Clonal mapping of Pinot Noir

Loss of source blocks in Adelaide Hills bushfires leads to clonal identification for Pinot Noir

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Trial site of the two Pinot Noir clones, MV6 clone (right side) and the D4V2 variant (left side) at the vineyard in Kuitpo

Introduction

Prior to the 2019-20 bushfires, Adelaide Hills Vine Improvement (AHVI) had the majority of Pinot Noir source blocks in South Australia, providing around 400,000 high health status cuttings to the Australian wine industry annually. The Cudlee Creek bushfire in December 2019 destroyed six out of 28 source blocks, significantly reducing the supply of high-demand clonal planting material to industry. While potential vineyards for new source blocks were identified, there was a lack of evidence on the provenance of the clonal material, specifically for the sought-after Pinot Noir clone D4V2.

Being aware of the release of the Chardonnay clonal map in 2018 by AWRI researchers, led by Dr Anthony Borneman, AHVII approached the AWRI to help clarify the identity of proposed alternate source blocks of Pinot Noir D4V2. A project proposal was developed and funding of $247,440 was provided by PIRSA's South Australian Wine Industry Development Scheme (SAWIDS), AWRI, Wine Australia and AHVII. The project aimed to identify genetic markers for individual Pinot Noir clones and to provide a way to identify potential sources of high-confidence germplasm for the re-establishment of source blocks within the Adelaide Hills.

> The project is a world-first application of state-of-the-art whole genome sequencing for the assessment of genetic diversity in Pinot Noir grapevines.

Sequencing a wide selection of germplasm

A broad range of Pinot Noir germplasm was sequenced by the AWRI, allowing the generation of a foundational sequence dataset of Pinot Noir clones, including key clones lost to bushfire damage. The project is a world-first application of state-of-the-art whole genome sequencing for the assessment of genetic diversity in Pinot Noir grapevines.

Leaf samples were obtained from a set of 197 grapevines representing 30 different Pinot Noir clones from across 21 sites. Within this set were six independent representatives of D4V2 and multiple representatives of most other clones. A subset of 96 vines was then selected

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that captured the major clonal groups. Genomic DNA was extracted from leaf samples of these 96 representative clones and subjected to genome sequencing followed by the clonal-marker identification pipeline developed by the AWRI. As a double-check, all samples were shown to have genetic backgrounds consistent with the targeted cultivar, Pinot Noir.

Identifying genetic differences and compiling a clonal family tree

A total of 3,208 marker single nucleotide polymorphisms (SNPs) were characterised across the 96 samples. The pattern of variation across these markers was used to uniquely identify each clonal sample and to then group samples into a phylogenetic network based upon their genetic similarity (Figure 1).

Genetic relationships were shown to be robust across multiple samples of the same clone. DNA typing data also supported known historic ancestry and/or geographic relationships, including:

- G8V7 (FPS 15) and G8V3 (FPS 13) both have origins in the Martini vineyard, California (1966) (https://fps.ucdavis.edu/fgrdetails.cfm?varietyid=1184)
- H7V15 (FPS 22) and D5V12 (FPS 19) being sourced from same vine (1974) (https://fps.ucdavis.edu/fgrdetails.cfm?varietyid=1184)
- UCD05 (FPS 05) and D4V2 (FPS 04) being derived from same stock via heat treatment (1965) (https://fps.ucdavis.edu/grapebook/winebook.cfm?chap=PinotNoir)
- Mariafeld, D2V5 (FPS 01A) and D2V6 (FPS 02A) having origins in Wädenswil, Switzerland (https://fps.ucdavis.edu/grapebook/winebook.cfm?chap=PinotNoir)
- MV6 and Mount Pleasant sourced from Mount Pleasant Vineyard, Hunter Valley, New South Wales (Nichols, 2006)

Understanding the outliers

Seven samples among clones 114, 115 and D4V2 (marked in red in Figure 1) were determined to be outliers compared to the consensus genetic profile associated with the expected clonal ID and may therefore represent instances in which the clone name has been mis-assigned. Given the importance of getting these predictions right, additional confirmation samples were processed for all of the outliers, which supported the results obtained for the original sample. Of particular note, the outlier set included all four samples of D4V2 from the AHVII source blocks, which displayed a genetic profile distinct from samples of D4V2 obtained from CSIRO and PIRSA and instead, clustered in a broad group with samples with origins in the Hunter Valley (MV6 and Mount Pleasant).

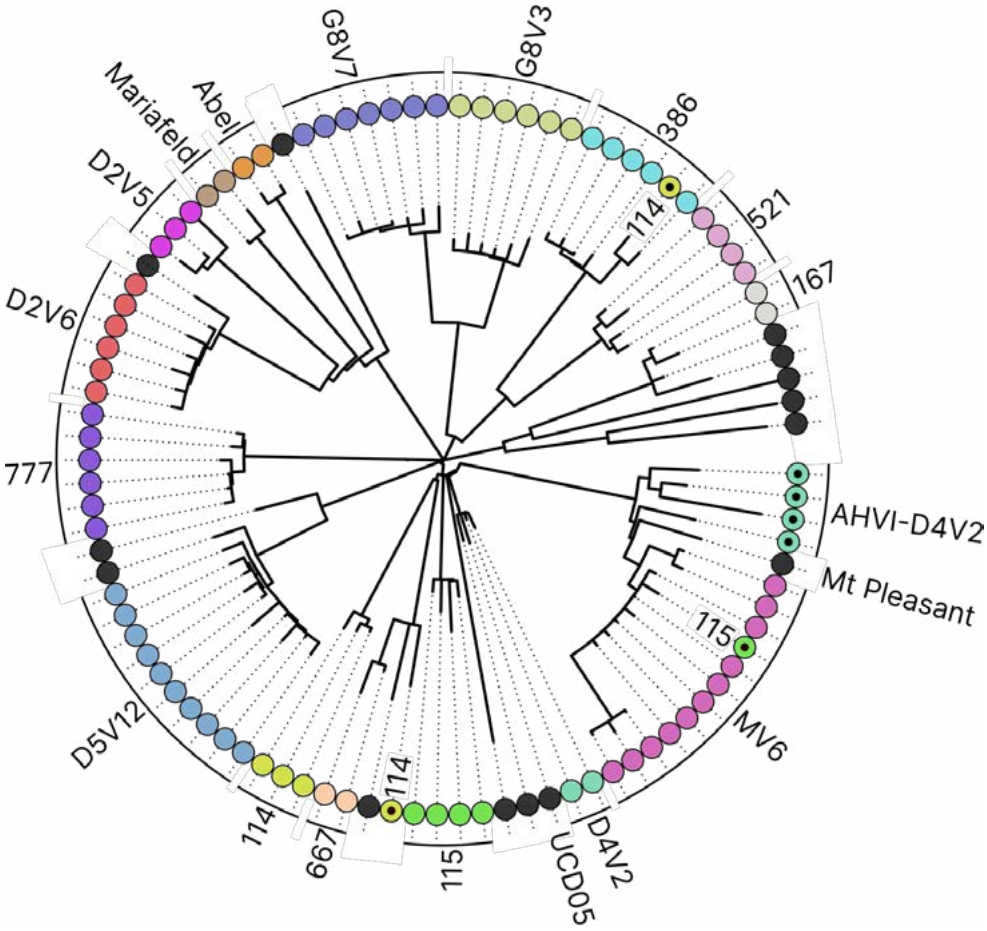

Figure 1. Compiled clonal phylogenetic results for Pinot Noir sequencing. Node tips are shaded according to existing clone annotation and are labelled by the clone designation. Outliers are marked with a black 'bullseye' and the supplied clonal name.

Which of the two conflicting D4V2 groups is correct?

It is likely that the D4V2 germplasm from CSIRO and PIRSA represents the true version of this clone in Australia. The Adelaide Hills Vine Improvement D4V2 (AHVI-D4V2) vines have been traced back to a non-certified source from the early 1970s. There is also a tight relationship between the CSIRO and PIRSA D4V2 and the SAVIA clone UCD05 (FPS05), which represents a heat-treated sibling of D4V2 (FPS04) and adds support for this group representing the correct D4V2.

If the Adelaide Hills Vine Improvement samples are not D4V2, what are they?

The genetic data points to the AHVI-D4V2 vines being closely related to Pinot Noir material originally obtained from the Mount Pleasant vineyard in the Hunter Valley. Interestingly, Mount Pleasant Pinot Noir has a rich history that stems from a wonderful genetic resource of vine material that was brought to Australia by James Busby way back in 1830. It was planted in the King's Paddock vineyard by Maurice O'Shea and was renamed to Mount Pleasant in 1921, with the grapes being used in his famous red wine blends.

However, the situation is not as simple as it seems, as the AHVI-D4V2 variant samples are neither a perfect match

to MV6 or the Mount Pleasant clone. Furthermore, the level of genetic diversity separating the various AHVI-D4V2 samples is much larger than expected if they are all cuttings of a single clone. In fact, rather than being one clone, it is likely that the original AHVI-D4V2 material was a mass-selected set of vines with ancestral origins from the Busby Collection of the 1830s.

Using the genetic information to inform future plantings

MV6 is considered a 'premium' clone and is the most widely planted Pinot Noir clone in Australia. The AHVI-D4V2 variant material, regardless of name, is also extremely sought after and its desirable characteristics may reflect the Mount Pleasant genetic ancestry uncovered by this study. This leads to the question as to what can be done for the existing AHVI-D4V2 variant resource? In this situation, individual samples from this mass-selected material have the potential to be specifically selected and propagated as new clonal material under different clonal designations, or simply used as a polyclonal planting material under a different name.

To move towards this, both MV6 and UCD05 mother vines, which are planted in the SA Vine Improvement Collection, will allow for some basic viticultural comparisons. Two sites near Kuitpo in the Adelaide Hills Wine Region are planted side-by-side with the AHVI-D4V2 variant and MV6 and are currently being investigated by Adelaide Hills VI. A range of characters such as growth habit, bunch and berry structure, maturity data and wine quality are being compared. This study will provide an indication of the attributes of the AHVI-D4V2 variant as a desirable clone of Pinot Noir and how it differs from MV6. Ultimately, this can lead to the establishment of a new clonal name that reflects its unique qualities that were sourced from the original Busby Pinot Noir collection.

Detailed knowledge of clonal genetics will enhance confidence in replanting investments, facilitate vineyard recovery and improve clonal selection for wine producers across the Australian wine regions. Using the same process, the next AWRI initiative 'The Thousand Genome project', which is being undertaken with support from Wine Australia, will stretch across hundreds of clones from dozens of varieties available to Australian growers. As we have seen in this project with Pinot Noir, many clones have been renamed multiple times and now we have the science to re-align similar clones and identify misnamed cultivars. It will be an excellent identification tool for one of the two pillars of certified propagation material – trueness to type – the other being high health status.

More details of the project's methodology, references and acknowledgements can be found in the Pinot Noir Clonal Mapping Project Final Report on the Wine Australia website: www.wineaustralia.com/research/projects/clonal-mapping-of-pinot-noir

In the Fresh Science session at the Australian Wine Industry Technical Conference 2022, Dr Bornemann was awarded both best viticulture presentation and best viticulturally focused poster for this work on clonal genetics. GW

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