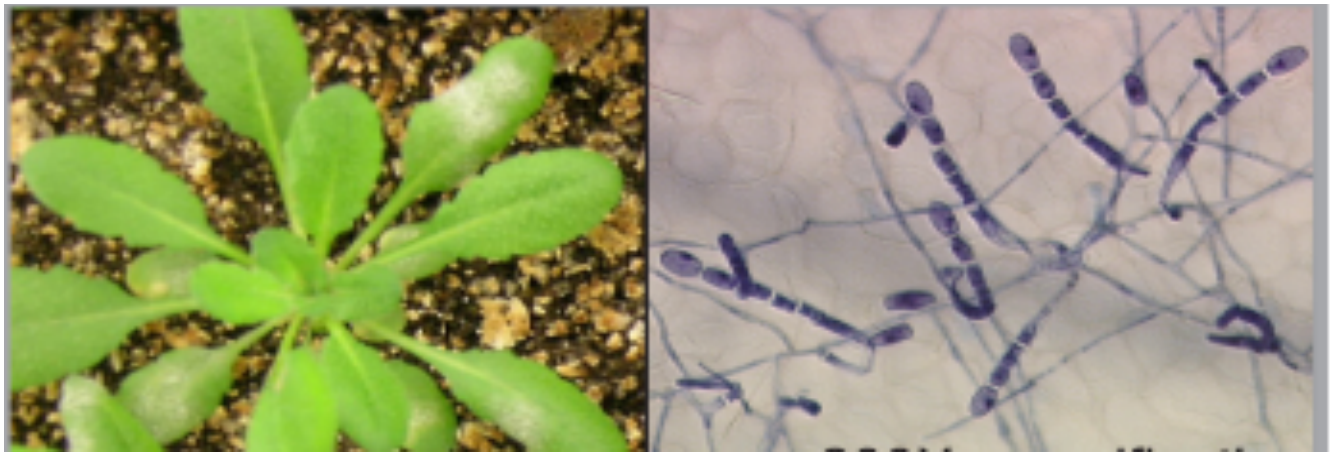
Resistance to powdery mildew in *Arabidopsis thaliana*

ANDREAS TATTIS

MSC THESIS REPORT



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Picture: Infected Arabidopsis leaves and microscopic view of the infection

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ABSTRACT

Powdery mildews (PM) are one of the most recognized plant diseases, caused by obligate biotrophic fungi belonging to the *Erysiphaceae*. They are responsible for many economically important diseases on a global scale.

PM have been well described extensively on the model plant *Arabidopsis thaliana*. Specifically, *Arabidopsis* can be infected by the following four PM species: *Erisyphe cruciferarum* (Koch and Slusarenko, 1990) *Golovinomyces cichoracearum* (Adam and Somerville, 1996), *Golovinomyces orontii* (Plotnikova *et al.*, 1998) and the tomato PM pathogen *Pseudoidium (*syn*. Oidium) neolycopersici* (Xiao *et al.*, 2001). The advantages of working with a model plant species have led to a remarkable breakthrough towards understanding the *Arabidopsis*-PM interactions. It is widely accepted that immunity to PM can be controlled by resistant (*R*) genes, which in turn have the ability to detect specific effectors of the fungal pathogen.

With the use of quantitative trait analysis (QTL) and DNA molecular markers, several dominant and semi-dominant resistant genes have been identified, with the *RPW8* locus representing the major QTL. However the limited durability and ephemerality of resistance genes due to continues coevolution with the pathogen gave rise to alternative strategies for breeding for resistance, such as the introduction of susceptibility genes through natural or induced loss of function mutations.

The *MLO* gene represents a benchmark due to its exceptional efficacy and longevity of the resistance. *Arabidopsis* triple mutant *atmlo2, atmlo6, atmlo12* - that are co-orthologs of the barley *Mlo* - exhibits full immunity to adapted PM.

In this thesis we focus on the findings concerning *Arabidopsis*-PM infection, resistance genes identified so far, host susceptibility factors and the molecular mechanisms underlying their function.

Additionally to literature study, we carried out experiments described in this thesis, concerning the identification through mapping of resistant genes of *Arabidopsis* against tomato PM *Oidium neolycopersici*. The experiments took place in Plant Breeding of Wageningen University & Research.

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CHAPTER 1: The infection of Powdery mildews on Arabidopsis

Arabidopsis thaliana

Arabidopsis thaliana is a dicotyledonous species that belongs to the *Brassicaceae* family and is native in Europe and Central Asia (Hoffmann 2002). It is considered a model plant for the study of growth and development in plants and the most important model plant when it comes to genetics and molecular research. This is due to the features that *Arabidopsis* possess, that make its study fast, cheap and convenient. In particular because of its homozygosity, self-fertile and rapid generation time, it can produce tens of thousands of offspring's in less than 2 months time, since it reaches from seed germination to mature seed production in about 6 weeks. In addition *A. thaliana* has a small genome size (132 Mbp), consisting of 5 chromosomes and including about 38000 loci of which 20000 are protein-coding genes (Arabidopsis Genome Institute 2000, Cheng et al 2017). Furthermore there are plenty of molecular tools available for its study plus the fact that it is a host to all groups of pathogens (Xiao *et al.*, 1997).

There are over 6000 accessions worldwide and due to natural polymorphisms between *A. thaliana* species, quantitative genetic methods have been applied widely, leading to the discovery of the genetic basis of several agronomic traits, including resistance. Additionally, it is easily transformed and receptive to mutagenesis by acquiring activation-tagging mutants that result in promotion of gene expression and at the same time knockout mutants with disruption of gene expression.

Mutagenesis can be achieved through Ethyl Methanesulfonate (EMS), which is the most commonly used chemical mutagen in plants mainly because of its successfulness and easiness to induce many random point mutations, exposuring traits of interest. On the other hand a more targeted method to suppress genes of interest effectively is using gene knockdown through the widely used RNAi mechanism. Specifically the mechanism behind this biological method relies in the use of double stranded RNA molecules blocking the expression of specific genes finally leading to post transcriptional gene silencing (Younis *et al.*, 2014). Gene silencing can be also be achieved through reverse genetics and the use of Virus-induced gene silencing (VIGS). This specific post-translational technique uses vectors that carry the target gene fragment that in turn produces dsRNA, causing RNA mediated

gene silencing. Additionally to the above transformation methods, CRISPR-Cas9 constitutes a hallmark in genome editing providing several applications in plant breeding industry.

Powdery mildews

Powdery mildews are plant pathogenic fungi that belong to the phylum Ascomycota forming the order of *Erysiphales* (Braun et al., 2008). They are obligate biotrophic phytoparasites, which invade epidermal cells and infect the aerial parts of higher plants. The main characteristics of the powdery mildew disease can be observed from distance as white powdery–like spots, formed by conidia and conidiophores, covering the flowers, leaves, stems and fruits of the infected plant. Moreover and in contrast with other more severe diseases, powdery mildew exhibits lack of systemic and root infection, slow increase and infrequent death of the host (Saharan et al., 2019).

The disease thrives particularly in temperate and humid climates, where it results in serious yield losses, up to 10-90% affecting also the quality of the product (Glawe et al., 2008). Powdery mildew has a wide host range including among others economically important staple crops such as cereals (wheat and barley), horticulture crops like tomato from the *Solanaceae* family, ornamental plants and members of the *Cucurbinaceae* family like squash and grapevine(Adam and Somerville, 1996).

Around 500 species of the fungi causing powdery mildew disease have been identified, which affect approximately 10000 different plants (Takamatsu et al., 2004)

While powdery mildew disease were recognized in 1973 by Linnaeus (Salmon et al.,2000), the first report in *Arabidopsis* powdery mildew dates back in the 1990's and since then a new chapter in research has begun (Micali et al., 2008).

There are four powdery mildew species that are pathogenic in *Arabidopsis thaliana* (Table 1). Specifically, *Erysiphe cruciferarum* (Koch and Slusarenko., et al 1990), *Golovinomyces cichoracearum* (Adam and Somerville et al.,1996), and *Golovinomyces oronti* (Plotnikova et al.,1998). The fourth one is the tomato powdery mildew *Oidium neolycopersici* that reproduces successfully on *Arabidopsis* (Xiao et al., 2001). The infection intensities differ across the different species with respect to environmental conditions (Table 1).

Table 1. Different features of four powdery mildew species infecting *Arabidopsis*. Information was obtained from the following publications: (Adam and Somerville, 1996; Plotnikova *et al.*, 1998; Micali *et al.*, 2008; Wu *et al.*, 2018) . N/A: Not Available; dpi: days post-inoculation; hpi: hours post-inoculation.

	G.cichoracearum UCSC1	G.orontii MGH	E.cruciferarum UEA1	O.neolycopersici
Genome size	174Mb	160Mb	N/A	120 Mb
Conidia size	26 TO 33 µm long	29 to 35 µm long	38 to 46 µm long	23 to 45 µm long
Number of conidia per conidiophore	16 to 19 µm wide	15 to 19 µm wide	13 to 16 µm wide	13 to 20 µm wide
Other hosts	Asteraceae, Cucurbitaceae	Brassicaceae, Solanaceae, Cucurbitaceae	Brassicaceae	Cucurbitaceae, Solanaceae
Conidium germination	1 to 2 hpi	1 to 2 hpi	N/A	3 to 5 hpi
Appresorium formation	6 to 10 hpi	5 hpi	N/A	6 to 8 hpi
Haustorium formation	10 to 14 hpi	14 to 16 hpi	N/A	N/A
Conidiophore formation	4 dpi	5 dpi	5 to 6 dpi	6 dpi
Reference	Adam and Somerville et al., 1996	Plotnikova et al., 1998	Koch and Slusarenko„ et al 1990	Xiao et al., 2001

It is revealed that powdery mildews have a large expanded genome, four times larger than other Ascomycete species, most of which consists of transposable elements (Spanu and Kämper, 2010).

The genome of powdery mildews species varies from 120Mb to 222Mb. Specifically, it has been found that the genome size of *Oidium neolycopersici* is 120Mb while the genome size of *Golovinomyces cichoracearum* and *Golovinomyces orontii* are 174Mb and 160Mb respectively. Worth mentioning is that comparing monocot and dicot powdery mildew, it has been shown that the dicot powdery mildew, like the ones infecting Arabidopsis, posses a much smaller effectorome (Wu *et al.*, 2018).

The effectorome is consistent of effector proteins that are molecules derived from the fungi inside the plant cell. These proteins manipulate the plant functions finally leading the fungus to invade the plant tissues and promote infection(Saur *et al.*, 2019).

Speaking of effector proteins, in the study performed by Wu et al. (2018), it was found that the monocot powdery mildew has an average number of genes encoding candidate

secreted effector proteins up to 661, while the equivalent to dicot powdery mildew has 116-175 genes. Additionally by comparing eight PM genomes it was found that 75% of identified genes are common to all genomes, while the remaining 25% of genes are consistent of lineage specific or biotype specific genes. (Wu *et al.*, 2018).

Based on the work of Christopher Ridout ,two powdery mildew effectors have been cloned, Avra10 and Avrk1 which were isolated by mapping from *Blumeria graminis hordei (Bgh),* and the corresponding R-proteins in barley are MLA10 and MLK1 respectively (Ridout *et al.*, 2006).

Since a lot of economically important plants are affected by powdery mildew, the scientific efforts to find means for controlling this disease are an ongoing endeavor. In order to achieve this, the main target and goal of producers focuses on the application of fungicides and for researchers the discovery and application of resistance varieties.

Life Cycle: Infection-Reproduction-Penetration

The Powdery mildew disease infection process, like other fungi, involves both a sexual and an asexual phase or either of them. For example, the asexual cycle of *Oidium neolycopersici* has been examined whereas the sexual cycle has not been observed.

Spores are the reproductive structures of the fungus and depending on the type of the fungus and the stage of its life cycle, spores can be sexual and asexual (Schulze-Lefert and Vogel, 2000). During asexual reproduction, conidia are produced within a few days after the infection begins. Somatic hyphae generate many conidiophores from which conidia are derived either singularly or in long chains (Figure1). This production of conidia will be continuous for as long as suitable environmental and host conditions takes place (Green, Carver and Gurr, 2002).

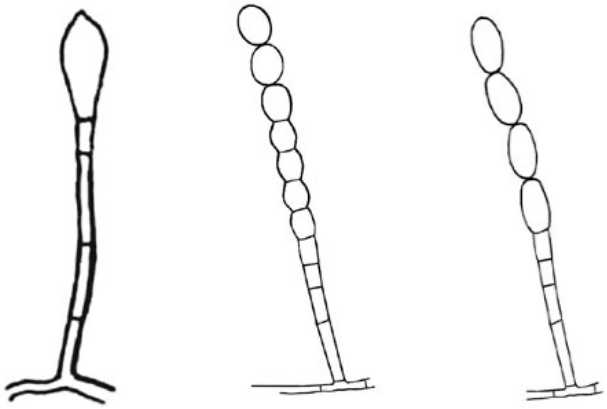

Figure 1. Picture on the left is conidiophore producing single conidium, while in the middle is conidiophore producing conidia in a chain. On the right side is a phenomenon called pseudo-chain in which single conidia are stick to each other and the result is not a true chain (Picture is copied from Heffer, Powelson, Johnson, & Shishkoff, 2006).

Sexual reproduction involves the recombination of the genetic material. The powdery mildew produces sexual fruiting bodies known as chasmothecium, which contains ascospores (Braun *et al.*, 2002). When the female and male gametangia come in contact, the antheridial nucleus is transferred to the ascogonium and plasmogamy takes place followed by karyogamy finally leading to the production of chasmothesium. Chasmothesia are placed either on the plants tissue surface or in the plant mycelium and when the conditions are favorable they germinate through the ascogonium and diffuse by rain, expelling their contents (Glawe, 2008).

When a conidium or ascospore lands on a leaf of the susceptible host, it will imbibe and germinate resulting in the production of a primary germ tube that elongates and differentiates forming a hyphae and an appressorium (Micali et al., 2008). The appressorium sticks in the host surface and produce a penetration peg in order to penetrate the epidermal cell.

In order for hyphal penetration to be accomplished, a combination of both a mechanical pressure and enzymatic activity is performed (Jones, Whipps and Gurr, 2001; Tucker and Talbot, 2001). Host plants may react to the penetration attempts by forming cell wall appositions, trying to eliminate the pathogen, through a physical and chemical block. Successful entry into the epidermal cell, results in a quick enlargement of the hypha and form a specialized feeding structure, the haustorium which is responsible, in biotrophic pathogens, for the absorption of amino acids and sugars (Hahn *et al.*, 1997; Voegele *et al.*, 2001). In figure 2, below, the infection cycle of powdery mildew is presented.

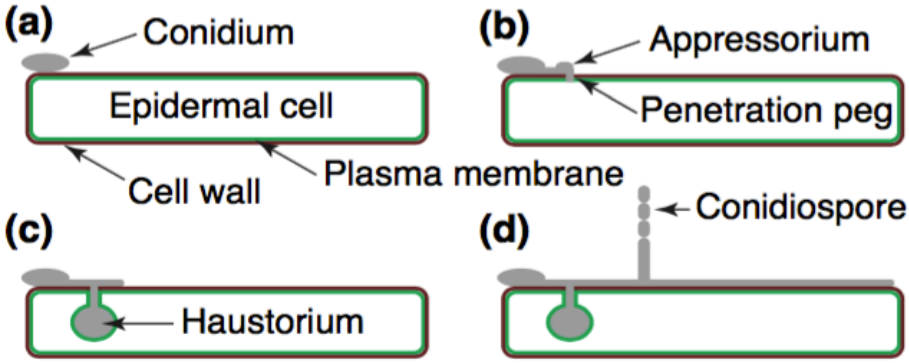

Figure 2. Powdery Mildew Life Cycle. In stage 1 the conidium lands on the leaf surface and germinates developing the appressorium at the tip of the germ tube. In stage 2 the appresorium, in order to promote the penetration of the leaf cuticle and epidermal cell wall, develops the penetration peg. Stage 3 involves the development of the haustorium, which is responsible for obtaining nutrients for further mycelial growth and in stage 4 the asexual life is complete with the maturation of the haustorium with extended lobes and the formation of conidiospores (Picture is copied from Koh, André, Edwards, Ehrhardt, & Somerville, 2005; Schulze-Lefert & Vogel, 2000).

Plant - pathogen interface

Plants are exposed to countless of plant pathogens. Despite that, most of them are non-host to the majority of the pathogens thus developing several mechanisms of protection.

Plants, throughout the ages of coevolution with pathogens, have developed a cell-autonomous multilayer immune system, allowing them to overcome pathogens (Dodds and Rathjen et al., 2010). The detection of the attacker constitutes a prerequisite for plants, in order for immunity to be achieved.

Once pathogens repress the mechanical barriers and secondary metabolites, the primary immune response is initiated by the pattern recognition receptors (PRRs). According to Jones and Dangl (2006), there are two layers of defense against the attackers. The first one is the detection of pathogen-associate-molecular-patterns (PAMPs) by the plant leading to PAMP-triggered immunity (PTI) and the second one involves the defense achieved by the effector-triggered immunity (ETI) (Jones and Dangl, 2006).

PRRs form the first group of host receptors and are responsible for the extracellular recognition of non-self molecules, known as PAMPs, also including flagellin and chitin. (Boller and Felix et al 2009). The defense is achieved by introducing signaling pathways against the pathogen, using defense response genes. This function leads to a signaling cascade, resulting in PTI. PTI is responsible for the induction of cell wall appositions and the production of the reactive oxygen species (Nürnberger and Lipka, 2005).

A very critical stage in plant infection is, among others, the formation, upon penetration, of cell wall appositions (CWA). CWAs are a mixture of components from which the most

known are cellulose, lignins, pectin, callose and phytoalexins. These components are responsible of providing physical and chemical protection against potential pathogens (Hardham et al., 2007 and Huckelhoven et al., 2007). Impending delay of CWAs is equated to enhance fungal infection (Assaad *et al.*, 2004).

Pathogens, in turn, have evolved effectors in order to suppress this immune response and enhance virulence (Cunnac et al., 2001). The suppression of PTI benefits the pathogen, leading to the process of effector-triggered-susceptibility (ETS).

Subsequently the recognition of the pathogen effectors by the plant receptors leads to a rapid response called ETI. These receptors comprise a group also called resistance (R) proteins. The majority of R proteins typically contain a nucleotide binding site and leucine-rich-repeat (NBS-LRR)(Głowacki, Macioszek and Kononowicz, 2011). Specifically the LRR domain is responsible for the effector recognition by involving a protein-protein interaction (Farham and Baulcombe et al., 2006, Sela et al., 2012). ETI, in turn, triggers a rapid cell death, known as hypersensitive response, of the infected and surrounding cells regulated by the salicylic acid pathway.

With regards to *Arabidopsis* - powdery mildew, host-pathogen interaction, examples of PAMPs are cell wall-derived chitin and the corresponding receptor is Chitin Elicitor Receptor Kinase (CERK1) that confers resistance to powdery mildew specie *G. cichoracearum (Wan et al., 2008).*

The structure and functional aspects of the fungal-plant interface are well described in a continued co-evolutionary four-phased model for plant immunity, which is called the zigzag model (Figure 3). The zigzag model, known by many as the “central dogma” in plant pathology, is an expository model providing a more unambiguous and precise aspect of host-pathogen interactions(Pritchard and Birch, 2014). Regarding this model, ETI puts selective pressure forcing the pathogen to produce new effectors. Those effectors in turn produce new receptors from the plant and this makes this cycle *ad infinitum* (Głowacki, Macioszek and Kononowicz, 2011).

Gene-for-Gene Resistance model

Most of the resistant genes that have been discovered so far operate according to the gene-for-gene model(Hammond-Kosack and Jones, 1997). According to this description of the interaction between the pathogen and the plant, for each resistant gene in the host there is a corresponding avirulence gene in the pathogen (Flor, 1971). This type of resistance seems to be widespread, occurring mostly in biotrophic pathogens, such as powdery mildew. This concept can also be linked with the effector – receptor model, in which, *R* genes are equivalent to receptors of effectors, that are encoded by genes in the pathogen, such as Avr genes (Mudgett, 2005; Ellis, 2006).

Resistance provided by the gene-for-gene model, as shown by various experiments, is associated with the hypersensitive response (HR) of plants (Hammond-Kosack, Jones and Jones, 1996). HR- based resistance is characterized by the rapid death of cells surrounding an infection in a way that restricts the further spread of the pathogens in other plant parts. In case of *Arabidopsis* - powdery mildew, the resistance is effective against one or a few genotypes of the pathogen, also known as race-specific. This is the main disadvantage of this resistance approach from the plant breeding point of view, since the continuous evolution over time of new pathogenic races makes the resistance non-durable, lasting no longer than 5-10 years (Király, Barna and Király, 2007).

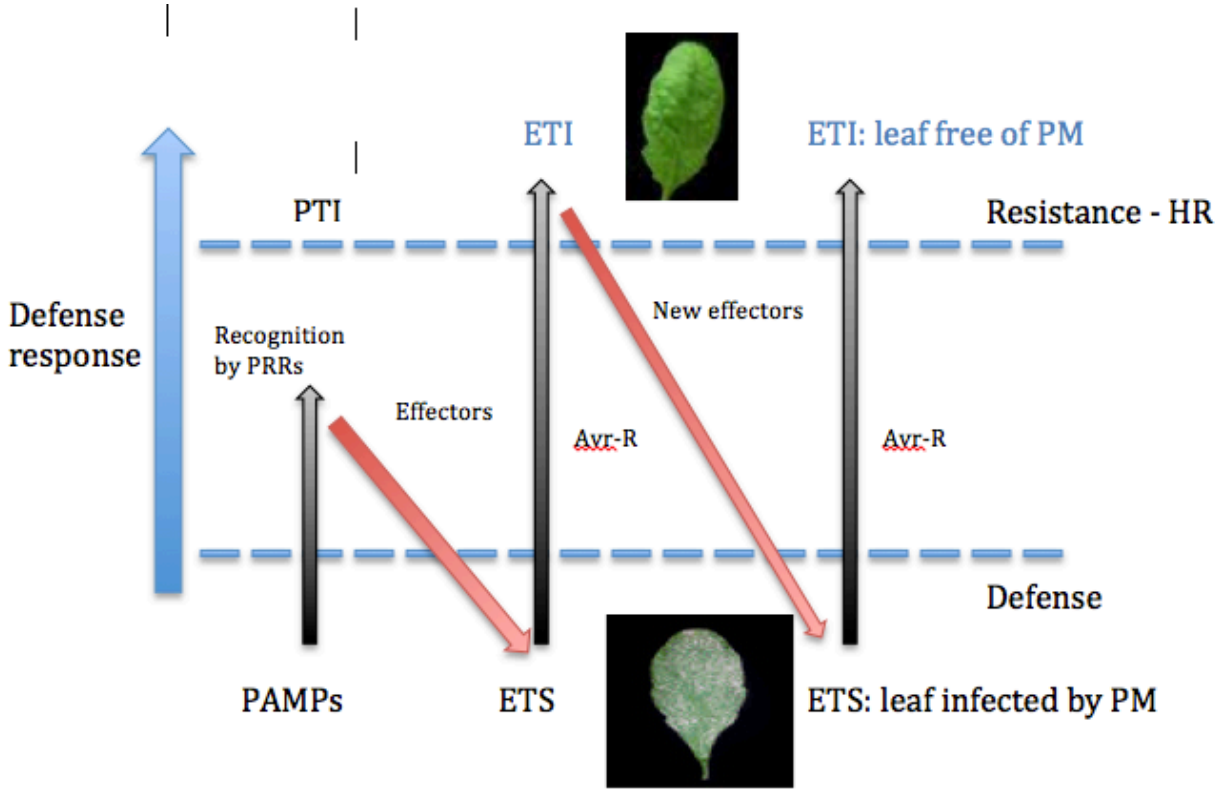

Figure 3. Overview of the plant immune system concerning resistance and susceptibility phases during the interaction with a pathogen. The four phases of the zig-zag model concerning the continuous "arms race" between the plant and a pathogen. In phase 1 pathogen patterns as PAMPS are recognized by plant receptors PRRs leading to activation of plant defense mechanisms such as PTI (PAMP-triggered immunity) and plant resistance, which is suppressed in phase 2 by pathogen effectors, resulting in ETS (effector-triggered susceptibility). Later on the corresponding plant R-proteins recognize pathogen effectors, that become avirulent (Avrs) in phase 3 promoting ETI (effector-triggered immunity). Lastly in phase 4 new pathogen effectors result again to ETS that will be followed by ETI and so on.

CHAPTER 2: Resistance

General Information

Resistance can be defined as the ability of the plant to reduce growth and/or development of the pathogen after contact has been initiated or established (Niks *et al.*, 2019). In order to measure the level of resistance, a comparison should be made between the quantity of the pathogen on the resistant plant and on a susceptible plant.

The first report of a plant resistance phenomenon was mentioned by Ward more than a century ago and concerned a resistant reaction to a rust pathogen that latter was characterized as the HR response (Ward, 1902).

The focus on breeding for resistance has been the introduction of dominantly or semi-dominantly inherited R-genes which constitute the second layer of defense and can recognize specific effectors, finally leading to ETI .The use of plant resistant genes in order to establish disease resistant varieties constitutes an alternative to other methods like pesticides and chemical controls. The genomic diversity that R proteins reveal, facilitates the recognition of a wide range of pathogen effectors (Głowacki, Macioszek and Kononowicz, 2011). However the ability of new evolving pathogen races to overcome R-genes and suppress host resistance leads to a vicious cycle which presupposes again the introduction of new R-genes (Brown, 1994).

The above situation can be reversed potentially through gene pyramiding by introducing multiple R-genes in the host (Crute and Pink, 1996).

In *Arabidopsis* - powdery mildew interaction besides the introduction of R-genes, two other possible strategies that confer resistance are available: non host resistance and loss of a susceptibility S-gene (Nürnberger and Lipka, 2005).

Types of Resistance

There are two main types of resistance: the innate resistance and the acquired resistance. The first one is expressed by plants in the following forms: the non-specific (general) and the specific resistance. Non-specific resistance, which is effective against various types of potential pathogens, includes among others non-host resistance, while specific resistance that is effective against only a few pathogens includes, the famous gene-for-gene resistance and gene silencing (Király, Barna and Király, 2007).

Classes of *R* genes

The significant evolution that plant resistance (*R*) genes have undergone over time in order to deal with pathogens is the reason behind their extended diversity and abundance in protein families and structures.

The prolonged use of bioinformatics tools lead the way to the detection of thousands of Resistance gene analogs (RGA) from sequenced plant genomes(Sekhwal *et al.*, 2015). RGA are a big class of potential (*R*) genes, that possess conserved domains and structure features. Despite the abundance in *RGA* and the great resource that is available, only a few (R) genes have been cloned and characterized so far in different plant species (Liu *et al.*, 2007; Sekhwal *et al.*, 2015).

Specifically based on the presence of different protein domains, *R* genes can be classified in 5 classes: (1) the best known , located in the cytoplasm is the NBS-LRR class (Hammond-Kosack and Jones, 1997); (2) the receptor-like kinases (RLK); (3) the receptor-like transmembrane proteins (RLP); (4) the serine-theorine kinase (STK); (5) the atypical *R* genes (Wang *et al.*, 2018). The atypical R genes belongs to a transmembrane (TM) class that possess an intra and extracellular loop that promotes the communication between both environments. Known R genes of this class in *Arabidopsis* are *RPW8.1, RPW8.2* and *Mlo gene* (Sekhwal *et al.*, 2015).

Resistance genes identified in *Arabidopsis* - powdery mildew pathosystem

In order to identify genes responsible for resistance to powdery mildew, several studies have been performed over the past years. Currently 13 genes have been identified and characterized named *RPW1* to *RPW14* with locus *RPW8* pointed as a key player and major Quantitative Trait Locus.

Table 2. Resistant genes identified so far in *Arabidopsis*, the corresponding accession and location.

Gene	Chromosome	Pathogen	Accession	Type of resistance
RPW1	2	*E.cichoracearum*	Kas-1	Semi-dominant
RPW2	3	*E.cichoracearum*	Wa-1	Semi-dominant
RPW3	3	*E.cichoracearum*	Te-0	Recessive
RPW4	4	*E.cichoracearum*	Stw-0	Semi-dominant
RPW5	5	*E.cichoracearum*	Su-0	Semi-dominant
RPW6	5	*E.cruciferarum*	Ms-0	Dominant
RPW7	3	*E.cruciferarum*	Ms-0	Allele of RPW8
RPW8	3	*E.cichoracearum*	Ms-0	Dominant

		E.cruciferarum		
RPW10	3	*E.cichoracearum*	Kas-1	Allele of RPW8
RPW11	5	*E.cichoracearum*	Kas-1	
RPW12	2	*E.cichoracearum*	Kas-1	
RPW13	3	*E.cichoracearum*	Wa-1	Allele of RPW8
RPW14	1	*E.cichoracearum*	Wa-1	Minor QTL

In a study performed by Adam and Somerville, five independent loci were shown to promote resistance to 5 different *Arabidopsis* accessions infected by a wild isolate of the powdery mildew *Erysiphe cichoracearum*. Specifically in accessions Kas-1, Wa-1, Stw-0 and Su-0 resistance to powdery mildew was governed by semi-dominant alleles *RPW1, RPW2, RPW4* and *RPW5* respectively, while in accession Te-0 resistance was encoded by the recessive allele *RPW3*, since in this case susceptibility is dominant and resistance response differs from the common HR. Papilla formation and consequently reduced fungal growth has been associated with these RPW loci, unlike cell death response that was not the case in this type of resistance (Adam and Somerville, 1996).

The location of the resistant loci has been determined through mapping studies with the use of sequence length polymorphism or microsatellite markers (Adam and Somerville, 1996).

Several researches in the following years led to the addition of several other RPW genes including *RPW6* through *RPW14*, that were identified and characterized (Xiao *et al.*, 1997; Wilson *et al.*, 2001).

Indicatively resistant F1 Hybrids, that were derived from a cross between susceptible *Arabidopsis thaliana* accession La-er and resistant accession Ms-0, revealed the presence of independent dominant genes controlling the resistance of Ms-0 accession to *Erysiphe cruciferarum* (Xiao *et al.*, 1997). Segregation in F_2 progenies revealed the presence of two dominant genes. One was *RPW6*, located on chromosome 5, and the other was *RPW7* located on chromosome 3.

Similar to previous studies, quantitative trait loci analysis was performed in the F_6 recombinant inbred line derived from a cross of resistant accession Kashmir-1 and susceptible Columbia gabrous (Col-gl1). The result was the identification and mapping of three unlinked powdery mildew resistant loci, *RPW10* through *RPW12*, that control resistance to *Arabidopsis* powdery mildew *Erysiphe cichoracearum* (Wilson *et al.*, 2001). *RPW10* is allelic to *RPW8*, validating the fact that *RPW8* is a key player among the RPW genes.

The cloned *R* gene, *RPW8* and its associated mechanisms

Additionally to previous findings, data from mapping of *RPW6* and *RPW7* was used this time with *Erysiphe cichoracearum*. This resulted in the identification of a dominant single locus, *RPW8*, mapped on chromosome 3 in the same position as *RPW7* (Figure 4).

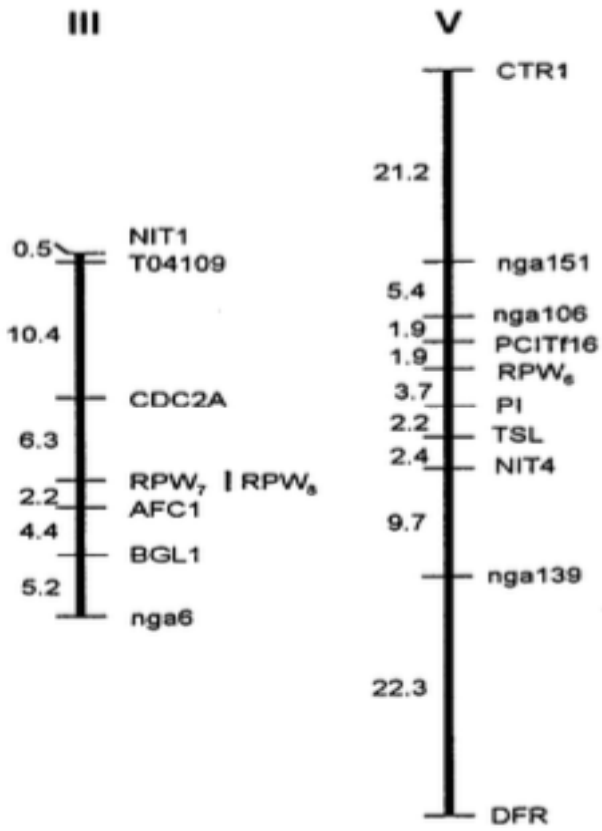

Figure 4. Genetic map displaying the genetic positions of *RPW6, RPW7, RPW8* in *Arabidopsis* accession Ms-0. *RPW6* and *RPW7* are both needed for resistance in *E.cruciferarum* while *RPW8* located at the same position as RPW7 conferred resistance to *E.cichoracearum*. Map distances are expressed in cM (Picture is copied from Xiao *et al.*, 1997).

Among many of the characterized R genes, *RPW8* is one of the most widely known atypical *R* genes, comprising natural resistance in Arabidopsis- powdery mildew pathosystem. The *RPW8* locus contains a coiled-coil (CC) motif and a putative N-terminal transmembrane domain (TM).

Several accessions were analyzed to date and resistance is either polygenic based on *RPW8* or a combination with the *RPW8* representing the major Quantitative Trait Locus (QTL) (Xiao *et al.*, 1997, 2005; Göllner *et al.*, 2008).

Mapping of *RPW8* revealed differences from the NBS-LRR resistant gene homologs proposing a different type of resistance. Specifically, it was found that in accession Ms-0, *RPW8* locus consists of two dominant genes *RPW8.1* and *RPW8.2* conferring resistance to

many powdery mildew pathogens, including isolates of *Erysiphe cichoracearum, Erysiphe cruciferarum and Erysiphe orontii* (Xiao *et al.*, 2001; Micali *et al.*, 2008). Col-0 *Arabidopsis* accession which is widely used as reference, lacks the genes *RPW8.1* and *RPW8.2* and thus is susceptible to all powdery mildew species (Xiao *et al.*, 2001).

Regarding *Oidium neolycopersici*, it was reported that *RPW8* mediated resistance is not functional suggesting that this tomato powdery mildew specie delivers most probably effectors, that are different from those of the *Erysiphales spp* that support *RPW8* mediate resistance, thus making them vulnerable to the disease (Göllner *et al.*, 2008). Moreover the non-discovery so far of any *RPW8* homologues in both cultivated and wild species of tomato support the above fact.

In contrast with the resistance, control by the locus *RPW1-RPW5, RPW8* locus lacks the NBS-LRR motifs as in most *R* proteins. However it has been shown that *RPW8* mediate resistance triggers the SA-depended HR response. This is associated with the accumulation of hydrogen peroxide and local cell death, upon pathogen attack (Xiao *et al.*, 2005). In addition *RPW8* mediated resistance requires also the classical components of NBS-LRR class such as *PHYTOALEXIN DEFICIENT 4 (PAD4), ENHANCE DISEASE SUSCEPTIBILITY 1 (EDS1) EDS5* and *NONEXPRESSOR OF PATJOGENESIS RELATED PROTEIN 1 (NPR1)*. However it has been confirmed that this is independent of the ethylene and jasmonic acid signaling pathways (Xiao *et al.*, 2005).

Except of *RPW8.1* and *RPW8.2*, homologues of *RPW8* (HR), also known as *RPW8*-like proteins, contribute to basal resistance to powdery mildew. Specifically three homologs of *RPW8* have been identified in Arabidopsis Ms-0 accession, designated as *HR1* (At3g50450), *HR2* (At3g50460), and *HR3* (At3g50470; Xiao et al., 2001) while powdery mildew-susceptible accession Col-0 also contains *HR4* (At3g50480). It has been showed recently that overexpression of all *HR* genes except *HR4* confers resistance to powdery mildew, while depletion of *HR2* or *HR3* results in enhanced susceptibility. The above indicate that only *HR1* to *HR3* likely contribute to basal resistance against powdery mildew pathogens (Berkey *et al.*, 2017).

Generally speaking, from a total of 360 *Arabidopsis* accessions, 76% were found to be resistant to *Erysiphe cruciferarum* than to *G.cichoracearum*(63%) (Adam *et al.*, 1999), while in an experiment performed by Gollner from 64 accessions, 26% were resistant to *G.orontii* (Göllner *et al.*, 2008).

Non-Host Resistance

It is widely accepted that in nature the majority of plants are usually healthy, despite the fact that they are constantly unprotected to potential pathogens. The above statement has as a result the disease being the exception and not the rule. The reason behind that is what we called non-host resistance (Lipka, Fuchs and Lipka, 2008).

By definition non-host resistance (NHR) includes the group of characteristics that a plant species exhibits in order to resist infection by all genetic variants of a pathogen, which makes it non-host to possible attackers (Thordal-Christensen, 2003). Although this type of plant resistance is the most durable and common type, due to its complex nature, there are not enough publications explaining the surrounding mechanisms. In later years the introduction of model interaction systems in *Arabidopsis* non-adapted powdery mildew empowered the discovery of key components and the extraction of several conclusions (Lipka *et al.*, 2010).

The phenomenon is related with pre-invasion entry control at the cell periphery and post-invasion immune responses based on HR. Specifically, experiments with the non-adapted powdery midlews *Blumeria graminis* and *Erysiphe pisi* in *Arabidopsis* proved the elimination of the pathogen up to 80-90%, while in rare cases that haustorium formation takes place, the plant defends itself by callose deposition and HR cell death, preventing further development of the pathogen (Collins et al., 2003, Lipka et al., 2005).

Pen Genes

The successful application of mutants in *Arabidopsis*, a groundbreaking discovery for NHR, revealed finally the mechanisms surrounding NHR (Lipka et al., 2010).

Arabidopsis thaliana entry control against non-adapted powdery mildews relies, according to Lipka et al. (2005) and Stein et al. (2006), on three characterized penetration mutants. These *Arabidopsis* mutants that are referred as *pen* mutants display increased penetration and haustorium formation by the non-host powdery mildew species, proving in this way that plants have the ability, through a controlled process, to eliminate fungal invasion in cell periphery.

Specifically mutants were developed through mutagenesis with Ethyl Methanesulfonate (EMS) in the non-host interaction of *Arabidopsis* and *Blumeria graminis* f.sp.*hordei (bgh)*, a grass powdery mildew fungus. Mutagenesis was applied for maintaining susceptibility in *Arabidopsis* and resulted in the identification of three *PEN* genes known as *PEN1, PEN2*,

PEN3 that are essential for non-host resistance, encoding a syntaxin protein, a glyxosil xydrolase and an ABC transporter respectively (Collins *et al.*, 2003; Consonni *et al.*, 2006).

In order to compare the level of *bgh* entry, an experiment was performed by Lipka et al. (2005) by exposing the fungus in 4 different combinations. Bgh was applied in wild type *Arabidopsis*, *pen1, pen2* single mutants and pen1 pen2 double mutants. Results have shown that single mutants *pen1* and *pen2* display entry rates up to 7 times higher than the wild type, while the double mutants exhibit 11 times higher penetration rates. These interactions led to the conclusion that *pen1* and *pen2* single mutants act in separate defense pathways and specifically *pen2* acts in association with *pen3* (Nürnberger and Lipka, 2005; Stein *et al.*, 2006). When the same experiment was repeated with inappropriate powdery mildew *Erysiphe pisi* and adapted powdery mildew *Golovinomyces orontii. E.pisi* shown enhanced invasive growth compared to *Bgh*, but similarly to *Bgh* failed to reproduce on *Arabidopsis* (Figure 5).

Comparative analysis in virulence level between *Bgh* and *E.pisi* led to the conclusion that *Arabidopsis* is at closer proximity with pea than barley(Lipka *et al.*, 2010).

In addition *G.Orontii* displays up to 80% invasive growth in wild type *Arabidopsis* compared to *Bgh* and *E.pisi* and no signs of change in invasiveness on either of the pen mutants (Figure 5). This suggests that *PEN 1* and *PEN 2* display entry limiting functions only in non-host powdery mildew pathosystems.

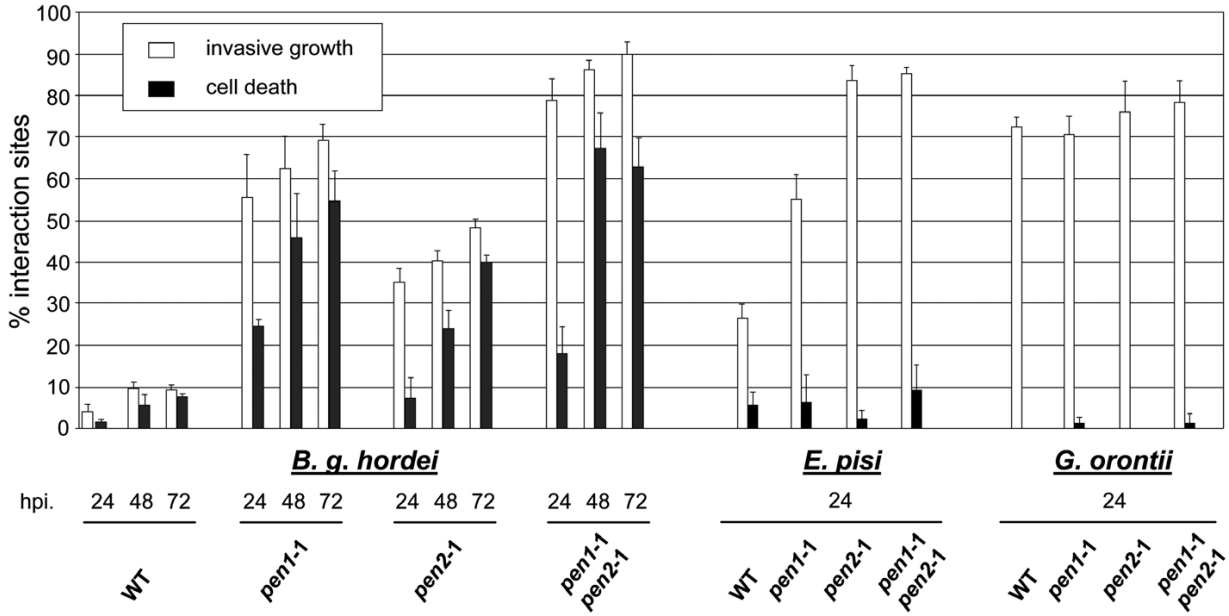

Figure 5. Graphic presentation of invasive growth and cell death at *Bgh*, *E.pisi* and *G.orontii* on WT *Arabidopsis, pen1, pen2* single mutants, and *pen1 pen2* double mutants (Picture is copied from Nürnberger & Lipka, 2005) .

Despite the fact that haustorial formation has been increased with the combination of mutations in all of these three genes, the overall susceptibility did not change because of successful post-entry cell death due to HR.

Post-haustorial NHR is controlled by 3 genes include *ENHANCED SUSCEPTIBILITY 1 (EDS1), PHYTOALEXIN DEFICIENT 4 (PAD4)* and *SENESCENCE ASSOCIATED GENE 101(SAG101).* Mutations in these three lipase-like proteins revealed reduction in HR response, allowing ectoplastic secondary hyphal growth and colony development of *Bgh* and *E.pisi* (Lipka *et al.*, 2005; Stein *et al.*, 2006).

Taking into account what has been mentioned above, we can summarize that Arabidopsis NHR to inappropriate powdery mildews depends on two effective defense systems: *PEN* genes ensuring pre-invasion resistance and *EDS1/PAD4/SAG101* regulating post-entry immunity (U. Lipka et al., 2010).

Particularly Schweizer proposed two possible models for NHR in consideration with the Zig-Zag model (Schweizer, 2007). According to the first one, the lack of evolution from the pathogens site may result in effectors that are ineffective or easily recognizable and thus suppression of PTI is not possible. The second model is based on the presence of multiple R-genes that successfully interact with pathogen effectors, leading eventually to durable resistance (Seifi *et al.*, 2014).

Pen Genes in other Crops

Appiano in her research showed through RNAi silencing that two syntaxin genes in tomato, *SlPEN1a* and *SlPEN1b,* are homologous of *Arabidopsis* genes and either have a major or minor role in penetration resistance. Specifically these genes are *AtPEN1*(major) and *AtSYP122*(minor) respectively. Both genes were studied with adapted powdery mildew *Oidium neolycopersici* and non-adapted powdery mildew *Bgh*. Results have shown that silencing *SlPEN1a* impacts the penetration resistance to both adapted and non-adapted powdery mildew's concluding that *SlPEN1a* is ortholog to *Arabidopsis AtPEN1* gene (Appiano *et al.*, 2015).

Still further research needs to be done in order to clarify the complete mechanisms of NHR to non-adapted pathogens and great attention must be given to the evolutionary relationship of the partners involved. This can be explained by the fact that in *Arabidopsis* interaction with adapted powdery mildew, effectors are responsible for invading the plant,

dealing successfully with both post and as well pre-invasion defenses (O'Connell and Panstruga, 2006).

Chapter 3: Resistance by modifying susceptibility genes

Susceptibility genes (S-genes)

For many years it was assumed that susceptible plants couldn't recover from disease. Breeding for resistance with *R* genes was the favored topic for research among the scientists in order to achieve immunity. However the limited durability of the *R*-Genes along with the potential for effectiveness against multiple pathogens led the way for scientists to search alternative ways for resistance. The above features of effectiveness and durability of resistance can be combined by focusing on the susceptibility genes (*S*-genes). The high success rate along with other advantages such as, higher durability and acceptance among other engineering tools, has established the application of *S*-genes over the resistance mediated by *R*-Genes (Pavan et al., 2010).

S-genes are actually genes of the plant that a pathogen manipulates for its own benefit, and can be considered all the genes that promote infection and susceptibility. This is actually the reason that they are named susceptibility genes (Van Schie and Takken, 2014). Specifically the term susceptibility gene was first introduced back in 2002, after the identification of the mutant *pmr6* conferring resistance to powdery mildew. At the moment over 100 *S*-genes are identified for pathogens and parasites (Van Schie and Takken, 2014).

The proteins that are encoded by *S*-genes, based on their operative nature, are essential by pathogens for two reasons: either for negatively regulating the plant defense response or as a necessity for growth and establishment of pathogens on the host plant (Pavan et al., 2010). Therefore loss of susceptibility of such *S*-genes results in immunity.

The majority of *S*-Genes discovered so far are in *Arabidopsis.* Resistance through *S*-Genes function is considered as non-host resistance and has also proved to be inherited recessively and broad spectrum(Zheng *et al.*, 2013; Gao *et al.*, 2015).

Application of S-genes into a breeding program

The first step is the identification of homologs at the crop of interest. At present the available information on genome sequence level especially in *Arabidopsis* has made the identification of homologs a relatively easy task.

The second step involves the investigation whether the identified *S*-gene is effective against the corresponding pathogen. This can be achieved by several methods such as silencing the

gene by RNA interference (RNAi), knocking out the gene, or overexpressing the candidate gene in order to see if a resistant plant ceases to be resistant.

After successfully accomplishing loss of function of the candidate *S*-gene, possible negative effects need to be checked including dwarfing, low fertility, yield reduction, and susceptibility to other pathogens.

Subsequently, and given that our candidate gene meets the criteria and therefore is promising, a stable mutation needs to be obtained. There are different strategies for creating a stable mutant. Wild relatives of the plant of interest are a great source of genetic variation and hence the natural mutations that occur are of great importance. One of the widely used approaches is crossing these wild species with commercial species and performing afterwards backcross breeding. Another option is the induction of chemical or physical mutations or knocking out the gene of interest either through virus-induced gene silencing (VIGS), RNAi or targeted genome editing.

Pathogen effectors as mentioned earlier are responsible for suppressing immunity. *S*-Genes from their part have the ability to code for effector targets that function as negative defense regulators or susceptibility factors (Pavan *et al.*, 2010). Effector targets can be plant factors that are crucial for the growth of the pathogen. In absence of the resistant protein, knocking down the effector target leads to resistance.

S-Genes Classification

There are 3 groups of *S*-genes based on the stage of infection according to Van Schie and Takken et al., 2014:

1) Genes allowing basic compatibility (pre-penetration), facilitating host recognition and penetration
2) Genes encoding negative regulators of immune signaling
3) Genes allowing sustained compatibility (post-penetration), fulfilling metabolic or structural needs and allowing pathogen proliferation

MLO-Mediated Resistance

One of the most recognized *S*-genes and also an astonishing report in disease resistant, involved in early pathogenesis, is the *MLO* (Mildew Locus O) gene that controls penetration of powdery mildew in *Arabidopsis* epidermal cells and acts as a negative regulator of plant defense responses. *MLO* was isolated in the same genetic screen with genes as *PMR4, PMR5,*

PMR6, PMR2 and is a prerequisite for conferring susceptibility to adapted powdery mildew species *G.cichoracearum* and *G.orontii* (Vogel and Somerville, 2000; Micali *et al.*, 2008).

The *MLO* gene was first discovered in Barley and was characterized and mapped on a later stage as a trans-membrane-anchored protein. Homologues of this gene family, act as susceptibility factors for the powdery mildew disease. Additionally to Barley, loss of function of MLO gene can result in resistance to both powdery mildew host plants *Arabidopsis* and tomato. This gene negatively regulates *PEN* genes (*PEN1/PEN2/PEN3*) that are associated with pathways for non-host resistance to powdery mildew in *Arabidopsis*. (Bhat *et al.*, 2005; Panstruga, 2005; Hardham, Jones and Takemoto, 2007).

MLO mutants were identified after the application of x-ray irradiation in Barley resulting in loss of susceptibility, and thus driving to the conclusion that *MLO* gene was knocked-out and plants carrying such a recessively inherited loss of function mutation are resistant to powdery mildew (Jørgensen, 1992).

Particularly in *Arabidopsis, MLO* genes are encoding by a family of 15 members, from which only three of them participate in powdery mildew susceptibility (*MLO2, MLO6, MLO12*) (Micali *et al.*, 2008). Inactivation through loss of function of *MLO2* results in incomplete resistance against the fungus and seems to prevent cell entry by 50%. Additionally in a study performed by Consonni et al., 2006 it was shown that double mutants *atmlo2/atmlo6* and *atmlo2/atmlo12* contribute to low levels of the powdery mildew growth while triple mutation in the phylogenetically closely related paralogs *MLO2, MLO6 and MLO12*, (*atmlo2/6/12*) results in the accomplishment of host immunity to powdery mildew(Consonni et al.,2006;Bai et al., 2007). (Figure 6)

Recent studies have shown that triple mutant *atmlo2/6/12* is not related only with resistance to powdery mildew, indicating that *MLO* proteins modulate the interaction of plants with other pathogens (Consonni *et al.*, 2006).

Figure 6. *Arabidopsis* plants five weeks after inoculation with powdery mildew *Golovinomyces oronti*. On the left side a heavy infected Col-0 wild type plant and on the right side a healthy triple mlo mutant in Col-0 genetic background (Consonni *et al.*, 2006).

Much alike to non-host resistance, *Mlo*-mediated resistance is independent of jasmonic acid (JA), and salicylic acid (SA) and ethylene (ETH) signaling, sharing the same histological mechanism of defense response (Trujillo *et al.*, 2004; Ellis, 2006).

Despite the fact that *Mlo*-based resistance is a very efficient method, some side-effects of the mutation need to be considered such as leaf cell death in older leaves and restrictions on its application in extreme environments (Hückelhoven, 2005).

Post-penetration S-Genes and their function

After successfully entering the epidermal cells and haustorium takes place, *S*-genes that belong to the second and third group are responsible for securing the interaction between the pathogen and the host. One of these genes is the *Enhanced Disease Resistance 1* (*EDR1*). The mutated gene *edr1* encodes a putative mitogen activated kinase kinase kinase regulating negatively SA-defense pathway. The mutated phenotype is associated with cell death at the infection site along with reduction in conidiophores, finally leading to MAMP-Triggered Immunity (Frye and Innes, 1998, 2007; Frye, Tang and Innes, 2001).

It has been proven that presence of EDR1 negatively affects RPW8 resistance gene by reducing RPW8 depended cell death upon pathogen attack (Xiao *et al.*, 2005).

The *edr1* mutation confers resistance to both *Arabidopsis* adapted powdery mildew *Golovinomyces cichoracearum* and the bacterial pathogen *Pseudomonas syringae* (Frye and Innes, 1998).

Similar to *EDR1, EDR2* encodes a START - lipid binding domain and acts in a common pathway with *EDR1* suppressing HR and SA defence responses. Double mutants exhibit similar resistant phenotypes to single mutant plants (Tang, Christiansen and Innes, 2005).

In contrast to *EDR1* and *EDR2* common function, *EDR3* gene seems to function independently.

Gao et al. (2015) showed that a natural *edr1* mutation in *Arabidopsis* accession C24 confers resistance to tomato powdery mildew *Oidium neolycopersici*. Nevertheless all *edr* mutants share the same connection with SA-mediated-resistance and programmed cell death.

Furthermore, additional susceptibility genes for powdery mildew in *Arabidopsis* have been identified. In an experiment performed by Vogel and Somerville, six powdery mildew resistant mutants were characterized (*pmr1-pmr6*) (Table 3).

PMR6 (Vogel *et al.*, 2002) and *PMR5* (Vogel *et al.*, 2004) are genes encoding a pectate lyase protein, thus loss of function in these genes confers resistance to powdery mildew species *Erysiphe cichoracearum* and *Erysiphe orontii*. Both mutants seem to carry alterations in plant cell wall composition by displaying increased pectin and uronic acid content, resulting in low levels of nutrient availability for the pathogen. The defense mechanism conferring resistance is independent of SA, ET and JA pathways. This can be explain by the fact that mutations in those defense pathways does not cause any changes on *pmr5* and *pmr6* mediated resistance (Micali *et al.*, 2008). Additionally both mutants confer resistance to two powdery mildew species but at the same time are susceptible to unrelated pathogens, thus leading to the conclusion that both proteins are considered to be a powdery mildew specific host susceptibility factor (Vogel *et al.*, 2002; Micali *et al.*, 2008).

On another note *Arabidopsis PMR4* mapped to chromosome 4, controls callose synthesis, which is induced by wounding or infection by the pathogen. *PMR4* belongs to the Glucan Synthase-like (GSL), gene family and was first introduced in a mutant screen for loss of susceptibility to adapted powdery mildew *Erysiphe cichoracearum* and *Erysiphe oriontii* (Huibers *et al.*, 2013). Overexpression of *PMR4* gene confers complete resistance to powdery mildew in *Arabidopsis* by high accumulation of callose deposits (Van Schie and Takken, 2014). Mutation in this gene (*pmr4*), surprisingly promotes resistance to powdery mildew by activation and increase of SA signaling pathway (Nishimura *et al.*, 2003). In contrast to *PMR5* and *PMR6, PMR4* and callose synthase negatively regulate the defense through the SA-pathway. Also callose synthase triggers successful defense responses against various types of pathogens, which makes it, unlike *PMR5* and *PMR6*, a more general basal defense mechanism located at the cell wall (Nishimura *et al.*, 2003).

In addition to previous findings, *pmr1* and *pmr2* located on chromosome 1 and *pmr3* located on chromosome 5 respectively also confer resistant to *Arabidopsis* powdery

mildew, *Erysiphe cichoracearum*. Except for *pmr1*, all other mutants were also resistant to *E.orontii,* whereas *pmr1* susceptibility indicates that the resistance of this mutant is specific. When challenging to unrelated pathogens like *P.parasitica*, surprisingly *pmr4* mutant showed resistance. Despite this, the susceptible phenotype of all mutants to *P.syringae* indicates that the resistance has a narrow spectrum. Segregation in F1 and F2 generation also proves that the resistance achieved by these mutations is recessive (Vogel and Somerville, 2000).

Additionally in an experiment performed by Huibers et al. (2013) silencing of *PMR4* by RNAi led in resistance to tomato powdery mildew *Oidium neolycopersici*, proving that *Arabidopsis PMR4* has functional orthologs in tomato (Figure 7) (Huibers *et al.*, 2013).

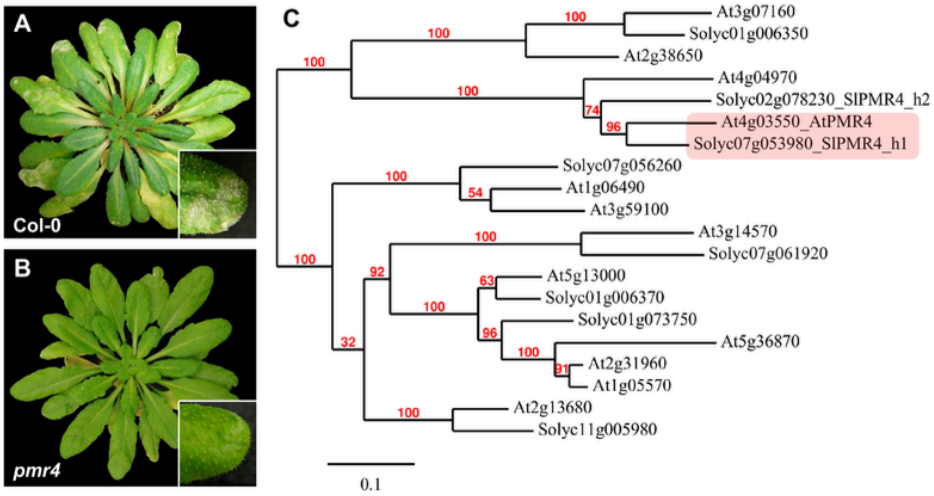

Figure 7. *Arabidopsis PMR4* resistance to *Oidium neolycopersici*. Col-0 and *pmr4* mutants 14 days after spraying with *Oidium neolycopersici*. Fungal spores are visible in Col-0 and not on *pmr4*, proving that *pmr4* is a susceptibility factor to *Oidium neolycopersici*. On the right a phylogenetic tree of *Arabidopsis PMR4* family-tomato *PMR4* orthologs. *SlPMR4* is the tomato ortholog of *AtPMR4* (Picture is copied from Huibers *et al.*, 2013).

In the same study, another *Arabidopsis* gene, *DMR1* which encodes a homoserine kinase, proved to act as *S*-gene for the tomato powdery mildew *O.neolycopersici*. Specifically silencing of this gene with RNAi resulted in low levels of sporulation in the mutated plants comparing to the parental lines after inoculation with *O.neolycopersici*. By using SGN (Sol Genomic Network), it was found that *AtDMR1* has 71% sequence identity with a single tomato gene, which was prove to be the tomato ortholog of *AtDMR1*, named *SlDMR1*. Pleiotropic effects have been observed in silenced *SlDMR1* plants and *dmr1 Arabidopsis* mutants that were smaller in size, unlike silenced *SlPMR4* plants that no pleiotropic effects were present(Huibers *et al.*, 2013).

Table 3. Known susceptibility genes to powdery mildew identified in *Arabidopsis.*

Gene	Encoded Protein	Powdery Mildew species	Specificity	Mechanism	Reference
EDR1	Mitogen-activated protein kinase	*G. cichoracearum*	Broad Spectrum	Salicylic Acid	C. A. Frye et al., 2001; Catherine A. Frye & Innes, 1998
MLO	Transmembrane protein	*G. cichoracearum* *G. orontii*	Broad Spectrum	Papillae	Consonni et al., 2006
PMR4	Callose synthase	*G. cichoracearum* *G. orontii*	Broad Spectrum	Salicylic Acid	Nishimura et al., 2003
PMR5	unknown	*G. cichoracearum* *G. orontii*	Broad Spectrum	Cell walls with pectin	J. P. Vogel et al., 2004
PMR6	pectate lysase-like	*G. cichoracearum* *G. orontii*	Broad Spectrum	Unknown	J. P. Vogel et al., 2002
PMR2	Transmembrane protein	*E. cichoracearum*	Broad Spectrum	Salicylic Acid	Consonni et al., 2006

Chapter 4: Wageningen Experiment – Mapping of Arabidopsis resistance to tomato powdery mildew

O. neolycopersici

O. neolycopersici is one of the two powdery mildew species classified in the *Oidium* genus that affects tomato (Kiss et al 2001), and one out of four that affects *A. thaliana* (Xiao et al 2001). Although is widely known as tomato powdery mildew, its host range is considered to include species of about 13 plant families (Lebeda et al 2014). The first report of this pathogen was in the mid 80's in several parts of the world and since then, tomato powdery mildew has been spread all around the world making it one of the most common diseases nowadays (Paternotte et al 1988). Having a worldwide distribution and rapid spread along with the diversification and the intensification of agriculture, *O. neolycopersici* impacts negatively on plant growth and causes profound yield and economical losses.

Resistance against *O. neolycopersici* in Tomato and *Arabidopsis*

Following genetic research on wild relatives of tomato, several resistant accessions have been characterized and nine loci have been mapped conferring resistance to *O.neolycopersici* (Seifi *et al.*, 2014). Resistant genes include 5 dominant monogenic genes (*Ol-1, Ol-3, Ol-4, Ol-5, Ol-6*), one recessive monogenic loci (*ol-2*) that was found to be a homologue to barley Mlo, and three quantitative trait loci. The exact location of the resistant genes along with the basis of the resistance is indicated in Table 4. Both the *Ol*-genes and the QTL's are associated with the hypersensitive response and papillae formation (Bai *et al.*, 2005).

Table 4. Resistant genes and QTL's identified in wild tomato species against powdery mildew

Gene	Location	Genetic basis	Resistance Mechanism
Ol-1	Chromosome 6	Dominant	Slow HR
Ol-3	Chromosome 6	Dominant	Slow HR
Ol-4	Chromosome 6	Dominant	Fast HR
Ol-5	Chromosome 6	Dominant	Slow HR
Ol-6	Chromosome 6	Dominant	Fast HR
Ol-2	Chromosome 4	Recessive	Papillae
Ol-gtl1	Chromosome 6	QTL	
Ol-gtl2	Chromosome 12	QTL	
Ol-qtl3	Chromosome 12	QTL	

In *Arabidopsis,* as we have mentioned earlier, resistance to *O.neolycopersici* has been confirmed through the *S*-gene function of *Arabidopsis* genes *PMR4* and *DMR1*. Mutants *pmr4* and *dmr1*, along with silencing of tomato orthologs *SlDMR1* and *SlPMR4*, appear to reduce the growth and multiplication of *O.neolycopersici* in *Arabidopsis* and Tomato respectively.

Research aim

The aim of the experiment concerned mapping of the resistance observed in *Arabidopsis thaliana* accessions Aa-0, Litva and Ts-2, against tomato powdery mildew.

Research questions applied:

- Which new markers can be used for mapping?
- In which chromosome is the candidate gene for resistance?
- Which marker is linked to the resistance gene?
- Which gene is responsible for the resistance?

Materials and Methods

In order to map the resistance observed in the *Arabidopsis thaliana* accessions that were resistant to tomato powdery mildew the following steps were performed. The first step was to apply a disease assay with *O. neolycopersici* in an F2 *Arabidopsis* population. The pathogen was maintained on tomato plants; specifically the source of fungal spores was infected tomato leaves. The second step was to isolate the DNA from an F2 population and genotype these plants with CAPS markers. The final step included genetic mapping, through marker data analysis, in order to identify the locus of the resistance gene.

Plant Material

There were 3 possible crosses between *Arabidopsis* accessions as shown in Tables 5,6,7. Depending on the germination rate, population size and the phenotypic evaluation of all 3 possible crosses the most promising cross was going to be chosen for further research.

1st experiment:

The first probable experiment to be performed was a cross of Litva *Arabidopsis* accession with the susceptible variety Col-0. Through mapping of this population 4 QTLs were found and analyzed in a previous experiment. The F1 of the cross was shown to have susceptible phenotype and the phenotypic segregation ratio in F2 generation was statistically analyzed with Chi-square Goodness of fit test hypothesizing resistance to be caused by a single recessive gene.

The aim was to confirm the results that were found. The main disadvantage was the absence of Litva accession from the Gramene SNP Query.

Table 5. 1st possible cross between *Arabidopsis* accessions

I				
Cross	**F1 Phenotype**	**segregation in F2 (R:S); (PX^2 test; 3:1)**	**segregation in F2 (R:I:S); (PX^2 test; 1:2:1)**	**segregation in F2 (R:S); (PX^2 test; 1:3)**
Litva(♀) x Col-0(♂)	Susceptible	(17:79); P<0.001	(17:51:28); P=0.235	(17:79); P=0.099

2nd experiment:

The second probable experiment to be performed was a cross of Ts-2 *Arabidopsis* accession with the susceptible variety Sha. The F1 of the cross was shown to have susceptible phenotype and the segregation in F2 confirmed with Chi-square Goodness of fit test that indicates that resistance is mainly of polygenic origin. The aim here is to identify the gene that is responsible for resistance against *O. neolycopersici.* The disadvantage in these cases is that there is only availability to Ts-1 in Gramene SNP Query and the fact that there are almost no seeds of Ts-2 (control) available.

Table 6. 2nd possible cross between *Arabidopsis* accessions

II				
Cross	**F1 Phenotype**	**segregation in F2 (R:S); (PX^2 test; 3:1)**	**segregation in F2 (R:I:S); (PX^2 test; 1:2:1)**	**segregation in F2 (R:S); (PX^2 test; 1:3)**
Ts-2(♀) x Sha(♂)	Susceptible	(72:24); P=0.814	(72:24:0); P<0.001	(72:24); P<0.001

3rd experiment:

In this case a cross between Aa-0 and the susceptible Col-0 resulted in a resistant F1. Segregation in F2 indicated that resistance is due to duplicate dominant epistasis, so mainly of polygenic origin. Specifically resistance is probably due to 2 dominant genes. The advantage here is the presence of Aa-0 in Gramene SNP Query and the availability of seeds. The aim in this case, similar to previous one is to identify the gene conferring resistance against powdery mildew *O. neolycopersici.*

Table 7.3rd possible cross between *Arabidopsis* accessions

III				
Cross	**F1 Phenotype**	**segregation in F2 (R:S); (PX^2 test; 3:1)**	**segregation in F2 (R:I:S); (PX^2 test; 1:2:1)**	**segregation in F2 (R:S); (PX^2 test; 1:3)**
Aa-0(♀) x Col-0(♂)	Resistant	(85:7); P<0.001	(85:7:0); P<0.001	(85:7); P<0.001

Sowing the seeds and transplantation

Arabidopsis plants of all 3 crosses sowed under short day conditions with 22˚C/18˚C day/night temperature in order to avoid early flowering of the plants (Table 8). After 2 weeks, plants of each germplasm were transplanted in trays in order to grow separately. A total of 800 plants from the 3 crosses along with the parents were transplanted two weeks after sowing.

Table 8. Seeds of all 3 possible experiments were sown in controlled conditions in a growth chamber. Based on the germination rate the plants were transplanted in 40-well trays after two weeks.

Experiment A				
Labeled	**Cross**	**Generation**	**Derived Plant**	**Seeds left**
A1	925 x Col-0	F3	F2-36	Left some seeds
A2	925 x Col-0	F3	F2-35	Left some seeds
A3	925 x Col-0	F3	F2-95	Left some seeds
A4	925 x Col-0	F3	F2-4	Left some seeds
A5	925 x Col-0	F3	F2-77	Left some seeds
A6	Control (Litva)			
A7	925 x Col-0	F3	F2-21	No seeds
Experiment B				
Labeled	**Cross**	**Generation**	**Derived Plant**	**Seeds left**
B1	6868 x Sha	F2	F1-1	Left some seeds
B2	6868 x Sha	F2	F1-2	Left some seeds
B3	6868 x Sha	F2	F1-3	Left some seeds
B4	6868 x Sha	F2	F1-4	Left some seeds
B5	6868 x Sha	F2	F1-5	Left some seeds
B6	Control (Ts-2)			No seeds left
Experiment C				
Labeled	**Cross**	**Generation**	**Derived Plant**	**Seeds left**
C1	10182 x Sha	F2	F1-1	Almost no seeds
C2	10182 x Sha	F2	F1-6	Almost no seeds
C3	10182 x Sha	F2	F1-7	Almost no seeds
C4	10182 x Sha	F2	F1-3	Almost no seeds
C5	10182 x Col	F2	F1-7	Left some seeds
C6	10182 x Col	F2	F1-4	Left some seeds
C7	10182 x Col	F2	F1-10	Left some seeds
C8	10182 x Col	F2	F1-3	Left some seeds
C9	Control (Aa-0)			very few seeds left
	Control (Sha)			few seeds left
	Control (Col-0)			Many seeds left

DNA isolation

Genomic DNA was isolated from all *Arabidopsis* plant leaves applying the protocol for 'dirty' genomic DNA isolation using a CTAB buffer, which is shown in Appendix.

Maintaining of the fungus

The isolate of *O.neolycopersici* (On-Ne), which is "The Netherlands' isolate" that has been collected in the 1990s (Lindhout, Pet and van der Beek, 1993) was maintained on susceptible tomato cultivar Moneymaker. The maintenance took place in a growth chamber at 20/2˚C with 70% relative humidity and a photoperiod of 16h.

Inoculation and Phenotyping

The inoculum was consistent of fungal spores from infected Moneymaker leaves. *Arabidopsis* plants of approximately 30 days old were inoculated by spraying fungal spores on the leaves. Each plant was exposed to the same amount of inoculum, in order to avoid variations in the degree of the infection as a result of differences in the proportion of the inoculum.

The concentration used in the inoculum was 50×10^6 which is high enough to ensure that susceptible plants will not escape infection.

The inoculation took place in the growth chamber, since key factors such as air humidity, temperature and light intensity were easier to manipulate in order to ensure as much as possible the development of the attacker. As a control the susceptible accession Col-0 and Sha were used together with the other plants.

The determination of infection phenotypes was performed qualitatively, by using the discrimination resistant or susceptible, approximately fourteen days post inoculation with *O.neolycopersici.*

Marker development

For the development of CAPS (Cleaved Amplified Polymorphic Sequence) markers we focused on experiment C and the cross between *Arabidopsis* accession Aa-0 and susceptible accession Col-0. The availability of Aa-0 in Gramene SNP Query and the availability in seeds were the main reasons behind the selection of this experiment. The genome sequence of

the crop of interest and the Blast tool[1] (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were great sources of information in order to achieve this aim.

CAPS markers were developed using the website Gramene SNP query[2] and by employing the sequence data available via TAIR[3]. In each of the 5 chromosomes of *Arabidopsis*, 5 markers were developed in such a way as to cover the whole chromosome. For CAPS markers, primers were designed using the website Primer3Plus[4], in order to match a unique flanking DNA sequence. NEBcutter V2.0 website[5] was then used in order to find combinations of Primers and restriction enzymes that through restriction fragment length polymorphism discriminate between the Aa-0 or Col-0 genotype. Blast was also used in order to ensure that the primers bind only to the complementary sequence in the genome and not to similar sequences elsewhere, in order to multiply specifically the desirable genetic loci. The list of the CAPS markers is presented in Table 9.

Results

Table 9. CAPS markers developed between Aa-0 and Col-0 genotypes for mapping powdery mildew *Oidium neolycopersici* resistance genes in *Arabidopsis.*

Chromosome	Region	Primer	Primer code	Sequence	Restriction Enzyme
1	1	Forward	ch1_r1_f	gcttcgaggaagctgagtgt	RSA1
1	1	Reverse	ch1_r1_r	tacagaagaaatcaatgcgtga	RSA1
1	2	Forward	ch1_r2_f	tcttctcctcctacgctcctc	HpyCH4 IV
1	2	Reverse	ch1_r2_r	cgccgaaacaattcttcaac	HpyCH4 IV
1	3	Forward	ch1_r3_f	catccaatgtccatctttttctaa	Hha1/Aa-0
1	3	Reverse	ch1_r3_r	gaggtggatgtttacattcctttt	Hha1/Aa-0
1	4	Forward	ch1_r4_f	acacaggccaaaatgcatag	Pst 1
1	4	Reverse	ch1_r4_r	cattacatgcttatgttgaacttcc	Pst 1
1	5	Forward	ch1_r5_f	tgagctcgtaacgatcatgg	Bsr I / Hinf I
1	5	Reverse	ch1_r5_r	taacaacactcccaggacca	Bsr I / Hinf I
2	1	Forward	ch2_r1_f	ggaagcacgagaatgaaagg	Nhe1
2	1	Reverse	ch2_r1_r	ttgctcaaccacaggaatga	Nhe1

[1] https://blast.ncbi.nlm.nih.gov/Blast.cgi

[2] https://archive.gramene.org/db/diversity/snp_query?species=arabidopsis&chr=&object_name=

[3] https://gbrowse.arabidopsis.org/cgi-bin/gb2/gbrowse/arabidopsis/

[4] https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi

[5] https://nc2.neb.com/NEBcutter2/

2	2	Forward	ch2_r2_f	gcgttcacaaggacgatttt	Hpa II
2	2	Reverse	ch2_r2_r	gaaggcgaaggagaatgaga	Hpa II
2	3	Forward	ch2_r3_f	ttcctaatcccaagcctgttt	Aci 1
2	3	Reverse	ch2_r3_r	ggcctcttgttactcttttgtagg	Aci 1
2	4	Forward	ch2_r4_f	ggttcacatcccatatgttgc	HpyCH4IV
2	4	Reverse	ch2_r4_r	cgctatgtgcctacggaaga	HpyCH4IV
2	5	Forward	ch2_r5_f	ttgtgaactggtttccatca	Dra I/MseI
2	5	Reverse	ch2_r5_r	tcccagttggcctcaatagt	Dra I/MseI
3	1	Forward	ch3_r1_f	gggggaggttatcagaatca	Dde 1
3	1	Reverse	ch3_r1_r	tctggcgaatcgttacaaaa	Dde 1
3	2	Forward	ch3_r2_f	tatccgagaaacgtcctcca	Aci 1
3	2	Reverse	ch3_r2_r	ccaataatgttacttctcagatttctc	Aci 1
3	3	Forward	ch3_r3_f	atgctcattcgcttccatgt	Nde I
3	3	Reverse	ch3_r3_r	agcgagaaagcgacaagcta	Nde I
3	4	Forward	ch3_r4_f	gtcgatcgtccacaggaagt	Xbal
3	4	Reverse	ch3_r4_r	aatcttcgagatttggggattt	Xbal
3	5	Forward	ch3_r5_f	ttgttggcaaattcggatg	Tag1
3	5	Reverse	ch3_r5_r	gcaagcaactcgctagtcaa	Tag1
4	1	Forward	ch4_r1_f	ttgagctttcctttagatcatca	Bfa 1
4	1	Reverse	ch4_r1_r	tccattaacaatcagtggatcg	Bfa 1
4	2	Forward	ch4_r2_f	ttattctctcgctcgccatt	Rsa 1
4	2	Reverse	ch4_r2_r	ttattctctcgctcgccatt	Rsa 1
4	3	Forward	ch4_r3_f	gaaaattgcgacaggagagg	Aci 1
4	3	Reverse	ch4_r3_r	gaagaatagaacgctgcaaaaa	Aci 1
4	4	Forward	ch4_r4_f	gcattctctctccactcagtctt	HpyCH4 IV
4	4	Reverse	ch4_r4_r	aaacagttatgagctgtcttgatga	HpyCH4 IV
4	5	Forward	ch4_r5_f	gcctcaatcctaaccgaatc	Ddel 1
4	5	Reverse	ch4_r5_r	tccaggaaggaatggaaaga	Ddel 1
5	1	Forward	ch5_r1_f	tgccatggatttaagatgaaga	Aci I/Aa-0, Pst1/Col-0
5	1	Reverse	ch5_r1_r	tttgctcttgcaatcctgaa	Aci I/Aa-0, Pst1/Col-0
5	2	Forward	ch5_r2_f	cggttctctcatagggttcc	Aci I
5	2	Reverse	ch5_r2_r	gaaaaattacaacataccaagcta ca	Aci I
5	3	Forward	ch5_r3_f	aaaaatttaaatcccgcacaa	DpnI
5	3	Reverse	ch5_r3_r	tccaagtgatgaccaaatgaa	DpnI
5	4	Forward	ch5_r4_f	ttccgggaaaatgctctactt	Dpn I
5	4	Reverse	ch5_r4_r	accaagcactgggatcagtc	Dpn I
5	5	Forward	ch5_r5_f	aaaatctagttaaccgtcacttaac ac	Dpn I/Aa-0, Sau96/Col-0
5	5	Reverse	ch5_r5_r	tgcaacatccggactacaga	Dpn I/Aa-0, Sau96/Col-0

Further explanation

The main aim in this thesis was the identification of resistant genes of *Arabidopsis thaliana* against tomato powdery mildew *Oidium neolycopersici* through interval mapping. The cross that was used in the experiment was the cross between *Arabidopsis thaliana* accession Aa-0 and susceptible accession Col-0. During the experimental phase in Wageningen the following steps were performed:

1. We sow the seeds of the following crosses
    a) Litva x Col-0
    b) Ts-2 x Sha
    c) Aa-0 x Col-0

    Based on several criteria and parameters that were mentioned and analyzed on the experimental part, we choose to proceed with the cross between Aa-0 x Col-0
2. After 2 weeks the plants were transplanted in trays
3. Genomic DNA isolation was performed to all the *Arabidopsis* plants (parental and progeny lines)
4. Plants were inoculated at 30 days by spraying fungal spores from infected Moneymaker leaves and the infection rates were measure qualitatively as R or S
5. Markers were developed in all 5 chromosomes for the experiment C (Aa-0 x Col-0), using bioinformatic tools as mentioned above in the Marker development section.

Theoretical section of the proceed of the planned experiment and expected results

Marker assisted selection procedure

In order to visualize the variation between the chromosomes of a DNA sequence, a combination of tools has been developed. A difference in the DNA sequence can be used as a marker, in order to stipulate that specific region of the DNA.

In order for a DNA marker to be useful in breeding programs it is essential that genetic differences between the two crossing parents occur and thus the marker must be polymorphic. In addition a reliable association between the marker and the trait of interest is highly depended from the distance between the two. For example the lower the distance between the marker and the trait of interest, the less the probability that recombination

may occur, during meiosis. According to that, the ideal genetic marker must be as close as possible or even a part of the gene of interest.

The development of markers include the following 3 techniques:

1. Digesting the DNA at the restriction sites using restriction enzymes.
2. Amplifying the DNA fragments by PCR, resulting in numerous copies of each specific fragment
3. Gel electrophoresis followed by staining with chemicals in order to separate and visualize the amplified products by size

In order to identify DNA regions that contain markers, related to the trait of interest linkage maps must be developed. Linkage or genetic maps are created based on statistical calculations of recombination frequencies between the markers. Specifically, for a linkage map to be developed the following steps must be performed

1. Development of a mapping population
2. Genotyping
3. Linkage analysis
4. Map construction

For a successful breeding program to be achieved the development of a mapping population is a very crucial step. It is essential that crossing parents with contrasting traits of interest have polymorphic markers. Several population types can be used that affect the mapping procedure. The most widely used are: homozygous parents of F2, recombinant inbred lines, backrossing in autogamous crops and F1 of heterozygous parents in allogamous crops(Collard *et al.*, 2005). In our case we performed mapping in F2 derived from homozygous parents as mentioned in the experimental part (Figure 8).

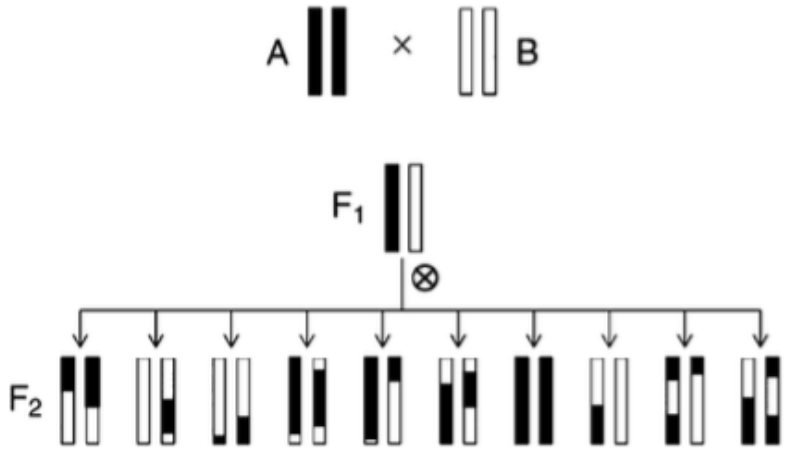

Figure 8. Development of a F2 mapping population. A female homozygous inbred line is crossed with a male homozygous inbred line. The heterozygous F1 plants are then selfed to develope the F2 seeds (Zhang, 2012)

The second step once the mapping population is genotyping, for example by DNA sequencing or RFLP analysis. High degree of heterozygosity in one or even better both of the parents is a necessary prerequisite.

DNA must be extracted from both parents and offsprings through a DNA isolation protocol. This allows us to record all individual genotypes of the mapping population. The next step towards the construction of a linkage map is the linkage analysis of markers. Specifically in this analysis, which is performed by a specific computer software, we correlate the markers that we have already developed to all individuals of the mapping population and the parents to determine those markers that tend to segregate in association with the trait of interest.

In order for this analysis to be performed, at first all genotypes of each individual must be recorded as A and B, for all markers. Based on the associate segregation of the markers we can observe which marker is closely related to other, by comparing marker pairs. The differences between the markers are calculated by analyzing the recombination frequencies between the pairs of markers. In the end when all the data are included in the analysis, a linkage map is constructed, demonstrating the most probable order of the markers.

For large number of markers, when the association cannot be observed manually, computer programs such us Mapmaker and JointMap are widely used (Lander *et al.*, 1987; Albini, Falque and Joets, 2003). These specific softwares are able to calculate all pairwise recombination frequencies of markers through LOD (Logarithm of Odds) score.

The LOD threshold for linkage is between 3 and 4, in order for a pair of markers to consider linked. This value is consistent with level of confidence about 95% with the other 5% representing false positive.

Table 10. Example of a linkage table demonstrating the linkage between resistance and specific markers.

	P1	P2	1	2	3	4	5	6	7	8	9	10	11	12
M1	AA	GG	AA	AA	AA	GG	GG	AA	GG	GG	AA	AA	AA	GG
M2	TT	CC	TT	TT	TT	TT	CC	CC	CC	TT	CC	CC	CC	TT
M3	TT	GG	GG	TT	GG	GG	TT	TT	GG	TT	TT	GG	TT	GG
M4	GG	AA	AA	GG	AA	AA	GG	GG	AA	GG	GG	GG	AA	AA
M5	TT	AA	TT	TT	TT	TT	AA	TT	AA	TT	AA	AA	TT	TT
M6	AA	CC	CC	AA	AA	AA	AA	AA	AA	CC	CC	AA	CC	CC
M7	CC	TT	TT	CC	CC	TT	TT	CC	TT	TT	CC	TT	CC	TT
Phen	**S**	**R**	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**S**	**S**	**R**	**S**	**R**

In the example above two homozygous parents were crossed, resulting in a RIL population, here showing 12 progenies. Both parents and progeny were genotyped for 7 different markers and were phenotyped according to their reaction to a specific disease resistance or susceptibility. Specifically all phenotypic data presented are marked as R (resistant) or S (susceptible).

When we compare the phenotypic data with the genotyping data of the markers, we can indicate whether there is a clear linkage between the marker and the phenotype. In this specific example, M3 is the only marker that its genotypic data (GG and TT) consistently follow the phenotypic data (R and S respectively).

In case recombinations occur between the associated data, then we can assume that there is some distance from the resistance gene. Recombination events, determine the distance between the markers and the gene of the trait of interest.

There are two terms referring to the distances of those recombinations. Hot spots are used when there are high levels of recombinations and thus larger genetic map distances and distance between the markers, in contrast to cold spots that refer to lower genetic map distances and markers that tend to be closer to one another.

After the linkage analysis has been performed and the recombination frequencies between the markers determined, the next step is to construct the linkage map by using those recombination frequencies. In order to convert the recombination frequencies into genetic

distance and thus providing a clearer estimation of the genetic distance, several methods can be applied, called mapping functions, with Haldane and Kosambi being the most widely used ones. Haldanes is based on the assumption of no interference meaning that the crossovers are independent of one another, whereas Kosambis is based on the assumption of positive interference allowing for positive crossovers to occur (Semagn, Bjørnstad and Ndjiondjop, 2006).

In conclusion, in order for a mapping experiment to be successful several parameters must be taken into account. Population size and composition is of great importance. The larger the mapping population the more accurate the map distances.

After the construction of the linkage map is finished and the order and the distances of markers have been assigned, the next step in a mapping experiment is phenotyping.

The purpose of the phenotyping experiment is to assign a trait value to each member of the mapping population. The higher the accuracy of the phenotyping, the more realistic the QTL (Quantitative Trait Loci) mapping results will be. A QTL is a locus-region on a chromosome in which there is statistical association with a certain phenotype that differentiates within a population and it requires that enough segregation exists for the traits in the mapping population. Specifically for detecting reliable and accurate QTLs large populations and repeated phenotyping are required. In simple terms the QTL mapping is a statistical analysis to investigate whether there is a correlation between a specific region on the chromosome and a certain phenotype (Kao, Zeng and Teasdale, 1999).

The final step of a successful breeding program is the QTL analysis. In this analysis a combination of phenotyping data, genotyping data and the linkage map are essential in order to detect QTLs and evaluate their importance.

At first the markers developed are used to divide the mapping population in several groups based on their marker genotype (A or/and B allele). In case of F2 population three groups are performed based on the presence of the A allele for parent 1, B allele for parent 2 and H (AB) in case of codominance of markers.

Then a statistical analysis in used to find significant differences between the phenotypic means of the groups. If so then this is an indication that the marker is associated with the QLT that is responsible for the trait of interest and a gene or more genes may influence the trait in that specific region. The above process is repeated for all markers.

Three different methods are used for detecting QTL's. The first one is single marker analysis in which one marker is involved at a time to find the link between the marker and the QTL(Edwards, Stuber and Wendel, 1987). An example is presented below (Figure 9).

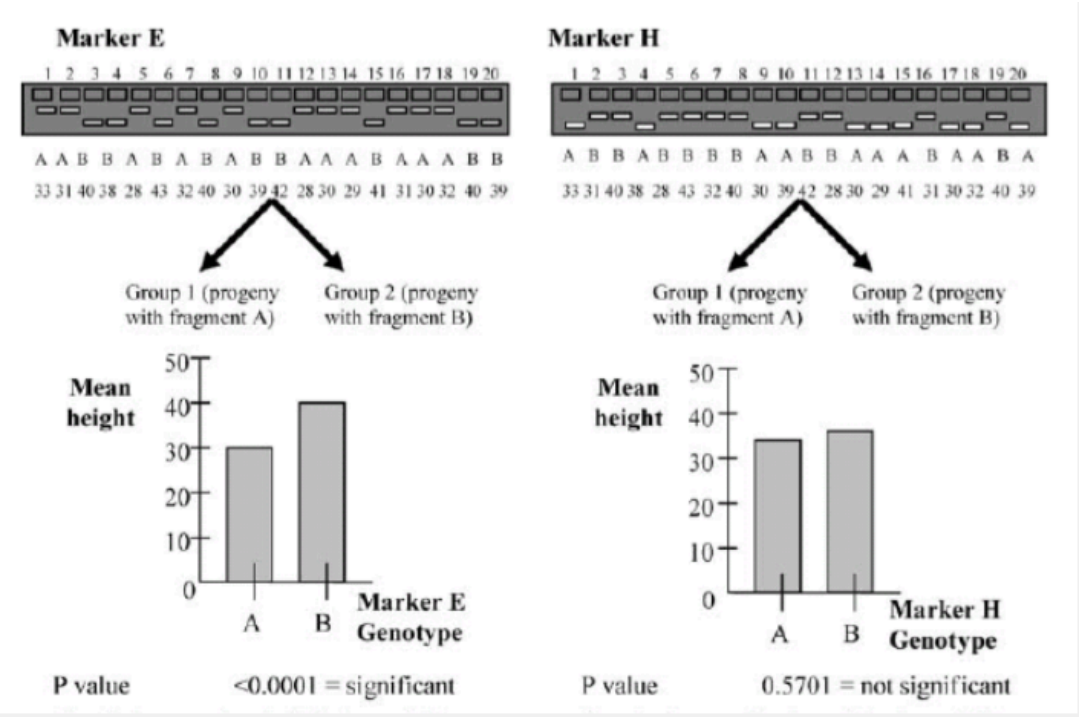

Figure 9. Principle of QTL mapping in single marker analysis. In the example above a significant difference (P value < 0.05) is observed between the two examined groups and that is an indication that marker E is linked to a gene with quantitative contribution to the trait. In contrast, no significant difference is observed in marker H, therefore this marker is not linked to the aforementioned gene (Collard *et al.*, 2005)

The second is the interval mapping that uses a pair of adjacent markers and a supposed QTL in between of those markers as presented in the figure below (Figure 10).

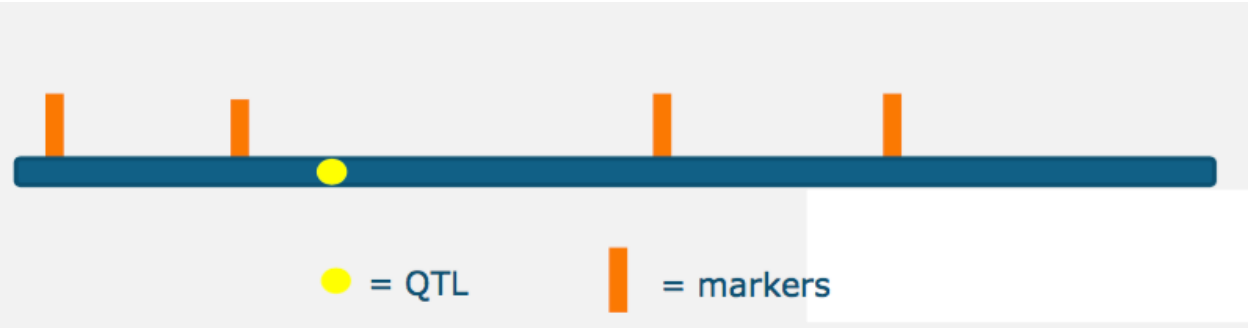

Figure 10. Interval mapping considers all position between markers possible QTL positions. It requires a linkage map and examines one QTL at a time.

Interval mapping posses higher power than single marker analysis and its principle is based on maximum likelihood approach that calculates likelihoods assuming a QTL/no QTL (Figure 11).

The third one mention as multiple QTL mapping is the most precise method of mapping allowing screening of multiple QTLs by using multiple marker intervals at the same time(Kao, Zeng and Teasdale, 1999).

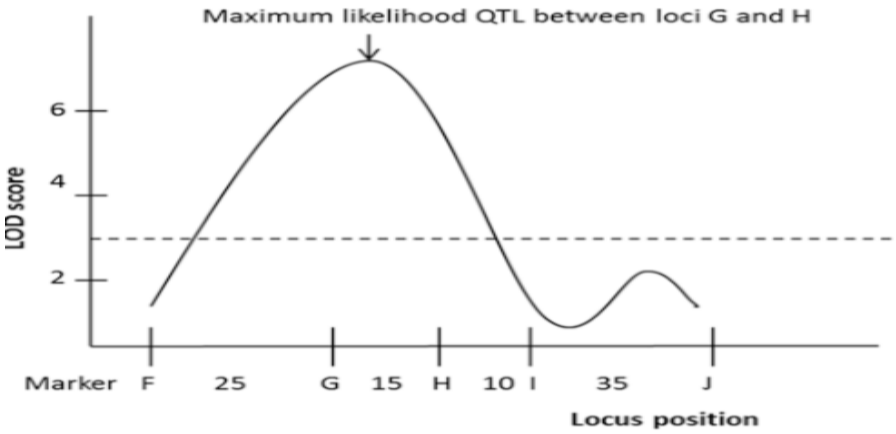

Figure 11. Principle of interval mapping (Lander and Botstein, 1989).

The above figure presents the LOD (logarithm of odds) profile for a particular chromosome or linkage group. The LOD curve achieves its maximum value somewhere between markers G and H, indicating that the QTL is present at that position. Also the QTL level of significance is defined from a LOD score being above the threshold value, which is between 3 and 4.

The even more precise location of a QTL can be achieved through fine-mapping. Several studies have shown that a marker in order to be informative must at least be 5cM away from the QTL. In most of the MAS experiments the average distance between markers and QTL varies between 5-20cM. So in order to increase the accuracy, fine mapping is performed with more number of markers within the suspected region (Boopathi, 2020).

General Discussion

Sources of Resistance

In order to achieve the introduction of resistance we need to ensure that sources of resistance are available during plant breeding (Polák and Bartoš, 2018). There are various approaches to introduce resistance nowadays, some of which are briefly explained further down. Cultivated crops were the first source of resistance breeding used. Prerequisite for this breeding method was the existence of an already resistant cultivar. The main disadvantage was low durability since the same source of resistance was used. Interspecific as well as intergeneric crosses were in the spotlight later on, with wild species being the example. Great care must be given for choosing a suitable source. An advantage of this method related to others is the large genetic variation that can be achieved, since pathogen and host are co-evolved for age. Disadvantages must also been taken seriously into account, since undesirable characteristics may arise, and barriers of crossability and sterility need to be resolved. Landraces, similar to wild species, are great source of resistance as they also display great genetic diversity (Niks *et al.*, 2019).

It has been worldwide accepted across the breeding community that the *Arabidopsis* plant comprises a successful model organism, which has benefited its members with lots of knowledge, scientific development and technological evolution, improving their work in many different levels.

Evolution and future perspectives of *R* genes and *S* genes

The interaction between *Arabidopsis* and powdery mildew has been a major topic across the breeding community, particularly with the use of plant accessions containing resistant genes that were crossed with susceptible *Arabidopsis* accessions, in order to achieve resistance. The straightforward application of several advance genetic methods along with a relatively small and simple genome sequence, made this model plant a pioneer in the area of breeding.

The introduction of *R* genes against various pathogens is of great interest for plant breeders and research has been going on for years in order to have a clear understanding of the evolutionary developed interactions between host and pathogens. Most of the *R* genes cloned so far encode a leucine rich repeat (LRR) region (Michelmore and Meyers,

1998). The most striking example of resistant gene in *Arabidopsis* powdery mildew pathosystem, conferring broad-spectrum resistance is the atypical *RPW8* locus from MS-0 accession, mapped on chromosome 3. Natural resistance analysis in *Arabidopsis* accessions revealed that resistance is either based on RPW8 or based of polygenic origin (Wilson *et al.*, 2001; Göllner *et al.*, 2008).

In case of polygenic resistance, it may occur by multiple genes that are all necessary for resistance or by combination of single genes, each of which independently provides resistance. The reason behind the lack of typical *R*-genes in *Arabidopsis*-powdery mildew resistance is partly due to the fact that the interaction between *Arabidopsis* and powdery mildew is recent, so both Avr and *R* gene pairs have limited time to evolve and also because *Arabidopsis* is not the primary host for powdery mildew (Schulze-Lefert and Vogel, 2000; Göllner *et al.*, 2008; Micali *et al.*, 2008).

Marker-assisted selection (MAS), and the use of DNA markers have improved the efficiency and accuracy of conventional plant breeding. Pathogens evolutionary potential is a big challenge for breeders and MAS reduce the reliance on laborious and time consuming screening procedures. Pyramiding multiple genes or several QTL's for a single disease resistance is a very important concept. This gives the opportunity to the host to simultaneously express more than one *R* gene, so that pathogen reproduction will be restricted in case those new avirulence genes evolve. Multiple *R* genes can be identified through transformation technologies by introducing these genes into the candidate plant either from wild relatives or sexually incompatible relatives.

Moreover, an alternative approach, which also is a challenge for breeders, is to use the knowledge that we have of PAMPs, effector proteins and *R* genes for improving resistance. Based on the work of Shen (Shen *et al.*, 2003), the identification of effectors that are responsible for strong virulence symptoms in plants can lead to the discovery of the most functional *R* genes for resistance.

Effectors demonstrate a multifunctional character by targeting many proteins involved in immunity, in order to obtain virulence. The challenge in the case of effectors is to investigate the functioning of pathogen effectors and improving the understanding of the interactions between plant and pathogens. This understanding could be of great significance in disease resistance breeding (Zhang *et al.*, 2022).

As most of the effectors often attack host plants, the recognition of the effector proteins will convert the host pathogens into non-host pathogens.

Furthermore resistance can be accomplished in different ways and each way has its own benefits and drawbacks. Except from the isolate presenting specific resistance, which presupposes the right combination of the resistant gene and the avirulence gene, recently the use of *S*-genes, specifically through the loss of function of these genes, is applied for obtaining a more durable non-host like resistance. Until now several *S*-genes have been characterized, with MLO gene being one of the best examples for powdery mildew penetration not only in *Arabidopsis* but also in tomato, barley, pepper and pea (Huibers *et al.*, 2013). *S*-gene can be considered a gene that contributes to microbial infection and loss of its function mutation can result in resistance. In order to identify S-genes we need to firstly identify the orthologous *S*-gene among the cultivated-economically important plants and secondly, once the function of the *S*-gene is confirmed, loss-of-function mutation needs to be applied through insertional mutagenesis or genetic transformation. The recessive nature of *S*-genes along with possible pleiotropic effects that often accompany their application are the main obstacles for the application of *S*-genes in plant breeding.

However the improved durability and the monitoring of pleiotropic effects with genetic transformation techniques such as TALENs (transcription activator-like effector nucleases) (Wood *et al.*, 2011) and CRISPR-Cas9 (Jiang *et al.*, 2013) manage to stimulate and consolidate their implementation. CRISPR-Cas9 and TALENs are genome-editing tools that are used extensively in the breeding industry. Specifically CRISPR-Cas9 at recent years is established as one of the most promising, if not the only, genome editing technology. This is being demonstrated by the huge number of publications on CRISPR-Cas9 technology in such short time from its very first applications in plants in 2013 (Belhaj, Chaparro-Garcia, Kamoun, & Nekrasov, 2013, (Shan *et al.*, 2013)).

So far this technology has been applied in model plants, such as *Arabidopsis* and tobacco, and in crop plants, including rice, wheat, maize and tomato. By minimizing possible ethical concerns and although improvements are necessary to be made to overcome several challenges, such as efficiency and specificity, this new biotechnological tool gives remarkable and numerous opportunities for increasing resistance, productivity, stress tolerance and vigor in plants in shorter time.

How the knowledge in *Arabidopsis* can benefit other crops like tomato

Obtaining resistance to *O. neolycopersici* in tomato represents an important trait in tomato breeding.

The main aim of experimenting in *Arabidopsis* was the discovery of at least a resistant gene or QTL responsible for the resistance to *O. neolycopersici.* The result of such experiments can be used in order to discover such genes in economically important cultivated crops, such as tomato. The identification of new *R* genes and their corresponding effectors, especially in model plants like *Arabidopsis,* will help to directly recognize these proteins and their interactions in other studied plants in order to improve resistance against several plant pathogens. Since the resistance conferred by R genes could eventually likely to be overcome turning the host susceptible in a short period of time, the introduction of a reverse genetic approach such as silencing the identified gene could also be used for a more durable and broad-spectrum resistance.

A first approach can be by investigating the existence of genes that are orthologous to the ones discovered in the model experimental plant. A second approach is to apply similar experimental approaches in tomato that were designed and were successful in *Arabidopsis*.

A lot of orthologous S genes were identified in potato by using *Arabidopsis* genome. This application open the way for genome editing in order to develop disease resistance that could be used for breeding in other crop species(Barka & Lee, 2022; Sun et al., 2016).

Two well know examples in *Arabidopsis* and tomato are the Arabidopsis PMR4 and DMR1 genes. RNAi silencing in these genes followed by phylogenetic analysis prove that both have orthologs in tomato. Loss of function of these orthologs will eventually lead to resistance and since no pleiotropic effects are present then the procedure is considered successful (Pavan *et al.*, 2010; Huibers *et al.*, 2013).

APPENDIX

Appendix I-Protocol for 'dirty' genomic DNA isolation using CTAB buffer

Composition CTAB buffer	1L
1 Molar Tris pH 7,5	100ml
5 Molar NaCl	140ml
MiliQ H_2O	740ml
0,5 Molar EDTA pH 8	20ml
2 % CTAB	20 gr

Disclaimer: When the DNA is extracted from leaf samples at the same day as harvesting, no procedure with liquid nitrogen is needed. Otherwise the material has to be treated with liquid nitrogen and grinded in the shaker for 60 seconds.

Step

1. Fresh young leaf disks of approximately 1x1 cm were collected in a 96 micronic tubes. Two steel balls were placed in every micronic tube. Samples were harvested on ice.
2. Add 2 x 250 CTAB extraction buffer with Rnase (per 1 ml CTAB 1 µL RNase) (2 mg/ml) is added to every individual leaf sample. Close the tubes tightly with caps.
3. Use a shaker to mix the leaf samples 3 x 180 seconds and check if the solution is intense green!
4. Place the micronic tubes and holder in a press and tight the nuts carefully to prevent the lids from popping off, and incubate the samples for 60 minutes in a water bath at 65°C.
5. Cool the samples in ice water for 45-60 minutes and still keep the samples in the press.

The following steps must be performed in a fume hood.

6. Add 250 µL chloroform isoamyl alcohol (24:1) , mix the suspension by inverting the tubes for approximately 40 times.
7. Separate the phases by centrifuging the samples at 4500 RPM for 15 min. After this pipet 400 µl of the watery phase into a new clean tube. Do not touch the pellet and the underlying phase.
8. Add 200 µL of isopropanol to the suspension and close the tubes with the caps. Mix the suspension by inverting the tubes briefly.
9. Centrifuge the samples for 30 minutes at 4500 RPM to get pellets on the bottom of the tubes. Hereafter you can briefly throw the supernatant away , the pellets will stay on the bottoms of the tubes.
10. Wash the pellets by adding 300 µL 70 % ethanol and centrifuge the samples 15 minutes at 4500 RPM.
11. Throw away the supernatant by inverting the tubes and dry the edges with clean towel. Now the samples have to dry for 2-3 hours (or during the night). No ethanol should be present anymore within the tubes, this would precipitate the DNA more.
12. Add 100 µL sterile MiliQ H_2O to the pellets and dissolve the DNA pellets by briefly vortexing or pipetting the suspension

REFERENCES

Adam, L. *et al.* (1999) 'Comparison of Erysiphe cichoracearum and E. cruciferarum and a

survey of 360 Arabidopsis thaliana accessions for resistance to these two powdery mildew pathogens', *Molecular Plant-Microbe Interactions*. doi: 10.1094/MPMI.1999.12.12.1031.

Adam, L. and Somerville, S. C. (1996) 'Genetic characterization of five powdery mildew disease resistance loci in Arabidopsis thaliana', *Plant Journal*. doi: 10.1046/j.1365-313X.1996.09030341.x.

Albini, G., Falque, M. and Joets, J. (2003) 'ActionMap: A web-based software that automates loci assignments to framework maps', *Nucleic Acids Research*. doi: 10.1093/nar/gkg594.

Appiano, M. *et al.* (2015) 'Identification of candidate MLO powdery mildew susceptibility genes in cultivated Solanaceae and functional characterization of tobacco NtMLO1', *Transgenic Research*. doi: 10.1007/s11248-015-9878-4.

Assaad, F. F. *et al.* (2004) 'The PEN1 syntaxin defines a novel cellular compartment upon fungal attack and is required for the timely assembly of papillae', *Molecular Biology of the Cell*. doi: 10.1091/mbc.E04-02-0140.

Bai, Y. *et al.* (2005) ' Tomato Defense to Oldium neolycopersici : Dominant OI Genes Confer Isolate-Dependent Resistance Via a Different Mechanism Than Recessive oI-2 ', *Molecular Plant-Microbe Interactions*, 18(4), pp. 354–362. doi: 10.1094/mpmi-18-0354.

Bai, Y. *et al.* (2007) ' Naturally Occurring Broad-Spectrum Powdery Mildew Resistance in a Central American Tomato Accession Is Caused by Loss of Mlo Function ', *Molecular Plant-Microbe Interactions*, 21(1), pp. 30–39. doi: 10.1094/mpmi-21-1-0030.

Barka, G. D. and Lee, J. (2022) 'Advances in S gene targeted genome-editing and its applicability to disease resistance breeding in selected Solanaceae crop plants', *Bioengineered*. doi: 10.1080/21655979.2022.2099599.

Belhaj, K. *et al.* (2013) 'Plant genome editing made easy: Targeted mutagenesis in model and crop plants using the CRISPR/Cas system', *Plant Methods*. doi: 10.1186/1746-4811-9-39.

Berkey, R. *et al.* (2017) 'Homologues of the RPW8 resistance protein are localized to the extrahaustorial membrane that is likely synthesized de novo', *Plant Physiology*. doi: 10.1104/pp.16.01539.

Bhat, R. A. *et al.* (2005) 'Recruitment and interaction dynamics of plant penetration resistance components in a plasma membrane microdomain', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.0500012102.

Boopathi, N. M. (2020) *Genetic mapping and marker assisted selection: Basics, practice and benefits, second edition*, *Genetic Mapping and Marker Assisted Selection: Basics, Practice and Benefits, Second Edition*. doi: 10.1007/978-981-15-2949-8.

Braun, U. *et al.* (2002) 'The taxonomy of the powdery mildew fungi', *The powdery mildews: a comprehensive treatise*.

Brown, J. K. M. (1994) 'Chance and selection in the evolution of barley mildew', *Trends in Microbiology*. doi: 10.1016/0966-842X(94)90650-5.

Collard, B. C. Y. *et al.* (2005) 'An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts', *Euphytica*. doi: 10.1007/s10681-005-1681-5.

Collins, N. C. *et al.* (2003) 'SNARE-protein-mediated disease resistance at the plant cell wall', *Nature*. doi: 10.1038/nature02076.

Consonni, C. *et al.* (2006) 'Conserved requirement for a plant host cell protein in powdery mildew pathogenesis', *Nature Genetics*. doi: 10.1038/ng1806.

Crute, I. R. and Pink, D. A. C. (1996) 'Genetics and utilization of pathogen resistance in plants', *Plant Cell*. doi: 10.1105/tpc.8.10.1747.

Edwards, M. D., Stuber, C. W. and Wendel, J. F. (1987) 'Molecular-marker-facilitated investigations of quantitative-trait loci in maize. I. Numbers, genomic distribution and types of gene action.', *Genetics*. doi: 10.1093/genetics/116.1.113.

Ellis, J. (2006) 'Insights into nonhost disease resistance: Can they assist disease control in agriculture?', *Plant Cell*. doi: 10.1105/tpc.105.040584.

Flor, H. H. (1971) 'Current Status of the Gene-For-Gene Concept', *Annual Review of Phytopathology*. doi: 10.1146/annurev.py.09.090171.001423.

Frye, C. A. and Innes, R. W. (1998) 'An arabidopsis mutant with enhanced resistance to powdery mildew', *Plant Cell*. doi: 10.1105/tpc.10.6.947.

Frye, C. A. and Innes, R. W. (2007) 'An Arabidopsis Mutant with Enhanced Resistance to Powdery Mildew', *The Plant Cell*, 10(6), p. 947. doi: 10.2307/3870681.

Frye, C. A., Tang, D. and Innes, R. W. (2001) 'Negative regulation of defense responses in plants by a conserved MAPKK kinase', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.98.1.373.

Gao, D. *et al.* (2015) 'Natural loss-of-function mutation of EDR1 conferring resistance to tomato powdery mildew in Arabidopsis thaliana accession C24', *Molecular Plant Pathology*. doi: 10.1111/mpp.12165.

Glawe, D. A. (2008) 'The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens', *Annual Review of Phytopathology*. doi: 10.1146/annurev.phyto.46.081407.104740.

Głowacki, S., Macioszek, V. K. and Kononowicz, A. K. (2011) 'R proteins as fundamentals of plant innate immunity', *Cellular and Molecular Biology Letters*. doi: 10.2478/s11658-010-0024-2.

Göllner, K. *et al.* (2008) 'Natural genetic resources of Arabidopsis thaliana reveal a high prevalence and unexpected phenotypic plasticity of RPW8-mediated powdery mildew resistance', *New Phytologist*. doi: 10.1111/j.1469-8137.2007.02339.x.

Green, J. R., Carver, T. L. W. and Gurr, S. J. (2002) 'The formation and function of infection feeding structures', in *The Powdery Mildews: A Comprehensive Treatise.*

Hahn, M. *et al.* (1997) 'A putative amino acid transporter is specifically expressed in haustoria of the rust fungus Uromyces fabae', *Molecular Plant-Microbe Interactions*. doi: 10.1094/MPMI.1997.10.4.438.

Hammond-Kosack, K. E., Jones, D. A. and Jones, J. D. G. (1996) 'Ensnaring microbes : The components of plant disease resistance', *New Phytologist*. doi: 10.1111/j.1469-8137.1996.tb04338.x.

Hammond-Kosack, K. E. and Jones, J. D. G. (1997) 'Plant disease resistance genes', *Annual Review of Plant Biology*. doi: 10.1146/annurev.arplant.48.1.575.

Hardham, A. R., Jones, D. A. and Takemoto, D. (2007) 'Cytoskeleton and cell wall function in penetration resistance', *Current Opinion in Plant Biology*. doi: 10.1016/j.pbi.2007.05.001.

Heffer, V. *et al.* (2006) 'Identification of Powdery Mildew Fungi anno 2006', *The Plant Health Instructor*. doi: 10.1094/phi-i-2006-0706-01.

Hückelhoven, R. (2005) 'Powdery mildew susceptibility and biotrophic infection strategies', *FEMS Microbiology Letters*. doi: 10.1016/j.femsle.2005.03.001.

Huibers, R. P. *et al.* (2013) 'Powdery Mildew Resistance in Tomato by Impairment of SlPMR4 and SlDMR1', *PLoS ONE*. doi: 10.1371/journal.pone.0067467.

Jiang, W. *et al.* (2013) 'Dealing with the Evolutionary Downside of CRISPR Immunity: Bacteria and Beneficial Plasmids', *PLoS Genetics*. doi: 10.1371/journal.pgen.1003844.

Jones, H., Whipps, J. M. and Gurr, S. J. (2001) 'The tomato powdery mildew fungus Oidium neolycopersici', *Molecular Plant Pathology*. doi: 10.1046/j.1464-6722.2001.00084.x.

Jones, J. D. G. and Dangl, J. L. (2006) 'The plant immune system', *Nature*. doi: 10.1038/nature05286.

Jørgensen, I. H. (1992) 'Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley', *Euphytica*. doi: 10.1007/BF00023919.

Kao, C. H., Zeng, Z. B. and Teasdale, R. D. (1999) 'Multiple interval mapping for quantitative trait loci', *Genetics*. doi: 10.1093/genetics/152.3.1203.

Király, L., Barna, B. and Király, Z. (2007) 'Plant resistance to pathogen infection: Forms and mechanisms of innate and acquired resistance', *Journal of Phytopathology*. doi: 10.1111/j.1439-0434.2007.01264.x.

Koch, E. and Slusarenko, A. (1990) 'Arabidopsis is susceptible to infection by a downy mildew fungus.', *The Plant Cell*, 2(5), pp. 437–445. doi: 10.1105/tpc.2.5.437.

Koh, S. *et al.* (2005) 'Arabidopsis thaliana subcellular responses to compatible Erysiphe cichoracearum infections', *Plant Journal*. doi: 10.1111/j.1365-313X.2005.02545.x.

Lander, E. S. *et al.* (1987) 'MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations', *Genomics*. doi: 10.1016/0888-7543(87)90010-3.

Lander, E. S. and Botstein, S. (1989) 'Mapping mendelian factors underlying quantitative traits using RFLP linkage maps', *Genetics*. doi: 10.1093/genetics/121.1.185.

Lindhout, P., Pet, G. and van der Beek, H. (1993) 'Screening wild Lycopersicon species for resistance to powdery mildew (Oidium lycoperiscum)', *Euphytica*, 72(1–2), pp. 43–49. doi: 10.1007/BF00023771.

Lipka, U. *et al.* (2010) 'Live and let die - Arabidopsis nonhost resistance to powdery mildews', *European Journal of Cell Biology*. doi: 10.1016/j.ejcb.2009.11.011.

Lipka, U., Fuchs, R. and Lipka, V. (2008) 'Arabidopsis non-host resistance to powdery mildews', *Current Opinion in Plant Biology*. doi: 10.1016/j.pbi.2008.04.004.

Lipka, V. *et al.* (2005) 'Plant science: Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis', *Science*. doi: 10.1126/science.1119409.

Liu, J. *et al.* (2007) 'Recent Progress in Elucidating the Structure, Function and Evolution of Disease Resistance Genes in Plants', *Journal of Genetics and Genomics*. doi: 10.1016/S1673-8527(07)60087-3.

Micali, C. *et al.* (2008) 'The Powdery Mildew Disease of Arabidopsis: A Paradigm for the Interaction between Plants and Biotrophic Fungi', *The Arabidopsis Book*. doi:

10.1199/tab.0115.

Michelmore, R. W. and Meyers, B. C. (1998) 'Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process', *Genome Research*. doi: 10.1101/gr.8.11.1113.

Mudgett, M. B. (2005) 'New insights to the function of phytopathogenic bacterial type III effectors in plants', *Annual Review of Plant Biology*. doi: 10.1146/annurev.arplant.56.032604.144218.

Niks, R. E. *et al.* (2019) *Breeding crops with resistance to diseases and pests*, *Breeding crops with resistance to diseases and pests*. doi: 10.3920/978-90-8686-882-7.

Nishimura, M. T. *et al.* (2003) 'Loss of a callose synthase results in salicylic acid-dependent disease resistance', *Science*. doi: 10.1126/science.1086716.

Nürnberger, T. and Lipka, V. (2005) 'Non-host resistance in plants: New insights into an old phenomenon', *Molecular Plant Pathology*. doi: 10.1111/j.1364-3703.2005.00279.x.

O'Connell, R. J. and Panstruga, R. (2006) 'Tête à tête inside a plant cell: Establishing compatibility between plants and biotrophic fungi and oomycetes', *New Phytologist*. doi: 10.1111/j.1469-8137.2006.01829.x.

Panstruga, R. (2005) 'Serpentine plant MLO proteins as entry portals for powdery mildew fungi', in *Biochemical Society Transactions*. doi: 10.1042/BST0330389.

Pavan, S. *et al.* (2010) 'Loss of susceptibility as a novel breeding strategy for durable and broad-spectrum resistance', *Molecular Breeding*. doi: 10.1007/s11032-009-9323-6.

Plotnikova, J. M. *et al.* (1998) 'Powdery mildew pathogenesis of Arabidopsis thaliana', *Mycologia*. doi: 10.2307/3761274.

Polák, J. and Bartoš, P. (2018) 'Natural Sources of Plant Disease Resistance and their Importance in the Breeding', *Czech Journal of Genetics and Plant Breeding*. doi: 10.17221/6255-cjgpb.

Pritchard, L. and Birch, P. R. J. (2014) 'The zigzag model of plant-microbe interactions: Is it time to move on?', *Molecular Plant Pathology*. doi: 10.1111/mpp.12210.

Ridout, C. J. *et al.* (2006) 'Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance', *Plant Cell*. doi: 10.1105/tpc.106.043307.

Saur, I. M. L. *et al.* (2019) 'Multiple pairs of allelic MLA immune receptor-powdery mildew AVR a effectors argue for a direct recognition mechanism', *eLife*. doi: 10.7554/eLife.44471.

Van Schie, C. C. N. and Takken, F. L. W. (2014) 'Susceptibility genes 101: How to be a good host', *Annual Review of Phytopathology*. doi: 10.1146/annurev-phyto-102313-045854.

Schulze-Lefert, P. and Vogel, J. (2000) 'Closing the ranks to attack by powdery mildew', *Trends in Plant Science*. doi: 10.1016/S1360-1385(00)01683-6.

Schweizer, P. (2007) 'Nonhost resistance of plants to powdery mildew-New opportunities to unravel the mystery', *Physiological and Molecular Plant Pathology*. doi: 10.1016/j.pmpp.2007.07.004.

Seifi, A. *et al.* (2014) 'Genetics and molecular mechanisms of resistance to powdery mildews in tomato (Solanum lycopersicum) and its wild relatives', *European Journal of Plant Pathology*. doi: 10.1007/s10658-013-0314-4.

Sekhwal, M. K. *et al.* (2015) 'Disease resistance gene analogs (RGAs) in plants', *International Journal of Molecular Sciences*. doi: 10.3390/ijms160819248.

Semagn, K., Bjørnstad, A. and Ndjiondjop, M. N. (2006) 'Principles, requirements and prospects of genetic mapping in plants', *African Journal of Biotechnology*.

Shan, Q. *et al.* (2013) 'Targeted genome modification of crop plants using a CRISPR-Cas system', *Nature Biotechnology*. doi: 10.1038/nbt.2650.

Shen, Q. H. *et al.* (2003) 'Recognition specificity and RAR1/SGT1 dependence in barley Mla disease resistance genes to the powdery mildew fungus', *Plant Cell*. doi: 10.1105/tpc.009258.

Spanu, P. and Kämper, J. (2010) 'Genomics of biotrophy in fungi and oomycetes-emerging patterns', *Current Opinion in Plant Biology*. doi: 10.1016/j.pbi.2010.03.004.

Stein, M. *et al.* (2006) 'Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration', *Plant Cell*. doi: 10.1105/tpc.105.038372.

Sun, K. *et al.* (2016) 'Silencing of six susceptibility genes results in potato late blight resistance', *Transgenic Research*. doi: 10.1007/s11248-016-9964-2.

Tang, D., Christiansen, K. M. and Innes, R. W. (2005) 'Regulation of plant disease resistance, stress responses, cell death, and ethylene signaling in Arabidopsis by the EDR1 protein kinase', *Plant Physiology*. doi: 10.1104/pp.105.060400.

Thordal-Christensen, H. (2003) 'Fresh insights into processes of nonhost resistance', *Current Opinion in Plant Biology*. doi: 10.1016/S1369-5266(03)00063-3.

Trujillo, M. *et al.* (2004) 'Mechanistic and genetic overlap of barley host and non-host resistance to Blumeria graminis', *Molecular Plant Pathology*. doi: 10.1111/J.1364-3703.2004.00238.X.

Tucker, S. L. and Talbot, N. J. (2001) 'Surface attachment and pre-penetration stage development by plant pathogenic fungi', *Annual Review of Phytopathology*. doi: 10.1146/annurev.phyto.39.1.385.

Tzeneva, V. A. *et al.* (2008) '16S rRNA targeted DGGE fingerprinting of microbial communities.', *Methods in molecular biology (Clifton, N.J.)*, 410, pp. 335–349. doi: 10.1007/978-1-59745-548-0_17.

Voegele, R. T. *et al.* (2001) 'The role of haustoria in sugar supply during infection of broad bean by the rust fungus Uromyces fabae', *Proceedings of the National Academy of Sciences of the United States of America*. doi: 10.1073/pnas.131186798.

Vogel, J. P. *et al.* (2002) 'PMR6, a pectate lyase-like gene required for powdery mildew susceptibility in Arabidopsis', *Plant Cell*. doi: 10.1105/tpc.003509.

Vogel, J. P. *et al.* (2004) 'Mutations in PMR5 result in powdery mildew resistance and altered cell wall composition', *Plant Journal*. doi: 10.1111/j.1365-313X.2004.02264.x.

Vogel, J. and Somerville, S. (2000) 'Isolation and characterization of powdery mildew-resistant Arabidopsis mutants', *Proceedings of the National Academy of Sciences*, 97(4), pp. 1897–1902. doi: 10.1073/pnas.030531997.

Wang, D. *et al.* (2018) 'Genetic signatures of plant resistance genes with known function within and between species', *Genetica*. doi: 10.1007/s10709-018-0044-9.

Ward, H. M. (1902) 'On the relations between host and parasite in the bromes and their brown rust, puccinia dispersa (erikss.)', *Annals of Botany*. doi: 10.1093/oxfordjournals.aob.a088874.

Wilson, I. W. *et al.* (2001) 'Quantitative trait loci analysis of powdery mildew disease resistance in the Arabidopsis thaliana accession Kashmir-1', *Genetics*.

Wood, A. J. *et al.* (2011) 'Targeted genome editing across species using ZFNs and TALENs', *Science*. doi: 10.1126/science.1207773.

Wu, Y. *et al.* (2018) 'Comparative genome analyses reveal sequence features reflecting distinct modes of host-adaptation between dicot and monocot powdery mildew 06 Biological Sciences 0604 Genetics', *BMC Genomics*. doi: 10.1186/s12864-018-5069-z.

Xiao, S. *et al.* (1997) 'Characterization of three loci controlling resistance of Arabidopsis thaliana accession Ms-0 to two powdery mildew diseases', *Plant Journal*. doi: 10.1046/j.1365-313X.1997.12040757.x.

Xiao, S. *et al.* (2001) 'Broad-Spectrum in Arabidopsis Resistance by RPW8', *Science*, 291(5501), pp. 118–120.

Xiao, S. *et al.* (2005) 'The atypical resistance gene, RPW8, recruits components of basal defence for powdery mildew resistance in Arabidopsis', *Plant Journal*. doi: 10.1111/j.1365-313X.2005.02356.x.

Younis, A. *et al.* (2014) 'RNA interference (RNAi) induced gene silencing: A promising approach of hi-tech plant breeding', *International Journal of Biological Sciences*. doi: 10.7150/ijbs.10452.

Zhang, S. *et al.* (2022) 'Action Mechanisms of Effectors in Plant-Pathogen Interaction', *International Journal of Molecular Sciences*. doi: 10.3390/ijms23126758.

Zhang, Y. M. (2012) 'F2 designs for QTL analysis', *Methods in Molecular Biology*. doi: 10.1007/978-1-61779-785-9_2.

Zheng, Z. *et al.* (2013) 'Loss of Function in Mlo Orthologs Reduces Susceptibility of Pepper and Tomato to Powdery Mildew Disease Caused by Leveillula taurica', *PLoS ONE*. doi: 10.1371/journal.pone.0070723.