Studying the function of a RxLR effector that targets the contact sites between the ER and the plasma membrane

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Phytophthora is a genus of pathogenic hemibiotrophic oomycetes that infects different species of plants, among which there are several cultivated plants. It is estimated that every year these oomycetes are responsible for millions of dollars of losses. Currently, the approach used to prevent Phytophthora infections is the massive use of pesticides that is pollutant and not fully effective. It is therefore necessary to develop new approaches to have an alternative for controlling these pathogens without the use of chemicals. To do so, first it is necessary to better understand the molecular interaction that enable these pathogens to successfully infect their hosts despite the activation of the plant immune system.

Throughout the of co-evolution between Phytophthora species and their hosts, plants have evolved their immune system to prevent infections, but oomycetes have also developed molecular mechanisms to impair the correct functioning of plant immunity, such as the secretion of effectors. Phytophthora species produce a specific type of effectors called RxLR effectors that are secreted into the extrahaustorial matrix and are supposed to be the main reason for the success of the infection. The current knowledge about the RxLR effectors indicates that, despite different effectors target different processes in plants, these proteins seem to have a tendency to interfere with cell-wall associated defences and vesicular transport. However, since the number of RxLR effector that have been characterised is limited, to develop new approaches to prevent Phytophthora infections, it would be necessary to learn more about this topic by characterising new RxLR effectors.

In this study we started to study four cDNA sequences encoding potential RxLR effectors of Phytophthora brassicae, a species that infects the plants of the Brassicaceae family. The aim was to identify at least one effector that is functional inside the host. To do so, the effectors were expressed in Nicotiana benthamiana plants, fused to different epitope tags, to assess their subcellular localisation and to realise a co-immunoprecipitation followed by a mass spectrometry to identify the target proteins. Furthermore, each effector was expressed in its natural host, Arabidopsis thaliana, to assess if its presence conditioned the development of the plant. The information gathered in this part of the project allowed us to select the effector that seemed the most promising in order to continue in its characterisation.

RxLR35 was chosen as preferred effector because it was shown to be localised in the contact sites between the ER and the plasma membrane, which seem to be involved in the perception of microbial pathogens. It was demonstrated that a constitutive expression of this effector in Arabidopsis thaliana plants renders the plants more susceptible to Phytophthora brassicae infection. Knowing that, it was tried to understand how RxLR35 could interfere with the function of EPCSs to negatively influence plant immunity.

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