Summary

Arabidopsis thaliana has developed into a major research subject within plant biology. Before this other plant species like tomato and tobacco, due to their economic importance, were important subjects of research. Owing to that their diseases have well been studied. Tobamo-viruses are common pathogens of many crop plants. Tomato/Tobacco Mosaic Virus (ToMV/TMV) was the first characterized tobamo-virus. Their diseases are characterized by mosaic symptoms on the leaves and growth arrest. This results into considerable economic and cosmetic damages in agronomically important plants.

To counteract upon pathogen infection, plants have developed two major lines of defense as described in **chapter 1**. The first line of defense encompasses passive and ubiquitously present barriers like cell wall, trichomes, cuticles and hairs to prevent entrance of pathogens. The second line of defense is reactive or inducible which relies on detection and recognition of the pathogen and mounts active defensive measures. This mechanism further splits into three types of defenses 1) innate immunity (non-host specific resistance); 2) pathogen- and host-specific resistance; and 3) post-transcriptional gene silencing.

The innate immunity refers to a non-host specific resistance which is a basal and general defense against general microbial elicitors known as pathogen-associated molecular patterns (PAMPs). These usually are derivatives of ubiquitous elements of pathogens. Recognition of these elicitors commonly takes place outside the cell by specialized plasma membrane anchored receptors known as pathogen recognition receptors (PRRs). These PRRs are divided into two types of receptors, receptor-like kinases (RLKs) and receptor-like proteins (RLPs). This type of plant defense response is also known as PAMPs triggered immunity (PTI).

The pathogen- and host specific resistance depends on specific avirulence (Avr) gene products produced by the pathogen and the recognition of these proteins by specific host resistance (R) gene products. This interaction between both the Avr and R proteins can take place either intra-cellularly or extra-cellularly. The *Avr* factors are also known as effectors and then the resistance anchored by the *R* genes is named as effectors-triggered immunity (ETI). This is robust and faster than the

PTI. This type of resistance is usually characterized by a hypersensitive response (HR) in plants.

Post-transcriptional gene silencing is also a non-host specific type of defense because no specialized and pathogen-specific host recognition proteins are required. Instead, the specific detection of pathogenic RNAs takes place using small 21-23 nucleotide RNAs derived from pathogenic double stranded RNAs.

It remains that both types of non-host specific defenses (innate immunity and post-transcriptional gene silencing) couldn't completely protect the plants against tobamo-virus infections. Due to that reason the studies related to pathogen and host specific resistance relying on the Avr and R proteins have gained more attention. This has contributed to understand the plant defense systems particularly against viral attacks. To date four ToMV resistance genes, one from tobacco (the *N* gene) and three from tomato (the *Tm-1*, *Tm-2* and *Tm-2*^{2} genes), have been isolated, characterized and transgenically expressed in both plant species. The *N* and *Tm-1* genes were only effective against ToMV-0 (the wild type) strains. Compared to that, the resistance of the *Tm-2* gene was longer lasting, but later on it was also broken due to co-evolution of its breaker strains. However, since almost five decades, the *Tm-2*^{2} gene has been conferring durable resistance to most of the tobamo-virus strains. ToMV isolates which were able to overcome its resistance are crippled. Interestingly the *Tm-2* and *Tm-2*^{2} genes are allelic. In addition to that their encoded R proteins also share a common Avr-factor, the movement protein (MP) of the ToMV pathogen. Both resistance gene products belong to the major class of R proteins, the CNL (CC-NBS-LRR) type of protein and they differ in only four amino acids. These differential amino acids are spread in two domains/regions (the NBS and the LRR) while the third domain (CC) was completely conserved. Compared to Tm-2^2 gene product, two of those mutations (Ile/Phe and Met/Ile) are present in the NBS region of the Tm-2 protein while the other two (Tyr/Asn and Ser/Thr) are in the LRR region. Despite this small number of variations, the difference in resistance specificity is remarkable. The combination of three aspects, sharing the MP as the Avr protein, the different locations of the mutations required to circumvent the resistances, and the different specificities, is intriguing. The roles of position and

differential amino acids between both proteins have been addressed in chapters 2 and 3. Domain shuffling experiments between the Tm-2 and Tm-2^2 gene products have been analyzed (**Chapter 2**). The results are consistent with the theory that the LRR domain regulates the specificity of R proteins by recognition of the Avr protein (the ToMV-MP). The NBS domain appears to be involved in the signal transduction rather than recognition events. In this way, the question related to the critical location of differential amino acids is solved. Moreover, the site-directed mutagenesis shows that in the Tm-2 background, only one amino acid replacement tyrosine (Tyr) instead of asparagine (Asn) which is located in the LRR region is responsible for the *Tm-2^2* resistance specificity (**Chapter 3**). Although in the same region another amino acid substitution serine (Ser) instead of threonine (Thr) also manifested a limited role in recognition of the ToMV-MP.

The *Tm-2^2* gene has been reported for functional expression in two plant species (tomato and tobacco) of the same family, Solanaceae. The transfer of its durable resistance to other plant species or families might be valuable. The extra-family gene transfer of the *Tm-2^2* gene has been studied in *Arabidopsis thaliana* which belongs to the Brassicaceae family (**Chapter 4**). This model plant has been useful to elaborate virus-plant interactions. An ecotype of *Arabidopsis*, Col-0 has been transformed and analyzed for resistance as well as differential time period of systemic viral infections. The crucifer-infecting tobamo-virus (TMV-Cg) is the most robust and rapidly infecting virus isolate in wild types. Col-0/*Tm-2^2* transgenic plants show ten days delayed systemic spread of the *Tm-2* breaker virus whereas the *Tm-2^2* breaker needed four extra days to systemically infect the plant. This suggests that the Tm-2^2 protein hampers the infection process of the tobamo-viruses. Although the introduction of virus resistance could not be fully achieved, this suggests expression of the *Tm-2^2* gene in *Arabidopsis*. Moreover, this study also indicates that the interaction of the R-protein with host factors, e.g. the guardee or down-stream signaling elements, might be critical for optimal functioning of the R-protein.

The *Tm-2^2* gene was introduced into the cultivated tomato almost five decades ago and still confers resistance to ToMV. It remains necessary, however, to search for

new or homologous resistances from-outside the agronomic gene pool in order to broaden the resistance sources for a variety of ToMV isolates. To address that issue, seventeen *Solanum* species have been analyzed for their resistance specificity to the *Tm-2* and *Tm-2²* breakers (**Chapter 5**). Based on this virus assay, six genotypes were selected for further molecular analysis using PCR amplification followed by DNA sequencing of the *Tm-2²* homologues. From all six genotypes genes could be isolated resembling the *Tm-2²* gene. Three of those *Tm-2²* homologues (*Sptm-3*, *Satm-2* and *Sctm-2*) isolated from *S. peruvianum*, *S. arcanum* and *S. chilense*, respectively, do not show any resistance. Two *Tm-2²* homologues, *ShTm-2* (*S. huaylasense*) and *SpiTm-2* (*S. pimpinellifolium*) show a *Tm-2* like resistance, while *ScoTm-2²* (*S. corneliomulleri*) displays a *Tm-2²* like resistance. Interestingly, the gene from *S. corneliomulleri* resembled the *Tm-2²* gene the most but encoded a truncated protein due to a 32bp insertion. The deduced truncated protein is 86 amino acids shorter at the C-terminus of the LRR region compared to the 861 amino acid long Tm-2² protein. Still, this gene conferred the *Tm-2²* like resistance specificity. The truncated gene from *S. corneliomulleri* might be a valuable addition to the available TMV resistance gene pool.

For future research, the results of the extra-family transfer of the *Tm-2²* gene and its truncated homologous gene might be valuable tools for plant molecular biologists. This may increase the TMV resistance gene pool and may lead to a further understanding of plant-virus interaction mechanisms.