hückelhoven and Remco Stam

Contributions

I performed all experiments in the study. I did the data analysis with help and input of all other authors. I wrote the initial draft of the manuscript and generated figures which were critically revised by the other authors. I finalised the manuscript after feedback from authors and reviewers.

2.3 Protocol for chemiluminescence based detection of ROS production in tomato

Summary

ROS production analysis is one of the widely used methods as it is one of the key indicator in plants for an activation of the basal immune response. The production happens during the initial few minutes after a plant encounters and perceives the pathogenic molecules. A chemiluminescence based assay can be performed for detection of ROS production with leaf discs on 96 well plates. This assay is commonly used for the model plant *Arabidopsis thaliana*. When performed with *S. chilense* leaf discs, however, the results were not reproducible. This might be due to the high production of compounds which potentially hinder the chemiluminescence reaction during the measurements. Therefore, two buffering systems, 2-(N-morpholino) ethanesulfonic acid (MES) and 3-(N-morpholino) propanesulfonic acid (MOPS) as well as water as control were tested. Leaf discs were incubated overnight in either 20mM MES (pH: 5.5), 20mM MOPS (pH 7.5) or water. The following day chemiluminescence based measurement in respective buffers was performed after elicitation with the flagellin peptide flg22. The data showed that the most reproducible and robust results were obtained with the MOPS buffered protocol.

Work is published in *protocol.ios*, (2021) DOI:10.17504/protocols.io.beeejbbe (non peer-reviewed)

Authors: Parvinderdeep S. Kahlon and Remco Stam

Contributions

I performed the method development assays with different buffering systems and wrote the protocol with input from Remco Stam.

2.4 Intraspecific diversity observed in the wild tomato species *Solanum chilense* in initial immune responses towards a glucan elicitor

Summary

Plants can sense pathogens through the perception of microbial-associated molecular patterns (MAMPs) and depending on the pathogen these MAMPs can vary. In this study a glucan elicitor laminarin was used which shares a structural homology with cell wall component of oomycetes. To evaluate if it can activate basal defence responses similar to *P. infestans* in *S. chilense* plants, RNAseq was performed on the plants from a central population, LA3111, of the wild tomato species *S. chilense*. At three hours after elicitation with laminarin or inoculation with *P. infestans* the plants' differentially expressed genes (DEGs) were compared. Key regulatory genes of reactive oxygen species (ROS) production and defence-related phytohormones pathways were shown to be differentially expressed. The DEGs from laminarin elicited samples showed a considerable overlap with DEGs from samples inoculated with *P. infestans.* This confirmed that laminarin is capable to trigger a subset of oomycete-associated defence responses. Further, basal immune responses upon elicitation with laminarin were studied in nine populations (83 plants) and a high diversity in ROS and ethylene (ET) production was observed within and between populations. Interestingly, significant differences in basal levels of the phytohormones salicylic acid (SA), abscisic acid (ABA), indoleacetic acid (IAA) and phaseic acid (PA) within and between populations were recorded. Generalised linear mixed models (GLMM) were generated to evaluate the effects of these components on resistance phenotypes observed in Publication II. Additive effects of induced ROS, ET and basal levels of ABA and PA showed the lowest Akaike information criterion (AIC) and lowest Bayesian information criteria (BIC) values which suggest it is the best fitting model. When considering individual immunity components, ET production upon elicitation with laminarin showed the strongest correlation with the resistance phenotype in the southern coastal populations. Finally, two plants from the southern coast population were tested and showed significantly higher disease severity upon chemical inhibition of ET as compared to controls.

Work is published in *bioRxiv* (2021) DOI: 10.1101/2021.06.25.449942 (non peer-reviewed)

Authors: Parvinderdeep S. Kahlon, Andrea Förner, Michael Muser, Mhaned Oubounyt, Michael Gigl, Richard Hammerl, Jan Baumbach, Ralph Hückelhoven, Corinna Dawid and Remco Stam

Contributions:

I performed the ROS production screening of all populations, ET for two populations and for other phytohormone evaluation I performed the treatment of plants with the help of Andrea Förner, I also extracted phytohormones from all samples with the help of Michael Gigl. I did data analysis and evaluation for ROS. I also performed data analysis and evaluation for ET production with Michael Muser and for other phytohormones (SA, jasmonic acid (JA), ABA, PA, IAA, dihydrophaseic acid (DPA)), with Michael Gigl. I performed qPCR on key regulators of ET and qPCR data evaluation of key regulators of ET, SA and JA with Michael Muser and Andrea Förner. I performed ET inhibition assays. I did the statistical analysis and generated GLMM for quantitative resistance. I wrote the first draft of the paper and generated the figures and tables (Figure 2-6; Table 1 and 2; Table S1, S4-S16) and after critical evaluation from other authors, I finalised the draft of the manuscript and submitted it to the preprint server.

2.5 Population studies of the wild tomato species *Solanum chilense* reveal geographically structured major gene-mediated pathogen resistance

Summary

Major gene-mediated (*R*) immunity in natural populations is shown in various plant-pathogen systems but is often studied in restricted host habitat coverage. In this study, major gene-mediated resistance against the fungal pathogen *C. fulvum* was evaluated covering the whole geographical habitat of *S. chilense*. The differences in resistance of two populations LA3111 (central) and LA4330 (southern highlands) of *S. chilense* against *C. fulvum* were recorded. Microscopic and phenotypic evaluation was preformed upon spray inoculation with *C. fulvum* on leaves of *S. chilense*. Further, the differences in resistance of five plants per population was confirmed using qPCR detection of pathogen load. The southern population LA4330 showed significantly higher pathogen load when compared to population LA3111.

Resistance to *C. fulvum* in tomato is based on gene-for-gene interaction among the avirulence (Avr) protein from the pathogen and plant resistance protein (Cf). Successful interaction results in hypersentive response (HR) which can easily be measured by a naked eye. Infiltration assays were performed on 15 populations with Avr4, Avr9 and apoplastic wash fluid (AF) containing a mix of all the Avr secreted by *C. fulvum* race 5 in the apoplast of a susceptible tomato. The results showed presence-absence variation in HR with complete loss of recognition at the edge of the species range. Further, presence-absence variation in the canonical region of major-resistance gene *Cf-4* (Cf-4 protein interacts with Avr4) was observed. The presence of the canonical region of *Cf-9* and absence of canonical region of allelic variant *9DC* (Cf-9 and 9DC protein interact with Avr9) was observed in nine tested populations. The presence of HR in plants upon infiltration of Avr4 showed no correlation with the presence of the canonical region of *Cf-4* leading to the conclusion that Avr4 recognition is possibly delivered by Cf-4 variants. With this study firstly, the complexity in the central population at the *Cf-4/Cf-9* locus was highlighted. Secondly, the possibilities of loss of recognition in the southern populations is hypothesised due to ecological factors and/or additionally trade-off mechanisms.

Work is published in *Proceedings of the Royal Society B*, 287. (2020) DOI:10.1098/ rspb.2020.2723.

Authors: Parvinderdeep S. Kahlon, Shallet Mindih Seta, Gesche Zander, Daniela Scheikl, Ralph Hückelhoven, Matthieu H. A. J. Joosten and Remco Stam

Contributions

I performed the infection assays with *C. fulvum* on LA3111 and LA4330 and performed qPCR for quantifying the pathogen load in the plants. I performed the infiltration assays with Avr9, Avr4 and mix of Avr for the populations LA1963, LA3111, LA2747, LA4330 and LA3786 and assisted Gesche Zander for populations LA1958, LA2746, LA2759, LA2931, LA3784 and additional plants for LA3111. I performed PCR to detect the canonical regions of *Cf-4* and *9DC* in all the populations presented in the manuscript and together with Shallet Mindih Seta, I also evaluated the *Cf-9* canonical region in all the populations. I wrote the initial draft and generated all figures and I generated tables (S1-S3, S5) for the manuscript. I corrected the manuscript after critical comments from the other authors and reviewers.

3. Discussion

Natural populations have long been used to explore polymorphisms in defence responses. Habitat adaptations can lead to diversity in defence responses in natural plant populations. While looking at the effect of environmental conditions on the outcome of resistance, Ogran *et al.* (2016) highlight two independent mechanisms to deliver resistance in two natural populations of *Eruca sativa* against *Spodoptera littoralis* depending on the habitat of the host. The mediterranean population had a high accumulation of glucosinolates and the desert population showed high trypsin proteinase inhibitor activity when challenged with the generalist herbivore *Spodoptera littoralis*. Another example is the resistance in *Senecio vulgaris* (groundsel) against *Erysiphe fischeri* that showed immense differences in phenotypes and different host lines possibly have evolved different survival strategies against the same pathogen leading to the conclusion that resistance was an outcome of the combined roles of the ecosystem and heterogeneity of the host (Beevan *et al.*, 1993). Further distinct pathways to activate immune signalling in *A. thaliana* were shown (Velásquez *et al.*, 2017) which can also be speculated to be based on distinct habitat adaptation of different accessions of *A. thaliana* originating from different geographical locations.

3.1 *S. chilense* as a model to study quantitative resistance against various pathogens

Variation in disease resistance was observed in geographically distinct populations of *S. chilense* against three filamentous pathogens (Stam *et al.*, 2017) and was proposed to be potentially due to effects of local environmental and habitat adaptations. In this dissertation, Publication II provides an in-depth evaluation of resistance to *P. infestans* and showed high diversity within and between population resistance against different isolates of *P. infestans*. Further, selection of *S. chilense* plants which were also tested against multiple isolates has shown no strong pathogen isolate effect overall leading to the conclusion of no clear signatures of host adaptations. The absence of adaptation to local pathogens can be due to a high tendency of *S. chilense* to outcross (Graham, 2005). Outcrossing across the species leads to higher gene flow and sweeping of the clear case of local adaptations (Kawecki and Ebert, 2004; Lenormand, 2002). Such mechanisms of unclear patterns of local adaptation have been highlighted between the nematode *Globodera pallida* and 12 wild potatoes from Peru (Gautier *et al.*, 2020). In Publication II, 7 isolates of *P. infestans* which included only one isolate from South America were tested. Therefore, it is a possibility that *S. chilense* plants show signatures of local adaptation when tested against locally isolated *P. infestans*.

Additionally, an interesting aspect to consider in future studies would be the population fragmentation and connectivity within the four geographically defined groups of *S. chilense* and their role in resistance outcome. Population fragmentation has been highlighted in resistance of *Plantago lanceolata* against *Podosphaera plantaginis* by Höckerstedt *et al.* (2018). The authors showed that well-connected populations exhibit higher fluctuation over the years in disease severity when compared to fragmented populations. The same study also showed that well-connected populations had a better response against pathogen attack when compared to fragmented populations. Data on resistance of *S. chilense* against *P. infestans* shown by Stam *et al.* (2017) and Publication II, partially supports this observation because on average high susceptibility is observed in populations from the southern highland group. These populations are expected to be more fragmented, in regard to distance and connectivity to the neighbouring populations. But to conclude the role of fragmentation further studies are needed with on-site data curation on the distance among different populations and possible connectivity among these populations within the four geographically distinct locations. Overall, Publication II in the dissertation showed basal resistance of *S. chilense* against *P. infestans* which represents the response against pathogen isolates which are non-local and un-adapted.

3.2 Quantitative resistance observed in *S. chilense* relies on multiple factors

Basal resistance is often multigenic and has been shown in different plant populations against *Phytophthora* spp.. For example, in European oak the resistance against *Erysiphe alphitoides* and *Phytophthora cinnamomi* (Bartholomé *et al.*, 2020). To evaluate the possible effects underlying the quantitative resistance in our system of *S. chilense* against *P. infestans*, an analytical approach was used and key immunity components of basal resistance were measured (Publication IV). The defence responses were evaluated upon elicitation with laminarin which is a glucan elicitor and structurally similar to components of the oomycete cell wall (Aroson *et al.*, 1967). Laminarin has been shown to activate early defence responses in *Nicotiana* spp. which are members of Solanaceae family (Meénard *et al.*, 2004; Wanke *et al.*, 2020). In publication IV, laminarin treated plants showed significant overlap in transcript levels of defence-related genes when compared to *S. chilense* plants treated with *P. infestans*.

S. chilense showed differences in laminarin-triggered ROS production. High variation within and between populations was observed but no correlation to the resistance phenotype was apparent (Publication IV). Such variations in ROS without a correlation to resistance phenotype

have been reported by Robert *et al.* (2019) in 58 accessions of *S. pimpinellifolium* in response to flg22 and flgII-28 peptides. Further, the defence-related phytohormones; ET, SA, JA, IAA, ABA, PA and DPA were also evaluated. For ET, a large variation in accumulation within and between the populations was observed. Higher ET accumulation in *S. chilense* plants was significantly correlated to *P. infestans* resistance phenotype. ET levels have also been shown to be of importance for *P. infestans* resistance in mature *Nicotiana benthamiana* plants (Shibata *et al.*, 2010; Matsukawa *et al.*, 2013). The potential of exogenous ET in activating defence responses in a resistant potato genotype was shown by Yang *et al.* (2020).

JA was not detected in our plant samples and laminarin-triggered SA was not significantly different than the basal levels. In *Nicotiana tabacum* and *A. thaliana* it has been shown that only sulphated laminarin can induce the SA pathway when compared to non-sulphated laminarin (Meénard *et al.*, 2004). Differences in basal levels of SA within and between populations were observed but not correlated to the resistance phenotype. In *Solanum tuberosum,* the lack of influence of basal SA in resistance against *P. infestans* has been reported, as transgenic lines lacking SA were not affected in resistance (Yu *et al.*, 1997). Although the basal level of SA in the plants has been shown to be prominent in defence against Citrus Exocortis Viroid (CEVd) or Tomato Spotted Wilt Virus (TSWV) (López-Gresa *et al.*, 2016). *S. chilense* harbours resistance against viruses (Scott and Schuster, 1991; Zakay, 1991) and we observed differences in basal levels of SA in our plants (Publication IV). It can be hypothesized that the basal SA levels and virus resistance in *S. chilense* are linked but this needs to be experimentally confirmed.

GLMMs were generated to test the effects of different measured immunity components for resistance. An additive effect of laminarin-dependent ROS production, ET accumulation and basal levels of ABA and PA provided the best fitting model. Interestingly, geographical trends were observed for different measured components. ET accumulation showed the strongest correlation to resistance in the coastal group followed by basal PA levels in central populations (Publication IV). The role of ET accumulation in resistance was further supported via an ET inhibition assay in two plants of the coastal population. The effect of ET in resistance in coastal populations might be an evolutionary benefit in these populations which can be due to adaptation to constant abiotic stress faced. Enhanced accumulation of *ACC*, a precursor of ET under salt stress, has been shown in the salt-tolerant *Brassicaceae* species *Cakile maritima* and *Thellungiella salsuginea* when compared to salt-stress sensitive *A. thaliana* (Ellouzi *et al.*, 2014). In addition to the direct role of ET in salt stress responses, it has been shown that ET is

important in modulating other phytohormones that play an important role in biotic and abiotic stresses (Vos *et al.*, 2015; Kazan, 2015). The coastal population LA2932 had the highest basal levels of ABA (Publication IV) and the same population also showed a strong correlation with induced ET production upon laminarin treatment and resistance to *P. infestans*. ABA in tomato plants has been reported to suppress resistance against *Oidium neolycopersici* and *B. cinerea* (Achuo *et al.*, 2006). ABA is also shown to be a negative regulator of ET further increasing the susceptibility of tomato plants as in the case against *B. cinerea* resistance (Sivakumaran *et al.*, 2016). In contrast to that, we observed a combined effect of ABA and ET in our GLMM model with a lower AIC/BIC scores (Publication IV).

Combined stress (biotic and abiotic) responses in plants, have been observed to lead to both positive and negative effects in the plant's phenotype, physiology, and resistance properties. In tomatoes, different abiotic stresses, such as drought and salt stress, have shown to increase resistance to the fungi *B. cinerea* and *O. neolycopersici* (Achuo *et al.*, 2006). High temperature stress in tomato leads to interference in Mi-1 mediated resistance against the root-knot nematode, *Meloidogyne* spp. (Dropkin, 1969). The complex interplay in biotic and salt stresses and clear effect of ET in interactions of tomato with the powdery mildew pathogen *O. neolycopersici* and salt stress has also been highlighted (Kissoudis *et al.*, 2017). Here, ET acts as a trade-off mechanism under combined stress making the plant more salt-tolerant but susceptible to powdery mildew. Geographically dependent levels of phytohormones in *S. chilense* was observed, indicating much complex mechanisms in different biotic and abotic stress tolerance and interplay among these phytohormones in geographically dependent manner can be expected. The complete mechanism behind the quantitative resistance observed in *S. chilense* against *P. infestans* (Publication II and IV) is still elusive. To completely entangle the mechanism behind the quantitative resistance observed, a transcriptomic and metabolic approached can be used. In the model species *A. thaliana,* these approaches have helped in understanding the mechanisms involved in resistance delivered by a complex quantitative trait locus against the bacterial pathogen *Xanthomonas campestris* (Delplace *et al.*, 2020).

3.3 *S. chilense* as a model to study qualitative resistance

Major gene-mediated resistance in wild tomatoes has been explored against the fungal pathogen *C. fulvum*. The first *Cf* resistance gene successfully cloned from the wild tomato species *S. pimpinellifolium* was *Cf-9* (Jones *et al*., 1994). *Cf* genes have shown to be complex at the genomic level (Parniske *et al.*, 1997). The presence-absence variation of two *Cf* genes,

Cf-4 and *Cf-9* in 15 populations of *S. chilense* were evaluated in this dissertation (Publication V). Interestingly, complete loss of recognition of *C. fulvum* race 5 Avr proteins in the southern populations was observed which was hypothesized in Publication V to be regulated due to changes in the co-receptors rather than the *Cf* genes themselves. The loss of recognition can be due to the absence of *C. fulvum* disease pressure, due to arid conditions in the southern region of the *S. chilense*. As co-receptors/co-regulators of Cf proteins are shown to be involved in different regulatory processes (Schwessinger *et al.*, 2011). The loss of recognition can also be due to extreme environmental conditions and potential trade-off mechanisms exerted by environmental stresses on the co-receptors. It will be interesting to further investigate different pathogen interactions in the southern populations as it can be possible that the loss of recognition of *Cf* genes is a trade-off to maintain resistance against a different pathogen that is more prevalent in the southern regions. Another possibility can be that *C. fulvum* inhabiting the local areas of *S. chilense* secrete different Avr proteins when compared to the race used in this study (Publication V). In that case, resistance might be mediated by different Cf proteins which do not recognise the currently tested Avr proteins.

In central and northern populations, we observed presence-absence variation at the genomic sequences of *Cf-4* and no direct correlation to corresponding Avr recognitions. The underlying reason remains unknown but due to the complex nature of *Cf* genes and a high number of recombinations, it can be proposed that the Avr4 recognition is regulated by allelic variation in *S. chilense.* An allelic variant of *Cf-9* called *9DC* is reported to also recognise the Avr9 protein in natural populations of *S. pimpinellifolium* (Van der Hoorn *et al.*, 2001). In future, to resolve the *Cf* gene complexity in *S. chilense* the repetitive sequences need to be resolved using long read sequencing methods to better understand the sequence diversity and evolutionary forces at play on this gene family.

3.4 Potential cross-talk among biotic and abiotic stress responses of *S. chilense*

Signatures of positive selection were reported on 16 genes involved in abiotic responses (such as salt stress, drought stress and cold stress) in 23 populations of *S. chilense* (Böndel *et al.*, 2018). High nucleotide diversity was observed at nonsynonymous sites of these genes when compared to reference genes, which indicates potential positive selection pressure. Local adaptation based on observed high nucleotide diversity at the edge of the species distribution was apparent at the southern coastal and highland populations with extreme environmental stresses. Similarly, studies on different biotic stress-regulated genes and proteins also show

differential evolutionary pressures depending on the pathway under study (Rose *et al.*, 2011) or depends on the population under study (Stam *et al.*, 2019a).

S. chilense has been widely studied for salt stress responses. Plants under salt stress showed no compromise in shoot growth when compared to non-treated plants (Martínez *et al.*, 2012; Kashyap *et al.*, 2020a). Further, it was shown that salt tolerance is obtained in *S. chilense* via osmotic stress regulation (Martínez *et al.*, 2014). In a comparative transcriptomic approach, it became clear that *S. chilense* likely achieves salt tolerance via interaction of several gene products involved in calcium, auxin, and ET signalling pathways (Kashyap *et al.*, 2020b). The positive role of ET accumulation was confirmed in resistance against *P. infestans* in this dissertation (Publication IV). In addition, calcium signalling is shown to plays an integral part in the basal defence responses in plants (Seybold *et al.*, 2019). Hence indicating that common signalling components play a role in *S. chilense* for different stress tolerance mechanisms.

The studies mentioned above together indicate a potential crosstalk among the biotic and abiotic stress-regulated molecular components in *S. chilense* and future studies on combined stress can provide a better understanding of the complex networks involved.

The role of complex plants networks involved in physiological and developmental changes and their implications in system biology is important to understand the mechanisms completely (Marshall-Colón and Kliebenstein, 2019). Decomplexification of these networks have been instrumental in basic understanding of evolutionary history by focusing on single gene candidates or gene families involved in specific stress response. The future steps may include, considering the networks involved in multiple responses (biotic and abiotic) as one entity and understanding the crosstalk among these responses and the genes involved in these interactions. As in natural habitats plants are expected to face both biotic and abiotic stress simultaneously. Determining the evolutionary forces in a network context for an individual's stress-tolerance and then combining it for multiple stresses and understanding the forces at play in the complex at the population level will be challenging (as discussed in Publication I).

3.5 *S. chilense*'s potential in bridging the gaps between evolutionary biology, molecular biology, and crop improvement

This dissertation (Publication II, IV and V) and former studies (Stam *et al.*, 2017; 2019a) on biotic interactions have shown that *S. chilense* is an amenable model system to study genomic and phenotypic diversity and the molecular mechanisms underlying these responses.

S. chilense resistance against *P. infestans* is proposed to be based on basal defence mechanisms and possibly on an advantageous effect of ET signalling in coastal population, that helps the plants to cope with salt stress. The same *S. chilense* southern populations show loss of recognition of Avr proteins of *C. fulvum* race 5. In addition, high complexity is expected at the resistance loci of *S. chilense* in the central and northern populations. Also, resistance response against *P. infestans* in *S. chilense* showed high diversity within populations. But, Southern highland populations were more susceptible on average when compared to other groups. Populations of *S. chilense* in the southern highlands due to their high susceptibility can be assumed to experience no strong *C. fulvum* or *P. infestans* pressure or the plants show specific adaptations to the local *C. fulvum* or *P. infestans* that could not be investigated using a non-adapted pathogen. Adaptation of pathogens to their environment is shown to greatly influence the fitness of the host (Laine, 2008). It will be important in future to elucidate these mechanisms against locally isolated pathogens to validate possible similarities and/or differences in the resistance mechanisms observed (Publications II, IV and V).

S. chilense is a wild relative of an economical important crop. It also provides a specific advantage of a well-elaborated model with known demography and defined evolutionary history. Finally, it possesses coping mechanisms against different abiotic and biotic stresses. All studies combined indicate that the species possess novel unexplored mechanisms in responses to combined environmental stresses. Future studies generating knowledge from combined stress responses and exploring complex networks will deepen our understanding on the coping mechanisms in wild tomatoes which can potentially define the usefulness of this species in crop protection programs.

4. Concluding remarks

In summary, this dissertation work shows quantitative and qualitative resistance against different pathogens. The work reports high diversity in defence related responses at different levels; firstly, resistance against *P. infestans*, secondly in laminarin-induced basal defence responses and thirdly in presence-absence variation of major-gene mediated resistance against *C. fulvum*. The study indicates the importance of the plant populations' habitat in delivering the resistance against two filamentous pathogens. Further, connecting plant responses and molecular defence mechanisms to pathogen virulence and plant resistance with the wild pathogen species from the same locations as *S. chilense* populations will shed more light to understanding the evolution of pathogen resistance and the role of local adaptations.

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