



FELLOWSHIP SUMMARY REPORTS

OECD-CRP research theme III: Transformational technologies and innovation

- Awarded participant:

Pablo Carbonell-Bejerano

- Project title:

De novo genome assembly and comparative genomics of somatic variants adaptable to climate change for cultivar innovation in grapevine

- Host institution:

Tübingen Max-Planck-Institute for Developmental Biology, Max Planck Society, Germany

- Host collaborator:

Prof. Dr. Detlef Weigel

- Fellowship dates:

From 01/09/2019 to 12/01/2019

Pablo Carbonell-Bejerano consents this entire report and/or parts of it to be posted on the Co-operative Research Programme's website.





1. Research project objectives and relevance

Perhaps even more as for many other crops, global warming is threatening the production of quality wine in classical winegrowing regions. Increasing temperature and drought episodes in these regions hasten grapevine phenology and alter grape ripening and composition. This trend is expected to continue in most traditional viticultural areas, affecting particularly intensely regions around the Mediterranean, including the Iberian Peninsula. High temperatures inhibit anthocyanin accumulation and speed up acidity loss in grape, which leads to excessively alcoholic wines that are unbalanced in colour and phenolic composition. Breeding for adaptation of grapevine cultivars to quality fruit production under future, more extreme climate conditions is a suitable strategy to deal with these negative consequences.

Importantly, winemaking involves a classical industry and traditional cultivars are often preferred by producers and consumers, which restricts breeding possibilities. Still, as an alternative breeding strategy, there is a long tradition to select new clones of established cultivars; these clones maintain the vast majority of attributes that make a varietal wine recognizable, but at the same time have new improved features due to somatic variation. Hence, **somatic variation** provides for the time being the only socio- and eco-friendly resource to increase trait diversity in elite wine cultivars, which can be exploited **to anticipate and adapt production to fluctuating consumer demands and changing environments using more sustainable cultivation systems**.

Tempranillo is the most widespread red wine cultivar in the Iberian Peninsula and the third in the world, In my previous work as postdoctoral researcher at the Institute for Grape and Wine Research (ICVV, Spain), I selected somatic variants of Tempranillo with ripening features adaptable to quality wine production under predicted future climate. Nonetheless, markers enabling clone identification are needed for the feasibility of this innovation to become generic towards cultivar improvement. Since somatic variants are usually near-isogenic lines, the identification of the causal somatic mutations is required to enable the development of reliable molecular markers for the protection of selected clones. Because grapevine cultivars are highly heterozygous and accumulate prominent genome structural variation upon vegetative propagation, obtaining a reference grade diploid assembly for the genome of Tempranillo is fundamental to understand its varietal attributes and to identify somatic mutations useful for intravarietal breeding in this relevant cultivar. In this context, I proposed the following two research objectives for this OECD-CRP fellowship:

Objective 1: *Produce a diploid-aware reference genome for the Tempranillo grapevine cultivar*

Objective 2: *Characterize at the molecular level the somatic variation of Tempranillo clones with ripening phenotypes suitable for quality wine production under warmer climate*

2. Objectives achievement status

The two proposed objectives were partially achieved:

1. The Tempranillo genome was sequenced and preliminary genome assemblies were produced.
2. Illumina whole-genome re-sequencing and RNA-seq transcriptome data of climate change-adaptable somatic variants of Tempranillo cultivar were studied and candidate responsible somatic mutations were identified.

Preliminary results were obtained so far and further research is ongoing to fully accomplish the objectives. On one hand, the fellowship award covered only 19 out of the 26 weeks estimated as the required timeframe to complete all the research. On the other hand, Objective 1 was delayed because, instead of one as initially proposed, two different sequencing technologies, PacBio and Oxford Nanopore, were used to sequence the Tempranillo genome.





This was decided to enable a more consistent genome assembly, gathering together the advantages provided by each sequencing technology. Bioinformatic analyses from all the sequencing data produced in the fellowship are still running to obtain the definitive Tempranillo genome assembly. This assembly will be used to reanalyse whole-genome resequencing and RNA-seq data of climate change adaptable variants to more consistently map the responsible somatic mutations.

3. Major achievements of the fellowship

1. The genome of Tempranillo grapevine cultivar was sequenced using last generation PacBio and Oxford Nanopore long-read technologies. Globally, the genome was covered in >190-fold depth with sequencing reads averaging >15,000 nucleotides of length. This involves a high-quality input data for subsequent bioinformatic genome assembly pipelines.
2. Preliminary genome assemblies from either PacBio or Oxford Nanopore sequencing data have been produced using bioinformatic pipelines developed to this aim. The results are highly promising in view that some quality parameters of the preliminary assemblies, including contig length, outperform those in the current version of the grapevine reference genome.
3. A bioinformatic pipeline is currently running to produce a definitive reference genome assembly for Tempranillo grapevine cultivar using all the long-read sequencing data generated during the fellowship.
4. Candidate genetic variation to be responsible for climate change adaptable phenotypes in Tempranillo somatic variants has been detected from preliminary analyses of whole-genome re-sequencing and RNA-seq transcriptome data. These analyses will be completed using the definitive Tempranillo genome assembly.

4. Work follow-up

- At least one publication in a high impact journal is envisaged. The generation of a Tempranillo reference genome assembly, the comparison of assembly features using either Nanopore or PacBio sequencing data and the identification of candidate mutations explaining new climate change adaptable ripening phenotypes are relevant results to be published. Hopefully the publication/s will be released within one year, once all the bioinformatic analysis can be completed.
- This fellowship involved a start point for collaborations between ICVV and Max-Planck-Institute. In fact, similar collaborative genome assembly and intravarietal studies have already been initiated for other grapevine cultivars. The host institution also showed interest in the applications of methods that they are developing to grapevine research.
- In the mid or long term, climate change adaptable somatic mutations identified thanks to this research could potentially lead to the development of protected intellectual property for future genome editing-mediated breeding.

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?

Tempranillo is a traditional grapevine cultivar suitable for the production of premium wines and recognized by producers and consumers. In fact, Tempranillo is the most widely grown wine cultivar in the Iberian Peninsula and the third in the world, occupying a total surface of 231,000 ha (OIV, 2017). The release of the reference-grade genome assembly for Tempranillo generated in this project opens up a plethora of opportunities for breeding in this relevant cultivar and therefore, for the improvement of the sustainability of vitivincultural agro-ecosystems.





This reference genome can serve as a base for the identification and certification of improved clones or somatic variants of Tempranillo. By selecting clones more adapted to raising stress situations and more tolerant to pests and diseases, precious resources can be saved and doses of environmentally hazardous pesticides and fungicides can be reduced. As a proof of concept, in the context of this OECD fellowship we identified candidate mutations leading to new phenotypes in Tempranillo somatic variants with ripening cycles adaptable to warmer climates. These results should encourage policymakers to promote similar clonal breeding research and agriculture policies to adapt autochthonous grapevine cultivars in the different OECD winegrowing regions by using improved clones. Moreover, our research shows a way to detect genetic variation specific of selected clones. Upon confirmation of the functional role for the candidate mutations that we detected, this knowledge can be exploited for the development of molecular markers intended to breeding or trade regulation purposes.

6. Relevance of this research to the objectives of CRP and CRP theme III:

Because of the economic value of its production and derived goods, grapevine constitutes the most important fruit crop in the world. While the vast majority of the vineyard surface is devoted to winemaking, this activity is very relevant for the economy of OECD countries as they produce >70% of the worldwide wine volume. Climate change is threatening the sustainability of these activities in most of these regions. Winemakers face unprecedented challenges: they must meet a growing demand for quality and recognizable wines, and at the same time, there is a need to make viticulture more sustainable by decreasing the waste of precious resources. In addition, cultivation and production need to become better adapted to current and future climates. This is the more challenging, as both conventional breeding and genetic modification result in cultivars that are unlikely to become widely accepted in the market. The only viable alternative is the use of spontaneous somatic variants. As a long-term alternative strategy, the identification of climate change adaptable somatic variants and the responsible somatic mutations can be exploited to adapt appreciated traditional cultivars to more extreme future conditions using sustainable agro-ecosystems. The availability of cultivar-specific and diploid-aware reference genomes as the Tempranillo genome assembly that we produced in this project can speed up intravarietal breeding programmes to identify improved somatic variants that enable a sustainable viticulture and the same time, keep the cultural and society recognizable values of traditional cultivars.

7. Satisfaction

This OECD fellowship was an excellent opportunity for me to engage a world leading laboratory in plant developmental biology and evolutionary genetics and genomics. During my stay there, I have acquired skills in cutting-edge methods, including long-read sequencing technologies and genome assembly bioinformatic analysis that put my research at the forefront of the grapevine genomics field. In addition, during this stay I built a collaboration network with expert researchers in the host entity that are developers of methodologies that can serve to boost my future research. Collectively, this experience increases my possibilities for the foundation of a successful genomics-assisted clonal breeding research line as a group leader in the next step of my scientific career.

Globally I find this programme very positive and there was no major practical problem with my fellowship. However, a more detailed justification of the fellowship duration could be provided to the awarded participants since for instance, in my case this time frame was insufficient to complete all the objectives.

8. Advertising the Co-operative Research Programme

- How did you learn about the Co-operative Research Programme?

I learnt about the project by internal emailing in my home institution.

- What would you suggest to make it more “visible”?





Co-operative Research Programme: Biological Resource Management for Sustainable Agricultural Systems

By encouraging the dissemination of fellowship results and their relevance in social media.

