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Scion genetics & improvement: development of new disease-resistance varieties



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1. Abstract

Fungicide applications for the control of the two most important diseases of winegrapes in Australia, powdery mildew and downy mildew, could be significantly reduced, or even eliminated, by the breeding of new winegrape varieties with reduced susceptibility to these pathogens. Using marker assisted selection technology, in combination with the rapid flowering microvine mutant, we have successfully combined powdery and downy mildew resistance loci from the wild North American species *Muscadinia rotundifolia* and the wild Chinese grape species *Vitis romanetii* to produce 2nd generation mildew-resistant premium winegrape varieties with stronger and more durable resistance to mildews.

2. Executive summary

The Wine Australia Strategic Plan 2015-2020 identified 'Increasing Competitiveness' as a major priority to increase the prosperity of the Australian Wine Industry. One of the strategies identified to improve the performance of Australian vineyards was through the availability of new grapevine varieties that tolerate biotic stressors, generated through plant breeding programs, informed by molecular methods to identify desirable genotypic traits. Through previous co-investment projects with Wine Australia, CSIRO has successfully bred new elite white and red selections that are resistant to powdery and downy mildew. Selected lines of these 1st generation (gen.) mildew-resistant varieties are currently being evaluated for vine performance and wine quality at three regional sites as part of WA-CSIRO SRA project CSP 1701-1.6.

The 1st gen. mildew-resistant winegrape varieties contain a single gene conferring powdery mildew resistance (*Run1*) and a single gene conferring downy mildew resistance (*Rpv1*) introgressed from the wild North American grape species *Muscadinia rotundifolia*. Although these 1st gen. varieties have been shown to be highly resistant to powdery and downy mildew in unsprayed vineyard trials in Nuriootpa, Irymple and Wagga Wagga, we are aware that mildew isolates have been identified in other grape-growing countries that may break the resistance conferred by these genes. To ensure the durability of resistance in these new varieties, it is necessary to combine at least two different resistance genes (loci) within the same variety because the likelihood of the pathogens overcoming both resistance genes simultaneously is extremely low.

The wild Chinese grape species *V. romanetii* has been reported to contain a single dominant locus (*Ren4*) that confers strong resistance to powdery mildew. Our research has confirmed that powdery mildew isolates, that are capable of breaking the resistance conferred by *Run1,* are unable to overcome *Ren4*-mediated resistance making it a good candidate for 'pyramiding' with *Run1* in 2nd gen. mildew-resistant varieties. Furthermore, we have discovered that the *Ren4* powdery mildew resistance locus is co-located with a strong downy mildew resistance locus (designated Rpv_{rom}) in the *V. romanetii* genome. This means that the *Ren4* and Rpv_{rom} loci co-segregate at a high frequency, effectively acting as a single locus in breeding populations, as in the case for the *Run1*/*Rpv1* locus from *M. rotundifolia*.

On the basis of these discoveries, a breeding strategy (Strategy 1) was designed based on crosses between previously generated 1st gen. mildew-resistant varieties containing the *Run1*/*Rpv1* locus and microvine breeding lines into which the *Ren4*/Rpv_{rom} locus from *V. romanetii* had been introgressed through a number of rounds of backcrossing. Using marker-assisted selection (MAS) we were able to screen over 11,000 seedlings to identify over 400 new 2nd gen. mildew-resistant varieties that were not only positive for both the *Run1*/*Rpv1 and Ren4*/Rpv_{rom} mildew resistance loci but also show four different types of berry characters - red skin, red flesh (tenturier), white skin and white skin with muscat character. All 2nd gen. varieties were successfully established in an unsprayed block of the SARDI vineyard in Nuriootpa for evaluation of viticultural and wine sensory properties.

Simultaneously with Strategy 1, an alternative breeding strategy (Strategy 3) was undertaken based on the use of a CSIRO female breeding line (15C002V0002) which is homozygous for the *Run1/Rpv1* locus which was crossed with *Ren4*/Rpv_{rom} microvine breeding lines. A total of 103 new 2nd gen. mildew-resistant winegrape varieties were selected using MAS from over 2,700 seedlings using breeding Strategy 3. However, approximately one third of the 2nd gen. varieties generated via

Strategy 3 was found to exhibit an unusual phenotype in the vineyard, characterised by very large numbers of bunches per vine and flowers per bunch and severely inhibited shoot elongation making them unsuitable for commercial wine production.

To expand the genetic (varietal) diversity of the 2nd gen. mildew-resistant varieties, a revised breeding strategy (Strategy 2) was devised which utilised female breeding lines containing both the *Run1*/*Rpv1 and Ren4*/*Rpv$_{rom}$* resistance loci that had been generated from Strategy 1 and Strategy 3 crosses. The availability of these new female 2nd gen. breeding lines enabled the use of any premium winegrape variety as a parent in the generation of mildew-resistant 2nd gen. progeny. Using Strategy 2, an additional 172 new 2nd gen. mildew-resistant winegrape varieties with either red skin, red flesh, white skin or white skin with muscat character were identified by MAS from the screening of over 4,000 seedlings. Significantly, these additional 172 varieties derived from crosses with a more diverse range of premium winegrape varieties that were not represented Strategy 1 or Strategy 3. To date, 65 Strategy 2 lines have been established in the SARDI vineyard with the aim to plant the remaining 107 lines in late 2022.

The enhanced resistance of 2nd gen. mildew-resistant varieties to powdery mildew and downy mildew, in comparison to 1st gen. mildew-resistant varieties, was demonstrated under identical conditions in laboratory assays. Furthermore, to the best of our knowledge these are the only winegrape varieties available anywhere in the world that combine the *Run1*/*Rpv1* resistance locus from *M. rotundifolia* and the *Ren4*/*Rpv$_{rom}$* resistance locus from *V. piasezkii*.

Fruit was harvested from a limited number of two and three year old 2nd gen. mildew-resistant varieties in season 2021/22. Harvest dates ranged from 25 February to 22 April and varieties displayed a range of bunch architectures from tight to loose. Sensory analysis undertaken on micro-scale ferments of fruit harvested from thirty-three 2nd gen. mildew-resistant varieties compared very favourably to wine made from five premium varieties sampled from the adjacent SARDI germplasm collection and fermented in the same way. Indeed, the average hedonic scale score calculated from a panel of four tasters, for wine from 28 of the 33 2nd gen. varieties was ranked equal or higher than the lowest-ranked premium variety and four 2nd gen. varieties produced wine of equal or higher preference to the highest ranked premium variety.

An additional aim was also initiated in Year 2 of the project to generate female breeding lines for use in future scion breeding projects that contain three different powdery mildew resistance loci (*Run1, Ren4 & Ren7*), together with the two downy mildew resistance loci (*Rpv1 & Rpv$_{rom}$*). The *Ren7* powdery mildew resistance locus from *V. piasezkii* was successfully introgressed into a microvine breeding line and then combined with the *Run1*/*Rpv1* and *Ren4*/*Rpv$_{rom}$* resistance loci. A total of 12 female 3rd gen. (*Run1*/*Ren4*/*Ren7*/*Rpv1*/*Rpv$_{rom}$*) breeding lines were successfully identified by MAS. In addition, all of these 3rd gen. breeding lines are positive for the muscat locus and four lines also contain the red-flesh locus which provide more options for future breeding projects. Four 3rd gen. microvine breeding lines commenced flowering before the end of the current project and were used in crosses with pollen from premium winegrape varieties to generate seven new 3rd gen. mildew- resistant varieties for future field evaluation.

The results of this project demonstrate the power of combining marker-assisted selection technology with the rapid breeding microvine mutant, to generate premium winegrape varieties, in a reasonably short period of time, which are essentially *V. vinifera*-like in growth and sensory characteristics but contain new highly desirable production and quality traits.

3. Background

The Australian wine industry is currently dominated by European cultivars that have little or no genetic resistance to powdery and downy mildew. As a result, grape producers rely on the frequent use of agrochemicals to minimise the potentially devastating impact of these pathogens on grape yield and quality. It is estimated that the cost of disease management and yield loss to the Australian wine industry (adjusted to 2020 figures) is in the order of A$92 million and A$76 million per year for powdery mildew and downy mildew, respectively (Scholefield and Morison 2010). Not only does this translate into increased production costs for growers, but there is also the potential impact of these chemicals on the health of beneficial organisms in the vineyard (Gadino et al. 2011) and vineyard workers (Le Moal et al. 2014), as well as increased carbon emissions generated from their frequent application. While the use of older contact fungicides based on sulfur and copper remain effective, the development of powdery mildew and downy mildew isolates with resistance to systemic fungicide chemistries introduced since the 1960s, presents a management problem in some viticultural regions in Australia (Hall 2017). The breeding of new winegrape varieties with genetic resistance to these two important pathogens would dramatically reduce the dependence on chemical inputs, leading to significant financial, health and environmental benefits for the Australian wine industry.

Previous Wine Australia-funded projects CSP 0904 and CSP 1303 have resulted in the development of new 1st generation (gen.) mildew-resistant winegrape varieties that contain a single gene conferring powdery mildew resistance (*Run1*) and a single gene conferring downy mildew resistance (*Rpv1*) introgressed from the wild North American grape species *Muscadinia rotundifolia*. Due to their close proximity in the genome, the *Run1* and *Rpv1* genes are inherited as a single locus (Feechan et al. 2013) and this *Run1*/*Rpv1* locus was introgressed into *Vitis vinifera* through a series of backcrosses undertaken by French breeder Alain Bouquet, working for the French National Institute for Agricultural Research (INRA) (Pauquet et al. 2001, Merdinoglu et al. 2003). In the case of powdery mildew, the resistance conferred by the *Run1/Rpv1* locus is found to be qualitative in most *V. vinifera* backgrounds with little or no hyphal development or sporulation, whereas the resistance against downy mildew can be considered as quantitative, typically reducing downy mildew sporulation by 70–80% under optimal infection conditions in laboratory assays (Feechan et al. 2013). Even so, the *Run1*/*Rpv1* locus is still capable of conferring strong resistance to downy mildew under field conditions (Dry et al. 2019).

The 1st gen. breeding program was based on crosses between a female backcross 5 *Run1*/*Rpv1* breeding line (VRH3294) (Pauquet et al. 2001) with a genome that is >98% *V. vinifera*, and a selection of premium red and white winegrape varieties. The 20 best performing 1st gen. red and white selections selected from over 1200 progeny planted in the Barossa Valley, based on yield, good acid at harvest and consistently high sensory scores of single vine micro-ferments, were then planted in replicated trials in the warm/hot regions of Irymple, Victoria and Wagga Wagga, NSW as well as the cooler region of Orange, NSW for further evaluation.

The initial performance of the 1st gen. white selections in Wagga Wagga was previously outlined in the final report of Wine Australia-funded project CSP 1402 'Evaluating and demonstrating new disease resistant varieties for warm irrigated regions' and a more comprehensive evaluation of the viticultural performance and sensory characteristics of the 20 best 1st gen. red and white selections and from the replicated trials in Irymple, Wagga Wagga and Orange, will be provided in the final report of Wine Australia-funded project CSA 1701-1.6 'Regional evaluation of new germplasm -

pathway to adoption'.

Pathogens such as powdery mildew and downy mildew are capable of undergoing rapid mutation in the field to produce new isolates that are capable of breaking the resistance conferred by single resistance genes. This has already been reported for the grapevine downy mildew resistance gene *Rpv3* in Europe (Peressotti et al. 2010). Thus, there is now increasing focus in winegrape and table grape breeding programs around the world on the development of mildew-resistant grape varieties with more durable resistance in the vineyard.

One of the most effective ways to increase the durability of resistance genes in the field is to combine or 'pyramid' resistance genes from different wild species, within the same plant (Mundt 2018). Generally, resistance proteins encoded by resistance genes from different sources, have evolved to recognise specific effector proteins secreted by invading pathogens and switch on plant defence mechanisms that stop infection. However, if this pathogen effector undergoes mutation in the field, it may no longer be recognised by the plant resistance protein and infection can occur. In this case the new pathogen isolate is said to have broken the resistance conferred by the resistance gene/locus.

By pyramiding resistance genes from different wild species, within the same variety, the likelihood of a single pathogen isolate undergoing simultaneous mutation of the different effectors recognised by the two different resistance proteins, is extremely low. Thus, many winegrape and table grape breeding programs around the world in France (Merdinoglu et al. 2018), Germany (Eibach et al. 2007), Italy (Cipriani et al. 2019), Chile (Agurto et al. 2017) and Brazil (Sánchez-Mora et al. 2017) are currently developing new mildew-resistant varieties containing at least two powdery mildew and two downy mildew resistance loci.

It has long been recognised that wild North American wild grapevine species represent an important source of resistance against a range of major grapevine pathogens. More recently however, it has become clear that many wild Chinese *Vitis* species also contain major resistance loci that confer strong resistance against powdery mildew and downy mildew, making them good candidates for pyramiding with the *Run1*/*Rpv1* locus (Dry et al. 2019).

Ren4 has been successfully introgressed into *V. vinifera* from the wild Chinese *Vitis* species *V. romanetii* and shown to segregate as a single dominant locus (Ramming et al. 2011; Mahanil et al. 2012). *Ren4* resistance was initially reported to be associated with high levels of penetration resistance and did not appear to be dependent on the induction of PCD (Ramming et al. 2011) suggesting it may have a different mode of action to the resistance conferred by *Run1*.

Another wild Chinese grapevine species, *V. piasezkii*, has also been shown to contain at least two powdery mildew *R* loci, designated *Ren6* and *Ren7*, on chr 9 and 19 respectively, which mediate a PCD-based resistance response (Pap et al. 2016). The *Ren6* resistance response was found to be even stronger than that mediated by the *Run1* locus, whereas the resistance response of *Ren7* genotypes was much slower than *Ren6,* resulting in a high percentage of penetrated epidermal cells in which either no PCD is observed or the PCD induction can be considered ineffective because the fungus is still able to produce a secondary hyphae (Pap et al. 2016). However, recent observations in California suggest that *Ren6*-mediated powdery mildew resistance may be less durable in the field than *Ren7*-mediated resistance (Dr Summaira Riaz, personal communication), making *Ren7* a better candidate for pyramiding strategies.

4. Project Aims and Performance targets

The main aim of this project was to breed 2nd gen. mildew-resistant scions combining powdery mildew and downy mildew resistance loci from different wild grapevine species to further enhance the strength and durability of mildew resistance in the vineyard. Progeny that carry two powdery mildew (*Run1, Ren4*) and two downy mildew (*Rpv1 and Rpv12 or Rpv$_{rom}$*) resistance loci will be selected by marker-assisted selection and transferred to the field for evaluation of viticultural performance and fruit (wine) quality.

The second aim of the project was to develop female breeding lines containing both powdery mildew (*Run1, Ren4*) and downy mildew (*Rpv1, Rpv12 or Rpv$_{rom}$*) resistance loci that could be used for the generation of scions in which disease-resistance traits are combined with desirable consumer traits from any susceptible premium winegrape variety.

Yr 1 (17/18)

	Output	Target date	Activities
A	2nd gen. disease resistant winegrapes with stacked mildew resistance loci (*Run1/Ren4/Rpv1/Rpv$_{rom}$*).	30/6/18	• Continue MAS of *Run1/Ren4/Rpv1/Rpv$_{rom}$* lines from crosses initiated in CSP 1301 until 8-10 flowering lines established. • Collect pollen from selected 1st generation RUN1/RPV1 lines (including red flesh, muscat character). • Commence pollination of *Run1/Ren4/Rpv1/Rpv$_{rom}$* female microvines with *Run1/Rpv1* pollen. Collect seed. • Commence embryo rescue and MAS with aim of selecting ~200 hermaphroditic talls containing *Run1/Ren4/Rpv1/Rpv$_{rom}$.*
B	2nd gen. disease resistant winegrapes with stacked mildew resistance loci (*Run1/Ren4/Rpv1/Rpv12*).	30/6/18	• Undertake selfing of selected *Ren4/Rpv12* microvine lines generated as part of CSP 1301. Collect seed. • Commence embryo rescue and undertake MAS to select *Run1/Ren4/Rpv1/Rpv12* lines to obtain 8-10 flowering lines.
C	Hermaphroditic microvines containing stacked resistance genes *Run1/Ren4/Rpv1/Rpv$_{rom}$* to be used to generate 3rd gen. disease resistant winegrape cultivars with desirable consumer traits	30/6/18	• Cross selected *Run1/Ren4/Rpv1/Rpv$_{rom}$* hermaphrodite microvine with *Run1/Rpv1* microvine. Collect seed. • Undertake embryo rescue and MAS to select 5-6 hermaphroditic microvines containing *Run1/Ren4/Rpv1/Rpv$_{rom}$*. Transfer selected lines to pots and allow to self.

Yr 2 (18/19)

	Output	Target date	Activities
A	2nd gen. mildew-resistant winegrapes with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1)* and Muscat flavour (*Mf1*) using selected 1st gen. (*Run1*/*Rpv1*) mildew resistant vines as pollen parents (STRATEGY 1)	30/6/19	• Continue pollination of *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* female microvines with pollen from selected 1st gen. mildew resistant (*Run1*/*Rpv1)* vines: ○ 37_14_11_04 (Red flesh) ○ 37_14_08_01 (Red flesh) ○ 39_18_06_02 (Muscat) ○ 39_17_03_03 (Muscat) ○ 39_17_09_01 (Muscat) • Continue embryo rescue & MAS with aim of selecting 250 fruiting talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* and segregating for red flesh and muscat characters for planting at Nuriootpa in 18/19.
B	2nd generation mildew-resistant winegrapes with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1*) and Muscat flavour (*Mf1*) using *REN4/ Rpv$_{rom}$ /Rf1* and *REN4/ Rpv$_{rom}$ /Mf1* genotypes as pollen parents (STRATEGY 3)	30/6/19	• Continue pollination of BC2 female tall *Run1/Run1/Rpv1/Rpv1* (15C002V0002) genotype with pollen from selected *Ren4/Rpv$_{rom}$ /Rf1* and *REN4/ Rpv$_{rom}$ /Mf1* hermaphrodite microvines. Collect seed. • Embryo rescue and MAS with aim of selecting 250 fruiting talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* and segregating for red flesh and muscat characters for planting at Nuriootpa in 2018/19.
C	Microvines containing stacked resistance genes *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* to be used to generate future generation disease resistant winegrape cultivars with desirable consumer traits (STRATEGY 4)	30/6/19	• Undertake embryo rescue and MAS to select at least five hermaphrodite microvines containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*. Transfer selected lines to pots and allow to self and collect seed. • Commence embryo rescue and MAS to selecting at least 5 hermaphrodite microvines homozygous for all four mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*)
D	3rd generation mildew-resistant winegrapes with three powdery mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1*) and Muscat flavour (*Mf1*) (STRATEGY 5)	30/6/19	• Pollinate microvines Vp003V006BC1-19, 18, 35 (containing *Ren7* powdery mildew locus from *V. piasezkii*) with pollen from picovine 06C008V0004. • Carry out embryo rescue and MAS to select VpBC2 female microvine progeny containing *Ren7* locus. • Transfer selected lines to pots to produce flowers for next cross with pollen from *Ren4/Rpv$_{rom}$ /Rf1* and *REN4/ Rpv$_{rom}$ /Mf1* hermaphrodite microvines generated in Activity B.

Yr 3 (19/20)

	Outputs	Target date	Activities
A	At least 100 2nd gen. mildew-resistant winegrape seedlings with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1)* and Muscat flavour (*Mf1*) using selected 1st gen. (*Run1*/*Rpv1*) mildew resistant vines as pollen parents (STRATEGY 1)	30/6/20	• Collect pollen from selected 1st generation mildew resistant (*Run1*/*Rpv1*) red-flesh and muscat vines in Nuriootpa. • Continue pollination of *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* female microvines. Collect seed. • Carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection using SNP panel.
B	At least 100 2nd gen. mildew-resistant winegrape seedlings with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$)* segregating for Red-flesh (*Rf1*) and Muscat flavour (*Mf1*) using *Ren4/Rpv$_{rom}$/Rf1* and *Ren4/Rpv$_{rom}$ /Mf1* and *Ren4/ Rpv$_{rom}$/Mf1* genotypes as pollen parents (STRATEGY 3)	30/6/20	• Ongoing collection of pollen from selected *Ren4/Rpv$_{rom}$/Rf1* and *Ren4/ Rpv$_{rom}$ /Mf1* hermaphrodite microvines. • Continue pollination of BC2 female tall *Run1/Run1/Rpv1/Rpv1* (15C002V0002) genotype. Collect seed. • Carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection using SNP panel.
C	2nd gen. mildew-resistant winegrapes with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$)* established in no-spray vineyards at SARDI Research station (Nuriootpa) and NSW-DPI research station (Orange).	30/6/20	• Propagate clonal copies of 2nd generation talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* in tissue culture. • Transfer in vitro plants to pots in glasshouse and grow to approx. 500 mm tall. • Plant seedlings in no-spray vineyards in Nuriootpa and Orange. • Training and pruning of 2nd generation selections planted in Nuriootpa in 18/19 season where necessary.
D	At least 10 microvines containing two powdery (*Run1/Ren4)* and two downy mildew (*Rpv1/ Rpv$_{rom}$)* resistance genes in homozygous state (STRATEGY 4).	30/6/20	• Continue to collect seed from selfing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* hermaphrodite microvines. • Carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection using SNP panel to select female and hermaphrodite microvines homozygous for *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* . • Establish microvines in GH to assess flowering potential.

E	2nd generation mildew-resistant winegrapes with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) established in no-spray vineyards at SARDI Research station (Nuriootpa) and NSW-DPI research station (Orange).	30/6/20	• Propagate clonal copies of 2nd generation talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* in tissue culture • Transfer in vitro plants to pots in glasshouse and grow to approx. 500 mm tall. • Plant seedlings in no-spray vineyards in Nuriootpa and Orange. • Training and pruning of 2nd generation selections planted in Nuriootpa in 18/19 season where necessary.
F	3rd gen. mildew-resistant winegrapes$^{\#}$ with three powdery mildew resistance loci (*Run1/Ren4/Ren7/Rpv1/ Rpv$_{rom}$*) segregating for Red-flesh (Rf1) and Muscat flavour (Mf1) (STRATEGY 5)	30/6/20	• Carry out embryo rescue on VpBC3 seeds and establish seedlings in tissue culture • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection to identify VpBC3 hermaphrodite microvines containing *Run1/Ren4/Ren7* and either Red-flesh or Muscat flavour. • Transfer selected lines to pots to produce flowers to collect pollen in preparation for cross with *Ren4/Ren4/Rpv$_{rom}$/Rpv$_{rom}$ /Run1/Run1/Rpv1/Rpv1* female microvines generated in STRATEGY 4

$^{\#}$ *Note: the 3rd generation is now being used to describe new varieties with three powdery mildew* resistance loci which is a new output added since the original Project plan was developed.

Yr 4 (20/21)

	Outputs	Target date	Activities
A	At least 150 new 2nd gen. mildew-resistant winegrape seedlings selected with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$)* (STRATEGY 1)	30/6/21	• Collect pollen from selected 1st generation mildew resistant (*Run1*/*Rpv1*) red skin, red-flesh and white varieties and use to pollinate *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* female microvines. • Collect pollen from selected *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* hermaphrodite microvines and use to pollinate emasculated 1st gen. mildew resistant (*Run1*/*Rpv1*) red skin, red-flesh and white varieties. • Collect seed and undertake embryo rescue to establish seedlings in tissue culture • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection using SNP panel.
B	2nd gen. mildew-resistant winegrape seedlings selected with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) established and maintained in no-spray vineyards at SARDI Research station (Nuriootpa).	30/6/21	• Propagate clonal copies of 2nd generation talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* in tissue culture. • Transfer in vitro plants to soil pots in glasshouse and harden off. • Grow to approx. 500 mm tall in flood tanks. • Plant seedlings in no-spray vineyards in Nuriootpa • Continue training and pruning of 2nd gen. selections previously planted at Nuriootpa.

C	Female/hermaphrodite microvines and talls containing stacked mildew resistance loci (*Run1*/*Rpv1*) and (*Ren4*/*Rpv$_{rom}$*) in homozygous state (STRATEGY 4).	30/6/21	• Complete genotyping of seedlings generated from selfing of *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* hermaphrodite microvines looking for genotypes with (*Run1*/*Rpv1*) and (*Ren4*/*Rpv$_{rom}$*) loci in homozygous state. • Continue to monitor previously selected genotypes with *R* loci in homozygous state for flowering potential. • Initiate new crosses involving parents containing RUN1/REN4/RPV1/RPV16 loci with different genetic backgrounds to try and increase recovery rates of female homozygous progeny. • Collect seed, carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA MAS using SNP panel. • Establish microvines in GH and plant talls in field at Nuriootpa to assess flowering potential. • Evaluate growth and flowering potential of previously generated Strategy 4 progeny. Propagate clonal copies of floriferous lines.
D	Microvines and talls with three powdery mildew resistance loci (*Run1/Ren4/Ren7*) and two downy mildew loci (*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1*) and Muscat flavour (*Mf1*) (STRATEGY 5)	30/6/21	• Carry out crosses between (*Ren4*/*Rpv$_{rom}$*/Ren7/*Rf1* or *Mf1* microvine parents and microvine parents containing (*Run1*/*Rpv1*) to generate breeding lines in which all 3 powdery mildew resistance loci have been combined. • Collect seed, carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection to identify microvines and talls containing three powdery mildew resistance loci *Run1/Ren4/Ren7/Rpv1/Rpv$_{rom}$* segregating for either Red-flesh or Muscat flavour. • Transfer selected lines to glasshouse or field to assess for vigour and flower production.

Yr 5 (21/22)

	Outputs	Target date	Activities
A	Approx 100 new 2nd gen. mildew-resistant winegrape seedlings with stacked mildew resistance loci.	30/6/22	• Collect pollen from an expanded range of premium susceptible red, red-flesh and white wine grape varieties and pollinate 2nd gen. *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* (a) female microvines and talls and (b) emasculated hermaphrodite microvines & talls. • Collect pollen from an expanded range of premium susceptible red, red-flesh and white wine grape varieties and pollinate 3rd gen. *Run1/Ren4/Ren7/Rpv1/Rpv$_{rom}$* (Strategy 5) female microvines. • Collect seed and undertake embryo rescue to establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA MAS using SNP panel.
B	Approx 100 2nd gen. mildew-resistant winegrape seedlings with stacked mildew resistance loci (*Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$*) planted in no-spray vineyards at SARDI Research station (Nuriootpa).	30/6/22	• Propagate clonal copies of 2nd generation talls containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* in tissue culture. • Transfer in vitro plants to soil pots in glasshouse and harden off. • Grow to approx. 500 mm tall in flood tanks. • Plant seedlings in no-spray vineyards in Nuriootpa. • Continue training and pruning of 2nd generation selections previously planted at Nuriootpa.
C	Evaluation of vine performance and wine quality of 2nd gen. mildew-resistant winegrape selections planted in no-spray vineyards.	30/6/22	• Organise installation of netting. • Monitor mildew susceptibility. • Collect viticultural data for Yr 3 fruiting vines e.g. bud burst date, flowering date, harvest date bunch number, bunch structure, berry wt, bunch wt, yield, pruning wt. • Collect berry chemical data at harvest using OenoFoss™. • Undertake micro-scale (~1kg) winemaking. • Undertake chemical analysis of wines using OenoFoss™. Evaluate wine sensory properties.
D	Female/hermaphrodite microvines and talls containing stacked mildew resistance loci (*Run1/Rpv1*) and (*Ren4/ Rpv$_{rom}$*) in homozygous state (STRATEGY 4).	30/6/22	• Continue embryo rescue on approx 900 seeds generated from crosses involving parents containing *Run1*/*Ren4*/*Rpv1*/*Rpv$_{rom}$* with different genetic backgrounds. • Undertake DNA extraction from leaf material sampled from seedlings and carry out DNA marker-assisted selection using SNP panel. Select lines which are female and homozygous for at least one of the resistance loci i.e. (*Run1/Rpv1*) or (*Ren4/Rpv$_{rom}$*) • Establish microvines in GH and plant talls in field at Nuriootpa to assess flowering potential. • Continue to evaluate growth and flowering potential of previously generated Strategy 4 progeny. Propagate clonal copies of floriferous lines.

E	Microvines and talls with three powdery mildew resistance loci (*Run1/Ren4/Ren7*) and two downy mildew loci (*Rpv1*/*Rpv$_{rom}$*) segregating for Red-flesh (*Rf1*) and Muscat flavour (*Mf1*) (STRATEGY 5).	30/6/22	• Continue crosses between (*Ren4*/*Rpv$_{rom}$*/Ren7/*Rf1* or *Mf1* microvine parents and microvine parents containing *Run1*/*Rpv1* to generate breeding lines in which all three powdery mildew resistance loci have been combined. • Collect seed, carry out embryo rescue and establish seedlings in tissue culture. • Sample leaf material and undertake DNA extraction. Carry out DNA marker-assisted selection to identify microvines and talls containing three powdery mildew resistance loci *Run1/Ren4/Ren7/Rpv1/Rpv$_{rom}$* segregating for either Red-flesh or Muscat flavour. • Transfer selected lines to glasshouse or field to assess for vigour and flower production.
F	Submission of final project report. Communication of results as applicable.	30/6/22	• Prepare final report. • Disseminate results contained in final project report to industry where possible e.g. AWRI Roadshows, industry seminars etc.

5. Methods

5.1 Microvine propagation and culture

5.1.1 Microvine propagation from cuttings

Microvine shoots were taken as semi-hard wood cuttings from mature plants and cut into small sections consisting of one node and one leaf. The cut section was dipped into plant rooting hormone (Gel Red; Growth Technology, WA, Australia) and placed into cardboard pots in a mixture of equal parts perlite and vermiculite. Cuttings were then placed into a misting propagation box (Sage Horticultural, Vic, Australia) for up to three weeks until roots could be seen growing out of the sides of the pot. Rooted cuttings were then potted up into soil and placed into the glasshouse to allow plants to mature.

5.1.2 De-flasking for glasshouse propagation

Microvines were sub-cultured into new tissue culture vessels and transfer into small soil pots after approximately four weeks. Tissue culture plants were carefully removed from the agar medium while minimising root damage and placed into the soil. To maintain humidity, a small plastic bag was placed over the plant and part of the pot, which was secured by a rubber band (Fig. 1). The plant was then transferred to the glasshouse under shade or in a plant growth room and allow to acclimatise by slowing removing the plastic bag over a three week period. Once the microvine had become established in the small pot, it was transplanted into a larger pot for maturation.

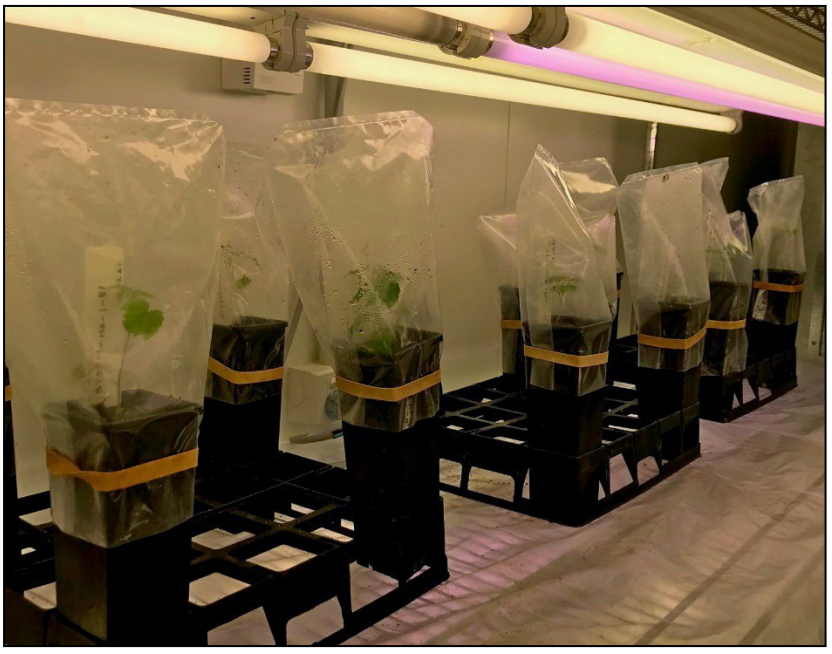


***Figure 1**. De-flasking of seedlings from tissue culture into soil. This step was usually done under a shade cloth in the glasshouse or in a plant growth room set at 24°C.*

5.2 Pollen collection and storage

All pollen from 1st gen. selections was collected from the SARDI vineyard, Nuriootpa. All varietal pollen was collected from two locations: Coombe Vineyard, Waite Campus, Urrbrae and SARDI vineyard, Nuriootpa. Pollen from the Turkish variety Bogazkére was collected from a vineyard at Lokum Wines, Dookie Vic. Grapevine inflorescences were ready to be picked when cap-fall was at approximately 50%. Flowers were then placed into small plastic containers, transported back to the laboratory and dried at 42 °C for a minimum of 24 h. Dried pollen was then left at room temperature for 15 min before being placed into plastic boxes containing gel orange desiccant. For long-term storage, pollen boxes were then placed into -80 °C freezers. Prior to use of frozen pollen, containers were removed from the -80 °C freezer and allowed to thaw for a minimum of 20 min at room temperature before being used for pollination.

5.3 Pollinations

Microvine pollinations were carried out in either glasshouses or growth rooms (Fig. 2). Female microvines were ready to be pollinated once flowers began to drop their caps (Fig. 3). Pollen was applied onto the stigmas with a small paintbrush. This was done twice a week until all inflorescences on a plant were pollinated. For field pollinations of female vines, inflorescences were bagged just before anthesis to prevent cross-pollination occurring (Fig. 4). Once cap-drop had reached 30%, either fresh or dried pollen was used to pollinate the flowers, and was repeated a couple of days later. Bags were removed a week after the last pollination. For pollination of hermaphrodite vines, inflorescences were emasculated, bagged and pollinated twice within seven days. Bags were removed a week after the last pollination.



Figure 2. *Growth room containing female microvines for pollination.*



***Figure 3**. Image of a female microvine inflorescence ready for pollination.*



***Figure 4**. Inflorescences on field vines bagged with paper or taffeta bags prior to anthesis to prevent cross-pollination.*

5.4 Seed germination

5.4.1 Seed collection and treatment

Pollinated grape bunches on both microvine and field vines were ready for harvest ~3 months after being pollinated (Fig. 5). Seeds were harvested from the berries by removing all pulp and skin. The outer membrane around the seed coat was also removed to improve germination by rubbing the seeds in a wire colander. Once seeds were cleaned, they were lightly coated in mancozeb fungicide before allowing them to dry at room temperature in petri dishes for a week. Seeds were surface-sterilised by shaking overnight in a fresh solution of 0.5 M hydrogen peroxide (8 mL per 10 seeds). Seeds were then rinsed four times in sterile water and transferred to a solution of 2.6 mM gibberellic acid (GA) (Sigma-Aldrich, St Louis, USA) where the GA solution was twice the volume of the seeds. After shaking overnight in the GA solution, seeds were rinsed four times in sterile water, placed into sterile 50 mL falcon tubes containing moist cotton wools plugs and stored at 4 °C.



Figure 5. *Female microvine breeding line with bunches following pollination.*

5.4.2 Embryo rescue

The recovery of microvine progeny from crosses involving microvine parents is poor without intervention to maximise seedling recovery. The embryo rescue method used was adapted from Chatbanyong and Torregrosa (2015). After a minimum period of three weeks in 4 °C cold storage, seeds were moved to a sterile laminar flow cabinet and split half along the ridge exposing the embryo using a scalpel blade. Seeds were then placed into petri dishes containing half-strength Murashige and Skoog (½MS) medium supplemented with activated charcoal (2.5 g/L) and the antibiotics Timentin (GoldBio, St Louis, USA) (200 mg/L) and Cefotaxime (Phytotech Labs, Kansas, USA) (200 mg/L). Petri dishes were sealed with Parafilm, covered with Alfoil and incubated at 27 ˚C. After approximately 10 days, seeds were exposed to low light to allow normal seedling development (Fig. 5). Once the cotyledons were fully emerged, seedlings were transferred to small tissue culture vessels containing ½MS for approximately three weeks. Plants destined for the glasshouse were sub-cultured onto rooting medium (Iocco et al. 2001) to initiate a more robust root system before transplanting into the glasshouse.



Figure 6*. Development of seedlings after 2 wks in the light on ½MS charcoal medium. This plate contains a mixture of tall and microvine seedlings.*

5.4.3 Germination on filter paper

Where only tall progeny were required to be recovered from a cross involving a microvine parent or where crosses did not involve a microvine parent, the application of the embryo rescue technique was not necessary. In these cases, seeds were germinated on sterile filter paper in petri dishes. After three weeks in cold storage, seeds were placed onto moist filter paper discs (Whatman No. 1) with approx. 30 seeds per petri dish (Fig. 7). To ensure seeds remained moist, four layers of Parafilm were used to seal plates before moving them to the growth room. Once seeds had germinated and seedlings had fully expanded cotyledons, they were moved to tissue culture vessels or directly to trays of FERTILPOT biodegradable pots (Fertil S.A.S., France) containing soil. These trays were then be placed in a plastic box (96 seedlings per box), covered in a plastic film and moved to a growth room 24 °C with 16 h of light (Fig. 8). Seedlings were kept humid for one week after transplanting by placing a plastic sheet on top of each box. Over the course of a week, the humidity was gradually decreased by lifting the sheet until it could be totally removed.



***Figure 7**. Grapevine seed germination on sterile filter paper.*



***Figure 8**. Seedlings germinated using sterile filter paper transferred to 96 well propagation trays.*

5.5 DNA marker analysis of seedlings

5.5.1 DNA extraction

DNA extractions were carried out on ~50 mg young leaf tissue by the Australian Genome Research Facility (AGRF). Fresh or frozen samples were sent in 96-well plates for processing. DNA was eluted in 60 µL of elution buffer and concentrations varied from 10-60 ng/µL.

5.5.2 Identification of Single Nucleotide Polymorphisms by Genotype-by-Sequencing

Genotype-by-sequencing (GBS) was used to identify novel Single Nucleotide Polymorphism (SNP) genetic markers linked to the *Ren7* locus. DNA was diluted to 10 ng/µL and sent to the Cornell University Biotechnology Resource Centre (Cornell University NY, USA). GBS analysis was undertaken on the two parents (*V. piasezkii* and picovine line 06C008V0003) used in the production of a *Ren7*-segregating population. After DNA sample preparation, genome sequencing was undertaken with an Illumina HiSeq 2000/2500. The raw sequence data were filtered and aligned to the grape reference genome - French-Italian Public Consortium (PN40024). SNPs were identified from the aligned tags using the TASSEL= GBS pipeline v3.0.166 (Glaubitz et al. 2014) and scored based on depth of coverage and genotypic statistics.

5.5.3 Single Nucleotide Polymorphism panel analysis

To identify plants with the desired traits of interest using marker-assisted selection (MAS), diluted DNA (1:10) was sent to AGRF for screening with two separate SNP panels; GrapeSet1.2 (Table 1) and Ren4.2 (Table 2) using the Agena Bioscience MassARRAY platform. SNP panel Grapeset1.2 enabled detection of the presence of the following loci: *Run1/Rpv1* powdery and downy mildew resistance, muscat flavour locus, flower sex locus and the red berry colour locus. The development of these SNP markers was described previously in Wine Australia projects CSP 0904 and CSP 1303. The Ren4.2 SNP panel was comprised of a selection of 11 SNPs covering the genomic region containing the *Ren4* and Rpv_{rom} loci as reported previously in Wine Australia Project CSP 1301.

5.5.4 Development of Cleaved Amplified Polymorphic Sequence markers for marker assisted selection of Ren4 progeny

Five new potential SNP markers associated with the *Ren4* locus were identified based on analysis of GBS data obtained as part of WA project CSP 1301. Genomic DNA from resistant line VrBC4-60 and susceptible line VrBC2-2 was extracted from approx. 20 mg leaf tissue according to the method of Zhang et al. (1998). PCR was carried out using MyTaq Red DNA Polymerase (Meridian Bioscience Inc., Ohio, USA) in a reaction mixture containing: 3 µL of 5x buffer, gDNA (10ng μl^{-1}), 0.7 µM forward primer, 0.7 µM reverse primer, 1.25 units DNA Taq polymerase and sterile water in a total volume of 15 µL. PCR cycling conditions were as follows: 95 ˚C for 1 min, followed by 34 cycles of 95 ˚C for 15 s, 60 ˚C for 15 s, 72 ˚C for 30 s. PCR products were electrophoresed on 1.2% agarose gel before fragments were excised. PCR fragments were purified using ISOLUTE II PCR and Gel Kit (Meridian Bioscience) according to the manufacturer's instructions. PCR products were ligated into the pDRIVE vector using the pDRIVE ligation kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. Ligation reactions (2 µL) were electroporated into DH5-alpha *E. coli* cells which were then plated onto Luria Broth (LB) Agar plates supplemented with 100 µM Ampicillin, 25 µM Isopropyl β-D-thiogalactoside and 40 µM 5-Bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside. Plates were left overnight at 37 °C. White colonies were then picked (six from each ligation) and inoculated into 4 mL LB supplemented with 100 µM Ampicillin and left shaking overnight 37 °C. Plasmid DNA was purified using the ISOLUTE II Plasmid Mini Kit as per manufacturer's instructions.

Positive clones were sequenced by Sanger sequencing (AGRF) with M13 primer. Sequences were analysed using the Geneious Prime program for editing and alignment to verify SNPs identified from GBS or any new potential SNPs for CAPS marker development. Sequence analysis confirmed the presence of SNPs and indicated those that could be converted into Cleaved Amplified Polymorphic Sequence markers. PCR products were subsequently amplified from genomic DNA extracted from progeny with and without the *Ren4* locus and digested with selected restriction enzymes to confirm that the restriction digest pattern was linked to the presence of the *Ren4* locus.

Table 1. *GrapeSet1.2 SNP panel used to screen 2nd gen. progeny for presence of loci associated with mildew resistance, muscat flavour, vine stature, flower sex and berry colour. The base highlighted in bold is linked to the trait.*

SNP ID	Genotype	SNP genotype	Grapevine trait
VVMT_14.1	***Run1/Rpv1****/run1/rpv1*	**A**/C	Resistant to PM & DM
	run1/rpv1/run1/rpv1	C/C	Susceptible to PM & DM
VVMT_16.2	***Run1/Rpv1****/run1/rpv1*	**A**/G	Resistant to PM & DM
	run1/rpv1/run1/rpv1	G/G	Susceptible to PM & DM
VVDXS_SNP5	***Dxs1****/dxs1*	**T**/G	Muscat flavour
	dxs1/dxs1	G/G	No Muscat flavour
VVMT_137.1	***H****/f*	**C**/A	Hermaphrodite
	f/f	A/A	Female
VVMT_66.2	***H****/f*	**A**/G	Hermaphrodite
	f/f	G/G	Female
VVMT_2.2	***B****/b*	**T**/C	Red (flesh/skin) berry
	b/b	C/C	White berry

5.5.5 Development of Cleaved Amplified Polymorphic Sequence markers for marker assisted selection of Ren7 progeny

Nine potential SNP markers associated with the *Ren7* locus were identified based on GBS data (Section 5.5.2). Genomic DNA from resistant line *Ren7* VpBC1_6 and the susceptible picovine parent 06C008V0003 was extracted from approx. 20 mg leaf tissue according to the method of Zhang et al. (1998). PCR reactions, cloning and sequencing analysis of amplified fragments were carried out as described in Section 5.5.4. Of the nine potential SNP markers analysed, two (Ren7_8941444 and Ren7_1086405) that subtended the *Ren7* locus were successfully converted into CAPS markers for detecting the presence of the *Ren7* locus.

5.5.6 Development of a red flesh-specific PCR marker

The PCR reaction was performed using the MyTaq HS Red DNA Polymerase, (Meridian Bioscience). Primer sequences were as follows: *VviMybA1* forward primer (CGTAATGTCCCATTCAGTGAG), *VviMybA1* reverse primer (CGGCATCTATTCAACCCTGC), *VviUbi* forward primer (AGTAGATGACTGGATTGGAGGT) and *VviUbi* reverse primer (GAGTATCAAAACAAAAGCATCG). Each primer was at a final concentration of 0.4 μM and 2.5 U Taq DNA polymerase was added per reaction with 20-60 ng of template in a reaction volume of 25 μL. The PCR amplification was performed using the following parameters 95 °C for 5 min followed by 95 °C 30 s, 60 °C 40 s, 72 °C 1 min for 35 cycles and then a final extension of 72 °C for 10 min. The DNA bands from PCR amplification were analysed by 1.2 % agarose gel electrophoresis in 0.5X TBE buffer.

***Table 2**. REN4.2 SNP panel used to screen 2nd gen. progeny for the presence of the Ren4/Rpv$_{rom}$ locus. Only progeny that were positive for all SNP markers were kept ensuring the whole locus was present. The base highlighted in bold is linked to the mildew resistance trait.*

SNP ID	Genotype	SNP genotype	Grapevine trait
Ren4_S18_20847957	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**G**/T	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	T/T	Susceptible to PM & DM
Ren4_S18_30305456	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**A**/G	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	G/G	Susceptible to PM & DM
Ren4_S18_30518780	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**T**/C	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	C/C	Susceptible to PM & DM
Ren4_S18_30597371	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**G**/T	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	T/T	Susceptible to PM & DM
Ren4_S18_30630529	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**G**/A	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	A/A	Susceptible to PM & DM
Ren4_S18_30960231	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**T**/C	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	A/A	Susceptible to PM & DM
Ren4_S18_31624834	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**T**/C	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	C/C	Susceptible to PM & DM
Ren4_S18_32016121	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**G**/C	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	C/C	Susceptible to PM & DM
Ren4_S18_32691612	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**T**/G	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	C/C	Susceptible to PM & DM
Ren4_S18_32838401	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**C**/A	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	C/C	Susceptible to PM & DM
Ren4_S18_33103639	***Ren4/Rpv$_{rom}$***/*ren4/rpv$_{rom}$*	**T**/G	Resistant to PM & DM
	ren4/rpv$_{rom}$/ren4/rpv$_{rom}$	G/G	Susceptible to PM & DM

5.6 Propagation of 2nd gen. progeny for field evaluation

Tissue culture seedlings with desired traits, as identified by MAS, were duplicated through in vitro sub-culture. Shoot tips were removed from an established plant and transferred to a new tissue culture vessel containing rooting medium (Iocco et al. 2001) to initiate a more robust root system before transplanting into the glasshouse. Once a sufficiently large root system had been established the plants were transferred to soil as outlined in section 5.1.2.

Once seedlings had been acclimatised in the glasshouse, they were transferred to full sun in flood tanks (Fig. 9). The flood tanks were filled daily with water that stands for 3 h before being emptied via a timer. Grapevines were maintained for a minimum of two months in the flood tank prior to planting in the field.

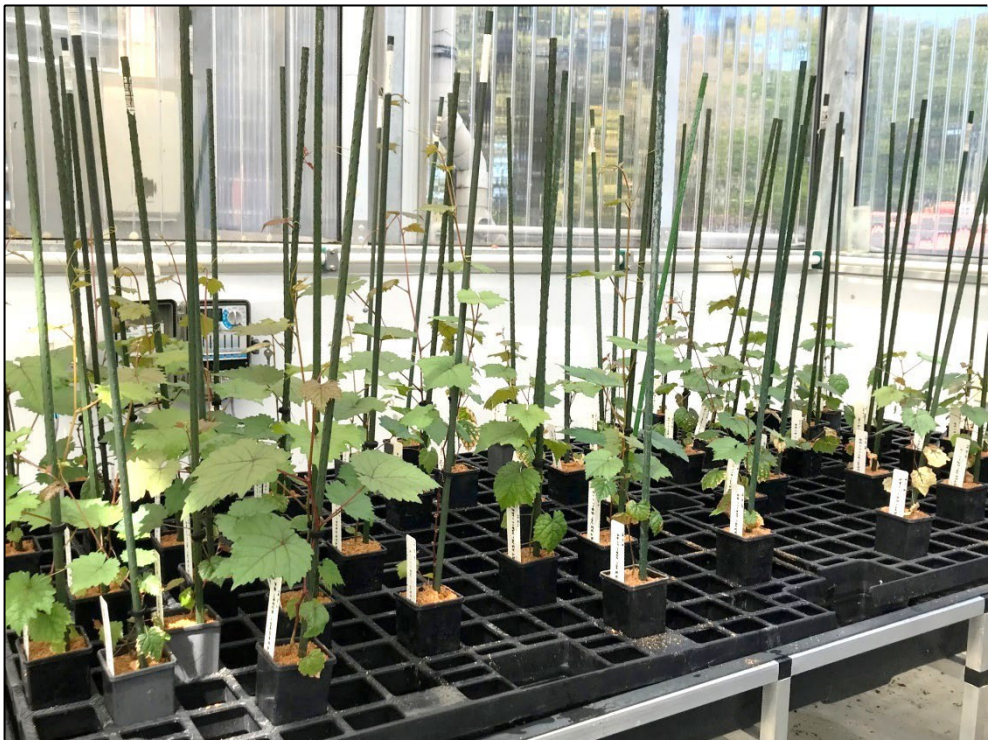


***Figure 9**. New 2nd gen. lines established in pots and growing in flood irrigated tanks in glasshouse until ready for planting in the field.*

All 2nd gen. and 3rd gen. vines were planted at the SARDI Research Station located in Nuriootpa. Field plantings were typically done from early summer to early autumn depending on when the plants were sufficiently mature. Vines were watered in with seaweed concentrate (Seasol, Vic, Australia) and protected with a plant guard for at least the first 12 months (Fig. 10). Where possible, new vines were trained to produce bilateral cordons, but in many cases, only unilateral cordons were produced. In these cases, the cordons were trained along the cordon wire and continued up to and along the catch wire. In the 2021/22 season the vines were also netted to protect the fruit from birds (Fig. 11).



Figure 10. *Planting out of new 2nd gen. mildew resistant varieties at SARDI Research Station, Nuriootpa.*



Figure 11. *Fruiting 2nd gen. mildew-resistant varieties protected by netting during fruit development in 2021/22 season.*

5.7 Pathogen infection assays

5.7.1 Powdery mildew

Erysiphe necator (isolate APC1) culture and inoculations were performed as previously described in Donald *et al*. (2002). Staining of fungal structures was performed as described previously (Koch and Slusarenko, 1990). Visualisation and scoring for programmed cell death (PCD) for at least 100 germinated powdery mildew conidia per leaf were performed as described previously (Feechan et al. 2011).

For detailed examination of *Ren4*-mediated resistance, detached leaves of the BC2-*Ren4* genotype were inoculated with spores from five different North American powdery mildew isolates: Musc4 and Musc5 collected from Georgia and LNYM, NY19 and NY1-137 collected from New York State (Feechan et al. 2015). Leaf samples were collected after 2 d for microscopic examination as described above.

5.7.2 Downy mildew

Plasmopora viticola inoculum was collected from an experimental vineyard on the Waite Campus, Adelaide and maintained on discs cut from *V. vinifera* cv. Cabernet Sauvignon leaves. Freshly cut leaf discs were placed onto moist filter paper in Petri dishes abaxial side up and inoculated with a 20 µL droplet of *P. viticola* suspension (5×10^4 sporangia/mL) overnight. After the droplet was removed, the leaf discs were maintained in a temperature-controlled incubator at 22-23 ˚C with a 16 h light / 8 h dark light cycle. A quantitative evaluation of sporulation was obtained 6 d after inoculation by measuring the number of sporangia per disc using a haemocytometer.

5.8 Vine management

Vine management was similar for both red and white selections. Fertiliser was applied once in spring and again near the end of the season. Vines were irrigated to a level to avoid water stress. The vines were sprayed against mites in spring. Weed control was conducted during the season with herbicide sprays in the vine row and the mid-row and frequent mowing.

5.9 Phenotyping and evaluation of field grown plants

Selected single vines were evaluated in the 2021/22 season for bunch number, date of berry maturity (22 °Bx), bunch architecture type, average bunch weight, average berry weight and total yield.

5.10 Berry and wine chemical analysis

5.10.1 Berry chemical analysis

Thirty berries were randomly picked from bunches. The berries were crushed in a plastic bag and 2 mL of must was transferred to a 2 mL Eppendorf tube and centrifuged for 1 min @ 13,000 rpm. Approximately 0.6 mL of the supernatant was analysed using an OenoFoss™ analyser for Brix, pH, total acidity, volatile acidity, alpha amino nitrogen, ammonia, tartaric acid, malic acid and density.

5.10.2 Wine chemical analysis

Approximately 1 mL of final wine was collected during winemaking and transferred into a 2 mL Eppendorf tube and centrifuged for 1 minute @ 13,000 rpm. Approximately 0.6 mL of the supernatant was analysed using an OenoFoss™ analyser for ethanol, pH, total acidity, volatile acidity, malic acid, wine density and glucose/fructose.

5.11 Micro-scale winemaking

Grape bunches were harvested at 22 °Bx or greater. Around 1.5 - 2 kg of grape bunches were transferred to a press seal bag (305 mm x 405 mm) with a tablespoon of dry ice and 1.2 mL potassium metabisulfite 100 mg/mL solution (based on 50 ppm for 60 % juice recovery). Berries were squeezed until all were disrupted and free running juice was visible. The juice was strained through a kitchen sieve and centrifuged for 2 min @ 1489 RCF to remove solids. Exactly 500 mL of juice was removed from the centrifuge bottle and transferred to a 500 mL Schott bottle with an airlock and silicon sampling septum. The juice was temperature adjusted for 1- 2 h before adding yeast, DAP and PVPP. The juice pH was measured and adjusted to pH 3.5 with tartaric acid. Must parameters were measured with an OenoFoss™ analyser and sugars were analysed using the Megazyme D-fructose/D-glucose Assay kit (Deltagen Catalogue # KFRUGL). The juice was inoculated with 10 mL of an overnight yeast culture (Maurivin PDM Yeast), 1 mL of 476 mg/mL DAP stock (200 ppm YAN) and 1 mL of PVPP 130 mg/mL (260 ppm PVPP). The juice was fermented at 18 °C, with 2 min shaking at 100 rpm every 2 h. Total sugars were measured every day. When the sugar concentration reached 2.5 g/L the wine was racked by transferring into a clear wine bottle, using a siphoning device under argon pressure with 500 µL copper sulfate (1 mg/mL) and 500 µL PMS (100 mg/mL) and left to settle for 7 d at 4 °C. The headspace was minimised by filling to the top with marbles. After cold settling, the wine was filtered through a 0.45 µm autoclaved ground water filter (Air-Met FTH-45) by using argon gas to push it into a combination of 200 mL, 100 mL and 50 mL amber bottles (Cospak). The bottles were sealed with Tampertell cap cello wadded caps (Cospak) and then sealed with wax. Finished wine analysis was done for each wine using the OenoFoss™ analyser. Wines were stored at 16 °C.

6. Results/Discussion

6.1 Further characterisation and fine mapping of the *Ren4* and *Rpv$_{rom}$* resistance loci from *V. romanetii*

6.1.1 Ren4-mediated powdery mildew resistance

Powdery mildew resistance conferred by *Ren4* was initially reported by Ramming et al. (2011) to be associated with high levels of penetration resistance and did not appear to be dependent on the induction of programmed cell death (PCD) as is the case for *Run1*-mediated powdery mildew resistance. To investigate this hypothesis, we collaborated with Dr Lance Cadle-Davidson (ARS, Cornell) to examine in more detail the mechanism of action of *Ren4*-mediated powdery mildew resistance and to test the isolate specificity of this resistance. A BC2-*Ren4* breeding line was inoculated with five different powdery mildew isolates collected in North America including the Musc4 and Musc5 isolates which had been shown previously to break *Run1*-mediated resistance (Feechan et al. 2015). The results of Figure 12 show that *REN4*-mediated powdery mildew resistance occurs post-penetration and may involve two different mechanisms; penetrated epidermal cells either undergo PCD or the haustoria becomes encased in callose thereby effectively blocking nutrient uptake (Fig. 12, panel b). The results also confirm that *Ren4* resistance is not broken by the Musc4 or Musc5 isolates that break *Run1*-mediated resistance (Fig. 12, panel a), confirming the suitability of the *Ren4* locus for pyramiding with the *Run1* locus in 2nd gen. mildew resistant varieties.

6.1.2 Fine mapping of the Ren4 and Rpv$_{rom}$ loci

In the previous Wine Australia project CSP 1301 we mapped the locations of the *Ren4* powdery mildew resistance locus and the *Rpv$_{rom}$* (formerly designated *Rpv16* in CSP 1301) downy mildew resistance locus from *V. romanetii* to adjacent regions on chromosome 18. Based on the information we had at the time, the relative size of these regions were ~1.65 Mbp for *Ren4* and ~1.1 Mbp for *Rpv$_{rom}$*. To improve the resolution of our mapping and, in so doing, identify genetic markers that would be more reliable during the marker-assisted selection (MAS) process, an additional two breeding populations were screened that had different sets of parents to the population used in CSP 1301. This included progeny obtained from the VrBC4 selfed cross generated as part of Strategy 4 (Section 6.4.1) and VrVa progeny obtained from a cross between VrBC4 and VaBC2 (a breeding line derived from a *V. amurensis* x microvine cross previously described in CSP 1301).

Screening lines from the selfed VrBC4 and VrVa populations, identified another seven lines which had new recombination points within this region that were informative in relation to the position of the *Ren4* and *Rpv$_{rom}$* loci on Chr. 18 (Fig. 13). Based on these data, the genomic region on Chr. 18 containing *Ren4* has been refined to ~0.67 Mbp and the region containing *Rpv$_{rom}$* to ~0.325 Mbp.

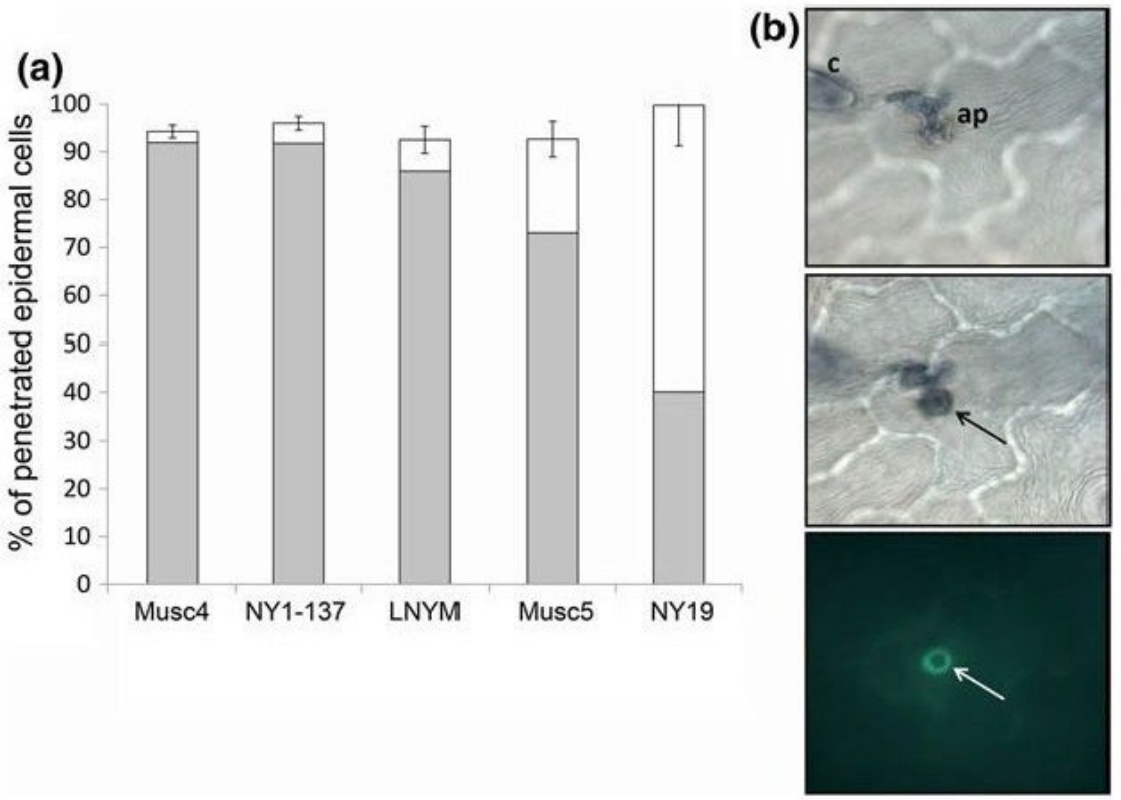


Figure 12. *Ren4-mediated resistance involves the induction of both programmed cell death and callose encasement of the haustorial complex. (a) results are expressed as the percentage of successful infections that resulted in either PCD induction (grey bar) or callose deposition (white bar) (b) photographs of infection by NY19 isolate. Top panel shows germinated conidium (c) and appressoria (ap) on the surface of the leaf. Middle panel is focussed below the appressorium to show the globular papillum (arrow). Bottom panel is the same view as middle panel but viewed using a blue light filter set to visualise callose deposition around the haustorial complex. Reproduced from Dry et al. (2019)*

***Figure 13**. Mapping the relative locations of the Ren4 powdery mildew resistance locus and the Rpv$_{rom}$ downy mildew resistance locus from V. romanetii. Screening of additional lines (marked with an asterisk) has identified 7 new recombinants that has allowed the region containing the Ren4 and Rpv$_{rom}$ to be further refined (highlighted with dotted lines) from that presented in WA final report CSP 1301 (coloured blocks at bottom of table).*

	PM	DM	Ren4_S18_20847957	Ren4_S18_21139144	Ren4_S18_26434758	Ren4_S18_30305456	Ren4_S18_30518780	Ren4_S18_30597210	Ren4_S18_30597371	Ren4_S18_30630529	Ren4_S18_30664367	Ren4_S18_30665201	Ren4_S18_30960231	Ren4_S18_31112827	Ren4_S18_31119885	Ren4_S18_31624834	Ren4_S18_31821750	Ren4_S18_31897588	Ren4_S18_32016121	Ren4_S18_32691612	Ren4_S18_32838401	Ren4_S18_33043251	Ren4_S18_33103639	Ren4_S18_33370745	Ren4_S18_340005468	Ren4_S18_34222141	Ren4_S18_34287210	Ren4_S18_34452432
Vr003V010BC4-8	R	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vr003V010BC4-53	R	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Vr003V010BC4-55	R	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Vr003V010BC4-7	R	R	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vr003V010BC4-60	R	R	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VrVa Her -7*	R	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
VrBC4S 40T_21_2*	S	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Vr003V010BC4-47	S	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
VrBC4S45_115*	S	R	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vr003V010BC4-25	S	S	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vr003V010BC4-50	S	S	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VrBC4S15_61*	S	R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Vr003V010BC4-29	R	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
VrBC4S30_71*	R	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
VrVaS-55*	S	n.t.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Vr003V010BC4-35	S	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Vr003V010BC4-37	S	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Vr003V010BC4-3	S	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VrVaS-127*	R	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-

Previously mapped region of *Rpv $_{rom}$*

Previous mapped region of *Ren4*

6.2 Breeding and selection of 2nd gen. mildew-resistant winegrape varieties using 1st gen. varieties as parents

6.2.1 Breeding Strategy 1

The steps involved in the breeding and selection of 2nd gen. mildew-resistant winegrape varieties using Strategy 1A are outlined in Figure 14. Hermaphrodite VrBC4 (*Ren4*/*Rpv*$_{rom}$) lines generated in Wine Australia project CSP 1301 were allowed to undergo self-pollination and seeds were screened by MAS to select for female microvines that were homozygous for the *Ren4*/*Rpv*$_{rom}$ locus. Using a parent that is homozygous for the *Ren4*/*Rpv*$_{rom}$ locus ensured that all 2nd gen. progeny would inherit this locus. A total of 17 female homozygous (*Ren4*/*Ren4*/*Rpv*$_{rom}$/*Rpv*$_{rom}$) microvine VrBC4S lines were selected by MAS and evaluated for flowering potential.

Flowering in many of the selected VrBC4S lines was poor and/or slower than anticipated. Indeed, the unexpected delay in flowering of the VrBC4S female lines led to the initiation of an alternative breeding strategy (Strategy 3) as described in Section 6.2.2. Seven VrBC4S female microvine lines were eventually selected based on flowering potential and propagated to produce approximately 100 VrBC4S breeding lines. These were pollinated with pollen collected from a selection of 1st gen. mildew-resistant varieties that had shown superior performance in relation to vine vigour and winemaking qualities (Table 3). The 1st gen. parents used as pollen donors in Strategy 1A crosses included with red flesh (Dunkelfelder), red skin (Shiraz, Tannat, Tempranillo), white skin (Riesling, Verdehlo) and white skin with muscat character (Frontignac) varieties (Table 3). Reciprocal crosses (Strategy 1B) were also carried out (Fig. 15) involving the pollination of selected emasculated 1st gen. varieties planted in the field, with pollen from homozygous hermaphrodite *Ren4*/*Rpv*$_{rom}$ microvines (Table 4).

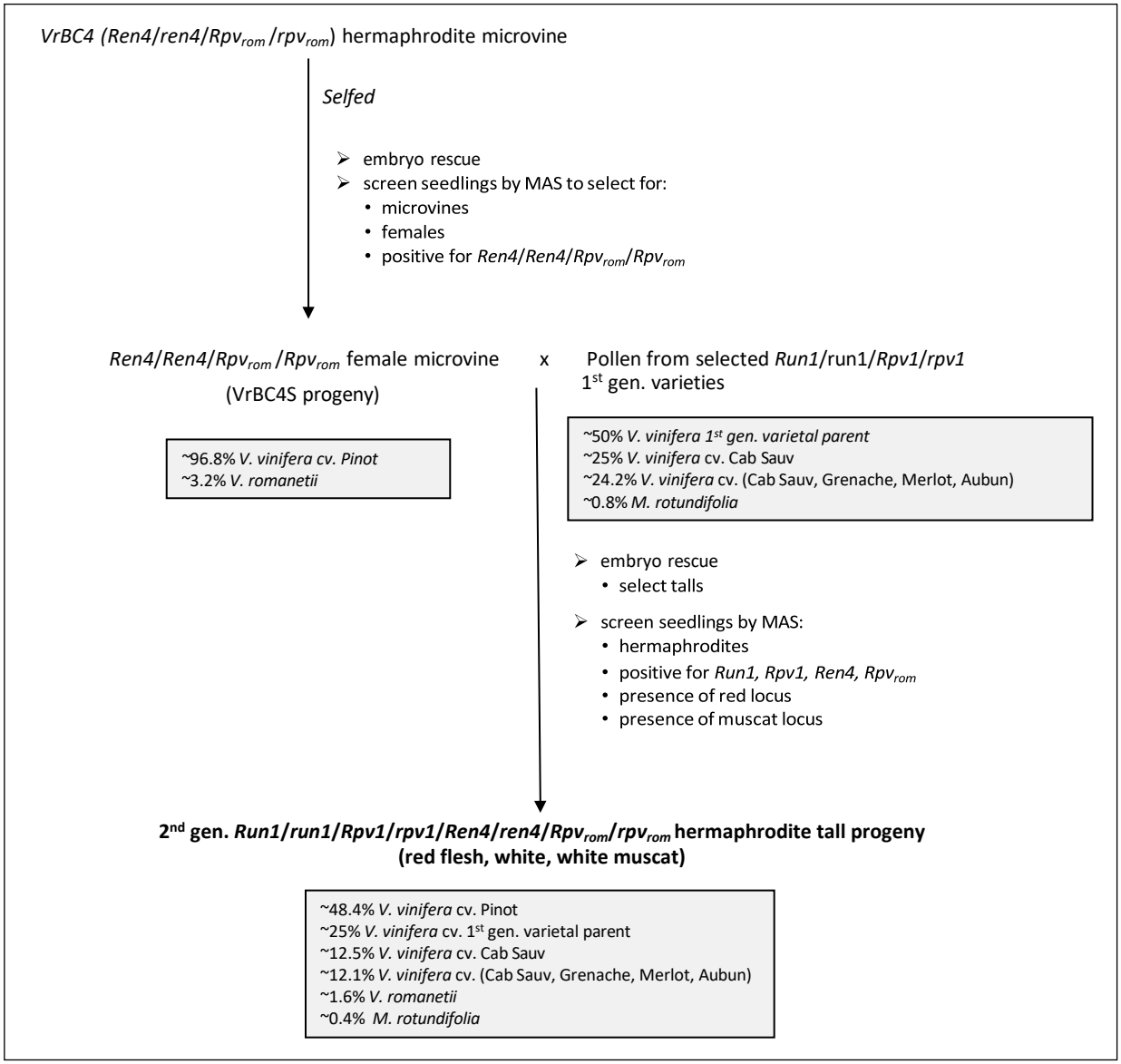


Figure 14. *Breeding Strategy 1A. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is also shown.*

***Table 3**. List of female breeding line/pollen source combinations used in Strategy 1A.*

Female (microvine)	Pollen donor (1st gen. variety)	1st gen. varietal parent
VrBC4S 40-138	39_08_05_01 (white)	Chardonnay
	39_08_05_02 (white)	Chardonnay
	37_14_05_02 (red flesh)	Dunkelfelder
	37_14_06_01 (red flesh)	Dunkelfelder
	37_14_08_01 (red flesh)	Dunkelfelder
	37_14_11_04 (red flesh)	Dunkelfelder
	37_14_12_02 (red flesh)	Dunkelfelder
	39_17_09_01 (muscat)	Frontignac
	39_18_06_02 (muscat)	Frontignac
	39_11_10_02 (white)	Riesling
	39_12_05_02 (white)	Riesling
	39_12_10_03 (white)	Riesling
	37_17_12_02 (red skin)	Shiraz
	37_25_03_04 (red skin)	Tannat
	37_25_06_03 (red skin)	Tannat
	37_24_07_01 (red skin)	Tannat
	37_24_11_01 (red skin)	Tannat
	37_16_09_04 (red skin)	Tempranillo
	39_16_06_03 (white)	Verdelho
	39_16_07_02 (white)	Verdelho
VrBC4S 42-75	37_14_05_02 (red flesh)	Dunkelfelder
	37_14_06_01 (red flesh)	Dunkelfelder
	37_14_08_01 (red flesh)	Dunkelfelder
	37_14_11_04 (red flesh)	Dunkelfelder
	37_14_12_02 (red flesh)	Dunkelfelder
	39_17_03_03 (muscat)	Frontignac
	39_17_09_01 (muscat)	Frontignac
	39_11_10_02 (white)	Riesling
	39_12_10_03 (white)	Riesling
	37_24_11_01 (red skin)	Tannat
VrBC4S 40-120	37_14_05_02 (red flesh)	Dunkelfelder
	37_14_08_01 (red flesh)	Dunkelfelder
	37_14_11_04 (red flesh)	Dunkelfelder
	39_17_09_01 (muscat)	Frontignac
	39_18_06_02 (muscat)	Frontignac
	39_12_10_03 (white)	Riesling
	37_17_12_02 (red skin)	Shiraz
	39_16_06_03 (white)	Verdelho
VrBC4S 40-173	37_14_11_04 (red flesh)	Dunkelfelder
	39_17_09_01 (muscat)	Frontignac
VrBC4S 42-46	37_14_11_04 (red flesh)	Dunkelfelder
	39_17_09_01 (muscat)	Frontignac
VrBC4S 42-63	37_14_08_01 (red flesh)	Dunkelfelder
	37_14_11_04 (red flesh)	Dunkelfelder
VrBC4S 40-130	37_14_11_04 (red flesh)	Dunkelfelder

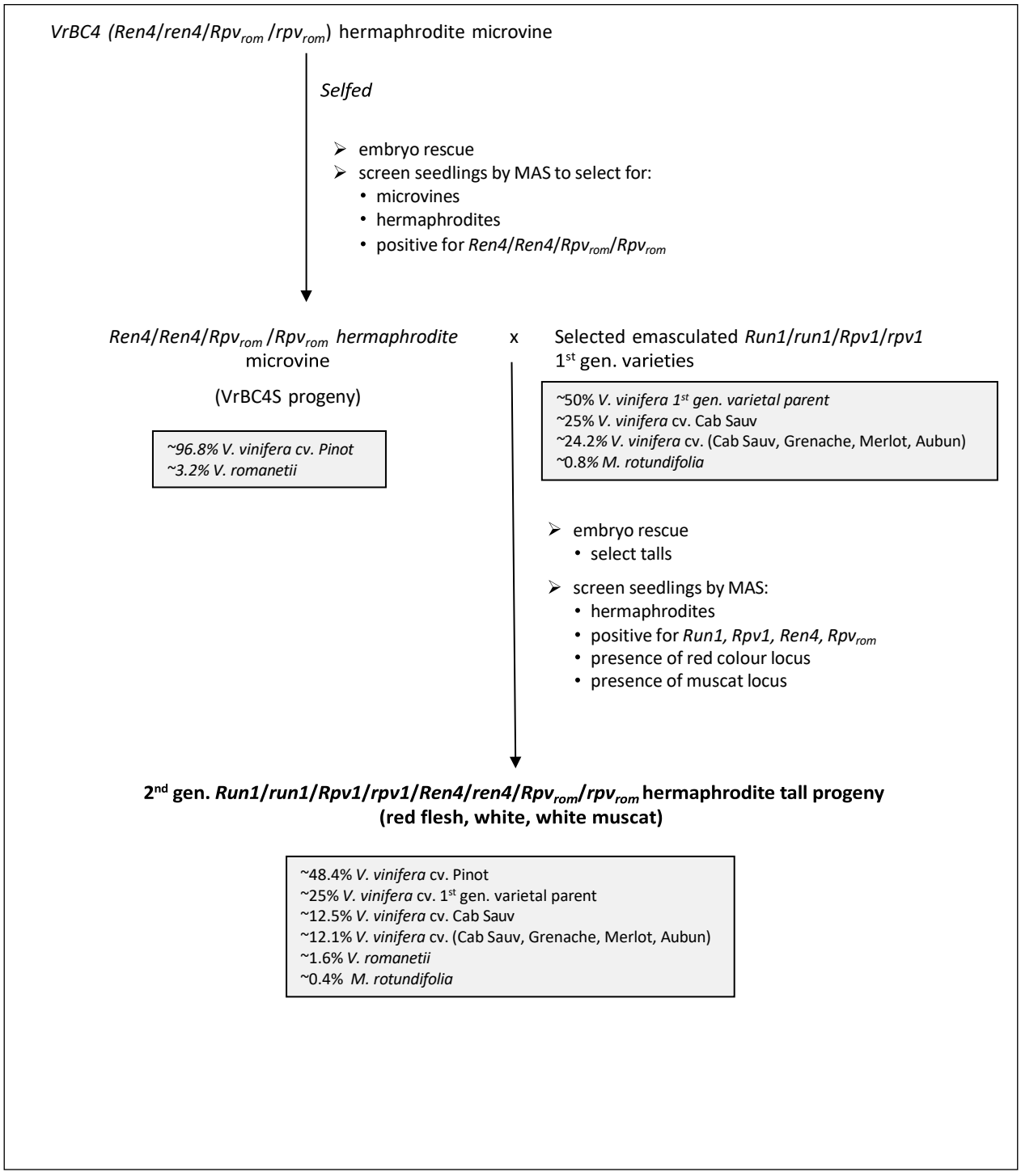


Figure 15. *Breeding Strategy 1B. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is also shown.*

***Table 4**. List of emasculated breeding line/pollen source combinations used in Strategy 1B.*

Female (1st gen.- emasculated)	1st gen. varietal parent	Pollen donor (microvine)
37_14_12_02 (red flesh)	Dunkelfelder	VrBC4S 42-23
39_11_10_02 (white)	Riesling	VrBC4S 42-23
37_17_12_02 (red skin)	Shiraz	VrBC4S 42-23
37_24_07_01 (red skin)	Tannat	VrBC4S 42-23
37_24_11_01 (red skin)	Tannat	VrBC4S 42-23
37_25_03_04 (red skin)	Tannat	VrBC4S 42-23
39_16_06_03 (white)	Verdelho	VrBC4S 42-23

A total of 18,812 seeds were collected from Strategy 1A and Strategy 1B crosses (Table 5). Seeds were germinated in tissue culture using an embryo rescue technique because of the reduced germination rates observed with standard germination techniques when one of the parents is a microvine. Germination rates for Strategy 1A seed (overall average 62%) were generally lower than for Strategy 1B seed (overall average 69%). However, a significant proportion of the germinated seeds from both Strategy 1A and 1B produced plants that were deformed and assessed to be non-viable and were discarded. Viable seedlings segregated as tall or microvine, which was assessed visually. For Strategy 1A seeds the segregation of tall : microvine plants was 57% : 43% in agreement with the predicted inheritance ratio of 50% : 50% for the microvine trait (Table 5). In the case of Strategy 1B seeds, more talls were recovered than expected (segregation ratio 67% : 33%) which probably reflects some cross contamination from self-pollenation in emasculated bunches, which would only produce tall progeny.

A total of 4,342 tall viable plants generated from Strategy 1 were selected for DNA extraction and MAS to identify hermaphrodites positive for the *Run1/Ren4/Rpv1/Rpv$_{rom}$* mildew resistance loci with either red flesh, red skin, white skin or muscat characters. There was good agreement between the predicted inheritance of these traits (Table 6) and the observed segregation of these traits as demonstrated for a subset of Strategy 1A progeny generated from crosses between microvine line VrBCs 40-138 and various different 1st gen. parents (Table 7). As expected, 100% of the progeny were found to be positive for the *Ren4*/*Rpv$_{rom}$* locus while all other traits segregated as a single dominant trait. The selected lines were sub-cultured in vitro to make a back-up copy and after ~ 4 weeks were deflasked into pots and grown in the glasshouse until they were ready for planting out in the field for evaluation.

Table 5 shows that from the 4,342 tall viable plants generated from Strategy 1, a total of 402 tall hermaphrodite 2nd gen. mildew-resistant (*Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv$_{rom}$/rpv$_{rom}$*) winegrape varieties were successfully established in the field for further evaluation. This represents a recovery rate of 9.2% compared to a theoretical recovery rate of 25% based on segregation ratios for flower sex and the *Run1/Rpv1* locus (Table 6). This reduced recovery of 2nd gen. lines for evaluation in the field reflects the loss of plants during: (a) *in vitro* propagation (b) transfer of *in vitro* plants to soil and (c) transfer of glasshouse plants to the field. When the total number of seeds collected from Strategy 1 crosses is taken into consideration, the number of 2nd gen. mildew-resistant lines established in the field for evaluation, was 2.1% of the total number of seed collected.

Table 5. *Summary of germination success using embryo rescue technique with Strategy 1A and Strategy 1B seeds. 'Percent germ.' represents the % of all seeds that germinated. 'Percent germ. talls' represents the percentage of germinated seedlings that are talls.*

Female	Seed No.	Total germ.	% germ.	Total non-viable	Micro.	Talls	% germ. talls	2nd gen. established in field
Strategy 1A								
VrBC4S 40-138	11,745	7,257	62	2,824	1,932	2,501	34	226
VrBC4S 42-75	2,666	1,647	62	530	455	662	40	61
VrBC4S 40-120	2,645	1,701	64	487	499	715	42	75
VrBC4S 42-63	420	214	51	114	48	52	24	2
VrBC4S 42-46	161	84	52	30	21	33	39	3
VrBC4S 40-130	100	65	65	26	15	24	37	2
VrBC4S 40-173	64	34	53	15	4	15	44	2
Total	17,737	10,968	62	4,011	2,970	3,987	36	371
Strategy 1B								
37_24_07_01	277	210	76	52	49	109	52	10
37_24_11_01	248	192	77	56	53	83	43	7
37_14_12_02	158	116	73	43	33	40	34	4
37_17_12_02	176	101	57	33	21	47	47	4
39_11_10_02	94	29	31	2	12	15	52	4
37_25_03_04	69	49	71	16	0	33	67	1
39_16_06_03	53	42	79	10	4	28	67	1
Total	1,075	739	68	212	172	355	48	31

***Table 6**. Expected inheritance ratios in breeding Strategy 1 crosses. As the Vvgai mutation that causes microvine stature acts as a dominant negative mutation, plants which have the genotype VvGAI/Vvgai are microvine in stature.*

Breeding Strategy	Parents	Phenotype	Locus	Genotype	VrBC4S (fem.) x 1st gen. variety	VrBC4S (herm.) x 1st gen. variety
1A	VrBC4S progeny	PM resistance	*Run1*	null	50% *Run1*/*run1 :* 50% *run1*/*run1*	
		DM resistance	*Rpv1*	null	50% *Rpv1*/*rpv1 :* 50% *rpv1*/*rpv1*	
		PM resistance	*Ren4*	*Ren4*/*Ren4*	100% *Ren4*/*ren4*	
		DM resistance	Rpv_{rom}	Rpv_{rom}/Rpv_{rom}	100% Rpv_{rom}/rpv_{rom}	
		Stature	*VvGAI*	*VvGAI*/*Vvgai* (microvine)	50% *VvGAI*/*VvGAI*: 50% *VvGAI*/*Vvgai*	
		Flower sex	*f*	*f*/*f (female)*	50% *h*/*f* : 50% *f*/*f*	
		Berry colour	*B*	*b*/*b (white)*	50% *B*/*b* : 50% *b*/*b*	
		Muscat flavour	Dxs1	*dxs1*/*dxs1* (neutral)	50% *Dxs1*/*dxs1*: 50% *dxs1*/*dxs1*	
1B	VrBC4S progeny	PM resistance	*Run1*	null		50% *Run1*/*run1 :* 50% *run1*/*run1*
		DM resistance	*Rpv1*	null		50% *Rpv1*/*rpv1 :* 50% *rpv1*/*rpv1*
		PM resistance	*Ren4*	*Ren4*/*Ren4*		100% *Ren4*/*ren4*
		DM resistance	Rpv_{rom}	Rpv_{rom}/Rpv_{rom}		100% Rpv_{rom}/rpv_{rom}
		Stature	*VvGAI*	*VvGAI*/*Vvgai* (microvine)		50% *VvGAI*/*VvGAI*: 50% *VvGAI*/*Vvgai*
		Flower sex (herm.)	*h*	*h*/*f (hermaphrodite)*		25% *h*/*h*: 50% *h*/*f* : 25% *f*/*f*
		Berry colour	*B*	*b*/*b (white)*		50% *B*/*b* : 50% *b*/*b*
		Muscat flavour	Dxs1	*dxs1*/*dxs1* (neutral)		50% *Dxs1*/*dxs1*: 50% *dxs1*/*dxs1*
1A & 1B	1st gen. mildew-resistant variety	PM resistance	*Run1*	*Run1*/*run1*		
		DM resistance	*Rpv1*	*Rpv1*/*rpv1*		
		PM resistance	*Ren4*	*null*		
		DM resistance	Rpv_{rom}	*null*		
		Stature	Vvgai	*VvGAI*/*VvGAI* (tall)		
		Flower sex (herm.)	*h*	*h*/*f (hermaphrodite)*		
		Berry colour	*B*	*B*/*b (red)* & *b*/*b* (white)		
		Muscat flavour	Dxs1	*Dxs1*/*dxs1* (muscat) & *dxs1*/*dxs1* (neutral)		

***Table 7**. Observed segregation rations in selected Strategy 1A progeny. Data shown is only for a sub-set of progenies obtained from crosses of the female microvine genotype VrBC4S 40-138 with a range of different 1st gen. parents. n/a = not applicable*

1st gen. male parent	1st gen. varietal parent	No. of plants genotyped	No. of plants positive for traits as detected by MAS					
			Herm.	Female	*Run1/Rpv1*	*Ren4/Rpv$_{rom}$*	Red flesh/skin berry	Muscat flavour
37_14_05_02	Dunkelfelder	34	17 (50%)	17 (50%)	16 (47%)	34 (100%)	18 (53%)	n/a
37_14_06_01	Dunkelfelder	8	5 (63%)	3 (37%)	3 (37%)	8 (100%)	5 (63%)	n/a
37_14_08_01	Dunkelfelder	56	29 (52%)	27 (48%)	28 (50%)	56 (100%	28 (50%)	n/a
37_14_11_04	Dunkelfelder	110	55 (50%)	55 (50%)	50 (45%)	110 (100%)	59 (54%)	n/a
37_14_12_02	Dunkelfelder	113	70 (62%)	43 (38%)	45 (40%)	113 (100%	61 (54%)	n/a
37_17_12_02	Shiraz	102	52 (51%)	50 (29%)	40 (39%)	102 (100%	53 (52%)	n/a
37_24_07_01	Tannat	88	45 (51%)	43 (49%)	41 (46%)	88 (100%)	41 (47%)	n/a
37_24_11_01	Tannat	66	35 (53%)	31 (47%)	37 (56%)	66 (100%)	41 (62%)	n/a
37_25_03_04	Tannat	30	17 (57%)	13 (43%)	15 (50%)	30 (100%)	19 (63%)	n/a
37_25_06_03	Tannat	19	10 (53%)	9 (47%)	11 (58%)	19 (100%)	10 (53%)	n/a
37_16_09_04	Tempranillo	51	25 (49%)	26 (51%)	23 (45%)	51 (100%)	26 (51%)	n/a
39_08_05_01	Chardonnay	91	46 (51%)	45 (49%)	34 (37%)	91 (100%)	n/a	n/a
39_08_05_02	Chardonnay	10	5 (50%)	5 (50%)	4 (40%)	10 (100%)	n/a	n/a
39_17_09_01	Frontignac	69	38 (55%)	31 (45%)	34 (49%)	69 (100%)	n/a	15 (22%)
39_19_06_02	Frontignac	10	4 (40%)	6 (60%)	5 (50%)	10 (100%)	n/a	5 (50%)
39_11_10_02	Riesling	34	16 (47%)	18 (53%)	15 (44%)	34 (100%)	n/a	n/a
39_12_05_02	Riesling	42	26 (62%)	16 (38%)	21 (50%)	42 (100%)	n/a	n/a
39_12_10_03	Riesling	7	5 (71%)	2 (29%)	4 (57%)	7 (100%)	n/a	n/a
39_16_06_03	Verdelho	138	77 (56%)	61 (46%)	59 (43%)	138 (100%)	n/a	n/a
39_16_07_02	Verdelho	60	27 (45%)	33 (55%)	34 (57%)	60 (1900%)	n/a	n/a

6.2.2 Breeding Strategy 3

In addition to Strategy 1, an alternative breeding strategy (Strategy 3) was also employed to develop 2nd gen. mildew-resistant varieties (Figs. 16 & 17). In Strategy 3, the *Run1*/*Rpv1* locus was sourced from the CSIRO breeding line 15C002V0002, which is a tall female, white-berried vine homozygous for the *Run1/Rpv1* locus. Breeding line 15C002V0002 displays prolific flowering in the field and berries derived from open-pollinated flowers of 15C002V0002 did not exhibit any undesirable aromatic characteristics. The parentage of breeding line 15C002V0002 is shown in Supplementary Fig. 2.

In Strategy 1, the red flesh and muscat loci were as sourced from selected 1st gen. parental lines. However, as line 15C002V0002 does not contain either of these two loci, it was necessary to introduce these berry quality loci through the microvine breeding parent. To do this it was first necessary to cross VrBC4S breeding lines with either the red flesh microvine breeding line 10C026V0005 or the muscat microvine breeding line 10C007V001 (Figs. 16 & 17) to produce *Ren4*/*ren4*/Rpv_{rom}/rpv_{rom} microvine breeding lines with either red flesh or muscat berry traits (Table 8) from which pollen could be collected to pollinate 15C002V0002 (Figs. 16 & 17).

A total of 4,864 seeds were collected from Strategy 3R and 3M crosses (Table 9). Seeds were germinated via the embryo rescue protocol as described for Strategy 1. Rates of germination ranged from 30-73%, which is similar to that observed for Strategy 1 seeds, which also involved a microvine parent. As observed with the germination of Strategy 1-derived seeds, a significant proportion of the germinated seeds from both Strategy 3R and 3M produced plants that were deformed and assessed to be non-viable and were discarded. Viable seedlings segregated as tall or microvine, which was assessed visually. All 1,236 viable tall Strategy 3 plants were selected for DNA extraction and MAS to identify hermaphrodites positive for the *Run1*/*Ren4*/*Rpv1*/Rpv_{rom} mildew resistance loci with either red flesh, white skin or white skin with muscat berry characteristics. The selected lines were sub-cultured before being deflasked and planted into pots in the glasshouse until they were ready for planting out in the field for evaluation.

Table 9 shows that from a total of 1,236 viable tall seedlings generated from Strategy 3, a total of 103 tall hermaphrodite 2nd gen. mildew-resistant (*Run1/run1/Rpv1/rpv1/Ren4/ren4/*Rpv_{rom}/rpv_{rom}) winegrape varieties were successfully established in the field for further evaluation. This represents a recovery rate of 8.3%, which is similar to the recovery percentage observed for Strategy 1 crosses (Table 5). When the total number of seeds collected from Strategy 3 is taken into consideration, the recovery of 2nd gen. mildew-resistant lines for field evaluation was 2.1% of the total number of seed collected which is also in close agreement with the recovery percentage observed for Strategy 1.

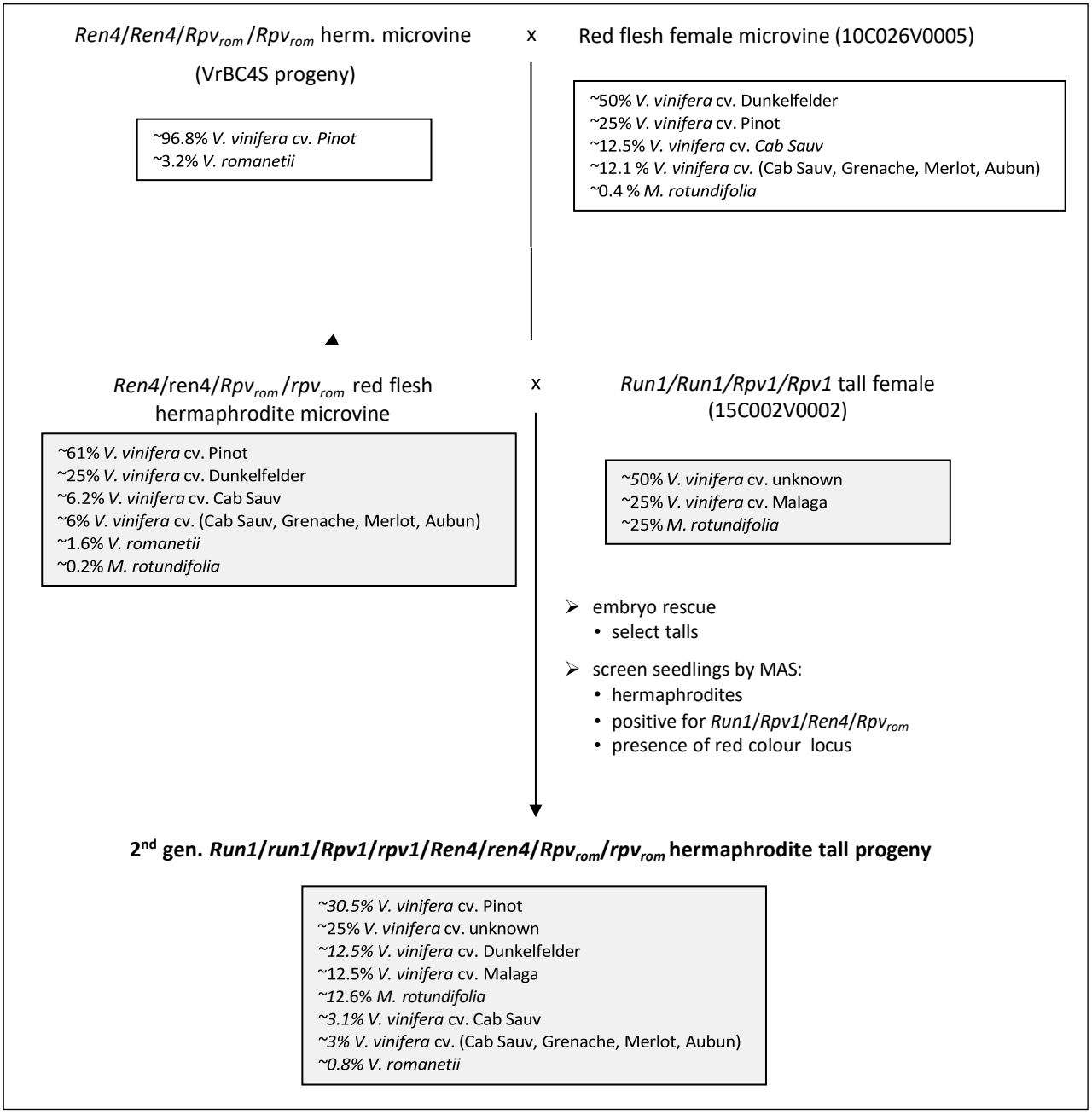


Figure 16. *Breeding Strategy 3R. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is also shown.*

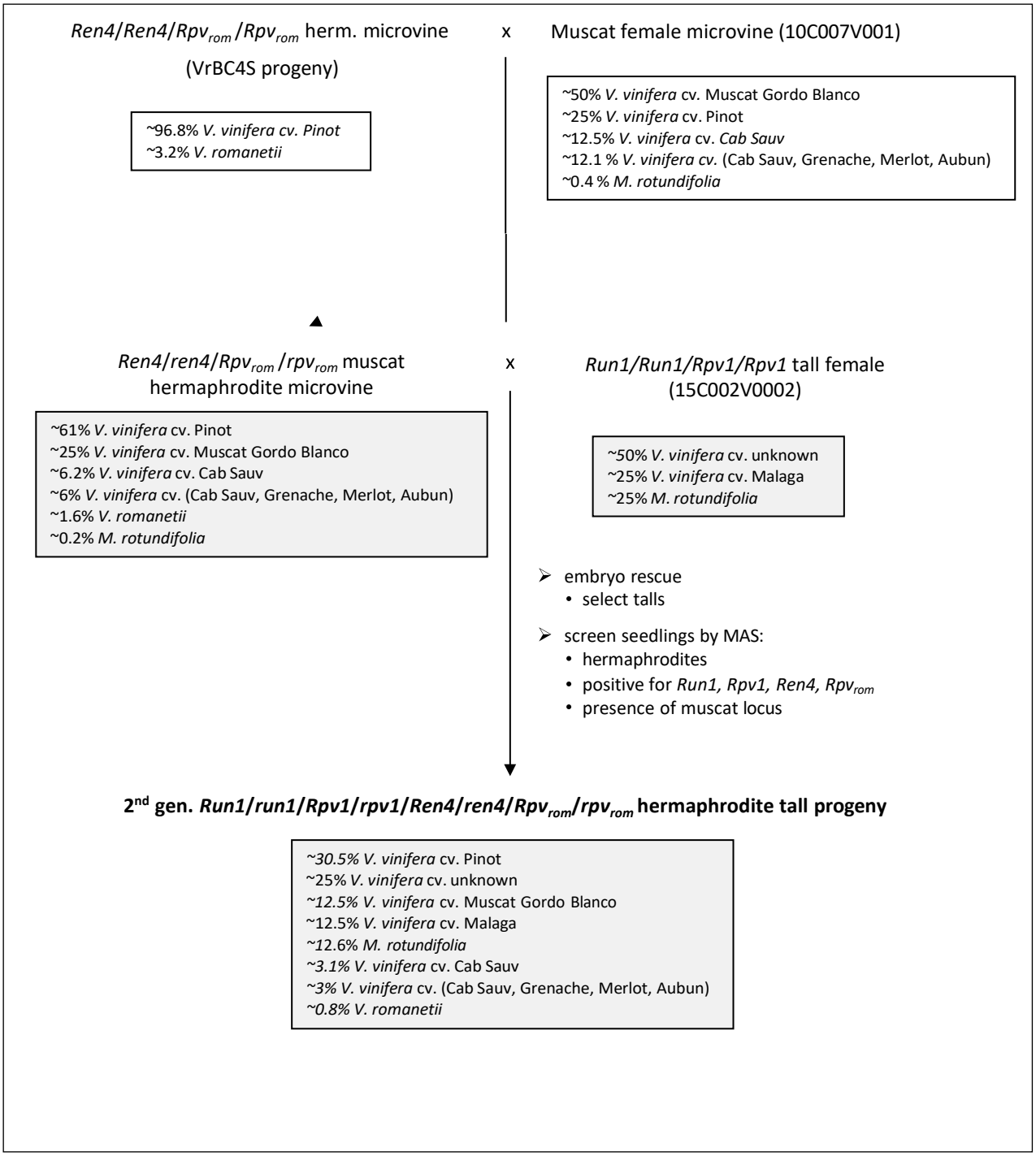


Figure 17. *Breeding Strategy 3M. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is also shown.*

***Table 8**. List of breeding line/pollen source combinations used in Strategy 3R and 3M.*

Female (tall)	Pollen donor (microvine)
Strategy 3R	
15C002V0002	Vr42F1R04_1 (red flesh)
	Vr42F1R04_4 (red flesh)
	Vr42F1R04_8 (red flesh)
	Vr42F1R04_25 (red flesh)
Strategy 3M	
15C002V0002	VrBC4S30F1_6 (muscat)
	VrBC4S30F1_10 (muscat)
	VrBC4S30F1_11 (muscat)

***Table 9.** Summary of germination success using embryo rescue technique with Strategy 3R and Strategy 3M seeds. 'Percent germ.' represents the % of all seeds that germinated. 'Percent germ. talls' represents the percentage of germinated seedlings that are talls.*

Female (Tall)	Pollen donors	Seed No.	Total germ.	% germ.	Total non-viable	Mv.	Talls	% talls germ.	2nd gen. established in field
Strategy 3R - red flesh pollinations									
15C002V0002	Vr42F1R04_1	955	282	30	94	43	145	15	20
	Vr42F1R04_4	678	382	56	111	120	151	22	10
	Vr42F1R04_8	291	137	47	47	40	50	17	4
	Vr42F1R04_25	137	91	66	55	24	12	9	2
	Total	2,061	892	43	307	227	358	17	36
Strategy 3M - muscat pollinations									
15C002V0002	VrBC4S30F1_6	350	236	67	84	24	128	37	7
	VrBC4S30F1_10	1,078	790	73	208	204	378	35	25
	VrBC4S40F1_11	1,375	868	63	301	195	372	27	35
	Total	2,803	1,894	68	593	423	878	31	67

6.3 Breeding and selection of 2nd gen. mildew-resistant winegrape varieties using 2nd gen. breeding lines crossed with premium winegrape varieties.

Breeding Strategies 1 and 3 are limited by the fact that only certain genotypes can be used as parents in the production of 2nd gen. mildew-resistant varieties because both parents are contributing either the *Run1*/*Rpv1* or *Ren4*/*Rpv*$_{rom}$ resistance loci. Thus, in order to expand the range of varietal parents that could be used for the production of 2nd gen. mildew-resistant varieties, it was necessary to develop 2nd gen. breeding lines that contained both the *Run1*/*Rpv1* and *Ren4*/*Rpv*$_{rom}$ resistance loci which could then be crossed with any other susceptible premium winegrape variety.

The first group of 2nd gen. female breeding lines to be recovered was selected from the original crosses used as part of Strategy 1 and Strategy 3 (Fig. 18). Thus, in addition to the 2nd gen. hermaphrodite varieties that were selected field evaluation, the MAS also identified microvine and tall female 2nd gen. breeding lines that contained both the *Run1*/*Rpv1* and *Ren4*/*Rpv*$_{rom}$ resistance loci in the heterozygous state. The selection of additional female 2nd gen. breeding lines used in this project is described in sections 6.3.3 and 6.4.

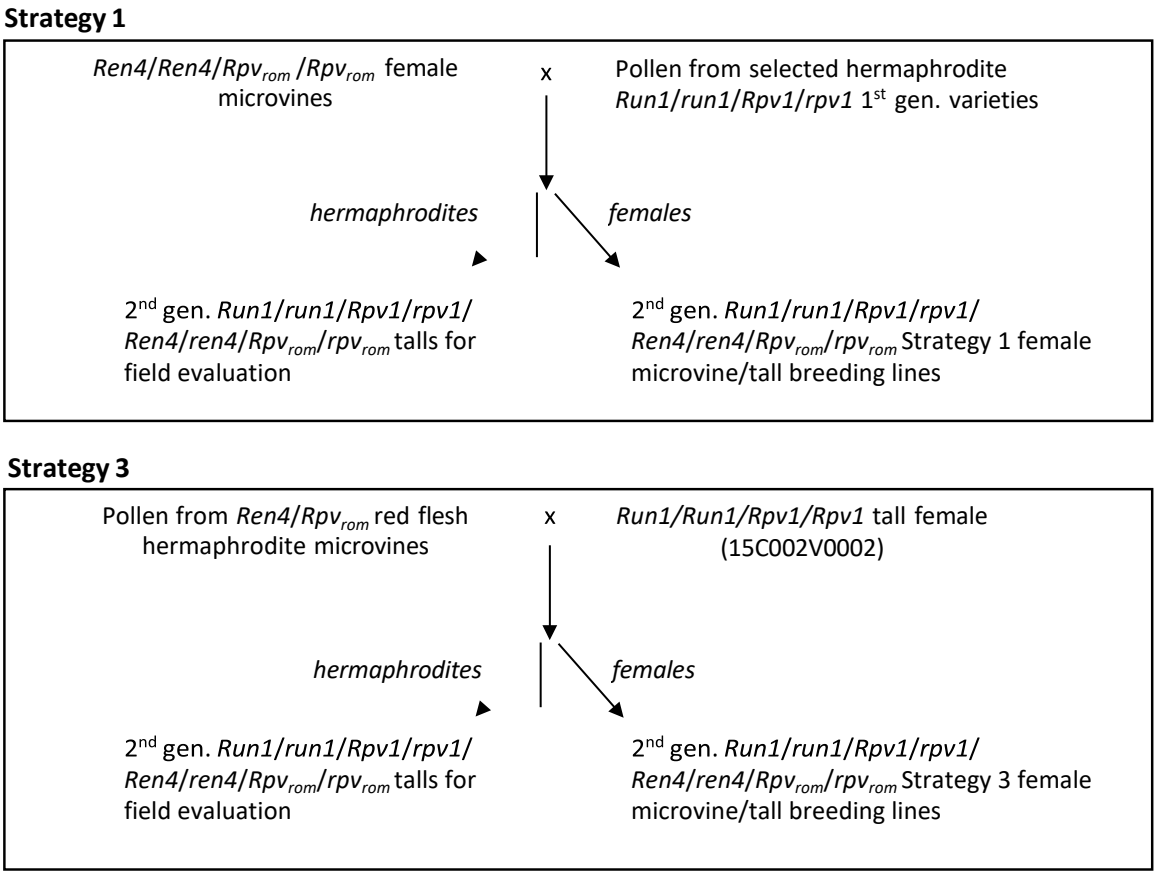


Figure 18. *Generation of 2nd gen. female breeding lines via Strategy 1 & 3.*

Table 10. *List of female microvine 2nd gen. breeding lines derived from Strategy 1 and Strategy 3 crosses. All breeding lines have the genotype: Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv_{rom}/rpv_{rom}. SNP-confirmed presence of the muscat locus is indicated. The variety name in brackets after the listed genotype of the female or male parents indicates the winegrape variety from which this parent was derived in the last cross.*

Breeding line name	Female parent	Male parent	Derived from which breeding Strategy?	Berry colour	Muscat
37140502_75_15	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x
37141202_138_166	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x
37140801_138_88	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x
37141202_138_9	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	white	x
37141202_138_58	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	white	x
39160603_138_11	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x
39160603_138_55	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x
15C2V2_10_05	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓
15C2V2_R04_1_77	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x
15C2V2_R04_1_81	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x

Table 11. *List of female tall 2nd gen. breeding lines derived from Strategy 1 and Strategy 3 crosses. All breeding lines have the genotype: Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv$_{rom}$/rpv$_{rom}$. SNP-confirmed presence of the muscat locus is indicated. Planting date and location in SARDI vineyard in Nuriootpa also indicated. The variety name in brackets after the listed genotype of the female or male parents indicates the winegrape variety from which this parent was derived in the last cross.*

Genotype name	Female Parent	Male Parent	Derived from which breeding strategy?	Berry colour	Muscat	Location	Planted
39170303_75_15	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	x	37_27_07_04	19/12/18
39170303_75_22	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	✓	37_27_06_04	19/12/18
15C2V2_6_11	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	37_27_11_01	22/03/19
15C2V2_6_42	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	37_27_10_02	22/03/19
15C2V2_6_74	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	37_27_06_01	19/12/18
15C2V2_6_100	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	37_27_05_02	19/12/18
15C2V2_6_103	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	37_27_05_04	19/12/18
15C2V2_11_12	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	37_27_07_01	19/12/18
15C2V2_R04_1_189	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	37_26_01_04	3/12/19
37140502_75_4	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	37_26_09_03	14/12/21
37140601_75_16	VrBC4S 42-75	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	37_26_13_01	16/02/22
37141202_138_8[1]	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	37_26_09_04	14/12/21
37141202_138_24[2]	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	37_26_04_03	14/12/21
37171202_138_27	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	37_26_01_01	16/02/21
37171202_138_43	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	37_26_05_01	08/01/21
37240701_138_19	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	37_26_05_02	08/01/21
37240701_138_24	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	37_26_05_03	08/01/21
37240701_138_112	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	37_26_02_03	14/12/21
37241101_75_36	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	37_26_02_04	14/12/21
37241101_120_23	VrBC4S 40-120	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	37_26_03_01	14/12/21
37250304_138_40	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	37_27_01_01	16/02/22
37250603_138_23	VrBC4S 40-138	37_25_06_03 (Tannat)	Strategy 1A	red skin	x	37_26_08_03	14/12/21

6.3.1 Development of a DNA marker specific for the red flesh trait

The generation of 2nd gen. breeding lines containing all four resistance loci was significant because it meant that any *V. vinifera* variety could theoretically be used as a pollen parent, including the pollination of a red flesh 2nd gen. breeding line with pollen from a red skin variety which was not possible in Strategy 1 or Strategy 3 crosses. As the SNP marker used to analyse Strategy 1 and Strategy 3 progeny (Table 1) could not differentiate between red skin and red flesh genotypes, it was necessary to develop a new genetic marker to specifically detect red flesh progeny. The red flesh berry phenotype is caused by a 480 bp duplication within the *VvmybA1* promoter (Röckel et al. 2020). The *VviMybA1* forward primer which spans across the promoter duplication junction and the *VviMybA1* reverse primer located within the coding region were designed to pick up the duplication associated with the red flesh berry phenotype giving rise to a 976 bp PCR product. The *VviUbi* forward and reverse primers were also included in the PCR reaction, which gave rise to a 174 bp product and acted as a control for DNA quality. The PCR reaction was performed as described in Methods section 5.5.6. Marker validation was undertaken on 137 grapevine DNA samples coming from 24 different genetic backgrounds with the red flesh phenotype. Figure 19 shows an example of the products obtained from the PCR reaction. All samples tested gave a positive result for the 174 bp amplification fragment for Ubiquitin indicating that the genomic DNA was of good quality. DNA samples which amplified the 976 bp product associated with the *VviMybA1* promoter duplication were scored as having the red flesh berry phenotype. There was a 100% association between the red flesh positive marker result and the red flesh phenotype (Table 12). All red skin/white flesh and white skin/white flesh genotypes had a negative score for the marker. These results indicate that the marker is a reliable predictor of the berry red flesh phenotype.

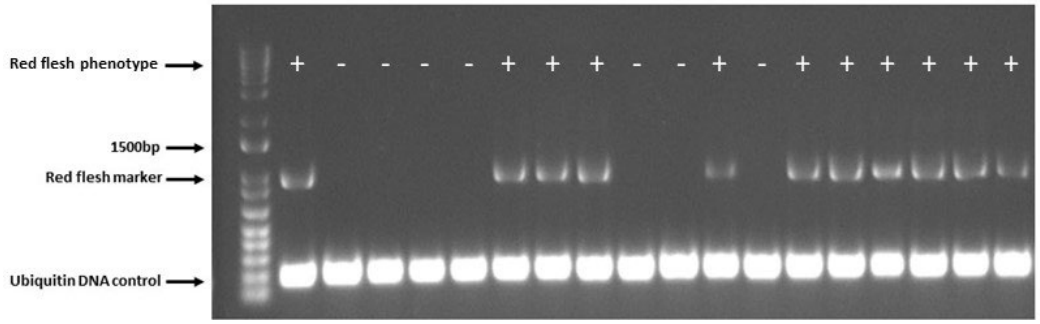


Figure 19. *Agarose gel analysis for the detection of the red flesh-specific PCR marker. Red skin/red flesh genotype is scored as positive when the upper PCR product (976 bp) is visible. The lower 174 bp Ubiquitin PCR product is amplified in all samples and serves as a quality control for the genomic DNA samples.*

Table 12. *Summary data of the PCR amplification across the VviMybA1 promoter duplication region that determines the red flesh berry phenotype. The PCR positive result (976 bp product) is only observed in DNA samples extracted from grapevines displaying the red skin/red flesh phenotype.*

Berry phenotype	**Sample No.**	***VviMybA1* PCR (+)**	***VviMybA1* PCR (-)**
red skin/red flesh	93	93	0
red skin/white flesh	34	0	34
white skin/white flesh	10	0	10

6.3.2 Breeding strategy 2

Two versions of breeding Strategy 2 were used as outlined in Figures 20-21. Strategy 2.1 utilised female microvine and tall 2nd gen. breeding lines generated through Strategy 1, whereas Strategy 2.3 used female microvine and tall breeding lines generated through Strategy 3. As a result, the genetic composition of 2nd gen. progeny derived from these two strategies is significantly different with the genome of Strategy 2.1 progeny comprising only ~ 1% DNA from the two wild grapevine species *M. rotundifolia* and *V. romanetii* whereas the approximately 6.7% of the genome of Strategy 2.3 progeny were derived from these two wild species (Figs. 20-21).

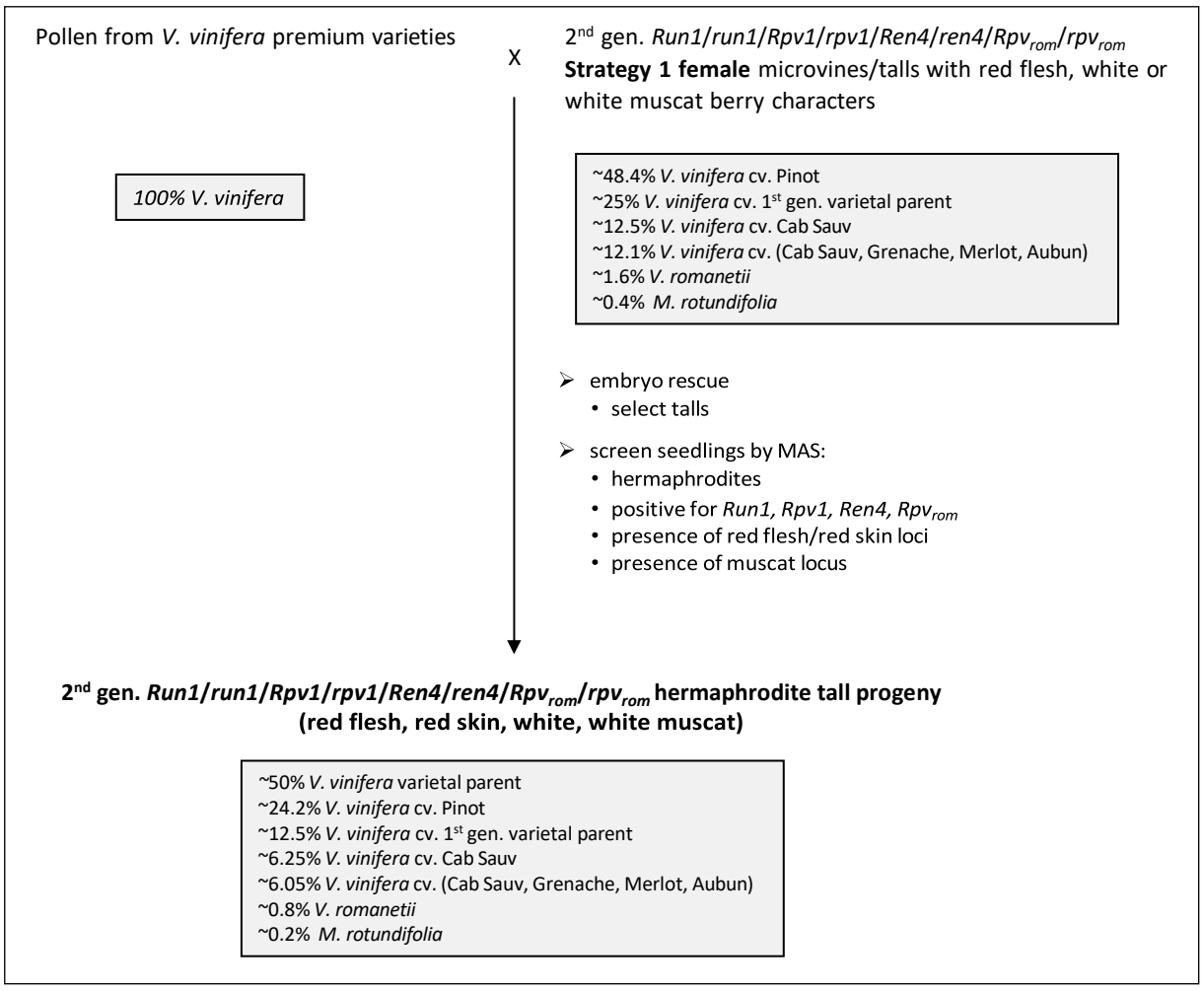


Figure 20. *Breeding Strategy 2.1. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is shown.*

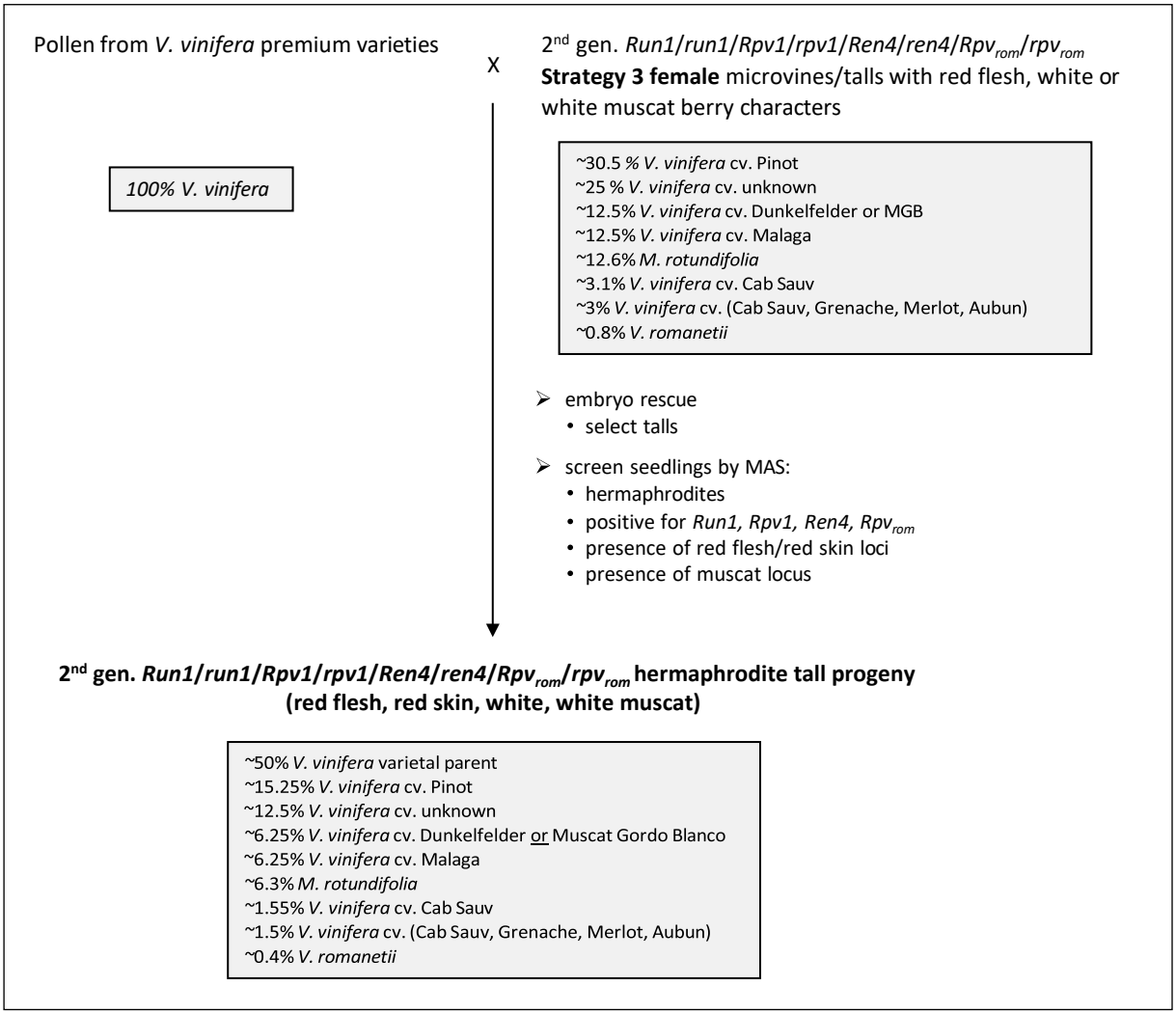


Figure 21. *Breeding Strategy 2.3. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is shown.*

The female microvine and tall Strategy 2 breeding lines were pollinated with pollen collected with a diverse range of mildew susceptible winegrape varieties, many of which had not been represented in breeding Strategy or Strategy 3 including Sauvignon Blanc, Cabernet Sauvignon, Grenache, Viognier, Malbec and Merlot (Tables 13-14). The Strategy 2.3 female breeding lines became available for pollination in the spring of 2020 whereas the Strategy 2.1 females were not available for pollination until the spring of 2021. The first round of pollinations were carried out exclusively on Strategy 2.3 breeding lines and resulted in the production of 2,937 seed which were germinated by embryo rescue (Table 15).

***Table 13**. List of female breeding line/pollen source combinations used in Strategy 2.1*

Female	Female (varietal background)	Pollen donor
Microvine		
37140502_75_15 (red flesh)	Dunkelfelder	Cabernet Franc
		Cabernet Sauvignon
		Malbec
		Merlot
		Shiraz
37141202_138_9 (white)	Dunkelfelder	Viognier
37141202_138_58 (white)	Dunkelfelder	Riesling
		Viognier
37141202_138_166 (red flesh)	Dunkelfelder	Cabernet Sauvignon
		Grenache
		Merlot
		Shiraz
37140801_138_88 (red flesh)	Dunkelfelder	Cabernet Sauvignon
		Shiraz
39160603_138_11 (white)	Verdelho	Sauvignon Blanc
39160603_138_55 (white)	Verdelho	Alicante Bouchet
		Viognier
39160702_138_9 (white)	Verdelho	Viognier
Tall		
39170303_75_22 (muscat)	*Frontignac*	Chardonnay
		Sauvignon Blanc
		Viognier
39170303_75_15 (white)	*Frontignac*	Durif
		Malbec
		Shiraz

***Table 14**. List of female breeding line/pollen source combinations used in Strategy 2.3*

Female	Female berry type	Pollen donor
Microvine		
15C2V2_10_05	muscat	Riesling
		Sauvignon Blanc
		Verdelho
15C2V2_R04_1_77	red flesh	Bobal
		Bogazkere
		Cabernet Franc
		Cabernet Sauvignon
		Grenache
		Malbec
		Sangiovese
		Shiraz
		Tempranillo
15C2V2_R04_1_81	red flesh	Bobal
		Shiraz
		Malbec
		Grenache
		Cabernet Sauvignon
		Cabernet Franc
		Merlot
		Mataro
		Tricaderia
Tall		
15C2V2_6_11	white	Cabernet Franc
		Durif
		Mataro
		Tempranillo
15C2V2_6_42	muscat	Durif
		Verdelho
15C2V2_6_74	muscat	Cabernet Franc
		Grenache
		Riesling
15C2V2_6_103	muscat	Chardonnay
15C2V2_11_12	white	Malbec
		Merlot
15C2V2_R04_1_189	red flesh	Malbec
		Shiraz

Table 15. *Summary of germination success using embryo rescue technique with Strategy 2.3 seeds. 'Percent germ.' represents the % of all seeds that germinated. 'Percent germ. talls' represents the percentage of germinated seedlings that are talls.*

Female	Seed No.	Total germ.	% germ.	Total non-viable	Mv.	Talls	% talls germ.	2nd gen. established in field
Microvine								
15C2V2_10_05 (muscat)	393	142	36	73	20	49	12	8
15C2V2_R04_1_77 (red flesh)	2,104	1,361	65	577	263	521	25	48
15C2V2_R04_1_81 (red flesh)	208	119	57	54	27	38	18	2
Total	2,705	1,622	60	704	310	608	22	58
Tall								
15C2V2_6_74 (muscat)	232	188	81	45	0	143	62	7

Germination rates for Strategy 2.3 seeds were similar to that observed previously with Strategy 1 (Table 5) and Strategy 3 (Table 9) seeds. Also, as observed Strategy 1 and Strategy 3 seeds, a significant proportion of the germinated seeds from Strategy 2.3 produced plants that were deformed and assessed to be non-viable and were discarded. Viable seedlings generated by crosses between 2nd gen. microvine breeding lines and tall *V. vinifera* varieties segregated as microvines or talls, which was assessed visually. However, where the female tall 2nd gen. breeding line 15C2V2_6_74 was used, only tall progeny were recovered as expected (Table 15).

In total, 751 tall viable Strategy 2.3 plants were sampled for DNA extraction and MAS to identify hermaphrodites positive for *Run1*/*Ren4*/*Rpv1*/Rpv_{rom} mildew resistance loci with either red flesh, red skin, white skin or white skin with muscat berry characteristics. From the genotyping of 608 tall viable plants, derived from microvine parents, a total of 58 2nd gen. mildew-resistant tall hermaphrodite plants were successfully established in the field for further evaluation. This represents a recovery rate of 9.5% compared to the theoretical recovery of 12.5% based on segregation ratios for flower sex, *Run1*/*Rpv1* locus and the Ren4/Rpv_{rom} locus (Table 15). In addition, seven 2nd gen. mildew-resistant tall hermaphrodite plants were recovered from Strategy 2.3 crosses involving the tall breeding line 15C2V2_6_74.

By the spring of 2021, Strategy 2.1 female microvines and talls were also available, in addition to the Strategy 2.3 females, for pollination with a diverse range of pollen donors (Table 13). A total of 3,903 Strategy 2.1 seeds and 2,464 Strategy 2.3 seeds were collected (Table 16). As the project was then in its final year, a decision was made to focus on the recovery of tall seedlings only because: (a) sufficient female microvine breeding lines had already been selected and (b) it would allow germination to be carried out by the simpler filter paper method (Section 5.4.3) rather than by the more time-consuming and labour-intensive method of embryo rescue. As observed for germination via the embryo rescue method, germination rates with the filter paper method varied widely depending on the parents (Table 16). However, the average rates of germination resulting in a tall seedling from the filter paper method were similar to those obtained for embryo rescue, once the microvine and non-viable seedlings had been taken into account.

Table 16. *Summary of germination success using filter paper method with Strategy 2.1 & 2.3 seeds. The germination data are for talls only as no microvines are recovered using the filter paper method.*

Female	Seed No.	Total germinated	% germination	2nd gen. ready for planting in 2022
Strategy 2.1 (Microvine)				
37140502_75_15 (red flesh)	303	72	24	10
37140801_138_88 (red flesh)	123	41	33	2
37141202_138_9 (white)	95	37	39	2
37141202_138_58 (white)	369	141	38	18
37141202_138_166 (red flesh)	91	35	38	2
39160603_138_11 (white)	250	60	24	2
39160603_138_55 (white)	91	32	35	6
Total	1,322	418	32	42
Strategy 2.1 (Tall)				
39170303_75_15 (white)	276	78	28	3
39170303_75_22 (muscat)	2,305	836	36	17
Total	2,581	914	35	20
Strategy 2-3 (Microvine)				
15C2V2_R04_1_77 (red flesh)	1,556	513	33	26
15C2V2_R04_1_81 (red flesh)	67	19	28	1
Total	1,623	532	33	27
Strategy 2-3 (Tall)				
15C2V2_6_11 (white)	55	33	60	2
15C2V2_6_42 (muscat)	115	94	82	2
15C2V2_6_100 (muscat)	255	102	40	5
15C2V2_6_103 (muscat)	15	9	60	2
15C2V2_11_12 (white)	104	40	38	1
15C2V2_R04_1_67 (white)	249	65	26	3
15C2V2_R04_1_189 (red flesh)	48	21	44	3
Total	841	364	40	18

Leaves were collected from Strategy 2 seedlings and DNA extractions and SNP genotyping undertaken as previously outlined. The *Run1*/*Rpv1* and *Ren4*/*Rpv*$_{rom}$ loci were detected in approximately 50% of the progeny as predicted for crosses between the 2nd gen. female breeding lines and mildew-susceptible winegrape varieties (Table 17). The same was also true for the inheritance of flower sex, apart from crosses involving winegrape varieties that are homozygous for the hermaphrodite flower trait (*h*/*h;* see Table 6) in which case all the resultant progeny are hermaphrodites. This was observed for crosses involving Chardonnay, Riesling and Sangiovese, which have previously been reported to be homozygous for the hermaphrodite allele (Antcliff 1980, Zho et al. 2021), but had not previously been reported for Malbec.

***Table 17**. Observed segregation rations in Strategy 2 progeny. Data are only presented for crosses where more than 20 progeny were available for genotyping.*

Male parent	Female parent(s)	No. of plants genotyped	No. of plants positive for traits detected by MAS			
			Herm.	Female	*Run1/Rpv1*	*Ren4/Rpv$_{rom}$*
Strategy 2-1						
Chardonnay*	Tall	26	26 (100%)	0 (0%)	7 (27%)	12 (46%)
Riesling*	Microvine	113	113 (100%)	0 (0%)	40 (35%)	52 (46%)
Sauvignon Blanc	Microvine/Tall	115	60 (52%)	55 (48%)	53 (46%)	44 (38%)
Viognier	Microvine/Tall	159	85 (53%)	74 (46%)	83 (52%)	68 (43%)
Cab Sauv	Microvine	32	18 (56%)	14 (44%)	15 (47%)	13 (40%)
Malbec*	Microvine/Tall	26	26 (100%)	0 (0%)	11 (42%)	12 (46%)
Merlot	Microvine	27	15 (55%)	12 (45%)	8 (30%)	18 (29%)
Shiraz	Microvine	21	12 (57%)	9 (43%)	12 (57%)	12 (57%)
Strategy 2-3						
Riesling*	Microvine/Tall	55	55 (100%)	0 (0%)	27 (49%)	26 (47%)
Bogazkere	Microvine	27	14 (52%)	13 (48%)	8 (30%)	18 (66%)
Cabernet Franc	Microvine/Tall	31	19 (61%)	12 (39%)	12 (39%)	15 (48%)
Cab Sauv	Microvine/Tall	135	78 (58%)	57 (42%)	65 (48%)	70 (52%)
Durif	Tall	22	7 (32%)	15 (68%)	12 (54%)	9 (41%)
Grenache	Microvine/Tall	241	121 (50%)	120 (50%)	105 (44%)	104 (43%)
Malbec*	Microvine/Tall	86	86 (100%)	0 (0%)	48 (56%)	41 (48%)
Sangiovese*	Microvine	23	23 (100%)	0 (0%)	10 (43%)	11 (48%)
Shiraz	Microvine/Tall	103	48 (47%)	55 (53%)	46 (45%)	33 (32%)
Tempranillo	Microvine	47	29 (62%)	18 (38%)	24 (51%)	20 (42%)

Marker-assisted selection of this second batch of Strategy 2 seed identified another 107 2nd gen. varieties for field evaluation (Table 16). Thus, in total, 172 new 2nd gen. mildew-resistant varieties were generated via Strategy 2, which are currently being maintained in the glasshouse in preparation for planting in late 2022.

6.3.3 Selection of new female tall 2nd gen. breeding lines derived from Strategy 2.

Strategy 2 crosses not only generated additional tall hermaphrodite 2nd gen. varieties for field evaluation (Tables 15-16), but also produced additional tall 2nd gen. female breeding lines with different parents to those breeding lines generated from Strategy 1 and Strategy 3 including Cabernet Sauvignon, Durif, Grenache, Merlot, Sauvignon Blanc, Trincaderia and Viognier (Table 18). These lines are currently being maintained in the glasshouse in preparation for planting in late 2022 to evaluate their flowering potential.

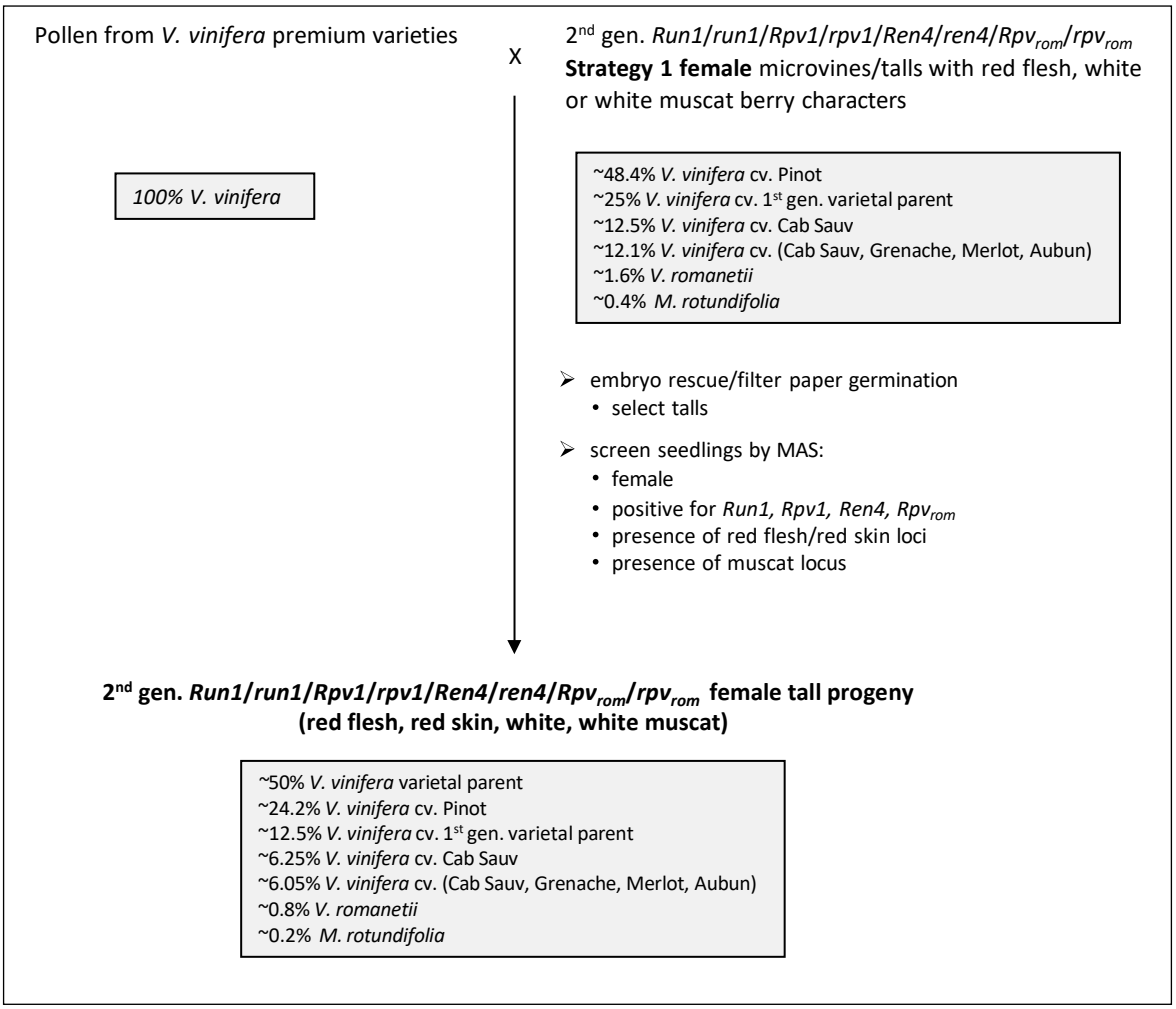


Figure 22. *Generation of tall 2nd gen. breeding lines via Strategy 2.1.*

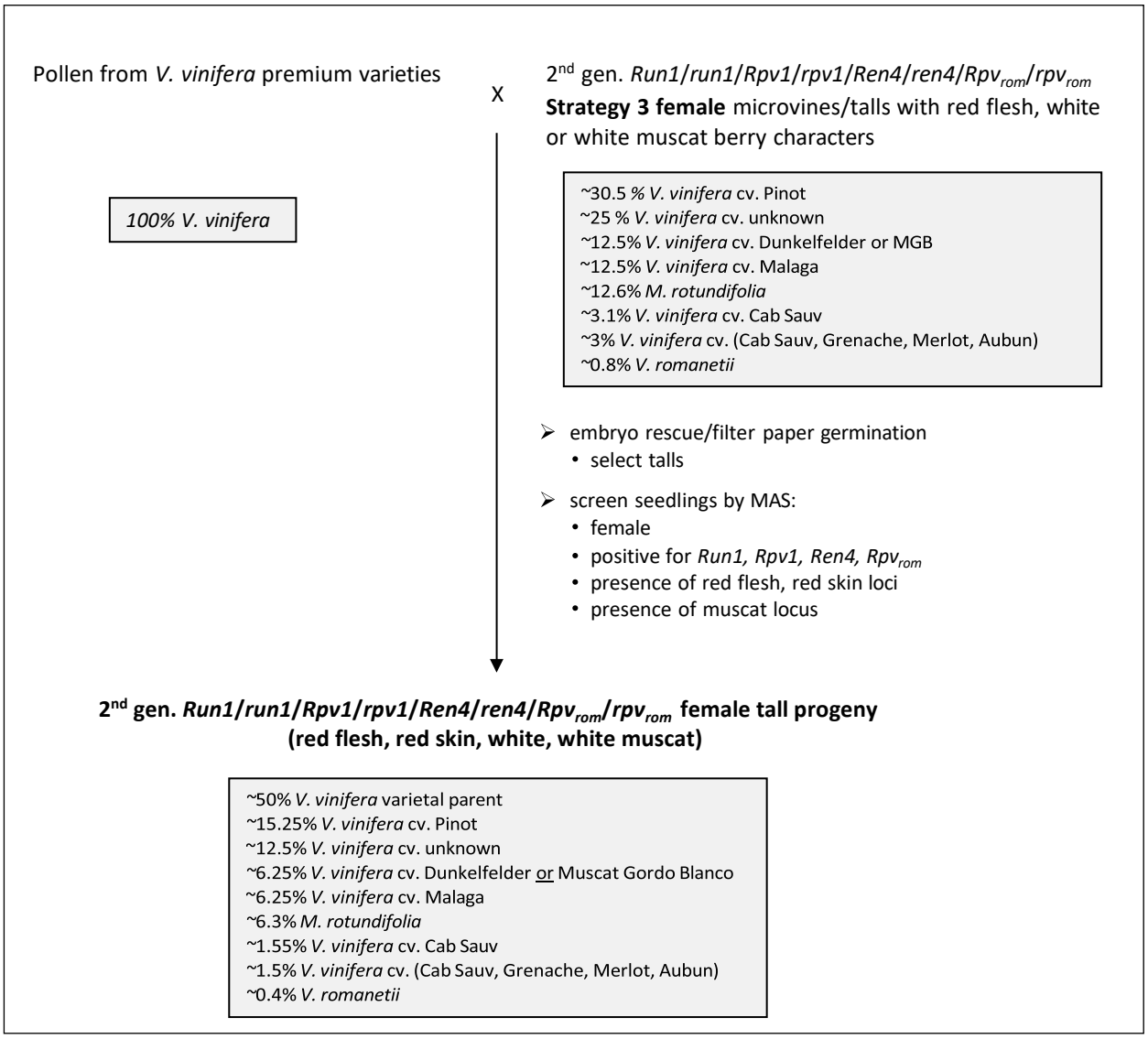


Figure 23. *Generation of tall 2nd gen. breeding lines via Strategy 2.3.*

Table 18. *List of female tall 2nd gen. breeding lines derived from Strategy 2 crosses. All breeding lines have the genotype: Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv$_{rom}$/rpv$_{rom}$. All lines are currently maintained in a CSIRO glasshouse on Waite campus. ST21M indicates derived from Strategy 2.1 involving a female microvine breeding line; ST21W indicates derived from Strategy 2.1 involving a female tall breeding line; ST23M indicates derived from Strategy 2.3 involving a female microvine breeding line; ST23W indicates derived from Strategy 2.1 involving a female tall breeding line. The male parent used in the Strategy 2 cross is also indicated.*

Genotype name	Female parent	Male parent	Berry colour
ST21M_ME_1_6	37140502_75_15 (red flesh)	Merlot	white
ST21M_ME_1_23	37140502_75_15 (red flesh)	Merlot	red flesh
ST21M_SB_1_1	39170303_75_15 (white)	Sauvignon Blanc	white
ST21M_SB_1_16	39170303_75_15 (white)	Sauvignon Blanc	white
ST21M_SB_1_7	39170303_75_15 (white)	Sauvignon Blanc	white
ST21M_SH_2_6	37140502_75_15 (red flesh)	Shiraz	red skin
ST21M_VI_1_4	39170303_75_15 (white)	Viognier	white
ST21M_VI_1_6	39170303_75_15 (white)	Viognier	white
ST21W_DU_1_3	39170303_75_15 (white)	Durif	red skin
ST21W_SB_1_8	39170303_75_22 (muscat)	Sauvignon Blanc	white
ST21W_VI_1_47	39170303_75_15 (white)	Viognier	white
ST21W_VI_1_5	39170303_75_15 (white)	Viognier	white
ST21W_VI_1_61	39170303_75_15 (white)	Viognier	white
ST23M_CS_3_1	15C2V2_R04_1_77 (red flesh)	Cabernet Sauvignon	red flesh
ST23M_CS_3_50	15C2V2_R04_1_77 (red flesh)	Cabernet Sauvignon	red flesh
ST23M_GR_3_11	15C2V2_R04_1_77 (red flesh)	Grenache	red skin
ST23M_GR_3_17	15C2V2_R04_1_77 (red flesh)	Grenache	red skin
ST23M_GR_3_22	15C2V2_R04_1_77 (red flesh)	Grenache	red skin
ST23M_GR_3_42	15C2V2_R04_1_77 (red flesh)	Grenache	red skin
ST23M_GR_3_46	15C2V2_R04_1_77 (red flesh)	Grenache	red flesh
ST23M_GR_3_48	15C2V2_R04_1_77 (red flesh)	Grenache	red flesh
ST23M_SH_4_34	15C2V2_R04_1_77 (red flesh)	Shiraz	red flesh
ST23M_TE_1_24	15C2V2_R04_1_77 (red flesh)	Tempranillo	red flesh
ST23M_TE_1_27	15C2V2_R04_1_77 (red flesh)	Tempranillo	white
ST23M_TE_1_33	15C2V2_R04_1_77 (red flesh)	Tempranillo	red flesh
ST23M_TE_1_40	15C2V2_R04_1_77 (red flesh)	Tempranillo	red flesh
ST23M_TR_1_8	15C2V2_R04_1_81 red flesh)	Trincaderia	red skin
ST23W_DU_4_11	15C2V2_6_42 (muscat)	Durif	red skin
ST23W_DU_4_7	15C2V2_6_42 (muscat)	Durif	red skin

6.4 Development of homozygous 2nd gen. mildew-resistant breeding lines for improved efficiency of production of 2nd gen. winegrape varieties

6.4.1 Breeding Strategy 4

Given the relatively low rate of recovery of 2nd gen. varieties for field evaluation (1.7 - 2.2 %; Tables 15-16) from the germination and MAS of seed generated using the 2nd gen. female breeding lines, an attempt was made to improve this recovery percentage for future breeding projects by generating 2nd gen. female breeding lines in which all mildew resistance loci were present in the homozygous (*Run1*/*Run1*/*Rpv1*/*Rpv1*/*Ren4*/*Ren4*/Rpv_{rom}/Rpv_{rom}) state rather than the heterozygous (*Run1*/*run1*/*Rpv1*/*rpv1*/*Ren4*/*ren4*/Rpv_{rom}/rpv_{rom}) state. This would potentially result in a four-fold increase in the recovery percentage of 2nd gen. varieties meaning fewer crosses would need to be undertaken and fewer seed collected. It would also reduce the cost of undertaking MAS by making it unnecessary to have to screen for the inheritance of the powdery mildew and downy mildew resistance loci.

The original strategy, designated as breeding Strategy 4, to generate homozygous female 2nd gen. breeding lines is shown in Figure 24. It was based on the selfing of hermaphrodite *Run1*/*run1*/*Rpv1*/*rpv1*/*Ren4*/*ren4*/Rpv_{rom}/rpv_{rom} microvine breeding lines and screening of progeny for females and talls that were homozygous for all mildew resistance loci. A total of 3,039 seed were collected from six hermaphrodite microvine breeding lines (Table 19). Germination of selfed seed using the embryo rescue technique ranged from 23 - 59% which is similar to that observed for out-crosses with Strategy 2 progeny (Table 16). Unfortunately, MAS did not identify any female breeding lines that were homozygous for all mildew resistance loci. However, four female 2nd gen. breeding lines (two microvine and two tall) were retained that were found to be homozygous for either the *Run1*/*Rpv1* loci or the Rpv_{rom}/rpv_{rom} loci (Table 20). The female microvine line ST4_A_87 shows good flowering potential while line ST4_09_91 has never flowered. The two tall female lines were planted in the field, but are not thriving and have not yet flowered.

6.4.2 Breeding S*trategy 4NEW*

Breeding Strategy 4, based on selfing of hermaphrodite microvines, proved unsuccessful in generating new female microvine breeding parents homozygous for all mildew resistance loci (Table 20). This may be related to in-breeding depression associated with the selfing step. Therefore, it was decided to initiate a modified breeding strategy (designated Strategy 4NEW; Fig. 25) involving crosses between parents containing all four mildew resistance loci in the heterozygous state (*Run1*/*run1*/*Rpv1*/*rpv1*/*Ren4*/*ren4*/Rpv_{rom}/rpv_{rom}), but which had different genetic backgrounds, to try and increase recovery rates of female homozygous progeny. Thus, pollen was collected from 2nd gen. Strategy 3 varieties that had been planted in the field in 2019 (Supplementary Table 1) and had commenced flowering in 2021. This pollen was used to pollinate microvine and tall female breeding lines originally derived from Strategy 1 crosses (Fig. 18).

A total of 732 seeds were collected from 14 different crosses involving multiple parental combinations (Table 21). Marker-assisted selection identified only two female breeding lines (Table 22) that were homozygous for all resistance loci (*Run1*/*Run1*/*Rpv1*/*Rpv1*/*Ren4*/*Ren4*/Rpv_{rom}/Rpv_{rom}). One of these lines (ST4_C_3_41) is a tall female and was planted in the field in December 2021 to evaluate flowering potential. The second homozygous breeding line, ST4_CM_2_3, is a microvine but it is not thriving and has not yet flowered. In addition to these two homozygous lines, 17 other 2nd gen. female breeding lines (nine microvine, eight tall) were identified that are homozygous for either the *Run1*/*Rpv1* or

Ren4/*Rpv*$_{rom}$ loci (Table 22). As the use of these female breeding lines, in particular talls, would still lead to a significant improvements in breeding efficiency compared to 2nd gen. breeding lines in which all mildew resistance loci are heterozygous, they should be evaluated for flowering potential. To this end, six of the eight tall female lines have been planted in the SARDI vineyard in Nuriootpa and the remaining two will be planted in the field in the Spring of 2022. It is also intended to evaluate the flowering potential of the nine female microvine breeding lines (Table 22) currently maintained in the CSIRO glasshouse on the Waite campus.

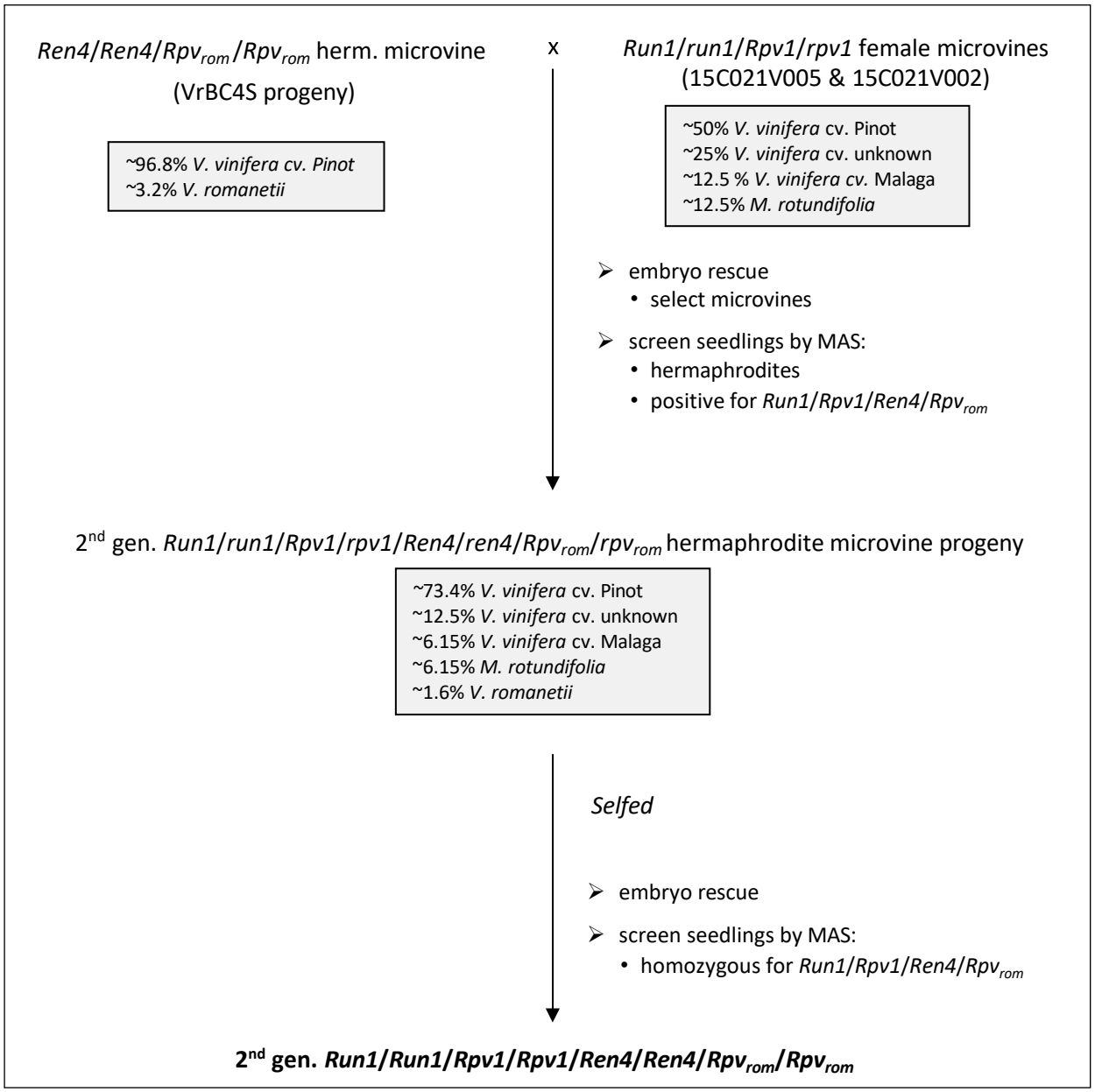


Figure 24. *Generation of homozygous female microvine 2nd gen. breeding lines via Strategy 4.*

***Table 19**. Summary of germination success using embryo rescue technique with Strategy 4 seeds.*

Hermaphrodite parent	Seed No.	Total germ.	% germ.
15C2V242-23_06	400	145	36
15C2V242-23_07	593	348	59
15C2V242-23_13	626	228	36
15C2V242-23_14	420	98	23
VrS21F1C02_01	1000	263	26
Total	3039	1082	35

***Table 20.** List of female microvine and tall 2nd gen. breeding lines derived from Strategy 4. Bold indicates where the mildew resistance loci are present in the homozygous state. n/a = not applicable*

Selfed parent	Genotype name	***Run1***	***Rpv1***	***Ren4***	***Rpvrom***	Stature	Berry colour	Location	Flowering as of June 2022?
15C2V242-23_07	ST4_A_87	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	Rpv_{rom}/Rpv_{rom}	Mv	white	n/a	Yes
15C2V243_23_09	ST4_09_91	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/ren4*	Rpv_{rom}/rpv_{rom}	Mv	white	n/a	No
15C2V242-23_13	ST4_07_53	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	Rpv_{rom}/Rpv_{rom}	Tall	white	37_26_04_01	No
15C2V242-23_13	ST4_09_113	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	Rpv_{rom}/Rpv_{rom}	Tall	White	37_26_03_02	No

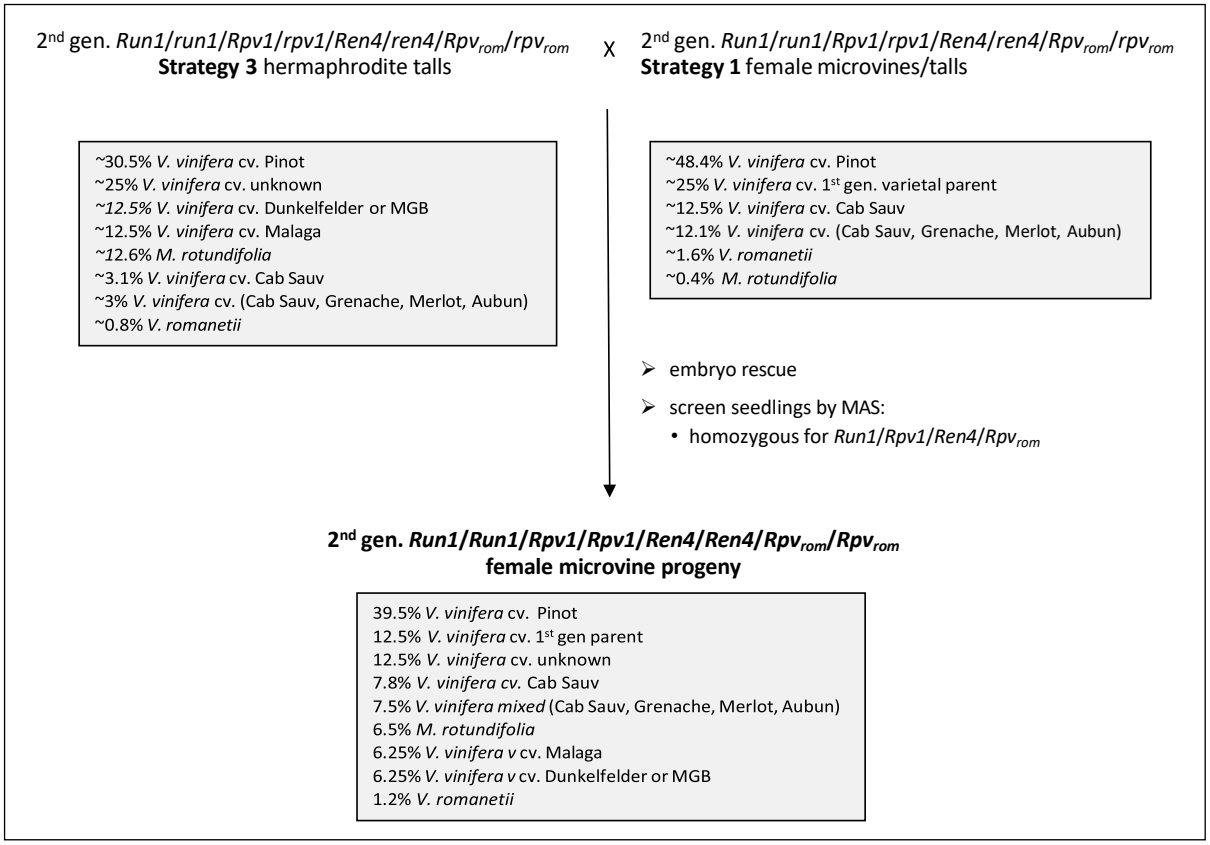


Figure 25. *Generation of homozygous female microvine 2nd gen. breeding lines via Strategy 4NEW. MGB = Muscat Gordo Blanco*

***Table 21**. List of female breeding line/pollen source combinations used in Strategy 4NEW crosses.*

Female parent	Stature	Berry colour	Male parent	Stature	Berry colour	Seed No.	Total germ.	% germ.
37140502_75_5	Mv	white	15C2V2_R04_1_30	Tall	red flesh	20	2	10
37140502_75_15	Mv	red flesh	15C2V2_R04_1_30	Tall	red flesh	10	10	100
37141202_138_31	Mv	white	15C2V2_R04_1_128	Tall	red flesh	51	13	25
37141202_138_47	Mv	white	15C2V2_R04_1_30	Tall	red flesh	13	5	38
37141202_138_47	Mv	white	15C2V2_R04_1_128	Tall	red flesh	30	19	63
37141202_138_58	Mv	white	15C2V2_R04_1_30	Tall	red flesh	17	12	71
39121003_75_39	Mv	white	15C2V2_R04_1_128	Tall	red flesh	7	3	43
39141202_138_9	Mv	white	15C2V2_R04_1_30	Tall	red flesh	36	12	33
39160603_138_10	Mv	white	15C2V2_R04_1_128	Tall	red flesh	57	22	39
39160702_138_10	Mv	white	15C2V2_R04_1_30	Tall	red flesh	34	25	74
39170303_75_15	Tall	white	15C2V2_6_15	Tall	white	82	59	72
39170303_75_15	Tall	white	15C2V2_R04_1_112	Tall	red flesh	84	48	57
39170303_75_22	Tall	muscat	15C2V2_6_15	Tall	white	270	116	43
39170303_75_22	Tall	muscat	15C2V2_R04_1_30	Tall	red flesh	21	18	86
					Total	732	364	50

Table 22. List of female microvine and tall 2nd gen. breeding lines derived from Strategy 4NEW crosses. Bold indicates where the mildew resistance loci are present in the homozygous state. n/a = not applicable

Genotype name	**Female parent**	**Male parent**	***Run1 locus***	***Rpv1 locus***	***Ren4 locus***	***Rpv$_{rom}$ locus***	**Berry colour**	**Location**	**Flowering as of June 2022**
Microvine									
ST4_CM_2_3	39160702_138_10	15C2V2_R04_1_30	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	n/a	Yes
ST4_CM_2_1	39160702_138_10	15C2V2_R04_1_30	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/ren4*	*Rpv$_{rom}$/rpv$_{rom}$*	white	n/a	No
ST4_CM_4_3	39160603_138_10	15C2V2_R04_1_128	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/ren4*	*Rpv$_{rom}$/rpv$_{rom}$*	white	n/a	No
ST4_CM_5_2	39141202_138_9	15C2V2_R04_1_30	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/ren4*	*Rpv$_{rom}$/rpv$_{rom}$*	white	n/a	No
ST4_CM_1_1	39160603_138_11	15C2V2_42_23_13	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	n/a	Yes
ST4_CM_1_2	39160603_138_11	15C2V2_42_23_13	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	n/a	No
ST4_CM_2_6	39160702_138_10	15C2V2_R04_1_30	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	n/a	No
ST4_CM_4_6	39160603_138_10	15C2V2_R04_1_128	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	n/a	No
ST4_CM_4_7	39160603_138_10	15C2V2_R04_1_128	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	n/a	No
ST4_CM_10_1	37141202_138_31	15C2V2_R04_1_128	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	n/a	No
Tall									
ST4_C_3_41	39170303_75_15	15C2V2_R04_1_112	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	37_26_11_03	No
ST4_C_4_1	37140502_75_5	15C2V2_R04_1_30	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/ren4*	*Rpv$_{rom}$/rpv$_{rom}$*	white	37_27_01_04	No
ST4_C_2_16	39170303_75_22	15C2V2_6_15	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	37_26_10_03	No
ST4_C_3_8	39170303_75_15	15C2V2_R04_1_112	*Run1/Run1*	*Rpv1/Rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	37_26_10_04	No
ST4_C_3_17	39170303_75_15	15C2V2_R04_1_112	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	37_27_01_02	No
ST4_C_3_24	39170303_75_15	15C2V2_R04_1_112	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	37_27_01_03	No
ST4_C_3_31	39170303_75_15	15C2V2_R04_1_112	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	37_26_11_02	No
ST4_CM_4_10	39160603_138_10	15C2V2_R04_1_128	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	red flesh	Glasshouse	n/a
ST4_CM_9_1	37141202_138_47	15C2V2_R04_1_128	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv$_{rom}$/Rpv$_{rom}$*	white	Glasshouse	n/a

6.5 Development of breeding lines to produce 3rd gen. winegrape varieties with three powdery mildew resistance loci.

Soon after the commencement of this project, we became aware that the French INRA ResDur winegrape breeding program was embarking on a breeding strategy to incorporate three powdery mildew resistance loci and three downy mildew resistance loci within new winegrape varieties, to maximise durability against these two pathogens (Merdinoglu et al. 2018).

While this was not an original aim of this project, it was decided in Year 2 that this project should also commence the preparation of breeding lines that could be used in future breeding projects to produce new winegrape varieties that contain three different powdery mildew resistance loci. Given the more widespread impact of powdery mildew infection on winegrape production in Australia, compared to downy mildew infection, this was considered to be a higher priority for Australian growers than the production of breeding lines that contain three different downy mildew resistance loci.

CSIRO had previously been involved in a collaboration with researchers at UC-Davis to genetically map and functionally characterise powdery mildew resistance originating from the wild Chinese grapevine species, *V. piasezkii*. The results of this work showed that the *V. piasezkii* genome contained at least two powdery mildew resistance loci, designated *Ren6* and *Ren7* (Pap et al. 2016). The *Ren6* resistance response was found to be stronger than that mediated by *Ren7*, but observations in California suggested that *Ren6*-mediated powdery mildew resistance may be less durable in the field than *Ren7*-mediated resistance (Dr Summaira Riaz, personal communication), making *Ren7* a better candidate for pyramiding strategies.

6.5.1 Development of Ren7 backcross breeding lines

In order to generate 3rd gen. mildew-resistant varieties with three powdery mildew resistance loci it was first necessary to undertake a backcrossing program with *V. piasezkii* to reduce the contribution of the *V. piasezkii* genome in the breeding lines. As reported in Pap et al. (2016) we had previously created an F1 population (VpF1) by crossing *V. piasezkii* accession DVIT2027 (previously imported from the UC-Davis germplasm collection, California, USA) with picovine line 06C008V0003. Prior to the commencement of this project, female VpF1 lines containing the *Ren7* powdery mildew locus were identified by MAS (data not shown) and backcrossed with picovine line 06C008V0004 to create VpBC1 lines. One of these female VpBC1 lines (Vp003V006BC1-19) was the starting point for further backcrossing undertaken in this project (Fig. 26).

Female VpBC2 line Vp003V006BC1-19 was pollinated with pollen from picovine 06C008V0004 to generate VpBC2 progeny. A total of 50 seedlings were obtained by embryo rescue of which 38 were genotyped using the two simple-sequence repeat (SSR) markers subtending the *Ren7* locus outlined in Pap et al. (2016). Based on these markers we were able to identify three microvine female VpBC2 lines containing the *Ren7* locus. Once these three VpBC2 lines had commenced flowering they were pollinated with pollen from microvine hermaphrodite lines generated via Strategy 3M (VrBC4S40F1_11; Table 9) and 3R (Vr42F1R04_1; Table 9) to produce VpBC3 seed (Fig. 26). A total of 2033 VpBC3 seeds were collected and germinated via embryo rescue (Fig. 26). A total of 322 microvine lines were selected for MAS which identified 5 VpBC3 *Ren4*/*ren4*/Rpv_{rom}/rpv_{rom} /*Ren7*/*ren7* red flesh hermaphrodite microvine progeny and 4 VpBC3 *Ren4*/*ren4*/Rpv_{rom} /rpv_{rom} /*Ren7*/*ren7* muscat hermaphrodite microvine progeny. These were established in the glasshouse to assess flowering potential.

6.5.3 Breeding Strategy 5

Breeding Strategy 5 is outlined in Figure 27. Pollen was collected from the two best flowering VpBC3 hermaphrodite lines containing either the red flesh locus (VpBC3_R04_1_146) or the muscat locus (VpBC3_11_44) and used to pollinate the 2nd gen. female microvine line 15C2V2_10_5 (Table 10). Approximately 1,600 seed were collected and subjected to embryo rescue and MAS. The inheritance of the *Ren7* powdery mildew locus was detected using CAPS markers as described in Section 5.5.2.

Marker-assisted selection identified nine microvine and three tall 3rd gen. female breeding lines that contained all three powdery mildew resistance loci (*Run1*, *Ren4* & *Ren7*) together with both downy mildew resistance loci (*Rpv1 & Rpv$_{rom}$*) (Table 23). In four of these 3rd gen. female breeding lines the *Ren4* and *Rpv$_{rom}$* loci were found to be present in the homozygous state. Furthermore, as a result of the fact that both parents contained the *Dxs* muscat locus, two of the 3rd gen. microvine breeding lines were also homozygous for the muscat locus. In addition, two of the 3rd gen. microvine breeding lines were found to contain both the muscat locus and the red flesh locus. This means that our breeding program has successfully combined three powdery mildew resistance loci, two downy mildew resistance loci, a red flesh locus and a muscat locus, from five different parents, into a female breeding line that is approx. 90% *V. vinifera* in genomic composition (Fig. 27). The microvine 3rd gen. breeding lines were established in the glasshouse and the three tall 3rd gen. breeding lines were planted in the field in Feb 2021 to assess flowering potential.

The original aim of this component of the project was to create 3rd gen. female breeding lines that could be crossed with any susceptible premium winegrape variety to produce new winegrape varieties of ~95% *V. vinifera* genomic composition with extremely durable resistance to powdery and downy mildew and unique sensory characteristics. When this activity was initiated in Year 2 of this project, it was not anticipated that it would be possible to commence the breeding and selection of 3rd gen. mildew-resistant winegrape varieties by the end of the project, because it was going to require at least three crosses to produce the 3rd gen. breeding lines from the germplasm available. However, fortunately, three of the 3rd gen. female microvine lines (SY5_B_25, SY5_B_107 & SY5_B_112) produced flowers before the end of this project (Table 23) and these were pollinated with pollen from four premium wine varietals: Shiraz, Cabernet Sauvignon, Malbec and Sauvignon Blanc.

Table 24 summarises the germination success and recovery of 3rd gen. tall hermaphrodite progeny from seed collected from Strategy 5 crosses. Seed numbers were low because of the small number of flowers available for pollination on the 3rd gen. breeding lines. However, seven tall hermaphrodite 3rd gen. mildew-resistant winegrape varieties were recovered which represents a frequency of 2.6% per germinated seedling compared to the theoretical percentage of 6%. In addition, nine new 2nd gen. mildew-resistant winegrape varieties were also recovered which lack the *Ren7* powdery mildew resistance locus. The seven 3rd gen. varieties are predicted to show a mixture of red flesh and red skin phenotypes based on marker analysis (Table 25). Two of the seven 3rd gen. varieties (Table 25) and one of the nine 2nd gen. varieties recovered from these Strategy 5 crosses (Supplementary Table 1) were planted in Nuriootpa in the 2021/22 season while the remainder are being maintained in the glasshouse until they can be planted later in 2022.

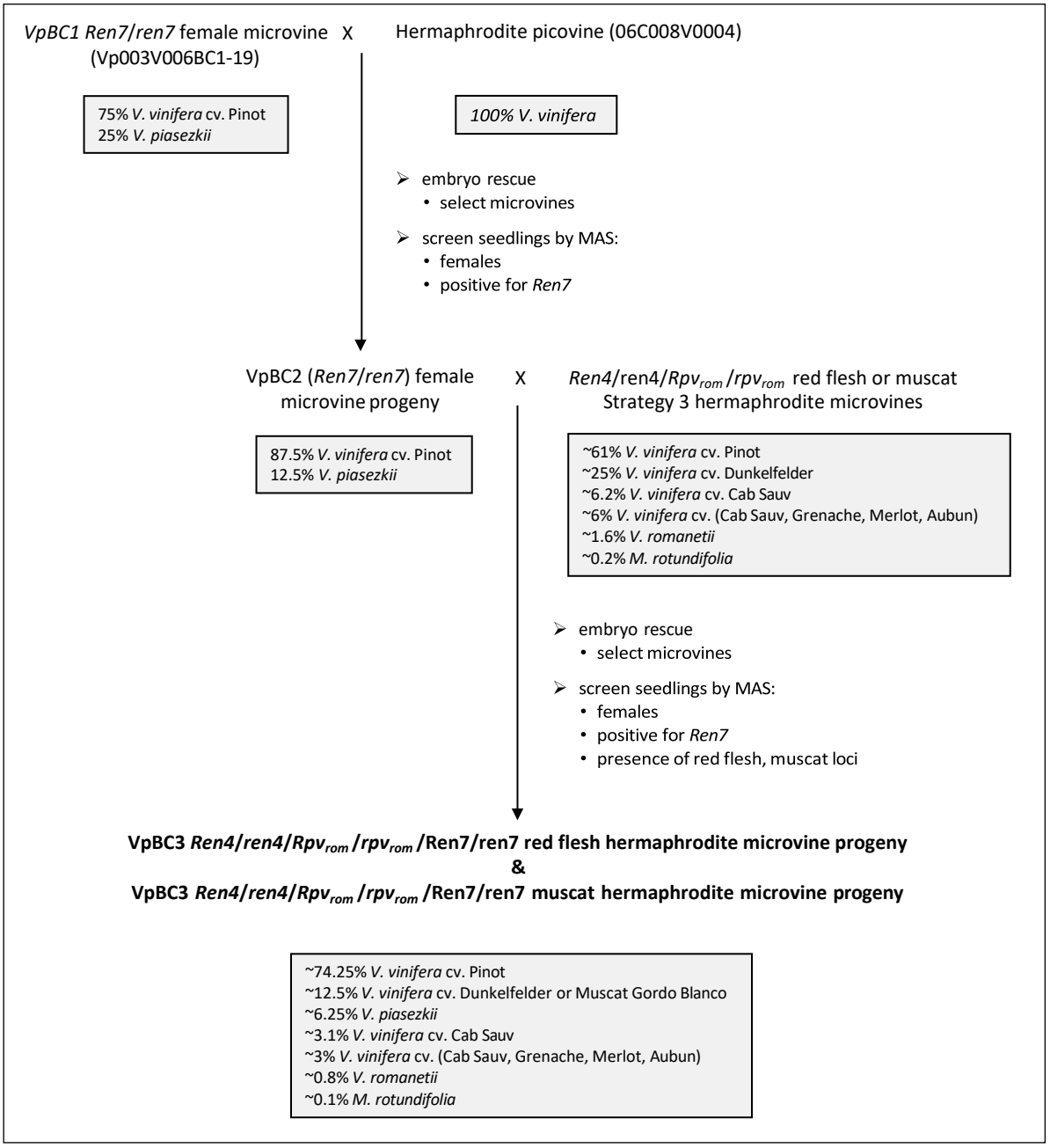


Figure 26. *Generation of VpBC3 breeding lines for use in breeding Strategy 5.*

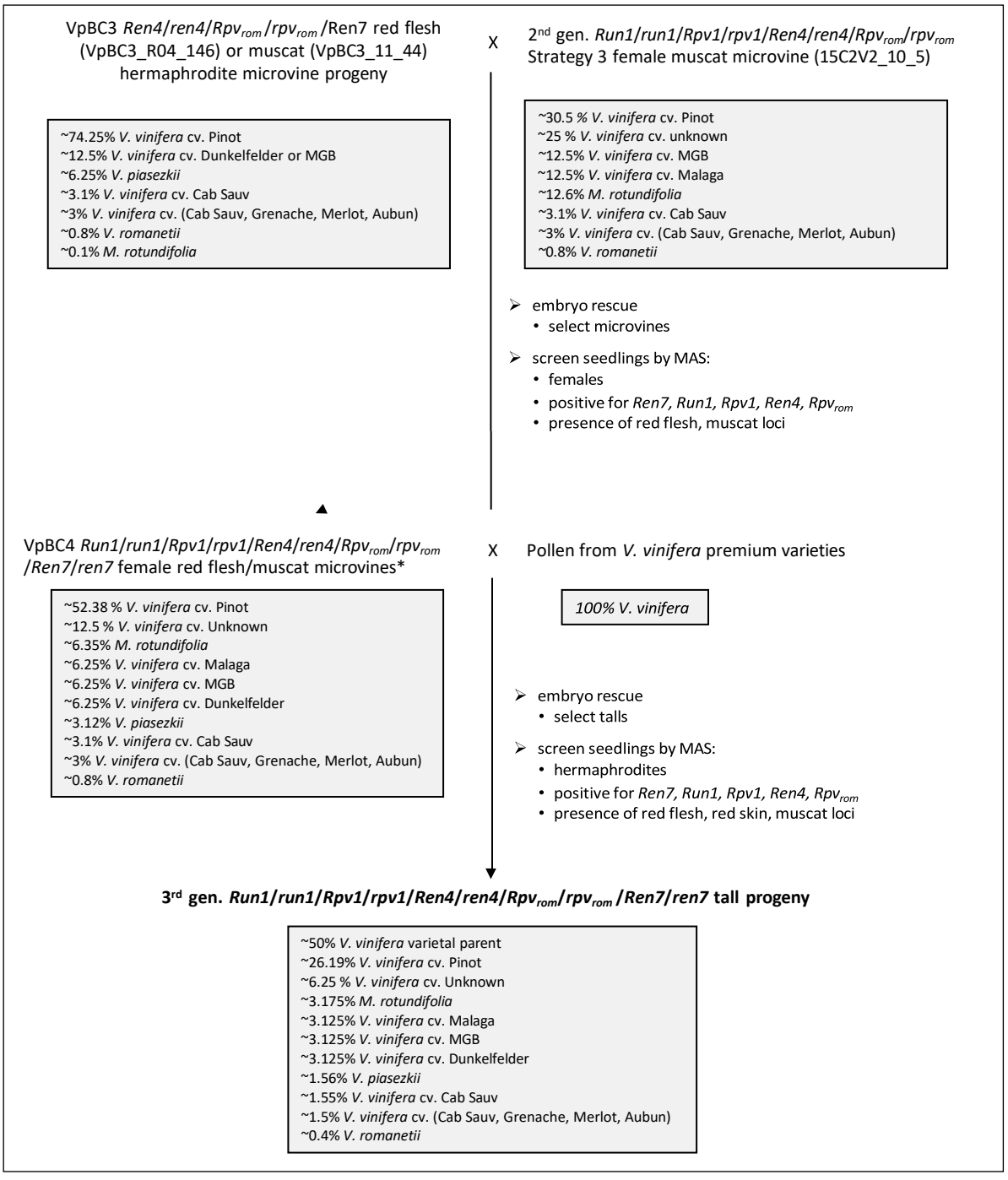


Figure 27. *Breeding Strategy 5. The estimated percent contribution of different parents to the genotype of the different breeding lines and progeny is also shown. Asterisk indicates that only the example of the cross involving VpBC4 microvine progeny that were heterozygous for the red flesh locus and the muscat locus is illustrated.*

***Table 23**. List of female microvine and tall 3rd gen. female breeding lines. Both female and male parents are microvines. Bold indicates where the loci are present in the homozygous state. Dxs is the wildtype muscat allele as outlined in Table 6.*

Genotype name	Female parent	Male Parent	*Run1 locus*	*Rpv1 locus*	*Ren4 locus*	*Rpv_{rom} locus*	*Ren7 locus*	Muscat locus	Berry colour	Location	Flowering as of June 2022?
Microvines											
SY5_A_11	15C2V2_10_5	VpBC3_11_44	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/Dxs*	white	Glasshouse	No
SY5_A_12	15C2V2_10_5	VpBC3_11_44	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/Dxs*	white	Glasshouse	No
SY5_A_40	15C2V2_10_5	VpBC3_11_44	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv_{rom}/Rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	white	Glasshouse	No
SY5_B_25	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	red flesh	Glasshouse	Yes
SY5_B_58	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv_{rom}/Rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	red flesh	Glasshouse	No
SY5_B_74	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*dxs/dxs*	white	Glasshouse	No
SY5_B_79	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv_{rom}/Rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	red flesh	Glasshouse	No
SY5_B_107	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	red flesh	Glasshouse	Yes
SY5_B_112	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	white	Glasshouse	Yes
Talls											
SY5_A_45	15C2V2_10_5	VpBC3_11_44	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	white	37_26_06_03	No
SY5_B_12	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/Ren4*	*Rpv_{rom}/Rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	white	37_26_06_03	No
SY5_B_82	15C2V2_10_5	VpBC3_R04_146	*Run1/run1*	*Rpv1/rpv1*	*Ren4/ren4*	*Rpv_{rom}/rpv_{rom}*	*Ren7/ren7*	*Dxs/dxs*	white	37_26_07_01	No

***Table 24**. Summary of germination success and recovery of 2nd gen. and 3rd gen. tall hermaphrodite progeny from Strategy 5 crosses. The genotype of 2nd gen. progeny is Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv_{rom} /rpv_{rom} whereas 3rd gen. progeny have the genotype Run1/run1/Rpv1/rpv1/Ren4/ren4/Rpv_{rom} /rpv_{rom} /Ren7/ren7. Malbec seeds were germinated via the filter paper method instead of embryo rescue which is why there are no microvine progeny recorded.*

Female parent (Mv.)	Male parent	Seed No.	Total germ.	% germ.	Mv.	Talls	3rd gen. progeny recovered	2nd gen. progeny recovered
SY5_B_25 (red flesh/muscat)	Cab. Sauv.	35	23	66	5	5	0	1
SY5_B_107 (red flesh/muscat)	Shiraz	255	146	57	26	50	2	1
SY5_B_107 (red flesh/muscat)	Malbec	211	86	41	-	86	5	7
SY5_B_112 (muscat)	Sauv. Blanc	42	10	24	1	3	0	0
	Total	543	265			144	7	9

***Table 25**. 3rd generation mildew-resistant varieties for field evaluation*

Location	Variety name	Female parent	Male parent	Colour	Planted
37_28_04_01	ST53M_SH_1_7	SY5_B_107	Shiraz	red flesh	14.12.21
39_08_06_01	ST53M_SH_1_54	SY5_B_107	Shiraz	red flesh	16.02.22
Glasshouse	ST53_MA_1_20	SY5_B_107	Malbec	red skin	n/a
Glasshouse	ST53_MA_1_29	SY5_B_107	Malbec	red skin	n/a
Glasshouse	ST53_MA_1_31	SY5_B_107	Malbec	red flesh	n/a
Glasshouse	ST53_MA_1_37	SY5_B_107	Malbec	red flesh	n/a
Glasshouse	ST53_MA_1_45	SY5_B_107	Malbec	red flesh	n/a

n/a = not applicable

6.6 Evaluation of 2nd gen. mildew resistant winegrape varieties

6.6.1 Comparison of mildew resistance in 2nd gen. varieties vs 1st gen. varieties

The addition of a second powdery mildew resistance locus (*Ren4*) and a second downy mildew resistance locus (Rpv_{rom}) in 2nd gen. mildew-resistant winegrape varieties, compared to 1st gen. mildew-resistant varieties is designed to not only increase the durability of mildew resistance in the vineyard, but to also increase the resistance level of these varieties. This is especially significant in the case of downy mildew because the *Rpv1* downy mildew resistance locus from *M. rotundifolia*, that is present in the 1st gen. varieties, does not confer complete resistance against downy mildew and a low level of sporulation is normally observed under high disease pressure conditions (Feechan et al. 2013).

To compare the relative levels of mildew resistance displayed by 2nd gen. varieties in comparison to 1st gen. varieties, cuttings of five different 1st gen. varieties were collected from the field in Nuriootpa and established as potted vines in the glasshouse, together with potted vines of seven 2nd gen. varieties which had yet to be planted in the field, and Cabernet Sauvignon to be used as a susceptible control. Leaves were collected at the same stage of development and used for the detached leaf powdery mildew or leaf disc downy mildew assays as described in Section 5.7.

The results of Figure 28 show that powdery mildew development is further reduced in 2nd gen. varieties compared to 1st gen. varieties with an average over 95% of the epidermal cells penetrated by the germinated powdery mildew spores initiating an effective PCD-based resistance response in 2nd gen. varieties compared to 31% in 1st gen. varieties. However, it is worth noting that the resistance response observed in 1st gen. varieties is still effective enough to prevent powdery mildew growth and sporulation under field conditions.

Similarly, downy mildew sporulation was further reduced in 2nd gen. varieties compared to 1st gen. varieties and the Cabernet Sauvignon control discs (Fig. 29). In comparison to the susceptible control variety Cabernet Sauvignon, sporulation was reduced by 92 - 97% (ave. 95.3%) in 2nd gen. varieties compared to 80 - 94% (ave. 88.8%) in 1st gen. varieties under these high disease pressure conditions. The increased resistance of 2nd gen. varieties to downy mildew infection, compared to 1st gen. varieties is visually illustrated in Figure 30.

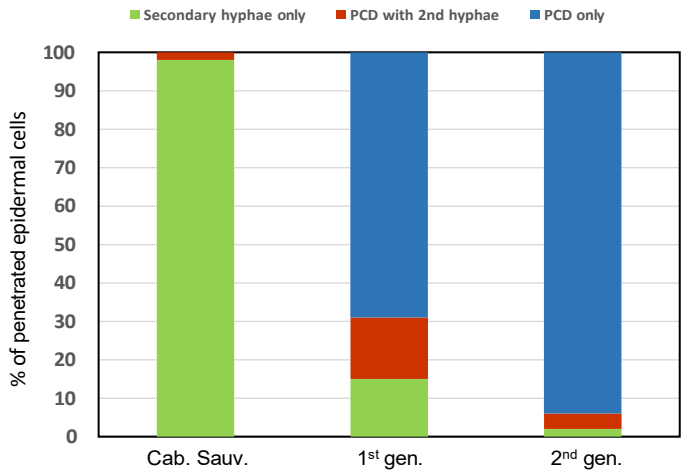


Figure 28. *Comparison of powdery mildew susceptibility of 1st gen. and 2nd gen. varieties under high disease pressure conditions. Detached leaves of five 1st gen. varieties, seven 2nd gen. varieties and the susceptible variety Cabernet Sauvignon were inoculated with the same powdery mildew inoculum and the level of infection detected 48 hpi. Results are averaged across all 1st and 2nd gen. varieties and are expressed as the % of germinated conidia that (a) resulted in a successful penetration of an epidermal cell and produced a secondary hyphae (b) successfully penetrated an epidermal cell and induced a programmed cell death (PCD) resistance response in the penetrated cell but the response was not rapid enough to prevent production of a secondary hyphae resistance response or (c) successfully penetrated an epidermal cell and induced a PCD resistance response that prevented any further hyphal growth.*

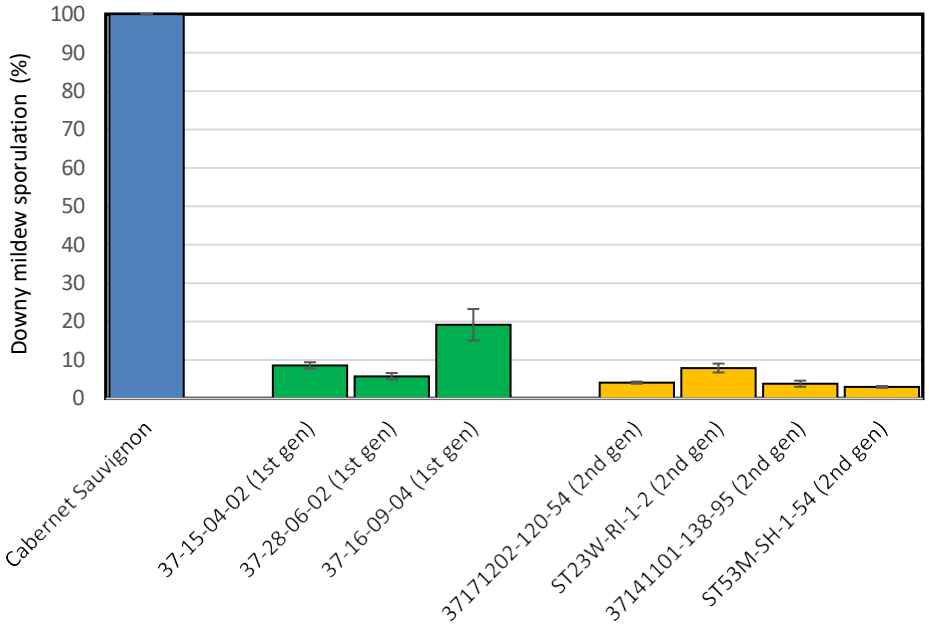


Figure 29. *Comparison of downy mildew susceptibility of 1st gen. and 2nd gen. varieties under high disease pressure conditions. Leaf discs from detached leaves of three 1st gen. varieties, four 2nd gen. varieties and the susceptible variety Cabernet Sauvignon were inoculated with the same downy mildew inoculum and the level of downy mildew sporulation measured 6 dpi. Results are expressed as the percent sporulation, in comparison*

to the Cabernet Sauvignon control, averaged across at least 2-4 independent assays for each genotype. Error bars are standard errors.

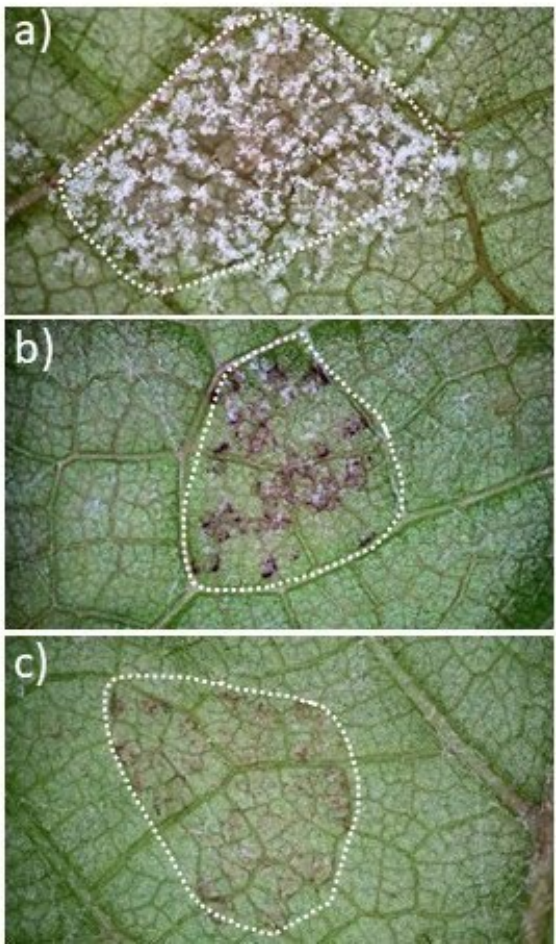


Figure 30. *Comparison of downy mildew susceptibility of 1st gen. and 2nd gen. varieties under high disease pressure conditions. Photos show representative levels of sporulation on leaf discs of (A) Cabernet Sauvignon (B) 1st gen. variety and (C) 2nd gen. variety 6 dpi with grapevine downy mildew. The brown regions indicate mesophyll cells that have undergone the PCD resistance response. The white dotted line indicates the area of inoculation with the downy mildew sporangia suspension. A low level of sporulation is still visible in the inoculated region of the leaf disc of the 1st gen. variety but virtually no sporulation is visible on the 2nd gen. variety.*

6.6.2 Vine performance in field

The full list of 2nd gen. mildew-resistant winegrape varieties generated by the different breeding strategies is shown in Supplementary Table 1. Of the 686 2nd gen. mildew-resistant winegrape varieties generated in this project, 571 have been established in the SARDI vineyard in Nuriootpa, while the remaining 115 are currently in the glasshouse awaiting planting. Table 26 shows a breakdown of the different types of 2nd gen. varieties generated and from which breeding strategy.

The 2nd gen. varieties vines currently established in the field for evaluation were planted between March 2019 - February 2022 (Supplementary Table 1). Weather data recorded at Nuriootpa weather station were obtained from the Bureau of Meteorology for the period October 2019 to April 2022 and are shown in Figure 31.

***Table 26**. Summary of all 2nd gen. varieties generated by the different breeding strategies used in this project.*

	White	White muscat	Red skin	Red flesh	Total
Strategy 1	144	23	89	146	402
Strategy 2	59	8	47	58	172
Strategy 3	48	44	0	11	103
Strategy 5	0	0	5	4	9
Total	251	75	141	219	686

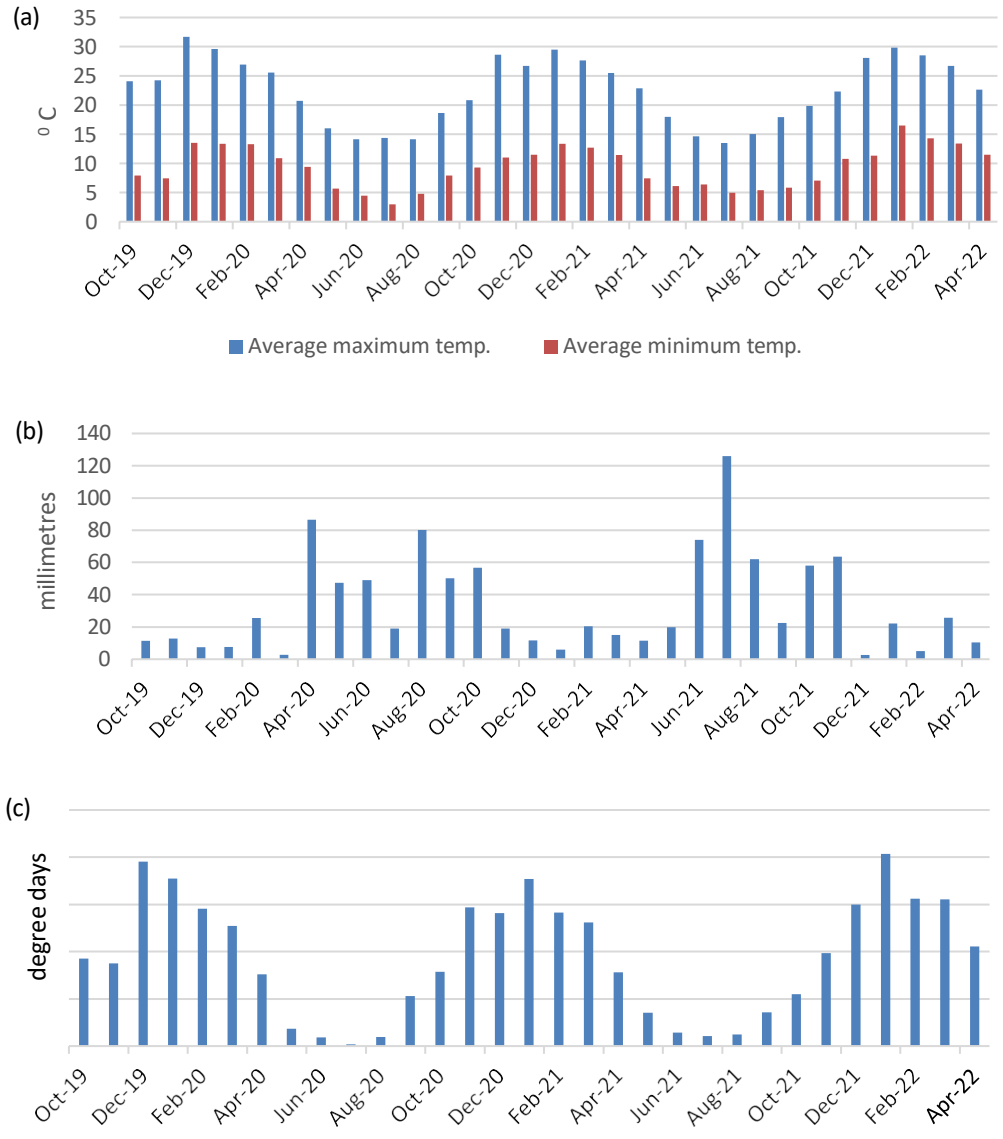


***Figure 31**. BOM weather station data near the planting site of 2nd gen. mildew resistant varieties. (a) Monthly average max. & min. temperature (b) Monthly average rainfall (c) Monthly growing degree days.*

Many vines planted in March 2019 produced fruit in their second year of growth (2020/21 season) but this was removed to encourage vine growth. In the 2021/22 season, it was observed that approx 37% of the Strategy 3R or 3M vines were showing an unusual flowering phenotype involving the production of 2-3 bunches per shoot with a very large number of flowers per bunch (Fig. 32). A number of these shoots lacked a growing tip and appear stunted. It appeared that the unusually high production of flowers had inhibited normal shoot elongation. Removal of the bunches did not stimulate any further shoot growth later in the season (data not shown). As this unusual flowering phenotype was only observed in Strategy 3 varieties it strongly suggests that the gene(s) responsible for this phenotype have been inherited from the female tall 15C002V0002 breeding line (Figs 16 & 17). Interestingly, 15C002V0002 breeding lines grown in the same vineyard, produce large number of flowers and bunches but shoot growth appears to be normal. However, as not all Strategy 3 progeny display this unusual phenotype, the activity of the gene(s) responsible which have been inherited from the 15C002V0002 breeding line, must be modulated by gene(s) donated from the male parent also.

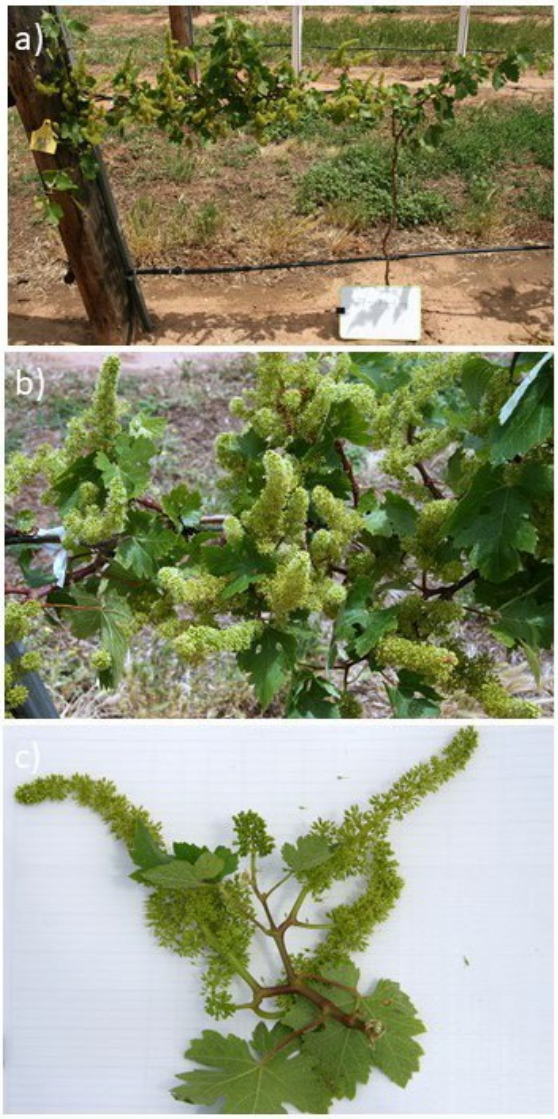


Figure 32. *Unusual flowering phenotype displayed by selected Strategy 3R or 3M 2nd gen. varieties. (a) 2nd gen. variety 15C2V2_11_181 (b) close-up of bunches (c) individual shoot showing three bunches on one shoot.*

In season 2021/22, fruit development was followed using weekly Brix measurements and fruit was harvested from a total of 41 2nd gen. white varieties. Of these, 16 were 3 year old vines planted in March 2019. In addition, because it was in the final year of the project, it was decided to also harvest fruit from 24 2 year old vines that demonstrated vigorous growth. At harvest, the total yield, bunch number and average berry weight were recorded (Supplementary Table 2) and photos were taken of bunch structure. If the total harvest weight was > 1.5 kg the fruit was also used for micro-scale fermentation (section 5.11). In addition, fruit was harvested from five established vines of premium winegrape varieties and the Strategy 3 breeding line 15C002V0002 that were growing at the same location to enable comparison of fruit and wine chemistry and sensory.

Harvest dates for the 2nd gen. white varieties were generally later than for the five premium varieties with 73% of 2nd gen. varieties harvested 2-6 weeks after the latest of the premium varieties. Given the young age of the 2nd gen vines, this may be more of a reflection of the limited capacity of the small canopies on these vines to provide sugar for ripening rather than a genetic trait associated with ripening time. It will be interesting to follow harvest dates as the vines continue to mature, as delayed ripening represents a potentially attractive trait for Australian growers dealing with climate change-mediated harvest compression.

Average yields for the 2nd gen. white varieties ranged from 0.65 - 5.93 kg per vine (Supplementary Table 2) but it is not valid to make comparisons between the different varieties because of the different ages of the vines and the fact than no attempt was made to standardise bud numbers at this early stage of development.

Average berry weight, a measure of berry size, is considered as an important trait of grapes that affects wine quality, with larger berries being the least desirable (Matthews and Nuzzo 2007). It is typically a genotypic trait. Average berry weights for the 2nd gen. white varieties harvested ranged from 0.46 – 2.0 g per berry (Fig. 33), which is slightly lower than for the premium varieties but again this may be more a function of vine age.

Bunch architecture is determined largely by inflorescence development prior to anthesis (Shavrukov et al. 2004). A more open bunch reduces contact between berries and allows greater airflow through the bunch, reducing the humidity after a rain event. These attributes assist in reducing the incidence of botrytis infection. Published studies indicate that the genetic control of bunch architecture is likely to be highly complex with many genes contributing minor effects (Dry et al. 2019). Thus, it is not currently feasible to select against tight bunch varieties at the seedling stage using MAS and this trait must be assessed at fruit development. Bunch architecture was recorded photographically for all 2nd gen. white varieties harvested in 2022. Significant variation was observed in bunch architecture ranging from tight to open (Fig. 34).

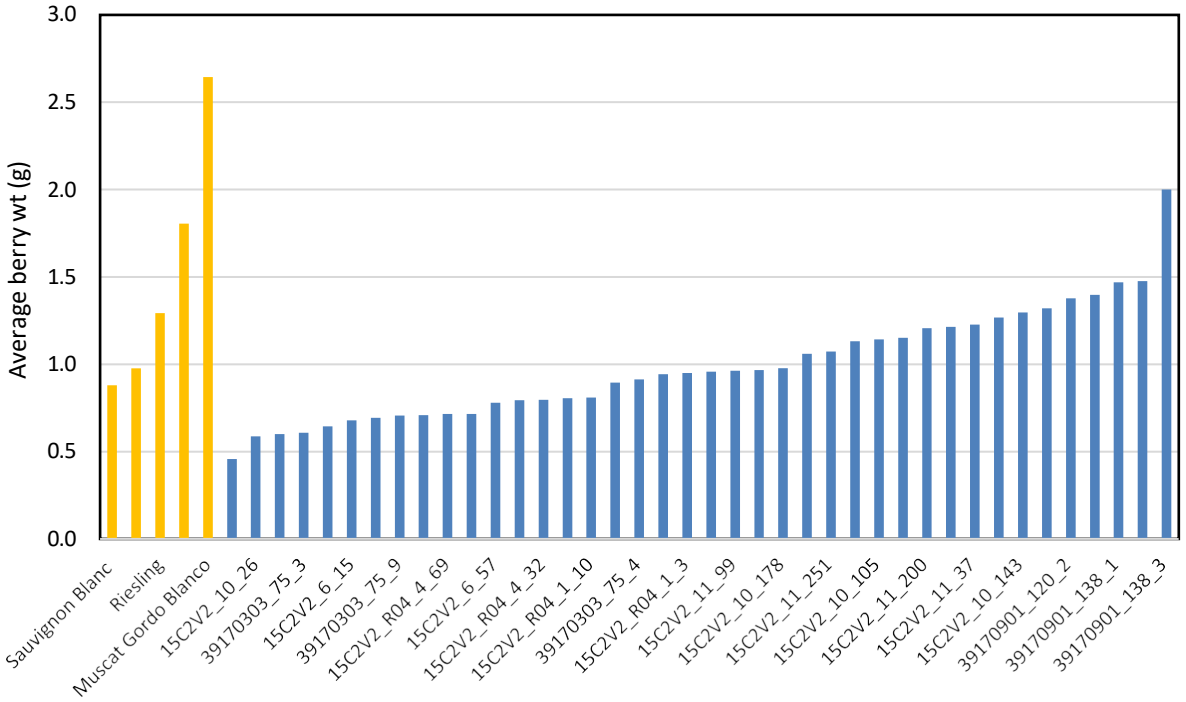


Figure 33. *Average berry weight of 2nd gen. mildew-resistant varieties harvested in 2021/22 season.*

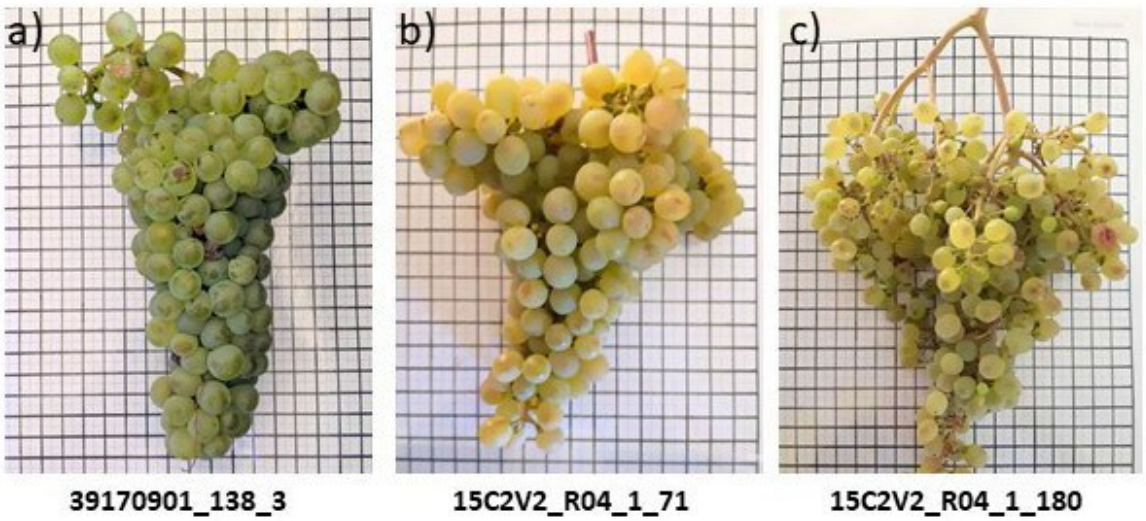


Figure 34*. Examples of different bunch architectures within the 2nd gen. mildew-resistant varieties harvested in 2022. (a) tight bunch (b) medium openness bunch and (c) open bunch*

6.6.3 Chemical analysis of berries

For winemaking, the levels of yeast assimilable nitrogen (YAN), organic acids and juice pH are important to avoid additions in the winery that increase the cost of wine production.

Nitrogen is a critical grape nutrient for yeast growth and fermentation activity and affects the rate and completion of fermentation, fermentation bouquet and wine style (AWRI 2022). YAN analysis provides information on the nitrogen status of grapes, musts and juices: specifically, the amount of nitrogen available for yeast to utilise during fermentation. In the winery, DAP is added if YAN is low, to prevent stuck ferments. A total of 39/40 2nd gen. varieties harvested were found to have YAN levels > 150 mg/L which is the minimum YAN requirement for low-risk fermentation of white wines (AWRI 2022).

Organic acids present in grape juice contribute to its sensory quality, because they ensure a balance between sweet and acid flavours, which confers palatability (Gurak et al. 2010). The pH of the berry juice/must affects the taste, sugar/acid balance and stability of wines (Yara, 2018) and tartaric acid is added to adjust the pH if it is too high. Thus, selections that have good acid levels at harvest will also reduce winery inputs. Tartaric acid levels in the 2nd gen. varieties ranged from 5.6 - 9.9 g/L compared to 6.8 - 10.7 g/L for the premium varieties (Supplementary Table 2). Malic acid levels in the 2nd gen. varieties ranged from 0 - 3.6 g/L compared to 0.2 - 3.8 g/L for the premium varieties (Supplementary Table 2).

The juice of harvested fruit of 2nd gen. varieties was found to be in the pH range 3.21 - 4.04 compared to 3.03 - 3.58 of the premium varieties (Supplementary Table 2). Indeed, 28/40 of the 2nd gen. varieties had a juice pH higher than all the premium varieties examined (Fig. 35). However, this is not surprising based on previous observations involving the field evaluation of 1st gen. varieties (Wine Australia Project CSP 1303) which showed that the pH of fruit at harvest decreased as the vines matured.

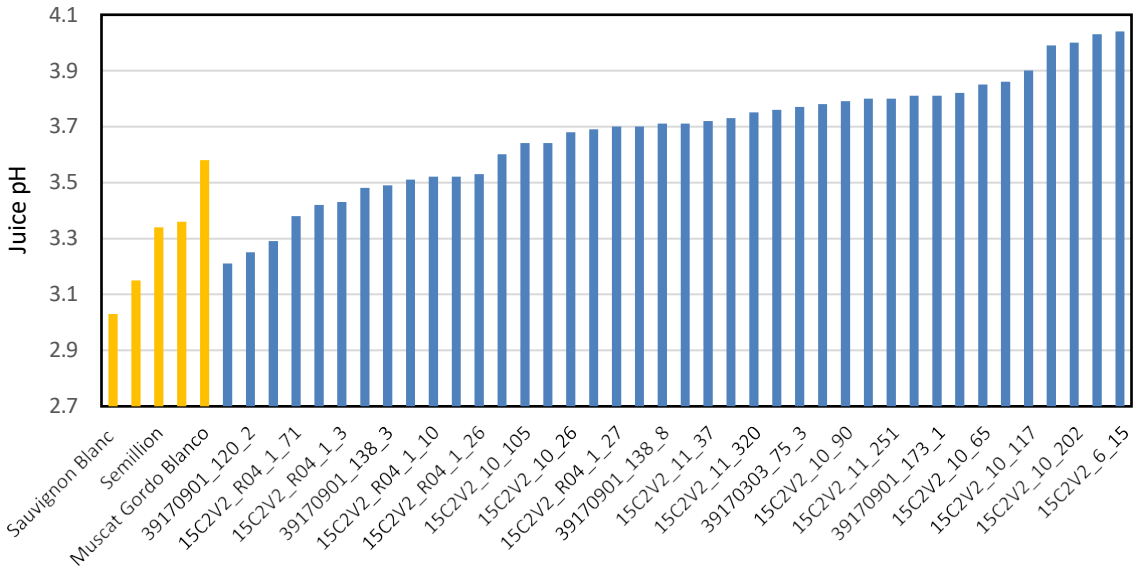


Figure 35. *Juice pH of 2nd gen. mildew-resistant varieties harvested in 2021/22 season.*

6.6.4 Chemical analysis of wine

Sufficient fruit (> 1.5 kg per vine) was harvested from 33/40 of the 2nd gen. varieties (Supplementary Table 2) to undergo micro-scale fermentation (Section 5.11). Chemistry data for these micro-scale ferments are shown in Supplementary Table 2.

Selections were harvested when they reached 22 °Bx so that sensory analysis could be carried out on wines of approximately the same alcohol content. Most of the wines had alcohol levels between 12-14.6% which was in the expected range for grapes of that sugar content. However, several of the late-ripening varieties had to be harvested at less than 22 °Bx, which led to alcohol levels of < 12% in the final wine. During the winemaking process, tartaric acid was added to adjust the pH at the start of the fermentation for those selections with high pH to a pH of ~3.5. If the pH was below this, then no pH adjustment was made.

Volatile acids (VA) in wine can lift wine aromas and add to the complexity of the flavour components. However, in quantities higher than the optimum, it has spoilage properties. The predominant volatile acid is acetic acid. Wine produced from harvested fruit of 2nd gen. varieties was found to have volatile acids levels in the range 0.08 - 0.29 g/L compared to 0.07 - 0.24 g/L for the wines made from the premium varieties (Supplementary Table 2).

6.6.5 Sensory analysis of wine

The amount of wine obtained from the 1.5-2 kg of white grape bunches was around 350 mL. Yeast fermentation usually took around 6-8 days. Four tasters comprising two winemakers and two experienced wine consumers were asked to assess the wines using a nine-point hedonic scale. The hedonic scale was originally developed for the purpose of measuring the food preferences of soldiers (Peryam and Girardot 1952) and has since been widely adopted widely to assess the average degree of liking or disliking of foods or consumer products (Wichchukit and O'Mahony 2015). The tasters were also asked to record any comments on taste and style (Supplementary Table 2).

The average hedonic scale score across the four panelists ranged from 4.25 - 7 for the 2nd gen. white varieties, which compared very favourably with the average hedonic scale score range for the premium varieties of 5 - 6.5. Indeed, 28/33 2nd gen. varieties produced wine of equal or higher preference ranking to the lowest ranked premium variety (Sauvignon Blanc) and 4/33 produced wine of equal or higher preference to the highest ranked premium variety (Muscat Gordo Blanco). Figure 37 shows visual representation of the descriptors used most frequently by the tasters in describing 2nd gen. white wines with an average hedonic scale >5. Not surprisingly, muscat character was frequently mentioned, as half of the varieties in this group tested positive for the muscat locus (Supplementary Table 2). Overall, the wines were generally assessed as being floral and fruity with notes of citrus, stonefruit, apricot etc. with Riesling-like characters.

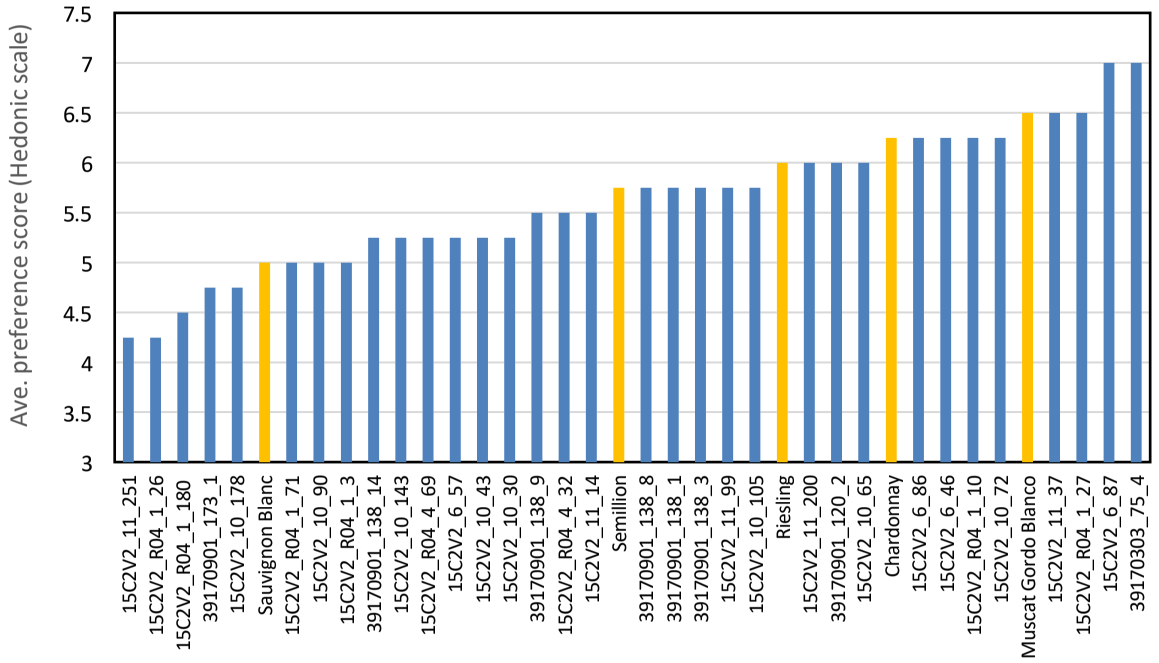


Figure 36. *Average preference score (Hedonic scale) of wines made from selected 2nd gen. mildew-resistant varieties harvested in 2021/22 season.*

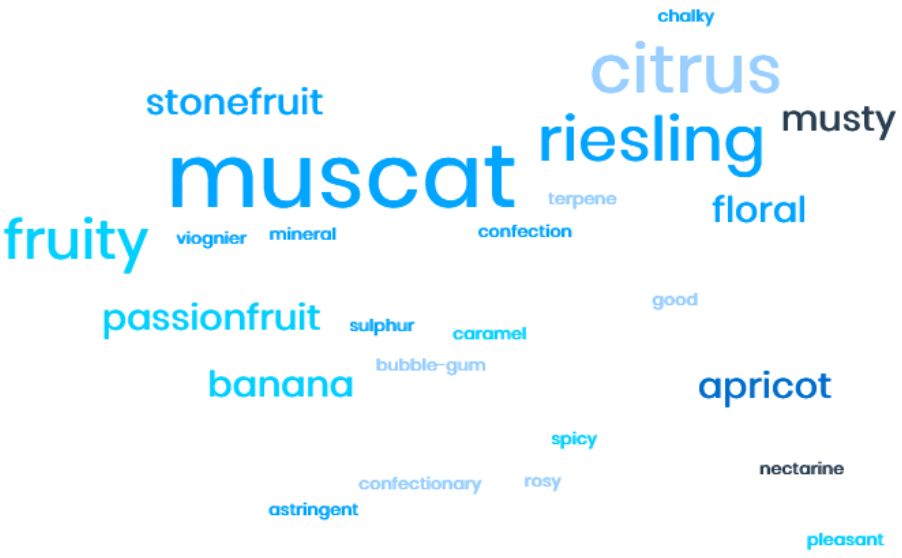


Figure 37. *Word cloud of sensory descriptors used to describe wines made from 2nd gen. mildew-resistant white varieties harvested in 2021/22 season.*

7. Outcomes/Conclusions

- At the commencement of this project it had been anticipated that a larger number of 2nd gen. mildew-resistant varieties would have been evaluated for viticultural production and wine sensory characteristics by project end. However, due to the slower than expected development of the female microvine breeding lines used in Strategy 1, the planting of 2nd gen. mildew-resistant winegrape varieties in the vineyard was delayed by approximately 12 months.

- The *Ren4* powdery mildew resistance locus from the wild Chinese species *V. romanetii* was shown to confer resistance against powdery mildew isolates that can break resistance conferred by the *Run1* locus from *M. rotundifolia* (Fig. 12), making it a good candidate for a pyramiding strategy with *Run1*, to develop new varieties with durable resistance to powdery mildew.

- The discovery that *Ren4*-mediated powdery mildew resistance also co-segregated, at a high frequency, with a strong downy mildew resistance locus (designated Rpv_{rom}), led to the development of the main breeding strategy (Strategy 1; Figs. 14 & 15) which was based on reciprocal crosses between previously generated 1st gen. mildew-resistant varieties established in the vineyard containing the *Run1*/*Rpv1* locus and microvine breeding lines that were homozygous for both *Ren4* and Rpv_{rom}.

- Breeding Strategy 1 generated 402 tall hermaphrodite 2nd gen. (*Run1*/*Ren4*/*Rpv1*/Rpv_{rom}) mildew-resistant winegrape varieties, from the MAS of over 11,000 seedlings (Table 5), with either red skin (89), red flesh (146), white skin (144) or white skin with muscat character (23). These 2nd gen. varieties were successfully established in an unsprayed block of the SARDI vineyard in Nuriootpa (Supplementary Table 1). Most of these vines have shown good growth and development in the field.

- An alternative breeding strategy (Strategy 3; Figs. 16 & 17) was also devised based on the use of a CSIRO female breeding line (15C002V0002) that is homozygous for the *Run1*/*Rpv1* locus crossed with *Ren4*/Rpv_{rom} microvine breeding lines bred to contain either the red flesh or muscat locus. Strategy 3 generated a total of 103 2nd gen. (*Run1*/*Ren4*/*Rpv1*/Rpv_{rom}) mildew-resistant winegrape varieties from the MAS of over 2,700 seedlings (Table 9), with either red-flesh (11), white skin (48) or white skin with muscat character (44), that were successfully established in the SARDI vineyard (Supplementary Table 1). Unfortunately, approximately one third of the 2nd gen. varieties generated via Strategy 3 were found to exhibit an unusual growth phenotype in the vineyard (Fig. 32), characterised by very large numbers of bunches per vine and flowers per bunch and severely inhibited shoot elongation making them unsuitable for commercial wine production.

- In addition to producing tall hermaphrodite 2nd gen. mildew-resistant winegrape varieties for field evaluation, breeding Strategy 1 and Strategy 3 also generated female microvine and tall 2nd gen. breeding lines containing both the *Run1*/*Rpv1 and Ren4*/Rpv_{rom} resistance loci in the heterozygous state (Fig. 18). The availability of these female 2nd gen. breeding lines allowed us to expand the diversity of premium winegrape varieties that could be used to generate new 2nd gen. mildew-resistant winegrape varieties (Figs. 20 & 21).

- Breeding Strategy 2 generated 172 tall hermaphrodite 2nd gen. (*Run1*/*Ren4*/*Rpv1*/Rpv_{rom}) mildew-resistant winegrape varieties from the MAS of over 4,000 seedlings (Tables 15 & 16), with either red skin (47), red-flesh (58), white skin (59) or white skin with muscat character (8). Strategy 2 breeding lines were pollinated with pollen collected from a more diverse range of premium winegrape varieties that had not been represented in breeding Strategies 1 or 3, such

as Cabernet Franc, Grenache, Malbec, Sauvignon Blanc and Viognier (Tables 13-14). To date, 65 lines have been established in the SARDI vineyard (Supplementary Table 1) and are showing good growth and development. The remaining 107 lines (Table 16) will be planted in the field in late 2022 (Supplementary Table 1).

- An additional 29 female tall 2nd gen. breeding lines with either red skin, red flesh or white skin were also selected from Strategy 2 crosses (Table 18) to expand the genetic (varietal) diversity of the 2nd gen. breeding lines. These will be planted in the field in late 2022 to evaluate their flowering potential.
- A strategy (Strategy 4) designed to breed female 2nd gen. lines that are homozygous for both the *Run1*/*Rpv1* and *Ren4*/*Rpv$_{rom}$* loci, based on selfing of heterozygous microvines, was unsuccessful most likely due to in-breeding depression. An alternative strategy (Strategy 4NEW), based on the crossing of parents with both the *Run1*/*Rpv1* and *Ren4*/*Rpv$_{rom}$* resistance loci in the heterozygous state, but with different genetic backgrounds, was also evaluated. Only two female homozygous *Run1*/*Rpv1*/*Ren4*/*Rpv$_{rom}$* progeny were recovered (Table 22). However, these lines are yet to be evaluated for flowering potential (Table 18).
- Good progress was made on an additional project aim, initiated in Year 2 of the project, to develop female breeding lines containing three different powdery mildew resistance loci (*Run1, Ren4 & Ren7*) and two downy mildew resistance loci (*Rpv1 & Rpv$_{rom}$*). The *Ren7* powdery mildew resistance locus from *V. piasezkii* was successfully introgressed into a microvine breeding line and then combined with the *Run1/Rpv1* and *Ren4/Rpv$_{rom}$* resistance loci. A total of 12 (nine microvine, three tall) female 3rd gen. breeding lines (containing three powdery mildew resistance loci) were obtained. In addition, all 3rd gen. breeding lines are positive for the muscat locus and four lines also contain the red flesh locus. Four 3rd gen. microvine breeding lines that had commenced flowering before the end of the current project were used in crosses with pollen from premium winegrape varieties which generated seven 3rd gen. mildew-resistant varieties for future field evaluation (Supplementary Table 1).
- The resistance of 2nd gen. mildew-resistant varieties to powdery mildew and downy mildew was shown to be enhanced in comparison to 1st gen. mildew-resistant varieties under identical conditions in laboratory assays. To the best of our knowledge these are the only mildew-resistant winegrape varieties available anywhere in the world that combine the *Run1*/*Rpv1* resistance locus from the wild North American grape species *M. rotundifolia* and the *Ren4*/*Rpv$_{rom}$* resistance locus from the wild Chinese grape species *V. romanetii*. This is significant because data obtained by the CSIRO team, in collaboration with US researchers, have demonstrated that powdery mildew isolates capable of breaking *Run1*-mediated resistance are not able to overcome *Ren4*-mediated resistance.
- Fruit was harvested from a limited number of two and three year old 2nd gen. mildew-resistant varieties in season 2021/22 (Supplementary Table 2). Harvest dates ranged from 25 February to 22 April and varieties showed a range of bunch architectures from tight to loose (Fig. 34).
- Sensory analysis was undertaken on micro-scale ferments of fruit harvested from 33 2nd gen. mildew-resistant varieties and compared to micro-scale ferments of fruit harvested from vines of five established premium white varieties in the SARDI germplasm collection (Supplementary Table 2). The average hedonic scale score for wine from these 2nd gen. varieties by a panel of four tasters, compared very favourably with the average hedonic scale for wine made from the

premium varieties, with over 80% of the 2nd gen. varieties ranking of equal or higher preference to the lowest ranked premium variety.

8. Recommendations

- Evaluation of the viticultural characteristics of the 571 2nd gen. mildew-resistant varieties planted in the SARDI vineyard in Nuriootpa needs to continue to identify those varieties with the best production potential. The focus should be on identifying those 2nd gen. mildew-resistant varieties with good yields, good levels of acidity at harvest and avoiding varieties with tight bunch architecture. Preference should also be given to varieties that ripen at different times of the season to existing premium varieties. Varieties that demonstrate acceptable viticultural production characteristics over at least two seasons should be put through micro-fermentation to assess wine sensory characteristics.
- The remaining 107 2nd gen. varieties and nine 3rd gen. varieties currently in the CSIRO glasshouse (Supplementary Table 1) need to be planted for field evaluation.
- The evaluation of the flowering potential of new 2nd gen. female breeding lines derived from breeding Strategy 2 (Table 18) and Strategy 4 (Table 22) needs to be completed. In the case of tall lines, this will involve planting in the SARDI vineyard in Spring 2022. The availability of tall 2nd gen. female breeding lines with good flowering potential will lead to increased breeding efficiencies for future breeding programs because: (a) all of the progeny generated from crosses in which both parents are tall can be evaluated for desirable traits rather than having to discard the 50% of the progeny that are microvines when using microvine breeding lines and (b) seed germination can be carried out using the less labour-intensive, filter paper method (Table 16) rather than having to use embryo rescue.
- Attention should be also be paid to any significant differences in susceptibility of 2nd gen. mildew-resistant varieties to bud and blister mites, as it is likely that sulfur sprays normally used to control powdery mildew may also be contributing to the control of these pests and the removal of these sprays from the normal spray program, when using 2nd gen. mildew-resistant varieties, may see an increase in mite damage.

Appendix 1: Communication

Scientific publications

- Dry IB, Davies C, Dunlevy JD, Smith HM, Thomas, MR, Walker AR, Walker RR, Clingeleffer PR (2022) Development of new wine-, dried- and table grape scions and rootstocks for Australian viticulture: past, present and future. Aust. J. Grape & Wine Research 28: 177-195.
- Dry IB, Thomas MR, Riaz S, Fuchs M, Sosnowski M (2019) Scion breeding for resistance to biotic stresses. Cantu, D. and Walker, M.A., eds. The Grape Genome (Springer Nature Switzerland: Cham, Switzerland) pp. 319–348.

Conference presentations

- Dry I, Holzapfel B, Thomas M. ' New strategies for the generation of disease-resistant premium winegrapes'. Talk presented at CRUSH the grape and wine science symposium, Waite Campus, September 2018.
- Rossouw G, Holzapfel B, Thomas M, Dry IB. 'Breeding and regional evaluation of mildew resistant grapevines in Australia'. Poster presented at 17th Australian Wine Industry Technical conference, Adelaide SA, July 2019.
- Rossouw G, Holzapfel B, Thomas M, Dry IB. 'Breeding and regional evaluation of mildew resistant grapevines in Australia'. Workshop presented at 17th Australian Wine Industry Technical conference, Adelaide SA, July 2019.
- Dry IB, Audeguin L, Cousins P, Harris B, Clark J. 'Update on breeding and evaluation of new mildew-resistant wine grape varieties for Australian conditions'. Workshop presented at 18th Australian Wine Industry Technical conference, Adelaide SA, June 2020.

Industry Journal articles / Interviews

- Holzapfel B, Thomas MR and Dry IB (2017) Evaluating and demonstrating new disease resistant varieties for warm irrigated areas. Grapevine Management Guide 2017-18.
- Ian Dry interviewed for ABC Catalyst television program on the development of disease-resistant premium winegrape cultivars, April 2018.
- Schoeler M, Rossouw G, Thomas M, Dry I Holzapfel B (2019) Breeding new resistant grapevine varieties. Grapevine management Guide 2018-19. NSW Dept Primary Industries.
- Ian Dry interviewed on ABC radio, South-East on breeding new disease-resistant winegrape varieties. July 2019.
- Ian Dry interviewed for article in Grapegrower and Winemaker Issue 676, 2020: 'Breeding in resilience: countering disease, heat and water stress in vine'. May 2020.
- Ian Dry interviewed for article in Grapegrower and Winemaker Issue 701, 2022: 'Seeing the light: innovation in powdery mildew treatment'. June 2022.

Industry presentations

- Presentation to Wine Australia Board on the CSIRO winegrape scion & rootstock marker-assisted breeding program (Adelaide, Nov 2017).
- Presentation to Sunraysia growers/winemakers on CSIRO breeding program for the generation of disease-resistant premium winegrapes (Mildura, Jun 2019).
- Presentation to Australian Grape & Wine National Wine Biosecurity Committee on new strategies for grapevine germplasm improvement (Adelaide, Apr 2021).
- Presentation to NSW Wine Industry Assoc. NSW Wine Innovation Forum on 'Development of new varieties for the Australian wine industry with improved resistance to mildew pathogens' (Griffith, Jun 2021).
- Presentation to Wine Australia Research Advisory Committee on new strategies for grapevine germplasm improvement (Adelaide, Oct 2021).
- Presentation in AWRI webinar series on 'Breeding and evaluation of new mildew-resistant winegrape varieties for Australian conditions.' (Adelaide, Oct 2021)
- Presentation to Board of Vinehealth Australia on breeding and evaluation of new mildew-resistant winegrape varieties for Australian conditions (Adelaide, Dec 2021).

Appendix 2: Intellectual Property

A Register of Background Intellectual Property was developed and submitted to Wine Australia at the time of project commencement.

All new 2nd gen. mildew-resistant varieties, 2nd gen. female breeding lines, 3rd gen. mildew-resistant varieties and 3rd gen. female breeding lines have been listed in the SRA IP register.

Appendix 3: References

Agurto M, Schlechter RO, Armijo G, Solano E, Serrano C, Contreras RA, Zuniga GE and Arce-Johnson P (2017) *RUN1* and *REN1* pyramiding in grapevine (*Vitis vinifera* cv. Crimson Seedless) displays an improved defense response leading to enhanced resistance to powdery mildew (*Erysiphe necator*). Front Plant Sci **8:** 758.

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Appendix 4: List of Supplementary Tables

Supplementary Table 1: List of all 2nd gen. mildew-resistant varieties generated in this project for field and wine evaluation.

Supplementary Table 2: Viticultural and wine sensory data for selected 2nd gen. mildew-resistant varieties planted in Nuriootpa

Supplementary Table 1. List of all 2nd gen. mildew-resistant varieties generated in this project for field and wine evaluation

Strategy 21M & Strategy 23M indicates female parent is a microvine, Strategy 21W & Strategy 23W indicates male parent is a tall. Location shows planting position at Nuriootpa or glasshouse on CSIRO Waite campus. The variety name in brackets after the listed genotype of the female or male parents indicates the winegrape variety from which this parent was derived in the last cross. n. a.= not applicable.

Location	Variety name	Female parent	Male parent	Breeding Strategy	Berry colour	Muscat	Planted
37_15_01_01	37171202_138_76	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	white	x	17/02/21
37_15_01_02	37171202_138_89	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	white	x	17/02/21
37_15_01_03	37240701_138_50	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	white	x	17/02/21
37_15_01_04	39160603_138_220	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_02_01	39080501_138_17	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_02_02	39080501_138_32	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_02_03	39080501_138_45	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_02_04	39080501_138_82	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_03_01	39080501_138_92	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_03_02	39080501_138_98	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_03_03	39080501_138_100	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_03_04	39080501_138_101	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_04_01	39080502_138_3	VrBC4S 40-138	39_08_05_02 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_04_02	39080502_138_10	VrBC4S 40-138	39_08_05_02 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_04_03	39080502_138_16	VrBC4S 40-138	39_08_05_02 (Chardonnay)	Strategy 1A	white	x	17/02/21
37_15_04_04	39111002_75_7	VrBC4S 42-75	39_11_10_02 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_05_01	39160603_138_216	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_05_02	39111002_75_15	VrBC4S 42-75	39_11_10_02 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_05_03	39111002_138_41	VrBC4S 40-138	39_11_10_02 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_05_04	39160603_138_211	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_06_01	39111002_4223_25	39_11_10_02 (Riesling)	VrBC4S 42-23	Strategy 1B	white	x	17/02/21
37_15_06_02	39120502_138_44	VrBC4S 40-138	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_06_03	39121003_75_49	VrBC4S 42-75	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_06_04	39121003_120_4	VrBC4S 40-120	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_07_01	39121003_120_5	VrBC4S 40-120	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_07_02	39121003_138_3	VrBC4S 40-138	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_07_03	39121003_138_12	VrBC4S 40-138	39_12_10_03 (Riesling)	Strategy 1A	white	x	17/02/21
37_15_07_04	39160603_120_1	VrBC4S 40-120	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21

37_15_08_01	39160603_120_14	VrBC4S 40-120	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_08_02	39160603_138_40	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_08_03	39160603_138_105	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_08_04	39160603_138_147	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_09_01	39160603_138_165	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_09_02	39160603_138_173	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_09_03	39160603_138_192	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_09_04	39160603_138_195	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_15_11_04	39111002_75_8	VrBC4S 42-75	39_11_10_02 (Riesling)	Strategy 1A	white	x	23/12/21
37_16_01_01	39120502_138_8	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_01_02	39120502_138_14	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_01_03	39120502_138_20	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_01_04	39120502_138_27	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_02_01	39120502_138_28	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_02_02	39120502_138_42	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_02_03	39121003_75_3	VrBC4S 42-75	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_02_04	39121003_75_43	VrBC4S 42-75	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_03_01	39121003_75_56	VrBC4S 42-75	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_03_02	39121003_120_2	VrBC4S 40-120	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_03_03	39121003_120_9	VrBC4S 40-120	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_03_04	39160702_138_53	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_04_01	39160603_120_10	VrBC4S 40-120	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_04_02	39160603_138_1	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_04_03	39160603_138_14	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_04_04	39160603_138_19	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_05_01	39160603_138_28	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_05_02	39160603_138_33	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_05_03	39160603_138_49	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_05_04	39160603_138_67	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_06_01	39160603_138_74	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_06_02	39160603_138_77	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_06_03	39160603_138_85	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_06_04	39160603_138_101	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_07_01	39160603_138_103	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_07_02	39160603_138_104	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_07_03	39160603_138_106	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21

37_16_07_04	39160603_138_116	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_08_01	39160603_138_151	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_08_02	39160603_138_158	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_08_03	39160603_138_159	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_08_04	39160603_138_161	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_09_01	39160603_138_167	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_09_02	39160603_138_174	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_09_03	39160603_138_180	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	8/01/21
37_16_09_04	39160603_4223_7	39_16_06_03 (Verdelho)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_16_10_01	39160702_138_2	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_10_02	39160702_138_20	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_10_03	39160702_138_30	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_10_04	39160702_138_32	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_11_01	39160702_138_50	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	8/01/21
37_16_11_02	39121003_75_47	VrBC4S 42-75	39_12_10_03 (Riesling)	Strategy 1A	white	x	8/01/21
37_16_11_03	39160702_138_26	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	17/02/21
37_16_11_04	39160702_138_35	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	17/02/21
37_16_12_01	39160702_138_36	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	17/02/21
37_16_12_02	39160702_138_45	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	17/02/21
37_16_13_01	39160702_138_48	VrBC4S 40-138	39_16_07_02 (Verdehlo)	Strategy 1A	white	x	17/02/21
37_16_13_02	39160603_138_203	VrBC4S 40-138	39_16_06_03 (Verdelho)	Strategy 1A	white	x	17/02/21
37_17_02_01	37140801_138_51	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_02_02	37140801_138_55	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_02_03	39170901_75_24	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_17_02_04	39170901_120_21	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_17_03_01	39170901_75_9	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_17_03_02	39170901_75_10	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_17_03_03	39170901_75_12	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_17_03_04	39170901_75_14	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_17_04_04	37140801_75_55	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_05_01	39170901_75_26	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_17_05_02	37141104_75_10	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_05_03	37141104_75_20	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_05_04	37141104_120_38	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_06_01	37141104_138_59	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_17_06_02	39170901_75_15	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20

37_17_06_03	39170901_75_19	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_17_06_04	39170901_75_20	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_17_07_02	37141202_4223_33	37_14_12_02 (Dunkelfelder)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_07_03	37171202_120_19	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	white	x	8/01/21
37_17_07_04	37171202_120_25	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	white	x	8/01/21
37_17_08_01	37171202_4223_3	37_17_12_02 (Shiraz)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_08_02	37171202_4223_16	37_17_12_02 (Shiraz)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_08_03	37240701_138_17	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	white	x	8/01/21
37_17_08_04	37240701_138_80	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	white	x	8/01/21
37_17_09_01	37240701_4223_2	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_09_02	37240701_4223_27	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_09_03	37241101_4223D_6	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_09_04	39080501_138_4	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	8/01/21
37_17_10_01	39080501_138_40	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	8/01/21
37_17_10_02	39080501_138_41	VrBC4S 40-138	39_08_05_01 (Chardonnay)	Strategy 1A	white	x	8/01/21
37_17_10_03	39111002_75_17	VrBC4S 42-75	39_11_10_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_10_04	39111002_138_18	VrBC4S 40-138	39_11_10_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_11_01	39111002_138_36	VrBC4S 40-138	39_11_10_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_11_02	39111002_138_45	VrBC4S 40-138	39_11_10_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_11_03	39111002_4223_5	39_11_10_02 (Riesling)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_11_04	39111002_4223_11	39_11_10_02 (Riesling)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_12_01	39111002_4223_27	39_11_10_02 (Riesling)	VrBC4S 42-23	Strategy 1B	white	x	8/01/21
37_17_12_02	39120502_138_2	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_13_01	39120502_138_3	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_17_13_02	39120502_138_4	VrBC4S 40-138	39_12_05_02 (Riesling)	Strategy 1A	white	x	8/01/21
37_18_02_01	37141104_173_1	VrBC4S 40-173	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	3/12/19
37_18_02_02	39180602_120_2	VrBC4S 40-120	39_18_06_02 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_02_03	39180602_138_4	VrBC4S 40-138	39_18_06_02 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_02_04	39170901_46_4	VrBC4S 42-46	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_03_01	39170901_138_5	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_03_02	39170901_138_6	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_03_03	39170901_138_7	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_03_04	39170901_138_8	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_04_01	39170901_138_9	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_04_02	39170901_173_1	VrBC4S 40-173	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_06_01	39170901_138_1	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19

37_18_06_02	39170901_138_14	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_06_03	39170901_138_20	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_06_04	39170901_138_3	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_08_01	39170901_120_3	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_08_02	39170901_120_4	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_08_03	39170901_120_9	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_08_04	39170901_120_10	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_10_01	39170901_46_5	VrBC4S 42-46	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_10_02	39170901_75_5	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_18_10_03	39170901_75_6	VrBC4S 42-75	39_17_09_01 (Frontignac)	Strategy 1A	white	x	3/12/19
37_18_10_04	39170901_120_2	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	3/12/19
37_19_03_03	37140801_63_2	VrBC4S 42-63	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	17/02/21
37_19_04_03	37140801_120_13	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_19_04_04	39170901_138_51	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_06_01	37140801_138_39	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_19_06_02	37140601_138_12	VrBC4S 40-138	37_14_06_01 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_19_06_03	37141104_138_41	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	white	x	24/03/20
37_19_06_04	39170901_138_47	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_08_03	39170303_75_50	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_08_04	39170901_138_35	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_10_02	39170901_138_48	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_19_10_03	39170901_120_40	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_10_04	39170901_120_41	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_11_01	39170901_138_14	VrBC4S 40-138	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_19_11_02	39180602_138_20	VrBC4S 40-138	39_18_06_02 (Frontignac)	Strategy 1A	white	x	24/03/20
37_19_11_03	39170901_46_4A	VrBC4S 42-46	39_17_09_01 (Frontignac)	Strategy 1A	white	x	24/03/20
37_19_11_04	39170901_120_15	VrBC4S 40-120	39_17_09_01 (Frontignac)	Strategy 1A	white	✓	24/03/20
37_20_01_03	39170303_75_3	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	✓	22/03/19
37_20_02_03	39170303_75_9	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	x	22/03/19
37_20_08_01	37141202_4223_32	37_14_12_02 (Dunkelfelder)	VrBC4S 42-23	Strategy 1B	white	x	17/02/21
37_20_09_04	39170303_75_3	VrBC4S 42-75	39_17_03_03 (Frontignac)	Strategy 1A	white	x	24/03/19
37_21_02_01	37140801_75_18	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_02_02	37140801_138_2	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_02_03	37140801_138_8	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_02_04	37140801_138_13	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_04_01	37141104_75_3	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19

37_21_04_02	37141104_120_2	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_04_03	37141104_120_20	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_04_04	37141104_120_31	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_05_01	37140801_138_15	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_05_02	37141104_63_2	VrBC4S 42-63	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_05_03	37141104_75_1	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_05_04	37141104_75_2	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_06_01	37141104_120_32	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_06_02	37141104_130_1	VrBC4S 40-130	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_06_03	37141104_138_6	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_06_04	37141104_130_10	VrBC4S 40-130	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_07_01	37141104_138_12	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_07_02	37141104_138_13	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_07_03	37141104_138_15	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	3/12/19
37_21_09_01	37140801_75_33	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_09_02	37140801_75_35	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_09_03	37140801_75_41	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_09_04	37140801_75_51	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_10_01	37140801_75_56	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_10_02	37140801_120_3	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_10_03	37140801_120_6	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_10_04	37140801_120_8	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_11_01	37140801_120_19	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_11_02	37140801_120_28	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_11_03	37140801_120_39	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_11_04	37140801_120_42	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_21_12_01	37140801_120_43	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_12_02	37140801_120_44	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_13_01	37140801_120_45	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_21_13_02	37140801_138_19	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_01_01	37140801_138_36	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_01_02	37140801_120_25	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_01_03	37140801_138_40	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_01_04	37140801_138_43	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_02_01	37140801_138_47	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_02_02	37140801_138_54	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20

37_22_02_03	37140801_138_58	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_02_04	37141104_75_11	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_03_01	37141104_120_3	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_03_02	37141104_120_40	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_03_03	37141104_120_45	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_03_04	37141104_120_51	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_04_01	37141104_138_23	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_04_02	37141104_138_25	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_04_03	37141104_138_35	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_04_04	37141104_138_37	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_05_01	37141104_138_40	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_05_02	37141104_138_44	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_05_03	37141104_138_47	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_05_04	37141104_138_56	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_06_01	37140601_138_8**	VrBC4S 40-138	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_06_02	37140801_75_18	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	24/03/20
37_22_06_03	37140502_75_50	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_06_04	37140502_75_79	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_07_01	37140502_75_80	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_07_02	37140502_75_85	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_07_03	37140502_75_91	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_07_04	37140502_75_94	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_08_01	37140502_120_2	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_08_02	37140502_120_14	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_08_03	37140502_120_17	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_08_04	37140502_120_18	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_09_01	37140502_120_24	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_09_02	37140601_75_9	VrBC4S 42-75	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_09_03	37140601_75_11	VrBC4S 42-75	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_09_04	37140601_138_8**	VrBC4S 40-138	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_10_01	37140601_138_10	VrBC4S 40-138	37_14_06_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_10_02	37140801_120_14	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_10_03	37140801_120_17	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_10_04	37140801_120_18	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_11_01	37140801_120_12	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_11_02	37140801_120_23	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21

37_22_11_03	37140801_138_58B	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_11_04	37141104_75_30	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_12_01	37141104_120_56	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_12_02	37141104_120_62	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_13_01	37141104_138_37B	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_22_13_02	37141104_138_39	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_01_01	37141104_138_42	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_01_02	37141104_138_75	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_01_03	37141104_138_94	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_01_04	37141104_138_95	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_02_01	37141202_138_10	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_02_02	37141202_138_98	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_02_03	37141202_138_99	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_02_04	37141202_138_100	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_03_01	37141202_138_108	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_03_02	37141202_138_119	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_03_03	37141202_138_132	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_03_04	37141202_4223_39	37_14_12_02 (Dunkelfelder)	VrBC4S 42-23	Strategy 1B	red flesh	x	8/01/21
37_23_04_01	37171202_120_21	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_04_02	31717202_120_37	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_04_03	37171202_138_53	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_04_04	37171202_138_67	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_05_01	37171202_138_68	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_05_02	37171202_138_75	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_05_03	37171202_138_83	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_05_04	37171202_138_86	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_06_01	37171202_138_88	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_06_02	37171202_138_90	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_06_03	37171202_4223_8	37_17_12_02 (Shiraz)	VrBC4S 42-23	Strategy 1B	red skin	x	8/01/21
37_23_06_04	37240701_138_2	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_07_01	37240701_138_21	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_07_02	37240701_138_62	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_07_03	37240701_138_66	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_07_04	37240701_138_76	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_08_01	37241101_138_9	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_23_08_02	37241101_138_10	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	8/01/21

37_23_08_03	37241101_138_18	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_08_04	37241101_138_43	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	8/01/21
37_23_09_01	37241101_4223D_21	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	8/01/21
37_23_09_02	37241101_4223D_31	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	8/01/21
37_23_09_04	37141202_138_122	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_10_01	37171202_138_12	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	8/01/21
37_23_10_04	37140502_75_53	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	8/01/21
37_23_11_01	37140502_75_82	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_11_03	37140502_75_47	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_11_04	37140502_75_90	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_12_01	37140502_120_10	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_12_02	37140502_120_15	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_13_01	37140502_120_22	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_23_13_02	37140502_120_26	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_01_01	37140502_120_27	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_01_02	37140502_120_33	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_01_03	37140502_120_38	VrBC4S 40-120	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_01_04	37140502_138_17	VrBC4S 40-138	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_02_02	37140502_138_71	VrBC4S 40-138	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_02_03	37140801_75_71	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_02_04	37140801_75_73	VrBC4S 42-75	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_03_01	37140801_120_10	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_03_02	37140801_120_20	VrBC4S 40-120	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_03_03	37140801_138_44	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_03_04	37140801_138_59	VrBC4S 40-138	37_14_08_01 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_04_01	37141104_75_33	VrBC4S 42-75	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_04_02	37141104_120_59	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_04_03	37141104_120_64	VrBC4S 40-120	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_04_04	37141104_138_27	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_05_01	37141104_138_70	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_05_02	37141104_138_76	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_05_03	37141104_138_84	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_05_04	37141104_138_90	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_06_01	37141202_138_100	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_06_02	37141202_75_2	VrBC4S 42-75	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_06_03	37141202_138_73	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21

37_24_06_04	37141202_138_86	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_07_01	37141202_138_110	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_07_02	37141202_138_118	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_07_03	37141202_138_127	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_07_04	37141202_138_131	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_08_01	37141202_138_133	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_08_02	37141202_138_137	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_08_03	37141202_138_140	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_24_08_04	37141202_4223_34	37_14_12_02 (Dunkelfelder)	VrBC4S 42-23	Strategy 1B	red flesh	x	17/02/21
37_24_09_01	37171202_120_1	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_09_02	37171202_138_11	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_09_03	37171202_138_40	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_09_04	37171202_138_42	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_10_01	37171202_138_65	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_10_02	37171202_138_73	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_10_03	37171202_138_85	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red flesh	x	17/02/21
37_24_10_04	37171202_4223_15	37_17_12_02 (Shiraz)	VrBC4S 42-23	Strategy 1B	red flesh	x	17/02/21
37_24_11_01	37240701_138_42	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_11_02	37240701_138_82	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_11_03	37241101_75_3	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_11_04	37241101_138_19	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_12_01	37241101_138_20	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_12_02	37241101_138_22	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_13_01	37241101_138_34	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_24_13_02	37241101_138_51	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_25_01_01	37241101_138_53	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	17/02/21
37_25_01_02	37241101_4223D_10	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	17/02/21
37_25_01_03	37241101_4223D_70	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	17/02/21
37_25_01_04	37141202_138_140	VrBC4S 40-138	37_14_12_02 (Dunkelfelder)	Strategy 1A	red flesh	x	17/02/21
37_25_02_01	37160904_138_2	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_02_02	37160904_138_7	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_02_03	37160904_138_19	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_02_04	37160904_138_23	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_03_01	37160904_138_25	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_03_02	37160904_138_28	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21
37_25_03_03	37160904_138_29	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	14/12/21

37_25_03_04	37250603_138_29	VrBC4S 40-138	37_25_06_03 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_04_01	37171202_120_45	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	14/12/21
37_25_04_02	37171202_120_53	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	14/12/21
37_25_04_03	37171202_120_54	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	14/12/21
37_25_04_04	37171202_120_55	VrBC4S 40-120	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	14/12/21
37_25_05_01	37171202_138_109	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	14/12/21
37_25_05_02	37240701_138_113	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_05_03	37240701_120_5	VrBC4S 40-120	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_05_04	37240701_120_7	VrBC4S 40-120	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_10_01	37140502_75_89	VrBC4S 42-75	37_14_05_02 (Dunkelfelder)	Strategy 1A	red flesh	x	14/12/21
37_25_10_02	37141104_138_100	VrBC4S 40-138	37_14_11_04 (Dunkelfelder)	Strategy 1A	red flesh	x	14/12/21
37_25_10_03	37240701_138_99	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_10_04	37250603_138_25	VrBC4S 40-138	37_25_06_03 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_11_01	37240701_138_118	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_25_11_02	37240701_4223_41	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_11_03	37240701_4223_44	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_11_04	37240701_4223_49	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_12_01	37240701_4223_58	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_12_02	37240701_4223_64	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_13_01	37240701_4223_85	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_25_13_02	37250603_138_17	VrBC4S 40-138	37_25_06_03 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_01_01	37241101_75_23	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_01_02	37241101_75_25	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_01_03	37241101_75_39	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_01_04	37241101_75_40	VrBC4S 42-75	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_02_01	37241101_120_8	VrBC4S 40-120	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_02_02	37241101_120_27	VrBC4S 40-120	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_02_03	37241101_138_83	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_02_04	37241101_138_86	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_03_01	37241101_138_95	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_03_02	37241101_138_103	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_03_03	37241101_138_111	VrBC4S 40-138	37_24_11_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_03_04	37241101_4223_9	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_28_04_02	37241101_4223_20	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_28_04_03	37241101_4223_24	37_24_11_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_28_04_04	37250304_138_4	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21

37_28_05_01	37250304_138_5	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_05_02	37250304_138_12	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_05_03	37250304_138_14	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_05_04	37250304_138_17	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_06_01	37250304_138_22	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_06_02	37250304_4223_5	37_25_03_04 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	14/12/21
37_28_06_03	37240701_138_105	VrBC4S 40-138	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	14/12/21
37_28_07_03	37160904_138_31	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	16/02/22
37_28_07_04	37160904_138_34	VrBC4S 40-138	37_16_09_04 (Tempranillo)	Strategy 1A	red skin	x	16/02/22
37_28_08_01	37171202_138_129	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	16/02/22
37_28_08_02	37171202_138_130	VrBC4S 40-138	37_17_12_02 (Shiraz)	Strategy 1A	red skin	x	16/02/22
37_28_08_03	37240701_120_6	VrBC4S 40-120	37_24_07_01 (Tannat)	Strategy 1A	red skin	x	16/02/22
37_28_08_04	37240701_4223_86	37_24_07_01 (Tannat)	VrBC4S 42-23	Strategy 1B	red skin	x	16/02/22
37_28_09_01	37250304_138_31	VrBC4S 40-138	37_25_03_04 (Tannat)	Strategy 1A	red skin	x	16/02/22
Glasshouse	ST21M_AB_1_2	39160603_138_55	Alichante Bouchet	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_AB_1_3	39160603_138_55	Alichante Bouchet	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_CF_1_3	37140502_75_15	Cabernet Franc	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21M_CS_2_6	37141202_138_166	Cabernet Sauvignon	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_CS_2_23	37141202_138_166	Cabernet Sauvignon	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_CS_3_3	37140502_75_15	Cabernet Sauvignon	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21W_DU_1_1	39170303_75_15	Durif	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21W_DU_1_2	39170303_75_15	Durif	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_MA_1_1	37140502_75_15	Malbec	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_MA_1_3	37140502_75_15	Malbec	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_MA_1_7	37140502_75_15	Malbec	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_MA_1_10	37140502_75_15	Malbec	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21W_MA_1_5	39170303_75_15	Malbec	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_ME_1_3	37140502_75_15	Merlot	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21M_ME_1_8	37140502_75_15	Merlot	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_ME_1_22	37140502_75_15	Merlot	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21M_RI_1_1	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_2	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_7	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_17	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_21	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_22	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.

Glasshouse	ST21M_RI_1_24	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_26	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_56	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_61	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_67	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_70	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_71	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_72	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_83	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_84	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_95	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_RI_1_101	37141202_138_58	Riesling	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_SB_1_5	39160603_138_11	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_SB_1_49	39160603_138_11	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_SB_1_50	39160603_138_11	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_SB_1_12	39170303_75_22	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_SB_1_46	39170303_75_22	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_SB_1_58	39170303_75_22	Sauvignon Blanc	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_SB_1_3	39170303_75_22	Sauvignon Blanc	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_SH_2_10	37140502_75_15	Shiraz	Strategy 2.1	red flesh	x	n. a.
Glasshouse	ST21M_SH_4_1_1	37140801_138_88	Shiraz	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_SH_4_1_4	37140801_138_88	Shiraz	Strategy 2.1	red skin	x	n. a.
Glasshouse	ST21M_VI_1_10	37141202_138_9	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_VI_1_11	37141202_138_9	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_VI_3_7	39160603_138_55	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_VI_3_12	39160603_138_55	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_VI_4_2	39160603_138_55	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21M_VI_4_2	39160603_138_55	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_3	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_4	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_9	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_11	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_43	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_52	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_59	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_64	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.

Glasshouse	ST21W_VI_1_71	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_76	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_82	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_85	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
Glasshouse	ST21W_VI_1_91	39170303_75_22	Viognier	Strategy 2.1	white	x	n. a.
37_14_01_01	ST23M_RI_1_27	15C2V2_10_05	Riesling	Strategy 2.3	white	x	16/02/22
37_14_01_02	ST23M_SB_1_4	15C2V2_10_05	Sauvignon Blanc	Strategy 2.3	white	x	16/02/22
37_14_01_03	ST23M_SB_1_12	15C2V2_10_05	Sauvignon Blanc	Strategy 2.3	white	x	16/02/22
37_14_01_04	ST23M_SB_1_13	15C2V2_10_05	Sauvignon Blanc	Strategy 2.3	white	x	16/02/22
37_15_10_01	ST23W_RI_1_11	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	✓	14/12/21
37_15_10_02	ST23W_RI_1_13	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	✓	14/12/21
37_15_10_03	ST23W_RI_1_2	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	x	14/12/21
37_15_10_04	ST23W_RI_1_23	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	x	14/12/21
37_15_11_01	ST23W_RI_1_28	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	x	14/12/21
37_15_11_02	ST23W_RI_1_29	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	✓	14/12/21
37_15_11_03	ST23W_RI_34	15C2V2_6_74 (Muscat)	Riesling	Strategy 2.3	white	✓	14/12/21
37_15_12_01	ST23M_RI_1_11	15C2V2_10_05	Riesling	Strategy 2.3	white	x	16/02/22
37_15_12_02	ST23M_RI_1_18	15C2V2_10_05	Riesling	Strategy 2.3	white	x	16/02/22
37_15_13_01	ST23M_RI_1_23	15C2V2_10_05	Riesling	Strategy 2.3	white	x	16/02/22
37_15_13_02	ST23M_RI_1_25	15C2V2_10_05	Riesling	Strategy 2.3	white	x	16/02/22
37_23_10_02	15C2V2_BK_3.2	15C2V2_R04_1_81	Bogazkere	Strategy 2.3	red flesh	x	8/01/21
37_23_10_03	15C2V2_BK_3.3	15C2V2_R04_1_81	Bogazkere	Strategy 2.3	red flesh	x	8/01/21
37_25_06_01	ST23M_CF_1_2	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red skin	x	14/12/21
37_25_06_02	ST23M_GR_1_46	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	14/12/21
37_25_06_03	ST23M_GR_1_66	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	14/12/21
37_25_06_04	ST23M_GR_1_73	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	14/12/21
37_25_07_01	ST23W_GR_1_28	15C2V2_R04_1_77	Grenache	Strategy 2.3	red skin	x	14/12/21
37_25_07_02	ST23M_MA_1_4	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_25_07_03	ST23M_MA_1_6	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	14/12/21
37_25_07_04	ST23M_MA_1_8	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_25_08_01	ST23M_MA_1_15	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_25_08_02	ST23M_MA_1_17	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	14/12/21
37_25_08_03	ST23M_MA_1_74	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	14/12/21
37_25_08_04	ST23M_MA_1_33	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_25_09_01	ST23M_MA_1_48	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	14/12/21
37_25_09_02	ST23M_MA_1_52	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21

37_25_09_03	ST23M_MA_1_58	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_25_09_04	ST23M_MA_1_59	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_28_06_04	ST23M_MA_1_7	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	14/12/21
37_28_07_01	ST23M_CF_1_5	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red skin	x	14/12/21
37_28_07_02	ST23M_MA_1_110	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	14/12/21
37_28_09_02	ST23M_BG_1_16	15C2V2_R04_1_77	Bogazkere	Strategy 2.3	red skin	x	16/02/22
37_28_09_03	ST23M_BG_1_28	15C2V2_R04_1_77	Bogazkere	Strategy 2.3	red flesh	x	16/02/22
37_28_09_04	ST23M_BO_1_30	15C2V2_R04_1_77	Bobal	Strategy 2.3	red flesh	x	16/02/22
37_28_10_01	ST23M_CF_1_5	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red skin	x	16/02/22
37_28_10_02	ST23M_CF_1_8	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red flesh	x	16/02/22
37_28_10_03	ST23M_CF_1_26	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red flesh	x	16/02/22
37_28_10_04	ST23M_CF_1_45	15C2V2_R04_1_77	Cabernet Franc	Strategy 2.3	red skin	x	16/02/22
37_28_11_01	ST23M_GR_1_25	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	16/02/22
37_28_11_02	ST23M_MA_1_2	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
37_28_11_03	ST23M_MA_1_19	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
37_28_11_04	ST23M_MA_1_42	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	16/02/22
37_28_12_01	ST23M_MA_1_53	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
37_28_12_02	ST23M_MA_1_78	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
37_28_13_01	ST23M_MA_1_81	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
37_28_13_02	ST23M_MA_1_84	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	16/02/22
39_08_01_01	ST23M_MA_1_86	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_01_02	ST23M_MA_1_90	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_01_03	ST23M_MA_1_98	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_02_01	ST23M_MA_1_99	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	16/02/22
39_08_02_02	ST23M_MA_1_101	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_02_03	ST23M_MA_1_104	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	16/02/22
39_08_03_01	ST23M_MA_1_122	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_03_02	ST23M_MA_1_125	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_03_03	ST23M_MA_1_126	15C2V2_R04_1_77	Malbec	Strategy 2.3	red skin	x	16/02/22
39_08_04_01	ST23M_MA_1_127	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_04_02	ST23M_MA_1_128	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_04_03	ST23M_MA_1_131	15C2V2_R04_1_77	Malbec	Strategy 2.3	red flesh	x	16/02/22
39_08_05_01	ST23M_SH_1_34	15C2V2_R04_1_77	Shiraz	Strategy 2.3	red flesh	x	16/02/22
39_08_05_02	ST23M_SH_1_36	15C2V2_R04_1_77	Shiraz	Strategy 2.3	red skin	x	16/02/22
Glasshouse	ST23M_CS_2_2	15C2V2_R04_1_81	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_4	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.

Glasshouse	ST23M_CS_3_7	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_15	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_16	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_25	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_28	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_32	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_35	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_42	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_43	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_61	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_65	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_CS_3_69	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_76	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_CS_3_83	15C2V2_R04_1_77	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_CS_2_13	15C2V2_R04_1_67	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_CS_2_14	15C2V2_R04_1_67	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_CS_2_36	15C2V2_R04_1_67	Cabernet Sauvignon	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_CH_1_4	15C2V2_6_100	Chardonnay	Strategy 2.3	white	✓	n. a.
Glasshouse	ST23W_CH_1_8	15C2V2_6_100	Chardonnay	Strategy 2.3	white	x	n. a.
Glasshouse	ST23W_CH_1_11	15C2V2_6_100	Chardonnay	Strategy 2.3	white	✓	n. a.
Glasshouse	ST23W_CH_1_17	15C2V2_6_100	Chardonnay	Strategy 2.3	white	✓	n. a.
Glasshouse	ST23W_CH_1_19	15C2V2_6_100	Chardonnay	Strategy 2.3	white	x	n. a.
Glasshouse	ST23W_CH_2_2	15C2V2_6_103	Chardonnay	Strategy 2.3	white	x	n. a.
Glasshouse	ST23W_CH_2_5	15C2V2_6_103	Chardonnay	Strategy 2.3	white	x	n. a.
Glasshouse	ST23W_DU_1_2	15C2V2_6_11	Durif	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_DU_1_7	15C2V2_6_11	Durif	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_DU_4_4	15C2V2_6_42	Durif	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_GR_3_15	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_GR_3_31	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_GR_3_50	15C2V2_R04_1_77	Grenache	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23W_MA_1_1	15C2V2_R04_1_189	Malbec	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_MA_1_5	15C2V2_R04_1_189	Malbec	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23W_ME_1_14	15C2V2_11_12	Merlot	Strategy 2.3	red skin	x	n. a.
Glasshouse	ST23M_SA_1_6	15C2V2_R04_1_77	Sangiovese	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_SA_1_7	15C2V2_R04_1_77	Sangiovese	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_SA_1_9	15C2V2_R04_1_77	Sangiovese	Strategy 2.3	red flesh	x	n. a.

Glasshouse	ST23M_SA_1_20	15C2V2_R04_1_77	Sangiovese	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_SH_4_6	15C2V2_R04_1_77	Shiraz	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_SH_4_19	15C2V2_R04_1_77	Shiraz	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_SH_4_29	15C2V2_R04_1_77	Shiraz	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23W_SH_2_9	15C2V2_R04_1_189	Shiraz	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23M_TE_1_8	15C2V2_R04_1_77	Tempranillo	Strategy 2.3	red flesh	x	n. a.
Glasshouse	ST23W_VE_1_7	15C2V2_6_42	Verdelho	Strategy 2.3	white	✓	n. a.
37_17_01_01	15C2V2_11_181	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_01_02	15C2V2_11_193	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_01_03	15C2V2_11_201	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_01_04	15C2V2_11_265	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_17_04_01	15C2V2_11_218	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_04_02	15C2V2_11_227	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_04_03	15C2V2_11_250	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_17_07_01	15C2V2_11_190	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	8/01/21
37_18_07_01	15C2V2_11_99	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_07_02	15C2V2_11_200	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_07_03	15C2V2_11_251	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_07_04	15C2V2_11_320	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_09_01	15C2V2_11_44	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_09_02	15C2V2_11_47	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	3/12/19
37_18_09_03	15C2V2_11_50	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_09_04	15C2V2_11_54	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_11_01	15C2V2_10_160	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_18_11_02	15C2V2_10_178	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_18_11_03	15C2V2_10_202	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_18_11_04	15C2V2_11_37	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	3/12/19
37_18_12_01	15C2V2_10_143	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_18_12_02	15C2V2_10_151	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_18_13_01	15C2V2_10_123	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_18_13_02	15C2V2_10_133	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_19_03_04	15C2V2_11_71	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_04_01	15C2V2_10_237	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	24/03/20
37_19_04_02	15C2V2_11_68	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_05_01	15C2V2_10_249	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	24/03/20
37_19_05_02	15C2V2_11_273	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20

37_19_05_03	15C2V2_11_134	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_05_04	15C2V2_11_322	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_07_01	15C2V2_11_118	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_07_02	15C2V2_11_131	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_07_03	15C2V2_11_153	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_07_04	15C2V2_11_154	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_09_01	15C2V2_11_137	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_09_02	15C2V2_11_141	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_09_03	15C2V2_11_175	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_09_04	15C2V2_11_212	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_12_01	15C2V2_11_144	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_12_02	15C2V2_11_192	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/20
37_19_13_01	15C2V2_11_279	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_19_13_02	15C2V2_11_296	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	x	24/03/20
37_20_01_01	15C2V2_6_57	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	22/03/19
37_20_01_02	15C2V2_6_86	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	✓	22/03/19
37_20_01_04	15C2V2_6_15	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	22/03/19
37_20_02_01	15C2V2_6_46	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	22/03/19
37_20_02_02	15C2V2_6_87	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	22/03/19
37_20_05_04	15C2V2_6_188	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	3/12/19
37_20_06_01	15C2V2_10_14	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_06_02	15C2V2_10_16	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_06_03	15C2V2_10_38	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_06_04	15C2V2_10_42	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_20_07_01	15C2V2_10_43	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_20_07_02	15C2V2_10_65	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_07_03	15C2V2_10_72	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_07_04	15C2V2_10_74	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_08_02	15C2V2_10_82	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	24/03/20
37_20_08_03	15C2V2_10_90	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_08_04	15C2V2_10_95	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	x	3/12/19
37_20_09_01	15C2V2_10_26	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	24/03/19
37_20_09_02	15C2V2_10_30	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	24/03/19
37_20_09_03	15C2V2_11_14	15C002V0002	VrBC4S30F1_11	Strategy 3M	white	✓	24/03/19
37_20_10_01	15C2V2_6_128	15C002V0002	VrBC4S30F1_6	Strategy 3M	white	x	24/03/19
37_20_12_02	15C2V2_10_98	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19

37_20_13_01	15C2V2_10_105	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_20_13_02	15C2V2_10_117	15C002V0002	VrBC4S30F1_10	Strategy 3M	white	✓	3/12/19
37_18_01_01	15C2V2_R04_4_88	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_18_01_02	15C2V2_R04_4_94	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_18_01_03	15C2V2_R04_25_3	15C002V0002	Vr42F1R04_25	Strategy 3R	white	x	3/12/19
37_18_01_04	15C2V2_R04_25_6	15C002V0002	Vr42F1R04_25	Strategy 3R	white	x	3/12/19
37_18_05_03	15C2V2_R04_1_123	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_18_05_04	15C2V2_R04_1_127	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_01_01	15C2V2_R04_4_40	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_19_01_02	15C2V2_R04_4_69	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_19_01_03	15C2V2_R04_4_84	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_19_01_04	15C2V2_R04_4_85	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_19_02_01	15C2V2_R04_1_143	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_02_02	15C2V2_R04_1_155	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_02_03	15C2V2_R04_1_180	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_02_04	15C2V2_R04_4_32	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	3/12/19
37_19_03_01	15C2V2_R04_1_114	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_03_02	15C2V2_R04_1_115	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	3/12/19
37_19_08_01	15C2V2_R04_4_30	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	24/03/20
37_19_08_02	15C2V2_R04_4_55	15C002V0002	Vr42F1R04_4	Strategy 3R	white	x	24/03/20
37_19_10_01	15C2V2_R04_8_68	15C002V0002	Vr42F1R04_8	Strategy 3R	white	x	24/03/20
37_20_04_03	15C2V2_R04_1_10	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	22/03/19
37_20_04_04	15C2V2_R04_1_26	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	22/03/19
37_20_05_02	15C2V2_R04_1_71	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	22/03/19
37_20_10_03	15C2V2_R04_1_27	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	24/03/19
37_20_10_04	15C2V2_R04_1_28	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	24/03/19
37_20_11_01	15C2V2_R04_1_3	15C002V0002	Vr42F1R04_1	Strategy 3R	white	x	24/03/19
37_21_01_01	15C2V2_R04_1_72	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	22/03/19
37_21_01_02	15C2V2_R04_1_112	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	3/12/19
37_21_01_03	15C2V2_R04_1_30	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	24/03/19
37_21_01_04	15C2V2_R04_1_126	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	3/12/19
37_21_03_01	15C2V2_R04_1_128	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	3/12/19
37_21_03_02	15C2V2_R04_1_136	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	3/12/19
37_21_03_03	15C2V2_R04_1_187	15C002V0002	Vr42F1R04_1	Strategy 3R	red flesh	x	3/12/19
37_21_03_04	15C2V2_R04_4_6	15C002V0002	Vr42F1R04_4	Strategy 3R	red flesh	x	3/12/19
37_21_08_02	15C2V2_R04_8_25	15C002V0002	Vr42F1R04_8	Strategy 3R	red flesh	x	24/03/20

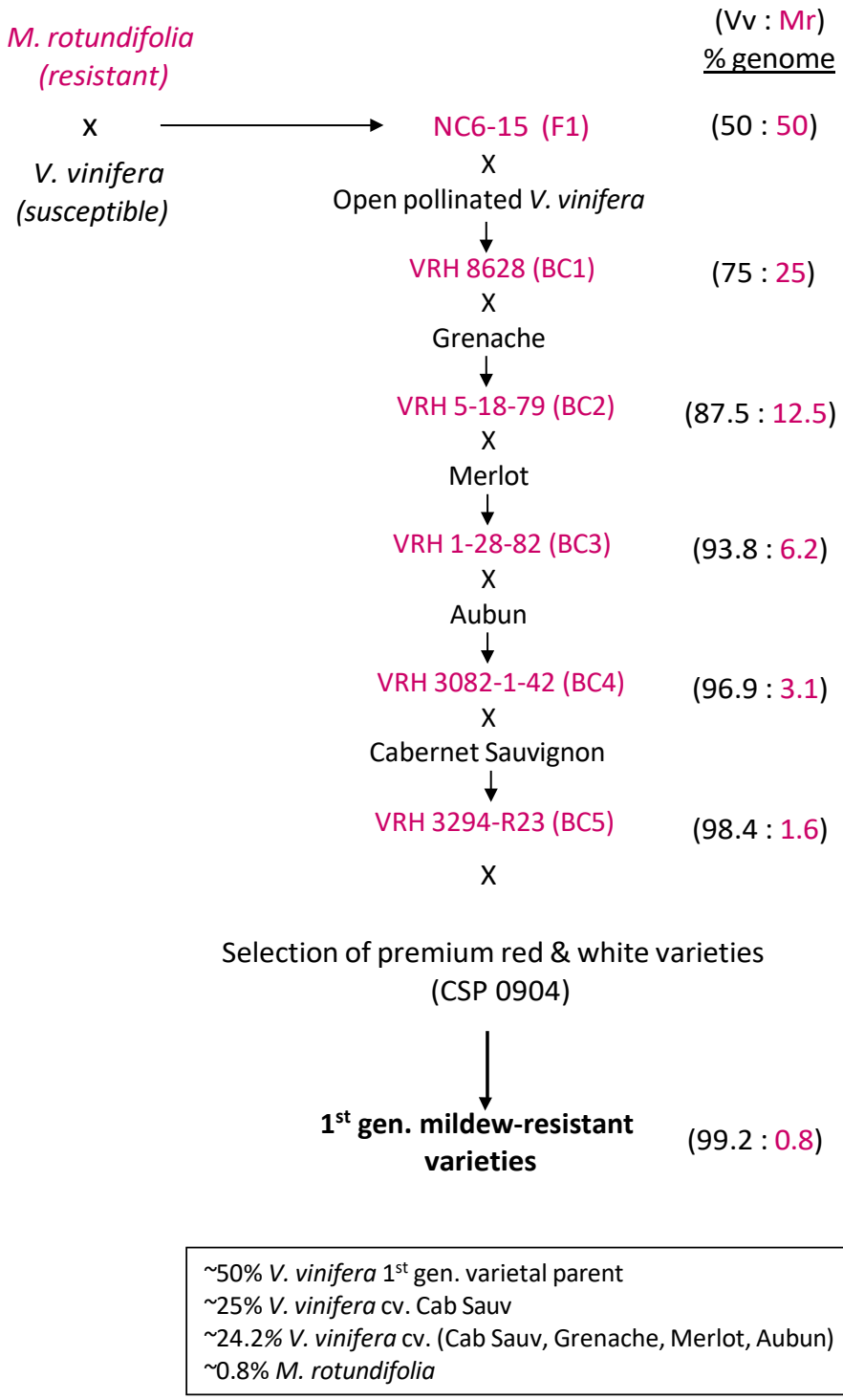
37_21_08_03	15C2V2_R04_8_27	15C002V0002	Vr42F1R04_8	Strategy 3R	red flesh	x	24/03/20
37_21_08_04	15C2V2_R04_8_70	15C002V0002	Vr42F1R04_8	Strategy 3R	red flesh	x	24/03/20
39_08_05_03	ST53M_SH_1_18B	SY5_B_107	Shiraz	Strategy 5	red flesh	x	16/02/22
Glasshouse	ST53_CS_4_1B	SY5_B_25	Cabernet Sauvignon	Strategy 5	red skin	x	n. a.
Glasshouse	ST53_MA_1_16B	SY5_B_107	Malbec	Strategy 5	red flesh	x	n. a.
Glasshouse	ST53_MA_1_19B	SY5_B_107	Malbec	Strategy 5	red flesh	x	n. a.
Glasshouse	ST53_MA_1_33B	SY5_B_107	Malbec	Strategy 5	red skin	x	n. a.
Glasshouse	ST53_MA_1_42B	SY5_B_107	Malbec	Strategy 5	red skin	x	n. a.
Glasshouse	ST53_MA_1_46B	SY5_B_107	Malbec	Strategy 5	red skin	x	n. a.
Glasshouse	ST53_MA_1_49B	SY5_B_107	Malbec	Strategy 5	red flesh	x	n. a.
Glasshouse	ST53_MA_1_58B	SY5_B_107	Malbec	Strategy 5	red skin	x	n. a.

Supplementary Table 2. Viticultural and wine sensory data for selected 2nd gen. mildew-resistant varieties planted in Nuriootpa

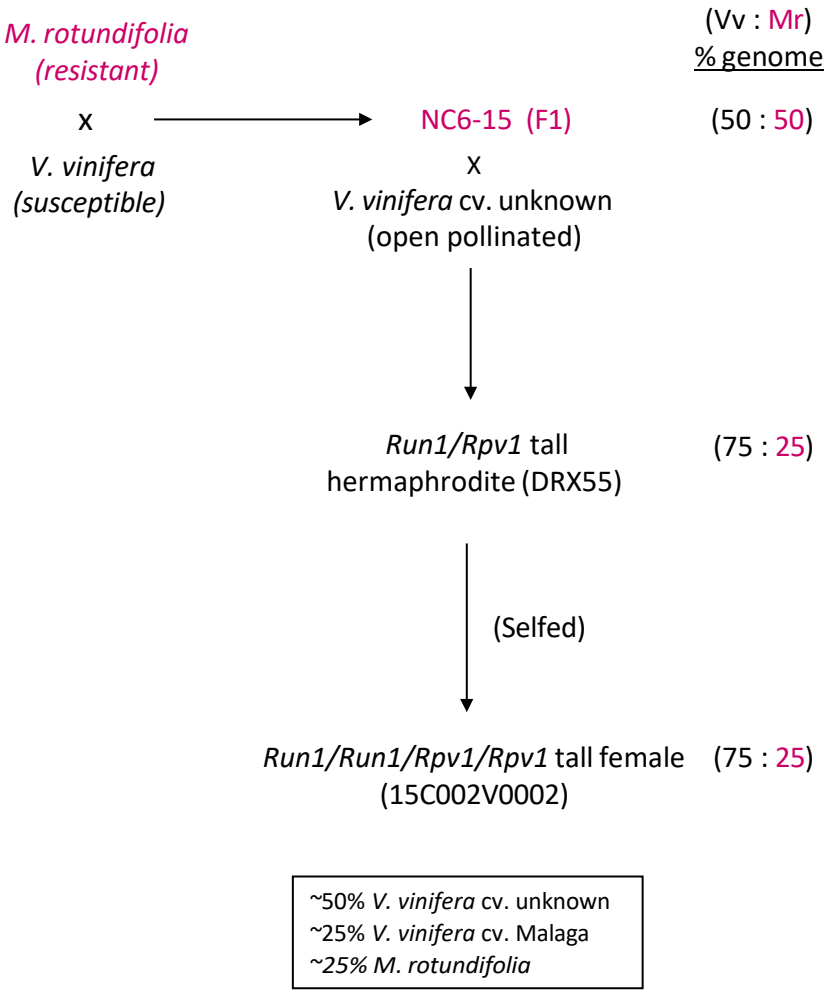
In addition to the 2nd gen. varieties, viticultural and wine sensory data was also collected for five premium wine grape varieties and the Strategy 3 breeding line 15C002V0002 that were also grown at the same location. Wine was only made from varieties from which > 1.5 kg of fruit was harvested. Fuuit and wine chemistry was measured using an OenoFossTM analyser. Wine tasters were asked to score the wines using a Hedonic scale: 9 = like extremely; 8 = like very much, 7 = like moderately; 6 = like slightly; 5 = neither like or dislike; 4 = dislike slightly; 3= dislike moderately; 2 = dislike very much; 1 = dislike extremely and invited to write comments about the wines. 2nd gen varieties are listed in order of average wine tasting score. n. a. = not applicable.

			Harvest data					Fruit chemistry data											Wine chemistry data						Sensory analysis							
																									Hedonic Scale 1-9					Wine tasting comments		
Variety name	Plant location	Muscat locus	Harvest date	Brix	Bunch no.	Yield (kg)	Ave. berry wt (g)	degrees Baumé	pH	TA (g/L)	Malic acid (g/L)	Tartaric acid (g/L)	TSS (g/L)	VA (g/L)	YAN (mg/L)	Alpha Amino (mg/L)	Gluconic acid (g/L)	Ammonia (mg/L)	Ethanol (%)	pH	TA (g/L)	Malic acid (g/L)	Gluc/Fruc (g/L)	VA (g/L)	Taster 1	Taster 2	Taster 3	Taster 4	Ave. score	Taster 1	Taster 2	Taster 4
Chardonnay	N23_13_P52		25/02/22	23.1	n. a.	n. a.	0.98	12.6	3.36	7.8	2.5	9.2	22.7	0.06	296	187	0.30	133	12.9	3.27	7.8	2.7	1.9	0.24	7	7	8	3	**6.25**			
Muscat Gordo Blanco	N24_4_P16		25/03/22	23.5	n. a.	n. a.	2.64	12.4	3.58	4.7	0.5	6.8	22.3	0	248	162	0.00	105	13.8	3.45	5.8	1.1	2.9	0.07	6	8	9	3	**6.5**			
Riesling	N23_9_P43		25/03/22	24.6	n. a.	n. a.	1.29	12.8	3.15	6.3	0.2	8	23	0.01	201	130	0.00	87	14.8	3.16	7.4	0.9	2.1	0.3	5	5	8	6	**6**			
Sauvignon Blanc	N23_10_P8		25/02/22	21.7	n. a.	n. a.	0.88	11.6	3.03	10.6	3.8	10.7	20.8	0.08	229	136	0.20	114	13.8	3.07	8.5	2.7	1.7	0.12	5	5	7	3	**5**			
Semillion	N23_9_P46		4/03/22	22.3	n. a.	n. a.	1.81	11.7	3.34	6.6	1.4	7.9	21	0.03	202	122	0.00	98	13.2	3.08	7.3	1.2	2.3	0.17	6	6	7	4	**5.75**			
15C002V0002	37_27_08_04		25/02/22	24.3	n. a.	n. a.	1.66	12.8	3.46	6.8	1.9	8.8	23	0.06	344	238	0.30	129	13.9	3.32	7	1.8	1.4	0.14	6	6	8	2	**5.5**			
15C2V2_6_87	37_20_02_02		18/03/22	19	42	1.83	0.71	9.9	3.6	5.3	0.6	7.1	17.8	0.05	255	194	0.00	74	11.7	3.44	6.1	0.7	1.8	0.09	5	7	8	8	**7**			great flavour, good mouthfeel, apricot
39170303_75_4	37_20_09_04	✓	1/04/22	22.5	60	3.03	0.91	11.9	3.69	4.3	1	7.3	21.5	0.04	190	142	0.00	59	13.7	3.43	6.2	0.9	1.6	0.24	6	7	9	6	**7**	hot, floral, good fruity taste	good	muscat, stonefruit
15C2V2_11_37	37_18_11_04	✓	4/03/22	21.6	29	4.26	1.23	11.3	3.72	4.7	1.7	6.2	20.4	0.05	238	184	0.00	66	13	3.33	7	1.3	1.9	0.15	6	7	7	6	**6.5**	fruity, floral, well balanced		mineral, acid
15C2V2_R04_1_27	37_20_10_03		18/03/22	27.7	44	2.50	0.46	14.5	3.7	4.4	0.1	9.4	26.1	0.1	239	180	0.00	71	14.6	3.33	5.9	0.4	2.6	0.15	6	7	6	7	**6.5**		textured	very much like Viognier
15C2V2_6_86	37_20_01_02	✓	4/03/22	23.6	21	2.30	1.40	12.5	3.82	4.5	1.8	7	22.5	0.07	255	192	0.00	78	14.5	3.61	7.1	2.3	0.7	0.19	6	8	6	5	**6.25**	rosy/floral/tropical, well balanced		acid, muscat
15C2V2_6_46	37_20_02_01		4/03/22	23.7	43	2.02	0.69	12.6	3.76	3.9	0	7.4	22.6	0.05	229	181	0.00	58	13.4	3.48	5.8	0.4	2	0.14	6	7	6	6	**6.25**			pleasant, floral, good mouthfeel
15C2V2_R04_1_10	37_20_04_03		25/02/22	24	29	1.54	0.81	12.9	3.52	6.1	1.3	7.6	23.2	0.03	290	207	0.00	102	14.5	3.44	6.2	1.6	1.4	0.09	7	5	6	7	**6.25**	passionfruit		very much like Sauv. Blanc
15C2V2_10_72	37_20_07_03	✓	1/04/22	20.6	111	4.30	0.94	10.8	3.86	3	0.4	5.8	19.5	0	256	203	0.00	65	12.3	3.4	6.1	0.7	1.9	0.18	6	7	6	6	**6.25**	very floral taste		acid, citrus
15C2V2_11_200	37_18_07_02	✓	18/03/22	26.1	35	4.02	1.21	13.2	3.99	5.4	2.4	9.5	23.7	0.04	268	253	0.10	19	13.3	3.48	5.7	0.8	1.7	0.12	5	7	7	5	**6**			strong muscat
39170901_120_2	37_18_10_04	✓	18/03/22	23.7	41	3.73	1.38	12.3	3.25	8.3	2.5	9.3	22.1	0.05	277	187	0.20	110	14.1	3.27	7.8	2.3	3.3	0.29	6	6	5	7	**6**	banana, tropical/citrus flavour, good acid		bubble gum/confectionary
15C2V2_10_65	37_20_07_02	✓	18/03/22	21.8	72	3.15	0.72	11.6	3.85	3.5	0	6.7	21	0.05	327	243	0.00	103	14.6	3.33	5.9	0.4	2.6	0.15	6	8	6	4	**6**			muscat
39170901_138_8	37_18_03_04		1/04/22	21.2	28	2.75	1.48	11.1	3.71	5.1	1.3	6.2	20	0.04	206	157	0.00	60	12.9	3.46	7.1	1.9	1.2	0.14	4	6	7	6	**5.75**			confection
39170901_138_1	37_18_06_01		18/03/22	23	25	2.22	1.47	12.2	3.8	4.8	2.1	7	21.9	0.03	270	200	0.00	86	14.1	3.41	6.6	1.7	1.8	0.13	7	6	6	4	**5.75**	muscat/floral flavour, well balanced		Riesling - "sweet" flavours
39170901_138_3	37_18_06_04		4/03/22	20.8	21	3.37	2.00	10.9	3.49	7	3.6	6.7	19.6	0.03	218	155	0.00	78	13.2	3.39	7.2	2.8	2.3	0.19	5	6	6	6	**5.75**			acid, good mouthfeel
15C2V2_11_99	37_18_07_01	✓	1/04/22	19.5	26	1.91	0.96	10.4	3.7	4.6	0.4	7.6	18.7	0.02	242	165	0.00	94	11.1	3.33	6.1	0.7	1.5	0.21	5	7	6	5	**5.75**	muscat aroma/flavour. Oily on palate		astringent, muscat
15C2V2_10_105	37_20_13_01	✓	25/02/22	24.6	37	1.57	1.14	13.1	3.64	5.1	1.6	7	23.6	0.05	225	169	0.00	69	14.8	3.39	6.4	1.8	1.9	0.21	6	7	8	2	**5.75**	very floral, fruity, well balanced	terpenes	
39170901_138_9	37_18_04_01		22/04/22	20.2	61	5.54	0.97	10.5	3.48	5.9	1.2	7.2	18.9	0.01	179	126	0.00	65	11.4	3.2	7	1.5	1.9	0.18	5	5	7	5	**5.5**			acid, floral, Riesling-like
15C2V2_R04_4_32	37_19_02_04		18/03/22	23.9	49	1.76	0.80	12.6	3.52	4.8	0.4	8.6	22.7	0.03	209	125	0.10	103	14.5	3.31	5.7	0.6	2.5	0.11	6	5	5	6	**5.5**	fruity, well balanced		Riesling-like, great mouthfeel
15C2V2_11_14	37_20_09_03	✓	25/03/22	20.7	18	2.66	1.06	11	3.64	4.2	0.5	6	19.8	0	156	114	0.00	52	11.9	3.37	5.5	0.8	3	0.15	6	5	7	4	**5.5**	floral, citrus	neutral	citrus, Riesling-like, caramel
39170901_138_14	37_18_06_02	✓	22/04/22	20.8	60	4.99	1.27	10.9	3.51	5.5	1.5	6.7	19.6	0	169	121	0.00	59	11.7	3.27	6.7	1.8	1.8	0.18	6	5	5	5	**5.25**	banana, passionfruit, tropical taste		muscat
15C2V2_10_143	37_18_12_01	✓	1/04/22	20.8	64	4.08	1.30	11	3.81	3.7	0.6	6.5	19.8	0.01	239	177	0.00	77	13	3.33	6.1	0.8	1.5	0.15	7	5	6	3	**5.25**	musty aroma/flavour, well balanced		muscat, sulphur
15C2V2_R04_4_69	37_19_01_02		8/04/22	16.8	70	2.43	0.72	8.4	3.42	5.8	0.3	8.2	15.2	0.02	237	144	0.00	113	9.8	3.11	7	0.3	1.5	0.14	3	6	6	6	**5.25**			stonefruit, tropical fruit
15C2V2_6_57	37_20_01_01	✓	18/03/22	19.8	55	3.18	0.78	10.4	3.78	4.2	1.2	7.6	18.7	0.04	206	149	0.00	70	12.4	3.27	6.6	0.9	2.3	0.11	6	3	8	4	**5.25**	musty aroma/flavour, oily, hot		strong muscat
15C2V2_10_43	37_20_07_01		4/03/22	22.9	72	2.85	0.79	12.1	3.73	4.8	1.2	7.1	21.8	0.05	286	210	0.00	93	14.3	3.51	6.2	1.4	1.5	0.2	5	7	5	4	**5.25**	apricot, nectarine	floral	muscat
15C2V2_10_30	37_20_09_02	✓	18/03/22	23.1	42	3.31	1.15	12.2	4.03	2.8	0.6	6.5	21.9	0.07	252	193	0.00	72	14.6	3.33	5.9	0.4	2.6	0.15	6	6	7	2	**5.25**	floral, citrus, fresh, well balanced	spicy	muscat
15C2V2_R04_1_71	37_20_05_02		8/04/22	21	36	5.92	0.81	10.8	3.38	6	0.1	8.9	19.5	0.01	259	178	0.00	100	12.8	3.28	5.9	0.6	1.7	0.1	6	5	3	6	**5**			Riesling-like, good mouthfeel
15C2V2_10_90	37_20_08_03	✓	1/04/22	23.9	31	2.15	0.90	12.7	3.79	4.2	0.9	7.9	22.9	0.02	187	134	0.00	65	13.4	3.32	6.7	1.2	2.2	0.15	6	4	5	5	**5**			strong muscat
15C2V2_R04_1_3	37_20_11_01		22/04/22	19.2	40	2.53	0.95	9.4	3.43	6.8	0.5	8.9	17	0	292	178	0.00	138	10.9	3.05	7	0.6	1.4	0.17	4	7	5	4	**5**		chalky	citrus, acid
39170901_173_1	37_18_04_02		8/04/22	20.5	63	2.34	1.32	10.6	3.81	2.6	0	5.6	19.2	0	247	164	0.00	101	13.1	3.39	5.8	0.5	2	0.15	5	5	5	4	**4.75**			neutral, butterscotch, full mouthfeel
15C2V2_10_178	37_18_11_02	✓	18/03/22	21.4	28	2.85	0.98	11.4	3.71	4.5	0.5	7.5	20.5	0	235	192	0.00	54	12.9	3.37	5.9	0.7	1.1	0.08	5	6	7	1	**4.75**	pale, watery		reduced/faulty?
15C2V2_R04_1_180	37_19_02_03		22/04/22	20.6	81	4.68	0.60	10.7	3.29	6.6	0.3	8.8	19.2	0.04	228	141	0.00	106	11	2.91	7.8	0.5	1.3	0.14	4	5	4	5	**4.5**			muscat, floral
15C2V2_11_251	37_18_07_03	✓	1/04/22	20.9	33	1.61	1.07	10.6	3.8	4.9	0.7	6.5	19.1	0	219	208	0.20	14	12.8	3.56	6.6	1.3	1.4	0.17	4	8	4	1	**4.25**			reduced/faulty?
15C2V2_R04_1_26	37_20_04_04		4/03/22	21.3	37	1.90	1.13	11.2	3.53	4.6	0.5	6.7	20.2	0.01	203	137	0.00	81	12.7	3.23	5.8	0.3	2	0.14	4	6	5	2	**4.25**	green/grassy		acid, citrus
15C2V2_11_320	37_18_07_04		25/02/22	24.9	25	1.19	1.21	13	3.75	4.6	1.2	7.1	23.4	0.06	261	203	0.00	71														
15C2V2_10_202	37_18_11_03		25/02/22	24.8	23	1.21	0.96	13.3	4	3.1	0.5	6.2	23.9	0.05	309	238	0.20	87														
39170303_75_3	37_20_01_03		1/04/22	20.5	34	0.88	0.61	10.8	3.77	4.2	1.3	7.8	19.5	0.05	145	111	0.00	41														
15C2V2_6_15	37_20_01_04		8/04/22	23.3	43	1.49	0.68	12.5	4.04	2	0	7.2	22.5	0	283	217	0.00	80														
39170303_75_9	37_20_02_03		25/03/22	20	24	0.65	0.71	10.2	3.21	7.8	1.3	9.9	18.4	0.08	173	105	0.00	84														
15C2V2_10_26	37_20_09_01		1/04/22	22.5	34	1.08	0.59	12	3.68	4.3	0.1	7.5	21.7	0	262	203	0.00	72														
15C2V2_10_117	37_20_13_02		18/03/22	23.9	38	0.78	0.64	12.7	3.9	3.8	0.6	6.6	22.8	0.06	245	199	0.00	56														

Appendix 5: Supplementary Figures



***Supplementary Figure 1**. Breeding strategy used for production of 1st gen. mildew-resistant varieties. Numbers in brackets show the estimated percent contribution of different parents to the genotype.*



***Supplementary Figure 2**. Breeding strategy used for generation of Strategy 3 breeding line 15C002V0002. Numbers in brackets show the estimated percent contribution of different parents to the genotype.*

Appendix 6: Staff

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