FELLOWSHIP SUMMARY REPORT

- Leandro Peña

- Subject: RESISTANCE TO PESTS AND DISEASES THROUGH MODULATING EMISSION OF VOLATILE ORGANIC COMPOUNDS IN CITRUS VARIETIES USING NEW BREEDING TECHNOLOGIES.

  THEME 3: TRANSFORMATIONAL TECHNOLOGIES AND INNOVATION: Novel and innovative technologies that achieve a step change.

  Topic: Advanced breeding tools – genetics and genomic technologies

- Host institution: Institute of Fruit tree and Tea Science (NIFTS) in National Agriculture and Bio-oriented Research Organization (NARO), Shizuoka 424-0292, Japan.

- Host collaborator: Takehiko Shimada

- Dates of the fellowship: June 2nd to July 13th, 2019

- I consent to this report being posted on the Co-operative Research Programme's website, or alternatively, a short paragraph about my fellowship which could be used anonymously.

1. What were the objectives of the research project? Why is the research project important?

The general objective of the project was to study the best strategies to get resistance phenotypes in citrus against devastating diseases through volatile organic compound (VOC) modulation by using New Breeding Techniques (NBTs). The information already available in our labs or that needed to be generated on the genome sequences of the citrus varieties of interest to afford cisgenesis or editing will be shared by our laboratories but kept as confidential for others until new resistant germplasm is generated. Our labs also launch to develop a new delivery system for CRISPR/Cas9 based genome editing using citrus.
It may make possible the generation of new improved citrus varieties, more resistant to diseases, and more acceptable to the consumers as they would be undistinguishable from natural mutants occurring in nature and/or orchards.

2. Were the objectives of the fellowship achieved?
Or are they on the way to being achieved?
If not, for what reasons? (The data or research is still ongoing or being analysed; technical reasons (e.g. equipment not working, adverse weather conditions, unexpected results, etc.; other reasons?)

They are in the way to be achieved. Based on recent results from our labs, we have decided to use a terpene synthase gene from citrus to generate cisgenic/intragenic citrus plants which may be more resistant to important citrus pathogens, including *Candidatus* Liberibacter asiaticus (CLas), bacterial pathogen associated to Huanglongbing (HLB), the most serious disease of citrus worldwide. Our rationale is that this new synthase has double activity, as mono- and also as sesqui-terpene synthase, and should work better than just a either mono- or sesqui-terpene synthase to generate resistance to pathogens. Preliminary in vitro experiments are reinforcing this. We have also decided the best strategy to over-express such terpene synthase gene in citrus without causing any excessive toxicity to plant cells but getting high enough accumulation. Additionally, we have studied and designed the best procedures to be able to generate crisprgenic plants in citrus, which both labs are currently implementing.

3. What were the major achievements of the fellowship? (up to three)

- We have decided to use a recently characterised terpene synthase gene from citrus to generate cisgenic/intragenic citrus plants which may be more resistant to important citrus pathogens.
- We have also planned the best strategy to over-express such terpene synthase gene in citrus without causing any excessive toxicity to plant cells but getting high enough accumulation.
- We have studied and designed the best procedures to be able to generate crisprgenic plants in citrus, which both labs are currently implementing.

4. Will there be any follow-up work?

My stay in Dr. Shimada's lab is allowing us to re-start collaboration on the use of VOCs and VOC genes to generate resistance to important citrus pathogens. Our past collaboration projects on the use of monoterpene synthases in transgenic citrus plants were rather advanced, with more than 10 scientific papers published during the last 8 years, and several field trials already set up in Brazil to test the best strategies, so we were in a situation in which we needed to discuss how to continue our works together considering the recent progress in the availability of tools and techniques to afford new biotechnologies with the aim of generating improved citrus varieties, more resistant to diseases, and more acceptable to the consumers.

After setting up a pipeline for the next 5 years, our aim is to generate not only scientific publications within the next coming years but also new citrus varieties more resistant to devastating citrus diseases, and more specifically to HLB.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Generation of new citrus varieties more resistant to the causal agents of HLB would represent a long-term solution for a problem which is causing huge economic losses in areas affected by the disease, thousands of job losses, currently dependent on the application of toxic insecticides (which are being applied weekly in attempts to control the insect vector of the CLas bacterium), and removal of millions of affected trees which is causing soil erosion. Moreover, the strategies we are proposing to afford are based on the use of genes/genome from citrus, in such a way that they would be more acceptable to the consumers as they would be undistinguishable from natural mutants occurring in nature and/or orchards. NBTs offer the only acceptable alternative in Europe and Japan to produce new varieties with efficient, durable and sustainable resistance to HLB.

6. How was this research relevant to:

- The objectives of the CRP?
- The CRP research theme?

This cross-disciplinary research project will provide outstanding possibilities in citrus for the use of NBTs as advanced breeding tools by using already available genetics and genomic technologies, which fits perfectly with theme 3 of the present call, TRANSFORMATIONAL TECHNOLOGIES AND INNOVATION: Novel and innovative technologies that achieve a step change, in the Topic: Advanced breeding tools – genetics and genomic technologies. This project may make possible the generation of new improved citrus varieties, more resistant to diseases.

7. Satisfaction

- Did your fellowship conform to your expectations? Yes.
- Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify. Not really.
- Did you encounter any practical problems? No.
- Please suggest any improvements in the Fellowship Programme. Improve publicity of the Programme through publication of calls in webpages of the most relevant scientific publishers/journals.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme? Internet and previous application (2001).
- What would you suggest to make it more “visible”? Mentioned above.
- Are there any issues you would like to record? This offers a good opportunity to establish collaborations with research groups from OECD countries, other than those inside Europe.

One paragraph non-scientific explanation of how your research might benefit society:

Global citrus industry is seriously threatened today by a disease called Huanglongbing (HLB). Currently its control is based on the elimination of symptomatic trees, aggressive insecticidal treatments and excellent nursery practices. However, long-term coexistence between a profitable and environmentally friendly citrus industry and the endemic presence of the HLB is virtually impossible. HLB is already causing billionaire losses in regions affected. The only long-term and sustainable way to achieve resistance to HLB is to incorporate genetic resistance in those varieties that are intended to be protected. We are affording it through the use of New Breeding Techniques (NBTs), so new HLB-resistant citrus varieties would be undistinguishable from natural mutants occurring in nature and/or orchards.