FELLOWSHIP SUMMARY REPORTS

Name:
Dr. José Ramón Úrbez-Torres

Subject title and theme number of your research fellowship:
"Investigating next-generation sequencing technology as a standard diagnostic tool for grapevine trunk disease fungal pathogens"

Transformational Technologies and Innovation (Theme 3)

Your host institution:
Instituto de Ciencias de la Vid y el Vino (ICVV) / Institute of Grapevines and Wine Science
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Spain

Host collaborator:
Dr. David Gramaje
Investigador Científico Titular del Consejo Superior de Investigaciones Científicas (CSIC)
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The dates of your fellowship:
June 15, 2023 – October 26, 2023
Original end of the fellowship was October 19, 2023. However, one week extension was given due to unforeseen circumstances that affected Dr. Úrbez-Torres during the week of August 14, 2023.

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

1. What were the objectives of the research project?

The aim of this research project was to investigate the potential use of next generation sequencing (NGS) technology for the detection of grapevine trunk diseases (GTD) fungal pathogens and to further study the possibility of its implementation and adoption in clean plant and import/export biosecurity programs. To address this main goal, the following objectives were proposed.

Objective 1. Develop a pipeline from sample preparation to data analyses using NGS for detection of grapevine trunk disease fungi from grapevine planting material.

Objective 2. Compare NGS detection of GTD fungi against currently available detection methods.

Objective 3. Potential implementation of NGS technology for detection of GTD fungi in clean stock programs and import/export biosecurity screening.

Why is the research project important?
Grapevines, with over 7 million ha cultivated, are one of the most extensively grown and economically important fruit crops in the world with grape-producing countries benefiting tremendously from the major economic boost that grape and wine industries provide. Grapevine trunk diseases (GTD), caused by over 130 fungal pathogens, are considered the main biotic factor limiting production and vineyards' lifespan worldwide and resulting in unsustainable economic losses for the grape and wine industry. GTD fungi occur in propagated nursery planting material and thus, they can be introduced in newly established vineyards. Most important, these pathogens can be routinely moved within and between countries. Because grapes are clonally propagated and worldwide distributed, with millions of plants moving each year between countries and continents, the movement of infected grapevine material represents a serious risk not only to growers but to countries' agriculture economies. Contrary to other grape pathogens (viruses and bacteria), there are currently no methods in place to detect GTD fungi in clean stock and import/export bio-security programs. Next-generation sequence (NGS) technology has been recently identified as an alternative to current DNA-based diagnostic tools in the above mentioned programs with success in the area of grapevine viruses detection. The proposed research is important as aims to develop an NGS pipeline for detection of GTD fungi, which will expand the number of grape pathogens that can be detected in one single test. This work aims to provide solutions to current gaps in molecular diagnostics and to provide science based evidences to support international efforts and influence decision makers in order to validate and standardize NGS as a diagnostic tool to be used in import/export programs.

2. Were the objectives of the fellowship achieved?

Objective one and objective two were achieved. Objective three was not fully completed. Dr. Úrbez-Torres's CRP research proposal was originally submitted for a duration of 24 weeks but the CRP was limited to 18 weeks. As per the timeline provided, objective three was scheduled to be completed during the last 8 weeks of the CRP. Accordingly, only two out of four sub-objectives within objective three were initiated, which included i) organizing a conference call to report on technologies and policies currently in place in different clean stock, import/export biosecurity programs and ii) identify limitations in current pathogen's detection technology and gaps in policies from the different programs.

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

- Completion of a research project relevant to the priorities established by the OECD-CRP. The main outcomes of this research were:
  - **i.** Development and implementation of a non-destructive methodology for sample collection and processing by micro-drilling live plant material. Internal vascular tissue were collected without compromising the plant health and viability. Tissue amounts were collected for DNA extraction and further process to detect and identify grapevine trunk diseases fungi using NGS.
  - **ii.** Development of NGS pipelines for four different genes, including the Internal Transcribe Spacer (ITS), beta-tubulin (*TUB*), translation elongation factor one alpha (*TEF*), and actin (*ACT*). Pipelines were successfully implemented in the detection of grapevine trunk diseases fungal pathogens from grapevines collected from commercial vineyards as well as from propagated nursery material. Up to 48 different GTD

fungal pathogens were detected using NGS. Different bioinformatic platforms were investigated and sequencing results from all four genes were compared. NGS pipeline using *TUB* gene and SCATA platform resulted in the best results for the detection of GTD fungal pathogens but also for the discrimination among GTD fungal species for which ITS gene was not capable to properly identify to species level.

iii. NGS results were compared against currently available methods for identification of GTD fungi, including traditional plating with morphological identification and droplet digital PCR (ddPCR). NGS resulted to be the most sensitive tool with capacity to identify to species level the largest number of GTD fungal species. NGS identified 48 GTD fungal species compared with 10 and seven different species by traditional and ddPCR methods, respectively.

In conclusion, NGS pipeline platforms for four different genes were successfully developed and implemented for the detection and identification of GTD fungal pathogens directly from grapevine tissue.

- The collaboration established and consequent knowledge gained and transferred between both participants (Dr. Úrbez-Torres and Dr. Gramaje) and institutions (Agriculture and Agri-Food Canada and The CSIC Institute of Grapevines and Wine Science).

- The CRP made possible to participate in several scientific conferences organized by both the host institution and relative close locations. Participation in these scientific conferences allowed me to meet scientists conducting research in related disciplines and establish new connections. As a result, a strong collaboration and partnership was developed with Professor Aleš Eichmeier from the Mendeleum Institute of Genetics at the Mendel University in Brno, who is conducting similar research.

4. Will there be any follow-up work?

- *Is a publication envisaged? Will this be in a journal or a publication? When will it appear?*
  Both participants (Dr. Úrbez-Torres and Dr. Gramaje) envision to prepare a peer reviewed publication in a scientific journal based on the results obtained from this CRP. Further analyses of the results will be needed to have solid data to publish and the hopes are that the manuscript can be completed within two years.

- *Is your fellowship likely to be the start of collaboration between your home institution and your host?*
  As a result of this CRP, my institution's commercialization office has already started conversations to developed an official and formal collaboration with the Institute of Grape and Wine Science (Dr. Gramaje) in the form of a letter of understanding or a Memorandum of understanding. In addition, my institution is also exploring the possibility to establish another formal partnership with Professor Eichmeier from the Mendel University in Czech Republic, which is also a country participanting in the OECD-CRP program.

- *Is your research likely to result in protected intellectual property, novel products or processes?*
  The research completed in this CRP will most-likely not result in protected intellectual property but will result in the development of a new process and methodology by expanding the usage of NGS for the detection of a large group of fungal pathogens. This work is expected to be published in an open access scientific journal.

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?
Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

This research resulted in the development of NGS pipelines to identify and detect grapevine trunk diseases fungal pathogens, which are known to cause important economic losses to the grape and wine industry worldwide. These fungi are also known to occur in a latent stage within the nursery propagated material, which represents a serious risk to growers as these pathogens can be routinely introduced in newly established vineyards and moved within and between countries. Current detection and identification methods for these pathogens are based on traditional taxonomy, which requires highly trained personnel or PCR-based methods, which require a multi-gene approach, which has been shown to be time-consuming and costly. Contrary to viruses and bacteria, one of the main reasons why clean plant programs and/or import/export programs still lack

certification of plant health status on GTD fungi is due to the lack of effective, accurate and sensitive detection tools. Clean plant and import/export programs' main goal is i) to ensure plant material provided to growers is free of specific pathogens and ii) the protection of countries' agriculture economies by detecting regulated/invasive pathogens. NGS technology has transformed research in many fields of biology. Among its many applications, it provides and alternate approach for the detection of plant pathogens and has the potential to be used as a routine diagnostic tool for a rapid and accurate diagnosis of pathogens. Currently, NGS is being implemented as an standard detection tool for grapevine viruses in several clean plant programs worldwide. In addition, NGS is being given consideration by crop protection organizations such as EPPO (European and Mediterranean Plant Protection Organization) and NAPPO (North American Plant Protection Organization) to be an alternative to improve current DNA-based detection tools and to be used as a routine and internationally validated diagnostic tool for virus detection in import/export biosecurity programs. Results from this research will add capacity to this technology to detect a much broader group of pathogens, which can be implemented in both clean plant and import/export programs. Early and rapid detection of plant pathogens, primarily regulated and/or invasive, is the first line of protection to regional and national agriculture economies. Introduction of new pathogens can cause untenable economic losses to agriculture, resulting in yield losses and increase of production costs, which eventually will lead to a price increment of the affected agriculture commodities to the public. Therefore, results from this research add to the need to develop new policies that allows the establishment of the latest available technologies in pathogen detection such as NGS to be implemented in critical biosecurity programs to protect national agriculture systems.

6. How was this research relevant to:

- *The objectives of the CRP?*
  The accomplishment of the proposed research objectives resulted in significant advances in knowledge, which are aimed to provide applied solutions to issues highlighted as priorities by the grape industry, but also by other commodities worldwide regarding the movement of contaminated plant material around the world and the risk this represents to agriculture economies. In addition, this project strengthened the current scientific knowledge available regarding the applications of NGS technology as standard diagnostic tool and contributed by adding the possibility to screen for a much broader group of plant pathogens. This research project filled gaps regarding the lack of standard and validated molecular detection methods for GTD fungal pathogens in clean stock programs and import/export biosecurity programs and addressed and provided solutions to two main problems that regulatory programs currently face and that limit the inclusion of these fungi in screening panels, including i) the lack of a non-destructive method for GTD fungal pathogen sampling and ii) the lack of a multiplexing approach capable to detect all 130 different fungi associated with GTD simultaneously. NGS technology is already being evaluated by policy makers as the future tool for standard diagnostics of plant pathogens and thanks to the successful advances obtained in using this technology for detection of grapevine viruses in quarantine programs, policy makers start contemplating the need to change current policies relevant to diagnostics and movement of plant pathogens to bring them up to speed with the fast changes occurring in science, and in particular in the field of molecular diagnostics. Therefore, results from this project supported the work already conducted in viral pathogen diagnostics and contributed to further influence policy decisions.
  This research project will allow the development of a long-term relationship between the two grapevine research leading institutes in Canada (Summerland Research and Development Centre) and Spain (Institute of Grapevine and Wine Science), with the possibility to establish a formal collaboration that will facilitate and strengthen future partnerships not only in the area of grapevine pathology but also in other fields of viticulture and oenology. Results from this project addressed common problems faced not only by Canadian and Spanish grapevine industries but also by grapevine and other commodity industries in the rest of OECD countries.

- *The CRP research theme?*
  This research proposal was submitted under Theme 3 *"Transformational Technologies and Innovation"* as it aims to use genetic and genomic technologies to provide tangible solutions to increasing concerns among countries regarding the global spread of crop pathogens and the need to improve pathogen detection systems relevant to biosecurity. This study provided NGS pipelines for the detection of GTD fungi on grapevines. Canada is at the forefront in the development of NGS methods for plant virus testing using dsRNA and

Canadian scientists along with the regulatory Canadian Food Inspection Agency (CFIA) are taking a leading role at the international level to further standardize and validate these new technologies. Results from this study will further support and contribute to maximize results in the Canadian import/export program. In addition, the grape and wine industry in Canada, represented by the Canadian Grapevine Certification Network (CGCN) and in partnership with CFIA and Agriculture and Agri-Food Canada (AAFC), is developing the first domestic grapevine clean plant program to limit the entrance of virus-infected propagative plant material into the production system. This research project will expand the screening of grapevine material by NGS to include GTD fungi with the overall objective to improve plant health.
In addition, this research also fell under Theme 2 *"Managing Risks in a Connected World"* as it aims to minimize the impact of GTD on vineyards by contributing to the development and selection of cleaner grapevine planting material in regard to GTD fungal pathogens. Growers' access to clean plant material is the first step to develop and implement a successful sustainable management strategy against pathogens. For other plant pathogens such as grapevine viruses or bacteria, clean plant programs exist or are under development to increase the availability of transplants free of those pathogens. There are also national and international policies that regulate the movement of these viruses and their plant hosts among countries. Unfortunately, there are currently no regulations or policies in place to prevent the movement of the fungi causing GTD, primarily because there are no standard or validated detection tools that can effectively detect these pathogens in such programs. Considering the significance of these pathogens and the imminent threat they pose to not only grapes, but many other valuable crops (tree fruits, small fruits, nut trees, ornamentals,…), continued research that delivers a standard multiplexing tool is imperative. Moreover, this research will contribute risk-assessment studies that engage all levels of government policy makers, which is critical for developing other strategies to minimize the impact of GTD at local, national and international levels.

7. Satisfaction

- *Did your fellowship conform to your expectations?*
  Yes, the CRP fellowship met all my expectations and allowed me to acquire critical knowledge but also to transfer knowledge to the host institution.

- *Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.*
  The OECD-CRP will definitely increase my career opportunities both within my institution but also internationally. The OECD-CRP is considered a highly prestigious award within my institution and will contribute to demonstrate innovation, recognition and impact on the research I am conducting. Innovation, Impact and Recognition are the three most important pillars for career success within my institution and thus, the OECD CRP will significantly contribute into my career progression within Agriculture and Agri-Food Canada. At the international level, the OECD-CRP has allowed me to develop new collaborations and partnerships that will undoubtably contribute to increase my career opportunities

- *Did you encounter any practical problems?*
  No, I did not encounter any problem. The OECD-CRP has tremendously supported my fellowship as it was cancelled in 2020 due to the pandemic. The OECD-CRP has done everything possible for me to complete my fellowship and deferred the funding until 2023 when I was finally allowed to travel for work by my institution.

- *Please suggest any improvements in the Fellowship Programme.*
  I have no suggestions to improve the program from a technical point as from my experience it has worked perfectly. Communications by OECD towards me have been outstanding and all my answers with respect technical questions were addressed in a very timely manner. I would like to thank the whole OECD-CRP team for making my fellowship happen but specially I would like to highlight the personal attention and support received from Nathalie Elisseou Léglise.
  The only thing to consider in future fellowships is to adjust the monetary support received to the current cost of living, including prices of travelling, lodging, meals, and incidentals as a result of the inflation faced worldwide.

8. Advertising the Co-operative Research Programme

- *How did you learn about the Co-operative Research Programme?*
  Through my Institution and employer, Agriculture and Agri-Food Canada. Every year our institution communicates to its scientists the opportunity to apply for the OECD-CRP program. I first heard about this program in 2017 and submitted my application in 2019.

- *What would you suggest to make it more "visible"?*
  The OECD-CRP fellowship can be announced in professional scientific associations. For instance, in my case as plant pathologist, it can be announced through the Canadian Phytopathological Society, The American Phytopathological Society, The International Society for Plant Pathology,… Similarly, it can be announced in other professional societies depending on the specific area of research.

- *Are there any issues you would like to record?*
  I experienced no issues through the entire process from proposal submission, award of the OECD-CRP and completion of the fellowship.