SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16107
STSM title: Characterization of new effectors of *Xanthomonas arboricola* pv. *pruni*
STSM start and end date: 05/02/2018 to 16/02/2018
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PURPOSE OF THE STSM

The purpose of this STSM has been to learn the details of an Arabidopsis mesophyll protoplast system for the identification of early suppresors of PAMP-triggered immunity initially described in Dr. Frederic Brunner's lab and modified and succesfully used in Dr. Guido Sessa's lab for the characterization of *Xanthomonas euvesicatoria* effectors. In my laboratory, I am planning to establish a new line of research for the identification of *Xanthomonas arboricola* pv. *pruni* effectors. In collaboration with Dr. Jaime Cubero from INIA, Madrid, the available genome information coming from his laboratory in the last few years will be used to identify interesting effectors and to initiate their functional characterization.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

Concerning the protoplast system, a three-days protocol was initially demostrated by Dr. Georgy Popov, and repeated in my hands on the next week, where key aspects of the protocol were discussed and fixed, and troubleshooting discussed. The protocol included isolation of protoplasts from Arabidopsis young leaves, mediated by an enzymatic cell-wall digestion, transfection of protoplast using the corresponding constructs, and evaluation of results. In our system, protoplast are transfected with a 35S:effector construct, a reporter construct (in our case, pFRK1:LUC, inducible by the PAMP-immunity elicitor flg22), and a pUBQ1:GUS construct for normalization. The protoplasts are then flg22- or mock-elicited and induction of the reporter gene is evaluated at several time-points after elicitation. Induction rate is calculated as (elicitor luciferase/elicitor GUS) / (mock luciferase/mock GUS). Key aspects of the protocol included gentle handling of the protoplasts and adequate temperature of transfection. Possible causes of artifacts were also discussed. To facilitate the correct understanding of the whole protocol, two reactions were done: a positive control using the AvrPto effector, and a negative control, using a 35S:GFP control.

On the other hand, we discussed during my stay a machine-learning approach recently used for

the identification of new effectors of *Xanthomonas euvesicatoria* (Teper et al, Mol Plant Pathol, 2016) and we initiated discussions with Dr. Tal Pupko, who developed the machine-learning approach, for the identification of new effectors of *Xanthomonas arboricola* using this strategy. In this context, I could understand the protocol to evaluate translocation using the hypersensitive response (HR)-inducing domain of AvrBs2 as a reporter, and the materials will be transferred to my laboratory in Spain for future use.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

In this STSM, the objective was to get experience in a particular technology. Using *Xanthomonas arboricola* effectors require cloning steps that can be performed in my laboratory at my arrival, so only positive and negative controls were assayed. In this sense, the results obtained were the expected: The positive control (AvrPto) yielded supression of immunity after flg22 addition, and the negative control, using a 35S:GFP construct, showed a clear induction of luciferase as a consequence of PAMP-triggered immunity activation.

FUTURE COLLABORATIONS (if applicable)

As explained above, we have initiate a collaboration with Dr. Tal Pupko for the identification of new effectors using a machine-learning approach and the available genome information of *Xanthomonas arboricola*.

Moreover, a collaboration with the host laboratory leaded by Dr. Guido Sessa has been established, and we start now with the project aimed to functionally characterize *X. arboricola* effectors, using the abovementioned protocols.