# New regulatory mechanisms in the plant-pathogen arms race

For its virulence activity, *Phytophthora* can use apoplastic and cytoplasmic effectors that can suppress the immune system of the plant. From the other side, plants exploit extracellular and intracellular receptors that can recognise the secreted effectors, triggering a cascade of reactions and lead to immunity. The intracellular receptors are also called R proteins. The effector recognition could be either direct or indirect. The latter involves a third protein, whose modification after effector interaction activates the response of R protein. My aim was to study the role of two exosyst components Sec3/Sec5 in the recognition of AVR3a forms by R3a protein in *N. benthamiana*. Sec3 and Sec5 can interact with both AVR3a, however, it has not elucidated whether Sec3 and Sec5 are required in the AVR3a effector recognition Moreover, I designed functional and stable HA-tags of R3a protein that could improve its detection and could be used in further studies about the mechanism of R proteins function.

To investigate the role of Sec3 and Sec5 exocyst components in plant immunity, Tobacco Rattle Virus (TRV) VIGS was used. The two biggest leaves of small 4-leaf-stage *N. benthamiana* plants were infiltrated with TRV:*Sec3* and TRV:*Sec5* constructs. As a negative control for the experiment TRV:*GFP* was utilised where the vector contained only the Green Fluorescent Protein. Three weeks later, silencing has spread systemically, so it could be feasible to estimate the approximate VIGS efficiency. In the other objective of my project, three constructs of R3a protein were designed each one tagged on a different position on the LRR domain. Custom mutagenic primers were used for substitution on the target area of R3a gene through PCR. Next, there was production of circular recombinant PCR products without template DNA and cloning into *E.coli* through Gateway™ cloning and subsequently transformation to *Agrobacterium tumefaciens* was followed with a electroporation method. The HA-tagged constructs were infiltrated in *N. benthamiana* to test whether they are functional. For the detection of the tagged protein, western blot analysis was conducted.

To test the VIGS efficiency the VIGS silenced plants were challenged by $AVR3a^{KI}$+R3a and Rd4+$AVR3a^{EM}$ (Rd4 is a mutated form of R3a able to recognise $AVR3a^{EM}$ form) and the cell death was scored. The results did not show any attenuation in the recognition of $AVR3a^{KI}$ while $AVR3a^{EM}$ did not give any response even in the control plants. However, the controls did not work properly so no safe conclusions could be made. In the second objective, to test the functionality of the HA constructs they were co-infiltrated with $AVR3a^{KI}$ and $AVR3a^{EM}$ forms. HA2 gave very strong responses, identical with the wild-type. HA1 and HA3 did not show very strong responses, though, much lighter compared to the control. The most challenging part of that objective was the detection of the HA tags through western blot. The western blot analysis was performed many times with appropriate adjustment at the incubation conditions with antibodies and the loading concentrations. Finally all the tagged proteins were detected even those which gave a weak response on the agroinfiltration.

It is still not clear how AVR3a forms are being recognized by R3a. The functional tools that have been proposed above can be exploited for further studies on the relocalization of immune proteins. The findings from both the experiments could provide a practical and theoretical contribution in the cytoplasmic function and activation of R proteins and may reveal new mechanisms that could be used to boost the production of resistant plants.

To investigate further *P. infestans* I applied for an internship, as a part of my MSc studies, and had the opportunity to come in the James Hutton Institute, carry out that project and deepen my knowledge in plant-microbe interaction under the supervision of Piers Hemsley and Paul Birch. I learnt new molecular techniques and was introduced in the world of proteomics that will be definitely useful in my future career. I would like to thank BSPP for that bursary and all the staff helped me to complete that project.

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