Schweizerische Eidgenossenschaft
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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 signals for a rapid activation of the defense system that blocks or limits the development of the bacteria in the organism.

The aim of this work is to characterize the function and role of these putative secreted proteins in the development of FD. In a first step, an analysis of the expression of the 11 secreted proteins in different hosts will allow the selection of the protein on which the rest of the internship will focus. In a second phase, the function of this selected protein will be characterized in detail in order to understand its function in the host plants and also in the insect vectors responsible for the dissemination of the disease.

Experimental approach

Agroscope implemented an experimental system for the transmission of FD in its quarantine environment on the Changins site. This rare and complex set-up enables several types of plants or insects to be infected according to the experimental needs. It will allow qPCR based studies of the expression of effectors in the various plant or insect hosts that participate in the disease cycle. A collection of DNA gathered over the last 10 years from different sites and host types will be used to analyze the genetic diversity of effectors in FD.

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. Particular attention will focus on effectors in which genetic variability has been detected during the DNA collection screening.

The effectors expressed in the plants will then be fused to a fluorescent protein to determine their intracellular localisation by fluorescence microscopy.

An ongoing project in the laboratory based on a yeast two-hybrid screen is underway to identify plant proteins likely to interact with these proteins secreted by the phytoplasma into host cells. We expect to get the results of these analyses before the beginning of the internship. These results will provide valuable information that will integrate and support the student's experimental work.

Indicative bibliography

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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	1260 Nyon (Changins) VD - Switzerland

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Start date: upon agreement