Nucleotide-binding and molecular interactions of plant disease resistance proteins

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CHAPTER 1

General introduction and outline

INTRODUCTION

Despite large number and variety of potential invaders like bacteria, viruses, fungi, insects and nematodes, plants are rather capable to distinguish between harmless and harmful. Successful recognition and subsequent effective defence against pathogenic intruders is feasible thanks to a sophisticated and multilayered immune system. Besides permanent protective barriers like the cuticle and cell walls, and besides responses induced upon disturbances of plant integrity and welfare, like basal defence mechanisms as secretion of antimicrobial enzymes and toxic secondary metabolites, plants evolved a complex innate immunity system. The first layer of this defence is mediated by specific Pathogen Recognition Receptors (PRR) that are able to sense conserved microbial molecules called Pathogen-Associated Molecular Patterns (PAMP). This type of non-host resistance is called PTI (PAMP-Triggered-Immunity) and is applied against non-adapted microorganisms and parasites (Jones and Dangl, 2006). Only specialized pathogens can overcome or suppress this resistance thanks to the development of specific virulence effectors. The emergence of (new) virulence is often correlated with the evolution of pathogen strains or races, and sometimes might have devastating effects on a host plant. Fortunately, to defeat these adapted enemies, plants evolved a counter strategy: a cultivar-specific second layer of innate immunity, mediated by resistance (R) proteins (Fig. 1). R proteins are able to directly or indirectly perceive pathogen-delivered effectors, previously referred to as avirulent proteins (Avr). Next, to arrest growth and further spreading of the pathogen, these specialized receptors initiate hypersensitive responses (HR) often associated with programmed cell-death. Since this resistance depends on the presence of both the plant R protein and the corresponding pathogen Avr effector, this type of immunity is called ETI (Effector Trigger Immunity) (Jones and Dangl, 2006) and its basis was initially described as the gene-for-gene hypothesis (Flor, 1942). Most of the R proteins that have been cloned over the last 20 years or so belong to the intracellular NB-LRR (Nucleotide Binding domain followed by Leucine-Rich Repeats) protein class (Leipe et al., 2004). They are proposed to work as a nucleotide binding/hydrolysing molecular switch, regulating signal transduction by conformational changes, and show diverse interactions with either recognition or signaling components (Takken et al., 2006; Tameling et al., 2006). To get insight in the molecular mechanism by

which NB-LRR proteins activate plant defence we analyzed their inter- and intramolecular interactions in relation to the nucleotide-binding state.

OUTLINE OF THIS THESIS

Chapter 2 presents a detailed introduction in the form of an overview of current knowledge about R protein structure, function and intra- and intermolecular interactions. Conclusions of this literature review led to the refined molecular switch model for R protein activation, which provided the basis for the rest of this thesis. This mechanistic model proposes that upon pathogen perception the NB-LRR protein-bound nucleotide is exchanged from ADP to ATP. This triggers a series of conformational changes, allowing the newly exposed NB domain to interact with downstream signalling partners and activate defence signalling.

In **Chapter 3** we investigated in more detail different aspects of NB-LRR protein interactions with signaling or recognition complex components and nucleotide-induced conformational changes. Using tomato I-2 R protein (conferring resistance to *F. oxysporum* (Simons et al., 1998; Houterman et al., 2009)) and three of its biochemically and phenotypically characterized mutants affected in nucleotide binding or hydrolysis, we demonstrated that the conformation of an R protein depends indeed on its nucleotide binding state. This conclusion is based on the identification of *Sl*Formin and *Sl*Trax, specific I-2 N-terminal yeast two-hybrid (Y2H) interactors that show distinct and often opposite I-2 binding preferences. Furthermore, the involvement of these interactors in I-2 mediated resistance has been studied.

In **Chapter 4** we provide insight into the dynamic interplay between specific R protein domains and their role in R protein functioning, using as an example the Mi-1.2 protein (conferring resistance to nematodes, white flies and aphids (Milligan et al., 1998; Rossi et al., 1998; Vos et al., 1998; Nombela et al., 2003)). This CC-NB-LRR protein contains an extended N-terminus, characteristic for solanaceous R proteins. We have been able to associate the negative and positive regulatory potential of this N-terminus on R protein mediated HR with specific regions. A secondary structure prediction of the N-terminus correlated these regions further with predicted (sub)domains. In addition, since the N-terminus was required either *in trans* or *in cis* to induce HR in various autoactivating mutants, we concluded that the Mi-1.2 activation mechanism consists of multiple steps, each involving

distinct intramolecular interactions. The smallest functional molecule that is able to trigger HR when over-expressed in *Nicotiana benthamiana* leaves was identified to be almost the full-length Mi-1.2 protein.

Chapter 5 presents to our knowledge the first example in which the nucleotide-binding state of a full-length R protein has been determined. We found that barley MLA27 protein (conferring resistance to powdery mildew (Seeholzer et al., 2010)) is stably bound to ADP, providing additional support for the switch model on R protein functioning. Correctly folded full-length R protein gives the opportunity to directly monitor its conformational changes during nucleotide dissociation or re-binding, what has been initiated in the same chapter. Moreover, it provides a positive control for further attempts, following these described for Rx (conferring resistance to Potato Virus X (Bendahmane et al., 1999)), to successfully produce active NB-LRR protein variants in heterologous expression systems.

Finally, in **Chapter 6** the results obtained in this thesis and their impact towards understanding NB-LRR protein functioning are discussed.

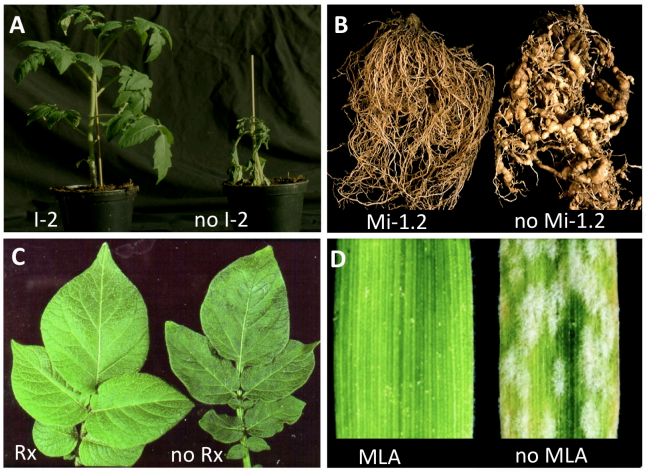

Fig. 1. Response of susceptible and resistant plants to infection with race-specific pathogens
Infection by specialized pathogens causes disease on a susceptible plant (on the right), while a resistant plant (on the left), carrying the particular pathogen-corresponding *R gene,* remains healthy, as the infection was successfully prevented. **A)** resistant (I-2) and susceptible (no I-2) plants of tomato (*Solanum lycopersicum*) infected with a *F. oxysporum* f. sp. *lycopersici* race 2*;* **B)** resistant (Mi-1.2) and susceptible (no Mi-1.2) plants of tomato (*S. lycopersicum*) infected with root-knot nematodes (*Meloidogyne* spp.) (http://www.nematology.umd.edu/images/eis143.jpg); **C)** resistant (Rx) and susceptible (no Rx) plants of potato (*Solanum tuberosum*) infected with a Potato virus X (http://vegetablemdonline.ppath.cornell.edu/Images/Potatoes/Virus_News/image005.png); **D)** resistant (MLA) and susceptible (no MLA) plants of barley (*Hordeum vulgare*) infected with *Blumeria graminis f. sp. hordei* (http://www.plantcell.org/content/16/12/3480.full.html-).

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