Schweizerische Eidgenossenschaft
Confédération suisse
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Eidgenössisches Departement für
Wirtschaft, Bildung und Forschung WBF

Agroscope

Master internship at Agroscope

Title

Analysis of Flavescence Dorée effectors

Introduction

Flavescence Doree (FD) is an emerging quarantine disease that arrived in Switzerland in 2004 in Ticino. It is caused by the phytoplasma *Candidatus* Phytoplasma vitis, and is transmitted from vine to vine mainly by the leafhopper *Scaphoideus titanus*. The insect is present in Ticino, in the Lake Geneva region and in Valais. Due to global warming, it is gradually expanding its territory northwards by a few kilometers each year. Its presence in new wine-growing areas is at the origin of the development of numerous epidemic outbreaks north of the Alps since. Insecticide control is used to progressively reduce the number of FD-positive vines in the areas recently affected by the epidemic. However, in areas where the disease has not been detected early enough, FD becomes established in wild plants in the landscape and its eradication is no longer an option.

Objectives

The Master's project is part of a larger effort by Agroscope to study the still poorly understood molecular mechanisms that lead to the development of devastating symptoms in susceptible grape varieties such as Cabernet dorsa and Pinot noir, while other grape varieties such as Merlot and Chasselas seem more tolerant.

The Virology, Bacteriology & Phytoplasmology research group of Agroscope has recently decoded the genome of FD phytoplasma (Debonneville et al. 2022). This genome reveals the presence of 11 secreted proteins, 4 of which have no equivalent in other organisms. Bacterial secreted proteins interfere with the host cell defense system to allow infection. However, some resistant hosts use them as an early infection signal for a rapid activation of the defense system that blocks or limits the development of the bacteria in the organism. A previous work has shown that some of these proteins are more expressed in insect vectors whereas others are more abundant in host plants. Some of them were recombinantly expressed in bacteria and purified in order to perform pulldown experiments to identify potential binders.

The aim of this work is to identify specific binders of the different protein candidates and to better understand the functions of these effectors in the development of the FD disease.

Experimental approach

Purified proteins bound on beads will be used to perform pulldown assays with plant (Vitis vinifera, Vicia faba) or insect (Scaphoideus titanus, Euscelidius variegatus) extracts prior to analysis by nanoLC-MS/MS with a LC gradient length adapted to the expected complexity of the sample. This will allow the identification of interacting proteins, as well as analysis of their binding specificity.

In parallel to these analytical works, the 11 FD secreted proteins will be cloned into expression vectors adapted to the production of stable (Arabidopsis) or transient (N. bentamiana, Vitis vinifera) transgenic plants for functional studies in planta. The effectors expressed in the plants will then be fused to a fluorescent protein to determine their intracellular localisation by fluorescence microscopy.

Indicative bibliography

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Bertazzon, N., Bagnaresi, P., Forte, V., Mazzucotelli, E., Filippin, L., Guerra, D., . . . Angelini, E. (**2019**). Grapevine comparative early transcriptomic profiling suggests that Flavescence doree phytoplasma represses plant responses induced by vector feeding in susceptible varieties. Bmc Genomics, 20(1), 526. doi:10.1186/s12864-019-5908-6

Bai, X., Zhang, J., Ewing, A., Miller, S. A., Jancso Radek, A., Shevchenko, D. V., . . . Hogenhout, S. A. (**2006**). Living with genome instability: the adaptation of phytoplasmas to diverse environments of their insect and plant hosts. J Bacteriol, 188(10), 3682-3696. doi:10.1128/jb.188.10.3682-3696.2006

Bai, X., Correa, V. R., Toruño, T. Y., Ammar, E. D., Kamoun, S., & Hogenhout, S. A. (**2009**). AY-WB phytoplasma secretes a protein that targets plant cell nuclei. Molecular Plant-Microbe Interactions, 22(1), 18-30. https://doi.org/10.1094/MPMI-22-1-0018

MacLean, A. M., Sugio, A., Makarova, O. V., Findlay, K. C., Grieve, V. M., Tóth, R., . . . Hogenhout, S. A. (**2011**). Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in Arabidopsis plants. Plant Physiol, 157(2), 831-841. doi:10.1104/pp.111.181586

Sugio, A., Kingdom, H. N., MacLean, A. M., Grieve, V. M., & Hogenhout, S. A. (**2011**). Phytoplasma protein effector SAP11 enhances insect vector reproduction by manipulating plant development and defense hormone biosynthesis. Proceedings of the National Academy of Sciences, 108(48), E1254-E1263. doi:10.1073/pnas.1105664108

Pecher, P., Moro, G., Canale, M. C., Capdevielle, S., Singh, A., MacLean, A., . . . Hogenhout, S. A. (**2019**). Phytoplasma SAP11 effector destabilization of TCP transcription factors differentially impact development and defence of Arabidopsis versus maize. PLoS Pathog, 15(9), e1008035. doi:10.1371/journal.ppat.1008035

Huang, W., MacLean, A. M., Sugio, A., Maqbool, A., Busscher, M., Cho, S.-T., . . . Hogenhout, S. A. (**2021**). Parasitic modulation of host development by ubiquitin-independent protein degradation. Cell, 184(20), 5201-5214.e5212. doi:https://doi.org/10.1016/j.cell.2021.08.029

Debonneville, C., Mandelli, L., Brodard, J., Groux, R., Roquis, D., & Schumpp, O. (**2022**). The Complete Genome of the "Flavescence Dorée" Phytoplasma Reveals Characteristics of Low Genome Plasticity. Biology, 11(7). doi:10.3390/biology11070953

Work environment, scientific and technical supervision

This internship in grapevine phytopathology will allow the candidate to become familiar with the physiology and molecular mechanisms of plant-microorganism interactions. It will be an opportunity to develop practical skills in molecular biology adapted to expression studies while keeping a close link with the problematic in the field and the affected vineyard plots in Switzerland.

These techniques include primer design, qPCR, cloning and microscopy. Inoculation experiments under controlled conditions include the manipulation of insect vectors and the cultivation of plants.

The student will work closely with a team of 3 people involved in the project under the supervision of the scientist in charge of the project. He/she will benefit from a dynamic research context in a multidisciplinary research team including 2 postdocs, 2 PhD students and a very collaborative and friendly team as well as a network of partners in the canton of Vaud and in Ticino.

The laboratory is equipped with excellent research facilities with greenhouses suitable for experiments including biosafety level 2 organisms, climate chambers and sites for experiments and field studies.

Information about Agroscope

Agroscope is the Swiss centre of excellence for agricultural research, and is affiliated with the Federal Office for Agriculture (FOAG). Agroscope makes an important contribution to a sustainable agriculture and food sector as well as to an intact environment, thereby contributing to an improved quality of life. Agroscope researches along the entire value chain of the agriculture and the food sector. Its goals are a competitive and multifunctional agricultural sector, high-quality food for a healthy diet, and an intact environment. In pursuing these aims, the research institute gears itself to the needs of its service recipients.

Agroscope deals with issues in the following spheres:

- Plant Breeding, Plant Production, Plant Protection and Plant Products;
- Livestock, Feed and Products of Animal Origin;
- Food and Nutrition;
- Cropping Systems, Protection of Natural Resources, Agricultural Economics and Agricultural Engineering.

Place of Work

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Start date: upon agreement