

Pseudomonas syringae caused by avrpt2



**ASSIGN
BUSTER**

‘ RIN 4 Orthologs’

- Maheen Rashid

Introduction

Plants are susceptible to a wide range of bacterial diseases. Depending on the origin of the infection, these diseases can be localized within the stems, leaves or the roots of the infected plant. When a human body is compromised, as a result of it being infected by a bacterial disease, the infection's symptoms can thus be visually identified. Likewise, when a plant contracts a bacterial disease, it displays several symptoms such as blights, cankers, galls, leaf spots, overgrowths, specks, scabs, or wilts [1]. One very important concern witnessed due to the health of a plant being compromised is the massive decrease in the overall crop production. Therefore, to avoid these sudden decreases in crop production, a system has to be devised that can provide plants with broad spectrum immunity against bacterial infections.

In order to resist pathogen invasions, plants have developed a form of immunity which is comprised of two modes: PAMP Triggered Immunity (PTI) and Effector Triggered Immunity (ETI). ‘ PAMP’ is a pathogen associated molecular pattern which is a microbial structure that is generally conserved in a specific class of microbes. As the name suggests, PAMP triggered immunity is the first line of defense which is triggered when pattern recognition receptors (PRRs) present on the plant cell's plasma membranes recognizes these PAMPs [2]. For example, the flagella are a broadly conserved bacterial feature; the recognition of certain components of flagella

by an FLS2 receptor in a plant cell induces a PTI response. PTI consequently provides the plant innate immunity against the non-self and restricts the growth of the pathogen [2]. There are, however, pathogens which are able to successfully survive PTI, these pathogens make use of effector molecules, instead of PAMPs, to invade a plant [3]. To counter these microbes, the plant has another more robust mode of immunity - the effector triggered immunity, which is induced when a pathogen injects effector molecules into the plant through its Type 3 Secretion System (T3SS). These Type 3 effector molecules are known to interfere with a plant's PTI by causing its suppression and at the same time these effector molecules cause effector mediated perturbations of certain plant host proteins which consequently elicits another line of a plant defense response: the effector triggered immunity [4].

In this research proposal, the main focus is on the virulence of *Pseudomonas syringae* caused by one of its effector molecules 'Avrpt2'. In the model plant *Arabidopsis thaliana*, it has been studied that *P. Syringae* uses its Type 3 secretion system to deliver Avrpt2 into the plant's cytosol. Once this effector molecule successfully enters into the cytosol it causes modification of the host protein, Arabidopsis RPM1 interacting protein 4 (AtRIN4). RIN4 consists of 3 structurally important domains: 3 terminal Cysteine residues which constitute the palmitoylation site required for anchoring RIN4 to the cell membrane and two NOI domains namely NOI1 and NOI2 which contain the cleavage sites for Avrpt2 [5]. The significance of studying RIN4 as a host protein lies in the fact that it is not only a multifunctional protein which regulates both branches of immunity PTI and ETI, but it is also a conserved

protein across the plant kingdom which enables the inspection of the RIN4 mediated immunity in plants other than *Arabidopsis*. Previous studies have demonstrated a 'Guard Hypothesis' according to which AtRIN4 is guarded by cognate R proteins, Rpm1 and RPS2, which are also localized at the plasma membrane by AtRIN4 which, under normal conditions, negatively regulates these R proteins [6]. In such a situation where *P. Syringae* injects Avrpt2 into a plant, Avrpt2 causes cleavage of RIN4 into 3 component fragments (ACP1, ACP2 and ACP3) [7], which further inhibit PTI but also, at the same time, the non membrane tethered fragment, ACP2, activates RPS2 which leads to a hypersensitive response culminating in a localized cell death at the point of infection. This localized cell death halts the microbial growth and allows the plant to survive the pathogen attack [6]. This also explains why it is important for AtRIN4 to keep it inactive in normal conditions, since the absence of AtRIN4 would mean ectopic activation of AtRPS2 leading to a continuous hypersensitive response. Evidently, this RPS2 mediated immune response is only present in those *Arabidopsis* plants which carry the RPS2 gene and are therefore resistant to those strains of *P. Syringae* which use the effector molecule Avrpt2 to infect the plant. Plants which do not contain RPS2 or contain a dysfunctional copy of RPS2 will not be able to trigger an effector triggered immune response due to the absence of this cognate protein that elicits the hypersensitive response. [8] The results thus far have indicated that AtRIN4 negatively regulates AtRPS2 by localizing it at the cell membrane and the cleavage of AtRIN4 by Avrpt2 releases a cytosolic fragment, ACP2, which triggers AtRPS2 to initiate ETI rendering a plant resistant to the infection caused by *P. Syringae*.

Studies have already shown that RIN4 is generally conserved across a wide variety of plants [9] and following the conservation data, the primary focus of this proposal is to explore if RIN4 orthologs isolated from other cash crops, such as rice, tomato, soybean, potato etc are functionally similar to AtRIN4 and to study whether these RIN4 orthologs are capable of suppressing AtRPS2 and if their ACP2 fragments can activate an AtRPS2 dependent immune response. Once functionally similar RIN4 orthologs are identified, this research will further lead to the introgression of AtRPS2 genes in crop plants that contain RIN4 orthologs capable of complementing AtRIN4. This is expected to be an effective method to elicit immunity in those crop plants susceptible to infections by *P. Syringae*.

Hypothesis

Conservation of RIN4 orthologs across a variety of plant leads to the hypothesis that these orthologs, from various important cash crops, should complement the function of AtRIN4. Exploring how RIN4 orthologs interact with bacterial effector molecules and AtRPS2 to elicit an RPS2 mediated immune response would enable the development of a method that can render resistance in plant species that are prone to infections caused by *P. syringe*.

Research Aims

Currently, research has shown that in the model plant, *Arabidopsis thaliana*, AtRPS2 is a resistance protein that guards AtRIN4 for effector induced perturbations [6]. The modification of AtRIN4 by Avrpt2, a type 3 bacterial effector derived from *P. syringae*, causes the activation of a robust RPS2

mediated immune response. Hence, the RPS2 protein provides a strong resistance to *Arabidopsis* against strains of *P. syringae* that infect the plant using Avrpt2 molecules. This project is aimed towards researching whether *Arabidopsis* RIN4 orthologs from 5 important cash crops, namely Rice, Tomato, Peach, Soybean and Lettuce, can function similarly by negatively regulating the resistance protein AtRPS2 and if the ortholog of the non-membrane tethered fragment (ACP2), which is released in the cytosol during RIN4 modification, can activate AtRPS2 and trigger the localised hypersensitive response which saves the plant from the pathogen attack.

Once functionally similar orthologs are identified, the the project would advance towards the introgression of AtRPS2 genes in those crop plants that contain RIN4 orthologs capable of complementing AtRIN4 and negatively regulating RPS2 but are still susceptible to diseases due to the absence of RPS2 proteins. Studying the interactions between Avrpt2, RIN4 orthologs, ACP2 orthologs and AtRPS2 can help mediate this form of immunity in cash crops containing RIN4 orthologs and therefore cater to the long term aim of inducing immunity and resistance in those cash crops which lose out on their crop production due to the devastating outbreaks of infections caused by *P. Syringae*.

Research Plan/Methodology

In order to determine if the isolated RIN4 orthologs function in a manner similar to *Arabidopsis* RIN4, the basic plan is to use the heterologous system of *Nicotiana Benthamiana* for the transient expression of RIN4 orthologs and AtRPS2. *Nicotiana Benthamiana* is an ideal plant system to study transient

expression of proteins since it's an easy system to use and it can be genetically transformed with great efficiency [10].

The research plan has been divided into two main objectives. Objective (1) deals with the interaction between RIN4 orthologs and AtRPS2, whereas Objective (2) is aimed towards studying the effect of ACP2 and ACP3 orthologs on the activity of AtRPS2.

Objective 1: According to the first task of this proposal, the project seeks to test if RIN4 orthologs can suppress the ectopic activation of AtRPS2 like AtRIN4 does. For this purpose the RIN4 orthologs were already isolated and amplified prior to the start of this project, hence now, the RIN4 copies will be cloned in a binary vector, pEARLEY Gate 104 which contains an N-terminal YFP tag. It has been previously shown that the membrane attachment of RIN4 is essential to suppress AtRPS2 and therefore, firstly, the sub cellular localization of the RIN4 orthologs will be determined by checking for YFP signals using confocal microscopy. This will also aid in the confirmation that the structure of the orthologs is similar to AtRIN4 which is why they also anchor at the plasma membrane. Secondly the AtRPS2 shall be cloned into another binary vector, pEARLEY gate 102 which contains a C-terminal CFP tag. Both these proteins will be expressed alongside each other in *N. Benthamiana* by first transforming these vectors in *Agrobacterium* and then infiltrating each of the *Agrobacterium* solutions into the abaxial surface of the *N. Benthamiana* leaves. In the situation that these orthologs suppress AtRPS2, no disease symptom will be apparent and the plant will look healthy. However, in case these orthologs fail to negatively regulate AtRPS2, a highly noticeable macroscopic cell death will occur on the leaves of *N. Benthamiana*

<https://assignbuster.com/pseudomonas-syringae-caused-by-avrpt2/>

which will indicate that the RPS2 mediated hypersensitive response has been triggered. This cell death response can be measured and further quantified by making use of Trypan blue staining (which stains dead cells) or ion leakage technique which measures the conductivity of ions that are leaked from the cell walls of dead plant cells.

Objective 2: Furthermore, the Avrpt2 cleaved fragments of orthologs of RIN4 that are equivalent to ACP2 and ACP3 will also be transiently expressed alongside AtRPS2 in *N. Benthamiana*. The methodology will be same as that of Objective 1. First orthologous ACP3 fragment shall be cloned into a binary YFP vector. This vector will then be used to transform *Agrobacterium* which will then be infiltrated with AtRPS2 in the *N. Benthamiana* leaves. Similarly the ACP2 equivalent fragment, cloned in another binary YFP vector, will be used to transform another solution of *Agrobacterium* and will be introduced into the *N. Benthamiana* leaves with AtRPS2. Similar to the approach in Objective 1, the fluorescence tags in the vectors will be used to monitor the localization of the ACP2 and ACP3 fragments and investigate their interactions with AtRPS2.

Expected Outcomes

Objective 1: According to the hypothesis put forward in this project, if RIN4 orthologs are able to suppress the ectopic activation of AtRPS2 the plant should not show any visible change in its phenotype. The leaves of *N. Benthamiana* will appear healthy and normal as there is no immunity being triggered inside the plant cells due to the suppression of RPS2. Also, the

RIN4 orthologs must necessarily be located at the plasma membrane when visualized under confocal microscopy.

Objective 2: If the cleaved fragments of RIN4 orthologs, ACP2 and ACP3 are equivalent to the AtRIN4 fragments then they must show similar interactions with AtRPS2 when expressed together in the leaves of *N. Benthamiana*.

Studies in *Arabidopsis* have indicated that AtACP3 is a membrane localized fragment which can partially suppress the activation of RPS2 whereas AtACP2 is a cytosolic fragment which can trigger the activation of RPS2.

Therefore, if the claim that these fragments isolated from RIN4 orthologs are functionally similar to AtACP2 and AtACP3 is true, then the cloned ACP3 fragment when infiltrated with AtRPS2 will show no plant cell death since RPS2 is suppressed by ACP3. Contrary to this suppression, when ACP2 ortholog is expressed in combination with AtRPS2, ACP2 will release the suppression off of RPS2 and a macroscopic HR will be observed on the leaves of *N. Benthamiana* validating the functional similarity of ACP2 and ACP3 orthologs to cleaved AtRIN4 fragments.

References

1. “ Bacterial Disease.” S in Plants and Crops. N. p., n. d. Web. 12 Oct. 2014. .
2. Chakravarthy, Suma, André C. Velásquez, and Gregory B. Martin. “ Abstract.” *National Center for Biotechnology Information* . U. S. National Library of Medicine, 09 Sept. 2009. Web. 12 Oct. 2014.
3. Jonathan D. G. Jones & Jeffery L. Dangl. “ The Plant Immune System.” *Nature*, 2006. Web. 11 Oct. 2014. [www. path. ethz. ch%2Feducation %2Fcourses%2Flecture_notes>](http://www.path.ethz.ch/education/courses/lecture_notes/).

4. Afzal, Ahmed J., Jin Hee Kim, and David Mackey. “ The Role of NOI-domain Containing Proteins in Plant Immune Signaling.” *BMC Genomics* 14. 1 (2013): 327. 2013. Web. 11 Oct. 2014.
5. Mackey D, Holt BF, Wiig A, Dangl JL: “ RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis.” *Cell* 2002, Web. 11 Oct. 2014.
6. Jones, Jonathan D. G, and Jeffery L. Dangl. Nature. com. Nature Publishing Group, 16 Nov. 2006. Web. 12 Oct. 2014. .
7. Afzal, Ahmed J. “ Separable Fragments and Membrane Tethering of Arabidopsis RIN4 Regulate Its Suppression of PAMP-Triggered Immunity.” National Center for Biotechnology Information. U. S. National Library of Medicine, 07 Oct. 2011. Web. 12 Oct. 2014. .
8. Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL:” Arabidopsis RIN4 Is a Target of the Type III Virulence Effector AvrRpt2 and Modulates RPS2-mediated Resistance.” *Cell*, 2003.
9. Belkhadir Youssef, Zachary Nimchuk, David A. Hubert, David Mackey, and Jeffery L. Dangl. “ Arabidopsis RIN4 Negatively Regulates Disease Resistance Mediated by RPS2 and RPM1 Downstream or Independent of the NDR1 Signal Modulator and Is Not Required for the Virulence Functions of Bacterial Type III Effectors AvrRpt2 or AvrRpm1.”. *The Plant Cell Online*, 2004.
10. Goodin, Michael M., David Zaitlin, Rayapati A. Naidu, and Steven A. Lommel. “ Molecular Plant-Microbe Interactions Journal – 21(8): 1015 – Abstract.” *Molecular Plant-Microbe Interactions Journal – 21(8): 1015 – Abstract*. N. p., 2008. Web. 12 Oct. 2014. .