Selection of Clones for the Australian National Nuclear Grapevine Collection

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Many vineyards in Australia have vines with virus infections, which affect both yield and quality and can cause incompatibility between stock and scion in grafted vines. The testing of vines in grapevine collections for viruses in the late 1990s using ELISA methods revealed that some vines, previously thought to be free of virus, were in fact infected with leafroll virus. RT-PCR testing of grapevine collections for viruses, was first conducted in Australia by SARDI at Nuriootpa in 1998 and this further confirmed the presence of virus infections. This gave an urgent stimulus for the development of a totally new system for the maintenance of germplasm in Australia. A study tour in 1998 by myself to visit most research institutes involved with germplasm collections and virus testing in the USA, Canada and Europe revealed that two different types of grapevine collections were held in most other countries:

- Genetic resource collections – these are maintained to retain genetic diversity of those varieties currently used commercially and to preserve varieties not presently used. The varieties held are also useful for ampelographical comparisons and for breeding. They typically contain a large number of clones – for example the largest collection, at Vassal (near Montpellier in France), has over 3,000 varieties represented by more than 7,000 clones. Although there may be clones in these collections with viruses, such as leafroll, the virus status of clones may not be an issue.
- Nuclear collections of elite clones distributed in vine improvement schemes – these contain a much smaller number of clones which must be certified free of important viruses by biological indexing before they can be held in the collection.

In Australia, the largest genetic resource collections are held by CSIRO at Merbein in Victoria and SARDI at Nuriootpa in South Australia. There are also other smaller collections held in other states (deLaine and Nicholas 2000). Genetic resource collections are considered an unsatisfactory site to hold virus-tested clones. At this time there were no nuclear collections in Australia and a successful application was made by SARDI to Grape and Wine Research and Development Corporation (GWRDC) to fund a project on sanitary selection with the objectives:

- to select elite clones free of important viruses by using three virus detection procedures, for all varieties and rootstocks likely to be used in future winegrape plantings in Australia.
- to make these clones available for establishment in a new nuclear collection in an isolated location, to supply base material for vine improvement schemes.
- to publish a catalogue for industry containing information on all clones held in the nuclear collection.

Grapevine viruses

Grapevine viruses and sanitary selection were recently reviewed with reference to Australia (Nicholas 2004) – see this source for more information. Leafroll viruses are considered the most detrimental with reported yield losses varying from minor to more than 50%. Leafroll viruses can also affect grape quality by causing delayed maturity, and reduced fruit colour of red varieties. The most common leafroll-associated virus detected in diagnostic RT-PCR tests in Australia is GLRaV-3 followed by GLRaV-1 and GLRaV-9 (Habili and Symons 2000), (Habili and Rowhani 2002). GLRaV-2, which is less common, is reported to be involved with the incompatibility of scions grafted onto 5BB Kober (Greif et al. 1995). Other leafroll viruses that are less common are GLRaV-4, which is found in the Sultana clones H4 and H5 and GLRaV-5 found in a clone of Tempranillo.

Rugose wood is a complex disease caused by viruses. It is characterised by symptoms on the woody cylinder beneath the bark of the trunk. Symptoms are uncommon on ungrafted rootstocks and scions, but may appear following grafting. They include: swelling above the graft union, with a marked difference in diameter between the scion and rootstock; and pits or grooves on the woody cylinder. Vine yield and vigour are often reduced. Budburst may be delayed and vines may decline or even die. Water stress in combination with rugose wood and leafroll viruses will increase the severity of symptoms.

Four different rugose wood disorders can be distinguished by biological indexing (Garau et al. 1997). These are Rupestris stem pitting, Kober stem grooving, corky bark and LN33 stem grooving. Rupestris stem pitting symptoms have been associated with the Foveavirus RSPaV, which is present in most Australian vineyards—this may not be of major economic significance for vines on their own roots (Reynolds et al. 1997).

The phloem-limited Vitivirus Grapevine virus A (GVA) is the agent of Kober stem grooving (Garau et al. 1994), (Chevalier et al. 1995). It can be symptomless in some varieties, but has been associated with Shiraz Disease in South Africa (Goszczynski and Jooste 2003) and Australia (Habili and Randles 2004). Symptoms

of Shiraz Disease include: delayed budburst; stunted growth; canes which never mature; and leaves with leafroll-virus-like symptoms that do not drop in winter.

Selection of elite clones

In the GWRDC funded project, over 300 candidate clones for the nuclear collection were initially selected in close consultation with the Australian Vine Improvement Association from existing clones in vine improvement schemes and other clones considered potentially useful from their known performance in trials. For each candidate, a vine was selected (mostly by using pre-existing RT-PCR data) from the genetic resource collections held by SARDI at Nuriootpa in South Australia, Department of Primary Industries at Irymple and CSIRO at Merbein in Victoria. Cuttings of the candidate were taken from this vine for the clone to undergo sanitary selection. This has involved rapid screening of the clones for viruses using both RT-PCR and ELISA (Figure 1) laboratory methods and the remaining ones have then been biologically indexed (Figure 2). The laboratory methods and indicators used to select clones for the Australian National Nuclear Grapevine Collection are given in Table 1. Results from all three methods are complementary and their combined use provides a more reliable indication of virus status.

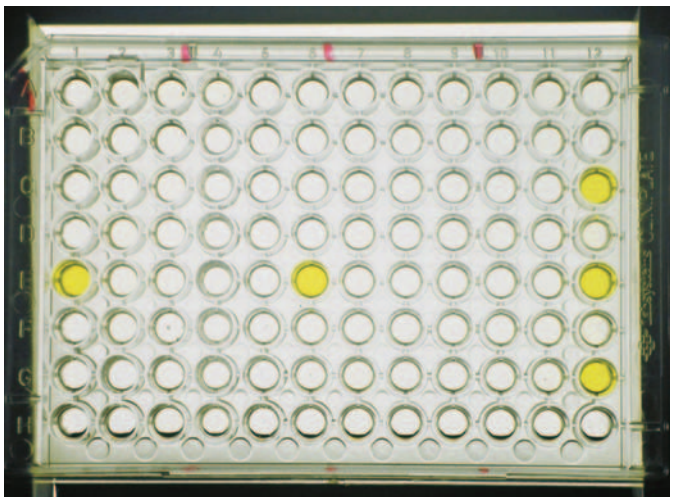

Fig. 1. An ELISA plate showing the positive reaction to Grapevine leafroll-associated virus 3 in yellow (5 of the 96 samples tested positive) – supplied by N Habili, Waite Diagnostics.

Fig. 2. In biological indexing, Kober stem grooving symptoms develop after inoculation of 5BB Kober with a clone which has GVA (seen after removal of bark).

Table 1. Virus detection methods and indexing indicators used for sanitary selection of elite clones for the Australian National Nuclear Grapevine Collection

Virus diseases	RT-PCR tests for pathogen	ELISA tests for pathogen	Biological indexing indicator
Leafroll			
Grapevine leafroll	GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, GLRaV-9, GRSLaV	GLRaV-1, GLRaV-3	Cabernet Franc
Rugose wood complex			
Rupestris stem pitting	RSPaV		Rupestris St George
Kober stem grooving	GVA		5BB Kober
Grapevine corky-bark	GVB		LN33
LN33 stem grooving			LN33
Fleck			
Grapevine fleck virus	GFkV-A, GFkV-B		Rupestris St George
Fanleaf			
Grapevine fanleaf virus	GFLV		Rupestris St George

Biological indexing involves grafting candidates and indicators together and the resultant vines are then grown for several years in the field to observe any virus symptoms i.e. indexing detects the disease rather than the associated virus. Indexing with woody indicators was used prior to the availability of laboratory methods. The early indexing work in Victoria was reported by Shanmuganathan and Fletcher (1980) and in South Australia (SARDI) by Cirami et al. (1998). Subsequent indexing in Australia was continued by G. Fletcher and by L. Krake from CSIRO (Krake et al. 1999). The biological indexing program for this project began in year 2000 and both G. Fletcher and L. Krake were initially closely involved. Vines being indexed were trellised to assist observation of symptoms. They have covered a 1.7 hectare area. SARDI has established a virus reference collection of vines with specific viruses at Loxton as has been done in Europe (Greif and Walter 1997). Each year that vines have been grafted for indexing at Loxton, positive control vines (i.e. vines with known viruses grafted to indicators) have been established for comparison with candidates to assist in identification of symptoms. Indexing in the GWRDC project will be completed in 2006.

Australian National Nuclear Grapevine Collection

The Australian Vine Improvement Association has recognised the unique quality of this material and established the Australian National Nuclear Grapevine Collection of 200 of these elite clones in 2004 in virgin land at the NSW DPI Research Station at Dareton (Kerridge 2005) see Figure 3. It is being managed under industry agreed protocols.

The vines have been checked by ampelographer George Kerridge for trueness to type. All vines in the nuclear collection will be periodically laboratory tested and checked visually to ensure that they remain free of viruses. The clones will be made available to vine improvement groups in Australia wishing to distribute them provided that it can be ensured that their continued integrity and future health status is not compromised.

As part of this project a catalogue is being finalised which contains more detailed information on the clones selected in the project for planting in nuclear collections. The catalogue be will available on the SARDI website http://www.sardi.sa.gov.au and will also be accessible via a link to the AVIA website http://www.avia.org.au/.

Fig. 3. Australian National Nuclear Grapevine Collection

The longer term benefits arising from the project will be that vineyards planted with propagation material sourced through vine improvement schemes will have superior health status and not be subject to the detrimental effects of virus infections, which include reduced graft take and rooting in the nursery, progressive decline of vines, reduced yield and quality of grapes and reduced longevity of plantings.

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