OECD CO-OPERATIVE RESEARCH PROGRAMME: BIOLOGICAL RESOURCE MANAGEMENT FOR SUSTAINABLE AGRICULTURAL SYSTEMS

Fellow
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Subject Title
Reconstruction of plant antiviral DICER functions in cell-free extracts and in the heterologous system *Saccharomyces cerevisiae*
THEME 3: TRANSFORMATIONAL TECHNOLOGIES AND INNOVATIONS

Host institution: Institute for Biochemistry and Biotechnology-Section Microbial Biotechnology-martin Luther University Halle-Wittemberg
Name of Host Collaborator: Prof. Sven-Erik Behrens
Duration of the fellowship: 8 weeks
Dates: **From:** 22 May **To:** 19 June
From: 24 November **To:** 21 December (2018)

I hereby consent to my report being posted on the Co-operative Research Programme's website

1. Objectives of the research project

With this research proposal, we would have liked to set-up an heterologous yeast system to dissect the activities of the main plant DICERS involved in antiviral silencing, i.e. DCL2 and DCL4. The use of a plant *in vitro* system (tobacco cell free extract) was planned to be explored in order to generate short non-coding RNAs effective in antiviral silencing.

Importance of the research project

OECD Member States and EU policies seek to reduce the reliance on pesticides for crop protection by the design and implementation of novel biotechnologies. One of the possible approaches involves non-transgenic nucleic acid-based technologies to induce resistance in plants against plant pathogens including viruses and their vectors (insects, fungi, nematodes). A yeast system that could recapitulate antiviral DICERS would enable a massive and low-cost production of specific antiviral non-coding RNAs vaccines.

2. Were the objectives of the fellowship achieved?

The main objectives have been mostly achieved. Indeed, we were able to generate vectors able to overexpress tagged version of *N. benthamiana* (Nb)DCLs into the *Saccharomyces cerevisiae*.

3. Major achievements of the fellowship

Here below the major (up to three) achievements obtained during the period of the fellowship:

a) We have generated a vector able to express a tagged version of *N. benthamiana* (Nb)DCL4. We have shown a regular expression by western blot analysis, we have identified the subcellular localization by indirect immunocytochemistry, and we have evaluated his functionality *in vivo* by NGS of short RNAs of viral origin (tombusvirus sub viral RNAs). During the fellowship, we were able to generate also an expression vector for a tagged version of NbDCL2 that will be used in alternative to that used to study NbDCL4. The expression and the analysis of DCL2 is now in progress at the CNR (Italy);
b) We were able to set up a technical protocol able to immunocapture the soluble fraction of tagged NbDCLs. The outputs of the protocol are at the moment under evaluation;

c) We have used of a plant *in vitro* system (tobacco cell free extract) as a control of the activities of NbDCL4 expressed in yeast. The tobacco cell free extract have been also largely and successfully used to identify short non-coding RNAs effective in antiviral silencing in plant.

4. Follow-up work

The data obtained from the tobacco cell free extract were used to identify viral short interfering RNAs, which were able to exert efficient antiviral activity *in planta*. These observations will be disseminated through communication in scientific publications. The publication will likely be released by the spring 2019.
The data from the expression of tagged NbDCLs into the heterologous system *S. cerevisiae* will be likely released through a communication in a European congress of virology taking place in spring 2019.

The fellowship will surely be the start of collaboration. In the short term, we will be involved in finalizing the publications. In the long term we will likely consolidate our collaborative research in developing novel non-transgenic strategies to protect plants against pathogens.

Hopefully, our research will result in protected intellectual property regarding processes, however the protection of the patents requires sponsors, more likely extra-European, that would support it.

5. Importance of the research project for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society.

With this proposal we would have liked to disclose the mechanisms of activity of plant DICERs, which are the core proteins that convert double stranded RNA of viral origin into short non -coding RNA. The latter represent the main antiviral RNA molecules *in planta*. In the short/mid term, this aim is surely of help for the scientific community that works worldwide in our field of science. In the long term period, our research activities would have a beneficial effect to the society in term of a small contribution for growing a culture of science.
Moreover, OECD Member States and EU policies seek to reduce the reliance on pesticides for crop protection by the design and implementation of novel biotechnologies. One of the possible approaches involves non-transgenic nucleic acid-based technologies to induce resistance in plants against plant pathogens including viruses and their vectors (insects, fungi, nematodes). Thus, we aimed to set-up an optimal system that could be translated into applications for massive and cost-effective production of natural antiviral molecules to be us for sustainable plant protection measures in agriculture.

6. Relevance of the research to:

the CRP objectives
The output of our research activities in part supported by the OECD Co-operative Research Programme will be of help for the scientific community engaged in our field of science and it will provide a small contribution to the entire society for growing a culture of science. Our activities are anchored in a scientific environment at academic level. We consider the above outputs to fulfil the main CRP objective to strengthen scientific knowledge. We are available to provide other relevant scientific information and advice that could inform future policy decisions related to the sustainable use of natural resources, in the areas of food, agriculture, forests and fisheries.

the CRP research theme "Transformational Technologies and Innovations"
Our research activity is engaged to develop novel non-transgenic biotechnologies that could help to protect plants from pathogens and other environmental stresses. Our activities are therefore trying to introduce innovations in our research activities also through using novel systems and models of study. Either the heterologous system *Saccharomices cerevisieae* or the plant *in vitro* systems should be considered innovative approaches to achieve knowledge. These approaches could be translated into applications that could gain weight in the context of agricultural sustainability and resilience in a resource and climate constrain. Accordingly, most OECD Member States and EU policies seek to reduce the reliance on pesticides for crop protection and we believe that our research proposal is in line with this idea.

7. Satisfaction

The OECD Co-operative Research Programme supported my staying at the host lab for eight weeks. The possibility to split it in two parts (four weeks each) denoted also a supportive approach by the OECD Research programme, which is functional to specific research activities carried out in different labs. The support has been fully conform to my expectations.
I am a permanent researcher at the Institute for Sustainable Plant Protection of the Italian Research Council, the largest Italian institution involved in research in plant protection. The successful participation to the OECD Co-operative Research Programme fellowship adds a distinctive element to my CV and therefore could increase my career opportunities.
Moreover, hopefully this experience could increase my personal visibility at OECD level, wich hopefully could be in turn a primary step for future involvement into a more interactive flux of scientific information and advices from/to OECD offices.

8. Advertising the Co-operative Research Programme

In 2006, I have been supported for the first time by the Co-operative program, for a collaborative research with a research group located in Hungary. I did learn about this programme from the research environment in which I work. I have the feeling that the programme is enough visible, even thought I am not aware about the numbers of successful applications / total application per year.
One issue: I would suggest to extend the eligibility to applicants that were already supported with a limit of exclusion of 2 years from the last support. This would encourage people interested like me (and many others) to plan an extension of the collaboration in the future 2-3 years.

Date: 31/12/2018

Dr. Vitantonio Pantaleo